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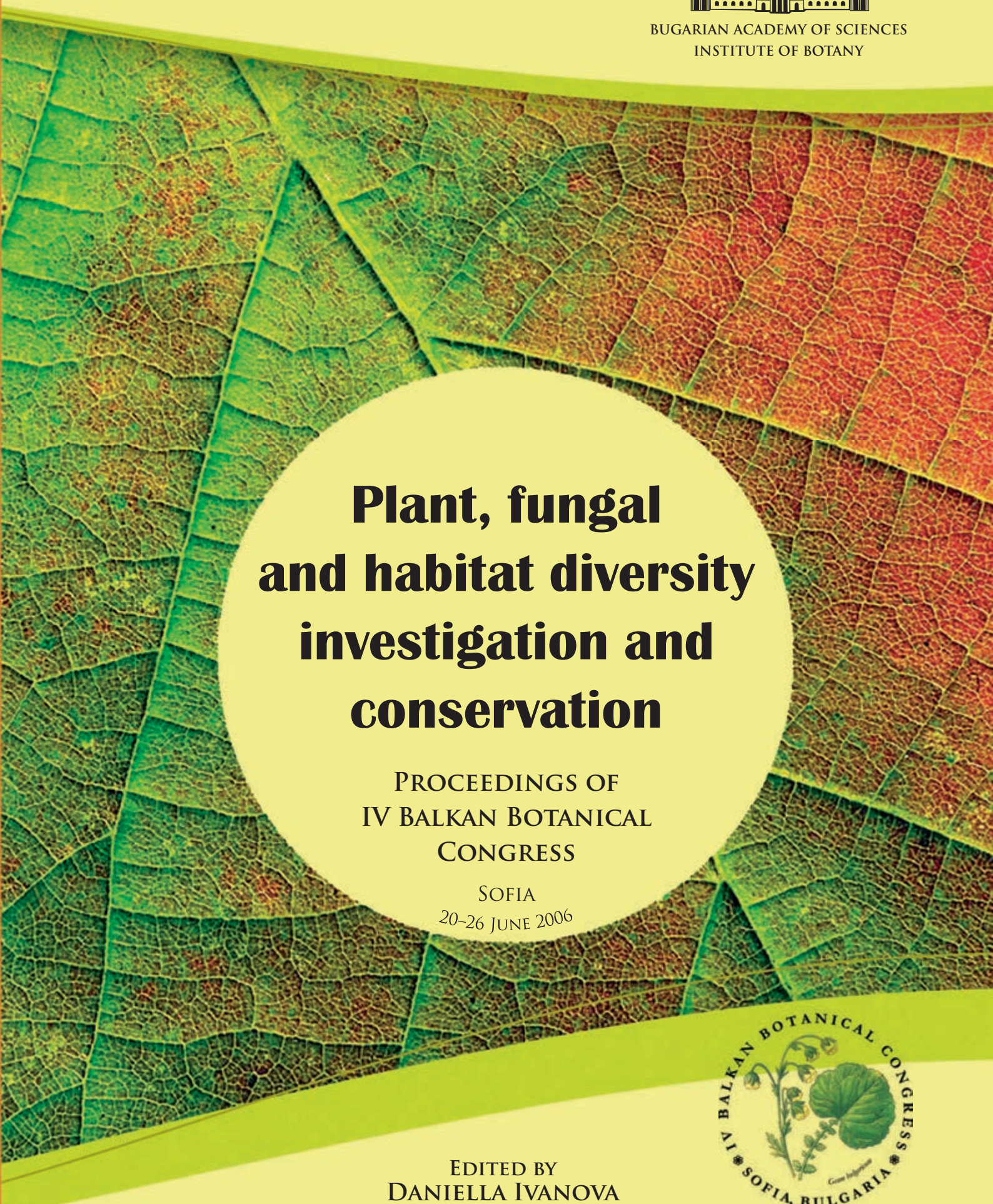

Plant, fungal and habitat diversity investigation and conservation

Proceedings of IV Balkan Botanical Congress

Sofia
20–26 June 2006

Edited by
Daniella Ivanova

IV Balkan Botanical Congress
Sofia, Bulgaria

Plant, fungal and habitat diversity investigation and conservation

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Published by Institute of Botany, Bulgarian Academy of Sciences,
Acad. G. Bonchev St., bl. 23, 1113 Sofia, Bulgaria
web site: www.bio.bas.bg/botany/

Edited by Daniella Ivanova

Page make-up by Simeon Simeonov

Citation: Ivanova, D. (ed.). 2009. Plant, fungal and habitat diversity investigation and conservation. Proceedings of IV Balkan Botanical Congress, Sofia, 20–26 June 2006. Institute of Botany, Sofia.

ISBN 978-954-9746-14-3

Printed by Prof. Marin Drinov Academic Publishing House
Acad. G. Bonchev St., bl. 6, 1113 Sofia, Bulgaria

PLANT, FUNGAL AND HABITAT DIVERSITY INVESTIGATION AND CONSERVATION

Proceedings of IV Balkan Botanical Congress

Sofia, 20–26 June 2006

INSTITUTE OF BOTANY **BULGARIAN ACADEMY OF SCIENCES**

ISBN 978-954-9746-14-3

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PREFACE

Dear colleagues, participants in the IV Balkan Botanical Congress!

What is this volume in front of You? This is the result of the efforts of more than 300 scientists from 29 countries!

It is a mosaic of scientific studies of different level of complexity – from profound scientific investigation to research of more popular character. The great efforts of the editors have not been in vain! Now we have a volume that is more or less unified in facts and graphic design. But still, for some facts the authors carry the complete responsibility.

The creative and friendly atmosphere and the constructive discussions in Sofia make me feel optimistic about the future of the Balkan botany!

Prof. D.Sc. Dimitar Peev
/President of IV Balkan Botanical Congress/

EDITORIAL FOREWORD

The Fourth Balkan Botanical Congress entitled "Plant, fungal and habitat diversity investigation and conservation" was held in Sofia, Bulgaria, on 20–26 June 2006. It was organised by the Institute of Botany of the Bulgarian Academy of Sciences (BAS) under the auspices of the President of BAS. The Congress reflected the broad interests in botany and succeeded in attracting a broad spectrum of specialists. Over 370 delegates from 29 countries took part in the event.

The present Proceedings contain a considerable part of the papers (oral and posters) of the Congress. They comprise 130 original articles and represent the range of botanical research topics presented during the meeting. Many of these contributions contain new scientific information and/or extend and improve the previous state of knowledge. The articles are grouped according to the 7 original sessions as follows:

- Scientific Area A — *Plant structure, function and development*;
- Scientific Area B — *Plant diversity – past and present*;
- Scientific Area C — *Plant ecology. Vegetation and habitat diversity*;
- Scientific Area D — *Fungal diversity*;
- Scientific Area E — *Plant and habitat conservation*;
- Scientific Area F — *Ethnobotany and phytochemistry*;
- Scientific Area G — *Botanical collections and education in botany*.

The editor made the necessary efforts to reach uniformity in the papers' style; however, the sole responsibility for the whole contents of the paper (views and statements) remains only to the author(s). Readers seeking further information are encouraged to contact the relevant author(s) directly.

According to the standards of our edition, citations of plant name authors follow Brummitt, R.K. & Powell, C.E. (eds). 1992. Authors of Plant Names. Royal Botanic Gardens, Kew. Titles of periodicals are abbreviated in conformity with the updated version of Botanico-Periodicum-Huntianum [Bridson, G.D.R. (compiler), Townsend, S.T. (editor), Polen, E.A. (editor) & Smith, E.R. (editorial assistant). 2004. BPH–2. Periodicals with botanical content. Constituting a second edition of Botanico-Periodicum-Huntianum. Vols 1 & 2. Hunt Institute for Botanical Documentation, Carnegie Mellon University, Pittsburgh]. Titles of periodicals not listed in BPH–2 are abbreviated according to the abbreviation standards there defined.

The editor would like to thank all the authors for their contributions to the Proceedings and for their collaboration for the improvement of paper quality. Also, financial support of numerous sponsors (shown on the outside back cover) is gratefully acknowledged.

Daniella Ivanova

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SCIENTIFIC AREA A

PLANT STRUCTURE, FUNCTION AND DEVELOPMENT

Root development of *Picea abies* seedlings in different substrata

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Abstract. The study shows the influence of used substratum types on root development of one-year-old seedlings in nursery plantation. The carried out sowing of the *Picea abies* seeds occurred in substratum of peat, Goc and humus taken from natural sources, which differed in texture and chemical composition. The experiment was applied in eight variations of pure or mixed combinations of substrata. During fertilization, the regular steps of nursering were applied and the development of seedlings in all variations followed. A morphometric root analysis was done at the end of vegetation. The results of this study confirmed that the used type of substratum has a significant influence to seedling root development.

Key words: specific containers, spruce, substratum

Introduction

For a successful forestation it is necessary to use the nursery plants which have well developed overground parts, but also must have a well developed root system. Well branched roots need veins of first, second and third orders, significant for operating the entire root system and will not eventually become stiff roots (2 mm) as they are responsible for water absorption and mineral supply from the soil. The substratum which is used in the production of plants plays one of the most important roles in the development of the roots.

This study shows how the used substrata and their mixtures affect the development of roots in one-year-old spruce seedlings which are produced in specific containers.

Material and methods

The study of the influence of different substrata to root development was done in the nursery of the Institute of Forestry, Belgrade.

The *Picea abies* (L.) H. Karst. seeds from the Krusevac region were sowed. Seed health condition before sowing was analysed, and a seed prevention treatment was completed (sterilization for 20 min with 30 % of H_2O_2 and rinse 3 times in sterile water). Substratum preventive treatment was also done with an organic – venturin and inorganic – cupersulfide medium.

Used substrata:

Peat – product from Ukraina;

Substratum Goc – product of Faculty of Forestry, Belgrade;

Substratum from two *P. abies* plantations (A & B) in Jastrebac Mt, Serbia region.

Substrata were used in different volumetric quantity (Table 1).

Table 1. Volumetric quantity of used substrata in %.

	Peat	Substratum Goc	Jastrebac A	Jastrebac B
I	100			
II	50		50	
III	50			50
IV		100		
V		50	50	
VI		50		50
VII			100	
VIII				100

The supplementary feeding and water sprinkling were done at the same time for the entire experiment.

In the pedological Laboratories of the Institute of Forestry and the Faculty of Forestry in Belgrade, physical and chemical properties of the soil were

analysed by standard pedological analyses (Tables 2, 3) (Veselinović & al. 2003; Šijačić-Nikolić & al. 2006).

Results have been processed by standard statistical methods.

Table 2. Physical properties of the substrata.

Substratum	Particle size of substratum					
	Coarse sand	Fine sand	Silt	Clay	Total sand	Total clay
	%					
Jastrebac A	35.60	31.70	15.10	17.60	67.30	32.70
Jastrebac B	42.40	27.20	13.60	16.80	69.60	30.40
Goc	3.83	32.97	19.20	18.80	56.00	44.00

Table 3. Chemical properties of the substrata.

Substratum	pH	Humus (%)	N (%)	P_2O_5 (mg/100)	K_2O (mg/100)	C–N
Peat	5.10	–	3.80	<20	<20	12.20
Jastrebac A	4.80	8.27	0.39	46.95	0.00	–
Jastrebac B	4.00	8.60	0.42	46.95	0.00	–
Goc	6.89	8.21	0.40	8.00	14.50	11.90

Results and discussion

Analysis of the effect of different substrata on the number of surviving and bent plants

The first control of the plants was done 4 weeks after seed sowing while the second was done at the end of the vegetative season. Phytopathological analysis of the bending plants was done during the first control of germination. The *Fusarium oxisporum* Seh. caused the bending of the seedlings (Lazarev 2005).

Data of the seedlings counted during the first control showed a higher percent of germinated plants in 100 % of Goc, 100 % of Jastrebac B, and mixture of 50 % of Goc and 50 % of Jastrebac B substrata. At the end of the vegetative season, a higher percentage of plants was found in 100 % of Jastrebac B, 100 % of Goc, and mixture of 50 % of Goc and 50 % of Jastrebac A substrata. The best results for germination and the greatest survival of plants was found in substrata containing 100 % of Goc or 100 % of Jastrebac B (Table 4).

Analysis of the influence of different substrata on the quality of the developed roots

In the study of the influence of different substrata on root development the following parameters were taken into consideration: length of the root; diameter of the root collar; the weight of the root biomass (fresh and air dried) (Veselinović & al. 2001).

Table 4. Percentage of germinated plants.

Substratum	After 4 weeks	At the end of vegetative season	Bent plants
I	34.8	58.0	1.2
II	23.2	53.6	1.08
III	23.2	42.02	0.04
IV	**40.6**	**65.2**	0.02
V	36.2	**62.3**	1.05
VI	**40.6**	43.5	1.7
VII	26.1	36.2	0.6
VIII	**42.0**	**60.9**	0.7

Substratum influence on root weight. Seedlings cultivated in mixture of 50 % of Peat and 50 % of Jastrebac A substratum or 50 % of Peat and 50 % of Jastrebac B attained the greatest weights with an average weight of 1.146–1.474 g (Table 5). The smallest weights were found in seedlings cultivated in 100 % of Jastrebac A and 100 % of Peat substratum. There is no influence of the other substrata on root weight.

Table 5. Root weight (fresh and air dry).

Substratum	Root biomass weight (g)	
	fresh	air dry
I	0.298	0.146
II	**1.474**	0.505
III	**1.146**	0.460
IV	1.041	0.381
V	0.467	0.239
VI	0.592	0.298
VII	0.297	0.171
VIII	0.547	0.324

Substratum influence on root length and diameter of root collar. The growth of the roots was worst in the pure Peat substratum (6.28 cm).

There were statistically significant improvements in the root's lengths in the 100 % Goc, and mixture of 50 % of Goc and 50 % of Jastrebac B substrata, and the greatest root length was found in the mixture of 50 % of Goc and 50 % of Jastrebac A (10.63 cm) (Table 6).

The worst results of the diameter of the root's collar occured in plants cultivated in pure Peat, Jastrebac A and Jastrebac B substrata. Statistically significant improvements of the root's collar occured in the Goc substratum and its mixtures. The best results were received in the mixture of 50 % of Goc and 50 % of Jastrebac B substratum.

Substratum has an influence on the shape of the root. Plants grown in the mixture of substrata contain-

ing Jastrebac A and Jastrebac B have quite well developed root system with well branched roots.

Table 6. Root collar and length in different substrata.

Substratum	Root collar	Root length
I	0.27 ± 0.04^{a}	6.28 ± 0.85^{a}
II	0.32 ± 0.02^{ab}	8.36 ± 0.51^{bc}
III	0.37 ± 0.03^{bcd}	7.93 ± 0.62^{ab}
IV	0.38 ± 0.02^{cd}	10.33 ± 0.489^{d}
V	0.32 ± 0.03^{abc}	10.63 ± 0.74^{d}
VI	0.43 ± 0.03^{d}	10.24 ± 0.66^{d}
VII	0.24 ± 0.04^{a}	10.29 ± 0.89^{cd}
VIII	0.27 ± 0.03^{a}	10.09 ± 0.79^{cd}

Multifactorial test in ranges – amounts recorded with the same letters in columns do not show a difference on a significance level of $p < 0.05$.

Conclusions

The results of the present study show that various organic substrata in the production of *P. abies* by containers were very influential on the formation of its roots.

The greatest numbers of surviving plants at the end of vegetation were those planted in 100 % of the Goc substratum.

The number of the bent plants in the studied substrata is significantly less than those in which acid content increased.

The achieved weight of the roots is best if the substratum in which they are cultivated is a combination of Peat with substratum Jastrebac A or Jastrebac B.

The most excellent length of the roots of *P. abies* is achieved with the use of 50 % of the Goc and 50 % of the Jastrebac A substratum.

Plants cultivated in 50 % of the Goc and 50 % of the Jastrebac B substratum achieved the greatest diameter of the root collar.

From the analysis it is evident that plants cultivated in different combinations of substrata from cultures of the *P. abies* on Jastrebac Mt (A & B) achieved the best results.

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Morphogenesis of some Siberian species of the genus *Dracocephalum* (*Lamiaceae*)

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Abstract. Morphogenesis of 5 species of *Dracocephalum*, growing in Siberia, has been studied. Eight phases of morphogenesis have been determined in accordance with those, characterized by Russian scientists.

Key words: *Dracocephalum*, life forms, morphogenesis, ontogenetic state, ontogeny

Introduction

Formation of life forms of an adult individual takes place in ontogeny. Morphological structure of the individual, which researchers regard as alteration of morphological phases, changes in ontogeny. Study of ontogeny of plants and determination of changes of morphological structures under definite ecological conditions allow understanding the ways of adaptation of individuals to growth conditions. To date, as a result of research of ontomorphogenesis of a great number of species, 16 phases of morphogenesis have been described. It has been established that for plants of one or some biomorphs a definite sequence of changing morphogenesis variants in ontogeny exists (Serebryakov & Chernysheva 1955; Ryberg 1959; Serebryakova 1971; Smirnova & Uranov 1976).

The genus *Dracocephalum* L. is the most abundant in montane areas of Siberia, Kazakhstan, Middle Asia and Mediterranean Sea where the species are found mainly in the middle and upper belts (Budantsev 1987). In Siberia there are 24 species of this genus (Malyshev 1992), belonging to 7 life forms (Nozirova 2004).

Ontomorphogenesis of taprooted plants such as: non-particulated semirosular annual *D. foetidum* Bunge and particulated herbaceous oligocarpic with semirosular shoots *D. nutans* L.; multicapitate taprooted particulated dwarf semishrub *D. fruticulosum* Steph., multicapitate taprooted particulated perennial plant with rosellate shoots *D. imberbe* Bunge; and *D. grandiflorum* L. has been studied.

Most of *Dracocephalum* species are xeric plants. *D. foetidum*, *D. imberbe* and *D. fruticulosom* are widespread in mountain desert-steppes and in various petrophytic sites: on dry rocky and talus slopes, sandy deposits of arid intermountain basins. They are also characterized by different altitudinal positions. *D. foetidum* is found in the middle and lower parts of mountain ridges. *D. imberbe* occurs in the middle mountain belt. *D. fruticulosum* is typical of the middle and upper mountain belts.

Some *Dracocephalum* species are spread in mesic and moderately xeric sites. *D. nutans* occurs in meadows, meadow-steppes, light-coniferous and birch forests and in various stony habitats on mountain slopes. *D. grandiflorum* is typical of higher altitudes. It occurs in alpine, subalpine meadows and in the tundra communities with lichens and *Betula rotundifolia* as well. Sometimes *D. grandiflorum* is found at timberline.

Material and methods

When studying ontomorphogenesis, methods developed by Serebryakova (1971) and Smirnova & Uranov (1976) were used.

Peculiarities of ontomorphogenesis of *D. foetidum* were studied in individuals growing near Naryn village in the Tuva Republic, in sandy steppe with a pre-

dominance of *Artemisia arenaria*, *Cleistogenes squarrosa*, *Potentilla acaulis*, *Panzerina lanata*.

Ontomorphogenesis of *D. fruticulosum* was studied in petrophytic mountain steppe with *Selaginella sanguinolenta* (*S. sanguinolenta*, *Carex rupestris*, *Caragana bungei*, *Artemisia frigida*, *Potentilla acaulus*) on the steep slope near Khairakan village (Tuva Republic).

The materials for the study of ontomorphogenesis of *D. nutans* and *D. imberbe* were collected in the South-Eastern Altai. Ontomorphogenesis of *D. nutans* was studied in the Dzhazator River basin in stonecrop meadow-steppe petrophyte community (*Sedum hybridum*, *Festuca valesiaca*, *Phleum phleoides*, *Dracocephalum nutans*, *Stipa capillata*, *Carex pediformis*).

Ontomorphogenesis of *D. imberbe* was described on pebbles near Dzhazator village.

The course of ontomorphogenesis of *D. grandiflorum* individual was studied by us in Ust–Kansk area of the Altai Mts near Kaisyn village in alpine fescue-low-herbaceous meadow (*Carex ledebouriana*, *Festuca ovina*, *D. grandiflorum*, *Oxytropis strobilacea*, *Dasystephana algida*, *Helictotrichon hookeri*).

Results and discussion

In ontogeny of the species studied 8 phases of morphogenesis were determined:

Primary shoot – a leading shoot, developing from a seed and having seed and secondary roots.

Primary bush – a bush as a result of branching of a leading shoot in its basal part.

A loose bush – a bush as a result of manifold branching of a leading shoot in its basal part; the root-system is mixed.

Tillering particle (a secondary bush) – a bush, as a result of division of caudex, which keeps branching. Tillering particles are spatially separated.

Not-tillering particle – a shoot that lost ability to branch.

A system of partial bushes and shoots – a morphological formation as a result of vegetative reproduction of an ovule parent; it is represented by only partial shoots and bushes, connected with each other by long communication rhizomes; the root-system is adventitious.

Partial bush – a bush emerged after destruction of communication rhizomes in a system of partial bushes and shoots; the root-system is adventitious.

Partial shoot – a shoot emerged after destruction of communication rhizomes in a system of partial bushes and shoots; the root-system is adventitious.

A comparison of the morphogenesis of the individuals on model dragonheads, forming different life forms, allowed determining 4 variants of the morphogenesis.

The 1st variant was described in ontogeny of taprooted semirosular annual *D. foetidum*. The sequence of morphogenesis phases is the following: primary shoot–primary bush (Fig. 1).

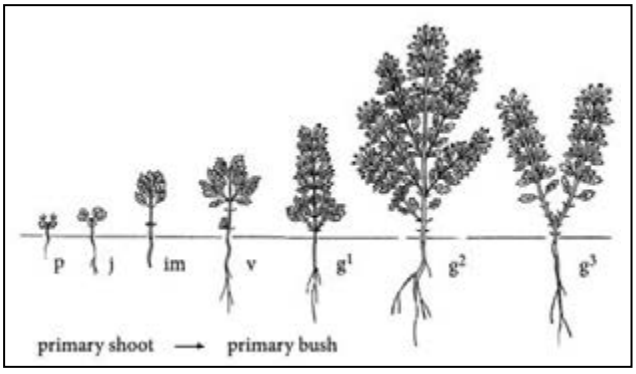

Fig. 1. Morphogenesis of taprooted annual plant *D. foetidum*: **p** – sprout; **j** – juvenile state; **im** – immature state; **v** – virginal state; **g¹** – young generative state; **g²** – adult generative state; **g³** – old generative state.

From the moment of germination up to virginal state, *D. foetidum* exists in the phase of a primary shoot. Formation of an upper rosellate shoot (Nukhimovskiy's term 1997) is typical for immature plants. In virginal ontogenetic state the shoot branches out. A primary bush consisting of 3–4 upper rosellate shoots is formed.

Young generative plants mainly represent one generative shoot and 1–4 upper rosellate vegetative shoots. Generative shoots are orthotropous, elongated. When practically all vegetative shoots become generative, the plants pass to middle-aged generative state. After fruiting the plant dies off.

The 2nd variant of morphogenesis was studied in ontogeny of taprooted herbaceous oligocarpic with semirosular shoots *D. nutans*. The individuals pass through the following stages: primary shoot–primary bush–loose bush–not-tillering particle (Fig. 2).

A stage of primary shoot is characteristic of the juvenile individuals and sprouts. Juvenile individuals are represented by a rosellate shoot. A primary bush is formed in immature ontogenetic state. Main and side shoots are formed monopodially. Due to length-

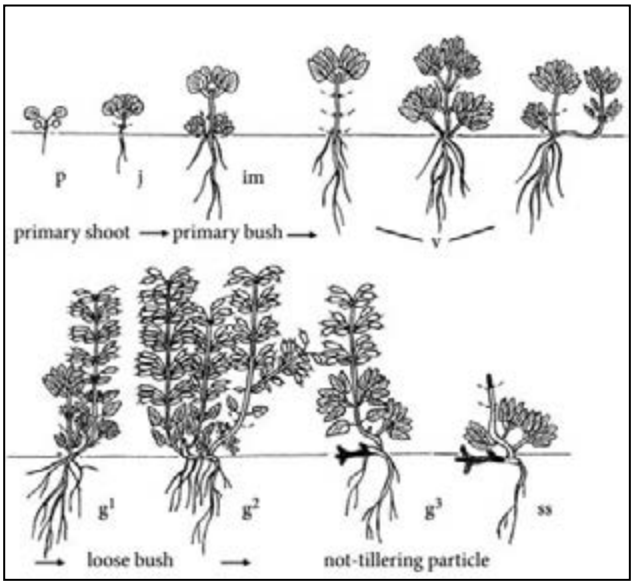

Fig. 2. Morphogenesis of taprooted herbaceous oligocarpic plant *D. nutans*: **p** – sprout; **j** – juvenile state; **im** – immature state; **v** – virginal state; **g¹** – young generative state; **g²** – adult generative state; **g³** – old generative state; **ss** – subsenile state.

ening of epicotyl the main shoot becomes upper rosellate and side shoots become rosellate. The root-system is mixed.

In the third year plants pass to virginal state. The primary bush consists of 3–5 upper rosellate shoots. Main and side shoots may fall, but do not root. The leading shoot comes into flower in the 3rd–4th year. After die-off of a peduncle monopodial growth changes to sympodial one. Young generative plants are represented by a loose bush which consists of 1–2 oblong dicyclic generative shoots and 1–8 upper rosellate vegetative shoots. In old generative state particulation of the bush takes place, as a result of which the most part of the bush dies off. Only particle which has a main root keeps safe. Particles have a small number of generative and vegetative shoots. The subsenile individual is characterized by unfolding from cauline buds 1–3 upper rosellate shoots. Senile individuals were not found in the coenopopulations studied.

The 3rd variant of morphogenesis: primary shoot–primary bush–loose bush–tillering particle–not-tillering particle was determined in ontogeny of some life forms of multicapitate taprooted dwarf semishrub *D. fruticulosum* (Fig. 3) and perennial plant with rosellate shoots *D. imberbe* (Fig. 4). The stage of a primary shoot in ontomorphogenesis of *D. fruticulosum* is not long and is characteristic of sprouts (Fig. 3).

The plants pass to juvenile state this or next year. In the next year change of growth of plants from mo-

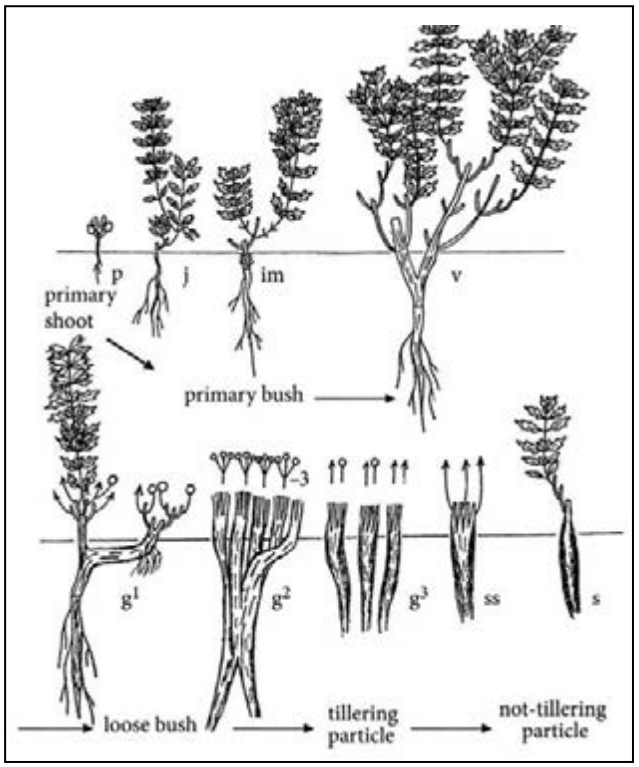

Fig. 3. Morphogenesis of multicapitate taprooted dwarf semishrub *D. fruticulosum*: **p** – sprout; **j** – juvenile state; **im** – immature state; **v** – virginal state; **g¹** – young generative state; **g²** – adult generative state; **g³** – old generative state; **ss** – subsenile state; **s** – senile state.

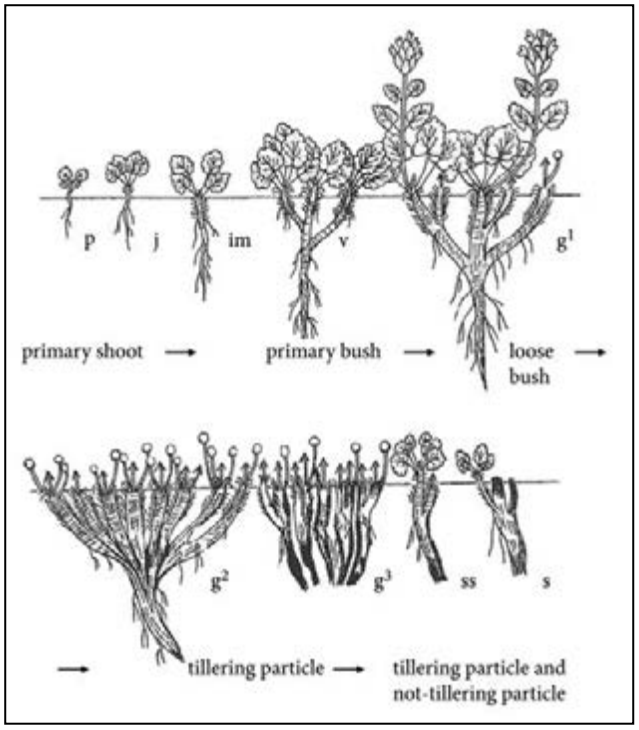

Fig. 4. Morphogenesis of multicapitate taprooted dwarf perennial plant *D. imberbe*: **p** – sprout; **j** – juvenile state; **im** – immature state; **v** – virginal state; **g¹** – young generative state; **g²** – adult generative state; **g³** – old generative state; **ss** – subsenile state; **s** – senile state.

nopodial to sympodial one takes place. The individuals of juvenile state form a primary bush which consists of 2–3 elongated shoots. By the fourth or fifth year plants of immature state have two or three elongated shoots of innovation. Caudex begins to form owing to thickening of the basal parts of innovation shoots (one node) of the previous years.

From the beginning of virginal state up to mature generative state the plants are represented by a loose bush. In virginal state the bush consists of 2 or 3 axes of innovation (systems of shoots of formation). Each of axes consists of 2–3 elongated shoots of innovation. Basal parts of shoots of the 2nd–4th orders lignify up to 2–4 nodes. Caudex becomes bicipitate. In middle-aged generative state the bush spreads out, the number of axes of innovation increases (up to 23), which is connected with unfolding of dormant buds on the basal part of caudex. All shoots of axes of innovation are in flowers.

A clone consisting of 3–5 viable tillering particles is formed in old generative state. Subsenile plants are represented by solitary not-tillering particles.

Though the given variant of morphogenesis is described for the species of different life forms, the time of passing through morphogenesis phases is different in all species studied.

Phase of a primary shoot of the individuals of *D. imberbe* is longer. After overground germination of a seed of *D. imberbe*, the phase of the primary shoot comes, it is over in the virginal state (Fig. 4).

The primary shoot is rosellate. Virginal plants are represented by a primary bush, consisting of 3–4 rosellate shoots. Growth is monopodial. In virginal state a multicapitate caudex consisting of 2–3 capitates is formed.

The loose bush of young generative plants consists of 2–3 polycarpic (Mikhailova's term 1971) and 2–3 vegetative rosellate shoots. The number of heads of the caudex increases up to 5. As a result of senile particulation of the caudex of *D. imberbe* and *D. fruticulosum*, old generative individuals of both are represented by 5–8 tillering particles forming a compact clone. Each particle is represented by 1–3 vegetative and 1–2 polycarpic shoots.

Subsenile plants are represented by separate not-flowering tillering and not-tillering particles. In senile state the individuals do not branch out.

Thus, the course of morphogenesis of the individuals of semishrub *D. fruticulosum* and perennial *D. imberbe* is analogous.

The 4th variant of morphogenesis: primary shoot–primary bush–system of partial bushes and shoots–partial bush–partial shoot was described in short-rooted–loose bushed perennial plant *D. grandiflorum* (Fig. 5).

Initial stages of ontomorphogenesis of *D. grandiflorum* individual run in phase of a primary rosellate shoot. Basal part of the shoot of the juvenile plants is retracted to the ground owing to contractile activity of the main root. A short epigeogenous rhizome is formed.

The primary bush is formed in plants of seed origin in virginal ontogenetic state; it consists of simple and branched rosellate shoots connected by epigeogenous horizontal rhizomes with a system of adventitious roots. The main root dies off. Virginal individuals of vegetative origin are formed during particulation in young and middle-aged generative states and are represented by partial bushes and shoots. Partial bushes

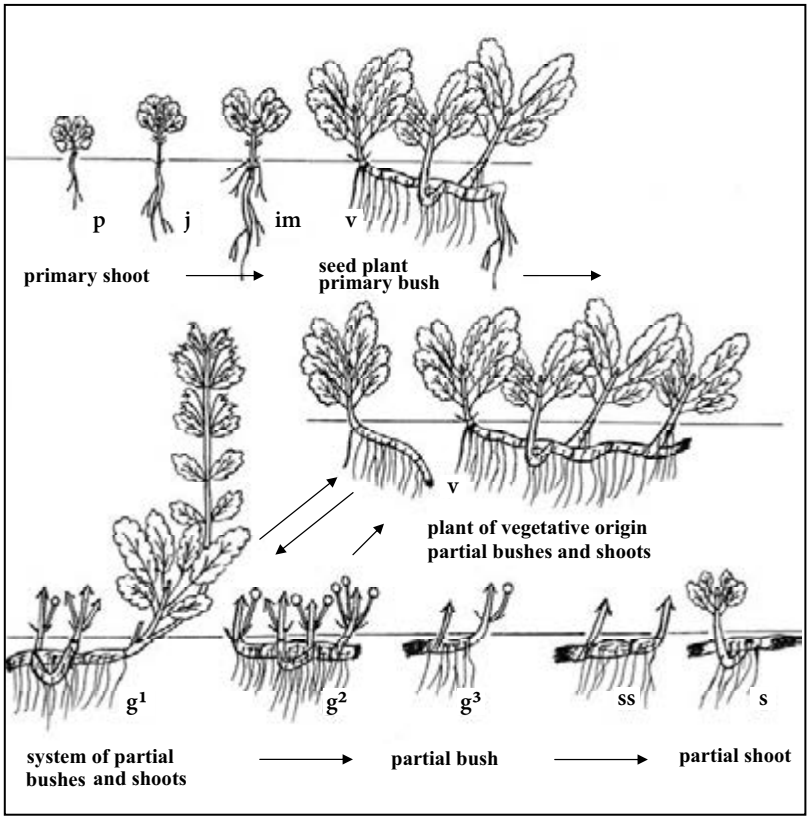

Fig. 5. Morphogenesis of short-rooted perennial plant *D. grandiflorum*: **p** – sprout; **j** – juvenile state; **im** – immature state; **v** – virginal state; **g¹** – young generative state; **g²** – adult generative state; **g³** – old generative state; **ss** – subsenile state; **s** – senile state.

consist of 2–3 rosellate shoots and 1–2 branches of epigeogenous rhizome.

The individuals, which have seed and vegetative origins, in young generative state branch out and particulate, forming a system of partial bushes and shoots. This system consists of 1–2 polycarpic and 2–4 vegetative rosellate shoots. Only side shoots of a polycarpic shoot become monocarpic shoots. Particulation is possible, which leads to destruction of the system of partial bushes and shoots and to formation of rejuvenated up to the virginal state filial individuals. The system of partial bushes and shoots is destroyed in old generative state. The individuals of this ontogenetic state are represented by partial bushes which consist of 1–2 polycarpic and 1–3 vegetative shoots. One or two rosellate shoots unfold from dormant buds on the rhizome of subsenile individuals. Senile plants are represented by partial shoots.

Comparison of the course of ontogeny of the *Dracocephalum* species with different life forms has shown that individuals of the semishrub *D. fruticulosum* and perennial *D. imberbe* pass through the same phases of morphogenesis. The life form of a semishrub is formed early in the ontomorphogenesis. The overground sphere of perennials dies off completely every year. Shoots of innovation of *D. imberbe* develop from buds located on the caudex, shoots of innovation of *D. nutans* – on the shoots located under ground or on the rhizome, like in *D. grandiflorum* individuals. The individuals with one life form pass through the same phases of morphogenesis.

Phases of morphogenesis do not always coincide with the course of ontogeny.

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Influence of heavy metals in algal cells of *Enteromorpha intestinalis* (*Ulvaceae*) and *Cladophora vagabunda* (*Cladophoraceae*)

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Abstract. Our research work, of a CNCSIS Romanian Research Grant, started in 2003 and had in view to establish the content of heavy metals for three red, three brown and four green macrophyte sea algae species, collected from nine stations placed on the Romanian Black Sea coast. This work extended on the cytological and physiological alterations suffered by the green sea algae exposed to high concentrations of heavy metals. In this paper we report the results for the species *Enteromorpha intestinalis* (*Ulvaceae*) and *Cladophora vagabunda* (*Cladophoraceae*). Morphological and cytological alterations observed in the algal thalli showed that long accumulations of heavy metals at cellular level have degenerative effect in time, accelerate cell ageing and change their morphology, being easy to notice the noxious effect of great concentrations of heavy metals, which exceed the possibilities of metabolism of algal cells.

Key words: alterations, *Cladophora vagabunda*, degenerative effect, *Enteromorpha intestinalis*, heavy metals, thallus

Introduction

Pollution is a man-made phenomenon, arising either when the concentrations of naturally occurring substances are increased, or when unnatural synthetic compounds (xenobiotics) are released into the environment. Its effects on algae may either inhibit or stimulate growth, so that the algae themselves become the cause for concern.

Some reviews of the effects of heavy metals on algae include those of Rai & al. (1981), Stokes (1981) and Davies (1983). Heavy metals may be used as algaecides, for example copper solutions may be added to reservoirs, although some algae are remarkably tolerant of heavy metal pollution. Many studies are contradictory, and it is still not possible to generalize about the influence of metals on individual species or communities.

From the anthropic factors which had act in the last decades, the increase of heavy metals' amount in the marine environment represents one of the most critical kind of pollution which had made deep alterations in the structure of community of organisms from north-west of Black Sea and Romanian Black Sea coast.

It is known that metallic ions' accumulation in the body of benthic organisms is influenced by a series of factors: variation of sea water physics–chemical parameters, ecophysiological abilities of marine organisms. Our research had in view to establish the content of heavy metals for three red, three brown and four green macrophyte sea algae species and also for some marine invertebrate and vertebrate, in order to accomplish the purpose of a CNCSIS Romanian Research Grant started in 2003. We had measured: Cd, Fe, Mn, Zn and Cr from the macrophytic sea algae and Cd, Fe and Cr from marine invertebrate and vertebrate. For the accumulation degree of heavy metals in macrophytic sea algae we could establish a great fluctuation, illustrated by yearly, seasonal and zone variations and by systematic affiliation of species, the greater values being found for *Cladophora* and *Enteromorpha*.

So, in this paper we report the results of our studies for two species of *Chlorophyta*: *Enteromorpha intestinalis* (L.) Link and *Cladophora vagabunda* (L.) C. Hoek exposed *in vitro* to high concentrations of heavy metals.

Material and methods

Measuring of heavy metals was made by atomic absorption spectrophotometry and identifications of cytological and physiological alterations of algal thallus were made by electronomicroscopy after *in vitro* cultivation of green algae exposed to higher concentrations of heavy metals than in force rates.

Enteromorpha and *Cladophora* samples were collected from different tidal zones at nine locations at Constanța beach. They were placed in different bags and frozen until use. The algal thalli were gathered from a rocky substratum, in an optimal physiological state, from the Romanian coast area of the Black Sea. They were transported in plastic bags, in refrigerating boxes and after the taxonomic identification they were *in vitro* cultivated with addition of different heavy metals in a higher concentration and processed by the electronomicroscopic technique.

In order to increase the efficiency of our microscopic observations we selected the most efficient nutritive medium for *in vitro* cultivation, namely a medium consisting of enriched Provasoli sea water (PES) with addition of phytohormones, 0.2 mg/l indolilacetic acid and 0.1 mg/l kinetin, for the control sample. We used also in four experimental variants heavy metals, Cu: 0.3 mg/l and 0.4 mg/l and Cd: 0.03 mg/l and 0.04 mg/l, for the other sample. The inoculation was made after a sterilization of samples in 5 % lactic acid solution for 20 minutes, in Erlenmeyer glasses with 50 ml PES medium. We used algal explants drawn from the basal region of young thalli and in every glass we put 7–8 explants. The nutritive medium was refreshed every week in the same conditions.

For the electronomicroscopic technique through transmission, the samples were cut in the shape of thallus fragments of 1 mm/1 mm and were prefixed in 0.1 N sodium cacodylate tampons with 2.5 % glutaraldehyde. After prefixation, the samples were washed in 0.1 N cacodylate tampon and fixed in solution of 2 % osmium tetraoxide. The specimens were washed again in 0.1 N cacodylate tampon to remove the osmium excess and dehydrated in serial baths of alcohol: 30°, 50°, 70°, 90°, 95°, 100°, 15–30 minutes each. The first three baths were executed at 4 °C and the rest were carried out at room temperature. In order not to affect the cell osmolarity all the solutions were prepared with filtered and sterilized sea water.

After the dehydration the samples were kept overnight in a mixture of propylene oxide with epoxidic resins of the type Epon 812 and DMP–30 as a hardening agent in order to introduce the warm resin polymerization. Subsequently the samples were placed in plastic capsules, covered with Epon 812 and then placed for polymerization in sterilizers at 67 °C for 46–60 hours.

The ultrafine sections of 400–600 Å were obtained with the aid of a Leica Ultracut–R ultramicrotome. They were placed on metallic grills and double contrasted with uranil acetate and lead citrate and then were observed under the performing electronic microscope Philips CM120.

Results

The alterations, made by pluricellular algae studied, were noticed analysing the growth of algal explants in optimum nutritive medium established by our previous studies (Doroftei & al. 2000, 2001, 2002; Sava & al. 2001, 2002) and for each of species studied we noticed alterations after only 7–10 days from *in vitro* cultivation, independently of the kind of heavy metal added.

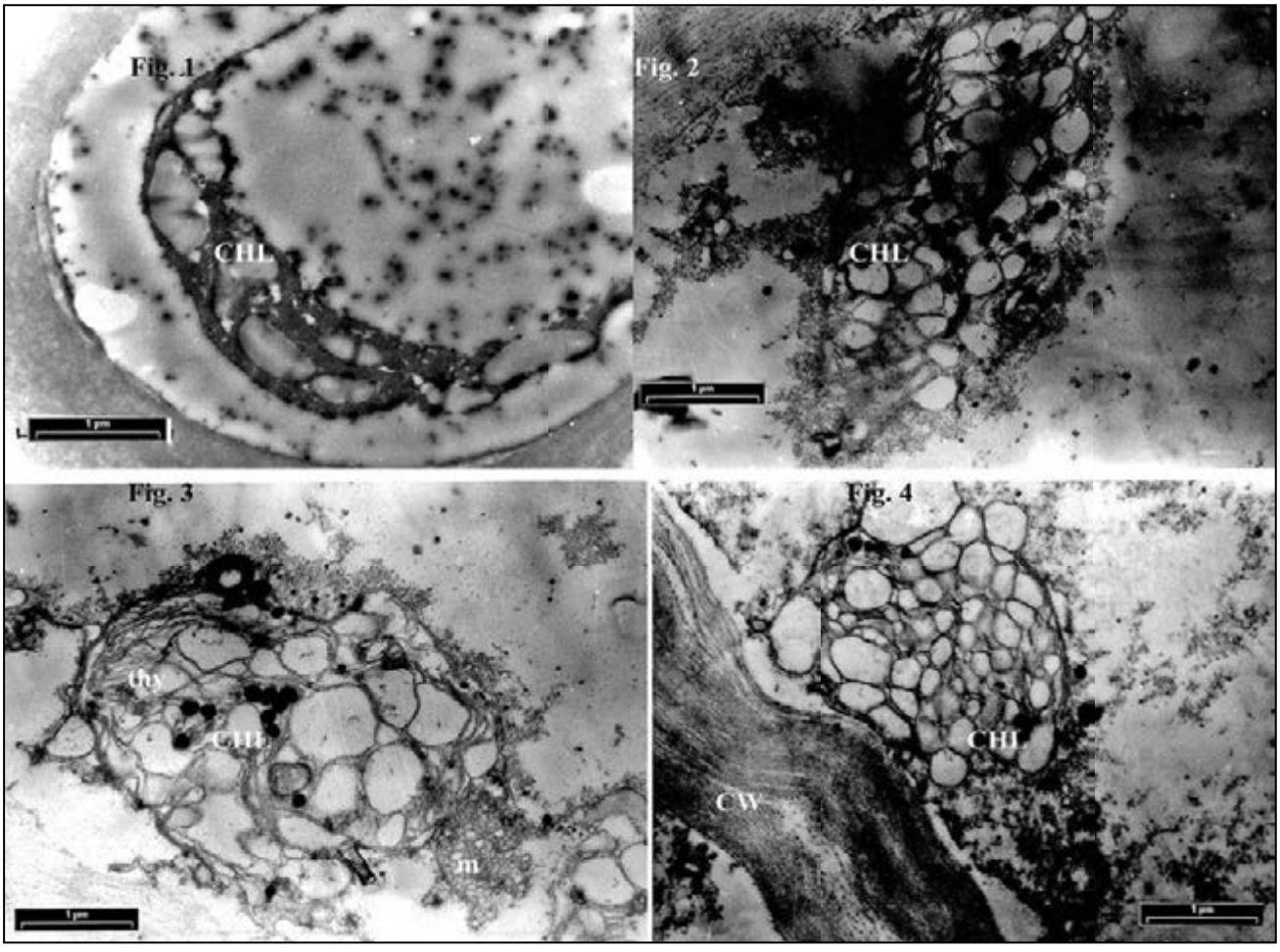
The analysis of micrographs after 10–14 days from the *in vitro* cultivation revealed for both studied species important alterations, particularly at chloroplast level. So, at the algal thallus level, we could notice frequently algal cells with chloroplast disorganizations and reduced size (Plate II, Fig. 3). Inside the chloroplasts we could remark an almost complete thylakoids' disorganization. The thylakoids are hypertrophied, unequally spaced, with very large spaces between them, without plastidial envelope parallelism (Plate I, Figs 2–4; Plate II, Figs 1,3,4; Plate III, Figs 1–3). Frequently we noticed sinuous, spaced thylakoids and between the thylakoids there were very numerous heavy metal sediments under the form of spherical particles with electron dense corpuscle appearance (Plate I, Figs 2–4; Plate II, Figs 1,4; Plate III, Figs 1,2). In some chloroplasts the thylakoids are winding in a network with irregular, de-

generative appearance, being noticed the thylakoids' thickening which did not appear in pairs (Plate I, Figs 2–4; Plate II, Fig. 1).

Through algal thallus length we noticed also a reduced number of cells where the pyrenoids were not present. Generally, in both species, the pyrenoids are small, with unclear outline with hardly visible pyrenosomes and massive, voluminous starch sheath (Plate II, Fig. 3; Plate III, Fig. 3). The synthesis of carbohydrates characterizes a reduced protein biosynthesis as a result of particular conditions of *in vitro* cultivation. On the other hand, starch depositing made difficult the cells' growth, being the signal of a reduced photosynthetic activity, with prevalent synthesis of carbohydrates. The most characteristic aspect remains the presence of the metallic particles inside the chloroplasts (Plate I, Figs 2–4; Plate II, Figs 1,4; Plate III, Figs 1,2). There were also obvious, for both algal species, the nuclear envelope alterations, the presence of diffuse heterochromatin distributed in the karyoplasm and the shape alteration of the nucleus with peripherical site and nuclear outline (Plate II, Fig. 2; Plate III, Fig. 4).

The cell wall was noticed to increase its thickness for the algal cells *in vitro* cultivated. After 30 days from the *in vitro* cultivation the heavy metals' presence in large concentrations determined the senescence and cellular degeneration, noticed distinctly in both species, in any region of their thalli, the lyses of their cellular content, the complete chloroplasts' alteration, as well as the starch reserves exhaustion, remaining visible sometimes the metallic particles, less numerous (Plate I, Fig. 1; Plate III, Fig. 3).

Plate I



Figs 1–4. TEM micrographs showing sections through *C. vagabunda*'s thallus:
1, cellular lyses with reduced chloroplast (Cd 0.03 mg/l added, 30 days' culture); **2–4,** disorganized chloroplasts with numerous heavy metal bodies (**2, 3,** Cu 0.4 mg/l added, 30 days' culture; **4,** Cu 0.3 mg/l added, 30 days' culture): **CHL** – chloroplast; **thy** – thylakoids; **m** – mitochondria; **CW** – cell wall.

Discussion

The results from the atomic absorption spectrophotometry obtained in our previous studies showed that the value of the heavy metals' *in vivo* concentration determined for the animal or vegetal organisms studied is higher than in the sea water, which justified the use of the hydrobyonts at the contamination identification (Sava & al. 2003).

Depending on their sensibility and heavy metal accumulation capacity, in the last decades these marine organisms were frequently used as indicators of this kind of pollution. In order to use a marine organism like bioaccumulator in monitoring programme, some conditions are necessary to be accomplished:

– to accumulate the pollutant without being killed by it;

– to be sedentary organism, for being representative for that marine area;

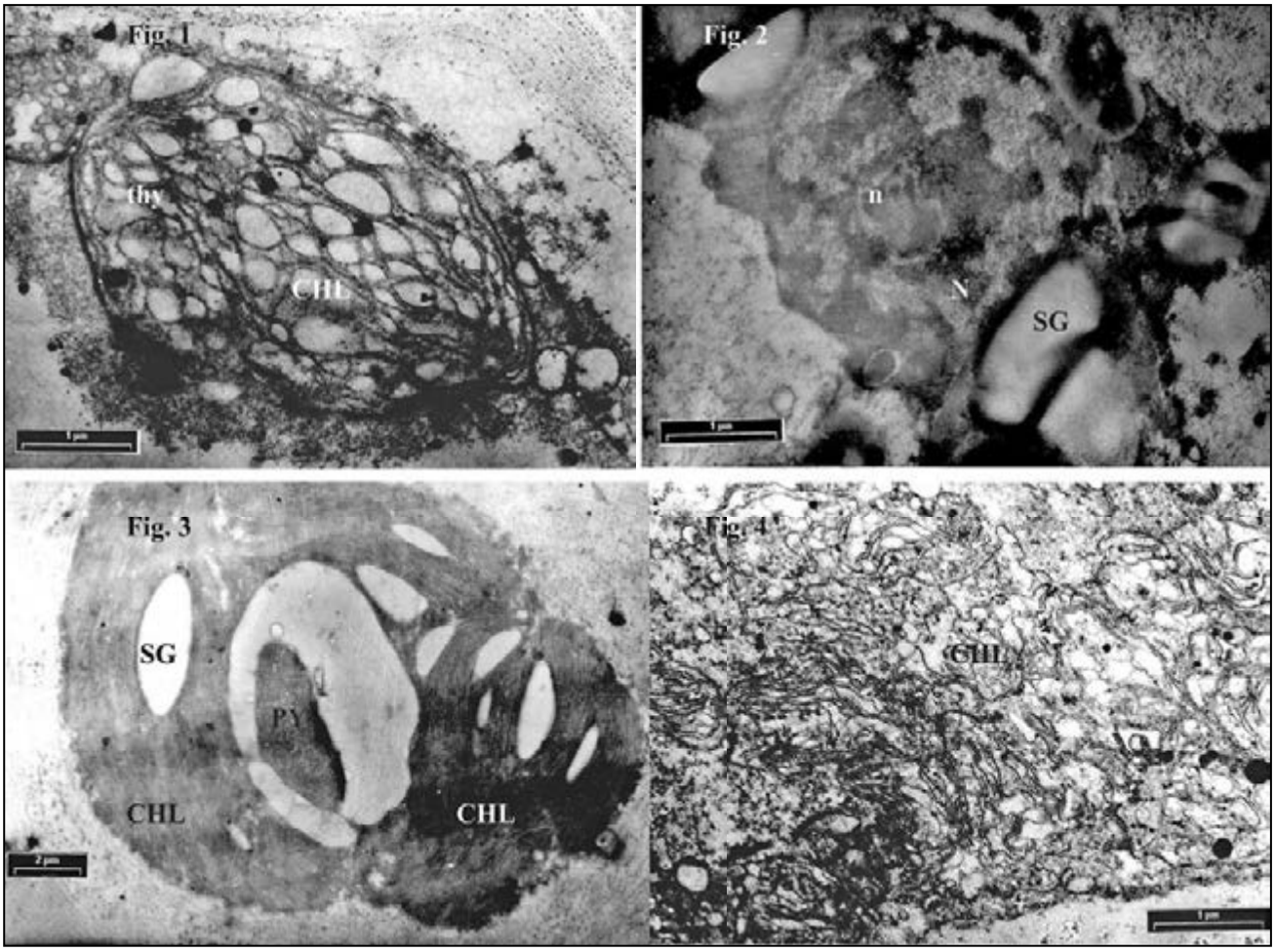
– to have a long period of life time;

– to have large size and to be present in enough quantity in order to assure the biological material for testing and analysing.

The macrophyte sea algae accomplish these conditions so that the variations of heavy metals' concentrations in their organisms can be used like indicators for reflecting in their turn the heavy metals' concentrations in the sea water.

The other studies of algal floras in mine drainage areas showed that metals decrease both diversity and pro-

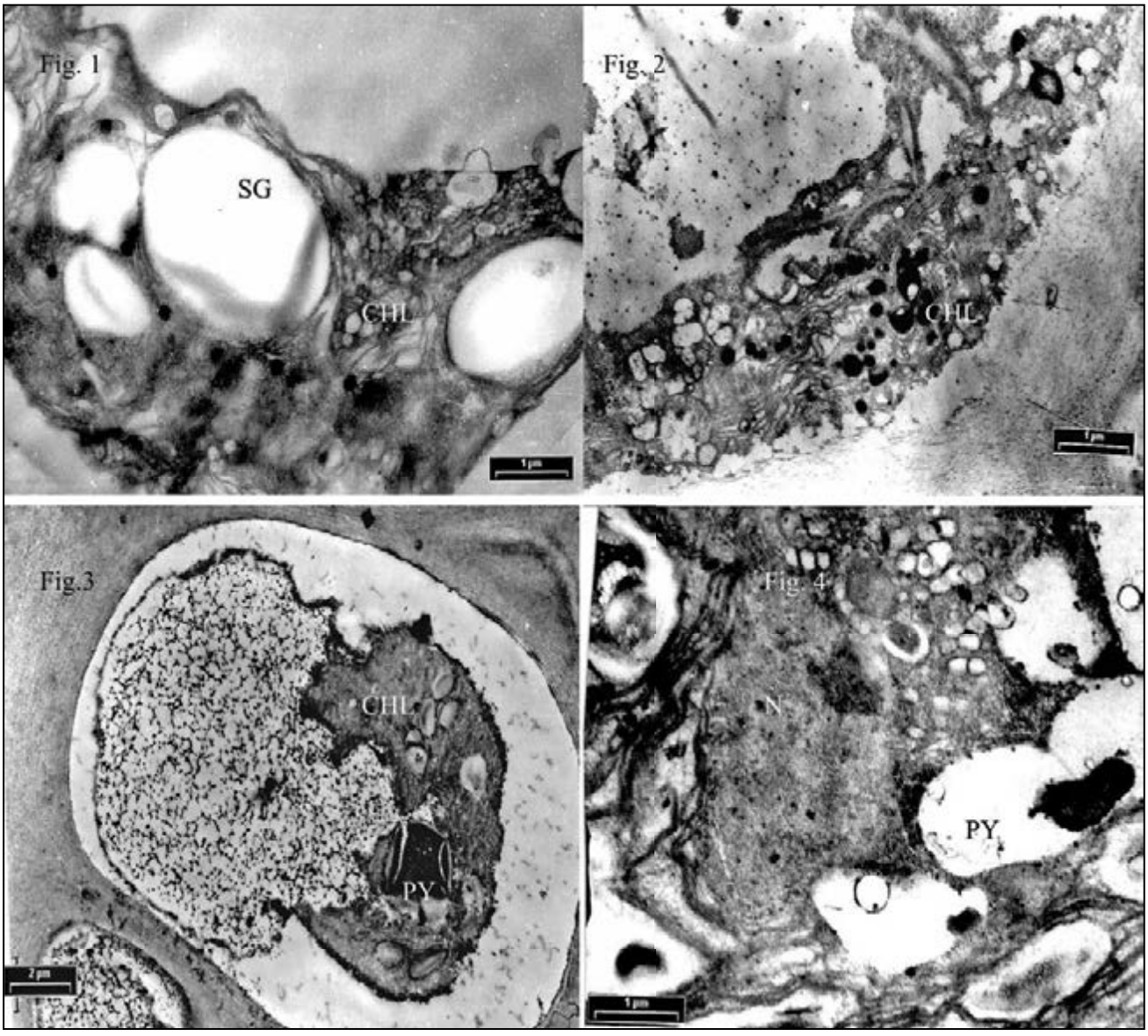
Plate II



Figs 1–2. TEM micrographs showing sections through *C. vagabunda*'s thallus:
1, disorganized chloroplast with numerous heavy metals bodies (Cu 0.3 mg/l added, 30 days' culture); **2,** nucleus with diffuse heterochromatin and visible nucleolus (Cd 0.04 mg/l added, 21 days' culture);
Figs 3–4. TEM micrographs showing sections through *E. intestinalis*' thallus:
3, reduced chloroplast with small pyrenoid and intraplastidial starch storage (Cd 0.04 mg/l added, 14 days' culture); **4,** disorganized chloroplast with numerous heavy metal bodies (Cu 0.3 mg/l added, 21 days' culture): **CHL** – chloroplast; **thy** – thylakoids; **N** – nucleus; **n** – nucleolus; **SG** – starch grain; **PY** – pyrenoid.

ductivity, and the cyanophytes and diatoms are generally less tolerant than the members of *Chlorophyceae*. Information on the mechanisms of tolerance, which includes membrane impermeability, extracellular chelation, or internal detoxification, is sparse and frequently contradictory. At similar external levels of copper, tolerant cells of *Chlorella* ssp. contained the same concentrations of copper as cells of non-tolerant strain, suggesting the lack of an exclusion mechanism (Butler & al. 1980). The authors suggested that exclusion was in part due to the association of heavy metals with algal extracellular products, with the resulting metal complex being more stable in tolerant strains. In *Scenedesmus* (*Chlorophyceae*), however, tolerance to copper did not apparently involve the production of extracellular chelators (Mierle & Stokes 1976); exclusion was apparently by a reduction in membrane permeability. Longer term experiments showed uptake was not the prime mechanism of tolerance (Stokes 1981). EM studies have located copper complexes in the nuclei of tolerant algae (Silverberg & al. 1976). Non-tolerant algae also accumulate copper in a similar manner, but it is accompanied by extensive

Plate III



Figs 1–4. TEM micrographs showing sections through *E. intestinalis*' thallus:
1–2, disorganized chloroplasts with numerous heavy metal bodies (**1,** Cu 0.3 mg/l added, 21 days' culture; **2,** Cu 0.4 mg/l added, 21 days' culture); **3,** cellular lyses with reduced chloroplast and small pyrenoid (Cd 0.03 mg/l added, 30 days' culture); **4,** nucleus with disorganized nuclear envelope and small nucleolus (Cd 0.03 mg/l added, 21 days' culture): **CHL** – chloroplast; **N** – nucleus; **PY** – pyrenoid; **SG** – starch grain.

membrane damage. In metal-sensitive strains of *Diatoma tenue* (*Bacillariophyceae*) lead and copper were incorporated into polyphosphate bodies, but damage to mitochondrial and vacuolar membrane also occurred (Sicko-Goad 1982).

In our present studies, all the cellular alterations, which appeared after 10–14 days from *in vitro* cultivation, became even more visible after 21–30 days, showing the fact that there is no regenerating potential for new algal thalli in the presence of high heavy metal concentrations. The heavy metals' presence at the cellular level is thus proved to be noxious, with certain negative critical consequences on the new algal thalli growth and development, such as on the algal fertility.

After 21–30 days the micrographs showed the protoplasm distortion accompanied by visible plasmolysis, the reducing of chloroplasts by only a few hypertrophied thylakoids, strongly thickened and unequally spaced out. It was also observed the presence of intraplastidial starch grains and smaller very numerous heavy metal bodies. It was not observed the presence of the pyrenoids at the chloroplast level. The number of the cellular organelles is reduced and the central vacuole is voluminous.

In conclusion, the morphological and cytological alterations observed in the algal thalli used in our heavy metal experiments showed that long accumulations at the cellular level had degenerative effect in time, accelerated the cellular senescence and altered the morphology of algal thalli, being easy to notice the noxious effect of great concentrations of heavy metals, which exceed the possibilities of metabolism of algal cells. No tolerance mechanisms occurred on studied species exposed to high heavy metals' concentrations. The *in vitro* cultivation is an experimental model which, at the cellular level, can get useful data about the marine medium's quality and the possibilities of its natural purifying and also about the bioindicator quality of some marine algal species, qualitatively and quantitatively prevalent for the Black Sea Coast.

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Ultrastructural aspects of *Porphyra leucosticta* (*Bangiaceae, Rhodophyta*) from the Romanian Black Sea Coast

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Abstract. The present paper reports the results of our research of the ultrastructure of *Porphyra leucosticta* (*Bangiaceae*, *Bangiales*) and completes a series of papers on the comparative ultrastructure of some *Chlorophyta* and *Rhodophyta* from the Romanian Black Sea Coast. Among the red algae, members of genus *Porphyra* have a life cycle that alternates between two morphologically dissimilar phases, a foliose gametophyte and a filamentous sporophyte. The lower cells are producing rhizoids to form a simple holdfast. At the ultrastructural level the specialized feature are: chloroplast envelope 2-layered, chloroplast ER absent, thylakoids unstacked, pit-connection, and storage product like floridean starch which lies freely in the cytoplasm.

Key words: chloroplast, chromatophore, parenchymatous, *Porphyra leucosticta,* thallus, thylakoid, ultrastructure

Introduction

An important component of the oceanic and marine vegetation is represented by the algae, among which the macrophyte ones are an important link in the trophic net and an important biologic resource. The algae research in all aspects is included in the objectives of international study programs, on multiple levels, of the biological productivity. All possibilities of amelioration or even diminishing of their multiplication in cases of "water blooming" are considered. It is opportune to study the ultrastructure of the marine algae cells under the present conditions when the quantity and quality of the algal representatives is diminishing on the Black Sea Coast.

Our researches stipulated the analysis of the algal cell ultrastructure in *Porphyra leucosticta* Thur., in order to complete the already existing data regarding the ultrastructure of the algal cell, as a result of the interest granted to this field by the teaching staff and the students of the Faculty of Natural Science – "Ovidius" University during the past years. *Porphyra leucosticta* is a blade-forming, none motile red alga that grows primarily on rocky, temperate shores around the world, so we found it on the Romanian shore of Black Sea. These algae play an important commercial role because they are a major source of food for humans throughout the world, but not in our country because of their small amounts.

Material and methods

The experimental part of researches was accomplished during the period July 2003 – April 2005 at the laboratory of electronic microscopy of "Ovidius" University of Constanța, founded in 1997. *Porphyra* samples were collected from different tidal zones at one location at Constanța beach. They were placed in different bags and frozen until use. The algal thalli were gathered from a rocky substratum, in an optimal physiological state, from the Romanian coast area of the Black Sea. They were transported in plastic bags, in refrigerating boxes and after the taxonomic identification they were processed by the electronomicroscopic technique through transmission. The samples were cut in the shape of thallus fragments of 1 mm/1 mm and were prefixed in 0.1 N sodium cacodylate tampons with 2.5 % glutaraldehyde. After the prefixation,

the samples were washed in 0.1 N cacodylate tampon and fixed in solution of 2 % osmium tetraoxide. The specimens were washed again in 0.1 N cacodylate tampon to remove the osmium excess and dehydrated in serial baths of alcohol: 30°, 50°, 70°, 90°, 95°, 100°, 15–30 minutes each. The first three baths were executed at 4 °C and the rest were carried out at room temperature. In order not to affect the cell osmolarity all the solutions were prepared with filtered and sterilized sea water.

After the dehydration the samples were kept overnight in a mixture of propylene oxide with epoxidic resins of the type Epon 812 and DMP–30 as a hardening agent in order to introduce the warm resin polymerization. Subsequently the samples were placed in plastic capsules, covered with Epon 812 and then placed for polymerization in sterilizers at 67 °C for 46–60 hours.

The semifine and ultrafine sections were obtained with the aid of a Leica Ultracut–R ultramicrotome. The semifine sections were plucked out of the bath with a thin wire loop and put on a degreased port-object blade. The colouration was made applying on the blade 2–3 drops of toluidine blue solution, then the blades were set back in the thermostat at 60 °C for 10–30 min, washed in tap water, then in 100 % acetone, rapidly passed through xylol, blotted, assembled and examined under the photonic microscope. The ultrafine sections of 400–600 Å were placed on metallic grills and double contrasted with uranil acetate and lead citrate. The grills were observed under the performing electronic microscope Philips CM120 and the images obtained were processed by a video camera, producing photographs on a Sony photo printer.

Results and discussion

Porphyra is a genus of red algae that grows primarily in an estuarine environment with a salinity of approximately 17 ppt and a temperature of less than 24 °C. The species of the *Porphyra* genus always show a very soft and thin thallus, more or less transparent, composed by a single layer of cells; the macroscopic thalli we find in nature are the gametophytes (Polne-Fuller & Gibor 1984). The sporophytes (*"Conchocelis"*) live perforating a variety of calcareous substrates, ranging from the shells of dead Molluscs to Barnacles to Annelids tubes (Cole & Sheath 1990). *Porphyra leucosticta* has leaf-like, rounded, soft and fragile thallus; diameter up to 20 cm; violet pink. It is an ephemeral species, found during spring in sheltered infralittoral environments, epiphyte, mostly on other red algae. It exists in two different forms; the easily visible thin blades, called the gametophyte stage, and a microscopic filamentous stage, called the *Conchocelis* phase.

The *Conchocelis* phase is present from April through the end of summer. This may be the evolutionary result of herbivores eating the blades during the summer, or temperature intolerance. *Porphyra* blades may be circular or linear in outline, and range in length from several to over 30 centimetres. The colours range from rose-pink to red or violet. A single thallus can show decolourated or whitish parts, together with deep red or violet patches: the latter represent both the female fertile parts (Plate I, Figs 2–4) and zones of direct reproduction (monospores), while the decolourated patches are the male parts (Plate I, Fig. 1).

On the thallose phase we can observe the spermatia producing in uncoloured cells on the median surface of the thallus (Plate I, Fig. 1). *Porphyra leucosticta* have red leafy parenchymatous thalli, with a little cell differentiation, uninucleate and presenting a star-shaped chromatophore (Plate I, Figs 2–4). The lower cells are producing rhizoids to form a simple holdfast. Growth is diffuse and may result in a leafy thallus of one cell layer (monostromatic).

Cell ultrastructure of the holdfast and vegetative and reproductive areas of the sexually mature blade was also in our aims. So, cell wall thickness decreased from very thick in holdfast (Plate III, Fig. 3) and non-dividing vegetative cells to thin in dividing vegetative cells. A two-layered wall surrounds the cells of *P. leucosticta.* The innermost of these two layers is comprised of electron-dense reticulated microfibrils embedded in an amorphous matrix, while the outermost layer represents mucilaginous, electron-transparent material (Brawley & Wetherbee 1981; Craigie 1990). Golgi-derived vesicles contribute to the formation of the electron-dense cell-wall layers, while mucilage sacs are responsible for the deposition of the electron-transparent ones. A thin cuticle invests the thallus (Tsekos 1981). In the *Bangiophycidae* the wall of the macroscopic phase is composed of a microfibrillar framework of β-1,3 linked xylan and recent reports have shown that the *Conchocelis* phase of these algae possesses cellulose as a wall component (Delivopoulos & al. 1999). An outer cuticle of β-1,4 linked mannan or

Plate I

Fig. 1

Fig. 2

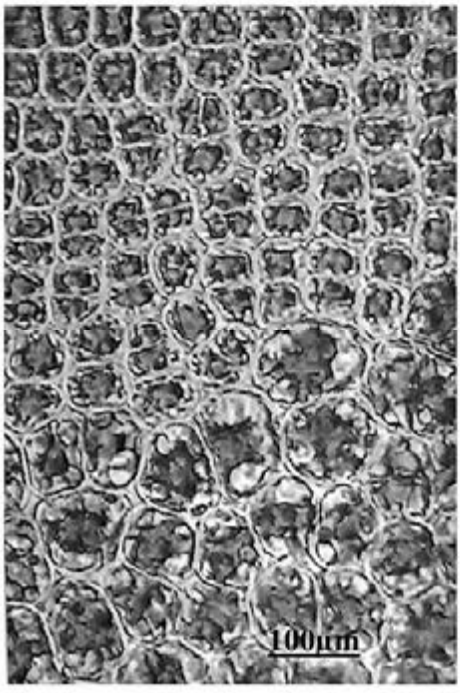


Fig. 3

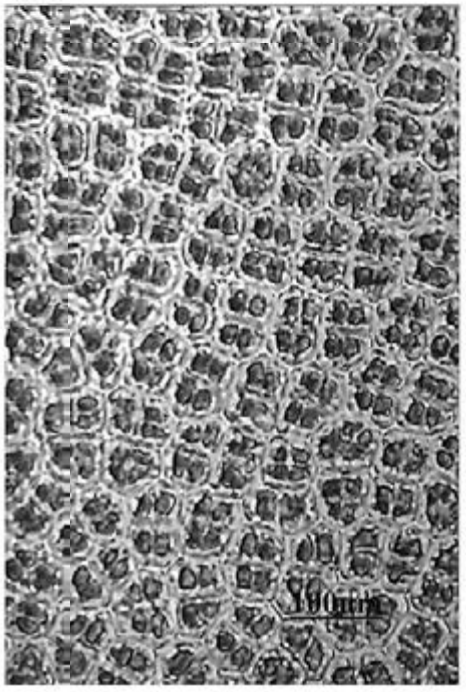


Fig. 4

Fig. 1. Decolourated patches of the *P. leucosticta* blade represented the male fertile parts showing the spermatia producing. Scale bar = 100 µm.
Figs 2–4. Surface view of the mature blade showing star-shaped chloroplasts. Scale bars = 100 µm.

protein has also been reported in some bangiophytes (Pueschel 1979). The pit-plug or pit-connection is a wall feature of the *Conchocelis* phase and is formed between two cells during division. The plugs are usually biconcave, with a central constriction (Brawley & Wetherbee 1981).

The plasmalemma appears highly irregular due to the intense exocytosis of Golgi vesicle and electron-dense masses are seen beneath the cuticle (Gantt & Conti 1965; Gantt & al. 1968).

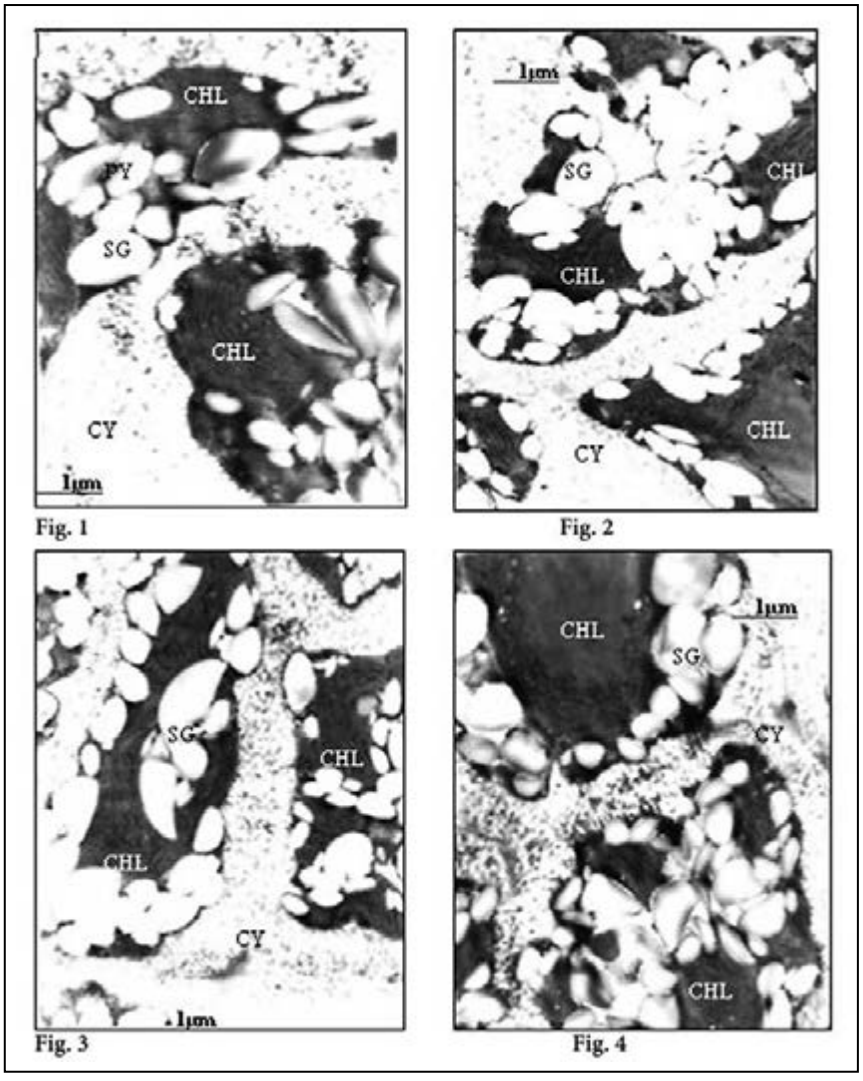
The structure and the organization of the rhodophyte thylakoids show a primitive homology with those of blue-green algae, although there are substantial differences in function resulting from the former's compartmentalization within bounding membrane (Staehelin & al. 1978). Phycobilisomes are present on the stromal surface of the lamellae (Talarico 1996). In the *Bangiophycidae* a pyrenoid is often present and the lamellae form a reticulum within the pyrenoid (Gibbs 1962; Tsekos & Reiss 1990).

The vegetative gametophytic cells of *P. leucosticta* possess lobed chloroplasts (Plate II, Figs 1–4) with parallel, within the lobes, thylakoids whose margins abut against the inner membrane of the envelope. Some thylakoids are crossing the pyrenoid (Plate II, Fig. 1) and the chloroplasts are encircled by a peripheral thylakoid. The intrapyrenoidal thylakoids appear to be lacking phycobilisomes. The presence of phycobilisomes in the thylakoids is more conspicuous particularly in the middle region of the algal thallus (Gantt 1986; Algarra & al. 1990). Lipid droplets (plastoglobuli) are presented in the stroma, often occurring as clusters between the thylakoids and within the pyrenoid (Waaland & al. 1974). It was evident that thylakoids are formed from unraveling coiled lamellar bodies (Lichtlé & Thomas 1976; Staehelin 1986). The starch stored is held in the cytoplasm, outside the chloroplast (Plate II, Figs 1–4; Plate III, Figs 1,2).

Dictyosomes exhibit intensive activity in the middle and mostly in the apical region of the thalli. The presence of mucilage sacs is characteristic, but mucilage sacs originate either from the endoplasmic reticulum or from concentric membrane bodies and ultimately discharge their content into the cell wall (Tsekos 1981).

There are numerous mitochondria in apical region and a small number in the basal region of the thallus (Plate III, Fig. 3), while the endoplasmic reticulum seems to be well developed in the middle and especially in the apical region of the thalli. There are also microbodies (2 μm in diameter).

Plate II



Figs 1–4. TEM micrographs showing sections through the median region of the thallus: **CHL** – chloroplast; **PY** – pyrenoid; **SG** – starch grain; **CY** – cytosol. Scale bars = 1 μm.

The cells of *Porphyra* thallus are uninucleate, the nucleus has medium size, with a double nuclear envelope, a ground substance or nucleoplasm, chromosomes and one or two nucleoli, which are densely staining concentrations of basophilic material rich of ribonucleoprotein (Plate III, Figs 1–4). The nuclear envelope is perforated by numerous pores which may or may not be arranged in a specific pattern. The membranes of the nuclear envelope are 7–8 nm thick, separated by a perinuclear cavity.

In conclusion, at the ultrastructural level the specialized features are: chloroplast envelope 2-layered; chloroplast ER absent; thylakoids unstacked; pit-connection or pit-plug in *Conchocelis* phase; storage product like floridean starch which lies freely in the cytoplasm.

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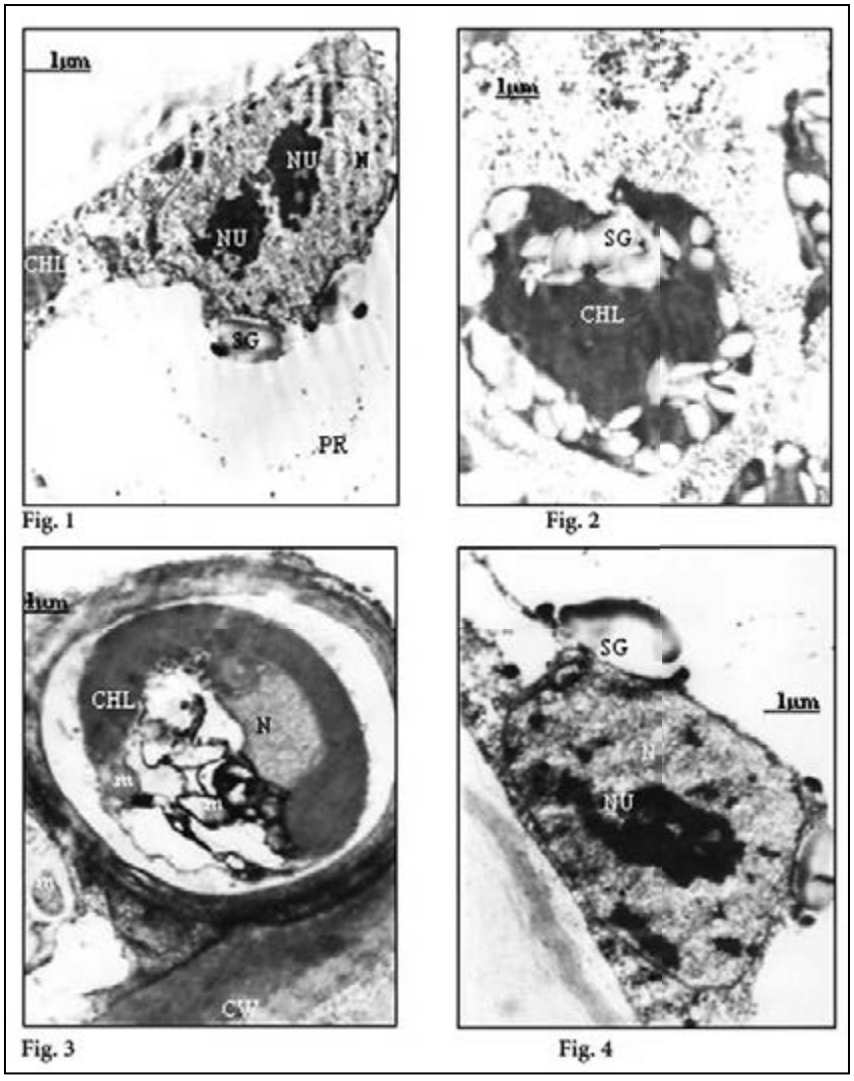
Plate III



Figs 1, 2, 4. TEM micrographs showing sections through the median region of the thallus. Scale bars = 1 µm.

Fig. 3. TEM micrograph showing a sections through the basal region of the thallus (one lobed chloroplast, mitochondria, nucleus and thick cell wall): **CHL** – chloroplast with parallel thylakoids; **CW** – cell wall; **N** – nucleus; **NU** – nucleolus; **m** – mitochondria; **PR** – polyribosomes; **SG** – starch grain. Scale bar = 1 µm.

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Variation in quantitative pollen parameters of *Pinus nigra* and *P. sylvestris* (*Pinaceae*) populations of Mts Pieria (N Greece)

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Abstract. A series of quantitative features (max. length of pollen, max. length and height of corpus, max. height and width of saccus, distance of sacci and width of corpus–saccus intersection) were measured on bulk samples of *Pinus nigra* and *P. sylvestris* pollen collected from three localities in the western side of Mts Pieria (N Greece). Pollen grains were acetolysed and treated with 10 % KOH. Measurements were performed on intact pollen grains, positioned in equatorial view. Discriminant analysis was applied to all the pollen features and relatively low percentages of pollen grains were incorrectly classified. The results are promising regarding the identification of the pollen of the two species, collected from pollen traps placed in W Pieria Mountains.

Key words: Greece, Pieria Mts, *Pinus nigra*, *Pinus sylvestris*, pollen

Introduction

Pinus nigra Arnold and *P. sylvestris* L. (*Pinaceae*) belong to the subgenus *Pinus* (Vidaković 1991) and both have bisaccate pollen grains, i.e. with two sacci (bladders), which are assigned to diploxylon-type (Punt & al. 1994). The forest vegetation on the western side of Pieria Mts is formed by the two pine species whereas *P. sylvestris* also forms the tree-limits.

Modern pollen deposition in the upper part of the western side of Pieria has been monitored since 1996, when transect of six pollen traps (P1–P6) was set up for the first time in Greece. The two species were included in a common pollen sum in all diagrams presented until now (Athanasiadis & al. 2002; Gerasimidis & al. 2006) due to the fact that they share a common pollen type (*Pinus sylvestis*-type).

Discriminant analysis has been used in order to distinguish closely related pollen taxa (Robbins & al. 1979; Kim & al. 1990; Panajiotidis & al. 2000). It has also been used to determine the pollen load of certain *Picea* species in Holocene pollen assemblages (Birks & Peglar 1980).

In this study a number of quantitative pollen parameters of *P. sylvestris* and *P. nigra* are subjected to discriminant analysis in an attempt to distinguish the two species. The study, which concerns the populations of both species found in western Mts Pieria, is the first step in the process of determining each species' pollen load to the content of the six pollen traps placed in Mts Pieria. For this reason, the chemical preparation of the pure pollen samples follows the one applied to the pollen content of the traps (Hicks & al. 1996) ensuring that in all cases the size of the quantitative pollen parameters measured is equally affected.

Material and methods

Fresh pollen material of *P. nigra* and *P. sylvestris* was collected from three localities, near pollen traps P2, P5 and P6, in western Mts Pieria, during the period of anthesis (June 2005). Bulk samples were chemically treated in the laboratory via acetolysis and hot 10 % KOH (Hicks & al. 1996). Fifty (50) intact pollen grains (PG) of each species were measured in equatorial view, under light and in some cases phase contrast microscopy, for the following parameters: maximum length of pollen grain (mLEN), maximum length and height of corpus (corLEN, corHEI), maximum height and width of saccus (sacWDTH, sacHEI), distance of sacci (sacDIST) and width of corpus–saccus intersection (csiWDTH). The pa-

rameters sacWDTH and sacHEI were measured on the left saccus of the pollen grain, while the sacDIST measured the length of the straight-line that connects the midpoints of the sacci. The data were subjected to discriminant analysis using the XLStat 7.5 package (Addinsoft, Brooklyn, NY).

Results and discussion

Pinus nigra shows relatively higher mean values than *P. sylvestris* concerning the total of pollen parameters measured (Table 1).

Table 1. Means, standard deviations and range of values of the quantitative pollen parameters measured. Structure coefficients estimate the correlation of the pollen parameters with DF1.

Pollen parameters	Structure coefficients	*Pinus nigra*	*Pinus sylvestris*
mLEN (max. length of pollen grain)	**0.63***	**94.06** ± 5.15 (***84.38–106.25***)	**88.83** ± 6.15 (***75.00–101.56***)
corLEN (corpus length)	**0.66**	**62.76** ± 4.40 (***53.13–72.66***)	**58.56** ± 4.22 (***46.88–67.19***)
corHEI (corpus height)	**0.36**	**44.58** ± 3.33 (***37.50–53.13***)	**42.95** ± 3.17 (***35.94–53.13***)
sacWDTH (saccus width)	0.16	**34.89** ± 3.01 (***35.94–50.00***)	**33.98** ± 3.31 (***32.81–50.00***)
sacHEI (saccus height)	**0.40**	**41.63** ± 3.17 ***(26.56–42.19)***	**40.84** ± 4.11 ***(25.00–45.31)***
sacDIST (distance of sacci)	**0.66**	**35.01** ± 3.35 ***(18.75–34.38)***	**32.41** ± 5.86 ***(14.06–32.81)***
csiWDTH (width of corpus–saccus intersection)	**0.21**	**28.92** ± 3.80 ***(28.13–40.63)***	**24.81** ± 4.58 ***(27.34–40.63)***

***Correlations in bold are significant at $p < 0.05$.**

The currently presented values of maximum length and corpus length of both species are considerably higher than those reported by Beug (2004). For example Beug reports mean maximum length values of 73.5 µm (62.2–83.7) and 68.7 µm (59.5–80.0) for two populations of *P. sylvestris* and 73.3 µm (62.5–83.3) for one of *P. nigra*. The considerable difference of these values from the ones presented in Table 1 could be attributed to the additional treatment of our samples with KOH which expands pollen grains as Hanks & Fairbrothers (1970) have shown.

One discriminant function (DF1), which explains the total of the observed variance, derived from the analysis since only two groups participate in the analysis. Three of the pollen parameters measured, namely maximum length of pollen grain, length of corpus and distance of sacci, have large structure coefficients, which means that they are strongly correlated to DF1 (Table 1). This allows the interpretation of the observed differences between the two species, regarding the discriminant scores of their pollen grains, in terms of size differences of those pollen parameters. The positive correlation of the three parameters with the DF1 indicates that cases (pollen grains) with high discriminant scores on DF1 have also high values for the three parameters. In Fig. 1 it can be seen that the vast majority of *P. nigra* pollen grains have higher (positive) discriminant scores on DF1 in contrast to *P. sylvestris* pollen grains which mostly have negative ones.

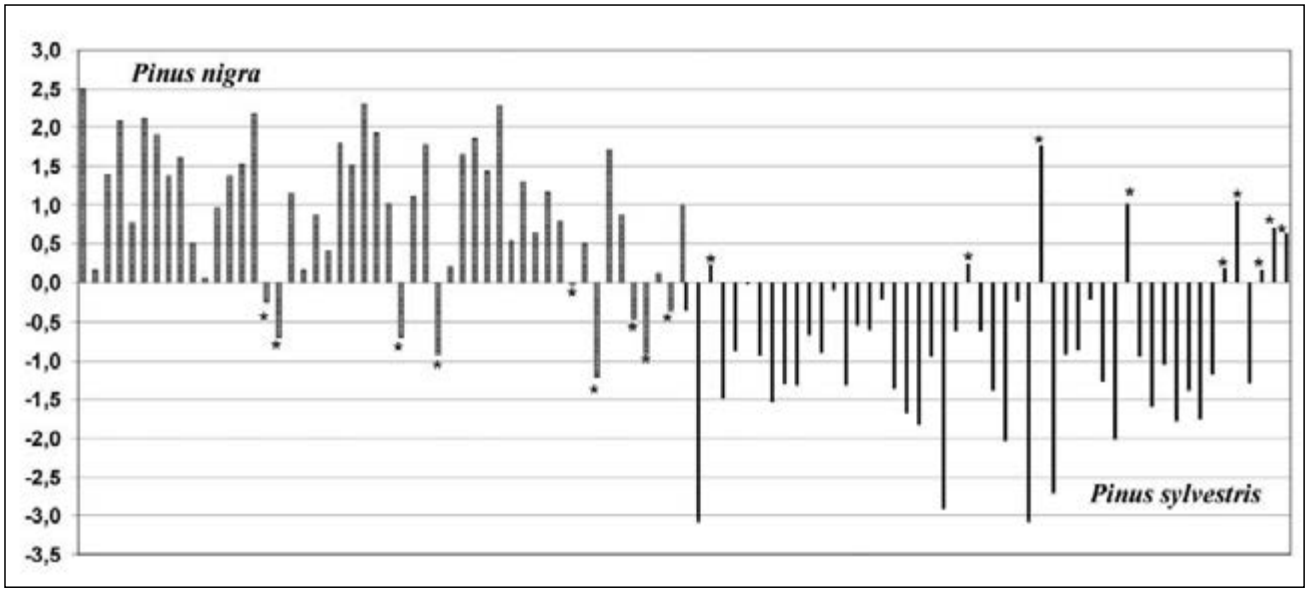

Fig. 1. Discriminant scores of the pollen grains of *P. nigra* and *P. sylvestris* on the single DF1 function. Columns marked with an asterisk represent pollen grains incorrectly classified.

Discriminant analysis acts as a dynamic classification technique which assigns each case (here the pollen grain) to one of the investigated species. The rate of misclassification (assignment of a pollen grain to a species other than the one it actually belongs to) is a measure of the taxonomic value of the pollen parameters used. The validity of the classification results of discriminant analysis is stronger when the assumption of equal within-groups covariance matrices is met (for a thorough explanation of this concept see Klecka 1980). The related Box's M test scored a value of 41.337 which is not significant at the p-level of 0.05 meaning that the null hypothesis of equality of the within-groups covariance matrices cannot be rejected.

The rate of misclassification of *P. nigra* and *P. sylvestris* pollen is identical (18 % or 9/50 pollen grains, Table 2). The results of this classification are promising as regards the identification of the investigated species using quantitative pollen parameters. It can be seen that although the independent pollen parameters measured are considerably overlapping (Table 1), their combined use offers an ability to discriminate pollen of the investigated species with a > 80 % probability (Table 2). The improvement is large considering that the by chance correct assignment of a pollen grain to the species it actually belongs is only 50 %.

Table 2. Classification of the pollen grains of the investigated pine species. The number and percentage of the correctly classified pollen grains is given on the main diagonal.

	to ***Pinus nigra***	to ***Pinus sylvestris***
from ***Pinus nigra***	**41** 82.00%	9 18.00%
from ***Pinus sylvestris***	9 18.00%	**41** 82.00%

The inclusion of additional variables, like cappa (cap) thickness for example, may improve the classification results achieved already helping thus to identify with lower error probability both species. The combined study with other features could also be helpful in improving classification results. Currently, the size and number of nodules (button-like or rod-like small bodies) found in the nexine of the central part of the saccus are being investigated (Klaus 1972). Klaus (1972) reported significant variation in size and number of nodules between *P. nigra* and *P. sylvestris* pollen.

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Anatomical study on stem-roots in three species of genus *Angelica* (*Apiaceae*)

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Abstract. An anatomical study on stem-roots of *Angelica sylvestris* and *A. pancicii* from Bulgaria and *A. archangelica* with foreign origin as medicinal and essential oil plants was carried out. In the studied sections of the stem-root a typical secondary structure of root in *A. archangelica* and *A. pancicii* was observed, while in *A. sylvestris* – a stem one. Many ruptures of parenchyma in the cortex, because of a contractive activity of the stem-root, were established. In the anatomy of stem-root of the studied species considerable differences were not found. The results of the discriminant analysis carried out allowed separation of the three species *Angelica* in two groups with regard to the size and number of the secretory canals.

Key words: anatomy, *Angelica*, *Apiaceae*, essential oil canals, stem-root

Introduction

Genus *Angelica* L. includes a number of valuable essential oil and medicinal species which are studied mainly in this respect (Chen & al. 1995; Bernard & Clair 1997; Wolski & al. 2001a, b). According to Denisova (1968) the stem-root in the species *Angelica* is an organ where essential oils accumulate.

In the last two decades besides *Angelica archangelica* L. and *A. sinensis* (Oliv.) Diels, other *Angelica* species were studied too as a source of components with medicinal importance (Mizuno & al. 1994; Liu & al. 1995). Up to now, the stem-root's anatomy was studied only in *Archangelica decurrens* Ledeb. (Denisova 1968), while fruit's anatomy was well-researched in many species of the genus *Angelica* (Hsu 1970 [cit. after Heywood 1971]; Pimenov & Kljuykov 2002).

In the Bulgarian flora this genus is presented with three species: *A. sylvestris* L., *A. pancicii* Vandas and *A. archangelica* (Peev 1982). According to Andreev (1984), *A. pancicii* is a rare species and Balkan endemic and *A. archangelica* is shown as an extinct species. Up to now, phytochemical, embryological and anatomical investigations on Bulgarian populations of *A. sylvestris* and *A. pancicii* were carried out (Paskov & al. 1954; Petkov 1982; Jankova-Tsvetkova 2004).

In the present work, the results of the first anatomical study of the stem-root of the three species *Angelica* are shown. The aim of the study was to reveal the anatomical peculiarities of this structure as a major source of valuable essential oils.

Material and methods

The anatomy of the stem-root in *A. sylvestris* and *A. pancicii* from Bulgaria (Vitosha Mt) and *A. archangelica* with foreign origin (Germany) was studied in microtome sections in an equal distance away from the leaf rosette. The sections were treated according to the generally accepted cytological techniques for glycerine-gelatinous and permanent microscope slides and stained with Heidenhain's haematoxylin. The size and number of the essential oil secretory canals of the three studied species were established. Discriminant analysis (Wilks' Lambda) of the obtained data was made using the WINDOWS STATISTICA.

Results and discussion

In the studied sections of stem-root of *A. archangelica* and *A. pancicii*, a typical secondary structure of root was observed (Plate I, Figs 1–2; Plate II, Fig. 1), while in *A. sylvestris* – a stem one (Plate II, Fig. 2). A well-developed secondary cortex and central cylinder with poorly developed mechanical tissue were found – typical for the *Apiaceae* family (Denisova 1968). In the parenchyma of the stem-root's cortex many rexigenous ruptures were observed (Plate I, Fig. 1). Such ruptures were also found by Denisova (1968) in other species of the family *Apiaceae*, as: *Archangelica decurrens*, *Cicuta virosa* L., *Sium latifolium* L., *Anthriscus sylvestris* (L.) Hoffm. She announced that these ruptures are formed as a result of the shortening activity of the stem-root, due to which it gets out to soil.

In well-developed parenchyma of the cortex in the studied species many essential oil and schizogenetic secretory canals were observed. Denisova (1968) showed the same case in *A. decurrens*. These canals are arranged in the right circles in other species of the family *Apiaceae* (*Daucus carota* L., *Hippomarathrum microcarpum* (M. Bieb.) B. Fedtsch., *Apium graveolens* L.), and the number of circles corresponds with the age of the plants (Denisova 1968).

According to Metcalfe & Chalk (1957), in the most members of *Apiaceae* family secretory canals are presented in secondary phloem of older roots. They also occur in the xylem of some species of the genera *Myrrhis* Mill. and *Opopanax* Koch, in the pericycle immediately within the cork of *Ferula communis* L. and in the secondary phloem.

We observed that the secretory canals in the three studied species of *Angelica* are solitary in the cortex of the stem-root, while Metcalfe & Chalk (1957) announced for anastomosing ones in the upper part of root in the family *Apiaceae*. In the stem-root's xylem,

Plate I

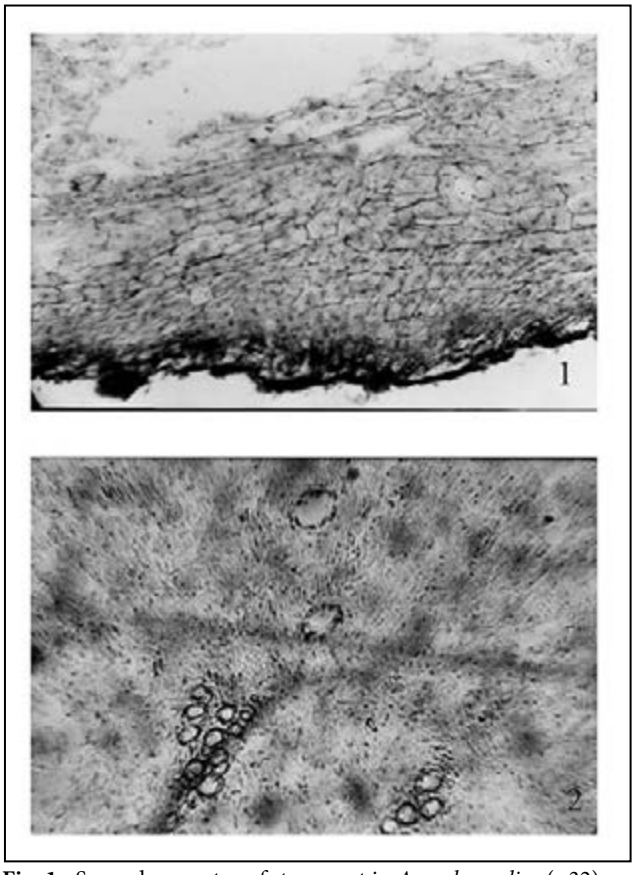


Fig. 1. Secondary cortex of stem-root in *A. archangelica* (×32).
Fig. 2. Secondary structure of stem-root in *A. pancicii* (essential oil canals and xylem with tracheal elements) (×160).

Plate II

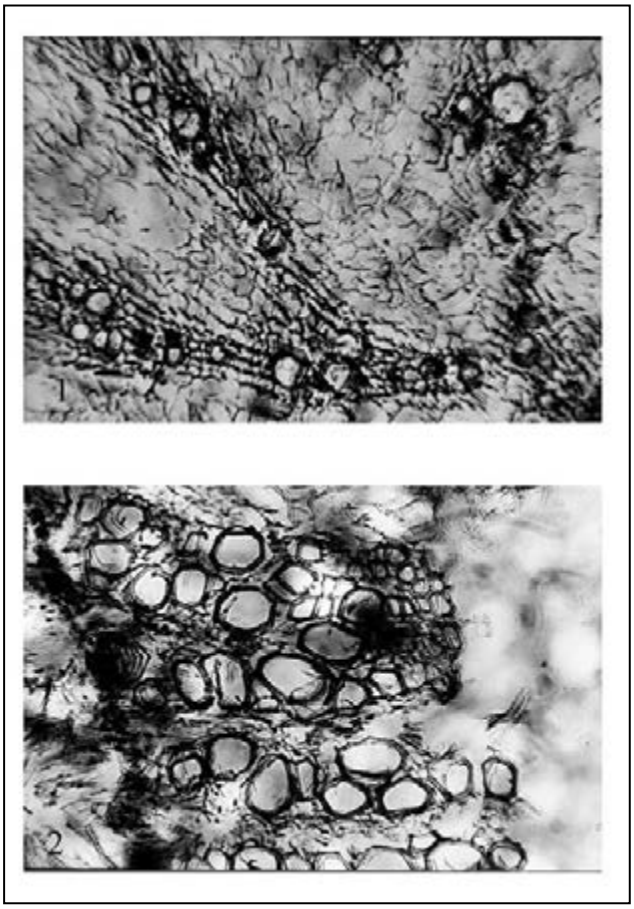


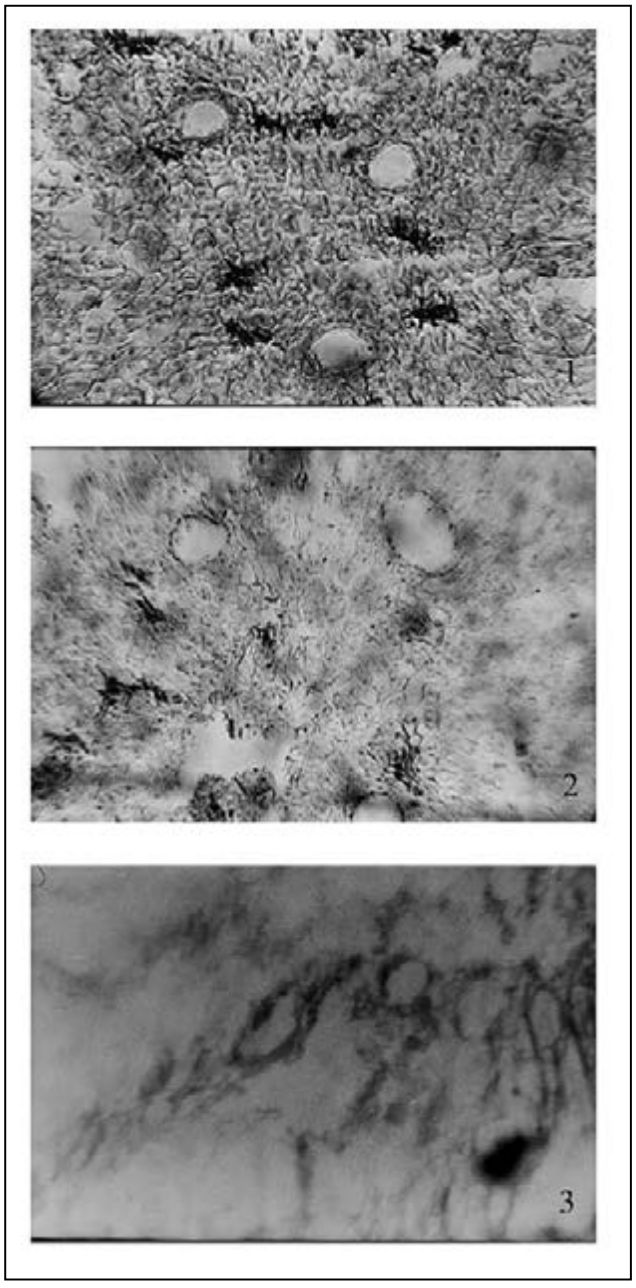
Fig. 1. Secondary structure of stem-root in *A. pancicii* (rayligneous parenchyma in the pith) (×160).
Fig. 2. Secondary xylem with tracheid elements and pith's cavity of stem in *A. sylvestris* (×160).

the tracheal elements were arranged in radial rows (Plate I, Fig. 2). A well-developed ray-ligneous parenchyma with traces of xylem of the primary radial vascular bundle was observed in the pith.

Our observations on the studied material showed that the essential oil secretory canals were similar in respect to their size and number (Plate III, Figs 1–3; Table 1). The difference in these two peculiarities measured between the Bulgarian species and *A. archangelica* is more evident than between *A. sylvestris* and *A. pancicii*.

The results of the discriminant analysis carried out allowed separation of the three species in two groups: the first with *A. sylvestris* and *A. pancicii*, and the second with *A. archangelica* (Table 2).

Plate III



Figs 1–3. Secretory essential oil canals in the parenchyma of the secondary cortex in:
1, *A. archangelica* (×160); **2,** *A. pancicii* (×160);
3, *A. sylvestris* (×160).

Table 1. Number and size of the essential oil canals of the stem-root in the three studied species of *Angelica*.

Species	Essential oil canals		
	number X±S	size 1 X±S (cm)	size 2 X±S(cm)
A. sylvestris	2.823 ± 1.891	0.072 ± 0.024	0.098 ± 0.038
A. pancicii	3.410 ± 2.337	0.077 ± 0.082	0.093 ± 0.023
A. archangelica	2.177 ± 3.086	0.060 ± 0.067	0.106 ± 0.115

Legend: X – average value; S – standard error; Size 1 – width of the essential oil secretory canals; Size 2 – height of the essential oil secretory canals.

Table 2. Results of the discriminant analysis at three studied *Angelica* species.

Group	Percent correct	*A. archangelica* p=.33333	*A. pancicii* p=.33333	*A. sylvestris* p=.33333
A. archangelica	80	24	3	3
A. pancicii	63.33333	5	19	6
A. sylvestris	60	9	3	18
Total	67.77778	38	25	27

Conclusion

The present anatomical study on the stem-root shows that there were not considerable differences between the three *Angelica* species. In relation to the size and number of the essential oil canals in the stem-root clearly expressed differences were not established either. The discriminant analysis on these features determines two groups of species: 1. *A. sylvestris* and *A. pancicii*; 2. *A. archangelica*.

On the basis of the results of the study we may conclude that besides *A. archangelica*, *A. sylvestris* and *A. pancicii* can also be used as a source of components with medicinal importance. New data were received, that clarify the relationships of the three species within the limits of the genus *Angelica*.

Acknowledgements. The authors wish to express their gratitude to Dr Lyuba Evstatieva of the Institute of Botany (BAS) for her kindness to give them the studied material of *A. archangelica*.

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Wolski, T., Najda, A., Ludwiczuk, A. & Dyduch, J. 2001a. *Umbelliferae* family plants (*Apiaceae*) as oil and coumarin materials. Part 1. The content and composition of essential oils obtained from the fruits of *Angelica* (*Archangelica officinalis* Hoffm.). – *Umbelliferae* Improv. Newslett., **11**: 4-7.

Histo-anatomical peculiarities of *Nepeta pannonica* (*Lamiaceae*) during the ontogenesis

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Abstract. The structure of vegetative organs (stem and leaf) from *Nepeta pannonica* was investigated. It was established that in the top of the stem the collenchyma strands are of a tangential type, becoming angular at the base. The foliar lamina is of hypostomatic type. The secretory hairs have glands consisting of a variable number of cells: hairs with unicellular gland are presented on the young leaf; hairs with a bicellular gland are the most frequent and these with octocellular gland are very rare. The general structure plan is in accordance with Metcalfe & Chalk's description for the *Lamiaceae* family. Some particular features, important for plant identification in pharmaceutics have been underlined.

Key words: leaf, medicinal plant, *Nepeta pannonica*, stem, structure

Introduction

Nepeta pannonica L. (syn.: *Nepeta nuda* L. ssp. *nuda*) is a common and cosmopolitan species growing in hay fields, glades and meadows. In spite of this some botanists have made confusion between it and *N. cataria* L. (catmint, catnip). *Nepeta cataria* is a common weed considered as antropophyllous and nitrophyllous species (Ciocârlan 2000).

Nepeta pannonica is a perennial plant, whose stem is 50–120 cm tall and which flourishes frequently in the months of June and July. Regarding the ecological growing conditions, *N. pannonica* is typically a plant of full sun to light shady places including woodlands, meadows and fencerows. It is frequent on full sunlight roadsides, in clear-felled and coppiced woodlands, scrubs and sometimes in derelict woodland, and in orchards (Pădure 2004). The corolla is longer than the calyx and visibly comes out from among the teeth of the latter. All the flowers of the inflorescence are usually hermaphrodite (Toma & Rugină 1998).

Biotherapy. The aerial parts of the plant, with flowers, are important in traditional medicine. They have a strong, penetrating smell. They are used in the treatment of the cough, tuberculosis and to treat other lung diseases, nervous disorders and for the stimulation of gastro-intestinal secretions.

Apiculture. Melliferous species. Quantity of nectar – 0.15–0.34 mg/inflorescence. Production of honey – 100–140 kg/ha. Medium economical-apicultural importance (Pârvu 1997).

In this paper, we wish to present a complete description of the structure of the stem and leaf of *N. pannonica*.

Material and methods

The vegetal material came from the Natural Reserve "Valea lui David" (county of Iaşi), one of the few steppe reserves in Romania, located near the city of Iaşi. This material was represented by the stem and leaves of *N. pannonica*.

The fixing and processing of the material were done according to the usual protocol of the Vegetal Morphology and Anatomy laboratory belonging to the Biology Department of the University "Al. I. Cuza" of Iaşi.

The sections were made transversal at the basis, middle and top levels of the stem. The obtained per-

manent preparations were analysed and photographed at the Novex optical microscope.

For the phytochemical studies the samples have been submitted to qualitative analysis by thin layer chromatography (TLC) in order to reveal the spectrum of flavonoid and polyphenolcarboxilic compounds using the following conditions:

- support: Kieselgel 60 F254 Fertigplatten Merck;
- mobile phase: ethyl acetate–formic acid–acetic acid–water (100:11:11:27);
- detection: spraying with NEU / examination in UV light;
- references: apigenin-7-glucoside, chlorogenic acid, caffeic acid, ferrulic acid.

The essential oil obtained from the flowering aerial parts was analysed by spectrometry (GC/MS).

Results and discussion

Stem. The epidermis is made up of slightly tangentially elongated cells whose inner and especially outer walls are thicker than the others; the outer wall is covered by a relatively thick cuticle. Here and there we find stomata which are visibly prominent over the epidermis, uniseriate tectorial hairs with a verrucose surface (Fig. 1) and secreting hairs with a unicellular (spherical) or bicellular gland (Fig. 2).

The cortex is differentiated in belts of angular collenchyma at the base (Fig. 3), becoming tangential type at the top at the level of the ribs and chlorenchyma areas in the rest of the cortex. In the latter areas, large hypodermic aeriferous cavities are visible. The innermost layer of the cortex is a primary endodermis (Fig. 4).

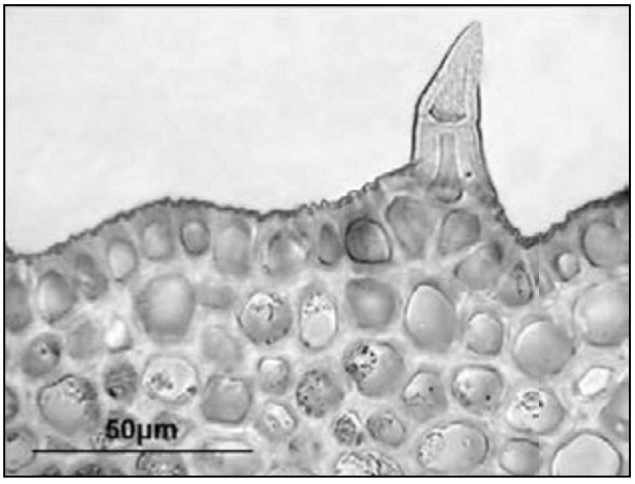

Fig. 1. Cross-section through the stem: epidermis cells and uniseriate tectorial hair could be observed.

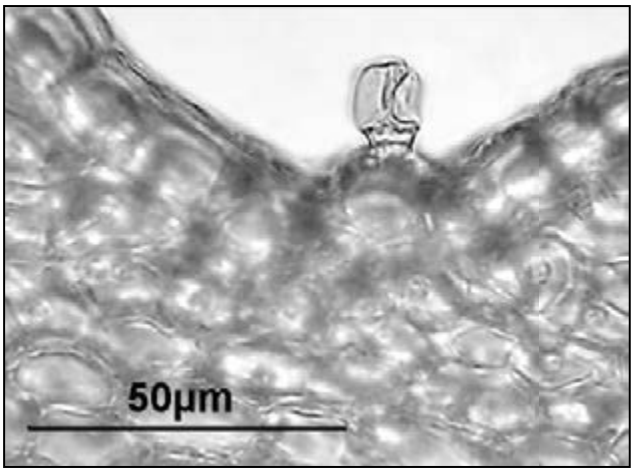

Fig. 2. Cross-section through the stem: secreting hair could be observed.

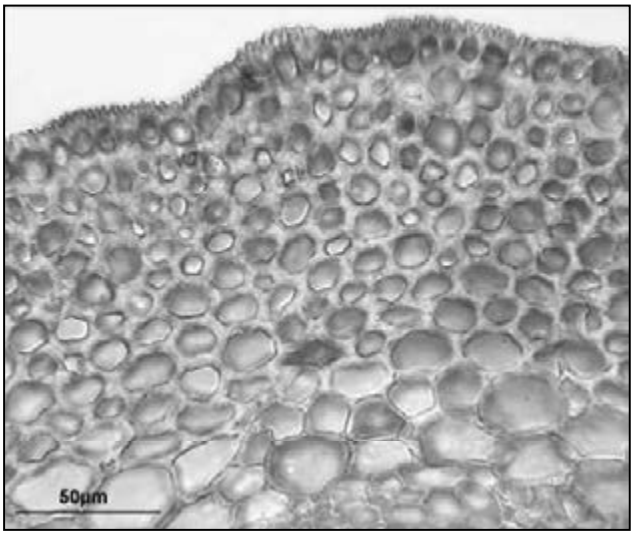

Fig. 3. Cross-section through the stem: angular collenchyma could be observed.

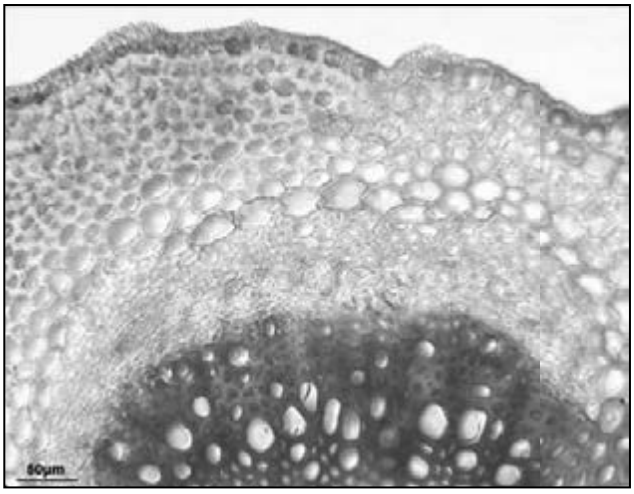

Fig. 4. Cross-section through the stem: angular collenchyma could be observed.

The central cylinder comprises 8 to 12 vascular bundles of the open collateral type, those which are at the level of the ribs being much larger and presenting – in a periphloemic position – few sclerenchymatic fibres (Fig. 5). The phloem presents sieve tubes, annex cells and parenchymal cells and the wood is made of radial lines of vessels, separated by libriform elements and by parenchymatous cells; the parenchyma is cellulosed in the primary structure and lignified in the secondary one.

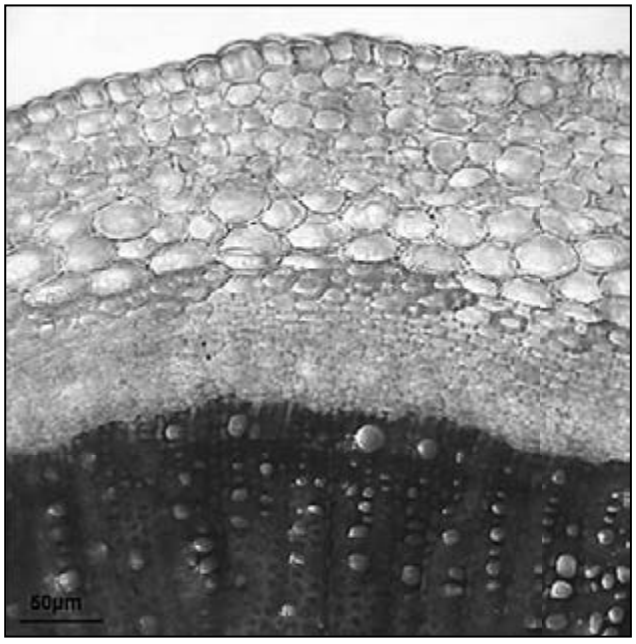

Fig. 5. Cross-section through the stem: central cylinder and periphloemic sclerenchyma could be observed.

The vascular bundles are separated by very large medullary rays made of cells whose walls are thickened and lignified between the wood sectors (Fig. 6).

The pith is thick, parenchymatous-cellulosic, of the spongy type; in the central part of the stem, the pith is resorbed, thus creating a relatively large aeriferous cavity (Fig. 7). On the three levels under analysis, the structure of the stem is secondary, being only the result of an activity of the cambium.

Leaf. The mesophyll of the lamina is differentiated in palisade tissue (with one layer on the upper face) and spongy tissue on the lower face, so the lamina has a dorsoventral bifacial structure. The foliar peculiarity important for plant identification in pharmaceutics is the structure of the secretory hairs: hairs with unicellular gland are presented on the young leaf; hairs with a bicellular gland are the most frequent and these with octocellular gland are very rare.

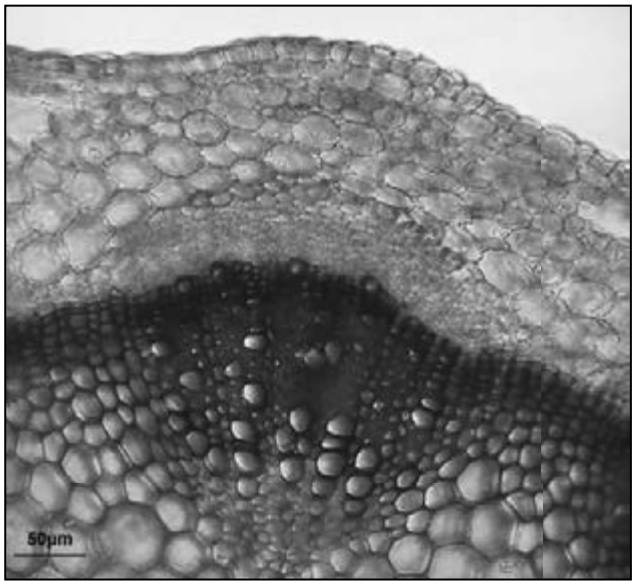

Fig. 6. Cross-section through the stem: vascular bundle could be observed.

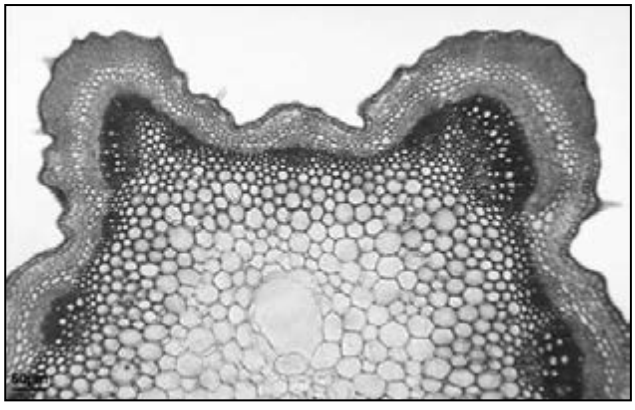

Fig. 7. Cross-section through the stem: meddulary aeriferous cavity could be observed.

Also, we report the results of phytochemical studies on aerial parts of *N. pannonica*.

The chemical composition was qualitatively and quantitatively investigated. The TLC has shown that flavonoids and polyphenols of caffeic acid type were present. The essential oil obtained from the flowering aerial parts was analysed by means of GC/MS. The major constituents were found to be sesquiterpenes (β-caryophyllene, caryophyllene oxide, β-bourbonene, germacrene G). The sesquiterpenes were accounted for 37.06 % and the monoterpenes – for 20.31 % of the sample.

Conclusions

The general structure plan corresponds to the one described by Metcalfe & Chalk (1950, 1988) for the *Lamiaceae* family. For the stem, on the three investigat-

ed levels, the variations are especially of a quantitative nature (the number of tectorial and secreting hairs increases from the base towards the top). The secreting hairs with octocellular glands are very rare.

Nepeta pannonica is typically a plant of full sun to light shady places including woodlands, meadows and fencerows. It is also found in grass habitats growing in sunlight, beside wastelands, waysides, and at the edge of pastures, encroaching small areas at the margins of arable fields or in vicinity of households (Pădure 2004).

The TLC has shown that flavonoids and polyphenols of caffeic acid type were present.

Acknowledgements. The authors would like to thank doctoral candidate Ms. Ramona Rotari for the help she has provided in creating the iconography.

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Nepeta (*Lamiaceae*) morphology and anatomy in Romania and their taxonomic significance

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Abstract. Morpho-anatomical studies of four *Nepeta* species from Romania are reported in this study. The investigated taxa featured useful taxonomic characters that can be used to establish interspecific relationships among *Nepeta* species. The morpho-anatomical characters of stem and leaves are proposed for taxonomical purposes. To assess more accurately the above relationships fruits and pollen were further investigated using morpho-anatomical techniques. The characters referred to are discussed in relation with their taxonomic significance between species.

Key words: characters, leaves, morpho-anatomy, *Nepeta*, nutlets, pollen, stem, taxonomy

Introduction

Morpho-anatomical studies of four *Nepeta* species named *N. cataria* L. (Sect. *Nepeta*), *N. nuda* L. ssp. *nuda* (Sect. *Orthonepeta*), *N. parviflora* M. Bieb. and *N. ucranica* L. ssp. *ucranica* (Sect. *Oxynepeta*) are reported in this study. *Nepeta cataria* and *N. nuda* are common and widespread plants; the other two species represented by *N. parviflora* (distribution area only in Dobrudja region) and *N. ucranica* (only in Transylvania Plain, in the centre of Romania) have a rare status within *The Vascular Plant Red List for Romania* (Dihoru & Dihoru 1994; Pădure & Bădărău 2001; Pădure 2002; Oprea 2005).

The flowers are hermaphrodite or unisexual; verticillasters in spike-like or in lax or dense, sometimes pedunculate cymes. Calyx cylindrical to ovoid, 15-veined, accrescent; teeth 5, subequal, the upper sometimes exceeding the lower ones. Corolla cylindrical, 2-lipped; tube slender, long, glabrous inside; upper lip patent, flat, 2-fid; lower lip 3-lobed. Stamens didynamous, parallel; anther-cells divergent, opening by a common slit. Nutlets dark brown to blackish, elliptic or ovate, trigonous or rounded trigonous in transverse section, and apical rounded, with a whitish or whitish-grey lateral straight or bilobed areole. The nutlets have a prominent or a slightly hidden areole, extending 1:10–2:10 of nutlet length; pericarp smooth or sculptured (Pădure 2005; Pădure & al. 2005).

Morphology, shape, colour and size of the vegetative organs may be used as a diagnostic character in species differentiation. Some common, usual features characterize the leaf and stem anatomy in *Lamiaceae*. Among these are stem indumentum (with glandular and non-glandular trichomes), lamina type, petiole structure, or complex organization of vascular bundles, stomata and collenchyma types, size and number of the vascular bundles, etc.

The morphology of nutlets or pollen (Tarnavschi & al. 1981) was used as a diagnostic character in classification (Wojciechowska 1966). Nutlet morphology in *Lamiaceae* has proved useful to varying degrees at different levels of the taxonomic hierarchy (Cantino & Sanders 1986). The importance of the morphology of nutlet surface has already been demonstrated for *Nepeta*. In recent times the importance of SEM in pericarp study and pollen surfaces has been demonstrated (Ryding 1992; Budantsev 1993a, b; Budantsev & Lobova 1997; Duletiæ-Lauševiæ & Marin 1999; Pădure 2005; Pădure & al. 2005).

The objective of this paper is to provide a detailed description of the stem, leaves (incl. petiole), nutlet morpho-anatomy and pollen morphology in four species of *Nepeta* in Romania. The original drawings and images of investigated species are presented for com-

parison. The mentioned features are useful as taxonomic characters that can be used to establish interspecific relationships among *Nepeta* species from Romania.

Material and methods

The species of *Nepeta* were collected from different populations within Romania: Cernavodă and Basarabi (Constanța County, 2002–2004) and Cluj on Dealul Sf. Gheorghe (Cluj County, 2000) for *N. cataria*; Băile Herculane (Caraș Severin, 2003) and Frăsinet (Vâlcea County, 2003) for *N. nuda*; Basarabi and Cotu Văii (Constanța County, 2001–2005) for *N. parviflora*; Frata–Vișinelu and Ploscoș–Valea Florilor (Cluj County, 2000) for *N. ucranica*. The material was fixed in a 50% or 70% ethanol mixture with 2% formalin, and 5% acetic acid (FAA). Voucher specimens of all species are kept at BUAG, BUC and BUCA Herbaria, Bucharest. Typically, plant leaves and stems of all ages for different populations were serially free-hand sectioned, stained in carmin-alaunat [carmin "Nacarat", 0.1% in 0.7% $AlK(SO_4)_2$] and green iodine, mounted in gelatinized glycerin. Stem and leaves epidermal peels were obtained from the middle region of leaf and stems in mature plants. The fragments were cleared with chloral hydrate. Afterwards, the sections were examined and photographed from permanent slides by MC–7 microscope at different magnifications. Gross morphology was studied using a binocular stereoscopic microscope. The anatomical studies of nutlets regarding the pericarp structures were represented by cross-sections in median zone and tangential one for exocarp aspect. The dried and mature nutlets were preserved in glycerol: 90° ethylic alcohol (1:1). For the morphological study of crystals from sclerenchyma layer in pericarp, the nutlets were smashed and inserted in hypochlorite for 24 hours. Original photos and drawings were made.

Results

We analysed general leaf and stem morphology, indumentum characteristics and all tissues in cross-section. Qualitative and quantitative data were obtained for a critical differentiation between species. Different types of indumentum could be recognized based on its morphology: pubescent in *N. cataria*, glabrescent in *N. nuda*, lanate-villose in *N. parviflora* and puberulent in *N. ucranica* (Fig. 1, left). We found on the same epidermic peels anizo-, actino- and diacytic stomata types grouped in stomatiferous zones (Fig. 1, right). The indumentum consisted of uniseriate and multicellular non-glandular and glandular trichomes (capitate and peltate type). The peltate trichomes lacked in stem epidermis of *N. parviflora*. Stem shape was rectangular in cross-section and 4-cornered with or without lacuna pith. The stem size ranged from 2170 μm in *N. nuda* to 4760 μm in *N. cataria*. The endodermis had primary structure with Casparian stripes on anticlinal walls of cells. Vascular bundle in *Nepeta* was open. The number of the vascular bundles varied from 4–(8)–16 mixed fascicles in *N. ucranica* to 4–8 in *N. cataria*, *N. nuda* and *N. parviflora*. The collenchyma was angular in all species to tangential type in *N. parviflora* (Pădure 2005).

Nepeta species have leaves with bifacial lamina, with dorsiventral structure, differentiated into palisade and spongy chlorenchyma. Lamina thickness, measured halfway between both cuticles ranged in cross-section from 106 μm in *N. cataria* to 233 μm in *N. ucranica*. The species presented 2–3 palisade layers (excepting *N. cataria*,

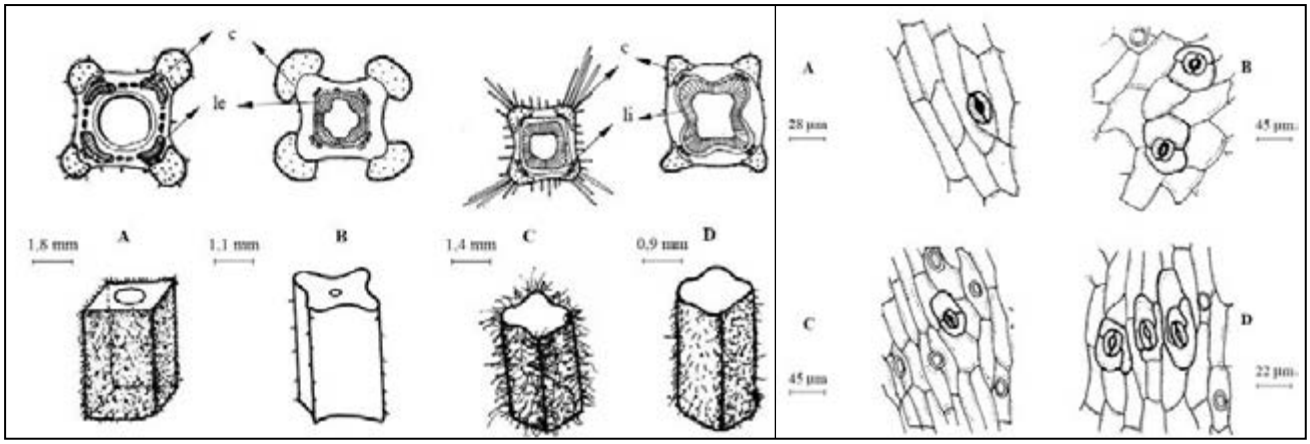

Fig. 1. The stem in cross-section and indumentum types (left); stomatiferous zones of stem epidermis (right): **A** – *N. cataria*; **B** – *N. nuda*; **C** – *N. parviflora*; **D** – *N. ucranica*; **c** – collenchyma; **le** – xylem; **li** – phloem.

one layer), to 2–4 spongy ones. The thickness of palisade tissue was 52 μm in *N. cataria* and 132 μm in *N. ucranica*. The adaxial and abaxial cuticles ranged from 2.4 μm in *N. cataria* to 7 μm in *N. parviflora* (Pădure 2004).

Stomata occurred on both surfaces in all species – amphistomatic lamina (excepting *N. cataria*, hypostomatic lamina). Stomata position (generally): cells adjacent to the guards cells and guard cells were the same level with unspecialized epidermal cells, or cells adjacent to the guards cells and guard cells raised in *N. parviflora*, due to increased hairiness. Frequently stomatal types were anomocytic or diacytic; atypical patterns with twin-stomata or actinocytic ones were present in all investigated species. The midrib of leaf did protrude in all species, more evident in *N. nuda*. Angular collenchyma (adaxially / adaxially) was 3-layered and its thickness was thicker adaxial in all species. The petiole was different-shaped: strongly flatted and lenticular in *N. nuda* or semicircular in *N. cataria*, *N. parviflora* and *N. ucranica*. The petiole presented a prominent adaxial ditch in all species or it lacked in *N. nuda*. Adaxial crests at the petiole level: strongly prominent in *N. parviflora* or absent in *N. nuda*. Angular collenchyma: discontinuous at the level of petiole in *N. parviflora* and continuous at the level of petiole – "hypodermal muff" – in *N. nuda* (Fig. 2). The trichomes: protective hairs (non-glandular trichomes) were present on both epidermises, unicellular type in *N. nuda* or multicellular (2–16-celled) in *N. parviflora*. They were shorter, very rare, usually absent (glabrous type) in *N. nuda* and long, dense (lanate-villose type) in *N. parviflora*. Secretory peltate glands present; capitate trichomes present, abaxially and adaxially in all species.

The original drawings and photos of nutlets are presented in Figs 3–4. In *Nepeta* species from Romania, two main types could be recognized based on surface ornamentation: smooth and sculptured. The exocarp ornamentation in tangential section was represented by polygonal cells with different shapes and thickening. The walls of the exocarp cells were rounded in *N. nuda* or straight in the others *Nepeta* species (Fig. 3).

The nutlet pericarp consisted of exocarp (with tubercles in *N. nuda*) and mesocarp (2–3-layered) and elongated bone cells (sclerenchyma region) enlarged in the innermost parts, occupying most of the pericarp. Numerous calcium oxalate crystals were observed in this region. The endocarp was a thin layer of small parenchymatous cells (Fig. 4A–E).

Nutlets are good characters for species recognition but the pollen tectum ornamentation appears to have potential value for classification at infrageneric levels (Jamzad 2001). *Nepeta* presents a bi-reticulate, rare perforate-reticulate (*Oxynepeta*) pollen tectum, perforated, with elliptical polar outline of the grains. Pollen grains of *N. cataria* were hexacolpate, subprolate to prolate spheroidal, with polar axis (P) 31–37 μm, equatorial axis (E) 24–27 μm; the pollen grains in *N. nuda* were prolate to subprolate, with P = 24–30 μm, E = 13–24 μm. In Sect. *Oxynepeta* the pollen grains of *N. parviflora* were prolate spheroidal to spheroidal or subprolate, with P = 27–28 μm, E = 21–24 μm; in *N. ucranica* the pollen grains were isopolar, ellipitical, with truncate poles, with P = 34–36 μm, E = 22–24 μm (Pădure 2005).

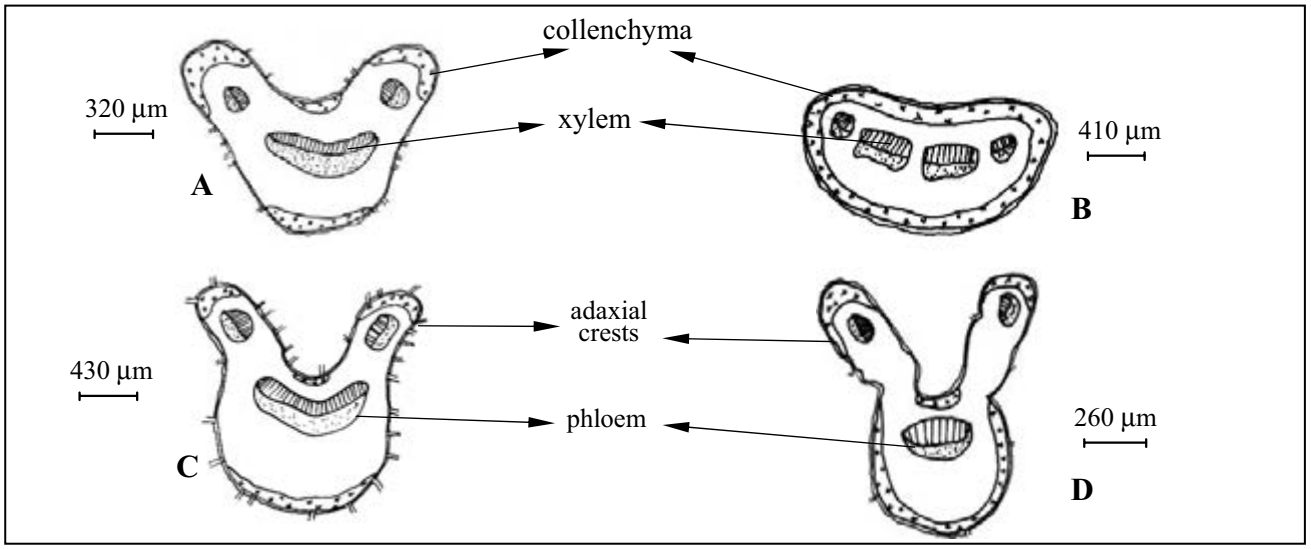

Fig. 2. The petiole shape: **A** – *N. cataria*; **B** – *N. nuda*; **C** – *N. parviflora*; **D** – *N. ucranica*.

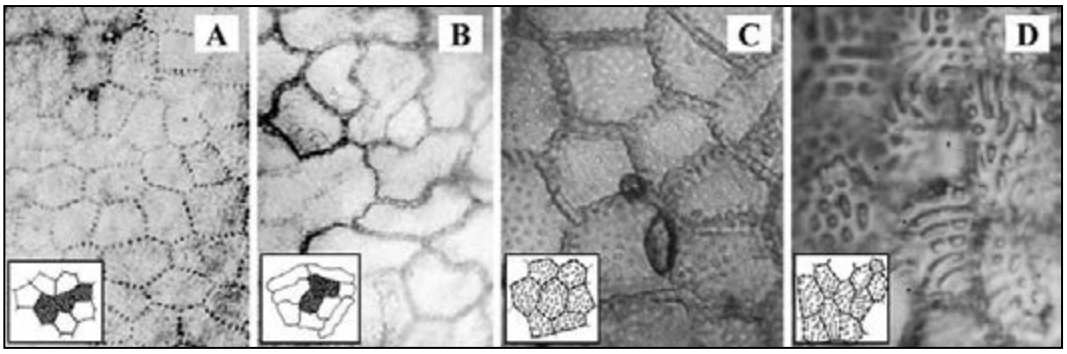

Fig. 3. The exocarp of nutlets in tangential section: **A** – *N. cataria*; **B** – *N. nuda*; **C** – *N. parviflora*; **D** – *N. ucranica* (oc. 12.5×; ob. 40×).

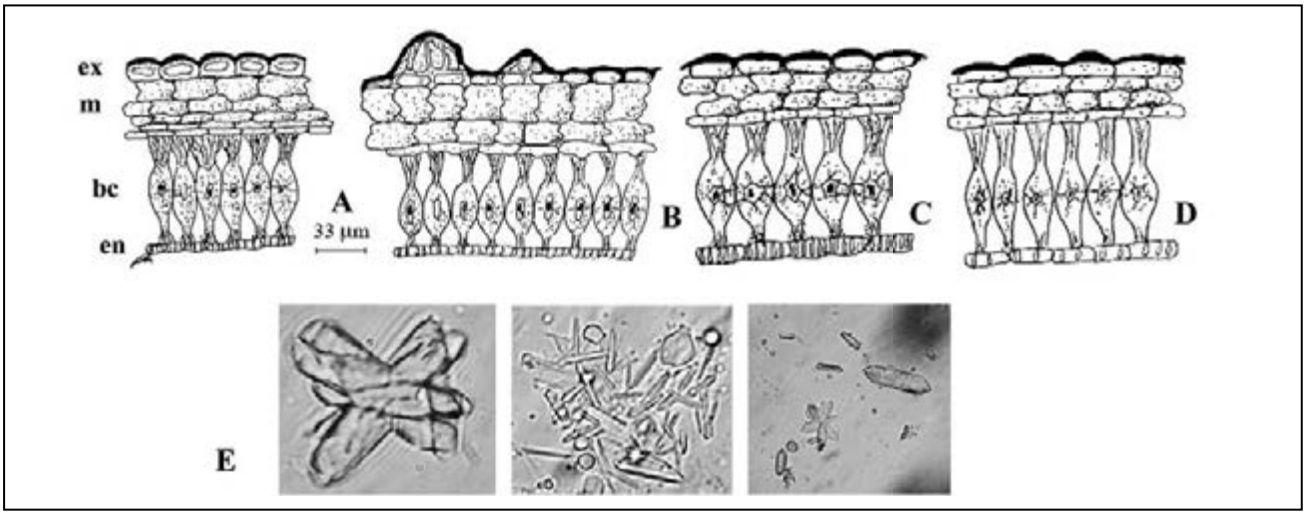

Fig. 4. The pericarp nutlets in cross-section: **A** – *N. cataria*; **B** – *N. nuda*; **C** – *N. parviflora*; **D** – *N. ucranica*; **ex** – exocarp; **m** – mesocarp; **bc** – layer of bone cells; **en** – endocarp; **E** – crystals.

Discussion

From the original results obtained in this study it can be concluded that morphological and anatomical characteristics of the leaves (incl. petiole), stem, nutlets and pollen are useful as taxonomic characters at the species level in *Nepeta* genus.

Depending on environmental conditions, the stem of *Nepeta* is remarkably variable in terms of length, diameter, branching pattern, indumentum type and colour (green to blue-violet). The stem epidermis in these species does not have uniform arrangement of cells. It presents narrow longitudinal zones with stomata, which we called stomatiferous zones; the epidermal cells are longitudinally elongated and have thin, primary cell walls. There are distinct subsidiary cells present around some guard cells and the arrangement is diacytic, anomocytic, anizocytic or actinocytic type. Untypical stomata named "heterodiacytic type" (Pădure in press) are noticed on stem epidermis (Fig. 1D, right). In this stomata type guard cells are surrounded by two unequal subsidiary cells distinct by epidermis cells. They are numerous mostly in *N. ucranica* stem epidermis. Subsidiary cells to the guard cells and guard cells more often rise. The stomatiferous zones contain typically 3–4 distinctive cells around the stoma complex. The trichomes are represented by non-glandular, multicelled and uniseriated hairs in all species. The glandular trichomes (secretory hairs) are represented by capitate and peltate in *N. ucranica*, and only by capitate hairs in *N. parviflora* epidermis.

The leaves are hypostomatic in *N. cataria* and amphistomatic in the others *Nepeta* species. Palisadic tissue is 2(3)-layered, except *N. cataria*, with one layer; tangential sections at the adaxial surface of leaf show rounded palisadic cells in all *Nepeta* species, except *N. cataria* with hexagonal cells. The stomata were anomocytic or diacytic; atypical patterns with twin-stomata or actinocytic ones are present in all investigated species.

The morphology of nutlets was investigated in four taxa of the genus *Nepeta* from Romania for the first time. Their size is 0.9–1.8 × 0.8–1.2 mm; they are elliptic or

ovate, trigonous or rounded trigonous in transverse section, and apically rounded, with a whitish or whitish-grey lateral straight or bilobed areole. The nutlets have a prominent or a slightly hidden areole, extending 1:10–2:10 of nutlet length. *Nepeta nuda* has an apical tuft of multicellular simple hairs. Similar thin hairs occur in *N. cataria* in very young nutlets, but later they are absent. The pericarp characteristics studied (shape and position of areoles, surface and colour of nutlets) are very useful in classification at taxonomic level in *Nepeta*.

Pollen grains of *Nepeta* are different-shaped: hexacolpate, subprolate to prolate spheroidal in *N. cataria*, prolate to subprolate in *N. nuda*, prolate spheroidal to spheroidal or subprolate in *N. parviflora*, and isopolar, ellipitical, with truncate poles in *N. ucranica*.

Based on morphological characters an original dichotomic key of *Nepeta* species is presented (Pădure 2005).

1a Hermaphrodite flowers arranged in dense glomerular cymes, ± sessile, corolla white; leaves triangular-ovate, base cordate, strongly crenate; nutlets smooth . . . . . ***N. cataria***

1b Flowers in dichazial pedunculate cymes, corolla violet; leaves ovate-oblong, base cordate or truncate, margin crenate-serrate; nutlets sculptured **2**

2a Gynomonoecious plants; stem canaliculate, glabrescent; corolla pale-violet; calyx tooth short; bracteoles half as long as calyx; pericarp nutlets with obvious tubercules and non-glandular trichomes at apical side . . . . . ***N. nuda***

2b Gynodioecious plants; tetrangular stems with plane sides, stem puberulent to lanate-villose; corolla blue-violet; calyx tooth longer, ± aristate; bracteoles much longer as ½ calyx lenght; pericarp slightly tuberculated **3**

3a Abaxial leaves surfaces (and stem) lanate; corolla shorter than calyx; blue-azure flowers in dichazial dense cymes; dark brown to blackish nutlets . . . . . ***N. parviflora***

3b Stem puberulent; leaves (abaxial) glabrous; corolla longer than calyx; blue-violet flowers arranged in lax dichazial cymes, sometimes with monochazium; brownish nutlets with glandular trichomes at the apical part . . . . . ***N. ucranica***

Acknowledgements. The author wishes to thank Dr V. Ciocârlan for providing advice and support in many areas and for critical reading of the manuscript; his research knowledge, technical understanding, good attitude and friendship improved the author's work and PhD experience.

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Variation of morpho-anatomical, biochemical, and physiological indicators in leaf apparatus of some ornamental plant species

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Abstract. Three species of genus *Pelargonium* (*P. zonale*, *P. radens* and *P. fragans*) and one of genus *Rosa* (*R. rubiginosa*) have been studied, investigating the morpho-anatomical characteristics of the leaf, the assimilating pigments' amount and the production of volatile oils. The biological parameters have shown evident variations. In *P. zonale* the quantitative differences expressed by the number of components of the oil are obvious: 49 in the vegetative stage and 161 in the flowering stage. In *R. rubiginosa* before flowering, 56 components have been found, among which predominant are eucalyptol and borneol. These compounds give a persistent and pleasant odour.

Key words: leaf morpho-anatomy, pigments, volatile oil

Introduction

Our research took into study two decorative plant genera widely cultivated in Romania for their decorative (by flowers and general aspect of the plants) and aromatic qualities (the plants produce volatile oils at foliar level). This last property is well known for the representatives of the *Pelargonium* genus and less for the ones of the *Rosa* genus. In these circumstances we have been interested in their aromatic properties, directed by the quantity and composition of the volatile oils secreted by special structures in the foliar apparatus so we can bring into discussion their uses in aromatherapy.

Material and methods

The biological material used for the present study consists of three species of *Pelargonium*: *P. zonale* (L.) L'Hér., *P. radens* H.E. Moore and *P. fragans* Willd., cultivated in the greenhouse of "Anastasie Fatu" Botanical Garden of Iași and, respectively, one species of genus *Rosa*: *R. rubiginosa* L., cultivated in the same location, in unprotected (free) areas. All species were cultivated in summer of 2004.

For *Pelargonium* species the experimental lot consisted in 15 individuals for each investigated species. Up to date, there have been taken two series of samples, before and during flowering period, respectively. Before taking the samples for the extraction of the essential oils, plants' heights have been measured and leaves have been counted, for a further contingent correlation with other biochemical and physiological parameters.

For the species of *Rosa* genus the analysed material was collected in specific phenophases during the summer of the year 2004 (from June to September): for the morphological and anatomical analyses, the material was collected when the first flower opened, and for the biochemical and physiological studies, in the period starting with the flowering until the formation of fruits and until senescence. The leaves analysed were

collected from the middle region of the yearly twig and from mature plants of more than 25 years old, which excludes the behavioural variations caused by their adaptation to the environment conditions.

The anatomical analyses (made in the Laboratory of Vegetal Morphology and Anatomy of the Faculty of Biology within the "Al. I. Cuza" University of Iaşi) aimed at:

a) Investigating the anatomy of the leaf lamina, near the middle vein, by the technique of hand sectioning and of double staining;

b) Investigating the morphology of the structures that secrete volatile oils on the fresh leaf surface, using the NOVEX microscope with a magnifying power of 200×; the photographic images were obtained and processed with a MINOLTA digital photocamera.

The biochemical and physiological analyses consisted in:

a) Determinations of foliar assimilator pigments – through spectrophotometric method (Boldor & al. 1983).

b) The extraction of volatile oils from fresh leaves using hydro-distillation method and a Clevenger device. The analysis of the quality was carried out using a gas chromatograph with a spectrometric mass detector Agilant 5973 and an auto sampler; the DB5 chromatographic column has a length of 25 m and an interior diameter of 0.25 mm. The separated compounds were identified by means of the Nist spectra database, and the peaks' position was confirmed by the Kovats indices.

Results and discussion

Anatomical characteristics of the leaf limb:

• In *P. zonale* the analysis of the foliar surface points out the existence of multicellular secretive hairs on the two sides of the limb; these formations have big dimensions compared to the component cells of the multicellular leg. The basis of the hair is situated between the epidermic cells, unicellular and polygonal (Fig. 1 – image a). The aspect that was not pointed out during the ontogeny of the analysed species was the morphological differences of the secretive hairs inducted by the age of the leaves.

The secretive structures of the *P. radens*' essential oil were found to be multiple-celled hairs located both in upper and in lower epidermis. The length of the hairs is variable, in accordance with the hair foot component cells (Fig. 1 – images b and c).

• In *R. rubiginosa* (Fig. 2) the anatomical analyses of the leaf limb aimed at identifying and describing the structures that secrete volatile oils; evidenced the presence of such structures on different segments of the leaf.

The rachis presents long tactile hairs and papillae-like structures in patches, with a headed ending, with a multicellular leg, with cells lined up in two parallel rows and with thin and cellulosic walls that remind the multicellular secretive hairs.

The limb of the leaflet: from a frontal perspective, the epidermis is made up of polygonal cells with straight lateral walls in the upper epidermis as well as in the lower epidermis. The anomocytic stomata are present only in the lower epidermis, so the limb is hypostomatic. In both epidermises, there are long, unicellular tactile hairs, with very thick walls and with an obtuse point. At the ends of the lamina, one can see multicellular secretive headed structures, made up of cells with very thin, cellulosic walls. All these secretive structures from the petiole, rachis and leaflets are multicellular secretive hairs. They are in much greater number on the inferior part of the limb. In the transversal section of median vein, one can see that it is obviously prominent on the inferior part of the limb, and that the epidermis presents tactile and secretive hairs (Gostin & al. 2000).

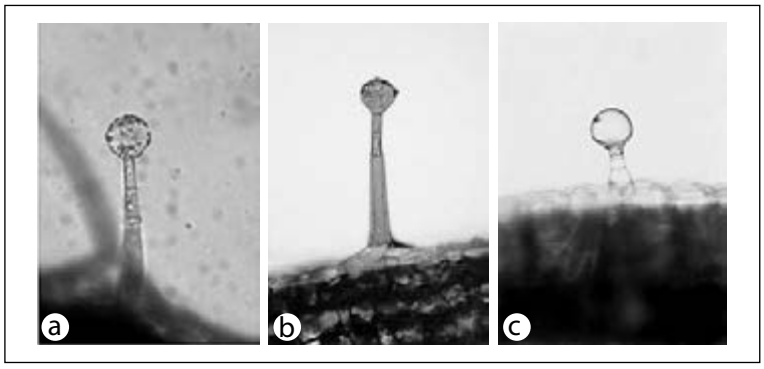

Fig. 1. Secretive hairs (multicellular hairs) in *P. zonale* (image **a**) and *P. radens* in the vegetative stage: longer hair foot (image **b**); shorter hair foot (image **c**) (×200).

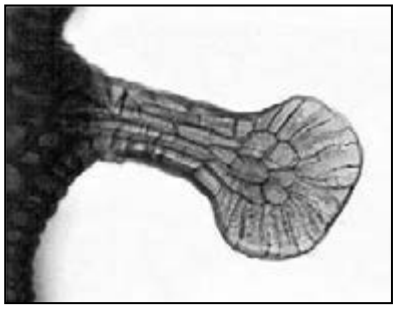

Fig. 2. *R. rubiginosa*: multicellular secretive hairs from the rachis of the leaf (×50).

The biochemical and physiological parameters:

➢ The leaf assimilating pigments' content:

• In *Pelargonium* species (Diagram 1)

The leaf assimilating pigments' content varies in correlation with the analysed taxon and the moment of analysis (phenophase).

Pelargonium radens and *P. zonale* have in both moments of analysis (before blooming and during blooming) a total leaf assimilating pigments' content higher then *P. fragans*. *Pelargonium zonale* has the highest content of **a** chlorophyll during the young period of the flowers (before flowering – 2.177 mg) and during flowering this content is not higher than the maximum value of *P. zonale*.

The **b** chlorophyll is more reduced then the **a** chlorophyll in all the cases of taxa that we have investigated, throughout all effected determinations. In *P. zonale* the **b** chlorophyll level drops at the second moment of analyses, reaching practically half (0.4756 mg) of its initial value (0.7577 mg). In the other two species there is a slight increase of this pigment in the flowering moment compared to the anterior one. The assimilating pigments' content reaches small values in all taxa in both phenophases. The ratio **a** chlorophyll / **b** chlorophyll is 3:1 in favour to **a** chlorophyll for *P. zonale* in both moments of analyses and slightly smaller in the other two species. The high content of **a** chlorophyll shows that these plants need light. Also we consider that the variation of assimilating pigments' content in the *Pelargonium* species indicates physiological efforts in the test plants to sustain a photosynthetic process (Burzo & al. 2004) adapted to their needs in both analyses moments (the increase of foliar surface by new organic substance synthesis in the vegetative stage and the growth of the reproducing apparatus in the flowering phenophase).

• In *Rosa rubiginosa* (Diagram 2)

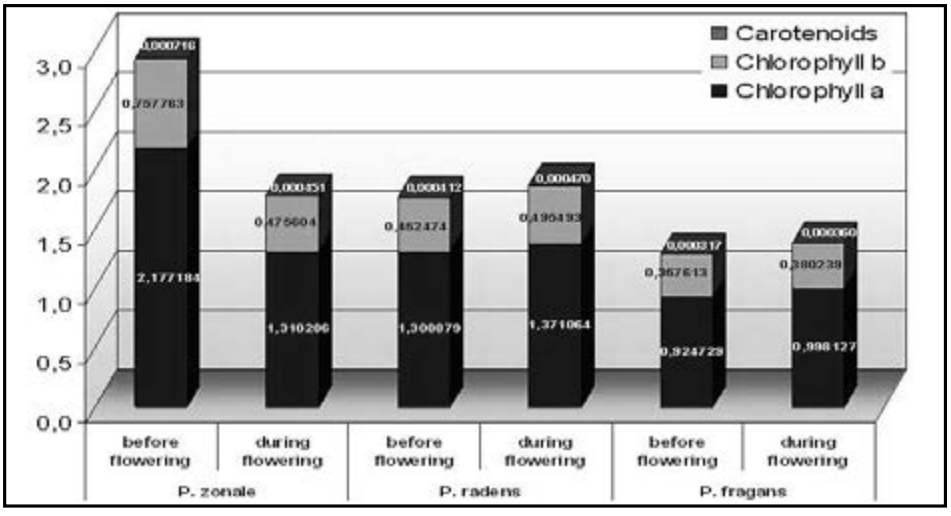

Diagram 1. The dynamics of photosynthetic pigments found in leaves of *P. zonale*, *P. radens* and *P. fragans* before and during the flowering period (mg/g fresh matter).

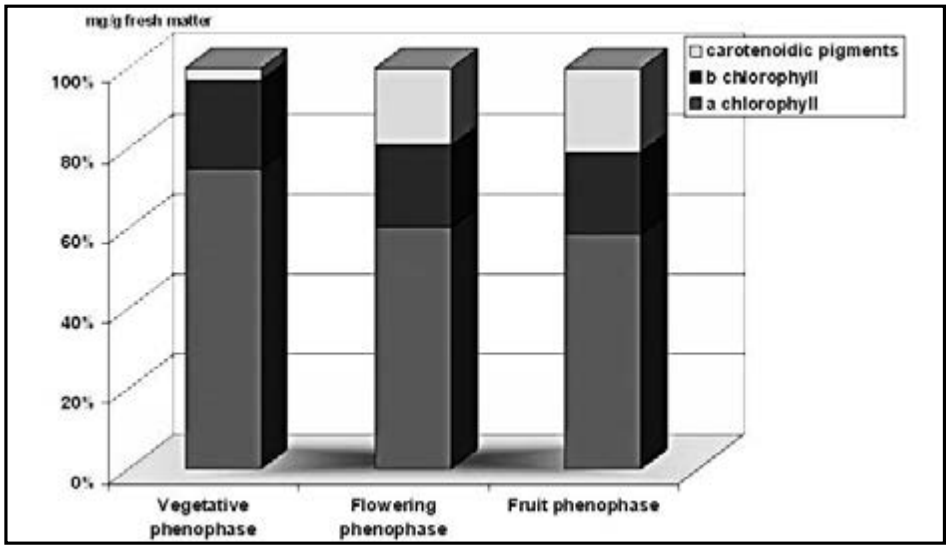

Diagram 2. The dynamics of photosynthetic pigments found in leaves of *R. rubiginosa* (mg/g fresh matter).

The quantity of **a** chlorophyll, the main photo-assimilating pigment within the photosynthesis process, varies according to the ontogenetic moment. The quantitative evolution of the **a** chlorophyll respects the progression of the phenophases in the ontogenetic cycle, with maximum biosynthesis, accumulation and usage points in the flowering phenophase (2.31 mg and 1.73 mg, respectively), when the biosynthesis needs of the test plants become maximum (it is the moment of formation of the reproductive system). Along the entire measurement period, the **b** chlorophyll has subunitary values; the biosynthesis curve has maximum points of biosynthesis in the vegetative phenophase (0.58 mg). The ratio between **a** chlorophyll and **b** chlorophyll remains constant during the entire time interval, close to the value 3:1 for the **a** chlorophyll. According to these values, we can characterize the taxon studied as being amenable to light. Carotenoidic pigments present subunitary values along the entire period analysed. In the vegetative phenophase, the investigated taxon present a content of carotenoidic pigments 10 times lower as compared to the rest of the period taken into account, and a maximum content in the flowering period.

We consider that the values registered are in accordance with the environment conditions in which the test plants lived, with a dimmer light in the first analysis interval (in spring) and with a stronger light, but also with an increase in the negative effects of solar radiations on plants in the summer stage (the flowering phenophase), when the light period and intensity increased (Burzo & al. 2004). At a later stage, in the fruit phenophase (corresponding to the beginning of the autumn), the carotenoidic pigments content decreases again, approaching the value registered in the spring stage (0.30 mg in the fruit phenophase, 0.32 mg in the vegetative phenophase, respectively). In the specialized literature, specialists state that there is a constant relation between the intensity of the photosynthetic process and the content of assimilating pigments. Thus, we can consider that the investigated taxon with European origin makes an increased metabolic effort in order to insure an intense photosynthesis process. This process can thus produce the organic substances needed in every stage of the ontogenetic cycle of the taxon under investigation.

➢ The dynamic of volatile oils:

• In *Pelargonium* species

The quantity dynamics of volatile oils in the *Pelargonium* leaves (Diagram 3), which is expressed in extraction percentage, shows a superior rhythm for all three species in the second analysed phenophase (flowering phenophase), respecting the specialized literature, according to which in the flowering period the plants produce a higher quantity of volatile oils (Burzo & al. 2005).

The quantitative dynamics of volatile oils in the leaves of investigated *Pelargonium* taxa (Diagram 4), expressed by the number of components detected for the *P. zonale*, shows a quantity three times higher in the flowering period compared to the phenophase anterior to the flowering moment (the vegetative phenophase). This situation can be found also in the *P. fragans* species, where in the flowering phenophase we found six times more compounds compared to the vegetative phenophase. In *P. radens* the situation is reversed, the number of compounds in the

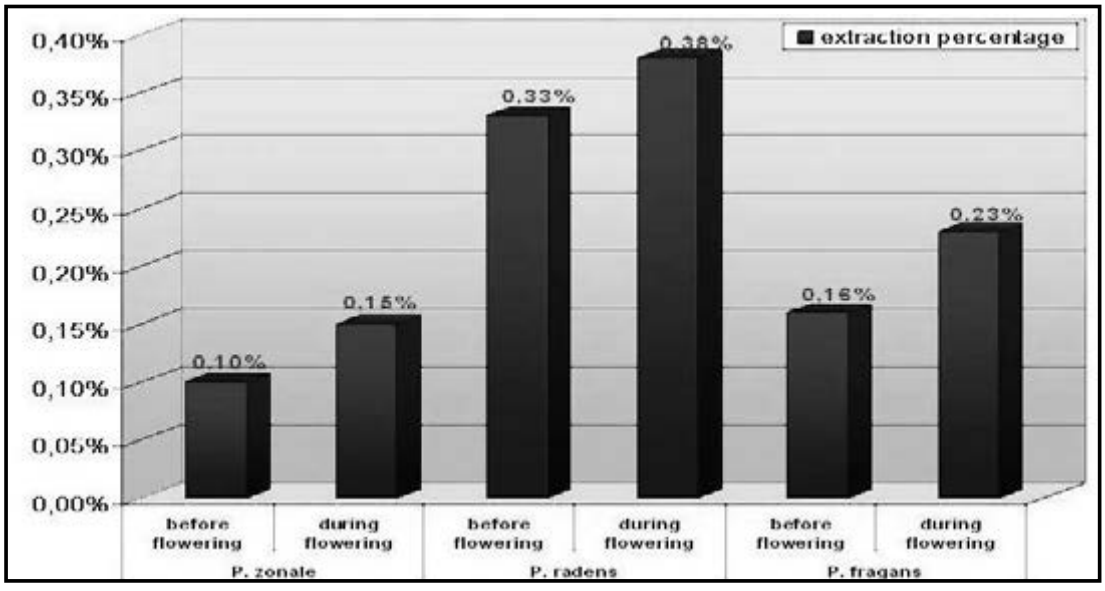

Diagram 3. Quantitative dynamics of the volatile oils (percentage).

vegetative phenophase (64 compounds) is higher than the one in the flowering period (44 compounds).

In *P. zonale* species there has been performed a comparison between the quality of the volatile oils in the two phenophases (Diagram 5) expressed in % between the compounds identified in the two moments of analyses. From the graphic representation we consider that the oil produced in the flowering phenophase is of a higher quality, having greater quantities of common identified components (myrcene, menthone, β-carotene, cedrol).

In *P. radens* the efficiency of the oil extraction was calculated at 0.33 % in the vegetative period and its quantity composition showed a higher number of compounds (64) (Diagram 6). In this species the most common ones are the citronellol (28.69 % of all identified compounds) and the menthone (27.30 %).

- In *Rosa rubiginosa*

The composition of the volatile oil in *R. rubiginosa* is presented in Diagram 7. *Rosa rubiginosa* presents on the leaf (on the inferior epidermis of the leaf limb,

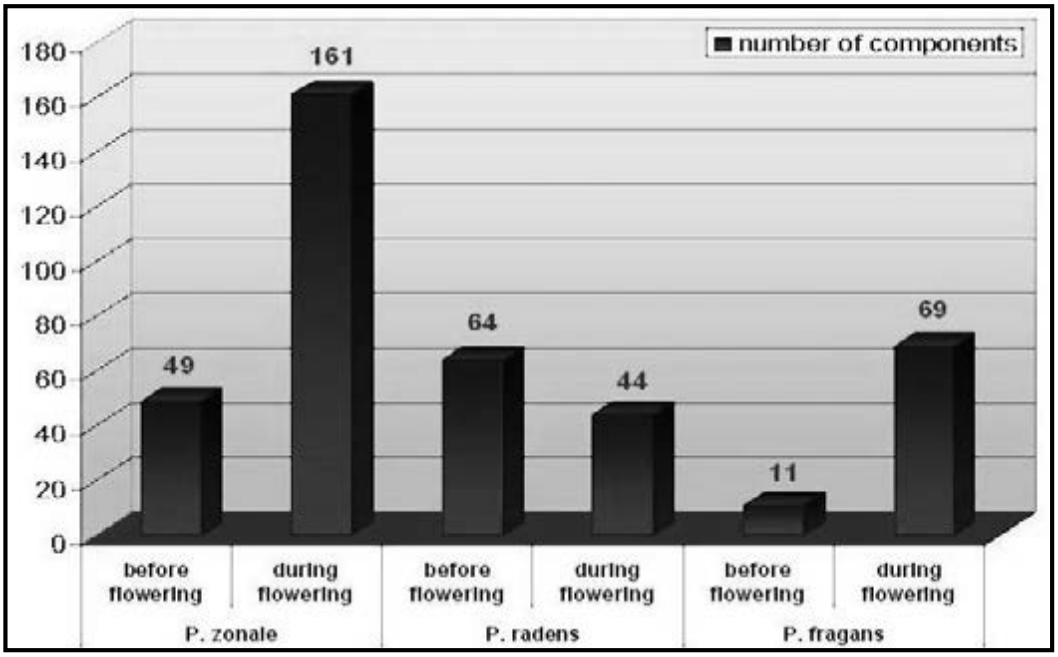

Diagram 4. Quantitative dynamics of the volatile oils (number of components).

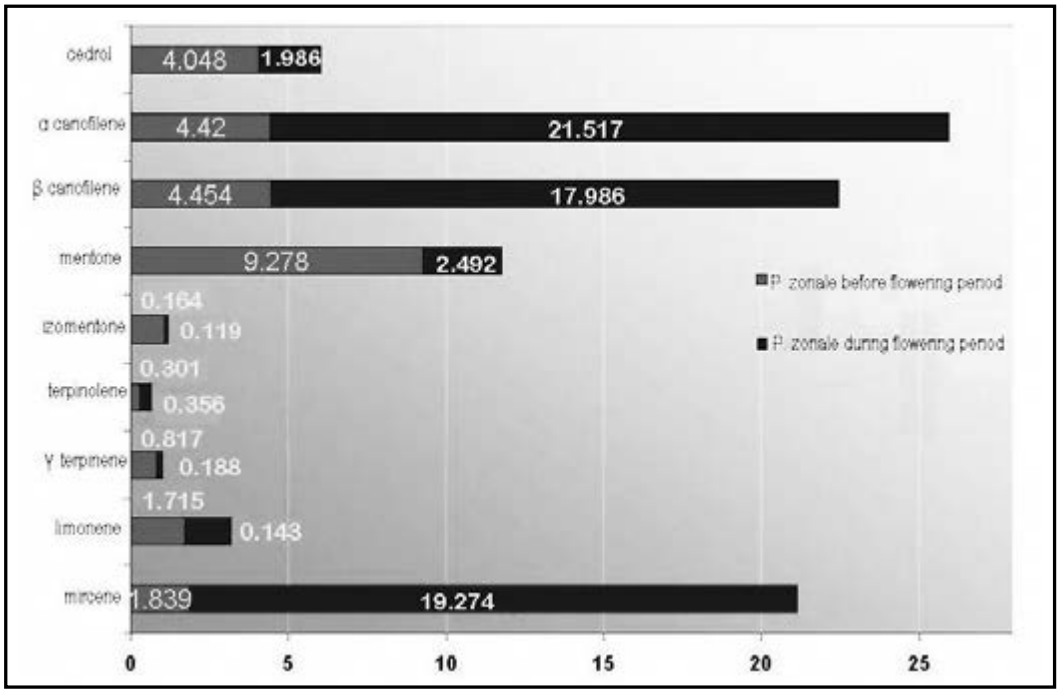

Diagram 5. Difference between the main components found in *P. zonale* volatile oils extracted before and during flowering period (%).

on the rachis and stipels) glandular hairs that produce a volatile oil with a scent of green apples (Gostin & al. 2000). About 56 compounds make up this oil, among which the most important are the eucalyptol (26.88 % of all the identified compounds) and borneol (15.29 %), compounds that confer to the oil a pleasant, persistent smell. We also mention the presence of some compounds that represent more than 1 % of all the isolated compounds: p-cymene (12.03 %), γ-terpinene (8.26 %), bornyl acetate (5.60 %), α-pinene (4.66 %), camphene (3.73 %), β-pinene (3.69 %), α-humulene (3.42 %) and β-caryophillene (3.40 %). The other compounds have values close to 1 % of all the identified and isolated compounds, or even smaller values.

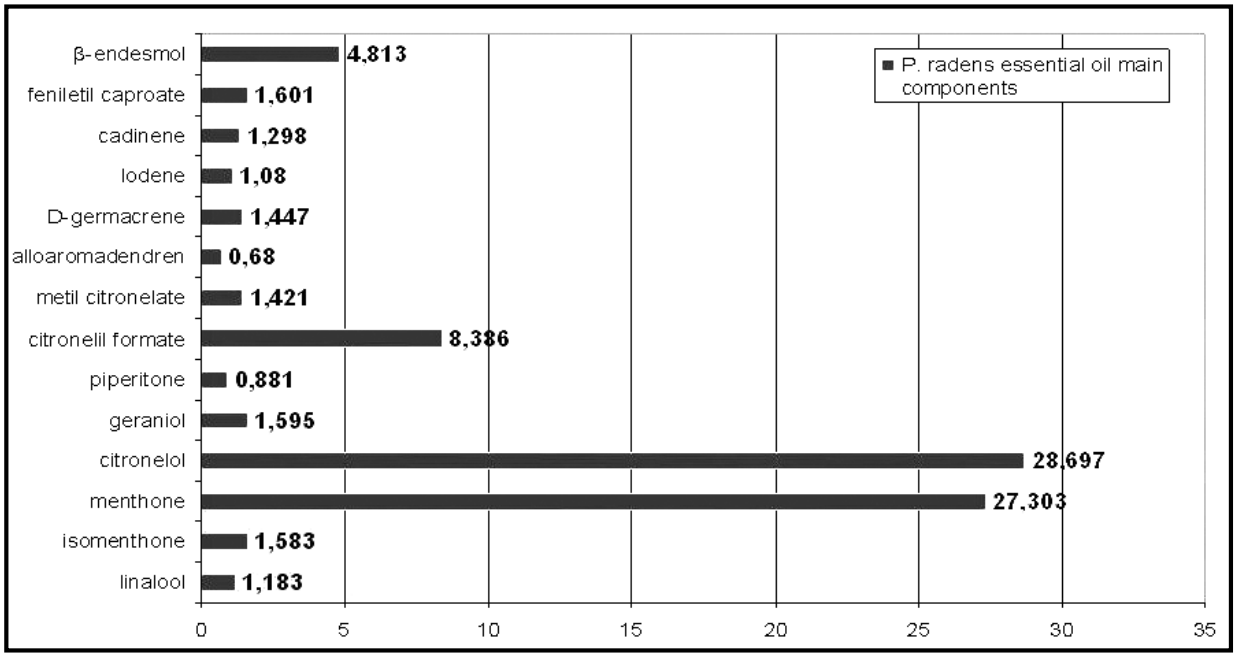

Diagram 6. *P. radens* essential oil main components (% of all identified compounds).

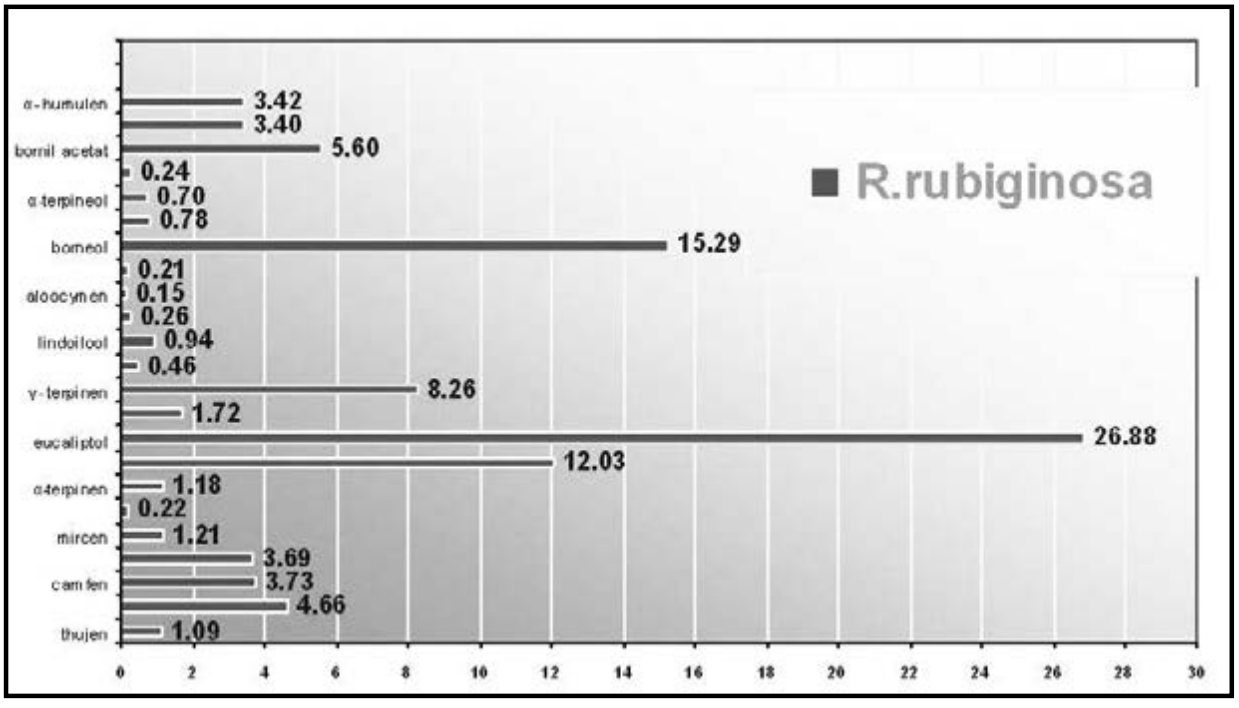

Diagram 7. The chart for the main components of volatile oils (% of all identified compounds) extracted from *R. rubiginosa*, cultivated in Iaşi Botanical Garden.

Conclusions

• The secretive structures for volatile oils in *P. radens* are multiple-celled glandular hairs, located both in the upper and in the lower epidermis. In both analysed genera there are multicellular secretive hairs on the two sides of the limb; the aspect that was not pointed out during the ontogeny of the analysed species were the morphological differences of the secretive hairs inducted by the age of the leaves.

• The practical data that have been obtained concerning the anatomy of the leaf for all studied species are in accordance with the data presented in the specialized literature.

• The variation of the biochemical and physiological indices analysed within the ontogenetic dynamics for the taxa under study present their adaptation to the vegetation conditions offered by the Botanical Garden in Iaşi. The interpretation of the data is strictly related to the climatic conditions in the summer of 2004, marked in free area of the garden by an increased drought, high temperatures, which meant that the test plants had to make great metabolic efforts for survival.

• For all three species of *Pelargonium* as well as for *Rosa rubiginosa*, the amount of pigments was found larger especially during the flowering period.

• The extraction efficiency of volatile oils from leaves of analysed plants has different values, according to the species and to the ontogenetic period; for all analysed species, the extraction efficiency is higher during the flowering period.

• The number of components of the volatile oils extracted is larger during the flowering period for the oils extracted from *P. zonale* and *P. fragans*, and lower for *P. radens*.

• The chemical composition of *P. zonale* essential oils extracted in the two different ontogenetic periods differs qualitatively, as well as quantitatively. There are different components in the oils extracted in the two ontogenetic periods, whereas the common components for the two oils have been found in different percentages.

• The volatile oils produced within the leaves by the species *R. rubiginosa*, with a specific qualitative and quantitative composition, give the taxon a special aromatic character that increases its ornamental, aromatic and, implicitly, commercial qualities.

Acknowledgements. This paper was realized with the support of The Research Basis with Multiple Users of the Faculty of Horticulture, in Agriculture Sciences and Veterinary Medicine University of Bucharest, Romania.

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Influences of interfered delayed senescence on morphological and anatomical changes in *Glycine max*

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Abstract. In this study, morphological and anatomical changes of *Glycine max* (soy bean plant), which appeared during the senescence, were investigated. For this study the plant specimens produced at Biological Department of Faculty of the Art and Sciences of Trakya University were used. The plants were divided into main and control experimental groups. Anatomical changes in these two groups of the plants were investigated. The data obtained from the experiments were evaluated and compared to previous studies.

Key words: anatomical changes, *Glycine max*, senescence

Introduction

Senescence is death of some plant cells, tissues or organs according to the developmental processes of the plant. It is a programmed result of development and also a critical activity in a plant's life. The senescence may be benefited by being used agriculturally.

In the organs where senescence takes place biochemical changes occur and catabolic reactions fasten. With these biochemical changes the chlorophyll molecules are broken down to form xanthophylls and carotenoids. As a result, the protein amount in the leaves gradually decreases. DNA and RNA are suspended and these molecules move to other parts of the plant with growth activity to be used for next season or next generations (Molish 1938).

Soybean seeds are of a paramount importance since they are used to obtain 250 different types of industrial products such as soybean milk, milk-powder, yoghurt, ice-cream, cheese, margarine, gasoline, ink, antibiotics, drugs, soap, insecticides, baby food and soybean oil. About 10 % of all car oils consumed in Belgium annually are obtained from soybean. In addition, soybean is the only food besides animal originated one, possessing the 8 amino acids humans can't synthesize and have to intake from outside.

In this study, we aimed to determine the anatomical changes that occur during senescence in the stem and leaves of *Glycine max* (L.) Merr.

Material and methods

The studied material was obtained in Trakya Agricultural Research Institute. The plants were grown in Botanical garden of the Biology Department. The seeds were planted along lines 40 cm apart leaving a distance of 20 cm between each seed. A total of 500 plantations were done inside experimental and control group parcels. The seed development was monitored daily. After 10 days of the seed plantation almost all seeds germinated. Following this period the plants were regularly given water and the wild grasses were picked to provide the plant healthy growth.

The growing plants were observed to flower towards 11 May 2003. Following the first flowering, the buds and flowers of plants of the experimental group were picked up every day in contrast to the plants of the control group. The changes occurring in the plants were observed and photographed. Stem and leaf samples were taken at regular intervals from both experimental and control plants for anatomical investigations. Some of these samples were used for laboratory studies, while others were kept in 70 % ethanol for further analysis.

The preparations for anatomical investigations were made from samples taken when the flowering started, during maturation and at the end of experiment. Cross sections taken using a razor blade for stem anatomy were stained with safranin and embedded in glycerin-jelly (50 % glycerin: 50 % gelatin). The permanent slides were investigated with light microscope and photographed using an Olympus CH 2 photomicroscope.

Results and discussion

Morphological comparisons were made between the plants of control group which underwent senescence during experiment and experimental plants whose senescence was prevented. It was observed that control group plants' growth reduced the seed formation and they became pale. These plants died to the end of 5th month. However, the stems of the plants of experimental group, whose senescence was prevented by picking their flowers everyday, got thicker compared to the control group. Also, their leaves were thicker and dark green and they gave more buds. We observed that the experimental group plants still continued to grow, when the control plants died. No anatomical differences were observed in cross sections of samples of both groups taken when flowering started until the time when control group plants developed seed. However, differences were established between the groups by August. The sections observed showed us that the stems of the experimental group plants had a more jagged structure than the control ones. The increase in numbers of the inner cell layers was determined to cause this difference. The plants of control group did not show such an increase (Figs 1, 2). In addition, the cells below epidermal tissues of the experimental plants increased in their sizes and they involved chloroplasts even when control group plants went yellow. Chloroplasts of the control group plants were observed to be damaged and some of the cells beneath epidermis turned into lamellar collenchymas (Figs 3, 4).

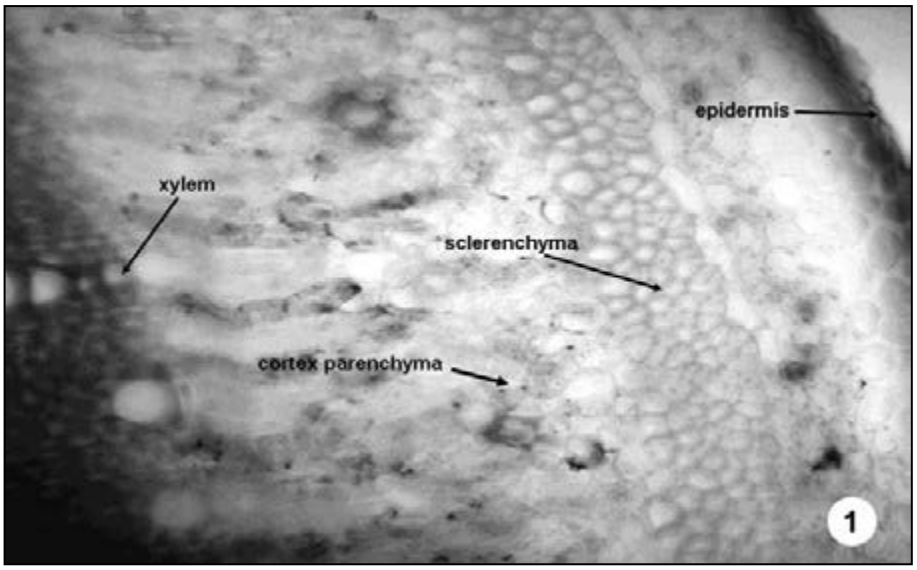

Fig. 1. Tranverse section of control group plants in August (×100).

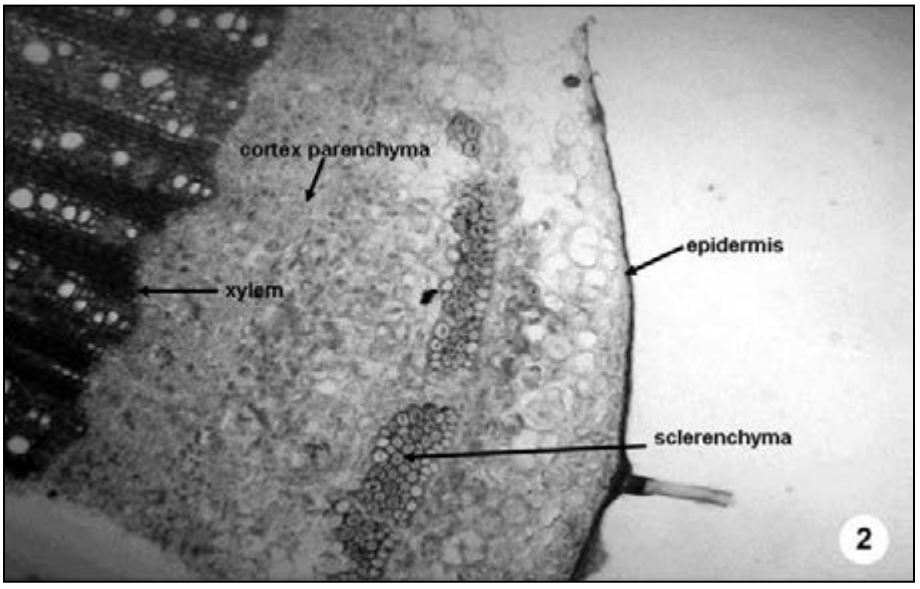

Fig. 2. Tranverse section of experimental group plants in August (×40).

Our anatomical and morphological findings were compared to those announced by other authors given below.

According to Molish (1938), the catabolic reactions fasten in organs where senescence takes place. As a result, the broken

down materials are moved to the stored organs or to other plant parts to be used in either next season or next generations. It has been observed that material accumulation was much more in experimental group stem cells of plants than control group.

Butler (1967) reported that chloroplasts were broken down, free ribosomes increased in number and membrane system of the cell aborted at the senescence. The turns to yellow in our control group are thought to be due to the break down of chloroplasts.

Önder and Yentür (1999) investigated the changes occurring during senescence with electron microscopy. They reported that structural deformations took place in organelles of cells during the senescence. Our light microscope observations confirm this finding.

Matile (1975) reported that various enzymes located in the vacuolar system broke macromolecules down. According to this idea these macromolecules could easily move to other parts of the plants.

Woolhouse (1967) stated that damages in the cell structures and organelles influenced the photosynthesis and respiration in a bad manner. Such an influence is seen as a decrease in photosynthesis. The respiration continues to be stable for some time but starts to decrease at the end of senescence following a rapid increase substance content of plant organs. Our morphological observations also confirm these statements.

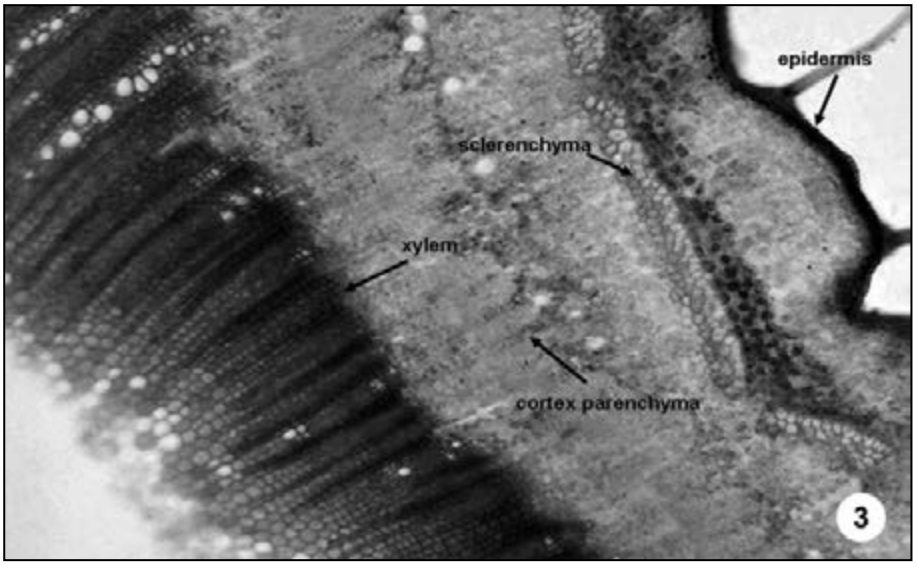

Fig. 3. Transverse section of mature plants of control group (×40).

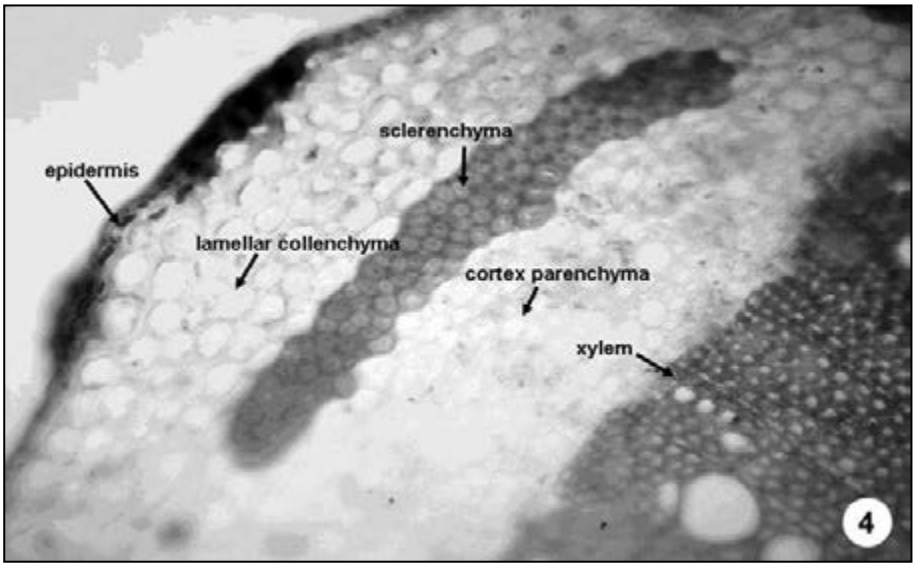

Fig. 4. Transverse section of mature plants of experimental group (×100).

Murneek (1926) reported that growth rate of the plants went slow with the start of senescence. He also reported that growth rate of the tomato plants, whose flowers were picked up, increased due to a delayed senescence. We observed in our study that the growth continued in our experimental plants, while it stopped in control group due to flowering and fruiting.

Leopold & al. (1959) retarded the death of soybean plants for one year delaying the senescence. Our experimental plants were still in growing process even when the control ones were about to mature. But they died when the temperature decreased during the cold season. We think that if suitable climatic conditions are provided it is likely for the plants to continue their growth.

This accumulation was observed in the parenchyma tissues in the plants of our experimental group. However, the accumulation was very little in control plants, probably because of transportation of synthesized materials to the fruits

and seeds. As such transportation is not seen in the experimental plants, these materials accumulate in stems. Our findings on this property are in accordance with the previous ones. We believe that our present findings will contribute to the further studies. We also think that new studies carried out on the senescence in different plant species will be useful to reveal in detail the peculiarities of the morphological and anatomical changes during this important process.

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Morphological characteristics of achenes in *Potentilla argentea* group (*Rosaceae*)

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Abstract. Achene morphology in taxa from *Potentilla argentea* group, i.e. *P. argentea* and *P. neglecta*, was examined with stereoscope and scanning electron microscope. Achenes of these taxa varied slightly in shape and colour, while marked differences among them appeared in the surface sculpture and in size, dimensions of aril, dorsal ridge and ribs. SEM analyses allowed determining two distinct morphological types of achenes: ruminate-reticulate and ruminate sculpture. The dimensions of achenes and the surface sculpture are very good features to separate *P. argentea* and *P. neglecta* as different species; however, these features may be a valuable taxonomical criterion only in combination with other ones.

Key words achenes, fruit wall sculpture, *Potentilla argentea* group, SEM

Introduction

Potentilla argentea group (*Rosaceae*) is a polyploid complex, the members of which may be either amphi- or apomictic; some diploids and most polyploids are obligate apomicts (Ball & al. 1968). However, according to Holm & Ghatnekar (1996) the diploid *P. argentea* L. is a sexual species and facultative apomict. Marklund (1933) recognized three species from *P. argentea* group, i.e. *P. argentea*, *P. neglecta* Baumg., and *P. calabra* Ten. Müntzing (1928, 1931, 1958) has shown that one of Marklund's species is usually diploid and another usually hexaploid, though the correlation is not perfect. They were classified as microspecies, subspecies or rarely varieties (Ascherson & Graebner 1904–1905; Wolf 1908; Szafer & Pawłowski 1955; Gerstberger 2002; Kurtto & al. 2004).

Potentilla argentea and *P. neglecta* have been described as independent species mostly on the basis of the number of leaflets on basal leaves, the pattern of pubescence of upper and lower surfaces of leaf blades (Wolf 1901, 1903, 1908; Juzepczuk 1941; Ball & al. 1968; Borhidi & Isépy 1965; Soják 1995). Whereas their trichomes are of the same type, the density being a very variable character, there is no ground for separating them as different species (Leht 1989).

It is known that fruits and seeds are very useful in identification and classification of plant taxa (Karcz 1996; Liu & Lin 1999; Fagúndez & Izco 2004; Maciejewska-Rutkowska & Bednarz 2004; Özcan 2004). Description of achenes from taxa of *P. argentea* group is limited to their length, shape and colour (Wolf 1908; Juzepczuk 1941; Kelley 1953; Szafer & Pawłowski 1955; Soják 1995). These features, however, are not sufficient to identify particular taxa.

Fruit in *Potentilla* named also achene is dry, not dehiscent and monospermous, small in size and brown in colour (Wolf 1908; Hegi 1922; Juzepczuk 1941; Kelley 1953; Szafer & Pawłowski 1955; Ball & al. 1968; Anderberg 1994; Soják 1995; Gerstberger 2002). This latter feature is a good criterion of its maturity, since an unripe fruit is more light brown than a mature one. Therefore, the purpose of the present paper was the complex morphometric analysis of the achenes from two taxa of *P. argentea* group together with their shape, colour and surface sculpture.

Material and methods

The following taxa from *P. argentea* group, i.e. *P. argentea* and *P. neglecta,* were analysed. Nomenclature of taxa was used according to Kurtto & al. (2004). Plant material originated from natural habitats in Poland.

Only mature, fully developed achenes, intensively brown in colour, were used in the investigations while distinctly smaller and deformed ones were discarded.

Colour of the achenes was determined in the day light on the basis of colour scale recommended by Berggren (1969). Dimensions – length, width and thickness of the achenes, width of the aril, width and thickness of the ribs and width and height of dorsal ridge were measured according to the description presented in Fig. 1. Morphometric analysis of the achenes except aril and rib dimensions was made using a stereoscope microscope Nikon SMZ 800 with millimetre scale (exact to 0.05 mm). At least 60 individual achenes for each taxon were analysed.

For scanning electron microscopy (SEM) samples were mounted on metal stubs, coated with technical gold (Pelco S.C 6 coating system), examined and photographed using a Tesla BS 340 scanning electron microscope. Shape of achenes, dimensions of aril and ribs as well as the pattern of surface sculpture of the fruit wall were analysed on 5 photographs for each taxon.

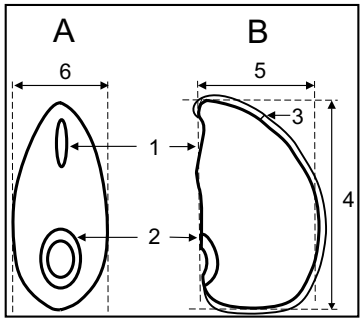


Fig. 1. Achene of *Potentilla* sp.: **A** – ventral and **B** – lateral view; **1** – style scare; **2** – aril around scare attachment; **3** – dorsal ridge; **4** – length; **5** – width; **6** – thickness [after Anderberg (1994), modified].

Obtained data were statistically analysed by the Student's test. A difference was considered statistically significant when $p < 0.01$.

Results

Achenes in *P. argentea* group are bilateral, shape ovate, laterally flattened, apex obtuse, curved, base obtuse (Plate I, Figs 1, 2).

Two types of achenes are distinguished: large, present in *P. argentea* and small, present in *P. neglecta* (Table 1). Achene colour varies slightly from brown in *P. argentea* to nut-brown in *P. neglecta* (Table 1).

Scare attachment, i.e. the point of achene attachment to the receptacle is surrounded by an aril slightly visible in *P. neglecta* or clearly – in *P. argentea*, with their respective widths being 10 μm and 50 μm (Table 2).

Table 1. Colour and dimension of achenes (in mm) in taxons of *P. argentea* group.

Taxa	Length	Width	Thickness	Colour	
				Achenes	Ribs
P. argentea	1.10 ± 0.01	0.84 ± 0.01	0.57 ± 0.01	brown	brown
P. neglecta	0.76 ± 0.02	0.64 ± 0.01	0.42 ± 0.01	nut-brown	brown

Table 2. Characteristics of achenes in taxons of *P. argentea* group (SEM).

Taxa	Aril		Dorsal ridge			Surface sculpture	Ribs (μm)	
		Width (μm)		Width (μm)	Thickness (μm)		Width	Height
P. argentea	clear	50	clear	40	20–40	reticulate	60	40
P. neglecta	unclear	10	unclear	20	20	ruminate-reticulate	20	20

A clear dorsal ridge about 40 μm wide and 20–40 μm thick was present in achenes of *P. argentea*, while unclear one, about 20 μm wide and 20 μm thick was observed in *P. neglecta* (Table 2).

Ribs seen at achene surfaces are brown in colour (Table 1). Distinct ribs, very sharp in shape were seen in achenes of *P. argentea* (Plate I, Figs 1A, B), or oval in shape – in *P. neglecta* (Plate I, Figs 2A, B). Width of ribs varied from 20 μm in *P. neglecta* up to 60 μm in *P. argentea* (Table 2). Similarly rib height was the lowest (20 μm) in *P. neglecta*, while the largest (40 μm) was in *P. argentea* (Table 2).

SEM analyses of the surface sculpture revealed two types of achenes:

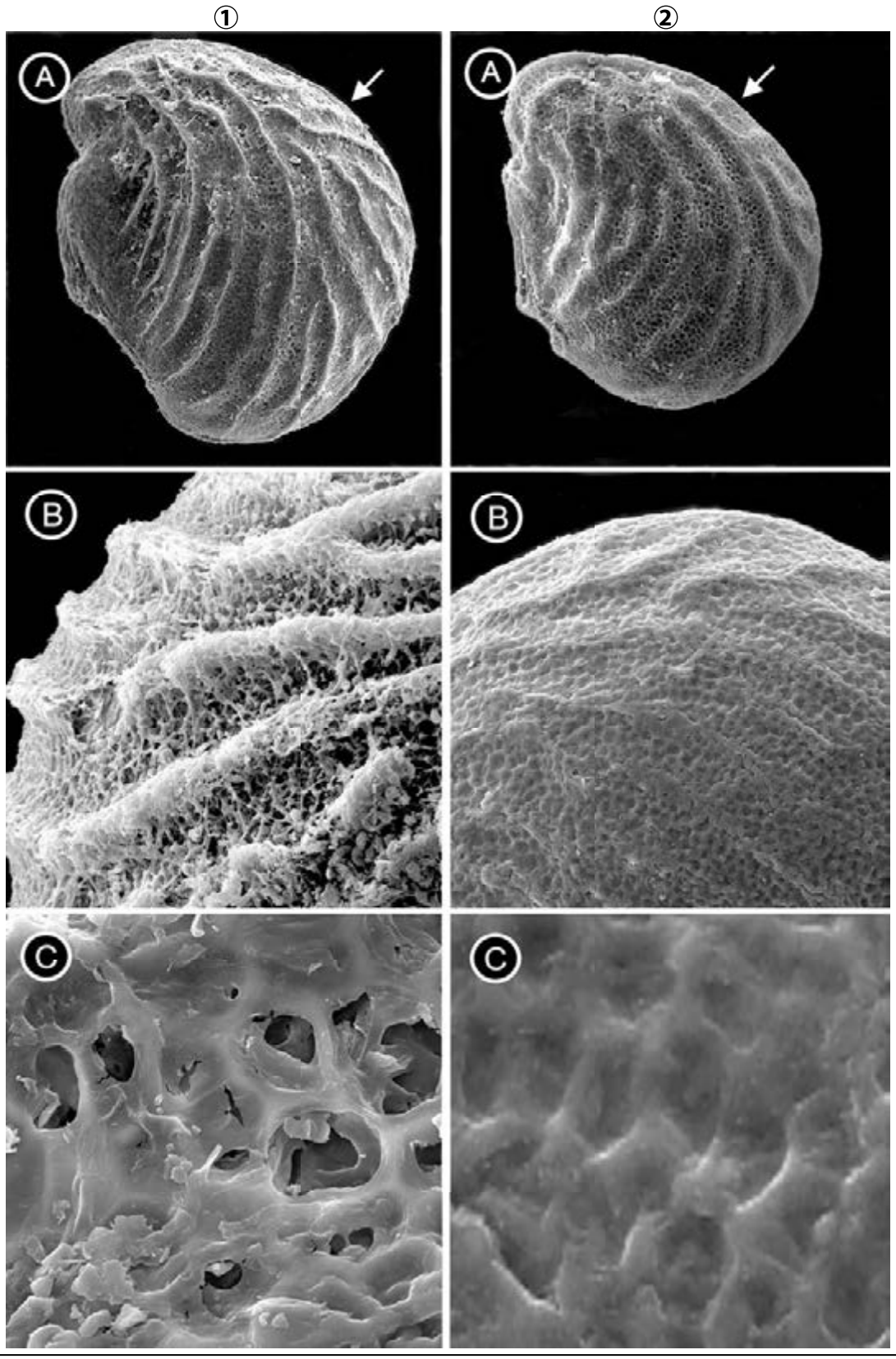
– with ruminate sculpture, characterized by oval ribs and epidermal cells partly destroyed and covered with numerous aggregates of some material (probably waxes, etc.), various in shape and size. This type of achenes was typical for *P. argentea* (Plate I, Figs 1B, C);

– with ruminate-reticulate sculpture; among distinct and sharp ribs well preserved epidermal cells, hexagonal in shape were visible. Achenes of this type were characteristic of *P. neglecta* (Plate I, Figs 2B, C).

Discussion

Potentilla argentea group has been treated in widely divergent ways by different authors, ranging from the mere recognition of *P. argentea* to the acceptance and description of a large number of distinct species (Kurtto & al. 2004). For example, Wolf (1908) divided the aggregate of *P. argentea* into *P. argentea* with twelve varieties. Later Juzepczuk (1941) distinguished *P. argentea* and *P. impolita* (sensu auct.; = *P. negecta*) and Gerst-

Plate I



Figs 1–2. Surface sculpture of the achenes of *P. argentea* group, at different magnifications – (**A**) ×500; (**B**) ×1000; (**C**) ×3000: **1,** *P. argentea* (on the left); **2,** *P. neglecta* (on the right). Arrows indicate dorsal ridge.

berger (2002) distinguished *P. argentea* with four varieties, but *P. negecta* was not recognized. Pedersen & Schou (1997) have shown that type material of *P. impolita* Wahlenb. is belonging to *P. inclinata* Vill.

A similarly narrow concept of species was presented by Marklund (1933) and Ball & al. (1968) according to whom *P. argentea* group consists of three separate species in the area of Europe, i.e. *P. argentea*, *P. calabra* and *P. neglecta*. The classification into three species proposed after Marklund (1933) and Ball & al. (1968) seems to be more satisfactory on a European scale, though some may be such on more local scales (Kurtto & al. 2004).

Our investigations of taxa from *P. argentea* group revealed differences in colour and shape of achenes as well as in their sizes. According to our measurements the lengths of achenes of *P. argentea* (1.1 mm) were similar to those described by Anderberg (1994) and Soják (1995) – 0.7–1.9 mm.

Scanning electron microscopic analysis of achenes from *P. argentea* group allowed distinguishing new additional features such as aril, dorsal ridge and rib dimensions, useful in taxonomy of this difficult collective species. These features of achenes in addition to the morphology of leaves (Wolf 1901, 1903, 1908; Juzepczuk 1941; Ball & al. 1968; Soják 1995) proved to be of high systematic importance in taxonomy of *Potentilla* species. The surface sculpture and the dimensions can be a good criterion in classification of taxa from *P. argentea* group.

The results of the present paper indicate significant differences between *P. argentea* and *P. neglecta*. Differences in the dimensions, the colour, the surface sculpture of achenes and in dorsal ridge dimensions are ground for separating *P. argentea* and *P. neglecta* as different species similarly to Marklund's (1933) and Ball's & al. (1968) idea.

Acknowledgements. The author would like to thank Dr Krzysztof Polański (Institute of Physics, University of Łódź) for taking the photographs.

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Fruit and seed morphological peculiarities of the critically threatened *Eriolobus trilobatus* (*Rosaceae*)

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Abstract. *Eriolobus trilobatus* is one of the rarest tree species of the European flora and it has been included in the Red lists of the countries in which it can be met. It is a valuable fruit and honey-bearing plant with high ornamental qualities and drought resistance. In the present study a description of fruits and seeds of *E. trilobatus* from two successive crops (2002, 2003) was made. The studied materials came from two localities – one of them in the Eastern Rhodopes (Bulgaria) in the region of Ivaylovgrad town and the other one in Northern Greece in the region of Dadia village. The size and the mass of fruits and seeds from both localities and crops have been compared. The basic indices of the quality of the seed materials – mass of 1000 seeds, seed yield, number of seeds in a single fruit, have been determined. A study is recommended on the possibilities for seed propagation of the species and its cultivation in the most southern and arid regions of Bulgaria.

Key words: Bulgaria, *Eriolobus trilobatus*, fruits, Greece, seeds

Introduction

Eriolobus trilobatus M. Roem. (= *Malus trilobata* (Labill.) C.K. Schneid., *Sorbus trilobata* (Labill.) Boiss., *Crataegus trilobata* Labill.) is a small tree of family *Rosaceae*, subfamily *Pomoideae.* It was described from the German botanist Roemer, who put it in a separate genus. It used to be referred to genus *Crataegus* and genus *Sorbus*. It is a thermophylous Mediterranean element of the Bulgarian flora (Assyov & al. 2002).

The tree is 6–10 m high, branches are spinousless, and the crown is usually stretched. Young twigs are purple and leaves are bare and glossy above, bright light-green below, thin and stiff, 5–8 cm long and up to 9–10 cm wide, with heart-like base, deep palmately 3-lobed with wide 2-lobed lateral lobes and simple or 3-lobed middle lobes. Flowers are about 3.5 cm in diameter, arranged in 6–8 umbel-like to raceme-like clusters. Petals are white, blunt serrated at the top. Fruit is oblong egg-like to globular, similar to wild apple fruit, light-yellow to dark-brown in colour, with stony cells around the core. The tree species propagates by seeds. The flowering begins in May and ends in June. The fruits ripen in October–November. They have good taste quality. The local human population uses the fruits for food in raw and canned form (Tashev 2001).

This species grows on dry and poor soils in xerothermic oak zone (up to 260 m alt.) under transitional Mediteranean climate. It stands very good the dry conditions – in the very dry 2000 year trees had full flowering and abundant fructification (Tashev 2001).

Geographic distribution of the species covers Eastern Mediterranean – Northeastern Greece, Syria, Lebanon, Israel and Southeastern Bulgaria (Valev 1973; Browicz 1982). The *E. trilobatus*' locality in Bulgaria is in the far north part of the species area of distribution and far out of its ecological optimum and consequently, its reproductive functions are embarrassed.

Eriolobus trilobatus is a plant with high conservation status in Bulgaria and abroad. It was included in the *Red Book of Bulgaria* under category "threatened to extinction" (Marinov 1984). It is protected by the Law on Biological Diversity of Bulgaria (2002). The index of species threatening (Itr) is 122 which refers to the critically threatened species of the Bulgarian flora (Peev 1981). The species has been included in the *Greek Plant Red Data Book* in category "vulnerable" (Christensen 1995). Its distribution in Greece was

studied by Browicz (1982), who determined 2 basic localities: 13–18 km along the Loutros–Pesani road, and 4 km SE of Mesti site. *Eriolobus trilobatus* is one of the rarest tree species of the European flora.

The first individual of *E. trilobatus* in Bulgaria was found in the area of Likana in May 25, 1955 (Stojanov & al. 1955). The authors mentioned that the locality found is about 70 km apart the localities of this species in West Thrace found by Dingler (the same author classified it as *M. trilobata* var. *rumelica* (Dingler) Schneid.).

Eriolobus trilobatus is exceptional drought-resistant tree species with abundant annual fructification. The discovery of this species in Bulgaria recommends its propagation for using as ornamental and fruit species in the most southern and arid regions of Bulgaria.

The aim of this study was to describe morphological peculiarities of fruits and seeds of *E. trilobatus* from two localities in Rhodopes Mts (Eastern) – in Bulgaria and Northern Greece.

Material and methods

The studied material was collected from two crops – 2002 and 2003, in localities in Bulgaria and Greece.

The locality in Bulgaria is in the area of Likana between the villages Svirachi and Belopolyane 6–7 km from the town Ivaylovgrad. It consists of 2 trees growing 1000 m between each other. There used to be a third tree which was destroyed during the road's construction. The trees are found in open places next to agricultural land. Other woody species growing in the locality are: *Quercus pubescens* Willd., *Phillyrea latifolia* L., *Carpinus orientalis* Mill., *Juniperus oxycedrus* L., *Coronilla emerus* L., *Cornus mas* L.

The locality in Greece is in the region of Dadia village (Northern Greece). It consists of about 15 individuals situated singly and rarely in small groups of 2–3 or more trees. The distances between the trees and groups are from 50 to 200 m. The individuals were found near the road Loitsos–Pesani in open coppice forests including mostly *Quercus pubescens*, *Q. frainetto* Ten., *Q. coccifera* L., *Fraxinus ornus* L., *Arbutus unedo* L., *A. andrachne* L., *Phillyrea latifolia* L., *Paliurus spina-christi* Mill. and other species.

The following parameters have been studied: fruit length and diameter; fruit stalk length; number of seeds in a single fruit; mass of a single fruit; seed length, width and thickness; mass of 1000 seeds; number of seeds in 1 kg; seed yield. Minimum 25 fruits and seeds of every locality and crop have been measured. The data were processed statistically. The relationships between fruit size and seed number in a single fruit were tested by mean of correlation and simple regression analysis.

Results and discussion

The results of the fruit length and diameter of fruits (Table 1) determine that the largest fruits originated from locality in Bulgaria crop 2003 – a mean length 22.6 mm and a mean diameter 23.9 mm. They were also the heaviest – the mean mass of a single fruit was 9.66 g. Similar values were obtained for the fruits from the locality in Greece crop 2002 – mean length 21.2 mm and mean diameter 24.7 mm. The data of the fruit diameter approximately concord to the values reported by other authors (Huxley 1992), where the lowest values were about 20 mm. The smallest fruits were measured in the locality in Greece crop 2003 – mean length 17.1 mm and mean diameter 17.6 mm.

Each fruit had a fruit stalk with the length from 25.6 to 33.5 mm (Table 1). No relationship was found between the length of fruit stalk and fruit length. For example, the small fruits of the Bulgarian locality crop 2002 had the longest fruit stalk – 33.5 mm, while the fruits of the same locality crop 2003 were the longest – 22.6 mm, but with the shortest fruit stalks – 25.6 mm.

In Table 2 the number of seeds in a single fruit is presented. A high percentage of seedless fruits and fruits with one seed were observed in the Bulgarian locality. The average number of seeds in a single fruit was more than 1 only in crop 2003. In the Greek local-

Table 1. Fruit characteristics of *E. trilobatus*.

Locality/ Crop	Length (mm)			Diameter (mm)			Fruit stalk (mm)			Average mass of 1 fruit ± s.e. (g)
	Mean ± s.e.	Min	Max	Mean ± s.e.	Min	Max	Mean ± s.e.	Min	Max	
Bulgaria										
2002	18.6±0.15	13	25	21.4±0.23	14	30	33.5±0.50	14	50	6.37±0.16
2003	22.6±0.39	17	28	23.9±0.54	19	38.5	25.6±0.89	14	42	9.66±0.16
Greece										
2002	21.2±0.14	16	29	24.7±0.16	17	32	33.1±0.60	15	60	8.14±0.16
2003	17.1±0.20	14	21	17.6±0.21	13	21	29.5±0.66	15	46	4.38±0.57

Legend: s.e. – standard error.

Table 2. Number of seeds in a single fruit.

Locality/ Crop	Bulgaria				Greece			
	2002		2003		2002		2003	
	Number	%	Number	%	Number	%	Number	%
Fruitless	208	71.6	122	27.6	140	12.7	10	3.6
Fruit with abortive seeds			25	5.6	21	1.9		0.0
Fruit with 1 seed	73	25.1	235	53.1	443	40.2	74	27.0
Fruit with 2 seeds	8	2.7	47	10.6	254	23.0	92	33.6
Fruit with 3 seeds	1	0.3	12	2.7	133	12.0	40	14.6
Fruit with 4 seeds			1	0.2	67	6.1	27	9.9
Fruit with 5 seeds	1	0.3	1	0.2	26	2.4	19	6.9
Fruit with 6 seeds					13	1.2	7	2.6
Fruit with 7 seeds					4	0.4	4	1.5
Fruit with 9 seeds					1	0.1	1	0.4
Total fruits	291	100.0	443	100.0	1102	100.0	274	100.0
Total seeds	97		698		1863		660	
Average number of seeds in a single fruit	0.33		1.5		1.69		2.4	

ity the seedless fruits were considerably fewer (from 3.6 % to 12.7 %); there have been fruits with different number of seeds reaching 9. The average number of seeds in a single fruit in both crops increased and even exceeded 2. These results showed that in the Bulgarian locality there are serious problems with pollination and development of seeds, because of the trees remoteness. The nearer distance between the individuals in the Greek locality is a prerequisite for successful fructification. The parthenocarpy is slightly presented.

No statistically significant relationship between the length of fruit and the number of seeds in a single fruit was found. Consequently the number of seeds in a single fruit did not depend on the size of fruit, but on the fructification and especially on the pollination.

The seeds of *E. trilobatus* are similar in shape and size to the seeds of *Malus sylvestris* L. No considerable differences in shape and colour of seeds in both localities and crops were found. They were prolonged ovate, pointed of both ends. One side of seed was nearly straight and the other was round, slightly domed. Some seeds were 3-sided. The seeds were red-brown to dark red-brown in colour. Some seeds were lighter red-brown with light orange hues, lightly glossy. The data presented similarity in size of seeds from the both localities and crops (Table 3). The seeds from the Bulgarian locality crop 2003 were a bit larger than those from the Greek locality crop 2002. The mass of 1000 seeds corresponded to the size of the seeds. It was the biggest – 47.07 g in the case of the biggest seeds, and the smallest – 28.53 g in the case of the smallest seeds. The number of seeds in 1 kg did not depend on their mass, because the highest number of seeds in 1 kg had been established in the case of the smallest and lightest seeds. Seed yield was too small especially in Bulgarian locality – only 0.26 %–0.38 %. The values of this parameter in the Greek locality were a bit higher and got near or exceeded 1 %–0.86 % for crop 2002 and 1.25 % for crop 2003. The low values of the seed yield in *E. trilobatus* can be explained by the small number of seeds having small size and mass and situated in comparatively fleshy and heavy fruit. Seed yield in other species from genus *Malus* is low too, for example in *M. sylvestris* – from 0.8 % to 1.5 % and in *M. pumila* Mill. – 0.75 % (Milev & al. 2004).

Conclusions

The fruits from the Bulgarian locality crop 2003 had the largest sizes.

No relationship between the length of fruit stalk and fruit length was found.

The highest percentage of seedless fruits was established in the Bulgarian locality. This shows that *E. trilobatus* has serious problems with pollination and development of seeds, because of the tree remoteness.

Statistically significant relationship between the length of fruit and number of seeds in a single fruit was not found.

Table 3. Seed characteristics of *E. trilobatus*.

Locality/ Crop	Seeds									Mass of 1000 seeds (g)	Number of seeds in 1 kg	Seed yield (%)
	Length (mm)			Width (mm)			Thickness (mm)					
	Mean ± s.e.	Min	Max	Mean± s.e.	Min	Max	Mean ± s.e.	Min	Max			
Bulgaria												
2002	6.4±0.06	5	7	4.5±0.06	4	5	2.3±0.02	2	2.8	35.42	28232	0.26
2003	7.2±0.09	6	9	5.0±0.12	4	9	2.5±0.05	2.1	4.8	47.07	21245	0.38
Greece												
2002	6.7±0.07	5.5	7.5	4.6±0.05	3	5	2.5±0.03	1.7	2.8	37.11	26947	0.86
2003	6.2±0.07	5	7	3.9±0.05	3	5	2.1±0.03	1.6	2.8	28.53	35050	1.25

Legend: s.e. – standard error.

There was a similarity in size of seeds from both localities and crops.

The seed yield was a bit higher in the Greek locality.

Further studies of seed propagation and cultivation in the southern and arid regions of Bulgaria have to be carried out.

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Variations in quantitative vessel element characters of *Cerasus avium* (*Rosaceae*) in Turkey

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Abstract. Variations in quantitative vessel element characters of the wood of *Cerasus avium* were investigated in relation to altitude and tree-ring width. According to multiple regression analysis using altitude and tree-ring width as independent variables, significant correlations were obtained at the 0.001 level for vessel density and vulnerability ratio, at the 0.01 level for mesomorphy ratio, and at the 0.05 level for vessel tangential diameter and element length. The number of vessels per group does not show any correlation with independent variables. Non-anatomical factors (altitude and tree-ring width) explain 25.4–50.1 % of variation for dependent variables.

Key words: altitude, *Cerasus avium*, ecology, tree-rings, wood anatomy

Introduction

Cerasus avium (L.) Moench is one of the native taxa in Turkey; it is distributed from sea level up to 1600 m alt. in deciduous forests of Euxine province (Browicz 1972). Euxine province, one of the two sub-provinces of Euro–Siberian Region, lies between Melet River in Ordu and Istranca Mt in Kırklareli along Black Sea Region of Turkey (Yaltırık & Efe 1989) (Fig. 1). In general, there is a humid climate type in this subprovince (Erinç 1996).

The basic and detailed studies in relation to ecological wood anatomy were carried out by different researchers (Carlquist 1966, 1975, 1977a, b, 1980; Baas 1973, 1976; Van der Graaff & Baas 1974; Baas & al. 1983; Carlquist & Hoekman 1985; Fahn & al. 1986). In recent years, many studies have been realized on this subject in different regions of the world (Zhang & al. 1992; Lindorf 1994, 1997; Noshiro & al. 1994, 1995; Noshiro & Suzuki 1995; Villar-Salvador & al. 1997; Mauseth 1999; Alves & Angyalossy-Alfonso 2000, 2002; Cooper & Cass 2001). Most of the studies mentioned above indicated some ecological trends in vessel element characters. It has been explained by Carlquist (1975) and examined by Baas (1976) in detail that anatomical diversity in wood is a function of selective pressures of environmental conditions such as water availability, temperature, etc. Two ratios based on vessel diameter, vessel density and vessel element length were formulated by Carlquist (1977b) and Carlquist & DeBuhr (1977) for ecological interpretation in wood anatomy: vulnerability (mean vessel diameter divided by mean vessel density) and mesomorphy (vulnerability multiplied by vessel element length). In his book, entitled "Comparative Wood Anatomy", Carlquist (1988) evaluated the significance of vulnerability and mesomorphy equations as compared to Hagen-Poiseuille equation in detail.

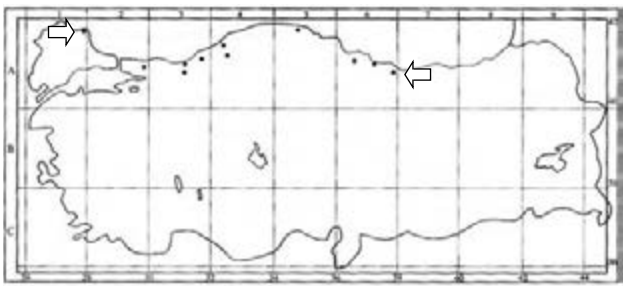

Fig. 1. Euxine province between arrows, and localities of wild cherry trees sampled in this province of Turkey (•).

As for Turkey, there are a small number of studies related to ecological wood anatomy. At first, Yaltırık (1968) studied anatomical characteristics of Turkish maples wood with relation to the humidity of

the sites. Şanlı (1977) examined anatomical features of beech woods related to altitude in the different regions of Turkey. After then, some studies mentioning the relationships between wood anatomical characters and ecology have been carried out in Turkey (Gerçek & al. 1998; Merev & Yavuz 2000; Yaman 2002; Serdar 2003). Because there have been a great variety of woody taxa and their habitats in Turkey, and a small number of these taxa have been studied so far in relation to ecological wood anatomy, Turkey has an important potential for this research area.

In the present study, the wood of *C. avium* was selected as research material because its altitudinal range in Euxine province is enough to investigate altitudinal trends. The variations in quantitative vessel element characters, vulnerability and mesomorphy ratios of the wood of this species were examined in relation to altitude and tree-ring width.

Material and methods

The mature wood specimens were extracted from 24 wild cherry trees located at different altitudes of Euxine subprovince (Table 1). During sampling, the trees with even-aged (about 50 years old) were preferred for minimizing the effect of tree stem diameter and age on quantitative anatomical characters of wood. The wood specimens were extracted from the same side (north) at breast height of trunks and later they were given shape to cubic. Prior to sectioning, the cubic-shaped woods were boiled, and later they were put in the solution of "glycerine–water–ethyl alcohol" during several days for softening. After these procedures, the transverse, tangential and radial sections were taken from cubic-shaped woods by using a Euromex sliding microtome. For preparation of wood sections and macerations, standard procedures were applied (Yaltırık 1971). Vessel diameter, vessel density (the number of vessels per mm^2) and the number of vessels per group were determined in transverse sections. Vessel diameter was measured based on lumen. Vessel element length was determined from macerated materials and measured from tip to tip (including the element tails). For quantification of vessel grouping, the method proposed by Carlquist (1988) was used. Twenty-five measurements were performed for the mean of each quantitative anatomical character.

In addition, increment core was extracted by using increment borer from breast height of trunk for each sample tree. Tree-ring widths belonging to last ten years were measured on the increment cores, and later their mean was calculated for each tree. Data gathered on altitude, tree-ring width, selected anatomical characters, vulnerability and mesomorphy ratios were analysed by correlation and multiple regression methods in the SPSS 9.0 program. In the multiple regression analysis, altitude and tree-ring width were considered as independent variables, and the selected wood anatomical characters, vulnerability and mesomorphy ratios were used as dependent variables.

Table 1. Some information for the localities of the selected sample trees of *C. avium* in Euxine province of Turkey.

RFD	FE	FTD	CST	LFCN	ALT	EXP	DT
Giresun	Ordu	Merkez	GOM	Kursuncali	980	North	19.06.2001
Giresun	Unye	Merkez	GUM	Cataltepe	180	North	20.06.2001
Amasya	Samsun	Ayvacik	ASA	Senpinar	210	Northwest	20.06.2001
Sinop	Ayancik	Goldagi	SAG	Cinarduzu	580	Northwest	21.06.2001
Zonguldak	Yenice	Kavakli	ZYK1A	4	410	North	10.08.2000
Zonguldak	Yenice	Kavakli	ZYK1B	4	410	North	10.08.2000
Zonguldak	Yenice	Kavakli	ZYK2	21	790	North	10.08.2000
Zonguldak	Yenice	Kavakli	ZYK3A	28	1350	Northeast	10.08.2000
Zonguldak	Yenice	Kavakli	ZYK3B	28	1350	Northeast	10.08.2000
Zonguldak	Ulus	Drahna	ZUD1	Nun deresi	890	Northeast	18.09.2000
Zonguldak	Ulus	Drahna	ZUD2	Nun deresi	1070	Northeast	18.09.2000
Zonguldak	Bartin	Yenihan	ZBY1	Kirazlik	480	Northeast	04.10.2000
Zonguldak	Bartin	Yenihan	ZBY2	Kirazlik	480	Northeast	04.10.2000
Zonguldak	Dirgine	Kozdere	ZDK	Elemen	1150	North	01.09.2001
Bolu	Akcakoca	Altincay	BAA1	Altincay	280	North	06.10.2000
Bolu	Akcakoca	Altincay	BAA2	Altincay	320	Northeast	06.10.2000
Bolu	Akcakoca	Altincay	BAA3	Altincay	340	Northeast	06.10.2000
Bolu	Duzce	Cicekli	BDC1	Yanik	940	Northwest	04.10.2001
Bolu	Duzce	Cicekli	BDC2	Yanik	940	Northwest	04.10.2001
Bolu	Duzce	Merkez	BDM	Merkez	220	North	04.10.2001
Adapazari	Izmit	Dilovasi	AID	Radar	480	North	03.10.2001
Istanbul	Demirkoy	Macara	IDM	Pirgoplu	180	Northeast	28.06.2001
Istanbul	Demirkoy	Kadinkule	IDK1	Kadinkule	650	North	29.06.2001
Istanbul	Demirkoy	Kadinkule	IDK2	Velika	470	North	29.06.2001

Abbreviations: RFD – Regional forest directorate; FE – Forest enterprise; FTD – Forest territorial division; CST – Code of sample tree; LFCN – Locality or forest compartment number; ALT – Altitude; EXP – Exposure; DT – Date of obtaining of wood specimen.

Results

Cerasus avium has a wood with semi-ring porous; however, it tends to diffuse porous in narrower rings. Tree-rings are distinct because of size differences in vessel diameter between latewood and earlywood in successive tree-rings, and flattened fibres at the end of latewood. Mean vessel tangential diameter is 53.61 μm (range 47.19–63.15 μm). Vessel density shows a range from 80.70 to 159.65; and its mean is 125.01. Mean vessel element length is 399.65 μm (range 346–448.40 μm). The number of vessels per group ranges from 1.34 to 2.01 on average, and its mean is 1.67. Vessels are solitary, or in radial multiples or clusters. Solitary vessels are oval to round, and grouped ones are almost angular in outline. There are well-developed helical thickenings on vessel wall, and vessel elements have simple perforation plates.

The means of vulnerability and mesomorphy ratios for *C. avium* are respectively 0.4458 and 178.08 in Euxine province. Vulnerability and mesomorphy ratios, and the quantitative data belonging to selected anatomical characters are given separately for each sample tree in Table 2. According to Pearson correlation analysis (Table 3), the correlation between two non-anatomical features (altitude and tree-ring width) is not statistically significant. However, vessel tangential diameter and vessel density show a significant correlation with altitude (correlation coefficients –0.514 and 0.463, respectively). There is no significant correlation between altitude and other two selected anatomical characters. In other words, while decreasing vessel diameter of *C. avium* wood from low to high altitudes in Euxine province, vessel density increases; however, there is not any significant relationship between vessel element length, the number of vessels per group and altitude. While only vessel density has a significant correlation with tree-ring width, other three anatomical characters do not show a relationship with this non-anatomical feature. The ratios of vulnerability and mesomorphy based on vessel diameter, vessel density and element length have significant correlations with both altitude and tree-ring width. In other words, vulnerability and mesomorphy ratios estimated for *C. avium* decrease from low to high altitude, and increase from narrow tree-rings to wider ones.

Table 2. Non-anatomical and selected anatomical characters of *C. avium* wood.

CST	ALT (m)	TRW (mm)	VTD (μm)	VMM^2	VEL (μm)	VGR	VUL	MESO
GUM	180	5.24	59.69	80.70	395.20	1.34	0.7396	292.29
IDM	180	1.95	54.37	151.70	416.40	1.67	0.3584	149.23
ASA	210	4.47	53.59	106.40	408.40	1.63	0.5036	205.68
BDM	220	6.11	51.49	83.15	346.00	1.62	0.6192	214.24
BAA1	280	5.47	52.32	126.70	446.40	1.75	0.4129	184.34
BAA2	320	8.78	63.15	97.20	424.40	1.70	0.6497	275.73
BAA3	340	3.01	55.66	128.75	405.20	1.87	0.4323	175.17
ZYK1A	410	2.75	55.43	129.30	431.20	1.85	0.4287	184.85
ZYK1B	410	2.49	54.50	134.20	445.20	1.63	0.4061	180.78
IDK2	470	4.28	54.93	134.80	394.00	1.70	0.4075	160.54
ZBY1	480	2.89	53.99	112.40	374.40	1.58	0.4803	179.84
ZBY2	480	3.01	53.76	119.20	448.40	1.58	0.4510	202.23
AID	480	1.30	51.92	142.35	403.60	1.64	0.3647	147.19
SAG	580	7.41	48.38	101.00	346.80	1.41	0.4790	166.10
IDK1	650	0.59	54.11	154.60	403.20	1.73	0.3500	141.12
ZYK2	790	3.36	55.86	148.20	407.20	1.96	0.3769	153.48
ZUD1	890	1.20	54.70	109.25	430.80	1.70	0.5007	215.70
BDC1	940	7.50	54.27	107.60	363.60	1.72	0.5044	183.39
BDC2	940	1.95	52.04	118.20	420.80	1.52	0.4403	185.27
GOM	980	1.59	54.63	120.80	394.40	1.70	0.4522	178.35
ZUD2	1070	2.08	48.00	145.60	361.60	1.63	0.3296	119.20
ZDK	1150	3.64	52.10	134.00	364.00	1.56	0.3888	141.51
ZYK3B	1350	3.54	50.60	154.60	404.80	1.70	0.3273	132.49
ZYK3A	1350	2.88	47.19	159.65	355.60	2.01	0.2956	105.10
MEAN		3.64	53.61	125.01	399.65	1.67	0.4458	178.08
S.D.		2.14	3.42	22.30	31.28	0.15	0.1055	43.36

Abbreviations: CST – Code of sample tree; ALT – Altitude; TRW – Tree-ring width; VTD – Vessel tangential diameter; VMM^2 – Vessel density (the number of vessels per mm^2); VEL – Vessel element length; VGR – The number of vessels per group; VUL – Vulnerability ratio; MESO – Mesomorphy ratio; S.D. – Standard deviation.

Table 3. Correlations of non-anatomical and selected wood anatomical characters, vulnerability and mesomorphy ratios of *C. avium*.

	ALT	TRW	VTD	VMM^2	VEL	VGR	VUL	MESO
ALT	1.000							
TRW	-0.260	1.000						
VTD	-0.514*	0.254	1.000					
VMM^2	0.463*	-0.609**	-0.396	1.000				
VEL	-0.341	-0.270	0.475*	0.114	1.000			
VGR	0.253	-0.207	-0.040	0.575**	0.124	1.000		
VUL	-0.520**	0.599**	0.619**	-0.939***	-0.025	-0.501*	1.000	
MESO	-0.584**	0.484*	0.754***	-0.851***	0.302	-0.427*	0.943**	1.000

***** = significant at the 0.001 level; ** = significant at the 0.01 level; * = significant at the 0.05 level.**
Abbreviations: as in Table 2.

In addition, the selected quantitative anatomical features, vulnerability and mesomorphy ratios have also correlations with one another. Vessel tangential diameter is positively correlated with vessel element length, vulnerability and mesomorphy ratios. Vessel density is positively correlated with the number of vessels per group, and negatively correlated with vulnerability and mesomorphy ratios. Besides, the number of vessels per group shows a negative correlation with vulnerability and mesomorphy ratios.

According to the multiple regression analysis using altitude and tree-ring width as independent variables, significant correlations were obtained at the 0.001 level for vessel density and vulnerability ratio, at the 0.01 level for mesomorphy ratio, and at the 0.05 level for vessel tangential diameter and element length (Table 4). The number of vessels per group does not show any correlation with independent variables. Determination coefficients (R^2) are 0.254–0.501 for significant dependent variables. In other words, non-anatomical factors (ALT and TRW) explain 25.4–50.1 % of total variation for significant dependent variables. For altitude, standardized regression coefficients are significant at the 0.01 level for mesomorphy ratio, and at the 0.05 level for vessel tangential diameter, vessel element length and vulnerability ratio. For tree-ring width, they are significant at the 0.01 level for vessel density and vulnerability ratio, and at the 0.05 level for mesomorphy ratio. The greatest standardized regression coefficients between dependent variables and non-anatomical factors are –0.524 for vessel density, 0.498 for vulnerability and –0.491 for mesomorphy. Change in the values of these dependent variables in respect to independent variables is plotted in Figs 2–4.

Table 4. Multiple regression analysis of selected anatomical characters, vulnerability, and mesomorphy ratios.

DV	R	R^2	F-ratio	Partial Regression Coefficient			Standardized Regression Coefficient	
				Constant	ALT	TRW	ALT	TRW
VTD	0.529	0.280	4.077*	55.667	-0.0044*	0.2060	-0.481	0.129
VMM^2	0.686	0.470	9.309***	132.452	0.0196	-5.4460**	0.327	-0.524
VEL	0.504	0.254	3.570*	443.567	-0.0372*	-5.6030	-0.440	-0.384
VGR	0.293	0.086	0.983	1.659	0.0000	-0.0107	0.214	-0.152
VUL	0.708	0.501	10.536***	0.427	-0.0001*	0.0244**	-0.390	0.498
MESO	0.678	0.460	8.930**	188.077	-0.0575**	7.2140*	-0.491	0.357

*** = significant at the 0.001 level; ** = significant at the 0.01 level;
* = significant at the 0.05 level.
Abbreviations: DV – Dependent variables; R – Multiple correlation coefficient; R^2 – Coefficient of determination; other abbreviations as in Table 2.

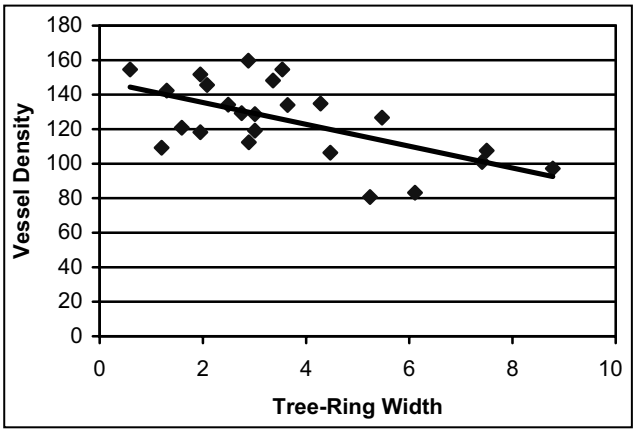

Fig. 2. Trend between vessel density and tree-ring width.

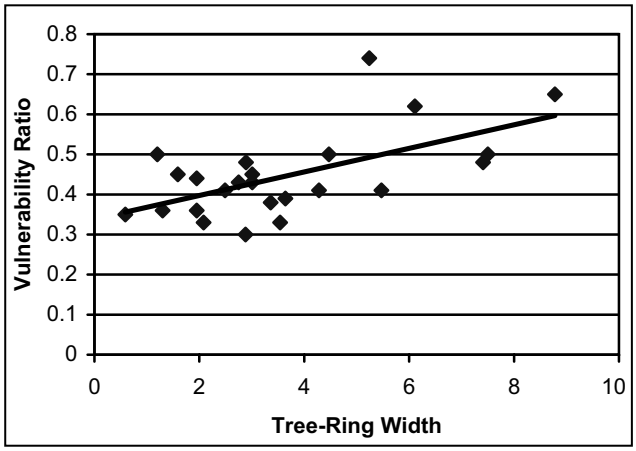

Fig. 3. Trend between vulnerability ratio and tree-ring width.

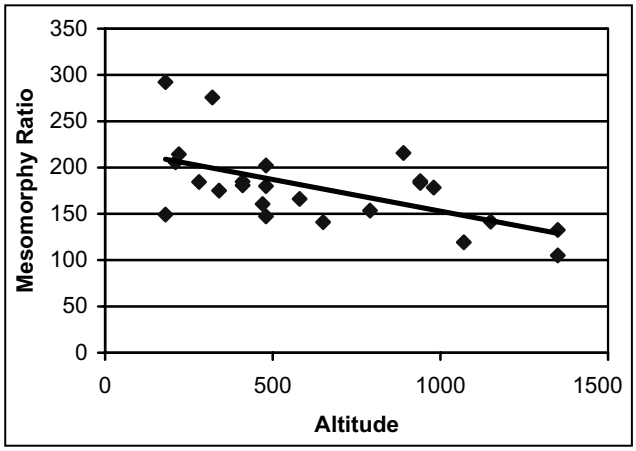

Fig. 4. Trend between mesomorphy ratio and altitude.

Discussion

Wood anatomy of *Rosaceae* family in China was studied by Zhang and Baas (1992), and wood structure of this family in relation to ecology, habit and phenology was evaluated in details by Zhang & al. (1992). In these articles, there were also one variety and four different samples of *C. avium* among the *Rosaceae* species studied. The mean values of some quantitative vessel characters in the woods of four different specimens of *C. avium* in China are 48–55 μm for vessel tangential diameter, 90–176 vessels per mm^2 for vessel density and 300–390 μm for vessel element length. The mean values in wood of *C. avium* var. *decumana* for the aforementioned vessel characters were 50 μm, 220 vessels per mm^2, and 390 μm, respectively (Zhang & Baas 1992). As for Turkish *C. avium* examined in the present study, the mean values are 53.61 μm for vessel tangential diameter, 125.01 for vessel density and 399.65 μm for vessel element length. According to IAWA Committee (1989), it may be classified as number 41 for vessel tangential diameter, number 50 for vessel density and number 53 for vessel element length.

Altitude has a significant effect on vessel tangential diameter, vessel element length, mesomorphy and vulnerability ratios of *C. avium* wood. While vessel tangential diameter and vessel element length tend to decrease with increasing of altitude in Euxine province, vulnerability and mesomorphy ratios tend to be lower values. Carlquist (1977b) introduced the concept of vulnerability to wood anatomists with regard to embolism of secondary xylem. Very narrow vessel diameter corresponds with low vulnerability values (<1), which reflect high conductive safety, while high vulnerability values (>3) are indicative of taxa having high conductive efficiency. The mean vulnerability value of *C. avium* in Euxine province is 0.4458. According to this vulnerability value, it can be explained that the wood of *C. avium* in this province shows high conductive safety. Furthermore, helical thickenings on vessel wall of *C. avium* may be considered as a feature raising conductive safety. Carlquist (1988) pointed out that the correlation between some quantitative vessel element features and altitude should be connected with factors of water availability and temperature, since altitude is not an ecological factor in itself. In Euxine province, while annual precipitation increases from sea level up to high altitude, mean annual temperature decreases. Among localities of sample trees, the amount of annual precipitation ranges from 764.30 mm up to 1583 mm, and mean annual temperature has a range between 7.15 °C and 13.48 °C (Yaman 2002). In spite of enough precipitation in Euxine province (humid climate), low temperatures may limit growth of trees at high elevation. Low temperatures at high elevations affect the viscosity of water both in soil and in stem (Çepel 1993), thus taking water up from soil and transport of water in secondary xylem may be negatively affected. The change in quantitative characters of vessel elements in *C. avium* associated with changing temperature along altitudinal gradients was consistent with the hypothesis of Roderick & Berry (2001). These authors pointed out that the effect of temperature on the viscosity of water will affect vessel size and / or number and therefore wood density. Recently, the physiological link between water viscosity and wood anatomy was examined in *Eucalyptus camaldulensis* by Thomas & al. (2004). Carlquist (1988) indicated that narrower vessel diameter and shorter vessel element length are related to conductive safety. Becoming narrower of vessel diameter and shorter of vessel element length of *C. avium* wood growing in high altitudes can be explained as an indication of conductive safety. Decreasing of mesomorphy value from low up to high altitudes shows that high elevations in Euxine province (despite not xeric sites) cause a xeromorphic effect on the wood of *C. avium* because of the low temperature. Mean mesomorphy value of *C. avium* in this province is 178.08. In general, this species in Euxine province is mesomorphic with respect to its wood anatomy; however, it tends to have xeromorphic wood features (narrower vessel diameter and shorter vessel element length) at high altitudes of this province as if it is in xeric site. As known, it was indicated that low values of mesomorphy ratio (<100) show xeromorphy, and its high values show mesomorphy with respect to wood anatomy (Carlquist 1977b).

There is not a significant correlation between altitude and tree-ring width in this research; however, tree-rings width significantly affects vessel density, vulnerability and mesomorphy ratios. Vessel density increases with narrowing of tree-rings in the wood of *C. avium* in Euxine province. This may be explained as the high altitudinal conditions that cause the narrowing of tree-rings also cause increase in vessel density. Positive correlations between vulnerability, mesomorphy and tree-ring width seem to be quite logical results when environmental conditions causing the narrowing of tree-ring are considered.

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The comparative floral structure of *Brexia*, *Parnassia* and some *Celastraceae*

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Abstract. The last molecular data have shown the existence of close relationship between *Brexia*, *Parnassia* and *Celastraceae* family, so the floral structure of these taxa, demonstrating the definite characters of likeness, is necessary to be examined. In spite of essential differentiating features of the habit of these plants, their flowers contain similar structures – nectaries-staminodes in *Brexia* and *Parnassia*, and nectarous disk in *Celastraceae*. Nectarous glandules in *Parnassia* flower are arranged on ends of the stem-like structures nested on petal-like platforms between stamens; secretory tissue in *Brexia* flower – on thread-like structures between stamens. Nectarous disk in *Celastraceae* flowers is a formation of complex morphological nature. It is formed in result of a blending of stamen bases, sepals and petals. This peculiarity places the *Celastraceae* family rather isolated. A building of detailed studies in floral development of these taxa is necessary.

Key words: *Brexia*, *Celastraceae*, floral structure, nectarous disk, *Parnassia*, relationship, staminodes

Introduction

Many years ago the botanists began to study the floral structure in many taxa of flowering plants. The floral structure characters are basic in current systems, although similar investigations for many taxa of *Rosidae* are not conducted yet (Matthews & Endress 2005a). To such non-investigated groups belong *Brexiaceae*, *Celastraceae* and *Parnassiaceae* families.

One of the first descriptions and illustrations of *Brexia* and *Parnassia* genera in comparison aspect belongs to Engler (1891). He included these taxa in *Saxifragaceae* family. The interest to these genera was stimulated by works of Arber (1913), Perrier de la Bathie (1942), Croizat (1947), and especially Bensel & Palser (1975a, b). Since many authors have begun to consider *Brexia* and *Parnassia* genera relating to *Celastraceae* family (Simmons 2004a, b), although final solution of the question is not adopted yet. In recent time, the system of *Magnoliophyta* has undergone a radical revision due to new molecular data (APG 2003). In particular, taxonomic relationships of the *Brexia* and *Parnassia* genera among *Saxifragales* or *Celastrales* orders are revised (Morgan & Soltis 1993), and now they are included in the *Celastraceae* family (Zhang & Simmons 2006). So, a necessity for further investigation of the floral structure and development in many taxa of *Saxifragales* and *Celastrales* orders exists, because greatest successes are achieved in molecular–genetic mechanisms of structural diversity of flowers (Tikhodeyev 2001), and floral morphogenesis is a convenient model for genes' expression studies. It is hoped that we will be able to throw over the necessary bridge between molecular and traditional systematic data.

Material and methods

Floral buds and flowers of *Brexia* were collected from living plant in the greenhouse of the Komarov's Botanical Institute (St.-Petersburg). Flowers of different species of *Parnassia* were received from the Herbaria of Main Botanical Garden (MHA), Moscow State University (MW) and Komarov's Botanical Institute (LE). The inflorescences and floral structure of some *Celastraceae* were examined on the basis of the living plant collection in Arboretum of MBG in Moscow and Arboretum of KBI in St.-Petersburg (Table 1). Plant material was fixed

and stored in 70 % ethanol. In the present work, were used the traditional methods of the morphological and anatomical studies, namely the binocular and light microscope MBB–1. Photographs were made using a digital camera "Sony Cyber–Shot DSC W–1".

Table 1. Taxa used in this investigation.

Taxa names	Floral buds	Flowers	Voucher
Brexia madagascariensis Thouars ex Ker Gawl.	+	+	Greenhouse of KBI
Parnassia bifolia Nekr.		+	LE
P. cabulica Planch. ex C.B. Clarke		+	MW
P. californica (A. Gray) Greene		+	MHA
P. chinensis Franch.		+	LE
P. crassifolia Franch.		+	LE
P. davidi Franch.		+	LE
P. delavayi Franch.		+	LE
P. dilatata Hand.-Mazz.		+	MHA, LE
P. faberi Oliv.		+	LE
P. filchneri Ulbr.		+	LE
P. fimbriata Banks		+	MHA
P. foliosa Hook. f. & Thoms		+	MHA, MW, LE
P. gansuensis T.C. Ku		+	MHA
P. glauca Raf.		+	MHA
P. kotzebuei Cham. ex Spreng.		+	MHA
P. laxmanni Pall.		+	LE
P. lutea Batal.		+	LE
P. mucronata Siebold & Zucc.		+	LE
P. monochorifolia Franch.		+	LE
P. montanensis Fernald & Rydb.		+	MHA
P. mysorensis Heyne ex Wall.		+	LE
P. nubicula Wall.		+	MHA
P. nummularia Maxim.		+	LE
P. oreophila Hance		+	MHA, LE
P. palustris L. s.l.	+	+	MHA, MW, LE, in nature
P. parviflora DC.		+	MHA
P. scaposa Mattf.		+	LE
P. setchuensis Franch.		+	LE
P. wightiana Will.		+	LE
Celastrus orbiculata Thunb.	+	+	Arboretum of MBG
Euonymus europaea L.	+	+	Arboretum of MBG
E. miniata Tolm.	+	+	Arboretum of KBI
E. verrucosa Scop.	+	+	Arboretum of BIN
Tripterygium regelii Sprague & Takeda	+	+	Arboretum of MBG

Abbreviations: KBI – Komarov Botanical Institute, St.-Petersburg; MBG – Main Botanical Garden, Moscow; BIN – Botanical Garden of Komarov Botanical Institute, St.-Petersburg (KBI); LE – herbarium in Komarov Botanical Institute; MW – herbarium of Moscow State University; MHA – herbarium of Main Botanical Garden, Moscow.

Results

Floral morphology of *Brexia madagascariensis* (Figs 1, 2)

The flowers of *Brexia* are disposed in axial dichasial inflorescences (simple, many-membered and umbellate), rather large (diameter 25–30 mm), actinomorphic and bisexual, 5-merous, widely-glassed in shape, on relatively long pedicels (about 7 mm). The torus of flower is short and planed. Calyx is with five fused sepals; each sepal is truncate-oviform. Corolla consists of five free, widely-oval petals. The flower consists of five free stamens, at one cycle with five thread-like (filamentous) staminodes. Between the bases of two neighbouring stamens the nectaries form specific thread-like struc-

Fig. 1. Floral buds of *Brexia madagascariensis* (orig.).

Fig. 2. Flower of *B. madagascariensis* (Foto G.D. Carr from: http://www. botany/hawaii/edu/faculty/carr/images/bre_mad.jpg).

ture, and the number of its appendages is six. Nectaries are each supplied by central vascular traces, as the stamens and consist of epidermis (almost deprived of stomas) and many-layered glandular tissue. Secretion of nectar is realized on the whole surface of the nectary across the cuticle of epidermal cells as well as the stomata. The gynoecium is syncarpous, formed by fusion of 5 carpels. The ovary is upper, 5-border and 5-locular. The wall of ovary is differentiated on two zones: outside phlobafen-content and inner with light parenchymal cells. Each loculus of the ovary comprises 3 anatropous ovules. Style is only one. Stigma is 5-lobed.

Nectaries of *Brexia* begin to be formed during the early stages of floral development because in non-mature dense flower buds such structures are fully formed (Fig. 3). Nectaries are retained after fertilization in developing fruit (Fig. 4).

Floral morphology of *Parnassia* (Fig. 5)

The flowers in *Parnassia* are rather large, solitary on scapes (leafless shoots) or on leafy shoots, forming from the rosette of bottom leaves. The flowers are bisexual, usually actinomorphic or weakly zygomorphic, 5-merous. The perianth is double, calyx is formed by five free sepals, corolla is represented by five free petals, smooth (*P. glauca*, *P. palustris*) or eyelash (*P. foliosa*) at the border. Following range is formed by the five nectaries-staminodes, alternated with five stamens, disposed in an inner whorl. The gynoecium is syncarpous at the base, but paracarpous and unilocular above. It is formed by the fusion of 3–4(5) carpels. The ovary is upper or half-inferior.

The staminodes consist of petaloid appendage, carrying tree or more stem-like structures, and finishing on their ends by sphere-like formations – nectarous glandules. The foot of the glandule is formed by strongly stretching cells, and its head – by stretching cells at the border and by small secretory cells in the centre. Such structure is retained after fertilization in order to be fulfilled the important metabolic functions in developing fruit.

Thus, morphological diversity of the flowers in *Parnassia* genus is defined by form and structure of petals and staminodes. Zygomorphy in the floral structure of different species is arisen in consequence of the asymmetry of perianth (especially petals) and gynoecium parts.

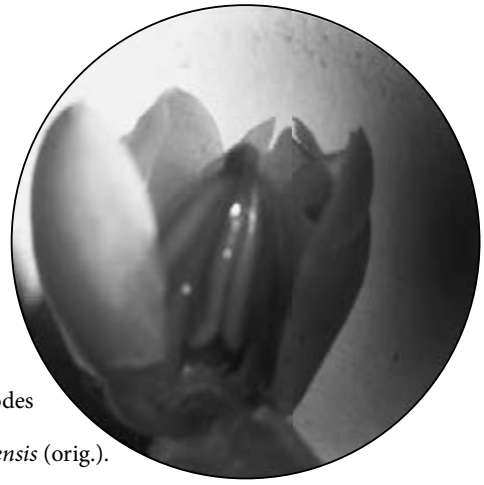

Fig. 3. Staminodes in floral bud of *B. madagascariensis* (orig.).

Fig. 4. Blooming flower of *B. madagascariensis* (orig.).

Floral morphology of some *Celastraceae* (Figs 6, 7)

Flowers are actinomorphic, bisexual or (rarely) unisexual, 3–5-merous, generally completed in cymose or (rarely) racemose inflorescences, terminals or axiles (Savinov 2004). Reduction in inflorescences to single flowers is known in some genera (e.g., *Psammomoya*). The general character of flowers in *Celastraceae* is the nectarous disk which usually fuses with sepals, petals and ovary base. Its structure is formed during the fusion of appendicular organs of flower: sepals, petals and stamens.

Fig. 5. Flower of *Parnassia palustris* (Foto A.S. Beer).

Fig. 6. Flowers of *Euonymus europaea* (orig.).

Fig. 7. Flowers of *E. miniata* (orig.).

Discussion

Floral architecture

Flowers of all studied taxa are actinomorphic, and they have a radial symmetry. The flowers of *Parnassia* are weakly zygomorphic (Martens 1936). It has been shown that the *Parnassia* flowers are laying as zygomorphic in floral morphogenesis (Pervuchina 1979). This is unusual character for *Brexia* and *Celastraceae*. Flowers of *Brexia* and *Parnassia* have some features in common with *Celastraceae* ones, including general architecture (Fig. 8), forming the staminodial protuberances between stamens. *Brexia* typically has the floral formula *K$_{(5)}$C$_5$A$_{5+5}$G$_{(\underline{5})}$; *Parnassia palustris* – *K$_5$C$_5$A$_{5+5}$G$_{(\underline{4})}$; very interesting species from SE Asia and Australia *Corynocarpus laevigata* J.R. & G. Forst. – *K$_5$C$_5$A$_{5+5}$G$_{(\underline{1})}$; "typical" *Celastraceae* – *K$_{4\text{-}5}$C$_{4\text{-}5}$A$_{3\text{-}5}$G$_{(\underline{2\text{-}5})}$. So, these taxa are isolated among others *Celastraceae*.

The nature of the nectariferous formations

Nectariferous disk of the representatives of *Celastraceae* in structural–functional relation differs very strongly from nectaries in *Brexia* and *Parnassia*. Its structure is of complex morphological nature, and it has very diverse forms (whole, lobed, explosive). At the same time, reduction of the stamens in this case is not excluded. This has been shown in early literature (Loesener 1942a, b; Berkeley 1953; Douglas 1957; Kartashova 1965; Robson 1965).

Reduction of androecium and nectaries-staminodes forming an inner androecial whorl in *Parnassia* flowers has been reported in some articles (Arber 1913; Eames 1961; Sharma 1968; Ku 1987). The globiferous filaments of the staminodes are each supplied by separate vascular traces that have been interpreted as derived from connected stamen-fascicles (Drude 1875; Arber 1913; Eames 1961; Bensel & Palser 1975a). On the basis of floral anatomy, Eames (1961) came to the conclusion that *Parnassia* does not belong to *Saxifragaceae* family. So, such way of reduction took place in *Brexia* (Fig. 9), and the structure is very different in other species of the genus (Schatz & Lowry 2004). Similar unusual formations have been reported in flowers of *Corynocarpus laevigata* (Kartashova 1965; Philipson 1987). Here we can see specific tongue-like nectaries with petaloid scales, alternating with stamens. According to Philipson (1987), such petaloid scales are equivalent to petals rather than to staminodes.

So, a petaloid transformation of the stamens is very usual in flowers of flowering plants. The conclusion now has confirmed on the basis of investigation of the teratic flowers and ABC genetic model of floral development. Nectaries of *Brexia* and *Parnassia* are very multifunctional organs and well retained in fruits.

Other features

These genera are united by their capsular fruits. Fruit in *Brexia* is upper syncarpous many-seeded loculicidal capsule, with 4–5 locules. Unusual characters of *Brexia* capsule are the alternated sclerenchymal and non-sclerenchymal zones in the pericarp (Yembaturova & Savinov 2006). Such structure may be discovered in some *Celastraceae*, for example in *Kokoo-*

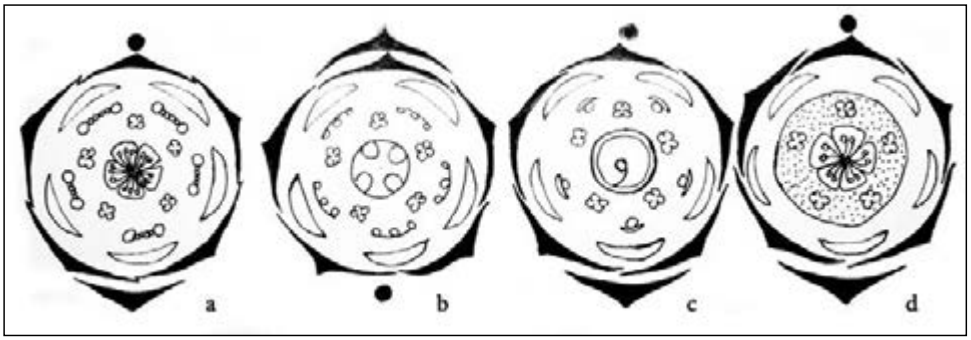

Fig. 8. Floral diagrams of *B. madagascariensis* (**a**), *Parnassia palustris* (**b**), *Corynocarpus laevigata* (**c**) and "typical" *Celastraceae* (**d**) (orig.).

na, *Lophopetalum* and *Microtropis* genera (original materials). Fruit in *Parnassia* is many-seeded loculicidal capsule dehiscent at apex. The fruit type is common in *Celastraceae* too. Other features include: early floral studies (Klopfer 1973); ecology of pollination (Martens 1936; Kozo-Poljanski 1947); embryological characters (Kamelina 1988; Tobe & Raven 1993). Finally, the genus *Brexia* and many representatives of *Celastraceae* are trees and shrubs, while different species of *Parnassia* genus have an herbaceous habit. However, among the near relatives of *Celastraceae* is fam. *Stackhousiaceae* with herbaceous habit of its representatives.

Relationships of these taxa

Among close relatives of *Celastraceae* is *Stackhousiaceae* almost completely including herbal plants. What may be said now about affinities of *Brexia*, *Parnassia* and *Celastraceae*? For example, Croizat (1947) described the possible evolutionary transition from flower of the *Brexia*-type to flower of the *Celastraceae*-type. For that he necessarily admits only insignificant changes in ovary and disk position.

So, in many current systems of the flowering plants the genera *Brexia* and *Parnassia* are considered as closely related to *Celastrales* order and *Celastraceae* family (Takhtajan 1997; Thorne 2000; Shipunov 2005), although this is very unusual in early systems (e.g., Cronquist 1988). This conclusion now is confirmed by the comparative morphology of the flowers (Matthews & Endress 2005b).

Conclusions

As a result of the study, the following common features of *Brexia*, *Parnassia* and *Celastraceae* can be summarized:

1. Syncarpous 4–5-carpellate gynoecium and 5-merous flower.

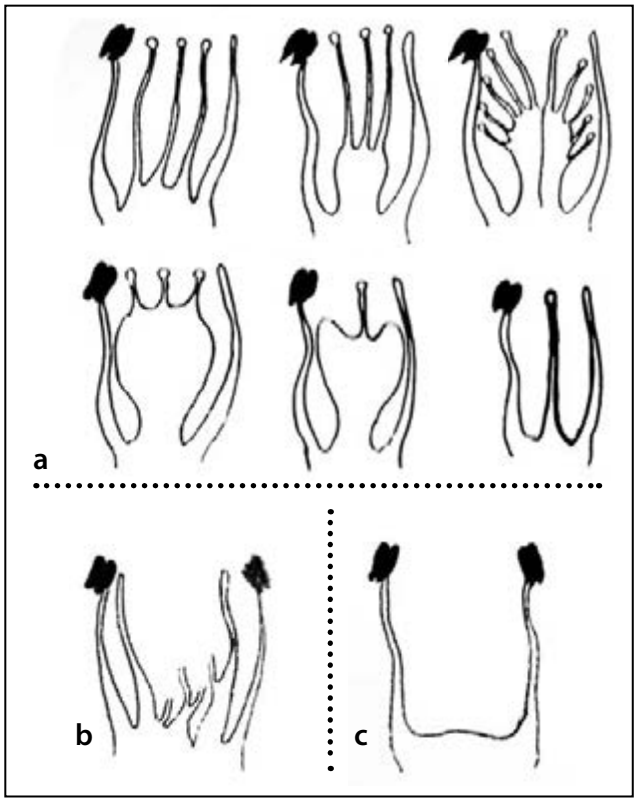

Fig. 9. The structure of androecium in *Parnassia* (**a**), *Brexia* (**b**) and some *Celastraceae* (**c**) (orig.).

2. Formation of nectariferous structure in the flower with irregular appendages (unusual only in *Celastraceae*) as outer circle of androecium.

3. Nectaries-staminodes and nectarous disk well retained in fruits.

4. Differentiation of androecium in *Parnassia* and *Brexia* flowers is similar. But this character is unusual in *Celastraceae* flowers.

Brexia and *Parnassia* differ from *Celastraceae* family by simple, staminodial nature of their nectaries. Nectarous disk in flowers of *Celastraceae* is formed by the base of stamens, petals and sepals and it has complex nature. This conclusion say to us that these taxa are concerned as isolated among other representatives of the *Celastrales*.

Acknowledgments. I am very grateful to A.K. Skvortsov (Main Botanical Garden RAS, Moscow, Russia), A.P. Melikian, D.D. Sokoloff (Moscow State University, Moscow, Russia) and N.N. Arnautov (Komarov Botanical Institute, St.-Petersburg, Russia) for helpful discussion and their interest to this paper. Many thanks are due also to Mark P. Simmons (Colorado State University, Fort Collins, USA), Peter K. Endress (Institute of Systematic Botany, University of Zurich, Zurich, Switzerland) and Dimiter Terziisky (University of Agriculture, Plovdiv and Sofia University, Sofia, Bulgaria) for discussions. Thanks also to S.S. and A.S. Beer (Moscow State University, Moscow, Russia) and E.Y. Yembaturova (Timirijazev's Agricultural Academy, Moscow, Russia) for advices.

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Wheat–*Thinopyrum* hybrids – production and characteristics

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Abstract. The species from the genus *Thinopyrum* are a rich source of genetic diversity which may be introgressed into cereals through different techniques. In order to develop intergeneric genetic material as a source for resistance to biotic and abiotic stress in wheat breeding, intergeneric hybrids of wheat with three species of *Thinopyrum* were obtained. The crossability and viability of hybrid seeds, the percentage of hybrid plants obtained and their fertility were established. The cytological analysis of F1 hybrids was carried out and it revealed some homoeologic pairing at diakinesis. This shows the possibility for recombinogenesis and alien introgression into wheat.

Key words: amphidiploid, backcrossing, chromosome pairing, crossability, *Thinopyrum*, *Triticum aestivum*, *T. durum*

Introduction

The wild relatives of *Triticum* are important sources of valuable biological properties for wheat breeding. *Thinopyrum intermedium*, *Th. junceum* and *Th. elongatum* were most widely used in hybridization with wheat. Their most important signs are the desease resistance, drought and salt resistance and the high protein content in the grain. Besides their use for wheat breeding, the hybrids with these species give important cytogenic information regarding the interactions between the various genomes of *Thinopyrum* and those of wheat. The purpose of this paper was to report the production and cytogenetic characteristics of different hybrids of wheat with *Th. intermedium, Th. junceum* and *Th. elongatum* and of some backcross derivatives.

Material and methods

The Bulgarian cultivars Sredets, Lozen 76, Tseveryana and 788 of *Triticum durum* (2*n* = 28, AABB) and Sadovo, Rusalka, Charodeyka of *T. aestivum* (2*n* = 42, AABBDD) were included in the hybridization. The cultivars Bezostaya, Chinese Spring and Mara were also included in the hybridization, because in these cultivars monosomic lines were created and besides the cv. Bezostaya is of great economic importance in Bulgaria. *Thinopyrum elongatum*, *Th. junceum* and three biotypes of *Th. intermedium* were used as father parents. The problems of the intergeneric hybridization of wheat with *Th. elongatum* related to a very low crossability and viability of hybrid seeds and hybrid sterility were overcome by including of already obtained amphidiploid of wheat with *Th. elongatum* which was used as a bridge in the hybridization.

Hybridization was performed by pollination of previously emasculated and isolated ears under greenhouse conditions, while backcross was carried out under field conditions. The cytological analysis was performed on temporary squash preparations stained with aceto-carmine according to the routine technique used for the purpose in the laboratory (Belcheva & Michailova 1991). A variation analysis (Lidanski 1988) and Student's t-test were used for the statistical data processing.

Results and discussion

Hybrids with *Thinopyrum intermedium*

A comparatively high degree of crossability of different cultivars of *T. aestivum* with *Th. intermedium* was observed (Table 1). The percentage of the obtained seed-setting varied in different cultivars and depended on their genotype. The cultivars Rusalka and Bezostaya produced the highest percentage of hybrid seeds, while the cv. Mara produced the lowest one.

Table 1. Produced and viable hybrid seeds from crosses of *T. aestivum* cvs. with *Th. intermedium* biotypes.

Crosses	Pollinated florets	Hybrid seeds		Germinated plants	
	No.	No.	%	No.	%
CS×Th.int(1)	132	28	21.21	24	86.67
M×Th.int(1)	218	7	3.89^{+++}	4	57.14^{+}
B×Th.int(1)	492	150	30.49^{++}	123	82.00
S×Th.int(1)	562	104	18.51	96	92.31
R×Th.int(1)	380	151	39.74^{+++}	129	85.43
S×Th.int(1)	562	104	18.51	96	92.31
S×Th.int(2)	384	142	36.98^{+++}	136	95.71
R×Th.int(1)	380	151	39.74	129	85.43
R×Th.int(2)	378	220	58.20^{+++}	201	91.36
B×Th.int(1)	492	150	30.49	201	123
B×Th.int(2)	314	117	37.26^{+}	103	88.03
B×Th.int(3)	146	41	28.08	26	63.41^{+}

(+++), (++) and (+) – Significant at: p=0.001, p=0.01 and p=0.05, respectively.
Abbreviations: CS – Chinese Spring; M – Mara; S – Sadovo; R – Rusalka; B – Bezostaya.

A marked influence on the percentage of produced hybrid seeds exerted the father form as well. The crosses with biotype No. 2 of *Th. intermedium* were characterized by the highest seed-setting. The explanation of this fact can be found in the polymorphism of *Th. intermedium* (Sharma & Ohm 1990). According to some authors (Shulindin & Naumova 1962; Panayotov 1974) the cultivars with a short vegetative period were characterized by better crossability. Our results were not in conformity with this suggestion. Particularly demonstrative in this regard were our data about the crosses of the cv. Mara. It is characterized by a short vegetative period but unlike the other early cultivars, such as Chinese Spring and Rusalka, it possessed lower crossability. A conclusion can be drown that the genes of crossability of the "Kr" series in the cv. Mara are in a homozygous dominant state (Kr1 Kr1 Kr2 Kr2) according to Krolow (1970) and Zeven (1987). Similar results were obtained also in the hybridization of this cv. with *Secale cereale* (Tyankova 2000, 2003). Still obscure is the question whether the genes of crossability of *T. aestivum* with the different species of *Triticinae* are the same. It is not clear also whether similar genetic factors exist in the other species of *Triticinae* as well. It is quite possible that genes of the same or opposite action on crossability with those of *T. aestivum* exist in the homeoallels of the father form – *Th. intermedium*. The possible existence of genes of crossability in *Thinopyrum* was assumed also by Sharma & Ohm (1990). Additional and special investigations will be required to clarify those questions.

The cytological investigation of the meiotic chromosome pairing in wheat–wheatgrass F1 hybrids showed considerable differences in the number and character of the chromosome associations in the various hybrid combinations (Table 2). The highest percentage of paired chromosomes and an average number of bivalents per cell were observed in the hybrid of the cultivars Chinese Spring and Rusalka and the lowest one – in the hybrid of cv. Sadovo. Rod bivalents are typical for all hybrid combinations and part of the ring ones was rather small, but the individual crosses differed also in this respect. The average number of ring bivalents was the highest for the hybrid of Chinese Spring (1.88) and the lowest – for that of Bezostaya (0.15).

Table 2. Type of chromosome pairing in F_1 *T. aestivum* × *Th. intermedium* hybrids.

Varieties	Bivalents				Polyvalents		Univalents			Paired chromosomes (%)
	Limes	Mav	±m	Ring Mav	Tri valents	Quadri valents	Limes	Mav	±m	
CS	6–13	9.45	0.30	1.88	0.91	0.03	13–32	20.85	0.69	51.36
R	5–13	8.37	0.32	1.70	0.73	0.10	16–30	22.67	0.65	46.02
M	4–13	7.45	0.21	0.78	0.51	0.02	20–32	25.71	0.40	39.36
B	3–11	7.00	0.38	0.15	0.28	0.05	16–36	26.78	0.48	35.81
S	2–9	5.70	0.29	0.83	0.47	0.03	24–38	29.07	0.57	30.79

Abbreviations: CS – Chinese Spring; R – Rusalka; M – Mara; B – Bezostaya; S – Sadovo.

The sterility of the hybrids in the varieties Bezostaya, Sadovo and Rusalka was surmounted by backcrossing. The results from backcrossing of the F1 hybrids showed that in different combinations the number of the produced viable seeds varied and depended on the genetic basis of the cultivars used and on the character of the backcross combination (Table 3). When the same wheat cultivar as that par-

Table 3. Production, viability and fertility of BC_1 generation.

Backcross combinations	Pollinated florets No.	BC_1– seeds		Germinated plants		Fertility of BC_1 No.
		No.	%	No.	%	
(B × Th. int) × B	586	2	0.34	0	0	–
(S × Th. int) × S	882	6	0.68	1	16.67	0 seeds
(R × Th. int) × R	920	7	0.76	3	42.86	2 plants 0 seeds 1 plant 7 seeds
(S × Th. int) × B	968	7	0.72	0	0	–
(R × Th. int) × B	620	5	0.81	2	40.0	0 seeds
(R × Th. int) × S	861	16	1.86	13	81.25	3 plants 0 seeds 5 plants 1–2 seeds 5 plants 16–38 seeds

Abbreviations: B – Bezostaya; S – Sadovo; R – Rusalka.

ticipating in the F1 hybrid was used in backcrossing, the percentage of the produced backcross seeds was low (0.34–0.76) and their viability expressed by the number of germinated seeds was very weak. When in backcrossing a cultivar different from that participating in the hybrid was used, the effect was up to a certain extent better. Similar conclusions were made by Laptchenko (1953) and Rusmini (1958). However, it is not valid for all variants. Very important was to combine suitable cultivars in one combination. In our case most suitable proved to be the combination (cv. Rusalka × *Th. intermedium*) × cv. Sadovo. In all variants in which the cv. Bezostaya participated, the seed-setting of backcross seeds was very low and in most cases the seeds were not viable. Obviously, this parameter – the increased quantity of seed-setting after pollination with another cultivar (Laptchenko 1953; Rusmini 1958) can be specified for the individual combination in confirmation of the results obtained by Panayotov (1973).

Hybrids with *Thinopyrum junceum*

The cultivar Bezostaya was included in hybridization with *Th. junceum*. The results from the hybridization showed considerably lower percent of hybrid seeds in comparing to those with *Th. intermedium*. However, the percentage of hybrid seeds obtained was relatively high compared to the evolutional distance between *Triticum* and *Thinopyrum* (Table 4). The lower percentage of seed-setting could up to a certain extent be due to the fact that selected biotypes of *Th. intermedium* characterized by better biological properties were used in the hybridization.

Table 4. Produced and viable hybrid seeds from crosses of *T. aestivum*, cv. Bezostaya, with *Th. junceum*.

Number of pollinated florets – 376
Number of hybrid seeds obtained – 18
Percent of hybrid seeds – 4.79
Germinated plants, number – 11
Germinated plants, percent – 61.11

The results from the meiotic analysis of the hybrid Bezostaya × *Th. junceum* were rather similar to those of the hybrid Bezostaya × *Th. intermedium* (Table 5). The bivalents could be the result from the allosyndesis between chromosomes of one or more genomes of *Th. intermedium* or of *Th. junceum*, respectively, with those of *T. aestivum* or could be due mainly to the autosyndesis of the chromosomes of *Thinopyrum*. The different opinions of various authors represent the difference in the evaluation of the genome interactions between wheat and the indicated species of *Thinopyrum*. A number of investigations have shown that both *Th. intermedium* and *Th. junceum* represent a segmental autoallohexaploid with established high ability of autosyndesis of their chromosomes (Pienaar 1990). However, taking into account the obtained results of chromosome pairing in one haploid of *Th. intermedium* (Dewey 1962) in which the maximum number of bivalents was 7 and the data about the meiotic pairing in the euhaploid of Chinese Spring (Kimber & Riley 1963), a conclusion may be drawn that under definite conditions in the hybrids of *T. aestivum* with *Th. intermedium* and *Th. junceum* certain allosyndetic pairing runs also between some of the wheat chromosomes and those of *Thinopyrum*. Therefore, introgression of genes from the genome of *Th. junceum* or *Th. intermedium* into the genome of wheat is possible.

Table 5. Number and character of bivalent and polyvalent associations in MI of PMC in F_1 Bezostaya × *Th. junceum* hybrid.

Number of studied cells – 30	
Bivalents:	Univalents:
Limes – 5^{II}–12^{II}	Limes – 13^{I}–30^{I}
Av. number, M – 24.07 ± m – 0.59	Av. number, M – 7.67 ± m – 0.30
Ring bivalents – 1.88	
Polyvalents:	
Trivalents – 0.81	
Paired chromosomes per cell (%) – 42.31%	

Hybrids of *T. durum* and *T. aestivum* with *T. durum/Th. elongatum* AD (2*n* = 42, AABBEE)

As it was already mentioned the hybrids with *Th. elongatum* were produced using an amphidiploid *T. durum*, cv. Steward/*Th. elongatum*, which was kindly provided to us by Prof. A. Mochizuki, Hyogo Agricultural College, Sasayama, Hyogo-ken, Japan. As it is evident from the Table 6, the wheat–*Th. elongatum* amphidiploid crossed comparatively well with different cultivars both of *T. durum* and *T. aestivum* with considerable variation of the genotypes. The highest percentage of seed-setting, the best viability of seeds and the highest percentage and number of F1 headed plants were characteristic of the cvs. Bezostaya 1 (*T. aestivum*) and Tseveryana (*T. durum*).

The hybrid plants of all the cultivars of *T. durum* and *T. aestivum* varied largely in fertility. The lowest was the number of grains from the hybrid plants of Bezostaya 1. Considerably higher was the number of grains from the hybrid plants of the different cultivars of *T. durum*. The hybrids of the variety Sredets and the variety 788 had the highest fertility and those of the variety Tseveryana had the lowest one.

Table 6. Viable seeds obtained from crosses of *T. durum* and *T. aestivum* cultivars with the amphidiploid *T. durum/Th. elongatum* (AABBEE) and the fertility of F_1 hybrid plants.

Crosses	Hybrid seeds (%)	Maturity reached by F1 plants (%)	Number of seeds in F1 hybrid plants	
			In main spike Mav ± m	Per plant Mav ± m
T. durum × AABBEE				
Sredets	10.36	16.70	28.3 ± 7.2	301.2 ± 4.6
Lozen 76	15.60	36.40	22.4 ± 3.5	120.5 ± 5.3
Tseveryana	6.90	18.20	17.3 ± 4.2	94.5 ± 3.8
788	11.70	71.40	26.4 ± 2.9	334.4 ± 4.2
T. aestivum × AABBEE				
Rusalka	7.50	0	–	–
Charodeyka	12.50	0	–	–
Bezostaya 1	26.70	92.31	21.4 ± 5.8	21.4 ± 5.76

Cytological analysis revealed that the hybrids had the expected chromosome number $2n = 35$ and the genome constitution AABBE for the hybrids of *T. durum*, and $2n = 42$, AABBDE for the *T. aestivum* hybrids. Meiotic analysis in MI of the hybrids of *T. durum*, cv. Lozen 76, showed that the chromosome associations of PMC were mainly bivalents (Table 7). Their maximal number in a large part of PMC was 14. The number of univalents varied from 5 to 15, and in 15 of all the 78 cells analysed (19.23 %) the number of univalents was lower than 7. On the other hand, multivalent associations were observed in the PMC. It was obvious that single chromosomes from the E genome of *Th. elongatum* were also involved in pairing in these cells. Meiosis in two cells was analysed in the diakinesis stage. One of the cells was with 7 univalents and 14 ring bivalents. The other cell, however, had only one univalent. All the other chromosomes were involved in pairing represented by two trivalents, five ring bivalents and nine rod bivalents. The high degree of pairing observed showed that, in fact, a large number of chromosomes of the E genome had taken part in pairing and probably allosyndesis had run between them and the individual chromosomes of the A or B genome of wheat despite the presence of Ph genes in the wheat medium. The low degree of chromosome pairing observed in MI could be due to the premature separation of bivalents and not to no-homology. It comes to support Mochizuki's opinion (1962) of the existence of homologous pairing between the chromosomes of *Th. elongatum* and wheat chromosomes. Therefore, the introduction of genetic material from *Th. elongatum* into wheat is possible.

In conclusion, the obtained results show that the amphidiploid *T. durum*/*Th. elongatum* can successfully be used in hybridization with different varieties of *T. durum* and *T. aestivum* with considerable genotype influence on the hybridization and fertility of hybrid plants. Chromosome pairing running in diakinesis is higher compared to the relatively lower degree of pairing in MI which creates the conditions for recombinogenesis and the possibility for alien introgression into wheat. Hybrids produced by us can be used as the starting material for the needs of wheat breeding.

Acknowlegments. The Bulgarian National Science Fund under contract B–1202/02 supported this research.

Table 7. Number and type of bivalent and polyvalent associations in MI of PMC in F1 hybrid of cv. Lozen 76 (AABBE).

Bivalents	Univalents	Polyvalents	Paired chromosomes per cell (%)
Limes: $6^{II} – 14^{II}$ Mav–11.07 ± m–0.49 Ring – 9.39 Rod – 1.68	Limes: $5^{I} – 15^{I}$ Mav–7.23 ± m–0.27	Trivalents Mav–0.75 ± m–0.28 Quadrivalents Mav–0.16 ± m–0.09 Sixvalents Mav–0.24 ± m–0.08	75.63
Number of cells with 7^{I}: 24 = 30.77% Number of cells with more than 7^{I}: 39 = 50% Number of cells with less than 7^{I}: 15 = 19.23% Number of cells analysed: 78			

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Cytogenetic effects induced by caffeine in *Cannabis sativa* (hemp) root meristems

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Abstract. In this paper, some cytogenetic effects induced by caffeine in meristematic cells of *Cannabis sativa* (hemp) root apexes are analysed. Mitotic index, percentual distribution of cell division phases, frequency and type of chromosome aberrations in hemp root meristems were quantified. At 0.01 % and 0.05 % caffeine, the cell division was stimulated, but starting with 0.1 % caffeine a depression of mitotic index appears, which directly increased with concentration. Prophases are numerically predominant. Between the concentration increase and incidence of chromosome aberrations a linear, direct relationship exists.

Key words: caffeine, chromosome aberrations, hemp

Introduction

The hemp (*Cannabis sativa* L.) is a dioecious, annual plant, but several monoecious varieties also exist. The leaves of female plants are covered with glandular hairs. At this level, in resin glands, the cannabinoids are synthesized. In many countries, the hemp cultivation is unlawful, especially regarding those varieties containing greater quantities of THC (tetrahydrocannabinol), the most important psychoactive component in marijuana (the mixture of female flowers, buds, and leaves). There are technical varieties with THC smaller than 0.3–0.5 %, furnishing fibres or seeds, with multiple uses. Although in Europe, for much time, only the Indian hemp was considered to synthesize hallucinatory substances, during last years these active principles were evidenced too in European hemp, especially under high temperature and prolonged drought.

The mutagenic studies in hemp are few. One of the directions of chemical mutagenesis could be, for example, the decrease of THC level, by the inactivation or blocking of THCA-synthetase. This fact is possible by induction of a mutation in the gene responsible for the synthesis of respective enzyme. THCA-synthetase, a monomere enzyme with 545 amino acids, catalyses the transformation reaction of cannabigerolic acid in tetrahydrocannabinolic acid. Due to the great importance of hemp, it is necessary to amplify the genotype and phenotype diversity, to optimize some desired traits. To this objective belongs the present study that analyses the effects induced by a chemical stressor – caffeine. The studies on chemical mutagenesis in hemp are few and relatively difficult, because of the processing particularities of biological material.

The approach of the study of cytogenetic effects induced by caffeine is justified also by the presence of this compound in tea and in coffee, the most widely consumed beverages, caffeine being suspected as having a potential genetic risk (Sax & Sax 1966).

Material and methods

As biological material, seeds of monoecious hemp (*C. sativa*) were used. Seven concentrations of caffeine were tested (0.01 %, 0.05 %, 0.1 %, 0.125 %, 0.25 %, 0.3 %, and 0.5 %). The duration of treatment was 12 hours. The results were compared with a control, maintained in the same conditions, but treated with distilled water. The germination was realized

in Petri dishes, on filter paper moistened with distilled water. The small roots, 10–12 mm in length, were fixed for 24 hours in absolute ethyl alcohol: glacial acetic acid mixture (3:1). After fixation, the roots may be kept in 70 % alcohol, at +4 °C. The staining was performed with Carr solution (modified carbolfuchsine, sorbitol containing), after a hydrolysis in 50 % HCl, for 8 minutes. To obtain the preparations, the small roots were placed on a glass slide in 45 % acetic acid. The meristematic apexes were detached then the squash method was used to obtain a good cell spreading. For each variant, five root preparations were analysed. The chromosome aberrations were scored in ana-telophases. For the calculation of the mitotic index (MI), the following formula was used: *MI = (number of dividing cells/number of analysed cells) × 100*. Microscope examination was made at MC–3 microscope, in strong light and by using a green filter, to amplify the contrast between chromosomes and cytoplasm.

Caffeine ($C_8H_{10}N_4O_2$; 1,3,7-trimethylxanthine; 1,3,7-trimethyl-2,6-dioxopurine; 7-methyltheophylline; 1-methyltheobromine) (Fig. 1) is a white powder, density=1.23, molecular weight=194.19, moderately soluble in organic solvents, melting point = 234–239 °C. From chemical point of view, caffeine is a purine alkaloid, from methyl-xanthine chemicals' group, together with theophylline and theobromine. These compounds have different biochemical effects and are found in plants in various proportions. They are different by the position of methyl groups in their structure and are easily oxidized to uric acid and other methyl-uric acids.

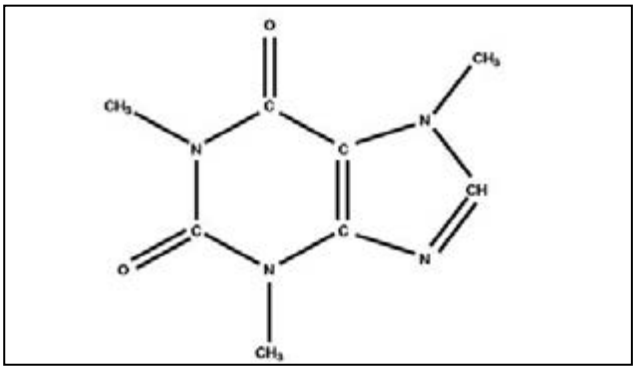

Fig. 1. Chemical structure of caffeine (from: www.coffee-tea.co.uk).

Results

Regarding mitotic index behaviour (Table 1, Fig. 2), at 0.01 % and 0.02 % caffeine a stimulation of cell division appeared, with 11 %, respectively 14 %, comparatively with control. A decrease of mitotic index is visible after 0.1 % caffeine treatment, continuous until maximum tested concentration, where this parameter has the minimum level. The cell division inhibition at variants treated with maximum tested concentrations (0.3 % caffeine and 0.5 % caffeine) is obvious, mitotic index being 8.20 %, respectively 7.05 %, values representing diminutions with 55 % for the first case, 72 % for the second, comparatively with control (18.23 %).

Out of the total dividing cells, the prophases are numerically prevalent (Table 1, Fig. 3), without being a division blocking in prophase, followed at relatively great distance by ana-telophases and metaphases. At 0.25 %, 0.3 %, and 0.5 % caffeine, the incidence of mitotic phases significantly and progressively diminishes, following the descendant line registered by mitotic index.

Table 1. Mitotic index and frequency of mitosis phases, after caffeine treatment of hemp seeds.

Variant	Total cells	Interphases		Mitotic index		Prophases		Metaphases		Ana-telophases	
		no.	%	no.	%	no.	%	no.	%	no.	%
Control	8790	7188	81.77	1602	18.23	878	9.99	373	4.24	351	4.00
0.01 % caffeine	8020	6404	79.85	1616	20.15	988	12.32	233	2.90	395	4.93
0.05 % caffeine	7907	5998	75.86	1909	24.14	1170	14.80	251	3.17	488	6.17
0.1 % caffeine	6835	5793	84.75	1042	15.25	803	11.75	74	1.09	165	2.41
0.125 % caffeine	7407	6358	85.84	1049	14.16	807	10.89	90	1.21	152	2.06
0.25 % caffeine	8135	7311	89.87	824	10.13	598	7.35	68	0.84	158	1.94
0.3 % caffeine	7324	6723	91.80	601	8.20	453	6.18	51	0.70	97	1.32
0.5 % caffeine	8011	7446	92.95	565	7.05	421	5.25	52	0.65	92	1.15

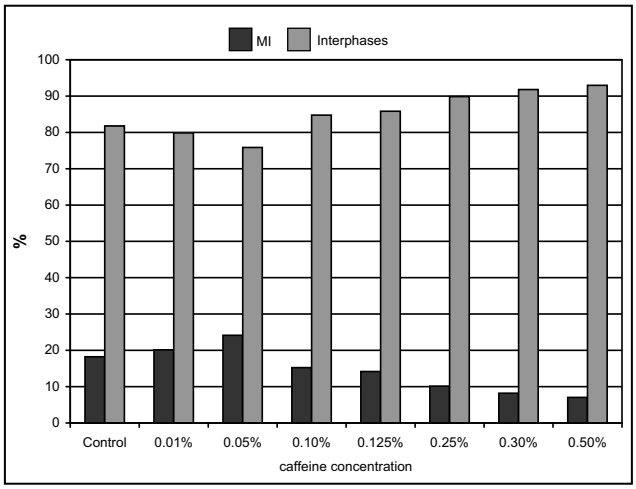

Fig. 2. Influence of caffeine treatment on mitotic index in hemp.

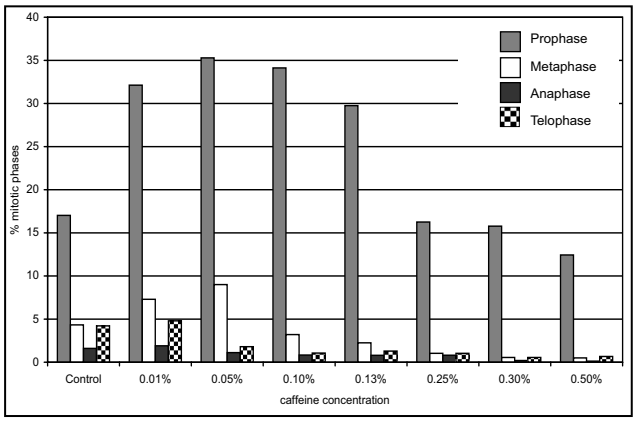

Fig. 3. Incidence of mitotic phases, after caffeine treatment.

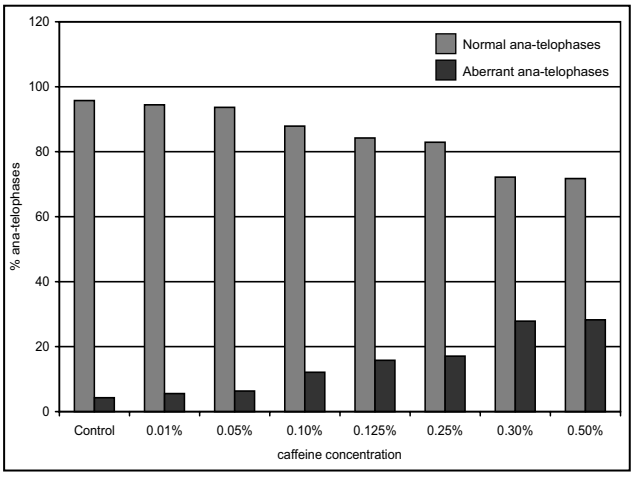

Fig. 4. Incidence of aberrant and normal ana-telophases in hemp root meristems, after caffeine treatment.

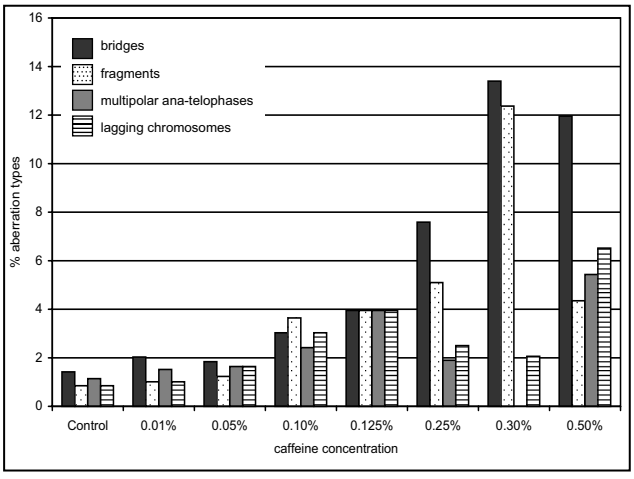

Fig. 5. Incidence of aberrations in total analysed ana-telophases, after caffeine treatment.

As shown in Fig. 4 and Table 2, which comparatively present the incidence of aberrant ana-telophases in the total of examined ones, after caffeine treatment, a linear relationship between the concentration increase and the number of aberrant ana-telophases is visible. The frequency of spontaneous aberrations, expressed by the value of distilled water treated control, was 4.27/100 ana-telophases. In caffeine treated variants, the percentual value of aberrant ana-telophases is markedly increased starting from 0.1 % caffeine and 0.125 % caffeine, where the increase was approximately 3 times greater than control, while at 0.3 % and 0.5 % caffeine, the aberration frequency is 6.5, respectively 6.7 times greater than the control value.

From the point of view of the frequency and types of chromosome aberrations (Fig. 5, Table 2), we can note the great percentage of fragments (12.37 % of the total of aberrant ana-telophases), at 0.3 % caffeine. This concentration has the stronger mutagen effect, inducing chromosome breakages and fragments, as well as an enough great number of lagging chromosomes (6.52 % in 0.5 % variant), and multiple bridges (11.96 % in 0.5 % caffeine variant, respectively 13.40 % in 0.3 % caffeine variant), fact that reveals a possible alteration at the level of chromosomal attachment and slidding on spindle fibres.

Table 2 includes the data on aberrant ana-telophases incidence, in the whole of examined ana-telophases, as well as the numerical and percentual situation of aberrations' types found in caffeine treated variants, comparatively with control.

Table 2. Frequency of chromosome aberrations in mitotic ana-telophases from hemp root meristems, after caffeine treatment.

Variant	Analysed ana-telophases	Normal ana-telophases		Aberrant ana-telophases		Aberration type							
						Bridges		Fragments		Multipolar ana-telophases		Lagging chromosomes	
		no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Control	351	336	95.73	15	4.27	5	1.42	3	0.85	4	1.14	3	0.85
0.01 % caffeine	395	373	94.43	22	5.57	8	2.03	4	1.01	6	1.52	4	1.01
0.05 % caffeine	488	457	93.65	31	6.35	9	1.84	6	1.23	8	1.64	8	1.64
0.1 % caffeine	165	145	87.88	20	12.12	5	3.03	6	3.64	4	2.42	5	3.03
0.125 % caffeine	152	128	84.21	24	15.79	6	3.95	6	3.95	6	3.95	6	3.95
0.25 % caffeine	158	131	82.91	27	17.09	12	7.59	8	5.10	3	1.90	4	2.50
0.3 % caffeine	97	70	72.16	27	27.84	13	13.40	12	12.37	0	0.00	2	2.06
0.5 % caffeine	92	66	71.74	26	28.26	11	11.96	4	4.35	5	5.43	6	6.52

Discussion

Mutagenic effects of caffeine were studied in prokaryotic as well as eukaryotic organisms, with variable results. In several cases it was considered as a DNA repair inhibitor (Sasaki & al. 1988, cited by Itoyama & Bicudo 2000). The researchers tested the mutagenic activity of caffeine and other purine analogues, in various biological systems, but the conclusions were not identical. For hemp, there are not experimental data regarding the mutagenic effects of caffeine on cell division and genetic material.

The caffeine, as well as other purine derivatives, can be incorporated in DNA macromolecule. In the next DNA replication, the initial nitrogenous base will be changed by a base analogue, because of some incorporation errors. To be mutagens and, therefore, incorporated in DNA macromolecule, it is required to action during DNA active synthesis. Caffeine is a "base analogue" of adenine, and in fact can sometimes be incorporated into a growing DNA chain, instead of adenine. Caffeine is considered by some authors as a weak mutagen, for this reason.

Because of lack of some identical or similar results on the mutagenic effect of caffeine in different biological systems, the mutagenic potential of caffeine and certain phenoxy-methyl-xanthine compounds is controversial and discussible. Kaul & Zutshi (1973) and Hernández & al. (1986) sustained the idea that these compounds induce chromosome aberrations, centric breakages, or sub-chromatid exchanges, and Sax & Sax (1966) highlighted the radiomimetic effect of caffeine from tea, coffee and Coca Cola on plant chromosomes. Caffeine produces mutations in bacteria, fungi, and insects, and induces chromosome aberrations in both onion root tips and human tissue culture cells (Sax & Sax 1966). Itoyama & Bicudo (2000) sustained that this compound has an inhibitive effect on DNA repair, stopping the repair of cytogenetic lesions, but does not determine an increase of micronuclei number, in the first ascendant phase of caffeine concentration curve.

Raicu & Stoian (1967) evidenced a great number of chromosome aberrations (bridges, lagging chromosomes, tetraploid cells) and a modified dynamics of cell cycle (prolongation) in *Vicia faba* L., under the impact of treatments with purine derivatives (adenine, adenosine, 3'-adenylic acid, xanthine, hypoxanthine), at 0.1–0.3 % concentrations. Tudose & Filimon (1972–1973) established the mutagenic effect of caffeine in wheat, where this purine derivative determined the increase of dividing cells and chromosome aberration frequency in mitotic ana-telophases. In relation to mutagenicity, even the results for the same organism in literature are occasionally antagonistic (Laranja & al. 2003).

The mechanism of caffeine action is not completely deciphered. It is possible that the methylated oxypurines (including caffeine) act as solubilizing agents and form molecular complexes, because of the existence of a close relationship between the solubilizing power of a chemical and its ability to produce chromosome aberrations. It seems, also, that these substances interact with DNA and alter its physical properties, in particular the denaturation temperature, fact that determines the increase of spontaneous rate of mutations.

Our results on effects induced by caffeine on nuclear material from hemp root meristems are, generally, in accordance with previous researches (Kihlman & al. 1971; Hernández & al. 1986; Maniu & al. 1998), in the sense of the confirmation of mutagenic effect of caffeine, by altering the chromosome structure. Thus, Hernández & al. (1986) confirmed the ability of caffeine to induce chromosomal aberrations in proliferating cells when they are incubated during G2 and mitotic prophase. In their experiments, caffeine (1–10 mM) induced chromosomal aberrations in a dose-dependent manner, and the treatment efficiency correlated linearly with the square of caffeine concentration. Maniu & al. (1998) evidenced aberrant ana-telophases and a relatively high frequency of micronuclei in the presence of caffeine. In our experiments, the micronuclei were not observed.

The chromosome deletions which take place in the presence of chemical tested compound lead to the chromosome fragments, single or multiple bridges (these are, generally, the result of the fusion of two chromosomes which suffered terminal deletions), visible between the two chromatid groups separated at the cell poles. Regarding the lagging chromosomes, these have lost the ability to attach to spindle fibres and can not participate at a normal division, determining disequilibrium between the daughter cells. The tri-, tetra-, or multipolar ana-telophases

are the consequence of defective formation of division spindle.

In interphases, especially in variants treated with the highest caffeine concentrations, binucleate cells were present, but their number was insignificant. Their presence is due to cytokinesis inhibition by caffeine, without affecting the chromosomes and nuclei division. This possible cytokinesis inhibition by caffeine in plants is recorded in literature (de la Peña & al. 1981; Hepler & Bonsignore 1990).

To complete the image of caffeine induced modifications in plant cell, we add other literature data. For example, Rybaczek & al. (2002) demonstrated that 1,3,7-trimethylxanthine can induce premature condensation of incompletely replicated chromosomes in root meristem cells, fact that is correlated with an increased level of protein phosphorylation, while Del Campo & al. (1997) confirmed that the treatment of the *Allium cepa* L. root meristems by two short caffeine exposures, spaced by 15 hours, leads to the appearance of acentric chromosomal segments which, after reconstitution, give rise to aneuploid nuclei containing unstable and broken DNA. Our previous studies (Truță & al. 2000) evidenced a moderate effect of caffeine and of a group of 8-(4R)-phenoxy-methyl-xanthine compounds on cytogenetical parameters in *A. cepa* for respective tested concentrations.

As conclusions, we can sustain that caffeine, in increasing concentrations and at the tested exposure time, induced, in *C. sativa* root meristems, a stimulation of mitotic index at smaller tested concentrations. The highest concentrations were inhibitive on mitotic index, in direct relation with the caffeine concentration increase. Also, the incidence of chromosome aberrations induced by caffeine was important, comparatively with control, especially at the greatest tested concentrations.

Acknowledgements. This paper was realized in BIOSTAR research program, with the financial support of the Ministry of Education and Research, Romania.

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The use of biological method of virotic testing to obtain plum-pox virus free apricot cultivars

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Abstract. Presently, the plum-pox virus is one of the most grave diseases of the apricot's cultivars. That is why, to obtain apricot cultivars with tolerance and genetic resistance to this virus as well as to introduce them in cultivation, have represented one of the important objectives of the research programme at the Fruit Research Station in Constanța. This paper presents the data regarding the susceptibility of some apricot cultivars and hybrids confronted by the natural infections with local plum-pox virus strains. The observations pursued to detect typical symptoms of the plum-pox virus attack, both on leaves (May–June) and also on fruits and stones during their maturity (July–September). There was a large difference of manifestation of the virus symptoms among the cultivars and hybrids and a high variability regarding the intensity of the attack on the vegetative organs.

Key words: apricot, genetic resistance, peach, plum-pox, symptom

Introduction

Nowadays, in Europe, plum-pox potyvirus (PPV) became one of the most severe diseases for all countries growing stone fruit species, because the diseased trees get lower productions, the fruits are qualitatively depreciated and they fall down 2–3 weeks before the ripening time.

Romania along with Hungary, Bulgaria, Croatia, Poland, Slovakia and Portugal are the countries where this virus is present from many years and where the level of the contamination is very high (Smith & al. 1994; Balan & Stoian 1995).

The researches concerning the epidemiology and spreading ways of this virus started after 1960 (Minoiu & Beligan 1990) and revealed that plum-pox disease is wide spread on plum and the strain isolated from this fruit species is classified as "chlorotic type".

On the apricot the disease is not so spread in commercial orchards and its local importance is depending on grown cultivars and rootstocks, climatic conditions and its specific epidemiology.

For these reasons the breeding and promotion in culture of new apricot cultivars genetically resistant or tolerant to this virus represent one of the most important objectives of the research programmes developed by Fruit Growing Research and Development Station in Constanța.

The current bibliography reveals many apricot cultivars resistant to plum-pox potyvirus (PPV) such as: Stella (Németh 1986), Harlayne (Dosba & al. 1988), Sunglo (Karayiannis 1995), etc.

This work goal is to present many data regarding some apricot cultivars and hybrids' sensibility to infection with local strains of PPV.

Material and methods

The biological material was represented by 535 apricot cultivars and hybrids, having different eco-geographic origins and preserved in the national apricot genotype collection at Fruit Growing Research and Development Station, Constanța. According their origins, the genotypes' distribution is as follows: 4 % from Asia; 26 % from Europe (among these 38 % are from Romania) and 32 % from America.

The national apricot genotype collection was established between 1986 and 1988 as follows: 465 genotypes in 1982; 49 cultivars in 1986 and 29 cultivars in 1988.

As regards the planting design, for each genotype 5 trees were planted, the trees being spaced at 4.5 × 4.5 m.

In the experimental orchard the appropriated technology required by apricot was applied.

Observation and evaluations were carried out for 3 years, during 2003–2006.

Under natural given condition, the inoculum's vectors were the aphids and the inoculum's sources were 2 apricot genotypes, Marculesti 42/25 and Mandula caiszi, infected by grafting.

The observation goal was to put in evidence the typical symptoms of plum-pox potyvirus, both on leaves (in May and June), also on fruit and stones at their maturity (from July to September).

The sensible genotypes were tested by grafting on biologic indicator "G.F. 305". The annual spread of the virus in the experimental orchard was recorded in all 3 years of the study, starting with the first year of fruit bearing.

Table 1. Severity of some apricot cultivars and hybrids on plum-pox potyvirus infection, FGRDS Constanța, 2003–2006.

No.	Tested cultivar/hybrid	Sensibility on			Infected after 1 year
		Leaves	Fruits	Stones	
1	2	3	4	5	6
1	Marculesti 16/7 A	+++	+++	+++	9
2	Marculesti 91	+	++	++	10
3	Marculesti 66/16A	(+)	+	+	11
4	Saturn	0	+	++	9
5	Sirena	++	++	++	11
6	Baneasa 26/16	++	+++	+++	10
7	Baneasa 28/10	+++	+++	+++	11
8	Baneasa 28/17	0	++	++	10
9	RR 20–54	+	++	++	11
10	NJ 535	(+)	(+)	(+)	11
11	Early Golden	+++	+++	+++	10
12	NJA 6	0	++	++	11
13	Reale d'Imola	+++	++	++	11
14	Baneasa 1/21	0	0	(+)	10
15	Screara	+++	+++	+++	10
16	Harcot	+	0	0	–
17	Mamaia	+++	+++	+++	9
18	Mandula cayszi	+++	+++	+++	11
19	Marculesti 42/27	+++	+++	+++	10
20	Mari de Tarnave	0	0	0	–
21	Joubert Foulon	(+)	+	+	11
22	Moniqui	(+)	(+)	(+)	10
23	Reliable	0	(+)	+	11
24	Callatis	(+)	+	(+)	11
25	Marculesti 42/25	+++	+++	+++	9
26	Cais de Olanda	0	++	++	10
27	D1R76T28	(+)	(+)	(+)	9
28	Stark Early Orange	0	(+)	0	10
29	Ananas	0	++	++	9
30	Sulmona	+++	+++	+++	9
31	Stella	0	0	0	–
32	NJA 48	0	0	0	–
33	Amal	0	(+)	(+)	11
34	Umberto	(+)	+	+	11
35	Canada 51144	0	+	++	9
36	Vivagold	0	0	0	–
37	Traian	0	0	0	–
38	Mari de Cenad	(+)	(+)	(+)	11
39	Excelsior	+++	+++	++	9
40	Best of Hungari	++	+++	+++	11
41	Canette	+++	+++	+++	9
42	Don Gaetano	++	+++	+++	11
43	Royal	(+)	+++	+++	10
44	Rosii timpurii	0	0	0	–
45	NJA 11	(+)	++	++	9
46	Wenacthee	++	+++	+++	10
47	Eten Bey	(+)	+	+	10
48	Neptun	++	++	++	10
49	Precoce de Colomer	(+)	(+)	0	11
50	Venus	(+)	++	++	9
51	Blenril	(+)	++	++	9
52	Alberge de Montgamet	+	++	+++	9
53	Slava Diurgna	+++	+++	+++	10
54	Litoral	(+)	++	++	10
55	Selena	(+)	++	++	10
56	NJA 19	+	++	++	10
57	Tonda di Tossiguano	+	++	++	11
58	Villand	0	(+)	0	10
59	Farmigdale	0	+	+	11
60	Early Bee	+	+++	+++	9
61	Tabriz Nakjavan	0	0	0	–
62	Baneasa 3	+	+	+	10
63	Piestany 8/35	++	++	+++	9
64	Marculesti 17/1	0	(+)	(+)	11
65	NJA 17	(+)	(+)	(+)	11
66	Marculesti 7/28	0	+	+	11
67	Racoszi Magyar caiszi	+++	+++	+++	9
68	Angounois	++	+++	+++	11

In the same time, in order to determine the severity of infection with plum-pox on vegetative organs, for each genotype 3 repetitions of 100 organs were assessed using the scale used in Table 1.

Results

Among all 535 apricot's genotypes (cultivars and hybrids), after 11 and respectively 14 years since the apricot collection was established, 104 genotypes (19.4 %) presented plum-pox symptoms on leaves, fruits and stones.

In the first year of observations 25 genotypes (24.1 %), in the second year 34 genotypes (32.6 %) and in the third year 45 genotypes (43.1 %) were infected (Table 1).

Table 1. Continuation.

1	2	3	4	5	6
69	Saianet 2–25–4	0	++	++	11
70	NJA 42	0	0	0	–
71	Busiereska	+++	+++	+++	10
72	Balmont	0	0	(+)	11
73	Piestany 8/42	++	++	++	9
74	Balsoi Pozdnii	++	++	++	9
75	Sephid	+	0	0	–
76	Marculesti 98	(+)	++	++	11
77	V 600–2030	0	(+)	0	9
78	R.R. 20–54	0	(+)	0	11
79	I.R. Viceroy 603–2	0	(+)	(+)	11
80	Montedoro	0	(+)	(+)	9
81	Kets pshor	0	0	0	–
82	Rakche karpo	0	(+)	(+)	9
83	B8R29AT109	0	0	0	–
84	Worley' peach	++	+++	+++	10
85	D1R75T28	(+)	(+)	(+)	9
86	De Costanjeni	0	+	+	9
87	Piestany 0/25	0	0	(+)	10
88	Baneasa 33/16	0	(+)	(+)	11
89	Favorit	(+)	(+)	(+)	11
90	De Silistra	++	+++	+++	9
91	Canino	0	(+)	(+)	11
92	ABC	0	(+)	(+)	9
93	Goldrich	0	0	0	–
94	Riland	0	0	0	–
95	Marculesti 19	(+)	(+)	++	11
96	V 631 2019	+++	+++	+++	9
97	Marculesti 9/5	0	0	0	–
98	Soiul 132	0	0	0	–
99	Marculesti 24/1	0	0	0	–
100	V 60102	(+)	+	+	10
101	NY 493	0	++	++	11
102	Baneasa 40/40	0	0	0	–
103	Mari de Marculesti	0	0	0	–

Legend: 0, without any symptoms; (+), very few symptoms; +, moderate symptoms; ++, heavy symptoms; +++, very heavy symptoms.

On the one hand, large differences in PPV symptoms' manifestation were noticed among the collected apricot cultivars and hybrids, and on the other hand, we observed an even higher variability of symptoms' manifestation on apricot vegetative organs.

The most severe symptoms were visible on fruits and stones and this fact reveals that observations carried out on leaves are not sufficient in order to identify the PPV potyvirus presence.

Otherwise all the cultivars evidenced as infected were the subject of the biological test by grafting on "G.F. 305" indicator. On the occasion of the biological test, the field results were confirmed and all infected cultivars and hybrids without visible symptoms were identified.

Discussion

The majority of apricot's genotypes (cultivars and hybrids) collected and preserved at Fruit Growing Research and Development Station, Constanţa are resistant or tolerant to PPV strains.

The genotypes Baneasa 1/21, Harcot, Vivagold, Traian, Rosii timpurii, Sephid, Kets pshor and Viland were evidenced as highly resistant to PPV potyvirus and were already used as genitors in the apricot breeding program.

Otherwise in order to restrain this dangerous virus spread, the chosen genitors were not introduced in the breeding programs without the certification of their "virus-free" status by indexation on biological indicators (i.e. G.F. 305).

The intensity of PPV symptoms on apricot vegetative organs reveals that local strains of PPV are less virulent than the strain isolated in South-east of France (PPV – Dideron strain) and than the one isolated in the North of Greece (PPV – Marcus strain).

The results of our work confirm some other reports in the literature, regarding some cultivar and their sensibility to this virus: Stella, Stark Early Orange, Sunglo and NJA 42 (e.g., Karayannis 1995).

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Some embryological features of two species of *Leucanthemum vulgare* complex (*Asteraceae*)

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Abstract. The peculiarities of the anthers, microsporogenesis and development of male gametophyte of *Leucanthemum praecox* and *L. vulgare* in Bulgaria have been studied. The anthers were tetrasporangiate with 4-layered anther wall. In *L. vulgare* some deviations during the meiosis in the microspore mother cells (MMCs) were observed. As a result of simultaneous microsporogenesis predominantly tetrahedral tetrads were formed. The mature pollen was 3-celled. This study revealed that the two *Leucanthemum* species differ in: morphology and behaviour of the anther's tapetum in the meiosis; characteristics and degree of deviations during the microsporogenesis; type of microspore tetrads and amount of sterile pollen in the anthers.

Key words: anther, embryology, *Leucanthemum*, meiosis

Introduction

The genus *Leucanthemum* Mill. belongs to the family *Asteraceae* (tribe *Anthemideae* Cass.). It consists of 33 species with distribution mainly in Central and Southern Europe (Bremer & Humphries 1993). Some species in *L. vulgare* s.l. are widespread as weeds. *Leucanthemum vulgare* s.l. has been a subject of intensive studies carried out in various parts of its area of distribution. They reveal that this taxon is a polyploid complex, represented by numerous cytotypes ranging from 2*x* to 12*x* (*x* = 9), but the most frequent of them are diploid (2*n* = 2*x* = 18), tetraploid (2*n* = 4*x* = 36) and hexaploid (2*n* = 6*x* = 54). The morphological, anatomical and karyological studies carried out till now show that the complex *L. vulgare* is taxonomically intricate (Villard 1971; Bremer & Humphries 1993). Data on the mode of reproduction of the genus *Leucanthemum* are very scarce and refer only to the *L. vulgare* s.l. (Villard 1971; Przywara 1974; Solntseva 1987). The embryological data concern mostly the meiosis and microsporogenesis.

In the Bulgarian wild flora the genus *Leucanthemum* is presented with 3 species included in *L. vulgare* complex. As a result of karyological investigation on Bulgarian populations of *L. vulgare* s.l. three well-defined species were established in Bulgaria (Kuzmanov & al. 1988). The diploid *L. praecox* (Horvatić) Horvatić with 2*n* = 18 is widespread, followed by tetraploid *L. vulgare* Lam. (2*n* = 36*)*, whereas hexaploid *L. pallens* (J. Gay) DC. (2*n* = 54) has a restricted distribution.

In the present work the results of the investigation on the male generative organs of *L. praecox* and *L. vulgare* s.str. as a major part of their reproductive system are shown. This work as a part of a PhD Thesis is an introduction to the first embryological study on the Bulgarian taxa of the complex *L. vulgare*. Further embryological investigations on new populations and on the hexaploid species *L. pallens* are in progress. Being relatively stable and more reliable than the morphological ones, the embryological characters may turn out useful in differentiation of the taxa within the complex *L. vulgare* and in the evaluation of relationships between them.

Material and methods

Comparative-embryological study on native Bulgarian populations was made: *L. praecox* – 2 populations with 2*n* = 18 (No. 041007 – Western Rhodopes, near Batak dam; No. 041208 – Western Stara planina Mts, Petrohan) and *L. vulgare* – 1 population with 2*n* = 36

(No. 041507, Central Stara planina Mts, Beklemeto). The material (flower buds and capitula) was collected periodically during July–August 2004–2005 and fixed in FAA mixture (formalin: glacial acetic acid: ethanol in a correlation 5:5:90 parts with 70 % ethanol). The material was treated according to the classical paraffin methods (Romeis 1948). Serial sections were made 10–18 μm thick and stained with Heidenhain's haematoxylin. The observations on the permanent slides were made with light microscope "Amplival".

Results and discussion

All features of the male generative organs shown in the following discussion are common to the genus *Leucanthemum*, unless the main differences between the species studied are mentioned specifically.

The anthers were tetrasporangiate and the 4-layered anther's wall developed centrifugally following the Dicotyledonous-type (Davis 1966). The anther's wall consisted of epidermis, endothecium, one middle layer and tapetum (Plate I, Figs 1, 2). Poddubnaya-Arnoldi (1982) and Solntseva (1987) showed that the middle layer in the family *Asteraceae* is ephemeral. We consider that this layer was not strongly ephemeral in the two studied species of *Leucanthemum*. In *L. praecox* its remnants might be observed at the stage of microspore tetrads while in *L. vulgare* they persisted in the anther's wall even after one-celled pollen stage. The endothecium developed fibrous thickenings that are typical for *Asteraceae* family (Poddubnaya-Arnoldi 1982; Solntseva 1987). However, they were not so clearly expressed like in other Bulgarian representatives of the tribe *Anthemideae*, i. e. the genera *Achillea* L., *Artemisia* L. and *Tanacetum* L. (Yurukova-Grancharova & al. 2002). Initially, the tapetum was of the secretory type and passed a long parietal phase. The tapetal cells contained a dense cytoplasm and a deeply staining nucleus. During the heterotypic division of the meiosis in microspore mother cells (MMCs) a multiplication of the nuclei began in the tapetal cells and after subsequent mitosis before the end of the heterotypic division in *L. praecox* they became 8–16 nucleate (Plate I, Fig. 3). In the two species studied a polyploidization in the nuclei of the tapetal cells run too. The multinucleate or polyploid tapetal cells were with different shape and size (usually polygonal) and in *L. praecox* they penetrated deeply into the anther's locule. In this species, the tapetum passed a long parietal phase and after one-celled pollen stage a transformation of the secretory tapetum into real periplasmodium proceeded. In *L. vulgare*, such transformation was not observed and the tapetum degenerated soon after the formation of 2-celled pollen grains in the anther. Przywara (1974) established an early degeneration of the tapetum and observed that the tapetal cells of tetraploid *L. vulgare* "are viable until the stage of primary pollen grain and afterwards they undergo the process of degeneration".

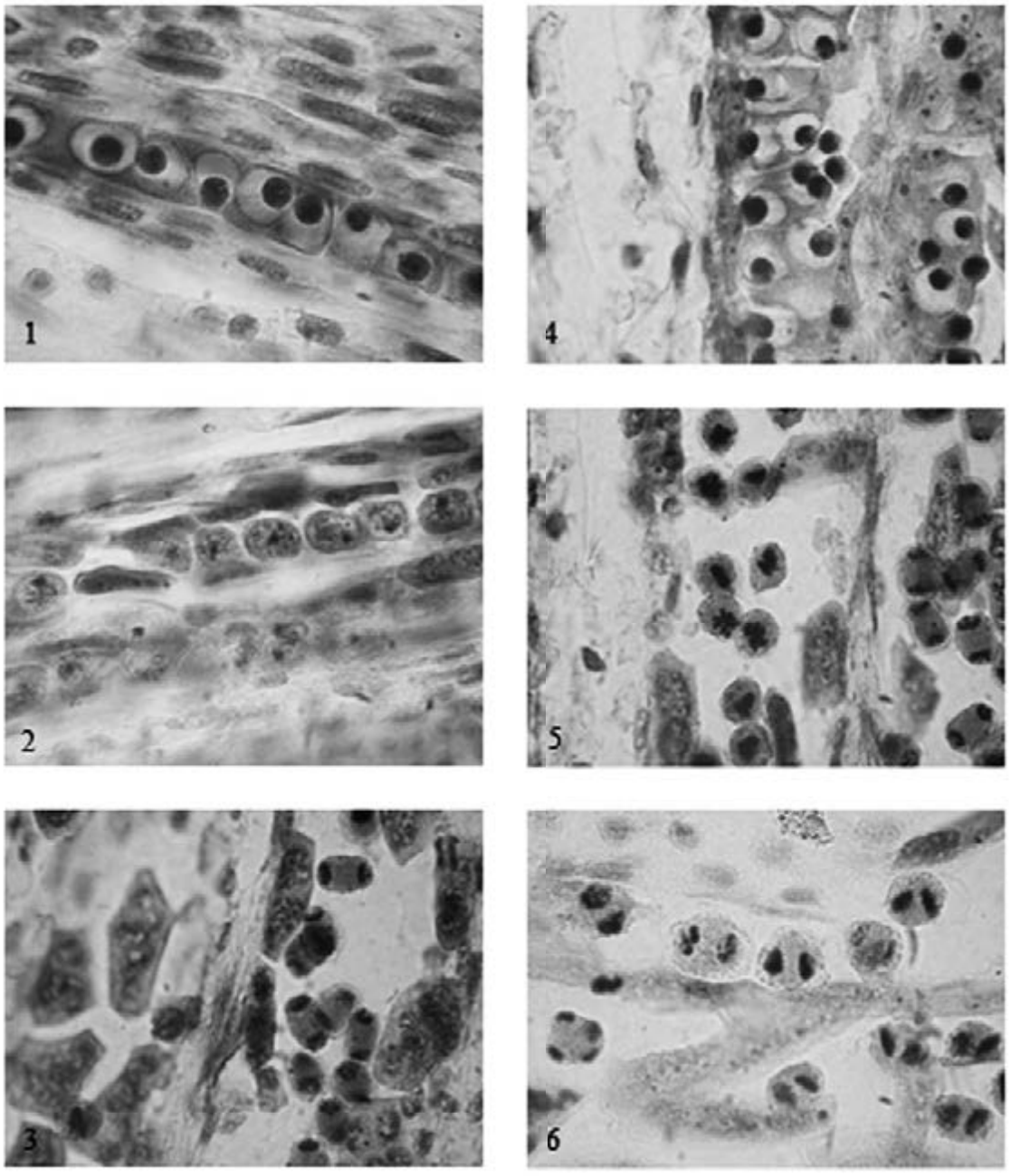
The sporogenous tissue was one- (Plate I, Fig. 1) or two-layered (Plate I, Fig. 4). The meiosis in *L. praecox* run normally and in the MMCs at metaphase I always 9 bivalents were counted (Plate I, Fig. 5). The same number of bivalents during the diakinesis was established by Favarger & Villard (1965) and Villard (1971) in the diploid *L. praecox*. Meiosis in the anthers of diploid taxa of the genus *Leucanthemum* was observed to be regular (Favarger & Villard 1965; Villard 1971).

In *L. vulgare* we observed that the chromosome associations in the MMCs were predominantly bivalents and single quadrivalents. Single quadrivalents during the first division of the meiosis in MMCs of this tetraploid species were noticed by Przywara (1974) in Poland and Villard (1971) in Switzerland.

During the meiosis in MMCs of *L. vulgare* some other deviations were observed: i. e. chromosomes out of the spindle; lagging chromosomes; bivalents and single quadrivalents at the diakinesis and metaphase I; anaphase bridges; abnormal disposition of the spindles during the homeotypic division (Plate I, Fig. 6). Almost the same deviations during the meiosis in MMCs showed Przywara (1974) in the tetraploid *L. vulgare* s.str. and hexaploid *L. montanum* DC. from Poland. In both studied species of *Leucanthemum* the meiosis in the MMCs proceeded asynchronously even in an anther locule (Plate I, Fig. 5; Plate II, Fig. 1). The comparative analysis showed that the asynchrony of the processes registered during the microsporogenesis in an anther and between neighbouring anther locules was more strongly expressed in the tetraploid *L. vulgare*.

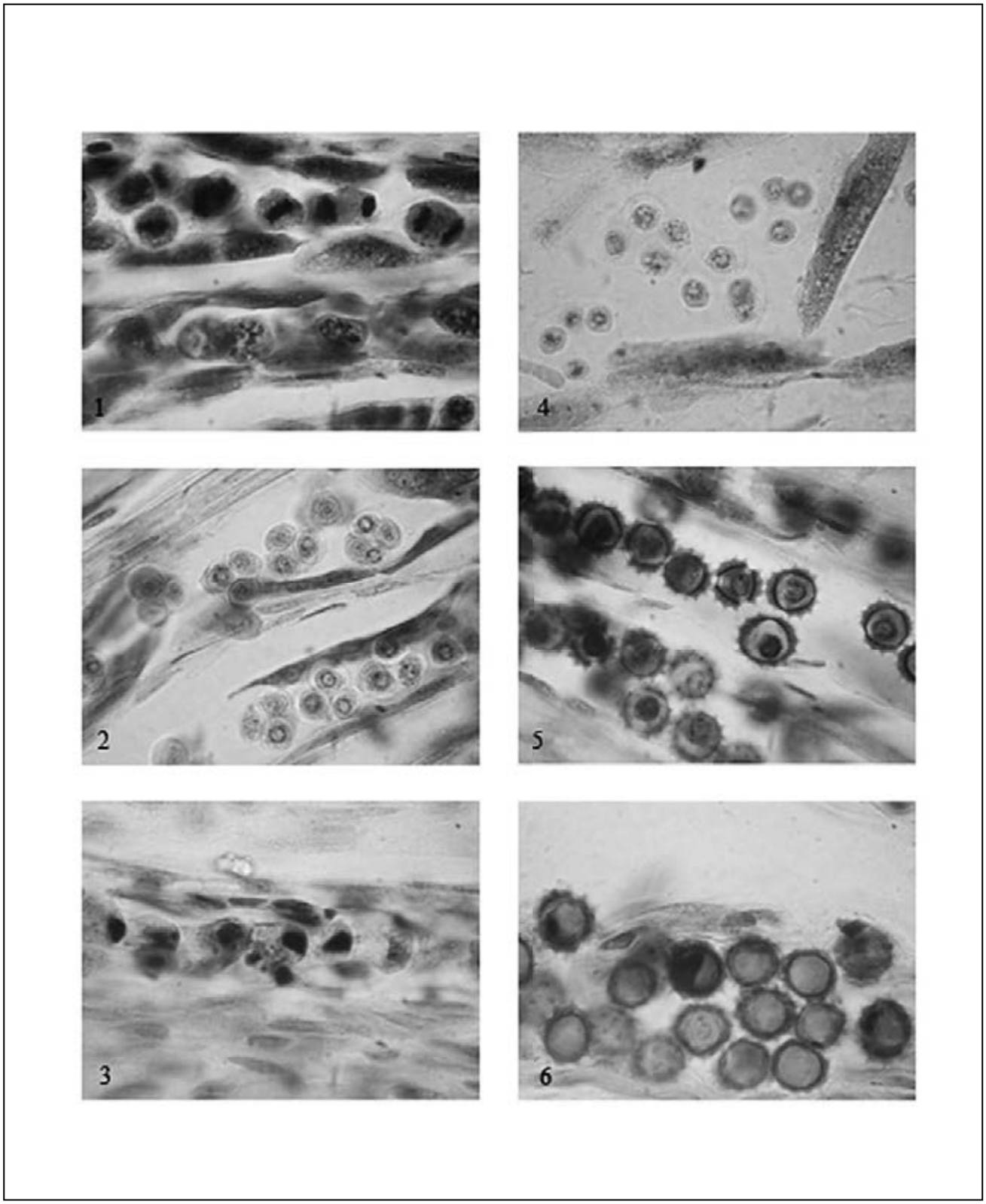
The microsporogenesis in both species studied run simultaneously. In the anthers of *L. praecox* predominantly tetrahedral tetrads and isobilateral ones were formed (Plate II, Fig. 2). Within the tetrads the four microspores were morphologically uniform and micronuclei were not found. A few abnormalities were observed during the various developmental stages in *L. vulgare*.

Plate I



Figs 1–6. Anther, microsporogenesis and male gametophyte in *L. praecox* and *L. vulgare*:
1, Four-layered anther wall and one-layered sporogenous tissue in *L. vulgare* (×400); **2,** Four-layered anther wall and prophase I in MMCs in *L. praecox* (×400); **3,** Multinucleate tapetal cells at anaphase I of the meiosis in *L. praecox* (×400); **4,** Two-layered sporogenous tissue in *L. praecox* (×400); **5,** Metaphase I in MMCs with 9 bivalents in *L. praecox* (×400); **6,** Deviations in the homeotypic division in MMCs in *L. vulgare* (×400).

Plate II



Figs 1–6. Anther, microsporogenesis and male gametophyte in *L. praecox* and *L. vulgare*:
1, An asynchrony during the meiosis in an anther locule and in two neighbouring locules in *L. vulgare* (×160); **2,** Tetrahedral microspore tetrads in *L. praecox* (×400); **3,** Degenerating MMCs during the meiosis in the anthers of *L. vulgare* (×160); **4,** Different types of microspore tetrads in *L. vulgare* (×400); **5,** Mature pollen in *L. praecox* (×400); **6,** Fertile and sterile pollen in *L. vulgare* (×400).

For example, the nuclear material in the sporogenous tissue accidentally degenerated and some MMCs disintegrated during meiosis accompanied by the breakdown of tapetal cells (Plate II, Fig. 3). Because of some disturbances during the microsporogenesis in *L. vulgare* different types of tetrads (Plate II, Fig. 4), unequivalent microspores and micronuclei within a tetrad as well as pentads were observed in the anthers.

The young microspores were released from the tetrads and very quickly became round within the anthers. After, they differentiated into one-nucleate pollen grains with dense cytoplasm and a conspicuous, centrally located nucleus. At this time, the pollen cytoplasm became vacuolated and the nucleus migrated from the centre toward the cell periphery. Afterwards, the pollen nucleus divided to form a larger vegetative cell and a smaller generative one that were darkly stained. The generative cell underwent a division to form two sperms with a crescent shape. Ultimately, the mature pollen was 3-celled, 3-colporate with echinate exine (Plate II, Fig. 5).

In *L. praecox* the pollen was viable and fertile. In some anthers of *L. vulgare* a degeneration of MMCs during the meiosis, as well as microspore tetrads and mature pollen grains were established. Besides, single microspores (1–2) within the tetrad were sterile, almost transparent without nucleus and cytoplasm or were dark stained and degenerating. By this reason, in some anthers of *L. vulgare* a little amount of viable and fertile pollen grains was formed. A pycnosis and subsequent degeneration of the whole anther content was observed in some florets of individual capitula of *L. vulgare*. As a result, such anthers became completely sterile. Because of all mentioned disturbances of the microsporogenesis and development of male gametophyte a large quantity of sterile pollen was formed in some anthers of *L. vulgare* (Plate II, Fig. 6).

Conclusion

The study on the male generative organs in native Bulgarian populations of the diploid *L. praecox* and tetraploid *L. vulgare* was carried out. The analysis of the microsporogenesis showed insignificant differences between both studied populations of *L. praecox*. In this respect some abnormalities were established in the population of *L. vulgare*. During this study some clearly expressed differences between the two species of *Leucanthemum* were found as regards the morphology and behaviour of the anther's tapetum during the meiosis in MMCs; the features and degree of deviations during the microsporogenesis; the types of microspore tetrads; the amount of sterile pollen grains in the anthers. The deviations of the normal meiosis established in *L. vulgare* leaded to sterile and degenerated pollen grains in some anthers with reduced vitality and fertility. Generally, in relation to the character and the level of the differences during the microsporgenesis and the peculiarities of the anther tapetum, the tetraploid *L. vulgare* differs clearly from the diploid *L. praecox*. The results of the present work enrich the embryological characteristics of the studied species, as well as the genus *Leucanthemum*, which are poorly studied embryologically.

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Embryological study of a hexaploid *Hieracium bauhinii* from Bulgaria

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Abstract. Embryological study on a hexaploid accession of *Hieracium bauhinii* with $2n = 6x = 54$ from Bulgaria was carried out. Various deviations during the meiosis in the microspore mother cells resulting in the formation of monads, dyads, polyads and sterile pollen were observed. The abnormal anthers and large quantity of sterile pollen lead to mostly unsuccessful pollination and fertilization. The embryo sac (ES) develops after *Polygonum* (monosporic) or *Allium* (bisporic) types. Usually the development of legitimate ES is suppressed by the rapid development of aposporous one or both types of ESs can co-exist in a single ovule. It was established that *H. bauhinii* is a facultative apomict, combining sexual mode of reproduction with strongly expressed somatic apospory and polyembryony.

Key words: apomixis, chromosome number, embryo sac, *Hieracium bauhinii*, male and female gametophyte

Introduction

Hieracium bauhinii Schult. (*H.* subg. *Pilosella*) has a wide distribution area ranging from Central and South Europe to South-West Siberia and Central Asia (Zahn 1923). It is among the most common representatives of the genus in Bulgaria, distributed in all floristic regions between 0–1500 m alt. The species occurs in a wide range of habitats: meadows, forest margins and openings, disturbed ground, urban areas. The taxonomy of the species has not been satisfactorily resolved yet. Taxonomic difficulties are caused by the occurrence of several ploidy levels (e.g. see Rotreklová 2004), apomixis and hybridization. However, very little is known about the microevolutionary processes and biology of the species. Especially valuable is the knowledge on the mode of reproduction which encouraged us to undertake this study.

The aims of the study were: 1. To reveal the mode of reproduction of the hexaploid *H. bauhinii*; 2. To establish the peculiarities of the embryological processes and structures.

Material and methods

A natural accession of *H. bauhinii* from Sofia district, Bulgaria was studied karyologically and embryologically. Living plants were collected and grown under glass for obtaining the chromosome number. Root-tips were cut, pretreated with 0.01 % colchicine for 90 min and fixed in ethanol: glacial acetic acid (3:1) for at least 2 h at room temperature. Hydrolyzation was conducted in 1N HCl at 60 °C for 20 min. Then root-tips were treated with HCl: ethyl ether (1:1) for 10 min at 60 °C, rinsed in distilled water and stained with haematoxylin after Gomori (Melander & Wingstrand 1953) for 30 min at 60 °C. Finally, root-tips were squashed in a drop of 45 % acetic acid and mounted in Euparal. Plant material for embryological studies (flower buds, capitula and achenes) was collected and fixed in formalin: glacial acetic acid: 70 % ethanol (5:5:90), embedded in paraffin and cut into 6–18 μm thick sections using conventional methods (Romeis 1948). The sections were stained with Heidenhain's haematoxylin and mounted in Canada balsam. Observations were made with light microscope "Amplival".

Results and discussion

Chromosome counting was done in 5 plants. They all proved to be hexaploid, with $2n = 6x = 54$.

The anthers are tetrasporangiate. The 4-layered anther wall develops according to the Dicotyledonous-type and consists of epidermis, endothecium,

a middle layer and tapetum (Plate I, Figs 1, 2). The endothecium does not develop the typical for *Asteraceae* fibrous thickenings. The middle layer is strongly ephemeral and degenerates early during the homeotypic division of meiosis in the microspore mother cells (MMCs). The anther tapetum is secretory, with a long parietal phase during which a multiplication of nuclei up to 4 (rarely 8) in its cells occurs (Plate I, Figs 1, 2). The secretory tapetum degenerates early and does not transform into amoeboid one which was established by Gentscheff (1937) in other polyploid species of *H.* subg. *Pilosella*.

The one-layered sporogenous tissue is poor, comprising a small number of cells which function directly as MMCs (Plate I, Figs 1, 2). Microsporogenesis in *H. bauhinii* as in the other apomictic species of *H.* subg. *Pilosella*, is characterized by numerous abnormalities. The first and foremost peculiarity is the lack of synchronization in MMCs development within an anther. In sexual *Hieracium* spp. MMCs are usually well synchronized within the same anther. However, this is not the case in apomictic taxa of the genus where MMCs in different phases – from leptotene to pachytene – can be found in a single anther during the heterotypic division of meiosis. In some anthers an early inhibition of the meiosis in MMCs (during heterotypic division) was observed.

Most frequently the following disturbances during the meiosis were found: different number of speeding or lagging chromosomes; chromosomes out of the spindle; chromosome bridges and asymmetrical dispositions of the spindles during the homeotypic division. As a result, the simultaneous microsporogenesis leads to different types of tetrads (Plate I, Fig. 3), monads, polyads, morphologically different microspores in the tetrads and micronuclei.

Degeneration processes, e.g. degeneration of MMCs, individual microspore in the tetrads or whole tetrads in some anther locules (Plate I, Figs 4, 5) begin early in the anther ontogenesis. It should be noticed that in the florets of many capitula abnormal anthers are formed initially (Plate I, Fig. 6). Due to early inhibition of the meiosis in MMCs and degeneration processes, the development of the male gametophyte is disturbed. Consequently, many anthers become completely sterile and the florets in capitula – functionally female. Lack or a low number of mature pollen grains was registered in the anthers. Most of the mature pollen grains are sterile, degenerated and defective. Only a small amount of the normal 3-celled pollen grains are fertile. The undevelopment and degeneration of anthers and high degree of sterile pollen grains, observed in the hexaploid *H. bauhinii*, were reported by Czapik (1994) in other taxa with gametophytic apomixis.

