ISSN 1120-4060

BOCCONEA — 21

Herbarium Mediterraneum Panormitanum

BOCCONEA

Edited on behalf of the International Foundation *pro Herbario Mediterraneo* by Francesco M. Raimondo & Werner Greuter

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Redazione di "Bocconea"
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Printed by Luxograph s.r.l., Piazza Bartolomeo da Messina, 2/E - Palermo
Registration at Tribunale di Palermo, n. 19 of 6 May 1991
ISSN 1120-4060
ISBN 978-88-7915-023-1

BOCCONEA — 21

Proceedings of the XI OPTIMA Meeting
Beograd, 5-11 September 2004

Edited by
Olja Vasić

Published under the auspices of OPTIMA
by the
Herbarium Mediterraneum Panormitanum

Citation of the papers:
Petrova, A. & Vladimirov, V. 2007: Recent (1994-2004) taxonomic studies on the Bulgarian flora. – Pp. 7-25 in: Vasić, O. (ed.), Proceedings of the XI OPTIMA Meeting, Beograd 5-11 Sept. 2004. – Bocconea **21.**

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Communications

Posters

Ana Petrova & Vladimir Vladimirov

Recent (1994-2004) taxonomic studies on the Bulgarian flora

Abstract

Petrova, A. & Vladimirov, V.: Recent (1994-2004) taxonomic studies on the Bulgarian flora. — Bocconea 21: 7-25. 2007. — ISSN 1120-4060.

For the period 1994-2004, despite financial constraints related to the economic and political changes in Bulgaria, some 400 papers dealing with the taxonomy, biosystematics and floristics of the vascular plants of the Bulgarian flora have been published. Taxonomic revisions of different groups, such as *Achillea*, *Cardamine*, *Centaurea*, *Crepis*, *Erysimum*, *Plantago* and *Veronica*, were based on modern biosystematic and allozyme or DNA supported analyses. Many of these studies have been carried out in cooperation with researchers and research teams from other countries, such as Austria, Denmark, Italy, Poland, Slovakia, Sweden, United Kingdom etc. Currently the Bulgarian flora consists of ca. 3900 species, 910 genera and 150 families. In the last decade many species new to science have been described and numerous taxa have been confirmed for the Bulgarian flora or recorded for the first time, e.g. 10 genera (*Acalypha, Ambrosia, Commelina, Cytinus, Duchesnea, Echinocystis, Myricaria, Rochelia, Sicyos, Sisyrinchium*) and two families (*Commelinaceae, Rafflesiaceae*). During the report period the 10th volume of Flora of Republic of Bulgaria has been published, including the taxonomic treatments of 10 families. Many taxa from *Pteridophyta, Asteraceae, Brassicaceae, Campanulaceae, Poaceae* etc. have been studied karyologically.

Introduction

Taxonomic and floristic studies on the Bulgarian flora have already a more than two-century long history. They started with investigations of foreign botanists, among which especially prominent is Czech botanist Josef Velenovský, the author of very first flora of the country, Flora Bulgarica (1891, 1898). Botanical explorations were later continued by enthusiastic Bulgarian botanists as Stefan Georgiev, Ivan Urumov, Bozhimir Davidov, Boris Achtarov, Nikolay Stojanov, Boris Stefanov, Daki Jordanov and many others, who left deep and long-lasting traces in Bulgarian botany.

The main research centres for taxonomic and floristic studies on the Bulgarian flora are: Institute of Botany of the Bulgarian Academy of Sciences, Department of Botany of the Faculty of Biology of Sofia University St. Kliment Ohridski, Department of Botany in the Agrarian University in Plovdiv, Botanical Garden of the Bulgarian Academy of Sciences, National Museum of Natural History of the Bulgarian Academy of Sciences and University of Forestry in Sofia.

For the period 1994-2004 about 400 papers have been published dealing with taxonomic, biosystematic and floristic studies on the Bulgarian flora. They can be divided into: a) studies on selected taxonomic groups for the multi-volume Flora of the Republic of Bulgaria, b) biosystematic studies on different taxonomic groups mainly for dissertations and diploma works, c) inventories of the floristic diversity of protected areas and certain floristic regions in the country, d) informations on the chorology of different taxonomic groups.

Floras, guides, surveys

The 10th volume of the Flora of the Republic of Bulgaria was published (Kožuharov & Kuzmanov 1995). It includes 10 families (*Acanthaceae*, *Scrophulariaceae*, *Globulariaceae*, *Gesneriaceae*, *Orobanchaceae*, *Lentibulariaceae*, *Plantaginaceae*, *Caprifoliaceae*, *Adoxaceae*, *Valerianaceae*), 38 genera and 239 species. Comparing these figures to the fourth edition of Flora of Bulgaria (Stojanov & al. 1966, 1967), there is an addition of 21 species, resulting from the description of new species in *Verbascum*, *Veronica* and *Linaria*, the discovery of species new for the country and the new specific status of 9 taxa.