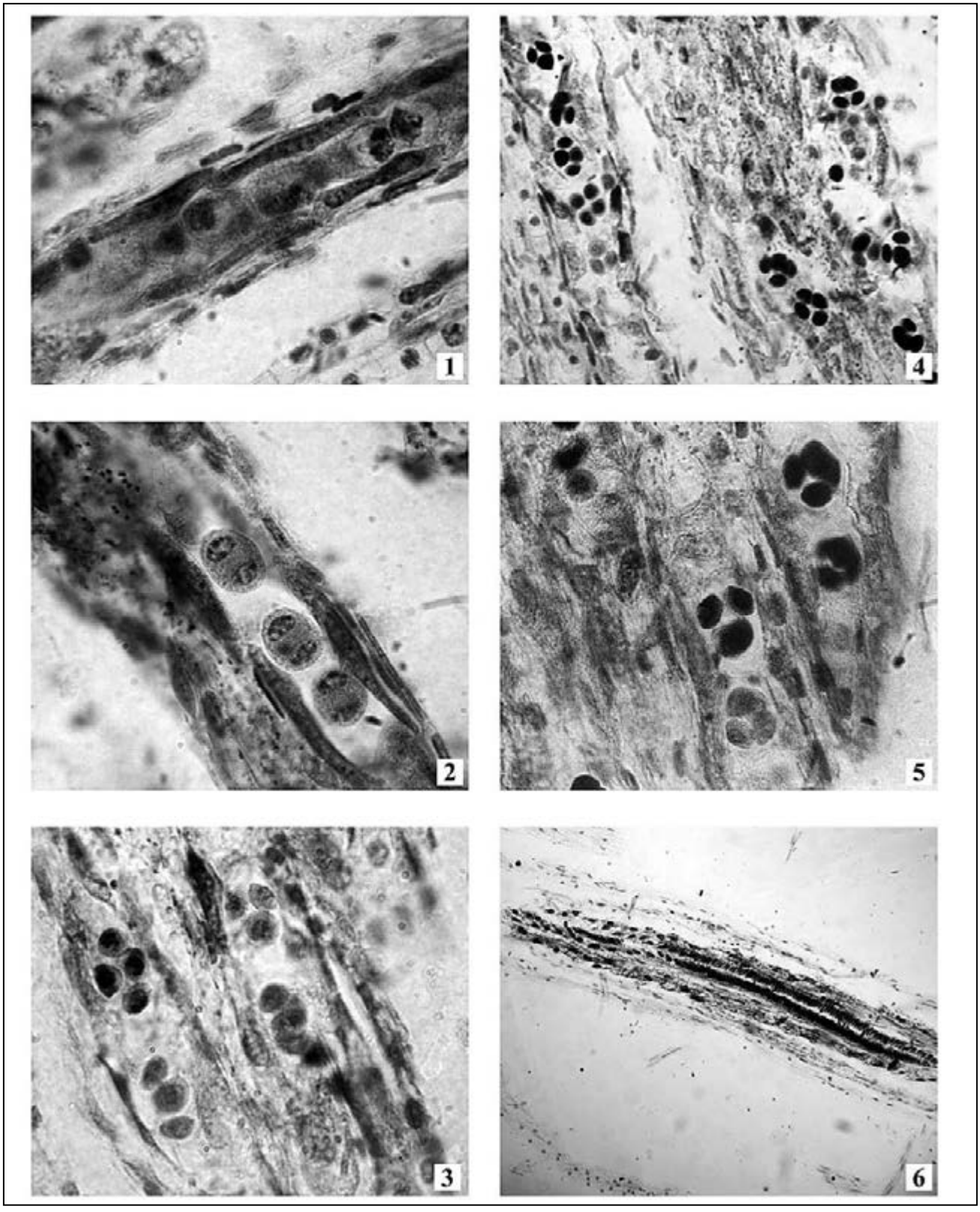
The ovule is anatropous, tenuinucellate and unitegmic. The archesporium is formed hypodermally and consists of a single cell which directly functions as a megaspore mother cell (Plate II, Fig. 1). The meiosis in megaspore mother cells results in a tetrad or dyad. Afterwards, the female gametophyte develops according to respectively the *Polygonum* (monosporic) or *Allium* (bisporic) type (Plate II, Figs 2, 3). The chalazal megaspore enlarges and becomes vacuolate, giving rise to one-nucleate ES whereas the sister megaspores degenerate. After 3 mitotic divisions, subsequently 2-, 4- and 8-nucleate ES is formed. The mature ES is typically differentiated and consists of 3-celled egg apparatus (egg cell and two synergids), two polar nuclei (after their fusion a central cell is formed) and 3-celled antipodal complex deeply disposed in the chalaze (Plate II, Fig. 4). The three antipodals in the ES are one-nucleate with linear or T-shaped arrangement. They often remain as 3 free nuclei and usually degenerate before fertilization and even before the polar nuclei fusion.

At the stage of 2-nucleate ES the one-layered nucellar epidermis is almost completely degenerated and the endothelium begins to differentiate from the innermost layer of the single integument. The endothelium consists of not typical radially lengthened cells and usually can not be clearly distinguished from the neighbouring somatic cells of the ovule.

Some deviations in the legitimate (meiotic) ESs were registered, such as atypical polarization of the nuclei in 4- and 8-nucleate ESs, lack of differentiation of the ES elements, two egg cells instead of one (Plate II, Fig. 5), elements of two ESs arranged in a row in the ovule (Plate II, Fig. 6), extrusion of the developing ES through the micropyle like a haustorium (Plate III, Fig. 1).

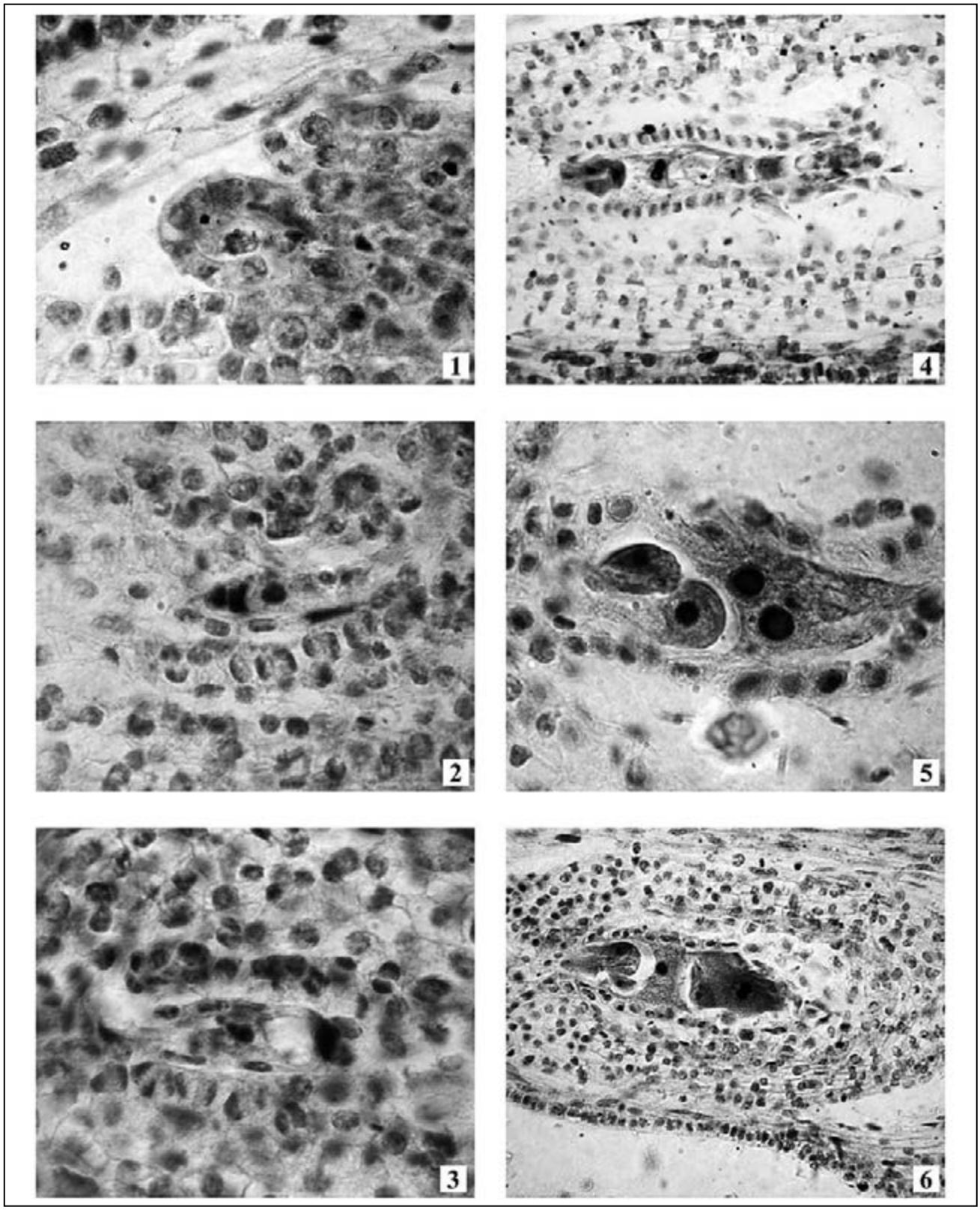
The above-mentioned features of the female gametophyte development are a precondition for its consequent degeneration. This was often observed in the ovules of individual florets. Most of these irregularities are not observed in amphimictic species but are characteristic for the apomictic taxa and seem to be more numerous in aposporous plants (Czapik 1994).

Plate I



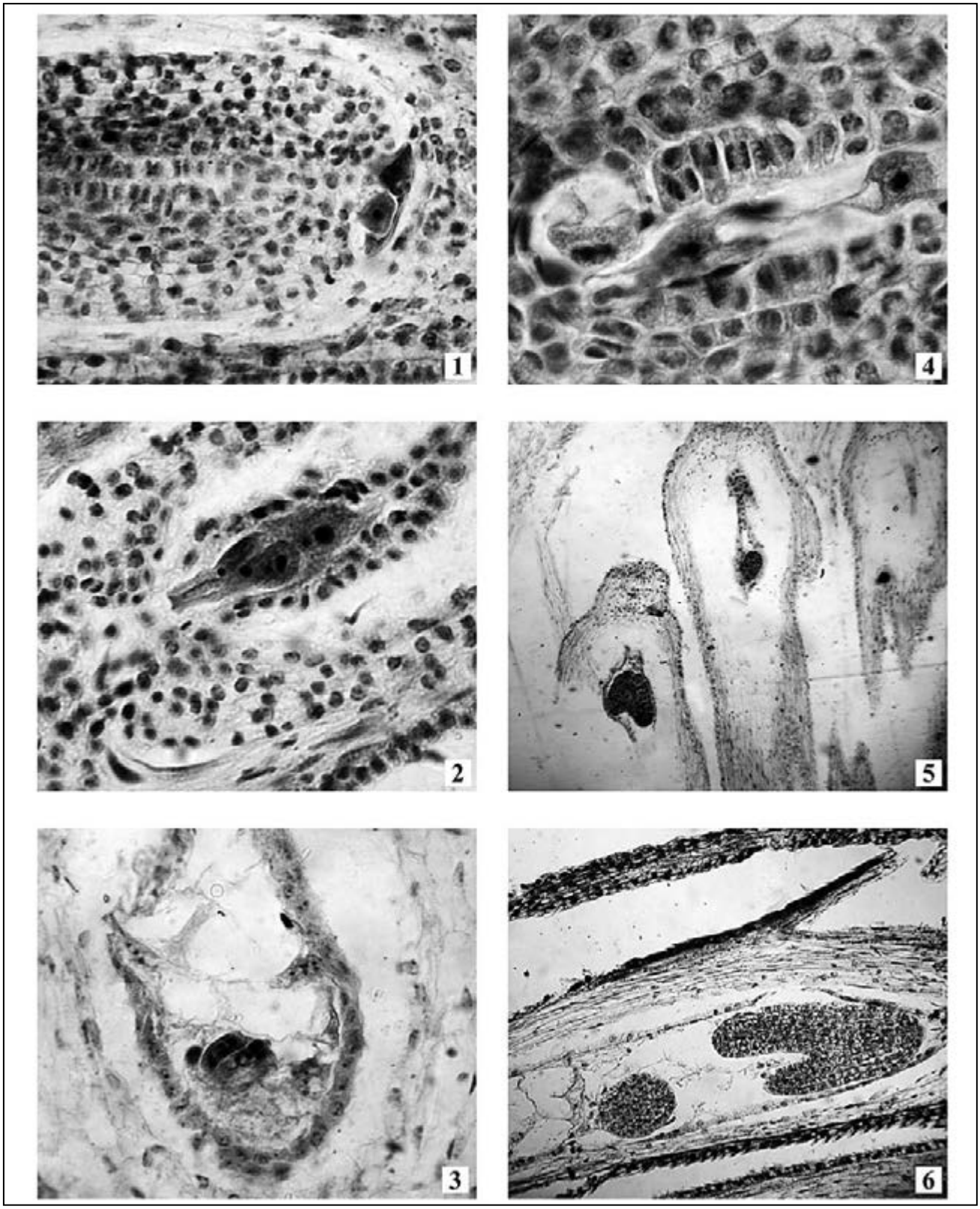
Figs 1–6. Anther and development of male gametophyte in *H. bauhinii*:
1, Four-layered anther wall with secretory tapetum and MMCs in prophase I (×400); **2,** Four-layered anther wall with secretory tapetum and MMCs in telophase I (×400); **3,** Different type of microspore tetrads (×400); **4,** Degenerating microspore tetrads (×160); **5,** Degenerating microspore tetrads (×400); **6,** An abnormal anther (×100).

Plate II



Figs 1–6. Ovule and development of female gametophyte in *H. bauhinii*:
1, Megaspore mother cell in prophase I (×400); **2,** One-nucleate ES after *Polygonum* type (×400); **3,** One-nucleate ES after *Allium* type (×400); **4,** Mature ES (×160); **5,** Two egg cells in an egg apparatus and two polar nuclei in ES (×400); **6,** Elements of two ESs in a row in the ovule (×100).

Plate III



Figs 1–6. Development of female gametophyte in *H. bauhinii*:
1, Extrusion of a legitimate ES through the micropyle (×160); **2,** Double fertilization in a legitimate ES (×160); **3,** Two-celled embryo (Asterad-type) and nuclear endosperm (×160); **4,** Co-existence of legitimate and aposporous ESs in an ovule (×400); **5,** Legitimate and aposporous embryos in neighbouring florets (×100); **6.** Legitimate and aposporous embryos in an ovule (×160).

The legitimate embryo and endosperm are formed after double fertilization (Plate III, Fig. 2). It was observed that the embryogenesis preceded endospermogenesis and the embryo developed after Asterad-type (Plate III, Fig. 3). Initially the endosperm was nuclear (Plate III, Fig. 3) and differentiated into cellular one after the globular stage of the embryo.

In fact, in the studied accession most ESs and seeds develop by apospory, a form of gametophytic apomixis. It is important to notice that this type of apomixis does not exclude sexual reproduction. Apospory in *H. bauhinii* begins after the initiation of the sexual reproduction. A somatic cell, termed aposporous initial cell, appears after the formation of the megaspore tetrad in the ovule. Positional differentiation of the aposporous initial (usually giving rise to an adventitious embryo) occurs more often adjacent to the cell undergoing sexual reproduction. The aposporous ES begins to develop from a somatic cell deeply disposed into the chalaze. It is not obligatory that the aposporous initials arise from the nucellus as in this species, since epidermal and integumentary origins of the initial cells have been also described (Koltunow & Grossniklaus 2003). The initial cell enlarges and undergoes mitosis resulting in the formation of an aposporous ES which was observed in different developmental stages. In *H. bauhinii* aposporous ESs can be formed independently in ovules where legitimate ESs do not develop. Also, sexual and apomictic ESs can co-exist in a single ovule, side by side in a competitive manner (Plate III, Fig. 4). Koltunow & al. (1998) showed that the structures formed during the apomixis were similar or varied significantly in form and position relative to the sexual counterparts in the ovule.

The structures formed during the sexual and aposporous ES development in *H. bauhinii* share common elements but numerous deviations also exist. The aposporous ES can be easily distingushed from the legitimate one by some specific features, such as: unusual number of nuclei; nuclei of various sizes with more than one nucleolus; lack of polarization of the nuclei in the young ES; increased number of polar nuclei (more than two). Usually, only one ES predominates in its development, leading often to early degeneration of the other. Turesson (1972) and Koltunow & al. (1998, 2000) described in aposporous species of *Hieracium* the degeneration of majority of sexual ESs before the stage of florets opening and anthesis.

In some ovules of *H. bauhinii* the unreduced aposporous ES typically develops faster than the sexual one and physically displaces it. An extrusion of the legitimate ES through the micropyle was observed and consequently, because of its degeneration, sexual reproduction ceased.

Apomixis frequently is associated with the expression of some mechanisms which limit self-fertilization (autogamy): self-incompatibility, dioecy, heterostyly. The autogamy was demonstrated in apomicts from *H.* subg. *Pilosella* – *H. aurantiacum* and *H. piloselloides* (Bicknell & al. 2003). Czapik (1996) reported the autonomous apomixis occurred in *Compositae* in dioecious *Antennaria* species as well as in pollen sterile *Hieracium* taxa and apomictic species of genus *Chondrilla*. Bicknell & Koltunow (2004) showed that *H.* subg. *Pilosella* was "a model system for aposporous plus autonomous gametophytic apomixis" which was proved in this study too.

The high amount of sterile pollen and the early presence of mature aposporous ESs in unopened capitula of *H. bauhinii* suggest autogamy is unlikely. Thus, the embryo and endosperm form without fertilization and the embryo develops by unreduced parthenogenesis.

Sometimes, both zygotic and parthenogenetic seeds can be formed not only in different capitula of an individual but also in neighbouring florets of the same capitulum (Plate III, Fig. 5). Moreover, co-existence of sexual and aposporic embryos (polyembryony) was found in a single ovule in the same or different developmental stages (Plate III, Fig. 6). Therefore, apomixis in the studied accession is facultative and agrees with the statement of Koltunow & al. (2000) that facultative apomicts "can produce seeds derived from both sexual and apomictic processes". The facultative apomixis is a mechanism in apomictic plants allowing them to also retain the capacity to reproduce sexually to a varying degree (residual sexuality). In addition, the studied species has a well developed means of vegetative reproduction by stolons which is very effective on a local scale.

Conclusions

The study showed that the hexaploid accession of *H. bauchinii* is amphi-apomictic, i.e. its reproductive system combines both strongly prevailing somatic apospory with sexual reproduction (residual sexuality).

Additionally, very effective vegetative reproduction by stolons is present. In most cases many florets or even whole capitula are functionally female. The apomictic embryo is a result of unreduced parthenogenesis and the endosperm develops autonomously. Despite the little quantity of fertile pollen double fertilization sometimes occurs and leads to formation of legitimate embryo and endosperm.

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Morphological variability in clonal cultures of *Tetrastrum heteracanthum*

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Abstract. The variability of taxonomical features such as length and number of spines and shape of the coenobium of *Tetrastrum heteracanthum* was studied. Clonal cultures, with species specific initial coenobium, were used. The variability in clonal cultures was traced at three different temperatures. The influence of nutritive solutions with different concentrations was investigated by intensive cultivating.

Key words: clone cultures, morphology, taxonomy, *Tetrastrum*, variability

Introduction

Tetrastrum heteracanthum (Nordst.) Chodat was described by Nordstedt as *Staurogenia heteracantha* and later was attached by Chodat to the genus *Tetrastrum*. Migula (1907) referred it to the genus *Crucigenia* as *C. heteracantha* (Nordst.) Kuntze, which was not adopted from the later authors. Major characteristic of this species is the presence on each cell of two asymmetrical, differently long spines. Many authors later described based on the special features such as length and thickness of the spines many infraspecific taxa: var. *dentatum* Playfair; f. *rectispina* R. Fisch.; var. *minor* Y.V. Roll; var. *homoiacanthum* Hub.-Pest.; var. *longispinum* Ahlstrom & Tiffany; f. *crassispinum* Hortob.; var. *crassispinum* Hortob.

Ahlstrom & Tiffany (1934) described on text and drawing given by Schiller (1924) the variety *longispinum*. Although they gave it such category and expressed an opinion that it may be only an ecological variety, regarding this variety the opinion of the later authors is different. Korshikov (1953) acknowledged its independence, while Komárek & Fott (1983) included it in the variability of the species.

Even more different are the opinions of the authors about the described from Huber-Pestalozzi (1929) var. *homoiacanthum*. While Komárek & Fott (1983) included it in the variability of the species, Hindák (1984) and Comas Gonzáles (1984), independently of each other, considered that the feature "two spines with the same length" is with sufficient authority and they gave it a species rang – *T. homoiacanthum* (Hub.-Pest.) Hindák comb. nova and *T. homoiacanthum* (Hub.-Pest.) Comas comb. nova.

Both taxa f. *crassispinum* and var. *crassispinum* described by Hortobágyi (1968, 1979) on the basis of the spine thickness are related to the synonymy of the species by Komárek & Fott (1983). *Tetrastrum asymmetricum*, which was reported by Hortobágyi (1967) and for which the presence of one asymmetrical spine on the cells is typical, also is enlisted by Komárek & Fott (1983) in the bounds of *T. heteracanthum*.

Having in mind the infraspecific taxa in *T. heteracanthum* proposed by different authors, as well as the different points of view about the taxonomical value of the special features of the spines (number, length and thickness), we set the task to trace their variability in laboratory cultures and in nature.

The purpose of the present study was to trace out the variability of the taxonomical features – length of the spines and their number on the cells. The experiments were conducted on clonal cultures under intensive cultivation in different nutritious media with concentration gradient. The influence of different temperatures was also evaluated.

Additionally to the special features of the species, the shape (the type) of the coenobium and its overlapping percentage in the clonal cultures, which gave additional information for the characteristics of the species, were studied.

Material and methods

The material for the study was received from the algal collection of the University Coimbra, Portugal. It was a strain of *T. heteracanthum* No. 1223 coming from Lagoa das Bracas. From it we isolated two clones with an initial coenobium corresponding to the both described types of coenobia, typical for the species. Clone No. 5083/6 was with initial coenobium type B – square coenobium, with four crosswise laying cells, with square open space in the centre of the coenobium and with two asymmetrical differently long spines on the cells. Clone No. 5083/8 was with initial coenobium type E – rhomboid coenobium, with four crosswise laying cells, with rhomboid open space in the centre of the coenobium and with two asymmetrical differently long spines on the cells (Fig. 1).

The clonal cultures were accomplished by using the method of the capillary pipette (Stein 1973) modified by Mladenov & Furnadžieva (1995). The initial clone was cultivated preliminarily in luministat with continuous light conditions of 7 klux, 24±1 °C temperature and continuous aeration. These clonal cultures (algae suspensions) were the initial material for intensive cultivation in nutritious solutions with different gradient of concentration.

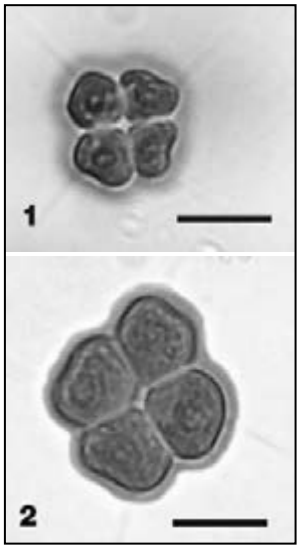

Fig. 1. Initial coenobium: **1,** for clone No. 5083/6; **2,** for clone No. 5083/8. Scale bars = 10 μm.

The intensive cultivation was conducted in a cultivating installation according to Dilov & al. (1972). It was completed in Ackerman test-glass in light conditions 15/9 light/dark and 12 klux. During this light period, the suspensions were blown with 100 l air/h/100 ml mixture enriched with 1 % CO_2. Three nutritious media were used: BBM (Archibald & Bold 1970), G_1 – Göttingen Basal Medium 1 (Schlösser 1982) and Z – Zehnder Medium (Staub 1961) – dissolved, natural and concentrated. We made the increase in concentration by adding 3 $g.l^{-1}$ NaCl to the nutritious medium whereas the decrease in concentration was done by 10× dissolving (without changing the concentration of the microelements).

The variability of the clonal cultures was studied in intensive cultivations under three different temperatures – minimum (16 °C), optimum (24 °C) and maximum (36 °C).

The microscopic investigation was accomplished by studying 500 coenobia for every variant of the experiment for reading of coenobium type; 50 coenobia were microscopically analysed for number and length of the spines.

Results

Length and number of spines on the cells

The length and the number of spines are used as taxonomical features to demarcate between the species and the infraspecific taxa in the genus. Therefore we were interested in knowing their variability not only in the nature, but also in the clonal cultures.

In our study of clonal cultures was established that in most cases the spines are unequal. The long spine is with size from 4 μm to 20 μm, the lowest value being found in G_1 and Z concentrated nutritious media and the highest in BBM normal (Table 1). The size of the short spine varied from 2 μm to 10 μm, the lowest value being found in G_1 and Z concentrated nutritious media, and the highest in the normal nutritious (Table 1). In G_1 dissolved nutritious we observed specimens with curved spines (Fig. 2). The sizes of the long and the short spines are bigger in clone No. 5083/8 (type E) compared to clone No. 5083/6 (type B).

Ahlstrom & Tiffany (1934) in their first monograph of genus *Tetrastrum*, based on data from different authors, noticed as characteristic features of *T. heteracanthum* the presence of (1)–2 spines on the cell, the length of the long spines being 8–24 μm, and that of the short spines 1–9 μm. Brunnthaler (1915) reported size of the short spine 8 μm, and for the long one – 14 μm.

Hortobágyi (1968), studying species from natural samples, established that the length of the long spine is 10.4–11.6 μm, and the short one is 4.5–5 μm. In natural samples from Asia, Ergashev (1979) reported for the long spines 8–24 μm, and for the short ones 1–9 μm.

Hindák (1988) in his investigations on *T. heteracanthum* noticed that the spines are two, but sometimes a

third, shorter spine is formed. The length of the long spine is (5)–15–25 μm, and for the short it is (2)–5–15 μm. For the natural samples from Ribeirão Preto (Brazil), Silva (1999) established that the length of the long spine is 6–7.5 μm, and the short is 1–2.5 μm.

The authors Komárek & Fott (1983) and John & al. (2002) rejected the taxonomical value of the length of the spines, included this feature in the variability of the typical variety and gave for the short spine 1–24 μm, and for the long spine 8–46 μm (Table 2).

In our study in intensive cultivation the length of the short spine varied from 2 to 10 μm, and of the long from 4 to 20 μm, which sizes are similar to those given by the other authors who investigated this species. An interesting fact is that in minimum and optimum temperature longer spines are developed, while in maximum temperature their length decreased (Table 3).

Table 1. Length of the spines on the cells of the coenobium in clones 5083/6 and 5083/8.

Medium	Length (μm)					
	Short spine			Long spine		
	min–max	x±σ	CV (%)	min–max	x±σ	CV (%)
Clone No. 5083/6						
1/10BBM	3.0–4.0	3.2±0.4	12.5	6.0–12.0	7.0±0.9	12.8
BBM	3.0–4.0	3.7±0.5	13.5	9.0–12.0	10.3±1.4	13.5
BBM+3 $g.l^{-1}$	3.0–4.0	3.5±0.4	11.4	6.0–12.0	7.0±0.9	12.8
$1/10G_1$	2.0–5.0	3.4±0.4	11.7	7.0–14.0	11.5±1.6	13.9
G_1	3.0–4.0	3.2±0.4	12.5	9.0–12.0	10.6±1.4	13.2
G_1+3 $g.l^{-1}$	2.0–5.0	3.2±0.4	12.5	4.0–6.0	6.2±0.8	12.9
1/10Z	4.0–10.0	5.4±0.7	12.9	6.0–18.0	10.0±1.4	14.0
Z	2.0–8.0	4.6±0.6	13.0	5.0–12.0	8.4±1.1	13.0
Z+3 $g.l^{-1}$	3.0–4.0	3.5±0.4	11.4	4.0–6.0	5.4±0.7	12.9
Clone No. 5083/8						
1/10BBM	2.0–7.0	4.8±0.6	12.5	10.0–20.0	16.8±2.3	13.6
BBM	2.0–9.0	4.5±0.6	13.3	10.0–20.0	15.4±2.1	13.6
BBM+3 $g.l^{-1}$	3.0–6.0	4.0±0.5	12.5	6.0–9.0	7.8±1.1	14.1
$1/10G_1$	2.0–4.0	3.0±0.4	13.3	6.0–10.0	8.3±1.1	13.2
G_1	3.0–6.0	4.9±0.6	12.2	6.0–12.0	9.4±1.3	13.8
G_1+3 $g.l^{-1}$	3.0–4.0	3.0±0.4	13.3	4.0–6.0	5.6±0.7	12.5
1/10Z	3.0–6.0	3.6±0.5	13.8	6.0–10.0	6.2±0.8	12.9
Z	3.0–6.0	3.3±0.4	12.1	6.0–11.0	6.7±0.9	13.4
Z+3 $g.l^{-1}$	2.0–4.0	3.2±0.4	12.5	4.0–6.0	4.8±0.6	12.5

Legend: min – minimum; max – maximum; x – average; σ – standard deviation; CV – coefficient of variation; BBM – Bold's Basal Medium; G_1 – Göttingen Basal Medium 1; Z – Zehnder Medium.

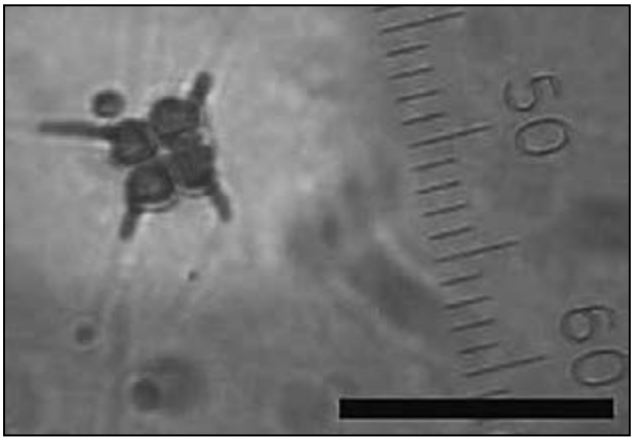

Fig. 2. Coenobium with curved spine under condition of G_1 dissolved nutritious medium. Scale bar = 10 μm.

Table 2. Size of the cell, short and long spine of *T. heteracanthum* according to different authors.

Authors	Size on the cell (μm)	Short spine (μm)	Long spine (μm)
Ahlstrom & Tiffany (1934)	2.0–11.5	1.0–9.0	8.0–24.0
Brunnthaler (1915)	4.0–8.0	8.0	14.0
Hortobágyi (1968)	3.7–5.6	4.5–5.0	10.4–11.6
Hindák (1988)	5.0–7.0	(2.0)–5.0–15.0	(5.0)–15.0–25.0
John & al. (2002)	2.0–11.5	1.0–24.0	8.0–46.0
Ergashev (1979)	3.5–12.0	1.0–9.0	8.0–24.0
Komárek & Fott (1983)	2.0–11.5	1.0–24.0	8.0–46.0
Silva (1999)	3.5–6.5	1.0–2.5	6.0–7.5

Table 3. Length of the short and long spines on the cells of the coenobium in clones 5083/6 and 5083/8 under different temperatures.

Clone	T° of growth	Length (μm)					
		Short spine			Long spine		
		min–max	x±σ	CV (%)	min–max	x±σ	CV (%)
No. 5083/6	T min	3.0–9.0	5.6 ±0.7	12.5	8.0–12.0	9.2±1.3	14.1
	T opt	3.0–9.0	3.7±0.5	13.5	4.0–20.0	10.3±1.4	13.5
	T max	2.0–6.0	3.4±0.4	11.7	6.0–15.0	10.0±1.4	14.0
No. 5083/8	T min	3.0–6.0	5.4±0.7	12.9	6.0–12.0	9.3±1.3	13.9
	T opt	2.0–9.0	4.5±0.6	13.3	10.0–20.0	15.4±2.1	13.6
	T max	2.0–6.0	3.3±0.4	12.1	5.0–12.0	7.7±1.0	12.9

Legend: T° – temperature; min – minimum; max – maximum; opt – optimum; x – average; σ – standard deviation; CV – coefficient of variation.

Coenobium types in the clone cultures

The types of coenobium, which the authors gave for this species, are two: square coenobium with square open space in the centre of the coenobium, and rhomboid coenobium with rhomboid open space in the centre of the coenobium.

In our study, even though in small percentage, we observed also the other types of genus *Tetrastrum* coenobia which are given in our earlier article (Velichkova & Kiryakov 2005). In our study of intensive cultiva-

tion process, which was applied to the three nutritious media with their variants for clone No. 5083/8, we observed that in the highest percentage was found the initial (rhomboid) type E, followed by the square coenobium type B (Fig. 3).

During the intensive cultivation process for the nutritious media with concentration gradient the results for clone No. 5083/6 showed that the initial (square) coenobium type B dominated, followed by the rhomboid coenobium type E (Fig. 4).

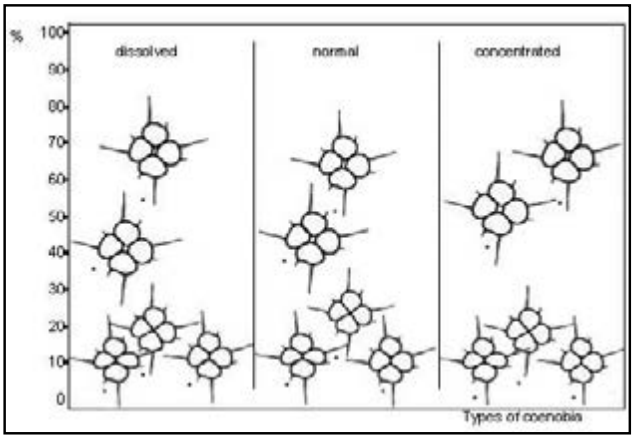

Fig. 3. Prevailing types of coenobia (in %) in intensive cultivation in nutritive medium BBM with different concentrations – clone 5083/8.

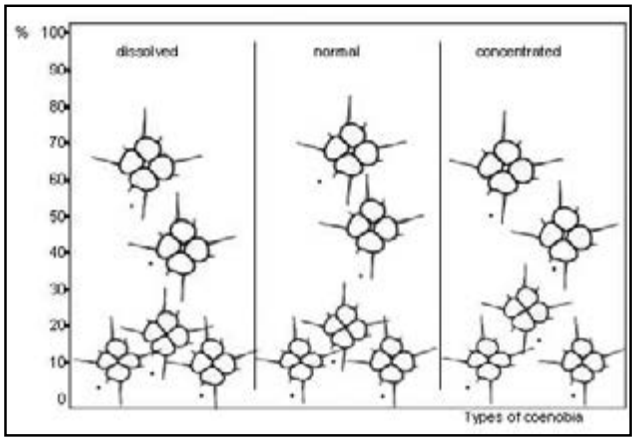

Fig. 4. Prevailing types of coenobia (in %) in intensive cultivation in nutritive medium BBM with different concentrations – clone 5083/6.

Discussion

On the natural samples from marsh Halleger, Hindák (1988) presented illustrations of *T. heteracanthum* (Abb. 15) in which the difference of the length of the long and the short spine was very obvious and led to correlation of 5:1, whereas in others it was only 5:4. This variability was also seen in our cultures.

Our results for the variability of the length of the spines in clonal cultures of *T. heteracanthum* during cultivation in different nutritious media and different temperatures showed about the length of the spines (short and long) no diversion to this established in the natural samples of the typical species. In both clonal cultures the maximum size for the long spine did not exceed 20 μm, and for the short – 10 μm. Ahlstrom & Tiffany (1934) and Korshikov (1953) recognized var. *longispinum* like various category of the species, in which the length of the long spine was over 46 μm, and of the short one over 22 μm. Expecting this we considered that var. *longispinum* must be independently saved.

About f. *crassispinum* and var. *crassispinum* described from Hortobágyi (1968, 1979), we will subscribe to Komárek & Fott's opinion (1983), who related to the variability of the species. In reference to var. *homoiacanthum*, which is characterized with two equal considerably longer than the coenobium spines, as we already noted, the opinions of the authors are rather different. From complete disregard of his taxonomical value Komárek & Fott (1983) raised it to a species rank.

In our cultures we also observed specimens with two considerably long, but unequal spines which did not exceed 10 μm and thus they are included in the limits of the typical species. Hindák (1984) raised the mentioned variety to the species rank and in the illustrations of his own material presented a specimen with two unequal in length spines, which may be due to different angle at which they are located, for that reason he gave a size over to 18 μm. In all our investigated cultures under different conditions no specimen was established with spines having this length. That is why we think that for this type of coenobium a taxonomical independence must be reserved, but with rank of variety given as described by the authors – *T. heteracanthum* (Nordst.) Chodat var. *homoiacanthum* Hub.-Pest.

Acknowledgements. The author thanks Prof. Ivan Kiryakov for helping in the preparation of the manuscript and for his critical comments. The author thanks for strain of *T. heteracanthum* No. 1223 from the collection of the University Coimbra, Portugal.

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In vitro selection of stabilized intergeneric wheat lines tolerant to abiotic stress

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Abstract. The purpose of this study was to investigate the response of intergeneric wheat–*Thinopyrum intermedium* hybrid lines to *in vitro* cultivation in control and drought stress conditions. Explants from immature embryos of different wheat cultivars and intergeneric hybrids were used in the experiment. *In vitro* selection at the level of calli from 4 genotypes using different PEG concentrations was performed. The most suitable genotypes and PEG concentrations specific for each of the examined genotypes were established. The *in vitro* technological approach presented allows selecting valuable wheat forms that may be efficiently used in various breeding programs.

Key words: drought tolerance, immature embryos, Polyethylene glycol, tissue culture

Introduction

Over the last years particular attention has been paid to wheat genetic improvement in order to increase grain yield and to breed new wheat forms resistant to biotic and abiotic stress. Wild plant species are a rich source of genetic material of valuable traits including resistance to abiotic stress which may be introduced into crop plants (McGuire & Dvorak 1981; Shannon & Noble 1990). The methods of plant tissue cultures allow both to examine directly the *in vitro* response of the plants and to identify the most tolerant genotypes. Besides, these methods make possible the performance of the selection at cell level, when the changes of the traits studied are more efficiently expressed at that level than at the level of regenerated plants. In cereals, wild species from genus *Thinopyrum* are a rich source of genes for resistance to abiotic stress. Therefore, the purpose of our study was to investigate the response of intergeneric wheat–*Thinopyrum intermedium* hybrid lines to *in vitro* cultivation in control and drought stress conditions. Thus, the selection of lines responsive to *in vitro* cultivation in stress simulating conditions would allow revealing genotypes tolerant to drought stress.

Material and methods

Explants from immature embryos of different wheat cultivars and intergeneric hybrids were used in the experiment. The hybrids were stabilized wheat lines obtained through intergeneric hybridization of three *Triticum aestivum* cultivars (Bezostaya, Sadovo and Rusalka) with the wild species *Thinopyrum intermedium* ($2n = 42$) (Tyankova 2000). The genome of *Th. intermedium,* as it is known, possesses genes for resistance to abiotic stress (McGuire & Dvorak 1981). Three of these lines and their parental cultivars were examined for their response to *in vitro* cultivation in control and in drought simulated conditions. For this purpose the immature embryos were cultivated on MS medium to which 0.5 mg/l kinetin, 3.5 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D), 150 mg/l glutamine and 1000 mg/l casein hydrolizate were added.

The isolated, 12–15 days old, semi-transparent immature embryos, 1.0–1.5 mm long were sterilized and planted on solid nutrient medium with the scutellum upward. The callus cultures were induced and maintained in the dark in thermostat TB–150. For regeneration the calli were illuminated with 2500 lx for 16 h per day. All stages of the *in vitro* cultivation proceeded at 25 °C. Each embryo was grown on a tube containing 10 ml nutrient medium. For regeneration 4–5 calli were grown in Erlenmeyer flask containing modified nutrient media on Murashige & Skoog (1962). To simulate water deficiency Polyethylene glycol (PEG) 6000 (Sigma) was added (5 % or 10 %). After 20–30 days cultivation the regenerants were transplanted in pots containing soil and perlite.

Variation analysis and Student's t-test were used for statistic processing of the data to prove the difference in the mean values of the parameters examined.

Results and discussion

As seen from Table 1, the callus induction observed in the wheat cultivars and lines varied between 81.90 % and 97.40 %. There was no significant variation among the genotypes tested. This indicated lack of genotype differences at that stage of embryo development and is in agreement with Stolarz & Lörz (1986) and Immonen (1992). The immature embryos cultivated on nutrient medium for callus initiation proliferated intensively and within a week accumulated non-organized homogenic cell mass. Some of the calli were white, of high water content and smooth surface. They had no morphogenic potential and were defined as calli of non-regenerative type.

The calli of the embryogenic type were of granular structure and yellowish in colour. Such calli normally express high regeneration ability. The data presented in Table 1 indicate that the genotypes studied differ in their morphogenic potential. Statistically proved higher frequency of the morphogenic calli compared to the control (cv. Sadovo) was observed in the three hybrid lines.

The yellowish calli of granular structure were planted to regenerate on a nutrient medium of properly balanced plant growth regulators (BAP, IAA and kinetin) (Zagorska & al. 1991). In our study the regenerant development proceeded along two parallel pathways: somatic embryogenesis and organogenesis. On regeneration medium the calli gave rise to numerous shoots and thin roots. Formation of leaves, leaf buds and shoots was also observed. The data in Table 1 suggest that the genotypes tested differ significantly in regeneration frequency and in propagation coefficients (number of regenerants that have emerged from one callus). Regeneration frequency, i.e. the percentage of embryogenic calli producing regenerants varied for the different genotypes. The wheat cultivar Rusalka and the three hybrid lines were of higher regeneration frequency compared to that of the standard for Bulgaria wheat cultivar Sadovo. The propagation coefficient of hybrid line 31 was higher than that of cv. Sadovo, while the propagation coefficients of cultivars Bezostaya and Rusalka were similar to that of cv. Sadovo. No correlation was observed between the degree of callus induction and the regeneration potential among the genotypes suggesting that both phases of the *in vitro* response are under independent genetic control, which is in agreement with other investigators (Henry & al. 1994a, b).

The standard wheat cultivar Sadovo and the three hybrid lines were tested for their response to simulated drought. At rooting, the regenerants were grown for tree weeks on the following nutrient media: VK1 – control, VK1 + 5 % PEG, VK1 + 10 % PEG. The frequency of well rooted, poorly rooted and dead plants was scored for each nutrient medium and genotype. Biometric data for regenerants' stem height and root length were also collected. The data presented show that the highest percentage of well rooted regenerants of all genotypes results from the control nutrient media (Table 2). Best rooting was observed in the wheat–wheatgrass hybrid line 31 followed by hybrid line 36. As it is also seen from Table 2, PEG in the nutrient medium suppressed rooting. In some cases the inhibitory effect of 5 % PEG was stronger than that of 10 % PEG. It may be due to the fact that 10 % PEG induces liquefaction of the nutrient me-

Table 1. Callus induction and regeneration of immature embryos of wheat (*T. aestivum*, cvs. Bezostaya, Sadovo and Rusalka) and its intergeneric hybrids.

Genotype	Embryos tested	Induced calli		Morphogenic calli	Regeneration frequency	Propagation coefficient
	(No.)	(No.)	(%)	(%)	(%)	
Sadovo	500	455	91.0	53.61±2.9	51.1±3.8	1.97±0.93
Bezostaya	500	440	88.8	56.38±3.8	47.3±1.7	1.63±0.60
Rusalka	500	470	94.0	48.12±3.1	59.3±1.3^{a}	1.45±0.28
WWGH 31	244	175	81.9	70.50±3.2^{b}	68.6±5.2^{b}	2.68±0.38^{a}
WWGH 36	500	487	97.4	69.81±1.4^{b}	59.4±2.4^{a}	1.62±0.79
WWGH 68	300	275	91.7	72.80±3.1^{b}	64.7±1.6^{a}	1.36±0.64

a, b – Significant at P=0.05, P=0.01, respectively.
Abbreviations: WWGH – wheat–wheatgrass hybrid line.

Table 2. Rooting frequency and certain biometric parameters in regenerants grown on control and selective media containing 5 % or 10 % PEG.

Genotype	Medium	Regenerants tested (No.)	Well rooted (%)	Poor rooted (%)	Mean No. of roots	Mean root length (cm)	Mean stem length (cm)
Sadovo	Control	29	14.4±2.9	76.5	6.0	1.6±0.8	16.4±4.6
	5 % PEG	25	3.2±1.1^{c}	80.1	10.4	0.8±0.3^{a}	14.6±1.7
	10 % PEG	19	6.2±1.8^{b}	71.2	14.4^{b}	1.0±0.6^{a}	12.5±1.6^{a}
WWGH 31	Control	47	64.5±7.8	21.7	3.9	3.2±0.8	17.1±3.1
	5 % PEG	37	23.6±4.2^{c}	44.7^{a}	21.8^{b}	2.0±0.8^{a}	13.4±3.0^{a}
	10 % PEG	31	43.9±3.9^{b}	28.9	19.3^{b}	4.5±2.6^{a}	14.1±4.8
WWGH 36	Control	48	26.8±2.4	54.6	11.4	2.2±2.4	15.4±4.8
	5 % PEG	44	16.8±1.6^{a}	58.2	14.1	1.1±1.7^{a}	14.4±3.9
	10 % PEG	54	18.7±3.2^{a}	51.5	9.8	1.5±3.2^{a}	15.0±4.5
WWGH 68	Control	26	16.7±3.6	67.3	7.2	1.7±0.7	19.8±2.7
	5 % PEG	31	8.2±2.8^{b}	71.4	6.1	1.0±0.9^{a}	14.3±3.7^{a}
	10 % PEG	22	11.2±1.7^{a}	63.6	11.3	1.8±1.3	16.6±2.6

a, b, c – Significant at P= 0.05, P= 0.01, P= 0.01, respectively.
Abbreviations: WWGH – wheat–wheatgrass hybrid line.

dium leading to reduced resistance against root growth and enhancing the access to available nutrients.

Almost all differences in root length among individual genotypes and variants are statistically significant, in contrast to the stem height. It may be due to the fact that at the time of rooting the stems of the regenerants are almost fully developed while root growth is still at an early stage of development. Besides, the degree of root development is strongly dependent on nutrient medium composition.

The fully developed regenerants were transferred from the tubes into pots containing soil and perlite (1:1) and kept further in a phytostatic chamber with continuous illumination and constant temperature (~25°C) to spend the winter and to adapt to non-sterile greenhouse regimen. The latter is important because the regenerants' adaptability potential had been suppressed under conditions of simulated drought and a considerable part of them failed to adapt to the altered conditions. The hybrid lines 31 proved to be of highest adaptability.

The data obtained show that the genotypes studied are comparatively tolerant to drought and may be used in different breeding programmes to increase wheat drought tolerance.

Acknowlegments. The Bulgarian National Science Fund under contract B–1202/02 supported this research.

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Growth and biochemical response of tobacco callus to abiotic stress condition

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Abstract. Growth, viability, izoenzyme intensity and proline content of unadapted and adapted (0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5 % NaCl) callus of *Nicotiana tabacum* cv. Krumovgrad 90 were examined in this study. Growth reduction of callus in higher NaCl concentrations (1.5 %) correlated with a decreased relative staining intensity of MDH isoenzymes. Increasing amounts of NaCl up to 1.5 % provoked progressive proline accumulation, while higher NaCl content (2.0 %) decreased it and callus growth was relatively low. The present results confirm the important role of MDH and proline in stress adaptive response as protective factors of the enzyme systems.

Key words: callus growth, *in vitro* culture, isoenzymes, MDH, *Nicotiana tabacum*, proline, salt stress

Introduction

Morphological and biochemical study of stress answer is a base for development of plants resistant to abiotic stress factors and an alternative approach for overcoming the damaging effects of environment pollution. Another important practical result is the possibility to obtain new biological indicators for the pollution and bioremediation of soils, plant biomass, etc. Application of *in vitro* model system for abiotic stress study considerably reduces the time for obtaining resistant forms. It is already established that positive correlation exists between the resistance of cell and callus culture, and the whole plants respectively (Yang & al. 1990; Kirti & al. 1991; Ochatt & al. 1998; Lombardi & al. 2004; Parida & Das 2005).

The present paper reports our study of the salinity influence on the biomass accumulation and biochemical parameters of *Nicotiana tabacum* calli.

Material and methods

Nicotiana tabacum (cv. Krumovgrad 90) was used as initial material. Stem segments 1–1.5 cm high were cultivated on Murashige & Skoog (1962) agar medium supplemented with 2 mg/l σ-naphthalene acetic acid and 0.5 mg/l kinetin for callus induction. After 20 days a part of the well developed calli were removed to fresh medium used as control and the rest part of them were placed on medium with 0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5 % NaCl. Callus weight was measured before and after 20 days treatment.

The relative callus growth rate was calculated by the following formula:

σ = A2-A1/A1, where A1 is the initial weight of the callus and A2 – callus weight after 20 days treatment.

The protein extract for isoenzyme analysis was prepared in the following way: callus, previously frozen at –40 °C, was grown in a glass mortar with 0.05 M Tris–HCl buffer, pH 7.2, containing protective supplements (Ryechter & Levak 1969). The ratio callus weight (g): volume buffer (ml) was 1/3. Vertical block polyacrylamide gel electrophoresis with gel size 1/90/7 mm was applied (Davis 1964). MDH isoenzymes were visualized following the recipe of Shaw & Prasad (1970). The enzyme activity was defined in Units/mg protein (U/mg protein) respectively (1 Unit = 1 mol.min^{-1}). The enzyme investigations were carried out with the use of UV–visible spectrophotometer SHIMADZU. The proline concentration was determined after the method of Bate (1973) described by Pahlich & al. (1983) on the ninhydrin reaction base.

Results and discussion

The relative growth rate of *N. tabacum* (cv. Krumovgrad 90) callus treated with increased NaCl concentrations and the control are presented in Figs 1 and 2. Structural differences were established according to NaCl concentrations: white, small-grained, compact callus at 0.5 % and 1.0 %; white, coarse-grained, soft, friable one at 1.5 % and brown, coarse-grained, soft callus at 2.0 % NaCl.

In spite of the variation of this index values for the separate repetition of callus growth some tendencies were pointed out:

- Intense callus growth exceeding the control was observed at 0.5 % NaCl treatment. This phenomenon could be explained with the positive effect of low NaCl concentrations, also determined in other plant species by Sabbah & Tal (1990);
- Callus increase was delayed at higher (1.0 %, 1.5 %, 2.0 %) NaCl quantities;
- Any growth was put on end at 2.5 % NaCl.

Malatedehydrogenase isozyme (MDH) spectra both in the control and in treated with NaCl (0.5–2.0 %) calli are given in Fig. 3. High staining intensity of MDH spectra in the control and in calli at 0.5–1.0 % NaCl was in positive correlation with the relative higher values of callus growth. The reduction in development of adapted with 1.5 % NaCl callus corresponded to decreased relative staining intensity of MDH isoenzymes. The results obtained for medium containing 2.0 % NaCl were of considerable interest. In three repetitions increased intensity of MDH izoenzyme staining compared with the control was observed. It can be connected with the dependence established by Weimberg (1967) of the activity of pea supernatant MDH in higher NaCl concentrations. Data obtained showed that MDH is an important indicator for energetic metabolism.

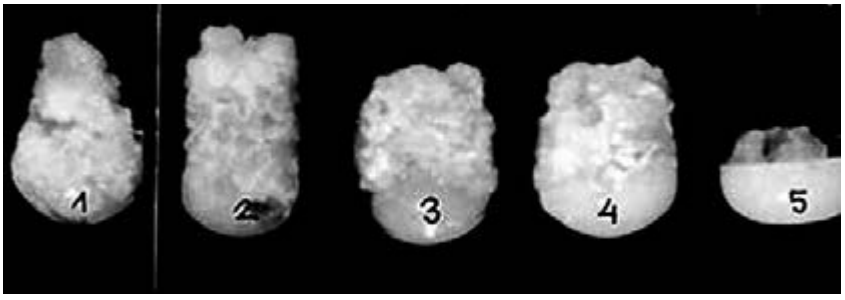

Fig. 1. Calli treated with NaCl: **1,** Control 0.0 %; **2,** 0.5 %; **3,** 1.0 %; **4,** 1.5 %; **5,** 2.0 %.

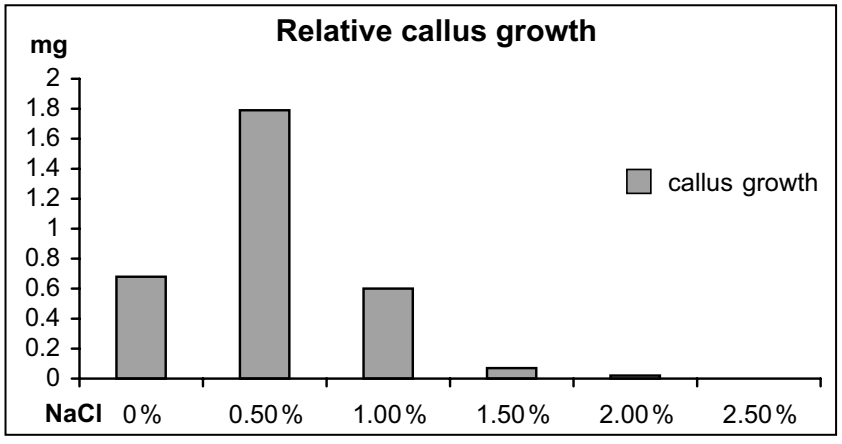

Fig. 2. Salinity effect of relative callus growth.

The comparative assessment of the above-mentioned callus behaviour with the quantitative changes in proline, another biochemical indicator studied, showed increased activity of this enzyme in the material *in vitro* adapted to salinity. Proline concentration in cv. Krumovgrad 90 callus, treated with NaCl, is presented in Fig. 4.

Progressive proline accumulation with the increase of NaCl (0.5 %, 1.5 %) in the nutrient medium was observed. In 1.5 % NaCl this amino

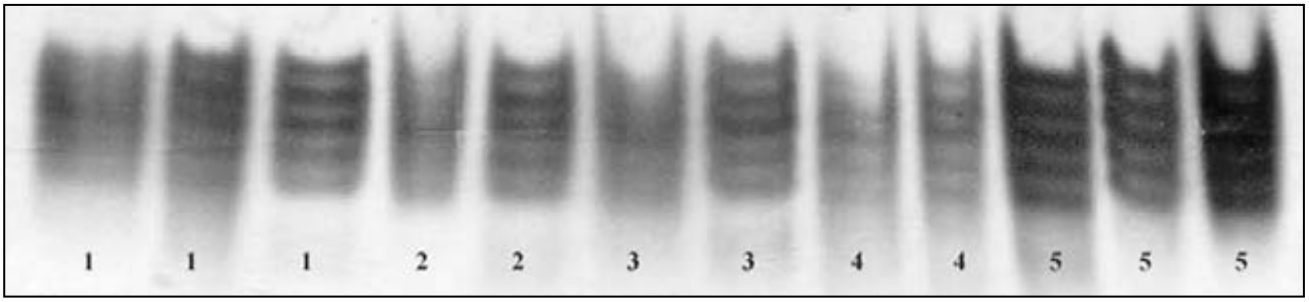

Fig. 3. Electrophoretic spectra of the MDH isozymes: **1,** Control; **2–5,** Calli treated with NaCl – 0.5 % (**2**); 1.0 % (**3**); 1.5 % (**4**); 2.0 % (**5**).

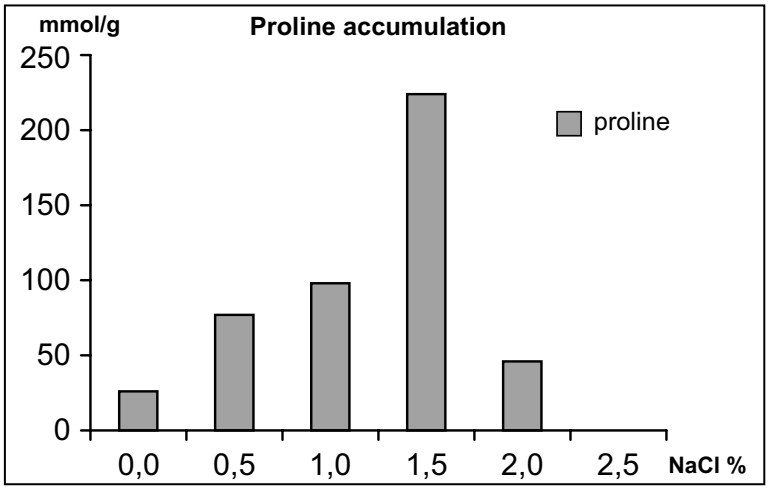

Fig. 4. Prolin accumulation in NaCl treated callus.

acid reached its peak and decreased content was established in higher (2.0 %) NaCl concentrations, accompanied by retardant callus growth. The results obtained indicated biochemical parameter of tobacco stress answer and confirmed the role of proline as a hopeful indicator for the possibility of plants to adapt to anthropogenic pollutans and stress factors, also established by other authors (Potluri & Devi Prasad 1994; Gangopadhyay & al. 1997; Roosens & al. 1999; Noaman & Ahmad 2004; Rai & al. 2006).

Our preliminary study is a part of *in vitro* screening of Bulgarian tobacco cultivar adaptation to salinity in order to create forms resistant to this factor. Our results confirm the importance of cell and tissue culture methods as quick and effective means for investigation of the adaptive stress response and resistance of tobacco towards anthropogenic influences. The biochemical parameters of the callus can be applied as indicators for pollution and stress influence of the medium.

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Somaclonal variation in *Rubus caesius* callus cultures

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Abstract. One of the advantages of callus cultures is the occurrence of genetic variations, the main source for new genotypes selection. The apical buds of *Rubus caesius* have shown an intensive dedifferentiation reaction and rapid callus development. A type of genetic changes has been detected in callus cultures, namely aneuploidy. There were selected not only somaclones with normal chromosome numbers, but also ones with different chromosome numbers.

Key words: aneuploidy, callus, micropropagation, *Rubus caesius*

Introduction

The indirect micropropagation with callus formation represents an alternative to select and perpetuate new cell lines, with different ploidy levels (Busby & Himelrick 1999). The purpose of the investigation was to initiate callus cultures at *Rubus caesius* L. and to study the relationship between totipotency expression of cultured cells and the changes in the level of ploidy. The modern approaches of plant propagation based on cell and tissue culture techniques are able to increase the efficiency of breeding processes. They offer opportunities for rapid clonal propagation of some unique genotypes. The unconventional techniques permit the multiplication and maintenance of these genotypes.

Material and methods

The axillary buds were sterilized with 70 % ethanol, after that with 0.5 % sodium hypochlorite for 10–15 minutes. After rinsing with sterile distilled water, the explants were transferred to MS medium containing 2 mg/l BAP and 0.2 mg/l IAA and maintained at 23 °C under 16 hours light cycle (Fiola & Schwartz 1986; Fiola & al. 1990).

The MS medium supplemented with 2 mg/l BAP and 0.2 mg/l IAA stimulated callus induction on young explants. The primary callus cultures of *R. caesius* were subcultured at an interval of 4 weeks and maintained several months in dedifferentiation state. The regenerative capacity of callus cultures was also tested (Table 1).

The heterogeneous proliferous structures were cytogenetically studied. For cytogenetical analyses, the callus was subjected to the following treatments: 2 hours in 0.2 % colchicine solution, then 2 hours in distilled water; fixed for 24 hours in Carnoy's solution; hydrolysed for 8 min in 50 % HCl at room temperature; stained in Carr solution. The slides were analysed at Nikon E600 Eclipse microscope, being photographied with Nikon CoolPix900.

Table 1. ***In vitro*** **reactions of** ***R. caesius*** **cultivated on different variants of MS medium.**

Variants of MS medium	Growth regulators		Reactions
	BAP	IAA	
I	0.2 mg/l	0.2 mg/l	–
II	2 mg/l	0.2 mg/l	Caulogenesis Callus
III	0.2 mg/l	2 mg/l	–
IV	2 mg/l	2 mg/l	Caulogenesis

Results

The induction of cell division in differentiated tissues resulting in callus cultures increases the chance of mutations (D'Amato 1978).

The inductive variants of basal medium MS have determined an intensive reaction of dedifferentiation in primary explants of *R. caesius*, finalized with the development of some heterogeneous proliferous structures (Plate I, Figs 1, 2).

The apical buds of *R. caesius* have shown an intensive reaction of dedifferentiation and rapid callus development. The primary callus cultures were white to pale yellow or green in colour, often with a nodular surface (Plate I, Fig. 3).

Callus formation may be accompanied by cell variation. Serial subcultures of callus pieces could show cytological differences, which lead to the establishment of different cell lines (Bayliss 1980).

In the case of *R. caesius*, the cell variability has been detected not only in primary callus cultures, but also during successive subcultures.

Discussion

In the primary callus cultures of *R. caesius* it was observed a variability in the chromosome number, in comparison with normal one *in vivo*, and $2n = 20$, 24 and 27 were established, instead of $2n = 28$ (Thompson 1997). Thus, the most frequent type of genetic changes detected in callus cultures was aneuploidy.

The decreases of chromosome number in some cells appear to be a consequence of imperfections of chromosomal replication during division of cells. After the second and third subculture a diminution of the chromosome number per cell was also observed (Plate II, Fig. 3).

The chance of mutations arising depends not only on number of subcultures, but also on type of tissues used for callus initiation and the kind of using regulators, such as synthetic cytokinins (variant II of MS medium).

In *R. caesius* there is a marked correlation between the production of mutations and the regenerative potential of callus cultures. Differences in the reaction of callus cultured on the same type of medium (MS, variant II) have been noticed, depending on the type of cell lines. The normal cell lines ($2n = 28$) retain a high regenerative potential (Plate II, Fig. 2), while the aneuploid cell lines are not regenerative (Plate I, Fig. 3; Plate II, Fig. 1).

Plate I

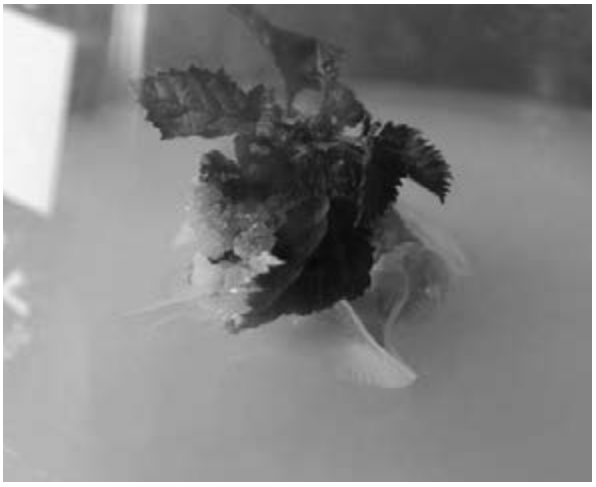

Fig. 1. Apical bud of *R. caesius*.

Fig. 2. Callus induction at *R. caesius*.

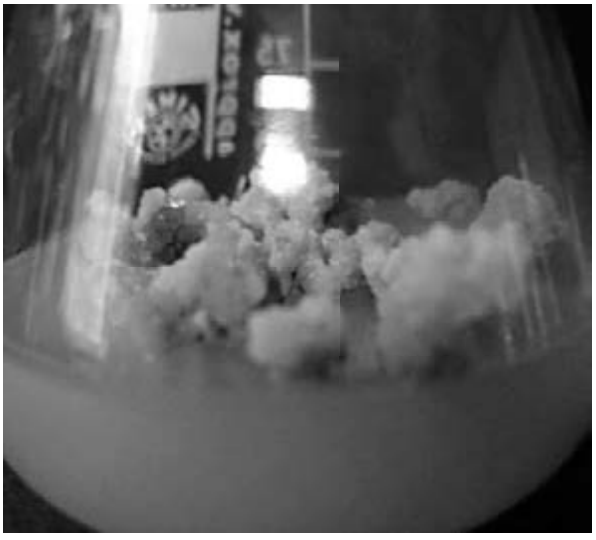

Fig. 3. Primary callus cultures.

Plate II

Fig. 1. Secondary callus cultures.

Fig. 2. Heterogeneous proliferous structures.

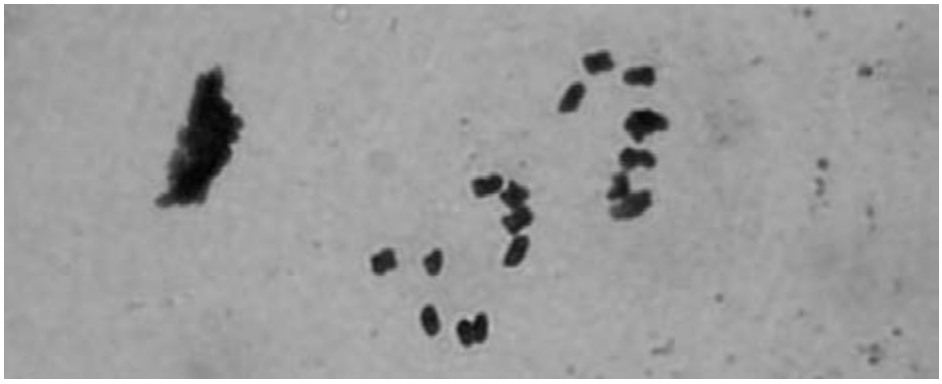

Fig. 3. Metaphase plate with $2n = 20$ in *R. caesius* callus.

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SCIENTIFIC AREA B

PLANT DIVERSITY – PAST AND PRESENT

Late Holocene environmental reconstruction based on pollen and diatom sediment record from the Central Sredna Gora Mountain, Bulgaria

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Abstract. The pollen and diatom record from Late Holocene sediments of fen in the Mt Central Sredna Gora (Bulgaria) was studied. It consists of 74 pollen taxa and 165 diatom species, varieties and forms; represents three biostratigraphic zones. The fen emerged during the Subatlantic period, which coincided with the Iron Age in Roman times, has been related ever since to an increased anthropogenic impact on vegetation. After 830±120 BP both vegetation and diatom succession showed last cooling phase in fen development. The vegetation succession and fluctuating development of autochthonous diatom communities were controlled by the local environmental conditions, other climatic changes and also strongly affected by the active anthropogenic activity.

Key words: Bulgaria, diatoms, Late Holocene, paleoecology, pollen

Introduction

The Holocene history of mountain ecosystems in Mt Sredna Gora has been recorded in the sediments of different type of wetlands. The intermediate position of Mt Sredna Gora as a geographical and floristic link between the Rilo–Rhodopean mountain massif and the Balkan Range makes it an important place in the Holocene history of vegetation studies. The region is strongly affected by the economic activity of man and is still poorly studied paleoecologically.

For Mt Central Sredna Gora, four forest succession phases have been established in the 10 025±225 BP period, and light has been thrown on the development of vegetation and main climatic changes until the formation of contemporary beech forests (Filipovitch 1992; Filipovitch & al. 1998). Based on diatom fossil record from sediments of the various wetlands in the area reconstruction of the paleoecological conditions during the last 10 000 years was made (Stancheva & Temniskova 2004).

The presented here study is focused on the Late Holocene paleocological changes in Mt Central Sredna Gora based on pollen and diatom record from the Barierata stratigraphic profile. The taxonomical composition of the diatom flora from the sediments of Barierata core already has been published (Stancheva 2001a, b). The rare diatom species, *Decussata hexagona* (Torka) Lange-Bert., known from a few localities in Europe, was reported from studied sediments (Stancheva & Temniskova 2006).

The goals of this paper are the following: first, to provide detailed biostratigraphical data on the Late Holocene pollen and diatom record from the sediments of Barierata core; second, to describe the local paleoecological situation in the forestless areas of Mt Sredna Gora based on the vegetation changes and succession of diatom communities, as well as to offer in retrospect a realistic evaluation of the anthropogenic changes.

Material and methods

Mt Sredna Gora lies to the south of the Balkan Range and Rearbalkan valleys, with a predominantly West-East orientation. The vegetation of the area falls into the European Deciduous Forests Zone, the Balkan (Illyrian) Province, Sredna Gora region. Beech and

oak forests dominate the contemporary vegetation (Bondev 2002).

The investigated fen Barierata is located at an altitude of 1000 m in the Mt Central Sredna Gora (Fig. 1). The Barierata fen has approximately 30 000 m^2 area without open water surface. It is crossed by stream surrounded by a few individuals of *Alnus glutinosa* (L.) Gaertn. The following species prevail in the vegetation cover of the Barierata wetland: *Plantago lanceolata* L., *Rumex acetosa* L., *Ranunculus acer* L., *Juncus compressus* Jacq., *J. effusus* L., *Holcus lanatus* L., *Myosotis sparsiflora* J.G. Mikan ex Pohl, *Poa bulbosa* L. var. *vivipara* Koch, *P. palustris* L., as well as the mosses *Calliergonella cuspidata* (Hedw.) Loeske, *Brachythecium rivulare* Bruch & Schimp. and *Ceratodon purpureus* (Hedw.) Brid.

The material studied was obtained from a sediment core taken with a Dachnowsky-type corer, with camera length 20 cm and diameter of 2 cm. The core length from fen Barierata was 70 cm with lithological composition as follows: 0–20 cm – peat with phytoremains; 20–50 cm – brown peat with sand; 50–70 cm – clay with a lot of sand and gravels. Layer with wood charcoal particles was found at 52 cm. The small size of charcoal particles (2 mm) did not allow the identification of the wood.

Radiocarbon dating of one sediment sample was carried out at the Leibniz Institute for Applied Geosciences GGA, Hannover, Germany.

The sediment samples for pollen analysis were taken at 5–10 cm intervals and were treated by standard methods including hydrochloric and hydrofluoric acids and acetolysis (Faegri & Iversen 1989). Identification of pollen and spores was performed using the reference collection of the Laboratory of Palynology, Institute of Botany, BAS, Sofia, the keys in Faegri & Iversen (1989), Moore & al. (1991), Chester & Raine (2001) and Beug (2004). The pollen percentage of arboreal (AP) and non-arboreal (NAP) pollen types is presented as a part of the total pollen sum (ΣP), including ΣP=AP+NAP–L=100 %. Spores of *Pteridophyta* and pollen grains of *Cyperaceae* and aquatic plants are excluded from the total pollen sum. The results of the palynological studies are presented in percentage pollen diagram (Fig. 2), on which the reconstruction of vegetation was based. Statistical processing of the data and plotting of the pollen diagram were made with the help of TILIA and TILIA·GRAPH software (Grimm 1991).

Samples for diatom analysis were taken at 1–3 cm intervals. Laboratory processing followed the technique

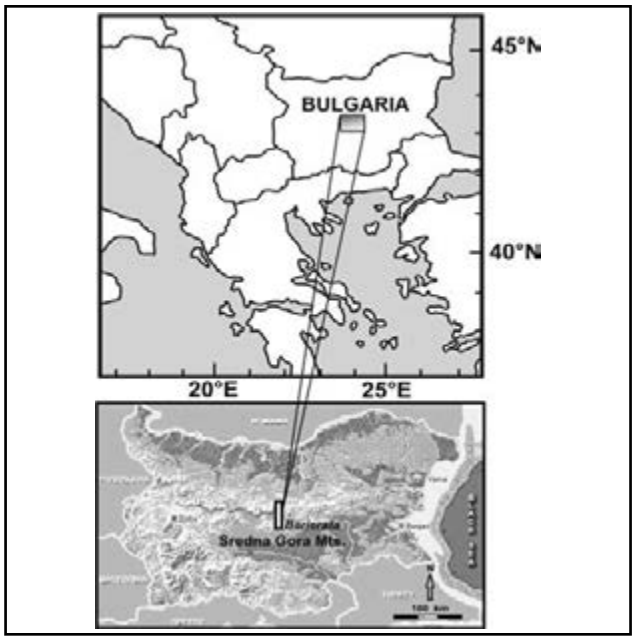

Fig. 1. Location map of the studied fen Barierata in Mt Central Sredna Gora, Bulgaria.

of Hasle & Fryxell (1970). 500 valves were counted per slide. The data for all diatoms that appear over 2 % are displayed in diagram. For the diatom taxa with known autecology, ecological spectra were determined based on literary sources with comparable data by the various authors. The percentage ratio of Diatom frustules/Chrysophycean stomatocysts was determined after Smol (1985). pH reconstruction is based on Index B (Renberg & Hellberg 1982). Ecological analysis and pH reconstruction were applied to samples with 500 counted diatom valves and were described in detail by Stancheva & Temniskova (2004). Diatoms were identified using Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Krammer (2000), and Lange-Bertalot (2001).

Results

Pollen analysis

The pollen analysis has shown 74 pollen taxa altogether: 24 from AP (trees and shrubs) group, 42 from the herbaceous group (NAP) and 8 local pollen taxa (L) comprising aquatic, marsh and spore plants. All identified taxa were elements of the contemporary vegetation. Apparently, the floristic composition has not changed since the Early Holocene. Quantitative correlation between the different taxa however had changed and this led to changes in the dominants of the plant communities (Filipovitch 1992; Filipovitch & al. 1998).

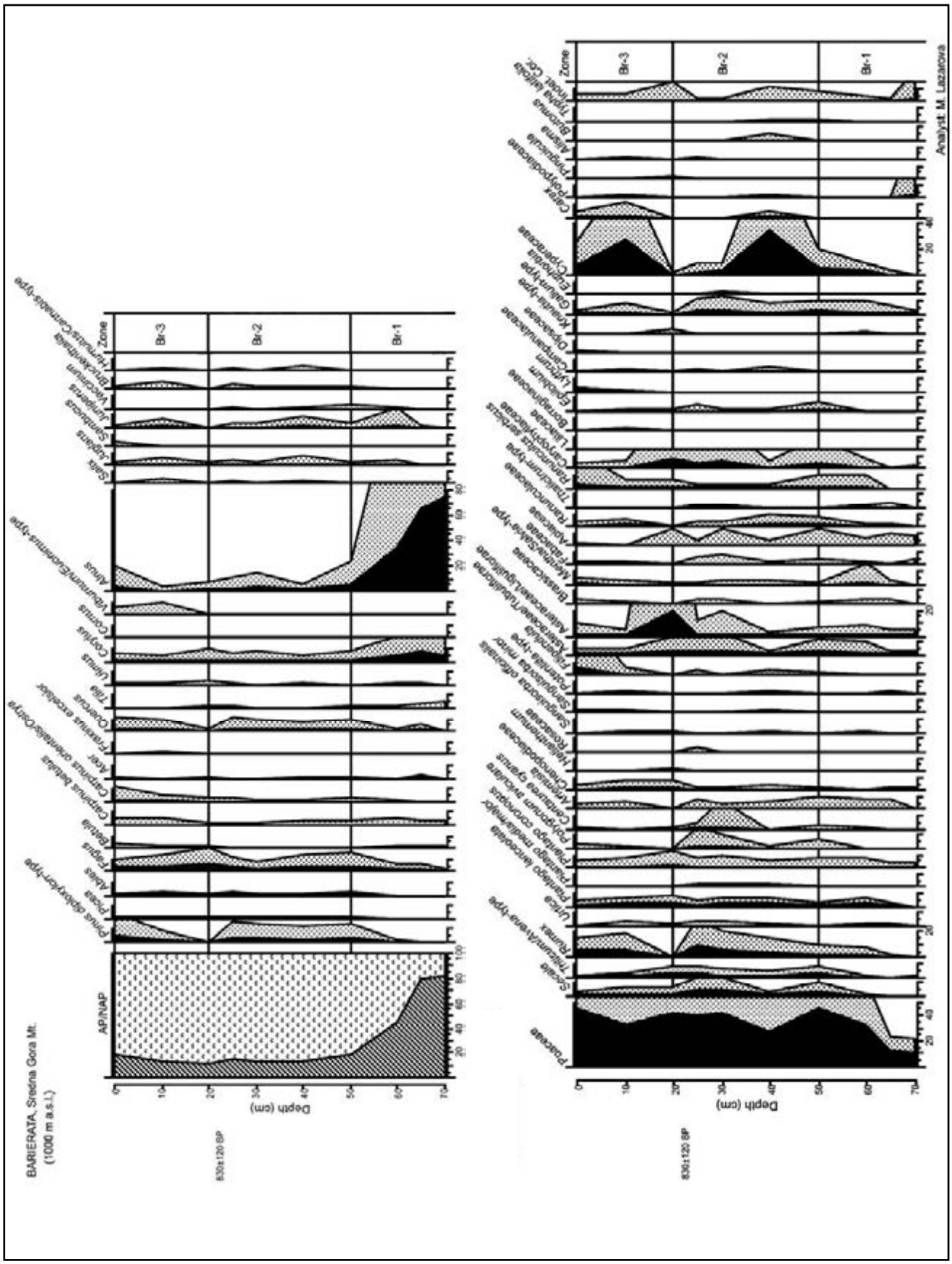

Fig. 2. Percentage pollen diagram of Barierata core.

Detached local pollen zones (LPZ) reflect the general stages in alteration of the vegetation in the studied region.

***LPZ Br-1* (70–50 cm)**

The arboreal taxa are dominant (AP) with maximum values for the entire profile ranging from 81.4 % to 44 %. Pollen from *Alnus* is registered with the highest participation and maximum values between 74.6 % and 34.3 % and it actually predetermines the trend of AP curve in the pollen diagram. Pollen from coniferous trees is not registered in this zone, except for *Pinus diploxylon*-type in insignificant amount and *Juniperus* with a maximal value 1.6 % at the end of the zone.

Fagus up to 2 % and *Quercus* up to 1.3 % participate significantly among the deciduous taxa. *Corylus* shows maximum values for the profile between 4 % and 7.5 %. The rest representatives of the deciduous are in insignificant amount less than 1 %.

The participation of non-arboreal taxa (NAP) is between 18.7 % and 56 %. *Poaceae* is dominant among them with up to 12 % in the lower two levels and a maximum value of 33.5 % at the end of the zone. *Rumex* up to 2.4 %, *Asteraceae/Tubuliflorae*, *Galium*-type up to 2.2 %, *Plantago lanceolata* up to 2 %, *Mentha/Salvia*-type up to 1.6 %, *Caryophyllaceae* up to 1.5 % show more considerable presence while *Apiaceae*, *Ranunculaceae*, *Chenopodiaceae*, *Artemisia* are under 1 %.

Cyperaceae up to 3.4 % and *Polypodiaceae* up to 2 % are registered from the local hydrophytes vegetation.

***LPZ Br-2* (50–20 cm)**

Sharp change in the ratio AP/NAP was registered. Non-arboreal (NAP) pollen dominates with values from 81.6 % to 86.7 %. Total participation of arboreal (AP) pollen reaches up to 18.4 %. An increase of *Pinus diploxylon* between 2.4 % and 3.3 % and a decrease of *Juniperus* to 0.8 % are registered compared to the previous zone. The amounts of *Picea* and *Abies* are insignificant. Representatives of deciduous *Alnus* decrease considerably from 4.7 % to 1 % and *Corylus* to 1.7 %. Increase of *Fagus* up to 4.8 % and *Quercus* up to 2.1 % is registered. *Juglans* and *Humulus/Cannabis* are with minimal values.

Poaceae is dominant from the NAP in this zone with values up to 45 % and local maximum of 28 % (at 40 cm). *Rumex* (3–7 %) and with local maximum of 10.4 % (at 25 cm), *Secale* up to 4.7 %, *Triticum/Avena* up to 3 %, *Asteraceae/Tubuliflorae* up to 4 %, *Caryophyllaceae* from 1.3 % up to 6.8 %, *Galium*-type up to 2.7 %, *Plantago lanceolata*, *Ranunculaceae* up to 2 %, *Centaurea cyanus* L. with local maximum up to 2.1 %, *Polygonum aviculare* L. up to 1.8 %, *Apiaceae* up to 1.3 %, *Chenopodiaceae*, *Artemisia* up to 1 %, etc. are well represented.

From the locals – *Cyperaceae* with 3–7 % and with local maximum of 37.5 % (40 cm) is registered. The presence of *Carex*, *Typha latifolia* L., *Pinguicula*, *Alisma*, *Butomus* is sporadic and with minimal values.

***LPZ Br-3* (20–0 cm)**

Non-arboreal taxa (NAP) are dominant between 80.6 % and 88.5 %. Arboreal taxa participate with 11.5 % to 19.4 %. After decrease in the beginning of the zone, *Pinus* reaches 4.6 % at the end of the zone, which is maximal for the profile. The lower boundary of the profile is characteristic by a drop of share of *Pinus* and growth of *Fagus* which reaches maximum values for the profile up to 6.2 %, whereupon it restores values from the previous zone. After minimal participation of *Quercus* and *Alnus* in the beginning of the zone, they reach up to 2.1 % and 4 %, respectively. *Corylus* participate with 1–2 %. The share of *Carpinus orientalis/Ostrya* increases up to 1.2 %, and share of *Viburnum/Euonymus* – up to 1 %. The rest of thermophilous deciduous taxa stay almost unaltered compared to *Br-2*.

Among the non-arboreal (NAP) pollen in this zone, *Poaceae* has its highest values between 32 % and 45 %. In the beginning of the zone *Asteraceae/Liguliflorae* (18.6 %) and *Caryophyllaceae* (9 %) show local maximums, and at the end of the zone *Filipendula* (6 %) and *Ranunculus serbicus* Vis. (3 %) show local maximums. *Plantago lanceolata* up to 2.5 % and *Chenopodiaceae* up to 1.7 % increase their share (maximums for the profile). Compared to zone *Br-2*, a slight decrease in participation of *Rumex* to 6 %, *Secale* to 2.5 %, *Asteraceae/Tubuliflorae* to 2.7 %, *Galium*-type to 1.8 %, *Ranunculaceae* to 1.3 %, *Centaurea cyanus* and *Polygonum aviculare* under 1 % is observed. *Triticum/Avena* up to 3 %, *Apiaceae* up to 1.3 %, *Artemisia*, etc., are with unaltered share.

The locals are represented by *Cyperaceae* up to 8.5 % with maximum of 28 % (10 cm), including *Carex* up to 1 %, *Pinguicula* and *Alisma* episodically.

Diatom analysis

A total of 165 diatom species, varieties and forms belonging to 41 genera were identified in the studied sediments. The stratigraphic distribution pattern and relative abundance of the most frequently occur-

ring diatoms in the sediments of Barierata core are presented in Fig. 3. Changes in the diatom species composition enabled us to distinguish three diatom assemblage zones (DAZ) representing different ecological conditions:

DAZ SGD1 (70–50 cm, corresponding to the *LPZ Br-1*)

The lowermost part of the zone was characterized by a poor diatom flora with high species diversity of aerophilous genus *Luticola* D.G. Mann. First planktonic species *Aulacoseira granulata* (Ehrenb.) Simonsen and *A. islandica* (O. Müll.) Simonsen appeared at 60 cm with single valves. Towards the end of the zone a species-rich diatom community has been dominated by benthic and epiphytic diatoms *Eunotia glacialis* F. Meister, *E. implicata* Nörpel-Schempp, Alles & Lange-Bert., *E. minor* (Kütz.) Grunow, *Encyonema gracile* Rabenh., *E. silesiacum* (Bleisch) D.G. Mann, *Gomphonema micropus* Kütz., *G. clavatum* Ehrenb., *G. parvulum* (Kütz.) Kütz., *Nitzschia hantzschiana* Rabenh., *Navicula elginensis* (W. Greg.) Ralfs, *Pinnularia borealis* Ehrenb., *P. subcapitata* W. Greg., *P. viridis* (Nitzsch) Ehrenb.

DAZ SGD2 (50–20 cm, corresponding to the *LPZ Br-2*)

In the interval 50–40 cm the tychoplanktonic diatoms *Aulacoseira alpigena* (Grunow) Krammer, *A. tenuior* Krammer, *Fragilariaforma virescens* (Ralfs) Williams & Round, *Staurosira construens* var. *subsalina* (Hust.) Andresen, Stoermer & Kreis and var. *venter* (Ehrenb.) Hamilton, *Tabellaria flocculosa* (Roth) Kütz., as well as *Aulacoseira* sp. 1, *Aulacoseira* sp. 2 dominated. *Eunotia incisa* W. Greg., *Encyonema minutum* (Hilse) D.G. Mann, *E. gracile*, *Surirella tenera* W. Greg. reached their first maximum in relative abundance.

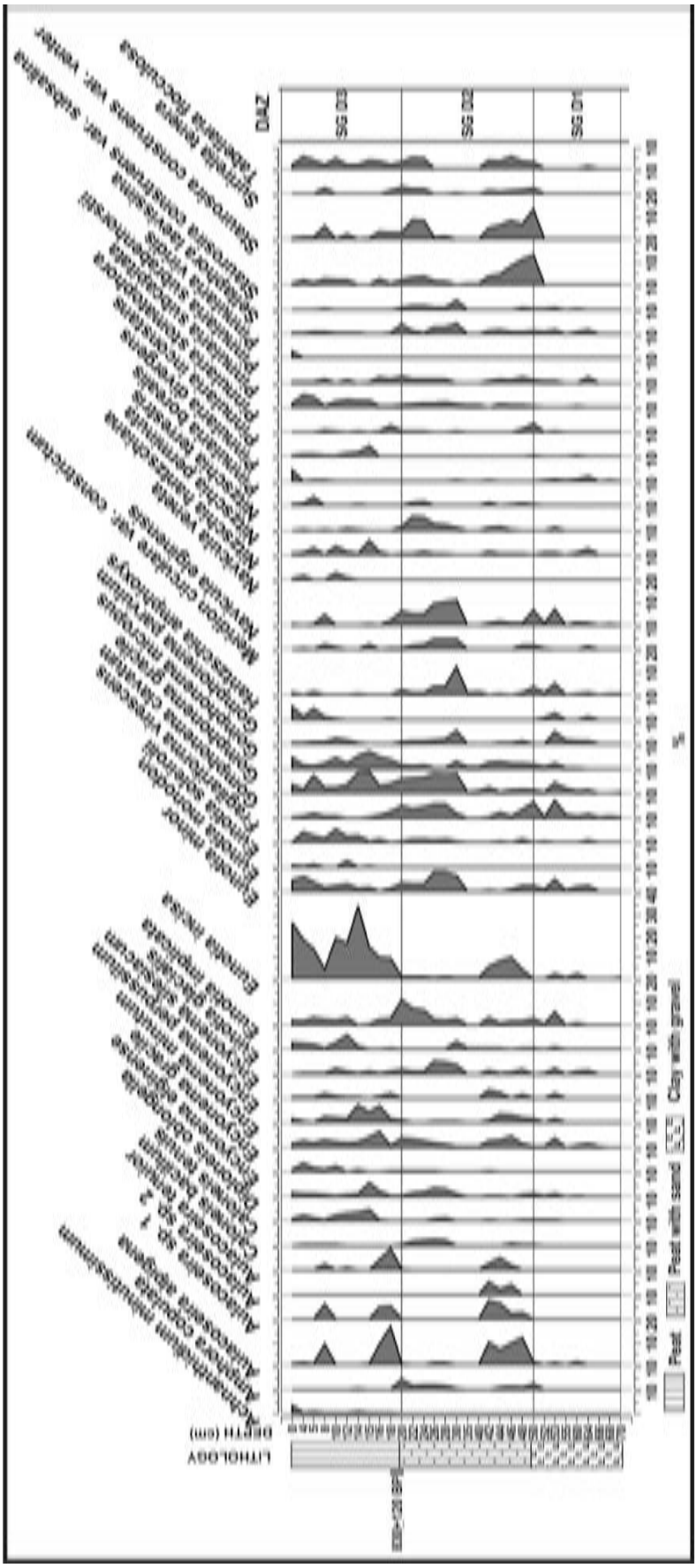

Fig. 3. Diatom percentage diagram of the most common diatoms in the sediments of Barierata core.

In the interval 40–30 cm the composition and structure of the diatom community abruptly changed. The species richness decreased and the most of the diatoms recorded in the preceding stage discontinued development. *Hantzschia amphioxys* (Ehrenb.) Grunow, *Navicula elginensis* and *Sellaphora laevissima* (Kütz.) D.G. Mann reached their highest relative abundance.

The interval 30–20 cm was characterized by a diverse diatom community dominated by benthic and epiphytic species: *Caloneis bacillum* (Grunow) Cleve, *Diploneis oblongella* (Nägeli) A. Cleve, *Encyonema silesiacum*, *Eunotia implicata*, *E. minor*, *Gomphonema micropus*, *G. clavatum*, *Hantzschia amphioxys*, *Navicula elginensis*, *Meridion circulare* var. *constrictum* (Ralfs) VanHeurk, *Nitzschia perminuta* (Grunow) Perag., *Pinnularia viridis*, *P. stomatophora* (Grunow) Cleve, *P. microstauron* (Ehrenb.) Cleve, *Sellaphora laevissima*.

DAZ SGD3 (20–0 cm, corresponding to the *LPZ Br-3*)

This zone was characterized by a rich and diverse diatom flora. In the peaty sediments *Eunotia incisa*, *E. implicata*, *E. glacialis*, *E. minor*, *E. monodon* Ehrenb., *Aulacoseira alpigena*, *A. tenuior*, *Aulacoseira* sp. 1, *Achnanthidium minutissimum* (Kütz.) Czar., *Tabellaria flocculosa*, *Gomphonema clavatum*, *G. gracile* Ehrenb., *Pinnularia stomatophora*, *P. subcapitata* dominated. The planktonic diatoms *Aulacoseira subarctica* (O. Müll.) E.Y. Haw. and f. *recta* (O. Müll.) Krammer, *Cyclotella meneghiniana* Kütz., *C. stelligera* Cleve & Grunow, *Stephanodiscus medius* Håk., and tychoplanktonic *Diatoma anceps* (Ehrenb.) Kirchn., *D. mesodon* (Ehrenb.) Kütz., were characteristic for the zone.

The aerophilic and closely specialized species, suited to inhabit acidic mountain wetlands as *Caloneis molaris* (Grunow) Krammer, *C. leptosoma* (Grunow) Krammer, *Platessa rupestris* (Krasske) Lange-Bert., *Sellaphora seminulum* (Grunow) D.G. Mann, *Chamaepinnularia soehrensis* var. *hassiaca* (Krasske) Lange-Bert., *Decussata hexagona* (Torka) Lange-Bert., *Stenopterobia curvula* (W. Smith) Krammer attained higher frequencies in this zone. The number and relative abundance of the north-alpine diatoms *Caloneis lauta* J.R. Carter & Bailey-Watts, *Frustulia crassinervia* (Bréb.) Lange-Bert. & Krammer, *Neidium bisulcatum* (Lagerst.) Cleve, *Rossithidium petersennii* (Hust.) Round & Bukhtiyarova, *Diatoma anceps* and *D. mesodon* increased towards the surface.

Diatom ecology

Only 6% of the identified diatom taxa have unknown ecology. Fig. 4 shows the proportion between different ecological groups, Diatom frustules/Chrysophycean stomatocysts ratio and pH values obtained by application of Index B. The ecological composition of the diatom flora was generally characteristic with dominance of oligohalobous-indifferent (57.8%), acidophilous-indifferent (44%), oligotrophic (25.9%), benthic (34.3%), and

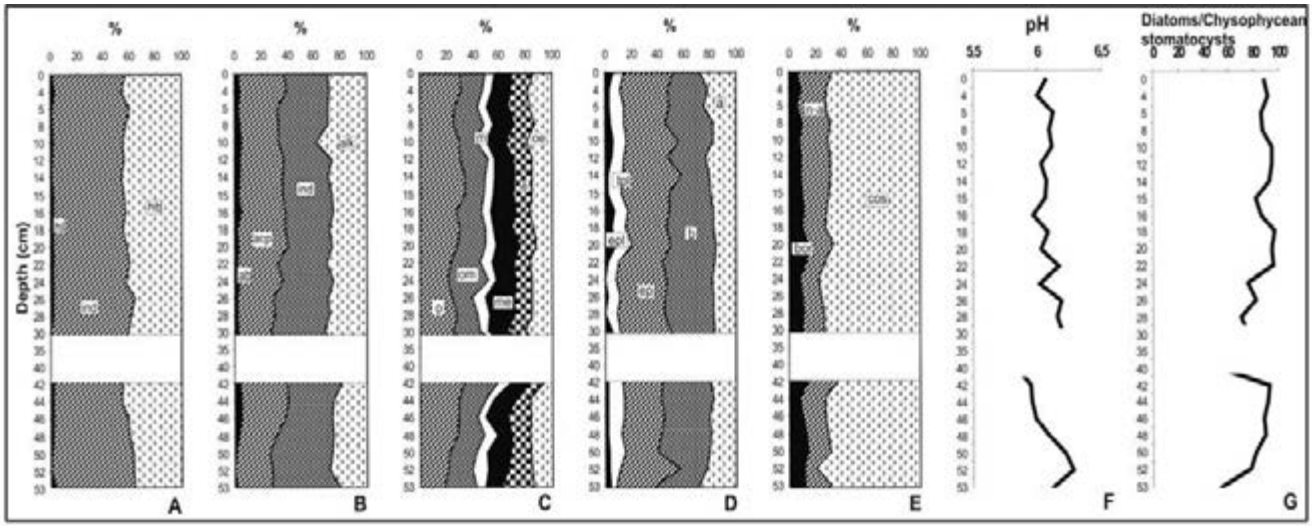

Fig. 4. Relative abundance of the diatom ecological groups, diatom-inferred pH values and ratio of Diatom frustules/Chrysophycean stomatocysts in the sediments of Barierata core.
Legend: A. Halobion diatom groups (**hl** – oligohalobous-halophilous; **ind** – oligohalobous-indifferent; **hb** – oligohalobous-halophobous); **B.** pH diatom groups (**ab** – acidobiontic; **acp** – acidophilous; **ind** – indifferent; **alk** – alkaliphilous); **C.** Trophic diatom groups (**o** – oligotrophic; **om** – oligo-mesotrophic; **m** – mesotrophic; **me** – meso-eutrophic; **e** – eutrophic; **oe** – oligo- to eutrophic); **D.** "Habitat" diatom groups (**epl** – euplanktonic; **tpl** – tychoplanktonic; **ep** – epiphytic; **b** – benthic; **a** – aerophilous); **E.** Geographic distribution (**bor** – boreal; **n-a** – north-alpine; **cos** – cosmopolitan); **F.** Diatom-inferred pH values; **G.** Percentage ratio of Diatom frustules/Crysophycean stomatocysts.

cosmopolite (66.4 %) diatoms. Based on Diatom frustules/Chrysophycean stomatocysts ratio several changes of environmental condition were distinguished (Fig. 4G). Diatoms gradually increased dominating over Chrysophycean stomatocysts in the intervals 53–42 cm (DAZ SGD1) and 30–20 cm (DAZ SGD2), because of the improvement of the hydrology of the habitat. Diatoms constantly dominated over Chrysophycean stomatocysts (80–97 %) in uppermost 20 cm of the sediments indicating last period with high water level. The active water reaction values for the sediments from Barierata core ranged from 5.84 to 6.28 (Fig. 4F). In the uppermost 30 cm of the sediments the pH is almost constant with values about 6.

Discussion

The obtained palynological results along with the analysis of autochthonous siliceous microfossils and radiocarbon give evidence for the assumption that sedimentation in the investigated region had started rather late (in the Subatlantic). The Subatlantic period, which commenced after 3000 BP and coincided with the Iron Age and Roman times, has been related to an increased anthropogenic impact on vegetation.

The earliest deposits from zone *Br-1* show vegetation dominated by tree taxa, with prevalence of *Alnus*, which is characteristic of humid habitats. Pollen grains of *Fagus* are also registered in the zone, apparently owing to a transfer from the higher-altitude mountain regions, considering the fact that it coincides in time with the period of the latest change in forest vegetation of Mt Sredna Gora. According to Filipovitch (1992) later than 1785 BP the high slopes of Mt Central Sredna Gora were covered by beech forest, while in the damp ravines and along the rivers communities of alder and willows had large distribution. The expansion of *Fagus* was accompanied with the formation of new peat bogs along the high mountain slopes, and of marshes along the medium-high and the low slopes (Filipovitch & al. 1998). This is an excellent adjustment with the presumed lowering of temperature and increase of humidity at the end of the Subboreal and during the Subatlantic (Bortenschlager 1982). Representatives of *Poaceae* dominated the herbage, with more significant participation of *Rumex*, *Asteraceae*/*Tubuliflorae*, *Galium*, *Plantago lanceolata*, *Mentha*/*Salvia*, *Caryophyllaceae*, *Apiaceae*, etc. Hydrophilous vegetation was generally poorly represented. Towards the end of the zone, the participation of *Alnus* dropped sharply from its maximum value of 74.6 % to 5 %.

The pioneer diatom flora that had developed during the period of *Alnus* dominance (DAZ SGD1) was poor in species composition with benthic character, which confirmed initially unfavourable hydrological conditions for the development of diatoms. A high presence of oligohalobous-halophilous, eutrophic, epithytic and aerophilous diatoms indicates low water level, strong connection with nutrient reach ground water and appearance of places with inundated surface at initial stage of fen developing. The emergence of planktonic species *Aulacoseira granulata* and *A. islandica* (60 cm) evidences a river impact on the fen. The sediments with reduced presence of *Alnus* showed a rich diatom flora, probably resulting from higher water levels. The rapid change in Diatom frustules/Chrysophycean stomatocysts ratio supported the changes in the important environmental gradients as pH, nutrients and water level due to formation of the wetland close to the stream.

In the next zone (*Br-2*), a sharp decrease of arboreal species reflects the reduced participation of *Alnus*. The latter could be explained by forest fires, as evidenced by charcoal particles at a depth of 52 cm. The change in *Alnus* participation could have been also related to anthropogenic activity. Such an assumption is supported by the reduction of the forest in the Mt Sredna Gora region in the beginning of A.D. According to Filipovitch (1992) at that period the inhabitants at the foot of Mt Sredna Gora started to use intensively the mountain slopes as pastures. The anthropogenic impact took expression in the destruction of *Alnus* communities, in order to extend the pastures and arable land, or to use the trees for timber in construction and ore-mining. The assumption that such changes were anthropogenically conditioned is supported by the parallel increase and maximum distribution of all indicating human influence anthropophytic elements (pasture, ruderal) (Behre 1981), such as: *Rumex*, *Polygonum aviculare*, *Centaurea cyanus*, *Asteraceae*/*Tubuliflorae*, *Poaceae*, *Caryophyllaceae*, as well as *Juglans* and *Humulus*/*Cannabis*. The cultivated gramineous species *Secale*, *Triticum*, *Avena* had marked an increase in that period, reaching their maximum values, which was certainly due to a more intensive agriculture and extension of arable lands in the region. In local vegetation, the representatives of *Cyperaceae* increased their participation to the maximum, probably under optimum water levels for their development. *Carex* and *Typha latifolia* also took part in these communities. Participation of *Butomus* (40 cm) and *Alisma* (25 cm) gives grounds

to the assumption that there were higher water levels. Single specimens of *Pinguicula* (40 cm) occurred in the marshy areas.

The idea of an improved hydrological regime in the fen after local deforestation is corroborated by the development of the tychoplanktonic diatom flora, characteristic for habitats with stagnant water. The diatom communitiy from DAZ SGD2 is dominated by *Aulacoseira alpigena*, an acidophilous species developing in mass numbers both in the shallow basins filled with *Juncus* spp. and in the deep oligotrophic lakes (Haworth 1988), as well as by tychoplanktonic species *Tabellaria flocculosa*, *Staurosira construens* var. *venter* and var. *subsalina*. Presumably, that was the highest water level throughout the entire history of the fen, evidenced by the fact that euplanktonic species *Asterionella formosa*, *Fragilaria crotonensis*, *Tabellaria fenestrata* were identified only there. The three latter species have fragile valves and their preservation is problematic, which comes to explain their low abundance in the sediments.

A sharp environmental change, probably related to general decreased humidity, put an end to the development of the tychoplanktonic diatom community that was replaced by a poor diatom flora, dominated by *Hantzschia amphioxys*, an euribiontic species, able to develop aerophytically even in the moist soil (Krammer & Lange-Bertalot 1988). The low content of diatom remnants in the sediments of that period (42–30 cm) can be explained by particular drying out of the fen surface. According to Velez & al. (2005) in peaty conditions, when peat might have dried, diatoms were not preserved, while pollen grains were. Changes in diatom community were followed by the strongly diminishing of the hydrophilous vegetation (after 30 cm), probably due to changes in the hydrological regime.

At the end of the zone (DAZ SGD2) water exchange in the fen once more improved and the fen was partially isolated from the ground waters by the peat accumulation. The new hydrological and trophic conditions brought about a change in the diatom flora, dominated by some acidophilous benthic species of the genera *Eunotia* Ehrenb. and *Pinularia* Ehrenb. Apparently, the river was another source of biogenic elements, which is corroborated by the presence of rheophilous species, such as *Meridion circulare* var. *constrictum* and *Amphora copulata* (Kütz.) Schoeman & R.E.M. Archibald. There were limited patches of open water, where *Tabellaria flocculosa*, *Staurosira construens* var. *venter* and var. *subsalina* throve.

After 830±120 BP (*Br-3*), vegetation characteristically showed a greater species diversity of the grassy taxa explained by an increase of herbage in general. The gramineous species retained their dominant position. In the beginning of the period, representatives of *Asteraceae*/*Liguliflorae* and *Caryophyllaceae* have their maximum participation, while at the end of the same period representatives of wet meadows increased in numbers: *Filipendula*, *Ranunculus serbicus*, *Plantago lanceolata* and *Chenopodiaceae*. Anthropophytes were present throughout, although in slightly lower numbers than in the preceding zone: *Rumex*, *Secale*, *Triticum*/*Avena*, *Centaurea cyanus*, *Polygonum aviculare*, *Plantago lanceolata*, *Asteraceae*/*Tubuliflorae*, etc. Generally, the composition of herbaceous communities remained unchanged, though with a greater taxa abundance. That was a period of formation of the contemporary plant communities in the region. The increase of *Pinus* in the latest pollen spectra apparently resulted from man-made plantations, dated to the beginning of the last century. In the vicinity of the investigated site communities of *Alnus* and *Salix* registered limited presence in the more humid habitats. Representatives of *Cyperaceae*, including *Carex*, extended again their distribution, reaching a second maximum of 28 % (at 10 cm), subsequently followed by a sharp drop in participation.

The peat sediments deposited about 830±120 BP (DAZ SGD3) had preserved a diatom community typical for mountain wetlands with high water level, acid water reaction and modest nutrient content. It was dominated by the tychoplanktonic *Aulacoseira*-flora as well as *Eunotia incisa*, a north-alpine species tolerating a low active water reaction (Krammer & Lange-Bertalot 1991a). The high percentage of north-alpine diatoms showed a lower water temperature during the latest stage of the fen development. The low temperatures had a favourable effect on a cold-stenothermal diatom flora in the studied mountain fen that reached its maximum species diversity. Diatom community was dominated by different representatives of the genera *Eunotia*, *Pinnularia*, *Frustulia* Rabenh., and *Stenopterobia* Bréb., known as closely specialized taxa, suited to live in progressively developed bogs. Changes in ecological composition of diatom communities from the sediments of studied fen showed that during its development the water level remained inconstant and sensitive to climatic changes. Water salinity in the fen varied from 0.01 to 0.3 g/l and the active water reaction ranged from 5.84 to 6.28. The water was poor in biogenic substances and with mild to low temperature.

The plant succession reflected in the pollen diagram, the succession of the diatom community and the emergence and development of the fen, all resulted from the environmental and climatic changes in the Holocene, but were also strongly affected by the anthropogenic activity, whether direct or not. Presented here data showed that after 2000 yr. BP, the steadily mounting anthropogenic impact brought about strong deforestation of vast areas, distribution of anthropophytic elements in the mountain flora and changes in the floristic composition of all plant communities. Provided integrated diatom-pollen based Late Holocene environmental reconstruction contributes to our understanding of the complicated natural and anthropogenic factors influenced Late Holocene history of the Central Sredna Gora Mountain.

Acknowledgements. The authors thank Prof. M. Frechen (Institut für Geowissenschaftliche Gemeinschaftsaufgaben, Hannover, Germany) for the radiocarbon date of the cores from Mt Sredna Gora and for the comments on the manuscript. We thank Dr Achim Bechtel (University of Leoben, Austria) for his assistance in the course of investigations. We are grateful to Dr Ivanka Stefanova (University of Minnesota, Minneapolis, USA) for the coring of the fen. We thank Prof. D. Temniskova (Sofia University, Sofia, Bulgaria) for the critical comments to improve the manuscript.

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Remarkable and newly recorded aeroterrestric cyanoprokaryotes and algae in Bulgaria

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Abstract. Studies on aerophytic and terrestric algae and Cyanoprokaryota from nature and after application of cultivation techniques are presented; 9 of the recorded taxa are firstly reported or firstly proved by cultures for the Bulgarian flora.

Key words: aeroterrestric algae, Bulgaria, cultures, Cyanoprokaryota, lichens

Introduction

The diversity and taxonomy of soil- and aerophytic algae and Cyanoprokaryota is still poorly known worldwide. In Southeast European countries including Bulgaria studies of edaphophytes and aerophytes are also scarce with some exceptions like investigations of cave algae (e.g., Petkoff 1939; Draganov & Dimitrova 1968; Draganov & Dimitrova-Burin 1980; Stoyneva & al. 2002) or the studies of soil algae and Cyanoprokaryota (e.g., Draganov 1964, 1968; Stoyneva 1991; Draganov & al. 1992). As taxonomic studies of aeroterrestric algae in most cases need special cultivation techniques under standard laboratory conditions, research in this field of algology is underrepresented if compared with aquatic research (Dindal 1990; Johansen & Shubert 2001).

During scientific cooperation projects between the algological working groups at the Botanical Institutes of Sofia and Innsbruck aerophytic algal material from different localities in Western Bulgaria was collected and together with soil samples taken in cultures. Among the algae investigated 9 were firstly recorded in cultures or new for the Bulgarian flora.

Material and methods

Soil samples were taken from the soil surface and 2 cm beneath it from the Boris Garden in Sofia (B). Incubation of soil material on agar-solidified media followed the method described in Ettl & Gärtner (1995). Water diluted soil material was sprayed over the surface of agar Petri dishes. After 6 to 8 weeks of cultivation at room temperature, on a north-faced window, purification treatments on agar plates were made according to Ettl & Gärtner (1995) and Andersen & Kawachi (2005). Clonal cultures were used for taxonomic investigation. Some of the isolated strains are kept in agar tubes for a long-time collection at the Botanical Institutes in Sofia and Innsbruck.

Aerophytic material was collected by scraping from rock surfaces of Erma Gorge in the vicinity of the town Trun (T) and from different sites in the cave Prohodna nearby Karloukovo village (K). Enrichment cultures were again made on agar plates, pure cultures were established by the same procedures as for soil algae.

Light microscopy was done with a Reichert Diapan and an Olympus SH2 microscope, photos taken with a ProGres C 10 digital camera system. Determinations follow the adequate references, as Geitler (1932), Ettl & Gärtner (1995), John & al. (2002), Wehr & Sheath (2003) and Komárek & Anagnostidis (1999, 2005).

Results and discussion

Below a commented list of isolated algae in alphabetical order is provided, where taxa newly recorded and proved in cultures for Bulgaria are marked with aster-

isks (*). Abbreviations of the localities follow the descriptions in Material and methods.

Cyanoprokaryota

Chroococcus tenax (Kirchn.) Hieron.

K, common in "Tintenstrichen" on walls of Prohodna cave, *ca*. 5 metres inside of entrance. Cells 13–17 µm in diameter.

****Chroococcus westii*** J.B. Petersen

K, nearby the entrance of Prohodna cave, between juvenile stages of lichens (e.g., *Dermatocarpon miniatum* (L.) Mann). Colonies usually of 2–4 cells, 14–15 µm, with granular content and conspicuous pink to violet colour. Envelopes distinctly striated.

Gloeocapsa aeruginosa Kütz.

K, between mosses and young thalli of *Dermatocarpon miniatum* in the entrance of Prohodna cave. A widespread aerophytic species on wet calcareous rocks and a main part of the "Tintenstrich" flora (Geitler 1932). It differs from *G. sanguinea* (C. Agardh) Kütz. by colourless envelopes and smaller cells (Jaag 1945; Komárek & Anagnostidis 1999). Colonies in our material were 12–15 µm in diameter.

Gloeocapsa cf. ***aeruginosa*** Kütz.

K, on moist walls near the entrance of Prohodna cave. Colonies 12–15 µm, differs from the type species by its granular envelopes.

Nostoc commune (Vaucher) Bornet et Flahault

T, tiny but macroscopically visible colonies in the surrounding of the jelly lichen *Collema cristatum* (L.) F. Weber ex F.H. Wigg. Cells short, barrel-shaped, 4.5–6.5 µm wide, heterocytes spherical.

Phormidium kuetzingianum (Kirchn.) Anagnostidis et Komárek

K, forming thin greyish layers on moist rocks together with *Gloeocapsa* spp. along a "Tintenstrich" nearby the entrance area of the cave Prohodna. Trichomes 3.5–4 µm wide, in thin sheaths.

Phormidium sp.

K, forms greyish-violet bundles of filaments, in culture without sheaths, resembles *P. violaceum* (Wallr. ex Gomont) Anagnostidis but differs in morphology of apical cell, without calyptra. Trichomes 5–7 µm wide, almost isodiametric.

****Tolypothrix tenuis*** var. ***calcarata*** (Schmidle) B.A. Whitton

K, in Prohodna cave together with crustaceous lichens (e.g., *Caloplaca xantholyta* (Nyl.) Jatta). The alga formed a greyish-blue to greenish-grey felt when moist. Cells 7–8 µm wide and 8–10 µm long, not constricted at cross walls, false branches single, subtended by 1-pored heterocytes. Sheath thin, close to the trichome and partially strongly calcified (Plate I, Figs 1, 2).

Tolypothrix sp.

K, forming dark greyish crusts between moss protonemata near the entrance of Prohodna cave. Trichomes 9–11 µm wide and 4–6 µm long, with firm sheath. Resembles a form of *T. byssoidea* (Hassall) Kirchn.

Xanthophyceae

****Chloridella*** sp.

B, cells in culture 7–9 µm wide, spherical, with thin and smooth cell wall; chloroplasts 1 to 2, cup-shaped, without pyrenoid; a characteristic oil droplet between the chloroplasts. Reproduction by 4 autospores. The isolated strain resembles *C. neglecta* (Pascher et Geitler) Pascher but differs in the number of chloroplasts. The similar species, *C. cystiformis* Pascher has a thickened, brownish cell wall (Pascher 1939).

****Heterococcus*** sp.

B, *Heterococcus* species are characteristic members of the soil algal flora; their determination is possible on culture studies which contain juvenile, adult and reproductive stages. The isolated material shows branched filaments, cells 5–6 µm wide and up to 18 µm long. Reproduction with zoospores and aplanospores, but still not properly documented due to need of long-time cultivation (Plate I, Figs 3–5).

Ulvophyceae

Trentepohlia aurea (L.) Mart.

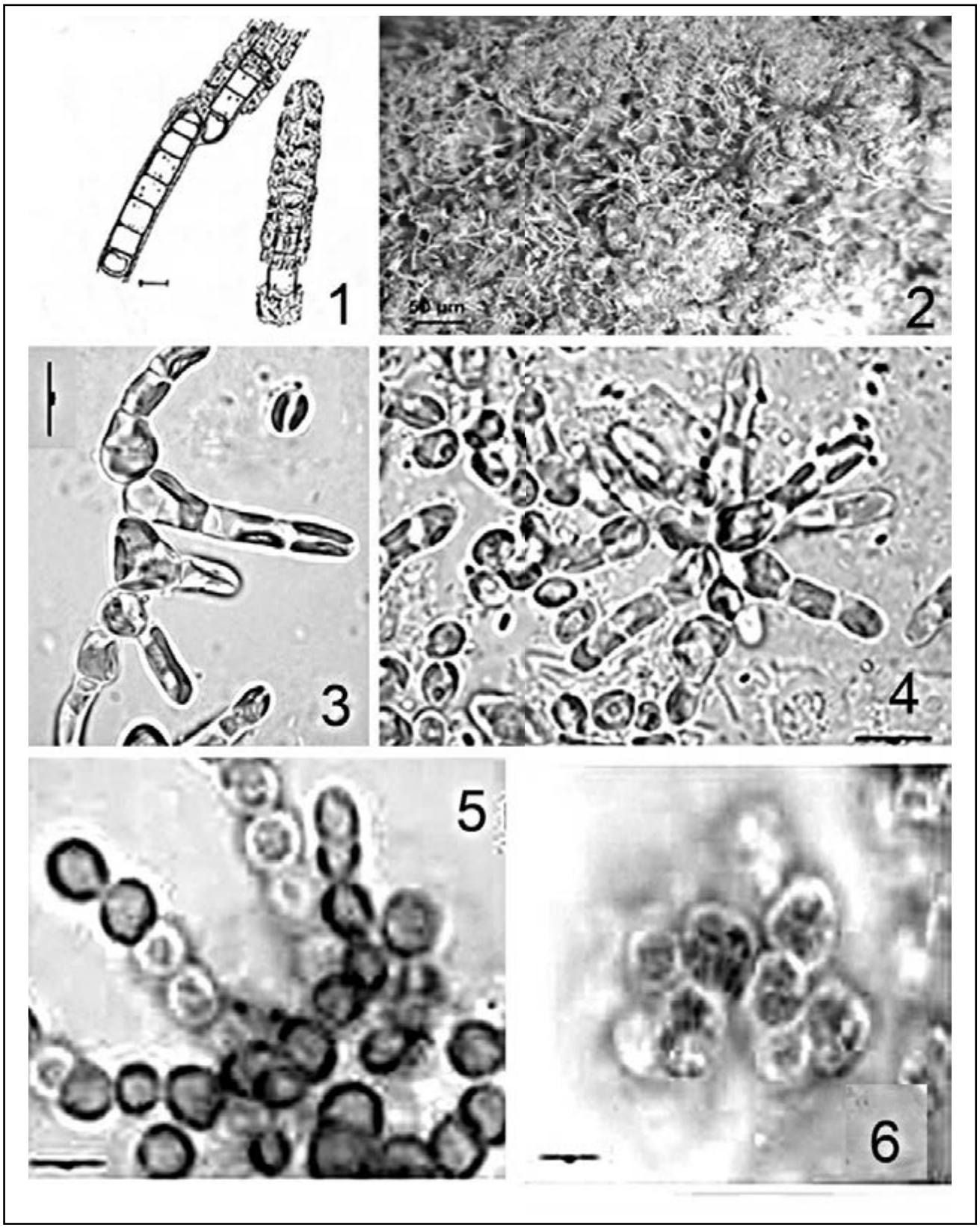
T, on moist calcareous rocks between small thalli of *Nostoc commune* (Vaucher) Bornet et Flahault and the lichen *Gyalecta jenensis* (Batsch) Zahlbr. Cells 10–25 µm wide, 20–35 µm long, apical cells with distinct pectose caps. Zoosporangia seldom observed in fresh material.

Chlorophyceae

****Chlorococcum ellipsoideum*** Deason et Bold

B, cells ellipsoidic or irregular, 7–8 µm wide and 11 µm long, chloroplast parietal with some incisions, one pyrenoid with smooth continuous starch sheath. Resembles the type (refer to descriptions by Ettl & Gärtner 1988) – Plate II, Figs 7, 8.

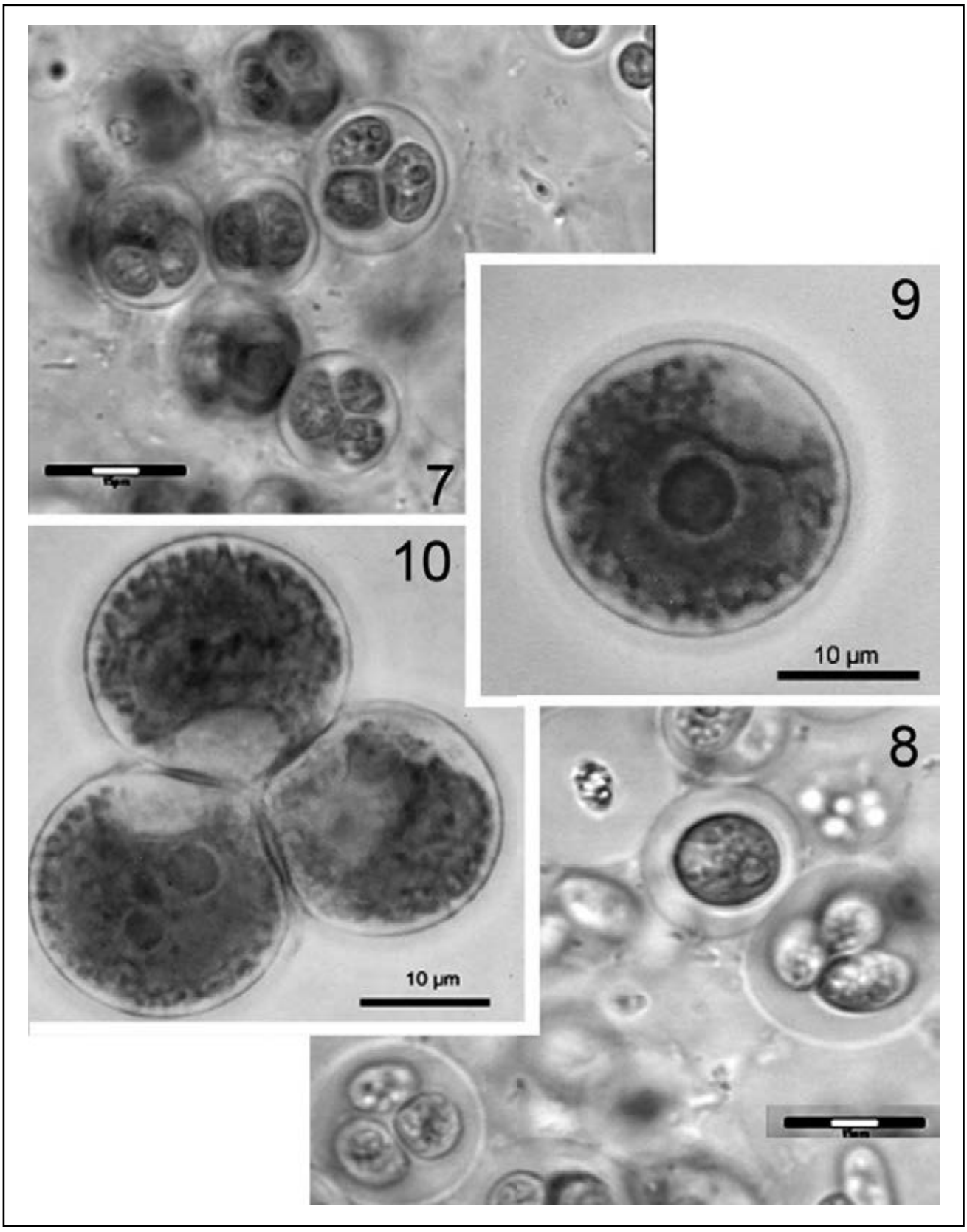
Plate I



Figs 1, 2. *Tolypothrix tenuis* var. *calcarata* from field material;
Figs 3–5. *Heterococcus* sp. from culture;
Fig. 6. *Desmococcus olivaceus* from culture.

Scale bars = 10 µm (Figs 1, 3–6) and 50 µm (Fig. 2).

Plate II



Figs 7, 8. *Chlorococcum ellipsoideum* from culture;
Figs 9, 10. *Trebouxia arboricola* from culture.
Scale bars = 10 µm (Figs 9–10) and 15 µm (Figs 7, 8).

Trebouxiophyceae

Apatococcus lobatus (Chodat) J.B. Petersen

T, on calcareous rock (wall of a tunnel), forming greenish layer. In agar culture this isolate forms short filaments of 3–4 cells, but no zoospores have been observed. Cells 6–14 μm in diameter.

****Chlorella minutissima*** Fott et Nováková

K, between mats of *Tolypothrix* spp. and moss protonemata on moist walls of the Prohodna cave nearby the entrance. Cells 2.5–4 μm in diameter.

****Chlorella trebouxioides*** Punčoch.

K, together with Cyanoprokaryota and layers of *Apatococcus lobatus* on moist rocks nearby the entrance to Prohodna cave. Resembles the original description of Punčochářová (1994), known from soil and bark (Hanagata & al. 1997). Cells 5–15 μm long and 3–12 μm wide.

****Desmococcus olivaceus*** (Persoon ex Acharius) J.R. Laundon

K, common on moist walls of Prohodna cave, between soredia of lichens and moss protonemata. Also found on a fragment of glass (!) on the ground at the same locality. This cosmopolitan aerophytic alga forms cuboidal cell packets, chloroplast parietal with lobed margin and a small sometimes badly visible pyrenoid. Cells 6–10 μm in diameter. The reproduction stages (aplanosporangia) with characteristic warty cell walls were observed (Plate I, Fig. 6).

Trebouxia arboricola Puym.

T, found in free living but also lichenized stage on moist rocks. Single cells without lichenization were also found in K on rocks of Prohodna cave among *Desmococcus olivaceus* layers. Seemingly widely distributed in Bulgaria, earlier recorded from bark (Vodenicharov & al. 1971) and on monuments (Gärtner & Stoyneva 2003). Cells 10–15 μm in diameter (Plate II, Figs 9, 10).

****Trebouxia*** cf. ***excentrica*** P.A. Archibald

K, only found in lichenized stage, perhaps from lichen soredia, together with *Chroococcus westii*, *Desmococcus olivaceus* and juvenile *Dermatocarpon miniatum*, at the entrance of Prohodna cave. The very distinct deeply incised chloroplast fills not more than 2/3 of the cell lumen. Cells 10–14 μm in diameter.

For future research, nature conservation and education it would be of great interest to conserve all the newly isolated algal strains for long-term maintenance in a culture collection under controlled environmental conditions.

Acknowledgements. This study was carried out with the financial support of the Austrian Research Fund (MOEL project No. 180).

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Notes on distribution of *Batrachospermum arcuatum* and *B. gelatinosum* (Batrachospermales, Rhodophyta) in Bulgaria

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Abstract. The freshwater red alga, *Batrachospermum arcuatum*, is recorded for the first time in the freshwater algal flora of Bulgaria. It was collected in nine localities (streams, karst spring and stone trough of fountains). A new locality of *B. gelatinosum* was also reported. The morphological characters of *B. arcuatum* and *B. gelatinosum* specimens were described. In addition, the morphological variation among *B. arcuatum* specimens from nine stream segments was examined and showed a west-east trend in both whorl and carposporophyte diameter. A proposal for protection of *B. arcuatum* and *B. gelatinosum* is presented.

Key words: *Batrachospermum*, *B. arcuatum*, *B. gelatinosum*, Bulgaria, conservation, distribution, stream

Introduction

More than 200 taxa of *Rhodophyceae* are known from freshwater habitats (Kumano 2002). Many of them, especially members of Batrachospermales, inhabited aquatic environments with good water quality, i.e. low nutrient and high oxygen concentration (Sheath & Hambrook 1990) and have been considered for protection in Europe and elsewhere (Knappe & al. 1996; Edlund & al. 1999). *Batrachospermum* section *Batrachospermum* is generally characterized by the following features: well-developed whorls, undifferentiated and straight carpogonial branches, non-pedicellate carpogonia with club- to urn-shaped trichogynes at various distances from the whorl axis (Starmach 1977; Kumano 1993; Vis & al. 1996a). Members of this section are widespread in arctic, boreal and temperate regions (Vis & al. 1996a). The distribution of macroscopic freshwater red algae in Bulgaria has been documented in a few publications from the beginning of 20th century and summarized by Vodeničarov & al. (1991). The most recent data refer to the distribution of *Hildenbrandia rivularis* (Liebm.) J. Agardh in Bulgaria (Stoyneva & al. 2002, 2003). Nine species, varieties and forms of *Batrachospermum* have been reported in Bulgaria but each only from a single location. Only *B. gelatinosum* (L.) DC. was repeatedly collected from streams and karst springs in different parts of the country (for references see Vodeničarov & al. 1991). In Bulgaria, four species and one variety attributable to *Batrachospermum* section *Batrachospermum* have been previously reported, including *B. gelatinosum* (as *B. moniliforme* Roth, *B. moniliforme* f. *densum* (Sirodot) Israelson), *B. anatinum* Sirodot (as *B. ectocarpum* Sirodot), *B. boryanum* Sirodot, *B. boryanum* var. *distensum* (Kylin) Israelson, *B. confusum* (Bory) Hassall (as *B. crouanianum* Sirodot) (Vodeničarov & al. 1991).

In this study we present data on the first record of *Batrachospermum arcuatum* Kylin in Bulgaria and information on a new locality for *B. gelatinosum*. We describe morphological characters of *B. arcuatum* and *B. gelatinosum* and also provide data on morphological variability and ecological preferences of *B. arcuatum*.

Material and methods

Nine locations with *B. arcuatum* and one with *B. gelatinosum* were sampled in Bulgaria (Fig. 1).

Site 1. Stone trough of fountain in the village of Ovcharovo (43°11'N, 27°01'E; elevation 112 m), estate of the town of Shumen.

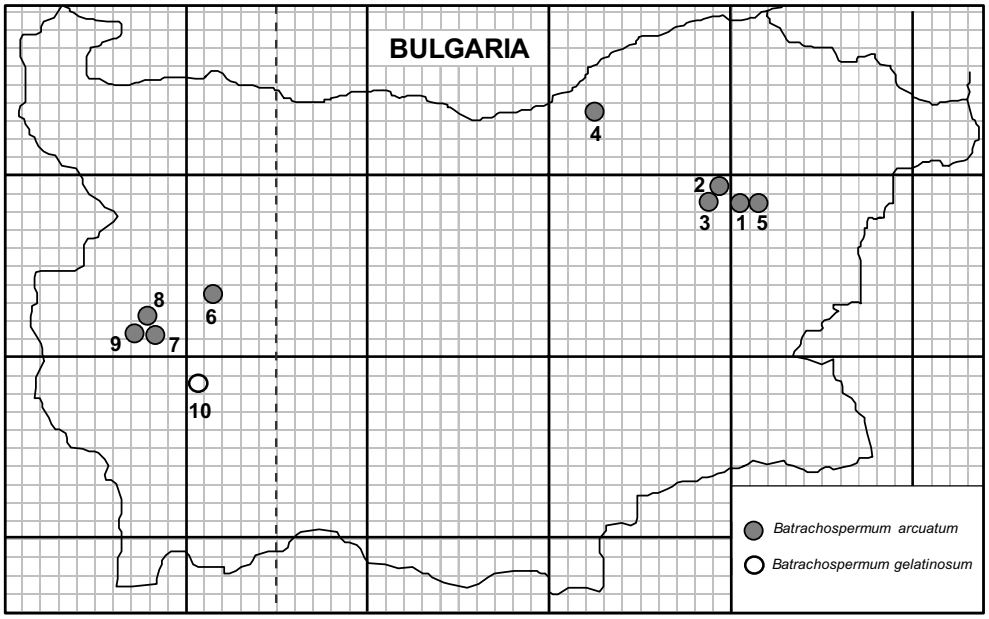


Fig. 1. UTM-grid map of Bulgaria with localities of *B. arcuatum* and *B. gelatinosum*.

Site 2. Stone trough of fountain west of the town of Shumen at the boundary of Shumensko plato Nature Park (43°21' N, 26°55' E; elevation 320 m).

Site 3. Stone trough of fountain near the village of Lozevo at the boundary of Shumensko plato Nature Park (43°20' N, 26°35' E; elevation 324 m).

Site 4. Byalata stena karst spring in Beli Lom River Gorge, Roussenski Lom Nature Park (43°39' N, 26°04' E; elevation 70 m).

Site 5. Stone trough of fountain near the village of Kostena reka (43°11' N, 27°09' E; elevation 259 m), estate of the town of Shumen.

Site 6. Matitsa Stream north of the village of Eleshnitsa below outlet of Toplika spring, Stara Planina Mts (42°45' N, 23°40' E; elevation 724 m), estate of the town of Sofia.

Site 7. Zheleznishka Stream west of the Zheleznitsa village in Vitosha National Park, Mt Vitosha (42°32' N, 23°22' E; elevation 960 m).

Site 8. Dragalevska Stream south of the village of Dragalevtsi in Vitosha National Park, Mt Vitosha (42°38' N, 23°19' E; elevation 920 m).

Site 9. Stream crossing Ofeliite locality before the run into Vladayska River, Vitosha National Park, Mt Vitosha (42°30' N, 23°15' E; elevation 1600 m).

Site 10. Malyovitsa stream above the village of Govedartsi at the boundary of National Park Rila, Rila Mts (42°15' N, 23°29' E; elevation 1268 m).

Samples were immediately fixed with 2–4 % formaldehyde. Maximum depth and width, temperature, pH, specific conductance, calcium hardness, nitrate and phosphorus concentration were measured at sites 1, 2 and 3 on 03.04.2006 and at site 9 on 07.07.2006 using C 200 Series Multiparameter Bench Photometers, Hanna Instruments Inc., USA (Table 1). *Batrachospermum arcuatum* female thalli from each location were measured for morphological characteristics in replicates of 30 (15 for carpogonia) using Amplival photomicroscope (Table 2). Characteristics of both *B. arcuatum* and *B. gelatinosum* were photographed with an Olympus PM–10 AK camera system. The specimens collected in this study were deposited in the algal collection of the Department of Botany of Sofia University "St. Kliment Ohridski", Bulgaria. The map (Fig. 1) was prepared by Bulgarian UTM Directory programme (Michev 1999). Nomenclature is given in Kumano (2002).

Table 1. Physical and chemical parameters measured at some study sites containing *B. arcuatum* in Bulgaria.
Site designations as given in Material and methods.

B. arcuatum site No.	Maximum width (m)	Maximum depth (cm)	Temperature (°C)	pH	Specific conductance (μScm^{-1})	Nitrate (NO_3^-) mgL^{-1}	Phosphorus (P) mgL^{-1}	Calcium hardness ($CaCO_3$) mgL^{-1}
Site 1	1.20	38	10.3	6.8	981	17.72	0.094	690
Site 2	1.10	26	10.8	7.6	285	17.72	0.896	170
Site 3	1.30	44	9.4	6.9	216	12.80	0.091	120
Site 9	3.40	60	7.4	7.2	46	0.62	0.420	10

Table 2. Median morphometric characteristics with range in parentheses of *B. arcuatum* from stream sites in Bulgaria.
Site designations as given in Material and methods.

B. arcuatum morphometric characteristics	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Whorl diameter (μm)	780 (390–1040)	780 (340–910)	533 (351–845)	748 (455–1170)	715 (325–1040)	442 (182–780)	482 (286–780)	390 (260–1040)	476 (156–780)
Main axis central cell length (μm)	442 (195–650)	342 (152–550)	463 (325–715)	343 (143–728)	280 (78–520)	182 (52–390)	417 (130–572)	180 (50–425)	130 (52–234)
Fascicle cell length (μm)	17 (10–25)	16 (10–25)	17 (10–25)	15 (10–25)	11 (10–25)	11 (10–24)	11 (10–25)	15 (10–25)	15 (10–25)
Carposporophyte – no. per whorl	1.2 (1–2)	1.2 (1–2)	2 (1–5)	4 (2–7)	2 (1–4)	2 (1–3)	1 (1–2)	1.1 (1–3)	1.8 (1–2)
Carposporophyte – diameter (μm)	130 (80–143)	116 (91–143)	104 (91–130)	79 (60–91)	104 (91–143)	91 (75–104)	85 (78–104)	75 (65–91)	116 (91–130)
Carpogonium – diameter (μm)	5.5 (5–7)	5.5 (5–6)	5.5 (5–7)	5.5 (5–6)	5.5 (5–6)	5.8 (5–9)	5.5 (5–6)	5.2 (5–8)	5.5 (5–6)
Carpogonium – length (μm)	27 (25–36)	25 (20–34)	24 (22–35)	24 (20–25)	29 (25–35)	26 (24–31)	21 (20–25)	22 (20–25)	25 (20–35)
Carpogonium – branch cell no.	10 (8–17)	8 (7–12)	10 (6–13)	9 (8–10)	6.5 (3–12)	6.5 (3–11)	5.5 (3–8)	7 (4–11)	7 (3–10)

Results

Batrachospermum arcuatum Kylin 1912
(Plate I, Figs 1–11)

Basionym: *Batrachospermum arcuatum* Kylin 1912: 22, fig. 7 a–e.

Description: Thalli are dioecious, consisting of barrel-shaped to globose whorls (Plate I, Figs 2, 3). Whorls are 182–1170 μm in diameter up to 2080 μm in some male thalli. The central cells of main axis are 52–728 μm in length with cortication consisting of cylindrical cells only. Spermatangia are terminal on fascicle (Plate I, Fig. 1). Mature female whorls contain 1–7 peripheral or rarely exserted carposporophytes. Carposporophytes are spherical, pedicellate, 60–143 μm in diameter. Gonimoblast filaments consist of 2–4 cylindrical cells. Carpogonia are 20–36 μm in length with clavate trichogyne 5–9 μm in diameter (Plate I, Figs 4–7). Carpogonium-bearing branches are undifferentiated, 3–17 cells in length. Carposporangia are obovoidal, 11–15 μm in length, 8–12 μm in diameter (Plate I, Figs 9, 10).

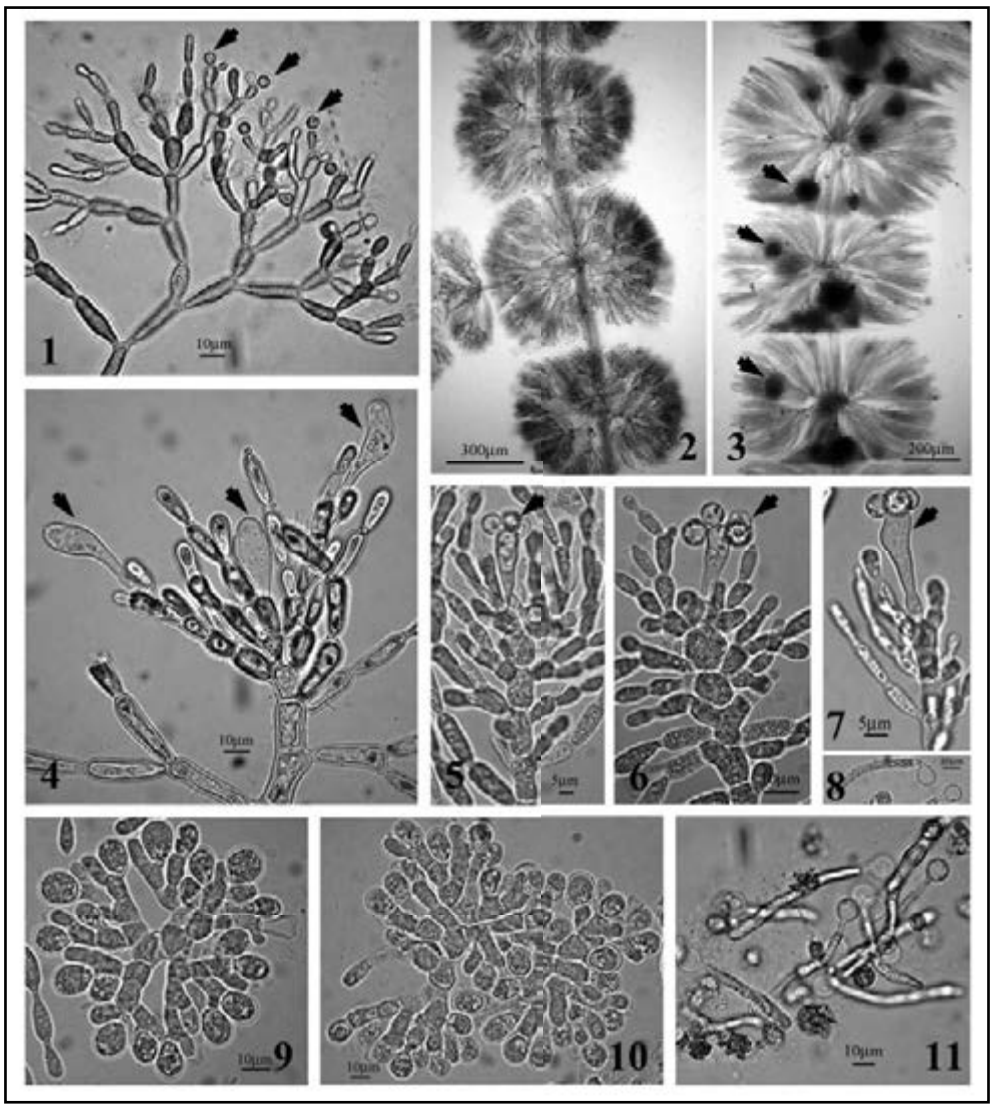
Bulgarian distribution: In this study *B. arcuatum* was collected from nine sites. **Site 1**: 03.06.2001, 05.06.2002, 10.06.2003, 06.06.2004, 20.06.2005, 03.04.2006; **Site 2**: 21.06.2005, 03.04.2006; **Site 3**: 21.06.2005, 03.04.2006; **Site 4**: 31.05.2001; **Site 5**: 28.07.2001; **Site 6**: 06.10.2001; **Site 7**: 20.10.2001; **Site 8**: 14.09.2002; **Site 9**: 15.06.1999, 10.06.2001, 15.06.2002, 20.07.2003, 10.08.2004, 12.06.2005.

Habitat: Thalli are epilithic on submerged stones in small mountain streams of Mt Vitosha, in spring-fed streams of the Stara Planina Mts and in a karst spring and stone trough of fountains in Northeastern Bulgaria. The water was well-aerated, clear and current velocity was moderate to high. Streams tended to vary in maximum width (3.8–6.2 m), maximum depth (60–87 cm) while the springs and stone trough of fountains were shallower (26–44 cm). The temperature was low (7.2–12.5 °C), pH was slightly acidic to slightly alkaline (6.8–7.6). The specific conductance (46 μScm^{-1}) and calcium hardness (10 mgL^{-1}) were low for streams whereas they were higher for springs (380–981 μScm^{-1}; 120–690 mgL^{-1}, respectively). The nitrate concentration ranged from 0.622 to 17.72 mgL^{-1}, and phosphorous concentration ranged from 0.09 to 0.89 mgL^{-1} (Table 1).

World distribution: Portugal, Sweden, Belgium, Crimea, Poland and Germany in Europe; Japan and Taiwan in Asia; Australia; USA and Mexico in North America.

Remarks: Both *B. arcuatum* and *B. anatinun* Sirodot are included in *B. arcuatum* group by Entwisle & Foard (1997) having carpogonia subtended by a relatively short filaments of unmodified cells. The morphometric study of *B. arcuatum* specimens, presented here, confirm the distinguishing qualitative features of this taxon, i.e. dioecious thalli and having regular cortication consisting of cylindrical cells. *Batrachospermum arcuatum* thalli have a wide range of variation within and among Bulgarian localities, and the ranges of morphometric characteristics overlap. The eastern localities tended to have individuals with larger mean plant size (whorl diameter and main axis central cell length) and larger carposporophyte diameter than those from more western locations. Exserted carposporophytes were found only in individuals from the three eastern locations (sites 1, 3, 5). The most conservative are the features of carpogonia, i.e. shape, length and diameter (Table 2).

Plate I



Figs 1–11. Morphological characteristics of *B. arcuatum* from locations in Bulgaria:
1, Fascicle tip with apical spermatangia (arrowheads) (Site 3); **2,** Male plant main axis with globose whorls (Site 3); **3,** Female plant main axis with confluent, barrel-shaped whorls containing numerous spherical carposporophytes (arrowheads) (Site 4); **4,** Carpogonia with clavate trichogyne (arrowheads) (Site 6); **5–7,** Fertilized carpogonium with clavate trichogyne (arrowheads) (Site 4); **9, 10,** Carposporophyte with short 2- to 3-celled gonimoblast filaments having apical carposporangia (Site 4); **8, 11,** Germinating carpospores (Site 4).

Batrachospermum gelatinosum (L.) DC. 1801
(Plate II, Figs 1–8)

Basionym: *Conferva gelatinosa* L. 1753: 1166.

Synonyms: *Batrachospermum moniliforme typicum* Sirodot: 1884: 211, pl. III, fig. 1; *B. densum* Sirodot 1884: 228, pl. XII, figs 1–2, pl. XIII, figs 1–11, pl. XIV, figs 1–8.

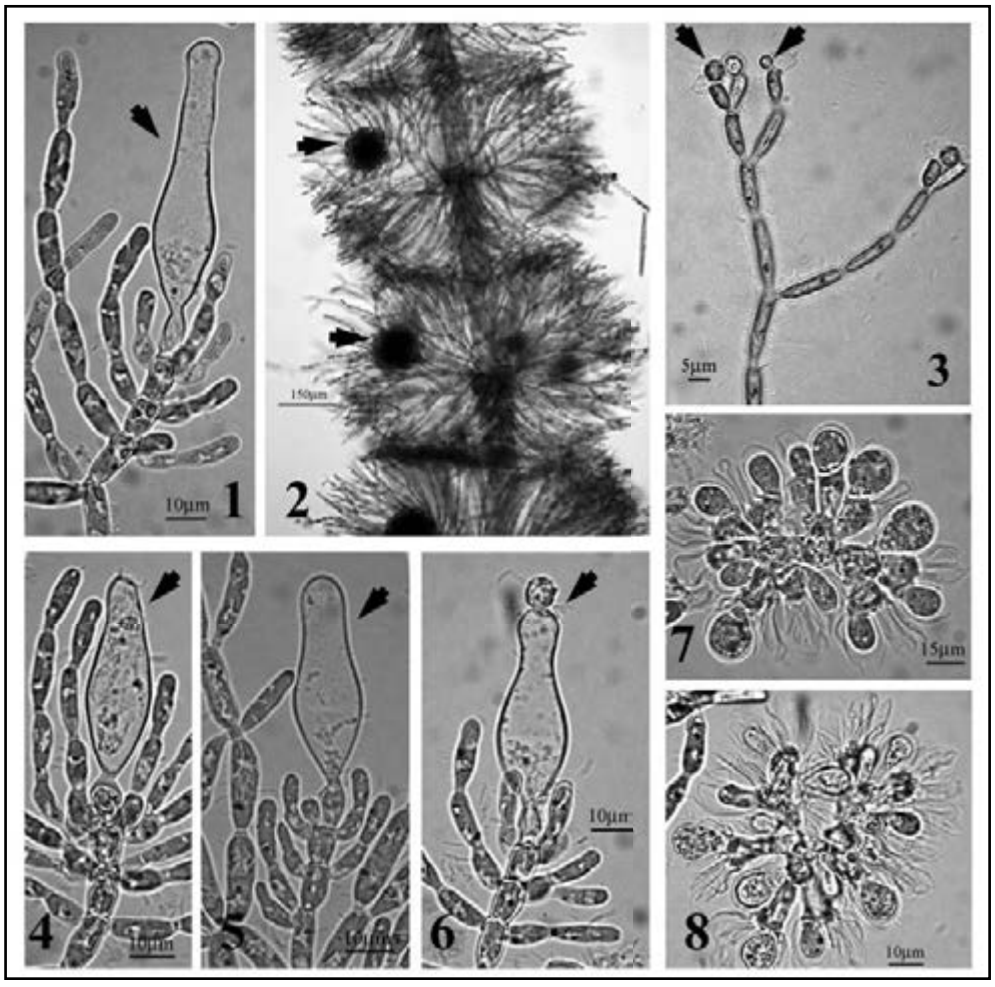
Description: Thalli are monoecious, consisting of confluent barrel-shaped whorls, 455–720 µm in diameter with 1–5 peripheral carposporophytes (Plate II, Fig. 2). The central cells of main axis are 130–325 µm in length with cortication consisting of cylindrical cells. Carposporophytes are spherical, pedicellate, 52–85 µm in diameter. Spermatangia are terminal on fascicle (Plate II, Fig. 3). Gonimoblast filaments consist of 2–4 cylindrical cells. Carpogonia are 48–68 µm in length with lanceolate to elongate-lanceolate trichogyne 12–15 µm in diameter (Plate II, Figs 1, 4–6). Carpogonium-bearing branch is composed of 3–6, undifferentiated cells. Carposporangia are obovoidal, 8–15 µm in length, 6–12 µm in diameter (Plate II, Figs 7–8).

Bulgarian distribution: In this study, *B. gelatinosum* was collected from **Site 10**: 06.11.2004.

Habitat: Thalli are epilithic on submerged stones in small mountain streams, Rila Mts, with width 3 m, maximum depth 45 cm and temperature 9 °C. The water was well-aerated, clear and current velocity was high.

World distribution: Known from temperate regions such as France, Sweden, Belgium, Poland, Germany, Bulgaria in Europe; India, Iraq, Korea, China, Japan in Asia; USA and Canada in North America.

Plate II



Figs 1–8. Morphological characteristics of *B. gelatinosum* from locations in Bulgaria: **1,** Carpogonium with elongate, lanceolate trichogyne (arrowhead); **2,** Main axis with barrel-shaped whorls containing numerous spherical carposporophytes (arrowheads); **3,** Fascicle with apical spermatangia (arrowheads); **4, 5,** Carpogonium with lanceolate trichogyne (arrowheads); **6,** Fertilized carpogonium with lanceolate trichogyne (arrowhead); **7, 8,** Carposporophyte with 2- to 3-celled gonimoblast filaments having apical obovoidal carposporangia.

Discussion

Detailed morphological description of newly recorded for Bulgaria species *B. arcuatum* showed similarities to the specimens from North American populations (Vis & al. 1996b). The morphometric study of *B. arcuatum* populations revealed a wide range of variation along the stream locations in Bulgaria and a west-east trend in the means of some morphological characteristics was observed. The lower altitude and longer growing season in eastern geographical locations may partially explain the larger plant size. In addition, the eastern *B. arcuatum* spring habitats were characterized by lower current velocity and higher concentration of nitrates and carbonates compared to western mountain stream habitats. It is known physical habitat conditions and nutrient content could affect gross morphology of some red freshwater algae (Sheath & Hambrook 1990).

The morphometric analysis presented here is based on one time sampling, therefore seasonal studies of *B. arcuatum* accompanied by environmental data collection are needed to correlate morphological variation to changes in habitat characteristics.

In Europe and North America, *B. arcuatum* inhabits waters with similar quality as in Bulgaria. For example, in Portugal this species was collected from waters with pH 7.2 and temperatures 12–15 °C, in North America – pH 7.4–7.9 and temperatures 3–16 °C (Reis 1974; Vis & al. 1996b). However, in Poland Starmach (1989) collected *B. arcuatum* from streams with lower pH (5.5–6.5) and somewhat lower temperatures (7–8 °C). Although this alga appears to have a restricted distribution in western North America, it is widespread in Europe (Vis & al. 1996b; Kumano 2002).

Unlike *B. arcuatum*, *B. gelatinosum* is wide spread in many countries and is the most common species of freshwater red algae in North America (Vis & al. 1996a, b; Kumano 2002). Both temperature and pH varied greatly for *B. gelatinosum* locations in Europe (1.5–20 °C, pH 6.5–8.5)

and North America (1–27 °C, pH 4.8–8.4) (Reis 1974; Starmach 1984, 1989; Sheath & al. 1986). The global distribution of this alga may be due to its ability to tolerate a wide range of stream chemical and physical characteristics (Vis & al. 1996b). This finding is in agreement with numerous records of *B. gelatinosum* from different floristic regions in Bulgaria (Vodeničarov & al. 1991).

In terms to assess *B. arcuatum* tolerance to human disturbance, the density of *B. arcuatum* populations from two stream segments under various human influences was monitored yearly from 2001 to present. *Batrachospermum arcuatum* from stone trough of fountain in Ovcharovo village (site 1) was threatened with damage by livestock during 2001–2002. Since the isolation of fountain by fence in 2003, the density of *B. arcuatum* population has increased from 2 to 18 individuals per m^2 and has remained stable to date. The second monitored *B. arcuatum* stream reach is located near the rest area at Ofeliite place in Vitosha National Park (site 9). The active human use, including horse breading damaged *B. arcuatum* habitat. As a result, from 2004 the density of *B. arcuatum* population in this stream has decreased from 25 to 4 individuals per m^2. The presented data showed high sensitivity of *B. arcuatum* to the human impact which endanger its population stability.

Despite the broad area surveyed in this study, most of the *B. arcuatum* and *B. gelatinosum* populations were found at the boundaries or within the National parks of Vitosha and Rila, as well as Nature Parks of Roussenski Lom and Shumensko plato. The established distribution pattern of both red algae is most likely due to the relatively low human impact and good water quality of those localities near protected natural areas. To date little is known about algal biodiversity of protected areas in Bulgaria (Temniskova & al. 2005), therefore investigations of algal flora in these areas should be a priority. *Batrachospermum arcuatum* and *B. gelatinosum* have been included in the red list of rare and threatened algae in other European countries and elsewhere (Knappe & al. 1996; Edlund & al. 1999). Because of the restricted distribution of *B. arcuatum* and *B. gelatinosum* to more pristine areas and the potential threat of their habitats in Bulgaria, we propose protection status for both red freshwater algae.

Acknowledgements. The authors are grateful to Mr. Stancho Stanchev and Mr. Evtim Topalov for the assistance in the course of investigations and especially for the help with collecting of the samples. We thank Mr. Christo Stanchev for logistic support.

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New bryofloristic records for the square A4 (Rize, Turkey)

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Abstract. This research was carried out in order to make a contribution to Rize bryoflora. The site under investigation is in the Colchic province of the Euro–Siberian floristic region in the Holarctic kingdom and is found in the A4 square according to grid system of Henderson for Turkey. In the study area, 38 taxa belonging to 32 genera and 18 families were determined as new bryofloristic records for this grid. Moreover, *Nardia scalaris*, *Pedinophyllum interruptum*, *Bucklandiella macounii* subsp. *alpinum*, *Pseudoleskea radicosa* var. *radicosa*, *Isothecium myosuroides* var. *brachythecioides* are recorded for second time only on mainland Turkey.

Key words: A4 square, bryophyte flora, new records, Rize, Turkey

Introduction

The bryophyte flora of Turkey has been studied by foreign botanists since 1860 and last 3 decades Turkish botanists are also studying this subject. But these researches were localized in small areas, for this reason the large part of Turkey has still not been studied in detail. Recent additions to the Turkish bryophyte flora such as: *Ctenidium molluscum* (Hedw.) Mitt. var. *condensatum* (Schimp.) E. Britton, *C. molluscum* var. *robustum* Boulay (Uyar 2003), *Didymodon sicculus* Cano & al., *Fissidens polyphyllus* Wilson ex Bruch, Schimp. & W. Gümbel, *Orthotrichum microcarpum* De Not., *Seligeria paucifolia* (Dicks.) Carruth. (Papp & Sabovljević 2003), *Hedwigia ciliata* (Hedw.) Ehrh. ex P. Beauv. var. *leucophaea* Bruch & Schimp. (Erdağ & al. 2003), *Syntrichia papillosa* (Wilson) Jur. (Erdağ 2003), *Pedinophyllum interruptum* (Nees) Kaal. (Keçeli 2004), *Riccardia latifrons* (Lindb.) Lindb. (Keçeli & al. 2004), *Pseudoleskeella rupestris* (Berggr.) Hedenäs & L. Söderstr., *Isothecium myosuroides* Brid. var. *brachythecioides* (Dixon) C.E.O. Jensen, *Eurhynchium hians* (Hedw.) Sande Lac. var. *rigidum* (Boulay) J.-P. Frahm (Uyar & Çetin 2004), *Harpanthus scutatus* (Web. & Mohr) Spruce, *Nardia scalaris* S.F. Gray, *Scapania subalpina* (Nees ex Lindenb.) Dumort, *Blindia caespiticia* (Web. & Mohr) C. Muell., *Taxiphyllum densifolium* (Lindb. ex Broth.) Reim. (Papp 2004), *Ptilidium pulcherrimum* (Weber) Hampe (Keçeli & Çetin 2005), *Isothecium holtii* Kindb. (Uyar & Ören 2005), *Didymodon bistratosus* Hébrard & Pierrot (Erdağ & Kürschner 2005), *Eremonotus myriocarpus* (Carrington) Pearson (Kürschner & Parolly 2006), *Telaranea europaea* Engel & Merr. (Keçeli & Abay 2007a), *Pallavicinia lyellii* (Hook.) Carruth. (Keçeli & Abay 2007b), *Bucklandiella microcarpa* (Hedw.) Bednarek-Ochyra & Ochyra (Abay & al. 2007), *Dicranum flexicaule* Brid. (Uyar & al. 2008) and three new species from west and south-west of Turkey, *Cinclidotus nyholmiae* Çetin (Çetin 1988), *Cinclidotus bistratosus* Kürschner & Lübenau-Nestle (Kürschner & Lübenau-Nestle 2000), *Orthotrichum leblebicii* Erdağ, Kürschner & Parolly (Erdağ & al. 2004) reflect that our knowledge about the bryophyte flora of Turkey is still incomplete. It was reported by Uyar & Çetin (2004) and Kürschner & Erdağ (2005) that there are approximately 3 species belonging to the class *Anthoceropsida*, 163 species and intraspecific taxa belonging to the class *Hepaticopsida* and 726 species and intraspecific taxa belonging to the class *Bryopsida*. In addition, recent studies on Turkish bryoflora showed that there are approximately 172 taxa of *Hepaticopsida* and 733 taxa of *Bryopsida.* In

other words, the number of bryophyte taxa in Turkey increases significantly by further investigations.

Up to now, several bryofloristic studies were carried out in localities close to the research area by Handel-Mazzetti (1909), Gökler & Öztürk (1989, 1992 a, b), Özdemir (1994, 1999), Baydar & Özdemir (1996), Özdemir & Baydar (1997), Gökler (1998), Özdemir & Çetin (1999), Abay (2006), but no detailed study has yet been made on the Bryophyte flora of Rize province of Turkey. Our main aim was to determine the bryophyte flora of the Rize province. That is why we have been studying on bryophyte flora in this region since 2004. It is hoped that this study will contribute to the bryophyte flora of Turkey and will be useful as a guide for future studies.

Site Description

The study area is located between 40°46'N – 41°02'N and 40°53'E – 41°12'E and within the A4 grid square according to the system adopted by Henderson (1961) for Turkey (Fig. 1). The research area under review is located in the Colchic section of the Euro–Siberian floristic region in the Holarctic kingdom (Zohary 1973).

The Kaçkars are important mountain chains in the area and part of the Pontic Alps. The Kaçkar Mts can be divided into three sections: Verçenik (3710 m) in the west, Kavron (3932 m) in the centre, and Altıparmak (3492 m) in the east. The area collected from bryophyte samples is covered with deciduous trees like: alder, sweet chestnut, hornbeam, beech, lime, box, etc., and also coniferous trees like *Picea orientalis* (L.) Link, up to 1800–2000 m alt. Above this altitude there is alpine-subalpine vegetation in between 2000–2300 meters, and finally above 2300 m rocky areas, composed of granite, cyanite, andesite, and diorite rocks are present (Fındık 2001). The study area is under the influence of oceanic climate with mean annual temperature of 14 °C and mean annual precipitation of 2313 mm. The seasonal precipitation regime for a year is as follows: autumn (A), winter (W), summer (S) and spring (S) – (AWSS) (Akman 1999).

Material and methods

Material for this paper was gathered from the forests of Rize province in August and September of 2004 and in June, August and September of 2005. For each of the species necessary notes on vegetation, habitat, and GPS coordinates were taken. Materials were deposited in herbarium of G. Abay (Çankırı) and herbarium of G. Uyar (Zonguldak).

The plant list was prepared according to Corley & al. (1981), Corley & Crundwell (1991), Koperski & al. (2000) for mosses and to Grolle (1983), Casas (1998) and Grolle & Long (2000) for liverworts.

The ecological characteristics (water requirements and substrates) and life forms of the bryophytes in this study have been assessed by using Dierßen (2001) and Mägdefrau (1982).

The taxa which were recorded for second time for the bryophyte flora of Turkey are indicated by asterisk (*) in the list. The station number, description of the habitat, dates and collector numbers were given in the plant list.

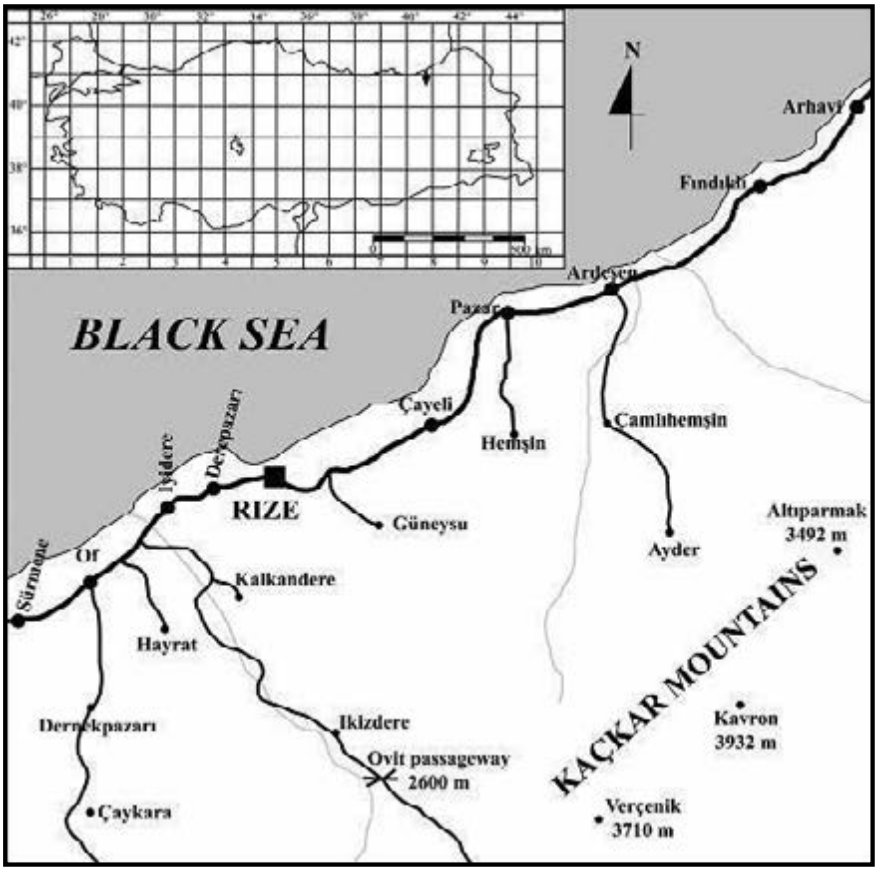

Fig. 1. Geographic location of the study area.

Results

A total of 551 bryophyte taxa (148 liverworts and 403 mosses) were collected during the study. After checking the relevant literature on the bryophyte flora of Turkey we concluded that 38 taxa belonging to 32 genera and 18 families are new bryofloristic records for A4 grid (Özdemir 2000). Moreover, *Pedinophyllum interruptum, Bucklandiella macounii* subsp. *alpinum*, *Pseudoleskea radicosa* var. *radicosa, Isothecium myosuroides* var. *brachythecioides* and *Nardia scalaris* are recorded for second time from Turkey (Frisvoll 1988; Keçeli 2004; Papp 2004; Uyar & Çetin 2004).

In the floristic list for each taxon, the numbers of the sites where they have been found are given, followed by the description of the habitat occupied in the study area. Table 1 lists the sites sampled, all of which belong to the study region.

Table 1. Details of the study sites.

Site number	Geographic coordinates and provinces	Altitude in meters above sea level (m)	Forest formation and common shrubs and herbs
1	2	3	4
1	40°57'18.0" N – 41°05'56.0" E Ayder high plateau, near Ayder stream	1245	PO, AG, FO, RP
2	40°57'20.4" N – 41°05'57.4" E Ayder high plateau, near Ayder stream	1231	AG, PO, RP, Rsp, PA
3	40°56'23.8" N – 40°58'57.3" E Palovit district, near stream	813	AG, BS, BS, PO, CS, TB, Tsp
4	40°56'29.7" N – 40°59'05.3" E Palovit district, near stream	835	AG, CB, BS, BS, PO, CS, TB
5	40°51'14.7" N – 41°00'51.4" E Elevit high plateau	1886	Rsp, RP, Asp
6	40°51'43.4" N – 40°56'40.1" E On the way of coming out from Çat, Kale–Hemşin direction, 50 m after passing the bridge, steep rocky slopes on the right	1310	AG, PO
7	40°53'00.1" N – 41°07'52.6" E Yukarı Kavrun plateau	2265	Meadows, peat places, uncovered area, near stream
8	40°51.089" N – 41°08.677" E Mezovit Pond province	2830	RC, Asp
9	40°57.255" N – 41°06.109" E Ayder high plateau	1350	PO, PA
10	40°57.382" N – 41°06.233" E Ayder plateau	1450	PO, AG, CS, RP, IC
11	40°57.163" N – 41°05.839" E Ayder high plateau, Fırtına stream	1180	AG, RP, Rsp, PA
12	40°57.114" N – 41°05.884" E Ayder high plateau, Fırtına stream	1190	AG, PO RP, SN, Rsp, PA
13	41°02'55.67" N – 41°00'18.06" E Between Çamlıhemşin and Topluca village	309	CS, PO, AG, CB, FO, BS, QH, RP
14	41°02'58.68" N – 41°00'20.12" E Between Çamlıhemşin and Topluca village	318	AG, BS, RP, CS, AC, PO
15	40°57'35.17" N – 41°05'10.29" E Between Ayder high plateau and Çamlıhemşin, Kalerindüzü	1100	PO, FO, AG, RP
16	40°57'53.70" N – 41°04'26.38" E Between Ayder high plateau and Çamlıhemşin	1034	AG, FO, PO
17	40°57'36.99" N – 40°56'46.14" E Between Çamlıhemşin and Çat, Meydan village	1260	BS, AG, SN
18	40°48'41.63" N – 40°56'14.91" E Between Çiçekli and Kale high plateaus	1815	PO, CA, RL, PT, AC
19	40°47'56.87" N – 40°54'24.55" E Verçenik high plateau	2016	RL, meadows
20	40°46'53.65" N – 40°53'38.93" E Verçenik Mountain	2540	Vsp, Asp
21	40°58'08.76" N – 41°11'34.73" E Avusor high plateau	2360	Rocky and meadows field
22	40°56'17.20" N – 41°12'01.10" E Kemerli Kaçkar Mountain, glacial lake	2680	Rocky field
23	40°54'21.87" N – 40°56'52.41" E Meydan village, Kolona provinces	990	AG, BS, PO, CS, RP, FO
24	40°51'37.29" N – 41°04'01.77" E Tirovit high plateau	2451	Meadows, uncovered area
25	40°55'34.53" N – 41°10'37.43" E Palokçur high plateau	2231	RC, VAL,VA meadows

Abbreviations: AC – *Acer cappadocicum*; **AG** – *Alnus glutinosa*; **Asp** – *Alchemilla* **sp.**; **BS** – *Buxus sempervirens*; **CA** – *Corylus avellana*; **CB** – *Carpinus betulus*; **CS** – *Castanea sativa*; **FO** – *Fagus orientalis*; **IC** – *Ilex colchica*; **LO** – *Laurocerasus officinalis*; **PA** – *Pteridium aquilinum*; **PO** – *Picea orientalis*; **PT** – *Populus tremula*; **QH** – *Quercus hartwissiana*; **RC** – *Rhododendron caucasicum*; **RL** – *Rhododendron luteum*; **RP** – *Rhododendron ponticum*; **Rsp** – *Rubus* **sp.**; **SN** – *Sambucus nigra*; **Tsp** – *Tilia* **sp.**; **VA** – *Vaccinium arctostaphylos*; **VAL** – *Veratrum album*; **Vsp** – *Vaccinium* **sp.**

The Floristic List

Marchantiopsida Stotler & Stotl.-Crand.

Aneuraceae H. Klinggr.

1. *Aneura pinguis* (L.) Dumort. – 7: on wet rock near stream bank, 02.09.2004, TK 2950.
2. *Riccardia chamaedryfolia* (With.) Grolle – 8: on rock crevices near water leakage, 02.09.2004, TK 2963.

Lophoziaceae Cavers

3. *Barbilophozia hatcheri* (A. Evans) Loeske – 1: on dead *Picea orientalis* trunk, 31.08.2004, TK 2811; 7: on rock, 02.09.2004, TK 2945 and on rock in stream bed, TK 2964.

Plagiochilaceae (Jörg.) Müll. Frib.

4. **Pedinophyllum interruptum* (Nees) Kaal. – 2: on wet rock in stream bed, 31.08.2004, TK 2822; 6: on rocks in shaded habitat, 01.09.2004, TK 2926.

Cephaloziellaceae Douin

5. *Cephaloziella hampeana* (Nees) Schiffn. – 2: on fallen and decaying logs, 31.08.2004, TK 2823.

Bryopsida (Limpr.) Rothm.

Archidiaceae Schimp.

6. *Archidium alternifolium* (Hedw.) Schimp. – 13: on rocks in shaded habitats, 11.06.2005, Abay 858.

Fissidentaceae Schimp.

7. *Fissidens dubius* P. Beauv. – 10: on soil near stream bed, 03.09.2004, Uyar 780.
8. *F. exilis* Hedw. – 14: on wet soil near stream bank, 11.06.2005, Abay 779.

Dicranaceae Schimp.

9. *Dicranodontium denudatum* (Brid.) E. Britton – 8: on wet soil in woodland, 02.09.2004, Abay 621; 9: on bark of trees, 03.09.2004, Uyar 764.
10. *Dicranoweisia crispula* (Hedw.) Milde – 20, 21, 23, 25: on rock near roadside, 14–15.06.2005, 30.08.2005, 01.09.2005, Abay 800, Abay 799, Abay 758, Uyar 831.
11. *Campylopus subulatus* Schimp. – 9: on soil in open or shaded habitats, 03.09.2004, Uyar 829.
12. *Oncophorus virens* (Hedw.) Brid. – 23: on wet soil in woodland, 30.08.2005, Abay 755.

Ditrichaceae Limpr.

13. *Ditrichum pallidum* (Hedw.) Hampe – 2: on bark of tree, 31.08.2005, Abay 650.
14. *D. pusillum* (Hedw.) Hampe. – 22: on rocks in woodland, 15.06.2005, Abay 797.

Encalyptaceae Schimp.

15. *Encalypta streptocarpa* Hedw. – 13: attached to rocks in water**,** 11.06.2005, Abay 833.

Pottiaceae Schimp.

16. *Tortula subulata* Hedw. var. *angustata* (Schimp.) Limpr. – 9: on humus soil in woodland, 03.09.2004, Uyar 812.
17. *Didymodon fallax* (Hedw.) R.H. Zander – 11: on rocks near stream bed, 03.09.2004, Uyar 823.
18. *Tortella densa* (Lorentz & Molendo) Crundwell & Nyholm – 8: on soil in open areas, 02.09.2004, Abay 628.
19. *T. nitida* (Lindb.) Broth. – 4: on bare rocks, 01.09.2004, Abay 590.

Grimmiaceae Arnott.

20. *Grimmia montana* Bruch & Schimp. – 18, 19: on bare rocks, 13–14.06.2005, Uyar 814, Uyar 814A.
21. *Bucklandiella macounii* (Kindb.) Bednarek-Ochyra & Ochyra subsp. *macounii* – 22: on bare rocks, 15.06.2005, Uyar 808.
22. **B. macounii* (Kindb.) Bednarek-Ochyra & Ochyra subsp. *alpinum* (E. Lawton) Bednarek-Ochyra & Ochyra – 21, 22, 24: on rocks near glacial lake, 15.06.2005, 30.08.2005, Abay 727, Abay 767, Abay 810.

Orthotrichaceae Arnott.

23. *Orthotrichum speciosum* Nees ex Sturm. – 3: on rocks in shaded habitat, 01.09.2004, Abay 607.
24. *O. urnigerum* Myrin – 18: on bare rocks, 13.06.2005, Uyar 822.

Leskeaceae Schimp.

25. **Pseudoleskea radicosa* (Mitt.) Kindb. & Macoun var. *radicosa* – 8: on rocks near pond, 02.09.2004, Uyar 796.
26. *Pseudoleskeella catenulata* (Schrad.) Kindb. – 5: on rocks in shaded habitat, 01.09.2004, Abay 582.
27. *P. nervosa* (Brid.) Nyholm. – 25: on rocks in shaded habitat, 01.09.2005, Abay 857; 23: on tree trunks, 30.08.2005, Abay 762.
28. *Leskea polycarpa* Hedw. – 7: on rocks in damp habitats, 02.09.2004, Abay 692.
29. *Pterigynandrum filiforme* Hedw. – 11: on tree barks, 03.09.2004, Abay 638.

Helodiaceae (M. Fleisch.) Ochyra

30. *Helodium blandowii* (F. Weber & D. Mohr.) Warnst. – 7: on rocks near stream bank, 02.09.2005, Abay 630.

Amblystegiaceae (Broth.) M. Fleisch.

31. *Campylium protensum* (Brid.) Kindb. – 9: on rocks nearby stream bed, 03.09.2004, Uyar 803.
32. *Calliergon stramineum* (Brid.) Kindb. – 8: on humus soil in woodland, 02.09.2004, Uyar 783.

Brachytheciaceae Schimp.

33. **Isothecium myosuroides* Brid. var. *brachythecioides* (Dixon) Braithw. – 9: on tree trunks, 03.09.2004, Uyar 800.
34. *Brachythecium erythrorrhizon* Bruch, Schimp. & W. Gümbel – 6: on rocks near stream bed, 01.09.2004, Abay 658.
35. *Eurhynchium schleicheri* (R. Hedw.) Jur. – 17: on fallen and decaying logs, 13.06.2005, Abay 722.

Plagiotheciaceae (Broth.) M. Fleisch.

36. *Isopterygiopsis pulchella* (Hedw.) Z. Iwats. – 9: on soil in woodland, 03.09.2004, Uyar 789.

Hypnaceae Schimp.

37. *Hypnum imponens* Hedw. – 12: on tree trunks, 03.09.2004, Abay 644; 11: on rocks in shaded habitats, 03.09.2004, Uyar 766.
38. *Ctenidium molluscum* (Hedw.) Mitt. var. *condensatum* (Schimp.) E. Britton – 15: on soil in woods, 12.06.2005, Uyar 819; 16: on rocks in woodland, 12.06.2005, Uyar 819A.

Discussion

We are to indicate that *Nardia scalaris* has been newly reported by Papp (2004) from Rize province, Ardeşen town, from Ayder to Kavron village, 6 km from Kavron, alpine-subalpine vegetation, 1740 m, on soil. Nevertheless, we collected it from Ayder plateau, 1350 m, under the *Picea orientalis* forest formation on sandy gravely soil. Although these two collecting localities for this species are not far-away, the difference of elevations between the first and the second recorded points is conspicuous.

As might be expected from climate, mesophytic bryophytes (41.0%) are dominant in these new records for A4 square. These are followed by hygrophytes (38.4%), xerophytes (17.9%) and then amphiphytes (2.5%) occurring in the region.

The predominant life form in these plants is weft (33.3%), and the other life forms observed in these plants are as follows: short turf (20.5%), mat (15.3%), tall turf (12.8%), cushion (10.2%), fan (5.1%), and tail (2.1%). In addition, according to substrates of the plants, major groups are epilithic (35.8%), helophytic (20.5%) and humicolous taxa (20.5%). The rest of taxa are epiphytic (12.8%) and epixylic (10.2%).

Acknowledgements. We gratefully acknowledge financial support of the Turkish Scientific and Technical Research Council (TBAG–2404).

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Balkan vascular flora in numbers

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Abstract. The present study is based on the five volumes of the edition *Flora Europaea*. It contains integrated species concept, critical opinion on the interpretation of variability in the local floras, standardization in presentation of information and at last, but not least – possibility for comparison of the materials from different parts of the areas, as well as serious references. According to the total number of species the Balkan countries are arranged in the following way: Yugoslavia (sensu *Flora Europaea*) – 4234; Greece – 3728; Bulgaria – 3189; Romania – 3023; Albania – 2681; Turkey (European part) – 1787. In the same way the number of Families, Genera and Subspecies has been calculated. The Balkan and the local endemic diversity are established too. The comparative analysis of these figures shows that the Balkan Peninsula can be considered as an effective speciation centre.

Key words: Balkan Peninsula, endemics, vascular flora

Introduction

Necessity of this investigation

The vascular flora of the Balkan Peninsula presents an exceptional interest, defined from its geographical position of a migration bridge between the East and the West, the varied relief with more than 300 habitat units (CORINE Biotopes Project 1991) and its geological history, at which the oldest fossil floras in Bulgaria are from Neogene, while the youngest floras are from Late Quaternary (Palamarev 2003).

The species diversity can be traced in some old publications (Pančić 1874; Halácsy 1901–1904; Stojanov & Stefanov 1924–1925); it is summarized (to some degree) by Turrill (1929) and Hayek (1927–1933).

In their works on the national floras Tan & Mullaj (2001), Tan & Strid (2001), Özhatay (2001), and Petrova (2001) report the taxonomic richness of their countries. Despite the similar titles and problems treated, these studies do not give a possibility for comparison because of different authors' emphases and interpretations.

So, at this moment we do not have information which in comparative plan can show what the relationships in the higher flora are in the different parts of the Balkan Peninsula, its speciation capacity, the trends in consolidation for the different taxonomic groups and the flora of the Peninsula as a whole. We will make an attempt to fill that gap.

General approach

The biosystematic works in the current Floras of Albania, Greece, Serbia, Turkey, Romania (partially), Bulgaria to some degree reflect the subjective characteristics of the national schools. That is why our study has chosen as a reliable source *Flora Europaea* (Tutin & al. 1964, 1968–1980). On this base we have a source, where common taxonomical scheme, equal interpretation of the variability in the range of the species, and a common graphical design are used.

Material and methods

The order, family, genus, species and subspecies have been recorded in special columns. If the taxon is pointed out for the whole Europe, all countries are pointed out. The typical subspecies is not pointed out; the non-typical subspecies are marked. The endemics at genus, species and subspecies levels are pointed out for the respective

country and are included in a special list. Their distribution with the accepted abbreviations is shown. If there are taxa with other signs, e.g., "extinct", "probable to occur", they are numbered in the described way. After the preparation of the initial tables with data in figures for the number of taxa from different categories, a logical review is made. The results from the comparative analysis are pointed out. They are relevant. Two coefficients are formed: 1. Coefficient of relevant taxonomic diversity (RTD fl.) and 2. Coefficient of the endemic relevant diversity (RTD end.).

The values of coefficients are formed as a sum of the number of families, number of genera, number of species and number of subspecies, divided on the area of the country, where:

$$\mathbf{RTD} = \frac{\Sigma \text{ taxa}}{\text{territory}} \leq 1$$

As higher its value is, as bigger is the diversity of taxa of the above pointed out levels, or occurrence of the different taxa is higher.

As lower its value is, as less is the taxonomic diversity – the flora is formed by a low number of families, genera, species and subspecies. In this respect the taxonomic diversity can be accepted as a visually more undiversified because of the low "occurrence" of different taxa.

Results and discussion

Taxonomical diversity

The number of taxa for the different countries is pointed out in increasing order of the sum of taxa, defined in *Flora Europaea* (Table 1).

As far as the different values of RTD fl. are concerned, the picture is the following:

- The territory between 40th and 44th parallel and 20th and 24th meridian has been characterized by highest values – i.e. the essential central part of the Balkan Peninsula.
- The territory between 44th and 46th parallel and 16th and 20th meridian or the north-west part of the peninsula is with lowest value.
- In direction North – North-East (for comparison) the value of RTD fl. goes to 0.018 between 44th and 48th parallel and 24th and 28th meridian.

Having in mind these proportions between the RTD fl. values, if we assume that all other conditions and events that define the taxonomic richness of the Balkan Peninsula are similar, then the conclusions are:

- Evolutionary "productivity" of the central zone of the Balkan Peninsula is the highest (Fig. 1).
- In the territories neighbouring that zone the productivity remains still essential. These territories reach the Dinaric Mts to the West; the north-western parts of Stara Planina Mts – to the North-East; the Central Rhodope Mts – to the East ; and Pind Mts – to the South.

Table 1. Taxonomic richness in the countries of Balkan Peninsula.

Countries	Area (km^2)	RTD fl.	Families	Genera	Species	Subspecies
Turkey (European part)	23 700	0.116	119	609	1787	234
Albania	28 750	0.136	135	743	2685	344
Romania	237 500	0.018	132	736	3023	346
Bulgaria	110 990	0.041	144	782	3189	422
Greece (continental part)	131 960	0.039	142	826	3728	525
FR Yugoslavia	213 800	0.027	145	869	4234	504

Abbreviation: RTD fl. – Coefficient of relevant taxonomic diversity.

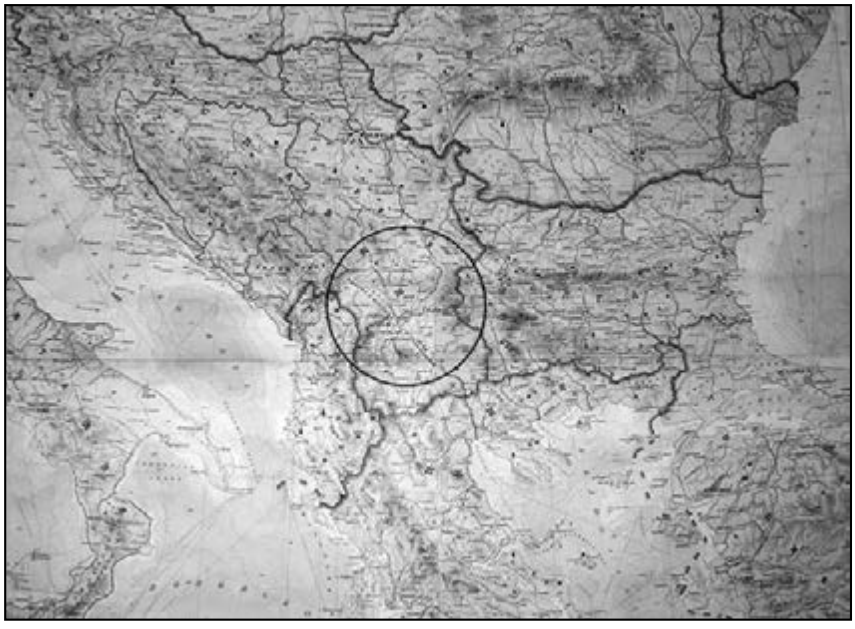

Fig. 1. The area of the maximal evolutionary "productivity" of Balkan Peninsula.

Endemic element

Analysis of RTD end. (Table 2)

The analysis of the RTD end. values shows the following tendencies:

- Relevant taxonomic diversity of endemic elements is highest in south-west and south-east parts of the central zone of the Balkan Peninsula.
- Lower (in different degrees) are the values defined in north-west and north-east parts of the Balkan Peninsula.
- The floristic richness does not coincide with the endemic richness. A notion can be made that in the south and south-west parts the speciation power on a species level is bigger and is related mainly to the mountain complexes of Olymp, Pind, Epir and their adjacent territories.

Table 2. Quantitative representation of endemic elements in the countries of Balkan Peninsula.

Countries	RTD end.	Species	Subspecies	Origin	
				Families	Genera
Turkey (European part)	0.010	98	30	35	78
Albania	0.030	621	67	60	226
Romania	0.004	574	70	91	207
Bulgaria	0.009	614	98	56	219
Greece (continental part)	0.010	949	110	59	254
FR Yugoslavia	0.007	1178	118	61	289

Abbreviation: RTD end. – Coefficient of endemic relevant diversity.

Spatial qualitative representation (Table 3)

Tan & Strid (2001) have proposed an interesting scheme for assessment of the spatial representation of the endemics. Due to non synchronization of the information which we have, we are using only two categories – local and Balkan endemics, as it is in *Flora Europaea*.

For coincidence of the distribution in different Balkan countries of certain percentage of Balkan endemics at species level we cannot point out their number. The local endemics are limited in terms of space and the number given here corresponds to their real distribution. Still, the notion for bigger richness of the Balkan endemics is confirmed. The numbers pointed out by Tan & Mullaj (2001), Tan & Strid (2001) and Petrova (2001) are very similar to those which are given in our calculation: e.g., 400 Balkan endemics for Albania, while in *Flora Europaea* they are 338; as well as 281 species for Bulgaria, respectively in *Flora Europaea* – 287; for Greece totally 740 endemic taxa, while in *Flora Europaea* – 811. The explanation for these differences can be found namely in the different interpretation of the variability. This fact forced us to work with *Flora Europaea*, and not with local floras, even if they are newer.

The total number of local endemics is impressive – about 750 species have quite limited distribution, good taxonomic status and present real adaptive lines in the conditions of mainly mountain extreme ecological niches. The sum of local and Balkan endemic species and subspecies can reach about 10 % from the total European vascular flora. This is serious evidence for the speciation capacity of the region.

Taxonomic representation

Although we are able to confirm the thesis for the existence of speciation centre on the Balkan Peninsula, it is determined that there is no an endemic family. That fact is supporting the circumstance that the Balkan flora is still a European flora.

With certainty it can be said that 28 genera from the vascular flora are endemic for the Balkan Peninsula. By increasing of the biosystematic knowledge, we are convinced that their number will increase, because there are still genus complexes which have artificial nature. Examples in that respect can be seen in family *Poaceae* and genus *Centaurea*. *Apiaceae* and *Brassicaceae* families comprise 4 endemic genera each, *Boraginaceae* and *Poaceae* – 3 genera each, *Fabaceae* and *Gesneriaceae* – 2 genera.

Table 3. Local and Balkan endemics in the countries of Balkan Peninsula.

Taxon	Albania		Bulgaria		Greece (continental part)		FR Yugoslavia		Romania		Turkey (European part)	
	Balk.	Loc.	Balk.	Loc.	Balk.	Loc.	Balk.	Loc.	Balk.	Loc.	Balk.	Loc.
Species	338	22	287	66	329	401	399	160	101	82	48	6
Subspecies	44	2	46	20	39	47	56	22	30	9	16	1
Total	382	24	332	86	367	444	354	182	131	91	64	7

Abbreviations: Balk. – Balkan endemics; Loc. – local endemics.

List of the genera

Apiaceae *Sclerochorton* Boiss. *Hladnikia* Rchb. *Stefanoffia* H. Wolff *Johrenia* DC. ***Apocynaceae*** *Rhazya* Decne. ***Asteraceae*** *Amphoricarpos* Vis. ***Boraginaceae*** *Macrotomia* DC. *Halacsya* Dörfl. *Trachystemon* D. Don ***Brassicaceae*** *Degenia* Hayek *Lepidotrichum* Velen. & Bornm. *Bornmuellera* Hausskn. *Andrzeiowskia* Rchb. ***Caryophyllaceae*** *Bolanthus* (Ser.) Rchb. ***Dioscoreaceae*** *Dioscorea* L.	***Dipsaceae*** *Morina* L. ***Ericaceae*** *Bruckenthalia* Rchb. ***Fabaceae*** *Petteria* C. Presl *Hammatolobium* Fenzl ***Geraniaceae*** *Biebersteinia* Stephan ***Gesneriaceae*** *Haberlea* Friv. *Jankaea* Boiss. ***Liliaceae*** *Strangweia* Bertol. ***Malvaceae*** *Kitaibela* Willd. ***Oleaceae*** *Forsythia* Vahl ***Poaceae*** *Festucopsis* (C.E. Hubb.) Melderis *Danthoniastrum* (Holub) Holub *Phacelurus* Griseb.

The presence of genera from families that differ in terms of evolutionary status and have different origin once again shows the "provoking" role of geological and historical reasons or factors which have led to that floristic complex – some of the genera are mainly related to recent floras (*Poaceae*, *Apiaceae*), but the representatives of *Gesneriaceae* show a little permissible, but real relation to relic floras of South-eastern China – Yunan. So, the possibility for interpretation of the origin of the Balkan endemic flora gains one dimension more. The following questions can be asked:

- Which family has the highest number of endemic species?
- Which genus has the highest number of endemic species?

The answer is the following:

Families are: *Asteraceae* ~ 497 endemic species; *Caryophyllaceae* – 185 endemic species and *Poaceae* – 147 endemic species.

Genera are: *Hieracium* (*Asteraceae*) – 191 endemic species; *Centaurea* (*Asteraceae*) – 106 endemic species and *Campanula* (*Campanulaceae*) – 77 endemic species.

For all the rest of the families and genera there is also an essential endemic element, for example in *Boraginaceae* – 121 endemic species, while genus *Festuca* (*Poaceae*) has 56 endemic species. The analysis of these species can throw additional light on the genesis and consolidation of the Balkan vascular flora.

Conclusions

1. In terms of richness of endemic elements at genus, species and subspecies level the Balkan Peninsula is a main speciation zone in South-east Europe.
2. The "core" of the speciation is in the mountains of the central parts of the Balkan Peninsula.
3. To the North-West and partially to the South the evolutionary capacity goes down. The flora is with a less number of endemic species and is more "monotonous".
4. The existing infraspecific variability gives a reason to predict the speciation of a high number of new taxa at a species rank. The processes of speciation are not slowed down.

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Flora of Akçakoca district (Düzce–Turkey)

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Abstract. The district of Akçakoca (Düzce) which covers mixed deciduous forests is situated in West Karadeniz (Black Sea) region in Turkey. 1510 plant specimens were collected during 20 field trips between the years 2001 and 2003. 103 families, 384 genera, 636 species, 13 subspecies, 8 varieties and 73 cultivated species (totally 657 taxa) were determined. The largest families are *Asteraceae* (66 species), *Fabaceae* (55 species) and *Poaceae* (39 species); the largest genera are *Trifolium* (15 species), *Euphorbia* (11 species) and *Veronica* (9 species). Phytogeographically, Euro–Siberian elements are the first with 163 species (28.95 %), Mediterranean elements are the second with 64 species (11.36 %) and Irano–Turanian elements are the third with 4 species (0.71 %). Endemism ratio is 1.24 % (7 species). Threat categories of species in the area are given.

Key words: Akçakoca, Düzce, Euro–Siberian, flora, Turkey

Introduction

The flora of Turkey which has 163 families, 1168 genera, 8988 species and 10 754 taxa (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000) is too rich because of its various climate, topographical structure, geographical and phytogeographical regions.

The flora of Akçakoca district (Düzce) which covers mixed deciduous forests is not as rich as expected owing to the location in Euro–Siberian region compared with both Mediterranean and Irano–Turanian phytogeographical regions.

The area is in the A3 according to Davis' grid-square system adopted for the *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985).

Akçakoca (Düzce) is situated in the West Karadeniz (Black Sea) region. The research area is surrounded with natural borders which are Karadeniz on the north, Kocaman river on the east, Kaplandede mount on the south and Melen river on the west (Fig. 1). It has a surface of *ca*. 250 km^2.

The altitude of area is between 0 m and 1168 m where the top of the Kaplandede mount is.

The area was not studied in detail but some collections were recorded in *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988).

However, the flora of area between Ereğli–Akçakoca–Yığılca which is near the Akçakoca district was studied by Cöbek in 1989 (MSc Thesis, unpubl.). The flora of Kaplandede mount which is the south border of Akçakoca was studied by Sazak in 1997 (MSc Thesis, unpubl.).

Climate. The ombro-thermic diagrams according to Gaussen are provided for Akçakoca, Alaplı, Ereğli districts and Düzce province (Cireli & al. 1973) (Fig 2). There is not dry or frosty time in Akçakoca (Fig. 2a). The total annual precipitation is 1089 mm and the average annual temperature is 14.2 °C in the area. The precipitation regime is Autumn–Winter–Summer–Spring. Akçakoca has oceanic climate (Akman 1990).

Akçakoca is consisting of Paleozoic massif which has sandstone and schist (Emiroğlu 1970), brown forest and alluvial soils, briefly (Dönmez 2000).

There are many streams and 42 villages in the area (Fig. 1). People's incoming is mainly cultivation of hazelnut. The forest areas are replaced by hazelnut gardens rapidly. This indicates disturbance in Euro–Siberian floristic composition in the research area.

Material and methods

The material given below is based on collections made by the authors between the years 2001 and 2003. Vouchers are deposited in HUB and Hb. Yıldırımlı. 1510 vascular plant specimens have been collected as a result of 20 field works.

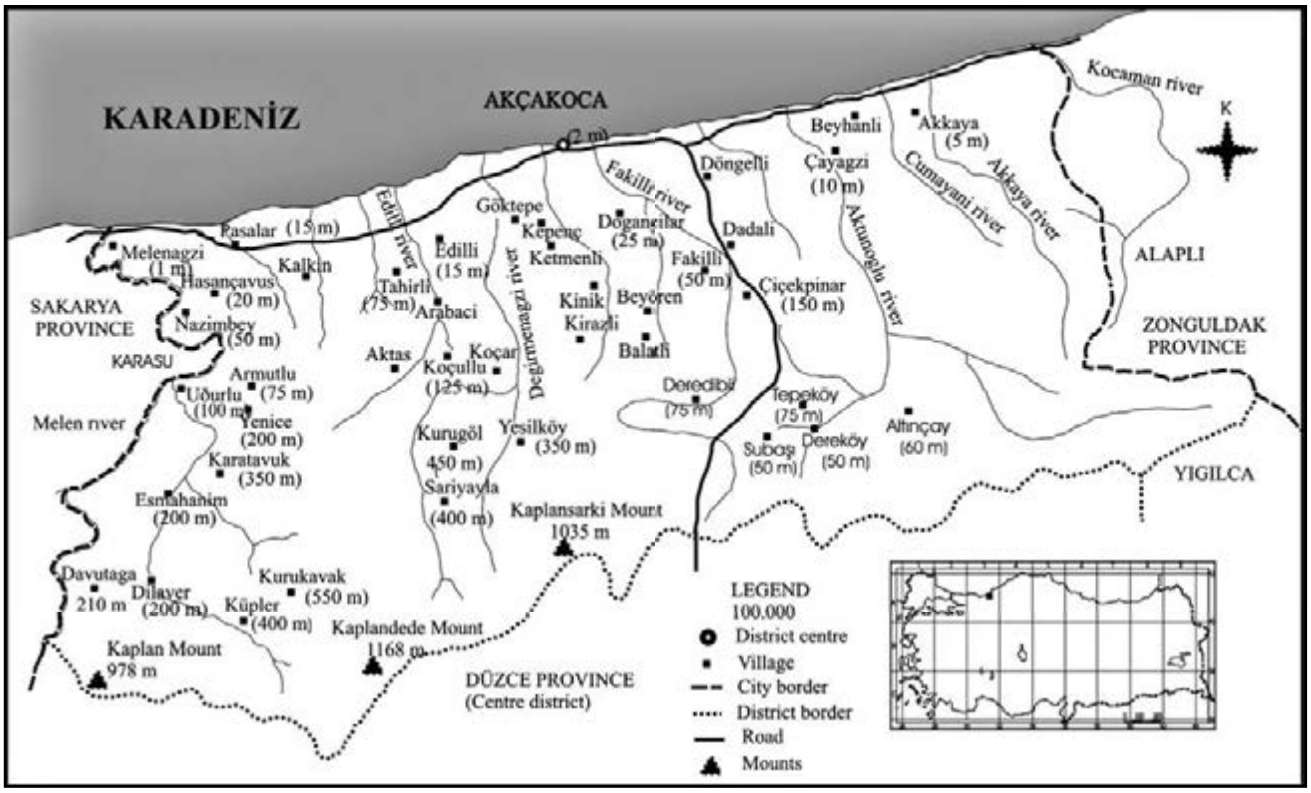

Fig. 1. Geographical map of Akçakoca district (modified Emiroğlu 1970).

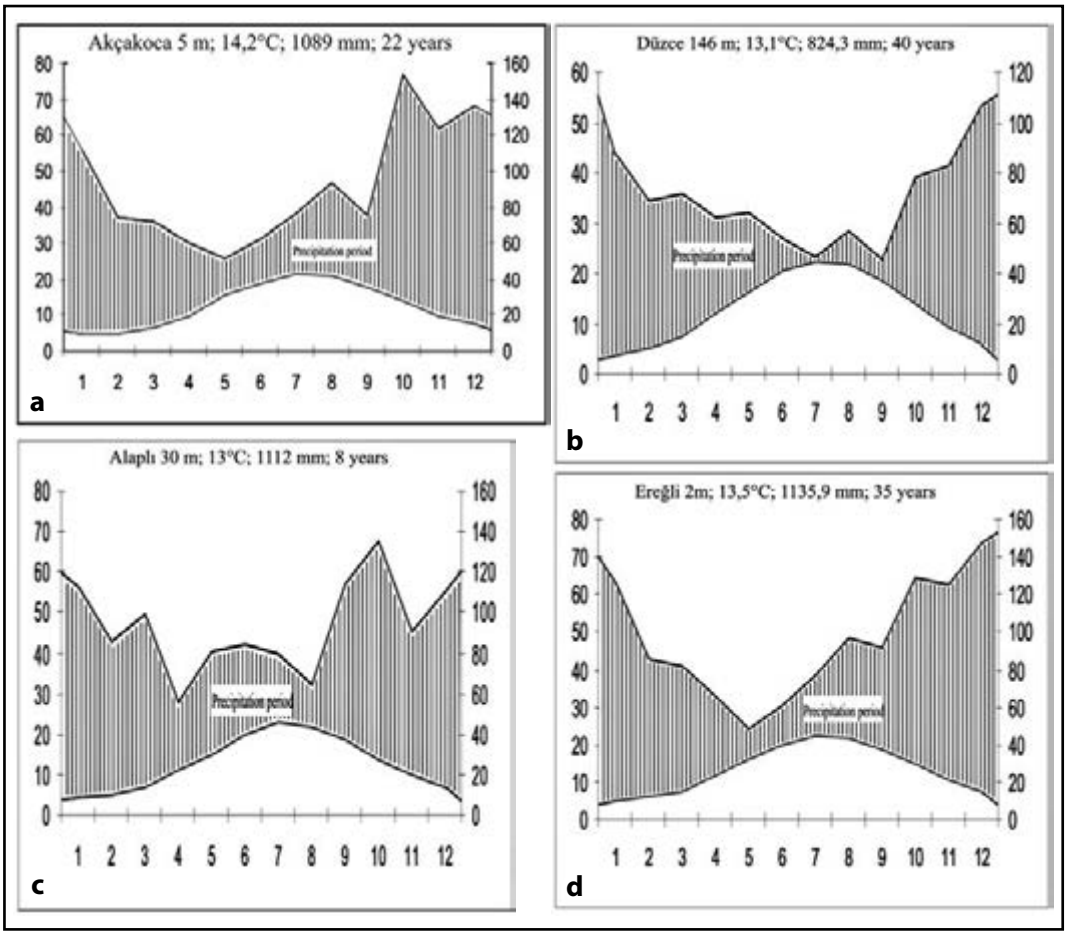

Fig. 2. Ombro-thermic diagrams of: **a** – Akçakoca district; **b** – Düzce province; **c** – Alaplı district; **d** – Ereğli district.

The identifications and the nomenclature were based mainly on *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988) and *Flora Europaea* (Tutin & al. 1964, 1968–1980). A complete list of the vascular plant taxa found in Akçakoca district is given according to Davis' *Flora*. Author abbreviations follow Brummitt & Powell (1992). The cultivated taxa are noted in the list by asterix; their Turkish names are given at the end of the citation. However, the cultivated taxa have not been considered in the floristic analysis.

Results

The Location Key

After the first locality "A3 Düzce: Akçakoca" terms are not repeated in the subsequent localities.

1. A3 Düzce: Akçakoca, Melenağzı village, Melen stream banks, marshy area, 0–10 m, 07.04.2001. **2.** Between Melenağzı and Nazımbey villages, under cultivated *Corylus*, edge of hedges, 1–50 m, 07.04.2001. **3.** Melenağzı village, southern slopes, under *Fagus orientalis* forest, 20 m, 07.04.2001. **4.** Armutlu village, under cultivated *Corylus* and *Fagus orientalis* forest, 75 m, 18.05.2001. **5.** Between Esmahanım and Uğurlu villages, under cultivated *Corylus* and *Fagus orientalis* forest, 100–200 m, 18.05.2001. **6.** Between Dilaver and Küpler villages, under cultivated *Corylus* and *Fagus orientalis* forest, 200–300 m, 18.05.2001. **7.** Armutlu village, south slopes, under *Fagus orientalis* forest, 75 m, 18.05.2001. **8.** Edilli village, under cultivated *Corylus* near the sea, stream banks, coasts, 1–5 m, 19.04.2001. **9.** Tahirli village, mouth of stream, along the streams, under *Fagus orientalis*, 1–25 m, 19.05.2001. **10.** Paşalar village, slopes, under cultivated *Corylus* and *Fagus orientalis* forest, 1–10 m, 19.05.2001. **11.** Küpler village, along the stream, under cultivated *Corylus*, 350–400 m, 07.06.2001. **12.** Around Kurukavak village, under cultivated *Corylus*, 500–550 m, 07.06.2001. **13.** Yenice village, along the road, under cultivated *Corylus*, 200–300 m, 07.06.2001. **14.** Karatavuk village, under cultivated *Corylus*, 300–350 m, 07.06.2001. **15.** Hemşin village, under cultivated *Corylus*, along road, 75–100 m, 07.06.2001. **16.** Koçullu village, under cultivated *Corylus*, 100–125 m, 09.06.2001. **17.** Sarıyayla village, under cultivated *Corylus*, along the road, 400 m, 09.06.2001. **18.** Gebekese village, under cultivated *Corylus*, 75–90 m, 09.06.2001. **19.** Between Koçullu and Sarıyayla villages, under cultivated *Corylus*, along the road, 100–350 m, 09.06.2001. **20.** Kurugöl village, roadsides, slopes, 450–550 m, 09.06.2001. **21.** Yeşilköy village, stream banks, 250–350 m, 09.06.2001. **22.** Around Doğancılar, pasture slopes, 1–50 m, 09.06.2001. **23.** Alaplı's border, around Kocaman, roadsides, meadow, 1–40 m, 10.06.2001. **24.** Around Doğancılar, scrub, pasture slopes, 10.06.2001. **25.** Between Çiçekpınar and Doğancılar villages, along the streams, soil mass, 5–20 m, 10.06.2001. **26.** Tepe village, under mixed deciduous forest (*Fagus orientalis*, *Carpinus betulus*, *Quercus petraea*), 45–75 m, 10.06.2001. **27.** Çayağzı stream banks, marshy area, red soils, 2–25 m, 10.06.2001. **28.** Around Şifalısu, forest pathsides, 200 m, 10.06.2001. **29.** Çiçekpınar village, water depot pathsides, hedges and pathsides, under cultivated *Corylus*, 150–200 m, 29.09.2001. **30.** Edilli village, forest, 5–50 m, 29.09.2001. **31.** Melenağzı village, along Melen stream, 1–50 m, 29.09.2001. **32.** Alaplı's border, along Kocaman stream, under mixed deciduous forest (*Fagus orientalis*, *Carpinus betulus*, *Quercus petraea*), 5–50 m, 29.09.2001. **33.** Around Doğancılar, opposed the hazelnut factory, scrub and groves, 1–50 m, 30.09.2001. **34.** Centrum, Yeni district, 1–5 m, 08.10.2001. **35.** Between Doğancılar and Çiçekpınar villages, roadsides, stream banks, under cultivated *Corylus*, 1–50 m, 24.02.2002. **36.** Düzce road, around Çiçekpınar, roadsides, 100–150 m, 24.02.2002. **37.** Kurukavak village, under cultivated *Corylus*, 550–600 m, 15.03.2002. **38.** Around Doğancılar, pasture, scrub, 1–50 m, 29.06.2002. **39.** Çiçekpınar village, along the streams, 20–30 m, 29.06.2002. **40.** Çiçekpınar village, around sand kiln, pasture, roadsides, 30 m, 29.06.2002. **41.** Alaplı's border, around Kocaman, towards Akçakoca, pasture slopes, 1–40 m, 30.06.2002. **42.** Melenağzı village, along Melen stream, marshy area, grassland, 1–40 m, 30.06.2002. **43.** Between Hasançavuş and Melenağzı villages, under cultivated *Corylus* and *Fagus orientalis* forest, 10–20 m, 30.06.2002. **44.** Nazımbey village, under cultivated *Corylus*, edge of hedges, 40 m, 30.06.2002. **45.** Çiçekpınar village, around sand kiln, *Corylus* fields, roadsides, 30 m, 30.06.2002. **46.** Around Doğancılar, pasture slopes, 1–50 m, 29.06.2002. **47.** Mouth of the Edilli stream, stream banks, coastal sands, 0 m, 30.06.2002. **48.** Uğurlu village, around Şifalısu, Uğurlu stream banks, under *Fagus orientalis* forest, 50–75 m, 22.07.2002. **49.** Esmahanım village, along the road, 100–125 m, 22.07.2002. **50.** Dilaver

village, Dere district, roadsides, stony area, 100–150 m, 22.07.2002. **51.** Uğurlu village, roadsides, under *Fagus orientalis* forest, 200–225 m, 22.07.2002. **52.** Küpler village, near Dere district, 260–300 m, 22.07.2002. **53.** Küpler village, along the stream, 350–370 m, 22.07.2002. **54.** Kurukavak village, along the road, 370–450 m, 22.07.2002. **55.** Kurukavak village, along the road, 500–575 m, 22.07.2002. **56.** Karatavuk village, along the road, slopes, hedge of cultivated *Corylus*, 400–450 m, 22.07.2002. **57.** Bazaar, roadsides, cultivated area, 1–10 m, 23.07.2002. **58.** Around Kale, slopes, scrub, pasture, 15–20 m, 23.07.2002. **59.** Alaplı's border, around Kocaman, coastal sands, 0 m, 23.07.2002. **60.** Çayağzı stream, hedge of cultivated *Corylus*, 0–25 m, 23.07.2002. **61.** Around Yalılar, coastal sands, 0 m, 23.07.2002. **62.** Around Yalılar, near the water depot, 2–5 m, 23.07.2002. **63.** Kurukavak village, under cultivated *Corylus*, 550–590 m, 23.07.2002. **64.** Kurugöl village, towards Sarıyayla village, under cultivated *Corylus*, hedges, 450–550 m, 26.10.2002. **65.** Towards Sarıyayla village, under *Fagus* forest, 350–450 m, 26.10.2002. **66.** Sarıyayla village, along road, 150–200 m, 500–550 m, 26.10.2002. **67.** Around Fakıllı cavern, groves, scrub, 50 m, 27.10.2002. **68.** Kaplandede mount, Kestanebayırı forest, along the road, 150 m, 27.10.2002. **69.** Summit of Kaplandede mount, under the mixed deciduous forest (*Fagus orientalis*, *Carpinus betulus*, *Quercus petraea*), 1000 m, 27.10.2002. **70.** Kaplandede mount, clearings in forest, 730 m, 28.10.2002. **71.** Under Cumayanı forest, 5 m, 28.10.2002. **72.** Kurukavak village, under cultivated *Corylus*, roadsides, 550 m, 07.10.2002. **73.** Centrum, 5 m, 11.10.2002. **74.** Around Doğancılar, pasture slopes, 5–10 m, 15.03.2003. **75.** Kurugöl village, under cultivated *Corylus*, 450 m, 15.03.2003, **76.** Along Melen stream, water area and grassland, under cultivated *Corylus*, 15–20 m, 15.03.2003. **77.** Melenağzı village, along the road, under cultivated *Corylus*, 10–15 m, 15.03.2003. **78.** Paşalar village, along the road, under cultivated *Corylus*, graveyard, 45–75 m, 16.03.2003. **79.** Yukarı district, around graveyard, 45 m, 06.04.2003. **80.** Around Çayağzı village, Çayağzı stream banks, near the coast, 0–40 m, 22.04.2003. **81.** Akkaya stream, picnic area, under cultivated *Corylus*, 5 m, 22.04.2003. **82.** Around Kocaman, pasture, 15 m, 22.04.2003. **83.** Centrum, bazaar, 5 m, 28.07.2003. **84.** Around Doğancılar, pasture slopes, 20–30 m, 10.05.2003. **85.** Between Doğancılar and Çiçekpınar villages, south slopes, scrub, 20 m, 10.05.2003. **86.** Entrance of Deredibi village, roadsides, 40–75 m, 10.05.2003. **87.** The new Düzce road, roadsides, 100–200 m, 10.05.2003. **88.** The old Düzce road, roadsides, 75–200 m, 10.05.2003. **89.** Subaşı village, under cultivated *Corylus*, roadsides, 40–50 m, 10.05.2003. **90.** Sortie of Subaşı village, around Dere village, 50–55 m, 10.05.2003. **91.** Subaşı village road, scrub, 50 m, 10.05.2003. **92.** Around Şifalısu, near forest, 200–250 m, 10.05.2003. **93.** Around Dere village, under cultivated *Corylus*, 50–75 m, 10.05.2003. **94.** Düzce road, Çiçekpınar village, roadsides, 40–75 m, 10.05.2003. **95.** Karaburun village, scrub, under forest, 50–60 m, 11.05.2003. **96.** Melenağzı village, along Melen stream, towards Nazımbey village, marshy area, cultivated *Corylus*, 1–50 m, 11.05.2003. **97.** Near Paşalar village, roadsides, under cultivated *Corylus*, 40–75 m, 11.05.2003. **98.** Centrum, cultivated *Corylus*, 15 m, 24.05.2003. **99.** Centrum, 5 m, 05.07.2003. **100.** Subaşı village, edge of cultivated *Zea mays*, 50 m, 26.07.2003. **101.** Around Akkaya stream, coastal sands, 0 m, 15.08.2002. **102.** Kaplandede mountain, slopes of Forest Ministry Local Organization's depot, forest, 200–700 m, 15.08.2002. **103.** Around Şifalısu, to Kaplandede mount, mixed forest (*Fagus orientalis*, *Carpinus betulus*, *Quercus petraea*), 250–500 m, 06.06.2003. **104.** Şifalısu area, towards Kaplandede mount, mixed forest (*Fagus orientalis*, *Carpinus betulus*, *Quercus petraea*), 250–500 m, 06.06.2003. **105.** Kocaman forest, picnic area, 30 m, 08.06.2003. **106.** Summit of Kaplandede mount and around tomb, mixed and deciduous forest, 110–1150 m, 07.06.2003. **107.** Edilli village, sandy, 1–50 m, 29.09.2001. **108.** Alaplı, around Shell, bank of road, 20 m, 08.06.2003. **109.** Around Orman işletmesi, coastal sandy, banks of stream, 1–5 m, 08.06.2003. **110.** Edilli village, coastal sandy, 1–50 m, 29.09.2001. **111.** Along Melen stream, meadow, *Corylus* fields, 15–20 m, 15.03.2003. **112.** Around Yalılar, 0–2 m, 23.07.2002. **113.** Edilli village, pasture slopes, 1–50 m, 29.09.2001. **114.** To Alaplı, pass Çayağzı village, limestone slope, 1–50 m, 08.06.2003. **115.** Edilli village, *Corylus* fields, 1–50 m, 29.09.2001. **116.** Edilli village, Mehmet Emin's garden, 20–30 m, 07.06.2003. **117.** Dadalı village, around harvest, 100–120 m, 12.08.2003. **118.** Altınçay village, *Corylus* fields, 15 m, 24.05.2003. **119.** To Alaplı, banks of road, around Karayolları, 10 m, 08.06.2003. **120.** Slopes of Kale, shrubs, 40–50m, 08.05.2006.

The Floristic List

Abbreviations: ADK – Aslı Doğru Koca, ISTO – the Herbarium of Istanbul University, Forest Faculty, D. – P.H. Davis, ŞY – Şinasi Yıldırımlı.

PTERIDOPHYTA

EQUISETACEAE ***Equisetum ramosissimum*** Desf. 62, ADK 1977; ***E. palustre*** L. 42, ADK 1739; 96, ADK 2307; ***E. arvense*** L. 48, ADK 1769; 31, ADK 1534, ŞY; ***E. telmateia*** Ehrh. 65, ŞY 28198, ADK; 95, ADK 2301; 2, ADK 1045.

ADIANTACEAE ***Adiantum capillus-veneris*** L. 65, ADK 2026, ŞY; 30, ADK 1489, ŞY.

HYPOLEPIDACEAE ***Pteridium aquilinum*** (L.) Kuhn 51, ADK 1824; 29, ADK 1461, ŞY; 2, ADK 1043; 88, ADK 2257.

ASPLENIACEAE ***Asplenium trichomanes*** L., Kaplandede mount, east side, near the water, rocky and moist area, 400–800 m, S. Sazak, ISTO 27530; ***A. adiantum-nigrum*** L. 68, ŞY 28231, ADK; 67, ADK 2062, ADK; ***Phyllitis scolopendrium*** (L.) Newman 48, ADK 1770; 67, ADK 2061, ŞY; 65, ADK 2028, ŞY.

ATHYRIACEAE ***Athyrium filix-femina*** (L.) Roth 60, ADK 1948; 88, ADK 2259.

ASPIDIACEAE ***Polystichum setiferum*** (Forssk.) Woyn. 51, ADK 1822; ***Dryopteris borreri*** Newman 64, ŞY 28156, ADK. Euro–Siberian element.

BLECHNACEAE ***Blechnum spicant*** (L.) Roth 30, ADK 1490, ŞY; 64, ADK 2003, ŞY.

SPERMATOPHYTA

GYMNOSPERMAE

PINACEAE ****Abies bornmuelleriana*** Mattf. 78, ADK 2158, köknar; ****Picea orientalis*** (L.) Link 79, ADK 2164, ladin; ****Cedrus deodora*** (Roxb.) G. Don 78, ADK 2157, sedir; ****Pinus pinea*** L. 104, ŞY 28588, fıstık çamı; ****P. sylvestris*** L. 56, ADK 1879, sarıçam.

TAXACEAE ****Taxus baccata*** L. 105, ŞY 28841, porsuk ağacı.

CUPRESSACEAE ****Cupressus sempervirens*** L. 78, ADK 2154, selvi; ***Juniperus sabina*** L. 60, ADK 1947; ***J. oxycedrus*** L. var. ***oxycedrus*** 58, ADK 1923.

ANGIOSPERMAE

DICOTYLEDONEAE

RANUNCULACEAE ***Helleborus orientalis*** Lam. 13, ADK 1280. Euxine element; ***Nigella damascena*** L. 58, ADK 1918; ***Actaea spicata*** L. 8, ADK 1269; ***Caltha polypetala*** Hochst. ex Lorent 1, ADK 1003; 2, ADK 1045; 35, ADK 1608; 5, ADK 1145; ***Clematis vitalba*** L. 20, ADK 1351; 17, ADK 1304; 32, ADK 1570, ŞY; 46, ADK 1680; ***Ranunculus constantinopolitanus*** (DC.) d'Urv. 8, ADK 1173; 2, ADK 1056; 15, ADK 1286; ***R. marginatus*** d'Urv. var. ***trachycarpus*** (Fisch. & C.A. Mey.) Azn. 84, ADK 2203; 85, ADK 2241; 97, ADK 2311; ***R. muricatus*** L. 4, ADK 1112; ***R. ophioglossifolius*** Vill. 96, ADK 2308; ***R. paludosus*** Poir. 8, ADK 1188.; ***R. polyanthemos*** L. 28, ADK 1460; ***R. repens*** L. 5, ADK 1125.

BERBERIDACEAE ***Epimedium pubigerum*** (DC.) G. Moreno & Decne. 10, ADK 1234; 83, ADK 1299A; 8, ADK 1200.

PAPAVERACEAE ***Chelidonium majus*** L. 53, ADK 1864; 84, ADK 2194. Euro–Siberian element; ***Glaucium leiocarpum*** Boiss. 59, ADK 1928; ***Papaver lacerum*** Popov 42, ADK 1736; 15, ADK 1292; ***P. rhoeas*** L. 15, ADK 1292A; ***P. dubium*** L. 85, ADK 2225; ***Corydalis caucasica*** DC., Kaplandede mount, west side, bank of road, under *Castanea*, 800–900 m, S. Sazak. Euxine element.

BRASSICACEAE ****Brassica oleracea*** L. 64, ADK 2016, kara lahana; ****B. rapa*** L. var. ***rapa*** 57, ADK 2386, şalgam; ***Raphanus raphanistrum*** L. 23, ADK 1395; 30, ADK 1503; ***Calepina irregularis*** (Asso) Thell. 80, ADK 2175; ***Rapistrum rugosum*** (L.) All. 41, ADK 1694; ***Cakile maritima*** Scop. 107, ADK 1505; ***Lepidium sativum*** L. subsp. ***sativum*** 92, ADK 2276, tere otu; ***L. virginicum*** L. 28, ADK 1453; ***Cardaria draba*** (L.) Desv. subsp. ***draba*** 98, ADK 2340; ***Thlaspi perfoliatum*** L. 80, ADK 2168; ***T. alliaceum*** L. 93, ADK 2281; 85, ADK 2247; ***Capsella bursa-pastoris*** (L.) Medik. 92, ADK 2275; 91, ADK 2261; ***Andrzeiowskia cardaminifolia*** (DC.) Prantl 8, ADK 1178; ***Erophila verna*** (L.) Chevall. subsp. ***verna*** 76, ADK 2142; ***E. verna*** (L.) Chevall. subsp. ***praecox*** (Stev.) Walters 74, ADK 2114; ***Nasturtium officinale*** R. Br. 16, ADK 1297; 12, ADK 1271; 1, ADK 1001; 5, ADK 1143; ***Rorippa sylvestre*** (L.) Bessey 17, ADK 1309; 23, ADK 1391; 39, ADK 1640; ***Barbarea vulgaris*** R. Br. 93, ADK 2280; 97, ADK 2321; 85, ADK 2243; ***Cardamine bulbifera*** (L.) Crantz 5, ADK 1142; ***C. quinquefolia*** (M. Bieb.) Schmalh. 2, ADK 1053; 37, ADK 1615. Euro–Siberian element; ***C. lazica*** Boiss. & Balansa, ŞY 28636, ADK; 103, ŞY 28595, ADK. Euxine element; ***C. impatiens*** L. var. ***impatiens*** 4, ADK 1115. Euro–Siberian element; ***C. impatiens*** L. var. ***pectinata*** (Pall.) Trautv. 5, ADK 1119; ***C. hirsuta*** L. 1, ADK 1032; 3, ADK 1075; ***Maresia nana*** (DC.) Batt. 9, ADK 1203; ***Alliaria petiolata*** (M. Bieb.) Cavara & Grande 8, ADK 1180; 5, ADK 1141; ***Sisymbrium altissimum*** L. 5, ADK 1320; 2, ADK 1059; ***S. irio*** L. 92, ADK 2274; ***S. officinale*** (L.) Scop. 41, ADK 1699.

CISTACEAE ***Cistus creticus*** L. 23, ADK 1393; 19, ADK 1336; 25, ADK 1432; 29, ADK 1487; 44, ADK 1725. Omni–Mediterranean element; ***C. salviifolius*** L. 8, ADK 1194; 9, ADK 1212; 5, ADK 1121; ***Helianthemum nummularium*** (L.) Mill. subsp. ***nummularium*** 85, ADK 2223.

VIOLACEAE ***Viola odorata*** L. 75, ADK 2126; 76, ADK 2138; 37, ADK 1621; 2, ADK 1065; ***V. sieheana*** W. Becker 37, ADK 1622; ***V. canina*** L. 2, ADK 1063; 35, ADK 1600; 8, ADK 1177; ***V. kitaibeliana*** Roem. & Schult. 84, ADK 2192.

POLYGALACEAE ***Polygala supina*** Schreb. 39, ADK 1634; 85, ADK 2237; 94, ADK 2293.

PORTULACACEAE ***Portulaca oleracea*** L. 57, ADK 1886.

CARYOPHYLLACEAE ***Arenaria serpyllifolia*** L. 86, ADK 2251; ***Minuartia imbricata*** (M. Bieb.) Woronow 106, ŞY 28661, ADK. Euxine element; ***Moehringia trinervia*** (L.) Clairv. 103, ŞY 28596, ADK; 106, ŞY 28663A, ADK; ***Stellaria media*** (L.) Vill. subsp. ***media*** 9, ADK 1202; 1, ADK 1039; 8, ADK 1185; ***S. holostea*** L. 2, ADK 1049. Euro–Siberian element; ***Myosoton aquaticum*** (L.) Moench 12, ADK 1272. Euro–Siberian element; ***Cerastium anomalum*** Waldst. & Kit. 80, ADK 2172; ***C. fontanum*** Baumg. subsp. ***triviale*** (Link) Jalas 1, ADK 1020; 1, ADK 1029; 111, ADK 2136; ***C. pumilum*** Curtis 8, ADK 1171; 81, ADK 2182; 4, ADK 1113; ***Spergularia bocconii*** (E. Scheele) Asch. & Graebn. 84, ADK 2189; ***Dianthus armeria*** L. subsp. ***armeria*** 23, ADK 1403. Euro–Siberian element; ***Saponaria officinalis*** L. 52, ADK 1842; ***Silene italica*** (L.) Pers. 60, ADK 1961; ***S. vulgaris*** (Moench) Garcke, Kaplandede mount, surrounding of fountain, bank of road, 970 m, S. Sazak, ISTO 27583; ***S. gallica*** L. 108, ŞY 28728.

ILLECEBRACEAE ***Herniaria hirsuta*** L. 109, ŞY 28780, ADK.

POLYGONACEAE ***Polygonum salicifolium*** Brouss. ex Willd. 31, ADK 1544; 171, ADK 1302. Mediterranean element; ***P. lapatifolium*** L. 42, ADK 1738; ***P. persicaria*** L. 73, ADK 2101, ŞY; 34, ADK 1592; 69, ADK 2075; ***P. hydropiper*** L. 64, ADK 2016, ŞY; ***P. maritimum*** L. 61, ADK 1982; 61, ADK 1983; ***P. mesembricum*** Chrtek 110, ADK 1522, ŞY; 67, ADK 2040, ŞY; ***P. aviculare*** L. 32, ADK 1573, ŞY; ***Rumex acetosella*** L. 106, ŞY 28677, ADK; ***R. crispus*** L. 25, ADK 1433; 12, ADK 1278; 44, ADK 1724; 65, ADK 2032, ŞY; ***R. conglomeratus*** Murray 51, ADK 1803; ***R. pulcher*** L. 119, ŞY 28759.

CHENOPODIACEAE ***Beta vulgaris*** L. var. ***cicia*** (L.) Moq. 57, ADK 2387, pazı; ***Chenopodium album*** L. subsp. ***album*** var. ***album*** 67, ADK 2041; 71, ADK 2086, ŞY; 32, ADK 1563, ŞY; ***C. album*** L. subsp. ***album*** var. ***microphyllum*** (Boenn.) Aellen 71, ADK 2085, ŞY; ***Atriplex tatarica*** L. var. ***tatarica*** 30, ADK 1412; ***A. hastata*** L. 30, ADK 1511; ***Salsola tragus*** L. 30, ADK 1510; 58, ADK 1921; 59, ADK 1940; 61, ADK 1984.

AMARANTHACEAE ***Amaranthus chlorostachys*** Willd. 25, ADK 1436; ***A. patulus*** Bertol. 67, ADK 2047, ŞY; ***A. lividus*** L. 99, ADK 2377; ***A. spinosus*** L. 31, ŞY 27226, ADK.

PHYTOLACCACEAE ***Phytolacca americana*** L. 23, ADK 1408; 64, ADK 2009, ŞY; 51, ADK 1821.

HYPERICACEAE ***Hypericum calycinum*** L. 18, ADK 1323; 12, ADK 1266. Euxine element; ***H. bithynicum*** Boiss. 11, ADK 1262; 18, ADK 1320; 15, ADK 1289; ***H. perforatum*** L. 46, ADK 1670; 44, ADK 1727; 50, ADK 1797; 22, ADK 1363.

MALVACEAE ***Malva sylvestris*** L. 23, ADK 1384; ***M. neglecta*** Wallr. 11, ADK 1263; ***Lavatera punctata*** All. 40, ADK 1641; ***Alcea setosa*** (Boiss.) Alef. 41, ADK 1704. Mediterranean element; ***Althea hirsuta*** L. 18, ADK 1328.

TILIACEAE ***Tilia argentea*** Desf. ex DC. 106, ŞY 28691; 39, ADK 1632.

LINACEAE ***Linum trigynum*** L. 4, ADK 1103; 10, ADK 1227; 42, ADK 1742. Mediterranean element; ***L. bienne*** Mill. 84, ADK 2217. Mediterranean element.

GERANIACEAE ***Geranium purpureum*** Vill. 8, ADK 1179; 5, ADK 1133; 13, ADK 1284; 53, ADK 1854; 48, ADK 1759; 60, ADK 1964; ***G. robertianum*** L. 4, ADK 1103; ***G. rotundifolium*** L. 11, ADK 1261; ***G. pyrenaicum*** Burm. f. 84, ADK 2197; ***G. pusillum*** Burm. 55, ADK 1872; ***G. dissectum*** L. 6, ADK 1153; ***G. asphodeloides*** Burm. subsp. ***asphodeloides*** 10, ADK 1236; 37, ADK 1614; 2, ADK 1055. Euro–Siberian element; ***G. cinereum*** Cav. subsp. ***subcaulescens*** (L'Hér. ex DC.) Hayek var. ***subcaulescens*** 4, ADK 1103A; ***Erodium cicutarium*** (L.) L'Hér. subsp. ***cicutarium*** 84, ADK 2196; ***E. acaule*** (L.) Bech. & Thell. 35, ADK 1598; 74, ADK 2117. Mediterranean element.

OXALIDACEAE ***Oxalis articulata*** Savign. 108, ŞY 28734, ADK; ***O. corniculata*** L. 32, ADK 1574, ŞY; 84, ADK 2217; 67, ADK 2039, ŞY.

BALSAMINACEAE ***Impatiens noli-tangere*** L. 31, ADK 1540, ŞY. Euro–Siberian element.

ACERACEAE ***Acer trautvetteri*** Medw. 106, ŞY 28645, ADK. Euxine element; ***A. campestre*** L. subsp. ***campestre*** 39, ADK 1638.

STAPHYLEACEAE ***Staphylea pinnata*** L. 12, ADK 1276; 5, ADK 1117; 21, ADK 1360; 85, ADK 2239.

VITACEAE ***Vitis vinifera*** L. 29, ADK 1479, ŞY, üzüm; ***Parthenocissus vitacea*** (Knerr) Hitchc. 73, ADK 2108.

RHAMNACEAE ***Paliurus spina-christi*** Mill. 22, ADK 1377; ***Frangula alnus*** Mill. subsp. ***alnus***, Akçakoca, Kühne 150. Euro–Siberian element.

AQUIFOLIACEAE ***Ilex colchica*** Pojark. 5, ADK 1147; 68, ADK 2056, ŞY. Euxine element.

FABACEAE ***Albizzia julibrissin*** Durazz. 49, ADK 1779. Hyrcano–Euxine element; ***Sophora jaubertii*** Spach 20, ADK 1341; 46, ADK 1672; 52, ADK 1835; 11, ADK 1248; 65, ADK 2024, ŞY. Euxine element; ***Chamaecytisus hirsutus*** (L.) Link 88, ADK 2256; 91, ADK 2270; ***Genista tinctoria*** L. 23, ADK 1386; 12, ADK 1267; 16, ADK 1300; 60, ADK 1959. Euro–Siberian element; ***Spartium junceum*** L. 23, ADK 1385; ***Argyrolobium biebersteinii*** P.W. Ball 46, ADK 1625; 23, ADK 1388A; 171, ADK 1313; 32, ADK 1577, ŞY; 72, ADK 2098, ŞY; ***Lupinus albus*** L. subsp. ***graecus*** (Boiss. & Spruner) Franco & Q.J.P. Silva, Akçakoca, Künhe 110. Mediterranean element; ***Robinia pseudoacacia*** L. 15, ADK 1279; 16, ADK 1294; 49, ADK 1791, akasya; ***Galega officinalis*** L. 52, ADK 1841; 59, ADK 1943; 51, ADK 1813; 48, ADK 1757; 46, ADK 1656. Euro–Siberian element; ***Astragalus glycyphyllos*** L. subsp. ***glycyphylloides*** (DC.) Matthews 46, ADK 1672A. Euro–Siberian element; ***Psoralea bituminosa*** L. 41, ADK 1695; 23, ADK 1416; 18, ADK 1322; 20, ADK 1347. Mediterranean element; ***Phaseolus vulgaris*** L. 57, ADK 1968, fasulye; ***Vigna unguiculata*** (L.) Walp. 57, ADK 1885, börülce; ***Cicer arietinum*** L. 46, ADK 1656, nohut; ***Vicia crocea*** (Desf.) B. Fedtsch. 106, ŞY 28669, ADK. Hyrcano–Euxine element; ***V. cassubica*** L., Kaplandede mount, west side, bank of road, under *Castanea*, 800–900 m, S. Sazak, ISTO 27519. Euro–Siberian element; ***V. cracca*** L. subsp. ***gerardii*** Gaudin 10, ADK 1240; ***V. villosa*** I.L. Roth subsp. ***eriocarpa*** (Hausskn.) P.W. Ball, from Düzce to Akçakoca, 250 m, D. 37476; ***V. laxiflora*** Brot. 94, ADK 2292. Mediterranean element; ***V. cuspidata*** Boiss. 84, ADK 2198. Mediterranean element; ***V. sativa*** L. subsp. ***nigra*** (L.) Ehrh. var. ***nigra*** 6, ADK 1156; ***Lathyrus aureus*** (Steven) D. Brândză 108, ŞY 28730. Euxine element; ***L. venetus*** (Mill.) Wohlf. 6, ADK 1151; 91, ADK 2271. Euro–Siberian element; ***L. laxiflorus*** (Desf.) Kuntze subsp. ***laxiflorus*** 5, ADK 1127; 18, ADK 1333; 11, ADK 1253; 6, ADK 1154; 10, ADK 1221; ***L. saxatilis*** (Vent.) Vis. 84, ADK 2219. Mediterranean element; ***L. nissolia*** L. 11, ADK 1249; ***Pisum sativum*** L. subsp. ***sativum*** var. ***sativum*** 84, ADK 2200, bezelye; ***Ononis spinosa*** L. subsp. ***leiosperma*** (Boiss.) Širj. 58, ADK 1913; ***Trifolium uniflorum*** L. 84, ADK 2194A. Mediterranean element; ***T. repens*** L. var. ***repens*** 9, ADK 1201; 11, ADK 1245; 84, ADK 2188; ***T. repens*** L. var. ***giganteum*** Lagr.-Foss. 20, ADK 1339A; 97, ADK 2317; 93, ADK 2283; ***T. nigrescens*** Viv. subsp. ***nigrescens*** 46, ADK 1653; ***T. nigrescens*** Viv. subsp. ***petrisavii*** (Clem.) Holmboe 25, ADK 1437; ***T. mesogitanum*** Boiss. 44, ADK 1728; 22, ADK 1368; 44, ADK 1729. Mediterranean element; ***T. campestre*** Schreb. 18, ADK 1329; 40, ADK 1644; ***T. patens*** Schreb. 97, ADK 2319; ***T. resupinatum*** L. var. ***resupinatum*** 97, ADK 2320; ***T. resupinatum*** L. var. ***microcephalum*** D. Zohary 25, ADK 1437; 18, ADK 1332; 22, ADK 1369; ***T. clusii*** Godr. & Gren. 9, ADK 1210; 11, ADK 1255; 46, ADK 1666; ***T. pratense*** L. var. ***pratense*** 53, ADK 1863; 5, ADK 1140; 69, ADK 2069, ŞY; ***T. caudatum*** Boiss., Kaplandede mount, summit, clearing of forest, 1168 m, S. Sazak. Endemic (LC); ***T. lucanicum*** Gasp. 85, ADK 2234; ***T. lappaceum*** L. 24, ADK 1421. Mediterranean element; ***T. arvense*** L. var. ***arvense*** 23, ADK 1388; 41, ADK 1708; ***T. angustifolium*** L. var. ***angustifolium*** 22, ADK 1361; ***T. alexandrium*** L. 84, ADK 2193; ***Melilotus officinalis*** (L.) Desr. 108, ŞY 28732, ADK; ***Medicago orbicularis*** (L.) Bartal. 84, ADK 2197; ***M. lupulina*** L. 55, ADK 1878, ŞY; 48, ADK 1754, ŞY; 84, ADK 2195; ***M. minima*** (L.) Bartal. var. ***minima*** 84, ADK 2192; ***M. polymorpha*** L. var. ***polymorpha*** 85, ADK 2231; ***M. polymorpha*** L. var. ***vulgaris*** (Benth.) Shinners 26, ADK 1445; 11, ADK 1251; 97, ADK 2322; 84, ADK 2196; ***M. arabica*** (L.) Huds. 1, ADK 1015; 84, ADK 2218; ***M. marina*** L. 61, ADK 1974, ŞY; ***M. littoralis*** Rohde ex Loisel. 9, ADK 1205; ***M. rigidula*** (L.) All. var. ***rigidula*** 84, ADK 2191; ***Dorycnium hirsutum*** (L.) Ser. 8, ADK 1183. Mediterranean element; ***D. pentaphyllum*** Scop. subsp. ***herbaceum*** (Vill.) Rouy 46, ADK 1671A; ***D. pentaphyllum*** Scop. subsp. ***anatolicum*** (Boiss.)

Gams 46, ADK 1671; ***D. graecum*** (L.) Ser. 18, ADK 1326; 10, ADK 1222; 20, ADK 1339; 12, ADK 1270; 51, ADK 1829. Euxine element; ***Lotus angustissimus*** L. 23, ADK 1392; ***L. corniculatus*** L. var. ***corniculatus*** 171, ADK 1313A; ***L. corniculatus*** L. var. ***tenuifolius*** L. 20, ADK 1348; 46, ADK 1651; 42, ADK 1717; ***Coronilla varia*** L. subsp. ***varia*** 20, ADK 1346; ***Hippocrepis unisiliquosa*** L. subsp. ***unisiliquosa*** 84, ADK 2189; 85, ADK 2236; ***Scorpiurus muricatus*** L. var. ***subvillosus*** (L.) Fiori 40, ADK 1642; 84, ADK 2190; 85, ADK 2228. Mediterranean element.

ROSACEAE ***Laurocerasus officinalis*** M. Roem. 2, ADK 1159; 52, ADK 1833; 78, ADK 2161; 98, ADK 2333; ***Prunus spinosa*** L. subsp. ***dasyphylla*** (Schur) Domin 58, ADK 1917; ***P.** × **domestica*** L. 49, ADK 1778, erik; ***P. divaricata*** Ledeb. subsp. ***divaricata*** 41, ADK 1705; 36, ADK 1612; 84, ADK 2191; 80, ADK 2177; ***Cerasus avium*** (L.) Moench 49, ADK 1772, kiraz; ***C. vulgaris*** Mill. 57, ADK 1885, vişne; ***Persica vulgaris*** Mill. 73, ADK 2105; 80, ADK 2178, şeftali; ***Rubus sanctus*** Schreb. 29, ADK 1472, ŞY; 30, ADK 1514, ŞY; ***R. discolor*** Weihe & Nees 18, ADK 1325; 12, ADK 1275; ***R. tereticaulis*** P.J. Müll. 1, ADK 1009; ***R. hirtus*** Waldst. & Kit. 68, ADK 2060, ŞY; 171, ADK 1303. Euro–Siberian element; ***Potentilla detommasii*** Ten. 22, ADK 1362; ***P. reptans*** L. 21, ADK 1357; 11, ADK 1265; 40, ADK 1646; 53, ADK 1853; 171, ADK 1310; ***Fragaria vesca*** L. 15, ADK 1187; 2, ADK 1042; 4, ADK 1092; 39, ADK 1747; ***F. × ananassa*** Duchesne 92, ADK 2277, çilek; ***Geum urbanum*** L. 60, ADK 1950; 48, ADK 1766. Euro–Siberian element; ***Agrimonia eupatoria*** L. 30, ADK 1513, ŞY; 42, ADK 1737; 52, ADK 1846; 58, ADK 1915; ***Sanguisorba minor*** Scop. subsp. ***muricata*** (Spach) Briq. 23, ADK 1419; ***Rosa canina*** L. 10, ADK 1225; 3, ADK 1078; 11, ADK 1259; ***R. damascena*** Mill. 23, ADK 1414A; 49, ADK 1783, gül; ***Mespilus germanica*** L. 58, ADK 1907; 7, ADK 1765. Hyrcano–Euxine element; ***Pyracantha coccinea*** M. Roem. 24, ADK 1429; 30, ADK 1515, ŞY; 66, ADK 2034, ŞY; 58, ADK 1922; ***Crataegus pentagyna*** Waldst. & Kit. ex Willd. 41, ADK 1685; 32, ADK 1564, ŞY. Euro–Siberian element; ***C. rhipidophylla*** Gand. 8, ADK 1175; ***C. microphylla*** C. Koch 10, ADK 1220. Hyrcano–Euxine element; ***Sorbus domestica*** L. 99, ADK 2373; ***Eriobotrya japonica*** (Thunb.) Lindl. 73, ADK 2104, yeni dünya; ***Cydonia oblonga*** Mill. 89, ADK 2263, ayva; ***Malus sylvestris*** Mill. subsp. ***orientalis*** (Uglitzk.) Browicz var. ***orientalis*** 10, ADK 1233; 29, ADK 1471, ŞY; ***M. sylvestris*** Mill. subsp. ***mitis*** (Wallr.) Mansf. 41, ADK 1702, elma; ***Pyrus communis*** L. subsp. ***communis*** 29, ADK 1471, ŞY; 10, ADK 1233; ***Chaenomeles speciosa*** (Sweet) Nakai 37, ADK 1617; ***Kerria japonica*** (L.) DC. 49, ADK 1773.

MYRTACEAE ***Myrtus communis*** L. subsp. ***communis*** 58, ADK 1919.

PUNICACEAE ***Punica granatum*** L. 67, ADK 2049, ŞY, nar.

LYTHRACEAE ***Lythrum hyssopifolia*** L. 42, ADK 1718; ***L. salicaria*** L. 61, ADK 1978; 46, ADK 1626, ŞY. Euro–Siberian element.

ONAGRACEAE ***Circaea lutetiana*** L. 50, ADK 1801; 51, ADK 1809; ***Epilobium hirsutum*** L. 54, ADK 1866; ***E. parviflorum*** Schreb. 65, ADK 2030, ŞY; 56, ADK 1881; ***E. montanum*** L. 10, ADK 1299; ***E. minutiflorum*** Hausskn. 54, ADK 1865; 44, ADK 1730. Euro–Siberian element.

CALLITRICHACEAE ***Callitriche stagnalis*** Scop. 108, ŞY 28720, ADK.

CUCURBITACEAE ***Cucurbita pepo*** L. 99, observation, bal kabağı; ***C. moschata*** Duchesne 60, ADK 1967; 49, ADK 1777, sakız kabağı; ***Cucumis sativus*** L. 99, observation, salatalık.

DATISCACEAE ***Datisca cannabina*** L. 65, ADK 2027, ŞY; 55, ADK 1870.

CRASSULACEAE ***Sedum pallidum*** M. Bieb. var. ***bithynicum*** (Boiss.) Chamb. 22, ADK 1375.

SAXIFRAGACEAE ***Saxifraga rotundifolia*** L. Around Kocaman and Çorakiçi mevkii, 210 m, 25.06.1987, Cöbek 1102. Euro–Siberian element; ***S. cymbalaria*** L. var. ***cymbalaria*** 84, ADK 2204; 86, ADK 2244.

GROSSULARIACEAE ***Ribes alpinum*** L. 103, ŞY 28607, ADK; 18, ADK 1325; ***R.* aff. *biebersteinii*** Berland. ex DC. 103, ŞY 28605, ADK. Euxine element.

APIACEAE ***Sanicula europaea*** L. 4, ADK 1101; 5, ADK 1132; 48, ADK 1756, ADK; ***Eryngium maritimum*** L. 110, ADK 1491, ŞY; 59, ADK 1938; ***E. creticum*** Lam. 58, ADK 1902; 46, ADK 1681. Mediterranean element; ***Chaerophyllum aromaticum*** L. 49, ADK 1760, ŞY; 52, ADK 1840, ŞY. Euro–Siberian element (Yıldırımlı & Koca 2003); ***Scandix pecten-veneris*** L. 85, ADK 2227; ***Smyrnium perfoliatum*** L. 89, ADK 2260; ***Crithmum maritimum*** L. 110, ADK 1492, ŞY; ***Oenanthe fistulosa*** L. 93, ADK 2282; 20, ADK 1350; 58, ADK 1899; 4, ADK 1093; ***O. pimpinelloides*** L. 18, ADK 1314; 39, ADK 1630; ***Aethusa cynapium*** L. 64, ADK 2013, ŞY; 65, ADK 2033, ŞY. Euro–Siberian element; ***Anethum graveolens*** L. 98, ADK 2330, dere otu; ***Conium maculatum*** L. 98, ADK 2368; ***Petroselinum crispum*** (Mill.) A.W. Hill 42, ADK 1719, maydanoz; ***Angelica sylvestris*** L. var. ***sylvestris***, Kaplandede mount, west side, bank of road, under *Castanea*, 800–900 m, S. Sazak, ISTO 27557. Euro–Siberian element; ***Peucedanum aegopodioides*** (Boiss.) Vandas 117, ADK 2388. Euro–Siberian element; ***Heracleum platytaenium*** Boiss. 120, ADK 2391. Endemic (LC); ***Torilis arvensis*** (Huds.) Link subsp. ***arvensis*** 62, ADK 1972; 52, ADK 1837; ***Daucus carota*** L. 59, ADK 1911; ***D. guttatus*** Sm. 29, ADK 1462, ŞY; 30, ADK 1493; 10, ADK 1235.

ARALIACEAE ***Hedera helix*** L. 33, ADK 1579, ŞY; 2, ADK 1062; 67, ADK 2046, ŞY.

CORNACEAE ***Cornus sanguinea*** L. subsp. ***sanguinea*** 33, ADK 1584; ***C. sanguinea*** L. subsp. ***australis*** (C.A. Mey.) Jáv**.** 10, ADK 1223; 11, ADK 1260; ***C. mas*** L. 74, ADK 2121; 35, ADK 1605. Euro–Siberian element.

CAPRIFOLIACEAE ***Sambucus ebulus*** L. 18, ADK 1321; 30, ADK 1507, ŞY; 41, ADK 1696; 53, ADK 1859. Euro–Siberian element; ***S. nigra*** L. 23, ADK 1407; 38, ADK 1624; 53, ADK 1855. Euro–Siberian element; ***Lonicera xylosteum*** L. 98, ADK 2340; ***Symphoricarpos albus*** L. 49, ADK 1782.

RUBIACEAE ***Asperula involucrata*** Wahlenb. 23, ADK 1390. Euxine element; ***A. taurina*** L. subsp. ***taurina*** 5, ADK 1139A; ***Galium rotundifolium*** L. 15, ADK 1288. Euro–Siberian element; ***G. verum*** L. subsp. ***verum*** 58, ADK 1908. Euro–Siberian element; ***G. album*** Mill. subsp. ***pycnotrichum*** (Heinr. Braun) Krendl 68, ADK 2054, ŞY; 20, ADK 1340. Euro–Siberian element; ***G. odoratum*** (L.) Scop. 5, ADK 1139. Euro–Siberian element; ***Rubia peregrina*** L. 26, ADK 1439; 30, ADK 1516, ŞY; 29, ADK 1474, ŞY; 23, ADK 1394; 8, ADK 1170; 58, ADK 1920; 59, ADK 1935. Mediterranean element; ***Cruciata laevipes*** Opiz 84, ADK 2214. Euro–Siberian element; ***Sherardia arvensis*** L. 84, ADK 2211; 8, ADK 1172; 4, ADK 1115A. Mediterranean element.

VALERIANACEAE ***Valerianella costata*** (Steven) Betcke 5, ADK 1134; 84, ADK 2210. Mediterranean element; ***V. turgida*** (Steven) Betcke 84, ADK 2210A.

DIPSACACEAE ***Dipsacus laciniatus*** L. 48, ADK 1761; 49, ADK 1784; ***Knautia degenii*** Borbás ex Formánek 109, ŞY 28781, ADK. Endemic (LC). Mediterranean element; ***Scabiosa atropurpurea*** L. subsp. ***maritima*** (L.) Archer 46, ADK 1663; 58, ADK 1904; 59, ADK 1927; 29, ADK 1485, ŞY.

ASTERACEAE ***Sigesbeckia orientalis*** L. 64, ADK 2001, ŞY; ***Bidens tripartita*** L. Kaplandede mount, bank of road, 900 m, S. Sazak; ***Xanthium strumarium*** L. subsp. ***cavanillesii*** (Schouw) D. Löve &

Dans. 31, ADK 1539, ŞY, ***Telekia speciosa*** (Schreb.) Baumg. 48, ADK 1768; 50, ADK 1794. Euro–Siberian element; ***Asteriscus aquaticus*** (L.) Less. 46, ADK 1654. Mediterranean element; ***Pallens spinosa*** (L.) Cass. 22, ADK 1371; ***Inula graveolens*** (L.) Desf. 32, ADK 1552, ŞY. Mediterranean element; ***Pulicaria dysenterica*** (L.) Bernh. 51, ADK 1830; 30, ADK 1498; 57, ADK 1892; ***Carpesium abrotanoides*** L. 64, ADK 2020, ŞY; ***Gnaphalium luteo-album*** L. 23, ADK 1409; ***G. sylvaticum*** L. Kaplandede mount, clearings of forest, 970 m, S. Sazak; ***Solidago virgaurea*** L. subsp. ***virgaurea*** 72, ADK 2096, ŞY; 115, ADK 1499, ŞY; ***Aster subulatus*** Michx. 29, ADK 1464, ŞY; ***Conyza canadensis*** (L.) Cronquist 9, ADK 1211; 10, ADK 1237; 8, ADK 1192; 29, ADK 1468, ŞY; ****Dicrocephala integrifolia*** (L. f.) Kuntze 67, ADK 2043, ŞY; ***Bellis perennis*** L. 1, ADK 1114; 2, ADK 1054; 8, ADK 1169; 9, ADK 1213; 35, ADK 1610. Euro–Siberian element; ***Senecio vulgaris*** L. 34, ADK 1593, ŞY; 1, ADK 1027; 77, ADK 2151; 83, ADK 2213; ***S. vernalis*** Waldst. & Kit. 31, ADK 1538, ŞY; ***Tussilago farfara*** L. 74, ADK 2119. Euro–Siberian element; ***Petasites hybridus*** (L.) Gaertn. 86, ADK 2252. Euro–Siberian element; ****Calendula officinalis*** L. 35, ADK 1607; ***Eupatorium cannabium*** L. 29, ADK 1467, ŞY; 32, ADK 1554, ŞY; 51, ADK 1857; 55, ADK 1868; 68, ADK 2057, ŞY. Euro–Siberian element; ***Anthemis cretica*** L. subsp. ***umbilicata*** (Boiss. & Huet) Grierson 51, ADK 1815; 48, ADK 1763; ***A. cotula*** L. 46, ADK 1667; ***A. tinctoria*** L. var. ***discoidea*** (All.) DC. 64, ADK 2012, ŞY; 49, ADK 1788; 94, ADK 2288; ***Otanthus maritimus*** (L.) Hoffmanns. & Link 59, ADK 1932; 61, ADK 1969. Mediterranean element; ***Chrysanthemum segetum*** L. 43, ADK 1723. Mediterranean element; ***Leucanthemum vulgare*** Lam. 56, ADK 1880. Euro–Siberian element; ***Tanacetum parthenium*** (L.) Sch. Bip. 53, ADK 1858; 64, ADK 2014, ŞY; ***T. corymbosum*** (L.) Sch. Bip. subsp. ***cinereum*** (Griseb.) Hayek 41, ADK 1713. Euro–Siberian element; ***Matricaria chamomilla*** L. var. ***recutita*** (L.) Grierson 171, ADK 1311; ***Tripleurospermum sevanense*** (Manden.) Pobed. 70, ADK 2077, ŞY; ***Artemisia vulgaris*** L. 71, ADK 2089, ŞY; ***Arctium minus*** (Hill) Bernh. subsp. ***minus*** 51, ADK 1825; ***A. minus*** (Hill) Bernh. subsp**.** ***pubens*** (Bab.) Arènes 67, ADK 2042, ŞY. Euro–Siberian element; ***Silybum marianum*** (L.) Gaertn. 22, ADK 1376. Mediterranean element; ***Cirsium vulgare*** (Savi) Ten. 51, ADK 1808; ***C. hypoleucum*** DC. 11, ADK 1257; 8, ADK 1174; 5, ADK 1129; 20, ADK 1353; 51, ADK 1831. Euxine element; ***C. arvense*** (L.) Scop. subsp. ***vestitum*** (Wimm. & Grab.) Petr. 40, ADK 1642; 69, ADK 2072, ŞY; ***Centaurea iberica*** Trevir. ex Spreng. 46, ADK 1650; 23, ADK 1383; ***Carthamus lanatus*** L. 46, ADK 1648; 50, ADK 1796; ***Scolymus hispanicus*** L. 46, ADK 1679. Mediterranean element; ***Cichorium intybus*** L. 49, ADK 1787; 39, ADK 1746; ***Tragopogon longirostris*** Bisch. ex Sch. Bip. var. ***longirostris*** 24, ADK 1430; ***T. dubius*** Scop. 119, ŞY 28744, ADK; 23, ADK 1379; ***Leontodon tuberosus*** L. 84, ADK 2216A. Mediterranean element; ***L. hispidus*** L. var. ***hispidus*** 114, ŞY 28803, ADK; 43, ADK 1722; ***Helminthotheca echioides*** (L.) Holub, near Akçakoca, Beug & Wagenitz, 203; ***Rhagadiolus stellatus*** (L.) Gaertn. var. ***edulis*** (Gaertn.) DC. 85, ADK 2224; ***Sonchus asper*** (L.) Hill subsp. ***glaucescens*** (Jord.) Ball 26, ADK 1440; 16, ADK 1295; 21, ADK 1356; 96, ADK 2304; ***S. oleraceus*** L. 32, ADK 1553, ŞY; ***Hieracium vagum*** Jord. 67, ADK 2048, ŞY; 29, ADK 1466, ŞY. Euro–Siberian element; ***Pilosella piloselloides*** (Vill.) Soják subsp. ***megalomastix*** (Nageli & Peter) P.D. Sell & C. West 114, ŞY 28804A, ADK; 103, ŞY 28589, ADK; ***P. × auriculoides*** (Láng) P.D. Sell & C. West 114, ŞY 28804, ADK; 106, ŞY28648, ADK; ***Lactuca saligna*** L. 8, ADK 1888; ***L. serriola*** L. 32, ADK 1558, ŞY. Euro–Siberian element; ****L. sativa*** L. 45, ADK 1743, marul; ***Mycelis muralis*** (L.) Dumoulin 51, ADK 1806; 64, ADK 2007, ŞY. Euro–Siberian element; ***Lapsana communis*** L. subsp. ***intermedia*** (M. Bieb.) Hayek 64, ADK 2008, ŞY; 50, ADK 1800, ADK; 16, ADK 1298; 15, ADK 1287; 10, ADK 1226; 4, ADK 1110; ***Taraxacum microcephaloides*** Soest 4, ADK 1107A; 9, ADK 1208A; ***T. scaturiginosum*** G.E. Haglund 32, ADK 1555, ŞY; 9, ADK 1208; 8, ADK 1176; 76, ADK 2137; 77, ADK 2149; ***T. macrolepium*** Schischk. 2, ADK 1052; 41, ADK 1690; 1, ADK 1014; ***T. buttleri*** Soest 35, ADK 1608A; ***Chondrilla juncea*** L. var. ***juncea*** 29, ADK 1465, ŞY; ***Crepis paludosa*** (L.) Moench 64, ADK 2021, ŞY; 30, ADK 1497, ŞY; 72, ADK 2097, ŞY. Euro–Siberian element; ***C. bithynica*** Boiss. 84, ADK 2216. Euro–Siberian element; ***C. reuterana*** Boiss. subsp. ***reuterana*** 97, ADK 2318. Mediterranean element; ***C. foetida*** L. subsp. ***rhoeadifolia*** (M. Bieb.) Čelak. 32, ADK 1557, ŞY; ***C. sancta*** (L.) Babc. 76, ADK 2135; 74, ADK 2111; 77, ADK 2152; ***C. vesicaria*** L. 96, ADK 2305; 84, ADK 2215.

CAMPANULACEAE ***Campanula lyrata*** Lam. subsp. ***lyrata*** 23, ADK 1389; 22, ADK 1560; 41, ADK 1709. Endemic (LC); ***C. latifolia*** L., Kaplandede mount, east side, bank of road, 900 m, S. Sazak, ISTO 27573. Euro–Siberian element; ***C. rapunculoides*** L. subsp. ***rapunculoides*** 55, ADK 1876; 4, ADK 1828; 69, ADK 2073, ŞY; ***C. rapunculoides*** L. subsp. ***cordifolia*** (C. Koch) Damboldt 52, ADK 1844; ***C. persicifolia*** L. 19, ADK 1334; 171, ADK 1307; 72, ADK 2102, ŞY. Euro–Siberian element; ***C. olympica*** Boiss. 60, ADK 1962. Euxine element; ***Legousia falcata*** (Ten.) Fritsch 26, ADK 1445. Mediterranean element; ***L. speculum-veneris*** (L.) Chaix 23, ADK 1382. Mediterranean element.

ERICACEAE ***Rhododendron ponticum*** L. subsp ***ponticum*** 7, ADK 1162; 5, ADK 1124; ***Erica arborea*** L. 7, ADK 1163; 29, ADK 1484, ŞY; 8, ADK 1186. Mediterranean element; ***Calluna vulgaris*** (L.) Hull, Kaplandede mount, west side, bank of road, 300–400 m, S. Sazak, ISTO 27538. Euro–Siberian element; ***Arbutus unedo*** L. 29, ADK 1585, ŞY; ***A. andrachne*** L. 4, ADK 1096; 10, ADK 1239; 3, ADK 1080; ***Vaccinium arctostaphylos*** L. 19, ADK 1337. Euxine element.

PRIMULACEAE ***Primula vulgaris*** Huds. subsp. ***sibthorpii*** (H. Hoffm.) W.W. Sm. & Forrest 37, ADK 1620. Euxine element; ***Lysimachia verticillaris*** Spreng. 2, ADK 1358; 46, ADK 1629. Hyrcano–Euxine element; ***Anagallis arvensis*** L. var. ***arvensis*** 10, ADK 1231; 9, ADK 1212; 11, ADK 1244; 18, ADK 1315; ***A. arvensis*** L. var. ***parviflora*** (H. Hoffm. & Link) Ces. 95, ADK 2299. Mediterranean element.

EBENACEAE ***Diospyros lotus*** L. 66, ADK 2037, ŞY; 49, ADK 1774; 67, ADK 2050; ****D. kaki*** L. 66, ADK 2036, ŞY, Trabzon hurması.

STYRACACEAE ***Styrax officinalis*** L. 7, ADK 1164.

OLEACEAE ***Fraxinus angustifolia*** Vahl. subsp. ***oxycarpa*** (M. Bieb. ex Willd.) Franco & Rocha Afonso 117, ADK 2385. Euro–Siberian element; ***Ligustrum vulgare*** L. 21, ADK 1359; 24, ADK 1427; 33, ADK 1582, ŞY. Euro–Siberian element; ****Olea europaea*** L. var. ***europaea*** 98, ADK 2345, zeytin.

APOCYNACEAE ****Nerium oleander*** L. 57 ADK 1898; ***Vinca major*** L. subsp. ***major*** 83, ADK 2128; ***V. major*** L. subsp. ***hirsuta*** (Boiss.) Stearn 6, ADK 1152.

ASCLEPIADACEAE ***Periploca graeca*** L. var. ***graeca***, west of Akçakoca, Wagenitz, 225. Mediterranean element.

GENTIANACEAE ***Blackstonia perfoliata*** (L.) Huds. subsp. ***perfoliata*** 20, ADK 1349; 44, ADK 1733; 39, ADK 1639; 46, ADK 1660; ***Centaurium erythraea*** Rafn subsp. ***erythraea*** 26, ADK 1446; 44, ADK 1732. Euro–Siberian element; ***C. erythraea*** Rafn subsp. ***turcicum*** (Velen.) Melderis 52, ADK 1847; 46, ADK 1675; ***C. pulchellum*** (Sw.) Druce 58, ADK 1896; 23, ADK 1418; 39, ADK 1636; ***Gentiana asclepiadea*** L. 72, ADK 2103, ŞY; 65, ADK 2025, ŞY. Euro–Siberian element.

CONVOLVULACEAE ***Convolvulus cantabrica*** L. 23, ADK 1398; 24, ADK 1426; 46, ADK 1661. Mediterranean element; ***C. arvensis*** L. 23, ADK 1380; 11, ADK 1264; 46, ADK 1659; ***Calystegia sepium*** (L.) R. Br. 11, ADK 1241; ***C. silvatica*** (Kit.) Griseb. 171, ADK 1302A; 15, ADK 1291; 60, ADK 1954; ****Ipomoea purpurea*** (L.) Roth 72, ADK 2095, ŞY.

CUSCUTACEAE ***Cuscuta campestris*** Yunck. 61, ADK 1973.

BORAGINACEAE ***Heliotropium dolosum*** De Not., 10 km east of Akçakoca, D. 3746; ***Myosotis ramosissima*** Rochel ex H. Schult. subsp. ***ramosissima*** 88, ADK 2254; ***M. arvensis*** (L.) Hill subsp. ***arvensis*** 18, ADK 1327; 26, ADK 1443; 171, ADK 1305; 11, ADK 1247. Euro–Siberian element; ***M. lazica*** Popov 64, ADK 2010, ŞY. Euxine element; ***M. sylvatica*** Ehrh. ex Hoffm. subsp. ***cyanea*** Vestergr. 4, ADK 1116; ***M. laxa*** Lehm. subsp. ***caespitosa*** (Schultz) Hyl. ex Nordh. 23, ADK 1378; ***Cynoglossum creticum*** Mill. 11, ADK 1252; 96, ADK 2309; ***C. montanum*** L. 11, ADK 1258; 20, ADK 1343; 46, ADK 1668. Euro–Siberian element; ***Lithospermum officinale*** L. 98, ADK 2329; 53, ADK 1856. Euro–Siberian element; ***L. purpurocaeruleum*** L. 85, ADK 2230. Euro–Siberian element; ***Echium italicum*** L. 41, ADK 1688. Mediterranean element; ***E. vulgare*** L. 20, ADK 1345; 55, ADK 1877; 41, ADK 1687; 69, ADK 2070, ŞY. Euro–Siberian element; ***Cerinthe minor*** L. subsp. ***auriculata*** (Ten.) Domac 68, ADK 2044, ŞY; ***Symphytum officinale*** L. 98, ADK 2327. Euro–Siberian element; ***S. tuberosum*** L. subsp. ***nodosum*** (Schur) Soó 10, ADK 1224; 2, ADK 1040; 95, ADK 2300. Euro–Siberian element; ***Trachystemon orientalis*** (L.) G. Don 4, ADK 1105. Euxine element; ***Anchusa undulata*** L. 9, ADK 1206. Mediterranean element.

SOLANACEAE ***Solanum nigrum*** L. subsp. ***schultesii*** (Opiz) Wessely 23, ADK 1405; 24, ADK 1431; 52, ADK 1845; 58, ADK 1999; ***S. alatum*** Moench 34, ADK 1589A, ŞY; ***S. dulcamara*** L. 50, ADK 1799; 52, ADK 1843. Euro–Siberian element; ****S. tuberosum*** L. 99, observation, patates; ****S. melongena*** L. 99, observation, patlıcan; ****Capsicum annuum*** L. 99, observation, biber; ****Lycopersicon esculentum*** Mill. 99, observation, domates; ***Physalis alkekengi*** L. 20, ADK 1354; 73, ADK 2107; ***Atropa belladona*** L., Kaplandede mount, summit, 1168 m, S. Sazak; Kaplandede mount, bank of road, 900 m, S. Sazak. Euro–Siberian element; ***Datura stramonium*** L. 25, ADK 1438; 31, ADK 1542, ŞY; ****Cestrum nocturnum*** L. 91, ADK 2268.

SCROPHULARIACEAE ***Verbascum blattaria*** L. 27, ADK 1451; 41, ADK 1692; ***V. xanthophoeniceum*** Griseb. 2, ADK 1048; ***V. pyramidatum*** M. Bieb. 23, ADK 1396; 41, ADK 1685. Euxine element; ***V. densiflorum*** Bertol. 42, ADK 1740. Euro–Siberian element; ***Scrophularia scopolii*** Hoppe ex Pers. var. ***scopolii*** 97, ADK 2313; 26, ADK 1444; 42, ADK 1735; ***Misopates orontium*** (L.) Raf. 23, ADK 1402; 42, ADK 1710; ***Linaria genistifolia*** (L.) Mill. subsp. ***genistifolia***, Kaplandede mount, surrounding of fountain, bank of road, 970 m, S. Sazak, ISTO 27567. Euro–Siberian element; ***L. iconica*** Boiss. & Heldr. 94, ADK 2294. Endemic (LC). Irano–Turanian element; ***Cymbalaria longipes*** (Boiss. & Heldr.) A. Chev. 34, ADK 1596, ŞY. Mediterranean element; ***Digitalis ferruginea*** L. subsp. ***ferruginea*** 48, ADK 1765; 46, ADK 1673. Euro–Siberian element; ***Veronica serpyllifolia*** L. 9, ADK 1214; 8, ADK 1182; ***V. arvensis*** L. 84, ADK 2207. Euro–Siberian element; ***V. persica*** Poir. 4, ADK 1111; 11, ADK 1256; 94, ADK 2113; 35, ADK 1604; ***V. filiformis*** Sm. 85, ADK 2173; 84, ADK 2208; 37, ADK 1613. Euxine element; ***V. hederifolia*** L. 80, ADK 2170; ***V. anagallis-aquatica*** L. subsp. ***anagallis-aquatica*** 18, ADK 1317; 42, ADK 1716; 14, ADK 1282. Irano–Turanian element; ***V. chamaedrys*** L. 84, ADK 2209. Euro–Siberian element; ***V. pectinata*** L. var. ***pectinata*** 4, ADK 1099; 9, ADK 1213A; ***V. officinalis*** L. 103, ŞY 28614, ADK. Euro–Siberian element; ***Parentucellia latifolia*** (L.) Caruel subsp. ***latifolia*** 9, ADK 1204; 86, ADK 2250; 85, ADK 2225. Mediterranean element; ***Bellardia trixago*** (L.) All. 22, ADK 1366.

OROBANCHACEAE ***Orobanche nana*** Noë ex Beck 51, ADK 1819; 84, ADK 2186.

VERBENACEAE ***Verbena officinalis*** L. 23, ADK 1413; ***Vitex agnus-castus*** L., *ca.* 10 km east of Akçakoca, D. 37457. Mediterranean element.

LAMIACEAE ***Ajuga reptans*** L. 5, ADK 1138; 9, ADK 1217; 4, ADK 1494; 8, ADK 1196. Euro–Siberian element; ***A. chamaepitys*** (L.) Schreb. subsp. ***chia*** (Schreb.) Arcang. var. ***chia*** 46, ADK 1664; ***A. chamaepitys*** (L.) Schreb. subsp. ***palaestina*** (Boiss.) Bornm. 39, ADK 1639; ***Teucrium chamaedrys*** L. subsp. ***chamaedrys*** 114, ŞY 28816; 99, ADK 2372. Euro–Siberian element; ***Scutellaria galericulata*** L. 31, ADK 1545; ***S. albida*** L. subsp. ***albida*** 23, ADK 1401; 51, ADK 1802. Mediterranean element; ***Lamium garganicum*** L. subsp. ***laevigatum*** Arcang. 4, ADK 1104. Euxine element; ***L. amplexicaule*** L. 85, ADK 2223. Euro–Siberian element; ***L. ponticum*** Boiss. & Balansa ex Boiss. 34, ADK 1591, ŞY. Endemic (LC). Irano–Turanian element; ***L. purpureum*** L. var. ***purpureum*** 8, ADK 1189; 1, ADK 1028; 35, ADK 1599. Euro–Siberian element; ***L. purpureum*** L. var. ***aznavourii*** Gand. ex Azn. 106, ŞY 28673, ADK; 79, ADK 2166; 94, ADK 2118. Endemic (CR). Euxine element; ***Galeobdolon luteum*** Huds. subsp. ***montanum*** (Pers.) R.R. Mill 5, ADK 1146. Euro–Siberian element; ***G. luteum*** Huds. subsp. ***luteum*** 93, ADK 2284. Euro–Siberian element; ***Sideritis montana*** L. subsp. ***montana*** 39, ADK 1748. Mediterranean element; ***Stachys thirkei*** C. Koch 22, ADK 1367; 18, ADK 1319; ***S. sylvatica*** L. 171, ADK 1306; 23, ADK 1399. Euro–Siberian element; ***S. annua*** (L.) L. subsp. ***annua*** var. ***lycaonica*** R. Bhattacharjee 61, ADK 1970. Irano–Turanian element; ***Melissa officinalis*** L. subsp. ***altissima*** (Sm.) Arcang. 51, ADK 1807. Mediterranean element; ***Glechoma hederacea*** L. 9, ADK 1213A; 5, ADK 1144; 8, ADK 1190. Euro–Siberian element; ***Prunella vulgaris*** L. 60, ADK 1952; 48, ADK 1758; 23, ADK 1410; 69, ADK 2076, ŞY; 51, ADK 1812. Euro–Siberian element; ***P. laciniata*** (L.) L. 22, ADK 1364; 24, ADK 1422, ŞY; 39, ADK 1631; 46, ADK 1647. Euro–Siberian element; ***Origanum vulgare*** L. subsp. ***viride*** (Boiss.) Hayek 41, ADK 1691; 46, ADK 1669; ***Satureja hortensis*** L. 66, ADK 2035, ŞY; ***Calamintha grandiflora*** (L.) Moench 69, ADK 2071, ŞY. Euro–Siberian element; ***C. sylvatica*** Bromf. subsp. ***sylvatica*** 72, ADK 2094, ŞY. Euro–Siberian element; ***C. nepeta*** (L.) Savi subsp. ***glandulosa*** (Req.) P.W. Ball 41, ADK 1707; 46, ADK 1665; 32, ADK 1551, ŞY; 58, ADK 1916; 48, ADK 1753; ***Clinopodium vulgare*** L. subsp. ***vulgare*** 48, ADK 1762; 29, ADK 1488, ŞY; 64, ADK 1996, ŞY; 69, ADK 2068, ŞY; ***Thymus longicaulis*** C. Presl subsp. ***longicaulis*** var. ***longicaulis*** 85, ADK 2222; 94, ADK 2291; ***Mentha pulegium*** L. 50, ADK 1793; 42, ADK 1741; 60, ADK 1949; 30, ADK 1527; ***M. aquatica*** L. 31, ADK 1547, ŞY; ***M. longifolia*** (L.) Huds. subsp. ***longifolia*** 55, ADK 1871. Euxine element; ***M. longifolia*** (L.) Huds. subsp. ***typhoides*** (Briq.) Harley var. ***typhoides*** 41, ADK 1686; ***M. spicata*** L. subsp. ***spicata*** 51, ADK 1818; ***M. spicata*** L. subsp. ***tomentosa*** (Briq.) Harley 31, ADK 1548, ŞY; ***Lycopus europaeus*** L. 31, ADK 1546, ŞY. Euro–Siberian element; ***Salvia forskahlei*** L. 29, ADK 1481, ŞY; 60, ADK 1960; 41, ADK 1689; 65, ADK 2022, ŞY; ***S. glutinosa*** L. 65, ADK 2023, ŞY. Euxine element; ***S. amplexicaulis*** Lam. 84, ADK 2200. Euro–Siberian element; ***S. verbenaca*** L. 30, ADK 1524, ŞY; 58, ADK 1926; 22, ADK 1370. Mediterranean element; ***S. verticillata*** L. subsp. ***verticillata***, between Akçakoca and Alaplı, 10 m, 16.06.1987, Cöbek 1190. Euro–Siberian element.

PLANTAGINACEAE ***Plantago major*** L. subsp. ***major*** 27, ADK 1450; ***P. coronopus*** L. subsp. ***coronopus*** 71, ADK 2083, ŞY; 61, ADK 1980. Euro–Siberian element; ***P. lanceolata*** L. 25, ADK 1436; 3, ADK 1079; 85, ADK 2240.

THYMELAEACEAE ***Daphne pontica*** L., between Akçakoca and Alaplı, 10 m, 16.06.1987, Cöbek 1193. Euxine element.

ELAEAGNACEAE ****Elaeagnus angustifolia*** L. 119, ŞY 28750, ADK; 99, ADK 2346, iğde.

LAURACEAE ***Laurus nobilis*** L. 2, ADK 1072; 10, ADK 1232; 32, ADK 1575, ŞY. Mediterranean element.

SANTALACEAE ***Osyris alba*** L. 58, ADK 1897. Mediterranean element.

LORANTHACEAE ***Viscum album*** L. 3, ADK 1087.

ARISTOLOCHIACEAE ***Asarum europaeum*** L., Kaplandede mount, under *Fagus orientalis*, bank of road, shadow, 900–1000 m,

S. Sazak. Euro–Siberian element; ***Aristolochia pontica*** Lam. 6, ADK 1148. Euxine element.

EUPHORBIACEAE ***Mercurialis annua*** L. 84, ADK 2213; 25, ADK 1435; ***M. perennis*** L. 5, ADK 1136. Euro–Siberian element; ***Euphorbia peplis*** L. 110, ADK 1528, ŞY; 59, ADK 1945. Mediterranean element; ***E. supina*** Raf. 32, ADK 1578, ŞY; ***E. villosa*** Waldst. & Kit. ex Willd. 113, ADK 1529, ŞY; 40, ADK 1645. Euro–Siberian element; ***E. pubescens*** Vahl, 10–15 km east of Akçakoca, D. 37494; ***E. stricta*** L. 4, ADK 1108; 8, ADK 1181; 5, ADK 1131; 1, ADK 1008; 2, ADK 1061; 93, ADK 2286; 97, ADK 2323. Euro–Siberian element; ***E. helioscopia*** L. 1, ADK 1031; 76, ADK 2140; ***E. exigua*** L. var. ***retusa*** L. 25, ADK 1434; ***E. peplus*** L. var. ***peplus*** 74, ADK 2115; ***E. paralias*** L. 71, ADK 2080, ŞY; 59, ADK 1941. Mediterranean element; ***E. seguieriana*** Neck. subsp. ***niciciana*** (Borbás ex Novák) Rech. f. 22, ADK 1374; 6, ADK 1150; 39, ADK 1745; ***E. amygdaloides*** L. var. ***amygdaloides*** 5, ADK 1123A; 14, ADK 1283; 95, ADK 2302; 94, ADK 2290; 81, ADK 2183. Euro–Siberian element.

URTICACEAE ***Urtica dioica*** L. 18, ADK 1324; 1, ADK 1037. Euro–Siberian element; ***Parieteria judaica*** L. 113, ADK 1518, ŞY.

CANNABACEAE ***Humulus lupulus*** L. 32, ADK 1561, ŞY. Euro–Siberian element.

MORACEAE ****Morus alba*** L. 99, ADK 2383, akdut; ****M. nigra*** L. 99, ADK 2384, karadut; ****Ficus carica*** L. subsp. ***carica*** 49, ADK 1776, incir.

ULMACEAE ***Ulmus minor*** Mill. subsp. ***minor*** 10, ADK 1228; 85, ADK 2235.

JUGLANDACEAE ****Juglans regia*** L. 49, ADK 1775, ceviz; ***Pterocarya fraxinifolia*** (Poir.) Spach, east of Akçakoca, ISTO 4286. Hyrcano–Euxine element.

PLATANACEAE ***Platanus orientalis*** L. 47, ADK 1750.

FAGACEAE ***Castanea sativa*** Mill. 26, ADK 1442; 29, ADK 1470, ŞY. Euro–Siberian element; ***Fagus orientalis*** Lipsky 26, ADK 1441; 29, ADK 1482, ŞY; 58, ADK 1910; 64, ADK 2002, ŞY. Euro–Siberian element; ***Quercus petraea*** (Matt.) Liebl. subsp. ***petraea*** 90, ADK 2267; ***Q. petraea*** (Matt.) Liebl. subsp. ***iberica*** (Steven ex M. Bieb.) Krassiln. 29, ADK 1483, ŞY; ***Q. cerris*** L. var. ***cerris*** 85, ADK 2238. Mediterranean element; ***Q. pubescens*** Willd. 60, ADK 1946A; 58, ADK 1909; ***Q. virgiliana*** Ten. 60, ADK 1946A; ***Q. ilex*** L. 32, ADK 1549. Mediterranean element.

CORYLACEAE ***Carpinus betulus*** L. 32, ADK 1559, ŞY. Euro–Siberian element; ***Corylus avellana*** L. subsp. ***avellana*** 48, ADK 1755. Euro–Siberian element; ****C. avellana*** L. subsp. ***pontica*** (G.C.T. Koch) W. Winkl. 63, ADK 1989; 43, ADK 1721; 35, ADK 1602; 10, ADK 1229, fındık; ****C. maxima*** Mill. 63, ADK 1988. Euro–Siberian element, fındık.

BETULACEAE ***Alnus glutinosa*** (L.) Gaertn. subsp. ***glutinosa*** 32, ADK 1550, ŞY; 30, ADK 1531, ŞY. Euro–Siberian element.

SALICACEAE ****Populus alba*** L. 29, ADK 1476, ŞY. Euro–Siberian element, akkavak; ****P. tremula*** L. 29, ADK 1475, ŞY. Euro–Siberian element, titrek kavak; ****P. nigra*** L. subsp. ***nigra*** 114, ŞY 28829, ADK, karakavak; ***Salix alba*** L. 47, ADK 1751; 29, ADK 1477, ŞY; 96, ADK 2303. Euro–Siberian element; ****S. babylonica*** L. 47, ADK 1752, salkım söğüt.

MONOCOTYLEDONEAE

ALISMATACEAE ***Alisma plantago-aquatica*** L 112, ADK 1979. Euro–Siberian element.

MUSACEAE ****Musa acuminata*** Colla 116, observation, muz.

ARACEAE ***Arum orientale*** M. Bieb. subsp. ***orientale*** 18, ADK 1316; 27, ADK 1449.

DIOSCOREACEAE ***Tamus communis*** L. subsp. ***communis*** 59, ADK 1931.

LILIACEAE ***Smilax excelsa*** L. 29, ADK 1463, ŞY; 28, ADK 1454; 58, ADK 1906; 2, ADK 1070; ***Ruscus aculeatus*** L. var. ***aculeatus*** 2, ADK 1066; ***R. aculeatus*** L. var. ***angustifolia*** Boiss. 5, ADK 1120; ***R. hypoglossum*** L. 7, ADK 1166; 5, ADK 1118. Euro–Siberian element; ***Asparagus aphyllus*** L. subsp. ***orientalis*** (Baker) P.H. Davis 58, ADK 1914. Mediterranean element; ***Polygonatum multiflorum*** (L.) All. 5, ADK 1137; ****Allium cepa*** L. 99, ADK 2389; ****A. sativum*** L. 24, ADK 1425; 46, ADK 1626; 42, ADK 1714; ****A. porrum*** L. 98, ADK 2338, pırasa; ***Scilla bifolia*** L. 37, ADK 1616. Mediterranean element; ***S. bithynica*** Boiss. 1, ADK 1038; 10, ADK 1238; 5, ADK 1130; 84, ADK 2187A; 81, ADK 2181; 8, ADK 1197. Euxine element; ***S. autumnalis*** L. 33, ADK 2390; ***Ornithogalum sphaerocarpum*** A. Kern. 20, ADK 1344; ***O. sigmoideum*** Freyn & Sint. 1, ADK 1023; 76, ADK 2131; ***Muscari armeniacum*** Leichtlin ex Baker 80, ADK 2169; 1, ADK 1005; 9, ADK 1207; 81, ADK 2180; ***M. comosum*** (L.) Mill. 84, ADK 2211. Mediterranean element; ***Lilium martagon*** L., Kaplandede mount, summit, 1168 m, S. Sazak, ISTO 27529; Kaplandede mount, under *Tilia* sp., bank of road, 800–900 m, 02.06.96, 20.07.96, 30.08.96, S. Sazak. Euro–Siberian element; ***Paris incompleta*** M. Bieb., 13, ADK 1281. Euxine element.

AMARYLLIDACEAE ***Galanthus elwesii*** Hook. f. 37, ADK 1619; 75, ADK 2127. Mediterranean element; ***G. nivalis*** L. subsp. ***nivalis*** 8, ADK 1167. Euro–Siberian element; ***Pancratium maritimum*** L. 59, ADK 1936. Mediterranean element.

IRIDACEAE ***Iris pseudacorus*** L. 109, ŞY 28795, ADK.

ORCHIDACEAE ***Cephalanthera rubra*** (L.) Rich. 20, ADK 1342. ***C. longifolia*** (L.) Fritsch 106, ŞY 28698, ADK. Euro–Siberian element; ***C. damasonium*** (Mill.) Druce 12, ADK 1268. Euro–Siberian element; ***Platanthera bifolia*** (L.) Rich. 24, ADK 1424. Euro–Siberian element; ***Ophrys oestrifera*** M. Bieb. subsp. ***oestrifera*** 11, ADK 1242; 84, ADK 2187; ***Serapias vomeracea*** (Burm. f.) Briq. subsp. ***orientalis*** Greuter 95, ADK 2295. Mediterranean element; ***Anacamptis pyramidalis*** (L.) Rich. 22, ADK 1372; 24, ADK 1423; 23, ADK 1427; 46, ADK 1676; ***Orchis coriophora*** L. subsp. ***coriophora*** 24, ADK 1423A; 22, ADK 1365; ***O. laxiflora*** Lam. 97, ADK 2312.

TYPHACEAE ***Typha domingensis*** Pers. 61, ADK 1985.

JUNCACEAE ***Juncus inflexus*** L. 61, ADK 1971; 52, ADK 1834; ***J. effusus*** L. 23, ADK 1412; ***J. bufonius*** L. 108, ŞY 28737, ADK; ***J. articulatus*** L. 108, ŞY 28738, ADK. Euro–Siberian element; ***Luzula forsteri*** (Sm.) DC. 106, ŞY 28695, ADK; 80, ADK 2174. Euro–Siberian element; ***L. sudetica*** (Willd.) DC. 3, ADK 1085; 84, ADK 2193. Euro–Siberian element; ***L. pallescens*** (Wahlenb.) Besser 103, ŞY 28619, ADK; 84, ADK 2210. Euro–Siberian element.

CYPERACEAE ***Cyperus esculentus*** L. 32, ADK 1569, ŞY; ***Carex otrubae*** Podp. 109, ŞY 28793, ADK. Euro–Siberian element; ***C. divulsa*** Stokes subsp. ***divulsa*** 103, ŞY 28621, ADK. Euro–Siberian element; ***C. pseudocyperus*** L., Kaplandede mount, east side, moisture area, 840 m, S. Sazak, ISTO 275221. Euro–Siberian element; ***C. pendula*** Huds. 109, ŞY 28792, ADK; 103, ŞY 28615, ADK; 23, ADK 1411. Euro–Siberian element; ***C. sylvatica*** Huds. subsp. ***sylvatica***, east of Akçakoca, Kühne 118. Euro–Siberian element; ***C. flacca*** Schreb. subsp. ***serrulata*** (Biv.) Greuter 114, ŞY 28837, ADK. Mediterranean element; ***C. pallescens*** L. var. ***pallescens*** 103, ŞY 28616, ADK.

POACEAE ***Brachypodium sylvaticum*** (Huds.) P. Beauv. 51, ADK 1823; 54, ADK 1867; 49, ADK 1786. Euro–Siberian element; ***Elymus elongatus*** (Host) Runemark subsp. ***elongatus*** 59, ADK 1939; ***E. pyc-***

nanthus (Godr.) Melderis 61, ADK 1981. Mediterranean element; ****Triticum aestivum*** L. 41, ADK 1693, buğday; ****Secale cereale*** L. var. ***cereale*** 85, ADK 2242, çavdar; ***Hordeum murinum*** L. subsp. ***glaucum*** (Steud.) Tzvelev 119, ŞY 28764, ADK; ***Bromus hordeaceus*** L. subsp. ***thominii*** (Hardouin) Maire & A. Weiller 103, ŞY 28624; 119, ŞY 28769, ADK. Mediterranean element; ***B. madritensis*** L. 108, ŞY 28739, ADK; 119, ŞY 28768, ADK; ***Avena barbata*** Pott ex Link subsp. ***barbata*** 119, ŞY 28762, ADK. Mediterranean element; ****A. sativa*** L. 41, ADK 1698, yulaf; ***Aira elegantissima*** Schur subsp. ***elegantissima*** 15, ADK 1290. Mediterranean element; ***Holcus lanatus*** L. 18, ADK 1331; 46, ADK 1628; 51, ADK 1810; 23, ADK 1412. Euro–Siberian element; ***Agrostis stolonifera*** L. 108, ŞY 28742, ADK. Euro–Siberian element; ***Phalaris paradoxa*** L. 108, ŞY 28741, ADK. Mediterranean element; ***Anthoxanthum odoratum*** L. subsp. ***odoratum*** 9, ADK 1239. Euro–Siberian element; ***Phleum bertoloni*** DC. 47, ADK 1749; ***P. subulatum*** (Savi) Asch. & Graebn. subsp. ***subulatum*** 49, ADK 1785; ***P. exaratum*** Hochst. ex Griseb. subsp. ***exaratum*** 46, ADK 1670; ***Festuca drymeja*** Mert. & Koch 103, ŞY 28626, ADK. Euro–Siberian element; ***Lolium perenne*** L. 103, ŞY 28631, ADK; 109, ŞY 28797, ADK. Euro–Siberian element; ***L. rigidum*** Gaudin var. ***rigidum*** 119, ŞY 28766, ADK; 103, ŞY 28628, ADK; ***Vulpia fasciculata*** (Forssk.) C. Fritsch 119, ŞY 28767, ADK; 103, ŞY 28629, ADK. Mediterranean element; ***Poa annua*** L. 8, ADK 1191; ***P. supina*** Schrad. 106, ŞY 28700, ADK; ***P. trivialis*** L. 103, ŞY 28625, ADK; ***P. pratensis*** L. 106, ŞY 28702, ADK; 103, ŞY 28627, ADK; ***P. angustifolia*** L. 114, ŞY 28835, ADK; ***P. bulbosa*** L. 106, ŞY 28703, ADK; ***Dactylis glomerata*** L. subsp. ***hispanica*** (Roth) Nyman 68, ADK 2052, ŞY; ***Cynosurus cristatus*** L. 103, ŞY 28630, ADK. Euro–Siberian element; ***C. echinatus*** L. 20, ADK 1338. Mediterranean element; ***Briza media*** L. 58, ADK 1901; ***B. maxima*** L. 15, ADK 1285; ***Phragmites australis*** (Cav.) Trin. ex Steud. 119, ŞY 28765, ADK; ***Cynodon dactylon*** (L.) Pers. var. ***villosus*** Regel, Alaplı sınırı, 59, ADK 1937; ***Echinochloa crus-galli*** (L.) P. Beauv. 70, ADK 2078, ŞY; 71, ADK 2091, ŞY; ***Digitaria sanguinalis*** (L.) Scop. 32, ADK 1567, ŞY; 31, ADK 1543, ŞY; ***D. ischaemum*** (Schreb. ex Schweigg.) Muhl. var. ***asiatica*** (Tzvelev) Tzvelev 30, ŞY 27215, ADK; ****Paspalum paspalodes*** (Michx.) Scribn., west of Akçakoca, Wagenitz & Beug, 209; ***Setaria viridis*** (L.) P. Beauv. 55, ADK 1875; 53, ADK 1862; ***S. glauca*** (L.) P. Beauv. 30, ADK 1520, ŞY; 70, ADK 2079, ŞY; ****Saccharum officinarum*** L. 100, ADK 2382, şekerkamışı; ***Sorghum halepense*** (L.) Pers. var. ***halepense*** 59, ADK 1934; 58, ADK 1912; 41, ADK 1703; ****S. bicolor*** (L.) Moench 118, ADK 2355, süpürgelik; ***Bothriochloa ischaemum*** (L.) Keng 58, ADK 1900; ****Zea mays*** L. subsp. ***mays*** 42, ADK 1715, mısır.

Discussion

The statistical results are given in Table 1.
The largest families are given in Table 2.
The largest genera are given in Table 3.

There are three phytogeographical regions in Turkey such as Euro–Siberian, Mediterranean and Irano–Turanian (Davis 1971). The research area is situated entirely in the Euro–Siberian region. The chorological aspect is concordant by high percentages of Euro–Siberian elements (28.95 %) (Table 4). The elements of the phytogeographical regions are given in Table 4.

There are 7 endemic species in the area. Compared to the rate of Turkish endemism (30–33 %), the endemism rate of Akçakoca district is low, because the climatic and topographical properties are uniform and similar to Euro–Siberian region of Europe. According to the Turkish *Red Data Book*, the IUCN threat categories of the endemic and vulnerable species are given in the Table 5 (Ekim & al. 2000; IUCN 2001). *Lamium purpureum* var. *aznavourii* occurs in the *Fagus orientalis* forest near the sea shore. *Campanula lyrata* and *Knautia degenii* species are widespread. *Linaria iconia* and *Lamium ponticum* species are found near the city because of anthropogenic factors. *Trifolium caudatum* is recorded from only one locality. The vulnerable *Symphytum tuberosum* is abundant between Akçakoca and Melenağzı. *Chaerophyllum aromaticum* is a new record for the flora of Turkey (Yıldırımlı & Koca 2003). This species is mainly distributed in Central and Eastern Europe, Balkan Peninsula and now collected in Akçakoca–Düzce–Turkey from two localities. *Heracleum platytaenium* is a rare and monocarpic species in the research area.

Table 1. Number of families, genera, species, infrageneric and cultivated taxa in the research area.

	Family		Genus		Species		Subspecies	Variety	Taxon	
	N	C	N	C	N	C			N	C
Pteridophyta	7	–	9	–	13	–	–	–	13	–
Spermatophyta	87	9	323	52	550	73	13	8	571	73
Gymnospermae	1	2	1	6	2	7		–	2	7
Angiospermae	86	7	322	46	548	66	13	8	569	66
Monocotyledoneae	11	1	59	7	85	11		1	86	11
Dicotyledoneae	75	6	270	39	463	55	13	7	483	55
Total	94	9	332	52	563	73	13	8	584	73
	103		384		636		13	8	657	

Abbreviations: N – Native; C – Cultivated.

Table 2. The largest 10 families in the research area.

	Family	Number of species	Number of taxa	Percentage for species (%)
1	*Asteraceae*	66	67	11.72
2	*Fabaceae*	55	61	9.76
3	*Poaceae*	39	39	6.92
4	*Lamiaceae*	35	40	6.21
5	*Brassicaceae*	25	27	4.44
6	*Rosaceae*	22	22	3.90
7	*Scrophulariaceae*	21	21	3.73
8	*Boraginaceae*	17	17	3.01
9	*Apiaceae*	16	16	2.84
10	*Liliaceae*	14	15	2.48
	Others	254	260	45.11
	Total	563	584	100

Table 3. The largest 10 genera in the research area.

	Genus	Number of species	Number of taxa	Percentage for species (%)
1	*Trifolium*	15	18	2.66
2	*Euphorbia*	11	11	1.95
3	*Veronica*	9	9	1.59
4	*Medicago*	8	9	1.42
5	*Geranium*	8	8	1.42
6	*Polygonum*	7	7	1.24
7	*Ranunculus*	7	7	1.24
8	*Vicia*	7	7	1.24
9	*Carex*	7	7	1.24
10	*Crepis*	6	6	1.06
	Others	477	494	84.72
	Total	563	584	100

Table 4. Distribution of the phytogeographical elements in the research area.

Phytogeographical regions	Number of species	Percentage (%)
Euro–Siberian	163	28.95
Mediterranean	64	11.36
Irano–Turanian	4	0.71
Multiregional or unknown	332	58.96
Total	563	100

Table 5. The endemics and vulnerable (non endemic) species and their threat categories in the research area (the rank is according to threat category).

Endemic or vulnerable (non endemic) species	Threat Category
Lamium purpureum var. *aznavourii*	Critically Endangered (CR)
Chaerophyllum aromaticum (non endemic)	Vulnerable (VU)
Symphytum tuberosum subsp. *nodum* (non endemic)	Vulnerable (VU)
Trifolium caudatum	Least Concern (LC)
Heracleum platytaenium	Least Concern (LC)
Knautia degenii	Least Concern (LC)
Campanula lyrata subsp. *lyrata*	Least Concern (LC)
Linaria iconia	Least Concern (LC)
Lamium ponticum	Least Concern (LC)

Acknowledgements. Specimens were collected during the research named "Flora and ethnobotany of the Akçakoca district (Düzce)" between the years 2001 and 2003. This research was supported by Hacettepe University Research Unit (Project No. 01.01.601.004). The authors thank Hacettepe University Research Unit for financial support.

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Species of international significance and their distribution in Kosovo

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Abstract. Kosovo is considered between the Balkan and European endemic floristic centres according to IUCN criteria, with two existing endemic floristic centres on its territory: Sharri National Park and Bjeshket e Nemuna Mts, and also two potential endemic floristic centres: Pashtriku and Koritniku. In this paper are presented 74 species of international significance until now known in Kosovo, out of an overall number of 1800 species already identified, and of 2500 expected species. About 200 endemic species are recorded in Kosovo and 74 of them are of international significance; in more than 90 % of cases they vegetate in at least one of the mentioned floristic centres.

Key words: endemics, floristic centres, red list, relicts, significance

Introduction

Kosovo, located in the Central Balkans, covers an area of 10 877 km^2, bounded by Serbia to the north and east, Macedonia to the southeast, Albania to the southwest, and Montenegro to the west. Topographically, it is an elevated basin enclosed by mountain ranges and hills. Most of the area of Kosovo consists of two plains: Kosovo and Dukagjini divided by a hilly ridge running north to south. The Shar Mts are a major range that form the southern border and are shared with Macedonia and Albania. The Albanian Alps form the western border and are shared with Montenegro and Albania.

Kosovo varies in elevation from 265 m to 2656 m a.s.l. with approximately 77 % of its area lying between 500 m and 1500 m. Higher altitude areas, above 1500 m, cover approximately 6 % of the area and are significant from a biodiversity standpoint (Schweithelm 2003). The soils are generally nutrient rich, providing a good growth medium for natural plants and agricultural crops.

The climate is influenced by its proximity to the Adriatic and Aegean Seas as well as the continental European landmass to the north. The overall climate is a modified continental type, with some elements of a sub-Mediterranean climate in the extreme south and an alpine regime in the higher mountains. Winters are cold with an average temperature in January and February of 0 °C and with significant accumulation of snow, especially in the mountains. Summers are hot, with extremes of up to 40 °C. The average annual rainfall in Kosovo is 720 mm but can reach more than 1000 mm in the mountains. The varied elevations, climatic influences, and soils within Kosovo provide a wide diversity of microhabitats to which plant and animal species are adapted (Mustafa & Hoxha 2004).

Material and methods

The results of this paper are the result of an investigation of the most important endemic and relict species living in Kosovo. The herbarium of existing determined flora, broad literature, a number of studies made in Kosovo during recent years, and the main international documents and programs such as CORINE have been consulted in order to prepare the List of species of international significance for Kosovo.

Results and discussion

The number of identified species in Kosovo is 1800, while the number of expected species is about 2500 (Krasniqi 1998). This figure shows that Kosovo with approximately 2 % of the Balkan territory contains about 30 % of its flora, and about 16 % of the whole European flora consisting of 12 500 species (Table 1).

Table 1. Flora of Kosovo in the Balkan Peninsula.

Country	Territory (km²)	No. of species	No. of endemics
Albania	28 748	3250	230
Bosnia and Hercegovina	51 129	3572	500
Bulgaria	110 912	3900	186
Greece	132 562	4992	850
Macedonia	25 713	3700	117
Serbia	88 361	3562	287
Montenegro	13 812	3136	223
Kosovo	10 887	1800 (2500)	200

The existence of two endemic floristic centres on its territory: Sharri National Park and Bjeshket e Nemuna categorises Kosovo as one of the 16 main floristic endemic centres of Europe (Stevanović & Vasić 1995).

From an overall number of 2500 expected species, the number of endemic species in Kosovo is about 200 (Krasniqi 1998), which includes endemic, endemic–relict and sub-endemic species. This shows that within 2 % of the Balkan territory, in Kosovo about 10 % of the endemics of the Balkans live (2059 species). From this number, those living only in Kosovo (Kosovo endemics) include 13 species. Below is the list with steno-endemic species of Kosovo: *Aconitum pentheri* Hayek, *Achillea alexandri-regis* Bornm. & Rudsky, *Diathus scardicus* Wettst., *Bornmuellera dieckii* Degen, *Sempervivum kosaninii* Praeger, *Sedum flexosum* Wettst., *Saxifraga scardica* Griseb., *Potentilla doerfleri* Wettst., *Verbascum scardicola* Bornm., *Thymus doerfleri* Ronniger, *Thymus rohlenae* Velen. ex Rohlena, *Centaurea albertii* Rexhepi and *Cynoglossum krasniqii* Wraber (Rexhepi 2000).

Regarding the diversity of plant associations, in Kosovo 139 plant associations are recorded, classified in 20 classes, 35 orders and 63 families (Mustafa 2004). This number is considered to vary, depending on the criteria used for determination of plant associations (Schweithelm 2003).

Of particular interest are endemic and endemic–relict plant associations (Mustafa 2004) such as: *Potentillo doerfleri–Juncetum trifidi*, *Ptilotricho–Bruckenthalio–Pinetum mughi*, *Polygalo–Genistetum hassertianae*, *Polygalo–Forsythietum europaeae*, *Potentillo–Fumanetum bonapartei*, *Pinetum heildreichii–Bruckenthalio–Ptilotrichum dieckii*, *Pinetum heildreichii–peucis scardicum*, *Pinetum heildreichii*, *Astero–Juniperetum europeae*, *Wulfenio–Pinetum peucis*, *Sedo–Bornmuellerietum dieckii* and *Onosmo–Scabietosum fumaroides* (Rexhepi 1986).

The first efforts to put under a legal and institutional protection some of the most important and threatened species in Kosovo were made in 1986, by the Institute for Nature Protection of Kosovo, with the Decision No. 239/86 that proposed a list of 27 species (Table 2) "to be considered as protected" (Hoxha & al. 2004).

Table 2. List of proposed "protected plant species".

1. *Taxus baccata* L.	15. *Daphne blagayana* Freyer
2. *Quercus trojana* Webb	16. *Ramonda serbica* Pančić f. *longipetiolata* Gajić
3. *Ulmus campestris* L.	17. *Paeonia decora* G. Anderson
4. *Acer heildreichii* Boiss. & Heldr.	18. *Paeonia corallina* Retz.
5. *Forsythia europaea* Degen & Bald.	19. *Waldsteinia geoides* Willd.
6. *Wulfenia carinthiaca* Jacq.	20. *Polygala doerfleri* Hayek
7. *Tulipa scardica* Bornm.	21. *Dioscorea balcanica* Košanin
8. *Trollius europaeus* L.	22. *Moltkea doerfleri* Wettst.
9. *Lilium albanicum* Griseb.	23. *Rhododendron ferrugineum* L.
10. *Dianthus scardicus* Wettst.	24. *Gentiana lutea* L.
11. *Fritillaria graeca* Boiss. & Spruner	25. *Draba korabensis* Kümmerle & Degen
12. *Narcissus poeticus* L.	26. *Leontopodium alpinum* Cass. subsp. *nivale* (Ten.) Tutin
13. *Rumex balcanicus* Rech. f.	27. *Aster albanicus* Degen
14. *Ilex aquifolium* L.	

Even if the proposed list of species seems appropriate, the criteria and scientific arguments used for compiling it are not clear, although a number of the species are endemics, relicts and some species are used for economic purposes (medicinal plants such yellow gentian). Furthermore a real practical implementation of this list and its effects was never done.

A list of 74 species of international significance in Kosovo has been prepared by us (Table 3). According to the floristic spectrum of these species, the dominant families are: *Asteraceae* with 30 species, *Caryophyllaceae* and *Rosaceae* with 5 species each, *Fabaceae* and *Violaceae* with 3 species each, while the other families are represented mostly with 2 and 1 species. The most represented genera are: *Hieracium* with 19 species, *Potentilla*, *Viola* and *Crepis* with 3 species each, etc.

Table 3. List of the species of international significance in Kosovo.

No.	Species	No.	Species
1.	*Pancicia serbica*	38.	*Cerastium neoscardicum*
2.	*Aristolochia merxmuelleri*	39.	*Dianthus behriorum*
3.	*Achillea alexandri-regis*	40.	*Dianthus scardicus*
4.	*Achillea corabiensis*	41.	*Silene retzdorffiana*
5.	*Amphoricarpus antarcticus*	42.	*Silene schmuckeri*
6.	*Aster albanicus*	43.	*Fumana bonapartei*
7.	*Centaurea kosaninii*	44.	*Sempervivum kosaninii*
8.	*Centaurea alba*	45.	*Dioscorea balcanica*
9.	*Crepis macedonica*	46.	*Cephalaria pastricensis*
10.	*Crepis bertiscae*	47.	*Euphorbia montenegrina*
11.	*Crepis albanica*	48.	*Astragalus fiale*
12.	*Hieracium cinerascens*	49.	*Genista hassertiana*
13.	*Hieracium doerfleri*	50.	*Trifolium wettsteinii*
14.	*Hieracium eremnophilum*	51.	*Ramonda serbica* f. *longipetulata*
15.	*Hieracium erythrocarpum*	52.	*Crocus kosaninii*
16.	*Hieracium gentile*	53.	*Crocus scardicus*
17.	*Hieracium gigantophyllum*	54.	*Stachys serbica*
18.	*Hieracium grossianum*	55.	*Narthecium scardicum*
19.	*Hieracium guentheri-beckii*	56.	*Tulipa scardica*
20.	*Hieracium hayekii*	57.	*Forsythia europaea*
21.	*Hieracium ipekanum*	58.	*Plantago reniformis*
22.	*Hieracium kobilicanum*	59.	*Polygala doerfleri*
23.	*Hieracium naegelianum*	60.	*Rumex balcanicus*
24.	*Hieracium panaeolum*	61.	*Aquilegia blecici*
25.	*Hieracium pseudocalycinum*	62.	*Malus florentina*
26.	*Hieracium schefferi*	63.	*Geum bulgaricum*
27.	*Hieracium semisylvaticum*	64.	*Potentilla visianii*
28.	*Hieracium thapsiformioides*	65.	*Potentilla montenegrina*
29.	*Hieracium tomassiniforme*	66.	*Potentilla doerfleri*
30.	*Hieracium trebevicianum*	67.	*Melampyrum doerfleri*
31.	*Halascya sendtneri*	68.	*Verbascum scardicola*
32.	*Moltkia doerfleri*	69.	*Wulfenia blecicii*
33.	*Solenanthus krasniqii*	70.	*Valeriana bertiscae*
34.	*Bormuellera dieckii*	71.	*Valeriana pancicii*
35.	*Draba korabensis*	72.	*Viola elegantula*
36.	*Thlaspi bellidifolium*	73.	*Viola grisebachiana*
37.	*Phyteuma pseudorbiculare*	74.	*Viola speciosa*

According to the IUCN (1994) categories of threat, the majority of the species belong to category R (Rare), while 4 species belong to category V (Vulnerable): *Achillea alexandri-regis*, *Aster albanicus* Degen, *Halascya sendtneri* Boiss. and *Silene retzdorffiana* Hayek (Fig. 1).

From the species of Kosovo, *Forsythia europaea* Degen & Bald., endemic and relict species growing only in Kosovo and some parts of northern Albania, is listed in the World Red List. In the European Red List (ERL) are listed 24 species or 31 % of total species of international significance of Kosovo.

In Annex IV (Animal and plant species of community interest whose conservation requires designation of special areas of conservation) of the HABITAT Directive 92/43/EEC (1992) from the species of flora of Kosovo only ramonda (*Ramonda serbica* Pančić f. *longipetulata* Gajić) is listed (Hoxha & al. 2004).

In Annex V of HABITAT Directive (Animal and plant species of community interest whose taking in the wild and exploitation may be subject to management measures) from the species of Kosovo only yellow gentian (*Gentiana lutea* L.) is listed (Hoxha & al. 2004).

According to CORINE (1990) the majority of species belong to Code 62, followed by Code 61, 36, etc. (Fig. 2). This confirms that about 46 % of species of international significance grow in zones in or above the upper timberline, as well as in zones of mountain limestone.

Fig. 3 shows the distribution of species of international significance in each of the 4 centres from which it can be concluded that more than 90 % of these species grow in one or more of these four centres and only 6 of them – in other parts of Kosovo such as Mirusha, Germia, etc. (Mustafa & Hoxha 2001).

Taking into consideration the number of endemic species, in Kosovo in the 4 identified floristic cen-

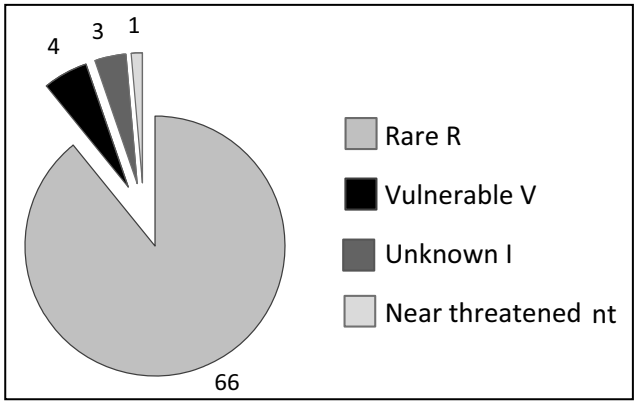

Fig. 1. Threat categories of species of international significance in Kosovo.

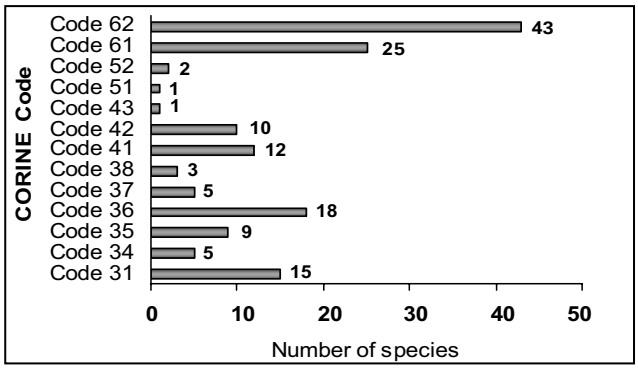

Fig. 2. Distribution of species according to CORINE (1990).

tres: Bjeshket e Nemuna, Sharri National Park, Pashtriku and Koritniku, the number of registered endemic species per 10 × 10 km varies from 40 up to 60, whilst in Bjeshket e Nemuna this number is more than 90 species (Hoxha & al. 2004) (Fig. 4).

We have to mention that two other mountains: Pashtriku and Koritniku are still not well studied, while Bjeshket e Nemuna and Sharri National Park need to finalise the complete inventory of their flora. This number of endemic species places Sharri National Park and Bjeshket e Nemuna among the main local endemic centres of the Western and Central Balkan Peninsula (Stevanović & Vasić 1995), and have all the attributes of diversity centres of global importance.

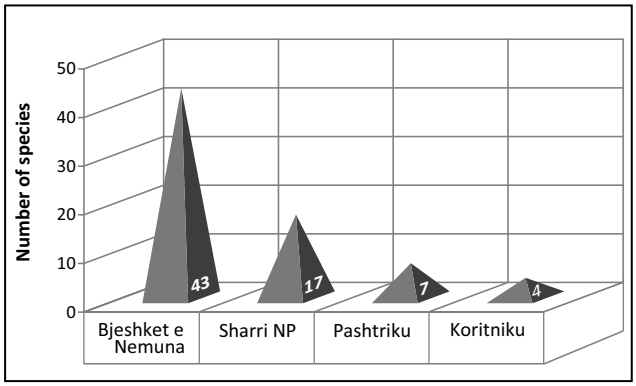

Fig. 3. Distribution of species of international significance in floristic centres of Kosovo.

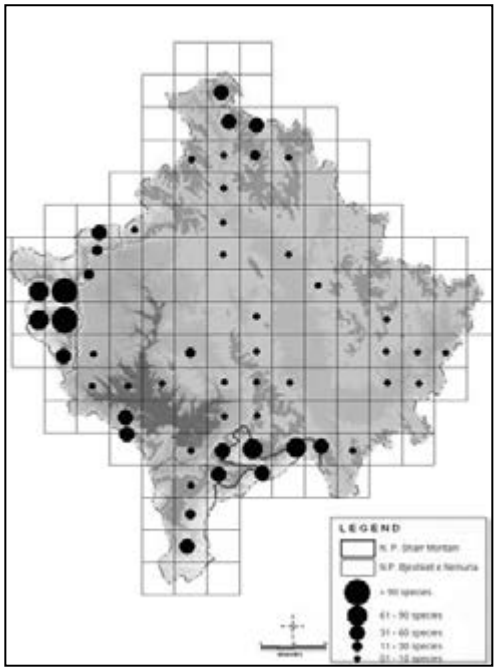

Fig. 4. Number of endemics in Kosovo shown on map of 10 × 10 km (prepared by Tomor Celaj, GIS, Ministry of Environment and Spatial Planning, from Stevanović & Vasić 1995).

Conclusions and recommendations

In this paper a list of 74 species of international significance in flora of Kosovo has been elaborated. Based on the number of endemic and relict species, and the level of damage to biodiversity, this list looks to contain a very limited number of species. The complete inventory of flora of Kosovo will give a clear picture of the species of international importance, with emphasis on threatened species and those threatened by extinction. This list of 74 species can be considered as the "nucleus" of a future complete Red List of Kosovo.

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The genus *Taraxacum* (*Asteraceae*) in Italy. II. Five new species of *Taraxacum* sect. *Erythrocarpa*

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Abstract: *Taraxacum* sect. *Erythrocarpa* is reported for the first time for Calabria (S Italy). After field, herbarium and laboratory studies, five new species belonging to that section are described as new to science: *T. calabricum* Aquaro, Caparelli & Peruzzi; *T. cescae* Aquaro, Caparelli & Peruzzi ($2n = 32$) – occurring also in C Italy; *T. kirschneri* Aquaro, Caparelli & Peruzzi ($2n = 24$); *T. optimae* Aquaro, Caparelli & Peruzzi and *T. pollinense* Aquaro, Caparelli & Peruzzi ($2n = 32$). Morphological, cytotaxonomical and distributional data are illustrated and discussed.

Key words: *Asteraceae*, karyology, Italy, *Taraxacum*, taxonomy

Introduction

According to Kirschner & Štěpánek (1985), *Taraxacum* F.H. Wigg. sect. *Erythrocarpa* Hand.-Mazz. (*Asteraceae*) is known to comprise 50–80 species, occurring mainly on the territories extending from the Eastern Mediterranean to Central Asia. The representatives of this section share usually the following features: plants often robust; outer involucral bracts lanceolate to broadly ovate with pale, often broad, scarious margin; achenes densely spinulose, usually red to dark brown, large (often larger than 4.5–5 mm, including long cylindrical cone), with a long rostrum. In Europe, only 24 species are known (Kirschner & Štěpánek 1985; Richards 1991; Sonck 1993; Kirschner & al. 2006–2007): *T. duriense* Soest and *T. malato-belizii* Soest (from Iberian peninsula); *T. pseudohoppeanum* Kirschner & Štěpánek (from Maritime Alps); *T. albomarginatum* A.J. Richards, *T. amborum* G.E. Haglund, *T. dialeptum* Sonck, *T. olympophilum* Sonck and *T. panhellenicum* Sonck (from Greece); *T. capricum* Soest (from France, Italy); *T. apulicum* Soest (from S Italy); *T. dorchocarpum* Soest, *T. janchenii* Kirschner & Štěpánek (= *T. hoppeanum* Griseb. & Schenk, *nom. illeg.*), *T. pindicola* (Bald.) Hand.-Mazz. and *T. poliochloroides* R. Doll (from Balkans); *T. calocephalum* Hand.-Mazz. and *T. poliochlorum* Dahlst. (from E Mediterranean); *T. erythrocarpum* Kirschner & Štěpánek (from W Carpathians); *T. pieninicum* Pawł. (from Pieniny Mts); *T. tauricum* Kotov (from Ukraine); *T. breve* R. Doll, *T. conicum* R. Doll, *T. desertorum* Schischk., *T. divulsiforme* R. Doll and *T. pseudophaleratum* R. Doll (from European Russia).

Until recently, only three species of *T.* sect. *Erythrocarpa* were quoted for Italy (Kirschner & Štěpánek 1985; Kirschner & al. 2006–2007): *T. apulicum*, *T. capricum* and *T. pseudohoppeanum*. Any of these species is quoted in Conti & al. (2005).

During a general field and herbarium revision of the genus *Taraxacum* in Calabria (S Italy) we identified several plants clearly referable to sect. *Erythrocarpa*. This contribution, dealing with the taxonomy of these plants, is part of a planned series of papers devoted to *Taraxacum* diversity and distribution in Italy (Aquaro & Peruzzi 2007; Aquaro & al. 2008).

Material and methods

The study was based on *exsiccata* from CLU and FI and on live plants collected during the years 2004–2006.

Karyological studies were carried out, after cultivation in the Botanic Garden of Calabria University. Root tips were pretreated with a 0.3 % colchicine solu-

tion and fixed in Carnoy; afterwards they were hydrolysed in 1N HCl solution and coloured with fuchsine; at the end, they were squashed in a 45 % solution of acetic acid for counting and observation of chromosomes. Karyotype formula according to Levan & al. (1964) was drawn out from measurements made on five somatic metaphase plates. A_1 (Intrachromosomal asymmetry index) and A_2 (Interchromosomal asymmetry index) were calculated according to Romero Zarco (1986).

Results and discussion

According to our morphological study, five apomict systematic units belonging to *Taraxacum* sect. *Erythrocarpa* are identified in the studied area of S Italy. None of them is identifiable with previously described taxa.

Two groups of robust plants from Mount Cocuzzo and Sila Massif seem related to *T. olympophilum* (here described as *T. cescae* and *T. optimae*, respectively), but they clearly differ from it in having ovate and uncorniculate outer bracts. Two other groups of robust plants from N Calabria and Sila Massif appear instead related to *T. calocephalum* (here described as *T. calabricum* and *T. kirschneri*, respectively), but they are easily distinguishable from it in having sparsely spinulose achene bodies. The latter group – characterized by small-sized plants coming from Pollino Massif (here described as *T. pollinense*) is instead similar to *T. amborum* from Greece and to *T. janchenii* from N Balkans, from which it differs however in several morphological features, notably in leaves, outer bracts, rostrum and achenes dimensions. All the five new systematic units are following, arranged in alphabetical order.

T. calabricum Aquaro, Caparelli & Peruzzi sp. nov. (Figs 1A–C)

Diagnosis: Planta 12–14 cm alta. Folia suberecta, luteo viridia, dense araneosa, plerumque 6.2–9.5 cm longa et 1.8–3.5 cm lata, profunde divisa; lobus terminalis obtuse triangularis (0.7 × 1 cm) vel lingulatus (0.7–1.3 × 0.1–0.2 cm); lobi laterales 4–7, anguste triangulari vel subdeltoidei, interdum positi asimmetrici, marginibus distalibus denticulatibus convexis vel concavis; interlobi dentati crispo-plicatuli; petiolus subalatus, purpureo-coloratus, 1.5–2.5 cm longus. Scapi foliis subaequilongi, purpurei, dense araneosi, 7.5–10 cm longi. Involucrum basi 1.3–2.5 cm diametro, squamis interioribus ad 11–18 mm longis et 1.5–2.5 mm latis; squamae exteriores 12–14, laxe patentes, interdum apice ricurvato, lanceolatae vel ovato-lanceolatae, acuminatae, ciliolatae, pro parte interiorae violaceae, membranaceo-marginatae (margines ad 0.2 mm lati), 5–6 mm longae, 2–3 mm latae; squamae interiores exterioresque saepe cornutae. Stigmata obscura, antherae polliniferae, grana pollinis diametro valde variantia. Achenium rubrum, superne breviter subdense spinulosum, 3.4 mm longum (pyramide exclusa) et 1 mm latum, in pyramiden cylindricam 0.8 mm longam subabrupte abiens. Rostrum 8 mm, pappus albus *ca*. 6 mm longus.

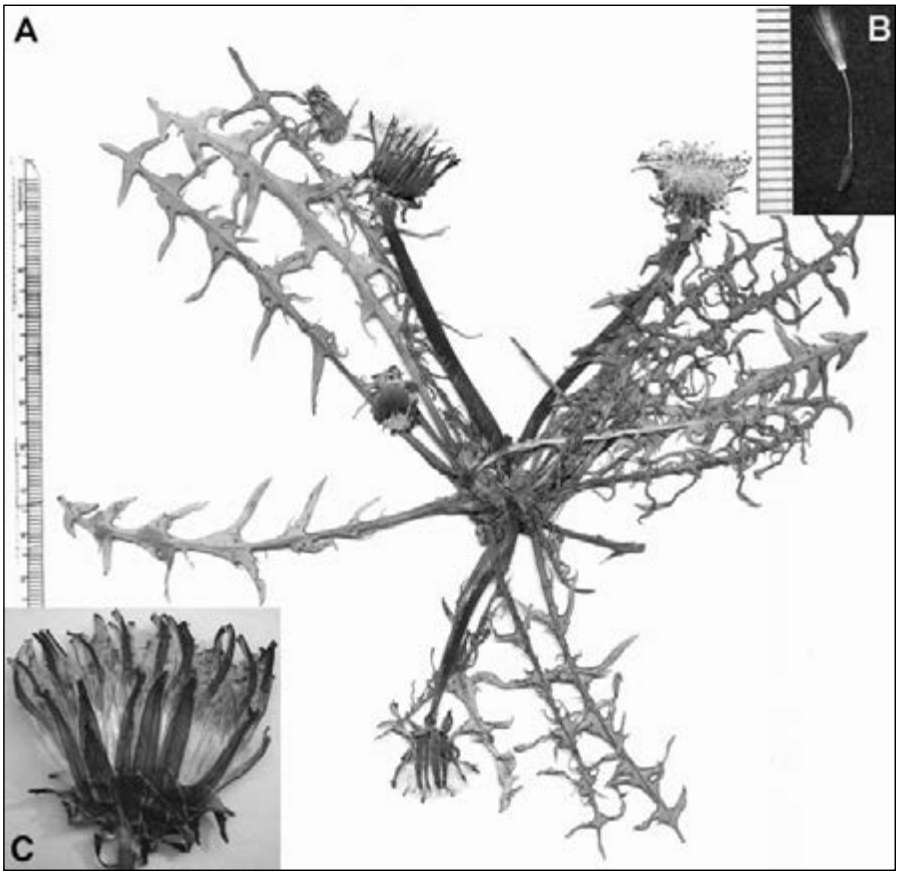

Fig. 1. *T. calabricum* sp. nov.: **A** – general view; **B** – particular of achene; **C** – particular of flowering capitulum. All plants scanned are parts of type collections.

Holotypus: Italy, Calabria – Parco dei Cappuccini, Carolei (Prov. Cosenza, Calabria), UTM 33S XD 05.45, 611 m alt., esp. E, subst. calcareo, 17.03.2002, *A. Romeo* (CLU, n. 4621, sub *T. officinale* Weber).

Paratypi: Italy, Basilicata – Massiccio del Pollino: il Visitone (Pz), versante Nord del Pollino, nei pressi di una depressione umida a margine della strada, 26.04.2006, *G. Aquaro, L. Peruzzi* & *D. Gargano* (CLU, n. 20914; FI, PI); Italy, Calabria – Massiccio del Pollino: tra la Madonnina e Colle del Dragone, Apr 2006, *G. Aquaro, L. Peruzzi* & *D. Gargano* (PI); Dirupata di Morano Calabro (Cs), 722 m alt., 15.04.1994, *P. Calvosa* & *L. Bernardo* (CLU, n. 4611, sub *T. laevigatum*).

Other specimens seen: Italy, Calabria – La Sila: verso Serra della Guardia; pascolo sul crinale, 1350 m, 07.05.1950, *Sarfatti* & *Corradi* (FI, sub *T. laevigatum*); La Sila: Lago Arvo, sponda settentrionale a E di Lorica, 1300 m, 09.05.1950, *Sarfatti* & *Corradi* (FI, sub *T. laevigatum*).

Description: Perennial medium-sized herb, 12–14 cm tall, densely hairy, with scapes more or less equalling the leaves. Leaves sub-erect, green-yellowish, 6.2–9.5 cm long and 1.8–3.5 cm wide, deeply lobed; terminal lobe obtuse-triangular (0.7 × 1 cm) to lingulate (0.7–1.3 × 0.1–0.2 cm); lateral lobes 4–7, narrowly triangular to sub-deltoid, sometimes asymmetrical; distal margin denticulate, either concave or convex; interlobes dentate and plicate; petiole purplish, narrowly winged, 1.5–2.5 cm long. Scape purplish, densely hairy, 7.5–10 cm long. Involucre 1.3–2.5 cm wide at the base, inner bracts 14–19, 11–18 mm long and 1.5–2.5 mm wide, with a dark and corniculate apex; outer bracts green (violet on the inner surface), 12–14, patent, sometimes with curved apex, lanceolate to ovate-lanceolate, ciliate at the margins, acuminate at the apex, 5–6 mm long and 2–3 mm wide, with membranous margin *ca.* 0.2 mm wide. Stigma dark green; anthers with pollen grains of variable size. Achenes deep red, with 6 ridges and 24 narrow spines in the upper portion; body 3.4 mm long and 1 mm wide; cone 0.8 mm; rostrum 8 mm; pappus white, *ca.* 6 mm long.

Ecology: mountain arid grasslands on both calcareous and siliceous substrates (600–1350 m).

Chromosome number: unknown.

Distribution: widespread in N Calabria (Fig. 2, stars).

Taraxacum calabricum is a species whose leaves remind also sect. *Erythrosperma*, but according to the features of achenes and outer bracts it falls in our opinion clearly within sect. *Erythrocarpa*. According to studies on Calabrian *Taraxacum* (Aquaro 2006), the two sections can be easily distinguished as following:

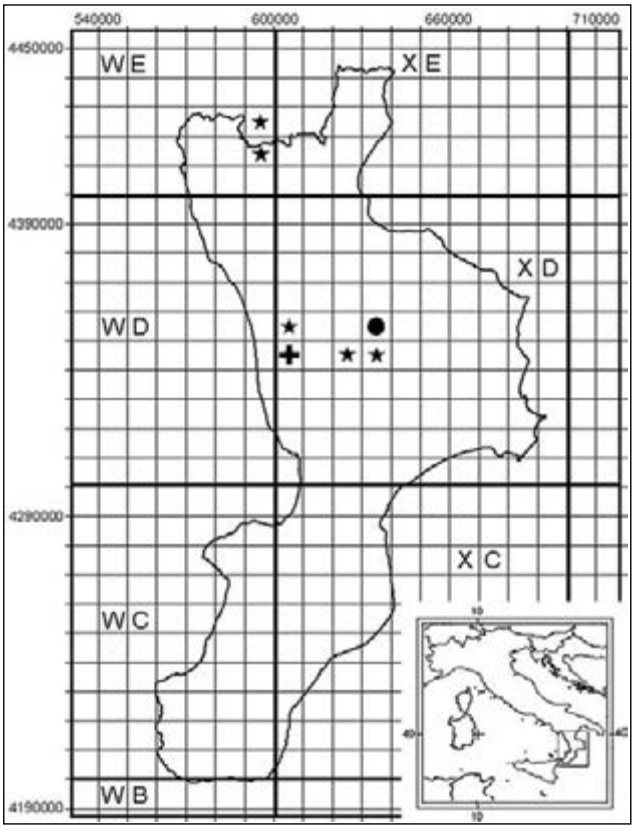

Fig. 2. Geographical distribution of *T. calabricum* sp. nov. (star), *T. cescae* sp. nov. (+) and *T. kirschneri* sp. nov. (circle).

– achene body 2.5–3(3.5) mm long and always less than 1 mm wide; involucre (6)10–14(20) mm wide; external bracts 1–2 mm wide. . . .sect. ***Erythrosperma***

– achene body 3–3.6 mm long and generally ≥1 mm wide; involucre (11)14–24(30) mm wide; external bracts (1)2–3 mm wide. . . . . sect. ***Erythrocarpa***

T. cescae Aquaro, Caparelli & Peruzzi sp. nov. (Figs 3A–C)

Diagnosis: Planta 25 cm alta. Folia suberecta, subolivaceo-viridia, sparse araneosa, plerumque 9.5–13 cm longa et 4–5 cm lata, profunde divisa; lobus terminalis acute triangularis vel hastatus, 1.7–3 cm longus, 1.2–3.2 cm latus; lobi laterales 7–8, anguste triangulares vel subdeltoidei, patentes vel subsagittati, positi saepe asimmetrici; marginibus distalibus denticulatibus convexis vel concavis; interlobi dentati crispo-plicatuli; petiolus subalatus, obscuro-viridis, 1–1.5 cm longus. Scapus purpureus, superne sparse

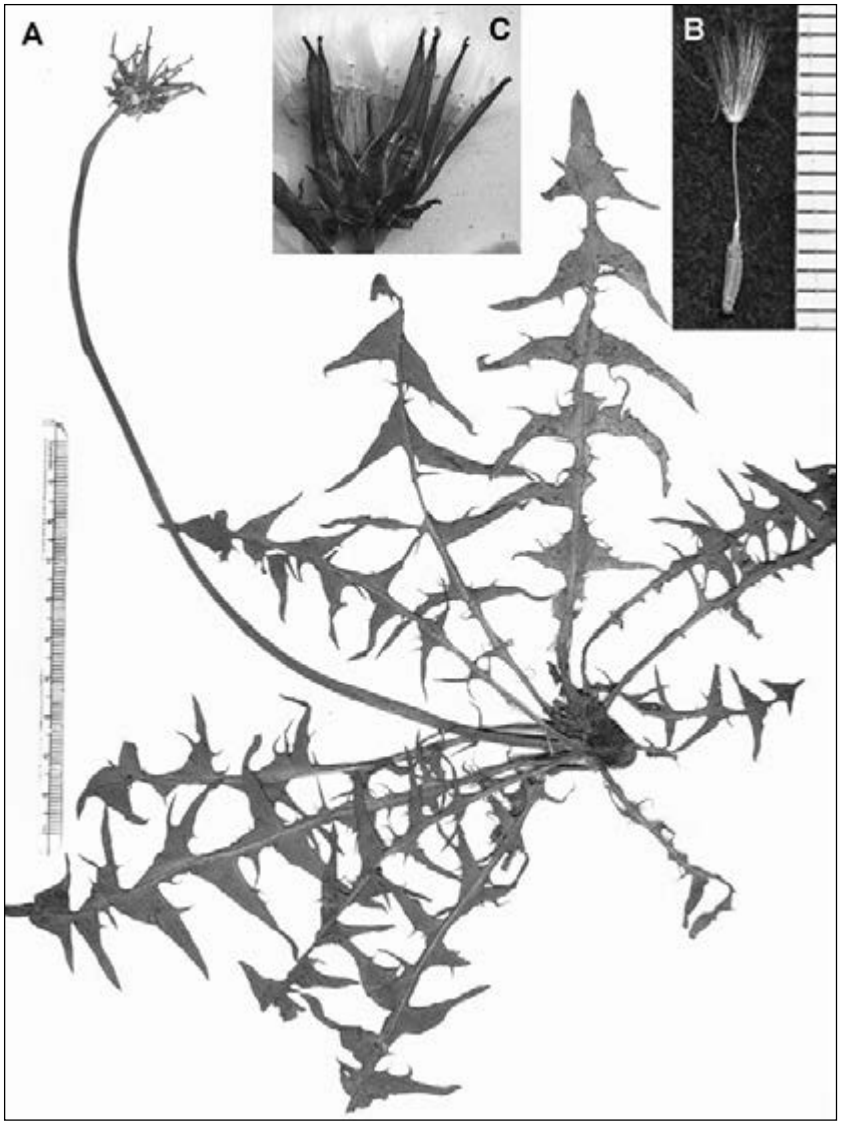

Fig. 3. *T. cescae* sp. nov.: **A** – general view; **B** – particular of achene; **C** – particular of fruiting capitulum. All plants scanned are parts of type collections.

araneosus, 24 cm longus. Involucrum basi 1–1.5 cm diametro, squamis interioribus ad 14–16 mm longis et 2 mm latis, apice oscuro raro calloso; squamae exteriores 9–12, subadpressae, ovatae, acuminatae, apice purpureo, ciliolatae, membranaceo-marginatae (margines ad 0.3 mm latae), 5–6 mm longae, 2–3 mm latae. Stigmata lutea, antherae polliniferae, grana pollinis diametro valde variantia. Achenium subpallide rufum, superne subdense spinulosum, 3–3.6 mm longum (pyramide exclusa) et 1 mm latum, in pyramiden cylindricam 0.8–1 mm longam subabrupte abiens. Rostrum 6–7 mm, pappus albus *ca*. 5–6 mm longi.

Holotypus: Italy, Calabria – Catena Costiera: Monte Cocuzzo, lungo la strada che dal Casello forestale sale in Cima, 1450 m, 16.06.2004, *G. Aquaro* & *F. Venneri* (CLU, n. 18132).

Paratypi: Italy, Marche – Gola del Furlo (provincia di Pesaro–Urbino), a margine strada su substrato calcareo, *ca*. 300 m, UTM 33T UJ 16.35, 14.04.2007, *L. Peruzzi* & *K.F. Caparelli* (FI, PI).

Description: Perennial medium-sized herb, 25 cm tall, scarcely hairy, with scapes longer than leaves. Leaves sub-erect, olive green, 9.5–13 cm long and 4–5 cm wide, deeply lobed; terminal lobe triangular, 1.7–3 cm long and 1.2–3.2 cm wide; lateral lobes 7–8, triangular to sub-deltoid, often asymmetrical; distal margin denticulate, either concave or convex; interlobes dentate and plicate; petiole dark green, narrowly winged, 1–1.5 cm long. Scape purplish, scarcely hairy, 24 cm long. Involucre 1–1.5 cm wide at the base, inner bracts 11–14, 14–16 mm long and 2 mm wide, with a dark and rarely corniculate apex; outer bracts green, 9–12, adpressed, with purplish apex, ovate, ciliate at the margins, acuminate at the apex, 5–6 mm long and 2–3 mm wide, with membranous margin *ca*. 0.3 mm wide. Stigma yellow; anthers with pollen grains of variable size. Achenes light red, with 10 ridges and short spines in the upper portion; body 3–3.6 mm long and 1 mm wide; cone 0.8–1 mm; rostrum 6–7 mm; pappus white, *ca*. 5–6 mm long.

Etymology: this species is dedicated to Prof. Giuliano Cesca (University of Calabria), specialist in the genus *Euphorbia* L. and devoted to the study of Calabrian flora, in occasion of his retirement.

Ecology: arid rocky places on limestones (300–1500 m).

Chromosome number: $2n = 32$ (Fig. 4A). Karyotype formula: $2n = 4x = 16m + 4m^{sat} + 4m$ (Fig. 4B); $A_1 = 0.25$, $A_2 = 0.20$.

Distribution: seemingly endemic to Central and Southern Italy. Calabrian distribution is shown in Fig. 2 ("+").

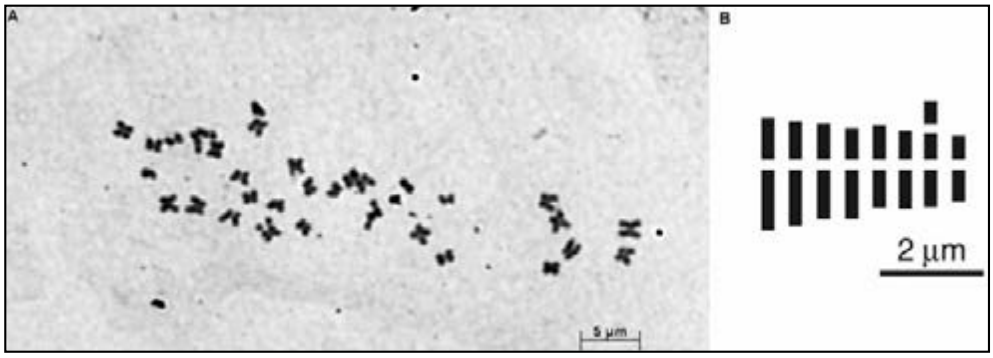

Fig. 4. *T. cescae* sp. nov.: **A** – root tips metaphase plate, $2n = 32$; **B** – haploid idiogram.

T. kirschneri **Aquaro, Caparelli & Peruzzi sp. nov.** (Figs 5A–B)

Diagnosis: Planta 13–15 cm alta. Folia suberecta, graminio-viridia, dense araneosa, plerumque 5.2–10.3 cm longa et 0.8–1.7 cm lata, lobata; lobus terminalis obtuse triangularis vel triangularis, 0.7–1.2 cm longus, 0.7–1.4 cm latus; lobi laterales 5–7, sagittati, patentes vel subsagittati, interdum positi saepe asimmetrici; marginibus distalibus saepe denticulatibus convexis; interlobi dentati crispo-plicatuli; Petiolus subalatus purpureo-colorato, 1.4–3.5 cm longus. Scapus viridis, dense araneosus, 7–15 cm longus. Involucrum basi 0.9–1.4 cm diametro, squamae interiores ad 10–14 mm longae et 1–2 mm latae, apice viridis, partim callosae vel corniculatae; squamae exteriores 13–16, laxe patentes interdum apice ricurvato lanceolatae, acuminatae apice fusco, ad 9–10 mm longae et 2–3 mm latae, saepe callosae. Stigmata lutea, antherae polliniferae, grana pollinis diametro valde variantia. Achenium stramineum (superne roseo-colorato), superne parce spinulis acutis, 2.8 mm longum (pyramide exclusa) et 0.6 mm latum, in pyramiden conicam 0.9 mm longam subabrupte abiens. Rostrum 9 mm, pappus albus *ca*. 5.5 mm longi.

Holotypus: Italy, Calabria – Sila, sulla strada che da Lorica va verso Camigliatello, 18.06.2004, *G. Aquaro* & *N.G. Passalacqua* (CLU, n. 18135).

Description: Perennial medium-sized herb, 13–15 cm tall, densely hairy, with scapes longer than leaves. Leaves sub-erect, light green, 5.2–10.3 cm long

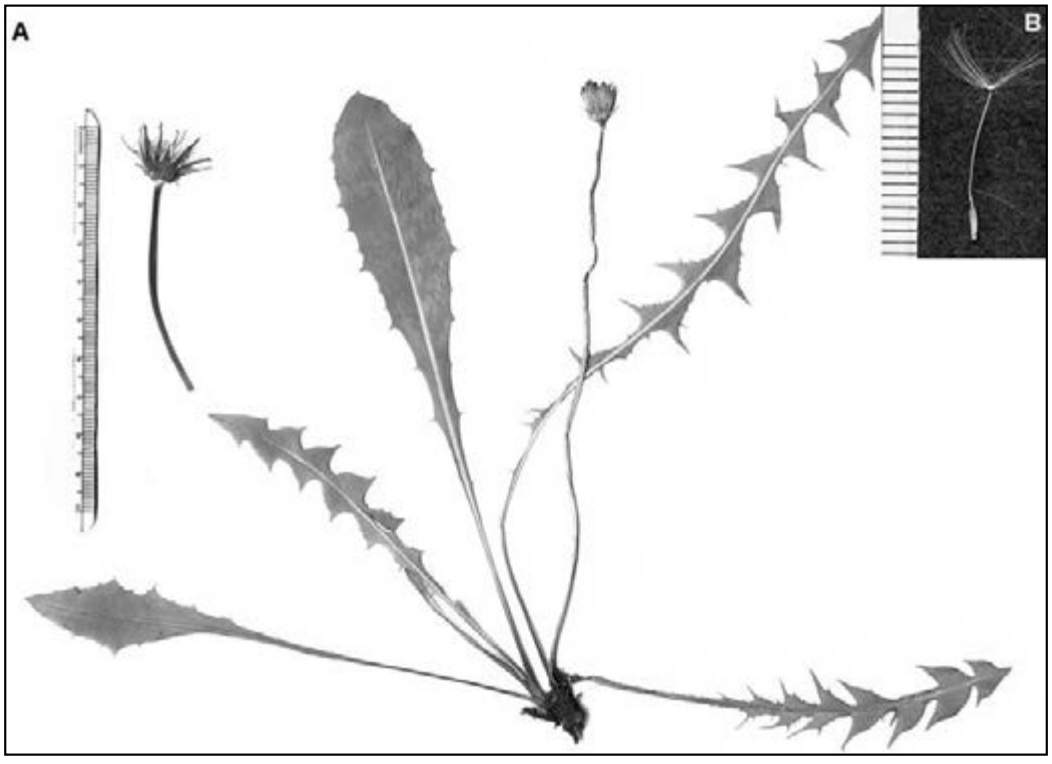

Fig. 5. *T. kirschneri* sp. nov.: **A** – general view; **B** – particular of achene. All plants scanned are parts of type collections.

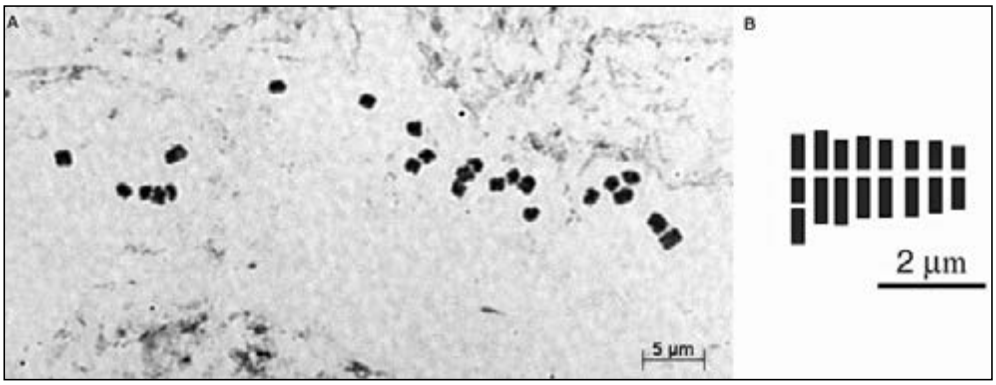

Fig. 6. *T. kirschneri* sp. nov.: **A** – root tips metaphase plate, $2n = 24$; **B** – haploid idiogram.

and 0.8–1.7 cm wide, lobed; terminal lobe obtuse-triangular to triangular, 0.7–1.2 cm long and 0.7–1.2 cm wide; lateral lobes 5–7, sagittate, often asymmetrical; distal margin often denticulate, convex; interlobes short, dentate and often plicate; petiole purplish, narrowly winged, 1.4–3.5 cm long. Scape green, densely hairy, 7–15 cm long. Involucre 0.9–1.4 cm wide at the base, inner bracts 12–13, 10–14 mm long and 1–2 mm wide, with a corniculate apex; outer bracts green, 13–16, patent, often curved at the apex, with dark apex, lanceolate, acuminate and often corniculate at the apex, 9–10 mm long and 2–3 mm wide. Stigma yellow; anthers with pollen grains of variable size. Achenes dark yellow with rose apex, with 10 ridges and *ca*. 30 spines in the upper portion; body 2.8 mm long and 0.6 mm wide; cone 0.9 mm; rostrum 9 mm; pappus white, *ca*. 5.5 mm long.

Etymology: this species is dedicated to Prof. J. Kirschner (Academy of Science, Průhonice – Czech Republic), specialist in the genus *Taraxacum*.

Ecology: mountain grasslands on siliceous substrate.

Chromosome number: $2n = 24$ (Fig. 6A). Karyotype formula: $2n = 3x = 3sm_{sat} + 21m$ (Fig. 6B); $A_1 = 0.25$, $A_2 = 0.17$.

Distribution: known only for Sila Massif (Fig. 2, circle).

T. optimae Aquaro, Caparelli & Peruzzi sp. nov. (Figs 7A–B)

Diagnosis: Planta 27–29 cm alta. Folia erecta, laete viridia vel gramineo-viridia, subglabra, plerumque 9.5–26 cm longa et 1.8–3.7 cm lata, lobata; lobus terminalis acute triangularis vel hastatus, 3.5–5 cm longus, 2.5–3 cm latus; lobi laterales 6, deltoidei, marginibus distalibus convexis; petiolus subalatus, 2.5–6 cm longus, purpureo-colorato, nervo mediano viridis raro purpureo-colorato. Scapus inferne viridis superne paulo brunnescens, glabrus, 27–28 cm longus. Involucrum basi 1.3–1.8 cm diametro, squamae interiores ad 14–16 mm longae et 1.5–1.8 mm latae; squamae exteriores 14–15, subadpressae, ovatae, acuminatae, apice obscuro, membranaceo-marginatae (margines ad 0.5 mm latae), ad 6–7 mm longae et 2.5–3 mm latae. Stigmata obscu-

Fig. 7. *T. optimae* sp. nov.: **A** – general view; **B** – particular of capitulum. All plants scanned are parts of type collections.

ra, antherae polliniferae, grana pollinis diametro valde variantia. Achenium ignotum.

Holotypus: Italy, Calabria – Prov. di Cosenza, Sila Grande *ca*. 13.5 km E-NE of Camigliatello Silano Macchialonga. Latitudine 39°21'59" N, Longitudine 16°36'11" E, 1510–1560 m, 11.06.1997, *Partecipanti VIII Iter Mediterraneum 0991/04* (CLU, n. 4619, sub *T*. cfr. *officinale*).

Description: Perennial medium/big-sized herb, 27–29 cm tall, glabrous, with scapes equalling or longer than the leaves. Leaves erect, light green, 9.5–26 cm long and 1.8–3.7 cm wide, lobed; terminal lobe triangular or hastate, 3.5–5 cm long and 2.5–3 cm wide; lateral lobes 6, deltoid; distal margin convex; petiole purplish, narrowly winged, 2.5–6 cm long, with central nerve green or rarely purplish. Scape green, purplish in the distal portion, glabrous, 27–28 cm long. Involucre 1.3–1.8 cm wide at the base, inner bracts 13, 14–16 mm long and 1.5–1.8 mm wide; outer bracts 14–15, sub-adpressed, ovate, acuminate and dark at the apex, 6–7 mm long and 2.5–3 mm wide, with membranous margin *ca*. 0.5 mm wide. Stigma dark green; anthers with pollen grains of variable size. Achenes unknown. Known only as herbarium material.

Etymology: the name of this species comes from OPTIMA (Organization for the PhytoTaxonomic Investigation of the Mediterranean Area), whose members first collected this species during the "VIII Iter Mediterraneum" in Calabria.

Ecology: mountain grasslands on siliceous substrate.

Chromosome number: unknown.

Distribution: known only for Sila Massif (Fig. 8, "+").

T. pollinense Aquaro, Caparelli & Peruzzi sp. nov. (Figs 9A–C)

Diagnosis: Planta 8.5–18 cm alta. Folia subprostrata, laete viridia vel gramineo-viridia, sparse araneosa, plerumque (1.7)3–7.5 cm longa et 0.5–1.9 cm lata, sublobata; lobus terminalis obtuse triangularis, 0.3–1 cm longus, 0.5–1.2 cm latus; lobi laterales 2–5, triangulares-deltoidei, marginibus distalibus convexis raro denticulatibus; interlobii sublati brevi, crispoplicatuli; petiolus alatus, subnullus vel 1.2 cm longus, nervo mediano viridis vel purpureo-colorato. Scapus paulo brunnescens, sparse araneosus, 6–17 cm longus. Involucrum basi 1–3 cm diametro, squamae interiores ad 12–16 mm longae et 1.5–2 mm latae, apice purpurascentes; squamae exteriores 13–16, subadpressae, ovatae, acuminatae, apice purpureo, membranaceo-marginatae (margines ad 0.3 mm latae), ad 4–6 mm longae et 1.5–3 mm latae. Stigmata obscure lutea, antherae polliniferae, grana pollinis diametro valde variantia. Achenium rubrum, superne breviter subdense spinulosum, 3–3.4 mm longum (pyramide exclusa) et 1 mm latum, in pyramiden cylindricam 0.7–1 mm longam subabrupte abiens. Rostrum 8–9 mm, pappus albus *ca*. 5–6 mm longi.

Holotypus: Italy, Calabria – Sommità del M. Pollino, 2229 m, valletta nivale, 29.06.2005, *G. Aquaro, L. Peruzzi* & *D. Gargano* (CLU, n. 18133; isotypes: FI, PI).

Paratypus: Italy, Calabria – M. Manfriana, Massicio del Pollino (Cs), *ca*. 1900 m, valletta nivale, 31.05.2005, *G. Aquaro, L. Peruzzi* & *D. Gargano* (CLU, n. 18134).

Other specimens seen: Italy, Calabria – Sommità del M. Pollino reg. alp., 22.06.1899, *Fiori* (FI, sub *T. apenninum*).

Description: Perennial medium/small-sized herb, 8.5–18 cm tall, pubescent on the adaxial surface of the leaves and densely hairy on the rachis; scapes much

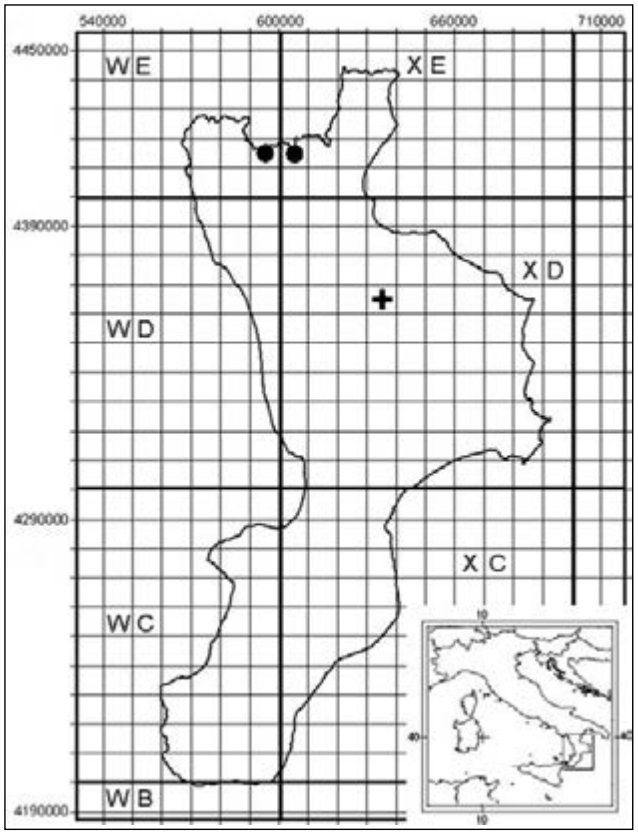

Fig. 8. Geographical distribution of *T. optimae* sp. nov. (+), *T. pollinense* sp. nov. (circle).

longer than the leaves. Leaves adpressed to soil, light green, (1.7)3–7.5 cm long and 0.5–1.9 cm wide, lobed (no more than 2/3 of the lamina); terminal lobe obtuse-triangular, 0.3–1 cm long and 0.5–1.2 cm wide; lateral lobes 2–5, triangular to deltoid; distal margin convex, rarely denticulate; interlobes short and sometimes plicate; petiole green to purplish along the rachis, winged, up to 1.2 cm long. Scape brown-reddish, sparsely hairy, 6–17 cm long. Involucre 1–3 cm wide at the base, inner bracts 13–16, 12–16 mm long and 1.5–2 mm wide, with a rose-reddish membranous apex; outer bracts 13–16, sub-adpressed, ovate, acuminate at the apex, 4–6 mm long and 1.5–3 mm wide, with membranous margin *ca*. 0.3 mm wide. Stigma dark green; anthers with pollen grains of variable size. Achenes light to dark red, with 11 ridges and few spines in the upper portion; body 3–3.4 mm long and 1 mm wide; cone 0.7–1 mm; rostrum 8–9 mm; pappus white, *ca*. 5–6 mm long.

Etymology: the name of this species comes from Mount Pollino (Pollino Massif), where the species was collected for the first time.

Ecology: snowbeds on limestones (1900–2200 m).

Chromosome number: $2n = 32$ (Fig. 10A). Karyotype formula: $2n = 4x = 4sm_{sat} + 20m + 4m^{sat} + 4m$ (Fig. 10B); $A_1 = 0.25$, $A_2 = 0.16$.

Distribution: known only for Pollino Massif (Fig. 8, circles).

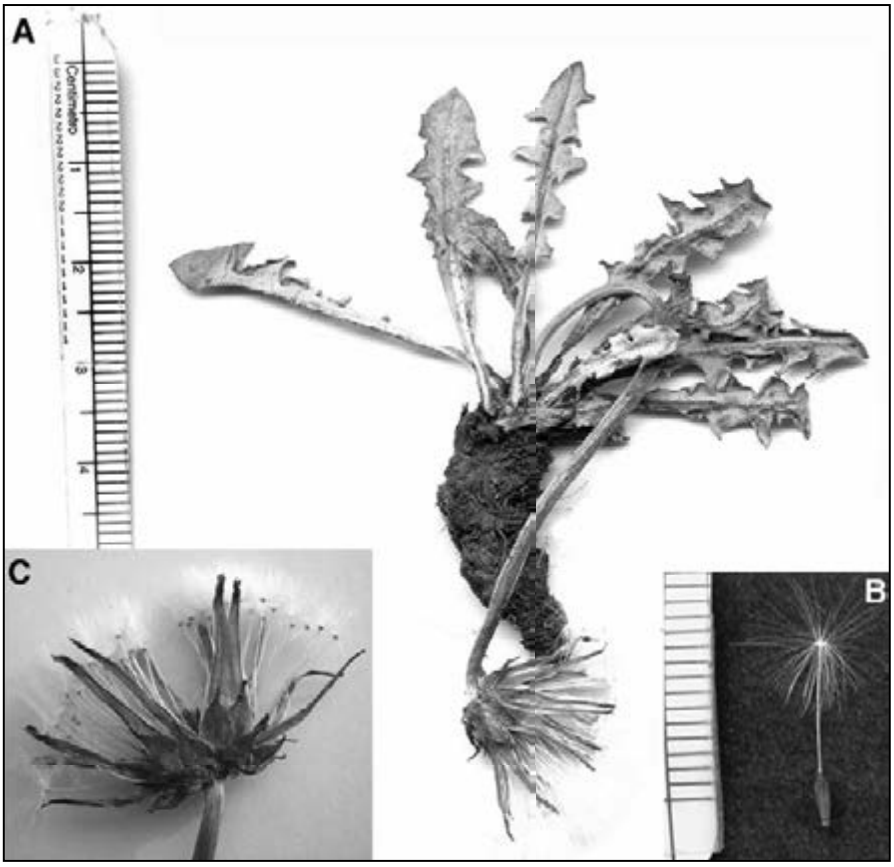

Fig. 9. *T. pollinense* sp. nov.: **A** – general view; **B** – particular of achene; **C** – particular of fruiting capitulum. All plants scanned are parts of type collections.

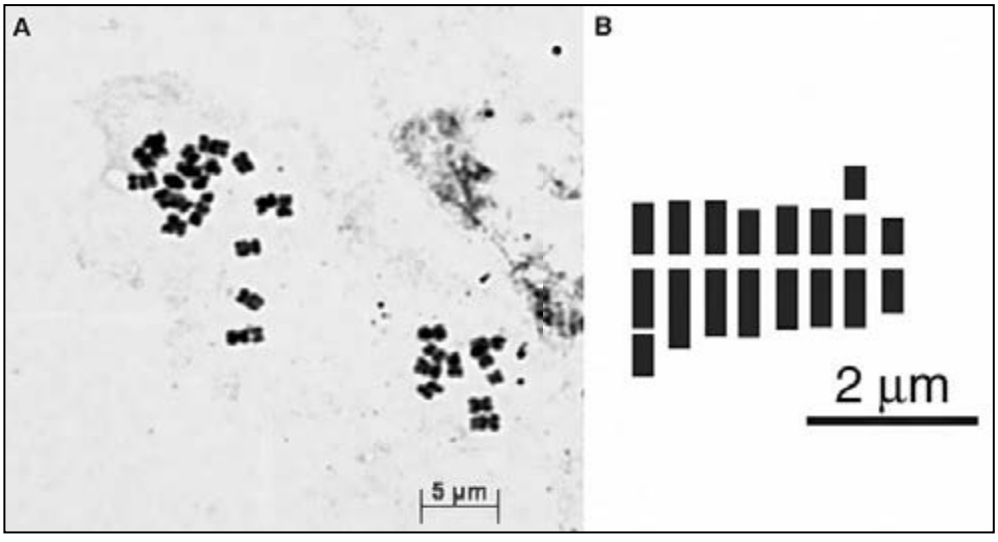

Fig. 10. *T. pollinense* sp. nov.: **A** – root tips metaphase plate, $2n = 32$; **B** – haploid idiogram.

Conclusions

Taraxacum sect. *Erythrocarpa* was here recorded for the first time in Calabria. In particular, it was possible to identify five distinct agamospecies in the studied area of S Italy (one of them, *T. cescae*, has been found also in Marche, C Italy). Four of them are big-sized plants, related to the E Mediterranean *T. calocephalum* (*T. calabricum* and *T. kirschneri*, both triploid with $2n = 24$ chromosomes) and to the Greek endemic *T. olympophilum* (*T. cescae*, $2n = 32$ and *T. optimae*). *Taraxacum pollinense*, with $2n = 32$ chromosomes, appear instead related to the Greek endemic *T. amborum* and to the N Balkan *T. janchenii*.

Acknowledgements. Many thanks are due to Prof. J. Kirschner and Dr. J. Štěpánek (Academy of Science, Průhonice – Czech Republic) for hosting two of the authors (GA & KFC) in Průhonice in September 2005, for providing precious bibliography and for helping on *Taraxacum* taxonomy.

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A new record for the flora of Turkey: *Ornithogalum boucheanum* (*Hyacinthaceae*)

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Abstract. A new record, *Ornithogalum boucheanum* (*Hyancinthaceae*) was added to the flora of Turkey with a specimen collected from European Turkey, A1(E): Edirne. Its morphological, karyological and palynological characteristics are given and its relationship with *O. nutans* is discussed. Chromosome number was found to be $2n = 56$. The pollen grains observed were oblate in shape, monocolpate and with reticulate exine.

Key words: flora of Turkey, new, *Ornithogalum boucheanum*

Introduction

The genus *Ornithogalum* L. has over 140 species throughout the world. It is a large genus centred in South Africa and the Mediterranean, with numerous species of horticultural note (Zahariadi 1980; Cullen 1984; Heywood 1993). According to *Flowers of Greece and the Balkans* (Polunin 1987) there are about 26 often similar-looking species in Balkan's area, 6 of which are endemic.

Ornithogalum is a taxonomically difficult genus, while its morphology is poorly correlated with variation in chromosome number and karyotype (Dalgıç & Özhatay 1997).

According to the *Flora of Turkey and the East Aegean Islands* (Cullen 1984; Davis & al. 1988; Özhatay 2000), the genus is represented in Turkey by 42 species; 15 of them are endemic to Turkey (Özhatay 2000). *Ornithogalum* is currently represented in European Turkey by 13 species. Dalgıç & Özhatay (1997) counted the choromosome number of *O. boucheanum*, but they did not determine it as a new record. After examination of the known bibliographical references (Webb 1966; Cullen & Ratter 1967; Lungeanu 1972; Cullen 1984; Dahlgren & al. 1985; Davis & al. 1988; Dalgıç & Ozhatay 1997), it became clear that this taxon is a new record for the flora of European Turkey.

This study was conducted especially for determination of *O. boucheanum* specimens which were collected from Edirne. Description of the species and its descriptive illustrations are given. Besides, *O. boucheanum* was investigated karyologically and palynologically.

Material and methods

The studied material of *O. boucheanum* was collected in 1989 and 2004, during floristic excursions in European Turkey. Voucher specimens were deposited in the Herbarium of Trakya University (EDTU). These specimens were determined according to the *Flora Europaea* (Zahariadi 1980) and the web pages of *Ornithogalum* species (www.hot.ee/sibullilled/ 2006; www.botanik.uni-karlsruhe.de/ 2006). The distribution of the determined taxon in European Turkey is presented in Fig. 1. The description of the species is given.

Fig. 1. The map of European Turkey showing the locations of *O. boucheanum* (■).

The chromosome preparations were made using a standard root-tip squash technique. Root tips for karyological analyses were pretreated with ABN for 24 hours at +4 °C, then fixed in Carnoy, i.e. 96 % ethyl alcohol: glacial acetic acid (3:1) for 24 hours. The root tips were hydrolysed with 1N HCl for 15 min at 60 °C. They were stained with Feulgen reagent for 2 hours in darkness at 25 °C. Dissected meristems were squashed and counterstained with aceto-orcein (Dalgıç & Özhatay 1997).

Pollen grains were examined by staining with lactophenol-anilin blue, they were swollen by 45 % acetic acid and stained with 2 % aceto-orcein for examination of the exine ornamentation. Besides, pollen slides were prepared according the methods described by Erdtman (1964). An Olympus photomicroscope with an apochromatic oil immersion objective (×100) and periplan eye piece (×10) were used in palynologic observations. The photographs were taken by the same Photomicroscope.

Results and discussion

Ornithogalum boucheanum **(Kunth) Asch. [syn.:** ***O. undulatum*** **Bouché,** ***O. nutans*** **subsp.** ***boucheanum*****]** (Figs 2–12)

(Subgen. *Myogalum* (Link) Baker)

Description

Scape is 35–50 cm long (Fig. 2). Bulbs are 30 × 35 mm, ovoid (Fig. 6), tunica is light brown, scapus is erect, cylindrical, light green. Leaves (Fig. 2) are 4–6, bright green, 38–50 cm × 10–20 mm, linear, canaliculated, the same length as scapus. The outer face of the leaves has white lines, edges are parallel and naked. Raceme is cylindrical with 20–30 flowers (Fig. 3). Bractae are 22–25 mm, lanceolate-acuminate, light brown and membrane-like structured. Pedicel is 5–10 mm, 10–13 mm in fruit. Outer tepal is 25–30 × 10 mm, inner tepal 20–25 × 8 mm, lanceolate, inner side is white toothed, outer side has a wide green band and acute apex. Filaments are 6–10 mm, membrane-like structured, wide, with wings. Anthers are 2, 4–5 mm, white or light yellow (Fig. 5). Ovarium is 5–9 mm, light green, conical-spherical, stilus is 4–7 mm (Fig. 4). Fruit is 18 × 10 mm, ovoid (Fig. 7). Seeds are 3–5, ovoid-orbicular, black and wrinkled (Fig. 8).

Other characteristics

Flowering time: April–May.

Growing habitat: near fields, bushy slopes.

Fig. 2. The general view of *O. boucheanum*.

Fig. 3. The flower of *O. boucheanum*.

Fig. 4. The pistil of *O. boucheanum* (×10).

Fig. 5. The stamen of *O. boucheanum* (×10).

Fig. 6. The bulb of *O. boucheanum*.

Fig. 7. The fruit of *O. boucheanum* (×7.5).

Fig. 8. The seeds of *O. boucheanum* (×20).

General distribution: Europe, South-East Balkans, Turkey.

Distribution in Turkey: European Turkey.

Distribution in European Turkey: A1(E) Edirne: Hacidanisment–Saridanisment village, 41°54' N, 26°49' E, 405 m alt., 30.04.1989, G. Dalgıç (EDTU 3150!); Karakasim: Tayakadin, 41°31' N, 26°37' E, 29 m alt., at the village entrance, 29.04.2004, G. Dalgıç & F. Dane (EDTU 9499!).

Suggested conservation status

The species seems to be very rare, local and near residential area; it should be classified as Vulnerable (Vu) (IUCN 2001).

Morphology

Ornithogalum boucheanum is morphologically closely related to *O. nutans*, but it differs from *O. nutans* with its diagnostic characters given in Table 1.

Table 1. Comparison between the diagnostic characters of *O. boucheanum* and *O. nutans*.

Diagnostic characters	*O. boucheanum*	*O. nutans*
Bulb	30–35 mm, ovoid	20–28 × 13–23 mm, ovoid
Scape	35–50 cm	20–60 cm
Leaves	Linear, canaliculated, the same length as scape or shorter	Linear parallel, almost as long as to longer than scape
Raceme	15–20 flowers	9–17 flowers
Pedicels	5–10 mm	20–40 mm
Flowers	All fertile	All fertile
Perianth segments	10–16 mm	20–31 mm
Ovary	Narrowly ovoid, longer than slender style	Ovoid to subglobose, shorter than slender style

Karyology

As a result of our investigations, the chromosome number of *O. boucheanum* is found to be $2n = 56$. The karyotype is defined as comprising 8 groups of chromosomes. These groups contain: 1 big centromere median (1st chromosome group); 2 big centromere submedian (2nd–3rd chromosome group), in 3rd group there are satellites at the end of the shorter arm of the 1st and 2nd chromosome; 3 medium centromere submedian (4th–5th–6th chromosome group); 2 small centromere median (7th–8th chromosome group) chromosomes (Figs 9, 10). *Ornithogalum boucheanum* is closely related to *O. nutans*, but differs from it with its basic chromosome number (*O. nutans* – $x = 7$; *O. boucheanum* – $x = 8$) and morphology (Dalgıç & al. 2004). While chromosome numbers of *O. nutans* are determined as $2n = 14$ and $2n = 35$, the chromosome number of *O. boucheanum* is determined as $2n = 56$ (Dalgıç & Özhatay 1997).

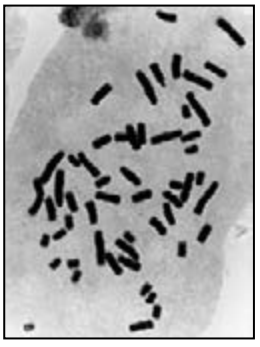

Fig. 9. The chromosome microphotograph of *O. boucheanum*, $2n = 56$ (×1000).

Fig. 10. The karyotype of *O. boucheanum*.

Pollen grain characteristics

The investigations on pollen grain characteristics showed that the pollen grains were oblate in shape, monocolpate (Fig. 11) and with reticulate exine (Fig. 12). Pollens were measured as P (polar axis): 70.84 ± 0.35 μm and E (equatorial axis): 50.27 ± 1.22 μm. It was observed that all the pollen grains were fertile after treatment with lactophenol-anilin blue as *O. nutans*, but they were bigger than *O. nutans* pollens. In *O. nutans* pollens, P = 64.02 ± 0.15 μm and E = 42.01 ± 0.20 μm. The exine ornamentation was reticulate in both species.

Acknowledgements. We would like to thank Prof. Dr. Neriman Özhatay who conducted the PhD thesis of G. Dalgıç for her help.

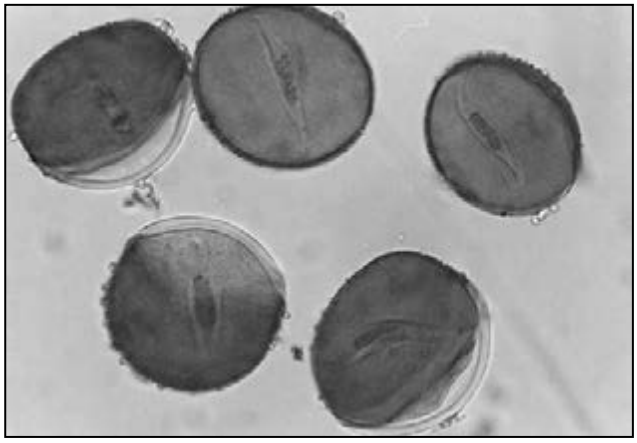

Fig. 11. Pollen grains of *O. boucheanum* (×400): equatorial view and aperture.

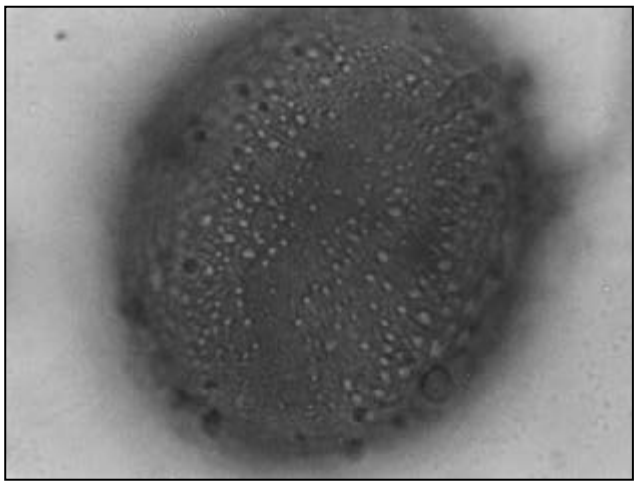

Fig. 12. Pollen grain of *O. boucheanum* (×1000): exine pattern.

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A cytotaxonomical study on endemic *Hesperis turkmendaghensis* (*Cruciferae* / Sect. *Hesperis*) in Turkey

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Abstract. *Hesperis turkmendaghensis* is described as a new species in 2005 from Turkey. This species grows naturally in Eskisehir province (Turkmen Dağı) in Central Anatolia in Turkey. In this study, the chromosome number of endemic *H. turkmendaghensis* belonging to Sect. *Hesperis* was determined. Its karyotype was studied by image analysis system. The chromosome number of the species was counted as $2n = 14$. Also, the chromosome morphology of *H. turkmendaghensis* was identified by calculating arm and centromeric index, the ratio length of its chromosome arms; its idiogram and karyogram were also made.

Key words: *Cruciferae*, *Hesperis*, karyotype, Turkey

Introduction

The genus *Hesperis* L. (*Cruciferae*) is distributed in the warm climate belt of Eurasia in South and Central Europe, Southwest Asia, the Caucasus, Russia and mountainous regions of Western China and Mongolia. This genus comprises almost 56 species throughout the world (Tzvelev 1959; Dvořák 1982; Duran & al. unpubl.) and 27 species in Turkey (Duran & Ocak 2005; Duran & al. unpubl.). Most species in Anatolia are confined to rather restricted areas of distribution. On the other hand, those occurring in moist areas are more widespread, especially in the Euro–Siberian phytogeographic region.

Hesperis turkmendaghensis was described by A. Duran and A. Ocak from Central Anatolia in 2005. The species grows under mixed forest, open forest and shady slopes in Turkmen Dağı in Eskisehir province (Duran & Ocak 2005). It belongs to Irano–Turanian phytogeographic region element. The population is not in good condition and approximately 300 specimens grow in a small area. Therefore, it should be graded as Critically Endangered (CR) (IUCN 2001).

The karyological researches on *Hesperis* taxa showed rather different chromosome numbers – $2n = 12, 14, 24, 26$ and 28. There are $2n = 24$ chromosomes in *H. alba* Mill. and *H. tristis* L., $2n = 14$ and $2n = 28$ chromosomes in *H. bicuspidata* Poir., $2n = 14, 24, 28$ chromosomes in *H. matronalis* L., $2n = 14$ chromosomes in *H. sylvestris* Crantz, *H. velenovskyi* (Fritsch) Fritsch, *H. sibirica* L., *H. steveniana* DC. and *H. pycnotricha* Borbás & Degen, $2n = 12$ chromosomes in *H. laciniata* All., *H. persica* Boiss. and *H. pendula* DC. (Löve & Löve 1961; Dvořák 1964, 1966a, b, c, 1973; Dvořák & Dadáková 1974, 1975, 1976; Tan & Iatrou 2001; Duran & Ocak 2005; Duran & al. unpubl.).

In this study, the chromosome number and karyotype of *H. turkmendaghensis* were identified for the first time.

Material and methods

The seeds of *H. turkmendaghensis* were obtained from Turkmen Dağı (Eskisehir) in Turkey. Seeds were germinated at room temperature on moist filter paper in Petri dishes. Root tips for karyotypic studies were pretreated with α-mono-bromonaphthalene at 4 °C for 16 hours, and were fixed in a mixture of ethanol: glacial acetic acid (3:1) for 24 hours in refrigerator. The root tips were hydrolysed with 1N HCl for 13 minutes at room temperature. They were stained with 2 % acetic orcein for 2 hours in squashed root meristems then were counterstained with 45 % acetic acid. We made permanent slides using the standard liquid nitrogen

method. We examined slides under Olympus BX–50 Photomicroscope using an oil immersion objective (100×). Photographs were taken with the same microscope. The idiogram was prepared with measurements taken on enlarged micrographs of five well spread metaphase plates. The length of long and short arm, arm ratio, centromeric index and relative chromosome length were measured by software image analysis (Bs200Pro Image Analysis Software) loaded on a personal computer. Chromosomes were classified using the nomenclature of Levan & al. (1964). Karyogram of the best metaphases and idiogram of this species were arranged in decreasing length.

Results and discussion

The cytological researches on the genus *Hesperis* show that chromosome numbers are $2n$ = 12, 14, 24, 26 and 28 (Manton 1932; Löve & Löve 1956, 1961; Dvořák 1964, 1966a, b, c, 1973, 1982; Dvořák & Dadáková 1974, 1975, 1976; Tan & Iatrou 2001).

The chromosome number and karyology of *H. turkmendaghensis* are being reported here for the first time. The chromosome number was determined to be $2n$ = 14 (Fig. 1). Total chromosome length is between 5.21–2.50 µm. Total lengths of chromosomes, arm ratio, centromeric index and relative chromosome length (according to Levan & al. 1964) are given in Table 1. The total length of the haploid set was 22.49 µm and the total measured length of the diploid chromosomes was 21.99 µm. The average chromosome length was calculated as 3.14 µm.

The karyotype analysis of *H. turkmendaghensis* determined the diploid chromosome number $2n$ = 14. According to this result, basic chromosome number is n = 7. The karyotype of the species consists of four median, two submedian and one subterminal chromosomes. The idiogram and karyogram designated the karyotype formula K ($2n$ = 14) = 4m+2sm+1st (Figs 2, 3).

We hope that this study will contribute to the future karyological studies on genus *Hesperis*.

Table 1. Measurements of somatic chromosomes in *H. turkmendaghensis*.

Chromosome / Pair No.	Chromosome arms (µm)		Total length (µm)	Arm ratio (L/S)	Relative length (%)	Chromosome type
	Long arm (L)	Short arm (S)				
1	3.00	2.21	5.21	1.36	10.04	m
2	1.91	1.34	3.25	1.43	6.09	m
3	2.42	0.77	3.19	3.14	3.50	st
4	1.91	0.74	2.65	2.59	3.36	sm
5	1.46	1.17	2.63	1.26	5.29	m
6	1.74	0.82	2.56	2.11	3.75	sm
7	1.41	1.09	2.50	1.28	4.98	m
Total length of haploid complement: 22.49 µm						

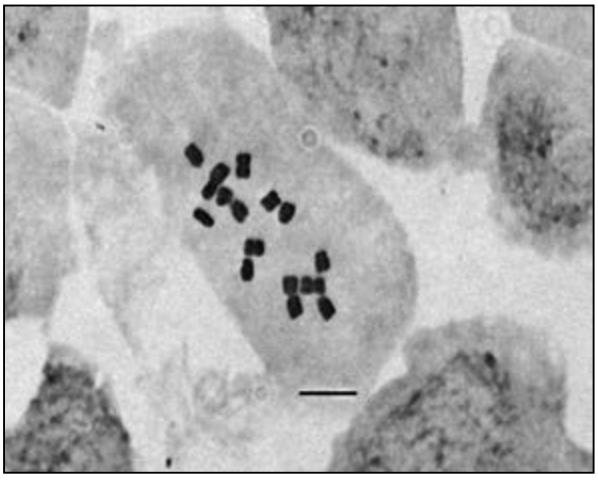

Fig. 1. Mitotic metaphase chromosomes in *H. turkmendaghensis*, $2n$ = 14. Scale bar = 5 µm.

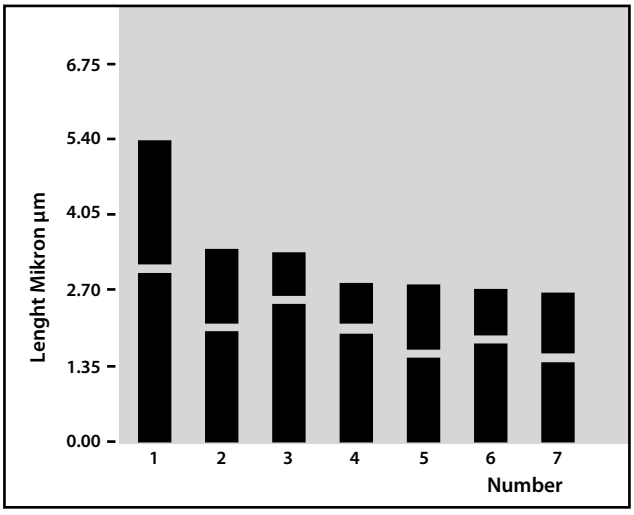

Fig. 2. Idiogram of *H. turkmendaghensis*.

Fig. 3. Karyogram of *H. turkmendaghensis*.

Acknowledgements. We express our thanks to TÜBITAK (Project No. TBAG–1748) and the Scientific Investigation Project to Coordinate of Selçuk University (Project No. 06701104) for financial support.

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Notes on some critical *Bupleurum* species from sect. *Aristata* in Bulgaria

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Abstract. This paper deals with comparative morphological and karyological investigations on 10 annual species of sect. *Aristata* of the genus *Bupleurum* in Bulgaria. The aim of the study is to illustrate the differences between closely related taxa, which often are determined incorrectly.

Key words: *Bupleurum* section *Aristata*, karyology, morphology

Introduction

The Eastern Mediterranean has high species diversity in annuals of *Bupleurum* (Snogerup 1972). The penetration of species from Asia Minor, as well as speciation in the Western Balkans is the reason on the Balkan Peninsula (including the Aegean islands) to occur about 80 % (26 species) of the European annuals of *Bupleurum* (Snogerup & Snogerup 2001). In the Iberian Peninsula there are only 7 annual *Bupleurum* species (Neves 2003). In the Western Mediterranean, where is the ancient origin centre of the genus, predominate the perennials of *Bupleurum*.

Thirteen annual *Bupleurum* species are presented in Bulgaria (1 of sect. *Bupleurum* and 12 of sect. *Aristata*), most of them have Balkan, Balkan–Pannonian or Balkan–Anatolian distribution areas. In the present work the Snogerup & Snogerup (2001) taxonomic scheme for the annual *Bupleurum* species is adopted. The section *Aristata* Godr. divides to: subsect. *Aristata* – the bracteoles more or less scarious, before and after flowering envelop the flowers, and subsect. *Juncea* Briq. – the bracteoles are herbaceous, erecto-patent to patent all through the flowering stage. For the differentiation of the species in the subsections high taxonomic value have the form and nervation of the bracteoles and the bracts, the length and the correlation between the umbel rays, and in some cases the characteristics of the mericarps and the styles.

Material and methods

The morphological investigation was done on personal gatherings during the period 2004–2005 and on specimens from Bulgarian herbaria SOM and SO. For showing in details the differences between close and often incorrectly determined taxa macrophotographs of bracts, umbel rays, mericarps, etc. were made.

The karyotypes were studied on mitotic metaphase plates obtained from root tips taken from seeds, fixed in ethanol: acetic acid (3:1), hydrolyzed in 1N HCl at 60 °C for 12 min, and stained with haematoxylin after Gomory (Melander & Wingstrand 1953).

Results and discussion

Morphological investigation

All taxa of subsect. *Aristata* – *B. odontites* L., *B. apiculatum* Friv., and *B. flavum* Forssk. were examined. In the subsect. *Juncea* comparatively are discussed pairs of species: *B. euboeum* Beauverd & Topali – *B. tenuissimum* L.; *B. aequiradiatum* (H. Wolff) Snogerup & B. Snogerup – *B. praealtum* L.; *B. pachnospermum* Pančić – *B. affine* Sadler.

- **Subsect. *Aristata***

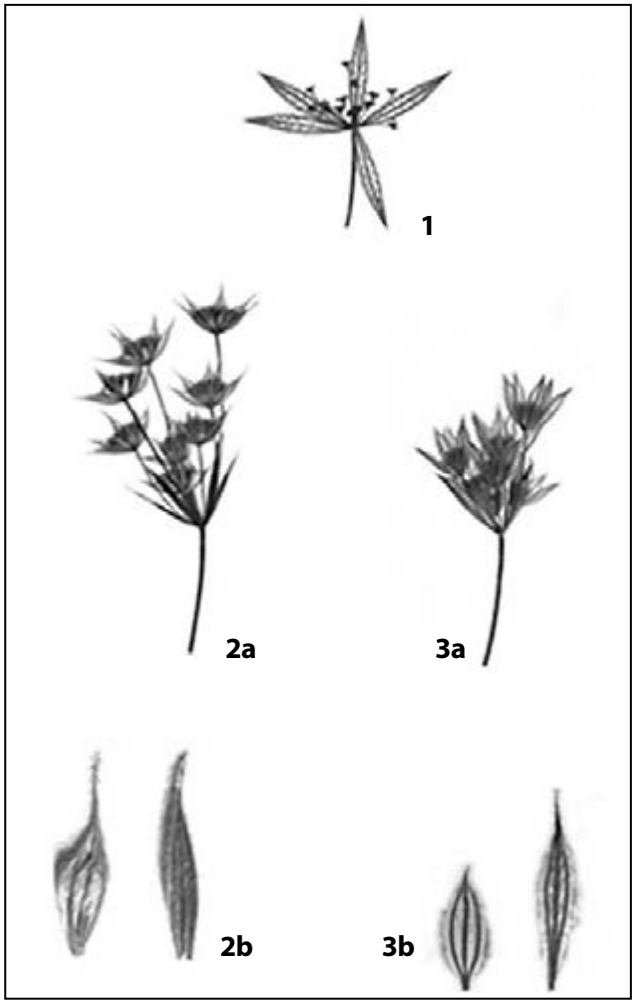
Subsect. *Aristata* is presented in Bulgaria with the species *B. odontites* (= *B. fontanesii* Guss.), *B. apicula-*

tum, and *B. flavum*. *Bupleurum baldense* Turra subsp. *gussonei* (Arcang.) Tutin was specified wrongly for Bulgaria from Assenov (1982). The Bulgarian specimens of this taxon belong to *B. apiculatum*.

Bupleurum odontites is easily distinguished by its lanceolate bracteoles and bracts of which the three main veins have many secondary veins in between, forming a unique reticulate nervation. The flower stalks are rather unequal (Plate I, Fig. 1).

Some problems raise the differentiation of *B. apiculatum* and *B. flavum*, which are sympatric species in the south-eastern parts of the Balkan Peninsula. Both species have elliptic bracteoles and lanceolate bracts, but the characteristics of the nervation differentiate them clearly.

Plate I



Figs 1–3. Macromorphological characters of the species of *Bupleurum* subsect. *Aristata*:
1, *B. odontites*, flowers and bracteoles; **2,** *B. apiculatum*: umbel rays (**a**); bracteole and bract (**b**); **3,** *B. flavum*: umbel rays (**a**); bracteole and bract (**b**).

The bracteoles of *B. apiculatum* have on top a distinct, 2–3 mm long awn; the middle of three main veins has very few inconspicuous short secondary veins or such are missing. The bracts are herbaceous, narrow lanceolate, with three inconspicuous parallel veins, without secondary veins and with narrow scarious strips on the periphery (Plate I, Figs 2a, b).

The bracteoles of *B. flavum* are mucronate on top or have an awn up to 1 mm long; the main veins are pronounced, forming elevated ridges; secondary veins are missing. The bracts are scarious, wide lanceolate, with three visible main veins, the middle one with indistinct secondary veins, on the periphery with wide scarious strip (Plate I, Figs 3a, b).

- **Subsect. *Juncea***

According to Assenov (1982) *B. tenuissimum* is represented in Bulgaria with 2 subspecies – subsp. *tenuissimum* and subsp. *gracile* (M. Bieb.) H. Wolff. The combination *B. tenuissimum* subsp. *gracile*, however, is illegitimate, because was founded on the name *B. gracile* (M. Bieb.) DC. (1830), non d'Urv. (1822), which is a subsequent homonym, so it was rejected and replaced with nomen novum *B. marschallianum* C.A. Mey. (1831). The taxon *B. marschallianum*, in fact, does not occur in Bulgaria.

Bupleurum euboeum (*B. marschallianum* auct., non C.A. Mey.) was reported for Bulgaria first by Snogerup & Snogerup (2001) on some materials from Philippopel (Plovdiv), 08.1889, Velenovský, deposited in PR and PRC collections. This new for Bulgaria taxon called for a critical review of the specimens in Bulgarian herbarium collections deposited under the names *B. tenuissimum*, *B. tenuissimum* subsp. *gracile*, and *B. marschallianum*. As a result of the revision *B. euboeum* was confirmed for the country. Herbarium specimens from two locations were found – Plovdiv town, 03.08.1893, S. Gheorghieff, SO 54376 (sub *B. marschallianum*) and the valley of Strouma River (Southern), nearby mineral bath of Marikostinovo village, Petrich district, 25.07.1977, B. Kouzmanov, SOM 135455 (sub *B. marschallianum*), rev. I. Assenov, 12.07.1978 (sub *B. tenuissimum* subsp. *gracile*). The second locality habitat was visited in 2004 and *B. euboeum* was rediscovered.

Because of the warty formations on the surface of the fruits *B. tenuissimum* and *B. euboeum* were related to a separate subsect. *Trachycarpa* (Lange) Briq. Despite the relation, the two species differ from one another in

their habit and characteristics of the fruits. The secondary sprays of *B. euboeum* (Plate II, Fig. 1a) are more or less equal along the whole length of the stem, while at *B. tenuissimum* (Plate II, Fig. 2a) the secondary sprays in the low third of the stem are much longer than those in the middle and the upper part of the stem.

Bupleurum euboeum – the fruit surface uniformly papilla-like rugulate, the ridges of the mericarps inconspicuous. The styles are 0.4–0.5 mm, surpass the radius of the stylopodium. At the fruit ripening the stylopodium is visible and stays over the mericarps (Plate II, Fig. 1b).

Bupleurum tenuissimum – the fruit surface partly rugulate, the mericarps ridges pronounced, almost aliform, verrucose. The styles are up to 0.2 mm, shorter than the radius of the stylopodium. At the fruit ripening the mericarps increase in their upper part and cover the stylopodium (Plate II, Fig. 2b).

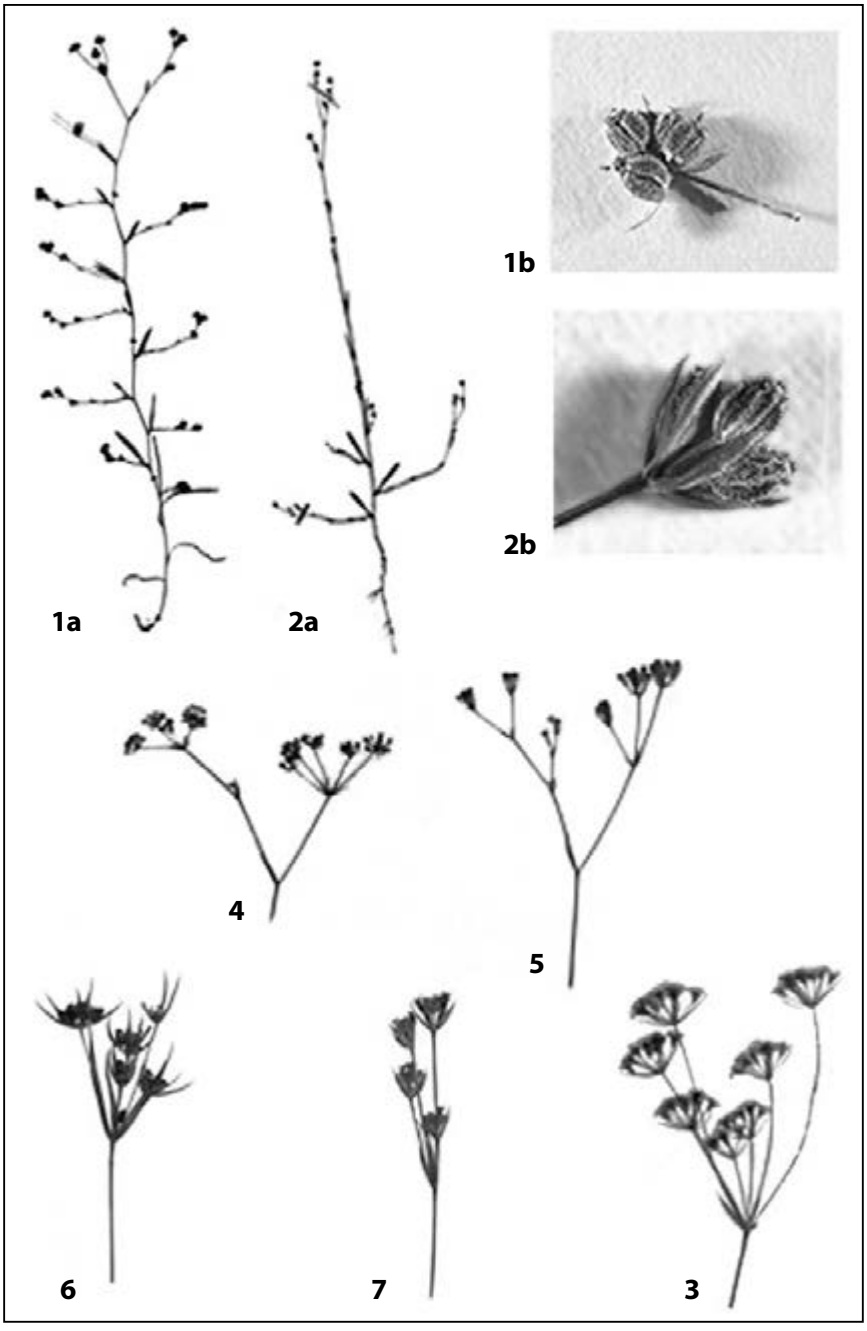
Recent taxonomic works on *B. commutatum* Boiss. & Balansa in Bulgaria accept its subdivision into 3 subspecies – subsp. *commutatum*, subsp. *aequiradiatum* (H. Wolff) Hayek (≡ *B. aequiradiatum*), and subsp. *glaucocarpum* (Borbás) Hayek (= *B. pachnospermum*).

Bupleurum commutatum s. str. is well distinguished from *B. aequiradiatum* and *B. pachnospermum* (considered until recently as its subspecies) by its comparatively unequal and tender umbel rays (Plate II, Fig. 3). The rays lengthen at the fruit ripening and because they are thin they bend more or less arch-like. *Bupleurum commutatum*, *B. aequiradiatum*, and *B. pachnospermum* show essential differences in the karyotypes morphology too.

The region's live materials observations determined morphological similarity between *B. aequiradiatum* and *B. praealtum* – the stem leaves with thick (keel-like) middle vein, bracteoles do not surpass the flowers and fruits, the umbel rays are equal (Plate II, Figs 4, 5).

Morphological similarity show also the pair of species *B. pachnospermum* and *B. affine* – habit characteristics, straight and powerful stem with monotype branching, secondary stems also straight and appearing in the axils of the middle stem leaves, sometimes

Plate II



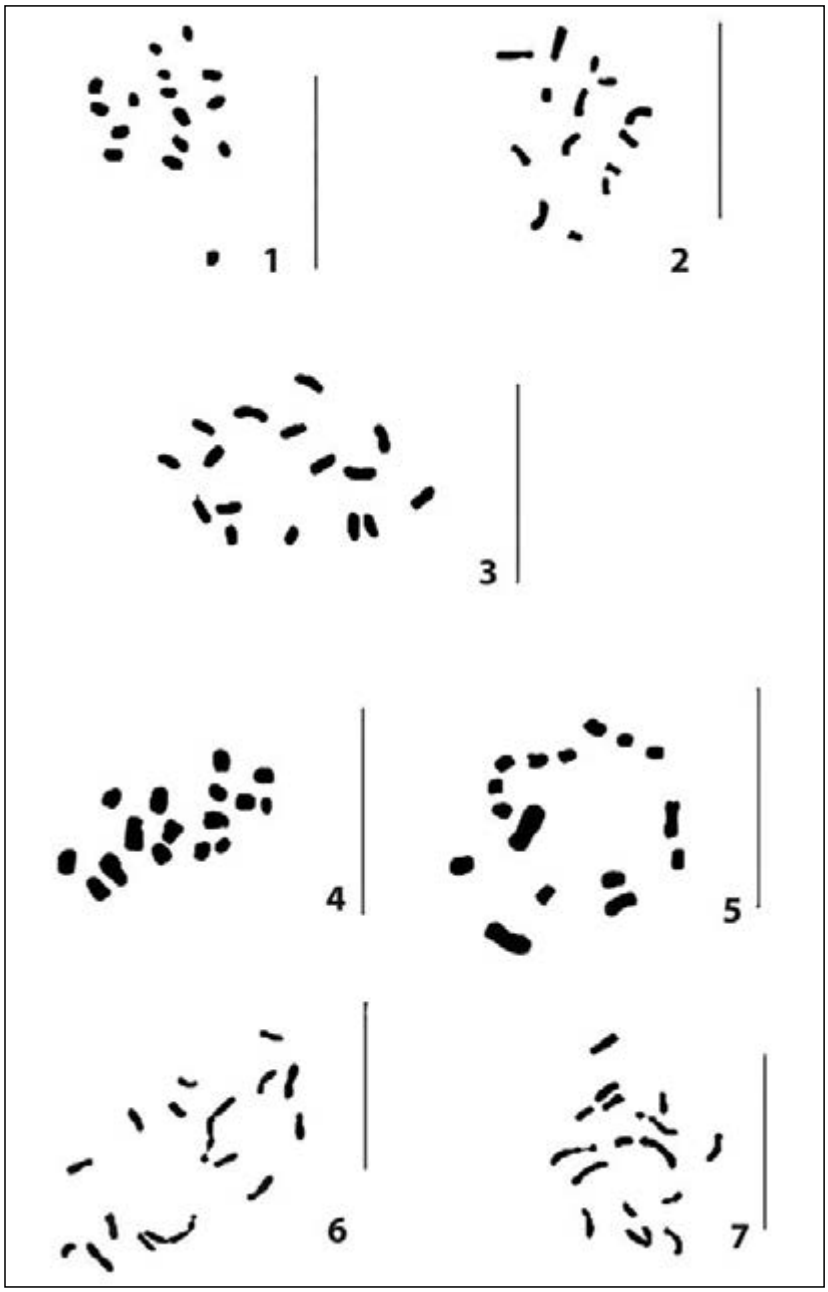
Figs 1–7. Macromorphological characters of the species of *Bupleurum* subsect. *Juncea*:
1, *B. euboeum*: habit (**a**) and fruits (**b**);
2, *B. tenuissimum*: habit (**a**) and fruits (**b**);
3–7, umbel rays – **3,** *B. commutatum*;
4, *B. aequiradiatum*; **5,** *B. praealtum*;
6, *B. pachnospermum*; **7,** *B. affine*.

even lower. The bracteoles form and length, number of umbel rays, as well as the fruit characteristics also show close relation (Plate II, Figs 6, 7).

Karyological investigation

Seven species of sect. *Aristata* were karyologically investigated. For six of them – *B. apiculatum*, *B. flavum*, *B. euboeum*, *B. commutatum*, *B. aequiradiatum*, and *B. pachnospermum* the chromosome numbers are reported here for the first time. For *B. affine* the investigation is the first one from Bulgarian population.

Plate III



- **Subsect. *Aristata***

Bupleurum apiculatum. 2*n* = 16 (Plate III, Fig. 1). Two populations were karyologically investigated: from Mt Golo Bardo, Radomir district, FN-60 and from Ploski village, Sandanski district, FM-81. The karyotype consisted of small, gradually differentiating in length short chromosomes without visible centromeres.

Bupleurum flavum. 2*n* = 14 (Plate III, Fig. 2). Two populations were karyologically investigated: from Dobrich village, Elhovo district, MG-66 and from Medovo village, Kableshkovo district, NH-42. The karyotype consisted of medium-sized and short chromosomes, most of them of submetacentric type.

Bupleurum odontites was not karyologically investigated from Bulgarian population. In the literature the diploid chromosome number 2*n* = 2x = 16 has been reported (Snogerup 1994).

- **Subsect. *Juncea***

Bupleurum euboeum. 2*n* = 16 (Plate III, Fig. 3). One population was karyologically investigated: from Marikostinovo village, Petrich district, FL-98. The karyotype consisted of nearly identical in length chromosomes of metacentric and submetacentric type. A pair of SAT-chromosomes was observed in some of the chromosome sets.

Bupleurum commutatum. 2*n* = 16 (Plate III, Fig. 4). Two populations were karyologically investigated: from Mt Chepun, Dragoman district, FN-65 and from the Bakadzhitsite hills, Yambol district, MG-79. The karyotype consisted of small chromosomes without distinct centromeres.

Bupleurum aequiradiatum. 2*n* = 16 (Plate III, Fig. 5). Two populations were karyologically investigated: from Smirnentsi village, Harmanli district,

Figs 1–7. Metaphase plates of: **1,** *B. apiculatum*, 2*n* = 16; **2,** *B. flavum*, 2*n* = 14; **3,** *B. euboeum*, 2*n* = 16; **4,** *B. commutatum*, 2*n* = 16; **5,** *B. aequiradiatum*, 2*n* = 16; **6,** *B. pachnospermum*, 2*n* = 16; **7,** *B. affine*, 2*n* = 16. Scale bars = 10 μm.

MG-02 and from Medovo village, Kableshkovo district, NH-42. A pair of chromosomes of submetacentric type comparatively longest, and a pair of shorter chromosomes of metacentric type were observed in the karyotype. The other six pairs of chromosomes were short, without visible centromeres.

Bupleurum pachnospermum. 2*n* = 16 (Plate III, Fig. 6). One population was karyologically investigated: from Mt Chepun, Dragoman district, FN-65. The karyotype was heterogeneous; it consisted of one pair of long acrocentric chromosomes with satellites, one pair of long chromosomes of metacentric type, five pairs of medium-sized submetacentric chromosomes and one pair of very short chromosomes, probably of metacentric type.

Bupleurum affine. 2*n* = 16 (Plate III, Fig. 7). Two populations were karyologically investigated: from Mt Chepun, Dragoman district, FN-55 and from Ushi village, Treklyano district, FN-21. The karyotype was heterogeneous; it consisted of one pair of long chromosomes of submetacentric type, one pair of acrocentric chromosomes with satellites, four pairs were medium-sized, gradually differentiating in length submetacentric chromosomes, and two pairs of short chromosomes, without distinct centromeres. Our result agrees with earlier report for this species (Cauwet-Marc 1976).

Bupleurum tenuissimum and *B. praealtum* were not karyologically studied from Bulgarian populations. In the literature the diploid chromosome number 2*n* = 2x = 16 has been reported for both species (Cauwet-Marc 1976).

Conclusion

As a result of the comparative morphological and karyological investigations it was established that the species *B. apiculatum* and *B. flavum* are well differentiated. *Bupleurum euboeum* was confirmed for the Bulgarian flora. *Bupleurum aequiradiatum* and *B. pachnospermum* are well differentiated species and even distinct from *B. commutatum*. *Bupleurum aequiradiatum* shows similarity to *B. praealtum*, and *B. pachnospermum* is closely related to *B. affine*.

Acknowledgements. The authors are grateful to R. Dimova for the English translation of the text and to C. Nikolov for the photos.

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Pollen morphological study on some Bulgarian and Turkish species of genus *Centaurea* s.l. (*Compositae*)

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Abstract. The present work includes some preliminary data from a comparative biosystematic investigation on genus *Centaurea* s.l. in the Bulgarian and Turkish floras. The pollen morphology and exine srtucture of eight taxa – *Centaurea derderiifolia*, *C. drabifolia* subsp. *detonsa*, *C. kernerana*, *C. moesiaca*, *C. parilica*, *C. polyclada*, *Cyanus pseudaxillaris* and *C. thirkei* were studied in order to specify their type and taxonomic importance. All taxa except the last one are endemics. The results show that the pollen shape, size, aperture and exine structure proved to be of high diagnostic value and could be used as additional characters to determine the species.

Key words: *Centaurea*, endemic taxa, pollen morphology, SEM, TEM

Introduction

Pollen morphology, base chromosome number and DNA sequences are the most important characteristics used to make a modern phylogenetic taxonomic scheme of subtribe *Centaureinae*, *Compositae* (Wagenitz & Hellwig 1996; Garcia-Jacas & al. 2001; Hellwig 2004). Wagenitz (1955) delimited eight *Centaurea* pollen types corresponding very well to the main taxonomic groups into this subrtibe. In the Turkish and Bulgarian floras grow about 250 species, more than 50 % of which are weakly investigated endemics.

The aims of this work were to examine the morphology of the pollen grains of eihgt taxa of subtribe *Centaureinae* (seven of them are endemics) and to determine their pollen type following Wagenitz (1955) as a step of their biosystematic study.

Material and methods

The research was based on material collected from different floristic regions of Bulgaria and Turkey, where the studied taxa are naturally distributed.

The vouchers are kept in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM) and in the Bartin Faculty of Forestry, Zonguldak University, Turkey.

The pollen morphology was studied in light microscope (LM) and scanning and transmission electronic microscopes (SEM and TEM). The material for the light microscopy was prepared after Wodehouse (1935) (W) and Erdtman (1943) (E). The preparation of the pollen grains for observation in SEM was accomplished according to Huttunen & Laine (1983). Observations were carried out with a SEM Leica S420 at 15 kV. For the TEM microscopy Glauert (1975) was followed. The morphological terminology of Erdtman (1943, 1952), Skvarla & Turner (1966), Skvarla & al. (1977) and Faegri & Iversen (1989) has been used.

The abbreviations in Turkish localities are in accordance with the Turkish map grid system (Davis 1975).

The abbreviations of the authors follow Brummitt & Powell (1992).

Results

Centaurea derderiifolia Wagenitz

Locality: Turkey–B7, Elazığ: Ensedere locality, above Bölükçalı village, Keban district, rocky slopes, 1200 m alt., 26.08.2003, leg. & det. Z. Kaya.

Distribution: Turkish endemic.

Pollen type: Trizonocolporate (Fig. 1).

Wagenitz's pollen type: Jacea.

Pollen shape: Spheroidal, P/E = 1.00 (W); suboblate, P/E = 0.79 (E).

Exine thickness: 1.69 μm (W); 4.68 μm (E).

Apertures: Colpi narrow and long, with definite boundaries. Colpus ends – acute. Colpus length 22–25 μm, width 1–2 μm. Pores definite. Porus length (P_l)/Porus width (P_w) = 1.37. The polar triangle is not definite.

Sculpture: Microechinate. Spinules short, *ca.* 0.5 μm. Density of the sculpture elements: 5–7 in 9 μm^2.

Centaurea drabifolia Sm. subsp. *detonsa* (Bornm.) Wagenitz

Locality: Turkey–C3, Isparta: Dedegöl Mt, cliffs and rocky slopes, 1600 m, 28.08.1999, leg. & det. Z. Kaya.

Distribution: Turkish endemic.

Pollen type: Trizonocolporate (Fig. 2).

Wagenitz's pollen type: Jacea.

Pollen shape: Spheroidal, P/E = 1.01 (W); subprolate, P/E = 1.21 (E).

Exine thickness: 2.34 μm (W).

Apertures: Colpi long and wide, with definite boundaries. Colpus ends – acute. Colpus length 30–31 μm, width 8–9 μm. Pores with definite boundaries. P_l/P_w = 0.8 (W); 0.88 (E). The polar triangle is large and regular.

Sculpture: Microechinate. Spinules short, *ca.* 0.4–0.85 μm. Density of the sculpture elements: 2–5 in 9 μm^2.

Centaurea polyclada DC.

Locality: Turkey–B1, Izmir: Caparli village, above Yamanlar district, Yamanlar Mt, rocky places, 700 m, 07.07.2000, leg. & det. Z. Kaya.

Distribution: Turkish endemic.

Pollen type: Trizonocolporate (Fig. 3).

Wagenitz's pollen type: Jacea.

Pollen shape: Sphaeroidal, P/E = 0.99 μm (W); sphaeroidal, P/E = 1.13 μm (E).

Exine thickness: 1.06 μm (W).

Apertures: Colpi long and wide, with definite boundaries. Colpus ends – acute. Colpus length 25–27 μm, width 9–10 μm. Pores with definite boundaries. P_l/P_w = 0.90 μm. The polar triangle is large and regular.

Sculpture: Microechinate. Spinules short, *ca.* 0.6–1.06 μm. Density of the sculpture elements: 6–9 in 9 μm^2.

Cyanus thirkei Sch. Bip.

Locality: Turkey–A3, Bolu: Arak village in forest of *Pinus nigra*, Mengen district, 900 m, 22.06.2004, leg. & det. Z. Kaya.

Distribution: Bulgaria, Moldavia, European Turkey, Asia Minor.

Pollen type: Trizonocolporate (Fig. 4).

Wagenitz's pollen type: Montana.

Pollen shape: Subprolate, P/E = 1.18 (W); sphaeroidal, P/E = 1.04 (E).

Exine thickness: 2.70 μm (W).

Apertures: Colpi long and wide, with definite boundaries. Colpus ends – acute. Colpus length 33–34 μm, width 9–10 μm. Pores with definite boundaries. P_l/P_w = 0.92 μm. The polar triangle is large and regular.

Sculpture: Scabrate. Density of the sculpture elements: 2–3 in 9 μm^2.

Centaurea moesiaca Urum. & Wagner

Locality: Bulgaria, Beklemeto, Stara planina Mts, 10.08.1995, leg. & det. S. Bancheva (Sh9535).

Distribution: Bulgarian endemic.

Pollen type: Trizonocolporate (Fig. 6).

Wagenitz's pollen type: Jacea.

Pollen shape: Spheroidal, P/E = 1.01 (W); subprolate, P/E = 1.2 (E).

Exine thickness: 2.8–4.3 μm (E).

Apertures: Colpi narrow and long, with definite boundaries. Colpus ends – acute. Colpus length 20.1 μm ± 1.2, colpus width 2.1 μm ± 0.3. Pores definite; pore length 3.3 μm ± 0.9. Coste colpi thickness 1.4 μm ± 0.5.

Structure: Tectate and cavate.

Sculpture: Echinate. Spinules *ca.* 2 μm. Density of the sculpture elements: 1–3 in 9 μm^2.

Intine: Thin, homogenous; Exine/Intine ≈ 3/1.

Centaurea kernerana Janka

Locality: Bulgaria, Ray chalet, Stara planina Mts, 08.08.1995, leg. & det. S. Bancheva (Sh9537).

Distribution: Bulgarian endemic.

Pollen type: Trizonocolporate (Fig. 7).

Wagenitz's pollen type: Jacea.

Pollen shape: Subprolate, P/E = 1.1 (W).

Exine thickness: 2.5–3.6 μm (E).

Apertures: Colpi narrow and long, with definite boundaries. Colpus ends – acute. Colpus length 24.6 μm ± 2.6, colpus width 2.1 μm ± 0.8. Pores definite; pore length 5.1 μm ± 1.5. Coste colpi thickness 2.4 μm ± 0.4.

Structure: Tectate and cavate (Fig. 8).

Sculpture: Microechinate. Spinules *ca.* 0.3–1 μm. Density of the sculpture elements: 1–3 in 9 μm^2.

Intine: Thin and homogenous; Exine/Intine ≈ 3/1.

Centaurea parilica Stoj. & Stef.

Locality: Bulgaria, Koynarite, Slavyanka Mt, 25.08.1995, leg. & det. S. Bancheva (Sh9532).

Distribution: Balkan endemic – Bulgaria and Greece.

Pollen type: Trizonocolporate (Fig. 5).

Wagenitz's pollen type: Jacea.

Pollen shape: Subprolate, P/E = 1.2 (E); prolate, P/E = 1.8 (W).

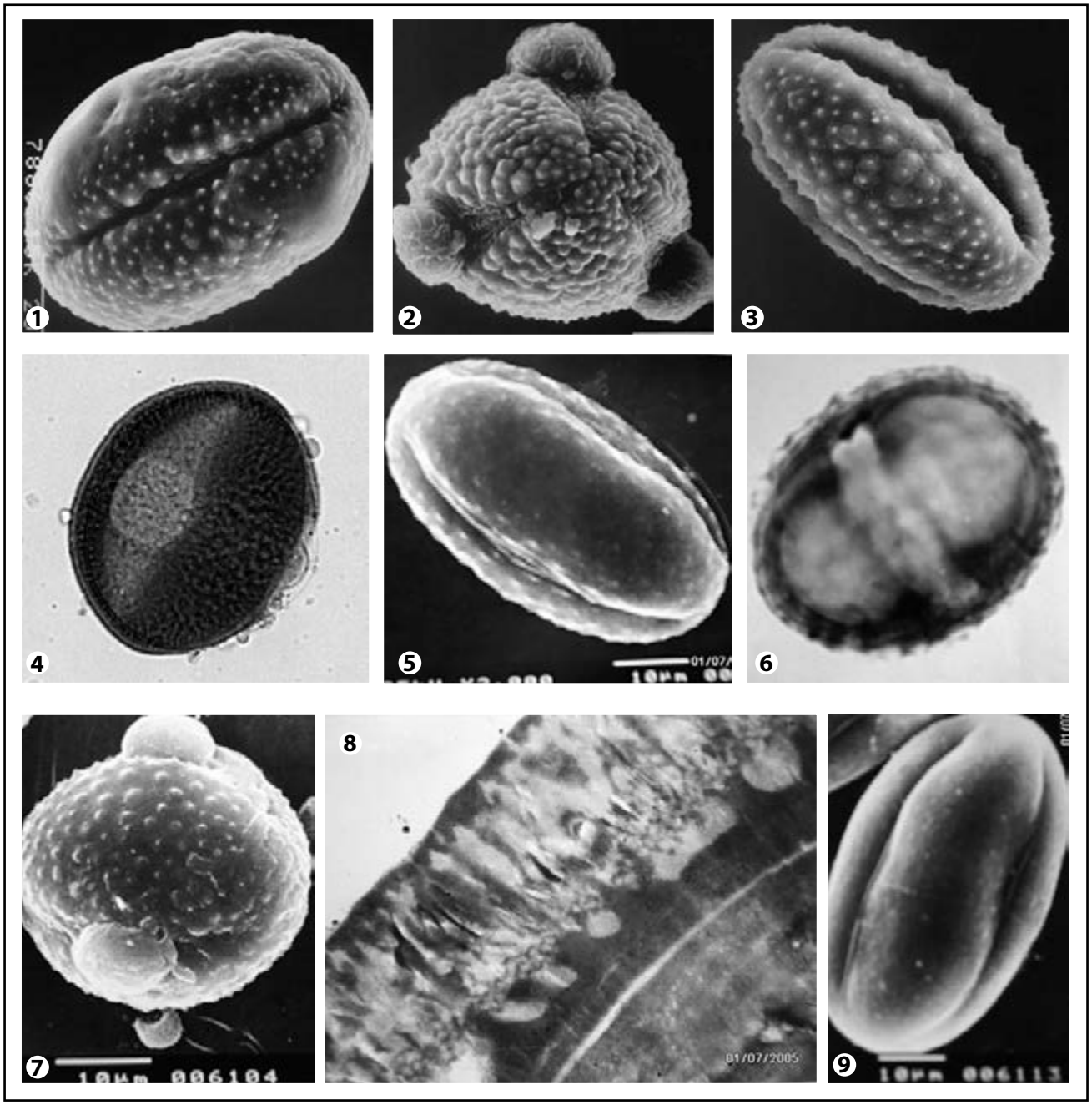
Exine thickness: 3.2–5 μm (E).

Apertures: Colpi narrow and long, with definite boundaries. Colpus ends – acute. Colpus length 27 μm ± 2.8, colpus width 3.1 μm ± 0.5. Pores definite; pore length 4.7 μm ± 0.9. Coste colpi thickness 2.9 μm ± 0.5.

Structure: Tectate and cavate.

Sculpture: Microechinate. Spinules *ca*. 0.2–0.4 μm. Density of the sculpture elements: 1–2 in 9 μm^2.

Intine: Thin; Exine/Intine ≈ 3/1.



Figs 1–9. Pollen grain of:
1, *Centaurea derderiifolia* (SEM); **2,** *C. drabifolia* subsp. *detonsa* (SEM); **3,** *C. polyclada* (SEM); **4,** *Cyanus thirkei* (LM); **5,** *Centaurea parilica* (SEM); **6,** *C. moesiaca* (LM); **7, 8,** *C. kernerana* (SEM and TEM); **9,** *Cyanus pseudaxillaris* (SEM).

Cyanus pseudaxillaris Stef. & Georgiev

Locality: Bulgaria, Thracian plain, Ovchi halmove, 25.05.1984, leg. & det. S. Bancheva (Sh9801).

Distribution: Bulgarian endemic.

Pollen type: Trizonocolporate (Fig. 9).

Wagenitz's pollen type: Montana.

Pollen shape: Prolate, P/E = 1.9 (E).

Exine thickness: In the equatorial area 3.3 μm ± 0.7; in the polar area 2.1 μm ± 1.2.

Apertures: Colpi narrow and long, with definite boundaries. Colpus ends – acute. Colpus length 43.1 μm ± 3.4, colpus width 2.3 μm ± 0.6. Pores definite; pore length 5.9 μm ± 1.2. Coste colpi thickness 2.4 μm ± 1.5.

Structure: Tectate. Ect/End ≈ 2/1.

Sculpture: Scabrate. Density of the sculpture elements: 3–4 in 9 μm^2.

Intine: Thin; Exine/Intine ≈ 3/1.

Discussion

Two of the investigated species belong to Wagenitz's Montana pollen type: *Cyanus pseudaxillaris* and *C. thirkei* (Figs 9, 4). Despite their affiliation to the same section *Napuliferi* of genus *Cyanus*, the mentioned taxa could be easily distinguished by some parametres of the pollen grains. The pollen shape of *C. thirkei* is subprolate (P/E = 1.18), whereas the pollen shape of *C. pseudoaxillaris* is prolate (P/E = 1.9). The exine thickness is respectively 2.70 μm in the first species and 3.3 μm in the second one. The aperture length and width, as well as the density of the sculpture elements are different too.

The remaining 6 taxa, *Centaurea derderiifolia*, *C. drabifolia* subsp. *detonsa*, *C. kernerana*, *C. moesiaca*, *C. parilica* and *C. polyclada* have Jacea pollen type (Wagenitz 1955). The exine sculpture of *C. moesiaca* is of echinate type (Fig. 6), with spinules *ca*. 2 μm, whereas the rest belong to the microechinate type. The density of the sculpture elements is highest in *C. polyclada* (Fig. 3). The pollen grains of *C. parilica* have subprolate to prolate shape and the thickest exine, 3.2–5 μm. The spinules of *C. kernerana* reach 1 μm, but the density of the sculpture elements is 1–3 in 9 μm^2. The pollen grains of *C. derderiifolia* and *C. drabifolia* subsp. *detonsa* have a similar density of the sculpture elements, but they differ by their shape and colpi length and width (Figs 1, 2).

The obtained results show that the pollen shape, size, aperture and exine structure possess high taxonomic value and could be used in determination of the *Centaurea* s.l. taxa.

Acknowledgements. This study is financially supported by an international collaboration programme between TÜBITAK and Bulgarian Academy of Sciences, project *Variation and distribution patterns in genus Centaurea s.l. (Asteraceae) in the Bulgarian and Turkish floras with emphasis of their conservation*.

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Studies on the morphology of achenes of some endemic *Centaurea* species of Turkey using scanning electron microscope

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Abstract. The detailed morphological features of achenes of twelve Turkish endemic *Centaurea* taxa were studied using scanning electron microscope. These taxa belong to the sections *Acrolophus (C. polyclada* and *C. consanguinea*), *Cynaroides* (*C. scrolelepis*, *C. kurdica*, *C. fenzlii*, *C. aladagensis*, *C. spicata*, *C. tomentella*, *C. haussknechtii*, *C. amonicola*) and *Ptosimopappus* (*C. ptosimopappa* and *C. ptosimopappoides*).

Key words: achenes, *Centaurea*, morphology, scanning electron microscope

Introduction

Genus *Centaurea* L. is represented by about 500 species in the world (Rendele 1976), but some authors state that there are 600 species distributed in Asia, North Africa and America (Heywood 1979; Hickey & King 1981). According to the species number *Centaurea* is the third genus in Turkey following the genera *Astragalus* L. and *Verbascum* L. Out of 190 species 117 are endemics. The ratio of endemism is 61.6 % (Wagenitz 1975; Davis & al. 1988; Güner & al. 2000; Duran & Duman 2002; Türkoğlu & al. 2003; Aytaç & Duman 2005; Uzunhisarcıklı & al. 2005), stressing the fact that the genetic centre of this genus lies in Turkey. The achene morphology is very important and is used to determine the systematic categories of this genus. In this study general structure of achenes and seed surface structure of 12 species of *Centaurea* were investigated. In particular the species of sections *Cyanaroides*, namely *C. tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss., *C. scrolelepis* Boiss. and *C. kurdica* Reich., resembling each other, show different achene morphology. At the same time section *Ptosimopappus* is an endemic one and is represented by 2 species in the Turkish flora. The detailed achene morphology of these species is presented here.

Material and methods

The achene specimens were collected during 2002–2005. The localities of the species are as follows:

- *C. polyclada*, Canakkale, Guzelyali, roadside and forest, 10 m alt., 01.07.2003;
- *C. consanguinea*, 40 km from Elazig to Karakocan, roadside, 08.07.2003;
- *C. scrolelepis*, 60 km from Mus to Elazig, roadside and steppe, 13.07.2002;
- *C. kurdica*, 40 km from Bingol to Elazig, roadside and steppe, 13.07.2002;
- *C. fenzlii*, Mus–Solhan, in front of Alparslan Government Farm, steppe, 12.07.2002;
- *C. aladagensis*, Nigde, Camardı–Aladaglar, 08.07.2003;
- *C. spicata*, Hatay, Dortyol, 180 m, 10.07.2003;
- *C. tomentella*, Malatya–Darende, in front of Highway Construction Station, 02.08.2002;
- *C. haussknechtii*, Adiyaman, 10 km from Kahta to Nemrut Dagi, roadside, 12.07.2003;
- *C. amonicola*, Osmaniye, Yarpuz, Yaglipinar Mt, forest, 1250 m, 10.07.2003;
- *C. ptosimopappa*, Hatay, Amanos Mts, Dortyol, 850–900 m, 10.07.2003;
- *C. ptosimopappoides*, Adana, Aladag, Karsanti – Pos Forest, 1100 m, 08.07.2003.

The length and the breadth were measured using digital compass; 20 achenes and means were taken. The surface structure was also recorded. The achenes from the herbarium samples were left in Polaron SC 502 gold dust and pictures were taken with the help of Jeol JSM–840 electron microscope (Vujičić & al. 1993; Juan & al. 1997, 1999, 2000). The plant samples were deposited in the Canakkale Onsekiz Mart University, Biology Department, Botany Laboratory (Celik: 2100–2350).

Results and discussion

Description of achenes

Centaurea polyclada DC. (Sect. ***Acrolophus***) (Fig. 1)

Achene light, obovate-rectangular, adpressed on both sides, apex semicircular and generally small, 1.6–5.0 ± 2.7 mm long, 1.2–1.5 ± 0.2 mm broad, without pappus or with small pappus; pappus 0.2–0.5 ± 0.2 mm long. Achene cells wavy, surface has simple sparse hairs, part attached to the capitulum slightly sunken (Uysal & al. 2005b).

Centaurea consanguinea DC. (Sect. ***Acrolophus***) (Fig. 2)

Achene changes between rotundate to obovate, upper part swollen, lower side adpressed, 3.0–3.5 ± 0.8 mm long, 1.9–2.2 ± 0.7 mm broad, with pappus; pappus (0.5–) 1.0–2.0 (–2.5) ± 0.9 mm long. Achene cells in straight lines, wall of middle and side cells not much thick, surface hairy and sides slightly wavy (serrate), part attached to the capitulum slightly narrows down with sunken shape (Celik & al. 2005).

Centaurea scrolepis Boiss. (Sect. ***Cynaroides***) (Fig. 3)

Achene oblong, slightly obovate to rectangular, swollen above, adpressed below, 6.8–7.5 ± 0.7 mm long, 2.2–2.5 ± 0.9 mm broad, with pappus; pappus 7.8–11.2 ± 2.2 mm long. Cells of achene in straight lines,

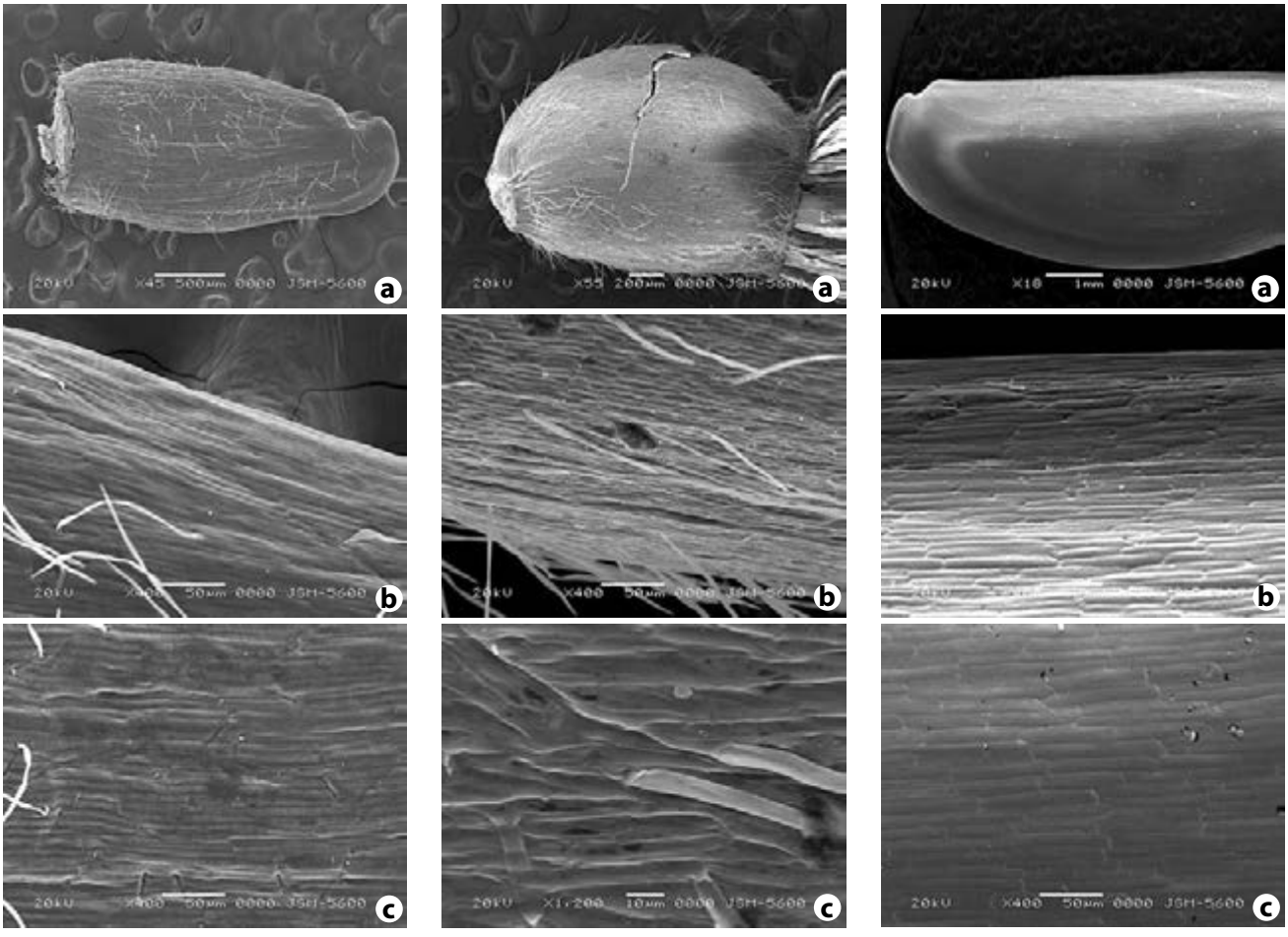

Fig. 1. *C. polyclada*: **a** – general outlook of achene; **b, c** – surface of achene.

Fig. 2. *C. consanguinea*: **a** – general outlook of achene; **b, c** – surface of achene.

Fig. 3. *C. scrolepis*: **a** – general outlook of achene; **b, c** – surface of achene.

wall of middle and side cells not much thick, surface hairy, part attached to the capitulum slightly narrows down with sunken shape (Uysal & al. 2005a).

Centaurea kurdica Reichardt (Sect. ***Cynaroides***) (Fig. 4)

Achene orbicular to widely ovate, upper side smooth, lower side slightly swollen, 6.0–7.3 ± 0.5 mm long, 1.8–2.1 ± 0.7 mm broad, with pappus; pappus 7.8–11.2 ± 1.8 mm long. Cells of achene in straight lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum slightly sunken (Uysal & al. 2005a).

Centaurea fenzlii Reichardt (Sect. ***Cynaroides***) (Fig. 5)

Achene oblong, swollen above, apex wide and circular, base blunt (obtuse), 5.8 ± 0.6 mm long, 3.8 ± 0.4 mm broad, with pappus; pappus 5.8–10.4 ± 1.2 mm long. Achene cells in oblique lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum semicircular.

Centaurea aladagensis Wagenitz (Sect. ***Cynaroides***) (Fig. 6)

Achene obovate, upper part adpressed, lower side swollen, apex circular, not wide, base blunt (obtuse), slightly twisted, 4.8 ± 1.2 mm long, 3.2 ± 0.7 mm broad, with pappus; pappus 5.8–10.4 ± 1.2 mm long. Cells of achene in straight lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum slightly sunken.

Centaurea spicata Boiss. (Sect. ***Cynaroides***) (Fig. 7)

Achene semiobovate, adpressed above, lower side swollen, apex circular, slightly narrowed, base asymmetrical and slightly twisted, 4.6 ± 1.1 mm long, 2.4 ± 0.4 mm broad, with pappus; pappus 6.8–9.7 ± 0.8 mm long. Cells of achene in straight lines, wall of middle and side

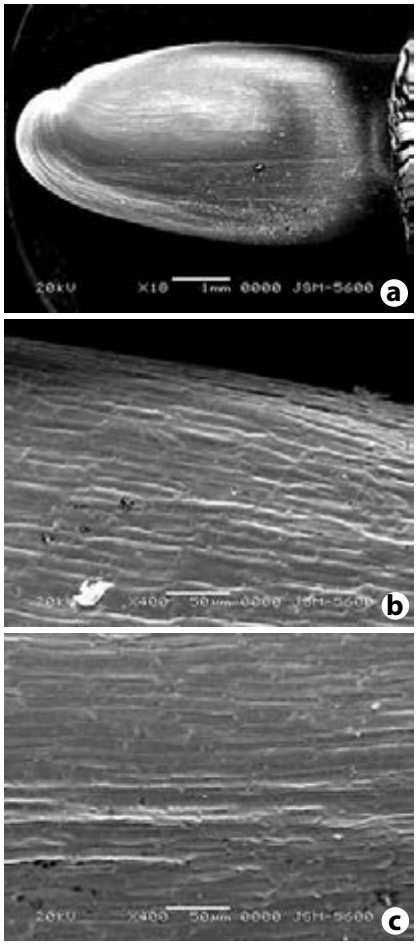

Fig. 4. *C. kurdica*: **a** – general outlook of achene; **b, c** – surface of achene.

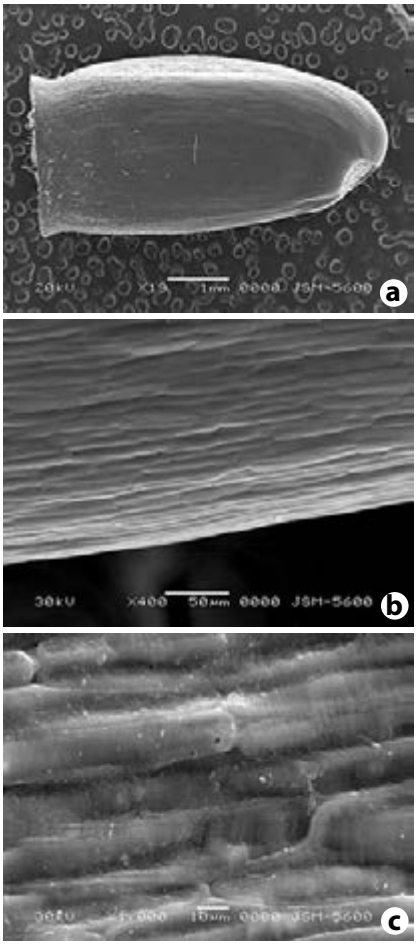

Fig. 5. *C. fenzlii*: **a** – general outlook of achene; **b, c** – surface of achene.

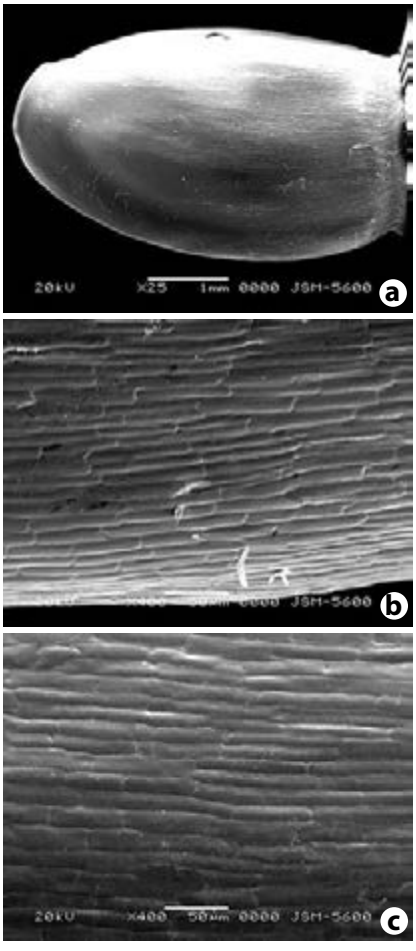

Fig. 6. *C. aladagensis*: **a** – general outlook of achene; **b, c** – surface of achene.

cells not much thick, surface not hairy, part attached to the capitulum slightly sunken (emarginate).

Centaurea tomentella **Hand.-Mazz. (Sect. *Cynaroides*)** (Fig. 8)

Achene wide rectangular, straight above, narrowing down below towards the apex, apex blunt, 5.8 ± 0.6 mm long, 3.8 ± 0.4 mm broad, with pappus; pappus 7.7–8.3 ± 0.3 mm long. Cells of achene in straight lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum slightly straight.

Centaurea haussknechtii **Boiss. (Sect. *Cynaroides*)** (Fig. 9)

Achene semiobovate, straight above, swollen but narrowing down below towards the apex, apex blunt, 5.7 ± 0.8 mm long, 3.9 ± 0.6 mm broad, with pappus; pappus 7.6–8.7 ± 0.5 mm long. Cells of achene in straight lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum slightly sunken.

Centaurea amonicola **Hub.-Mor. (Sect. *Cynaroides*)** (Fig. 10)

Achene obovate, swollen above, straight below, narrows towards the apex, apex blunt, 5.8 ± 0.6 mm long, 3.7 ± 0.4 mm broad, with pappus; pappus 7.7–9.3 ± 0.4 mm long. Cells of achene in straight lines, wall of middle and side cells thick, surface not hairy, part attached to the capitulum slightly sunken.

Centaurea ptosimopappa **Hayek (Sect. *Ptosimopappus*)** (Fig. 11)

Achene obovate, adpressed above, swollen below, narrows towards the apex, apex blunt, 4.6 ± 0.9 mm long, 3.7 ± 0.6 mm broad, with pappus; pappus 3.8 ± 0.8 mm long. Cells of achene in unarranged lines, wall of middle and side cells not much thick, surface not hairy, part attached to the capitulum exactly semicircular.

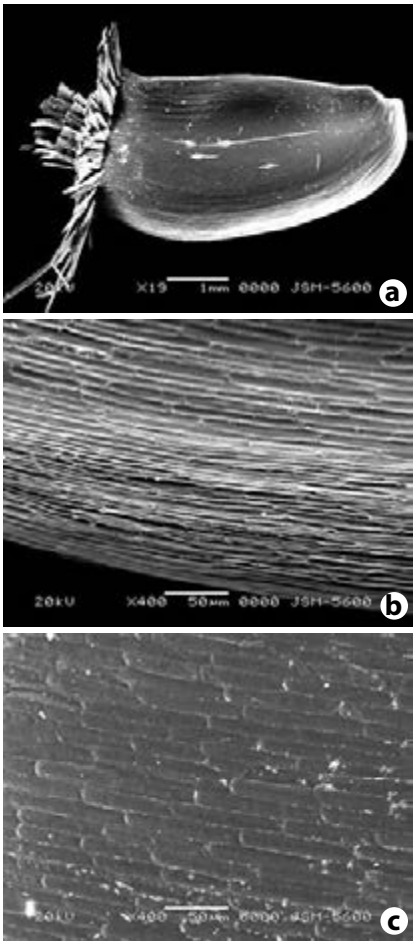

Fig. 7. *C. spicata*: **a** – general outlook of achene; **b, c** – surface of achene.

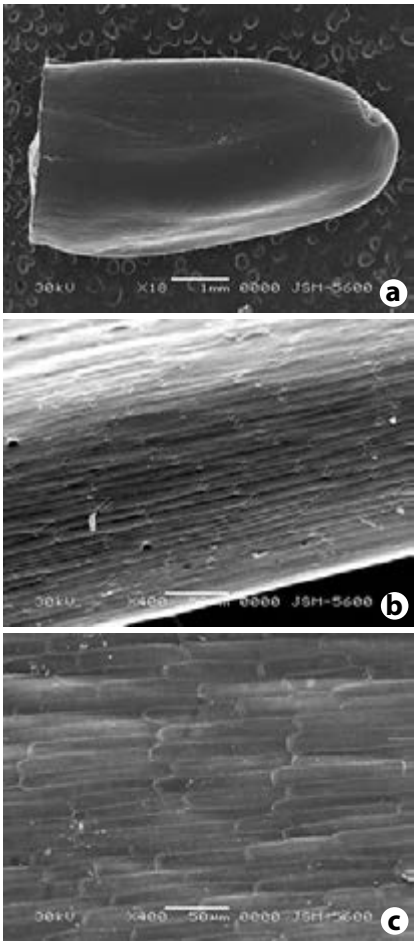

Fig. 8. *C. tomentella*: **a** – general outlook of achene; **b, c** – surface of achene.

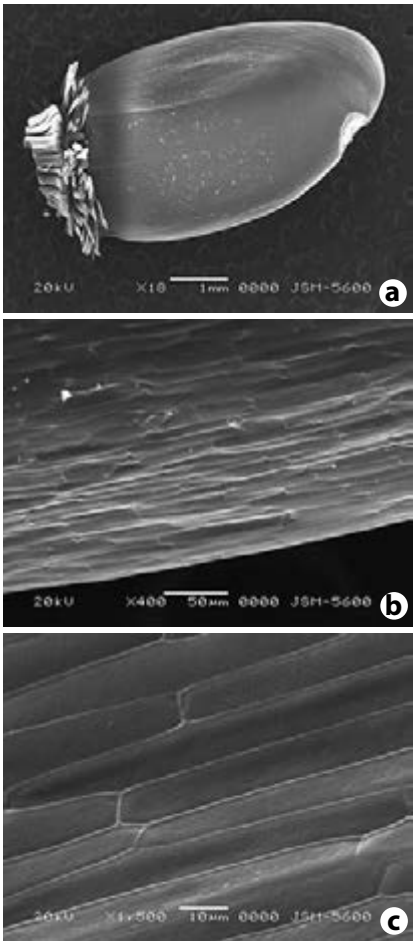

Fig. 9. *C. haussknechtii*: **a** – general outlook of achene; **b, c** – surface of achene.

***Centaurea ptosimopappoides* Wagenitz (Sect. *Ptosimopappus*)** (Fig. 12)

Achene rectangular, swollen above, sunken below and slightly narrows towards the apex, apex circular (rotundate), 5.6 ± 1.9 mm long, 3.4 ± 0.6 mm broad, with pappus; pappus deciduous, 3.8 ± 0.8 mm long. Cells of achene in unarranged lines, wall of middle and side cells not much thick, surface with simple sparse hairs, part attached to the capitulum exactly semicircular.

In this paper morphology of achenes of the sections *Cynaroides*, *Ptosimopappus* and *Acrolophus* has been presented. These findings will be used in the preparation of an identification key for these sections.

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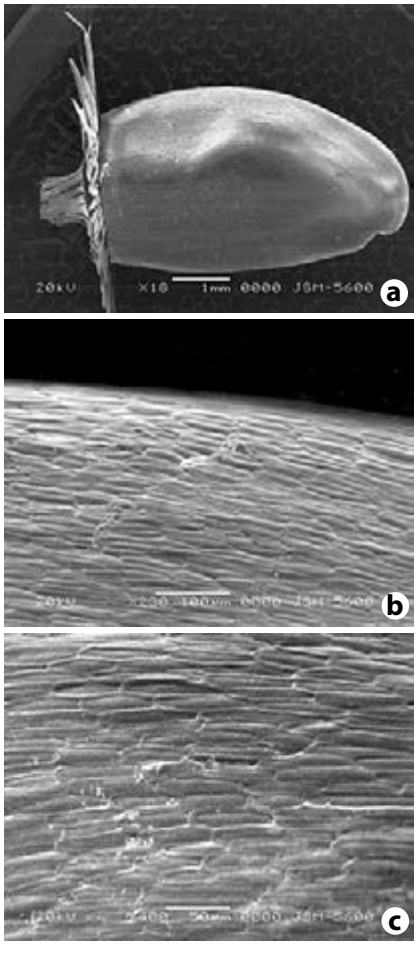


Fig. 10. *C. amonicola*: **a** – general outlook of achene; **b, c** – surface of achene.

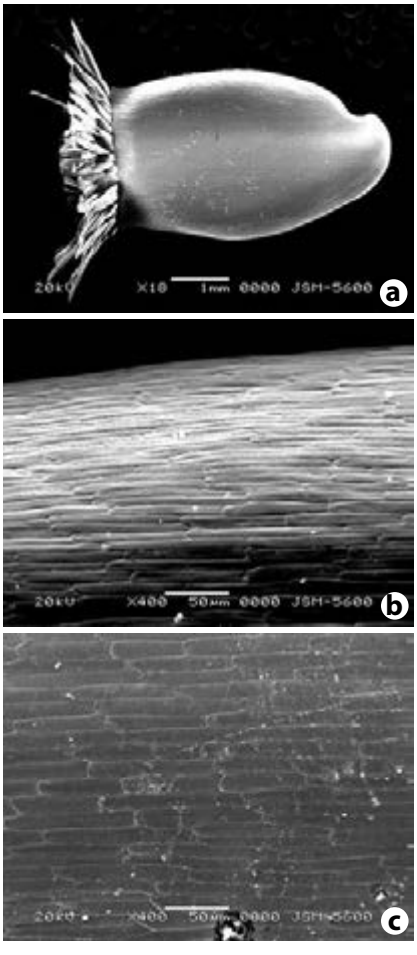
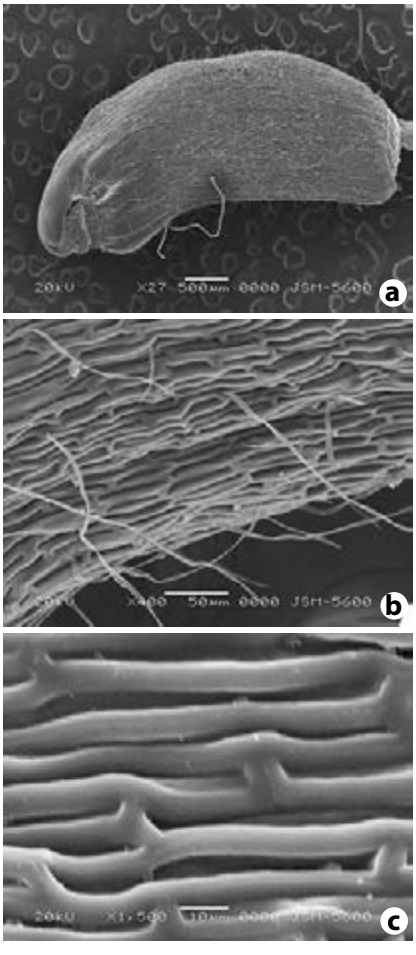


Fig. 11. *C. ptosimopappa*: **a** – general outlook of achene; **b, c** – surface of achene.



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Taxonomic relations among some Turkish serpentine endemic *Alyssum* (*Brassicaceae*)

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Abstract. Metal hyperaccumulators are plants capable of extracting certain metals and non-metals from the soil and accumulating them in above-ground tissues to relatively high concentrations. *Alyssum* is the represent of Ni-hyperaccumulator genus with many of its taxa. *Alyssum floribundum*, *A. peltarioides* subsp. *peltarioides*, *A. peltarioides* subsp. *virgatiforme*, *A. virgatum*, *A. caricum* are some of the serpentine endemics of Turkish *Alyssum*. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE) method is most widely used due to its validity and simplicity for describing genetic structure of plant collections. RAPD–PCR is also used in molecular systematics and constitution of plant genome maps successfully. In this study some of the *Alyssum* taxa and the relations among them were identified by RAPD–PCR and SDS–PAGE methods.

Key words: *Alyssum,* RAPD–PCR, SDS–PAGE

Introduction

Serpentine soils derived from a wide range of ultramafic rock types are widely distributed around the world. Serpentines in Turkey are abundant and their floras are rich in species; many of them are endemic to these soils. They are characterized by high levels of nickel (Ni), cobalt (Co) and chromium (Cr), low levels of nitrogen (N), phosporus (P), potassium (K), calcium (Ca), and a high magnesium/calcium (Mg/Ca) quotient. These extreme chemical properties render serpentine soils uninhabitable for most plant species but also comprise a major selective force in the evolution of endemic serpentine taxa. The Ni-hyperaccumulation trait is mainly found in members of the family *Brassicaceae*, especially in the genera *Alyssum* L. and *Thlaspi* L. (Mengoni & al. 2003). So the data obtained from molecular studies revealing the genetic interrelations in these genera would be helpful to evaluate the genetic basis of Ni-hyperaccumulation.

Because of environmental influence, phenotypic traits in many cases fail to serve as unambiguous markers for systematics and diversity analysis. Molecular markers successfully developed during the last two decades have largely overcome the problems that are associated with phenotype-based classification. Initially, isozymes and Restriction Fragment Length Polymorphisms (RFLPs) served as reliable markers for genetic analyses in plants. But PCR based techniques developed in recent years such as Random Amplified Polymorphic DNAs (RAPDs), Inter Simple Sequence Repeats (ISSRs), Amplified Fragment Length Polymorphisms (AFLPs), and Simple Sequence Repeats (SSRs), also called microsatellites, provide DNA markers that are dispersed throughout plant genomes and are easier to reproduce and analyse (Awasthi & al. 2004).

Different methodologies using molecular markers are widely used to analyse the pattern of variation within and among natural populations of tree species. Among the various maker systems, RAPDs are one of the most popular DNA-based approaches. They are the least technically demanding and offer a fast method for providing information from a large number of loci, particularly in species where no study has previously been undertaken. Moreover, the diversity assessed with RAPDs is comparable with that obtained with allozymes or RFLP (Fontaine & al. 2004).

Among biochemical techniques, Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS–PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm. Seed storage proteins have been used as genetic

markers in four major areas: 1) analyses of genetic diversity within and between species, 2) plant domestication in relation to genetic resources conservation and breeding, 3) genome relationship and 4) as a tool in crop improvement. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants. This method can also be used as a promising tool for distinguishing cultivars of particular crop species (Ghafoor & al. 2000).

Material and methods

Plant material

Plant materials used in this study are: *A. floribundum* Boiss. & Balansa, *A. peltarioides* Boiss. subsp. *peltarioides*, *A. peltarioides* subsp. *virgatiforme* (Nyár.) T.R. Dudley, *A. virgatum* Nyár., and *A. caricum* T.R. Dudley & Hub.-Mor. (from collections of N. Adıgüzel) (Table 1). These taxa are some of the serpentine endemics of Turkish *Alyssum* (Dudley 1965).

Table 1. Plant material used in the study.

*Species	Localities	No.
A. caricum	Muğla: Fethiye	4655
A. peltarioides ssp. *peltarioides*	Konya: Çamlık	6337
A. peltarioides ssp. *virgatiforme*	Erzincan: Refahiye	4178
A. floribundum	İçel: Fındıkpınarı	3975
A. virgatum	Kütahya: Gediz	3540

*Seeds and other plant parts are from N. Adıgüzel's collection.

DNA isolation and RAPD amplification

Total DNAs of the species were isolated according to CTAB method (Clark 1997). The PCR amplifications were performed as described by Williams & al. (1990). Fourteen primers were used for the amplifications carried out in Techne (UK) Progene Thermocycler. 15 µl of reaction products were separated alongside molecular weight markers (100 bp DNA ladder) by electrophoresis, on 1.5 % agarose gels containing ethidium bromide. The gels were photographed under UV light and the amplification patterns were examined.

Protein extraction and gel electrophoresis

Protein extraction was performed according to Saraswati & al. (1993). Electrophoresis was carried out following the Laemmli method (Laemmli 1970). Each run included known molecular weight marker proteins (Fermentas). Proteins on the gel were fixed and stained with Comassie Brilliant Blue G–250 as described by Demiralp & al. (2000).

Data analysis

In the study of overall genetic variation, fragments that were readable and reproducable were used. Bands were scored as either present (1) or absent (0) for all species studied. Common band analysis was conducted using the computer programme UPGMA based on the Nei's genetic distance to determine the genetic distance values between species (Nei 1972).

Results and discussion

Five *Alyssum* taxa used in this study were examined for their relationships. The morphological differences of four species are indicated in Table 2. RAPD–PCR and SDS–PAGE analyses were used in order to differentiate the taxa. For RAPD analysis, 14 primers were used for the amplification of the DNA. RAPD bands were ranging from 200 bp to 1500 bp in size. Some of the bands were monomorphic, while others showed at least one polymorphism (Fig. 1). Dendrograms were constructed using UPGMA computer program.

Table 2. Morphological characters of the four *Alyssum* species used in the study.

	A. floribundum	*A. peltarioides*	*A. virgatum*	*A. caricum*
Stem	up to 100 cm	up to 75 cm	up to 75 cm	up to 40 cm
Leaves of steril shoots	strongly bicoloured	concoloured	concoloured	bicoloured
Fruits	obovate, subundulate, glabrous	obovate or rotundate, strongly undulate, glabrous	obovate or rotundate, strongly undulate, glabrous	broadly obcordate or orbicular, subundulate, glabrous
Seeds	narrowly winged	wingless or very narrowly winged	wingless or very narrowly winged	winged

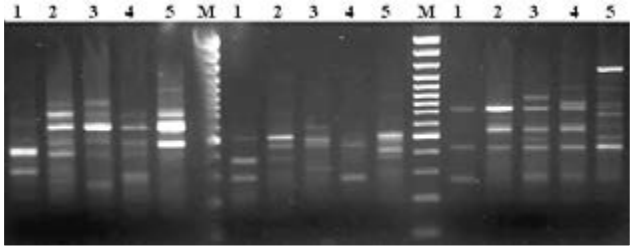

Fig. 1. RAPD fragment patterns of *Alyssum* species generated by the primers LA12, A7, OPB5, respectively:
1, *A. floribundum*; **2,** *A. peltarioides* subsp. *peltarioides*; **3,** *A. peltarioides* subsp. *virgatiforme*; **4,** *A. virgatum*; **5,** *A. caricum*; **M** – DNA size marker.

Topology obtained from RAPD electrophoretic analysis of five taxa of *Alyssum* is presented in Fig. 2. According to the dendrogram *A. caricum* had the genetic distance 76 %, 80 %, 75 %, 52 % with *A. floribundum*, *A. peltarioides* subsp. *peltarioides*, *A. peltarioides* subsp. *virgatiforme* and *A. virgatum*, respectively (Table 3).

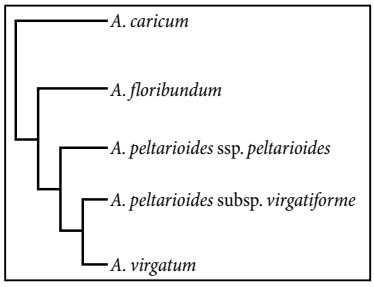

Fig. 2. Dendrogram generated by UPGMA clustering of RAPD data.

Table 3. Distance data between *Alyssum* taxa (result of PCR).

Pop ID	*A. floribundum*	*A. peltarioides* ssp. *peltarioides*	*A. peltarioides* ssp. *virgatiforme*	*A. virgatum*	*A. caricum*
A. floribundum	**				
A. peltarioides ssp. *peltarioides*	0.56	**			
A. peltarioides ssp. *virgatiforme*	0.63	0.46	**		
A. virgatum	0.60	0.54	0.43	**	
A. caricum	0.76	0.80	0.75	0.52	**

Alyssum caricum differs from the other *Alyssum* species with its shorter stem, broadly obcordate or orbicular fruit and broader winged seed.

Alyssum peltarioides has two subspecies. Morphological differences of the two subspecies are as follows:

- *A. peltarioides* subsp. *peltarioides*

Inflorescences condensed, sparingly branched, not more than 5 cm long and few-fruited; fruiting stems low or decumbent, 5–10 cm.

- *A. peltarioides* subsp. *virgatiforme*

Inflorescences widely spreading, strongly branched, 10–20 cm long and many-fruited; fruiting stems arcuate-ascending or erect, 25–75 cm.

The analysis of seed proteins showed that both subspecies had similar protein pattern. Seed protein composition determined by SDS–PAGE is shown in Fig. 3. The dendrogram constructed using genetic distances is shown in Fig. 4 and distance data of these taxa are given in Table 4.

These four *Alyssum* species are very close to each other. It is very difficult to separate them morphologically without leaves of sterile shoots and mature fruits. *Alyssum virgatum* is the most similar species to *A. peltarioides*. It differs from *A. peltarioides* in having the leaves usually conduplicate, the petals with dense indumentum.

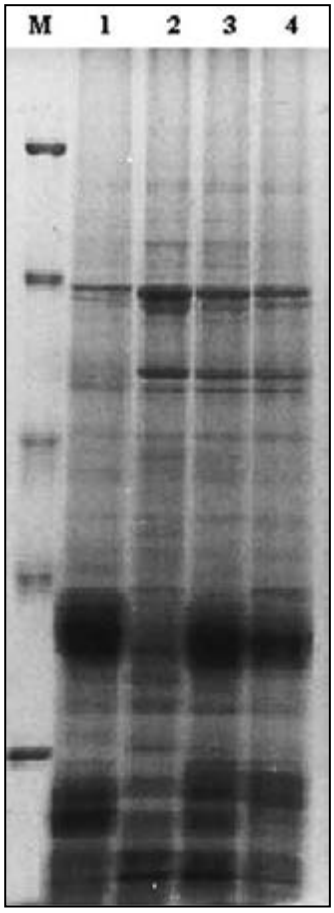

Fig. 3. SDS–PAGE profile of *Alyssum* species: **1,** *A. caricum*; **2,** *A. floribundum*; **3,** *A. peltarioides* subsp. *peltarioides*; **4,** *A. peltarioides* subsp. *virgatiforme*; **M** – protein size marker.

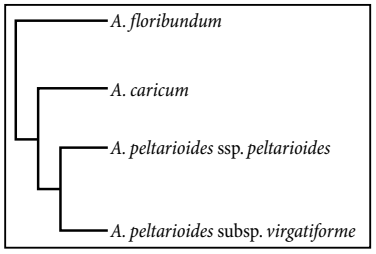

Fig. 4. Dendrogram generated by UPGMA clustering of SDS–PAGE data.

Table 4. Distance data between *Alyssum* taxa (result of SDS–PAGE).

Pop ID	*A. caricum*	*A. floribundum*	*A. peltarioides* ssp. *peltarioides*	*A. peltarioides* ssp. *virgatiforme*
A. caricum	**			
A. floribundum	0.90	**		
A. peltarioides ssp. *peltarioides*	0.35	0.90	**	
A. peltarioides ssp. *virgatiforme*	0.40	0.81	0.12	**

The results obtained from RAPD–PCR and SDS–PAGE support the discrimination by morphological data. It is obvious that these species are very close to each other but the differences at DNA profiles (Fig. 2) support their being separate taxa.

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Isozyme investigation on some taxa of Mediterranean *Centaurea* (*Compositae*)

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Abstract. The genetic diversity of four Mediterranean *Centaurea* species (*C. sphaerocephala*, *C. napifolia*, *C. solstitialis* subsp. *schowii* and *C. nicaeensis*) belonging to *Jacea* group (*sensu* Garcia-Jacas & al. 2000) was investigated using isozymes. Seven loci from four enzyme systems (IDH, MDH, PGM and PGI) were analysed. Allele frequencies and genetic variability for each locus in each population were calculated. A total of seventeen alleles was identified, two of them exclusive to *C. sphaerocephala* and *C. solstitialis* subsp. *schowii* at *Mdh-1* and *Pgm-1* loci, respectively. The highest intrapopulation variability was found in *C. sphaerocephala* and *C. nicaeensis*. The genetic distances among all taxa examined are clearly expressed. The obtained dendrogram shows two groups – the first one includes the yellow flowering species, while the second group – the purple flowering taxa.

Key words: *Centaurea*, genetic diversity, isozyme analysis, weeds

Introduction

The isozyme analysis is one of the most important methods in the evaluation of the variability and genetic similarity among populations and taxa (Soltis & Soltis 1990). In this work four steno-Mediterranean *Centaurea* taxa are investigated: *C. sphaerocephala* L., *C. napifolia* L., *C. solstitialis* subsp. *schowii* (DC.) Dostál and *C. nicaeensis* All. The first two species belong to subgen. *Seridia* (Juss.) Czerep., the others – to subgen. *Solstitiaria* (Hill) Dobrocz. (Dostál 1976). The pollen grains of all investigated taxa are *Jacea* type (*sensu* Wagenitz 1955) and consequently Garcia-Jacas & al. (2000) include them to *Jacea* group. *Centaurea sphaerocephala* inhabits sandy grounds near the sea in the W Mediterranean region, while the other three taxa are weeds with high invasive capacities.

The main goals of the present study are to describe allelic variation at isozyme loci of selected enzyme systems and to study the genetic relationships among the taxa.

Material and methods

Centaurea napifolia, *C. sphaerocephala*, *C. nicaeensis* and *C. solstitialis* subsp. *schowii* were collected from their natural localities in Sicily (Table 1). Voucher specimens were deposited in the *Herbarium Mediterraneum* of Palermo (PAL). For each population, a sample of 20 individuals was tested.

The following enzyme systems were examined: IDH – isocitrate dehydrogenase (E.C.1.1.1.42), MDH – malate dehydrogenase (E.C.1.1.1.37), PGI – phosphoglucoisomerase (E.C.5.3.1.9), PGM – phosphoglucomutase (E.C.2.7.5.1).

Fresh young leaves were crushed in 200 μl buffer containing TrisHCl, pH 7.5, and 1 % reduced glutath-

Table 1. Origin of the studied material.

Taxon	Locality	Date	Collector
C. napifolia	Sicily, town of Mazara	02.03.2006	S. Bancheva, E. Schimmenti, G. Scafidi
C. nicaeensis	Sicily, Ficuzza Natural Reserve, Gorghi Tondi Locality	03.03.2006	S. Bancheva, E. Schimmenti, G. Scafidi
C. solstitialis subsp. *schowii*	Sicily, Ficuzza Natural Reserve, Gorghi Tondi Locality	03.03.2006	S. Bancheva, E. Schimmenti, G. Scafidi
C. sphaerocephala	Sicily, town of Alcamo	02.03.2006	S. Bancheva, E. Schimmenti, G. Scafidi

ione. Crude extracts were absorbed on paper wicks and stored at -80 °C until use. For the complete methodology see Bancheva & al. (2006).

Data analysis

For each locus the resulting zymograms were interpreted as allelic frequencies.

For all populations levels of allozyme diversity were estimated: the mean number of alleles per locus (A), the mean percentage of polymorphic loci (P), observed (Ho) and expected heterozygosity (He) calculated from allelic frequencies according to the Hardy-Weinberg law. Chi square test was used to evaluate the signification of the deviation from the Hardy-Weinberg law.

Wright (1951) fixation (F) index was calculated as F = 1-Ho/He.

For each population gene diversity H, as described by Nei (1973), was calculated.

Genetic distances among populations were estimated from allelic frequencies (Nei 1978) using BIOSYS–2 (Swofford & Selander 2000).

Dendrogram was computed from the identity matrices using UPGMA method.

Results

Allele frequencies

Seven loci (*Idh-1*, *Idh-2*, *Mdh-1*, *Pgm-1*, *Pgm-2*, *Pgi-1*, *Pgi-2*) were revealed from the four investigated enzymes. In total seventeen alleles in these loci were detected in the enzyme systems. *Pgi-1* locus was monomorphic for all populations. The allelic frequencies for each population are reported in Table 2.

The two loci belonging to PGM system together with *Mdh-1* and *Pgi-2* loci are very polymorphic. They have three different alleles for each locus.

In *C. sphaerocephala* and *C. solstitialis* subsp. *schowii*, two exclusive alleles at the loci *Mdh-1* (allele c) and *Pgm-1* (allele c), respectively, were found.

Genetic variability

The mean proportion of polymorphism (P95 and P99), the mean number of alleles per locus (A), the observed (Ho) and expected (He) heterozygosity for each population are shown in Table 3.

The mean number of alleles per locus ranges from 1.4 in *C. napifolia* to 1.9 in *C. sphaerocephala*.

Centaurea sphaerocephala and *C. nicaeensis* have the highest polymorphism (71.4 %); the lowest value was found in *C. napifolia* (28.6 %).

The mean heterozygosity index (He), which is a measure of intra-population variability, showed highest values in the populations of *C. sphaerocephala* and *C. nicaeensis* (0.359). The lowest values were observed in *C. napifolia* (0.158).

In the examined populations, the observed heterozygosity was higher than expected, as revealed by the negative values of F.

For each locus and for each population the coefficient of gene diversity (H) ranges from 0 at the monomorphic loci to 0.685 at the *Mdh-1* locus (Table 4). The last locus, together with *Pgm-2*, shows the highest values in all examined species because of their high level of polymorphism.

Table 2. Frequencies of allozymes of 5 enzymatic systems detected in 8 *Centaurea* populations.

Locus	Allele	*C. napifolia*	*C. solstitialis* subsp. *schowii*	*C. nicaeensis*	*C. sphaerocephala*
Mdh-1	a	0.5	0.5	0.5	0.417
	b	0.5	0.5	0.5	0.292
	c	0	0	0	0.292
Idh-1	a	1	0	0.278	0.5
	b	0	1	0.722	0.5
Idh-2	a	1	0	0	1
	b	0	1	1	0
Pgm-1	a	1	0.389	0.611	0.727
	b	0	0.056	0.389	0.273
	c	0	0.556	0	0
Pgm-2	a	0.273	0.188	0.5	0
	b	0.591	0.438	0.5	0.5
	c	0.136	0.375	0	0.5
Pgi-1	a	1	1	1	1
Pgi-2	a	1	1	0	0
	b	0	0	0.556	0.773
	c	0	0	0.444	0.227

Table 3. Average number of alleles per locus (A), average polymorphism P95 and P99, mean heterozygosity (Ho – observed, He – expected) and fixation index (F) for each population.

Population	A	P95	P99	Mean heterozygosity		F
				Ho	He	
C. napifolia	1.4	28.6	28.6	0.247	0.158	–0.55
C. solstitialis subsp. *schowii*	1.7	42.9	42.9	0.280	0.253	–0.10
C. nicaeensis	1.7	71.4	71.4	0.413	0.359	–0.15
C. sphaerocephala	1.9	71.4	71.4	0.571	0.359	–0.59

Table 4. Nei's (1973) gene diversity for each population at each locus.

Taxon / Locus	*C. napifolia*	*C. solstitialis* subsp. *schowii*	*C. nicaeensis*	*C. sphaerocephala*
Idh-1	0	0	0.425	0.524
Idh-2	0	0	0	0
Mdh-1	0.524	0.529	0.529	0.685
Pgm-1	0	0.569	0.503	0.416
Pgm-2	0.584	0.675	0.529	0.524
Pgi-1	0	0	0	0
Pgi-2	0	0	0.523	0.368

Genetic relationships

Table 5 shows the matrix of genetic distances and genetic identities: the greatest values of distances were found between *C. napifolia* and *C. nicaeensis* and between *C. solstitialis* subsp. *schowii* and *C. sphaerocephala*.

The obtained dendrogram (Fig. 1) shows two groups – the first one includes the yellow flowering species, while the second group – the purple flowering taxa.

Table 5. Matrix of Nei's distance (above diagonal) and identity (below diagonal).

	Taxon	1	2	3	4
1	***C. napifolia***	–	0.549	0.620	0.281
2	***C. solstitialis* subsp. *schowii***	0.577	–	0.270	0.660
3	***C. nicaeensis***	0.538	0.764	–	0.370
4	***C. sphaerocephala***	0.755	0.517	0.690	–

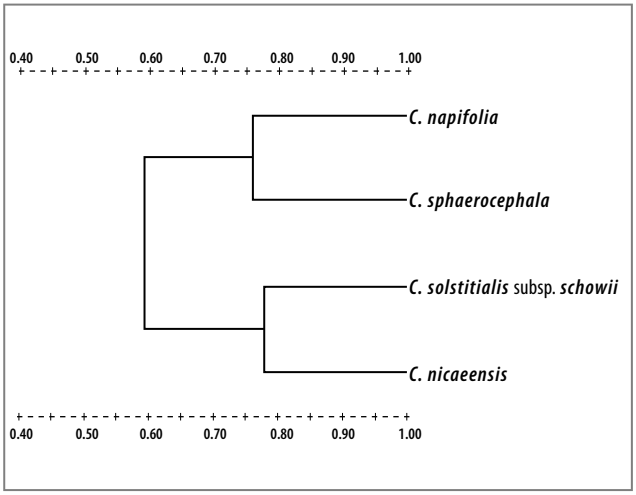

Fig. 1. Dendrogram (UPGMA method) showing the relationships among 8 populations of the *Centaurea jacea* group based on Nei's genetic identities.

Discussion and conclusion

The highest polymorphism (71.4 %) was detected in *C. sphaerocephala* and *C. nicaeensis*. The first one occurs in extreme ecological conditions – on the sands and sandy ground near sea. The second species is a weed for agriculture. According to the over-dominance hypothesis, the fitness of the population and the ecological plasticity should generally increase with the increasing of the heterozygous loci (Ginzburg 1979; Turelli & Ginzburg 1983; Mitton 1990). Heterozygous individuals often exhibit higher variability, greater developmental stability, and higher growth rates than the homozygous ones (Seager & Ayala 1982; Carson 1987; Mitton 1990). Moreover, the high level of observed heterozygosity indicates that there is an "excess" of outcrossing compared to the expectation under Hardy-Weinberg equilibrium, so we could conclude that in these populations there is a very effective self-incompatibility.

The genetic diversity found in *C. solstitialis* subsp. *schowii* (A 1.7, P99 42.9, Ho 0.280) is similar to the results by Sun (1997) about *C. solstitialis* in USA, where the species is a very successful colonizing weed (A 1.85, P99 40.0, Ho 0.169). The most important contribution to the high level of genetic variation and to this pattern of genetic structure appears to be its outbreeding system and anthropogenic factors in seed dispersal (Sun 1997).

In the population of *C. napifolia*, the polymorphism is rather low (28.6 %). The species is an annual weed and it is probably self-compatible. According to Brown & Marschall (1981) some colonizing species and weeds are often depauperate in genetic variation within populations.

The presence of alleles fixed in different species indicates lack of gene flow and a very good reproductive isolation among all studied taxa.

The grouping in the dendrogram reflects the Dostál's subdivision (1976) in two subgenera: subgen. *Solstitiaria* which includes the yellow flowering species and subgen. *Seridia* comprising the purple flowering taxa.

The genetic distances among all examined taxa are clearly expressed. So the plant speciation of the studied groups could be occurring in remote time.

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Limits and type of the variation in samples of species from genera *Crocus* (*Iridaceae*) and *Colchicum* (*Liliaceae*) from Bulgaria

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Abstract. Five samples from *Colchicum autumnale*, 4 samples from *Crocus flavus* and 3 samples from *Crocus veluchensis* have been subjected to variation and cluster analyses, Pearson's correlation and multiple regression. The limits of the variation on species level are discrete. The variation coefficients are predominantly within a moderate degree. The cluster analysis demonstrates the presence of a clone structure. The measured samples are far from the line of the full similarity. The Pearson's corelation is low. The multiple determination is high. The proportion between the levels of the binary correlation and the multiple determination confirms already proven hypothesis about the compensative role of the multivariate correlation.

Key words: cluster analysis, *Colchicum*, *Crocus*, multiple determination, Pearson's correlation, variation coefficient

Introduction

The present work is a continuation of the authors' interest to apply statistical analysis for species with different genetic structures. The information about limits and type of variation in panmict species is rich and gives possibilities for comparison. However, the same information about clonal structures is insufficient and needs more investigation.

Material and methods

Twelve samples from *Crocus veluchensis* Herb., *C. flavus* Weston and *Colchicum autumnale* L. have been investigated. Each sample contained thirty individuals. The morphometric features were measured with accuracy of up to 0.01 mm. The rate of dispersion for the separate cases was measured by variation coefficient in percentage (CV %) following a five-degree scale (Mamaev 1968): CV up to 7.0 % – very low; 7.1 % > CV > 12.0 % – low; 12.1 % > CV > 20.0 % – moderate; 20.1 % > CV > 40.0 % – high; CV over 40.1 % – very high. In order to determine the rate of correlation between two or more variables Pearson's correlation analysis and Multiple regression were used. The rate of Pearson's correlations (r) between the features was measured following a five-degree scale (Gatev 1980): r up to 0.30 – weak; 0.31 > r > 0.50 – moderate; 0.51 > r > 0.70 – significant; 0.71 > r > 0.90 – high; r over 0.91 – very high. The rate of multiple determination (R^2) was measured according to the three-degree scale: R^2 up to 0.50 – low; 0.51 > R^2 > 0.75 – high; R^2 over 0.76 – very high (Lidanski 1988) In order to determine the rate of similarity cluster analysis through calculation of the Euclidean distance was used (StatSoft 1995).

Three samples of *C. veluchensis* were collected in Western Rhodopes Mts as follows: "Karachoumak" locality – 1687 m alt. (sample 1); "Kartela" locality – 1681 m alt. (sample 2); around dam "Batak", 1136 m alt. (sample 3). Sixteen morphometric features were measured as follows: x_1–stem length; x_2–flower length; x_3–spate length; x_4–spate width; x_5–external perianth length; x_6–external perianth width; x_7–internal perianth length; x_8–internal perianth width; x_9–stamen length; x_{10}–anther length; x_{11}–corm height; x_{12}–corm width; x_{13}–leaf length; x_{14}–leaf width; x_{15}–leaves' number; x_{16}–flowers' number.

The origin of the four samples of *C. flavus* was as follows: Vitosha Mt, Bistritsa village, paragliding ar-

ea – 953 m alt. (sample 1); Vitosha Mt, Bistritsa village, "Gergyov Dol" locality – 866 m alt. (sample 2); Vitosha Mt, Bistritsa village – 899 m alt. (sample 3); Lozen Mt, "Urvich" locality – 903 m alt. (sample 4).

Fourteen features were measured as follows: x_1–stem length; x_2–flower length; x_3–spate length; x_4–spate width; x_5–external perianth length; x_6–external perianth width; x_7–internal perianth length; x_8–internal perianth width; x_9–stamen length; x_{10}–anther length; x_{11}–leaf length; x_{12}–leaf width; x_{13}–leaves' number; x_{14}–flowers' number.

The origin of the five samples of *C. autumnale* was as follows: Pirin Mts, "Kroushe" locality – 1079 m alt. (sample 1); Pirin Mts, Ribarnika – 888 m alt. (sample 2); Vitosha Mt, at the foots of Chiprovitsa peak – 780 m alt. (sample 3); Vitosha Mt, at the foots of Bogdana peak – 874 m alt. (sample 4); Vitosha Mt, Bistritsa – 941 m alt. (sample 5). Twelve features were measured as follows: x_1–flower length; x_2–sheath length; x_3–sheath width; x_4–external perianth length; x_5–external perianth width; x_6–internal perianth length; x_7–internal perianth width; x_8–stamen length; x_9–anther length; x_{10}–corm height; x_{11}–corm width; x_{12}–flowers' number.

Results

Crocus veluchensis

Variation analysis

The values of the standard deviations formed a continuous row. A more substantial dispersal was found within the features describing lengths. These were the following: x_1–stem length; x_2–flower length; x_3–spate length; x_{13}–leaf length. The variability found related to the moderate and high degree (Table 1). Out of the total number of variation coefficients 50 % were within the high degree, 46 % were within the moderate degree and 4 % – within the low degree. No coefficients were found within the very low or within the very high variability degree. The lowest variability was registered for x_7–internal perianth length and x_{10}–anther length. High variability was registered for x_1–stem length; x_4–spate width; x_{14}–leaf width; x_{15}–leaves' number.

Pearson's correlation coefficients (r)

The degree of binary correlation between the features is low. The biggest percentage of the couples of features fell within the weak (64 %) and the moderate (20 %) degree of binary correlation. The most significant binary correlation was measured for the couples of features: x_2x_3–flower length/spate length, x_3x_5–spate length/external perianth length, x_3x_6–spate length/external perianth width, x_3x_7–spate length/internal perianth length, x_5x_7–external perianth length/internal perianth length, x_6x_{12}–external perianth width/corm width, x_7x_8–internal perianth length/internal perianth width, $x_{15}x_{16}$–leaves' number/flowers' number. It was established that a higher binary correlation is registered for couples of features describing lengths.

Multiple determination (R^2)

A high degree of the multiple determination between the studied features was distinctly expressed (Table 2). Out of the total number of multiple coefficients 50 % were within the very high degree, 44 % – within the high degree and 6 % – within the low degree. The features x_2–flower length and x_3–spate length showed a very high degree of multiple determination. The feature x_4–spate width had weak multiple determination.

Table 1. Variation coefficients of *C. veluchensis*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}	x_{15}	x_{16}
1	32.85	15.74	15.24	32.31	13.92	17.40	10.81	18.04	24.41	15.62	20.14	24.99	25.88	39.15	28.11	34.87
2	31.41	14.54	14.06	22.04	15.07	23.53	13.33	17.69	23.57	11.41	16.20	17.97	15.01	25.71	25.94	17.66
3	38.71	16.23	15.55	29.33	15.38	20.34	18.21	25.44	19.51	17.85	22.35	23.40	15.46	20.89	24.55	33.90

Table 2. Coefficients of a multiple determination (R^2) of *C. veluchensis*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}	x_{15}	x_{16}
1	0.76	0.75	0.80	0.48	0.71	0.61	0.70	0.63	0.68	0.78	0.75	0.76	0.60	0.67	0.70	0.74
2	0.70	0.86	0.81	0.49	0.70	0.81	0.86	0.81	0.77	0.67	0.76	0.80	0.86	0.64	0.74	0.58
3	0.81	0.87	0.92	0.77	0.90	0.81	0.89	0.83	0.56	0.88	0.75	0.65	0.90	0.72	0.75	0.35

Cluster analysis

The obtained cluster similarity between the studied samples is relatively low (Fig. 1). This fact may be explained by the clonal structure of the studied samples. The samples from the same altitude stood closely within the cluster. These ones had similar metrical characteristics.

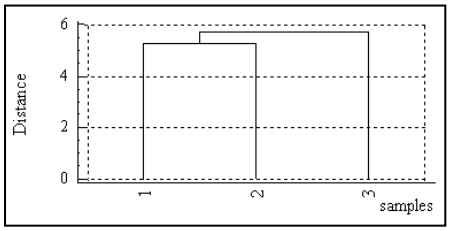

Fig. 1. Distance among samples of *C. veluchensis*.
Sample **1** – Western Rhodopes Mts, "Karachoumak" locality;
sample **2** – Western Rhodopes Mts, "Kartela" locality;
sample **3** – Western Rhodopes Mts, around dam "Batak".

Crocus flavus

Variation analysis

With the smallest dimensions of more of the features was characterized sample 1 (Vitosha Mt, paragliding area) and with the greatest – sample 4 ("Urvuch" locality). The biggest dispersal of values from the standard deviation has been registered for the following features describing lengths: x_1–stem length; x_2–flower length; x_3–spate length; x_{11}–leaf length. The rest of the features showed stability. The established variability was related to the moderate and low variation degree (Table 3). Out of 56 variation coefficients, 25 % fell within the low rate, 45 % – within the moderate one, 16 % – within the high degree and 14 % – in the very high degree. The feature x_5–external perianth length was characterized by low variability for all four samples. By very high variability degree for all four samples were characterized features x_1–stem length and x_{14}–flowers' number.

Pearson's correlation coefficients (r)

The biggest percentage of the couples of features fell within the weak (70 %) and moderate (22 %) degree of binary correlation. The most significant binary correlation was measured for the following couples: x_1x_{11}–stem length/leaf length; x_2x_3–flower length/spate length; x_2x_{11}–flower length/leaf length; x_3x_{11}–spate length/leaf length; x_5x_7–external perianth length/internal perianth length; $x_{13}x_{14}$–leaves' number/flowers' number. High binary correlation was registered for couples of features describing lengths.

Multiple determination (R^2)

The multiple determination coefficients were within the high (39 %) and very high (32 %) degrees (Table 4). A bigger percentage of those coefficients have been found within the low degree (16 %) in comparison to *C. veluchensis*. The biggest percentage of multiple coefficients within the very high degree was characteristic for sample 1 (Vitosha Mt, paragliding area). The features x_7–internal perianth length and x_{11}–leaf length had the highest multiple determination. The lowest multiple determination was registered for feature x_4–spate width.

Cluster analysis

The samples collected from the zone around the village of Bistritsa showed compactness in the assessment

Table 3. Variation coefficients of *C. flavus*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}
1	40.37	13.36	11.15	13.24	11.05	19.06	12.61	22.21	24.05	19.38	19.16	19.50	35.35	54.66
2	40.17	10.10	11.63	16.13	9.90	12.28	10.43	14.20	12.19	10.98	20.63	19.05	24.27	47.05
3	57.85	10.91	9.67	18.54	10.81	17.62	10.27	14.91	17.33	11.98	14.50	19.07	19.81	41.20
4	50.87	14.77	16.77	15.67	7.68	15.35	8.02	18.38	16.18	13.60	22.58	39.80	27.32	47.70

Table 4. Coefficients of a multiple determination (R^2) of *C. flavus*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}
1	0.80	0.83	0.77	0.47	0.79	0.71	0.85	0.67	0.64	0.62	0.86	0.60	0.90	0.89
2	0.46	0.75	0.59	0.54	0.88	0.50	0.84	0.73	0.39	0.48	0.64	0.13	0.65	0.69
3	0.76	0.48	0.50	0.45	0.71	0.48	0.69	0.54	0.35	0.30	0.77	0.42	0.66	0.70
4	0.68	0.81	0.80	0.49	0.75	0.68	0.87	0.60	0.27	0.55	0.86	0.50	0.81	0.83

of their morphometric similarity (Fig. 2). The sample 4 from the mountain of Lozen was distinguished at an insignificant Euclidean distance. Samples 1 and 2 from Bistritsa collected at the same altitude showed a significant similarity with respect to their morphometric characteristics. A similarity was established for the clusters of *C. flavus* and *C. veluchensis*.

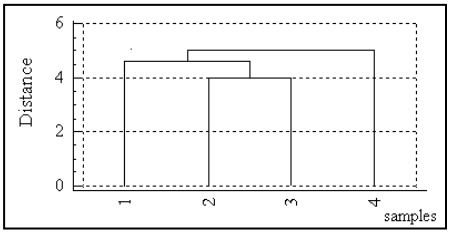

Fig. 2. Distance among samples of *C. flavus*.
Sample **1** – Vitosha Mt, Bistritsa village, paragliding area; sample **2** – Vitosha Mt, Bistritsa village, "Gergyov Dol" locality; sample **3** – Vitosha Mt, Bistritsa village; sample **4** – Lozen Mt, "Urvich" locality.

Colchicum autumnale

Variation analysis

The values of standard deviations formed a continuous row. The biggest dispersion was shown for lengths of the measured features. These were: x_1–flower length; x_2–sheath length; x_4–external perianth length; x_6–internal perianth length; x_{10}–corm height. The variability was within the moderate and high degree – 53 % of the coefficients fell within the moderate degree, 37 % – within the high, 7 % – within the very high and 3 % – within the low degree (Table 5). The features x_5–external perianth width and x_{12}–flowers' number had the highest values of variation coefficients for all samples. The lowest values were registered for features x_1–flower length and x_4–external perianth length. Relatively the most variable was sample 4 (Bogdana peak) – 58 % of the features were within the high and very high degree. The less variable was sample 3 (Chiprovitsa peak) for which 75 % of the features fell within the low and moderate degree.

Pearson's correlation coefficients (r)

The biggest percentage was registered for the couples of features within the weak (47 %) and moderate (30 %) degree of binary correlation. The most significant binary correlation has been established for the couples of the following features: x_1x_2–flower length/sheath length; x_4x_6–external perianth length/internal perianth length; x_5x_7–external perianth width/internal perianth width.

Multiple determination (R^2)

The multiple determination between the features studied was distinctly expressed (Table 6). Out of the total number of multiple determination coefficients 11.7 % were within the low, 40 % – within the high and 48.3 % – within the very high degree. The biggest percentage of multiple coefficients within the very high degree was registered for sample 4 (Bogdana peak). With a very high multiple determination were features x_4–external perianth length and x_6–internal perianth length for which from all the samples we obtained coefficients within the very high degree. Relatively the lowest multiple determination was shown by feature x_9–anther length.

Table 5. Variation coefficients of *C. autumnale*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}
1	16.57	13.63	21.60	16.00	22.83	16.21	23.98	13.37	19.82	16.96	19.49	46.22
2	11.64	20.53	18.27	14.23	23.43	15.52	25.67	21.11	18.42	24.45	19.86	31.51
3	15.00	15.63	30.56	12.07	22.02	14.56	19.98	15.76	13.90	16.94	19.96	53.38
4	16.93	22.42	23.54	13.45	20.12	14.55	24.39	22.26	15.66	16.99	22.78	59.97
5	12.92	17.28	30.62	12.34	21.23	14.06	22.27	18.88	15.47	20.27	28.48	51.74

Table 6. Coefficients of a multiple determination (R^2) of *C. autumnale*.

Features / Samples	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}
1	0.76	0.78	0.64	0.82	0.88	0.83	0.87	0.51	0.60	0.43	0.54	0.43
2	0.62	0.63	0.6	0.88	0.81	0.90	0.71	0.57	0.60	0.41	0.63	0.68
3	0.46	0.57	0.78	0.90	0.84	0.9	0.75	0.51	0.38	0.69	0.61	0.90
4	0.86	0.78	0.71	0.91	0.83	0.87	0.82	0.46	0.39	0.60	0.80	0.77
5	0.73	0.66	0.73	0.85	0.71	0.95	0.76	0.77	0.54	0.81	0.87	0.80

Cluster analysis

The clustering of the samples coresponded to their origin (Fig. 3). A significant morphometric similarity was found between samples 3 and 5. Sample 4 was within the same group. All of them were collected from Vitosha Mt, while the samples from Pirin Mts formed a separate cluster.

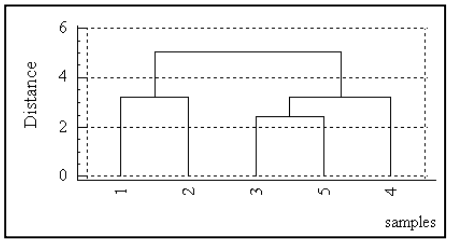

Fig. 3. Distance among samples of *C. autumnale*.
Sample **1** – Pirin Mts, "Kroushe" locality; sample **2** – Pirin Mts, Ribarnika; sample **3** – Vitosha Mt, at the foots of Chiprovitsa peak; sample **4** – Vitosha Mt, at the foots of Bogdana peak; sample **5** – Vitosha Mt, Bistritsa.

Conclusions

1. For the three investigated species a predomination of the moderate variability was found, but with differences in the ratios within the other degrees. For *C. veluchensis* the ratio between the moderate and high degrees was almost equal. About *C. flavus* the moderate degree was predominant and the low degree was represented up to a considerable extent. For *C. autumnale* the situation was similar to the one for *C. veluchensis*. We consider that this type of relations may be explained by study of a greater number of samples. The values of the variation coefficients formed continuous rows for all three species. The variability had not discrete degree of the feature complex. A higher level of variability was established for features describing lengths.

2. The Pearson's corelation was low. The stability of the morphometric complex was lower and it may be supposed that there is a greater plasticity of the morphometric features and hence a greater relative adaptability of the studied clones. A higher relative Pearson's correlation was found for couples of features describing lengths. It is possible that this dependence might be of functional nature.

3. The multiple determination (R^2) was high for all three species. We interpret this fact as evidence for stability of the morphometric complex, a good level of adaptability resulting from the selection pressure at the ecological conditions. The low degree of Pearson's correlation is compensated by the significantly higher multiple determination. That syndrom has been ascertained by us for many other cases (Delcheva 2002; Delcheva & Peev 2002).

4. The common cluster was formed by similar morphometric features for the three species (Fig. 4).

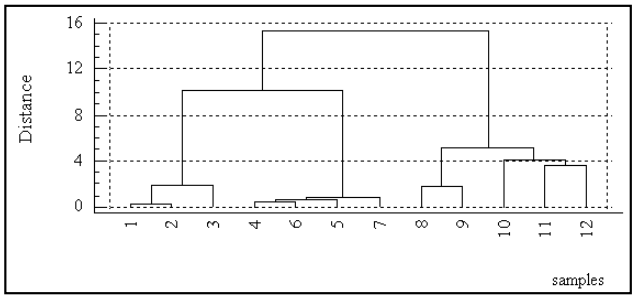

Fig. 4. Distance among all samples.
Samples **1, 2, 3** – *Crocus veluchensis*; samples **4, 5, 6, 7** – *Crocus flavus*; samples **8, 9, 10, 11, 12** – *Colchicum autumnale*.

Definitely, clusters from the samples per genera (*Colchicum*, *Crocus*) were formed. The genera clusters were characterized by a lower degree of similarity in contrast to the ones for the separate species. The species clusters were characterized by a higher degree of similarity within the generalized cluster in contrast to the similarity degree found for the separate samples. Such fact might be explained by filling of the row of values upon which the degree of interruption and probable variability decreases. The two species of *Crocus* have similar morphometric characteristics due to clonal nature of the samples.

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Morphometrical variability in Bulgarian *Galanthus elwesii* (*Amaryllidaceae*)

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Abstract. *Galanthus elwesii* has a wide horizontal (in all floristic regions) and vertical (from 0 to 1500 m alt.) distribution in Bulgaria. Plant material from 29 different localities has been collected according to the statistical requirements. Eight characters have been measured and calculated using STATISTICA 6.0 software package. The comparative analysis shows small degrees in the variability (CV from 6.1 % to 29.4 %) with prevalence of the low and extremely low degrees (80 % of the coefficients of variation). According to the cluster analysis samples are morphometrically homogeneous, with comparatively high similarity. Only one sample from Rhodope Mts is discrete and different.

Key words: *Galanthus elwesii*, morphometry, variation

Introduction

Galanthus elwesii Hook. f. (*Amaryllidaceae*) is distributed in the Balkan Peninsula, the Aegean Islands, Ukraine and Turkey (Davis 1999). In Bulgaria it has a wide horizontal (in all floristic regions) and vertical (from 0 to 1500 m alt.) distribution (Kozhuharov 1992). *Galanthus elwesii* has been subject of numerous taxonomic revisions, but still there is not consideration between authors about the species concept. One of the main reasons that lead to taxonomic confusion is that some main characters are lost, when the plant material is pressing and drying. This makes difficult the taxonomic revisions and necessitates a study of living plants. Delipavlov (1968, 1971) and Artjushenko (1970) consider that *G. elwesii* and *G. graecus* Orph. ex Boiss. are distributed in Bulgaria. In the following works (Brickell 1984; Davis 1997, 1999) after taxonomic investigations authors conclude that *G. graecus* has to be in the synonymy of *G. gracilis*, first described by Čelakovsky (1891). This opinion has been adopted lately by Delipavlov & Cheshmedzhiev (2003). At the same time other authors (Webb 1978, 1980; Kamari 1982; Kozhuharov 1992) consider that all these taxa belong to one species – *G. elwesii,* first described by Hooker in 1875 (Webb 1978). Infraspecific variability of this species is performed with a number of subspecies. There are some papers that examine the problem with variability of *G. elwesii*. Kamari (1982) examines the variation of some morphological characters among species of genus *Galanthus* growing in Greece. About *G. elwesii* and *G. graecus* she concludes that they belong to one species, highly variable in all morphological characters. Korkut (1994) makes a research on variability of some characters of *G. elwesii* compared between different samples from one population. He concludes that there is great variability of all measured characters, except length of inner perianth segments.

The comparison of the morphological characters from different origins of *G. elwesii* has to show the character of morphometrical variability and whether values of measured characters are discreet, or there is continuous variability between samples from different origins.

Material and methods

Plant material from 29 different localities in Bulgaria has been collected according to the statistical requirements (Table 1). The study was carried out on live material. All plants were in flowering period, with bulbs. The species has been determined according Kozhuharov (1992) and Webb (1980).

Table 1. Studied samples from *G. elwesii*.

No.	Sample code	Locality
1	028a	NE Bulgaria: Obrochiste village, by the road to Balchic town, oak forest, 219 m; E exposition, 20.02.2002.
2	029a	NE Bulgaria: Tsarkva village near Dobrich town, oak forest, 117 m; E exposition, 21.02.2002.
3	0210	NE Bulgaria: Shumensko plateau, *Carpinus orientalis* forest near the monument, 454 m; W exposition, 21.02.2002; 28.02.2004.
4	0211	NE Bulgaria: west slopes right of the road Omurtag–Turgovishte, deciduous forest, 301 m; W exposition, 21.02.2002; 26.02.2004.
5	0216	NE Bulgaria: Tervel town, locality under the wall; NE exposition, 20.02.2002.
6	0218	NE Bulgaria: Tervel town, Dan Kula locality; NE exposition, 20.02.2002.
7	046	NE Bulgaria: deciduous forest between Kalinata and Vasilevo villages, Chernata Gora locality, 300 m; NE exposition, 27.02.2004.
8	047	NE Bulgaria: oak forest between Sirakovo and Surnino villages, right on the road to Surnino; NE exposition, 27.02.2004.
9	048	NE Bulgaria: Shumensko plateau, oak forest at the beginning of Bunata Reserve, on the left of the road, 555 m; NE exposition, 28.02.2004.
10	049	NE Bulgaria: Penev Grob locality between Kyulevcha and Markovo villages, *Carpinus orientalis* forest, 343 m; NE exposition, 28.02.2004.
11	0410	NE Bulgaria: the plateau above Madarsky Konnik locality, *Carpinus orientalis* forest, 50 m before the stronghold, left of the road, 450 m; NW exposition, 29.02.2004.
12	0411	NE Bulgaria: before Koprivets village, right on the road, *Carpinus orientalis* forest, 265 m; SW exposition, 29.02.2004.
13	0412	Danube Valley: the valley of Chernelka River between Kurtozhabene and Gortalovo villages, near bushes, 175 m; SE exposition, 29.02.2004.
14	0213	Danube valley: Vladimirovo village, near the Monastery, bushes, 350 m; NE exposition, 22.02.2002.
15	0212	E Forebalkan: Sevlievo town, *Tilia* forest near the road to Momina Salza hut, 400 m; NE exposition, 22.02.2002.
16	044	E Forebalkan: Veliko Turnovo town, slopes near the main road from Veliko Turnovo to Turgovishte, near bushes, 295 m; NW exposition, 26.02.2003.
17	031	E Forebalkan: Yoglav village, Kamuka locality, near the forest; N exposition, 26.02.2003.
18	024	E Stara planina Mts: Karnobat town, Markela locality, before the town, 219 m; E exposition, 18.02.2002.
19	023	E Stara planina Mts: Sliven town, near the road to Karandila, oak forest, 848 m; W exposition, 18.02.2002.
20	0418	W Stara planina Mts: Baba peak, in the forest, 1500 m; NE exposition, 01.02.2003.
21	0419	Sofia region: Makotsevo village, Sinigerov Dol locality, *Carpinus orientalis* forest, 650 m; NE exposition, 18.03.2004.
22	0221	Sofia region: Kokalyane village, near the Urvich stronghold, 610 m; NW exposition, 05.03.2002.
23	032	Znepole region: Trun town, Mogilata locality, 550 m; NW exposition, 25.03.2004.
24	042	Mesta Valley: Hadzhidimovo village, St. Dimitar chapel, deciduous forest, 450 m; SW exposition, 19.02.2004.
25	041	Pirin Mts: Musomishte village, Karacheto locality, 550 m; E exposition, 19.02.2004.
26	0413	Sredna Gora Mts: Nivata locality near the river Mativir, Ihtiman town, 600 m; N exposition, 28.05.2004.
27	034	W Rhodopes: Belovo–Yundola road, over Yadenica River, 776 m; NE exposition, 19.03.2003.
28	035	Central Rhodopes: Ruen village near Plovdiv city, deciduous forest near St. Ilia chapel, 600 m; NE exposition, 19.03.2002.
29	0222	Central Rhodopes: Bachkovo village, near the road from Monastery to children's camp, 485 m; NE exposition, 09.03.2002.

The following morphometrical characters were investigated:

1. Stem – length (cm)
2. Leaf – length and width (cm)
3. Spatha – length (cm)
4. Perianth segments
   4.1 Outer perianth segments – length and width (cm)
   4.2 Inner perianth segments – length and width (cm).

Over 30–50 specimens from any sample were collected by random sampling and mean values of all characters were calculated. All values were statistically significant (p-value ≤ 0.05). The measurements were taken with accuracy 0.1 cm. Observed morphological characters were compared by mean values of different samples. Degrees of variability were defined according the scale of Coefficient of variation in Peev (2001): 0%–10% – extremely low; 10.1%–20% – low; 20.1%–30% – transpose; 30.1%–40% – moderate; 40.1%–50% – rising; 50.1%–60% – high rising; 60.1%–70% – significant; 70.1%–80% – high; 80.1%–90% – very high; over 90% – exceptional variability.

Cluster analyses, Scatterplots and Correlation analyses were performed using the STATISTICA for Windows 6.0 StatSoft (1996) software package.

Results and discussion

Variation in measured characters among samples

The comparative morphometrical analysis of measured characters shows small degrees in the variability. Coefficients of variation are from 6.1% to 29.4%, with prevail of the low degrees – 72.8% of the samples. 7.8% are with extremely low and 19.4% with transitional variation. From measured characters, with lowest values of varia-

bility are the length and width of inner and outer perianth segments (from 6.1% to 24.5%) and most variable is width of leaf – from 15.3% to 28.9%. Among samples with lowest degrees are these from Northwest Bulgaria – samples 028a, 029a, 049 (Table 2).

Table 2. Mean (cm) /first line/ and Coefficient of variation CV% /second line/ of measured characters from investigated samples of *G. elwesii*.

Samples code	Width of leaf	Length of leaf	Length of spatha	Length of stem	Length of outer perianths	Width of outer perianths	Length of inner perianths	Width of inner perianths
023	0.596	10.416	2.63	10.493	1.651	0.773	0.95	0.504
	21.6	24.1	13.2	21.7	16.8	20.8	13.4	18.5
024	0.623	10.008	2.844	9.463	1.879	0.847	0.973	0.532
	27.0	24.3	17.7	22.1	10.2	18.9	11.1	17.7
028a	0.937	14.608	3.386	12.632	2.087	1.323	1.106	0.702
	25.2	13.0	13.0	17.7	7.8	24.5	6.4	12.4
029a	0.959	17.66	3.162	13.393	2.078	1.141	1.087	0.731
	19.8	9.5	14.1	14.1	6.1	8.1	7.7	9.8
0210	0.635	12.592	2.816	11.64	1.572	0.922	0.923	0.485
	15.3	17.8	13.6	18.2	14.0	13.9	10.4	16.6
0211	0.828	17.126	3.179	14.774	1.77	0.944	0.933	0.528
	21.6	14.6	15.8	14.7	16.0	11.9	14.0	19.2
0212	0.731	16.47	3.256	13.128	1.613	0.796	0.836	0.466
	21.2	19.4	15.8	19.9	11.5	12.3	13.3	16.3
0213	0.638	13.604	2.917	10.104	1.578	0.743	0.915	0.422
	19.5	16.3	14.9	20.6	14.8	13.9	18.2	15.5
0216	1.002	15.609	3.721	12.288	1.954	0.879	0.988	0.451
	18.9	15.7	18.4	22.4	10.6	16.8	15.7	13.4
0218	1.053	17.041	3.629	13.363	2.086	0.832	1.115	0.511
	28.9	14.6	14.1	18.8	18.3	18.5	12.2	21.7
0221	0.671	12.757	2.827	12.822	1.713	1.183	0.985	0.588
	17.1	22.4	16.8	20.1	11.8	23.1	9.3	11.0
0222	0.687	13.433	2.987	13.474	1.699	0.929	0.972	0.579
	18.0	22.2	12.6	21.7	12.2	23.2	7.9	17.4
031	0.714	13.368	2.542	12.564	1.919	1.093	1.006	0.632
	19.0	13.2	15.8	17.9	11.1	18.3	8.3	11.8
032	0.77	15.526	2.684	12.77	1.936	1.026	0.992	0.584
	17.1	14.3	12.6	18.6	10.7	16.0	8.4	14.0
034	0.967	20.641	3.5	15.909	2.175	1.381	1.187	0.806
	24.6	19.3	19.6	20.1	13.6	20.8	9.1	16.0
035	0.621	14.806	2.87	12.614	2.065	1.153	1.052	0.643
	18.3	19.8	16.9	21.9	11.6	13.6	8.3	11.5
041	0.598	11.466	3.372	11.894	2.016	1.150	1.000	0.638
	22.1	25.8	20.4	25.6	16.5	17.1	16.6	13.1
042	0.471	7.4	2.793	8.83	1.907	1.059	1.02	0.589
	25.3	18.5	14.5	18.0	12.7	20.8	14.0	14.3
044	0.738	13.735	2.965	12.626	1.944	1.065	1.026	0.606
	16.3	19.5	12.4	18.2	12.8	14.6	9.7	15.2
046	0.65	10.709	2.53	10.976	1.804	1.107	0.97	0.6
	21.5	13.9	16.0	14.6	14.7	21.8	11.5	18.3
047	0.853	11.77	3.067	11.22	2.203	1.243	1.077	0.68
	26.2	14.2	20.6	15.4	15.3	21.0	11.6	18.7
048	0.892	12.798	3.166	12.71	2.13	1.298	1.06	0.652
	20.9	15.2	17.9	17.0	13.3	15.9	9.9	13.9
049	0.516	9.614	2.44	9.252	1.806	0.982	0.958	0.516
	23.3	14.4	12.3	15.1	9.2	14.8	9.0	13.8
0410	0.65	10.60	2.66	9.93	1.88	1.06	0.99	0.57
	23.0	16.0	12.5	15.4	13.0	15.2	11.1	13.6
0411	0.95	15.696	3.478	13.696	2.27	1.35	1.13	0.734
	27.7	12.7	15.2	16.4	11.2	16.3	10.2	13.1
0412	0.787	10.102	2.917	9.623	2.051	1.232	1.053	0.670
	29.4	19.8	16.5	18.6	13.8	18.9	11.7	18.9
0413	0.893	11.741	2.922	11.757	2.133	1.261	1.083	0.685
	21.5	17.3	16.8	16.8	12.0	16.6	10.0	13.8
0418	1.091	10.959	3.302	11.625	2.107	1.393	1.175	0.784
	24.9	18.8	13.9	20.6	11.3	16.1	10.2	15.5
0419	0.47	9.88	2.275	9.92	1.885	0.875	1.005	0.57
	23.0	23.1	17.6	20.5	10.5	14.8	9.9	14.1

Table 3 performs variation in the average values of measured characters and limit values where over 50% of the samples exist.

Limits of variation in measured characters are compared with the limits published in previous works (Artjuschenko 1970; Delipavlov 1971; Brickell 1984; Zeybek & Sauer 1995; Davis 1999) (Table 4). Width of leaf, inner, other perianth segments and length of inner perianth segments show values, corresponding to both species *G. elwesii* and *G. gracilis* (*G. graecus*) according to these authors. According to Brickell (1984) our values for length of outer perianth segments (1.6–2.3) refer to *G. gracilis*, but according to others – they are for both species. Our values of length of leaf (7.4–20.6) according to Davis (1999), Brickell (1984) and Zeybek & Sauer (1995) are limits for both species, but Artjuschenko (1970) and Delipavlov (1971) do not report values like these about length of leaf. Length of stem with limit values 8.8–14.7 Davis (1999) refers to both species, Artjuschenko (1970) – to *G. elwesii*, Brickell (1984) – to *G. gracilis*.

Table 3. Limit values of measured characters and values where over 50% of the samples exist.

Character (cm)	Width of leaf	Length of leaf	Length of spatha	Length of stem	Length of outer perianths	Width of outer perianths	Length of inner perianths	Width of inner perianths
limits	0.5–1.1	7.4–20.6	2.3–3.7	8.8–4.7	1.6–2.3	0.7–1.4	0.8–1.2	0.4–0.8
>50%	0.6–0.9	11.0–15.0	2.8–3.3	10.5–12.8	1.8–2.1	0.9–1.2	1.0–1.1	0.5–0.7

Table 4. Limits of variation in some morphological characters according to five authors.

Author	Species	Width of leaf	Length of leaf	Length of stem	Length of outer perianths	Width of outer perianths	Length of inner perianths	Width of inner perianths
Davis	*G.elwesii*	(0.5–)0.6–3.1(3.5)	(4.8–)5.5–25(–28)	9.0–18	1.8–2.3(–2.6)	1–1.5(–1.7)	1–1.2	0.6–0.7
(1999)	*G.gracilis*	(0.25–)0.3–0.9(–1.2–2.2)	(2.3–)5.5–16(–24)	6–10(–12)	1.8–2.3(–2.6)	0.8–1.4	1–1.2	0.6–0.7
Artjuschenko	*G.elwesii*	1.5–3	7.0–10	10.0–13	2	1.3	1.1	0.6
(1970)	*G.graecus*	0.5–0.7	–7	7.0–9.0	2.5	0.7	1.1	0.5
Delipavlov	*G.elwesii*	1.7–2.2	6.0–9.0		2.5–2	1.2–1.5		
(1971)	*G.graecus*	0.5–1	5.0–6.0	15–20	1.7–2.2	0.8–1	0.9–1.1	0.5–0.6
Zeybek & Sauer	*G.elwesii*	0.7–1.8(–2.8)	15–18		2	0.7–0.9	0.7–0.9	0.3–0.5
(1995)	*G.gracilis*	(0.4–)0.5–0.6	(5.5)9–17		(1.2–)1.8–2.2	0.8–1.2	0.8–1.1	0.3–0.5
Brickell	*G.elwesii*	(0.6–)1.3–2.5(–3)	(7.5–)12–20(–32)	12.0–28.0	2.0–2.7	(0.9–)1.2–1.9	1–1.5	0.5–0.8
(1984)	*G.gracilis*	0.3–0.5(–0.7)	(5–)8–11(–15)	8.0–14.0	1.5–2.6	0.7–1.0	0.7–1.1	0.3–0.5

Correlation analysis

Simple linear correlation (Pearson r) shows great correlation (between 0.92 and 0.84) between width of outer and width of inner perianth segments, length of leaf and length of stem, length of outer and length of inner perianths and width and length of inner perianth segments. The lowest values of correlation are between length of leaf and width of outer perianth segments – 0.08 (Table 5).

Scatterplots show that investigated samples are from one species, rather homogeneous, according to the investigated features (Fig. 1). Almost all points form one shape, like "cloud". Some seven samples are separated – 0212, 0213, 0216, 0218, 042, 0418, 032. The pictures of the normal distribution are indicator for the clone-population structure of this species.

Cluster analysis

Cluster analysis from 29 different origins of *G. elwesii* based on nine morphological characters is shown in Fig. 2. Single linkage method and Euclidian distances are used when clustering. Five clusters are formed. Cluster 1 included samples with the lowest values of measured characters. Their variables are under mean values for those characters. These samples occurred in dry, open places or in light *Carpinus orientalis* forests and near bushes. Cluster 3 included samples that have values above mean value for every character. Most of them grow in oak forests with rich soils, or near rivers on alluvial soils and enough moisture. Samples in clusters 2 and 4 have changeable values of investigated characters and middle position between 1st and 3rd clusters. All these four clusters have a great similarity and small linkage distance from the overall similarity line. Only cluster 5 is different from the others. It has a single sample – 034 from Western Rhodope Mts near Yadenitsa River above Belovo town. All measured characters of this sample are with high, above mean values. Another special feature is that leaves are longer than stem. In all other cases the stem is longer.

Conclusion

The study of variability of 29 samples of *G. elwesii*, grown in Bulgaria, shows low and continuous variability according measured characters. These undiscreet values are typical for clone-population structure of one species. Scatterplots show that population structure prevails at this stage of development of *G. elwesii* clone-populations. According to the cluster analysis samples are morphometrically homogeneous, with comparatively high similarity. Only the sample 034 from the Rhodopes is discrete and different. The results confirm that investigated clone-populations belong to one species with no differentiation on subspecies. Only according to environmental conditions (mostly the moisture) clone-populations from different origins are arranged in two main groups.

Table 5. Correlation coefficients between measured characters (*shows positive correlation).

Variable	Width of inner perianths	Length of inner perianths	Width of outer perianths	Length of outer perianths	Length of spatha	Width of leaf	Length of leaf
Length of stem	0.33	0.36	0.29	0.26	0.60*	0.61*	0.90*
Length of leaf	0.16	0.27	0.08	0.2	0.63*	0.61*	
Width of leaf	0.47*	0.68*	0.46*	0.61*	0.78*		
Length of spatha	0.24	0.47	0.26	0.45			
Length of outer perianths	0.79*	0.88*	0.76*				
Width of outer perianths	0.92*	0.77*					
Length of inner perianths	0.84*						

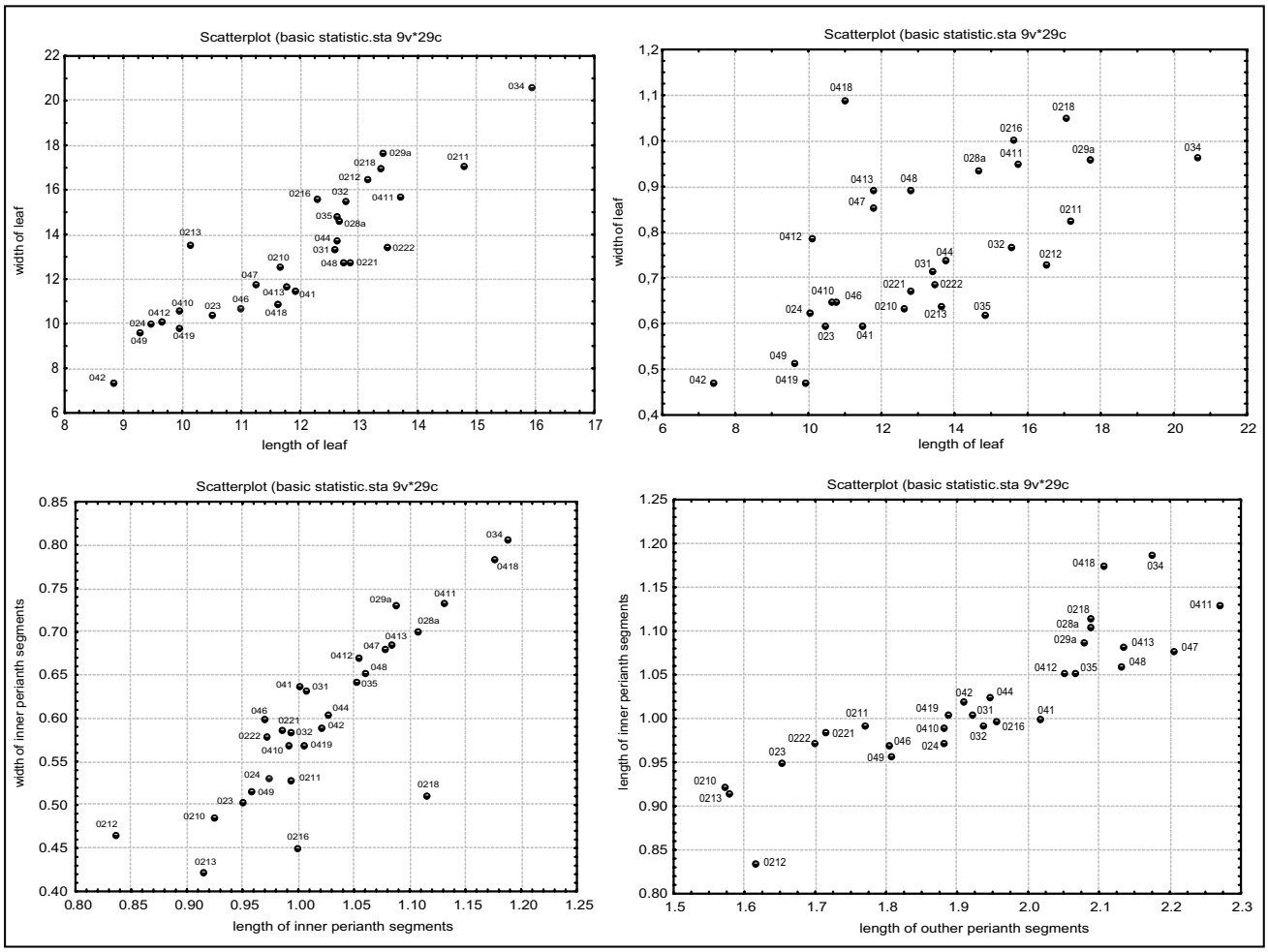

Fig. 1. Scatterplots of measured characters from investigated samples of *G. elwesii*.

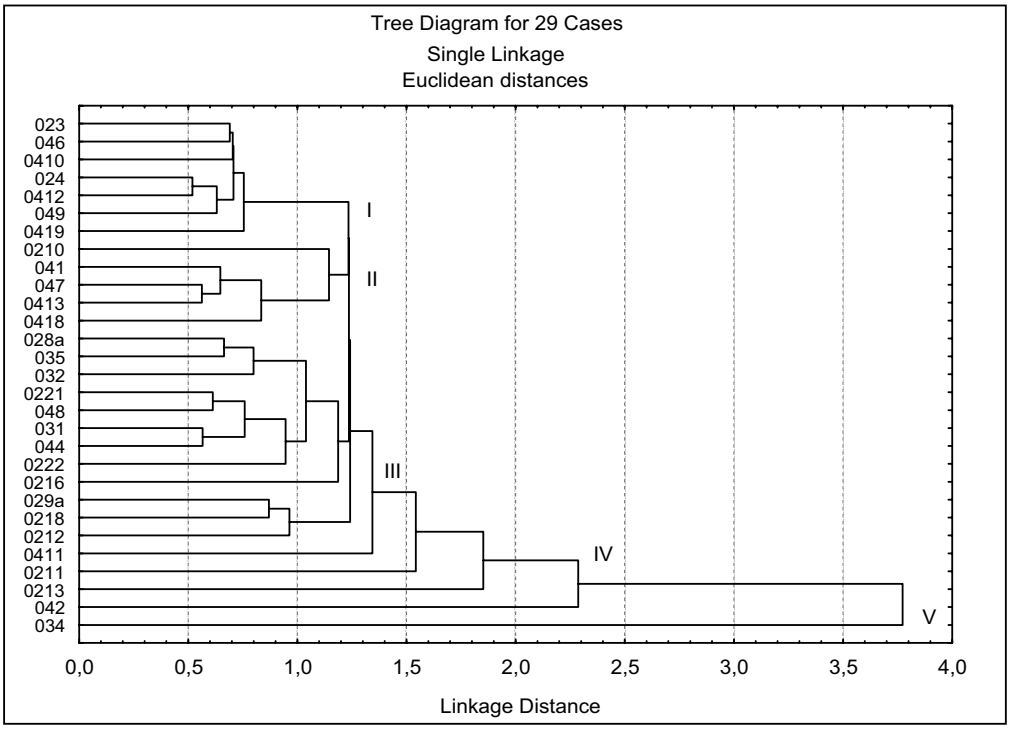

Fig. 2. Cluster analysis for 29 samples of *G. elwesii*. Nine morphological characters are used when clustering.

Acknowledgements. The author is indebted to Prof. Dr. Dimitar Peev for the helpful discussions and encouragement during this work.

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A new section (Sect. *Heleolinum*) of the genus *Linum* (*Linaceae*) for Turkey

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Abstract. Section *Heleolinum* is a new section of the genus *Linum*, described by Egorova in 1998. It includes one species which has two subspecies: *L. seljukorum* subsp. *seljukorum*, Turkish endemic and *L. seljukorum* subp. *barsegianii*, Armenian endemic. The dichotomic key of the sections, which are found in Turkey, is given. Detailed morphological characteristics with line drawings and a distribution map of *L. seljukorum* subsp. *seljukorum* are presented.

Key words: identification key for sections, *Linum seljukorum*, section *Heleolinum*, Turkish flora

Introduction

So far the genus *Linum* was represented by 5 sections in the *Flora of Turkey and the East Aegean Islands* (Davis 1967) – Sect. *Cathartolinum* Griseb., Sect. *Linastrum* (Planch.) Winkl., Sect. *Syllinum* Griseb., Sect. *Dasylinum* (Planch.) Juz., and Sect. *Linum* (= Sect. *Eulinum* Griseb.). The section *Dasylinum* is characterized by having hairy leaves, often large pink or white flowers, short pedicels, hairy sepals, coherent petals, hairy or glabrous capsule. It is represented by 8 species in the *Flora of Turkey* (Davis 1967): *L. olympicum* Boiss. (endemic), *L. hirsutum* L., *L. unguiculatum* P.H. Davis (endemic), *L. densiflorum* P.H. Davis, *L. hypericifolium* Salisb., *L. pubescens* Banks & Sol., *L. anisocalyx* P.H. Davis (endemic), and *L. seljukorum* P.H. Davis.

Davis (1967) mentioned *L. seljukorum* in the Flora with the remark "A very distinctive species, perhaps deserving a separate section; its small petals, homostyly, and sepals ± equal to the capsule are not found elsewhere in Sect. *Dasylinum*". According to Davis Turkish specimens from Konya region and Armenian specimens from regions around the mountain Ararat are both *L. seljukorum*. New gatherings from Armenia (Ararat region, near Ararat village) showed that there are several different characteristics from *L. seljukorum*, and Gabrielian & Dittrich (1992) described Armenian specimens as a new species, *L. barsegianii* Gabrieljan & Dittrich. They placed this new species together with *L. seljukorum* in the Sect. *Halolinum* described by Egorova (1973). Because the "*halolinum*" epithet was previously used by Planchon (1847) and does not match botanical nomenclatural rules, the name of the section was changed as *Heleolinum* T.V. Egorova (Egorova & Gabrielian 1998). Later, Egorova changed the rank of *L. barsegianii* into a new combination of *L. seljukorum* subsp. *barsegianii* (Gabrieljan & Dittrich) T.V. Egorova (Svetlova 2007).

With the addition of this new section to Turkish Flora, the diagnostic key for the sections of genus *Linum* is proposed. The description and detailed hand drawn morphological characteristics, as well as the distribution area of *L. seljukorum* subsp. *seljukorum* are given.

Material and methods

The specimens of *L. seljukorum* were collected from different localities from Aksaray–Konya regions in 2005. They are kept in Marmara University, Faculty of Arts and Sciences Herbarium (MUFE). Specimens found in different herbaria in Turkey were also examined. Description of *L. seljukorum* is given according to the *Flora of Turkey* and all these studied specimens. Line drawings were hand drawn.

Results

A new identification key of the sections of the genus *Linum* in Turkey was elaborated.

Identification key of genus *Linum* in Turkey

1. Usually annuals; leaves opposite; petals white, less than 5 mm ..... ***L. cathartolinum*** (Sect. ***Cathartolinum***)

1*. Rarely annuals; leaves alternate (or the lowest rarely opposite); petals usually coloured; if white, then more than 5 mm **2**

2. Annuals with small yellow flowers (petals 3–8 mm); capsule 2–3 mm..... Sect. ***Linastrum***

2*. Perennial, or if annual or biennial, then flowers and capsules larger **3**

3. Petals yellow or white, coherent below; stem with ridges decurrent from the leaf bases; stipular glands often present; sepals usually keeled, at least in fruit ..... Sect. ***Syllinum***

3*. Petals blue, lilac, pink or white, free or coherent; stem terete; stipular glands absent; sepals not keeled..... **4**

4. Pedicels short, hairy like the sepals; petals coherent or free; capsule usually hairy **5**

5. Petals more than 10 mm, lilac, pink or white, coherent, heterostylic ..... Sect. ***Dasylinum***

5*. Petals less than 10 mm, blue, free, homostylic..... ..... ***L. seljukorum*** (Sect. ***Heleolinum***)

4*. Pedicels long, glabrous like the sepals; petals always free, blue, lilac or white; capsule glabrous .... Sect. ***Linum***

Sect. *Heleolinum* T.V. Egorova: Annual herbs. The leaf margins glandular-ciliate. Flowers small and blue, petals free, homostylic, fruiting pedicels short. The margins of the sepals glandular-ciliate. Stigmas linear-clavate. Sepals ± equal to the capsules.

This section is represented by one species with two subspecies and they are identified by the following characters:

***L. seljukorum* subsp. *seljukorum*:** Stem 6–20 cm, straight, greyish with blue flowers, calyx shorter or equal to capsule. Distributed around Tuz Lake in Konya. Turkish endemic.

***L. seljukorum* subsp. *barsegianii*:** Stem 10–35 cm, slightly curved, straw coloured with 5–14 pinkish flowers, calyx longer than capsule. Distributed around Ararat Mt. Armenian endemic.

Description of *L. seljukorum* subsp. *seljukorum*

L. seljukorum P.H. Davis in Notes R.B.G. Edinb. 22: 147, t. 7 (1957) (Fig. 1)

Annual herb, 3–25 cm. Stems ascending, usually branched and ending into unilateral cymes. The upper half of stems softly hirsute, the lower half sparsely hirsute or glabrous. Basal leaves obovate-spathulate, fleshy, glabrous or sparsely hirsute, not glandular margined. Median cauline leaves ovate-oblong or lanceolate, 4.5–15 mm long, 1–4 mm broad, 3-nerved, pilose-villous with glandular-ciliate margins. Flowers homostylic; sepals 4–5 mm, obtuse, acute, densely villous with glandular-ciliate margins. Petals 7–8 mm, blue, free, nerves bifurcated in the upper half. Stigmas linear-clavate. Capsule 4 mm, beaked, ovate-globose, the apex sparsely pilose. Carpels 5, ovary 10-locular. Seeds elliptic, light brown-yellowish, 2.4–2.5 mm. Fl. 6–9. Dry mud at edge of saline marshes, *ca*. 1000 m.

Distribution of *L. seljukorum* subsp. *seljukorum* (Figs 2, 3)

Type: Turkey **C4** Konya – Konya to Kaşinhan, 1000 m, 07.09.1947, Davis 14777 (holo. E!, iso. K!).

B4 Konya: Aslım arid marshes, *ca*. 1010 m, 09.08.1960, E. Yurdakulol, ANK 51!; **B4** Konya: Cihanbeyli, Bulok Lake, *ca*. 1010 m, 04.08.1960, Khan, Prance, Ratchiffe, ANK 445!; **B4** Konya: Cihanbeyli, Tuz Lake near Cihanbeyli, 10.08.1971, E. Özhatay & C.M. Rogers, MUFE 4211!; **B4** Aksaray: Sultanhanı salt marshes, (Niğde), 910 m, 02.08.1986, E. Leblebici 6817, Ö. Seçmen, ISTE 68100!; **B4** Konya: Cihanbeyli, Bulok Lake environs, 1000 m, 13.08.1993, M. Koyuncu 10653, AEF 17944!; **B4** Aksaray: Sultanhanı, 940 m, 18.07.1996, M. Vural 7576, N. Adıgüzel, M. Öztekin, GAZI!; **B4** Konya: Tuz Lake, Aksaray–Eskil (near Selam cemetery), 950 m, 14.07.1997, M. Aydoğdu, AEF 3844!; **B4** Konya: Gölyazı–Tersakan, 980 m, 22.07.1997, GAZI 7929!; **B4** Aksaray: Aksaray–Konya road, 2 km to Sultanhanı, 23.06.2005, 940 m, N. & E. Özhatay 10320, N. Şafak, MUFE!; **B4** Aksaray: East of Eskil, 24.06.2005, 950 m, N. & E. Özhatay 10345, N. Şafak, MUFE!

Endemic; Ir.–Tur. element.

Acknowlegements. This work was supported by the Marmara University Research Project (FEN–054/230804).

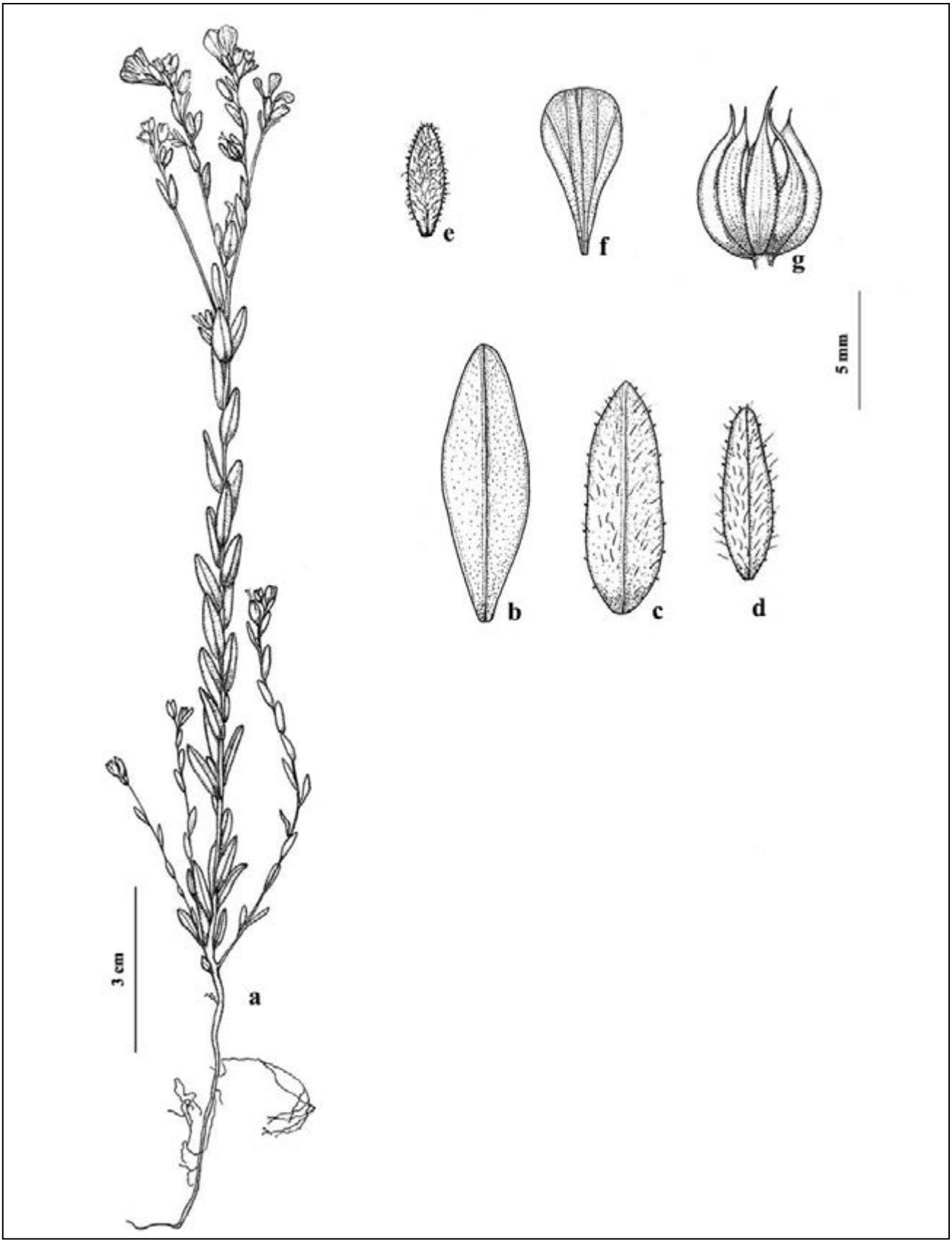

Fig. 1. *Linum seljukorum* subsp. *seljukorum*: **a** – plant; **b, c** – basal leaves; **d** – median cauline leaf; **e** – sepal; **f** – petal; **g** – capsule [E.Ö. 10345 (MUFE)].

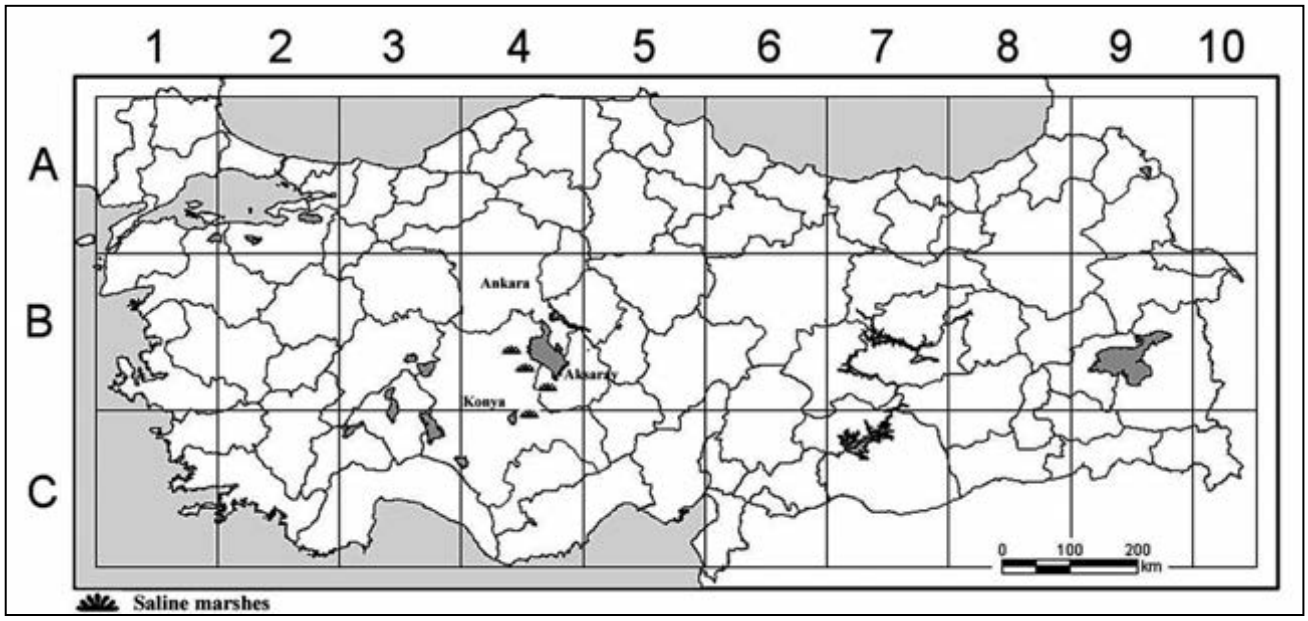

Fig. 2. Distribution map of *L. seljukorum* in Turkey.

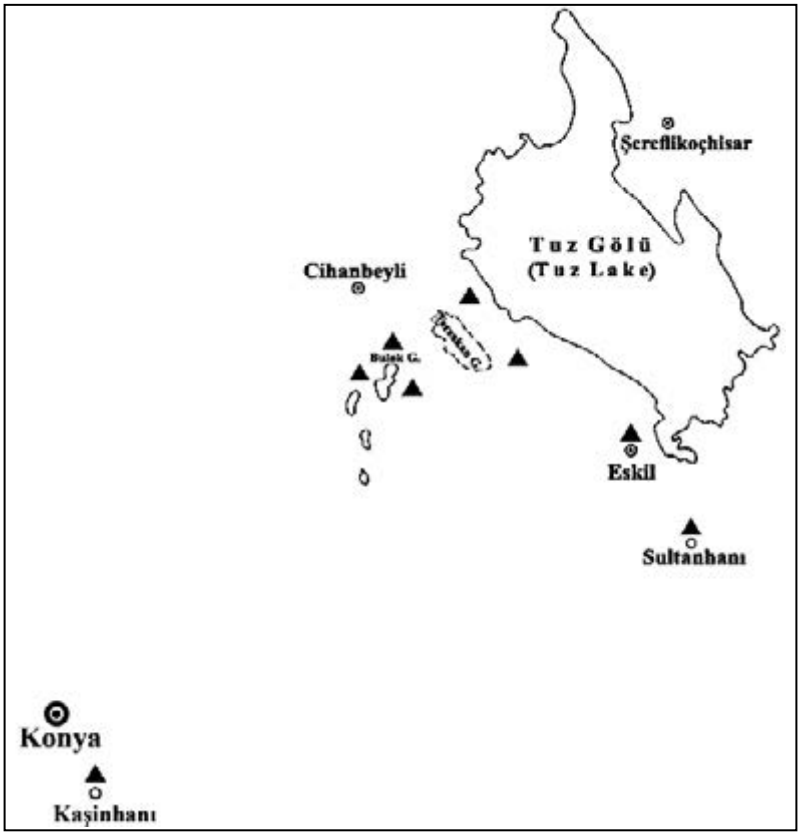

Fig. 3. Distribution map of *L. seljukorum* (▲) in Konya–Aksaray region.

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The rediscovery of *Galium huber-morathii* (*Rubiaceae*): a local endemic species of Bozkır (Konya / Turkey)

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Abstract. This study is based on morphological investigation of *Galium huber-morathii*. The species is a narrow endemic recorded from a very limited area of Bozkır (Konya / Turkey). Huber-Morath was the first botanist to collect specimens of this species, from the Konya: Bozkır region in 1948. Until we collected it in 2003, no one seems to have reported a second collection. Some deficiences dealing with *G. huber-morathii* were eliminated, and the description was prepared again. Distribution, habitat and ecology, flowering time, altitude and threat category of this plant were given.

Key words: Bozkır, *Galium*, Konya, local endemic, Turkey

Introduction

In terms of species numbers, *Galium* L. (*Rubiaceae*) is represented by *ca*. 400 taxa. It is the richest genus in Turkey. *Galium* is represented with 105 species; 51 of them are endemic. The rate of endemism is about 49 % (Davis & al. 1988; Güner & al. 2000).

Material and methods

In the scope of the project called "The Flora of The Region Among Bozkır–Çumra Apa Dam and Hadim (C4 Konya)", and founded by Scientific Research Projects Coordination Office (BAP–2002/120 and BAP–2006/067010589), the local endemic taxon *Galium huber-morathii* Ehrend. & Schönb.-Tem. was collected from Bozkır area. The first specimens of this species were collected during an expedition by Huber-Morath to the area around Bozkır, South Anatolia in June 1948. These specimens were described as a new species by F. Ehrendorfer and E. Schönbeck-Temesy in 1979. In the 7th volume of *Flora of Turkey and the East Aegean Islands* the authors indicated that, although allied to them, *G. huber-morathii* is distinct from *G. tmoleum* Boiss. and *G. mite* Boiss. & Hohen. (Ehrendorfer & Schönbeck-Temesy 1982). Some deficiences dealing with *G. huber-morathii* were eliminated, and the description was prepared again in this research.

The specimens collected were identified with the help of *Flora of Turkey and the East Aegean Islands* (Ehrendorfer & Schönbeck-Temesy 1982; Davis & al. 1988; Güner & al. 2000). The Herbarium of KNYA was used to check some of the specimens. The authors of species names are written according to Brummitt & Powell (1992). The plant specimens prepared for herbarium collections have been stored in the Herbarium of KNYA.

Results and discussion

Description

The following description is based on our own observations and that given in the *Flora of Turkey* (Ehrendorfer & Schönbeck-Temesy 1982).

Galium huber-morathii Ehrend. & Schönb.-Tem. (Fig. 1)

Type: Turkey C4 Konya – Konya to Bozkır, gorge of Çarşamba River, 74 km from Konya, limestone rocks, 1060 m, 14.06.1948, *Huber-Morath* 9336 (holo. Hb. Hub.-Mor.! Iso WU!)

Perennial with woody rhizome. Stems numerous, (10–) 20–35 (–45) cm, lignescent at base, weak, fragile, erect to ascending, glabrous, ± shining; internodes ± equal, (1.5–) 2–5 cm. Leaves in whorls of (4–) 6, subpetiolate, glabrous, ± shining; lamina 10–25 × 1–2 mm, narrowly elliptic, ± linear, acuminate to mucronate,

with hyaline apex, margins flat and smooth (Fig. 2). Inflorescence ovoid, with monochasial ultimate branches, glabrous; peduncles (10–) 15–30 mm, pedicels filiform, 1.5–4 mm; lowermost bract leaf-like, upper reduced or absent. Corolla white, 2.5 (–3) mm in diameter, nearly rotate, tube 0.2–0.4 mm, lobs ovate, apiculate, 1–1.5 mm (Fig. 3). Anthers 0.3–0.5 mm, cylindrical, yellowish-green. Ovary adpressed-hairy at base. Mericarp ± single, broadly ellipsoid, 1–1.5 mm, rugulose, glabrescent or minutely hairy. Flowering May–June. Limestone rocks, *ca*. 1200 m.

Distribution

[Turkey] C4 Konya: Bozkır, Maviboğaz, gorge of river Çarşamba, limestone rocks, 37°16'N, 32°16'E, 1200 m, 09.07.2003, O. Tugay 3343.

As a result of our field studies, this species, which was known only from its type locality, has been collected from the locality of gorge of Çarşamba River in Bozkır as given in the *Flora of Turkey*. However, according to our studies, we have observed the existence of the species at 8th km of the Bozkır–Maviboğaz road on the rocks. The populations were healthy in both localities.

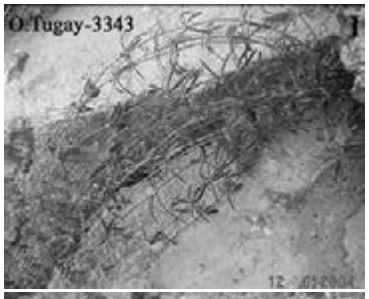

Fig. 1. Habitat of *G. huber-morathii*.

Fig. 2. Leaves of *G. huber-morathii*.

Fig. 3. Flowers and fruits of *G. huber-morathii*.

Habitat and ecology

Known locality is on limestone rocks in the gorge of Çarşamba River (Fig. 1). The vertical distribution is between 1000–1200 m. It shares its habitat with *Equisetum ramosissimum* Desf., *Cheilanthes marantae* (L.) Domin, *Ceterach officinarum* DC., *Ephedra major* Host, *Fibigia eriocarpa* (DC.) Boiss., *Clypeola ciliata* Boiss., *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Viola odorata* L., *Viola heldreichiana* Boiss., *Smyrnium connatum* Boiss. & Kotschy, *Campanula buseri* Damboldt, *Androsace maxima* L., *Vinca herbacea* Waldst. & Kit., *Neatostema apulum* (L.) Johnst., *Andrachne telephioides* L., *Allium lycaonicum* Siehe ex Hayek, and *Fritillaria crassifolia* Boiss. & A. Huet subsp. *crassifolia*.

Threat category

According to Ekim & al. (2000), this species is classified as "endangered (EN)". In this article, we have reviewed this assessment using the most recent version of the IUCN Red List Categories (IUCN 2001). *Galium huber-morathii* is an endemic species known only from the type gathering. The range of this species is limited to a single location and its area of occupancy is estimated to be less than 5 km (criteron B2a), the mature individual plants number being less than 250 (criteron C). Therefore it can be included in CR (Critically Endangered) category (IUCN 2001).

Acknowledgements. We thank to the Curators of KNYA, GAZI and ANK, who allowed us to study their other *Galium* specimens. We also thank to Scientific Research Projects Coordination Office (BAP–2002/120 and BAP–2006/067010589) for financial support.

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Delphinium balcanicum (*Ranunculaceae*): a contribution to the flora of Turkey

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Abstract. In this study, *Delphinium balcanicum* (*Ranunculaceae*) was added to the flora of Turkey with a specimen collected from European Turkey, A1(E): Tekirdağ, Edirne, Çanakkale. A short description of the species and its distribution are given. Its relationships with *D. venulosum* and *D. hellenicum* are discussed.

Key words: *Delphinium balcanicum*, flora of Turkey

Introduction

Delphinium L. is a difficult genus, many of its taxa being connected by intermediates, possibly of hybrid origin. Several species centred in the Caucasus reach their limit in NE Anatolia (Davis 1965). It is usually widespread in Europe, Anatolia, the Balkans and Mediterranean region. There are 25 species of this genus in Europe (Pawłowski 1993) and 6 species in the Balkans (Polunin 1987). 22 species of *Delphinium* in *Flora of Turkey and the East Aegean Islands* are recorded, 4 of which are endemic. Only 2 *Delphinium* species are present in European Turkey (Davis 1965). During our ongoing efforts to further study the flora of European Turkey, we came across some interesting *Delphinium* specimens that were collected in 1987–1988 from Edirne, Çanakkale and Tekirdağ by Dane. Following Davis (1965), Polunin (1987) and Pawłowski (1993) this specimen was determined as *Delphinium balcanicum*. After examination of the known bibliographical references (Davis 1965; Webb 1966; Davis & al. 1988; Alpınar 1993; Özhatay & al. 1994, 1997, 2000; Güner & al. 2000; Dane & Dalgıç 2001), it became clear that this taxon is a new record for the flora of European Turkey. But in recent years, although the presence of this species in Thrace had been recorded by Pawłowski (1993), recorded specimens are needed that belong to this species. For this reason in this study the presence of *D. balcanicum* in European Turkey is exposed depending to specimens.

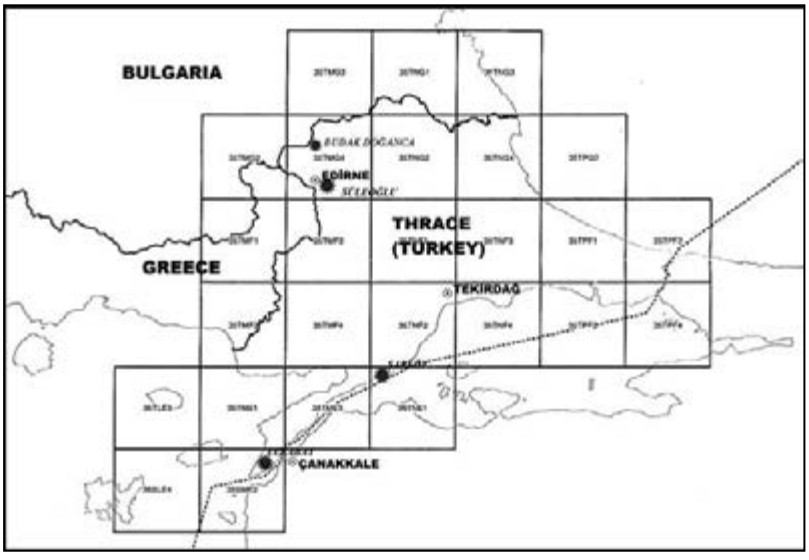

Fig. 1. The localities of *D. balcanicum* in European Turkey.

Material and methods

The studied *D. balcanicum* material was collected in 1987 and 1988, during floristic excursions in European Turkey. Voucher specimens were deposited in the Herbarium of Trakya University (EDTU). These specimens were determined according to the *Flora Europaea* (Pawłowski 1993), *Flowers of Greece and the Balkans* (Polunin 1987) and *Flora of Turkey* (Davis 1965). The distribution of the determined taxon in European Turkey is presented in Fig. 1.

Results

Delphinium L.

Perennial, biennial or annual herbs. Flowers zygomorphic, borne in racemes. Leaves alternate, palmately divided. Perianth biseriate, sepals and petals are petaloid. Sepals 5, the posterior spurred. Petals 4, in 2 dissimilar pairs, the upper pair with nectariferous spurs inserted into the sepal spur. Stamens in 8 spirally arranged series. Follicles 3(–5).

This genus includes 3 sections: 1. Sect. *Delphinastrum* DC.; 2. Sect. *Delphinium* (*Delphinellum* DC.); 3. Sect. *Staphisagria* DC.

Delphinium balcanicum belongs to Sect. *Delphinium*.

Delphinium balcanicum Pawł. (Figs 1–3)

Annual. Stem 20–40 cm, slender, sparsely branched, with blue-violet flowers and usually upward-pointing spurs; with cylindrical spike. Uppermost leaves linear entire, lower usually tripartite. Inflorescence mostly very dense (rarely more or

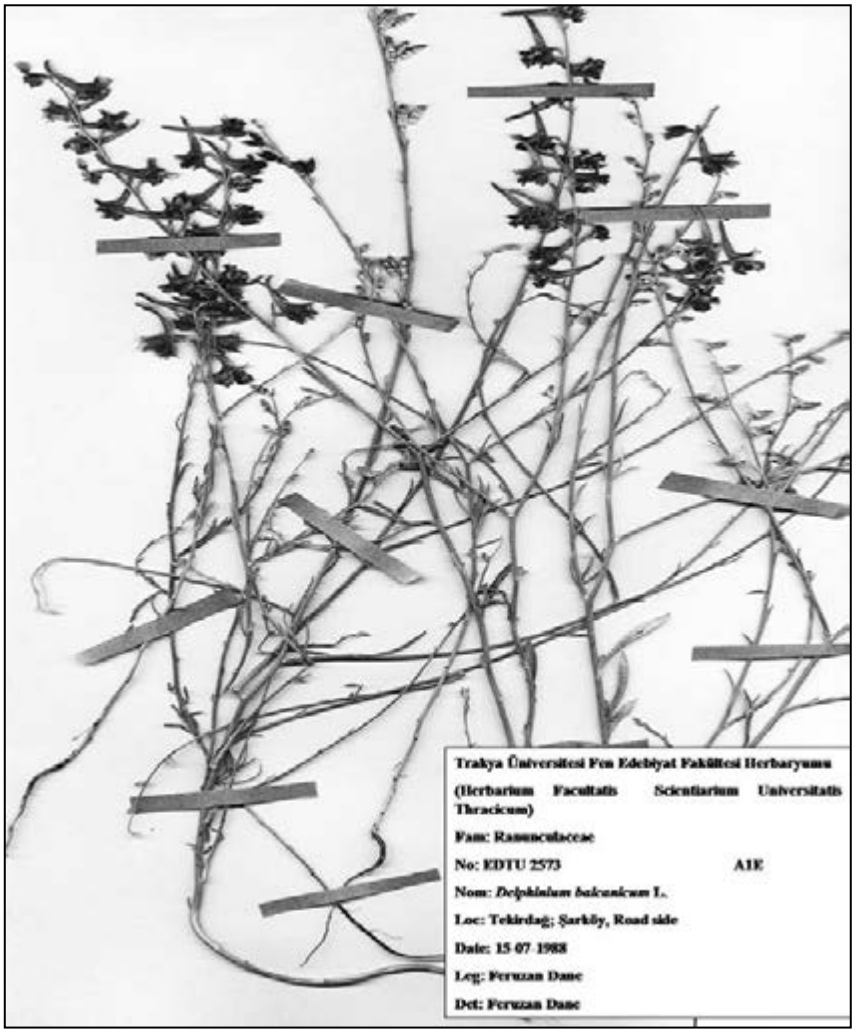

Fig. 3. Herbarium material of *D. balcanicum*.

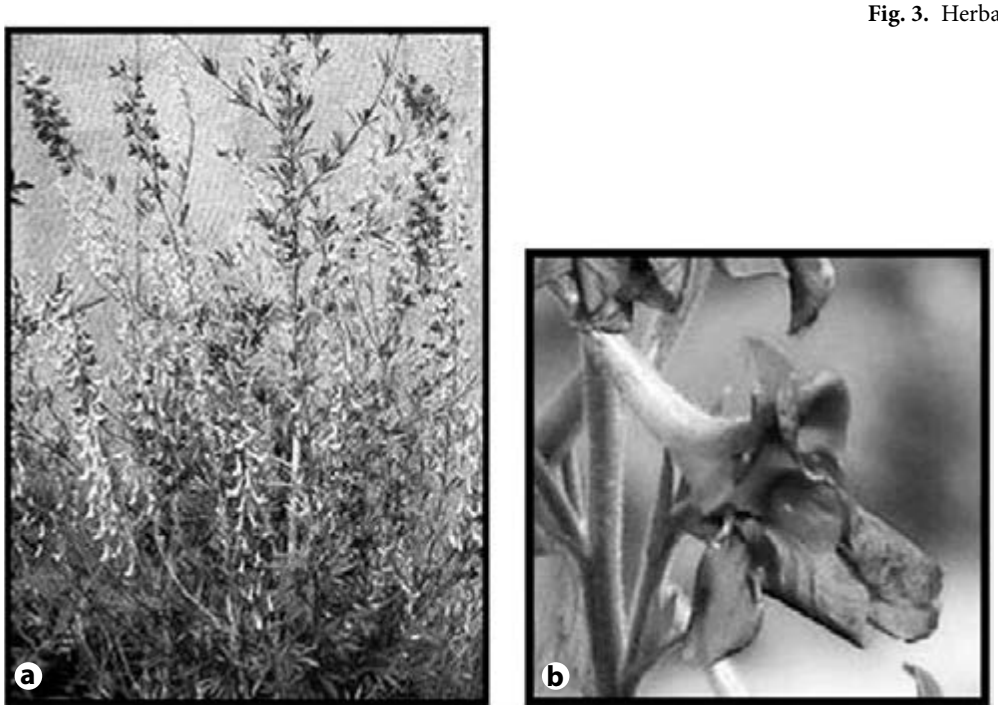

Fig. 2. *D. balcanicum*: **a** – general view; **b** – flowers.

less lax). Flowers with long spreading hairs. Blade of inner lateral perianth segments like a ping-pong bat, the outside of outer segments is hairy. Perianth segments 6–9 mm, patent-pilose. Spur up to twice as long as perianth segments. Follicles 5–7 (–9) mm, 2–3 times as long as wide, densely covered with rather long patent hairs. Open grund, cultivated places (Polunin 1987; Pawłowski 1993).

General distribution: Balkan peninsula – Bu, Gr, Ju, Tu.
Distribution in Turkey: European Turkey.
Distribution in European Turkey: A1(E) Çanakkale: Eceabat, Seddulbahir, 40°11'02" N, 26°21'23" E, 0 m alt., 07.07.1987, leg. & det. F. Dane (EDTU 1733); A1(E) Edirne: Budak Doğanca, 41°45'37" N, 26°20'33" E, 98 m alt., 08.07.1988, F. Dane (EDTU 2398); A1(E) Edirne: Süleoğlu, road side, 41°46'02" N, 26°54'43" E, 156 m alt., 14.07.1988, F. Dane (EDTU 2469); A1(E) Tekirdağ: Sarköy, road side, 40°36'58" N, 27°06'03" E, 0 m alt., 15.07.1988, F. Dane (EDTU 2573).

Discussion

The morphology of the collected specimens of this species is identical with that given by Pawłowski (1964). *Delphinium balcanicum* is closely related to *D. venulosum* Boiss. (which was recorded in Turkey B5 Kayseri), but differs from it with its simple pilose stems, very dense inflorescence and follicles that are 7–9 mm long while follicles are 5–6 mm in *D. venulosum* (Davis 1965). And also it is closely related to *D. hellenicum* Pawł. that spreads in Greece, but differs from it in type of hairs and blade of inner lateral perianth segments. *Delphinium hellenicum* is hairless and the blade of its inner lateral perianth segments is heart-shaped at base. *Delphinium balcanicum* has long spreading hairs and the blade of the inner lateral perianth segments is rounded.

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Distribution and habitat features of the endemic *Jurinea* species for Turkey

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Abstract. In this study, the descriptions of 6 endemic *Jurinea* species collected from different localities in Turkey and studied morphologically were reviewed in the light of the literature data and our observations. The distributions of the endemic taxa are as follows: *J. brevicaulis* and *J. cadmea* have local distribution; *J. alpigena*, *J. ancyrensis* and *J. cataonica* have regional, and *J. pontica* has larger distribution. The photos of some taxa were presented. IUCN red list categories of the taxa were evaluated based on literature data and our field observations. Also their map of distribution was given.

Key words: endemism, IUCN red list categories, *Jurinea*, Turkish flora

Introduction

Turkey has very rich flora with about 10 000 taxa and 33 % of endemism. The genus *Jurinea* Cass. is distributed in large areas of the world from West of Europe to Central Asia. It especially spreads in the Mediterranean and Irano–Turanian phytogeographic regions. The genus is represented by 200 species in the world. Turkey is one of the main centres of diversity for *Jurinea* (Danin & Davis 1975). The genus comprises 19 taxa occuring mainly in the Mediterranean and Irano–Turanian regions (Danin & Davis 1975; Duman & Aytaç 1999). Generally, *Jurinea* species grow on stony calcareous cliffs, seashores, gypsum fields, rocky mountain summits, open woods and shrubs, steppes, fallow fields, sandy beaches, forests, rocky slopes, and on maritime limestone cliffs in Turkey. There are 6 endemic taxa of *Jurinea*, and the endemism value is about 31.57 % in Turkey. The endemic taxa of the genus are localized especially in Bozdağ, Akdağ, Sünnice Mts, East and West Anatolia. In this study, some distributional and morphological data for all 6 endemic *Jurinea* species were given. We also reviewed their IUCN red list categories previously evaluated by Ekim & al. (2000), using the most recent version of the IUCN Red List Categories (IUCN 2001).

Material and methods

The plant samples were collected from different localities in Turkey during field trips from 2004 to 2005. The specimens were stored at different herbaria (KNYA, GAZI, HUB) in Turkey. The collecting localities of the species are as follows:

- *J. cadmea* – B2 Izmir: Ödemiş, Bozdağ, rocky mointain summits, 1530 m, 05.07.2005, B. Doğan 1004;
- *J. alpigena* – A4 Karabük: Yenice, Keltepe, 1500 m, 10.07.2005, B. Doğan 1015;
- *J. pontica* – A3 Sakarya: Sapanca Lake, 40–50 m, 09.07.2005, B. Doğan 1011; A4 Çankırı: Çankırı–Ilgaz road, 3 km, gypsum steppes, 1100 m, 10.07.2005, B. Doğan 1016;
- *J. brevicaulis* – B7 Erzincan: Erzincan–Çayırlı road, Akdağ, 1550 m, 07.08.2005, B. Doğan 1028;
- *J. ancyrensis* – B7 Elazığ: Harput, 1450 m, 03.06.2005, B. Doğan 1021; B7 Elazığ: Baskil, the village of Hacı Mustafa, 1850 m, 03.08.2005, B. Doğan 1022;
- *J. cataonica* – B7 Erzincan: Erzincan–Çayırlı road, Akdağ, 1750 m, 07.08.2005, B. Doğan 1029.

The specimens were studied morphologically, and the descriptions of the species were reviewed in

the light of the literature data and our observations. IUCN red list categories of the species were evaluated through our field observations and the literature data (Danin & Davis 1975; Ekim & al. 2000; IUCN 2001).

Results and discussion

Jurinea cadmea Boiss. (Figs 1, 2)

Dwarf acaulescent rosulate perennial. Leaves pinnatisect or rarely lyrate-pinnatisect, lateral segments 5–7 × 2–3 mm, oblong, obtuse, apiculate, with undulate margin, green to grey, pitted above, white-arachnoid beneath. Capitula sessile or rarely on short scape up to 8 cm. Involucre globose, 10–20 mm broad; phyllaries 5-seriate, outer reflexed, arachnoid, 2–3 × 1–2 mm, inner erect, 13–14 × 2 mm, glabrous. Flowers lilac-pink. Achenes 4–5 mm, longitudinally striate. Pappus 2 times as long as achene, hairs scabrous. Fl. 6–8. Rocky mountain summits. 1070–2100 m.

We determined this species from one location in Bozdağ (Izmir). Its area of occupancy was very small, but the population had approximately 100 individuals. It is known also from Izmir (Danin & Davis 1975). Taking these data into consideration, this species was graded as "Vulnerable VU". Our evaluation agrees with Ekim & al. (2000).

J. alpigena C. Koch (Fig. 1)

Rosulate perennial, with woody base. Stems/peduncles simple, arachnoid-floccose, 15–40 cm × 1–4 mm, with (0–)1–3 small leaves. Rosette leaves petioled, entire to pinnatisect, 4–9 cm, green, sparsely arachnoid-pilose and pitted-glandular above, white and densely arachnoid beneath. Capitula solitary. Involucre hemispherical, 15–30 mm broad; phyllaries 4–5-seriate, ± adpressed, outer 10–12 × 3 mm, inner 12–16 × 1–1.5 mm.

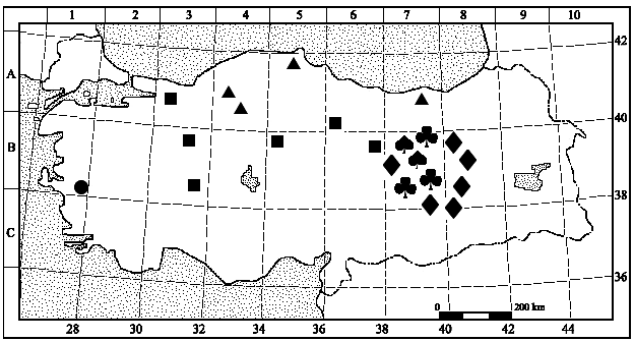

Fig. 1. Distribution map of: *Jurinea cadmea* (●), *J. alpigena* (▲), *J. pontica* (■), *J. brevicaulis* (♠), *J. ancyrensis* (♣), *J. cataonica* (♦).

Fig. 2. *J. cadmea* in the wild.

Flowers bright purple to pink. Achenes nearly smooth. Pappus hairs barbellate. Fl. 5–7. Rocky and sandy beaches, rocky slopes, and fallow fields. Up to 2740 m alt.

We determined this species from Keltepe (Karabük). The population had approximately 200 individuals. *Jurinea alpigena* is known in Kırklareli, Bolu, and Sinop districts from about 10 locations in an area covering 10 000–35 000 km^2. These data taken into consideration, this species was graded as "Lower risk LR (Ic)" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

J. pontica Hausskn. & Freyn ex Hausskn. (Fig. 1)

Perennial. Stems 20–70(–125) cm, usually branched above, diameter ranging from 2–6 mm at base to 1–2 mm above, leafy throughout. Radical leaves 3–12 cm, pinnatisect, lobes narrowly linear with revolute magrin; median cauline leaves decurrent, pinnatisect, 1.5–7 cm; uppermost leaves entire, linear (2–25 × 1–2 mm), longer ones decurrent. Stem and leaf indumentum as in *J. consanguinea*. Capitula usually many (5–15), borne on (1–)5–20 cm branchlets. Involucre subglobose, 15 × 10–20 mm; phyllaries 6–7-seriate; outer 2–4 × 1 mm; median 4–7 × 1 mm, herbaceous, arachnoid, with glabrous, black, reflexed to erect tips; inner 10–12 × 1–2 mm, glabrous, spinescent and erect. Flowers purple. Achenes smooth to longitudinally striate, 3 mm. Pappus hairs barbellate. Fl. 6–8. Woods, steppes, fields and slopes. 50–1450 m.

Jurinea pontica is classified as "Lower risk LR (lc)" according to the recent *Red Data Book of Turkish Plants* (Ekim & al. 2000). It is distributed in Sakarya, Bilecik, Çankırı, Amasya, Samsun, Eskişehir, Kayseri, Sivas, Konya, and Sivas provinces (Danin & Davis 1975). Its observed population is very rich and its extent of occurrence is more than 20 000 km^2. Popula-

tions are not distant from each other, and negative extreme fluctuations in extent of occurence, area of occupancy, and number of individuals are not expected. We agree with Ekim & al. (2000).

J. brevicaulis Boiss. (Figs 1, 3)

Perennial. Stems 15–25 cm, erect, adpressed-tomentose, 1–3 mm in diameter, emerging from a thickened base built up of woolly leaf bases. Stems mostly corymbosely branched above with 2–5 capitula solitary on simple 3–7 cm branches, leafy throughout; upper leaves involucrate. Cauline leaves oblong-lanceolate, attenuate at base, 25–90 × 3–10 mm, lower ones petiolate, glandular-pitted, slightly papillose-arachnoid above, densely arachnoid beneath. Involucre obconical with tapering base, 25–35 × 20–25 mm; phyllaries 5–6-seriate, floccose, adpressed and lanceolate, outer unarmed, inner tapering to a 3–9 mm spine. Flowers purplish-pink. Achenes smooth. Pappus hairs plumose, 2–3 times as long as achenes. Fl. 8. Stony igneous hillsides, steppes, bare slopes. 1400–1900 m.

This species is known from more than 10 locations in Gümüşhane and Erzincan provinces. In addition, its observed population is very rich. Therefore, it should be classified as "Lower risk LR (lc)" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

J. ancyrensis Bornm. (Figs 1, 4)

Perennial. Stems 15–40 cm, procumbent, usually simple, villous, woolly with ± straight hairs at base, slender (2 mm diam.), densely leafy throughout. Leaves oblong-linear, 2.5–6 mm broad, greyish lanate, sessile, shortly subcordate and auriculate at base, not involucrate, 15–25 × 2.5–4 mm, patent to reflexed. Capitula solitary. Involucre obconical with rounded base, 30–35 × 20–30 mm; phyllaries many, 7–8-seriate, lax, slender, 2–30 mm, lanceolate-acicular, tapering to a slender 5–10 mm spine with adpressed crisp hairs, outermost short, patent. Flowers lilac. Achenes smooth, coronulate. Pappus hairs plumose. Fl. 7. Igneous slopes, over-grazed steppes. 1100–1400 m.

We determined this species from Elazığ. The population has approximately 150 individuals. *Jurinea ancyrensis* is known in Malatya, Tunceli and Adıyaman districts from about 10 locations in the area covering 5000–25 000 km^2. Taking these data into consideration, this species should be graded as "Lower risk LR (Ic)" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

J. cataonica Boiss. & Hausskn. (Figs 1, 5)

Perennial. Stems 30–45 cm, erect, glabrescent or canescent, woody and woolly with crisped hairs at base, 2–4 mm in diameter, densely leafy throughout. Cauline leaves lanceolate, acute, (25–)50–75 × (3–)12–20 mm, patent to deflexed with subcordate-auriculate base, green and glabrescent, sometimes tomentellous beneath; uppermost leaves linear-lanceolate, much shorter and narrower, not involucrate. Capitula borne in corymbs, 2–7 together on 2–5 cm branches. Involucre narrowly obconical with a rounded base, 20–25 × 10–15 mm; phyllaries 6-seriate, tightly adpressed, lanate, ending in a 1 mm spine. Flowers purple. Achenes longitudinally striate. Pappus hairs plumose. Fl. 7–9. Rocky limestone slopes. 900–1800 m.

Fig. 3. *J. brevicaulis* in the wild.

Fig. 4. *J. ancyrensis* in the wild.

Fig. 5. *J. cataonica* in the wild.

This species is known from more than 10 locations in Erzincan, Tunceli, Sivas, and Bitlis provinces. In addition, its observed population is very rich. Therefore, it should be classified as "Lower risk LR (lc)" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Acknowledgement. We would like to thank to Selçuk University (BAP–Project No. 05401046) for financial support during this study.

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Some endemic species known from Amanos and Bolkar (Cilician Taurus) mountains

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Abstract. Amanos Mountain Range and Bolkar Mts, two branches of Anatolian Diagonal, are intersection of the Mediterranean and Irano–Turanian phytogeographical region and differentiation regions of some genera. These mountains have very rich flora and are important endemism centres of Turkey. In this study, 8 endemic species were collected and studied morphologically, and their descriptions were reviewed in the light of the literature data and our observations. These species are *Linum empetriflorum*, *Prenanthes glareosa*, *Polygala inexpectata*, *Draba haradjanii*, *D. acaulis*, *Achillea monocephala*, *Heracleum pastinaca* and *Silene haradjanii*. IUCN red list categories of these taxa were evaluated based on literature data and our field observations. Also, their distribution map and some photos were presented.

Key words: endemism, IUCN red list categories, Turkish flora

Introduction

The Amanos Mountain Range is an interesting area, occupying an intersection of the Mediterranean phytogeographical region and the Anatolian Diagonal, with many Euro–Siberian phytogeographical region enclaves. The area is very rich in endemic plants. The concept of the Diagonal was first proposed by P.H. Davis, who defined it as an oblique belt running from the north-east to the Anti-Taurus; it then divides into two, with one branch to the Amanus (Amanos Dağları), the other to the Cilician Taurus (Davis 1971). 33% of the total number of species growing in Turkey are found along the Diagonal, while 5% are more or less restricted to it. One explanation for the present richness of the species is neo-endemism and distribution patterns of the plants related to the Diagonal (Ekim & Güner 1986). In this study, the descriptions and IUCN red list categories of 8 endemic species for Amanos and Bolkar Mts are reviewed.

Materials and methods

The plant samples were collected from different localities of Bolkar and Amanos Mts during field trips from 2000 to 2005. The specimens were stored at GAZI and KNYA herbaria in Turkey. The collecting localities of the species are as follows:

- *Achillea monocephala* – C5 Mersin: Çamlıyayla, Cocakdere–Cehennemdere border, Manastır area, *Cedrus libani–Pinus nigra–Juniperus excelsa* mixed forest, clearings, 1700–1800 m, 12.07.2004, M. Dinç 2204 (KNYA);
- *Draba acaulis* – C5 Konya: Ereğli, Aydos Mt, Yazıgöl plateau, alpine steppe, 2600 m, 24.06.2000, M. Dinç 719 (KNYA);
- *Draba haradjianii* – C6 Osmaniye: Zorkun plateau, Kel Dazı hill, rock crevices, 1850 m, 05.07.2003, A. Duran 6290 & M. Sağıroğlu (GAZI);
- *Heracleum pastinaca* – C5 Niğde: Ulukışla, Bolkar Mts, between Gümüş village and Karagöl, steppe, 2600 m, 26.08.2004, A. Duran 6889 (GAZI);
- *Linum empetrifolium* – C5 Mersin: Aslanköy, Cocakdere, Gökviraj, rocky areas, steppe, 1900–2100 m, 06.06.2003, M.Dinç 1594 & H.H. Doğan (KNYA);
- *Polygala inexpectata* – C5 Karaman: Ayrancı, between Çatköy and Akpınar, steppe, 1550 m, 13.06.2005, M. Dinç 2364 (KNYA);
- *Prenanthes glareosa* – C5 Niğde: Ulukışla, Bolkar Mts, between Gümüşköy and Karagöl, 2600 m, 16.08.2004, A. Duran 6880 (GAZI);
- *Silene haradjianii* – C6 Hatay: Dörtyol, Topaktaş plateau, Mığrı, clearings, 1800 m, 16.07.2004, Y. Menemen 1268 & A. Duran (GAZI).

The specimens were studied morphologically, and the descriptions of the species were reviewed in the light of the literature data and our observations. IUCN red list categories of the species were evaluated based on our field observations and the literature data (Coode & Cullen 1965, 1967; Davis 1967, 1972; Huber-Morath 1975; Jeffrey 1975; Erik 1981; Davis & al. 1988; Gemici 1992; Ekim & al. 2000; IUCN 2001)

Results and discussion

Draba acaulis **Boiss.** (Fig. 1)

Perennial herbs with long leafy columniform caudiculi, forming rounded tufts. Scapes very short, up to 1.5 cm, pubescent. Leaves soft, overlapping, persistent, papery when dried, pubescent with long soft hairs at margins, oblong-linear, 3.5–5 × 0.8–1.2 mm. Racemes terminal, 3–5 flowered, pubescent throughout. Pedicels 2–5 mm in flowering, up to 12 mm in fruiting. Sepals elliptic-lanceolate, rugose, 2–2.5 × 1–1.5 mm, usually glabrous, rarely sparsely hairy. Petals yellow, 4–5.5 × 2–2.5 mm, clawed, with conspicuous brownish veins. Ovary with 12–32 ovules. Siliculae oblong-elliptic, inflated, 3.5–4.5 × 1.3–1.6 mm, densely pubescent.

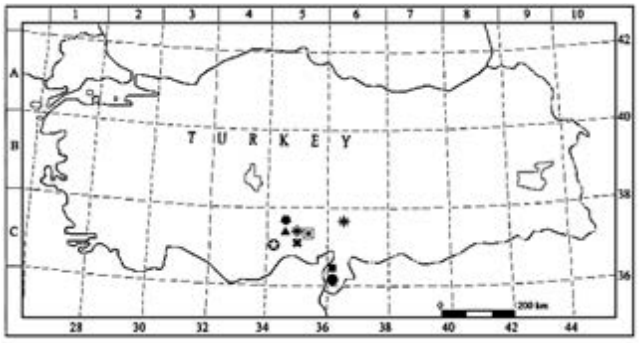

Fig. 1. Distribution map of: *Achillea monocephala* (✖), *Draba acaulis* (▲), *Draba haradjianii* (■), *Heracleum pastinaca* (✳), *Linum empetrifolium* (▣), *Polygala inexpectata* (✪), *Prenanthes glareosa* (⬟), *Silene haradjianii* (●).

This species has been known from three localities in Bolkar Mts (Coode & Cullen 1965; Erik 1981; Gemici 1992). We determined it from fourth locality in the area. According to our observations, its populations are very rich and extent of occurrence is estimated as 5000–20 000 km^2. In the light of these data, this species should be graded as "Vulnerable VU". Our evaluation is the same as Ekim & al. (2000).

Draba haradjianii **Rech.** (Figs 1, 2)

Perennial herbs, forming rounded tufts. Caudiculi leafy only at base, with withered leaves remaining from previous year below. Leaves oblong-obovate to oblanceolate, canescent, soft textured, overlapping, persistent, 5–9 × 1–3 mm, with densely 3–5 branched dendroid hairs. Scapes 2–20 cm, striate, with sparsely dendroid hairs lower ¼, without hairs upper part. Flowers yellow. Racemes loose, terminal, 4–20 flowered. Pedicels 3–10 mm in flower, to 20 mm in fruit. Petals yellow, *ca*. 4 mm. Ovary with 16–24 ovules. Siliculae oblong-ellipsoid, 5–6 × 1.8–2.1 mm, glabrous.

Draba haradjianii has been known only from the type collection until now (Coode & Cullen 1965). It was classified as "data deficient DD" in the *Red Data Book of Turkish Plants* (Ekim & al. 2000). We determined it from a second location near the type locality in Amanos Mts. This species naturally grows only in rock crevices. Population is very poor (approximately 15–20 individuals) and the distribution area is very small. We think that this species should be classified as "critically endangered CR".

Fig. 2. *D. haradjianii* in the wild.

Polygala inexpectata **Peşmen & Erik** (Figs 1, 3)

Canescent, caespitose perennial herbs with strongly branched woody rootstock. Stems ascending, 2.5–7 cm, densely crispate-pubescent. Leaves sessile, ovate-lanceolate to elliptic, 2.5–13 × 1–5 mm, densely crispate-pubescent. Racemes mostly terminal, rarely lateral, cylindrical, 2–4.5 cm, 6–20 flowered. Bracts 2 for each pedicel, 2.5–3 mm, 1-nerved, purplish, lanceolate, pubescent, completely membranous, cadu-

cous, lower shorter or equalling pedicels, upper overtopping pedicels. Flowers pinkish-purple, nodding at anthesis. Pedicels 1–3 mm, pubescent, purple. Sepals overtopping corolla, pubescent outside, outer oblong-lanceolate, green, 2–4 × 1–2 mm, with membranous margins, inner petaloid, obovate, 6–7 × 3–4 mm, with purplish mucro up to 0.3 mm, pinkish in flower, pinkish-white or white-membranous with conspicuous green nerves in fruit. Corolla 4–5 mm, tube incurved, lateral lobes oblong, 2–3 mm, middle lobe dark purple and multifid. Anthers yellow. Capsule (immature) obcordate, 4–4.5 × 3–3.5 mm, with wing 1 mm broad, sparsely pilose. Style 1 mm.

This species has been known from three locations in Ereğli (Konya) district of Bolkar Mts until now (Davis & al. 1988). We determined it from fourth locality near the others. The population, however, is poor. Also, its extent of occurrence is 100–5000 km^2. In the light of these data, it should be classified as "endangered EN" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Fig. 3. *P. inexpectata* in the wild.

Linum empetrifolium **(Boiss.) Davis** (Fig. 1)

Dwarf alpine caespitose plants. Suffrutescent caudex branched. Stems numerous, 2–6 cm long, ascending or procumbent, 1–3 flowered. Leaves loosely imbricate, minutely scabrid, ericoid, linear, thick, increasing in size upwards 3–7 × 0.5–1.5 mm, revolute, prominently 1-nerved, the upper often with mucronulate tips. Pedicels 5–10 mm, apparently recurved in fruit. Sepals 4–4.5 × 2–4.5 mm, prominently 3–5-nerved, outer oblong-elliptic, subacute, sometimes acute and minutely mucronulate, inner oblong-orbicular, obtuse with scarious margins. Petals 9–12 mm. Capsule 6–7 mm.

Linum empetrifolium has been known from two localities near to each other in Bolkar Mts (Davis 1967). As it has not been recorded since 1896, it was classified as "data deficient DD" according to the *Red Data Book of Turkish Plants* (Ekim & al. 2000). We determined it from a third location near the type locality in Bolkar Mts. This species is restricted to rocky places. Population is very poor (approximately 10–15 individuals) and the distribution area is very small. Therefore, we think that this species should be classified as "critically endangered CR".

Silene haradjianii **Chowdhuri** (Fig. 1)

Loosely tufted suffruticose perennial herbs. Flowering stems erect, densely leafy at base, 20–80 cm long, glaucous, swollen at nodes, sometimes purplish, glabrous below, viscid above. Basal leaves oblanceolate to spathulate, 12–20 × 2–9 mm, glabrous, glaucous, scabrit to minutely ciliate at margins, acuminate, sometimes purplish. Cauline leaves gradually decrease in size towards inflorescence, 7–15 × 1–5 mm, oblanceolate to linear, glaucous, sometimes purplish. Inflorescence 1–2 flowered, viscid. Bracts linear to subulate, 3–5 mm, with shortly ciliate scarious margins, sometimes purplish. Pedicels 5–40 mm long, viscid, glabrous, glaucous, sometimes purplish. Calyx 20–30 mm long, 10-nerved, glabrous, purplish at anthesis, constricted around anthophore and dirty-white or straw coloured at fruiting time. Calyx teeth 2–3 mm long, triangular, scarious and ciliolate at margins. Petals 22–28 mm, exceeding calyx, purplish. Styles 3. Capsule oblong, included in calyx, 14–16 mm long. Anthophore glabrous, striate, 9–12 mm long.

Until now, this species has been known from two localities near to each other in Amanos Mts (Coode & Cullen 1967). As it has not been recorded since 1906, it was classified as "data deficient DD" according to the *Red Data Book of Turkish Plants* (Ekim & al. 2000). We determined it from a third location near the type locality in Amanos Mts. The population has approximately 100 individuals and its area of occupancy is estimated to be less than 10 km^2. However, there is not enough data about other populations. We think that this species should be classified as "critically endangered CR".

Heracleum pastinaca **Fenzl** (Fig. 1)

Small, slender alpine perennial, procumbent or ascending, caudices clothed by papery petiole bases. Stems

2–8 cm, less than 1 mm in diameter. Leaves mostly basal, ternate-pinnate, ± puberulous, lamina narrowly triangular-oblong, 2–3 cm, the lower pair of pinnae distant, petiolulate and trisect, the terminal pinna ternate with a petiolate terminal segment; ultimate segments obovate-orbicular, dentate to shortly incised. Rays 2–3, unequal, 1–2.5 cm. Bracts and bracteoles absent. Flowers white or pink, regular. Ovary subglabrous. Fruits obovate, 5–7 × 4.5–6 mm, very narrowly winged, glabrous; dorsal vittae filiform, *ca*. 1/2 as long as mericarp; commissural vittae 2(–4), short and divergent.

Heracleum pastinaca grows in Niğde and Adana district of Bolkar Mts, and in Berit Mt (Kahramanmaraş). It was classified as "Lower risk LR (cd)" according to the *Red Data Book of Turkish Plants* (Ekim & al. 2000). Since its observed population is rich, extent of occurrence is more than 20 000 km^2 and populations are distant from each other, we agree with Ekim & al. (2000).

Achillea monocephala **Boiss. & Balansa** (Fig. 1)

Perennial herbs. Stems 20–45 cm high, cylindrical, striate, densely tomentose when young, sparsely hairy to glabrescent later. Leaves filiform, tomentose to glabrescent, median cauline 6–22 × 0.6–1 mm, bent downwards, linear, pinnatisect, segments loosely to densely imbricate, indistinctly 3-lobed or undivided, lobes orbicular, spinulose-denticulate, 0.6–1 mm. Capitula 1–8, peduncles 2.5–12 cm long. Corymbs 2–5 cm wide. Involucre hemispherical to depressed, 4–6 × 4–7 mm, truncate, rarely rounded at base. Phyllaries ovate to lanceolate, subacute or acute, densely tomentose, outer carinate and brown margined, inner scarious margined. Ligules 8–14, shallowly 3-lobed at apex, sulphur yellow, 1–2.5 mm. Disc flowers 50–100.

Until now, this species has been known only from the type locality (Huber-Morath 1975). We determined it from a second locality on Bolkar Mts. The population includes at least 500 individuals. There are not estimated extreme negative fluctuations in number of individuals and extent of occurence since its distribution area is under protection. Therefore, we think that *A. monocephala* should be classified as "endangered (EN)". Our evaluation is the same as Ekim & al. (2000).

Prenanthes glareosa **(Schott & Kotschy ex Boiss.) C. Jeffrey** (Figs 1, 4)

Creeping stoloniferous herbs 5–15 cm. Leaves mostly basal, lyrate-pinnatisect, glaucous, glabrous, often

Fig. 4. *P. glareosa* in the wild.

pink-tinged, 1.5–7.5 × 1–3.5 cm, the lowest one distinctly petiolate, uppers with widely winged petioloid base, a broadly elliptic, ovate, reniform or suborbicular, cordate, sinuate-dentate, rounded sometimes shortly acuminate terminal lobe 1–3.5 × 1–3.5 cm, and usually a few much smaller lateral lobes; upper few, reduced. Teeth gland-tipped. Inflorescence scapose or not, usually branched, 1–5-capitulate, glabrous. Involucre 8–13 mm; phyllaries 8–10, 3-seriate, glabrous, outer 2–4 mm long, shorter than half length of the inner ones. Flowers pale blue or lilac, 1.2–1.4 cm, hairy about mouth. Achenes 6–7.2 mm, pale brown, columnar, slightly compressed, smooth, ribs narrow, rounded. Pappus 5.5–7.5 mm, minutely scabrous, white.

Prenanthes glareosa grows in different localities in Bolkar Mts (Jeffrey 1975). We determined it from another locality in its extent of occurrence. The population is rich according to our observation. It is now known from more than five locations and its extent of occurrence is estimated as 5000–20 000 km^2. In the light of these data, this species is graded as "Vulnerable VU". Our evaluation is the same as Ekim & al. (2000).

Acknowledgements. We would like to thank to Selçuk University (BAP–Project No: 06701107) for financial support during this study.

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Rare and endemic species from Van and Hakkari provinces, East Anatolia/Turkey and their threat categories

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Abstract. In this study, 151 endemic and rare taxa distributed in Van and Hakkari provinces in East Anatolia were determined. Threat categories of some endemic species were discussed and changed according to *Red Data Book of Turkish Plants* and to IUCN criteria.

Key words: endemic, threat categories, Turkey

Introduction

Van and Hakkari provinces are situated in East Anatolia. This area belonging to Irano–Turanian phytogeographic region has a high topography attaining over 2000 m and much mountainous. Many of the high mountains of East Anatolia are limestone. Mt Cilo (4168 m) in Hakkari is largely dolomitic, and on the north side of this mountain a glacier still exists. The young volcanic mountains especially erupted during Quaternary. Mt Süphan (4058 m) and Mt Nemrut (3050 m) near Van Lake and Mt Tendürek (3533 m) in north of Van Lake are high mountains of Quaternary volcanic formation (Davis 1965). In these mountains, above 3000 m, snow lies throughout the summer. Winter temperatures are quite low in the highlands of East Anatolia (Davis 1965).

The mountains and valleys in Van and Hakkari provinces are very rich in terms of endemics. There are 12 "Important Plant Areas" in these provinces. These are: Artos, Buzul and Ikiyaka, Erek, Ispiriz, Pirreşit, Süphan and Tendürek mountains, Çatak, Mukus, Şemdinli, Zapsuyu valleys and Yüksekova (Özhatay & al. 2003).

Mt Artos is situated in south side of Gevaş (Van), 3537 m altitude. 48 endemic and rare taxa are distributed in this area.

Buzul and Ikiyaka are situated in southeast side of Hakkari, 4168 m altitude. At least 50 endemic taxa are distributed in this area.

Çatak valley is situated in south of lake Van, between Gürpınar (Van) and Pervari (Siirt). 1195 taxa were found in this area, 95 of them are endemic.

Mt Erek is situated in southeast of lake Van, 3250 m altitude. 780 taxa were found in this area, 118 of them are endemic.

Mt Ispiriz is situated in east of Van. 50 endemic and rare taxa are distributed in this area. 9 of them are restricted to this area. They are very narrow endemics which are known only from few localities.

Mukus valley is situated in south of lake Van and between Gevaş (Van) and Pervari (Siirt). 30 endemic taxa are distributed in this area.

Mt Pirreşit is situated in northeast of lake Van, 3110 m altitude. 828 taxa were found in this area, 75 of them are endemic.

Mt Süphan is situated in north of lake Van, 4058 m altitude. 802 taxa were found in this area, 57 of them are endemic.

Şemdinli valley contains slopes of Şemdinli and Hacıbey rivers (Hakkari). It is known that 23 endemic taxa were found in this area.

Mt Tendürek is situated in northeast of lake Van, 3533 m altitude. 17 endemic and rare taxa were found in this area.

Yüksekova is situated in southeast of Turkey. It contains marshy areas and pastures around of Yüksekova (Hakkari). 44 rare and endemic taxa were found in this area.

Zapsuyu valley (Hakkari) is situated in southeast of Turkey. 40 endemic and rare taxa were found in this area (Özhatay & al. 2003).

Material and metods

During the period 2001–2004 the authors collected numerous plant specimens from Van and Hakkari provinces, also some endemic and rare plants were photographed. Collected plants were determined according to *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000). Threat categories were defined according to *Red Data Book of Turkish Plants* (Ekim & al. 2000) and to IUCN (2001). All herbarium specimens collected from these provinces were deposited at GAZI and VANF.

Results and discussion

In this study, 151 endemic and rare taxa were determined (see Appendix 1). 101 of 151 are endemics and the others are rare plants for these provinces.

According to our field observations, threat categories of some species are discussed. *Cephalorrhynchus rechingerianus* Tuisl and *Tanacetum argyrophyllum* (K. Koch) Tzvelev var. *polycephalum* (Sch. Bip.) Grierson are considered as DD (Data Deficient) categories in *Red Data Book of Turkish Plants* (Ekim & al. 2000). These two taxa were collected by the authors from Van and Hakkari provinces.

Cephalorrhynchus rechingerianus was collected in nine localities (criterion B2a). It is common in these provinces. It grows on rocky slopes, at 1800–2600 m alt. Its threat category should be VU (Vulnerable) (IUCN 2001).

Tanacetum argyrophyllum var. *polycephalum* grows on stream sides, rocky slopes and screes. It is poorly known from only one locality, Çatak valley (Van) (criterion B2a). Mature individuals within the population were fewer than 50 (criterion D) (IUCN 2001). Threat category of this taxon should be CR (Critically Endangered).

Astragalus chaldiranicus Kit Tan & Sorger is known only from one locality in Van province and is restricted to this area. It grows on wet meadows and gypsaceous hills at 2200 m (Davis & al. 1988). This species is locally abundant at Çaldıran, but it has been reaping on flowering time, thus it is under threat. Its threat category is VU (Vulnerable) (Ekim & al. 2000).

Astragalus davisii D.F. Chamb. & Matthews grows in mountain pastures and scrubs at 1900–3200 m. This species is very beautiful and distinct. Features of fruits are unknown in *Flora of Turkey*. We collected the fruiting and flowering materials in Van province and studied these materials. Legumes are oblong-ovate, densely white tomentose. This endemic species is quite common and abundant in Van province but it is restricted to this area. Its threat category is NT (Near Threatened) (Ekim & al. 2000).

Centaurea vanensis Wagenitz is known only from Van in Turkey and also in Iran. It grows on stony slopes and steppes, at 1750–2750 m. IUCN category is not suggested to *C. vanensis* in *Red Data Book of Turkish Plants*. According to our observations, this species is found in few localities in this province. Its threat category should be stated as VU (Vulnerable) (IUCN 2001).

Rosa pisiformis (D.H. Christ) Sosn. is distributed in a few provinces in East and Northeast Anatolia. This endemic species has been used as an ornamental plant in gardens in Hoşap (Van province). Also its fruits have been used for decoration by Hoşap people. It is quite threatened in the wild in this area.

Salvia kronenburgii Rech. f. is known only from several gatherings. It is restricted to Van province. It grows on shale hills, slopes at 1850–2500 m. Individuals are healthy and abundant at their habitats. This endemic species is quite attractive with its whitish corollas and pale green broadly campanulate calyces. Its threat category is VU (Vulnerable) (Ekim & al. 2000). It generally grows at the main roadsides therefore its habitat can be threatened by road restorations.

Fritillaria minima Rix is restricted only to Van province. It grows in snow patches on limestone. There is no threat for this endemic species. It grows at high altitudes, 2650–3000 m; its threat category is VU (Vulnerable) (Ekim & al. 2000).

The list of threat categories of endemic and rare species distributed in Van and Hakkari provinces is given at the end of this paper (Appendix 1).

Appendix 1.

Apiaceae (=*Umbelliferae*)

1- Eryngium bornmuelleri Nábělek. . . . . ----- ***"NT"***
2- E. wanaturi Woron. . . . . ----- ***"VU"***
3- Ferulago angulata Boiss. . . . . ----- ***"VU"***
4- F. bernardii L.. . . . . ----- ***"VU"***
5- F. platycarpa Boiss. & Balansa . . . . . END. ***"LC"***
6- Grammosciadium cornutum (Nábělek) Towns. . . . . ----- ***"VU"***
7- Heracleum crenatifolium Boiss. . . . . END. ***"NT"***
8- Laserpitium carduchorum Hedge & Lamond . . . . . END. ***"NT"***
9- Malabaila lasiocarpa Boiss. . . . . END. ***"LC"***
10- Pimpinella aromatica M. Bieb. . . . . ----- ***"VU"***
11- Sium sisarum L. var. ***lancifolium*** (M. Bieb.) Thell. . . . ----- ***"VU"***
12- Trigonosciadium viscidulum Boiss. & Hausskn. . . . . END. ***"VU"***

Asteraceae (=*Compositae*)

13- Achillea cappadocica Hausskn. & Bornm. . . . . END. ***"LC"***
14- A. schischkinii Sosn. . . . . END. ***"LC"***
15- Centaurea gigantea Sch. Bip. ex Boiss. . . . . ----- ***"VU"***
16- C. hakkariensis Wagenitz . . . . . END. ***"VU"***
17- C. handeli Wagenitz. . . . . ----- ***"VU"***
18- C. karduchorum Boiss.. . . . . END. ***"NT"***
19- C. nemecii Nábělek . . . . . ----- ***"VU"***
20- C. persica Boiss.. . . . . ----- ***"VU"***
21- C. saligna (K. Koch) Wagenitz . . . . . END. ***"LC"***
22- C. vanensis Wagenitz . . . . . ----- ***"VU"***
23- Cephalorrhynchus rechingeranus Tuisl . . . . . ----- ***"VU"***
24- Cousinia bicolor Freyn & Sint.. . . . . END. ***"LC"***
25- C. boissieri Buhse . . . . . ----- ***"VU"***
26- C. grandis C.A. Mey. . . . . ----- ***"VU"***
27- C. vanensis Hub.-Mor. . . . . END. ***"LC"***
28- Crepis bupleurifolia (Boiss.) Freyn & Sint. . . . . END. ***"LC"***
29- C. hakkarica Lamond . . . . . END. ***"EN"***
30- Echinops pungens Trautv. var. ***adenoclados*** Hedge . . . END. ***"NT"***
31- Inula helenium L. subsp. ***vanensis*** Grierson . . . . . END. ***"NT"***
32- I. inuloides (Fenzl) Grierson . . . . . ----- ***"VU"***
33- Jurinea cataonica Boiss. & Hausskn. . . . . END. ***"LC"***
34- Leontodon oxylepis Boiss. & Heldr. var. ***divaricatus*** (Boiss.) Kupicha. . . . . END. ***"LC"***
35- Scorzonera davisii Lipsch.. . . . . END. ***"VU"***
36- S. semicana DC.. . . . . END.? ***"LC"***
37- Senecio cilicius Boiss.. . . . . END. ***"LC"***
38- S. eriospermus DC. var. ***crambeifolius*** Boiss. . . . . END. ***"LC"***
39- S. paucilobus DC. . . . . ----- ***"VU"***
40- Tanacetum argyrophyllum (K. Koch) Tzvelev var. ***polycephalum*** (Sch. Bip.) Grierson . . . . . ----- ***"CR"***
41- T. cadmeum (Boiss.) Heywood subsp. ***orientale*** Grierson . . . . . END. ***"LC"***
42- T. nitens (Boiss. & Noë) Grierson . . . . . END. ***"LC"***
43- T. zahlbruckneri (Nábělek) Grierson . . . . . END. ***"LC"***
44- Taraxacum fedtschenkoi Hand.-Mazz.. . . . . ----- ***"VU"***
45- Tragopogon aureus Boiss. . . . . END. ***"LC"***
46- Tripleurospermum callosum (Boiss. & Heldr.) E. Hossain . . END. ***"LC"***

Boraginaceae

47- Alkanna froedinii Rech. f. . . . . END. ***"LC"***
48- Onosma bracteosum Hausskn. & Bornm. . . . . END. ***"LC"***
49- Paracaryum cristatum (Schreb.) Boiss. subsp. ***cristatum*** END. ***"LC"***
50- Rochelia disperma (L. f.) K. Koch var. ***microcalycina*** (Bornm.) J.R. Edm. . . . . END. ***"LC"***

Brassicaceae (=*Cruciferae*)

51- Aethionema froedinii Rech. f.. . . . . ----- ***"LC"***
52- Alyssum filiforme Nyár. . . . . END. ***"LC"***
53- A. pateri Nyár. subsp. ***pateri*** . . . . . END. ***"LC"***
54- A. pateri Nyár. subsp. ***prostratum*** (Nyár.) T.R. Dudley . . END. ***"LC"***
55- Aubrieta parviflora Boiss.. . . . . ----- ***"VU"***
56- Draba rosularis Boiss. . . . . END. ***"LC"***
57- Erysimum sintenisianum Bornm. . . . . END. ***"LC"***
58- Isatis aucheri Boiss. . END. ***"LC"***
59- I. candolleana Boiss. . . . . END. ***"LC"***
60- I. cappadocica Desv. subsp. ***macrocarpa*** (Jaub. & Spach) P.H. Davis. . . . . ----- ***"VU"***

Campanulaceae

61- Campanula coriacea P.H. Davis. . . . . END. ***"LC"***

Caryophyllaceae

62- Cerastium kotschyi Boiss. . . . . ----- ***"VU"***
63- Dianthus lactiflorus Fenzl. . . . . END. ***"NT"***
64- D. masmenaeus Boiss. var. ***glabrescens*** Boiss.. . . . . END. ***"LC"***
65- D. recognitus Schischk.. . . . . END. ***"NT"***
66- Gypsophila bitlisensis Barkoudah . . . . . END. ***"NT"***
67- Silene capitellata Boiss. . . . . END. ***"LC"***
68- S. sclerophylla Chowdhuri . . . . . END. ***"LC"***

Celastraceae

69- Euonymus latifolius (L.) Mill. subsp. ***cauconis*** Coode & Cullen . . . . . END. ***"NT"***

Dipsacaceae

70- Cephalaria speciosa Boiss. & Kotschy . . . . . END. ***"LC"***
71- Pterocephalus kurdicus Vatke var. ***kurdicus***. . . . . ----- ***"VU"***
72- P. kurdicus Vatke var. ***viscosissimus*** Bornm.. . . . . ----- ***"VU"***

Euphorbiaceae

73- Euphorbia cardiophylla Boiss. & Heldr. . . . . END. ***"LC"***
74- E. sanasunitensis Hand.-Mazz. . . . . END. ***"NT"***

Fabaceae (=*Leguminosae*)

75- Astragalus achundovii Grossh. . . . . ----- ***"VU"***
76- A. brachycarpus M. Bieb.. . . . . ----- ***"VU"***
77- A. campylosema Boiss. subsp. ***campylosema*** . . . . . END. ***"LC"***
78- A. chaldiranicus Kit Tan & Sorger . . . . . END. ***"VU"***
79- A. compactus Lam. . . . . END. ***"LC"***
80- A. davisii D.F. Chamb. & Matthews . . . . . END. ***"NT"***
81- A. pinetorum Boiss.. . . . . ----- ***"LC"***
82- A. pulchellus Boiss. . . . . ----- ***"VU"***
83- A. rechingeri Širj. . . . . END. ***"NT"***
84- A. subsecundus Boiss. & Hohen.. . . . . ----- ***"VU"***
85- Hedysarum vanense Hedge & Hub.-Mor. . . . . END. ***"VU"***
86- Lathyrus brachypterus Čelak. var. ***haussknetchtii*** (Širj.) P.H. Davis . . . . . END. ***"LC"***
87- Onobrychis cappadocica Boiss.. . . . . END. ***"LC"***
89- O. sulphurea Boiss. & Balansa var. ***pallida*** (Boiss. & Kotschy) Hedge . . . . . END. ***"LC"***

Appendix 1. Continuation.

88- O. sulphurea Boiss. & Balansa var. ***sulphurea*** . . . . . END. ***"VU"***
90- O. sulphurea Boiss. & Balansa var. ***vanensis*** Hedge . . . END. ***"NT"***
91- Oxytropis kotschyana Boiss. & Hohen.. . . . . ----- ***"VU"***
92- Trifolium longidentatum Nábělek . . . . . END. ***"NT"***
93- Trigonella plicata (Boiss. & Balansa) Boiss. . . . . END. ***"LC"***

Fagaceae

94- Quercus petraea (Matt.) Liebl.
subsp. ***pinnatiloba*** (K. Koch) Menitsky . . . . . END. ***"LC"***

***Hypericaceae* (=*Guttiferae*)**

95- Hypericum pseudolaeve Robson . . . . . END. ***"LC"***

***Gramineae* (=*Poaceae*)**

96- Stipa kurdistanica Bor . . . . . ----- ***"VU"***

Illecebraceae

97- Paronychia kurdica Boiss.
subsp. ***haussknechtii*** Chaudhri . . . . . END. ***"LC"***

Iridaceae

98- Iris aucheri (Boiss.) Stapf . . . . . ----- ***"VU"***
99- I. iberica Hoffm. subsp. ***lycotis*** (Woronow) Takht. . . . ----- ***"VU"***
100- I. sari Schott ex Baker . . . . . END. ***"LC"***

***Lamiaceae* (=*Labiatae*)**

101- Cyclotrichium glabrescens
(Boiss. & Kotschy ex Rech. f.) Leblebici . . . . . END. ***"NT"***
102- C. stamineum (Boiss. & Hohen.) Manden. & Scheng. . . . ----- ***"VU"***
103- Marrubium parviflorum Fisch. & C.A. Mey.
subsp. ***oligodon*** (Boiss.) Seybold . . . . . END. ***"LC"***
104- Micromeria cremnophila Boiss. & Heldr.
subsp. ***anatolica*** P.H. Davis . . . . . END. ***"LC"***
105- Phlomis armeniaca Willd. . . . . END. ***"LC"***
106- Salvia dichroantha Stapf . . . . . END. ***"LC"***
107- S. kronenburgii Rech. f.. . . . . END. ***"VU"***
108- Stachys iberica M. Bieb. subsp. ***iberica***
var. ***densipilosa*** Bhattacharjee . . . . . END. ***"LC"***
109- Thymus fedtschenkoi Ronniger
var. ***handelii*** (Ronniger) Jalas . . . . . END. ***"NT"***
110- T. pubescens Boiss. & Kotschy ex Čelak.
var. ***cratericola*** Jalas . . . . . END. ***"NT"***

Liliaceae

111- Allium pseudoampeloprasum Miscz. ex Grossh. . . . END. ***"NT"***
112- A. shatakiense Rech. f. . . . . END. ***"NT"***
113- A. stearnianum M. Koyuncu, Özhatay & Kollmann
subsp. ***vanense*** Kollmann & M. Koyuncu . . . . . END ***"NT"***
114- A. tchihatschewii Boiss.. . . . . END. ***"LC"***
115- Bellevalia fominii Woronow . . . . . ----- ***"VU"***
116- B. longistyla (Miscz.) Grossh. . . . . ----- ***"VU"***
117- B. rixii Wendelbo . . . . . END. ***"EN"***
118- Fritillaria imperialis L.. . . . . ----- ***"VU"***
119- F. michailovskyi Fomin . . . . . END. ***"NT"***
120- F. minima Rix . . . . . END. ***"VU"***
121- F. zagrica Stapf. . . . . ----- ***"VU"***
122- Gagea tenera Pascher . . . . . ----- ***"VU"***
123- Ornithogalum alpigenum Stapf . . . . . END. ***"NT"***

Linaceae

124- Linum mucrotanum Bertol.
subsp. ***orientale*** (Boiss.) P.H. Davis . . . . . ----- ***"VU"***

Lythraceae

125- Lythrum anatolicum Leblebici & Seçmen . . . . . END. ***"CR"***

Malvaceae

126- Alcea apterocarpa (Fenzl) Boiss. . . . . END. ***"LC"***

Paeoniaceae

127- Paeonia kesrouanensis Thiébaut . . . . . ----- ***"VU"***

Papaveraceae

128- Papaver clavatum Boiss. & Haussk. ex Boiss.. . . . . END. ***"LC"***
129- P. curviscapum Nábělek . . . . . ----- ***"VU"***

Plumbaginaceae

130- Acantholimon bracteatum (Girard) Boiss.
var. ***capitatum*** (Sosn.) Bokhari. . . . . END. ***"LC"***
131- Limonium vanense Kit Tan & Sorger . . . . . END. ***"VU"***

Polygonaceae

132- Polygonum rottboellioides Jaub. & Spach . . . . . ----- ***"VU"***

Primulaceae

133- Dionysia bornmuelleri (Pax) Melch. . . . . ----- ***"VU"***
134- Primula davisii W.W. Sm. . . . . END. ***"VU"***

Ranunculaceae

135- Delphinium carduchorum Chowdhuri & P.H. Davis . . END. ***"NT"***
136- D. cyphoplectrum Boiss.
var. ***vanense*** (Rech. f.) P.H. Davis. . . . . END. ***"NT"***
137- D. macrostachyum Boiss. ex Huth. . . . . ----- ***"VU"***
138- Ranunculus fenzlii Boiss.. . . . . END. ***"LC"***
139- Ranunculus munzurensis Erik & Yild. . . . . END. ***"NT"***
140- Thalictrum sultanabadense Stapf. . . . . ----- ***"VU"***

Rhamnaceae

141- Frangula alnus Mill.
subsp. ***pontica*** (Boiss.) Davis & Yalt.. . . . . END. ***"LC"***

Rosaceae

142- Cerasus mahaleb (L.) Mill. var. ***alpina*** Browicz . . . . END. ***"VU"***
143- Potentilla anatolica Peşmen . . . . . END. ***"LC"***
144- Rosa pisiformis (D.H. Christ) Sosn. . . . . END. ***"NT"***

Rubiaceae

145- Galium uliginosum L.. . . . . ----- ***"VU"***

Rutaceae

146- Haplophyllum buxbaumii (Poir.) G.Don
subsp. ***mesopotamicum*** (Boiss.) C.C. Towns . . . . . ----- ***"VU"***
147- H. schelkovnikovii Grossh.. . . . . ----- ***"VU"***

Scrophulariaceae

148- Rhynchocorys kurdica Nábělek . . . . . END. ***"NT"***
149- Scrophularia libanotica Boiss.
var. ***urartuënsis*** R.R. Mill . . . . . END. ***"LC"***
150- Verbascum kurdicum Hub.-Mor. . . . . END. ***"LC"***
151- Veronica orientalis Mill.
subsp. ***carduchorum*** P.H. Davis ex M.A. Fisch.. . . . . END. ***"NT"***

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Halophytic endemics of Turkey

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Abstract. According to the plant diversity studies there are 406 halophytic taxa spreading in coastal and inland halophytic areas of Turkey. 65 taxa are endemic to Turkey; 63 of them are inland, 1 is coastal and 1 occurs in both inland and coastal saline environments. Phytogeographically, 54 are Irano–Turanian, 1 is Mediterranean, 1 is Euxine, 2 are East Mediterranean and 7 are imperfectly known. *Asteraceae* (with 17 endemics) and *Chenopodiaceae* (with 10 endemics) have the highest number of halophytic endemic taxa. According to IUCN and *Red Data Book of Turkish Plants* the status of halophytic endemics is as follows: EN 5, CR 8, LR (cd) 4, LR (lc) 26, LR (nt) 3, VU 17, and there is no any risk of threat for 2 of the taxa.

Key words: endemic, halophytic, Turkey

Introduction

There are a number of different halophyte definitions. The most accepted one is the ecological definition favoured by Flowers (1975), Flowers & al. (1986) and Jennings (1968, 1976). In this definition halophytes are explained as plants that complete their life cycle in saline environments. There are generally accepted limitations to regard a habitat as saline. Other definitions depend on the amount of salt present in soil. For example according to Chapman (1974) species capable of tolerating 0.5 % or more NaCl are regarded as halophytes. According to these definitions, the studies till today had been investigated and the endemic plant taxa that are accepted as halophytes are listed in Table 1.

There are many salt affected soils in Turkey, but the major halophytic areas are as follows: Tuz Lake Basin, Kızılırmak Delta, Çukurova (Seyhan, Ceyhan Deltas), Göksu Delta, Gediz Delta, Iğdır Plain, Acıgöl and many lagoons distributed in the Aegean and Mediterranean coasts.

Being in the North Hemisphere Temperate Zone, Turkey has an unusual biological structure, where we can observe the situation simply by observing its ecological diversity and high endemism ratio that forms the biological diversity. First of all on the territory of Turkey three main phytosociological regions meet: Euro–Siberian (around Black Sea Coast and surrounding mountains), Irano–Turanian (Central, East and Southeast Anatolia) and Mediterranean (Mediterranean coast and mountains) (Fig. 1).

In addition to this, topographic diversity of the country (altitude varies from 0 m to 5137 m), geological and geomorphologic structure, climatic differences between the regions (continental, oceanic and Mediterranean climates) (Akman & Ketenoğlu 1986) maintain further ecological diversity. As a result of all these factors Turkey is one of the leading countries for known plant endemism with about 33 %.

The richest family in endemism in Turkey is *Asteraceae* having a total of 430 species, 40 % of which are endemic. Of the 400 species of *Fabaceae*, 41 % are endemic, and of the 306 *Lamiaceae* species 57 % are endemic.

Material and methods

Flora of Turkey and the East Aegean Islands (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000) has some information about the ecological requirements of nearly all the taxa distributed in Turkey. Also there are some works especially on the halophytes in Turkey: Wagenitz (1959), Beyce (1960), Birand (1961), Zeybek (1969), Yurdakulol (1974), Zeybek & al. (1976), Tatlı & İstanbulluoğlu (1987), Gehu & Uslu (1989), Yurdakulol & Ercoşkun (1990), Öztürk & al. (1995), Yurdakulol & al. (1996), Güvensen & Öztürk (2003), Aydoğdu & al. (2004), Çolak & Sorger (2004), Hamzaoğlu & al.

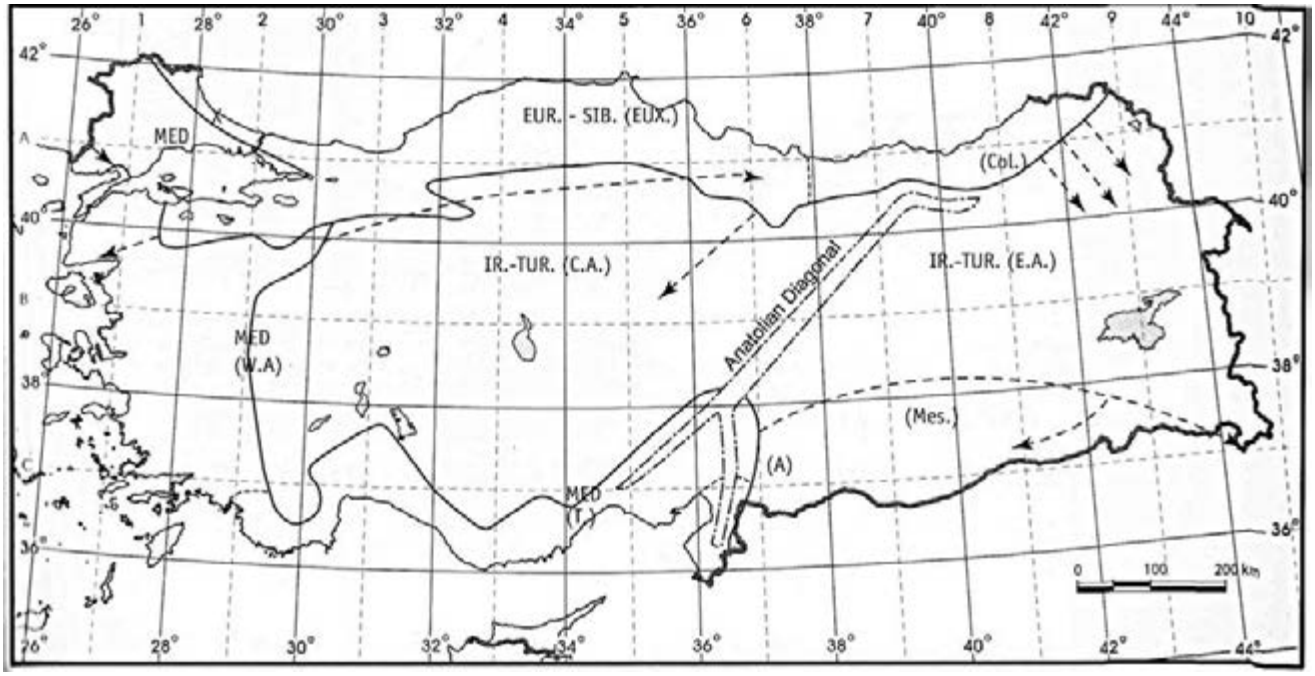

Fig. 1. Map of phytogeographical regions in Turkey (Davis 1971).

(2005), Yaprak (2006). And there are some new halophytic taxa that have been published recently by Freitag & al. (1999), Freitag & Duman (2000), Dönmez & Mutlu (2004), and Vural & al. (2006). Herewith we reviewed the known halophytic endemic plants in Turkey using the literature mentioned above. We also determined their life form, chorotype, ecotype and IUCN threat categories according to Ekim & al. (2000).

Results

As a result of this review, it was found that 406 halophytic taxa are distributed in Turkey and 65 of them are endemic. Endemic halophytic taxa that were determined are listed in Table 1, with comments on their life form, chorotype, ecotype and IUCN threat categories (Ekim & al. 2000). The threat categories of the endemic halophytes of Turkey are as follows: LR(cd) 4, LR(lc) 26, LR(nt) 3, VU 17, CR 8, and EN 5. There are 2 taxa which do not have any risk of threat. Almost all of the endemic halophytes of Turkey belong to the inland saline areas. There is only 1 taxon from coastal areas, and 1 taxon having both coastal and inland distribution. Phytogeographically most of the taxa belong to Irano–Turanian phytogeographical region which covers most of the central part of Turkey. The phytogeographical regions and the number of taxa they have are as follows: Irano–Turanian 54, East Mediterranean 2, Mediterranean 1, and Euxine 1. There are also 7 taxa that do not belong to any of the phytogeographical regions or their distribution is imperfectly known. The families that are rich in endemic halophytic taxa and the number of taxa belonging to these families are as follows: *Asteraceae* 17, *Chenopodiaceae* 10, *Plumbaginaceae* 6, and *Liliaceae* 6 (Fig. 2).

Discussion

Most of the inland saline habitats in Turkey are distributed in Central Anatolia, which is a closed basin, surrounded by mountains, especially Tuz Lake basin. Being adapted microhabitats make dispersal difficult

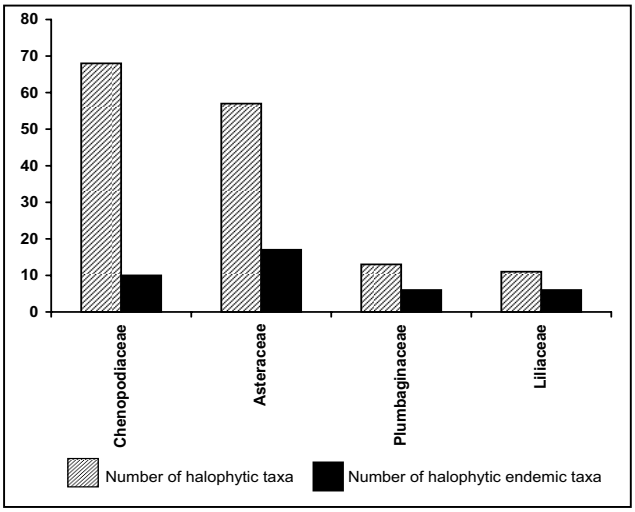

Fig. 2. The families rich in halophytes and halophytic endemics.

for endemics and this makes the endemism ratio of the inland halophytes higher.

Coastal saline habitats are not as diverse as inland saline habitats because the flora and vegetation structure of coastal saline habitats are more homogeneous than their inland representatives. Floating experiments show us that most of the coastal halophytes can disperse by sea. Because of those properties, most of the endemics belong to the inland saline environments.

Table 1. List of the endemic halophytic plants in Turkey.

Families	Taxa	IUCNTC	Inland	Coastal	Chorotype
1	2	3	4	5	6
Apiaceae	*Bupleurum heldeichii* Boiss. & Balansa	LR(lc)	X		Ir–Tur
	B. sulphureum Boiss. & Balansa	LR(lc)	X		Ir–Tur
	B. turcicum Snogerup	LR(nt)	X		Ir–Tur
Asteraceae	*Achillea aleppica* DC. subsp. *zederbaueri* (Hayek) Hub.-Mor.	LR(lc)	X		Ir–Tur
	A. goniocephala Boiss. & Balansa	LR(lc)	X		Ir–Tur
	A. sieheana Stapf	VU	X		Ir–Tur
	Centaurea halophila Hub.-Mor.	EN	X		Ir–Tur
	C. tuzgoluensis Aytaç & H. Duman	CR	X		Ir–Tur
	Cousinia birandiana Hub.-Mor.	LR(lc)	X		Ir–Tur
	C. caesarea Boiss. & Balansa		X		Ir–Tur
	C. halysensis Hub.-Mor.		X		Ir–Tur
	C. humilis Boiss.	CR	X		Ir–Tur
	C. iconica Hub.-Mor.	LR(cd)	X		Ir–Tur
	Onopordum anatolicum (Boiss.) Eig	LR(lc)	X		Ir–Tur
	O. davisii Rech. f.	LR(nt)	X		Ir–Tur
	O. polycephalum Boiss.	LR(lc)	X		Ir–Tur
	Scorzonera hieraciifolia Hayek	LR(lc)	X		Ir–Tur
	Senecio salsuginea H. Duman & Vural	CR	X		Ir–Tur
	Taraxacum farinosum Hausskn. & Bornm.	LR(lc)	X		Ir–Tur
	T. mirabile Wagenitz	VU	X		–
Boraginaceae	*Anchusa leptophylla* Roem. & Schult. subsp. *tomentosa* (Boiss.) Chamb.	LR(lc)	X		Ir–Tur
Brassicaceae	*Alyssum macropodum* Boiss. & Balansa var. *macropodum*	LR(lc)	X		Ir–Tur
	A. pateri Nyár. subsp. *pateri*	LR(lc)	X		Ir–Tur
	Lepidium caespitosum Desv.	VU	X		Ir–Tur
Caryophyllaceae	*Silene salsuginea* Hub.-Mor.	EN	X		Ir–Tur
Chenopodiaceae	*Cyathobasis fruticulosa* (Bunge) Aellen	VU	X		–
	Kalidium wagenitzii (Aellen) Freitag & G. Kadereit	EN	X		–
	Microcnemum coralloides (Loscos & Pardo) Font Quer subsp. *anatolicum* Wagenitz	VU	X		Ir–Tur
	Petrosimonia nigdeensis Aellen	LR(cd)	X		–
	Salsola anatolica Aellen	LR(lc)	X		Ir–Tur
	S. cyrenaica (Maire & Weiller) Brullo subsp. *antalyensis* Freitag & Duman	CR		X	Med
	S. grandis Freitag, Vural & N. Adıgüzel	CR	X		Ir–Tur
	S. stenoptera Wagenitz	LR(lc)	X		Ir–Tur
	Suaeda cucullata Aellen	VU	X		–
	S. prostrata Pall. subsp. *anatolica* Aellen	VU	X		–
Fabaceae	*Astragalus karamasicus* Boiss. & Balansa	LR(lc)	X		Ir–Tur
	A. micropterus Fisch.	LR(lc)	X		Ir–Tur
	Sphaerophysa kotschyana Boiss.	LR(cd)	X		Ir–Tur
	Trigonella isthmocarpa Boiss. & Balansa	VU	X		Ir–Tur
Guttiferae	*Hypericum salsugineum* N. Robson & Hub.-Mor.	VU	X		Ir–Tur
Iridaceae	*Gladiolus halophilus* Boiss. & Heldr.	VU	X		Ir–Tur

Table 1. Continuation.

1	2	3	4	5	6
Lamiaceae	*Salvia halophila* Hedge	VU	X		Ir–Tur
	Thymus leucostomus Hausskn. & Velen. var. *leucostomus*	LR(nt)	X		Ir–Tur
	Wiedemannia orientalis Fısch. & Mey.	LR(lc)	X		Ir–Tur
Liliaceae	*Allium nevsehirense* M. Koyuncu & Kollmann	LR(lc)	X		Ir–Tur
	A. scabriflorum Boiss.	LR(lc)	X		Ir–Tur
	A. sieheanum Hausskn. ex Kollmann	LR(lc)	X		Ir–Tur
	A. stylosum O. Schwarz	LR(lc)	X		E.Med
	A. vuralii Kit Tan	VU	X		Ir–Tur
	Asparagus lycaonicus P.H. Davis	EN	X		Ir–Tur
Plumbaginaceae	*Acantholimon halophilum* Bokhari	VU	X		Ir–Tur
	Limonium anatolicum Hedge	VU	X		Ir–Tur
	L. effesum (Boiss.) Kuntze	VU	X	X	E.Med
	L. iconicum (Boiss. & Heldr.) Kuntze	LR(lc)	X		Ir–Tur
	L. lilacinum (Boiss. & Balansa) Wagenitz	LR(lc)	X		Ir–Tur
	L. tamaticoides Bokhari	EN	X		Ir–Tur
Poaceae	*Festuca rubra* L. subsp. *pseudorivularis* Markgr.-Dann.	CR	X		Eux
	Puccinellia bulbosa (Grossh.) Grossh. subsp. *caesarea* Kit Tan	CR	X		–
Ranunculaceae	*Consolida glandulosa* (Boiss. & A. Huet) Bornm.	LR(lc)	X		Ir–Tur
	C. stenocarpa (P.H. Davis & Hossain) P.H. Davıs	LR(lc)	X		Ir–Tur
	Delphinium venulosum Boiss.	LR(lc)	X		Ir–Tur
	Nigella turcica Dönmez & Mutlu	CR	X		Ir–Tur
Scrophulariaceae	*Verbascum campestre* Boiss. & Heldr.	LR(cd)	X		Ir–Tur
	Verbascum helianthemoides Hub.-Mor.	VU	X		Ir–Tur
	Verbascum pyroliforme (Boiss. & Heldr.) Kuntze	VU	X		Ir–Tur

Abbreviations: IUCNTC – IUCN Threat Categories: LR(cd) – Lower risk (conservation dependent); LR(lc) – Lower Risk (least concern); LR(nt) – Lower Risk (near threatened); VU – Vulnerable; CR – Critically Endangared; EN – Endangered; Chorotypes: Ir–Tur – Irano–Turanian; Med – Mediterranean; E.Med – East Mediterranean; Eux – Euxine.

Acknowledgements. The authors are grateful to Şenol Alan, Prof. Dr. Osman Ketenoğlu, S. Tuğrul Körüklü, Prof. Dr. Ender Yurdakulol and Ministry of Agriculture for their kind help.

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Natural hybrids of *Rumex* subgenus *Rumex* (*Polygonaceae*) in Bulgaria

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Abstract. On the basis of the available literature, herbarium specimens and personal accessions, the information about the distribution of the known natural hybrids of *Rumex* subg. *Rumex* in the country (*R. obtusifolius* × *R. patientia*, *R. palustris* × *R. stenophyllus*, *R. conglomeratus* × *R. sanguineus* and *R. crispus* × *R. patientia*) was updated. The present study confirmed the distribution of *R. crispus* × *R. obtusifolius* for the flora of Bulgaria. New natural hybrids *R. conglomeratus* × *R. crispus*, *R. confertus* × *R. obtusifolius*, *R. cristatus* × *R. obtusifolius*, *R. palustris* × *R. obtusifolius* and *R. patientia* × *R. pulcher* were established for the first time in Bulgaria and their localities were reported. The aim of this study was to review the known chorological data about natural hybrids of *R.* subg. *Rumex* in Bulgaria.

Key words: chorology, maps, natural hybrids, *Rumex* subgenus *Rumex*

Introduction

In the recent classification, the genus *Rumex* L., which contains about 250 species, is subdivided into three subgenera. The largest subgenus *Rumex* includes about 150 species (Datta 1952), among which are some of the most widely spread plants all around the world (Rechinger 1949). The natural hybridization in *R.* subg. *Rumex* is a process revealed in high frequency in this group of vascular plants. This is confirmed by the fact that, in historical aspect, the description of new hybrid combinations is a process, continuing till now. Practically, all possible hybrid combinations of the taxa in the subgenus have been described. A great part of the hybrids are described using herbarium materials (very often single sheets). In theoretical aspect, the recognition of hybrid forms has a significant importance for the differentiation of the taxa, due to the fact that *Rumex* hybrids are very often regarded as species by some authors (Rechinger 1932).

The natural hybridization in *R.* subg. *Rumex* in the Bulgarian flora has not been purposefully researched so far. The data about the distribution of natural hybrids on the territory of Bulgaria is outdated and incomplete. The first hybrids for the country were reported by Urumov (1901), Širjaev (1922), Stojanov & Stefanov (1924), Stojanoff (1932), Rechinger (1933), and later on by Anchev (1984). In most cases, the data about the interspecific hybrids are rather generalized and incomplete – separated localities were reported more than 50 years ago, and never confirmed afterwards or there were no herbarium materials of Bulgarian origin deposited. Some of the hybrids were discovered in the last few decades (Panov 1987). The hybrids of *R.* subg. *Rumex*, described and specified for the Bulgarian flora, have not provided a sufficiently complete idea about the amount and spreading of the natural hybrids in the country. The lack of systematic researches in the group is slowing down the knowledge about *R.* subg. *Rumex* and particularly about its hybrids in Bulgaria. Perhaps due to this reason natural hybrids are often identified and deposited as species in the Bulgarian herbaria.

The present study was based on literature data, herbarium samples and field studies and its purpose was to update and present the available information regarding the spreading of some natural hybrids of *R.* subg. *Rumex* in Bulgaria.

Material and methods

The new material used in this study was collected in 2003–2005 from natural habitats in the country. The collections were based on the transect method. The samples of the hybrid forms were deposited in herbari-

um SOA. The collections from SOM, SOA and SO were revised. For some critical taxa with no existing herbarium specimens in the national herbaria, voucher specimens from the herbaria of Vienna University (WU) and Vienna Natural History Museum (W) were used for comparison. The chorological information has been mapped in a relational data base by the software program "dSOA" (Stoyanov 2003). The data were presented according to Kozhuharov & al. (1983). The maps were divided in the floristic regions and subregions as accepted in the multivolume edition *Flora Reipublicae Popularis Bulgaricae*.

Results and discussion

The hybrid samples deposited at the Bulgarian herbaria are limited in number, in most cases individual samples. Too often the hybrid forms have been deposited as species and their hybrid appurtenance was determined as a result of revisions. Five of the known hybrid combinations were not confirmed with herbarium materials.

***Rumex obtusifolius* L. × *R. patientia* L.** (Fig. 1)

Panov (1987) provided data for this hybrid for the region of Sofia. The information is supported by a hybrid sample: Sofia region – residential area Geo Milev, 550 m alt., 28.08.1986, FN-83 (coll. Panov), SOM 146058.

Comparative samples: SO 17602 (Austria inferior, Rechinger, 1892); W 05670 (Chiswick, Middlesex, J. Lousley, 1942); WU 853 (Bulgaria, Klisura, Velenovský, 1887).

New data: Northeast Bulgaria – in the village Mechka, district of Rousse, 40 m alt., 16.08.2005, MJ-25 (TzR), SOA 56953; Danubian plane – 5–6 km south of Pleven, 116 m alt., 25.06.2004, LJ-00 (TzR), SOA 56977; West Frontier Mts – after the village Kamenichka Skakavitsa along the road to Gueshevo, uncultivated land and pastures, 1402 m alt., 19.06.2005, FM-27 (TzR), SOA 56945; Belasitsa Mt – between the villages Klyuch and Scrut, 300 m alt., 19.06.2005, FL-68 (TzR), SOA 56951; Rila Mts – after the village of Saparevo, along the road under a pine forest, 760 m alt., 19.06.2005, FM-98 (TzR), SOA 56952; Sredna Gora Mts (West) – over the town of Klisura, along Stryama river in the Kosharite locality, 740 m alt., 03.07.2005, KH-92 (TzR), SOA 56953; Rhodopes Mts (Central) – along the road between Narechenski Bani and Hvoyna village, 680 m alt., 12.06.2005, LG-03 (TzR), SOA 56961; after the village Chokmanovo towards Arda river, 973 m alt., 22.07.2005, LG-10 (TzR), SOA 56946; Rhodopes Mts (East) – ruderal places to the village of Mandritsa, 100 m alt., 15.07.2005, MF-28 (TzR), SOA 56947; Thracian Lowland – along Klokotnitsa river in the village Klokotnitsa, 135 m alt., 04.07.2004, LG-74 (TzR), SOA 56974.

This hybrid combination is widely spread in Macedonia, Bosna, Croatia (Rechinger 1943).

***Rumex crispus* L. × *R. patientia* L.** (Fig. 2)

Stojanoff (1932) provided data about the spreading of the hybrid in the country for the Thracian Lowland on the basis of one herbarium sample, initially determined by Střibrný as *R. obtusifolius* and later revised by Rechinger, belonging to the hybrid combination – Thracian Lowland: Ad Papazli, bei Philipopolis, 140 m alt., 04.05.1915, LG-46 (Rechinger), SOM 17837.

Comparative samples: SO 17607 (Austria inferior, Rechinger); SOA 5768 (Austriae inferior, Rechinger, 1930); WU 2636 (Austriae inferioris, Teyber, 1911);

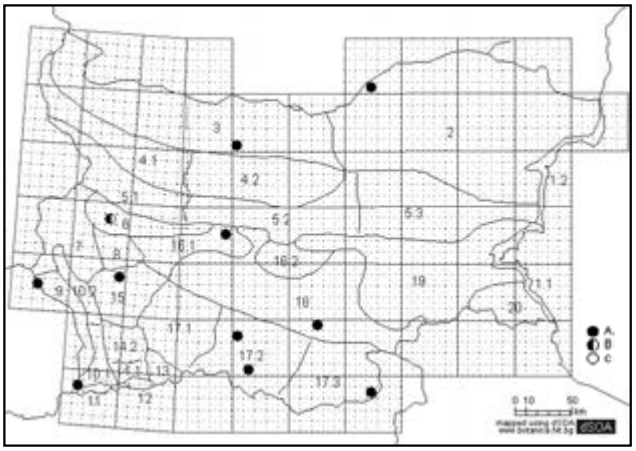

Fig. 1. Distribution map of *R. obtusifolius* × *R. patientia*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

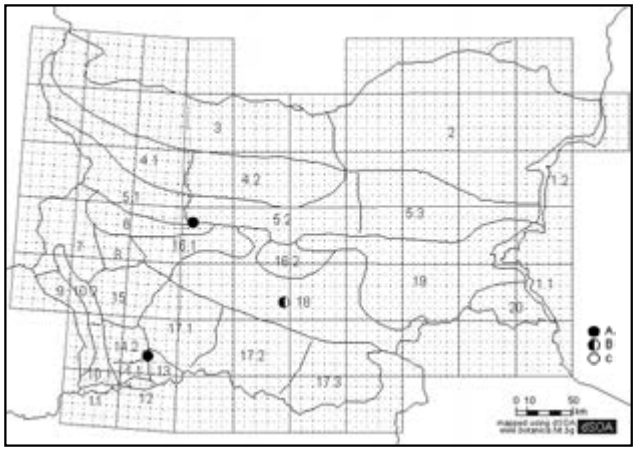

Fig. 2. Distribution map of *R. crispus* × *R. patientia*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

WU 2438 (Austriae, Rechinger, 1890); W 1965–7592 (Banatus, 1914, Prodan).

New data: Stara planina Mts (Central) – ruderal places around village Zlatitsa, 696 m alt., 03.07.2005, KH-63 (TzR), SOA 56973; Valley of Mesta River – the village Gospodintsi, ruderal places, 560 m alt., 17.06.2005, GM-21 (TzR), SOA 56980.

Rumex palustris Sm. × ***R. stenophyllus*** Ledeb. (Fig. 3)

Stojanoff (1932) provided the only literature record about this hybrid in Bulgaria for the mouth of Kamchia river. The information has been confirmed by a single herbarium sample, initially determined by Davidov as *R. pulcher*, later revised by Rechinger as *R. palustris* × *R. stenophyllus*, belonging to the hybrid combination – Black Sea Coast (North): Kamchia, 0 m alt., 02.08.1898, NH-76 (coll. Davidov), SOM 17849.

Comparative samples: W 01243 (Oesterreich Wien, 220–230 m, T. Barta, 2000); W 15360 (Hungaria orientalis, Rechinger, 1894); W 1966–85 (Austria, Melzer, 1964); W 1973–28948 (Austria, Rechinger, 1923); SOM 17796 (Hungaria: prope pag. Galos, Rechinger); SO 17537 (Hungaria orientales, V. Borbás) sub *R. limosus* × *odontocarpus*.

New data: Northeast Bulgaria – along Danube River at Rousse town, together with the parent forms, 29 m alt., 16.08.2005, MJ-15 (TzR), SOA 56957; Danubian plane – along the Danube coast at the village Archar, 20 m alt., 06.09.2004, FP-75 (TzR), SOA 56972.

Rumex crispus L. × ***R. obtusifolius*** L. s.l. (Fig. 4)

This hybrid combination is regarded as one of the most widely spread in Europe. The polymorphism established in it has been marked by many authors (Rechinger 1932; Klimeš 1993) and is due to the variability in the group of *R. obtusifolius*, which, according to the authors, is organized in 4 subspecies or varieties, showing great diversity.

Literature data showed that the hybrid is distributed in the middle part of Stara Planina Mts over the town Klisura (Velenovský 1891; Stojanov & Stefanov 1924) and the village of Hainito (Urumov 1901) under the name *R. pratensis* Mert. & W.D.J. Koch (= *R. crispus* × *R. obtusifolius*), without specifying its hybrid appurtenance. Later on, it was reported for the Rhodopes Mts (without specifying a definite locality) by Stojanov & Stefanov (1924), but there are no samples deposited in the Bulgarian herbaria under this name. As a result of revisions, four samples of Bulgarian origin, belonging to the hybrid combination, were determined: Sofia region – grassy places in the green areas along the "G. Traykov" blvd., close to Perlovska river, 550 m alt., 29.09.1986, FN-83 (coll. Panov), SOM 146059, sub *R. crispus* × *stenophyllus*; Vitosha Mt – over the village Bosnyak, 1073 m alt., 26.06.2005, FN-70 (coll. D. Dimitrov), SOM 161970, sub *R. stenophyllus*; Belasitsa Mt – in meadow at the village Samuilovo, Petrich region, 300 m alt., 19.05.1951, FL-78 (coll. N. Stojanov, B. Achtarov), SOM 92069, sub *R. sanguineus*; Pirin Mts (South) – 1970 m alt., GL-29, 08.1989 (coll. D. Stoyanov), SO 149811, sub *R. obtusifolius* ssp. *transiens*.

Comparative samples: SOA 5682 (Suecica, Lagerkranz, 1915); SOA 5762 *R. crispus* × *silvestris* (Austria inferior, Rechinger, 1927); SOM 113050, 113052 (Thuringia: Schlusingen, Haussknecht, 1883, 1889); SOM 109223 (Goeteborg, ad Gulbergsan, Ohlsen, 1928); SOM 109224 (Suecicae, Hasslow, 1934); SOM 141920 (Finland, Er. Reinikka, 1976); WU 2636 (Austria, Rechinger, 1905); s.n. (Wien, Rechinger, 1891); W 1993–01251 sub *R. crispus* (Caucasus, V. Vašák, 1975); W 2006–14353 sub *R. crispus* (Armenia, Oganesian, 2006).

New data: Danubian plane – Pleven, grassy places to the North, outside the town, 100 m alt., 25.06.2003, LJ-01 (TzR), SOA 56975; Stara planina Mts (Central) – in the village Tsarkvishte, 780 m alt., 03.07.2005, KH-

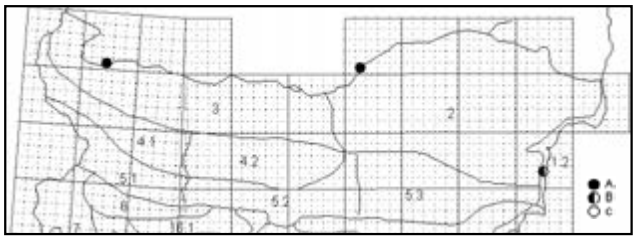

Fig. 3. Distribution map of *R. palustris* × *R. stenophyllus*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

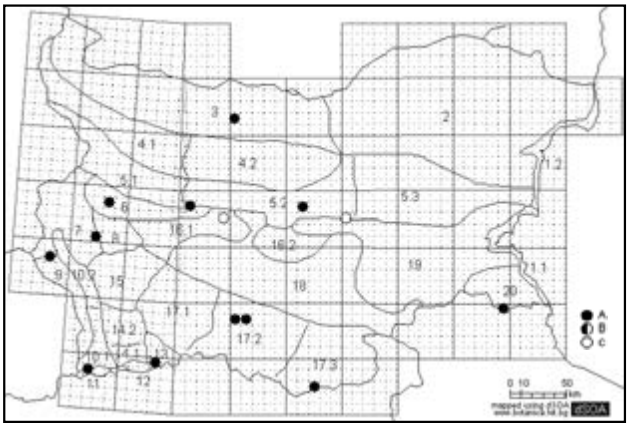

Fig. 4. Distribution map of *R. crispus* × *R. obtusifolius*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

63 (TzR), SOA 56965; the Shipchenski pass, together with the parent forms, 1185 m alt., 16.08.2005, LH-63 (TzR), SOA 56982; West Frontier Mts – along the road at the village Vratsata, Kyustendil region, 799 m alt., 19.06.2005, FM-38 (TzR), SOA 56966; Valley of Mesta River – along the road from the village of Sadovo to Ilinden, 793 m alt., 17.06.2005, GL-39 (TzR), SOA 56944; Rhodopes Mts (Central) – in the village Yugovo, damp places, 680 m alt., 17.07.2004, LG-13 (TzR), SOA 56948; along the paths in the village Hvoyna, 680 m alt., 22.07.2005, LG-03 (TzR), SOA 56949; Rhodopes Mts (East) – uncultivated land in the village of Tihomir, 519 m alt., 14.07.2005, LF-77 (TzR), SOA 56960; Strandzha Mt – along Churka river after the village Brashlyan, 348 m alt., 04.07.2004, NG-44 (TzR), SOA 56968.

Rumex conglomeratus Murray × ***R. crispus*** L. (Fig. 5)

This hybrid combination has not been reported in the literature nor deposited as herbarium samples of Bulgarian origin.

Comparative samples: SOA 5763 (Austria, Rechinger, 1927); WU 00430 (Iter Chilense, Rechinger, 1987); W 6326 (Iter Graecum: Epirus, 1200–1400 m, Rechinger, 1956); W 1949–10778 (Burgenland, Rechinger, 1924); W 1979–13090 (Macedonia, Rechinger, 1972).

New data: Black Sea Coast (South) – along the mouth of Veleka river, together with parent forms, 5 m alt., 03.07.2004, NG-85 (TzR), SOA 56978; in the dense forest of Arkutino locality, 10 m alt., 03.07.2004, NG-68 (TzR), SOA 56970; Znepole region – between the villages Golyam Vurbovnik and Maluk Vurbovnik, 420 m alt., 19.06.2005, FM-67 (TzR), SOA 56955; Sredna Gora Mts (East) – along the road in the village Zelenikovo, 420 m alt., 04.06.2005, LG-49 (TzR), SOA 56962; Rhodopes Mts (East) – uncultivated land in the village Odrintsi, 90 m alt., 15.06.2005, MF-28 (TzR), SOA 56956; along the river in the town Momchilgrad, 452 m alt., 14.06.2005, LG-60 (TzR), SOA 56954; a swamp place under the Perperikon fortress, 480 m alt., 14.06.2005, LG-71 (TzR), SOA 56950; at the road to the village Zhelezino, 396 m alt., 14.06.2005, MF-19 (TzR), SOA 56963; Thracian Lowland – after the village Rakovski around irrigation canals, 160 m alt., 04.06.2005, LG-27 (TzR), SOA 56964; ruderal places around the village Brezovo, 240 m alt., 04.06.2005, LG-38 (TzR), SOA 56967.

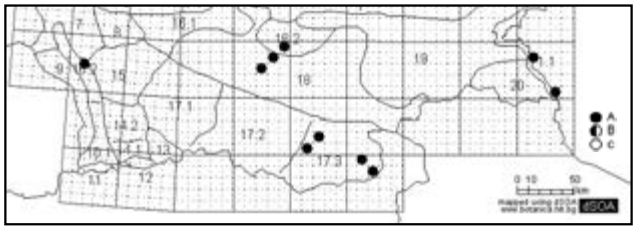

Fig. 5. Distribution map of *R. conglomeratus* × *R. crispus*:
A – new data; **B** – herbarium specimens; **C** – literature data.

A widely spread hybrid form famous for Romania (Prodan 1952), Greece (Snogerup & Snogerup 1997), Macedonia, Herzegovina (Rechinger 1943). The expanded geographic area during the last decades is probably related to the cosmopolitan spreading of the parent forms.

Rumex patientia L. × ***R. pulcher*** L. (Fig. 6)

The hybrid is new for the country. There were neither literature data nor deposited materials with Bulgarian origin.

New data: Valley of Mesta River – in the village of Gospodintsi, along the river, together with the parent forms, 560 m alt., 17.06.2005, GM-21 (TzR), SOA 57073; Sredna Gora Mts (East) – the village of Zelenikovo, 420 m alt., 04.06.2005, LG-49 (TzR), SOA 57072; Rhodopes Mts (East) – Momchilgrad town, along the river, together with the parent forms, 452 m alt., 14.06.2005, LG-60 (TzR), SOA 57071.

Data about the broad spreading of the hybrid in Greece were given by Snogerup & Snogerup (1997).

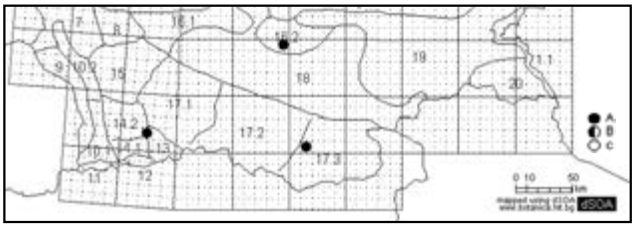

Fig. 6. Distribution map of *R. patientia* × *R. pulcher*:
A – new data; **B** – herbarium specimens; **C** – literature data.

Rumex conglomeratus Murray × ***R. sanguineus*** L. (Fig. 7a)

This hybrid was reported by Širjaev (1922) for the Preobrazhenie Monastery (together with the parent forms) and Stojanov & Stefanov (1924) with locality near Turnovo. There were no materials deposited of this hybrid form of Bulgarian origin.

Comparative samples: W 1996–05882 (England, Lousley, 1942); W 1964–14471 (Austria, Rechinger, 1896).

New data: Black Sea Coast (South) – in the thick forest at Ropotamo river, 1.5 m alt., 03.07.2004, NG-68 (TzR), SOA 56969; Rhodopes Mts (East) – about 10 km west from Ivaylovgrad town, along oak forests, 170 m alt., 15.07.2005, MF-38 (TzR), SOA 56985.

Rumex confertus Willd. × ***R. obtusifolius*** L. (Fig. 7b)

There were no literature data or materials deposited of this hybrid from Bulgaria.

Comparative samples: WU 1894 (Galicae, rev. Rechinger, 1930); W 1935–1585 (Latvia, Rechinger, 1933).

New data: Northeast Bulgaria – Shoumen district, along the road to village Struino, together with the parent forms, 239 m alt., 16.08.2005, MJ-80 (TzR), SOA 56958.

This highly sterile hybrid with abortive blossoms and underdeveloped valves was identified according to the description by Rechinger (1932).

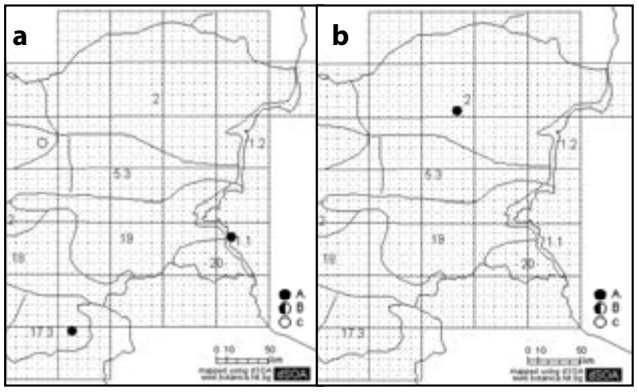

Fig. 7. Distribution map of: **a)** *R. conglomeratus* × *R. sanguineus*; **b)** *R. confertus* × *R. obtusifolius*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

Rumex cristatus DC. × ***R. obtusifolius*** L. (Fig. 8a)

The hybrid is new for Bulgaria. There were no literature data or materials deposited.

New data: Rhodopes Mts (East) – at the village Mandritsa, ruderal places and pastures, together with the parent forms, 100 m alt., 15.06.2005, MF-28 (TzR), SOA 56983.

Rumex palustris Sm. × ***R. obtusifolius*** L. (Fig. 8b)

This hybrid was established for first time for Bulgaria.

Comparative samples: WU 1565 (Austria, Figert, 1895); WU (Suecica, Murrbek, 1887).

New data: Sredna Gora Mts (West) – above Klisura, along Stryama river, together with the parent forms, 740 m alt., 03.07.2005, KH-92 (TzR), SOA 56981; Thracian Lowland – along Stryama river after the village of Kalekovets, 160 m alt., 04.06.2005, LG-27 (TzR), SOA 56979.

A hybrid between the both taxa is known for Greece (Snogerup & Snogerup 1997).

The surveys show dependence between the spreading of the parent species and the hybrid derivatives – hybrids between ruderal and broadly flexible in ecological aspect species are more widely spread, while the hybrid combinations between species with more limited spreading are established only in places where the spreading of the parent species is sympatric.

Fig. 8. Distribution map of: **a)** *R. cristatus* × *R. obtusifolius*; **b)** *R. palustris* × *R. obtusifolius*: **A** – new data; **B** – herbarium specimens; **C** – literature data.

Conclusion

The present survey is a contribution to the problems of natural hybridization between the taxa of *R.* subg. *Rumex* in Bulgaria. On the basis of data in the country from the literature and herbarium sheets chorological maps of the known hybrids from the subgenus were made for a first time. The information accumulated and processed so far enriched the chorological information for the natural hybrids of *R.* subg. *Rumex*, object of the present survey. New hybrid combinations were established on the territory of the country: *R. conglomeratus* × *R. crispus, R. confertus* × *obtusifolius*, *R. cristatus* × *obtusifolius*, *R. palustris* × *R. obtusifolius* and *R. patientia* × *R. pulcher*. The presence of *R. crispus* × *R. obtusifolius* in the Bulgarian flora was confirmed. The observations showed that the diversity of natural hybrids is bigger than the known till now, so further researches on the natural hybridization of *R.* subg. *Rumex* in Bulgaria are necessary.

Acknowledgements. This study was financially supported by the National Council of Scientific Research (Project VUB 10/5) and the SYNTHESYS Project (AT–TAF–2666) which is financed by European Community – Research Infrastructure Action under the FP6 "Structuring the European Research Area" Programme.

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Chorology and critical notes on genus *Orobanche* (*Orobanchaceae*) in Bulgaria

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Abstract. New collected material of genus *Orobanche* sect. *Orobanche* subsect. *Glandulosae* and both specimens existing in Bulgarian herbaria and data published before are revised and used for creation of maps. One new subspecies (*O. alba* subsp. *xanthostigma*) and two forms (*O. alba* f. *lutescens* and f. *rubiginosa*) are reported as new taxa in the flora of Bulgaria. The chorological data about the species and intraspecific taxa are reconsidered. The host plants are discussed comparing both herbarium data and literature reviewed.

Key words: chorology, determination key, distribution map, *Glandulosae*, host plants, intraspecific taxa, *Orobanche*, parasitic plants

Introduction

In the Bulgarian flora are known four species of genus *Orobanche* L. sect. *Orobanche* subsect. *Glandulosae* (Beck) Teryokhin – *O. alba*, *O. reticulata*, *O. pancicii* and *O. serbica*. *Orobanche alba* is a polymorphic species which is not fully examined in Bulgaria. The aim of this study is to revise and represent the known chorological data about the members of subsect. *Glandulosae* in Bulgaria as well as to analyze the information about their host plants.

Material and methods

The new material used in this study was collected during 2002–2005 in Bulgaria. An examination of specimens maintained in the Bulgarian herbaria – SOM, SOA, SO was carried out. The chorological data were processed in UTM-grid, according to Kozhuharov & al. (1983) and presented in abbreviated MGRS code. Using these data further maps were created by dSOA computer program (Stoyanov 2003). The collections were cited and grouped as follow: floristic region (in **bold**), MGRS coordinates, locality, altitude, host plant, date, author, herbarium acronym and number. The floristic regions as described and numbered by *Flora Reipublicae Popularis Bulgaricae* (vols 3–9) and *Flora Reipublicae Bulgaricae* (vol. 10) were shown in the maps (Figs 1–5). The Floras and determination books were featured with abbreviations: CBVF (Dimitrov 2002); FB1, FB3 (Stojanov & Stefanov 1925, 1948), FB4 (Stojanov & al. 1967), FV (Kitanov 1963), FD (Kitanov 1980), FRB (Delipavlov 1995), KPB (Cheshmedzhiev 2003), KVPB (Kozhuharov 1992). The author's names were featured with abbreviations as accepted in the International Plant Names Index (2005), except: GS – G. Stoychev, KS – K. Stoyanov, TzR – Tz. Raycheva.

Results and discussion

Orobanche alba Steph. (Figs 1, 2)

New and unpublished data: **1.1**: (f. *maxima*, f. *rubra*); **11**: (f. *capitata*); **12**: (f. *capitata*); **13**: GL-39. 3 km in North from the frontier-post Ilinden, 550 m, 17.06.2005 (KS) SOA 56985; **14.1**: GM-10. Pirin village, 750 m, host *Thymus*, 18.06.2005 (KS) SOA 57056; (see also f. *communis*, f. *maxima*, f. *rubra*); **17.3**: MF-07. Above Choukoura River, 1400 m, 09.07.1930 (T. Georgiev) SOA 10428; MF-19. Crossway Kroumovgrad–Kobilino–Ivaylovgrad, in oak forest, 460 m, 14.07.2005 (KS) SOA 56986; MG-11. Rocky terrain in Kartal Konak locality, on the land of Vulche Pole village, 299 m, 28.06.1940 (Kitan.) SO 68515; (see also f. *rubiginosa* and subsp. *xanthostigma*).

This species is reported for: 1.2 (T. Georgiev 1937; Velen. 1891, 1898), 2 (Baev 1947; FD; Davidov 1905; T. Georgiev 1937; Urum. 1904, 1905b, 1909), 4.1 (Urum. 1902, 1917, 1935a), 4.2 (T. Georgiev 1937; Urum. 1897, 1912, 1913a, b, 1926, 1928; Velen. 1898), 5.1 (T. Georgiev 1937; Urum. 1901a, 1905a, b, 1906, 1909, 1913b, 1926, 1935a), 5.2 (Baev 1947; Neichev 1908; T. Georgiev 1937; Toshev 1903; Urum. 1901b, 1926, 1928), 6 (Urum. 1905b, 1909; Velen. 1891), 7 (Baev 1947; T. Georgiev 1937; Toshev 1903; Urum. 1905b, 1913a, 1935b), 8 (FV; T. Georgiev 1937; Urum. 1905b, 1929a, 1930, 1935b; Velen. 1891), 9 (Urum. 1904, 1935b), 10.2 (Urum. 1935b), 14.2 (T. Georgiev 1937), 15 (T. Georgiev. 1937; Urum. 1906, 1935b), 16 (T. Georgiev 1937), 16.1 (Urum. 1929a), 17.1 (Urum. 1906; T. Georgiev 1937), 17.2 (Stransky 1921; T. Georgiev 1937; Urum. 1912, 1913b; Velen. 1898), 18 (T. Georgiev 1937; Urum. 1908, 1912, 1926; Velen. 1891, 1898). The literature data without herbarium sheets are found for the regions: 3 (Urum. 1902, 1912, 1917, 1926, 1928, 1935a), 5.3 (Urum. 1905b, 1909; Velen. 1891) and 19 (Urum. 1912, 1926).

This species is indicated for the whole country in the Floras and keys (FB1; FB4; KVPB; FRB; KPB; CVFB) up to 2000 m alt. There are missing data for region 20. The own collections and herbarium data confirm the regions 1, 2, 4.2, 5.1, 5.2, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17 and 18. This species is usual on grassy lands, shrubs and meadows (FRB; KPB), alpine and subalpine pastures (Velen. 1891), parasitizing on *Lamiaceae* (FB1; PFB; T. Georgiev 1937; FB4; KVPB; FRB; KPB) – *Thymus* (FRB). The herbarium sheets and own collections confirm the host plants *Thymus* spp., *Origanum vulgare*, *Acinos suaveolens* and *Satureja* spp. (Fig. 1).

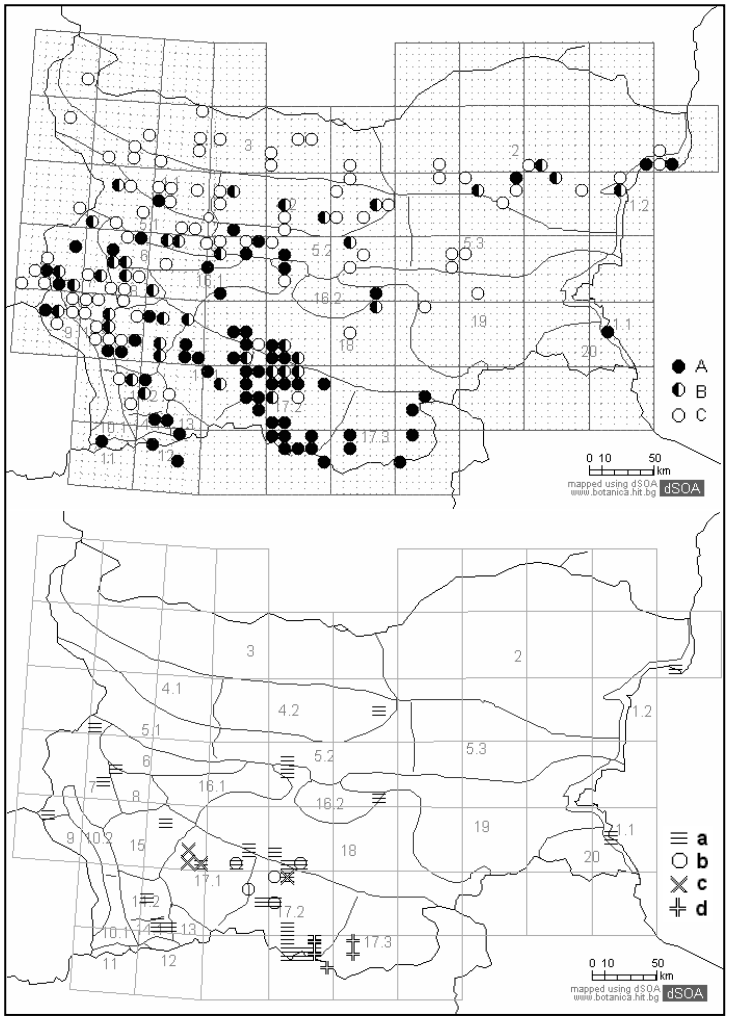

Fig. 1. *O. alba*: Distribution data: **A** – new and unpublished data; **B** – confirmed data; **C** – data from literature; Host plants: **a** – *Thymus*; **b** – *Acinos*; **c** – *Origanum*; **d** – *Satureja*.

O. alba var. *alba* f. *alba*

Syn.: *Orobanche alba* L. var. *substenophylla* T. Georgiev. Lectotypus: **14.2**: FM-93. Razlozhki Souhodol (Mehomiiski Souhodol), 2000 m (T. Georgiev) SOA – missing!

The description of the lectotype above coincides to the description of f. *alba* which is spread in the area of the species. The known host plants are *Thymus*, *Acinos* and *Satureja*.

O. alba var. *alba* f. *campanulata* Beck

This form is not confirmed by herbarium sheets and is indicated for the regions 6, 8, 16 (T. Georgiev 1937; FB4; FRB).

O. alba var. *alba* f. *capitata* Beck

New data: **1.2**: NJ-90. Balchik, 199 m, 20.05.1901 (Davidov / T. Georgiev) SOM 69580; PJ-10. Kalekairyak, 40 m, host *Thymus*, 18.06.2004 (KS) SOA 56987; **2**: NH-29. Nevsha, 100 m, 18.05.1902 (A. Yavashov / T. Georgiev) SOM 69604; NJ-90. Povelianovo (Imirler), Padina (Kopuschii), etc., 200 m, 06.1913 (Davidov / T. Georgiev) SOM 69603; **4.2**: LH-46. Sevlievo, 230 m, 05.1910 (? / T. Georgiev) SOM 69585; LH-87. Above Samovoden, 181 m, 1900 (Urum.) SOM 69574, 69619, 69620; (Urum.) SOM 69574; **5.1**: FN-78. Petrohan, 1444 m, 01.08.1903 (Dren.) SOM 69605; GN-07. Refuge Pursheviteа, 1300 m, 05.09.2004 (KS) SOA 56988; GN-08.

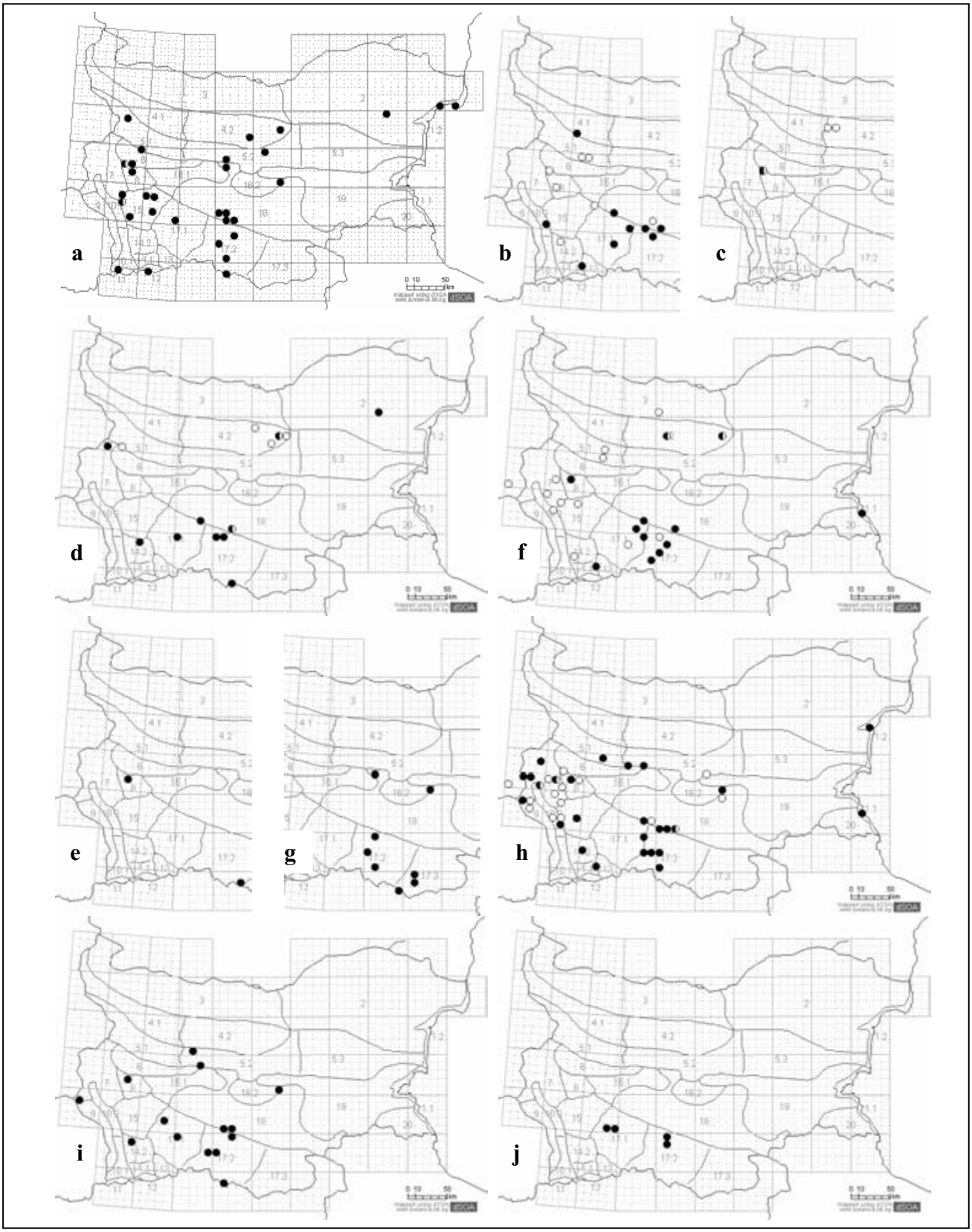

Fig. 2. Intraspecific taxa of *O. alba*:
Figs a–h – subsp. *alba*: **a** – f. *capitata*; **b** – f. *communis*; **c** – f. *lactea*; **d** – f. *longibracteata*; **e** – f. *lutescens*; **f** – f. *maxima*; **g** – f. *rubiginosa*; **h** – f. *rubra*; **i** – var. *bidentata*; **j** – subsp. *xanthostigma*.

Vilya Glava, 14.07.1930 (Stoj. / Acht.) SOM 69628; **5.2**: LH-12. Pochivaloto, 1300 m, 14.06.2003 (KS) SOA 56343; 1400 m, host *Thymus*, 28.07.2003 (KS) SOA 56989; 07.07.2004 (KS) SOA 56990; LH-64. Gabrovo, Sokolut, 329 m, 30.07.1930 (A. Yurkovskiy) SOM 69589; Malousha peak, 329 m, 28.07.1928 (A. Yurkovskiy) SOM 69590; **11**: FL-78. Belasitsa, 500 m, 10.05.1980 (IB) SOM 139654 (sub *Lathraea squamaria*); **12**: GL-18 Mt Slavyanka (Alibotush) – summer post, 1200 m, 07.1932 (Dren.) SOM 69570; **17.1**: GM-45. Park Kleptouza, 810 m, host *Thymus*, 05.07.2003 (KS) SOA 56991; **17.2**: LF-18. Mochoure, 1200 m, host *Thymus*, 03.09.2005 (KS) SOA 56992; LG-02. Below Chepelare, 1100 m, 31.05.2003 (KS) SOA 56993; LG-23. Luki, 660 m, 16.06.1965 (M. Popova / ?) SOA 24493, 24494; LG-25. Asenovgrad (Stanimaka), 300 m, 27.04.1914 (I. Mrkvichka / T. Georgiev) SOM 69618; 06.05.2004 (KS) SOA 56994; near Asenova Krepost, 400 m, host *Thymus*, 09.05.2004 (KS) SOA 56995; 08.05.2002 (KS) SOA 56997; **18**: LG-06. Purvenets, 250 m, host *Thymus*, 16.05.2004 (O. Todorov / KS) SOA 56998; LG-16. Plovdiv – Dzhendema, 164 m, 06.1896 (Stříbrný) SOA s.n.; LH-80. Above Ayazmoto, 250 m, 23.05.1943 (A. Yurkovskiy) SOM 69589; 01.06.2004 (KS) SOA 56999.

This form is reported and confirmed by herbarium sheets for the regions 6 (FB4; FRB), 8 and 15 (T. Georgiev 1937). The new data add the regions 1.2, 2, 4.2, 5.1, 5.2, 11, 12, 17.1, 17.2 and 18. The herbarium sheets and own collected materials confirm a vertical distribution between 40 m and 2000 m alt. The confirmed host plants are *Thymus* and *Acinos*.

O. alba var. *alba* f. *communis* Beck

New records: **10.2**: FM-75. Blagoevgrad (Gorna Dzhoumaya), 650 m, 01.05.1930 (Davidov / T. Georgiev) SOM 69598; **14.1**: GM-20. Between Dobrotino and Popovi Livadi, 1120 m, host *Thymus*, 17.06.2005 (KS) SOA 57000; **17.1**: KG-63. Beglika, 1600 m, 07.1935 (N. Antonov / T. Georgiev) SOA 19071; KG-85. South from Bratsigovo, 599 m, 05.08.1930 (I. Mrkvichka / T. Georgiev) SOM 69584; **17.2**: LG-05. Zdravets hut, 1200 m, 17.06.1973 (St. Dimitrov) SOA 33083 (sub *O. rapum-genistae*); Akademik hut, 393 m, 22.05.2004 (M. Lacheva / KS) SOA 57001; LG-25. Above Asenovgrad, 300 m, 18.05.1983 (Delip.) SOA 38274; host *Acinos suaveolens*, 11.05.2003 (KS) SOA 56345; LG-14. Narechenski Bani, on rocky slope, 913 m, 02.07.2005 (KS) SOA 57002.

Indicated as widespread in the area of the species (FB1; FB4; FRB). This form is reported for the region 5.1 and 18 (T. Georgiev 1937). Chorological records without confirming herbarium sheets are found for the regions 8, 14.2 and 15 (T. Georgiev 1937). This form is vertically distributed between 260 m and 1600 m alt. Confirmed host plants of this form are *Thymus* and *Acinos*.

O. alba var. *alba* f. *lactea* Beck

This form is indicated for the regions 4.2 and 6 (T. Georgiev 1937; FB4; FRB), and confirmed by one herbarium sheet from region **6**: FN-72. Lyulin – meadows, host *Thymus*, 06.1924 (Stoj. & Stef.) SOA 10431.

O. alba var. *alba* f. *longebracteata* Beck

New and unpublished data: **2**: NJ-10. Novi Pazar, 156 m, 16.05.1905 (Davidov / T. Georgiev) SOM 69577; **7**: FN-55. The southern slope of Chepun, above Dragoman, 710 m, host *Thymus*, 30.06.2004 (KS) SOA 58140. **14.2**: GM-03. Dolen Yalovarnik, 1740 m, 23.07.1951 (Jordanov / Kitan.) SO 68524 (left specimen – sub *O. rapum-genistae* rev. Delip. sub *O. gracilis*); **17.1**: KG-54. Below Kleptuza peak, 900 m, 02.07.2004 (KS) SOA 57003; **18**: KG-86. Above Sinitovo, 400 m, host *Thymus*, 01.06.2005 (KS) SOA 57004, 57005, 57006.

Reported for 4.2 (Urum. 1912, 1913a, b, 1928) and 17.2 (FB1; FB4; FRB). Indicated for 5.1 (T. Georgiev 1937), 5.2 (FB1; FB4; FRB) and 6 (FB4; FRB). The confirmed vertical distribution of this form is between 156 m and 1740 m alt. The only known host plant of this form is *Thymus*.

O. alba var. *alba* f. *lutescens* (Boreau) Beck (1890) Monogr. Orob. 212

New data: **8**: FN-81. Hotel Moreni, 1800 m, host ?*Thymus*, 25.07.2004 (KS) SOA 57007; **17.2**: LF-38. Above Zlatograd near Presoka, 941 m, host *Satureja cinerea*, 28.07.2005 (KS) SOA 57008. This form is new for the flora of Bulgaria.

O. alba var. *alba* f. *maxima* Beck

New data: **1.1**: NG-67. Cape Karaagach, 15 m, host *Thymus*, 15.07.2003 (GS / KS) SOA 57010; **8**: FN-81. Kladnitsa, 1154 m, 26.06.2004 (GS / KS) SOA 57011, 57012; **14.1**: GM-20. Refuge Popovi Livadi, 1420 m, 18.05.2005 (TzR / KS) SOA 57013; **18**: KG-86. Elenski peak, 400 m, host *Thymus*, 01.06.2005 (KS) SOA 57014.

This form is reported for: 4.2 (Urum. 1912, 1913a; FB1), 17.1 (T. Georgiev 1937; FRB) and 17.2 (T. Georgiev 1937; FB4; FRB). Indicated for the regions: 3 (Urum. 1912; FB1; FRB), 5.1 (Urum. 1913b; FB1), 5.2 (FB4; FRB), 7 (Urum. 1935a; FRB), 10.2 (Urum. 1935a), 14.2 (H. Uhlich pers. comm.) and 15 (Urum. 1935a; FRB). The confirmed vertical distribution is between 15 m and 1420 m alt. Established host plants are *Acinos suaveolens* and *Thymus*.

O. alba var. *alba* f. *rubiginosa* (A. Dietr.) Beck (1890) Monogr. Orob. 212

New data: **5.2**: LH-12. Pochivaloto, 1300 m, 14.06.2003 (KS) SOA 56343; 07.07.2004 (KS) SOA 57015, SOA 57016; **17.2**: LG-14. Narechenski Bani, 913 m, host *Thymus*, 02.07.2005 (KS) SOA 57017; LG-02. Choukata near Bogoutevo, 1100 m, host *Acinos suaveolens*, 31.05.2003 (KS) SOA 57018, 57019; LG-10. Chokmanovo, 973 m, host *Thymus*, 15.06.2003 (Y. Guteva / KS) SOA 56359, 57021; **17.3**: LF-47. between Gorski Izvor, Koushla and Granchitsa, 440–560 m, host *Satureja pilosa*, 29.07.2005 (KS) SOA 57022, 57023, 57024; LF-68. Gorsko Dyulevo – Chorbadzhiisko, 500 m, host *Satureja cinerea*, 14.07.2005 (KS) SOA 57025; LF-69. Gorsko Dyulevo, 400 m, host *Satureja cinerea*, 14.07.2005 (KS) SOA 57026, 57027; host *Thymus*, 14.07.2005 (KS) SOA 57028; **18**: LH-80. Park Ayazmoto, 250 m, host *Thymus*, 01.06.2004 (KS) SOA 57029; 420 m, 05.05.2005 (KS) SOA 57030.

This form is new for the Bulgarian flora. The Bulgarian keys lead incorrectly to *O. rapum-genistae* (T. Georgiev 1937; FB4) or *O. pancicii* (KVPB; FRB) instead of this form.

O. alba var. *alba* f. *rubra* (Hook.) Beck

New and unpublished data: **1.1**: NG-67. Kiten, 15 m, 23.05.1963 (Cheshm.) SOA 18591; 27.05.1963 (Delip.) SOA 18587, 18588, 18589, 18590; Chouka peak, 20 m, 27.05.1963 (D. Gramatikov) SOA 18586; Cape Karaagach, 15 m, host *Thymus*, 15.07.2003 (GS / KS), SOA 57009; **1.2**: NH-78. Mt Avrenska above Galata, 140 m, 07.05.1901 (Davidov / T. Georgiev) SOM 69627; **5.1**: GN-24. Mourgash peak, Stanchiv Preslap locality, 1101 m, 12.07.1952 (Acht. & Velchev) SOM 90538; **5.2**: KH-63. Below Golyam Kordun peak, 1650 m, 16.07.1952 (Acht. & Velchev) SOM 91851; KH-83. Maluk Vurtop, Kamenitsa near Klisoura, 740 m, 07.1920 (S. Baev / Acht.) SOM 69576; **14.1**: GM-20. Up from Dobro Pole above Pirin village, 1800 m, 25.07.1935 (Acht.) SOM 69677, 69680; **17.1**: KG-84. Ravnogor, 1300 m, 20.07.1949 (?) SOA 18612 (sub *O. rapum-genistae*).

This form is reported for: 7 (FB1; T. Georgiev 1937; Urum. 1913a, 1935b), 8 (T. Georgiev 1937; Urum. 1935b), 9 (Urum. 1935b), 14.2 (T. Georgiev 1937), 15 (Urum. 1935b), 17.2, 18 (FB1; T. Georgiev 1937; Urum. 1912). It is indicated for the regions: 10.2 (Urum. 1935b), 16 (T. Georgiev 1937) and 19 (FB1; Urum. 1912).

The Floras indicate the taxon too generally as widespread in the species area (FB4; FRB), but data lack for 14 subregions. This form is vertically distributed between 15 m and 1900 m alt. The taxon is very often identified incorrectly as *O. purpurea* Jacq. (because of confusion with *O. purpurea* Hook., nom. amb.) or as *O. gracilis* Sm. (because of the corolla hue). The confirmed host plant is *Thymus*.

O. alba var. *bidentata* Beck

New and unpublished data: **5.2**: KH-75. Teteven, 498 m, 1910 (Urum. / Stoj.) SOM 104240; KH-83. Maluk Vurtop, Kamenitsa, 740 m, 07.1920 (S. Baev / Acht.) SOM 69576 (the 4th sample left to right); **8**: FN-81. Bai Krustyu locality, 1300 m, 19.08.2003 (KS) SOA 57057; hotel Moreni, 1800 m, host *Thymus*, 25.07.2004 (KS) SOA 57031; **9**: FM-28. Gurlyano –Vrattsa, 1000 m, 19.06.2005 (KS) SOA 57032; **14.2**: FM-93. Refuge P. Yavorov, 1700 m, host ?*Thymus*, 14.07.2003 (TzR. / KS) SOA 56418; **17.1**: GM-36. Starina locality, near the road, 1090 m, host *Origanum vulgare*, 27.07.2003 (KS) SOA 57033; KG-54. Sivata voda locality, 900 m, host *Thymus*, 900 m, 02.06.2004 (KS) SOA 57034; **17.2**: KG-92. Zaburdo, 1373 m, host *Thymus*, 28.08.2005 (TzR / KS) SOA 57035; LF-18. Peak Lishava Chuka SW from Chepintsi, 1134 m, 05.09.2005 (KS) SOA 57036; LG-02. Chepelare, 1090 m, host *Acinos suaveolens*, 31.05.2003 (KS) SOA 57037; LG-15. Markovo, 400 m, host *Thymus*, 01.06.2003 (KS) SOA 57038; LG-24. Near Chervenata Stena Reserve, 460 m, 26.06.2004 (KS) SOA 57039; **18**: LG-25. Asenovgrad, 300 m, 4.05.2003 (KS) SOA 57040; 300 m, host *Thymus pulegioides*, 06.05.2004 (KS) SOA 57041; LH-80. Park Ayazmoto, 250 m, host *Thymus*, 01.06.2004 (KS) SOA 58141.