Two editions of the Conspectus of the Bulgarian vascular flora (Dimitrov 2001, 2002c) have been issued. They provide distribution maps and floristic data for more than 3800 species recorded in the country until 2002.

Very recently, the Key to the Plants of Bulgaria (Delipavlov & Cheshmedzhiev 2003) has been published. It includes keys for the families, genera, species and subspecies of all vascular plants in the country, altogether more than 3800 wild growing as well as 528 of the most widespread introduced and cultivated species. Furthermore, data on the distribution of all species and subspecies in the country is provided.

Biosystematic studies

Several taxonomic groups have been subject of comprehensive biosystematic studies applying a number of modern research methods. The Bulgarian representatives of *Achillea* sect. *Filipendulinae* have been studied (Nedelcheva 1996, 1998). The occurrence of 6 species, 4 varieties and 1 hybrid has been established. It has been found that *Achillea biebersteinii* was erroneously reported for the country. The specific status of the Bulgarian endemic *A. thracica* has been confirmed. Karyological data for the taxa have proven valuable for making taxonomic decisions (Nedelcheva 1995, 1995a). Phytochemical data show heterogeneity and polymorphism among the species with respect to their flavonoid aglycones and confirm their significance as ecological markers for habitats and levels of xerophytisation (Ivancheva & Nedelcheva 1995). Recent biosystematic studies of all Bulgarian taxa of *Achillea* were carried out by Saukel & al. (2003), based on karyological data, phytochemical and DNA analyses. The total of 19 species are reported for the flora of the country, one of them, *A. asplenifolia*, for the first time. The importance of hybridization even between phylogenetically distant species is documented by analysis of a hybrid swarm. A general survey on the basis of *nr*DNA and *cp*DNA sequences demonstrates that *Achillea* is a monophyletic genus.

Centaurea s. l. is one of the most diverse genera of the *Compositae* in Bulgaria. Two sections - *Cyanus* (now genus *Cyanus*) and *Lepteranthus* (*Centaurea* subg. *Jaceae*) have been studied by Sharkova-Bancheva (1999) based on comparative morphological, karyological and pollen-morphological data. It has been established that 16 species (8 in each section) occur in Bulgaria. *Centaurea pichleri* has been reported as new for the country (Bancheva & Denchev 2000), whereas the occurence of *C. phrygia* has not been confirmed. Karyological data shows that two basic chromosome numbers occur in sect. *Cyanus*, but only one (x = 11) in sect. *Lepteranthus*. Most of the species are diploid and only a few tetraploid (Bancheva 1998, 1999; Sharkova 1996; Sharkova & Peev 1997). These studies are now expanded to other sections of the genus. A taxonomic revision has been carried out for 7 species of sect. *Napulifera* (genus *Cyanus*) distributed in Bulgaria and the Balkan peninsula (Bancheva & Raimondo 2003) based on comparative morphology, pollen morphology (SEM data) and karyology. Two basic chromosome numbers have been established for the section: x = 11 for *C. pseudoaxilaris* and x = 10 for the other species. Data of pollen morphology support the specific status of *C. pseudoaxilaris* and *C. tuberosus*. New nomenclatorial combinations are proposed. *C. tuberosus* is reported for the first time for Greece.

A set of analytical techniques, haematoxylin staining, Giemsa-C-banding, Feulgen cytophotometry with scanning densitometry and video-based image analysis as well as propidium iodide (PI) flow cytometry, have been applied to a number of populations of the genus *Crepis* (Dimitrova & al. 1999; Dimitrova & Greilhuber 2000, 2001) together with 'classical' comparative morphological and karyological methods. Dimitrova & Greilhuber (2000) found a positive correlation between C-value and karyotype asymmetry, and concluded that evolutionary advancement in the Bulgarian *Crepis* species is correlated with lower chromosome numbers, lower C-values and higher chromosome symmetry.

Biosystematic studies in *Campanula* by Ančev (1994) have revealed dysploidy and relic or sometimes recent intra- and interspecific hybridization followed by the formation of polyploids as the main evolutionary patterns in the genus. The author based his conclusions on comparative morphological, chorological, karyological and pollen-morphological data and on informations concerning reproductive biology.

Extensive studies have been carried out in *Brassicaceae* by Ančev and collaborators. Four new species have been described: three in *Erysimum* (Ančev & Polatschek 1998) and one in *Alyssum* (Ančev & Uzunov 2002). Furthermore, Anchev (1997) discusses speciation patterns in the family and provides taxonomic notes on *Erysimum* (Anchev 1995) as well as trichome morphology, their diversity and evolutionary trends within *Alyssum* (Ančev 2000).

Data on relationships and evolutionary mechanisms within *Astragalus* subgenus *Cercidothrix* were reported by Pavlova (2003).

Karyological studies

More than 600 species of different families have been studied karyologically in order to obtain chromosome numbers and characterize karyotypes. The results have been published in more than 60 papers (Pavlova & Tosheva 2001, 2002, 2003, 2004; Petrova &

Stanimirova 2001, 2002, 2003; Vladimirov & Szeląg 2001, 2001a; Anchev & Goranova 2002, 2003; Pavlova & al. 2002b; Ivanova 2003; Stoyanova 2003; Tosheva 2003; for the period 1990-2000 see Petrova 1996, 2002). These studies were concentrated mainly on groups of current taxonomic interest such as *Asteraceae* (*Centaurea*, *Cichorioideae*), *Boraginaceae*, *Brassicaceae*, *Campanulaceae*, *Caryophyllaceae*, *Cyperaceae*, *Fabaceae*, *Lamiaceae*, *Liliaceae*, *Poaceae*, etc.

Chemotaxonomic studies

Several groups of chemical compounds, iridoids, flavonoids, terpenoids, have been used as taxonomic markers in order to interpret the evolutionary relationships between different taxonomic groups. *Veronica* and *Plantago* were studied by Taskova & al. (1997, 1997a, 1998, 2002, 2002a, 2002b) and Taskova (1999) with respect to the quantitative and qualitative occurrence of iridoid glucosides in species of the two genera. The results confirm the phylogenetic relationships between the two genera. Treatment of *Veronica* sect. *Pseudolysimachion* as a separate genus is well supported, whereas the generic treatment of *Plantago* subg. *Psyllium* is refuted.

The analysis of the content of iridoids, flavonoids and terpenoids has been carried out in *Lamiaceae* and other families (Taskova & al. 1997b). Chemotaxonomic studies on flavonoids were done in *Euphorbia* (Ivancheva & al. 1997), *Achillea* (Ivancheva & Stancheva 1996, 1996a) and *Geranium* (Ivancheva & Petrova 2000), on steroidal sapogenins in *Asparagus* and *Ruscus* (Nikolov & Gussev 1997) and on alkaloids in *Genista* (Christov & Evstatieva 2001).

Molecular studies

Isozyme polyacrylamide gel electrophoresis has been applied to several representatives of the tribes *Poeae* and *Triticeae* of the *Poaceae* in order to address different taxonomic problems. Within tribe *Poeae* the results support recognition of subtribe *Sclerochloinae* (Angelov 1999, 2000). Studies in *Festuca* reveal that the Balkan endemics *F. hercegovinica*, *F. oviniformis*, *F. hirtovaginata* and *F. thracica* are well differentiated “narrow” species which exhibit high levels of “hidden” infraspecific genetic variation (Angelov 1998, 2002, 2002a, 2003). Electrophoretic data support the taxonomic distinctness of subsect. *Festuca* and subsect. *Saxatiles* (Angelov 1998) as a mesomorphic and a xeromorphic group, respectively, as was already suggested by Kožuharov (1982). Within *Elymus* s. l. isozyme data reveal that *Elytrigia*, *Psammopyrum* and *Trichopyrum* are better lumped within a “broad” genus *Elymus*, whereas *Agropyron* should be considered as a separate genus (Angelov 2001a, 2003b); *Elymus varnense* is shown to be a well differentiated species (Angelov 2001). The taxonomic position of *Dasypyrum* and *Peridictyon* is not yet well resolved within the tribe *Triticeae* (Angelov 2002b, 2003c, 2003d, 2003e). Nevertheless, *Peridictyon* should be treated as a separate genus (Angelov 2000a, 2003a).

Certain relationships within *Veronica* previously suggested on the basis of morphological and karyological characters are now supported by a combination of molecular and chemical data (Taskova & al. 2004).

Embryological and anatomical studies

Embryological studies provide taxonomically useful data, especially in apomictic groups. Extensive relevant investigations were carried out, mainly in the BAS Institute of Botany, on the following *Compositae* genera: *Achillea*, *Artemisia*, *Cicerbita*, *Crepis*, *Lapsana*, *Leontodon*, *Sonchus*, *Tanacetum*, *Xanthium*, etc. (Jurukova-Grancharova 1994, 2000; Jurukova-Grančarova 1997; Yurukova- Grancharova 2004; Robeva & Yurukova-Grancharova 1995, 2001; Robeva & al. 1995; Terziiski & al. 1995, 1996, 1997, 1998; Jurukova-Grancharova & al. 2001; Yurukova-Grancharova & al 2002; Yurukova-Grancharova & Baldjiev 2003), on *Apiaceae* (Yankova & Robeva 2003), *Lamiaceae* (Yurukova-Grancharova & Daskalova 1995, 2002; Daskalova 2002, 2004a, 2004b; Daskalova & Yurukova-Grancharova 1996), *Thalictrum* (Daskalova 1997, 2000, 2004), *Gentiana* (Bicheva & al. 2004), and on *Iberis* (Yurukova-Grancharova & al. 2004). These studies concern the pecularities of micro- and macrosporogenesis, male and female gametophytes as well as embryosac types. Relevant data are very useful for revealing the mode of reproduction, particularly important in rare, endangered and endemic taxa.