This variety is indicated for 16 (T. Georgiev 1937; FB4; FRB) without evidence of herbarium sheets. The vertical distribution of this taxon is between 250 m and 1800 m alt. The known host plants are *Acinos*, *Thymus* and *Origanum vulgare*.

O. alba subsp. *xanthostigma* Rätzel & Uhlich (2004) Feddes Repert. 115 (1–2): 189-211

New data: **17.1**: GM-35. Between Yundola and Avramovo, 1300 m, host *Origanum vulgare*, 27.07.2003 (KS) SOA 57042; GM-45. Sivata Voda locality, 1100 m, host *O. vulgare*, 05.07.2003 (KS) SOA 57043; 900 m, host *O. vulgare*, 02.06.2004 (KS) SOA 57044; **17.2**: LG-13. Forest near Yugovo, 680 m, host *O. vulgare*, 17.07.2004 (TzR / KS) SOA 57058; LG-14. Narechenski Bani, 913 m, host *O. vulgare*, 02.07.2005 (KS) SOA 57045.

This subspecies is new for the flora of Bulgaria. The Bulgarian keys determine it incorrectly as *O. rapum-genistae* (T. Georgiev 1937), *O. pancicii* (FB4; KVPB; FRB) or *O. amethystea* (KPB).

Orobanche reticulata Wallr. (Fig. 3)

New and unpublished data: **1.1**: NG-67. Kiten, 15 m, 27.05.1963 (Cheshm.) SOA 18606; **1.2**: PJ-10. Kalekairyak, 80 m, host *Cirsium*, 07.09.2003 (KS) SOA 57046; **12**: GL-28. Mt Slavyanka (Alibotush), 1300–1400 m, 23.06.1929 (Dren. / ?) SOM 69785; **18**: KG-86. Below Elenski peak, 340–400 m, host ?*Cirsium*, 01.06.2005 (KS) SOA 57047; (see also subsp. *pallidiflora*); **20**: NG-36. Zvezdets (Gyok-Tepe), 320–330 m, 17.07.1934 (Jordanov sub *O. cernua*) SO 68498.

This species is reported for the regions: 3 (FRB; T. Georgiev 1937), 5.1, 6 (CVFB; FB1; FB4; FRB; T. Georgiev 1937; Urum. 1909; Velen. 1891), 7 (FB4; T. Georgiev 1937), 14 (CVFB; FB4; FRB), 14.2 (T. Georgiev 1937), 15 (CVFB; FB1; FRB; Velen. 1891) and 17.2 (FB1). It is indicated without confirming material for: 2 (CVFB; FD; FRB; T. Georgiev 1937), 4 (CVFB; FB1; FB3; FB4; FRB; T. Georgiev 1937; Velen. 1891), 4.2 (Urum. 1898) and 8 (CVFB; FB4; FRB; FV; T. Georgiev 1937; Urum. 1930).

The own collections and the herbarium data confirm the regions 1, 3, 5.1, 6, 7, 12, 14.2, 15, 17.2, 18, and an altitude between 15 m and 2000 m. This species can be found on grassy and rocky slopes, in shrubs, up to the subalpine area. The records in literature indicate host plants of *Asteraceae* (KPB) – *Cirsium, Carduus* (FB1; FB4; PFB; T. Georgiev 1937) and *Dipsacaceae* (FB1; FB4; PFB; KPB; T. Georgiev 1937) – *Knautia, Scabiosa* (FRB; PFB). The herbarium sheets and the own collections confirm the hosts *Achillea pectinata* and *Cirsium*.

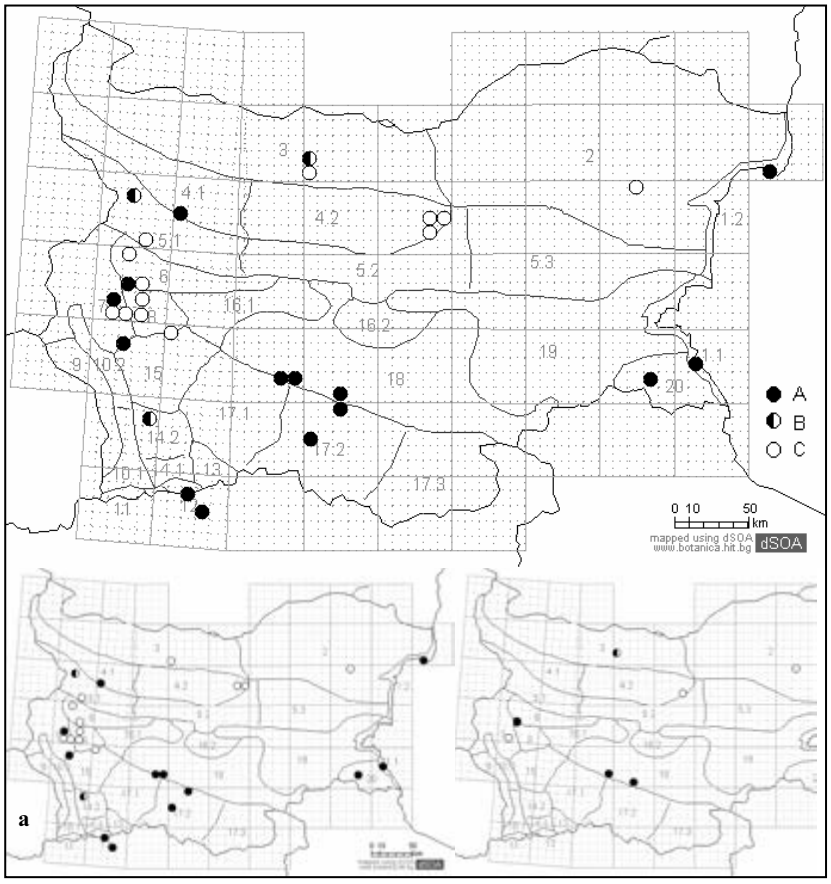

Fig. 3. *O. reticulata*: **a** – subsp. *reticulata*; **b** – subsp. *pallidiflora*.
A – new and unpublished data; **B** – confirmed data; **C** – data from literature.

O. reticulata subsp. *reticulata*

This subspecies is confirmed for 1, 5.1, 6, 7, 8, 12, 14.2, 15, 17.1, 17.2, 18, 20. The literature data indicate it for the regions 2, 3, 4, 6, 5.1, 8, 14, 15 (KPB) or as widespread (FRB).

O. reticulata subsp. *pallidiflora* (Wimm. & Grab.) Beck

New data: **18**: LG-25. Asenovgrad, 300 m, host *Cirsium*, 29.05.2003 (KS) SOA 57048; host *Achillea pectinata*, 06.05.2004 (KS) SOA 57049; KG-96. Novo Selo, 200 m, host *Cirsium arvense*, 11.05.2003 (K. Kishelov / KS) SOA 57050; 15.05.2003 (KS). SOA 56349.

This subspecies is reported for the region 3 (FRB; KPB; T. Georgiev 1937). It is indicated for 2 (T. Georgiev 1937), 4 (FRB; KPB), 4.1 (Urum. 1898), 7 and 8 (T. Georgiev 1937) without confirmation.

The key in FRB leads incorrectly to *O. alba* instead to this subspecies.

Orobanche serbica **Beck & Petrovič** (Fig. 4)

Syn.: *O. serbica* var. *laxiflora* T. Georgiev. Lectotypus: **7**: FN-55. On the chalky slopes of Mt Chepan, above Dragoman, 710 m, 20.06.1930 (Stoj. / T. Georgiev) SOA 49665 – !.

This species is confirmed only from two localities of Znepole region (**7**) – Mt Chepan above Dragoman and Mt Zemen (CVFB; FB1; FB4; FRB; T. Georgiev 1937; KPB; KVPB; Urum. 1913a, 1935b; Kovachev 1984) on altitudes between 600 m and 1000 m. The unconfirmed data are from: 2 (Urum. 1912), 3 (FB1; Urum. 1917, 1926, 1935a; Kovachev 1984), 4 (CVFB; FB1; FRB; KPB; KVPB; Kovachev 1984), 4.1 (Urum. 1917, 1926, 1935a), 4.2 (Urum. 1912, 1913a, 1928, 1935a), 5.1 (CVFB; FB1; FRB; KPB; KVPB; Urum. 1917, 1935a), 6 (Urum. 1913b, 1926; FB1; Kovachev 1984), 8 (Urum. 1930), 9 (Urum. 1913b, 1935b), 15 (Urum. 1935b), 18 (CVFB; KPB; KVPB; FB1; FRB; Urum. 1912, 1913b, 1929b; Kovachev 1984), 19 (CVFB; FB1; KPB; KVPB; Urum. 1912).

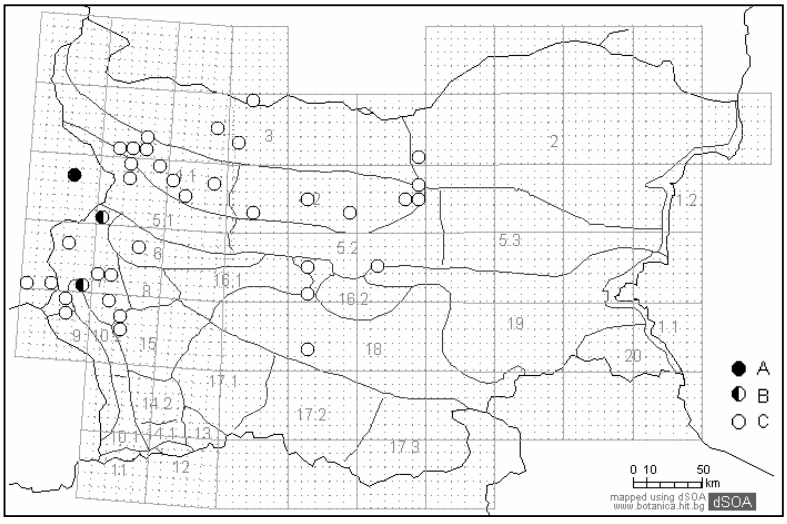

Fig. 4. *O. serbica*: **A** – new and unpublished data; **B** – confirmed data; **C** – data from literature.

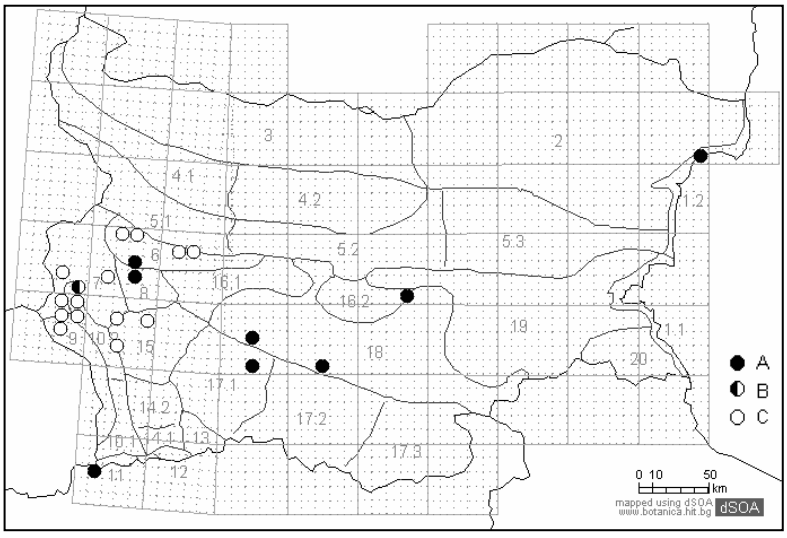

Fig. 5. *O. pancicii*: **A** – new and unpublished data; **B** – confirmed data; **C** – data from literature.

As mentioned by T. Georgiev (1937), Urumov indicates the species for many localities in Bulgaria but without confirmation of herbarium material. One herbarium sheet (SOM 69740) is revised by Delipavlov as *O. serbica* but it tallies with the description of *O. minor* Sutt. Probably the other indicated localities are based on incorrectly identified *O. alba* or *O. minor.* This rare species is endemic for the Balkan Peninsula, with a few known localities. The only confirmed host plant for this species is *Artemisia* (KVPB) – *A. alba* [= *A. camphorata* (FB1; FB4; PFB; T. Georgiev 1937; Kovachev 1984); = *A. lobelii* (FRB)] (KPB). The unproved hosts *Genista*, *Chamaecytisus*, *Alchemilla* (Kovachev 1984) are indicated, probably on the basis of incorrectly determined *O. minor* and *O. crenata*, or by the plants in neighbourhood.

Orobanche pancicii **Beck** (Fig. 5)

New and unpublished data: **17.1**: KG-75. Peshtera, 750 m, 29.06.2005 (KS) SOA 57051; **18**: KG-77. On the hills above Pazardzhik (Tatar Pazardzhik), 215 m, 19.04.1914 (I. Mrkvichka) SOA 48504; LG-25. Asenovgrad, 300 m, host ? *Pistacia terebinthus*, 29.05.2003 (KS) SOA 57052; host *Cephalaria flava*, 29.05.2003 (KS) SOA 57053; 01.05.2003; LH-80. Park Ayazmoto, 250 m; 01.06.2004 (KS) SOA 57054, 57055.

This species is reported for the regions: 1.2 (CVFB; FB3; FB4; FRB; KPB; KVPB; T. Georgiev 1937), 6 (CVFB; FRB; KPB; KVPB; Urum. 1912), 7 (CVFB; FRB; KPB; KVPB; Urum. 1913a, b, 1935b), 8 (CVFB; FB4; FRB; FV; KPB; KVPB; T. Georgiev 1937; Urum. 1930; Velen. 1891), 11 (CVFB; KPB). It is indicated for: 9 (Urum. 1913b, 1935b); 10 (KVPB; KPB; CVFB), 10.2 (Urum. 1913a, 1935b), 15 (Urum. 1935b), 20 (FB3); in altitude up to 1800 m. The herbarium sheets and the own collections confirm the regions 1.2, 6, 7, 8, 11, 16.2, 17.1 and 18, between 190 m and 1800 m alt.

The indicated host plants are *Ligustrum vulgare* (FB1; FB4; FRB; PFB; T. Georgiev 1937), *Euonymus latifolia* (FB4; FRB; T. Georgiev 1937), *Dipsacaceae* (KVPB) – *Scabiosa* (FRB; KPB) – *S. leucophylla* (FB1; FB4; PFB; T. Georgiev 1937). One herbarium sheet (in SOA) has a twig of *Euonymus*. The other sheets have not data about the host plant. The own collections confirm the host *Cephalaria*. One plant was found near the roots of *Pistacia terebinthus*.

A summary of the specified chorological data is shown in Table 1. The known host plants are given in Table 2. On the basis of the specified data an identification key was compiled.

Table 1. Distribution of *O.* subsect. *Glandulosae* in Bulgaria – comparison by floristic regions (the new data are signed with *).

No.	Taxon	Floristic regions indicated	Floristic regions confirmed	New data	Altitude (m)
1.	*O. alba*	3, 4.1, 5.3, 19	4.2, 5.1, 5.2, 6, 7, 8, 9, 10.2, 14.2, 15, 16.1, 17.2, 17.2, 18	1.1, 11, 12, 13, 14.1, 17.3	15–2000
	f. *campanulata*	6, 8, 16	–	–	–
	f. *capitata*	–	6, 8, 15	1.2, 2, 4.2, 5.1, 5.2, 11, 12, 17.1, 17.2, 18	40–2000
	f. *communis*	8, 14.2, 15	5.1, 18	10.2, 14.1, 17.1, 17.2	260–1600
	f. *lactea*	4.2	6	–	–
	f. *longebracteata*	5.1, 5.2, 6	4.2, 17.2	2, 7, 14.2, 17.1, 18	150–1740
	f. *lutescens**		–	8, 17.2	900–1800
	f. *maxima*	3, 7, 5.1, 5.2, 10.2, 14.2, 15	4.2, 7, 17.1, 17.2	1.1, 8, 14.1, 18	15–1420
	f. *rubiginosa**		–	5.2, 17.*, 17.3, 18	400–1400
	f. *rubra*	10.2, 16, 19	7, 8, 9, 14.2, 15, 17.2, 18	1, 5.1, 5.2, 14.1, 17.1	15–1900
	var. *bidentata*	16	–	5.2, 8, 9, 14.2, 17.1, 17.2, 18	300–1800
	subsp. *xanthostigma**		–	17.1, 17.2	900–1300
2.	*O. reticulata*	2, 4.2, 6, 17.1, 17.3	3, 4.2, 5.1, 7, 8, 14.2, 15, 17.2	1, 12, 18, 20	15–2000
	subsp. *reticulata*	2, 3, 4.2, 6, 7	5.1, 8, 14.2, 15, 17.1, 17.2	1, 12, 18, 20	15–2000
	subsp. *pallidiflora*	2, 4.1, 7, 8	3	18	100–300
3.	*O. serbica*	2, 3, 4, 5.1, 6, 8, 9, 15, 18, 19	7	–	700–1000
4.	*O. pancicii*	6, 9, 10.2, 15, 20	1.2, 7, 8, 11	17.1, 17.2, 18	190–1800

Table 2. Host plants of *O.* subsect. *Glandulosae* in Bulgaria (new data are signed with *).

No.	Taxon	Indicated host plants	Confirmed host plants
1.	*O. alba*	*Lamiaceae: Thymus*	*Lamiaceae: Thymus, Origanum vulgare*, Acinos suaveolens*, Statureja cinerea*, S. pilosa**
	f. *campanulata*		–
	f. *capitata*		*A. suaveolens, Thymus*
	f. *communis*		*A. suaveolens, Thymus*
	f. *lactea*		*Thymus*
	f. *longibracteata*		*Thymus*
	f. *lutescens**		*Thymus, S. cinerea*
	f. *maxima*		*A. suaveolens, Thymus*
	f. *rubiginosa**		*Thymus, A. suaveolens, S. pilosa*
	f. *rubra*		*Thymus*
	var. *bidentata*		*A. suaveolens, O. vulgare, Thymus*
	subsp. *xanthostigma**		*O. vulgare*
2.	*O. reticulata*	*Asteraceae*: *Cirsium, Carduus*; *Dipsacaceae*: *Knautia, Scabiosa*	*Asteraceae: Achillea pectinata*, Cirsium* spp.
	subsp. *reticulata*		*Cirsium*
	subsp. *pallidiflora*		*Achillea pectinata*, Cirsium arvense*
3.	*O. serbica*	*Asteraceae*: *Artemisia – A. alba*; *Fabaceae*: *Genista*, *Chamaecytisus*; *Rosaceae*: *Alchemilla*	*Asteraceae*: *Artemisia alba*
4.	*O. pancicii*	*Oleaceae*: *Ligustrum vulgare*; *Celastraceae*: *Euonymus latifolia*; *Dipsacaceae*: *Scabiosa*	*Celastraceae*: *Euonymus latifolia*; *Dipsacaceae*: *Cephalaria; ?Anacardiaceae: ?Pistacia terebinthus**

Identification key of genus *Orobanche* subsect. *Glandulosae* in Bulgaria

1. Stigma brightly yellow and remains yellow after the bloom 2

1*. Stigma red, purple or whitish, sometimes yellow but in this case it darkens after bloom and the host plant is from *Lamiaceae* 3

2. Corolla 18–26 mm long, often with straight part of the dorsal line. Filaments inserted on 2–2.5 mm up of the corolla base. Upper lip lobes bent outwards. Stigma often with red aureole ..... ***O. pancicii***

2*. Corolla 17–19 mm long, with uniformly curved dorsal line. Filaments inserted on 3–5 mm up of the corolla base. Upper lip lobes not bent outwards. Stigma without red aureole ..... ***O. serbica***

3. Calyx parts with unclear nerves; become dark brown *in sicco*. Corolla yellow with violet nerves and deeply incised upper lip. Filaments inserted 2–4 mm up of the corolla base; in the upper half with diluted glandular hairs. Host plants from *Asteraceae* . . . . . ***O. reticulata*** . . . a

   a. Upper lip intense violet or purple . . . subsp. ***reticulata***

   a*. Upper lip white or pale-yellowish . . . . subsp. ***pallidiflora***

3*. Calyx parts with 1–3 nerves, *in sicco* light brown. Corolla rose-reddish or yellowish with slightly incised upper lip. Filaments inserted less than 3 mm of the corolla base, with dense glandular hairs on the top half. Host plants from *Lamiaceae* . . . . . ***O. alba*** . . . b

   b. Calyx parts long about 7 mm, entire, equal to the half of the corolla tube. Corolla with uniformly curved dorsal line. Stigma brightly yellow . . . . . .subsp. ***xanthostigma***

   b*. Calyx parts 8–20 mm long, usually entire, sometimes bidentate, usually equal with the corolla tube. Corolla with straight part in the dorsal line. Stigma usually red or purple, rarely yellow or white . . . . . subsp. ***alba*** . . . c

   c. Calyx parts bidentate. Teeth often dissimilar. Bracts equal to the corolla. . . . . var. ***bidentata***

   c*. Calyx parts monodentate. . . . . var. ***alba*** . . . d

   d. Stigma yellow or white .....e

   d*. Stigma red, purple or pink .....f

   e. Whole plant yellow as the stigma . . . . . f. ***lutescens***

   e*. Corolla reddish or red, coloured as the type . . . . . f. ***rubiginosa***

   f. Bracts vastly longer than the corolla . . . . . f. ***longibracteata***

   f*. Bracts not longer than the corolla .....g

   g. Corolla 25–30 mm long .....f. ***maxima***

   g*. Corolla shorter than 20 mm .....h

   h. Spike rotundate or ovate, compact, with no many flowers . . . . . f. ***capitata***

   h*. Spike elongated, cylindrical, usually with many flowers . . i

   i. Corolla pale yellow or white . . . . . j

   i*. Corolla pink or purple to red . . . . . k

   j. Corolla white . . . . . f. ***lactea***

   j*. Corolla pale yellow. . . . . f. ***alba***

   k. Spike dense, elongated ovate, short, with a little flowers. Stem thin . . . . . f. ***communis***

   k*. Spike ± lax, cylindrical, elongated with many flowers. Stem thick l

   l. Corolla short, long almost as wide . . . f. ***campanulata***

   l*. Corolla 1.5–2 times longer than wide . . . . . f. ***rubra***

Conclusion

The big intraspecific diversity of *O. alba* and *O. reticulata* shows that the hue of stigma is not a reliable criterion for identification of the species in this section. Although *O. alba* is found to be distributed in the whole country according to Floras and keys, practically records or herbarium sheets from some regions are missing. *Orobanche alba* f. *rubiginosa, O. alba* f. *lutescens* and *O. alba* subsp. *xanthostigma* are new taxa for the flora of Bulgaria. The new data add 3 regions (5 subregions) to the distribution area of *O. alba.* The taxa of *O. alba* subsp. *alba* are revised. One of the known forms, *O. alba* f. *campanulata*, is not confirmed by herbarium material. Three regions (4 subregions) are added to the distribution area of *O. reticulata,* and the data about *O. reticulata* subsp. *pallidiflora* are specified to 2 regions. *Orobanche serbica* is found only in two isolated localities of Znepole region. Two regions are added to the distribution area of *O. pancicii*. The review of existing herbarium data about host plants of genus *Orobanche* subsect. *Glandulosae* in Bulgaria presents that occasionally the information is not sufficient. A large part of the specimens are not supported by information concerning host plant. In other cases the information on the specimen label is not proved with enclosed host. Probably most of the data about host plants are based on the plants found in neighbourhood to the parasite. The information obtained in the study shows a clear specificity according to the host plants. *Orobanche alba* parasitizes only on *Lamiaceae*, *O. reticulata* – only on *Asteraceae*. The highest specificity displays the endemic *O. serbica* which parasitizes only on *Artemisia alba*. The information about the host of *O. pancicii* is not clear and needs research in future.

Aknowledgements. The study was supported by SYNTHESYS Project (AT–TAF–3133) which is financed by European Community – Research Infrastructure Action under the FP6 "Structuring the European Research Area" Programme.

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Variability of cultivated populations of the European plane

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Abstract. To research the morphological and genetic variability of European plane, this study applies the comparative morphological analysis of the European plane (*Platanus* × *acerifolia*) cultivated populations in the area of Belgrade city. The study results show that the cultivated populations of European plane in the area of Belgrade city are characterized by a high degree of individual and group variability of a series of characters, such as crown form, types of bark, straightness of stem, stem anatomic structure, percentage of characters of Oriental and American plane, characters of leaves and inflorescences.

Key words: individual and group variability, *Platanus* × *acerifolia*

Introduction

Variability, as one of the main characteristics of living beings, is the initial material for natural and planned selection (stabilizing, directed and disruptive). Along with inheritance and selection, variability is one of the three main factors of natural and anthropogenically directed evolution of organisms. Depending on the causal factors, variability can be divided into: modifications – phenotype changes occurring as the consequence of environmental factor, and mutations – phenotype changes occurring as the consequence of the genotype changes (Isajev & Šijačić-Nikolić 2003). The study of individual and group variability of trees has multiple significances both for taxonomy and for genetic study, and the study results are increasingly applied in the production of planting material. Previous studies of the local populations of European plane indicate the pronounced variability of many characteristics (Đukić 1992).

Material and methods

European plane in Serbia is a fast-growing, long-living and adaptable species, favourable for the establishment of plantations and for the cultivation in urban environmental conditions (Jovanović 1950, 1991; Petrović 1950; Tucović 1970). Most likely, it originates from England, where it was created about 1640 by crossing the Oriental plane (*Platanus orientalis* L.) and American plane *(Platanus occidentalis* L.). This interspecific hybrid is climatically much more resistant than both of its parents (Jovanović 1991). It is a mesophilous–xerothermic species (Letić 2002). It tolerates urban conditions and dust. It justifies the planned crossing of allochthonous species with the domestic, more or less closely related tree species.

To research the morphological and genetic variability of European plane, this study applies the comparative morphological analysis of the European plane (*Platanus* × *acerifolia* Willd.) cultivated populations in the area of Belgrade city (Knežević 2005). The study includes the analysis of crown form, characteristics of dead bark, trunk anatomic structure, straightness of stem, characteristics of flowering, inflorescences and fruits on the selected test trees.

Results

The results of the study of variability of the cultivated European plane populations in the area of Belgrade city show a high variability of the analysed characters. Depending on phenotype characteristics, it is possible to define several different phenogroups.

There are 4 phenogroups of crown form: trees with wide, wide conical, conical, and narrow conical crowns (Fig. 1). The trees with wide crowns have vigorously developed lateral branches, which are most often horizontally directed. The phenotype with narrow conical crowns is the rarest phenotype. The trees have monopo-

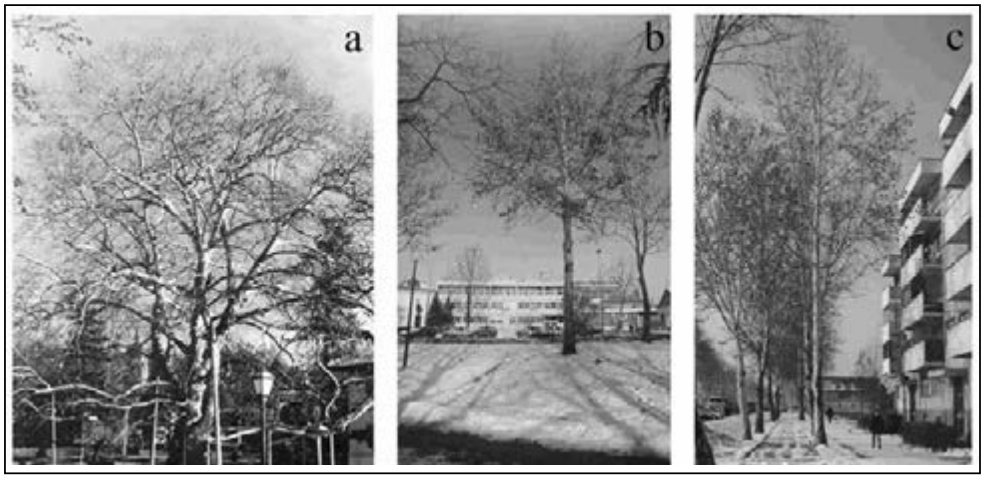

Fig. 1. European plane phenogroups according to crown form: **a** – wide; **b** – wide conical; **c** – conical crown.

dial stems, narrow crowns and thin branches. They are characterized by fast height growth, and their diameter increment is the lowest compared to other phenogroups. They are suitable for solitary planting and for smaller groups in urban conditions.

Depending on the characteristics of dead bark on the trunk, there are trees with smooth, shallowly fissured and fissured bark (Fig. 2). In addition to aesthetic attributes, the trees with fissured bark have a slightly higher diameter increment.

In the populations, there are two phenotypes of stem straightness: trees with monopodial, full and straight stems and trees with forking (low, medium and high) (Fig. 3).

There are two phenogroups of the anatomic structure of stems: with and without anatomic defects. The trees with anatomic defects (Fig. 4) can be classified into two groups: trees with the outgrowth along the stem due to the multiplication of dormant buds or short shoots, type *mazerika*, and trees with the stem deformations, due to the non-uniform function of the cambium, type *lignotuber*. The trees with defects of stem structure are characterized also by a sharp taper and by reduced competitiveness in the closed canopies. The trees with bur texture are used for the production of ornamental veneers. Dormant buds are formed on the stems in the form of characteristic outgrowths. Such trees are especially valuable because of their ornamental texture, which is best visible on the tangential section. The trunks differ in anatomic structure from other plane trees by the valuable bur texture.

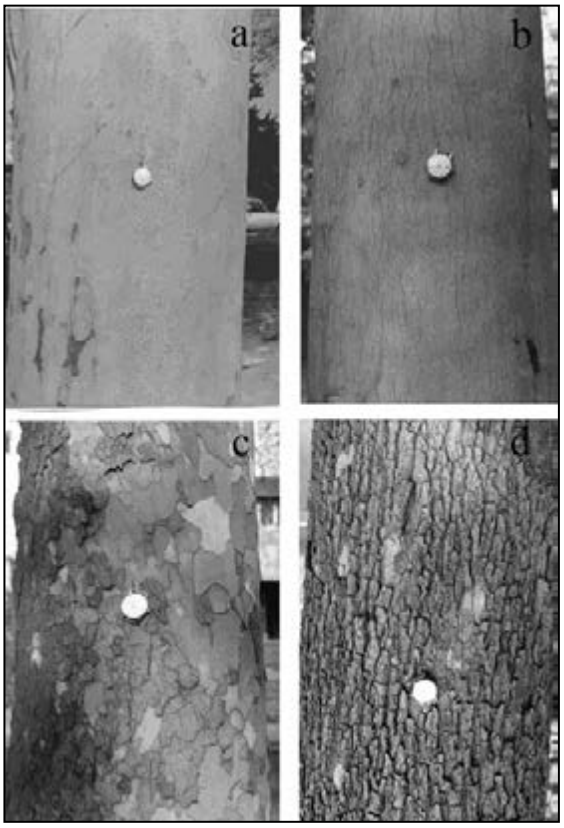

Fig. 2. European plane phenogroups according to dead bark: **a, b** – smooth; **c** – shallow fissured; **d** – fissured bark.

During the morpho-physiological analysis of trees at one locality, a very low percentage of parent phenotypes were recorded: 2.7 % of type *occidentalis* and 3.7 % of type *orientalis*, determined based on the leaf characteristics or by the number and structure of seed heads (Tucović & al. 1997).

Fig. 3. European plane phenogroups according to trunk straightness: **a** – monopodial, full and straight trunks; **b** – trees with forking.

The study shows major variability of clusters, seed heads (Fig. 5). 15 % of analysed trees have simultaneous occurrence of two types of inflorescences and fruits, i.e. heterocarpy (Fig. 6). The smaller fruits are formed after leafing and the first flowering and they are the result of two-phase growth of shoots (Fig. 7). First spring flowering is simultaneous with leafing and in spring the twigs form larger seed heads. The second, summer flowering occurs after the full leafing and smaller seed heads are formed on summer twigs. The repeated flowering, i.e. the second yield, is an ancestral or an ancient character, inherited from the ancestors. It characterizes the old species originating from the warmer regions where there are no climate and other disharmonies during the growing season. Warm summers with uniform temperature, moisture and other environmental factors create the conditions for the second flowering. The phenomenon of two types of inflorescences and fruits affects the germination percentage, the seeding density in nurseries, and it also enables the raising of cultivars with two yields. In addition to theoretical, this has a practical significance (Knežević & Tucović 2003).

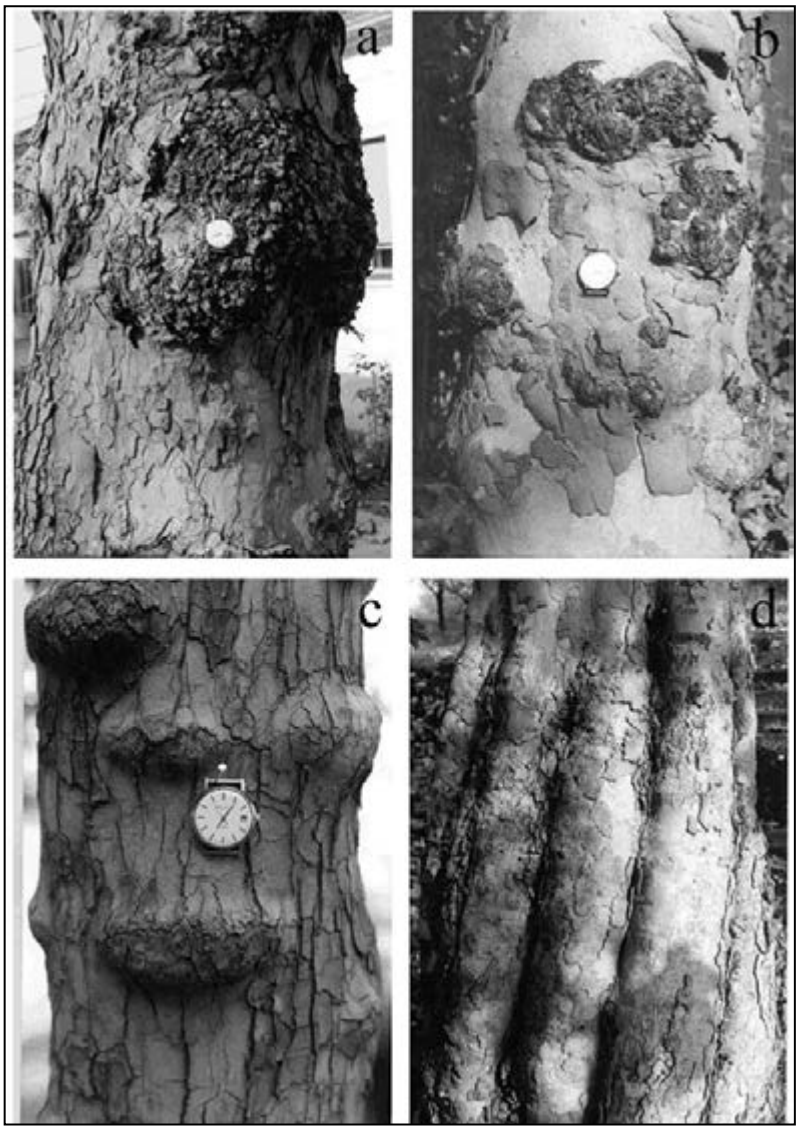

Fig. 4. Phenogroups of European plane as per trunk structure abnormality: **a, b** – bur trees; **c** – trees with elliptic outgrowths; **d** – trees with vertical ribs.

Conclusion

The study results show that the cultivated populations of European plane in the area of Belgrade city are characterized by a high degree of individual and group variability of a series of characters, such as crown form, types of bark, straightness of stem, stem anatomic structure, percentage of characters of Oriental and American plane, characters of leaves and inflorescences. Based on the growth elements, height, diameter, and heights expressed in percentages of crown circumference, European plane in the area of Belgrade city is characterized by fast and stable growth. Although it grows here in the unfavourable conditions of urban environment, surrounded by concrete, exposed to drought, high temperatures and severe air pollution, it has good phenotype characters of the cultivated populations, which proves its high adaptability.

European plane, because of the abundant yield, numerous and small fruits collected in globular clusters (seed heads), is classified in the category of tree species whose recombination system enables a very high level, not only of the potential variability, but also of the free genetic variability. Thanks to the recombinations, the economically significant properties are readily realized, so they can be subjected to natural or empirical selection. The definition of different phenogroups is the starting point for the production of European plane seedlings intended for landscaping or for the establishment of special purpose plantations of this very significant and interesting tree species.

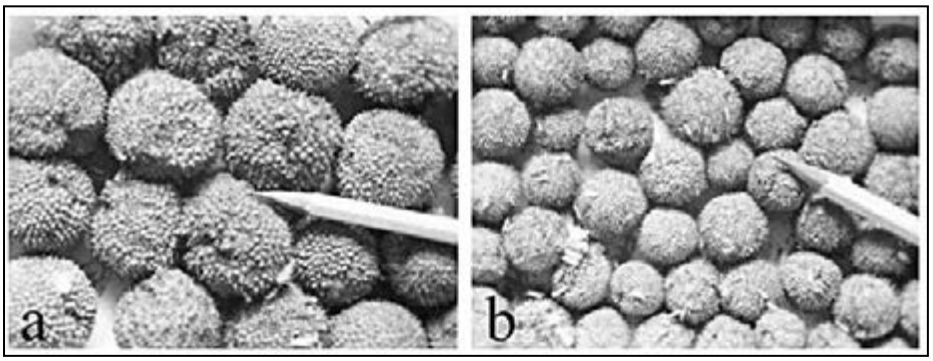

Fig. 5. Variability of seed heads on the same European plane tree in the first (**a**) and the second (**b**) more or less summer flowering.

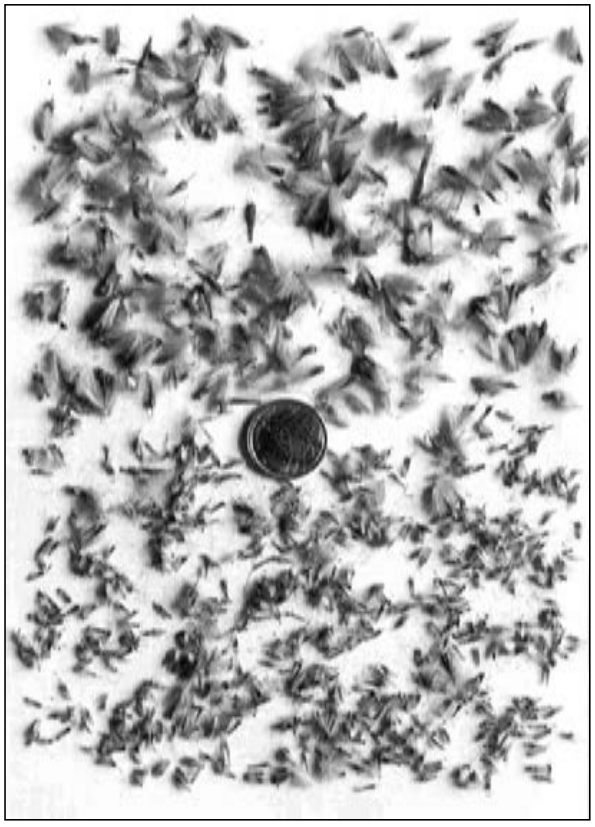

Fig. 6. Comparative characteristics of fruits from the first (spring) and the second (summer) flowering, i.e. European plane heterocarpy.

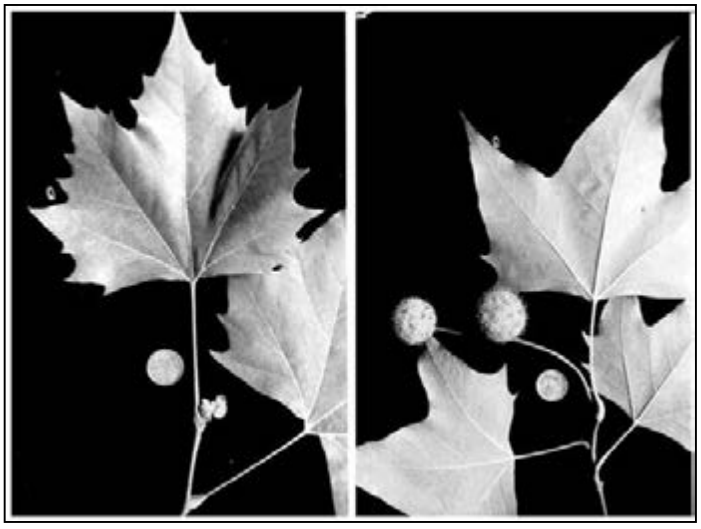

Fig. 7. The second phase of flowering and formation of inflorescences on one of test trees.

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Analysis of seed and seedling characters of different ginkgo parent trees

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Abstract. Seven ginkgo genotypes, appearing individually on Belgrade greenspaces, have been analysed. Standard statistical parameters show significant genetic variability of the analysed genotypes. Genetic variability of chosen individuals is much higher than it can be observed by parent morphological traits. Collection of data of ginkgo genetic variability in the area of Belgrade city is a base for the conservation and sustainable use of the genetic potential. Also, variability of seed and seedling trait quality can be used as a marker for the estimation of further plant development.

Key words: analysis, genetic variability, ginkgo, seedlings, seeds

Introduction

Ginkgo (*Ginkgo biloba* L.) is the oldest and the most resistant tree species on the planet, originating from China, about 250 million years old, from the Mesozoic period Triassic. It survived four ice ages, two atomic bombs on Japan, the devastating fire in Tokyo, as well as the numerous attacks by insects, fungi and air pollution. Today ginkgo is a stenoendemic of the east part of China, while in other parts of China, Japan and Korea it is considered a secondary wild species. In many countries, such as in Serbia, ginkgo is an allochthonous species, introduced by man (Vilotić 2004).

On the territory of Serbia, ginkgo occurs in green areas mainly as individual trees or in smaller groups, very rarely in tree rows. In the wider zone of Belgrade, after Vilotić (2004), there are 94 trees. There are no written data on the origin of ginkgo seeds and seedlings on the territory of Serbia, but it is supposed that seeds and seedlings were not brought from Asia, but that they are of European origin (parks in England, Czech Republic, Slovakia, Germany, etc.). All the old specimens are protected by the law.

It is known that ginkgo wood is used in wood industry, leaf – for the extracts with medicinal character, and seeds – in nourishment or as a drug. Also, ginkgo is very suitable for cultivation in shelterbelts, multiple purpose plantations, or in urban conditions. Bearing in mind its multiple benefit value, the interest for this species has been increasing in Serbia in the recent years.

Material and methods

The study of individual and group variability of trees has multiple significance both for taxonomic and for genetic studies, and the study results have been increasingly applied in the production of nursery stock (Stilinović & Tucović 1975). The study of ginkgo genetic, morphological and physiological variability is based on the analysis of seeds and seedlings of different parent trees selected in the area of Belgrade city.

Depending on the yield quantity as the major selection criterion, 7 parent trees were selected in autumn 2005 and the seeds were collected (Fig. 1). After the removal of the seed coats, the dimensions of the clean seeds were measured on the sample of 100 seeds. The following morphometric parameters were analysed: seed length (mm), seed width (mm) and seed mass (g).

The seeds were sown in autumn in plastic tubs type "Plantagrah" in the mixture of peat, sand and forest humus (2:2:1). During winter and early spring, the tubs were stored in the greenhouse of the Faculty of Forestry in Belgrade, and they were transferred to the open in May. Seed germination percentage was determined in the conditions of the greenhouse.

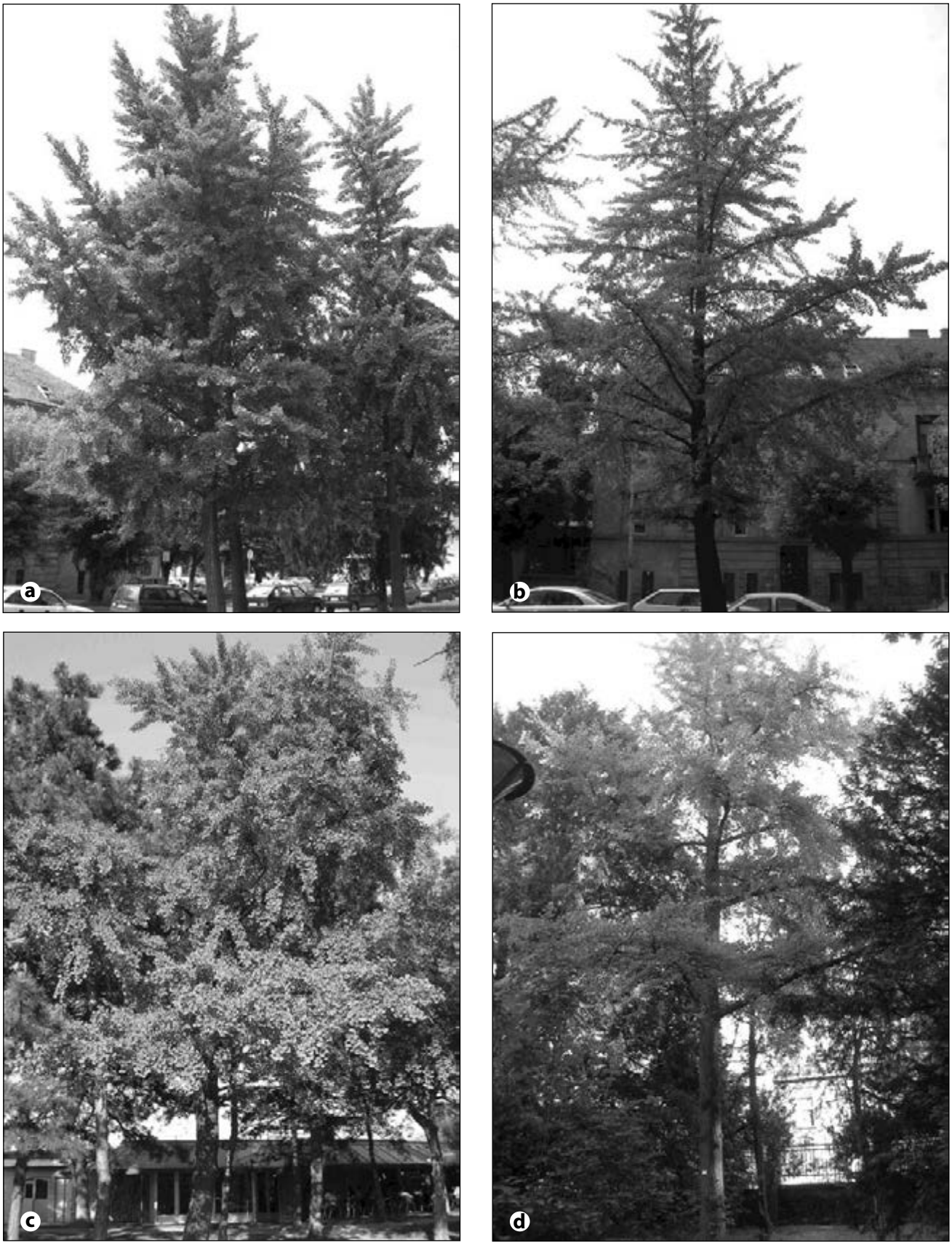

Fig. 1. Some of the selected ginkgo parent trees on the area of Belgrade city: **a** – tree number 1; **b** – tree number 3; **c** – tree number 4; **d** – tree number 6.

The analysis of seedlings was performed on the sample of 30 plants and it covered the following morphometric characters: height of above-ground part (mm), root collar diameter (mm) and average number of leaves. The collected data were computerized by the programme "Statistica 6.0". The statistical significance of differences between the mean values of the analysed morphometric seed and seedling characters of parent trees was determined by Student's t-test at the 95 % probability level (Isajev & Šijačić-Nikolić 2003).

Cluster analysis was performed to estimate the genetic distance between the selected parent trees based on the morphometric characters of seeds and seedlings (Šijačić-Nikolić 2001).

Results and discussion

Based on the study results of the analysis of morphometric characters of seeds from 7 ginkgo parent trees (Table 1), it can be concluded that the differences between the mean values in most cases are statistically significant for all three analysed characters (Šijačić-Nikolić & al. 2006).

Seed mass is the least variable character, and seed width has the highest variability. At the level of the genotypes it can be concluded that parent trees 1, 2 and 3 have the greatest seed length and seed width, and they are the lowest in ginkgo 6. Ginkgo 1 has the highest seed mass (2.22 g), and the trees 2 and 3 have lower values of seed mass (only 0.60 g and 0.99 g, respectively), although their dimensions are relatively high.

In addition to seed dimensions and forms more or less typical for the species (Fig. 2a), there are a number of seeds ("stones") which show some deviations, most often regarding the seed form, size and structure (Fig. 2b).

The germination of ginkgo seeds is hypogeal. There are two cotyledons. The cotyledons remain underground having the function of haustoria, absorbing organs, which are one of the characteristics of the most primitive woody species of gymnosperms. The seedlings have massive stems and fan-like two-part first leaves which have a characteristic venation (Stilinović 1985).

Based on the presented results of seed germination percentage of 7 ginkgo parent trees (Table 2), it can be concluded that these results agree with the reference data according to which ginkgo germination percentage ranges from 20 % to 80 % (Vilotić 2004). Such a high variability of seed germination percent-

Table 2. Results of seed germination percentage of 7 ginkgo parent trees.

Parent tree	Germination percentage (%)
1	66.04
2	0
3	13.20
4	39.62
5	28.30
6	26.42
7	50.94

Table 1. Results of the analysis of seed morphometric characters of 7 ginkgo parent trees.

Seed length (mm)									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	20.76	0.70	–						
2	16.20	0.62	–47,87*	–					
3	16.79	0.81	–38.59*	5.18*	–				
4	11.09	0.74	–93.17*	–55.79*	–50.03*	–			
5	12.31	0.62	–85.02*	–43.20*	–41.14*	12.08*	–		
6	8.41	0.70	–127.25*	–84.40*	–77.98*	–27.19*	–40.48*	–	
7	13.33	0.79	–72.08*	–28.79*	–30.43*	21.75*	10.72*	44.08*	–
Seed width (mm)									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	16.31	1.93	–						
2	12.09	0.72	–20.15*	–					
3	13.10	0.92	–3.21*	8.13*	–				
4	7.18	0.89	–45.46*	–43.09*	–45.01*	–			
5	7.52	0.80	–41.59*	–41.16*	–44.13*	2.70*	–		
6	6.21	0.86	–47.09*	–52.30*	–51.80*	–8.16*	–11.27*	–	
7	7.07	0.67	–44.95*	–48.80*	–49.55*	–1.03	–4.70*	8.48*	–
Seed mass (g)									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	2.28	0.50	–						
2	0.60	0.11	–28.83*	–					
3	0.99	0.19	–20.52*	17.87*	–				
4	1.97	0.19	–5.60*	61.23*	34.97*	–			
5	2.04	0.19	–4.17*	65.43*	38.58*	23.56*	–		
6	1.25	0.15	–18.30*	35.09*	10.10*	–31.11*	–33.03*	–	
7	1.97	0.15	–5.34*	77.75*	38.45*	1.29	–3.84*	35.99*	–

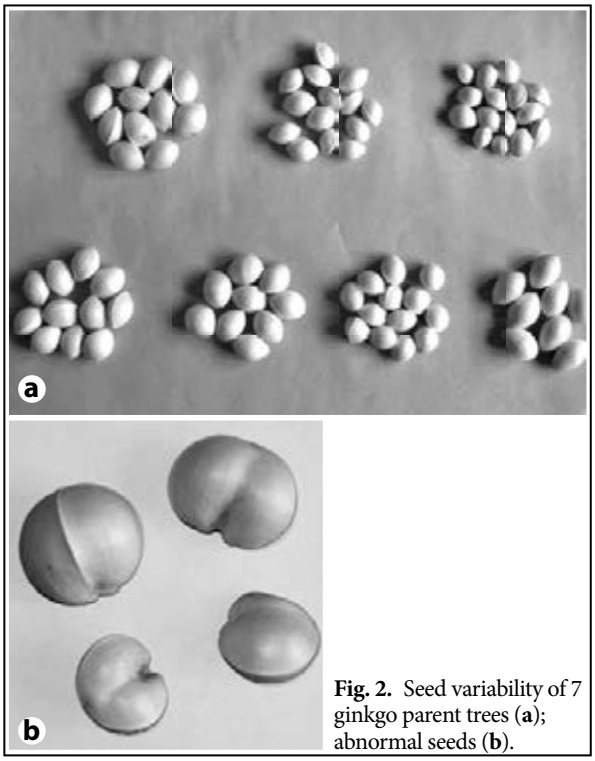

Fig. 2. Seed variability of 7 ginkgo parent trees (**a**); abnormal seeds (**b**).

age can be the consequence of inbreeding, as this is a dioecious species, or the consequence of the pollination system.

Genotypes 1 and 7 have the highest germination percentage. Genotype 2 did not germinate at all, while the germination percentage of genotype 3 was only 13.20 %. The analysis of data shows the correlation between the relatively low seed mass of parent trees 2 and 3 and low, i.e. no germination percentage of these seeds, which is most probably the consequence of the pollination conditions, i.e. the absence of the sufficient quantity of pollen.

Some deviations from normal seedling were observed during the seedling development of different ginkgo parent trees, such as: abnormal germination, the appearance of twins and the appearance of epigeal germination (Fig. 3).

The presented results of the analysis of seedling morphometric characters of 7 ginkgo parent trees (Table 3) show that the differences between the mean values of parent trees are in most cases statistically insignificant. All three analysed seedling characters have the highest mean values of parent trees 1 and 7, and the lowest mean values of parent trees 5 and 6.

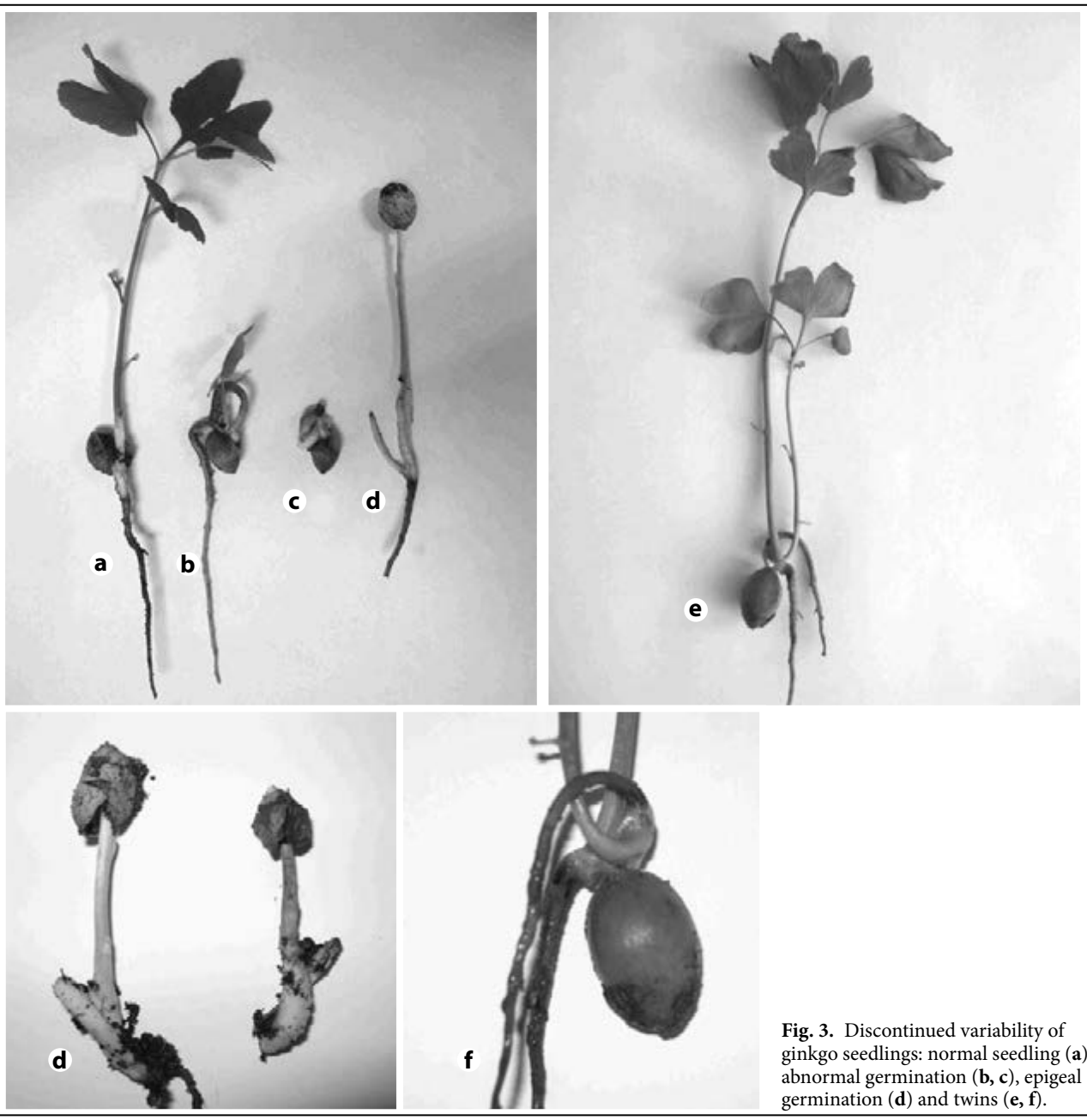

Fig. 3. Discontinued variability of ginkgo seedlings: normal seedling (**a**), abnormal germination (**b, c**), epigeal germination (**d**) and twins (**e, f**).

Table 3. Results of the analysis of seedling morphometric characters of 7 ginkgo parent trees.

Height of above-ground part (mm)									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	129.00	28.22	–						
2	0	0	0	–					
3	0	0	0	0	–				
4	125.65	15.19	–0.42	0	0	–			
5	117.50	39.81	–0.91	0	0	–0.88	–		
6	115.75	22.84	–1.56	0	0	–1.96	–0.18	–	
7	141.75	30.91	1.27	0	0	1.91	2.09	2.83*	–
Root collar diameter (mm)									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	2.54	0.36	–						
2	0	0	0	–					
3	0	0	0	0	–				
4	2.47	0.45	–0.50	0	0	–			
5	2.29	0.32	–2.32*	0	0	–1.41	–		
6	2.34	0.47	–1.57	0	0	–0.99	0.40	–	
7	2.46	0.37	–0.66	0	0	–0.06	1.32	0.88	–
Number of leaves									
Parent tree	Mean value	Standard deviation	t – value						
			1	2	3	4	5	6	7
1	4.60	0.68	–						
2	0	0	0	–					
3	0	0	0	0	–				
4	4.45	0.61	–0.82	0	0	–			
5	3.70	0.65	–3.59*	0	0	–3.29	–		
6	3.60	0.51	–4.59*	0	0	–4.67*	–0.52	–	
7	4.45	0.68	–0.61	0	0	0	4.27*	4.68*	–

The Cluster analysis (Fig. 4) shows that the selected ginkgo parent trees are grouped in two homogeneous groups. The first homogeneous group consists of trees 5 and 6 with which trees 4 and 7 are grouped, and the second group are trees 2 and 3. Tree 1 is grouped at the greatest genetic distance with these two homogeneous groups. It is interesting that the trees 1 and 7, which have the highest mean values of the analysed seed and seedling morphometric characteristics, are grouped at the greatest genetic distance, which proves the specificity of the genotypes of these parent trees.

Conclusion

The results of the seed and seedling morphometric analyses of 7 ginkgo parent trees from the area of Belgrade city show the presence of major genetic variability. The genetic variability of the selected parent trees is considerably higher than it could be concluded from the simple observation of morphological variability. The research at this level shows a high genetic potential of parent trees 1 and 7, as well as of other parent

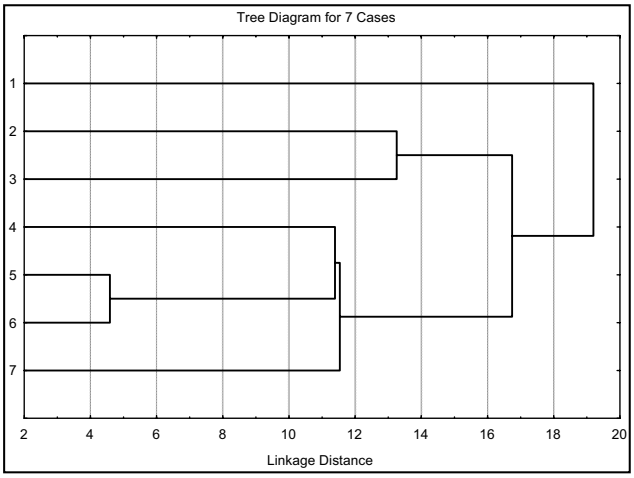

Fig. 4. Cluster analysis tree diagram based on the seed and seedling morphometric characteristics of 7 ginkgo parent trees.

trees, except trees 2 and 3. The highest number of the analysed characters has a quantitative character and it is governed by polymeric genes whose effect is additive.

The determined genetic variability is the initial base for the study of the genetic potential of ginkgo parent trees, their conservation and directed utilization. Also, the variability of seed quality and seedling characters can be the indicator of further seedling development, which emphasizes the significance of such research for the advancement of the technology of seed and plant production of this interesting tree species.

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Contribution to the adventive flora of Kalimnos (East Aegean, Greece)

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Abstract. At present, the known adventive flora of Kalimnos consists of 50 species, 33 of which are new records for the island. Some of these taxa are fully naturalized and widespread throughout the inhabited part of the island, whereas others are rare and casual, probably escaping from cultivation. Chorological analysis shows that most of the adventive species are of American origin, followed by those of Asiatic, African and Mediterranean origin. There are also some Cosmopolitan and Tropical species, whereas the origin of six taxa is unknown or uncertain.

Key words: adventive, Aegean, Greece, Kalimnos

Introduction

Kalimnos is an island situated in the South-East Aegean. It covers an area of approximately 110 km^2. The island is very rocky and the highest peak is Mt Profitis Ilias, with an altitude of 676 m. It is inhabited by approximately 16 400 people, most of them living in the town of Pothia, which is very densely populated.

A number of botanists have in the past occupied themselves with the study of the flora of Kalimnos. As resulting from previous investigations, as well as our own recent research, the known flora of the island amounts to more than 700 taxa. However little was known so far about the adventive flora of the island. The main publication on the flora of Kalimnos is that of Hansen (1980), who reports a total of 15 adventive species, from previous records, as well as his own observations. Two more adventive species are reported by Tan (1982) and Tan & Panitsa (2000). In the present contribution, we list a total of 50 adventive taxa from Kalimnos, 33 of which are new to the island.

Material and methods

The following list of the adventive species originates from the floristic investigation of the first author on the island, dated from March 1998 until May 2006 and also from data that were kindly provided by Dr Th. Raus, regarding his own collections together with H. Sipman in 2000, as well as collections made by R.M. Burton in 1988 and 1989. We also include all the previous records, as well as a specimen of *Kochia scoparia* (L.) Schrad., collected by Kraft, kept at the Botanical Museum of Lund.

The nomenclature of the species is according to *Flora Hellenica* (Strid & Tan 1997, 2002), *Med-Checklist* (Greuter & al. 1984, 1986, 1989), *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985) and *The European Garden Flora* (Walters & al. 1986; Cullen & al. 1997). The chorological elements are mostly according to Pignatti (1982). The species are given in alphabetical order and each is followed by the chorological element, as well as the localities and dates of collections or observation. The localities mentioned in the floristic list of the adventive taxa are shown on the map in Fig. 1.

Abbreviations and symbols

*	New record for Kalimnos
?	Unknown or uncertain chorological element
AH	A. Hansen (1980)
FAe	Flora Aegaea (Rechinger 1943)
FH1	Flora Hellenica, Vol. 1 (Strid & Tan 1997)
obs.	Observation (not collected)
R&S	Th. Raus & H. Sipman
RMB	R.M. Burton
SZ	Sevasti Zervou

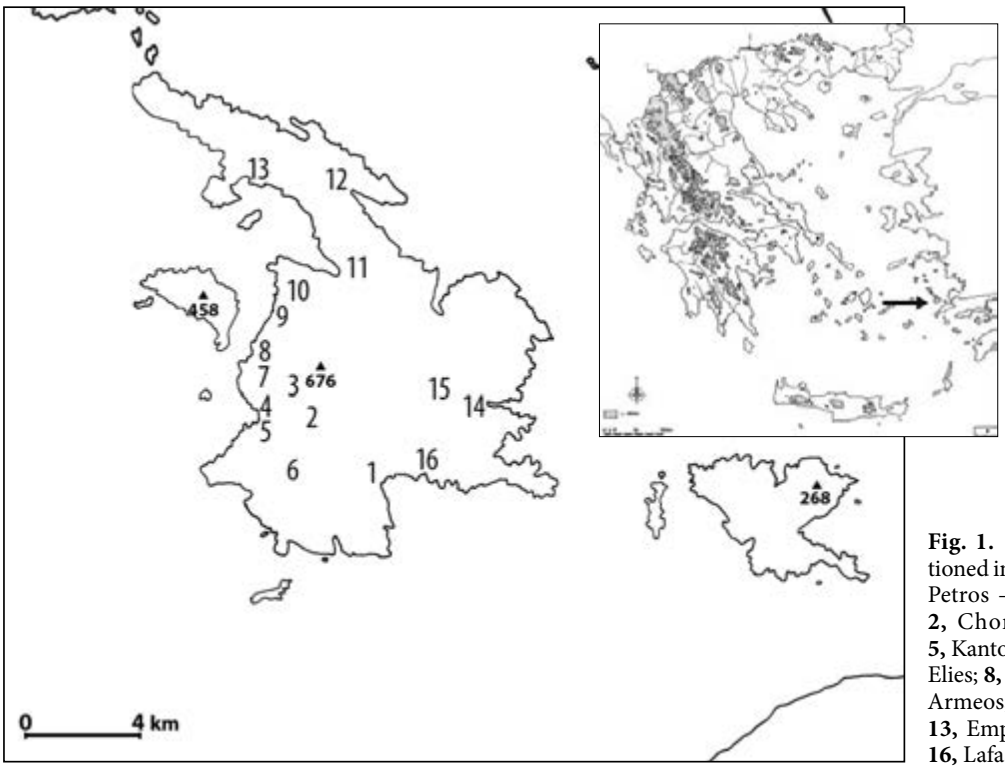

Fig. 1. Localities in Kalimnos, mentioned in the floristic list: **1,** Pothia – Ag. Petros – Ag. Varvara – Chrisocheria; **2,** Chora; **3,** Tsoukalio; **4,** Linaria; **5,** Kantouni; **6,** Argos; **7,** Platis Gialos – Elies; **8,** Mirties; **9,** Sikia; **10,** Masouri – Armeos; **11,** Arginonta; **12,** Palionisos; **13,** Emporios; **14,** Vathis; **15,** Metohi; **16,** Lafasi.

Floristic list

1. **Abutilon theophrastii* Medik. – Palaeotrop. – Sikia, 25.09.2001 (SZ obs.).
2. **Aeonium arboreum* (L.) Webb & Berthel. – Macarones. – Sikia, 30.04.2006 (SZ obs.); Pothia, 29.04.2006 (SZ obs.).
3. **Agave americana* L. – N-Amer. – Palionisos, 20.08.1998 (SZ obs.).
4. **Ailanthus altissima* (Mill.) Swingle – China – Mirties, 20.09.2000 (R&S obs.); Kantouni, 20.04.2001 (SZ obs.); Linaria, 20.04.2001 (SZ obs.). Naturalized.
5. **Amaranthus albus* L. – N-Amer. – Pothia, 27.02.1998 (SZ 482); Metohi, 21.09.2000 (R&S 24121).
6. **Amaranthus blitoides* S. Watson – N-Amer. – Vathis, 24.09.1989 (RMB 89.13), 17.08.1999 (SZ 1846), 21.09.2000 (R&S 24111); Mirties, 20.09.2000 (R&S 24096); Masouri, 20.09.2000 (R&S 24097).
7. *Amaranthus cruentus* L. – Neotrop. – Linaria, 21.09.1989, RMB 89.6 (Tan & Panitsa 2000).
8. **Amaranthus hybridus* L. – N-Amer. – Vathis, 17.08.1999 (SZ 1842a), 21.09.2000 (R&S 24110).
9. **Amaranthus quitensis* Kunth – S-Amer. – Vathis, 21.09.2000 (R&S 24112).
10. **Amaranthus retroflexus* L. – N-Amer. – Vathis, 17.08.1999 (SZ 1842b), 21.09.2000 (R&S obs.).
11. *Amaranthus viridis* L. – S-Amer. – Kantouni (AH); Pothia (AH), 30.09.1989 (RMB 89.30); Emporios, 23.09.1989 (RMB 89.10); Vathis, 17.08.1999 (SZ 1854), 20.09.2000 (R&S 24070), 16.11.2001 (SZ 2383a); Metohi, 21.09.2000 (R&S 24120). Naturalized, found throughout the inhabited part of Kalimnos.
12. **Apium graveolens* L. – Paleotemp.? – Lafasi, 26.04.2006 (SZ obs.). Escaped from cultivation.
13. **Aptenia cordifolia* (L. f.) Schwantes – S-Afr. – Path from Linaria to Platis Gialos, 01.03.1998 (SZ 572).
14. *Arundo donax* L. – C-Asiat. – FAe, Arginonta (AH).
15. **Aster squamatus* (Spreng.) Hieron. – Neotrop. – Pothia, 24.09.1989 (RMB 89.18); Kantouni, 20.04.2001 (SZ obs.); Masouri, 25.09.2001 (SZ 2370).
16. **Calendula officinalis* L. – ? – Vathis, 28.04.2006 (SZ 2620). Escaped from cultivation, found at a road margin inside the village.
17. *Cannabis sativa* L. – C-Asiat. – Pothia (AH).
18. **Cardiospermum halicacabum* L. – Trop. – Chrisoheria, 29.09.1989 (RMB 89.28).
19. **Carpobrotus edulis* (L.) N.E. Br. – S-Afr. – Sikia, 25.09.2001 (SZ obs.).

20. **Chasmanthe vittigera* (Salisb.) N.E. Br. – S-Afr. – Above Platis Gialos, 01.03.1998 (SZ 582). Obviously escaped from cultivation. To our knowledge, this seems to be the first record of this species from the Aegean islands as a whole and the second from Greece. There is another very recent record from the small Ionian island of Kastos (Chousou-Polydouri & Yannitsaros, in press).
21. *Chenopodium ambrosioides* L. – Neotrop. – FH1, Vathis, Davis 67901 (Tan 1982), 24.09.1989 (RMB 89.15); Pothia, 11.08.1998 (SZ 853), 21.09.2000 (R&S 24104).
22. *Cicer arietinum* L. – Pont.? – Arginonta (AH).
23. **Citrullus lanatus* (Thunb.) Matsum. & Nakai – Palaeotrop. – Masouri, 25.09.2001 (SZ obs.).
24. *Conyza bonariensis* (L.) Cronquist – S-Amer. – AH (Grierson 1975); Ag. Petros, 27.02.1998 (SZ obs.); Ag. Varvara, 24.06.1998 (SZ 796); Vathis, 17.08.1999 (SZ 1850); Masouri, 17.09.2000 (R&S 24024); Kantouni, 20.09.2000 (R&S 24074). Naturalized and widespread throughout the island.
25. **Conyza canadensis* (L.) Cronquist – N-Amer. – Vathis, 20.02.1999 (SZ 933), 17.08.1999 (SZ 1843, 1849); Metohi, 21.09.2000 (R&S 24122). Naturalized.
26. *Coriandrum sativum* L. – SW-Med. – Pothia (AH).
27. *Datura innoxia* Mill. – S-Amer. – Pothia (AH).
28. *Datura stramonium* L. – Cosmop. – Pothia (AH); Vathis (AH), 20.02.1999 (SZ obs.); Kantouni, 16.11.2001 (SZ obs.). Naturalized.
29. **Eleusine indica* (L.) Gaertn. – Termocosmop. – Masouri, 06.08.2005 (SZ 2548).
30. **Iris germanica* L. – ? – Chora, 27.04.2006 (SZ obs.); Argos, 27.04.2006 (SZ obs.); Sikia, 30.04.2006 (SZ obs.).
31. **Kochia scoparia* (L.) Schrad. – C-Asiat. – Masouri, 05.10.1991 (Kraft s.n.). Specimen not seen, found on the website of the Botanical Museum of Lund.
32. *Lens culinaris* Medik. – ? – Pothia (AH).
33. **Linum grandiflorum* Desf. – N-Afr. – Emporios, 16.03.2002 (SZ 2438). A new adventive species for Greece, probably escaped from cultivation. There is a very small population at the margin of a fallow field in Emporios.
34. **Lobularia maritima* (L.) Desv. – Stenomedit. – Vathis, 20.04.2002 (SZ 2475). Cultivated in gardens and escaping.
35. **Lycopersicon esculentum* Mill. – C-S-Amer. – Masouri, 25.09.2001 (SZ obs.). Cultivated as a vegetable and escaping.
36. **Matthiola incana* (L.) R. Br. – Stenomedit. – Between Platis Gialos and Elies, 01.03.1998 (SZ 578). Cultivated in gardens and escaping.
37. **Medicago arborea* L. – N-E-Med. – Metohi, 26.04.2006 (SZ obs.); Sikia, 30.04.2006 (SZ obs.). Escaped from cultivation.
38. *Melia azedarach* L. – India-China – Kantouni (AH), 20.04.2001 (SZ obs.). Naturalized, according to Hansen (1980).
39. **Mirabilis jalapa* L. – S-Amer. – Metohi, 21.09.2000 (R&S 24125); Vathis, 02.12.2001 (SZ 2387).
40. *Nicotiana glauca* Graham – S-Amer. – Pothia (AH), 27.02.1998 (SZ obs.); Armeos, 28.02.1998 (SZ obs.); Masouri, 25.06.1998 (SZ obs.), 20.09.2000 (R&S obs.); Mirties, 16.11.2001 (SZ obs.); Emporios, 17.11.2001 (SZ obs.). A widespread, naturalized species, found in almost every part of the island, mainly on roadsides.
41. *Nicotiana virginica* L. – Amer. – Ciferri (1944) recorded this species from Kalimnos (in Hansen 1980), but its presence needs confirmation, as it has not been located since.
42. **Opuntia ficus-barbarica* A. Berger – Neotrop. – Between Platis Gialos and Elies, 01.03.1998 (SZ obs.).
43. **Ornithogalum arabicum* L. – S-Med. – Pothia, 29.04.2006 (SZ 2621). Escaped from cultivation.
44. *Oxalis pes-caprae* L. – S-Afr. – Pothia, (AH), 27.02.1998 (SZ 415); Armeos, 28.02.1998 (SZ obs.); Kantouni, 01.03.1998 (SZ obs.); Chrisoheria, 19.02.1999 (SZ obs.); Vathis (AH), 20.02.1999 (SZ obs.); Arginonta (AH). Fully naturalized and widespread throughout the inhabited part of the island.
45. **Parthenocisus* sp., Armeos, 30.04.2006 (SZ 2641).
46. *Ricinus communis* L. – Palaeotrop. – Pothia (AH), 27.02.1998 (SZ obs.); Masouri, 28.02.1998 (SZ obs.), 20.09.2000 (R&S obs.); Vathis, 20.02.1999 (SZ obs.). Naturalized.
47. *Setaria adhaerens* (Forssk.) Chiov. – Subcosmop. – Pothia (AH); Sikia, 25.08.1998 (SZ 858); Tsoukalio, 28.11.1998 (SZ 898b); Vathis, 17.08.1999 (SZ 1851); Masouri, 17.09.2000 (R&S 24028). Naturalized.
48. **Triticum* sp., Pothia, 25.04.2002 (SZ 2476).
49. **Tropaeolum majus* L. – S-Amer. – Emporios, 30.04.2002 (SZ 2529). Cultivated in gardens and escaping.
50. **Vicia faba* L. – ? – Emporios, 08.03.2002 (SZ 2419).

Chorological analysis – discussion

As mentioned above, the chorological elements are given mostly according to Pignatti (1982). In order to make the chorological spectrum (Fig. 2) of the adventive flora of Kalimnos, we put together the chorological elements into larger groups, shown in Table 1. For the chorological analysis we took under consideration 48 taxa, since 2 were not possible to be identified beyond genus (*Parthenocisus* sp. and *Triticum* sp.).

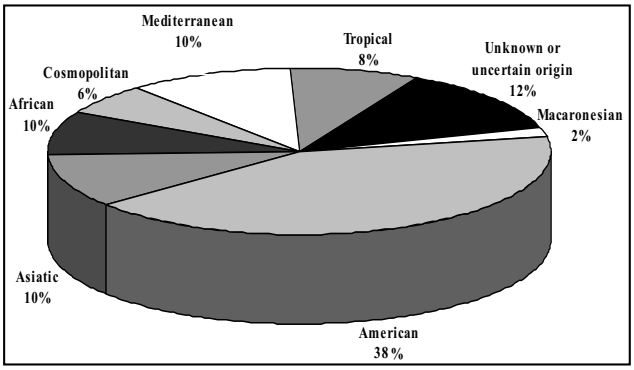

Fig. 2. The chorological spectrum of the adventive flora of Kalimnos.

Table 1. The number of adventive taxa included in each group of chorological elements, as well as their percentage.

Groups of chorological elements	Number of taxa	%
American (N-S-C-Amer., Neotrop.)	19	38
Asiatic (C-Asiat., India, China)	5	10
Mediterranean (N-E-S-W-Med., Stenomedit.)	5	10
African (N-S-Afr.)	5	10
Tropical (Trop., Palaeotrop.)	4	8
Cosmopolitan (Cosmop., Termocosmop., Subcosmop.)	3	6
Macaronesian	1	2
Unknown/Uncertain origin	6	12
Total	**48**	**96%**

From the chorological spectrum (Fig. 2), it is clear that the majority of the adventive species (38 %) originates from America. In this category we find some species that seem to be fully naturalized in Kalimnos, such as *Conyza bonariensis*, *Nicotiana glauca* and *Amaranthus viridis*.

The taxa originating from Asia, Africa and the Mediterranean region are 10 % each, followed by the Tropical ones (8 %). Among the African species, there is *Linum grandiflorum*, recorded for the first time as an adventive species for Greece. This species probably escaped from cultivation and its presence is casual.

A very interesting South African species that escapes from cultivation is *Chasmanthe vittigera* which is recently found as an adventive in Greece.

Acknowledgements. The authors would like to thank Dr Th. Raus (Berlin) for the kind offer of data regarding his own collections in Kalimnos together with H. Sipman, as well as collections made by R.M. Burton.

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Bulgarian Botanical Files for risk assessment of impact of genetically modified plants on the Bulgarian native flora

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Abstract. Bulgarian Botanical Files were developed to assess the spontaneous gene flow from genetically modified plants (GMP) to the native flora of Bulgaria. The risk of gene transfer from 60 cultivated plants to their native wild forms and relatives was evaluated. Floristic, ecological and biological information was summarized in dispersal codes $D_{pdf.}$ Cultivation of 29 species could not cause negative effect. For 26 species it could be expected significant negative effect due to existence of wild forms and relatives. For three species it may be expected minimal and/or local effect on the spontaneous flora while for two ones local to significant negative effect could be expected in the contact areas.

Key words: Bulgarian native flora, genetically modified plants, risk assessment

Introduction

To regulate the utilization of genetically modified organisms (GMO) and to respond to public concerns, Cartagena Protocol on biosafety, which regulates safety transfer, use and release of GMO, was signed in 2002. The importance of the problem could be illustrated by the finding that 12 out of 13 world's most important crops hybridize with their wild relatives (Ellstrand 2001, 2003; Ellstrand & al. 2002).

Studies, so called Botanical Files (BF), analysing spontaneous gene flow from crops to native flora were carried out by de Vries & al. (1992) and Ammann & al. (1996). The authors stated that the spontaneous gene transfer could be realized by dispersal of pollen, seeds and other diaspores from cultivated to wild plants. Codes for assessment of these processes were introduced: for gene dispersal by pollen (D_p), gene dispersal by seeds and other diaspores (D_d) and a qualitative code D_f, indicating frequency of occurrence. An integral code D_{pdf} serves as a tool for assessment of the ecological effect of gene flow from cultivars to wild species.

The main purpose of UNEP–GEF project for Bulgaria was to establish regulatory and administrative frame for safety transfer use and release of GMO. Within this project Bulgarian Botanical Files (BBF) for risk assessment of genetically modified (GM) crops and their impact on biodiversity were developed.

Methodology

The basic definitions and terms used in the existing treatments were accepted (de Vries & al. 1992; Ammann & al. 1996). All data collected were incorporated into a Botanical File for each species in a standard format following the items: 1. Introduction, modes and areas of cultivation, origin; 2. Escape; 3. Wild relatives; 4. Hybridization; 5. Conclusion.

The codes D_p and D_d were determined according to de Vries & al. (1992). Spontaneous gene dispersal by pollen (D_p) concerns the crossing among plants of a species, interspecific and intergeneric hybridization. Seven categories were accepted for D_p with values from 0 to 5 and 9, when there is not reliable evidence. Spontaneous gene dispersal by seeds and other diaspores (D_d) reflects the mode and capacity of diaspore dispersion. Six categories (from 0 to 4 and 9) for code D_d were accepted.

For lack of standard agrostatistical references such as *Hoofdafdeling Landbouwstatistieken* (1989), *Landbouwtelling* (1989) and chorological treatment in Bulgaria equivalent to *Atlas van de Niderlandse Flora* (1985, 1989), used by de Vries & al. (1992), the method for determina-

tion of the frequency of occurrence of species in the wild (code D_f) was adapted. We analysed the distribution of totally 4233 taxa (Kozhuharov & al. 1992) in 26 floristic regions of Bulgaria and frequency of each taxon was determined according to number of floristic regions where it has been found (Table 1). Categories of D_f:

- 0: The species is not native for Bulgarian flora and it is not found out of cultivation;
- 1: The species is native for Bulgarian flora, extremely rare to rare, occurs in 1–3 floristic regions or it is not native for Bulgarian flora but it is found out of cultivation;
- 2: The species is native for Bulgarian flora, rare to less rare, occurs in 4–10 floristic regions;
- 3: The species is native for Bulgarian flora, commonly to widely distributed, occurs in 11–26 floristic regions;
- 9: Distribution of the species is unknown or uncertain.

Protocol for making BBF

Choice of plant species: BBFs were developed for 60 cultivated plants (main crops, medicinal and essential oil plants) selected according to their economic and ecological importance.

Table 1. Categories of the code D_f according to the frequency of occurrence of taxa in the Bulgarian flora.

Category of D_f		Occurrence frequency class in Bulgarian flora	Number of taxa	% of total number of taxa in Bulgarian flora
1	The species is extremely rare to rare	1	843	39
		2	454	
		3	355	
2	The species is rare to less rare	4	312	29
		5	261	
		6	211	
		7	149	
		8	142	
		9	76	
		10	67	
3	The species is commonly to widely distributed	11	51	32
		12	35	
		13	29	
		14	30	
		15	10	
		16	6	
		17	7	
		18	9	
		19	4	
		20–25	0	
		26	1182	

Data collection: Taxonomic and floristic sources used: the Chorological card-index of the Institute of Botany; data from Bulgarian Herbaria; thesises, reviews, monographs, articles, unpublished data and observations; personal communications. The list of all wild forms and relatives was based on main treatments of the Bulgarian flora (Jordanov 1970–1979; Velchev 1982–1989; Kozhuharov 1992, 1995; Delipavlov & Cheshmedzhiev 2003) and *Flora Europaea* (Tutin & al. 1968–1980, 1993). The possibility for crossing and hybridization was evaluated according to Zhukovski (1964), Zohary & Hopf (1993), Heywood & Zohary (1995), Gabrielian & Zohary (2004). For identification of crops and cultivars, regions and areas of cultivation we summarized information from various sources (Stefanoff & Kitanov 1962; Popov & Dimitrov 1983; Kitanov 1986; Topalov & al. 1989; Yankulov 2000; Kartalov & al. 2004), official documents of Ministry of Agriculture and Forestry (MAF), the Official cultivars' lists, and an inquiry on municipal level (162 municipalities) assisted by MAF.

Distribution maps: Electronic maps presenting crops distribution, their wild forms and relatives were made by software MAPPAD 2.0. Topographical data were transformed into geographic coordinates. Distribution of crops (2000–2004) by municipalities was presented by the geographic coordinates of the respective municipal centre. Maps were included in BBF only for species of potential local ecological effect.

Determination of codes (D_p), (D_d) and (D_f): Data collected for each species (crop) were assigned to the respective categories of codes D_p, D_d and D_f. Due to insufficient information in Bulgaria about "running wild" of cultivated forms and existence of natural hybrids with wild relatives (codes D_p and D_d), any relevant data for these processes were also used.

Assessment of ecological risk: Modified combinations of the integral codes D_{pdf} were applied to assess ecological risk for biodiversity of Bulgaria caused by GM crops (Table 2).

Table 2. Combinations of the integral codes D_{pdf}.

Ecological risk	Code D_{pdf}
No effect	0.0.0; 0.0.1; 1.0.0; 0.3.0; 2.0.0; 3.0.0
Minimal / local	3.0.1; 4.0.1; 4.3.0; 4.3.1
Significant	3.3.3; 4.3.2; 4.3.3; 4.4.2; 4.4.3; 5.0.0; 5.0.1; 5.0.3; 5.3.1; 5.3.2; 5.3.3

Results and discussion

Development of BBF methodology made possible evaluation of the risk for negative ecological effect of GM crops on native flora of Bulgaria. Information about the 60 studied species is presented in Table 3.

Twenty nine cultivated plants (48.3 %) could not cause a negative ecological effect as they originate from the New World or Asia and have no wild relatives and hybrids in Bulgarian flora. Genetically modified forms of soybean and maize are most cultivated plants throughout the world. These two crops could not influence negatively native Bulgarian flora. Nevertheless, a potential risk for human health exists due to possibility for gene flow from GM crops intended to create industrial biochemicals to crops grown for human consumption (Ellstrand 2001, 2003).

Twenty six cultivated plants (43.3 %) could cause significant negative ecological effect on spontaneous flora of Bulgaria. This group includes economically important crops as *Avena sativa*, *Secale cereale*, *Beta vulgaris*, *Brassica napus*, *Medicago sativa*, *Prunus avium*, *P. domestica*, *Rubus idaeus*, *Vitis vinifera*. Most of the rest (essential oil and medicinal plants) are facultative crops, genetically not significantly differing from their wild forms/relatives. For this reason, there is a significant risk for gene transfer from their GM forms to native flora of Bulgaria.

Five cultivated plants (8.3 %), *Coriandrum sativum*, *Foeniculum vulgare*, *Sinapis alba*, *Glaucium flavum* and *Salvia officinalis*, could cause minimal and/or local negative ecological effect on the native flora of Bulgaria. Wild forms of the first 4 species and the wild relative of the last one have a limited distribution in Bulgaria. Hence, locally gene flow could be expected in the regions where cultivars are grown near by natural populations of the species. Potato *Solanum tuberosum* belongs to the same group. It hybridizes with species *S. dulcamara* and *S. nigrum*, which are elements of the native flora of Bulgaria, but no viable hybrid seeds are obtained (McPartlan & Dale 1994). For this reason no negative ecological effect of potato on native flora could be expected.

Acknowledgements. This study was carried out by the financial support of UNEP–GEF project for Bulgaria. The authors are thankful to AgroBioInstitute and Acad. A. Atanasov for the useful cooperation.

Table 3. Code D_{pdf} and evaluation of risk for biodiversity of Bulgaria caused by cultivation of genetically modified forms of the investigated species.

	Cultivated plant	Usage	Code	Risk
1	*Leucojum aestivum* L.	medicinal, ornamental	4.4.2	significant
2	*Coriandrum sativum* L.	medicinal and aromatic, food (spice)	4.3.1	local
3	*Daucus carota* L. var. *sativa* Hoffm.	food (vegetable, spice)	4.3.3	significant
4	*Foeniculum vulgare* Mill.	medicinal and aromatic	4.3.2	local
5	*Pastinaca sativa* L.	food (vegetable, spice)	4.3.3	significant
6	*Chamomilla recutita* (L.) Rauschert	medicinal and essential oil	4.4.3	significant
7	*Helianthus annuus* L.	oil plant, food, fodder, melliferous	0.0.0	no effect
8	*Lactuca sativa* L.	food (vegetable)	2.0.0	no effect
9	*Silybum marianum* L.	medicinal	4.4.3	significant
10	*Brassica napus* L.	oil plant, fodder, melliferous	5.3.2	significant
11	*B. oleracea* L.	food (vegetable), ornamental	1.0.0	no effect
12	*Raphanus sativus* L.	food (vegetable)	4.3.3	significant
13	*Sinapis alba* L.	food (spice), medicinal, oil plant	4.3.1	local
14	*Beta vulgaris* L.	food, fodder	4.3.2	significant
15	*Cicer arietinum* L.	food	0.0.0	no effect
16	*Glycine max* (L.) Merr.	food, oil plant	0.0.0	no effect
17	*Lens culinaris* Medik.	food	0.0.0	no effect
18	*Medicago sativa* L.	fodder	5.3.3	significant
19	*Phaseolus vulgaris* L.	food, medicinal	0.0.0	no effect
20	*Trigonella foenum-graecum* L.	food (spice), medicinal	0.3.0	no effect
21	*Hypericum perforatum* L.	medicinal, ornamental	4.4.3	significant
22	*Lavandula angustifolia* L.	essential oil, medicinal, melliferous, ornamental	0.0.1	no effect
23	*Melissa officinalis* L.	medicinal, essential oil, melliferous	4.3.3	significant
24	*Mentha arvensis* L.	essential oil, medicinal, melliferous	3.3.3	significant
25	*M. piperita* L.	essential oil, medicinal, melliferous	1.0.0	no effect
26	*M. spicata* (L.) Huds.	food (spice), medicinal, essential oil, melliferous	3.3.3	no effect
27	*Ocimum basilicum* L.	essential oil, medicinal, melliferous, food (spice), ritual	0.0.0	no effect
28	*Salvia officinalis* L.	medicinal, essential oil, melliferous, anti-erosion, ornamental	3.0.1	local
29	*S. sclarea* L.	essential oil	4.3.3	significant
30	*Satureja hortensis* L.	food (spice), essential oil, melliferous	0.3.0	no effect
31	*Allium cepa* L.	food (vegetable, spice)	0.0.0	no effect
32	*A. porrum* L.	food (vegetable, spice)	5.0.0	significant
33	*A. sativum* L.	food (vegetable, spice), medicinal	0.0.0	no effect

Table 3. Continuation.

	Cultivated plant	Usage	Code	Risk
34	*Althaea officinalis* L.	medicinal, melliferous	5.0.3	significant
35	*Gossypium hirsutum* L.	fibrous, oil plant	0.0.0	no effect
36	*Glaucium flavum* Crantz	medicinal, ornamental	4.0.1	local
37	*Avena sativa* L.	food, fodder	5.0.0	significant
38	*Hordeum vulgare* L.	food, fodder	3.0.0	no effect
39	*Panicum miliaceum* L.	food, fodder	0.0.0	no effect
40	*Secale cereale* L.	food, fodder	5.3.1	significant
41	*Triticum aestivum* L.	food, fodder	3.0.0	no effect
42	*T. dicoccum* Schübl.	food, fodder	3.0.0	no effect
43	*T. durum* Desf.	food, fodder	0.0.0	no effect
44	*T. monococcum* L.	food, fodder	5.3.2	significant
45	*T. turgidum* L.	food, fodder	0.0.0	no effect
46	*Zea mays* L.	food, fodder, medicinal	0.0.0	no effect
47	*Fragaria* × *ananassa* Duchesne	food	0.0.0	no effect
48	*Prunus avium* L.	food	4.3.3	significant
49	*P. domestica* L.	food	5.0.1	significant
50	*Rosa* × *bifera* (Poir.) Pers.	essential oil, food, medicinal	1.0.0	no effect
51	*Rosa* sp.	medicinal, food	4.3.3	significant
52	*Rubus idaeus* L.	food, medicinal	5.3.3	significant
53	*Atropa bella-donna* L.	medicinal	4.3.3	significant
54	*Capsicum annuum* L.	food, medicinal	0.0.0	no effect
55	*Datura stramonium* L.	medicinal	4.3.3	significant
56	*Lycopersicon esculentum* Mill.	food	0.0.0	no effect
57	*Solanum melongena* L.	food	0.0.0	no effect
58	*S. tuberosum* L.	food, fodder	1.0.0	no effect
59	*Valeriana officinalis* L.	medicinal	4.3.3	significant
60	*Vitis vinifera* L.	food, oil plant	4.3.2	significant

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SCIENTIFIC AREA C

PLANT ECOLOGY. VEGETATION AND HABITAT DIVERSITY

On the distribution patterns of *Quercus crenata*: implications of its W Mediterranean and Transadriatic outposts for the epionthology of *Q. suber* and *Q. cerris*

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Abstract. *Quercus crenata* is a hybridogenic species (*Q. suber* × *cerris)* whose morphological variability, distribution patterns and phylogenetic relationships are still imperfectly known. Its range is centred in Mediterranean Italy. It occurs often far from stands of *Q. suber*, reaching the E coasts of the Adriatic Sea as S as Albania. It is also recorded surprisingly far from the W range limits of *Q. cerris,* in Andalusia. Despite of its phenotypic variability, historical cadastral sources and toponymy suggest a traditional clustering of this taxon along with *Q. suber* in Italy, suggesting a larger spread of the *Q. suber* – *Q. crenata* complex along the Adriatic coast in a recent past. Late Quaternary macrofossil records of *Quercus* cfr. *suber* from Greece suggest a previous unexpected larger range of the complex reaching the Aegean region at East, where it has apparently been affected by a subrecent retreat.

Key words: phytogeography, *Quercus crenata*, toponyms

Introduction

Quercus crenata Lam. (syn.: *Q. pseudosuber* Santi: cfr. Pignatti 1982) is a hybridogenic species (Q. *cerris* × *Q. suber* Borzí: cfr. Fiori 1969) whose morphological variability and distribution patterns are only imperfectly known. The hybrid nature of *Q. crenata* in populations from Central Italy was supported (see Schirone & al. 1995; Bellarosa & al. 1996, 2005) by means of molecular analysis (seed storage proteins resolving, restriction fragment length polymorphism – RFLP – of nuclear ribosomal genes and nuclear internal trabscribed spacers – ITS – sequencing).

Quercus crenata belongs to *Quercus* L. subgen. *Cerris* sensu Schwarz (1936–1939). It is a semi-evergreen taxon encompassing several introgressive forms between *Q. suber* L. and *Q. cerris* L. (Schirone & al. 1989). Leaves are coriaceous (as in *Q. suber*), shortly lobated and mucronate with an intermediate shape between *Q. suber* and *Q. cerris*. They are persistent throughout the winter and are shed in spring, just before the new leaves develop. The cupules are hemispheric with long, narrow and curled scales, very similar to those of *Q. cerris*. Aborted acorns are very frequent. The trees can reach 20 m height. The bark ranges from moderately to completely corky. In Italy populations grow at different elevations (0–1100 m) mostly on more sunny sites in the North, where regeneration is usually poor (Armiraglio & al. 2003) or absent (Cristofolini & Crema 2005).

Results

Results based on a morphometric analysis of the *Q. crenata* species–complex (Cristofolini & Crema 2005) show that populations in Southern Italy and Sicily should retain a larger morphological heterogeneity than northern ones. According to this, the authors suggest the former to be offspring of ongoing hybridization processes (*Q.* × *pseudosuber* Santi) while the latter, far away from the range limits of one of the putative parental species, *Q. suber*, to be legacy of more ancient events of introgression (*Q. crenata* Lam. s.s.).

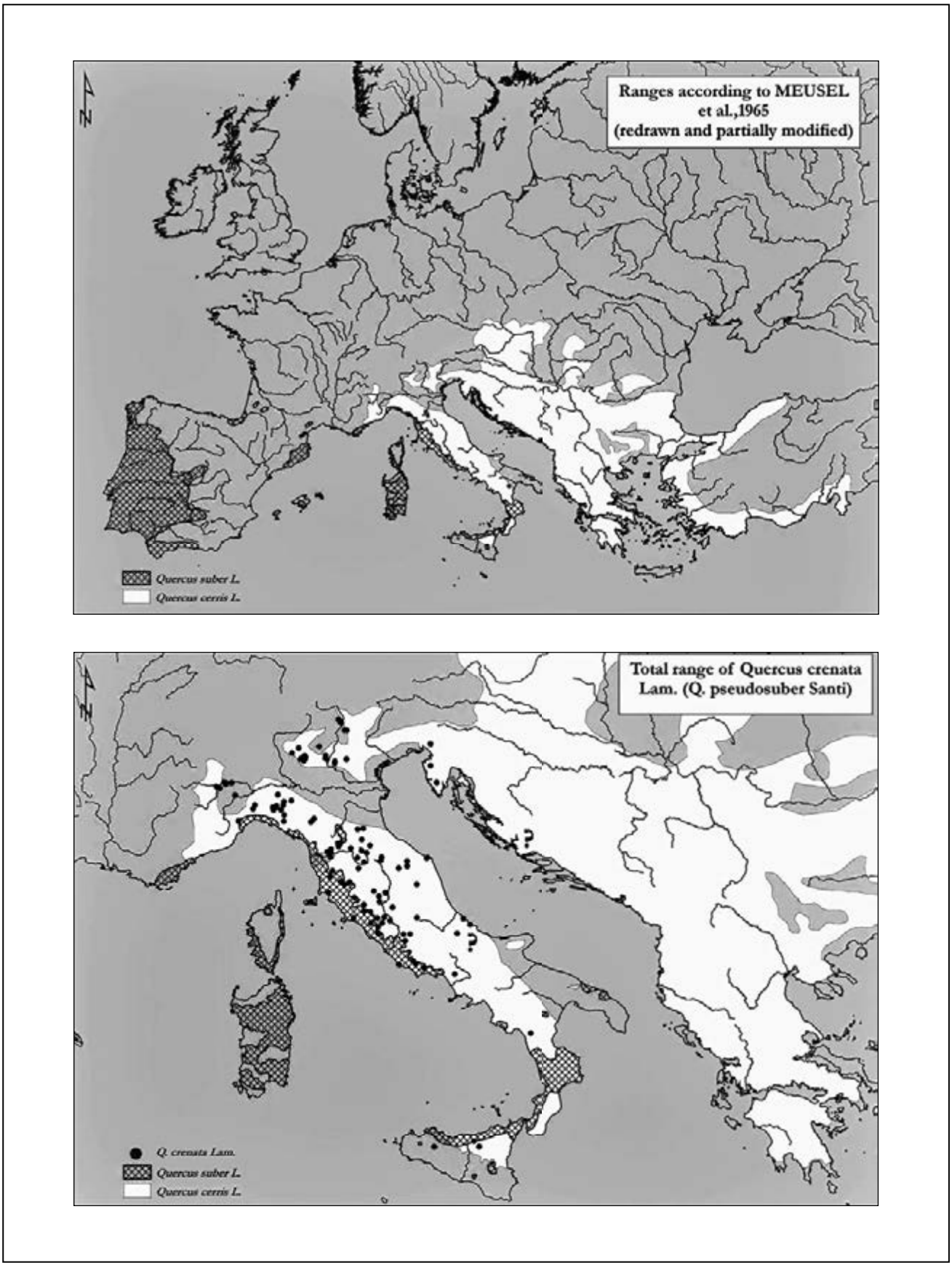

Fig. 1. Compared distributions of *Q. cerris*, *Q. suber* and *Q. crenata* (Africa omitted).

Whether or not this variability might justify the risky nomenclatural split proposed by these authors, the range-bulk of a *Q. crenata* s.l. is apparently centred in the Italian peninsula within territories belonging to the Mediterranean and Submediterranean floristic domains (sensu Walter & Straka 1970). It often occurs far from stands of both parental species, reaching the eastern coasts of the Adriatic Sea in Slovenia and Istria. An outstanding record is reported very far from the western range limits of *Q. cerris* in Andalusia (Camus 1936–1954).

While *Q. suber* is concentrated in the West Mediterranean regions, the range bulk of *Q. cerris* is located in Eastern Europe, reaching the forest steppe of the Anatolian highlands.

The range of the putative hybrid *Q. crenata* s.l. is shifted towards the North and East, reaching the Forealps and the northern districts of the Adriatic coast (*Q. adriatica* Simonk.: cfr. Fiori 1969).

At present peninsular Italy is the only territory in Europe where the ranges of these three species do overlap.

Its occurrence along the Adriatic coast of the Balkans is highly controversial. Older records report *Q. crenata* and its parent species, *Q. suber*, both in Dalmatia and Albania (De Visiani 1842–1852, 1872). According to Camus (1936–1954) the populations of *Q. suber* in Dalmatia should rather be referred to *Q. crenata*. Records from Spain, Albania and Greece (Camus 1936–1954; Greuter & al. 1986 and Schwarz 1993) were not reported by Jalas & Suominen (1976) and not accepted by contemporary local floras (Demiri 1983; Paparisto 1988), or are yet to be confirmed. In Croatia as well, *Q. crenata* has been deleted from the national check list (Nikolić 1996). This species is now confirmed only for Slovenia (Cristofolini & Crema 2005), while *Q. suber* is at present considered to be absent all along the coasts of the Balkans (Fig. 1).

Despite of its phenotypic variability, historical cadastral sources and toponymy suggest a traditional clustering of this taxon along with *Q. suber* throughout peninsular Italy and emphasize a long-lasting scarcity of population of *Q. suber* – *Q. crenata* complex along the Adriatic coast (Fig. 2).

However toponyms suggest a larger spread in the East of Italy of at least *Q. suber* in previous times.

Historical documents from the beginning of the 20th century report the persistence of large stands of *Q. suber* in the territory of Brindisi (Apulia) as late as the end of the thirties.

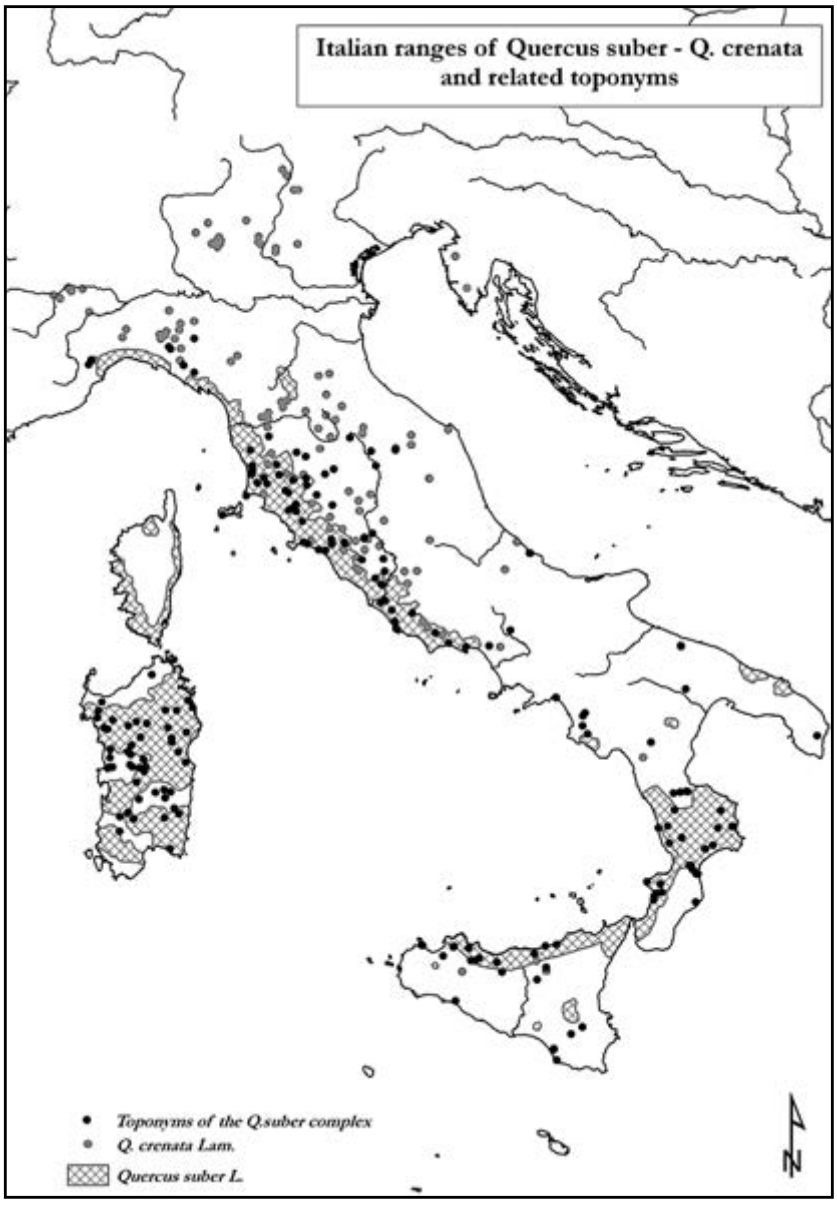

Fig. 2. Toponymy related to *Q. suber* compared with ranges of *Q. suber* and *Q. crenata* in Italy.

Discussion

The controversial records from the Balkans suggest that *Q. crenata* s.l. experiences at present the consequences of an ongoing retreat at its easternmost range limits, at least since the onset of historical times.

This retreat seems to parallel the retreat of one of its putative parental species, *Q. suber,* across the same timespan, at both sides of the Adriatic Sea. According to remnants now deposited at Crecchio, a location in Abruzzi at 42° latitude along the Middle–Adriatic coast, a last stand was completely destroyed as early as in the 6th century. In Greece macroremains of *Q. suber* (along with *Corylus* and *Pinus pinaster*) from the region of Athens, dating back not earlier than to the Epiglacial or early Holocene, along with linguistic remains (*phellos* = cork or the cork-oak) and the record reported by Grisebach (1877), suggest a previous unexpected larger range of the complex reaching the Aegean region at East, where it has apparently been affected by a subrecent retreat.

Whether or not this retreat might be mostly due to anthropogenic influence (subrecent deforestation), in the long run this process seems to be also controlled by the climatic changes of the late Quaternary and the last glacial cycle, which might have affected the whole *Q. suber* complex at easternmost limits of its European range.

Quercus cerris does not seem to be affected at same extent. Its apparently higher resilience to climatic changes enabled it to reach the Forealps during the recover of the forests Holocene reafforestation.

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Vegetation diversity mapping and conservation value assessment of reed beds and wet meadows in lakes of Western Greece

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Abstract. Lakes Trichonis and Lysimachia, both NATURA 2000 sites, belong to the western chain of Greek wetlands running along the coast of the Ionian Sea. The study of reed bed and wet meadow vegetation diversity at the plant community and habitat type levels, revealed 27 syntaxa (11 associations and 16 communities) that correspond to 8 habitat types. The vegetation units were studied and mapped using GIS. The conservation value for each community and corresponding habitat types were assessed using numerical scales for the most frequently used criteria, such as diversity, rarity, threat, naturalness and replaceability. Multi-Criteria Evaluation (MCE) and GIS techniques were used to estimate the total conservation value of each community and habitat type. From the results it appears that the conservation value assessment maps of NATURA 2000 sites could be used as an effective management and monitoring tool in long-term conservation strategies.

Key words: diversity, GIS, Greece, reed beds, wet meadows

Introduction

Lakes Trichonis and Lysimachia are included in the Greek part of the European Ecological Network NATURA 2000, designated as one of the proposed Sites of Community Importance (pSCI). The NATURA 2000 Ecological Network in Greece consists of 359 sites categorized as follows: 208 sites have been designated Sites of Community Importance (SCIs), 120 sites as SPAs (Special Protection Areas) and 31 have been designated as both SCIs and SPAs (Dimopoulos & al. 2006). Both lakes are characterized by high diversity in vegetation units and habitat types (Annex I, Directive 92/43/EEC).

In the last five decades, the wetlands of W Greece with high ecological and nature conservation value, have suffered significantly from human activities (ratio of extant to extinct wetlands in the western chain is 1:2.5, with significant loss in the south) (Psilovikos 1992). Consequently, the assessment of the conservation status of the studied lakes could be used as an effective management and monitoring tool in long-term conservation strategies.

The vegetation diversity of lakes Trichonis and Lysimachia (NW Greece) was little known until recently. Descriptive information on the vegetation of the two lakes originated from Koussouris & Diapoulis (1982), Koumpli-Sovantzi (1983) and Koumpli-Sovantzi & Vallianatou (1985). The objectives of this paper are to: a) record and quantify the diversity of vegetation units and habitat types in both lakes; b) map the distribution pattern of the wet meadows and reed thicket vegetation; c) assess and map the conservation status of each vegetation unit applying a multi-criteria approach.

Study area

Lakes Trichonis (T) and Lysimachia (L) are situated in Western Greece. Lake Trichonis (38°34'N, 21°30'E) is the largest natural lake in Greece in terms of size (area: 96.9 km^2, perimeter: 51 km, max water depth: 58 m) and water volume (3×10^9 m^3). Lake Lysimachia (38°34'N, 21°23'E) is a shallow fresh-water lake with max water depth less than 9 m (2.8 km to the west of Lake Trichonis) and an area of 13.2 km^2 and perimeter 17 km (Fig. 1). The two lakes are connected the one to the other, as well as to the River Acheloos via a ditch (A) and a canal, respectively (Fig. 1). Both lakes are of tectonic origin, resulting

from the post-alpine tectonic subduction created mainly by two fault systems of E–W and NW–SE direction. A secondary fault system positioned perpendicularly to the above, defines the area's hydrographic network of mainly seasonal flowing water channels and small streams. In addition, the presence of the crossed fault systems and the tectonic plates of the Pindos geotectonic zone favour the selective movement of groundwater and the formation of springs and sinkholes that all contribute to the water reserves of the hydrological basin.

Material and methods

To identify the vegetation units, 426 relevés were sampled according to the Braun-Blanquet method (Westhoff & van der Maarel 1978; Kent & Coker 1994); 177 relevés were made in reed thickets and 249 in wet meadows vegetation. The total data set of relevés was classified by means of TWINSPAN (Hill 1979). For the phytosociological interpretation and syntaxonomic assignment of the vegetation units, we mainly used Horvat & al. (1974), Raus (1980), Mucina (1997), as well as Weber & al. (2000) for applying nomenclatural rules.

The assessment of the conservation significance of the studied vegetation is based on the evaluation system suggested by Dimopoulos & al. (2000). Five criteria (Diversity, Rarity, Threat, Naturalness and Replaceability) were used to score and estimate the total conservation value of each syntaxonomic unit. The numerical values for each criterion were assigned according to the 10-point scale given by Dimopoulos & al. (2000). The average criterion value of the polygons for each vegetation unit is given in Table 2. The software ArcView GIS Version 3.1 was used for the spatial distribution of the vegetation communities and to produce a vegetation map. Following this procedure five maps were created, each one forming a separate layer in the GIS. Multi-Criteria Evaluation (MCE) and the corresponding software ArcView GIS were used to evaluate the plant community conservation significance using the combined influence of the five criteria (Carver 1991; Eastman 1997).

The ratings for a pairwise comparison matrix (containing the lower half of a symmetric matrix) between the criteria were used to calculate a best fit set of weight. The pairwise comparison matrix and the rating scale for the criteria are according to a 9-point continuous scale, ranging from 1/9 to 9 (extremely less to extremely more important), given in Boteva & al. (2004). Highest importance was assigned to Naturalness, Rarity and Diversity in the pairwise comparison matrix (Boteva & al. 2004). These factors are considered to account for the majority of the ecological value of a community type (Smith & Theberge 1986; Usher 1986; Dimopoulos & al. 2000). Each criterion map (diversity, naturalness, threat, etc.) was multiplied by its weight and summed to generate a map of the conservation score for each vegetation unit. The calculated weight for each criterion is as follows: Diversity – 0.3030, Rarity – 0.3298, Naturalness – 0.2663, Threat – 0.0633, Replaceability – 0.0377.

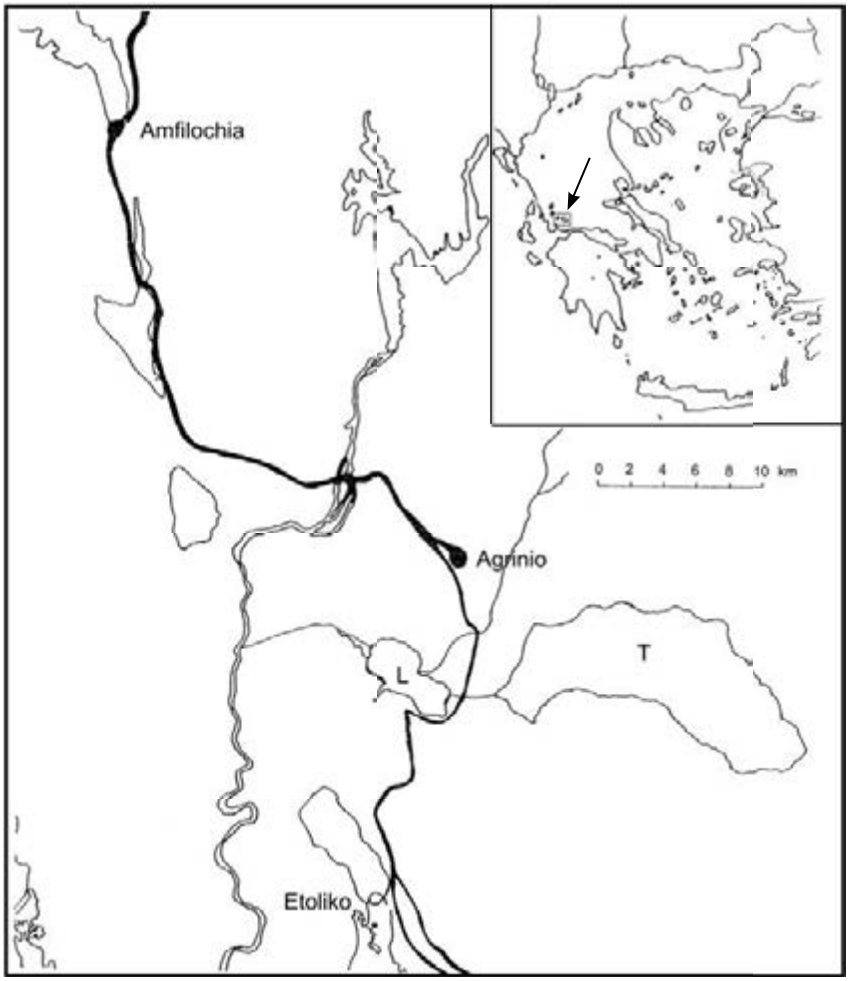

Fig. 1. Map of lakes of Trichonis (**T**) and Lysimachia (**L**).

Results

Analysis of the 426 relevés on wet meadows and reed thickets vegetation resulted in the identification of the 27 vegetation units attributed at different syntaxonomic ranks (Table 1). Of the 27 distinguished vegetation units, 24 occur in Lake Trichonis and 16 in Lake Lysimachia (Table 1). The *Phragmition* and *Trifolion resupinati* are the most variable alliances in the reed thickets and wet meadows vegetation, respectively (Table 1). These vegetation units represent 8 habitat types (Annex I, Directive 92/43/EEC), of which the habitat type "Calcareous fens with *Cladium mariscus* and species of *Caricion davallianae* (7210)" is a priority one for conservation at the European level.

Evaluation scores of the Diversity, Rarity, Naturalness, Threat and Replaceability criteria for each vegetation unit are shown in Table 2.

A vegetation map, as well as a map showing the conservation significance of the wet meadows and reed thickets vegetation in the lakes Trichonis and Lysimachia were produced but not presented here for technical reasons.

The MCE analysis showed that the following syntaxonomic units per lake have high conservation value:

I) Lake Lysimachia: a) *Carex acutiformis* comm. in the NW of the lake; b) *Ranunculus marginatus* comm. in the SE of the lake; c) *Cyperetum longi* in the NE and NW of the lake; d) *Trifolium resupinatum* comm. in the NW of the lake.

II) Lake Trichonis: a) *Trifolium resupinatum* comm. in the NW, N, E, W, SE and SW of the lake; b) *Ranunculus marginatus* comm. in the N and NW of the lake; c) *Alopecuro–Ranunculetum marginati* in the NW and S of the lake; d) *Cladietum marisci* in the S and SE of the lake; e) *Veronica anagallis-aquatica* comm. in the NE of the lake; f) *Sparganietum erecti* in the SW and NE of the lake.

These vegetation units are characterized by the highest conservation value due to the high score of the threat criteria. Furthermore, the highest value of the diversity criterion, especially for the *Trifolium resupinatum* comm., *Ranunculus marginatus* comm. and *Alopecuro–Ranunculetum marginati*, in combination with the low value of the replaceability criterion, could explain the highest conservation score for these vegetation units. *Scirpoides holoschoenus* comm. and *Paspalum paspaloides* comm. are the vegetation units with the lowest total conservation score; this is due to the low scores for the threat and naturalness criteria of these communities, which are not very sensitive to environmental changes.

Discussion

The highest number of vegetation units is observed in Lake Trichonis (Table 1), due to its large surface area and the different trophic status in the two lakes which may also explain the difference in the number of vegetation units (Rørslett 1991). Trichonis is an oligotrophic lake which supports the development of a great variety of vegetation units. The low diversity in vegetation units observed in Lake Lysimachia is due to rapid water level fluctuation which influences plant and vegetation diversity (Rørslett 1991). The *Phragmito–Magnocaricetea* is characterized by the highest number of syntaxa (9) in the reed thicket vegetation and the *Molinio–Arrhenatheretea* is the most variable class of the wet meadow vegetation with seven (7) syntaxa.

The GIS–MCE technique is a very useful tool for identifying sites for habitat and species management and conservation (Potter & al. 1993; Cook & Norman 1996; Bian & West 1997; Brown & al. 1998; Lee & al. 2001; Lurz & al. 2001). From the criteria used in the present study, it is evident that threat and diversity have the maximum value in vegetation units with high conservation scores, in contrast to the replaceability criterion which appears to have low values. The calculation of each criterion's weight is an important element in order to create maps with the conservation score of the vegetation units using MCE (Boteva & al. 2004).

Regarding the evaluation of the conservation significance of the vegetation units in both lakes, it is evident that *Carex acutiformis* comm., *Ranunculus marginatus* comm., *Cyperetum longi*, *Trifolium resupinatum* comm., *Alopecuro–Ranunculetum marginati*, *Cladietum marisci*, *Veronica anagallis-aquatica* comm. and *Sparganietum erecti*, have the maximum conservation score. From these vegetation units, *Cladietum marisci* and its corresponding priority habitat type (7210) should be managed properly with the aim of conservation, in the sense of the Directive 92/43/EEC.

In conclusion, knowledge of the diversity of the vegetation units and habitat types of both wetlands, in combination with their inclusion in the Hellenic NATURA 2000 network, impose their conservation through the application of rational management measures. The conservation significance of the vegetation units and the corresponding habitat types could be used for plan-

Table 1. Vegetation units of lakes Trichonis (T) and Lysimachia (L) with their corresponding high-rank syntaxa and habitat types ((h): Hellenic habitat types).

Habitat types (Annex I, Dir. 92/43/EEC) & Hellenic habitats	High-rank syntaxa (Class, Order, Alliance)	Vegetation units	Lakes	
			T	L
72A0 (h)	*Phragmito–Magnocaricetea* Klika in Klika & Novák 1941 *Phragmitetalia* Koch 1926 *Phragmition communis* Koch 1926	*Phragmitetum australis*	+	+
		Phragmites australis–Calystegia sepium comm.	+	+
		Phragmitetum australis solanetosum dulcamarae		+
		Scirpetum lacustris	+	+
		Scirpus lacustris comm.	+	+
		Sparganietum erecti	+	
		Typha domingensis comm.	+	
		Typha domingensis–Phragmites australis comm.	+	+
		Phragmites australis–Ludwigia peploides subsp. *montevidensis* comm.		+
		Scirpetum maritimi	+	
72A0 (h)	*Phragmito–Magnocaricetea* Klika in Klika & Novák 1941 *Nasturtio–Glycerietalia* Pignatti 1953 *Sparganio–Glycerion fluitantis* Br.-Bl. & Siss. in Boer 1942	*Veronica anagallis-aquatica* comm.	+	
72A0 (h)	*Galio–Urticetea* Passarge ex Kopecký 1969 *Calystegietalia sepium* Tüxen 1950 ex Mucina 1993 nom. mut. *Calystegion sepium* Tüxen ex Oberdorfer 1957	*Arundini donacis–Calystegietum sepium*	+	+
7210	*Phragmito–Magnocaricetea* Klika in Klika & Novák 1941 *Phragmitetalia* Koch 1926 *Phragmition communis* Koch 1926	*Cladietum marisci*	+	
72B0 (h)	*Phragmito–Magnocaricetea* Klika in Klika & Novák 1941 *Nasturtio–Glycerietalia* Pignatti 1953 *Magnocaricion elatae* Koch 1926	*Carex acutiformis* comm.	+	+
		Iris pseudacorus comm.	+	
		Cyperetum longi	+	+
1410	*Juncetea maritimi* Tüxen 1951 *Juncetalia maritimi* Br.-Bl. 1931 *Juncion maritimi* Br.-Bl. 1931	*Juncus acutus* comm.	+	
3150	*Lemnetea* Bólos & Masclans 1955 *Lemnetalia minoris* Bólos & Masclans 1955 *Lemnion minoris* Bólos & Masclans 1955	*Lemnetum minoris*	+	
	Potamogetonetea Klika in Klika & Novák 1941 *Potamogetonetalia* Koch 1926 *Nymphaeion albae* Oberd. 1957	*Nymphaeetum albae*	+	
32B0 (h)	*Bidentetalia tripartitae* Br.-Bl. & Tx. ex Klika & Hadač 1944 *Bidentetea tripartiti* Tx. & al. ex von Rochow 1951 *Bidention tripartitae* Nordhagen 1940	*Ludwigia peploides* subsp. *montevidensis–Papsalum paspaloides* comm.		+
3290 (h)	*Potentillo–Polygonietalia* Tx. 1947 *Molinio–Arrhenatheretea* Tüxen 1937 *Paspalo–Agrostidion semiverticillatae* Br.-Bl., Roussine & Negre 1952	*Lythrum junceum–Poa trivialis* subsp. *sylvicola* comm.	+	
		Paspalum paspaloides comm.	+	+
6420 (h)	*Molinio–Arrhenatheretea* Tüxen 1937 *Trifolio–Hordeetalia* Horvatic 1963 *Trifolion resupinati* Mikevski 1957	*Equisetum palustre* comm.	+	+
		Trifolium resupinatum comm.	+	+
		Alopecuro–Ranunculetum marginati Typicum	+	
		Ranunculus marginatus comm.	+	+
6420 (h)	*Molinio–Arrhenatheretea* Tüxen 1937 *Holoschoenetalia* Br.-Bl. (1931) 1947 *Molinio–Holoschoenion* Br.-Bl. ex Tchou 1948	*Scirpoides holoschoenus* comm.	+	+

ning conservation strategies on the wet meadow and reed thicket vegetation and prioritize areas of interest for the application of management measures. Scientifically-based monitoring of the diversity at species and habitat types level, combined with the development and application of GIS techniques, are both constitutive elements of an effective management plan.

Acknowledgements. We thank Sandy Coles (M.Sc.) for linguistic revision and improvement of the manuscript.

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Table 2. Evaluation of the criteria Diversity (D), Rarity (Ra), Naturalness (N), Threat (Th), Replaceability (Re) for each vegetation unit.

Vegetation units	No. of mapping polygons	Th	Re	D	N	Ra
Phragmitetum australis	10	3	8	3	8	2
Phragmites australis–Calystegia sepium comm.	18	3	8	5	8	2
Phragmitetum australis solanetosum dulcamarae	2	5	4	4	6	4
Scirpetum lacustris	14	3	8	3	8	5
Scirpus lacustris comm.	8	3	8	5	8	2
Sparganietum erecti	5	5	4	4	8	3
Typha domingensis comm.	5	3	8	3	8	5
Typha domingensis–Phragmites australis comm.	8	3	8	5	8	5
Arundini donacis–Calystegietum sepium	4	3	8	4	2	5
Phragmites australis–Ludwigia peploides subsp. *montevidensis* comm.	1	4	6	3	6	7
Scirpetum maritimi	5	5	4	5	8	3
Veronica anagallis-aquatica comm.	4	5	4	5	8	8
Cladietum marisci	3	5	6	5	8	3
Carex acutiformis comm.	3	5	4	4	8	8
Iris pseudacorus comm.	4	5	4	4	8	8
Cyperetum longi	6	5	4	5	8	3
Juncus acutus comm.	4	3	3	4	4	8
Lemnetum minoris	3	3	2	1	6	3
Nymphaeetum albae	3	3	2	1	6	3
Ludwigia peploides subsp. *montevidensis–Papsalum paspaloides* comm.	1	4	6	4	4	8
Lythrum junceum–Poa trivialis subsp. *sylvicola* comm.	5	4	4	6	4	8
Paspalum paspaloides comm.	19	2	4	6	4	8
Equisetum palustre comm.	4	3	4	6	4	8
Trifolium resupinatum comm.	13	5	4	6	4	8
Alopecuro–Ranunculetum marginati Typicum	2	5	4	6	4	8
Ranunculus marginatus comm.	7	5	4	6	4	8
Scirpoides holoschoenus comm.	11	2	4	6	4	8

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Secondary metabolites: tools for stress protection in plants

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Abstract. The protective role of secondary metabolites against environmental constraints is substantiated by studying various plant – abiotic stress stimuli systems. It was shown that phenylamides, conjugates of hydroxycinnamic acids and polyamines, are induced in tobacco and bean leaves, subjected to water excess and heat shock, respectively. ROS-scavenging ability of phenylamides was evidenced. Anthocyanins were found to dramatically accumulate in cotton leaves due to Na/K unbalance; shifts from mono- to orthodihydroxy substitution in the B-ring of anthocyanin aglycone was established, with this conferring a higher ROS-scavenging capacity of the molecule. The protective effect of a gaseous secondary metabolite, isoprene, against ozone fumigation and heat shock was shown. The ROS-scavenging ability of isoprene was demonstrated. Altogether, the data provide evidence that secondary metabolites can be involved in the non-enzymatic plant defence strategy by their antioxidant and antiradical properties.

Key words: anthocyanins, antioxidants, isoprene, phenylamides, reactive oxygen species (ROS)

Introduction

In addition to primary metabolites, such as amino acids, fatty acids, nucleotides, carbohydrates, cytochromes, chlorophylls, and metabolic products of the anabolic and catabolic pathways which occur in all plants and have the same metabolic functions, plants also contain a large variety of compounds, named secondary metabolites (Heldt 1999). The plant physiologist Albrecht Kossel was the first to coin the term "secondary" for these seemingly non-functional compounds, stating that "while primary metabolites are present in every living cell capable of dividing, the secondary metabolites are present only incidentally, and are not of paramount significance for plant life" (Kossel 1891). Although occurring in all living organisms – from bacteria and fungi to mammals, secondary metabolites are particularly largely distributed in plants. As sedentary organisms incapable of fleeing from unfavourable cues and lacking an immune system, plants have evolved the strategy of exploiting an extremely diversified network of constitutive and inducible secondary metabolites, allowing them to deter, avoid or tolerate stress constraints, to complete their life cycle and survive. Thus, secondary metabolites play a major role in the adaptation of plants to their constantly changing environment and the generation of diversity at the organism level (Bourgaud & al. 2001; Osbourn

& al. 2003; Grotewold 2005). More than 100 000 secondary compounds have been described displaying a large complexity of chemical types, structures and biological implications. Functions of secondary metabolites comprise attractants, such as colour pigments and scents, repellents such as antifeedants against insects and herbivores, and toxins that affect microbial agents. They can also act as signals in plant–plant, plant–herbivore and plant–microbe interactions, and are components of the signal-transducing cascades in plants (Yang & al. 1997; Baldwin & al. 2002; Hadacek 2002; Foley & Moore 2005). Some secondary metabolites absorbing light and UV radiation act as photoprotectants, while high molecular compounds such as lignin stabilize cell walls thus constituting a physical anti-stress barrier (Dixon & Paiva 1995; Steyn & al. 2002; Edreva 2005). Recently, increasing amount of evidences substantiates the functioning of secondary metabolites as antioxidants assisting the plant to cope with oxidative stress arising in stress situations (Grace & Logan 2000; Gould & al. 2002; Grassmann & al. 2002).

The aim of the present paper was to summarize our data on the involvement of secondary metabolites in several plant – abiotic stress systems, by assuming that these molecular species could counteract the state of oxidative stress arising in a harmful, hostile environment. Phenylamides in tobacco and bean, anthocyanins in cotton and isoprene in reed were the subject of our research.

Phenylamides are typical bifunctional compounds produced by the conjugation of polyamines with hydroxycinnamic acids (Fig. 1), combining the properties of both parent compounds. The cationic character conveyed by the polyamines can determine an interfering in pH, ion and osmotic cell balance, as well as interactions with negatively charged loci in nucleic acids, proteins, cell walls and membranes, this yielding a structure stabilizing effect. The hydroxycinnamic acid moiety of phenylamides can confer an ability to form dimers catalysed by peroxidase – H_2O_2 system, and free radical scavenging ability. Thus, when bound to cell walls, phenylamides could bridge and cross-link polysaccharide chains contributing to cell wall consolidation; as free radical scavengers they may counteract oxidative stress (Bors & al. 1989; Facchini & al. 2002). The involvement of phenylamides in the responses of plants to pathogenic agents has long been reported and later confirmed (Martin-Tanguy 1985; Bouchereau & al. 1999), while paucity of data is available on the phenylamides in relation to abiotic challenges.

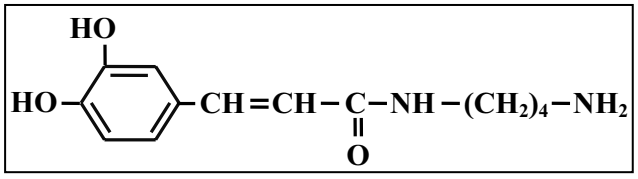

Fig. 1. A phenylamide conjugate of caffeic acid and putrescine (caffeoylputrescine).

Red pigments of various chemically unrelated types (red carotenoids, betacyanins, anthocyanins) are largely reported to accumulate in the vegetative parts of plants in stress conditions, such as low temperature, excess light, UV radiation, mineral unbalance, etc., with anthocyanins being recognized as the most important group of stress-responsive red coloured compounds (Ibdah & al. 2002; Steyn & al. 2002; Han & al. 2003; Hormaetxe & al. 2004). Light attenuating, UV-screening, osmoregulatory and ROS-scavenging properties of these compounds were proposed as a rationale of putative protective functions (Chalker-Scott 1999; Steyn & al. 2002; Hughes & al. 2005). An abnormal red colouration of cotton growing in the Aegean region of Turkey and other countries has long been observed but the type and putative function of the red pigments remained unknown.

An emphasis was laid on the isoprene (C_5H_8, 2-methyl-1,3-butadiene), a gaseous metabolite emitted in the atmosphere by a range of plant species, including reed (Kesselmeier & Staudt 1999). In the last decade intensive research has been devoted to this highly reactive volatile organic compound. Besides being implicated in the tropospheric chemistry by a series of complex interactions (Fehsenfeld & al. 1992), isoprene was assigned an important physiological role relevant to defence performance in plants (Sharkey & Singsaas 1995; Loreto & al. 2001; Sharkey & al. 2001).

Materials and methods

Experimental design

In our research several model systems were used embracing plant species of different genera and stress factors of various origin.

Tobacco (*Nicotiana tabacum* L.) – water excess. Heavy rainfalls during the late vegetation periods when tobacco leaves become "over-riped" caused the formation of dot-like necrosis on leaf lamina named

"sharilka", referred to as a physiological disorder typical for Oriental tobacco (Edreva & al. 1972a, b). Presence of phenylamides was tested in "sharilka"-affected leaves, healthy leaves serving as controls.

Bean (*Phaseolus vulgaris* L.) – heat shock. Plants were exposed to 50°C for 5 h, this resulting in leaf wilting (Edreva & al. 1998). Phenylamides were determined in leaves of high temperature-treated bean plants, using non-treated controls.

Cotton (*Gossypium hirsutum* L.) – Na^+ overaccumulation. Leakage of K and accumulation of Na^+ in the soil brought about K depletion and Na^+ overaccumulation in the leaves of plants that was accompanied by an abnormal red colouration of leaves (Edreva & al. 2002; Yağmur & al. 2003). Anthocyanins content and pattern were analysed in reddening cotton leaves, green leaves serving as controls. Antioxidant and antiradical capacity was also determined.

Reed (*Phragmites australis* L.) – ozone fumigation and heat shock. Leaves of reed plants emitting isoprene and those in which isoprene was specifically inhibited by fosmidomycin were used. Ozone fumigation caused damage symptoms (flecking) on the leaves of isoprene-deficient plants, while not affecting the isoprene-emitting ones (Loreto & al. 2001). Similarly, in isoprene-deficient leaves heat shock produces severer damage than in isoprene-evolving leaves (Velikova & Loreto 2005; Velikova & al. 2005). Contents of hydrogen peroxide (H_2O_2) and lipid peroxides in reed leaves were analysed as markers of oxidative stress.

The ability of isoprene to quench $^1O_2$ was evaluated in isoprene-emitting reed leaves as compared to leaves in which isoprene synthesis was previously inhibited (Velikova & al. 2004), while the $^1O_2$ quenching ability of phenylamides was determined using synthetic compounds (Velikova & al. 2007).

Details on the plant material and stress treatments are given in the corresponding above-cited papers.

Methods

Phenylamides were analysed by thin layer chromatography (Delétang 1974). $^1O_2$ quenching ability of phenylamides was tested *in vitro* by using Rose Bengal photosensitizer as a $^1O_2$ generator, and a Hansatech oxygen electrode. The $^1O_2$ quenching ability of isoprene emitting- and inhibited reed leaves was similarly determined (Velikova & al. 2004). Anthocyanins were analysed by HPLC (High Performance Liquid Chromatography) according to Woodall & Stewart (1998). Antioxidant capacity was estimated by the FRAP (ferric reducing antioxidant power) assay (Benzie & Strain 1999), and antiradical capacity – by the $DPPH^•$ (2,2-diphenyl-1-picrylhydrazyl radical) test (Brand-Williams & al. 1995). Lipid peroxide content was determined by the malondialdehyde assay (Heath & Packer 1968), and hydrogen peroxide content – as described by Velikova & al. (2004).

Results and discussion

Phenylamides

In tobacco leaves exposed to water excess ("sharilka") we detected phenylamides as conjugates of the polyamines spermidine and putrescine with ferulic and caffeic acids as hydroxycinnamic acid components. Impressively, phenylamides lacked in the healthy leaves being induced by the stress imposed (Edreva & al. 1995). Similarly, induction of phenylamides following abiotic stress was reported for tobacco leaves suffering K^+, Ca^{2+}, Mg^{2+} and P deficiency (Delétang 1974) or fumigated with ozone (Langebartels & al. 1991), as well as for S-starved or heat-treated tobacco cell suspension cultures (Klapheck 1983; Königshofer & Lechner 2002). In our experiments we identified the same polyamine and hydroxycinnamic acid components of phenylamides as those reported in the above tobacco – stress systems.

In a different plant – abiotic stress couple, namely bean – heat shock we have also established formation of phenylamides. High temperature treatment of the plants induced the appearance of conjugates of putrescine, spermidine and cadaverine with ferulic, p-coumaric and caffeic acids in the leaves (Edreva & al. 1995), with this being the first report on the occurrence of phenylamides in bean. Cadaverine is an uncommon polyamine characteristic for *Leguminosae* plants (Flores 1990), and was found to highly accumulate in a free form in the leaves of heat-treated bean plants (Edreva & al. 1998). The appearance of cadaverine as a conjugate in stress conditions may be relevant to its versatile employment in the protective mechanisms of bean plants.

Our data and those cited above point that the induction of phenylamides is not confined to a certain plant or a specific abiotic challenge, as also shown by the formation of phenylamides following herbicide application in spinach (Suzuki & al. 1981) and heat

treatment of alfalfa (Königshofer & Lechner 2002), but can be commonly involved in the responses of plants to abiotic stresses. Given the largely reported involvement of phenylamides in biotic stress situations (Flores & Martin-Tanguy 1991; Facchini & al. 2002), these secondary metabolites could be regarded as tools in a general mechanism triggered by plants to withstand adverse stress constraints of various origin. It can be assumed that the multiplicity of properties conferred to the molecule of conjugates by their parent compounds may underlie a protective role. Since oxidative stress is recognized as a primary event in plant exposed to harmful environment (Foyer & Noctor 2005), the ROS-scavenging ability of phenylamides may be of considerable protective importance by contributing to the regulation of ROS level and prevention of ROS-induced damage. *In vitro* scavenging of free radicals by phenylamides was reliably demonstrated (Bors & al. 1989; Son & Lewis 2002), and supposed to be due to the hydroxicinnamic acid moiety (Bors & al. 1989). By *in vitro* experiments for the first time we provided evidence that phenylamides scavenge singlet oxygen, a ROS particularly damaging to the photosynthetic apparatus. Potentiation of the $^1O_2$ scavenging ability of the conjugates relative to both components of their molecule was established (Velikova & al. 2007). A view is developed that conjugation is a means by which plants moderate the high cytotoxicity proper to free hydroxycinnamic acids, keeping or even potentiating their beneficial characters (Martin-Tanguy 1997). Detailed *in vivo* experiments are needed to ascertain the exact mechanisms of phenylamide involvement in stress protection of plants.

Anthocyanins

We reported that the abnormal red colouration of leaves of cotton was provoked by Na/K imbalance and Na^+ overaccumulation in the soil and plants (Yağmur & al. 2003). It was demonstrated that the red colour of cotton leaves is due to the tremendous accumulation of anthocyanins and not to other red-coloured chemical types, whereas in the green leaves very low levels of anthocyanins were presented (Edreva & al. 2006). Of particular interest is the different character of the main aglycones in red leaves (cyanidin) and green ones (malvidin) which are distinguished by distinct substitution pattern of the B-ring, namely presence of o-dihydroxy grouping in cyanidin and one hydroxy/two

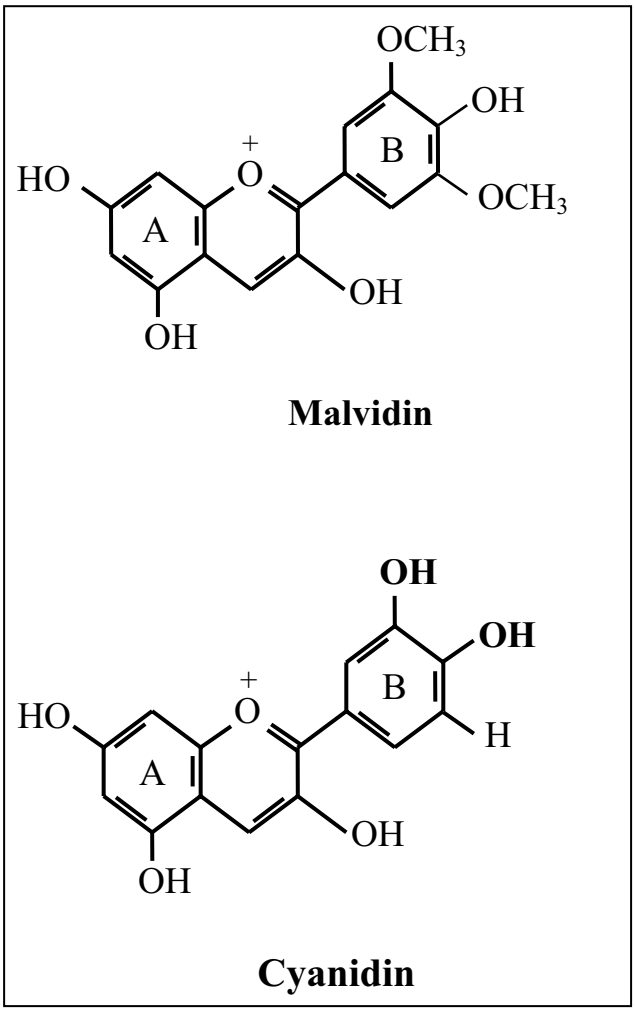

Fig. 2. Structural formulae of the main anthocyanin aglycones in the green (malvidin) and red (cyanidin) cotton leaves.

methoxy groups in malvidin (Fig. 2). The increase of Na^+ level in plants was shown to result in overproduction of toxic oxygen species, particularly the strongly damaging hydroxyl free radical ($OH^•$) (Alia & al. 1993). Since anthocyanins are recognized as free radical scavengers by *in vitro* tests (Kähkönen & Heinonen 2003; Kim & al. 2003), their protective role in cotton reddening could be reasonably supposed. In support to this assumption we have shown that extracts of red cotton leaves performed higher antioxidant and antiradical capacity than extracts of green leaves (Table 1). The data are in line

Table 1. Antioxidant and antiradical capacities (μmol g^{-1} fm) in red cotton leaves. Green leaves served as controls.

Variants	Antioxidant capacity	Antiradical capacity
Green leaves	12.07	1.70
Red leaves	16.00	2.29

with findings that cyanidin glycosides possess higher antioxidant and antiradical activity than malvidin glycosides (Aaby & al. 2004). This character is common for o-diphenols (Rice-Evans & al. 1997), and can be accounted for by the facilitated electron exchange in the o-dihydroxy – o-quinoid transitions. Hence, the shift from malvidin to cyanidin aglycones, i.e. to o-dihydroxy substitution in the B-ring of anthocyanins during cotton leaf reddening can contribute to an increased protective capacity against oxidative stress in the red cotton leaves. A shift from mono- to o-dihydroxy substitution in the B-ring of flavonoids has been observed in UV-tolerant rice, mustard, *Arabidopsis* and *Petunia* plants as a response to UV-radiation (Markham & al. 1998; Olsson & al. 1998; Ryan & al. 2001, 2002); this has been proposed as a mechanism used by plants to overcome the UV-induced oxidative stress. Our results (Edreva & al. 2006) are in line with the above findings, suggesting that the hydroxylation pattern of the B-ring in anthocyanins can also be important in stress protection.

Isoprene

By using an efficient model system of reed leaves normally emitting isoprene, and leaves in which isoprene biosynthesis was previously inhibited, we were able to demonstrate a differential effect of stress factors in both situations. Ozone fumigation and high temperature exposure produced severe damage to the photosynthetic performance of isoprene-deficient leaves while stress-induced injury was attenuated in isoprene-evolving leaves (Loreto & Velikova 2001; Velikova & Loreto 2005). We hypothesized that the stress tolerance in isoprene-emitting leaves can be mediated by antioxidant interactions of isoprene, interfering with events leading to functional and structural cell damage. The contents of H_2O_2 and lipid peroxides were assessed by assuming that both metabolites are recognized as markers of oxidative stress in plants, with the formation of malondialdehyde (MDA) being indicative of membrane fragmentation (Heath & Packer 1968). When ozone fumigation and heat shock were imposed, enhancement of both metabolites was registered in isoprene-inhibited leaves whereas the isoprene-emitting ones sustained lower leaves of H_2O_2 and MDA. Moreover, in control (stress-free) leaves isoprene inhibition *per se* brought about a certain degree of oxidative stress (Loreto & Velikova 2001; Velikova & Loreto 2005). Altogether the data point to the ability of isoprene to counteract oxidative stress possibly by ROS-scavenging mechanisms. In fact, higher $^1O_2$ scavenging capacity of isoprene-evolving reed leaves as compared to isoprene-deficient leaves was clearly demonstrated (Velikova & al. 2004). Similarly to carotenoids, conjugated double bond structure of isoprene (Fig. 3) may quench $^1O_2$ by facilitating energy transfer and heat dissipation (Cantrell & al. 2003); thus isoprene can act as a mean to elevate the antioxidant power of cell. Moreover, as a small lipophilic molecule isoprene may assist in hydrophobic lipid–lipid, lipid–protein and protein–protein interactions. If allocated to membranes, it may contribute to membrane stabilization with this providing an additional protective mechanism (Sharkey & Yeh 2001).

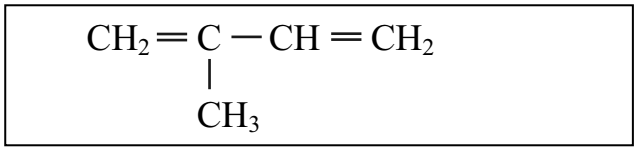

Fig. 3. Chemical structure of isoprene.

In conclusion it can be stated that secondary metabolites constitute a remarkably flexible biochemical system integrated into a complex network of functions that have evolved both divergently or convergently over millions of years of plant evolution. Their involvement in the non-enzymatic defence strategy can play a major role in plant adaptation to the environmental challenges and the generation of diversity at the organism level (Shirley 1996; Bourgaud & al. 2001). The plant protection that secondary metabolites can confer and the benefits of their use as pharmaceuticals and health-promoting nutraceuticals in animals and humans substantiate, according to Dixon & Steele (1999), their being "a gold mine for metabolic engineering".

Acknowledgments. The financial support of the National Science Fund (project SS–1503/05), NATO Reintegration Grant (No 981279/2004–2007), and Bilateral projects within the framework agreement between Italian National Research Council and Bulgarian Academy of Sciences (2004–2006), as well as between TÜBITAK, Turkey, and Bulgarian Academy of Sciences (2000–2002) is gratefully acknowledged.

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The role of woody species in the secondary succession under monitored conditions (Białowieża Forest, NE Poland)

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Abstract. The role of selected woody species in the course of secondary succession was analysed on the basis of 30 years long observations carried on permanent plots. After 10 years the first individuals of woody species became higher than herbs. After 15 years withdrawal of the meadow species under the pressure of developing trees was observed. After 15–20 years tenacious components of forest established shrub layer.

Key words: ornithochores, permanent plots, pioneer species, Poland, primeval forest, succession

Introduction

Secondary succession interested many investigators as a process of restoration of forest phytocoenoses destroyed by natural agencies or man's activity (Burrows 1990; Luken 1990; Glenn-Lewin & al. 1992; etc.). These problems have for a long time been in the field of interest of the Białowieża Geobotanical Station of Warsaw University staff and co-workers (Faliński 1986a, b, 1998; Falińska 1989, 2003; Adamowski & Knopik 1996; Pabjanek 2003).

This paper covers a small section of studies that have been ongoing for 30 years in the Garden. The role of selected woody species in the secondary succession was analysed on the basis observations carried out on permanent plots for over 30 years. The aim of the study was to establish patterns in the occurrence of woody species in the course of secondary succession.

Material and methods

The Experimental Ecological Garden of the Geobotanical Station of Warsaw University is located in the middle of the Białowieża Clearing. It was established on fertile ground cleared by the cutting of oak–lime–hornbeam forest (*Tilio–Carpinetum typicum*) at the end of 17th century (Faliński 1986a).

The Experimental Garden, created in 1974, occupies an area of 1.2 ha and is divided on sectors. Sectors C and E are used for observation of secondary succession. Each sector is divided into 22 plots (6.25 m × 1.6 m), separated from each other with paths 30 cm wide. The plot set is surrounded by an isolating belt and the fence, protecting experimental fields from external factors.

The areas are observed every year during the period of maximum plant growth (end of June – beginning of July), with the aim to determinate for each plot separately the overall species composition, community structure and appearance of tree seedlings and undergrowth (all were mapped during the first 20 years). The basic investigation in the successive years comprised phytosociological records, separate for each plot. The presence of individual plants in observation plots, and their abundance and sociability on the Braun-Blanquet scale were recorded, and additionally their abundance on Londo's scale. A detailed description of the study procedure may be found in the earlier papers by Faliński (1986a, b) and Adamowski & Knopik (1996).

Using archival materials collected at the Białowieża Geobotanical Station, the authors analysed the role of selected woody species in the course of secondary succession on permanent plots in sector C. The present

sector C had been used as an arable field. The authors deal with the quantitative and qualitative composition of woody species found in the observation plots during the period 1974–2005.

Results

1. Cover of the herb layer remained at a level higher than 90 % during the first 18 years after cessation of cultivation (Fig. 1) and subsequently began to decrease rather steeply. This decrease may be linked to withdrawal of meadow species under the influence of shading by trees (Fig. 2).
2. Starting with the 25th year after cessation of cultivation, a stabilisation of the herb layer is observed despite a further regression of meadow species (Fig. 2). The end of the downward tendency of herb layer coverage index change may be linked to the spread of pre-forest species, especially of *Melampyrum nemorosum* (Fig. 2).

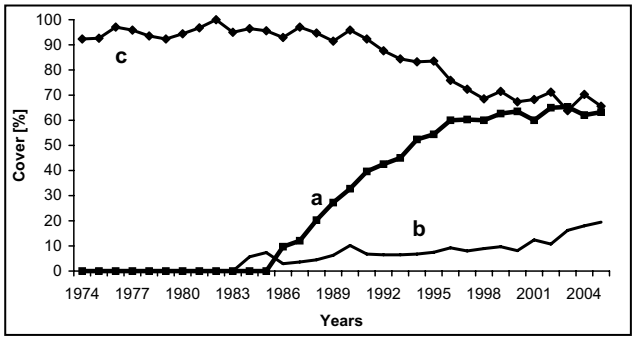

Fig. 1. The changes in the vegetation cover in the course of secondary succession on abandoned field: **a** – tree layer; **b** – shrub layer; **c** – herb layer.

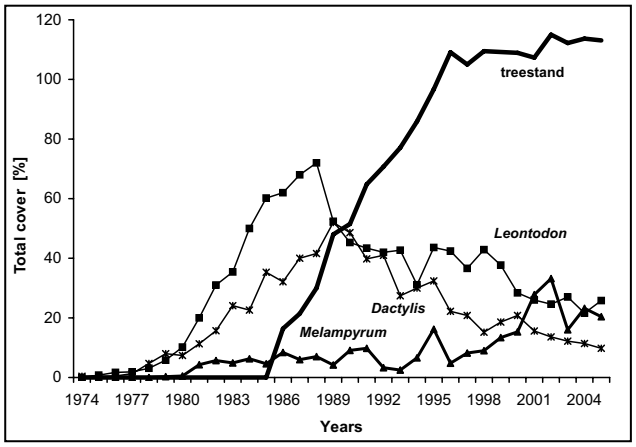

Fig. 2. The changes in the cover of meadow species and pre-forest species in the course of secondary succession on abandoned field.

3. During 31 years of observations, a total of 50 tree and shrub taxa appeared on the experimental plots.
4. First seedlings of light-seed pioneer trees (*Betula*, *Populus*, *Salix*) and of forest trees with winged seeds (*Acer*, *Fraxinus*) appeared already in 1975 (Fig. 3a), but for many years their share in the herb layer was insignificant. Individuals of arborescent species have grown taller than the herb layer level in the 10th year after cessation of cultivation and they have formed the tree layer in the 12th year (Fig. 1).
5. In the 14th year after cessation of cultivation (1987), first seedlings of an ornithochorous tree species (*Sorbus aucuparia*) appeared, and in the following years the share and species richness of this group increased conspicuously (Fig. 3a). In spite of the massive appearance of species which are spread by animals, only common oak *Quercus robur* has hitherto entered the shrub layer. It is interesting that oak seedlings appeared most abundantly between the 23rd and 27th observation year (Fig. 3b).
6. Cover of the shrub layer still remains at a low value; more pronounced increase tendencies in recent years are caused by outward growth of several oldest specimens of *Tilia cordata*, by numerous hornbeam and lime individuals reaching the shrub lev-

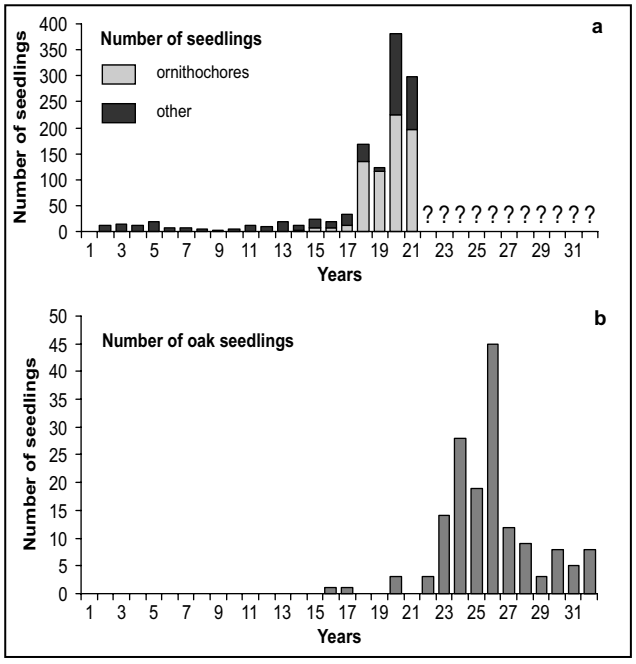

Fig. 3. The changes in the number of seedlings occurring on the permanent plots during the secondary succession: **a** – number of all seedlings; **b** – number of common oak seedlings.

el limit height and by vegetative encroachment of aspen onto the experimental plots (Fig. 4).

7. Individuals of goat willow, birch and maple formed the shrub layer only during several years, but eared sallow played a major role in this layer for many years (Fig. 4). Currently the shrub layer is composed of 10 species.
8. The first to enter the tree layer were pioneer species: *Salix caprea, Betula pendula, Populus tremula*, and slightly later (1991 – 18th year) also *Acer platanoides*. In recent years (2002 – 29th year), *Tilia cordata* entered this layer as well. Currently, the tree layer is formed by 5 species. However, thirty one years after cessation of cultivation the tree stand is still dominated by pioneer species (Fig. 5).
9. After an initial period of rapid growth, cover of the tree layer began to stabilise 23 years after cessation of cultivation at the level of 60–65 % (Fig. 1).
10. In recent years, an inhibition of growth and gradual withering away of goat willow individuals has been observed, while individuals of silver birch and aspen remain in good health.
11. Juvenile individuals of species which are spread both by birds and by the wind tend to concentrate under the tree canopy. This tendency is relatively stronger in the case of ornithochores (Fig. 6).
12. Individuals of goat willow, aspen, silver birch and Norway maple have reached the ability to reproduce generatively, although in the case of the maple seed production remains at a low level. In 2005, fly honeysuckle *Lonicera xylosteum* flowered for the first time.
13. In recent years (1999, 2002), herbaceous forest species (*Convallaria majalis, Anemone nemorosa*, respectively) appeared under the tree canopy; however, they still have an insignificant share in the herb layer.

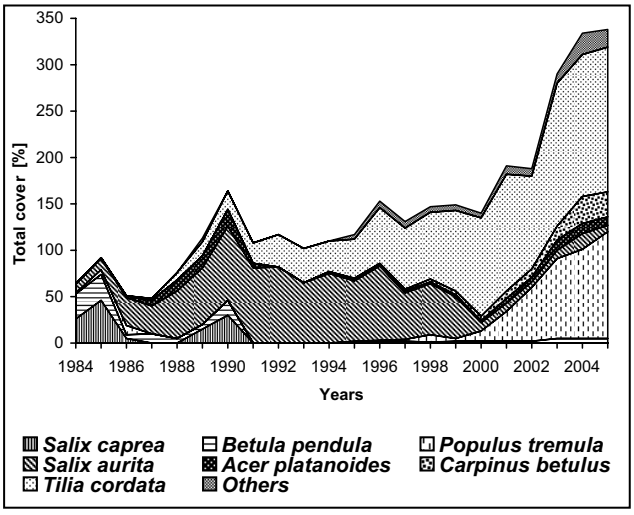

Fig. 4. Cover of particular species in shrub layer in the course of secondary succession on abandoned field.

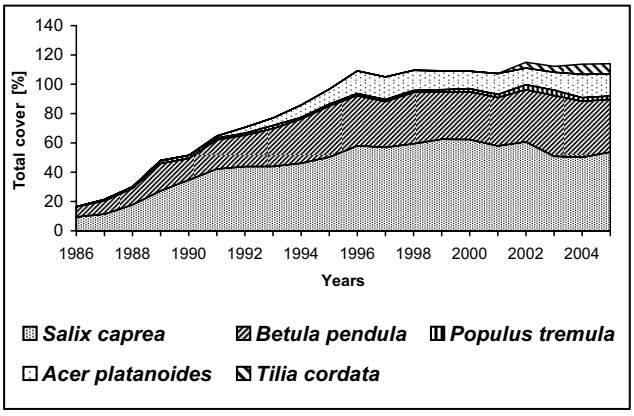

Fig. 5. Cover of particular species in treestand in the course of secondary succession on abandoned field.

Discussion

Tree species play a decisive role in the course of secondary succession in the moderate climate forest zone. By growing taller than herbaceous plants, they shape lighting and humidity condition under the canopy of their crowns, they compete with herbaceous plants for nutritional compounds, and they deposit tree litter, thus influencing soil properties (Glenn-Levin & al. 1992; Ejrnæs & al. 2003; Howard & Lee 2003).

This influence of tree crowns which grow outward and throw shade on lower vegetation layers is conspicuous in our studies through the recession of heliophile meadow species after *ca*. 20 years of succession duration (Fig. 2). Similar results have been obtained by Howard & Lee (2003) in their studies on temporal patterns of vascular plant diversity in New Hampshire.

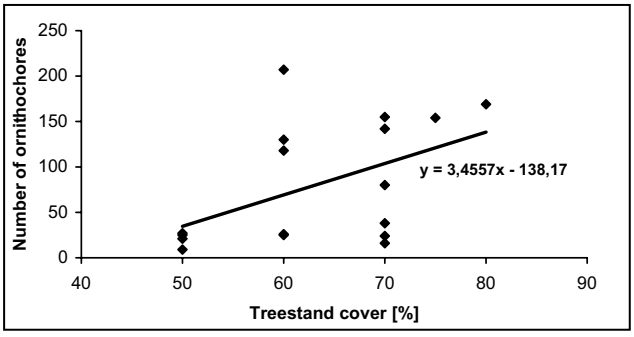

Fig. 6. Relationship between treestand and number of ornithochores occurring on the permanent plots.

Already less than thirty years after cessation of cultivation, heliophile tree species start to decrease their share in the herb layer, while after >80 years they disappear almost entirely. An increase of coverage index value for tree species after *ca*. 15 years of secondary succession has also been observed by Prach & Pyšek (2001) in the Czech Republic. Both of these values are close to the ones derived from our observations.

The space released by heliophile plants is occupied by tree and shrub seedlings and by pre-forest herbaceous species such as *Geum urbanum*, *Anthriscus sylvestris* and especially *Melampyrum nemorosum* (Fig. 2). Spreading of the latter species which is a parasite of tree roots is linked to the progressing growth of tree root systems.

In the course of observations it was established that trees which grow taller than the herb layer level are convenient roosting sites for seed-spreading birds, leading to a concentration of seedlings and juvenile individuals of ornithochoric species under the crowns of pioneer trees and in their vicinity (Adamowski & Knopik 1996). During recent 10 years, seedlings of these species have started to appear also on study plots which were not previously shaded by crowns of pioneer trees, but the interdependence between coverage index value of the tree layer and the number of juvenile individuals of ornithochoric species on individual plots is still visible (Fig. 6). Shading of the herb layer by tree crowns and decrease of herb layer coverage index value lead to an increase in number of appearing seedlings also in the case of other tree species such as maple, lime and hornbeam. The number of appearing seedlings of these woody species began to increase nearly simultaneously with the appearance of a tree layer, i.e. *ca*. 13 years after cessation of cultivation (Adamowski & Knopik 1996). The inhibitory influence of a compact herb layer in abandoned fields on seedling recruitment has been mentioned e.g. by Prach & Pyšek (2001). In our observations this inhibitory effect was especially conspicuous in the period 1979–1983 – i.e. between the 6^{th} and 10^{th} year after cultivation had been abandoned. Since that time, seedlings of pioneer species appear sporadically. Judging from our observations, it is, however, difficult to agree entirely with the opinion of Prach & Pyšek (2001) – in the first years after cessation of cultivation, in spite of considerable compactness of the herb layer, favourable conditions have obviously existed for the growth of seedlings, both of pioneer trees and of permanent forest components. A high percentage of these seedlings has survived and they gave rise to the current tree layer. The appearance of compact birch thickets reaching a height of *ca*. 5 m within 10 years has been observed on abandoned fields in Białowieża Forest (Pabjanek 2003).

Fruiting of maple trees observed in recent years is a symptom of the gradual transition of the formed forest community to autochtonous seeding. The role of autochtonous seeding in later stages of pine forest series succession was stressed by Faliński (1986a, b). Brzeziecki (2000), who wrote on life strategies of forest trees, listed maple as a competition-enduring species with delayed reproduction. However in our studies maple has flowered as the first of permanent tree stand components. Its growth rate at the juvenile stage, when full lighting is available, is not much slower than that of pioneer species.

In the nearest future, the following events may be expected: decrease in goat willow share in the tree layer, transition of successive individuals of permanent forest species into the tree stand and their flowering, increase of shrub layer coverage index value due to more and more numerous individuals of lime, hornbeam and oak reaching a specific height, as well as the entrance of hazel, Guelder rose, buckthorn and other shrub species into the shrub layer. The herb layer will witness an increase in share of already present herbaceous forest species and the appearance of subsequent ones.

On the basis of our studies, tree species can be considered as succession promoters in the sense given to the term by Falińska (1989). Their action consists in: shading the herb layer and tree litter deposition, thus dislodging heliophile meadow species; increasing the number and diversity of appearing seedlings, especially of species spread by birds; finally reproducing generatively such permanent forest components as maple, lime or hornbeam.

Acknowledgements. In the first place we are grateful to Prof. J.B. Faliński, who sadly passed away recently, for his helpful suggestions and countless stimulating discussions. We wish to cordially thank Prof. A.U. Warcholińska for many years of common observations in the Experimental Garden of the Białowieża Geobotanical Station as well as for making available unpublished material from studies on secondary succession. We would like also to thank Dr Łukasz Pulaski for his help with English translation.

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Contribution to the phytosociological study of low-altitude *Buxus sempervirens* (*Buxaceae*) formations (Mt Olympos, Greece)

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Abstract. Low altitude formations of evergreen boxwood (*Buxus sempervirens*), found at the foot of Mt Olympos, were phytosociologically approached. Shrublands of evergreen boxwood are classified into the class *Rhamno–Prunetea spinosae* as *Buxus sempervirens–Phillyrea latifolia*–community due to the significant stability and coverage of *Phillyrea latifolia.* The evergreen boxwood dominates also in the shrub understory of local *Platanus orientalis* woodlands. These formations are classified into the suballiance *Platanenion orientalis* of the alliance *Populion albae* as *Platanus orientalis–Buxus sempervirens*–community. The appearance of evergreen boxwood in the investigated site can be correlated with the historic background of the wider region as from antiquity man has favoured boxwood.

Key words: boxwood, *Buxus sempervirens*, Greece, Mt Olympos

Introduction

Boxwood (*Buxus sempervirens* L.) is an evergreen sub-Mediterranean shrub or small tree, which is widely distributed throughout Southern Europe, Northern Africa and Western Asia (Bonnier 1934).

Boxwood is marked for the large ecological range of the areas where it appears either in the form of shrublands or as an element of the shrub understorey of different forest communities. It expands from low altitudes, as in the case studied here, up to 1400 m (Horvat & al. 1974). In certain areas the establishment of *B. sempervirens* shrublands is related to serpentine soils, while in others this type of soil is avoided and lime-rich ones are clearly preferred (Bergmeier 1990). The ability of boxwood for adaptation and growth in different environmental conditions is manifested through the marked ecological and floristic differentiation of the various vegetation units in which it participates (Horvat & al. 1974).

This study aims at the phytosociological investigation of the *B. sempervirens* formations in the northeast foot of Mt Olympos, which are part of the wider area of the ancient sacred city of Dion.

Investigated area

The investigated area is located south to southwest of Dion village and *ca.* 10 km south of the city of Katerini, at the edges of the northeastern foot of Mt Olympos (Fig. 1). The

Fig. 1. Map showing the location of the investigated area.

terrain is of low inclination, the area has a general north-east exposition and an altitudinal range of 120–170 m.

The geological background of the area is composed of rough-grained materials, such as scree and talus cones, mainly of calcareous breccio-conglomerates, with elements of various size, loose to cohesive, with clayey and carbonate cement (I.G.M.E. 1978–1983), formed by the disintegration of the rocks of Mt Olympos.

The climate of the area is characterized as typically Mediterranean with two clearly different annual periods: one warm, dry period (summer) and one mild, wet period (Grigoriadis & al. 2005).

The vegetation of eastern Olympos, which is the surrounding background of the investigated area, is shaped by the following main successive formations (Dafis 1989). The vegetation of the plains and partly of the hilly terrain, situated above the coastal vegetation, has been destroyed and replaced by arable fields. In the foot of Mt Olympos and up to an altitude of 300–400 m a well developed ecosystem of evergreen broadleaves, belonging to *Adrachno–Quercetum ilicis*, is encountered. Above this zone, forests of *Pinus nigra* are expanding accompanied by scattered stands of *Fagus sylvatica*. *Pinus leucodermis* dominates the forest vegetation of the high altitudes of Mt Olympos starting its expansion from 1100 m (scattered stands) and ending up to *ca.* 2500 m where it forms the treelimits just below the alpine vegetation. Azonal vegetation dominated by *Platanus orientalis* is found near ravines, as in the investigated area which lies on the plain-hilly terrains where as mentioned before the natural vegetation has been intensely altered or completely destroyed.

Material and methods

Fifteen (15) plots with a size ranging from 100 m^2 to 250 m^2 were selected in May of 2004. Relevés were made in all plots (Tables 1, 2) using the Braun-Blanquet method (Braun-Blanquet 1964).

Identification and nomenclature of vascular plants were based on *Flora Hellenica* (Strid & Tan 1997, 2002), *Med-Checklist* (Greuter & al. 1984–1989), *Mountain Flora of Greece* (Strid 1986; Strid & Tan 1991) and *Flora Europaea* (Tutin & al. 1968–1980, 1993).

The vegetation table was processed with the SORT 3.9 software package (Ackermann & Durka 1997). The classification of plant taxa as diagnostic of the vegetation units (syntaxa) was based on Bergmeier (1990), Mucina (1997), Karetsos (2002), Korakis (2003), Fotiadis (2004) and others.

Results

The studied boxwood formations are the lowest ones, regarding the altitude, reported in Greece so far. The interpretation of their phytosociological data resulted in two communities, which are classified in different classes. The shrublands of *B. sempervirens* are classified into the class *Rhamno–Prunetea spinosae* as *Buxus sempervirens–Phillyrea latifolia*–comm. (Table 1) and the *Platanus orientalis* woodlands with the dominance of *B. sempervirens* in their shrub understory are classified into the class *Populetea albae* as *Platanus orientalis–Buxus sempervirens*–comm. (Table 2).

The syntaxonomic synopsis of the investigated vegetation is:

Class: *Rhamno–Prunetea spinosae* Riv.-God. & Bor. Carb. 1961

Community: *Buxus sempervirens–Phillyrea latifolia* –community

Class: *Populetea albae* Br.-Bl. 1962

Order: *Populetalia albae* Br.-Bl. 1931 ex auct. (in Br.-Bl. 1931 non valid. publ.)

Alliance: *Populion albae* Br.-Bl. 1931 ex auct. (in Br.-Bl. 1931 non valid. publ.)

Suballiance: *Platanenion orientalis* (I. & V. Kárpáti 1961) Bergmeier (1990)

Community: *Platanus orientalis–Buxus sempervirens*–community

Discussion

The *Buxus sempervirens–Phillyrea latifolia*–comm. comprises shrublands that grow on flat terrain in an altitudinal range of 120–170 m. The shrub storey is concrete having coverage of 100 % and a height of 2–3 m. The dense coverage of shrubs reduces the presence of herbaceous plants resulting in an herb understorey with coverage no more than 10 %. For the same reason the number of herbaceous species recorded in the floristic composition of the community is rather low having a mean value of 19 (10–28) species per relevé.

The investigated community was compared with the *Buxus sempervirens–Phillyrea latifolia*–comm., described from Kato Olympos and assigned in *Rhamno–Prunetea spinosae* by Bergmeier (1990), since one of the main attributes of both communities is the co-dominance of *Phillyrea latifolia* in the shrub storey. The investigated vegetation unit not only was identified as *Buxus sempervirens–Phillyrea latifolia*–comm.,

Table 1. ***Buxus sempervirens–Phillyrea latifolia*–comm.**

Plot number		1	2	3	4	5	6	7	8	9	10	11
Coordinates of plot	N	40° 08' 36"	40° 08' 36"	40° 08' 50"	40° 08' 49"	40° 08' 47"	40° 08' 49"	40° 08' 50"	40° 08' 49"	40° 08' 5"	40° 08' 52"	40° 08' 51"
	E	22° 29' 33"	22° 29' 31"	22° 29' 24"	22° 29' 25"	22° 29' 30"	22° 29' 29"	22° 29' 3"	22° 29' 32"	22° 29' 34"	22° 29' 32"	22° 29' 13"
Size of plot (m²)		250	200	150	150	150	150	150	150	150	150	100
Altitude (m)		130	125	120	121	121	120	132	151	165	165	169
Cover of shrubs (%)		100	100	100	100	100	100	100	100	90	95	100
Cover of herbs (%)		30	10	10	10	10	10	10	10	10	10	10
Height of shrubs (m)		3	3	2.5	2.5	3	2	2.5	3	3	3	3
***Buxus sempervirens-Phillyrea latifolia*-comm.**												
Buxus sempervirens	S/H	4/2	4/1	4/1	4/1	./+	3/+	3/.	4/1	4/1	3	4/.
Phillyrea latifolia	S/H	2/1	2/+	2/+	2/+	3/1	2/+	3/.	2/1	3/1	3/+	2/.
Diagnostic species of *Rhamno-Prunetea spinosae, Prunetalia spinosae*												
Ruscus aculeatus		3	1	3	3	2	3	2	2	1	1	1
Asparagus acutifolius	S/H	./2	./+	./+	./+	./+	./+	+/2	./+	./+	./+	+/+
Crataegus monogyna	S/H	+/.	+/+	./+	./r	./+	+/+	+/.	./+	./r	./r	+/+
Paliurus spina-christi	S/H	.	.	.	.	.	./+	+/.	.	.	.	.
Prunus spinosa	S/H	.	./+	.	./r	.	.	+/.	./r	.	.	.
Sanguisorba minor ssp. *muricata*		r	.	.	.	.	.	.	r	.	.	.
Celtis australis		r	.	r	.	.	.	.	.	.	.	.
Diagnostic species of *Querco-Fagetea, Quercetalia pubescentis*												
Quercus coccifera	S/H	4/2	3/.	4/1	4/+	3/+	3/+	2/.	2/1	2/+	3/1	2/.
Carpinus orientalis	S/H	./+	./+	./+	2/+	2/1	+/+	.	./r	+/1	./1	1/.
Fraxinus ornus	S/H	./+	./+	./+	+/r	+/.	.	+/.	+/.	./+	./r	.
Brachypodium sylvaticum		r	.	+	.	r	.	.	r	r	.	r
Carex flacca ssp. *flacca*		+	+	+	+	.	+	.	.	.	+	+
Cotinus coggygria		r	r	.	.	.	.	.	r	.	.	.
Cyclamen hederifolium		r	r	.	.	.	.	.	r	.	+	+
Teucrium chamaedrys		+	+	+	+	.	+	.	r	.	r	.
Satureja vulgaris		.	.	.	.	r	r	.	.	.	.	+
Scutellaria columnae ssp. *columnae*		r	+	.	+	+	.	.	.	r	.	.
Silene italica ssp. *italica*		.	.	.	.	+	r	.	.	.	.	.
Diagnostic species of *Quercetea(-alia) ilicis*												
Osyris alba	S/H	./3	./3	+/3	./3	./2	./2	.	./+	+/1	./+	./1
Pistacia terebinthus	S/H	./+	./+	./+	./r	+/+	.	./+	./+	./+	./+	./r
Other companion species												
Platanus orientalis	T/S/H	./+/	.	./+/.	.	.	.	.	.	.	.	.
Juniperus oxycedrus ssp. *oxycedrus*		+	r	.	1	.	.	.	+	+	1	.
Anacamptis pyramidalis		.	r	.	.	.	.	.	.	r	.	.
Cercis siliquastrum	S/H		./r	.	.	.	.	+/.	.	.	.	.
Asphodelus aestivus		r	r	r	r	.	r	.	.	r	r	r
Geranium robertianum ssp. *purpureum*		.	r	.	.	+	r	r	.	.	.	.

Table 1. Continuation.

Plot number		1	2	3	4	5	6	7	8	9	10	11
Pyracantha coccinea	S/H	.	.	.	.	.	.	+/.	.	.	.	.
Satureja alpina		r	.	.	.	.	.	.	r	.	.	r
Vincetoxicum fuscatum		.	r	.	.	.	.	.	r	.	.	.
Hedera helix ssp. *helix*		r	.	.	r	.	.	.	.	.	.	.
Ephedra foeminea		.	+	.	+	.	.	.	.	.	.	.
Clematis flammula		.	r	.	.	r	.	.	.	.	.	.
Ballota nigra		.	r	.	.	.	.	.	r	.	.	.
Rubia peregrina		+	.	.	.	.	.	.	r	.	.	.
Anemone pavonina		r	.	.	.	.	+	.	.	.	.	.

Pyrus amygdaliformis S 2:+, *Tamus communis* 3:r, *Ranunculus neapolitanus* 5:r, *Arabis sagittata* 8:r, *Asplenium onopteris* 5:+, *Buglossoides purpurocaerulea* 3:r, *Cardamine graeca* 4:r, *Carthamus lanatus* 5:r, *Colutea arborescens* 1:r, *Crucianella angustifolia* 10:r, *Dorycnium herbaceum* 5:r, *Dracunculus vulgaris* 3:r, *Ephedra foeminea* S 2:1, *Hypericum perforatum* 11:r, *Lonicera etrusca* 5:r, *Lotus aegeus* 4:+, *Medicago arborea* 1:r, *Melica ciliata* 10:r.

Table 2. ***Platanus orientalis–Buxus sempervirens*–comm.**

Plot number		12	13	14	15
Coordinates of plot	N	40° 08' 58"	40° 08' 54"	40° 08' 52"	40° 08' 50"
	E	22° 29' 32"	22° 29' 53"	22° 29' 55"	22° 29' 51"
Size of plot (m²)		250	250	250	250
Altitude (m)		117	110	121	140
Cover of trees (%)		80	80	80	80
Cover of shrubs (%)		70	70	70	5
Cover of herbs (%)		15	15	20	30
Height of trees (m)		14	15	16	10
***Platanus orientalis-Buxus sempervirens*-comm.**					
Platanus orientalis	T/S/H	5/./+	5/./.	5/+/+	5/+/.
Buxus sempervirens	S/H	4/+	3/+	3/1	+/+
Diagnostic species of *Populetea albae, Populion albae*					
Juglans regia		.	r	r	r
Carex pendula		+	.	.	.
Dracunculus vulgaris		+	+	+	.
Diagnostic species of *Rhamno-Prunetea spinosae, Prunetalia spinosae*					
Asparagus acutifolius	S/H	./1	./1	./+	./+
Crataegus monogyna	S/H	./+	./+	./+	./+
Palurus spina-christi	S/H	./r	.	./r	./+
Clematis vitalba	S/H	+/1	./+	./1	./+
Ruscus aculeatus		2	1	+	1
Sanguisorba minor ssp. *muricata*		.	+	+	+
Rubus sanctus		+	+	+	+
Tamus communis		+	+	+	.

Table 2. Continuation.

Plot number		12	13	14	15
Diagnostic species of *Querco-Fagetea*, *Quercetalia pubescentis*					
Quercus coccifera	S/H	./r	./r	.	./+
Fraxinus ornus	S/H	.	./r	.	./r
Brachypodium sylvaticum		+	+	.	+
Cyclamen hederifolium		+	+	r	.
Teucrium chamaedrys		+	+	.	+
Satureja vulgaris		+	+	+	+
Scutellaria columnae ssp. *columnae*		+	.	.	+
Silene italica ssp. *italica*		.	r	.	r
Viola alba ssp. *denhardtii*		r	+	.	+
Eupatorium cannabinum ssp. *cannabinum*		.	r	r	.
Buglossoides purpurocaerulea		+	+	.	.
Diagnostic species of *Quercetea*(*-alia*) *ilicis*					
Phillyrea latifolia	S/H	+/r	./+	./+	./+
Pistacia terebinthus	S/H	./r	./+	./r	.
Diagnostic species of *Festuco-Brometea*					
Asphodelus aestivus		+	r	+	+
Cynosurus echinatus		.	r	+	.
Muscari neglectum		r	+	+	+
Melica ciliata		r	r	.	.
Diagnostic species of *Galio-Urticetea*					
Geranium robertianum ssp. *purpureum*		+	+	l	+
Galium aparine		+	+	+	.
Other companion species					
Pyracantha coccinea	S/H	+/.	.	.	./+
Vitis vinifera		+	r	+	r
Euphorbia helioscopia		r	+	l	+
Catapodium rigidum		.	r	+	+
Orlaya daucoides		+	+	+	+
Anemone pavonina		+	+	+	+
Arenaria serpylifolia		.	.	+	+
Thlaspi perfoliatum		.	r	r	.
Geranium rotundifolium		.	.	+	+
Hypericum perforatum		.	r	.	r
Crepis sancta		.	r	r	.
Crucianella angustifolia		.	+	+	+
Ficus carica		r	r	r	.
Satureja menthifolia		.	r	.	+
Carduus pycnocephalus		.	r	r	.
Rhagadiolus stellatus		+	+	.	.
Bromus sterilis		.	+	r	.
Lolium rigidum		.	r	.	.
Alyssum murale		.	+	.	r
Poa pratensis		.	.	+	+

Lactuca serriola 14:+, *Nigella arvensis* 15:r, *Ranunculus neapolitanus* 15:r, *Anagallis arvensis* 15:+, *Satureja alpina* 15+, *Galium spurium* 12:r, *Quercus pubescens* 12:r, *Sambucus nigra* 12:r, *Silene viridiflora* 12:r, *Celtis australis* 12:r, *Smilax aspera* 12:r, *Carex divulsa* 13:+, *Bellis perennis* 13:r, *Arabis sagittata* 13:r, *Dasypyrum villosum* 13:r, *Poa bulbosa* 13:r, *Origanum vulgare* 13:+, *Lapsana communis* ssp. *communis* 14:+. *Stellaria media* 14:r, *Cerastium brachypetalum* ssp. *roeseri* 14:r, *Clypeola jonthlaspi* 14:r, *Arum maculatum* 14:+, *Sherardia arvensis* 14:+, *Torilis arvensis* 14:+, *Geranium columbinum* 15:+, *Lactuca viminea* 15:+, *Satureja juliana* 15:r, *Teucrium polium* ssp. *capitatum* 15:r.

but it was also assigned to the same class with the one from Kato Olympos since species of *Rhamno–Prunetea spinosae* are significantly present in both, with *Ruscus aculeatus, Asparagus acutifolius* and *Crataegus monogyna* prevailing.

The presented *Buxus sempervirens–Phillyrea latifolia*–comm. is directly assigned to the *Rhamno–Prunetea spinosae*. On the contrary, the same community described by Bergmeier (1990) in Kato Olympos, as well as two other units of boxwood shrublands (*Cotoneastro nebrodensis–Buxetum sempervirens* association and *Buxus sempervirens–Daphne oleoides*–comm.) were assigned to the suballiance *Buxo–Syringenion* of the alliance *Berberidion vulgaris* of the class *Rhamno–Prunetea spinosae*. Lack of the corresponding diagnostic species did not allow a more detailed classification of the studied community.

The *Platanus orientalis–Buxus sempervirens*–comm. comprises stands of plane situated on flat surfaces in an altitudinal range of 110–140 m. The tree storey has coverage of 80 % and a mean height of 15 m. The coverage of the shrub understorey, which is dominated by *B. sempervirens*, fluctuates intensely (5–70 %). The herb storey has a variable coverage (15–40 %). The community has a significantly larger floristic composition than that of *Buxus sempervirens–Phillyrea latifolia*–comm., with a mean value of 47 (40–52) species per relevé.

Bergmeier (1990) assigned the formations of *Platanus orientalis* from Kato Olympos and the East Mediterranean in general to the suballiance *Platanenion orientalis*, the alliance *Populion albae*, the order *Populetalia albae* and the class *Populetea albae*. Forests of *P. orientalis* from sites in N Greece were accordingly classified by Korakis (2003), Fotiadis (2004), Chochliouros (2005). The same syntaxonomy is adopted in the present study where the formation of *P. orientalis* is classified as *Platanus orientalis–Buxus sempervirens*–comm. on the basis of the participation of boxwood in the shrub understorey.

The dominance of *P. orientalis* determines the syntaxonomic status of the community since only a few of the diagnostic species of the relative syntaxa are present, mainly *Juglans regia*, *Carex pendula* and *Dracunculus vulgaris*. On the contrary, the community bears many species of the *Rhamno–Prunetea spinosae* which characterize phytosociologically the adjacent *Buxus sempervirens–Phillyrea latifolia*–comm. Thus, the *Platanus orientalis–Buxus sempervirens*–comm. can be considered as the ecotone between the boxwood shrublands and the stands of *P. orientalis* which develop in places with better soil conditions without the presence of boxwood.

The boxwood participates in the understorey of various Greek forest units such as *Pinus nigra*, *Carpinus orientalis*, *Quercus trojana*, *Ostrya carpinifolia*, *Fagus sylvatica* (Horvat & al. 1974; Korakis 2003). With the exception of the present study, boxwood has been reported as element of the shrub understorey of *P. orientalis* stands only once in the *Platanus orientalis–Corylus avellana*–comm. found in Mt Vermio (Chochliouros 2005).

The classification of *P. orientalis* stands is in general difficult due to the fact that they represent azonal formations with large geographical and ecological variation while their floristic composition is influenced by that of the given zonal vegetation in the inside of which they develop (Horvat & al. 1974). The present investigated community comprises several species of *Querco–Fagetea* and *Quercetalia pubescentis* reflecting the above-mentioned complex floristic composition of the *P. orientalis* woodlands. Apart from those species that characterize different forest communities, the presence of those related to human activity, mainly pastoral, as those of *Festuco–Brometea* (*Asphodelus aestivus*, *Cynosurus echinatus*, *Muscari neglectum*, *Poa bulbosa*, *Melica ciliata*), the nitrophilous of *Galio–Urticetea* (*Geranium robertianum*, *Galium aparine*) and several companion species (*Vitis vinifera*, *Ficus carica*, etc.), is also noticeable.

The human impact on the natural vegetation of the area manifests itself in space and time. Three villages (Karitsa, Dion, Platanakia) are found today in the vicinity of the studied area while back in antiquity the nearby sacred city of Dion played a dominant role in the area. According to Horvat & al. (1974) boxwood is included in the "anthropo-zoogen" shrubs of the (sub)-Mediterranean vegetation belt. Moreover, it seems that boxwood was specially favoured by man who has transported it to places outside its natural occurrence since antiquity (Decocq & al. 2004). Theophrastus repeatedly refers to the growth of boxwood in the area of Mt Olympos. All these facts in conjunction with Theophrastus' report on the use of *B. sempervirens* wood in curving small statues and idols support the hypothesis that the development of boxwood shrublands is related to the ancient sacred city of Dion (Theophrastus 1998).

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The high-rank syntaxa of semi-natural grasslands in Straldzha–Aytos phytogeographic region

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Abstract. The study region is situated in the Southeast Bulgaria. A total of 344 relevés collected in 2004–2005 were classified by TWINSPAN. The resulting vegetation types were checked by statistical tendency of joint occurrence of species in vegetation in the Cocktail method within JUICE software. The resulting groups were plotted onto a DCA ordination diagram. Under discussion are the semi-natural grasslands on alliance level within classes *Festuco–Puccinellietea*, *Phragmito–Magnocaricetea*, *Molinio–Arrhenatheretea*, *Festuco–Brometea* and *Helianthemetea guttati*. Class *Festuco–Brometea* revealed the highest diversity on the alliance level.

Key words: Bulgaria, classification, phytocoenology, vegetation

Introduction

Semi-natural grasslands are among the most valuable ecosystems within agricultural landscapes. Earlier vegetation studies carried out by the Bulgarian researchers were mostly performed using the dominance approach. Despite some recent investigations (Meshinev & al. 2005), this vegetation is still insufficiently evaluated in terms of the Braun-Blanquet phytosociological approach in Bulgaria. This makes its insertion within the European context (Rodwell & al. 2002) difficult. The aims of this study are (i) to describe the semi-natural grassland vegetation in the Straldzha–Aytos phytogeographic region and (ii) to classify this vegetation into phytosociological alliances in the context of the European phytosociological classification system.

Material and methods

The Straldzha–Aytos phytogeographic region is situated between 42–43° N and 26–27° E, in the Southeast Bulgaria. It includes parts of the districts of Sliven, Yambol and Bourgas. The relief is predominantly plain and hilly. Agricultural lands dominate the area. The climate of the region is transitional between continental and Mediterranean. The mean annual temperature for the period 1954–1993 measured in the town of Yambol was 12.2 °C, and the mean annual precipitation (for the same period and town) was 545 mm (Velev 1997).

Field investigations were carried out throughout the years 2004 and 2005. Vegetation samples were taken from all available grasslands found in the region. All visible conspicuous vegetation and habitat types within each place were sampled. The aquatic, the weed and the man-made communities have not been subject of this study. Methods of the Zürich-Motnpellier school (Westhoff & van der Maarel 1980) were applied for vegetation descriptions. The total number of collected relevés was 344. The standard relevé area was 16 m^2 (Chytrý & Otýpková 2003) with the exceptions of thirteen relevés having an area of 100 m^2. The vascular plant and bryophyte cover was estimated using the nine-grade Braun-Blanquet scale (van der Maarel 1979).

The vascular plant nomenclature follows Kozhuharov (1992) and the bryophyte nomenclature follows Natcheva & Ganeva (2005). Nomenclature of the syntaxa follows Rodwell & al. (2002).

The data set was entered and stored in the TURBOVEG data base (Hennekens & Schaminée 2001) and than exported into JUICE software (Tichý 2002). A polythetic, divisive classification that uses two-way indicator species analysis (TWINSPAN, Hill 1979) was applied. The resulting vegetation types roughly corresponded with the major, ecologically definable vegeta-

tion types. These vegetation types were then checked by statistical tendency of species to have a joint occurrence in vegetation by the Cocktail method (Bruelheide 1995), using the phi (Φ)-coefficient (Chytrý & al. 2002), which was standardized to an equal size for all groups. On the base of results of this analysis, some relevés were moved within the groups. The species in the table were sorted according to their fidelity to vegetation type. Species with fidelity above 30 (Φ>0.30) in any group were regarded as diagnostic. Syntaxonomical interpretation of each group was made after referring to the appropriate literature (e.g., Chytrý & Tichý 2003; Horvat & al. 1974; Micev 1957; Micevski 1970). All undetermined groups were removed from the subsequent analysis. All resulting clusters were plotted onto a DCA (Detrended Correspondence Analysis) ordination diagram, with square-root transformation of the data and with down weighting of the rare species.

Results and discussion

The amount of 175 phytosociological relevés is classified into six alliances. The alliances with their diagnostic species are shown in Table 1. Two of them represent vegetation in the wetlands, one belongs to the halophyte vegetation and three syntaxa represent dry grasslands. The results show high degree of diversity on alliance level in the studied phytogeographic region. It is determined by the variety of environmental conditions.

The area is one of the few in the country where inland saline habitats occur. *Puccinellion limosae* (Cluster 3 in Table 1) is a relatively species poor vegetation type. The total cover of the herb layer is between 65 % and 95 %. The dominant species are *Camphorosma monspeliaca* or *Puccinellia convoluta*.

The habitats of more mesophytic character and thus much appropriate for agriculture are converted in arable lands. As a result the areas occupied by such kind of semi-natural vegetation are much reduced. Two classes are established with the following alliances: *Scirpion maritimi* (Cluster 2 in Table 1) grows in mild terrain depressions and is very rare in the studied region. It is one of the pioneer helophytic communities, which is characterized by the dominance of *Bolboschoenus maritimus*.

The meadow vegetation, documented by 24 relevés mainly from the northern part of the region, is assigned to *Trifolion resupinati* (Cluster 1 in Table 1). It occupies wet soils on river flood plains and other high water-table sites, between 150 m and 350 m a.s.l. Average number of species per relevé is 24.6.

Due to the considerably hot and dry climate the xerophytic vegetation types prevail in the region. Most widespread class *Festuco–Brometea* is represented by two alliances.

Festucion valesiacae (Cluster 4 in Table 1) is wide distributed in the region. It includes closed communities with dominance of *Festuca valesiaca*, *Dichantium ischaemum*, *Chrysopogon gryllus* or *Stipa capillata*. It is documented by 93 relevés.

Saturejon montanae (Cluster 5 in Table 1) occurs on calcareous places in the northwestern part of the region and covers a small area. Most relevés, assigned to this alliance, are from Karabair hill. It is relatively open vegetation, with high stone cover. Average number of species per relevé is 30.7.

Another type of the dry grasslands belongs to *Helianthemetea guttati*. *Trifolion cherleri* (Cluster 6 in Table 1) is an alliance with a submediterranean character, developing on dry soils and sunny expositions. Floristically, it is a relatively species rich community with an average of 30 plant species per relevé.

Table 1. Shortened synoptic tables with fidelities (Φ values multiplied by 100).

Group No.	1	2	3	4	5	6
No. of relevés	24	3	12	93	16	24
Trifolium pratense	**84.5**	---	---	---	---	---
Ranunculus sardous	**80.4**	10.2	---	---	---	---
Holcus lanatus	**76.2**	---	---	---	---	---
Orchis elegans	**76.2**	---	---	---	---	---
Trifolium patens	**67.4**	---	---	---	---	---
Festuca pratensis	**65.8**	14.5	---	---	---	---
Prunella vulgaris	**64.3**	---	---	---	---	---
Anthoxanthum odoratum	**64.0**	---	---	---	---	---
Cirsium arvense	**61.1**	---	---	---	---	---
Ononis arvensis	**61.1**	---	---	---	---	---
Cynosurus cristatus	**61.1**	---	---	---	---	---
Lotus corniculatus	**58.0**	---	---	---	---	---
Galium debile	**54.2**	---	---	---	---	---
Rhinanthus rumelicus	**54.2**	---	---	---	---	---
Carex distans	**53.5**	22.4	---	---	---	---
Lysimachia nummularia	**50.5**	---	---	---	---	---
Alopecurus rendlei	**50.5**	---	---	---	---	---
Mentha spicata	**50.5**	---	---	---	---	---
Juncus gerardii	**50.3**	---	1.2	---	---	---
Calliergonella cuspidata	**46.6**	---	---	---	---	---
Orchis laxiflora	**46.6**	---	---	---	---	---

Table 1. Continuation.

Group No.	1	2	3	4	5	6
No. of relevés	24	3	12	93	16	24
Moenchia mantica	**46.6**	---	---	---	---	---
Rumex acetosa	**42.4**	---	---	---	---	---
Carex tomentosa	**42.4**	---	---	---	---	---
Bromus secalinus	**42.4**	---	---	---	---	---
Lolium perenne	**41.7**	---	25.5	---	---	---
Daucus carota	**38.8**	---	---	---	---	---
Juncus effusus	**37.8**	---	---	---	---	---
Ophioglossum vulgatum	**37.8**	---	---	---	---	---
Leucanthemum vulgare	**37.8**	---	---	---	---	---
Holoschoenus vulgaris	**37.8**	---	---	---	---	---
Alopecurus myosuroides	**37.8**	---	---	---	---	---
Rhinanthus angustifolius	**37.8**	---	---	---	---	---
Dactylis glomerata	**36.9**	---	---	---	---	8.5
Carex hirta	**35.6**	29.8	---	---	---	---
Verbena officinalis	**32.6**	---	---	---	---	---
Carex otrubae	**32.6**	---	---	---	---	---
Festuca nigrescens	**32.6**	---	---	---	---	---
Ornithogalum narbonense	**32.6**	---	---	---	---	---
Gladiolus imbricatus	**32.6**	---	---	---	---	---
Plantago lanceolata	**32.3**	---	21.0	9.4	---	6.0
Filipendula vulgaris	**31.0**	---	---	---	---	1.2
Teucrium scordium	**---**	**100.0**	---	---	---	---
Juncus articulatus	12.0	**83.7**	---	---	---	---
Potentilla reptans	19.0	**80.4**	---	---	---	---
Juncus compressus	---	**79.1**	---	---	---	---
Rorippa prolifera	---	**76.0**	---	---	---	---
Bolboschoenus maritimus	---	**73.2**	---	---	---	---
Sanionia uncinata	---	**73.2**	---	---	---	---
Eleocharis palustris	---	**70.7**	---	---	---	---
Elymus repens	---	**69.4**	---	---	---	---
Plantago major	10.0	**60.0**	---	---	---	---
Gratiola officinalis	23.8	**58.2**	---	---	---	---
Eleocharis acicularis	---	**54.2**	---	---	---	---
Alisma plantago-aquatica	---	**54.2**	---	---	---	---
Mentha pulegium	---	**50.0**	---	---	---	---
Oenanthe silaifolia	27.4	**33.5**	---	---	---	---
Trifolium hybridum	13.8	**31.6**	---	---	---	---
Chamomilla recutita	---	---	**86.4**	---	---	---
Camphorosma monspeliaca	---	---	**84.5**	---	---	---
Hordeum hystrix	---	---	**79.1**	---	---	---
Puccinellia convoluta	---	---	**73.4**	---	---	---
Lepidium ruderale	---	---	**61.1**	---	---	---
Trifolium retusum	---	---	**59.8**	0.5	---	---
Scorzonera laciniata	---	---	**57.3**	---	---	---
Cynodon dactylon	---	---	**54.3**	---	---	7.3
Plantago coronopus	---	---	**54.2**	---	---	---
Artemisia santonicum	---	---	**52.0**	---	---	---
Bromus mollis	---	---	**50.9**	---	---	---
Scleranthus polycarpos	---	---	**50.1**	---	---	---
Eragrostis pilosa	---	---	**46.6**	---	---	---
Gypsophila muralis	---	---	**46.6**	---	---	2.4

Group No.	1	2	3	4	5	6
No. of relevés	24	3	12	93	16	24
Sedum caespitosum	---	---	**45.6**	---	---	2.1
Taraxacum sp.	---	---	**45.3**	---	---	---
Puccinellia distans	---	---	**37.8**	---	---	---
Plantago tenuiflora	---	---	**37.8**	---	---	---
Spergularia marina	---	---	**37.8**	---	---	---
Plantago bellardii	---	---	**36.2**	---	---	---
Thymus striatus	---	---	---	**48.7**	27.6	29.8
Centaurea diffusa	---	---	---	**48.4**	---	1.8
Potentilla neglecta	---	---	---	**48.4**	---	1.8
Petrorhagia prolifera	---	---	---	**46.7**	---	15.6
Medicago rigidula	---	---	---	**44.9**	---	---
Herniaria hirsuta	---	---	---	**44.4**	---	10.5
Centaurea caliacrae	---	---	---	**42.0**	---	---
Astragalus onobrychis	---	---	---	**38.2**	3.6	---
Trifolium scabrum	---	---	---	**35.1**	9.4	9.1
Festuca valesiaca	---	---	---	**34.7**	27.3	18.9
Nigella arvensis	---	---	---	**31.7**	---	---
Carthamus lanatus	---	---	---	**31.5**	---	1.9
Achillea setacea	1.8	---	---	**30.1**	5.6	---
Sideritis montana	---	---	---	---	**82.2**	---
Anthyllis vulneraria	---	---	---	---	**80.2**	---
Echinops ritro	---	---	---	---	**74.5**	---
Sedum acre	---	---	---	---	**73.7**	---
Grimmia pulvinata	---	---	---	---	**72.2**	---
Helianthemum salicifolium	---	---	---	---	**70.4**	---
Paronychia cephalotes	---	---	---	---	**69.3**	---
Medicago rhodopaea	---	---	---	---	**65.5**	---
Weissia wimmeriana	---	---	---	---	**65.5**	---
Teucrium polium	---	---	---	15.2	**65.3**	4.6
Ajuga chamaepytis	---	---	---	---	**63.6**	---
Leontodon crispus	---	---	---	---	**56.2**	6.6
Inula aschersoniana	---	---	---	---	**56.1**	---
Scleranthus annuus	---	---	---	---	**54.9**	---
Achillea clypeolata	---	---	---	5.5	**54.8**	---
Euphorbia myrsinites	---	---	---	14.6	**53.4**	---
Crupina vulgaris	---	---	---	---	**53.2**	---
Hippocrepis ciliata	---	---	---	---	**52.7**	---
Agropyron cristatum	---	---	---	---	**52.7**	---
Jurinea consanguinea	---	---	---	---	**52.7**	---
Melica ciliata	---	---	---	---	**52.2**	---
Fumana procumbens	---	---	---	---	**50.4**	---
Alyssum tortuosum	---	---	---	---	**49.3**	---
Chrysopogon gryllus	---	---	---	---	**48.6**	16.1
Didymodon acutus	---	---	---	---	**48.4**	---
Linum tenuifolium	---	---	---	---	**47.9**	---
Pleurochaete squarrosa	---	---	---	---	**47.9**	---
Paliurus spina-christi	---	---	---	---	**46.6**	---
Acinos arvensis	---	---	---	---	**46.4**	---
Minuartia caespitosa	---	---	---	7.5	**46.3**	---
Syntrichia ruralis	---	---	---	4.1	**44.4**	16.4
Rhodax canus	---	---	---	---	**42.6**	---

Table 1. Continuation.

Group No.	1	2	3	4	5	6
No. of relevés	**24**	**3**	**12**	**93**	**16**	**24**
Asperula cynanchica	---	---	---	28.2	**42.5**	---
Convolvulus cantabrica	---	---	---	---	**40.3**	17.6
Hypericum rumeliacum	---	---	---	3.7	**38.8**	3.1
Allium flavum	---	---	---	---	**38.4**	---
Centaurea ovina ssp. *besserana*	---	---	---	---	**37.6**	---
Koeleria brevis	---	---	---	---	**36.8**	---
Koeleria nitidula	---	---	---	2.7	**36.2**	28.6
Bombycilaena erecta	---	---	---	9.6	**34.1**	12.6
Koeleria penzesii	---	---	---	---	**33.5**	7.1
Potentilla pedata	---	---	---	0.1	**32.2**	---
Orlaya grandiflora	---	---	---	---	**31.7**	6.2
Trifolium campestre	---	---	---	14.7	---	**72.7**
Trifolium arvense	---	---	---	17.7	---	**68.8**
Rumex acetosella	---	---	---	---	---	**64.6**
Lotus angustissimus	---	---	---	---	---	**61.1**
Psilurus incurvus	---	---	---	2.7	---	**59.4**
Anthemis ruthenica	---	---	---	13.0	---	**58.2**
Logfia minima	---	---	---	---	---	**56.7**
Scleranthus perennis	---	---	---	17.3	---	**56.0**
Trifolium angustifolium	---	---	---	7.3	---	**55.7**
Stachys angustifolia	---	---	---	---	---	**50.5**
Vulpia ciliata	---	---	---	---	---	**49.3**
Achillea crithmifolia	---	---	---	---	---	**46.0**
Ceratodon purpureus	---	---	---	14.0	---	**45.7**
Jasione heldreichii	---	---	---	---	---	**42.4**
Linaria pelisseriana	---	---	---	---	---	**42.4**
Potentilla argentea	---	---	---	---	---	**41.7**
Aira elegantissima	---	---	---	---	---	**41.0**
Trifolium cherleri	---	---	---	---	---	**40.5**
Trifolium strictum	---	---	---	---	---	**37.8**
Galium tenuissimum	---	---	---	8.2	---	**36.8**
Chondrilla juncea	---	---	---	27.3	---	**34.5**
Ornithogalum umbellatum	---	---	---	0.4	11.4	**33.5**
Hieracium praealtum	---	---	---	---	---	**33.1**
Hypochaeris radicata	3.1	---	---	---	---	**33.0**
Centaurium erythraea	---	---	---	---	---	**32.6**
Verbascum adrianopolitanum	---	---	---	---	---	**32.6**
Brachythecium albicans	---	---	16.6	---	---	**31.1**
Hieracium hoppeanum	---	---	---	---	---	**30.8**
Trifolium striatum	---	---	---	12.6	---	**30.3**
Poa sylvicola	**42.0**	**70.9**	---	---	---	---
Eryngium campestre	---	---	---	**48.5**	---	**47.9**
Dichantium ischaemum	---	---	---	**39.9**	**31.1**	10.6
Euphorbia cyparissias	---	---	---	---	**38.1**	**31.1**
Sanguisorba minor	---	---	---	---	**30.3**	**47.4**

Diagnostic species for the clusters (defined as those with Φ>0.30) are bold and ranked by decreasing Φ values, i.e. decreasing fidelities to each cluster. Negative Φ values are not shown.

Syntaxonomical synopsis

Class *Festuco–Puccinellietea* Soó 1968
- Order *Puccinellietalia* Soó 1940
  - Alliance *Puccinellion limosae* Soó 1930 corr. Wendelberger 1943

Class *Phragmito–Magnocaricetea* Klika in Klika & Novák 1941
- Order *Bolboschoenetalia maritimi* Hejný in Holub & al. 1967
  - Alliance *Scirpion maritimi* Dahl & Hadač 1941

Class *Molinio–Arrhenatheretea* Tüxen 1937
- Order *Trifolio–Hordeetalia* Horvatić 1963
  - Alliance *Trifolion resupinati* Micevski 1957

Class *Festuco–Brometea* Br.-Bl. & Tüxen ex Br.-Bl. 1949
- Order *Festucetalia valesiacae* Br.-Bl. & Tüxen 1943
  - Alliance *Festucion valesiacae* Klika 1931
  - Alliance *Saturejon montanae* Horvat 1962

Class *Helianthemetea guttati* (Br.-Bl. in Br.-Bl., Roussine & Nègre 1952) Rivas Goday & Rivas-Martínez 1963 em. Rivas-Martínez 1978
- Order *Helianthemetalia guttati* Br.-Bl. in Br.-Bl., Molinier & Wagner 1940
  - Alliance *Trifolion cherleri* Micevski 1970

The DCA was used to visualize the diversity of the vegetation in the studied region. The established alliances can be recognized in the ordination space. The DCA-ordination diagram (Fig. 1) shows that wet and dry vegetation types are well separated along the first ordination axis. This reveals that the most important factor for vegetation diversity in the region is the soil moisture. The halophyte alliance *Puccinellion limosae* is clearly separated along the second axis. But the second most important environmental factor cannot be found out only by the species data. However, *Festucion valesiacae* and *Trifolion cherleri* are not well differentiated along the first and second axis. This fact corresponds to the opinion of Micevski (1978) that the communities of alliance *Trifolion cherleri* belong to the class *Festuco–Brometea*. *Saturejon montanae* is well separated from previous two alliances occupying the right portion of the diagram.

Acknowledgements. This study is a part of PhD Thesis. The author would like to thank her supervisor Dr Iva Apostolova (Institute of Botany, BAS) for the useful comments on the manuscript, Dr Anna Ganeva (Institute of Botany, BAS) for the determination of the collected bryophytes and Mr. Kaloyan Ivanov (Cleveland State University, Cleveland, USA), who kindly reviewed the English text.

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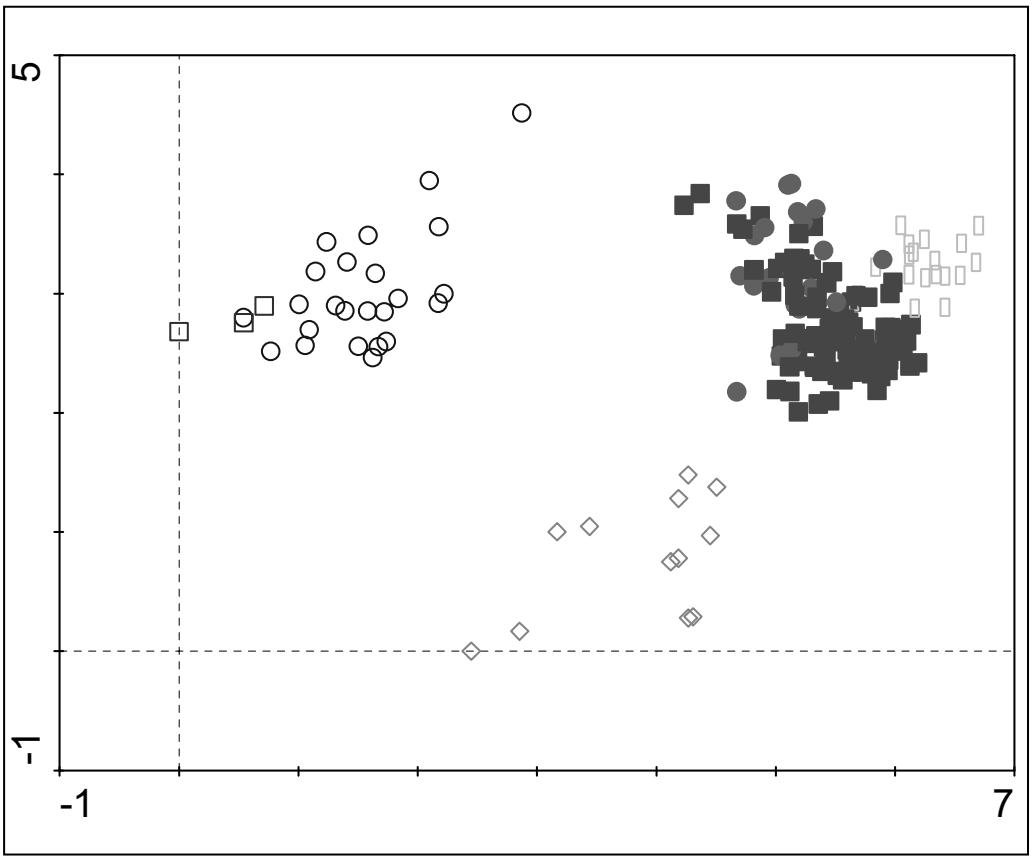

Fig. 1. The DCA-ordination diagram of alliances of semi-natural grasslands in Straldzha–Aytos phytogeographic region.
Legend:
◇ *Puccinellion limosae*;
□ *Scirpion maritimi*;
○ *Trifolion resupinati*;
■ *Festucion valesiacae*;
▯ *Saturejon montanae*;
● *Trifolion cherleri*.

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Contribution to the phytosociological research of *Pinus nigra* forests of the Troodos mountain of Cyprus

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Abstract. The National Forest Park of Troodos at Cyprus is dominated by pure and mixed stands of *Pinus halepensis* ssp. *brutia* and *P. nigra* ssp. *pallasiana*. *Pinus nigra* ssp. *pallasiana* extends from altitudes 1200 m and 1400 m in northern and southern aspects, respectively, up to the summit of the mountain. A phytosociological research of the *P. nigra* ssp. *pallassiana* forests was conducted by applying the Braun-Blanquet method in 31 relevés. *Pinus nigra* ssp. *pallassiana* forests were syntaxonomically assigned as *Pinus nigra* ssp. *pallasiana* – *Scutellaria cypria* var. *cypria* community in the *Quercetea pubescentis* class, *Querco–Cedretalia* order and *Cephalorrhyncho–Pinion* alliance. In addition, two sub-communities, one transitional state, one variance and three faces were distinguished.

Key words: Cyprus, *Pinus nigra*, phytosociological research, Troodos Mountain

Introduction

Phytosociological research in Cyprus and mountainous areas in particular is very limited. Troodos Mountain is the main massif of Cyprus and is considered of great importance, because of its scientific and ecological value. Moreover, Troodos Mountain attracts many visitors for recreation from Cyprus as well as from abroad. These are the main reasons which necessitate the systematic research of the vegetation of the Troodos Mountain.

The aim of this paper is the contribution to the study of the forest vegetation and of the European Black Pine Forests (*Pinus nigra* Arnold ssp. *pallasiana* (D. Don) Holmboe) inside the National Forest Park of Troodos (NFPT) in particular.

Material and methods

Study area

The study area is limited by the borders of the NFPT, which is located at the centre of the Troodos range. The later extends from the northwestern to the southeastern part of Cyprus. The study area covers a surface of 9029 ha which corresponds to 0.97 % of the total surface of the island. The centre of the study area is located at 34°56' N and 32°52' E (Georgiou & al. 2000).

The study area is almost posted at the centre of the ophiolithic complex of Troodos. The dominant rocks are distinguished in two categories. Harzburgite and dunite lie at the central section of the area. These are ultramafic rocks (>70 % ferromagnesian minerals) and 50–80 % of their minerals have been altered into serpentine. In the later, concentration of asbestos is locally found. Peripherally, the gabbro is the dominant rock, but other cumulate rocks are also present, as duntite, wehrlite, pyroxenite and granite. The intensive tectonic activity and the abrupt lifting episodes of the Troodos range during its formation caused fragmentation of its rocks to large extent. The above-mentioned geologic events have contributed to the increasing water penetration and retentivity of ground (Department of Geological Survey of Cyprus 1997; Tsintidis & al. 2002).

Meteorological data were available for the stations of Platania (1120 m), Pano Amiantos (1360 m), Prodromos (1380 m) and Troodos Square (1725 m) and concern the decade 1991–2000. Climate is characterized by a dry and hot period during summer, while the most of precipitation falls during winter. The three first meteorological stations are assigned to the Csa climatic type, according to Köppen (Flokas 1994), indicating Mediterranean climate with dry, hot summers and mild winters. The station of Troodos Square is assigned to the Csb climatic

type, indicating Mediterranean climate with dry, warm, short summers and mild winters. For the bioclimatic classification Emperger pluviothermic quotient and his corresponding climatic diagram, as it has been modified by Daget (1977) for the Eastern Mediterranean, was applied. The station of Troodos Square is assigned to the subhumid, extremely cold stage and the rest of the stations to the subhumid, very cold stage.

Although NFPT covers a surface of only 1 % of Cyprus, it is characterized by its rich flora and the high rank of endemism. The number of taxa of the study area comes up to 786, which is equal to 40 % of the total flora of the island (1907 taxa), while about 52 % (72 taxa) of the endemic taxa of Cyprus are found in Troodos mountain, too (Georgiou & al. 2000).

The vegetation of NFPT is dominated by forest ecosystems (89 %), mainly of *Pinus halepensis* ssp. *brutia* and *P. nigra* ssp. *pallasiana*, as well as *Juniperus foetidissima*, in pure or mixed stands. Much smaller surface is covered by evergreen woodlands and riparian vegetation (Georgiou & al. 2000).

Research method

For the phytosociological research of the vegetation the Braun-Blanquet method (Braun-Blanquet 1964; Westoff & van der Maarel 1973; Athanasiadis 1986) was applied in 31 relevés during the years 2003 and 2004.

Systematic identification of the taxa was done according to *Flora of Cyprus* (Meikle 1977, 1985), while *Endemic Plants of Cyprus* (Tsintidis 1995) and *Trees and Shrubs of Cyprus* (Tsintidis & al. 2002) were used on the side. For the nomenclature *The Cyprus Flora in Checklist Format* (Della 1999) was used, too.

Distinguishing of the vegetation units was done according to the floristic–statistical method of the School of Zürich–Montpellier (Manual table method) (Braun-Blanquet 1964; Westoff & van der Maarel 1973; Athanasiadis 1986).

Nomenclature of the units of vegetation as well as their assignment to Braun-Blanquet's taxonomic system follows Barbéro & Quézel (1979), Quézel & Barbéro (1985) and Mucina (1997).

Results and discussion

European Black Pine forests

The interpretation of the phytosociological data of the study area resulted in *Pinus nigra* ssp. *pallasiana* – *Scutellaria cypria* var. *cypria* community. In addition, two sub-communities, one transitional state, one variance and three faces were distinguished (Table 1).

The syntaxonomic synopsis of the investigated vegetation is as follows:

Quercetea pubescentis Doingt Kraft ex Scamoni & Passarge 1959
Querco–Cedretalia libani Barbéro, Loisel & Quézel 1974
Cephalorrhyncho–Pinion Barbéro & Quézel 1979
Pinus nigra ssp. *pallasiana – Scutellaria cypria* var. *cypria* community
Quercus alnifolia – Teucrium kotschyanum sub-community
Pterocephalus multiflorus ssp. *multiflorus* variance
Berberis cretica – Sorbus aria ssp. *cretica* sub-community
Alyssum troodi and *Hypericum confertum* ssp. *stenobotrys* face
Epilobium angustifolium and *Viola sieheana* face

Pinus nigra ssp. *pallasiana – Scutellaria cypria* var. *cypria* community (Table 1, relevés 1–31)

The *Pinus nigra* ssp. *pallasiana – Scutellaria cypria* var. *cypria* community is represented by 31 relevés and is distinguished by the dominance of *P. nigra* ssp. *pallasiana* in the storey of trees. Moreover, *S. cypria* var. *cypria*, *Pteridium aquilinum*, *Rubia tenuifolia* and *Limodorum abortivum* are present frequently.

As a rule, the structures of *P. nigra* stands consist of three storeys (storey of trees, shrubs and herbaceous species), while in two relevés shrubs are completely absent. The tree canopy ranges from 40 % to 60 %, except one relevé (80 %). In some cases shrubs coverage ranges from 10 % to 35 %, while in the most relevés coverage does not exceed 5 %. The storey of shrubs is composed mainly of the species *P. nigra* ssp. *pallasiana*, *Quercus alnifolia*, *Berberis cretica*, *Sorbus aria* ssp. *cretica, Arbutus andrachne*, *Rosa canina* ssp. *dumetorum* and *Juniperus foetidissima*. Usually, herbaceous species cover a very small percentage of the ground, while in few cases the coverage reaches up to 50 %. Regeneration of *P. nigra* ssp. *pallasiana* is often observed, but in few numbers.

Stands of *P. nigra* ssp. *pallasiana* occur from altitudes 1200 m up to 1800 m, mainly on ultramafic rocks and sometimes on gabbros. The inclination of the ground ranges from 9 % to 70 %, while in the most cases is gentle (25–55 %). Black Pine stands mainly occur in northern–northwestern to northern–northeastern aspects, in the middle of the slopes, in hollows as well as in bulges.

The dominance of the above-mentioned aspects as favourable for the occurrence of *P. nigra* stands is also because of several other reasons, as eroded stony soils, very steep slopes and the recently abandoned asbestos mine (220 ha).

Table 1. ***Pinus nigra*** **ssp.** ***pallasiana*** **–** ***Scutellaria cypria*** **var.** ***cypria*** **community (1–11:** ***Quercus alnifolia*** **–** ***Teucrium kotschyanum*** **sub-community; 9–11: Variance from** ***Pterocephalus multiflorus*** **ssp.** ***multiflorus*****; 22–31:** ***Berberis cretica*** **–** ***Sorbus aria*** **ssp.** ***cretica*** **sub-community; 22–28: Face from** ***Alyssum troodi*** **and** ***Hypericum confertum*** **ssp.** ***stenobotrys*****; 29–31: Face from** ***Epilobium angustifolium*** **and** ***Viola sieheana*****; 12–21: Transitional state).**

STOREY	A/A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	CONSTANCY
	No. OF SPECIES	16	9	9	12	14	10	8	10	16	19	14	16	11	16	15	14	20	14	23	14	22	11	10	16	12	10	5	16	10	14	14	
	ALTITUDE (m)	1431	1400	1216	1510	1650	1370	1414	1660	1338	1327	1311	1465	1458	1424	1320	1570	1458	1760	1528	1500	1686	1728	1660	1684	1700	1775	1764	1738	1704	1724	1718	
	ASPECT	WNW	NNW	N	NNW	SSW	SW	NNE	WNW	NNW	WNW	WNW	NNW	NNW	NNW	NNW	W	NNW	SSE	SW	NW	SE	NNE	NNE	W	W	SSW	N	NNE	E	WNW	E	
	INCLINATION (%)	36	47	14	32	27	9	58	47	36	47	47	36	47	36	23	70	27	27	9	70	9	36	47	58	36	27	32	47	18	27	14	
	COVERAGE OF TREES (%)	45	50	60	50	35	40	50	40	60	60	60	45	50	50	80	40	45	50	50	20	65	40	40	40	50	40	60	40	60	60	50	
	COVERAGE OF SHRUBS (%)	20	25	25	1		15	20	1	20	35	20	15	20	5	5	15	15	5	15	15	10	10	1	5	5	5	10	3	5	5	5	
	COVERAGE OF HERBS (%)	6	5	12	5	3	5	3	3	5	8	5	8	5	10	5	5	10	6	30	5	25	13	8	8	4	10	1	15	10	20	50	
	MAX. HEIGHT OF TREES (m)	15	18	17	20	15	16	15	14	17	24	16	15	14	13	8	13	15	20	16	10	20	12	12	13	15	16	12	16	22	16	22	
	MAX. DIAMETER (cm)	39	48	42	54	70	53	56	44	47	51	58	43	32	39	20	56	36	43	43	41	59	65	41	50	58	43	39	49	50	38	52	
	DAY	15	08	14	16	16	04	08	16	03	03	03	17	09	10	03	17	14	23	14	09	19	22	22	18	18	18	18	23	23	18	23	
	MONTH	06	06	05	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	06	05	06	06	06	06	06	06	06	06	06	06	
	YEAR	04	04	03	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	03	04	04	04	04	04	04	04	04	04	04	
	Pinus nigra **ssp.** ***pallasiana*** **-** ***Scutellaria cypria*** **var.** ***cypria*** **community**																																
T	*Pinus nigra* ssp. *pallasiana* C.-P.	3	4	4	4	3	3	4	3	4	4	4	3	4	4	5	3	3	4	4	2a	4	3	3	3	4	3	4	3	4	4	4	31
S	*Pinus nigra* ssp. *pallasiana*	.	2a	.	+	.	+	.	+	.	+	.	2a	.	.	.	2a	.	2a	+	.	.	2b	+	+	2a	2a	+	+	2a	.	+	
H	*Pinus nigra* ssp. *pallasiana*	+	+	+	r	.	1	.	.	r	r	.	.	.	.	.	r	r	r	.	.	r	+	r	r	r	+	r	1	.	r	1	
	Pteridium aquilinum	1	+	.	1	.	.	.	.	.	1	+	.	+	1	1	1	+	2a	2b	.	1	.	.	.	r	.	.	r	2a	1	3	19
H	*Scutellaria cypria* var. *cypria* C.-P.	r	+	.	.	r	+	.	.	.	r	+	.	.	r	.	r	1	+	.	1	.	+	+	+	1	.	.	.	.	.	.	16
S	*Rubia tenuifolia* Q.i.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	15
H	*Rubia tenuifolia*	.	+	+	1	+	.	.	1	r	1	r	.	.	1	.	.	2m	.	2m	+	2m	.	.	1	1	.	.	.	.	.	.	
	Limodorum abortivum Q.i.	+	+	.	+	.	r	r	.	.	+	.	.	.	.	.	.	(r)	r	+	.	.	.	.	+	.	.	.	+	+	(r)	(+)	14
	Quercus alnifolia **-** ***Teucrium kotschyanum*** **sub-community**																																
S	*Quercus alnifolia*	2a	2b	2a	.	.	2a	2a	.	2b	2b	2b	2a	2b	1	.	2a	2a	.	.	2a	.	.	.	.	.	.	.	.	.	.	.	11
H	*Quercus alnifolia*	1	2m	2a	1	r	1	1	+	2m	2m	2m	2m	1	1	+	1	1	r	+	1	1	r	+	r	r	.	.	r	+	(r)	.	
S	*Arbutus andrachne* Q.a.	2a	2a	2a	.	.	2a	2a	.	1	1	(+)	+	+	.	.	.	.	.	(+)	+	+	.	.	.	.	.	.	.	.	.	.	9
H	*Arbutus andrachne*	.	+	.	(r)	.	+	r	.	r	.	.	.	r	.	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
	Salvia willeana C.-P.	+	.	r	+	r	.	.	+	+	1	+	1	1	1	.	1	1	r	1	.	1	.	.	.	.	.	.	.	.	.	.	.	8
	Cistus creticus var. *creticus* C.-M.	(+)	.	1	.	r	1	r	.	.	.	r	+	.	r	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	6
	Teucrium kotschyanum Q.a.	r	r	.	.	.	.	+	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
	Clinopodium vulgare C.-M.	(+)	+	.	r	.	.	.	.	r	+	+	.	+	.	+	.	1	.	+	.	.	.	.	.	.	.	.	.	.	.	r	r	6
S	*Rubus sanctus* R.-P.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	3
H	*Rubus sanctus*	1	.	.	2a	.	.	.	.	.	1	.	1	r	1	r	+	+	.	2a	+	+	.	.	.	.	.	.	.	.	.	.	.	
	Variance from ***Pterocephalus multiflorus*** **ssp.** ***multiflorus***																																
	Pterocephalus multiflorus ssp. *multiflorus*				.	.	.	.	.	1	r	1	1	.	2m	2m	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	3
	Paeonia mascula T.-G.	.	.	.	.	.	.	.	.	1	+	r	.	r	+	.	.	1	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	3
S	*Juniperus oxycedrus*	.	.	.	.	.	.	.	.	1	(+)	.	.	.	1	+	+	+	.	2a	.	.	.	.	.	.	.	.	.	.	.	(+)	.	2
H	*Juniperus oxycedrus*	.	.	.	.	.	.	.	.	+	.	.	.	.	+	+	.	r	.	r	.	.	.	.	.	r	.	.	.	.	.	.	.	
S	*Rosa canina* ssp. *dumetorum* R.-P.	.	.	.	.	.	.	.	.	+	+	.	.	1	+	.	.	1	.	+	1	.	.	.	.	.	.	.	.	.	+	.	+	2
H	*Rosa canina* ssp. *dumetorum*	.	.	.	.	.	.	.	.	r	+	.	.	+	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	
	Berberis crectica **-** ***Sorbus aria*** **ssp.** ***cretica*** **sub-community**																																
S	*Berberis cretica* C.-P.	.	.	.	.	.	.	.	.	.	(+)	.	(+)	(+)	(+)	+	+	2a	+	2a	1	2a	.	+	.	+	.	.	+	.	.	+	11
H	*Berberis cretica*	.	.	.	.	r	.	.	.	.	.	.	1	+	.	1	+	1	+	1	+	1	+	+	r	1	r	+	1	r	r	1	
S	*Sorbus aria* ssp. *cretica* Q.p.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	+	.	1	(+)	.	.	1	.	.	+	+	.	2a	+	10
H	*Sorbus aria* ssp. *cretica*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	+	1	r	r	r	+	.	+	.	1	+	
	Scorzonera troodea	r	.	.	1	+	.	.	.	.	.	.	r	.	1	.	.	.	+	r	.	1	+	.	1	1	2a	r	1	1	2m	+	10
	Euphorbia cassia ssp. *rigoi* C.-P.	.	.	.	.	.	.	.	.	.	.	.	+	1	1	.	.	.	+	+	.	1	r	r	.	.	1	.	2a	r	.	1	7
T	*Juniperus foetidissima* D.-F.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2a	.	.	.	.	.	.	.	.	.	.	.	.	7
S	*Juniperus foetidissima*	+	.	.	.	(+)	.	.	(+)	.	.	.	.	.	.	.	+	+	.	.	+	+	.	+	.	.	+	.	.	.	+	+	
H	*Juniperus foetidissima*	.	.	.	.	.	.	.	(r)	.	.	.	r	.	.	.	r	r	r	.	r	+	.	.	r	.	.	.	r	.	r	+	

Table 1. Continuation.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	Face from *Alyssum troodi* and *Hypericum confertum* ssp. *stenobotrys*																																
	Alyssum troodi	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	+	2a	2a	2a	+	+	r	r	.	.	.	7
	Hypericum confertum ssp. *stenobotrys*		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2a	1	r	+	2a	.	1	.	.	.	6
	Crepis fraasii Q.p.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	1	1	.	.	.	.	.	3
	Face from *Epilobium anqustifolium* and *Viola sieheana*																																
	Epilobium angustifolium E.a.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	1	2a	2b	3
	Viola sieheana	.	.	.	2m	.	.	.	.	r	.	.	r	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	+	1	2m	2a	3
	Characteristic species of *Cephalorrhyncho - Pinion* Barbéro & Quézel 1979																																
S	*Prunus avium*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
H	*Prunus avium*	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	2
S	*Rosa chionistrae*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1
H	*Rosa chionistrae*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	1
S	*Cotoneaster racemiflorus* var. *numularius*				.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	(+)	.	.	.	.	.	.	.	.	.	.	.	.	1
H	*Cotoneaster racemiflorus* var. *numularius*				.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	1
	Cephalorrhynchus cyprius	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	2
	Characteristic species of *Quercetalia ilicis* Br.-Bl. 1936 em. Rivas-Martinez 1975																																
S	*Styrax officinalis*	2a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	(+)	.	.	.	.	.	.	.	.	.	.	.	.	.	(+)	3
H	*Styrax officinalis*	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	Lonicera etrusca	.	.	.	.	.	.	.	.	r	+	.	r	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	5
	Rubia laurae	.	.	.	.	.	.	.	.	.	.	.	2m	.	.	.	1	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.	4
	Other species																																
	Quercus coccifera ssp. *calliprinos*			.	.	.	.	.	.	r	.	r	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
S	*Rhus corriaria*	2a	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
H	*Rhus corriaria*	1	.	.	.	.	.	(+)	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
	Anthemis plutonia	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	r	.	+	.	.	.	+	.	.	.	+	.	.	.	5
	Teucrium cyprium var. *cyprium*			.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	r	.	r	.	r	.	5
	Thlaspi cyprium	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	r	.	.	.	+	.	.	.	.	.	.	1	.	.	.	4
	Centaurea aegialophila	(r)	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	+	.	.	.	.	.	.	.	.	.	.	4
	Saponaria cypria	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	r	.	.	+	.	.	.	4
	Asphodelus aestivus	.	.	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	3
	Turritis laxa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	1	.	.	.	.	.	.	.	2
	Crepis reuteriana	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	2
	Compositae sp.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	2

Species occuring only in one relevé: *Ornithogalum chionophilum* 47(r), *Poterium sanquisorba* ssp. *dictiocarpos* 37(r), *Orchidaceae* sp. 76 (1), *Genista sphacelata* var. *crudelis* 56 (+), *Platanthera chlorantha* ssp. *holmboe* 57 (r), *Hypochaeris glabra* 75 (r), *Rosa canina* ssp. *canina* 15(+), *Crataegus monogyna* 10(1), *Pistacia terebinthus* 17(+), *Cistus salviifolius* 6(1), *Turritis laxa* 17(+), *Crepis reuteriana* 9(r), *Compositae* sp. 10 (r).

Q.p.: *Quercetea pubescentis* C.-P.: *Cephalorrhyncho - Pinion* T.-G.: *Trifolioum - Geranietea* D.-F.: *Daphno - Festucetea*
Q.a.: *Quercion alnifoliae* R.-P.: *Rhamno - Prunetea* E.a.: *Epilobietea angustifolii*
Q.i.: *Quercetalia ilicis* C.-M.: *Cisto - Micromerietea julianae*

***Quercus alnifolia – Teucrium kotschyanum* sub-community** (Table 1, rel. 1–11)

This sub-association is distinguished by the dominance of *Quercus alnifolia* in the storey of shrubs with coverage values ranging from 13–35 % (except 2 relevés), while *Arbutus andrachne* is present in great degree, too. Moreover *Salvia willeana*, *Teucrium kotschyanum*, *Cistus creticus* var. *creticus* and *Clinopodium vulgare* occur frequently.

Referring to the storey of trees, no differentiation is observed comparatively with the *P. nigra* ssp. *pallasiana – Scutellaria cypria* var. *cypria* community. In contrast, the number of the herbaceous species is reduced as well as their cover values. Among the species of the herb storey *Q. alnifolia* seedlings are dominant.

Quercus alnifolia – Teucrium kotschyanum sub-community is represented by 11 relevés and mainly occurs in north–northwestern to west–northwestern aspects between altitudes 1200–1650 m. This association mainly occurs in quite steep or medium inclination convex slopes.

In this sub-community a variance from *Pterocephalus multiflorus* ssp. *multiflorus* is distinguished.

Variance from *Pterocephalus multiflorus* ssp. *multiflorus* (Table 1, rel. 9–11)

Beside *Pterocephalus multiflorus* ssp. *multiflorus* this variance is distinguished by the constant occurrence

of *Paeonia mascula* and the presence of *Rosa canina* ssp. *dumetorum* and *Juniperus oxycedrus* in shrubby or herbaceous form. Tree canopy is 60 % and shrubs cover a percentage of 20 % to 35 % of the ground. The maximum height of trees ranges between 16–24 m, which are high values for the study area.

This variance is represented by 3 relevés and occurs in western to northern aspects at altitudes around 1350 m, in gentle to medium slopes and mainly in hollow sites. Moreover, the *P. multiflorus* ssp. *multiflorus* variance is linked to gabbros in contrast with the majority of the rest of the relevés, which occur on ultramafic substrata.

***Berberis cretica – Sorbus aria* ssp. *cretica* sub-community** (Table 1, rel. 22–31)

The *Berberis cretica – Sorbus aria* ssp. *cretica* sub-community is represented by 10 relevés and is distinguished by the presence of *B. cretica* and *S. aria* ssp. *cretica* in shrubby or herbaceous form, while *Q. alnifolia* is restricted to herbaceous form in low numbers and cover values. In addition, *Scorzonera troodea* and *Euphorbia cassia* ssp. *rigoi* are important elements of the vegetation, while *J. foetidissima* is present frequently in shrubby and herbaceous form. Tree canopy does not show any difference with the *Pinus nigra* ssp. *pallasiana – Scutellaria cypria* var. *cypria* community, but coverage of shrubs is much reduced. In addition, regeneration of *P. nigra* occurs more frequently.

The *Berberis cretica – Sorbus aria* ssp. *cretica* sub-community occurs at altitudes above 1600 m, in every aspect and exclusively on ultramafic substrata. This sub-community is linked with gentle to medium inclination slopes in hollow sites.

Two faces are distinguished in this sub-community: one from *Alyssum troodi* and *Hypericum confertum* ssp. *stenobotrys* and the other one from *Epilobium angustifolium* and *Viola sieheana*.

Face from *Alyssum troodi* and *Hypericum confertum* ssp. *stenobotrys* (Table 1, rel. 22–28)

The face from *A. troodi* and *H. confertum* ssp. *stenobotrys* is represented by 7 relevés. The cover of the tree canopy ranges between 40–60 %, while its maximum height is considered relatively low for the area as it does not exceed 17 m in most of the relevés.

This face occurs at altitudes from 1650 m to 1800 m, in relatively gentle to medium inclination slopes independently of aspect or relief.

Face from *Epilobium angustifolium* and *Viola sieheana* (Table 1, rel. 29–31)

This face is represented by 3 relevés in quite dense stands. The tree canopy is 50 % and 60 %, while its maximum height ranges from 16 m to 22 m, which are high values for the area.

The face of *E. angustifolium* and *V. sieheana* occurs at altitudes around 1700 m, in gentle slopes and mainly in hollow sites.

Transitional State (Table 1, rel. 12–21)

Between *Quercus alnifolia – Teucrium kotschyanum* and *Berberis cretica – Sorbus aria* ssp. *cretica* sub-communities a transitional state is observed. This state is represented by 10 relevés and is characterized by the gradual decrease of the species of the former sub-community and the simultaneous increase of the species of the latter one.

Referring to the two sub-communities, no differentiation of the coverage of the tree canopy is observed, while in the other storeys coverage is similar to one or the other sub-community.

The transitional state occurs at altitudes from 1300 m to 1700 m mainly in western to northwestern aspects. The inclination ranges from gentle to medium and there is no any specific preference to the relief type.

Conclusion

Generally, the European Black Pine forests are quite sparse as well as the regeneration of *P. nigra* ssp. *pallasiana*. Probably, poor regeneration is due to the great thickness of the litter because of the aridity. The altitude and rock type seem to be the main factor for the distinction of the two sub-communities. In each of them the units of lower rank linked to available moisture as is modified by topography (aspect, inclination, relief).

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Artemisia campestris inland vegetation type in the "NATURA 2000" network site "Limnes Vegoritida – Petron" (GR 1340004)

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Abstract. *Artemisia campestris* dominates in sand dune communities along the north Greek coasts. This taxon has also been found to dominate in inland grassland communities in Northwest Greece, in the area around Lakes Vegoritida and Petron. The substrate of this area is calcareous and the soil is shallow and detrital. The floristic composition of the vegetation around the lakes, which is dominated by *A. campestris*, was investigated by means of 53 relevés, which were recorded using the Braun-Blanquet method. These relevés were analysed by means of numerical methods (two-way indicator species analysis and detrended correspondence analysis). Five relevé groups were distinguished, which were considered on the basis of their floristic composition to comprise three vegetation units. The distinction of the three vegetation units was also supported by the ordination diagram. The biotic and chorological spectra of the five relevé groups are provided and discussed. The *A. campestris* vegetation of the study area is classified within the Mediterranean tall grass and *Artemisia* steppes habitat type according to the EUNIS Habitat Classification and in the *Festuco–Brometea* class.

Key words: classification, *Festuco–Brometea*, Northwest Greece, ordination, plant communities, steppe-like vegetation

Introduction

Artemisia campestris L. is an Eurasiatic species, which characterizes steppic grassland communities. In Greece it dominates in sand dune communities, along the coasts of the northern part of the country (Babalonas 1979; Vasiliou 2000; Sýkora & al. 2003). It has been recorded in grey dune communities, such as the *Ephedro distachyae–Silenetum subconicae* and the *Artemisia campestris–Leymus racemosus* community (Sýkora & al. 2003). However, *A. campestris* also grows in inland dry grasslands (Horvat & al. 1974; Ellenberg 1988; Sýkora & al. 2003; Davies & al. 2004). In Greece, inland communities dominated by *A. campestris* were reported in the area around Lakes Vegoritida and Petron (Northwest Greece) (Pirini 2001; Pirini & Babalonas 2002). This steppe-like vegetation in the Balkan Peninsula is considered as a refugium for relict taxa originating from the tertiary period (Horvat & al. 1974; Ellenberg 1988; Zgaga 2005).

The aim of the present study was to investigate the floristic composition of the inland vegetation type dominated by *A. campestris* in the area around Limnes Petres and Vegoritida and to analyse it phytogeographically.

Material and methods

Study area

The study area is located in Northwest Greece and is confined by coordinates 40°44' to 40°47' N and 24°41' to 24°47' E. *Artemisia campestris* vegetation type occurs mainly in the northern part of Lake Petron and the southern part of Lake Vegoritida. In these areas *A. campestris* vegetation type occurs few meters from the lakes' shores and up to the feet of the surrounding hills. It is restricted mainly in areas where the substrate is sandy and gravelly and the slope is low or medium.

The climate of the area is submediterranean–continental, with a dry period during the summer and with harsh winter (Walter & Lieth 1964).

The geological substrate is calcareous. Especially in the areas where the *A. campestris* occurs, the soil is rich in detritus, which was formed by the erosion of the surrounding hills.

The study area is part of a Greek NATURA 2000 Network site (GR–1340004, Limnes Vegoritida–Petron).

Relevés data and analysis

In total 53 relevés were conducted in the *A. campestris* vegetation type, according to the Braun-Blanquet method (Braun-Blanquet 1964; Dierschke 1994). In each relevé the slope, exposition, altitude and total vegetation cover were recorded. Species cover abundance was recorded using the 7-grade scale of Braun-Blanquet.

Vegetation data were entered into TURBOVEG version 2.32a (Hennekens & Schaminée 2001) database. The phytosociological table was processed in JUICE version 6.3 (Tichý 2002). Taxa occurring in one or two relevés were omitted before the analyses.

The classification of the relevés was performed applying the TWINSPAN method (Hill 1979). Three pseudospecies cut levels were selected (0, 5, 25) and two levels of divisions were applied.

Detrended correspondence analysis (DCA; Hill & Gauch 1980) was used as the ordination method and was applied with the help of CANOCO version 4.5 software (ter Braak & Šmilauer 2002). Cover abundances of taxa were square-root transformed and passive explanatory variables were used in order to help the interpretation of the diagram. As explanatory variables the biotic and chorological spectra of each relevé were used. The above-mentioned spectra for each relevé were weighted by the relative cover abundance of taxa. The latter was calculated on the basis of the power transformed values of species cover abundance percentages, using as power the value 0.2.

For each vegetation unit the biotic and chorological spectra were calculated using as weight the relative cover abundances of taxa in each vegetation unit. The ranking of taxa in biotic forms and chorotypes was made mainly according to Pignatti & al. (2005), while for those taxa which are not included in the above-mentioned work, data from floras and other sources were used (e.g., Greuter & al. 1984–1989; Strid 1986; Strid & Tan 1991, 1997, 2002).

The nomenclature of taxa follows Strid & Tan (1997, 2002), Greuter & al. (1984–1989), Strid (1986), Strid & Tan (1991) and Tutin & al. (1968–1980, 1993).

Results

The TWINSPAN classification resulted in five relevé groups (Table 1). At the first level of division the 1st and 2nd relevé groups were distinguished from the next three ones. The second level of division resulted in the distinction of the 1st, 2nd and 5th relevé groups, while the division of the relatively large relevé group, which remained, resulted in the distinction of the 3rd and 4th relevé groups. On the basis of the floristic differentiation, the five relevé groups are considered to comprise three vegetation units. The first and second vegetation units are represented by the 1st and 2nd relevé groups, respectively, while the third vegetation unit consists of the 3rd, 4th and 5th relevé groups.

The first vegetation unit consists of the relevés conducted at Lake Vegoritida. It is positively differentiated by ruderal species, such as *Crepis setosa*, *Conyza canadensis*, *Cirsium candelabrum* and *Psoralea bituminosa*. Additionally, it is negatively differentiated by the absence of a group of species, which occurs in all the other vegetation units. This vegetation unit occurs in relatively lower altitudes than the rest of the units.

The second vegetation unit occurs in areas with relatively high inclination, but with more or less stabilized substrate. It is differentiated by species characteristic of the typical grasslands of the area, which have been classified within the *Stipa capillata* community by Pirini (2001).

The third vegetation unit is well differentiated from the previous two ones by taxa such as *Medicago minima*, *Avena fatua*, *Bromus tectorum* and *Arenaria serpyllifolia*. It grows on a more gravelly soil and on relatively more gentle slopes than the previous vegetation units. It is divided in two main subunits, which correspond to the 3rd and 5th groups of Table 1. The differentiation between these two subunits is relatively weak and may be attributed to the finer texture of soil and the smaller gravel size of the 5th group. The 4th group represents an intermediate vegetation type between the above-mentioned subunits.

In the DCA diagram (Fig. 1) the distinction between the first two vegetation units and the third one is clear. The first vegetation unit appears as an outlier in the diagram, occurring at the leftmost part of axis 1. Axis 1 is also responsible for the distinction between the second and the third vegetation unit. The differentiation inside the third vegetation unit (groups 3–5) may be attributed to axis 2.

Table 1. Relevés table of the *A. campestris* vegetation type. Taxa occurring in one or two relevés were omitted. Boxes indicate differential taxa.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Relevé No.	52	53	51	43	42	48	4	3	49	46	2	6	7	1	8	45	47	44	50	5	31	41	36	34	32	23	35	30	20	25	17	28	15	29	26	27	40	39	24	23	38	13	22	18	19	11	21	9	14	12	10	37	16
Relevé area (m²)	40	40	50	40	40	50	40	40	40	50	40	40	40	40	40	50	40	50	40	30	30	30	30	30	30	20	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Altitude	515	520	515	512	512	580	640	610	576	575	605	630	600	600	660	575	575	595	630	600	580	595	590	585	585	580	590	605	595	590	578	580	590	583	580	578	572	585	590	585	572	580	620	600	590	572	585	600	585	585	572	585	590
Exposition	SE	E	SE	N	SE	–	S	S	–	S	S	W	S	SE	SE	SE	–	SE	E	N	SE	W	E	S	SE	SE	NE	E	SE	S	E	E	E	E	E	E	E	E	S	SE	SE	E	E	E	E	SE	SE	SE	SE	E	SE	SE	SE	E
Slope (%)	20	20	25	5	20	–	40	10	–	10	5	50	5	20	50	10	–	50	50	5	20	15	10	5	15	10	10	35	10	15	10	15	10	10	5	5	5	100	200	155	100	15	100	500	5	5	500	300	500	5	500	455	155	100
Total cover (%)	500	800	750	800	400	800	650	700	400	650	800	800	600	700	700	500	850	650	500	700	750	900	700	700	600	500	600	700	700	700	900	600	850	500	600	700	500	650	600	800	400	900	405	455	800	404	555	700	700	600	400	455	
Crepis setosa	+	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Silene otites	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Conyza canadensis	+	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium candelabrum	r	.	r	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Psoralea bituminosa	+	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Centaurea grisebachii	+	.	+	.	+	+	+	+	r	+	.	+	.	r	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Petrorhagia illyrica	.	+	+	.	.	+	.	.	+	.	+	+	+	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cichorium intybus	.	.	+	.	+	.	+	+	.	+	+	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stipa capillata	.	.	.	.	.	r	+	1	.	.	1	2	.	+	+	+	r	r	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Echinops sphaerocephalus	.	.	.	.	.	+	+	+	+	+	.	.	+	+	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Scabiosa argentea	.	.	.	.	.	+	+	.	.	+	.	+	+	.	+	r	+	+	.	+	.	+	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Consolida regalis	.	.	.	.	.	.	.	+	.	+	.	r	.	.	r	r	r	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carlina corymbosa	.	.	.	.	.	.	+	+	.	+	.	+	.	.	+	+	.	+	+	.	.	.	.	.	.	.	.	.	.	.	+	.	r	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.
Avena barbata	.	.	+	.	.	.	+	.	.	.	r	.	.	.	+	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Taraxacum officinale	.	.	.	.	+	+	.	.	+	+	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stachys officinalis	.	.	.	.	.	.	+	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Centaurea salonitana	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Echinops microcephalus	.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Allium sphaerocephalon	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dichanthium ischaemum	.	.	.	+	.	.	+	+	+	+	1	.	1	1	2	+	+	+	.	.	+	.	r	.	+	.	.	1	.	+	.	.	+	1	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Xeranthemum inapertum	.	.	.	.	.	+	+	+	.	1	+	+	+	+	.	.	.	.	.	+	+	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Medicago minima	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	+	1	+	1	1	1	1	1	+	+	1	1	.	.	1	+	+	+	.	+	2	1	1	1	+	1	+	1	+	+	+	+
Avena fatua	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	+	.	+	+	+	+	+	+	1	+	.	+	+	.	+	+	+	+	+	+	+	.	+	+	.	.	+	.	.	.
Bromus tectorum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	r	.	r	+	+	.	.	+	.	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arenaria serpyllifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	+	.	+	+	.	+	+	.	.	+	+	+	.	+	.	+	+	1	+	+	.	+	+	.	+	+
Gnaphalium luteo-album	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	+	+	+	.	+	+	+	+	+	+	+	+	+	.	.	+	+	.	+	+	.	+	.	+	+	.	+	+	+	.
Vulpia ciliata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	.	.	.	+	.	.	.	.	.	+	+	+	+	+	.	+	.	+	+	.	+	.	+	.	.	.
Acinos alpinus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	+	+	.	+	+	+	+	+	.	+	.	.	.	.	+	.	.	.	.	.	.	+	.	+	+	+	.	+	.	+	.	.
Crepis sancta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	r	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	+	+	.	+	+	.	.
Leontodon crispus	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	+	.	.	.	r	+	r	+	r	.	+	.	+	.	r	.	+	.	.	r	.	+	.	+	.	.	+	.	+
Bromus intermedius	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	+	.	+	.	+	.	.	.	.	.	+	.	+	+	.	+	+	+	+	+	.	+	.	+	+	+	+

Table 1. Continuation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Linaria simplex	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	+	.	.	+	+	+	.	.	+	+	.	.	.	+	.	.	.	.	.	.	+	.	.	.	r	+	.	.	.	.	.	+
Onobrychis arenaria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	r	.	+	+	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+	.
Minuartia glomerata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	+	+	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	+	.
Brachypodium distachyon	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	+	.	.	+	.	+	.	+	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Lens nigricans	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	.	.	.	+	+	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Medicago turbinata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	+	+	.	.	+	.	+	.	+	+	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.
Psilurus incurvus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	+	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trigonella monspeliaca	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	.	.	.	+	+	+	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Erodium cicutarium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	+	.	+	.	r	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Linum bienne	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Arthemis arvensis	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+	+	+	+	+	.	.	+	.	.	.	r	+	r	+	.	.	.	.	.	.	.	.	.	.	.	r	.	.
Petrorhagia prolifera	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	.	.	.	.	r	.	.	.	.	.	+	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Arrhenatherum palaestinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	+	.	+	.	+	.	+	+
Valerianella rimosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	+	+	+	+	.	+	+	+	+
Lathyrus cicera	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	+	+	.	+	+	.	.
Euphorbia taurinensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	r	.	.	.	.	.
Hypericum rumeliacum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.
Cruciata pedemontana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Crupina crupinastrum	.	.	.	.	.	.	+	+	.	.	+	.	+	+	+	.	.	+	.	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.	.	.	.	+	.	+	+	+	r	.	+	.	+	.	.
Silene conica	.	.	.	.	.	.	.	.	.	+	.	+	.	+	.	.	.	.	.	.	+	+	.	.	+	.	+	.	.	.	+	r	.	+	.	.	.	+	+	+	+	.	.	+	.	.	.	.	.	.	+	+
Verbascum leucophyllum	.	.	.	.	+	.	+	.	.	+	.	.	+	.	r	+	+	+	.	.	+	.	r	.	.	.	.	.	r	.	+	.	+	.	.	.	+	+	+	+	+	.	.	r	.	.	.	+	+	+	+	+
Euphorbia myrsinites	.	.	.	.	.	.	.	+	+	.	+	.	+	.	+	+	+	+	.	.	.	.	.	.	+	.	.	.	+	.	+	.	+	.	.	.	.	.	.	.	.	.	r	.	+	.	+	+	+	+	+	+
Micromeria cremnophila	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	+	r	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.	.	+	.	r	+	+	1	1	+	+	.
Thymus sibthorpii	.	.	.	.	.	.	.	+	.	.	.	+	.	.	+	.	+	.	.	.	+	+	1	.	+	+	+	+	.	1	+	+	1	+	.	1	+	+	1	.	.	+	+	+	+	1	+	+	+	1	+	1
Helianthemum salicifolium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	.	.	+	+	+	+	1	+	+	.	.	.	+	+	.	1	+	+	.	.	+	.	.	.	.	.	.	+	.
Koeleria macrantha	.	.	.	.	.	.	.	+	.	.	+	+	.	+	.	+	+	+	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.	.	+	+	+	.	.	+	+	.	.	+	.	+
Marrubium peregrinum	.	.	.	.	.	.	+	.	+	+	.	+	.	r	+	.	+	.	.	.	.	+	+	.	r	.	.	.	.	r	.	+	.	.	.	.	+	+	.	.	+	r	.	.	+	+	.	+	.	.	.	.
Eryngium campestre	.	.	.	.	.	.	.	+	+	.	+	+	1	+	+	+	+	r	+	+	.	+	+	+	+	.	+	+	+	+	+	+	+	+	+	+	+	+	+	.	.	+	+	.	.	+	+	+	+	+	+	+
Cynodon dactylon	.	.	.	.	.	.	1	.	.	.	+	.	.	.	.	.	+	+	+	.	.	+	.	+	+	.	1	.	+	+	.	.	.	.	.	1	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+	.
Artemisia campestris	1	3	2	3	2	4	3	3	1	3	3	3	4	4	2	3	3	3	2	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	4	4	3	2	3	2	2	3	2	2	2	2	2	3	2	2	2	2
Teucrium polium	+	+	.	.	.	+	+	+	+	+	+	+	+	.	+	+	.	+	+	.	+	+	+	.	+	.	+	+	+	1	+	+	1	+	+	+	+	+	1	+	+	+	+	1	+	1	+	.	+	.
Erysimum pussilum	+	.	.	r	.	+	r	.	+	.	+	.	+	.	r	+	+	+	.	.	+	.	+	.	.	+	+	+	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dasypyrum villosum	r	+	.	.	.	r	+	.	.	+	.	+	.	r	r	.	.	r	.	.	+	.	.	.	+	+	+	+	+	+	+	+	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melica ciliata	+	+	+	.	+	.	+	+	.	+	.	.	+	.	+	+	+	+	.	.	+	.	.	.	+	.	+	.	.	+	.	.	.	+	.	.	+	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.
Chondrilla juncea	+	.	1	.	+	+	.	.	+	+	.	+	.	+	+	.	+	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	r	+
Asperula purpurea	.	.	.	.	+	.	+	+	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+	+	.	.	.	+	.	+	.	+	.	+	.	.	.	.	.	1	+	1	1	+	.	.	.	.
Orlaya kochii	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	+	+	.	.	.
Artemisia alba	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	r	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	+	1	.	.	+	r	+	r	.	+	r	.	.	r	.	.
Aegilops triuncialis	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+	.	.	.	.	+	+	.	+	+	.	.	.	.	.	.
Ononis pusilla	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	r	+	.	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+	r	.	.	.
Aurinia saxatilis	1	.	+	.	.	.	.	+	.	.	+	.	1	.	.	.	.	.	.	.	+	.	+	.	+	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Calamintha nepeta	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	.	.	.	+	.	.	.	.	.
Satureja montana	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	r	.	.	.	.	r	.	r	.	.	.	+	.	.	.	.	.	.	.	r
Teucrium chamaedrys	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Thesium humile	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	+	+	.	.	.	.	.	.	+	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Herniaria incana	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	+	.	.	.
Chrysopogon gryllus	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	r	.	.	.	.	1	.	.	.	.	r	r	.	.	.	+	.	.	.
Poa bulbosa	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	+	.	+	.	.	.	.	.	.	+	.	+	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Hippocrepis ciliata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.

Table 1. Continuation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Achillea chrysocoma	+					r						+	+																									+	+														+
Carthamus lanatus							+	+							+																																						
Trifolium campestre																									1							+			+			+				1								+		
Petrorhagia thessala				+	+	r								+											+														+												
Thesium linophyllon																																																			
Asperula rumelica															+					+												+	+	+																	
Stachys iva																									r						r																				
Fumana procubens									+																																										
Crucianella graeca										+					+																																				
Medicago orbicularis																					+		+		+		+																								
Astragalus sericophyllus																							+											+																	
Sedum urvillei																									+					r																					
Phleum arenarium			+				+				+																+																								
Hypochoeris cretensis					+																																+				+											
Potentilla recta						+																	+		+				+																						
Papaver rhoeas																								+																											
Scabiosa webbiana																									+		+					+																		
Galium verum		+																	+																																
Bromus ramosus																											+				+																		
Desmazeria rigida																											+										+												
Euphorbia helioscopia																																					+	+											

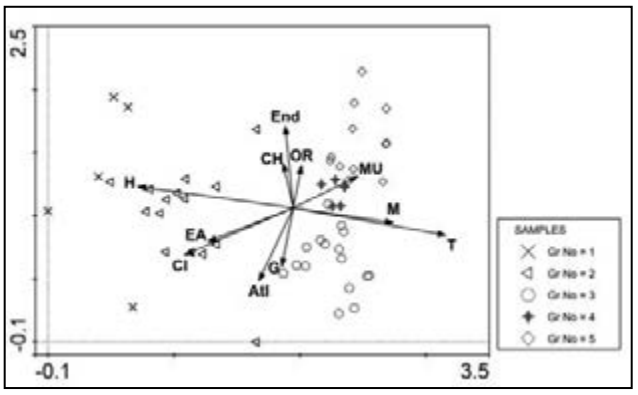

Fig. 1. Ordination biplot of the first two DCA axes. Arrows represent biotic forms and chorotypes of taxa.
Abbreviations: H – hemicryptophytes; **CH** – chamaephytes; **T** – therophytes; **G** – geophytes; **End** – Balkan and Greek endemics; **M** – Mediterranean; **OR** – Southeast European and Mediterranean mountainous taxa; **EA** – Eurasiatic; **Atl** – Atlantic; **CI** – circumboreal; **MU** – multizonal taxa.

The intermediate status of the 4th group is also indicated by the position of its relevés in the DCA diagram. The length of the first and second axis is 2.685 and 2.142, respectively. The eigenvalues are 0.371 and 0.167, respectively. Both axes explain 17.4 % of the total inertia (3.091).

The first axis is strongly correlated with the occurrence of therophytes, Meditteranean species and hemicryptophytes. Axis 2 is positively correlated with the occurrence of endemic (Balkan or Greek) species, as well as with the occurrence of chamaephytes and mountainous species (SE Europe and Meditteranean), while it is negatively correlated with the occurrence of geophytes and atlantic species. Therefore, axis 1 possibly represents the differentiation between the three vegetation units, which may be attributed to different soil and microclimate conditions, as well as to spatial differentiation (1st group consists of all the relevés made at Lake Vegoritida), while axis 2 may represent a gradient related with soil texture and gravel size.

In Figs 2 and 3 the biotic and chorological spectra of the relevé groups are presented. The biotic spectra of the relevé groups clearly revealed the predominance of hemicryptophytes in the first two vegetation units and the predominance of therophytes in the third vegetation unit. Furthermore, differences between the vegetation units are also shown from their chorological spectra, the most important of which concern the higher percentage of Eurasiatic species in the second vegetation unit and the higher percentage of Mediterranean species in the third vegetation unit.

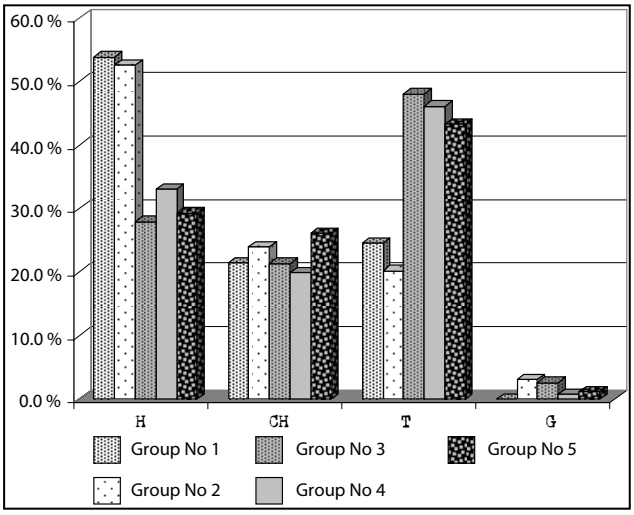

Fig. 2. Biotic spectra of the five relevé groups, weighted by the relative cover abundance of taxa.
Abbreviations: H – hemicryptophytes; **CH** – chamaephytes; **T** – therophytes; **G** – geophytes.

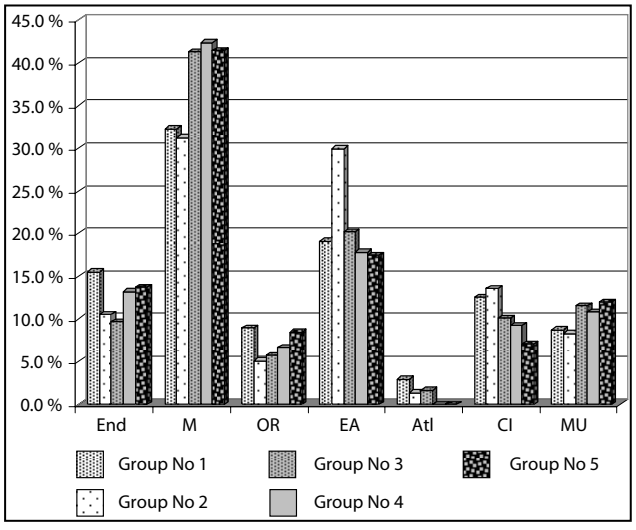

Fig. 3. Chorological spectra of the five relevé groups, weighted by the relative cover abundance of taxa.
Abbreviations: End – Balkan and Greek endemics; **M** – Mediterranean; **OR** – Southeast European and Mediterranean mountainous taxa; **EA** – Eurasiatic; **Atl** – Atlantic; **CI** – circumboreal; **MU** – multizonal taxa.

Discussion

The floristic composition of the *A. campestris* vegetation type of the study area is strongly differentiated from that of the grey dune communities occurring along the north Greek coasts, in which *A. campestris* also dominates. The common species between these two vegetation types, such as *Teucrium polium*, *Eryngium campestre*, *Cynodon dactylon*, *Dasypyrum villosum*, *Chondrilla juncea*, *Anthemis arvensis*, *Cichorium intybus*, *Arenaria serpylifolia* and *Medicago minima*, have a relatively wide ecological amplitude, occurring in different grassland and ruderal vegetation types. *Artemisia campestris* itself is not a typical species of coastal sand dune vegetation as it is commonly found in dry, base rich inland grasslands of *Festuco–Brometea* or *Koelerio–Corynephoretea* (*Sedo–Scleranthetea*) (Ellenberg 1988; Sýkora & al. 2003).

The *A. campestris* vegetation of the study area may be classified within the Mediterranean tall grass and *Artemisia* steppes habitat type according to the EUNIS Habitat classification (Davies & al. 2004). This habitat type is classified within the dry grassland vegetation. Indeed, according to the diagnostic species of the European vegetation classes provided by Mucina (1997), *Festuco–Brometea* is the most richly represented class in the phytosociological table of the *A. campestris* vegetation type of the study area.

The occurrence of *A. campestris* vegetation type in the study area, in which several species with a Southeast Asian distribution appear, as well as the neighboring grassland community of *Festuca valesiaca–Stipa capillata* form a steppe-like sward vegetation around Lakes Vegoritida and Petron. The study area may be considered as a refugium for light-loving steppe plants (Horvat & al. 1974; Ellenberg 1988; Zgaga 2005), which may have been preserved in the area because of the limestone substrate, which forms a karst landform, the detrital soil and/or the human influence on the vegetation cover.

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Plant communities, habitats and ecological changes in the vegetation on the territory of three protected areas along the Danube River

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Abstract. The objects of these researches – the eastern part of the island of Belene and the central part of "Kalimok–Brushlen" Protected Locality have been affected by a great anthropogenic degradation, which have happened during the second half of the 20th century. These changes altered the dominating plant communities and habitats of these protected areas. The former marshes are an object of big project for the restoration of their water regime.

Key words: Danube River, plant communities, syntaxa, wetlands

Introduction

During the summer of year 2004 at project: "Restoration of Wetlands and Reduction of Pollution", a scientific exploration of the two territories of flooding – the eastern part of the island of Persina (Belene) (within the boundaries of Persina Natural Park, which also include a "Persina Marshes" Maintained Reserve and "Persina–East" Natural Monument) and part of "Kalimok–Brushlen" Protected Locality – was carried out. The main objectives of the task are: determination of the syntaxonomic, hence habitat diversity of the territories and the changes into the vegetation after the changes in the water regime. Both territories are located on the Bulgarian riverside of the Danube, within some of the former floodplains – Svishtov–Belene (143 km^2) and Pobrezhie (100 km^2). Characteristic for both objects is the great anthropogenic degradation they have been subjected to, as a result from various reasons. For the island of Belene, that has been the construction of the prison and the dyke related to it, protecting the adjacent farm lands from flooding. For the region of the former marshes of Brushlensko and Kalimok, it has been the unsuccessful drainage of the lands for the purpose of their cultivation, and the successive construction of fishponds, which also proved economically inefficient. All these events happened during the second half of the 20th century. They have dramatically altered the picture of the dominating communities and habitats. Parts of these changes have remained hidden, as long as published data about the vegetation of the two objects, especially from the more distant past, are rather insufficient.

There can be found data on the vegetation and the flora of the island of Belene in the works of Stoyanov (1927, 1947, 1948), Bonchev (1929), Kochev & Yordanov (1981), Kochev & Tsolova (1984), Todorov (1984), Tzonev (2002), Tzonev & Šumberová (2004). The most complete one is the final report of Dister & al. (2001). The data on the vegetation of "Kalimok–Brushlen" are even scarcer than those on Belene. The region was briefly described by Bonchev (1929), Kochev & Yordanov (1981), Kochev & al. (1986) and Baeva (1991), the final report of WWF (Dister & al. 2001).

Material and methods

The surveys of the vegetation were carried out in accordance with the principles and methods of the sigmatic school (Braun-Blanquet 1964; Westhoff & van der Maarel 1973; Mueller-Dombois & Ellenberg 1974). More than 100 relevés were made for the purposes of

the investigation in the territories planned for flooding. For the purposes of the work on site, the 7-degree scale of Braun-Blanquet (1933) was used. It was transformed (according to Westhoff & van der Maarel 1973) for the purpose of the statistical processing into the 9-degree scale of Barkman & al. (1964). This scale is presented also in the diagnostic tables (see Tables 1–7). The floristic dissimilarity into the groupes of relevés was calculated through the formula: d=1-S, where S is similarity by means of the index of Horn (1966). During the tabular processing, the relevés were arranged in the succession determined by the classification (cluster) dendrogram (Podani 1993 – program SYNTAXA). The syntaxa were determined according to the rules of ICPN (Weber & al. 2000) and cited literature source (Sanda & al. 1999; Chytrý & Tichý 2003; Rodwell & al. 2002; etc.). The determined syntaxa were referred to as belonging to different habitats, according to EUNIS habitat classification (Davies & Moss 1997 – on-line version 2004) and the Council Directive 92/43/EEC (1992).

Results and discussion

C1.3 Permanent eutrophic lakes, ponds and pools

This habitat (Table 1) includes natural and semi-natural basins (canals and other basins) with standing, eutrophic water, rich in floating vegetation. According to Kochev & Tsolova (1984) all tree marshes on the island (Peschina, Murtvoto and Doulyova Bara) and the bigger pools of Nova Cherna fishponds represented mainly this habitat in the past. Now it is presented chiefly by the big and deep drainage canals, which do not run dry in summer. The habitat and its corresponding syntaxa belong to Annex I of the Directive 92/43/EEC with the code **3150 Natural eutrophic lakes with *Magnopotamion* and *Hydrocharition*–type vegetation**. The syntaxa which were established in the region belong to the following subtypes:

C1.31/ P–22.41 Free-floating vegetation of eutrophic waterbodies

This subtype occurs mainly in the canals which belong to the next syntaxa:

Ass. *Lemnetum minoris* – this association is established in some smaller canals on Belene Island and in the region of "Kalimok–Brushlen".

Ass. *Salvinio–Spirodeletum polyrhizae* – this is a dominant sytaxon of the habitat type of the floating on the water surface communities especially in the big and deep canals.

Ass. *Lemnetum gibbae* – this association was established to be of a very limited (2 m^2) distribution – in dried pools inside the moist meadows in the region of "Kalimok–Brushlen".

Ass. *Lemno–Utricularietum vulgaris* – this association was found in a small canal in the region of "Kalimok–Brushlen".

C1.33/ P–22.43 Rooted floating vegetation of eutrophic waterbodies

This subtype is presented as small spots inside the above-mentioned subtype. The reason is the heavily worsened conditions for its existence in both territories. According to Kochev & Yordanov (1981) and Kochev & Tsolova (1984), the open water surface of the three big marshes on the Belene Island was covered by this habitat type. During the 70's, the monocoenoses of *Nymphaea alba* in Doulyova Bara were the biggest in the whole country, and those of *Trapa natans* in Murtvoto marsh have covered an area of more than 400–500 dca. Now both species are extinct from the flora of Belene, and in "Kalimok–Brushlen" only single specimens or small groups survived.

Ass. *Nymphoidetum peltatae* – this syntaxon was very common and with bigger distribution in the past as in the marshes of Belene Island and Nova Cherna. *Nymphoides peltata* is a flexible species, but the association exists only on very small spots in the canals of "Kalimok–Brushlen".

Ass. *Nymphaeetum albae* – only one community from the central drainage canal of "Kalimok–Brushlen", in zone "West" was defined as this association.

C3.2 Water fringing reedbeds and tall helophytes other than canes

The main syntaxa dominated by tall graminoid helophytes are included in this habitat (Table 2). According to Bonchev (1929), Kochev & Yordanov (1981), Kochev & Tsolova (1984), this habitat type formed big communities in the past there. But the degradation processes in the marshes are connected with its big range now. Now all marshes on Belene Island and the basins of the fishponds are covered by this vegetation.

Ass. *Scirpetum lacustris* – some small phytocoenoses of this association cover were established in both investigated regions.

Table 1. C1.3 Permanent eutrophic lakes, ponds and pools.

Number of columns	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	**Const.**
Number of relevés	11	23	27	31	32	41	59	60	62	64	67	69	70	71	73	56	51	52	33	37	65	
Area of relevés (sq.m.)	10	1	5	2	25	2	4	2	4	4	2	2	3	9	4	0,7	0,2	0,3	9	1	10	
Cover of relevés (%)	50	80	50	90	90	70	100	80	60	80	95	100	100	95	95	80	70	70	80	60	80	
Diagnostic species of the associations																						
Salvinia natans	7	8	2	8	9	7	6	2	8	8		6	7	7	9	5			2	7	2	V
Lemna minor	5	5	2	5	7	5	3	8	2	2	9	6	7	6					2			IV
Lemna trisulca			8	7	7	7	7		5			7	8	8	5					2		III
Spirodela polyrhiza			2	2				2		2	2	2	2	6		2						III
Lemna gibba														6			8	8				I
Utricularia vulgaris															2	8						I
Nymphaea alba														2					9			I
Nymphoides peltata																				5	9	I
Lemnion, Utricularion, Lemnetalia, Lemnetea																						
Hydrocharis morsus-ranae				2	2	2		2	3	5			2	2	2	5			2			III
Azolla caroliana							8	3				7		7								I
Ceratophyllum demersum			6	2		3	6															I
Nymphaeion, Potametalia, Potametea																						
Potamogeton lucens										3						2				2		I
Trapa natans					2																	I
Other species																						
Oenanthe aquatica	2		2	2	2			2		2											2	II
Phragmites australis				2					2	2									2			I
Rorippa amphibia														2	2	2	2					I
Alisma plantago-aquatica	5									5												I
Sparganium erectum	2								5													I
Teucrium scordium																2		2				I
Riccia fluitans												6				2						I
Typha angustifolia										2											2	I
Typha latifolia		2																				I
Myosotis palustris																				2		I
Agrostis alba																		2				I
Persicaria minor									3													I
Catabrosa aquatica										3												I
Schoenoplectus lacustris																					2	I

1-15 Ass. *Salvinio-Spirodeletum polyrhizae* Slavnic 1956 – **1,** Murtvoto marsh; **2,** Peschinsko marsh; **3-6,** Nova Cherna – canals in zone "West"; **7-15,** canals in the fishponds in zone "East"; **16 Ass. *Lemno-Utricularietum vulgaris*** Soo 1928 – Nova Cherna – canal in zone "East"; **17, 18 Ass. *Lemnetum gibbae*** Miyawaki & Tuxen 1960 – Nova Cherna – dried pools in the meadows of zone "East"; **19 Ass. *Nymphaeetum albae*** Vollmar 1947 – Nova Cherna – canal in zone "West"; **20, 21 Ass. *Nymphoidetum peltatae*** (Allorge 1927) Muller & Gors 1960 – Nova Cherna – canals and the bottom of the fishponds in zone "East".

Ass. *Typhetum angustifoliae* – this is the second as a summarized area of this habitat type in the marshes of Belene Island and "Kalimok–Brushlen" (basins No. 6 and No. 2). *Typha angustifolia* goes before the expansion of the reed, when the basins become dry.

Ass. *Phragmitetum communis* – this is the commonest association on both territories. The biggest area with reed is in Peschina marsh, there is a wide belt in Murtvoto marsh, and in almost all basins of the fishponds of Nova Cherna.

Ass. *Typhetum latifoliae* – it was established south of the Peschina marsh.

Ass. *Typhetum laxmani* – its presence only in Kalimok was noted.

Ass. *Sparganietum erecti* – the communities where this species is a dominant were noted in some

small canals in the former fishponds "Kalimok–Brushlen".

***Iris pseudacorus* comm.** – the communities dominated by *Iris pseudacorus* in the Kadunovi Bari marsh in "Persina–East" are an interesting fact. The dominant is 1.80–2.00 m tall. Other tall plants (bushes and herbs) participate in these communities.

Ass. *Glycerietum aquaticae* – this association was established in dried canals in zone "East" of the former fishponds of Nova Cherna.

Table 2. C3.2 Water fringing reedbeds and tall helophytes other than canes.

Number of columns	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	**C**
Number of relevés	17	36	42	43	44	55	58	75	19	3	12	13	29	57	74	21	22	54	93	66	**o**
Area of relevés (sq.m.)	100	25	25	25	25	25	25	25	100	25	25	25	25	25	25	100	100	100	100	25	**n**
Cover of relevés (%)	100	90	90	100	100	100	100	100	100	70	80	80	80	80	100	100	100	100	80	70	**s**
																					t.
Diagnostic species of the associations																					
Phragmites australis	8	8	8	9	9	8	9	8						2	2	7		2		2	IV
Typha angustifolia								3	7	7	8	8	7	8	9				2		III
Typha latifolia			2													8					I
Schoenoplectus lacustris					2		2		7				7				8	7		3	II
Iris pseudacorus		2				6						2						7	8		II
Glyceria aquatica							2													8	I
Phragmito-Magnocaricetea, Phragmitetalia, Phragmition																					
Stachys palustris	2	3		2	2	3	5	3	6		5		3				6	3			III
Lycopus europaeus	2		2	2	2			2	2	2							3				II
Galium palustre		3	3				3	2			2							2			II
Alisma plantago-aquatica		2								2		2			2		2				II
Sparganium erectum											5	2					2				I
Phalaris arundinacea		5				3												6		3	I
Oenanthe aquatica										3	2	2									I
Sium latifolium		2									2						3				I
Other species																					
Cirsium arvense	3	3	2	5	3	3	3						2	2				2	2		III
Potentilla reptans	2	3	2		3		2		2	2			2			2	2	2			III
Lythrum salicaria	3	2						2	5		2	2		2		2					II
Lysimachia vulgare		5	2	5		3		5										2			II
Agrostis alba			3		5	3		2										5		2	II
Calystegia sylvatica	3		2	2	2														2		II
Xanthium strumarium										7							3		2		I
Persicaria mitis									3			2					2				I
Bolboschoenus maritimus		3						2		2			6								I
Gratiola officinalis							2											3			I
Symphytum officinale			2											2				2			I
Euphorbia lucida	2																		5		I
Elymus repens	5												2								I
Periscaria hydropiper										2					2						I
Salvinia natans														2	6	2					I
Rumex crispus													2								I
Althaea officinalis						2											2	2			I
Butomus umbellatus											3										I
Glechoma hederacea	7		5		2																I
Bidens frondosa																	3		2		I
Teucrium scordium													2					3			I
Epilobium parviflorum			3														2				I
Sambucus deborensis			2																		I
Tanacetum vulgare	2		2																		I
Ranunculus acris																	3				I

Table 2. Continuation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Rorippa amphibia										2				2							I
Sonchus arvensis subsp. *uliginosus*			2																		I
Eleocharis palustris										2			2								I
Myosoton aquaticum	2																				I
Glycyrrhiza echinata													2						2		I
Lycopus exaltatus																		2			I
Rumex palustris						3												2			I
Lysimachia nummularia										2											I
Amorpha fruticosa								2											2		I
Oenanthe fistulosa			2																		I
Solanum dulcamara			3																		I
Viola arvensis			2																		I
Myosotis scorpioides			3																		I
Inula britannica					2																I
Hydrocharis morsus-ranae							2														I
Drepanocladus aduncus													7								I
Lemna minor							2														I
Salix alba								2													I

1-8 Ass. *Phragmitetum communis* (Gams 1927) Schmale 1939 – **1,** Peschina marsh; **2, 3, 4, 5,** Nova Cherna – fishponds in the zone "West"; **6, 7, 8,** fishponds in the zone "East"; **9-15 Ass. *Typhetum angustifoliae*** Pingnatti 1953 – **9, 10,** Peschina marsh; **11, 12,** Murtvoto marsh; **13,** Nova Cherna – the fishponds in the zone "West"; **14, 15,** Nova Cherna – fishponds in the zone "East"; **16 Ass. *Typhetum latifoliae*** Lang 1973 – **16,** Peschina marsh; **17, 18 Ass. *Scirpetum lacustris*** Schmale 1939 – **17,** Peschina marsh; **18,** Nova Cherna – fishponds in the zone "East"; **19 *Iris pseudacorus* comm.** – **19,** Persina-East – Kadunovi Bari marsh; **20 Ass. *Catabrosetum aquaticae*** Kaiser 1926 – **20,** Nova Cherna – canal near to zone "East".

C3.24/ P–53.14 Medium tall non-graminoid waterside communities

These communities (Table 3) grow in the big, periodically dried basins, but can survive in the mud. They occur in zones not covered by the reed and reedmace of the Murtvoto and Peschina marshes and in some of the deeper parts of Nova Cherna fishponds. According to Stoyanov (1948), on Belene Island they were distributed only in the periphery of the marshes. Kochev & Yordanov (1981) described community of *Oenanthe aquatica* in the periphery of Murtvoto marsh, but in Peschina there is already an area of near 150 ha. In the end of the 70's, the succession went towards the enlargement of these communities. Now it was finished in Peschina, but in the Murtvoto marsh and the Nova Cherna fishponds it is passing to the last phase before the communities of tall helophytes.

Ass. *Rorippo amphibiae–Oenanthetum aquaticae* – this association presents the habitat in both regions in their open-water areas. Beside both main dominants more interesting are the survival hydrophytes in these phytocoenoses as *Potamogeton* spp., *Ceratophyllum submersum* L., *Nymphaea alba* L. (in "Kalimok–Brushlen"), *Nymphoides peltata* (S.G. Gmelin) O. Kuntze, *Salvinia natans* L.

E5.4/ P–37.13 /p/ Continental tall herb communities of humid meadows

About the distribution of this habitat type (Table 4) on Belene Island in the past there are indirect data. Described by Stoyanov (1948) "altoherboses" from the island belong to this habitat type. According to him, they often grew up on the places of destroyed forest communities. A similiar situation exists now on the island, but in more restricted areas. This habitat is included in Directive 92/43/EEC with code **6430 Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels**. The described relevés are divided into two provisional associations:

Ass. *Euphorbio lucidi–Bolboshoenetum maritimi* nom. prov. – this provisional association was found mostly in the western and south-western parts of the Peschina marsh. The height of the upper herb floor is near 1.20 m.

Ass. *Glycyrrhizo echinatae–Bolboschoenetum martimi* nom. prov. – this syntaxon was found only in the region of "Kalimok–Brushlen", fragmentary in the zone "East" and wider areas has in some former basins of the fishponds. A characteristic species is *Bolboschoenus martimus* (L.) Pallas, which appears on weak salty soils.

Table 3. C3.2 Medium tall non-graminoid waterside communities.

Association *Rorippo amphibiae-Oenanthetum aquaticae* (Soo 1928) Lohmeyer 1950			
Number of columns	1	2	3
Number of relevés	26	46	50
Area of relevés (sq.m.)	100	4	25
Cover of relevés (%)	80	70	80
Diagnostic species of the association			
Rorippa amphibia	7	8	7
Oenanthe aquatica	6	3	7
Oenanthion, Oenanthetalia			
Alisma plantago-aquatica	2	2	
Sparganium erectum			5
Phragmito-Magnocaricetea			
Typha angustifolia	6		
Phragmites australis			3
Schoenoplectus lacustris			2
Other species			
Persicaria hydropiper	3		
Salvinia natans	3		
Nymphoides peltata	7		
Potamogetom gramineus	2		
Agrostis stolonifera		5	3
Myosotis scorpiodes			3
Lemna minor			2
Hydrocharis morsus-ranae			2
Lemna trisulca			2

Localities and dates of the relevés: 1, Murtvoto marsh – 14.07.2004; **2, 3,** Nova Cherna – zone "East" – 03.08.2004.

Table 4. E5.4 Continental tall herb communities of humid meadows.

Number of columns	1	2	3	4	5	6	7	8	9	**Const.**
Number of relevés	20	24	25	45	48	78	79	80	81	
Area of relevés (sq.m.)	100	100	100	25	100	100	100	100	100	
Cover of relevés (%)	100	100	100	100	100	100	100	100	100	
Diagnostic species of the associations										
***Glycyrrhyzo echinatae-Euphorbietum lucidae* nom. prov.**										
Glycyrrhiza echinata		5	2			3	2		2	III
Matricaria trichophylla	2	2	3							II
Euphorbia lucida	7	7	7							II
***Lycopo exaltatae-Bolboschoenetum maritimae* nom. prov.**										
Bolboschoenus maritimus		5		3	5	3	3	5		IV
Lycopus exaltatus					3	5	2	5	3	III
Veronico longifoliae-Lysimachion vulgaris										
Stachys palustris	6			2	2	2	2	3	3	IV
Lythrum salicaria			2		2			2	3	III
Phalaris arundinacea				2	2			3		II
Symphytum officinale							2			I
Euphorbia palustris							2			I

Table 4. Continuation.

	1	2	3	4	5	6	7	8	9	
Molinetalia, Molinio-Arrhenatheratea										
Agrostis alba		6	6	7	7	5	8	7	7	V
Potentilla reptans	2	2			2	5	3	3		IV
Elymus repens	7	5	6	7			3	7		IV
Lotus corniculatus	2					5		5	5	III
Ranunculus repens		3	2		2					II
Lysimachia nummularia		2	5							II
Glechoma hederacea		3	5							II
Rumex crispus	2		2							II
Taraxacum officinale						2				I
Trifolium repens		5								I
Poa pratensis							2			I
Daucus carota						2				I
Other species										
Cirsium arvense	5	7	3	5	2	5	3	2	2	V
Lycopus europaeus	3	2		3	2	5		2		IV
Althaea officinalis	3	2					2	2	3	III
Tanacetum vulgare	2	5	2			5				III
Lathyrus latifolius	2	2				5		2		III
Medicago lupulina	2		5			2				II
Phragmites australis				2	2	2				II
Sonchus arvensis subsp. *uliginosus*						5		2	2	II
Galega officinalis		2						2		II
Trifolium fragiferum subsp. *bonannii*							7		6	II
Mentha arvensis		3	2							II
Lythrum virgatum			2						6	II
Iris pseudacorus			3				3			II
Schoenoplectus lacustris				3	7					II
Mentha aquatica					5		2			II
Xanthium strumarium		3								I
Gratiola officinalis					5					I
Sonchus oleraceus	6									I
Arctium lappa	2									I
Conyza canadense	2									I
Erigeron acer		2								I
Plantago major		3								I
Thalictrum lucidum		2								I
Inula britannica					7					I
Trifolium echinatum						7		2		I
Linaria vulgaris						2				I
Trigonella coerulea						2				I
Erodium cicutarium						2				I
Melilotus alba						2				I
Vicia varia									2	I
Calamgrostis epigejos									8	I

Localities and dates of the relevés: 1, 2, 3, Surroundings of Peschina marsh – 14.07.2004; **4, 5,** Nova Cherna – meadows in zone "East" – 03.08.2004; **6, 7, 8, 9,** Nova Cherna.

E1.9B3/ P–64.71 Pannonic pioneer dune grassland

This habitat includes (Table 5) inland-continental dune – sand-alluvial deposits. On Belene Island these are so called "gredi" or "grindi" and separate the marshes. Stoyanov (1948) writes that they are the most xerophyte (highest and driest part of the island) variant of the vegetation on the island. On the other hand, the species complex shows us more mesophyte conditions in the past. This can explain the presence of *Allium angulosum* L. (a clearly mesophyte species) south of Doulyova Bara. As a secondary result from human activities is their strong degree of ruderalisation, mostly after the grazing and trampling by domestic animals. Because of that they syntaxonomically belong to the ruderal vegetation, but the habitat is included in Directive 92/43/EEC with code **2340 Pannonic inland dunes.**

Table 5. E1.9B3 Pannonic pioneer dune grasslands.

Association *Tribulo-Tragetum* Soo & Tymar 1955													
Number of columns	1	2	3	4	5	6	7	8	9	10	11	12	**Const.**
Number of relevés	7	8	9	10	14	83	84	85	87	88	89	90	
Area of relevés (sq.m.)	100	100	100	100	100	100	100	10	4	4	4	100	
Cover of relevés (%)	70	70	70	80	70	80	70	60	40	60	60	50	
Diagnostic species of the association													
Cynodon dactylon	8	8	8	8	8	7	7	8	6	8	7	7	V
Tribulus terrestris		2				2			4	2	5	5	III
Tragus racemosus	6				5		7				6	7	III
Verbascum banaticum						2		2	2	2	3	2	III
Euphorbia chamaesyce	3	2		2	3						2		III
Eragrostion													
Eragrostis minor	5	3		7	3	4			5	5			III
Setaria pumila					2	7			3	2	6		III
Portulaca oleracea						2	2			2	3		II
Chenopodietea, Eragrostietalia													
Chenopodium album						3	5					2	II
Salsola ruthenica						2							I
Polygonum arenastrum										2			I
Conyza canadensis												2	I
Other species													
Matricaria trichophylla	5	3		2	3	2	2	3	2	2		3	V
Plantago lanceolata var. *eriophylla*	5		7	5	5		2	3	2	2	3	3	V
Bromus tectorum		2			2								I
Artemisia scoparia						2		3					I
Trifolium hirtum						2	2						I
Silene conica								2				2	I
Bromus mollis			2										I
Trigonella monspeliaca	2												I
Scleranthus annuus		2											I
Medicago minima			2										I
Hordeum murinum			2										I
Bromus arvensis						2							I
Crepis setosa						2							I
Trifolium arvense							2						I
Petrorhagia prolifera								2					I
Chondrilla juncea								2					I
Allium angulosum								1					I
Amaranthus albus									2				I
Amorpha fruticosa												1	I

Localities and dates of the relevés: 1, 2, 3, 4, "Greda" southeast from Murtvoto marsh; **5,** "Pcheligreda" – 13.07.2004; **6, 7, 8,** north of Peschina marsh; **9, 10, 11, 12,** south of Murtvoto marsh – 11.08.2004.

Ass. *Tribulo–Tragetum* – this association was found exclusively on the sand dunes between the big marshes on the island. They need special measures for conservation. Near Peschina they have been used as a quarry and are partially destroyed.

E6.22/ P–15.A2 Ponto-Sarmatic salt steppes and salt marshes

These are mesophyte meadows (Table 6), which occur along the periphery of the water basins and are under shorter, spring flooding. Later, they preserve a high level of the underground water and this is the reason for the processes of saltification, which is demonstrated by the presence of a complex of facultative halophytes, such as *Juncus gerardii* Loisel., *Trifolium fragiferum* L., *Mentha pulegium* L. These meadows are used as pastures and often their floristic complex includes many ruderal species. This habitat belongs to the included in Directive 92/43/EEC as a priority for preservation with code **1530 Pannonic salt steppes and salt marshes**.

Ass. *Trifolio fragiferii–Cynodonetum* – the association includes pasture coenoses. On Belene Island they have a transitional position between the dunes (E1.9B3) and tall-herb communities (E5.4) and are limited in the western part of Peschina marsh. The areas of their distribution in the central part of "Kalimok–Brushlen" are wider than in Belene. The intensive grazing is the reason for their strong ruderalisation and aridisation.

G1.1112 Eastern European poplar-willow forests

In this habitat, forest communities are included, mainly in the region of Belene Island, but they are nearly of a bush-tree aspect (Table 7), because of the dominant role that plays the aggressive American species *Amorpha fruticosa* L. In the region of "Kalimok–Brushlen", some small (several hectars) and young communities were found, dominated by willow. They have originated secondarily in the basins, during the process of the draining. The riverine fo-

rests are defined as priority for conservation habitat from the Directive 92/43/EEC with code **91E0 Mixed ash-alder alluvial forests of temperate and Boreal Europe (*Alno–Padion*, *Alnion incane*, *Salicion albae*)**. The islands of Nikopol – Belene group are very important place for the preservation of these forests, but the dikes around the island have destroyed the natural water regime of "Persina–East" and it is the main reason for the invasion of *Amorpha fruticosa*, which can not endure the longer flooding. One of the positive purposes of the project will be the restoration of the normal water regime of these forests and the support of the natural regeneration of the willow. The described communities on Belene belong to the next provisional syntaxon:

Ass. *Amorpho–Salicetum albae* nom. prov. – Stoyanov (1948) described the willow forests on Belene Island very completely in 9 different variants (associations), which according to the species composition can be defined as variants of the association *Salici–Populetum* (Tuxen 1931) Mejler-Drees 1936. Similar to the described by Stoyanov (1948) forests are preserved now only in the outer part of dike of the Danube and on some of the islands in Persina Natural Park – Kitka, Milka, etc. Now the biggest part of the island (especially the eastern part) is covered by the phytocoenoses with a big prevalence of *Amorpha fruticosa*. "Pesina–East" was completely conquered, where the willow is subdominant in the communities formed by this species.

Table 6. E6.2 Continental inland saline grasslands and herb-dominated habitats.

Association *Trifolio fragiferii-Cynodonetum* Br.-Bl. & Bolos 1958										
Number of columns	1	2	3	4	5	6	7	8	9	**Const.**
Number of relevés	1	2	38	39	40	49	53	63	76	
Area of relevés (sq.m.)	100	100	100	100	100	100	100	100	100	
Cover of relevés (%)	90	95	100	100	100	100	100	100	90	
Diagnostic species of the association										
Trifolium fragiferum	6	5	6	5	8	8	6	7	7	V
Cynodon dactylon	8	8		5	6	5	7		7	IV
Lolium perenne			9	7	8	7	7	6	7	IV
Loto-Trifolenion, Agropyro-Rumicion, Agrostietalia stoloniferae, Plantaginetea majoris										
Trifolium repens	2	5	2		2	2	2	3	5	V
Lotus corniculatus	6	5	3	2	3	5		5	3	V
Mentha pulegium			3	2	3	2		2	5	IV
Potentilla reptans	2	5	2			2			5	III
Juncus gerardii			2	2	5	2		2		III
Ranunculus repens	2	3				2			2	II
Agrostis stolonifera						2		7		II
Inula britannica				2					3	II
Elymus repens								7		I
Mentha longifolia									2	I
Other species										
Verbena officinalis	3	2	2	2	2		2	3	2	V
Taraxacum officinale		6		3		3	3	5	2	IV
Galega officinalis	2	2			2	3		5		III
Xanthium strumarium	2	2		2		2			3	III
Matricaria trichophylla	7	7					2		2	II
Cirsium arvense	2						3	3	2	II
Plantago lanceolata	2	2					5		2	II
Medicago lupulina		2		2			3	2		II
Poa pratensis				3		6		6	3	II
Lycopus europaeus					2	2		5	3	II
Bromus arvensis			2	5	5				3	II
Lycopus exaltatus				2	2	3			3	II

Table 6. Continuation.

	1	2	3	4	5	6	7	8	9	
Achillea millefolium	7						2		2	II
Bromus mollis	2	2						5		II
Cichorium inthybus	2	3					2			II
Cirsium vulgare	2	2					2			II
Bolboschoenus maritimus					2	2		2		II
Ononis arvensis						2		2	3	II
Dipsacus laciniatus	2	2								II
Althaea officinalis		3					2			II
Carduus acanthoides		2					2			II
Torilis arvensis		2					2			II
Oenanthe fistulosa			2		2					II
Ranunculus sardous					2				2	II
Rorippa prolifera					2				2	II
Schoenoplectus lacustris						2			2	II
Teucrium scordium						2			3	II
Daucus carota							2	3		I
Trifolium campestre	2									I
Centaurea calcitrapa		2								I
Onopordum acanthium		2								I
Gratiola officinalis						2				I
Setaria pumila						3				I
Glycyrrhiza echinata								3		I
Erigeron acer									2	I
Mentha aquatica						2				I
Rumex conglomeratus									2	I
Trifolium arvense							2			I
Convolulus arvensis							2			I
Agrimonia eupatoria							2			I
Carex divisa								3		I
Cyperus fuscus									2	I
Cirsium creticum									2	I

Localities and dates of the relevés: 1, 2, southwest from Peschina marsh – 07.07.2004; **3, 4, 5,** Nova Cherna – near zone "East" – 02.08.2004; **6, 7,** Nova Cherna – near zone "East" – 03.08.2004; **8,** Nova Cherna – near zone "East" – 04.08.2004; **9,** near Staro selo village – 05.08.2004.

The other established habitat types are artificial according to their origin. They cover big areas, but have not nature-conservation importance. Such are: G1.C/ P–83.321 /*Populus*/ plantations; G1.C/ P–83.324 /*Robinia*/ plantations; G1.C/ P–83.3251 Plantations of sweet tree (*Gleditschia triacanthos*); I1.1 Arable land and marked gardens; I1.55 Fallow inundated fields with annual and perennial weed communities.

Conclusions

We can conclude that of the greatest importance in these territories are the riverine forests (G1.1) and standing eutrophic waterbodies covered with typical hydrophyte communities (C1.3). The latter have become practically extinct on both territories and have survived only in the canals of "Kalimok–Brushlen". In spite of the fact that these syntaxa belong to this habitat, they are inside a technical construction. This decreases their conservation value. In spite of the big changes in the riverine forests on the island now they have the biggest conservation value. After them are the unique for the Belene Island sand dunes (E1.9B3). They can not restore after the destruction – extraction of sand, the afforestation, the ploughing, and the grazing. The eutrophic canals (C1.32), which save many typical hydrophytes, are threatened by the cleaning and other technical activities. The habitat of the non-graminoid hygrophytes (C3.24) is a poorer variant of the typical hydrophytes (C1.3). Quick suc-

Table 7. G1.1 Middle European /*Salix alba*/ forests.

Association *Amorpho-Salicetum albae* nom. prov.								
Number of columns	1	2	3	4	5	6	7	**Const.**
Number of relevés	4	5	15	16	82	86	92	
Area of relevés (sq.m.)	100	100	100	100	100	100	200	
Cover of relevés (%)	80	100	100	100	90	90	100	
Diagnostic species of the association								
Salix alba	8	7	5	7	3	9	6	V
Amorpha fruticosa	7	9	9	8	5	7	7	V
Erigeron annuus	3	3	5	6		3	2	V
Salicion albae, Salicetalia purprureae, Salicetea purpureae								
Rubus caesius	6	3	5	3	7		5	V
Galium aparine	3	5		2	7	5	7	V
Humulus lupulus	6	3			6		5	III
Calystegia sepium	3	2					3	II
Populus alba	3	3						II
Lysimachia nummularia	5					2		II
Populus x canadensis				2				I
Solanum dulcamara						3		I
Sambucus nigra					5			I
Other species								
Agrostis stolonifera	6		6	2		3		III
Cucubalus baccifer		5		2		2	3	III
Bidens frondosa	6					2	2	II
Iris pseudacorus	2					2	2	II
Lythrum salicaria	2					2	2	II
Lysimachia vulgaris	2			2			2	II
Glechoma hederacea	2		2	2				II
Ulmus laevis	2	2			2			II
Elymus repens		5	7				2	II
Cirsium arvense		2	5				2	II
Cornus sanguinea			6	3	2			II
Althaea officinalis			2	2			2	II
Urtica dioica	3				2			II
Aristolochia clematitis		3			5			II

Table 7. Continuation.

	1	2	3	4	5	6	7	
Conium maculatum		5					2	II
Arctium minus		3			2			II
Symphytum officinale		3					3	II
Torilis arvensis			2	2				II
Silene alba				2	2			II
Hypnum cupressiforme				2		4		II
Morus alba					3	2		II
Galium album	3							I
Clematis vitalba	2							I
Drepanocladus aduncus	4							I
Asparagus maritimus		2						I
Rumex crispus		2						I
Dipsacus laciniatus			2					I
Tanacetum vulgare			2					I
Erigeron acer			2					I
Bromus arvensis			2					I
Valeriana officinalis				2				I
Lathyrus latifolius				3				I
Carex divisa				2				I
Populus canescens					9			I
Myrrhoides nodosa					2			I
Chelidonium majus					2			I
Oenanthe aquatica						2		I
Stachys palustris						2		I
Lycopus europaeus						2		I
Alisma plantago-aquatica						2		I
Sium latifolium						2		I
Potentilla reptans						3		I
Rumex conglomeratus						2		I
Phragmites australis							2	I

Localities and dates of the relevés: 1, north bank of Murtvoto marsh – 07.07.2004; **2,** "Persina-East" – 07.07.2004; **3, 4**, northwest of Peschina marsh – 14.07.2004; **5,** north from Peschina marsh, near to the Danube dike – 11.08.2004; **6,** north bank of Peschina marsh – 11.08.2004; **7,** "Persina-East" – 17.08.2004.

cessional changes take part in it and there is a threat of their replacing by the reed and reedmace. Some rare hydrophytes occur in it and it is a breeding area for protected birds (ducks, terns). Tall-herb (E5.423) and salt (E6.22) meadows are nearly equal in terms of conservation value. The conservation value of the reedmace and reed communities is little (C3.2) and one of the purposes of this project is their decrease in the regions.

Acknowledgements. The author is grateful to Dr Pavel Zehtindzhiev, Mr. Vesko Koev, Mr. Stoyan Mihov, Mr. Yordan Kutsarov and Mrs. Tihomira Lazarova for their support. The research was funded by GEF TF 050709 project.

Synoptic sheme of both investigated regions

Cl. ***Lemnetea minoris*** Tuxen 1955
Ord. ***Lemnetalia minoris*** Tuxen 1955
All. ***Lemnion minoris*** Tuxen 1955
Ass. ***Lemnetum minoris*** Th. Muller & Gors 1960
Ass. ***Salvinio–Spirodeletum polyrhizae*** Slavnic 1956
Ass. ***Lemnetum gibbae*** Miyawaki & J. Tuxen 1960
Ord. ***Lemno–Utricularietalia*** Passarge 1978
All. ***Utricularion vulgaris*** Passarge 1964
Ass. ***Lemno–Utricularietum vulgaris*** Soo 1928
Cl. ***Potametea*** Klika in Klika & Novak 1941
Ord. ***Potametalia*** Koch 1926
All. ***Nymphaeion albae*** Oberdorfer 1957
Ass. ***Nymphoidetum peltatae*** (Alloirge 1927) Muller & Gors 1960
Ass. ***Nymphaeetum albae*** Vollmar 1947
Cl. ***Phragmito–Magnocaricetea*** Klika in Klika & Novak 1941
Ord. ***Phragmitetalia*** Koch 1926
All. ***Phragmition communis*** Koch 1926
Ass. ***Scirpetum lacustris*** Schmale 1939
Ass. ***Typhetum angustifoliae*** Pignatti 1953
Ass. ***Phragmitetum communis*** von Soo 1927
Ass. ***Typhetum latifoliae*** Lang 1973
Ass. ***Typhetum laxmani*** Nedelcu 1968
Ass. ***Glycerietum aquaticae*** Hueck 1931
Iris pseudacorus **community**
Ord. ***Oenanthetalia aquaticae*** Hejny in Kopecky & Hejny 1965
All. ***Oenanthion aquaticae*** Hejny ex Neuhausl 1959
Ass. ***Rorippo amphibiae–Oenanthetum aquaticae*** (Soo 1928) Lohmeyer 1950
Cl. ***Molinio–Arrhenatheretea*** Tuxen 1937
Ord. ***Molinetalia*** Koch 1926
All. ***Veronico longifoliae–Lysimachion vulgaris*** (Passarge 1977) Balatova-Tulackova 1981
Ass. ***Euphorbio lucidi–Bolboshoenetum maritimi*** **nom. prov.**
Ass. ***Glycyrrhizo echinatae–Bolboschoenetum martimi*** **nom. prov.**
Cl. ***Chenopodietea*** Br.-Bl. in Br.-Bl., Roussine & Negre 1952 em. Lohmeyer, J. & R. Tuxen ex Matuszkiewicz 1962
Ord. ***Eragrostietalia*** J. Tuxen ex Poli 1966
All. ***Eragrostion*** Tuxen ex Oberdorfer 1954
Ass. ***Tribulo–Tragetum*** Soo & Tymar 1955
Cl. ***Plantaginetea majoris*** Tuxen ex Preising in Tuxen 1950
Ord. ***Agrostietalia stoloniferae*** Oberdorfer in Oberdorfer & al. 1967
All. ***Agropyro–Rumicion crispi*** Nordhagen 1940
Suball. ***Loto–Trifolienion*** Westhoff & van Leeven ex Vicherek 1973
Ass. ***Trifolio fragiferii–Cynodonetum*** Br.-Bl. & Bolos 1958
Cl. ***Salicetea purpureae*** Moor 1958
Ord. ***Salicetalia purpureae*** Moor 1958
All. ***Salicion albae*** Soo 1930
Ass. ***Amorpho–Salicetum albae*** **nom. prov.**

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Plant communities of *Pinus sylvestris* forests in West Rhodopes, NE Macedonia, Greece

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Abstract. In Greece, *Pinus sylvestris* forests are restricted in the northern part, forming either pure or mixed stands with *Fagus sylvatica*, *Picea abies* and *Betula pendula*. In the Greek part of West Rhodopes, they replace partly *Fagus* and *Picea* forests as a result of human impact. However, permanent communities with *P. sylvestris* can be found on relatively poor soils. These forests have been studied using the Braun-Blanquet method, with the help of twenty-one relevés. Syntaxonomical comments, as well as information about the site characteristics, structure and synecology of the distinguished vegetation units are given.

Key words: Northeast Greece, *Pinus sylvestris*, pioneer forest, Rhodopes

Introduction

Pinus sylvestris is the most extensively distributed pine in the world. It occurs naturally in a large part of the Eurasian continent. Its distribution ranges in latitude from 37° N to 70°20' N, while its altitudinal distribution ranges from the sea level to about 2700 m. In the southern part of its distribution (Southern Europe, Asia Minor), it is a high altitude mountain tree, growing at 1200–2700 m alt. (Hegi 1981; Vidacović 1991; Mátyás & al. 2004).

Its distribution in Greece is restricted to the mountains in the northern part of the country (Pindos: Valia Kalda, Olimbos, Pieria, Vermio, Voras, Lailias, Orvilos, Rhodopes) (Gerasimidis 1985; Eleftheriadou 1992; Christensen 1997; Tsiripidis & al. 2004).

Phytosociological studies of *P. sylvestris* forests are up today very scarce. According to Horvat & al. (1974), the occurrence of *P. sylvestris* forests in Mts Balkan, Rila, Pirin and Rhodopes (particular in their western part) is azonal. However, extended pioneer forests of *P. sylvestris* occur in the same area, as a result of human impact. The first phytosociological research on these forests in Greece was conducted by Zoller & al. (1977), with the publication of just two relevés from Central Rhodopes and Mt Lailias. Gamisans & Hebrard (1980), based on 4 relevés, described a vegetation unit and they considered that it closely resembled the *Vaccinio–Pinetum sylvestris* Penev 1960, which occurs in Bulgaria. Gerasimidis (1985) published 25 relevés from Mts Lailias and Pieria.

Material and methods

Study area

The research area is located in West Rhodopes and comprises two sites of the Greek "Natura 2000" Network (GR1140002 – Rodopi (Simyda), GR1140003 – Periochi Elatia). In this area, *P. sylvestris* forms pure or mixed stands with spruce or beech. The differentiation of natural vegetation of the area clearly follows an altitudinal gradient. *Pinus sylvestris* forests occur above the deciduous oak zone and in the same altitudinal zone with *Betula pendula* (west part of the study area), *Fagus sylvatica* s.l. or *Picea abies* forests. Along the streams of constant water flow in the study area, especially at the northern part, azonal stands of *Alnus incana* ssp. *incana* dominate.

Human impact on the vegetation of Rhodopes area started as early as *ca*. 1440 B.C. In the 2nd century B.C., fir and spruce were largely replaced by birch-oak forests. When human activity again decreased, beech invaded the area, and birch gave way to pine, fir and spruce (Athanasiadis & al. 1993). In historical times, nomadic Sarakatsans used to set fires in the wider area of Rhodopes, in order to clear the land for grazing (Müller 1929). Today, human impact is less intense and includes woodcutting and grazing by cows.

Geologically the area belongs to the Rhodope massif (Mountrakis 1985). The bedrock consists of acid igneous rocks (granites, granodiorites, monzonites) (I.G.M.E. 1983).

The climate of the area could be classified in the Dbf climatic type according to Köppen's classification, which is humid continental climate with a harsh winter, a short hot period in summer and a rather uniform distribution of rainfall during the year (Eleftheriadou 1992; Tsiripidis & al. 2004).

Data analysis

In total 21 relevés were conducted in the *P. sylvestris* forests of West Rhodopes according to the Braun-Blanquet method (Braun-Blanquet 1964; Dierschke 1994). In each relevé the slope, exposition, altitude, relief, tree layer cover, shrub layer cover and herb layer cover were recorded. For the species cover abundance, the 9-grade scale of Braun-Blanquet was used (Barkman & al. 1964).

Relevés data were entered into TURBOVEG version 2.32a database (Hennekens & Schaminée 2001). Taxa occurring in two or one relevés were omitted before the analyses. Classification was performed using TWINSPAN method (Hill 1979). Four pseudospecies cut levels were used (0, 5, 25, 50) and two levels of divisions were applied. TWINSPAN was applied with the help of JUICE version 6.3 software (Tichý 2002). In the relevés table, taxa were reshuffled manually in order to obtain a better presentation of the differentiation of the vegetation units.

Classification was tested with the help of detrended correspondence analysis (DCA; Hill & Gauch 1980). In DCA cover abundances of taxa were square-root transformed.

The nomenclature of taxa follows Tutin & al. (1968–1980, 1993); Greuter & al. (1984–1989); Strid (1986) and Strid & Tan (1991, 1997, 2002).

Results and discussion

Relevés were conducted from 1070 up to 1560 m altitude, mainly at south, southeast and west expositions, and on slopes with inclination of 0–60 %. The geological substrate of all relevés consists of acid igneous rocks (granites, granodiorites). The tree layer (cover 50–80 %, height 17–32 m) is dominated by *P. sylvestris*. Furthermore, *Picea abies* occurs in all the three vegetation layers, while *Betula pendula*, *Quercus petraea* ssp. *medwediewii* and *Fagus sylvatica* appear only sporadically. The shrub layer (cover 1–35 %) consists mainly of *Juniperus communis* ssp. *communis*, *P. sylvestris*, *P. abies*, *F. sylvatica* and *Rosa canina.* The herb layer is rich in species (18–74 species per relevé), presenting a cover (15–)30–90 %. In this layer, there is regeneration mainly from *P. abies* ssp. *abies*, *J. communis* ssp. *communis*, *Chamaecytisus eriocarpus*, *Corylus avellana*, *R. canina* and less from *P. sylvestris*, *Q. petraea* ssp. *medwediewii*, *F. sylvatica*, *Prunus cerasifera*, *Rubus idaeus*. Worth mentioning is the low natural regeneration of *P. sylvestris* in the major part of the studied forests.

The TWINSPAN classification resulted in the distinction of three relevé groups, which were classified in two main vegetation units (Table 1).

In the DCA diagram (Fig. 1), the distinction of the first vegetation unit was clear and may be attributed to the first axis. The latter axis had eigenvalue equal to 0.316, while the eigenvalue of the second axis was 0.103. The length of the first two axes was 2.225 and 1.730 SD. Total inertia of the species data were equal to 1.478. Both the first two DCA axes explained 28.3 % of the total species variance.

First axis, which was responsible for the distinction of the two main vegetation units, seems to represent a microclimate gradient from the warmer and drier sites of the first vegetation unit to the cooler and more humid ones of the second vegetation unit. This microclimate differentiation may be attributed to the lower altitude and the south or east exposition of the relevés of the first unit.

Second axis is difficult to be ecologically interpreted, but it contributes to the discrimination of the two subunits of the second vegetation unit. It may be related with a differentiation in the soil properties, such as a higher nitrogen or organic matter content in the soils of the first subunit or the gentler slope of the relevés of the same subunit.

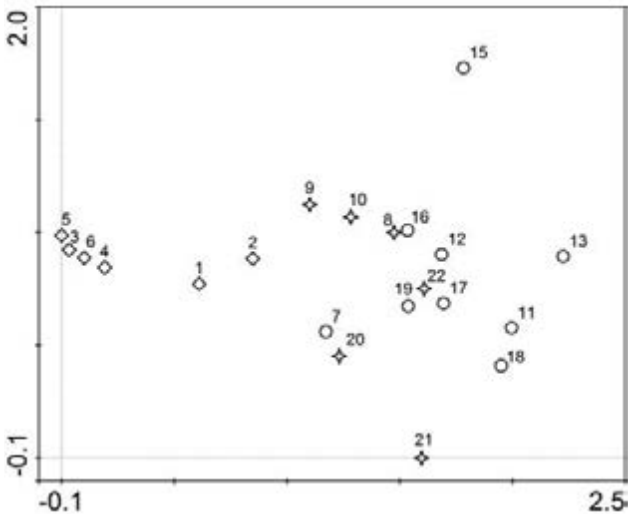

Fig. 1. Ordination biplot diagram of the first two DCA axes. Different symbols indicate vegetation units and subunits: unit 1 (diamond), subunit 2a (star), subunit 2b (circle).

The description of the distinguished vegetation units and subunits is given below.

Unit 1: *Pinus sylvestris* unit. It is differentiated by the presence of *P. sylvestris* in the herb layer, as well as by a group of 17, mainly light-demanding, xerophytic species. Its classification is difficult, due to the absence of the *Vaccinio–Piceetea* and *Vaccinio–Piceetalia* species.

Pinus sylvestris dominates in the tree layer with a cover 60–80 %, while some individuals of *Betula pendula* appear sporadically. The shrub layer presents a cover 10–30 % and it is characterized by the presence of *P. sylvestris* and *R. canina*. Several other woody species appear in the shrub layer, from which *J. communis* ssp. *communis* presents a high constancy. The herb layer has a cover ranging from 15 % to 60 %.

This unit is restricted at lower altitudes (1070–1330 m), usually at south to east expositions (83 %), on slopes with inclination of 15–45 % and on soils which derive from granodiorites. It is distributed in the southeastern part of the research area, where the site conditions are worse.

Unit 2: *Picea abies* ssp. *abies*–*Pinus sylvestris* unit. The presence of *P. abies* ssp. *abies* in the shrub and herb layer differentiates this unit. Furthermore, it is differentiated from unit 1 by the occurrence of a group of 12 species. This unit could be classified in the *Vaccinio–Piceetea* and *Vaccinio–Piceetalia*.

In the tree layer (cover degree 50–75 %), *P. sylvestris* dominates, while other woody species (*P. abies*, *B. pendula*) occur sporadically. The shrub layer appears comparatively poor, with a cover less than 10 %, except some stands, where the invasion of *P. abies* in the shrub and herb layer is obvious. The occurrence of *P. abies* in this layer is abundant, while *P. sylvestris* and *B. pendula* are less common. The herb layer has a cover (25–)40–90 %.

Unit 2 occurs at higher altitudes, from (1150–) 1330 to 1530 m, usually at west to northeast expositions (60 %), on slopes with inclination of 0–45(–60) % and on soils which derive mainly from granodiorites. It is distributed in the northwestern, central and eastern part of the research area.

Subunit 2a: With *Fagus sylvatica*. It occurs usually at west expositions (50 %) and on slopes with mild inclination of 0–30(–40) %, in the northwestern and central part of the research area.

Subunit 2b: With *Vaccinium vitis-idaea*. It occurs usually at west to northeast expositions (64 %) and on slopes with inclination of 20–45(–60) %, in the central and eastern part of the research area.

Based on the relevés data and the physiography of the area, it is clear that the occurrence of the unit 1 is associated with lower altitudes and mainly south expositions. It is distributed in the southwestern part of the area, where the site conditions are worse. Additionally, the participation of a group of light-demanding, xerophytic species, as well as the presence of *P. sylvestris* in all three layers and the simultaneous absence of *P. abies* from the species composition, indicate that this unit may represent the final succession stage of these forests, which may be characterized as permanent community.

Unit 2 is associated with higher altitudes and usually west to northeast expositions. It is distributed in the northwestern, central and eastern part of the research area, where the site conditions are better. The scattered presence of *P. sylvestris* in shrub and herb layer as well as the simultaneous presence mainly of *P. abies* and less of *F. sylvatica* and *Q. petraea* ssp. *medwediewii* suggest the pioneer character of these forests. *Pinus sylvestris* forests of this unit may be considered as a progressive stage of forest succession, which finally may lead to *Picea abies* forests unless human interaction prevents succession.

Table 1. Vegetation units distinguished in *Pinus sylvestris* forests in West Rhodopes. I – *Pinus sylvestris* unit; II – *Picea abies* ssp. *abies–Pinus sylvestris* unit.

Vegetation units		I						II														
								With *Fagus sylvatica*						With *Vaccinium vitis-idaea*								
Relevé number		**6**	**5**	**4**	**3**	**2**	**1**	**21**	**20**	**10**	**22**	**8**	**9**	**16**	**7**	**15**	**19**	**18**	**12**	**11**	**17**	**13**
Relevé area (m2)		300	300	300	300	300	300	300	300	300	300	300	300	350	300	300	400	300	300	300	300	400
Altitude (m)		1070	1130	1250	1200	1330	1140	1380	1460	1360	1430	1350	1330	1490	1150	1440	1480	1560	1510	1220	1260	1530
Aspect		S	E	S	SE	S	W	W	S	W	E	–	W	W	NE	SW	S	W	S	NW	N	NW
Slope (%)		30	45	15	30	15	30	40	30	10	15	0	10	35	45	50	30	30	60	40	45	20
Cover tree layer (%)		65	60	60	70	70	80	65	65	65	55	75	65	70	60	70	70	70	60	50	65	55
Cover shrub layer (%)		15	10	30	30	10	10	3	3	5	1	2	3	20	8	20	3	3	35	5	7	15
Cover herb layer (%)		60	40	15	30	50	30	70	60	60	85	55	40	40	25	75	30	35	60	85	90	85
Relief (S: slope, P: plane, R: ridge)		S	S	R	S	P	S	S	S	R	R	P	P	S	S	S	S	S	S	R	S	S
Geological substrate (G: granodiorite)		G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
Height of trees (m)			17			25	24	22	25	26	25	29	23	23	22	27	19	24	30	25	32	28
Diameter of trees (cm)			22			37	30	31	34	44	42	31	34	33	30	38	32	34	44	33	45	36
Species number		51	74	38	41	48	60	45	36	62	33	37	52	40	38	35	18	23	41	29	42	27
Brachypodium sylvaticum ssp. *sylvaticum*	6	A	1	+	A	A	1	.	.	+	.	.	1	.	.	.	.	.	.	.	+	.
Pinus sylvestris	4	1	1	1	A	1	1	.	.	.	+	.	.	.	1	.	+	.	.	+	.	.
Rosa canina	4	+	1	+	+	+	+	.	+	.	.	.	.	.	.	.	.	.	+	.	.	.
Briza media	6	M	M	+	1	.	r	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.
Carex caryophyllea	6	1	M	+	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphorbia cyparissias	6	1	+	.	+	+	+	.	.	1	.	.	r	.	.	.	.	.	.	.	r	.
Galium aparine	6	1	r	+	r	.	r	.	1	.	.	.	.	.	.	.	.	.	.	.	r	.
Anthoxanthum odoratum	6	1	1	.	.	r	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Scabiosa triniifolia	6	.	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dianthus cruentus	6	+	+	r	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa bulbosa	6	r	r	+	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Crataegus monogyna	6	.	r	r	r	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bellardiochloa variegata	6	+	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla micrantha	6	1	r	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Centaurea affinis	6	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sanguisorba minor ssp. *muricata*	6	r	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Plantago holosteum	6	.	+	+	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphorbia myrsinites	6	r	r	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca valesiaca	6	+	+	1	1	+	+	.	.	1	+	r	M	.	.	.	.	.	.	.	.	.
Teucrium chamaedrys ssp. *chamaedrys*	6	1	1	.	+	1	1	.	.	1	.	1	1	.	+	.	.	.	.	.	.	.
Prunus cerasifera	6	.	1	1	r	r	+	.	.	r	.	r	+	.	.	.	.	.	.	.	.	.
Knautia ambigua	6	.	1	.	.	r	+	.	.	r	.	.	+	.	.	.	.	.	.	.	.	.

Table 1. Continuation.

		6	5	4	3	2	1	21	20	10	22	8	9	16	7	15	19	18	12	11	17	13
Picea abies ssp. *abies*	4	.	.	.	.	.	.	+	.	1	1	.	.	B	.	B	+	+	3	+	1	A
Picea abies ssp. *abies*	6	.	.	.	.	r	.	.	+	1	1	r	+	A	+	1	A	M	1	1	1	1
Hieracium sparsum	6	.	.	.	.	.	.	1	+	1	M	1	1	1	r	+	A	M	+	.	+	+
Vaccinium myrtillus	6	.	.	.	.	.	.	1	.	1	1	1	.	A	.	1	1	A	3	B	A	3
Hieracium erythrocarpum	6	.	.	+	.	.	.	1	.	1	.	1	+	r	M	+	1	M	.	+	1	+
Aremonia agrimonoides ssp. *agrimonoides*	6	.	.	.	.	r	r	.	1	1	.	r	1	M	.	M	.	.	M	+	+	r
Veronica officinalis	6	.	.	.	.	+	.	1	1	1	1	A	1	1	.	.	.	.	r	.	+	1
Euphorbia amygdaloides ssp. *amygdaloides*	6	.	.	.	.	.	.	.	.	1	.	.	.	1	.	1	.	.	1	1	1	1
Pyrola chlorantha	6	.	.	.	.	.	r	.	.	1	1	+	1	1	.	1	.	.	.	.	+	+
Veronica chamaedrys ssp. *chamaedrys*	6	.	.	.	.	.	.	M	1	.	1	.	.	r	.	.	r	1	.	.	+	.
Fagus sylvatica	4	.	.	.	.	.	.	.	+	+	.	.	+	.	.	.	.	.	+	+	+	+
Campanula persicifolia	6	.	.	.	.	.	+	.	1	r	.	.	r	+	.	.	.	1	r	.	.	.
Hypochoeris maculata	6	.	.	.	.	r	.	.	.	1	.	r	1	r	.	r	.	+	.	.	.	.
Fagus sylvatica s.l.	6	.	.	.	r	.	+	1	1	+	.	r	+	+	r	.	.	.	.	.	1	.
Rubus idaeus	6	.	.	.	.	1	+	A	1	.	1	.	r	1	.	.	.	.	.	.	.	.
Mycelis muralis	6	.	.	+	.	.	.	+	1	1	.	1	+	.	.	.	.	.	.	.	1	.
Pyrola minor	6	.	.	.	.	.	.	.	1	1	M	.	A	.	.	1	.	.	.	.	.	.
Epilobium angustifolium	6	.	.	.	.	+	.	.	r	.	.	r	+	r	.	.	.	.	r	.	.	.
Campanula patula	6	.	.	.	.	.	.	+	.	1	+	.	.	.	.	.	.	+	.	.	.	.
Agrostis capillaris	6	.	.	.	.	.	.	+	.	.	+	.	1	.	.	.	.	.	.	.	.	.
Galium verum ssp. *verum*	6	.	.	.	.	.	.	.	.	.	r	+	+	.	.	.	.	.	.	.	.	.
Vaccinium vitis-idaea	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	3	B	3
Viola riviniana	6	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	r	1	1	1	.
Pinus sylvestris	1	4	4	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Pinus sylvestris	6	r	1	1	+	.	.	.	.	+	.	.	1	.	+	.	.	.	r	+	1	.
Fragaria vesca	6	A	1	1	1	A	1	1	A	A	1	.	M	M	1	M	+	.	1	1	+	+
Calamagrostis arundinacea	6	+	+	+	.	B	1	A	1	A	B	B	.	A	A	B	A	B	3	3	1	3
Deschampsia flexuosa	6	+	.	1	1	B	A	4	1	B	B	B	1	A	A	1	A	B	A	3	3	.
Corylus avellana	6	+	1	.	+	+	+	+	+	1	r	r	1	+	+	.	+	r	+	+	+	.
Luzula luzuloides	6	.	.	.	r	B	A	A	A	B	3	A	B	1	1	1	.	1	1	B	3	B
Genista carinalis	6	+	A	1	1	r	+	+	.	+	.	r	1	.	1	.	1	+	+	1	B	1
Dactylis glomerata	6	+	1	1	+	1	1	M	1	M	1	+	1	1	.	1	.	.	1	.	+	.
Chamaecytisus eriocarpus	6	+	1	1	+	+	1	1	.	+	+	r	+	+	+	.	1	+	.	.	.	r
Juniperus communis ssp. *communis*	4	1	1	B	.	1	+	+	.	+	.	+	+	.	1	+	+	+	1	+	+	.
Juniperus communis ssp. *communis*	6	+	+	1	1	1	.	.	.	1	+	.	1	+	+	1	1	.	+	1	+	+
Poa nemoralis	6	+	1	1	1	1	1	.	1	.	1	+	.	+	+	+	.	+	.	.	.	r
Rosa canina	6	1	1	1	1	1	1	+	1	1	.	+	+	+	+	.	.	.	+	.	.	.
Orthilia secunda	6	.	r	.	.	1	.	1	.	M	1	M	A	M	M	1	.	.	+	.	+	1
Hieracium macranthum ssp. *testimoniale*	6	+	1	1	1	1	+	.	.	.	+	.	1	1	.	r	1	.	+	.	r	.
Trifolium alpestre	6	1	+	.	.	.	1	1	.	1	.	r	1	.	1	+	.	.	1	.	1	.
Quercus petraea ssp. *medwediewii*	6	+	.	+	+	+	+	.	.	.	.	.	r	r	1	.	r	.	.	r	1	.
Hypericum perforatum	6	+	1	.	.	r	.	+	.	+	+	.	+	1	.	+	.	.	.	.	1	.
Thymus sibthorpii	6	1	1	+	1	.	+	r	.	r	.	.	1	.	r	.	.	.	+	.	.	.
Pteridium aquilinum	6	B	.	.	.	1	+	.	3	+	.	.	.	1	.	1	+	.	A	.	.	.
Digitalis viridiflora	6	1	.	.	.	.	1	.	+	.	+	.	r	1	.	.	.	.	1	1	+	.
Hieracium olympicum	6	.	r	.	.	+	.	.	+	.	.	.	.	.	+	.	.	.	+	+	1	r
Poa pratensis ssp. *angustifolia*	6	1	.	.	+	+	+	.	.	.	.	.	.	1	.	1	.	.	.	.	.	1

Table 1. Continuation.		**6**	**5**	**4**	**3**	**2**	**1**	**21**	**20**	**10**	**22**	**8**	**9**	**16**	**7**	**15**	**19**	**18**	**12**	**11**	**17**	**13**
Primula veris	6	+	r	.	.	.	1	+	.	+	.	.	.	.	1	.	.	.	.	r	.	.
Monotropa hypopitys	6	.	r	.	r	.	.	.	.	.	.	+	+	1	+	.	.	1	.	.	.	.
Genista sagittalis	6	.	.	.	.	+	.	.	.	r	.	.	1	r	.	.	+	.	.	.	r	r
Satureja vulgaris	6	.	.	.	.	+	+	.	.	.	.	+	r	.	.	.	.	.	+	.	+	.
Hieracium racemosum	6	.	.	r	.	.	+	.	.	.	.	r	.	.	.	r	.	.	+	.	r	.
Cruciata glabra	6	.	.	.	.	.	1	M	.	+	.	.	.	.	.	.	.	+	1	+	.	.
Hypericum cerastoides	6	.	+	.	r	.	.	.	.	.	.	.	.	r	.	r	.	.	.	.	+	.
Betula pendula	1	1	1	.	.	.	.	.	.	A	.	1	.	.	1	.	.	.	.	.	.	.
Rumex acetosella	6	.	+	.	.	.	+	.	.	.	+	.	.	+	.	.	.	.	+	.	.	.
Goodyera repens	6	.	.	.	.	r	.	+	.	.	.	1	.	.	.	.	+	+	.	.	.	.
Hieracium murorum	6	.	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.	.	.	.	1	+
Festuca rubra	6	.	.	.	.	.	+	.	.	1	.	.	1	.	.	+	.	.	.	.	.	.
Hieracium pilosella	6	.	.	.	.	.	.	1	.	.	1	.	.	.	+	.	.	.	.	.	.	+
Geum urbanum	6	.	r	.	.	r	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.
Brachypodium pinnatum	6	.	.	.	.	.	.	.	.	1	.	+	.	.	.	4	.	.	M	.	.	.
Lathyrus pratensis	6	.	.	.	.	.	+	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.
Ajuga reptans	6	.	.	.	.	r	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
Rubus canescens	6	+	.	.	r	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.
Asplenium adiantum-nigrum	6	r	.	.	.	r	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prunus avium	6	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Stellaria graminea	6	.	r	.	.	.	1	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Astragalus glycyphyllos	6	.	r	.	.	.	.	.	.	+	.	.	r	.	.	.	.	.	.	.	.	.
Urtica dioica	6	.	.	+	.	.	.	.	+	.	.	r	.	.	.	.	.	.	.	.	.	.
Cystopteris fragilis	6	.	.	.	.	r	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Myosotis sylvatica	6	.	.	.	.	.	+	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Leontodon hispidus ssp. *hispidus* var. *hispidus*	6	.	.	.	.	.	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.
Hieracium bauhini	6	.	.	.	.	.	.	1	.	+	.	.	.	.	.	.	.	.	+	.	.	.