The ultrastructural differentiation of the foliar epidermis, cuticles, stomata and indumentum of most of the extant as well as fossil Euro-Mediterranean (incl. Bulgarian) taxa of *Fagaceae* was studied by Uzunova & al. (1997). The authors outline the main evolutionary trends in the family. Similar analyses of the leaf epidermis in Bulgarian taxa of *Origanum* have also provided taxonomically useful data (Uzunova & Stoyanova 2003). An anatomical study of the fruits of two *Angelica* species has been carried out by Yankova (2004).

Terziiski & Cheshmedzhiev (1994) and Češmedžiev & Terzijski (1997) have analysed the ultrastructural organisation of the seed surface in some species of *Allium*, *Nectaroscordum*, etc.

Palynological studies

The characteristics of the pollen and spores have been studied in a number of groups. Most of the investigations have been carried out with electron microscope techniques to make even small differences between the taxa better visible.

An atlas with morphological descriptions and illustrations of the spores of extant pteridophytes native to Bulgaria was prepared by Ivanova & al. (2003). Its main objective is to facilitate taxonomic and paleopalynological studies.

SEM studies on fossil and recent macro- and microspores have shown that the only representative of *Isoetes* in Bulgaria is *I. lacustris* (Stefanova & Ivanova 2000).

For taxonomic purposes palynological studies have been carried out in *Achillea* (Nedelcheva 1998), *Amaryllidaceae* (Borisova & Slavomirova 1994), *Astracantha* (Pavlova & Berge 1995), *Astragalus* (Pavlova & al. 1994, 1995), *Brassicaceae* (Anchev & Deneva 1997), *Centaurea* (Sharkova-Bancheva 1999), *Gentiana* (Kožuharova & al. 1995; Kožuharova & Božilova 2001), *Oxytropis* (Pavlova & Berge 1997) and *Tamus* (Slavomirova 1999).

Population studies

Statistical population studies have been performed in *Carex* (Stoeva & Popova 1995; Stoeva & al. 2002), *Saxifraga* (Peev & Delcheva 1995; Delcheva & Peev 1997, 2002, 2002a; Delcheva 2000), *Dianthus microlepis* (Peev & al. 1995) and *Dactylis glomerata* (Peev & al. 1996). Peev (2002) has summarized his long-lasting studies on the morphometric variability of significant characters in clones and populations of different taxonomic groups in relation to different elevations and habitats.

Floristic and chorological studies

A significant proportion of the studies on the Bulgarian flora have been devoted to floristics and chorology. Comprehensive investigations of the central part of the Danube plain were done by Tzonev (2002) that document 1272 species and subspecies from 113 families of vascular plants. Furthermore, Tzonev (2000, 2002) gives new chorological data for 58 Bulgarian taxa. A new alien species for the whole Balkan peninsula is *Cyperus strigosus* (Tzonev & al. 2003).

Comprehensive studies have been carried out on the floristic diversity of protected areas, the Central Balkan National Park, the Rila National Park (Evstatieva & Hardalova 2000; Gussev & al. 2000; Meshinev & al. 2000; Peev & al. 2000) and several other reserves. Gussev & al. (1997) present a detailed analysis of the flora of the Vitanovo Reserve in Strandža Mt. highlighting the remarkable conservation value of the area. A total of 642 species of vascular plants has been documented, 26 of which are Tertiary relics, 9 are Balkan endemics and 23 have a conservation status.

The flora, vegetation and phytogeographic relationships of the Uzunbudžak Biosphere Reserve, within the Strandža Nature Park have been studied by Gussev & al. (2004). The authors provide data about the considerable diversity of floristic elements (37), preglacial relics (52), Bulgarian and Balkan endemics (24) and taxa of concern for conservation (63).

Numerous other protected areas have been investigated: Dzhendema Reserve within the Central Balkan National Park (Vassileva & al. 2003), Dupkata and Likana in the Eastern Rhodopes (Petrova & al. 2001), Aldomirovsko Blato marsh (Apostolova & al. 2001), Dolna Topčija and Ostrica (Pavlov & Dimitrov 2001, 2002) as well as Vălči Dol in the eastern Rhodopes, Pobiti Kamăni in East Bulgaria, the Marica part of the Central Rila Reserve, the Bjala Krava Reserve in Stara Planina, the Kaliakra Reserve and the Maričini Ezera (see Petrova 2002).

Meshinev & al. (1994) report 208 naturally occuring species for the sand dunes in the region of the summer resort Slunčev Briag (Sunny Beach). Of these species 33 % exhibit secondary expansion, and a few invasive species also have become established. Such studies can be very important for tracing the changes in the distribution of anthropophytes if repeated regularly.

The distribution of vascular plants in Bulgaria relative to floristic regions and habitat diversity is discussed by Meshinev & Apostolova (1998). Velchev (1998) considers the floristic diversity of limestone areas and Pavlova & al. (1997, 2002a, 2003, 2004) on serpentines.