***Species in two relevés*:** *Viola tricolor* ssp. *macedonica* (6) 1: M, 3: r; *Muscari comosum* (6) 1: 1, 2: r; *Koeleria cristata* (6) 1: 1, 2: r; *Silene viridiflora* (6) 1: 1, 18: +; *Phleum montanum* (6) 1: +, 2: +; *Eryngium campestre* (6) 1: +, 2: r; *Ajuga genevensis* (6) 1: r, 2: +; *Luzula multiflora* ssp. *multiflora* (6) 2: 1, 4: r; *Genista lydia* (6) 2: +, 3: +; *Viola sieheana* (6) 2: +, 18: +; *Agrimonia eupatoria* (6) 2: r, 4: +; *Rosa agrestis* (6) 2: r, 7: 1; *Lonicera xylosteum* (6) 2: r, 7: r; *Trifolium repens* ssp. *repens* (6) 2: r, 9: 1; *Helianthemum nummularium* (6) 2: r, 14: r; *Juniperus oxycedrus* ssp. *oxycedrus* (4) 3: 1, 4: +; *Hypericum olympicum* (6) 3: +, 4: r; *Hieracium jankae* (6) 3: r, 14: +; *Dryopteris filix-mas* (6) 5: +, 7: r; *Betula pendula* (6) 5: +, 14: r; *Quercus petraea* ssp. *medwediewii* (4) 6: +, 13: +; *Silene atropurpurea* (6) 6: +, 18: r; *Carex digitata* (6) 6: r, 14: 1; *Trifolium medium* ssp. *balcanicum* (6) 6: r, 20: r; *Trifolium pratense* (6) 7: +, 9: 1; *Salix caprea* ssp. *caprea* (6) 7: r, 12: +; *Polypodium vulgare* (6) 7: r, 14: 1; *Asplenium trichomanes* (6) 7: r, 14: +; *Geranium robertianum* ssp. *robertianum* (6) 8: 1, 9: r; *Vicia tenuifolia* (6) 9: 1, 12: +; *Lotus corniculatus* (6) 9: +, 12: +; *Ranunculus sartorianus* (6) 9: r, 10: r; *Fraxinus ornus* (6) 9: r, 14: r; *Picea abies* ssp. *abies* (1) 10: 1, 16: 1; *Leontodon hispidus* ssp. *hispidus var. glabratus* (6) 11: r, 12: +; *Hieracium pavichii* (6) 12: +, 14: 1; *Betula pendula* (4) 12: +, 14: +; *Festuca varia* (6) 13: 1, 19: 1; *Luzula luzulina* (6) 13: 1, 21: +; *Rosa pendulina* (6) 14: r, 19: 1; *Abies borisii-regis* (4) 18: +, 20: +;

***Species in one relevés*:** *Satureja majoranifolia* (6) 1: 1; *Rubus canescens* (4) 1: +; *Ostrya carpinifolia* (6) 1: +; *Rubus hirtus* (6) 1: +; *Orlaya daucorlaya* (6) 1: r; *Potentilla argentea* (6) 1: r; *Stachys angustifolia* (6) 1: r; *Carlina vulgaris* (6) 2: 1; *Rorippa thracica* (6) 2: 1; *Platanthera chlorantha* (6) 2: 1; *Prunella laciniata* (6) 2: 1; *Anthemis tinctoria* ssp. *parnassica* (6) 2: +; *Malus domestica* (4) 2: +; *Dorycnium pentaphyllum* (6) 2: r; *Valerianella locusta* (6) 2: r; *Cirsium ligulare* (6) 2: r; *Hieracium pilosissimum* (6) 2: r; *Campanula lingulata* (6) 2: r; *Quercus pubescens* (6) 2: r; *Carpinus betulus* (6) 2: r; *Potentilla inclinata* (6) 2: r; *Koeleria pyramidata* (6) 3: +; *Valeriana officinalis* (6) 3: +; *Moehringia trinervia* (6) 3: r; *Juniperus communis* ssp. *communis* (1) 4: +; *Plantago lanceolata* (6) 4: r; *Linaria genistifolia* (6) 4: r; *Thymus longicaulis* ssp. *chaubardii* (6) 5: +; *Bromus benekenii* (6) 5: +; *Prunus cerasifera* (1) 5: +; *Corylus avellana* (4) 5: +; *Malus sylvestris* (6) 5: r; *Dactylorhiza sambucina* (6) 6: 1; *Achillea millefolium* (6) 6: +; *Vicia tetrasperma* (6) 6: +; *Viola reichenbachiana* (6) 6: +; *Vicia lathyroides* (6) 6: r; *Cerastium fontanum* ssp. *vulgare* (6) 6: r; *Vicia sativa* ssp. *nigra* (6) 6: r; *Saxifraga carpetana* ssp. *graeca* (6) 6: r; *Silene waldsteinii* (6) 6: r; *Chaerophyllum hirsutum* (6) 7: +; *Prunella vulgaris* (6) 8: M; *Rumex aquaticus* (6) 8: 1; *Epilobium montanum* (6) 8: 1; *Euphorbia stricta* (6) 8: +; *Hypericum maculatum* ssp. *immaculatum* (6) 8: +; *Cardamine bulbifera* (6) 8: r; *Agrostis castellana* (6) 9: 1; *Pimpinella saxifraga* (6) 9: +; *Chaerophyllum aureum* (6) 9: +; *Campanula rapunculoides* (6) 9: +; *Epipactis helleborine* (6) 9: r; *Corallorhiza trifida* (6) 10: r; *Centaurea stenolepis* (6) 11: r; *Hypochoeris radicata* (6) 12: 1; *Ornithogalum kochii* (6) 13: r; *Arrhenatherum elatius* ssp. *elatius* (6) 14: r; *Moneses uniflora* (6) 15: 1; *Prenanthes purpurea* (6) 15: +; *Asplenium cuneifolium* (6) 15: +; *Hypericum spruneri* (6) 15: r; *Athyrium filix-femina* (6) 15: r; *Thymus* species (6) 15: r; *Festuca koritnicensis* (6) 18: +; *Moehringia pendula* (6) 19: B; *Pinus nigra* (4) 19: +; *Abies borisii-regis* (6) 19: +; *Rubus saxatilis* (6) 19: +; *Cotoneaster nebrodensis* (6) 19: r; *Mentha longifolia* (6) 20: r; *Salvia glutinosa* (6) 20: r; *Deschampsia cespitosa* ssp. *cespitosa* (6) 21: B.

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The Romanian endemic *Dianthus gelidus*: plant community composition and habitat characteristics

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Abstract. In a total vascular flora of 3300 species, Romania has 42 *Dianthus* species, 12 of which are endemic to the country and 25 are in the Red List. Many *Dianthus* species are confined to the Carpathian chain, e.g. *D. gelidus*, a rare and clonal species from the alpine areas. This paper compares the plant associations and habitat characteristics where *D. gelidus* grows in its major sites within the more alpine areas of the Romanian Carpathians, discussing similarities and differences. The ecological requirements of this endemic subspecies are discussed not only in the context of the anthropogenic pressures that threaten its populations, but also of the management of protected areas.

Key words: community, *Dianthus gelidus*, endemic, habitat, Romanian Carpathians

Introduction

Within Romania, the alignment of the South-East Carpathian chain causes marked deviations and perturbations to the regional and local circulation of the air masses and hence to the temperature and precipitation regimes on slopes with different aspects, summits and sectors of particular massifs (Mihăilescu 1969). These climatic differentiations in turn cause partition of the vegetation, soil types, water regime, etc. The high montane zone is defined as the unfavourable area for forest development, i.e. >1900 m altitude in the Southern and >1750 m altitude in the Northern Carpathians. The climate is severe and humid: precipitation exceeds evaporation for the whole year; the minimum absolute temperature may fall to -25 °C (exceptionally to -38 °C), with 250–265 frost days; the annual mean wind speed is >6–7 m/s. Snow coverage can last 180–220 days/year and the thickness of the successive snow layers can reach 7–8 m, redistributed from the Northwest slopes to the upper parts of Southern and Eastern slopes or to valley bottoms and depressions resulting in even deeper snow that may last until July or August. Very active pedogenetic processes take place relatively quickly in alpine grasslands, since for much of the year the soil is frozen and covered by thick snow. Here soil develops on a thin rocky layer derived from erosion, having a relatively simple soil profile: the upper horizon (12–20 cm) comprises plant roots and organic material intermixed with rock fragments – decomposition rates are low due to low temperature and excess soil moisture. Below this horizon, organic content is much reduced and the soil gradually (at 50–70 cm depth) gives way to the compact parent material (Caine 1974; Retzer 1974; Puiu 1980).

Plants from the alpine areas show adaptations to these extreme conditions that allow them to resist strong winds whilst increasing their surface area for absorption of nutrients, etc., e.g. small stems and leaves, well-developed subterranean organs (especially in plants from mobile and semi-fixed screes). Therefore, in these areas hemicryptophytes prevail (Coldea 1997; Körner 1999).

Material and methods

Dianthus gelidus Schott, Nyman & Kotschy [syn. *D. glacialis* Haenke ssp. *gelidus* (Schott, Nyman & Kotschy) Tutin] (*Caryophyllaceae*) is endemic to the Romanian Carpathians, perennial, diploid ($2n = 30$) and flowers in July–August. It is a microtherm species, limited by altitude to alpine and subalpine areas where

it grows typically in tundra plant associations subject to strong wind, covered with snow for 7–8 months/ year (Williams 1893; Prodan 1953).

Amongst other themes, the present research focused on the population ecology and phytosociology of *D. gelidus*. We researched the geographical and ecological distribution of *D. gelidus* species using literature and fieldwork. None of the published research had focused on the variation in the plant associations within which *D. gelidus* occurred in different mountain ranges, focussing rather on general floristics (Puşcaru & al. 1956; Nyárády 1958; Beldie 1967; Puşcaru-Soroceanu & Puşcaru 1969; Stancu 2002; Nicolae 2005), or on phytosociology at the Romanian level (Coldea 1997). Human impact on alpine areas is increasing in the last decade combined with climate changes. The plant associations where *D. gelidus* grows occur as fragments with scattered distributions and are vulnerable to human impact. We attempt to understand the protection measures that are necessary for the plant associations containing *D. gelidus*.

In the period 2002–2005, field survey was carried out during the flowering season of *D. gelidus* mainly in subalpine and alpine areas of three Massifs (Retezat, Făgăraş, Bucegi). The distribution of *D. gelidus* was confirmed and new localities identified using GPS. Data from the literature and herbaria were gathered from all Massifs where *D. gelidus* grows. Following fieldwork we cannot confirm any localities from Parâng and Rodna Massifs, where we believe *D. gelidus* is very rare or has disappeared. We classified the communities following Braun-Blanquet (1964), Weber & al. (2000) and key Romanian phytosociological literature (Coldea 1997; Cristea & al. 2004; Sanda & al. 2005). *Dianthus gelidus* is recognised as a regional characteristic species (Westhoff & van der Maarel 1973).

Soil was extracted from areas with and without *D. gelidus* and chemical analyses performed in a specialist laboratory. The Consort 532 pH/Conductivity/°C was used for a regional record of pH and temperature.

Based on summary synthetic tables, the similarities among plant associations from Bucegi (Obârşia Ialomiţei, Coştila, Caraiman, Babele, Vârful cu Dor), Făgăraş (Buda, Râiosu, Bâlea) and Retezat (Păpuşa) Massifs were established using Ward's linkage and Correlation Coefficient Distance.

Results

Dianthus gelidus is found in the Romanian Carpathians in some plant associations at an altitude of 1800–2500 m. These associations occur on high plateaux, summits (with steep or moderate slopes) and in glacial cirques (with mobile, semi-fixed or fixed screes), on limestone and/or siliceous substrates with a shallow soil profile (10–30 cm depth). The associations can be either heliophilous (in areas with high light intensity) or sciaphilous (in shade, adapted to low light intensity); and in terms of soil moisture xero-mesophilous (dry to moist), mesophilous (neither arid nor wet soils) or meso-hygrophilous (moist to damp) (Table 1).

The plant associations with *D. gelidus* are small in extent (Table 1). In these plant associations, *D. gelidus* is scattered in small areas. In the *Saxifragetum moschatae–aizoidis* Boşcaiu 1971 association from Făgăraş Massif it forms the subassociation *dianthetosum glacialis* Voik & Erika Schneider-Binder 1978 (Doniţă & al. 2005). Accompanying species are mainly hemicryptophytes (Fig. 1) and diploids (Fig. 2), reflecting the relict character of these associations (Favarger 1964).

Among the recorded species, some are: a) endemic to the Romanian Carpathians (*Achillea schurii*, *Cerastium transsilvanicum*, *Leontodon montanus* ssp. *pseudotaraxaci*, *Papaver pyrenaicum* ssp. *corona-sancti-stephani*, *Thymus pulcherrimus*) (Coldea 1997); b) endemic to the Carpathians (*Kobresia myosuroides*, *Loiseleuria procumbens*, *Pinguicula vulgaris*); or c) vulnerable within the Carpathians (*Astragalus frigidus*, *Carex rupestris*, *Nigritella nigra* ssp. *rubra*, *Oxytropis carpatica*, *Salix retusa*) (Witkowski & al. 2003).

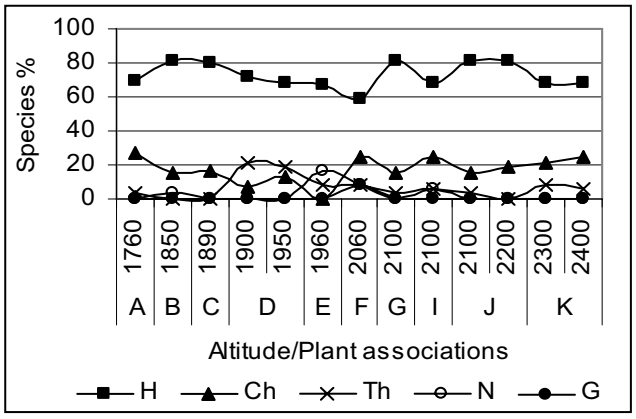

Fig. 1. Correlation of Raunkiaer life-forms with plant associations and altitude from every association.
Abbreviations:
Plant associations: A – *Potentillo chrysocraspedae–Festucetum airoidis*; **B, E** – *Cetrario–Loiseleurietum procumbentis*; **C** – *Violo declinatae–Nardetum*; **D** – *Cerastio lerchenfeldiani–Papaveretum*; **F, I** – *Salicetum retuso–reticulatae*; **G** – *Saxifragetum moschatae–aizoidis*; **J** – *Seslerio–Festucetum versicoloris*; **K** – *Achilleo schurii–Dryadetum*;
Life-forms: H – hemicryptophyte; **Ch** – chamaephyte; **Th** – therophyte; **N** – nanophanerophyte; **G** – geophyte.

Table 1. Biotope characteristics of the plant associations studied (after Coldea 1997).

Association	Extent (ha)	Altitude (m)	Location	Substrate	Soil profile	pH	Soil moisture	Light	a) Wind b) Snow cover	Mean annual temperature (°C)	Mean annual precipitation (mm)
Salicetum retuso–reticulatae	10–50	1800–2400	Steep slopes, cornices	Jurassic limestone	Skeletal superficial, rich in humus	6.7–7.3	Xero-mesophilous	Heliophilous	a) strong b) long	1.0 – -2.5	1250–1400
Cerastio lerchenfeldiani–Papaveretum	10–100	1800–2200	Glacial cirques	Crystalline limestone, amphybolite	Skeletal, rendzina	6.0–7.5	Xero-mesophilous	Heliophilous	a) strong b) long	0.0 – -1.5	1300–1450
Cardaminopsio neglectae–Papaveretum	10–50	1900–2200	Glacial cirques	Crystalline limestone, amphybolite	Skeletal, rendzina	5.8–7.5	Xero-mesophilous	Sciaphilous	a) strong b) long	0.0 – -1.5	1300–1450
Saxifragetum moschatae–aizoidis	~ 100	1850–2200	Mobile and semi-fixed screes	Limestone, siliceous	Skeletal, rendzina	5.8–7.2	Meso-hygrophilous	Sciaphilous	a) strong b) long	0.0 – -1.5	~ 1300
Primulo–Caricetum curvulae	1000–5000	1980–2500	High plateaux	Siliceous	Ranker	4.2–4.4	Meso-hygrophilous	Sciaphilous	a) strong b) long	0.5 – -2.5	1350–1450
Potentillo crysocraspedae–Festucetum airoidis	150–200	1850–2500	High plateaux, summits (moderate slopes)	Siliceous, rarely calcareous	Ranker alpine skeletal, deep to superficial	4.0–4.5	Mesophilous	Helio–Sciaphilous	a) strong b) prolonged freeze	3.0 – -2.5	~ 800
Cetrario–Loiseleurietum procumbentis	~ 10	2000–2500	High plateaux, summits (moderate slopes)	Siliceous	Podzol humic ferruginous skeletal	4.0–4.5	Mesophilous	Sciaphilous	a) strong b) prolonged freeze	0.0 – -1.5	1300–1450
Oxytropido carpaticae–Elynetum	100–200	1850–2000	High plateaux, summits (moderate slopes)	Jurassic limestone	Ranker and protorendzina superficial	4.8–5.8	Meso-xerophilous	Heliophilous	a) strong – snow removed b) prolonged freeze	1.0 – -2.5	1350–1450
Achilleo schurii–Dryadetum	10–50	1700–2200	High moderate slopes	Crystalline limestone, amphybolite	Rendzina and pararendzina rich in humus	6.6–6.8	Meso-xerophilous	Heliophilous	a) strong – snow removed b) prolonged freeze	1.5 – 0.0	1250–1400
Seslerio–Festucetum versicoloris	500–1000	2100–2500	Summits, screes, cornices	Jurassic limestone	Superficial rendzinas rich in humus	7.0–7.4	Meso-hygrophilous	Helio–Sciaphilous	a) +/- protected b) long	1.9	1400–1450
Seslerio bielzii–Caricetum sempervirentis	1000–2000	1650–2200	Summits, screes, cornices	Jurassic limestone	Superficial proto- and pararendzinas rich in skeletal	6.2–7.0	Meso-hygrophilous	Helio–Sciaphilous	a) +/- protected b) long	2.0 – -1.5	1200–1450

The dendrogram based on the records from different Massifs (Fig. 3) shows that the three Massifs are very different to one another in their floristic composition, but they are very similar in their flora.

Assessment of the conservation status of these associations (Table 2) showed high biodiversity value. The habitats are endemic to Romania and although within National or Natural Parks, the level of real protection is low. The very restricted patch size makes them very vulnerable to uncontrolled tourism, intensive grazing, habitat degradation/fragmentation, and invasive species, with climate change increasingly important.

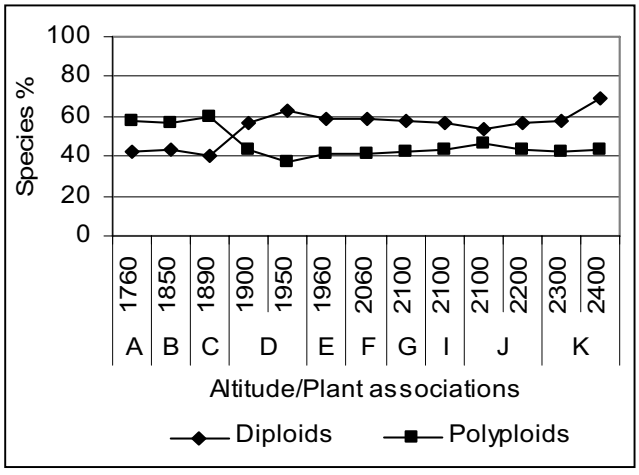

Fig. 2. Occurrence of diploids and polyploids at different altitudes in the plant associations studied.
Abbreviations: as in Fig. 1.

Discussion

Endemic *D. gelidus* is a regionally characteristic species in plant communities of high conservative value. Although these habitats occur outwith Romania, they comprise many endemic and rare species. The biotopes are very vulnerable to the smallest physical disturbance, with increased fragmentation of these already naturally fragmented habitats causing marked and possibly irreversible disturbance of the narrowly distributed plant association. Any impact upon the plant communities can bring about their disappearance. As threats to plants have increased, so has interest in the technique of plant population viability analysis (PVA). In Romania, PVAs have been used to assess the future status of populations or management strategies, comparing populations and identifying the most sensitive life stage. Hence we started population studies on *D. gelidus*, to identify the status of plant associations containing *D. gelidus*. These plant associations belong to habitats that are endemic to Romania and which require urgent conservation and protection measures.

Acknowledgements. We express our thanks to Dr Mihaela Paucă-Comănescu for her scientific advice and support, to Rodica Iosif for assistance in field trips and in the lab and to both of them for friendship. Special thanks go to Acad. N. Boşcaiu for his scientific advice and his passion for plant communities. Many thanks to Dr T.H. Sparks (CEH Monks Wood) for statistical help.

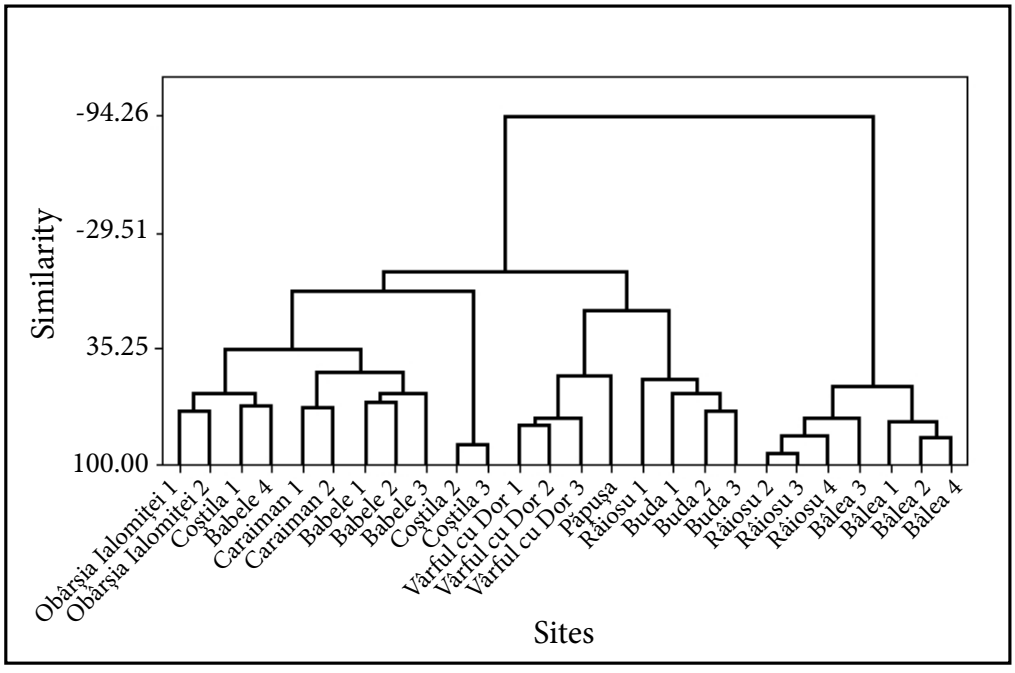

Fig. 3. Dendrogram with Ward's linkage and Correlation Coefficient Distance on the records from three South-East Carpathian Massifs.

Table 2. Conservation status of the studied plant associations (after Doniță & al. 2005).

Association	EUNIS	Romanian Habitats	Conservation Value	Habitats Directive
Salicetum retuso–reticulatae	F2.1211	R3616 South-Eastern Carpathian dwarf shrubs with alpine willows (*Salix retusa*, *S. reticulata*)	High Many endemic and rare species	6170 Alpine and subalpine calcareous grassland
	F2.2915 Carpatho–Balkan *Dryas* mats	R3617 Dwarf shrubs with *Dryas octopetala*	High Many endemic and rare species	–
Cerastio lerchenfeldiani–Papaveretum	H2.44 Carpathian calcareous screes	R6109 SEn Carpathian mobile and semi-mobile calcareous screes with *Papaver corona-sancti-stephani*, *Cerastium lerchenfeldianum* and *C. transsilvanicum*	High Endemic habitat	8120 Calcareous and calschist screes of the montane to alpine levels (*Thlaspietea rotundifolii*)
Cardaminopsio neglectae–Papaveretum	H2.44 Carpathian calcareous screes	R6107 SEn Carpathians mobile and semi-mobile calcareous screes with *Cardaminopsis neglecta*, *Papaver corona-sancti-stephani* and *Doronicum carpaticum*	High Endemic habitat	
Saxifragetum moschatae–aizoidis	H2.44 Carpathian calcareous screes	R6106 SEn Carpathians semi-fixed calcareous scree and rock communities with *Cerastium arvense* ssp. *calcicolum*, *Saxifraga moschata* and *S. aizoides*	High Endemic habitat	
	H2.44 Carpathian calcareous screes	R6108 SEn Carpathians reduced mobility and high humidity calcareous screes with *Rumex scutatus*, *Saxifraga moschata*, *S. aizoides* and *Doronicum columnae*	High Endemic habitat	
Primulo–Caricetum curvulae	E 4.34 Alpigenous acidophilous grasslands	R3602 SEn Carpathian grasslands with *Carex curvula* and *Primula minima*	Low (High where *Pedicularis exaltata* occurs)	–
Potentillo crysocraspedae–Festucetum airoidis	E 4.3432 Carpathian *Festuca airoides* grasslands	R 3604 SEn Carpathian grasslands with *Festuca supina* and *Potentilla ternata*	High Endemic habitat	–
Cetrario–Loiseleurietum procumbentis	F2.211 Alpide dwarf azalea heaths	R3101 South-East Carpathians dwarf azalea heaths (*Loiseleuria procumbens*)	High Reduced distribution areas with difficult survival conditions	4060 Alpine and boreal heaths
Oxytropido carpaticae–Elynetum	E4.42 Wind edge (*Kobresia myosuroides*) swards	R 3601 SEn Carpathian grasslands with *Kobresia myosuroides* and *Oxytropis carpatica*	High Endemic habitat	6170 Alpine and subalpine calcareous grasslands
Achilleo schurii–Dryadetum	F2.2915 Carpatho–Balkan *Dryas* mats	R3617 Dwarf shrubs with *Dryas octopetala*	High Many endemic and rare species	–
Seslerio–Festucetum versicoloris	E4.4392 East Carpathian calciphilous stepped grasslands	R3605 SEn Carpathian grasslands with *Festuca versicolor* and *Sesleria rigida* ssp. *haynaldiana*	High Endemic habitat	6170 Alpine and subalpine calcareous grasslands
Seslerio bielzii–Caricetum sempervirentis	E4.4392 East Carpathian calciphilous stepped grasslands	R3612 SEn Carpathian grasslands with *Carex sempervirentis* and *Sesleria bielzii*	High Endemic habitat	

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The vegetation of forest and shrubland with creeping *Pinus heldreichii* in "Elikodromio", Olympus National Park, Greece

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Abstract. The area "Elikodromio", at an altitude of 2000 m a.s.l. (near-by the "Spilios Agapitos" refuge) within the National Park of Mt Olympus, consists of a mosaic of vegetation with unusual physiognomy. The vegetation was studied phytosociologically using twenty relevés. The data analysis revealed three vegetation units: 1) *Campanula rotundifolia* s.l.–*Pinus heldreichii* unit (*P. heldreichii* of typical growth form); 2) *Buxus sempervirens–Pinus heldreichii* unit (*P. heldreichii* with creeping-shrub growth form that resembles the "Krummholz" form of *Pinus mugo*); and 3) *Genista radiata–Buxus sempervirens* unit. The area's vegetation is described and commented phytosociologically and ecologically on.

Key words: avalanches, Krummholz, Mt Olympus, *Pinus heldreichii*

Introduction

Mt Olympus is known worldwide as much for its ecological characteristics and incomparable natural beauty as for its role in ancient Greek mythology and history. For these reasons, Mt Olympus was the first area in Greece assigned a special protection regime and declared a National Park in 1938. The core of the National Park is located on the eastern slopes of the mountain and covers an area of *ca*. 4000 ha. UNESCO declared the National Park a biosphere reserve in 1981, and the European Union has included Mt Olympus in the Important Bird Fauna Areas (79/409/EC) inventory and the Natura 2000 Network (92/43/EC).

The study area was especially chosen to highlight the particular vegetation present in the area as it presents phytosociological, ecological and aesthetic interest.

Study area

The study area is located on the eastern slopes of Mt Olympus at an altitude of 1850–2000 m close to the mountain refuge "Spilios Agapitos" and more specifically in the area around the heliport (in greek *Elikodromio*). The E4 long-distance European path crosses the study area.

The substrate of the study area is composed of dolomitic limestones, as is the majority of the mountain (Strid 1980; IGME 1983). Intense filtering of precipitation through the rock mass is characteristic of the mountain massif, and explains the complete lack of surface water and springs at altitudes above 1200 m during the summer months, despite frequent precipitation.

Due to the absence of climatic stations on the mountain, the area's climate cannot be described accurately. According to the mean estimated yearly air temperature of Greece (Flocas & al. 1983), the mean yearly air temperature of the area is *ca*. 4 °C, while the mean temperature during the vegetation period is 10 °C. During winter, much of the precipitation falls in the form of snow that covers the area for much of the year. During the vegetation period thunderstorms with hail are also frequent. According to the wardens (pers. comm.) of the "Spilios Agapitos" and "Christos

Kakkalos" refuges, it is also worth noting that creeping snow masses and avalanches affect the area frequently.

The vegetation of the wider area comprises stands of *Pinus heldreichii*. Physiognomically, the study area's vegetation is characterized by a complex of vegetation units with different growth forms that have distinct boundaries: 1) pure stands of *P. heldreichii* of the typical tree form with old individuals of heights up to 25 m; 2) shrublands of *P. heldreichii* of creeping form usually less than 4 m height, that increase parallel to the slope; and 3) shrublands mainly of the woody *Buxus sempervirens* and the occasional presence of *P. heldreichii*. In some plots next to the E4 path, *Atropa bella-donna* was found within the above formation.

Material and methods

Twenty representative sampling plots were chosen within the above vegetation units throughout the study area. Relevés were taken from each plot using the Braun-Blanquet method (Braun-Blanquet 1964; Westhoff & van der Maarel 1973; Athanasiadis 1986) during 2002 and 2003. Abiotic data were also recorded for each relevé such as: altitude, exposition, mean slope gradient, and total cover of the herb, shrub and tree layers. The herb layer included all the herb and woody taxa with heights less than 50 cm. The shrub layer included all woody taxa with heights between 50 cm and 5 m, and the tree layer contained all woody taxa with heights over 5 m. The relevés were of size 50 (–100) m^2 in the shrublands, 100 m^2 in the area with the creeping-shrub form of *P. heldreichii*, and (250–) 300 m^2 in adjacent stands of the typical tree form of *P. heldreichii*. The location of each sampling plot is shown in Fig. 1.

The data collected were analysed with the aim of recording the structure and floristic composition of the vegetation units described, as well as the ecological characteristics of their habitats.

Taxa identification and nomenclature follow: Tutin & al. (1968–1980, 1993), Strid (1980, 1986), Strid & Tan (1991, 1997, 2002).

Analysis of the data presented in Table 1 was performed using the software SORT 3.8 for phytosociological data analysis (Ackermann & Durka 1997). Class diagnostic taxa were identified following Mucina (1997), who in turn followed the nomenclature of Barkman & al. (1986).

Results and discussion

Three vegetation units were identified in the study area: 1) *Campanula rotundifolia* s.l.–*Pinus heldreichii* (tree form) unit; 2) *Buxus sempervirens–Pinus heldreichii* (creeping-shrub form) unit; and 3) *Genista radiata–Buxus sempervirens* unit. *Pinus heldreichii* dominates the first two units in tree or creeping-shrub growth form, with significant presence of the shrubby taxa *Juniperus communis* subsp. *hemisphaerica* and *Cotoneaster integerrimus.* In the *Genista radiata–Buxus sempervirens* unit, *B. sempervirens* co-dominates with *G. radiata*.

A large number of taxa has been observed, common to all three identified units (*Festuca varia*, *Sesleria robusta*, *Achillea holosericea*, *Galium mollugo* group, *Iberis sempervirens*, *Fragaria vesca*, *Physospermum cornubiense*, *Thalictrum minus* subsp. *saxatile*, *Daphne oleoides*, etc.) with similar constancy and cov-

Fig. 1. View of the area where the "Krummholz" form of *P. heldreichii* is observed. The long distance European path E4 is crossing the area and the relevés' position is marked.

er values in each unit. This fact provides strong indication for the common origin of the three vegetation units from stands of *P. heldreichii* and that their differentiation is due to deforestation that led to increased insolation, soil erosion and exposure of the bedrock. Following this degradation, the vegetation adapted to the newly created ecological conditions resulting in the study area's present physiognomy and floristic composition. The presence of *Atropa bella-donna*, a taxon that occurs in logged or deforested areas, together with other taxa that demonstrate similar ecological conditions, such as *Fragaria vesca* and *Epilobium angustifolium*, is a further indication arguing to the assumption that the area was previously covered by forest. Based on their diagnostic species (Mucina 1997), the common taxa belong phytosociologically to various classes such as *Erico–Pinetea*, *Querco–Fagetea*, *Daphno–Festucetea*, *Festuco–Brometea*, *Elyno–Seslerietea*, *Epilobietea angustifolii*, *Juncetea trifidi* and *Thlaspietea rotundifolii*.

It is worth noting that in all vegetation units taxa were found with high constancy and cover values which are regarded as diagnostic taxa of classes characterizing alpine–sub-alpine vegetation (*Elyno–Seslerietea*, *Juncetea trifidi*), such as *Achillea holosericea*, *Genista radiata* and *Festuca varia*, or shrubs and forests of the temperate zone (*Querco–Fagetea*, *Erico–Pinetea*, *Quercetea pubescentis*) such as *Sesleria robusta*, *Buxus sempervirens*, *Physospermum cornubiense* and *Poa nemoralis*. This indicates that the wider area is located within the transition zone between the alpine–sub-alpine vegetation and vegetation of warm temperate areas.

Based on Ellenberg & al. (1994) species-indicators, most of the common taxa are found both in areas with abundant sunlight (or relatively shaded sites) and in areas with slightly acidic to alkaline soils, poor in available nitrogen and nutrients. This is consistent with the general ecological characteristics of the study area, as in none of the distinguished units deep-shaded areas are formed within the vegetation, thus the presence of light-loving taxa is favoured. Additionally, even in neighbouring areas of *P. heldreichii* stands, the open canopy (50–60 % cover) allows direct sunlight to reach the ground. The area's substrate (dolomitic limestone) also favours the formation of slightly acidic or alkaline soils (Papamichos 1996). Lastly, according to Ellenberg & al. (1994), soil nitrogen content may be regarded as an indicator of the general availability of soil nutrients. Therefore the study area's nitrogen poor soils are also poor in available nutrients.

Species indicators belonging to the differentiating taxa groups within units show similar ecological characteristics to those of their common species indicators. Therefore, the study area's three units are distinguished chiefly by their physiognomic and floristic rather than ecological characteristics. In addition, the small number of phytosociogical relevés does not allow the use of strictly phytosociological terminology.

1) *Campanula rotundifolia* s.l.–*Pinus heldreichii* (tree form) unit

This unit is characterized by *P. heldreichii* and *Sesleria robusta*, two diagnostic taxa of the class *Erico–Pinetea* to which this unit is assigned. Phytosociologically, the unit resembles the *Pinus heldreichii–Genista radiata* community (*Pinion peuce* Horvat 1950, *Erico–Pinetalia* Horvat 1959, *Erico–Pinetea* Horvat 1959) as described by Habeck & Reif (1994) from the same area. It is distinguished from the other two units, especially the *Buxus sempervirens–Pinus heldreichii* (creeping shrub form) unit, by a group of eighteen (18) differentiating taxa (Table 1).

Of the diagnostic taxa of the other classes (Table 1), taxa of the *Daphno–Festucetea* class show high constancy and cover values. Phrygana and mountain meadow vegetation of the limestone mountains of mainland Greece and the Aegean Islands belong to this vegetation class. The *Festuco–Brometea* has the same number of diagnostic taxa as the *Daphno–Festucetea* but with lower constancy and cover values. Additionally, the presence of diagnostic taxa of the *Querco–Fagetea* is regarded as significant. These diagnostic taxa are numerous but they participate within the unit with small cover values and constancy.

The unit is distinguished within *P. heldreichii* stands that possess "typical growth form" trees with straight trunks and heights of up to 25 m. The cover of the tree layer, the sole taxon of which is *P. heldreichii*, ranges from 50 % to 60 %. The shrub layer has covers of 10–20 %, and the herb layer covers of 75–95 %. The unit is composed of 89 taxa, and the mean average number of taxa per relevé is 48.

2) *Buxus sempervirens–Pinus heldreichii* (creeping shrub form) unit

The unit is characterized by *P. heldreichii* in its creeping-shrub form, and by *Sesleria robusta*, two diagnos-

tic taxa of the class *Erico–Pinetea* to which this unit is assigned. It is distinguished from the other two units, especially the *Campanula rotundifolia* s.l.–*Pinus heldreichii* unit, by a group of ten (10) differentiating taxa (Table 1).

Of the diagnostic taxa of the other classes (Table 1), this unit has the highest in number, cover and constancy values of *Festuco–Brometea* taxa, while the diagnostic taxa of *Daphno–Festucetea* and *Querco–Fagetea* are as described above.

The presence in this unit of taxa belonging to classes that characterize rock-loving chasmophytic vegetation (*Thlaspietea rotundifolii* and *Asplenietea trichomanis*) is noteworthy. The abundance of bare rocky substrate favours the presence of chasmophytic taxa such as *Jovibarba heuffelii*, *Asplenium adiantum-nigrum* and *A. trichomanes*.

This unit is found in sites of *P. heldreichii* in its creeping-shrub form. The taxon's peculiar growth form is characterized by badly formed individuals of less than 4–5 m in height with their trunks running parallel to the ground and very much resembles the "Krummholz" form of *Pinus mugo* found in areas with very strong winds and heavy snowfall.

The cover of the tree layer ranges from 0–5 % (*P. heldreichii* individuals of creeping-shrub form but with heights of over 5 m). The shrub layer has covers of 50–80 %, and the herb layer covers of 80–90 %. The unit is composed of 89 taxa, and the mean average number of taxa per relevé is 39.

Stands of individuals with this growth form are characterized by large openings and severe soil erosion. It is worth noting that large surface of the relevés is bare rock (10–35 %).

Continuous creeping snow masses and avalanches, both past and present, have resulted in the deforestation of the study area and the formation of its present appearance. Traces of landslips in the area are still visible even today. Thus, the present creeping-shrub form of this unit can be explained by the continuous effects of creeping snow masses and avalanches in combination with the likely concentration of large amounts of snow on the tree crowns. This obliges the trees to develop under the constant stress of excessive weight on their crowns, hence their deformation.

3) *Genista radiata–Buxus sempervirens* unit

The third unit is characterized by the presence of *B. sempervirens*, *Sesleria robusta*, and the occasional appearance of *P. heldreichii* shrubs. The unit is distinguished by a group of fourteen (14) differentiating taxa (Table 1).

Of the classes' diagnostic taxa occurring in this vegetation unit, those belonging to classes characteristic of rock vegetation, as well as to synanthropic vegetation (*Epilobietea angustifolii*) such as *Atropa bella-donna,* are of special interest. It is not possible to assign this unit into any specific vegetation class. It has to be pointed out, however, that the unit does share some floristic elements with the *Buxus sempervirens–Daphne oleoides* community (*Buxo–Syringenion* (Fukarek ex) Bergmeier stat. nov. et valid., *Berberidion vulgaris* Br.-Bl. 1950, *Prunetalia spinosae* Tx. 1952, *Rhamno–Prunetea spinosae* Riv.-God. & Bor. Carb. 1961) identified on Kato Olympus by Bergmeier (1990).

The shrubs have heights of over 2 m and the shrub layer's cover ranges from (0–) 5–40 %. The herb layer has covers of 50–80 %. The frequent presence of large rocks within the relevés is characteristic of this unit (rock covers range from 20–50 %) and therefore may be considered lithophytic. The total number of taxa in this unit is 94 and the mean average number of taxa per relevé is 34.

In the last unit, an important feature is the presence of *A. bella-donna* at its floristic composition. The species *A. bella-donna* enters plant communities in areas previously covered with closed-canopy stands that have, for some reasons, been destroyed (logging, forest road construction, etc.) and the humus-rich soils were exposed to direct sunlight. Drosos (1977) reports that the occurrence of *A. bella-donna* units on alkaline limestone soils is rare as they prefer acidic, medium-textured (clay-sand) soils, lacking calcium but rich in nitrogen and available nutrients. Based on the fact that *A. bella-donna* units are differentiated both floristically and ecologically, two different "subunits" could be identified within this unit. Nevertheless, the small number of relevés results to ambiguity as it comes to the correctness of its further division. In Table 1, the two "possible" subunits are noted.

Table 1. Vegetation units identified in the area of "Elikodromio" on Mt Olympus. I. *Campanula rotundifolia* s.l.–*Pinus heldreichii* unit; II. *Buxus sempervirens–Pinus heldreichii* unit; and III. *Genista radiata–Buxus sempervirens* unit.

Vegetation units			I				II						III									
													With *Atropa bella-donna*						Without *A. bella-donna*			
Plot number			15	17	13	12	4	16	2	1	3	14	18	9	19	5	10	11	8	6	7	20
Elevation (m a.s.l.)			1990	2000	1990	1950	1850	1895	1993	1955	1870	1990	1990	1900	1895	1890	1870	1870	1900	1993	1910	1940
Inclination (%)			60	60	65	65	55	45	45	45	70	70	70	75	70	65	65	75	80	75	80	80
Exposition			NE	SSE	ENE	SE	E	SE	E	SE	E	ESE	SE	SE	ESE	SE	SE	E	SE	SE	SE	SE
Size of plot (m2)			300	300	300	250	100	100	100	100	100	100	500	500	500	100	500	500	500	500	500	500
Number of species			50	42	50	51	32	43	33	38	35	52	47	26	40	34	29	30	33	35	42	25
Cover of trees (%)			50	50	60	50	5	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cover of shrubs (%)			20	10	15	20	70	60	80	80	70	50	100	300	400	5	100	350	15	-	10	10
Cover of herbs (%)			95	80	75	90	90	90	90	80	85	90	800	600	600	600	700	700	500	600	700	500
Day			4	4	4	8	4	5	3	3	4	4	5	6	5	5	8	8	6	5	6	5
Month			8	8	8	7	7	8	7	7	7	8	8	7	8	7	7	7	7	7	7	8
Year			2003	2003	2003	2002	2002	2003	2002	2002	2002	2003	2003	2002	2003	2002	2002	2002	2002	2002	2002	2003
Campanula rotundifolia s.l.			1	1	1	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ranunculus sartorianus			+	+	1	1	.	.	r	.	.	.	.	.	.	.	.	.	.	r	.	.
Rhinanthus rumelicus			r	+	1	1	.	r	.	.	.	1	.	.	.	+	.	.	.	.	.	.
Carduus tmoleus		Da.–Fe.	+	+	1	+	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.
Aremonia agrimonioides		Q.–F.	r	+	r	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Carum rupestre		Da.–Fe.	1	1	.	1	.	.	1	.	.	.	.	r	.	.	.	.	.	.	.	.
Bupleurum falcatum ssp. *cernuum*		T.–G.	.	1	r	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+
Poa media		J. t.	.	+	r	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Leontodon hispidus ssp. *hispidus* var. *hispidus*			.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphorbia amygdaloides ssp. *heldreichii*			1	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium rotundifolium			1	.	+	.	r	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.
Leontodon hispidus ssp. *hispidus* var. *glabratus*			r	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epipactis thessala			.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Monotropa hipopitys			.	r	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prenanthes purpurea		Q.–F.	r	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphorbia sp. 1			.	r	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anthyllis vulneraria			.	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carlina acaulis ssp. *simplex*			r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pinus heldreichii	T	Er.–Pi.	4	3	4	3	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pinus heldreichii	S		2	2	2	2	4	4	4	4	4	3	.	.	1	.	.	1	+	.	+	1
Pinus heldreichii			+	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	1
Juniperus communis ssp. *hemisphaerica*	S	Da.–Fe.	1	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Juniperus communis ssp. *hemisphaerica*			1	1	2	3	3	1	2	2	2	2	1	.	.	.	.	.	1	.	r	2
Cotoneaster integerrimus	S	R.–P.	1	.	.	.	.	.	.	+	.	.	.	1	.	.	.	.	.	.	.	.

Table 1. Continuation.

			15	17	13	12	4	16	2	1	3	14	18	9	19	5	10	11	8	6	7	20
Cotoneaster integerrimus			1	.	r	1	1	2	2	+	.	1	.	1	r	r	.	.	.	.	.	.
Sorbus aria s.l.	S	Q. p.	.	.	.	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	+
Sorbus aria s.l.			.	.	r	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chamaecytisus polytrichus		Fe.–Br.	.	.	r	1	+	1	1	+	1	1	r	.	.	.	.	.	.	r	r	.
Centaurea pindicola			.	1	.	2	r	+	r	r	.	r	r	.	.	.	.	+	.	.	.	.
Bromus cappadocicus ssp. *lacmonicus*			1	2	+	.	.	1	.	1	.	1	1	.	.	.	.	.	.	.	1	.
Sedum ochroleucum			r	1	.	1	.	.	.	1	r	1	r	.	.	.	.	.	.	+	.	.
Hieracium murorum group			+	1	2	1	.	r	.	.	+	.	.	.	r	.	.	.	.	.	.	.
Brachypodium sylvaticum ssp. *sylvaticum*		Q.–F.	2	2	.	1	.	.	.	.	2	.	.	.	2	.	.	.	.	.	.	.
Calamagrostis varia		E.–S.	1	.	2	.	.	3	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Jovibarba heuffelii		A. t.	.	.	.	.	+	2	+	+	+	1	.	.	.	.	.	.	.	.	+	.
Cotoneaster nebrodensis	S	R.–P.	.	.	.	.	1	.	+	.	1	.	.	.	.	.	.	.	.	.	.	+
Cotoneaster nebrodensis			.	.	.	.	.	1	.	.	.	r	.	.	.	.	.	.	r	.	.	.
Hippocrepis comosa		Fe.–Br.	.	.	.	.	1	.	+	r	2	+	.	.	.	.	.	.	.	r	.	.
Asplenium trichomanes ssp. *inexpectans*		A. t.	r	.	.	.	+	r	.	1	1	r	r	.	r	.	.	.	.	.	.	.
Viola rupestris		Fe.–Br.	.	.	.	1	.	r	.	r	1	+	.	.	.	.	.	.	.	.	.	.
Minuartia attica ssp. *attica*			.	.	.	r	.	r	.	r	.	+	.	.	.	.	.	.	.	.	.	.
Onobrychis alba		Fe.–Br.	.	.	.	.	.	.	.	r	r	.	.	.	.	.	.	.	.	.	.	.
Thymus sp. 1			.	.	.	.	.	.	r	r	.	.	.	.	.	.	.	.	.	.	.	.
Thymus boissieri			.	.	.	.	r	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.
Asplenium adiantum-nigrum		A. t.	.	.	.	.	.	r	.	.	r	.	.	.	.	.	.	.	.	.	.	.
Buxus sempervirens	S	Q. p.	.	.	.	1	.	2	.	1	.	.	.	2	3	1	2	3	2	.	2	.
Buxus sempervirens			.	.	.	.	2	3	2	2	2	2	2	2	1	1	2	.	2	r	1	1
Aurinia corymbosa			.	.	.	.	+	r	+	r	.	1	1	1	+	1	1	1	+	1	1	1
Genista radiata		E.–S.	.	.	.	.	.	.	.	.	+	.	1	2	2	+	1	1	r	.	.	2
Brachypodium pinnatum		Fe.–Br.	.	.	.	.	.	.	.	.	.	.	2	1	+	2	2	2	1	1	.	.
Verbascum eriophorum			.	.	.	.	.	.	.	.	.	r	.	+	1	+	+	+	+	.	+	+
Asperula aristata ssp. *thessala*			.	.	.	.	.	.	.	.	.	+	+	1	.	.	1	+	r	.	r	.
Origanum vulgare ssp. *vulgare*		T.–G.	.	.	.	.	.	.	.	.	.	+	+	.	+	r	.	.	.	.	1	1
Achnatherum calamagrostis		T. r.	.	.	.	.	.	.	.	.	.	.	1	.	+	.	.	.	.	.	.	2
Atropa bella-donna	S	Ep. an.	.	.	.	.	.	.	.	.	.	.	2	.	1	.	.	.	.	.	.	.
Atropa bella-donna			.	.	.	.	.	.	.	.	.	.	1	r	r	2	2	1	.	.	.	.
Mercurialis ovata		Q. p.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	1	.	.	.	+
Euonymus verrucosus		Q.–F.	.	.	.	.	.	.	.	.	.	.	.	+	r	.	.	+	1	.	.	.
Sanguisorba minor ssp. *muricata*			.	.	.	.	.	.	.	.	.	.	r	.	r	.	.	r	.	.	.	.
Arabis collina			.	.	.	.	.	.	.	.	.	.	.	r	.	+	.	.	.	.	.	.
Polygala nicaeensis ssp. *mediterranea*			.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	r	+	r	+
Veronica orsiniana ssp. *orsiniana*			.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	+	+	r	.
Carum graecum ssp. *graecum*			.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	r	+
Rubus idaeus	S	Ep. an.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Rubus idaeus			1	1	+	r	.	.	.	.	.	r	.	1	1	2	1	1	+	1	1	.
Rosa canina	S	R.–P.	.	.	.	.	1	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.
Abies × *borisii-regis*	S	Q.–F.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Abies × *borisii-regis*			.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sorbus aucuparia	S		.	r	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Ostrya carpinifolia	S	Q. p.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rosa arvensis		Q.–F.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.
Rosa sp. 1			.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.
Festuca varia		J. t.	2	2	3	2	3	3	3	3	3	3	3	2	2	2	1	1	2	3	2	2
Sesleria robusta		Er.–Pi.	3	2	1	1	2	2	1	2	2	1	1	2	+	+	1	1	r	2	2	.
Achillea holosericea		E.-S.	r	1	+	1	r	+	r	+	+	1	1	+	1	r	+	1	.	1	1	1

Table 1. Continuation.		15	17	13	12	4	16	2	1	3	14	18	9	19	5	10	11	8	6	7	20
Galium mollugo group		+	1	+	1	1	1	1	+	1	1	1	1	1	1	1	1	1	1	1	.
Iberis sempervirens		1	r	1	1	1	1	1	+	1	.	+	1	1	1	1	1	1	1	1	1
Fragaria vesca	Ep. an.	3	3	2	2	1	2	1	1	1	2	2	1	1	1	2	.	1	+	1	.
Physospermum cornubiense	Q.–F.	1	r	+	2	.	1	r	r	.	1	1	+	1	1	1	r	r	+	1	1
Thalictrum minus ssp. *saxatile*		+	.	1	1	r	1	.	+	.	+	1	1	1	1	1	2	+	+	1	1
Daphne oleoides	Da.–Fe.	r	1	1	1	1	r	2	1	2	1	r	r	+	.	.	.	1	.	+	r
Acinos alpinus ssp. *meridionalis*	Da.–Fe.	2	1	1	1	1	.	.	r	1	+	.	.	r	.	r	+	r	1	r	+
Hieracium pannosum		1	2	1	.	+	1	+	.	+	+	1	1	1	.	.	.	r	1	1	+
Teucrium chamaedrys	Fe.–Br.	.	.	.	+	.	1	+	.	.	1	1	1	1	+	1	2	1	1	2	1
Silene vulgaris ssp. *prostrata*		1	1	+	r	.	1	.	.	r	1	+	.	r	1	r	1	.	.	1	.
Alyssoides utriculata ssp. *utriculata*		r	.	+	.	.	+	+	+	1	.	.	2	1	.	1	1	1	1	1	.
Linaria peloponnesiaca var. *parnassica*		1	1	.	.	.	.	.	.	.	.	1	+	r	1	1	1	1	1	1	.
Sedum album		1	r	1	.	.	+	.	1	r	1	.	.	.	r	.	.	r	1	+	.
Clinopodium vulgare ssp. *arundanum*		1	r	.	1	.	1	.	.	.	1	1	.	.	1	1	1	+	.	.	.
Campanula glomerata	Fe.–Br.	.	1	.	.	.	+	.	.	.	1	.	.	r	.	r	r	+	r	.	.
Hieracium hoppeanum ssp. *pilisquamum*		r	1	+	+	.	1	.	.	.	1	.	.	.	.	.	.	.	r	+	.
Carex sp. 1		.	.	+	.	.	.	+	.	.	r	.	.	1	r	1	.	2	.	1	.
Poa nemoralis	Q.–F.	1	.	1	.	.	.	+	.	1	1	.	.	.	1	.	.	1	.	1	.
Silene multicaulis ssp. *multicaulis*	T. r.	.	+	.	.	.	+	r	.	1	.	.	.	1	r	.	.	.	.	r	1
Hypericum perforatum	Fe.–Br.	.	.	.	.	r	+	.	1	.	1	1	.	.	.	.	.	.	1	.	.
Thymus sibthorpii		.	.	.	r	.	1	.	+	.	1	.	.	.	.	.	.	.	1	+	.
Thesium linophyllon ssp. *montanum*		.	.	.	.	r	.	.	.	.	1	.	.	r	r	.	.	r	.	.	.
Sideritis scardica		.	.	.	r	.	.	.	.	.	1	+	.	.	.	.	.	.	1	1	.
Marrubium thessalum		r	.	.	1	.	1	.	.	.	.	r	.	.	.	.	.	.	+	.	.
Linum hirsutum ssp. *spathulatum*		.	1	.	.	.	.	r	.	r	.	.	.	.	.	.	.	.	.	r	r
Teucrium montanum ssp. *helianthemoides*		.	.	.	r	.	.	r	r	.	r	+	.	.	.	.	.	.	.	.	.
Selinum silaifolium		.	r	1	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	r	.
Epilobium angustifolium	Ep. an.	+	.	1	.	.	.	.	.	.	.	.	.	.	1	+	.	.	.	.	.
Mycelis muralis	Q.–F.	1	.	.	r	.	.	.	.	.	.	.	.	+	.	r	.	.	.	.	.
Scabiosa columbaria ssp. *balcanica*		.	.	1	+	.	.	.	.	.	r	.	.	r	.	.	.	.	.	.	.
Acinos alpinus ssp. *alpinus*	E.–S.	.	.	.	.	.	1	.	.	.	.	+	.	.	1	.	.	.	.	.	.
Helianthemum nitidum		.	.	+	r	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca valesiaca	Fe.–Br.	.	.	.	.	.	.	.	+	.	+	.	.	.	.	.	.	.	r	.	.
Mentha sp. 1		.	.	.	.	.	1	.	.	.	.	1	.	r	.	.	.	.	.	.	.
Hieracium cymosum ssp. *heldreichianum*		.	2	.	.	.	.	.	r	+	.	.	.	.	.	.	.	.	.	.	.
Linum catharticum	Fe.–Br.	.	.	r	.	r	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.
Linum tenuifolium		.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.
Stipa bromoides		.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1	.
Cirsium arvense		r	.	.	.	.	.	.	.	.	.	r	.	.	.	.	r	.	.	.	.

Species in two or one plots: *Arabis glabra* r(7), 1(11); *Astragalus depressus* +(16), r(18); *Lotus corniculatus* +(12), r (14); *Myosotis sylvatica* ssp. *cyanea* r(13), +(10); *Saxifraga sempervivum* r(12), r(4); *Dianthus integer* ssp. *minutiflorus* (T. r.) r(1), r(6); *Viola graeca* 1(12), r(2); *Polystichum lonchitis* (T. r.) r(15); *Carex* sp.2 1(15); *Cerastium banaticum* ssp. *speciosum* r(15); *Hieracium* sp.1 +(15); *Hieracium* sp.2 +(15); *Erigeron acer* r(15); *Fagus sylvatica* ssp. *sylvatica* (Q.–F.) r(15); *Hieracium waldsteinii* +(15); *Luzula sylvatica* +(13); *Orchis* sp.1 r(13); *Euphorbia* sp.2 r(13); *Coronilla varia* r(13); *Veronica chamaedrys* 1(12); *Poa* sp.1 1(16); *Ceterach officinarum* +(1); *Stipa pennata* ssp. *pulcherrima* r(1); *Ligusticum olympicum* r(1); *Draba lasiocarpa* (Fe.–Br.) +(14); *Arabis sagittata* r(14); *Cerastium* sp.1 r(14); *Eryngium amethystinum* (Fe.–Br.) 1(18); *Laserpitium siler* ssp. *laeve* +(18); *Bellardiochloa variegata* +(18); *Salvia ringens* +(18); *Trifolium repens* r(18); *Centaurea* sp.1 r(18); *Euphrasia salisburgensis* (E.–S.) r(18); *Potentilla recta* r(18); *Silene* sp.1 r(19); *Saponaria bellidifolia* r(8); *Asplenium ruta-muraria* (A. t.) +(6); *Aubrieta thessala* r(6); *Tragopogon balcanicus* r(7).

The abbreviations mean "diagnostic species of": **Da.–Fe.**: *Daphno–Festucetea* Quézel 1964; **Q.–F.**: *Querco–Fagetea* Br.-Bl. & Vlieger in Vlieger 1937; **T.–G.**: *Trifolio–Geranietea* Müller 1962; **J. t.**: *Juncetea trifidi* Hadač 1946; **Er.–Pi.**: *Erico–Pinetea* Horvat 1959; **R.–P.**: *Rhamno–Prunetea* Rivas Goday & Borja Carbonell 1961; **Q. p.**: *Quercetea pubescentis* Doing-Craft ex Scamoni & Passarge 1959; **Fe.–Br.**: *Festuco–Brometea* Br.-Bl. & R. Tx. in Br.-Bl. 1949; **E.–S.**: *Elyno–Seslerietea* Br.-Bl. 1948; **A. t.**: *Asplenietea trichomanis* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberd. 1977; **T. r.**: *Thlaspietea rotundifolii* Br.-Bl. 1948; **Ep. an.**: *Epilobietea angustifolii* R.Tx. & Preising ex von Rochow 1951.

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Environmental responses of vegetation composition along an altitudinal–climatic gradient of Western Crete, Greece

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Abstract. In the context of an intra-Mediterranean project aiming towards the study of the complex environmental degradation occurring in calcareous areas, the role of a number of environmental and anthropogenic factors on plant community composition along a climatic–altitudinal gradient of Western Crete was evaluated. Even though human activities have acted upon plant community composition, it was climate (as expressed via altitudinal changes) that was found to play the most important role in the distribution of plant species and vegetation units along the gradient. Leguminous flora was proved to act as an indicator of grazing impact on vegetation, either by being under- or over-represented.

Key words: altitudinal–climatic gradient, correspondence analysis, fire, grazing, legumes, Mediterranean ecosystems, species richness

Introduction

Despite the fact that they constitute only 1.2 % of the terrestrial ecosystems of the world, Mediterranean-type ecosystems rank second after the tropical forests for their biodiversity (Cowling & al. 1996). Although water deficit is the main stress for their biota, humans have affected and shaped Mediterranean-type plant communities since the Neolithic era, in particular at the Mediterranean Basin (Aschmann 1973). Their impact was a relatively slow and cumulative process of modifying natural ecosystems by means of cultivation, logging and grazing. However, after the Second World War major changes have been recorded at these ecosystems (Antrop 1993; Arianoutsou 2001). Extensive rural migration, agricultural intensification with new machinery, excess use of fertilizers, irrigation technology, international commerce and pressure for tourist development are among the major trigger factors of changes occurred in the traditional land use patterns. These changes have often resulted in land degradation and desertification (Fantechi & Margaris 1986).

Environmental Responses of the Mediterranean Ecosystems (ERMES) was the title of an EU project, aiming towards the study of the complex environmental degradation that is perceived to be occurring in limestone and marl areas near the Mediterranean Sea (Imeson & al. 1996). For the project purposes, an inter-Mediterranean gradient of regions of increasing aridity from west (Spain) to east (Greece and Israel) was established, including local climatic gradients per region.

In this context, one of the goals of the research, which took place at the Greek sites, was the evaluation of the role played by a number of environmental and anthropogenic factors on plant community composition along the particular climatic gradient (Western Crete) established.

Material and methods

Study sites

Crete is a mountainous island which presents an impressive variety of natural, semi-natural and cultural landscapes (Rackham & Moody 1996). Due to large topographical variation, the island has a wide range of climatic types, from sub-tropical to alpine. It was on the western part of the island that a climatic–altitudinal gradient of sites has been established, with the

altitude varying from 50 m to 1050 m a.s.l. The gradient consisted of seven sites found within the limits of Mediterranean climate, ranging from sub-humid to semi-arid conditions. All sites were situated on south-facing slopes overlying limestone rocks. The major disturbance agent acting upon those sites was livestock grazing, while fire had occasionally been interacting with it. Plant communities in all sites of lower altitude (50 m to 700 m) were dominated by seasonal dimorphic shrubs, such as *Sarcopoterium spinosum*, *Coridothymus capitatus*, and *Genista acanthoclada* (phryganic shrublands), while plant communities of upland sites were dominated by the evergreen sclerophyllous shrub *Quercus coccifera* (maquis shrublands).

Data collection

Patches with rather homogeneous vegetation cover were considered for sampling. At each patch a 2×25 m^2 plot has been established. All plant species growing within the plot have been recorded. Field campaign took place at three months intervals during the period between June 1996 and June 1997, so as to cover as much floristic elements as possible.

Data analysis

The ordination of the plots against the prevailing environmental and anthropogenic factors was performed with the application of CANOCO (version 4.0 for Windows software) for Canonical Correspondence Analysis (ter Braak 1987). The factors and the nature of their scaling are shown in Table 1.

Plant species nomenclature

Plant species nomenclature follows Turland & al. (1993).

Results

In total, 120 plant taxa of 38 families have been recorded. The higher species richness was recorded at the two sites of higher elevation (Fig. 1). The vast majority of the recorded taxa were herbaceous annuals. In terms of plant family spectrum, *Leguminosae* (22 taxa) and *Compositae* (14 taxa) were the families with the highest number of taxa. *Compositae* did not present any particular trend against altitude. This was not the case for *Leguminosae* that met their minimum richness in the lowland sites.

The results of the Canonical Correspondence Analysis (CCA) are shown in Table 2 and Fig. 2. The set of variables chosen explains 54.62 % of the species data variance (sum of all canonical eigenvalues vs. sum of all unconstrained eigenvalues). From the values of canonical coefficients against the Axes 1 and 2, it can be inferred that the ordination along the first axis is clearly shaped by the altitudinal gradient, while for the second axis 'grazing' is mainly to be accounted for the result.

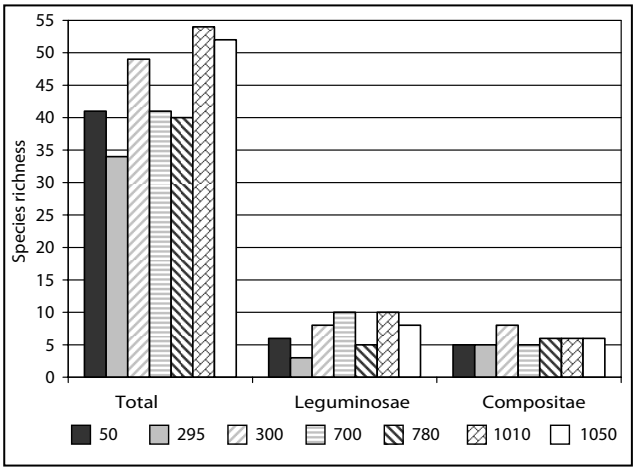

Fig. 1. Total plant species richness and number of recorded taxa of *Leguminosae* and *Compositae* families along the climatic–altitudinal gradient of Western Crete. Numbers in the legend refer to the altitude of the respective study site along the gradient.

Table 1. The variables included in the Canonical Correspondence Analysis (CCA) and the nature of their scaling.

Environmental variables	Code name	Nature of scale
Altitude	ALTITUDE	ordinal (m)
Grazing	GRAZING	nominal (0: only summer, 1: all year)
Fire incidents	FIRE	nominal (0: no, 1: yes)
Slope inclination	SLOPE	ordinal (°)

Table 2. The results of the Canonical Correspondence Analysis (CCA) as given by CANOCO 4.0 for Windows.

Axis variable	Axis 1	Axis 2	Total inertia
Eigenvalues	0.529	0.290	1.686
Species–environment correlations	0.967	0.913	
Cumulative percentage variance			
of species data	31.4	48.6	
of species–environment relations	42.3	65.6	
Canonical coefficients of variables			
Altitude	-1.230	-0.552	
Slope	0.278	0.356	
Grazing	0.166	-0.970	
Fire	0.156	0.767	
Sum of all unconstrained eigenvalues			1.686
Sum of all canonical eigenvalues			0.921

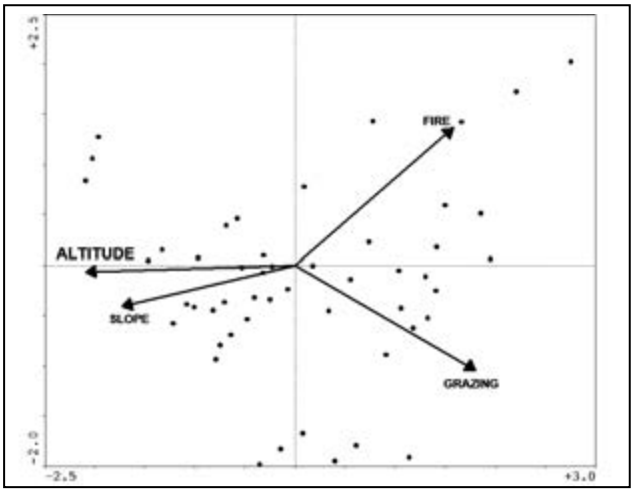

Fig. 2. Biplot ordination of plant species after Canonical Correspondence Analysis (CCA) of the vegetation composition data along the climatic–altitudinal gradient of Western Crete. Each circle represents one of the 120 recorded taxa.

Discussion

Changes in terms of vegetation composition along the gradient were found to be gradual, since the vast majority of taxa were present in more than one site. This seems to be a general observation in reports from other altitudinal gradients (Whittaker & Niering 1975; Auerbach & Schmida 1993). The gradual character of vegetation change lies on the fact that these changes are the result of plant species differential response to climatic variables. Floristic changes occur at the population level, through alterations of the relative abundance of the species, changing, in turn, community composition and structure (Miles 1981; McDonald & al. 1996).

After 'altitude', it was 'grazing' that was proved to play a major role in plant species ordination. 'Grazing' was included in the analysis in a nominal way, distinguishing sites grazed throughout the year from sites grazed only during the summer months, primarily due to the altitude (Papanastasis & al. 2003). The plant group mostly affected by grazing regime seems to be that of legumes. *Leguminosae* and *Compositae* were the two plant families presenting the highest number of species throughout the sites of the gradient. Still, the pattern of their richness versus altitude differed, probably due to different ecological attributes of their members (e.g., anemochory vs. zoochory, respectively [Kazanis & Arianoutsou 2004]). Species number of *Compositae* did not vary much along the gradient, whereas for legumes there was a trend of increasing species richness with altitude, in the contrary of what was expected, according to several data from Greece, reviewed by Arianoutsou & Thanos (1996).

Furthermore, Bergmeier & Matthäs (1995) reported that the altitudinal distribution of some species in Western Crete was broader than that reported by Turland & al. (1993). According to our data, there are 13 taxa that have been recorded from higher altitudes than reported by Turland & al. (1993). They are all herbs except of *Smilax aspera*, a woody climber. Three of them are legumes (*Trifolium angustifolium*, *Scorpiurus muricatus* and *Hippocrepis unisiliquosa*). This over-representation of legume species at sites of higher elevations might have been related to the fact that livestock animals are known to disperse legume seeds (Russi & al. 1992). The most prominent scenario is that seeds are dispersed by animals at the higher elevations during the summer months and onwards, they readily germinate and establish in wither months, when livestock animals are absent. On the contrary, legume species richness is under-represented in lowland sites because of their high palatability by livestock animals (Legg & al. 1998) that remain present all year long, preventing them from establishing adequately. This under-representation is more evident in recently burned sites, where legumes would have been expected to dominate (Arianoutsou & Thanos 1996).

Acknowledgements. This work was financially supported by the European Union, DG XII, Environment and Climate Program, under the project ERMES II, ENV4–CT95–0181. The authors would like also to thank Prof. V.P. Papanastasis for sharing with them his experience on the management and the land-use implications of Western Crete rangelands and Mr. S. Kyriakakis for his valuable help in the identification of some plant taxa.

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Variability of two-year-old seedlings of Moesian beech as the base of improvement

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Abstract. The analysis of several morphological features and phenotype characteristics of seedlings in the juvenile test with 10 half-sib lines of Moesian beech provides the guidelines for further breeding and production of planting material for urban coenoses and the establishment of special purpose plantations of this species. The seedlings were cultivated in the area, which is more or less ecologically homogeneous, so the environmental effect as the site factor can be eliminated, and the variability can be considered as a result of the differences in genotypes. The study results of Moesian beech half-sib line progeny can serve as the base for the selection of parent trees.

Key words: *Fagus moesiaca*, phenotype characteristics, seed sources

Introduction

Beech forests in Serbia are characterized by ecological and taxonomic diversity, which distinguishes them from beech forests of Europe. The devastation and intensive felling of beech forests in the 19th century decreased the area of the best phenotype and genotype stands and deteriorated the ecological conditions of beech forest development. In Serbia today only 25 % of the territory is covered with forests (Janković & Nikolić 1998). Moesian beech is the most widely distributed species and it represents the national wealth (Josifović 1973; Vukićević 1996). In addition to great significance of Moesian beech in forestry, there is its application in urban coenoses where, in addition to other uses, it also has an ornamental significance.

The synthesis of new cultivars is essential for landscape architecture and horticulture, because of the improved aesthetic properties, increased or decreased productivity, higher resistance to diseases, insect attacks, air pollution, as well as the optimal adaptation in urban coenoses (Ocokoljić & Anastasijević 2004a, b). In the above cases, selection is based on the morphological and physiological parameters. The significance of morphometric research of half-sib line growth is confirmed by the papers of numerous authors (Tucović & Stilinović 1981; Tucović 1983) who studied forest and ornamental trees and shrubs. The experimental results indicate the need to detect, before mass production, the parent trees with the best combining capacity, i.e. the trees with the highest quality and the most productive progeny.

Material and methods

The methods applied in the study are the analytic methods of tree breeding: mass selection (by selection of seed forests) and individual selection (by selection of plus trees) in order to conserve the best inheritance material for future reproduction. The main criteria and the aim of individual selection were the phenotype characteristics of trees and seed quality. The selection and marking of Moesian beech parent trees in Topčider was performed in 2001. Altogether 10 trees were selected and they were numbered and marked in the field. The general grades of their phenotype value and seed yield range between 1 and 5, where 1 stands for the poorest phenotype and yield and 5 denotes the best phenotype and yield at the level of the culture. All 10 selected trees were evaluated by 5 points.

For the research of generative progeny, we collected the seed of 10 selected trees in the phase of

full (technical) ripeness. After a short period of drying, the seeds were stratified and kept until spring. The seeds in the stratification were controlled once a week. Based on the observed germination, the stratification was stopped at the beginning of the spring. In April 2004, the seeds were sown in beds after the previous soil preparation. The beds were formed by block system with random arrangement of half-sib families in each repetition and divided into four equal blocks. The spacing between seed rows was 20 cm and between seeds in the row – 15 cm. Intensive tending measures were applied during the cultivation period.

The beds were established in the nursery in the suburbs of Belgrade. Geographical position and the co-ordinates are: 44°48' N and 20°28' E. The nursery is on the gentle north-west slope, at the altitude of 112 m. Based on the subspontaneous flora, the site of the sample plot belongs to the site of the climatogenic community *Quercetum frainetto–cerris* Rud. The fringe region of Belgrade, i.e. the south margin of Pannonia, which is the site of the nursery, is the site of the subassociation *Quercetum frainetto–cerris aculeatetosum* Jov. – the community of Hungarian oak and Turkey oak with butcher's broom, variation with hornbeam, as a natural part of this forest community (Jovanović 2000). Climate characteristics of the site where the half-sib families were cultivated correspond to the characteristics of the climate of Belgrade. The detailed soil analysis, with physical and chemical properties, was based on the open profile. The soil type is chernozem, lessivé, on loess and loesslike sediments.

To establish the criteria of future individual selection, we performed the comparative analysis of growth elements and recorded the special phenotype characteristics of the progeny of 10 test trees during two vegetation growth periods, for the seed yield of 2003. The following growth elements were analysed: variability of seedling height, diameter in root collar, number of formed branches, and number of formed leaves by the middle of the second vegetation period.

All the quantitative analyses were carried out on a sufficient number of samples. Diameter measurement of root collar was performed by micrometer to 0.1 mm, and the height was measured with a ruler from the root collar to the tip of the terminal bud, to 1 mm. The data were statistically processed by Excel with the computation of the basic statistical parameters and the corresponding correlations.

Results and discussion

The comparative analysis of seedlings of 10 half-sib lines aged two years, seed yield 2003, included: growth elements (as described above) and the appearance of special phenotype characteristics.

At the end of the second vegetation period, the average height at the population level was 23.10 cm. The lowest average height at the individual level was measured in the family number 7 (19.50 ± 1.15 cm), and the maximal average height was measured in the family number 6 (27.60 ± 1.48 cm). The biometric analysis was applied to prove that the seedling variability obtained by morphometric analysis was statistically confirmed and all relevant statistical parameters are presented in Table 1.

The analysis of average height increment in two vegetation periods shows that the average height of the greatest number of families ranged about the mean value of all families (Table 1). The families' number 1 and 4 showed the pronounced deviation from the mean value. The exceptionally low increment of the families 1 and 7 could be characterized as dwarf or semidwarf growth. The breeding program for dwarf phenotype uses the plants with very poor growth as the initial material. Based on the above observations, it can be concluded that this is the expression of genotype variability.

The practical experience shows that root diameter in root collar is an important indicator of root development, which by all means affects the development of the whole plant. Taking into account the above fact, the morphometric analysis in the juvenile test included also the analysis of root collar diameter in the analysed family progeny aged two years, seed yield 2003. The results of these analyses were processed at the population and individual levels (Table 1).

The average root collar diameter at the population level was 4.20 mm. The minimal root collar diameter at the individual level was measured in the family number 10 (3.51 ± 0.16 mm), and the maximal diameter was measured in the family number 8 (6.12 ± 1.86 mm).

The analysis of seedling growth in the nursery during two years and the values of statistical parameters (Table 1) show the expressed variability of diameter increment. As the height and diameter growth is genetically determined, it can be concluded that the variability of genetic predispositions of the analysed test trees is expressed. The study results indicate that there are significant differences of the measured parameters among 10 analysed families, but not between the repe-

Table 1. Statistical parameters for properties of the growth elements of 10 half-sib lines of Moesian beech.

Statistical parameter / Tree	Characteristics	$\bar{x} \pm S_{\bar{x}}$	$S \pm S_S$	$V \pm S_V$	Coefficients of linear correlation
1	a) height (cm)	20.02 ± 0.79	3.96 ± 0.55	19.77 ± 2.77	a – b/ 0.11
	b) diameter (mm)	4.37 ± 0.19	0.98 ± 0.01	22.39 ± 3.14	a – c/ 0.14
	c) formed leaves (No)	5.00 ± 0.33	1.65 ± 0.23	33.17 ± 4.66	b – c/ 0.27
2	a) height (cm)	24.26 ± 1.56	7.79 ± 1.09	32.13 ± 4.51	a – b/ 0.88
	b) diameter (mm)	4.28 ± 0.23	1.15 ± 0.16	26.81 ± 3.76	a – c/ 0.00
	c) formed leaves (No)	11.60 ± 1.30	6.52 ± 0.51	56.20 ± 7.89	b – c/ 0.11
3	a) height (cm)	23.04 ± 1.41	7.09 ± 0.99	30.79 ± 4.32	a – b/ 0.80
	b) diameter (mm)	3.99 ± 0.21	1.07 ± 0.15	26.77 ± 3.76	a – c/ 0.10
	c) formed leaves (No)	11.16 ± 0.86	4.37 ± 0.60	38.77 ± 5.44	b – c/ 0.53
4	a) height (cm)	25.90 ± 1.24	6.22 ± 0.87	24.01 ± 3.37	a – b/ 0.08
	b) diameter (mm)	4.29 ± 0.15	0.77 ± 0.11	18.05 ± 2.13	a – c/ 0.38
	c) formed leaves (No)	10.48 ± 0.99	4.96 ± 0.70	47.40 ± 6.66	b – c/ 0.72
5	a) height (cm)	22.78 ± 1.25	6.23 ± 0.87	27.36 ± 3.84	a – b/ 0.14
	b) diameter (mm)	4.06 ± 0.15	0.77 ± 0.11	18.99 ± 2.67	a – c/ 0.46
	c) formed leaves (No)	10.28 ± 1.07	5.35 ± 0.75	52.05 ± 7.31	b – c/ 0.64
6	a) height (cm)	27.60 ± 1.48	7.40 ± 1.04	26.82 ± 3.77	a – b/ 0.13
	b) diameter (mm)	4.20 ± 0.17	0.86 ± 1.20	20.49 ± 2.88	a – c/ 0.37
	c) formed leaves (No)	11.60 ± 1.49	7.44 ± 1.04	64.12 ± 9.00	b – c/ 0.46
7	a) height (cm)	19.46 ± 1.15	5.77 ± 0.81	29.64 ± 4.16	a – b/ 0.58
	b) diameter (mm)	3.62 ± 0.11	0.54 ± 0.07	14.81 ± 2.08	a – c/ 0.67
	c) formed leaves (No)	11.04 ± 0.74	3.67 ± 0.51	33.23 ± 4.67	b – c/ 0.64
8	a) height (cm)	22.21 ± 1.42	7.13 ± 100	32.09 ± 4.51	a – b/ 0.00
	b) diameter (mm)	6.12 ± 1.86	9.31 ± 1.31	152.32 ± 21.39	a – c/ 0.80
	c) formed leaves (No)	10.08 ± 0.95	4.75 ± 0.66	47.13 ± 6.62	b – c/ 0.09
9	a) height (cm)	23.48 ± 1.56	7.81 ± 1.09	32.27 ± 6.67	a – b/ 0.57
	b) diameter (mm)	3.80 ± 0.17	0.85 ± 0.12	22.43 ± 3.15	a – c/ 0.77
	c) formed leaves (No)	11.96 ± 1.40	7.18 ± 1.00	62.07 ± 8.43	b – c/ 0.74
10	a) height (cm)	22.38 ± 1.79	8.96 ± 1.26	40.07 ± 5.62	a – b/ 0.88
	b) diameter (mm)	3.51 ± 0.16	0.83 ± 0.11	23.59 ± 3.31	a – c/ 0.58
	c) formed leaves (No)	9.60 ± 1.08	5.42 ± 0.76	54.49 ± 7.93	b – c/ 0.58

titions, which confirms the hypothesis that the differences in root collar diameter are primarily genetically conditioned.

To determine the correlation between height and root collar diameter in the progeny of the analysed Moesian beech families, the coefficients of linear correlation were calculated at the level of all families (Table 1). The identified positive correlations show that root collar diameter increases with the increase of seedling height.

At the end of the second vegetation period, the average number of formed leaves at the population level was 10. The minimal number of formed leaves at the individual level was measured in the family number 1 (5.00 ±0.33), and the maximal number of formed leaves was measured in the family number 10 (27.0 ±1.43). The analysis of the average number of formed leaves at the end of the second vegetation period shows that the greatest number of families had the average number of leaves ranging about the mean value of all families (Table 1). The families' number 1 and 10 showed the pronounced deviation from the mean value.

To determine the correlation between height and root collar diameter and the number of formed leaves in the progeny of the analysed Moesian beech families, the coefficients of linear correlation were calculated (Table 1). The correlation analysis of two-years-old seedling height and the number of formed leaves showed the positive correlation of these elements, with the average coefficient of linear correlation r = 0.43. The correlation analysis of two-years-old seedling root collar diameter and the number of formed leaves shows that seedlings with larger root collar diameters form greater numbers of leaves. The above correlations indicate that the effect of root collar diameter on the number of formed leaves is greater than the effect of seedling height.

The analysis of phenotype characteristics of individuals within the same half-sib line and also between

families in the juvenile test of 10 half-sib lines shows the variability of leaf colour and dimensions, branching forms and types. The seedlings with different leaf pigmentation are few. The greatest number of seedlings belongs to the type *atroviridis*, i.e. their leaves are dark green, and also there is a greater number of the type *viridis* with pale green leaves.

According to branching habit, there are phenogroups with monopodial growth and with forked growth. During the second year, the progeny of the selected test trees developed the individuals with early branching, which indicates that this property is under strong genetic control. Based on the study results, half-sib lines 1, 4 and 6 had the exclusively monopodial growth. A high percentage of forked seedlings (above 50%) were recorded in the families 3 and 10. Taking into account that Moesian beech is the species, which can be applied in tree rows, its forked growth, especially the low forking (forking in the juvenile test, i.e. at the age of two years, is a definite sign of future low forking), is not a desirable property. In this sense, the individuals with monopodial growth should be selected for line planting.

In the breeding program for dwarf phenotype, the initial material should be the plants with poor growth. In the analysis of half-sib lines during two vegetation periods, family 7 showed the extremely poor growth of all cultivated individuals. Dwarf or semidwarf growth was also recorded in some individuals of the families 2, 5 and 10. Dwarf plants can have the prostrate habit types, i.e. the cultural forms – *prostrata*, *repens*, *horizontalis* and *procumbens*. The habit of the type *prostrata* occurred in three seedlings of different half-sib lines.

In addition to dwarf individuals with the leaves typical for the species, there were four individuals with the leaves of *atropurpurea* type.

Bearing in mind that the individuals with special phenotype characteristics occur rarely, the observed variability of leaf colour and form, growth and branching habit is highly significant. The genotypes with special phenotype characteristics should be fixed by selection and the selected genotypes should be propagated by vegetative reproduction.

Conclusions

The experimental results indicate the need to detect, before mass production, the parent trees with the best combining capacity, i.e. the trees with the highest quality and the most productive seeds.

The comparative analysis of half-sib family seedlings enabled the identification of the extreme material, which is significant for further reproduction. All the analysed seedling properties in the field test showed the variability both between individual families and within each family. The seedlings were cultivated in the more or less ecologically homogeneous area, so the factor of environmental effect can be eliminated and the observed variability can be considered as a result of genotype differences. The analyses included the quantitative properties, which are controlled by polymer genes, i.e. by numerous multiple genes whose effects are added. As the fathers are not known, because the reproductive material originates from free pollination, the good genetic constitution of test trees–mothers can be accepted because the results of the analysed properties occurred in their half-sib progenies.

Taking into account that the individuals with special phenotype characteristics occur rarely, the observed variability in leaf colour and form, as well as in the growth and branching habit is extremely significant. The results of the study of Moesian beech half-sib progeny can serve as the basis for the selection of parent trees, which can be the source of reproduction material for the production of seedlings with desired characteristics.

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Effect of low temperatures on the fructification of Turkish hazel

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Abstract. The breeding of ecologically, ornamentally or economically significant trees is scientifically and economically justified. Planned selection can single out the trees and ensure good-quality seed material. However, the quality and the scope of seed production are affected by numerous factors. This paper points to the effect of climatic factors, especially low temperatures at the beginning of the growth season during the time of the flowering phenophase, on the seed yield of Turkish hazel in Novi Sad. The causes of the lower seed yield and the reduced seed quality of the studied Turkish hazel trees are related to the variability of climate parameters during the study period, but also to the genotype of parent trees.

Key words: *Corylus colurna*, seed yield, selection

Introduction

The fact that out of 300 000 plant species on the Earth, only a small number of species (*ca*. 200) are cultivated more or less widely, and not more than 15 to 20 species are cultivated widely for food, emphasizes the importance of the analysis of the domestification syndrome. The domestification syndrome characterizes a small number of species which have economically significant (desired) properties that ensure the economic gain (Vukićević 1996; Jovanović 2000). For this reason, the orientation to genetic and physiological analysis of the economically significant plant properties is justified. The classification of species based on genetic and ecological criteria ensures a more rational and faster conversion of the potential plant variability into free variability, available to special purpose and natural selection. Attention should be focused on the study of plant ontogenesis, aiming at better and more rational programming and the selection of initial populations and their improvement, all in the function of conservation and enhancement of the available genetic richness (Zhuchenko 1988). A special and an extremely important field of study is the effect of technological measures on the cultivated populations, as well as the adaptation of species to the concrete conditions of cultivation.

Woody species simultaneously inhabit two environments: aerial and edaphic. The edaphic environment, just as the air, is very changeable, but the interaction of trees and shrubs in them is based on general biological laws (Tucović 1976). One of the laws indicates that none of the climate-edaphic factors of life or nature forces: light, warmth, air, mineral elements, can be replaced by anything else. The effect of environmental factors on the life of woody species is not uniform. Environmental factors can weaken or stimulate the life phenomena. The climate-edaphic factors affect the growth, development and productivity of plants through the soil and climate conditions. Each of the factors affects in a special way, depending on the dose. To decrease or to eliminate the effect of these factors on numerous tree properties, it is necessary to study their effect continuously.

Starting from the above facts, we analysed the Turkish hazel seed yield and seed quality on green spaces of Novi Sad, in the aim to determine the effect of climate factors on fructification.

Material and methods

Seven Turkish hazel trees in Novi Sad are object of the study. The group of analysed trees belongs to the population in the oldest and largest park in the city, in Futoški Park. The altitude of the locality is 78 m. The soil belongs to the type of alluvial soil, but it has been largely anthropogenised. The site belongs to the community of willows and poplars in the widest sense (*Saliceto–populetum* sensu lato). The climate is temperate continental, but with significant characteristics of semi-arid climate. Mean annual temperature is 11.1 °C. Mean annual precipitation is 603.1 mm.

The study methods are individual selection and analytic method of tree breeding, and the entire study was performed by taking into account the fact that the selection and utilisation of Turkish hazel trees as seed sources enable the supply of good quality seed material, necessary for the production of planting material for the needs of urban coenoses, as well as for the synthesis of the new varieties significant for fruit growing.

The reconnaissance in the field and the comparative analysis were performed during 4 consecutive years, with the analysis of the seed from the seed years (2001 and 2004). The comparative analysis of yield was performed by recording the fruit length, fruit width, fruit thickness, nut mass, core mass, thickness of pericarp and by assessing the seed fullness. The fruit characteristics are analysed on the samples of 100 fruits from each tree, for each seed year, in four repetitions. 400 fruits were selected from each tree, and their fullness was assessed by sectioning. All the quantitative data were processed statistically, with the determination of the limits of variability, mean values, standard deviation, variation coefficients and mean errors for each of the calculated statistical parameters.

Results and discussion

As the analysed trees are situated in Futoški Park in Novi Sad, we shall present the position of Novi Sad, which makes the transition from the conditions of the Adriatic coast climate to the thermal conditions of the completely continental areas. Such a specific position of Novi Sad enables the assessment of the reaction of Turkish hazel on the effect of low and extremely low temperatures at the beginning of the vegetation period. We use the data of the Republican Hydro-meteorological Institute on mean daily and absolute minimal temperatures in February. Air temperatures during February are important, because it is the period when Turkish hazel most often flowers (Ninić-Todorović 1990; Ocokoljić & Ninić-Todorović 2003). In both study years, 2001 and 2004, the flowering phenophase occurred during February.

As the yield and quality do not depend only on mean daily temperatures and absolute minima, but also on other numerous factors, this paper analyses the complex effect of low temperature on Turkish hazel fructification during 4 consecutive years. The yield was especially analysed in 2001 and 2004, which were the years of abundant yield. The seeds were categorized according to the quality, based on the morphometric analysis and the analysis of seed fullness (Fig. 1).

The study data on seed properties point to the effect of low temperatures at the beginning of the vegetation period on Turkish hazel seed quality. Extremely low temperatures in mid February 2004 caused the lower seed quality of all trees. Namely, although 2004 was the year of abundant yield, with the maximal seed yield of all 7 Turkish hazel trees, the analysis of seed fullness shows the average seed fullness at the level of the population amounting to 66.6 % which is by 25.4 % less than in 2001, which was also classified as one of the years of abundant yield. The effect of low temperatures during February on Turkish hazel fructification was confirmed by the comparative analysis of seed quality in 2001 and 2004. The average nut length at the population level in 2001 was 16.40 mm which is by 0.20 mm more than the average nut length in 2004. The comparative analysis of other parameters also shows that the values of the fruits ripened in 2001 were larger, i.e.: nut width 0.31 mm; nut thickness 0.10 mm; nut mass 0.20 g; core mass 0.10 g and pericarp thickness 0.28 mm. The differences are even greater at the individual level (Fig. 2).

To determine the correlation of the analysed parameters of Turkish hazel nuts, the coefficients of linear correlation were calculated for both years of abundant yield (2001 and 2004) for the following: nut length and width, nut length and thickness, nut width and thickness, nut length and mass, nut width and mass, as well as nut mass and core mass (Table 1). The coefficients of correlation between all study parameters are very high. The calculated positive correlations in both seed years indicate that with the increase of nut length all other parameters also increase, and that with the increase of total nut mass, core mass also increases.

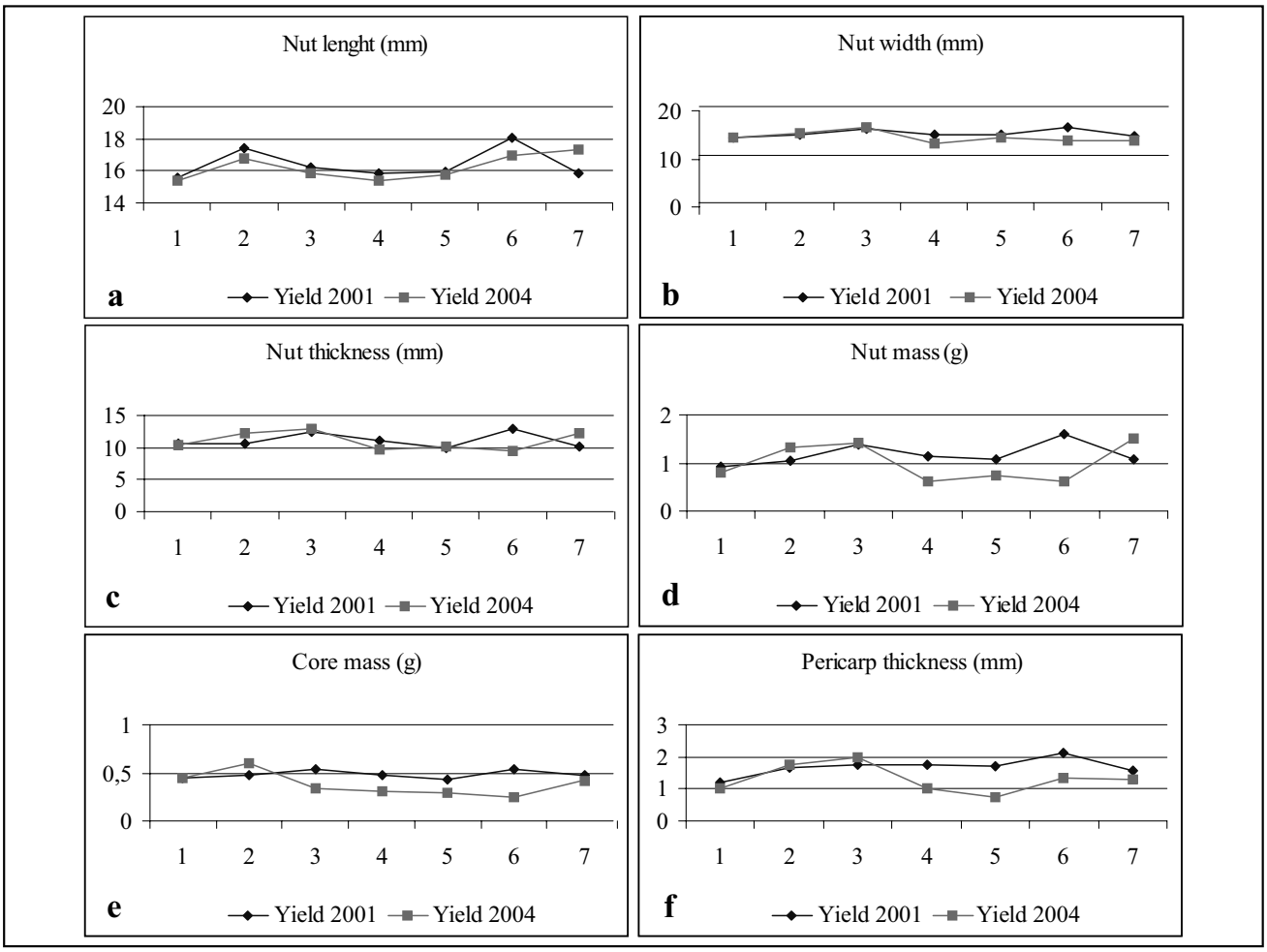

Fig. 1. Biometric properties (**a** – **f**) of the fruits of 7 Turkish hazel trees in Novi Sad.

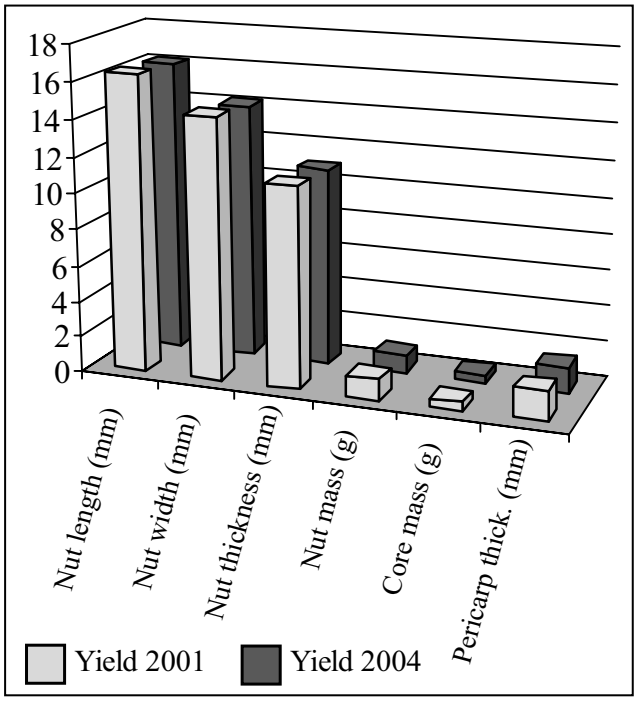

Fig. 2. Average value for properties of the fruits of Turkish hazel trees population in Novi Sad.

Table 1. Coefficients of linear correlation (r) for the analysed properties of Turkish hazel fruits.

Coefficients of linear correlation (r) between:	Yield 2001	Yield 2004
nut length and width	0.24	0.45
nut lenght and nut thickness	0.35	0.50
nut width and nut thickness	0.55	0.68
nut lenght and nut mass	0.38	0.53
nut width and nut mass	0.65	0.64
nut mass and core mass	0.71	0.66

The economic and horticultural values of woody species are higher if their cultivation is possible in different ecological conditions. Consequently, it is important to study the Turkish hazel reactions to meteorogenic factors.

Conclusions

The analysis of several morpho-physiological properties of the nuts of seven Turkish hazel trees in Novi Sad proves the adverse effect of low temperatures at the beginning of the vegetation period on fructification and seed quality. As it is known, only the good quality and well-studied seed material enables the establishment of cultural communities of forest trees, and also can serve as the initial material for the production of planting stock used in urban coenoses and for the synthesis of the new varieties significant for fruit growing.

Extremely low temperatures at the beginning of the vegetation period have a significant effect on the yield and fullness of Turkish hazel seeds. The study data on tree reactions to the changes of climate factors contribute not only to the study of the genetic–physiological mechanisms of resistance, but also to the definition of the parameters for the selection of parent trees, as well as for the definition of the strategy of synthesis of new Turkish hazel selections. The dendrological significance of such observations is great, especially if they are multiannual, because they can directly affect the generative propagation.

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Productive types of spruce–fir forests on Mt Zlatar

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Abstract. Mixed forests of spruce and fir occupy a relatively small area compared to the total forest area in Serbia, but by their productivity and conservation, they belong to the class of the most valuable forests. By the research of the wider area of Mt Zlatar on 15 experimental fields, on two extremely different geological layers with three soil types, ass. *Abieti–Piceetum serbicum* with only one subassociation (*typicum*) is described. On the basis of phytocoenological and pedological researches of the investigated area, five ecological-vegetation units are designated. Based on productive and developmental characteristics, they are synthesized in 2 productive forest types: Forest type 1 – high spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on calcomelanosol, medium deep calcocambisol and medium deep dystric cambisol, and Forest type 2 – high spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on deep calcocambisol and deep dystric cambisol.

Key words: ecological differentiation, fir, productive type, spruce, Zlatar

Introduction

Mixed forests of spruce and fir cover a relatively small area compared to the total area under forests in Serbia. However, by their productivity and conservation, they belong to the class of our most valuable forests. The percentage of these forests in the growing stock of Montenegro is considerably higher, therefore they have a higher management significance there (Matović 2005).

In Serbia, mixed spruce and fir forests are mostly situated on the mountains Zlatar, Golija, Kopaonik, Mokra Gora, Stara Planina, and to a less extent they occur on Mt Zlatibor (Murtenica), Tara, Radočelo and other mountainous massifs, most often in favourable edaphic conditions, on deeper acid brown and brown calcareous soils. The stands have good canopy closure, spruce and fir trees are vital and their productivity is good. In Montenegro, these forests are most represented in the areas of Pljevlja, Žabljak, Kolašin, Berane and Rožaj (Matović 2005).

Spruce and fir forests in Serbia and Montenegro most often occur as climate-regional forests at the transition between mixed forests of spruce, fir and beech and pure mountainous forests of spruce, but they also grow in the ranges of mixed forests of fir–beech and fir–spruce–beech, in general at the elevations between 1000 and 1600 metres. In Montenegro and West and Southwest Serbia, these forests grow at somewhat lower altitudes than in Central and East Serbia, which is primarily the consequence of spruce descending to the belt of fir and beech (Matović 2005).

The percentage of mixed spruce and fir forests is considerably higher in the east part of Zlatar, where due to orographic conditions, they occur in mosaics with pure spruce forests and mixed forests of spruce, fir and Scots pine. In the west part of Zlatar, these forests grow on smaller areas, most often within the larger complexes of mixed forests of spruce, fir and beech.

Material and methods

In the wider area of Zlatar, altogether 15 sample plots were established, average area being about 0.32 ha.

The following data were taken on each sample plot:

- orographic (altitude, exposure, slope and GPS coordinates);
- collected data for geological, pedological and phytocoenological study;
- collected basic taxation data by the method usually applied on sample plots;
- 32 trees were measured for the dendrometric analysis.

The data collected for geological, pedological and phytocoenological studies were processed by standard methods.

The taxation elements were processed separately for each sample plot. Height curves were constructed for each tree species per sample plots, and Prodan's function was applied for fitting. Current diameter increment was fitted by the cubic parable. Volume was calculated by double-entry volume tables for fir and spruce for the area of Kopaonik (Banković & al. 2003, 2004). Current volume increment was calculated by the "method of diameter increment". The collected field data for stem analysis were computed by a special program package.

Results

Phytocoenological study

Only one forest community – spruce and fir forest (*Abieti–Piceetum serbicum*) was identified in all the study stands, with one subassociation: *typicum*.

Abieti–Piceetum serbicum typicum Mišić & al. occurs both on the calcareous bedrock and on hornstone veins, which is primarily more the consequence of the significant effect of altitude, mountainous climate and edificators on the pedogenetic processes than of the bedrock.

The tree layer has different degrees of closure from 0.7–1.0, which is the result of different management procedures in the past. The following edificators are dominant in the tree layer: fir (*Abies alba*) and spruce (*Picea abies*), and individually Scots pine (*Pinus silvestris*), beech (*Fagus moesiaca*), aspen (*Populus tremula*), birch (*Betula pendula*) and sycamore maple (*Acer pseudoplatanus*).

Shrub layer is mainly composed of the seedlings of fir (*Abies alba*), spruce (*Picea abies*), fly honeysuckle (*Lonicera xylosteum*) and hazel (*Corylus avellana*). Along with these species, red-berried elder (*Sambucus racemosa*), aspen (*Populus tremula*), mezereum (*Daphne mezereum*), beech (*Fagus moesiaca*), dog rose (*Rosa pendulina*) and mountain ash (*Sorbus aucuparia*) have significant degrees of presence. Sallow (*Salix caprea*), sycamore maple (*Acer pseudoplatanus*), birch (*Betula pendula*) and ivy (*Hedera helix*) occur individually.

Ground flora in this community consists of 70 species. The characteristic set of ground flora is composed of: *Vaccinium myrtillus*, *Mycelis muralis*, *Dryopteris filix-mas*, *Geranium robertianum*, *Polygonatum verticillatum*, *Hieracium murorum*, *Deschampsia flexuosa*, *Anemone nemorosa*, *Oxalis acetosella*, *Stellaria nemorum*, *Euphorbia amygdaloides*, *Prenanthes purpurea*, *Asperula taurina*, *Ajuga reptans*, *Aremonia agrimonioides*, *Sanicula europaea*, *Gentiana asclepiadea*, *Rubus idaeus*, *Luzula luzuloides*, *Fragaria vesca*, *Symphytum tuberosum*, *Veronica chamaedrys*, *Rubus hirtus*. Along with the above-mentioned species of ground vegetation, the following species have a significant degree of presence: *Festuca vallesiaca*, *Galium silvaticum*, *Athyrium filix-femina*, *Erythronium dens-canis*, *Cardamine bulbifera*, *Epilobium montanum*, *Asarum europaeum*, *Glechoma hirsuta*, *Solidago virgaurea*, *Geum urbanum*, *Saxifraga rotundifolia*, *Viola sylvatica*, *Calamagrostis arundinacea*, *Carex sylvatica*, *Campanula patula*, *Orobanche* sp. The individual appearance of about 30 species of ground flora is also significant.

According to Mišić & al. (1985) and Obratov (1992), on Mt Zlatar also, only one association was identified in the mixed spruce and fir forests (*Abieti–Piceetum serbicum typicum*).

Bedrock and soil

Two different types of parent rock were determined in the study stands on the mountain Zlatar: limestone and diabase-hornfels formation. Parent rock had a significant effect on pedogenetic processes, but the formation of different soil types was also affected by vegetation (acidifying tree species), relief, climate factors (mountainous climate, high amounts of precipitation), etc.

The following soil types occur in the study stands: on limestones, black soil (calcomelanosol) and brown calcareous soil (calcocambisol), and on acid siliceous rocks – acid brown soil (dystric cambisol). All profiles are characterized by a deep layer of organic litter, which indicates a slow decomposition of organic matter and a slow cycling of nutrients in the ecosystem.

- **Calcareous black soil (calcomelanosol)**

These soils, in the typical forms of shallow solum, in the study colluvial black soils are 45–60 cm deep. Their texture is loam to clay loam, with a high content of skeleton. They are well structured, permeable and aerated, and very humous soils with a wide C/N ratio.

Chemical characteristics are mild acid to neutral reaction, high total adsorption capacity and high de-

gree of basic cation saturation. The content of potassium is within medium to good supply, and phosphorus is deficient in all the study profiles.

- **Brown calcareous soil (calcocambisol)**

The depth of solum in the study profiles ranges from 40 cm to 90 cm. Their textural class is that of heavy soils, except in the cases when the detritus from which the bulk of the soil mass was formed is of siliceous origin. The skeleton contents in these profiles range from skeletal soils to absolutely skeletal soils. The skeleton content increases with the solum depth.

Chemical characteristics are acid to mild acid reaction. In all profiles, pH value increases with depth, which is the consequence of the greater presence of calcareous skeleton in the deeper layers. The exception is SP 14, where active acidity is more intense in the deeper layers than in the surface layers. This is the consequence of the higher presence of hornfels in the skeleton, which left more residues from which the soil was formed, and of the more intensive biological base accumulation in surface layers by the herbaceous vegetation.

According to critical values by AL method, the potassium content ranges from poor to medium supply, and phosphorus is deficient in all the study profiles.

This soil type is divided into two varieties:

- medium deep brown calcareous soils;
- deep brown calcareous soils.

- **Acid brown soil (dystric cambisol)**

The depth of solum in these soils ranges from 60 cm to 100 cm. The skeleton content is different, the deeper soil profiles are without skeleton, or are poorly skeletoid, while the percentage in the shallower profiles (60–70 cm) is 50–80 %. Particle size distribution differs from sandy loam to clay. Acid brown soils are found on hornstone veins in the calcareous massif of Zlatar. At some sites, the characteristics of acid brown soils were also affected by the surrounding calcareous material, i.e. the calcareous water, which brings the soil alkaline elements, and in some cases, by the colluvial process, the pieces of calcareous rocks and soil material originating from limestone detritus.

Chemical properties are acid reaction, low degree of basic cation saturation of adsorptive complex. In most analysed profiles, total adsorption capacity decreases with depth, which is the consequence of the lower presence of humus substances. Only in the profiles of the sample plots 1 and 11, in deeper layers, the total adsorption capacity is somewhat higher than in the surface layer, but in these cases this is the result of a considerably heavier textural class of the cambic horizon. In surface layers, they are humous to very humous soils.

The humus accumulation horizon is deep, which is the result of higher altitudes, less favourable climate conditions for the mineralization of the organic matter to the final products of decomposition, and simultaneously also of the chemical nature of the organic litter, which originates from the acidifying coniferous species. Along with the general macroclimate conditions and the unfavourable chemical nature of the organic matter, the rate of mineralization of the organic matter is additionally slowed by the microecological conditions, which spruce and fir create by their deep shade.

Organic matter is characterized by a wide carbon/nitrogen ratio, which is also conditioned by macro- and microecological conditions and by the chemical nature of the organic matter under these stands. This means that nutrient cycling in the study ecosystems is slow but it meets fully the demands of spruce and fir.

Based on the critical values of AL method, the supply of plant available potassium in profile 1 and 15 is good, while in other profiles it is poor to medium. However, it is satisfactory for the demands of oligotrophic and acidophilous species. Phosphorus is highly deficient in all profiles. Low quantities of phosphorus are determined only in the humus accumulation horizon, while in the deeper horizons they are lower than 1 mg/100 g.

This type and variety of acid brown soils on hornstones is divided into two forms:

- medium deep acid brown soil;
- deep acid brown soil.

Ecological differentiation of the sites

Based on phytocoenological and pedological research, the study stands are classified into five ecological units:

1. Ecological unit I – high spruce and fir forest *(Abieti–Piceetum serbicum typicum)* on calcareous black soils;
2. Ecological unit II – high spruce and fir forest *(Abieti–Piceetum serbicum typicum)* on medium deep, skeletal, brown calcareous soils;

3. Ecological unit III – high spruce and fir forest *(Abieti–Piceetum serbicum typicum)* on deep brown calcareous soils;
4. Ecological unit IV – high spruce and fir forest *(Abieti–Piceetum serbicum typicum)* on medium deep, skeletal, acid brown soils;
5. Ecological unit V – high spruce and fir forest *(Abieti–Piceetum serbicum typicum)* on deep acid brown soils.

In the definition of ecological units, the decisive effects were the edaphic factors, primarily the different soil types, soil depth and skeletalness.

Productive differentiation of the sites

The productive differentiation of the sites must be based on a great number of objectively selected parameters. In the previous research, the most frequently applied parameters of productive site differentiation were: the average values of the condition in the ecological units (N, G, V, I_v, p_i), mean values (h_g, h_{gmax}, d_g, d_{gmax}) and the ranges of growth elements obtained by the analysis of mean stand and dominant trees.

The average values of the condition in the ecological units (N, G, V, I_v, p_i) have a limited application, especially the number of trees and volume increment. Basal area and volume, as the parameters of the productive differentiation, can be reliable to a certain degree, if the study stands are characterized by an integrated management treatment in the past.

Mean values (h_{gmax}, d_{gmax}, h_g, d_g) have a limited application also, because these are the calculated values and they are under the effect of the stand silvicultural treatment in the past, especially the mean stand diameter. The most representative parameter, and also under the lowest effect of management treatment in this group, is the mean height of dominant trees and it is today most often applied in the productive site differentiation, especially in even-aged forests. Vučković (1989) reports that the mean height of dominant trees (the so-called upper height) compared to the mean stand height is considerably less dependent on stand conditions and maintenance, and therefore also on the so-called "calculation ranges".

The ranges of growth elements obtained by the analysis of mean stand trees also have a limited application, because they are under the influence of the stand structural characteristics. The ranges of growth elements of dominant trees are considerably less affected by stand conditions, so they can be a rather objective indicator, but only in even-aged stands.

The study stands are approximately equally stocked, well maintained and even-aged in the narrow sense (at the level of the sample plot), which enables the implementation of many of the above parameters. The basic problem in the productive site differentiation, in the concrete case, is the fact that this is a mixed forest, and that the stands in the near past were subject to different management systems, damage by snow and windthrow, and insect pest calamities.

The parameters of all three groups were implemented in the productive site differentiation of the study stands, i.e.: the average values of the condition in the ecological units (N, V, I_v), mean values (h_{gmax}, d_{gmax}) and the ranges of growth elements obtained by the analysis of dominant trees (Tables 1, 2).

The standard mathematical-statistical methods are applied in the concrete study. Each ecological unit is observed as a treatment, and each sample plot as the repetition within the treatment.

The analysis of covariance and the analysis of variance (Hadživuković 1991) should show if there are statistically significant differences in the values of the observed characters, and Duncan's test should show between which treatments (ecological units) the differences are statistically significant. The covariate in the analysis of covariance is the number of trees per hectare.

Mean values of the treatments (ecological units) per study parameters are presented in Tables (separately for the average values of the stand condition and the mean values, and separately for the ranges of growth elements obtained by the analysis of dominant trees).

The results of the analysis of covariance of basal area show that there are no statistically significant differences in the values of this character. Duncan's test shows that there are no statistically significant differences between I, IV, II and II, III, V ecological units. The second ecological unit is in between, and it does not show the statistically significant difference compared to all other ecological units (Table 3).

The results of the analysis of covariance of volume show that there are no statistically significant differences in the values of the study parameter. Duncan's test shows that there are no statistically significant differences between I, IV, II and III, V ecological units (Table 3).

Table 1. The average values of the condition and the mean values in the ecological units.

Sample plot	N (pcs/ha)			G (m^2/ha)			V (m^3/ha)			I_v (m^3/ha)			d_{gmax} (cm)		h_{gmax} (m)	
	fir	spruce	total	fir	spruce	total	fir	spruce	total	fir	spruce	total	fir	spruce	fir	spruce
High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on calcareous black soils (Ecological unit I)																
4	710.7	78.5	851.2	45.064	3.571	52.523	546.804	43.071	637.359	6.125	0.637	7.602	41.5	39.2	26.1	27.8
12	310.0	563.3	895.2	19.412	35.785	56.288	272.072	511.697	799.030	4.110	6.407	10.684	42.0	39.2	30.1	31.6
16	655.5	306.2	961.7	31.433	19.190	50.623	432.024	262.160	694.184	6.305	4.380	10.685	33.7	38.3	26.8	29.7
average	558.8	316.0	902.7	31.969	19.516	53.145	416.967	272.309	710.191	5.513	3.808	9.657	39.1	38.9	27.7	29.7
High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on medium deep, skeletal. brown calcareous soils (Ecological unit II)																
3	510.6	53.2	574.5	39.756	4.293	45.548	530.556	59.615	611.793	7.632	1.193	9.242	47.8	51.4	29.7	33.5
6	325.0	250.0	606.8	28.491	20.979	51.984	382.231	278.432	693.933	4.546	3.914	8.897	46.0	46.3	28.5	30.2
10	109.9	534.0	680.6	9.912	35.372	48.666	132.206	474.802	653.190	2.028	7.434	10.204	45.2	42.2	28.5	30.8
average	315.2	279.1	620.6	26.053	20.214	48.733	348.331	270.950	652.972	4.735	4.180	9.448	46.3	46.6	28.9	31.5
High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on deep brown calcareous soils (Ecological unit III)																
5	464.5	26.6	505.9	44.127	2.270	47.587	641.272	30.693	687.612	7.969	0.701	9.011	51.0	48.0	31.5	32.1
8	433.9	232.8	672.4	40.257	21.490	62.132	588.393	299.546	893.101	7.669	4.071	11.823	49.5	47.5	31.6	32.1
14	415.8	261.2	711.3	41.348	29.633	72.925	630.292	453.125	1111.386	5.978	4.953	11.180	52.0	54.9	33.3	36.3
average	438.1	173.5	629.9	41.911	17.798	60.881	619.986	261.121	897.366	7.205	3.242	10.671	50.8	50.2	32.2	33.5
High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on medium deep, skeletal. acid brown soils (Ecological unit IV)																
7	180.2	530.0	742.0	13.025	40.048	56.102	180.537	542.357	770.065	2.209	7.933	10.777	46.2	44.0	29.0	30.4
11	555.8	416.2	987.3	23.259	26.266	49.694	298.723	359.060	659.325	5.996	5.730	11.736	36.1	39.2	26.7	30.1
15	736.7	58.5	835.1	45.249	3.871	50.744	635.046	50.590	707.259	9.783	0.743	10.843	39.2	47.3	28.8	31.1
average	490.9	334.9	854.8	27.177	23.395	52.180	371.435	317.336	712.216	5.996	4.802	11.119	40.5	43.5	28.2	30.5
High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on deep acid brown soils (Ecological unit V)																
1	390.1	26.8	422.6	41.764	3.618	45.975	627.614	52.545	688.679	9.925	0.749	10.796	51.2	50.9	32.7	33.2
2	310.3	56.0	390.1	34.312	6.236	43.408	544.693	94.406	682.151	8.489	1.390	10.433	52.5	51.3	34.9	36.3
9	595.2	35.7	631.0	60.966	3.478	64.444	1012.556	51.896	1064.452	11.333	1.032	12.365	49.2	50.9	35.1	35.5
average	431.9	39.5	481.2	45.681	4.444	51.276	728.288	66.282	811.761	9.916	1.057	11.198	51.0	51.0	34.2	35.0

Abbreviations: N – number of trees per hectare; G – stand basal area per hectare; V – stand volume per hectare; I_v – stand volume increment per hectare; d_{gmax} – stand quadratic mean diameter (dominant); h_{gmax} – mean height (dominant).

Table 2. The ranges of growth elements obtained by the analysis of dominant trees in ecological units (OP = observation field).

Ecological units		OP	Age														
			20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
			Diameter growth (mm)					Height growth (m)					Volume growth (dm^3)				
Fir	I	4	58.5	179.5	269.5	332.0	378.0	5.0	14.0	20.4	25.6	29.8	9.2	163.2	573.3	1098.7	1609.5
		12	44.3	110.0	201.5	299.5	375.0	3.9	11.1	17.4	24.1	28.5	3.6	59.9	297.7	867.6	1612.0
		16	57.0	138.8	205.8	274.0	341.0	4.3	11.4	17.9	23.3	27.6	6.6	81.5	308.8	744.3	1433.5
	II	3	69.5	245.8	339.8	418.8	470.3	5.4	17.0	23.9	28.7	31.7	11.3	370.7	1045.8	1991.4	2839.5
		6	30.8	175.5	295.5	377.0	424.8	2.8	9.9	19.2	24.9	29.3	3.5	111.8	567.5	1319.0	1955.6
		10	29.3	165.3	275.5	362.0	427.0	3.8	13.1	20.7	26.8	30.0	2.7	134.0	590.8	1361.7	2144.4
	III	5	46.0	190.0	290.8	352.5	431.0	3.8	12.2	20.2	25.4	30.3	4.5	165.4	631.3	1223.6	2191.3
		8	113.3	259.0	370.3	442.5	483.5	8.1	18.1	25.0	30.3	33.8	40.7	442.2	1285.0	2268.2	3105.4
		14	109.8	231.3	312.5	377.5	426.8	8.0	18.0	23.6	28.5	31.8	33.0	351.2	919.5	1629.7	2366.3
	IV	7	13.8	160.8	257.8	334.8	392.3	2.0	9.5	17.4	23.3	28.3	1.3	97.8	430.9	1003.2	1722.9
		11	73.3	163.5	212.8	268.3	353.3	7.2	15.5	19.9	24.4	30.0	15.9	180.8	382.5	745.4	1492.2
		15	66.3	154.3	211.5	271.3	337.8	5.1	13.2	17.7	22.5	27.9	9.0	114.4	312.5	699.6	1361.8
	V	1	42.5	144.0	269.8	350.5	417.0	3.6	10.5	19.2	25.6	31.9	3.1	81.9	525.7	1264.2	2243.3
		2	38.5	144.0	285.8	387.0	456.5	3.9	10.9	19.4	25.4	30.9	2.5	82.5	564.8	1363.4	2342.4
		9	80.5	198.5	293.5	387.5	455.8	7.2	15.2	23.5	30.3	34.8	19.3	233.3	750.8	1722.9	2777.7
Spruce	I	4	12.3	98.0	168.8	245.0	326.8	2.4	8.1	15.2	21.5	25.9	1.0	30.7	177.3	507.8	1052.2
		12	111.0	224.5	276.0	331.3	377.3	8.0	17.7	24.4	29.7	33.7	40.7	340.3	752.5	1318.6	1939.9
		16	110.5	201.5	299.8	373.5	419.5	7.2	15.7	23.1	28.9	31.4	33.9	234.1	787.1	1534.1	2155.9
	II	3	100.0	238.5	338.5	435.0	512.0	8.0	17.3	24.5	30.8	34.5	32.7	357.4	1039.0	2185.4	3155.3
		6	72.3	188.8	269.0	345.0	412.0	4.8	13.1	20.2	25.6	29.1	10.6	152.5	512.4	1065.2	1644.6
		10	7.3	99.3	181.8	266.8	346.3	2.0	9.1	17.4	22.8	27.7	0.7	38.1	229.6	661.8	1307.2
	III	5	62.8	206.0	267.3	300.8	346.5	4.4	14.2	20.2	25.0	29.4	8.4	224.0	559.9	870.1	1377.5
		8	34.8	73.9	175.8	285.3	343.8	2.7	5.3	10.7	18.8	25.1	2.6	13.2	131.4	618.4	1153.2
		14	103.0	248.0	351.5	473.3	528.8	5.0	15.8	23.8	30.3	35.0	32.8	431.7	1213.2	2505.3	3444.1
	IV	7	41.8	186.3	282.3	355.8	421.3	3.7	12.4	21.1	26.8	31.7	4.8	160.2	625.5	1286.6	2044.4
		11	62.8	132.5	185.3	248.5	350.8	6.0	14.4	19.8	25.2	30.7	10.3	106.7	279.3	617.8	1407.8
		15	86.0	173.0	265.0	320.5	396.3	6.4	11.3	19.3	23.2	26.9	20.9	125.2	489.3	955.3	1716.6
	V	1	22.0	124.0	241.8	306.3	386.8	2.4	9.5	18.5	24.0	28.7	1.2	53.4	417.9	916.1	1892.1
		2	21.0	97.3	211.8	296.8	371.0	2.2	8.9	19.3	27.2	31.3	0.9	28.3	337.1	949.8	1743.1
		9	72.5	141.0	265.5	333.0	383.0	3.8	9.1	18.7	23.0	29.3	8.6	62.4	513.7	1016.2	1706.8

The results of the analysis of covariance of volume increment show that there are no statistically significant differences in the values of this character. Duncan's test shows a great heterogeneity between ecological units, but the values of volume increment per ecological unit is distributed by the same order as the volume and basal area. Such heterogeneity between ecological units is the consequence primarily of the great impact of management on current volume increment; consequently in the productive site differentiation it is better to apply the average volume increment (Table 3).

The results of the analysis of covariance of mean diameters of dominant fir trees show that there are no statistically significant differences in the values of this character. Duncan's test shows that there are no statistically significant differences between I, IV, II, V and II, III, V ecological units. The results of the analysis of covariance for mean diameters of dominant spruce trees show that there are no statistically significant differences in the values of this character. Duncan's test shows that there are no statistically significant differences between I, IV, II and IV, II, III, V ecological units. As the mean diameter of dominant trees is under a great impact of management, it is not an objective parameter in the productive site differentiation (Table 4).

The results of the analysis of covariance of mean heights of dominant fir trees show that there are no statistically significant differences in the values of this character. Duncan's test shows that there are no statistically significant differences between I, IV, II and III, V ecological units. The results of the analysis of covariance of mean heights of dominant spruce trees show that there are no statistically significant differences in the values. Duncan's test shows that there are no statistically significant differences between I, IV, II; II, III and III, V ecological units. Mean heights of dominant trees are under the lowest impact of management treatment compared to all other applied parameters, which classifies them as the most objective parameters in the productive site differentiation (Table 4).

The results of the analysis of variance of the development of fir height show that in the first 80 years there are no statistically significant differences in the values of this character, while at the age of 100 years, the difference becomes significant. Duncan's test shows that there are no statistically significant differences between ecological units till the age of 40 years. From the age of 60 years, the differences become significant, and in the greatest number of cases, there are no differences between I, IV, II and II, III, V ecological units (Table 5).

The results of the analysis of variance of the development of spruce height show that there are no statistically significant differences in the values of this character, which is also shown by Duncan's test for ecological units (Table 6).

Table 3. Analysis of covariance G, V and I_v total.

Ecological unit	G						V						I_V					
	F	P	Dun.				F	P	Dun.				F	P	Dun.			
I	2.4	0.13	x				3.4	0.06	x				5.9	0.01	x			
IV			x						x							x		
II			x	x					x							x	x	
III				x						x							x	
V				x						x								x

Abbreviations: G – stand basal area per hectare; V – stand volume per hectare; I_v – stand volume increment per hectare; F – Ratio test; P – productive site differentiation; Dun. – Duncan test.

Table 4. Analysis of covariance d_{gmax} and h_{gmax} for spruce and fir.

Ecological unit	d_{gmax}										h_{gmax}									
	fir						spruce				fir						spruce			
	F	P	Dun.				F	P	Dun.				F	P	Dun.					
I	1.8	0.22	x				1.5	0.29	x				4.5	0.03	x				1.9	0.19	x			
IV			x						x	x					x						x			
II			x	x					x	x					x						x	x		
III				x						x						x						x	x	
V			x	x						x						x							x	

Abbreviations: d_{gmax} – stand quadratic mean diameter (dominant); h_{gmax} – mean height (dominant); F – Ratio test; P – productive site differentiation; Dun. – Duncan test.

Table 5. Analysis of variance of fir height development.

Ecological unit	Age																							
	20					40				60				80				100						
	F	P	Dun.				F	P	Dun.				F	P	Dun.				F	P	Dun.			
I	0.8	0.54	x				1.0	0.48	x				2.5	0.11	x				3.0	0.08	x	x			4.3	0.03	x			
IV			x						x						x						x						x			
II			x						x						x	x					x	x					x	x		
III			x						x							x					x							x		
V			x						x						x	x					x	x						x		

Abbreviations: F – Ratio test; P – productive site differentiation; Dun. – Duncan test.

Table 6. Analysis of variance of spruce height development.

Ecological unit	Age																							
	20					40				60				80				100						
	F	P	Dun.				F	P	Dun.				F	P	Dun.				F	P	Dun.			
I	1.0	0.47	x				0.7	0.64	x				0.3	0.91	x				0.2	0.95	x				0.0	1.00	x			
IV			x						x						x						x						x			
II			x						x						x						x						x			
III			x						x						x						x						x			
V			x						x						x						x						x			

Abbreviations: F – Ratio test; P – productive site differentiation; Dun. – Duncan test.

The development of diameter and volume both for spruce and for fir shows similar regularities as the development of height and it can be explained by the similar method from the aspect of the analysis of variance.

The phenomenon that the study spruce trees have similar development per diameter, height and volume in all ecological units can primarily be explained by the bioecological characteristics of this species. Although the ecological units differ, especially by the soil depth and skeletalness, spruce develops equally well, primarily because of its disk-like root system. It can be concluded that spruce is in its ecological optimum in all ecological units. It is not the case with fir and it is characterized by slow development in ecological units with shallower and more skeletal soils (I, IV and II), which is primarily the consequence of fir root system, which requires deeper soils for successful development.

Discussion and conclusion

The synthesis of the ecological and productive-development studies creates the conditions for the definition of the forest productivity types.

The study of productivity was based on five parameters (G, V, I_v, d_{gmax} and h_{gmax}) based on which it was concluded as follows:

- there are no statistically significant differences between I, IV and II ecological units except by current volume increment;
- also, there are no statistically significant differences between III and V ecological units, except by current volume increment;
- between II and III, V ecological units, for both tree species, there are no statistically significant differences per G and d_{gmax}.

In the study of tree development, three parameters (diameter growth, height growth and volume growth) were applied at the ages of 20, 40, 60, 80 and 100 years. Based on these parameters, the following was concluded:

- there are no statistically significant differences for fir at the age of 20 years. From the age of 40 years, in most cases, there are no differences between I, IV, II and II, III, V ecological units;
- there are no statistically significant differences between ecological units for spruce as per any of the parameters.

Based on the above productive-development study, it can be concluded that ecological units are differentiated in two groups: I, IV, II and III, V ecological units. The second ecological unit by some parameters groups with III and V ecological units, but by most parameters, it shows a higher homogeneity with I and IV ecological units. This refers especially to the mean heights of dominant trees, which are not significantly affected by management treatment, and which are today increasingly applied in the productive site differentiation.

Based on the above, two productive forest types have been preliminarily differentiated in the study stands:

1. Productive forest type 1 – High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on calcareous black soils, medium deep, skeletal, brown calcareous soils and medium deep acid brown soils;
2. Productive forest type 2 – High spruce and fir forest (*Abieti–Piceetum serbicum typicum*) on deep brown calcareous soils and deep acid brown soils.

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Evaluation of the resources of medicinal plants in Mt Lozenska

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Abstract. The resources of 24 species from 137 established ones in Mt Lozenska (the western part of Mt Sredna Gora) were investigated. Evaluation of the resources showed that only 10 species are economically important – 2 species permitted for gathering for personal and economic use (with annual quota) and 8 species free for gathering. A comparative analysis of the resources in the forest belts has been made. Recommendations for the population use and the protection of some areas have been laid out.

Key words: Bulgaria, medicinal plants, Mt Sredna Gora, protection, resources

Introduction

The medicinal plants as important element of the bioresources of Bulgaria are an object of the Biodiversity Support Program (1994). In this connection the distribution and the state of the populations of the medicinal plants in Mt Lozenska have been previously studied (Vitkova & Gyurova 2002).

Mt Lozenska is a part of Mt Sredna Gora (western) and is situated near to Sofia; this fact results in the increase of anthropogenic pressure on the biodiversity. The mountain is not a high one but it is suitable for tourism, camping and gathering of medicinal plants.

The aim of the present study consists in the establishment of the medicinal plant resources and the existing possibilities for their balanced use and protection.

Mt Lozenska: area – 80 km^2; length – 18 km; max width – 6 km; max altitude – 1190 m. The forest belts in Mt Lozenska were described for the first time by Ganchev (1961).

Material and methods

The present investigation was carried out in the period of April 1999 – June 2000. Terrains at altitude between 600 m and 1190 m on the northern, southern and eastern slopes of the mountain have been studied. No western slopes have been outlined, because some part of the mountain in that direction is occupied by Dzherman villa zone.

Seventy localities in the different parts of the mountain and forest belts were investigated. Detailed studies in 30 populations of 24 species designated as economically important were carried out.

The altitude, area of the populations, projected cover (%) of the species or number of the plants per m^2, production (g/m^2), operative reserves (fresh material) kg were examined (Table 1). The medicinal plants resources were established after the *Methods for determination of the medicinal plants reserves* by Shreter & al. (1986).

The projected cover of the species in the studied populations or number of the plants per m^2 were established by 50 plots with area 20 m^2. The production g/m^2 was obtained by the method of the report stages for species set up branch-populations and the method of the pattern specimens for species seed propagation only (Shreter & al. 1986). The phytomass obtained from 100 plots with area 1 m^2 or 25(50) patterns was weighed in a green state. The main results were processed by means of variational statistical methods. Mean mathematical values (M) of the production were calculated. In the most cases error (m) does not exceed 10 %, rarely reaching 15 %.

Table. 1. Resources of medicinal plants in Mt Lozenska.

Group	Species	Habitat code	Forest belt	Location	Altitude (m)	Area of locality (ha)	Projected cover (%), or number of plants/m²	Production (g/m²)	Operative reserve (fresh material) kg
1	2	3	4	5	6	7	8	9	10
II group – species forbidden for economic use but permitted for gathering for personal needs	1. *Adonis vernalis* L.#		xob	1	650	2	25	8.44±0.86 herba	33.60
	2. *Asarum europaeum* L.	9130 PAL.CLASS.: 41.13	bb	2	1050	5	15	84.22±8.13 herba 27.17±2.05 rhizoma	509.70 180.50
	3. *Polygonatum odoratum* (Mill.) Druce		xob	3	700	5	4	26.11±2.78 radix	41.10
III group – species permitted for gathering for personal and economic use (with annual quota)	1. *Primula veris* L.		xob	4	750	0.6	15	8.16±0.99 herba 27.16±4.62 rhizoma	5.60 16.13
			hbb	5	900	3	5	14.58±1.68 herba 40.96±4.77 rhizoma	16.83 47.13
	2. *Carlina acanthifolia* All.*		bb	6	900	10	0.7/m²	10.58±1.18 radix	822.00
	3. *Galium odoratum* (L.) Scop.	9130 PAL.CLASS.: 41.13	bb	7	1000	3	30	43.77±4.82 herba	307.20
	4. *Stachys officinalis* (L.) Trev.* (= *Betonica officinalis*)	6510 PAL.CLASS.: 38.252	mob	8	850	3.5	35	67.45±7.01 herba	654.27
		6510 PAL.CLASS.: 38.252	bb	9	1100	5	35	96.31±8.85 herba	1375.50
IV group – species free for gathering	1. *Achillea millefolium* group*		xob	10	900	3	25	76.20±8.50 herba	444.50
	2. *Agrimonia eupatoria* L.*		mob	11	800	1	70	243.70±16.70 herba	1472.10
	3. *Allium ursinum* L.	9130 PAL.CLASS.: 41.13	bb	2	1000	0.6	30/m²	27.20±4.32 herba	112.62
	4. *Centaurium erythraea* Rafn.		xob	12	650	0.4	4/10m²	9.33±1.30 herba	26.92
	5. *Cotinus coggygria* Scop.		mob	13	850	1	10	28.35±2.70 herba	23.00
	6. *Dryopteris filix-mas* (L.) Schott	9130 PAL.CLASS.: 41.13	bb	14	900	0.05	20	263.62±17.70 rhizoma	22.83
	7. *Euphrasia* spp. diversa		xob	15	850	1	38/m²	27.80±2.70 herba	223.80
	8. *Galium verum* L.*	6510 PAL.CLASS.: 38.252	mob	8	850	10	30	29.80±3.67 herba	673.80
		6510 PAL.CLASS.: 38.252	bb	9	1100	5	35	96.31±8.85 herba	1375.50
	9. *Geranium macrorrhizum* L.	9130 PAL.CLASS.: 41.13	bb	14	950	2	15	182.50±18.91 herba 64.00±11.07 rhizoma	434.04 125.58
			mob	16	800	1	5	78.13±12.30 herba 66.95±8.81 rhizoma	26.81 24.67
	10. *Hypericum perforatum* L.		bb	6	900	10	3	21.56±2.05 herba	52.40

Table 1. Continuation.

1	2	3	4	5	6	7	8	9	10
	11. *Juniperus communis* L.*	5130 PAL.CLASS.: 31.88	bb	6	900	5	4/100m²	3.76±0.22 galbuli	166.00
	12. *Origanum vulgare* L.		xob	17	700	1	40	33.50±2.78 herba	112.00
	13. *Polypodium vulgare* L.		xob	3	700	0.5	5	27.59±2.63 rhizoma	5.59
			hbb	18	900	5	5	67.25±4.88 rhizoma	143.73
	14. *Rosa* spp. diversa*		hbb	19	900	2.5	2/100m²	13.96±0.98 fructs	299.50
	15. *Thymus* spp. diversa*		xob	15	800	2.5	25	67.73±6.41 herba	343.18
			hbb	20	900	5	40	56.83±6.70 herba	868.60
	16. *Vaccinium myrtillus* L.*		bb	21	950	10	25	49.90±3.44 herba	1075.50
	17. *Veronica officinalis* L.		xob	22	900	50	2	51.35±5.10 herba	411.50

Habitat codes: 6510 – Lowland hay meadows; 9130 – *Asperulo–Fagetum* beech forests; 5130 – *Juniperus communis* formations on heaths or calcareous grasslands.

Forest belts: xob – xerothermic oak belt; **bb** – beech belt; **hbb** – hornbeam–beech belt; **mob** – mesophilous oak belt.

Locations: 1 – Krusteto locality; **2** – peak Polovrak; **3** – "St. Nikola" Monastery locality; **4** – above Pancharevo Lake (western); **5** – peak Mala Lalina Mogila; **6** – Vlakovete locality; **7** – Lovdzhiyska Cheshma locality; **8** – Benta locality; **9** – Kulni Dol locality; **10** – between Krusteto and Vlakovete locality; **11** – valley of Rekata River below peak Sharbanitsa; **12** – valley of Iskur River, Bubreka locality; **13** – north of Dzherman Monastery; **14** – valley of Rakita River below peak Goli Rid; **15** – summer houses zone Passarel; **16** – middle parts of the valley of Gabra River; **17** – Kaleto locality; **18** – valley of Rekata River; **19** – Dulgopolyanski Rid locality; **20** – Durvodeletsa locality; **21** – peak St. Petka; **22** – Dzhelepitsa locality.

* – species for economic use; # – CITES.

The taxonomical affiliation of the species is given according to Kozhuharov (1992). The habitat types were described according to the *Handbook for identification of habitats of European importance* (Kavrakova & al. 2005).

The investigated plants have been found to be associated with the four groups by the Law on Biological Diversity (2002) and Order No RD 173/2005 of the Ministry of Environment and Waters in Bulgaria: I group – species forbidden for gathering; II group – species forbidden for economic use but permitted for personal needs; III group – species permitted for gathering for personal and economic use (with annual quota); IV group – species free for gathering.

Results and discussion

The economically valuable populations were found mainly on the northern slopes of the mountain at an altitude between 670 m and 1110 m in the xerothermic oak, beech, hornbeam–beech and mesophilous oak belts. We have established a great diversity of medicinal plants which occupied various ecological niches in the four belts. A total of 112 species, or 82 % of all medicinal plants found in Mt Lozenska, has been identified on the northern slopes of the mountain.

The complex of ecological conditions together with the general plant cover determine to a great extent the differences of the amount phytomass among the different populations of some of the examined species (*Primula veris*, *Stachys officinalis*, *Geranium macrorrhizum*, *Polypodium vulgare*).

In the xerothermic oak belt on the northern slopes *Adonis vernalis*, a group II species, formed an extensive population (2 ha) in Krusteto locality. An extensive population (3 ha) of *Primula veris* – III group species – has been found on the peak Mala Lalina Mogila (1177 m alt.), in a phytocoenosis composed mainly by *Corylus avellana*, *Crataegus monogyna*, *Fragaria vesca*, *Glechoma hederacea*, *Helleborus odorus*, *Viola odorata*. One of the big populations of *Origanum vulgare* (1 ha), identified in Mt Lozenska, was in the xerothermic belt on the northern slopes, under peak Kaleto.

Vlakovete locality in the beech belt was a vast open space at an altitude of about 900 m, covered by shrubs and herbaceous plants. Here the species belonging to III group – *Carlina acanthifolia* formed populations of economic importance. A population of *Geranium macrorrhizum* was spread on the northeastern slopes on the peak Goli Rid (963 m alt.) on an area of 2 ha.

On the northern slopes in the mesophilous oak belt north of Dzherman Monastery, *Cotinus coggygria* has formed a small population of high abundance and limited operative reserve in the forest of *Carpinus betulus* and *Quercus dalechampii*.

Population (5 ha) of *Asarum europaeum* was found under peak Polovrak in the beech belt and it was with economically important reserves. Less extensive populations of the species occurred in Lanchevica locality (0.1 ha) and also on the northern slope of peak Bachoul (0.2 ha). A population of *Galium odoratum* on an area of 3 ha was spread along the road from Lovdzhiyska Cheshma locality to Lozenski Monastery in the forest of *Fagus sylvatica* and *Carpinus betulus*.

There was a large population of *Vaccinium myrtillus* in the region of Lozenski Monastery. It was spread intermittently as far as peak Popov Dyal (Dzhelepitsa locality) and occurred both in pure and mixed forests of *Fagus sylvatica*. Our observations have shown that this species was not very fertile in the Mt Lozenska, because fructification is influenced by forest canopy.

The southern slopes of the mountain are comparatively dry and some parts of them are occupied by agricultural lands. In the xerothermic oak belt *Polygonatum odoratum* and *Polypodium vulgare* have formed more extensive populations with good resources in the area of St. Nikola Monastery.

Mention deserves the fact that *Stachys officinalis* occurred in 88% of the investigated localities of the mesophilous oak belt on the southern slopes but with different abundance. In Kulni Dol locality the species took part in the composition of herbaceous phytocoenosis of 5 ha.

On the eastern slopes population of *Veronica officinalis* (50 ha) was established in Dzhelepitsa locality in the forest of *Quercus cerris*.

Evaluation of the resources of 24 species from 137 species established in Mt Lozenska showed that only 10 are economically important with operative reserves over 300 kg – 2 of the group III and 8 of the group IV (Table 1). The maximum operative reserves were determined as follows: herbal reserves being between 343 kg of Herba Thymi serpylli and 1472 kg of Herba Agrimoniae. The species *Stachys officinalis*, *Galium verum*, *Achillea millefolium* group, *Asarum europaeum*, *Geranium macrorrhizum* and *Vaccinium myrtillus* take interstitial place. Limited operative reserves were established for *Adonis vernalis*, *Primula veris*, *Centaurium etythraea* and *Cotinus coggygria*. Rhizome reserves were maximum 125 kg of Rhizoma Geranii macrorrhizi and for the radix reserves up to 822 kg of Radix Carlinae. The quantity of the fruit reserves was maximum 299 kg of Fructus Rosae, and Galbuli Juniperi up to 166 kg.

Only one species of the group I – *Anemone sylvestris* – has been found with some plants in the region of peak Polovrak.

The reserves of the II group species are considerably limited. One species of this group was included in CITES – *Adonis vernalis*.

An adverse anthropogenic impact on the medicinal plants was observed. Ten populations of 8 species were found in the 3 habitats of European significance /6510 – Lowland hay meadows; 9130 – *Asperulo–Fagetum* beech forests; 5130 – *Juniperus communis* formations on heaths or calcareous grasslands/ (Table 1).

Conclusion

Characteristics of the resources of the medicinal plants in Mt Lozenska showed that they are limited. For the populations which are parts of the habitats of European significance and the regions with a rich plant diversity (such as peaks Polovrak, Lalina Mogila, Goli Rid, Urvich) the gathering of medicinal plants have to be carried out warily. The limited medicinal plant resources impose the application of the rotational principle during their exploitation.

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Dependence of Douglas-fir height on geographic characteristics of provenances in East Serbia

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Abstract. Douglas-fir is the most common and dominant tree in North America occurring in nearly all forest types, competes well on most parent materials, aspects, and slopes. The latitudinal range of Douglas-fir is the greatest of any commercial conifer of western North America. Its native range is extending from 19° to 55° N latitude. In order to examine its adaptability, growth and wood quality, the Institute of Forestry in Belgrade established the experiment at two localities in Serbia. So as to assess Douglas-fir variability, this paper analyses the dependence of height on the provenance latitude, longitude and altitude.

Key words: Douglas-fir, geographic characteristics, height, provenances, Serbia

Introduction

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] was first discovered by the botanist Archibald Menzies (who is honored in the botanical name) in 1791 near Vancouver, and it was introduced in England by Scot, David Douglas in 1827, who is honored by the common name. One of the most important timber trees in North Coast Douglas-fir is adapted to a moist, mild climate; it grows larger and faster than Rocky Mountain Douglas-fir or Continental. Douglas-fir is the second-tallest tree in the world, as well as the largest member of the *Pinaceae* of North America than other species in the genus. It is also one of the world's best timber producers and yields more timber than any other tree in North America.

Douglas-fir is the most popular introduced species in Europe. Trees of 60–75 m or more in height and 1.5–2 m in diameter are common in old growth stands. It commonly lives more than 500 years and occasionally more than 1000 years.

Since its introduction in Europe followed by numerous tests (about 1850), Douglas-fir (*P. menziesii*) has always been the subject of continuous research: Hanson & Robinson (1963), Schober (1963), Haddock & al. (1967), Hermann & Lavander (1968), Hermann & Ching (1975), Rehfeldt (1983), Monserud (1984), Hermann (1985, 1987), Brodie & Walstad (1987), Kleinschmit & Bastien (1992), Lavadinović (1996), Lavadinović & Koprivica (1996). As an exceptionally successful species in its country of origin (USA) and with high economic value as an introduced species, it has found its place in a mosaic of sites in Europe. In order to examine its adaptability, growth and wood quality, the Institute of Forestry in Belgrade established the experiment at two localities in Serbia. So as to assess Douglas-fir variability, this paper analyses the dependence of height on the provenance latitude, longitude and altitude.

Material and methods

The material for the experiment was the collection of seeds from 27 Douglas-fir provenances collected by the Centre for forest seed in Georgia (USA) from the entire natural area of the species (Table 1).

At the age of fifteen, in the provenance test Tanda in East Serbia, plant heights were measured in all the fifteen block repetitions. Sample plot Tanda is in the management unit Stol of the Forest Administra-

tion Bor (city in the east part of Serbia), on the site of Hungarian oak (*Quercetum frainetto cerris* Rud.). Geographic coordinates of the sample plot are 44°14' N and 22°09' E, at the elevation of 370 m, southeast exposure. Parent rock is granite, and the soil is acid brown.

In order to test the effect of latitude, longitude and altitude on the variability of plant height, the methods of simple and multiple regression and correlation were applied.

Results

Table 1 presents average heights of tested Douglas-fir provenances, with geographic characteristics.

Average plant height of all the provenances is 4.00 m, standard deviation 0.94 m, and the coefficient of variation 23.5 %. The provenance 19 (Washington 204-09) has the lowest height (2.11 m), and the trees of the provenance 31 (Washington 205-02) are the highest (5.47 m). There are significant differences between tree heights.

Table 1. Geographic characteristics and plant height of tested Douglas-fir provenances.

Provenance		Our sign	Latitude (°)	Longitude (°)	Altitude (m)	Plant height (m)
Oregon	205-15	1	43.7	123.0	750	3.90
Oregon	205-14	2	43.8	122.5	1200	4.01
Oregon	202-27	3	45.0	122.4	450	4.81
Oregon	205-38	4	45.0	121.0	600	4.77
Oregon	204-34	6	45.0	121.0	1050	4.95
Oregon	205-16	7	44.0	123.0	150	4.87
Washington	205-31	8	48.8	121.5	450	4.93
Washington	204-07	9	49.0	119.0	1200	2.53
Oregon	205-13	10	43.8	122.5	1050	4.20
Oregon	205-18	11	44.2	122.2	600	4.63
Oregon	202-22	12	42.5	122.5	1200	3.91
Oregon	202-21	14	42.4	123.7	300	3.99
Washington	202-17	15	47.6	121.7	600	4.31
Oregon	201-10	16	44.5	119.0	1350	2.98
Washington	204-06	17	49.0	120.0	750	2.82
Oregon	202-19	18	45.3	123.8	300	4.84
Washington	204-09	19	49.0	119.3	900	2.11
Oregon	205-11	20	45.0	123.0	150	4.75
Oregon	205-45	21	44.0	122.0	900	4.48
Oregon	202-31	24	44.3	118.8	1500	2.39
Oregon	205-29	26	42.6	122.8	900	3.98
Oregon	205-08	27	42.7	122.5	1050	3.46
Oregon	205-22	28	45.0	121.0	750	4.49
Oregon	204-18	29	44.5	119.0	1500	2.24
Oregon	204-04	30	45.0	121.5	900	3.67
Washington	205-02	31	47.7	123.0	300	5.47
Oregon	205-17	32	44.0	124.0	450	4.59

As all the plants in the experiment grow in equal site conditions, plant height was correlated to the geographic characteristics of the provenances. Table 2 presents the simple coefficients of linear correlation between the variables: Y – plant height, X_1 – latitude, X_2 – longitude, X_3 – altitude.

Partial coefficients of correlation of the second order are:

$r_{yx_1.x_2x_3} = 0.0085$; $r_{yx_2.x_1x_3} = 0.3670$; $r_{yx_3.x_1x_2} = -0.3298$.

The coefficients of correlation show that there is a significant dependence of the provenance plant height on longitude and altitude, whereas the dependence on latitude is insignificant.

Table 2. Correlation matrix.

Variable	Y	X_1	X_2	X_3
Y	1.000	−0.162	0.741***	−0.714***
X_1		1.000	−0.456**	−0.133
X_2			1.000	−0.674***
X_3				1.000

Legend: ** – significant at the level p<0.01; *** – significant at the level p<0.001; Y – plant height; X_1 – latitude; X_2 – longitude; X_3 – altitude.

Dependence of Douglas-fir height (Y) on latitude (X_1): The correlation matrix (Table 2) shows that the coefficient of linear correlation between height and latitude is not statistically significant. In order to complete the above data, the main parameters of parabolic regression and correlation have been given:

$$Y = -171.80 + 7.743125\ X_1 - 0.085085\ X_1^2 \quad (1)$$

SE = 0.90 m, $R^2 = 0.0827$, R = 0.2876

$F > F_{0.05}$

The results of fitting have been presented in Fig. 1.

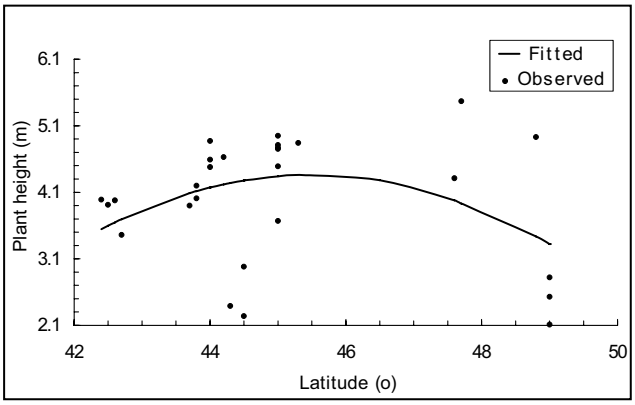

Fig. 1. Dependence of Douglas-fir height on latitude.

Dependence of Douglas-fir height (Y) on longitude (X_2): This dependence is clearly expressed. The parameters of parabolic regression and correlation are as follows:

$$Y = -2135.14 + 34.8538\,X_2 - 0.141938\,X_2^2 \quad (2)$$

SE = 0.56 m, $R^2 = 0.6416$, R = 0.8000

$F < F_{0.001}$

The dependence has been shown in Fig. 2.

With the increase of the provenance longitudes, plant height also increases (parabolically). The provenances ranging between the longitudes of 121° and 124° have significantly higher plants than the provenances with the longitude below 120°. The dependence has been explained 64 %.

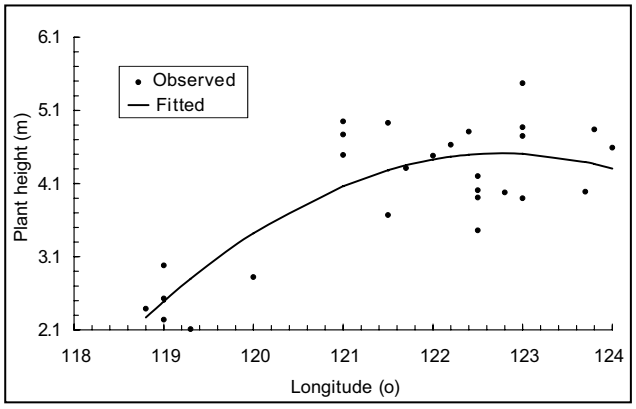

Fig. 2. Dependence of Douglas-fir height on longitude.

Dependence of Douglas-fir height (Y) on altitude (X_3): This dependence is also clearly expressed. The parameters of the parabolic regression and correlation are as follows:

$$Y = 4.96 - 0.000469\,X_3 - 0.00000075\,X_3^2 \quad (3)$$

SE = 0.67 m, $R^2 = 0.4855$, R = 0.6968

$F < F_{0.001}$

The dependence has been shown in Fig. 3.

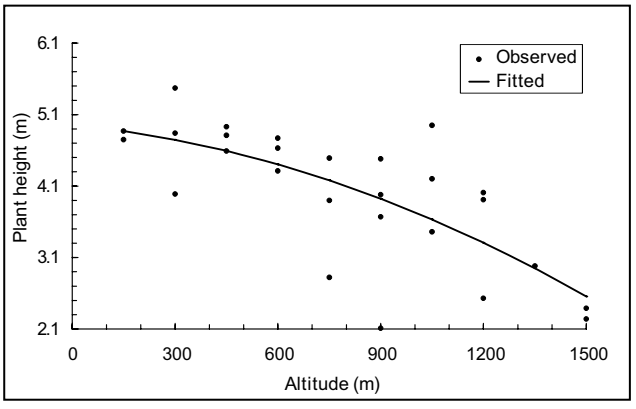

Fig. 3. Dependence of Douglas-fir height on altitude.

With higher altitudes of provenances, plant height decreases (parabolically). The highest plants belong to the provenances with lower altitudes, but the difference is not great below 1200 m. Here, of course, as well as in other diagrams, the deviations from the general law can be observed, because of the simple regression and correlation. We have already seen (Table 2) the significant collinearity of independent variables (latitude and longitude, i.e. longitude and altitude). Consequently, several models of multiple regression and correlation were also applied.

Dependence of Douglas-fir height (Y) on latitude (X_1), longitude (X_2) and altitude (X_3): Additive and substitution models have been applied for this dependence. It is interesting that the results of additive models are better.

The best results are produced by the model where the effect of independent variables is expressed by the second order parabola:

$$Y = -2401.30 + 6.47336\,X_1 - 0.070007\,X_1^2 + 36.8398\,X_2 - 0.150314\,X_2^2 \quad (4)$$

SE = 0.42 m, $R^2 = 0.7985$, R = 0.8936

$F < F_{0.001}$

As all the coefficients of regression in the model (4) are not statistically significant, the method of stepwise regression has also been applied. The result is the model without latitude (X_1):

$$Y = -2777.99 + 45.60846\,X_2 - 0.186812\,X_2^2 - 0.002741\,X_3 - 0.00000118\,X_3^2 \quad (5)$$

SE = 0.48 m, $R^2 = 0.7317$, R = 0.8554

$F < F_{0.001}$

Additive model with the multiplication of independent variables is also interesting:

$$Y = -5358 + 0.001964\,X_1X_2 - 0.000355\,X_1X_3 + 0.000121\,X_2X_3 \quad (6)$$

SE = 0.53 m, $R^2 = 0.6824$, R = 0.8261

$F < F_{0.001}$

Regression models (4, 5 and 6) show that height variation of plants of different provenances can be explained 70 % to 80 % by the collective effect of latitude, longitude and altitude.

Conclusion

Height measuring of Douglas-firs (at the age of fifteen) of different provenances, in the experiment in East Serbia, showed significant differences.

Based on the analysis of plant height dependence on the provenance latitude, longitude and altitude (geographical characteristics), the following was concluded:

– the effect of latitude on plant height is insignificant;

– plant height increases (parabolically) with the increase of longitude;

– plant height decreases (parabolically) with higher altitudes.

The model of multiple regression and correlation explains about 80 % of the total variability of the tested plant heights, whereas the provenance longitude and altitude have the decisive effect on plant height.

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Collection, identification and ethnopharmacology of medicinal flora of Koshk and Dehsard area in Kerman province in Iran

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Abstract. Cold season habitats have special ecological and biological characteristics that have established and improved plant species with medicinal characteristics. Some of the most important in Kerman are Southwestern cold season rangelands in the protected area Koshk–Dehsard. During 2003–2005 species were collected from different localities of this area; 100 species of them were more important from the point of view of traditional uses, 100 species belonging to 28 families have medicinal value. About 66 % of the medicinal species belong to 6 families – *Fabaceae* (21 species), *Asteraceae* (13), *Brassicaceae* (9), *Apiaceae* (8), *Lamiaceae* (7), *Liliaceae* (6). The largest medicinal genus is *Astragalus* with 12 species. The abundant medicinal species is *Artemisia sieberi*. Persian and local names along with localities of distribution, altitude, herbarium number, therapeutic effect, method of consumption and utilizable organs for any species were collected. Most of the plants were therophytes and with herbal form.

Key words: Iran, medicinal plants, plant species

Introduction

The beginning of systematic science is not specified correctly. Chinese, Egyptian and Assyrian old tribes have classified crops and described them according to their needs. Considering different properties of the plants and their use, especially drug plants, the need for their description and classification in turn has caused the elaboration of plants' classification. About 3000 B.C., the Chinese emperors used drug plants and talked about their properties.

Generally, what is today known as drug plants' collecting and introducing science is based on contemporary plant systematics. It can be clearly declared that systematic science is an active and endless science and it is the basis for work on the drug plants. The necessity of selecting and performing the plan was the proposition of Kerman County Natural Resources Administration for collecting, identifying and introducing drug plants.

The history of region phytology

The phytologist/herbalist has came to Iran with the aim of collecting plants since 19th century, but collecting plants from Kerman was not considered as much until when Bornmüller made some trips to Iran including Kerman. He collected a lot of plants from mountain high regions such as Joopar Mt and Lalezar Mt and some other summits during his trip to Kerman. Bornmüller was one of the few phytologists who were not afraid of going high in the mountains to collect plants.

After Bornmüller other herbalists such as Parsa, Rechinger, Esfandiari and others have done collections from regions near to the district under study, located some kilometres from it. In the second half of the 20th century Zohary proceeded to investigate Iran's vegetation units and passed through Sirjan (which has the nearest situation to the under study region), where he conducted some studies.

Edmonson, Asadi and Miller also collected plants from Khabr Mt which is located some kilometres from the under study region. Due to high mountains in vicinity such as Lalezar and Khabr, which have richer plant cover, phytologists have not granted collecting plants from this region the first priority and by now its flora was unknown.

Region introduction

The studied region with extent of 40 000 hectares is located between 28°38'–28°50' N and 56°32'–56°45' E. The North district of this region is Koshk, which has important heights such as Koshk peak (3273 m), Goldar peak (2500 m), Roogodar peak (2500 m). In the southern part is Dehsard with important heights such as Gelian peak (2763 m) and Dehsard peak (2321 m).

This area is a young tectonic region under the Dehsard fault, which compared with other quartz sedimentary domains, is of lower extent and width; due to the activity of this fault zone the geomorphology of this region is under progress and assumed in young stage.

Calcium bicarbonate exudations occur in Dehsard fault zone. Rainfall in this district occurs in the late winter which is the main part of precipitations of rain occurring from December till April. Of course, we have been witnesses of fluctuations and irregularities in this respect.

The annual evaporation based on Dehsard station is 2889.4 mm, the greatest amount of which takes place in July. The region maximum temperature is mainly during June, July and August and the minimum temperature occurs during January and February. In August the wind speed is reaching to maximum, and the atmospheric pressure – to minimum. Maximum sun hours take place in May till August, and minimum in February. The frost days are observed mainly in December, January, February, and especially in March.

The presence of elevated mountain ranges and low and smooth plains is of significant importance for the morphology of the area. The mountains' altitudes reduce toward South and the general inclination of the region is mild, from North to South. Generally the region under study has very steep slopes in mountain foots because of the location and limitation of the surrounding mountains which gradually pass from a slightly mild slope in middle slope.

Material and methods

With regard to this study, all the plant species were collected from all points of the region. Collection was performed in all seasons, especially in spring, several times a week. Collected samples were complete as far as possible, namely they had root, tuber (in the case of tuberous plants) or rhizome, stem, leaves, flower and fruit; in the case of tree and woody species, they had a part of branch or stem. Then the samples were pressed and after drying they were attached on herbarium standard sheets.

Immediately after collecting each sample, the following parameters were considered: collecting place, local name, date, altitude, importance, ecological and plantation characteristics, growth spectrum, time of flowering and ripening of fruits.

The identification and the nomenclature of the species were mainly based on *Flora Orientalis* (Boissier 1867–1884), *Flora Iranica* (Rechinger 1963–2001), *Flora of Iran* (Vols 3–43), *Flora of West Pakistan* (Nasir & Ali 1970–1985), as well as on Ghahreman (1978–2005), Maassoumi (1986–1995), and Mozafarian (1994). One sample from each species was delivered to Islamic Azad University herbarium.

After identifying all the species, a study on the drug species was conducted. The necessary information about these plants (such as: their scientific name, local name, dispersion, treatment effect, use method and the organs under use) was based on enhanced information from villagers and tribes of the region, personal information, study of drug plants, related books (Moatar & Shareat 1990; Rezaie & al. 1990–2004) (Table 2).

On the basis of Raunkiaer's point of view the biological spectra of the species were divided into 6 groups – Phanerophytes, Chamaephytes, Cryptophytes, Hemicryptophytes, Therophytes and Geophytes (Table 1). The basis of this method depends on how the plant copes with environmental conditions in unfavourable growth season; it is also based on the situation of those buds which produce new stalks and branches after unfavourable atmospheric conditions.

Table 1. Plant biological spectra of drug plants.

Therophytes	33 species
Chamaephytes	25 species
Hemicryptophytes	19 species
Geophytes	12 species
Phanerophytes	9 species

Table 2. List of detected drug species in the region.

Scientific name	Biological spectrum	Flowering	Fruit maturity	Locality altitude	Stand property *	Organs under use **	Voucher no.
1	2	3	4	5	6	7	8
GYMNOSPERMAE							
Ephedraceae							
Ephedra strobilacea	Phanerophyte	May	Late spring	2400	*4–8*	*9*	102
ANGIOSPERMAE							
DICOTYLEDONES							
Anacardiaceae							
Pistacia atlantica	Phanerophyte	June	September–October	2100	*1*	*5–6*	104
Apiaceae							
Bunium cylindricum	Geophyte	May	May–June	2300	*2–3*	*5–6–7*	105
Bunium persicum	Geophyte	May	June–July	2400	*2–3*	*5–6–7*	106
Carum carvi	Hemicryptophyte	May	June	2300	*2–3*	*9*	107
Echinophora platyloba	Hemicryptophyte	August	September–October	2100	*4–5*	*9*	108
Pimpinella khorasanica	Hemicryptophyte	June	July–August	2200	*4–8*	*9*	111
Scandix stellata	Therophyte	May	May–June	2200	*2–3*	*9*	112
Torilis arvensis	Chamaephyte	April	May	2000	*5*	*9*	113
Torilis nodosa	Therophyte	April	May	2050	*5*	*9*	114
Asteraceae							
Achillea millefolium	Hemicryptophyte	June	August	2300	*4–8*	*2–3–4*	116
Acroptilon repens	Hemicryptophyte	June	July	2350	*6*	*8*	118
Arctium lappa	Hemicryptophyte	June	August	2350	*6*	*1–3*	121
Artemisia aucheri	Chamaephyte	Autumn	Late autumn	2450	*6*	*2–3–4*	122
Artemisia sieberi	Chamaephyte	Autumn	Late autumn	1900	*1*	*2–3–4*	123
Calendula persica	Therophyte	May	June	1900	*1*	*3–4*	124
Centaurea iberica	Therophyte	June	June–July	1900	*1*	*8*	128
Centaurea solstitialis	Therophyte	May–June	June–July	1950	*1*	*4*	129
Cichorium intybus	Hemicryptophyte	June	August	2100	*3*	*1–3*	131
Cousinia stocksii	Geophyte	July	August	2200	*4–8*	*9*	132
Cirsium congestum	Hemicryptophyte	June	July	2050	*5*	*1*	133
Echinops aucheri	Hemicryptophyte	June	July–August	2000	*7*	*8–5*	136
Scariola orientalis	Chamaephyte	June	September	2100	*2–3*	*8*	145
Boraginaceae							
Lappula spinocarpos	Therophyte	March–April	May	1950	*5*	*8*	153
Nonnea caspica	Therophyte	May	May–June	2250	*2*	*8*	154
Onosma microcarpum	Hemicryptophyte	June	June–July	2300	*6*	*8*	155
Onosma stenosiphon	Hemicryptophyte	May	June	2150	*10*	*8*	156
Brassicaceae							
Alyssum alyssoides	Therophyte	April–May	May	2350	*8*	*9*	160
Alyssum heterotrichum	Therophyte	April	May	2250	*2–3*	*9*	161
Alyssum inflatum	Chamaephyte	May	June	2200	*8*	*9*	162
Cardaria draba	Hemicryptophyte	May	June	2250	*2–3*	*9*	166
Clypeola aspera	Therophyte	April–May	June	2250	*2–3*	*9*	168
Descuriana sophia	Therophyte	April–May	May–June	2100	*2–3*	*9*	170
Eruca sativa	Therophyte	May	May–June	1900	*1*	*9*	171
Malcolmia africana	Therophyte	June	June	2250	*4–8*	*9*	174
Sisymbrium officinalis	Therophyte	May–June	July	2000	*5*	*9*	175
Caryophyllaceae							
Silene conoidea	Therophyte	May	June	2000	*5*	*9*	181
Vaccaria pyramidata	Therophyte	May	June–August	1950	*5*	*9*	182
Chenopodiaceae							
Chenopodium album	Therophyte	June	June–July	1900	*1*	*3–6*	184

Table 2. Continuation.

1	2	3	4	5	6	7	8
Chenopodium novopokrovskyanum	Therophyte	July–August	September–October	2050	*1*	*3–6*	185
Noaea mucronata	Chamaephyte	August	September	2400	*4–8*	*8*	186
Convolvulaceae							
Convolvulus arvensis	Chamaephyte	May–June	June–July	2000	*7*	*1–3–4–6*	187
Dipsacaceae							
Scabiosa flavida	Therophyte	June–August	July–August	1900	*7*	*9*	192
Scabiosa kermanensis	Chamaephyte	June	July	1900	*7*	*9*	193
Euphorbiaceae							
Euphorbia buhsei	Chamaephyte	Spring	June	2250	*2–3*	*6*	194
Euphorbia gedrosiaca	Geophyte	May–June	July	3100	*6*	*6*	195
Fabaceae							
Astragalus aureus	Chamaephyte	May	June	2300	*2–3*	*9*	196
Astragalus campylanthus	Chamaephyte	May	June	2250	*2–3*	*9*	197
Astragalus cephalantus	Chamaephyte	May	June–July	2000	*7*	*9*	198
Astragalus dactylocarpus	Chamaephyte	May	June	2200	*2–3*	*9*	199
Astragalus eremophilus	Chamaephyte	May	June	2200	*2–3*	*9*	200
Astragalus fasciculifolius	Phanerophyte	May	June	2300	*6*	*9*	201
Astragalus glaucacanthos	Chamaephyte	June	July	2200	*2–3*	*9*	202
Astragalus ledinghamii	Chamaephyte	May	June	2000	*7*	*9*	203
Astragalus mucronifolius	Hemicryptophyte	April	May	2250	*4–8*	*9*	204
Astragalus porphyrophysa	Chamaephyte	June	June–July	2300	*6*	*9*	205
Astragalus schistocalyx	Chamaephyte	June	July	3100	*6*	*9*	206
Astragalus squarrosus	Chamaephyte	June	June–July	2100	*6*	*9*	207
Cicer arietinum	Therophyte	June	July	2150	*4–8*	*5–6*	208
Glycyrrhiza glabra	Chamaephyte	June	June–July	2000	*5*	*1*	210
Medicago sp.	Hemicryptophyte	June	June–July	1900	*2*	*3*	211
Medicago radiata	Therophyte	May–June	June–July	2000	*5*	*3*	212
Medicago sativa	Hemicryptophyte	June	July	2000	*5*	*3*	213
Sophora alopecuroides	Hemicryptophyte	June	July	2150	*3–4–8*	*9*	214
Vicia arintabensis	Therophyte	May	June	2000	*5*	*5–6*	215
Vicia michauxii	Therophyte	June	June–July	2000	*5*	*5–6*	216
Vicia peregrina	Therophyte	May–June	June–July	2000	*5*	*5–6*	217
Fumariaceae							
Fumaria asepala	Therophyte	May–June	June–July	2150	*3*	*9*	218
Geraniaceae							
Erodium cicutarium	Therophyte	April–May	June	2250	*2–3*	*9*	219
Juglandaceae							
Juglans regia	Phanerophyte	May	September–October	2400	*6*	*3–5*	222
Lamiaceae							
Marrubium astracanicum	Chamaephyte	June	July	1900	*7*	*2–3–4*	224
Marrubium crassidens	Chamaephyte	June	July	2000	*7*	*2–3–4*	225
Mentha longifolia	Chamaephyte	June	July	2000	*5*	*2–3–4*	226
Nepeta ispahanica	Therophyte	May	June	2000	*9*	*2–3–4*	227
Stachys inflata	Chamaephyte	May	July	2200	*2–3*	*8*	229
Teucrium polium	Chamaephyte	June	July	2200	*2–3*	*2–4*	230
Ziziphora tenuior	Therophyte	May	June	2000	*7*	*8*	231
Malvaceae							
Malva neglecta	Therophyte	May–June	July	1900	*7*	*8*	232
Moraceae							
Morus alba	Phanerophyte	April–May	Spring	2000	*5*	*1–3–4*	233
Papaveraceae							

Table 2. Continuation.

1	2	3	4	5	6	7	8
Papaver glaucum	Therophyte	May	June	2200	*2–3*	*5–6*	238
Plantaginaceae							
Plantago lanceolata	Hemicryptophyte	May–June	June–July	2000	*9*	*9*	241
Resedaceae							
Reseda alba	Hemicryptophyte	April	May	2170	*2–3*	*9*	248
Rosaceae							
Amygdalus communis	Phanerophyte	May	August–September	2030	*5*	*5–6*	250
Amygdalus lycioides	Phanerophyte	May	August	2150	*2–3*	*9*	251
Crataegus sp.	Phanerophyte	May	June	2000	*5*	*5*	253
Sanguisorba minor	Therophyte	June	July	2150	*2–3*	*9*	254
Salicaceae							
Salix alba	Phanerophyte	May	May	1900	*9*	*3–4*	258
Solanaceae							
Hyoscyamus senecionis	Geophyte	Spring	Summer	2250	*2–3*	*3–6*	262
Zygophyllaceae							
Peganum harmala	Hemicryptophyte	June	July	1900	*1*	*5–6*	266
MONOCOTYLEDONES							
Ixioliriaceae							
Ixiolirion tataricum	Geophyte	April	June	2400	*2–3*	*9*	267
Liliaceae							
Allium sp.	Geophyte	May	June	2200	*4–8*	*3–7*	272
Allium atroviolaceum	Geophyte	April	May	2100	*4–5*	*3–7*	273
Allium scabriscarpum	Geophyte	April	May	2200	*10*	*3–7*	274
Allium schoenoprasum	Geophyte	April–May	May	2400	*4–8*	*3–7*	275
Allium stamineum	Geophyte	May–June	July	2200	*4–8*	*3–7*	276
Colchicum sp.	Geophyte	Autumn	Autumn	2200	*4–8–10*	*4–6–7*	277
Poaceae							
Phalaris minor	Therophyte	May	June	2300	*10*	*9*	289
Polypogon monspeliensis	Therophyte	May	June	2250	*2–3*	*9*	293

* **1, plain zones; 2, sandy and clay soils; 3, gravel hills; 4, foot of a mountain; 5, farms' margins; 6, mountain lands; 7, lands; 8, semi mountains; 9, humid lands; 10, cliffy zones.**

** **1, root; 2, stem; 3, leaf; 4, flower; 5, fruit; 6, seed; 7, bulb; 8, all; 9, plant organs have different applications in various regions.**

Results and discussion

The studied area is of a relatively good richness in the case of drug plants, so that among 200 collected plants, about 100 species (50 %) are of drug value. Of them, 88 are dicotyledons, 9 – monocotyledons and 1 is gymnosperm.

It is note worthy that among 58 drug plant families present in the county, 28 families are identified and collected in the region under study (Table 2).

About 66 % of medicinal species belong to 6 families, namely *Fabaceae* (21 species), *Asteraceae* (13), *Brassicaceae* (9), *Apiaceae* (8), *Lamiaceae* (7), *Liliaceae* (6) (see Table 2). The largest medicinal genus is Astragalus with 12 species. The most abundant medicinal species is *Artemisia sieberi* Besser.

It is note worthy that including drug plants and non-drug plants therophytes have devoted themselves the maximum percentage, *ca*. 34 % (Table 1).

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A survey of vegetation types in south of Iran (southwest of Kerman)

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Abstract. On the basis of prevalence of species and external appearance of relatively homogeneous, congenial botanical units and on the basis of the prevalence grade of 1 or 2 species and in some cases 3 species, 12 vegetation types were recognized: 1 – *Artemisia*–Grass; 2 – *Pistacia–Daphne–Sageretia*; 3 – *Pistacia–Amygdalus–Artemisia* (the dominant species occupying 2/3 of the area); 4 – *Pistacia–Amygdalus–Ephedra*; 5 – *Pistacia–Peganum–Artemisia*; 6 – *Peganum–Artemisia*; 7 – *Artemisia–Euphorbia–Astragalus*; 8 – *Pistacia–Amygdalus–Bunium*; 9 – *Artemisia–Astragalus*; 10 – *Artemisia–Acanthophyllum–Acantholimon*; 11 – *Amygdalus–Convolvulus–Ebenus*; 12 – *Allium–Vicia*. The extent, location and associated important species for each type were noted separately.

Key words: botanical unit, Iran, Kerman, vegetation types

Introduction

The correct use of resources and plant reserves of a given zone necessitates complete knowledge of plant species in this zone, having information about their status in forming vegetation cover. The selection of the related zone is based on the richness of the vegetation, as well as on the lack of studies about this zone. In this plan, the vegetation types and species have been determined by conducting field studies and the indexes of existing plants in the region and different vegetation types have been prepared.

It must be noted that identifying the vegetation types and plant populations is a useful task which prepares the background for conducting basic researches/studies and suitable methods of using them. This plan was performed based on the proposition of the Natural Resources Administration of Kerman County to determine the pattern of plants' dispersion in different regions of the zone.

Study area

The area under study is located southwest of Kerman and has southern and northern extent, so that the more we go to the south, the warmer weather is. In the southern part the main vegetation cover is under the influence of warm climate, and in the more northern parts – under the influence of colder climate conditions.

Generally, this zone is a mountain region, having at least 2000 m altitude and maximum 3300 m above sea level.

Several seasonal rivers are running in this zone. The mountains are mainly from limestone and orbitoline belonging to the second ages.

The main soil pH is 7–8.5, and the soils have a lot of calcium carbonate in them. The presence of ultrabasic configurations such as serpentines results in reduction of vegetation cover percentage.

The weather in the studied area is mainly considered as dry and semidry according to the Demortin method. Ombrothermic climatic curve has been used to determine the climate properties of the zone under study, which actually shows the degree of dryness and humidity and also the duration of dry and humid period during the year. It indicates that the period November–April is humid, and the rest period is dry.

The winds are considered as an important factor on the water conditions and on the climate, because they have some effect on temperature, evaporation and rainfall.

To determine the information regarding wind and atmospheric pressure, Baft station has been used. The main direction of the winds has been from West and South-West. The atmospheric precipitations of this zone and the more southern parts are different having 3 atmospheric effects, which in turn are divided as follows: Western atmospheric front which is from the high latitudes of Mediterranean Sea and Pacific Ocean and causes the most rainfalls in the months January, February, March and April; North and West-North climatic front that causes cool in the Northern zones, especially some kilometres from the northern part of the study area; Southern climatic front in the southern parts, resulting from the Asian seasonal winds that cause higher temperatures and flood-like summer rainfalls during some years.

Material and methods

In this survey, during the years 2003–2005, a base map and also topographic maps (scale 1:50 000) were used for determination and separation of the vegetation types from each other. For this purpose, on the basis of prevalence of species and external appearance of relatively homogeneous, congenial botanical units and on the basis of the prevalence grade of one or two species and in some cases three species, each of the units was named after primary diagnosis and by many measurements in different directions.

The extent or the boundaries of the determined types with the use of natural features, roads and amount lines on the topographical plan, has been specified based on visual observations.

The identification and nomenclature of the species were mainly based on *Flora Orientalis* (Boissier 1867–1884), *Flora Iranica* (Rechinger 1963–2001), *Flora of Iran* (Vols 3–43), *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988), *Flora of the U.S.S.R.* (Komarov & al. 1934–1964), *Flora of Iraq* (Towsend & Guest 1966–1988), *Flora of West Pakistan* (Nasir & Ali 1970–1985), as well as on Mobaien (1975–1995), Ghahreman (1978–2005, 1993–1994), Maassoumi (1986–1995), Mozafarian (1994, 2000), Sabeti (1994), Mirtadjadini (1996), and Chalbian & Sharifnia (2003).

Results and discussion

Altogether 200 species belonging to 47 families and 145 genera were recognized in the area. Life form, flowering and fruiting time, locality and local uses of plants were designated for each species. 65 % of the species belong to 7 families: *Asteraceae*, *Fabaceae*, *Poaceae*, *Apiaceae*, *Boraginaceae*, *Lamiaceae*, and *Liliaceae*. The largest family is *Asteraceae* with 35 species, and the largest genus is *Astragalus* with 13 species. The majority of the elements are Irano–Turanian.

The results of plant species' identification and sampling indicate that there are more than 200 naturally occurring species, dispersed in 12 vegetation types.

Table 1 presents the vegetation types established. The associated important plant species for each vegetation type are given in Table 2.

Generally the biological spectrum and the appearance of plants in each region indicate the climate of that region; the presence or absence of a biological form indicates the difference of the climate of that region with other region. Similar climate has similar biological spectrum, and if biological spectra of two regions are similar, we should be confident that the climate of those regions and their ecological conditions are very much similar.

In the studied region, the biological spectrum shows that the therophytes (with 35.5 %) are the largest and cryptophytes and halophytes (with 1 %) are the smallest groups of life forms. The relatively high percentage of weeds and the low percentage of halophytes indicate shortage of water resources and permanent and seasonal rivers.

Also, for the present time, *Artemisia sieberi*, *Pistacia atlantica* and *Amygdalus scoparia* are the dominant species growing at altitudes from 1800 m to 2400 m a.s.l. (Table 1). This vegetation type is predominating over the other types; it has minimum rainfall per year, desert dry and semidry climate with tolerant strength of high temperatures and it occupies 2/3 of the region.

Among the vegetation types, there are changes from the plain part of the studied region to the mountain parts. It seems that the most important factor is the altitude in such a manner that when moving ahead from plain regions to mountain regions, cushion species and perennial and annual herbaceous are added, as well.

Table 1. Vegetation types in the studied area.

Vegetation type name	Extent (ha)	Location	Altitude (m a.s.l.)	Appearance physiognomy	Dominant species
Artemisia–Grass	2000	North	1800–2000	Fruticose and grass field	*Artemisia sieberi–Bromus tectorum–Hordeum glaucum*
Pistacia–Daphne–Sageretia	1200	North–North-East	2000–2400	Tree and shrub field	*Pistacia atlantica–Daphne mucronata–Sageretia thea*
Pistacia–Amygdalus–Artemisia	10 000	North–North-East–West	1800–2400	Tree and fruticose field	*Pistacia atlantica–Amygdalus scoparia–Artemisia sieberi*
Pistacia–Amygdalus–Ephedra	700	North-East	2000–2500	Tree and shrub field	*Pistacia atlantica–Amygdalus scoparia–Ephedra strobilacea*
Pistacia–Peganum–Artemisia	600	East	1800–2000	Tree and fruticose field	*Pistacia atlantica–Peganum harmala–Artemisia sieberi*
Peganum–Artemisia	400	East	1800–2000	Fruticose and grass field	*Peganum harmala–Artemisia sieberi*
Artemisia–Euphorbia–Astragalus	5000	North and West	2500–3500	Fruticose and grass field	*Artemisia aucheri–Euphorbia gedrosiaca–Astragalus schistocalyx*
Pistacia–Amygdalus–Bunium	400	West	2200–2500	Tree and fruticose field	*Pistacia atlantica–Amygdalus scoparia–Bunium persicum*
Artemisia–Astragalus	2000	West and North-West	1800–2100	Fruticose and grass field	*Artemisia sieberi–Astragalus cephalanthus*
Artemisia–Acanthophyllum–Acantholimon	1000	West	2100–2400	Fruticose and grass field	*Artemisia sieberi–Acanthophyllum glandulosum–Acantholimon austrotiranicum*
Amygdalus–Convolvulus–Ebenus	5000	South-East and East	2000–2400	Shrub and fruticose field	*Amygdalus lycioides–Convolvulus fruticosus–Ebenus stellatus*
Allium–Vicia	800	South-East	2000–2200	Fruticose and grass field	*Allium stamineum–Vicia aintabensis*

Table 2. List of associated important species for each vegetation type.

Vegetation type name	Associated important species
Artemisia–Grass	*Erodium oxyrrhynchum–Poa* spp.*–Geranium rotundifolium*
Pistacia–Daphne–Sageretia	*Artemisia sieberi–Acer monspessulanum*
Pistacia–Amygdalus–Artemisia	*Filago arvensis–Onosma microcarpum–Alyssum inflatum–Malcolmia inflatum–Cardaria draba–Erodium cicutarium*
Pistacia–Amygdalus–Ephedra	*Daphne mucronata–Sageretia thea–Acer monspessulanum*
Pistacia–Peganum–Artemisia	*Erodium oxyrrhynchum–Stipa barbata*
Peganum–Artemisia	*Erodium oxyrrhynchum–Orobanche aegyptiaca–Stipa barbata*
Artemisia–Euphorbia–Astragalus	*Tulipa biflora–Galium spurium*
Pistacia–Amygdalus–Bunium	*Bunium cylindricum–Onosma microcarpum–Ixiolirion tataricum–Taeniatherum crinitum–Papaver glaucum*
Artemisia–Astragalus	*Astragalus campylanthus–Alyssum inflatum*
Artemisia–Acanthophyllum–Acantholimon	*Convolvulus fruticosus–Euphorbia buhsei*
Amygdalus–Convolvulus–Ebenus	*Convolvulus arvensis–Acanthophyllum glaudulosom–Acantholimon austrotiranicum–Chenopodium album–Chenopodium novopokroskyanum*
Allium–Vicia	*Allium atroviolaceum–Vicia michauxii–Cicer arietnum–Medicago radiata–Medicago lupulina–Sophora alopecuroides–Torilis arvensis–Turgenia biflora*

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Degradation of montane forests of beech in Southwest Serbia

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Abstract. The degradation of montane forests of beech is presented in different site conditions in Southwest Serbia. Various forms of degradation on limestones and on acid siliceous rocks are especially emphasized. The phases of degradation differ essentially depending on the different conditions of parent rock and soil characteristics. The field of research is the wider area of Southwest Serbia (Plateau) with the hills of the surrounding mountains, so that it can be stated that it represents the Southwest Serbia.

Key words: beech, degradation, site, Southwest Serbia

Introduction

Mixed broadleaf–coniferous and beech forests (*Fagetum moesiacae montanum*) were under great negative impacts and they were massively destroyed. Beech formed mixed forests with spruce and fir on the shallow karst of Southwest Serbia. Still, beech forests, as the members of paleogenetic forests in this district, remained for a somewhat longer time and on larger areas. They were also protected and saved by cattle breeders because of the supply of fuelwood, and beech has high coppice vigour. Beech forests grow on the cooler exposures on limestones, while the warm sides are degraded to meadows and pastures. This study presents the base for the determination of the directions of beech forest degradation in different site conditions. Also, the intensities of degradation are different, as the consequence of various ecological conditions and zoo-anthropogenic impacts.

Methods

Taking into account the complexity of the research, this study applies different methods for the determination of the characteristics of climate, soil, recent forest vegetation, methods of regressive successions – degradations, as well as the reconstruction of the natural potential vegetation.

Results

Site characteristics

This community, as the most represented community in the study area, has a very wide ecological range of distribution. Regarding the altitude, beech stands range at the altitudes of 1000 m to 1600 m. They also occur at lower altitudes in the form of smaller stands and tree groups, but in general, at the lower altitudes near the villages, the forests are completely destroyed. Now the pioneer tree species have occupied these sites in the progressive succession. The best stands, which are the subject of research, grow at the altitudes to about 1280 m, which is also the optimal belt of the beech forest range in this geographical belt. At the altitudes higher than 1450 m beech occurs in the form of partially maintained stands on the west, southwest and south exposures. As for the slope, in this area beech grows on different slopes and positions, on the ridges, steep slopes, plateaux, but it is best preserved on the longer sides of the moderate slopes facing the north. In general, it

can be stated that it grows on the limestone bedrock, on acid quartz conglomerates, sandstones and on diabase-hornstones. Based on this and based on other differences, which are the consequences of other different conditions, the community is divided into two subassociations: *calcicolum* and *silicicolum*. On limestones, beech stands grow on calcareous black soil (calcomelanosol) and on brown calcareous soil (calcocambisol), as the dominant type on which beech grows; and on acid bedrocks, beech stands appear on dystric cambisols.

The floristic composition of the community consists of 182 species, which indicates that beech community is very rich. The tree layer consists of 10 tree species; the shrub layer consists of 25 species of shrubs and there are 147 species in the layer of ground vegetation. The wealth of plant species in individual relevés is the consequence of the poor stand condition, the presence of the species of grass communities due to the broken canopy.

Characteristics of the community

The stand stocking is from 0.5 to 0.9, which indicates that the stands are degraded or recently regenerated, so the crowns have not succeeded to cover the ground area. The values of tree height range from 8 m to 26 m. Mean diameter ranges from 14 cm to 28 cm. The mean spacing between the trees amounts at the most to 4 m, taking into account the good coppice vigour. Of the tree species in the tree layer, wild cherry [*Prunus avium* (L.) L.] is somewhat more significant regarding the degree of presence, but it appears in the form of individual trees. *Acer platanoides* L., *Betula pendula* Roth, *Carpinus betulus* L., *Picea abies* (L.) H. Karst, *Pyrus pyraster* (L.) Burgsd., *Populus tremula* L., *Salix caprea* L., and *Ulmus carpinifolia* Gled. also occur individually in some relevés.

At places where degradation is more intensive, the response to the increased light from above is the mass occurrence of the species in the shrub layer. In the shrub layer in all relevés, but with varying significance, *Fagus moesiaca* (Maly) Domin, *Corylus avellana* L. and *Rosa pendulina* L. occur as the signs of degradation and the greater light intensity. *Crataegus monogyna* Jacq. occurs somewhat less frequently.

In the layer of ground flora, *Aremonia agrimonioides* DC. has the highest degree of presence, and *Anemone nemorosa* L., *Fragaria vesca* L., *Glechoma hirsuta* Waldst. & Kit., and *Helleborus odorus* Willd. have lower degrees of presence.

Regressive successions

Beech community on acid siliceous rocks (*Fagetum moesiacae montanum silicicolum*) occurs in mosaics with the community *Populo–Betuletum* and the pioneer community of birch *Betuletum verrucosae,* most often in the belt at the altitude of 1050–1200 m. In the belt of villages, beech community succeeded to survive in the forms of smaller forests and village forests. Here beech forests are degraded with a significant percentage of aspen, birch and hazel. There is almost not one maintained stand which could represent the true natural beech forests in their optimal conditions. The community of aspen and birch is a form of degradation of montane beech forests, which has almost disappeared due to the permanent anthropogenic impact. It grows on the deeper soils, pseudogleys, luvisols, dystric cambisols to mild eutric cambisols, less exposed to the sun, where the evaporation of soil moisture is less intensive. Its presence is considerable in the sheltered river valleys, where there is sufficient moisture and favourable climate currents, but without strong wind. It is not present on the south slopes of the smaller rivers. On very deep pseudogleys, the community is present also on mildly sloping warmer sides, but the belt above the river valleys, with warmer conditions and shallower dystric cambisols, is already the zone of the potential community of sessile oak and Turkey oak. On the deeper soils above the rivers and streams, the community of aspen and birch with a significant percentage of hazel represents the final stage of degradation of forest vegetation.

The degradation of beech community *Fagetum moesiacae montanum silicicolum* (Table 1, left column) results in *Populo–Betuletum* on eutric rankers, cambisols and colluviums, and in *Betuletum verrucosae* on dystric cambisols and dystric rankers. The final stages of the regressive successions in the above-mentioned conditions are as follows: meadows and pastures of the type of meadow and pasture community of brome-grass and dog's tail grass *Brometo–Cynosuretum*, matweed *Nardetum strictae* and the pastures of fescue grass and cuscus-grass *Festuco–Chrysopogonetum grylli,* and on the warm exposures, the community of meadows and pastures of matweed *Nardetum strictae* and pastures of fescue grass and cuscus-grass *Festuco–Chrysopogonetum grylli*.

Table 1. Scheme of regressive successions and degradation phases of the community *Fagetum moesiacae montanum*.

Devastation (canopy opening, foddering, clearing)	
↓	↓
On sheltered, cooler north and northeast exposures, moister soils, pseudogleys and deep dystric cambisols the stands become opened, aspen and birch enter the tree layer	On brown calcareous soils (calcocambisol), on deeper calcareous black soils (calcomelanosol), the devastation leads to massive development of hazel (*Corylus avellana*) in the shrub layer with hawthorn (*Crataegus monogyna*) and dog rose (*Rosa pendulina*)
↓	↓
Further devastation, elimination of beech	Devastation of the tree layer
↓	↓
Aspen and birch create the community (*Populo–Betuletum*)	Hazel invades the entire area Formation of hazel *Coryletum avellanae* growth
↓	↓
Further devastation (canopy opening, pasturage)	Felling, destruction, threading, pasturage and other zoo-anthropogenic impacts
↓	↓
Aspen is massively regenerated by coppicing, it dominates in the lower positions, in the medium position with birch and hazel, hazel growths are in the upper position	Disappearance of hazel growths and the development of juniper *Juniperus communis* among the remaining groups of hazel
↓	↓
Formed areas of aspen, birch and hazel in the lower positions, where forest degradation ends on deep acid brown soils and pseudogleys	Formation of juniper community *Juniperetum communis*
↓	↓
Further degradation by clearing, excessive pasturage, threading, etc. zoo-anthropogenic impacts in the medium and higher positions of the slopes	Felling of juniper for village demands, pasturage, threading, burning of juniper
↓	↓
Community of meadows and pastures (community of brome-grass and dog's tail grass type *Brometo–Cynosuretum*, community of matweed *Nardetum strictae* and pastures of fescue grass and cuscus-grass *Festuco–Chrysopogonetum grylli*)	Formation of pastures, types *Festucetum, Cariceto–Brometum erecti, Rhinantho–Cynosuretum cristati*

The regeneration of this community is good in favourable edaphic and climate conditions, thanks to the high coppice vigour of aspen and birch, so that the degradation does not succeed as far as meadow and pasture community. In the belt, much more above the rivers, on dystric soils, which are less deep than pseudogleys and luvisols, beech degradation does not stop with the community *Populeto–Betuletum*, but proceeds through hazel brushland (*Coryletum avelanae*) to the meadows and pastures. The communities of meadows and pastures, which result from the further degradation of forests, are the community of brome-grass and dog's tail grass, type *Brometo–Cynosuretum*, the community of matweed *Nardetum strictae* and the pastures of fescue grass and cuscus-grass *Festuco–Chrysopogonetum grylli*.

Out of the rocky gorges, valleys and depressions, the potential vegetation of the lower belt of this part of the Plateau, at the altitude of 1050–1200 m, on the slopes up to 40°, is *Fagetum moesiacae montanum calcicolum*. This is the area which is composed of calcareous black soils (calcomelanosol) and brown calcareous soils (calcocambisol). The degradation of this community (Table 1, right column) by overfelling proceeds through the stands of beech with a percentage of wild cherry (*Prunus avium*) and aspen (*Populus tremula*). The shrub layer consists of the significant percentage of *Corylus avellana*, *Rosa pendulina*, *Crataegus monogyna*, *Viburnum lantana* L., *Evonymus*, *latifolius* (L.) Mill., etc. Further degradation, on brown calcareous soils and on brownised calcareous black soil, results in hazel *Coryletum avellanae* growths. The constant negative zoo-anthropogenic impacts transform the hazel growths into the community of juniper *Juniperetum communis*. Further regression to pastures leads by juniper burning, pasturing and cattle threading to pure poor pastures. The final stages of regression are the pastures of the types *Festucetum*, *Danthonietum calycinae*, *Cariceto–Brometum erecti*, *Rhinantho–Cynosuretum cristati*.

Conclusion

The degradation of beech forests develops by different intensities and in different directions on limestones and on acid siliceous rocks. On limestones, the degradation develops faster to hazel growths, without transient phases. In the degradation on siliceous bedrock, the transient phase is the forest of aspen with birch and hazel. In the upper positions, hazel invades the area directly.

This community regenerates well in favourable edaphic and climate conditions, thanks to the high coppice vigour of aspen and birch, so the degradation does not proceed as far as meadow and pasture communities.

The final stages of regressive successions in the above-mentioned conditions are meadows and pastures of the type meadows and pastures community of brome-grass and dog's tail grass *Brometo–Cynosuretum*, and matweed *Nardetum strictae*, etc.

The soil condition is less favourable on the calcareous bedrock because the forest degradation is more intensive from the aspect of the capacity of forming the tree layer of aspen and birch which would protect the soil against erosion.

The degradation of this community by overfelling develops through the beech stands with a percentage of wild cherry (*Prunus avium*) and aspen (*Populus tremula*) to hazel *Coryletum avellanae* growth. Further degradation develops to juniper *Juniperetum communis* community, and then the regression proceeds to pastures, types *Festucetum*, *Danthonietum calycinae*, *Cariceto–Brometum erecti*, *Rhinantho–Cynosuretum cristati.*

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Populational strategy of *Allium* species (*Alliaceae*)

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Abstract. Biomorphs, ontogenesis, pattern of vegetative propagation and of that by seeds, structures of coenopopulations and ways of self-preservation have been studied in 16 species of the genus *Allium* growing in the steppes, desertificated steppes and sandy steppes of Kazakhstan and Southern Siberia. The interrelationship between the type of ontogenetic development and coenopopulation structure was established. Populational strategy of species was described and competitive, reactive, tolerant and tolerant-reactive species were identified.

Key words: *Allium*, coenopopulation, life form, ontogenesis, plant strategies

Introduction

Cognition of the mechanisms of sustainable development of natural systems is based on a comprehensive study of species biology. This allows evaluating the role of different species in phytocoenoses and distinctiveness of their populational strategy. Strategy of a species life is a combination of adaptive mechanisms that makes it possible to grow together with different organisms and to hold a certain position in appropriate biocoenoses (Grime 1974, 1979; Rabotnov 1975, 1985). Moreover, this notion is applied to the organisms (animals and plants), their populations and a species as a whole. There are primary and secondary types of strategies described in detail by Grime (1974, 1979).

Smirnova (1987) considers it necessary, when describing a type of populational strategy of the species, to distinguish phytocoenotic potencies and positions of the species in the coenosis and to determine integral (general) and differential (specific) characters of the strategy.

Competitive ability, tolerance and reactivity belong to the integral characters (properties). Accordingly species in which one of the characters prevails may be assigned to competitive, tolerant and reactive ones. Integral properties can be characterized with the help of specific characters, a part of which is typical of an individual or coenopopulation element and a part – to a coenopopulation (Smirnova & al. 1976; Smirnova 1987; Zaugolnova & al. 1988). In this article the populational strategy of the genus *Allium* L. species is characterized with the use of the following differential characters proposed by Smirnova: 1) type and duration of individual ontogenesis; 2) reproductive ability and types of vegetative propagation and spread; 3) type of self-preservation of a coenopopulation; 4) type of ontogenetic and spatial structure of a coenopopulation; and 5) density.

Species of the genus *Allium*, the objects of study, are widespread in Eurasia (Stearn 1978, 1992). They occur in all botanical–geographical zones and belts, both in the plains and mountains. Most of onions are of little importance in the plant cover composition. Being assectators they can form an aspect during blooming (*A. anisopodium* Ledeb., *A. pallasii* Murray, *A. senescens* L., *A. giganteum* Regel). Only some species (*A. polyrhizum* Turcz. ex Regel, *A. altaicum* Pall., *A. schoenoprasum* L., *A. microdictyon* Prokh., *A. ursinum* L.) are edificators or dominants and codominants in the communities (Bykov 1962; Ellenberg 1988).

Material and methods

The objects of study were 16 species of onions growing in different biomes in Siberia and Kazakhstan and forming various life forms (Table 1). When studying ontogenesis of the *Allium* species, a concept of discrete ontogeny developed by Russian botanists (Rabotnov 1950, 1969; Uranov 1975; Smirnova & al. 1976; Gatsuk & al. 1980; Zaugolnova & al. 1988) was taken. It is based on singling out in the process of individual

Table 1. Brief characteristics of the species studied.

No.	Species	Life form	Habitats studied
1.	*A. altaicum*	laxicaespitose rhizomatous-bulbous, monocentric	Altai Mts, Chikhachev Range, stony steppe
2.	*A. bidentatum*	densely caespitose, monocentric	Kazakhstan, Tarbagatai Range, dry steppe
3.	*A. caeruleum*	bulbous with short stolones, unmanifestly polycentric	Kazakhstan, Chu-Iliiskiye Mts, bushy meadow
4.	*A. caesium*	bulbous with short stolones, unmanifestly polycentric	Kazakhstan, Alakolskaya Hollow, sandy steppe
5.	*A. caespitosum*	long-rhizomatous, manifestly polycentric	Kazakhstan, Zaisanskaya Basin, sandy desert
6.	*A. fetisowii*	bulbous, monocentric	Kazakhstan, Northern Tyan-Shan, Sogetinskiye Mts, mountain steppe
7.	*A. galanthum*	laxicaespitose rhizomatous-bulbous, monocentric	Kazakhstan, Chu-Iliiskiye Mts, desertificated montane steppe
8.	*A. nutans*	laxicaespitose rhizomatous-bulbous, unmanifestly polycentric	Altai Mts, meadow steppe
9.	*A. obliquum*	rhizomatous-bulbous, monocentric	Altai Mts, meadow steppe
10.	*A. pallasii*	bulbous, monocentric	Altai Krai, sandy steppe
11.	*A. polyrhizum*	densely caespitose, monocentric	Eastern Zabaikalye, Toreiskiye Lakes, true steppes
12.	*A. prostratum*	densely caespitose, monocentric	Eastern Zabaikalye, Dauria, stony steppe
13.	*A. rubens*	densely caespitose, monocentric	Altai Krai, meadow steppe
14.	*A. sabulosum*	bulbous with long stolones, manifestly polycentric	Kazakhstan, Sands of Taukumy, haloxylon desert woodlands on hilly sands
15.	*A. senescens*	laxicaespitose rhizomatous-bulbous, unmanifestly polycentric	Eastern Zabaikalye, Dauria, meadow steppe
16.	*A. tulipifolium*	bulbous, monocentric	Altai Krai, desertificated steppe

development the stages characterized by quantitative indicator characters. Ontogenetic structures of coenopopulations were determined using 10–80 plots of size 1 m^2 as the base. The plots were laid out by chance or in a regular way on the transects 1 m wide. In the mountains, transects were arranged lengthwise and crosswise of the slope (Smirnova & al. 1976; Zaugolnova & al. 1988).

Description of the structures of coenopopulations of different species was supported by a notion of the base spectrum of coenopopulations (Zaugolnova & al. 1988). Density was calculated on the basis of individual abundance per unit of place of growth.

Results and discussion

Life form and types of individual ontogenesis

Diversity of life forms determined by both historical development of species and present conditions of habitation is characteristic of the genus *Allium* species (Cheryomushkina 2004). As our study has shown, individuals with different life forms develop in the same way. Six types of ontogenesis have been determined in onions.

I type. In the species with bulbous (*A. tulipifolium* Ledeb., *A. fetisowii* Regel) and rhizomatous-bulbous (*A. obliquum* L.) monocentric biomorphs the ontogenesis is complete or shortened due to absence of final stages of the development. No vegetative propagation. Duration of the ontogenesis does not exceed 20–25 years. A pregenerative period is long and ranges from 8 to 12 years.

II type. In the species with densely caespitose rhizomatous (*A. bidentatum* Fisch. ex Prokh., *A. polyrhizum*) and densely caespitose rhizomatous-bulbous (*A. prostratum* Trevir., *A. rubens* Schrad. ex Willd.) monocentric biomorphs duration of the ontogenesis is 50 and more years. They come into flower in the 6^{th}–9^{th} year. Vegetative propagation occurs at the end of the ontogenesis: a compact clone is formed (senile particulation by: Rabotnov 1975). Ramets are of the same ontogenetic age or older, live for a long time. Individuals are vegetatively immobile.

III type. It occurs in laxicaespitose rhizomatous-bulbous (*A. altaicum*, *A. galanthum* Kar. & Kir.) as well as in bulbous (*A. pallasii*) monocentric species. Duration of the ontogenesis is different and depends on the life form (Table 2). Duration of the pregenerative period is 8–12 years. Vegetative propagation occurs in the middle of the generative period. Several generations of the ramets are observed over the complete ontogenesis. Rejuvenation of the ramets is absent or insignificant (at 1–2 ontogenetic stages). The species are vegetatively immobile.

IV type. It is described in laxicaespitose rhizomatous-bulbous unmanifestly polycentric species (*A. senescens*, *A. nutans* L.). Long-lasting ontogenesis from 50 to 100 years is typical of them. They come into flower in the 5^{th}–7^{th} year. Ontogenesis of a seed individual is incomplete and finishes with repeated particulation in the young and middle-age generative states.

Table 2. Some ontogenetic characteristics of the *Allium* species.

Species	Beginning of vegetative propagation	Number of generative shoots of mature generative individuals	Presence of senile individuals
A. altaicum	g_2	20–22	absent
A. bidentatum	g_3	16–21	present
A. caeruleum	im	1–3	absent
A. caesium	j	1–4 (6)	absent
A. caespitosum	g_2	1–2 (5)	present
A. fetisowii	absent	1	absent
A. galanthum	g_2	6–18 (28)	present
A. nutans	g_2	2–6	present
A. obliquum	absent	1	present
A. pallasii	g_2	2–4 (12)	absent
A. polyrhizum	g_3	3–6 (34)	present
A. prostratum	g_3	2–7	present
A. rubens	g_3	3–9	present
A. sabulosum	im	1–2	absent
A. senescens	g_2	2–8	present
A. tulipifolium	absent	1	absent

Abbreviations: g_2 – mature generative individuals; g_3 – old generative individuals; j – juvenile individuals; im – immature individuals.

Ramets are capable of being insignificantly rejuvenated. The species have little vegetative mobility.

V type. It is characteristic of bulbous species (*A. caesium* Schrenk, *A. caeruleum* Pall.) with short stolons. Vegetative propagation begins in the pregenerative period. The ontogenesis of a seed individual is complete and consists of ontogenesis of the genet and repeated shortened ontogenesises of ramets. Rejuvenation of ramets is deep (up to the juvenile or immature stages). The ontogenesis is long-lasting. The individuals are vegetatively immobile or demonstrate limited mobility.

VI type. It is typical of long-rhizomatous (*A. caespitosum* Siev. ex Bong. & C.A. Mey.) and bulbous species (*A. sabulosum* Stev. ex Bunge) with long stolons. The ontogenesis of a seed individual is incomplete, vegetative propagation begins in the pregenerative period and continues for a long time. Deep rejuvenation up to the juvenile (*j*) or immature (*im*) stages is observed. Ontogenesis lasts for an indefinitely long time. The vegetative mobility is well-defined.

Reproductive ability and types of vegetative propagation and spread, a way of self-preservation of the coenopopulation

The role of vegetative propagation and that by seeds in self-preservation of the coenopopulation changes significantly in onion species. Self-preservation by seeds is characteristic of *A. obliquum*, *A. tulipifolium*, *A. bidentatum*, *A. rubens*. Individuals of *A. obliquum* and *A. tulipifolium* form from 407 to 1173 seeds (Table 3). In individuals of densely caespitose plants seed productivity is significantly lower: 20–28 seeds. Vegetative propagation is either absent or represents senile particulation. Smirnova (1987) considers the latter to be of no importance in coenopopulation self-preservation.

The contribution of propagation by seeds is also great in *A. pallasii*, *A. altaicum*, *A. nutans*, *A. senescens*. At the same time the importance of non-specialized vegetative propagation increases. Individuals of *A. pallasii* and *A. altaicum* form compact clones. Senescence of the individuals appeared vegetatively occurs at the same time with senescence of the clone without increase of individual numbers. Such vegetative propagation promotes prolongation of the complete ontogenesis due to lengthy generative period of individuals. Diffuse clones up to 1 m in diameter are formed in *A. nutans* and *A. senescens*. Repeated branching and

Table 3. Some populational characters of the species studied.

Species	Types of ontogenesis	Seed productivity, items/individual	Number of ramets	Degree of ramet rejuvenation	Way of coenopopulation self-preservation
A. fetisowii	I	305–645	absent	absent	by seeds
A. obliquum	I	407–1140	absent	absent	by seeds
A. tulipifolium	I	459–1173	absent	absent	by seeds
A. bidentatum	II	42–72	2–3	absent	by seeds
A. polyrhizum	II	29–68	2–3	absent	by seeds
A. prostratum	II	32–97	2–5	absent	by seeds
A. rubens	II	66–78	2–5	absent	by seeds
A. altaicum	III	150–658	2–4	1 ontogenetic state younger	mainly by seeds
A. galanthum	III	288–540	2–3	1 ontogenetic state younger	mainly by seeds
A. pallasii	III	128–256	2–3	1 ontogenetic state younger	mainly by seeds
A. nutans	IV	200–640	3–6	1–2 ontogenetic states younger	by seeds, vegetative
A. senescens	IV	198–602	3–5	1–2 ontogenetic states younger	by seeds, vegetative
A. caeruleum	V	absent	8–16 (25)	3–4 ontogenetic states younger	vegetative
A. caesium	V	4–62	4–7	3–4 ontogenetic states younger	vegetative
A. caespitosum	VI	4–25	5–8	3–4 ontogenetic states younger	vegetative
A. sabulosum	VI	42–294	7–12	3–4 ontogenetic states younger	vegetative

arrangement of ramets far apart weaken the inhibitory effect of the old parts on the young ones. This way of vegetative propagation makes an important contribution to coenopopulation self-preservation and increase in abundance of both ripe generative and young (virginal (*v*) and generative (g_1) individuals.

Predominance of vegetative self-preservation and almost complete absence of that by seeds is typical of *A. caesium*, *A. caeruleum*, *A. caespitosum* and *A. sabulosum*. In *A. caesium* and *A. caeruleum* vegetative propagation is due to deeply rejuvenated diaspores: bulblets and those in inflorescences. Efficiency of vegetative reproduction is connected with the habitation conditions and gradually drops to the middle of the generative period. Such a type of propagation leads to formation of compact or weakly diffuse clones just in the pregenerative period and domination of them in a coenopopulation. *Allium caespitosum* and *A. sabulosum* form diffuse clones due to long stolons and rhizomes. Spread centres represent deeply rejuvenated ramets. Hence, it may be stated that the way of self-preservation of coenopopulations of these species is determined significantly by the type of ontogenesis.

Type of ontogenetic and spatial structure of a coenopopulation; ecological density

Young individuals prevail in *A. tulipifolium*, *A. fetisowii*, *A. obliquum* coenopopulations. High seed productivity and considerable duration of the pregenerative period (up to 8–12 years) result in a high density of young plants in a coenopopulation. Absolute maximum may fall on any of ontogenetic groups of the pregenerative fraction. Its position is connected with intensity of individual die-off and various durations of ontogenetic states. A drastic drop of the curve from juvenile to virginal group is determined by great elimination of juvenile plants (Fig. 1).

Species density ranges on the average from 19.4 to 44.2 individuals per 1 m^2. Spatial structure of the coenopopulations is group. Regular frutification and seed germination near the maternal plant form small weakly fluctuating aggregations represented by the individuals of all ontogenetic groups.

In *A. bidentatum*, *A. rubens*, *A. prostratum* and *A. polyrhizum* coenopopulations absolute maximum falls on old generative (g_3) and subsenile (*ss*) individuals. Formation of a sod and its subsequent particulation at the end of the generative period lead to a significant extension of general duration of ontogenesis due to slow development of postgenerative individuals (Fig. 2).

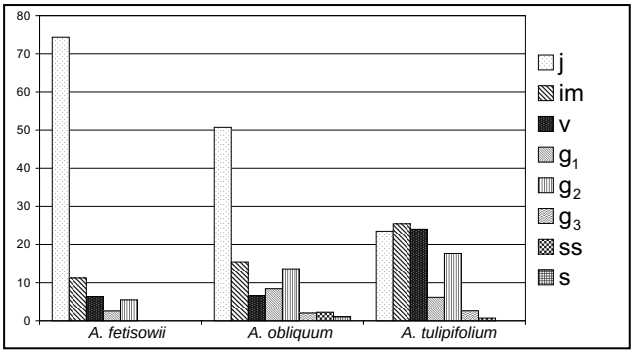

Fig. 1. Ontogenetic spectra of coenopopulations of the species: *A. fetisowii*, *A. obliquum*, *A. tulipifolium* (%).
Abbreviations: **j** – juvenile individuals; **im** – immature individuals; **v** – virginal individuals; $\mathbf{g_1}$ – young generative individuals; $\mathbf{g_2}$ – mature generative individuals, $\mathbf{g_3}$ – old generative individuals; **ss** – subsenile individuals; **s** – senile individuals.

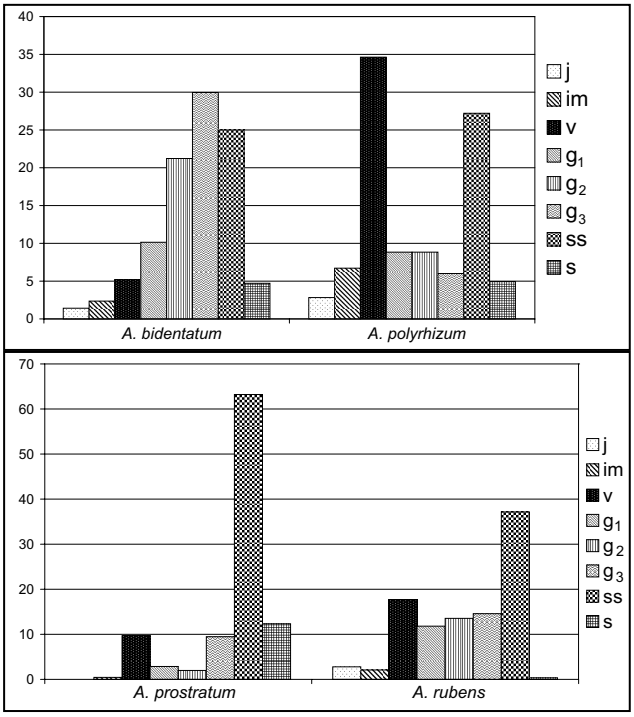

Fig. 2. Ontogenetic spectra of coenopopulations of the species: *A. bidentatum*, *A. polyrhizum*, *A. prostratum*, *A. rubens* (%).
Abbreviations: **j** – juvenile individuals; **im** – immature individuals; **v** – virginal individuals; $\mathbf{g_1}$ – young generative individuals; $\mathbf{g_2}$ – mature generative individuals, $\mathbf{g_3}$ – old generative individuals; **ss** – subsenile individuals; **s** – senile individuals.

In the left part of the spectrum local maxima are mainly noted in virginal plants, which is connected with low seed productivity, irregular renewal by seed and poor survival of juvenile plants in arid conditions of the steppe communities. Thus, distribution of individuals in coenopopulations of these species takes the form of a single- or double-peaked curve.

Density, on the average, varies over wide limits among populations of both one species and different species (from 8.7 in *A. prostratum* to 45.8 in *A. polyrhizum*). Spatial structure of coenopopulations is diffuse. Germination and bursts of numbers of young individuals occur only in a free territory.

Allium galanthum, *A. altaicum* and *A. pallasii* coenopopulations have an ontogenetic spectrum on the left, with absolute maximum on one of the pregenerative groups. Maximum in the young part of the spectrum is more often noted in juvenile plants, which is determined by high germination (Fig. 3).

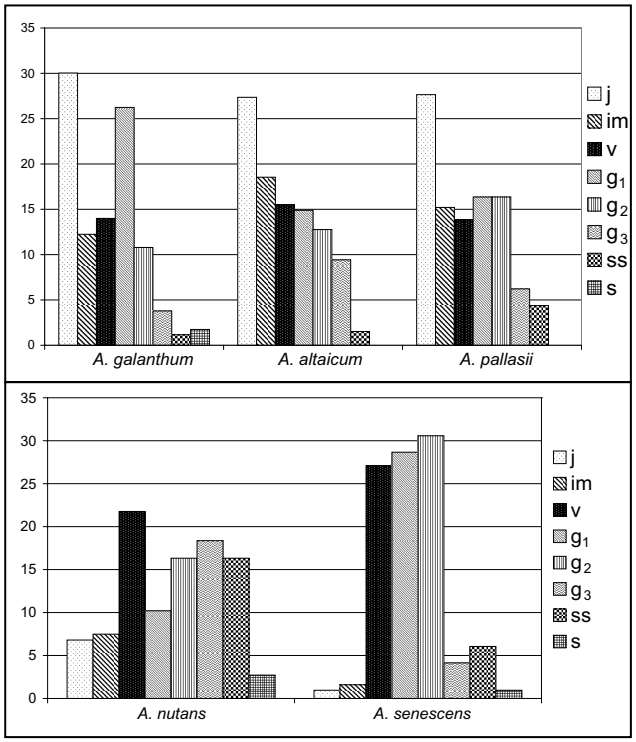

Fig. 3. Ontogenetic spectra of coenopopulations of the species: *A. galanthum*, *A. altaicum*, *A. pallasii*, *A. nutans*, *A. senescens* (%).
Abbreviations: j – juvenile individuals; **im** – immature individuals; **v** – virginal individuals; $\mathbf{g_1}$ – young generative individuals; $\mathbf{g_2}$ – mature generative individuals, $\mathbf{g_3}$ – old generative individuals; **ss** – subsenile individuals; **s** – senile individuals.

Density in *A. galanthum* and *A. altaicum* does not exceed 2.5–13.6 individuals per 1 m^2, whereas in *A. pallasii* it amounts to 48.3 individuals. Spatial structure is diffuse-group. Immobile adults are arranged compactly and retain the territory for a long time. Seed productivity leads to diffuse distribution of young plants whose place of removal from the maternal plant is determined by the direction of blowing winds during seed ripening (*A. galanthum*, *A. altaicum*, *A. pallasii*).

The ontogenetic spectrum of *A. nutans* and *A. senescens* coenopopulations is bimodal. Absolute maximum falls on either one of the groups of the left or right part of the spectrum, or, as in *A. senescens*, on mature generative individuals. Similar distribution appears as a consequence of a mixed type of self-preservation of coenopopulations and great length of individual life in mature generative (g_2) status. Local ups in the spectrum are possible on young plants due to good seed renewal and insignificant rejuvenation of ramets. A rise in the right part of the spectrum occurs due to increase in abundance of old generative plants or subsenile plants. Slow die-off of old individuals is determined by enough reserve of nutrients in the rhizome and bulb (Fig. 3).

Density of individuals per unit of area varies from 3 to 15 per 1 m^2. Spatial structure of coenopopulations is diffuse-group. Sizes of aggregations and their limits are determined by the character of dissemination and turfness of soil. Young plants are concentrated around an adult forming seeds. Viable sprouts appear as a rule in a free space.

The ontogenetic spectrum of *A. caesium* and *A. caeruleum* coenopopulations is left-side. Pregenerative individuals prevail in them. Absolute maximum in the spectrum falls on juvenile plants. Formation of vegetative deeply rejuvenated diaspores at the early stages of ontogenesis sharply increases abundance of this group (from 40 to 89 %). The generative fraction in the ontogenetic spectrum greatly varies (from 0.5 to 20 %). Short life of individuals and die-off at any stage of the ontogenetic development because of extermination by plant feeders result in decrease of adults in the population (Fig. 4).

As a rule, intensity of formation of bulblets in a bulb falls to the middle of the generative period. They are the most abundant in immature and virginal states (from 2–4 to 8–12 in *A. caeruleum*). This way of propagation leads to formation of compact or weakly diffuse clones with low density (e.g., 13.3 clones per 1 m^2) already in the pregenerative period and to their predominance in the coenopopulation.

The ontogenetic spectrum of *A. caespitosum* and *A. sabulosum* coenopopulations is left-side too. Young individuals of vegetative origin prevail in coenopopulations. Absolute maximum in the spectrum falls on different ontogenetic groups and depends on a life form. Juvenile and immature individuals prevail in *A. sabulosum* because of a deeper rejuvenation of ramets

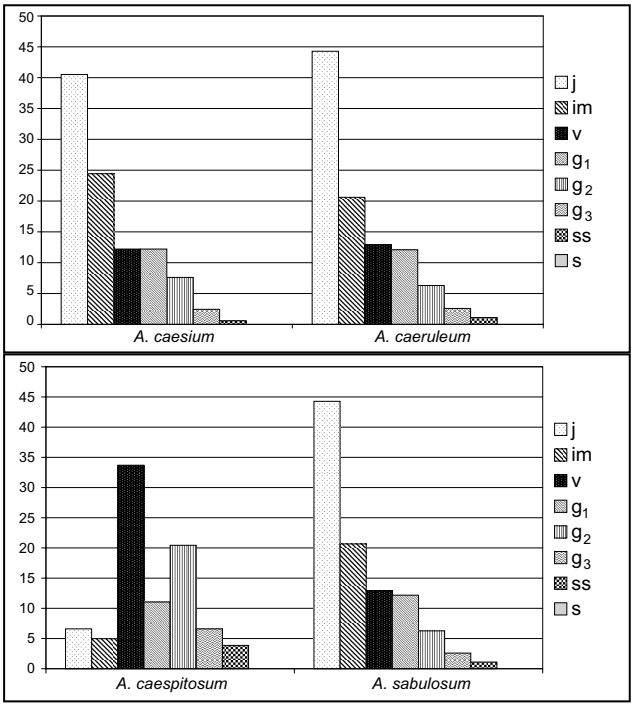

Fig. 4. Ontogenetic spectra of coenopopulations of the species: *A. caesium*, *A. caeruleum*, *A. caespitosum*, *A. sabulosum* (%).
Abbreviations: j – juvenile individuals; **im** – immature individuals; **v** – virginal individuals; $\mathbf{g_1}$ – young generative individuals; $\mathbf{g_2}$ – mature generative individuals, $\mathbf{g_3}$ – old generative individuals; **ss** – subsenile individuals; **s** – senile individuals.

and their early appearance in ontogenesis; in *A. caespitosum* virginal individuals prevail (Fig. 4).

Density of individuals is not high: on the average from 7 to 13 individuals per 1 m^2. Spatial structure of coenopopulations is diffuse and is determined by vegetative propagation and pattern of spread in sandy substrate.

The analysis of development pattern of 16 onion species in different conditions of habitation and structure of their coenopopulations has revealed the diversity of populational strategy of the species studied.

Competitive species (*A. polyrhizum*, *A. bidentatum*)

A monocentric type of biomorph is characteristic of them. As an element of a coenopopulation first appears a seed individual, then a compact clone and at the end of ontogenesis – a particle. The ontogenesis is lengthy, belongs to type II, rarely III. Density of the individuals is maintained by: 1) propagation by seeds, and 2) mature or senile particulation. Spatial structure is diffuse [seed self-preservation and diffuse-group (mixed way of self-preservation)]. Ontogenetic spectra are right-side and bimodal, sometimes left-side as in *A. microdictyon* Prokh. (Smirnova 1987). The species possessing the above-mentioned characters are dominants and codominants in phytocoenoses or prevail in seasonal synusia.

Reactive species (*A. altaicum*, *A. galanthum*, *A. caesium*, *A. caeruleum*, *A. pallasii*)

Reactivity of onions manifests itself in high seed and vegetative productivity, quickened pace of growth and development in ontogenesis. They form monocentric and unmanifestly polycentric biomorphs. Adult compact and weakly diffuse clones represent a centre of the effect on the environment. Individuals of the reactive species develop by types III and V of the ontogenesis. Duration of the ontogenesis types varies. Density of individuals is high. Ontogenetic spectra are left-side. Spatial structure is diffuse and diffuse-group. Reactive species, as a rule, dominate in disturbed phytocoenoses (*A. caeruleum*) and seasonal synusia (*A. pallasii*) or form monodominant communities on mounds like *A. altaicum*.

Tolerant species (most of onions: *A. prostratum*, *A. senescens*, *A. rubens*, *A. nutans*)

Monocentric and unmanifestly polycentric biomorphs are formed in them. A slow pace of growth and development in youth, mature and senile particulation increase duration of the ontogenesis of types II and IV. Self-preservation of coenopopulations occurs either by seeds or in a mixed way. In spite of high potential seed productivity, loss of sprouts hinders abundant replenishment of coenopopulations with young plants. In the coenopopulations of tolerant species absolute maximum in the bimodal spectrum is observed on the right. Diffuse-group and group spatial structures formed due to diffuse and compact clones existing for a long time allow tolerant species to withstand a competition with stronger species.

Tolerant species occupy, as a rule, a subordinate position in the phytocoenosis. Their density is not great, but abundance of adult individuals is enough for preservation of sustainable status of coenopopulations.

Tolerant-reactive species (*A. tulipifolium*, *A. fetisowii*, *A. obliquum*, *A. sabulosum*, *A. caespitosum*)

They form monocentric and manifestly polycentric biomorphs. Reactivity shows itself in active seed or only vegetative renewal. Ontogenesis of the species of type I is not lengthy. Vegetative propagation of

the species of type VI of ontogenesis is performed by deeply rejuvenated primordia capable to move in space. Tolerance of monocentric species is expressed in a slow pace of young individual development. Manifestly polycentric species have a lengthy ontogenesis; vegetative mobility is mainly determined by moving substrate. Ontogenetic spectra are of the left-side type; spatial structure is group and diffuse-group. Most of species of such behaviour are not competitive.

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Serpentine flora of some regions in Serbia

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Abstract. The investigations of serpentine flora have been carried out at some localities in the Western, Central and Southern Serbia. The aims of the researches were to point out serpentine endemics, serpentine site indicators and facultative serpentinophytes, to compare recent and previous investigations on serpentines and to establish the degree of similarity between serpentine floras. According to Sørensen's index of similarity the localities in Central and Western Serbia are the most like, while the localities in the southern and western part have the smallest degree of similarity. This is caused by geographical position, climate, geological characteristics, vegetation, etc.

Key words: facultative serpentinophytes, regional endemics, serpentine flora, serpentine site indicators, trans-regional endemics

Introduction

Serpentine is one of the important factors that influence floristic diversity. Besides the edaphic, the plant species-level response (autecology), and the plant community-level effect (synecology) are, after Brady & al. (2005), the most important factors for serpentine plant species growing and adaptation.

In the soils developed on serpentine, the chemical and physical characteristics are unfavourable for many plant species. Serpentine soils are often deficient in essential plant nutrients such as nitrogen (N), potassium (K) and phosphorus (P). Also serpentine soils are characterized by low calcium/magnesium ratios with Ca at significantly lower concentrations relative to surrounding areas (Brady & al. 2005). Due to these characteristics, serpentine distinguishes the presence of smaller number of plant species in comparison with limestone (Harrison & al. 2000; Batianoff & Singh 2001; Green & al. 2003). Plant species adapted to serpentine soils often possess somewhat distinct morphology from closely related species not adapted to serpentine sites (Cooke 1994). The edaphic specialization and plant adaptation to serpentine soils are phylogenetically and geographically widespread (Brady & al. 2005).

At the same time, high levels of genetic differentiation were detected between the populations within one region, as well as between the populations of different regions (Patterson & Givnish 2004). Plant populations growing on serpentine have strong divergent selection; the subsequent genetic differentiation of the populations renders them reproductively isolated, and in extreme cases, results in ecological speciation (Schluter 2001). Stevanović & al. (2003) indicate the importance of serpentine habitats as centres for floristic differentiation and speciation.

According to Stevanović & Janković (2001) plants growing on serpentine are serpentinophytes, specific adapt plant species and subspecies. Related to plants' preference for serpentine there are several groups of serpentinophytes: facultative serpentinophytes, significant serpentine site indicators and endemic species. Within endemics Stevanović & al. (2003) named three groups of endemics, also related to preference for serpentine: obligate serpentine endemics, facultative serpentine endemics and accidental serpentine endemics. According to the same authors, in the flora of Serbia there are 43 obligate serpentine endemics from three different categories related to their distributional range: trans-regional endemics (TRE), regional endemics (RE) and locally distributed (steno) endemics (LE).

In this paper serpentine flora of some regions in Serbia is presented. The investigations have been carried out in the western part of Serbia on Divčibare. After completed investigations of flora of Divčibare we compared serpentine flora of this area with flora of several other regions – in the western part of Serbia: Tara Mt, Ozren Mt near Sjenica and Zlatibor Mt; Cen-

tral Serbia: Goč Mt and Studena Mt near Kraljevo and southern part of Serbia: Kosovo and wider surrounding of Miruša River (Metohija). The total area or some parts of these regions are covered by serpentine. Comparing the flora of these regions we determined their degree of similarity.

Material and methods

The investigations of serpentine flora of Divčibare have been carried out during 4 years. The results of these investigations were compared with existing data of flora of several other regions in Serbia.

Collected plant materials were determined by standard floristic methods. The samples have been compared with exsiccates of Nature History Museum Herbarium (BEO) and Herbarium of Faculty of Biology (BEOU).

To compare serpentine flora of Divčibare and flora of the other investigated regions in Serbia available floristic literature about these regions was used.

For analysis of the similarity of flora of researched areas Sørensen's similarity index (Sørensen 1948) was used. This index was calculated whereby next equation:

$$IS_s = 2C/A+B \times 100$$

where **C** presents number of common species of flora of two area which are compared; **A** and **B** are the total species number from one, respectively the other locality. The index implies by the percentage (%).

Results

Serpentine is one of the very important factors that influence floristic diversity of investigated areas. Stevanović & al. (2003) indicate the importance of serpentine habitats as centres for floristic differentiation and speciation. Serpentine sites are distinguished by high endemic species presence.

According to recent researches (Popović 2005) flora of Divčibare consist of 413 taxa. Among them there are 4 obligate serpentine endemics from trans-regional endemics (TRE) category: *Gypsophila spergulifolia* Griseb., *Alyssum markgrafii* O.E. Schulz ex Markgr., *Potentilla australis* Krašan subsp. *malyana* Novák and *Euphorbia glabriflora* Vis. The other serpentinophytes belong to the groups of facultative serpentinophytes and significant serpentine site indicators. The species *Cheilanthes marantae* (L.) Domin, *Asplenium cuneifolium* Viv. and *Echium rubrum* Jacq. are significant serpentine site indicators. Facultative serpentinophytes of flora of Divčibare are: *Teucrium montanum* L., *Potentilla arenaria* Borkh., *Chrysopogon gryllus* Trin., *Daphne blagayana* Freyer, *Erica carnea* L., *Scleranthus dichotomus* Schur, *Hypericum barbatum* Jacq., *Thymus jankae* Čelak., *Ranunculus montanus* Willd. f. *serpentini* Novák, *Veronica jacquinii* Baumg., *Plantago holosteum* Scop. and *Minuartia verna* (L.) Hiern.

Gajić (1984) named presence of about 630 taxa in flora of Goč Mt. Out of that number 4 taxa are trans-regional endemics (TRE): *Eryngium serbicum* Pančić, *Alyssum markgrafii*, *Potentilla australis* subsp. *malyana* and *P. visianii* Pančić, and 2 are obligate serpentine endemics from the regional endemics (RE) category: *Alyssum montanum* L. subsp. *serbicum* Novák and *Helleborus serbicus* Adamović. On Goč Mt 14 serpentinophytes are facultative and significant serpentine site indicators. The facultative serpentinophytes on Mt Goč are the same as in flora of Divčibare. Besides *Cheilanthes marantae*, *Asplenium cuneifolium* and *Echium rubrum*, the species *Asplenium adulterinum* Milde as serpentine site indicator is presented.

Flora of Tara Mt contains about 958 taxa (Gajić 1992). Among them 4 taxa are trans-regional endemics (TRE): *Halacsya sendtneri* (Boiss.) Dörfl., *Alyssum markgrafii*, *Potentilla australis* subsp. *malyana* and *Euphorbia glabriflora*. On Tara Mt facultative serpentinophytes and significant serpentine site indicators are the same as in flora of Divčibare.

According to Pavlović (1955) on Ozren Mt there are 311 taxa. Three taxa are trans-regional endemics (TRE): *Halacsya sendtneri*, *Stachys chrysophaea* Pančić and *Potentilla australis* subsp. *malyana*, and 1 is regional endemic (RE): *Alyssum montanum* subsp. *serbicum*. On Ozren Mt *Alyssum murale* Waldst. & Kit. var. *variabile* Nyár. is the only taxon from the group of significant serpentine site indicators. The other taxa are facultative serpentinophytes: *Cerastium moesiacum* Friv. f. *serpentini* Novák, *Scleranthus dichotomus* var. *serpentini*, *Asplenium adiantum-nigrum* L., *Thymus jankae* var. *subacicularis* Borbás, *Ranunculus montanus* f. *serpentini*, *Iris reichenbachii* Heuff.

Flora of Zlatibor Mt consists of about 355 taxa (Pavlović 1951). Among them 5 taxa are trans-regional endemics (TRE): *Halacsya sendtneri*, *Potentilla australis* subsp. *malyana*, *P. visianii*, *Verbascum bosnense* K. Malý and *Euphorbia glabriflora*, and 1 is obligate serpentine endemic from the regional endemics (RE) category: *Linaria rubioides* Vis. & Pančić. The significant

serpentine site indicators of Zlatibor Mt are: *Cheilanthes marantae*, *Echium rubrum* and *Euphorbia serpentini* Novák. The other taxa belong to the group of facultative serpentinophytes: *Scleranthus dichotomus* var. *serpentini*, *Asplenium adiantum-nigrum*, *A. cuneifolium*, *Ranunculus montanus* f. *serpentini*, *Iris reichenbachii*, *Plantago holosteum*, *Minuartia verna*, *Teucrium montanum*, *Daphne blagayana* and *Erica carnea*.

According to Tatić (1969), on Studena Mt there are 359 taxa. Out of that number 3 are trans-regional endemics (TRE): *Alyssum markgrafii*, *Potentilla australis* subsp. *malyana* and *Euphorbia glabriflora*. Species *Helleborus serbicus* is regional endemic (RE). In flora of Studena Mt there are 3 significant serpentine site indicators: *Cheilanthes marantae*, *Echium rubrum* and *Silene paradoxa* L. Facultative serpentinophytes of this region are: *Asplenium cuneifolium*, *Scleranthus dichotomus* var. *serpentini*, *Iris reichenbachii*, *Plantago holosteum*, *Minuartia verna*, *Teucrium montanum*, *Daphne blagayana*, *Erica carnea*, *Juniperus oxycedrus* L., *Carex humilis* Leyss., *Hypericum barbatum* and *Linum hologynum* Rchb.

Rexhepi (1979) named presence of 202 taxa on serpentine sites of Kosovo. Among them trans-regional endemics are: *Alyssum markgrafii*, *Fumana bonapartei* Maire & Petitm., *Halacsya sendtneri*, *Potentilla australis* subsp. *malyana*, *P. visianii*, *Euphorbia glabriflora* and *Stachys chrysophaea*. In Kosovo *Asplenium cuneifolium* Viv., *Cheilanthes marantae*, *Silene paradoxa*, *Alyssum murale* Waldst. & Kit. and *Echium rubrum* are significant serpentine site indicators. Facultative serpentinophytes are: *Silene longiflora* Ehrh., *Thymus jankae*, *Dorycnium germanicum* Rouy, *Carex humilis*, *C. laevis* Kit., *Hypericum barbatum*, *Scabiosa leucophylla* Borbás, *S. fumaroides*, *Veronica jacquinii*, *Linum hologynum*, *L. tauricum* Willd. var. *serbicum* (Podp.) Hayek, *Cotinus coggygria* Scop., *Poa badensis* Haenke, *Festuca pančićiana* (Hack.) Beldie, *Hippocrepis comosa* L., *Convolvulus cantabricus* L., *Potentilla hirta* L. var. *zlatiborensis* Novák, *Minuartia verna*, *Agropyrum cristatum* (Schreb.) P. Beauv., *Sedum album* L., *Plantago holosteum*.

According to the researches of The Institute for nature protection of Serbia (1996) in wider surrounding of Miruša River there are 359 taxa; 9 of them are trans-regional endemics: *Alyssum markgrafii*, *Aristolochia merxmuelleri* Greuter & E. Mayer, *Euphorbia glabriflora*, *Potentilla visianii*, *P. australis* subsp. *malyana*, *Genista hassertiana* (Bald.) Bald., *Fumana bonapartei*, *Halacsya sendtneri*, *Stachys chrysophaea*. The species: *Allysum montanum* subsp. *serbicum*, *Polygala döerfleri* Hayek, *Forsythia europaea* Degen & Bald., *Aster albanicus* Degen, *Centaurea kosaninii* Hayek and *Veronica andrasovszkyi* Jáv. are regional endemics. Three taxa are significant serpentine site indicators: *Asplenium cuneifolium*, *Cheilanthes marantae* and *Silene paradoxa*. Facultative serpentinophytes are: *Juniperus oxycedrus*, *Plantago holosteum* and *Teucrium montanum*.

Discussion

For the analysis of similarity of investigated floras one of important facts is that serpentine covers total or parts of these regions. Serpentine covers total area of Divčibare, Ozren Mt, Zlatibor Mt, Studena Mt and investigated part of Kosovo. The other researched regions Goč Mt, Tara Mt and wider surrounding of Miruša River distinguish geological variety. Except the localities in the southern part of Serbia, the climate of all investigated regions is temperate continental. In the southern part Mediterranean influence is more expressive.

On the basis of the Sørensen's index of similarity (IS_s) it is possible to determine resemblance of total flora of investigated regions.

Because of geological variety flora of Goč Mt is richer than flora of Divčibare. In spite of this, the number of common taxa of these two regions is big (314). On the basis of data about total number of taxa in flora of Divčibare and Goč and their common taxa, the Sørensen's index of similarity (IS_s) could be calculated. It amounts 60.21 %, and indicates high degree of similarity between the floras of these areas.

Thanks to geological variety Tara Mt is floristically richer than Divčibare. However, a significant degree of similarity of floras of Divčibare and Tara Mt exists, what the Sørensen's index of similarity shows. It amounts 47.70 %.

Flora of Ozren Mt is distinguished by smaller number of taxa in comparison with flora of Divčibare. The number of common taxa in floras of both regions is 146. The Sørensen's index of similarity is 40.33 %.

Divčibare is richer in taxa than Zlatibor Mt. 175 taxa are common in floras of both areas, so the Sørensen's index of similarity amounts 45.57 %, what points out significant degree of similarity of these floras.

The flora of Studena Mt near Kraljevo is poorer than the flora of Divčibare. As the common number of taxa for these regions is 179, the Sørensen's index of similarity is 46.98 %. There is big similarity between floras of Studena Mt and Divčibare.

The Sørensen's index of similarity for floras of Kosovo and Divčibare is 19.53 %. It indicates low degree of similarity between the floras of these areas. Such situation could be explained by different geographical position of Kosovo and Divčibare, and thereby different climate influences.

For wider surrounding of Miruša River and Divčibare, the Sørensen's index of similarity is 30.01 %. It indicates low degree of similarity between the floras of both areas. Namely, this low similarity degree is caused by geographical position of these regions and the most important factor is the isolation of wider surrounding of Miruša River.

In Table 1 different groups of serpentinophytes in all researched regions are presented.

On the basis of data from Table 1 we can conclude that obligate serpentine endemics (TRE and RE) are the most numerous at wider surrounding of Miruša River. In this region there are 15 obligate serpentine endemics, in Kosovo 7, on Goč Mt and Zlatibor Mt 6, on Divčibare, Tara Mt, Ozren Mt and Studena Mt 4 obligate serpentine endemics. The explanation for these numbers of obligate serpentine endemics is the isolation of wider surrounding of Miruša River. Namely, this area presents basin which is surrounded by mountains, and one of the features responsible for the distribution and abundance of obligate serpentine endemics is isolation. Number of regional endemics is low in all investigated areas except at wider surrounding of Miruša River.

The total number of serpentinophytes is approximately the same, except in the case of Kosovo. In Kosovo large areas are covered by serpentine. The climate influences on this region are different in comparison with the other investigated areas. The influence of Mediterranean climate is more expressive on Kosovo than on the other researched regions. In serpentine flora of Kosovo there are more Mediterranean floristic elements.

From Table 2 it could be noticed that there are 5 trans-regional serpentine endemics which grow only in one of investigated areas: *Gypsophila spergulifolia* (grows on Divčibare); *Eryngium serbicum* (Goč Mt); *Verbascum bosnense* (Zlatibor Mt); *Aristolochia merxmuelleri* and *Genista hassertiana* (wider surrounding of Miruša River). Species *Fumana bonapartei* is presented at two localities (Kosovo and wider surrounding of Miruša River). Only one taxon – *Potentilla australis* subsp. *malyana*, is presented at all investigated localities.

Table 1. Serpentinophytes in the floras of investigated regions.

Localities	TRE	RE	Serpentine site indicators	Facultative serpentinophytes	Σ
Divčibare	4	–	3	12	19
Goč Mt	4	2	4	10	20
Tara Mt	4	–	3	10	17
Ozren Mt	3	1	1	6	11
Zlatibor Mt	5	1	3	9	18
Studena Mt	3	1	3	12	19
Kosovo	7	–	5	20	32
Miruša	9	6	3	3	21

Abbreviations: TRE – trans-regional endemics; RE – regional endemics.

Table 2. Trans-regional endemics from investigated regions.

Taxa	D	G	T	O	Z	S	K	M
Gypsophila spergulifolia	+	–	–	–	–	–	–	–
Alyssum markgrafii	+	+	+	–	–	+	+	+
Potentilla australis subsp. *malyana*	+	+	+	+	+	+	+	+
Euphorbia glabriflora	+	–	+	–	+	+	+	+
Eryngium serbicum	–	+	–	–	–	–	–	–
Potentilla visianii	–	+	–	–	+	–	+	+
Halacsya sendtneri	–	–	+	+	+	–	+	+
Stachys chrysophaea	–	–	–	+	–	–	+	+
Verbascum bosnense	–	–	–	–	+	–	–	–
Fumana bonapartei	–	–	–	–	–	–	+	+
Aristolochia merxmuelleri	–	–	–	–	–	–	–	+
Genista hassertiana	–	–	–	–	–	–	–	+

Abbreviations: D – Divčibare; G – Goč Mt; T – Tara Mt; O – Ozren Mt near Sjenica; Z – Zlatibor Mt; S – Studena Mt; K – Kosovo; M – Miruša.

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Dendroflora species for biological recultivation by afforestation, remediation and post-mining landscaping

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Abstract. The paper presents the research results of biological recultivation by afforestation of deposols formed in the process of open cut coal mining in the Kolubara lignite basin. In addition to the dominant role of revitalisation of the areas degraded by mining, many tree and shrub species can also be used for the production of biomass by energy plantations and for cleaning up the contaminated soils from different pollutants (phyto-remediation).

Key words: afforestation, aquatic plants, biological recultivation, trees and shrubs, phytoremediation

Introduction

One of the most aggressive disturbances in the natural landscapes and ecosystems is open-cast exploitation of mineral raw materials, especially coal. Rapid changes of land use and the increasing damage to the ecosystem are the challenges that demand complex solutions, requiring the management of the entire space and the mass of overburden above the coal deposits. The land rehabilitation and restructuring offer numerous possibilities of land restoration, for the goals and demands of both human and natural communities.

To mitigate the damaging effects of open-cast mines, thermoelectric power stations and plants for coal processing, strip mining should be accompanied by the biological rehabilitation of minespoil banks and the reclamation of the degraded landscape. It should not be insisted on the authentic restoration of the previous forms, contents and functions, because other ambiance landscape values can also be created depending on the modified ecological, social and other conditions.

Short overview on biological recultivation in the world

The first attempts of recultivation of devastated areas and land management occurred in Germany in mid 19th century, and in USA and England at the beginning of the 20th century, but the scientific research and the more extensive application of recultivation and land management measures started after the Second World War. The most significant results in this field were achieved in Germany where more than 40 000 hectares of mined land were recultivated and managed. Along with Germany, there is also a series of other examples.

For example, in USA, this is regulated by the Federal Surface Mining Control and Reclamation Act of 1977. Thanks to these laws, there are very good examples of successfully revitalised lands: West Pennsylvania (Aharrah & Hartman 1973), South Indiana (Byrnes & Miller 1973; Medvick 1973; Miles & al. 1973), Pennsylvania (Davis 1973), Ohio (Funk 1973), Kansas (Geyer 1973), and West Virginia – Elkins (Hodgson & Townsend 1973; Thurman & Sencindiver 1986).

In Great Britain, there are special laws of land planning and management in England, Wales, and in Scotland. Recently, very significant results have been achieved in the reclamation of regions damaged by mining in the past – North England (Chadwick 1973), Yorkshire, North Derbyshire and Doncaster (Lindley & Mansfild 1979).

There are also remarkable works in Denmark (Schlätzer 1973).

In the former socialist countries, the most remarkable results (in addition to the former GDR) have been achieved in the former Czechoslovakia (Jonaš 1973).

The objective of this work was to present the results of biological recultivation of the lands degraded by opencast mining in the Kolubara–Tamnava basin, using methods and phytoremediation by trees for "soils" and by aquatic plants for polluted waters.

Material and methods

The research included two groups of problems:

a. **Biological recultivation, bioremediation and landscaping of the areas degraded by opencast mining** – survey of areas with different tree species and age classes, characteristics of deposols, development of tree species on two different types of deposols (diameter and heights at the age of 10 years);

b. **Purification of the aquatic ecosystems with different plant species** – insights of basic characteristics of constructed wetlands, their components and the best plants for water phytoremediation were given according to different literature data. Based on the previous studies and the experiments and studies by the authors of this paper, *Canna indica* L. was tested for its ability to remove different pollutants from water as an ornamental plant with a high potential of biomass production.

Results and discussion

a. Biological recultivation and phytoremediation by afforestation and post-exploitation landscaping

To mitigate the harmful consequences of the development of opencast mines, mining should be followed by the biological rehabilitation and by management of the degraded landscape in order to establish different vegetation on deposols.

Recultivation is a complex of mining-technical, engineering, reclamation, agricultural and forestry works, directed to the transformation of industrially degraded lands into an area favourable for agriculture and forestry, recreation, various forms of water storages, capital and housing construction and other purposes.

Recultivation generally means the re-establishment of plant communities in the area after coal mining. It is not necessary, or even possible to restitute by recultivation the previous landscape and to create the identical state as before coal extraction. Any forms of land use can be supported, either the existing ones or any other desired forms. It is important that the selected land use satisfies the demands of the local population, natural sites, as well as the properties of the newly formed soil, post-exploitation stratigraphy and the costs.

Biological recultivation in Serbia – the example of Kolubara–Tamnava basin

The area of Kolubara–Tamnava lignite basin is characterised by impressive works on revitalisation and management of degraded lands. Until 1997 the recultivated area amounted to 1298 hectares (Table 1). Forests occupy 74.7 % of the total recultivated area, farmland 23 %, orchards 1.3 %, and nurseries 1 %. Such initial ratios of different ecosystems are justified if it is recognised that forest ecosystems are the most significant for environmental protection. A more significant proportion of agricultural ecosystems are planned with the further process of coal extraction and biological recultivation.

The first afforestation of minespoil banks started in 1957 by planting predominantly black locust seedlings, and the first project of biological recultivation based on previous ecological, phytocoenological and soil studies was made in 1977 (Institute of Forestry, Belgrade).

Depending on micro-ecological conditions and the deposol type, numerous conifer and broadleaf species were implemented in afforestation.

Table 2 shows that the largest area is occupied by pure plantations of Austrian pine and Scots pine (27.7 %), and by group selection mixtures of broadleaves and conifers (23.2 %). The percentage of group mixtures of coniferous plantations is 11.1 % of total

Table 1. Biological recultivation in Kolubara Basin performed until 1997.

Type of recultivation	Mine fields			Totally
	"A"+"B"	"D"	"Tamnava–East"	ha
Recultivation by afforestation (forests)	**301**	**610**	**60**	**971**
Agricultural recultivation (farmland and arable land)	40	235	23	298
Orchards	6	11	–	17
Nurseries	–	12	–	12
Totally agricultural recultivation	46	258	23	327
TOTALLY BIOLOGICAL RECULTIVATION	**347**	**868**	**83**	**1298**

Table 2. Survey of areas by tree species and age classes.

Tree species	Totally		Age		
	hectares	%	< 5 year	6–10 year	> 10 year
Pure plantations of Austrian and Scots pines	262.00	27.7	88.00	34.00	140.00
Pure plantations of larch	33.00	3.4	10.00	6.00	17.00
Pure plantations of Douglas-fir	13.00	1.3	–	–	13.00
Pure plantations of Weymouth pine	21.00	2.1	–	–	21.00
Pure plantations of oaks	23.00	2.3	–	13.00	10.00
Pure plantations of maple	29.00	3.0	2.00	13.00	14.00
Pure plantations of black locust	82.00	8.4	7.00	–	75.00
Pure plantations of other broadleaves	78.00	7.9	48.00	20.00	10.00
Mixed stands of conifers	109.00	11.1	–	12.00	97.00
Mixed stands of broadleaves	93.00	9.6	15.00	40.00	38.00
Mixed stands of conifer and broadleaves	228.00	23.2	–	63.00	165.00
Totally	**971.00**	**100.0**	**170.00**	**201.00**	**600.00**

afforested area, and mixed plantations of broadleaves account for 9.6 %. Other broadleaves – lime, alder, Siberian elm, birch, etc., account for 7.9 %, black locust 8.4 % of the total afforested area, while other species account for 1.3 % to 3.4 % of the total plantation area.

Besides the mentioned species, the following species were experimentally planted in the process of biological recultivation by afforestation: *Gymnospermae* (*Pseudotsuga menziesii* (Mirb.) Franco, *Picea pungens* Engelm., *P. abies* (L.) H. Karst., *Larix europaea* Lam., *Pinus wallichiana* A.B. Jacks. & DC., *P. strobus* L., *P. ponderosa* Douglas ex P. & C. Lawson, *P. nigra* J.F. Arnold, *P. silvestris* L., *Chamaecyparis lawsoniana* (A. Murray) Parl.) and *Angiospermae* (*Liriodendron tulipifera* L., *Ulmus pumila* L., *Quercus borealis* Michx., *Q. robur* L., *Betula pendula* Roth, *Alnus glutinosa* (L.) Gaertn., *Populus* × *euramericana* (Dode) Guinier, *Tilia* sp., *Robinia pseudoacacia* L., *Acer negundo* L., *A. platanoides* L., *A. saccharinum* L., *A. pseudoplatanus* L., *Fraxinus excelsior* L., *F. americana* L.).

In the process of coal extraction with powerful excavators, the overburden was deposited mostly by non-selective methods. Previously natural soils were replaced by minespoil banks, originating from different geological layers. Pontian sands and the heavy Pliocene clay are most often found on the surface of the spoil banks. The "soils" formed in this way belong to the class of anthropogeneous – technogenic soils, type deposols.

They have very variable properties, which is the consequence of different initial characteristics of the deposited material. Generally, all of them are characterised by a very low amount of humus and organic matter and by a weak acid to neutral reaction.

A relatively great number of species is applied in the afforestation of deposol minespoil banks, not only because of the great variability of micro-ecological conditions on small areas, conditioned by unselective mining spoil dumping, but also because of the tendency to enrich the landscape aesthetics of the forest ecosystems, which should be a leisure and recreation zone in the post-exploitation period (Dražić 2000, 2001a, b, 2003; Dražić & al. 2003; Dražić & Bojović 2004).

Development characteristics of tree species in forest plantations

The survey of diameter measurements shows that, at the age of 10 years, on the deposol of lighter mechanical composition, alder attains the greatest values of diameter (12.3 cm), larch (11.7 cm), Weymouth pine and Douglas-fir (8.6 cm), Scots pine (8.3 cm) and birch (8.1 cm). Austrian pine has the smallest diameter (7.3 cm).

On the more heavily-textured deposol, the state is somewhat different. Alder also attains the largest diameter (10.0 cm), then Weymouth pine (9.2 cm) and Douglas-fir (9.1 cm). Larch is in the fourth position (8.6 cm), and finally Scots pine and Austrian pine with equal diameters (6.7 cm).

As for tree height, on the lighter-textured deposol, the greatest values are attained by larch (10.80 m),

then alder (10.00 m) and birch (9.40 m). Scots pine (7.10 m), Weymouth pine (6.85 m), Douglas-fir (6.50 m) and Austrian pine (4.60 m) have considerably lower heights.

On the more heavily-textured deposol, larch and alder have identical heights (10.00 m), then Weymouth pine (8.00 m), Douglas-fir (6.55 m), Austrian pine (4.85 m) and Scots pine (4.50 m).

Alder on both deposol types attains the same height. On the more heavily-textured deposol, Weymouth pine attains a notably superior height. Austrian pine and Douglas-fir attain a slightly greater height on this soil type.

All the implemented tree species in afforestation have a high survival percentage after planting, very good dynamics of diameter, height and volume development, but there are differences among the species on the identical deposols, and the differences in the development of each species on different deposols.

Monitoring of the development of planted trees and shrubs makes it possible for each type of deposol to make the optimal selection of species for afforestation.

Development characteristics of tree species in parks established on deposol

Along with the research of tree development characteristics in forest plantations, the study also included the dynamics of development of numerous species growing in groups in the park plantings on the deposol of lighter mechanical composition. This analysis is especially significant because of the potential widening of the spectrum of tree species in the further forest-biological recultivation.

The survey of diameter measurements shows that, at the age of 10 years, the largest-diameter coniferous species are: sequoia (10.7 cm) and Arizona cypress (10.6 cm), then Bhutan pine (10.4 cm), western yellow pine (9.4 cm), Atlas cedar (7.3 cm), Lawson's cypress (6.9 cm), incense cedar (6.3 cm), Caucasian fir (4.8 cm) and finally Serbian spruce (4.4 cm). Mean value of diameter for conifers is 7.9 cm.

Birch reached the largest diameter (11.9 cm) of all the broadleaf species, which is at the same time the largest diameter of all the study species in the park plantation. Birch is followed by Siberian elm (11.5 cm), American red oak (5.1 cm) and tulip tree (4.4 cm). Average value of diameter for broadleaf species is 8.2 cm.

In the study park planting, conifers reach averagely lower height (5.00 m) than broadleaf species (6.10 m). The highest tree species at this age is Atlas cedar (6.00 m), then Lawson's cypress (5.67 m), Bhutan pine (5.05 m), sequoia (5.00 m), incense cedar (4.03 m), western yellow pine (4.00 m) and Caucasian fir (3.00 m).

The highest broadleaf species is birch (8.00 m), then Siberian elm (6.33 m), red oak (5.50 m) and finally tulip tree (4.42 m).

The research results show that despite great difficulties caused by unselective casting of overburden and the absence of previous technical recultivation and planned shaping of the area for forest-biological recultivation, excellent results of successful afforestation and dynamics of development of different broadleaf and conifer tree species have been achieved.

It is possible to create extraordinary anthropogenic forest ecosystems, of rich colours, multi-functional values, enriched by aquatic and meadow-grassland ecosystems, which make an excellent base of further spontaneous evolution of the wealth of plant and animal biodiversity.

In the past decades many researchers have used different tree species as vegetation cover of the degraded lands (post-mining site reclamation) to remove some toxic substances. The tree species used for these purposes prevent pollution spreading by wind and water, both from the land surface and the transport of contaminants in the deeper layers of the degraded soils (Pulford & Watson 2003). It is reported that the plants growing on polluted soils have about 20–50 % lower biomass than the control. Although plant growth on the polluted soil is lower, such plants could be used both for cleaning up the soil and for the production of biomass that would subsequently be used for energy (McElroy & Dawson 1986). The main characteristic of trees that makes them suitable for phytoremediation is their large biomass, both above and below ground. Fallen leaves, dead tree roots and the root exudates add the organic matter to the surface layers, promoting nutrient cycling, soil aggregation and water holding ability. The transpiration stream removes large amounts of water from the soil, decreases the downward flow through the soil, and so reduces the leaching losses. The growing of trees in degraded lands influences positively the physical and chemical stabilisation of the soils. The most frequently applied trees are: willow, poplar, and many other tree species. The promising solution is the use of fast-growing species,

a short rotation coppice system with harvests every 5 years. The large willow breeding programme in Sweden and the UK could produce different clones that have suitable characteristics for phytoremediation, as well as high biomass production, high metal uptake and translocation to the above-ground parts of trees, and tolerance. The plants, on the other hand, increase the diversity, activity and multiplication of different soil microorganisms which have a very important role in soil remediation. The symbiotic life of plants and microorganisms enhances the potential remediation ability of both. The damaged lands in most cases have the problems with polluted water. To prevent the spreading of polluted water to the surroundings, it is necessary to use water plants which can clean up such water.

b. Possibility of purifying aquatic ecosystems by different plant species

Long-term practice of removing heavy metals and other pollutants from industrial wastewater was often reduced to processes like precipitation and flocculation followed by sedimentation and disposal of removed sludge (Ensley 2000). With the ecological awareness raising and with the need for new water treatment technologies more harmonised with the environment, the scientists and researchers have found many alternative solutions which can help in management, conservation and purification of waters. One of the new technologies are the constructed wetlands in which the removal of pollutants is achieved by different natural processes (House & al. 1999; Verhoeven & Meuleman 1999). In these alternative systems, the organic and inorganic matter, nutrients, pathogens, heavy metals and other pollutants (Knight & al. 1993; Naranjo & al. 1993; Peterson & Teal 1996; Redding & al. 1997) are removed from wastewater thanks to the symbiotic relationships between their basic components – aquatic plants (Peterson & Teal 1996), microorganisms (Perkins & Hunter 2000), algae, substrate and water.

Aquatic plants together with microorganisms make the core of these systems of alternative wastewater purification. They stabilise the substrate and slow down the water velocity. They eliminate pollutants from wastewater with their roots extracting mineral materials directly from water. The removed elements such as carbon, nutrients and trace elements are then incorporated in plant tissues. These plants aerate water as well, by oxygen input directly from the atmosphere to water through their stems and roots. Their stems and roots also provide the sites for microbial attachment.

Upon accumulating heavy metals and other pollutants, the plants are harvested, removed and disposed to appropriate places (Flathman & Lanza 1998; Zhu & al. 1999). Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov & al. 1995; Flathman & Lanza 1998). Dushenkov & al. (1995) explain that the translocation of heavy metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of the contaminated plant residues needing disposal. In contrast, Zhu & al. (1999) suggest that the efficiency of the process can be increased by using plants which have a higher ability to absorb and translocate metals within the plant.

Dushenkov & Kapulnik (2000) describe the ideal plants for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable for plants to produce significant amounts of biomass or root surface area.

The most often used aquatic plants in constructed wetlands and other plants used for phytoremediation of polluted waters are: *Phragmites communis* Trin., *Schoenoplectus lacustris* (L.) Palla, *Typha latifolia* L., *Carex* spp., *Iris pseudoacorus* L., *Caltha palustris* L., *Lemna minor* L., *Sagitaria sagittifolia* L., *Scirpus palustris* L., *Canna indica* L., *Lythrum salicaria* L., *Juncus effusus* L., *Menyanthes trifoliata* L., *Symphytum officinalis* L., *Mentha aquatica* L., *Alisma plantago-aquatica* L., *Gladiolus palustris* Gaudin, *Phalaris arundinacea* L., *Eichhornia crassipes* (Mart.) Solms, *Populus* spp., *Salix* spp., *Taxodium distichum* (L.) Rich., etc.

For the purpose of our experiments we have chosen *Canna indica* (Indian shot), an herbaceous perennial with the potential of removing the pollutants from water as well from the air and soil. *Canna indica* is grown hydroponically in nutrient solutions with different concentrations of lead. It is tested for biomass production, uptake of nutrients and heavy metals, and other significant parameters. Some preliminary results are derived and we expect that the results at the end of the growth season will show that *C. indica* can be used in Serbia not only as an ornamental plant, but also as a plant with a great potential of biomass production and with a potential of removing heavy metals and other pollutants from water.

Conclusions

There is a large number of different plant species which are suitable for biological recultivation of the areas degraded by opencast mining, remediation of contaminated soils, and treatment of wastewaters and conservation of aquatic ecosystems.

In addition to a great contribution in revitalising the damaged sites, to different degrees and in different ways, recultivation can also contribute to the designing of aesthetically pleasing landscapes with great ambience values and more versatile land uses.

Acknowledgements. The paper is a result of the research project financed by the Ministry of Science and Environmental Protection of the Republic of Serbia.

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The vascular flora of *Quercus ithaburensis* ssp. *macrolepis* (*Fagaceae*) forest remnants found in Thrace (NE Greece)

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Abstract. The present research investigates the vascular flora of the unique valonia oak (*Quercus ithaburensis* ssp. *macrolepis*) forest remnants found in Thrace (Northeastern Greece). These well-defined forest remnants comprise pure valonia oak stands as well as mixed with *Juniperus excelsa*. Vascular taxa from both stand types were collected during the year 2005. The floristic catalogue provides information about the collection sites, life duration, life form and chorological distribution of each taxon. According to the life duration and life form spectra most species are annual therophytes. The chorological spectra show that Mediterranean and sub-Mediterranean elements are predominant.

Key words: chorological spectrum, Greece, life duration, life form, Thrace, valonia oak

Introduction

The Mediterranean endemic *Quercus ithaburensis* Decne. ssp. *macrolepis* (Kotschy) Hedge & Yalt. (valonia oak) is found in xerothermic sites of various soil types (Pantera 2001). Historical evidence bears witnesses of extended valonia oak forests dominating in low or medium elevated areas, i.e. areas experienced intense anthropogenic activities. Inevitably, the anthropogenic impact on distribution of valonia oak was dramatic, resulted in severe limitations of its expansion (Papadopoulos & al. 2002). Specifically, in the region of Thrace (Northeastern Greece) pure valonia oak forests assemble in the lowlands and semi-mountainous areas of Nipsa and Doriko (prefecture of Evros), occupying a total area of 500 ha. There are also isolated individual trees spread in the lowlands of Alexandroupoli, Palagia, and Avanta (prefecture of Evros), and agricultural lands of Proskynites, Nea Santa and Gratini (prefecture of Rodopi) (Pantera & Papanastasis 2003).

Research on the flora of forest habitats in Greece is limited (Eleftheriadou 1992; Tsiripidis & Athanasiadis 2003). For valonia oak forests the only available data are sporadically found in phytosociological studies. The main objectives of the study were to record and analyse the floristic composition of valonia oak forests found in Evros, which constitutes one of the northernmost limits of their expansion in Greece and Mediterranean as well. Floristic catalogue is the main deliverable of the present work.

Material and methods

The research was conducted in the valonia oak forest, found close to Nipsa and Doriko (40°56'26" N, 26°00'43" E and 40°55'26" N, 25°58'20" E) north of Alexandroupoli (Thrace, Northeastern Greece) (Fig. 1). Plant material was collected from seven sites of Nipsa and Doriko areas (Table 1) in May 2005.

The climate is characterized by a 4.5-months xerothermic period (mid of May – end of September) and according to the Emberger's bioclimatic consideration the winters are sub-humid and rainy. The parent rock material is mainly limestone of the Perirodopian zone, and the soils are of loam to clay-loam type. In the study area, valonia oak is found on hilly areas (158–223 m alt.) of mostly southern to eastern exposition, and in gently slopes surfaces (10–35 %). There are evidence that valonia oak forests were once spread in the cultivated, plain areas that surround the hills; nowadays, only aged and declined remnant trees, but of imposing presence, are sporadically found in these areas.

Vegetation is characterized by the dominance of *Q. ithaburensis* ssp. *macrolepis*, occasionally found in mixed stands with *Juniperus excelsa*.

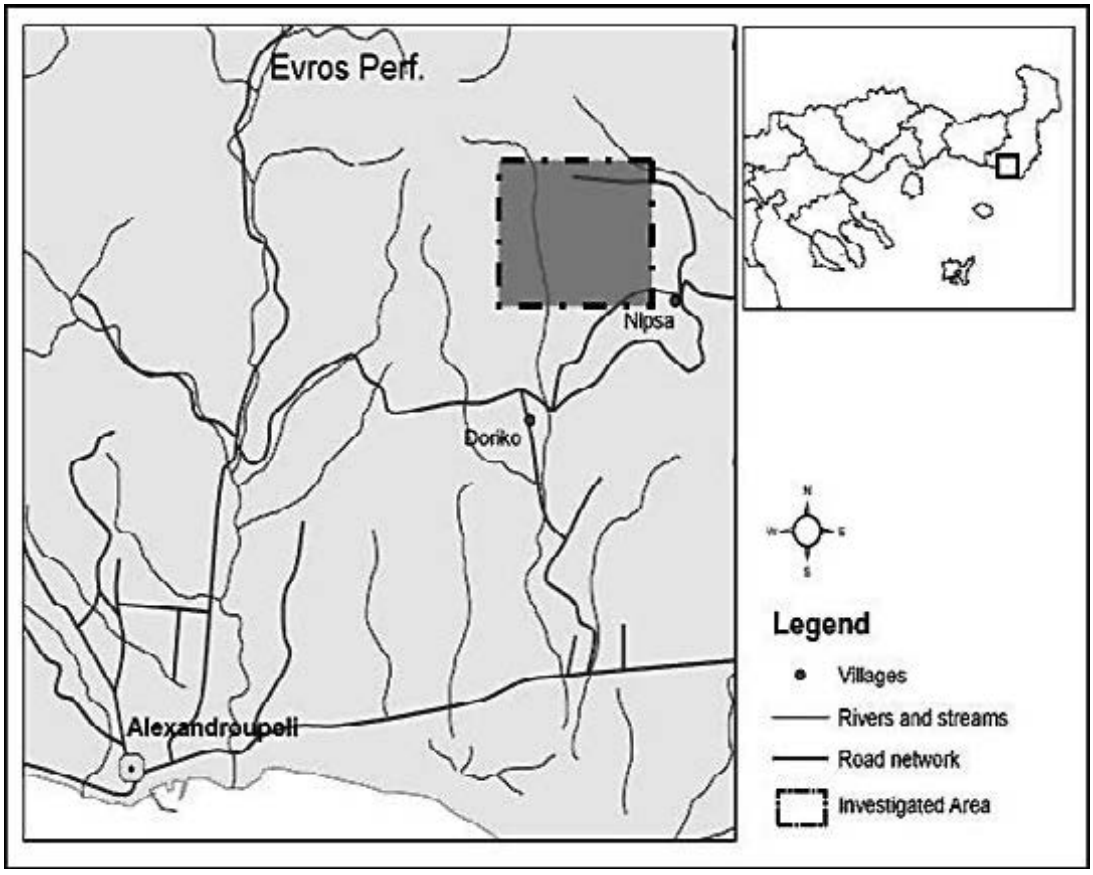

Fig. 1. Location of the research area.

Table 1. Location of plant collection sites.

	Collection sites						
	1	2	3	4	5	6	7
Latitude (N)	40°56'26"	40°56'18"	40°56'21"	40°56'24"	40°56'30"	40°56'25"	40°55'26"
Longitude (E)	26°00'43"	26°00'33"	26°00'28"	26°00'08"	25°59'87"	25°59'52"	25°58'20"
Altitude (m a.s.l.)	205	205	205	223	229	223	158

The identification of taxa was made by means of *Flora Europaea* (Tutin & al. 1968–1980, 1993) and *Flora Hellenica* (Strid & Tan 1997, 2002).

Families, genera and species were arranged alphabetically within 4 major groups of vascular plants, viz. *Pteridophyta*, *Gymnospermae*, *Dicotyledoneae* and *Monocotyledoneae*. Species nomenclature follows *Flora Hellenica* (Strid & Tan 1997, 2002), *Med-Checklist* (Greuter & al. 1984–1989), *Mountain Flora of Greece* (Strid 1986; Strid & Tan 1991), and *Flora Europaea* (Tutin & al. 1968–1980, 1993). Chorological origin was determined via the Oberdorfer's identification system for Europe (Oberdorfer 1990).

Information about (a) collection site (in parentheses), (b) ecological form, (c) biotic form and (d) phytogeographical distribution (codified by Oberdorfer) accompanies each identified taxon of the floristic catalogue. Chorological spectrum of the recorded taxa was constructed based on Raunkiaer's method (Raunkiaer 1910). In the final stage, the typical floristic catalogue was constructed.

Results and discussion

The selected data revealed that the flora of valonia oak forest in Thrace consists of 131 taxa, 36 families and 98 genera. The group of dicotyledonous plants comprises the majority of the families (28), followed by the monocotyledonous (5), gymnosperms (2) and pteridophytes (1) (Table 2).

The majority of the plants are annual herbs (42.8 %), followed by perennial herbs (32.8 %) and woody plants (21.4 %) (Table 3). The dominance of annual plants, in contrast to other forest vegetation units of Northern

Greece (Tsitsoni & Karagiannakidou 1992; Theodoropoulos & Athanasiadis 1993; Eleftheriadou & al. 1998; Chasapis & al. 2004; Fotiadis & Athanasiadis in press), indicates that the (present) valonia oak forest in Thrace is degradated. The dominance of annual plants is probably due to the relatively low tree canopy (30–70 %) and shrub canopy (30–70 %) coverage. Also, the percentage of woody plants (21.4 %), in the life duration spectrum, is in accordance with the typical forest character of the collection sites. Their percentage becomes higher (27.8 %) if the species stability in the seven collection sites (in terms of Tüxen & Ellenberg 1937) is taken under consideration (perennial herbs: 37.6 %, annual herbs 31.8 %, and biennial herbs: 2.8 %).

Table 2. Floristic catalogue.

Pteridophyta	
Polypodiaceae	*Asplenium ceterach* L. subsp. *ceterach*: (1, on rocks) P, H, med-smed (subatl)
	A. onopteris L.: (5, on rocks) P, H, med (-atl)
	A. trichomanes L. subsp. *trichomanes*: (1, on rocks) P, H, eurassubozzean
	Pteridium aquilinum (L.) Kuhn subsp. *aquilinum*: (5, on rocks) P, G, (no-)eurassubozean
Spermatophyta	
Gymnospermae	
Cupressaceae	*Juniperus excelsa* M. Bieb.: (1-6) Ar, P, osmed-kont
	J. oxycedrus L. subsp. *oxycedrus*: (1-7) Fr, P, med-smed
Pinaceae	*Pinus halepensis* Mill. subsp. *brutia* (Ten.) Holmboe: (2) Ar, P, omed
Angiospermae	
Dicotyledoneae	
Aceraceae	*Acer campestre* L.: (5) Fr/Ar, P, smed-gemabkont
	A. monspessulanum L.: (1, 4) Fr/Ar, P, smed
Anacardiaceae	*Pistacia terebinthus* L.: (6) Fr, P, med
Betulaceae	*Carpinus orientalis* Mill.: (4) Ar/Fr, P, osmed
Boraginaceae	*Alkanna tinctoria* Tausch subsp. *tinctoria*: (5) P, H, med
	Lithospermum arvense L.: (1, 2) A, T, smed (gemabkont)
	L. purpurocaeruleum L.: (6) P, H, smed
	Myosotis ramosissima Rochel subsp. *ramosissima*: (7) A, T, smed-euras
Campanulaceae	*Campanula cervicaria* L.: (1-4) P, H, gemabkont-smed
Caprifoliaceae	*Lonicera etrusca* Santi: (2, 5) Fr, P, med
Caryophyllaceae	*Cerastium brachypetalum* Pers. subsp. *roeseri* (Boiss. & Heldr.) Nyman: (2) A, T, med-omed
	C. glomeratum Thuill.: (7) A, T(H), eurassubozean
	C. semidecandrum L.: (2) A (P), T (H), smed-subatl
	Dianthus pinifolius Sm. subsp. *pinifolius*: (2) P, H, osmed
	Silene italica (L.) Pers. subsp. *italica*: (1-4, 6-7) P, H, smed (gemabkont)
	Stellaria media (L.) Vill.: (1, 7) A(B), T(H), cosmo
	S. pallida (Dumort.) Piré: (7) A, T, smed-gemabkont
Cistaceae	*Cistus creticus* L. subsp. *creticus*: (1-6) Fr, P, med
	Helianthemum aegyptiacum (L.) Mill.: (1, 5) A, T, med-euras
	Tuberaria guttata (L.) Fourr.: (1, 5) A, T, med-smed-atl
Compositae	*Achillea millefolium* L. subsp. *millefolium*: (6, 7) P, H(Ch), no-eurassubozean
	Anthemis arvensis L. subsp. *arvensis*: (3) A, T, eurassubozean-med
	A. tinctoria L. subsp. *parnassica* (Boiss. & Heldr.) Franzén: (1, 3, 4, 6, 7) P, H, osmed
	Carthamus lanatus L. subsp. *lanatus*: (2-4, 6, 7) A, T, med
	Centaurea cf. *affinis* Friv. subsp. *affinis*: (1) P, H, balc-Rm
	Crepis foetida L.: (1, 4, 5) A, T, smed-subatl (kont)
	C. sancta (L.) Babc.: (2, 3, 7) A, T, med-smed
	Crupina vulgaris Cass.: (2) A, T, euras
	Filago vulgaris Lam.: (3) A, T, med-smed-euras
	Hieracium bauhini Besser: (1, 4) P, H, euras(kont)
	Lactuca serriola L.: (7) P(A), H(T), smed-euras

Table 2. Continuation.

	Lapsana communis L.: (2) A(P), T(H), eurassubozean-smed
	Leontodon crispus Vill. subsp. *crispus*: (1, 4, 5) P, H, eurassubozean(smed)
	Rhagadiolus stellatus (L.) Gaertn.: (5-7) A, T, med
	Taraxacum officinale agg.: (1, 4, 5, 7) P, H, no-euras(subozean)
Convolvulaceae	*Convolvulus althaeoides* L. subsp. *tenuissimus* (Sibth. & Sm.) Stace: (2, 3, 5, 7) P, H, osmed
Cruciferae	*Alyssum minus* (L.) Rothm.: (1, 3) A, T, med-kont
	A. umbellatum Desv.: (7) A, T, omed-osmed
	Calepina irregularis (Asso) Thell.: (1) A(B), T(H), med(kont)
	Clypeola jonthlaspi L. subsp. *microcarpa* (Moris) Arcang.: (2) A, T, med
	Draba muralis L.: (7) A(P), T(H), smed
	Erophila verna (L.) Chevall: (2, 5) A, T, euras
	Erysimum crassistylum Presl: (1, 3, 7) B, H, balc-it
	Sisymbrium officinale (L.) Scop.: (7) A, T, euras-smed
	Thlaspi perfoliatum L. subsp. *perfoliatum*: (1, 2) A, T, smed-euras
Euphorbiaceae	*Euphorbia myrsinites* L. subsp. *myrsinites*: (1-3, 7) P, Ch, (o)med
Fagaceae	*Quercus coccifera* L.: (4) Ar/Fr, P, med
	Q. ithaburensis Decne. subsp. *macrolepis* (Kotschy) Hedge & Yalt.: (1-7) Ar/Fr, P, omed
	Q. pubescens Willd.: (1-3, 5-7) Ar/Fr, P, smed
Geraniaceae	*Geranium columbinum* L.: (5) A, T, euras-smed
	G. molle L. subsp. *molle*: (1, 7) A, T, (med) smed (subatl)
	G. robertianum L. subsp. *purpureum* (Vill.) Nyman: (1-6) A, T, smed
Guttiferae	*Hypericum montbretii* Spach: (6) P, H, osmed-kont
	H. perforatum L.: (4) P, H, eurassubozean-smed
Labiatae	*Lamium amplexicaule* L. subsp. *amplexicaule*: (1, 6) A, T, euras-smed(med)
	Phlomis fruticosa L.: (2-5) Fr, P, med
	Prunella laciniata (L.) L.: (5) P, H, smed
	Salvia officinalis L.: (3) P, Ch, med
	Satureja vulgaris (L.) Fritsch: (5) P, H, euras-smed,circ
	S. juliana L.: (2, 3) P, Ch, med
	Teucrium capitatum L.: (1-3, 7) P, Ch, med
	T. chamaedrys L. subsp. *chamaedrys:* (1, 3, 4, 6, 7) P, Ch, smed-med
	Thymus longicaulis C. Presl: (1-3) P, Ch, osmed
Leguminosae	*Colutea arborescens* L.: (1, 6) Fr, P, smed
	Hippocrepis emerus (L.) Lassen subsp. *emeroides* (Boiss. & Spruner) Lassen: (3, 4, 6) Fr, P, (o)smed
	Lathyrus aphaca L.: (5, 7) A, T, omed-smed
	Medicago arabica (L.) Huds.: (7) A, T, med
	M. coronata (L.) Bartal.: (7) A, T, med
	M. tuberculata Willd.: (3) A, T, med
	Onobrychis aequidentata (Sm.) d' Urv.: (1, 2) A, T, omed
	Trifolium angustifolium L.: (1) A, T, smed
	T. arvense L.: (2) A, T, eurassubozean-smed
	T. campestre Schreb.: (4) A, T, smed-subatl
	T. hirtum All.: (7) A, T, smed
	T. stellatum L.: (1, 7) A, T, smed
	T. subterraneum L.: (1) A, T, smed
	Vicia hirsuta (L.) Gray: (2) A, T, euras-smed
	V. lutea L. subsp. *lutea*: (1, 6, 7) A(P), T(H), med-smed(-atl)
	V. tetrasperma (L.) Schreb.: (7) A, T, euras-smed
	V. villosa Roth subsp. *varia* (Host) Corb.: (4) A, T, smed-med
	V. villosa Roth subsp. *villosa*: (6) A, T, smed-euras(-kont)
Oleaceae	*Fraxinus ornus* L.: (1, 2) Ar, P, osmed
	Jasminum fruticans L.: (3, 6, 7) Fr, P, omed
	Phillyrea latifolia L.: (1-6) Ar/Fr, P, med

Table 2. Continuation.

Papavaraceae	*Fumaria kraliki* Jord.: (1) A, T, europkont
Polygonaceae	*Rumex patientia* L. subsp. *orientalis* (Bernh.) Danser: (1) P, H, osmed-gemabkont
Ranunculaceae	*Clematis flammula* L.: (3) Fr, P, smed
	Ranunculus constantinopolitanus (DC.) d'Urv.: (3-7) P, H, osmed
Rhamnaceae	*Paliurus spina-christi* Mill.: (1-3, 5-7) Fr, P, osmed
Rosaceae	*Crataegus monogyna* Jacq.: (6) Fr, P, smed (-subatl)
	Malus trilobata (Labill.) C.K. Schneider: (5) Fr, P, omed
	Potentilla recta L.: (5-7) P, H, omed (kont)
	Prunus spinosa L. subsp. *dasyphylla* (L.) C.K. Schneider: (5) Fr, P, smed-euras(subatl)
	Pyrus amygdaliformis Vill.: (3, 5-7) Ar, P, med
	Rosa canina L.: (2, 3, 5, 6) Fr, P, eurassubozean-smed
	R. gallica L.: (2) Fr, P, med-kont
	Rubus sanctus Schreb.: (7) Fr, P, med-smed
	Sanguisorba minor Scop.: (2, 4, 5) P, H, euras(med)
Rubiaceae	*Cruciata pedemontana* (Bellardi) Ehrend.: (1, 6) A, T, smed
	Galium aparine L.: (2, 3, 6, 7) A, T, euras(subozean)
	G. verum L. subsp. *verum*: (5, 7) P, H, euras
	Sherardia arvensis L.: (2, 3, 5-7) A, T, med-euras
Scrophulariaceae	*Verbascum humile* Janka: (3, 5-7) B (P), H, balc (Bu-Tu-Gr)
	V. cf. *longifolium* Ten.: (4) B (P), H, balc-it
	Veronica triloba (Opiz.) Wiesb.: (1-6) A, T, smed
Umbelliferae	*Eryngium campestre* L.: (1-3, 6) P, H, europkont-med-smed
	Orlaya daucoides (L.) Greuter: (1, 2, 4-7) A, T, med
	Scandix pecten-veneris L. subsp. *pecten-veneris*: (1, 5, 7) A, T, med-smed
	Tordylium maximum L.: (1) B (A), H (T), smed-med
Valerianaceae	*Valerianella pumilla* (L.) DC.: (1, 2) A, T, med
Violaceae	*Viola tricolor* L. subsp. *tricolor*: (2) A, T, prealp
Monocotyledoneae	
Araceae	*Dracunculus vulgaris* Schott: (1, 6) P, G, med
Cyperaceae	*Carex distachya* Desf.: (1, 3, 5) P, H, med
	C. flacca Schreb. subsp. *flacca*: (2-4, 6, 7) P, G, eurassubozean-smed
Dioscoreaceae	*Tamus communis* L. subsp. *communis*: (4, 5) P, G, smed (atl)
Gramineae	*Brachypodium sylvaticum* (Huds.) P. Beauv. subsp. *sylvaticum*: (3, 4, 7) P, H, eurassubozean-smed
	Bromus sterilis L.: (1, 5-7) A, T, smed
	Dactylis glomerata L.: (1, 3, 5-7) P, H, cosmo
	Hordeum murinum L. subsp. *leporinum* (Link) Arcang.: (3) A, T, med
	Poa bulbosa L.: (1, 4, 6, 7) P, H, med-smed (kont)
Liliaceae	*Asparagus acutifolius* L.: (1-7) Fr, Ch, med
	Muscari neglectum Guss.: (1, 3, 4, 7) P, G, med-smed
	Ornithogalum cf. *montanum* Cirillo: (1, 2) P, G, osmed
	Ruscus aculeatus L.: (1, 3-5, 7) P, G, smed

Table 3. Life-duration, life-form and chorological distribution of the flora of the valonia oak forests in Thrace.

Life duration	
Trees/shrubs	21.4 %
Perennial herbs	32.8 %
Biennial herbs	3.1 %
Annual herbs	42.8 %

Life form	
Phanerophytes	20.6 %
Chamaephytes	5.3 %
Geophytes	5.3 %
Hemicryptophytes	26.0 %
Therophytes	42.8 %

Chorological distribution	
Mediterranean	37.4 %
Sub-Mediterranean	35.9 %
Eurasiatic	17.6 %
Continental	2.3 %
Alpine	0.8 %
North	1.5 %
Cosmopolitical	1.5 %
Balkan	3.1 %

Therophytes (42.8 %) dominate the life-form spectrum, followed by hemicryptophytes (26 %) and phanerophytes (20.6 %) (Table 3). The high percentage of therophytes, seen in the life-form spectrum, reflects the degradation of the valonia oak forest. According to Tsitsoni & Karagiannakidou (1992), the high percentage of therophytes in the forests of *Pinus halepensis* is explained by the type of climate (sub-humid with mild winters). Also, phytosociological units of the *Quercetalia ilicis* vegetation zone or of the arid subzone of *Quercetalia pubescentis* show relatively high therophyte percentages, like in *Q. coccifera* shrublands (39 % hemicryptophytes, 30.9 % therophytes – Theodoropoulos & Athanasiadis 1993), in *Q. trojana* forests (39.56 % hemicryptophytes, 31.45 % therophytes – Eleftheriadou & al. 1998), and in *Q. pubescens* forests (40.51 % hemicryptophytes, 34.36 % therophytes – Fotiadis & Athanasiadis in press). In *Q. frainetto*, *Q. petraea* ssp. *medwediewii*, *Tilia tomentosa*, and *Fagus sylvatica* forests, found in colder areas than the investigated valonia oak forest, hemicryptophytes predominate over therophytes (Theodoropoulos & Athanasiadis 1993; Fotiadis & Athanasiadis in press).

Mediterranean (med, 37.4 %), and sub-Mediterranean (smed, 35.9 %) species dominate in the chorological spectrum, while Eurasiatic elements (euras, 17.6 %) are far in the third place (Table 3). Balkanic and sub-Balkanic species are as low as 3.1 %. This is probably due to the fact that the area of research is not isolated. A subsequent in-depth analysis of the chorological spectrum revealed that the 25.3 % of the Mediterranean (med) and sub-Mediterranean (smed) elements are of eastern origin, while none western elements are present in the valonia oak forest of Thrace. The latter result is in accordance to the east-Mediterranean character of the dominant species (*Q. ithaburensis* ssp. *macrolepis*).

Sub-Mediterranean elements are predominant in the oak forests of Northern Greece (Theodoropoulos & Athanasiadis 1993; Eleftheriadou & al. 1998; Fotiadis & Athanasiadis in press), while Mediterranean elements are predominant in the kermes oak (*Q. coccifera*) shrublands and alepo pine (*Pinus halepensis*) forests of Chalkidiki (Tsitsoni & Karagiannakidou 1992; Theodoropoulos & Athanasiadis 1993). It seems that the sub-Mediterranean elements dominate the forests of *Quercetalia pubescentis* zone, while Mediterranean elements dominate the forests of *Quercetalia illicis* zone – zones defined by Dafis (1973) and Athanasiadis (1986). Probably, the research area is found in the transient zone between *Quercetalia ilicis* and *Quercetalia pubescentis* zones, as the percentage difference of its Mediterranean and sub-Mediterranean elements is quite low (1.5 %).

Acknowledgements. This paper is a part of the research project "Ecology of valonia oak (*Quercus ithaburensis*) forests in Greece" in the framework of the program "Archimedes II–Environment – Aid of Researching Teams in the Technological Educational Institutes" (EPEAEK II) that are partly financed by the European Community Fund (ECF) at 75 % and by National Attendance at 25 %.

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New localities of the vulnerable species *Eriolobus trilobatus* (*Rosaceae*) in northeastern Greece

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Abstract. *Eriolobus trilobatus*, the only species of the monotypic genus *Eriolobus* (*Rosaceae*, *Maloideae*), is one of the rarest trees of the Greek flora and is listed as "Vulnerable" in the *Red Data Book of Rare and Threatened Plants of Greece*. It is an east Mediterranean species having a sparse distribution with several disjunctions. It extends from Lebanon and Syria, through Anatolia, to northeastern Greece and southeastern Bulgaria where the single European localities occur. In this area there are two confirmed reports for Greece and one for Bulgaria. The localities of five new discovered populations of *E. trilobatus* in Greece are presented in this paper as well as notes on habitat and vegetation of the sites.

Key words: plant distribution, threatened species

Introduction

Eriolobus trilobatus is the only species of the monotypic genus *Eriolobus* (*Rosaceae*, *Maloideae*) and one of the rarest trees occurring in Greece and Bulgaria, the countries where the European populations are found. It has been listed as "Vulnerable" in the *Red Data Book of Rare and Threatened Plants of Greece* (Phitos & al. 1995).

The taxonomic status of the species was rather ambiguous in the past as has been discussed by Browicz (1969), and the nomenclature chronologically included the following names: *Crataegus trilobata* Poir., *Pyrus trilobata* (Poir.) DC., *Sorbus trilobata* (Poir.) Heynh., *Eriolobus trilobatus* (Poir.) M. Roem., *Cormus trilobata* (Poir.) Decne. and *Malus trilobata* (Poir.) C.K. Schneid.

Eriolobus trilobatus, "bragania" according to the local name given in Evros, is a small, deciduous tree up to 10 m tall. For a species of the *Rosaceae* family, it is characterised by its late flowering in May–June and because of the large size of the white flowers (diameter up to 4 cm) it can easily be recognised during this period. It is a valuable tree due to the sweet edible fruits it produces late in September, which people pick and store.

From a chorological viewpoint *E. trilobatus* is an East Mediterranean element whose geographical distribution appears to be extremely interesting. It was discovered in 1787 by Labillardière in Lebanon and following this, during the 19th century it was recorded in isolated sites in Anatolia. Today, the known distribution of the species in Asia includes West and South Anatolia, Syria, Lebanon and North Israel, while in Europe its distribution encompasses the east section of Greek Thrace (Evros Prefecture) and southeastern Bulgaria (Browicz 1972; Vulev 1973; Boratyński & al. 1992), even though the indigenous status of the Bulgarian population has been under question (Terpó 1968).

The first European recording was in 1876 by Dingler (1883) who found the plant in Greek Thrace, west of Alexandroupolis, between the villages Makri and Maronia. Two more occurrences were discovered in the district of South Evros in 1961 (by Ball and Wagestaffe) and 1972 (by Stamatiadou), whereas the single Bulgarian stand was found in 1954 in the eastern Rhodope Mountains near the Greek border (Stojanov & al. 1955).

Browicz (1982) in his survey in Evros during June 1979 did not find the references made by Dingler, Ball and Wagestaffe, but was able to confirm the stand re-

ferred to by Stamatiadou north of the village Loutros in the vicinity of Pesani. In his survey he also discovered a new station of *E. trilobatus* 4 km southeast of Mesti.

In the present study, an initial recording was made of the locations of the European distribution of *E. trilobatus* and data were provided on its biotope.

Material and methods

The present recording was based on field-work carried out during the month of May in the years 2004–2006. Information relating to the existence of *E. trilobatus* was collected through interviews with people who work in the countryside and the forest, e.g. loggers, foresters, shepherds, etc. The recording of the co-ordinates for the positions of trees, groups of trees and small thickets was executed with the use of *Garmin* satellite navigator. The co-ordinates of the localities are provided in the Datum *hddd. ddddd* WGS 84.

Results and discussion

From the present research, five new areas of distribution were located and they are provided in Fig. 1, together with the three former reports. Detailed recordings of the populations (except Mesti) were also made (Fig. 2). In total, 71 locations of one or more individuals of *Eriolobus trilobatus* were recorded; they are presented in Table 1.

All locations are found in the collinar zone, in maquis or deciduous scrub or in open thermophilous oakwoods and pine forests.

Nipsa stand

The population is located north of the Nipsa settlement in coppice oakwood or mixed pine–oak stands on S, SE and E slopes between an altitude of 170–220 m. The substrate is acid siliceous and the dominant vegetation consists of low and open mixed *Quercus pubescens – Quercus ithaburensis* ssp. *macrolepis* stands or *Pinus halepensis* ssp. *brutia – Quercus pubescens – Quercus ithaburensis* ssp. *macrolepis* stands. In the understory *Phillyrea latifolia*, *Carpinus orientalis*, *Pistacia terebinthus*, *Juniperus oxycedrus*, *Arbutus andrachne* and *Cistus creticus* dominate, but also *Rhus coriaria*, *Paliurus spina-christi*, *Colutea arborescens*, *Pyrus amygdaliformis*, *Tamus communis*, *Asparagus acutifolius* and *Clematis vitalba* are present.

The individuals of *E. trilobatus*, which were observed, have a height of between 1–10 m and a diameter of between 2–45 cm.

Pesani I stand

The population is located north of the village of Loutros, near the Pesani settlement and is also referred by Browicz (1982). It is found on S, SW and W slopes between an altitude of 210 and 330 m. The substrate is acid siliceous and the dominant vegetation is low forest consisting of *Pinus halepensis* ssp. *brutia* and *P. nigra* ssp. *nigra* plantations and high, dense scrub of *Phillyrea latifolia*, *Arbutus andrachne*, *Rhus coriaria*, *Fraxinus ornus* and *Cistus creticus*. Also scattered *Quercus frainetto* individuals occur.

The individuals of *E. trilobatus*, which were observed, have a height of between 2.5–9 m and a diameter of between 4–40 cm.

Pesani II stand

The population, which consists of a few young individuals, is located near Pesani Bridge at an altitude of 380 m, in open scrub vegetation.

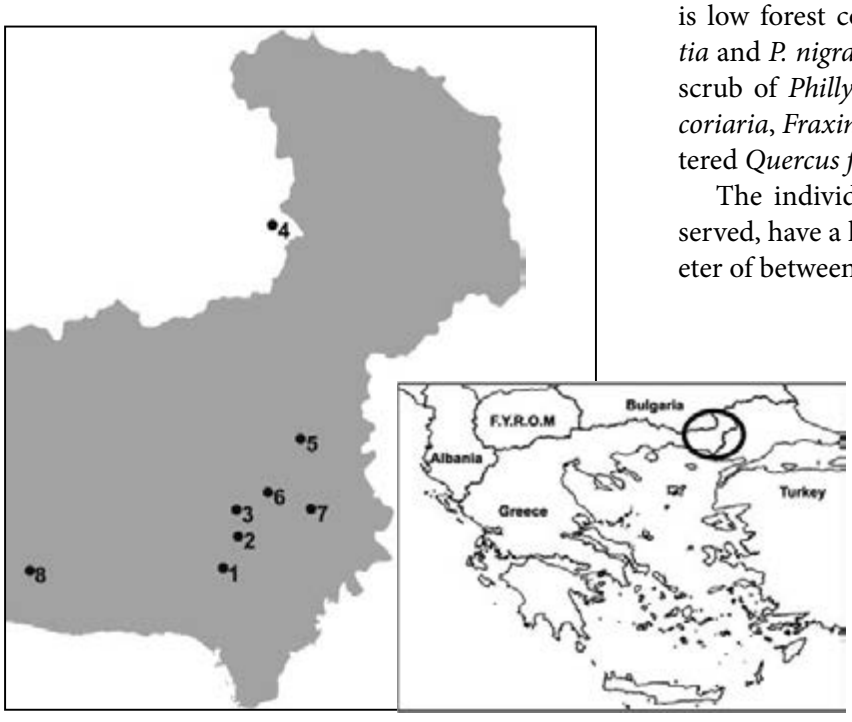

Fig. 1. Distribution of *E. trilobatus* stands: **1,** Nipsa; **2,** Pesani I; **3,** Pesani II; **4,** Mandritsa (Bulgaria); **5,** Dadia; **6,** Lefkimi I; **7,** Lefkimi II; **8,** Mesti.

Fig. 2. Detailed maps of *E. trilobatus* distribution in the recorded stands. Numbers as in Fig. 1.

The individuals of *E. trilobatus*, which were observed, have a height of between 3–4 m and a diameter of between 5–8 cm.

Bulgaria stand

The Bulgarian population consists of two mature individuals found in the vicinity of Mandritsa, at an altitude of 180–210 m.

Dadia stand

This stand includes one individual that occurs west of Dadia village at an altitude of 166 m. It grows in *Pinus halepensis* ssp. *brutia* forest where scattered *Quercus pubescens* individuals are found. The shrub layer consists of *Phillyrea latifolia* and *Pistacia terebinthus*.

The individual observed has a height of 9 m and a diameter of 16 cm.

Table 1. Location of the recordings.

ID	LAT	LONG	STAND
101	40.93421	26.01892	NIPSA
102	40.93715	26.02103	NIPSA
103	40.93736	26.02175	NIPSA
104	40.93749	26.02142	NIPSA
105	40.94096	26.02280	NIPSA
106	40.94390	26.02227	NIPSA
107	40.94523	26.02182	NIPSA
108	40.94714	26.02022	NIPSA
109	40.94816	26.01926	NIPSA
110	40.94723	26.02014	NIPSA
201	41.00197	26.05611	PESANI I
202	40.99353	26.05433	PESANI I
203	40.99430	26.05182	PESANI I
204	40.99243	26.04316	PESANI I
205	40.99247	26.04286	PESANI I
206	40.99249	26.04274	PESANI I
207	40.99281	26.04258	PESANI I
208	40.99267	26.04237	PESANI I
209	40.99282	26.04198	PESANI I
210	40.99301	26.04187	PESANI I
211	40.99299	26.04670	PESANI I
212	40.99304	26.04709	PESANI I
213	40.99106	26.04919	PESANI I
214	40.99097	26.04919	PESANI I
215	40.99093	26.04915	PESANI I
216	40.99085	26.04920	PESANI I
217	40.99090	26.04938	PESANI I
218	40.99082	26.04954	PESANI I
219	40.98974	26.05142	PESANI I
220	40.85480	26.07116	PESANI I
221	40.98898	26.05300	PESANI I
222	40.98933	26.05485	PESANI I
223	40.98880	26.05547	PESANI I
224	40.98861	26.05570	PESANI I
225	40.98847	26.05550	PESANI I
226	40.98844	26.05557	PESANI I
227	40.98816	26.05590	PESANI I
228	40.98784	26.05640	PESANI I
229	40.98761	26.05644	PESANI I
230	40.98756	26.05645	PESANI I
231	40.98626	26.05851	PESANI I
232	40.98623	26.05855	PESANI I
233	40.98603	26.05869	PESANI I
234	40.98613	26.05895	PESANI I
235	40.98690	26.05980	PESANI I
236	40.98795	26.05998	PESANI I
237	40.98822	26.06086	PESANI I
238	40.98827	26.06101	PESANI I
239	40.98829	26.06088	PESANI I
240	40.98828	26.06104	PESANI I
241	40.98814	26.06163	PESANI I
242	40.98826	26.06171	PESANI I
243	40.98776	26.06220	PESANI I
244	40.98770	26.06214	PESANI I
245	40.98721	26.06217	PESANI I
246	40.98591	26.06217	PESANI I
247	40.98663	26.06442	PESANI I
248	40.98762	26.06469	PESANI I
249	40.98868	26.06566	PESANI I
250	41.02681	26.05167	PESANI II
301	41.02840	26.05254	PESANI II
401	41.45936	26.12935	BULGARIA
402	41.44681	26.14551	BULGARIA
501	41.13191	26.18199	DADIA
601	41.05452	26.11154	LEFKIMI I
602	41.05351	26.11417	LEFKIMI I
603	41.05392	26.11594	LEFKIMI I
604	41.05246	26.11639	LEFKIMI I
605	41.05241	26.11644	LEFKIMI I
606	41.05083	26.11905	LEFKIMI I
701	41.02642	26.19934	LEFKIMI II

Lefkimi I stand

The population is located northwestward of the village of Lefkimi in low pine forest and high scrub. It is found on S and SE slopes between an altitude of 180 m and 270 m. The substrate is metamorphic, mainly schist and the vegetation in the tree layer consists of *Pinus halepensis* ssp. *brutia*, *Quercus pubescens* and scarce *Q. frainetto*, while in the shrublayer *Phillyrea latifolia*, *Pistacia terebinthus*, *Arbutus andrachne*, *Fraxinus ornus*, *Sorbus domestica*, *S. torminalis* and *Cistus creticus* dominate.

The individuals of *E. trilobatus*, which were observed, have a height of between 3–8 m and a diameter of between 4–15 cm.

Lefkimi II stand

This stand includes one mature tree, which is encountered at the edge of Lefkimi village at an altitude of 168 m. The individual observed has a height of 10 m and a diameter of 43 cm.

From the results of this research, it appears that *E. trilobatus* presents a relatively wide but particularly sparse distribution in the central and southern section of the Evros Prefecture. Its distribution generally follows the altitudinal zone of 150–350 m and perhaps reaches inside the borders of Bulgaria. Its population, according to reports by the local inhabitants, has decreased in the last decades, despite the fact that traditionally it has been protected from logging due to its edible fruits.

The location and mapping of the other existing European populations of the species in Greece and Bulgaria, the estimation of possible threats, as well as the planning of *in situ* and *ex situ* conservation measures must be set as objectives for future research.

Acknowledgements. The present study was funded by the research project "PYTHAGORAS II": KE 1329–1. The authors would like to thank Stefan Avramov for his contribution in the location of the Bulgarian population.

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Contributions to the flora of Ballıkayalar National Park (Gebze, Kocaeli / Turkey)

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Abstract. Ballıkayalar Valley is placed at the southern side of Kocaeli Peninsula in Turkey that has some national richness, natural beauty, interesting geomorphology and attractiveness. For this reason, it has been reserved as a national and natural park in 1994. Our study was carried out between 2002 and 2006 years. According to the results of floral study, 225 taxa belonging to 60 families were found. In the studying area, the most common taxa were belonging to the *Fabaceae*, *Poaceae*, *Asteraceae*, *Lamiaceae*, *Rosaceae* families. The important elements of phrygana, macchia and pseudomacchia are the most common in the district. It is important that the majority of the plants which are in the valley, belong to the *Lamiaceae*, *Rosaceae* and *Fabaceae* families, which include some medicinals.

Key words: Ballıkayalar Valley, flora, Turkey

Introduction

The studied area is located at the south of Kocaeli Peninsula and is 8-km distant from Gebze District (Fig. 1). Owing to its floral and natural attractiveness, Ballıkayalar locality has been distinguished as a Nature Park since the 1994. The debt of the valley is 70–80 m, its width is 40–80 m and its length is 1.5 km. While the elevations are nearly 90 m at the beginning of the valley, they are falling down to 50 m at the end. Ballıkayalar Stream consists of some small streams such as Aren Stream, Ayvalık Stream and Degirmen Stream. They begin at the slopes near Denizli village. Some sport activities such as rocky climbing, nature walking, caving and picnic taking are being made in the valley (Anonymous 1995; www.kultur.gov.tr).

Material and methods

For determining the flora of the district, some plant specimens were collected periodically from the studied area in various vegetation seasons between the years 2002 and 2006. These specimens were dried according to herbarium techniques and stuck on pieces of carton.

Plant materials were identified using *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000). The confirmation and comparison of identified plant specimens were done using both Marmara University Herbarium (MUFE) and Istanbul University Pharmacy Faculty Herbarium (ISTE) records.

At first, families, genera and species were ordered according to *Flora of Turkey and the East Aegean Islands* and then they were ordered alphabetically. Studied materials have been deposited in MUFE Herbarium.

Results and discussion

There are two papers related to Ballıkayalar Valley before our study. The first report is prepared in 2004; it includes only 64 taxa belonging to 20 families (Anonymous 2004). The second report is published in 2006, including 416 taxa belonging to 74 families (Akaydın & al. 2006).

In our study, 225 taxa belonging to 60 families have been determined between 2002 and 2006 as additional records for Ballıkayalar Valley.

The plant families, which are represented by the highest number of species, are listed as follows: *Fabaceae* 30, *Poaceae* 21, *Asteraceae* 19, *Lamiaceae* 15, *Caryophyllaceae* 12, *Rosaceae* 12. The genera with highest number of species are: *Trifolium* 8, *Sedum* 5, *Medicago* 5, *Silene* 4, *Geranium* 4, *Veronica* 4, *Euphorbia* 3,

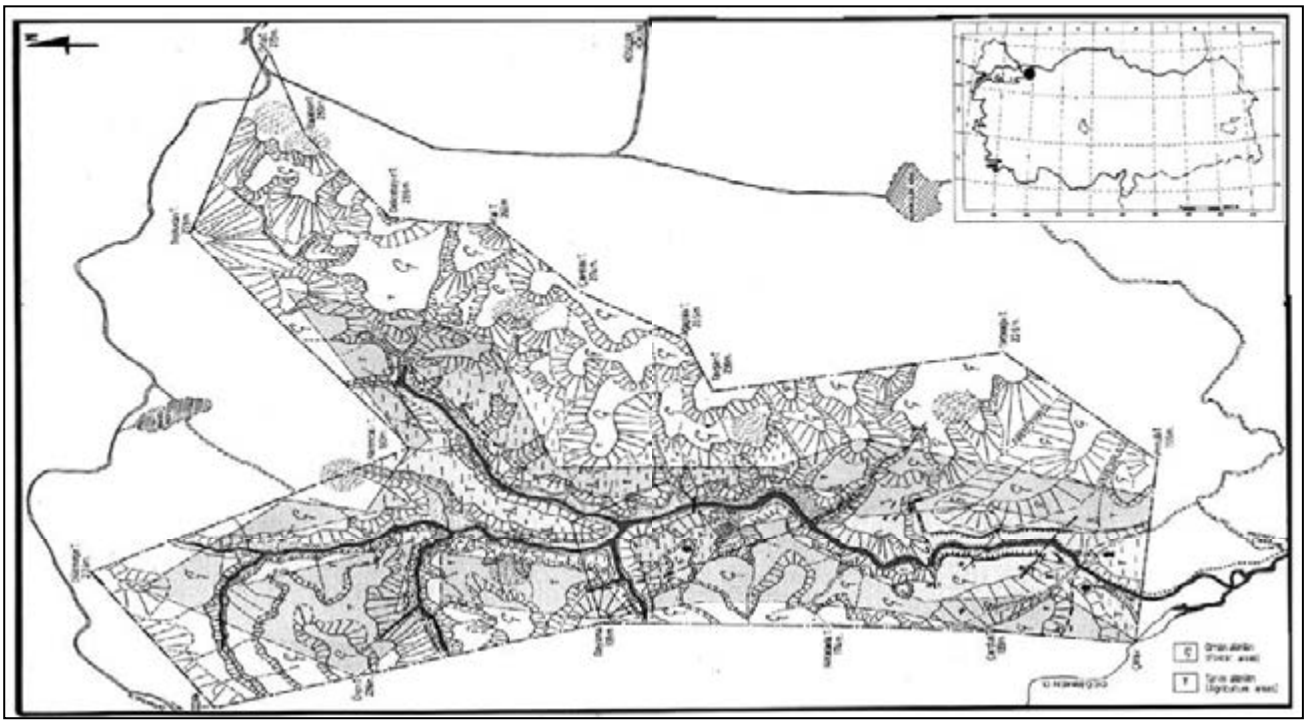

Fig. 1. The geographical position of Ballıkayalar Valley in Gebze, drown by Cengiz Kayacılar.

Taraxacum 3, *Hypericum* 3, and *Salvia* 3. Plants belonging to both Euro–Siberian and Mediterranean climates are the majority in the study area. These results indicate that the study area is also a transition zone between Mediterranean and Euro–Siberian regions.

The floristic list of Ballıkayalar Valley is:

Pteridophyta

Hypolepidaceae

1. *Pteridium aquilinum* (L.) Kuhn

Spermatophyta

Gymnospermae

Pinaceae

2. *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe

Angiospermae

Dicotyledoneae

Aceraceae

3. *Acer campestre* L. subsp. *campestre*
4. *Acer negundo* L.

Anacardiaceae

5. *Rhus coriaria* L.

Apiaceae (=Umbelliferae)

6. *Ammi visnaga* (L.) Lam.
7. *Caucalis platycarpos* L.
8. *Conium maculatum* L.
9. *Foeniculum vulgare* Mill.

Apocynaceae

10. *Vinca major* L.

Asclepiadaceae

11. *Periploca graeca* L.

Asteraceae (=Compositae)

12. *Anthemis cotula* L.
13. *Anthemis cretica* (L.) Nyman subsp. *pontica* (Willd.) Grierson
14. *Carthamus dentatus* Vahl
15. *Cichorium pumilum* Jacq.
16. *Cirsium arvense* (L.) Scop.
17. *Cirsium vulgare* (Savi) Ten.
18. *Conyza canadensis* (L.) Cronquist
19. *Crepis sancta* (L.) Babc.
20. *Echinops microcephalus* Sm.
21. *Echinops ritro* L.
22. *Leontodon asperrimus* (Willd.) Ball
23. *Leontodon cichoraceus* (Ten.) Sanguin.
24. *Sonchus asper* (L.) Hill subsp. *glaucescens* (Jord.) Ball
25. *Urospermum picroides* (L.) F.W. Schmidt
26. *Taraxacum officinale* Weber
27. *Taraxacum palustre* (Sm.) DC.
28. *Taraxacum vulgare* (Lam.) Schrank
29. *Tussilago farfara* L.
30. *Xanthium spinosum* L.

Boraginaceae

31. *Anchusa strigosa* Labill.
32. *Echium plantagineum* L.
33. *Lithospermum purpurocaeruleum* L.
34. *Myosotis sicula* Guss.
35. *Trachystemon orientalis* (L.) G. Don

Brassicaceae (=Cruciferae)

36. *Calepina irregularis* (Asso) Thell.
37. *Capsella rubella* Reut.
38. *Neslia apiculata* Fisch.
39. *Rorippa sylvestris* (L.) Besser
40. *Sinapis arvensis* L.

Campanulaceae

41. *Asyneuma rigidum* (Willd.) Grossh. subsp. *rigidum*

Caprifoliaceae

42. *Lonicera caprifolium* L.
43. *Lonicera etrusca* Santi var. *etrusca*
44. *Sambucus nigra* L.

Caryophyllaceae
45. *Cerastium diffusum* Pers. subsp. *diffusum*
46. *Cerastium glomeratum* Thuill.
47. *Dianthus leptopetalus* Willd.
48. *Minuartia anatolica* (Boiss.) Woron. var. *polymorpha* McNeill
49. *Minuartia verna* (L.) Hiern.
50. *Petrorhagia prolifera* (L.) Ball & Heywood
51. *Saponaria officinalis* L.
52. *Silene alba* (Mill.) Krause
53. *Silene dichotoma* Ehrh. subsp. *sibthorpiana* Rchb.
54. *Silene italica* (L.) Pers.
55. *Silene vulgaris* (Moench) Garcke
56. *Stellaria media* L.
Chenopodiaceae
57. *Atriplex tatarica* L. var. *tatarica*
58. *Chenopodium album* L. subsp. *album* var. *album*
Cornaceae
59. *Cornus mas* L.
Corylaceae
60. *Carpinus orientalis* Mill. subsp. *orientalis*
Crassulaceae
61. *Sedum acre* L.
62. *Sedum hispanicum* L. var. *hispanicum*
63. *Sedum rubens* L.
64. *Sedum sediforme* (Jacq.) Pau
65. *Sedum telephium* L.
Cynocrambaceae
66. *Theligonum cynocrambe* L.
Euphorbiaceae
67. *Chrozophora tinctoria* (L.) A. Juss.
68. *Euphorbia amygdaloides* L.
69. *Euphorbia chamaesyce* L.
70. *Euphorbia seguieriana* Neck. subsp. *niciciana* (Borbás ex Novák) Rech. f.
Fabaceae (=Leguminosae)
71. *Calicotome villosa* (Poir.) Link
72. *Cercis siliquastrum* L.
73. *Coronilla varia* L.
74. *Hippocrepis unisiliquosa* L. subsp. *unisiliquosa*
75. *Hymenocarpus circinnatus* (L.) Savi
76. *Lathyrus laxiflorus* (Desf.) Kuntze subsp. *laxiflorus*
77. *Lotus corniculatus* L.
78. *Lupinus angustifolius* L.
79. *Medicago disciformis* DC.
80. *Medicago minima* (L.) Bartal. var. *minima*
81. *Medicago radiata* L.
82. *Medicago rigidula* (L.) All. var. *rigidula*
83. *Medicago sativa* L. var. *sativa*
84. *Melilotus alba* Desr.
85. *Ononis viscosa* L. subsp. *breviflora* (DC.) Nyman
86. *Robinia pseudoacacia* L.
87. *Scorpiurus muricatus* L. var. *subvillosus* (L.) Fiori
88. *Securigera securidaca* (L.) Degen & Dörfl.
89. *Sophora jaubertii* Spach
90. *Trifolium angustifolium* L.
91. *Trifolium campestre* Schreb.
92. *Trifolium pauciflorum* d'Urv.
93. *Trifolium pratense* L. var. *pratense*
94. *Trifolium repens* L. var. *macrorrhizum* (Boiss.) Boiss.
95. *Trifolium repens* L. var. *repens*
96. *Trifolium resupinatum* L. var. *resupinatum*
97. *Trifolium scabrum* L.
98. *Vicia cracca* L. subsp. *stenophylla* Velen.
99. *Vicia hybrida* L.
100. *Vicia sativa* L. subsp. *nigra* (L.) Ehrh. var. *nigra*
Fagaceae
101. *Quercus infectoria* Olivier subsp. *infectoria*
102. *Quercus pubescens* Willd.
Geraniaceae
103. *Geranium columbinum* L.
104. *Geranium molle* L.
105. *Geranium robertianum* L.
106. *Geranium purpureum* Vill.
Hypericaceae (=Guttiferae)
107. *Hypericum bithynicum* Boiss.
108. *Hypericum calycinum* L.
109. *Hypericum montbretii* Spach
Juglandaceae
110. *Juglans regia* L.
Lamiaceae (=Labiatae)
111. *Ballota nigra* L. subsp. *anatolica* P.H. Davis
112. *Lamium amplexicaule* L.
113. *Mentha longifolia* (L.) Huds.
114. *Micromeria myrtifolia* Boiss. & Hohen.
115. *Prunella laciniata* (L.) L.
116. *Prunella vulgaris* L.
117. *Salvia frigida* Boiss.
118. *Salvia sclarea* L.
119. *Salvia viridis* L.
120. *Scutellaria albida* L. subsp. *albida*
121. *Sideritis montana* L. subsp. *montana*
122. *Stachys annua* (L.) L. subsp. *annua* var. *annua*
123. *Stachys byzantina* C. Koch
124. *Stachys sylvatica* L.
125. *Teucrium polium* L.
Lauraceae
126. *Laurus nobilis* L.
Linaceae
127. *Linum hirsutum* L. subsp. *anatolicum* (Boiss.) Hayek
128. *Linum tenuifolium* L.
Loranthaceae
129. *Viscum album* L. subsp. *album*
Moraceae
130. *Morus alba* L.
131. *Morus nigra* L.
Oleaceae
132. *Fraxinus excelsior* L.
133. *Olea europaea* L. var. *europoea*
134. *Olea europaea* L. var. *oleaster* (Hoffmanns. & Link) DC.
Onagraceae
135. *Epilobium parviflorum* Schreb.
Orobanchaceae
136. *Orobanche caryophyllacea* Sm.
137. *Orobanche minor* Sm.
Plantaginaceae
138. *Plantago media* L.
Platanaceae
139. *Platanus occidentalis* L.
Polygonaceae
140. *Polygonum equisetiforme* Sibth. & Sm.
141. *Polygonum lapathifolium* L.
142. *Rumex acetosella* L.
143. *Rumex scutatus* L.
Primulaceae
144. *Primula vulgaris* Huds. var.*vulgaris*
Punicaceae
145. *Punica granatum* L.
Ranunculaceae
146. *Helleborus orientalis* Lam.
147. *Nigella damascena* L.
Rhamnaceae
148. *Rhamnus alaternus* L.

Rosaceae
149. *Crataegus monogyna* Jacq. subsp. *monogyna*
150. *Cydonia oblonga* Mill.
151. *Fragaria vesca* L.
152. *Geum urbanum* L.
153. *Malus sylvestris* Mill. subsp. *orientalis* (A. Uglitzk.) Browicz var. *orientalis*
154. *Potentilla argentea* L.
155. *Potentilla reptans* L.
156. *Prunus mahaleb* L.
157. *Prunus spinosa* L. subsp. *dasyphylla* (Schur) Domin
158. *Pyrus amygdaliformis* Vill.
159. *Rosa sulphurea* Aiton
160. *Sanguisorba officinalis* L.

Rubiaceae
161. *Cruciata taurica* (Willd.) Ehrend.
162. *Galium aparine* L.
163. *Rubia tinctorum* L.

Rutaceae
164. *Ruta montana* (L.) L.

Salicaceae
165. *Populus* × *canadensis* Moench
166. *Populus tremula* L.
167. *Salix alba* L.
168. *Salix caprea* L.

Saxifragaceae
169. *Saxifraga hederacea* L. var. *hederacea*

Scrophulariaceae
170. *Digitalis ferruginea* L.
171. *Kickxia spuria* (L.) Dumort. subsp. *integrifolia* (Brot.) R. Fern.
172. *Scrophularia scopolii* Hoppe ex Pers. var. *scopolii*
173. *Verbascum xanthophoeniceum* Griseb.
174. *Veronica chamaedrys* L.
175. *Veronica cymbalaria* Bodard
176. *Veronica lysimachioides* Boiss.
177. *Veronica persica* Poir.

Simaroubaceae
178. *Ailanthus altissima* (Mill.) Swingle

Solanaceae
179. *Solanum nigrum* L. subsp. *nigrum*

Thymelaeaceae
180. *Daphne pontica* L.

Tiliaceae
181. *Tilia argentea* Desf. ex DC.

Ulmaceae
182. *Ulmus minor* Mill. subsp. *canescens* (Melville) Browicz & Ziel.

Valerianaceae
183. *Valeriana dioscoridis* Sibth. & Sm.
184. *Valerianella coronata* (L.) DC.
185. *Valerianella rimosa* Bastard

Verbenaceae
186. *Verbena officinalis* L.

Zygophyllaceae
187. *Tribulus terrestris* L.

Monocotyledoneae

Araceae
188. *Arum maculatum* L.

Cyperaceae
189. *Carex flacca* Schreb. subsp. *serrulata* (Biv.) Greuter
190. *Carex pendula* Huds.
191. *Cyperus longus* L.

Lemnaceae
192. *Lemna minor* L.

Liliaceae
193. *Asphodeline lutea* (L.) Rchb
194. *Allium paniculatum* L. subsp. *paniculatum*
195. *Allium peroninianum* Azn.
196. *Asparagus aphyllus* L. subsp. *orientalis* (Baker) P.H. Davis
197. *Ornithogalum umbellatum* L.
198. *Ruscus aculeatus* L. var. *angustifolius* Boiss.
199. *Ruscus hypoglossum* L.
200. *Smilax aspera* L.
201. *Smilax excelsa* L.

Orchidaceae
202. *Anacamptis pyramidalis* (L.) Rich.

Poaceae (=Gramineae)
203. *Aegilops biuncialis* Vis.
204. *Aegilops triuncialis* L.
205. *Alopecurus myosuroides* Huds. var. *tonsus* (Blanche ex Boiss.) R.R. Mill
206. *Avena sativa* L.
207. *Avena wiestii* Steud.
208. *Bothriochloa ischaemum* (L.) Keng.
209. *Briza humilis* M. Bieb.
210. *Briza maxima* L.
211. *Bromus scoparius* L.
212. *Bromus sterilis* L.
213. *Cynodon dactylon* (L.) Pers. var. *dactylon*
214. *Cynosurus echinatus* L.
215. *Echinochloa crus-galli* (L.) P. Beauv.
216. *Eragrostis minor* Host
217. *Hordeum murinum* L. subsp. *murinum* var. *murinum*
218. *Lolium perenne* L.
219. *Lolium rigidum* Gaudin var. *rigidum*
220. *Melica ciliata* L. subsp. *ciliata*
221. *Phleum montanum* C. Koch
222. *Poa trivialis* L.
223. *Setaria verticillata* L. var. *verticillata*

Typhaceae
224. *Typha angustifolia* L.
225. *Typha latifolia* L.

Acknowledgements. Thanks are due to Prof. Dr. Sabri Sümer for his kind help.

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SCIENTIFIC AREA D

FUNGAL DIVERSITY

Biodiversity and conservation in the Pigelleto, Mt Amiata (Italy)

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Abstract. The Pigelleto Natural Reserve is situated southeast of Mt Amiata (Siena, Central-Italy). It includes a relict nucleus of *Abies alba* at low altitude, probably an autochthonous ecotype. The high biodiversity level and conservation value of the area are supported by this contribution to the knowledge of vascular and mycological flora. The observations on vascular flora, well studied in the past, led to the discovery of few new elements. Among the species of macrofungi (nearly 160) collected for the first time during the last studies (2005–2006), there are some interesting entities because of their threat status.

Key words: macrofungi, Pigelleto Natural Reserve, Red Lists, vascular flora

Introduction

This work was carried out in the Pigelleto Natural Reserve situated on Mt Amiata (Fig. 1) as part of the Life04NAT IT/000191 Project concerning the conservation of the *Abies alba* Mill., probably an autochthonous ecotype. The study aims at improving the knowledge of the natural heritage which this area offers and the publication of the final data is in progress.

The Pigelleto Natural Reserve, established by the Tuscan Region in 1996, extends over 862 hectares in the municipal area of Piancastagnaio in the Province of Siena. It runs along the ridge which connects the volcanic cone of Mt Amiata to the calcareous group of Mt Civitella, at an altitude of 600 to 969 m.

The main object of our study is the silver fir, one of the most interesting natural components in the area under investigation. It constitutes natural woods together with deciduous species such as *Fagus sylvatica* L. and *Quercus cerris* L.

The fir woods of the Pigelleto are important because they represent the relict nucleus of probably autochthonous silver firs growing at a low altitude on a pre-Apennine massif (De Dominicis & Loppi 1992). In fact this species, even if assisted by man through silviculture, generally finds its optimal environment at higher altitudes in the Apennines (De Dominicis & Loppi 1992).

Fig. 1. Localization of the Pigelleto Natural Reserve.

In this work we analyse the data relative to surveys carried out in 2005–2006 aimed at increasing the knowledge of the natural heritage in the Pigelleto, in particular the vascular flora and macrofungi, intended as all fungi which produce visible fruit bodies of more than a millimetre in size (Arnolds 1981).

Material and methods

Observations were carried out from April 2005 to May 2006. The frequency of observation for macrofungi varied from a minimum of once a month in the periods of lesser fungi production to a maximum of three times a month in the autumn when, as usual in our region, the conditions for an abundant carpophore production were optimal.

For both plants and fungi, the study of the samples was carried out preferably on fresh material but also on dry samples as per the standard macro- and microscopic techniques.

The nomenclature of the vascular species mentioned follows Pignatti (1982), while that of the fungus species refers principally to the Italian check-list of Basidiomycetes (Onofri & al. 2005); in second place to the Dutch list (Arnolds & al. 1995), in particular for the Ascomycetes. Citations of authors follow Brummitt & Powell (1992).

The vascular and fungi species which were studied are preserved in the *Herbarium Universitatis Senensis* (SIENA).

Results

The vascular flora, of which we report only the new records, totals 412 species belonging to 230 genera. Of these, 407 were already registered in the past (Angiolini & al. 1994) and their presence was largely reconfirmed during the present research.

The species reported for the first time were *Carex remota* L., *Cephalanthera longifolia* (Huds.) Fritsch, *Epipactis microphylla* (Ehrh.) Sw., *Salix alba* L. and *S. caprea* L.

The past mycoflora list (Perini & al. 1995, 2004, 2005; Salerni & Perini 2003; unpubl. data), appears enriched with nearly 160 species of macrofungi collected for the first time during the recent studies (the publication of the final data is in progress). These include 36 mycetes interesting because of their status of threat.

Discussion

The many vascular flora species of interest to naturalists and conservationists which can be found in the Reserve have already been indicated and amply commented upon in Angiolini & al. (1994). Among the five new vascular species found in the Reserve it is interesting to note that two are tree species (*Salix alba* and *S. capraea*), raising the number of phanerophytes in the Reserve to 66 (16.01 % of the total flora). Both these species occur, together with *Carex remota,* in the areas with considerable water stagnation. The finding of *Cephalanthera longifolia* and *Epipactis microphylla* bring the number of taxa of the *Orchidaceae* family to 15. These are mostly species connected to forests, with a nearly total absence of orchid types which thrive in grasslands given the scarcity of open and sunny areas.

International experts in nature protection and conservation have set up a "*Red List*" of rare and endangered species of wild flora and fauna. The whole family of *Orchidaceae*, and not only single genera or species, are included in this List (Haber 1976). This happened because *Orchidaceae* – due to their rarity level and high endemizing capabilities – had been included in the red lists of endangered/protected species at world (CITES, Bern Convention) and national level (Conti & al. 1992, 1997) and protected under several international statements (IUCN 1994).

From a mycological point of view, among the nearly 160 species of macrofungi never collected before 2005 in the Pigelleto Natural Reserve, we found 36 species included in the *Red Lists* (or proposed for the *Red Lists*) of several European countries because of their rarity due to natural or anthropic intervention (Malta – Schembri & Sultana 1989; Holland – Arnolds & al. 1995; Norway – Bendiksen & al. 1997; Italy – Venturella & al. 1997; Estonia – Järva & al. 1998; Denmark – Stoltze & Pihl 1998; Austria – Krisai-Greilhuber 1999; Sweden – Gärdenfors 2000; Russia – Kovalenko 2000; Lithuania – Lygis 2000; Finland – Rassi & al. 2001). These are reported in Table 1.

Among these, *Amanita eliae* Quél., the ectomycorrhizal species linked to deciduous woods and *Pholiota flammans* (Batsch) P. Kumm., which grows on decomposing wood of conifers, are to be noted; they are the only two included in the preliminary list, drawn up by the Mycological Working Group of the Italian Botanical Society, of the 23 species considered rare and/or at risk in Italy (Venturella & al. 1997).

Table 1. List of fungi included in the *Red Lists* (or proposed for the *Red Lists*) of signal European countries.

	M.	H.	N.	I.	D.	E.	R.	A.	L.	S.	F.
Amanita eliae Quél.		×		×	×			×			
A. submembranacea (Bon) Gröger		×									
Antrodia xantha (Fr.) Ryvarden		×									
Astraeus hygrometricus (Pers. : Pers.) Morgan		×									
Clavulina rugosa (Bull.) J. Schröt.		×									
Clitocybe sinopica (Fr.) P. Kumm.					×						
Cortinarius castaneus (Bull.) Fr.		×									
C. multiformis Fr. var. *coniferarum* M.M. Moser		×									
C. rigens (Pers.) Fr.		×									
Craterellus cornucopioides (L.) Pers.		×									
Cystoderma carcharias (Pers.) Fayod		×									
Cystolepiota pulverulenta (Huijsman) Vellinga		×									
Entoloma clypeatum (L.) P. Kumm.								×			
Exidia recisa (Ditmar) Fr.		×									
Gymnopilus junonius (Fr.) P.D. Orton			×					×			
Helvella crispa (Scop. : Fr.) Fr.	×										
Hygrophorus eburneus (Bull.) Fr.		×									
Hysterangium stoloniferum Tul.					×						
Inocybe bongardii (Weinm.) Quél.		×									
I. cincinnata (Fr.) Quél. var. *major* (S. Petersen) Kuyper							×				
I. obscurobadia (J. Favre) Grund & D.E. Stuntz		×									
Lactarius blennius (Fr.) Fr.									×		
L. fuliginosus (Fr.) Fr.		×									
Mycena stipata Maas Geest. & Schwöbel					×						
Pholiota flammans (Batsch) P. Kumm.		×		×							
P. scamba (Fr.) M.M. Moser		×									
Pluteus romellii (Britzelm.) Sacc.							×				
Polyporus tuberaster (Jacq.) Fr.			×			×				×	
Psathyrella conopilus (Fr.) A. Pearson & Dennis			×								
Russula foetens (Pers.) Fr.		×									
R. postiana Romell							×				
Scleroderma verrucosum (Bull. : Pers.) Pers.										×	
Tapinella atrotomentosa (Batsch) Jbutara		×									
Trametes versicolor (L.) Lloyd											×
Tricholoma album (Schaeff.) P. Kumm.		×									
T. virgatum (Fr.) P. Kumm.		×									

Abbreviations: M. = Malta; H. = Holland; N. = Norway; I. = Italy; D. = Denmark; E. = Estonia; R. = Russia; A. = Austria; L. = Lithuania; S. = Sweden; F. = Finland.

The presence of this considerable group of species considered as at risk of extinction or anyway in danger in a number of countries, principally Holland (Table 1) emphasizes the conservation role of the Pigelleto. The majority of these species are abundant in the area being studied, a fact which may depend on their distributional area as well. Even so, we believe it opportune to constantly monitor their presence in the future in order to find any decrease in their presence and act to curb it by identifying and eliminating the disturbances which are causing it. Given the close connection between fungi and the environment in which they live, the conservation of the macrofungi biodiversity is directly linked to the protection of the habitat. For this reason the maintenance of the habitat in which the threatened fungi species were found is desirable, since their presence in the Red Lists of other European countries would lead us to suppose that they are particularly sensitive to natural and anthropic stress.

The area of the Pigelleto could therefore be considered as an optimal centre for the *in situ* conservation of species which are elsewhere at risk and it could fit into the Important Plant Areas (IPA) of the European Plant Conservation Strategy (Smart & al. 2002), which does not refer to the Kingdom *Plantae* alone.

Acknowledgements. We would like to thank Pier Giuseppe Montini of the Amiata – Val d'Orcia Consortium of Mountain Municipalities, Marcello Miozzo of DREAm Italia and Renato Scapigliati of Abies Alba Co-operative Society, for their kind collaboration and helpfulness.

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Ecology and distribution of species from genus *Tulostoma* (*Gasteromycetes*) in the Republic of Macedonia

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Abstract. This paper contains results on the subject of the ecology and distribution of the genus *Tulostoma* in the Republic of Macedonia. Insufficient data about the distribution of four species (*Tulostoma brumale*, *T. caespitosum*, *T. fimbriatum*, *T. melanocyclum*) have been published so far; therefore the aim of this work is to provide a more comprehensive review on the ecology and distribution of this systematic category in the Republic of Macedonia. The species *T. squamosum* is new for the mycobiota of the Republic of Macedonia. A total number of five species from the genus *Tulostoma* have been found.

Key words: distribution, ecology, reported species, R. Macedonia, *Tulostoma* spp.

Introduction

Systematic research on the genus *Tulostoma* Persoon 1974 : Persoon 1801 (*Basidiomycota*, *Gasteromycetes*, *Tulostomataceae*) has not been conducted up till now in the Republic of Macedonia, and there are rather few mycological papers concerned with individual species of this genus only, from one or two localities in the Republic of Macedonia. Publications making reference to individual species of *Tulostoma* genus are as follows: *T. brumale* (Rusevska & Karadelev 2004), *T. caespitosum* (Wright 1987), *T. fimbriatum* (Lindtner 1939; Pilát & Lindtner 1939) and *T. melanocyclum* (Tortić 1988). Consequently, the objective of this paper is to present a more comprehensive list about the ecology and distribution of the *Tulostoma* species in the Republic of Macedonia.

The distinctive features of this genus are the following: a typical *Tulostoma* gastrocarp comprises a spore-sac or peridium and a stem. The spore-sac is usually a globose-depressed to hemispheric receptacle. It develops hypogeously, and upon maturation of the spores the elongation of the stem pushes it above the soil surface, and at the same time an apical opening appears – the stoma (mouth) – that allows spore dispersion. The peridium is composed of two distinct layers, not always easy to distinguish: the exoperidium and the endoperidium. Gleba pulverulent, yellowish brown. Capillitium simple or branched, septate, eventually with swollen septa, where disarticulation usually takes place, showing thick walls without pores. Spores globose to ovoid, smooth or with different kind of ornamentation, such as spiny, warty, reticulate, etc.

Material and methods

The sources of this list are published papers, exsiccates deposited in different collections, and research notes of the present authors. Some of the specimens, as mentioned above, have been preserved at the Natural History Museum in Belgrade (BEO), Botanical Department, Faculty of Science in Zagreb (ZA) and in the National Collection of Fungi, FUNGI MACEDONICI (exs. MAK) at the Institute of Biology, Faculty of Natural Science, Ss Cyril and Methodius University in Skopje. All species without any fungaria marks in the text have been collected and determined by the present authors.

The identification of *Tulostoma* species has been made according to Pilát (1958), Jülich (1984), Breitenbach & Kränzlin (1986), Wright (1987), Miller & Miller (1988), Pegler & al. (1995), Hansen & Knudsen (1997), Calonge (1998) and Krieglsteiner (2000). In a few cases, the names of the species have been modified according

to *Index Fungorum* (2005). The species are reported alphabetically. Under each fungal species, data on the geographical distribution, altitude, forest association, data source and previous publications are provided.

Results

Of about twenty species of *Tulostoma* genus identified in Europe, to date the following five species have been recorded in the Republic of Macedonia, presented in a list with the localities, altitude, association, datum, exsiccate (exs.) and previous published data:

1. *Tulostoma brumale* Pers.

Bogdanci: Gjavato vill., Gjurov Dol, 100 m, *Querco cocciferae–Carpinetum orientalis*, 17.12.1988, exs. MAK 88/4705; **Galichica Mt**: Ohrid side, near spring, 1400 m, on grass, 22.11.2002, exs. MAK 02/3042; **Kichevo**: Izvor vill., 500 m, 23.03.2004, exs. MAK 04/793; **Kumanovo**: 400 m, *Querco–Carpinetum orientalis macedonicum*, 20.03.2003, exs. MAK 03/3059; between Zubovce vill. and Shuplji Kamen vill., 400 m, *Querco–Carpinetum orientalis macedonicum*, 15.04.2003, exs. MAK 03/1427; Zubovce vill., Bislim, 500 m, degraded forest, 07.03.2004, exs. MAK 04/3976; D'lga, 400–450 m, *Querco–Carpinetum orientalis macedonicum*, 12.03.2003, exs. MAK 03/3837; **near Skopje**: Vodno, 900 m, in meadow, 04.05.2003, exs. MAK 03/3095; 500–1000 m, *Querco–Carpinetum orientalis macedonicum*, 20.10.2002, exs. MAK 02/3000; 400 m, in meadow with *Juniperus*, 01.01.2003, exs. MAK 03/2928; between Sredno Vodno and Matka, 900 m, 04.2004, exs. MAK 04/3975; **Skopje – Veles road**: St. Jovan Veterski monastery, 150–200 m, *Juniperus excelsa* forest, 23.10.2002, exs. MAK 02/2879; **Skopska Crna Gora Mt**: between Gornjane vill. and Blace vill., 700 m, degraded oak forest, 25.01.2003, exs. MAK 03/3234.

2. *Tulostoma caespitosum* Trab. apud Sacc.

Rabrovo vill., Strumica, leg. Wilson (Herb. Lloyd n° 15.425, BPI) – Wright (1987).

3. *Tulostoma fimbriatum* Fr.

Belasica Mt: Koleshino vill., between Daravica river and Baba river, 350 m, *Quercus* and *Castanea* forest, 01.01.2003, exs. MAK 03/2893; **Bogdanci**: 150 m, meadow in *Querco cocciferae–Carpinetum orientalis*, 12.12.2005, exs. MAK 05/5516; Kuchalat, 200–300 m, *Querco cocciferae–Carpinetum orientalis*, 02.01.2003, exs. MAK 03/4751; Gorni Bolovan, 200 m, *Querco cocciferae–Carpinetum orientalis*, 10.08.2002, exs. MAK 02/3741; **near Krivolak**: Orlovo Brdo, 150–225 m, 16.03.1939, 20.03.1939, Lindtner (1939), published as *T. granulosum* Lév. M.; **Kumanovo**: 400 m, *Querco–Carpinetum orientalis macedonicum*, 20.03.2003, exs. MAK 03/1403; Dobroshane vill., 350 m, mixed forest, 10.04.2005, exs. MAK 05/4866; **near Skopje**: Vodno, 450 m, pasture, 09.10.1937, exs. BEO, Pilát & Lindtner (1939), published as *T. granulosum*; **Prespa**: Nakolec vill., 850 m, *Salicetum*, 12.06.2003, exs. MAK 03/3254; **Ruen Mt**: Staro Nagorichane vill., 600 m, degraded habitat (*Paliurus spina-cristi*, *Quercus pubescens*), 30.11.2002, exs. MAK 02/1424; Zhegljane vill., 800 m, *Pinus* plantings, 18.11.2002, exs. MAK 02/2913.