A large part of relevant publications is devoted to floristic surveys of different parts of the country and contains new chorological informations. This is very important for documenting the present distribution of taxa in the country and tracing the changes and dynamics of the flora (Delipavlov & Cheshmedzhiev 1997; Delipavlov 1998; Dimitrov & Pavlova 2000, 2002; Tzonev 2000, 2002; Dimitrov & Tzonev 2001, 2002; Dimitrov & al. 2001, 2003; Kožuharov & al. 2001, 2002; Nyagolov & al. 2002; Pavlova & Nedelcheva 2001; Petrova & al. 2002, 2002a, 2003, 2003a, 2004; Bancheva & al. 2002, 2002a, 2002b, 2004; Dimitrov 2002, 2002a, 2002b, 2002c; Gussev & al., 2002, 2004; Kostadinova & Dimitrov 2002; Milanova & Gussev 2002; Sopotlieva & Petrova, 2002; Uzunov & al. 2002; Zieliński & al. 2002; Dimitrov & Assyov 2003; Dimitrov & Sidjimova 2003; Gerasimova & Petrova 2003; Gerasimova & al. 2003; Petrova 2003, 2004, 2004a, 2004b; Tashev 2003, 2003a; Uzunov & Gussev 2003; Vladimirov 2003; Dimitrov & Stoyanov 2003; Ivanova 2003a, 2004; Assyov & Vassilev 2004; Bancheva & Delcheva 2004; Bergman & al. 2004; Dimitrov & Vutov 2004; Nyagolov 2004; Stoyanov 2004; for the period until 2000 see Petrova 2002).

A number of foreign botanists has contributed to the Bulgarian flora: from the Czech Republic: J. Štěpánková, J. Kirschner, J. Štěpánek, P. Tomšovic, K. Sutory, Z. Kaplan (Štěpánková 1994; Kirschner & Štěpánek 1998; Ančev & Tomšovic 1999; Sutory 2000; Kirschner & Kaplan 2001; Šumberová & al. 2004; Tzonev & Šumberová); from Slovakia; K. Marhold (Marhold & al. 1996; Marhold & Ančev 1999); from Austria: D. Albach, M. Fischer, J. Greilhuber, A. Polatschek (Fischer & Peev 1995; Ančev & Polatschek 1998, 2003; Dimitrova & Greilhuber 2000, 2001; Albach & Vladimirov 2002); from Denmark: Kit Tan (Tan & Vladimirov 2001) and from Poland: Z. Szeląg, J. Zielinski (Vladimirov & Szeląg 2001, 2001a; Zielinski & al. 2002). In this context one should also mention the contribution of Bulgarian botanists to floras of other countries: Pavlova & Kožuharov (1995), Dimitrov (1998) and Ivanova & Piękos-Mirkowa (2003).

As a result of all the studies on the flora of Bulgaria new reports on the distribution of more than 450 taxa have been available. Two families (*Commelinaceae*, *Rafflesiaceae*), 10 genera (*Acalypha*, *Ambrosia*, *Commelina*, *Cytinus*, *Duchesnea*, *Echinocystis*, *Myricaria*, *Rochelia*, *Sicyos* and *Sisyrinchium*) and 70 species have been recorded for the first time in the country. Fifteen taxa for the Bulgarian flora have been re-confirmed or re-discovered. All this improves the national information status significantly.

The taxonomic studies on different groups have led to the description of 30 new taxa (Tab. 1) and to proposals of new combinations or status (Pavlova & Kozhuharov 1994; Delipavlov & Cheshmedzhiev 1997).

General comments on taxonomic studies in the period 1994-2004 and future challenges

The beginning of the period considered coincides with the early years of the political, economic and social changes in the country which have influenced the development of plant taxonomic studies. Financial constraints have been the major difficulty for such research activities. In most of the botanical centers the research facilities are out-of-date, the access to modern literature is very expensive and therefore limited, and the research

Table 1. List of newly described species.