4. *Tulostoma melanocyclum* Bres.

Kumanovo: 400 m, *Querco–Carpinetum orientalis macedonicum*, 20.03.2003, exs. MAK 03/836; between Zubovce vill. and Shuplji Kamen vill., 400 m, *Querco–Carpinetum orientalis macedonicum*, 15.04.2003, exs. MAK 03/1198; **near Prilep**: Pletvar, exs. ZA, SKO, Tortić 1988; **Prespa**: Nakolec vill., 850 m, *Salicetum*, 12.06.2003, exs. MAK 03/1395; **Ruen Mt**: Staro Nagorichane vill., 600 m, degraded habitat (*Paliurus spina-cristi*, *Quercus pubescens*), 30.11.2002, exs. MAK 02/1208.

5. *Tulostoma squamosum* Pers.

Gradsko: Stobi, 150–200 m, meadow, 30.12.2003, exs. MAK 03/3646; **Kichevo**: Izvor vill., 500 m, 23.03.2004, exs. MAK 04/3955; **Kumanovo**: 400 m, *Querco–Carpinetum orientalis macedonicum*, 20.03.2003, exs. MAK 03/1220; between Zubovce vill. and Shuplji Kamen vill., 400 m, *Querco–Carpinetum orientalis macedonicum*, 15.04.2003, exs. MAK 03/1185; D'lga vill., 650–700 m, *Querco–Carpinetum orientalis macedonicum*, 12.03.2003, exs. MAK 03/1226; **Ruen Mt**: Staro Nagorichane vill., 600 m, degraded habitat (*Paliurus spina-cristi*, *Quercus pubescens*), 30.11.2002, exs. MAK 02/332; 600 m, at roadsides, near arable land, 25.10.2005, exs. MAK 05/821; beside Pchinja river, 550 m, *Querco–Carpinetum orientalis macedonicum*, 18.11.2003, exs. MAK 03/2939; **near Skopje**: Vodno, 500–1000 m, *Querco–Carpinetum orientalis macedonicum*, 20.10.2002, exs. MAK 02/1372; Markov Manastir monastery, 250 m, meadow, 06.01.2003; **Skopje – Veles road**: St. Jovan Veterski monastery, 150–200 m, *Juniperus excelsa* forest, 23.10.2002, exs. MAK 02/1203.

Discussion

Of about twenty species of *Tulostoma* genus known in Europe, to date in the Republic of Macedonia the following five species have been recorded: *T. brumale*, *T. caespitosum*, *T. fimbriatum*, *T. melanocyclum*, and *T. squamosum*. The localities where these fungi were found are presented on Figs 1, 2, 3 and 4.

Tulostoma brumale is psammophilous or humicolous, but does not grow in truly desert zones, but rather in areas with vegetation protection, or on sandy soils among herbs, mosses, etc., generally solitary in large numbers, frequently mixed with other species in the same population, particularly with *T. melanocyclum* (Wright 1987). According to Krieglsteiner (2000), this is the most common species of the *Tulostoma* genus in Europe. In the Republic of Macedonia it grows on soil or sandy soil and is found in following associations: *Querco–Carpinetum orientalis macedonicum*, *Querco cocciferae–Carpinetum orientalis*, *Juniperus excelsa* forest and in meadows. As regards its vertical distribution, it is found between 100 m and 1400 m altitude, registered in more than ten localities. Fruiting all the year, our material was collected in I, III–V and X–XII. According to Karadelev (2000), this species is included in the Preliminary Red List for the Republic of Macedonia, as a rare species, but judging by the unpublished data, a conclusion may be drawn that it is not a rare species (Fig. 1).

Tulostoma fimbriatum grows on sandy soil, both in open areas, forests and gardens (Calonge 1998). According to Wright (1987), this is a typical European species, and probably the most common one. For the Republic of Macedonia, there are only few data; therefore a conclusion on its frequency cannot be drawn. According to the existing data, it is not a very common species (Fig. 2). *Tulostoma fimbriatum* has been found in the following associations: *Querco–Carpinetum orientalis macedonicum*, *Querco cocciferae–Carpinetum orientalis*, *Quercus* and *Castanea* forest, *Pinus* plantings, *Salicetum* and in meadows. The first data for this species in Republic of Macedonia were published by Lindtner (1939) and Pilát & Lindtner (1939), from Vodno and Orlovo Brdo (near Krivolak), respectively. The new data are from the following localities: Kumanovo, the mountains of Ruen and Belasica, then Bogdanci and Prespa, between 150 m and 850 m altitude. This species is fruiting in autumn and winter (Calonge 1998). The data presented in this paper are from the following months: I, III, IV, VI, VIII, XI and XII.

Tulostoma caespitosum (Fig. 3) grows on sandy soil, fruiting during autumn and winter (Calonge 1998).

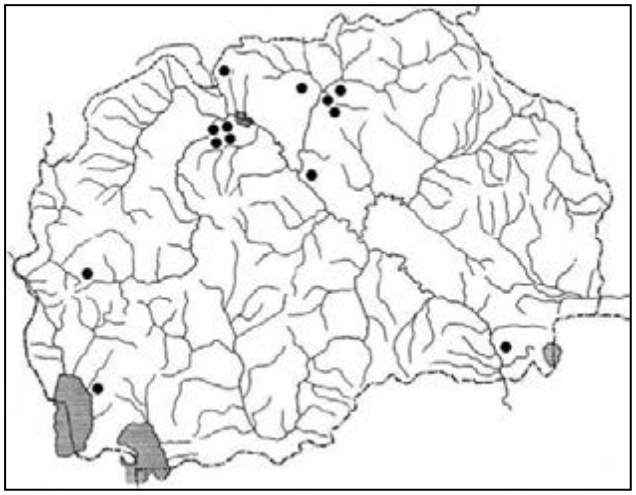

Fig. 1. Distribution map of *T. brumale*.

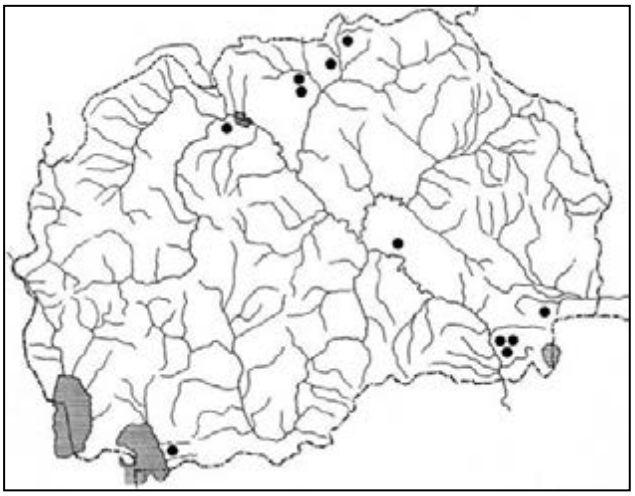

Fig. 2. Distribution map of *T. fimbriatum*.

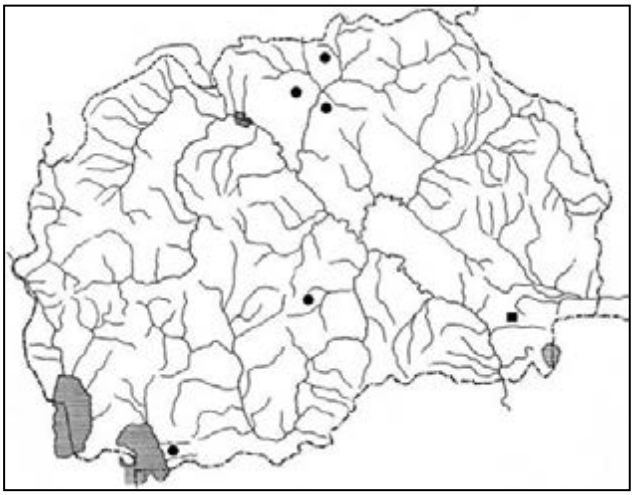

Fig. 3. Distribution map of *T. caespitosum* and *T. melanocyclum*: ■ – locality of *T. caespitosum*; ● – localities of *T. melanocyclum*.

There is only one published data for this species, by Wright (1987), Rabrovo vill., Strumica, leg. Wilson (Herb. Lloyd n° 15.425, BPI). Research conducted till now has not yet approved this species.

Tulostoma melanocyclum is a typically European species, particularly Southern, which grows on clayish soil with sand, amongst herbaceous vegetation (Wright 1987). This is a rather frequent species in West and South Europe (Wright 1987). In the Republic of Macedonia five localities have been recorded: Prilep (Tortić 1988), Ruen Mt, Kumanovo and Prespa (Fig. 3); therefore a conclusion on its distribution cannot be made. It is common to encounter it together with *T. brumale*, from which can be differentiated by the nature of the exoperidium, the colour of endoperidium, the spores and the capillitial septa (Wright 1987). It is found in the following associations: *Querco–Carpinetum orientalis macedonicum* and *Salicetum*, between 400–850 m altitude. The species is fruiting from II to XII, usually IV and X (Wright 1987). The data presented here are from the following months: III, IV, VI and XI. According to Karadelev (2000), this species is incorporated as a rare species in the Preliminary Red List for the Republic of Macedonia.

Tulostoma squamosum (Fig. 4) grows on calcareous soils among herbaceous vegetation, it is an infrequent although widely distributed species in most of Europe, principally Southern and Western (Wright 1987). There are no published data about this species for the Republic of Macedonia yet. It has been recorded in eleven localities, mostly in *Querco–Carpinetum orientalis macedonicum*, but also in *Juniperus excelsa* forest and in meadows. Concerning its vertical distribution, it is found at 150–1000 m altitude. This species is fruiting all the year, in the Republic of Macedonia it is collected in the following months: I, III–IV and X-XII. This is first record of this species for the Republic of Macedonia.

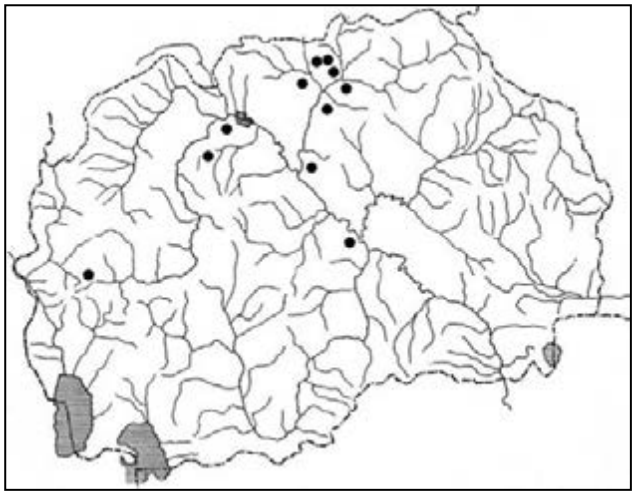

Fig. 4. Distribution map of *T. squamosum*.

Acknowledgements. We are indebted to Biology Students' Research Society for their financial help in organizing field trips for collection of scientific material and to Stole Stojanov for his effort in material collecting activities.

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Second contribution to hallucinogenic fungi in the Republic of Macedonia

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Abstract. To date a total of 30 fungi species with hallucinogenic properties have been recorded in the Republic of Macedonia. These species belong to the genera *Claviceps*, *Elaphomyces*, *Panaeolus*, *Psathyrella*, *Gymnopilus*, *Amanita*, *Pluteus*, *Psilocybe*, *Stropharia*, *Hygrocybe*, *Mycena*, *Rickenella* and *Vascellum*. Due to the presence of various compounds in their fruit bodies, these fungi can cause the syndromes psilocin–psilocybin, muscarine and ergotism. This article contains a list of data concerning the distribution and ecology of these species in the Republic of Macedonia. Most of the species are widespread, and grow on various substrates, such as soil, dung and enriched soil. There are representatives that are lignicolous, some are hypogeic, and one species is parasitic.

Key words: distribution, ecology, hallucinogenic fungi, R. Macedonia

Introduction

To date a total of 30 fungi species with hallucinogenic properties have been recorded in the Republic of Macedonia. According to Guzmàn (1983) and Guzmàn & al. (2000), the species that belong to this group of fungi can be divided into four smaller groups, according to the neurotropic compounds present in their fruit bodies:

1. Species that contain psilocin, psilocybin, baeocystin and nor-baeocystin and cause the psilocin–psilocybin syndrome (species that belong to the genera *Psilocybe*, *Gymnopilus*, *Panaeolus*, *Copelandia*, *Hypholoma*, *Pluteus*, *Inocybe*, *Conocybe*, *Paneolina*, *Rickenella*, *Agrocybe*, *Galerina* and *Mycena*);
2. Species that contain the ibotenic acid and its derivatives: muscimol and muscazone and cause the muscarine syndrome (*Amanita muscaria* var. *muscaria*, *A. muscaria* var. *regalis* and *A. pantherina*);
3. Species that contain ergotine derivatives and cause the ergotism (5 species from the genus *Claviceps* and 2 species from the genus *Cordyceps*);
4. Species that have been used as hallucinogenic in sacred rituals in Mesoamerica, but to date no adequate chemical studies about their neurotropic properties have been made (8 species from the genus *Boletus*, 6 species from the genus *Russula*, 3 species from the *Gasteromycetes*: *Lycoperdales* and *Phallales*).

In this article the authors have made an attempt to draft a complete list of the registered and published hallucinogenic species of fungi in the Republic of Macedonia. According to the published data, as well as to the results of the authors' investigations, out of the 30 species of registered hallucinogenic fungi, 23 species from the genera: *Panaeolus*, *Psathyrella*, *Gymnopilus*, *Hygrocybe*, *Pluteus*, *Psilocybe*, *Stropharia*, *Rickenella* belong to the psilocybin fungi from the first group; 3 species from the genus *Amanita* belong to the second group and cause the muscarine syndrome; 1 species from the genus *Claviceps* that belong to the third group and causes the ergotism, and 3 species that belong to the fourth group and belong to the genera *Elaphomyces* and *Vascellum*.

Material and methods

The material was collected in various forest associations, as well as in pastures, meadows, at roadsides,

etc., growing on various substrates: soil, enriched soil, dung, wood debris, under soil, etc. The species were identified during the field trips, or later, in the Mycological Laboratory at the Institute of Biology, Faculty of Natural Science and Mathematics, microscopically, using reagents, such as Melzer's reagent, KOH, sulfovaniline, etc. All the data were put in the MAC FUNGI database, while most of the species were preserved in the National Mycological Collection FUNGI MACEDONICI, placed in the Mycological Laboratory at the Faculty of Natural Science and Mathematics. For identification were used the following identification keys and field guides: Guzmàn (1983), Moser (1983), Breitenbach & Kränzlin (1991, 1995, 2000), Stamets (1996), Kriegelsteiner (2000a, b) and Dänke (2001). The nomenclature and synonymy follows *Index Fungorum* (2005).

Results

A list of hallucinogenic fungi, with their ecology and distribution in the Republic of Macedonia is presented. These data are provided by authors' field investigations as well as by 20 publications (see legend).

Ascomycota

Ascomycetes

Clavicipitaceae

Claviceps purpurea (Fr.) Tul.

This ascomycete is a parasite on rye (*Secale cereale*). It is found in rye plantations throughout the country.

Elaphomycetaceae

Elaphomyces granulatus Fr.

Ograzhden Mt, Suvolak, 800 m, 02.05.1990, *Festuco heterophyllae–Fagetum*, under soil, exs. MAK 90/4708; **Jakupica Mt**, Cheples, 1300–1400 m, 12.07.1999, *Calamintho grandiflorae–Fagetum*, under soil, exs. MAK 99/2454[10,17,18].

Elaphomyces reticulatus Vittad.

Pelister Mt, vill. Brajchino, 1000 m, October 2002, *Quercetum frainetto–cerris*, under soil[18]; **Galichica Mt**, Pljuska, 1000 m, October 1991, *Quercetum frainetto–cerris*, under soil[18]; **Kozhuf Mt**, Smrdliva Voda, 800 m, October 1985, *Festuco heterophyllae–Fagetum*, under soil[18].

Basidiomycota

Gasteromycetes

Lycoperdaceae

Vascellum pratense (Pers.) Kreisel

Kozhuf Mt, between summer resort "Mihajlovo" and Crvena Zemja, 1200–1400 m, 10.07.2005, at roadsides, soil, exs. MAK 05/5053; between summer resort "Mihajlovo" and Lokvichki, 09.07.2005, beech forest, soil, exs. MAK 05/4998; **Pelister Mt**, from PTT resort to mountain house Kopanki, 1340–1610 m, 21.09.1983, meadow, soil; **Kumanovo**, near vill. Miladinovci, *Pinus* plantings, soil; **Osogovski Planini Mt**, vill. Konopnica, 1100 m, 03.09.2002, meadow, soil; **Skopska Crna Gora Mt**, between vill. Gornanje and vill. Blace, 700 m, 25.01.2003, degraded oak forest, soil; Katlanovo, St. Bogorodica Monastery, 200 m, 23.10.2002, *Querco–Carpinetum orientalis*, soil, exs. MAK 02/2885; **near Skopje**, Vodno, between St. Pantelejmon and Sredno Vodno, 800 m, 12.10.1998, meadow, soil, exs. MAK 98/1963[19]; Matka, 250–300 m, 25.10.1998, meadow, soil, exs. MAK 98/1884; **Bistra Mt**, Mavrovo, Bunec, 1350–1400 m, 25.09.1998, *Calamintho grandiflorae–Fagetum*, soil, exs. MAK 98/1814[14]; **Ruen Mt**, vill. Staro Nagorichane, 600 m, 30.11.2002, degraded stands (*Paliurus*, *Q. pubescens*, *Crataegus*), soil, exs. MAK 02/2932.

Basidiomycetes

Bolbitiaceae

Panaeolus acuminatus (Schaeff.) Quél.
(syn. *Panaeolus rickenii* Hora)

Near Skopje, Katlanovska Banja, 250–350 m, *Querco–Carpinetum orientalis*, soil, PRM[1,5]; **Galichica Mt**, vill. Stenje, 900 m, 10.10.2000, *Quercetum frainetto–cerris,* soil, exs. MAK 00/4631.

****Panaeolus ater*** (J.E. Lange) Kühner & Romagn.

Veles, vill. Gorno Vranovci, 08.11.1998, *Querco–Castanetum*, soil, under *Quercus*, exs. MAK 98/1861.

Panaeolus campanulatus (L.) Quél.
(syn. *Panaeolus campanulatus* var. *sphinctrinus* (Fr.) Quél.)

Near Skopje, Katlanovska Banja, 250–350 m, *Querco–Carpinetum orientalis*, soil, PRM[1,5].

Panaeolus fimicola (Pers.) Gillet

Pelister Mt, between Palisnopje and hotel "Molika", 1400 m, 19.10.2005, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 05/5583.

Panaeolus olivaceus F.H. Møller

Prespa, vill. Nakolec, 850 m, 20.06.2003, *Salicetum*, soil, exs. MAK 03/3255.

Panaeolus papilionaceus var. ***papilionaceus*** (Bull.) Quél.

Pelister Mt, around Kopanki, 1600 m, September 2002, *Digitali viridiflorae–Pinetum peuces*, dung[15,17,18]; vill. Lavci, 800–900, 18.10.2004, mixed forest, dung, exs. MAK 04/4996; **Skopska Crna Gora Mt**, vill. Ljubanci, Zgurovci, 800 m, 09.10.2005, meadow, dung, exs. MAK 05/5475; **Kozhuf Mt**, Dudica, 2100 m, 12.06.2005, pasture, dung, exs. MAK 05/5489; **Strumica**, Monospitovsko Blato, 250 m, 14.10.2000, *Periploco graecae–Alnetum glutinosae*, dung, exs. MAK 00/4630; **Galichica Mt**, vill. Stenje, 900 m, 10.10.2000, *Quercetum frainetto–cerris*, dung, exs. MAK 00/4629; **Berovo**, 850 m, May, 2004, meadow, dung, exs. MAK 04/4009[20]; **near Skopje**, Vodno, 950 m, 17.05.2002, *Pinus* plantings, dung, exs. MAK 02/2637[17,18]; **Jablanica Mt**, between vill. Gorna Belica and vill. Visni, 15.06.2006, meadow, dung, exs. MAK, 06/2388.

Panaeolus reticulatus Overh.

Pelister Mt, vill. Rotino, 1000 m, 26.10.2004, meadow, soil, exs. MAK 04/4960.

Panaeolus semiovatus var. ***semiovatus*** (Sowerby) S. Lundell & Nannf.

(syn. *P. semiovatus* (Sowerby) S. Lundell & Nannf.; *Anellaria semiovata* (Sowerby) A. Pearson & Dennis)

Pelister Mt, around vill. Trnovo 1000 m, 26.05.2002, meadow, dung[15]; around Kopanki, 1650 m, September, 2002, meadow, dung[17,18]; Golemo Ezero, 2400 m, 08.10.2001, high mountain pasture, dung, exs. MAK 01/87[15]; **Skopska Crna Gora Mt**, dung, ZA[5]; vill. Ljubanci, Las, 800–900 m, 01.05.2006, meadow, dung, exs. MAK 06/1907; **Osogovski Planini Mt**, Ponikva, near Children's resort, 1400 m, 13.06.2005, meadow, *Fagus* and *Quercus* forest, dung, exs. MAK 05/841; **Shar Planina Mt**, Ceripashina, 1950 m, 14.07.1995, meadow, dung**; Kitka Mt**, 1000–1200 m, 11.05.2002, meadow, *Festuco heterophyllae–Fagetum*, dung, exs. MAK 02/2673[20]; **Strumica**, vill. Koleshino, 300 m, 14.10.2001, meadow, *Periploco–Alnetum glutinosae*, dung (coprophilous), MAK 01/566[20].

Panaeolus sphinctrinus (Fr.) Quél.

(syn. *Panaeolus campanulatus* var. *sphinctrinus* (Fr.) Quél.)

Jakupica Mt, vicinity of mountain hut Karadzica, 1400, conifer plantation (*Pinus nigra*, *P. sylvestris*, *P. strobus*, *Larix* spp., *Picea* spp.), dung, herb. Sy.[4,5]; **Osogovski Planini Mt**, Ponikva, 1500–1600 m, 20.09.2001, *Calamintho grandiflorae–Fagetum*, dung; **Pelister Mt**, Gjavato, old road to Bitola, 1150 m, 24.04.2002, small forest of *Alnus glutinosa*, dung, exs. MAK 02/2487[20]; **Strumica**, Grlieva Ceshma, 30.04.2002, *Osmunda* stand, grassy places near *Osmunda* stands, dung, exs. MAK 02/2667[20]; **Veles**, vill. Gorno Vranovci, 08.11.1998, *Festuco heterophyllae–Fagetum*, dung, exs. MAK 98/1786[20].

Panaeolus subbalteatus (Berk. & Broome) Sacc.

Pelister Mt, on the foot of the mountain, 750 m, meadow, soil[20].

Coprinaceae

Psathyrella candolleana (Fr.) Maire

Jakupica Mt, *Festuco heterophyllae–Fagetum,* herb. Sy.[4,5]; Cheples, 1300–1400 m, 10.07.1999, *Calamintho grandiflorae–Fagetum*, trunk of *Fagus*[10]; Cheples, 1300–1400 m, 11.07.1999, *Calamintho grandiflorae–Fagetum*, enriched soil, exs. MAK 99/2455; **Shar Planina Mt**, Jelak, 1750 m, *Abieti–Piceetum scardicum*, enriched soil[20]; *Calamintho grandiflorae–Fagetum*, on *Fagus*[17]; **Ruen Mt**, vill. Staro Nagorichane, 300 m, 03.06.2004, meadow, enriched soil, exs. MAK 04/4011[20]; **Skopje**, MANU, near the river Vardar, 250 m, 14.04.2002, enriched soil, exs. MAK 02/2601[20].

Cortinariaceae

Gymnopilus junonius (Fr.) P.D. Orton

(syn. *Gymnopilus spectabilis* (Fr.) Sing.)

Kitka Mt, near mountain house Kitka, 800–900 m, October 1999, *Orno–Quercetum petraeae*, trunk of *Quercus*; Bistra Mt, Mavrovo, Bunec, 1350–1400 m, 25.09.1998, *Calamintho grandiflorae–Fagetum*, log of *Fagus*, exs. MAK 98/1978[8,17,18].

Gymnopilus sapineus (Fr.) Maire

Pelister Mt, Kopanki, 1650 m, 21.09.2002, *Digitali viridiflorae–Pinetum peuces*, trunk of *Pinus peuce*, exs. MAK 02/3136[15].

Pluteaceae

Amanita muscaria var. ***muscaria*** (L.) Lam.
(syn. *Amanita muscaria* (L.) Lam.)

Pelister Mt, around mountain house Kopanki, 1640–1700 m, 03.10.2001, *Digitali viridiflorae–Pinetum peuces*, soil[15,17,20]; between mountain house Kopanki and Palisnopje, 1640–1540 m, 04.10.2001, *Digitali viridiflorae–Pinetum peuces*, soil[15,17,20]; Kopanki, 1650 m, 06.10.2000, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 00/4580[15,17]; Caparska Preseka, 1500 m, 08.10.2001, *Digitali viridiflorae–Pinetum peuces*, soil[15,17,20]; vill. Magarevo[20]; river Rotinska Reka[20]; hotel "Molika", soil[20]; Begova Cheshma, 1400–1500 m, *Gentiano luteaea–Pinetum peuces abietetosum*, soil, ZA[2,5,15,20]; Begova Cheshma, 1400–1500 m, *Digitali viridiflorae–Pinetum peuces*, soil, ZA[2,5,15,17,20]; Golema Livada, soil[20]; **Bistra Mt**, Mavrovo, Bunec, 1050–1100 m, *Calamintho grandiflorae–Fagetum*, soil[5,17]; vicinity of Mavrovo Lake, 1300–1400 m, summer–autumn 1998–1999, *Calamintho grandiflorae–Fagetum*, soil[8,17]; Mavrovo, Bunec, experimental plot, 1350–1400 m, 25.09.1998, *Calamintho grandiflorae–Fagetum*, soil[8,17]; vill. Galichnik, Kuliche, 1360 m, 08.08.2002, *Pinus* plantings, soil[16]; Mavrovo, 1400 m, *Fago–Abietetum meridionale*, soil[18]; **Kozhuf Mt**, Smrdliva Voda, 800 m, 24.10.2005, beech forest, soil, under *Betula*, exs. MAK 05/5548; between vill. Konjsko and vill. Huma, 700–750 m, 23.09.1995, *Pinus* plantings, soil; between vill. Majdan and Tribor, 800–1400 m, 08.10.1998, *Abieti–Fagetum* with *Pinus nigra* and *Pinus sylvestris*, soil; *Fagetum*, soil, ZA[5,17]; Krusha, 1000 m, *Abieti–Fagetum*, soil[20]; vill. Konjari, 1100 m, *Fagus* and *Pinus nigra* forest, soil[18,20]; Belantrok locality, 1250 m, *Abieti–Fagetum*, soil, ZA[5,17]; Smrdliva Voda, 1000 m, *Calamintho grandiflorae–Fagetum*, soil[18,20]; **Jakupica Mt**, bellow Begovo Pole, 1600 m, *Pinetum mughi silicicolum*, soil, herb. Sy., ZA[4,5,17]; Preslap, 1000 m, *Quercetum frainetto–cerridis*, soil, herb. Sy., ZA[4,5,17]; **Kitka Mt**, 1000–1300 m, *Festuco heterophyllae–Fagetum*, soil, herb. Sy., ZA[4,5,17]; vicinity of mountain hut Karadadzica, 1400 m, conifer plantings (*Pinus nigra*, *P. sylvestris*, *P. strobus*, *Larix* spp., *Picea* spp.), soil, herb. Sy., ZA[4,5,17]; **Krushevo**, 1300–1400, planted *Pinus nigra* stands, soil[5,17]; 1300–1400 m, *Festuco heterophyllae–Fagetum*, soil[5,17,18]; 1300–1400, conifer plantings, soil[5,17]; **Belasica Mt**, above vill. Bansko, 20.10.1996, *Pinus sylvestris* plantings, soil; Kavadarci, vill. Vitachevo, 28.09.1998, *Pinus* plantings, soil[20]; **Osogovski Planini Mt**, Ponikva, 1500–1600 m, 20.09.2001, *Calamintho grandiflorae–Fagetum*, soil, exs. MAK 01/805[17,18,20]; **Gevgelija**, Bolovan, 150 m, 20.11.1997, *Juglando–Platanetum orientalis*, soil.

****Amanita muscaria*** var. ***regalis*** (Fr.) Sacc.
(syn. *Amanita regalis* (Fr.) Michael)

Kozhuf Mt, vill. Konjsko, 600 m, 23.10.2005, *Pinus nigra* plantings, soil, exs. MAK 05/5551.

Amanita pantherina (DC.) Krombh.

Pelister Mt, vill. Brajchino, 1200 m, 15.08.2002, *Festuco heterophyllae–Fagetum*, soil; July 2001, *Pinetum peuces*, soil[12]; Golema Livada, 1300 m, mixed forest (*Betula*, *Populus*, *Pinus peuce*, *Abies*), soil[17,20]; Begova Cheshma, 1400–1500 m, *Digitali viridiflorae–Pinetum peuces*, soil, ZA[2,5,15,17]; **Veles**, vill. Smilovci, 21.09.1996, oak forest, soil[20]; vill. Oraov Dol, Kadiica, 18.06.1993, beech forest, soil; Kavadarci, vill. Vitachevo, 28.09.1998, *Pinus* plantings, soil[20]; vill. Lavci, 800–900 m, 18.10.2004, mixed forest, soil, exs. MAK 04/4984; **Skopje**, Hipodrom, 240 m, 07.11.1993, meadow, soil[20]; **near Skopje**, Katlanovo, 16.10.2002, *Pinus* plantings, soil; Katlanovo, 250 m, oak-hornbeam forest, soil[17,20]; vill. Bulachani, 700 m, 02.06.2002, oak forest, soil, exs. MAK 02/916; **Kozhuf Mt**, between vill. Konjsko and vill. Huma, 700–750 m, 23.09.1995, *Orno–Quercetum petraeae*, soil; Visoka Chuka, 1100 m, 20.06.1998, *Festuco heterophyllae–Fagetum*, soil[17,20]; near river Slivka, 800 m, 12.07.2004, *Fagetum*, soil[20]; road to Asan Cheshma, 750–1350 m, 11.07.2004, beech forest, soil, exs. MAK 04/4151[20]; Mala Rupa, 1000 m, 16.07.2004, mixed forest (*Fagus*, *Pinus*, *Abies*), soil[20]; Resa, 700–800 m, 14.07.2004, *Quercetum*, soil[20]; footpath to vill. Huma, 800 m, 05.10.1996, beech forest, soil; Smrdliva Voda, 800 m, 24.10.2005, beech forest, soil; *Fagetum*, soil[5,17]; way to Mala Rupa, 1000 m, 16.07.2004, mixed forest (*Fagus*, *Pinus*, *Abies*), soil, exs. MAK 04/4152; between summer resort "Mihajlovo" and Cvrstec, 1250–1500 m, 13.07.2005, beech forest, soil; **Bistra Mt**, vicinity Mavrovo Lake, 1300–1400 m, summer–autumn 1998–1999, *Calamintho grandiflorae–Fagetum*, soil[8,17]; Mavrovo, Bunec, experimental plot, 1350–1400 m, 25.09.1998, *Calamintho grandiflorae–Fagetum*, soil[8,17]; between vill. Lazaropole and vill. Tresonche, 1300 m, 12.07.2003, *Festuco heterophyllae–Fagetum*, soil[14,16,17]; Mavrovo, *Abies* forest, soil[17]; **Belasica Mt**, above vill. Bansko, 20.10.1996, *Fagetum*, soil; **Ograzhden Mt**, Suvi Laki, 1100–1300 m, 19.10.1996, beech-pine forest, soil; **Galichica Mt**, vill. Oteshevo, 900 m, 09.11.2002, oak forest, soil[20]; **Kichevo**, between vill. Tuin and vill. Popovjani, Shumjak, 800 m,

05.11.2002, oak forest, soil; **Bigla Mt**, 04.06.2005, oak forest, soil, exs. MAK 05/5351; **Skopska Crna Gora Mt**, vill. Rashtak, 01.10.1998, oak forest, soil; *Fagus* forest, soil[17]; above vill. Chucher – Sandevo, 600 m, 06.10.1998, degraded oak forest (*Querco–Carpinetum orientalis*), soil; **Kumanovo**, vill. Vince, 700–800 m, 27.10.2002, meadow, soil[17,20]; **Osogovski Planini Mt**, Ponikva, 1500–1600 m, 20.09.2001, *Calamintho grandiflorae–Fagetum*, soil, exs. MAK 01/806[17,20]; Ponikva, 13.06.2005, *Fagus* and *Quercus* forest, soil, exs. MAK 05/1577; **Kitka Mt**, Preslap, 1000 m, 24.06.1994, deciduous forest (*Fagus*, *Carpinus betulus*, *Quercus cerris*), soil; **Krushevo**, *Fagus* forest, soil[17]; **Jakupica Mt**, *Fagus* forest, soil[17]; Preslap, 1000 m, *Quercetum frainetto–cerridis*, soil, herb. Sy.[4,5,17]; **Plachkovica Mt**, *Fagus* forest, soil[17]; **Shar Planina Mt**, vill. Gorno Jelovce, July 1998, beech forest, soil[9,17]; **Nidze Mt**, Chemerikata, 1640 m, July 2002, *Pinetum sylvestris*, soil, exs. MAK 02/888[13]; **Karadzica Mt**, oak forest, soil[17].

Pluteus cervinus var. ***cervinus*** P. Kumm.
(syn. *Pluteus atricapillus* (Batsch) Fayod)

Pelister Mt, from PTT resort to mountain house Kopanki, 1340–1610 m, 21.09.1983, *Digitali viridiflorae–Pinetum peuces*, soil[15]; vill. Malovishte, St. Ana Monastery, 1200–1400 m, 22.07.1989, *Festuco heterophyllae–Fagetum*, *Fagus*[6,7]; vill. Lavci, 800–900 m, 18.10.2004, mixed forest, rotten wood of *Alnus* spp., exs. MAK 04/4993; around Kopanki, 1500–1700 m, 19.10.2005, *Digitali viridiflorae–Pinetum peuces* with *Fagus*, soil; **Jakupica Mt**, 1300 m, 08.07.1999, *Calamintho grandiflorae–Fagetum*, log of *Fagus*, exs. MAK 99/2047[10]; vill. Gorno Vranovce, 08.11.1998, *Festuco heterophyllae–Fagetum*, log of *Fagus*, exs. MAK 98/1857[20]; *Fagus*, herb. Sy.[3,4,5]; on *Quercus*, herb. Sy.[3,4,5]; **Ograzhden Mt**, Ezhovo Brdo, 1100–1300 m, 12.07.2000, mixed forest (*Fagus*, *Pinus sylvestris* and *P. nigra*), rotten branches of *Fagus*, exs. MAK 00/1469[11]; below Baltova Chuka, 1620 m, 20.07.2000, *Calamintho grandiflorae–Fagetum*, on *Fagus*[11]; **Shar Planina Mt**, river Ljubotenska Reka, 1400–1600 m, 17.07.1997, *Calamintho grandiflorae–Fagetum*, *Fagus*, exs. MAK 97/2962[9]; river Ljubotenska Reka, 1400–1600 m, 13.07.1997, *Calamintho grandiflorae–Fagetum*, on *Fagus*[9]; Ljuboten, 1500–1800 m, *Fagetum subalpinum serbicum*, on *Fagus*; PRM 490785, 490781, 490757[1,5]; **Bistra Mt**, Mavrovo, Bunec, experimental plot, 1350–1400 m, 25.09.1998, *Calamintho grandiflorae–Fagetum*, soil[8]; **Karadzica Mt**, Shashkovica, 1500–1600 m, 29.07.1997, *Calamintho grandiflorae–Fagetum*, on *Fagus*; **Kichevo**, between vill. Tuin and vill. Popovjani, Shumjak, 800 m, 10.10.2002, oak forest, *Quercus*; between vill. Tuin and vill. Popovjani, Shumjak, 800 m, 05.11.2002, oak forest, *Quercus*; **near Skopje**, Vodno, near vill. Krushopek, 800 m, 30.10.2004, at roadsides, soil, exs. MAK 04/4147[19,20]; **Skopje**, between vill. Ilinden and vill. Jurumleri, 250 m, 22.04.1999, *Populus nigra* and *Betula* plantings, *Populus*, exs. MAK 99/3930; **Demir Kapija**, Stojkova Chuka to Kaludjerska Chuka, 450–600 m, 21.05.2005, *Querco–Carpinetum orientalis* with *Pinus* plantings, root of broadleaved tree; **Skopska Crna Gora Mt**, Preslap, 1280 m, *Calamintho grandiflorae–Fagetum*, on *Fagus*[5]; Ramno, 1450–1500 m, *Calamintho grandiflorae–Fagetum*, on *Fagus*[5]; **Kozhuf Mt**, on *Fagus*[6]; between summer resort "Mihajlovo" and Cvrstec, 1250–1500 m, 13.07.2005, beech forest, rotten wood of *Fagus*, exs. MAK 05/5019; Visoka Chuka, 1000 m, July 1998, *Festruco heterophyllae–Fagetum*, on *Fagus*; **Nidze Mt**, Kopanki, 1400 m, 16.07.2002, mixed forest (*Pinus sulvestris*, *Abies*, *Populus tremula*), soil, exs. MAK 02/5671[13].

Pluteus salicinus (Pers.) P. Kumm.

Jakupica Mt, *Fagus*, herb. Sy.[3,4]; Cheples, 1400–1500 m, 11.07.1999, *Calamintho grandiflorae–Fagetum*, stump of *Fagus*; exs. MAK 99/2450[17,18]; **Ograzhden Mt**, Ezhovo Brdo, 1100–1300 m, July 2000, mixed forest (*Fagus*, *Pinus nigra*, *P. sylvestris*), rotten branches of *Fagus*[17,18]; **Kozhuf Mt**, between summer resort "Mihajlovo" and Vasov Grad, 12.07.2005, mixed forest (*Fagus*, *Quercus*, *Pinus*), on *Fagus*[17,18]; vill. Konjari, 1000 m, 13.10.2000, *Festuco heterophyllae–Fagetum*, rotten wood of *Fagus,* exs. MAK 00/4634[17,18]; Veles, vill. Gorno Vranovci, 08.11.1998, *Querco–Castanetum*, *Castanea*, exs. MAK 98/4313; **Bistra Mt**, dam Mavrovo, 1100–1300 m, 24.10.2000, beech forest *Fagus*, exs. MAK 02/3759[20]; Galichica Mt, Pljuska, 1000 m, 10.10.2001, *Quercetum frainetto cerris*, soil, exs. MAK 01/850[17,18].

Strophariaceae

Psilocybe coprophila (Bull.) P. Kumm.

Jakupica Mt, vill. Gorno Vranovce, 700–800 m, 08.11.1998, *Festuco heterophyllae–Fagetum*, dung, exs. MAK 98/3919[17,18]; **Shar Planina Mt**, Jelak, 1700–1800 m, 16.10.2000, *Piceetum excelsae–subalpinum scardicum*, dung, exs. MAK 00/2732.

Psilocybe inquilina (Fr.) Bres.
(syn. *Psilocybe muscorum* (P.D. Orton) M.M. Moser)

Pelister Mt, above vill. Slivnica, 1050 m, 21.04.2002, *Quercetum frainetto cerris*, soil, moss *Rhacomitrium*, exs. MAK 02/2656[17,18].

Psilocybe montana (Pers.) P. Kumm.
(syn. *Psilocybe physaloides* (Bull.) Quél.)

Pelister Mt, around Kopanki, 1600 m, 19.10.2005, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 05/5321; between mountain house Kopanki and Palisnopje, 1640–1540 m, 04.10.2001, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 01/143[15,17,18]; **Prespa Lake**, Strict Nature Reserve "Ezerani", 850 m, 18.04.2002, meadow, soil, exs. MAK 02/2653[17,18].

Psilocybe phyllogena (Peck) Peck
(syn. *Psilocybe rhombispora* (Britzelm.) Sacc.)

Kozhuf Mt, Mihajlovo to Chvrstec, 1250–1500 m, 13.07.2005, beech forest, leaves remnants, exs. MAK 05/5268; near river Stara Reka, 18.07.2005, beech forest, leaves remnants; around Mihajlovo, 1200 m, 12.07.2005, beech forest, leaves remnants, exs. MAK 05/4942, revised by G. Guzmàn; **Jablanica Mt**, from vill. Visni to vill. Gorna Belica, 15.06.2006, beech forest, leaves remnants, exs. MAK 06/392; between vill. Gorna Belica and vill. Vevchani, 15.06.2006, beech forest, leaves remnants, exs. MAK 06/2322.

Psilocybe subcoprophila (Britzelm.) Sacc.

Katlanovo, St. Jovan Veterski Monastery, 150–200 m, 23.10.2002, *Juniperus excelsa* forest, dung, exs. MAK 02/2871[17,18].

Psilocybe subviscida var. ***velata*** Noordel. & Verdun
(syn. *Psilocybe bullacea* (Bull.) P. Kumm.)

Pelister Mt, around vill. Trnovo, 1200 m, 26.05.2002, *Festuco heterophyllae–Fagetum* with *Populus tremula*, leaves remnants of *Populus tremula*[17,18]; vill. Rotino, 1050 m, 25.04.2002, *Pinus peuce* plantings, fallen branch of *Pinus peuce*[15,17,18]; **Prespa Lake**, Strict Nature Reserve "Ezerani", 22.04.2002, *Salicetum*, meadow, close to *Salix purpurea*, exs. MAK 02/2652[17,18]; Strict Nature Reserve "Ezerani", 21.04.2002, *Salicetum*, meadow, close to *Salix purpurea*, exs. MAK 02/2664[17,18].

Stropharia coronilla (Bull.) Fr.

Bogdanci, 150 m, 12.12.2005, meadow in *Querco cocciferae–Carpinerum orientalis*, soil, Kozarnik, 200 m, 03.01.2006, meadow in *Querco cocciferae–Carpinetum orientalis*, soil; **Pelister Mt**, between hotel "Molika" and mountain house Kopanki, 1100 m, 26.04.2002, meadow with *Juniperus communis* and *Pinus peuce*, sandy ground, pure soil, exs. MAK 02/2503[15,17,18]; **near Skopje**, Vodno, 750–1000 m, 16.10.2002, *Querco–Carpinetum orientalis*, soil, exs. MAK 02/2801[17,18]; Vodno, near vill. Krushopek, 800 m, 28.10.2004, meadow, soil; Vodno, near vill. Sopishte, 800 m, 04.12.2005, *Pinus nigra* plantings, soil; Vodno, near vill. Krushopek, 11.10.2005, meadow, soil; **Osogovski Planini Mt**, vill. Konopnica, 1200 m, 05.06.2003, *Orno–Quercetum petraeae*, soil[20]; vill. Konopnica, 1200 m, 05.06.2003, *Festuco heterophyllae–Fagetum*, soil[20]; **Skopska Crna Gora Mt**, above vill. Chucher – Sandevo, 600 m, 06.10.1998, degraded oak forest (*Querco–Carpinetum orientalis*), soil; vill. Brodec, 1000 m, 06.10.1998, *Querco–Carpinetum orientalis*, soil; near vill. Ljubanci, Orlovac, 400 m, 21.10.2004, meadow, soil; vill. Ljubanci, Zgurovci, 800 m, 02.10.2005, meadow, soil; **Demir Kapija**, vill. Miletkovo, 08.10.1998, meadow, soil; vill. Chiflik, October 2004, meadow, soil; **Bistra Mt**, Mavrovo, Bunec, experimental plot, 1350–1400 m, September 1998, *Calamintho grandiflorae–Fagetum*, soil[8]; Mavrovo, Bunec, 1100–1300 m, 24.10.2000, meadow, soil, exs. MAK 02/3749; **Gevgelija**, vill. Stojakovo, 80 m, December 1993, meadow, soil[18]; **Kozhuf Mt**, vill. Konjari, 1100 m, October 2000, *Fagus* and *Pinus nigra* forest, dung[18].

Tricholomataceae

Hygrocybe psittacina (Schaeff.) P. Kumm.

Karadzica Mt, mixed fir and beech forest, soil[17]; **Jakupica Mt**: Babina Rupa, 1900–2000 m, 13.07.1999, mountain pasture, soil[10]; near the pick Solunska Glava, 2300–2400 m, 13.07.1999, mountain pasture, soil; exs. MAK 99/2343[10]; **near Skopje**, Vodno, 800 m, 20.10.2002, *Querco–carpinetum orientalis*, soil, exs. MAK 01/2881[17]; Vodno, 750–1000 m, 16.10.2002, *Querco–carpinetum orientalis*, soil, exs. MAK 02/2792[17]; **Pelister Mt**, around Palisnopje, 1500 m, 06.10.2001, *Digitali viridiflorae–Pinetum peuces* with *Fagus*, soil, exs. MAK 01/37[15]; **Kozhuf Mt**, river Konjska Reka, 400 m, 20.05.2002, *Juglando–Platanetum orientalis*, soil, exs. MAK 02/2634[20].

Rickenella fibula (Bull.) Raithelh.
(syn. *Gerronema fibula* (Bull.) Singer)

Korab Mt, around Kepi Bard, 2600 m, 04.09.2005, high mountain pasture, wet place soil, among mosses, exs. MAK 05/5085; **Veles**, vill. Gorno Vranovci,

07.11.1998, *Querco–Castanetum*, meadow, soil, exs. MAK 98/4326; **Jakupica Mt**, grassy places, soil, herb. Sy.[4,5]; vill. Gorno Vranovci, November 1998, meadow, soil; river Babuna, between vill. Teovo and river Brezica, 300–350 m, 17.05.2003, at roadsides, soil[20]; **Pelister Mt**, Caparska Preseka, 1300 m, 30.09.2002, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 02/3570[15]; between mountain house Kopanki and Palisnopje, 1640–1540 m, 04.10.2001, *Digitali viridiflorae–Pinetum peuces*, soil, exs. MAK 01/154[15,20].

Legend:

[1] Pilát & Lindtner (1939); [2] Tortić (1968); [3] Tortić & Cekova (1975); [4] Sylejmani (1980); [5] Tortić (1988); [6] Karadelev (1993); [7] Karadelev (1995); [8] Karadelev (2000); [9] Karadelev & al. (2002a); [10] Karadelev & al. (2002b); [11] Karadelev & al. (2002c); [12] Karadelev & al. (2002d); [13] Karadelev & Rusevska (2002); [14] Karadelev & al. (2003b); [15] Karadelev & al. (2003a); [16] Karadelev & Rusevska (2004); [17] Karadelev & al. (2004); [18] Karadelev & Spasikova (2004a); [19] Rusevska & Karadelev (2004); [20] Karadelev & Spasikova (2004b).

MAK = Macedonian National Mycological collection (FUNGI MACEDONICI); PRM = Collection of fungi at the National Museum in Prague, The Czech Republic; ZA = Collection of fungi at the Botanical department, Faculty of science, Zagreb, Croatia; herb. Sy. = private herbarium of Sami Sylejmani.

Discussion

For the past several years, the hallucinogenic fungi in the Republic of Macedonia have been collected and observed, and completely reviewed for the first time in Karadelev & Spasikova (2004a). In the past reviews, as well as in this one, the authors have made attempts to draft a final list of hallucinogenic species of fungi, relying on several sources (Koike & al. 1981; Guzmàn 1983, 2005; Ohenoja & al. 1987; Stamets 1996; Guzmàn & al. 2000). As it appeared, this was not at all simple. Many of the sources mentioned are contradictory to one another, bringing confusion about the actual hallucinogenic properties of some species. However, in this paper we emphasize the ecology and distribution of these fungi in the Republic of Macedonia, not their chemical composition.

Considering the fact that this group of fungi has been investigated mainly through systematic investigation of fungi in the Republic of Macedonia, the localities where these fungi have been found have not been visited regularly and not by identical number of times. As the aspect of macrofungi changes with the seasons, it is obvious that the frequency and quantity of these species should be established by more intensive research.

A total number of 30 species of fungi were recorded. These fungi belong to 12 genera, from which three species belong to *Ascomycota* (*Claviceps purpurea*, *Elaphomyces granulatus*, *E. reticulatus*), while the rest of the species belong to *Basidiomycota*, where only *Vascellum pratense* belongs to the class *Gasteromycetes,* the others belong to the class *Basidiomycetes*. Among these species, the genus *Panaeolus* is presented with the largest number of species (total of 10 species), while the other genera are presented with less representatives, as follows: *Psilocybe* (6), *Amanita* (3), *Elaphomyces*, *Gymnopilus* and *Pluteus* (2 species each), and the genera *Claviceps*, *Stropharia*, *Hygrocybe*, *Psathyrella*, *Rickenella* and *Vascellum* are presented with 1 species each. According to our investigations and previously published data, the most frequent of these species are *Amanita muscaria* var. *muscaria*, *A. pantherina* and *Pluteus cervinus* var. *cervinus*, while the species *Amanita muscaria* var. *regalis*, *Elaphomyces granulatus*, *Gymnopilus junonius*, *G. sapineus*, *Panaeolus ater*, *P. acuminatus*, *P. fimicola*, *P. olivaceus*, *P. reticulatus*, *Psilocybe inquilina* and *Ps. subcoprophila* have occurred at only one locality each in the Republic of Macedonia.

The following species are **lignicolous**: *Gymnopilus junonius* (*Quercus*, *Fagus*), *G. sapineus* (*Pinus peuce*), *Pluteus cervinus* var. *cervinus* (*Fagus*, *Quercus*, *Alnus*, *Populus*, roots of broadleaved trees), *P. salicinus* (*Fagus*, *Castanea*), *Psathyrella candolleana* (Fagus), *Psilocybe phyllogena* (leaves remnants of *Fagus*), *Ps. subviscida* var. *velata* (*P. peuce*, *Populus tremula*). The following species are **terricolous**: *Amanita muscaria* var. *muscaria*, *A. muscaria* var. *regalis*, *A. pantherina*, *Hygrocybe psittacina*, *Panaeolus acuminatus*, *P. ater*, *P. fimicola*, *P. campanulatus*, *P. olivaceus*, *P. reticulatus*, *P. subbalteatus*, *Psilocybe montana*, *Ps. inquilina*, *Rickenella fibula*, *Stropharia coronilla* and *Vascellum pratense*; while the species *Panaeolus ater*, *P. fimicola*, *P. papilionaceus* var. *papilionaceus*, *P. semiovatus* var. *semiovatus*, *P. sphinctrinus*, *Psilocybe coprophila* and *Ps. subcoprophila* were found growing on **dung (coprophilous)**. Two species were found growing **under soil (hypogeic)** (*Elaphomyces granulatus*, *E. reticula-*

tus) and *Claviceps purpurea* is a **parasite** on rye (*Secale cereale*).

These species were found in various forest associations, mostly **beech**: *Calamintho grandiflorae–Fagetum*, *Festuco heterophyllae–Fagetum*; **oak**: *Quercetum frainetto–cerris*, *Orno–Quercetum petraeae*, *Querco–Castanetum*; **other deciduous forests**: *Juglando–Platanetum orientalis*, *Salicetum albae–fragilis*, *Alnetum glutinosae*; **conifer**: *Digitali viridiflorae–Pinetum peuces*, *Pinus sylvestris* plantings, *Pinetum mughi silicicolum*, forest of *Pinus nigra*, *Gentiano luteaea–Pinetum peuces abietetosum*, *Pinetum sylvestris*, *Abieti–Piceetum scardicum*, *Piceetum excelsae–subalpinum scardicum*, *Juniperus excelsa* forest; **mixed deciduous and conifers forests**: *Fagus* and *Pinus nigra*, *Abieti–Fagetum*, *Populus nigra* and *Betula* plantings, etc., as well as at **roadsides**, ***Osmunda regalis* stands**, **meadows** and **mountain pastures**.

The species *Panaeolus ater*, *P. fimicola*, *P. olivaceus*, *P. reticulates* and *Amanita muscaria* var. *regalis* are new data for the Republic of Macedonia.

Most of the species cause the psilocin–psilocybin syndrome (23), while only three (all from genus *Amanita*) cause the muscarine syndrome, *Claviceps purpurea* causes the ergotism and *Elaphomyces granulatus*, *E. reticulatus* and *Vascellum pratense* belong to the group of fungi used in sacred rituals. According to some sources, some of these fungi are with questioned hallucinogenic properties. All fungi, about which the authors have found literature data for their hallucinogenic properties, including Internet sources, are taken into consideration in this paper.

Conclusion

Total number of 30 species is provided here. These species belong to the genera *Claviceps*, *Elaphomyces*, *Panaeolus*, *Psathyrella*, *Gymnopilus*, *Amanita*, *Pluteus*, *Psilocybe*, *Stropharia*, *Hygrocybe*, *Mycena*, *Rickenella* and *Vascellum*. The species *Amanita muscaria* var. *muscaria*, *A. pantherina* and *Pluteus cervinus* var. *cervinus* are most frequent, while the species *Amanita muscaria* var. *regalis*, *Elaphomyces granulatus*, *Gymnopilus junonius*, *G. sapineus*, *Panaeolus ater*, *P. acuminatus*, *P. fimicola*, *P. olivaceus*, *P. reticulatus*, *Psilocybe phyllogena*, *Ps. inquilina* and *Ps. subcoprophila* have occurred very seldom in the Republic of Macedonia. These species are terricolous, lignicolous, coprophilous, hypogeic and parasites. The list provides the distribution of these species in the Republic of Macedonia, where a large number of new localities are presented, while 5 species are new for the Republic of Macedonia.

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Macrofungi of Goksu River Basin (Adiyaman–Turkey)

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Abstract. This study was based on macrofungi specimens collected on field trips to Goksu River Valley between 2001 and 2005. One hundred and three taxa from 27 families and 2 classes have been identified. Eleven of them belonged to *Ascomycetes* and 92 to *Basidiomycetes.*

Key words: Adiyaman, Goksu, macrofungi, taxonomy, Turkey

Introduction

Goksu River is a branch of river Firat and has a valley of about 140 km long. Ninety (90) km of it take place in Adiyaman province and the rest in Kahramanmaras and Malatya provinces.

The climate of the area is Mediterranean according to Emberger's formula (Akman 1999) and falls mainly into the Irano–Turanean phytogeographical sector within the holarctic floral kingdom. The forest vegetation of the valley consists mainly of *Pinus*, *Quercus*, *Juglans*, *Paliurus*, *Pistacia*, *Morus*, *Nerium* spp. along the slopes and *Platanus*, *Salix*, *Populus* and *Tamarix* spp. along the river bank and stream sides.

To date, Kaya & al. (2004) and Kaya (2005) conducted investigations in Besni and Golbasi districts, respectively. The study aimed to determine the macrofungal distribution in Goksu River Valley.

Material and methods

Macrofungi sampling studies were conducted between 2001 and 2005 mostly in the spring and autumn due to the suitability of the climatic conditions for carpophore formation. Necessary morphological and ecological characteristics of the specimens were recorded and colour slides or digital photographs of them were taken in their natural habitats. Then the fruit bodies were taken to the laboratory and spore prints were obtained. After a microscopic investigation, they were identified using the following literature: Phillips (1981), Moser (1983), Breitenbach & Kränzlin (1984–2000), Cappelli (1984), Miller & Miller (1988), Candusso & Lanzoni (1990), Ellis & Ellis (1990), Buczacki (1992), Jordan (1995), Pegler & al. (1995), Antonin & Noordeloos (1997), and Bessette & al. (1997).

The specimens are kept in Gaziantep University, Adiyaman Education Faculty.

Results

103 macrofungi taxa belonging to 27 families and 55 genera were identified. Their localities, habitats, collection dates, collector's name and herbarium numbers are given in the list below. "K" indicates the collector Kaya, followed by the personal herbarium number.

ASCOMYCETES

Morchellaceae

1. ***Mitrophora semilibera*** (DC. ex Fr.) Lév.
Tut – Havutlu village, floodplain, 600 m, 04.04.2003, K. 1964.

2. ***Morchella deliciosa*** (Fr.) Boud.
Besni – Buruncayir village, among poplar, 780 m, 03.04.2004, K. 2499.

3. ***M. elata*** (Fr.) Boud.
Tut – Yaylimli village, floodplain, 550 m, 24.04.2002, K. 1637.

4. ***M. esculenta*** (L.) Pers. var. ***rigida*** Krombh.
Tut – Boyundere village, floodplain, 580 m, 01.03.2002, K. 1498.

5. ***M. esculenta*** (L.) Pers. var. ***vulgaris*** Pers.
Besni – Sambayat village, mixed wood, 630 m, 10.04.2004, K. 2503.

6. ***Verpa conica*** Sw. ex Pers.
Besni – Buruncayir village, among poplar, 780 m, 03.04.2004, K. 2501.

Helvellaceae

7. ***Helvella lacunosa*** Afzel. ex Fr.
Besni – Buruncayir village, among poplar, 780 m, 03.04.2004, K. 2500.

8. ***H. leucopus*** Pers.
Tut – Meryemusagi village, river bank, among poplar, 650 m, 13.04.2003, K. 1994.

9. ***H. queletii*** Bres.
Tut – Yaylimli village, among poplar, 550 m, 24.04.2002, K. 1638.

10. ***Paxina acetabulum*** (L. ex St. Amans) Kuntze
Besni – Sambayat village, mixed wood, 630 m, 10.04.2004, K. 2502.

11. ***P. leucomelas*** (Pers.) Kuntze
Adiyaman – Borgenek village, Cement factory campus area, pine forest, 510 m, 03.04.2004, K. 2496.

Basidiomycetes

Hymenochaetaceae

12. ***Phellinus igniarius*** (L. : Fr.) Quél.
Besni – Sarikaya village, on *Salix* spp., 480 m, 07.12.2003, K. 2491.

13. ***P. tuberculosus*** (Baumg.) Niemelä
Tut – Yaylimli village, river bank, on willow stump, 550 m, 22.04.2003, K. 2062.

Polyporaceae

14. ***Bjerkandera adusta*** (Fr.) P. Karst.
Tut, on decaying *Populus* stump, 1080 m, 22.04.2003, K. 2059.

15. ***Cerrena unicolor*** (Bull. : Fr.) Murrill
Tut – Gurlevik, on *Quercus* trunk, 1100 m, 29.04.2003, K. 2142.

16. ***Fomes fomentarius*** (L. : Fr.) Fr.
Tut – Yalankoz village, on *Quercus* stump, 780 m, 30.10.2002, K. 1920.

17. ***Funalia troqii*** (Berk.) Bond & Singer
Tut – Boyundere village, on *Populus* stump, 580 m, 22.04.2003, K. 2060.

18. ***Polyporus arcularius*** Batsch. : Fr.
Adiyaman – Borgenek village, steppe, 560 m, 03.04.2004, K. 2494.

19. ***P. rhizophilus*** (Pat.) Sacc.
Tut – Yalankoz village, steppe, 780 m, 29.04.2003, K. 2146.

20. ***P. squamosus*** (Huds.) Fr.
Besni – Buruncayir village, on willow stump, 780 m, 03.04.2004, K. 2498.

21. ***Trametes versicolor*** (L. : Fr.) Lloyd
Tut – Yaylimli village, on *Quercus* remains, 570 m, 06.05.2003, K. 2259.

Ganodermataceae

22. ***Ganoderma applanatum*** (Pers.) Pat.
Adiyaman – Yeniköy village, on *Salix* stump, 530 m, 15.02.2001, K. 1145.

23. ***G. lucidum*** (Fr.) P. Karst.
Besni – Sarikaya village, on *Salix* stump, 480 m, 07.12.2003, K. 2490.

Schizophyllaceae

24. ***Schizophyllum commune*** Fr.
Tut – Boyundere village, on *Quercus* stump remains, 600 m, 22.04.2003, K. 2061.

Nidulariaceae

25. ***Crucibulum leave*** (Huds. : Pers.) Kambly
Tut – Akçatepe village, on decaying *Vitis* remains, 650 m, 29.04.2003, K. 2144.

26. ***Cyathus olla*** Batsch : Pers.
Adiyaman – Borgenek village, Cement factory campus area, 510 m, 30.10.2002, K. 1693.

Tulostomataceae

27. ***Tulostoma brumale*** Pers. : Pers.
Besni – Tasliyazi village, pine forest, 780 m, 25.04.2005, K. 2763.

Geastraceae

28. ***Geastrum rufescens*** Pers.
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3431.

Lycoperdaceae

29. ***Bovista plumbea*** Pers. : Pers.
Tut – Ciftlik village, steppe, in grasses, 650 m, 13.04.2003, K. 1999; Ogutlu village, in grasses, 650 m, 22.04.2003, K. 2064.

30. ***Lycoperdon molle*** Pers. : Pers.
Tut – Yalankoz village, under *Quercus* spp., 800 m, 29.04.2003, K. 2143.

31. ***L. perlatum*** Pers.
Golbasi – Ozan village, pine forest, 850 m, 01.12.2002, K. 1955.

Rhizopogonaceae

32. ***Rhizopogon luteolus*** Fr.
Tut – Akcatepe village, among shrub, 850 m, 30.10.2002, K. 1917.

Suillaceae

33. ***Suillus luteus*** (L. : Fr.) Roussel
Tut, cemetery, under *Pinus* spp., 1000 m, 22.04.2003, K. 2058.

34. ***S. granulatus*** (L. : Fr.) Kuntze
Adiyaman – Borgenek village, under *Pinus* spp., 510 m, 13.04.2004, K. 2005.

Paxillaceae

35. ***Paxillus involutus*** (Batsch) Fr.
Besni – Buruncayir village, among poplar, 780 m, 02.12.2003, K. 2481.

Gomphidiaceae

36. ***Chroogomphus rutilus*** (Schaeff. : Fr.) O.K. Miller
Besni – Tasliyazi village, pine forest, 780 m, 06.12.2003, K. 2485.

37. ***Gomphidius maculatus*** (Scop.) Fr.
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3430.

Hygrophoraceae

38. ***Hygrophorus camarophyllus*** (Alb. & Schwein. : Fr.) Dumée, Grandjean & Maire
Golbasi – Harmanli village, pine forest, 750 m, 02.12.2003, K. 2477.

39. ***H. chrysodon*** (Batsch : Fr.) Fr.
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3432.

Tricholomataceae

40. ***Clitocybe gibba*** (Pers.) P. Kumm.
Besni – Tasliyazi village, pine forest, 780 m, 25.04.2005, K. 2764.

41. ***Collybia dryophila*** (Bull.) P. Kumm.
Adiyaman – Borgenek village, under *Pinus* spp., 510 m, 13.04.2003, K. 2007.

42. ***C. luteifolia*** Gillet
Besni – Tasliyazi village, pine forest, 780 m, 06.12.2003, K. 2488.

43. ***Cripinellis stipitaria*** (Fr.) Pat.
Besni – Tasliyazi village, pine forest edge, 06.12.2003, K. 2487.

44. ***Cystoderma terrei*** (Berk. & Broome) Harm.
Golbasi – Aktoprak village, pine forest, 780 m, 13.12.2005, K. 3435.

45. ***Hemimycena pithya*** (Fr.) Dörfelt
Besni – Tasliyazi village, pine forest, 780 m, 06.12.2003, K. 2489.

46. ***Lepista nuda*** (Bull. : Fr.) Cooke
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3427.

47. ***Macrocystidia cucumis*** (Pers.) Joss.
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3434.

48. ***Marasmius oreades*** (Bolton : Fr.) Fr.
Tut – Yaylimli village, river bank, in grass, 550 m, 14.04.2003, K. 2004.

49. ***Melanoleuca excissa*** (Fr.) Singer
Tut – Kaslica village, in grass, 1000 m, 13.04.2003, K. 2001.

50. ***Mycena purpureofusca*** (Peck) Sacc.
Golbasi – Harmanli village, pine forest, 750 m, 14.11.2003, K.2438.

51. ***Omphalina pyxidata*** (Bull. : Fr.) Quél.
Tut – Boyundere village, river bank, among grasses, 580 m, 01.03.2003, K. 1500.

52. ***O. velutipes*** Ort.
Tut – Ciftlik village, river bank, among grasses, 600 m, 04.04.2003, K. 1961.

53. ***Tricholoma fracticum*** (Britzelm.) Kreisel
Golbasi – Aktoprak village, pine forest, 850m, 14.11.2003, K. 2437.

54. ***T. populinum*** (Britzelm.) Kreisel
Erkenek – Sakaltutan picnic area, among poplar, 1250 m, 14.11.2003, K. 2435.

55. ***T. terreum*** (Schaeff. : Fr.) P. Kumm.
Golbasi – Ozan village, pine forest, 850 m, 01.12.2003, K. 1956.

56. ***Xeromphalina fellea*** Maire & Malençon
Golbasi – Aktoprak village, pine forest, 850 m, 02.12.2005, K. 2480.

Pleurotaceae

57. ***Lentinus tigrinus*** (Bull. : Fr.) Fr.
Tut – Ciftlik village, river bank, on willow remains, 630 m, 04.04.2003, K. 1962.

58. ***Pleurotus eryngii*** (DC. : Fr.) Quél.
Tut – Gurlevik, on *Ferula* remains, 1100 m, 29.04.2003, K. 2143.

59. ***P. ostreatus*** (Jacq. : Fr.) P. Kumm.
Adiyaman – Durak village, on *Salix* stump, 450 m, 12.01.2001, K. 1096; Tut – Havutlu village, river bank, on *Salix* stump, 600 m, 04.04.2003, K. 1963.

Entolomataceae

60. ***Entoloma sinuatum*** (Bull. ex Fr.) P. Kumm.
Tut – Akcatepe village, among shrub, 650 m, 29.04.2003, K. 2145.

61. ***E. rusticoides*** (A. Gillet) Noordel.
Besni – Tasliyazi village, pine forest edge, 780 m, 06.12.2003, K. 2484.

62. ***E. sericeoides*** (J.E. Lange) Noordel.
Golbasi – Ozan village, pine forest edge, 850 m, 01.12.2002, K. 1957.

Pluteaceae

63. ***Pluteus romelli*** (Britzelm.) Sacc.
Tut – Meryemusagi village, river bank, on *Populus* remains, 650 m, 13.04.2003, K. 1996.

64. ***Volvariella bombycina*** (Schaeff. : Fr.) Singer
Tut – Yaylimli village, on *Populus* stump remain, 550 m, 06.05.2003, K. 2258.

65. ***V. speciosa*** (Fr. : Fr.) Singer
Adiyaman – Durak village, floodplain, among grasses, 450 m, 12.01.2001, K. 1093; Borgenek village, floodplain, among grasses, 490 m, 23.01.2001, K. 1128.

Agaricaceae

66. ***Agaricus campestris*** L. : Fr.
Adiyaman – Durak village, river bank, manured meadow, 450 m, 13.01.2001, K. 1094.

67. ***Leucoagaricus leucothites*** (Vittad.) Wasser
Dogansehir – Kapidere village, in grass among poplar, 1100 m, 22.10.2005, K. 2822.

Lepiotaceae

68. ***Macrolepiota excoriata*** (Schaeff. : Fr.) Wasser
Tut – Yalankoz village, steppe, 800 m, 30.10.2002, K. 1919; Golbasi – Aktoprak village, forest edge, 850 m, 13.12.2005, K. 3429.

Coprinaceae

69. ***Coprinus atramentarius*** (Bull. : Fr.) Fr.
Tut – Meryemusagi village, river bank, under *Populus* spp., 650 m, 13.04.2003, K. 1995.

70. ***C. auricomus*** Pat.
Tut – Boyundere village, floodplain, in grass, 580 m, 06.05.2003, K. 2255.

71. ***C. comatus*** (Müll. : Fr.) Gray
Tut – Boyundere village, river bank, on sandy soil, 580 m, 01.03.2003, K. 1499; Tut – Yalankoz village, among shrubs, 800 m, 30.10.2002, K. 1918.

72. ***C. disseminatus*** (Pers. : Fr.) Gray
Dogansehir – Kapidere village, woody debris, 1100 m, 22.10.2005, K. 2819.

73. ***C. micaceus*** (Bull. : Fr.) Fr.
Tut – Ciftlik village, on *Populus* stump, 630 m, 13.04.2003, K. 1998; Dogansehir – Kapidere, on *Salix* stump, 1100 m, 22.10.2005, K. 2824.

74. ***C. niveus*** (Pers. : Fr.) Fr.
Adiyaman – Borgenek village, on dung, 530 m, 03.04.2004, K. 2497.

75. ***C. plicatilis*** (Curtis : Fr.) Fr.
Tut – Yaylimli village, in grass, 550 m, 24.04.2002, K. 1640.

76. ***Panaeolus guttulatus*** Bres.
Tut – Ogutlu village, on woody debris, 650 m, 22.04.2003, K. 2063.

77. ***P. olivaceus*** Møller
Tut – Yaylimli village, on manured soil, 550 m, 24.04.2002, K. 1643.

78. ***P. papilionaceus*** (Bull. : Fr.) Quél.
Tut – Yaylimli village, river bank, on manured sandy soil, 550 m, 24.04.2002, K. 1641; Boyundere village, floodplain, 580 m, 06.05.2003, K. 2256.

79. ***P. sphinctrinus*** (Fr.) Quél.
Tut – Boyundere village, floodplain, in grass, 580 m, 06.05.2003, K. 2257.

80. ***Psathyrella candolleana*** (Fr. : Fr.) Maire
Tut, garden, on woody debris, 1050 m, 22.04.2003, K. 2036.

Bolbitiaceae

81. ***Agrocybe cylindraceae*** (DC. : Fr.) Maire
Tut – Yaylimli village, on *Populus* stump remains, 550 m, 24.04.2003, K. 1639.

82. ***A. dura*** (Bolton) Singer
Tut – Kaslica village, steppe, in grasses, 1000 m, 13.04.2003, K. 2002.

83. ***A. splendida*** Clemençon
Adiyaman – Borgenek village, floodplain, in grasses, 03.04.2004, K. 2493.

84. ***Bolbitius vitellinus*** (Pers. : Fr.) Fr.
Tut – Yalankoz village, on manured straw, 800 m, 29.04.2003, K. 2147.

85. ***Conocybe lactea*** (J.E. Lange) Métrod
Tut – Kaslica village, in grasses on manured soil, 1000 m, 13.04.2003, K. 2000.

86. ***C. moseri*** Watl. var. ***moseri***
Tut – Boyundere village, floodplain, in grass, 580 m, 13.04.2003, K. 2003.

87. ***C. subovalis*** Kühn. & Watling
Adiyaman – Borgenek, steppe, among grasses, 550 m, 18.03.2001, K. 1272.

88. ***C. tenera*** (Schaeff. : Fr.) Fayod
Tut – Meryemusagi village, in grass, 650 m, 13.04.2003, K. 1992.

Auriscapiaceae

89. ***Lentinellus omphalodes*** (Fr.) P. Karst.
Golbasi – Aktoprak village, pine forest, on pine twigs, 13.12.2005, K. 3426.

Strophariaceae

90. ***Hypholoma fasciculare*** (Huds. : Fr.) P. Kumm.
Dogansehir – Kapidere village, mixed wood, 1100 m, 22.10.2005, K. 2818.

91. ***Pholiota aurivella*** (Batsch. : Fr.) P. Kumm.
Dogansehir – Kapidere village, on *Salix* stump, 1100 m, 22.10.2005, K. 2823.

92. ***P. graminis*** (Quél.) Singer
Dogansehir – Kapidere village, mixed wood, 1100 m, 22.10.2005, K. 2825.

93. ***P. squarrosa*** (Pers. : Fr.) P. Kumm.
Dogansehir – Kapidere village, on *Juglans* spp., 1100 m, 22.10.2005, K. 2827.

94. ***Stropharia coronilla*** (Bull. : Fr.) Quél.
Tut – Boyundere village, steppe, 550 m, 01.03.2002, K. 1501.

95. ***Tubaria conspersa*** (Pers.) Fayod
Adiyaman – Yenikoy village, floodplain, on plant remains, 530 m, 15.02.2001, K. 1146.

96. ***T. hiemalis*** Romagn. ex Bon
Tut – Meryemusagi village, woody debris, 650 m, 13.04.2003, K. 1993.

Cortinariaceae

97. ***Hebeloma crustuliniforme*** (Bull. : Fr.) Quél.
Erkenek – picnic area, under mixed wood, 1100 m, 14.11.2003, K. 2422.

98. ***H. sinapizans*** (Paulet : Fr.) Gillet
Dogansehir – Kapidere village, mixed wood, 1100 m, 22.10.2005, K. 2825.

99. ***Inocybe bongardii*** (Weinm.) Quél. var. ***piscidora*** (Donadini & Riousset) Kuyper
Adiyaman – Borgenek village, under *Pinus* spp., 510 m, 13.04.2003, K. 2006.

100. ***I. fastigiata*** (Schaeff. : Fr.) Quél.
Meryemusagi village, floodplain, under poplar, 650 m, 13.04.2003, K. 1997.

101. ***I. geophylla*** (Sowerby) P. Kumm. var. ***geophylla*** (Sowerby) P. Kumm.
Besni – Tasliyazi village, pine forest, 780 m, 06.12.2003, K. 2486.

102. ***I. geophylla*** (Sowerby) P. Kumm. var. ***lilacina*** Gillet
Golbasi – Aktoprak village, pine forest, 850 m, 13.12.2005, K. 3433.

Russulaceae

103. ***Lactarius deliciosus*** (L. : Fr.) Gray
Golbasi – Harmanli village, pine forest, 750 m, 14.11.2003, K. 2439.

Discussion

In this study, 103 macrofungi taxa from 27 families and 55 genera were identified. Eleven of them have belonged to *Ascomycetes* and 92 to *Basidiomycetes*. Twenty eight (27.2 %) of the taxa are edible, 55 (53.4 %) inedible and 20 (19.4 %) poisonous. When compared, these results show similarities with the findings of the studies carried out in neighbouring regions (Isiloglu & Oder 1995; Kaya & al. 2004; Kaya 2005).

Although 28 of the 103 species are edible, only four species (*Coprinus comatus*, *Agaricus campestris*, *Pleurotus eryngii* and *P. ostreatus*) were found to be eaten by local residents. Since, the species which are not familiar to them are considered to be poisonous and they avoid collecting and consuming any other mushrooms they don't know.

Members of *Morchella* are not known by locals, either, but during spring they are collected by the mushroom hunters of companies which collect and export the fruit bodies.

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Contributions to the macrofungi flora of Muş (Turkey) province

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Abstract. This study was carried out on macrofungi samples collected in Muş province between 1998 and 2000. As a result of field and laboratory studies, 68 species belonging to 21 families were identified.

Key words: macrofungi, Muş, mycoflora, taxonomy, Turkey

Introduction

Muş is an East Anatolian vilayet with a surface area of 8196 km^2, located mainly in B9 according to Davis' grid square system.

The climate of the area is Mediterranean according to the Emberger's formula (Akman 1999). The rainfall in the region is 825.5 mm and the annual average temperature is around 9.3 °C.

Phytogeographically the area is in the Irano–Turanean flora sector. Main vegetation types are forests and anthropogenic steppes. The forest area is mainly characterized by *Quercus* L., especially *Q. infectoria* subsp. *boissieri* Reut. at higher portions of the area. At lower portions and stream sides *Populus* L. and *Salix* L. spp. form the main clamps of trees.

According to the review of the current literature, two researches on edible (Kaya 2000a) and poisonous (Kaya 2000b) mushrooms, growing in the area, were carried out.

The aim of the study was to determine the macrofungi flora of the province and to make a contribution to the macrofungal flora of Turkey.

Material and methods

The specimens of the study were collected during field trips in Muş province between 1998 and 2000. Morphological and ecological characteristics of the macrofungi were recorded and they were photographed at their natural habitats. The specimens were then taken to the laboratory and spore prints were obtained. After necessary microscopic investigations, they were identified with the help of Phillips (1981), Moser (1983), Breitenbach & Kränzlin (1984–2000), Ellis & Ellis (1990), Buczacki (1992), Pegler & al. (1995) and Antonin & Noordeloos (1997).

The specimens are kept in Gaziantep University, Adiyaman Education Faculty.

Results

Species identified from the research area are listed below. "K" indicates Kaya (collector's name) and the numbers indicate the personal herbarium numbers.

ASCOMYCETES

Helvellaceae

1. ***Helvella acetabulum*** (L. : Fr.) Quél.
Kizilagac, Harman village, under mixed wood, 1300 m, 15.05.2000, K. 1012.

2. ***H. leucopus*** Pers.
Bulanik, under *Populus* sp., 1450 m, 09.05.1999, K. 878.

3. ***Paxina leucomelas*** (Pers.) Kuntze
Muş – Alparslan farm, pine forest, 1250 m, 16.05.2000, K. 1029.

Morchellaceae

4. ***Mitrophora semilibera*** (DC. ex Fr.) Lév.
Bulanik, floodplain, under *Populus* sp., 1450 m, 17.05.2000, K. 1040.

5. ***Morchella elata*** Fr.
Bulanik, floodplain, under *Populus* sp., 1450 m, 09.05.1999, K. 879.

6. ***M. esculenta*** (L.) Pers.
Bulanik, floodplain, under *Populus* sp., 1450 m, 17.05.2000, K. 1038.

Pyronemataceae

7. ***Geopora arenicola*** (Lév.) Kers.
Bulanik, stream bank, damp woody debris, 1450 m, 17.05.2000, K. 1041.

Basidiomycetes

Ganodermataceae

8. ***Ganoderma applanatum*** (Pers. ex Wallr.) Pat.
Varto, Tepe village, on *Salix* stump, 1540 m, 21.06.1998, K. 527.

Hymenochaetaceae

9. ***Inonotus dryadeus*** (Pers. : Fr.) Murrill
Haskoy, Buvetli village, on *Salix* stump, 1320 m, 20.06.1998, K. 519.

10. ***I. hispidus*** (Fr.) Karst.
Sogucak village, on *Juglans* sp., 1400 m, 23.10.1998, K. 710.

11. ***Phellinus igniarius*** (L. : Fr.) Quél.
Varto, Tepe village, on *Salix* stump, 1540 m, 21.06.1998, K. 529.

12. ***P. tuberculosus*** (Baumg.) Niemalä
Malazgirt, on *Prunus* sp., 1550 m, 03.05.1998, K. 236.

Polyporaceae

13. ***Bjerkandera adusta*** (De Willd. : Fr.) Karst.
Muş, Tandogan village, on *Populus* stump, 1300 m, 01.06.1998, K. 463; Alparslan farm, 02.10.1998, K. 591.

14. ***Cerrena unicolor*** (Fr.) Murrill
Kizilagac, Harman village, on *Quercus* stump, 1300 m, 15.05.2000, K. 1014.

15. ***Fomes fomentarius*** (L. : Fr.) Kick
Malazgirt, on *Salix* trunk, 1550 m, 21.10.1998, K. 650; Muş – Alparslan farm, on *Salix* sp., 1250 m, 16.05.2000, K. 1031.

16. ***Funalia trogii*** (Berk.) Bond. & Singer
Tandogan village, on *Populus* stump, 1300 m, 02.05.1998, K. 224; Mercimekkale village, 21.06.1998, K. 537.

17. ***Pycnoporus cinnabarinus*** (Jacq. : Fr.) Karst.
Haskoy, Ucdam village, on *Quercus* stump, 1350 m, 02.05.1998, K. 205.

18. ***Trametes versicolor*** (Fr.) Pilát
Haskoy, Ucdam village, on *Quercus* sp., 1350 m, 02.05.1998, K. 202.

Schizophyllaceae

19. ***Schizophyllum commune*** Fr.
Korkut, Altinova village, on *Quercus* stump, 1400 m, 25.04.1998, K. 153; Muş – Sogucak village, on dead *Prunus* branch, 1400 m, 16.05.2000, K. 1032.

Sclerodermataceae

20. ***Scleroderma verrucosum*** (Bull.) Pers.
Haskoy, Buvetli village, around *Quercus* sp., 1350 m, 20.06.1998, K. 516.

Suillaceae

21. ***Suillus luteus*** (L.) Gray
Muş, Alparslan farm, under *Pinus* sp., 14.10.1999, K. 940.

Rhizopogonaceae

22. ***Rhizopogon luteolus*** Fr.
Muş, Alparstlan farm, pine forest, 1250 m, 16.05.2000, K. 1030.

Nidulariaceae

23. ***Cyathus olla*** Batsch : Pers.
Muş, Sogucak village, on dead twigs, 1400 m, 17.05.2000, K. 1033.

Tricholomataceae

24. ***Collybia dryophila*** (Bull. : Fr.) P. Kumm.
Muş, Karlidere village, under *Quercus* sp., 1350 m, 01.06.1998, K. 492.

25. ***C. marasmioides*** (Britzelm.) Bresinsky & Stangl
Muş, Sogucak village, under mixed wood, 01.06.1998, K. 486.

26. ***Marasmius rotula*** (Scop. : Fr.) Fr.
Kizilagaç, Harman village, in grass, 1300 m, 15.05.2000, K. 1013.

27. ***Mycena acicula*** (Schaeff. : Fr.) P. Kumm.
Bulanik, floodplain, on decaying bark, 1450 m, 17.05.2000, K. 1037.

28. ***M. galericulata*** (Scop. : Fr.) Gray
Haskoy, around the highway, under *Quercus* sp., 1350 m, 02.05.1998, K. 216.

29. ***M. hiemalis*** (Osbeck : Fr.) Quél.
Tandogan village, on *Populus* stump, 1300 m, 01.06.1998, K. 467.

30. ***Tricholoma fracticum*** (Britzelm.) Kreisel
Muş Sugar Factory Campus area, under *Pinus* sp., 1300 m, 22.10.1998, K. 711.

31. ***T. populinum*** J.E. Lange
Muş – Alparslan farm, on *Populus* stump, 1250 m, 22.10.1998, K. 669.

Pleurotaceae

32. ***Lentinus cyathyformis*** (Schaeff.) Bres.
Varto, Tasdibek village, on *Populus* stump, 1600 m, 21.06.1998, K. 522.

33. ***L. tigrinus*** (Bull. : Fr.) Fr.
Malazgirt, on *Salix* stump, 1550 m, 17.05.2000, K. 1042.

Entolomataceae

34. ***Entoloma undatum*** (Fr.) Mos.
Korkut, Alazli village, woody debris, 1400 m, 13.06.1999, K. 889.

Lepiotaceae

35. ***Lepiota oreadiformis*** Vel.
Haskoy, Elmabulak village, meadow, 1350 m, 17.05.1998, K. 298.

36. ***Macrolepiota excoriata*** (Schaeff. : Fr.) Wass.
Muş, Alparslan farm, under *Pinus* sp., 1250 m, 14.10.1999, K. 941.

Pluteaceae

37. ***Pluteus romelli*** (Britzelm.) Sacc.
Muş, Tandogan village, on decaying *Populus* remains, 1300 m, 16.05.2000, K. 1036.

38. ***Volvariella bombycina*** (Schaeff. : Fr.) Singer
Muş, Alparslan farm, on *Populus* stump, 1250 m, 03.10.1998, K. 592.

39. ***V. murinella*** (Quél.) M.M. Moser
Muş, Sogucak village, under mixed wood, 1400 m, 01.06.1998, K. 487.

40. ***V. speciosa*** (Fr. : Fr.) Singer
Malazgirt, Gulkoru village, 1600 m, 17.05.2000, K. 1045.

Agaricaceae

41. ***Agaricus silvicola*** (Vittad.) Peck
Bulanik, floodplain, under *Populus* sp., 1450 m, 09.05.1999, K. 875.

42. ***A. xanthoderma*** Genev.
Muş Sugar Factory Campus area, in grasses, 1300 m, 19.09.1999, K. 904.

Coprinaceae

43. ***Coprinus apthosus*** Fr.
Bulanik, Gulluova village, stream bed, 1500 m, on sandy soil, 20.05.1998, K. 365.

44. ***C. atramentarius*** (Bull. : Fr.) Fr.
Korkut, Altinova village, among poplar, 1400 m, 13.06.1999, K. 893.

45. ***C. auricomus*** Pat.
Korkut, Alazli village, woody debris, 1400 m, 13.06.1999, K. 889.

46. ***C. comatus*** (Muell. : Fr.) Pers.
Malazgirt, along highway, under poplar, 1550 m, 17.05.2000, K. 1043.

47. ***C. domesticus*** (Bolt. : Fr.) Gray
Muş, Tandogan village, woody debris, 1300 m, 14.10.1999, K. 937.

48. ***C. micaceus*** (Bull. : Fr.) Fr.
Muş, Yarpuzlu village, on *Salix* stump, 1300 m, 14.10.1999, K. 934.

49. ***C. kuehneri*** Ulje & Bas
Muş, Sogucak village, under mixed wood, 1400 m, 01.06.1998, K. 473.

50. ***C. niveus*** (Pers. : Fr.) Fr.
Haskoy, Gokyazı village, on cow dung, 1350 m, 26.04.1998, K. 173.

51. ***Panaeolus fimicola*** (Fr.) Gill
Haskoy, Buvetli village, meadow, 1350 m, 17.05.1998, K. 305.

52. ***Psathyrella candolleana*** (Fr. : Fr.) Maire
Bulanik, stream bank, around *Populus* sp., 1450 m, 17.05.2000, K. 1039.

53. ***P. marcescibilis*** (Britzelm.) Singer
Bulanik, Karaagil village, among leaf litter, 1450 m, 19.05.1998, K. 348.

54. ***P. spadiceogrisea*** (Schaeff. : Fr.) Maire
Bulanik, floodplain, woody debris, 1450 m, 19.05.1998, K. 355.

Bolbitaceae

55. ***Agrocybe cylindracea*** (DC. : Fr.) Maire
Malazgirt, on *Populus* sp., 1550 m, 21.10.1998, K. 649; Varto, Akpinar village, on *Populus* sp., 1550 m, 22.10.1998, K. 659.

56. ***A. paludosa*** (J.E. Lange) Kühn. & Romagn.
Bulanik, Sogutlu village, meadow, 1500 m, 20.05.1998, K. 367.

57. ***A. pediades*** (Fr.) Singer
Muş – Karlidere village, meadow, 1350 m, 01.06.1998, K. 493.

58. ***A. semiorbicularis*** (Bull. ex St. Amans) Fay
Haskoy – Gokyazı village, meadow, 1350 m, 26.04.1998, K. 172.

59. ***Conocybe lactea*** (J.E. Lange) Métrod
Haskoy, Buvetli village, meadow, 1350 m, 26.04.1998, K. 169; Koguktas village, in manured meadow, 17.05.1998, K. 303; Malazgirt Gulkoru village, on manure enriched soil, 17.05.2000, K. 1047.

60. ***C. rickeniana*** Singer ex P.D. Orton
Muş, Sogucak village, meadow, 1400 m, 01.06.1998, K. 482.

61. ***C. tenera*** (Schaeff. : Fr.) Fay
Haskoy, Kockdy village, meadow, 1350 m, 17.05.1998, K. 301.

Strophariaceae

62. ***Pholiota graminis*** (Quél.) Singer
Muş, Tandogan village, in wet meadow, 1300 m, 14.10.1999, K. 938.

63. ***P. gummosa*** (Lasch : Fr.) Singer
Varto, on *Salix* remains, 1450 m, 22.10.1998, K. 657.

64. ***Stropharia coronilla*** (Bull. : Fr.) Quél.
Malazgirt, Gulkoru village, meadow, 1600 m, 17.05.2000, K. 1046.

65. ***S. semiglobata*** (Batsch : Fr.) Quél.
Korkut – Altinova village, meadow, 1400 m, 25.04.1998, K. 156.

Cortinariaceae

66. ***Hebeloma crustuliniforme*** (Bull. : Fr.) Quél.
Muş, Yarpuzlu village, around *Populus* sp., 1300 m, 14.10.1999, K. 935.

67. ***Inocybe splendens*** R. Heim. var. ***phaeoleuca*** (Kühn.) Kuyper
Muş, Yarpuzlu village, under *Populus* sp., 1300 m, 14.10.1999, K. 936.

68. ***I. splendens*** R. Heim. var. ***splendens*** (Kühn.) Kuyper
Muş, Tandogan village, on loamy soil under poplar, 1300 m, 14.10.1999, K. 939.

Discussion

In previous studies which were carried out on edible (Kaya 2000a) and poisonous (Kaya 2000b) mushrooms, 47 macrofungi species have been recorded from the research area. With the addition of the findings of this study, this number increased to 98. Among these species 10 (10 %) belonged to *Ascomycetes* and 88 (90 %) to *Basidiomycetes*. When compared, these results bear similarities with the findings of the studies carried out in neighbouring regions (Demirel 1996; Kaya 2001).

The most widespread families in the region are *Coprinaceae*, *Tricholomataceae* and *Polyporaceae*, respectively.

Only three species – *Pleurotus eryngii*, *P. ostreatus* and *Agaricus campestris* – are consumed in all regions of the province, but *P. eryngii*, which is known as "Heliz Mushroom" almost equivalent with the term "Mushroom", is the only economically important one. During spring this mushroom is sold along the highways by the villagers or in city centres by mobile sellers.

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Macrofungi of the Parangalitsa Biosphere Reserve in Rila Mts, Bulgaria

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Abstract. Data on the species diversity of macrofungi in the Parangalitsa Biosphere Reserve in Rila Mts are reported. Mycological observations were carried out during the period 1998–2005 in old coniferous and beech forests. A total of 307 taxa (298 species, 8 varieties and 1 form) from larger ascomycetes and basidiomycetes were recorded. The macrofungi belonged to 12 orders, 55 families and 133 genera. Four species are new to Bulgaria. Among all macrofungi, 226 species are reported for the first time from the Parangalitsa Biosphere Reserve. Fourteen species are of high conservation importance.

Key words: biodiversity, macrofungi, old forests, Parangalitsa

Introduction

Parangalitsa Biosphere Reserve is situated in the south-west Rila Mts on the territory of the Rila National Park. Parangalitsa is one of the first Bulgarian reserves. It was declared as reserve by Order No. 8517 of 30.06.1933, issued by the Ministry of Agriculture and State Properties in order to preserve the unique nature complex of old virgin spruce forests (Nedyalkov & Nikolov 1986; Georgiev 1993). Data on macrofungi of the Parangalitsa Biosphere Reserve have been published by Rosnev & Stoichev (1985), Stoichev & Dimcheva (1987), Kuthan & Kotlaba (1989), Gyosheva (1996), Drumeva-Dimcheva & Gyosheva-Bogoeva (1998). A total of 79 species of ascomycetes and basidiomycetes have been reported (Table 1).

The present work includes data on the species diversity, ecological-trophic structure and conservation value of macrofungi found in the Parangalitsa Biosphere Reserve so far. The greatest number of materials was collected by us during the period 1998–2005. The main purpose of the investigation was to make contemporary inventory of macrofungi in the old coniferous and mixed forests in the reserve.

Study area

Parangalitsa Biosphere Reserve lies in the most upper reaches of the river Blagoevgradska Bistritsa (42°15' N, 20°15' E) and covers 1509 ha. A buffer zone of 1258.7 ha is also differentiated.

The geological base is formed of south-Bulgarian granites. Soil cover consists of brown mountain forests soils and mountain-meadow peat soils. The climate is typical mountainous. From a floristic point of view the reserve is very interesting: 30 species are endemics and relicts for the Balkan Peninsula, 6 species – for Bulgaria. Parangalitsa Biosphere Reserve is representative for the coniferous belt. Pure and mixed *Picea* forests with *Abies alba*, *Pynus sylvestris*, *P. peuce* and *Fagus sylvatica* cover the major part (75.4 %) of the territory [Acidophilous *Picea* forests of the montane to alpine levels (*Vaccino–Piceetea*) (9410)].

The oldest trees are more over 350 years. There are some windthrow spots in the spruce forests (18.6 ha). In the subalpine belt the communities of *Pinus mugo* prevail. The alpine communities are poorly developed (Nedyalkov & Nikolov 1986; Georgiev 1993).

Material and methods

Field studies in the Parangalitsa Biosphere Reserve were carried out by the tracking method during the period 1998–2005. The objects of mycological observations were pure and mixed *Picea abies* forests and *Fagus sylvatica* forests between 1400–2500 m alt. in the reserve (chiefly on the territory between the rivers Blagoevgradska Bistritsa and Haidoushka). The nomenclature of macrofungi follows Kirk & al. (2001). The conservation status of the species is based on the official *Red List of fungi in Bulgaria* (Gyosheva & al. 2006). The old forest indicator fungal species were proposed by Parmasto & Parmasto (1997), Tortič (1998), and Nitare (2000).

Results and discussion

Species diversity of macrofungi

The total number of currently known macrofungal taxa from the Parangalitsa Biosphere Reserve is 307 (298 species, 8 varieties and 1 form). In comparison, about 350 taxa are known so far from the Rila National Park (Gyosheva & Denchev 2000) and about 300 taxa – from the Rila Monastery Nature Park (Gyosheva 2003).

The greatest number of macrofungi (277 taxa) was found during the present investigation (1998–2005).

The fungi belong to *Ascomycota* (19 species) and to *Basidiomycota* (279 species, 8 varieties, 1 form), and are related to 12 orders, 55 families and 133 genera. Order *Agaricales* dominated by the number of species (164).

Table 1. Checklist of macrofungi in the Parangalitsa Biosphere Reserve.

Taxa	Plant communities				ETG**	Species from present study	Sources
	1	2	3	4			
Ascomycota							
Helotiales							
Cudoniaceae							
1. *Cudonia confusa* Bres.	+	+			St	+	
Helotiaceae							
2. *Ascocoryne sarcoides* (Jacq.) J.W. Groves & D.E. Wilson			+		LeS	+	
3. *Bisporella citrina* (Batsch. : Fr.) Korf & S.E. Carp.			+		LeS	+	
4. *Hymenosciphus calyculus* (Sowerby) W. Phillips			+		LeS	+	
5. *Neobulgaria pura* (Fr.) Pers.			+		LeS	+	Rosnev & Stoichev 1985
Hyaloscyphaceae							
6. *Lachnellula subtilissima* (Cooke) Leotiacaev Dennis		+			LeS	+	
Leotiaceae							
7. *Leotia lubrica* (Scop. : Fr.) Pers.			+		Hu	+	
Pezizales							
Discinaceae							
8. *Gyromitra infula* (Schaeff. : Fr.) Quél.	+	+			LeS	+	
Helvellaceae							
9. *Helvella crispa* Fr.			+		Hu	+	
Pezizaceae							
10. *Peziza badia* Pers.		+			Hu	+	
Pyrenomataceae							
11. *Humaria hemisphaerica* (F.H. Wigg. : Fr.) Fuckel		+			LeS	+	
12. *Scutellinia scutellata* (L.) Lambotte		+	+		Hu, LeS	+	Rosnev & Stoichev 1985
13. *Otidea onotica* (Pers. : Fr.) Fuckel			+		Hu	+	
Sarcoscyphaceae							
14. *Pithya vulgaris* Fuckel		+			LeS	+	
Xylariales							
Diatrypaceae							
15. *Diatrype disciformis* (Hoffm. : Fr.) Fr.			+		LeS	+	

	Taxa	Plant communities				ETG**	Species from present study	Sources
		1	2	3	4			
	Xylariaceae							
16.	*Hypoxylon fragiforme* (Pers. : Fr.) Kickx			+		LeS	+	Rosnev & Stoichev 1985
17.	*H. multiforme* (Fr. : Fr.) Fr.			+		LeS	+	
18.	*H. serpens* (Pers. : Fr.) Fr.			+		LeS	+	
19.	*Xylaria hypoxylon* (L. : Fr.) Grev.			+		LeS	+	Rosnev & Stoichev 1985
	Basidiomycota							
	Agaricales							
	Agaricaceae							
20.	*Agaricus arvensis* Schaef. : Fr.		+			Hu	+	
21.	*A. augustus* Fr.		+			Hu	+	
22.	*A. silvicola* (Vittad.) Peck		+			Hu	+	
23.	*Chlorophyllum rhacodes* (Vittad.) Velinga		+	+		Hu	+	
24.	*Lepiota clypeolaria* (Bull. : Fr.) P. Kumm.		+	+		Hu	+	
25.	*L. magnispora* Murrile		+	+		Hu	+	
26.	*Macrolepiota procera* (Scop. : Fr.) Singer		+	+		Hu	+	
	Bolbitiaceae							
27.	*Hebeloma crustiliniforme* (Bull. : Fr.) Quél.			+		Mr	+	
28.	*Naucoria cerodes* (Fr.) P. Kumm.		+			Br	+	
	Clavariaceae							
29.	*Macrotyphula fustilosa* (Holmsk. : Fr.) R.H. Petersen			+		LeS	+	
	Coprinaceae							
30.	*Coprinus plicatilis* (Curtis : Fr.) Fr.			+	+	Mr	+	
	Cortinariaceae							
31.	*Cortinarius brunneus* (Pers. : Fr.) Fr.	+	+			Mr	+	
32.	*C. bulbosus* (Sowerby : Fr.) Fr.	+	+			Mr	+	
33.	*C. cinnabarinus* Fr.			+		Mr	+	
34.	*C. cinnamomeobadius* (R. Hry) M.M. Moser	+	+			Mr	+	
35.	*C. cinnamomeus* (L. : Fr.) Fr.	+	+			Mr	+	
36.	*C. duracinus* Fr.			+		Mr	+	
37.	*C. elegantior* (Fr.) Fr.	+	+			Mr	+	
38.	*C. glaucopus* (Schaeff. : Fr.) Fr.		+	+		Mr	+	
39.	*C. multiformis* (Fr.) Fr.			+		Mr	+	
40.	*C. multiformis* var. *coniferatum* M.M. Moser		+			Mr	+	
41.	*C. obtusus* (Fr. : Fr.) Fr.	+	+			Mr	+	
42.	*C. privignoides* R. Hry		+			Mr	+	
43.	*C. purpurascnes* (Fr. : Fr.) Fr.		+	+			+	
44.	*C. sanguineus* (Wulfen : Fr.) Fr.	+	+			Mr	+	
45.	*C. semisanguineus* (Fr.) M.M. Moser		+			Mr	+	
46.	*C. torvus* (Fr. : Fr.) Fr.			+		Mr	+	
47.	*C. venetus* (Fr. : Fr.) Fr. var. *montanus* M.M. Moser		+			Mr	+	
48.	*Creptodus applanatus* (Pers.) P. Kumm. var. *subglobiger* Singer	+	+			LeS	+	
49.	*C. variabilis* (Pers. : Fr.) P. Kumm.		+	+		LeS	+	
50.	*Galerina hypnorum* (Schrank : Fr.) Kühner	+	+			Br	+	
51.	*G. marginata* (Batsch : Fr.) Kühner		+			LeS	+	
52.	*Gymnopilus hybridus* (Fr. : Fr.) Maire	+	+			LeS	+	
53.	*G. penetrans* (Fr. : Fr.) Murrill	+	+			LeS	+	
54.	*Inocybe flocculosa* (Berck.) Sacc.			+		Mr	+	
55.	*I. geophilla* (Sowerby : Fr.) P. Kumm.		+	+		Mr	+	
56.	*I. geophilla* var. *lilacina* (Peck) Gillet		+	+		Mr	+	
57.	*I. lacera* (Fr. : Fr.) P. Kumm.		+			Mr	+	

Taxa	Plant communities				ETG**	Species from present study	Sources
	1	2	3	4			
58. *I. obscura* (Pers.) Gillet	+	+			Mr	+	
59. *I. posterula* (Britzelm.) Sacc.		+	+		Mr	+	
60. *I. umbrina* Bres.	+	+			Mr	+	
61. *Leucocortinarius bulbiger* (Alb. & Scwein. : Fr.) Singer		+	+		Mr	+	
Entolomataceae							
62. *Clitopilus prunulus* (Scop. : Fr.) Fr.		+	+		Hu	+	
63. *Entoloma vernum* Lundell		+		+	Mr	+	
Hydnangiaceae							
64. *Laccaria amethystina* Cooke		+	+		Mr	+	
65. *L. laccata* (Scop. : Fr.) Fr.		+	+		Mr	+	
Lycoperdaceae							
66. *Bovista plumbea* Pers. : Pers.				+	Hu	+	
67. *Handkea excipuliformis* (Scop. : Pers.) Pers.		+	+		Hu	+	
68. *Lycoperdon echinatum* Pers. : Pers.			+		Hu	+	
69. *L. molle* Pers. : Pers.		+	+		Hu	+	
70. *L. perlatum* Pers. : Pers.	+	+	+		St	+	
71. *L. pyriforme* Schaeff. : Pers.		+	+		LeS	+	
72. *L. umbrinum* Pers. : Pers.		+	+		Hu	+	
Marasmiaceae							
73. *Armillaria mellea* (Vahl. : Fr.) P. Kumm.	+	+	+		LeS, LeP	+	Rosnev & Stoichev 1985
74. **Gerronema brevibasidiatum* (Singer) Singer	+				Br	+	
75. *Marasmiellus ramealis* (Bull. : Fr.) Singer			+		LeS	+	Rosnev & Stoichev 1985
76. *Marasmius alliceus* (Jacq. : Fr.) Fr.			+		LeS	+	Rosnev & Stoichev 1985
77. *M. androsaceus* (L. : Fr.) Fr.	+	+			Ad, LeS	+	
78. *M. bullardii* Quél.	+	+			Ad, St	+	
79. *M. oreades* (Bolton : Fr.) Fr.				+	Mr	+	
80. *M. quercetum* Britz.			+		LeS	+	
81. *M. rotula* (Scop. : Fr.) Fr.			+		LeS	+	
82. *Oudemansiella mucida* (Schrad. : Fr.) Höhn.			+		LeS	+	Rosnev & Stoichev 1985
83. *Rhodocollybia butyraceae* (Bull.) Antonín & Noordel.	+	+	+		St	+	
84. *R. butyraceae* f. *asema* (Fr. : Fr.) Antonín, Halling & Noordel.	+	+	+		St	+	
85. *Strobilurus esculentis* (Wulfen : Fr.) Singer	+	+			S	+	
86. *Xerula radicata* (Relhan : Fr.) Fr.			+		St	+	
Nidulariaceae							
87. *Crucibulum laeve* (Huds. : Pers.) Kambly			+		LeS	+	
88. *Cyathus striatus* (Huds. : Pers.) Hoffm.			+		LeS	+	
Pleurotaceae							
89. *Pleurotus pulmonarius* (Fr.) Quél.			+		LeS	+	Kuthan & Kotlaba 1989
Pluteaceae							
90. *Amanita battarae* (Boud.) Bon		+			Mr	+	
91. *A. citrina* (Schaeff.) Pers.			+		Mr	+	
92. *A. muscaria* (L. : Fr.) Hook.	+	+			Mr	+	
93. *A. pantherina* (DC. : Fr.) Krombh.		+	+		Mr	+	
94. *A. rubescens* (Pers. : Fr.) Gray	+	+	+		Mr	+	
95. *A. spissa* (Fr.) P. Kumm. var. *excelsa* (Fr.) Konrad & Maubl.	+	+			Mr	+	
96. *A. vaginata* (Bull. : Fr.) Vittad.	+	+	+		Mr	+	
97. *Pluteus atromarginatus* (Konrad) Kühner		+			LeS	+	Rosnev & Stoichev 1985
Schizophyllaceae							
98. *Schizophyllum commune* Fr. : Fr.			+		LeS	+	Rosnev & Stoichev 1985
Strophariaceae							
99. *Hypholoma capnoides* (Fr.) P. Kumm.	+	+			LeS	+	

Taxa	Plant communities				ETG**	Species from present study	Sources
	1	2	3	4			
100. *H. fasciculare* (Huds. : Fr.) Quél.	+	+	+		LeS	+	Rosnev & Stoichev 1985
101. *H. sublateritium* (Scaeff.) Quél.			+		LeS	+	Rosnev & Stoichev 1985
102. *Kuehneromyces mutabilis* (Schaeff. : Fr.) Singer & A.H. Sm.		+	+	+	LeS		Rosnev & Stoichev 1985
103. *Pholiota astragalina* (Fr.) Singer	+	+			LeS	+	
104. *Ph. flammans* (Batsch : Fr.) P. Kumm.	+	+			LeS	+	Rosnev & Stoichev 1985
105. *Ph. squarrosa* (Weigel : Fr.) P. Kumm.	+	+	+		LeS	+	Rosnev & Stoichev 1985
106. *Psilocybe montana* (Pers. : Fr.) P. Kumm.				+	Hu	+	
107. *Stropharia aeruginosa* (Curtis : Fr.) Quél.		+			Hu	+	
108. *S. semiglobata* (Batsch : Fr.) Quél.				+	C	+	
Tricholomataceae							
109. *Clitocybe clavipes* (Pers. : Fr.) P. Kumm.		+			St	+	
110. *C. fragrans* Sowerby : Fr.	+	+			Ad. St	+	
111. *C. gibba* (Pers. : Fr.) P. Kumm.		+	+		St, Hu	+	
112. **C. incilis* (Fr.) Quél.		+			Hu	+	
113. *C. metachroa* (Fr. : Fr.) P. Kumm.	+	+			Ad, St	+	
114. *C. nebularis* (Batsch : Fr.) P. Kumm.	+	+	+		Hu	+	
115. *C. odora* (Bull. : Fr.) P. Kumm.			+		St	+	
116. *C. phillophila* (Pers. : Fr.) P. Kumm.		+			St	+	
117. *C. subcordispora* Harmaja			+		Hu	+	Gyosheva 1996
118. *C. umbilicata* (Schaeff. : Fr.) P. Kumm.		+			LeS	+	
119. *Collybia cirrhata* (Pers.) Quél.	+	+			M	+	
120. *C. cookei* (Bres) J.D. Arnold	+	+			M	+	
121. *Cystoderma carcharias* (Pers.) Fayod		+			St	+	
122. *C. cinnabarinum* (Alb. & Schwein.) Fayod		+	+		St	+	
123 **Fayodia gracilipes* (Britz.) Bres. & Stangl	+				Hu	+	
124. *Hemimycena lactea* (Pers. : Fr.) Singer	+	+			Hu, LeS	+	
125. *Hygrocybe conica* (Scop. : Fr.) P. Kumm.				+	Hu	+	
126. *Hygrophorus agathosmis* (Fr.) Fr.	+	+			Mr	+	
127. *H. capreolarius* (Kalchbr.) Sacc.		+	+		Mr	+	
128. *H. chrysodon* (Batsch. : Fr.) Fr.			+		Mr	+	
129. *H. cossus* (Sow. : Fr.) Fr.			+		Mr	+	
130. *H. discoideus* (Pers. : Fr.) Fr.			+		Mr	+	
131. *H. eburneus* (Bull. : Fr.) Fr.			+		Mr	+	
132. *H. erubescens* (Fr. : Fr.) Fr.		+			Mr	+	
133. *H. piceae* Kühner	+	+			Mr	+	
134. *H. pudornius* (Fr.) Fr.	+	+			Mr	+	
135. *Lepista inversa* (Scop. : Fr.) Pat.	+	+	+		St	+	
136. *L. nuda* (Bull. : Fr.) Cooke			+		Hu	+	
137. *Leucopaxillus amarus* (Alb. & Schwein : Fr.) Kühner	+	+	+		Hu	+	
138. **Lyophyllum boudieri* Kühner & Romagn			+		Hu	+	
139. *L. connatum* (Schumah. : Fr.) Singer		+	+	+	Hu	+	
140. *Megacollybia platyphylla* (Pers. : Fr.) Kotl. & Pouzar		+	+		LeS	+	Rosnev & Stoichev 1985
141. *Melanoleuca melaleuca* (Pers. : Fr.) Murrill		+		+	Hu	+	
142. *Mycena adonis* (Bull. : Fr.) S.F. Gray	+				Br	+	
143. *M. alcalina* (Fr. : Fr.) P. Kumm.	+	+			LeS	+	
144. *M. arcangeliana* Bres.			+		LeS	+	
145. *M. aurantiomarginata* (Fr. : Fr.) Quél.	+	+			Ad, St	+	
146. *M. cinerella* (P. Karst.) P. Karst.	+	+			Ad, St	+	
147. *M. clavicularis* (Fr.) Gill.	+				LeS	+	
148. *M. crocata* (Schrad.) Fr.			+		Fd, St	+	
149. *M. epipterygia* (Scop. : Fr.) Gray	+	+			Ad, St	+	

Taxa	Plant communities				ETG**	Species from present study	Sources
	1	2	3	4			
150. *M. epipterygia* var. *viscosa* (Secr. ex Maire) Ricken	+	+			LeS	+	
151. *M. flvoalba* (Fr.) Quél.				+	Hu	+	
152. *M. galericulata* (Scop. : Fr.) Schaeff.			+		LeS	+	
153. *M. galopus* (Pers. : Fr.) P. Kumm. var. *nigra* Rea	+	+			Ad, St	+	
154. *M. haematopus* (Petr. : Fr.) P. Kumm.			+		LeS	+	Kuthan & Kotloba 1989
155. *M. laevigata* (Lasch : Fr.) Quél.	+				LeS	+	Kuthan & Kotloba 1989
156. *M. leptocephala* (Pers. : Fr.) Gillet	+	+			Ad, St	+	
157. *M. leptophyla* (Peck) Sacc			+		LeS	+	
158. *M. maculata* P. Karst.	+	+			St, LeS	+	
159. *M. metata* (Fr. : Fr.) P. Kumm.	+	+			Ad, St	+	
160. *M. pelianthina* (Fr. : Fr.) Quél.			+		Fd, St	+	
161. *M. polygramma* (Bull.) Gray		+	+		LeS	+	
162. *M. pura* (Pers.) Sacc.		+	+		St	+	
163. *M. renati* Quél.			+		LeS	+	Rosnev & Stoichev 1985
164. *M. rorida* (Scop. : Fr.) Quél.	+	+			LeS	+	
165. *M. rosea* (Bull.) Gramberg			+		St	+	
166. *M. rosella* (Fr.) P. Kumm.	+	+			Ad, St	+	
167. *M. sanguinolenta* (Alb. & Schwein. : Fr.) P. Kumm.		+	+		St, LeS	+	
168. *M. vulgaris* (Pers. : Fr.) P. Kumm.	+	+			Ad, At	+	
169. *Omphalina griseopallida* (Dezm.) Quél.				+	Hu	+	
170. *O. grossula* (Pers.) Singer	+	+			LeS	+	
171. *Panellus mitis* (Pers. : Fr.) Singer	+	+			LeS	+	Rosnev & Stoichev 1985
172. *P. serotinus* (Schrad. : Fr.) J.G. Kühner			+		LeS	+	Rosnev & Stoichev 1985
173. *P. stipticus* (Bull. : Fr.) P. Karst.			+		LeS	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
174. *Phaeolepiota aurea* (Matt. : Fr.) Maire			+	+	Hu	+	Stoichev & Dimcheva 1987
175. *Phyllotopsis nidulans* (Pers. : Fr.) Singer		+			LeS	_	Rosnev & Stoichev 1985
176. *Pseudoclitocybe cyathiformis* (Bull. : Fr.) Singer		+	+		Hu	+	
177. *Rickenella fibula* (Bull. : Fr.) Raithelh.		+		+	Br	+	
178. *R. swartzii* (Fr. : Fr.) Kuyper	+	+			Br	+	
179. *Tricholoma atrosquamosum* (Chevall.) Sacc.		+	+		Mr	+	
180. *T. saponaceum* (Fr. : Fr.) P. Kumm.		+			Mr	+	
181. *T. sulphureum* (Bull. : Fr.) P. Kumm.			+		Mr	+	
182. *Tricholomopsis rutilans* (Schaeff. : Fr.) Singer	+	+			LeS	+	Rosnev & Stoichev 1985
183. *Xeromphalina campanella* (Batsch : Fr.) Maire	+	+			LeS	+	
Boletales							
Boletaceae							
184. *Boletus appendiculatus* (Schaeff. : Fr.) Secr.			+		Mr	+	
185. *B. edulis* Bull. : Fr.	+	+	+		Mr	+	
186. *B. erythropus* (Fr. : Fr.) Krombh.	+	+	+		Mr	+	
187. *B. calopus* Fr.		+			Mr	+	
188. *B. chrysenteron* Bull.		+	+		Mr	+	
189. *B. subtomentosus* Pers. : Fr.		+	+		Mr	+	
190. *Chalciporus piperatus* (Bull. : Fr.) Bataille	+	+			Mr	+	
191. *Strobilomyces strobilaceus* (Scop. : Fr.) Berck.			+		Mr	+	
Goniophoraceae							
192. *Goniophora puteana* (Schumach. : Fr.) P. Karst.		+			LeS	+	Rosnev & Stoichev 1985
Gomphidiaceae							
193. *Chroogomphus helveticus* (Singer) M.M. Moser	+	+			Mr	+	
194. *Ch. rutilus* (Schaeff. : Fr.) O.K. Mill.		+			Mr	+	
195. *Gomphidius glutinosus* (Schaeff. : Fr.) Fr.	+	+			Mr	+	

	Taxa	Plant communities				ETG**	Species from present study	Sources
		1	2	3	4			
	Hygrophorpsidaceae							
196.	*Hygrophoropsis aruantiaca* (Wulfen : Fr.) Maire	+				LeS	+	
	Paxillaceae							
197.	*Paxillus atrotomentosus* (Batsch : Fr.) Fr.		+			LeS	+	Rosnev & Stoichev 1985
198.	*P. involutus* (Batsch : Fr.) Fr.		+	+		Mr	+	
199.	*P. panuoides* (Fr. : Fr.) Fr.	+	+			LeS	+	Rosnev & Stoichev 1985
	Suillaceae							
200.	*Suillus luteus* (L. : Fr.) Roussel		+			Mr	+	
201.	*S. vaiegatus* (Schwartz : Fr.) Richon & Roze		+			Mr	+	
	Cantharellales							
	Cantharellaceae							
202.	*Cantharellus cibarius* Fr. : Fr.		+	+		Mr	+	
203.	*C. lutescens* (Pers. : Fr.) Fr.		+			Mr	+	
204.	*C. tubaeformis* (Bull. : Fr.) Fr.	+	+			Mr	+	
205.	*Craterellus cornucopioides* (L. : Fr.) Pers.		+	+		Mr	+	
	Clavulinaceae							
206.	*Clavulina cinerea* (Bull. : Fr.) J. Schröt.		+	+		Hu	+	
207.	*C. coralloides* (L. : Fr.) J. Schröt.	+	+			Hu	+	
208.	*C. rugosa* (Bull. : Fr.) J. Schröt.		+	+		Hu	+	Kuthan & Kotlaba 1989
	Hydnaceae							
209.	*Hydnum repandum* L. : Fr.	+	+	+		Mr	+	
	Dacrymycetales							
	Dacrymycetaceae							
210.	*Calocera cornea* (Batsch : Fr.) Fr.			+		LeS	+	
211.	*C. viscosa* (Pers. : Fr.) Fr.	+	+			LeS	+	Rosnev & Stoichev 1985
212.	*Dacrymyces stillatus* Nees : Fr.	+	+			LeS	+	
	Hymenochaetales							
	Hymenochaetaceae							
213.	*Coltricia perennis* (L. : Fr.) Murrill		+			LeS	+	
214.	*Hymenochaete cruenta* (Pers. : Fr.) Donk		+			LeS		Rosnev & Stoichev 1985
215.	*Onnia tomentosa* (Fr. : Fr.) P. Karst.	+				LeS		Rosnev & Stoichev 1985
216.	*Phellinus hartigii* (Albesch. & Schnabl) Pat.		+			LeS		Rosnev & Stoichev 1985
217.	*Ph. pini* (Brot.) Bondartsev & Singer		+			LeS, LeP	+	
	Schizoporaceae							
218.	*Oxyporus populinus* (Schum. : Fr.) Donk			+		LeS		Rosnev & Stoichev 1985
219.	*Schizopora paradoxa* (Schrad. : Fr.) Donk			+		LeS		Rosnev & Stoichev 1985
	Phallales							
	Geastraceae							
220.	*Geastrum pectinatum* Pers. : Pers.			+		Hu	+	
	Ramariaceae							
221.	*Ramaria abietina* (Pers. : Fr.) Quél.	+	+			St, IeS	+	
222.	*R. aurea* (Schaeff.. : Fr.) Quél.	+	+	+		Hu	+	
223.	*R. corrugata* (P. Karst.) Schild		+			Hu	+	
224.	*R. flaccida* (Fr.) Bourdot		+			St, Hu	+	
225.	*R. flava* (Schaeff. : Fr.) Quél.		+	+		Hu	+	
226.	*R. pallida* (Schaeff.) Ricken				+	Hu	+	
	Polyporales							
	Albatrellaceae							
227.	*Albatrellus ovinus* (Schaeff. : Fr.) Kotl. & Pouzar		+			Hu	+	

	Taxa	Plant communities				ETG**	Species from present study	Sources
		1	2	3	4			
	Fomitopsidaceae							
228.	*Fomitopsis pinicola* (Sw. : Fr.) P. Karst.	+	+			LeS, LeP	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
229.	*F. rosea* (Alb. & Schwein. : Fr.) P. Karst.	+	+			LeS	+	Rosnev & Stoichev 1985
230.	*Postia stiptica* (Pers. : Fr.) Jülich	+	+			LeS	+	Rosnev & Stoichev 1985
	Ganodermataceae							
231.	*Ganoderma applanatum* (Pers.) Pat.			+		LeS, LeP	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
	Gloeophyllaceae							
232.	*Gloeophyllum abietinum* (Bull. : Fr.) P. Karst.	+	+			LeS	+	Rosnev & Stoichev 1985
233.	*G. odoratum* (Wulfen : Fr.) Imazeki	+	+			Les	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
234.	*G. sepiarium* (Wulfen : Fr.) P. Karst.	+	+			LeS	+	Rosnev & Stoichev 1985
235.	*G. trabeum* (Pers. : Fr.) Murrill		+			LeS		Rosnev & Stoichev 1985
	Hapalopliaceae							
236.	*Bjerkandera adusta* (Wulld. : Fr.) P. Karst.		+	+		LeS	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
237.	*Ishnoderma benzonium* (Wahlenb. : Fr.) P. Karst.	+	+			LeS	+	Rosnev & Stoichev 1985
	Meripilaceae							
238.	*Antorida heteromorpha* (Fr. : Fr.) Donk	+	+			LeS	+	
239.	*A. serialis* (Fr. : Fr.) Donk	+	+			LeS	+	Rosnev & Stoichev 1985
240.	*A. sinuosa* (Fr. : Fr.) P. Karst.	+	+			LeS		Rosnev & Stoichev 1985
241.	*A. xantha* (Fr. : Fr.) Ryvarden		+	+		LeS		Rosnev & Stoichev 1985
242.	*Physisoporinus sanguinolentus* (Alb. & Schwein.) Pilát	+				LeS		Rosnev & Stoichev 1985
	Meruliaceae							
243.	*Meruliopsis taxicola* (Pers. : Fr.) Bondartsev	+	+			LeS		Rosnev & Stoichev 1985
244.	*M. tremellosus* Schardl : Fr.			+		LeS		Kuthan & Kotlaba 1989
245.	*Phlebia radiata* Fr. : Fr.			+		LeS	+	
	Polyporaceae							
246.	*Amylocystis lapponicus* (Romell) Bondartsev & Singer	+				LeS		Rosnev & Stoichev 1985
247.	*Datronia mollis* (Sommerf. : Fr.) Donk.			+		LeS		Rosnev & Stoichev 1985
248.	*Fomes fomentarius* (L. : Fr.) J.J. Kickx			+		LeS, LeP	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
249.	*Lenzites betulina* (L. : Fr.) Fr.			+		LeS		Rosnev & Stoichev 1985
250.	*Phaeolus schweinitzii* (Fr. : Fr.) Pat.		+			LeP	+	Rosnev & Stoichev 1985
251.	*Polyporus brumalis* (Pers. : Fr.) Fr.			+		LeS	+	
252.	*P. cilliatus* Fr.			+		LeS		Kuthan & Kotlaba 1989
253.	*P. varius* (Pers. : Fr.) Fr.			+		LeS	+	Rosnev & Stoichev 1985
254.	*Pycnoporus cinnabarinus* (Jacq. : Fr.) Fr.			+		LeS	+	Rosnev & Stoichev 1985
255.	*Sceletocutis amorpha* (Fr. : Fr.) Kotl. & Pouzar		+			LeS		Rosnev & Stoichev 1985
256.	*Trametes hirsuta* (Wulfen : Fr.) Pilát			+		LeS	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
257.	*T. pubescens* (Schumach. : Fr.) Pilát			+		LeS		Rosnev & Stoichev 1985
258.	*T. versicolor* (L. : Fr.) Lloyd			+		LeS	+	Rosnev & Stoichev 1985
259.	*Trichaptum abietinum* (Dicks. : Fr.) Ryvarden	+	+			LeS	+	Rosnev & Stoichev 1985
260.	*T. fuscoviolaceum* (Ehrenb. : Fr.) Ryvarden		+			LeS	+	Rosnev & Stoichev 1985
261.	*Tyromyces caesius* (Schrad. : Fr.) Murrill	+	+			LeS	+	Rosnev & Stoichev 1985
262.	*T. ptychogaster* (Ludw.) Donk	+				LeS		Rosnev & Stoichev 1985
	Steccherinaceae							
263.	*Antrodiella hoehnelii* (Bres.) Niemalä			+		LeS		Rosnev & Stoichev 1985
264.	*A. semisupina* (Berk. & M.A. Curtis) Ryvarden			+		LeS		Rosnev & Stoichev 1985

	Taxa	Plant communities				ETG**	Species from present study	Sources
		1	2	3	4			
	Russulales							
	Bondarzewiaceae							
265.	*Heterobasidion annosum* (Fr. : Fr.) Bref.	+	+			LeP	+	Rosnev & Stoichev 1985
	Hericiaceae							
266.	*Dentipellis fragilis* (Pers. : Fr.) Donk			+		LeS		Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989
267.	*Hericium coralloides* (Scop. : Fr.) S.F. Gray			+		LeS	+	
268.	*H. flagellum* (Scop.) Pers.	+				LeS	+	
	Russulaceae							
269.	*Lactarius aurantiacus* (Pers. : Fr.) Gray	+	+			Mr	+	
270.	*L. blennius* (Fr.) Fr.			+		Mr	+	
271.	*L. blennius* var. *viridis* (Schrad.) Quél.			+		Mr	+	
272.	*L. deliciosus* (L. : Fr.) Gray		+			Mr	+	
273.	*L. deterrimus* Gröger	+	+			Mr	+	
274.	*L. fluens* Boud.			+		Mr	+	
275.	*L. glyciosmus* (Fr. : Fr.) Fr.			+		Mr	+	
276.	*L. lignyotus* Fr.	+	+			Mr	+	
277.	*L. pallidus* (Pers. : Fr.) Fr.			+		Mr	+	
278.	*L. scorbiculatus* (Scop.) Fr.	+	+			Mr	+	
279.	*L. vellereus* (Fr. : Fr.) Fr.		+	+		Mr	+	
280.	*L. zonarioides* Kühner & Romagn.	+	+			Mr	+	
281.	*Russula aeruginea* Fr.		+	+		Mr	+	
282.	*R. azurea* Bres.	+	+			Mr	+	
283.	*R. cyanoxantha* (Schaeff.) Fr.		+	+		Mr	+	
284.	*R. delica* Fr.		+	+		Mr	+	
285.	*R. emetica* (Schaeff. : Fr.) Pers.	+	+			Mr	+	
286.	*R. farinipes* Komell.			+		Mr	+	
287.	*R. fellea* (Fr. : Fr.) Fr.			+		Mr	+	
288.	*R. foetens* (Pers. : Fr.) Fr.		+	+		Mr	+	
289.	*R. fragilis* (Pers. : Fr.) Fr.		+	+		Mr	+	
290.	*R. integra* (L.) Fr.		+	+		Mr	+	
291.	*R. laurocerasii* Melzer		+	+		Mr	+	
292.	*R. lutea* (Huds. : Fr.) S.F. Gray		+	+		Mr	+	
293.	*R. mustelina* Fr.		+			Mr	+	
294.	*R. nigircans* (Bull.) Fr.		+	+		Mr	+	
295.	*R. ochroleuca* (Pers.) Fr.	+	+	+		Mr	+	
296.	*R. queletii* Fr.	+	+			Mr	+	
297.	*R. sanguinea* (Bull.) Fr.	+	+			Mr	+	
298.	*R. xerampelina* (Schaeff.) Fr.	+	+			Mr	+	
	Stereaceae							
299.	*Stereum hirsutum* (Willd. : Fr.) Gray			+		LeS	+	Kuthan & Kotlaba 1989
300.	*S. sanguinolentum* (Alb. & Schwein.) Fr.		+			LeS	+	Rosnev & Stoichev 1985
	Thelephorales							
	Bankeraceae							
301.	*Sarcodon imbricatus* (L. : Fr.) P. Karst.	+	+			Mr	+	
	Tremellales							
	Exidiaceae							
302.	*Exidia plana* (Wigg. & Schleich.) Donk			+		LeS		Kuthan & Kotlaba 1989
303.	*Pseudohydnum gelatinosum* (Scop. : Fr.) P. Karst.		+			LeS	+	Rosnev & Stoichev 1985; Kuthan & Kotlaba 1989

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		1	2	3	4			
304.	*Tremiscus helvelloides* (DC. : Pers.) Donk	+	+			LeS	+	Rosnev & Stoichev 1985
	Tremellaceae							
305.	*Tremella encephala* Pers. : Fr.		+			LeS	+	
306.	*T. foliaceae* Pers. : Fr.	+	+			LeS	+	
307.	*T. mesenterica* Retz. : Fr.			+		LeS	+	
	Total:	**112**	**202**	**154**	**14**		**278**	

Legend: * – New to Bulgaria species; **Plant communities: 1,** pure ***Picea abies*** **forests; 2,** mixed ***Picea abies*** **forests (*Picea abies*+*Pinus sylvestris*+*Abies alba*+*Pinus peuce*); 3, *Fagus sylvatica* forests; 4, grassy communities; **ETG (Ecological-trophic groups): Ad – needle-debris saprotrophs; Fd – leaf-debris saprotrophs; S – cone saprotrophs; St – litter saprotrophs; Hu – humus saprotrophs; LeS – wood saprotrophs; Br – moss saprotrophs; C – coprotrophs; M – fungal saprotrophs; Mr – mycorrhizal fungi; LeP – wood parasites.**

The most abundant were the following families: *Tricholomataceae* (73 species and 2 varieties), *Russulaceae* (29 species and 1 variety), *Cortinariaceae* (17 species). The richest genera are: *Mycena* (24 species and 2 varieties), *Russula* (18 species), *Cortinarius* (15 species and 2 varieties), *Lactarius* (11 species and 1 variety). During the field investigations, 226 taxa were found as new to the Parangalitsa Biosphere Reserve. Four species are new to Bulgaria: *Clitocybe incilis* (Fr.) Quél., *Fayodia gracilipes* (Britz.) Bres. & Stangl, *Gerronema brevibasidium* (Singer) Singer and *Lyophyllum boudieri* Kühner & Romagn. The list of all macrofungi found in the reserve so far is given in Table 1. The new to Bulgaria species were asterisked (*).

The distribution of macrofungi in the studied plant communities of the Parangalitsa Biosphere Reserve is also given in Table 1. One hundred and twelve (112) taxa have been registered in pure spruce forests, 202 taxa – in mixed spruce forests and 154 taxa – in beech forests. In the period of investigation the following macrofungi were determined as dominant species regarding the number of fruit bodies and frequency: *Amanita rubescens*, *Armillaria mellea*, *Clitocybe nebularis*, *Cortinarius purpurescens*, *Hydnum repandum*, *Hygrophorus piceae*, *Lactarus aurantiacus*, *L. scorbiculatus*, *Marasmius bulliardii*, *Mycena alcalina*, *M. maculata*, *Ramaria aurea*, *Rhodocollybia butyraceae*, *Sarcodon imbricatus* (in spruce forests) and *Boletus chrysenteron*, *Clitocybe odora*, *Cortinarius glaucopus*, *Hydnum repandum*, *Hygrophorus eburneus*, *Hypholoma fasciculare*, *Inocybe geophilla*, *Lactarius blennius*, *Mycena galericulata*, *M. rosea*, *Ramaria flava*, *Russula cyanoxantha*, *R. fellea* (in beech forests).

Ecological-trophic structure

Macrofungi from 11 ecological-trophic groups were determined in studied plant communities in the Parangalitsa Biosphere Reserve (Table 1): needle-debris saprotrophs – 12 species, leaf-debris saprotrophs – 3 species, cone saprotrophs – 1 species, litter saprotrophs – 33 species, humus saprotrophs – 46 species, moss saprotrophs – 6 species, coprotrophs – 1 species, fungal saprotrophs – 2 species, wood saprotrophs – 111 species, mycorrhizal fungi – 98 species and wood parasites – 7 species. Wood saprotrophs dominated by the number of species. 48 species of them were found in pure spruce forests, 68 species – in mixed spruce forests and 60 species – in beech forests. Eight species wood rotting fungi indicator for old and primeval spruce and beech forests have been found in the Parangalitsa Biosphere Reserve (Parmasto & Parmasto 1997; Tortič 1998; Nitare 2000): *Amylocystis lapponica* (*Picea*), *Dentipellis fragilis* (*Fagus*), *Fomitopsis rosea* (*Picea*), *Hericium coralloides* (*Fagus*), *H. flagellum* (*Abies, Picea*), *Ishnoderma benzoinum* (*Picea*, *Abies*), *Phellinus nigrolimitatus* (*Picea*, *Abies*, *Pinus*), *Phaeollus schweinitzii* (*Pinus peuce*, *Picea*).

Comparatively high was the species diversity in the group of mycorrhizal fungi in the forest communities of the reserve (Table 1). 75 species of them were found in coniferous and 48 species in beech forests. Among the biotrophs, 4 species are with high economic importance (Rosnev & Stoichev 1985): *Armillaria mellea*, *Fomes fomentarius*, *Fomitopsis pinicola* and *Heterobasidion annosum*.

Species of conservation significance

The old virgin forests in Europe are very important habitats concerning the conservation of fungi (Arnolds 1998). On the territory of the Parangalitsa Biosphere Reserve there are distributed 14 species from the official *Red List of fungi in Bulgaria* (Gyosheva & al. 2006): Endangered (EN) – 7 species (*Amylocystis lapponica*, *Antrodia heteromorpha*, *Hericium flagellum*,

Hygrophorus erubescens, *Leotia lubrica*, *Leucocortinarius bulbiger* and *Tremiscus helvelloides*); Vulnerable (VU) – 3 species (*Macrotyphula fistilosa*, *Otidea onotica* and *Strobilomyces strobilaceus*); Near Threatened – 3 species (*Fomitopsis rosea*, *Hericium coralloides* and *Phyllotopsis nidulans*). Eleven species of conservation significance were founded by us during the present investigation (Table 1). Seven species have been registered on decay wood, including windthrow spots.

Conclusion

The presented data suggest the conclusion that the Parangalitsa Biosphere Reserve is extremely interesting in respect to fungi. This conclusion is supported by the macrofungal diversity and especially by the established species with conservation value in the reserve. It is very likely to determine the Parangalitsa Biosphere Reserve, especially the old spruce forests, as Important Fungus Areas site in Bulgaria on the basis of the further mycological explorations.

Acknowledgements. We are grateful to specialists in Rila National Park Directorate for their help in the accomplishment of the field investigations.

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The application of GC/MS for chemotaxonomy of agarical fungi from genera *Clitocybe* and *Lepista*

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Abstract. Volatile and polar fractions of seven macromycete species from the genera *Clitocybe* and *Lepista* (*Agaricales*, *Tricholomataceae*) were analysed by GC/MS. The primary metabolites, which dominated in the polar fraction, appeared to be suitable for taxonomic conclusions in the investigated macromycetes. We succeeded to identify more than 100 compounds; the main groups of the identified compounds were hydrocarbons, fatty acids and their esters, terpenoids (volatile fractions), oxidized fatty acids derivatives, free amino acids and sugars (polar fractions). From the results obtained we made some chemotaxonomic conclusions.

Key words: *Clitocybe*, GC/MS, *Lepista*, polar components, volatiles

Introduction

Macromycetes of the genera *Clitocybe* and *Lepista* are almost cosmopolitan; they grow in different forest and herbaceous ecosystems. In these genera there are valuable edible species, but on the other hand, some *Clitocybe* species contain muscarine and its isomers (Genest & al. 1968). The fungi of both genera display some common morphological characteristics of their fruiting bodies and, based on this fact, Bigelow & Smith (1969) included *Lepista* as a section in the genus *Clitocybe*. However, this point of view was not generally accepted (Singer 1986). Because of the significant similarities, several species are of ambiguous taxonomic position: some authors consider them as belonging to the genus *Lepista* while others believe they are members of the genus *Clitocybe*, e.g. *Clitocybe nebularis* (Batsch : Fr.) P. Kumm. [= *Lepista nebularis* (Batsch : Fr.) Harm.], *Lepista gilva* (Pers. : Fr.) Roze [= *Clitocybe gilva* (Pers. : Fr.) Gill.], *Lepista inversa* (Scop.) Pat. [= *Clitocybe inversa* (Scop.) Quél.] (Moser 1983; Ryman & Holmasen 1992; Courtecuisse & Duhem 1995; Svrček 1998; Enderle 2004). Metabolomic studies could provide valuable information and help answer the questions about the position of the above-mentioned species: closer to genus *Lepista* or to genus *Clitocybe*. They will also reveal the chemical similarities and differences between the two genera.

GC/MS is a very suitable method for investigation of complex mixtures of organic compounds, such as mushroom extracts. However, the analysis is not a true quantification because the ion currents generated depend on the characteristics of the investigated compounds. For this reason the data obtained from the GC/MS analyses can be used for the characterization of the biodiversity and for comparisons between the same groups of compounds in related organisms. The results can characterize the investigated organisms, indicate the presence of known biologically active compounds and allow some chemotaxonomic conclusions.

In this article, we report the results of the GC/MS analyses of volatile and polar compounds of the fruit bodies of 5 macromycetes species of the genus *Clitocybe*: *C. clavipes* (Pers. : Fr.) P. Kumm., *C. alexandri* (Gill.) Konr., *C. fragrans* (With. : Fr.) P. Kumm., *C. nebularis* (Batsch : Fr.) P. Kumm., *C. ododra* (Bull. : Fr.) P. Kumm., and 2 of the genus *Lepista*: *L. inversa* (Scop.) Pat. and *L. nuda* (Bull. : Fr.) Cooke. They are widespread in forest ecosystems in Bulgaria and in Europe, with the only exception of *C. alexandri*.

Material and methods

Collection of the samples

The macromycetes were collected, as follows: *C. alexandri*, *C. clavipes*, *C. fragrans*, *L. inversa* in Rila Mts in the locality of Borovets, 1200 m alt.; *C. odora*, *C. nebularis*, *L. nuda* in Mt Osogovo, Kyustendil district, around the chalet of Iglika, 1640 m alt., in October 2004.

Extraction

The fresh fruit bodies of macromycetes were cut into small pieces and consecutively extracted with methanol, methanol-chloroform (1:1) and chloroform. The extracts were combined, water was added and the chloroform layers were removed. The water-methanol layers were extracted twice with *n*-butanol. Data about the weights and yields are represented in Table 1.

Isolation and analysis of volatile compounds

A part of the chloroform extract (about 300 mg) was subjected to a four-hour distillation–extraction in a Lickens–Nickerson apparatus (Hendriks & al. 1981). The volatile compounds were extracted from the distillate with diethyl ether (yields of volatiles in Table 1). They were analysed by GC/MS on a GC Hewlett Packard 6890 + MS 5973 (Hewlett Packard, Palo Alto, California, USA). A HP5–MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies, Wilmington, Delaware, USA) was used. The ion source was set at 250 °C and the ionization voltage was 70 eV. The temperature was programmed from 40 °C to 280 °C at a rate of 6 °C.min^{-1}. Helium was used as a carrier gas.

Table 1. Weights of macromycetes extracted, extracts and volatiles.

Macromycetes species	Macromycetes (g)	Chloroform extract (mg)	Butanol extract (mg)	Volatiles (% of the chloroform extract)
C. clavipes	150	800	200	11.2
C. fragrans	27	155	90	4.8
C. alexandri	170	640	80	3.1
L. inversa	130	360	680	22.2
C. odora	100	580	1350	22.0
C. nebularis	13	253	504	6.5
L. nuda	89	123	272	3.3

Analysis of polar compounds

A part of the *n*-butanol extract (5 mg) was dissolved in 50 μL dry pyridine and 75 μL of bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was added. The mixture was heated at 80 °C for 30 min and analysed by GC/MS. The silylated extract was investigated by GC/MS on the instrument, described above, with a capillary column HP–5 (23 m × 0.2 mm, 0.5 μm film thickness, Agilent Technologies, Wilmington, Delaware, USA). As a carrier gas helium was used with a temperature program 100 °C – 315 °C at 5 °C.min^{-1} and a 10-min hold at 315 °C.

Identification of compounds

The identification was accomplished using computer searches on commercial libraries. In some cases, when identical spectra had not been found, only the structural type of the corresponding component was proposed based on its mass spectral fragmentation. Some components remained unidentified because of the lack of authentic samples and library spectra of the corresponding compounds.

Results and discussion

The volatile fractions were obtained and analysed as described above. The results obtained are summarized in Table 2. The main groups of compounds in the volatile fractions are hydrocarbons, ethers, chlorinated hydrocarbons, acids, esters, alcohols, aldehydes, ketones, terpenoids, N-containing compounds.

Hydrocarbons are important constituents of the waxes, covering the plant tissues. They defend plants from drought, injury, some predators, etc. Probably they possess identical functions in macromycetes. In all investigated samples the aliphatic hydrocarbon composition is similar – the main hydrocarbons are saturated and not branched, but their concentrations strongly differ. In the investigated samples we found aromatic hydrocarbons in three of the samples investigated, but significant relative concentrations of these compounds appeared only in *C. nebularis*.

Halogen-containing hydrocarbons are rarely found in living organisms, incl. Basidiomycetes (de Jong 1997). Chlorinated compounds have been detected previously in *L. inversa* and suggested for use as taxonomic markers (Boustie & al. 2005). However, we found chlorinated compounds only in two of the investigated macromycetes – *C. fragrans* and in

lower concentrations in *C. clavipes*. The chlorinated compounds identified are almost entirely ethane derivatives, the main one being identified as 1,1,2,2-tetrachloroethane. Such compounds can play the role of allelochemicals (antibacterial and antifungal compounds, food deterrents, etc.).

Alcohols are known as allelochemicals in plants, taking part in many plant–insect relationships. We found traces of alcohols in the volatiles of three of the investigated macromycetes and only in *C. alexandri* they were in significant relative concentrations. Phenol, even in a low concentration, might serve in macromycetes as disinfectant. It was found only in *C. nebularis* and *C. clavipes*. In the investigated species we found only alcohols containing eight carbon atoms. 1-Octen-3-ol is characteristic for many mushrooms and is called "mushroom alcohol". It was recently found to have antifungal activity (Okull & al. 2003). The main alcohol in *C. alexandri* was 2-ethylhexanol.

The carbonyl compounds (aldehydes and ketones) were present in the analysed macromycetes in low concentrations. They are important allelochemicals, active even at low concentrations.

Free acids are widely distributed in living organisms. They have some defensive functions and serve as an energetic source for the organisms. The highest concentrations of fatty acids were present in the volatiles of *L. nuda*, followed by *C. alexandri*. The main acidic component in *C. nebularis* appeared to be acetic acid (75.4 % of total volatiles), and probably it is a defensive agent in this organism. The relatively high concentration of pentadecanoic acid in the volatiles of *L. nuda* is an indication for the presence of associated bacteria. Benzoic acid and polyunsaturated fatty acids were found only in *C. clavipes* and probably possess some defensive functions.

Significant part of the fatty acids was found as esters. While ethyl esters, which are rare in nature, could be artifacts, formed during extractions, the methyl esters are evidently natural products, because methanol was not used in the extraction procedures.

Terpenoids are often found in fungi, incl. *Clitocybe* and *Lepista* species (Abraham 2001; Jansen & de Groot 2004). In *C. clavipes* we found traces of monoterpenes and two isomeric diterpenes, probably acids. Higher concentrations of sesquiterpenes are present in *C. alexandri*, but part of them remained unidentified. Squalene was the only terpenoid in *C. odora*. Of interest is the presence 3-methyl butanoic acid only in *C. alexandri*, and it is the main acid in this species. It is found as an ester in another species – *C. clavipes* and indicates the first stages of terpenoid biosynthesis in these macromycetes.

N-containing compounds are characteristic for many macromycetes, but in the investigated volatiles we found such compounds only in *L. nuda* and *C. odora*. It is important, in order to use mushrooms for food, to mention the presence of traces of aniline and its derivative in *L. nuda*.

It is evident that the analysis of volatile fractions gives some hints concerning the taxonomy of *Clitocybe* and *Lepista*. However, the information is not enough to solve the existing taxonomic problems. Recently we found that in some higher plants part of the primary metabolites are even more suitable for such investigations (unpublished results). Now we demonstrate that the same is true for some macromycetes (*Clitocybe* and *Lepista*).

The polar fractions of the investigated fungal species were analysed by GC/MS after silylation. The derivatization was necessary in order to increase the volatility of the polar fraction, which is not enough for gas chromatographic analysis. The results obtained are represented in Table 3. Polyols were present in higher concentrations in *L. nuda* and *L. inversa*. Probably they are characteristic for *Lepista* genus mainly and are absent or appear in lower concentrations in

Table 2. Volatile constituents of *Clitocybe* and *Lepista* species (% of the Total ion Current, GC/MS).

Compounds	*C. clavipes*	*C. fragrans*	*C. alexandri*	*L. inversa*	*C. odora*	*C. nebularis*	*L. nuda*
Alkanes	0.2	3.5	1.0	12.8	15.7	–	0.1
Aromatic hydrocarbons	0.7	–	0.8	–	–	19.9	–
Chlorinated compounds	0.2	5.9	–	–	–	–	–
Alcohols and phenols	<0.1	–	7.4	–	–	<0.1	<0.1
Ethers	1.4	1.4	–	–	–	–	<0.1
Carbonyl compounds	0.2	1.0	0.2	–	–	2.4	<0.1
Acids	3.0	3.5	19.8	7.6	3.4	75.4	28.6
Esters	0.4	1.7	0.3	1.3	0.6	–	<0.1
Esters of acetic and phenylacetic acids	1.9	–	0.9	–	–	–	<0.1
Ethyl esters of fatty acids	41.5	0.7	47.9	50.2	10.2	–	33.9
Terpenes	1.4	–	6.3	–	4.5	–	<0.1
Nitrogen-containing compounds	–	0.1	–	–	–	–	12.0
Others	0.2	0.5	–	–	7.3	–	–
Total % of identified compounds	52.6	17.6	84.6	71.9	42.9	97.7	74.5

Table 3. Constituents of the polar fraction of *Clitocybe* and *Lepista* species (% of the Total ion Current, GC/MS).

Compounds	*C. clavipes*	*C. fragrans*	*C. alexandri*	*L. inversa*	*C. odora*	*C. nebularis*	*L. nuda*
Alcohols and ethers	0.5	0.5	–	1.4	–	–	2.3
Fatty acids	3.8	1.3	1.7	6.7	2.8	28.2	12.7
Hydroxy fatty acids	7.8	2.8	2.5	0.6	4.0	1.0	<0.1
Amino acids	12.2	2.4	9.4	4.2	–	9.0	2.5
Phosphoric acid and derivatives	33.5	8.4	5.6	2.6	2.0	6.7	20.6
Other acids	1.3	<0.1	0.9	<0.1	<0.1	<0.1	2.4
Esters	–	–	0.2	1.6	–	8.6	
Carbohydrates	18.9	70.5	76.6	22.4	5.1	16.7	42.0
Nitrogen-containing compounds	5.4	0.9	0.5	14.7	84.1	4.0	13.5
Total % of identified compounds	83.4	86.8	97.4	52.4	98.4	74.2	98.0

Clitocybe spp. Hydroxy acids are produced by biological oxidation of fatty acids. We found that *L. nuda* and *L. inversa* contain much lower relative concentrations of these acids, while *C. nebularis* has average concentration of them. The concentrations of the free amino acids varied strongly and could not give any taxonomic information.

Dehydroabietic acid was found only in *C. clavipes* and *C. nebularis*. Carbohydrates, as expected, were found in all investigated species. In all cases there are significant concentrations of monosaccharides. It is interesting to note the total absence of disaccharides in *C. nebularis* and *C. clavipes*, another similarity between these two species.

Among the polar compounds, urea appeared to be the main component of the polar fraction of *C. odora* and probably is a good taxonomic marker for this species. It is known that in *Clitocybe* spp. often there are significant amounts of urea (Singer 1986). It was in much lower amounts in *L. nuda* and *L. inversa*, while in the rest of the *Clitocybe* species the concentrations were insignificant.

Obviously, the amount of data obtained was large and for this reason a hierarchic cluster analysis procedure was applied to investigate the variability of the chemical composition of the macromycetes and to find out how this variability corresponded to the mycological classification. The dendrograms were based on squared Euclidean distances with clustering achieved using the Ward's method. When the cluster analysis was applied taking into consideration both volatile and polar constituents (Fig. 1a), *C. nebularis* was closer to *L. inversa* than to the remaining investigated macromycetes. Of special interest is *C. alexandri*. According to the dendrogram, *C. alexandri* was closer to *L. nuda* than to any other species analysed.

Further, cluster analysis was applied to different groups of compounds. Based on volatiles only (Fig. 1b), *C. alexandri* and *L. nuda* were clustered together again. This is a strong indication that *C. alexandri* has a special taxonomic position between the genera *Clitocybe* and *Lepista*. *Clitocybe nebularis* appeared to be between the two investigated genera. Cluster analysis based on oxidized fatty acids in polar fraction (Fig. 1c) demonstrated significant similarity between *C. alexandri* and *L. inversa*, which is in accordance with the hypothesis concerning the special position of *C. alexandri*.

The cluster analysis based on the free fatty acids (in polar fraction) (Fig. 1d) divided the investigated macromycetes into two groups, corresponding to the accepted existence of two genera – *Clitocybe* (*C. clavipes*, *C. odora*, *C. fragrans*) and *Lepista* (*L. nuda*, *L. inversa*, *C. alexandri*). Contrary to the existing opinions *C. alexandri* is closer to genus *Lepista* than to genus *Clitocybe*. There are indications that *C. fragrans* and *C. clavipes* are very close and probably form a specific group in genus *Clitocybe*.

Acknowledgements. Partial financial support of this work by the National Science Fund (Bulgaria), Contract X–1415, is gratefully acknowledged. The authors thank Mrs Antonova for running the GC/MS analyses.

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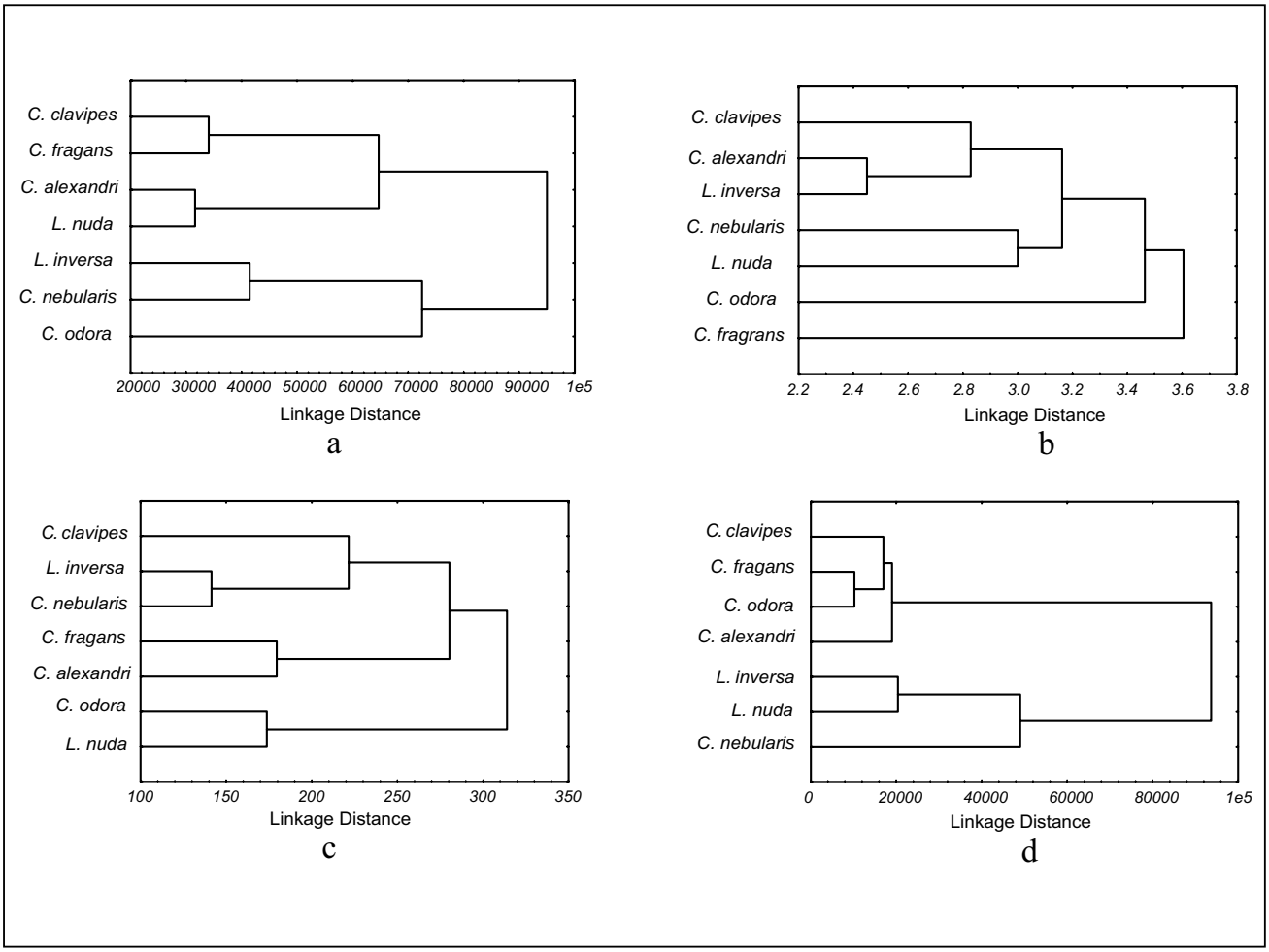

Fig. 1. Dendrograms, generated with STATISTICA for Windows (StaSoft Inc., 1995): **a** – Dendrogram based on both volatile and polar compounds; **b** – Dendrogram based on volatiles only; **c** – Dendrogram based on hydroxy fatty acids; **d** – Dendrogram based on fatty acids.

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Genus *Peziza* (*Pezizaceae*) in Bulgaria

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Abstract. As a result of taxonomic revision of the herbarium specimens of discomycetes fungi belonging to the genus *Peziza*, deposited at the Mycological Collection of the Institute of Botany (SOMF), 26 species were established. Six of them are new to the country: *Peziza ammophila*, *P. badiofusca*, *P. hortensis*, *P. lobulata*, *P. nivalis*, and *P. ostracoderma*. Key to the species, short morphological descriptions and distribution for new to the country species are given. Correct names are introduced for 9 species, wrongly determined and deposited in SOMF under other names.

Key words: Bulgaria, key to the species, *Peziza*, revision

Introduction

Genus *Peziza* Fr. (*Pezizaceae*, *Pezizales*, *Ascomycetes*) is a large genus, including probably over 100 species world-wide (according Spooner 2001). It includes operculate species, those in which the ascus dehisces via an apical lid or operculum.

The modern genus *Peziza* is now restricted to the almost all large operculate *Discomycetes* which have fleshy apothecial fruit bodies, iodine-positive unitunicate asci and complex excipules build primarily of large globose cells and interwoven hyphae. Because of the former broad application of this generic name there are thousands of species referred to it. Of these, only a small proportion are truly *Peziza* in the restricted sense (Rifai 1968; Korf 1972, etc.). Moreover, many species referred to *Peziza* have not been critically reexamined. There is no world monograph, and much revision work in the genus is needed.

Purposeful investigations on species composition, ecology and distribution of the fungi from genus *Peziza* are not carried out in Bulgaria. About 35 species are published till now in our country, as some of them are reported by their synonyms, belonging mainly to the genera *Plicaria* Fuckel and *Galactinia* (Cooke) Boud.

Material and methods

The investigations of 84 herbarium specimens belonging to the genus *Peziza*, collected from different floristic regions of Bulgaria and deposited at the Mycological Collection of the Institute of Botany (SOMF), are carried out. Asci, ascospores, and paraphyses were observed and measured after rehydrating of dried material in 10 % KON. Squash mounts were made in Melzer's reagent. Fifty spores and 10 asci from each specimen were measured.

Determination, nomenclature, and taxonomy of the species are in accordance with Seaver (1961), Moser (1963), Smitska (1975), Dennis (1978), Smitskaya (1980), Breitenbach & Kränzlin (1984), Dissing (2000), Spooner (2000, 2001).

Key to the Bulgarian species of *Peziza*

1. Ascospore's coat smooth 2
1*. Ascospore's coat ornamented 14
2. Ascospores without oil drops 3
2*. Ascospores with oil drops 13
3. Ascospores up to 17.5 µm long 4
3*. Ascospores longer than 17.5 µm 9
4. Apothecia sessile 5

4*. Apothecia stalked 7

5. Ascospores 7.5–10 µm wide 6

5*. Ascospores 10–12.5 µm wide . . . . . **19. *P. nivalis***

6. Apothecia dark-violet. On old fireplaces. . . . . .**16. *P. lobulata***

6*. Apothecia pale fawn. On walls and on soil . . . . . .**22. *P. repanda***

7. Apothecia on rotten wood. . . . . **18. *P. micropus***

7*. Apothecia on soil. 8

8. Apothecia up to 5.7 cm in diameter, on stalk, up to 1 cm long. On calcareous soil, wet wood, etc. . . . . **9. *P. cerea***

8*. Apothecia up to 1.7 cm in diameter, on stalk, longer than 1 cm. On humus soil . . . . . **24. *P. varia***

9. Ascospores 7.5–10 µm wide. 10

9*. Ascospores 10–12.5 µm wide . . . **15. *P. hortensis***

10. Apothecia on dung. 11

10*. Apothecia on other substrata 12

11. Apothecia 1.2–3.6 cm in diameter, outside gray-yellow, slightly powdery. On dung and soil . . . . . .**25. *P. vesiculosa***

11*. Apothecia 0.2–1 cm in diameter, outside yellow-brown. On cow dung . . . . . **14. *P. fimeti***

12. Apothecia 1.5–3.5 cm in diameter, sink into the sand, with short, stem-like base . . . . . **1. *P. ammophila***

12*. Apothecia 0.5–1 cm in diameter, sessile. On soil. . . . . **2. *P. ampliata***

13. Ascospores with one big oil drop. Apothecia on soil . . . . . .**3. *P. amplissima***

13*. Ascospores with two oil drops. Apothecia on old fireplaces . . . . .**26. *P. violacea***

14. Ascospore's coat warty. 15

14*. Ascospore's coat spinulose. 24

15. Ascospores 5–7.5 µm wide 16

15*. Ascospores 7.5–10 (–12.5) µm wide 17

16. Apothecia white, pink to violet. On calcareous soil . . . . . **11. *P. domiciliana***

16*. Apothecia brown-violet. On old fireplaces . . . . . .**21. *P. praetervisa***

17. Ascospores 10–15 µm long 18

17*. Ascospores longer than 15 µm. 19

18. Ascospores with one central oil drop. Apothecia outside dark-brown with orange-brown hymenium . . . . . **7. *P. badiofusca***

18*. Ascospores with one or two oil drops. Apothecia outside red-brown with dark-brown hymenium . . . **20. *P. ostracoderma***

19. Ascospores without oil drops . . **4. *P. arvernensis***

19*. Ascospores with oil drops 20

20. Ascospores with warty-reticulate coat and one big and several smaller oil drops. . **5. *P. badia***

20*. Ascospores with distinct warts on the coat and with two oil drops. 21

21. Ascospores up to 17.5 µm long 22

21*. Ascospores longer than 17.5 µm. 23

22. Apothecia up to 4.5 cm in diameter, outside whitish, slightly powdery with ochraceous-brown hymenium. Paraphyses filled with violet granules . . . . . **13. *P. emileia***

22*. Apothecia up to 3 cm in diameter, outside fawn-brown with red-brown hymenium. Paraphyses with yellowish content . . .**17. *P. michelii***

23. Ascospores ornamented by fine oblong warts. Paraphyses clavate at the apex, up to 5 µm wide. . . . . **6. *P. badioconfusa***

23*. Ascospores ornamented by simple warts. Paraphyses with clavate apex up to 7.5–10 µm wide. . . . . .**10. *P. depressa***

24. Apothecia on old fireplaces. Ascospores without oil drops . . . . . **12. *P. echinospora***

24*. Apothecia on soil. Ascospores with oil drops . . .25

25. Apothecia up to 3.6 cm in diameter. Ascospores with two large oil drops, ornamented by big, rough, semispherical short spines. Paraphyses branched . . . . . **23. *P. succosa***

25*. Apothecia up to 1.8 cm in diameter. Ascospores with one big and several smaller oil drops, ornamented by small, regularly disposed spines. Paraphyses simple . . . . **8. *P. brunneoatra***

New species *Peziza* for Bulgaria

Peziza ammophila Durieu & Mont., Expl. Sci. Alg., Bot. Atlas, tab. 28, 1847 (Plate I, Fig. 2)

Apothecia 1.5–3.5 cm in diameter, at first entirely immersed in the sand appearing as holes in the ground, scattered, deep cup-shaped with irregular or often star-shaped margin, extended below into a stem-

like base, consisting of sand, mixed by fungus mycelium; apothecia outside entirely encrusted of sand; hymenium brownish, yellowish into the rim. Asci 260–350 × 15–17.5 (–20) µm, cylindrical, J+, 8-spored. Ascospores (15–) 17.5–20 × (5–) 7.5–10 µm, ellipsoidal, unicellular with smooth coat, hyaline. Paraphyses cylindrical, enlarged above up to 7 µm, clinging together and not very distinct.

Substratum and distribution in Bulgaria: on sandy soil, Black Sea coast (Southern), between Kiten and Primorsko, 15.11.1962, leg. Müller, SOMF 2829.

Peziza badiofusca (Boud.) Boud., British Cup-fungi, p. 16, 1960 (Plate I, Fig. 1)

Apothecia 1.3–2.1 cm in diameter, sessile, single or gregarious, ochraceous-brown, unicolorous with slightly warty rim. Asci 300–350 × 10–15 µm, cylindrical, J+, 8-spored. Ascospores 10–15 × 7.5–10 µm, ellipsoidal, unicellular with one central oil drop and coat, ornamented by small, regularly disposed warts, hyaline. Paraphyses cylindrical, septate, apex clavate, up to 5–7.5 µm.

Substratum and distribution in Bulgaria: on soil, Sredna Gora Mts (Mt Lozenska, Urvich locality), 28.08.1975, leg. C. Hinkova, SOMF 13 545 (sub *Peziza michelii* Dennis).

Peziza hortensis P. Crouan & H. Crouan, Fl. Finistère, p. 53, 1867 (Plate I, Fig. 5)

Apothecia 0.4–1 cm in diameter, sessile, single or gregarious, at first closed, globose, later expanded, shallow discoid with notched rim, unicolorous, pale ochraceous-brown. Asci 280–290 × 12.5–15 µm, cylindrical, J+, 8-spored. Ascospores 17.5–20 × 10–12.5 µm, ellipsoidal, unicellular, without oil drops and with smooth coat, hyaline. Paraphyses cylindrical, septate, apex slightly clavate up to 2.5–5 µm.

Substratum and distribution in Bulgaria: on soil, Vitosha region (Mt Vitosha, Krapets locality), 10.07.1969, leg. B. Aleksandrov, SOMF 8208.

Peziza lobulata (Velen.) Svrček, Česká Mykol., **30**(3–4), p. 130, 1976 (Plate I, Fig. 4)

Apothecia 1–1.9 cm in diameter, sessile, cup-shaped with notch rim, black-violet, outside with big warts, unicolorous. Asci 200–230 × 7.5–10 µm, cylindrical, J+, 8-spored. Ascospores 10–15 × 7.5–10 (–12.5) µm, ellipsoidal, with rounded or narrowed ends, unicellular, thin-walled with smooth coat, without oil drops, hyaline. Paraphyses filiform, apex clavate, up to 5 µm wide, predominantly convolute, fill with yellowish-brown oil content.

Substratum and distribution in Bulgaria: on soil, Rila Mts (near the Zavrachitsa chalet), 28.07.1962, leg. C. Hinkova, SOMF 4448 (sub *Peziza sepiatra* Cooke).

Peziza nivalis (R. Heim & L. Remy) M.M. Moser, Israel J. Bot., **23**, p. 162, 1974 (Plate I, Fig. 6)

Apothecia 1.6–2.3 cm in diameter, sessile, single or gregarious with inside convoluted rim, concolorous, hazelnut to chestnut-brown. Asci 220–280 × 15 (–17.5) µm, oblong-cylindrical, J+, 8-spored. Ascospores (12.5–) 15 × 10–12.5 µm, late ellipsoidal, unicellular, without oil drops, with smooth coat, hyaline. Paraphyses with clavate apex up to 5 µm wide, septate, downwards irregular, in some places uneven wide.

Substratum and distribution in Bulgaria: on soil, Pirin Mts (Vasilevska Mandra locality), 27.04.1970, leg. B. Aleksandrov, SOMF 8241.

Peziza ostracoderma Korf, Mycologia, **52**, p. 650, 1961 (Plate I, Fig. 3)

Apothecia 0.7–2 cm in diameter, sessile, single, at first deep cupulate, later expanded to almost flat, outside smooth, red-brown, downwards slightly downy; hymenium dark brown, smooth. Asci 220–290 × 10–12.5 µm, cylindrical, J+, 8-spored. Ascospores 12.5–15 (–17.5) × 7.5–10 µm, ellipsoidal, with one or two oil drops, unicellular and with coat, covered by short dot-like warts, hyaline. Paraphyses cylindrical, septate, apex clavate, up 5–7.5 µm wide.

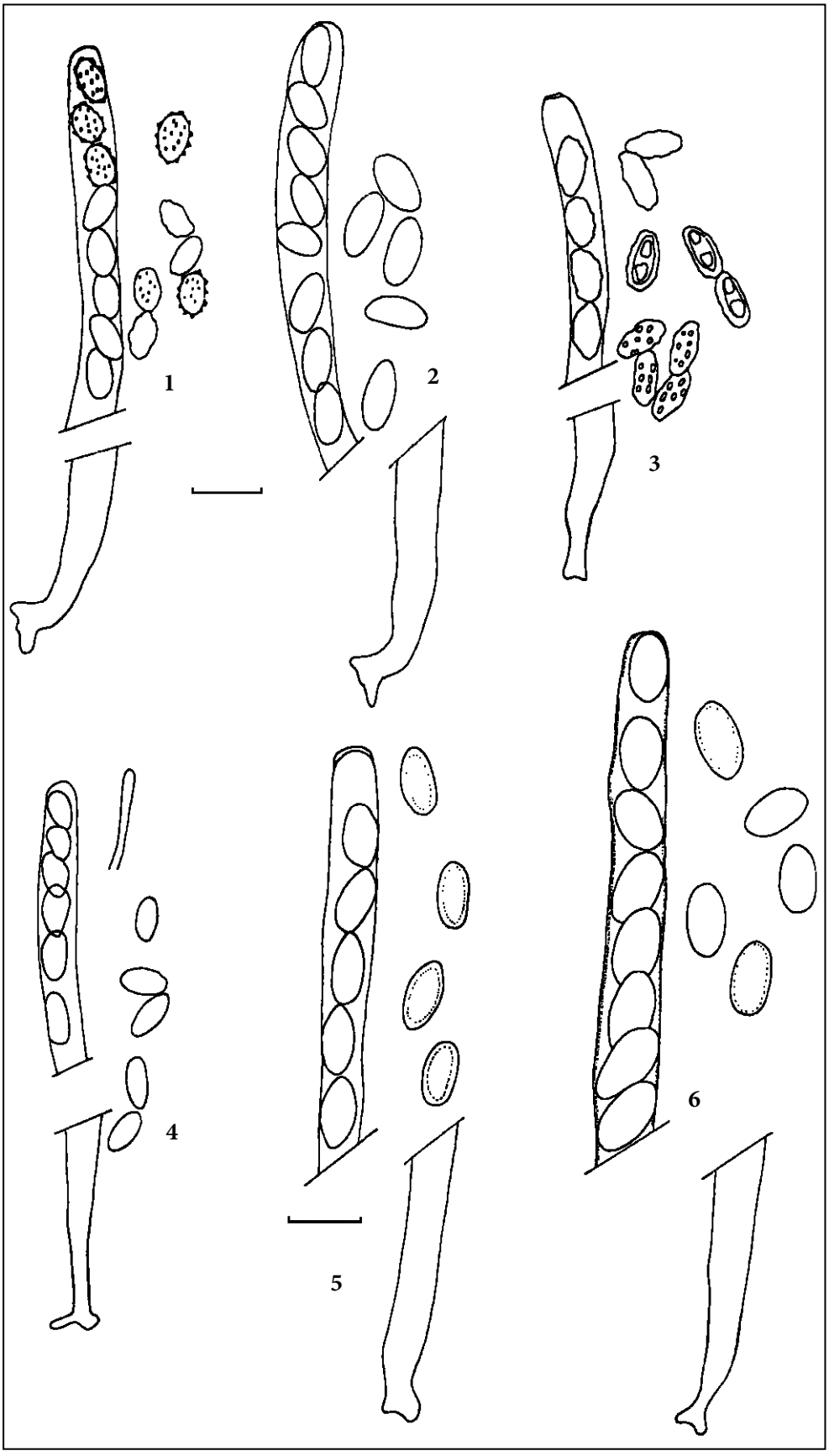
Substratum and distribution in Bulgaria: on soil, Vitosha region (Mt Vitosha, between Zheleznitsa and Kovachevtsi villages, under *Alnus glutinosa*), 30.08.1969, leg. B. Aleksandrov, SOMF 8477 (sub *Peziza atrovinosa* Cooke & Gerard); Sredna Gora Mts (above Kokalyane village), 07.09.1976, leg. C. Hinkova & M. Drumeva, SOMF 13 580 (sub *Peziza atrovinosa*).

Note: *P. atrovinosa* is a North-American species (Seaver 1928) and morphologically is much closed to *P. ostracoderma*, but differs mainly by its larger apothecia.

Results

As a result of the first taxonomic revision of all available Bulgarian specimens from genus *Peziza*, stored in SOMF, 26 species were established.

Plate I



Figs 1–6. Asci and ascospores of:
1, *P. badiofusca*;
2, *P. ammophila*;
3, *P. ostracoderma*;
4, *P. lobulata* (and paraphysis);
5, *P. hortensis*;
6, *P. nivalis*.
Scale bars = 20 µm.

Six of them are new to the country: *P. ammophila*, *P. badiofusca*, *P. hortensis*, *P. lobulata*, *P. nivalis* and *P. ostracoderma*.

Two species – *P. brunneoatra* and *P. michelii* – are included in the *Red List of Bulgarian Fungi* (Gyosheva & al. 2006) with category "Endangered" (EN).

The correct names of 9 species, wrongly determined and deposited in SOMF under different names, are given: *P. badiofusca*, *P. brunneoatra*, *P. emileia*, *P. lobulata*, *P. michelii*, *P. ostracoderma*, *P. praetervisa*, *P. succosa* and *P. violacea.* Correct names of 7 species deposited in SOMF under their synonyms are introduced: *P. arvernensis* (sub *P. sylvestris* (Boud.) Sacc. & Traverso), *P. badia* (sub *Plicaria badia* (Pers.) Fuckel), *P. cerea* (sub *Plicaria muralis* (Sowerby) Rehm), *P. domiciliana* (sub *P. adae* J. Sadler ex Cooke), *P. echinospora* (sub *P. antracophila* Dennis), *P. fimeti* (sub *Plicaria fimeti* (Fuckel) Rehm), *P. succosa* (sub *Galactinia succosa* (Berk.) Sacc.).

Established taxa belong to the following ecological-trophic groups (according Arnolds 1981 and Gyosheva & Vassilev 1994): **Humus saprotrophs** – 17 species: *P. ammophila*, *P. ampliata* Pers., *P. amplissima* Fr., *P. arvernensis* Boud., *P. badia* Pers. ex Mérat, *P. badioconfusa* Korf, *P. badiofusca*, *P. brunneoatra* Desm., *P. depressa* Pers., *P. domiciliana* Cooke, *P. emileia* Cooke, *P. hortensis*, *P. michelii*, *P. nivalis, P. ostracoderma*, *P. succosa* Berk., *P. varia* (Hedw.) Fr.; **Carbotrophs** (on old fireplaces) – 4 species (*P. echinospora* P. Karst., *P. lobulata*, *P. praetervisa* Bres., *P. violacea* Pers. : Fr.). **Coprotrophs** (on excrements) – 1 species (*P. fimeti* (Fuckel) Seaver); **Wood saprotrophs** – 1 species (*P. micropus* Pers. : Fr.).

Two species, *P. cerea* Sowerby : Fr. and *P. repanda* Pers. : Fr., develop as well on soil as on other substrata – wet walls and wood, calcareous soil, etc. One species – *P. vesiculosa* Bull. develops on soil and on dung.

Four species are established from new localities (*P. badioconfusa*, *P. brunneoatra*, *P. echinospora*, *P. succosa*).

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New data of anamorphic fungi from Bulgaria

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Abstract. The work contains new data about species composition and distribution of the anamorphic fungi for Bulgaria. One genus and five species are reported for the first time from the country. Five host plants of anamorphic fungi, new for Bulgaria, are also recorded. The correct names of two anamorphic fungi species are given.

Key words: anamorphic fungi, Bulgaria, *Sirosporium*

Introduction

New data on the anamorphic fungi of Bulgaria are represented. The genus *Sirosporium* Bubák & Serebrian. apud Bubák and the species *S. antenniforme* (Berk. & M.A. Curtis) Bubák & Serebrian., *Ascochyta siemaszkoi* Melnik, *Cladosporium gentianae* Lobik, *Fusicladium nebulosum* (Ellis & Everh.) Ritschel & U. Braun and *Septoria longispora* Bondartsev are reported as new taxa for Bulgaria. Five fungus-host combinations, new for the country, are also recorded. The correct names of *Asteromella gentianellae* (C. Massal.) Petr. and *Fusicladium pomi* (Fr.) Lind are given.

On the basis of the investigated materials short descriptions and illustrations of the taxa new to Bulgaria are provided. All researched specimens are deposited in the Mycological Collection of the Institute of Botany, Bulgarian Academy of Sciences (SOMF).

Results

New taxa for Bulgaria

Ascochyta siemaszkoi Melnik, Novosti Sist. Nizsh. Rast., **12**: 205, 1975 (Fig. 1)

Leaf spots amphigenous, circular or irregular, up to 1 cm in diameter, single, grey-brownish, paler in the centre. **Pycnidia** numerous, epiphyllous, immersed into the tissues, pale brown, spherical to flattened-spherical, 160–175 µm in diameter. **Conidia** longish-cylindrical with rounded ends, straight, rarely slightly curved, aseptate or 1–2-septate, hyaline, guttulate, (20–) 25–37.5 (–45) × 7.5–9 µm.

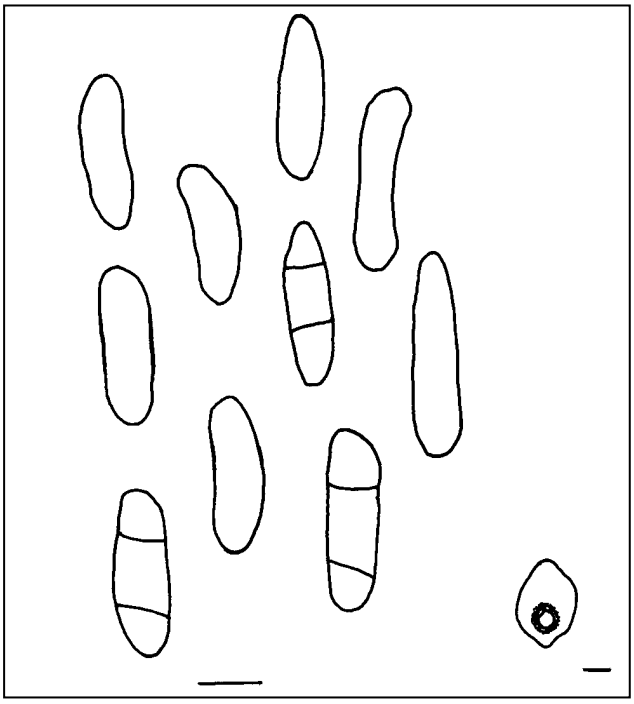

Fig. 1. Pycnidium and conidia of *Ascochyta siemaszkoi*. Scale bars = 10 µm.

On leaves of *Cirsium arvense* (L.) Scop., Forebalkan (Eastern): Veliko Turnovo, 20.10.2005, leg. S. Milanova, det. G. Bakalova (SOMF 25 990).

Cladosporium gentianae Lobik, Bolezni Rast., **17**(3–4): 189, 1928 (Fig. 2)

Leaf spots irregular, single or confluent, yellowish. **Mycelium** superficial, occasionally immersed; hyphae branched, septate, often constricted at the septa, 5–10 µm wide, cells 5–11 µm long, pale brown.

Conidiophores solitary or in loose fascicles, simple, septate, erect or decumbent, straight to curved, slightly geniculate-sinuous, 75–130 × 4–7 µm, not constricted at the septa, smooth, brown, usually paler towards the apex, conidiogenous loci terminal and intercalary, protuberant, thickened, darkened, 1–1.5 µm diam. **Conidia** straight, widely or narrowly ellipsoidal, obovoid, sometimes subglobose or cylindrical, apex rounded or somewhat attenuated, 8–17.5 × 4–7.5 µm, aseptate or 1 (2–3)-septate, usually non constricted at the septa, pale olivaceous-brown, smooth or slightly verruculose, hila protuberant, thickened and darkened, 1–1.5 µm diam.

On leaves of *Gentiana asclepiadea* L., Balkan Range (Western): under Kom Peak, 01.07.1986, E. Sameva (SOMF 25 991).

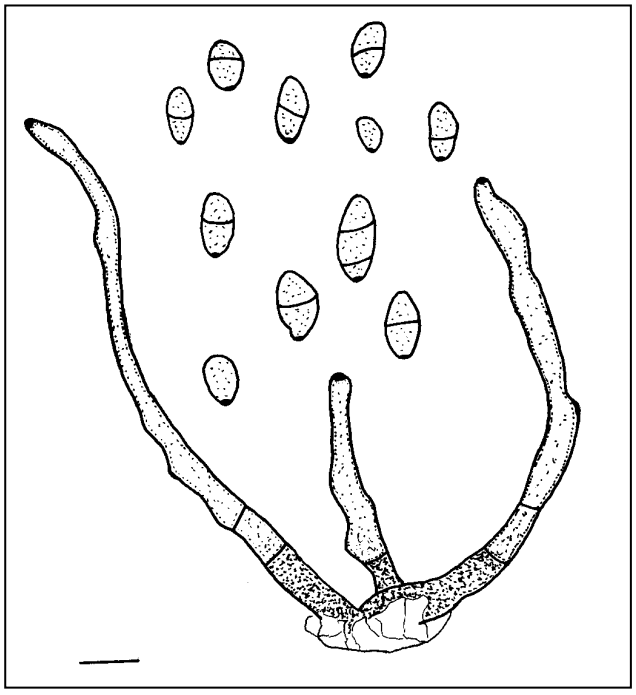

Fig. 2. Conidiophores and conidia of *Cladosporium gentianae*. Scale bar = 10 µm.

Fusicladium nebulosum (Ellis & Everh.) Ritschel & U. Braun, A monograph of *Fusicladium* s. lat. (*Hymenomycetes*): 67, 2003 (Fig. 3)

Leaf spots amphigenous, circular or irregular, 1–1.5 cm, single, cinnamon-brown. **Colonies** amphigenous, punctiform or irregular, single or confluent, black. **Conidiophores** reduced to conidiogenous cells. **Conidia** solitary, fusiform to ellipsoidal, straight to somewhat curved, one-celled or with 1 septum near the base, rarely 2-septate, pale olivaceous-brown, verruculose, pointed at the apex, truncate to somewhat convex at the base, (10–) 12.5–15 (–17.5) × (4–) 5–6 µm; hila unthickened, not or only slightly darkened.

On leaves of *Fraxinus excelsior* L., Forebalkan (Eastern): Pavlikeni, 12.07.1960, leg. M. Markov, det. G. Bakalova (SOMF 8113).

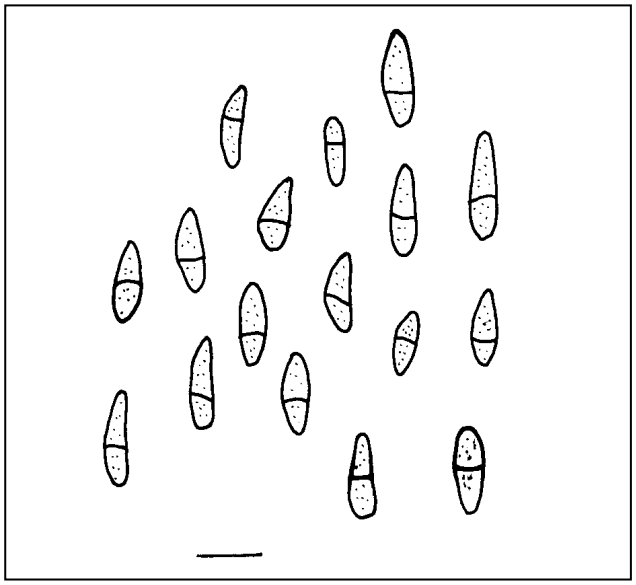

Fig. 3. Conidia of *Fusicladium nebulosum*. Scale bar = 10 µm.

Septoria longispora Bondartsev, Tr. Imp. Bot. Sada, **26**: 46, 1910 (Fig. 4)

Leaf spots amphigenous, rounded or irregular, 0.1–0.5 cm in diameter, mainly single, rarely confluent, ochraceous-brownish, paler in the centre. **Pycnidia** epiphyllous, 1–3 on spot, globose or flattened globose, thick-walled, dark brown, 90–200 µm in diameter, with circular ostiole, indistinctly limited, about 50 µm in diameter, surrounded by darker cells. **Conidia** filiform, straight or irregular curved, tapering at the ends or rounded at the base, apically acuminate, with 2–5 septa, hyaline, (80–) 100–125 (–140) × 2.5–3 µm.

On leaves of *Convolvulus arvensis* (L.) Scop., Forebalkan (Eastern): Veliko Turnovo, 20.10.2005, leg. S. Milanova, det. G. Bakalova (SOMF 25 992).

Sirosporium Bubák & Serebrian. apud Bubák, Hedwigia, **52**: 272-273, 1912

For generic description see Ellis (1971).

S. antenniforme (Berk. & M.A. Curtis) Bubák & Serebrian., Hedwigia, **52**: 272, 1912 (Fig. 5)

Spots on the upper leaf surface irregular, confluent, pale brown to brown, indistinctly delineated, surrounded

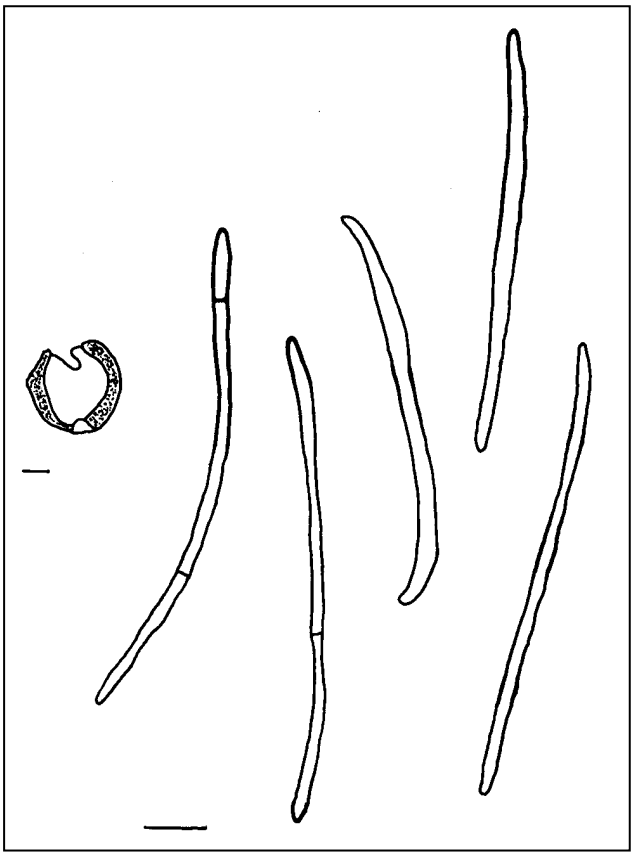

Fig. 4. Pycnidium and conidia of *Septoria longispora*. Scale bars = 10 µm.

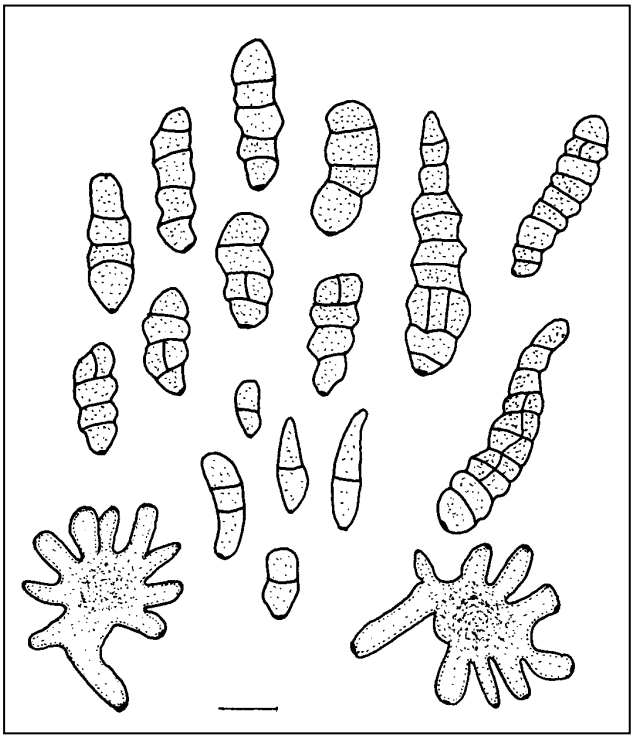

Fig. 5. Conidiophores and conidia of *Sirosporium antenniforme*. Scale bar = 10 µm.

by yellowish halo. **Colonies** hypophyllous, effuse, dark brown to black, velvety, dense and darker in the central part, somewhat loose and paler in the periphery. **Conidiophores** in dense fascicles, ascending, straight or slightly curved, short, subcylindrical, pale brown to brown, rounded and paler at the apex, aseptate, conidial scars conspicuous, usually terminal, thickened and darkened, smooth to slightly rough-walled, (10–) 12–22 × 4.5–6.5 µm. **Conidia** straight or curved, often obclavate, some narrowly or widely cylindrical or nearly fusiform, with 1–17 transverse and several longitudinal and oblique septa, usually constricted at the septa, smooth or verruculose, pale brown, 12–60 (–77) × 5.5–18 (–20) µm; hilum frequently protruding, 1–2 mm thick.

On leaves of *Celtis glabrata* Steven, Northeast Bulgaria: Rousse district, near Ivanovo village, 21.09.2003, leg. E. Genova, fungus comm. & det. E. Sameva (SOMF 25 993).

Species with new hosts in Bulgaria

Ascochyta cyrcaeae Bubák & Picb., Ann. Mycol., **35**: 142, 1937
On leaves of *Oenothera biennis* L., Rila Mts: near Dolna Banya, 26.09.1935, leg. K. Drenovski, fungus comm. & det. E. Sameva (SOMF 25 994).

A. doronici Allesch., Hedwigia, **36**: 162, 1897
On leaves of *Serratula tinctoria* L., Northeast Bulgaria: near Suvorovo, 22.07.1904, leg. B. Davidov, fungus comm. & det. E. Sameva (SOMF 25 995).

A. verbascina Thüm., Contr. Fl. Litor. №343; Sacc., Syll. Fung., **3**: 402, 1884
On leaves of *Verbascum banaticum* Schrad., West Frontier Mts: Mt Osogovo (above Kyustendil, near St. Luca Monastery), 07.07.2001, E. Sameva (SOMF 25 996).

Colletotrichum dematium (Pers.) Grove, J. Bot., **56**: 341, 1918
On leaves of *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz-Bip., Rila Mts: near Rila Monastery, 05.11.1973, leg. S. Vanev, det. E. Sameva (SOMF 20 100).

C. truncatum (Schwain) Andrus & W.D. Moore, Phytopathology, **25**: 121, 1935
On leaves of *Lupinus hartwegii* Lindley, Thracian Lowland: Karlovo, 29.08.2002, E. Sameva (SOMF 25 997).

Corrected names of species published from Bulgaria

Asteromella gentianellae (C. Massal.) Petr., Hedwigia, **65**: 253, 1925 (as *Phyllosticta gentianellae* Massal. – Klika 1929; Hinkova 1960)
On leaves of *Gentiana asclepiadea* L., Balkan Range (Western): under Kom Peak, 01.07.1986, E. Sameva (SOMF 25 998).

Fusicladium pomi (Fr.) Lind, Dan. fung.: 521, 1913 (as *F. dendriticum* (Wallr.) Fuckel – Malkov 1905)
On leaves of *Malus dasyphylla* Borkh., Mt Belasitsa: near Belasitsa chalet, 27.06.1974, G. Bakalova (SOMF 25 999).

Acknowledgements. The authors extend their thanks to Dr E. Genova and to the Curator of SOM for the specimens put at their disposal.

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Taxonomic study of the cercosporoid hyphomycetous fungi in Bulgaria

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Abstract. The work contains the summarized data about species composition, distribution and specialization of the parasitic cercosporoid hyphomycetous fungi in Bulgaria. One hundred and thirteen taxa (101 species and 12 varieties) of twelve genera were determined. Seven genera, twenty species and one variety are new for Bulgaria. *Ramularia jordanovii* and *R. periplocae* were found only on Bulgarian territory.

Key words: Bulgaria, cercosporoid hyphomycetous fungi, new taxa, specialization

Introduction

Cercosporoid hyphomycetous fungi pertain to the formal taxonomic group of the anamorphic fungi. They are known only from the asexual reproductive state (**anamorph**). One small part of them are with experimentally proven sexual phase (**teleomorph**).

In recent years the hyphomycetous fungi have been object of extensive taxonomical, ecological and chorological researches. They are phytopathogenic and cause characteristic leaf spots diseases on cultivated and economically important wild plants.

Material and methods

Scientific research was realized by complex mycological methods including field investigation (tracking method) and also laboratory studies using comparative-morphological, microscopic and statistic methods. Squash mounts were made in lactophenol. Fifty conidia from each specimen were measured, but when only one herbarium specimen from the species was available, one hundred conidia were measured. Data of the feature's length and width were treated by statistic methods. Voucher specimens for all findings were deposited in the Mycological Collection of the Institute of Botany, Bulgarian Academy of Sciences (SOMF).

Determination, nomenclature and taxonomy of the species are in accordance with Vassiljevsky & Karakulin (1937), Chupp (1953), Deighton (1976, 1987), Braun (1993, 1995a, b, 1998), Braun & Melnik (1997), Shin & Kim (2001).

Results

During the investigation of 368 herbarium specimens of cercosporoid hyphomycetous fungi, collected in different floristic regions of Bulgaria, 113 taxa (101 species and 12 varieties) of 12 genera were established; 7 of them are new for the country (in the "List of the taxa" they are marked by an asterisk).

List of the taxa

Cercospora Fresen.
C. armoraciae Sacc.
C. barbareae (Sacc.) Chupp
C. beticola Sacc.
C. bizzozeriana Sacc. & Berl.
C. cruciferarum D.E. Ellis & Everh.
C. cynoglossi J.M. Hook
C. depazeoides (Desm.) Sacc.
C. echii G. Winter
C. elongata Peck
C. galegae Sacc.
C. malvicola D.E. Ellis & G. Martin
C. mercurialis Pass.

C. olivascens Sacc.
C. phaseolina Speg.
C. plantaginis Sacc.
C. plumbaginea Sacc. & D. Sacc.
C. taurica Tranzshel
C. violae Sacc.
C. zonata G. Winter

Cercosporella Sacc**.** emend. Deighton
C. coronillae Karak.
C. prolificans (D.E. Ellis & Holw.) Sacc.
C. virgaureae (Thüm.) Allesch.

Mycovellosiella Rangel
M. bellynckii (Westend.) Constant.
M. dulcamarae (Peck) U. Braun
M. ferruginea (Fuckel) Deighton

Passalora Fr.
P. avicularis (G. Winter) Crous, U. Braun & E.F. Morris
P. bolleana (Thüm.) U. Braun
P. bupleuri (Pass.) U. Braun
P. campi-silii (Speg.) U. Braun
P. dubia (Riess) U. Braun
P. malkoffii (Bubák) U. Braun
P. microsora (Sacc.) U. Braun
P. punctum (Delacr.) Petzoldt
P. rosicola (Pass.) U. Braun

****Phacellium*** Bonord.
P. alborosellum (Desm.) U. Braun
P. episphaerium (Desm.) U. Braun

Phaeoramularia Muntañola
P. punctiformis (Schltdl.) U. Braun

****Pseudocercospora*** Speg.
P. handelii (Bubák) Deighton
P. ramischiae U. Braun & Melnik

****Pseudocercosporella*** Deighton
P. angustana (Ferraris) U. Braun
P. magnusiana (Allesch.) U. Braun
P. pastinacae (P. Karst.) U. Braun

Ramularia Unger
R. abscondita (Fautrey & F. Lamb.) U. Braun
R. adoxae P. Karst.
R. agrestis Sacc. var. *agrestis*
R. agrimoniae Sacc.
R. ajugae (Niessl) Sacc.
R. alnicola Cooke var. *alnicola*
R. alpina (C. Massal.) Nannf.
R. aplospora Speg.
R. archangelicae Lindr.
R. atropae Allesch.
R. beccabungae Fautrey
R. bulgarica Bubák & Picb.
R. caduca (W. Voss) U. Braun
R. calthae Lindr.
R. cardamines Syd.
R. carneola (Sacc.) Nannf.
R. chaerophylli Ferraris
R. chamaedryos (Lindr.) Gunnerb.
R. coccinea (Fuckel) Vestergr.
R. cylindroides Sacc. var. *angustispora* U. Braun & Chevassut
R. cylindroides Sacc. var. *cylindroides*
R. cynarae Sacc.
R. cynoglossi Lindr.
R. didyma Unger var. *didyma*
R. doronici Pass. & Thüm.
R. echii Bondartsev
R. evanida (J.G. Kühn) Sacc.
R. filaris Fresen var. *filaris*
R. gei (A.G. Eliasson) Lindr.
R. geranii Fuckel var. *erodii* Sacc.
R. geranii Fuckel var. *geranii*
R. grevilleana (Tul. & C. Tul.) Jørst. var. *grevilleana*
R. heraclei (Oudem.) Sacc.
R. hyperici U. Braun & Scheuer
R. inaequale (Preuss) U. Braun
R. jordanovii Vanev & Bakalova
R. lactea (Desm.) Sacc.
R. lamii Fuckel var. *lamii*
R. lamii Fuckel var. *minor* U. Braun
R. lampsanae (Desm.) Sacc.
R. lysimachiae Thüm.
R. macrospora Fresen.
R. macularis (J. Schröt.) Sacc. & Syd.
R. marrubii C. Massal.
R. medicaginis Bondartsev & Lebedeva
R. moehringiae Lindr.
R. mulgedii (Bubák) Bubák
R. nagornyi Karak.
R. periplocae Vanev
R. plantaginis D.E. Ellis & G. Martin
R. pratensis Sacc. var. *pratensis*
R. primulae Thüm.
R. ranunculi-montani (C. Massal.) U. Braun
R. rhabdospora (Berk. & Broome) Nannf.
R. rosea (Fuckel) Sacc. var. *rosea*

R. rubella (Bonord.) Nannf.
R. rumicis Kalchbr. & Cooke
R. salviae-pratensis Pellic. & U. Braun
R. sambucina Sacc.
R. sennensis Gonz. Frag.
R. silvestris Sacc.
R. simplex Pass.
R. tanaceti Lind
R. telekiae Bubák & Wróbl.
R. tricherae Lindr.
R. urticae Ces.
R. variabilis Fuckel
R. veronicae Fuckel

****Spermosporina*** U. Braun
S. alismatis (Oudem.) U. Braun

****Stenella*** Syd.
S. lythri (Westend.) J.L. Mulder

****Thedgonia*** B. Sutton
T. ligustrina (Boerema) B. Sutton

Two species – *Ramularia jordanovii* (Fig. 1) and *R. periplocae* (Fig. 2) were found only on the Bulgarian territory.

Twenty species and one variety were determined as new to Bulgaria – *Cercospora barbareae*, *C. cynoglossi*, *C. elongata*, *C. phaseolina*, *C. plumbaginea*, *C. taurica*, *Cercosporella prolificans*, *Passalora bupleuri*, *P. punctum*, *Pseudocercospora handelii*, *P. ramishiae*, *Pseudocercosporella angustana*, *P. magnusiana*, *P. pastinacae*, *Ramularia abscondita*, *R. alpina*, *R. geranii* var. *erodii*, *R. nagornyi*, *R. ranunculi-montani*, *R. salviae-pratensis*, *Stenella lythri*.

One hundred and eighty-two (182) species of wild plants were established as hosts of cercosporoid hyphomycetous fungi in Bulgaria. They belong to 104 genera of 41 families (Fig. 3).

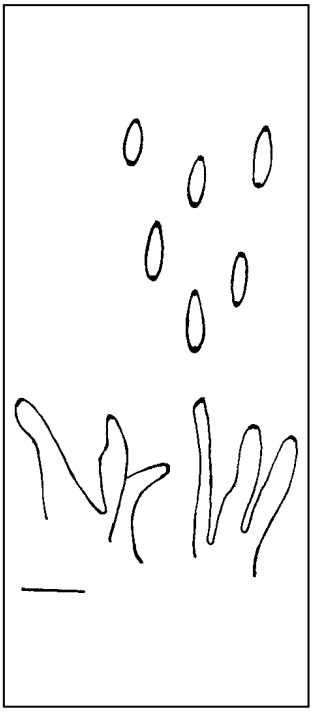

Fig. 1. Conidiophore fascicles and conidia of *Ramularia jordanovii*. Scale bar = 10 µm.

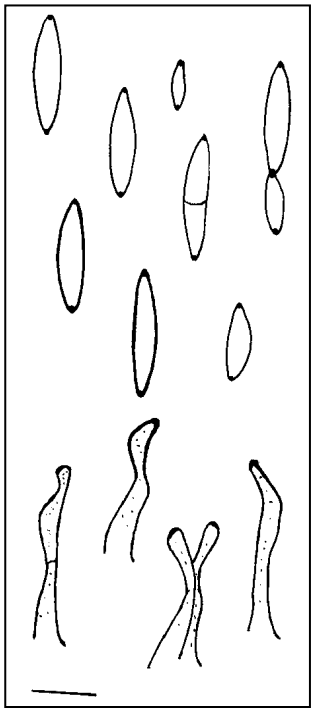

Fig. 2. Conidiophores and conidia of *Ramularia periplocae*. Scale bar = 10 µm.

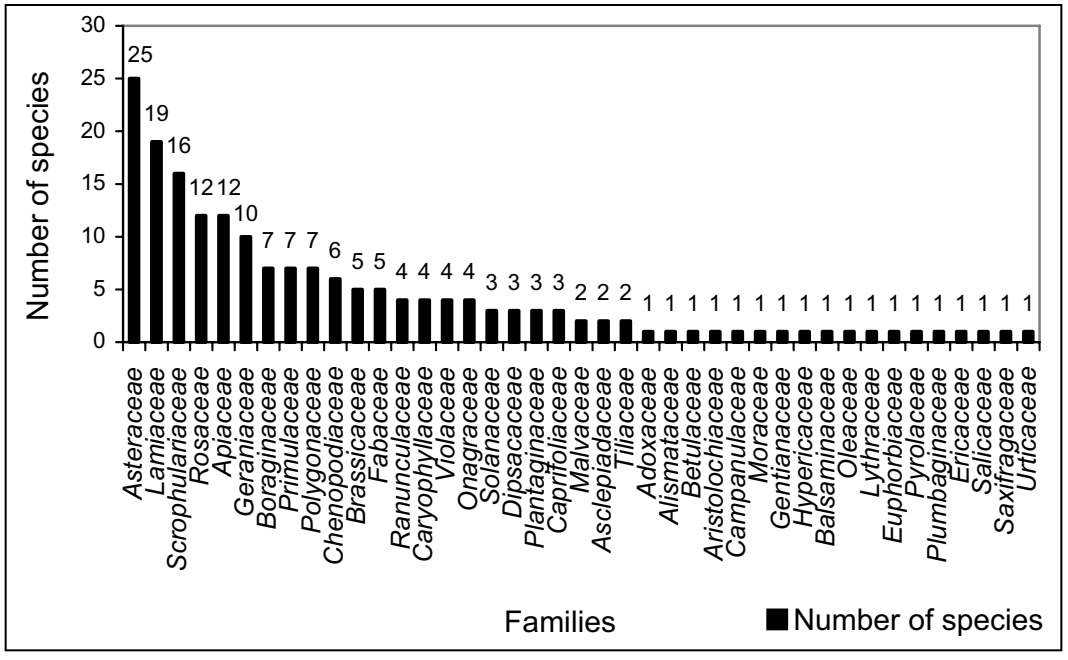

Fig. 3. Number of species in the host families of the cercosporoid hyphomycetous fungi.

Thirteen species of host plants are new to the country: *Ajuga pyramidalis* L. (for *Ramularia ajugae*), *Carduus nutans* L. (for *R. cynarae*), *Chaerophyllum hirsutum* L. (for *R. chaerophylli*), *Lamium garganicum* L. (for *R. lamii* var. *lamii*), *Marrubium peregrinum* L. (for *R. marrubii*), *Picris echioides* L. and *P. hieracioides* L. (for *R. inaequalae*), *Plantago media* L. (for *R. plantaginis*), *Rumex conglomeratus* L., *R. obtusifolius* L. and *R. sanguineum* L. (for *R. rubella*), *R. crispus* L. and *R. obtusifolius* (for *R. pratensis* var. *pratensis*), *R. sanguineum* (for *R. rubella*), *R. alpinus* L. (for *R. rumicis*), *Veronica polita* Fries (for *R. veronicae*).

Ten species of host plants are new to the science: *Angelica pančiči* Vandas (for *Ramularia archangelicae*), *Cerastium caespitosum* Gilib. (for *Phacellium alboroselum*), *Digitalis ferruginea* L. and *Verbascum abietinum* Borbás (for *Ramularia variabilis*), *Geranium reflexum* L. (for *R. geranii* var. *geranii*), *Pulmonaria rubra* Schott (for *R. cylindroides* var. *angustispora*), *Ranunculus arvensis* L. and *R. serbicus* Vis. (for *R. ranunculi-montani*), *Scrophularia aestivalis* Griseb. (for *R. carneola*), *Veronica serpyllifolia* L. (for *R. coccinea*).

Based on the specialization of the cercosporoid hyphomycetous fungi in Bulgaria, 3 groups were recognized – species specialization (16 taxa), genus specialization (54 taxa), and family specialization (43 taxa).

In accordance with the extent of the cercosporoid hyphomycetous fungi were determinated 3 groups, too (Fig. 4).

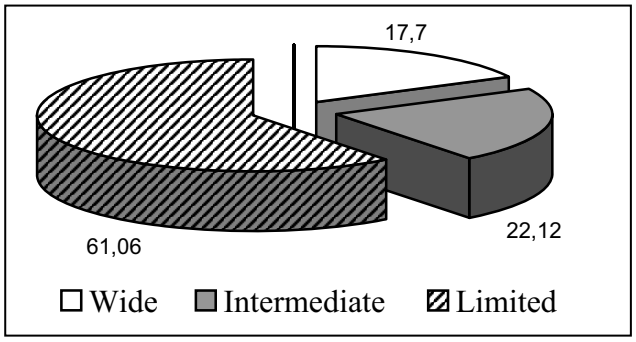

Fig. 4. Extent of a distribution of the cercosporoid hyphomycetous fungi in Bulgaria.

Proven anamorph-teleomorph connections for cercosporoid hyphomycetous fungi in Bulgaria belong to the genera *Mycosphaerella* Johanson and *Sphaerella* Rabenh. (Table 1).

Acknowledgements. The authors extend their thanks to Paulina Georgieva for the technical help.

Table 1. Anamorph-teleomorph connections.

Anamorphs	Teleomorphs
Passalora bolleana	*Mycosphaerella bolleana* (Kendrik & DiCosmo 1979)
P. punctum	*M. anethi* (Petzoldt 1989)
P. rosicola	*M. rosicola* (Kendrik & DiCosmo 1979)
Pseudocercospora ramischiae	*M. pyrolae* (Braun & Melnik 1997)
Pseudocercosporella angustana	*M. taraxaci* (Braun 1995b)
Ramularia aplospora	*M. alchemillae* Vasiljevsky (Braun 1998)
R. atropae	*M. montellica* (syn.: *M. atropae*) (Braun 1998)
R. evanida	*M. gentianae* (Braun 1998)
R. grevilleana var. *grevilleana*	*M. fragariae* (Braun 1998)
R. inaequale	*M. hieracii* (Braun 1998)
R. lactea	*M. violae* (Braun 1998)
R. lysimachiae	*M. lysimachiae* (Braun 1998)
R. sambucina	*M. ebulina* (Braun 1998)
R. tricherae	*Sphaerella silvatica* (syn.: *Mycosphaerella scabiosae*) (Braun 1998)
R. urticae	*M. superflua* (Braun 1998)
R. variabilis	*M. mariae* (syn.: *M. variabilis*; *M. digitalis-ambiguae*) (Braun 1998)

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Fungal diseases of caraway (*Carum carvi*) in Bulgaria

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Abstract. The incidence of fungal diseases on caraway (*Carum carvi*) was investigated at 4 localities of Bulgaria in the period 2001–2005. The most frequently occurring pathogens were *Phomopsis diachenii*, *Erysiphe heraclei*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Ascochyta carvi*, *A. phomoides*, and *Stemphylium botryosum*. *P. diachenii* caused the most devastating and widely distributed disease.

Key words: *Carum carvi*, fungal diseases, *Phomopsis diachenii*

Introduction

The investigation was carried out within the framework of a bilateral research project between Institute of Genetics (IG), Bulgarian Academy of Sciences, Sofia, Bulgaria and the Institute for Resistance Research and Pathogen Diagnostics, Federal Centre for Breeding Research on Cultivated Plants, Aschersleben, Germany.

The aim of the study was to establish the occurrence of fungal pathogens causing the most distributed and damaging diseases on caraway in the period 2001–2005.

Material and methods

Field experiments for observations of fungal disease incidence were carried out at the Institute of Genetics (Sofia region), at Dobroudzha Agricultural Institute (DAI) near General Toshevo (Northeast Bulgaria) and at two Field Experimental Stations: in Bagrentsi village near Kyustendil (Znepole region) and in Stamboliyski near Plovdiv (Thracian Lowland) in the period 2001–2005. The trials were set-up by the block method in two replications each block with twenty plants. One annual ('Sprinter') and three biennial ('Rekord', 'Konczewicki', and 'Arterner') cultivars of caraway (*Carum carvi* L.) were involved in the study. Examinations for disease occurrence were made 2–3 times during the growing season and specimens were collected. The causal agents were isolated from different plant parts. Potato dextrose agar (PDA) was used for initial isolation and subsequent culturing and storage of the strains. The identification of fungi was made on the basis of their morphological and cultural characters.

Results

Phomopsis diachenii was established on caraway for the first time in 2001 (Rodeva & Gabler 2004). Since then it has been the most harmful and widely distributed pathogen of this crop. The main symptoms were umbel browning and the drooping of the diseased umbellets and umbels. The disease quickly spread downward, causing stem necrosis and eventually death of the plant (Fig. 1). The pathogen produced black pycnidia and two types of conidia (alpha and beta) on infected plant tissues as well as on nutrient media. Alpha conidia less abundant than beta conidia, oval to elliptical with slightly pointed apices, 2–3 guttulate, 5–15 × 1.6–4 µm. Beta conidia straight or curved, eguttulate, 10–31 × 0.7–2.5 µm (Fig. 2). *P. diachenii* showed slight variation within and some variation between strains. Colonies zonate, gray with dark grayish-green patches or dark gray-violet from above, mycelium scarce. Pycnidia scattered or in concentric rings, superficial (Fig. 3A). *P. diachenii* was consistently found on caraway plants examined at all four localities in the period 2001–2005.

Fig. 1. Typical symptoms, caused by *Phomopsis diachenii* on caraway.

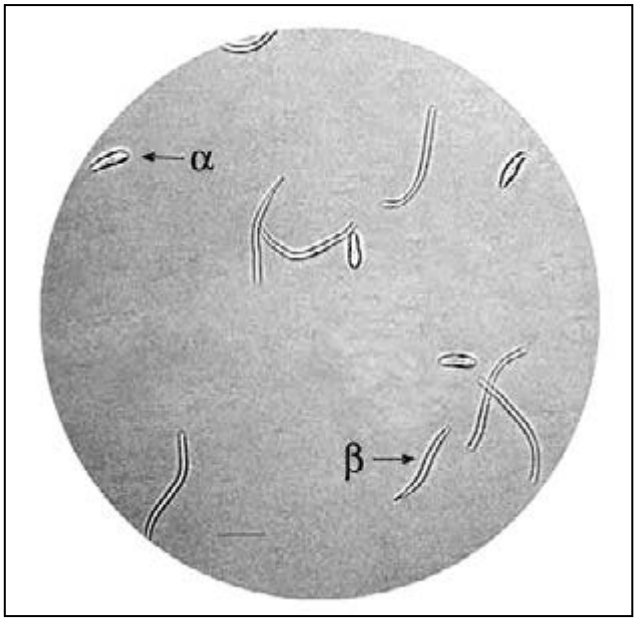

Fig. 2. Alpha and beta conidia of *Phomopsis diachenii*. Scale bar = 10 µm.

Besides *P. diachenii* several other *Phomopsis* spp. differing from it were found. In 2003, a *Phomopsis* sp. was isolated from the stem bases of the diseased plants in Bagrentsi. This was characterised by small pycnidia bearing mainly α-conidia 5–8.5 × 2–5 µm and very rarely β-conidia 13–21 × 1–2 µm. The fungus formed whitish rose colonies on PDA (Rodeva & Gabler 2004).

Another *Phomopsis* sp. was obtained from leaf tissue of caraway at IG (Rodeva & Gabler 2006). It differed from all other *Phomopsis* strains in pycnidial, conidial and colony morphology. Colonies zonate with radial strands, light gray to gray from above, reverse cream. Conidiomata developed big, rotund, multiloculate bodies (Fig. 3B). Abundant α-conidia, elliptical with slightly pointed apices, multiguttulate, 7–11 × 2.6–3.9 µm. Scarce β-conidia, straight or curved, 15–28 × 0.5–1.6 µm (Fig. 4).

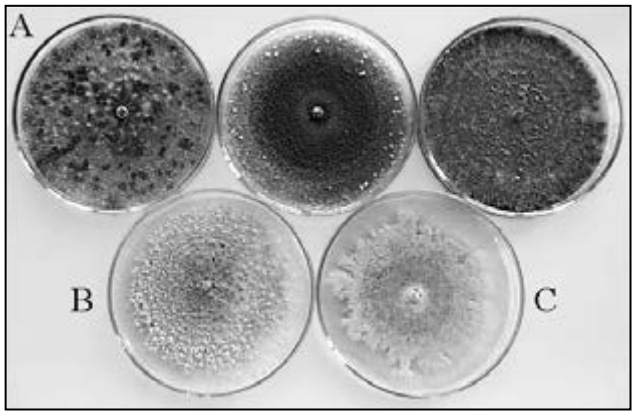

Fig. 3. Growth of *Phomopsis* spp. on PDA: **A** – Three strains of *P. diachenii* from diseased umbels; **B** – *Phomopsis* sp. from leaf tissue; **C** – *P. diachenii* of ascospore origin.

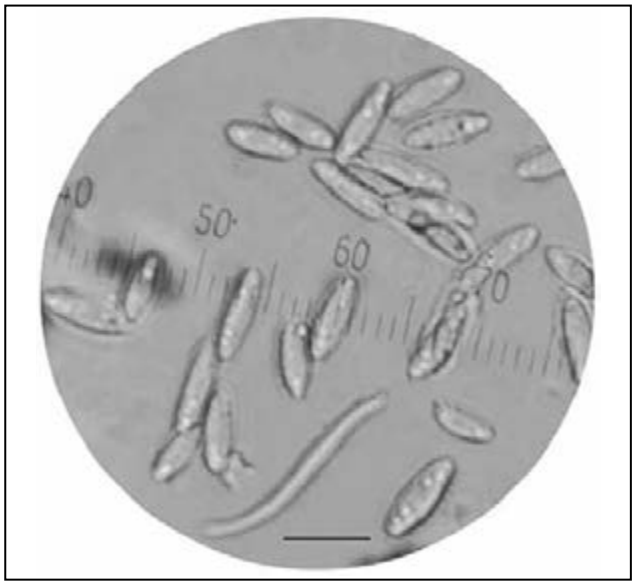

Fig. 4. Alpha and beta conidia of *Phomopsis* sp. from leaf tissue. Scale bar = 10 µm.

One specimen from annual caraway bearing pycnidia of *P. diachenii* formed perithecia in moist chamber after 4–5 weeks of incubation at 22 °C. Well-developed, globose, often clustered, perithecial ascomata

were found embedded in stem tissues adjacent to the pycnidia. Numerous asci were formed in the perithecia. The ascospores were unicellular, colourless, ellipsoidal, guttulate, papillate, with rounded ends, 10–15 × 4–6 µm (n = 100) (Fig. 5). This fungus was assigned to *Diaporthe angelicae* (Berk.) D.F. Farr & Castl. [syn.: *Diaporthopsis angelicae* (Berk.) Wehm.] (Rodeva & Gabler 2004).

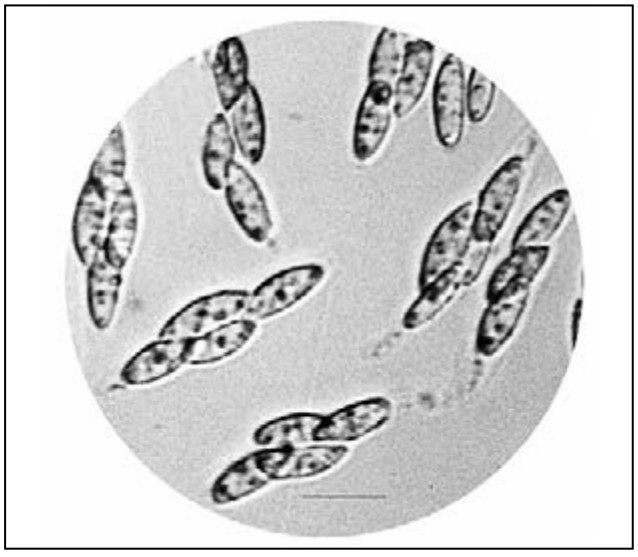

Fig. 5. Ascospores of *Diaporthe angelicae*. Scale bar = 10 µm.

The colonies developed from ascospores showed some morphological differences by comparison with the colonies of *P. diachenii* obtained from pycnidia. They were dirty rose to grayish from above, reverse light brown (Fig. 3C). Alpha conidia less abundant than beta conidia, elliptical with slightly pointed apices, 9–12 × 2.8–3.4 µm. Beta conidia straight or curved, eguttulate, 20–33 × 1.5–2 µm.

Erysiphe heraclei DC. was found as causal agent of powdery mildew on caraway at all four localities every one year. The symptoms were most obvious on the umbels, which were covered by white powder consisted by numerous cylindrical conidia, 25–44 × 10–15.6 µm (Figs 6, 7). Many cleistothecia developed late in the growing season and served in fungal survival. They contained asci with three to five elliptical ascospores.

Botrytis cinerea Pers. : Fr. and *Sclerotinia sclerotiorum* (Lib.) de Bary were often isolated from stems and leaves showing necroses (Fig. 8A). At first colonies of both fungi showed similar morphology developing fast spreading white mycelium. *S. sclerotiorum* strains formed a white mass called sclerotial initials, mainly at the periphery and the centre of the colony 5–6 days after inoculation, which melanized within 1–2 weeks. The mature sclerotia were round-shaped and variable in size, greater than 2 mm in diameter and were easily detached from the mycelium (Fig. 8B). *B. cinerea* produced more but smaller irregularly shaped sclerotia, arranged in approximately concentric circles. Unlike *S. sclerotiorum* strains, sclerotia were enveloped in a

Fig. 6. Powdery mildew caused by *Erysiphe heraclei*.

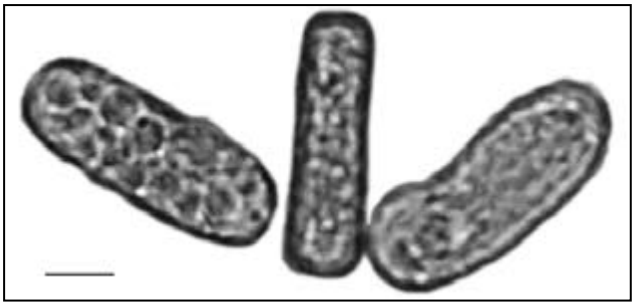

Fig. 7. Conidia of *Erysiphe heraclei*. Scale bar = 10 µm.

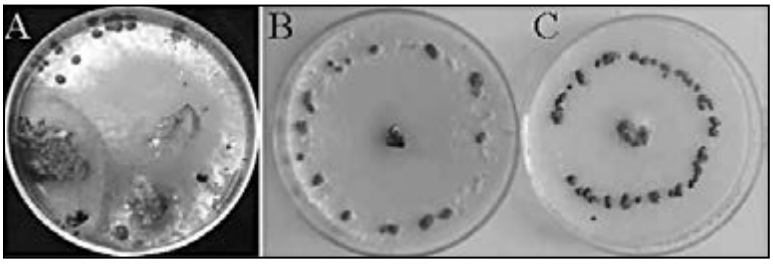

Fig. 8. Colonies of *Sclerotinia sclerotiorum* and *Botrytis cinerea*: **A** – Isolation of both fungi from stem and leaf tissue; **B** – *S. sclerotiorum*; **C** – *B. cinerea*.

hyphal weft and firmly attached to the subtending hyphae and agar medium. They melanized 2–3 weeks after inoculation. Besides sclerotia *B. cinerea* produced conidial sporulation (Fig. 8C).

Ascochyta carvi Ondřej and *A. phomoides* Sacc. were isolated from leaf specimens taken at IG and DAI, respectively. On PDA *A. carvi* produced scarce mycelium and great number of pycnidia mainly with two-cells conidia. *A. phomoides* developed gray-brown colonies with abundant cottony mycelium. The great deal of its conidia was one-cell.

At all localities single caraway plants were totally dead and it was established *Verticillium dahliae* Kleb. as causal agent. Microsclerotia were observed on the roots and on the stem bases. They were also formed on PDA together with scarce mycelium.

Stemphylium botryosum Wallroth was often isolated from different above-ground plant parts. Its sexual form *Pleospora herbarum* (Persoon) Rabenhorst was found on the diseased plant tissues as well as on PDA.

Alternaria spp. [*A. alternata* (Fr.) Keissler, *A. tenuissima* (Kunze) Wiltshire, *A. radicina* Meier, Drechsler & Eddy] and *Fusarium* spp. [*F. avenaceum* (Fr.) Sacc., *F. oxysporum* Schlecht., *F. solani* (Mart.) Sacc., *F. sporotrichoides* Sherb.] were also isolated from caraway but had minor importance for the crop cultivation.

Discussion

P. diachenii was previously reported as an aggressive pathogen on caraway (Ondřej 1997; Gabler & Ehrig 2000; Kusterer & al. 2002; Rodeva & Gabler 2004). In Poland this fungus has not been found yet (Machowicz-Stefaniak & al. 2003; Gabler & Machowicz-Stefaniak 2004). Besides *P. diachenii* we have found several other *Phomopsis* spp. on this crop. It has been found that more than one *Phomopsis* species could occur on a single host plant (Mostert & al. 2001; Farr & al. 2002).

Anthracnose caused by *Mycocentrospora acerina* (Hart.) Deighton, and white mould caused by *S. sclerotiorum*, are major problems in caraway production in Netherlands (Evenhuis & al. 1995; Evenhuis 1997; Evenhuis & Verdam 1997; Evenhuis & al. 1999). *M. acerina* was reported on wild growing caraway in Bulgaria (Vanev 1988). During the years of the study we could not find this pathogen in any region where the experiments were carried out.

In Bulgaria *Erysiphe cichoracearum* DC. was established on *C. carvi* and *E. heraclei* on many others *Apiaceae* hosts (Fakirova 1991). We have found only *E. heraclei* as causal agent of powdery mildew of caraway. *E. heraclei* was reported in Czech Republic (Odstrčilová & al. 2002), in Germany and Poland (Gabler & Machowicz-Stefaniak 2004).

Ondřej (1983) found 6 species belonging to genus *Ascochyta* on *Apiaceae* hosts. On caraway he reported *A. phomoides* and a new species *A. carvi*. Vanev & Bakalova (1991) established *A. phomoides* on *Aegopodium podagraria* as a new host plant for this fungus.

Odstrčilová & al. (2002), studying the fungi that contaminated the seeds of umbelliferous, reported many fungal species on caraway including *Alternaria* spp., *Ascochyta carvi*, *Botrytis cinerea*, *Erysiphe heraclei*, *Fusarium* spp., *Mycocentrospora acerina*, *Phomopsis* sp., *Sclerotinia sclerotiorum*, *Septoria carvi*, *Stemphylium botryosum*.

Septoria carvi Syd. has been found in both countries Germany and Poland but with greater importance for the second one (Machowicz-Stefaniak & al. 2003; Gabler & Machowicz-Stefaniak 2004). In Bulgaria neither *S. carvi* nor *S. umbelliferarum* Kalchbr. were established (Vanev & al. 1997).

The investigation on fungal diseases of caraway revealed that *Phomopsis diachenii*, *Erysiphe heraclei*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Ascochyta carvi*, *A. phomoides*, and *Stemphylium botryosum* were the most frequently found pathogens. Among them *P. diachenii* caused the most devastating and widely distributed disease.

Acknowledgements. Financial support by the Bulgarian National Science Fund (project B–1301) is gratefully acknowledged.

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Mycelial interactions within and between *Botrytis cinerea* and *Sclerotinia sclerotiorum* strains

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Abstract. *Botrytis cinerea* and *Sclerotinia sclerotiorum* cause diseases of many economically important crops. An investigation was undertaken on mycelial interactions within and between both fungi isolated from different host plants on potato dextrose agar. It was found that all interactions within *S. sclerotiorum* and some within *B. cinerea* were compatible showing contiguous growth of aerial mycelium. Both mycelial-free space and dark interaction line were observed as incompatible reaction between some of *B. cinerea* strains. Cultivated together both fungi are incompatible and no development occurred at the contact zone. The growth of *B. cinerea* was limited by *S. sclerotiorum*.

Key words: *Botrytis cinerea*, mycelial interactions, *Sclerotinia sclerotiorum*

Introduction

Botrytis cinerea Pers. : Fr. is a necrotrophic fungal pathogen that attacks over 200 different plant species (Elad 1997). The disease is manifested by necrotic areas with extensive fungal growth and sporulation giving characteristic appearance of grey mould. The related necrotroph *Sclerotinia sclerotiorum* (Lib.) de Bary affects more than 350 plant species causing important diseases known by more than 60 names, perhaps the most common is white mould (Purdy 1979; Bolton & al. 2006). Kohn & al. (1990) defined a plate system that allowed mycelial compatibility between strains of *S. sclerotiorum* to be determined. Using the same procedure the strains of this fungus were separated into mycelial compatibility groups (MCG) (Kohn & al. 1991; Ford & al. 1995; Durman & al. 2003). Similar technique was applied to study *in vitro* mycelial interactions among members of a soil microfungal community (Stahl & Christensen 1992). *B. cinerea* and *S. sclerotiorum* are soil inhabiting, have common host plants, similar requirements for resources and other niche overlaps. Their interactions are potentially important determinant of spatial distributions and functioning of their populations.

The aim of the investigation was to characterize strains of both fungi and to study the mycelial interactions within and between them.

Material and methods

Fungal strains were isolated from different plant parts of naturally infected cultural and wild growing hosts of *B. cinerea* and *S. sclerotiorum*. Potato dextrose agar (PDA) was used for their initial isolation and subsequent culturing and storage. Nine strains of *B. cinerea* included in the experiments were isolated from *Capsicum annuum* L. (*Bc*1), *Carum carvi* L. (*Bc*2), *Phaseolus vulgaris* L. (*Bc*3), *Triticum aestivum* L. (*Bc*4), *Anethum graveolens* L. (*Bc*5), *Chaerophyllum bulbosum* L. (*Bc*6), *Angelica pančičii* Vandas (*Bc*7), *Opopanax bulgaricum* Vel. (*Bc*8), and *Seseli annuum* L. (*Bc*9). The strains of *S. sclerotiorum* were isolated from *Capsicum annuum* (*Ss*1) and *Carum carvi* (*Ss*2, *Ss*3).

Strains were morphologically characterized on the basis of colony radial growth, colour, mycelium density and the following characteristics of mature sclerotia: number, arrangement, shape and size.

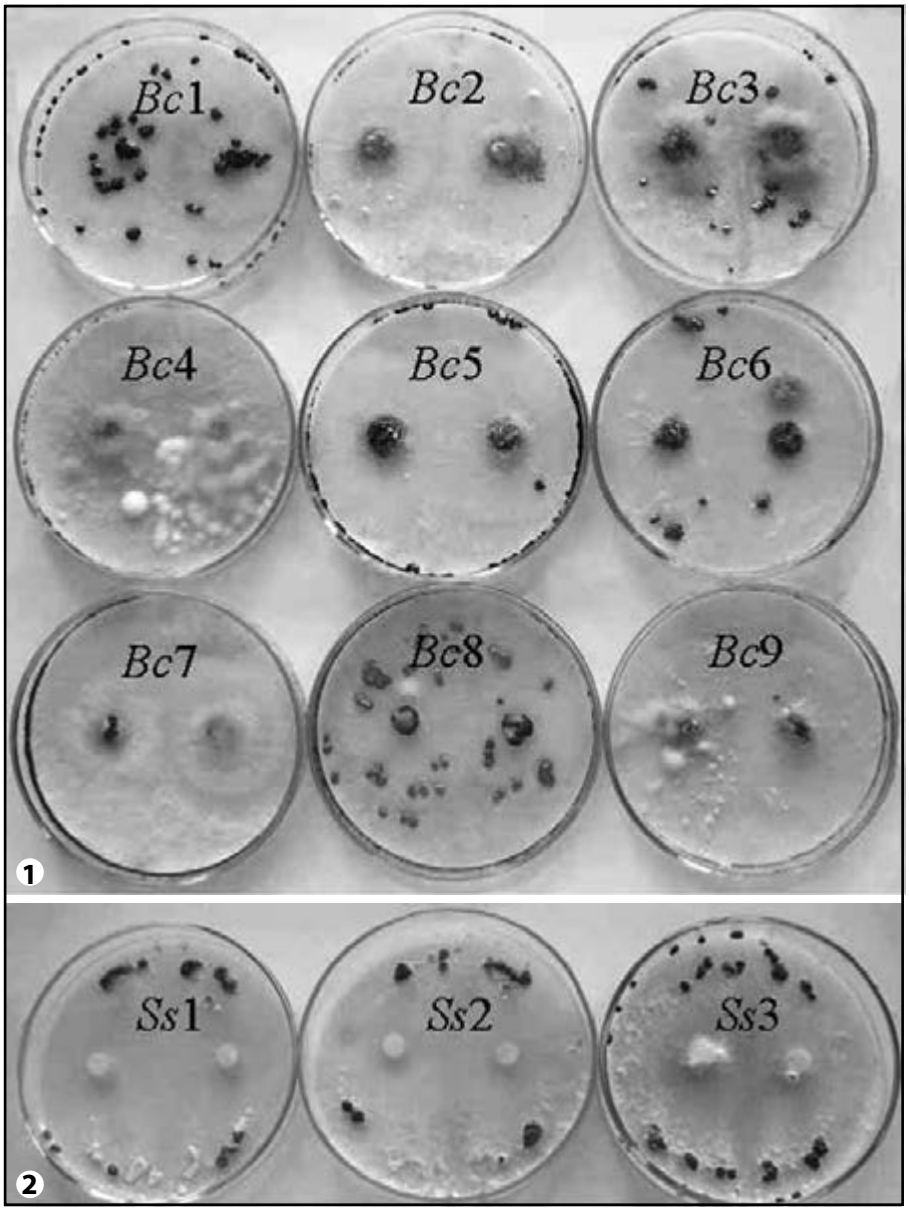
Mycelial compatibility was determined directly using the technique of Kohn & al. (1990). Each strain was grown on PDA for 5 days before pairing. For the pairing experiments 5-mm discs of mycelial inoculum removed from the growing colony margins were placed 3.5 cm apart on PDA in 9-cm petri dishes, one pairing per dish and incubated in the dark at room temperature. Strains were paired in all combinations – with itself and with each of remain-

ing ones. All treatments were replicated three times. Mycelial compatibility was macro- and microscopically recorded 4 and 14 days after inoculation. Pairings were scored as compatible when the two strains merged to form one colony with no distinct interaction zone and as incompatible when the two strains failed to grow together and a thin mycelial-free space remained between them or when a dark interaction line was observed at the zone of contact.

Results

In all combinations mycelium from paired discs grew through each other with no discernible reaction for the first 48 h. The colonies of *B. cinerea* and *S. sclerotiorum* showed similar morphology developing fast spreading white mycelium. *S. sclerotiorum* strains formed a white mass called sclerotial initials, mainly at the periphery and the center of the colony 5–6 days after inoculation, which melanized within 1–2 weeks. The mature sclerotia were round-shaped and variable in size, greater than 2 mm in diameter and were easily detached from the mycelium. *B. cinerea* produced more but smaller irregularly shaped sclerotia, arranged mainly at the periphery of the colony. Unlike *S. sclerotiorum* strains, sclerotia were enveloped in a hyphal weft and firmly attached to the subtending hyphae and agar medium. They melanized 2–3 weeks after inoculation. Besides sclerotia *B. cinerea* produced conidial sporulation. Two morphological types of *B. cinerea* were defined according to cultural aspects onto agar media: type with sclerotia – *Bc*1, *Bc*3, *Bc*5, *Bc*6, *Bc*8 and mycelial sporulating type – *Bc*2, *Bc*4, *Bc*7, *Bc*9 (Plate I, Fig. 1). Classifying all strains of *B. cinerea* into distinct groups was difficult.

Plate I



Figs 1–2. Self-self pairings showing compatible reaction on PDA 14 days after inoculation:
1, *B. cinerea*;
2, *S. sclerotiorum*.

All self-self pairings of *B. cinerea* and *S. sclerotiorum* were compatible (Plate I, Figs 1, 2).

After 4 days, pairs of *B. cinerea*, which were mycelially compatible, showed contiguous growth of white mycelium through the reaction zone. In incompatible combinations, no development of white aerial mycelium occurred. Both mycelial-free space and dark interaction line were observed. In the first case a clear, uncolonized area was left between mycelia at the zone of contact. In the second one pigmented incompatible line developed in those regions. Of the 9 strains of *B. cinerea* tested *Bc*1 was mycelially compatible only with itself and incompatible with all others showing dark interaction line with four of them (*Bc*2, *Bc*3, *Bc*4, *Bc*5) and mycelial-free space with the rest ones (*Bc*6, *Bc*7, *Bc*8, *Bc*9) at the zone of contact (Table 1, Plate II, Fig. 3).

Table 1. Mycelial interactions within *Botrytis cinerea* strains.

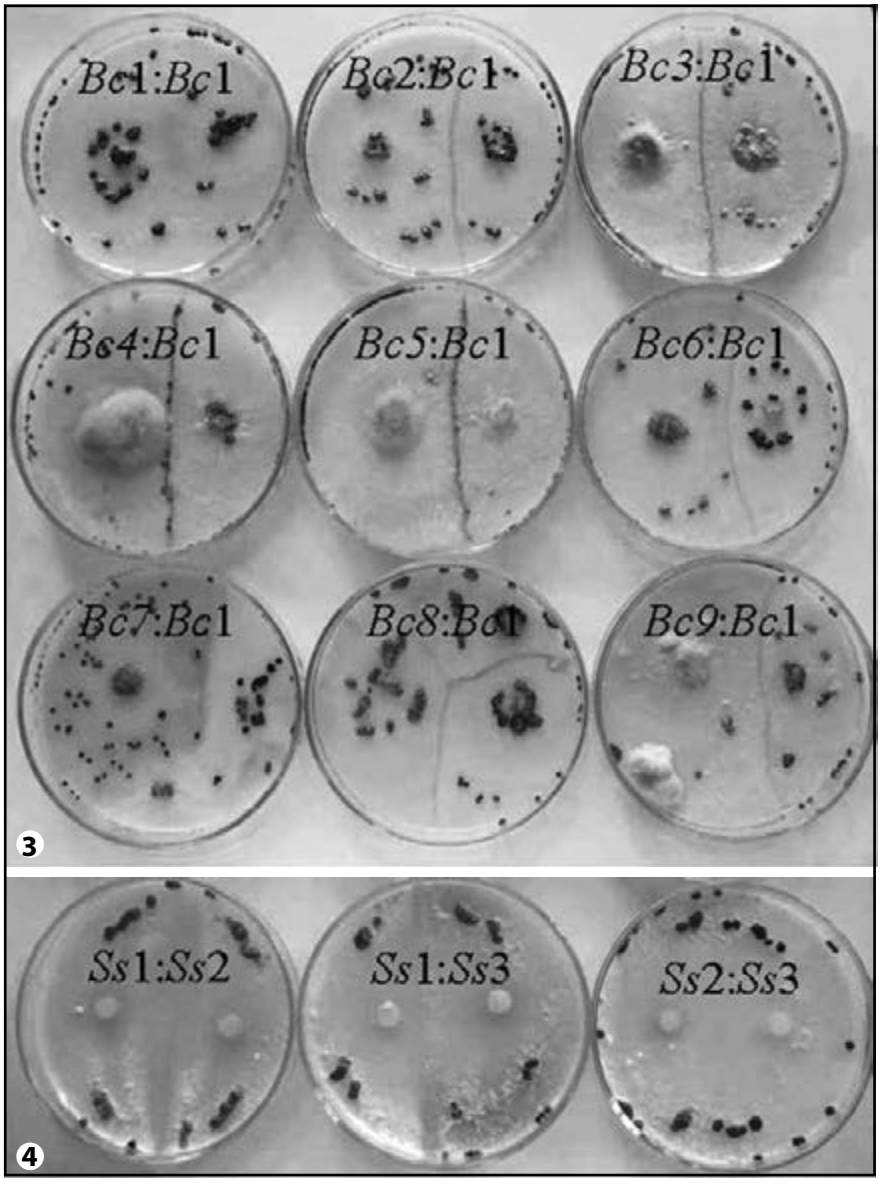
Strains	1	2	3	4	5	6	7	8	9
1	[a]C	[b]DIL++	DIL+++	DIL+++	DIL+++	[c]MFS	MFS	MFS	MFS
2	DIL++	C	C	DIL++	MFS	MFS	C	C	C
3	DIL+++	C	C	DIL+++	DIL+++	C	C	MFS	C
4	DIL+++	DIL++	DIL+++	C	C	C	DIL+	DIL++	DIL++
5	DIL+++	MFS	DIL+++	C	C	C	DIL+	DIL+	DIL++
6	MFS	MFS	C	C	C	C	MFS	MFS	MFS
7	MFS	C	C	DIL+	DIL+	MFS	C	DIL++	MFS
8	MFS	C	MFS	DIL++	DIL+	MFS	DIL++	C	C
9	MFS	C	C	DIL++	DIL++	MFS	MFS	MFS	C

Legend: [a]C = compatibility; [b]DIL = dark interaction line with different intensity +, ++, +++; [c]MFS = mycelial-free space.

The studied *S. sclerotiorum* strains showed mycelial compatibility. Hyphae intermingled without any reactions suggesting antagonism (Plate II, Fig. 4).

Invasive interactions were observed between *S. sclerotiorum* and *B. cinerea* with one mycelium partially replaced the other. The growth of all *B. cinerea* strains tested was limited by every one of the three strains of *S. sclerotiorum* and mycelial-free space appeared (Fig. 5).

Plate II



Figs 3–4. Mycelial interactions within *B. cinerea* and *S. sclerotiorum* strains on PDA 14 days after inoculation: **3,** Incompatible mycelial interactions of strain *Bc*1 with the rest strains of *B. cinerea*. Dark interaction line with *Bc*2, *Bc*3, *Bc*4 and *Bc*5 and mycelial-free space with *Bc*6, *Bc*7, *Bc*8 and *Bc*9 at the zone of contact; **4,** Compatible mycelial interactions of *S. sclerotiorum* strains.

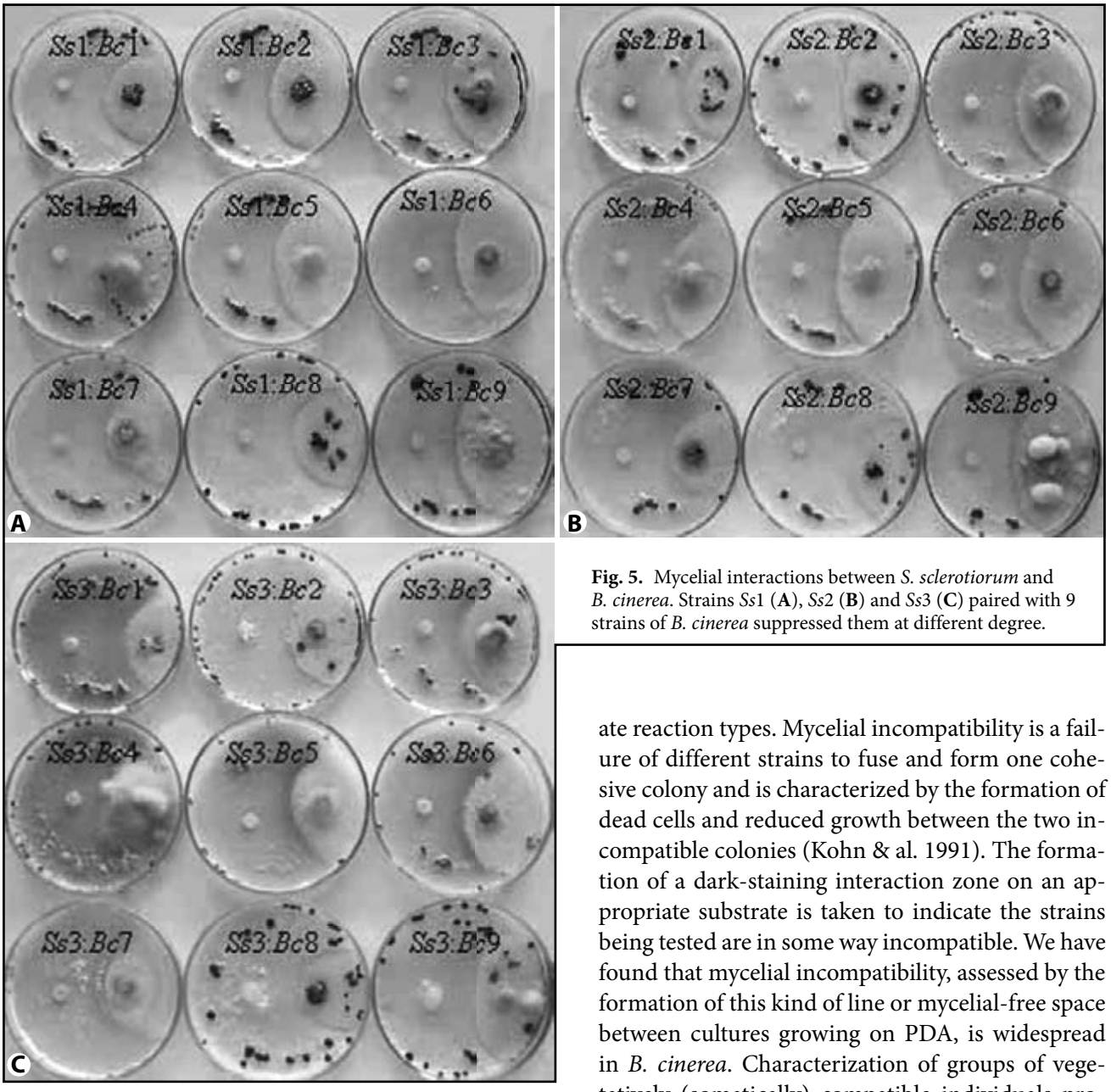


Fig. 5. Mycelial interactions between *S. sclerotiorum* and *B. cinerea*. Strains *Ss*1 (**A**), *Ss*2 (**B**) and *Ss*3 (**C**) paired with 9 strains of *B. cinerea* suppressed them at different degree.

Discussion

One of approaches used with filamentous fungi to investigate the ability of vegetative (somatic) hyphae of strains to interact examines the zone of contact between strains. The types of reaction between mycelia of fungal cultures give an indication of the degree of antagonism between them and probably of their genetic relationship. If hyphae of mycelia intermingle then the relationship is probably very close. Alternatively, when a clear, uncolonized area is left between mycelia, they are incompatible. Between these two extremes there are many intermediate reaction types. Mycelial incompatibility is a failure of different strains to fuse and form one cohesive colony and is characterized by the formation of dead cells and reduced growth between the two incompatible colonies (Kohn & al. 1991). The formation of a dark-staining interaction zone on an appropriate substrate is taken to indicate the strains being tested are in some way incompatible. We have found that mycelial incompatibility, assessed by the formation of this kind of line or mycelial-free space between cultures growing on PDA, is widespread in *B. cinerea*. Characterization of groups of vegetatively (somatically) compatible individuals provides a powerful approach for species subdividing into discrete populations in filamentous ascomycetous fungi (Caten 1972). The existence of mycelial incompatibility within *B. cinerea* strains indicates that hyphal fusions are not common in the *B. cinerea* populations and suggests presence of genetically isolated units of the species.

Kohn & al. (1990) examined mycelial incompatibility in 31 strains of *S. sclerotiorum* isolated from a variety of hosts and geographic areas and found that each of 21 strains were mycelially incompatible with all others, each representing a MCG made up of one strain. Among the 10 remaining strains there

were 4 MCGs, each group contained two to three strains. Ford & al. (1995) studying the mycelial compatibility of 6 strains of *S. sclerotiorum* resolved them into four MCGs; one group contained 3 strains and the other 3 belonged to separate groups. Among 140 strains Durman & al. (2003) distinguished 50 different MCGs, 27 of which consisted of two or more strains. Only few pairings scored as incompatible, showing reaction line, whereas most of the other incompatible pairings presented other discontinuities on the interaction zone. Kohn & al. (1991) suggested that MCGs represented genetically different individuals and each MCG was genotypically unique. Our strains showed mycelial compatibility and pairings in all combinations grew together and formed a confluent colony.

Stahl & Christensen (1992) studied *in vitro* interactions among 7 members of a soil microfungal community other than *S. sclerotiorum* and *B. cinerea*. All of interactions between two colonies of the same fungus resulted in some degree of self-inhibition in spite of the fact that both mycelia in intraspecific interactions were started as hyphal tips from the same stock culture and were presumably very similar, if not identical, genetically. In our experiments all strains of *B. cinerea* and *S. sclerotiorum* were compatible with themselves and self-self pairings merged to form one colony with no distinct interaction zone.

Growth of all *B. cinerea* strains tested was affected by the presence of *S. sclerotiorum* strains in paired cultures. *B. cinerea* colonies showed suppressed development at different degree by all three strains of *S. sclerotiorum*. The higher competitive activity of *S. sclerotiorum* against *B. cinerea* was reported by other authors (Machowicz-Stefaniak 1998).

These preliminary results were obtained with a restricted collection of strains and must be confirmed. Nevertheless, the biological experimental procedures applied here could be of particular interest to characterize the strains and to study the mycelium interactions between them. Mycelial incompatibility exhibited by *B. cinerea* strains suggested that genetic heterogeneity exists within the species.

Acknowledgements. Financial support by the Bulgarian National Science Fund (projects B–1301 and B–1515) is gratefully acknowledged.

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Activity of pectinase and cellulases in submerged cultures of *Claviceps purpurea*

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Abstract. The pharmacological effects of ergot alkaloids, the discovering of new compounds and the difficulties in synthetically production have determined the intensification of researches about *Claviceps purpurea* who has the capacity to synthesize these alkaloids. *In vivo*, at the early stage of infection (e.g., 3 days after inoculation), the degradation of pectin was greater than that of cellulose in the cell walls of the same infected host tissues, indirectly suggesting that the pectinases may be secreted earlier or exert higher activities than cellulases. Our study refers to the activity of a pectinase and enzymes of cellulase complex in submerged cultures of *C. purpurea*.

Key words: cellulases, *Claviceps purpurea*, pectinases, phytohormones, vitamins

Introduction

Between the producers of biological active substances, *Claviceps purpurea* (Fr.) Tul., a ubiquitous pathogen of cereals and grasses, causing Ergot disease, is considered as having one of the most complex biological and biochemical behaviours. The fungus study presents, out of theoretical importance, a very great practical interest, because the ergot alkaloids and their derivatives are potentially therapeutic agents for Parkinson malady, achromegaly, amenorrhea–galactorrhea, suppression of postpartum lactation, treatment of breast cancer and possibly cancer of the prostate, migraines and the symptoms associated with them, orthostatic circulatory perturbations, senile cerebral insufficiency, hypertension as well as other affections in which their antibacterial, hypolipemic and immune-regulator effects are studied (Desai & al. 1983; Surdu & al. 2005).

In vivo, this pathogen is well equipped to degrade the monocotyledonous cell wall, although the lack of detectable host defence reactions and the long survival of the host cells indicate that the secretion of cell-wall-degrading enzymes is well controlled (Kang & Buchenauer 2000; Tudzynski & Scheffer 2004). Because of the composition of cell walls, it is possible that some exoenzymes to be involved in host tissue colonisation, with the role to degrade the glucurono-arabino-xylans, the pectins, and the cellulose.

This paper presents the results of our study on activity of some hydrolases (pectinase, enzymes of cellulase complex), at *C. purpurea* strains of different alkaloid type, in conditions of submerged cultivation: control cultures and cultures with addition of growth regulators, vitamins, and phytohormones. Some vitamins from B complex and certain phytohormones are presented *in vivo* in the complex medium offered by the host ovary to fungal parasite.

Material and methods

The analysed biological material is represented by the supernatant of submerged cultures of *C. purpurea* strains. The strains were encoded depending on the predominant alkaloid type: T1 and T2 – ergotamine type strains; S1 and S2 – ergocristine type strains; P1 and P2 – ergocryptine type strains**.** The samples were collected 48 h, 52 h, and 56 h after medium inoculation with conidia obtained on agar medium. These intervals were chosen to register the modifications spe-

cific for conidia formation and conidia germination. To evidence the influence of some growth regulators on the activity of studied enzymes, the culture medium was supplemented with vitamin solutions (biotin – Bi, folic acid – FA, nicotinic acid – NA), and phytohormones (indole-3-acetic acid – IAA, gibberellic acid – GA).

Pectins are large polysaccharide molecules, made up (mainly) of chains of several hundred galacturonic acid residues. Enzymes from this pectinase group include polygalacturonases, pectin methyl esterases, and pectin lyases. These pectinase enzymes act in different ways on the pectins found in the primary cell walls and in the middle lamella.

In this paper, we studied the polygalacturonase activity (EC 3.2.1.15). The principle of the used method consists in quantification of galacturonic acid resulted after the hydrolytic action of the polygalacturonase. In strong acid medium and at warm, some furfurolic acids are formed, which, in the presence of anthrone, give a specific colour (its intensity is proportional to the enzyme activity), spectrophotocolorimetrically determinable (Spiro 1966).

The polygalacturonase activity was expressed in international units (U):μmols galacturonic acid decomposed per hour, in optimum reaction conditions, per 1 ml analysed biological material.

The studied enzymes of cellulase complex are endoglucanase (EC 3.2.1.4), that splits the intern bonds of cellulose macromolecule, exoglucanase (EC 3.2.1.91), that acts on cellulose by splitting cellobiose units from unreducing extremity of strand, and cellobiase (EC 3.2.1.21), that catalyses the hydrolysis of 1,4-β-glucosidic bonds. The synergetic action of these enzymes leads to the split of native cellulose.

In principle, the method for the cellulase determination consists in evaluation of reducing glucides resulted from the action of enzymes of cellulase complex on specific substrate (Pettersson & Porath 1966). The reducing glucides were determined with 3,5-dinitrosalycilic acid, which is reduced to 3-amino-5-nitrosalycilic acid, orange coloured. The colouration intensity, colourimetrically determined, is proportional with enzyme activity (Miller 1959).

For the calculation of the obtained results, a standard curve was constructed, using a glucose solution of known concentration. The specific enzyme activities were expressed in mg glucose reported to protein amounts from supernatants of analysed culture media.

Results and discussion

In conditions of submerged cultivation, the ergoline alkaloid producing strains belonging to *C. purpurea* species have the longer cultivation period, comparatively with other microorganisms producing secondary metabolites. To study the pectinase and the enzymes of cellulase complex from culture media, in the conidia formation and conidia germination stages, the interval from 48 h to 56 h was established for biochemical determinations.

The pectinase activity displays an increase tendency in tested control strains, excepting S2 ergocristine strain, with spontaneous flora ascendance and the single strain adapted on Ergo type rye just one year (Fig. 1). Generally, in dynamics, the enzyme has the most reduced activities at 52 h, excepting the T1 ergotamine strain and P1 ergocryptine strain, in which the smaller values of enzymatic activity were registered in the first 48 h.

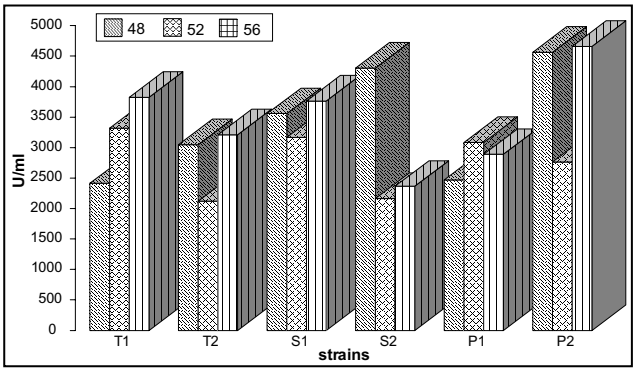

Fig. 1. The dynamic of pectinase activity at control *C. purpurea* strains.

The addition of indole-3-acetic acid in culture medium determined a marked increase of pectinase activity in ergotamine strains, but moderate in those of ergocryptine type (Fig. 2). On the contrary, the gibberellic acid had a diminished action comparatively to indole-3-acetic acid, in the case of ergotamine strains, but superior, both to these and to controls, in the case of ergocryptine strains.

At all studied strains, especially in 52 h – 56 h interval, the biotin addition induced significant increases of pectinase activity (Fig. 3). In ergocryptine strains, large variations of enzyme activity were registered.

The folic acid addition (Fig. 3) had no effect on pectinase activity of ergocryptine strains, but in ergotamine and ergocristine strains, this compound

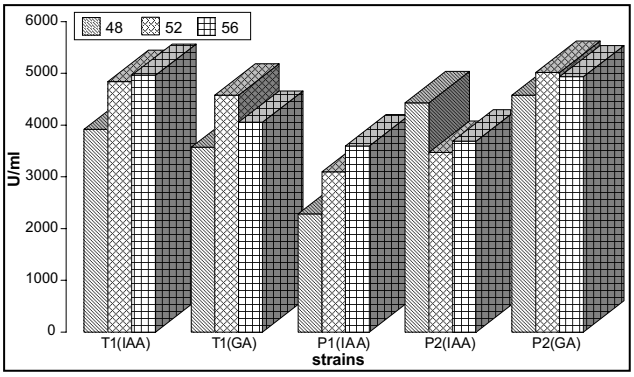

Fig. 2. The dynamic of pectinase activity at *C. purpurea* strains cultivated on medium with **indolilacetic acid** and **gibberellic acid** addition.

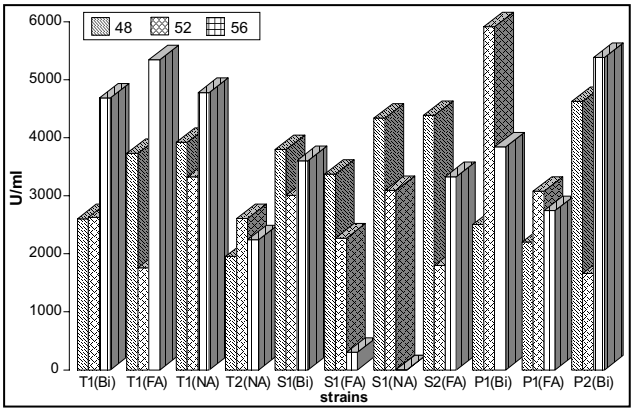

Fig. 3. The dynamic of pectinase activity at *C. purpurea* strains cultivated on medium with **biotine**, **folic acid** and **nicotinic acid** addition.

determined manifestations similar to those induced by biotin.

Regarding nicotinic acid, its action is different in the ergotamine strains (Fig. 3). The T1 strain, with ergotamine ascendance, reacts to vitamins action, in general, and especially to nicotinic acid, by higher enzymatic activities. A similar behaviour is observed for S1 strain, with common origin with the two ergotamine strains, which, in their turn, have an ergocryptine ascendance. Therefore, it is a manifestation of the tendency of origin strains.

The investigated strains are generally stimulated by the presence of growth stimulators, vitamins or hormones, to produce pectinases. The maximum enzymatic activity amplitude is at certain culture ages, depending on the strain characteristics as well as on the predominant alkaloid type of the strains.

The analysis of the results regarding extracellular endoglucanase activity of control strains evidenced that the enzyme is inactive in ergotamine strains and also in S1, strain with ergotamine ascendance (Fig. 4). The enzyme is present in culture liquid of the other strains and displays similar dynamics in S2 and P2 strains, with a general tendency to activity decrease.

The addition of growth stimulators determined the endoglucanase activation at ergotamine strains. A decrease of enzyme activity was registered for endoglucanase dynamics, in the investigated time interval (Fig. 5). For ergocryptine strains, the indole-3-acetic acid addition determined a diminution of endoglucanase, while the gibberellic acid had a positive influence on strain enduglucanase production, especially at 52 h culture age.

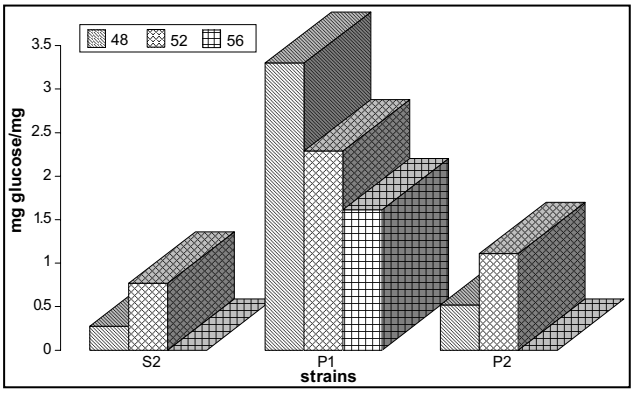

Fig. 4. The dynamic of endoglucanase activity at control *C. purpurea* strains.

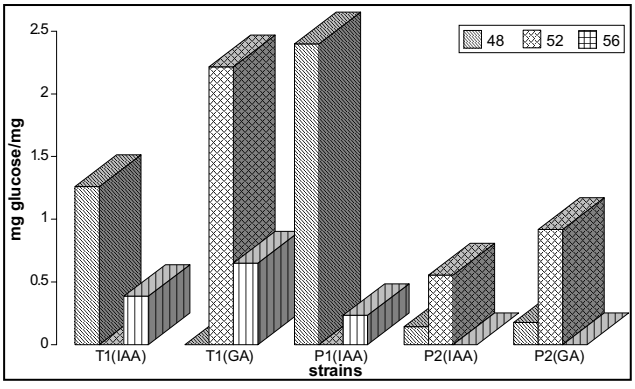

Fig. 5. The dynamic of endoglucanase activity at *C. purpurea* strains cultivated on medium with **indolilacetic acid** and **gibberellic acid** addition.

In the case of vitamins, their addition produced sporadically endoglucanase activation, without significant surpasses of the control values (Fig. 6).

The presence of endoglucanase only in few strains of control variant and the increase of strain number in the case of vitamins or hormones addition sug-

gest that only certain strains, in specific condition, have the capacity to produce this enzyme of cellulase complex.

At investigated control strains, exoglucanase is present in small quantities. One exception is the T2 strain that at the age of 52 h has an approximately 4 times higher activity than the other variants (Fig. 7).

The indole-3-acetic acid addition determined a decrease of exoglucanase activity, evidenced in 52 h – 56 h interval, at T1 and P2 strains; respectively in the first interval, at P1 strain (Fig. 8). If in culture medium the gibberellic acid is added, the exoglucanase is active in 48 h – 52 h interval, at T1 and P2 analysed strains.

The added vitamins allow the ergotamine and ergocryptine cultures to produce exoglucanase in significant higher quantities than the ergocristine strains (Fig. 9). The stronger exoglucanase stimulation appeared in the case of folic acid addition. This compound induced the T1 and P1 strains to produce this enzyme during whole-investigated interval. Also, the T2 and S2 capacity is amplified, such as these strains present maximum exoglucanase at 52 h.

The capacity to produce exoglucanase, evidenced in tested strains, is influenced by the addition of some compounds which, depending on their nature, can efficiently act at different ages, for a short period.

Cellobiase is present in all studied control strains (Fig. 10). At T2 and S2 strains great activities of this enzyme were registered at 52 h.

The indole-3-acetic acid addition reduced the cellobiase activity at T1 strain, while the gibberellic acid addition determined an inverse behaviour (Fig. 11). The presence of the indole-3-acetic acid in culture medium stimulated the cellobiase activity in P1 strain.

The vitamins have a positive influence on cellobiase activity, for all studied strains, in the first cultivation period (Fig. 12).

The biotin stimulated the enzyme activity especially at T1 and S1 strains, whereas the nicotinic acid had the most evident influence, but on short time, in investigated interval.

The vitamins influence the cellobiase biosynthesis. This enzyme is present and active in control cultures, for all investigated alkaloid types.

Regarding the cellulase complex activity, the strains have a different reaction to the effectuated additions, fact that leads to two suppositions: or the enzymes exist and are activated in certain conditions, or they are synthesized at certain ages of the growth and development cycle of *C. purpurea* cultures.

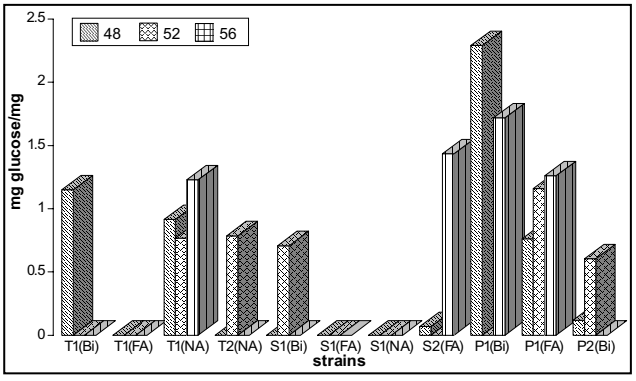

Fig. 6. The dynamic of endoglucanase activity at *C. purpurea* strains cultivated on medium with **biotine**, **folic acid** and **nicotinic acid** addition.

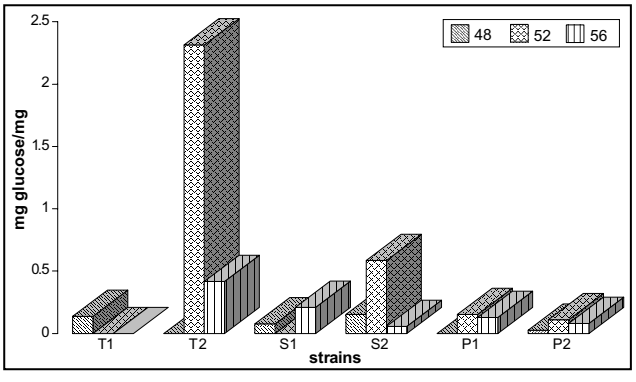

Fig. 7. The dynamic of exoglucanase activity at control *C. purpurea* strains.

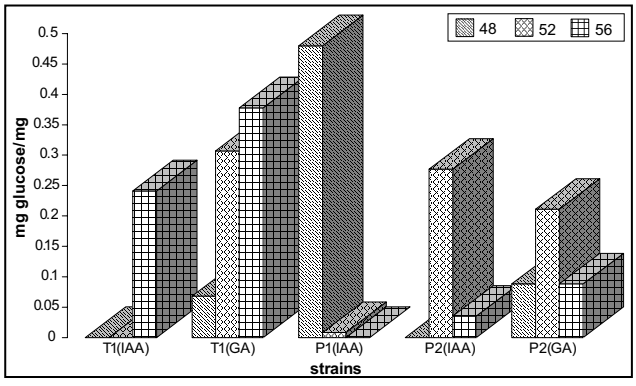

Fig. 8. The dynamic of exoglucanase activity at *C. purpurea* strains cultivated on medium with **indolilacetic acid** and **gibberellic acid** addition.

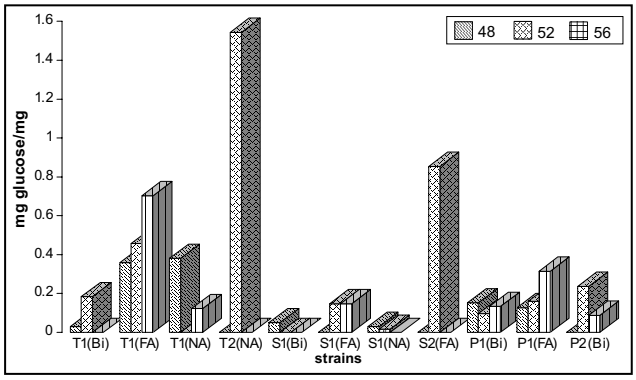

Fig. 9. The dynamic of exoglucanase activity at *C. purpurea* strains cultivated on medium with **biotine**, **folic acid** and **nicotinic acid** addition.

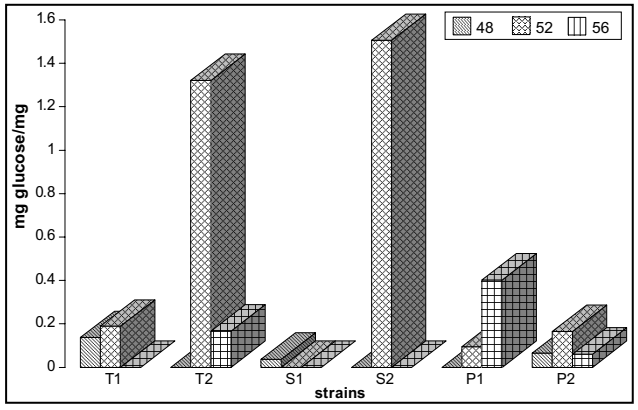

Fig. 10. The dynamic of cellobiase activity at control *C. purpurea* strains.

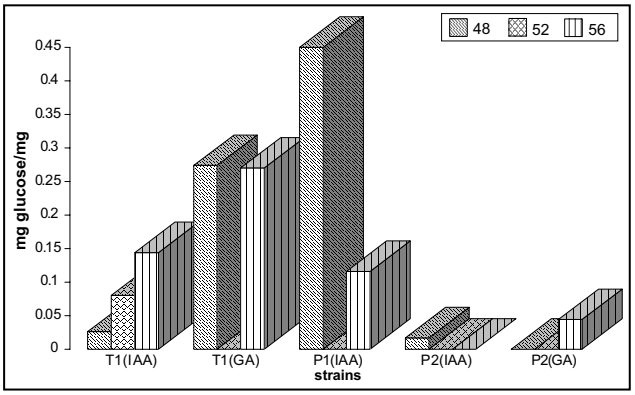

Fig. 11. The dynamic of cellobiase activity at *C. purpurea* strains cultivated on medium with **indolilacetic acid** and **gibberellic acid** addition.

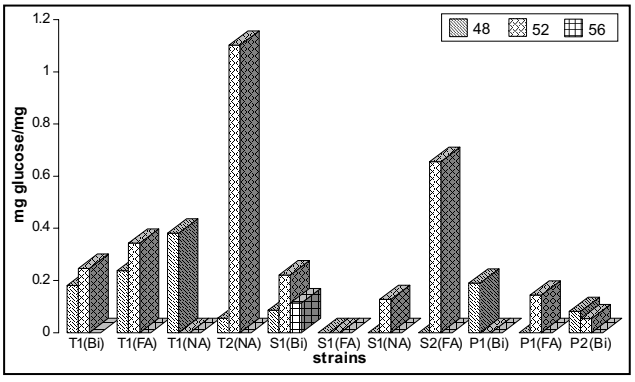

Fig. 12. The dynamic of cellobiase activity at *C. purpurea* strains cultivated on medium with **biotine**, **folic acid** and **nicotinic acid** addition.

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The dynamics of soluble proteins and proteinases in submerged culture of *Claviceps purpurea*

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Abstract. The cultures belonging to *Claviceps purpurea* strains of different alkaloid type have a specific growth rhythm, evidenced by their development stage (mycelium, conidia, mycelium and conidia), at a certain age, in identical culture conditions. The protein amount varies in mycelium, conidia and liquid culture media, depending on strain age and alkaloid type of the strain. Exocellular proteinase has a reduced activity in the case of ergotamine and ergocristine strains; the ergocryptine strains constantly have, in liquid culture, proteinases well represented by their activity.

Key words: *Claviceps purpurea*, proteinases, soluble proteins, submerged cultures

Introduction

During host infection, the plants and the pathogens secrete a variety of exocellular proteins, some of which having a contribution to pathogenesis. These proteins include a number of hydrolytic enzymes that are produced during different stages of infection, such as cutinases, pectinases, produced by phytopathogen agents (Oeser & al. 2002; Li & al. 2003), and proteases, produced by pathogen microorganisms (Naglik & al. 2003). The exocellular proteins can play a role in the molecular dialogue established between parasite and host and contribute to their interrelationship (Esquerré-Tagayé & al. 2000). Many of phytopathogen microorganisms produce exocellular proteinases, which, together with other enzymes, have an important role in pathogenesis (Valueva & Mosolov 2004).

This paper presents the dynamics of proteinase activity and of protein amount in submerged cultures of *Claviceps purpurea* (Fr.) Tul. strains of different alkaloid type (ergotamine, ergocristine, ergocryptine). The respective inoculi are used for artificial infection of rye ears, the aim of this procedure being the sclerotia production.

Material and methods

The biological material is represented by *C. purpurea* sclerotia, harvested from *Ergo* rye cultivar. The biological material was selected from Microbiology Department Collection of Biological Research Institute of Iaşi. The strains subjected to biochemical determinations were noted depending on predominant alkaloid type: T203, T217, T224 – ergotamine producing strains; P204, P209 – ergocryptine producing strains; and S203, S219 – ergocristine producing strains. The asepsized fragments of sclerotia belonging to the three alkaloid types were placed on T2 agar medium (Strnadová & Kybal 1974). The cultures were incubated at 28 °C, for 18–21 days. To obtain submerged cultures, the colonies formed on agar media were inoculated in liquid medium (Wack & al. 1983). The flasks were 3–5 days incubated, at 24 °C, under stirring.

The biochemical determinations were effectuated at 48 h, 52 h, and 56 h, after conidia inoculation in culture media. It is accepted that two or three cell cycles take place, and the chosen intervals are those that precede and follow the sporulation.

In identical conditions, the cultures have a proper growth rhythm. The sporulation is not synchronous, and the mycelium capacity to form conidia also is dif-

ferent. In certain cases (T224, P204, P209), the conidia layer separated by centrifugation was very thin, such as the biochemical analyses were effectuated on mycelium with a poor conidial charge. In other situations (T203, S203, S219), the conidia amount was predominant, while the mycelium quantity was negligible.

The separation of conidia from mycelium was realized by centrifugation. At the top of test tubes, in a variable size layer, the conidia were disposed, whereas the mycelium at the bottom of test tubes was separated as sediment. The mycelium, conidia and culture liquid were subjected to biochemical determinations.

The protein extraction from mycelium and conidia was realized with Na_2HPO_4 buffer, pH=4, and to quantify the soluble protein, the Lowry method was used (Lowry & al. 1951). The protein amount from mycelium and conidia was expressed in g% dry matter, and in mg/ml in the case of culture liquid. The Kunitz method was used to determine the proteinase activity (Kunitz 1947). The specific activity of these enzymes was expressed in μmol tyrosine/g protein, for mycelium and conidia, and in μmol tyrosine/mg protein, for culture liquid.

Results and discussion

The stages of the growth process – the conidia germination, the vegetative growth, the conidia forming – are accompanied by variation of protein level. Thus, during germination, the proteosynthesis rate increases and a greater quantity of protein is formed, whereas the sporulation is characterized by relatively constant values of the proteins (Carlile & Watkinson 1994). Taking into consideration these aspects, the analysis of results regarding the mentioned biochemical parameters is realized in relation with the growth particularities of *C. purpurea* submerged cultures, belonging to the strains quantitatively and qualitatively characterized by their capacity to produce specific alkaloids.

In some species of *Claviceps*, a marked increase of proteinase activity was registered during conidia formation, and a decrease during their germination; also, some exocellular proteinases are more active than the endocellular ones (Rehacek & al. 1974). Kregar & al. (1986) observed, after the study of extra- and intracellular proteinases from strains of *C. purpurea* grown in submerged culture, a maximum of intracellular proteinase activity at 6 days after cultivation, whereas the exocellular proteinase activity continued to increase on whole fungus growth period. In ergotamine strain mycelium, the dynamics of proteinase activity is similar (Fig. 1), with ample variations in T217 strain, and more reduced in T224. The maximum enzymatic activity was registered at 48 h (11.078 μmol Tyr/g protein, for T217), and at 56 h (5.548 μmol Tyr/g protein, for T217). In P204 ergocryptine strain, an increase of this enzyme was noted, at the same time with culture ageing.

The dynamics of conidial proteinase activity (Fig. 2) varied depending on culture age and strain alkaloid type. In conidia of ergotamine and ergocristine strains, the enzymatic activity is reduced for all intervals, with the exception of S219 strain, at 48 h, which has an intense enzymatic activity – 23.792 μmol Tyr/g protein. Between all alkaloid types, it seems that P209 ergocryptine strain has a maximum proteinase activity at 48 h – 32.43 μmol Tyr/g protein. After this moment, the enzymatic activity diminishes at the same time with culture ageing. The maximum proteinase activity of this strain can be explained by the mixture of conidia traces and mycelium of tested biological material.

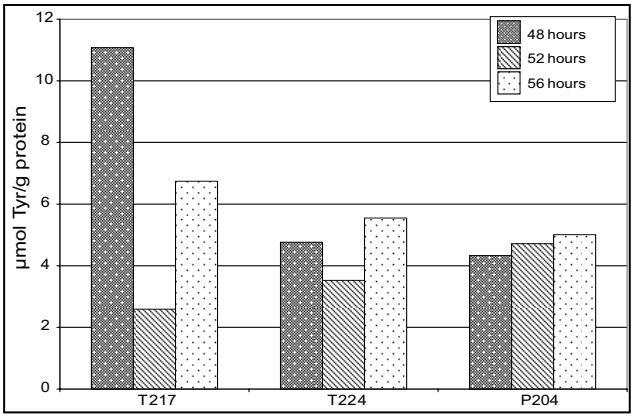

Fig. 1. Proteinase activity in *C. purpurea* mycelium.

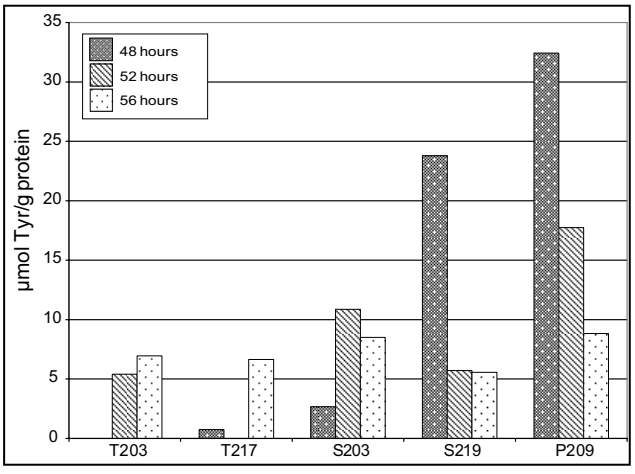

Fig. 2. Proteinase activity in *C. purpurea* conidia.

The proteinase activity is different in the culture liquid of the different alkaloid strains (Fig. 3). At ergotamine strains, the enzymatic activity is completely different. Thus, in T203 strain, the enzymatic activity registered a marked decrease, from 48 h to 52 h (from 0.017 µmol Tyr/mg protein to 0.002 µmol Tyr/mg protein), moment after which the activity significantly increased and exceeded the value registered at 48 h. In T224 strain, the proteinase activity progressively increased from the first to the last interval of analysis, so that the maximum value is noted at 56 h (0.027 µmol Tyr/mg protein). For the T217 strain, the enzymatic activity diminished from 48 h to 56 h, when its value was zero.

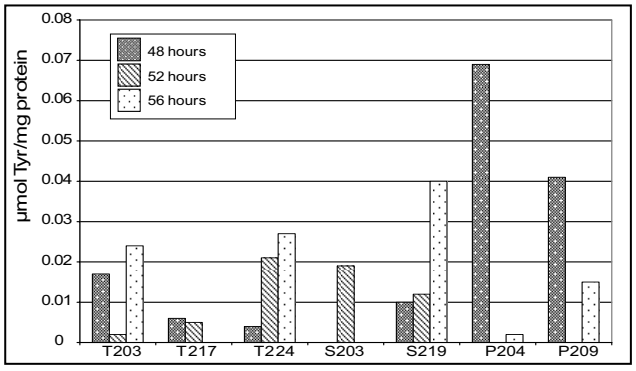

Fig. 3. Proteinase activity in *C. purpurea* culture liquid.

The S203 ergocristine strain displayed proteinase activity only at 52 h, at the other two moments the enzymatic enzyme being absent. For S219 strain, it is visible a relatively constant enzymatic activity at 48 h and 52 h, and a significant increase at 56 h (0.04 µmol Tyr/mg protein), value almost double comparatively with ergotamine strains in the same moment of determination.

The ergocryptine strains have an identical proteinase activity: at 48 h this activity is relatively high (0.069 µmol Tyr/mg protein, for P204 strain, and 0.041 µmol Tyr/mg protein, for P209 strain), at 52 h it is absent, while at 56 h the enzymatic activity increases, having a value of 0.002 µmol Tyr/mg protein, for P204 strain, respectively 0.015 µmol Tyr/mg protein, for P209 ergocryptine strain.

The T217 and T224 ergotamine strains have an identical behaviour regarding protein biosynthesis at the three time intervals (Fig. 4). The maximum quantity for both strains was obtained at 52 h after inoculation (18.43 g % dry matter, for T217 strain, and 14.00 g % dry matter, for T224 strain). For T217 strain, the obtained value is explained by the intense germination of conidia that, in this moment, can not be separated from mycelium.

The dynamics of protein biosynthesis in P204 ergocryptine strain (Fig. 4) is similar to that of ergotamine strains, namely the maximum value is registered at 52 h, too (10.74 g % dry matter); however, this value is inferior to those obtained for ergotamine strains. The P209 ergocryptine strain had a specific behaviour that is, at 52 h, the protein amount diminished comparatively with the first 48 h after inoculation and registered the maximum value at 56 h (6.51 g % dry matter). Generally, the mycelial protein level is higher in ergotamine strains, at 48 h and 52 h.

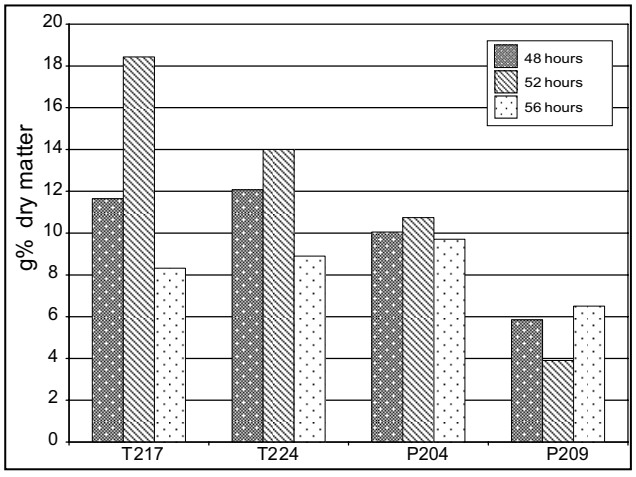

Fig. 4. Protein amount in *C. purpurea* mycelium.

In literature is mentioned that the conidia formation is accompanied by the decrease of protein amount (Carlile & Watkinson 1994), fact evidenced by our results in the case of T217 strain. In this strain, the conidia production was extremely small at 52 h, reason for which the protein determination was made only in mycelium containing conidia traces, therefore the value is enough high (Fig. 5). Different from the results obtained for mycelium, in conidia of ergotamine strains, the maximum protein amount was registered at 48 h after inoculation (11.68 g % dry matter, for T217 strain, and 11.72 g % dry matter, for T224 strain).

In S203 strain, the protein amount displayed ample variations, the maximum value being registered at 56 h after inoculation (24.2 g % dry matter). The other analysed ergocristine strain presented a diminution of protein level, in direct relation with the culture ageing.

In liquid culture medium (Fig. 6), the protein amount displayed marked variations, both between the three alkaloid types and in the strains belonging to

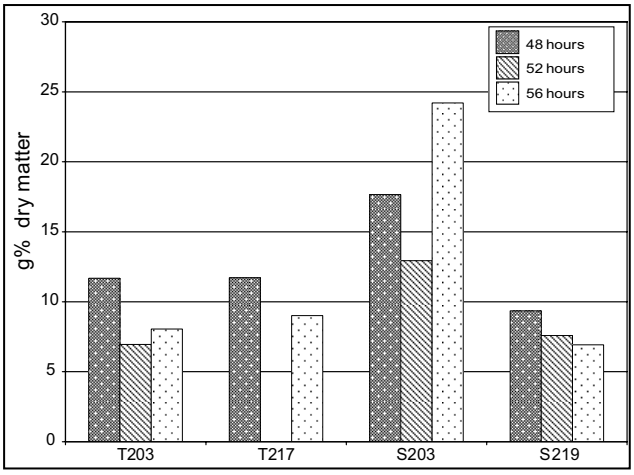

Fig. 5. Protein amount in *C. purpurea* conidia.

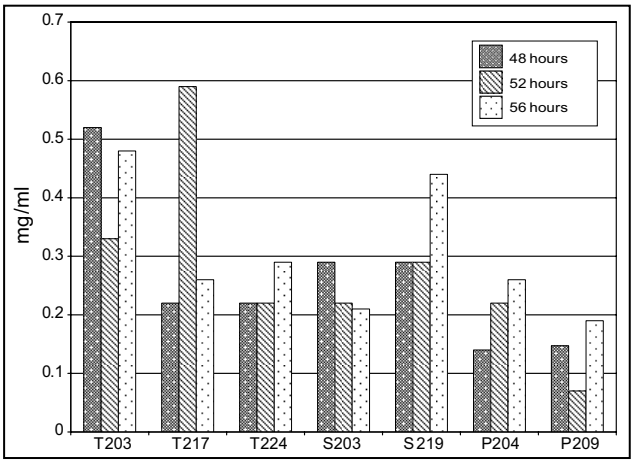

Fig. 6. Protein amount in *C. purpurea* liquid culture medium.

the same alkaloid type. In T203 ergotamine strain, the dynamics of exocellular protein biosynthesis is similar to that of conidia, but in T217 strain the profile is the same with that of the mycelium. For T224 ergotamine strain, at 48 h and 52 h, the protein level remains constant, and then it increases at 56 h after inoculation.

The two analysed ergocristine strains have at 48 h the same protein quantity (0.29 mg/ml), value which decreases at S203 strain in the next two intervals, but increases at S219 strain (0.44 mg/ml, at 56 h).

In the ergocryptine strains, the exocellular protein levels are identical at 48 h, moment after which the protein quantity increases (P204) or decreases (P209), at 52 h; at 56 h, the registered values were 0.26 mg/ml, for P204, respectively 0.19 mg/ml, for P209 strain.

As conclusions, we can sustain that the analysed *C. purpurea* strains have a proper biosynthetic capacity, determined by the expression of different genetic information of each strain.

The intracellular proteinase activity of mycelium and conidia presents maximum values especially at 48 hours from inoculation. The exocellular proteinase activity is reduced in ergotamine and ergocristine strains, but it is enough well represented in ergocryptine strains.

The protein amount of mycelium, conidia and liquid culture depends on the culture age and the alkaloid type of *C. purpurea*. The protein amount is greater in the mycelium of ergotamine strains than in that of ergocryptine strains; the exocellular protein has an individual dynamics adequate of each alkaloid type.

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Nucleic acids content of conidia and bioproductive features of some *Claviceps purpurea* (*Clavicipitaceae*) strains

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Abstract. The presence of *Claviceps purpurea* ergot fungus induces important modifications in the rye metabolism. 6–10 days post infection the conidia appear, being protected in the honey-dew. Samples of honey-dew have been prelevated from infected rye ears, 14 days age. We made analyses on conidia from agarised medium and from submerged cultures. The DNA and RNA level varies depending on alkaloid type of strains. Some correlations were established between the particularities of alkaloid biosynthesis and the nucleic acids content.

Key words: *Claviceps purpurea*, conidia, ergot alkaloids, nucleic acids, sclerotia production

Introduction

The *Claviceps purpurea* (Fr.) Tul. strains are interesting from the theoretical point of view, because of some less studied aspects of the biology of this species, but also from the practical point of view. From the economical point of view, the *C. purpurea* species is in attention for the losses that are produced in the rye cultures, not so much because of the rye production decrease but because the infection with sclerotia reduces much its quality, through the presence of ergot alkaloids which determine noxious effects on the central nervous system of mammals. Moreover, manifests of ergotised rye made bread were present in the Medieval Ages, the epidemic being known as The fire of St. Anthony causing death or invalidism of thousands of people.

On the other side, these alkaloids are exploited on a large scale for their pharmacological value (Taber 1985; Tudzynski & al. 2001; Keller & Tudzynski 2002). Because of the structural homology with the neurotransmitters of serotonin and dopamine type, the ergot alkaloids act as potentiators or antagonists at the situs of this biogenic amines, finding applications in treatment of a large variety of clinical conditions (post partum haemorrhage, headaches, senile cerebral insufficiency, Parkinson disease). Studies show that the pharmacological activities of the ergot alkaloids depend on the identity of the groups that give specificity to the compounds and on the bond state between C_9 – C_{10} (Floss 1973).

In the last decades both cases have kept a constant interest on the fungus, because of the development of a new defence strategy against this fungus and of the strain improvement for biotechnological reasons need a detailed knowledge of the fungus biology, physiology and genetics.

Kobel & Sanglier (1986) sustain that the conidiogenesis and alkaloid biosynthesis necessary genetic information normally exist simultaneously. But this two processes take place in different medium conditions and for this reason are rarely observed at the same time. The genic determinants expression is not taking place at the same time. The favourable conditions for the sphacelial developing are not proper for the alkaloid biosynthesis. The ergot alkaloids synthesis is a complex process, that involves a large number of factors and phases. There exists a large quantity of detailed informations regarding the biochemical aspects of the ergot alkaloid biosynthesis way, there is a restricted knowledge of the participating genes.

We consider that the bioproductive characteristics of the *C. purpurea* strains are influenced by the conidia produced by mycelium *in vivo* or *in vitro.* We decided to evaluate the nucleic acids quantity which is found

in the honey-dew protected conidia formed on the rye ears after its artificial infection, as well as in the conidia formed on agarised medium and in the submerged cultures, used for the host plant artificial infection.

Material and methods

From the sclerotia fragments, after their sterilizing and placing on the Petri plates with T_2 agarised medium (Strnadová & Kybal 1974) we obtained *C. purpurea* colonies. The conidia formed on the surface of some 24–28 days old colonies were harvested and analysed. Other colonies were inoculated in liquid medium (Wack & al. 1983), in Erlenmeyer flasks. The submerged cultures were incubated at a temperature of 24 °C in stirring conditions. The samples were prelevated at 48 h, 52 h, and 56 h old cultures.

Some submerged cultures were used as inoculi for the artificial infection of the rye ears. The conidia formed by the parasite are protected by a honey-dew. Drops are eliminated on the floral surface. The samples were collected 12–14 days from the rye ears infection.

The conidia formed in the submerged cultures or those present in the honey-dew were separated through centrifugation.

The nucleic acids were analysed by spectrophotometrical method, based on reaction of ribose with orcinol, in the case of RNA determination, and on reaction of deoxyribose with diphenylamine, for the quantification of DNA (Herbert & al. 1974).

For the characterization of strains alkaloidal biosynthesis potential, each sclerotia half was analysed for the total alkaloid quantity and the ergopeptid alkaloid types determination.

For the determination of the total alkaloid amount, the method based on alkaloid extraction with a methanolic solution of tartric acid and extract purification with zinc acetate was used. The extract reacts with the sulfuric solution of para-dimethyl-aminobenzaldehyde, forming a blue compound, measurable at photocolorimeter. The alkaloid spectrum was determined by thin layer chromatography, on silica gel plates, impregnated with formaldehyde. The mobile phase was the ethyl ether. The identification of the spots has been made in UV light, at $\lambda = 256$ nm.

It is necessary to make some specifications, in order to facilitate the understanding of the presented data. The sclerotia were coded according to the predominant alkaloid (T for ergotamine; S for ergocristine; P for ergocryptine). This symbol is followed by a number. The sclerotium and its descendance have the same code. The values of the total alkaloid content (coded as CAT) and the alkaloid spectrum represent the average of the determinations made upon 10 sclerotia, randomly chosen, from each sample group.

Results and discussion

The literature informations which have as key words *Claviceps purpurea* and nucleic acids are dominated by researches referring to alkaloid biosynthesis genic determinism, as well as by plant host – pathogen interaction aspects. There is relatively few data regarding the quantitative representation of them in the case of the species strains or of the different *C. purpurea* growth stages. Taber (1985) considers that at the mentioned species the nucleic acids are represented by 0.5 % DNA and 5 % RNA, and the DNA guanine/citosine proportion is 52.6 %, characteristic for the ascomycetes. The nucleic acids quantity variation is related to the monopotassic phosphate.

Sočič & al. (1985) associate the RNA quantity diminution, which takes place in the middle of the exponential growth stage, with the transition from the sphacelial growth to the submerged culture sclerotial growth. For this reason, the authors consider the RNA to be a good biochemical indicator for the optimum activity of the producing organism.

Recent studies, effectuated on sclerotia from hybrid or monoparental *C. purpurea* strains of different alkaloid types, evidenced some interesting tendencies regarding nucleic acid amount and RNA/DNA ratio, as well as the relationship established between DNA amount and total alkaloid level. The ANOVA test evidenced that the higher percentual average of ergotoxine alkaloids was registered for the sclerotia belonging to 10.18–11.54 mg/g DNA class. These sclerotia also displayed the higher average of total alkaloid content (Surdu & al. 2005).

Our results refer to the nucleic acids quantitative aspects in the conidia of *C. purpurea* fungus, formed in different cultivation and growth conditions.

The conidia, produced by the colonies formed on the T_2 agarised medium, have different DNA and RNA amounts (Fig. 1), the variation limits of these values being more extensive than at the ergocryptine type strains. The DNA amount is situated at the level indicated by Taber (1985), the strains differences being

relatively reduced. The ergocristine type strains which have a uniformity of DNA amount are to be noticed. The ergotamine type strains, especially those with ergocryptine origin, have different DNA amounts. The RNA amount variation is extensive, especially at the ergocryptine type strains.

The level of alkaloid biosynthesis of sclerotia from which the analysed colonies have derived is variable, the differences being more extensive at the ergotoxine ones than at the ergotamine ones. We point out that an increased alkaloid level is joined by a small value of the ratio between the DNA and RNA amount (P–209, S–203), the P–204 strain being situated at the opposite pole, where the decreased total alkaloids level is associated with the highest ratio value of the two nucleic acids.

The biochemical analyses made on the biological material represented by the submerged culture elements (mycelia and conidia) were effectuated at 4 hours intervals for most accurate determination of the produced modifications. It is known that at the fungi the development of the component elements is not synchronous. Prior experience, based on the microscopical observations, but also on the dynamics of some biochemical indicators, permitted us to consider that the duration of a cell cycle is 16 hours. From this reason, the first determination took place after three cell cycles, when the material amount was sufficient for analysis (Surdu 1998). The inoculum culture can be used after 72–96 hours of submerged cultivation, the macroscopic evaluation being completed by the microscopical observations.

The *C. purpurea* strains are different through their ability of producing conidia, the number and type of these being different at the ergotoxine and ergotamine strains. Normally, compared to the ergocristine type strains, and especially ergotamine type strains, the ergocryptine type strains produce a relatively small number of conidia. Carlile & Watkinson (1994) consider that the filamentous fungi produce two conidia types, with different functions and adaptations. Thus, the survival spores have great sizes, as a result of the nutritive reserve accumulation which allows them to surpass longer periods, until they germinate, as a reaction to the specific stimuli, or as result of a gradual decrease of latency. Their number generally is smaller than that of dispersion spores. These dispersion spores have smaller dimensions, a limited latency capacity, and they easily germinate in nutrient presence.

The *C. purpurea* strains have proper capacities to produce conidia. The conidia number and type are different for ergotoxine and ergotamine strains. Usually, the ergocryptine strains produce a relatively reduced number of macroconidia, whereas the ergotamine strains produce predominantly microconidia. The ergocristine strains produce macro- and microconidia in variable proportions, depending on strain (Surdu 1998).

The DNA quantity of the conidia formed in the submerged culture is different, remarking the constant reduced values of the ergotamine type strains and, on contrary, the significantly higher values of the ergotoxine strains, during the analysis (Figs 2, 3, 4). The differences of the conidia that result from same age cultures, but of different alkaloid type, as well as the DNA amount variations – extensive at some ergotoxine strains (S–218, P–209), insignificant at the ergotamine ones – can be caused by the different strains capacity of predominantly producing one of the two types of spores.

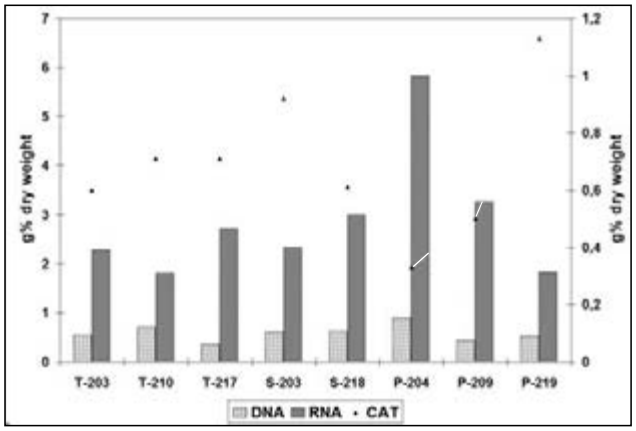

Fig. 1. DNA, RNA and total alkaloid amount from conidia (CAT) formed on T_2 agarised medium – *C. purpurea* strains of different alkaloid types.

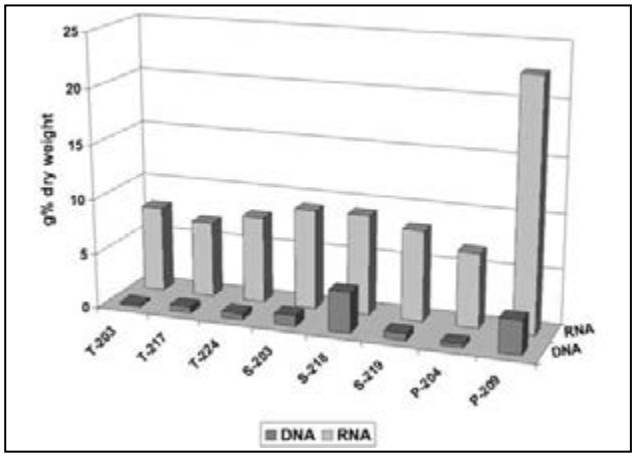

Fig. 2. DNA and RNA amount from *C. purpurea* conidia formed in submerged culture at 48 h age.

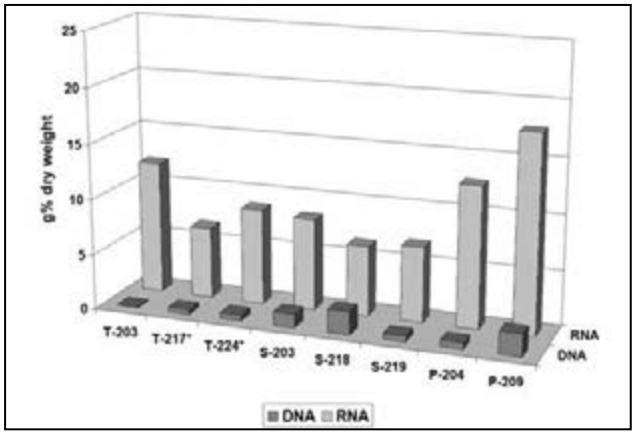

Fig. 3. DNA and RNA amount from *C. purpurea* conidia formed in submerged culture at 52 h age.

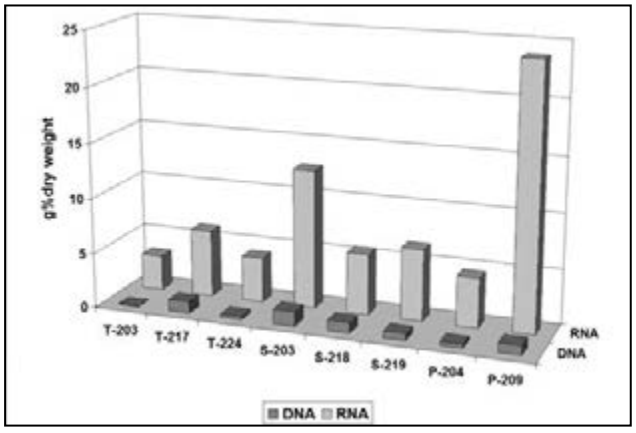

Fig. 4. DNA and RNA amount from *C. purpurea* conidia formed in submerged culture at 56 h age.

The relatively constant DNA amount of the ergotamine strains and some ergocristine ones suggests that these predominantly produce survival spores, which do not fast germinate. Our assumption is based on the fact that, corresponding to Carlile & Walkinson (1994) affirmations, DNA synthesis is not taking place in latent state. The registered dynamics of the ergocryptine type strains and some ergocristine type strains proves the fact that these germinate in order to form new mycelia. During the sporulation, a diminishing of the DNA amount is observed, process which takes place gradually (S–219 and P–209 strains) or suddenly (P–204 strain), probably in consequence to a better events synchronization during the culture development and growth period.

From the information provided by Carlile & Walkinson (1994) results that the filamentous fungi mitosis is a quick process, and the DNA synthesis lasts for 20 minutes, period which represents approx. 15 % of its duplication. In the nuclear population of the filamentous fungi the mitosis is not synchronous, and the hyphae grow apically and ramify laterally.

The RNA amount is represented in a different way (Figs 2, 3, 4). The variations are determined by the alkaloid type but also by their age. Therefore, at the ergotamine and ergocristine type strains the RNA amount is more reduced comparatively with the ergocryptine type strains. At most of the same aged cultures, the variations of the RNA amount are not very extensive, at 48 hours the values being comparable. There exists a tendency of the RNA amount to diminish after 56 hours.

Of course, there are some exceptions represented by larger RNA quantities, comparatively with the average value of all strains, fact that emphasizes the characteristics of ergocryptine strains. The presence of RNA different types in conidia is considered as normal, they being in great part as inactive forms. The starting of RNA synthesis, as a result of conidia germination induction, takes place very fast (approximately 15 minutes) (Carlile & Watkinson 1994). Our results suggest that the RNA synthesis is fast and intense in ergotoxine producing strains, the amount registered in their case being 3 times greater than in ergotamine strains. These RNA quantity increases take place at different ages, reason for which we consider that the cell cycle duration is different at investigated strains.

The microscopic aspect of honey-dew conidia is more uniform than that of the conidia from submerged cultures. The conidia are oval-elongate, of different thickness, the majority with one nucleus. If two or three nuclei are present, they are equal or unequal. The differences regarding conidia morphology and structure, conidia produced by strains of different alkaloid types, can be explained by the strain origin. Some strains have the origin in strains with identical biochemical features, while other strains come from strains predominantly producing another alkaloid than that synthesized by origin sclerotium of tested strain. The descendance tendency to display the origin strain characteristics is ascertained both microscopically and biochemically. In this way we too explain the deviation of some ergotamine and ergocristine strains from the specific manifestations, they being similar to origin strain, namely to ergocryptine type.

This situation is also illustrated for conidia produced by sphacelial mycelium, formed in rye ovaries. In the first post infection days, the ascospores or, in this case, the conidia from submerged cultures germinate and form a mycelium in ovary wall. The hyphae have a growth conducted to the ovary outside and produce, to the ovary surface, conidia protected by fine honey-dew drops (Taber 1985). In favourable conditions, the exudate appears after 6–8 days post infection, its presence being abundant 8–14 days post infection. The *C. purpurea* strains produce different exudate quantities, some strains being earlier, and other later from the point of view of this manifestation.

RNA and DNA quantities from honey-dew conidia are very different (Fig. 5), and the ratio of the two nucleic acids has very large variability limits. The biosynthetic capacity of origin sclerotia of tested strains is also different. At T–215 and T–217 strains with ergocristine origin, there is an inverse proportional ratio between DNA amount and sclerotium biosynthetic potential, expressed by total alkaloid amount. For the T–202 and T–208 strains, with ergotamine or ergocryptine origin, a direct proportional relation exists between the two tested parameters.

The RNA/DNA ratio represents the expression of the two nucleic acids, these values being different in conidia draw from different culture media. Thus, the variation limits are between 2.55–7.59 in conidia taken from agarised medium, at the inferior limit being the ergotamine type strains and at the upper limit being ergocryptine type strains or strains with ergocryptine ascendance.

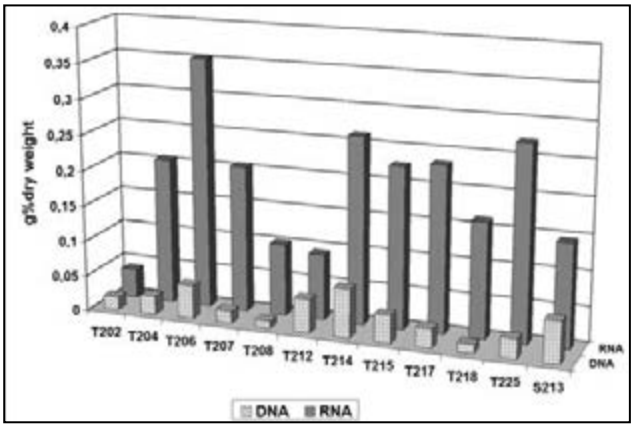

Fig. 5. DNA and RNA amount in *C. purpurea* conidia from honey-dew.

The majority of conidia from honey-dew are originated in ergotamine strains, but their origins, like we mentioned before, are different. The variation limits of RNA/DNA ratio values are between 1.94–12.46.

In submerged culture we found that the RNA/DNA ratio value could be a sensitive indicator of somatic development of organisms, of protein synthesis capacity of the cell, offering an estimation of growth rate and also, of metabolic state of the culture. The use of this ratio is based on the fact that the DNA remains in relative constant concentration in the variable conditions of the environment, while the RNA content is different, registering the highest concentration in the tissues with faster growth rate or with a high rate of protein synthesis. The RNA levels are actively regulated, as a response to requirements of protein synthesis and by nutrients and energy disposabilities. Because of the conservative nature of the DNA and abundance of rRNA, which represents 70–80 % from total RNA, the RNA/DNA ratio reflects, in this case, the modifications at the rRNA level (Table 1).

Table 1. Values of RNA/DNA ratio in conidia from submerged cultures – *C. purpurea* strains of different alkaloid types.

Culture age (h)	*Claviceps purpurea* strains							
	T–203	T–217	T–224	S–203	S–218	S–219	P–204	P–209
48	41.37	11.95	14.09	8.98	2.44	12.50	19.88	7.37
52	80.13	14.07	15.43	6.56	3.06	12.47	20.89	8.74
56	80.75	5.83	13.70	9.40	5.73	10.55	14.16	27.40

It can be noticed the low and close value of the RNA/DNA ratio in ergocristine producing strains. The highest value and, also, large variations of the ratio have been found in ergocryptine type strains, and especially ergotamine, depending on culture age.

Analysing the behaviour of some strains according to biosynthetic potential of descendance from conidia obtained in submerged culture, for which we have mentioned the RNA/DNA ratio value, we can notice that their manifestations are different. For the S–218 strain, from conidia with the smallest RNA/DNA ratio, resulted a descendance with a higher biosynthetic potential compared with origin sclerotium (0.61 g % total alkaloids in sclerotium, and the average value of total alkaloids for sclerotial descendance 0.86 g %). Not only quantity, but also the ratio between alkaloids has been modified, especially

for ergotoxine alkaloids (their ratio in sclerotium is 30:60:10, and for descendance is 35:35:30). Comparatively with ergocristine strain, the RNA/DNA ratio value at T–224 ergotamine producing strain is high, without any significant variation in the considered interval. The descendance from these conidia has productive traits like origin sclerotium of culture, by quantitative and qualitative point of view (the quantity of total alkaloids is of 0.65 g % in sclerotium, and the average value of total alkaloids quantity of descendance is 0.69 g %, both being represented by ergotamine).

In conclusion, the large variation of the RNA/DNA ratio value is determined by the genetic traits of the strains, by their origins, by the molecular synthesis required in a specific stage of development, the synthesis which presumes the specific activation and repression of the genes, with influence upon the quantitative level of RNA, and by conidia type. To these factors we could add the redundant level of some nucleotide sequences in DNA. We presume that in the most of the tested strains the low value of the RNA/DNA ratio, in ergotamine strains, it is because of repetitive DNA, a characteristic for eukaryotic genomic organization, its quantity being, as usual, higher in evolved taxa. In fungal genome it is 13 % (Raicu 1997). It seems like in the case of less evolved species, the repetitive DNA quantity variations are reduced and also, there is a positive correlation between total DNA and repetitive DNA.

Acknowledgements. This paper was realised in BIOSTAR research program, with the financial support of the Ministry of Education and Research, Romania.

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Invasive and potentially invasive parasite neomycetes from Romania

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Abstract. Our studies have been focused on *Peronosporales*, *Erysiphaceae*, *Uredinales* and *Ustomycetes*. We identified 83 parasite neomycetes on exotic and native plant species from Romania. Most of them are originated from Asia and America and were introduced in Romania with their hosts: *Erysiphe russelii*, *Ustilago oxalidis*, *Albugo amaranthi*, *Albugo portulacae*, *Puccinia xanthii*, etc. We also mention the presence of *Basidiophora kellermanni* on *Iva xanthifolia*, an alien invasive plant in East Romania, and *Puccinia komarowii* on *Impatiens parviflora*, also an alien invasive plant in Romania. Many neomycetes are parasite on cultivated plants producing important economic damages. On spontaneous plants we identified very aggressive invasive neomycetes as *Erysiphe vanbruntiana* on *Sambucus* species, *Melampsoridium hiratsukanum* on *Alnus* sp., *Puccinia lagenophorae* on wild *Senecio* species. Some neomycetes are parasite on cultivated plants as well as on spontaneous ones (*Puccinia malvacearum*, *P. xanthii*).

Key words: alien, *Erysiphaceae*, invasive, *Peronosporales*, Romania, *Uredinales*, *Ustomycetes*

Introduction

Neomycetes are alien (adventive, allochthonous) fungi. Usually, their introduction is a result of a not intentional human activity. Many of them came together with their host, either economic plants (e.g., fungi parasite on *Nicotiana tabacum*, *Helianthus annuus*, *Zea mays*) or weeds. Other fungi could have arrived here because of some natural factors, often unknown. In the new conquered area, neomycetes could be harmful, giving rise to economical problems or threatening native biodiversity. For this reason it is very important to have complete data about them. With few exceptions (Negrean 2003; Negrean & Drăgulescu 2005), in Romania there are no studies regarding parasite neomycetes. However, there are chorological papers for some neomycetes (Tănase & al. 1997; Negrean & Anastasiu 2005). At the European level available data are from Germany (Scholler 1996), and Austria (Essl & Rabitsch 2002).

Material and methods

The data have been obtained from literature, herbarium materials and as a result of own field observations along many years. For each taxon we registered: taxonomy, origin, host, Romanian distribution, the author(s) and the year for the first mention in our country.

The fungi nomenclature is according to *Index Fungorum*. The host-plants nomenclature is according to *Flora României* (Săvulescu 1952–1976) and *Flora Europaea* (Tutin & al. 1964–1980; Tutin & al. 1993). The international abbreviations for herbaria followed Holmgren & al. (1990).

Results and discussion

On different cultivated or spontaneous host-plants in Romania, we identified 83 parasite neomycetes taxa listed below:

Peronosporales*: *Basidiophora entospora Roze & Cornu (on *Conyza canadensis* (L.) Cronquist), ***B. kellermannii*** (Swingle ex Sacc.) G.W. Wilson (on *Iva xanthifolia* Nutt.), ***Peronospora destructor*** (Berk.) Casp. ex Berk. (on *Allium cepa* L. and *A. fistulosum* L.), ***P. ducometi*** Siemaszko & Janke (on *Fagopyrum esculentum* Moench, cult.), ***P. manshurica*** (Naumov) Syd. (on *Glycine max* (L.) Merr.), ***P. tabacina*** D.B. Ad-

am (on *Nicotiana* spp., cult.), ***Plasmopara halstedii*** (Farl.) Berl. & De Toni /incl. *P. angustiterminalis* and *P. carthami*/ (on *Helianthus*, *Xanthium*, *Carthamus*, *Dimorphotheca*, *Ambrosia*), ***Pl. skvortzovii*** Miura (on *Abutilon theophrasti* Medik.), ***Pseudoperonospora cannabina*** (G.H. Otth) Curzi (on *Cannabis sativa* L. s.l.), ***Ps. cubensis*** (Berk. & M.A. Curtis) Rostovzev (on cultivated *Cucurbitaceae*).

Ascomycotina*, *Erysiphaceae*:** ***Arthrocladiella mougeotii (Lév.) Vassilkov (on *Lycium barbarum* L.), ***Erysiphe begoniicola*** U. Braun & S. Takam. (on *Begonia* spp.), ***E. catalpae*** Simonyan (on *Catalpa* spp., cult.), ***E. cichoracearum*** DC. var. ***latispora*** U. Braun (on *Asteraceae*, *Heliantheae*, adventive and cultivated in Europe), ***E. diffusa*** (Cooke & Peck) U. Braun & S. Takam. (on *Glycirrhiza echinata* L.), ***E. euonymi-japonici*** (Vienn.-Bourg.) U. Braun & S. Takam. (on cultivated *Euonymus* spp.), ***E. flexuosa*** (Peck) U. Braun & S. Takam. (on *Aesculus hippocastanum* L.), ***E. howeana*** U. Braun (on *Oenothera* spp.), ***E. magnicellulata*** U. Braun var. ***magnicellulata*** U. Braun (on *Phlox* spp.), ***E. necator*** Schwein. (on *Vitis* spp.), ***E. palczewskii*** (Jacz.) U. Braun & S. Takam. (on *Caragana arborescens* L.), ***E. platani*** (Howe) U. Braun & S. Takam. (on *Platanus* spp.), ***E. pseudacaciae*** (P.D. Marchenko) U. Braun & S. Takam. (on *Robinia* spp.), ***E. rayssiae*** (Mayor) U. Braun & S. Takam. (on *Baptisia australis* R. Br.), ***E. russelii*** (Clinton) U. Braun & S. Takam. (on *Oxalis* spp.), ***E. sambuci*** Ahmad (on *Sambucus ebulus* L.), ***E. syringae*** Schwein. (on *Oleaceae*), ***E. vanbruntiana*** (W.R. Gerard) U. Braun & S. Takam. (on *Sambucus* spp.), ***Oidium abelmoschi*** Thüm. (on *Abelmoschus esculentus* (L.) Medik.), ***O. calanchoeae*** Lüstner ex U. Braun (on *Kalanchoe spathulata* DC.), ***O. caricae-papayae*** J.M. Yen /= *O. caricae* F. Noack/ (on *Carica* spp.), ***O. chrysanthemi*** Rabenh. (on *Dendranthema indicum* (L.) Des Moul.), ***O. eucalypti*** Rostr. (on *Eucalyptus cladocalyx* F. Muell.), ***O. hiratae*** U. Braun (on *Catalpa bignonioides* Walter), ***O. hortensiae*** Jørst. (on *Hydrangea macrophylla* (Thunb.) DC.), ***O. pedilanthi*** R. Mathur, B.L. Mathur & Bhargavan (on *Pedilanthus* spp.), ***O. pharbitis*** Sandu (on *Ipomoea purpurea* Roth), ***O. primulae-obconicae*** Ciocan & Calnegru (on *Primula obconica* Hance), ***Ovulariopsis eliadei*** Negru (on *Nelumbo nucifera* Gaertn.), ***Podosphaera mors-uvae*** (Schwein.) U. Braun & S. Takam. (on cultivated and spontaneous *Ribes* spp.), ***Uncinula celtidis*** Shvartsman & Kusnezova (on *Celtis* spp.), ***U. delavayi*** Pat. (on *Ailanthus altissima* (Mill.) Swingle).

Basidiomycetes*, *Teliomycetes* (*Uredinales*):** ***Cronartium ribicola A. Dietr. (on *Pinus* spp., *Ribes* spp.), ***Cumminsiella mirabilissima*** (Peck) Nannf. (on *Mahonia aquifolium* (Pursh) Nutt., cult.), ***Melampsoridium hiratsukanum*** S. Ito & Hirats. f. (on *Alnus incana* (L.) Moench subsp. *incana*), ***Puccinia antirrhini*** Dietel & Holw. (on *Antirrhinum majus* L.), ***P. balsamitae*** (F. Strauss) Rabenh. (on *Balsamita major* Desf., *Tanacetum balsamita* L.), ***P. buxi*** DC. (on *Buxus sempervirens* L.), ***P. callistephi*** Săvul. (on *Callistephus chinensis* (L.) Nees), ***P. carthami*** Corda (on *Carthamus tinctorius* L.), ***P. dracunculina*** Fahrend. (on *Artemisia dracunculus* L.), ***P. helianthi*** Schwein. (on *Iva xanthiifolia*, *Helianthus* spp.), ***P. hemerocallidis*** Thüm. (on *Hemerocallis* spp.), ***P. horiana*** Henn. (on *Dendranthema indicum* (L.) Des Moul.), ***P. komarovii*** Tranzschel (on *Impatiens parviflora* DC.), ***P. lagenophorae*** Cooke (on *Calendula*, *Senecio*), ***P. malvacearum*** Bertero ex Mont. (on *Malvaceae*), ***P. pelargonii-zonalis*** Doidge (on *Pelargonium zonale* (L.) Aiton), ***P. physalidis*** Peck (on *Physalis alkekengi* L.), ***P. schroeteri*** Pass. (on *Narcissus poeticus* L.), ***P. sorghi*** Schwein. (on *Zea mays* L.), ***P. xanthii*** Schwein. (on *Xanthium* spp.), ***Uromyces appendiculatus*** F. Strauss (on *Phaseolus*, *Vigna*), ***U. beticola*** (Bellynck) Boerema, Loer. & Hamers (on *Beta vulgaris* L.), ***Uromyces lupinicolus*** Bubák (on *Lupinus* spp.).

Basidiomycetes*, *Ustomycetes* (*Ustilaginales*):** ***Entyloma borraginis Cif. (on *Borago officinalis* L.), ***E. calendulae*** (Oudem.) de Bary (on *Calendula* spp.), ***E. dahliae*** Syd. & P. Syd. (on *Dahlia* spp.), ***Glomosporium amaranthi*** Hirschh. (on *Amaranthus* spp.), ***Melanopsichium pennsylvanicum*** Hirschh. (on *Polygonum* spp.), ***Sphacelotheca destruens*** (Schltdl.) J.A. Stev. & Aar. G. Johnson (on *Panicum miliaceum* L.), ***S. reiliana*** (J.G. Kühn) G.P. Clinton (on *Sorghum*, *Zea*), ***S. sorghi*** (Ehrenb. ex Link) G.P. Clinton (on *Sorghum* spp.), ***Tilletia caries*** (DC.) Tul. & C. Tul. (on *Triticum* spp.), ***T. laevis*** J.G. Kühn (on *Triticum* spp.), ***T. secalis*** (Corda) J.G. Kühn (on *Secale cereale* L.), ***Urocystis eranthidis*** (Pass.) Ainsw. & Sampson (on *Eranthis hyemalis* (L.) Salisb.), ***U. magica*** Frost (on *Allium cepa* L.), ***U. occulta*** (Wallr.) Rabenh. (on *Secale cereale* L.), ***Ustilago crameri*** Körn. (on *Setaria italica* (L.) P. Beauv.), ***Us. hordei*** (Pers.) Lagerh. (on *Hordeum* spp.), ***Us. maydis*** (DC.) Corda (on *Zea mays* L.), ***Us. oxalidis*** Ellis & Tracy (on *Oxalis* spp.), ***Us. raciborskiana*** Siemaszko & Wróbl. (on *Fallopia aubertii* (L. Henry) Holub).

For some invasive or potentially invasive parasite neomycetes we present chorological data:

Basidiophora entospora Roze & Cornu – in our country it was reported for the first time in 1932 by Săvulescu and Rayss (Săvulescu & Rayss 1932) and distributed in exsiccata HMR (no. 306). Until 1970 it was reported only 5 times. It is parasite on *Conyza canadensis* (L.) Cronquist (= *Erigeron canadensis* L.), adventive plant with North American origin. The host is fairly common but the parasite does not occur very frequently. Of the three specimens deposited in BUCM and examinated by us, only on one the fungus was present (Constantinescu & Negrean 1983: 266). We add new chorological data to those of Constantinescu & Negrean (1983): **HD**: Halta Geoagiu, 45°53'08" N, 23°14'59" E, 200 m, 07.06.1986, G. Negrean [BUCM 98.045]. **MH**: Gara Orşova, 14.05.1979, G. Negrean [BUCM 53.620]. **DJ**: Cernele, 18.05.1977, G. Negrean [BUCM 48.942]. **VL**: Muntele Cozia, Valea Păuşa, 29.06.1976, G. Negrean & O. Constantinescu [BUCM 46.101]. **TR**: Nanov, malul Vedei, 01.05.1979, G. Negrean [BUCM 55.926]; Pădurea Brânceni, 25.06.1980, G. Negrean [BUCM 56.132]. **PH**: Gara Câmpina, 08.06.1980, G. Negrean [BUCM 56.056]; Gara Floreşti–Prahova, 08.06.1980, G. Negrean [BUCM 56.052]; Buda (Săvulescu & Săvulescu 1964: 37), 23.05.1932, T. Săvulescu & T. Rayss [HMR 306; BUCM 639] (Săvulescu 1932; Bontea & Constantinescu 1968: 128); Bordeni E, 23.05.1980, G. Negrean [BUCM 55.819]. **IF**: Moara Vlăsiei, Pădurea Surlari, 44°40'10" N, 26°13'35" E, 90 m, 21.05.1982, G. Negrean [BUCM 70.312]; Gara Greci, 18.05.1980, G. Negrean [BUCM 55.837]; Greci, Pădurea Brânzeasca, 18.05.1980, G. Negrean [BUCM 55.853]; Moldoveni, 08.05.1977, G. Negrean [BUCM 48.750]; Jilava NE, in locis ruderalis, 44°20'56" N, 26°06'15" E, 73 m, 30.04.2000, G. Negrean [BUC; SOM]. **Mun. Bucureşti**: Bucureşti [BUCM] (Săvulescu & Rayss 1932); Herăstrău (Săvulescu & Săvulescu 1964: 37); Pădurea Boldul–Crețuleasca, 44°30'32" N, 26°11'33" E, 83 m, 23.05.1982, G. Negrean [BUCM 70.354]. **GR**: Gara Grădiştea, 44°12'28" N, 26°09'58" E, 50 m, 30.05.1982, G. Negrean [BUCM 72.187]; Comana, Lunca Neajlovului, 01.05.1977, G. Negrean & O. Constantinescu [BUCM 48.798]; Gara Comana, 44°10'15" N, 26°08'56" E, 48 m, 12.05.1999, G. Negrean [BUCM 136.501]; Comana S, Valea Gurbanului, incultivated fields, 23.03.2004, G. Negrean (3964). **IL**: Lehliu (Săvulescu & Săvulescu 1964: 37). **CT**: Mangalia N, Halta Popasul Căprioarelor, 27.05.1981, G. Negrean [BUCM 59.289]; Cernavodă S, 29.05.1980, G. Negrean [BUCM 55.933]. **TL**: Enisala E, Cetatea Heraclea, 44°52'35" N, 28°51'02" E, 1 m, 01.06.1984, G. Negrean [BUCM 82.947]. **GL**: Blăjeru, 10.06.1978, G. Negrean [BUCM 51.879]; Hanul–Conachi, 10.06.1978, G. Negrean [BUCM 51.868]. **IS**: Tîrgul–Frumos, Şorogari, Valea Lungă (Săvulescu & Săvulescu 1964: 37); Şorogari, 21.07.1932, Oescu & Rădulescu [BUCM 638] (Oescu & Rădulescu 1932: 638); Rediu N, Ferma Institutului Agronomic, 47°13'53" N, 27°30'12" E, 163 m, 24.05.1992, G. Negrean [BUCM 124.388]. **SV**: Gara Coşna, 47°22'12" N, 25°09'00" E, 858 m, 03.09.1982, G. Negrean [BUCM 72.951].

Basidiophora kellermannii (Swingle ex Sacc.) G.W. Wilson is parasite on *Iva xanthifolia* Nutt. Chorology: **IS**: Rediu, 47°13'14" N, 27°30'20" E, 90 m, 24.05.1993, G. Negrean [BUCM 127.044]; Iaşi, cartier Păcurari, 47°10'23" N, 27°32'20" E, 44 m, 29.09.1988, G. Negrean [BUCM 127.046].

Plasmopara skvortzovii Miura is parasite on *Abutilon theophrasti* Medik. Constantinescu & Negrean (1983) reported this taxon as new for Europe. It is from Asia, as its host. Chorology: **CL**: Căscioarele, 44°06'15" N, 26°28'31" E, 17 m, 07.10.1983, G. Negrean [HMR 3097] (Negrean 1984: 35).

Erysiphe catalpae Simonyan is from North America. The hosts are cultivated plants. In South Romania, where the climate is warmer, it seems to be invasive. Matrix: *Catalpa bignonioides* Walter – **MH**: Orşova, centrum, 44°27'30" N, 22°24'28" E, 15.09.2005, G. Negrean (6779) [BUC]; Defileul Dunării, Dubova, centrum, 44°37' N, 22°12' E, 15.09.2005, G. Negrean (6831) [BUC]; Schela–Cladovei NW, 44°37' N, 22°36' E, *ca.* 70 m, 17.09.2005, G. Negrean (6922) [BUC].

Erysiphe russelii (Clinton) U. Braun & S. Takam. (= *Microsphaera russelii* Clint.; *Oidium oxalidis* McAlp.) – introduced from North America in Europe on yellow flowered species of *Oxalis* (subgen. *Xanthoxalis*). In Romania it is parasite on:

Oxalis corniculata L. – **AG**: Piteşti, 44°51'45" N, 24°52'43" E, 268 m, 09.11.1985, G. Negrean [BUCM 92.867]. **Bucureşti**, 03.10.1973, G. Negrean [BUCM 44.118]; idem, 09.08.1975 [BUCM 44.119]; Cartier Ciurel, in caldaria, 04.02.1992; Cişmigiu, 44°36'07" N, 26°05'31" E, 71 m, 05.09.1987, G. Negrean [BUCM 92.867].

Oxalis europaea Jord. – **MM**: Sighetul–Marmaţiei, 47°56'27" N, 23°53'52" E, 270 m, 23.08.1994, G. Negrean [BUCM 130.922]. **SM**: Oraşul–Nou, Dealul Mujdeni, Pădurea Mujdeni, 47°51'03" N, 23°15'06" E, 150 m, 16.10.1983, G. Negrean [BUCM 80.497]. **GJ**: Târgu–Jiu, 08.07.1977, G. Negrean [BUCM 69.610]. **VL**: Mănăstirea Cornet, 45°23'15" N, 24°18'09" E, 340 m, 03.08.1987, G. Negrean [BUCM 104.630]. **IF**: Buftea NE, Pădurea Bufteian-

ca, in *Quercetum cerris*, 44°36'26" N, 25°59'22" E, 106 m, 16.06.1985, G. Negrean [BUCM 88.937]. **NT**: Piatra-Neamţ, 46°56'12" N, 26°26'48" E, 340 m, 02.09.1985, G. Negrean [BUCM 90.669].

Uncinula delavayi Pat. is native in China, as its host, *Ailanthus altissima* (Mill.) Swingle. Docea (1970) reported it from Bucharest. Being very rare, we indicate the specimens from BUCM: Bucureşti, Institutul Agronomic, 26.06.1966 and 25.06.1974, E. Docea (BUCM 56.085]; Spitalul Colţea, 26.06.1974, O. Constantinescu [BUCM 69.704]. Braun (1987), in the last world monography, mentions this taxon only from the locus classicus in China, where it was collected in 1987.

Melampsoridium hiratsukanum S. Ito & Hirats. f. was published by Pricop (2003). It is parasite on *Alnus* sp. from Asia and America. In Europe, this taxon was reported from Scotland, Finland, Estonia, Italy, Poland and Austria (Wołczańska 1999; Piątek & Ronikier 2001; Riegler-Hager & al. 2003). It is very invasive and harmful for *Alnus* species. Chorological data from Romania: Matrix:

Alnus incana (L.) Moench subsp. *incana* – **CJ**: Munţii Muntele Mare, Bazinul Someşului Cald, Ic Ponor, 46°37' N, 22°52' E, 24.09.2005, G. Negrean (6960) [BUC; LI]; Călata S, 46°37' N, 23°01' E, 26.09.2005, G. Negrean (7023) [BUC; LI]. **GJ**: Cloşani N, Valea Mare, Lunca Motrului, sub Baraj, in *Alnetum*, 45°07' N, 22°50' E, 08.09.2005, G. Negrean (6704) [BUC; LI]. **PH**: Sinaia, Parcul Cazinoului, 45°21'15" N, 25°33'12" E, 845 m, 28.08.2004, G. Negrean (GN: 5213) [BUC; LI], 45°21'15" N, 25°33'23" E, 815 m, 29.08.2004, G. Negrean (GN: 5121) [BUC]; Sinaia, Lunca Prahovei, 45°22'00" N, 25°32'50" E, 850 m, 09.06.2003, G. Negrean (3708a) [BUC; LI]; idem, 10.07.2003, G. Negrean [BUC; LI]; Lunca Prahovei sub Zamora, 22.08.2004, M. & G. Negrean (5095); Cumpătul, 22.08.2004, M. & G. Negrean (5098) [BUC; LI]; Sinaia, Cumpătul, Lunca Prahovei, 45°22'00" N, 25°32'50" E, 830 m, 10.07.2003, G. Negrean (3708a) [BUC; LI]; Sinaia, Cumpătul, in *Alnetum*, 45°22'11" N, 25°33'08" E, 860 m, 10.07.2003, G. Negrean & M. Negrean (5098) [BUC; LI]; Sinaia, Valea Prahovei, the bridge to Cumpătul, 45°21'35" N, 25°33'12" E, 825 m, 28.08.2004, G. Negrean (5213) [BUC; LI]. **SV**: Gura Humorului, 47°23' N, 25°53' E, 500 m, 16.08.2002, C. Pricop [I] (Pricop 2003). **BC**: Slănic Moldova, 46°15' N, 26°25' E, 420 m, 12.08.2002, C. Pricop [I].

Puccinia komarovii Tranzschel is from Central Asia, alien in Europe. In Romania it is parasite on *Impatiens parviflora* DC., subspontaneous in Botanical Garden of Cluj-Napoca, Valea Pârîul Ţiganilor, 46°51'46" N, 23°35'20" E, 347 m, 05.07.1993, G. Negrean [BUCM 129.306]. In the *Monographie* of Săvulescu (1953) it is not present.

Puccinia lagenophorae Cooke is from Australia. First record for Europe (France) belongs to Mayor (1962). In Romania it was found in 1973 and published by Negrean & Fodor (1990), on *Senecio squalidus* and *Calendula officinalis*. Scholler (1994) describes the history of this taxon in Europe. Chorology:

Calendula officinalis L. – **GR**: Gara Comana, 44°10'21" N, 26°08'53" E, 47 m, 25.07.1991, G. Negrean [BUCM 123.151] (Negrean & Fodor 1990: 72).

Senecio squalidus L. – **HR**: Rugăneşti, 46°18'18" N, 25°04'30" E, 405 m, 17.10.1991, G. Negrean [BUCM 123.151]. **HD**: Munţii Ţarcu–Petreanu, Valea Rîul Mare, 45°20'42" N, 22°44'12" E, 911 m, 30.07.1993, G. Negrean [BUCM 129.448]; Valea Netiş, 45°21'37" N, 22°44'46" E, 890 m, 04.08.1993, G. Negrean [BUCM 129.690]; Munţii Retezat, Barajul Gura Apei, 45°19'58" N, 22°43'10" E, 988 m, 16.10.1996, G. Negrean [BUCM 132.412]; Munţii Piule – Piatra–Iorgovan, Muntele Piule, 30.09.1974, G. Negrean [BUCM 69.527)]; Muntele Vulcan, 46°04' N, 22°57' E, *ca*. 1000 m, 24.05.1973, G. Negrean [BUCM 130.012]. **MH**: Munţii Mehedinţi, Muntele Grabanâc, 44°53'10" N, 22°29'30" E, 1050 m, 13.08.1994, G. Negrean [BUCM 131.069]. **VL**: Muntele Buila, Stâna La Oale, 25.09.1980, G. Negrean [BUCM 57.374]; Schitul Pahomie, 25.09.1980, G. Negrean [BUCM 68.666]. **PH**: Gara Buşteni, 13.10.1978, G. Negrean [BUCM 69.526] (Negrean & Fodor 1990: 72); Poiana–Ţapului, 04.08.1977, G. Negrean [BUCM 130.013]. **SV**: Depresiunea Dorna, Gara Poiana–Ştampei, 47°18'43" N, 25°07'54" E, 910 m, 18.08.1992, G. Negrean [BUCM 124.884].

Senecio vulgaris L. – **SM**: Dindeşti, 47°32' N, 22°19' E, 19.09.1978, G. Negrean [BUCM 69.529]. **SJ**: Şimleul–Silvaniei, 03.09.1977, G. Negrean [BUCM 69.454]. **MH**: Defileul Dunării, Staraş, in locis ruderalis, 16.09.2005, G. Negrean (6860) [BUC; LI]. **PH**: Buşteni, Lunca Prahovei, 45°24' N, 25°32' E, 13.10.1978, G. Negrean [BUCM 69.528]; Sinaia, 03.10.1975, G. Negrean & O. Constantinescu [BUCM 69.525].

Puccinia malvacearum Bertero ex Mont. is from South America. It was described from Chile in 1832 (Dupias 1971). In Europe it was recorded for first time in 1869 from Spain and France. It extended very fast. It is parasite on over 40 species from *Malvaceae* family. In our country it is parasite on: *Alcea pallida* (Willd.) Waldst. & Kit. subsp. *pallida* (= *Althaea pallida* Willd.) – 4 choronyms, *A. rosea* L. (= *Althaea rosea* (L.) Cav.) – 45 choronyms, *Althaea cannabina* L. – 1 choronym, *A. hirsuta* L. –

8 choronyms, *A. officinalis* L. – 14 choronyms, *A. sulphurea* Boiss. – 1 choronym, *Lavathera thuringiaca* L. – 7 choronyms, *Malva neglecta* Wallr. – 42 choronyms, *M. pusilla* Sm. (= *M. rotundifolia* L.) – 25 choronyms, *M. sylvestris* L. (incl. *M. erecta* C. Presl) – 77 choronyms, *M. verticillata* L. (incl. *M. crispa* (L.) L.) – 6 choronyms. The oldest specimen from Romania is on *Malva sylvestris*, Bradul, 21.09.1879, M. Fuss (Săvulescu 1953). The first record in Romania is in 1903 (Constantineanu 1903: 227).

Puccinia pelargonii-zonalis Doidge is from South Africa. First record in Europe is in 1962 (France). From Romania it is reported by Bechet & Marcu (1977), from Timişoara, on *Pelargonium zonale* (L.) Aiton. Chorological data: **CJ**: Cluj-Napoca, 18.06.1977, E. Szász & V. Cristea [BUCM 49.688]. **CT**: Mangalia, Hotel Diana, 27.05.1981, G. Negrean [BUCM 59.310]. **TL**: Tulcea, 45°10'45" N, 28°47'58" E, 10 m, 17.10.1982, G. Negrean [HMR 3059] (Negrean 1984: 22).

Melanopsichium pennsylvanicum Hirschh. (= *Melanopsichium austro-americanum* (Speg.) Beck) is from South America; reported for the first time in Romania by Săvulescu (1955). It is parasite on annual species of *Polygonum*. We present some chorological data. Matrix:

Polygonum hydropiper L. – **DJ**: Filiaşi, 09.10.1963 (Rîncu & Stănoiu 1970: 44).

Polygonum lapathifolium L. s.l. – **SM**: Satu-Mare, 22.09.1955, leg. K. Vánky, det. T. Săvulescu [BUC 233.085]; Dindeşti, 47°32' N, 22°19' E, 08.09.1975 and 14.09.1976, G. Negrean [BUCM] (Negrean 1982: 592). **MH**: Ieşelniţa, Balta, 44°43' N, 22°24'E, 15.09.2005, G. Negrean (6789) [BUC; LI]. **BH**: Oradea, Băile 1 Mai, 21.09.1955 (Săvulescu 1957: 830). **PH**: Pădurea Plopeni, 02.10.1981, G. Negrean [BUCM]. **IF**: Buftea NE, Pădurea Bufteianca, Valea Mocanului, 44°36'00" N, 25°59'06" E, 100 m, 03.10.1982, G. Negrean [BUCM 73.585]. **TL**: Delta Dunării, malul gârliţei Matiţa, 25.10.1953, T. Săvulescu [HMR 1568; BUCM 21.223] (Săvulescu 1955 /HMR/; Săvulescu 1955: 830, 1957: 825; Bontea & Constantinescu 1968: 812); Gârla Perevalovca, at its flow into Sf. Gheorghe channel, 26.09.1954, T. Săvulescu [BUCM 21.221] (Săvulescu 1955: 830, 1957: 825).

Ustilago oxalidis Ellis & Tracy is from North America. From Romania it is known since 1928 (Săvulescu 1957). It is parasite on:

Oxalis europaea Jord. – **MM**: Baia-Mare S, 05.09.1957, leg. N. Roman, det. G. Negrean [BUCM 81.701]. **BN**: Tiha–Bârgăului SE, Dealul Strâmbei, 47°13'08" N, 24°47'36" E, 645 m, 01.10.1987, G. Negrean [BUCM 106.285]; Prundul–Bârgăului SW, Dealul Runcul, 47°12'44" N, 24°44'06" E, 628 m, 12.10.1988, G. Negrean [BUCM 111.129]. **HD**: Petroşeni, in coemeterio, 45°24'48" N, 23°22'46" E, 632 m, 26.08.1988, G. Negrean [BUCM 110.391]. **SB**: Cisnădioara, 45°42'16" N, 24°06'43" E, 525 m, 07.09.1992, G. Negrean [BUCM 125.306]. **GJ**: Târgu–Jiu, Parcul Brâncuşi, 45°02'34" N, 23°16'11" E, 205 m, 29.09.1984, G. Negrean [BUCM 86.061]. **IF**: Buftea NE, Păd. Bufteianca, 44°36'26" N, 25°59'22" E, 106 m, 16.06.1985, G. Negrean [BUCM 88.907]. **NT**: Mănăstirea Bistriţa, 46°57'28" N, 26°17'26" E, 398 m, 01.09.1985, G. Negrean [BUCM 90.467]; Piatra-Neamţ, 46°55'24" N, 26°21'47" E, 320 m, 31.08.1985, G. Negrean [BUCM 90.330). **SV**: Clit, 47°45'00" N, 25°51'19" E, 465 m, 18.09.1987, G. Negrean [BUCM 105.688); Rădăuţi, in coemeterio, 47°50'14" N, 25°54'26" E, 380 m, 16.07.1988, G. Negrean [BUCM 109.564]; Zamostea E, Pădurea Luncii, 47°51'52" N, 26°14'58" E, 280 m, 13.07.1988, G. Negrean (BUCM 109.304).

Oxalis stricta L. – **MS**: Sighişoara, Krügberg, 01.08.1957, K. Vánky, rev. T. Săvulescu [HMR 1634; BUC 233.218] (Săvulescu 1959; Bontea & Constantinescu 1968: 850).

Conclusions

In Romania we identified 83 parasite neomycetes from main groups: *Peronosporales*, *Erysiphaceae*, *Uredinales*, *Ustilaginales*.

The first record of an alien fungus for Romania belongs to J.C. Baumgarten, before 1840.

The number of neomycetes increased permanently. At the end of the 19th century 8 species were reported. Until 1940 their number increased to 20. Between 1941 and 1960, to those 20, other 15 were added. The biggest number of neomycetes was recorded in the period 1960–1980 (22 taxa). From 1981 to 1983, 10 more species were added to the list of alien fungi.

Regarding the origin of neomycetes, 42.68 % (35 taxa) are from Asia and 40.24 % (33 taxa) are from America.

Many alien fungi are parasite on cultivated plants (65.85 % – 54 taxa) and only few are parasite on spontaneous plants (9.75 % – 8 taxa). 20 alien fungi are parasite on alien plants. These could be used for biological control of invasive alien plants.

Two new species are indicated in this paper for the first time in Romania: *Basidiophora kellermannii* and *Puccinia komarovii*.

Few species are recorded for Europe only from Romania: *Plasmopara skvortzovii*, *Puccinia callistephi*, *P. hemerocallidis*, *P. physalidis*.

Acknowledgements. We thank Dr O. Constantinescu and Dr M. Petrescu for allowing us to publish some unedited materials, as well as to Prof. Dr. Aurelia Crişan for the deposited materials from BUCM. This work is a result of a CNCSIS grant (contract No. 33469/2005).

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SCIENTIFIC AREA E

PLANT AND HABITAT CONSERVATION

Village grasslands of Romania – an undervalued conservation resource

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Abstract. Nature conservation in Romania has focussed on the mountains and the Danube Delta, with most botanical attention on old growth forest and montane habitats. In comparison, semi-natural anthropogenic habitats have largely been ignored. Recent surveys of grasslands in the submontane zone of Transylvania and Moldavia have revealed large areas of great biodiversity importance at a European level, containing Continental, Dacic–Pannonic and Mediterranean floristic elements. These "meadow-steppe" grasslands represent a vital area of a habitat that has disappeared from much of Europe following agricultural intensification. In addition, many wetlands within this agricultural landscape remain hydrologically intact, with an almost natural zonation of habitats. This paper describes the communities and flora, and stresses the urgency to protect a resource that is now almost unique in Europe and very vulnerable to pressures to increase the output of farmland following Romanian accession to the EU.

Key words: anthropogenic biodiversity, conservation, grassland, Romania

Introduction

Romania has a remarkable diversity and richness of wildlife, with many areas of high value at a continental or world level. In terms of its vascular flora, there are 3297 species and 498 subspecies (Ciocârlan 2000), of which some 38 % (1235 species and 203 subspecies) are nationally threatened (Oltean & al. 1994). From early in the twentieth century, nature conservation activity has designated National Parks and other protected areas, with renewed activity since 1990. However, information from the Ministry of Environment and Water Management on protected areas in Romania reveals that the overwhelming majority of designated land is in the higher parts of the Carpathian mountain chain or associated with the Danube and its Delta. In particular, national parks are largely confined to higher altitudes (ancient forest and montane habitats). The focus for biodiversity protection has been on these relic areas of wilderness, of which Romania holds a resource of international standing.

With such wild riches, it is perhaps not surprising that less attention had been given to those Romanian habitats shaped by human activity, such as semi-natural anthropogenic grasslands. Since 1990, more botanists from Western Europe have visited Romania. These botanists generally come from a landscape where true wilderness has largely been eradicated and where much of the plant diversity value resides in species-rich semi-natural habitats originally created (or modified) by agriculture and forestry. More recently in these countries (e.g. the United Kingdom and the Netherlands), even these rich anthropogenic habitats have been hugely degraded through agricultural intensification (Fuller 1987) and a vigorous programme of ecological restoration and species recovery has begun. Confronted with Romanian anthropogenic grasslands (especially old hay-meadows), foreign botanists have been filled with wonder that such remarkable, floristically rich vegetation survives and stimulated to publicise these treasures widely (Akeroyd 2002, 2006, 2007; Akeroyd & Patton 2005). Delight and concern for these habitats is not at all confined to outsiders, however, and in recent discus-

sions with its British equivalent, the Romanian Ecological Society identified these grasslands as second only to the natural forest resource in requiring concerted ecological research and nature conservation action (Prof. A. Vădineanu pers. comm.). The present paper is a preliminary contribution towards this goal.

Methods

Individually, since 1997 the authors have made numerous visits to many parts of Romania, studying the flora and vegetation, increasingly focussing on the grasslands of the Saxon lands in the counties of Braşov, Mureş and Sibiu (Mountford & Akeroyd 2005; Akeroyd 2006, 2007; Akeroyd & Page 2006). The present paper concentrates on this more detailed work in southern Transylvania, but also draws upon data gathered from other grasslands in the Carpathian foothills zone of Transylvania and Moldavia, including the counties of Suceava and Neamț in Moldavia.

The core study area of the Saxon lands lies to the north of the Transylvanian Alps (Carpații Meridionali) in the lower hill country of the Târnave plateau. In terms of European biogeographical regions, the area is Continental though influenced by the Alpine region to the south and showing floristic trends toward both the Pannonic and Steppic regions (Doniță & al. 2005). The study area lies at the mid-nemoral level, i.e. the zone of mixed deciduous forest with *Carpinus betulus* L. most prominent, but with areas of *Fagus sylvatica* L. on higher ridges and oaks (*Quercus* spp.) at lower altitudes: *Q. petraea* (Matt.) Liebl. with prominent *Lathyrus hallersteinii* Baumg. near Sibiu and the Târnava river, and *Q. robur* L. with *Melampyrum bihariense* A. Kern. toward the Olt valley in the south (Doniță & al. 1992; Mountford & Akeroyd 2005). However, much of this study area (and corresponding areas in Moldavia) has been partially cleared of forest to produce grasslands, with a flora derived from fragmentary ancient grassland on naturally open ground (e.g. steep slopes, eroding river banks, etc.). The meadows and pastures of the Saxon lands comprise a mixture of Central and South-east European steppic elements, together with more widespread Continental species, as well as some localised sub-Mediterranean elements (Akeroyd 2002, 2006). The rich floral (and faunal) diversity of this district derives from the persistence of traditional farming, which has given rise to a landscape that is approximately a third woodland, a third grassland and a third arable (Akeroyd 2006).

During most of our visits, the purpose of the botanical recording was to prepare floristic accounts of particular parishes or communes where nature conservation action was being considered. The initial data therefore largely comprised species lists for areas of uniform vegetation, annotated with abundance estimates. Increasingly, however, data have been gathered from stands using traditional phytosociological relevés (Braun-Blanquet 1964) in an attempt to understand the vegetation and its pattern (Oroian & al. 2004, 2005; A. Jones & J.R. Akeroyd unpubl.). In addition ordination studies of stands of grassland in relation to soil and land use are in progress (A. Jones unpubl.). Although our work has largely used Tutin & al. (1964–1980, 1993) as its benchmark for taxonomy, the authors here have also used the *Flora of Romania* (Ciocârlan 2000) and indicated in square brackets the modern Romanian usage where that differs from *Flora Europaea*. Further insights into the distribution of species in southern Transylvania were gained from Drăgulescu (2003) and Sămărghițan (2005), both of which also provide invaluable information on the plant communities within which each species typically occurs.

Since 1990, there have been several major works on the habitats and vegetation of Romania, building on the strong earlier tradition of phytosociological investigation. The present paper uses Sanda & al. (2001) and Doniță & al. (1992, 2005) as the key references, and authorities for individual syntaxa are presented in Tables 1 and 2. Doniță & al. (2005) have the additional advantage of cross-referencing vegetation associations in Romania to the habitats classified by EUNIS (Davies & Moss 1999), CORINE (European Communities 1991), the Palaearctic Habitats system (Devillers & al. 1999), Emerald and Natura 2000. We have used the habitat nomenclature and interpretation of Doniță & al. (2005) as the most up-to-date account of Romanian habitats and their correspondence to Europe-wide systems.

Results

The results of the vegetation classification are outlined in three tables. Table 1 lists the dry grassland habitats observed in the Saxon villages area, categorised according to the modern Romanian system and lists the corresponding EUNIS type given by Doniță & al. (2005). The habitat types are divided into steppic types (where *Brachypodium pinnatum* (L.) P. Beauv., *Bromus erectus* Huds., *Carex humilis* Leyss., *Chrysopogon gryllus* (L.)

Table 1. Steppic and drier mesotrophic grassland habitats recorded, after Doniță & al. (2005), cross referenced to EUNIS (Davies & Moss 1999) [Where there is no EUNIS number/name, equivalent CORINE number/name is used – in square brackets].

Romanian habitat number and name	EUNIS number and name
STEPPIC GRASSLANDS	
R3404 Pontic–Pannonic grasslands with *Festuca rupicola* and *Koeleria macrantha*	**E1.2C1** Pannonic loess steppes
R3406 Dacic–Sarmatic grasslands with *Carex humilis*, *Stipa joannis* and *Brachypodium pinnatum*	**E1.D2** Ponto–Sarmatic steppes
R3408 Dacic grasslands with *Bromus erectus*, *Festuca rupicola* and *Koeleria macrantha*	[34.322 Middle European *Bromus erectus* dry grassland]
R3409 Pontic grasslands with *Stipa lessingiana*, *S. pulcherrima* and *S. joannis*	[Correspondence not clear to either CORINE or EUNIS]
R3411 Dacic–Balkanic grasslands with *Chrysopogon gryllus* and *Festuca rupicola*	**E1.551** Lowland savory–*Chrysopogon* dry grasslands
R3414 Pontic–Pannonic grasslands with *Festuca valesiaca*	[34.312 Central European steppe grassland]
DRIER MESOTROPHIC GRASSLANDS	
R3802 Dacic–Getic meadows with *Arrhenatherum elatius*	**E2.233** Carpathian submontane hay meadows
R3803 South-Eastern Carpathian grasslands with *Agrostis capillaris* and *Festuca rubra*	**E1.721** Nemoral *Agrostis–Festuca* grasslands, i.e. of deciduous forest zone
R3804 Dacic–Getic grasslands with *Agrostis capillaris* and *Anthoxanthum odoratum*	**E1.721** Nemoral *Agrostis–Festuca* grasslands

Table 2. Phytosociological associations of dry grassland recorded, after Doniță & al. (2005) and Sanda & al. (2001).

FESTUCO–BROMETEA Br.-Bl. & R. Tx. ex Klika & Hadač 1944
- *Stipo pulcherrimae–Festucetalia pallentis* I. Pop 1968
  - *Bromo–Festucion pallentis* Zólyomi 1966
    - *Chrysopogon–Caricetum humilis* Zólyomi (1950) 1958
  - *Thymo comosi–Festucion rupicolae* Pop 1968
    - *Carici humilis–Stipetum joannis* Pop & Hodian 1985
- *Festucetalia valesiacae* Br.-Bl. & R. Tx. ex Br.-Bl. 1949
  - *Festucion valesiacae* Klika 1931 [synonym *Festucion rupicolae* Soó (1929 n.n.) 1940 corr. Soó 1964]
    - *Medicagini minimae–Festucetum valesiacae* Wagner 1941
    - *Festucetum rupicolae* Burduja & al. 1956
    - *Thymo pannonici–Chrysopogonetum grylli* Doniță & al. 1992
    - *Salvio nutanti–nemorosae–Festucetum rupicolae* Zólyomi 1958
  - *Stipion lessingianae* Soó 1942
    - *Stipetum pulcherrimae* Soó 1947
    - *Stipetum lessingianae* Soó (1927 n.n.) 1947
- *Brometalia erecti* Br.-Bl. 1936
  - *Cirsio–Brachypodion* Hadač & Klika in Klika & Hadač 1944
    - *Thymo comosi–Caricetum humilis* (Zólyomi 1931) Morariu & Danciu 1974 [Related to *Cariceto humilis–Brachypodietum pinnati* Soó (1942) 1947]
    - *Rhinantho rumelici–Brometum erecti* Sanda & Popescu 1999

MOLINIO–ARRHENATHERETEA Tx. 1937
- *Arrhenatheretalia* Pawl. 1928
  - *Arrhenatherion* Koch 1926
    - *Arrhenatheretum eliatoris* Br.-Bl. ex Scherrer 1925
  - *Cynosurion* R. Tx. 1947
    - *Festuca rubrae–Agrostetum capillaris* Horvat 1951
    - *Anthoxantho–Agrostetum capillaris* Silinger 1933

Trin., *Festuca rupicola* Heuff., *F. valesiaca* Schleich. ex Gaudin, *Koeleria macrantha* (Ledeb.) Schult. and *Stipa* species are prominent), and more mesotrophic, yet still dry, situations where the dominants include *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. & C. Presl and *Festuca rubra* L. Table 2 interprets these habitats in terms of the vegetation associations (and other levels of the phytosociological hierarchy) to which they correspond. Table 3 repeats the procedure for those grasslands that occur in moist situations or associated with stream floodplains and spring-lines, separately listing the hay-meadows of the *Molinio–Arrhenatheretea* and the highly restricted vegetation types around springs (mostly *Caricion davallianae*).

Finally, Table 4 presents a partial and selective catalogue of species with some nature conservation interest, either because they are rare, uncommon or threatened in Romania or in Europe generally (Cerovsky 1995) or because they are attractive and declining species whose presence might be of wider interest and importance for eco-tourism to the region. Table 4 also draws attention to non-grassland communities that are associated with the meadows

Table 3. Wet grassland and wetland. I. Habitats and II. Phytosociological associations recorded [Other legend as Tables 1 & 2].

I. Habitats	
Romanian habitat number and name	**EUNIS number and name**
R3710 Dacic meadows with *Molinia caerulea*	**E3.511** Calcicolous purple moor-grass meadows
R3712 Dacic communities with *Deschampsia cespitosa* and *Agrostis stolonifera*	**E2.233** Carpathian submontane hay meadows
R3715 Danubian–Pontic meadows with *Agrostis stolonifera*	**E2.251** Ponto–Pannonic mesophile hay
R3716 Danubian–Pontic meadows with *Poa pratensis, Festuca pratensis* and *Alopecurus pratensis*	**E2.251** Ponto–Pannonic mesophile hay
R5405 Eutrophic South-East Carpathian fens with *Carex flava* and *Eriophorum latifolium* [At higher altitudes, **R5406** Eutrophic SE Carpathian fens with *Carex flava* and *Blysmus compressus* may occur]	**D4.153** Middle European yellow sedge fens
II. Phytosociological associations	
MOLINIO–ARRHENATHERETEA Tx. 1937	
Molinietalia Koch 1926	
Molinion caeruleae Koch 1926	
Junco–Molinietum Priesing 1951	
Agrostion stoloniferae Soó (1933) 1971 [Some fragmentary stands referable to the *Calthion* R. Tx. 1937]	
Agrostetum stoloniferae (Ujvarosi 1941) Burduja 1956 [*Agrostio stoloniferae–Deschampsietum cespitosae* Ujvarosi 1947 may also be present]	
Ranunculo repentis–Alopecuretum pratensis Ellmauer 1933	
Agrostideto–Festucetum pratensis Soó 1949	
SCHEUCHZERIO–CARICETEA FUSCAE Tx. 1937	
Caricetalia davallianae Klika 1934	
Caricion davallianae Klika 1934	
Carici flavae–Eriophoretum latifolii Soó 1944 [Trend to *Carici flavae–Blysmetum compressi* Coldea 1997]	

Table 4. Selected species of nature conservation interest recorded in the village grasslands of Romania and the phytosociological syntaxa within which they typically occur. Based upon Sanda & al. (2001), Drăgulescu (2003) and Doniță & al. (2005), supported by fieldwork from the research reported here (Akeroyd 2002; Jones & Akeroyd unpubl.; Oroian & al. 2004, 2005; Mountford & Akeroyd 2005). Taxonomy as Tutin & al. (1964–1980, 1993) with synonyms from Ciocârlan (2000) indicated in square brackets, i.e. […].

A. Class *FESTUCO–BROMETEA*:	*Scorzonera purpurea* L.	*Stipa pennata* L.	*Thesium linophyllon* L.
A1 Order *Festucetalia valesiacae*:	*Adonis vernalis* L.	*Brassica elongata* Ehrh.	*Campanula sibirica* L.
	Chamaecytisus banaticus (Griseb. & Schenk) Rothm.	*Chrysopogon gryllus*	*Inula ensifolia* L.
	Hypericum elegans Stephan ex Willd.	*Iris pumila* L.	*Linum flavum* L.
	Orchis militaris L.	*Thesium linophyllon* L.	*Verbascum phoeniceum* L.
A1.1 Alliance *Festucion valesiacae*:	*Astragalus austriacus* Jacq.	*Astragalus dasyanthus* Pall.	*Crambe tataria* Sebeók
	Echium russicum J.F. Gmel. [*E. maculatum*]	*Ferulago sylvatica* (Besser) Rchb.	*Jurinea mollis* (L.) Rchb.
	Linum hirsutum L.	*Orchis tridentata* Scop.	*Orlaya grandiflora* (L.) Hoffm.	*Salvia nutans* L.
	Seseli pallasii Besser	*Trinia ramosissima* (Fisch. ex Trevis.) Koch	*Viola ambigua* Waldst. & Kit.
A1.2 Alliance *Stipion lessingianae*:	*Iris pumila*	*Salvia transsilvanica* (Schur ex Griseb.) Schur	
A2 Order *Brometalia erecti*:	*Linum flavum* L.		
A2.1 Alliance *Cirsio–Brachypodion*:	*Inula ensifolia*		
A3 Order *Brachypodio–Chrysopogonetalia* (Horvatic 1958) Boşcaiu 1972 [including *Danthonio–Brachypodion* Boşcaiu 1972, etc.]			
	Carlina acanthifolia All.	*Chrysopogon gryllus*	*Ferulago sylvatica*	*Salvia nutans*
	Trinia ramosissima			
B. Alliance *Festucion vaginatae* Soó 1929 [Within **Class *FESTUCETEA VAGINATAE*** Soó 1968]			
	Astragalus austriacus	*Chrysopogon gryllus*	*Linum hirsutum* L.	*Verbascum phoeniceum* L.
C. Alliance *Geranion sanguinei* R. Tx. 1961 [Within **Class *TRIFOLIO–GERANIETEA SANGUINEI*** Th. Müller 1961]			
	Dictamnus albus L.	*Echium russicum*	*Lembotropis nigricans* (L.) Griseb.	
	Linum flavum	*Prunus tenella* Batsch [*Amygdalus nana*]		
	[& other species of ***FESTUCO–BROMETEA*** and ***Prunetalia***]			

and pastures and which contain species of interest, specifically the sunny fringe communities of woodland edges belonging to the *Geranion sanguinei* alliance. This ecotonal vegetation is especially species-rich and attractively colourful, including not only its own distinctive flora but also elements derived from the grassland and the woodlands with which they form a habitat mosaic.

Such full information is not available for the other meadows that have been surveyed during this work,

e.g. within the Bârsa Mare valley (Braşov county) and the Stulpicani district of Suceava county. These meadows are similarly species-rich and beautiful, but lack the more distinctive steppic and sub-Mediterranean elements present in the core of the Saxon lands. Examples surveyed include more vegetation of the *Molinietalia* (both *Molinion caeruleae* and *Calthion palustris*) in which *Colchicum autumnale* L., *Dactylorhiza* spp., *Gladiolus imbricatus* Mill., *Parnassia palustris* L. and *Trollius europaeus* L. are often prominent. Similar communities, with *Alopecurus pratensis* L., *G. imbricatus*, *Sanguisorba officinalis* L. and *T. europaeus*, occur locally in damp, often north-facing meadows in the north-eastern part of the Saxon lands and eastward where Mureş, Braşov and Harghita counties meet (J.R. Akeroyd & al. unpubl.). Communities of the *Arrhenatheretalia* with *Orchis morio* L. and *O. coriophora* L. are present, locally with a clear transition to particular alliances, e.g. the *Cynosurion cristati* or possibly the *Polygono–Trisetion* Br.-Bl. & Tx. em. Marsch. 1947. The floristic composition of these non-steppic meadows is altogether more submontane and Continental, with links to meadow types in mountain districts elsewhere, especially in Central Europe. Some of the associations are rare and threatened in South-eastern Europe.

Discussion

The nature conservation interest of these habitats and their component communities can be problematic to assess objectively against agreed standards, since in Romania such anthropogenic habitats have often been neglected or undervalued by such evaluations, which (perhaps understandably) place greatest importance in communities and habitats with some claim to be "natural". The assessment given by Doniță & al. (2005) reckons that most of the habitats identified here for the Saxon lands or other important meadow areas have only moderate or reduced conservation value, except in limited examples where particular nationally rare species are found. Indeed, the only habitat that they list as having consistently high conservation value is the Eutrophic South-East Carpathian fens with *Carex flava* L. (R5405), which is noted as a priority habitat under the Emerald scheme. Such small-sedge fens are extremely restricted in extent in the Saxon lands, and represent islands of spring-fed wetland in a sea of drier grasslands.

However, we would strongly argue that the "village and farm" grasslands of Romania may well represent the most extensive, and thus most important, old grasslands in Europe. Different communities and species assemblages occur in Transylvania, in Moldavia, in Maramureş and in the low to middle altitudes of the Carpathians (Doniță & al. 1992, 2005; Sanda & al. 2001). Taken together as a national biodiversity resource, these grasslands are certainly of international significance. We maintain that their development over millennia of settlement and traditional regimes of cutting, grazing and burning of scrub (on slopes) makes them not only a key biodiversity resource but also a fascinating artefact of human history, comparable in value with the painted monasteries of southern Bucovina, the fortified Saxon churches of Transylvania and the historic Dacian remains of Sarmizegetusa. Not only do they contain significant pockets of rare plant species, but they also support considerable general floristic richness, along with rare fauna such as the globally threatened *Crex crex* L., as well as other birds, insects and amphibians listed on Annexe II of the EU Habitats Convention.

As yet, we have provisionally allocated the grasslands of the Saxon lands to some 10 associations of steppic swards and at least 3 types of dry mesotrophic grassland and 4 or more damp or wet grasslands. However, we acknowledge that the complex combination of land-forms and management history land means that the precise limits of phytosociological communities in our study areas are difficult to set. This complexity is most apparent where mosaics in response to gradient, soil depth, soil moisture and aspect are further affected by human disturbance as, for example, where swards dominated by *Chrysopogon gryllus* indicate "secondary steppe".

Nonetheless, the floristic value of these village grasslands can be partly demonstrated through examination of Table 4, using distributional information from Tutin & al. (1964–1980), Oltean & al. (1994), Cerovsky (1995), Ciocârlan (2000), Drăgulescu (2003), and Sămărghițan (2005). Useful comparisons with the Romanian situation can be gained from Slovakia (Cerovsky & al. 1999) and Serbia (Stevanović 1999), as well as national biodiversity summaries held on websites, e.g. European Environment Agency (www.eea.europa.eu) and Plant Talk (www.plant-talk.org). The European context alone may be inadequate, e.g. some species have a distribution that straddles Eastern Eu-

rope and Western Asia (Davis & al. 1965–1985), and reference to the "Pontic–Sarmatian" steppic elements (Stefureac 1965) should also be made.

Most of the species listed in Table 4 are limited to East-central and Southeast Europe, including the Balkan peninsula and are most frequent in Romania, Hungary, Ukraine, Bulgaria and southwest Russia, though some extend into Austria and even to southern France or Spain. Several occur in Russia and northeast Anatolia or the Turkish interior, e.g. *Crambe tataria* and *Echium russicum* [*E. maculatum*] (on Annexe II of the EU Habitats Directive). *Crambe tataria* is found all the way to east Kazakhstan but extends no further west than north-eastern Italy. *Salvia transsilvanica* is endemic to Romania, but many other species are decidedly local and declining. There is a strong suggestion that the Romanian populations represent a key relict of species and assemblages that have declined in neighbouring countries. Thus *Adonis vernalis* is threatened over most of its range in Central and South-eastern Europe; *Brassica elongata* and *E. russicum* are both critically endangered in Slovakia (*E. russicum* also in the Czech Republic) whilst *Salvia nutans*, one of several related Eurasian steppic taxa, is critically endangered in Hungary and extinct in Serbia. Species of more southerly distribution in Europe include *Chrysopogon gryllus*, *Orchis coriophora*, *O. tridentata*, and *Orlaya grandiflora*.

The importance of this resource and especially of the examples in the Saxon Lands requires some protective action. However, it is crucial that any such action has active local support and participation, and that it does not threaten the livelihoods of the traditional farmers. Hence, probably the best way forward is to consider a large (95 000 ha) Natura 2000 site supported by the Romanian Ministry of Environment and Water Management and designated under EU law – as suggested by *Fundația Adept* (www.fundatia-adept.org). Longer-term consideration might be given to Category V Protected Landscape status (IUCN, CNPPA & WCMC 1994), but it would be important that such a protected area covered the entire resource, rather than separate fragments of 50–1000 ha (Cristea 1995). Ongoing botanical and zoological studies by scientists from the Universities of Cluj, Sibiu, and Târgu Mureș indicate that the Saxon lands comprise a prime candidate area for Natura 2000 designation. Biodiversity conservation must be accompanied by viable farm livelihoods for those who manage Romanian hay-meadows. Indeed sensitive, non-intensive farming can protect biodiversity and promote sustainable development. Farming subsidies for traditionally managed grassland combined with education programmes and training courses in producing and marketing high quality foods may well be the most practical and successful option (Akeroyd 2006; Akeroyd & Page 2006).

Acknowledgements. Our thanks go to *Fundația Adept* for funding the fieldwork of UK and Romanian researchers, through the good offices of Nat Page and Jim Turnbull. JRA would like to thank Dr Andrew Jones, Dr Silvia Oroian, Ing. Mariana Hiriţiu and Dr Mihaela Sămărghiţan for access to unpublished data. JOM is grateful to Dr Doru Banaduc and Prof. Constantin Drăgulescu (both Lucian Blaga University, Sibiu) for their advice, and Marilena Onete (Institute of Biology of the Romanian Academy) for great help with access to relevant Romanian literature and its translation.

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Baseline survey as a tool in informing the management of protected areas in Romania

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Abstract. To manage a national park effectively, one needs to know what biota it holds, where they occur and what their environmental needs are. Despite past excellent research, good coverage of all biota is hard to achieve in a big national park. The BCM project tried to achieve a pragmatic approach to survey in three parks including Piatra Craiului that would: be a model for Romanian protected areas; characterise all the habitats in the parks; be robust to surveyors with limited taxonomic or field skills; be consistent between parks, allowing managers to compare the results of their management, and provide a consistent baseline against which change due to management or external factors could be assessed. This paper describes an approach that was flexible, but provided a basis for a rigorous quantitative assessment of biodiversity and ecology. The approach has been used as a platform for a multitude of studies, resulting in a huge increase in knowledge for Piatra Craiului and other parks.

Key words: baseline survey, Carpathians, management plan, monitoring, national park, research programme

Introduction

The case in favour of biodiversity conservation and sustainable use of natural resources has been argued many times, both at a local and at an international scale, not least through the *Convention on Biological Diversity*, signed by 150 government leaders at the 1992 Rio Earth Summit. Changes in socio-economic and political frameworks as well as altered environmental conditions (e.g. climate change) mean that the case for conservation has to be frequently repeated, re-interpreted and reinforced. However, among several unchanging principles is that effective implementation of conservation action depends on knowledge, i.e. what biota are present, what conditions these biota require, whether the conditions are presently suitable and what intervention (if any) is required to maintain or re-establish suitable conditions.

Protected areas, such as national parks, pose particular problems. Their biodiversity value cannot be readily moved if conditions change. The value society puts on the park means that it will receive more visitors, both tourist and educational, than "ordinary" rural areas. The park may border intensively exploited land, whose management for profit can have an impact on the protected area. As well as the natural (wildlife) resources, the park may possess mineral, timber and agricultural potential that other stakeholders will be anxious to exploit. In Europe many national parks include areas of cultural landscape. When such anthropogenic habitats are brought within a refuge, conflicts may arise between the need to maintain continuity of traditional management and the desire to emphasise wilderness and the governing role of natural processes.

In all such cases, and confronted with numerous other problems, the protected area will require a management plan to ensure that biodiversity is conserved or enhanced. The plan must have clear and measurable objectives with target habitats and species. This plan will in turn depend for its efficacy on characterising the habitats and vegetation associations of the national park and relating these to present and future en-

vironmental conditions. This is the role of a baseline survey and the monitoring schemes that derive from it – without knowing what biota the park has, where the biota are and how they are changing, any management applied is, at best, guesswork and, at worst, wasted effort.

Romanian Biodiversity Conservation Management Project

From 1990 Romania not only ratified the major international biodiversity conventions but also created its own *Biodiversity Conservation Strategy and Action Plan*. To meet the plan aims, in 1999 the Romanian government (supported by GEF – World Bank Global Environmental Facility) funded the *Biodiversity Conservation Management Project* (BCMP) to be run by the Ministry of Waters, Forests and Environmental Protection (MWFEP – now Ministry of Environment & Water Management) and the National Forest Authority (NFA). One objective of the *BCMP* was to establish effective, sustainable conservation management in the Carpathians, which might then act as models for best practice at other existing and potential protected areas in Romania (MWFEP 1999). Three areas were chosen to test different management strategies:

- Retezat National Park Biosphere Reserve – with extensive pristine mountain forest and alpine ecosystems;
- Vanatori–Neamț Forest Park, to test biodiversity-friendly sustainable forest management, within a region of natural mixed hill-forest and meadows.
- Piatra Craiului–Bucegi Natural Park (*ca*. 100 000 ha) with *ca*. 3400 ha of pristine ecosystems, surrounded by productive forestry and agriculture. Piatra Craiului was later declared a National Park and is the focus of the research reported here.

MWFEP commissioned the UK *Centre for Ecology and Hydrology* and *Komex International* to a) develop survey approaches for baseline biodiversity of the 3 model areas (Patriquin & al. 2000); and b) advise on the creation of a Biodiversity Information Management System (Moss & Harding 2000). Within *BCMP*, baseline survey was to help identify conservation priorities, guide conservation planning and management, and incorporate monitoring systems. The approach had to address known threats, e.g. over-grazing, tourism impacts, hunting, etc. As well as the operational manual and report to MWFEP and NFA (Patriquin & al. 2000), a summary of the approach is given by Mountford & al. (2005).

The *BCMP* tried to provide a pragmatic approach to gathering enough data for Piatra Craiului, etc. that would a) be a model for Romanian protected areas; b) characterise the habitats throughout the parks; c) be robust to surveyors with limited taxonomic and field skills; d) be consistent between parks, allowing managers to compare the efficacy of their management; and e) provide a rigorous baseline against which change due to management or external factors could be gauged and quantified.

The baseline survey itself began in 2000 and continued until 2003. The results of the surveys in the three parks have been reported in a series of publications. The present paper focuses on Piatra Craiului where there has been an attempt to review the output of the survey approach and where a full scale biodiversity monitoring plan has been drafted and implemented (Pop 2005). The paper confines it attention to the botanical, phytosociological and broad habitat components of the surveys, outlining the methods used, assessing how practical they proved and how they can influence both park management and the growth of research. It should be noted, however, that the output of the survey also included much information on animal groups, with many articles derived from such work (Pop & Vergheleț 2003; Pop 2006; Pop & Hanganu 2006).

Methods

By 2000, Piatra Craiului had received scientific study, though most of this was focussed on particular research sites, rather than the park as a whole. In contrast, the goal of the new survey was to create a rigorous and geographically representative baseline, allowing changes due to management or other factors to be quantified, and management action to be targeted. Literature referring to all three parks and their surroundings was reviewed and species checklists derived, highlighting taxa with designated conservation status and those that were ecologically important. Existing information on habitats and communities was related to the EUNIS habitat classification (Davies & Moss 1999). Detailed base-maps for the study areas

were to be compiled at 1:20 000 scale from forest cover maps, land use maps and topographic maps to allow development of a GIS.

Sampling Piatra Craiului: where shall we survey?

Although a comprehensive survey covering all parts of the park would be the ideal, the size of the park and the limited availability of surveyors made this impractical. Instead the approach attempted to include representative areas of all habitats from all parts of the park. For this purpose, a provisional habitat map was constructed from all available data, and used to plan the strategy. As survey results accrued, changing estimates of the location and extent of each habitat would be used to redistribute survey effort. The original recommendation (Patriquin & al. 2000) was that sample plots for vegetation (and fauna) be selected randomly using numbered grid cells on the 1:20 000 base-maps, each of 1 km^2 and subdivided into four 25 ha sub-cells. Following an initial selection, very remote cells or those that posed a real safety risk were removed from the list of survey-cells, and substitutes chosen checking all sample-cells against the provisional habitat map to ascertain whether there was any sampling bias. From cells recorded in the first year, a representative subset could be used for longer-term monitoring. The survey-cell was intended to serve as a broad sampling unit, within which the various elements of the ecological surveys would be conducted. Square plots should be accurately established within the survey-cell, using the base-maps and GPS to find the centre-point and then measuring off with a hip-chain and compass the edges and corners of the plot. Markers would be placed at regular intervals and key reference points to allow the plot to be subdivided into a grid, as well as to aid in refinding the plot.

Sampling Piatra Craiului: what shall we survey?

Although all biodiversity elements have value, scarcity of resources meant that priority had to be given to data that could be applied directly to informing management of the resource. The recommended approach was based on a general inventory of ecosystems, habitats and selected associated taxa, with more detailed surveys of biota considered important or valued, i.e.: 1) designated as of conservation importance; 2) characteristic of the park; 3) restricted distribution; 4) declining status; 5) ecological importance (keystones, etc.); 6) monitoring or scientific value; and 7) economic or social importance.

Sampling Piatra Craiului: when shall we survey?

The first year was to focus on revising forest cover and the provisional habitat map, as well as an inventory of selected biota for monitoring and detailed study. Subsequent years would include this monitoring, expand the overall coverage and also focus on valued ecosystem components and management-related issues. The main botanical effort occurred during June–September (2000–2003) when access to higher altitudes was simpler, but subsequent studies focussed on the spring flora at lower altitudes.

Vegetation survey

The core components of the baseline survey were vegetation recording to provide the habitat characterisation and ornithological survey. As habitats were recorded within sample-cells, they were classified by altitude, aspect, soil type, etc., allowing habitat suitability maps for important species to be derived later. Within each habitat type delineated, a 30 m transect would be established as the centre-line of a rectangular macro-plot (20 m × 30 m), which could in turn be used to survey tree density, cover, and species composition (Fig. 1). In forest or scrub, recording would include:

- Five 1 m^2 quadrats (total sampled area 5 m^2) at 5 m intervals on transect: all species < 1 m tall (vascular plants, bryophytes, lichens) with percentage cover estimated.
- Three plots of 3.1 m radius (total sampled area 90 m^2) located at 5 m, 15 m and 25 m points on the transect: record species in the 1–3 m (tall shrub) stratum.
- Entire macro-plot (total sampled area 600 m^2): cover of tree species and number of individuals of each species, allowing tree density to be estimated.

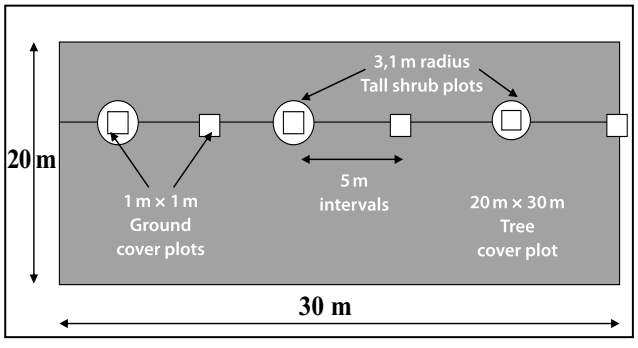

Fig. 1. Baseline Survey: recommended design of vegetation transects in Macro-plot 20 m × 30 m (after Patriquin & al. 2000).

Where a stratum (e.g. trees or tall shrub) is missing, the relevant plots would be omitted. Mueller-Dombois & Ellenberg (1974) advocate a similar design and sampled areas.

Recommended statistical analyses of vegetation data

Initial averaging of the replicated samples from each transect would produce a single summary relevé to be used in all subsequent analysis. The survey of Piatra Craiului would produce a large body of vegetation and environmental data requiring initial exploration to establish broad patterns, e.g. through ordination in CANOCO (ter Braak & Šmilauer 1998). Such a multivariate approach could indicate relationships or patterns worthy of detailed investigation and analysis. Species indicator values can be used in the analysis, either those derived for Central Europe by Ellenberg (1988) for temperature (T), light (L), soil reaction (R), fertility (N) and soil moisture (F), or those more widely used in Romania (Sanda & al. 2003) for moisture (U), temperature (T) and soil reaction (R). Such values can be used in monitoring since where the mean indicator values of the species complement shows a clear consistent change over time, this might provide a warning of acidification (e.g. mR) or fertilisation (mN) – see Stevens (2004).

Results

The approaches advocated in the *Operational Manual* (Patriquin & al. 2000) were scientifically-based and derived from techniques widely applied in similar landscapes elsewhere. However, testing of the approach in Piatra Craiului (and indeed the other parks) showed that full application of the baseline survey methodology could encounter problems, arising almost entirely from a shortage of expert surveyors. The description given here states where the advocated methods proved impractical and where their inherent flexibility allowed progress. It may be assumed that the methods outlined above were applied as described unless otherwise stated.

Survey preparation: literature, maps and aerial photographs

Preparation in Piatra Craiului gained from a recent and detailed doctoral study of the flora and vegetation (Mihăilescu 2001). The preliminary checklist was augmented with information on the habitat requirements of the species (e.g. Ellenberg 1988). Two types of map, forest cover (1:5000) and topographic (1:25 000), were used in planning the baseline survey, together with aerial photographs (at scales 1:7500, 1:10 000 and 1:15 000 from 1968, 1979 and 1991) to aid identification of major habitat types in the park. Practical difficulties in using these photographs were met especially in the high alpine zone where cloud cover meant that rock faces and screes could not always be identified. The preliminary base-map of EUNIS habitats was constructed using the 1:25 000 topographic map, and annotated with different colours corresponding to 9 major habitat types selected for special attention. The survey team found it easier to define "local types" adapted from EUNIS. This difficulty may have arisen partly from misunderstanding of the EUNIS system and partly from the fact that Romanian habitats had then not been fully characterised within EUNIS, since rectified by Doniță & al. (2005). The nine types (with EUNIS codes) were: a) grasslands used as hay fields (*Cynosurion*) mainly at the foot of the mountain [E2]; b) alpine and subalpine pastures (*Potentillo–Nardion*, *Seslerion*, etc.) [E4.3, E4.4]; c) rock faces, grasslands and scrub of alpine zone [F2.2, F2.3, E4, etc.]; d) calcareous scree [H2.4]; e) *Fagus sylvatica* forest [G1.6]; f) mixed *Fagus*–coniferous forest [G4.6]; g) *Picea abies* forest [G3.1]; h) *Pinus mugo* scrub [F2.4]; and i) gorges and canyons.

Sampling design, strategy, sample location and survey components

The recommended stratified random sampling approach was used for the total park area of 14 800 ha (i.e. 592 squares of 500 m × 500 m). Codes for these squares were entered on a database, and labelled with the main habitat type for that area identified from aerial photographs, etc. 60 sample plots were randomly selected, representing *ca*. 10 % of the park. In addition to this core stratified random sample, further plots were located more subjectively in areas known to have important biodiversity and which had not been well covered by the random selection. All the selected sample plots were marked on the base-map. A permanent marker was installed in the plot centre, with adjacent back-up painted marks on the bark of a tree or exposed rock.

The Park Administration decided that some components of the survey prescribed by the *Operational Manual* must be adapted since they believed the quality of the surveyors available was not sufficiently high to fully apply the methodology. This decision resulted in alteration to the original survey schedule, with different components of the survey being undertaken in different years. The GEF requirement to use volunteers, rather than paid contract researchers, resulted in further delays for training in field skills and taxonomy. The baseline survey ran more slowly than planned with some work on the various components, including vegetation survey, going on over four years (2000–2003). In 2000, the forest cover maps were revised, suitable sites for "ecotone" transects and permanent/detailed vegetation plots were identified and attention was paid to habitats not properly demarcated by the aerial photographs (e.g. alpine meadows).

Vegetation survey on Piatra Craiului: successes and problems

The vegetation survey followed the methodology proposed in the *Operational Manual*, with the Park team managing 40 volunteers of very varied knowledge (university professors, PhD students, undergraduate students, etc.). Any apparent discrepancies in data quality could be at least partially remedied by:

I. Experts conducting a limited representative resurvey to apply quality control;
II. Preliminary multivariate data analysis could use "field teams" as a variable and help identify any significant associations between recorders and the presence or absence of particular species;
III. Where differences in data quality are found, separate analyses can be performed on the higher quality data, especially if these cover the full range of habitats.

All selected 60 random sample plots were surveyed together with a further 40 more subjectively chosen sample plots. These 100 sample plots contained 258 transects, i.e.:

- 80 transects in the subalpine and alpine area (including alpine meadows, dwarf pine scrub, scree slopes and rocky faces);
- 32 in the *Picea abies* forests (including old stands, young stands, harvested areas, clearings inside the spruce forests, edge of the forest);
- 44 in mixed forest (with the same range of locations as before);
- 34 in mountain meadows (including grazed and ungrazed meadows);
- 20 in hay fields (included areas with bushes inside the hay fields);
- 30 in marshes with a further 18 along the riparian zone of rivers and streams.

Vegetation survey on Piatra Craiului: analysis

It is clearly desirable to classify the vegetation samples in terms of plant communities (Mihăilescu 2001) and habitats (Doniță & al. 2005) known to occur in the Carpathians. It will be important also to ascertain whether as yet undescribed syntaxa occur in Piatra Craiului, and whether these are revealed by analysis. A new syntaxon has already been described, i.e. *Carici remotae–Calthetum laethae* Coldea (1972) 1978 *ligularietosum sibiricae* nova subass. (Alexiu & Stancu in Pop & Vergheleț 2003). Independent investigative analysis of the vegetation data will be required firstly, classifying Piatra Craiului samples in terms of their species assemblages and then comparing the results with established syntaxonomy. The second stage of analysis should relate vegetation composition to environmental factors. These vegetation analyses are under way, and a first version of the habitats map has been constructed in a GIS (Fig. 2).

Piatra Craiului botanical studies: outputs

Botanical studies of Piatra Craiului have added 94 new taxa to the vascular plant list, which now totals 1178 taxa (1059 species, 113 species, 6 varieties). One of these (*Woodsia pulchella* Bertol.) is new to the Romanian flora. The rigour of the survey and preparation has also shown that 10 taxa previously noted are not actually part of the Romanian flora. The detailed baseline survey has allowed a *Red List of Higher Plants in Piatra Craiului National Park* to be created, as well as identification and mapping of the major "hotspots" for important plant species, both known and new. The key working outputs are the habitat map and electronic database, but many published outputs are already available on the flora and ecology of Piatra Craiului (Appendix 1).

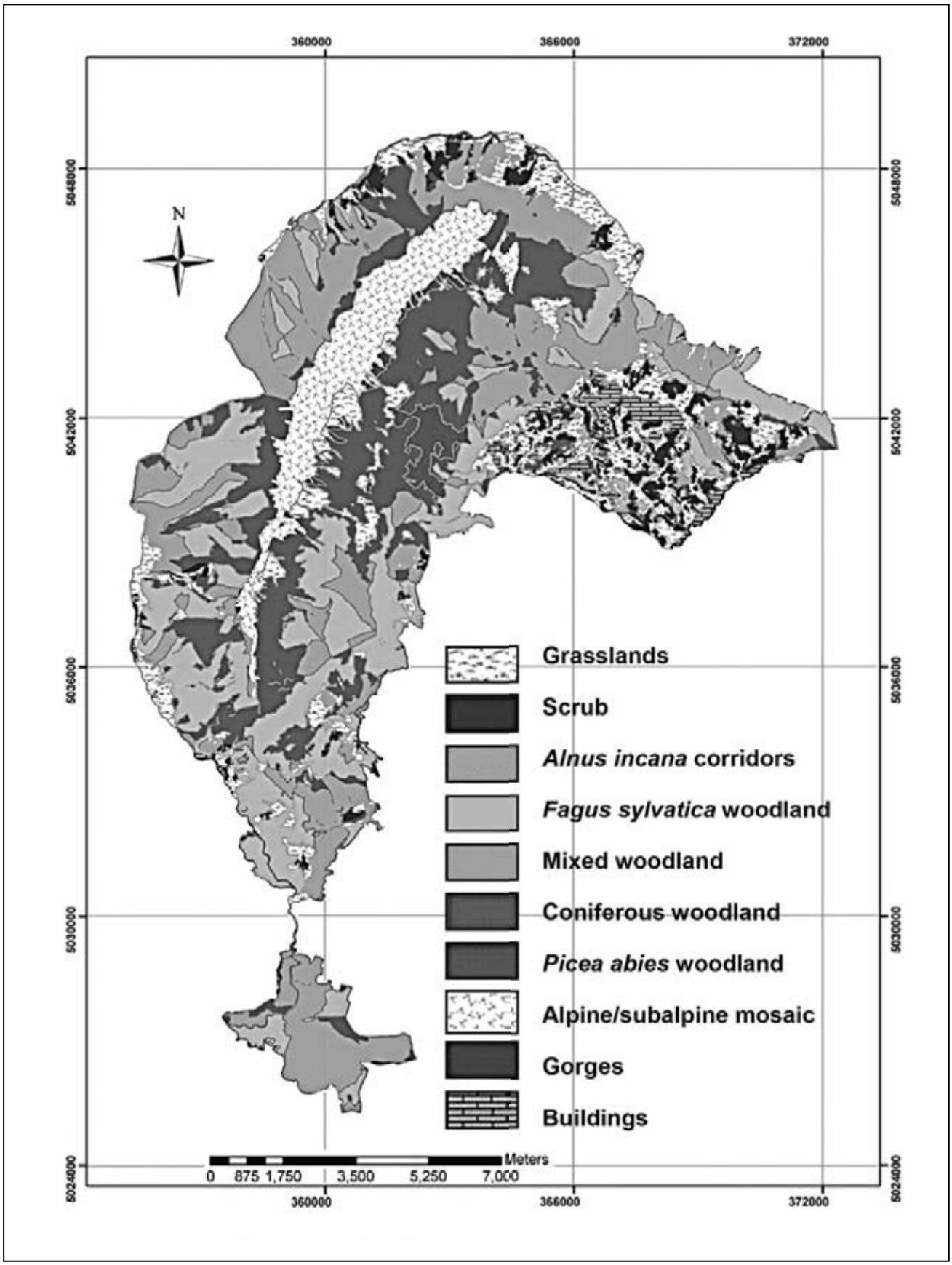

Fig. 2. Map of the main habitats of Piatra Craiului National Park (Romania). After Pop & Vezeanu in Pop (2006).

Appendix 1. Botanical studies in Piatra Craiului National Park (NP) as published in: 1) Pop & Vergheleț (2003); 2) Pop (2006); and 3) Pop & Hanganu (2006).

1) Volume **1** (2003):

Alexiu, V. & Stancu, D. *Carici remotae–Calthetum laethae* Coldea (1972) 1978 *ligularietosum sibiricae* nova subass. in the Brusturet Gorges (Piatra Craiului). Pp. 94-97.

Ciocârlan, V. & Pop, O. *Woodsia pulchella* Bertol. in Romanian flora. Pp. 84-86.

Ciocârlan, V. & Pop, O. Annotations on the Piatra Craiului Massif flora. Pp. 98-99.

Dihoru, G., Ștefănuț, S., Walfish, R. & Pop, O. Bryophyte flora of the Piatra Craiului Massif. Pp. 68-83.

Marcoci, C.N. New systematic and ecological data regarding the lichens of Piatra Craiului NP. Pp. 63-67.

Marușca, T., Pop, O., Vodă, A. & Vergheleț, M. Evaluation of grazing pressure on the Piatra Craiului National Park's pastures and management issues. Pp. 130-141.

Pop, O. Ethnobotanical note of Piatra Craiului NP. Pp. 150-158.

2) Volume **2** (2006):

Alexiu, V. & Stancu, D.I. Flora and vegetation succession as a result of beech deforestation in Dragoslovenilor Valley (Piatra Craiului NP). Pp. 103-107.

Dihoru, G. & Pop, O.G. New locations with *Musci* (*Bryophyta*) in Piatra Craiului NP. Pp. 80-87.

Marcoci, C.N. Macro-lichens of Dâmbovicioara Basin (Piatra Craiului NP). Pp. 94-99.

Onete, M., Ștefănuț, S., Pop, O.G. & Mountford, J.O. Geographical and ecological distribution of the narrow endemic and relict *Dianthus callizonus* in Piatra Craiului Massif. Pp. 134-143.

Pop, O.G. Identification of Important Plant Areas (IPAs) within Piatra Craiului NP. Pp. 108-133.

Pop, O.G. & Vezeanu, C. Mapping of the main habitats in the Piatra Craiului NP. Pp. 144-151.

Ștefănuț, S. *Frullania parvistipula* Steph. in the Piatra Craiului NP. Pp. 88-94.

Ududec, R.V. & Pop, O.G. Short note: new plant species and new locations of some species for Piatra Craiului NP. Pp. 100-102.

3) Volume **3** (2006):

Ciocârlan, V., Danciu, M., Pop, O.G & Indreica, A. New plant taxa and new location of some important plant species for Piatra Craiului NP. Pp. 86-90.

Danciu, M., Pop, O.G. & Indreica, A. The vegetation of two important habitats along the rivers of Piatra Craiului NP (alder corridors and *Myricaria germanica* scrub). Pp. 91-99.

Mihăilescu, S. Habitat identification for the proposal of Piatra Craiului NP as a Natura 2000 site. Pp. 108-113.

Onete, M. & Păunescu, A. Preliminary studies on the clonality of *Dianthus callizonus* species. Pp. 103-107.

Păunescu, A. Conservation of the endemic plant species *Cerastium transsilvanicum* Schur from Piatra Craiului Massif, using *in vitro* techniques. Pp. 100-102.

Pop, O.G. Development of a biodiversity monitoring program for the Romanian protected area. Experiences in Piatra Craiului NP. Pp. 229-247.

Ștefănuț, S. & Pop, O.G. Hornworts and liverworts of the Piatra Craiului NP. Pp. 72-85.

Discussion

The approaches advocated in the *BCMP Operational Manual* (Patriquin & al. 2000) derived much both from Romanian science, and from application and adaptation of methods applied successfully in similar habitats abroad. The project had to integrate historical and new survey data into a GIS that could then function as a management-support tool for the Park Administration into the future. The recommended methods were pragmatic and flexible, but provided a basis for rigorous quantitative assessment of the biodiversity and ecology through standard statistical approaches and tests.

In a national park the size of Piatra Craiului, with its precipitous terrain, good coverage of the distribution of biota is hard to achieve. The output of the surveys to date should be assessed and judged in the context of what the baseline survey was designed to achieve. Survey information has to be representative and rigorous, so that real trends and patterns could be distinguished from mere artefacts of sampling, e.g. locating survey points along frequented trails. Without such rigour, local factors can confuse the results and the Park Administration will be unable to make the right choices confidently.

Much of the information gathered is not only useful as an inventory for the Park but also in scientific terms (Appendix 1). However, these outputs alone can neither satisfy the *BCMP* objectives nor be used confidently to inform present and future management of the Park. Those elements of the baseline survey, such as the vegetation study, that were conducted in a statistically rigorous manner can be used to characterise the communities/habitats and, to some extent, their extent. The next crucial stage of the survey must be to build on the vegetation analysis through: a) relating the communities to measured environmental variables; b) revising the vegetation map; c) relating this map and the communities to current management practices; d) relating results to the ecological requirements of communities and species; and e) revising the manage-

ment plan where necessary to conserve and enhance biodiversity. Refinement of the management plan is accompanied by the monitoring plan (Pop 2005) selecting monitoring plots on the basis of: 1) having representative communities/habitats; and/or 2) current management practices; and using the monitoring results to both assess the success of management and provide early warning of habitat change. *BCMP* tried to put the management of Romanian biodiversity on a firm basis, allowing sustainable management of biodiversity within individual parks and the Carpathians as a whole.

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High Nature Value farmland: scientific challenges

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Abstract. High nature value farmlands (HNVF) are a priority in the European Union's environment policy in 2007–2013. Further to this policy, Bulgaria developed a National Agri-Environmental Programme. One of its main measures concerns HNVFs which still contain localities of species and habitats of conservational significance, and need practical measures for protection against the high anthropogenic pressure. Biodiversity experts face a challenge: to define the criteria of HNVF selection, to define areas and particular farms that meet the set criteria, to make an initial inventory and a large-scale mapping of the sites, to define restoration and maintenance regimes, and to monitor the results of the measures applied.

Key words: agriculture, environment, EU priority

Introduction

Under the conditions of contemporary civilization environmental changes have brought about grave threats to biological diversity on a global scale. Some important political documents and programmes have been developed, targeting practical measures for curbing the adverse effects. At a national level, many countries in the world, including Bulgaria, have developed their own Strategies, Laws, Programmes and Networks of Protected Territories. In synthesis, one could say that some thoroughly developed international and national systems for the protection of natural values now exist. It suffices to mention the internationally organised NATURA – 2000, IBA, IPA, Pan-Parks, etc., as well as the National Parks, Strict Nature Reserves, Managed Nature Reserves, Nature Parks, Protected Territories, etc. at the national level.

They all share one characteristic: the choice of location and their spatial borders are usually determined by the best preserved or relatively well preserved nature complexes!

But outside their limits still remain considerable territories in the zones of intensive anthropogenic activity, where most of the natural biological diversity exists. A typical example to this end is the intensively farmed zones with prevalence of arable lands. Some elements of the natural or semi-natural environment remain within their limits, namely field boundary strips, brooks, rivers or other water reservoirs and wetlands, stony terrains, meadows and pastures, etc., where many representatives of biological diversity have chosen to live.

Baldock & al. (1993, 1995) described the general characteristics of low-input farming systems in terms of biodiversity and management practices and introduced the term **"High Nature Value – farmland"**. Most of these farming systems are characterised by low stocking densities, low use of chemical inputs and often labour-intensive management practices, such as shepherding.

For over 15 years the European Forum on Nature Conservation and Pastoralism (EFNCP) has shown concern for the fate of biodiversity in the regions with intensive farming. It brings together ecologists, nature conservationists, farmers, and policy makers. This non-profit network exists to increase the understanding of high nature conservation and cultural value of certain farming systems.

Discussion

The need in measures to prevent the loss of high nature value farmlands is widely acknowledged now.

In May 2003, this was recognised by the European ministers of environment in Kiev. In their final resolution (UN/ECE 2003) they came out with the following statement on agriculture and biodiversity: "By 2006,

the identification, using agreed common criteria, of all high nature value areas in agricultural ecosystems in the pan-European region will be complete. By 2008, a substantial proportion of these areas will be under biodiversity sensitive management, by using the appropriate mechanisms such as rural development instruments, agri-environmental programmes and organic agriculture, *inter alia*, intended to support their economic and ecological viability. By 2008, financial subsidy and incentive schemes for agriculture in the pan-European region will take conservation and sustainable use of biodiversity under consideration".

Maintaining a good conservation status is the key to reaching the 2010 target of halting the loss of biodiversity.

The EU has a strong environmental regulatory approach, as well as high-level political commitments (for example, the Birds Directive, Habitats Directive and Water framework Directive). Environmental integration into all EU policies is an objective of principle of the EU policy. The European Environment Agency (EEA) reported "…the continuing search for efficiency, lower costs and increased scale of production is resulting in substantial pressure on the environment, landscapes and biodiversity, particularly in the most intensively farmed areas. At the same time, agriculture remains essential to the maintenance of many landscapes (EEA/2003)".

The European Agricultural Fund for Rural Development (EAFRD) regulation emphasises three priority areas have been set for the 2007–2013 programming period:

1. **Biodiversity and preservation of high nature value farming and forestry systems;**
2. **Water;**
3. **Climatic change.**

The three EU-level environmental priorities are in accordance with the surveys of EEA and BirdLife-International (2004) showing that current land use trends, specifically by the agricultural sector, are one of the main reasons for environmental decline in rural areas.

The Rural Development budget is unlikely to be large enough so as to meet the cost of delivering fully against all objectives associated with it.

Under the current rural development regulation adopted in 2003 (Reg. 1783/2003), the Member States are obligated to set in place agri-environmental schemes. Support can be granted to farmers for environmentally-friendly measures, including conservation of high nature value farmed environments which are under threat.

In the period of pre-accession to EU, Bulgaria has developed a National Agri-environmental Programme (NAEP) expected to meet the priorities underlying the European regulation documents.

The main goals of the Programme are:

- Preserving the rich natural heritage and improving the quality of life for the people;
- Conservation of biodiversity and protection of natural resources;
- Compensatory payments for farmers in a manner beneficial for the environment;
- New employment opportunities in rural areas;
- New markets for quality products produced in an environmentally-friendly way.

Within the framework of common goals NAEP has defined seven Specific objectives, of which Objective No. 5 states:

"Maintain biodiversity by encouraging the conservation of high nature conservation farmland (semi-natural habitats), which is under threat from changing land use, agricultural intensification and/or abandonment".

Apparently, some important primary documents exist at a political level, aimed at priority protection of high nature value farmland in the period 2007–2013.

Along with this, a number of organisational, administrative and scientific issues have not found their solution yet and the expectations are that along with the government institutions the scientific community will contribute significantly to the fulfilment of the proposed ideas.

An important issue is the criteria for selection of the concrete location of high nature value farmland. Along these lines, the European Commission is now developing a Joint Project between DG Agriculture, DG JRC, DG Environment, Eurostat, and EEA, aimed at evolving selection indicators at a European level. The Project has been finalized at the end of 2006. Apparently, the criteria proposed by the Commission will have priority, which does not exclude the need of their detailed adaptation at a national level.

We could point out in this respect the significance of the endemic species and habitats, the species and

habitats reflecting the specific biogeographical elements, such as steppe, Mediterranean and Sub-Mediterranean, South Euxinian, etc.

Particularly important is the preparation of a distribution map of HNV – farmland. Meantime, JRC jointly with EEA finalized a map of the potential locations of HNV – farmland in Europe. This map is based on CORINE – Landcover (2000) selected mapping units. Developed on a 1:100 000 scale, it would offer only a tentative idea of their distribution and cannot reveal their real boundaries. It will be of importance to develop some large-scale, at least 1:25 000 maps at a national level. Along these lines, it would be of particular importance for Bulgaria to complete the thorough inventory and mapping of grasslands, so far conducted on one-third of their entire area (Meshinev & al. 2005).

Strategically, the Government institutions should delimit the total area of HNV – farmland in a sophisticated scheme, in which it could fully or partially overlap with the locations in NATURA – 2000, IBA, IPA, and with the territories in the national ecological network. Within the same complicated scheme the experts should select and propose the most suitable modes for agricultural practices ensuring sustainable protection of the natural values in any specific situation.

The precise fixing of the opening stage, training of farmers, monitoring of results, and criteria for success of the measures, discovering the reasons for unfavourable results, have so far rather posed more questions than given clear answers.

Apparently, this calls for some dedicated joint efforts on the part of the Government institutions and biodiversity experts so as to resolve the obtaining issues and to carry out in practice the idea for protection of the high nature value farmlands.

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European red-listed bryophyte species collected during the expeditions of the Hungarian Natural History Museum in Serbia between 2000–2006

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Abstract. 26 bryophyte species (2 hepatics and 24 mosses) red-listed in Europe were reported earlier from Serbia. During our field trips between 2000 and 2006 34 populations of 15 species red-listed in Europe were discovered and 12 of them were recorded for the first time in Serbia. In this paper the exact details concerning the localities of the European red-listed species found in Serbia are summarized.

Key words: bryophytes, European red-listed, Serbia

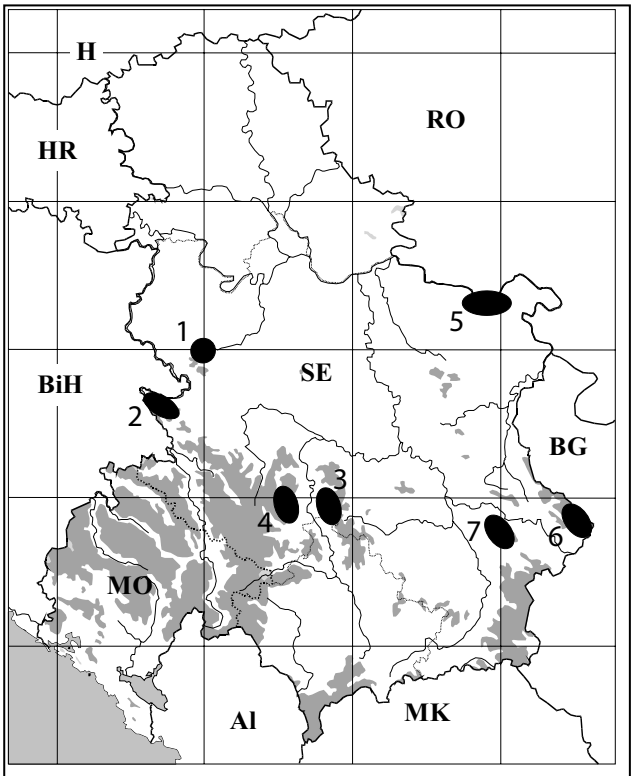
Introduction

To further explore the cryptogam flora of national parks and protected areas of Serbia several collecting trips were organized by the Hungarian Natural History Museum and the Institute for Nature Conservation of Serbia in the past five years. Main habitat types such as fens, stream valleys, forests, and grasslands developed on various bedrocks were visited, and bryophytes and lichens were collected from different substrates (soil, calcareous and non-calcareous rocks, tree barks and decaying wood). The elaboration of the complete set of bryophyte specimens collected in Petnica region, Tara National Park, Kopaonik Mts, Golija–Studenica Biosphere Reserve and Djerdap National Park and a part of the collected material from Stara Planina and Suva Planina had been finished. The location of investigated areas can be seen on Fig. 1.

108 bryophyte taxa (30 hepatics, 78 mosses) were reported for the first time in Serbia (Papp & Sabovljević 2001, 2002; Papp & al. 2004, 2006; Papp & Erzberger 2005). As recently the bryophyte flora of Serbia comprises 118 hepatics and 521 mosses on the bases of the check-lists made by Sabovljević & Stevanović (1999) and Sabovljević (2000a) with the addition of recently published new species from the country (Sabovljević 1998, 1999, 2000b, 2002, 2003a, b; Sabovljević & al. 1999; Sabovljević & Stevanović 2000; Papp & Sabovljević 2001, 2002; Sabovljević & Cvetić 2001, 2003; Veljić & al. 2001; Papp & al. 2004, 2006; Pócs & al. 2004; Grdovic 2005; Papp & Erzberger 2005) our work expanded the known inventory by 25 % (hepatics) and 15 % (mosses) of Serbia. Altogether 89 hepatics (75 % of the whole hepatic flora of Serbia) and 368 mosses (70 % of the whole moss flora of Serbia) were recorded in the investigated areas.

According to Sabovljević & Stevanović (1999) and Sabovljević (2000a) 26 bryophyte species (2 hepatics and 24 mosses) red-listed in Europe (ECCB 1995) were reported earlier from Serbia. During our field trips 34 populations of 15 species red-listed in Europe were discovered and 12 of them were recorded for the first time in Serbia. New data on the distribution of red-listed and rare species improve the correct evaluation of the threat status of these species at a European level. In this paper the exact details concerning the localities of European red-listed species found in Serbia are summarized.

The specimens are preserved in the Herbarium of the Hungarian Natural History Museum, Budapest



The location of the investigated areas.
(H = Hungary, RO = Romania, HR = Croatia, BiH = Bosnia-Herzegovina, MO = Montenegro, SE = Serbia, BG = Bulgaria, MK = FYR Macedonia, Al = Albania)
1 = Petnica region, 2 = Tara NP, 3 = Kopaonik NP, 4 = Golija-Studenica Biosphere Reserve, 5 = Djerdap NP, 6 = Stara Planina Mts, 7 = Suva Planina Mts

Fig. 1. Map of the investigated areas.

(BP) and in the Herbarium of the Botanical Museum Berlin–Dahlem (B).

Nomenclature of the species follows Erzberger & Papp (2004) and Koperski & al. (2000).

Enumeration

Anomodon rostratus (Hedw.) Schimp.

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This submediterranean, montane species (Düll 1985) occurs on shaded calcareous rocks, in rock crevices.

The first record of *A. rostratus* in Serbia was in Petnica, W Serbia (Papp & Sabovljević 2001), the second in Tara National Park (Papp & Sabovljević 2002) and the third in Mt Avala, C Serbia (Sabovljević & Cvetić 2003). The fourth record in Serbia is reported from Djerdap National Park (Papp & al. 2006). In SE Europe it was reported from Bulgaria, Romania (Düll 1985). It is known also from several localities in Slovenia (Martinčič 2003).

Exact localities: 1) Petnica region (W Serbia), Pocibrava rivulet, on calcareous rocks by the rivulet, 230–250 m, 31.03.2000. 2) Tara National Park, Biljeske Stene, Zvijezda reserve area, *Piceeto–Abieto–Fagetum*, 1080 m, 08.07.2000. 3) Djerdap National Park, north of Ploče towards Mali Štrbac hill, on shaded limestone rocks, 390 m, 44°37'01.2" N, 22°17'26.5" E, 12.07.2004; Djerdap National Park, east of Mali Štrbac towards Golo brdo hill, dolina, on shaded limestone rocks, 447 m, 44°37'51.6" N, 22°19'13.8" E, 14.07.2004.

Brachythecium geheebii Milde

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This species is a subcontinental-montane element (Düll 1985) and a European endemic (ECCB 1995). It occurs on shaded, slightly basic rocks in the high mountains of Central Europe and Norway (Frey & al. 1995; Düll 1985). It is recorded for the first time in Serbia from Golija–Studenica Biosphere Reserve (Papp & Erzberger 2005). Then it was found in Stara Planina Mts. It was reported earlier from Montenegro (Sabovljević & Stevanović 1999). In SE Europe it was reported from Bulgaria, Romania (Düll 1985), Montenegro and Slovenia (Pavletić & Pulević 1975; ECCB 1995).

Exact localities: 1) Golija Biosphere Reserve, Šaronje village, surroundings of Odvraćenica (N of Mt Kula), *Piceeto–Abieto–Fagetum*, on volcanic rock, 1680 m, 43°16'52.3" N, 20°19'56.9" E, 07.07.2003. 2) Stara Planina Mts, Babin Zub, boulder scree and basis of great, permian red sandstone rocks, 1580 m, 43°22'28.8" N, 22°36'59.1" E, 25.06.2005.

Bryum neodamense Itzigs. ex Müll. Hal

It is a vulnerable species (V) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This is a subarctic element (Düll 1985). It is recorded for the first time in Serbia in Stara Planina Mts from the bank of a small pool with *Warnstorfia exannulata*, *Aulacomnium palustre*. From SE Europe it is reported only from Croatia and Bulgaria (Düll & al. 1999). In Bulgaria it is known only from Mt Vitosha (Natcheva & Ganeva 2005).

Exact locality: Stara Planina Mts, between Babin Zub and Midžor, at the peak of Tupanar, temporary small pool in subalpine grassland, 1903 m, 43°24'00.9" N, 22°38'55.5" E, 30.06.2005.

Buxbaumia viridis **(Moug. ex Lam. & DC.) Brid. & Nestl.**

It is included in the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats 1979), and in the European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (1992). It is vulnerable species (V) according to the *Red Data Book of European Bryophytes* (ECCB 1995).

This boreal, montane species (Düll 1984) lives on large, well-decayed wood in constantly humid forests. It is an indicator species of old-growth forests. It was reported earlier from two localities in Serbia and one in Montenegro (Sabovljević & al. 1999), later on it was collected in Tara National Park (Papp & Sabovljević 2002) and in Golija–Studenica Biosphere Reserve (Papp & Erzberger 2005). It can be found in almost all the SE European countries (Bosnia–Herzegovina, Bulgaria, Croatia, Greece, Montenegro, Romania, Serbia, Slovenia) (Düll 1984; Martinčič 2003). But it has scattered distribution.

Exact localities: 1) Tara National Park, Predov krst, *Piceeto–Abieto–Fagetum,* 1060 m, 06.07.2000. 2) Tara National Park, Tepih Livada, Crveni potok reserve area, on decaying wood, 1050 m, 07.07.2000. 3) Tara National Park, Biljeske Stene, Zvijezda reserve area, *Piceeto–Abieto–Fagetum*, 1080 m, 08.07.2000. 4) Golija Biosphere Reserve, Šaronje village, surroundings of Odvraćenica (N of Mt Kula), *Piceeto–Abieto–Fagetum*, on volcanic rock, 1680 m, 43°16'52.3" N, 20°19'56.9" E, 07.07.2003. 5) Golija Biosphere Reserve, N of Odvraćenica, branch of Crna reka downwards from Radulovac, in the source area and along the stream, on decaying wood, 1735–1690 m, 43°17'33.0" N, 20°20'41.0" E, 11.07.2003.

Drepanocladus lycopodioides **(Brid.) Warnst.** (RT–ECCB 1995)

It is in the category of regionally threatened species (RT) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This is a boreal species living in wet meadows. It is first recorded in Serbia from Kopaonik Mts (Papp & al. 2004). In SE Europe it was reported from Romania (Düll 1985). It was also known from Slovenia (Martinčič 2003).

Exact locality: Kopaonik Mts, Jankove bare meadow, *Sphagnetum*, 1800 m, 43°19.114' N, 20°46.546' E, 09.07.2002.

Encalypta microstoma **Bals.-Criv. & De Not.**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This subarctic-subalpine element (Düll 1984) is recorded for the first time in Serbia in Stara Planina Mts. It is very rare in the higher mountains of Europe (Nyholm 1998). From SE Europe it is known only from Bulgaria in Rila Mts (Düll & al. 1999; Natcheva & Ganeva 2005).

Exact locality: Stara Planina Mts, between Babin Zub and Midžor, subalpine grassland with sandstone rock outcrops and small temporary streams, 1593 m, 43°22'45.1" N, 22°37'39.5" E, 28.06.2005.

Grimmia caespiticia **(Brid.) Jur.**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This is a subarctic-subalpine element (Düll 1984) and known as a rare species. It can be found on acidic rocks in alpine mountains of Europe, Asia and Greenland (Nyholm 1998). Its first record in Serbia is from Golija–Studenica Biosphere Reserve (Papp & Erzberger 2005). Then it was found in Stara Planina Mts. It was reported earlier from Montenegro (Sabovljević & Stevanović 1999). In SE Europe it was known also from Bulgaria and Romania (Düll 1984). And it was recorded in Turkey (Düll 1984).

Exact localities: 1) Golija Biosphere Reserve, Šaronje village, Jankov kamen, on exposed volcanic rock, 1755 m, 43°20'00.0" N, 20°16'28.7" E, 08.07.2003. 2) Stara Planina Mts, Crnovrska reka at Baltaberilovac village, on exposed, black schistose sandstone rocks, 488 m, 43°24'03.3" N, 22°29'44.0" E, 27.06.2005.

Lophozia ascendens **(Warnst.) R.M. Schust.**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This boreal, montane leafy hepatic (Düll 1983) lives on large, well-decayed logs in constantly humid forests; therefore, it is considered an indicator of old-growth forests.

Lophozia ascendens was found in Tara National Park for the first time in Serbia (Papp & Sabovljević 2002), later we have also collected it in Kopaonik National Park (Papp & al. 2004), and in Golija–Studeni-

ca Biosphere Reserve we have reported on its third locality (Papp & Erzberger 2005). Then it was found in Stara Planina Mts. In SE Europe it was reported from Bulgaria and Romania (Düll 1983).

Exact localities: 1) Tara National Park, Tepih Livada, Crveni potok reserve area, on decaying wood, 1050 m, 07.07.2000. 2) Kopaonik Mts, Jankove bare, *Sphagnetum* and *Piceetum*, on decaying wood, 1800 m, 43°19.114' N, 20°46.546' E, 09.07.2002. 3) Kopaonik Mts, Brzecka reka at Metodje Reserve, towards a geyser, on decaying wood, 1490 m, 43°18.710' N, 20°51.001' E, 12.07.2002. 4) Kopaonik Mts, Jaram under Karaman vrh, near Rtanj, *Piceetum*, on decaying wood, 1810 m, 43°18.248' N, 20°49.757' E and 43°17.434' N, 20°49.209' E, 13.07.2002. 5) Golija Biosphere Reserve, N of Odvraćenica, branch of Crna reka downwards from Radulovac, in the source area and along the stream, on decaying wood, 1735–1690 m, 43°17'33.0" N, 20°20'41.0" E, 11.07.2003. 6) Stara Planina Mts, Babin Zub, Zubska reka stream below the hotel, 1700 m, 43°22'23.7" N, 22°36'58.2" E, 26.06.2005.

Paraleucobryum sauteri **(Bruch & Schimp.) Loeske**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This suboceanic-montane element (Düll 1984) is recorded for the first time in Serbia in Stara Planina Mts. From SE Europe it is known from Bulgaria (only a pre-1956 literature record from Mt Vitosha) and Bosnia–Herzegovina, Montenegro, Croatia, Slovenia (Düll & al. 1999; Martinčič 2003; Natcheva & Ganeva 2005).

Exact locality: Stara Planina Mts, Babin Zub, boulder scree and crevices of great rocks, permian red sandstone, 1581 m, 43°22'28.8" N, 22°36'59.1" E, 25.06.2005.

Pseudoleskea saviana **(De Not.) Latzel**

It is in the category of regionally threatened species (RT) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This species occurs on shaded volcanic rocks, but sometimes it is found on tree bark mainly at the base of trunks. It is a rare, continental-subalpine species, which can be found in the Alps and also more towards the east in Romania, Bulgaria, Greece and Turkey (Düll 1985). From Slovenia it was reported by Martinčič (2003). It was also collected in Kopaonik Mts (Papp & al. 2004) and in Golija–Studenica Biosphere Reserve (Papp & Erzberger 2005). Then it was found in Stara Planina Mts.

Exact localities: 1) Kopaonik Mts, Vodopad Jelovarnik, *Fagetum*, on limestone rock, 1250 m, 43°16.202' N, 20°51.433' E, 10.07.2002. 2) Kopaonik Mts, Jaram under Karaman vrh, near Rtanj, *Piceetum*, on the bark of *Juniperus*, 1810 m, 43°18.248' N, 20°49.757' E and 43°17.434' N, 20°49.209' E, 13.07.2002. 3) Golija Biosphere Reserve, Šaronje village, surroundings of Odvraćenica (N of Mt Kula), *Piceeto–Abieto–Fagetum*, on volcanic rock and on the bark of *Fagus,* 1680 m, 43°16'52.3" N, 20°19'56.9" E, 07.07.2003. 4) Stara Planina Mts, Babin Zub, Zubska reka stream below the hotel, 1700 m, 43°22'23.7" N, 22°36'58.2" E, 26.06.2005.

Rhynchostegiella teneriffae **(Mont.) Dirkse & Bouman**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This atlantic, submediterranean, montane species (Düll 1985) lives on rocks usually near water.

It was recorded for the first time in Serbia at Petnica, W Serbia, by Papp & Sabovljević (2001). The second record of this rare European species in Serbia is from Djerdap National Park (Papp & al. 2006). In SE Europe it was known from Bosnia–Herzegovina (Pavletić 1955), Slovenia (Martinčič 2003) and Romania (Düll 1985). And it was reported from Turkey (Düll 1985).

Exact localities: 1) Petnica region (W Serbia), Pocibrava rivulet, on calcareous rocks by the rivulet, 230–250 m, 31.03.2000. 2) Djerdap National Park, Brnjička reka at Brnjica village, 104 m, 44°38'12.6" N, 21°44'42.5" E, 13.07.2004.

Rhynchostegium rotundifolium **(Brid.) Schimp.**

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This submediterranean-subatlantic species (Düll 1985) lives on shaded, humid rocks, rock walls, rock crevices, sometimes on decaying logs. In the Golija–Studenica Biosphere Reserve it was collected from shaded limestone rock crevices along a stream at Studenica monastery (Papp & Erzberger 2005). In SE Europe it was recorded in Romania (Düll 1985). From Slovenia it was reported by Martinčič (2003), from Bulgaria by Natcheva & Ganeva (2005).

Exact locality: Golija Biosphere Reserve, Studenica monastery and along a stream towards Godović village, on shaded limestone rock, 453 m, 43°29'16.2" N, 20°31'48.9" E, 09.07.2003.

Taxiphyllum densifolium (Broth.) Reimers

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This pontic, montane species (Düll 1985) lives in humid habitats, on shaded rocks.

It was recorded for the first time in Serbia in Djerdap National Park. It was found on the limestone rocks in the dolinas. It has quite large populations here, numerous patches can be found in several dolinas between Ploče and Mali Štrbac (Papp & al. 2006). Then it was found very recently in Suva Planina Mts. This is its second locality in Serbia.

It was described from the forests of the Caucasus Mts by Brotherus in 1892 (Reimers 1940). It is sporadically found in the Czech Republic, Poland, Hungary and Russia, and in SE Europe it was reported only from Romania (Düll 1985). It was discovered recently in Turkey in the Asian part of Black Sea coast (Papp 2004).

Exact localities: 1) Djerdap National Park, north of Ploče towards Mali Štrbac hill, on shaded limestone rocks, 390 m, 44°37'01.2" N, 22°17'26.5" E, 12.07.2004. 2) Djerdap National Park, east of Mali Štrbac towards Golo brdo hill, dolina, on shaded limestone rocks, 447 m, 44°37'51.6" N, 22°19'13.8" E, 14.07.2004. 3) Suva Planina Mts, at Bonjanine vode near Gornja Studena village, on shaded limestone rocks, 965 m, 43°13'02.5" N, 22°07'08.2" E, 10.07.2006.

Weisia levieri (Limpr.) Kindb.

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This atlantic, mediterranean species (Düll 1984) lives on soil at dry, exposed places. Its first record in Serbia is from Petnica, W Serbia. It was reported as *Weisia longifolia* (Papp & Sabovljević 2001), but later in 2003 the specimen (BP 167572) was revised by Papp. From SE Europe it was known from Monetengro (Düll 1984; Sabovljević & Stevanović 1999). From Slovenia it was reported by Martinčič (2003), from Bulgaria by Natcheva & Ganeva (2005).

Exact locality: Petnica region (W Serbia), Dračić village, in meadows, 300–350 m, 01.04.2000.

Weisia squarrosa (Nees & Hornsch.) C. Müll.

It is in the category of rare species (R) according to the *Red Data Book of European Bryophytes* (ECCB 1995). This subatlantic species (Düll 1984) occurs on humid, acidic soil.

It was recorded for the first time in Serbia at Petnica, W Serbia, by Papp & Sabovljević (2001). In SE Europe it was reported from Romania (Düll 1984). From Slovenia it was reported by Martinčič (2003).

Exact locality: Petnica region (W Serbia), Dračić village, in meadows, 300–350 m, 01.04.2000.

Acknowledgements. The authors are grateful to the Institute for the Protection of Nature of Serbia and the leadership and staff of national parks visited for their support of the field surveys. Many thanks are due to Duška Dimović, Belgrade, for her essential help in the organization of the field trips.

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The bryoflora of Albania: chorology, conservation issues

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Abstract. Albania represents one of the less studied areas in Europe, as far as bryophytes are concerned. The first check-list for the bryoflora of this country has been published recently. Albanian bryoflora consists of 327 taxa (3 hornworts, 86 liverworts, and 238 mosses), even though more are certainly present. In this paper a preliminary report on the characteristics of this bryoflora is presented. Temperate species appears to be the most important eco-chorological type (28.75 %), followed by boreal and subboreal types (22.94 %). Other types dominate particular areas. This is in accord with one previous study based only on liverworts. Possible environmental threats due to fast economic growth and conservation issues are eventually considered, also in relation to a possible use of bryophytes in biomonitoring.

Key words: Albania, bryophytes, chorology, conservation, liverworts, mosses

Introduction

Albania represents one of the less known areas in Europe, as far as bryophytes are concerned (ECCB 1995). Even though distributional studies were recognized as a priority by the Biodiversity Strategy and Action Plan for Albania (Bego & Koni 1999), in the case of bryophytes these have been hampered so far by the lack of a resident bryologist. Only very recently a check-list for this country has been prepared (Colacino & Sabovljević 2006). This check-list will be also available, in the near future, on a dedicated website (www.bryology.eu). The Albanian bryoflora consists of 327 taxa (3 hornworts, 86 liverworts, and 238 mosses) representing 308 species, 141 genera and 62 families. The actual number is likely to be higher given the morphological and climatic diversity of the country (for a brief outline of Albania's geography, climate, soils, flora, etc. see Colacino 2004). This is reflected also in its floristic diversity (3250 species of vascular plants on a surface of 28 748 km^2). Albanian vascular flora is characterised by a strong Balkan and Mediterranean character (25 % and 24 % of total flora, respectively. See Bego & Koni 1999; Vangjeli 2002). About 3.8 % of this flora is threatened based on red-list data (Colacino 2004, based on data from Vangjeli & al. 1995). In the case of bryophytes it is still too early for a red-list, as more inventory and mapping work are necessary, some considerations on conservation issues, however, are briefly discussed. The present, fast, economic development Albania is undergoing, is causing some problems in the management of natural resources and in the control of environmental pollution. Some of these problems will be briefly outlined also. The possible use of bryophytes in biomonitoring is also proposed.

Methods

In this paper a preliminary analysis of some of the characteristics of the Albanian bryoflora is presented. This is based on the check-list by Colacino & Sabovljević (2006), based on literature reports. The eco-chorological types of Düll (1983, 1984, 1985, 1992) have been used. The problems in using this type of data are known: areas are not well defined, nor their limits are indicated, as correctly outlined by Schumacker (1988). In any case, they are the only ones available for the whole of Europe, and their widespread use makes possible the comparison of different areas. A chorological spectrum for the whole of Albania is presented, and in addition, some areas within that country will be compared to the bryoflora of Italian Regions (Aleffi & Cortini-Pedrotti 2002). In this comparisons, given the preliminary nature of this analysis, only some

areas (Shkodra, Tirana, Vlora, Korça) and chorological types will be considered: (sub)arctic-(sub)alpine, (sub)boreal, (sub)Mediterranean, and (sub)oceanic. In this case, chorological data have been aggregated in classes of frequency (classes indicated in the pertinent figures). This analysis takes into account some of the physical–geographical and climatic subdivisions of Albania too. Possible species to consider for inclusion in a future red-list are indicated based on records from adjacent countries, in particular Montenegro.

Results

Albanian bryoflora (Fig. 1) appears to have a prevalence of temperate types species (28.75 %), which are widely represented in the northern hemisphere, compared to 15.2 % for Italy, 17.1 % for Spain, and 23.1 % for Greece (all data for Italy, Spain and Greece – from Cortini-Pedrotti 1996), followed by boreal and subboreal types with 22.94 %, and with similar values in the other countries considered (22 % in Italy, 22.5 % in Spain, 23.2 % in Greece).

Among the mountain boreal elements are *Lophozia ventricosa* (Dicks.) Dumort., collected by Kümmerle in 1916–1917 (Szepesfalvy 1926) on Mt Korabi (Dibra) at 2300 m alt., *Anomodon longifolius* (Schleich. ex Brid.) Hartm., collected in the Vermosh area (Albanian Alps) at 1100 m alt. by Dörfer in 1914 (Baumgartner 1915). Of particular interest is the presence of just one eu-oceanic bryophyte, the moss *Rhynchostegium alopecuroides* (Brid.) A.J.E. Smith, not present in Italy, and collected by Petrov in 1958 on Mount Ostrovica (Korça) at 1730 m alt. (Petrov 1960). As we can expect, Italy and Greece also present a very low percentage of eu-oceanic species (1.8 % and 0.9 %, respectively), while in Spain they arrive at 5.5 %. Another interesting record is *Cheilothela chloropus* (Brid.) Lindb. ex Broth., a disjunctive oceanic-Mediterranean type moss found in Shkodra (and Dalmatia) by Höhnel in 1885–1891 (Höhnel 1893). Oceanic-Mediterranean elements mark the transition from Mediterranean climate areas to those with a more atlantic influence (Cortini-Pedrotti 1996). The oceanic elements s.l. (including suboc, oc-submed, suboc-submed, and euoc) are 22.95 % in Albania, compared to 30.8 % in Italy, 47.2 % in

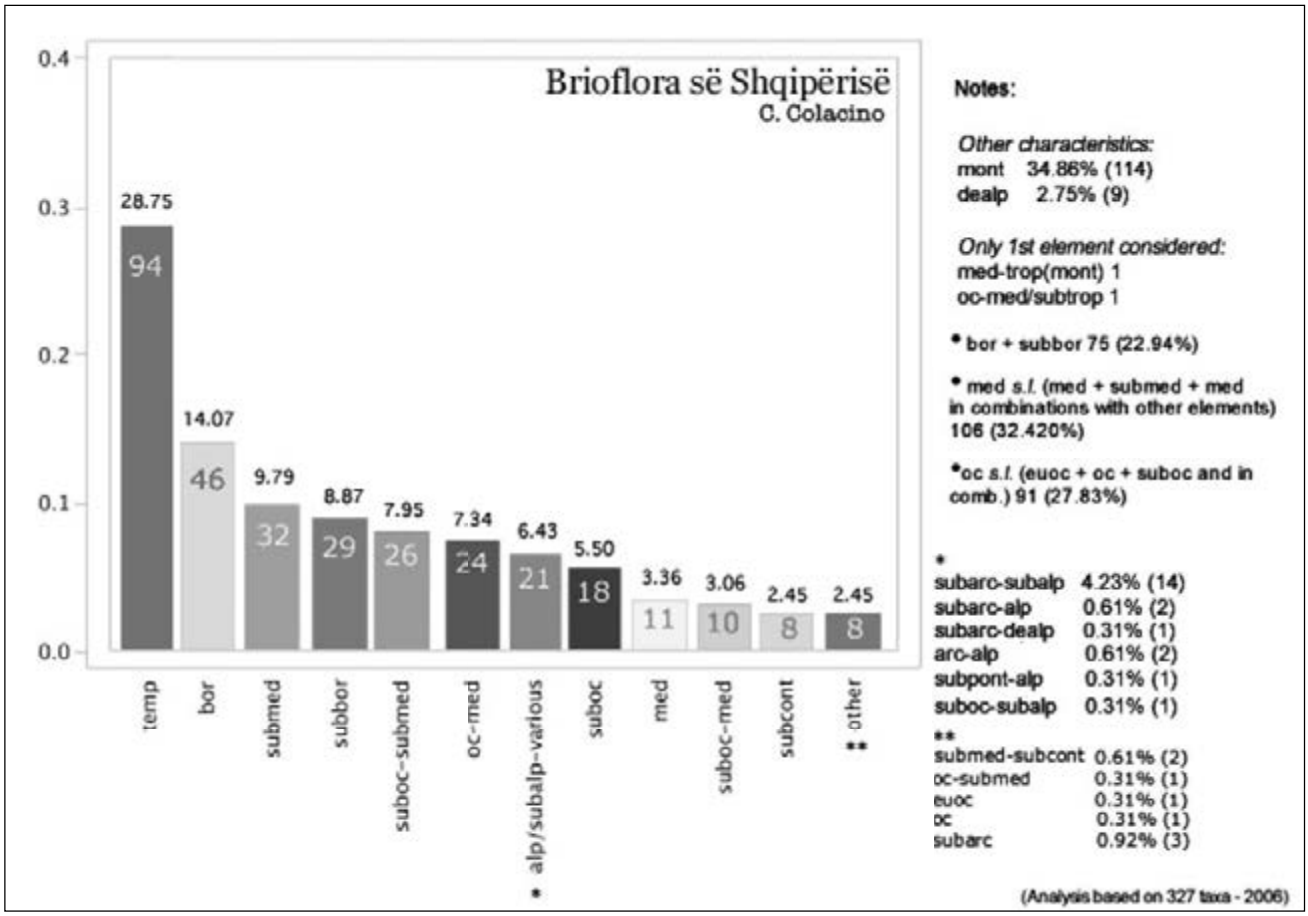

Fig. 1. Chorological analysis of Albanian bryoflora based on the check-list by Colacino & Sabovljević (2006).

Spain, and 46.8% in Greece. Strictly Mediterranean and submediterranean elements are 13.5% in Albania, 9.5% in Italy, 9.1% in Spain, and 12.2% in Greece. Eventually, the important chorological element of (sub)alpine-(sub) arctic types reaches 6.43% in Albania, 4.5% in Greece, 11.6% in Spain, and 19.6% in Italy, not surprisingly.

Different areas within Albania will be considered now, as a way to get a more detailed picture of the bryoflora of Albania. These areas will be compared, as an example, to the distribution of the same elements in Italy at the regional level. The data, aggregated into classes, have been mapped as a way to allow comparisons between different areas in the two countries. Only four of the main administrative divisions in Albania (Prefectures) will be considered (Shkodra [Scutari], Tirana, Korça, and Vlora [Valona]).

In Fig. 2 (sub)arctic-(sub)alpine elements are considered. It has been already noted above that, for the whole of the country, these are relatively less important. Considering their distribution at the regional level, however, it is possible to observe that at least in one area (Korça) their values are comparable to those of some Italian Regions which include Alpine areas (Piedmont, Lombardy). Among the arctic-alpine elements, *Jungermannia polaris* Lindb. collected by Petrov (1960) on Mt Ostrovica (Korça) at 1760–1800 m alt., but also by Bischler & al. (1980) at a much lower altitude (200–300 m) in the coastal area of Albania, and *Pohlia atropurpurea* (Wahlenb.) H. Lindb. collected near Renci (Shkodra) (Höhnel 1893).

In the case of boreal and subboreal elements (22.94% for Albania, and similar values for Italy, Spain and Greece), their actual distribution is variable, with higher values, among the prefectures considered, in Korça and Shkodra (Fig. 3), and lower values in the southern ones closer to the coast (Vlora) which have values comparable to the lowest in Italy (Basilicata).

The same area, characterised by the lower values for boreal elements (Vlora), has the highest values in Albania for oceanic elements (Fig. 4), values not attained anywhere in Italy (at regional level), and closer to those recorded for Greece, for instance.

In the case of the Mediterranean elements (Fig. 5), overall higher in Albania than in Italy, Greece, or Spain, they prevail even more clearly in areas characterised by a more distinct Mediterranean climate (compare to Fig. 6).

This short assessment would not be complete of course without a note on endemic species and on species of conservation interest. According to ECCB (1995)

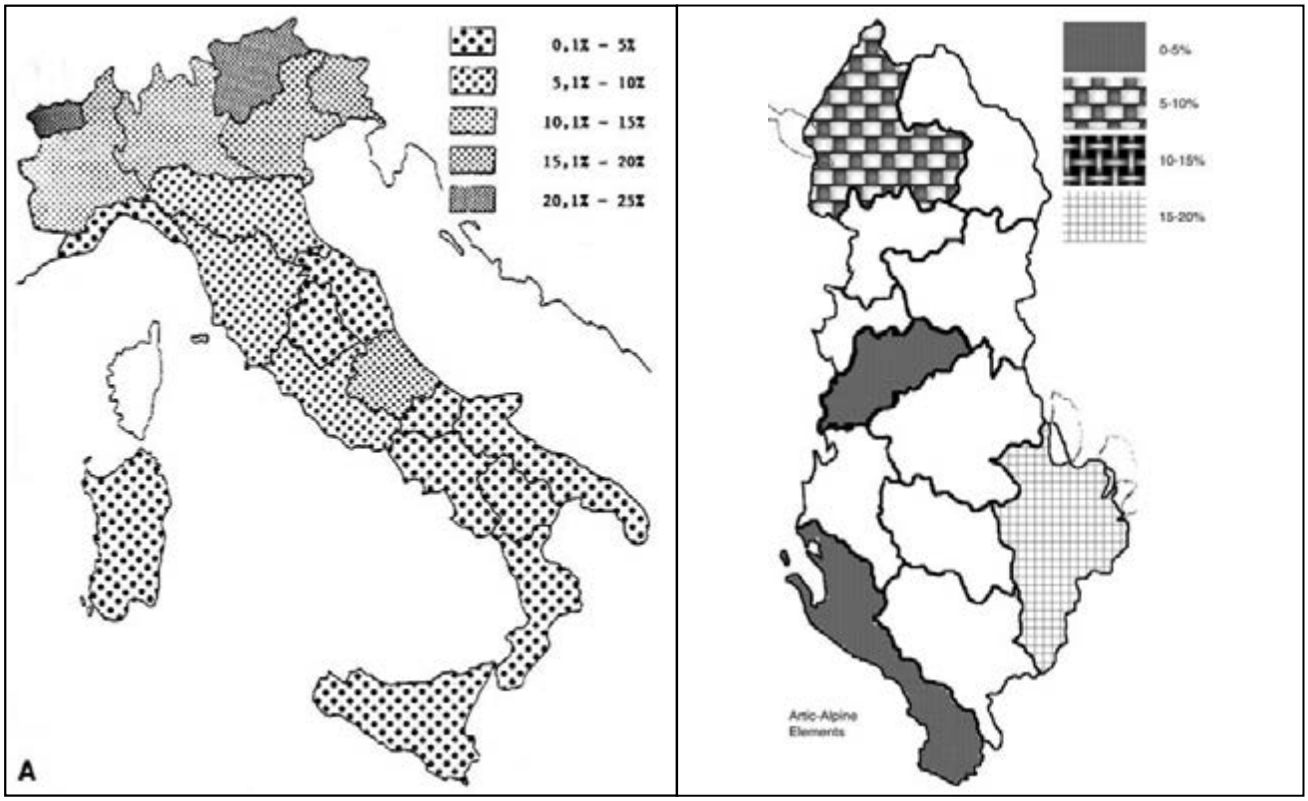

Fig. 2. (Sub)arctic-(sub)alpine elements in Italy and Albania. (**A** – from Aleffi & Cortini-Pedrotti 2002).

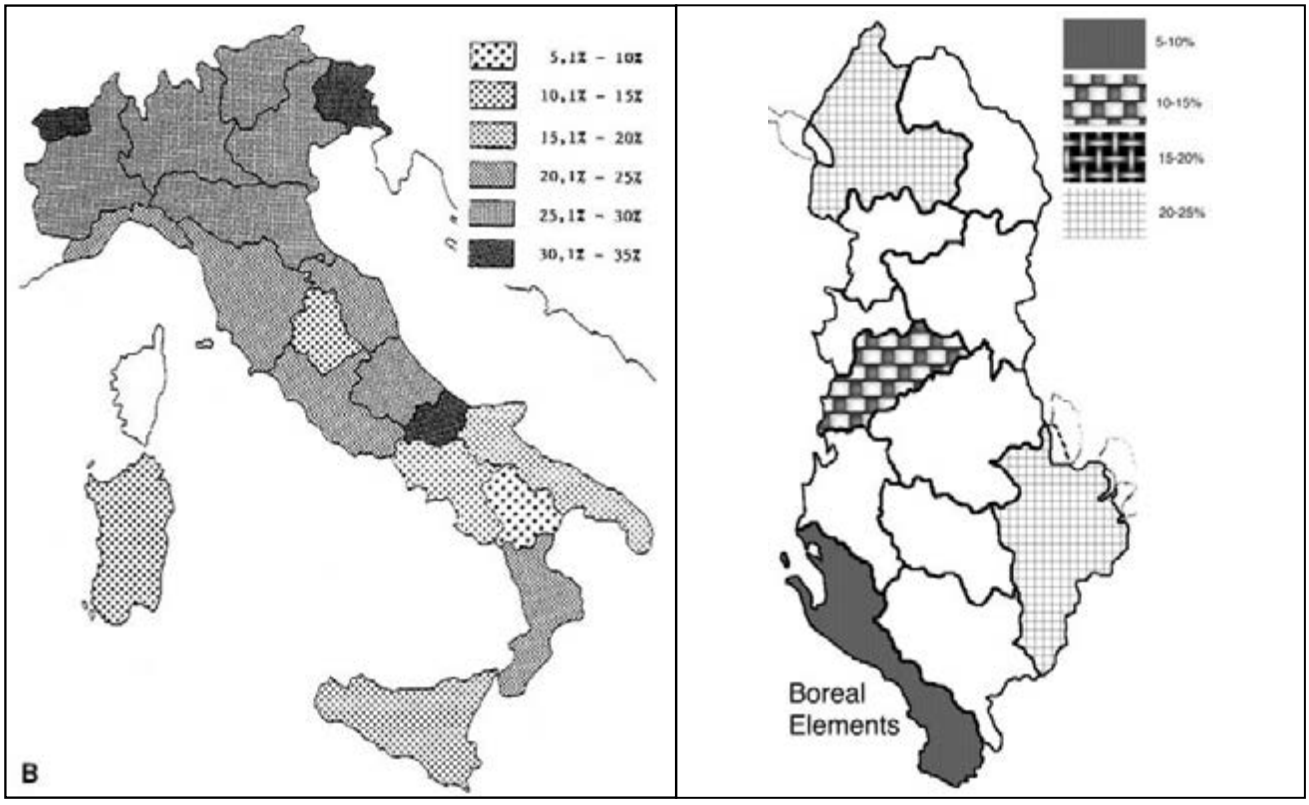

Fig. 3. (Sub)boreal elements in Italy and Albania. (**B** – from Aleffi & Cortini-Pedrotti 2002).

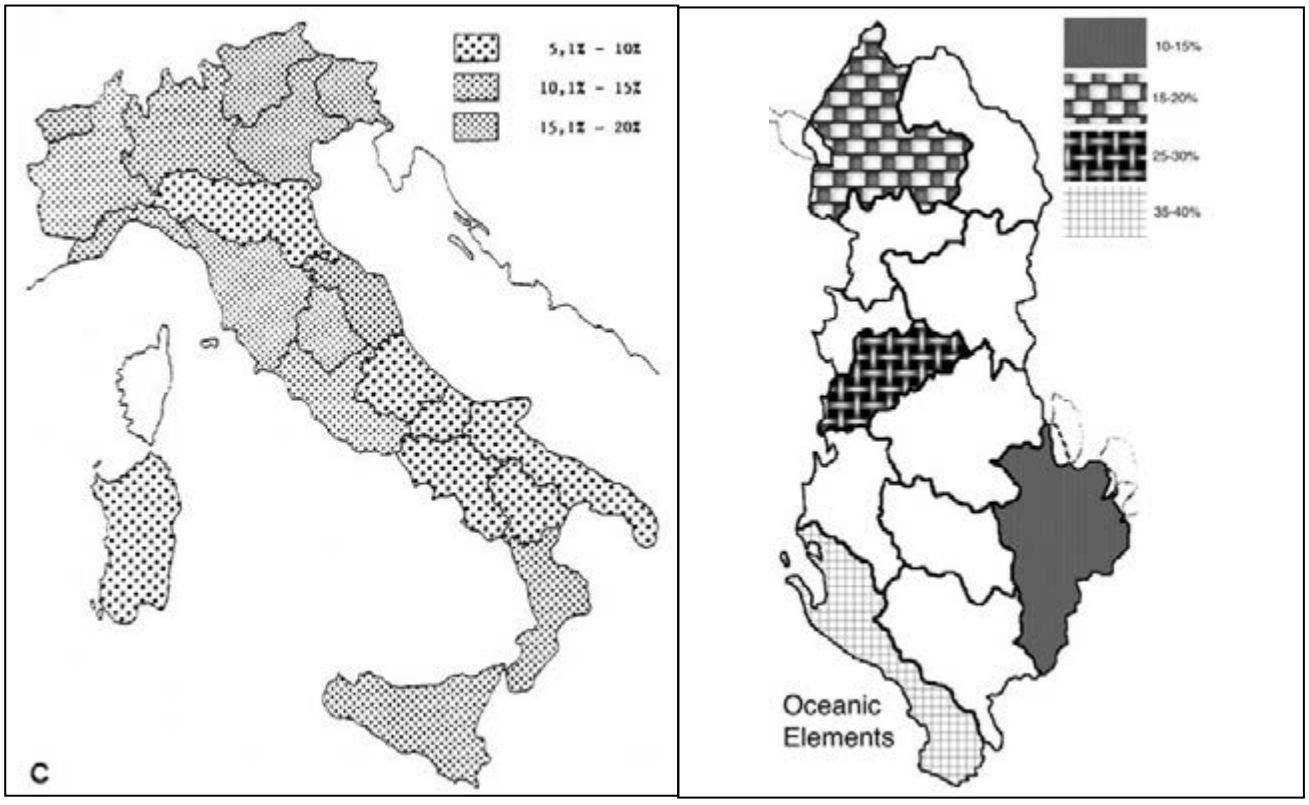

Fig. 4. (Sub)oceanic elements in Italy and Albania. (**C** – from Aleffi & Cortini-Pedrotti 2002).

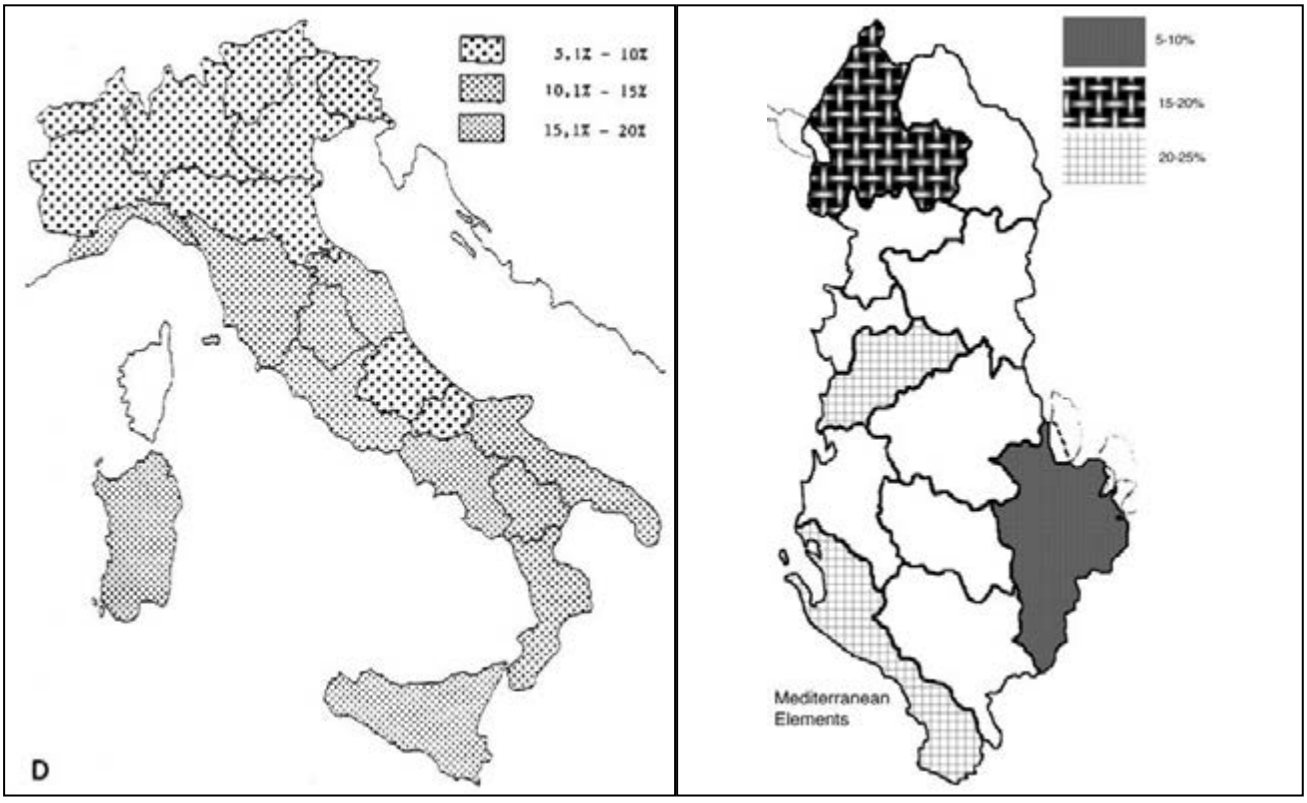

Fig. 5. (Sub)Mediterranean elements in Italy and Albania. (**D** – from Aleffi & Cortini-Pedrotti 2002).

there are nine bryophyte species of particular conservation interest in Albania; the only locally endemic species, the rare (R) liverwort *Frullania illyrica* Grolle, is now included in *F. inflata* Gottsche, with a wider distribution, and whose status is Vulnerable (V) according to Schumacker & Váňa (2005). There are four other liverworts, *Athalamia spathysii* (Lindenb.) S. Hatt., *Mannia triandra* (Scop.) Grolle, *Riccia trabutiana* Steph., all with the status of rare according to the European Red List, and the insufficiently known (K) *Marchantia palacea* Bertol. (but not threatened – NT, according to Schumacker & Váňa 2005). Among the mosses, *Neckera cephalonica* Jur. & Unger. (K) (considered an endemic for the Iberian Pensinsula, Greece, and Albania), *Buxbaumia viridis* (Moug. ex Lam. & DC.) Moug. & Nestl. (V), and *Tortula solmsii* (Schimp.) Limpr. (R), the latter collected in Albania only once, more than a century ago (Höhnel 1893). The ninth bryophyte species mentioned by ECCB (1995) is *Tortula lingulata* Lindb. (K), which is known from a single locality that Colacino & Sabovljević (2006) found to be actually outside the present borders of the country (and excluded therefore from the Albanian bryoflora). Other species should be considered also, especially those mentioned in red lists for neighbouring countries, such as Serbia (or better Kosova, when its status will be agreed on and data will become available), Montenegro, Greece, and Macedonia. Data from Montenegro will be considered here as an example (Sabovljević 2004). Among the species considered as critically endangered (CR) in Montenegro (and dwelling also in Albania) there is *Buxbaumia viridis*, while *Buxbaumia aphylla* Hedw. is considered endangered (EN); among those marked as vulnerable (VU) there are *Encalipta ciliata* Hedw., *Pseudoleskea saviana* (De Not.) Latzel, and *Schistidium agassizii* Sull. & Lesq. ex Sull. Among the liverworts, the CR *Athalamia hyalina* (Sommerf.) S. Hatt. and *Mannia androgyna* (L.) A. Evans, the EN *Jungermannia gracillima* Sm., the VU *Cephaloziella turnerii* (Hook.) Müll.Frib., *Leiocolea collaris* (Nees) Schljakov, and *L. turbinata* (Raddi) H. Buch. Of course, these are merely suggestions, as the inventory and mapping of Albanian bryophytes, as already stated, are to be completed before a red list can be put forward.

The environmental situation in Albania: a few notes

Some industrial plants, built in the 1960s are obsolete; others have been abandoned in the post-1990 period. All these have provoked several environmental hazards (see for instance Bego & Koni 1999, and UNEP 2000) and are threat factors for the flora of the

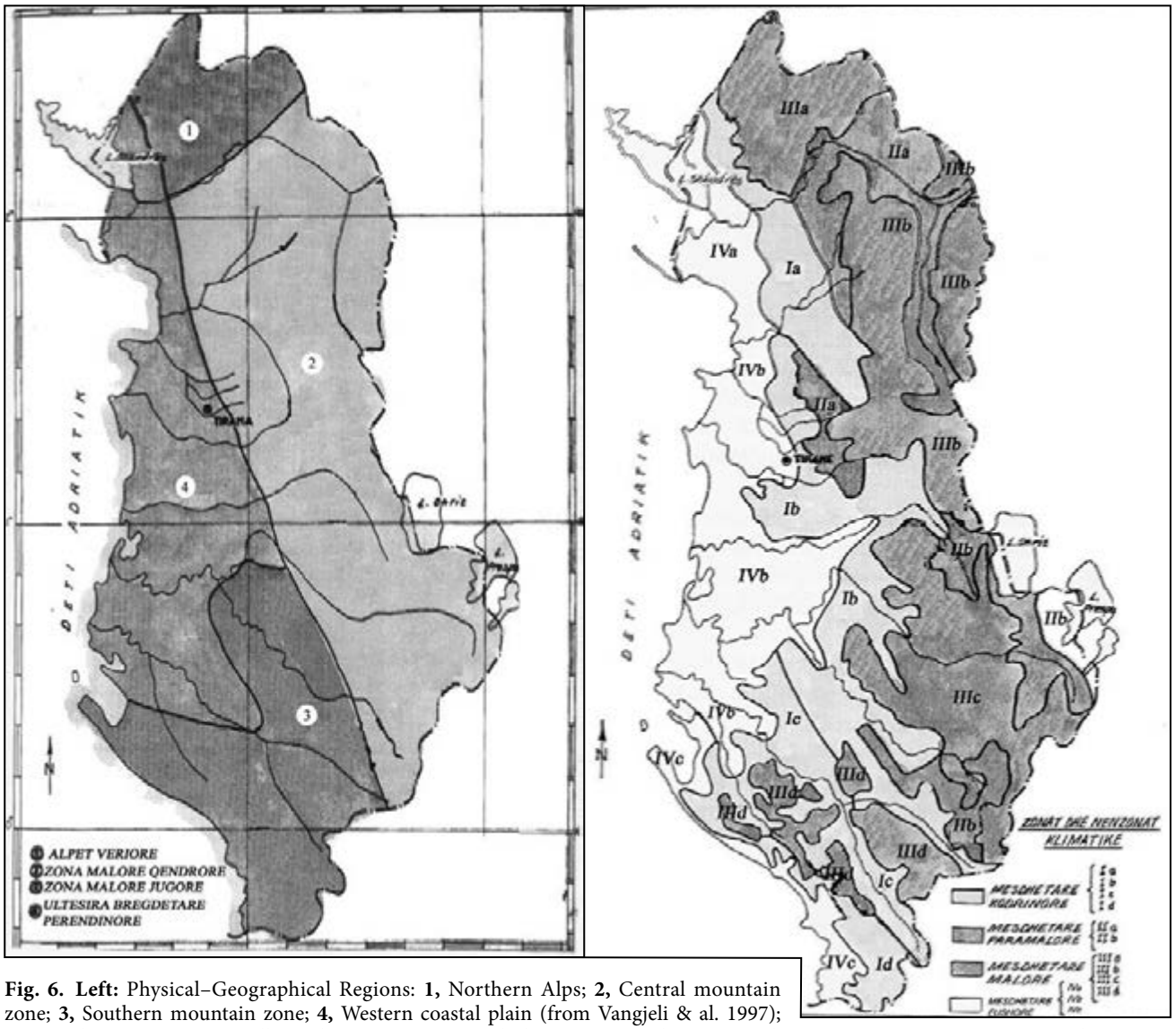

Fig. 6. Left: Physical–Geographical Regions: **1,** Northern Alps; **2,** Central mountain zone; **3,** Southern mountain zone; **4,** Western coastal plain (from Vangjeli & al. 1997); **Right:** Climatic Zones: **I** – Hilly Mediterranean or Central Plateau (Çermënike); **II** – Mediterranean Pre-Mountain; **III** – Mediterranean Mountain; **IV** – Lowland Mediterranean (from Vangjeli & al. 1997. Modif.).

country. Examples are the industrial plants of Elbasan, which affect a large area once used also for agriculture, and two disused and abandoned plants (one of fertilizers, the other of chemicals) in an area now densely populated, near Durazzo (Fig. 7) (UNEP 2000). It should be added, however, that the whole of the country does not present a severe case of environmental pollution with the exception of areas like Kukës, Rubik, Laç, Elbasan, Tirana, and Vlora. There, emissions of SO_2, H_2SO_4, CO, NH_3, NO_x, smog, as well as powders are present and no programs are under way to monitor the levels of these pollutants.

Bryophytes (and lichens) could be useful (and cost-effective) tools to assess, for instance, air pollution in Albania. It is important also to consider the effect of these threat factors on habitat loss and degradation; these studies are still quite rare (see Leone & al. 2003, as an example, for the Karavasta Lagoon National Park).

Discussion

Albanian bryoflora is relatively unknown, even though some areas have been investigated more than others (e.g., Tirana and Shkodra). The eco-chorology of Albanian bryophytes shows a prevalence of temperate elements, followed by boreal ones. This is in accord with the only one earlier study, limited to liverworts, which considered the chorology of Albanian bryoflora (Bischler & al. 1980), and which found a prevalence of non-Mediterranean elements (58 %

Fig. 7. Abandoned Chemical Plant near Durazzo [Durrës] (Aug. 2002) (Photo C. Colacino).

over 59 liverwort species considered), even though it was carried out exclusively in the coastal areas (with Mediterranean type climate) of Albania. A preliminary analysis of some areas within the country shows that the distribution of chorological elements varies widely. Mediterranean types, however, never achieve predominance anywhere (at least at this level of analysis). This ample variation is to be expected given the mountainous nature of the country and its climatic, and geomorphological diversity (Fig. 6), as well as because of its floristic diversity (Albania is an important meeting area between the Mediterranean, the central European, and the Pontic floristic elements, for vascular plants).

Given the relatively small size of the country, anyway, differences in elevation seem to be the most important factor in determining climatic differences, rather than latitude. The northern Alps, as well as the Korça area to the SE, show a relatively high number of arctic-alpine species, probably of relict nature. The same areas show also the highest concentration of boreal and subboreal element types, these distributions are linked to the areas with higher elevations. In contrast, oceanic element types have higher values in central and coastal areas (Tirana and Vlora). Mediterranean element types, finally, seem to come out with higher values to the west side of the country, and lower values to the east, and their occurrence also is apparently linked to differences in elevation. Albania is likely to have a comparatively high taxonomic diversity in bryophyte flora, as it is the case for its vascular flora, notwithstanding its small size and extension (about 350 km N–S, and 150 E–W). A more complete inventory and mapping of the country are needed, however, to demonstrate this feature. Priority should be given to areas characterised by habitat loss and degradation (most lowland areas), and to protected areas (Natural Parks, Reserves, etc.). Cooperation with the many and experienced vascular plants botanists of Albania is also to be considered to speed up the study of the bryoflora focusing it into the different vegetation types, and within the floristic regions of the country (see Fig. 8), as excellently done, for instance, for the recent check-list of Bulgarian liverworts and hornworts (Ganeva & Natcheva 2003). The need for local bryologists is evident, and indeed a young botanist from the Faculty of Natural Sciences of the University of Tirana is now been trained in bryology, in cooperation with the Laboratorio di Briologia of the University of Basilicata. The first resident bryologist ever for this country. This should allow to progress the inventory, as well as the mapping of Albanian bryoflora, and envisage a possible future use of bryophytes in biomonitoring programs.

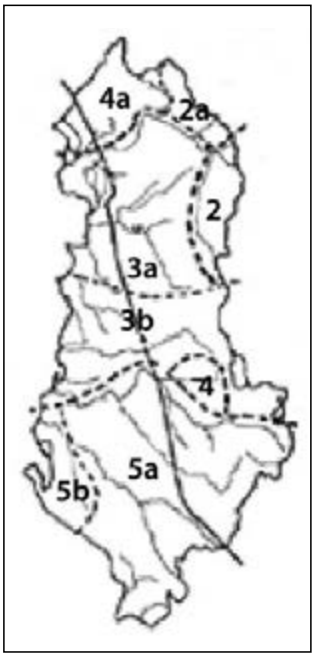

Fig. 8. Floristic regions of Albania (P. Hoda pers. com.)

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"Hot spots" within the high-mountain floras as the sectors of the integrity of the rare species (on the model of the Ukrainian Carpathians)

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Abstract. The brief results of the comparative study of the "hot spots" within the high-mountain flora of the Eastern Carpathians are presented. The results of the geographic–ecological study of the relict species *Primula farinosa* and its relict community *Caricetum davallianae* are discussed. The results of the study of biological–ecological peculiarities of *Anemone palmata* are presented for confirmation of necessity of union of the efforts of different specialists for generalisation of the protection of the "hot spots".

Key words: endemics, high-mountain floras, "hot spots", rare taxa and communities

Introduction

The Convention on Biological Diversity, signed in Rio Summit in 1992, Valencia in 1994, and Pan-European Strategy of Biological and Landscape Diversity accepted in Sofia at the International Meeting "Environment for Europe" in 1995, aimed to protect the rare species and lanscapes. If to take into consideration the unprecedented rates of extinction of plants, identification of areas of major diversity is very desirable. Therefore, we regard as very actual the problem of the "hot spot areas" which are regarded as the sectors with an acceptional concentration of species and a high rate of endemism that is in great danger of destruction (Médail & Quézel 1997).

Material and methods

Our treatment was based on the results of the comparative study of the "hot spots" within the high-mountain flora of the Eastern Carpathians at the territory of Ukraine and Romania (analysis of *ca*. 70 rare species common to these countries and of more than 20 common high-mountain communities including 5 to 20 rare species). Besides, we presented the results of the geographic–ecological and coenotic study of *Primula farinosa* being relict and rare in the Carpathians. At last, the biological–ecological peculiarities of *Anemone palmata* were presented as a model of the complex study of the "hot spots". We used in our paper the generally accepted methods of the population study.

Results

Despite the published important data on the centres of plant diversity for the whole world (e.g., Bevill & Louda 1999; Channell & Lomolino 2000), these and other extremely important syntheses did not embrace the data on the "hot spots" in the mountain floras of Europe which are the large reservoirs of the endemic and relict species, a lot of which are rare or threatened and have to be in a centre of a peculiar attention.

Conducting the study of the "hot spots" in the limits of the Eastern Carpathians, we realise the significant difficulties, especially with incorporation of our knowledge into the general data on the "hot spots" within the Carpathians, inconsistent understanding of the floristic peculiarities of their Regions, the fluctuating taxonomic status of many taxa and sometimes subjective definition and delimitation of endemic and

rare taxa. Nevertheless, we regard this study as the inevitable step of the development of the concept of the protection and conservation of the plant diversity.

The data on the specific richness of the flora of the vascular plants of the Carpathian Mountain System and its parts (our results, but also Chopik 1976; Coldea & Plamada 1976; Malinovski 1980; Coldea 1990; Ivan & al. 1993; Cristea 1995; Tasenkevich 1998; Malinovski & al. 2002) are presented here (Table 1).

Table 1. Numbers of taxa of vascular plants within the floras of the Carpathians.

Regions	Numbers		
	taxa	endemics	rare
Carpathians	4000	220	450
Eastern Carpathians	3000	150/50	350
Ukrainian Carpathians (UC)	2000	80/30	250
High Mountains of UC	1000	60/20	200

As happened, the whole flora of the Carpathians includes *ca*. 4000 species and subspecies (with 220 endemic taxa), the flora of the Eastern Carpathians – 3000 taxa (with 150 endemics), the flora of the Ukrainian part of the Carpathians – 2000 taxa (with 80 endemics including 30 narrow Eastern Carpathian taxa) and the high-mountain flora of the Ukrainian part of the Carpathians – 1000 taxa (with 60 endemics including 20 Eastern Carpathian taxa). Meanwhile, within the flora of the Carpathians, there are *ca*. 450 rare taxa, Eastern Carpathians – 350 taxa, Ukrainian Carpathians – 250 species and the high-mountain flora of the Ukrainian Carpathians – *ca*. 200 rare taxa. It means that the level of rarity everywhere is higher than the level of endemism, and it is comparatively high exactly within the high-mountain flora of the Ukrainian Carpathians. This phenomen is explained by the fact that a lot of high-mountain taxa, being extremely rare at the territory of Ukraine, are not endangered and sometimes not rare at the territory of the other parts of the Carpathians.

Therefore, we regard as expedient to present here the brief results of our comparative analysis of the rare high-mountain taxa at the territories of Ukraine and Romania carried out by us together with the Romanian colleagues. Within the Ukrainian part of the Carpathians, there are *ca*. 150 endangered or vulnerable taxa, and the similar data on the rare taxa are within the Romanian part of the Carpathians, besides, *ca*. 80 high-mountain taxa are common in the both floras.

The results of our study of 51 endangered or vulnerable taxa from these territories (mainly from Chernogora, Svidovets, Marmarosh, Gorgany and Chyvchyn in Ukraine, Fagarash, Piatra Craiului, Bucegi, Rodna and Retezat in Romania – Ziman & al. 1998) are presented below (Table 2).

Table 2. Geographic and biomorphological peculiarities of the model rare taxa within the high-mountain floras of Ukraine and Romania.

Species	1	2	3	4	5	6	7	8	9
Achillea lingulata Waldst. & Kit.		E	1	2	ph	sr	s	sr	ar
A. schurii Sch. Bip.	e	Ca	1	2	ph	sr	s	lr	ar
Aconitum hosteanum Schur	e	Ca	1	1	ph	sr	s	c	tr
A. jacquinii Rchb.	e	Ca	2	1	ph	nr	s	sr	ar
A. tauricum Wulfen subsp. *nanum* (Baumg.) Gáyer	e	Ca	2	2	ph	nr	s	sr	ar
Anemone narcissiflora L.		HA	2	2	ph	sr	m	sr	ar
Anthemis carpatica Willd.	r	E	1	1	ph	sr	s	sr	ar
Aquilegia nigricans Baumg.		E	1	1	ph	sr	s	c	tr
A. transsilvanica Schur	e	Ca	1	1	ph	sr	s	c	tr
Aster alpinus L.	r	C	1	2	ph	sr	s	sr	ar
Astragalus krajinae Domin	e	C	2	2	ph	nr	s	lr	ar
Bartsia alpina L.	r	C	2	2	ph	nr	s	lr	ar
Biscutella laevigata L.	r	E	1	2	ph	sr	s	c	tr
Callianthemum coriandrifolium Rchb.	r	E	1	1	ph	sr	m	sr	ar
Campanula carpatica Jacq.	e	Ca	1	2	ph	sr	s	sr	ar
Cardaminopsis ovirensis Wulfen	r	E	1	1	ss	sr	s	lr	tar
Carex davalliana Sm.	r	E	2	2	ph	sr	s	sr	ar
Coeloglossum alpinum Schur	e	Ca	1	2	ph	nr	s	b	ar
Dianthus speciosus Rchb.		Ca	1	2	ph	sr	s	c	tr
Draba aizoides (L.) Britt.	r	E	1	1	ss	nr	m	c	tar
Dryas octopetala L.	r	C	1	2	ss	nr	m	lr	tar
Gentiana acaulis L.		E	2	1	ph	sr	s	lr	ar
G. lutea L.	r	EA	2	1	ph	sr	s	c	tr
G. verna L.	r	E	1	1	ph	nr	s	lr	ar
Hedysarum hedysaroides (L.) Schinz & Thell.	r	E	1	2	ss	nr	s	lr	tar
Helianthemum grandiflorum Scop.		E	2	2	ph	nr	s	sr	tar
Leontopodium alpinum (L.) Cass.	r	E	1	1	ph	sr	s	sr	ar
Lloydia serotina (L.) Rchb.	r	HA	1	2	ph	nr	s	b	ar
Loiseleuria procumbens (L.) Desv.	r	HA	1	2	ss	nr	s	lr	ar
Minuartia oxypetala Woł.	e	Ca	1	1	ss	nr	s	lr	tar
Nigritella nigra Rchb.	e	Ca	1	1	ph	sr	s	b	ar
Oxyria digyna (L.) Hill	r	HA	2	2	ph	nr	s	sr	ar
Pedicularia oederi Vahl	r	HA	1	1	ph	sr	s	lr	ar
Phyteuma confusum A. Kern.	e	Ca	2	2	ph	sr	s	sr	ar
Primula halleri J.F. Gmel.	r	E	1	1	ph	sr	m	sr	ar
P. minima L.	r	E	2	2	ss	nr	m	lr	ar
P. verna L.	r	EA	2	2	ph	sr	m	sr	ar
Ranunculus thora L.	r	E	1	1	ph	nr	s	sr	ar
Rhodiola rosea L.	r	HA	2	1	ph	nr	s	lr	ar
Salix alpina Scop.	r	E	1	2	ss	nr	s	lr	ar
S. reticulata L.	r	HA	1	1	s	nr	s	lr	ar

Table 2. Continuation.

Species	1	2	3	4	5	6	7	8	9
S. retusa L.	r	E	1	1	s	nr	s	lr	ar
Saussurea porcii Degen	e	Ca	2	2	ph	sr	s	sr	ar
Saxifraga aizoides L.	r	HA	1	2	ss	nr	s	sr	ar
S. androsacea L.	r	EA	1	2	ss	nr	s	lsr	ar
S. bryoides L.	r	E	1	1	ss	sr	s	sr	ar
S. luteo-viridis Schott & Kotschy	r	E	1	1	ss	nr	s	sr	ar
Veronica alpina L.	r	C	1	2	ph	nr	s	lr	ar
V. aphylla L.	r	E	1	2	ss	nr	s	lsr	ar
V. bellidioides L.		E	1	1	ph	sr	s	sr	ar
V. fruticans Jacq.		E	2	2	ss	nr	s	lr	ar

Legend: 1: e – endemics; r – relicts; 2: HA – holarctic area; C – circumpolar area; E – Europe; EA – Eurasia; Ca – Carpathians; 3: 1–2 – relative categories of rarity in Ukraine; 4: 1–2 – the same in Romania; 5: ph – perennial herbs; ss – semishrubs; s – shrubs; 6: sr – semirosetteous shoots; nr – non-rosetteous shoots; 7: s – sympodial renewal; m – monopodial renewal; 8: c – caudex; sr – short rhizomes; lr – long rhizomes; b – bulbs; 9: tr – tap-roots; ar – adventitious roots; tar – mixed tap-adventitious roots.

Within these taxa, relicts predominate (31), and they are characterised by the disjunctive, mainly European (22), Eurasian (4), Circumpolar (4) and Holarctic (8) areas. Meanwhile, within this list, the Carpathian endemics are not numerous (12) and most of them are the narrow geographical races or subspecies of the polymorphic taxa having wide areas, and most of species grow in few localities. Within our list, 17 species (viz. *Aster alpinus*, *Biscutella laevigata*, *Campanula carpatica*, *Coeloglossum alpinum*, *Veronica alpina*, *V. aphylla*, etc.) in Ukraine are regarded as endangered, but in Romania they are vulnerable, meanwhile, the species more rare in Romania than in Ukraine are few (viz. *Aconitum anthora* subsp. *jacquinii*, *Gentiana acaulis*, *G. lutea*, *Rhodiola rosea*).

By the biomorphological peculiarities the perennial herbs (polycarpics) predominate (35 taxa), semishrubs are 14, and small shrubs are only two (*Salix* species). Many semishrubs (e.g., *Loiseleuria procumbens*, *Salix herbacea* and others) are non-rosetteous creeping plants characterised by long branched rhizomes lignified on almost all length and adventitious roots, but so called primitive semishrubs (with lignified basal parts of shoots only – viz. *Cerastium cerastoides*, *Dryas octopetala*, *Hedysarum hedysaroides*, *Rumex scutatus*, etc.) have semirosetteous above-ground shoots, mainly long rhizomes and mixed root system (having tap and adventitious roots). At last, few semishrubs (viz. *Draba aizoides*, etc.) are characterised by semirosetteous shoots, branched caudex and tap-roots. The characteristic features of all rare high-mountain shrubs and semishrubs are depressed reproductive renewal, rather intensive vegetative propagation and uncomplete age spectra of their populations. Most perennial herbs are characterised by adventitious roots only, and within them the semirosetteous plants (25 taxa) and plants with short rhizomes (21) predominate. Perennial herbs having tap-roots are not numerous (*Aconitum moldavicum* ssp. *hosteanum*, *Biscutella laevigata* and *Gentiana lutea* only) and all of them are characterised by semirosetteous shoots and branched caudex. The majority of the rare high-mountain species have sympodial renewal of shoots and species with monopodial main axis are few (*Anemone narcissiflora*, *Draba aizoides*, *Dryas octopetala*, *Primula halleri*, *P. minima*).

As a whole, the rare high-mountain species within the Carpathian floras of Ukraine and Romania are characterised by such common characters as the local, small, sometimes fragmented populations, mainly not complete age spectra, low ability to reproductive propagation, low indeces of renewal and replacing.

The next part of our study is a brief comparative analysis of the "hot spots" or localities including several rare species within the same high-mountain Carpathian communities at the territories of Ukraine and Romania. There are *ca*. 20 associations having 5 to 20 (sometimes more) endangered or vulnerable species (Coldea 1997; Malinovski & Krichfalushyi 2002; and others), and within them 11 include 10 to 25 rare taxa (Table 3).

Table 3. List of the Carpathian high-mountain plant associations in Ukraine and Romania having 10 to 25 rare taxa.

Associations	Type	Number of taxa	
		rare	common rare
Saxifrago (*paniculatae*)–*Festucetum versicoloris* Wal. 1933	relict	22	12
Festucetum amethystinae (Domin) Coldea 1984	rare	20	10
Achilleo (*schurii*)–*Dryadetum* (Beldie) Coldea 1984	endemic	22	12
Cystopteridetum fragilis Oberd. 1938	rare	20	5
Festucetum pictae Krajina 1933	rare	15	3
F. saxatilis Domin 1933	rare	15	10
Salicetum retuso–reticulatae Br.-Bl. 1926	relict	15	5
Primulo (*minima*)–*Caricetum curvulae* Br.-Bl. 1926 em. Oberd. 1957	rare	10	5
Rhododendro (*myrtifolii*)–*Vaccinietum carpaticae* Puskaru & al. 1956	endemic	10	5
Salicetum herbaceae Br.-Bl. 1931	relict	10	5
Salicetum kitaibelianae Coldea 1985	rare	10	3

There are two endemic, three relict and six rare associations, and within them, the most significant for protection are the associations and their localities including 20 or more rare taxa. Most of them are habituated to the subalpine or alpine limestones situated close to the mountain summits (at 1700–2000 m alt.). So the subalpine endemic association *Festucetum amethystinae* is the richest within them because it includes more than 85 taxa of vascular plants, and *ca*. 20 of them are rare (viz. *Aster alpinus*, *Leontopodium alpinum*, *Ranunculus thora*, *Aconitum jacquinii*, *Rhodiola rosea*, etc.), with 10 rare taxa common to the communities in Ukraine and Romania. In Ukraine it is located at Chernogora (Turkul, Rebra) and Svidovets (Dragobrat, Bliznitsa), in Romania at Rodna, Bucegi, Fagarash and some other Ranges. The subalpine relict association *Saxifrago* (*paniculatae*)*–Festucetum versicoloris* includes 85 taxa (with 22 rare ones – viz. the forementioned *Aster alpinus*, *Leontopodium alpinum*, *Rhodiola rosea*, but also *Draba aizoides*, *Coeloglossum alpinum*, *Hedysarum hedysaroides*, etc.) and with 12 rare taxa common to the communities in Ukraine and Romania. Its localities are situated at Chernogora (Turkul–Shpytsy) and Svidovets (Dragobrat, Bliznitsa, Heryshaska) in Ukraine and at Rodna, Fagarash, Retezat, Craiului in Romania. The next model community is the endemic association *Achilleo (schurii)–Dryadetum* including *ca*. 50 species with 22 rare taxa and with 12 taxa common to the communities in Ukraine and Romania. Beside dominant *Achillea schurii*, there are such endangered taxa as *Antennaria carpatica*, *Aster alpinus*, *Primula halleri* and *Phyteuma confusum*. Its localities are situated in Ukraine only at Svidovets (Dragobrat–Herishaska) and in Romania at Rodna, Fagarash and Bucegi.

Here we discuss briefly the recently published results of the comparative study of a mountain species which is rare in the Carpathians and frequent in the Alps. It is *Primula farinosa* which our finding (Ziman 1964) in the Ukrainian Carpathians is unique. Its plants grew in the Jasinian depression located between Chernogora, Svidovets and Gorgany Ranges at the altitude 700 m, in the southern slightly sloping peat bog with the predomination of *Carex davalliana*. According to the data of our observations, its population in 1962 included about 70 plants, in 1964 more than 90 plants. There were seedlings and young plants of *P. farinosa*, therefore, the age spectrum of population was complete. Unfortunately, in 1990 the plants of *P. farinosa* disappeared from this locality (Ziman & Vainagyi 1991), and afterwards we studied the populations of this species in other parts of its disjunctive area, in Slovakia close Brezno and in Romania close Tuznady (Ziman & al. 2001).

According to the literature (Hultén 1958; Valentine & al. 1972; Chopik 1976; Widmer 1981; etc.), *P. farinosa* is a circumpolar, arctic-montane glacial relict plant which area includes most countries of Europe, the northern part of Asia (from Siberia to Kamchatka) and north of North America. In North, Central, Eastern and South Europe there is a number of its localities, and this species is not rare in the Alps (Austria and Switzerland).

Within the Carpathian Mountain System, *P. farinosa* was known from nine localities in Poland (Pawłowski 1963), and at present time its unique locality exists (Beskids Zadezky, in the southern foot of the Radziejowa Range, close Javorka – Zaboklicka 1964) and till our days it is under monitoring (Zarzycki & Kaźmierczakowa 1993). In Czechia Dostál (1950) noted several *P. farinosa* localities in the Western Carpathians and Northern Moravia, but at present time it grows in the unique locality in the Western Carpathians, close Trshinets (Slavik 1990). In Slovakia *P. farinosa* occurred also in several localities (Nevucky 1943; Dostál 1950; Vološčuk 1996 – mainly in the Fatra and Low Tatras), and the unique standing of this species is confirmed – in the Low Tatras, close Brezno, locality Helpa (Háberová 1968; Ziman & Vainagyi 1991). In Romania recently were noted eight localities of *P. farinosa* (Morariu 1964; Sanda & Popescu 1971), and, according to the modern data (Cristea 1995; Coldea 1997; etc.), it is preserved in seven of them (Ciuc Basin – Tusnadu and Iara, Barsei Land – Stupini and Harman, Valley Uzului – Sat Cinod).

As a whole, *P. farinosa* disappeared from the flora of Ukraine, its localities became unique in Poland, Slovakia and Czechia, and they are few in Romania. We have to claim that these days *P. farinosa* evidently is endangered and disappearing species within the Carpathian Mountain System.

Primula farinosa grows everywhere in the similar ecological conditions, at the euthrophic bogs or boggy meadows at 700–800 m alt., within the association *Caricetum davallianae* Dutoit 1924. *Carex davalliana* is not too rare plant, but the association *Caricetum davallianae* is regarded as rare and relict because of its disjunctive area and the close lists of the characteristic

and constant species which everywhere include about 20 taxa (e.g., *Carex flava*, *C. panicea*, *Eriophorum latifolium*, *Molinia coerulea*, *Gymnadenia conopsea*, *Epipactis palustris*, *Equisetum palustre*, *Succisa pratensis*, *Parnasia palustris*, *Caltha laeta*, *Myosotis palustris*, *Polygala amara*, and some other taxa) within which the most interesting, in our opinion, is *Gentiana verna* which locality was and continues to be the unique in Ukraine and the Ukrainian Carpathians.

We have to note that plants of *P. farinosa* were introduced twice into the Ukrainian locality Heredzhivka: in 1990 from the Brezno population (Slovakia) and in 2000 from the Tuzhnady population (Romania), but both attempts were unsuccessful. We regard the detailed comparative study and the protection of all discussed localities of *P. farinosa* as extremely topical because of the tendencies of their disappearance.

We regard it would be better to realise a detailed study at least of several rare and threatened species within mountain floras of Europe as models for conclusions and generalisations on their protection.

In our opinion, the approaches of such work would be the multidisciplinary studies of species which are under threat of disappearing. The union for this purpose of the efforts of specialists on genetics, population biology, comparative morphology, ecology, phytocoenology is desirable and maybe necessary.

For confirmation of this idea we present briefly the results of our complex study of biological–ecological peculiarities of *Anemone palmata* in the Western Mediterranean realised by the team of botanists from France, Ukraine, Austria, USA and Spain (Médail & al. 2002) which we regard as a model for discussing of the "hot spots".

Our object *A. palmata* is one of the rarest and the most endangered taxa in France but it is not rare in Spain, Portugal and Morocco. As happened, in South-Eastern France (Provence) it is in the extreme periphery of *A. palmata* area (only five localities), but the western and south-western parts of the Iberian Peninsula are its core area, and *ca*. 40 populations of this species are known there.

According to the results of our study of 12 living populations of *A. palmata*, the essential biological and ecological characters of all five French populations are contrasted with those of the seven populations from E Spain. After study of about 20 individuals from each population by 13 morphometric characters, the significance of the differences between the French and Spanish plants was checked by analysis of variance (one-way ANOVA). The characters which proved to be significant were used for a Principal Component Analysis for more precise differentiation of the populations.

We established that the populations of *A. palmata* from SE France are characterised by unbalanced age spectrum, predominance of subsenile or senile plants, very low reproductive success and predominance of the vegetative propagation, but six (from seven) populations in E Spain have complete age spectrum with rather high number of seedlings and other young plants.

The next Correspondence Analysis realised by us discriminated the French and Spanish populations according to the substrate and composition of their plant communities (everywhere open grasslands or sclerophyllous chaparrals and low maquis with predomination of *Cistus albidus* and *Ulex parviflorus* on calcareous, dolomitic or siliceous substrates are situated). The list of species for the communities with *A. palmata* includes 200 species within which *ca*. 50 species are common to the French and Spanish communities.

Besides, according to data of our karyological study of seven populations, the French plants are diploid ($2n = 16$), and the Spanish plants are tetraploid ($2n = 32$).

As a result, our study showed that *A. palmata* is a rare and endangered species at its northernmost limit, in France (so called "old rare species" sensu Huenneke 1991), and some specific biological characteristics of the French *A. palmata* populations partly explain their rarity in France. Meanwhile, in the W and SW Iberian Peninsula *A. palmata* is rather frequent, and there is no danger of its extinction.

Discussion

We regard the establishing of the "hot spots" within the high-mountain flora of the vascular plants of the Eastern Carpathians as an important step to solve the problem of the plant conservation and the search of new approaches to the understanding of the History of the flora of the Carpathians and other mountains within Europe.

We believe it is desirable to arrange the detailed population study of the all localities of *Primula farinosa* throughout the Carpathian Mountain System, to compare them with not rare populations in Austria or Switzerland and only after that to carry out the active

measurements to protect and improve the state of the existing in the Carpathians localities and communities.

Like Morariu (1964), Stebbins (1980), Fiedler & Ahouse (1992) and Schemske & al. (1994), we regard that the understanding of the patterns of rarity constitutes a crucial topic in conservation of plant populations, and we believe in the expediency to pay the peculiar attention to the comparative study of the rare plants and related widespread congeners, desirably from the point of view of their reproductive biology, species life attributes, geography, ecology and genetic structure (Snyder & al. 1994; Médail & Verlaque 1997).

Acknowledgements. The authors are grateful to all co-authors of our recently published papers, especially to F. Ehrendorfer from Austria, G. Coldea and V. Cristea from Romania, M. Boscaiu from Spain and F. Médail from France. We are also pleased to thank F. Hamor from Ukraine, Carpathian Biosphere Reserve for his valuable support of our collecting of plants.

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Flora and vegetation of the national landmarks Kutina Pyramids and Stob Pyramids

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Abstract. Upon field studies in the Stob Pyramids Protected Area, a number of 198 plant species from 132 genera and 46 families were established. The flora of the Kutina Pyramids Protected Area consists of 210 vascular plants from 155 genera and 54 families. A total of 2 Bulgarian endemics (*Cerastium velenovskyi* and *Muscari vandasii*) and 6 Balkan endemics (*Achillea depressa*, *Achillea clypeolata*, *Cerastium petricola*, *Scabiosa triniaefolia*, *Hypericum rumeliacum*, and *Crucianella graeca*) were determined in the Stob Pyramids. A total of 1 Bulgarian endemic (*Galium rigidifolium*), 3 Balkan endemics (*Dianthus moesiacus*, *Chamaecytisus jankae*, and *Scabiosa triniaefolia*) and 1 CITES species (*Himantoglossum caprinum*) were determined in the Kutina Pyramids. The vegetation of both protected areas is described.

Key words: endemics, flora, vegetation

Introduction

The flora and vegetation of the natural landmarks Stob Pyramids and Kutina Pyramids have not been investigated so far. Some information on the geology, landscape, area, and location was reported by Popov (1961), Toshkov & Vihodcevsky (1964), Stojanov & al. (1968).

Material and methods

The flora and vegetation of the Stob Pyramids Protected Area was studied in the spring, summer, and autumn of 2003–2004. The investigations in the Kutina Pyramids Protected Area were carried out in the summer and autumn of 2005 and in the spring of 2006. The floristic samples were determined after Kozhuharov (1992, 1995), Jordanov (1963–1979), and Velchev (1982, 1989). The transect method was used for the investigation of the flora and vegetation of those two protected areas.

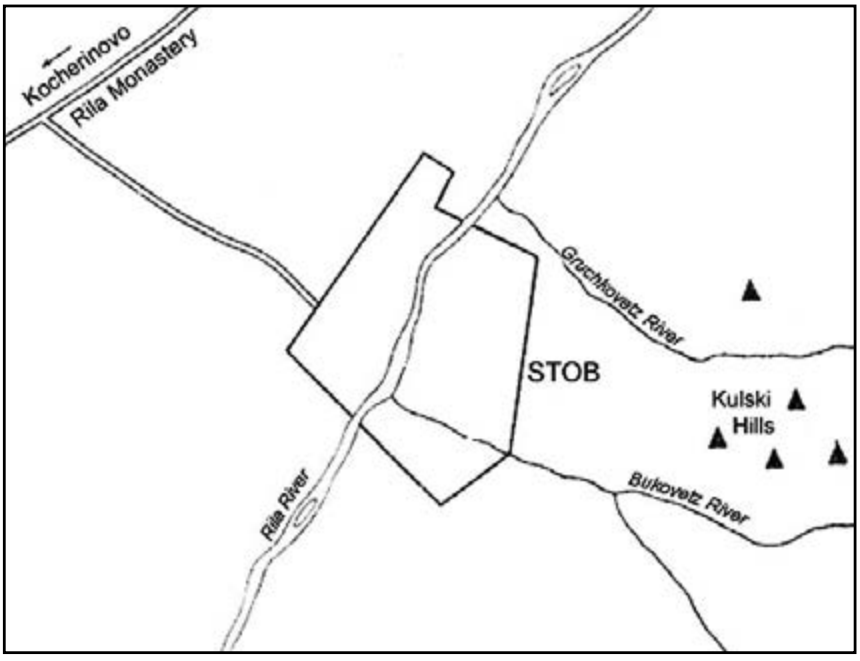

Fig. 1. Stob Pyramids Protected Area map.

Results and discussion

Stob Pyramids

The Pyramids were designated natural landmark under Ordinance No. 378/05.02.1964 under the National Environmental Protection Act with an area of 7.5 ha (Fig. 1). Located above the village of Stob, district of Kyustendil, east from the village between the Bukovetz

and Gruchkovetz gullies, these pyramids comprise the localities Kulski Rid and Tzurkvishteto. Three groups of pyramids are located on the south slope of Kulski Rid. These ones were shaped by water and temperature.

The pyramids from the first group are shorter (4–5 m). The middle group is more clearly shaped (10–12 m), and some of those pyramids have a sort of hats of flat rocks. The third group of pyramids is the most beautiful; they are very steep and scattered. Local people named them Svatovete (The Matchmakers) and Bratyata (The Brothers). Some other sharp pyramids are located on the north side of Kulski Rid.

Newly formed pyramids can be seen on the north side of the Gruchkovetz gully. Extremely picturesque are those that can be viewed from the road from Kocherinovo to the Rila Monastery.

The tree vegetation on the south side of the Stob Pyramids Protected Area consists of *Pinus nigra*, *Quercus dalechampii – Paliurus spina-christi.* The north slope is more wooded and humid. It is covered by the community of *Acer campestre* + *Quercus dalechampii* + *Betula pendula*. The following trees and bushes also occur: *Fraxinus ornus*, *Syringa vulgaris*, *Ligustrum vulgare*, *Colutea arborescens*, *Sorbus torminalis*, *Crataegus monogyna*, *Pyrus amygdaliformis*, *P. elaeagrifolia*, *Ulmus minor*, *Clematis vitalba*.

The herbaceous vegetation there is dominated by *Melica uniflora*, *Dactylis glomerata*, *Poa nemoralis*, *Brachypodium sylvaticum*, and *Cyclamen hederifolium*. Next to the middle group of pyramids is the community of *Ferulago campestris*. It also includes *Goniolimon tataricum*.

The open parts of Kulski Rid are covered with a herbaceous community of therophytes: *Trachynia distachya* + *Vulpia myuros* + *Taeniatherum caput-medusae* + *Aira capillaris* + *Psilurus incurvus*.

Conservation-important species are *Goniolimon tataricum*, *Erodium hoefftianum* and *Cerastium velenovskyi*. *Achillea clypeolata*, *A. depressa*, *Crucianella graeca*, *Cerastium petricola* and *Scabiosa triniifolia* are Balkan endemics. *Cerastium velenovskyi* is a Bulgarian endemic.

The full list of the species is given in Appendix 1.

Appendix 1. Flora of the Stob Pyramids Protected Area

Polypodiophyta

Aspleniaceae

1. *Asplenium adiantum-nigrum* L.
2. *Asplenium trichomanes* L.

Pinophyta

Cupressaceae

3. *Juniperus communis* L.
4. *Juniperus oxycedrus* L.

Pinaceae

5. *Pinus nigra* Arnold

Magnoliophyta

Anacardiaceae

6. *Cotinus coggygria* Scop.

Apiaceae

7. *Caucalis platycarpos* L.
8. *Eryngium campestre* L.
9. *Ferulago campestris* (Besser) Grecescu
10. *Orlaya grandiflora* (L.) Hoffm.
11. *Tordylium maximum* L.
12. *Torilis arvensis* (Huds.) Link

Asteraceae

13. *Achillea clypeolata* Simonk.
14. *Achillea depressa* Janka
15. *Achillea millefolium* L.
16. *Anthemis tinctoria* L.
17. *Carduus nutans* L.
18. *Carduus uncinatus* M. Bieb.
19. *Carlina vulgaris* L.
20. *Centaurea calcitrapa* L.
21. *Centaurea stoebe* L.
22. *Chondrilla juncea* L.
23. *Cichorium intybus* L.
24. *Conyza canadensis* (L.) Cronquist
25. *Crepis pulchra* L.
26. *Crupina vulgaris* Cass.
27. *Echinops microcephalus* Sibth.
28. *Echinops sphaerocephalus* L.
29. *Filago vulgaris* Lam.
30. *Hieracium caespitosum* Dumort.
31. *Inula brittanica* L.
32. *Inula conyza* L.
33. *Inula ensifolia* L.
34. *Inula hirta* L.
35. *Lactuca quercina* L.
36. *Scorzonera hispanica* L.
37. *Scolymus hispanicus* L.
38. *Senecio jacobaea* L.
39. *Tragopogon dubius* Scop.
40. *Xeranthemum annuum* L.

Betulaceae

41. *Carpinus orientalis* Mill.

Boraginaceae

42. *Onosma arenarium* Waldst. & Kit.

Brassicaceae

43. *Alyssum alyssoides* (L.) L.
44. *Alyssum corymbosioides* Formánek
45. *Alyssum murale* Waldst. & Kit.
46. *Arabis turrita* L.
47. *Cardamine hirsuta* L.
48. *Draba muralis* L.

Campanulaceae

49. *Campanula glomerata* L.
50. *Campanula trachelium* L.

Caryophyllaceae

51. *Cerastium petricola* Pančić
52. *Cerastium tenoreanum* Ser.
53. *Cerastium velenovskyi* Hayek
54. *Dianthus armeria* L.
55. *Dianthus pinifolius* Sibth. & Simonk.
56. *Herniaria hirsuta* L.
57. *Minuartia hirsuta* (M. Bieb.) Hand.-Mazz.
58. *Moenchia mantica* (L.) Bartl.
59. *Petrorhagia prolifera* (L.) P.W. Ball & Heywood

Chenopodiaceae

60. *Chenopodium vulvaria* L.

Cistaceae

61. *Helianthemum nummularium* (L.) Mill.

Convolvulaceae

62. *Convolvulus cantabrica* L.

Crassulaceae

63. *Sedum acre* L.
64. *Sedum cepaea* L.
65. *Sedum hispanicum* L.

Appendix 1. Continuation.

66. *Sedum maximum* (L.) Suter
67. *Sedum pallidum* M. Bieb.
68. *Sedum sartorianum* Boiss.

Cuscutaceae
69. *Cuscuta approximata* Bab.

Dipsacaceae
70. *Scabiosa columbaria* L.
71. *Scabiosa triniaefolia* Friv.

Euphorbiaceae
72. *Euphorbia cyparissias* L.
73. *Euphorbia helioscopia* L.

Fabaceae
74. *Astragalus hamosus* L.
75. *Astragalus onobrychis* L.
76. *Colutea arborescens* L.
77. *Coronilla varia* L.
78. *Genista carinalis* Griseb.
79. *Genista depressa* M. Bieb.
80. *Genista tinctoria* L.
81. *Lathyrus laxiflorus* (Desv.) Kuntze
82. *Lathyrus sphaericus* Retz.
83. *Medicago minima* (L.) Bart
84. *Onobrychis alba* (Waldst. & Kit.) Desv.
85. *Onobrychis lasiostachya* Boiss.
86. *Robinia pseudoacacia* L.
87. *Trifolium alpestre* L.
88. *Trifolium arvense* L.
89. *Trifolium diffusum* Ehrh.
90. *Trifolium dubium* Sibth.
91. *Trifolium nigrescens* Viv.
92. *Trifolium patens* Schreb.
93. *Trifolium purpureum* Loisel.
94. *Trifolium scabrum* L.
95. *Trifolium striatum* L.
96. *Vicia hirsuta* (L.) Gray
97. *Vicia lathyroides* L.

Fagaceae
98. *Quercus dalechampii* Ten.

Gentianaceae
99. *Centaurium erythraea* Rafn

Geraniaceae
100. *Erodium cicutarium* (L.) L'Hér.
101. *Erodium hoefftianum* C.A. Mey.
102. *Geranium molle* L.

Hypericaceae
103. *Hypericum olympicum* L.
104. *Hypericum rumeliacum* Boiss.

Iridaceae
105. *Crocus chrysanthus* (Herbich) Herbich

Juncaceae
106. *Luzula forsteri* (Sm.) DC.

Lamiaceae
107. *Ballota nigra* L.
108. *Clinopodium vulgare* L.
109. *Marrubium peregrinum* L.
110. *Salvia virgata* Jacq.
111. *Stachys germanica* L.
112. *Teucrium chamaedrys* L.
113. *Teucrium polium* L.
114. *Thymus longicaulis* C. Presl

Liliaceae
115. *Allium flavum* L.
116. *Allium pulchellum* Don.
117. *Gagea arvensis* (Pers.) Dumort.
118. *Muscari comosum* (L.) Mill.
119. *Muscari vandasii* Velen.
120. *Ornithogalum comosum* L.
121. *Scilla autumnalis* L.

Oleaceae
122. *Fraxinus ornus* L.
123. *Ligustrum vulgare* L.
124. *Syringa vulgaris* L.

Onagraceae
125. *Epilobium hirsutum* L.

Orobanchaceae
126. *Orobanche aegyptiaca* Pers.
127. *Orobanche gracilis* Simonk.

Papaveraceae
128. *Papaver dubius* L.
129. *Papaver rhoeas* L.

Plantaginaceae
130. *Plantago lanceolata* L.
131. *Plantago subulata* L.

Poaceae
132. *Aegilops neglecta* Req. ex Bertol.
133. *Agrostis capillaris* L.
134. *Aira elegantissima* Schur
135. *Avenula compressa* (Heuff.) Sauer & Chmelit.
136. *Brachypodium sylvaticum* (Huds.) P. Beauv.
137. *Bromus squarrosus* L.
138. *Bromus sterilis* L.
139. *Bromus tectorum* L.
140. *Cleistogenes serotina* (L.) Gould
141. *Cynodon dactylon* (L.) Pers.
142. *Cynosurus cristatus* L.
143. *Cynosurus echinatus* L.
144. *Dactylis glomerata* L.
145. *Dasypyrum villosum* (L.) Cand.
146. *Dichanthium ischaemum* (L.) Roberty
147. *Elymus repens* (L.) Gould
148. *Festuca valesiaca* Schleich. ex Gaudin
149. *Koeleria mitruschi* Ujhelyi
150. *Koeleria nitidula* Velen.
151. *Melica ciliata* L.
152. *Melica uniflora* Retz
153. *Phleum phleoides* (L.) Karst.
154. *Poa bulbosa* L.
155. *Poa nemoralis* L.
156. *Psilurus incurvus* (Gouan) Schinz & Thell.
157. *Stipa capillata* L.
158. *Taeniatherum caput-medusae* (L.) Nevski
159. *Trachynia distachya* (L.) Link
160. *Ventenata dubia* (Leers.) Cosson
161. *Vulpia myuros* (L.) C.C. Gmelin

Polygonaceae
162. *Bilderdykia convolvulus* (L.) Dumort.
163. *Polygonum aviculare* L.
164. *Rumex acetosella* L.
165. *Rumex pulcher* L.

Plumbaginaceae
166. *Goniolimon tataricum* (L.) Boiss.

Primulaceae
167. *Cyclamen hederifolium* Aiton

Ranunculaceae
168. *Clematis vitalba* L.
169. *Ranunculus ficaria* L.

Rhamnaceae
170. *Paliurus spina-christi* Mill.

Rosaceae
171. *Crataegus monogyna* Jacq.
172. *Potentilla argentea* L.
173. *Potentilla laciniosa* Waldst. & Kit. ex Nestler
174. *Potentilla micrantha* Ramond ex DC.
175. *Potentilla neglecta* Baumg.
176. *Potentilla obscura* Willd.
177. *Pyrus amygdaliformis* Viel.
178. *Pyrus elaeagrifolia* Pall.
179. *Sorbus torminalis* (L.) Crantz

Rubiaceae
180. *Crucianella graeca* Boiss.
181. *Cruciata laevipes* Opiz
182. *Cruciata pedemontana* (Bell.) Ehrhend.
183. *Galium divaricatum* Pourret. ex Lam.
184. *Galium lucidum* All.
185. *Galium pseudoaristatum* Schur
186. *Galium verum* L.

Santalaceae
187. *Comandra elegans* (Roch. ex Reichenb.) Rchb. f.

Saxifragaceae
188. *Saxifraga graeca* Boiss.

Scrophulariaceae
189. *Verbascum phoeniceum* L. subsp. *flavidum* (Boiss.) Bornm.
190. *Veronica austriaca* L. subsp. *jacquinii* (Baumg.) Maly
191. *Veronica chamaedrys* L.
192. *Veronica hederifolia* L.
193. *Veronica triphyllos* L.

Ulmaceae
194. *Ulmus minor* Mill.

Valerianaceae
195. *Valeriana officinalis* L.
196. *Valerianella carinata* Loisel.
197. *Valerianella coronata* (L.) DC.

Violaceae
198. *Viola arvensis* Murr.

Kutina Pyramids

These Pyramids are designated natural landmark under Ordinance No. 927/12.04.1962 by the Main Forestry Board, with an area of 12.5 ha (Fig. 2). Under the present forestry project, the protected area is 29 da and is Subsection 352 of the State Forestry of Sofia.

The pyramids are located northeast from the village of Kutina, at the foothills of Mt Mala Sofia, in the Golemiya Dol locality. They are made of clayey-sandy soil. There are several groups, and the tallest group is on the left side by the bright sandstone cliffs. Isolated groups of shorter pyramids are also located along the ravine on the right from the side of Kutina village. Two more groups are located before the small lake.

The tree vegetation in the big ravine is mixed deciduous and includes hygrophytes and mesophytes such as *Salix alba*, *Populus nigra*, *Sambucus nigra*, *Acer negundo*, *Betula pendula*, *Euonymus europaeus*, *Juglans regia*, *Ulmus minor*, *Cornus mas*, *Quercus polycarpa*, *Robinia pseudoacacia*, and *Prunus avium*.

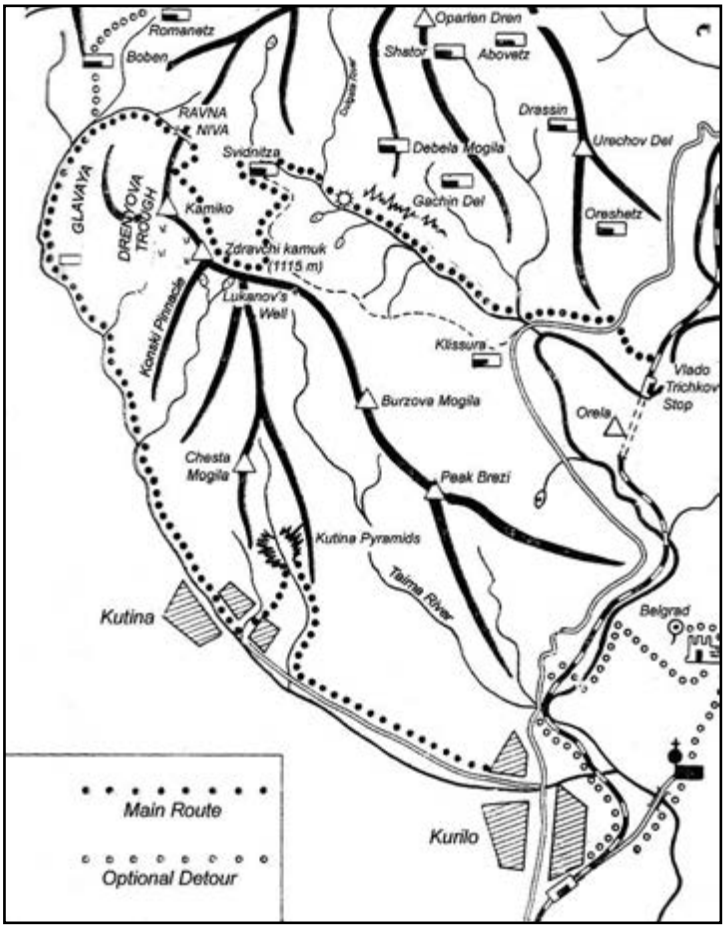

Fig. 2. Kutina Pyramids Protected Area map.

Among the herbaceous species are *Geum urbanum*, *Urtica dioica*, *Poa nemoralis*, *Clinopodium vulgare*, *Stellaria media*, *Dryopteris filix-mas*, *Viola reichenbachiana*, *Hieracium umbellatum*, *Alliaria petiolata*, *Potentilla micrantha*, *Corydalis solida*, *Buglossoides purpurocaeruleum*, *Campanula trachelium*, and *Mycelis muralis*.

The big lake is surrounded by hygrophytes like *Alisma plantago-aquatica*, *Ceratophyllum demersum*, *Typha latifolia*, *Lemna trisulca*, *L. minor*, *Potamogeton crispus*, *Elodea canadensis*, *Juncus conglomeratus*, *Rumex crispus*, and *Galega officinalis*.

The humid meadow north of the lake is covered with the association of *Agrostis capillaris* + *Poa compressa*.

Among the Fabaceous are *Melilotus officinalis*, *Dorycnium herbaceum*, *Astragalus glycyphyllos*, *Ononis arvensis*, *Medicago lupulina*, *Astragalus cicer*, and *Vicia sativa*.

The bushes are mostly *Prunus spinosa* and *P. cerasifera*. Among the other herbaceous species are *Crepis biennis*, *Eryngium campestre*, *Cephalaria transsylvanica*, *Cichorium inthybus*, *Picris hieracioides*, *Origanum vulgare*, *Plantago major*, *Leontodon crispus*, and *Peucedanum alsaticum*.

There is an artificial forest of *Pinus nigra* north of the big ravine.

In the open rocky and sandy places in the west part of the protected area is the association of *Festuca valesiaca* + *Dichanthium ischaemum* + *Chrysopogon gryllus* + *Stipa capillata*. The Poaceous are represented also by *Koeleria schurii* and *Melica ciliata*, and the Fabaceous by *Dorycnium herbaceum*, *Astragalus onobrychis*, *Trifolium arvense*, *Genista tinctoria*.

Among the various grass species are *Ferulago sylvatica*, *Scabiosa triniaefolia*, *Sanguisorba minor*, *Arabis sagittata*, *Peucedanum arenarium*, *Crupina vulgaris*, *Eryngium campestre*, *Centaurium erythraea*, *Inula ensifolia*, *Potentilla pedata*, *Campanula trachelium*, and *Dianthus capitatus* subsp. *andrzejowskianus*.

Himantoglossum caprinum (16 specimens) is in the CITES Appendices. The Balkan endemics are *Chamaecytisus jankae* and *Scabiosa triniaefolia*.

The full species list is given in Appendix 2.

Appendix 2. Flora of the Kutina Pyramids Protected Area

Polypodiophyta

Aspidiaceae
1. *Dryopteris filix-mas* (L.) Schott

Athyriaceae
2. *Cystopteris fragilis* (L.) Bernh.

Equisetophyta

Equisetaceae
3. *Equisetum arvense* L.
4. *Equisetum ramosissimum* Desv.

Pinophyta

Pinaceae
5. *Pinus nigra* Arnold

Magnoliophyta

Aceraceae
6. *Acer negundo* L.

Alismataceae
7. *Alisma plantago-aquatica* L.

Apiaceae
8. *Chaerophyllum bulbosum* L.
9. *Chaerophyllum sylvaticum* L.
10. *Daucus carota* L.
11. *Eryngium campestre* L.
12. *Falcaria vulgaris* Bernh.
13. *Ferulago sylvatica* (Besser) Rchb.
14. *Orlaya grandiflora* (L.) Hoffm.
15. *Peucedanum alsaticum* L.
16. *Peucedanum arenarium* Waldst. & Kit.
17. *Physospermum cornubiense* (L.) DC.
18. *Tordylium maximum* L.
19. *Torilis nodosa* (L.) Gaertn.

Araliaceae
20. *Hedera helix* L.

Asteraceae
21. *Arctium lappa* L.
22. *Anthemis tinctoria* L.
23. *Bidens tripartita* L.
24. *Carduus candicans* Waldst. & Kit.
25. *Centaurea biebersteinii* DC.
26. *Centaurea cuneifolia* Sibth. & Simonk.
27. *Chondrilla juncea* L.
28. *Cichorium inthybus* L.
29. *Crepis biennis* L.
30. *Crupina vulgaris* Cass.
31. *Echinops sphaerocephalus* L.
32. *Filago vulgaris* Lam.
33. *Hieracium bauchinii* Bess.
34. *Hieracium umbellatum* L.
35. *Hypochaeris radicata* L.
36. *Inula ensifolia* L.
37. *Inula hirta* L.
38. *Inula oculus-christi* L.
39. *Inula salicina* L.
40. *Lapsana communis* L.
41. *Leontodon crispus* Vill. subsp. *crispus*
42. *Leucanthemum vulgare* Lam.
43. *Mycelis muralis* (L.) Dumort.
44. *Picris hieracioides* L.
45. *Scorzonera hispanica* L.
46. *Senecio vernalis* Waldst. & Kit.
47. *Sonchus oleraceus* L.
48. *Tussilago farfara* L.
49. *Xeranthemum cylindraceum* Sibth. & Simonk.

Betulaceae
50. *Betula pendula* Roth
51. *Carpinus orientalis* Mill.

Boraginaceae
52. *Buglossoides purpurocaerulea* (L.) I.M. Johnst.
53. *Echium vulgare* L.

Brassicaceae
54. *Alliaria petiolata* (Bieb.) Cav. & Grande
55. *Alyssum desertorum* Stapf.
56. *Alyssum strigosum* Banks & Sol.
57. *Arabis glabra* (L.) Bernh.
58. *Arabis sagittata* (Bertol.) DC.
59. *Erophila verna* (L.) Chevall.
60. *Erysimum diffusum* Ehrh.
61. *Thlaspi alliaceum* L.

Campanulaceae
62. *Campanula bononiensis* L.
63. *Campanula lingulata* Waldst. & Kit.
64. *Campanula patula* L.
65. *Campanula rapunculoides* L.
66. *Campanula trachelium* L.

Caryophyllaceae
67. *Dianthus capitatus* Balb. ex DC. subsp. *andrzejowskianus* Zapał.
68. *Dianthus moesiacus* Vis. & Pančić
69. *Holosteum umbellatum* L.
70. *Lychnis coronaria* (L.) Desr.
71. *Petrorhagia illyrica* (L.) P.W. Ball & Heywood
72. *Petrorhagia prolifera* (L.) P.W. Ball & Heywood
73. *Silene italica* (L.) Pers.
74. *Silene otites* Simonk.
75. *Silene vulgaris* (Moench) Garcke
76. *Stellaria graminea* L.
77. *Stellaria media* (L.) Vill.

Celastraceae
78. *Euonymus europaeus* L.
79. *Euonymus verrucosus* Scop.

Ceratophyllaceae
80. *Ceratophyllum demersum* L.

Convolvulaceae
81. *Convolvulus arvensis* L.
82. *Convolvulus cantabrica* L.

Cornaceae
83. *Cornus mas* L.
84. *Cornus sanguinea* L.

Crassulaceae
85. *Sedum caespitosum* (Cav.) DC.
86. *Sedum maximum* (L.) Suter

Cyperaceae
87. *Carex caryophyllea* Latourr.
88. *Carex divisa* Huds.
89. *Eleocharis palustris* (L.) R. Br.

Dipsacaceae
90. *Cephalaria transsylvanica* (L.) Roem. & Schult.
91. *Knautia arvensis* (L.) Coult.
92. *Scabiosa ochroleuca* L.
93. *Scabiosa triniaefolia* Friv.

Euphorbiaceae
94. *Euphorbia cyparissias* L.

Fabaceae
95. *Astragalus cicer* L.
96. *Astragalus glycyphyllos* L.
97. *Astragalus onobrychis* L.
98. *Chamaecytisus jankae* (Velen.) Rothm.
99. *Chamaecytisus lejocarpus* (A. Kern.) Rothm.
100. *Coronilla varia* L.
101. *Dorycnium herbaceum* Vill. var. *macedonicum* (Degen & Dörfl.) Kuzmanov
102. *Galega officinalis* L.
103. *Genista tinctoria* L.
104. *Lathyrus niger* (L.) Bernh.
105. *Lathyrus tuberosus* L.
106. *Lembotropis nigricans* (L.) Griseb.
107. *Lotus corniculatus* L.
108. *Medicago lupulina* L.
109. *Medicago minima* (L.) Bartl.
110. *Melilotus officinalis* (L.) Pall.
111. *Onobrychis alba* (Waldst. & Kit.) Desv. subsp. *calcarea* (Vandas) Ball
112. *Ononis spinosa* L.
113. *Robinia pseudoacacia* L.
114. *Trifolium arvense* L.
115. *Trifolium hirtum* All. var. *hirtum*
116. *Trifolium montanum* L.
117. *Trifolium pratense* L.
118. *Vicia cassubica* L.
119. *Vicia sativa* L. complex

Fagaceae
120. *Quercus cerris* L.
121. *Quercus polycarpa* Schur
122. *Quercus rubra* L.

Gentianaceae
123. *Centaurium erythraea* Rafn

Geraniaceae
124. *Geranium columbinum* L.
125. *Geranium sanguineum* L.

Hippocastanaceae
126. *Aesculus hippocastanum* L.

Hydrocharitaceae
127. *Elodea canadensis* Michx.

Appendix 2. Continuation.

Hypericaceae
128. *Hypericum perforatum* L.

Iridaceae
129. *Iris germanica* L.

Juglandaceae
130. *Juglans regia* L.

Juncaceae
131. *Juncus effusus* L.

Lamiaceae
132. *Acinos suaveolens* (Sibth. & Simonk.) G. Don
133. *Clinopodium vulgare* L.
134. *Lycopus europaeus* L.
135. *Mentha spicata* L.
136. *Origanum vulgare* L.
137. *Prunella vulgaris* L.
138. *Salvia amplexicaulis* Lam.
139. *Sideritis montana* L.
140. *Teucrium chamaedrys* L.

Lemnaceae
141. *Lemna minor* L.
142. *Lemna trisulca* L.

Liliaceae
143. *Allium flavum* L.
144. *Allium sphaerocephalum* L.
145. *Anthericum liliago* L.
146. *Asparagus officinalis* L.
147. *Colchicum autumnale* L.
148. *Muscari comosum* (L.) Mill.
149. *Ornithogalum pyrenaicum* L.

Linaceae
150. *Linum tenuifolium* L. var. *rigidum* Podp.

Lythraceae
151. *Lythrum salicaria* L.

Oleaceae
152. *Fraxinus ornus* L.
153. *Ligustrum vulgare* L.

Onagraceae
154. *Epilobium roseum* Schreb.

Orchidaceae
155. *Himantoglossum caprinum* (M. Bieb.) Spreng.

Papaveraceae
156. *Corydalis solida* (L.) Schwarz

Plantaginaceae
157. *Plantago major* L.
158. *Plantago subulata* L.

Poaceae
159. *Agrostis capillaris* L.
160. *Brachypodium pinnatum* (L.) P. Beauv. subsp. *pinnatum*
161. *Brachypodium sylvaticum* (L.) P. Beauv.
162. *Chrysopogon gryllus* (L.) Trin.
163. *Cynosurus cristatus* L.
164. *Dactylis glomerata* L.
165. *Danthonia alpina* Vest
166. *Dichanthium ischaemum* (L.) Roberty
167. *Elymus hispidus* (Opiz) Meld. subsp. *hispidus*
168. *Festuca valesiaca* Schleich. ex Gaudin
169. *Phleum phleoides* H. Karst. var. *blepharoides* Asch. & Graebn.
170. *Poa compressa* L.
171. *Poa nemoralis* L.
172. *Stipa capillata* L.

Polygonaceae
173. *Rumex acetosella* L.
174. *Rumex crispus* L.
175. *Rumex sanguineus* L.

Potamogetonaceae
176. *Potamogeton crispus* L.

Ranunculaceae
177. *Clematis vitalba* L.

Rosaceae
178. *Agrimonia eupatoria* L.
179. *Crataegus monogyna* Jacq.
180. *Cydonia oblonga* Mill.
181. *Filipendula vulgaris* Moench
182. *Fragaria vesca* L.
183. *Geum urbanum* L.
184. *Malus sylvestris* Mill.
185. *Potentilla micrantha* Ramond ex DC.
186. *Potentilla neglecta* Baumg.
187. *Potentilla obscura* Willd. var. *obscura*
188. *Potentilla pedata* Willd.
189. *Potentilla reptans* L.
190. *Prunus avium* L.
191. *Prunus cerasifera* Ehrh.
192. *Prunus spinosa* L.
193. *Pyrus sativa* Lam. & DC.
194. *Rosa canina* L.
195. *Rubus caesius* L.
196. *Sanguisorba minor* Scop.

Rubiaceae
197. *Asperula cynanchica* L.
198. *Asperula purpurea* (L.) Ehrend.
199. *Galium aparine* L.
200. *Galium rigidifolium* Krendl.
201. *Galium verum* L.

Salicaceae
202. *Populus nigra* L.
203. *Salix alba* L.

Santalaceae
204. *Thesium arvense* Horv.

Scrophulariaceae
205. *Veronica hederifolia* L.
206. *Veronica polita* Fr.

Typhaceae
207. *Typha angustifolia* L.
208. *Typha latifolia* L.

Urticaceae
209. *Urtica dioica* L.

Violaceae
210. *Viola reichenbachiana* Jord. ex Bor.

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Plant species of conservation concern at Mt Chepun (Western Bulgaria)

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Abstract. The data present the results of the authors' field studies, complemented with the available literature data and the available information from the Bulgarian herbaria. Totally 52 plant species of conservation concern are found at Mt Chepun. The number of Bulgarian (3) and Balkan endemic species (33) is significant. Twenty one species are included in the Red Data Book of Bulgaria – 3 are endangered and 18 are rare. Data are presented for the populations of some species of conservation concern.

Key words: Mt Chepun, species of conservation concern

Introduction

Chepun mountain is a calcareous massif, situated in Western Bulgaria, south of the main ridge of the Balkan Range. In a morphogeographical respect the mountain is a part of West Balkan Range. The highest point is Petrovski Krust peak – 1206 m alt. The terrain is typically karst. The northern slopes of the mountain are occupied mainly by tree and shrub plant complexes. On the steep and rocky south slopes mainly shrub and grass communities are developing, rich in species of conservation concern.

According to the floristic division of Bulgaria Mt Chepun belongs to Znepole floristic region.

Material and methods

For studying the localities and status of the populations of the species of conservation concern at Mt Chepun the transect method has been used. The species have been identified according to the main sources for the Bulgarian flora: *Flora Reipublicae Popularis Bulgaricae* (Jordanov 1963–1979; Velchev 1982, 1989; Kozhuharov 1995); *Field Guide to the Vascular Plants in Bulgaria* (Kozhuharov 1992); *Key to the Plants of Bulgaria* (Delipavlov & Cheshmedjiev 2003). The authors' observations have been completed by literature data (Velchev 1962; Angelova 2004) and data from the Bulgarian herbaria (SOM, SO, SOA). The conservation status and endemism are defined according to *Red Data book of the P. R. Bulgaria* (Velchev 1984), *Atlas of Bulgarian endemic plants* (Petrova 2006), and *Conspectus of the Bulgarian vascular flora* (Assyov & Petrova 2006).

Results and discussion

In the studied region the finds of 18 rare and 3 threatened with extinction species of higher plants have been defined. The endemic element is presented by 3 Bulgarian and 33 Balkan endemics. In the Bulgarian Law on Biological Diversity (2002) 18 species are included with a conservation status (Table 1).

Two of the species are with category in *1997 IUCN Red List of Threatened Plants* (Walter & Gillet 1998): *Astragalus wilmottianus* (Rare) and *Tulipa urumoffii* (Vulnerable). In the Bern Convention (1997) two species are included: *Bromus moesiacus* and *Himantoglossum caprinum*. In the Convention on International Trade in Endangered Species (CITES) (1993) *Dactylorhiza cordigera*, *Galanthus elwesii*, *Gymnadenia conopsea*, *Ophrys cornuta*, *Orchis purpurea*, *O. tridentata*, and *Sternbergia colchiciflora* are included.

Table 1. Plant species of conservation concern at Mt Chepun.

Species	Red Book	Endemics		Bulgarian Biodiversity Law	Personal observations	Literature data	Herbarium specimens
		Balkan	Bulgarian				
Acanthus balcanicus Heywood & I. Richardson *		+			+		+
Achillea ageratifolia (Sm.) Benth. *		+			+	+	+
A. clypeolata Sm. *		+			+	+	+
Allium cupani Raf.	Rare					+	+
Anemone sylvestris L. *	Endangered			App. 3	+	+	+
Astragalus wilmottianus Stoj. *	Rare	+		App. 3	+	+	+
Asyneuma anthericoides (Janka) Bornm. *		+			+	+	+
Bunium ferulaceum Sibth. & Sm.	Rare						+
Bromus moesiacus Velen. *			+	App. 2, 3	+		
Cachrys alpina M. Bieb. *	Rare			App. 3	+	+	+
Centaurea chrysolepis Vis. *		+			+	+	+
Chamaecytisus calcareus (Velen.) Kuzmanov *		+			+		
Ch. jankae (Velen.) Rothm. *		+			+		+
Corothamnus rectipilosus (Adam.) Skalická *		+			+	+	+
Crucianella graeca Boiss.		+				+	
Daphne cneorum L.	Rare						+
Dianthus pelviformis Heuff.		+				+	+
Edraianthus serbicus (A. Kern.) Petrovič *	Rare	+		App. 3	+	+	+
Eryngium palmatum Pančić & Vis.	Rare	+		App. 3	+		
Erysimum comatum Pančić	Rare	+		App. 3		+	+
Festuca stojanovii (Acht.) Kožuharov		+					+
Fritillaria orientalis Adams	Rare			App. 2, 3			+
Galanthus elwesii Hook. f.				App. 3			+
Genista subcapitata Pančić *		+			+	+	+
Goniolimon tataricum (L.) Boiss. *	Endangered			App. 2, 3	+	+	+
Himantoglossum caprinum (M. Bieb.) Spreng.				App. 3		+	
Hypericum rumeliacum Boiss. *		+			+	+	+
Jurinea tzar-ferdinandii Davidov *	Rare	+		App. 2, 3	+	+	+
Laserpitium siler L.	Rare				+		+
Malcomia orsiniana (Ten.) Ten. subsp. ***angulifolia*** (Boiss. & Orph.) A. Stork	Rare			App. 3		+	
Melampyrum scardicum Wettst.		+					+
Minuartia bosniaca (G. Beck) K. Malý		+					+
M. bulgarica (Velen.) Griseb.		+				+	+
M. rhodopaea (Degen) Kožuharov & Kuzmanov	Rare	+					+
Nonea atra Griseb. *		+			+		
Onosma heterophylla Griseb.	Rare						+
Ophrys cornuta Steven				App. 3	+		
Polygala hospita Heuff. *	Rare				+	+	+
Scabiosa triniifolia Friv.		+				+	
Sempervivum erythraeum Velen.		+				+	
Senecio macedonica Griseb.		+				+	+
Silene fabarioides Hausskn. *		+			+		+
Stachys cretica L. subsp. ***bulgarica*** Rech. f.				App. 3		+	
S. milanii Petrovič		+					+
Tragopogon balcanicus Velen.	Rare	+				+	+
T. pterodes Pančić *		+			+		+
Trifolium dalmaticum Vis.		+				+	+
T. velenovskyi Vandas		+					+
Trinia glauca (L.) Dumort. subsp. ***carniolica*** (A. Kern. ex Janch.) H. Wolff *	Rare			App. 3	+	+	
Tulipa urumoffii Hayek *	Endangered		+	App. 3	+	+	+
Verbascum eriophorum Godr.	Rare	+		App. 3		+	
V. formanekii Borbás ex Formánek			+			+	+

* **Species with data for their populations.**

Status of the populations of some species of conservation concern

Acanthus balcanicus population is of low number of individuals, and is represented by comparatively small groups of plants in shrub communities on the southern slopes of the mountain.

Achillea ageratifolia occurs mainly on the ridge parts and southern slopes of the mountain, in stony and rocky places. The species forms dense population with high number of individuals.

Achillea clypeolata occurs on a mass scale in grassy and shrubby stony places. The population is with a mosaic structure, composed by groups with a high number of individuals. The species is a main element of the grassy complexes, which are peculiar for the calcareous terrains.

Anemone sylvestris is distributed mainly on the northern and north-western slopes of the mountain, on more moist soils, mainly on the border between grassy and shrubby communities. The population is represented by single individuals and generally is of low number.

Astragalus wilmottianus is distributed on stony terrains of southern and western exposure, together with other species of conservation concern – *Genista subcapitata*, *Chamaecytisus jankae*, *C. calcareus*, *Tragopogon pterodes*, *Corotamnus rectipilosus*, etc. The population is with a mosaic structure and occupies comparatively large area. The species is hardly competitive. In the last tree years a decrease of approximately 1/3 of the number and area of the population has been observed as a result of forestry fire and the following invasion of ruderal plants.

Asyneuma anthericoides forms population of comparatively not high number of individuals (some dozens of plants) in stony places at the foot of Petrovski Krust peak.

Bromus moesiacus is represented by single plants or small groups of individuals on the southern slopes of the mountain, on shallow and dry soils.

Cachris alpina has population of comparatively low number of individuals, not more than 250 plants, localised mainly on the southern and south-western slopes of Petrovski Krust peak.

Centaurea chrysolepis is a comparatively widely distributed species in the region. The population is with a high number of individuals, composed by single plants or small groups of 5–10 individuals.

Chamaecytisus calcareus is distributed in open stony places on the southern slopes of the mountain. It forms dense populations together with other species of genus *Chamaecytisus*, with *Genista subcapitata*, *Corothamnus rectipilosus* and perennial grassy species.

Chamaecytisus jankae occurs on the western and southern slopes below the peak Petrovski Krust. It forms dense populations with a high number of individuals. This is the typical species for calcareous terrains, forming complexes with other calciphilous species.

Corothamnus rectipilosus has fragmented and not high numerous population, occurring south of Petrovski Krust peak, on stony terrains which are comparatively difficult to access. The species forms complexes with the species of genus *Chamaecytisus* and annual grassy species.

Edraianthus serbicus forms fragmented population with a high number of individuals, composed by groups of 30–100 specimens. The species is localised on the southern and western stony and rocky parts of the mountain.

Genista subcapitata occurs together with species of the genus *Chamaecytisus*, *Corothamnus rectipilosus*, etc., on the slopes of south-western exposure. The population occupies an essential area and is of high number of individuals and high density.

Goniolimon tataricum is distributed in grassy stony places in the lower parts of the slopes of southern exposure. The population is of low number of individuals.

Hypericum rumeliacum is widely distributed, mainly in the lower and flatter parts of the mountain. The population is of high number of individuals and high density.

Jurinea tzar-ferdinandii occurs at the foot of Petrovski Krust peak and forms comparatively dense population, composed by large groups of many individuals. The species appears as a dominant in the vegetation communities.

Nonea atra occurs in stony places on the slopes of western and southern exposure. The population is represented by single plants.

Polygala hospita is distributed mainly in the lower parts of the southern slopes, in open shrubby communities. The population is with a mosaic structure, in some places with a significant number of individuals and high density.

Silene fabarioides occurs on stony terrain and shallow soils. The population is formed by single individuals with a low number and density.

Tragopogon pterodes is localised mainly on the southern and western slopes under Petrovski Krust peak. The population is of high number of individuals.

Trinia glauca subsp. *carniolica* is represented by population of single plants at the stony parts of the mountain, mainly in the region south of Petrovski Krust peak. It participates in the composition of communities, including species from the genus *Chamaecytisus*, *Fumana procumbens*, *Helianthemum nummularium*, *Anthyllis vulneraria*, *Lotus corniculatus*.

Tulipa urumoffii is of limited distribution in grassy stony places on the southern slope below the peak Petrovski Krust, on comparatively difficult to access terrain. The population is formed by single plants or groups of few individuals, and in some places the number and density are significant, which presupposes good reproduction capabilities when significant antropogenic pressure is lacking.

Conclusion

As a result of this study, considerable number of conservation important species was found on the territory of Mt Chepun. This is a reason for its acceptance as an area of high conservation value and a valuable argument for its announcement as a protected territory.

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Some endemic *Centaurea* species of Turkish flora

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Abstract. Turkey has very rich flora with about 10 000 taxa and 33 % of endemism. The genus *Centaurea* is one of the biggest genera of the flora of Turkey, and its endemism ratio is fairly high with about 62 %. The endemic taxa of the genus are especially localised in Taurus Mountains, Amanos Mountains, East Anatolia and Çoruh Valley. In this study, the descriptions of 12 endemic *Centaurea* species, collected from different localities in Turkey and studied morphologically, were reviewed in the light of the literature data and our observations. These species are *Centaurea amanicola*, *C. ptosimopappa*, *C. saligna*, *C. huber-morathii*, *C. fenzlii*, *C. pyrrholephara*, *C. armena*, *C. woronowii*, *C. lycopifolia*, *C. paphlagonica*, *C. tomentella* and *C. psephelloides*. The photos of some taxa of them were presented. IUCN red list categories of the taxa were evaluated based on literature data and our field observations. Also, a distribution map is given.

Key words: *Centaurea*, endemism, IUCN red list categories, Turkish flora

Introduction

Turkey is one of the main centres of diversity for the genus *Centaurea* L. (Wagenitz 1986). *Centaurea* is the third largest genus in Turkey. It has 187 taxa in 34 sections occurring mainly in the Mediterranean and Irano–Turanian regions (Wagenitz 1975; Davis &. al. 1988; Güner 2000; Duran & Duman 2002). Generally, *Centaurea* species grow on stony calcareous cliffs, vineyards, roadsides, seashores, gypsum fields, open woods and shrubs, waste places, steppe, fallow fields, maquis, sandy beaches, forests, dry meadows, rocky slopes and on maritime limestone cliffs in Turkey. There are 112 endemic taxa of *Centaurea* and the endemism value is about 62 % in Turkey. The endemic taxa of the genus are especially localised in Taurus Mountains, Amanos Mountains, East Anatolia and Çoruh Valley. In this study, some distributional and morphological data for 12 endemic *Centaurea* species were given. We have also reviewed their IUCN red list categories previously evaluated by Ekim & al. (2000), using the most recent version of the IUCN Red List Categories (IUCN 2001).

Material and methods

The plant samples were collected from different localities in Turkey during field trips from 1999 to 2005. The specimens were stored at different herbaria in Turkey. The collecting localities of the species are as follows:

- *C. amanicola* – C6 Osmaniye: Amanos Mts, Mitisin summer pasture, open *Pinus nigra* forest, 1350 m, 19.08.2005, M. Dinç 2486; C6 Osmaniye: between Yarpuz–Yaylapınar, open forest, 1300 m, 06.07.2003, A. Duran 6317;
- *C. lycopifolia* – B5 Kayseri: between Yahyalı–Kapuzbaşı, rocky crevices, 750 m, 19.05.2001, M. Dinç 1098; C6 Osmaniye – Fenk Yayla, *Pinus* forest, 1000–1250 m, M. Dinç 1153;
- *C. fenzlii* – B8 Muş: Malazgirt, forest horticulture, 22.06.1999, A. Duran 4668;
- *C. pyrrholephara* – A8 Bayburt: between Bayburt–Aşkale, Mt Kop, 2000 m, 31.06.1999, A. Duran 4801;
- *C. woronowii* – A9 Artvin: between Rabat church–Bulanık village, 1300 m, 29.06.1999, A. Duran 4769;
- *C. tomentella* – C6 Kahramanmaraş: between Kahramanmaraş–Göksun, Püren tunnel, 1500 m, 15.08.2004, A. Duran 6862;

- *C. armena* – B7 Erzincan: Üzümlü, Mt Keşiş, 2500 m, 03.07.1999, A. Duran 4840;
- *C. saligna* – B9 Van: between Tatum–Van, 70 km, 2100 m, 31.07.2004, A. Duran 6817;
- *C. huber-morathii* – C5 Kayseri: Yahyalı, Çamlıca (Faraşa) village, 1350–1500 m, 04.07.2003, A. Duran 6251;
- *C. ptosimopappa* – C6 Osmaniye: between Osmaniye–Zorkun Yaylası, 1050–1200 m, roadsides, 21.07.2001, M. Dinç 1157; ibid., 05.07.2003, A. Duran 6306;
- *C. psephelloides* – B7 Erzincan: between Kemaliye–Taşyol, limestone rocks, 980 m, 30.05.2003, A. Duran 6127;
- *C. paphlagonica* – B5 Yozgat: between Çayıralan–Elçi, 1600 m, 14.08.2004, A. Duran 6853.

The specimens were studied morphologically, and the descriptions of the species were reviewed in the light of the literature data and our observations. IUCN red list categories of the species were evaluated based on our field observations and the literature data (Wagenitz 1975; Davis & al. 1988; Ekim & al. 2000; Güner 2000; IUCN 2001).

Results and discussion

Centaurea amanicola Hub.-Mor. (Fig. 1)

Perennial, 40–100 cm tall, erect, branched only above with 6–8 capitula in a raceme. Leaves papery when dry; lower sparsely hirsute, upper thinly tomentose, entire or denticulate to repand-dentate; basal oblong-cordate, petiolate, lower and median oblong to broadly lanceolate, lower sessile with narrow base, median upper decurrent. Involucre 27–35 × 25–30 mm, ovoid to subglobose. Appendages large, totally concealing basal part of phyllaries, brown (outer whitish), broadly triangular to orbicular (6–8 (–9) mm broad without cilia), cilia 12–16 (–20) on each side (3–6 mm), terminal spinule 5–8 mm. Flowers purple. Achenes 6 mm; pappus 8–9 mm. Fl. 6–7. *Pinus* forest, 900–1300 m.

We determined this species from two locations in Amanos Mts. Its area of occupancy was very small, but population size observed was about 250 individuals. It is known from Amanos and Bolkar mountains (Wagenitz 1975) from about 10 locations in the area covering 5000–25 000 km^2. Taking these data into consideration, this species graded as "Vulnerable VU". Our evaluation agrees with Ekim & al. (2000).

Centaurea ptosimopappa Hayek (Figs 1, 2)

Semishurb, 100–180 cm tall, with ascending to erect glabrous branches. Leaves firm, almost leathery (persisting through winter), glabrous on both surfaces, lanceolate-spathulate to obovate, narrowed into a petiole, 12–15 × 2.5–3 cm; leaves of flowering shoots much smaller and narrower, uppermost partly enveloping capitula. Capitula on ± inflated peduncles. Involucre 18–25 × 8–16 mm, narrowly ovoid (contracted towards apex), phyllaries very numerous, glabrous, adpressed. Appendage a minute deciduous 0.3–0.5 mm spinule. Flowers yellow, marginal not radiant. Achenes 4–5 mm; pappus very deciduous, *ca.* 4 mm. Fl. 7–10. *Pinus* and *Quercus* forest, 150–1400 m.

We determined this species from Amanos Mts. The observed population size was about 250 individuals.

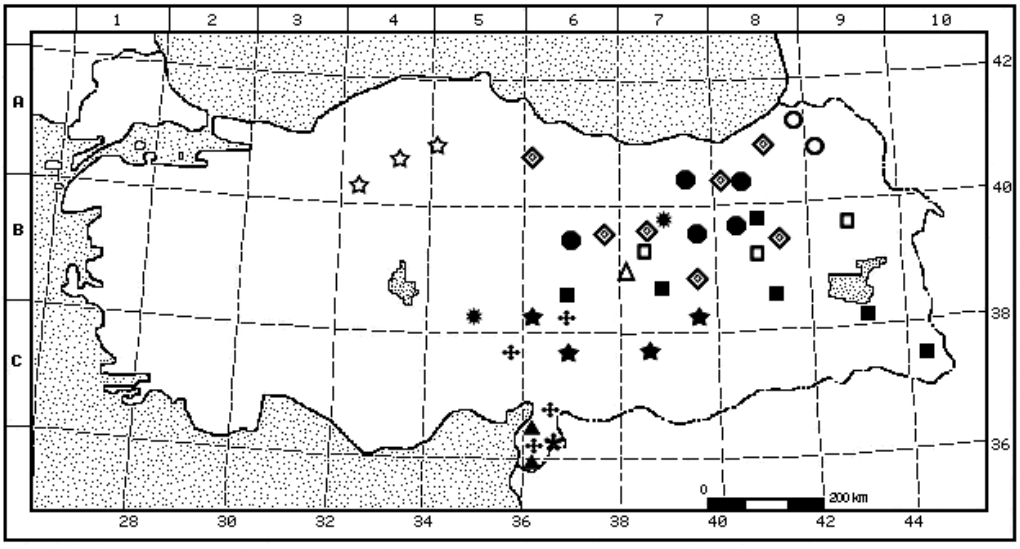

Fig. 1. Distribution map of: *C. amanicola* (★), *C. ptosimopappa* (▲), *C. saligna* (■), *C. huber-morathii* (✹), *C. fenzlii* (□), *C. pyrrholephara* (◈), *C. armena* (●), *C. woronowii* (○), *C. psephelloides* (△), *C. tomentella* (★), *C. lycopifolia* (✢), *C. paphlagonica* (☆).

It is known in Hatay, Adana and Osmaniye district of Amanos Mts from about 10 locations in the area covering 5000–25 000 km^2. Taking these data into consideration, this species graded as "Vulnerable VU" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Centaurea saligna **(C. Koch) Wagenitz** (Fig. 1)

Perennial, stems 20–70 cm tall, erect or slightly bent at base, simple or with several one-headed branches. Leaves firm, scabrous with septate hairs, nerves elevated; lower leaves lanceolate, narrowed into a short petiole, median and upper oblong-lanceolate (rarely linear-lanceolate), sessile with half clasping base or shortly decurrent, uppermost ± enveloping capitula. Involucre 28–30 × 25–35 mm, nearly globose. Appendages very large, totally concealing basal part of phyllaries, strawy, irregularly lacerate to ciliate, ending in a short 1–4 mm mucro. Flowers yellow, marginal not radiant. Achenes 6–8 mm; pappus 16 × 25 mm, plumose. Fl. 7–8. Steppe, rocky slopes, 1400–2000 m.

Centaurea saligna is classified as "Lower risk LR (lc)" according to the recent *Red Data Book of Turkish Plants* (Ekim & al. 2000). It is distributed in Erzurum, Kahramanmaraş, Elazığ, Muş, Bingöl, Van and Hakkari provinces in East and Southeast Anatolia (Wagenitz 1975). Since its observed population is very rich, extent of occurrence is more than 20 000 km^2, populations are not distant from each other, and negative extreme fluctuations in extent of occurrence, area of occupancy and number of individuals is not expected, we agree with Ekim & al. (2000).

Centaurea huber-morathii **Wagenitz** (Figs 1, 3)

Perennial, stems erect, 50–90 cm, simple or with 1–2 long branches, naked above. Leaves rather densely adpressed tomentose; basal withered at flowering time, lower and median pinnatipartite with 6–9 (in lower) to 3–4 pairs of linear-lanceolate lateral segments, 7–11 mm broad, decurrent along rachis, entire or partly (in lower leaves) with a single basicope lobe, terminal segment similar to lateral or (in median leaves) slightly larger, upper leaves oblanceolate, often 1–2 lobes on each side. Involucre 25–30 × 18–30 mm, oblong or subglobose, truncate at base. Appendages large, totally concealing basal part of phyllaries, hyaline whitish to light brown, nearly orbicular to broadly triangular, 10–14 mm broad, slightly decurrent, with numerous 1–1.5 mm cilia. Flowers lilac-pink, marginal radiant. Achenes 4.5–5 × 1.2–1.3 mm, pappus 11–13 mm, inner row 4–5 mm. Fl. 6–7. Alt. 1400–2000 m.

Mature achene size of *C. huber-morathii* was reported here for the first time. Until now, this species has been known only from two locations in Erzincan and known as endemic to East Anatolia (Wagenitz 1975). We found *C. huber-morathii* at third locality in Kayseri remote from Erzincan. Although its extent of occurrence is estimated more than 5000 km^2, it is known from only three locations, the populations are severely fragmented and the population observed in Kayseri is very poor. In the light of these data, this species graded as "Endangered EN".

Fig. 2. *C. ptosimopappa* in the wild.

Fig. 3. *C. huber-morathii* in the wild.

Centaurea fenzlii Reichardt (Fig. 1)

Biennial with thickened taproot. Stems erect 40–120 cm tall, branched in upper part with few large capitula. Leaves firm, scabrous; basal ovate-roundate, slightly cordate at base, petiolate, lower similar with broadly winged petiole, median and upper elliptical to linear, sessile and shortly decurrent. Involucre 30–40 × 30–50 mm. Appendages very large, totally concealing basal part of phyllaries, straw-coloured, firm, nearly orbicular with numerous 1–3 mm cilia (terminal shorter, inconspicuous). Flowers yellow. Achenes *ca*. 6 mm; pappus 6–10 mm. Fl. 6–7. Steppe, forest, fallow fields, 1150–1820 m.

This species is known from more than 10 locations in East Anatolia. In addition, its observed population is very rich. Therefore, it should be classified as "Lower risk LR (lc)" as in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Centaurea pyrrhoblephara Boiss. (Fig. 1)

Perennial with sterile shoots and several stems with 1–2 capitula, 20–50 cm. Leaves floccose-tomentose; basal and lower lyrate with very large broadly lanceolate terminal segment and 1–2 (3) pairs of small lateral segments (leaves of sterile shoots sometimes undivided); median pinnatilobate or simple, only small bract-like leaves in upper1/3 or 1/2 of the stem. Involucre 20–25 × 15–25 mm. Appendages very large (totally concealing basal part of phyllaries), nearly orbicular, centre straw-coloured, magrin brown with numerous cilia (3–5 mm). Flowers rose-purple, marginal strongly radiant. Achenes *ca*. 7 mm; pappus 4–7 mm. Fl. 6–7. Rocks (mainly limestone) and slopes, 1700–2300m.

Centaurea pyrrhoblephara grows in East and Northeast Anatolia (Wagenitz 1975) and is known from about 20 locations. Therefore, we agree with Ekim & al. (2000) classifying it as "Lower risk LR (lc)".

Centaurea armena Boiss. (Fig. 1)

Perennial, stem very short, 0.5–3 cm, simple or with few short branches; capitulum nearly sessile in a rosette of often very large leaves (up to 25 cm). Leaves ± densely hirsute with septate hairs; very variable, lyrate with (1–) 2–6 pairs of lateral segments and a triangular terminal segment or more rarely pinnatipartite or undivided. Involucre 20–27 × 18–30 mm, nearly globose. Appendages large, concealing basal part of phyllaries or nearly so, straw-coloured to light brown, erect or reflexed, ovate to triangular, with 6–20 cilia (2–5 mm) on each side. Flowers yellow, marginal not radiant. Achenes 5–6 mm; pappus 1–2 mm. Fl. 6–7. Mountain slopes, 1950–2600 m.

Centaurea armena is known from about 10 locations in East and Northeast Anatolia (Wagenitz 1975). Its extent of occurrence is estimated to be more than 20 000 km^2. Also, its observed population is very rich. Because of these data, we agree with its classification "Lower Risk (LR, lc)" in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Centaurea woronowii Bornm. (Fig. 1)

Perennial with short, rather stout rhizome. Stems erect or ascending, 8–20 cm tall, simple or mostly with several branches as long as stem. Leaves ± densely grey-tomentose, lower and median lanceolate or spathulate in outline and pinnatilobate to lyrate with 2–5 pairs of lobes or repand-dentate or partly entire, upper smaller, sessile and more often entire. Involucre 13–17 × 7–12 mm nearly cylindirical to cup-shaped. Appendages decurrent with a narrow brown border 0.2–0.5 mm broad, cilia 1–2 mm. Flowers purple to blackish-violet, marginal slightly radiant with linear to filiform lobes. Achenes 4–5 mm; pappus 0.5–1 mm. Fl. 4–7. Stony slopes, rocks, 700–1500 m.

Centaurea woronowii is confined to an Irano–Turanian enclave in Northeast Anatolia and is recorded from only three locations in Çoruh environs (Wagenitz 1975). We found it in fourth location near to other localities. Although it is known from only four locations, its extent of occurrence is estimated as 5000–20 000 km^2. Also, the observed population was rich. In the light of these data, this species should be graded as "Vulnerable VU". Our evaluation agrees with Ekim & al. (2000).

Centaurea psephelloides Freyn & Sint. (Figs 1, 4)

Perennial with woody rootstock with sterile shoots and flowering stems 20–35 cm tall with 1 capitulum (very rarely 2). Leaves adpressed floccose-tomentose; basal and lower long-petiolate, undivided, lanceolate to oblong and irregularly toothed or lyrate with a terminal segment of similar shape and 1–2 pairs of small lanceolate lateral segments; other stem leaves few, much smaller, lanceolate to linear-lanceolate. Involucre 17–21 × 15–17 mm, cup-shaped. Appendages rather large (concealing most of phyllaries), ovate to triangular, hyaline, straw-coloured, brownish in centre, with numerous short 0.5–1 mm cilia. Flowers pink,

marginal radiant. Achenes 6–8 mm; pappus 4–5 mm. Fl. 5–6. Chasmophytic.

Centaurea psephelloides has been known only from the type collection until now and was classified as "data deficient DD" according to the recent *Red Data Book of Turkish Plants* (Ekim & al. 2000). We determined it from a second location near the type locality in Bayburt (Fig. 1). Its population is very poor (approximately 15–20 individuals) and the distribution area is very small. Taking these data into consideration, we think that this species should be classified as "critically endangered CR".

Fig. 4. *C. psephelloides* in the wild.

Centaurea tomentella **Hand.-Mazz.** (Fig. 1)

Perennial, stem erect, 25–80 cm. Leaves firm, lower sparsely hairy, others ± tomentose; basal oblong-cordate, petiolate, lower lanceolate with winged petiole dilated at base (sometimes a pair of lobes at petiole in basal or lower leaves), median lanceolate or broadly lanceolate, shortly decurrent, upper lanceolate, small, sessile. Capitula arranged in a spike or raceme (peduncules up to 8 cm). Involucre *ca*. 30 × 25–30 mm, ovoid to globose. Appendages very firm, straw-coloured, totally or nearly totally concealing basal part of phyllaries, narrowly triangular, 4–5 mm broad, with 6–10 cilia on each side (5–8 mm) and ending in a 9–17 mm spine. Flowers purple; pappus *ca*. 8 mm. Fl. 7. Dry slopes, follow fields, 650–950 m.

Centaurea tomentella is known from Adıyaman, Kahramanmaraş and Malatya environs (Wagenitz 1975). Its extent of occurrence is estimated more than 20 000 km^2. Also, the observed population is rich. But the species populations are severely fragmented. Because of these data, we agree with its classification "Lower Risk (LR, nt)" in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Centaurea lycopifolia **Boiss. & Kotschy** (Figs 1, 5)

Perennial, stem ascending or decumbent, 15–40 cm, simple or with few long branches, stem and branches thickened below capitula. Leaves sparsely pilose with septate hairs, very variable; basal and lower lyrate with a ± triangular terminal segment and several pairs of lanceolate lateral segments, cauline leaves with trian-

Fig. 5. *C. lycopifolia* in the wild.

gular to lanceolate, irregularly toothed (rarely nearly entire) terminal segment and 1–3 pairs of lateral segments or undivided. Involucre 17–25 × 10–20 mm, ovoid to subglobose, arachnoid. Appendages rather large, concealing most of basal part of phyllaries, brown, triangular with 3–8 spinules on each side (2.5–4.5 mm), terminal scarcely different. Flowers yellow. Achenes 5–6 mm; pappus 1–2 mm. Fl. 5–7. Macchia, shrubs, rocks, 50–2000 m.

This species grows in South Anatolia. It was classified as "Lower Risk, near threatened (LR, nt)" by Ekim & al. (2000). Since its extent of occurrence is more than 20 000 km^2, we think that it should be classified under "Lower Risk". But it was reported that *C. lycopifolia* was common in the Amanus, where numerous collections were known (Wagenitz 1975). Moreover, observed populations in both Amanus and Aladağlar are very rich. Consequently, we think that it should be classified under "Lower Risk, least concern (LR, lc)".

Centaurea paphlagonica (Bornm.) Wagenitz (Fig. 1)

Perennial with woody rootstock, stem 10–55 cm, erect, simple or branched in upper part (with 1–4 capitula), whitish, winged for most of its length (wings 1–4 mm broad). Leaves hirsute with septate hairs; leaves of sterile shoots lanceolate, entire, lower and median lyrate with lanceolate terminal segment and 2–3 pairs of lateral segments, or pinnatipartite with 4–6 pairs of linear-lanceolate lateral segments, lower leaves petiolate, median decurrent like upper lanceolate leaves. Involucre 21–30 × 15–30 mm, globose to cup-shaped. Appendages large, concealing most of phyllaries, brown (darker in centre), triangular, with 8–15 cilia (2–4 mm) on each side, decurrent with a lacerate magrin, ending in a terminal 2–4 mm spinule. Flowers yellow, marginal not radiant. Achenes 5–5.2 × 1.6–2.2 mm; pappus 8–11 mm, brownish-purple, plumose, inner row 0.5–1 mm. Fl. 7–8. Subalpine meadows, *ca*. 1700 m.

Mature achene size of *C. paphlagonica* was reported here for the first time. Since its extent of occurrence is estimated to be about 20 000 km^2 and its observed population is rich, we agree with its classification "Lower Risk (LR, nt)" in the *Red Data Book of Turkish Plants* (Ekim & al. 2000).

Acknowledgements. We would like to thank to Selçuk University (BAP–Project No: 06701106) for financial support during this study.

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Data on the flora of Albania

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Abstract. The authors made a report on their field trips to five Albanian mountains (Griba Mts, Ostrovica Mts, Prokletije Mts and Tomor and Kulmakes Mts) in 2004 and 2005. They listed 8 new species for the Albanian flora and gave a short description on their European distribution. Also the significance of the occurrence of these species in Albania is discussed.

Key words: Albania, flora, Griba, Ostrovica, Prokletije

Introduction

Albania is one of the countries, which possess the richest flora in Europe, at the same time it is perhaps the less known area from the botanical point of view. In the recent past, the complete flora work of the country was issued (Paparisto & al. 1988; Qosja & al. 1992, 1996; Vangjeli & al. 2000; and Vangjeli 2003), which discusses 3235 species altogether (including more cultivated species). However, some facts are conspicuous – comparing of the taxon-list with that of the previous Albanian identification book (Demiri 1983) indicates very significant deviations in certain cases; the *Red Data Book* of the country (Vangjeli & al. 1995) reflects the not satisfactory knowledge on the areas of certain species; additional 12 species new to the flora of Albania were published in the recent past (Desfayes 2004). All these facts incite further study on the flora.

Material and methods

The authors organised field trips to Albania in 5 occasions in the years of 2004 and 2005. The collections targeted the following areas (see Fig. 1):

1. Tomor Mts (23.05.2004 – 26.05.2004, participants: Z. Barina, Cs. Németh).
2. Kulmakes and Tomor Mts, Maliçikes (08.08.2004 – 12.08.2004, participants: Z. Barina, Cs. Németh, D. Pifkó).
3. Griba Mts (Mali i Gribes) (01.05.2005 – 05.05.2005, participants: Z. Barina, G. Király, D. Pifkó).

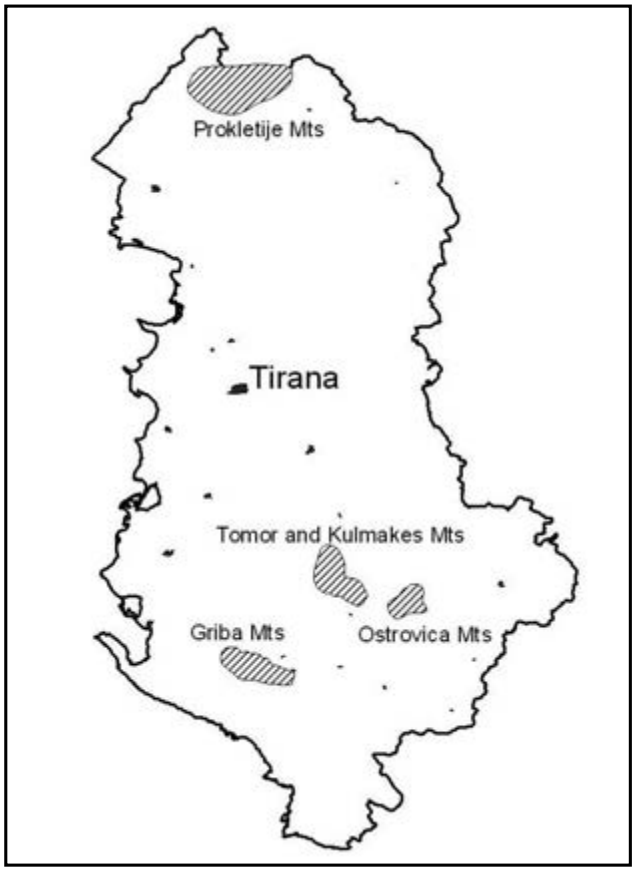

Fig. 1. The location of the mountains in Albania, mentioned in the paper.

4. Prokletije Mts (29.05.2005 – 04.06.2005, participants: Z. Barina, D. Pifkó).
5. Ostrovica Mts (Mali i Ostrovices) (03.07.2005 – 07.07.2005, participants: Z. Barina, D. Pifkó, D. Schmidt).

The geographical names referred in the paper are based on the denominations of the topographical map (1:50 000 scale) of Albania in Russian language and the denominations of the geological map of Albania in Albanian language.

The collected plants are deposited in the Herbarium Generale of Hungarian Natural History Museum, Botanical Department (BP).

Results

New species to the flora of Albania on the basis of Demiri (1983), Paparisto & al. (1988), Qosja & al. (1992, 1996), Vangjeli & al. (2000) and Vangjeli (2003):

Aphanes floribunda (Murb.) Rothm.

Location: Albania, District of Berat (Rrethi i Beratit), Tomor Mts: near village Vodice, in the valley of Vodice Stream. Coordinates: 40.690090 N, 20.034890 E, 130 m (leg. Z. Barina, Cs. Németh; 23.05.2004).

The species occurs in the Mediterranean region from Sardinia to Turkey (Tutin & al. 1968: 64; Pignatti 1982: 601), but it is not mentioned by Micevski (1998: 1002-1004) from the neighbouring Macedonia. Its further occurrences can be expected from other Mediterranean regions of Albania.

Carex digitata L.

Location: Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: Mt Zorgjit (Maja e Zorgjit, 1663 m), south of Theth, on limestone rocks. Coordinates: 42.385070 N, 19.761850 E, 1490 m (leg. Z. Barina, D. Pifkó; 30.05.2005).

No former data exist from Albania, however, it is mentioned by Strid & Tan (1991: 856-857) from more locations from the northern part of Greece.

Eranthis hyemalis Salisb.

Location: Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): northeast of Bença (Bënçë), northern slope of Mt Dutihe (1429.1 m), in closed grassland. Coordinates: 40.262010 N, 19.980910 E, 1223 m (leg. Z. Barina, G. Király, D. Pifkó; 02.05.2005).

In our opinion the occurrence of the species is native in the above location; its turn-up is expected from other mountains of Albania as well. It is also present in the southern part of Serbia (Gajić in Sarić 1992: 280-282) near the Albanian border. However, it is not mentioned by Micevski (1985) from Macedonia, and the populations in Croatia reported by Franjić (1992) are probably not native. Up to now, it has not been found because of its very early flowering; we came across with it at the beginning of May, above 1400 m altitude, we found only its specimens with fruit.

Himantoglossum adriaticum H. Baumann

Location: Albania, District of Skrapar (Rrethi i Skraparit), Ostrovica Mts (Mali i Ostrovices): western part of village Polena, by the roadside. Coordinates: 40.494980 N, 20.281540 E, 921 m (leg. Z. Barina, D. Pifkó, D. Schmidt; 03.07.2005).

The species was known from the surroundings of the Istrian Peninsula (Baumann 1978); our data expands the area of the species significantly to the south.

Melilotus graecus (Boiss. & Spruner) Lassen

Location: Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): north of Bença (Bënçë), near the Bridge Bençës; in Mediterranean scrubland. Coordinates: 40.258900 N, 20.002220 E, 400 m (leg. Z. Barina, G. Király, D. Pifkó; 02.05.2005).

Its previous occurrences were known exclusively from Greece (Širajev 1928; Tutin & al. 1968: 151); especially it can be found in the southern part of the country in more locations (Peloponnesos, Crete), but it is present also near the Albanian border, in the area of Pindos (Tan & Iatrou 2001: 188).

Orobanche pubescens d'Urv.

Location: Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): between Tepelenë and Bença, *ca*. 2 km south of Tepelena, bank of stream Bença. Coordinates: 40.280270 N, 20.014120 E, 168 m; being parasite on *Pastinaca* (leg. Z. Barina, G. Király, D. Pifkó; 04.05.2005).

It is native in Southeast Europe; it was known from Greece and the area of former Yugoslavia, too (Tutin & al. 1972: 290).

Sedum amplexicaule DC.

Location: Albania, District of Korçë (Rrethi i Korçës), Ostrovica Mts: Mt Komorru (1605 m), *ca*. 2 km southwest of Çemericë, in *Fagus sylvatica* forest. Coordinates: 40.540630 N, 20.464010 E, 1493 m (leg. Z. Barina, D. Pifkó, D. Schmidt; 06.07.2005).

Of the neighbouring countries this species is present also in Macedonia (Micevski 1998: 1054) and Greece (Strid & Tan 2002), furthermore, as for Strid & Tan (2002) "Almost throughout Greece". It is new to the Albanian flora; its turn-up is expected also from other regions of South Albania.

Viola chelmea Boiss. & Heldr.

Locations:

1. Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: pass Pejes (qafa Pejes), 2.5 km north of Okol, near "Buni i Gropazt"; in rocky grassland on limestone. Coordinates: 42.440940 N, 19.775510 E, 1734 m (leg. Z. Barina, D. Pifkó; 31.05.2005).
2. Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: Uroçishe Rukijes, between pass Pejes (qafa Pejes) and the Albanian–Montenegrin frontier; in rocky grassland on limestone (leg. Z. Barina, D. Pifkó; 31.05.2005).
3. Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: between Theth and Rogam, southeastern part of Mt Alis (2471 m), near pass "Valbona" (qafa Valbones); in rocky grassland on limestone. Coordinates: 42.407330 N, 19.808640 E, 1789 m (leg. Z. Barina, D. Pifkó; 31.05.2005).

The subspecies *chelmea* occurs only in the Peloponnesos Peninsula (Tan & Iatrou 2001: 210), however, subspecies *vratnikensis* Gáyer & Degen emerges in Croatia (Degen 1914), and Micevski (1995: 514) indicates the species also from Macedonia (without mentioning the subspecies) from the area of the near Šar Planina.

Red Data List species:

Further on some supplements are added to the *Red Data Book of Albania* (Vangjeli & al. 1995); the species found during our field trips, which were supposed to be extinct from Albania and some other endangered species are listed below.

Astragalus baldaccii Degen

Locations:

1. Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): near the peak of Mt Dutihe (1429.1 m); on limestone rocks. Coordinates: 40.264860 N, 19.961250 E, 1320 m (leg. Z. Barina, G. Király, D. Pifkó; 03.05.2005).
2. Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): *ca*. 3 km east of Kendrevicës (maja e Kendrevicës, 2121.4 m) around the 1721 m high peak; on limestone rocks. Coordinates: 40.276010 N, 19.880950 E, 1680 m (leg. Z. Barina, G. Király, D. Pifkó; 03.05.2005).

The species was described by Árpád Degen (1896) from the collection of Baldacci, originated from the near Nemërçkë Mountains ('Hab. in saxosis alpinis montis Nimerčka jugo Karajan supra Diovisda distr. Pogoni Albaniae') in 1896. Apart from this, it is known also from Greece (Tutin & al. 1968: 121), and as for Micevski (2001: 1184-1185) it is present from more locations in Macedonia. In the opinion of Vangjeli & al. (1995) it may be extinct from Albania (Ex?).

Its present locality is situated 40 km northwest of the range of Nemërçkë, supposedly the species is present in the *locus classicus* even today, and its turn-up can be expected from the near mountains as well.

Berberis vulgaris L.

Locations:

1. Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: Mt Xharaput (2216.6 m), *ca*. 3 km north of Okol, near "Buni i Gropazt"; in rocky grassland on limestone. Coordinates: 42.443790 N, 19.767360 E, 1750 m (leg. Z. Barina, D. Pifkó; 31.05.2005).
2. Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: Uroçishe Rukijes, near the Albanian–Montenegrin border, in rocky grassland on limestone (leg. Z. Barina, D. Pifkó; 31.05.2005).
3. Albania, District of Skrapar (Rrethi i Skraparit), Ostrovica Mts: northeast of village Backë, on the southeastern side of Frengu. Coordinates: 40.524050 N, 20.414500 E, 1796 m (leg. Z. Barina, D. Pifkó, D. Scmidt; 04.07.2005).
4. Albania, District of Skrapar (Rrethi i Skraparit), Ostrovica Mts: *ca*. 4 km northwest of Çemerica, at the base of the peak of 2383 m. Coordinates: 40.555650 N, 20.449230 E, 1889 m (leg. Z. Barina, D. Pifkó, D. Schmidt; 05.07.2005).

As for Vangjeli & al. (1995: 29) it is supposed to be extinct (Ex?), however, on the basis of our data it is probable that it is present also today in several mountains of Albania.

Gymnospermium altaicum (Pall.) Spach subsp. *scipetarum* (E. Mayer & Pulević) Kit Tan & Mullaj

Location: Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): on the west side of the ridge between the peaks maja e Trushnices

(1815.2 m) and Komtiri (1224 m), above village Zlezi. Coordinates: 40.297280 N, 19.906210 E, 1190 m (leg. Z. Barina, G. Király, D. Pifkó; 04.05.2005). On limestone, presumably in the place of a former oak forest, on few square metres.

A species of wide distribution, with rather scattered occurrences in Europe, and with more subspecies separated on the base of the morphology of leaves. Subsp. *scipetarum* occurs in Montenegro (Rumija Mts, Mayer in Greuter & Raus 1983: 278), as well as in Albania. Formerly it was known in Albania only from the surroundings of Kruja (Krujës) and Elbasan (Shëmil) (Paparisto & al. 1988: 291; Vangjeli & al. 1995; Tan & Mullaj in Greuter & Raus 2001: 319-320).

Petteria ramentacea (Sieber) C. Presl

Location: Albania, District of Tepelenë (Rrethi i Tepelenës), Griba Mts (Mali i Gribes): between Tepelenë and Bënçë, in the valley of stream Bënçë. Coordinates: 40.280270 N, 20.014120 E, 168 m (leg. Z. Barina, D. Pifkó, G. Király; 04.05.2005).

Its closest occurrence is known from the nearness of Logara Pass. Former data on the species in Albania are known from there and the northern part of the country (Vangjeli & al. 1995).

Plantago reniformis Beck

Location: Albania, District of Shkodër (Rrethi i Shkodrës), Prokletije Mts: by the well of Valbona River, above village Rogam; in grassy clearing. Coordinates: 42.409920 N, 19.822490 E, 1422 m (leg. Z. Barina, D. Pifkó; 02.06.2005).

An endemic species known from the south part of Serbia as well as North Albania, which is indicated in a single point of Greece as well (Gustavsson 1978: 17). It is known from Prokletije Mts (Josifović 1974; Vangjeli & al. 1995); our datum is a specification of the former indications.

Discussion

The authors publish 8 new species to the flora of Albania. Of these, on the basis of the formerly known data the occurrences of *Carex digitata* and *Sedum amplexicaule* were expected also in the area of Albania. More species were known also from one of the neighbouring countries and our data are the specification of the area of these taxa (*Aphanes floribunda*, *Eranthis hyemalis*, *Melilotus graecus*, *Orobanche pubescens*, *Viola chelmea*). *Himantoglossum adriaticum* was found a long distance from the previously known localities, significantly expanding this area, thus making probable the novel occurrences within the enlarged area getting known.

We managed to find *Astragalus baldacci* and *Berberis vulgaris*, which were supposed to be extinct from Albania, in more localities; besides this, we publish new occurrences of three further Red Data Book species as well as the specification of their localities (*Gymnospermium altaicum*, *Petteria ramentacea*, *Plantago reniformis*).

Acknowledgements. We would like to thank to Anila Paparisto for her help in obtaining the special literature and to Gergely Király, Csaba Németh and Dávid Schmidt for their help in the fieldwork.

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The endemic *Limonium calabrum* (*Plumbaginaceae*): population survey at Copanello (Calabria, S Italy)

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Abstract. The study deals with the distribution of the endemic *Limonium calabrum* in the central part of the Calabrian Ionian Coast (Copanello promontory). The population consists of about 2900 individuals and shows an asymmetrical distribution. In order to describe the distribution of the species at local scale the geomorphology, geology and environmental context are analysed. The demography of the population together with limited distribution area, eco-physiologic specialisation, apomixis, and especially the persistent anthropic pressure are the main threat factors that justify the need of protection actions and long-term monitoring.

Key words: anisotropy, asymmetric area, chorology, *Limonium calabrum*

Introduction

Copanello (Figs 1, 3) is a rocky promontory about 2 km long belonging to Stalettì municipality in Catanzaro province (Calabria, S Italy). The local geomorphology is represented by bluff 0–15 m high as part of a cliff up to 300 m a.s.l. Geological substrate is represented by acid rocks (quartz-diorite, granite, gneiss) covered by a calcareous sedimentary layer through a transition zone formed by conglomerate complexes (Marchetti & al. 1968). The thermo-pluviometric station of Soverato Marina (Ciancio 1971), the closest to Copanello, shows a typically Mediterranean climate (Fig. 1).

Limonium calabrum Brullo (Fig. 2) is a triploid apomictic agamospecies (Brullo & al. 1990) belonging to *L. minutiflorum* (Guss.) Kuntze group (Pignatti 1982; Brullo & al. 2001). This chasmophyte, halophyte species, in the investigated area, is one of the vascular plants better adapted to live close to the coast. Rosulate hemicryptophyte endemic of southern Calabria shows a distribution area divided in four disjunctions, three of which on the Thyrrenian coast (Scilla, Capo Vaticano, Tropea) and one (Copanello) on the Ionian coast (Fig. 1) (Pignatti 1982; Brullo & al. 2001). The species is reported as very rare by Pignatti (1982) and VU by Conti & al. (1997). *Limonium calabrum* lives on the dioritic and conglomerate substrata and never on sand, anyhow rare because of the coastal erosion (Niccoli & Procopio 1995), nor on clasts.

The promontory was altered by anthropic action. The bluff was excavated for fish-breeding (Gatti & Gatti 1982; Zinzi 1986), a railway, roads, galleries, bridges, many constructions built invading the habitat of the species. Actually the area is under landscape and environment protection (Zinzi 1997; Alcaro & Giacobbe 1999) and is included in the Nature 2000 Site – IT9300184 Scogliera di Staletti (Regione Calabria 2003). Aim of the work is a complete census of the population of this rare species and to describe spatial pattern of the local distribution. The data could be applied for the scope of the environmental monitoring as a base for sustainable management of the species in order to prevent and avoid further alteration.

Methods

The individuals were counted during 1999. Plant groups were mapped in scale 1:2000, following the corresponding coast-line and verifying the geographic position with GPS. In this way were identified the main groups (stands) of plants distanced not less than 100 m. Inside each stand were observed homogeneous groups of plants (sites) with distance between the sites about 1 m. Stands and sites were numbered in N–S direction. All relative measures for

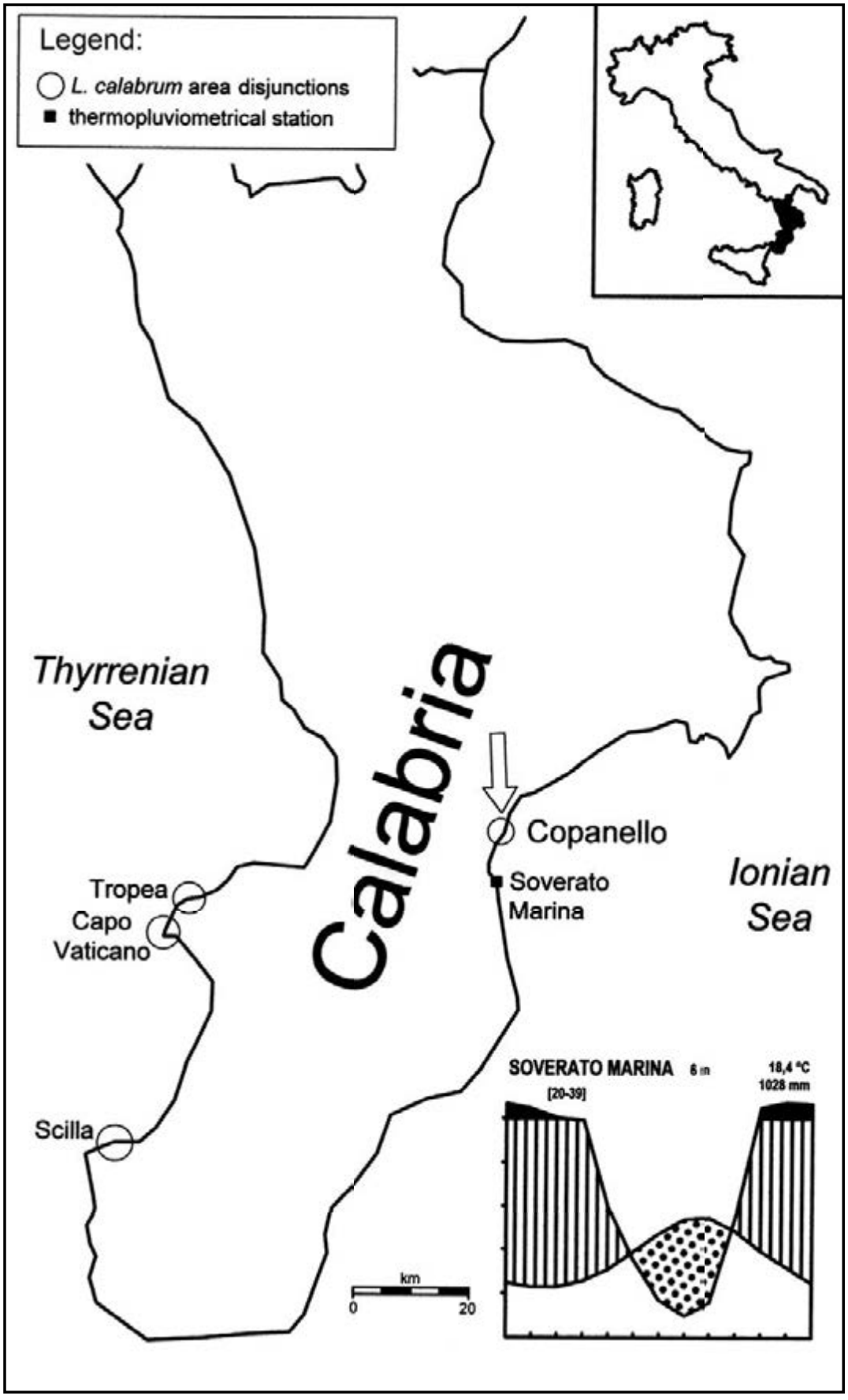

Fig. 1. Calabria. *L. calabrum* area disjunctions and Soverato Marina climogram (data Ciancio 1971) according to Walter & Leith (1960).

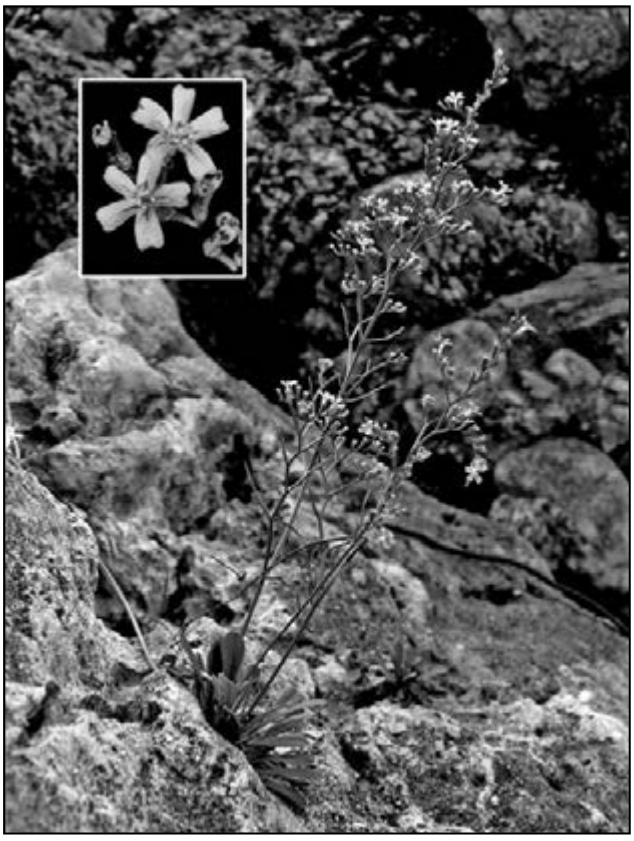

Fig. 2. *L. calabrum* flowering. Particular of flowers inside box.

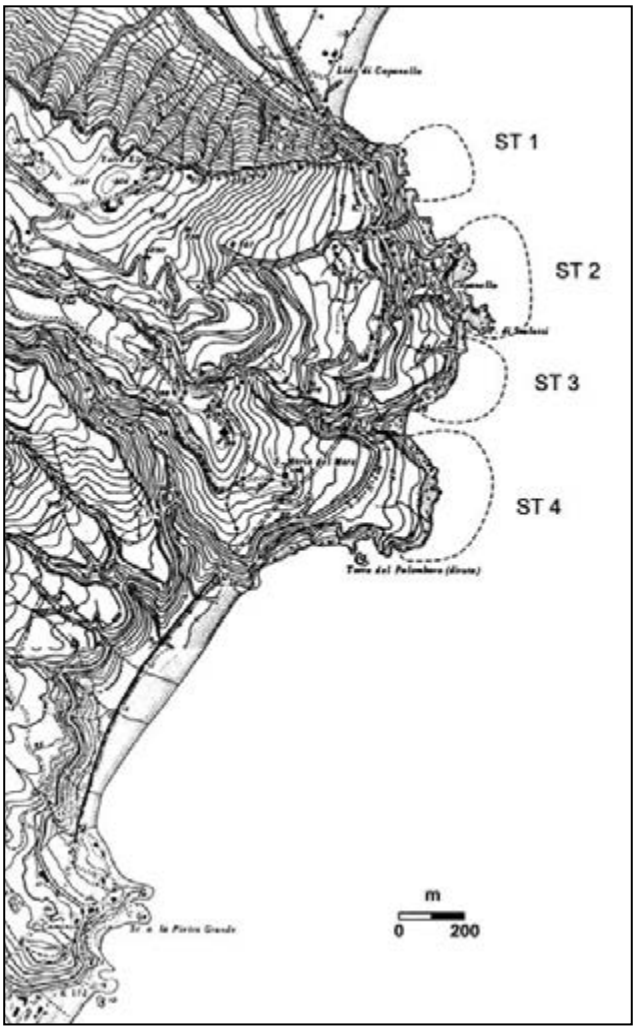

Fig. 3. Topographic map (1:10 000) of Copanello promontory (Carra 1959 a, b modified). The traced lines delimit the stands of *L. calabrum*.

statistical analysis were extrapolated on the base of the produced local distribution map. Due to the ecological peculiarities of the species (occupying a narrow strip of the coast) the area of distribution is considered asymmetrical (Zunino & Zullini 1999) and the analysis of the spatial pattern of the local distribution was statistically treated as distributed along a line (not a surface as real).

The study includes presence/absence analysis along the line of distribution and were calculated the percentage of plants by stands and sites, density, second degree polynomial tendency line and the data scattering respect to average by standard deviation. The linear distance between extremities of the distribution area is called Potential Longitudinal Distribution (PLD). PLD includes sites (where plant is present) and between sites, sectors of coast where species is absent (anisotropies). The sum of linear length of sites is called Real Longitudinal Distribution (RLD). For better characterisation of quanti-qualitative relationship between plants micro-distribution and environmental condition and definition of its potential habitat are individuated 5 main types of geomorphology (geomorphotypes) as shown in Fig. 7. The final step of the analysis includes application of some indices for interpretation of the anthropic impacts.

Results

The population of *L. calabrum* consist of 2895 plants and the data by stands and sites are summarised on the Table 1.

The population is distributed in 4 stands and 38 sites (Table 1; Figs 3, 4). The quantitatively most significant stand is Stand 2 with 40 % of plants (Fig. 5), followed by Stand 3 (29 %) and Stand 4 (24 %). Stands data are relatively scattered respect to average (723.8 plants/stand) as shown by standard deviation (407.1 about 56.2 % respect to average). The density of plants inside stands reach the maximum value in Stand 2 (3.18 plants/m) and in Stand 3 (2.56 plants/m); the

Table 1. Population of *L. calabrum* at Copanello.

Stand 1 pl= 198; l= 245.3; d=0.81					
sites	n	pl	l	d	gt
	1.1	40	7.4	5.4	D
	1.2	43	9.3	4.6	D
	1.3	6	5.6	1.1	A
	1.4	8	5.6	1.4	A
	1.5	32	14.8	2.2	A
	1.6	44	22.2	2.0	A
	1.7	25	40.7	0.6	A

Stand 2 pl=1179; l= 370.8; d=3.18					
sites	n	pl	l	d	gt
	2.1	450	22.2	20.3	E
	2.2	175	44.4	3.9	D
	2.3	20	7.4	2.7	D
	2.4	100	20.4	4.9	D
	2.5	55	11.1	4.9	D
	2.6	68	18.5	3.7	D
	2.7	100	18.5	5.4	D
	2.8	11	7.4	1.5	A
	2.9	160	33.3	4.8	D
	2.10	40	25.9	1.5	D

Stand 3 pl= 833; l= 324.8; d= 2.56					
sites	n	pl	l	d	gt
	3.1	130	37.0	3.5	B
	3.2	200	13.0	15.4	B
	3.3	58	22.2	2.6	B
	3.4	10	16.7	0.6	B
	3.5	17	22.2	0.8	B
	3.6	11	40.7	0.3	E
	3.7	52	33.3	1.6	E
	3.8	15	7.4	2.0	E
	3.9	260	40.7	6.4	E
	3.10	80	22.2	3.6	E

Stand 4 pl= 685; l= 538; d= 1.27					
sites	n	pl	l	d	gt
	4.1	17	16.7	1.0	C
	4.2	22	18.5	1.2	D
	4.3	17	18.5	0.9	D
	4.4	7	13.0	0.5	D
	4.5	17	5.6	3.0	D
	4.6	380	14.8	25.7	D
	4.7	94	13.0	7.2	D
	4.8	4	5.6	0.7	D
	4.9	14	18.5	0.8	D
	4.10	110	100.0	1.1	D
	4.11	3	11.1	0.3	C

Stands [average = 723.8 plants/stand; standard deviation = 407.1 (56.2 % respect to average)] and sites [average = 72.6 plants/site; standard deviation = 101.3 (132.9 % respect to average)], consistence [n – site; l – linear length (m); pl – plants; d – density (plants/m); gt – geomorphotype (Fig. 7A-B-C-D-E)].

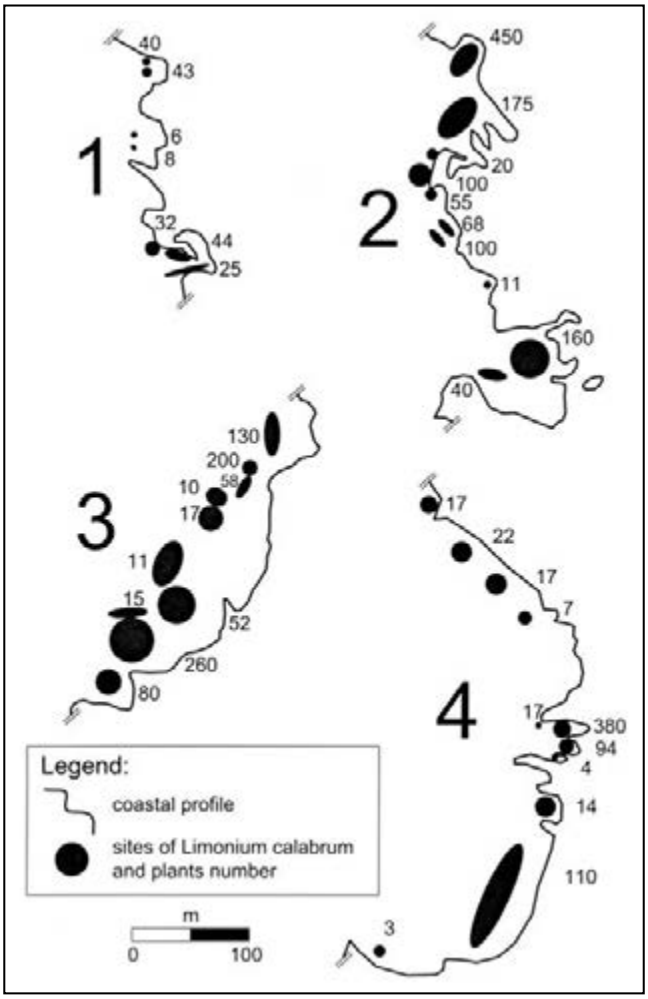

Fig. 4. Stands of *L. calabrum* reported along the coast line following the map in scale 1:2000 of the Copanello promontory. Black areas are proportional to the occupied surface.

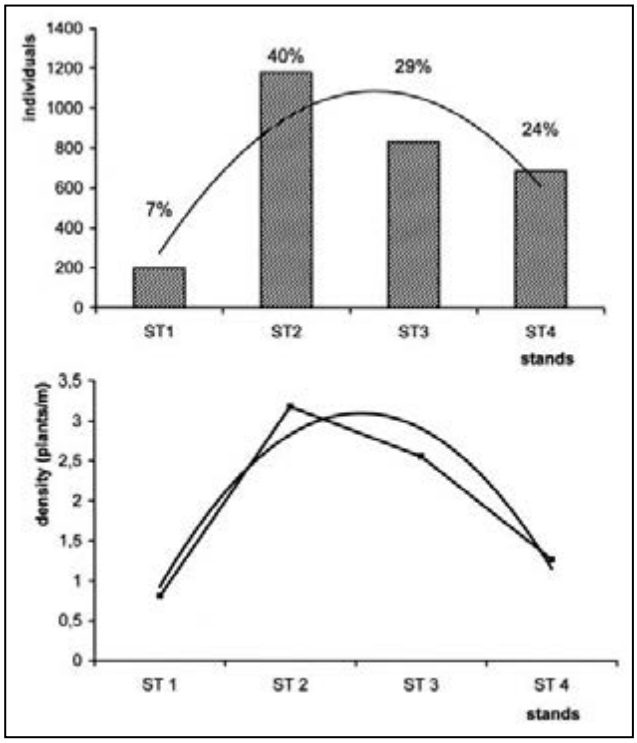

Fig. 5. Stands of *L. calabrum* at Copanello. Percentage of individuals, density (plants/m) and second degree polynomial tendency lines (parabolas).

density second degree polynomial tendency line shows a higher value in the central part of distribution area (Table 1, Fig. 5). The richest sites are Site 2.1 (450 plants), Site 4.6 (380 plants), Site 3.9 (260 plants) and Site 3.2 (200 plants). These sites contain 44.5 % (1290 plants) of whole population. The distribution pattern by sites is more scattered than stands respect to average (76.2 plants/site) as shown by standard deviation (101.3 equivalent about to 132.9 % respect to average) (Table 1; Figs 4, 6).

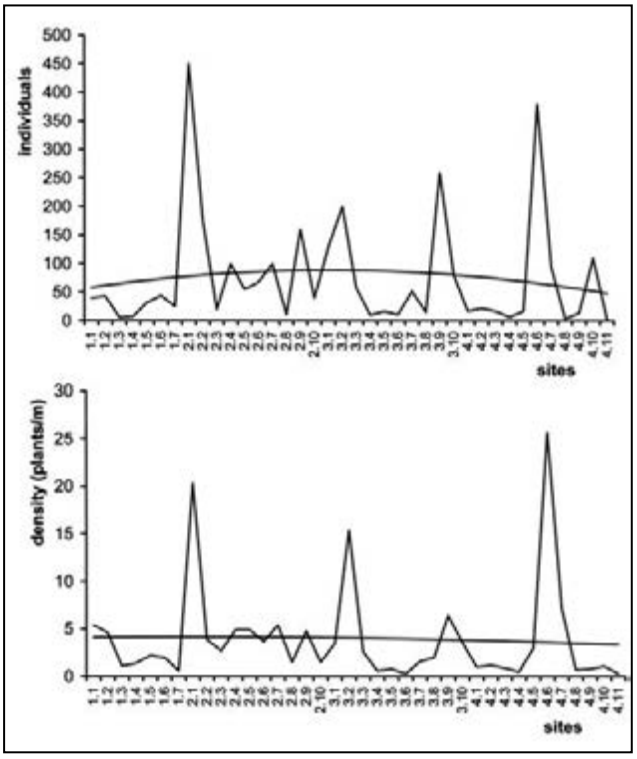

Fig. 6. *L. calabrum* individuals and density (plants/m) in the 38 sites censussed at Copanello and second degree polynomial tendency lines (parabolas).

The density of plants inside sites is higher in Site 4.6 (25.7 plants/m), Site 2.1 (20.3 plants/m) and Site 3.2 (15.4 plants/m); the density second degree polynomial tendency line shows a small decrease in the southern part of the distribution area (Fig. 6).

The linear distance between extremities of the distribution area (PLD) is 1998.9 m; the sum of linear length of sites (RLD) is 805.6 m.

On the promontory 5 main different bluff geomorphotypes have been found (Fig. 7). Type A shows a rise up to 2–5 m a.s.l. and is characterised by slight depression behind coast line (10–30 m) and hosts Sites 1.3, 1.4, 1.5, 1.6, 1.7, 2.8 – totally 126 plants (Table 2). Type B shows a rather flat trend

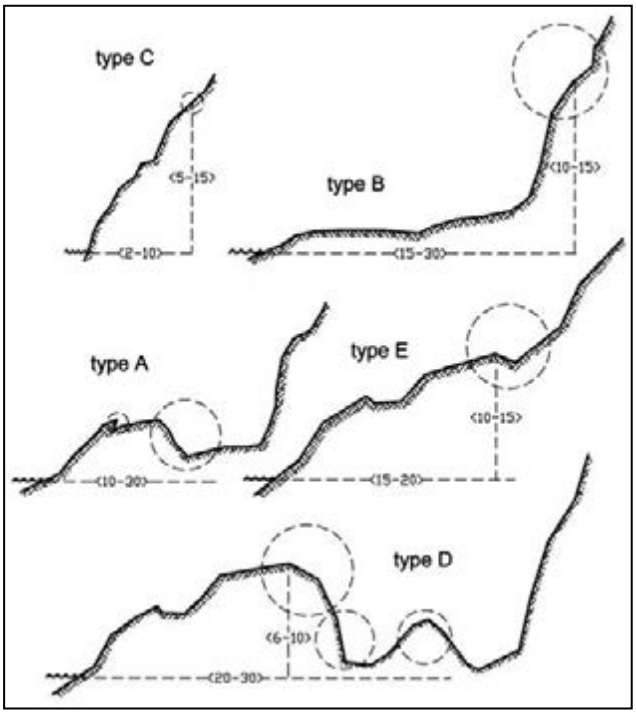

Fig. 7. Bluff geomorphological types found at Copanello. Traced circles show the position of *L. calabrum* plants. Distances are expressed in metres.

Table 2. Plants distribution on different bluff geomorphological types.

Type	Sites	Plants n°	Plants %	RLD %
A	1.3, 1.4, 1.5, 1.6, 1.7, 2.8	126	4.4	12
B	3.1, 3.2, 3.3, 3.4, 3.5	415	14.3	13.8
C	4.1, 4.11	20	0.7	3.4
D	1.1, 1.2, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.9, 2.10, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10	1466	50.6	50.1
E	2.1, 3.6, 3.7, 3.8, 3.9, 3.10	868	30.0	20.7
	total	**2895**	100	100

RLD – real longitudinal distribution sum of linear length of sites (805.6 m).

(1–2 m a.s.l.) opened to sea water stagnation and salt sedimentation about 15–30 m inward; a 10–15 m tall rocky wall rises abruptly (angle 65°) where are hosted plants (Sites 3.1, 3.2, 3.3, 3.4, 3.5; 415 plants). Type C shows a steep trend (angle 60–65°) that rises up to 5–15 m in a short distance (2–10 m) – Sites 4.1 and 4.11; 20 plants. Type D shows a first small rise (1–3 m a.s.l., 2–5 m from coast line), inwards a higher rise (6–10 m a.s.l., 10–20 m from coast line) and in between an area of salt sedimentation – Sites 1.1, 1.2, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.9, 2.10, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10; 1466 plants. Type E shows a type D trend with a first small rise (1–3 m a.s.l.,

2–5 m from coast line), inwards a second one, bigger than the first (6–10 m a.s.l., 10–20 m from coast line) and in between an area of salt sedimentation; from the second rise top a slight depression can be found and the cliff rises – Sites 2.1, 3.6, 3.7, 3.8, 3.9, 3.10; 868 plants (Fig. 7).

On type D bluff (1466 plants; 50.6 %) is found the highest number of plants. Comparing data to RLD, types D (50.6 % plants; 50.1 % of RLD) and B (14.3 % plants; 13.8 % of RLD) contain plants proportionally to RLD share; for types A (4.4 % plants; 12 % of RLD) and C (0.7 % plants; 3.4 % of RLD) the rate is less than proportional, while is more than proportional for type E (30 % of the population on the 20.7 % of RLD). The data shows type E as the most suitable bluff geomorphological type for the species in the study area (Table 2).

Table 3. Entity and typology of the anisotropy on *L. calabrum* distribution at Copanello.

Anisotropies	Category	m
1.1–1.2	human infrastructure	2.6
1.2–1.3	clasts	46.2
1.3–1.4	vertical bluff	5.1
1.4–1.5	vertical bluff	76.9
1.5–1.6	human infrastructure	5.1
1.6–1.7	human infrastructure	3.8
1.7–2.1	clasts/human infrastructure	300
2.1–2.2	human infrastructure	17.9
2.2–2.3	human infrastructure	11.5
2.3–2.4	human infrastructure	5.4
2.4–2.5	bluff conformation	3.8
2.5–2.6	bluff conformation	16.7
2.6–2.7	bluff conformation	3.8
2.7–2.8	human infrastructure	43.6
2.8–2.9	human infrastructure	51.3
2.9–2.10	human infrastructure	7.7
2.10–3.1	vertical bluff	105
3.1–3.2	vertical bluff	12.8
3.2–3.3	vertical bluff	1.3
3.3–3.4	vertical bluff	7.7
3.4–3.5	vertical bluff	1.3
3.5–3.6	human infrastructure	23.1
3.6–3.7	human infrastructure	2.6
3.7–3.8	human infrastructure	9.0
3.8–3.9	human infrastructure	1.3
3.9–3.10	human infrastructure	10.3
3.10–4.1	beach	115
4.1–4.2	vertical bluff	32.1
4.2–4.3	vertical bluff	21.8
4.3–4.4	vertical bluff	21.8
4.4–4.5	clasts	82.1
4.5–4.6	bluff conformation	9.0
4.6–4.7	bluff conformation	1.3
4.7–4.8	bluff conformation	3.8
4.8–4.9	bluff conformation	30.8
4.9–4.10	bluff conformation	28.2
4.10–4.11	vertical bluff	71.8
	average	**32.3**
	total anisotropy	**1193.3**

Related to PLD and RLD is evident that between 38 sites can be found 37 sectors of coast where species is absent (anisotropies). Anisotropies have been measured (linear length between sites) and gathered in 5 categories (beaches, vertical bluff, bluff conformation, clasts, human infrastructure). «Beaches» and «vertical bluff» (over 65° angle) for different reasons hinder plant life and they are the only totally natural causes of anisotropy. «Bluff conformation» is a digitated coastal profile excavated for fish-breeding; indentations of coast hinder plant life as vertical bluff. During construction of public infrastructures (railway, roads, galleries) materials extracted were systematically thrown away on the underlying coast; «clasts» includes all these materials. The category «human infrastructure» includes all buildings found on the PLD (Table 3). For each category have been calculated its ratio with total anisotropy (1193.3 m) and PLD (1998.9) (Table 4).

Data summarised in Table 4 show that there are no plants of *L. calabrum* in 59.7 % of PLD. While 60.4 % of the anisotropy is due to anthropic actions and the category «clasts» is the most important cause of anisotropy (35.9 %). Some indices linked to presence–absence analysis are given.

Table 4. Causes of anisotropy in the distribution area of *L. calabrum* gathered in categories. Qualitative and quantitative analysis (A_{tot} = total anisotropy = 1193.3 m; PLD = potential longitudinal distribution = 1998.9 m).

Anisotropy causes	n°	m	% A_{tot}	% PLD
Beaches	1	115	9.6	5.8
Vertical bluff	11	357.6	30.0	17.9
Total natural causes	*12*	*472.6*	*39.6*	*23.7*
Bluff conformation	8	97.4	8.2	4.9
Clasts	3	428.2	35.9	21.4
Human infrastructure	14	195.1	16.3	9.8
Total anthropic causes	*25*	*720.7*	*60.4*	*36.1*
Total anisotropies	**37**	**1193.3**	**100**	**59.7**

$PLD = RLD + A_{tot} = 805.6 + 1193.3 = 1998.9$ m

In which: PLD = potential longitudinal distribution; RLD = real longitudinal distribution (sum of sites linear length); A_{tot} = total anisotropies (sum of linear distances between sites).

$I_{is} = RLD/PLD = 805.6/1998.9 = 0.4$

In which I_{is} = distribution isotropy index, a value expressing quantitatively the presence of species on PLD.

$I_{anis} = 1 - I_{is} = A_{tot}/PLD = 1193.3/1998.9 = 0.6$

In which I_{anis} = distribution anisotropy index (discontinuity degree).

$I_{antr} = A_{antr}/PLD =$
$= (\text{Bluff Conformation} + \text{Clasts} + \text{Human infrastructure})/PLD =$
$= (97.4 + 428.2 + 195.1)/1998.9 =$
$= 720.7/1998.9 = 0.36$

In which: I_{antr} = anthropisation index (anthropic pressure degree on the species distribution area due to human infrastructures or permanent substrate modifications); different from the homonym V (ratio between potential vegetation types and real vegetation types of an area; Colombo & Malcevschi 1996); A_{antr} = anthropic anisotropy (sum of linear length of discontinuities of anthropic origin).

Conclusions

The population of *L. calabrum* living on the Copanello promontory is fragmented and shows heterogeneous density. The small distribution area, fragmentation, eco-physiologic specialisation, apomixis, and the persistent anthropic pressure are factors that justify the need of protection actions. The long-term monitoring of distributive evolution of the studied population is therefore indicated in the future to preserve this rare endemic plant species applicable also as a bio-indication for environmental status of the coastal belt of the promontory.

Acknowledgements. Thanks to Dr Lia Pignotti, Dr Carmen Gangale and Dr Dimitar Uzunov for help in English translation and advices for the structure of this work.

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Alien vascular plants in Dobrogea (Romania) and their impact on different types of habitats

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Abstract. In different types of habitats from Dobrogea (including Danube Delta) we identified 140 neophytes and 9 archaeophytes; about half of them are from America and arrived here accidentally. Among neophytes, 30 taxa have invasive status, but only few are aggressive having a negative impact on different ecosystems: *Ailanthus altissima*, *Amorpha fruticosa*, *Azolla filiculoides*, *Bidens frondosa*, *Conyza canadensis*, *Elodea nuttallii*, *Lindernia dubia*, *Paspalum paspalodes*.

Key words: Dobrogea, ecosystems, impact, invasive alien plants, Romania

Introduction

Dobrogea (including Danube Delta) is a very important region for plant (about 2000 taxa) and habitat diversity (wetlands, dry grasslands, calcareous stony slopes, thermophilous woodlands, dunes and sands habitats, etc.). Here there are 20 endemic taxa and over 200 rare plants, many of them included in IUCN Red list, Bern Convention, Habitats Directive (*Campanula romanica*, *Centaurea jankae*, *C. pontica*, *Salicornia veneta*, *Achillea thracica*, *Aldrovanda vesiculosa*, *Alyssum borzaeanum*, *Colchicum fominii*, *Liparis loeselii*, *Marsilea quadrifolia*, *Moehringia jankae*, *Paeonia tenuifolia*, *Potentilla emilii-popii*, *Ruscus aculeatus*, *Salvinia natans*, *Serratula lycopifolia*, *Trapa natans*, *Typha minima*, *Zostera marina*) (Sârbu 2003). Biosphere Reserve Danube Delta and National Park Măcin Mountains are the main protected areas. According to Law No. 5/2000, in Dobrogea there are also 82 natural scientific reserves. Unfortunately, in this region there are many opportunities for alien plants: favourable climate for thermophilous elements, numerous gates and pathways (harbours, train stations, railways network, roads, Danube River, Danube – Black Sea Channel).

Among the first alien plants reported from Dobrogea are: *Heliotropium curassavicum*, *Coronopus didymus*, *Diplotaxis erucoides*, *Petunia parviflora* (Kanitz 1879–1881), *Amaranthus deflexus*, *Urtica pilulifera* (Brândză 1898), *Elodea canadensis* (Macovei & Scriban 1905), *Lycium barbarum* (Grecescu 1909) and *Azolla filiculoides* (Pallis 1916). The last records are: *Bellardia trixago* (Ciocârlan & Costea 2004), *Chloris barbata* and *Impatiens balsamina* (Anastasiu & Negrean 2005).

Material and methods

A comprehensive database with neophytes recorded from Dobrogea (including Danube Delta) has been done. The data were obtained from literature, herbarium sheets and as a result of own field observations along many years. For each taxon we registered: family, life form, origin, time of immigration, abundance, invasive status, type of habitats where the species was identified, way of introduction, the impact on natural ecosystems and the economic impact. We also listed the archaeophytes from Dobrogea. The nomenclature of species is according to *Flora Europaea* (Tutin & al. 1964–1980, 1993) and *Flora României* (Săvulescu 1952–1976).

The terminology and definitions recommended by Richardson & al. (2000) and Pyšek & al. (2004) were used to establish the status of alien plants in our country.

It must be mentioned that, generally, the appreciations of degree of naturalisation and invasiveness are subjective, as well as the appreciations on impact, depending on our perception related to these phenomena.

Results and discussion

In Dobrogea region we identified 140 alien plants taxa belonging to 43 families (Table 1). Among these, 36 taxa are distributed exclusively in Danube Delta. Few families are well represented: *Asteraceae* (23 taxa), *Amaranthaceae* (16 taxa), *Poaceae* (14 taxa), *Solanaceae* (10), *Fabaceae* (9 taxa), *Brassicaceae* (8 taxa), *Chenopodiaceae* (7 taxa). The others families are represented by one or two taxa.

Most neophytes from Dobrogea came from America – 69 taxa (49.28 %). Among these, about half of them belong to North-American species with 41 taxa (29.28 %). Mediterranean species follow those American, but at a distance, with only 24 representatives (17.14 %). Neophytes originated from Asia rank third with 16 taxa (11.42 %). 13 neophytes are from Europe (9.28 %); these came either from Eastern Europe (*Lychnis chalcedonica*, *Salsola acutifolia*, *S. collina*, *Scilla siberica*), from Central, Western and South-Western Europe (*Euphorbia leptocaula*, *Geranium sibiricum*, *Erucastrum gallicum*, *Apium graveolens*, *Elymus athericus*, *Hordeum marinum*, *Vicia lutea*, *Cytisus scoparius*) or from Anatolia and the Caucasus (*Sophora jaubertii*). Other elements have a low representation. We mention the presence in Danube Delta of an Australian taxon – *Chenopodium pumilio*.

The analyses of life forms revealed the dominance of therophytes (90 taxa – 64.28 %), compared with other categories. They are followed by hemicryptophytes with 18 taxa (12.85 %). The good representation of helohydrophytes (6 taxa – 4.28 %), comparing with other life forms, is explained by large water surfaces existing in this region. Phanerophytes are 12 taxa (8.57 %), 4 being trees, 5 shrubs and 3 vines. Chamaephytes and geophytes are weakly represented, generally fewer than 4 %.

Regarding the introduction, the most neophytes from Dobrogea are not intentional (99 taxa – 70.71 %), as a result of human activity, brought by animals, water, wind, etc. (97 taxa) or as a result of hybridisation (2 taxa: *Achillea roseo-alba* and *Amaranthus* × *budensis*). We mention that the not intentional introductions are favoured by the numerous gates that ensure international commercial relations of our country (Constanța, Sulina, Tulcea, Brăila and Galați harbours, railway stations), as well as by the existing transport net (fluvial and terrestrial, connecting Romania with South and East Europe as well as with Asia, etc.). 41 neophytes (29.28 %) were introduced deliberately as forestry, horticultural or agricultural plants and they escaped in different types of ecosystems.

Among 140 neophytes identified in Dobrogea, 4 taxa have been mentioned from a single locality or 2–3 localities, but their presence has not been re-signalled in the last 50 years. For this reason we consider them extinct (*Cyperus esculentus*, *Diplotaxis erucoides*, *Saccharum ravennae*, *Urtica pilulifera*). Other 24 neophytes have been indicated from a single location, but re-signalled afterwards. Many neophytes are rare, being indicated from few localities (48 taxa – 35.29 %). Only few neophytes are common (22 taxa) or locally abundant (12 taxa).

The analyses of naturalisation status reveal the most neophytes become spontaneous only casually (76 taxa – 54.28 %). These either escaped from culture, or penetrate accidentally and they are not able to produce new stable populations on long term, in the new conditions. We consider that only 34 taxa among analysed neophytes are naturalised, being able to reproduce and to sustain populations without human direct intervention. Among neophytes, 30 are invasive: *Acer negundo*, *Ailanthus altissima*, *Amaranthus albus*, *A. crispus*, *A. hybridus*, *A. retroflexus*, *Ambrosia artemisiifolia*, *Amorpha fruticosa*, *Artemisia annua*, *Azolla filiculoides*, *Bidens frondosa*, *Chamaesyce maculata*, *Conyza canadensis*, *Cuscuta campestris*, *Echinocystis lobata*, *Elodea nuttallii*, *Erigeron annuus* subsp. *annuus*, *Galinsoga parviflora*, *Iva xanthifolia*, *Lindernia dubia*, *Lycium barbarum*, *Matricaria discoidea*, *Paspalum paspalodes*, *Phytolacca americana*, *Robinia pseudacacia*, *Sorghum halepense*, *Veronica persica*, *Xanthium italicum*, *X. spinosum*, *X. strumarium*.

If the most naturalised and invasive alien plants are limited to the anthropic habitats, some of them penetrate semi-natural or natural ecosystems often having a negative impact. Among these, some seems to be very aggressive:

Ailanthus altissima is present in all types of habitats from Dobrogea. We identified it in costal dunes and sand habitats (EUNIS code B1) from Agigea Natural Reserve, dry grasslands (EUNIS code E1) from Dobrogea Plateau, Moesian Christ's thorn brush (EUNIS code F3) from Southern Dobrogea. From Danube Delta *A. altissima* was reported in sand habitats at Letea and Sulina (Dihoru & Negrean 1976).

Alcea rosea is often in anthropic habitats (EUNIS code I, J and X21), but we met it in dry grasslands

(EUNIS code E1) as well as in sand habitats with *Ephedra distachya* (EUNIS code B1.4) from Agigea Natural Reserve.

Amorpha fruticosa is a real competitor for the native plants of riverine scrubs, even forming a subassociation: *Salicetum triandrae* Malcuit 1929 subass. *amorphosum fruticosae* Borza 1954 (Doniță & al. 2005). It is very frequent in poplar galleries (EUNIS code G1.365) and almond willow-osier scrub (EUNIS code F9.121).

Azolla filiculoides covers large water surfaces threatening the communities of *Chara* and *Nitella* (EUNIS code C1.141, C1.142), of *Lemna minor*, *L. trisulca*, *Spirodela polyrhiza*, *Wolffia arrhiza* (EUNIS code C1.221), of *Salvinia natans* and *Marsilea quadrifolia* (EUNIS code C1.225), the communities with *Potamogeton perfoliatus*, *P. gramineus* and the communities with *Trapa natans*.

Bidens frondosa is frequent in riverine scrubs from Danube Delta.

Conyza canadensis is much spread but without an evident impact on native flora. We recorded it in dry grasslands (EUNIS code E1), as well as in costal dunes and sand habitats (EUNIS code B1) at the seashore.

Elodea nuttallii was reported in 1998 from Danube Delta (Ciocârlan & al. 1998). It develops important populations in communities with *Potamogeton perfoliatus*, *P. gramineus*, *P. natans*, as well as in communities with *Nymphaea alba*, *Trapa natans* and *Nuphar lutea*. It seems *Elodea nuttallii* replaces *E. canadensis*.

Lindernia dubia is invasive in natural ecosystems from Sacalin Islands (Ciocârlan 1994).

Paspalum paspalodes, even if not so spread, is very aggressive replacing native species as *Typha latifolia*, *Phragmites australis*, etc. We recorded it from communities with *Bolboschoenus maritimus* and *Schoenoplectus tabernemontani*, communities with *Typha angustifolia* and *T. latifolia* (EUNIS code C3.231/232), communities with *Phragmites australis* (EUNIS code C3.21).

Xanthium italicum seems to be more present than *X. strumarium*. It invades habitats as costal dunes and sand habitats (EUNIS code B1), dry grasslands (EUNIS code E1), inland saline grass and herb-dominated habitats (EUNIS code E6).

Xanthium spinosum is common in all types of habitats. We recorded it frequently on costal dunes and sand habitats (EUNIS code B1), as well as in associations of *Scolymus hispanicus* and *Ecbalium elaterium* from the Black Sea seashore.

From the economic point of view, we appreciate that about 24 neophytes from Dobrogea are harmful and 35 neophytes are potentially harmful. They are present in crops, ornamental cultures or on railways' embankments, along the roads, pathways, in railways stations, harbours. Their elimination, either mechanically or chemically, involves usually high costs.

Some neophytes from Dobrogea were included in National Red Lists (Oltean & al. 1994; Dihoru & Dihoru 1994): *Geranium sibiricum* (Ex), *Glinus lotoides* (R), *Petunia parviflora* (R), *Sophora jaubertii* (V/R).

Among archaeophytes, we recorded the next taxa: *Abutilon theophrasti* (frequent), *Agrostemma githago* (scattered), *Cannabis sativa* ssp. *spontanea* (frequent), *Cardaria draba* ssp. *draba* (frequent), *Centaurea cyanus* (frequent), *Consolida orientalis* (scattered), *Peganum harmala* (rare), *Portulaca oleracea* ssp. *oleracea* (frequent) and *Tanacetum parthenium* (scattered). All these species are usually present in artificial habitats (EUNIS code I and J) and they have not an evident economic impact.

Table 1. Neophytes recorded from Dobrogea (Romania).

No.	Taxon	Family	Origin	Life form	Distribution	Introduction	Abundance	Invasive status	Impact on natural ecosystems	Economic impact
1.	***Acer negundo*** L. ♀ ♂	ACE	AmN	PhM	D	I2	c	A3	U	d
2.	***Achillea roseo-alba*** Ehrend.	AST	Sm	H	D	N2	r	A1	U	nd
3.	***Acorus calamus*** L.	ARA	As	H	DD	I2	sc	A2	U	nd
4.	***Ailanthus altissima*** (Miller) Swingle	SMB	As	PhM	D,DD	I2	c	A3	I	d
5.	***Alcea rosea*** L.	MLV	Med (?)	H	D	I2	sc	A2	I	pd
6.	***Amaranthus acutilobus*** Uline & Bray	AMA	AmN	T	D	N1	r	A2	U	pd
7.	***Amaranthus albus*** L.	AMA	AmN	T	D,DD	N1	c	A3	U	d
8.	***Amaranthus blitoides*** S. Watson	AMA	AmN	T	D,DD	N1	r	A2	U	d

Table 1. Continuation.

No.	Taxon	Family	Origin	Life form	Distribution	Introduction	Abundance	Invasive status	Impact on natural ecosystems	Economic impact
9.	***Amaranthus crispus*** (Lesp. & Thev.) N. Terrac.	AMA	AmS	T	D,DD	N1	c	A3	U	d
10.	***Amaranthus deflexus*** L.	AMA	AmS	T	D,DD	N1	sc	A2	U	d
11.	***Amaranthus emarginatus*** Moq. ex Uline & W.L. Bray	AMA	Trop	T	DD	N1	sc	A1	U	pd
12.	***Amaranthus hybridus*** L. s.l.	AMA	AmN	T	D,DD	N1	c	A3	U	d
13.	***Amaranthus palmeri*** S. Watson	AMA	AmN	T	D	N1	s	A1	U	pd
14.	***Amaranthus powellii*** S. Watson s.l.	AMA	AmN&S	T	DD	N1	sc	A2	U	d
15.	***Amaranthus quitensis*** Kunth	AMA	AmS	T	D	N1	s	A1	U	pd
16.	***Amaranthus retroflexus*** L.	AMA	AmN	T	D,DD	N1	c	A3	U	d
17.	***Amaranthus rudis*** Sauer	AMA	AmN	T	D	N1	s	A1	U	pd
18.	***Amaranthus spinosus*** L.	AMA	AmN	T	D	N1	s	A1	U	pd
19.	***Amaranthus tamariscinus*** Nutt.	AMA	AmS	T	D	N1	s	A1	U	pd
20.	***Amaranthus viridis*** L.	AMA	AmS	T	D	N1	r	A1	U	pd
21.	***Amaranthus* × *budensis*** Priszter	AMA	×	T	D	N2	s	A1	U	pd
22.	***Ambrosia artemisiifolia*** L.	AST	AmN	T	D	N1	c	A3	PI	d
23.	***Ambrosia coronopifolia*** Torrey & A. Gray	AST	AmN	H	DD	N1	r	A1	U	pd
24.	***Ambrosia trifida*** L.	AST	AmN	T	D	N1	sc	A1	U	pd
25.	***Amorpha fruticosa*** L.	FAB	AmN	PhN	D,DD	I2	c	A3	I	d
26.	***Apium graveolens*** L. s.l.	API	EuW&S	TH	D,DD	N1	r	A2	PI	nd
27.	***Artemisia annua*** L.	AST	AsC&SW	T	D,DD	N1	c	A3	PI	d
28.	***Atriplex micrantha*** Ledeb.	CHN	As	T	D	N1	r	A1	U	nd
29.	***Azolla filiculoides*** Lam.	AZL	AmN	HH	D,DD	N1	la	A3	I	d
30.	***Bellardia trixago*** (L.) All.	SCR	Med	T	D	N1	s	A1	U	nd
31.	***Bidens connata*** Muhl. ex Willd.	AST	AmN	T	DD	N1	s	A2	PI	pd
32.	***Bidens frondosa*** L.	AST	AmN	T	DD	N1	r	A2	PI	pd
33.	***Bidens vulgata*** Greene	AST	AmN	T	DD	N1	c	A3	PI	d
34.	***Biscutella auriculata*** L.	BRA	Med	T	D	N1	s	A1	U	nd
35.	***Borago officinalis*** L.	BOR	Med	T	D	N1	r	A1	U	nd
36.	***Brachyactis ciliata*** Ledeb.	AST	As	T	DD	N1	sc	A1	U	nd
37.	***Cardiospermum halicacabum*** L.	SAP	Trop	T	D	N1	s	A1	U	nd
38.	***Cenchrus incertus*** M.A. Curtis	POA	Am	T	D	N1	r	A2	PI	pd
39.	***Chamaesyce canescens*** (L.) Prokh.	EUP	Sm	T	D	N1	sc	A1	U	nd
40.	***Chamaesyce maculata*** (L.) Small	EUP	AmN	T	DD	N1	sc	A3	PI	nd
41.	***Chenopodium ambrosioides*** L.	CHN	AmTrop	T	D	N1	sc	A2	PI	pd
42.	***Chenopodium foliosum*** (Moench) Ascherson	CHN	Med	T	D	N1	r	A1	U	nd
43.	***Chenopodium multifidum*** L.	CHN	AmS	H	D	N1	r	A1	U	nd
44.	***Chenopodium pumilio*** R. Br.	CHN	Australia	T	DD	N1	s	A1	U	nd
45.	***Chloris barbata*** Sw.	POA	Am	T	D	N1	s	A1	U	nd
46.	***Citrullus lanatus*** (Thunb.) Mansfeld	CUC	AfNW	T	D	I2	r	A1	U	nd
47.	***Cladium mariscus*** (L.) Pohl ssp. ***martii*** (Roem. & Schult.) Soó	CYP	AsC,Med	H	DD	N1	sc	A1	U	nd
48.	***Consolida ajacis*** (L.) Schur	RAN	Med	T	D	I2	sc	A2	U	nd
49.	***Conyza canadensis*** (L.) Cronq.	AST	AmN	T	D,DD	N1	c	A3	PI	nd
50.	***Coronopus didymus*** (L.) Sm.	BRA	AmS	T-TH	D,DD	N1	r	A1	U	nd
51.	***Cuscuta campestris*** Yuncker	CUS	AmN	T	D,DD	N1	sc	A3	PI	d
52.	***Cyperus esculentus*** L.	CYP	Trop	T-H	DD	N1	se	A1	U	nd
53.	***Cyperus odoratus*** L.	CYP	Trop	T-H	DD	N1	s	A1	U	nd
54.	***Cytisus scoparius*** (L.) Link s.l.	FAB	EuW,S&C	PhN	D	I2	la	A2	PI	nd
55.	***Datura stramonium*** L.	SOL	Am	T	D,DD	I2	c	A2	U	pd
56.	***Diplotaxis erucoides*** (L.) DC.	BRA	Med	T	DD	N1	se	A1	U	nd

Table 1. Continuation.

No.	Taxon	Family	Origin	Life form	Distribution	Introduction	Abundance	Invasive status	Impact on natural ecosystems	Economic impact
57.	***Echinocystis lobata*** (Michx) Torrey & A. Gray	CUC	AmN	T	DD	I2	sc	A3	U	pd
58.	***Eclipta prostrata*** (L.) L.	AST	AmTrop	T	D	N1	sc	A1	U	nd
59.	***Elaeagnus angustifolius*** L.	ELE	AsTemp	PhN	D,DD	I1	sc	A2	U	pd
60.	***Eleusine indica*** (L.) Gaertner	POA	Trop	T	D	N1	sc	A2	U	pd
61.	***Elodea canadensis*** Michx	HDC	AmN	HH	D,DD	N1	sc	A2	PI	pd
62.	***Elodea nuttallii*** (Planchon) St John	HDC	AmN	HH	D,DD	N1	la	A3	I	pd
63.	***Elymus athericus*** (Link) Kerguélen	POA	EuW&S	G	D,DD	N1	r	A2	U	nd
64.	***Erigeron annuus*** (L.) Pers. ssp. ***annuus***	AST	AmN	TH	D	N1	c	A3	I	pd
65.	***Erigeron annuus*** (L.) Pers. ssp. ***strigosus*** (Willd.) Wagenitz	AST	AmN	TH	D	N1	c	A3	I	pd
66.	***Eruca vesicaria*** (L.) Cav. s.l.	BRA	Med	T	D	N1	r	A1	U	nd
67.	***Erucastrum gallicum*** (Willd.) O.E. Schulz	BRA	EuC&SW	T-H	D	N1	r	A1	U	nd
68.	***Euphorbia leptocaula*** Boiss.	EUP	Eu (Pt)	H	DD	N1	se	A1	U	nd
69.	***Euphorbia peplus*** L.	EUP	Cs	T	D	N1	la	A2	U	nd
70.	***Fallopia aubertii*** (Louis Henry) J. Holub	PLG	As	PhLi	D	I2	r	A1	U	pd
71.	***Fallopia baldschuanica*** (Regel) J. Holub	PLG	As	PhLi	D	I2	r	A1	U	nd
72.	***Ficus carica*** L.	MOR	Med	PhN	D	I2	r	A1	U	nd
73.	***Fimbristylis bisumbellata*** (Forssk.) Bubani	CYP	Med	T	DD	N1	r	A1	U	nd
74.	***Galinsoga parviflora*** Cav	AST	AmS	T	D,DD	N1	c	A3	PI	d
75.	***Geranium sibiricum*** L.	GER	Eua	H	D	N1	r	A2	U	nd
76.	***Glinus lotoides*** L.	MOL	Med	T	D,DD	N1	sc	A1	U	nd
77.	***Helianthus annuus*** L.	AST	AmN	T	D,DD	N1	r	A1	U	nd
78.	***Heliotropium curassavicum*** L.	BOR	AmS	H	DD	N1	la	A1	U	nd
79.	***Hemerocallis fulva*** L.	LIL	As	H	D	I2	r	A1	U	nd
80.	***Hordeum jubatum*** L.	POA	AmN,As	T	DD	N1	sc	A1	U	nd
81.	***Hordeum marinum*** Huds.	POA	EuW&S	T	DD	N1	r	A2	U	nd
82.	***Hypecoum imberbe*** Sm.	PAP	Med	T	D	N1	s	A1	U	nd
83.	***Impatiens balsamina*** L.	BLS	As (India E)	T	D	I2	r	A1	U	nd
84.	***Ipomoea lacunosa*** L.	CNV	AmN	T	D	N1	s	A1	U	nd
85.	***Iva xanthifolia*** Nutt.	AST	AmN	T	D	N1	la	A3	PI	d
86.	***Lathyrus sativus*** L.	FAB	Med	T	D,DD	I2	r	A1	U	nd
87.	***Lemna minuta*** Kunth.	LMN	Am	HH	DD	N1	s	A1	U	nd
88.	***Lens nigricans*** (Bieb.) Godron	FAB	Med	T	D	N1	s	A1	U	nd
89.	***Lindernia dubia*** (L.) Penell	SCR	AmN	T	DD	N1	la	A3	I	pd
90.	***Lychnis chalcedonica*** L.	CRY	Eu (RussiaE&C)	H	DD	I2	r	A1	U	nd
91.	***Lycium barbarum*** L.	SOL	As	PhN	D	I2	la	A3	PI	d
92.	***Lycopersicon esculentum*** Miller	SOL	AmS	T	D	I2	sc	A1	U	nd
93.	***Matricaria discoidea*** DC.	AST	Am,As	T	D	N1	c	A3	PI	pd
94.	***Matthiola longipetala*** (Vent.) DC. s.l.	BRA	Med	T	DD	I2	r	A1	U	nd
95.	***Medicago sativa*** L. ssp. ***sativa***	FAB	Med	H	D,DD	I2	sc	A2	PI	nd
96.	***Morus alba*** L.	MOR	As (China)	PhM	D	I2	c	A2	PI	d
97.	***Nicotiana alata*** Link & Otto	SOL	AmS	T	D	I2	sc	A1	U	nd
98.	***Nigella sativa*** L.	RAN	Med	T	D	I2	sc	A1	U	nd
99.	***Oenothera parviflora*** L.	ONA	AmN	TH	D	I2	sc	A2	U	nd
100.	***Oxalis corniculata*** L.	OXL	AmN&C	H	DD	N1	sc	A2	U	pd
101.	***Panicum capillare*** L.	POA	AmN	T	D,DD	N1	sc	A2	U	pd
102.	***Panicum dichotomiflorum*** Michx	POA	AmN	T	D	N1	r	A2	U	pd
103.	***Parthenocissus inserta*** (A. Kerner) Fritsch	VIT	AmN	PhLi	D	I2	sc	A2	U	nd
104.	***Paspalum paspalodes*** (Michx) Scribner	POA	Trop	H	DD	N1	la	A3	I	d

Table 1. Continuation.

No.	Taxon	Family	Origin	Life form	Distribution	Introduction	Abundance	Invasive status	Impact on natural ecosystems	Economic impact
105.	***Petunia integrifolia*** (Hooker) Schinz & Thell.	SOL	AmS	T	D	I2	r	A1	U	nd
106.	***Petunia parviflora*** A.L. Juss.	SOL	AmS	T-H	DD	N1	la	A2	U	nd
107.	***Phalaris canariensis*** L.	POA	Canare	T	D	I2	r	A1	U	pd
108.	***Phytolacca americana*** L.	PHT	AmN	T	DD	I2	sc	A3	U	pd
109.	***Polygonum pensylvanicum*** L.	PLG	AmN	T	D	N1	s	A1	U	nd
110.	***Raphanus raphanistrum*** L. ssp. ***landra*** (Moretti ex DC.) Bonnier & Layens	BRA	Med	T	D	N1	r	A1	U	nd
111.	***Reseda alba*** L.	RSD	Med	T-H	D	I2	r	A1	U	nd
112.	***Robinia pseudacacia*** L.	FAB	AmN	PhM	D	I2	c	A3	I	pd
113.	***Ruta graveolens*** L.	RUT	Med	Ch	D	I2	r	A1	U	nd
114.	***Saccharum ravennae*** (L.) Murray	POA	Med	H	DD	N1	se	A1	U	nd
115.	***Sagittaria lancifolia*** L.	ALI	As	HH	DD	N1	s	A1	U	nd
116.	***Sagittaria trifolia*** L.	ALI	As	HH	DD	N1	r	A1	U	nd
117.	***Salsola acutifolia*** (Bunge) Botsch	CHN	Eua (RussiaSE, Ukraina SE & KazakhstanW)	T	DD	N1	r	A1	U	nd
118.	***Salsola collina*** Pallas	CHN	Eua (RussiaE&C)	T	DD	N1	r	A1	U	nd
119.	***Salvia reflexa*** Hornem.	LAM	AmN	T	D	I2	r	A1	U	nd
120.	***Scilla siberica*** Haw.	LIL	Eua (Russia)	G	D	I2	r	A1	U	nd
121.	***Senna obtusifolia*** (L.) Irwin & Barneby	CSL	Trop	T	D	N1	s	A1	U	nd
122.	***Sesbania exaltata*** (Raf.) Cory.	FAB	Am	T	D	N1	s	A1	U	nd
123.	***Setaria faberi*** Herrm.	POA	AsE	T	D	N1	s	A1	U	nd
124.	***Silene pendula*** L.	CRY	Med	T	D	I2	r	A1	U	nd
125.	***Sisymbrium irio*** L.	BRA	Sm	T	DD	N1	r	A1	U	nd
126.	***Solanum carolinense*** L.	SOL	AmN	H	D	N1	s	A1	U	nd
127.	***Solanum rostratum*** Dunal.	SOL	AmN	T	D	I2	s	A1	U	nd
128.	***Solanum triflorum*** Nutt. ssp. ***ponticum*** (Prodan) Negrean & Dihoru	SOL	AmN	T	D,DD	N1	la	A2	U	nd
129.	***Solanum tuberosum*** L.	SOL	AmS	T	D	I2	r	A1	U	nd
130.	***Sophora jaubertii*** Spach	FAB	Eua (Anat, CaucW)	H	D	I2	la	A2	U	nd
131.	***Sorghum halepense*** (L.) Pers.	POA	AfN, AsSW	H	D,DD	N1	c	A3	U	d
132.	***Tragopogon porrifolius*** L. s.l.	AST	Med	T-TH	D	I2	r	A1	U	nd
133.	***Urtica pilulifera*** L.	URT	Sm	T	D	N1	se	A1	U	nd
134.	***Vicia lutea*** L. s.l.	FAB	EuW&S	T	D	N1	sc	A2	U	nd
135.	***Xanthium italicum*** Moretti	AST	Med	T	D,DD	N1	c	A3	I	d
136.	***Xanthium orientale*** L.	AST	Am	T	DD	N1	r	A2	I	nd
137.	***Xanthium saccharatum*** Wallr.	AST	AmS	T	D	N1	r	A2	PI	nd
138.	***Xanthium spinosum*** L.	AST	AmS	T	D	N1	c	A3	I	d
139.	***Xanthium strumarium*** L.	AST	Am	T	D,DD	N1	c	A3	I	d
140.	***Zea mays*** L. ♀ ♂	POA	Am	T	D	I2	r	A1	U	nd

Abbreviations: ***Origin:*** **Af** – Africa; **Am** – America; **As** – Asia; **Eu** – Europe; **Eua** – Eurasia; **Cauc** – Caucasus; **Anat** – Anatolia; **Cs** – Cosmopolite; **Temp** – Temperate; **Trop** – Tropical; **Med** – Mediterranean; **Sm** – Submediterranean; **Pt** – Pontic; **N** – North; **E** – East; **S** – South; **W** – West; **C** – Centre (central). ***Life form:*** **Ch** – Chamaephyte; **G** – Geophyte; **H** – Hemicryptophyte; **HH** – Helohydrophyte; **PhLi** – Liana; **PhM** – Macrophanerophyte; **PhN** – Nanophanerophyte; **T** – Therophyte; **TH** – Hemitherophyte. ***Distribution:*** **D** – Dobrogea; **DD** – Danube Delta. ***Way of introduction:*** **I2** – intentional, escaped; **N1** – not intentional dispersed; **N2** – not intentional, hybrid. ***Abundance:*** **s** – single locality; **r** – rare; **sc** – scattered; **la** – locally abundant; **c** – common; **se** – one locality or more, but the plant has not been signalled for more than 50 years. ***Invasive status:*** **A1** – casual (an alien plant that reproduces occasionally in an area, but requires repetitive introductions for its persistence); **A2** – naturalised (an alien plant that reproduces constantly and sustains populations over several life cycles without direct human intervention); **A3** – invasive (an alien plant that produces reproductive offspring, often in large numbers, at considerable distances from the parental plants and over large areas). ***Impact on natural ecosystems:*** **U** – unknown; **PI** – potentially invasive; **I** – invasive. ***Economic impact:*** **d** – harmful; **nd** – not harmful; **pd** – potentially harmful.

Conclusions

In Dobrogea's flora we identified 149 alien plants taxa. 140 of these are neophytes and 9 are archaeophytes. *Asteraceae*, *Amaranthaceae* and *Poaceae* are the families with the highest weight in the structure of neophytes from Dobrogea. Over 60 % of the neophytes are therophytes. Many of them are not intentionally introduced (70.71 %) and they are from America (49.28 %). Among the invasive species only few are aggressive in natural and semi-natural ecosystems: *Ailanthus altissima*, *Alcea rosea*, *Amorpha fruticosa*, *Azolla filiculoides*, *Bidens frondosa*, *Conyza canadensis*, *Elodea nuttallii*, *Lindernia dubia*, *Paspalum paspalodes*, *Xanthium italicum*, *X. spinosum*. Four neophytes are included in the National Red Lists.

Acknowledgements. This work is a result of a CNCSIS grant (contract No. 33469/2005).

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Plant functional traits in relation to seedling recruitment and light conditions in sub-Mediterranean oak forests of Greece

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Abstract. We present the first results of a database containing plant functional traits in sub-Mediterranean oak forests of Greece. Data collected so far concern deciduous oak forests of NW Greece (Bourazani, Epirus), subjected to overgrazing by ruminants and non-ruminants for the last 30 years. To associate seedling recruitment capacity with certain functional traits, fruit and seed mass of 19 plants (14 woody and 5 herbaceous taxa) were measured. Preliminary germination results of these plants are furnished. Specific Leaf Area (SLA) values for 16 plants were determined and correlated with the prevailing Photosynthetic Active Radiation (PAR) under canopy and open-light conditions. The perspectives of developing a predictive tool of functional traits on the resistance and resilience of deciduous oak woodlands towards overgrazing are discussed.

Key words: fruit mass, functional traits, Greece, Photosynthetically Active Radiation (PAR), seed mass, Specific Leaf Area (SLA)

Introduction

Predicting the response of vegetation to environmental and land use changes remains a matter of major concern in recent theoretical and applied ecological research (McIntyre & al. 1999a, b). Plant functional types are diagnostic tools for disturbance processes and elaboration of long-term strategies in ecosystem management and ecological restoration projects (Gondard & al. 2003).

Specific Leaf Area (SLA) is a key variable in our understanding of forest ecosystem function (Ellsworth & Reich 1993; Pierce & al. 1994; Landsberg & Waring 1997). The ratio of leaf surface area to leaf mass describes the efficiency with which the leaf captures light and is inversely correlated with maximum photosynthetic rate (Marshall & Monserud 2003). Dispersule size and shape, as well as seed mass are good indicators of plants' response to disturbance, such as herbivory or overgrazing, and their competitive strength (Cornelissen & al. 2003). From the functional point of view, grazing acts as a filter of succession (De Bello & al. 2005). Pigs disturb the soil creating sites for seedling establishment and favouring early successional short-lived species (Chapin & al. 1997).

Objectives of this study are to: a) set the basis of creating a predictive plant functional pattern of species' response to different grazing regimes in sub-Mediterranean oak forests of NW Greece; b) record literature data and measure 17 functional plant attributes; c) correlate the monthly values of Photosynthetic Active Radiation (PAR) in light and under-canopy conditions with the calculated SLA of 16 plant species; d) measure seed and fruit mass, as regenerative traits in 19 plant species (14 tree and 5 herbaceous taxa) and compare them to find possible associations with seedling recruitment capacity.

Study area

The studied sub-Mediterranean deciduous oak forests are located in Bourazani area, 12 km NW of the municipality of Konitsa (Epirus, NW Greece) and approximately 5 km from the Greek–Albanian borders. The climate of the area (classified in the sub-humid bioclimatic zone) is sub-Mediterranean (average annual precipita-

tion: 700 mm, rainy cold winters: most rainfall in November–December). The bedrock consists mainly of flysch of paleogenic origin which is substituted locally by limestone covered by shallow rendzina soils (IGME 1973). The present forest vegetation consists of mixed deciduous woodlands with a high proportion of oak species (*Quercus pubescens*, *Q. frainetto*, *Q. cerris*, *Q. trojana* and more rarely *Q. petraea* subsp. *medwediewii* and *Q. coccifera*), accompanied by *Carpinus orientalis*, *Fraxinus ornus* and *Ostrya carpinifolia* (Tsaliki & al. 2005). The research is carried out in an enclosed forest area (112 ha) grazed for over 30 years and divided into two fenced parts (one is grazed by ruminants: 86 ha, and the other by wild boars: 26 ha). Hence, in this area three types of grazing regime have been practiced since 1974: a) ruminant overgrazing, b) wild-boar overgrazing and c) non grazed forests outside the fences (grazing practised sporadically by goats).

Material and methods

The list of 112 taxa occurring in the deciduous oak forests of the studied area was compiled in order to build a functional traits database. The recorded functional attributes are distinguished in three groups and listed below:

- Group I (vegetation) – life-form (Raunkiaer), growth form, plant height at maturity, evergreen/deciduous, annual/biennial/perennial, Specific Leaf Area (SLA), leaf size, leaf dry mass;
- Group II (regeneration) – fruit mass, seed mass, fruit type, dispersal mode, dispersal unit, flowering season, vegetative regeneration;
- Group III (morphology) – fruit, seed and leaf scans/pictures.

In the studied grazed sites, on the basis of their cover-abundance, 23 plant taxa were considered for plant trait measurements, including fruit mass, seed mass, Specific Leaf Area (SLA) and seedlings recruitment capacity measurements (Table 1). For SLA measurements standard methods were followed (Garnier & al. 2001); the SLA value for each taxon was estimated as the average of 20 measurements. Fruits and leaves were selected and stored from spring to autumn of 2005; for fruit and seed mass measurements, the field collections and experimental work follow the methodology proposed by Cornelissen & al. (2003) with samples of 100 fruits per taxon from selected populations. For both fruit and seed mass, the value was calculated as the average of 100 measurements.

Table 1. Functional attributes of the 23 species occurring in overgrazed deciduous oak woodlands.

No.	Taxon	SLA (g/cm^2)	Fruit mass (g)	Seed mass (g)	Emergent seedlings %	Dispersal mode
1	***Quercus frainetto***	121.51	1.7951	0.421	0	Accumulation*
2	***Quercus cerris***	132.14	5.4037	1.5935	0	Accumulation*
3	***Quercus pubescens***	192.34	2.4127	0.8265	0	Accumulation*
4	***Quercus trojana***	117.16	6.12	1.157	0	Accumulation*
5	***Phillyrea latifolia***	83.11	0.0177	0.0503	0	Accumulation*
6	***Fraxinus ornus***	141.99	0.0343	0.0248	22.5	Anemochory
7	***Carpinus orientalis***	228.05	0.0124	0.0062	0	Anemochory
8	***Juniperus oxycedrus***	96.00	0.1912	0.0011	0	Unassisted
9	***Paliurus spina-christi***	–	0.1923	0.0240	5	Anemochory
10	***Cercis siliquastrum***	155.24	–	0.0304	2	Endo-zoochory
11	***Crataegus monogyna***	182.55	0.3289	0.0046	0	Endo-zoochory
12	***Acer monspessulanum***	185.67	0.0369	0.0102	0	Anemochory
13	***Arbutus unedo***	63.64	–	–	–	Endo-zoochory
14	***Pistacia terebinthus***	147.27	0.0527	0.0101	30.77	Endo-zoochory
15	***Cotinus coggygria***	262.86	–	–	0	Endo-zoochory
16	***Sorbus torminalis***	178.62	–	–	–	Unassisted
17	***Clematis vitalba***	–	0.0041	0.0028	0	Anemochory
18	***Dorycnium hirsutum***	–	0.0670	0.0041	23.26	Endo-zoochory
19	***Psoralea bituminosa***	–	0.0222	0.0179	9.5	Endo-zoochory
20	***Briza humilis***	–	0.0038	0.0016	5	Exo-zoochory
21	***Bromus benekenii***	–	0.0104	0.0011	8	Exo-zoochory
22	***Trifolium arvense***	–	0.0005	0.0003	2.86	Anemochory
23	***Helleborus odorus* subsp. *cyclophyllus***	118.01	–	–	–	Endo-zoochory

Accumulation*: dispersal by hoarding (dispersal of seeds buried by mammals); (–): measurement not made.

To estimate the amount of light received by the potentially established seedlings and adult individuals of the studied taxa in the field, 3 micro-climatic stations were installed in the grazed and non-grazed areas and data were collected by two PAR sensors under shadow and in the light. The mean monthly PAR values were correlated with the mean SLA values in order to graphically represent the variation of the growth potential of the studied taxa.

Seed samples of 19 plant species (Table 1) were collected during the seed mass collections. The seeds were wet-stratified (2–6 °C) for one month in the dark. Afterwards, seed samples (10 to 30 seeds) were placed at constant 22 °C (10 h daily light) and emerged seedlings were recorded for six months. The seedling recruitment capacity (% percentage of the number of emerged seedlings divided by the number of seeds in

a sample) was measured. Raunkiaer's (1934) classification system was followed for the assignment of the considered taxa in life-forms (Fig. 1).

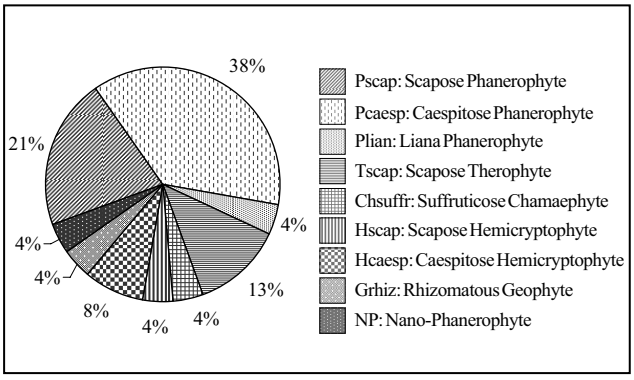

Fig. 1. Life-form spectrum of the 23 taxa used for plant trait measurements.

Results

The plant taxa of the overgrazed sites are banded into the life-form spectrum of Fig. 1, with the highest percentage represented by caespitose Phanerophytes.

The calculated SLA values (g/cm^2), fruit mass (fresh weight in g), seed mass (g) and seedling recruitment capacity, as well as the dispersal mode of 23 taxa are recorded and qualitatively related to the seed mass values (Table 1). For example, a heavy fleshy seed of *Quercus frainetto* is dispersed by hoarding (accumulation), while a tiny *Trifolium arvense* seed is dispersed by wind (anemochory).

Fig. 2 presents a graph of SLA values for 16 plant taxa in relation to the PAR values, as they fluctuate for a time period of 16 months under gap and shadowed conditions. *Cotinus coggygria* appears to have the highest SLA value of the tree taxa, while *Quercus pubescens* (192.34 g/cm^2) has the highest value among *Quercus* species. *Quercus trojana* (the most sclerophyllous of the *Quercus* species) shows the lowest SLA (117.16 g/cm^2). The most sclerophyllous tree taxa *Phillyrea latifolia*, *Arbutus unedo* and *Juniperus oxycedrus* are characterized by the lowest SLA values. PAR mean value in the light (50.61 uE) is much higher than PAR under canopy (8.90 uE) for this time period. PAR shows maximum values from end of March to the end of April, under canopy and in light conditions.

In Fig. 3, fruit fresh weight values of 19 selected plant species are compared to seed mass values of the same species and correlated with seedling recruitment capacity values. *Crataegus monogyna*, *Juniperus oxycedrus*, *Paliurus spina-christi* and all *Quercus* species have much higher fruit mass values than seed mass values. The first germination results showed an increased recruitment capacity for plant species that do not exhibit great variation between their fruit and seed masses. Such species are *Dorycnium hirsutum*, *Pistacia terebinthus*, *Fraxinus ornus*, *Psoralea bituminosa* and *Bromus benekenii*.

Discussion

PAR levels differ much in the field and under different light conditions. The useful for photosynthesis light, which can be absorbed by leaves, has a crucial role on

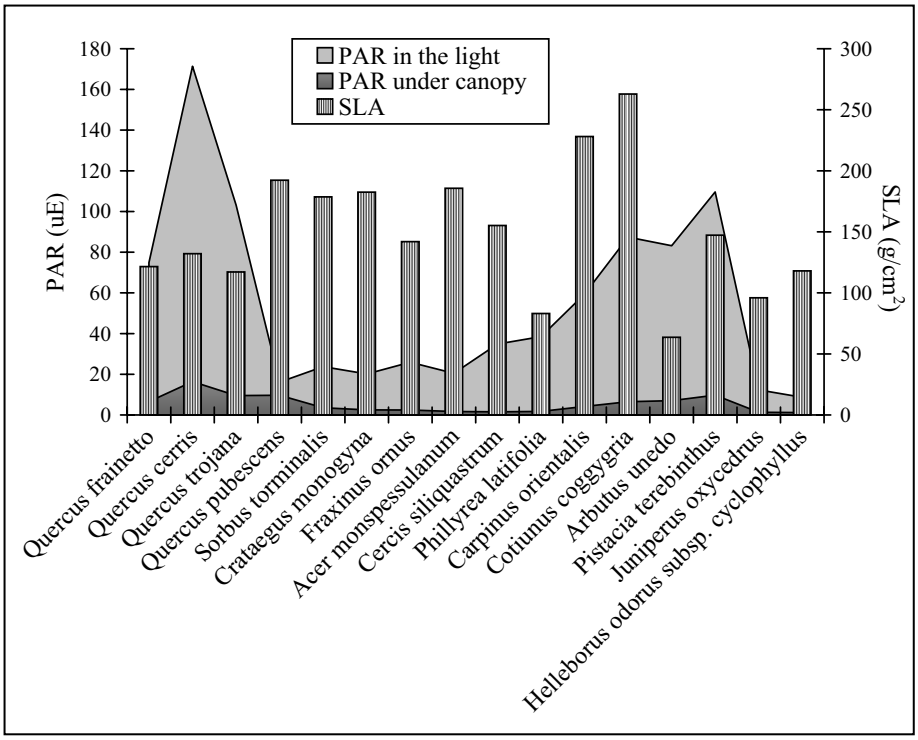

Fig. 2. Specific Leaf Area values (g/cm^2) of 16 plant species in grazed sites of the study area and Photosynthetically Active Radiation values (uE) in light and under canopy.

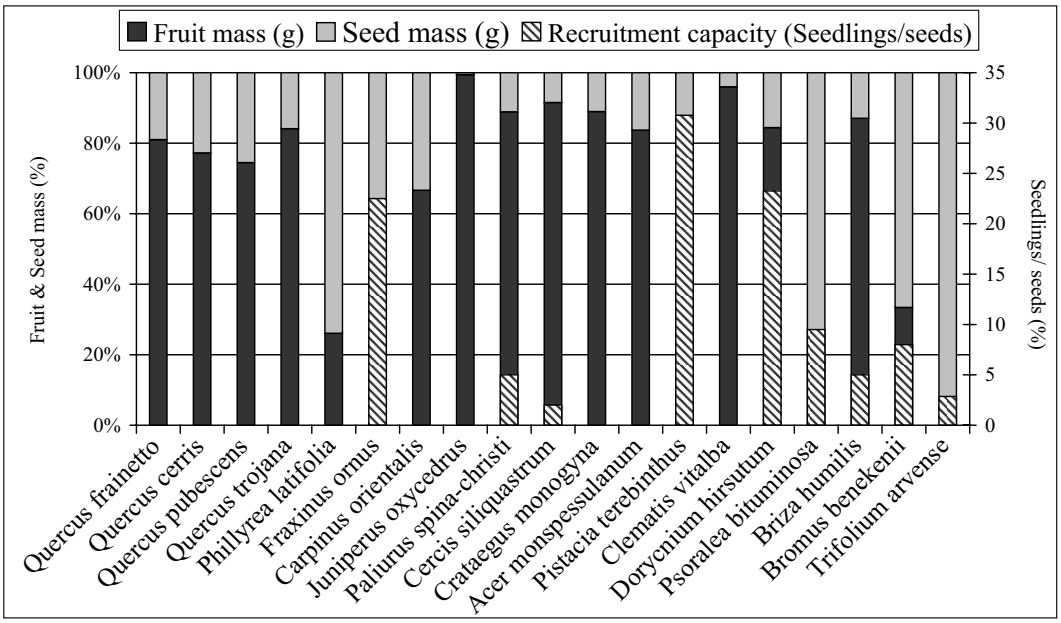

Fig. 3. Fruit mass (%), seed mass (%) and seedling recruitment capacity (%) for 19 plant taxa in overgrazed sites of the study area.

plant communities' structure as it affects the potential emergence and growth of certain plant taxa. PAR has been related with the vertical structure and position of the leaves for plant taxa such as *Fraxinus ornus*, as well as with seedlings relative growth rate of certain species, in order to explore their capacity to rehabilitate damaged environments.

Management practices or disturbances, such as overgrazing, affect dramatically the variation along gradients of plant communities. The mixed oak forests of the study area represent such a case. Overgrazed forests inside the fence have developed a critically different structure and floristic composition compared to the forests outside fences. Our research sphere is crucial to be considered as a developing study of creating plant and ecological traits predictive patterns of species competition under different grazing regimes. Therefore, SLA measurements are continued in the other two grazing regimes of our study area.

Seed mass, dispersal mode, dispersule shape and size and resprouting capacity are described as satisfactory indicative traits of plant species response to disturbance (Chapin & al. 1993; Díaz & al. 1999; Weiher & al. 1999; Lavorel 2002; Lavorel & Garnier 2002). Although our germination results indicate a potential pattern, they do not yet enable us to reach in a definite conclusion correlating clearly fruit and seed mass to seedlings recruitment under stable conditions; the experiments are going to be repeated and also to be continued for the other grazing regimes.

Further investigations are in progress, as variation is observed in disturbance regimes, floristic composition, vegetative and regenerative attributes of plant species in the under study sub-Mediterranean forest ecosystems. Data collection will continue in non-grazed woodlands to compare the derived functional response groups in grazed and non-grazed conditions. The creation of a database and the determination of plant functional types for these ecosystems remain important.

Acknowledgements. We thank Sandy Coles (M.Sc.) for linguistic revision and improvement of the manuscript. Thanks are also due to Mr Georgios Tassos for his valuable help and accommodation facilities provided for carrying this research within the study area of the "Environmental Park of Bourazani".

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Comparative analysis of vegetative methods of ginkgo reproduction

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Abstract. The research includes four methods of auto-vegetative reproduction of ginkgo: by softwood, hardwood, root and callus cuttings. The initial material for cuttings was three trees of different ages and the roots of one-year-old seedlings produced from the seeds of the same parent trees. The results obtained by the method of callus cuttings show essential differences in the rooting percentage, as well as in the development of the root system and the shoot. Further research should attempt the reproduction by callus cuttings under artificial mist, because in addition to other advantages, this reproduction method would have an economic justification, taking into account the multiplication factor.

Key words: cuttings, ginkgo, reproduction

Introduction

Trees like ginkgo are efficient sinks of pollutants, which justifies ginkgo cultivation on green spaces of urban coenoses (Vukićević 1996; Jovanović 2000). The number of aged trees is high, both in the world and in our country. On the territory of Serbia, there are 55 registered protected trees, which are more than 100 years old (Vilotić 2004). By all parameters, ginkgo belongs to the class of the most resistant and the most beautiful species, wherefore it is cultivated on green spaces throughout the world. Also there is an increasing trend of ginkgo plantation growing for the production of foliage for pharmaceutical industry. In China 5000 ha have been under plantations for leaf production since 1990, but also for the production of seeds (Cohen & Bartlik 1998).

Trees are generally thought to be propagated exclusively by seeds or by grafting, with the exception of poplars and willows, in which hardwood cuttings are standard methods of reproduction (Grbić 2000). According to the available references, ginkgo is in the group of trees, which are reproduced by seeds in nurseries. For this reason, the aim of this study is to analyse, by parallel experiments, the possibility of ginkgo reproduction by four types of cuttings, as well as to investigate, by comparative analysis, the effect of the age of parent trees on rooting capacity.

Material and methods

The analysed softwood and hardwood cuttings originate from three trees of different ages (two very old trees, which are protected, and one younger tree). Root cuttings are taken from the roots of one-year-old seedlings produced from the seeds of the same parent trees. Callus cuttings are taken from callus shoots developed on root cuttings.

Softwood cuttings are formed in a usual way. After the cuttings are collected, they are treated with Rhizopon AA (powdery indole-butyric acid) in concentration 1 %. The following data are recorded: rooting percentage, number and length of primary roots, number of shoots, diameter and length of the longest shoot.

Hardwood cuttings are taken during winter, cut up into lengths of 10 cm, treated with the solution of fungicide (Benomil) and then stored till April. In mid April, they are inserted in the rooting bed without treating with phytohormones. The following parameters are measured in autumn: rooting percentage, number and length of primary roots, length and diameter of the longest shoot, and the number of formed shoots.

Root cuttings are taken during the optimal period, in December. In April, the experiment is established by insertion of cuttings in the tubs, vertically with the proximal part 1 cm outside the compost. A polythene

foil is placed 20 cm above the cuttings. The development of cuttings is monitored every ten days (10th day, 20th day, 30th day and 60th day) and the changes on the proximal cut are recorded. The above-ground part is evaluated based on the scale Grbić (1992).

The percentage of active cuttings is calculated as the percentage i.e. the number of cuttings with the grade above nought. The number of shoots is recorded at the moment when most of the shoots are ready for cutting and application in the experiment with callus cuttings.

Callus cuttings are taken in June from the shoots of root cuttings longer than 10 cm. They are inserted in the compost mixture of peat and sand, ratio 4:1 and after that covered with a polythene foil. Before insertion, the cuttings are treated with phytohormone Rhizopon AA in concentration 1 %, pursuant to the usual procedure applied to softwood cuttings. The following data are recorded: rooting percentage, number of formed primary roots, length of primary roots, and length of the longest shoot.

Results and discussion

The analysis of the percentage of rooted cuttings shows the differences among the trees. The old-age trees proved to be the trees that can be used as the initial material for this method of vegetative propagation. At the end of the growing season, the rooted cuttings of all trees were recorded, as well as the appearance of callusing without the growth of the above-ground part, and the growth of the above-ground part without rooting. The percentage of rooted cuttings ranged from 40 to 61, if all trees and repetitions are taken into account. The absolute range of rooting percentage at the level of the experiment was 21. The comparison of quantitative and qualitative parameters of rooted cuttings shows the distinction of the old trees. The differences between the percentages of rooted cuttings shown by the results of the analysis of variance confirm the high significance level between the trees, and there are no significant differences between the repetitions. By the Duncan's multiple range tests, all mean values of rooting percentage are classified into 3 homogeneous groups. There is no overlapping between the homogeneous groups. The group with a significantly higher rooting percentage consists of the cuttings of the old ginkgo tree No. 3.

The number of formed primary roots on the rooted cuttings varied from 1 (in all trees) to 5 in the old tree No. 2. The mean number of the formed primary roots at the level of the experiment was 2. The analysis of variance shows that there are no significant differences between trees and repetitions. By the Duncan's multiple range test, all mean values are classified into one and the same homogeneous group, which confirms that there are no significant differences in the number of formed primary roots between the younger tree and the old trees.

The absolute length of primary roots for all trees and repetitions varied from 5.0 cm in ginkgo 2 to 32.4 cm in ginkgo 1. The mean values of the length of primary roots ranged from 14.9 cm for tree 2 to 16.5 cm for tree 3. The results of the analysis of variance show the highest significance level of the differences between the trees. There are no significant differences between the repetitions. The multiple range test classifies all the mean values of the length of primary roots into the same homogeneous group.

The number of formed shoots on softwood cuttings varied from 0 in all trees to 4 in ginkgo 2. The mean number of formed shoots at the level of the experiment was 1.7. The results of the analysis of variance show that the differences between the trees and repetitions are not significant. Duncan's multiple range test classifies all the mean values of the number of formed shoots into one and the same homogeneous group.

Mean value of the diameter of longest shoot at the level of the experiment was 4.32 mm. The maximal shoot diameter of 5.8 mm was measured for the cuttings of the old ginkgo 3, and the minimal diameter of 3.0 mm was measured for the cuttings of ginkgo 2. The analysis of variance for the diameter of the longest shoot shows minimally significant differences between the trees and insignificant differences between the repetitions. Duncan's multiple range test classifies all the mean values of diameter of the longest shoot into the same homogeneous group.

The absolute values of shoot length are: maximum 14.5 cm in the cuttings from ginkgo 3 and minimum 5 cm in the cuttings from trees 1 and 2. The mean value of shoot length for all trees and repetitions was 8.7 cm. The analysis of variance shows that the differences in shoot length between the trees are at a relatively high level. There are no significant differences between repetitions. Duncan's multiple range test classifies the values of shoot length into one homogeneous group.

The correlation analysis of six analysed parameters shows that positive correlation is 40.83 % and negative correlation is 35.83 %, and there was no correlation in 23.34 % of cases. The comparative analysis of correlation coefficients proves the correlation with the high level of significance between the length of primary roots and the length of the longest shoot, and also between the length of primary roots and the diameter of the longest shoot. The number of primary roots affects the length of roots variably, depending on the trees. The high negative significance levels of correlations are confirmed by the correlations of the percentage of rooted cuttings and the number and length of primary roots.

Based on the comparative analysis of the mean value of the study parameters of the trees which were the initial material for softwood cuttings, it can be concluded that the reproduction by softwood cuttings produced positive results regardless of the age of parent trees.

The percentage of rooted cuttings is determined by the assessment of the quality and quantity of changes on the cuttings. At the end of the growing season, rooted cuttings were recorded for all trees, which took part in the experiment. The percentage of rooted cuttings ranged from 63 to 87 if all trees and repetitions are taken into account, with the absolute range of 24. The differences between the percentages of rooted cuttings presented by the results of the analysis of variance confirm the highest significance level between the trees. There are no significant differences between the repetitions. The multiple range test classifies the percentages of rooted hardwood cuttings into two homogeneous groups. There is no overlapping. The group with significantly higher rooting percentage consists of the cuttings of the older ginkgo 3.

The number of formed primary roots on rooted cuttings after the end of the growing season varied from 3 for the trees 1 and 3 to 15 for ginkgo 2. The mean number of formed roots on all trees was 7.3; with minimal mean value 4.5 for the younger tree 1 and the maximal 8.4 for the old tree 3. The result of the analysis of variance confirms the lower significance level of the differences between the trees and the insignificant differences between the repetitions. The multiple range test classifies the number of formed roots in the same homogeneous group.

The absolute amount of root length at the level of the experiment ranged from 5.5 cm for ginkgo 3 to 41.5 cm for the same tree. The mean value of root length ranged from 7.33 cm for ginkgo 3 to 8.31 cm for ginkgo 2. The analysis of variance shows that there are no significant differences between trees and repetitions. The multiple range test classifies the lengths of formed primary roots into two homogeneous groups. The groups overlap. The group with significantly longer primary roots consists of the cuttings of the older tree 2 and the younger tree 1.

The number of formed shoots on hardwood cuttings after the end of the growing season varied from 0 for all trees to 3 for the younger tree. The mean number of formed shoots for all trees was 1.26; with the minimal mean value 1.0 for ginkgo 3 and the maximal 1.6 for ginkgo 1. The result of the analysis of variance confirms the lower significance level of the differences between the trees and insignificant differences between the repetitions. The multiple range test classifies the numbers of formed shoots in two homogeneous groups which overlap. The group with significantly higher values consists of the cuttings of the younger tree.

The maximal shoot diameter of 4.8 mm and the minimal diameter of 3.1 mm were measured for the cuttings from the same younger tree. For all trees that participated in the experiment, the differences in the diameter of the longest shoot are at the highest significance level, which is confirmed by the results of the analysis of variance. The multiple range test classifies the diameters of the longest shoot into one homogeneous group.

The extreme values for the shoot length are: maximum 15.8 cm for ginkgo 3 and minimum 5.0 cm for ginkgo 1. The mean value of shoot length at the level of the experiment is 10.3 cm. The analysis of variance confirms that there are no significant differences in the height of the above-ground parts between the trees and repetitions. The multiple range test classifies shoot lengths into one homogeneous group.

It was also recorded that in all trees there are cases of root formation, but there are no cases of bud growing, i.e. the above-ground part remains undeveloped.

Correlation analysis covers the following parameters: percentage of rooted cuttings, root length, number of roots, diameter of the longest shoot, length of the longest shoot and number of formed shoots for all analysed trees. There are altogether 39.88 % negative correlations and 60.12 % positive correlations. The result was not obtained in six cases, i.e. there was no correlation. It should be emphasised that the maximal positive correlation was between root length and the diameter of the longest shoot.

The comparative analysis of the study parameters regarding the age of the trees used as the initial material for cuttings shows that the age of parent trees does not affect the quality of rooted cuttings and the rooting percentage.

The percentage of active cuttings was determined by the qualitative evaluation of cuttings, and by the assessment of quantitative changes on the cuttings. There were no active cuttings on the 10th day of experiment. On the 20th day of the experiment, 30 % of the total inserted cuttings were active cuttings. The analysis of variance shows the highest significance level between the trees; there are no significant differences between the repetitions. Duncan's multiple range test classifies the percentages of active cuttings on the 20th day in two homogeneous groups. There was an overlapping between the groups. The group with a significantly higher percentage of active cuttings consists of the cuttings of ginkgo 3 and the younger tree 1. On the 30th day of the experiment, 33 % of the total inserted cuttings were active cuttings. Mean value of the percentage of active cuttings is higher by 3 compared to the 20th day. In this term, the younger tree has higher percentage. The analysis of variance confirms the highest significance level between the trees; there are no significant differences between the repetitions, just as on the 20th day. Duncan's multiple range test classifies the percentages of active cuttings on the 30th day in three homogeneous groups without overlapping. The group with a significantly higher percentage of active cuttings consists of the cuttings of the younger tree 1. On the 60th day of the experiment, all the trees had active cuttings. The percentage of active cuttings ranged from 20 to 58 at the level of the experiment. Mean value of the percentage of active cuttings was 52. This percentage is higher by 19 compared to the 30th day. On the 60th day, the disproportion between the degree of development and the number of active cuttings was lower. The results of the analysis of variance on the 60th day were identical as in the previous two terms: 20th day and 30th day. The Duncan's multiple range test classifies the percentages of active cuttings on the 60th day in three homogeneous groups without overlapping. As in the previous two terms, the group with a significantly higher percentage of active cuttings consists of the cuttings of the younger tree 1.

The number of formed shoots per cutting after two months of the experiment varied from 0 for all trees to 3 for trees 1 and 3. The mean number of formed shoots for all trees was 1.7. On the 60th day of experiment, the most represented cuttings were those with one (30.7 %) and two formed shoots (22.5 %). The result of the analysis of variance confirms the lower significance level of the differences between trees and insignificant differences between the repetitions. Duncan's multiple range test classifies the numbers of formed shoots into one homogeneous group.

The experiment with root cuttings points to the potential reproduction by this type of cuttings during the summer period, and also to the formation of callus and callus shoots, which can increase highly the multiplication factor.

The percentage of rooted cuttings ranged from 21 for ginkgo 3 to 41 for another old ginkgo 2 if all trees and repetitions are taken into account. The differences between the percentages of rooted cuttings according to the analysis of variance confirm the highest significance levels between the trees, and there are no significant differences between the repetitions. Mean values are distributed in 3 groups, without overlapping. The group with significantly high percentages of rooted cuttings contains the cuttings of ginkgo 2.

At the end of the growing season, the number of formed roots on rooted cuttings varied from 1 for all trees to 22 for ginkgo 2. The mean number of formed roots for all trees and repetitions was 6.6. The result of the analysis of variance confirms the highest significance levels in the number of formed roots between the trees; the differences between the repetitions are insignificant. Mean values are distributed in the same homogeneous group.

The absolute value of root length for all trees and repetitions ranged from 0 cm for ginkgo 3 to 6.7 cm for ginkgo 2. The mean value of root length per trees ranged from 2.09 cm (ginkgo 1) to 6.5 cm (ginkgo 2). The analysis of variance shows the highest significance level of the differences between the trees (0.000000), and there are no significant differences between the repetitions (0.966690). The mean values are distributed in two groups which overlap. The group with significantly longer roots contains the cuttings of ginkgo 2.

The extreme values of shoot length at the level of the experiment are: maximum 8.9 cm for ginkgo 2 and minimum 0.5 cm for another old ginkgo 3. The mean value of shoot length for all trees is 4.7 cm. If the trees are compared by the mean values of shoot length the decreasing order is as follows: ginkgo 2 (7.8 cm), gink-

go 3 (3.3 cm) and ginkgo 1 (2.95 cm). It was also recorded that in all trees there are cases of callus formation or root formation, but there are no cases of bud sprouting, i.e. the above-ground part remains undeveloped. Also, ginkgo 3 forms the above-ground part and sprouts, but there is no root formation or there is only callus formation. The analysis of variance shows that the differences in the height of above-ground part are at the highest significance level between the trees, and there are no significant differences between the repetitions. Duncan's multiple range test classifies the mean values in two homogeneous groups which overlap. The group with significantly longer roots contains the cuttings of ginkgo 2.

Correlation analysis covers the following four parameters: percentage of rooted cuttings, number of roots, length of primary roots, and shoot length per tree species and for all analysed trees. There are altogether 30.96 % negative correlations and 69.04 % positive correlations. The comparative analysis of the correlation coefficients shows the proportion of correlations, with a high significance level between the numbers of roots and shoot length, and also between root length and the length of the above-ground part. The correlation analysis shows that the root number and root length significantly affect the development of the above-ground part, but at the different levels depending on the tree.

Based on the study results, it can be concluded that there are essential differences in rooting percentage, but also in the development of root system and the above-ground part, so this method of reproduction produced the poorest results in the comparative experiments with four types of cuttings.

Conclusions

Of the four tested methods of ginkgo auto-vegetative reproduction, only callus cuttings did not produce good results. The failure of rooting of callus cuttings is not necessarily the final result. In this sense, the rooting under artificial mist should be attempted, with the heating of the compost, because this favours the root development. Further experiments with phytohormones could also produce good results.

Root cuttings, as opposed to stem cuttings, have restricted utilisation in clonal reproduction. This method requires the lifting of plants or root parts during the plant resting stage, which is considerably more difficult than the collection of stem cuttings. Still, the propagation by root cuttings is justified because of the formation of callus tissue and callus shoots.

The results of the experiment with softwood cuttings show the capacity of ginkgo reproduction by this method of auto-vegetative reproduction. By the analysis of study parameters of rooted cuttings from the trees used as initial material, it can be concluded that the age of parent trees does not affect the rooted cutting quality and rooting percentage. The evaluation of quality and quantity of the changes on softwood cuttings confirms that both old ginkgo parent trees can be used as the initial material for this method of vegetative reproduction.

As the reproduction by hardwood cuttings has many advantages compared to other methods of reproduction, the results obtained in the parallel experiments with this type of cuttings are especially significant. The comparative analysis of the study parameters regarding the age of the trees used as the initial material for cuttings shows that the age of parent trees does not affect the quality of rooted cuttings and the rooting percentage. The results of the experiment show that this method of vegetative reproduction can be recommended as very successful for ginkgo trees of great age.

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A quantitative approach to the phytogeography of the mire species of the Italian flora

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Abstract. An attempt to survey the chorological structure in the mire flora of Italy is presented. A set of 151 species of vascular plants recorded in the Italian mire sites has been selected on the basis of available references in literature. Distributional data and chorotype-attributes for this selection of Italian mire species have been processed, using a geostatistical approach. The obtained core-areas for distinct chorotypes might be interpreted as sources for reiterated events of persistence and later spread of different stocks of mire species under changing climatic scenarios. On this basis the analysis provides evidence for mechanisms and pathways of colonisation processes by palustrine species and for the genesis of the present-day mire communities.

Key words: chorology, Italian mire species, quantitative phytogeography

Introduction

South of the Alpine region, mires are scattered as relic isolates throughout the Italian peninsula. Their flora exhibits different degrees of impoverishment and decreases along the N–S geographical gradient. The study aims to detect patterns of distribution of the Italian mire species in Europe in order to provide some outlines to the reconstruction of a hypothetical model of their colonisation history in the Italian peninsula.

Material and methods

A set of 151 species of vascular plants recorded in the Italian mire sites has been selected on the basis of their indicator values of mire habitats according to sources in literature (Pott 1995; Ellenberg 1996). Presence/absence data *per* administrative district in Europe (Meusel & al. 1978) and chorotype-attributes for this selection of mire species of the Italian flora have been processed, using geostatistical methods ("kriging"). These interpolation techniques quantify the spatial structure of the data creating surfaces for points with the same values.

Ranges are described according to the presence/absence of the selected species in Administrative Districts of Europe (Countries), being more detailed individual ranges for each species not yet available in literature sources.

Results

Species richness

The isolines (Fig. 1) indicate geographical patterns of the floristical similarity between Italian mires and mires in the other European regions solely on the basis of the Italian stock of mire species. Presence/absence data *per* administrative district in Europe show the bulk of highest concentration located in SW Europe. The gradient of similarity follows a track protruding towards North (Scandinavian Peninsula). More significant differences in the floristic composition of mires is to be detected with the easternmost and westernmost territories of Europe. The Pyrenean Mountains and the Dinarides have apparently been efficient barriers for floral exchanges between Italy and surrounding territories while the Alps seem not to be acting in the same way.

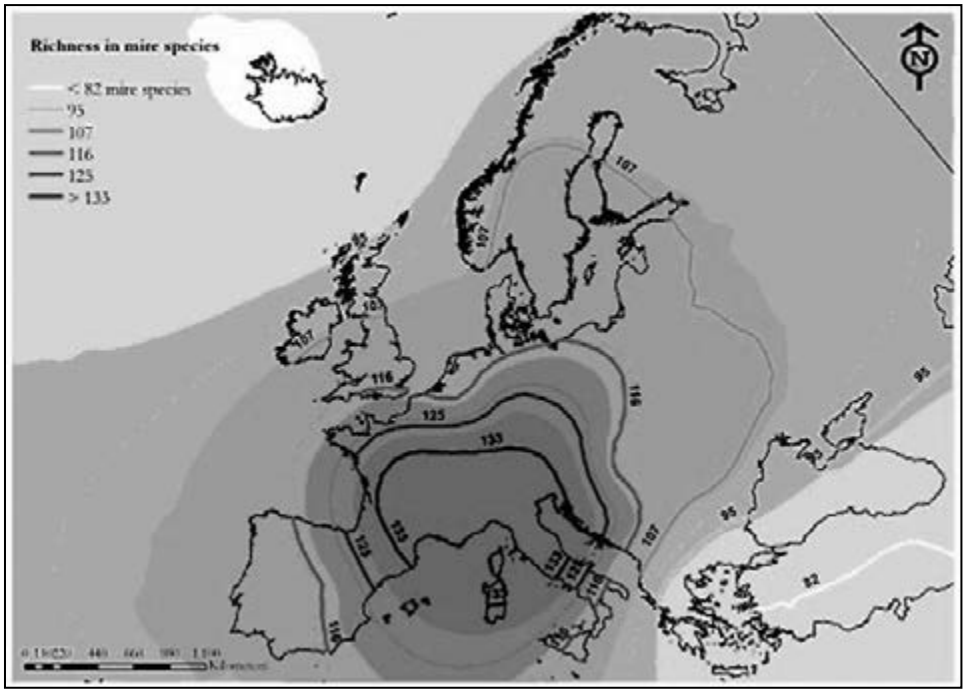

Fig. 1. Geographic variations in the species richness of mire species of the Italian set in Europe.