No	Species	Region	Publication
1	*Alyssum orbelicum* Ančev & Uzunov	Pirin Mts	Anchev, M. & Uzunov, D. 2002: Phytol. Balcan. 8(1): 26.
2	*Bromus parilicus* Petrova, Kozuharov & Ehrend.	Slavjanka Mt.	Petrova, A., Kozhuharov, S. & Ehrendorfer, F. 1997: Bocconea 5(2): 775.
3	*Cnicus bulgaricus* Panov	Belasica Mt.	Panov, P. 1996: Fitologiya 48: 9
4	*Epipactis spiridonovii* Devillers-Tersch. & Devillers	Pirin Mts	Devillers, P. & Devillers-Terschuren, J. 1996: Nat. Belg. 76(3): 87.
5	*Erysimum pirinicum* Ančev & Polatschek	Rila Mts	Ančev, M. & Polatschek, A. 1998: Ann. Natürhist. Mus. Wien 100B: 733.
6	*E. pseudoatticum* Ančev & Polatschek	Pirin Mts	Ančev, M. & Polatschek, A. 1998: Ann. Natürhist. Mus. Wien 100B: 726.
7	*E. slavjankae* Ančev & Polatschek	Slavjanka Mt.	Ančev, M. & Polatschek, A. 1998: Ann. Natürhist. Mus. Wien 100B: 729.
8	*Festuca achtarovii* Velchev & Vassilev	Mesta r. valley	Velchev, V. & Vassilev, P. 2002: Phytol. Balcan. 8(2): 185.
9	*F. calcarea* Velchev ex Denchev	Vračanska Mt.	Velchev, V. 2001: Phytol. Balcan. 8(1): 11; Denchev, C. 2004: Willdenowia 34: 78.
10	*F. maleschevica* Velchev & Vassilev	Maleševska Mt.	Velchev, V. & Vassilev, P. 2002: Phytol. Balcan. 8(2): 187.
11	*F. staroplaninica* Velchev	C Balkan Mt.	Velchev, V. 2001: Phytol. Balcan. 8(1): 5.
12	*F. vandovii* Velchev ex Denchev	Mesta r. valley	Velchev, V. 2001: Phytol. Balcan. 8(1): 9; Denchev, C. 2004: Willdenowia 34: 78.
13	*Hieracium ancevii* Szeląg	Pirin Mts	Szeląg, Z. 2001: Feddes Repert. Spec. 112(1-2): 12.
14	*H. kittanae* Vladimirov	C Rhodopi Mts	Vladimirov, V. 2003: Bot. J. Linn. Soc. 143: 214.
15	*Minuartia intermedia* Panov	Black Sea	Panov, P. 1996: Fitologiya 48: 5
16	*M. janevii* Panov	Struma r.	Panov, P. 1996: Fitologiya 48: 6.
17	*M. strandjensis* Panov	Strandža Mt.	Panov, P. 1996: Fitologiya 48: 7.
18	*Oenothera bulgarica* Delip.	Rila Mts	Delipavlov, D. 1998: Thaiszia J. Bot. 8:
19	*Oxytropis kozhuharovii* D. Pavlova, D. Dimitrov & M. Nikolova	Pirin Mts	Pavlova, D., Dimitrov, D. & Nikolova, M. 1999: Willdenowia 29: 69.
20	*Parietaria eronea* Panov	Danube Plain	Panov, P. 1996: Fitologiya 48: 4.
21	*P. rhodopaea* Panov	C Rhodopi Mts	Panov, P. 1996: Fitologiya 48: 3.
22	*Taraxacum ambitiosum* Kirshner & Štěpánek	Vitoša Mt.	Kirshner, J. & Štěpánek, J. 1998: A Monograph of *Taraxacum* Sect. *Palustria*: 176.
23	*Taraxacum lentum* Kirshner & Štěpánek	Lozenska Mt.	Kirshner, J. & Štěpánek, J. 1998: op. c. 216.
24	*T. melancholicum* Kirshner & Štěpánek	C Rhodopi Mts	Kirshner, J. & Štěpánek, J. 1998: op. c. 172.
25	*T. obuncum* Kirshner & Štěpánek	Lyulin Mt.	Kirshner, J. & Štěpánek, J. 1998: op. c. 165.
26	*T. strictum* Kirshner & Štěpánek	Lozenska Mt.	Kirshner, J. & Štěpánek, J. 1998: op. c.154.
27	*T. subudum* Kirshner & Štěpánek	Lozenska Mt.	Kirshner, J. & Štěpánek, J. 1998: op. c. 225.
28	*T. suspectum* Kirshner & Štěpánek	Vitoša Mt.	Kirshner, J. & Štěpánek, J. 1998: op. c. 162.
29	*Thymelaea bulgarica* Cheshm.	C Balkan Mt.	Cheshmedziev, I. 1997: Bocconea 5(2):
30	*Vicia jordanovii* Velchev	C Balkan Mt.	Velchev, V. 2001: Phytol. Balcan. 8(1): 3.

infrastructure is not sufficiently developed. Moreover, plant taxonomy has not been among the research priorities in the country and this has lead to a decrease of interest among students in taxonomy and general botany. Competition and the pressure for fast careers have lead to early specialization of young scientists and a lack of broader training necessary for the development of good taxonomists. At the same time some very positive trends have become effective, such as the increased mobility of scientists across Europe and the establishment of international research projects.

Many new challenges have to be faced by contemporary taxonomists, especially in a country in transition. There is a strong demand for re-considering the utility of plant taxonomy. Plant systematics should become more useful to a wider range of end-users. Practically, this means i.a. that reliable species inventories for territories of interest should be provided quickly and in a user-friendly way. Links with efforts to conserve biodiversity should be strengthened in order to respond to the political demands of the Convention on Biological Diversity and other biodiversity related documents and initiatives. The dialogue with other scientific disciplines should be re-vitalized and multi-disciplinary training of young researchers should be improved.