Chorotype analysis

Chorotypes according to Oberdorfer (1990) have been clustered in fewer more general categories (Fig. 2) as follows:

Arctic–Alpine (8 species): (some representatives) *Bartsia alpina*, *Carex microglochin*, *Juncus arcticus*, *J. filiformis*, *J. triglumis*, *Tofieldia pusilla*.

Atlantic (4 species): *Scutellaria minor*, *Potamogeton polygonifolius*, *Cirsium dissectum*, *Allium ericetorum*.

Circumboreal (26 species): (some representatives) *Swertia perennis*, *Scheuchzeria palustris*, *Parnassia palustris*, *Menyanthes trifoliata*, *Liparis loeselii*, *Epilobium palustre*.

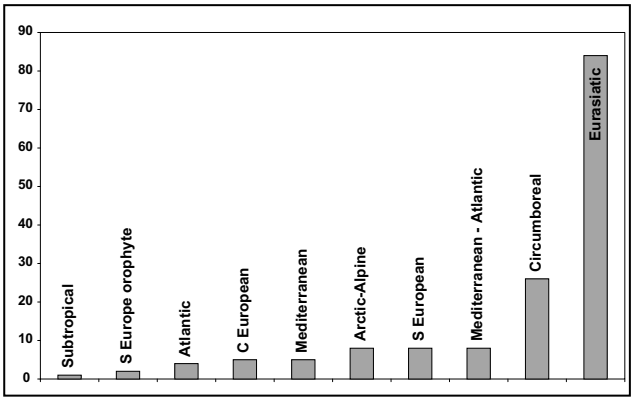

Fig. 2. Chorological repartition of the set of selected species of Italian mire flora.

Eurasiatic (84 species): (some representatives) *Tofieldia calyculata*, *Pinguicula alpina*, *Gentiana pneumonanthe*, *Eriophorum latifolium*, *Carex echinata*, *C. davalliana*, *C. cespitosa*, *C. appropinquata*.

Subtropical (1 species): *Caldesia parnassifolia*.

Central European (5 species): (some representatives) *Myosotis nemorosa*, *Laserpitium prutenicum*, *Gladiolus palustris*.

South European (8 species): (some representatives) *Succisella inflexa*, *Juncus subnodulosus*, *J. acutiflorus*, *Carex acutiformis*.

Mediterranean (5 species): (some representatives) *Dorycnium rectum*, *Dactylorhiza saccifera*.

Mediterranean–Atlantic (8 species): (some representatives) *Spiranthes aestivalis*, *Hydrocotyle vulgaris*, *Carex punctata*, *Anagallis tenella*.

Mediterranean Orophyte (2 species): *Pinguicula leptoceras*, *Carex frigida*.

The same interpolation method ("kriging") has been used in the study of the spatial pattern of variation in richness of mire species belonging to distinct chorotypes. The distribution patterns of each chorotype is represented using lines (isocores) which connect points with the same number of species belonging to the same chorotype, from higher values (dark color) to lower ones (light color).

Herewith the core-areas of 3 ecologically contrasting stocks (Mediterranean, Arctic–Alpine, and Atlantic–Mediterranean) are outlined.

MEDITERRANEAN CHOROTYPE (Fig. 3). Ecological implications embedded in the current definitions of chorotypes are deleted by the common prevailing hydrophytic demand of the species. This is particularly significant in the case of the so-called Mediterranean stock. This suggests that only thermicity (or the length of the growing season) might be crucial. On this basis the core-area of the Italian mire species belonging to the Mediterranean chorotype in Europe seems to encompass refugial sites for these species under previous colder climatic conditions, when they might have been restricted to the southernmost district of SW Italy. The species with core area in the S Ionian regions (Greece, Turkey, S Italy) might be the ones with the highest affinities for the mid-Holocene climatic condition.

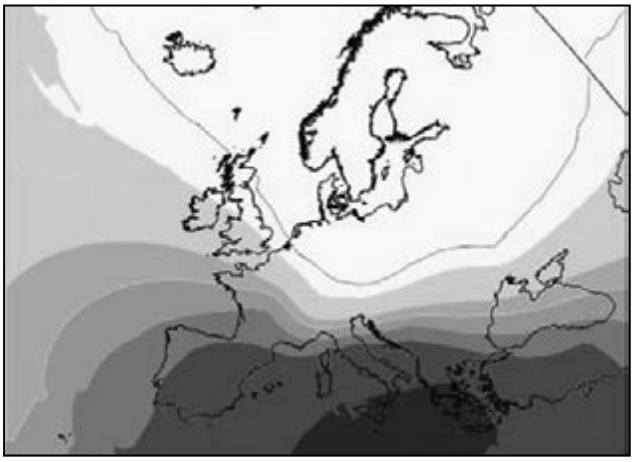

Fig. 3. Core area of Mediterranean Chorotype.

ARCTIC–ALPINE CHOROTYPE (Fig. 4). This pattern of isolines emphasises the area where the most cold-tolerant mire species of the Italian flora are scattered. It seems to be the result of a modern range reassessment of a previous "northern" species stock which survived during the last pleniglacial (Fig. 5) in periglacial areas along the Atlantic coast down to NW Italy. In postglacial times this stock gave origin to the fragmented Arctic–Alpine geoelement starting from the refugial areas of the N Atlantic *nunatakker*, along the coasts of Aquitania and reaching the areas peripheral to the former alpine ice-sheet.

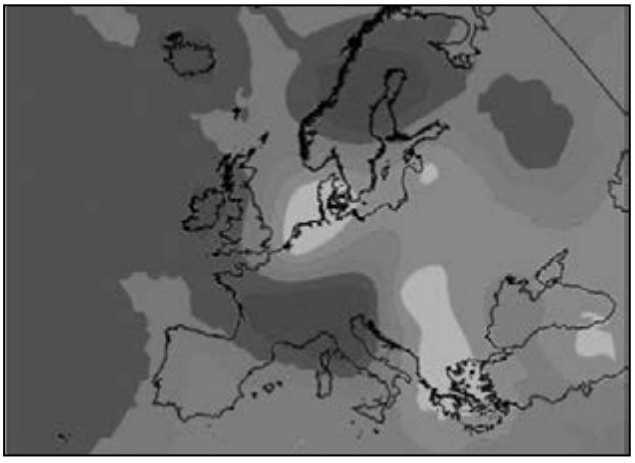

Fig. 4. Core area of Arctic–Alpine Chorotype.

Fig. 5. Paleoenvironmental scenario in Europe at the last glacial maximum (18 000 years B.P.) (redrawn from Walter & Straka 1970).

MEDITERRANEAN–ATLANTIC CHOROTYPE (Fig. 6). This pattern has its core area in the territories of the Iberian Peninsula and Southern France, extending to Italy only at the latitude of the middle Tyrrhenian district. These territories have been less affected by the unfavourable climatic conditions of the last pleniglacial than the N Atlantic districts and therefore might suggest to have acted as refugial areas for fen species during that time.

Discussion

The core-areas for distinct chorotypes obtained with this method emphasise geographical patterns which allow inferences about events of spread, retreat and relictuality consistent with major events of environmental changes in Europe during Late Quaternary (Fig. 5).

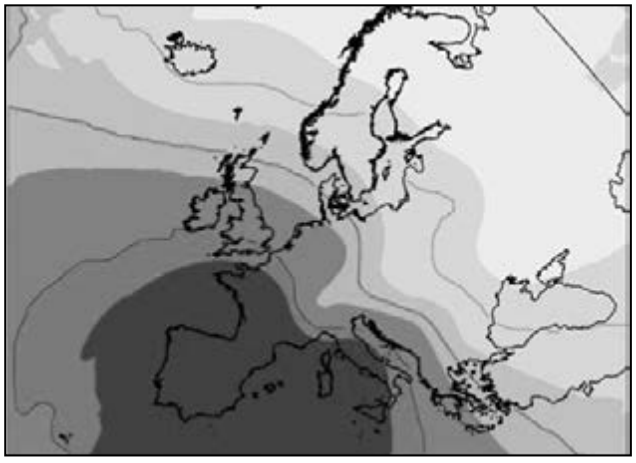

Fig. 6. Core area of Mediterranean–Atlantic Chorotype.

On this basis the analysis stresses the "autochtony" in Italy of the arctic–alpine stock, whose disjunction is likely to have originated by the postglacial disappearance of stepping stones at west, which might have occurred first after the disappearance of the European ice-sheet.

Moreover the smoothing north–south gradient reveals a track of continuity of suitable sites for mire species and still active species flux between Italy and Northern Europe.

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SCIENTIFIC AREA F

ETHNOBOTANY AND PHYTOCHEMISTRY

Ethnomedicinal uses of the wild vascular plants from European Turkey (Turkish Thrace)

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Abstract. 138 wild vascular plants used for medicinal purpose, belonging to 71 genera of 49 families, were recorded in the European Turkey (Çatalca, Kırklareli, Tekirdağ provinces). The list was developed alphabetically by botanical name (family), followed by local name, part used and ethnomedicinal uses. It was found that 18 taxa are commonly used for different and several remedies by local people. On the other hand 10 plant species have been used very locally and their usage has been recorded for the first time in Turkey.

Key words: ethnomedicinal uses, European Turkey, food plants, Turkey, wild plants

Introduction

European Turkey (Turkish Thrace) occupies a small part of Europe and Turkey, and has a surface area 23 500 km^2. It is situated north of the Sea of Marmara, which is connected to the Black Sea and Aegean Sea via the Bosphorus and Dardanelles, respectively (Fig. 1); together the sea and straits separate Europe from Asia. In comparison with Turkey's general topography, Turkish Thrace generally has low elevations.

In the NE of the region, the Istranca Mountain range (Yıldız Mts) extends into SE Bulgaria; its

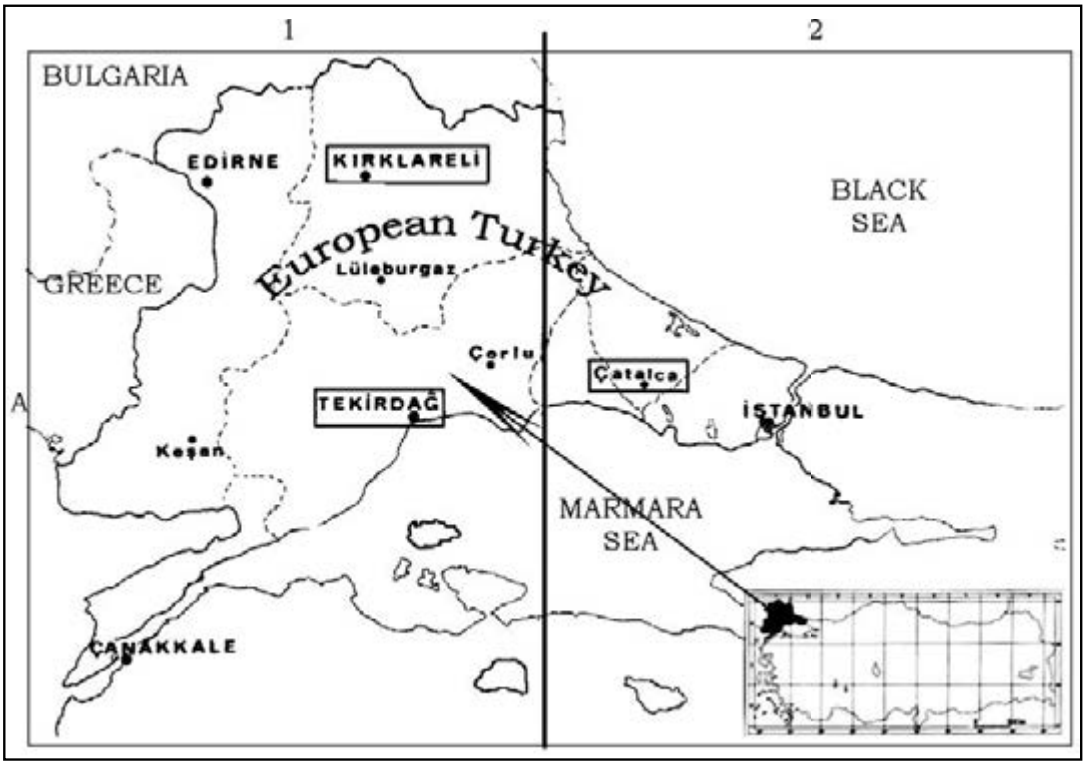

Fig. 1. Map of European Turkey, investigated areas marked with outlines.

highest point is the Mahya Dağ (1035 m). This range borders the Black Sea and represents a low continuation of Anatolia's northern Black Sea mountains. The range is largely composed of schists and is covered with forest vegetation, the humid northern slopes supporting *Fagus orientalis* forest and *Rhododendron ponticum* schrub.

In the SW of the region, the Ganos Mts (Tekir Mts) and Koru Mt (which continue southwards into the Gelibolu Peninsula) have typical Eastern Mediterranean landscape with *Pinus brutia* forest and macchia vegetation. The greater part of it is occupied by gently undulating lowland of less than 200 m elevation, drained by the Ergene (a tributary of Meriç) and almost entirely cultivated.

Floristic composition and endemism

The flora of European Turkey has attracted the attention of numerous botanists since the beginning of the 19th century. Clarke, Formánek, Aznavour and Grisebach are main contributors, whilst many additional species were collected by army personnel (Bulgarian, Russian and British) during the wars 1912–1923.

From 1950s onwards Turkish botanists have collected many specimens and made numerous records, of which the most valuable collections and records were perhaps made by A. Baytop and her colleagues from ISTE Herbarium.

Today ISTE houses specimens of nearly 1960 taxa collected from European Turkey representing *ca*. 80 % of its vascular flora. The other herbarium keeping a large collection of specimens from this region is EDTU (Thrace University Herbarium, in Edirne).

The number of species recorded in the flora of European Turkey (Hayek 1924–1933; Turill 1924; Webb 1966), *Flora of Turkey and the East Aegean Islands* with its supplements (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000) and several other published papers (Özhatay 2001) together with the specimens kept in ISTE and EDTU is about 2350; including subspecies and varieties – about 2550 taxa (Özhatay 2001).

It is not comparable, of course, with the richer parts of Asiatic Turkey, but from so small area, where there is scarcely any land above 1000 m, where endemics are few and where the native vegetation has been in large part destroyed, a smaller total might have been expected. The explanation lies in the wide range of climate and the high diversity of habitats found within the area. European Turkey is in consequence the meeting-point of several floristic elements, notably the Mediterranean element, the Balkan–Central European element, the Sarmatian element and the Euxine element.

G.V. Aznavour (1861–1920)

Prof. Dr. Eduard Formánek (1845–1900)

Over 300 species that are regarded as nationally rare or threatened occur within the area. Of these some 50 species are more abundant here than elsewhere on earth. Several habitats within the area are of high national or even international importance to nature conservation, most notably sand dunes on the Black Sea shoreline, flooded 'longoz' forests, humid Thracian–Euxinian forests on the northern slopes of the Istranca Mts, coastal lagoons, wetlands, fragments of heathland and dry calcareous grasslands exhibiting characteristics typical of the middle European steppes.

Material and methods

This paper is based on data obtained from the following thesises and projects:

- Master Thesises: Akalın, E., Medicinal and edible wild plants of Tekirdağ province in European Turkey, 1993 (Supervisor: Prof. K. Alpınar); Ecevit, G., An ethnobotanical study in Çatalca, Istanbul in European Turkey, 2003 (Supervisor Prof. Dr. N. Özhatay).
- Project supported by IU. Research Foundation, Kültür, Ş., An ethnobotanical investigation in Kırklareli (European Turkey), 2004.

Data collected in the course of interviews were taken from local healers, farmers, and people, local open markets who shared with us their knowledge about use of plants in folk medicine. About 850 informants were interviewed using standardised questionaries. The plant specimens were collected and identified following *Flora of Turkey and the East Eagean Islands*. Voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul (ISTE).

Results and discussion

The result of the medicinal uses of the plants occurring in European Turkey is presented in Table 1.

Information on 138 wild plant species belonging to 49 families used by local people for treating different disorders was gathered. Among them 21 species are also used as food. The families represented by the highest number of species in the study are as follows: *Rosaceae* (22 taxa), *Lamiaceae* (21 taxa) and *Asteraceae* (17 taxa).

The higher frequency of the use of green parts of plants in preparation of herbal remedies is largely due to the fact that they can be collected easily and they are readily available. Roots, bulbs, tubers and particularly seeds and barks were used to a much smaller extent.

Local people choose to use herbal remedies mainly for the treatment of gastrointestinal complaints such as stomachache, abdominal pain, hemorrhoids and diarrhea. On the other hand, skin problems such as abscess, eczema, wounds fissures, warts and burns are also the most frequent complaints treated with herbal remedies.

Infusion and especially decoction are generally chosen for internal administration. For external application either fresh or dried material can be applied directly or after being cooked in a poultice form.

Most of the informants stated that they have learned usage of these medicinal plants from their parents and elderly relatives. The herb is recommended to the patient by another patient who had previous experience with it.

We determined that same plants are used for several disorders in many villages. It is more common to use only one species in each remedy.

According to the result of survey 138 wild vascular plants are being used for herbal remedies. The most well known and common species are: *Cotinus coggyria*, *Hypericum perforatum*, *Rosa canina*, *Sambucus ebulus*, *S. nigra*, *Malva sylvestris*, *Matricaria chamomilla* var. *recutita* and *Achillea millefolium*. Usage of the following species could be very dangerous in over dosage: *Datura stramonium*, *Euphorbia nicaeensis* and *Arum orientale*.

Plants used for medicinal treatment in the European part of Turkey are grouped into 3 main groups.

Group I. "Plants used as internally": 35 taxa as analgesic, spasmolitic and antipyretic, 41 taxa as stomachic, 43 taxa for cold, flu and cough, 38 taxa for urinary ailment, 23 taxa for asthma, shortness of breath (dyspnoea), 27 taxa for diabetes, 22 taxa for cardiac and vein disorders, 19 taxa for digestive systems, intestinal disorders, constipation and diarrhea, 23 taxa for hemorrhoids and 45 taxa for other different treatments.

Group II. "Plants used as externally": 39 taxa for skin disorders, 17 taxa as antirheumatic, 10 taxa anti-inflammatory, 7 taxa antifungal and the rest of them (34 taxa) for different disorders.

Group III. "Unusual usage": there are 10 taxa either their usage is for the first time recorded in Turkey or they have different usage – *Clematis vitalba* (for toothache), *Ferulago confusa* (for speechless and depression), *Fraxinus ornus* ssp. *ornus* (for earache), *Rosa canina* (for swelling), *Lithospermum officinale* (for night-time wetting of children), *Ruscus aculeatus* var. *aculeatus* (for night-time wetting of children), *Orchis purpurea* (to remove warts), *Heliotropium europaeum* (for contact dermatitis and itching), *Linum tenuifolium* (for diabetes), *Cirsium creticum* ssp. *creticum* (for mushroom poisoning).

Table 1. List of wild vascular plants used for medicinal purpose in European Turkey.

Botanical names & family	Local names	Parts used	Uses & references	Notes
Acer campestre L. ssp. *campestre* (*Aceraceae*)	Akça ağaç	Cortex	Anti-inflammatory[B] (ext.)	
Achillea millefolium L. ssp. *pannonica* (Scheele) Hayek (*Asteraceae*)	Ayvadana, dişotu, civanperçemi, krannavaz, kurpotu, ronağvaç, sporiş, sporiyiş	Herbs	Antiseptic[C] (int.), wound cleaner[C] (int.), antihemorrhoids[C] (int.), gynecology[C] (int.), uroclepsia[C] (int.), cancer[C] (int.), nephritis[C] (int.), antirheumatic[C] (int.), back ache[C] (int.), cardiopathy[C] (int.), migraine[C] (int.), dizziness[C] (int.), antiemetic[C] (int.), epistaxis[C] (int.), eye strain[C] (int.), menstrual regularity[C] (int.)	
		Leaves	Skin disorders[C] (ext.), antiseptic[C] (ext.), cancer[C] (int.), stomachic[C] (int.), headache[C] (int.) analgesic[C] (int.), haemostatic[C] (ext.)	
		Flowers	Diarrhea[C] (int.), stomachic[C] (int.), vasodilator[C] (int.)	
A. setacea Waldst. & Kit. (*Asteraceae*)	Ayvadani, mayasılotu	Flowers	Analgesic[A] (int.), diarrhea[A] (int.), antihemorrhoids[A] (int.)	
Acinos arvensis (Lam.) Dandy (*Lamiaceae*)	Kayrak çayı	Herbs	Cold[C] (int.), flu[C] (int.), cough[C] (int.)	
Agrimonia eupatoria L. (*Rosaceae*)	Guatr otu	Herbs	Goitre[C] (int.)	
Alcea setosa (Boiss.) Alef. *(Malvaceae)*	Hatmeçiçeği	Flowers	Cough[A] (int.)	
Alnus glutinosa (L.) Gaertn. ssp. *glutinosa* (*Betulaceae*)	Kızıl ağaç	Flowers	Prostate[B] (int.), antihemorrhoids[B] (ext.), skin disorders[B] (ext.)	
Ammi visnaga (L.) Lam. (*Apiaceae*)	Gıvır	Flower rays	Toothache[A] (ext.)	
Anagallis arvensis L. var. *caerulea* (L.) Gouan (*Primulaceae*)	Kantariyeotu	Herbs	Abdominal pain[A] (int.)	
Anthemis chia L. (*Asteraceae*)	Papatya	Capitulum	Stomachic[A] (int.), inflammation[A] (int.), swellings[A] (int.), antipyretic[B] (int.), gynelogical ailments[B] (ext.)	
A. cretica L. ssp. *tenuiloba* (DC.) Grierson (*Asteraceae*)	Papatya	Herbs	Stomachic[C] (ext.)	
A. tinctoria L. var. *tinctoria* (*Asteraceae*)	Sarı papatya	Flowers	Abdominal pain of children[A] (int.)	
		Herbs	Antihemorrhoids[C] (ext.), cough[C] (int.)	
Artemisia absinthium L. (*Asteraceae*)	Acı pelin, acı pelinotu, pelinotu, pelin	Leaves	Stomachic[A] (int.), antidiabetic[A,C] (int.), malaria[C] (int.), antihypertensive[C] (int.), skin disorders[C] (ext.)	
		Herbs	Abortive[C], stomachic[C] (int.), appetizer[C] (int.), blood depurative[C] (int.), antidiabetic[C] (int.), uterus cyst[C] (int.), tuberculosis[C] (int.), antihypertensive[C] (int.)	
A. annua L. (*Asteraceae*)	Acı pelin, pelinotu	Herbs	Stomachic[A] (int.), antidiabetic[A] (int.)	
		Leaves	Tuberculosis[A] (int.)	
Arum italicum Mill. (*Araceae*)	Domuz yandıran, gabarcık, yandıran, yılan kılıcı, yılan yastığı, yılancık	Tubers	Antihemorrhoids with flowers[B] (int.), gynelogical ailments[B] (int.), cancer[B] (int.), skin disorders[B] (int.), stomachic[B] (int.)	
		Flowers	Antihemorrhoids[B] (int.)	
A. maculatum L. (*Araceae*)	Yılan yastığı yılanotu,	Tubers	Antihemorrhoids[C] (int.)	
		Leaves	Inflamed wounds[C] (ext.)	
A. orientale M. Bieb. (*Araceae*)	Yılanyastığı	Tubers	Stomachic[A] (int.)	
		Fruits (mature)	Eczema[A] (int.)	
Ballota nigra L. ssp. *anatolica* P.H. Davis (*Lamiaceae*)	Grip otu	Leaves	Cold[C] (ext.), flu[C] (ext.)	
Bellis perennis L. (*Asteraceae*)	Papatya	Capitulums	Cough[B] (int.)	
Capsella bursa-pastoris (L.) Medik. (*Brassicaceae*)	Çoban çadırı, çoban torbası, kedi tırnağı	Fruits	Tuberculosis[C] (int.), urinary ailment[B] (int.)	
Carduus acanthoides L. ssp. *acanthoides* (*Asteraceae*)	Küçük kenger	Herbs	Antihemorrhoids[C](int.), antidiabetic[C] (int.), kidney stones[C] (int.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
C. nutans L. ssp. *leiophyllus* (Petr.) Stoj. & Stef. (*Asteraceae*)	Çakır dikeni, deve dikeni, eşek dikeni, eşek gengeri	Herbs	Antihemorrhoids[C](int.), antidiabetic[C] (int.), urinary ailment[C](int.)	Food
C. pycnocephalus L. ssp. *albidus* (M. Bieb.) Kazmi (*Asteraceae*)	Eşekdikeni, eşekgengeri	Flowers	Antihemorrhoids[A] (int., ext.)	
Centaurium erythraea Rafn ssp. *erythraea* (*Gentianaceae*)	Kesik otu, kırmızı kantaron, mavi kantaron, pembe Kantıron	Herbs	Stomachic[B] (int.), skin disorders[B] (ext.), asthma[C] (int.), antirheumatic[C] (ext.)	
		Flowers	Stomachic[A] (int.)	
Chelidonium majus L. (*Papaveraceae*)	Mayasılotu, sarılık otu, sultan otu, temraotu, yaraotu	Latex	Skin disorders[C] (ext.), haemostatic[C] (ext.)	
		Herbs	Hepatitis[C] (int.)	
		Leaves	Antirheumatic[C] (ext.)	
Cirsium creticum (Lam.) d'Urv. ssp. *creticum* (*Asteraceae*)	Deve dikeni, yıldız otu	Fruits	Mushroom poisoning[B] (int.)	
Cistus salviifolius L. (*Cistaceae*)	Pamuk otu	Leaves	Skin disorders[C] (ext.)	
Clematis vitalba L. (*Ranunculaceae*)	Akbağ, diş otu, kedi bağırsağı, sarmaşık	Stems (peeled)	Analgesic[B] (ext.)	
Convolvulus arvensis L. (*Convolvulaceae*)	Kaplumbağa gerdanı, Sarmaşıkotu	Herbs	Diuretic[C] (int.)	
Cornus mas L. (*Cornaceae*)	Kızılcık	Cortex	Antifungal[C] (ext.), antipyretic[C] (int.)	Food
		Fruits	Cough[B,C] (int.), diarrhea[B,C] (int.), antifungal[C] (ext.), antipyretic[C] (int.)	
		Seeds	Antidiabetic[B] (int.)	
Corylus avellana L. var. *avellana* (*Corylaceae*)	Fındık, yabani fındık	Cortex	Prostatitis[C] (int.)	
Cotinus coggyria Scop. (*Anacardiaceae*)	Tetere, tetra, tetra otu, tetre	Roots	Skin disorders[B] (ext., int.)	
		Leaves	Stomachic[A,B,C] (int.), antidiabetic[B,C] (int.), antihemorrhoids[B,C] (ext.), eye allergy[B] (ext.), asthma[B,C] (int.), analgesic[B] (int.), skin disorders[A,C] (ext.), goitre[B] (int.), fungal infection[B] (ext.), fracture[C] (ext.), urinary diseases[C] (int.), cardiac diseases[C] (int.), nephritis[C] (int.), cancer[C] (int.), antihypertensive[C] (int.), cough[C] (int.), abdominal pain[C] (int.), numbness of arm[C] (int.), vasodilator[C] (int.), anthrax[C] (ext.), enteritis[C] (int.), mouth wounds[C] (ext.)	
Crataegus monogyna Jacq. ssp. *monogyna* (*Rosaceae*)	Alıç,alişan çalısı, arıç, cadı dikeni, yemişgen, yemişgen çalısı, yemişken dikeni	Herbs	Sedative[B] (int.), cardiac disorders[B] (int.)	Food
		Flowers	Cough[C] (int.), tuberculosis[C] (int.), stomachic[C] (int.), asthma[C] (int.), cardiac diseases[C] (int.)	
		Fruits	Tension[B] (int.)	
		Thorns	Snakebites[C] (ext.)	
C. pentagyna Waldst. & Kit. (*Rosaceae*)	Galagun, yemişgen, yemişken dikeni	Flowers	Cold[C] (int.), flu[C] (int.), nephritis[C] (int.), asthma[C] (int.), cough[C] (int.), cardiac diseases[C] (int.)	
Crepis vesicaria L. (*Asteraceae*)	Papatya	Capitulums	Cold[B] (int.)	
C. zacintha (L.) Babc. (*Asteraceae*)	Mayasılotu	Herbs	Antihemorrhoids[C] (int.)	
Cynodon dactylon (L.) Pers. var. *dactylon* (*Poaceae*)	Ayrıkotu	Roots	Shortness of breath[A] (int.), urinary ailment[A,C] (int.), anti-inflammatory[A,C] (int.), diuretic[C] (int.)	
Datura stramonium L. (*Solanaceae*)	Dişotu, patlangıç, süzgeçotu, taraklıot tatula, tatul	Leaves	Skin disorders[C] (ext.), asthma[C] (int.)	
		Flowers	Asthma[B] (int.), skin disorders[B] (ext.)	
		Seeds	Toothache[C] (ext.)	
Ecballium elaterium (L.) A. Rich. (*Cucurbitaceae*)	Acı kavun, acıdülek, gargadüleği, gargadüneği, kargadüleği, şeytan kavunu, yabani kavun, yaban düleği	Roots	Antihemorrhoids[A,B,C] (int.), analgesic[A] (int.), eczema[A] (int.), jaundice[A] (int.), uroclepsia[C] (int.)	
		Fruits	Analgesic[B,C] (int.), sinusitis[B,C] (int.), jaundice[B,C] (int.), antihemorrhoids[B,C] (int.) antirheumatic[C] (ext.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
Equisetum ramosissimum Desf. (*Equisetaceae*)	At kuyruğu, kırkkilit, minare otu	Herbs	Urinary ailment[B] (int.), vein disorders[B] (int.)	
E. telmateia Ehrh. (*Equisetaceae*)	At kuyruğu, biğirgan, çamotu, dereotu, suotu, dokuzbuğmalı, eklemotu, ekliot, kırkanahtar, kırkkilit, minare otu, tilki kuyruğu	Herbs	Tapeworm[A] (int.), hemorrhoids[A] (int.), urinary ailment[A,C] (int.), prostate[B,C] (int.), nephritis[C] (int.), cardiac disease[C] (int.), arteriosclerosis[C] (int.), asthma[C] (int.)	
Eryngium campestre L. var. *virens* Link (*Apiaceae*)	Deve dikeni, yıldız otu	Herbs	Urinary ailment[B] (int.)	
Euphorbia nicaeensis All. ssp. *glareosa* (Pall. ex M. Bieb.) A.R. Sm. var. *lasiocarpa* Boiss. (*Euphorbiaceae*)	Sütlüot	Latex	Skin disorders[C] (ext.)	
Fagus orientalis Lipsky (*Fagaceae*)	Gayın ağacı, kayın	Leaves	Hepatitis[C] (int.)	
Ferulago confusa Velen. (*Apiaceae*)	Sarıçiçek	Flowers	Speechless and depression[A] (int.)	
Fraxinus ornus L. ssp. *ornus* (*Oleaceae*)	Dişbudak, dışbudak, dişturbak, duşbudak, duştubak	Cortex	Analgesic[B] (int.), antirheumatic[B] (int.)	
		Fruits		
Hedera helix L. (*Araliaceae*)	Sarmaşık	Stems	Antidiabetic[C] (int.)	
		Cortex	Blood depurative[C] (int.)	
		Leaves	Antidiabetic[C] (int.), blood depurative[C] (int.)	
Heliotropium europaeum L. (*Boraginaceae*)	Temraotu	Flowers	Contract dermatitis and itching[A] (ext.)	
Helleborus orientalis Lam. (*Ranunculaceae*)	Kara ot	Leaves	Anti-inflammatory[B] (ext.)	
Heracleum sphondylium L. ssp. *ternatum* (Velen.) Brummitt (*Apiaceae*)	Devesil	Roots	Hemorrhoids[A] (int.), stomachic[A] (int.)	
Hyoscyamus albus L. (*Solanaceae*)	---	Seeds	Tapeworm[A] (int.)	
Hypericum perforatum L. (*Hypericaceae*)	Ada çayı, alaçayı, kalp otu, kantaron, kantaron çayı, kantariyon, kantaryon, kanturçiçeği, kantül, kesik otu, mide otu, sarı kantaron, sarıcayüz, sarıot	Herbs	Shortness of breath[A] (int.), antidiabetic[C] (int.), antihypertensive[C] (int.), cold[C] (int.), enteritis[C] (int.), antifungal[C] (int.), cardiac diseases[C] (int.), arteriosclerosis[C] (int.), antihemorrhoids[C] (ext.), asthma[C] (int.), insomnia[C](int.), uroclepsia[C] (for babies) (int.), gall bladder ailments[C] (int.), facial paralysis[C](int.), chest diseases[C] (int.), internal haemorrhage[C] (int.), anti-inflammatory[C] (int.), tuberculosis[C] (int.), pharyngitis[C] (int.), urinary ailment[B,C](int.), sedative[B] (int.), skin disorders[A,B,C] (ext.), stomachic[A,B,C] (int.), antirheumatic[B](ext.), toothache and earache[A] (ext.)	
		Flowers	Skin disorders[C] (ext.), stomachic[C] (int.), antidiabetic[C] (int.), enteritis[C] (int.)	
Inula britannica L. (*Asteraceae*)	Sarıçiçek	Flowers	Analgesic[A] (int.)	
Juniperus oxycedrus L. ssp. *oxycedrus* (*Cupressaceae*)	Ardıç, ardıçkatranı	Cones	Stomachic[B] (int.)	
		Seeds	Antihemorrhoids[B] (ext.)	
		Pix (tar)	Skin disorders[C] (ext.)	
Linum tenuifolium L. (*Linaceae*)	---	Herbs	Antidiabetic[C] (int.)	
Lithospermum officinale L. (*Boraginaceae*)	Tavşan darısı	Fruits	Enuresis[B] (int.)	
Lupinus albus L. ssp. *albus* (*Papilionaceae*)	Yahudi baklası	Seeds	Antidiabetic[A] (int.)	Dangerous
Lychnis coronaria (L.) Desr. (*Caryophyllaceae*)	Tavşan kulağı, haydutotu, kesikotu	Leaves	Skin disorders[C] (ext.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
Malus sylvestris Mill. ssp. *orientalis* (Uglitzk.) Browicz var. *orientalis* (*Rosaceae*)	Domuz elması, elma, ekşi elma, yabani elma, yabani ekşi	Leaves	Antidiabetic[C] (int.)	Food
		Fruits	Earache[C] (ext.), antidiabetic[C] (int.), blood depurative[C] (int.), skin disorders[C] (int.)	
Malva sylvestris L. (*Malvaceae*)	Ebe gömeci, ebem gümeci	Roots	Abortive[C] (int., ext.)	Food
		Herbs	Cough[B] (int.), urinary[C,B] (int.), stomachic[A] (int.), flu[A] (int.), asthma and shortness of breath[C] (int.), cough[C] (int.), gynelogical ailments[C] (int.)	Food
		Leaves	Anti-inflammatory[A,B,C] (ext.,int.), analgesic[A] (int., ext.)	Food
		Flowers	Cardiac[A] (int.), intestinal disorders[A] (int.), cancer[A] (int.), flu[C] (int.), cold[C] (int.), cough[C] (int.)	
Matricaria chamomilla L. var. *recutita* (L.) Grierson (*Asteraceae*)	Papatya	Flowers	Cough[C] (int.), asthma[C] (int.), tuberculosis[C] (int.), stomachic[C] (int.), enteralgia[C] (int.), cold[C] (int.), flu[C] (int.), urinary ailment[C] (int.), anti-inflammatory[C] (int.), carminative[C] (int.), sore throat[C] (ext.), skin disorders[C] (ext.), to take away bad breath of mouth[C] (ext.), women's diseases[C] (ext.)	
Medicago orbicularis (L.) Bartal. (*Fabaceae*)	Tirfil, tirfilotu	Fruits	Abdominal pain[C] (int.)	
Melissa officinalis L. ssp. *officinalis* (*Lamiaceae*)	Arıotu, oğulotu	Herbs	Shortness of breath[A,C] (int.), cough[A] (int.), cancer[C] (int.), cardiac and vein diseases[C] (int.), stomachic[C] (int.), nephritis[C] (int.), forgetfulness[C] (int.), antidiabetic[C] (int.), cold[C] (int.), flu[C] (int.), bronchitis[C] (int.), enteritis[C] (int.)	
Mentha longifolia (L.) Huds. ssp. *typhoides* (Briq.) Harley var. *typhoides* (*Lamiaceae*)	Dere nanesi, yabani nane	Leaves	Abdominal pain[C] (int.), diarrhea[C] (int.)	
M. pulegium L. (*Lamiaceae*)	Kırçayı, nane, yaban nanesi	Leaves	Stomachic[B] (int.), urinary ailment[B] (int.), flu[B] (int.)	Food
Mespilus germanica L. (*Rosaceae*)	Muşmula, yabani muşmula	Leaves	Cough[C] (int.), cold[C] (int.), flu[C] (int.)	Food
		Fruits	Diarrhea[C] (int.)	
Oenanthe pimpinelloides L. (*Apiaceae*)	Güre maydanoz, kaz ayağı	Herbs	Sedative[B] (int.), analgesic[B] (int.)	Food
Ononis spinosa L. ssp. *leiosperma* (Boiss.) Širj. (*Fabaceae*)	Kaplıca, kimya otu, kuşkonmaz, yağlıca	Roots	Skin disorders[B] (ext.), diuretic[B] (int.), urinary ailment[C] (int.)	
Orchis purpurea Huds. (*Orchidaceae*)	Hasancık, hıdrellez çiçeği, tukuk çiçeği	Tuber	Skin disorders[B] (ext.)	
O. vulgare L. ssp. *hirtum* (Link) Ietsw. (*Lamiaceae*)	Keklik, kekikotu, keklikotu, yer kekiği	Herbs	Cold[C] (int.), flu[C] (int.), abdominal pain[C] (int.), urinary ailment[C] (int.), stomachic[C] (int.)	Food
Origanum vulgare L. ssp. *vulgare* (*Lamiaceae*)	Dağ kekiği, dereotu, uzun kekik, keklikotu, kırçayı	Herbs	Abdominal pain[A,C] (int.), antidiabetic[B] (int.), cold[B] (int.), flu[B] (int.), abdominal pain[B] (int.)	
Paliurus spina-christi Mill. (*Rhamnaceae*)	Çakırdikeni, kara çalı	Fruits	Tuberculosis[B] (int.), cough[A] (int.), stomachic[A,C] (int.)	
Papaver rhoeas L. (*Papaveraceae*)	Borcanka, gelincik, gelincikotu	Herbs	Antirheumatic[C] (int.)	Food
		Leaves	Tonic[C] (int.)	
		Flowers	Antihemorrhoids[B] (int.), bronchital calmative[C] (int), cough[C] (int.), sore throat[C] (int.), immunotonic[C] (int.), galaktagog[C] (int.), epistaxis[C] (ext.)	
Petasites hybridus (L.) Gaertn. (*Asteraceae*)	Kabalak, kalabak otu, kalpakotu, kara kafes, konştrakt	Leaves	Skin disorders[B,C] (ext.), analgesic[B] (ext.), amnesia[B] (int.) (with *Melissa officinalis*)	
		Roots	Thrush[B] (ext.)	
Pimpinella anisum L. (*Apiaceae*)	Anason	Fruits	Sedative[C] (int.), soporific[C] (int.)	
Pistacia terebinthus L. ssp. *terebinthus* (*Anacardiaceae*)	Kokarağaç, menengiç ağacı	Leaves	Stomachic[C] (int.), hemorrhoids[C] (int.), psoriasis[C] (int.)	
		Cortex	Stomachic[C] (int.)	
Plantago lanceolata L. (*Plantaginaceae*)	Bobvitsa, damarlıot, damarotu, kesikotu, sinirliot, sinirotu	Leaves	Cough[C] (int.), bronchitis[C] (int.), expectorant[C] (int.), skin disorders[B,C] (ext.), sedative[B] (int.), gastritis[B], bee bites[C] (ext.), tuberculosis[C] (int.), stomachic[C] (int.), asthma[C] (int.), haemostatic[C] (ext.)	Food
		Seeds	Antihemorrhoids[B] (int.), diarrhea[B] (int.)	
		Leaves and seeds	Stomachic[B] (int.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
P. major L. ssp. *intermedia* (Gilib.) Lange (*Plantaginaceae*)	Damarotu, damarlıot, sinirliot, sinirotu, sinirli yaprak	Roots	Tuberculosis[B] (int.)	Food
		Leaves	Tuberculosis[C] (int.), stomachic[A,C] (int.), skin disorders[A,C] (ext.)	
		Seeds	Cancer[C] (int.)	
P. major L. ssp. *major* (*Plantaginaceae*)	Damarotu, damarlıot, bobvitsa, kara kabarcık, kesikotu, keskinotu, sinirliot, sinirotu,	Herbs	Blood depurative[C] (int.)	Food
		Leaves	Skin disorders[B,C] (ext.), sedative[B] (int.), boils[C] (ext.), antidiabetic[C] (int.), haemostatic[C] (ext.), goitre[C] (ext.), asthma[C] (int.), cold[C] (int.), flu[C] (int.)	
		Flowers	Diarrhea[B] (İnt.)	
		Seeds	Cancer[C] (int.)	
Polygonum equisetiforme Sibth. & Sm. (*Polygonaceae*)	Dereotu	Herbs	Antihemorrhoids[A] (int., ext.)	
P. lapathifolium L. (*Polygonaceae*)	Dere biberi, dereotu, deve sürdeği	Herbs	Antihemorrhoids[C] (ext.), itching[C] (ext.), eczema[C] (ext.), antifungal[C] (ext.)	
Polypodium vulgare L. ssp. *vulgare* (*Polypodiaceae*)	Tatlı papra	Herbs	Stomachic[C] (int.), prostatitis[C] (int.), antitussif[C] (int.), sedative[C] (int.)	
Populus tremula L. (*Salicaceae*)	Titrek kavak	Leaves	Antidiabetic[B] (int.), asthma[C] (int.), wart[C] (ext.)	
		Young shoots	Analgesic[B] (int.)	
		Cortex	Nephritis[C] (int.)	
Prunus divaricata Ledeb. ssp. *ursina* (Kotschy) Browicz (*Rosaceae*)	Güvem	Fruits	Antidiabetic[C] (int.), cold[C] (int.), flu[C] (int), asthma[C] (int), nephritis[C] (int.)	Food
P. spinosa L. ssp. *dasyphylla* (Schur) Domin (*Rosaceae*)	Güvem, güvem tikeni, veskruş	Roots	Liver diseases[B] (int.)	Food
		Fruits	Antidiabetic[A,B,C] (int.), asthma[B] (int.), cardiac diseases[C] (int.), nephritis[C] (int.), cold[C] (int.), flu[C] (int.), bronchial calmative[C] (int.), embolism[C] (int.)	
Pteridium aquilinum (L.) Kuhn (*Hypolepidaceae*)	Papra, tatlı papra	Herbs	Antirheumatic[C] (ext.)	
Pyrus elaeagnifolia Pall. ssp. *elaeagnifolia* (*Rosaceae*)	Ahlat, yaban ağlatı, yaban armudu	Fruits	Nephritis[C] (int.), antidiabetic[C] (int.)	Food
Quercus cerris L. var. *austriaca* (Willd.) Loudon (*Fagaceae*)	Meşe, palamut meşesi	Cortex	Antifungal[C] (ext.)	
		Leaves	Prostatitis[C] (int.)	
Ranunculus marginatus d'Urv. var. *marginatus* (*Ranunculaceae*)	Sevdaçiçeği, sevdaotu, suçiçeği	Roots	Itching[A] (ext.)	
R. muricatus L. (*Ranunculaceae*)	Ayakotu, bacakotu, çayır otu	Whole plant	Antirheumatic[A,B] (ext.)	
		Leaves	Analgesic[C] (ext.), oedema[C] (ext.)	
Rosa canina L. (*Rosaceae*)	Gözkıvıştran, gül bubusu, gül buğucuğu, gültikeni, köpekgülü, kuşburnu, öküz gözü, öküz götü, yabangülü, yabani gül	Leaves	Biliary disorders[A] (int.), cold[C] (int.), flu[C] (int.), cough[C] (int.), itching[C] (int.), nephritis[C] (int.), eczema[C] (int.)	Food
		Fruits	Antidiabetic[A] (int.), urinary ailment[B] (int.), skin disorders[B] (int.), stomachic[A,B] (int.), antirheumatic[B] (int.), cardiac diseases[B] (int.), cough[B] (int.), immunotonic[C] (int.), cancer[B] (int.), tuberculosis[B] (int.), asthma[A,B,C] (int.), analgesic[A] (int.), antihemorrhoids[A] (int.)	
		Branches and petals	Swelling[B]	
		Petals	Cold[C] (int.), flu[C] (int.), cough[C] (int.), bronchitis[C] (int.)	
R. gallica L. (*Rosaceae*)	Orman gülü	Flowers	Diarrhea[C] (int.)	
Rosmarinus officinalis L. (*Lamiaceae*)	Kuşdili	Herbs	Dyspepsia[C] (int.), antihypertensive[C] (int.), vasodilator[C] (int.), appetizer[C] (int.)	
Rubia tinctorum L. (*Rubiaceae*)	Boya kökü, broş, gözotu, kökboya, yer boyası, yapışkanotu	Leaves	Cataract[C] (ext.), abortive[C] (int.)	
Rubus canescens DC. var. *canescens* (*Rosaceae*)	Böğürtlen, kapina, karamuk	Roots	Cardiac diseases[C] (int.)	Food
		Leaves	Haemostatic[C] (ext.)	
R. canescens DC. var. *glabratus* (Godr.) Davis & Meikle (*Rosaceae*)	Böğürtlen, kapina, kupina, kuşüzümü	Roots	Antidiabetic[B] (int.), diarrhea[B] (int.)	Food
		Leaves	Immunotonic[C] (int.), cold[C] (int.), flu[C] (int.)	
		Fruits	Wounds[C] (int.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
R. discolor Weihe & Nees (*Rosaceae*)	Ahududu, böğürtlen, böğürtlen dikeni, çoban kapina, kapina, karamama, karamuk, kösteği, özmenek	Roots	Antidiabetic[B] (int.), urinary ailment[B] (int.), tuberculosis[B] (int.), nephritis[C] (int.), prostatitis[C] (int), fertility[C] (int.)	Food
		Leaves	Nephritis[C] (int.), skin disorders[C] (ext.), diarrhea[C] (int.)	
		Fruits	Nephritis[C](int.), skin disorders[B] (ext.), to facilitate of birth[B] (int.)	
R. hirtus Waldst. & Kit. (*Rosaceae*)	Karamuk	Roots	Nephritis[C] (int.), prostatitis[C] (int.)	
R. sanctus Schreb. (*Rosaceae*)	Böğürtlen, karamama	Roots	Tooth diseases[B] (ext.), cancer[B] (int.), stomachic[A] (int.)	
		Leaves	Antidiabetic[A] (int.), analgesic[A] (int.), asthma[B] (int.)	
		Fruits	Skin disorders[B] (ext.), liver diseases[B]	
R. tereticaulis P.J. Müll. (*Rosaceae*)	Böğürtlen, karamık	Leaves	Anti-inflammatory[A] (ext.)	
Rumex crispus L. (*Polygonaceae*)	Acı labada, labada konştrak, yabani labada, tatlı labada	Leaves	Skin disorders[B,C] (ext.)	Food
R. cristatus DC. (*Polygonaceae*)	Lapuşa	Roots	Itching[A] (ext.)	
Ruscus aculeatus L. var. *aculeatus* (*Liliaceae*)	Deve çalısı, enir, tavşan elması, tavşan memesi, tavşanotu, yaban mersini	Roots	Kidney stones[C] (int.), nephritis[C] (int.)	
		Herbs	Varices[B] (int.)	
		Fruits	Analgesic[B] (int.), enuresis[B] (int.)	
Salix alba L. (*Salicaceae*)	Salkımsöğüt, söğüt, söğüt ağacı	Cortex	Eczema[C] (ext.), antifungal[C] (ext.)	
		Leaves	Stomachic[C] (int.), headache[C] (int.), malaria[C] (ext.)	
Salvia fruticosa Mill. (*Lamiaceae*)	Adaçayı	Herbs	Stomachic[A] (int.), tuberculosis[A] (int.), sedative[A] (int.), perspiration[A] (int.), anti-inflammatory[A] (int.)	
S. verticillata L. ssp. *amasiaca* (Freyn & Bornm.) Bornm. (*Lamiaceae*)	Adaçayı	Leaves	Cardiovascular diseases[C] (int.), abdominal pain[C] (int.)	
Sambucus ebulus L. (*Caprifoliaceae*)	Ademotu, bizga, haptovina, karabubu, memer, mülver, mürver, pıyırgan, pıyran, piran, sultan otu, yer mülveri	Roots	Antirheumatic[A] (ext.), analgesic[C] (ext.), hemorrhoids[C] (int.)	
		Herbs	Antihemorrhoids[B] (ext.), antirheumatic[A,B,C] (ext.), snakebites[C] (ext.), stomachic[C] (int.), skin disorders[C] (ext.)	
		Leaves	Anti-inflammatory[A] (ext.), anthelmintic[A,C] (ext.), analgesic[A] (ext.), snakebites[C] (ext.), anthrax[C] (ext.), fleafuge[C] (ext.)	
		Flowers	Antihaemorrhoids[A] (int.), analgesic[A] (int.), swellings[A] (int.), urinary ailment[B] (int.), asthma[B] (int.), cough[B,C] (int.)	
		Fruits	Fungal diseases[B] (int.), malaria[B] (ext.), skin disorders[B] (ext.), antihemorrhoids[A,B,C] (int.), stomachic[C] (int.)	Food
S. nigra L. (*Caprifoliaceae*)	Ağaç mülveri, mülver, mürver, mürver çiçeği, mürver ağacı	Roots	Antirheumatic[B] (ext.)	Food
		Herbs	Swellings[B] (ext.)	
		Cortex	Skin disorders[C] (ext.)	
		Fruits	Asthma[C] (int.)	
		Seeds	Antihemorrhoids[C] (int.)	
		Flowers	Shortness of breath[A] (int.), antirheumatic[A] (ext.), paralytic[A] (ext.), prostate[B] (int.), diuretic[B] (int.), antidiabetic[B] (int.), cough[C] (int.), tuberculosis[C] (int.), whooping cough[C] (int.), asthma[C] (int.), antihemorrhoids[C] (int.), expectorant[C] (int.), cold[C] (int.)	
Scilla bifolia L. (*Liliaceae*)	Karga soğanı	Bulbus	Skin disorders[C] (int.), lumbago[C] (ext.)	
Sedum album L. (*Crassulaceae*)	Kayakoruğu, kulakotu	Leaves	Earache[A] (ext.)	
Sideritis montana L. ssp. *montana* (*Lamiaceae*)	Karaçay, tilki kuyruğu	Herbs	Cough[A,C] (int.), cold[C] (int.), flu[C] (int.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
S. scardica L. ssp. *scardica* (*Lamiaceae*)	Adaçayı, başak çayı, bazlak çayı, çiçek çayı, karlık çayı, karlı çay, kırçayı, kuyruk çayı, kuyruklu adaçayı, pazlak çayı, taşlık çayı, tilki kuyruğu	Herbs	Bronchitis[C] (int.), cough[C] (int.), cold[C] (int.), flu[C] (int.), kidney stones[C] (int.), stomachic[C] (int.), antiemetic[C] (int.)	
Sinapis arvensis L. (*Brassicaceae*)	Hardal otu, hardal, sarı hardal	Roots	Stomachic[B] (int.)	
		Branches	Antirheumatic[B] (ext.)	
		Seeds	Cough[B] (int.), antirheumatic[B,C] (int.)	
		Leaves	Cough[C] (int.)	
Sonchus asper (L.) Hill ssp. *glaucescens* (Jord.) Ball (*Asteraceae*)	Sütlengeç	Latex	Cuts[C] (ext.), skin disorders[C] (ext.)	
Sorbus aucuparia L. (*Rosaceae*)	Üvez	Leaves	Prostatitis[C] (int.), cancer[C] (int.), diarrhea[C] (int.)	
		Fruits	Antihemorrhoids[C] (int.)	
S. domestica L. (*Rosaceae*)	Börtlücan, üvez	Cortex	Stomachic[C] (int.)	Food
		Leaves	Prostatitis[C] (int.), antidiabetic[A,C] (int.), nephritis[C] (int.), gall bladder ailments[C] (int.), diuretic[C] (int.), diarrhea[C] (int.), kidney stones[C] (int.), cholesterol lowering[C] (int.), stomachic[A] (int.)	
		Fruits	Diarrhea[C] (int.)	
S. torminalis (L.) Crantz var. *torminalis* (*Rosaceae*)	Böğürtlecen	Leaves	Antidiabetic[C] (int.), stomachic[C] (int.)	Food
Teucrium chamaedrys L. ssp. *chamaedrys* (*Lamiaceae*)	Kısacık mahmut	Leaves	Diuretic[C] (int.), kidney stones[C] (int.), diarrhea[C] (int.), abdominal pain[C] (int.)	
T. polium L. (*Lamiaceae*)	Kekik, mayasılotu	Herbs	Antihemorrhoids[A] (int.), abdominal pain[C] (int.), cold[C] (int.), flu[C] (int.)	
T. scordium L. ssp. *scordioides* (Schreb.) Maire & Petitm. (*Lamiaceae*)	Kantariyeotu, kesikotu	Herbs	Skin disorders[A] (ext.)	
Thymus atticus Čelak. (*Lamiaceae*)	Keklikotu	Herbs	Abdominal pain[A] (int.)	
T. longicaulis C. Presl ssp. *longicaulis* var. *longicaulis* (*Lamiaceae*)	Keklikotu	Herbs	Abdominal pain[A] (int.), stomachic[A] (int.), diarrhea[A] (int.)	
T. longicaulis C. Presl ssp. *longicaulis* var. *subisophyllus* (Borbás) Jalas (*Lamiaceae*)	Keklikotu, kekikotu, kekik, kekikçayı	Herbs	Cold[C] (int.), flu[C] (int.), abdominal pain[C] (int.), cough[C] (int.), nephritis[C] (int.), antidiabetic[C] (int.), cholesterol lowering[C] (int.), stomachic[C] (int.), enteritis[C] (int.), kidney stones[C] (int.), cardiac disease[C] (int.), sedative[C] (int.), loosing of weight[C] (int.), insomnia[C] (int.), anti-inflammatory[C] (int.), anthelmintic[C] (int.)	
		Fruits	Toothache[C] (ext.)	
T. sibthorpii Benth. (*Lamiaceae*)	Kekik, keklik otu	Herbs	Stomachic[B] (int.), prostate[B] (int.), urinary ailment[B] (int.)	Food
T. zygioides Griseb. var. *zygioides* (*Lamiaceae*)	Çobanpoyu, kekik, keklikotu	Herbs	Stomachic[A] (int.), analgesic[A] (int.), antidiabetic[B] (int.)	
Tilia argentea Desf. (*Tiliaceae*)	Ihlamur	Roots	Cold[B] (int.)	Food
		Leaves	Antirheumatic[B] (ext.)	
		Flowers	Urinary ailment[A] (int.), analgesic[A] (ext.)	
		Flowers and bracts	Sedative[B] (ext.), cold[B] (int.)	
T. platyphyllos Scop. (*Tiliaceae*)	Ihlamur	Flowers	Asthma[C] (int.), cough[C] (int.), abdominal pain[C] (int.), cold[C] (int.), flu[C] (int.)	
Tribulus terrestris L. (*Zygophyllaceae*)	Çobankaldıran, çobanotlatan, devebağırtan	Herbs	Diuretic[C] (int.), cholesterosis[C] (int.)	
		Fruits	Urinary ailment[A] (int.), cardiac disorders[A] (int.)	
Typha angustifolia L. (*Typhaceae*)	Papur, sazlık	Spike hairs	Skin disorders[C] (ext.)	

Table 1. Continuation.

Botanical names & family	Local names	Parts used	Uses & references	Notes
Ulmus minor Mill. ssp. *canescens* (Melville) Browicz & Ziel. (*Ulmaceae*)	Kara ağaç	Cortex	Skin disorders[C] (ext.)	
U. minor Mill. ssp. *minor* (*Ulmaceae*)	Kara ağaç	Bark	Skin disorders[B] (ext.)	
		Flowers	Skin disorders[B](ext.)	
Urtica dioica L. (*Urticaceae*)	Isırgan, sırgan	Roots	Nephritis[C] (int.), stomachic[C](int.), baldness[C] (int.), prostatitis[C] (int.), urea[C](int.)	
		Herbs	Antirheumatic[C] (int.), prostatitis[C] (int.), antihemorrhoids[C] (int.), antihypertensive [C] (int.), embolism[C] (int.), cancer[C] (int.), nephritis[C] (int.), stomachic[C] (int.), tonic[C] (int.) laxative[C] (int.), varicosity[C] (ext.), baldness[C] (ext.), kidney stones[C] (int.), enteritis[C] (int.)	
		Leaves	Stomachic[B,C] (int.), dandruff[B] (ext.), goitre[B] (int.), women's diseases[B] (int.), urinary ailment[B] (int.), antirheumatic[B] (int.), cardiac and vein diseases[B] (int.), itching[B] (int.), skin disorders[C] (ext.), bronchitis[C] (int.), cough[C] (int), asthma[C] (int.), tonic[C] (int.), cancer[C] (int.), antiemetic[C] (int.), analgesic[C] (ext.), oedema[C] (ext.)	
		Seeds	Lung diseases[C] (int.), cancer[C] (int.), expectorant[C] (int.)	Food
U. urens L. (*Urticaceae*)	Isırganotu	Herbs	Antihemorrhoids[A] (int.), cancer[A] (int.), inflammation[A] (int.), skin disorders[A] (ext.), antirheumatic[A] (ext.), inflammation[A] (ext.)	Food
		Leaves	Antirheumatic[A] (ext.), antihemorrhoids[A] (int.), analgesic[A] (int.)	
		Seeds	Cancer[A] (int.), tuberculosis[A] (int.)	
Viola sieheana Becker (*Violaceae*)	Mor menekşe	Flowers	Cold[C] (int.), flu[C] (int.), cough[C] (int.)	Food
Viscum album L. ssp. *album* (*Loranthaceae*)	Burç, çekem, çekim, gevele, gökçe otu, ökse otu	Branches	Sedative[B] (int.), asthma[B] (int.), cardiac diseases[B] (int.)	
		Leaves	Antihypertensive[C] (int.), splenopancreatic diseases[C] (int.), sterility (woman)[C] (int.), galaktagog[C] (int.), cardiovascular diseases[C] (int.), asthma[C] (int.)	
		Fruits	Dizziness[C] (int.)	
Vitis sylvestris C.C. Gmel. (*Vitaceae*)	Kuş üzümü, yabani asma, yabani üzüm	Cortex	Hair tonic[C] (ext.)	
		Leaves	Baldness[C] (int.)	

Abbreviations: Uses: int. – internal uses; ext. – external uses; References: [A] – Akalın & Alpınar (1994); [B] – Ecevit & Özhatay (2004, 2006); [C] – Kültür (2007, 2008).

Acknowledgements. The authors are grateful to Prof. Dr. Asuman Baytop for the permission to use her photos and İlker Genç for his valuable help to prepare this paper. This present work was supported by the Research Fund of Istanbul University (project numbers: 491/230791, 1646, T–1175/01112001).

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Medicinal plants in folk medicine of taiga zone of Russia: peculiarities of use and resources

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Abstract. Experience of herbal medicine remains insufficiently studied. Materials are gathered since 1981 with method of questioning, structured observation and residents' interrogation. 93 species from 41 families are used in folk medicine; *Asteraceae* with 11.5 %, *Ranunculaceae* – 8.3 %, *Lamiaceae* – 6.2 % out of the total number of species are prevailing. 25 % of the species have original ways of use or are marked for the first time as folk medicinal plants. More than 50 species used in folk medicine are rare and listed in the *Red Book of Kirov region* (mostly from *Orchidaceae*). To decrease operational press on the species it is necessary to provide their protection and develop cultivation.

Key words: folk medicine, medicinal plants, resources, southern taiga, treatment

Introduction

Nowadays the population of different countries uses plants as efficient source of chemical substances which can be used for producing of new medicines, safe preventive and medicinal remedies, which are essential when reaching the principle "health for everybody" (Ramaswamy 2005). During the studies of useful plants it is necessary not only to reveal the species composition but also to estimate the resources to work out the recommendation on organizing non-exhausting rational use of them.

In taiga zone of Russia the studies of folk medicinal plants started in 18th century (Pallas 1776) and continued due to few researchers (Krylov 1886; Skalozubov 1904; Utkin 1931; Deryabina 1965; Grom 1965; Vollosovich 1965; Bush 1984; Ilyina 2000). The end of 20th century is the start of the period of general summarizing of gathered material, which is realized in monograph *Plant resources of USSR* (Sokolov 1984–1996) and reference book *Wild useful plants of Russia* (Budantsev & Lesiovskaya 2001) assembled by collaborators of Botanical Institute of Russian Academy of Sciences (Saint-Petersburg). In these works, which are the most complete in Russia at the moment, huge literary material on chemical composition and application of medicinal plants is summarized. About 2200 medicinal plant species are defined in Russia now and 135 of them are permitted for use in official medicine (Budantsev 2005). In Bulgarian phytotherapy 250 species are used (Stoeva 2004).

Study area

Kirov region was selected as a model region because it is situated in three vegetative subzones (middle and southern taiga, coniferous deciduous forests) and its ecological-coenotic and socio-economic parameters are typical for taiga regions of Russia.

Kirov region occupies 120.8 thousand km² in the eastern part of European Russia between 56°03' and 61°04' N and 46°43' and 53°56' E. The extension of the region from northern to southern border is 570 km and from western to eastern is 440 km.

The climate is continental. Average annual air temperature varies from 0.5 °C in the North to 2.7 °C in the South. Average annual rainfall is from 400 mm in the South and South-East to 600 mm in the North-West. General character of the region is flat but it is notable for significant ruggedness and hilliness. Prevalent heights of the plain are 150–180 m a.s.l. Basic part of the region's territory (77.9 %) is covered by podzolic soil types. Most of soils are of heavy loamy and clay granulometric composition (59.8 % of the area).

Oblongness of the territory in meridional direction and corresponding latitudinal modification in solar flux level determined the presence of 3 vegetative subzones: middle and southern taiga, coniferous deciduous forests.

The territory of Kirov region belongs to Ural–West-Siberia taiga province of Euro–Asian taiga area (Gribova & al. 1980; Aleksandrova & al. 1989). Forests are spread irregularly. In northern parts of the region forests occupy 75–85 % of the area and 7–20 % – in central and southern parts; bilberry fir woods prevail – 12 %, bilberry birch woods are 6.1 %, and majanthemum bilberry birch woods – 5.2 %. Swamps occupy about 5 %, meadows – 2 % of the area.

According to Takhtadzhian's (1978) floristic zoning Kirov region flora belongs to Holoarctic plant kingdom, Boreal subkingdom, circumpolar region, North-European province. Flora of the region if to consider adventive and cultural plants counts 1509 species of vascular plants (Tarasova 2005). Leading place by number of genera is occupied by families: *Asteraceae* (48 genera), *Poaceae* (44 genera), *Umbelliferae* (30 genera), *Rosaceae* and *Brassicaceae* (Egoshina 1999). The set of leading genera and presence of more than half of the species in them stresses boreal-moderate flora character and includes the leading genera of boreal-moderate floras indicated by Tolmachyov (1970).

Material and methods

The material about use of medicinal plants in folk medicine and estimation of raw material resources was gathered during the expeditions in Kirov region in the period 1981–2004.

The gathering of material aiming to reveal medicinal plant species in folk medicine and peculiarities of their use was held by analyses methods based on long-term contacts and profound interviews widely used in ethnographic researches (Mironov 1970; Kharamzin 2001), as well as individual questioning of inhabitants. More than 3000 countrymen of all ages, mainly old-residents of distant settlements took part in this part of the research.

The method of questionary was also used. It was assembled according to recommendations of Monteverde (1948) and included the following items: plant name, growing and collection area, habitat, brief morphological plant characteristic, name of applied parts of the plants, methods and terms of collection, methods of drying, processing and storage, illnesses during which the plant is applied, form, way of preparation, dose, treating duration. In cases of difficulties in defining the species its description was made and the specimen was collected. 13380 questionaries were sent. Main addresses are of workers of game management, forestry and agriculture spheres, biology and ecology teachers of rural schools. Average age of the questioned people is 50–60. Questionary return is 3684 pieces. A plant was included in the list of the used ones in Kirov region if it was mentioned by at least 10 % respondents.

For comparative estimation of peculiarities of plant use in Kirov and other regions the literary sources mentioned in the "Introduction" and some others were used (e.g., Jordanov & al. 1973; Pahlov 1993; Yakovlev & Blinova 1999).

Folk medicine plant resources estimation was held in two points.

Point 1 included revealing of basic places of vegetation, ecological-coenotic characteristics, eye estimation of abundance, popularity and resources.

All studied plant species were divided into four resource groups according to resource size, exploitation loading influence and spreading character (Egoshina 1989).

First group includes widely spread species whose resources are significant and thus collection does not damage population state (e.g., *Betula verrucosa*, *Pinus syivestris*, *Filipendula ulmaria*, etc.).

Second group includes forest and meadow species which have limited spreading, exact ecological confines, inhabit inviolate communities and experience intense exploitation by population (*Vaccinium vitis-idaea*, *Origanum vulgare*, *Hypericum perforatum*, *Juniperus communis*, *Arctostaphylos uva-ursi*, *Achillea millefolium*, *Rosa majalis* and *R. acicularis*).

The third group is composed mainly by ruderal and segetal species (*Urtica dioica*, *Tussilago farfara*, *Tanacetum vulgare*, *Gnaphalium uliginosum*, *Plantago major* and *Leonurus quinquelobatus*).

The fourth group includes rare and endangered (in consequence of immoderate exploitation and habitat destruction) medicinal plant species. These are basically species at the bounds of their natural habitats. The most valuable of them are *Adonis sibiricum* and *Paeonia anomalia*.

For the second point a modified scale (Table 1) was created for approximate definition of raw material resources on the basis of the scale by Polozhij & al. (1988).

Plant resources of categories 1 and 2 are industrial, 3 – can be the objects of limited collection, 4 – local collection, 5 category – non-commercial, collection is usually intolerable and/or inexpedient.

Table 1. Scale of approximate definition of raw material resources.

Resources category	Category «price»			
	Use scale	Spreading and tract area	Abundance in the tracts (%)	Approximate exploitation stocks of raw material (tons per 100–150 thousand km²)
1	For food and chemical–pharmaceutical industry of the country	Widely spread, covering significant areas	10–50 and more	More than 10
2	For regional food and chemical–pharmaceutical industry and pharmaceutical system	Rather widely spread, covering insignificant areas	5–30	1–10
3	For regional food industry, pharmaceutical system and personal use of inhabitants	Limited spread, insignificant areas	5–50	0.5–1.0
4	Collection for personal use of inhabitants	Tracts are fragmentary and small, not bigger than 1 hectare	1–10	0.1–0.5
5	Collection is inexpedient and/or inadmissible	Rarely found, do not form tracts	do not form dense tracts	Less than 0.1 t or absent

Results

The collection of data on plants allowed to define 93 species (6.2 % of the total number of species in the regional flora) (Table 2) which are used in folk medicine but not applied in the official one, or are used differently in folk medicine than in the official. Folk medicine plants of Kirov region belong to 41 families. The largest number of used species is in families *Asteraceae* – 11.5 %, *Ranunculaceae* – 8.3 %, *Lamiaceae* – 6.2 %, *Caryophyllaceae* – 5.2 %, *Rosaceae* – 4.1 %, *Fabaceae* – 4.1 %, *Orchidaceae* – 4.1 %, *Campanulaceae* – 4.1 %, *Hypericaceae* – 4.1 %, *Primulaceae* – 3.1 %. One species used in folk medicine in each of 24 families was also defined. Comparison of compositions of ten leading families of Kirov region flora and set of used in folk medicine has shown that they do not match. Probably significant distinction in families' composition shows purposeful selection of species used in folk medicine.

As a result of comparison of medicinal plants application in Kirov region and other regions the following was determined: application of 34.4 % of plant species coincide with the experience of other folk medicines, use of 9.4 % of the species is narrower than in other folk medicines. Application of 31.2 % of the plant species has common and original features: *Calypso bulbosa*, *Campanula trachelium*, *Chamaenerion angustifolium*, *Coronaria flos-cuculi*, *Daphne mezereum*, *Dianthus deltoides*, *Dracocephalum thymiflorum*, *Geum rivale*, *Moneses uniflora*, *Platanthera bifolia*, *Pulsatilla patens*, *Pyrola rotundifolia*, *Scleranthus annuus*, *Trientalis europaea*, *Veratrum lobelianum*. 25 % of the species have quite original ways of application or are marked as folk medicinal for the first time (*Actaea erythrocarpa*, *Campanula latifolia*, *Centaurea sumensis*, *Dryopteris austriaca*).

The most popular medicinal plants in Kirov region folk medicine are *Campanula glomerata*, *Carlina biebersteinii*, *Centaurea sumensis*, *Chamaenerion angustifolium*, *Chimaphila umbellata*, *Equisetum fluviatile*, *Hypericum maculatum*, *Mentha arvensis*, *Moneses uniflora*, *Paeonia anomala*, *Potentilla argentea*, *Polygala comosa*, *Sedum telephium*.

Overground phytomass is most often (66.1 %) used, underground phytomass is used more rarely (12.9 %) as are inflorescences and blossoms (7.3 %). The most rarely used are fruits, seeds (5.6 %), leaves, fronds (4.8 %) and bark (1.6 %).

Terms of raw material collection are traditional. Herbage, leaves, blossoms, inflorescences are collected during blossoming; rhizosomes – during fruit maturing.

Preparation forms of remedies vary – juice, water and milk decoction, steam, extract, tincture, ointment, powder, fumigation, fresh plants. Water decoctions are prepared by pouring of raw material with cold water, then boiling, cooling and filtering. Milk decoctions are prepared similarly, but used while hot or warm. To prepare extract raw material is poured with boiling water, covered with lid, kept under lid for half an hour and more. Steams are made by keeping of water and dry raw material mixture in hermetically closed vessel in the oven for not less than 3–4 hours or during the night. Raw material for compresses is warmed in hot water, milk or

buttermilk. Vodka in the ratio of 1:5 – 1:10 to raw material is usually used as a base of tinctures. Tinctures are kept in a warm place for 5–7 days. Usually fresh raw material, but not dried, is used for tinctures. For ointment preparation inner adipose is used. The most valuable is wild animal adipose (badger, bear, raccoon dog), but it is possible to use lard. The only case when vegetable oil is used is the preparation of *Symphytum officinale* ointment. Ointments are kept in a warm place for 12–16 hours, then filtered and stored in a cool place. For fumigation tufts of dry herbage are burnt above patient's bed for 5–10 minutes. This way of treatment is accompanied with prayers or incantations.

The most popular forms of remedies are water decoction (37.4 %), tinctures (14.2 %), water extracts (9.0 %) and use of fresh plants (9.7 %).

Dosage of used plants is conditional: handful, pinch, spoonful, glass. Probably, if to take into consideration that there is centuries-old experience and the effect of most of plants on the organism is mild, these doses can be considered permissible.

Among wild medicinal plants of folk medicine forest plants prevail (39.8 %), there is large number of upland (25.8 %), flood-land meadows and flood-land shrub thickets (16.1 %), weeds (segetal and ruderal) (7.5 %). Plants of swamps and ponds are characterized with minimum quantity (5.4 %).

By resources, exploitation influence and spreading 40.9 % of the species belong to the second resort group, 31.2 % – to the fourth, 17.2 % – to the first, and 10.8 % – to the third.

67.8 % of wild medicinal plant species of folk medicine can be the objects of industrial or limited collection and have the resources of categories 1–3. At the same time 29 plant species used in folk medicine are rare and need protection. This makes more than ¼ of all angiosperm species listed in the *Red Book of Kirov region* (Bolshakov 2001). The following rare plant species are most often used in folk medicine: *Centaurea sumensis, Calypso bulbosa*, *Paeonia anomala*, and *Pulsatilla patens*. Populations of these species are declining under the influence of collection.

Table 2. Medicinal plants used in folk medicine of Kirov region: resort characteristics and application peculiarities.

No.	Species	Growing area	Resort group of species	Stock category	Use	Remedy form, used part or organ
1	*Acinos arvensis*	2	2	3	Antiphlogistic, expectorate during colds	Water decoction; overground part
2	*Actaea erythrocarpa*	1	4	4	Nervous diseases	Tincture; rhizosomes
3	*Actaea spicata*	1	4	4	Immunemodulator (to treat impaired patients)	Tincture; rhizosomes
4	*Adonis sibirica*	1	4	5	Heart and gastrointestinal tract diseases	Water decoction; overground part
5	*Agrimonia eupatoria*	2	2	2	Larynx diseases, laryngitis	Water decoction; overground part
6	*Alisma plantago-aquatica*	5	2	1	Binding agent	Dry powder; overground part
7	*Anemonoides ranunculoides*	1	2	1	Internal – nervous diseases; external – rubbing during arthritis, arthrosis, osteochondrosis and waist-sacrum radiculitis	Tincture; rhizosomes
8	*Antennaria dioica*	1	1	1	Haemostatic during uterus haemorrhage	Water decoction; overground part
9	*Anthemis tinctoria*	2	3	3	Jaundice during hepatitis and other liver diseases, face skin bleach	Milk, water decoction; overground part; inflorescence
10	*Astragalus danicus*	2	2	3	Tonic; takes away tiredness and restores strength	Water decoction; fresh overground phytomass
11	*Athyrium filix-femina*	1	1	1	Antihelmintic, insecticide, abortive agent; internal – during arthritis, arthrosis	Water decoction; tincture; hot compresses; rhizosomes
12	*Atragene sibirica*	1	2	2	Permanent headaches	Water and milk decoction; overground part; inflorescense
13	*Cacalia hastata*	1	2	1	Veterinary – fastens placenta exit	Water decoction; overground part
14	*Calluna vulgaris*	4	4	5	Heart diseases; hypertension	Water decoction; overground part
15	*Calypso bulbosa*	1	4	5	Heart diseases	Fresh stem and blossoms
16	*Campanula cervicaria*	3	2	4	Sedative agent for kids; enuresis	Water steams, apartment fumigation; overground part
17	*Campanula glomerata*	2	2	4	Inflammatory diseases (incl. Erysipelatos) and throat; alcoholism	Water decoction; overground part

Table 2. Continuation.

No.	Species	Growing area	Resort group of species	Stock category	Use	Remedy form, used part or organ
18	*Campanula latifolia*	3	4	4	Post-natal period; in veterinary – fastens placenta exit and lactation intensification	Water decoction; blossoming shoots
19	*Campanula trachelium*	1	2	4	Mastitis and other diseases of nursing women, gonorrhea; in veterinary – fastens placenta exit, cow mastitis	Water decoction; blossoming shoots
20	*Carlina biebersteinii*	2	4	4	Children's nervous diseases; fright, stuttering, enuresis	Apartment fumigation; water decoction; overground part
21	*Centaurea sumensis*	1	4	4	All female diseases; complications after childbirth; relief of post-natal period	Water decoction; overground part; tincture
22	*Chamaenerion angustifolium*	1	1	1	Headaches (incl. vasomotor), diuretic, reduces arterial pressure	Water decoction; overground part, fresh young shoots
23	*Chimaphila umbellata*	1	4	3	Edemas and swellings; hypertension, salts accumulation; kidney and gall-bladder stones; prolapse of viscera; kidney diseases	Water decoction; overground part; tincture
24	*Cirsium heterophyllum*	1	1	1	Breath relief; phlegm remove; relief of general condition of seriously ill patients with high temperature	Water decoction; overground part
25	*Coronaria flos-cuculi*	2	1	1	Headaches; insomnia; children's high excitability	Water decoction; overground part; pillows
26	*Cuscuta europaea*	6	3	2	Uterus and other viscera prolapse	Baths; water decoction; overground part
27	*Cynoglossum officinale*	2	4	4	Insecticide	Powder; overground part
28	*Cypripedium calceolus*	1	4	5	Headaches; children's fright	Water decoction; overground part fresh leaves – external
29	*Dactylorhiza fuchsii*	1	4	5	Immunemodulator; raises potency	Fresh and dry pips; powder
30	*Daphne mezereum*	1	4	4	Toothache; umbilical cord inflame; vertigo	Fruit tincture and extract; fresh bark
31	*Delphinium elatum*	1	4	4	Revulsive; wound healing	Compresses; overground part
32	*Dianthus deltoides*	2	2	1	Headaches; insomnia and uneasy sleep; sedative for kids and ill	Pillows; water decoction; overground part
33	*Dianthus superbus*	3	2	1	Headaches; insomnia and uneasy sleep; sedative for kids and ill	Fumigation; pillows; water decoction; overground part
34	*Diphasiastrum complanatum*	1	4	4	Insecticide for parasites of head and skin of men and animals	Water decoction; overground part
35	*Dracocephalum thymiflorum*	2	4	4	Colds; respiratory tract inflammation; external – arthritis, arthrosis	Inhalation; herbage steam with oat straw
36	*Drosera anglica*	4	4	4	Colds; children's nervous diseases; wart reduction	Water decoction; external – fresh herbage and juice
37	*Drosera rotundifolia*	4	2	3	Colds; children's nervous diseases; wart reduction	Water decoction; fresh overground part
38	*Dryopteris austriaca*	1	2	2	Arthritis, arthrosis, osteochondrosis and waist-sacrum radiculitis; waist osteochondrosis	Compresses and envelopment from fresh overground parts
39	*Dryopteris carthusiana*	1	2	2	Arthritis, arthrosis, osteochondrosis and waist-sacrum radiculitis; waist osteochondrosis	Compresses and envelopment from fresh overground parts
40	*Dryopteris filix-mas*	1	1	1	Antihelminthic, diarrhea; fronds – arthritis, arthrosis, osteochondrosis and waist-sacrum radiculitis; waist osteochondrosis	Water decoction; rhizosomes; hot compresses and envelopment from fresh overground parts
41	*Equisetum fluviatile*	5	1	1	Arthrosis	Baths; hot compresses; overground part
42	*Equisetum hyemale*	1	2	3	Arthrosis	Water decoction; overground part
43	*Equisetum sylvaticum*	1	1	1	Diuretic	Fresh and roasted young sporiferous shoots

Table 2. Continuation.

No.	Species	Growing area	Resort group of species	Stock category	Use	Remedy form, used part or organ
44	*Filipendula ulmaria*	3	1	1	Arthritis, arthrosis, osteochondrosis and waist-sacrum radiculitis; waist osteochondrosis; tonsillitis, colds, inflammation of lungs and pleura; hypertension; auditory nerve neuritis, nervous diseases; preventive measures for oncological diseases, stomach and gastrointestinal tract disorders; infectious diseases; tea substitute	Tincture; water decoction; overground part and underground part; blossoming stems
45	*Filipendula vulgaris*	3	4	3	Similarly to *Filipendula ulmaria*. But is considered to be less efficient	Tincture; water decoction; overground part and underground part; blossoming stems
46	*Gentiana cruciata*	2	4	3	Choleretic, binding, antiphlogistic; sterility	Water decoction; overground and underground parts
47	*Geranium pratense*	2	2	1	Arthrosis, traumatic arthritis; children's fright	Baths; hot compresses; apartment fumigation; overground part
48	*Geum rivale*	3	2	1	Treating "omphalos" and "overdo"	Water decoction; overground part
49	*Geum urbanum*	3	2	2	Treating swellings and other ovary diseases, women "overdo"	Bath; water decoction; overground part
50	*Hierochloe odorata*	1	4	4	Headaches; gastrointestinal tract diseases	Water decoction; overground part
51	*Hypericum elegans*, *H. hirsutum*, *H. maculatum*, *H. perforatum*	2	2	2	Children's fright and enuresis; energizer; external – wound healing, seeds – laxative	Water decoction; overground part; tincture; ointment; blossoms, seeds
52	*Inula britannica*	2	2	3	Immunemodulator; external – wound healing	Fresh herbage, water steams, ointment, rhizosomes
53	*Iris pseudacorus*	5	4	4	Choleretic, cordial, immunemodulator	Water decoction; all parts of plant
54	*Juncus effusus*	4	2	1	Old men and children's enuresis	Water decoction; overground part – internal; baths
55	*Juncus filiformis*	4	2	2	Menstrual cycle disorders; uterus haemorrhage	Water decoction; overground part
56	*Larix sibirica*	1	2	2	Resin – gastrointestinal tract diseases and gum, wound healing, cones – waist-sacrum radiculitis; waist osteochondrosis	Resin – tincture; unripe cones
57	*Lathyrus pratensis*	2	1	1	Colds	Water decoction; overground part
58	*Leucanthemum vulgare*	2	1	1	Antipyretic, antiphlogistic, abortive at early pregnancy	Tincture, extract; overground part and/or inflorescence
59	*Linnaea borealis*	1	2	1	Sedative, soporific, cough, injury, hypodermic haematomas	Tincture, water decoction; overground part
60	*Lonicera pallasii*	1	2	3	Hypertension	Tincture; fruit confiture
61	*Lysimachia vulgaris*	2	2	2	Binding and stabilizing agent during gastrointestinal tract disorders	Water decoction; overground part; tincture
62	*Lythrum salicaria*	3	2	2	Gastrointestinal tract diseases, different inflammations	Water decoction; overground part
63	*Matteuccia struthiopteruis*	1	2	2	Antihelmintic, insecticide; fronds – arthritis, arthrosis	Water decoction; rhizosomes; hot compresses; fresh fronds
64	*Melampyrum nemorosum*	3	4	5	Children's fright	Apartment fumigation; overground part
65	*Mentha arvensis*	2	3	1	Colds, hypertension, badly healing wounds; slag eduction; scrofulous	Water decoction; overground part
66	*Mentha longifolia*	5	4	4	Colds, hypertension, badly healing wounds; slag eduction; scrofulous, sedative	Inhalation; steams; water decoction; overground part and oat straw
67	*Moneses uniflora*	1	4	5	Cardiac stimulation, hypertension, insomnia, men stimulant, eases women menopause passing; causes dreams of erotic content	Water decoction, tincture; fresh overground oart
68	*Myosotis palustris*	3	2	2	Myocarditis	Water decoction; overground part
69	*Nymphaea candida*	5	4	4	Heart pains	Water decoction; blossoms

Table 2. Continuation.

No.	Species	Growing area	Resort group of species	Stock category	Use	Remedy form, used part or organ
70	*Paeonia anomala*	3	4	5	Petals – children's diarrhea; rhizosomes – during women diseases, irregular painful periods, menopause	Water decoction, petals; tincture, underground part
71	*Parnassia palustris*	3	2	2	Myocarditis, gonorrhea, scrofulous, in veterinary – cow mastitis	Water decoction; overground part hot compresses, baths
72	*Platanthera bifolia*	1	4	4	Men stimulant, intensifies potency; menopause, immunemodulator for old men and children	Fresh plants (all parts); powder, water or milk decoction pips, water decoction; blossoms
73	*Polygala comosa*	2	2	2	Sedative during neurosis, children's fright, viscera prolapse, liver and heart diseases, hypertension, oncology diseases	Water decoction and tincture; overground part
74	*Populus tremula*	1	1	1	Analgetic, wound healing, aseptic, binding	Bark, powder and water decoction
75	*Potentilla argentea*	2	2	2	Gynecology inflammation, viscera prolapse, "overdo", liver, kidney, heart, gastrointestinal tract diseases, analgetic	Baths and water decoction; overground part and/or all parts
76	*Primula macrocalyx*	3	4	5	Immunemodulator, heart pains, softening agent during inflammations of upper airways	Water decoction; fresh overground part
77	*Prunella vulgaris*	2	1	1	Throat rinse during inflammations	Dilute tincture; milk and water decoction; overground part
78	*Pteridium aquilinum*	1	4	4	Binding, antihelmintic; cough; arthritis, arthrosis, waist-sacrum radiculitis; waist osteochondrosis	Water decoction; overground part; hot compress
79	*Pulsatilla patens*	1	4	4	Insomnia, nervous diseases; headaches; intestinal diseases; depigmentation	Water decoction; overground part tincture; thick boiled out decoction
80	*Pyrola rotundifolia*	1	2	2	Cordial stimulator	Water decoction; overground part; tincture
81	*Ranunculus sceleratus*	2	2	1	Irritant and revulsive; arthritis, arthrosis	Water decoction; fresh overground part; compresses
82	*Scleranthus annuus*	6	3	2	Viscera prolapse	Water decoction; bath; overground part
83	*Sedum telephium*	3	2	3	Traumatic arthritis; wound healing; gonadotropic, men stimulant, delays menopause and eases it; digestive system diseases	Water decoction; overground part, tincture; hot baths and compresses; fresh juice
84	*Senecio vulgaris*	2	3	2	In gynecology – haemorrhage	Water decoction; overground part
85	*Spergula arvensis*	6	3	2	Viscera prolapse	Water decoction; overground part; bath
86	*Stachys palustris*	3	2	2	Diuretic, antiphlogistic	Water decoction; overground part
87	*Symphytum officinale*	6	3	3	Trombophlebitis, varicose veins; in veterinary – fastens fracture knitting	Ointment from fresh underground part, white-egg and vegetable oil or inner adipose
88	*Thlaspi arvense*	6	3	2	Analgetic, revulsive, waist-sacrum radiculitis; waist osteochondrosis	Hot compresses, overground part; tincture, seeds
89	*Trientalis europaea*	1	1	1	Uterus fibroma, women sterility caused by different reasons; prevents miscarriages	Water decoction; overground part
90	*Trifolium aureum*	6	3	3	Headaches, colds, tonsillitis, insomnia	Water decoction; fresh overground part, steams, pillow; overground parts
91	*Trifolium pratense*	2	1	1	Headaches, colds	Water decoction; overground part
92	*Tripleurospermum perforatum*	6	3	1	Colds; jaundice during hepatits	Water decoction; inflorescence
93	*Veratrum lobelianum*	3	2	3	Scab, gastrointestinal tract disorder, rheumatism and other diseases with back and joint pains – ointment from rhizosomes and cut stems of wild rosemary	Water decoction; overground part; ointment, overground parts; blossoming stems of wild rosemary

Main places of vegetation are marked the following way: 1, forests, edges; 2, upland meadows; 3, flood-land meadows and shrubs thickets in flood-lands; 4, swamps; 5, rivers, lakes and other ponds; 6, weed (ruderal, segetal).

Conclusion

93 species of wild plants are used in folk medicine of Kirov region (6.2 % of the total number of species of vascular plant flora). 25.0 % of the species used in folk medicine of the region have quite original ways of application: *Calypso bulbosa*, *Campanula trachelium*, *Moneses uniflora*, *Platanthera bifolia*, etc. For the first time the use of *Actaea erythrocarpa*, *Campanula latifolia*, *Centaurea sumensis*, *Dryopteris austriaca* was defined. The most popular medicinal plants are *Chimaphila umbellata*, *Moneses uniflora*, *Carlina biebersteinii*, *Potentilla argentea*, *Chamaenerion angustifolium*, *Polygala comosa*, *Campanula glomerata*. These species can become top-priority objects of pharmaceutical researches. It is likely that during further studies of plants used in folk medicine their curative properties are confirmed.

More than half of species (67.8 %) have stocks of categories 1–3 and can be the objects of industrial collection, while 29 plant species used in folk medicine are rare and need protection. For decline of exploitation load on these species it is essential to organize their protection, population conditional monitoring and to work out the technologies of cultivation.

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Ethnobotany of Çan (Çanakkale) from Turkey

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Abstract. The city of Çan with an area of 887 km^2 is situated in the state of Çanakkale lying in the northwest of Marmara Region of Turkey. The city has a population of 53 thousand. An ethnobotanical survey was undertaken during 2002–2003. Our investigations revealed that 102 plant taxa belonging to 46 families are used by the local dwellers in this area for different purposes. *Salvia officinalis*, *Mentha arvensis* and *Tilia rubra* are used medicinally, 51 taxa as food and spices, 3 as dye plants, 4 for firewood purposes and 8 as ornamentals. However, majority of the taxa are consumed on multipurpose basis and the plant parts consumed mostly are leaves.

Key words: Çanakkale, ethnobotany, Turkey

Introduction

A large number of plants are consumed by humans for different purposes. The wild plant foods have played a key nutritive role in the cuisines of rural populations all over the world. At the same time the healing power of plants has always been very important for human well being, and most of our modern medicine is based on our traditional knowledge. Healing practices with plants are ongoing in many countries, and provide basic health care especially for the rural communities. Due to the renewed interest in ethnobotany especially over the past decade, it has become important that we establish a proper knowledge base of these plants, bringing together information on their ecology, habitat and distribution. Indigenous resource management strategies will prove of great help in this connection. Interest in plants in Turkey is also increasing and a lot of work is being carried out. Several publications have been made by different investigators notable among them being: Gümüş (1994), Işık & al. (1995), Sayar & al. (1995), Çubukçu & al. (1996), Alpınar (1999), Çelik & al. (1999), Ertuğ (2000), and Uysal & al. (2005). This statement has been highlightened by Başer & al. (1986).

This paper describes ethnobotanical aspects of the widely distributed major plants in Çan, situated in the state of Çanakkale lying in the northwest of Marmara Region of Turkey (Fig. 1). It covers an area of 887 km^2 and includes 1 muncipality with 51 villages. The population is 53 000 and these include migrants from different areas (14 villages), Pomak (4 villages), Yörük (6 villages), Manav (16 villages), and mixed population (11 villages) (Anonymus 1973). More than 50 % of these are farmers rest deal in animal raising and forest products.

Fig. 1. Map of surveyed area and sites of information.

Material and methods

Our studies were conducted during 2002–2003 in the city of Çan from Çanakkale. In all 9 villages on the basis of cultural differences were surveyed (Fig. 1). These villages are Bostandere, Kalburcu, Karakoca, Hacılar, Büyükpasa, Derenti, Terzialan, Etili and Yuvalar. Field investigations included surveys of local markets and interviews with 71 villagers. Collections of ethnobotanical data were made mainly in and around the rural areas noting the precriptions for their uses, local names, and parts used, distribution, and informant number and collection number. The specimens were identified with the help of *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis & al. 1988; Güner & al. 2000) and were deposited in the Herbarium of Faculty of Science & Arts, Çanakkale Onsekiz Mart University under the collector numbers as (Ismet Uysal, Uys.; Nehir Avcıoğlu, Avc.).

Results and discussion

Our investigations revealed that 102 plant taxa belonging to 46 families are used by the local dwellers in this area for different purposes. Out of these 70 are herbs, 12 shrubs and 20 trees. These belong to *Rosaceae* (11), *Lamiaceae* (10), *Asteraceae* (7), *Apiaceae*, *Cruciferae*, *Poaceae* (5 each), *Fabaceae*, *Liliaceae* (4 each), *Caprifoliaceae*, *Malvaceae* (3 each), *Caryophyllaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Fagaceae*, *Oleaceae*, *Pinaceae*, *Polygonaceae*, *Urticaceae*, *Vitaceae* (2 each), *Araceae*, *Betulaceae*, *Boletaceae*, *Cornaceae*, *Corylaceae*, *Crassulaceae*, *Cupressaceae*, *Elaeagnaceae*, *Ericaceae*, *Hypericaceae*, *Juglandaceae*, *Lythraceae*, *Moraceae*, *Papaveraceae*, *Plataginaceae*, *Platanaceae*, *Portulacaceae*, *Punicaceae*, *Ranunculaceae*, *Rhamnaceae*, *Salicaceae*, *Scrophulariaceae*, *Tiliaceae*, *Typhaceae*, *Verbenaceae*, *Violaceae* and *Zygophyllaceae* (1 each). Out of 102 taxa: 65 are used medicinally, 51 as food and spices, 3 as dyes, 4 as fuel, 8 as ornamentals and 15 for other purposes.

The demographic features of the reference persons were recorded and majority of these were above an age of 50 (56.3 %), 78.9 % being women and house wives, with primary school education and were married. They have been living in these villages for generations. Mostly they work in the city centre but at the same time try to earn their livelihood from carpet making, animal raising and selling of plants in the markets. House wives were more dealing with plants (Table 1).

Use of plants on the basis of different organs: 28 % leaves, 17 % fruit, 16 % stem, 15 % above-ground, 10 % flower and 10 % root, 3 % cone and 1 % seed. The leaves are mostly used in the plants. The information was gathered from 71 persons; they were using, collecting or selling the plants. The number of collectors used was the highest. Almost all of these were above their mid ages, because their experience is much more than young people (Table 1). They preferred random testing.

Abies nordmanniana Spach subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen, *Arum maculatum* L., *Artemisia absinthium* L., *Cornus mas* L., *Ecballium elaterium* (L.) A. Rich., *Eryngium campestre* L., *Hypericum perforatum* L., *Juglans regia* L., *Lythrum salicaria* L., *Marrubium vulgare* L., *Origanum majorana* L., *O. vulgare* L. var. *hirtum* (Link) J.H. Ietswaart, *Petroselinum crispum* (Mill.) Nyman, *Planta-*

Table 1. Classification of the informants according to their demographic features.

	Demographic features	Number	Percentage
Age of informants, years	Less than 19	1	1.4
	Between 19 and 31	7	9.9
	Between 31 and 49	23	32.4
	Over 50	40	56.3
Level of education	Illiterate	2	2.8
	Literate[1]	10	14.1
	Graduated from elementary or middle school	59	83.1
Marital status	Unmarried	4	5.6
	Married	66	93.0
	Widowed	1	1.4
Employment status[2]	Employment	19	26.8
	Not working or unemployment	52	73.2
Residence[3]	In large city	9	12.7
	In town or village	62	87.3
Duration of residence in the survey area	Less than 10 years	5	7.1
	More than 10 years	66	92.9
Gender of informants	Female	56	78.9
	Male	15	21.1

[1] – Literate indicates that the informant can read and write, but has not attended any school.
[2] – Since agriculture is the main livelihood, most of the informants work in their fields; hence percentages described here should not be accepted as an indication of the unemployment status of the region.
[3] – Place where the informant has lived for longest period of his or her life.

go lanceolata L., *Rosa canina* L., *Rubus canescens* DC., *Rumex patientia* L., *Salvia fruticosa* Mill., *S. officinalis* L., *Sambucus nigra* L., *Tribulus terrestris* L., *Urtica dioica* L., *Vitis vinifera* L. taxa differ from other publications are used as medicinal plants (Doğan & al. 2005; Özgen & al. 2005; Özgökçe & Özçelik 2005; Şimşek & al. 2005).

Anethum graveolens L., *Foeniculum vulgare* Mill., *Oenanthe pimpinelloides L.*, *Helianthus tuberosus* L., *Lactuca sativa* L., *Boletus edulis* Bull., *Viburnum opulus* L., *Stellaria media* (L.) Vill., *Chenopodium album* L., *Spinacia oleracea* L., *Cornus mas* L., *Corylus avellana* L., *Brassica oleracea* L., *Eruca sativa* Mill., *Lepidium sativum* L., *Sinapis alba* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Elaeagnus angustifolia* L., *Vicia faba* L., *Castanea sativa* Mill., *Juglans regia* L., *Mentha arvensis* L., *Thymus longicaulis* C. Presl subsp. *chauhardii* (Boiss. & Heldr. ex Rchb. f.) J. Jalas, *Allium cepa* L., *Allium sativum* L., *Saccharum officinarum* L., *Hibiscus esculentus* L., *Olea europaea* L. subsp. *europaea*, *Zea mays* L., *Rumex patientia* L., *Portulaca oleracea* L., *Mespilus germanica* L., *Pyrus elaeagnifolia* Pall., *Rosa* sp. L., *Punica granatum* L., *Malus sylvestris* Mill. var. *mitis* (Wallr.) Mansf., *Rubus canescens* DC., *Vitis vinifera* L. and *V. sylvestris* C.C. Gmel. taxa were reported by all informants as food plants. *Matthiola fruticulosa* (L.) Maire and *Phragmites australis* (Cav.) Steud. taxa were reported by some informants as ornamental plant. *Chamaecytisus austriacus* Link and *Spartium junceum* L. taxa were reported by some informants as broom plant. *Asphodelus aestivus* Brot. and *Platanus orientalis* L. taxa were reported by some informants as local belief.

In conclusion we can say that leaves and fruits although used as foods are mainly evaluated as medicinal plants especially *Salvia officinalis*, *Mentha arvensis* and *Tilia rubra*. Most important plants among 102 taxa collected during this survey and their uses are given in Table 2.

Table 2. List of plants used as foodstuff or as a remedy in Çan.

Latin names	Local names	Part(s) used	Uses	Preparation / application	Informant number and collection number
Apiaceae					
Eryngium campestre L.	Çakır-Boğa dikeni	Stem	Kidney problems	Boiled in the water and drunk	42 Uys. 2
Araceae					
Arum maculatum L.	Yılan cücüğü	Roots	**1.** Headaches **2.** Haemorrhoids	**1.** Roots left on fire then placed in a cloth and put on head **2.** Roots of hazelnut bigness taken in the morning and evening	**1.** 14 **2.** 22 Avc. 35
Asteraceae					
Artemisia absinthium L.	Pelin otu	Leaves	Diabetes	Boiled in the water and drunk	32 Uys. 6
Caprifoliaceae					
Sambucus ebulus L.	Kokar-Nazlı -Karabaş ot	Leaves	**1.** For cardiodynia and as tension regulator **2.** For stomach pain	**1.** Boiled in the water and drunk **2.** Leaves put in hot water for softening, and then placed on the stomach	**1.** 19,45 **2.** 19,55 Avc. 67
Caryophyllaceae					
Dianthus caryophyllus L.	Arnavut karanfili	Whole	Ornamental plant	Outdoors and indoors	1,2 Uys. 16
Crassulaceae					
Sedum telephium L.	Dam koruğu	Whole	Ornamental plant	Outdoors	53 Avc. 70
Cucurbitaceae					
Ecballium elaterium (L.) A. Rich	Acı dülek	Fruits, latex	**1.** Sinusitis and allergy **2.** Haemorrhoids	**1.** Allergy area is covered with latex. Fruit juice used for sinusitis, but dose is very important **2.** Fruits grinded, latex mixed with flour and then consumed on an empty stomach in the morning	**1.** 49 **2.** 55 Avc. 68

Table 2. Continuation.

Latin names	Local names	Part(s) used	Uses	Preparation / application	Informant number and collection number
Clusiaceae					
Hypericum perforatum L.	Kantoron, sarıca otu, sarıca yüz otu	Herb	**1.** Stomach pain and ulcer **2.** Calm down **3.** Haemorrhoids	**1.** As infusion with linden, mint, thyme and camomile, then drunk as tea **2.** As decoction, flowers left in hot water, tea **3.** Above-ground parts boiled and diseased parts kept in it	**1.** 2,34,54 **2.** 2,8,17 **3.** 63 Avc. 61
Lamiaceae					
Mentha arvensis L.	Nane	Leaves	Against colds	Leaves are boiled and water drunk as tea	1–71Avc. 11
Melissa officinalis L.	Melisa, oğul otu	Above-ground	**1.** Reduces cholesterol and regulates blood pressure **2.** Overcome heart beating and as calm down agent **3.** Bee collecting	**1.** Boiled flowers/leaves drunk on an empty stomach in the morning **2.** Leaves, flowers, branches boiled, drunk as tea **3.** Plants left near the hive	**1.** 19,21 **2.** 2,39 **3.** 39 Avc. 32
Salvia fruticosa Mill.	Boş-Boz şaplağı, morşablo	Above-ground	**1.** Lower the fever, for tonsillitis **2.** Heart problems **3.** Cough and throat problems	**1.** Green herb boiled with thyme and water drunk **2.** Dry leaves boiled with linden and drunk as tea **3.** Plant without roots boiled and sugar added and drunk as tea	**1.** 15 **2.** 21 **3.** 5,32,38,61,62 Avc. 30,47
Teucrium chamaedrys subsp. *lydium* O. Schwarz	Mayasıl, mahmutotu	Leaves, flowers	Haemorrhoids	Boiled in the water and drunk	34,53,57 Avc. 73
Oleaceae					
Phillyrea latifolia L.	Pırnal, pınar	Whole	**1.** Haemorrhoids **2.** Broom and as hedge **3.** Fodder	**1.** Buds taken on an empty stomach in the morning **2.** Branches used **3.** Leaves and fruits used	**1.** 23,28 **2.** 7,11,14,18 **3.** 11 Avc. 12
Plantaginaceae					
Plantago lanceolata L.	Sinirli ot, damarlı ot	Leaves, flowers	**1.** Against boils **2.** Stomach pain	**1.** Leaves heated and crushed and placed on wound **2.** Flowers boiled and drunk	**1.** 2,49,63 **2.** 19 Avc. 69
Polygonaceae					
Rumex patientia L.	Akıllı labada	Leaves	**1.** Dysentery **2.** Haemorrhoids	**1.** Leaves boiled and water drunk **2.** Leaves boiled and water drunk	**1.** 41 **2.** 51 Avc. 3
Rumex tuberosus L.	Kuzu kulağı	Stem, leaves	Tension, kidney stone	Eaten fresh or added to salad	1,2,3,9,12,63,66 Avc. 17
Punicaceae					
Punica granatum L.	Nar	Flowers	For blood pressure	Flowers boiled and water drunk	53 Uys. 39
Ranunculaceae					
Nigella sativa L.	Karaca otu, çörek otu	Above-ground	Cleaning of utensils	Dry plants used to rub utensils	43 Uys. 40
Rhamnaceae					
Paliurus spina-christi Mill.	Karaçalı	Leaves	Diabetes and burning pain during urination	Leaves boiled, waited for over night, remained water filtrated, and then drunk before breakfast	55 Avc. 60
Rosaceae					
Crataegus orientalis Bieb.	Alıç, yemişen tikeni	Fruits	Throat softening	Fruits boiled and water drunk	61,62 Uys. 41

Table 2. Continuation.

Latin names	Local names	Part(s) used	Uses	Preparation / application	Informant number and collection number
Cydonia oblonga Mill.	Ayva	Leaves, fruits	**1.** Cough and throat pain **2.** Lip cracks	**1.** Leaves boiled and water drunk **2.** Seeds boiled and rubbed cold on lips	**1.** 29,44,61 **2.** 61 Avc. 14
Mespilus germanica L.	Muşmula	Fruits, leaves	**1.** Dysentery **2.** Kidney stone	**1.** Leaves boiled and water drunk **2.** Fruits left in water and water drunk for 3–5 days	**1.** 29 **2.** 41 Uys. 42
Potentilla reptans L.	Beş parmak otu, tülü	Above-ground	Recovery of burns	Chopped with wild carnation and evil eye herb roasted on salt less butter, strained, put on burns	51,52,53 Avc. 72
Prunus spinosa L.	Güvem	Fruits	Diabetes	Fruits consumed	1 Uys. 42
Pyrus elaeagnifolia Pall.	Cepni armutu, ahlat, kırahlat, yabani armut	Fruits, leaves	**1.** Diabetes **2.** Guater **3.** Pickle **4.** Syrup	**1.** Pickles, water drunk everyday **2.** Leaves boiled and water drunk. **3.** 1 bucket of fruits boiled with half bucket of water, strained, sugar added to make syrup **4.** Fruits left in salt water for 1 month	**1.** 1,41,50 **2.** 57 **3.** 12 **4.** 11,12,41,50,57 Avc. 62
Rosa canina L.	Deli gül, kuşburnu, öküz götü	Roots, fruits	**1.** Throat pain **2.** Kidney stone	**1.** Fruits boiled and water drunk **2.** Roots boiled and water drunk morning and evening, on an empty stomach	**1.** 17,28,38,61,63 **2.** 17,22,28,38,48,49,61,63 Avc. 43,59
Rosa, Old Rose "Gallica Roses" (cultivated form)	Gül	Petals	Syrup and jam	Jam: 1 kg water, 1.5 kg sugar, 1 handful rose petals, boiled. Jam taken from the heat syrup made according to water, with rose petals, salt of lemon added, boiled, and then sugar added and boiled again	1–71 Avc. 55
Rubus canescens DC.	Böğürtlen	Fruits	Mouth wounds	Fruits eaten	1 Uys. 43
Sanguisorba minor L.subsp. *muricata* (Spach) Briq.	Nazar otu	Leaves	Burns	Used like *Potentilla reptans*	51,52,53 Avc. 58
Scrophulariaceae					
Verbascum sinuatum L.var. *sinuatum*	Balık otu, sığır kuyruğu	Whole	**1.** Ornamental plant **2.** Fishing	**1.** Pot and garden plant **2.** Flower branches left on the water till fishes come to the top	**1**. 3 **2**. 61,62,65Uys. 45
Tiliaceae					
Tilia rubra DC. subsp. *caucasica* (Rubr.) V. Engler.	Ihlamur	Leaves, flowers	**1.** Throat pain **2.** Make rice balls	**1.** Flowers and leaves boiled and water drunk **2.** Fresh leaves used to make stuffed dish	**1**. 1–71 **2**. 51 Uys. 46
Typhaceae					
Typha angustifolia L.	Papur	Stem	Pack saddle	Stem used with *Phragmites australis* to make pack saddle	64 Uys. 47
Urticaceae					
Urtica dioica L.	Isırgan	Whole	**1.** Haemorrhoids, diabetes and cancer **2.** Stop hair fall **3.** Rheumatic pains **4.** Stomach and intestine trouble	**1.** Plant boiled and water drunk **2.** Plant boiled and hairs washed with water **3.** Plant put on pain area **4.** Above-ground parts of plant boiled in water left to cool down and water drunk on an empty stomach in the morning with a spoon of honey	**1**. 1,31,35,50,65,67,68 **2**. 1 **3**. 48,50,65 **4**. 63 Avc. 26
Urtica pilulifera L.	Kara ısırgın	Above-ground	Heart trouble, cancer, piles	Plant boiled and water drunk	19,69 Avc. 28

Table 2. Continuation.

Latin names	Local names	Part(s) used	Uses	Preparation / application	Informant number and collection number
Verbanaceae					
Vitex agnus-castus L.	Ayıd, ayıt	Seeds, branches, leaves	**1.** Basket	**1.** Fresh branches shaped out for basket making	**1**. 16
			2. Diabetes and hypertension	**2.** Seeds dried, pressed in coffee mill and 1 tea spoon taken on an empty stomach in the morning	**2**. 23,30
			3. Sweating of babies	**3.** Green leaves placed on the body of baby	**3**. 59
					Avc. 63
Violaceae					
Viola odorata L.	Kır Menekşesi	Leaves	Burn healing	Used like *Potentilla reptans*	51,52,53
					Uys. 48
Zygophyllaceae					
Tribulus terrestris L.	Çoban çökerten	Leaves	Kidney stone	Leaves dried, boiled and water drunk	19,38
					Uys. 51

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Some local plant names and uses in Akçakoca (Düzce) and other part of Turkey

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Abstract. The richness of Turkish flora reflects to the local plant names and their miscellaneous uses. Some Turkish local plant names and their uses were compiled in the floristic excursions between 1978–1985 and 2000–2005 years during the study of Akçakoca's flora. The 50 collected plants belong to 35 families. These plant species have 57 Turkish local names. Out of them, 37 species are used for various purposes. The people have used these plants as medicinal (16 species), as edible (7 species) and for other purposes (14 species). The uses of 7 out of 50 plant species are reported for the first time; 1 out of 50 is probably an interesting record. This knowledge is getting lost rapidly due to the human impact such as immigration, modernity, etc.

Key words: Akçakoca (Düzce), ethnobotany, local names, plant uses, Turkey

Introduction

In the recent years, a lot of research is realised about Turkish ethnobotany (Keskin & Alpınar 2002; Ertuğ 2004; Özgen & al. 2004; Yıldırımlı 2004). The important factor of this increase is raised of number of studies of Turkish flora and revision. But obtained data are not carried into effect in Turkey. Both the richness of Turkish flora and ethnobotanical flora are coincidence. In this research, a profile of Turkish ethnobotanical flora is emphasised.

Material and methods

Knowledge has been compiled from all over Turkey during floristic trips between 1978–1985 and 2000–2005. The plant species are enumerated in alphabetical order at every category level in the findings. Author's abbreviations follow Brummitt & Powell (1992). Obtained ethnobotanical data are ordered subsequently: family, genus and species names, local name, ethnobotanical uses, locality, collector number and herbaria. Some of them are discussed in results. The cited specimens are kept in HUB, KNYA and Hb. Yıldırımlı. (*) indicates the first records, (**) – the interesting property from Turkey.

Results

Abbreviations: **ADK** – Aslı Doğru Koca; **ŞY** – Şinasi Yıldırımlı; **HUB** – the herbarium of Hacettepe University in Ankara; **KNYA** – the herbarium of Selçuk University in Konya; **Hb. Yıldırımlı** – the private herbarium of Ş. Yıldırımlı in Ankara.

PTERIDOPHYTA

Aspidiaceae

Dryopteris borreri Newman, **eğelti**, forage, A3 Düzce: Akçakoca, Kurugöl village, towards Sarıyayla village, under *Corylus* agriculture fields, hedges, 450–550 m, 26.10.2002, ŞY 28156, ADK (HUB, Hb. Yıldırımlı).

Hypolepidaceae

Pteridium aquilinum (L.) Kuhn, **eğelti**, **gölgelik**, summer houses are covered by ferns, A3 Düzce: Akçakoca, Uğurlu village, roadsides, in *Fagus orientalis* forest, 200–225 m, 22.07.2002, ADK 1824 (HUB, Hb. Yıldırımlı).

Sinopteridaceae

Cheilanthes fragrans (L. f.) Sw., diuretic, C5 Içel: above Işıktepe (Seydişıh) village, maquis, calcareous rocks, 150–500 m, 19.04.1985, ŞY 7594 (HUB, Hb. Yıldırımlı); **kayaotu**, C1 Muğla: Bodrum, between Türkbükü–Bodrum, *Pinus brutia* and mixed forest, 200–500 m, 15.03.1984, ŞY 5883 (HUB, Hb. Yıldırımlı).

SPERMATOPHYTA

GYMNOSPERMAE

Pinaceae

****Pinus nigra*** Arn. subsp. ***nigra*** var. ***caramanica*** (Loudon) Rehder, **karaçam**, the sap is obtained with primitive distillation method. This sap is mixed in milk and honey, then drunk. This mixture is well to pulmonary, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, 1500–1600 m, 08.06.2003, M. Dinç 1701 & H.H. Doğan (KNYA), the sap of phloem, called "**kamalak**", is well to pulmonary. As the sap of the phloem is mixed in milk and honey and drunk before breakfast. It must have breakfast after an hour, C6 Osmaniye: Zorkun pasture, 1600 m, 03.06.2005, M. Dinç 2628 (KNYA).

ANGIOSPERMAE
DICOTYLEDONEAE

Anacardiaceae

****Rhus coriaria*** L., **sumak**, leaves are boiled and used to foot fungus. Fruits are used as spice, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, *Pinus nigra* forest and forest clearings, 1500–1600 m, 08.06.2003, M. Dinç 1726 & H.H. Doğan (KNYA).

Asteraceae

Scorzonera cinerea Boiss., **bozkanak**, the latex obtained from roots is used as mastic, C5 Içel: Aslanköy, Cocakdere, Kıltır mount, alpine steppe, 2000–2100 m, 06.07.2003, M. Dinç 2010 (KNYA).

Berberidaceae

****Berberis crataegina*** DC., **karamuk**, the roots are boiled and drunk then it is well to haemorrhoids. The leaves and fruits are eaten, C5 Içel: Aslanköy, Cocakdere, around Gökviraj, alpine steppe, 1900–2100 m, 03.07.2003, M. Dinç 1867 (KNYA).

Betulaceae

Carpinus betulus L., **gürgen**, it is used as fuel and construction, A3 Düzce: Akçakoca, Alaplı's border, along Kocaman stream, in mixed deciduous forest (*Fagus orientalis*, *Castanea sativa*, *Quercus petraea*), 5–50 m, 29.09.2001, ADK 1559, ŞY (HUB, Hb. Yıldırımlı).

Boraginaceae

****Anchusa leptophylla*** Roem. & Schult. subsp. ***leptophylla***, **kızılcık**, roots are pressed and this mixture is used against the bite of scorpion and bee, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, *Pinus nigra* forest and clearings of forest, 1500–1600 m, 08.06.2003, M. Dinç 1724 (KNYA).

Caprifoliaceae

Sambucus ebulus L., **şahmelik**, the branches are used as brush, A3 Düzce: Akçakoca, Gebekese village, under *Corylus* agriculture fields, 75–90 m, 09.06.2001, ADK 1321 (HUB, Hb. Yıldırımlı).

Sambucus nigra L., **düdüklük**, the branches are used as toys because their hollow like whistle, A3 Düzce: Akçakoca, Alaplı's border, around Kocaman, towards Akçakoca, pasture slopes, 1–40 m, 30.06.2002, ADK 1696 (HUB, Hb. Yıldırımlı).

Convolvulaceae

Convolvulus arvensis L., **gergeşik otu**, A3 Düzce: Akçakoca, Küpler village, along the stream, under *Corylus* agriculture fields, 350–400 m, 07.06.2001, ADK 1264 (HUB, Hb. Yıldırımlı).

Ericaceae

Rhododendron ponticum L., **orman gülü**, **kuma yaprağı**, leaves used for not borning bread, A3 Düzce: Akçakoca, Armutlu village, south slopes, in *Fagus orientalis* forest, 75 m, 18.05.2001, ADK 1162 (HUB, Hb. Yıldırımlı).

Euphorbiaceae

****Euphorbia rigida*** M. Bieb., **sütleğen**, the latex is used against the bite of scorpion, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, *Pinus nigra* forest and forest clearings, 1500–1600 m, 08.06.2003, M. Dinç 1716 & H.H. Doğan (KNYA).

Fagaceae

Castanea sativa Mill., **karakabuk**, A3 Düzce: Akçakoca, Tepe village, in mixed deciduous forest (*Fagus orientalis*, *Castanea sativa*, *Quercus petraea*), 45–75 m, 10.06.2001, ADK 1442 (HUB, Hb. Yıldırımlı).

Hypericaceae

Hypericum scabrum L., **kantaron**, it is used for stomach, ulcer and internal and external injuries, C5 Içel: Aslanköy, Cocakdere, around Gökviraj, rocks, steppe, 1900–2100 m, 06.06.2003, M. Dinç 1582 & H.H. Doğan (KNYA).

Lamiaceae

Cyclotrichium origanifolium (Labill.) Manden. & Scheng., **nane ruhu**, dried leaves are used as tea against gastritis, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, *Pinus ni-*

gra forest and forest clearings, 1550–1600 m, 04.07.2003, M. Dinç 1940 & H.H. Doğan (KNYA).

Sideritis libanotica Labill. subsp. ***linearis*** Bentham, **bozçay**, dried leaves are used as tea against gastritis and relax, C5 Içel: Aslanköy, Cocakdere, around Ardıçlıoluk, clearings of forest, 2000–2100 m, 04.07.2003, M. Dinç 1906 (KNYA).

Oleaceae

Fraxinus angustifolia Vahl subsp. ***angustifolia***, **çınar**, A3 Düzce: Akçakoca, Dadalı village, around harvest, 50–55 m, 05.07.2003, ADK 2385 (HUB, Hb. Yıldırımlı).

Phillyrea latifolia L., **kesme**, C5 Içel: Above Işıktepe (Seydişıh) village, maquis, calcareous rocks, 150–500 m, 19.04.1985, ŞY 7586 (HUB, Hb. Yıldırımlı).

Oxalidaceae

Oxalis pes-caprae L., **dirfil**, C1 Muğla: Bodrum, between Türkbükü–Bodrum, *Pinus brutia* and mixed forest, 200–500 m, 15.03.1984, ŞY 5881 (HUB, Hb. Yıldırımlı).

Plantaginaceae

Plantago major L., **yara otu**, **şimsek otu**, leaves are heated and then used to inflammation. Leaves are boiled and drunk against eczema, A3 Düzce: Akçakoca, banks of Çayağzı stream, marshy, red soils, 2–25 m, 10.06.2001, ADK 1450 (HUB, Hb. Yıldırımlı).

Platanaceae

Platanus orientalis L., **kavlan**, A3 Düzce: Akçakoca, mouth of the Edilli stream, banks of stream, 5 m, 30.06.2002, ADK 1750 (HUB, Hb. Yıldırımlı), **karaçınar**, C2 Denizli: Sarayköy, Babadağ, Karabebek vineyard, 1000 m, 16.07.1983, ŞY 5499 (HUB, Hb. Yıldırımlı).

Polygonaceae

Rumex angustifolius Campd. subsp. ***angustifolius***, **labada**, C4 Konya: Taşkent, around Harzıdın, steppe, 1500–1750 m, 21.07.1985, ŞY 8436 (HUB, Hb. Yıldırımlı).

Rumex conglomeratus Mur., **efelek**, **üfelek**, **labada**, leaves are cooked with onion and rice then eaten, otherwise cooked as "dolma", A3 Düzce: Akçakoca, Uğurlu village, roadsides, in *Fagus orientalis* forest, 200–225 m, 22.07.2002, ADK 1803 (HUB, Hb. Yıldırımlı).

Rumex scutatus L., **kuzukulağı**, leaves are used as food, C1 Muğla: Bodrum, between Türkbükü–Bodrum, *Pinus brutia* and mixed forest, 200–500 m, 15.03.1984, ŞY 5880 (HUB, Hb. Yıldırımlı).

Plumbaginaceae

****Plumbago europaea*** L., **sirken**, it is used for dying of string, B4 Ankara: Elmadağ, *ca*. 1000 m, 19.09.1982, ŞY 4506 (HUB, Hb. Yıldırımlı).

Primulaceae

Cyclamen cilicium Boiss. & Heldr. var. ***cilicium***, **deve tabanı**, tuber is well to diabetis, C5 Içel: Aslanköy, Cocakdere, around Şahinkayası, clearings of *Pinus nigra* forest, 1500 m, 12.06.2005, M. Dinç 2324 & H.H. Doğan (KNYA).

Rhamnaceae

Paliurus spina-christi Mill., **karaçalı**, C2 Denizli: Honaz, Menteş village, Bağarası, along Dinana stream, 600 m, 27.05.1985, ŞY 8059 (HUB, Hb. Yıldırımlı).

Rosaceae

Crataegus orientalis Palas ex M. Bieb. subsp. ***orientalis***, **koyunalıçı**, C4 Konya: Taşkent, around Harzıdın, steppe, 1500–1750 m, 21.07.1985, ŞY 8442 (HUB, Hb. Yıldırımlı).

Laurocerasus officinalis Roem., **taflan**, fresh fruits are eaten, A3 Düzce: Akçakoca, between Melenağzı and Nazımbey villages, under *Corylus* agriculture fields, edge of hedges, 1–50 m, 07.04.2001, ADK 1159 (HUB, Hb. Yıldırımlı).

Mespilus germanica L., **töngel**, **muşmula**, fresh fruits are eaten, A3 Düzce: Akçakoca, Armutlu village, south slopes, in *Fagus orientalis* forest, 75 m, 18.05.2001, ADK 1765 (HUB, Hb. Yıldırımlı).

*****Persica vulgaris*** Mill., **şeftali**, dried leaves are pulverised by grinding in mortar and this powder mixed with olive then this mixture is well to haemorrhoids as external and internal, C5 Içel: Aslanköy, 1450 m, cultivated, 08.10.2005, M. Dinç 2505 (KNYA).

Sorbus domestica L., **avaz**, fresh fruits are eaten, A3 Düzce: Akçakoca, Dadalı village, around harvest, 50–55 m, 05.07.2003, ADK 2373 (HUB, Hb. Yıldırımlı).

Rubiaceae

Asperula serotina (Boiss. & Heldr.) Ehrend., **karıncaotu**, C4 Konya: Taşkent, along stream side, Kulakbendi–Ditren–Aktaş–Kireçocağı–Eşeninsuyu–Sugözü–Ekşielma upland, *Abies*, *Cedrus* forest, 1500–1750 m, 22.07.1985, ŞY 8485 (HUB, Hb. Yıldırımlı).

Galium spurium L. subsp. ***spurium***, **kaynaş**, C3 Isparta: Eğirdir, Sivri mount, rocky and stony places, 900–1200 m, 25.05.1985, ŞY 7973 (HUB, Hb. Yıldırımlı).

Solanaceae

Hyoscyamus aureus L., **tatula**, it is well to injuries, C9 Mardin: Cizre, along Dicle stream, towards Iraq bor-

der, pasture, 400 m, 14.04.1983, ŞY 4648 (HUB, Hb. Yıldırımlı).

Hyoscyamus reticulatus L., **köpekgülü**, C4 Konya: Ankara road, around of engine factory, banks of road, 1000 m, 01.06.1985, ŞY 8147 (HUB, Hb. Yıldırımlı).

Solanum dulcamara L., **köpeküzümü**, B4 Ankara: Elmadağ, *ca*. 1000 m, 19.09.1982, ŞY 4491 (HUB, Hb. Yıldırımlı).

Staphyleaceae

Staphylea pinnata L., **patlak ağacı**, **bilezik**, **tespih**, seeds are died, dried and used as toy by children, A3 Düzce: Akçakoca, around Kurukavak village, under *Corylus* agriculture fields, 500–550 m, 07.06.2001, ADK 1276 (HUB, Hb. Yıldırımlı).

Styracaceae

Styrax officinalis L., **tesbih**, C4 Antalya: Dedetürbelinaz upland pasture, 1150 m, 19.05.1984, ŞY 6182 (HUB, Hb. Yıldırımlı).

Thymelaeaceae

Daphne sericea Vahl, **develik**, C5 Içel: above Işıktepe (Seydişıh) village, maquis, calcareous rocks, 150–500 m, 19.04.1985, ŞY 7587 (HUB, Hb. Yıldırımlı).

Urticaceae

Urtica dioica L., **ısırgan**, seeds are pulverised by grinding in mortar and this powder is mixed with honey and then is eaten. It is used for stomach cancer. Other way this mixture is applied to the knee against rheumatism. The whole plant is added in barrel filled water and waited one month and then used as pesticide, C5 Içel: Aslanköy, Cocakdere, around Gökviraj, alpine steppe, 1900–2100 m, 03.07.2003, M. Dinç 1855 (KNYA).

Zygophyllaceae

Peganum harmala L., **üzerlik**, A5 Sinop: Boyabat, castle, rock, 400–500 m, 18.08.1985, ŞY 8663 (HUB, Hb. Yıldırımlı).

MONOCOTYLEDONEAE

Araceae

Arum orientale M. Bieb., **yılan ağusu**, A3 Düzce: Akçakoca, Gebekese village, under *Corylus* agriculture fields, 75–90 m, 09.06.2001, ADK 1316 (HUB, Hb. Yıldırımlı).

Liliaceae

Allium scorodoprasum L., **körmen**, it is gathered before flowering time and cooked or used as salad, C5 Içel: Aslanköy, Cocakdere, around Katıyayla, mixed forest (*Juniperus–Abies–Cedrus*), 1700–1900 m, 06.07.2003, M. Dinç 2026 & H.H. Doğan (KNYA).

Asphodeline rigidifolia (Boiss.) Baker, **çiriş otu**, leaves are boiled or roasted then eaten as purgative, C5 Içel: Aslanköy, Cocakdere, around Çağaloluk, stream, 1650–1750 m, 06.06.2003, M. Dinç 1512 & H.H. Doğan (KNYA).

Muscari comosum (L.) Mill., **yumurta çili**, whole plant, water and eggs put into a kettle and boiled. Turning blue eggs are used as toys by children, A3 Düzce: Akçakoca, around Doğancılar, field slopes, 20–30 m, 10.05.2003, ADK 2211 (HUB, Hb. Yıldırımlı).

Ruscus aculeatus L., **süpürge çalısı**, branches are tied with a stripe and used as brush, A3 Düzce: Akçakoca, between Esmahanım and Uğurlu villages, under *Corylus* agriculture fields and *Fagus orientalis* forest, 100–200 m, 18.05.2001, ADK 1120 (HUB, Hb. Yıldırımlı).

Poaceae

Sorghum bicolor (L.) Moench, **süpürge otu**, stem is cleaned from seeds by knife and used as brush, A3 Düzce: Akçakoca, Altınçay village, *Corylus* gardens, 15 m, 24.05.2003, ADK 2355 (HUB, Hb. Yıldırımlı).

Discussion

Seven out of 37 useful plants are reported for the first time and 1 is an interesting record from Turkey. The use of *Pinus nigra* is reported for the first time as pulmonary. *Rhus coraria* is used to foot fungus while this species is used as spice. The roots of *Berberis crataegina* are used against haemorrhoids whereas it is known that roots of this species contain dye. *Anchusa leptophylla* is called "kızılcık" in Içel, Aslanköy though the name of "kızılcık" is used for *Cornus*. The roots of this species are used against bite of scorpion and bee. This knowledge is reported for the first time. In Munzurdağları (Erzincan, Tunceli) the leaves of *Anchusa strigosa* are used for stomach ulcer (Yıldırımlı 1985). *Euphorbia* species have a lot of uses as medicinal, fishing, economical, etc. (Yıldırımlı 1991); it is reported for the first time to be used against bite of scorpion. The use of *Plumbago europaea* is reported for the first time as dye (Anonymous 1991). It is an interesting property that the leaves of *Persica vulgaris* are well to haemorrhoids. The use of *Urtica dioica* as pesticide is very interesting and reported for the first time.

Acknowledgements. Some specimens were collected during the research named "Flora and ethnobotany of the Akçakoca District (Düzce)" in the period 2001–2003. This research was supported by Hacettepe University Research Unit (Project No. 01.01.601.004). The authors thank Hacettepe University Research Unit for financial support.

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Cytotoxic activity of Bulgarian *Galanthus* species

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Abstract. Genus *Galanthus* (*Amaryllidaceae*) is represented by two species in Bulgaria – *G. nivalis* and *G. elwesii*, distributed in different parts of the country. In the present study, the plant material was collected from Northeast Bulgaria, where both species grow together, under the same environmental conditions. *Galanthus* species are of great interest with their content of *Amaryllidaceae* alkaloids. This is a group of alkaloids with a wide range of biological activities, used for treatment of Alzheimer disease, Poliomyelitis and different nervous disorders. Total alkaloid mixtures from both species were screened for cytotoxic activity and the results were compared. Cytotoxic activity was determined using brine shrimp (*Artemia salina*) lethality bioassay.

Key words: alkaloids, cytotoxic activity, *Galanthus*

Introduction

Genus *Galanthus* L. (*Amaryllidaceae*) comprises 18 species, distributed in Europe, Asia Minor and the Near East (Davis 1999). The species biosynthesise pharmacologically active alkaloids, so called *Amaryllidaceae* alkaloids, which are known to exhibit different pharmacological effects such as antitumor, acetylcholinesterase inhibitory and cytological activities. Some of alkaloids have been used in the treatment of Alzheimer disease, Poliomyelitis and different nervous disorders (Bastida & Viladomat 2002).

The genus is represented by two species in Bulgaria – *Galanthus nivalis* L. and *G. elwesii* Hook. f. The first species has limited distribution mainly in Eastern Bulgaria and its alkaloid profile is poorly investigated (Sidjmova & al. 2003). The second is wide spread and presented with three subspecies (Kozhuharov 1992). A survey of alkaloid profiles on 16 *G. elwesii* populations shows that crinine type alkaloids, haemanthamine or crinine are predominant alkaloids in most of the populations. Galanthamine type alkaloids have been found only in a few populations (Berkov & al. 2004). There are data that alkaloids possess cytotoxic activity (Bastida & Viladomat 2002).

A number of studies have demonstrated the use of the brine shrimp larvae *Artemia salina* (nauplii) assay to screen plants having cytotoxic activity. The method appears to be convenient, rapid, inexpensive and low toxic amounts are sufficient to perform the test (Solis & al. 1993; Quignard & al. 2003; Krishnaraju & al. 2005).

The object of the present study was to assess the cytotoxic activity on total alkaloid fractions of *G. nivalis* and *G. elwesii* using Brine shrimp (*Artemia salina*) lethality assay.

Material and methods

Plant material

Plant material (aerial parts) from *G. elwesii* and *G. nivalis* was collected near to Obrochishte village, in Northeastern part of Bulgaria, where both species grow under the same environmental conditions. Collection is realised in February 2006, when plants are in flowering period. Plant material was dried at room temperature.

Isolation of total alkaloid fractions

Powdered plant material (40 g of each sample) is individually extracted with 3 % H_2SO_4 (2 h shaking at room temperature). Obtained extracts were filtrated and concentrated mixtures were extracted with EtOAc three times to eliminate lipophilic compounds. The water solutions were basified with 25 % ammonia to

pH 10–11 and extracted three times with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo*. The dry extracts were dissolved in methanol to check for occurrence of alkaloids.

TLC analysis

TLC conditions used for the division of alkaloid fractions: chloroform/methanol/25 % ammonia (11:1:0.6, v/v/v). Alkaloid fractions were spotted on Merck aluminum sheets Kiselgel 60 F_{254} (0.2 mm thin layer, 10 × 20 cm). Compounds were visualised after spraying with Dragendorff's reagent.

Sample preparation

Total alkaloid fractions (50 mg) of both *Galanthus* species were first dissolved in 1 ml dimethylsulphoxide (DMSO). Serial dilutions were made to obtain four concentrations – 1000, 100, 10 and 1 µg/ml.

Preparation of saline solution

Artificial sea water (saline solution) was prepared by dissolving 20 g of sea salt in 1 l of distillated water.

Hatching of brine shrimp larvae

Brine shrimp eggs (*Artemia salina*) were incubated at 27±1 °C in a conical shaped vessel (1 L), containing saline solution under constant aeration and an incandescent lamp for 48 h.

Brine shrimp lethality assay

After hatching, active brine shrimp larvae (nauplii) free from eggshells were collected with a Pasteur pipette. Ten nauplii were introduced into vials containing 5 ml saline solution and graded concentrations (ranging from 1 to 1000 µg/ml) of the tested alkaloid fractions. Experiments were conducted along with control contain 5 ml saline solution, 0.1 ml DMSO and 10 nauplii. The number of affected or dead shrimps at each concentration of the fractions was counted under binocular microscope after 24 hours. Data were analysed with the Finney computer program to determine the LC_{50}.

Results and discussion

Two total alkaloid fractions from aerial parts of *G. nivalis* and *G. elwesii* were checked for cytotoxic activity. TLC analyses showed that both species contain different type of alkaloids. Alkaloid spots of *G. nivalis* and *G. elwesii* had different TLC behaviour (Rf values) – Fig. 1. Galanthamine was not found in the alkaloid fractions of both species. This observation is consistent with the literature data (Berkov & al. 2004).

The results of the cytotoxicity test using the brine shrimp assay are presented in Table 1. The degree of lethality was found to be directly proportional to the concentration of the alkaloid fractions. Maximum mortalities took place at the highest concentration whereas the least mortalities were at the lowest concentration. Total alkaloid fractions of *G. nivalis* and *G. elwesii* showed significant cytotoxicity with LC_{50} 3.80 and 0.36 µg/ml, respectively. These results are promising because LC_{50} values are less than 1000 µg/ml, which is indicative of the presence of potent cytotoxic components. The alkaloid fraction of *G. elwesii* showed higher activity. Isolation and spectroscopic identification of the compounds from these *Galanthus* species are in progress.

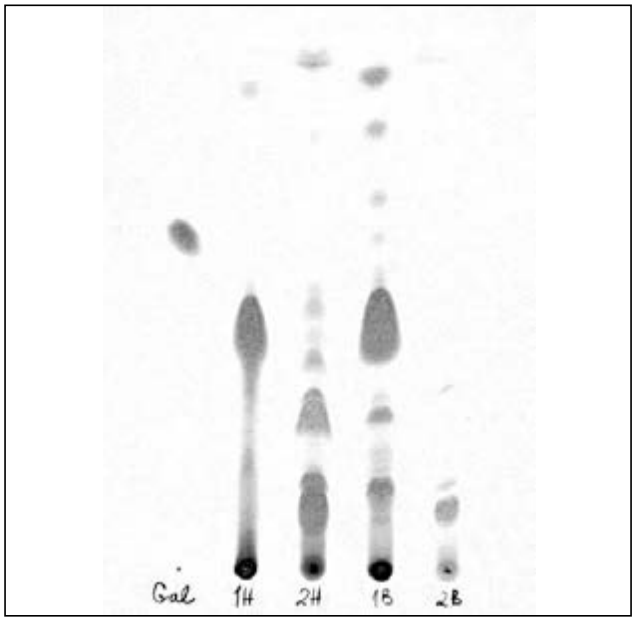

Fig. 1. TLC profiles of alkaloid fractions of *Galanthus* species (**1H** – *G. elwesii*, herba; **2H** – *G. nivalis*, herba; **1B** – *G. elwesii*, bulbs; **2B** – *G. nivalis*, bulbs; **gal** – galanthamine).

Table 1. The mean LC_{50} values ± S.D. for alkaloid fractions screened against brine shrimp larvae.

Plant species	LC_{50}[a] ± S.D.[b] (µg/ml)
Galanthus elwesii	0.36 ± 1.29
Galanthus nivalis	3.80 ± 3.59

a – Lethal concentration for 50 % of *Artemia salina* nauplii.
b – Mean of three measurements (10 nauplii per concentration; dead nauplii were counted).
S.D. – Standard deviation.

Conclusion

The brine shrimp lethality assay represents a rapid, reliable, convenient method for assessment of bioactivity of plant extracts.

This significant lethality of alkaloid fractions of *Galanthus* species to brine shrimp due to the presence of cytotoxic components is a reason for further antitumor activity investigations.

Acknowledgements. The financial support of this work by the National Science Fund, Bulgaria (Project B–1504) is gratefully acknowledged.

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Variation in contents of hypericin and flavonoids in *Hypericum maculatum* (*Hypericaceae*) from Lithuania

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Abstract. This study, carried out in 2004–2005, describes the variation of hypericin and flavonoid contents in different accessions of *Hypericum maculatum*. Flowering tops of *H. maculatum* were collected and analysed for hypericin and flavonoids using HPLC. The contents of hypericin ranged from 0.35–0.95 mg/g; flavonoid contents varied as follows: hyperoside – 16.66–40.89 mg/g, quercitrin – 0.00 to 1.07 mg/g and quercetin – 1.46–4.96 mg/g. The study indicated that flavonoid rutin was absent from the flavonoid pattern of *H. maculatum*, or present only in trace amounts (0.00–0.67 mg/g), however, *H. maculatum* is one of the most important sources of hyperoside. The accessions of *H. maculatum*, which accumulated high levels of flavonoids, seem to be promising for further breeding.

Key words: flavonoids, *Hypericaceae*, *Hypericum maculatum*, hypericin

Introduction

Hypericum maculatum Crantz is a perennial plant of the family *Hypericaceae* L. Plant species of the genus *Hypericum* L. are well known for their use in traditional medicine due to their therapeutic efficacy. In raw material of these species many groups of secondary metabolites, possibly with pharmacological action, have been identified. The main constituents of *Hypericum* species are: naphtodianthrones, primarily represented by hypericin and pseudohypericin; flavonoids, e.g. quercetin, hyperoside, rutin or quercitrin (Bombardelli & Morazzoni 1995). They support regeneration of human body cells, provide disinfection, and act as antidepressant, antibacterial, antiviral, and anti-inflammation tools (Kitanov & Nikolov 1991). Such large spectrum of the applicable functions depends on the quantity of each substance.

As reported by many authors, the contents of secondary metabolites vary not only between species but also within species depending on eco-geographical conditions of habitats (Brantner & al. 1994; Kitanov 1995; Osińska & Weglarz 2000; Pluhár & al. 2000; Bagdonaitė & al. 2001; Walker & al. 2001; Bagdonaitė & Radušienė 2002; Radušienė & Bagdonaitė 2002; Smelcerovic & al. 2006). The interaction of environmental conditions and plant sexual breeding leads to the emerging of ecotypic diversity. The wild ecotypes selected for their contents of active components are often used in cultivation (Galambosi 1993), as the cultivation of medicinal plants presents many advantages compared with the harvesting from the wild.

In case of *Hypericum*, the ecotypic selection is usually carried out by the means of phytochemical study of the flowering tops which are collected at full bloom period.

The objective of the present study was to characterise the variation in contents of secondary metabolites with the stress on hypericin and flavonoids in the flowering tops of *H. maculatum* accessions established in the field collection.

Material and methods

Plant material

Eight samples of *H. maculatum* were collected from different natural habitats in Lithuania in 2002 and transferred into the field collection of the Institute of Botany, Vilnius, Lithuania. Each accession was as-

signed with the collection number: 382, 405, 407, 409, 417, 418, 420 and 422. The herbarium vouchers (No. 68103, 68110, 68113, 68120, 68123, 68129, 68138 and 68159) of the accessions were deposited at the Herbarium of the Institute of Botany (BILAS). The plants were grown under the same cultivated field conditions on soil with P_2O_5 contents 202.9 mg/kg, K_2O – 213.9 mg/kg, pH – 5.42 and organic matter – 2.21 %. The flowering tops of 30 cm in length were collected in the 2nd and 3rd years of cultivation, in July 2004 and 2005. The harvested plant material was dried in a room for ten days at an ambient air temperature, then packed in paper bags and kept in dry and dark environment at a room temperature.

Extraction procedures

Samples of 0.5–1.0 g each of dried flowering tops of *H. maculatum* with moisture content of 10.0 % were mechanically ground to obtain a homogeneous drug powder and extracted with 96 % EtOH (50 ml) for 72 h, at a room temperature. The prepared samples were kept in the dark in a refrigerator until used. Conversion of protohypericin is performed by exposure to light for 30 minutes before analysis by HPLC (Kurth & Spreemann 1998; Michelitsch & al. 2000). 1 ml portion from each of the fresh drug extracts was taken up for HPLC analyses of hypericin. Each of 1-ml aliquot of the extracts was diluted with 19 ml of EtOH for flavonoids analyses. The content of hypericin and flavonoids was assessed in total 8 field accessions of *H. maculatum*.

Chemicals

All solvents for HPLC analysis were of HPLC grade and purchased from Roth, Karlsruhe (Germany). Rutin, hyperoside, quercetin, quercitrin and hypericin were obtained from Roth.

HPLC analysis

Calibration solutions in the concentration range of 0.5 to 100.0 μg/ml were prepared from stock solution of rutin, hyperoside, quercetin, quercitrin and hypericin in methanol (100.0 μg/ml).

Identification of flavonoids

Rutin, hyperoside, quercetin and quercitrin were detected employing the modified method of Liu & al. (2000). For this purpose HPLC "Waters 2690" with UV detector "Waters 2487" on "XTerra RP 18" 3.5 μm column (150 × 3.9 mm) was used. Ten microlitres of each sample were injected. Compounds in the column were separated with 5 % 0.1 % trifluoracetic acid ($C_2HF_3O_2$) in water (solvent A) and 95 % 0.1 % $C_2HF_3O_2$ in acetonitrile (solvent B), using a gradient elution programme: 0–45 min 95–55 % A, 5–45 % B; 45–50 min 55 % A, 45 % B; 50–55 min 55–95 % A, 45–5 % B, the flow rate was 0.4 ml/min. The column temperature was 20 °C. The elution was monitored at 360 nm (Kirakosyan & al. 2004; Kovacs & al. 2004) and the obtained data were compared with those of the authentic samples of the respective flavonoids.

Identification of hypericin

Hypericin was detected according to the modified method of Pharmeuropa (2004). For this purpose HPLC "Waters 2690" with UV detector "Waters 2487" on "CC 125/4 Nucleosil 100–5 C18" column (125 mm) was used. Ten microlitres of each sample were injected. The elution program was isocratic. The mobile phase was ethyl acetate/15.6 g/l NaH_2PO_4/methanol (39:41:160). The flow rate was 1.0 ml/min. The column temperature was 20 °C. The elution was monitored at 590 nm and the obtained data were compared with those of authentic samples of hypericin.

Statistical analysis

In order to determine whether there is statistically significant difference between the obtained values, one-way analyses of variance (ANOVA) were used with STATISTICA software package. The Student's test and significance level p=0.05 were employed in the data processing. The cluster analysis was applied to compare and group accessions according to their content of bioactive constituents.

Results and discussion

The genus *Hypericum* has received considerable interest from scientists, as it is a source of a variety of biologically active compounds including hypericin (Çirak & al. 2006). For many years, the red pigment hypericin was considered as the main active constituent of *Hypericum* and standardisation of *Hyperici herba* products has been based mainly on the quantification of this component as marker compound (Constantine & Karchesy 1998). Thus, hypericin has importance from the point of view of quality control. According to the literature references, the species of *Hypericum* differ in the composition and amounts of flavonoids as well as dominant compounds. Therefore, the studies on

flavonoids are important in the identification of interspecific hybrids as well (Mártonfi & al. 1996, 2001; Crockett & al. 2005).

In Lithuania, only *H. perforatum* is officially regarded as medicinal plant. Therefore, considering the pharmacological significance of hypericin and flavonoids and their possible use in therapeutics, it is important to find additional sources of these natural compounds. Our previous studies (Bagdonaitė & Radušienė 2002) indicated that wild populations of *H. maculatum* are characterised by high variability of the amount of secondary metabolites and can serve as the main source of genotypes for the breeding of new cultivars. In the present study, 8 accessions of *H. maculatum* were analysed for the quantity of 5 compounds. The contents of hypericin, rutin, hyperoside, quercitrin and quercetin are presented in Table 1. The content of hypericin varied between 0.35 and 0.86 mg/g in 2004 and between 0.50 and 0.95 mg/g in 2005. The levels of hypericin in flowering tops of *H. maculatum* were higher in 2005, however, the difference was not statistically significant (p=0.12). The quantitative variability of hypericin among *H. maculatum* accessions was quite high. As shown in Fig. 1, hypericin reached the highest concentrations in the accessions No. 418 (0.86 mg/g in 2004 and 0.92 mg/g in 2005) and No. 409 (0.68 mg/g in 2004 and 0.95 mg/g in 2005); while in the accession No. 417 it was present only at low concentrations (0.35 mg/g in 2004 and 0.50 mg/g in 2005).

Table 1. Statistical data and results of *t*-test for secondary metabolites of *H. maculatum*.

Compounds (mg/g)	2004				2005				t	p
	M	Min	Max	St. D.	M	Min	Max	St. D.		
Hypericin	0.58	0.35	0.86	0.154	0.71	0.50	0.95	0.157	-1.66	0.12
Rutin	0.30	0.00	0.67	0.186	0.46	0.26	0.58	0.113	-2.07	0.06
Hyperoside	32.06	26.29	36.66	4.026	31.74	16.66	40.89	8.641	0.09	0.93
Quercitrin	0.58	0.00	0.73	0.237	0.72	0.37	1.07	0.227	-1.26	0.23
Quercetin	2.03	1.46	3.06	0.497	3.90	2.21	4.96	0.837	-5.43	0.00*

Abbreviations: M – average; Min – minimum; Max – maximum; St. D. – standard deviation; t – Student Statistics; p – level of significance.

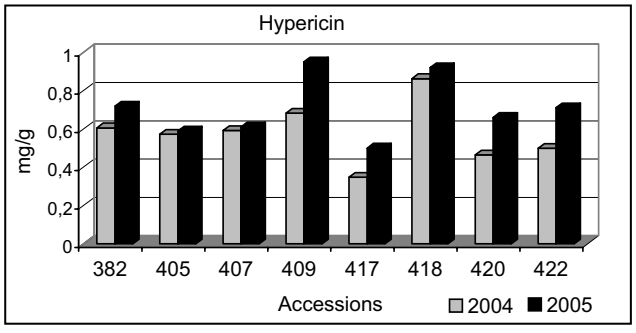

Fig. 1. Content of hypericin in *H. maculatum* accessions.

According to the literature references, the content of hypericin in the herb of *H. maculatum* from Slovenia ranges within 0.10–1.10 mg/g (Umek & al. 1999) and 0.50–1.00 mg/g from Bulgaria (Kitanov 2000). Basically, these data correspond with our data. The content of hypericin in *H. maculatum* reported by Brantner & al. (1994) and Osińska & Weglarz (2000) were higher than ours (0.40–2.30 mg/g and 1.50 mg/g, respectively). Girzu & al. (2000), Mártonfi & al. (2006) and Smelcerovic & al. (2006) have reported lower content of hypericin in *H. maculatum* (0.20 mg/g; 0.03–0.34 mg/g and 0.20 mg/g, respectively) than obtained by this study. The quantitative data reported by different authors vary significantly. This can be caused by the geographical latitude of plant origin and methods of sampling and analyses employed.

In a typical flavonoid spectrum of *H. maculatum*, hyperoside, quercitrin and quercetin are the major components. As shown in the Table 1, *H. maculatum* is characterised by high variability in the amount of flavonoids. The study indicated that the flavonoid rutin was absent from the flavonoid pattern of *H. maculatum*, or present only in a trace amounts (0.00–0.67 mg/g in 2004 and 0.26–0.58 mg/g in 2005). This is in agreement with our previous study (Radušienė & Bagdonaitė 2002) and the studies of *H. maculatum* growing in Bulgaria (Kitanov & Nikolov 1991), Slovenia (Umek & al. 1999) and Slovakia (Mártonfi & al. 2006).

The content of hyperoside in *H. maculatum* varied between 26.29 and 36.66 mg/g in 2004, and between 16.66 and 40.89 mg/g in 2005 (Table 1). The levels of hyperoside in flowering tops of *H. maculatum* did not differ statistically between 2004 and 2005 (p=0.93). The level of hyperoside in the examined *H. maculatum* accessions was significantly higher than that reported by Umek & al. (1999). The researchers indicated that hyperoside content ranged from 16.0–19.0 mg/g. The results of our study correspond with the findings of Smelcerovic & al. (2006) who pointed out that *H. maculatum* is the most important source for obtaining hyperoside.

In our study the quercitrin levels in the flowering tops of *H. maculatum* varied from 0.00 to 0.73 mg/g in 2004 and from 0.37 to 1.07 mg/g in 2005, while quercetin – 1.46–3.06 mg/g and 2.21–4.96 mg/g, respectively (Table 1). The quercitrin levels did not significantly differ between 2004 and 2005 (p=0.23). However, sta-

tistically significant differences of quercetin content in different years (p=0.00) was observed. In all studied *H. maculatum* accessions, the higher levels of quercetin were obtained in 2005. The contents of quercitrin (0.40–0.60 mg/g) and quercetin (1.50–1.80 mg/g) reported by Umek & al. (1999) were lower than our ones.

The results obtained by this study testify to the strong between-accession variability of flavonoids' content in *H. maculatum*. Therefore, the accessions were analysed by the means of cluster analysis. For the representation of the dendrogram the Euclidean Furthest Neighbour Method was chosen, as this gives the best reflection of the clusters. Each line representing an accession, totally 3 well-separated clusters were recognised (Fig. 2). The first cluster, which includes the accession No. 422, is the most distant in the dendrogram. The distinction of this accession is predetermined by the lowest level of flavonoids. The five accessions, No. 407, No. 409, No. 417, No. 418 and No. 420, of the second cluster are characterised by high contents of hyperoside, quercetin and quercitrin. The third cluster consists of two accessions, No. 382 and No. 405, containing a lower than the average quantity of hyperoside and quercitrin. However, much of quercetin was identified in these accessions.

In the current study, we observed the variation in concentrations of hypericin and flavonoids in different accessions of *H. maculatum* grown in the field in both years. According to our results, some *H. maculatum* accessions seem to be superior. Thus, a considerable advance in the plant breeding could be expected by further studies of the accessions Nos. 409 and 418, which proved to have high contents of flavonoids and hypericin. Smelcerovic & al. (2006) have reported significant differences in the contents of active compounds between the samples collected from the same location, which suggest that the genetic factors may play a significant role here.

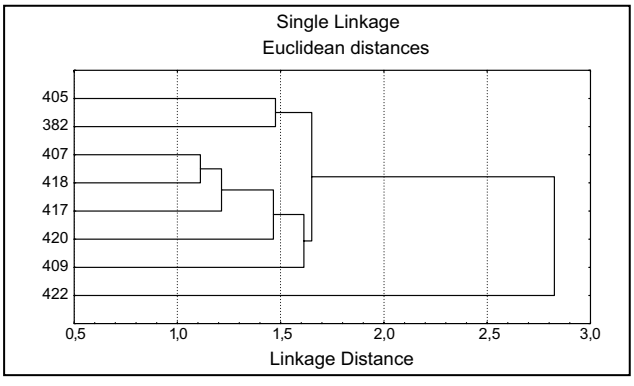

Fig. 2. The hierarchical cluster analysis dendrogram of *H. maculatum* accessions according to the content of flavonoids.

Hypericum maculatum is the species with the most similar phytochemical profiles to those of *H. perforatum* (Kitanov 1995; Osińska & Weglarz 2000; Crockett & al. 2005) and both species could successfully be used as source plants for obtaining the crude drug, *Hyperici herba*. The high contents of hyperoside and hypericin in *H. maculatum* encourage the cultivation and utilisation of this species in Lithuania as well.

Acknowledgements. The authors gratefully acknowledge the financial support of this study rendered by the Kaunas University of Medicine, Kaunas, Lithuania.

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Preliminary investigation of stress-induced modifications in *Veronica krumovii*'s flavonoid and volatile composition

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Abstract. The changes in the flavonoid and volatile metabolism using three types of stress-induced agents have been studied in *Veronica krumovii* (*Scrophulariaceae*). After application of mechanical damaging, chemical treatment with CuSO4 and fungi attack simulation with chitosan solution, modifications in normal compositions of flavonoids and volatile compounds have been observed. The elicitors increased the flavonoid formation after 24th hour of their application. The chitosan solution appeared to possess the strongest effect among the three stress reagents. Volatile composition reacted with an increase of the number of alcohol and ester compounds after stress treatment.

Key words: flavonoids, stress agents, volatile compounds

Introduction

The increased accumulation of flavonoids has been demonstrated in several species in response to variety of biotic and abiotic stimuli (elicitors) (Carlson & Dolphin 1981; Tumová & al. 2002). There are evidences that chitin (Pare & al. 1992; Liu & al. 1993; Akiyama & al. 1994), heavy metals (Tumová & al. 1998; Nikolova & al. 2005a) and several types of wounding (Treutter 2001) induce flavonoid biosynthesis. At the same time the volatile constituents are of interest, because such compounds often take part in the plant–plant and plant–insect relationships (De Moraes & al. 1998; Agrawal 2000; Bruin & Dicke 2001). These data provoked our interest to examine the changes in flavonoid and volatile metabolism in *Veronica krumovii* Peev (Peev) after application on different elicitors. The species is a part of *V. chamaedrys* group which includes also *V. chamaedrys* L., *V. vindobonensis* (M.A. Fisch.) M.A. Fisch. and *V. orbelica* (Peev) Peev. The species have been studied for presence of surface flavonoids. Apigenin, luteolin, and their methyl derivatives have been reported (Nikolova & al. 2005b). While the flavonoid composition of the above-mentioned species has been studied, their volatile compounds have not been investigated.

In the present study we analysed the modifications in *V. krumovii*'s flavonoid and volatile composition using three types of stress-induced agents – fungi attack (chitosan), mechanical damaging and chemical treatment with $CuSO_4$.

Material and methods

Plant material

The plant samples were collected from different places of the wild population of *V. krumovii* at the downhill of Mt Lyulin during flowering stage in May 2005. The taxonomical determination of the species followed Peev (1995).

The control samples were collected before the application of the stress treatment. The investigations were subjected on define parts of time (after 30 minutes, 6 hours, 24, 48, 72, 96 and 120 hours) for flavonoid analysis and after 30 minutes, 6 and 24 hours for volatile.

Treatment of the plants

Plants were sprayed with solutions of chitosan and $CuSO_4$ with concentration 1 g/l. A chitosan solution was prepared by dissolving chitosan in 0.1 M acetic acid and adjusting the pH to 5.6 with 2 N NaOH.

For mechanical damage plants were trampled by legs and small pieces of them were cut by knife.

Sample preparation

Two grams of air-dried (not ground) plants were rinsed with 20 ml acetone for 2 min. After evaporation of acetone, the dried extracts were dissolved in 200 µl methanol.

Quantitative flavonoid analysis

0.3, 0.6 and 0.9 µg/spot of apigenin (stock solution 1.5 µg/µl) were applied together with 30 µl from *V. chamaedrys* exudates with unknown concentrations on Merck aluminum sheets Kiselgel 60 F_{254} (0.2 mm thin layer, 10 × 20 cm). Toluene–dioxin–acetic acid (95:25:4) was used for the development of plates. Migration distance – 90 mm. Compounds were visualised after spraying with $FlCL_3$ reagent. The TLC plates were scanned and the images were analysed by QuantiScan 2.1® Biosoft software. The content of the compounds in the samples was calculated by comparison of densitogram peak areas of the samples with those of the three standards with known quantities, placed on the same plate.

Qualitative flavonoid analysis

Flavonoid aglycones were identified by direct TLC comparison with markers. Two TLC systems on two different substrates (polyamid and silica gel) were used for confirming of the identification of flavonoids. System 1: polyamide DC–11 plates, toluene–MeCOEt–MeOH 60:25:15 v/v/v; system 2: silica gel, toluene–dioxane–acetic acid 95:25:4 v/v/v. Chromatograms were viewed under UV light at 336 nm before and after spraying with "Naturstoffreagenz A", 1% solution of diphenylboric acid–ethanolamine complex in methanol.

Volatile analysis

20 g of fresh plant material have been subjected to distillation–extraction in a Licken–Nickersson apparatus for 4 h. The volatiles were extracted with diethyl ether and analysed by GC/MS with a Hewlett Packard gas chromatograph 6890 equipped with a Hewlett Packard MS 5973 detector (Hewlett Packard, Palo Alto, California, USA). A HP5–MS capillary column was used (30 m × 0.25 mm, 0.25 µm film thickness, Agilent Technologies, Wilmington, Delaware, USA). Helium was used as a carrier gas and the temperature programme was 40 °C to 280 °C at 6 °C.min^{-1}; a 10 min hold was applied. The ion source was set at 250 °C and the ionization voltage was 70 eV. The GC/MS investigation was based on the interpretation of the mass spectral fragmentation followed by comparisons of the spectra obtained with those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 (Hewlett Packard, Palo Alto, California, USA) were also applied. In the cases when the spectra of some isomers were very similar and these compounds could not be identified unambiguously, comparisons of the GLC retention times, obtained under the same conditions, were used. When there were no suitable authentic samples and spectra for comparison, no identification was made. Only the unambiguously identified compounds were reported in a table.

Results and discussion

Flavonoid analysis

The qualitative and quantitative flavonoid profiles of 42 samples of *V. krumovii* were analysed. The samples were collected during the different time after application of three types of stress-induced agents – mechanical damaging, solutions of chitosan and $CuSO_4$.

Apigenin was the main flavonoid aglycone in the exudates of *V. krumovii* and its content was examined the most exactly. Accumulation of apigenin was increased after application of the three stress-induced agents and got to its maximum on 24th hour. After that apigenin content was decreased. On 120th hour the content of apigenin was lower than in the control sample (Fig. 1). The accumulation of luteolin follows the same tendency. The chitosan solution appeared to possess the strongest effect between the three stress reagents. This result would be explained with data that phenylalanine ammonia–lyase (main enzyme in flavonoid byosinthesis) is strongly induced by chitin and its derivative chitosan (Pare & al 1992).

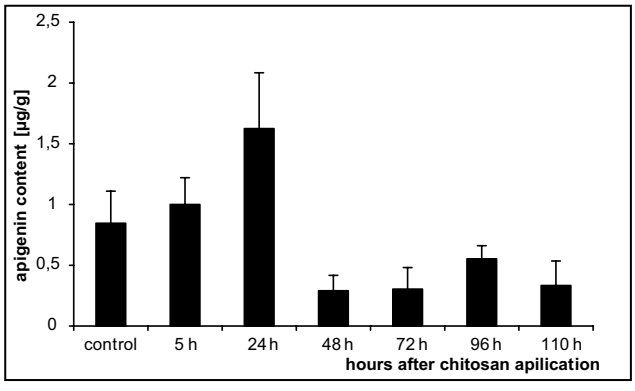

Fig. 1. Apigenin content in *V. krumovii* samples after chitosan application.

Additional flavonoid aglycones such as luteolin, luteolin 3'-methyl ether, apigenin 4'-methyl ether, apigenin 7-methyl ether and apigenin 7,4'-dimethyl ether were observed also in the acetone exudates of *V. krumovii*. The synthesis of methylated flavonoid derivatives was repressed or remained unchangeable in the beginning of stress situation. After 24th hour the accumulation of methoxylated flavonoids was increased and around 72nd hour most of compounds reached own maximum. The accumulation of apigenin 7,4'-dimethyl ether shows most significantly enhancement.

Volatile analysis

The analyses of the controlled and stressed samples revealed very complex composition of the volatiles from the investigated *Veronica* samples. More than fifty compounds were identified clearly (Table 1). The major chemical groups are: alcohols, ethers, acids, esters, terpenes, ketones, aldehydes, saturated hydrocarbons, amines, amides and heterocyclic hydrocarbons. With exception of the last three groups of compounds, the others persist into the unstressed (control) samples. These three groups are represented by only one compound for the mechanical stress and for the chitosan treatment. We suggest that is not accurate to define them as biomarkers. There are no data about their persistence after CuSO4 treatment. It is interesting the increasing number of compounds from different chemical groups in comparison with the controlled sample. There are five alcohols with high quantity concentration after the application of every one of the three stress treatments. In the investigated unstressed samples there are two. The literature data (Ngoh & al. 1998; Weissbecker & al. 1999; Smart & Blight 2000) show evgenol and similar alcohols to play important role into the plant defensive system as repellents or as attractants for parasitoids, even to possess direct contact toxicity against herbivorous insects. The similar conclusions could be achieved for the acids and especially their acetates, which we found in the stressed samples. They take important role into the process of plant defence as attractants for parasitoids or to possess strong antimicotic effect (Mattiaci & al. 2001; Filonow 2002).

Table 1. Volatile compounds of *V. krumovii* before and after application on three stress agents.

Compounds	Mechanical injury			Chitosan solution			$CuSO_4$ solution			Control
	30'	6 hours	24 hours	30'	6 hours	24 hours	30'	6 hours	24 hours	
Alcohols (total)	18.5	10.1	36.6	24.7	12.5	21.6	14.5	13	19.8	33.9
2,3-Butanediol	0.8	0.8	1.5	–	0.4	2.5	0.4	0.4	0.7	<0.1
Eugenol	<0.1	<0.1	–	0.7	<0.1	<0.1	0.5	0.4	<0.1	–
4-Hydroxy-benzeneethanol	–	–	–	–	2.9	–	–	–	–	–
2,6-Dihydroxynaphthalene	17.7	8.8	35.1	23.3	9.2	19.1	13.6	12.2	17.8	33.9
1,7-Dihydroxynaphthalene	–	0.5	<0.1	0.7	–	–	–	–	1.3	–
Ethers (total)	0.8	0.7	1.4	0	–	2.6	0.4	0.3	0.4	1.7
1,1-Diethoxyethane	0.8	0.7	1.4	–	0.4	–	0.4	0.3	0.4	1.7
2-Etoxybutane						2.6				
Acids (total)	3.4	5.1	1.3	11.1	2.4	8.8	17.9	7.8	0.9	<0.1
Benzoic acid	1	–	–	–	–	–	–	–	–	–
Octanoic acid	<0.1	<0.1	–	<0.1	1.2	–	1.7	1.2	<0.1	–
Nonanoic acid	<0.1	<0.1	–	<0.1	<0.1	–	0.6	0.7	<0.1	–
Decanoic acid	<0.1	<0.1	–	<0.1	<0.1	–	1	1.6	<0.1	–
Tetradecanoic acid	0.6	1.5	1.3	1.3	–	–	2	<0.1	0.9	–
Pentadecanoic acid		0.7	–	<0.1	–	–	0.5	–	–	–
Hexadecanoic acid	<0.1	<0.1	–	9.8	0.4	6.3	11.7	4.3	–	<0.1
Palmitoleic acid		1	–	–	–	–	–	–	–	–
Octadecanoic acid	0.6	0.7	–	<0.1	0.8	–	0.4	–	–	–
Oleic acide	1.2	–	–	–	–	–	–	–	–	–
Linoleic acid	–	1.2	–	–	–	–	–	–	–	–

Table 1. Continuation.

Compounds	Mechanical injury			Chitosan solution			$CuSO_4$ solution			Control
	30'	6 hours	24 hours	30'	6 hours	24 hours	30'	6 hours	24 hours	
1,2,3,4,4a, 9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenecarboxylic acid	–	–	–	–	–	2.5	–	–	–	–
Esters (total)	0.4	2.3	<0.1	–	3.9	–	–	–	0.5	–
Benzene acetic acid, d-amino methyl ester	–	–	<0.1	–	–	–	–	–	0.5	–
Hexadecanoic acid methyl ester	–	–	–	–	1.7	–	–	–	–	–
Oleic acid methyl ester	–	–	–	–	1.6	–	–	–	–	–
Linoleic acid methyl ester	–	–	–	–	0.6	–	–	–	–	–
N-Phenyl-glycine ethyl ester	0.4	1.3	–	–	–	–	–	–	–	–
α-Amino-benzeneacetic acid methyl ester	–	1	–	–	–	–	–	–	–	–
Terpens (total)	2.5	2.7	12.5	15.7	<0.1	3.9	3.9	11.6	16.3	4.3
Hexahydrofarnesyl acetone	0.4	0.4	0.6	1	<0.1	–	0.4	0.6	1.2	–
Phytol	2.1	2.3	11.9	14.7	–	3.9	3.5	11	15.1	4.3
Ketones (total)	<0.1	1.2	1.2	3.5	1.8	–	–	–	1.3	6.2
Acetophenone	–	–	–	–	1.1	–	–	–	–	–
1,1-(6-Methoxy-2,5-benzofurandiyl)bis-ethanone	<0.1	1.2	1.2	3.5	0.7	–	–	–	1.3	6.2
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	–	<0.1	–	–	–	–	–	–	–	–
Hydrocarbons (total)	9.4	19.2	23.8	26.1	27.5	20.4	48.21	28.1	18.6	41.9
Pentadecane	–	<0.1	<0.1	–	0.4	–	<0.1	<0.1	–	–
Heptadecane	–	–	–	–	0.5	–	–	–	–	–
Nonadecane	–	<0.1	<0.1	<0.1	–	–	–	0.6	0.9	
Eicosane	–	–	–	–	–	–	0.3	–	–	–
Heneicosane	–	–	–	0.5	–	–	0.9	0.8	0.8	<0.1
Docosane	–	–	–	0.7	–	–	1.7	–	–	2.1
Tricosane	–	0.6	0.9	1	1	1.6	3.11	2.3	0.8	3.6
Tetracosane	0.5	1.2	1.5	2.2	1.6	2.5	4.9	2.2	0.9	5.4
Pentacosane	1.7	2.3	3.7	4.8	2.9	3	6.7	3	3.4	7.1
Hexacosane	1.6	3.1	4.2	3.9	2.5	3.1	6	4.3	2.5	7.7
Heptacosane	1.9	3.5	5.2	6.1	3.5	3.5	6.6	5.3	4.2	7.5
Octacosane	1	1.7	2.2	2.1	3	2.4	4.1	2.8	1.6	3.9
Squalene	–	1.2	1.1	–	–	2.2	–	–	0.7	–
Nonacosane	1.6	2.8	3.5	3.9	4.9	2.1	5.9	2.1	2	4.6
Triacontane	0.6	0.9	<0.1	0.9	2.8	–	2.6	1.8	0.8	<0.1
Hentriacontane	0.5	1.9	1.5	–	4.4	–	3.9	1.5	–	<0.1
Dotriacontane	–	–	–	–	–	–	1.5	1.4	–	–
Amines (total)	–	<0.1	–	–	7.7	5.2	–	–	–	–
Aniline	–	<0.1	–	–	–	–	–	–	–	–
N-phenyl-1-naphthalenamin	–	–	–	–	7.7	5.2	–	–	–	–
Amides (total)	–	–	<0.1	–	–	–	–	–	–	–
N-phenyl-formamide	–	–	<0.1	–	–	–	–	–	–	–
Heterocyclic hydrocarbons (total)	–	–	–	–	<0.1	–	–	–	–	–
Benzothiazole	–	–	–	–	<0.1	–	–	–	–	–

Conclusions

The results showed that flavonoid and volatile metabolism is affected by different kind of elicitors. The chitosan solution appeared to possess the strongest effect between the three stress reagents. The maximum level in the synthesis of apigenin was determined to be at 24th hour and for methylated flavonoids at 72nd hour.

The volatile contents of *V. krumovii* reacted under the effect of the stress treatment with increase of the total number of the compounds, which are playing important role into the plant defence, in comparison with the unstressed samples. The number and the amount of the alcohols and esters should be suggested as biomarkers into future investigations.

All these evidences could be used in future monitoring of the possible environmental danger over the populations of *V. krumovii* in Bulgaria.

Acknowledgements. The financial support of this work by the National Science Fund, Bulgaria (Project B–1404) is gratefully acknowledged.

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Volatile constituents of *Phlomis lycia* (*Labiatae*)

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Abstract. As a part of our work on the screening of odoriferous plants of Greece, we have investigated the essential oil composition of *Phlomis lycia*, collected in Kalimnos Island (East Aegean, Greece) in May 2002 (sample A) and May 2003 (sample B). Semi-crushed air-dried aerial parts were subjected to hydrodistillation for 3 h and the obtained oils were analysed using GC/FID and GC/MS. The two oils were similar regarding the qualitative pattern but displayed some quantitative differences. The oils were characterised by the abundance of germacrene D. Other main metabolites were aromadendrene, limonene, α-pinene (sample A oil), β-caryophyllene, α-pinene and limonene (sample B oil).

Key words: East Aegean, essential oil, germacrene D, Greece, Kalimnos Island

Introduction

The genus *Phlomis* L. (*Labiatae*) comprises about 100 species of the Mediterranean region and Western Asia (Meikle 1985). According to Greuter & al. (1986) 11 *Phlomis* species are present in Greece.

Phlomis species are perennial herbs or shrubs, pilose or tomentose, with or without glands, simple, entire or crenate-dentate leaves, few- to many-flowered verticillasters, tubular or campanulate calyces and 2-lipped, purple, pink or yellow corollas. The stamens are 4, didynamous and the nutlets trigonous, glabrous or pubescent (Huber-Morath 1982).

Phlomis lycia D. Don belongs to Sectio *Phlomis* subsectio *Dendrophlomis*. It is a shrub up to 1.5 m high with densely tomentose leaves especially beneath, 1–2, 6–12-flowered verticillasters, linear-lanceolate bracteoles, densely white-lanate calyx, yellow corolla and grabrous nutlets. Its known distribution includes SW Anatolia and the East Aegean Islands of Strongili (Kastellorizo group), Simi, Tilos and Kalimnos (Fig. 1), where it grows in

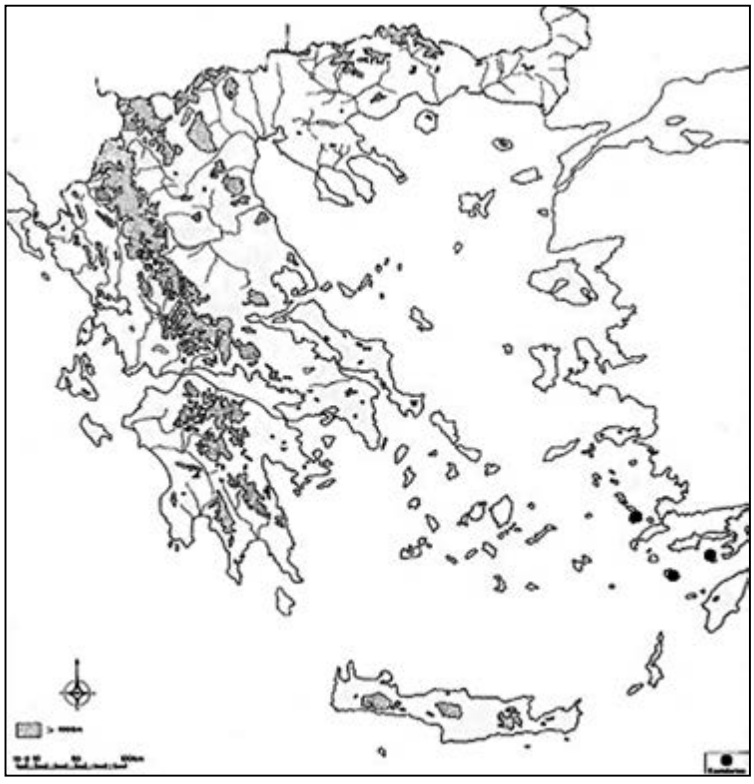

Fig. 1. Known distribution of *P. lycia* in Greece.

macchia, *Quercus* scrub, *Pinus brutia* forest, serpentine cliffs from sea level up to 900 m (Huber-Morath 1982). In Kalimnos *P. lycia* grows on rocky limestone places with phrygana vegetation from Pothia to Vathis.

Material and methods

Plant material

The aerial parts of *P. lycia* were collected during the flowering period from Kalimnos Island in May 2002 (sample A) and in May 2003 (sample B). Voucher specimens collected by Sevasti Zervou and Ioannis Bazos bearing the initials SZ and IB, respectively, are kept at their herbaria at the University of Athens (ATHU). *Specimens seen*: Nomos Dodekanisou, Kalimnos Island, between Pothia and Vathis, phrygana vegetation on limestone, 13.04.1998 (SZ 718) and 09.04.2001 (SZ 2291); ibid., 17.03.2003 (IB 4241). Nomos Dodekanisou, Kalimnos Island, Pothia, hill of Agia Varvara, 17.04.1999 (SZ 1058).

Isolation of essential oil

The dried aerial parts were subjected to hydrodistillation for 3 h, using a modified Clevenger-type apparatus and the resulting oils were dried over anhydrous sodium sulphate and stored at 4 °C until analysis.

Gas chromatography

GC analyses were carried out using a SRI 8610C GC/FID system, equipped with DB–5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm) and connected to a FID detector. The injector and detector temperature was 280 °C. The carrier gas was He, at flow rate of 1.2 ml/min. The thermal program was 60 °C to 280 °C at a rate of 3 °C/min. Two replicates of samples were processed in the same way.

Gas chromatography–Mass spectrometry

GC/MS analyses were performed on a Hewlett Packard 6890–5973 GC/MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (200 °C). The transfer line temperature was 250 °C. Helium was used as a carrier gas (1 ml/min), and the capillary column used was HP 5MS (30 m × 0.25 mm; film thickness 0.25 μm). The temperature programme was the same with that used for the GC analyses; split ratio 1:10. The injected volume was 1 μl.

Identification of the components

The identification of the components was based on comparison of their relative retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley libraries and literature (Adams 2001).

Results and discussion

Thirty-eight compounds determined and identified by GC and combined GC/MS, which accounted for 99.6–99.7 % of the total compositions of each oil, are reported in Table 1.

Table 1. Composition of *P. lycia* essential oils.

Constituents	RRI	Sample A	Sample B
α-thujene	927	tr	tr
α-pinene	936	8.9	13.4
sabinene	972	tr	tr
β-pinene	976	tr	tr
3-octanone	979	tr	–
myrcene	989	tr	tr
α-phellandrene	1003	tr	tr
α-terpinene	1016	tr	tr
p-cymene	1024	tr	tr
limonene	1029	10.4	8.2
(Z)-β-ocimene	1037	2.3	tr
(E)-β-ocimene	1048	tr	tr
terpinolene	1086	tr	0.8
linalool	1097	tr	1.8
nonanal	1101	tr	tr
α-cubebene	1345	8.8	2.8
α-ylangene	1368	tr	tr
α-copaene	1371	5.2	2.3
β-bourbonene	1381	tr	1.1
β-cubebene	1384	4.5	2.4
β-elemene	1386	tr	tr
α-gurjunene	1405	tr	tr
β-caryophyllene	1414	6.8	17.1
β-copaene	1427	tr	tr
aromadendrene	1436	10.0	6.1
α-humulene	1449	tr	tr
(E)-β-farnesene	1453	6.4	3.7
γ-muurolene	1473	tr	tr
germacrene D	1479	26.7	31.9
valencene	1491	tr	–
viridiflorene	1493	tr	tr
bicyclogermacrene	1494	5.0	4.1
α-muurolene	1495	tr	tr
α-cuprenene	1501	–	tr
β-bisabolene	1502	2.1	1.2
γ-cadinene	1507	tr	tr
7-epi-α-selinene	1516	2.6	1.5
δ-cadinene	1517	tr	1.3
Total		**99.6%**	**99.7%**

Legend: RRI – relative retention indices, relative to C_9-C_{23} n-alkanes on the HP 5MS column; Sample A – collection 2002; Sample B – collection 2003; tr – trace (<0.1%).

In *P. lycia* oils, α-pinene (8.9 % and 13.4 %, in Sample A and Sample B, respectively) and limonene (10.4 % and 8.2 %, respectively) were the principal monoterpenes, which accounted for 21.6 % and 24.2 % respectively of the whole oils. Sesquiterpenes constituted the main derivatives of the essential oils (78.1 % and 75.5 %, respectively), with germacrene D (26.7 % and 31.9 %, respectively) being the predominant component.

The two oils were similar regarding the qualitative pattern, however many differences can be noted in the percentage distribution of sesquiterpenes; α-cubebene 8.8 % in sample A oil, 2.8 % in sample B oil, β-caryophyllene 6.8 % and 17.1 %, respectively and aromadendrene 10.0 % and 6.1 %, respectively.

Previous studies on the volatiles from *Phlomis* species of Greek origin include *P. fruticosa* L., *P. lanata* Willd., *P. cretica* C. Presl and *P. samia* L. The essential oil from the flowers of *P. fruticosa* of Greek origin, collected from the island of Kythnos (Tsitsimi & al. 2000), was characterised by the presence of germacrene D (17.8 %), γ-bisabolene (12.6 %), α-pinene (8.9 %) and (E)-caryophyllene (8.7 %), while the composition of the essential oil from the fresh aerial parts of *P. fruticosa*, collected in Central-east Peloponnese in Greece (Aligiannis & al. 2004), was comparable with germacrene D (21.4 %), α-pinene (12.6 %), β-caryophyllene (12.6 %), linalool (8.0 %) and (Z)-γ-bisabolene (7.1 %) found as the major compounds.

In the essential oil of the endemic *P. lanata* growing in Crete (Couladis & al. 2000), α-pinene (25.41 %) was the dominating component followed by limonene (15.67 %) and (E)-caryophyllene (8.76 %).

The oil from the fresh aerial parts of *P. cretica* collected at the village Zaros in the island of Crete (Aligiannis & al. 2004), was characterised by the presence of germacrene D (20.1 %), β-caryophyllene (17.3 %), α-pinene (9.4 %), linalool (7.5 %), limonene (7.1 %) and cis-β-ocimene (5.4 %). The most abundant constituents of *P. cretica* verticillasters oil, obtained from air-dried plants collected from the Prefecture of Chania in Crete (Basta & al. 2006), were germacrene D (34.0 %), germacrene B (11.0 %) and (E)-caryophyllene (9.2 %), whereas in the leaf oil, germacrene D was the dominating component (47.9 %) followed by α-pinene (11.2 %) and γ-curcumene (7.3 %).

In the oil of *P. samia*, collected on Mt Parnon in Peloponnese, (E)-β-farnesene (20.7 %), germacrene D (6.3 %) and β-caryophyllene (5.8 %) were found to be the major components (Aligiannis & al. 2004).

In most cases the essential oils of Greek studied *Phlomis* species are characterised by the dominance of sesquiterpenes with germacrene D and β-caryophyllene being among the principal ones.

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Effect of some biofertilisers and natural minerals on the essential oil composition of *Salvia officinalis*, Dalmatica origin

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Abstract. *Salvia officinalis* (sage, *Lamiaceae*) is known for its essential oil present in the leaves, which makes it an important crop. Numerous reports have appeared on its chemical composition at different soil conditions. The summary effect of modern biological fertilisers – azotobacter, vermicompost and natural zeolite substrate was investigated on the essential oil composition of *Salvia officinalis*, growing in greenhouse. The study included six variants of them. The results showed that biological fertilisers increase considerably variability of the quality of the essential oil and less – its quantity.

Key words: azotobacter, fertilisers, natural zeolite substrate, *Salvia officinalis*, vermicompost

Introduction

One of the major concerns in today's world is the pollution and contamination of soil. The use of chemical fertilisers and pesticides has caused tremendous harm to the environment and human health. At the same time, the biologically active bioproducts, biofertilisers, natural minerals, and environmentally friendly products, used in most countries now, increase the soil fertility. They improve the nutrient status or ability of soil to supply nutrients for plant growth under favourable environmental conditions such as light, temperature and physical conditions of soil. The use of these biofertilisers and natural minerals affects positively the soil texture. It is one of the important properties of soil, since it influences aeration, permeability and water retention capacity.

Among the major benefits from biofertilisers and natural minerals' using are the replacement of chemical nitrogen and phosphorus significantly, the biological activation of the soil and the restoring of the natural soil fertility. These bioproducts guarantee soil sterility, avoid development of the pathogens and assure good protection against weeds. Finally they stimulate plant growth and increase the crop yield (Stoilov 1986; Stojanov & al. 1986; Manolov & Stoilov 1995). Successful results have been obtained from the application of biofertilisers and natural minerals in cultivation of different vegetables, flowers, strawberry's cultures and citruses (Stojanov & al. 1986). The cultivation of the medicinal plants is a very appropriate process for the application of the above-mentioned biologically active bioproducts, but there are not enough investigations on it (Evstatieva & al. 2001).

Recently several new biofertilisers and natural minerals are being used widely in the agricultural practice. Such are:

1. Nitrogen-fixing bacteria of the genus *Azotobacter* (strain *Azotobacter* BG No. 1619) found in soil and water that convert atmospheric nitrogen to a stable or biologically available form for the plant nutrient.

2. Vermicompost (also called Worm Compost, Vermicast, or Worm Manure) which is the excretion product of the breakdown of organic matter by some species of earthworms as *Eisenia fetida* and *Lumbricus rubellus*. Vermicompost is a natural fertiliser and soil conditioner. It possess all of the necessary nutrient compounds in times more than the richest natural soil (Stancheva 2003). It increases

the yields and quality of crops about 20–30 % with. Introduced into soil it assures nutrients for the cultivated plants for 3–5 years.

3. Zeolite substrate – this mineral substrate is designed for growing up of a wide range of agriculture plants in the conditions of a greenhouse. It is made out of natural mineral (as a name "Hydrocult" from "Beli Plast" deposit, Bulgaria) and contains all necessary nutrients for plant growth in the form of minerals. As a medium for plants growth it has a large store of nutritives, good physical properties, sufficient granule solidity, long-term operation without further use of mature or nutrient solution (2–3 years), purity of weeds, sterility and good aesthetic looks (Herchey & al. 1980; Weber & al. 1983; Ivanova & al. 1997).

The object of this study was to investigate the effect of some modern biological fertilisers on the essential oil composition.

Material and methods

Vegetatively propagated parts of cv. *Salvia officinalis*, Dalmatica origin, were transplanted to 1 kg plastic pots and were grown in a greenhouse during 2002–2004. The study included six variants/experiments of fertilisation (fertilisation with standard *Azotobacter* inoculated directly on leaves (A_L) and into greenhouse soil (A_S), Vermicompost – directly on the leaves (V_L) and Vermicompost – 10 g added in 1 kg greenhouse soil (V_S), pure zeolite substrate (Z) and zeolite substrate and greenhouse soil in 1:1 mixture (Z_S), in comparison with an untreated greenhouse soil as a control sample (K). Experiments consisted of 4 replications of fertilisation during 2002–2004.

Samples of leaves for chemical investigation were collected in the time of blossoming (at the flowering stage) of the second year.

The air-dried leaves of each variant/experiment were subjected to hydrodistillation in Clevenger type apparatus for 2 hours. The oil was evaluated qualitatively by GC analysis. The components were identified during odour trapping and compared with those of the pure standards. GC analyses were carried out on Rue Unicam Gas Chromatograph.

GC and GC/MS analyses: GC analyses were carried out on HP 5890 gas chromatograph (FID), carrier gas nitrogen, linear velocity 25 cm/s, fused silica capillary column HP 1, 30 m × 0.25 mm, d_f = 0.25 µm. The injector and detector temperatures were 260 °C; column temperature was programmed from 50 °C to 230 °C at a rate of 4 °C/min, and 15 min at 260 °C. GC/MS analyses were performed using an HP 6890 instrument. All the GC conditions and the capillary column used were as described above, but the carrier gas was He.

Results

The chemical composition of the studied sage oils can be seen in Table 1. The table contains only components in amount of more than 1 %, which constituted above 90 % of the oil samples (with exception of a sample A_L).

The analysed oils were characterised by high content of α- and β-thujone. These compounds were found in concentrations 32.2 % and 6.5 %, respectively, in the

Table 1. The chemical composition of the studied sage oil in 6 different fertilisations.

sample	1	2	3	4	5	6	7	8	9	10	11	12	13	total	% e.o.
K	0.70	8.16	26.70	2.63	12.76	4.39	0.87	11.01	1.38	8.11	7.10	0.63	7.90	90.09	0.80
A_L	1.05	4.20	37.11	3.32	12.03	4.08	–	3.98	–	4.83	8.76	0.30	8.10	87.76	1.10
A_S	0.72	6.36	41.33	3.70	9.44	2.69	–	7.64	–	6.07	7.27	0.91	7.01	93.14	1.16
V_L	1.20	4.32	40.78	8.48	8.18	2.19	–	4.72	0.50	5.10	8.59	1.15	6.08	90.79	0.96
V_S	1.20	8.28	27.70	3.15	9.22	2.87	–	12.42	–	6.54	12.86	0.83	9.59	94.66	1.14
Z	1.18	6.82	34.94	3.74	6.71	1.66	–	6.49	–	7.39	10.85	0.86	10.68	91.32	1.10
Z_S	0.94	6.41	26.04	6.24	6.07	3.83	–	9.22	–	8.40	13.22	1.07	9.37	90.81	1.01
Essential oil of cv. *S. officinalis* growing in the open air (Peshevski & al. 1997)															
	1.4	11.0	32.2	6.5	15.3	4.5	1.3	4.3	–	4.0	–	–	–	–	–

Legend:
Components: 1, β-pinene; **2,** 1,8-cineole; **3,** α-thujone; **4,** β-thujone; **5,** camphor; **6,** borneol; **7,** bornyl acetate; **8,** β-caryophyllene; **9,** aromadendrene; **10,** α-humulene; **11,** viridiflorol; **12,** sesquiterpene alcohol; **13,** manool;
K – Untreated greenhouse soil as a control sample; **A_L** – *Azotobacter* inoculated directly on leaves; **A_S** – *Azotobacter* inoculated into greenhouse soil; **V_L** – Vermicompost – directly on the leaves; **V_S** – Vermicompost – 10 g added in 1 kg greenhouse soil; **Z** – Pure zeolite substrate; **Z_S** – Zeolite substrate and greenhouse soil in 1:1 mixture.

plant growing in the open air (Peshevski & al. 1997). Their amounts significantly decreased to 26.7 % and 2.63 % under greenhouse conditions (standard sample K). The content of camphor was also reduced from 15.3 % to 12.8 %. 1,8-cineole slightly decreased from 11.0 % to 8.16 % in plants from the greenhouse. Further, β-caryophyllene and α-humulene were components, whose concentrations increased considerably in comparison with plants growing in greenhouse and open air. Thus, the concentration of β-caryophyllene was changed from 4.3 % to 11.0 %, and α-humulene – from 4.0 % to 8.11 %.

It was observed that the oil content of the all studied samples was almost with permanent quantity of 0.96–1.16 %, while the quantity of individual components varied significantly depending on the fertilisers used for treatment. The content of α-thujone in the sample Z_S (zeolite substrate: soil = 1:1) and the standard sample K was almost identical. It increased to 41.33 % and 40.78 % when the soil was fertilised with *Azotobacter* in soil (sample A_S) and Vermicompost on leaves (sample V_L) in comparison with standard sample (sample K) – 26.7 % and plants from the open air – 32.2 %.

β-thujone was affected mainly from Vermicompost on leaves – 8.48 % (sample V_L) and Zeolite substrate in soil – 6.24 % (sample Z_S).

The concentration of camphor in sample A_L (12.03 %) was similar to this in the standard sample K (12.76 %). Zeolite substrate in soil (Z_S) and pure Zeolite substrate (Z) affected to a great extent the content of camphor whose quantity was two times less (6.07 % and 6.71 %) in comparison with a standard sample K – 12.76 %.

Manool and viridiflorol were components which have not been found previously in oils from cultivated plants of *S. officinalis* of different origins in Bulgaria. These two compounds were determined now for the first time in plants from greenhouse (sample K) in relatively high concentrations – 7.9 % and 7.1 %, respectively.

The new components for the Bulgarian cultivated sage dominated in the plants treated by Zeolite containing variants (Z and Z_S) and Vermicompost in soil (V_S). Thus, viridiflorol was found to be 10.85 %, 13.22 %, and 12.86 %, respectively, compared to the standard sample K – 7.10 %. Manool was found in amount 10.68 %, 9.37 %, 9.59 %, respectively, while in a standard sample K it was 7.9 %.

The influence of different biological fertilisers on the quantity of some *S. officinalis* oil components provides opportunity to improve the oil quality in general. Depending on the preferred concentration of individual components in the oil, the respective fertiliser could be used. The results could be used for modification of the oil composition in accordance with pharmaceutical and perfumery requirements. As an example the camphor is widely used in numerous medicinal preparations because of its biological activity. Treatment of the leaves with *Azotobacter* gives plants with highest amount of this component (sample A_L 12.03 %). Thujone was found to possess antioxidant activity. From this point of view fertilisation of the soil by *Azotobacter* or spraying the leaves by Vermicompost gave plants with highest percentage of thujone.

Good quality Dalmatian sage oil is considered when it contains α- and β-thujone in concentration above 50 % and camphor bellow 20 % (Guenther 1949; Putievsky & al. 1992). Because of the toxicity of these monoterpene ketones (Tisser & Balacs 1995) later a standard was accepted, which allowed 18–43 % α-thujone and 3–8.5 % β-thujone. With respect to these requirements all the studied samples corresponded to this standard. From this point of view all of them were with a good quality.

Acknowledgements. The authors extend their thanks to the National Science Fund of Bulgarian Ministry of Education and Science for their support.

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Lignan production in cell cultures of *Linum tauricum* ssp. *tauricum*

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Abstract. Callus and suspension cultures were created from *Linum tauricum* ssp. *tauricum* using standard methods. The two main lignans: 6-methoxypodophyllotoxin (MPTOX) and 4'-demethyl-6-MPTOX were identified in the cultures. Both compounds, isolated for the first time from the intact plant, were identified by HPLC, UV and ^{1}H NMR. As a result of optimisations of growth media, a stable growth and production of both compounds were achieved. Elicitation by extracellular methyl jasmonate not only increases the biosynthesis of both compounds, but also changes the ratio in comparison with the intact plant.

Key words: cell cultures, lignans, *Linum tauricum*

Introduction

Antitumor activity is undoubtedly the most clinically relevant property of lignans, the semisynthetic derivatives of the aryltetralin lignan podophyllotoxin (PTOX), etoposide, teniposide and etopophos being currently in use (Mohagheghzadeh & al. 2002). Presently aryltetralin lignans are actually derived exclusively from roots and rhizomes of several *Podophyllum* species. Due to their restricted natural abundance and the important pharmacological application of these active compounds, identification of new sources or establishment of rational *in vitro* synthesis is very important for the production of therapeutic candidates for cancer chemotherapy. Plant *in vitro* cultivation has several advantages over collecting plants from fields. These are: production under controlled and reproducible conditions, independence of geographical and political factors. There is no necessity of application of harmful to the nature herbicides and insecticides. Another advantage is the fact that undifferentiated plant cells, maintained in a liquid medium possess a high metabolic activity due to which considerably high yields of secondary products can be achieved in short terms (one to three weeks) of cultivation. Elicitation is a biotechnologically established approach to enhance the effectiveness of an *in vitro* system for the production of a given metabolite. Exogenously supplied methyl jasmonate (MeJa) has been reported to induce various secondary metabolites' production in different cell cultures, which was proven to be due to the *de novo* transcription of genes, such as phenylalanine ammonia lyase, known to be involved in the chemical defence mechanisms of plants (Schmitt & Petersen 2002).

Linum tauricum Willd., occurring in the Balkan and Crimean regions, belongs to the section *Syllinum* of the genus *Linum* (*Linaceae*) and according to the *Flora of the People's Republic of Bulgaria* (Petrova 1979) has four subspecies: *serbicum* (Podp.) Petrova, *bulgaricum* (Podp.) Petrova, *tauricum* and *linearifolium* (Lindem.) Petrova. After elucidation of the phytochemical composition of the green parts of the intact plant, conventional *in vitro* cultures of the taxon *L. tauricum* ssp. *tauricum* were initiated. The objective of this study was to quantify and stimulate lignan accumulation in cell cultures of *L. tauricum* ssp. *tauricum*, which were initiated from us for the first time.

Material and methods

Plant material

The seeds from *L. tauricum* ssp. *tauricum* were collected near Varna town (Bulgaria) in July 2003. A

voucher specimen is deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM 126 657).

Plant cultures

Seeds were germinated under sterile conditions on hormone free MS–medium (Murashige & Skoog 1962) in continuous light. Sterile grown seedlings were used for initiation of callus cultures. Cell suspension cultures were derived from the callus cultures by transferring 5 g callus cells to 50 ml medium in a 300 ml Erlenmeyer flask. Standard medium for callus and suspension cultures was G48 (MS–medium containing kinetin 2 mg l^{-1}, 2,4-dichlorophenoxyacetic acid 0.1 mg l^{-1} and indoleacetic acid 0.2 mg l^{-1}). Callus cultures were transferred every 3 weeks into 100 ml fresh medium. Calli were incubated under permanent light. The suspension cultures were subcultivated every 12 days by transferring 5 g wet cells with a perforated spoon into 50 ml fresh medium. Suspension cultures were grown in dark on a gyratory shaker at 120 rpm.

Extraction and isolation of lignans

A fine powder (0.2 g) of the lyophilised plant material was extracted with methanol (2 ml) in an ultrasonic bath (two times for 30 s). Distilled water (6 ml) was added, and the pH was adjusted to 5.0 by o-phosphoric acid. After adding of β-glucosidase (1 mg), the sample was incubated at 35 °C for 1 h. Methanol (12 ml) was added and the mixture was incubated for another 10 min at 70 °C in an ultrasonic bath. After centrifugation, the supernatant was used directly or stored at -18 °C for HPLC.

HPLC analysis

Separation was performed using a GROM–SIL 120 ODS–5ST column with guard column (250 mm long, 4.6 mm i.d. and 40 mm long, 4.6 mm i.d., respectively; Grom Company, Herrenberg, Germany) and a gradient programme with water (A) and acetonitrile (B) as eluents as follows: 0 min – 25 % B, 0.8 ml/min, 25 min – 38 % B, 0.8 ml/min, 43 min – 43 % B, 1 ml/min, 46 min – 55 % B, 1 ml/min, 48 min – 70 % B, 1 ml/min, 50 min – 25 % B, 1 ml/min, 55 min – 25 % B, 0.8 ml/min. Retention times were for 4'-demethyl-6-methoxypodophylotoxin and for 6-methoxypodophyllotoxin at 28.82 min and 39.74 min, respectively (Fig. 1).

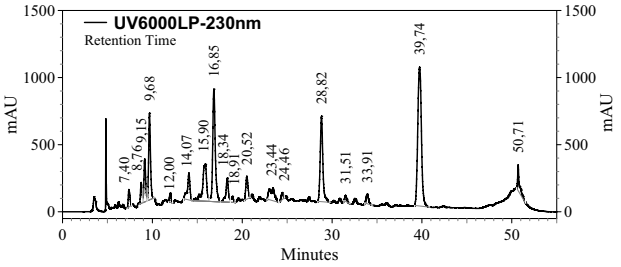

Fig. 1. HPLC of an extract from callus of *L. tauricum* ssp. *tauricum* showing the two compounds isolated 4'-demethyl-6-methoxypodophyllotoxin at Rt 28.82 min and 6-methoxypodophyllotoxin at 39.74 min, respectively.

Elicitation with extracellular methyl jasmonate

The elicitation with extracellular methyl jasmonate was performed at the 3rd day of culture. Three groups of MeJa treated suspensions were studied – with concentrations of the elicitor 50 μM, 100 μM and 150 μM in the nutrition media. A parallel control group without elicitor treatment was monitored. Every second day the biomass was harvested, suction filtrated and the fresh weight determined. After the establishment of a constant biomass weight, its dry weight was determined and the growth index (GI) was calculated following the formula: GI = Mi/Mo, where GI is growth index, Mi is the mass of suspension cells, obtained at the day of harvest, and Mo is the putative mass of the suspension cells on the 1st day of culture. Investigation was carried out in order to determine the independent impact on the suspensions of ethanol, needed to dissolve methyl jasmonate in the nutrition media (0.024 % v/v for 100 μM MeJa in 50 ml medium, 0.012 % v/v – for 50 μM MeJa and 0.036 % v/v – for 150 μM MeJa). For this reason the behaviour of two groups of suspensions with 0.024 % v/v and 0.036 % v/v ethanol in the nutrition media was investigated.

Results and discussion

The podophyllotoxin derivatives 4'DM-6MPTOX and 6MPTOX have been isolated and identified by NMR and UV as the two main lignans in the aerial parts of *L. tauricum* ssp. *tauricum* (Konuklugil & al. 2007). Here we report the identification of both derivatives as the main lignans in cell cultures of *L. tauricum* ssp. *tauricum*. 4'DM-6MPTOX is interesting with its activity; the pharmacological investigation of its cytostatic properties has demonstrated activity 2 to 3.5 times higher than that of the referent antineoplastic drug etoposide (Vasilev & al. 2005). There is no report in

the literature related to an elicitation experiment with extracellular MeJa for the enhancement of the production of the aryltetralin derivative 4'DM-6MPTOX in a plant cell *in vitro* system and no other elicitation experiments with a *L. tauricum* ssp. *tauricum* cell suspension culture. The PTOX derivatives 4'DM-6MPTOX and 6MPTOX were isolated and identified as the two main lignans in the callus and shoot cultures. The two substances were however produced only in traces in the suspension cultures of this species. Experiments with extracellular MeJa have led to a significant increase of the synthesis of both lignans in suspension culture. A comparison of fresh and dry weight curves between control/ethanol treated suspensions (Fig. 2) shows that the dry weight maximum is always achieved prior to the fresh weight maximum, which could be attributed to the slower water accumulation (respectively achievement of fresh weight) by the vacuole. The ethanol impact on the growth dynamics of the suspensions shows that the maximal values of fresh weight, dry weight and growth index are achieved simultaneously during the culture period in all control and treated groups. It can be seen that ethanol, in concentrations 0.024 % v/v and 0.036 % v/v in the nutrition medium, causes no retardation on the growth of the suspensions. The growth index maximums show that the putative biomass of the control has increased 7.5 times, the 0.024 % v/v EtOH treated suspension increases its biomass 6.96 times and the 0.036 % v/v EtOH treated suspension – only 4.6 times. This leads to a conclusion that the 0.024 % v/v concentration of ethanol has a considerably smaller inhibiting impact on growth rate, compared to 0.036 % v/v.

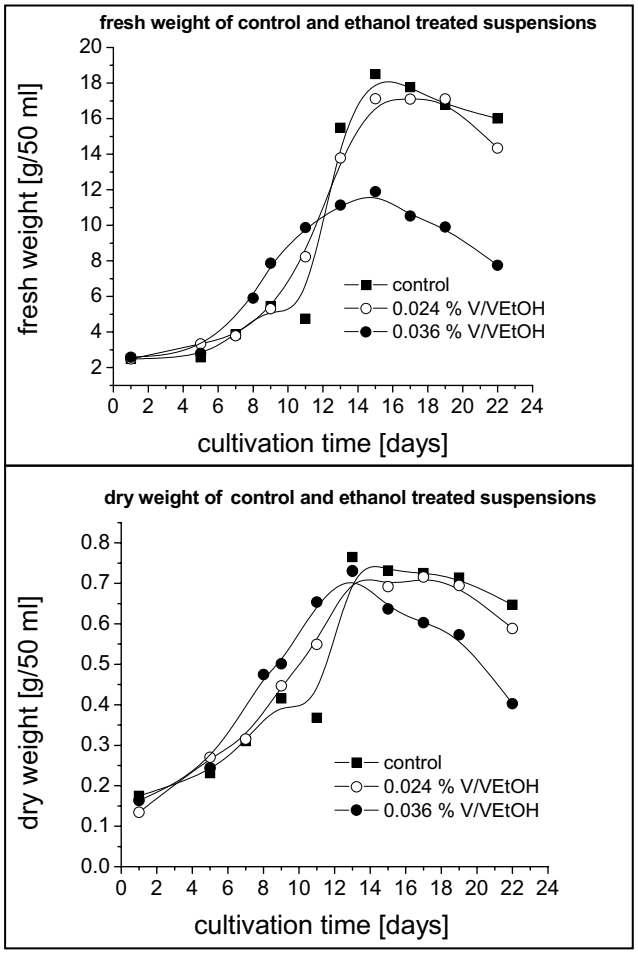

Fig. 2. Dynamics of biomass formation of *L. tauricum* ssp. *tauricum* cell suspension culture.

Most favourable proves to be the concentration of the elicitor 100 μM in the nutrition medium, which leads to substantial increase of the levels of both lignans from traces, reaching a maximum of 0.1180 mg/g dw and 0.1250 mg/g dw for 4'DM-6MPTOX and 6MPTOX, respectively (Table 1). Moreover, there is a commensurable ratio between the two compounds in 100 μM MeJa elicited suspensions. The lower elicitor concentration (50 μM) proves to be insufficient for synthesis of both lignans, and stimulates biosynthesis only of 6MPTOX up to 0.0443 mg/g dw. A concentration of 150 μM extracellular methyl jasmonate also induces the production only of 6MPTOX in a level of 0.0570 mg/g dw, which could be attributed to the inhibiting impact of higher concentration of ethanol or/and MeJa. The enhancement of the biosynthesis, from traces to almost equal levels of 4'DM-6MPTOX and 6MPTOX, respectively, is an indication that lignans in *L. tauricum* ssp. *tauricum* may be plant defence compounds as reviewed by Harmatha & Dinan (2003). The results of the present study indicate that addition of extracellular methyl jasmonate can not only increase the biosynthesis of

Table 1. Levels of the two main aryltetralin lignans 4'DM-6MPTOX and 6MPTOX, in suspension cultures before and after the elicitor treatment with the three concentrations of extracellular methyl jasmonate.

L. tauricum **ssp.** *tauricum*	4'DM-6MPTOX (mg/g dw)	6MPTOX (mg/g dw)
Suspension without elicitation	traces	traces
Suspension with 100 μM MeJa elicitation	0.1180	0.1250
Suspension with 50 μM MeJa elicitation	traces	0.0443
Suspension with 150 μM MeJa elicitation	traces	0.0570
Suspension with 0.024% v/v ethanol	traces	traces
Suspension with 0.036% v/v ethanol	traces	traces

Abbreviations: 6MPTOX – 6-methoxypodophyllotoxin; 4'DM–6MPTOX – 4'-demethyl-6-methoxypodophyllotoxin.

both 4'DM-6MPTOX and 6MPTOX in a *L. tauricum* ssp. *tauricum* grown suspension culture, but can also change the ratio of both compounds, in comparison with the intact plant and callus cultures, towards the more valuable 4'DM-6MPTOX. Moreover, our experiment shows that due to the 100 μM MeJa impact, the pharmacologically more valuable 4'DM-6-MPTOX reached a concentration, commensurable to the more common 6MPTOX. Note that, in the intact plant and in the non-elicited callus and shoot cultures, the levels of 4'DM-6MPTOX were almost twice as lower than of 6MPTOX.

Besides a compound with a trimethoxylated (6-methoxypodophyllotoxin) pendant ring also a 4'-demethyl analogue occurs. The 4'-demethyl-6-methoxypodophyllotoxin was isolated previously only from cell cultures of *Linum flavum* L. (also section *Syllinum*), but it is not a main lignan there. The detection of both PTOX–derivatives, 6-methoxypodophyllotoxin and 4'-demethyl-6-methoxypodophyllotoxin, in *L. tauricum* confirms again the hypothesis of Broomhead & Dewick (1990) that the presence of aryltetralin lignans is typical for the section *Syllinum* of the genus *Linum*.

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Phytochemical investigation of *Astragalus ponticus*

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Abstract. Seven known compounds were isolated from the aerial parts of *Astragalus ponticus* (*Fabaceae*). Based on chemical and spectral methods, the structures of the compounds were established as 3-O-methyl-D-chiro-inositol (D-pinitol) (**1**), eriodictyol-7-O-glucoside (**2**), eriodictyol-7-O-rhamnoside (**3**), quercetin-3-O-rhamnoside (quercitrin) (**4**), quercetin-3-O-arabinoside (avicularin) (**5**), quercetin-3-O-rutinoside (rutin) (**6**) and quercetin-3-O-galactoside (hyperoside) (**7**). This is the first reported occurrence of eriodictyol-7-O-rhamnoside and avicularin in genus *Astragalus* and D-pinitol, eriodictyol-7-O-glucoside and quercitrin in *A. ponticus*.

Key words: *Astragalus ponticus*, D-pinitol, *Fabaceae*, flavonoids

Introduction

Astragalus L., the largest genus in the family *Fabaceae*, is represented by 27 species in the flora of Bulgaria (Kozhuharov 1992). Many *Astragalus* species are used in traditional medicine as diuretic, tonic, antiperspirant, antihypertensive and diabetic (Tang & Eisenbrand 1992; Sinclair 1998).

Astragalus ponticus Pall. is a species mostly distributed throughout the temperate regions of the world, located principally in Southeastern Europe (Bulgaria, Romania), the Caucasus and Southwestern Asia. In a previous paper we reported the isolation of five flavonoids from ethyl acetate extract of this plant (Krasteva & al. 1999).

The aim of this study was to determine other compounds of the aerial parts of *A. ponticus*.

Material and methods

General

Melting points are uncorrected. UV spectra were recorded on a "WPA–LIGHTWAVE" spectrometer with diagnostic shift reagents. Optical rotation was measured with a Perkin–Elmer 343 polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on Bruker DPX–400 and Bruker AMX–400 instruments using TMS as internal standard. EIMS, HRMS and FABMS were carried out on Varian MAT CH_7A, Finnigan MAT 711 and Finnigan MAT CH_5DF spectrometers, respectively. Thin-layer chromatographic study (TLC) was carried out on silica gel plates (Kieselgel G, F_{254}, type 60, Merck, Germany), using the solvent systems: EtOAc/HCOOH/H_2O (100:10:40), $CHCl_3$/MeOH (9:1), BuOH/AcOH/H_2O (4:1:1) and Me_2CO/$CHCl_3$/MeOH/H_2O (75:10:10:5). The spots were visualised by spraying with NTS/PEG reagent (for flavonoids) and aniline/hydrogen phthalate reagent, followed by heating at 110 °C (for sugars). Column chromatography (CC) was carried out with Sephadex LH–20 (Pharmacia, Sweden) and Polyamid S (Riedel-de Haën, Germany). Preparative TLC was performed on silica gel plates (Kieselgel 60, 0.5 mm thick, Merck, Germany), eluted with S_1 (EtOAc/AcOH/H_2O, 100:10:40) and S_2 (EtOAc/MeCOEt/HCOOH/H_2O, 5:3:1:1).

Plant material

The aerial parts of *A. ponticus* were collected during the flowering period (June 2000) in North Bulgaria. The plant was identified by Dr D. Pavlova from the Department of Botany, Faculty of Biology, Sofia University, where voucher specimen has been deposited (SO 95177).

Extraction and isolation

The air-dried plant material (600 g) was extracted with 70 % ethanol under reflux. After the removal of EtOH the aqueous extract was consecutively treated with $CHCl_3$, EtOAc and n-BuOH. The EtOAc extract (9 g) was chromatographed on a Sephadex LH–20 column (MeOH) to give two main fractions (I and II). Fraction I was purified by chromatography on Sephadex LH–20 column (MeOH), followed by preparative TLC with solvent system S_1 to afford **4** (7 mg) and **5** (8 mg). Fraction II was rechromatographed on a Polyamid S column with H_2O–EtOH gradient (0–90 % EtOH) and further purified by preparative TLC in S_1 to give **2** (11 mg) and **3** (5 mg). The BuOH extract (12 g) was subjected to separation and purification by silica gel ($CHCl_3$/MeOH/H_2O, 98:72:9) and Sephadex LH–20 columns (MeOH) to yield two main fractions (A and B). The fraction A was further purified by preparative TLC in solvent system S_2 to afford **6** (34 mg) and **7** (9 mg). From fraction B, after treating with methanol at room temperature, yielded **1** (90 mg) that was separated by filtration and submitted to recrystallisation from ethanol.

3-O-methyl-D-chiro-inositol (D-pinitol) (**1**): White to off-white crystals, sweet in taste, well soluble in water; mp 186–188 °C, $[\alpha]_D^{20}$ + 65° (C = 0.5, H_2O); EIMS 70eV, *m/z* (rel. int.): 176 $[M\text{-}H_2O]^+$, 158 $[M2\text{-}H_2O]^+$, 162 $[M\text{-}CH_3OH]^+$, 144 $[M\text{-}CH_3OH\text{-}H_2O]^+$, 116 (base peak); FABMS (negative) *m/z*: 193 $[M\text{-}H]^-$, FABMS (positive) *m/z*: 195 $[M+H]^+$; HREIMS *m/z* 194. 07922 (calcd. 194. 07904 for $C_7H_{14}O_6$); ^1H and ^{13}C NMR: Table 1.

Eriodictyol-7-O-glucoside (**2**): White powder; mp 172–175 °C; UV (MeOH) λ_{max}: 275, 325 sh; +NaOMe: 278, 318; +NaOAc: 278, 330; +NaOAc/H_3BO_3: 278, 336; +$AlCl_3$: 284, 320; +$AlCl_3$/HCl: 284, 320.

Table 1. ^{13}C and ^1H NMR data of D-pinitol [D_2O, δ (ppm), J in H_2].

Position	δ_C	δ_H
1	74.30	4.01 (m, overlapping)
2	72.65	3.82 (dd, 10.0; 2.7 Hz)
3	85.59	3.35 (dd, 10.0; 9.8 Hz)
4	74.95	3.65 (dd, 9.9; 9.8 Hz)
5	73.38	3.76 (dd, 9.9; 2.6 Hz)
6	74.51	4.01 (m, overlapping)
3-OCH3	62.51	3.60 (s)

Eriodictyol-7-O-rhamnoside (**3**): White powder; UV (MeOH) λ_{max}: 271, 323 sh; +NaOMe: 272, 317; +NaOAc: 273, 337; +NaOAc/H_3BO_3: 273, 335; +$AlCl_3$: 284, 321; +$AlCl_3$/HCl: 284, 320.

Quercetin-3-O-rhamnoside (*quercitrin*) (**4**): Yellow powder; mp 180–183 °C; UV (MeOH) λ_{max}: 260, 355; +NaOMe: 275, 402; +NaOAc: 268, 364; +NaOAc/H_3BO_3: 265, 373; +$AlCl_3$: 275, 427; +$AlCl_3$/HCl: 275, 405.

Quercetin-3-O-arabinoside (*avicularin*) (**5**): Yellow powder; UV (MeOH) λ_{max}: 258, 357; +NaOMe: 271, 406; +NaOAc: 268, 390; +NaOAc/H_3BO_3: 262, 372; +$AlCl_3$: 270, 420; +$AlCl_3$/HCl: 264, 400.

Acid hydrolysis

Each glycoside (1 mg) was dissolved in 5 ml of 2N HCl/MeOH (1:1) for 1 h under reflux. The aglycones and sugars were identified by TLC with authentic samples.

Results and discussion

Solvent partition and repeated chromatographic purification of the ethyl acetate and butanol fractions and precipitate resulted in the isolation of seven known compounds (**1–7**) in pure form (Fig. 1).

In the EIMS, compound **1** gave a molecular ion peak $[M]^+$ at m/z 194, and significant peaks at m/z 176 $[M\text{-}H_2O]^+$, 158 $[M\text{-}2H_2O]^+$, 162 $[M\text{-}CH_3OH]^+$, 144 $[M\text{-}CH_3OH\text{-}H_2O]^+$, 116, 87 (base peak), and 73. The $[M]^+$ was confirmed by negative FABMS with a $[M\text{-}H]^-$ ion at m/z 193, and by positive FABMS with a $[M+H]^+$ ion at m/z 195. From the HRMS the molecular formula could be deducted as $C_7H_{14}O_6$.

^1H NMR data for **1** revealed the presence of two pairs of double doublets at: δ 3.35 (dd, $J_{2,3}$ = 10.00 Hz, $J_{3,4}$ = 9.80 Hz, H-3), δ 3.65 (dd, $J_{3,4}$ = 9.80 Hz, $J_{4,5}$ = 9.90 Hz, H-4), δ 3.76 (dd, $J_{4,5}$ = 9.90 Hz, $J_{5,6}$ = 2.60 Hz, H-5) and δ 3.82 (dd, $J_{2,3}$ = 10.00 Hz, $J_{1,2}$ = 2.70 Hz, H-2), a broad signal at 4.01 (2H, m, H-1, H-6 overlapping) and a three-proton singlet at 3.60 due to one methoxy group. The six ^{13}C NMR signals at δ 85.59, 74.95, 74.51, 74.30, 73.38, 72.65, and 62.51 (OCH_3) demonstrated the presence of a inositol monomethyl ether (Angyal & Odier 1983) (Table 1). The attachment of the methoxy group to C-3 was clear from the observation of ^1H,^{13}C long-range correlations between C-3 (δ_C 85.59) and the methoxy protons (δ_H 3.60) and between H-2 (δ_H 3.82) and the carbon C-3. The positive value of the optical rotation for **1**, $[\alpha]_D^{20}$ + 65°

(C = 0.5, H_2O) [lit. $[\alpha]_D^{20}$ + 65 °], is indicative of 3-O-methyl-D-chiro-inositol (Angyal & Odier 1983; Sridhar & al. 2006).

All the signals have been unambiguously confirmed by means of the ^{1}H-^{1}H COSY, HETCOR and ^{1}H,^{13}C long-range correlations NMR experiment.

Compounds **2** and **3** were obtained as amorphous white powders. The UV spectra of **2** and **3** showed an intense Band II absorption with only a shoulder or low intensity peak representing Band I, typically for flavanones (Mabry & al. 1970). The UV spectral data of the compounds with diagnostic shift reagents suggested the likely presence of **7,** substituted flavanone glycosides with free hydroxyl groups at 5, 3' and 4'-positions (Mabry & al. 1970; Markham 1982). Acid hydrolysis of **2** and **3** afforded the same genin, D-glucose and L-rhamnose. In addition, the compounds were co-chromatographed on TLC with authentic samples. On the basis of these results the compound **2** was identified as eriodictyol-7-O-glucoside and **3** as eriodictyol-7-O-rhamnoside.

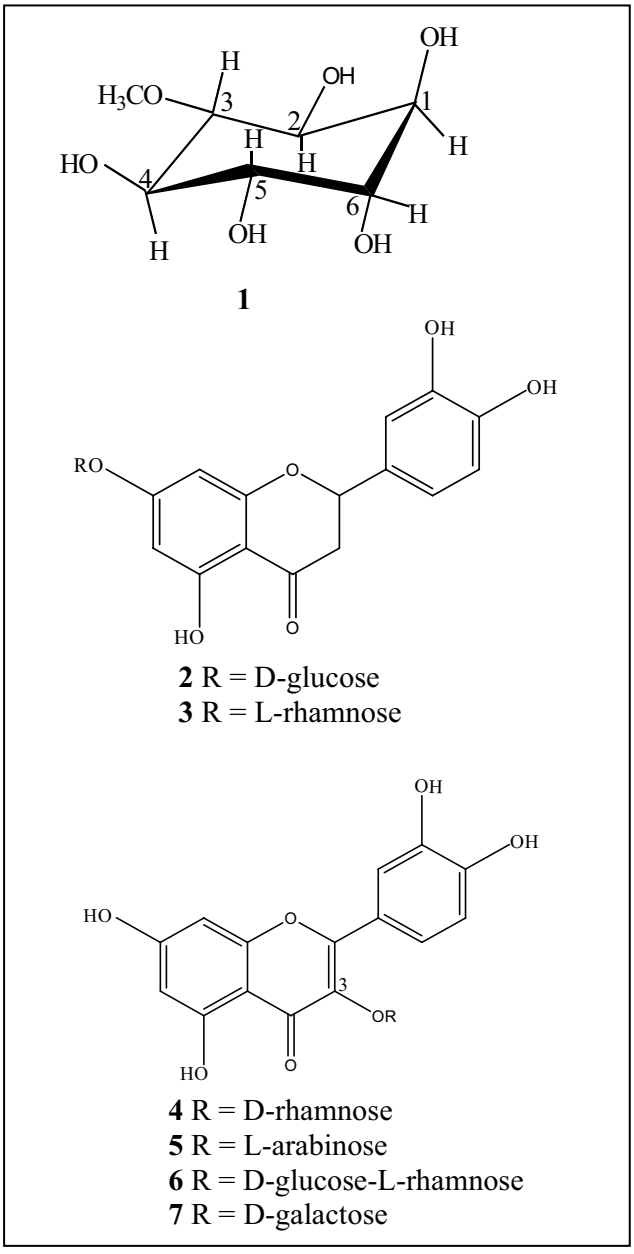

Fig. 1. Chemical structures of isolated compounds.

Compounds **4–5** are yellow powders. Their UV spectral properties indicated a 3-substitued quercetin derivates and the presence of free hydroxyl groups at positions 5, 7 and 3', 4' (Mabry & al. 1970; Markham 1982). The compounds were also identified by acid hydrolysis and direct comparison with authentic samples as quercetin-3-O-rhamnoside (quercitrin) (**4**), and quercetin-3-O-arabinoside (avicularin) (**5**).

In previous study, we identified quercetin-3-O-rutinoside (rutin) and quercetin-3-O-galactoside (hyperoside) in the ethyl acetate extract of *A. ponticus* (Krasteva & al. 1999). The present study resulted in the isolation of the same compounds (**6–7**) from the butanol extract of the plant.

This is the first report on identification of eriodictyol-7-O-rhamnoside and avicularin in genus *Astragalus* and D-pinitol, eriodictyol-7-O-glucoside and quercitrin in *A. ponticus*.

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Evaluation of antimicrobial activity of the macrofungus *Suillus bellinii*

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Abstract. In this study, ethyl acetate, acetone, chloroform and ethanol extracts of *Suillus bellinii* (*Boletaceae*) were tested for antimicrobial activity by Disc Diffusion method against some Gram-positive and Gram-negative bacteria and against yeast cultures. According to our results, the extract prepared from the macrofungus has effectively high antibacterial activity against the Gram-positive and Gram-negative bacteria, but it had no antifungal activity against the yeast cultures used in this study.

Key words: antimicrobial activity, *Suillus bellinii*

Introduction

Many antibiotics in clinical use developed from fungal and actinomycete metabolites. Large-scale screening programs of the 1940s for the detection of antibiotic activity included a variety of fleshy basidiomycetes (Benedict & Brady 1972; Board & Lovelock 1975). A number of more recent reports recorded additional general observations of microbial antagonism with basidiomycetes (Espanshade & Griffith 1966; Cochran 1978). Unfortunately, the identities of the basidiomycete metabolites responsible for the antimicrobial effects are still unknown in most instances.

The polyacetylenes are the most extensively characterised group of antagonistic mushroom constituents. More than 50 of these unsaturated antibiotic substances are known from one or more species of *Aleurodiscus*, *Clitocybe*, *Coprinus*, *Cortinellus*, *Marasmius*, *Merulies*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella* and *Tricholoma*. Other known antagonist compounds from basidiomycetes include the phenolic metabolites (Benedict & Brady 1972; Cochran 1978).

Although there are many investigations on S*uillus bellinii* (Inzenga) Watling in different subjects (Isikov & Kuznetzov 1990; Campanile & al. 2004), antimicrobial activity of this macrofungus has not been previously investigated. Therefore, our aim was to determine the antimicrobial effects of the extracts of S. *bellinii* against various microorganisms.

Material and methods

Macrofungal material

S*uillus bellinii* was collected from Uludag Mt, Bursa–Turkey in 1997. The macrofungus was identified by Prof. Dr. Fahrettin Gucin, Fatih University, Faculty of Science and Arts, Department of Biology, Istanbul. A voucher specimen has been deposited at the herbarium of Department of Biology, Uludag University, Bursa–Turkey.

Microorganisms

In this study, the following microorganisms were used: *Aeromonas hydrophila*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Serratia marcescens*, *Bacillus cereus*, *B. subtilis*, *B. brevis*, *B. sphaericus*, *B. megaterium*, *Mycobacterium smegmatis*, *Micrococcus luteus*, *M. flavus*, *Staphylococcus aureus*, *S. epidermidis*, *Alcaligenes faecalis*, *A. eutrophus*, *Salmonella typhi*, *S. typhimurium*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Erwinia amylovora*, *Xanthomonas campestris*, *Pseudomonas extorquens*, *P. fluorescens*, *P. aeruginosa*, *P. putida*, *Kluyveromyces fragilis*, *Candida albicans*, *Rhodotorula rubra*, *Aspergillus oryzae*, *A. flavus*, *Botrytis*

cineriae, *Fusarium oxysporium*, *Streptomyces murinus* and *Nocardia cornea*. Test microorganisms were obtained from culture collection of Ege University, Faculty of Science, Basic and Industrial Microbiology Department.

Preparation and antimicrobial activity of extracts

The material was ground to a fine powder. 15 g of this material were subjected to Soxhlet extraction for 12 h each using 150 mL of the following solvents: ethyl acetate, acetone, chloroform and ethanol. The extracts were kept at +4 °C (Khan & al. 1988; Dulger & Gonuz 2004).

In vitro antimicrobial studies were carried out by the Agar–Disc Diffusion method against test microorganisms. Mueller Hinton Agar (OXOID) was used as the most suitable medium for antimicrobial activity studies. The sterilised medium at 45–50 °C was poured into petri dishes. Agar depth was 4 mm. For 90 mm diameter plates 25 mL of medium were used. According to this method, ethanol, ethyl acetate, acetone and chloroform extracts were impregnated as 4 discs in ranging concentrations from 50 µL. Then all discs were dried at 50 °C and placed into the bacteria and yeasts petri dishes. Each disc was 6 mm in diameter. For each experiment a fifth disc, which contained only solvent, was used as control disc. As reference, antibiotics AK30 (= Amikasin) for bacteria and NY100 (= Nystatin) were used. Experiments were repeated three times and the results were expressed as average values.

Bacterial and yeast cultures were suspended in 4–5 mL Brain Heart Infusion Broth (OXOID). Bacteria were incubated at 37 °C for 2–5 h. Yeast cultures were incubated at 30 °C for 5–7 h. When a visible turbidity was obtained at the end of this time, the turbidity of bacterial suspension was adjusted against MacFarland Standart Tube [0.5] with physiologic serum and inoculation was performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. The entire Mueller Hinton Agar surface was streaked in 3 different directions by rotating the plate 60° angles after each streaking. Yeast cultures were inoculated into Mueller Hinton Agar (10^2 cfu/mL). All petri dishes after inoculation were allowed to dry for 15–20 min at room temperature. For bacteria (at 35 °C) and yeasts (at 30 °C), inhibition zone diameters were measured after 24–48 hours using Agar–Disc Diffusion method (Collins & Lyne 1987; NCCLS 1993).

Spore suspensions of filamentous fungi and actinomycetes were cultured on Sabouraud's Dextrose agar (10^5 cfu/mL) by plate dilution techniques using Thoma & Howard slides (Board & Lovelock 1975; Mitrokotsa & al. 1993; Favel & al. 1994). It was observed that Agar–Disc Diffusion method was generally not suitable for filamentous fungi and actinomycetes. Therefore this method was used after modification. In this experiment, the solutions (from 10 to 200 µg/mL) were added into the medium after autoclaving. Erythromycine (15 µg/mL) was used as a comparison antibiotic against filamentous fungi. The antibiotic was added into the medium. The evaluation of filamentous fungi and actinomycetes was carried out by means of reproduction on the medium and reduction of the colony numbers at the end of the seven days (Collins & Lyne 1987).

Results and discussion

The zone diameters of the plates after incubation are given in Table 1. According to our findings, all of the extracts of *S. bellinii* have been found to be ineffective against all *Bacillus*, *Salmonella* and *Micrococcus* species, *Escherichia coli*, *Enterobacter aerogenes*, *Listeria monoctogenes*, *Erwinia amylovora* and *Pseudomonas extorquens*. The various extracts of *S. bellinii* have been determined to be less effective than that of AK30 used as comparison antibiotic against *Staphylococcus aureus*, *S. epidermidis*, *Serratia marcescens*, *Alcaligenes faecalis*, *A. eutrophus*, *Xanthomonas campestris*, *Pseudomonas putida*, *P. aeruginosa*, *P. extorquens* and *P. fluorescens*. Notably, all the extracts except for chloroform extract have been found to be more active especially against *Citrobacter freundii* than that of the standard comparison antibiotic AK30. Notably, the aid-fast bacterium *Mycobacterium smegmatis* is more suspectible to extracts. The ethanol extracts have higher antibacterial activity against *Aeromonas hydrophila*, *Proteus vulgaris* and *Klebsiella pneumoniae* than those of the other extracts as compared to standard antibacterial antibiotic AK30.

The extracts of *S. bellinii* have no antiyeast activity. The extracts were found to be ineffective against *Kluyveromyces fragilis*, *Rhodotorula rubra* and *Candida albicans*, as compared to standard antibiotic Nystatin.

Table 2 shows that the colony numbers of filamentous fungi and actinomycetes were reduced between 99.95 % and 99.98 % for the concentrations of 10 µg/mL and 50 µg/mL of the related compounds after the incubation whilst the concentrations 100 µg/mL and 200 µg/mL of these compounds inhibited filamentous fungi and actinomycetes growth completely. All results showed that colony numbers were reduced because of the activity of the compounds contained in the extracts.

Inhibition zone diameters around control disc were measured between 0–1 mm.

The macrofungus differ significantly in its activity against tested microorganisms. These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multi-layered structure and the yeast cell wall is quite complex (Yao & Moellering 1995). In addition, microorganisms' variable sensitivity to chemical substances relates to different resistance levels between the strains (Çetin & Gürler 1989).

According to the literature, ethanol extract was the most effective extract in Disc Diffusion method on macrofungi (Broadbent 1966; Benedict & Brady 1972). In Table 1, antimicrobial activities of ethanol extracts were higher than others. So, it can be said that solvent of the fungal compounds showing antimicrobial activity is ethanol.

As a result, the extracts prepared from the macrofungus have effectively high antibacterial activity against the Gram-positive and Gram-negative bacteria, but they have no antifungal activity against the yeast cultures used in this study.

Table 1. Antimicrobial activity of the extracts of *S. bellinii* on some bacteria and yeasts.

Tested microorganisms	Zones of inhibiton (mm)				Comparison antibiotic
	Acetone	Chloroform	Ethyl acetate	Ethanol	AK30/NY100
Aeromonas hydrophila ATCC 7966	15.7	16.6	15.8	23.2	21.2
Listeria monocytogenes ATCC 19117	–	–	–	–	20.6
Escherichia coli ATCC 11230	–	–	–	–	17.2
Enterobacter aerogenes ATCC 13048	–	–	–	–	18.6
Proteus vulgaris ATCC 8427	17.6	14.0	17.2	23.0	18.0
Serratia marcescens NRRL 3284	12.2	12.6	13.8	134	20.0
Bacillus cereus ATCC 7064	–	–	–	–	16.2
Bacillus subtilis ATCC 6633	–	–	–	–	16.4
Bacillus sphaericus	–	–	–	–	20.0
Bacillus brevis ATCC 9999	–	–	–	–	18.6
Bacillus megaterium	–	–	–	–	20.4
Mycobacterium smegmatis CCM 2067	23.2	25.8	22.4	28.2	18.2
Micrococcus luteus LA 2971	–	–	–	–	24.4
Micrococcus flavus ATCC 14452	–	–	–	–	20.0
Staphylococcus aureus ATCC 6538P	–	12.2	–	13.6	24.2
Staphylococcus epidermidis NRRL B–4877	–	–	–	14.2	23.2
Alcaligenes faecalis CCM 3763	–	–	–	14.0	19.8
Alcaligenes eutrophus	–	–	11.2	12.8	20.2
Salmonella typhi ATCC 19430	–	–	–	–	20.6
Salmonella typhimurium CCM 5445	–	–	–	–	19.2
Klebsiella pneumoniae UC57	14.0	17.8	16.2	23.4	20.0
Citrobacter freundii ATCC 8090	23.2	–	25.0	29.0	20.0
Erwinia amylovora	–	–	–	–	19.4
Pseudomonas putida	–	9.8	–	11.2	20.2
Pseudomonas extorquens	–	–	–	–	18.6
Pseudomonas fluorescens	–	–	–	10.8	20.4
Pseudomonas aeruginosa ATCC 27853	–	–	11.8	13.2	19.6
Xanthomonas campestris	8.8	–	–	11.8	20.2
Candida albicans ATCC 10231	–	–	–	–	19.8
Kluyveromyces fragilis ATCC 8608	–	–	–	–	17.8
Rhodotorula rubra DSM 70403	–	–	–	–	18.2

Legend: (–) – no inhibition zones.

Table 2. Antimicrobial activity of the extracts of *S. bellinii* on some filamentous fungi and actinomycetes.

		The colony numbers after incubation*				
Tested microorganisms	**Concentrations (µg/ml)**	**Ethyl acetate**	**Acetone**	**Chloroform**	**Ethanol**	**Erythromycin (15 µg/ml)**
Aspergillus oryzae	10	48	76	39	42	
	50	21	45	21	23	
	100	–	–	–	–	15
	200	–	–	–	–	
Aspergillus flavus	10	70	52	54	36	
	50	39	21	22	17	
	100	–	–	–	–	18
	200	–	–	–	–	
Botrytis cineriae	10	44	56	52	31	
	50	23	18	27	13	
	100	–	–	–	–	15
	200	–	–	–	–	
Fusarium oxysporium	10	36	64	34	22	
	50	21	29	18	9	
	100	–	–	–4	–	16
	200	–	–	–	–	
Streptomyces murinus	10	50	45	32	34	
ISP 5091	50	28	23	20	18	
	100	–	–	–	–	NT
	200	–	–	–	–	
Nocardia cornea	10	44	38	25	18	
IFO 14403	50	21	14	16	11	
	100	–	–	–	–	NT
	200	–	–	–	–	

Legend: (*) – data are the average of n = 3 experiments; (–) – no growth; (NT) – not tested.

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Antimicrobial activity of some *Agaricus* species from Turkey

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Abstract. In this study, the ethanolic extracts obtained from *Agaricus macrosporus*, *A. essetei*, *A. xanthodermus*, *A. praeclaresquamosus*, *A. impudicus, A. arvensis*, and *A. langei* have been investigated for their antimicrobial activity against *Bacillus subtilis*, *B. cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhimurium*, *Serratia marcescens*, *Debaryomyces hansenii*, *Kluyveromyces fragilis* and *Rhodotorula rubra* by disc diffusion method. As a result of the study, we have found that all macrofungi revealed strong antimicrobial activity against all the test microorganisms, especially the yeast cultures.

Key words: antimicrobial activity, macrofungi

Introduction

Large-scale screening programs from the 1940s for the detection of antibiotic activity included a variety of fleshy basidiomycetes (Broadbent 1966; Espanshade & Griffith 1966; Benedict & Brady 1972). A number of more recent reports recorded additional general observations of microbial antagonism with basidiomycetes (Cochran 1978). Unfortunately, the identities of the basidiomycete metabolites responsible for the antimicrobial effects are still unknown in most instances.

The polyacetylenes are the most extensively characterised group of antagonistic mushroom constituents. More than 50 of these unsaturated antibiotic substances are known from one or more species of *Aleurodiscus*, *Clitocybe*, *Coprinus*, *Cortinellus*, *Marasmius*, *Merulies*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella* and *Tricholoma*. Other known antagonist compounds from basidiomycetes include phenolic metabolites (Benedict & Brady 1972; Cochran 1978).

In addition, more recently, polysaccharides derived from mushrooms have emerged as an important class of bioactive substances. Many medicinal and therapeutic properties are attributed to polysaccharides present in basidiomycetes. They were shown to modulate the immune system, to have hypoglycemic, antithrombotic, antibiotic, anti-tumor and antiviral activities, to lower the blood pressure and the concentration of blood lipids, to inhibit inflammation and microbial action, etc. (Jong & Birmingham 1993).

In this study, we aimed to determine the antimicrobial activity of some *Agaricus* species. Therefore, extracts obtained from these basidiomycete members were tested for antimicrobial activity against representative Gram-positive, Gram-negative bacteria, as well as yeasts.

Material and methods

Macrofungal materials

Seven species of *Agaricus* [*A. macrosporus* (F.H. Møller & Jul. Schäff.) Pilát, *A. essetei* Bon., *A. xanthodermus* Genev., *A. praeclaresquamosus* A.E. Freemann, *A. impudicus* (Rea) Pilát, *A. arvensis* Schaeff. : Fr. and *A. langei* (F.H. Møller & Jul. Schäff.) Maire] were collected from different localities in Turkey. Voucher specimens of the macrofungi were deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey, and identified by Yusuf Uzun.

Microorganisms

In this study, the following microorganisms were used: *Bacillus subtilis*, *B. cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhimurium*, *Serratia marcescens*,

Debaryomyces hansenii, *Kluyveromyces fragilis*, and *Rhodotorula rubra*. Test microorganisms were obtained from culture collection of Canakkale Onsekiz Mart University, Faculty of Science, Basic and Industrial Microbiology Department.

Preparation and antimicrobial activity of extracts

The material was ground to a fine powder. Fifteen g of those materials were subjected to Soxhlet extraction for 12 h each using 150 mL of the ethanol solvent. The extracts were kept at 4 °C (Khan & al. 1988; Dülger & al. 2004).

In vitro antimicrobial studies were carried out by the Agar–Disc Diffusion method against test microorganisms (Board & Lovelock 1975; Collins & Lyne 1987; NCCLS 1993; Favel & al. 1994). Mueller Hinton Agar (OXOID) was used as the most suitable medium for antimicrobial activity studies. The sterilised medium at 45–50 °C was poured into petri dishes. Agar depth was 4 mm. For 90 mm diameter plates 25 mL of medium were used. According to this method, ethanol extracts were impregnated as discs in ranging concentrations from 50 µL. Then all discs were dried at 50 °C and placed into bacteria and yeasts petri dishes. Each disc was 6 mm in diameter. For each experiment one disc, which contained only solvent, was used as control disc. As reference, antibiotics GM 10, SCF 105, AK 30, TS 25, NY 100, CLT 10 and KETO 20 were used. Experiments were repeated three times and the results were expressed as average values.

Bacterial and yeast cultures were suspended in 4–5 mL Brain Heart Infusion Broth (OXOID). Bacteria were incubated at 37 °C for 2–5 h. Yeast cultures were incubated at 30 °C for 5–7 h. When a visible turbidity was obtained at the end of this time, the turbidity of bacterial suspension was adjusted against MacFarland Standard Tube [0.5] with physiologic serum and inoculation was performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. The entire Mueller Hinton Agar surface was streaked in 3 different directions by rotating the plate 60° angles after each streaking. Yeast cultures were inoculated into Mueller Hinton Agar (10^2 cfu/mL). All petri dishes after inoculation were allowed to dry for 15–20 min at room temperature. For bacteria (35 °C) and yeasts (30 °C), inhibition zone diameters were measured after 24–48 h using Agar–Disc Diffusion method (Collins & Lyne 1987; NCCLS 1993).

Results and discussion

Table 1 shows antimicrobial activities of the macrofungi extracts, and the inhibition zones formed by standard antibiotic disks are indicated in Table 2.

From the results obtained, *Agaricus macrosporus*, *A. impudicus* and *A. arvensis* have been found to be ineffective against *Bacillus subtilis* ATCC 6633. In addition, *A. impudicus* was ineffective against *Bacillus cereus* ATCC 7064. The extracts of macrofungi have been determined to be less effective than all comparison antibiotics against all bacteria cultures. Notably, extracts of macrofungi, which have antagonistic effect against *Proteus vulgaris* ATCC 6899, have been found to be more active than the standard comparison antibiotic SCF 105.

The extracts of macrofungi have shown antiyeast activity. The extracts of *A. impudicus* and *A. langei* have been found to be more active against *Rhodotorula rubra* DSM 70403 than the standard comparison antibiotic KETO 20. Separately, the extracts of *A. praeclaresquamosus* have been found to be more active against *Debaryomyces hansenii* DSM 70238 than the standard comparison antibiotic NY 100.

Inhibition zone diameters around control disc were measured between 0–1 mm.

According to the literature, ethanol extract was the most effective extract in disc diffusion method on macrofungi (Broadbent 1966; Benedict & Brady 1972). So, we used ethanol solvent in our study.

The macrofungi differ significantly in their activity against tested microorganisms. These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multi-layered structure and the yeast cell wall is quite complex (Yao & Moellering 1995). In addition, microorganisms' variable sensitivity to chemical substances relates to different resistance levels between the strains (Çetin & Gürler 1989).

As a result of our study, *Agaricus* species have antimicrobial activity against some Gram (+) and Gram (-) bacteria and yeasts. All the extracts showed more antifungal activities than antibacterial activities.

Table 1. Summary of antimicrobial activity of studied macrofungi.

Macrofungus species	Zones of inhibiton (mm)*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A. macrosporus	6.0	10.0	8.6	10.0	12.3	11.6	13.5	8.5	10.3	10.0	10.0	12.5	10.3	10.0
A. impudicus	6.0	6.0	9.0	9.6	10.3	13.0	11.6	7.6	9.5	9.3	11.0	14.0	10.3	15.3
A. essetei	12.0	12.0	8.6	10.0	10.0	10.0	12.0	11.3	10.0	12.0	12.0	10.0	8.6	10.0
A. arvensis	6.0	14.6	11.0	12.0	10.0	10.0	10.3	11.0	10.0	11.3	13.0	12.0	11.5	12.5
A. langei	12.3	12.0	11.0	13.6	12.0	16.3	11.6	11.0	13.0	12.0	13.0	14.3	12.3	16.0
A. praeclaresquamosus	11.3	10.0	12.0	11.0	12.0	12.0	11.0	11.0	12.0	10.0	11.0	16.5	12.0	11.0
A. xanthodermus	11.0	10.0	12.0	11.6	10.3	13.0	11.3	12.3	11.0	10.6	11.0	13.0	12.0	11.3
Ethanol (control)	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Legend: 1, ***Bacillus subtilis*** **ATCC 6633; 2,** ***B. cereus*** **ATCC 7064; 3,** ***Enterobacter aerogenes*** **ATCC 13048; 4,** ***Escherichia coli*** **ATCC 10536; 5,** ***Klebsiella pneumoniae*** **CCM 2316; 6,** ***Micrococcus luteus*** **ATCC 9341; 7,** ***Proteus vulgaris*** **ATCC 6899; 8,** ***Staphylococcus aureus*** **ATCC 6538P; 9,** ***S. epidermidis*** **NRRL B–4877; 10,** ***Salmonella typhimurium*** **CCM 5445; 11,** ***Serratia marcescens*** **NRRL 3284; 12,** ***Debaryomyces hansenii*** **DSM 70238; 13,** ***Kluyveromyces fragilis*** **ATCC 8608; 14,** ***Rhodotorula rubra*** **DSM 70403; (*) – data are the average of n = 3 experiments; (–) – no inhibition zones.**

Table 2. Antimicrobial activities of some standard antibiotics.

Tested microorganisms	Zones of inhibiton (mm)*						
	GM 10	SCF 105	AK 30	TS 25	NY 100	CLT 10	KETO 20
Bacillus subtilis ATCC 6633	23.0	31.0	16.4	31.0	NT	NT	NT
Bacillus cereus ATCC 7064	22.0	20.0	16.2	7.0	NT	NT	NT
Enterobacter aerogenes ATCC 13048	15.0	20.0	18.6	27.0	NT	NT	NT
Escherichia coli ATCC 10536	20.0	25.0	17.2	6.0	NT	NT	NT
Klebsiella pneumoniae CCM 2316	17.0	21.0	20.0	27.0	NT	NT	NT
Micrococcus luteus ATCC 9341	20.0	30.0	24.4	35.0	NT	NT	NT
Proteus vulgaris ATCC 6899	15.0	11.0	20.0	23.0	NT	NT	NT
Salmonella typhimurium CCM 5445	16.0	23.0	24.2	27.0	NT	NT	NT
Staphylococcus aureus ATCC 6538P	25.0	28.0	23.2	30.0	NT	NT	NT
Staphylococcus epidermidis NRRL B–4877	15.0	21.0	19.2	6.0	NT	NT	NT
Serratia marcescens NRRL 3284	20.0	27.0	20.0	31.0	NT	NT	NT
Debaryomyces hansenii DSM 70238	NT	NT	NT	NT	16	18	14
Kluyveromyces fragilis ATCC 8608	NT	NT	NT	NT	18	18	16
Rhodotorula rubra DSM 70403	NT	NT	NT	NT	18	16	22

Legend: GM 10 – Gentamycin (10 mcg); SCF 105 – Sulbactam (30 μg) + Cefaperazona (75 μg); AK 30 – Amikasin; TS 25 – Trimethoprim (1.25 mcg) + Sulfamethoxazole (23.75 mcg); NY 100 – Nystatin (100 μg); CLT 10 – Clotrimazole (10 μg); KETO 20 – Ketacanazole (20 μg); (*) – data are the average of n = 3 experiments; (NT) – not tested.

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Antimicrobial activity of some macrofungi from Turkey

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Abstract. In this study, the ethanolic extracts obtained from *Macrolepiota procera*, *Coprinus comatus*, *Volvariella speciosa*, *Polyporus squamosus* and *Funalia trogii* have been investigated for their antimicrobial activity against *Bacillus subtilis*, *B. cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhimurium*, *Serratia marcescens*, *Debaryomyces hansenii*, *Kluyveromyces fragilis* and *Rhodotorula rubra* by disc diffusion method. As a result of the study, we have found that all macrofungi revealed strong antimicrobial activity against all the test microorganisms, especially the yeast cultures.

Key words: antimicrobial activity, *Coprinus comatus*, *Funalia trogii*, *Macrolepiota procera*, *Polyporus squamosus*, *Volvariella speciosa*

Introduction

Many antibiotics in clinical use were developed from fungal and actinomycete metabolites. During the last decades several pathogenic microorganisms developed resistance to the available antibiotics. Infections by multidrug resistant isolates of *Candida* spp., *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* spp., *Enterococcus* spp. and *Escherichia coli*, among others, became more and more frequent stimulating the search for new antibiotics with novel mechanisms of action (Kotra & Mobashery 1998; Morschhäuser & al. 2000; Sandven 2000; Thomson & Moland 2000).

The first investigations on the potential of basidiomycetes as sources of antibiotics were performed by Anchel, Hervey and Wilkins in 1941 (Sandven 2000), when they examined extracts of fruiting bodies and mycelia culture from over 2000 species. They succeeded in the isolation and identification of pleuromutilin (Kavanagh & al. 1950), a diterpene that is especially useful for the treatment of mycoplasm infections in animals (Brizuela & al. 1998) and served for the development of the first commercial antibiotic of basidiomycete origin.

The polyacetylenes are the most extensively characterised group of antagonistic mushroom constituents. More than 50 of these unsaturated antibiotic substances are known from one or more species of *Aleurodiscus*, *Clitocybe*, *Coprinus*, *Cortinellus*, *Marasmius*, *Merulies*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella* and *Tricholoma*. Other known antagonist compounds from basidiomycetes include phenolic metabolites (Benedict & Brady 1972; Cochran 1978).

In this study, we aimed to determine the antimicrobial activity of some macrofungi species, which are members of basidiomycetes' subdivision. Extracts were tested for antimicrobial activity against representative Gram-positive, Gram-negative bacteria, as well as yeasts.

Material and methods

Macrofungal material

Macrolepiota procera (Scop.) Singer, *Coprinus comatus* (O.F. Müll.) Pers., *Volvariella speciosa* (Fr.) Singer, *Polyporus squamosus* (Huds.) Fr., and *Funalia trogii* (Berk.) Bondartsev & Singer were collected from different localities in Turkey. Voucher specimens of the macrofungi were deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey, and identified by Yusuf Uzun.

Microorganisms

In this study, the following microorganisms were used: *Bacillus subtilis*, *B. cereus*, *Enterobacter aerogenes*, *Es-*

cherichia coli, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus*, *S. epidermidis*, *Serratia marcescens*, *Debaryomyces hansenii*, *Kluyveromyces fragilis*, and *Rhodotorula rubra*. Test microorganisms were obtained from culture collection of Canakkale Onsekiz Mart University, Faculty of Science, Basic and Industrial Microbiology Department.

Preparation and antimicrobial activity of extracts

The material was ground to a fine powder. Fifteen g of those materials were subjected to Soxhlet extraction for 12 h each using 150 mL of the ethanol solvent. The extracts were kept at 4 °C (Khan & al. 1988; Dulger & al. 2005).

In vitro antimicrobial studies were carried out by the Agar–Disc Diffusion method against test microorganisms (Board & Lovelock 1975; Collins & Lyne 1987; NCCLS 1993; Favel & al. 1994). Mueller Hinton Agar (OXOID) was used as the most suitable medium for antimicrobial activity studies. The sterilised medium at 45–50 °C was poured into petri dishes. Agar depth was 4 mm. For 90 mm diameter plates 25 mL of medium were used. According to this method, ethanol extracts were impregnated as discs in ranging concentrations from 50 µL. Then all discs were dried at 50 °C and placed into bacteria and yeasts petri dishes. Each disc was 6 mm in diameter. For each experiment one disc, which contained only solvent, was used as control disc. As reference, antibiotics GM 10, SCF 105, AK 30, TS 25, NY 100, CLT 10 and KETO 20 were used. Experiments were repeated three times and the results were expressed as average values.

Bacterial and yeast cultures were suspended in 4–5 mL Brain Heart Infusion Broth (OXOID). Bacteria were incubated at 37 °C for 2–5 h. Yeast cultures were incubated at 30 °C for 5–7 h. When a visible turbidity was obtained at the end of this time, the turbidity of bacterial suspension was adjusted against MacFarland Standard Tube [0.5] with physiologic serum and inoculation was performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. The entire Mueller Hinton Agar surface was streaked in 3 different directions by rotating the plate 60 ° angles after each streaking. Yeast cultures were inoculated into Mueller Hinton Agar (10^2 cfu/mL). All petri dishes after inoculation were allowed to dry for 15–20 min at room temperature. For bacteria (35 °C) and yeasts (30 °C), inhibition zone diameters were measured after 24–48 h using Agar–Disc Diffusion method (Collins & Lyne 1987; NCCLS 1993).

Results and discussion

Table 1 shows antimicrobial activities of the macrofungi extracts, and the inhibition zones formed by standard antibiotic disks are indicated in Table 2.

From the results obtained, *Polyporus squamosus* have no an important effect against all bacteria cultures. The extracts of macrofungi have been determined to be less effective than all comparison antibiotics against all bacteria cultures. Notably, extracts of macrofungi *Coprinus comatus*, *Volvariella speciosa* and *Macrolepiota procera*, which have antagonistic effect against *Proteus vulgaris* ATCC 6899, have been found to be more active than that of the standard comparison antibiotic SCF 105. However, the antibacterial activity of the other macrofungi extracts was equal to the standard comparison antibiotic SCF 105 against *Proteus vulgaris* ATCC 6899. All macrofungi extracts were ineffective against *Serratia marcescens* NRRL 3284.

The extracts of macrofungi have shown antiyeast activity. They have been determined to be effective in different ranging against the yeast cultures. Separately, the antiyeast activity of the extract of *Coprinus comatus* was equal to the comparison antibiotic KETO 20 against *Debaryomyces hansenii* DSM 70238.

Inhibition zone diameters around control disc were measured between 0–1 mm.

According to the literature, ethanol extract was the most effective extract in disc diffusion method on macrofungi (Broadbent 1966; Benedict & Brady 1972). So, we used ethanol solvent in our study.

The macrofungi differ significantly in their activity against tested microorganisms. These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multi-layered structure and the yeast cell wall is quite complex (Yao & Moellering 1995). In addition, microorganisms' variable sensitivity to chemical substances relates to different resistance levels between the strains (Çetin & Gürler 1989).

As a result, the macrofungi used in this study have antimicrobial activity against some Gram (+) and Gram (-) bacteria and yeasts.

Table 1. Summary of antimicrobial activity of studied macrofungi.

Macrofungus species	Zones of inhibiton (mm)*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Volvariella speciosa	12.0	11.0	10.0	10.0	9.6	12.0	12.0	13.0	10.0	12.0	10.0	12.6	11.5	11.3
Coprinus comatus	11.0	9.6	9.3	11.0	10.0	12.6	14.0	12.0	14.0	10.0	11.0	14.0	9.3	10.0
Polyporus squamosus	10.3	10.3	9.0	10.0	10.0	9.0	11.3	9.0	9.0	10.3	9.6	11.0	12.0	10.3
Macrolepiota procera	10.0	10.6	11.0	10.3	11.6	13.0	12.3	11.0	11.3	12.3	10.0	12.0	9.6	13.0
Funalia trogii	12.0	10.3	11.0	11.6	12.6	13.3	10.6	11.0	12.0	10.6	11.0	13.0	12.3	14.0
Ethanol (control)	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Legend: 1, ***Bacillus subtilis*** **ATCC 6633; 2,** ***B. cereus*** **ATCC 7064; 3,** ***Enterobacter aerogenes*** **ATCC 13048; 4,** ***Escherichia coli*** **ATCC 10536; 5,** ***Klebsiella pneumoniae*** **CCM 2316; 6,** ***Micrococcus luteus*** **ATCC 9341; 7,** ***Proteus vulgaris*** **ATCC 6899; 8,** ***Staphylococcus aureus*** **ATCC 6538P; 9,** ***S. epidermidis*** **NRRL B–4877; 10,** ***Salmonella typhimurium*** **CCM 5445; 11,** ***Serratia marcescens*** **NRRL 3284; 12,** ***Debaryomyces hansenii*** **DSM 70238; 13,** ***Kluyveromyces fragilis*** **ATCC 8608; 14,** ***Rhodotorula rubra*** **DSM 70403; (*) – data are the average of n = 3 experiments; (–) – no inhibition zones.**

Table 2. Antimicrobial activities of some standard antibiotics.

Tested microorganisms	Zones of inhibiton (mm)*						
	GM 10	SCF 105	AK 30	TS 25	NY 100	CLT 10	KETO 20
Bacillus subtilis ATCC 6633	23.0	31.0	16.4	31.0	NT	NT	NT
Bacillus cereus ATCC 7064	22.0	20.0	16.2	7.0	NT	NT	NT
Enterobacter aerogenes ATCC 13048	15.0	20.0	18.6	27.0	NT	NT	NT
Escherichia coli ATCC 10536	20.0	25.0	17.2	6.0	NT	NT	NT
Klebsiella pneumoniae CCM 2316	17.0	21.0	20.0	27.0	NT	NT	NT
Micrococcus luteus ATCC 9341	20.0	30.0	24.4	35.0	NT	NT	NT
Proteus vulgaris ATCC 6899	15.0	11.0	20.0	23.0	NT	NT	NT
Salmonella typhimurium CCM 5445	16.0	23.0	24.2	27.0	NT	NT	NT
Staphylococcus aureus ATCC 6538P	25.0	28.0	23.2	30.0	NT	NT	NT
Staphylococcus epidermidis NRRL B–4877	15.0	21.0	19.2	6.0	NT	NT	NT
Serratia marcescens NRRL 3284	20.0	27.0	20.0	31.0	NT	NT	NT
Debaryomyces hansenii DSM 70238	NT	NT	NT	NT	16	18	14
Kluyveromyces fragilis ATCC 8608	NT	NT	NT	NT	18	18	16
Rhodotorula rubra DSM 70403	NT	NT	NT	NT	18	16	22

Legend: GM 10 – Gentamycin (10 mcg); SCF 105 – Sulbactam (30 µg) + Cefaperazona (75 µg); AK 30 – Amikasin; TS 25 – Trimethoprim (1.25 mcg) + Sulfamethoxazole (23.75 mcg); NY 100 – Nystatin (100 µg); CLT 10 – Clotrimazole (10 µg); KETO 20 – Ketacanazole (20 µg); (*) – data are the average of n = 3 experiments; (NT) – not tested.

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Effects of air pollution on allergic properties of pollens (*Triticum aestivum*)

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Abstract. To study the effect of air pollution on allergic properties of wheat pollens (*Triticum aestivum*), aquatic extracts of pollens were collected from clean and polluted environment, and were used for performing an experiment on guinea-pigs aged 3 months. This was done by eye and nose tests and intradermal injection. After 15 to 20 minutes, animal reaction towards extract of 2 testable groups was appeared by sneeze, cough and itch of eyes. In that point of intradermal injection, after mentioned time as well as signs of itch, red Flare was appeared. In all cases, reaction to polluted extract is stronger than to clean or unpolluted extract. Blood factors such as blood sugar, Eosinophils and IgE have increased in the allergic mode.

Key words: air pollution, allergic properties, wheat pollens

Introduction

Plants which belong to *Compositae* have analogous and like kinds and types that spread world wide and they have been from upper Kertase period as yet. All plants of this division are grassy and rarely wooden. These plants have special shape and form that is called wheaten mode (gramini form). Graminis are grasses aged one or several years which cause to congested, pressed growings. Rhizome of wheats that is agent of stability and durability of plants in few years has accompaniment branches and growth (Sympodic) and various aerial stalks are caused by it yearly.

Pollen grains of wheat divisions always blow in flower before ovule. So pollination in these plants is like cross. Pollination is done by wind or indirectly.

Ancient information about allergy that is available relates to Galen and Hippocrates. They have detected that some people sneeze in the vicinity of plants and flowers but they were not aware about its quality. Today we know that some of allergic patiences such as asthma, hay fever, migraine are intensified by some groups of plants. Scientists determine a different nature for pollen allergies. Heslop-Harrison (1968) has assigned the situation of allergen proteins, in exine of pollen grains especially near to growing holes. Knox (1984) announced the collection of allergen ingredients related to pollen in the cover of pollen. According to Hoavik's opinion (1985), part of pollen allergies has glycoprotein structure that for example causes the allergic reactions to pollen seeds of juniper.

Material and methods

Method for preparing the pollens to study with scanning electron microscope

For studies of scanning electron microscope, after full drying of samples, they were dried by critical point dryer (Balz Rsunion, CPD) by dioxide carbon, then after attaching on the aluminum base, were covered by covering unit of golden and were studied and photographed using scanning electron microscope (SEM).

Method of preparing the pollens for direct observation

One of current methods for morphological studies of pollen grains is steulize method that was used in 1960 by Swedish scientist Erdtman for the first time. Natural mature pollens and pollens collected from regions with polluted air were prepared by steulize method and were compared under microscope.

Preparing the pollen extract

To study allergic effects of pollen grains and considering the allergens, pollen extract is needed. So, differ-

ent amounts of extract seed were weighted and were positioned in salt phosphate buffer. This pollen mixture was shaken at 4 °C for 24 h, and after centrifugation (4600 gr in 50 min), produced fluid was kept at -20 ° C until its use.

Testable animals

Since some scientists have focused on sensitivity of guinea-pigs towards allergen agents and also on external allergic similarity of animal with human, in this study guinea-pigs aged 3 months were used.

Experiments performed for studying the allergic figures

1. Intradermal experiment: to perform that trial, we have injected extract of pollens by insulin syringe in abdomen or thigh in which there are many joint tissues. In the point of injection, local edema (wheal) like an embossment and with obvious borders was produced. In experiments having positive result, after 10 to 20 minutes red halo (Flare) was appeared in that point. Assay of wheal and Flare was done according to (mm) as a criterion in evaluating the allergic reaction in extract.

2. Experiment of nose and eyes: in this method, few drops of extract were poured on the nose and eye mucus of guinea-pig by a glass pipette. The effect of pollen allergic reaction was studied by observing the nose mucus, severity and number of sneeze, stimulation and exudation of nose and also appearing the reactions such as itch, inflammation, more blood and histamine. Control test was done by pouring distilled water in nose and other eye, every 2 hours.

3. Assay of blood factors: 8 hours after injection, approximately 5 cc of blood was got from the heart of guinea-pig and by using clinical methods; blood factors such as number of white blood cells, blood sugar and IgE were studied and compared with existing guinea-pig.

Results

Study of allergic reaction by extracts

Injecting different amounts of pollen extract related to testable plant to guinea-pig causes allergic symptoms such as sneeze, trembling of body, teardrop and reddening the eyes. Deadly concentration of extract was 10 % concentration of extract that causes to allergic mode, for pollen extracts of clean air and for pollen extracts of polluted air was 7.2 % and 4.2 %, respectively. Injection of phosphate buffer (0/5 cc) as control to guinea-pigs of control group, have not lead to positive skin reaction.

Results earned from skin test

Intradermal injection of pollens causes to itch firstly and after half hour leads to inflammation and reddening in the point of injection. Then it maximises after 1 hour. Amount of inflammation in each 2 cases of pollen extract, related to polluted and unpolluted air, was intensified as compared with clean samples. Also, red and inflammation rate in polluted samples was increased as compared with unpolluted samples, and inflammation has continued few days later (Fig. 1).

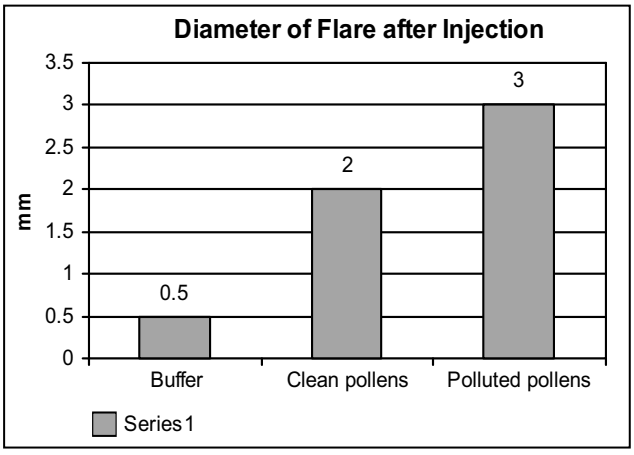

Fig. 1. Diameter of Flare after intradermal injection.

Results from assay of blood factors

IgE rate in blood is one of best indices related to allergic reactions. Study of IgE rate showed that its rate was increased because of the injection of pollen extract as compared with injection of phosphate buffer without extract (Fig. 2). Also, we have observed high improvement in P lower than 0/05 ($P<0.05$) by comparing IgE in pollen extract of polluted air with pollen extract of clean air.

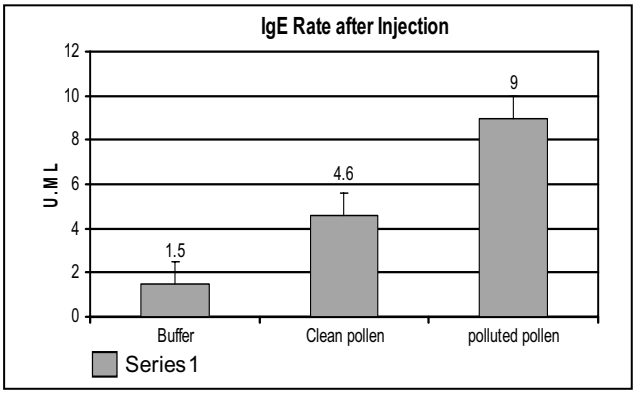

Fig. 2. Immunoglobulin E rate after intradermal injection.

Blood sugar rate in guinea-pigs was 120 mg% naturally, 193 mg% in allergic mode in comparison with pollen extract of clean air and 240mg in pollen extract of polluted air, in which we can observe high increase in P lower than 0/05 (P<0.05) (Fig. 3). Study of Neutrophils' and Eosinophils' rate shows that their rate has been increased by injecting the pollen extract as compared with its rate in blood serum of animal before injection and also as compared with injection of buffer. Neutrophils' and Eosinophils' rates in pollen extracts of polluted air have developed as compared with extracts of clean air (Fig. 4).

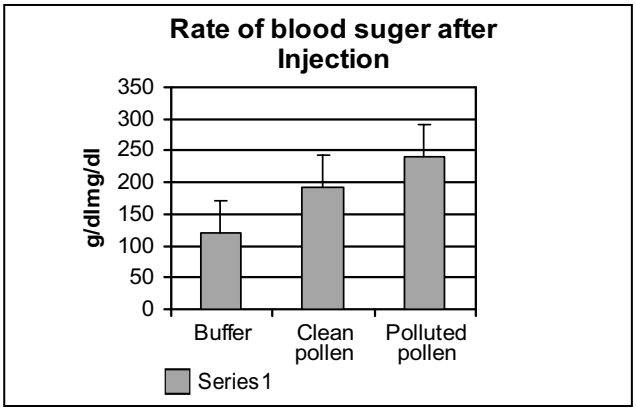

Fig. 3. Blood sugar rate after intradermal injection.

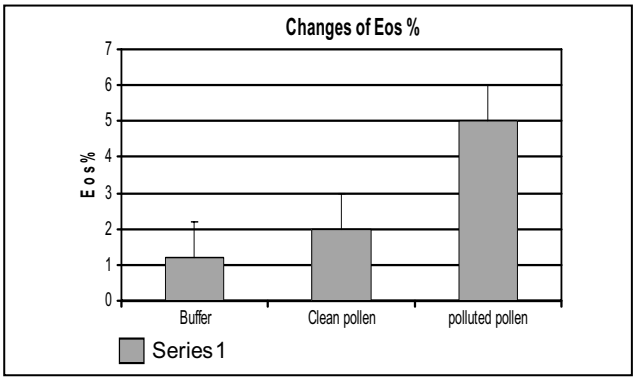

Fig. 4. Eosinophils' rate after intradermal injection.

Discussion

Allergic patiences are damages that humans have been confronted with them and always paid so much value to detect, appease and cure them. Today, human civilisation factor, creating different sources of pollution in environment and also applying the special management has opened new door to allergen; these factors have few scientific confidence and lead to waste human resources.

Allergic reaction in one kind of pollen relates to frequency and power of its allergen (DiGioacchino & al. 2001). With total results earned by skin test of breathing and eyes, power of allergic reaction in pollens of plants such as pine (Taylor & al. 2002), silvery cypress (Tajadod & Majd 2000), red rose and meadow (DiGioacchino & al. 2001), pennyroyal and thyme (Grote & al. 2001) was confirmed.

Pollen grains of grasses are one of important allergen sources world wide (Márquez & al. 1997). Abandoning the ingredients related to pollen grain in grasses causes to increase the allergens in environment and allergic reactions, after long periods of moisture especially in spring and summer (Grote & al. 2001).

Using the mentioned tests and confirmed experiences on guinea-pigs by scientists, in this study also allergen of wheat pollen is confirmed.

Severe Eosinophil of blood (Suphioglu 2000) was introduced as a proof about creating the allergic reactions in body. According to this matter, increasing the Eosinophils of guinea-pigs after injection of pollen extracts is one of the reasons for its allergic reactions.

Increasing of blood factors such as blood sugar and IgE are proofs for allergic reactions caused by plant pollens.

In current decade, this matter has drawn many scientists' attention that pollens act as a receiver and vector from different kinds of environmental pollution. With having the narrow and many holes in their exine, they also attract the mineral elements and other environmental pollutions and influence on sensitive animals as a store centre of many kinds of allergic ingredients (Cerceau-Larrival 2005).

In recent years, some researches were done about allergic properties of pollen grains in Iran and the effects of environmental pollution on power of allergic reactions by pollens. Some of these searches include: studies of DiGioacchinno & al. (2001) about pollens of meadow in Iran, plane and slap in Tehran; about mercury (Tajadod & Majd 2000); about thyme and spearmint (Suphioglu 2000). Recent study showed that in all experiments, effects of allergens of polluted pollens have been more specified and more severe in comparing with pollens collected from uncontaminated regions.

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SCIENTIFIC AREA G

BOTANICAL COLLECTIONS AND EDUCATION IN BOTANY

The role of the collection "*ex situ*" in the protection of threatened medicinal plants

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Abstract. A total of 33 wild medicinal species, distributed in Bulgaria with different status of protection have been collected and conserved under *ex situ* field collection. More than 80 % of the species sprouted successfully, flowered and fructified. Suitable methods for sustainable propagation were made. The seeds and/or vegetative parts of *Sideritis scardica*, *Rhodiola rosea*, *Acorus calamus*, *Primula veris*, etc., produced in the conditions of the collection, were used to distribute them for cultivation in the country and allowed a possibility for sustainable utilization of their resources in the practices.

Key words: collection "*ex situ*", threatened medicinal plants

Introduction

Many medicinal plant species are becoming scarce in the world. Wild populations of medicinal plants can be depleted by over-collecting and unsustainable management practices. The survival of medicinal plant populations and species can also be affected by depletion and conversion of natural habitat, pollution and climate change, competition with invasive species and other factors that influence ecosystems, species interactions and population dynamics.

The collection of wild medicinal plants in Bulgaria has long-established traditions. Bulgarian medicinal plants are considered to be among the highest quality plants in the world, owing to the specific ecological conditions. More than 200 medicinal drugs from more than 150 medicinal species are exported every year. Annually, about 15 000 tones of herbs are collected from nature and exported to over 40 countries (Evstatieva & Hardalova 2004). The large biological resources and the quality of the medicinal drugs derived from them have made Bulgaria a leader in the export list of Europe (Lange 1998).

As a result of the over-exploitation and destructive harvesting techniques there is a big threat for the biodiversity and the wild medicinal plant populations. Overexploitation of related species such as *Rhodiola rosea, Sideritis scardica*, etc., has led to genetic erosion of these plants in some native habitats. Consequently, 61 of the medicinal species are protected by the Law on Biological Diversity (2002) and 36 are under special regime of protection and use.

Protection and preservation of medicinal plants is of great importance, not only for the Bulgarian flora but also for the World one. One of the best ways for combining these contrary two activities, without being in conflict, is by cultivation of the species.

Ex situ conservation plays an important role in medicinal plant protection by preserving seeds and germplasm of threatened species.

Literature data about successful introduction and cultivation of some threatened medicinal plants as *Sideritis scardica*, *Acorus calamus*, *Ruta graveolens*, *Alchemilla* spp., *Atropa belladonna*, *Valeriana officinalis*, *Inula helenium*, etc., as well as for the selection of some of them were founded (Evstatieva 1982, 1996, 2002, in press; Evstatieva & al. 1990, 1992, 1996; Evstatieva & Stoyanova 1994; Protitch & Evstatieva 1994; Dimitrova & al. 1996; Evstatieva & Popova 1998; Evstatieva & Koleva 2000).

Set up of "*ex situ*" collection of medicinal plants started in 1982 in the Botanical Garden of the Institute of Botany with the financial support of Bilkokoop (Velchev & Evstatieva 1983), which continues till now.

More recently the Institute of Botany has initiated a project "Conservation of threatened medicinal plants by creation and maintenance of collection *ex situ*". This project was founded by the Ministry of Environment and Waters for a three year period (2003–2005).

The aim of our studies was to recommend approaches and actions that will support the conservation and sustainable use of medicinal plants by *ex situ* conservation. To obtain this aim it was necessary to cover the following requirements:

- To create experimental field collections for threatened and rare medicinal plants for *ex situ* preservation.
- To obtain information for their growth and development, reproductive biology, phenology, ecology and life cycle.
- To appreciate acclimatization and introduction possibility of these plants for specific agro-cultural conditions in Bulgaria.
- To supply plant material (seeds, seedlings, etc.) for propagation, species re-introduction, ecosystem restoration, agronomic improvement, research and education.

Material and methods

The cultivation has been done in three places: an experimental field close to village Lozen, Sofia region, a greenhouse near to the Institute of Botany in Sofia (670 m alt.), and the experimental field "Beglika", located in the Rhodopes Mts (41°50'57" N, 24°07'08" E), 1525 m alt.

Ecological, biological and chemical characteristics of about 50 wild medicinal plant species were taken into cultivation. Seeds, parts of plants or whole plants from well developed and productive genotypes of their populations, distributed in Bulgaria with different degree of protection have been collected and experimentally cultivated. For each taxon 3–4 individuals have been taken, taking care not to affect the population in the collecting areas.

Results

The experimental successful cultivation in "*ex situ*" field collection concerns 33 wild medicinal species, distributed in Bulgaria (Table 1). With respect to their conservation status, 42 % of them are protected by the Law on Biological Diversity and 58 % – under special regime for conservation and use.

More than 80 % of the species sprouted successfully, flowered and fructified. The adaptation of some species was characterized as poor and growth and development very slow, specifically as *Gentiana lutea*, *Haberlea rhodopensis*, *Cyclamen coum*, *Ruscus aculeatus*, *Taxus baccata*. Some other species (21 %) showed high, and others (64 %) – very high degree of adaptation to the new cultural conditions (Table 2).

The percentage of germination of the medicinal species as *Sideritis scardica, S. syriaca, Primula veris, Rubia tinctorum*, is higher than the germination of the species growing in the nature.

For the first time suitable methods for sustainable propagation of *Sideritis scardica*, *Rhodiola rosea*, *Acorus calamus, Menyanthes trifoliata* and *Primula veris* have been made. The seeds, seedlings and/or vegetative parts of the plants, provided by the collection, were used to cultivate them in the country and gave a possibility for sustainable utilization of their resources in the practices.

The experimental cultivation of these species has been done in order to obtain information on their ecology, reproductive biology, phenology and life cycle. They were used as selected criterion for acclimatization and introduction of these plants for specific agro-cultural conditions in Bulgaria.

Conclusion

Stable collection of threatened medicinal plants with sustainable possibility for propagation was created. It plays an important role in medicinal plant conservation of 33 valuable threatened by preserving seeds and germplasm and by supplying plant material for propagation, species re-introduction, ecosystem restoration, agronomic improvement, research and education.

The collection recommends approaches and actions that will support the conservation and sustainable use of medicinal plants while ensuring social equity.

A serious success is the solution of the problem with seed germination and vegetative propagation by tips of rhizomes of *Rhodiola rosea, Menyanthes trifoliata*, *Acorus calamus*.

Maintaining collection *ex situ* conditions provide the year round access to the gene resources. It stimulates the development of effective propagation methods of the given species for biological agriculture.

The improved propagation materials from these investigated medicinal species, adapted to cultivation, are delivered by the Institute of Botany, as well as a technology for their successful cultivation in the country. The reintroduction into the wild nature is possible.

The cultivation of valuable threatened medicinal plants will provide an important source of income for the local people, which in turn may produce incentives for the conservation of these species and their natural habitats.

Acknowledgements. This study was performed with the financial support of the Ministry of Environment and Waters, the National Science Fund (Projects No. 4639/04; 1532/05) and Bilkokoop.

Table 1. List of medicinal plants with protected status in the *ex situ* collection.

No.	Species	Protected by the Biodiversity Protection Law (2002)	Under special regime of protection and use	Included in the Red List of Bulgaria (2005)		
				CR	EN	VU
1	*Acorus calamus* L.	+		+		
2	*Adonis vernalis* L.		+			
3	*Alchemilla vulgaris* complex		+	+	+	+
4	*Althaea officinalis* L.		+			
5	*Artemisia santonicum* L.		+			+
6	*Atropa belladonna* L.		+			+
7	*Aquilegia nigricans* Baumg.	+				+
8	*Betonica officinalis* L.		+			+
9	*Convallaria majalis* L.		+			
10	*Cyclamen coum* Mill.	+				
11	*Frangula alnus* Mill.		+			
12	*Galanthus elwesii* Hook.	+			+	
13	*Galanthus nivalis* L.	+			+	
14	*Gentiana lutea* L.	+			+	
15	*Glaucium flavum* Crantz		+			
16	*Glycyrrhiza glabra* L.	+			+	
17	*Gypsophylla paniculata* L.		+		+	
18	*Haberlea rhodopensis* Friv.	+				
19	*Helichrysum arenarium* (L.) Moench		+			
20	*Hyssopus officinalis* L. ssp. *aristatus* (Godr.) Briq.		+			
21	*Inula helenium* L.		+			
22	*Leucojum aestivum* L.		+			+
23	*Menyanthes trifoliata* L.	+			+	
24	*Nymphaea alba* L.	+			+	
25	*Primula veris* L.		+			
26	*Rhodiola rosea* L.	+		+		
27	*Rubia tinctorum* L.		+			
28	*Ruta graveolens* L.	+			+	
29	*Ruscus aculeatus* L.		+			
30	*Sideritis scardica* Griseb.		+		+	
31	*Sideritis syriaca* L.	+		+		
32	*Taxus baccata* L.	+			+	
33	*Valeriana officinalis* L.		+			

Abbreviations: CR – Critically Endangered; EN – Endangered; VU – Vulnerable.

Table 2. List of threatened medicinal plants in the *ex situ* collection successfully introduced into cultivation.

No.	Species	Adaptation			Utilization in the practice
		Poor	High	Very high	
1	*Acorus calamus* L.			+	+
2	*Adonis vernalis* L.		+		
3	*Alchemilla vulgaris* complex			+	+
4	*Althaea officinalis* L.			+	+
5	*Artemisia santonicum* L.			+	+
6	*Atropa belladonna* L.			+	
7	*Aquilegia nigricans* Baumg.			+	
8	*Betonica officinalis* L.			+	
9	*Convallaria majalis* L.			+	
10	*Cyclamen coum* Mill.	+			
11	*Frangula alnus* Mill.			+	
12	*Galanthus elwesii* Hook.			+	
13	*Galanthus nivalis* L.			+	
14	*Gentiana lutea* L.	+			
15	*Glaucium flavum* Crantz			+	+
16	*Glycyrrhiza glabra* L.		+		
17	*Gypsophylla paniculata* L.		+		
18	*Haberlea rhodopensis* Friv.	+			
19	*Helichrysum arenarium* (L.) Moench		+		
20	*Hyssopus officinalis* L. ssp. *aristatus* (Godr.) Briq.			+	
21	*Inula helenium* L.			+	+
22	*Leucojum aestivum* L.			+	+
23	*Menyanthes trifoliata* L.		+		
24	*Nymphaea alba* L.		+		
25	*Primula veris* L.		+		
26	*Rhodiola rosea* L.			+	+
27	*Rubia tinctorum* L.			+	+
28	*Ruta graveolens* L.			+	+
29	*Ruscus aculeatus* L.	+			
30	*Sideritis scardica* Griseb.			+	+
31	*Sideritis syriaca* L.			+	+
32	*Taxus baccata* L.	+			
33	*Valeriana officinalis* L.			+	+

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Threatened plant genetic resources with Balkan area of distribution in the Czech Republic; Potential for their *in situ*/on farm conservation

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Abstract. Some threatened wild plant species with a potential use in agriculture are monitored in the wild and in cultivation. Projects of their "*in situ*" or "on farm" conservation are elaborated. Valuable landraces of fruit and field/horticultural crops from collections in gene banks are returned to cultivation using the "on farm" method. Many of the species are distributed in the Balkan Peninsula, with the margin of their coherent area of distribution in the south eastern part of the Czech Republic. Among them there are fragments of landraces or minor crops (e.g. *Cicer arietinum*, *Glycyrrhiza glabra*) and also neglected fruit trees (e.g. *Sorbus domestica*, *Mespilus germanica*). Selected wild species are monitored – *Chamaecytisus albus*, *Ch. virescens*, *Dorycnium germanicum* etc. *Verbascum speciosum* and *Tordylium maximum* are the species for which "*in situ*" conservation projects are running.

Key words: *in situ* conservation, on farm conservation, threatened species, wild species

Introduction

The National Programme on plant genetic resources conservation and utilization is focused on agricultural crops. However, crop wild relatives and other potentially utilisable plants get more and more attention in terms of their conservation and use for broadening of the species-pool.

Many of the species we are interested in are distributed in the Balkan area, with the margin of their coherent area of distribution in the south eastern part of the Czech Republic (Pannonian region). Some threatened wild plant species with a potential use in agriculture, or as ornamentals, are monitored "*in situ*". The proposals for suitable management and projects of their "*in situ*" or "on farm" conservation are elaborated. Among studied species *Astragalus excapus*, *Chamaecytisus albus*, *Ch. virescens*, *Dorycnium germanicum* are included.

The project of repatriation of critically endangered species in the Podyjí National Park (SE of the Czech Republic) was started in 2002 in collaboration with the National Park administration, mainly for *Verbascum speciosum* and *Tordylium maximum*. Seeds of the target species were collected and are stored in the Gene Bank. Seedlings obtained from cultivated plants are repatriated to their original localities.

Valuable landraces of fruits and field crops are returned to cultivation using the "on farm" method. Among them there are fragments of landraces or minor crops (e.g. *Cicer arietinum*, *Glycyrrhiza glabra*) and also neglected fruit trees (e.g. *Sorbus domestica*, *Mespilus germanica*). New orchards and field plantations have been recently established.

The nomenclature of plant names in the paper was unified according to Kubát & al. (2002).

Material and methods

Based on a literature search (Dostál 1989; Slavík & al. 1995; Čeřovský & al. 1999) and on previous research projects (Ševčíková & Holubec 2000; Holubec

& al. 2003; Vymyslický & al. 2003) rare and threatened species for the Czech Republic, with an area of distribution in the Balkans, were selected (*Astragalus excapus*, *Dorycnium germanicum*, *Glycyrrhiza glabra*, *Chamaecytisus albus*, *Tordylium maximum*, *Trifolium fragiferum* and *Verbascum speciosum*). They are mostly from the family *Fabaceae* and represent crop wild relatives or potentially useful species for agriculture.

Monitoring of these species was performed in the south eastern part of the Czech Republic (Southern Moravia); some of them were also monitored in Central Bohemia or in other areas). South Moravian localities are located in the Pannonian biogeographical province with a continental climate. Most of the localities are included in protected landscape areas or small area nature reserves (Vymyslický & al. 2007).

Monitoring was performed once a year from May to September, both on permanent plots and on the whole population. The following characteristics were recorded: number of plants per 4 m^2 plot, number of plants per locality, ontogenetic phases and healthy conditions of the species, factors causing threatened status of the species. If possible, seeds were collected for future storage in the Gene Bank and possible use in future repatriation. All the localities, that were found and revised, were recorded in the database.

Phytosociological relevés

One typical locality for each species was selected. Phytosociological relevés were recorded at 4 m^2 plot for each species. Braun-Blanquet scale was used for relevés recording. Numbers of species per plot and changes of the situation were acquired. Shannon diversity index for each relevé and year was calculated using the mathematical formula defined in MS Excel. The index is increased either by having additional unique species, or by having greater species evenness.

Results

In this chapter, some studied species and the results that have been found during their monitoring are presented. Comparison of Shannon indices and number of species is presented in Table 1.

Astragalus excapus

In the Czech Republic, it occurs at several steppe localities in the lowland regions. These localities are threatened by abandonment of the traditional management (sheep grazing and hay making). The two biggest localities in the Czech Republic (Pouzdřany and Radobýl steppes) have been monitored every year since 2001. The influence of the newly introduced sheep grazing on the population size and dynamics is studied. Both populations expand every year. Seeds of this species were collected and first attempts at its cultivation and potential use in horticulture were made.

Lathyrus sativus

It is an old marginal crop found in the warmer parts of the Czech Republic with its centre of cultivation in the south eastern part. At present it is very rarely cultivated. Seeds of cultivated plants were collected in 1990; afterwards they were sown and described in detail. The species has a big potential in agriculture and nowadays is cultivated in skansens (open air museums). The multiplied seeds are distributed to interested parties.

Tordylium maximum

The species has only three localities at present in the SE part of the Czech Republic. All the localities are critically endangered due to lack of gaps in the canopy. Since 2002, seeds of the species have been collected, seedlings were cultivated and then they were transplanted to the original sites. The localities are monitored every year and populations increase in size, fortunately.

Verbascum speciosum

At present it occurs at two localities in the SE part of the Czech Republic, the same as *Tordylium maximum*. The populations are threatened by the abandonment of traditional management (grazing) and by hybridization with *V. lychnitis*. Since 2002, the small plot experiments on the site with sod removal and sowing of seeds have been evaluated. Both populations are increasing in size and the repatriation process seems to be successful. The species has very great ornamental value, because of 2–3 m tall long-flowering stems with numerous dense branches.

Glycyrrhiza glabra

In the 19th century it was cultivated on large areas on terraces in vineyards of the Pannonian region. It was used in confectionery. At present only frag-

ments of former populations survive. Within the "on farm" cultivation project, the rhizomes of the species had been transplanted into field plantations and the quality of rhizomes for confectionery has been evaluated.

Chamaecytisus albus

It occurs on steppes in the SE part of the Czech Republic (Pannonian region). Only a few localities are known at present. They are greatly endangered by the invasion of *Robinia pseudacacia* and by wild rabbit grazing. The attempts to cultivate it have been successful since 2001. Seeds of the species have good germination; the plants are very ornamental during the flowering period.

Mespilus germanica

The species originated from the Balkan Peninsula. It is cultivated in warmer areas of the Czech Republic, mainly in the SE part. At the beginning of the 1990's, only old fruit trees survived in abandoned orchards. Nowadays the species is included in the "on farm" conservation project in the Podyjí NP.

Comparison of Shannon indices and number of species

Botanical diversity is often evaluated by Shannon or Evenness indices. These indices were calculated for all plots of all the monitored species (Table 1). There was no correlation between the Shannon index value and the number of species in a relevé, because the other important criterion is the value of dominance. Rich vegetation types, which have lower dominance levels, tend to have the highest Shannon/Evenness indices.

Table 1. Comparison of Shannon indices and number of species. Target species are in bold letters. Highest values are marked by bold.

Species	Shannon Index (average)	Number of species (average)
Astragalus austriacus	1.72	21
Astragalus excapus	2.07	24
Astragalus onobrychis	1.47	28
Chamaecytisus albus	1.88	21
Chamaecytisus virescens	1.84	21
Corothamnus procumbens	**2.31**	29
Dorycnium germanicum	**2.21**	**35**
Genista pilosa	1.44	25
Genista sagittalis	1.91	**30**
Glycyrrhiza glabra	1.85	23
Medicago minima	1.48	18
Medicago prostrata	2.03	20
Oxytropis pilosa	2.12	28
Trifolium fragiferum	2.00	19
Trifolium retusum	2.06	24
Trifolium striatum	1.40	19
Trigonella monspeliaca	**2.20**	22
Average	1.91	24

Conclusion

Most of the monitored species are well protected within protected areas, but it is necessary to maintain suitable management and gather more relevant information concerning these rare and endangered species. Only occasional disturbances can be observed (wild animals, people's footpaths, etc.). Decline in species number was observed during dry seasons (2004, 2007). On the contrary, increase of species number was recorded during wet seasons (2006, 2008). Species number as the measure of good state of the vegetation/locality can be used only by some species. For evaluation of the state of vegetation on each locality it is better to use the Shannon diversity index combined with species richness data instead of only species richness data. Species growing on extreme localities tend to have bigger fluctuations of their populations, but are better protected against human impact. Fortunately, by no one of the monitored species locality evincible negative trends of their population have been observed.

Acknowledgements. This work was conducted in the frame of the research project of the National Agency for Agricultural Research, financed by the Ministry of agriculture of the Czech Republic, No. 1G46066: "Conservation of plant biodiversity in the system of sustainable agriculture and landscaping". The authors thank Mr David Sharp for English correction.

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Collecting of plant genetic resources in Balkan Peninsula

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Abstract. Several joint collecting missions have been organised to the Balkans since 1988 to present. Seeds were collected, regenerated and plants evaluated on breeding useful characters. In the Bulgarian mission 73 samples were collected with prevailing *Triticeae* (54), and *Fabaceae* (11). In 2003 the mission was undertaken to Istria, Croatia and following samples were collected: *Triticeae* 18, other grasses 80, *Fabaceae* 80, medicinal/meadow species 95. On the Macedonia/Albania border 20 *Triticeae* species were collected in 1996. A systematic collecting activity was performed in Slovenia. The regions of Alps, Pannonia and pre-Alpine transition to the Balkans were visited and 960 accessions were collected on 93 locations.

Key words: collecting, evaluation, landraces, *Fabaceae*, *Triticeae*, wild germplasm

Introduction

Balkan Peninsula is very rich in wild flora, including crop wild relatives and unique cultivated germplasm still traditionally used as food and technological crops. The preservation of biodiversity and natural variation within species has become a global concern. Natural variation is essential to the evolutionary process and to long-term species survival. Genetic diversity ensures that no two members of a species or population are genetically identical, and that no individual carries all the possible trait variants in the particular species. The diversity within a species allows it to survive and adapt to new environments, new pests, and changing climates (Rieber 1998).

Genetic resources are essential source of genes for breeding. Breeding based on crossing of advanced cultivars is effective but due to the narrow genetic base of cultivated material genes are soon exhausted. Old landraces and obsolete cultivars have much broader genetic base being usually diverse populations of various types. Most of landraces have disappeared in the process of their replacement by growing bred and more advanced cultivars. Obsolete cultivars were usually long maintained by breeders and some of them were accepted to germplasm collections already a long time ago. In spite of so called germplasm erosion, some landraces and obsolete cultivars were regionally long persisting in cultivation because of their unique properties, local traditions and habits. Such refuges are still possible to find in some remote regions.

Wild plants, especially crop ancestors and botanically related species are other valuable sources of genetic diversity for breeding. They possess much broader genetic base than cultivated materials including traits not known in cultivated plants. However, their use requires more breeding work. In addition, there are many wild plants useful as food, for agriculture, pharmacology, animal feeding, industry, etc. These can diversify the spectrum of economic plants.

Original role of gene banks was to safe bred and cultivated materials at national and international levels. In the recent 15–20 years, there is a raising tendency to gather wild genetic resources to make them available for taxonomic research, breeders and other users (von Bothmer & Seberg 1995). The Czech Gene Bank organised or participated in many collecting activities within the country and abroad (Holubec & al. 2003).

Material and methods

Collecting missions are usually multicrop with a prior attention to certain group. Landraces and obsolete cultivars are of higher attention, but their finding is rather rare. Good chances have only collecting of fruit landraces (Holubec & Vymyslický 2005).

Seed collecting is preferred to vegetative samples. Seeds are collected for bulk samples in particular locality. If possible, seeds are sampled from larger amount of plants and from various microclimatic niches within the locality (von Bothmer & Seberg 1995). Data on locality, including GPS, geography and ecology are recorded to a collecting database. Voucher specimen for herbarium is collected if possible and if it needs further determination (Holubec 2005; Holubec & al. 2005).

Collected seeds are cleaned and dried (to 5–9 % of relative humidity) according to required standards for gene bank storage. Germination tests are made on smaller amount of seeds to save the original materials. The seeds are stored in glass jars with silicagel in active collection for short term and use (-5 °C) and in the base collection for long-term conservation (-18 °C). A part of original sample is used for regeneration and associated characterisation and evaluation. Technologies used in the Seed Bank of Endangered Plant Species follow Ellis & al. (1985).

Samples that are included in the national germplasm collection receive national accession number and passport data are recorded to the information system EVIGEZ. After repeated evaluation the received data are coded according to particular descriptor lists and included into description part of information system.

Results

The Czech Gene Bank organised 14 joint collecting missions within the territory of the Czech Republic (Ševčíková & Holubec 2000) and in bordering regions in cooperation with Slovakia, Slovenia, Poland and Austria. In total 3432 of seed samples were collected. The Gene Bank participated in 12 collecting missions abroad. Out of them 6 were directed to the Balkan Peninsula (Table 1) and were organised on the mutual basis.

Bulgaria, 1988. The first Balkan joint mission was organised by IGR Sadovo. It was aimed at collecting of *Aegilops* spp. The expedition route went along the southern border of Bulgaria from the Black Sea up to Strouma River valley. The collecting sites were within Strandzha, Sakar, Rhodopes, Southern Pirin, Slavyanka, Belasitsa and Rila mountains. Six species out of 9 native to Bulgaria were found and collected. They were partly on dry steppes in submediterranean vegetation and in secondary habitats along roads and weedy in fields. *Aegilops speltoides* together with *Triticum boeoticum* were found in Mt Sakar that belongs to the northern limit of distribution for both species.

Table 1. The number of collected seed samples within Balkan Peninsula.

Plant group/Genus	BGR 1988	BGR/GRC 1993	GRC 1995	MAK/ALB 1996	HRV 1998	SVN 1999	SVN 2000	SVN/HRV 2001	HRV/GRC 2002	GRC 2003	BGR 2004	HRV/SVN 2005
Triticeae	**54**	**7**	**15**	**18**					**18**			
Aegilops	36	4	9	9					10			
Triticum	2											
Hordeum	3	1	1	1					2			
Secale	1			1								
Dasypyrum	4	2	3	6					4			
Taeniatherum	5			1								
Perennial *Triticeae*	3	1	2	1		3			3			
Grasses	**3**			**3**		**98**	**84**	**139**	**79**			**4**
Fabaceae	**11**		**1**		**2**	**101**	**60**	**109**	**85**	**3**	**3**	**1**
Other wild spp.	**5**	**1**			**1**	**91**	**104**	**131**	**108**	**1**	**1**	**6**
Cultivars												**44**
Total	**73**	**9**	**15**	**22**	**3**	**293**	**248**	**379**	**289**	**4**	**4**	**55**

Abbreviations: BGR 1988 – Bulgaria 1988; BGR/GRC 1993 – Bulgaria/Greece 1993; GRC 1995 – Greece 1995; MAK/ALB 1996 – Macedonia/Albania 1996; HRV 1998 – Croatia 1998; SVN 1999 – Slovenia 1999; SVN 2000 – Slovenia 2000; SVN/HRV 2001 – Slovenia/Croatia 2001; HRV/GRC 2002 – Croatia/Greece 2002; GRC 2003 – Greece 2003; BGR 2004 – Bulgaria 2004; HRV/SVN 2005 – Croatia/Slovenia 2005.

While the other *Aegilops* species were in seeds, *Ae. speltoides* was in late flowering. An unusual morphological variation was found in the locality Goleshovo: *Ae. neglecta* with reduced upper florets to rachis only. Diseases are usually rare to find in the wild. Infestation by *Blumeria graminis* was seen on spikes of *Ae. lorentii* in Paril and Melnik (Fig. 1).

Fig. 1. Infestation of *Ae. lorentii* spike by powdery mildew (*Blumeria graminis*) in Melnik area, Bulgaria.

Middle Balkan, 1993–1996. Individual herbarium and seed collecting. Samples of *Aegilops* and other, preferably annual *Triticeae* species were collected within Mediterranean ephemeral vegetation and on secondary habitats. The most interesting was a collection of *Dasypyrum villosum* from Korab Mts possessing morphologically different types from altitudes 400 m to 1640 m.

Slovenia, 1999. The mission was organised by KIS Ljubjana. The region covered NW district from the three border point to the valley of Sava River, between Karavanke and N Julian Alps and partly Primorie. The route had high altitudinal amplitude from the sea level to 2400 m in the Triglav region. The mission was devoted to collecting of fodder plants, grasses and legumes. The highest share of collected samples had clovers (*Trifolium pratense*, *T. medium*, *T. repens*, *T. montanum*, *T. hybridum*) and other *Fabaceae* genera such as *Lotus*, *Medicago*, *Anthyllis*, *Melilotus*, *Chamaecytisus*, *Hippocrepis* and *Dorycnium*. From grasses group the most often collected species was *Phleum pratense*. One sample of wild beet (*Beta maritima*) was found in the former salt pans.

Slovenia, 2000. The mission lead to Southern Julian Alps and to the region of Slovenian Karst: Mala Gora, Kočevski Rog, Bela Krajina and Menišija, the region within the tectonical fold dividing Alpine massif from Balkan. The southeastern part represents a typical karst relief. The vegetation of the area was: thermophilous oak and hornbeam forests, steppes, meadows, pastures and agricultural land. The flora was very rich. Collecting of genetic resources in this region was devoted mainly to leguminous, medicinal, and aromatic plants. The collecting was performed on 21 localities. Altogether 256 seed samples of genetic resources were collected. Among interesting samples there were following: *Danthonia alpina*, *Trisetum distichophyllum*, *Linum hirsutum*, *Coronilla vaginalis*, *Gentiana lutea*, *Genista radiata*, *Dianthus barbatus*.

Slovenia, 2001. The expedition route went to SW Slovenia, Primorie and Brkini, west of Slovenian Karst. The most western parts were salt marshes on the sea coast and old abandoned salt pans. Several species of perennial *Triticeae* were found: *Thinopyrum intermedium*, *Elytrigia littoralis* and *Leymus arenarius*. In addition wild beet, *Beta maritima* was collected. The vegetation of the eastern part of the area was: thermophilous oak forests, submediterranean steppes, pastures and agricultural land. Collecting of genetic resources in this region was devoted mainly to grasses, medicinal, and aromatic plants. The collecting was performed on 27 localities. Altogether 294 seed samples of genetic resources were collected.

Among the more valuable species it is possible to list: *Panicum capillare*, *Satureja montana*, *Ruta graveolens*, *Allium* spp., *Carthamus lanatus*, *Lavandula angustifolia*, *Foeniculum vulgare*.

Croatia, 2002. The whole Istrian Peninsula was visited and a representative cut through the flora was received. Following regions were visited: Učka Mts (Vojak 1396 m). The vegetation represented beech forests, mountain meadows and historically planted *Pinus mugo* around the summit. Croatian Karst was distributed through the entire peninsula and was covered by submediterranean vegetation dominated by therophytes and shrubs, rich in annual *Trifolium* and *Medicago* species. The southern tip of the peninsula (Kamenjak and Premantura) was covered by Mediterranean macchia and therophyte grasslands. Halophytic communities were on the coast as well as in salt marshes: *Lotus creticus*, *Dorycnium hirsutum*, *Securigera securidaca*, and *Triticeae* grasses: *Aegilops geniculata*, *Ae. neglecta*, *Dasypyrum villosum*, *Taeniatherum asperum*, and ephemeral grasses *Trachynia rigida*, *Monerma cylindrica*, *Parapholis incurva*. During the expedition 15

localities were visited and 282 seed samples of genetic resources were collected.

Slovenia, 2005. The expedition aimed at collecting of landraces and obsolete cultivars. It took place in the Pannonian region Prekmurje close to the Hungarian border. It is a lowland area with intensive agriculture, but many landraces and obsolete cultivars have been maintained up today. On the both sides of border in Hungary and Slovenia there is a mixed population with its own cultural traditions and ways of agriculture. The farmers are proud of their tradition and maintain landraces for preparing of traditional meals, for technical purposes (making of brooms, basket and sandal weaving, Fig. 2) and for pumpkin oil pressing (Fig. 3). During the expedition 12 localities were visited and 54 seed samples of genetic resources were obtained from farmers. Only ten items were wild species. The real historical landraces were coloured and long-cob maize, beans, broom sorghum, oil pumpkins, marble cabbage, buckwheat, rustic tobacco and some cereals.

Fig. 2. Farmers use traditional maize landraces for basket and sandal weaving in Slovenia.

Fig. 3. Regional landraces of maize and oil pumpkin in northern Slovenia.

Characterisation and evaluation of collected samples

All collected samples were divided according to crop groups and sent to responsible curators. *Triticeae* tribe species are maintained in the Gene Bank, Prague – Ruzyně, *Fabaceae* and other dicotyledonous meadow and steppe species are maintained in the Research Institute for Fodder Crops, Troubsko (Vymyslický & al. 2003).

All *Triticeae* species were sown for regeneration. About 93 % of samples of annual species germinated and provided seeds. Germination of perennial species was bellow 50 %. Basic morphological description was made according to descriptor lists and data were included into the germplasm information system EVIGEZ.

In following years phytopathological evaluation of the material was elaborated. Resistance to stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*P. recondita*), stripe rust (*P. striiformis*), and powdery mildew (*Blumeria graminis* f. sp. *tritici*) was tested in the field and greenhouse conditions (Fig. 4). In addition, *Epichloë typhina* was observed in perennial species trials. Infestation of cereal aphids *Metopolophium dirrhodum*, *Rhopalosiphum padi* and *Sitobion avenae* was studied in the field.

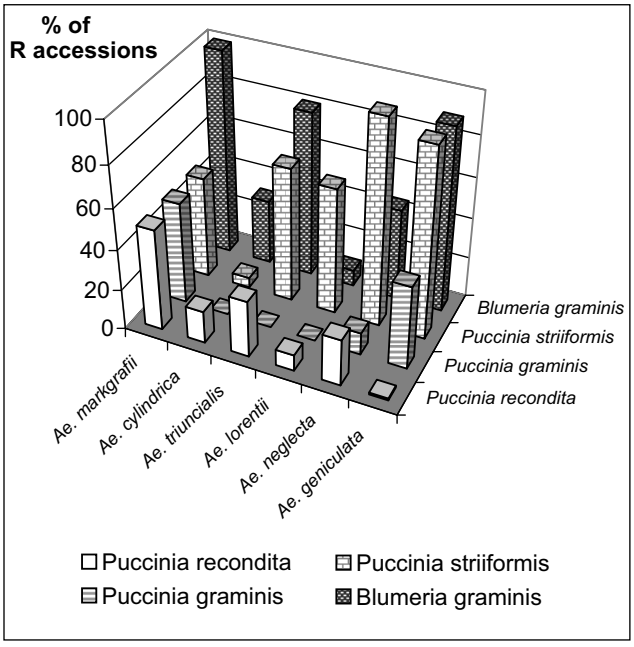

Fig. 4. The percentage of completely resistant *Aegilops* accessions to powdery mildew, stem, leaf and stripe rusts.

Totally, 294 seed samples of *Fabaceae* and other meadow species have been collected by members of the Research Institute for Fodder Crops since 1999. Up to now, 101 seed samples have been sown to be regenerated. Many wild plant species, especially weeds form persistent seed bank (Baskin & Baskin 1998) and the fact complicates regeneration and reintroduction of the species. So, only 34 of them germinated and 14 of them were morphologically described and evaluated according to descriptor's lists. This data are stored at database system of the gene bank called EVIGEZ. 10 seed samples have been stored at gene bank. From the point of view of morphological and resistance evaluation as perspective species for further use in agriculture or horticulture *Vicia grandiflora*, *Trifolium angustifolium*, *Dorycnium hirsutum*, *Carthamus lanatus* and *Psorallea bituminosa* can be considered.

Altogether 1394 seed samples were collected within the Balkan countries.

Acknowledgements. The contribution is based on projects MZe CR QC0063, 1G46066 and MSMT 2005-6/29.

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Status of the electronic documentation system in the Herbarium of Agricultural University – Plovdiv (SOA)

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Abstract. SOA herbarium contains more than 100 000 herbarium sheets. Analysing the existing systems and standards, an electronic herbarium documentation system is created. The used software is described and the capacities of the documentation system are demonstrated. The structure of the database is presented and described according to the data blocks and data management levels. On the basis of the stored information in the data management system, the count of types and total count of records, divided by varied criteria, are presented.

Key words: biodiversity collections, botanical software, chorological data, database, documentation, herbarium

Introduction

The Herbarium of Agricultural University – Plovdiv (SOA, according to *Index Herbariorum*) holds valued specimens. Some of them are collected about one century ago as presented by different authors (Delipavlov & al. 1997).

SOA consists of more than 100 000 specimens of Bulgarian vascular flora, which are collected from various floristic regions. The accessions hold information for the plant diversity in Bulgaria and the Balkan Peninsula. That is why although the physical access to the herbarium materials is limited, very often direct search of information by definite criteria is needed. About 100 000 exsiccates of vascular plants are stored in boxes and shelves according to the index of "*Genera Syphonogamarum ad Systema Englerianum*" by De Dalla Torre & Harms (1907). In the database we used the taxonomic system accepted in *Flora Reipublicae Popularis Bulgaricae*.

Software description

The program module (dSOA) of the database is compiled in the Botanical Department of Agricultural University (Stoyanov 2003). It requires low system resources – Windows 95 on Pentium 100 with 16 MB RAM, 2 MB free disk space and VGA are enough to work with the databases. This program does not require any external software for data management. It allows to create unlimited number of thematic relational databases. The user interface of the program module (Fig. 1) is realised in Bulgarian language. The both data windows offer the both table and form view of the records (Fig. 1–A, C). To reach compatibility with other systems is predicted that dSOA system could export and import tables in CSV (Comma Separated Values) format. They could be processed by any office application. The specimen data can be exported to hypertext or plain text documents too, as well as to any user-defined format or template. For example some data could be exported as a Java-midlet and be read on the field using any mobile device (Fig. 1–F). dSOA could prepare complete report by number of stored records and their relation to users, families, genera, authors and type specimens. The program module is able to display the distribution of selected specimens as UTM map (Fig. 1–E).

Database structure and data description

The structure of the documentation system is shown in Fig. 1 and Fig. 2. The database is multi-file realised.

The user access (Fig. 2, box 1) is realised in 3 levels – administrator ("root"), registered users and unregistered users ("guest"). The users have the permis-

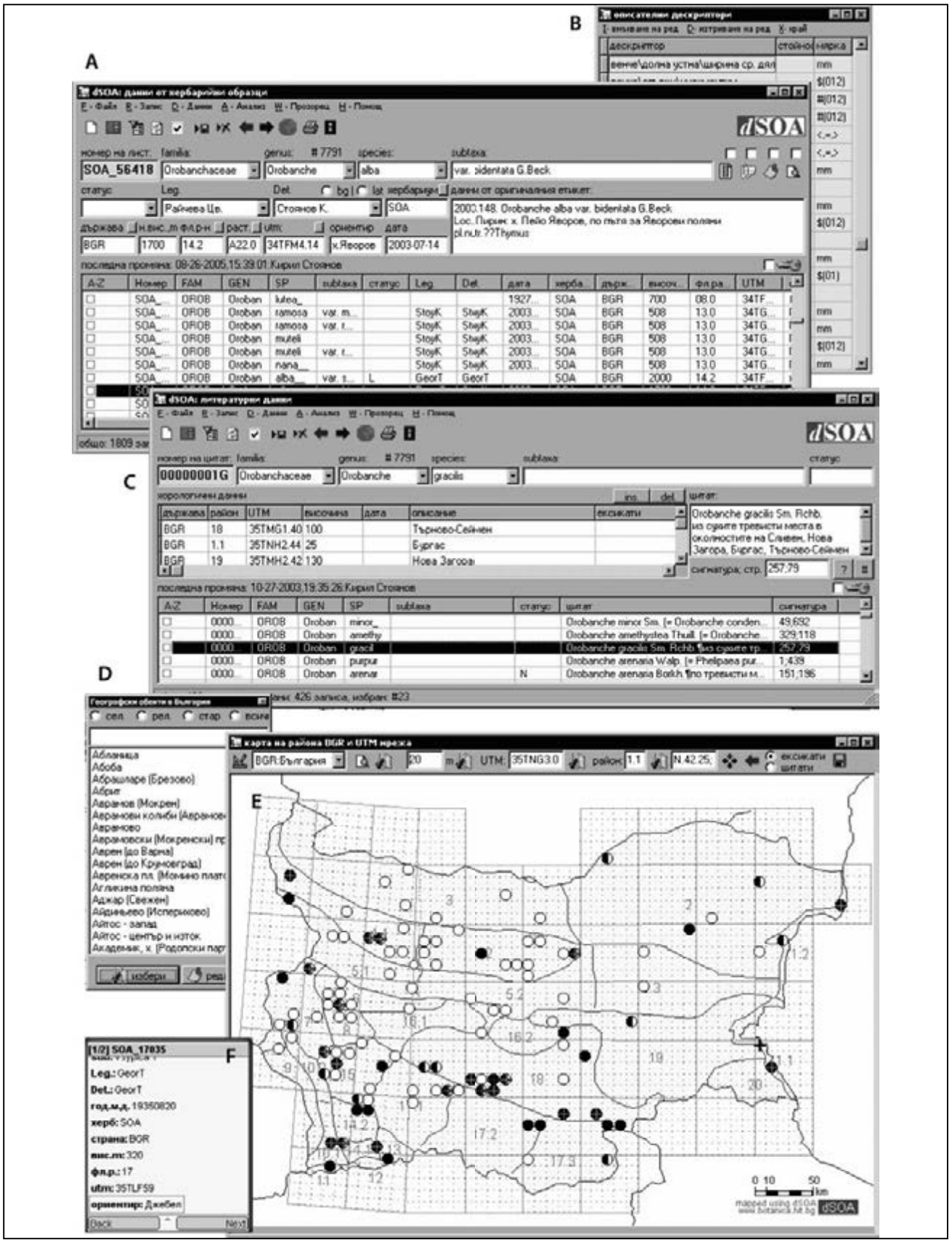

Fig. 1. Screenshots of the herbarium management software: **A** – herbarium data form; **B** – additional description data; **C** – bibliographic data form; **D** – geographic gazetteer; **E** – map drawing form; **F** – data exported in Java midlet.

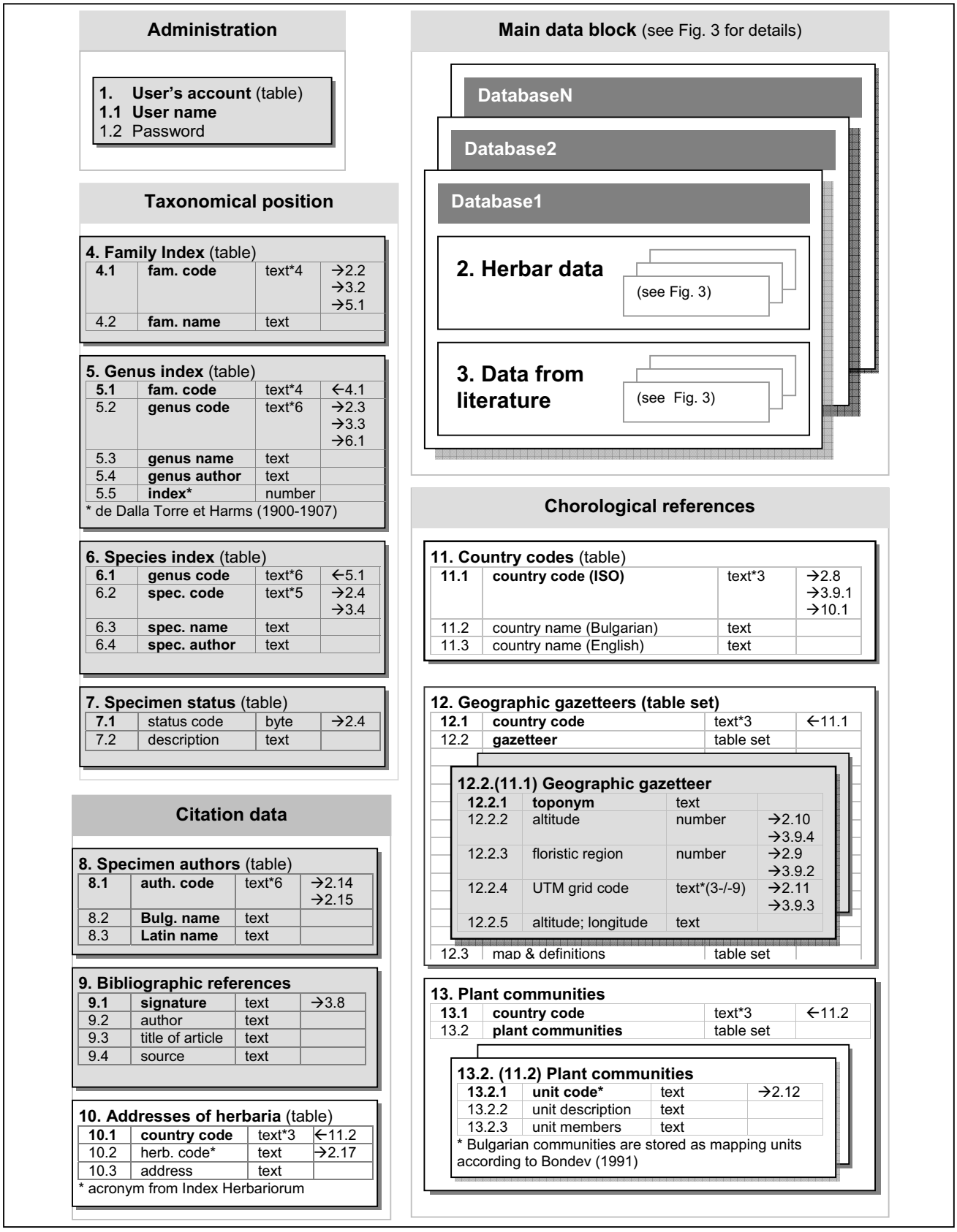

Fig. 2. Functional chart – major structure of the database in SOA herbarium.
Numbers in **bold** show the primary index keys in the tables. Field names in bold show the required fields in any record. Left arrow shows input from related field. Right arrow shows output to related field.

sion to store data and to lock records. The access of the "guests" is free but without permission to write data.

The accepted storage system in the herbarium is used according to the standard of Bisby (1994). The taxonomic description is divided in 3 indexed tables as follow: family (Fig. 2, box 4), genus (Fig. 2, box 5) and species (Fig. 2, box 6). These tables are available in the user interface as menus. The real datum in the fields is the index code from the tables. This division is realised in aim to avoid typing errors which is indispensable if the data are stored manually above and to make the main data file more compact. The field of intraspecific taxa remains manually entered because of the big intraspecific diversity.

Three index tables are created to enter citation data. The first one (Fig. 2, box 8) describes the authors of the herbarium sheets spelled in Bulgarian and Latin alphabet. The index code allows viewing the names of the authors with both alphabets. The second index (Fig. 2, box 9) describes the literature sources and allows searching floristic data from the literature. The acronyms from *Index Herbariorum* (Holmgren & Holmgren 1998) are stored in unlinked file available as a list (Fig. 2, box 10).

All the geographic reference data are stored in separate data files according to the country name. The names of the countries are stored in a table as Bulgarian and English names indexed as 3-letter ISO standard codes (Fig. 2, box 11). This international coding is accepted in many herbaria, botanical gardens and genebanks (van Hintum 1995; Walter 1995). The country index is used as a trigger for the other two stacks of tables in this group – gazetteers and plant communities (Fig. 2, boxes 12, 13). The first one is physically related to the main data set and consists of toponyms, altitudes, numbers of the floristic regions, UTM (MGRS) grid codes and geographic coordinates. The gazetteer sheet is related to map definition sheet. The alphanumeric codes of the UTM grid sets (50 × 50 km) from Atlas Florae Europeae Project (2002) and the grid (10 × 10 km) for Bulgaria (Kozhuharov & al. 1983) are accepted as geographic characterisation. The map of 20 floristic regions which is accepted in *Flora Reipublicae Popularis Bulgaricae*, vols 3–10 (1963–1995) is used for description of Bulgarian floristic data, as well. The altitude is shown in metres above the sea level. The plant communities in Bulgaria are stored as mapping units according to Bondev (1991).

The main data block is realised as a stack of thematic databases. All the databases are built on the basis of two tables – data from herbarium sheets (Fig. 2, box 2) and data from publications (Fig. 2, box 3). The user can switch between different thematic databases on the fly. The system keeps in each record from the main block information about the date, the name of last editor and the level of accessibility of the record.

The plant accessions (about 100 000) and fungal samples (about 5000) are assigned by their herbarium sheet number. This number is used in the scientific publications describing the specimens. We accept the serial number of herbarium sheet as an unique number or primary key index in database. In this way we answer to the both requirements: searching by serial number and limiting the record/number duplication. The data fields as family, genus, species, status (type specimens), geographic data, authors' names and last editor data are linked to the tables described above (Fig. 3, box 2). The date format is "year-month-day" in case to simplify searching. The fields of the intraspecific taxa, data from the original label and landmark have to be stored manually from the keyboard. Because of the diversity of the data, which supports the herbarium sheets, every record can be linked to external files and additional data fields. The additional data fields are defined in a descriptor list. The external files consist of one text file for the revision notes, one file for additional notes about the specimen and one table of other external files, incl. images. This organisation allows to expand the data structure according to the features of the stored taxa. The stored data is searchable by the content of the main fields and by existence of additional files.

The chorological data in the literature are structured in another order. Each floristic record for a taxon contains a list of localities sometimes with citation of herbarium sheets. It means that each record in the database (Fig. 3, box 3) must have included table for the chorology and herbarium sheets (Fig. 3, box 3, 3.9). The other fields are linked as the fields in the herbarium records. The field "signature/page" (Fig. 3, box 3, 3.8) is linked to the bibliographic reference table (Fig. 2, box 9).

This organisation of the database allows comparison between data from literature and data from herbarium sheets. It makes possible to create comparative distribution maps of any taxon (Fig. 1–E).

The table of users' accounts and the direct manual edition of the main data block (for rescue purposes) are accessible only for the administrator. The registered users are allowed to store data in the all other tables. The data levels are as follow:

1. Index data: families, genera, species, authors, countries, herbaria, bibliography.
2. Geographic gazetteer: storage of the toponym data.
3. Main data: records from separate herbarium sheets and records from publications.
4. Data export to external files as CSV tables, text files, maps and other users' formats.

The unregistered users have not permission to change the data except the revision notes.

Main data block

2. Herbarium specimen data (table) – descriptor list for separate herbarium sheet

2.1	**Number** of the herbarium specimen	text	
2.2	Family code	text (4 letters)	←4.1
2.3	Genus code	text (6 letters)	←5.2
2.4	Species code	text (6 letters)	←6.2
2.5	Intraspecific taxon	text (free text)	
2.6	Status	byte	←7.1
2.7	**Original label data**	memo	
2.8	Country code ISO	text (3 letters)	←11.1
2.9	Floristic region number	decimal number	←12.2.3
2.10	Altitude (m)	decimal number	←12.2.2
2.11	UTM grid (from D.)	text	←12.2.4
2.12	Plant community	text	←13.2.1
2.13	Landmark	text	
2.14	Collector's name (Leg.)	text	←8.1
2.15	Author's code (Det.)	text	←8.1
2.16	Date of collecting	YYYY-MM-DD	
2.17	Herbarium code		←10.2
2.18	Last editor (name, date, time) and user's key	text	←1.1
2.19	Additional fields (external descriptor list)	included table	
2.20	External files:	2.20.1. Revision notes 2.20.2. Additional notes	2.20.3. External files images or text

3. Data from literature (table) – descriptor list for separate card

3.1	**Number** of the card	text	
3.2	Family code	text (4 letters)	←4.1
3.3	Genus code	text (6 letters)	←5.2
3.4	Species code	text (6 letters)	←6.2
3.5	Intraspecific taxon	free text	
3.6	Status	byte	
3.7	**Original data**	memo	
3.8	Signature/page	text	←9.1
3.9	**Chorology**	included table	

3.9. Chorology

3.9.1	Country code	text (3 letters)	←11.1
3.9.2	Floristic region	decimal number	←12.2.3
3.9.3	UTM grid	text (6-9 letters)	←12.2.4
3.9.4	Altitude	decimal number	←12.2.2
3.9.5	Date	YYYY-MM-DD	
3.9.6	Description	text	
3.9.7	Herbarium sheets	text	←→2.1

Fig. 3. Functional chart of the main data block (continues from Fig. 2).

Overview of the herbarium data stored till now

The stored data till now contain 7004 records holding information about 75 families, 275 genera and 845 species, collected by 40 authors. The count of stored records divided by families is shown in Table 1. The herbarium contains 210 types (Fig. 4). The stored data cover the 20 floristic regions, mainly from the Rhodopi Mts, Thracian Lowland, Black Sea Coast and Balkan Range (Fig. 5). The territory of Bulgaria is presented by 399 UTM (MGRS) squares 10 × 10 km (Fig. 6). The bigger part of the specimens is collected from the lowland's belt (Fig. 7). The most intensive collecting season is between May and July, followed by August and September (Fig. 8). The stored records show that the bigger part of the stored specimens is collected between the 40s and 70s of the 20th century (Fig. 9).

Table 1. Count of the stored herbarium data divided by families.

Family	Genera	Species	Countries	Floristic regions	UTM squares	Authors	Types	Years	Sheets
Alismataceae	2	3	1	6	9	6	0	1910-1996	18
Alliaceae	1	5	1	5	9	1	14	1969-1990	15
Amaranthaceae	1	17	2	10	35	8	1	1910-1996	400
Anacardiaceae	3	6	3	7	12	8	0	1951-1996	36
Apiaceae	4	4	1	3	3	5	8	1918-1990	8
Aquifoliaceae	1	1	1	3	4	4	0	1957-1994	18
Araceae	1	1	1	1	1	1	1	1978	1
Araucariaceae	1	1	1	0	0	1	0	1965	1
Aristolochiaceae	2	5	3	7	10	5	0	1910-1995	28
Asteraceae	5	15	34	10	6	7	34	1893-1963	34
Brassicaceae	3	4	1	2	3	2	5	1975-1994	7
Butomaceae	1	1	1	2	2	3	0	1923-1980	6
Buxaceae	1	1	2	4	4	4	0	1967-1984	5
Callitrichaceae	1	7	6	8	23	2	0	1905-1994	76
Campanulaceae	1	1	1	1	1	1	1	1914	1
Cannabaceae	2	2	1	5	5	5	0	1905-1995	16
Caprifoliaceae	1	1	1	1	1	1	3	1976-1978	3
Caryophyllaceae	10	82	2	20	155	14	6	1899-2002	792
Celastraceae	2	6	2	7	12	6	0	1949-1991	42
Cephalotaxaceae	1	1	1	1	1	1	1	1972	1
Chenopodiaceae	14	42	5	15	69	9	1	1910-2002	288
Clethraceae	1	3	1					1897-1931	7
Colchicaceae	2	3	1	3	3	3	6	1964-1975	6
Convallariaceae	1	1	1	1	1	1	1	1992	2
Crassulaceae	2	6	1	3	2	2	10	1933-1968	10
Cupressaceae	6	16	3	7	10	6	0	1949-1997	55
Cyperaceae	10	43	5	15	65	7	0	1905-1995	336
Dipsacaceae	2	2	1	2	2	2	4	1920-1923	4
Empetraceae	1	1	2	1		2	0	1860-1961	7
Ephedraceae	1	3	2	2	6	4	0	1952-1986	17
Ericaceae	2	2	1	0	0		0	1927	2
Euphorbiaceae	4	35	2	18	83	11	1	1905-1998	371
Fabaceae	6	7	2	3	3	6	7	1885-1932	9

Table 1. Continuation.

Family	Genera	Species	Countries	Floristic regions	UTM squares	Authors	Types	Years	Sheets
Fagaceae	1	14	4	9	28	9	0	1949-2001	102
Hyacinthaceae	1	1	1	1	1	1	1	1928	1
Hydrocharitaceae	3	3	2	1	3	3	0	1949-1979	13
Hypericaceae	1	2	1	2	2	3	5	1914-1993	5
Iridaceae	2	3	1	4	2	5	3	1959-1986	50
Juncaginaceae	1	1	1	2	3	3	0	1910-1957	9
Lamiaceae	3	3	1	2	2	3	4	1923-1969	4
Liliaceae	4	7	1	7	9	4	13	1929-2004	16
Linaceae	1	1	1	1	1	1	1	1923	1
Loranthaceae	3	3	2	7	12	6	0	1951-1996	45
Malvaceae	1	1	1	1	1	1	1	1994	1
Molluginaceae	2	2	2	3	3	3	0	1973-1997	4
Monotropaceae	1	1	2	4	6	5	0	1840-1983	17
Moraceae	3	5	3	4	5	3	0	1963-1980	21
Najadaceae	1	3	1	1	3	3	0	1951-1993	9
Nyctaginaceae	1	1	1	1	1	1	0	1963	2
Orobanchaceae	2	34	16	17	95	24	6	1834-2004	382
Phytholaccaceae	1	2	2	5	5	5	0	1905-1976	7
Pinaceae	7	25	4	8	14	8	0	1949-1992	66
Poaceae	95	261	6	20	227	19	5	1893-1998	2850
Polygalaceae	1	8	1	9	20	6	0	1949-1996	41
Polygonaceae	7	40	5	15	66	9	0	1910-2002	202
Portulacaceae	3	3	1	9	12	6	0	1905-1984	20
Potamogetonaceae	1	8	1	5	13	4	0	1915-1992	40
Pyrolaceae	1	7	2	9	32	14	0	1842-1905	122
Ranunculaceae	1	1	1	1	1	2	2	1940	2
Rosaceae	3	9	1	6	7	3	10	1900-1964	11
Rubiaceae	1	1	1	1	1	1	1	1898	1
Santalaceae	3	9	3	13	25	6	0	1905-2000	66
Scrophulariaceae	4	8	1	7	13	11	9	1864-1977	40
Sparganiaceae	1	3	1	4	5	2	0	1949-1985	7
Staphylleaceae	1	2	1	4	6	5	0	1949-1975	19
Taxaceae	1	1	1	4	4	1	0	1949-1977	8
Taxodiaceae	3	3	1	6	6	4	0	1949-1976	10
Thymelaeaceae	2	3	1	3	3	2	15	1816-1989	15
Typhaceae	1	8	2	4	11	6	3	1910-1998	35
Ulmaceae	3	9	2	7	12	4	0	1955-2000	27
Urticaceae	2	6	3	6	16	6	0	1905-2000	27
Violaceae	1	4	1	2	2	2	21	1916-1974	21
Vitaceae	1	2	1	2	2	1	10	1978	10
Zannichelliaceae	1	1	1	4	7	3	0	1953-1979	22
Zosteraceae	1	2	1	2	2	2	0	1953-1966	2

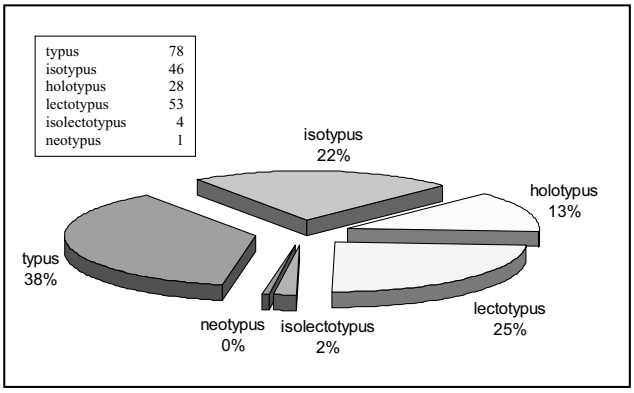

Fig. 4. The type specimens in the Herbarium of Agricultural University – Plovdiv (SOA).

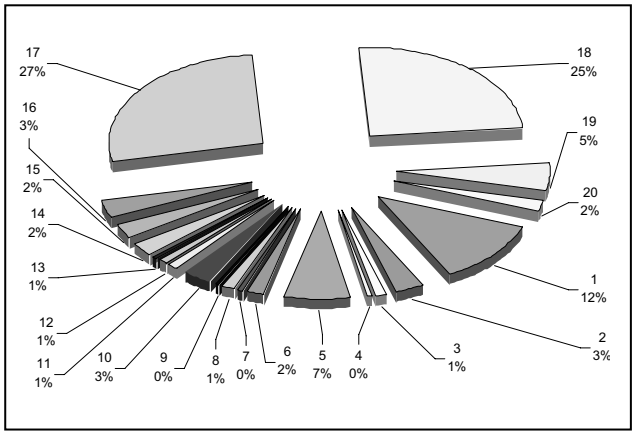

Fig. 5. Horizontal distribution (by floristic regions) of the stored specimens.
1, Black Sea Coast, **2,** Northeast Bulgaria; **3,** Danubian plain; **4,** Forebalkan; **5,** Balkan Range; **6,** Sofia region; **7,** Znepole region; **8,** Mt Vitosha; **9,** West Frontier Mts; **10,** Valley of Strouma River; **11,** Mt Belasitsa; **12,** Mt Slavyanka; **13,** Valley of Mesta River; **14,** Pirin Mts; **15,** Rila Mts; **16,** Mt Sredna gora; **17,** Rhodopi Mts; **18,** Thracian Lowland; **19,** Toundzha Hilly Country; **20,** Mt Strandzha.

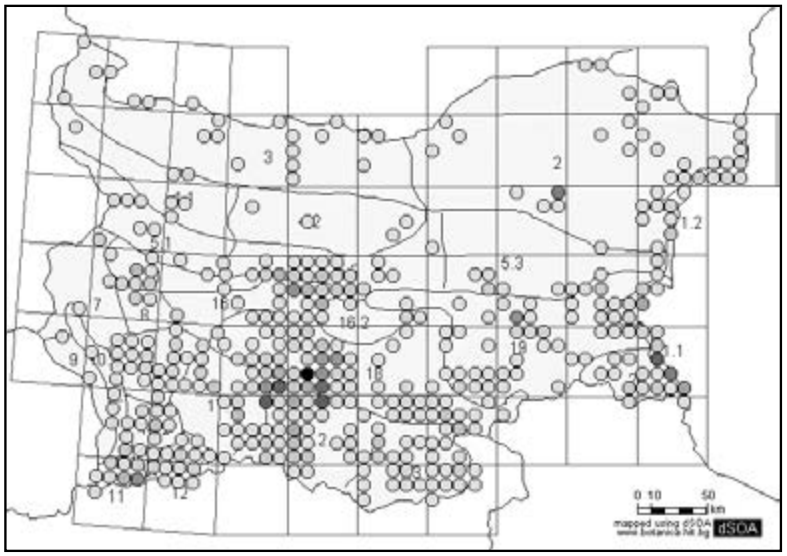

Fig. 6. UTM map of the localities – the localities with more specimens are darker.

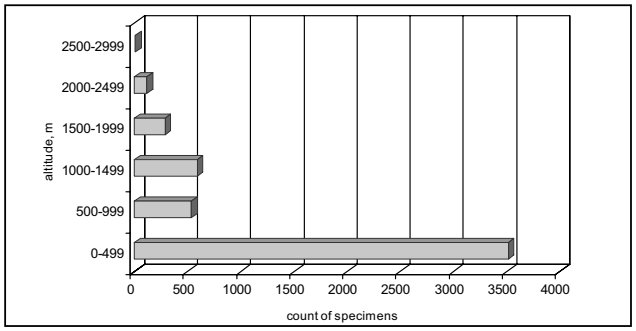

Fig. 7. Vertical distribution of the stored specimens.

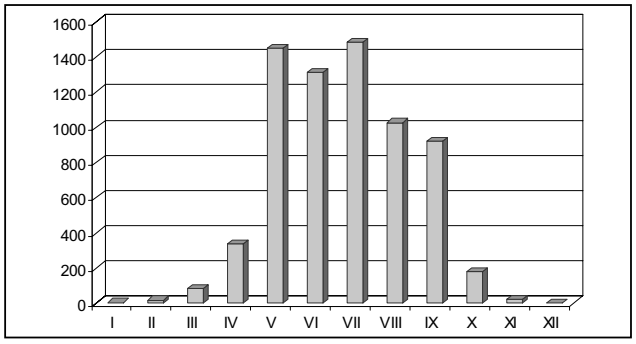

Fig. 8. Count of specimens according to the months.

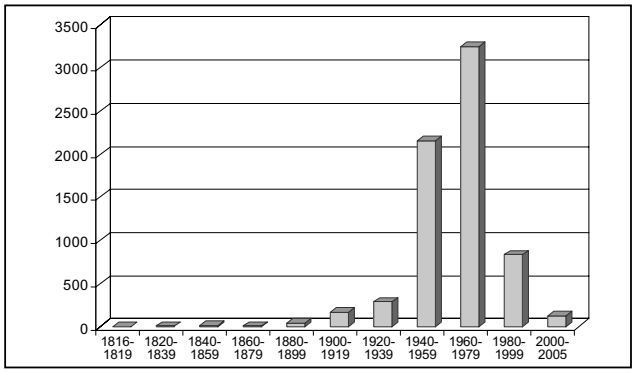

Fig. 9. Count of specimens according to 20-year periods.

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ISBN 978-954-9746-14-3