Plant taxonomy may be difficult and expensive in Bulgaria nowadays, but it is in no way old-fashioned, boring or even useless. On the contrary, for all those who are fascinated and inspired by the diversity of plant life, it is a very challenging, motivating and exciting field of work and hobby.

Acknowledgements

The authors express special thanks to the unknown reviewers for the helpful comments on the manuscript.

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G. Bedini, M. Ansaldi & F. Garbari

Mapping and demography of endangered plants in the Apuan Alps, NW Tuscany, Italy

Abstract

Bedini, G., Ansaldi, M. & Garbari, F.: Mapping and demography of endangered plants in the Apuan Alps, NW Tuscany, Italy. — Bocconea 21: 27-44. 2007. — ISSN 1120-4060.

The Apuan Alps, a massif located between La Spezia and Lucca on the Tyrrhenian coast of NW Tuscany, harbors a diverse flora, rich in biogeographically significant endemic and relic species. Intense exploitation of the marble outcrops and traditional agropastural practices involving fire management of pastures are the major risk factors for the survival of many species, particularly those linked to marble substrates. Distribution of the populations of 8 rare and relic species (*Anagallis tenella, Daphne alpina* subsp. *alpina*, *Horminum pyrenaicum, Biscutella cichoriifolia, Centaurea montis-borlae, Rhododendron ferrugineum, Biscutella apuana, Centaurea arachnoidea*) was mapped in the field and the density and seed production of the populations were recorded. Updated distribution maps were obtained through GIS software. Preliminary, qualitative observations on the extent to which suitable habitats are colonized by the studied species are reported.

Introduction

The Apuan Alps, with their peaks reaching almost 2000 m, mark the skyline of NW Tuscany in close proximity to the Tyrrhenian Sea. The mountain range is located between La Spezia and Lucca and extends about 55 km in length and about 25 km in width. The main watershed runs approximately parallel to the coastline with NW-SE orientation. The territory is a protected area managed by the Regional Natural Park of Apuan Alps.

The summit part of the range mainly consists of marble and other carbonate rocks. Sharp erosion profiles shape them like true alpine peaks and they have been described as "instantly petrified stormy sea" (Repetti 1833).

On the other hand, the basal part mainly consists of Palaeozoic schists of a siliceous nature. Minor rock outcrops include serpentines, marly clays, and sandstones (Carmignani & al. 2001).

The mountain range is a barrier to western winds blowing from the sea, and the winds have to rise to pass over the peaks, where the temperature is lower. Upon cooling down, the water vapor collected over the sea, condenses and then discharges as rainfall or snowfall. For this reason, the summit area receives more rainfall than the surrounding plains, following a distinctive pattern (Rapetti & Vittorini 1994) (Fig. 1).

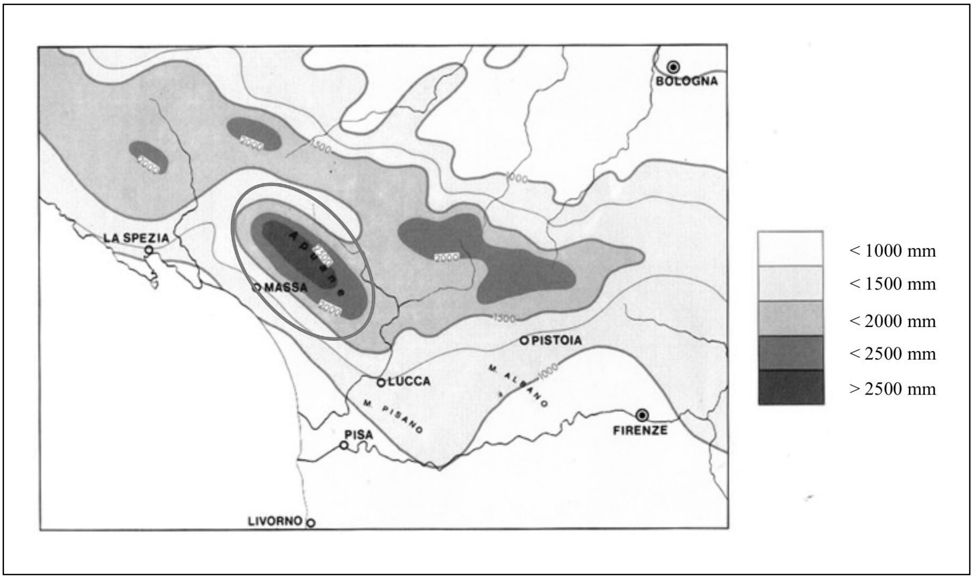

Fig. 1. Rainfall chart of N-W Tuscany. The Apuan region lies approximately within the ellipsoid.

From the climatic point of view the main watershed, parallel to the coastline, separates two main zone, the sea-side slope with higher temperatures and lower rainfall, and the inland slope where temperatures are lower and the rainfall is higher.

Another interesting feature is the presence of deep gorges carved in the sea-side slopes. At their bottom, dense shade, mild temperatures and protection from cold northerly winds determine an oceanic type climate, an outstanding feature in a Mediterranean area.

Due to the superimposition of lithologic, geomorphologic, geographic and climatic factors, combined with biogeographic history, the present Apuan flora includes a high number of taxa, which represent several floristic chorotypes that have reached the area with the migration waves preceding and following the glaciations.

The total of 1672 floristic units are reported for the area, with a high proportion of Eurasiatic elements (29%) followed by Eurimediterranean (18%), Arctic-alpine (12%), Orophytes (10%), and widely distributed units (9.5%). The endemics are about 5%, and also a small but important proportion of Atlantic species (3.5%) is present (Vaira & al. 2005).

Some Arctic-alpine and orophilous species are of special interest as microthermic relics, and demonstrate the role of this territory as a refugial area during glaciations. These taxa include *Valeriana saxatilis*, *Geranium argenteum*, *Rhododendron ferrugineum* (Ansaldi & al. 2004), *Menyanthes trifoliata*, *Eriophorum latifolium*, *Hutchinsia alpina*, *Homogyne alpina*, *Huperzia selago*, *Empetrum nigrum*, *E. hermaphroditum*, *Daphne alpina* subsp. *alpina* and *Horminum pyrenaicum*. Most of these reach the southern limit of their range in the Apuan Alps and in the Tuscan-Aemilian Apennine (Ferrarini 1970).

Deep gorges with an oceanic climate are refugial localities for Atlantic species such as *Hymenophyllum tunbrigense*, *Vandenboschia speciosa*, *Anagallis tenella* and *Euphorbia* *hyberna* subsp. *insularis*, some of which grow here at the eastern border of their ranges (Ferrarini 1970a, 1977; Tomei & al. 1985; Marchetti 2004).

The endemic species prefer the summit area of the massif. If only the summit flora is considered, the percentage of endemism rises to 10.1% (Tomaselli & Agostini 1994). Some endemic species, like *Globularia incanescens* and *Salix crataegifolia*, are of palaeogenic origin. Other endemics have been categorized by Bechi & al. (1996) as patroendemics (*Polygala carueliana*, *Athamanta cortiana*), *s*chizoendemics (*Astrantia pauciflora* subsp. *pauciflora*, *Buphthalmum salicifolium* subsp. *flexile*, *Carex ferruginea* subsp. *macrostachys*, *Carum appuanun* subsp. *appuanum*, *Leontodon anomalus*, *Moltkia suffruticosa*, *Santolina leucantha*, *Silene lanuginosa*, *Biscutella apuana*), apoendemics (*Galium palaeoitalicum*, *Artemisia nitida*, *Festuca apuanica*), while some are of uncertain linkage (*Centaurea montis-borlae*, *Cerastium apuanum*, *Rhamnus glaucophylla*, *Thesium sommieri*).

From the Apuan flora 105 species are listed in the RLT - Red List for Tuscany (Conti & al. 1997; Vaira & al. 2005). They have been designated as priority species for conservation projects in a Memorandum of Understanding (MoU) signed by the Department of Botanical Sciences of Pisa and the Apuan Alps Regional Natural Park. The goals set by the MoU include: (1) inventory and monitoring of populations of the priority species growing in the Park, (2) assessment of the risk category according to the IUCN criteria (IUCN 2001), (3) identification of risk factors for plant population, (4) *ex situ* conservation in the Pisa Botanic Garden seed bank, one of the nodes of RIBES Network (Rete Italiana Banche germoplasma per la conservazione Ex Situ), and (5) scientific research on reproductive biology. The present paper is a report on the first group of plants studied within the framework of the MoU (Tab. 1).

Materials and methods

The studied species were mainly selected from the RLT species growing in the Park. In agreement with the members of the Park Board, we were focused on: 2 of 3 CR species, 4 of 32 VU species, and on 2 strictly endemic species not included in the Red List (Tab. 1).

Their range was first checked from literature (Ansaldi & Bartelletti 1996; Ansaldi & al. 1994; Arrigoni 1956; Arrigoni & al. 1977; Baroni 1908; Bartelletti & al. 1996; Bazzichelli 1963; Bechi 1992; Bechi & al. 1996; Bertoloni 1819, 1833-1854; Bolzon 1894-1895; Caruel 1860; Garbari & Del Carratore 1993; Del Prete 1976; Del Prete & Miceli 1981; Del Prete & Tomaselli 1981; De Maria 1988; Ferrarini 1964, 1966, 1967, 1972, 2001; Ferrarini & Marchetti 1978, 1994; Ferrarini & al. 1997; Ferrarini & Rolla 1977; Fiori 1923-1929; Foggi & Rossi 1996; Lombardi & al. 1998; Marchetti 1973-1974, 1983; Moggi & al. 1987; Pacifico & al. 1996, 2000; Parlatore 1848-1896; Pellegrini 1942; Pichi Sermolli 1970; Pignatti 1982; Signorini 1993; Simi 1851; Soldano 1978, 1978a, 1984; Tutin & al. 1964-1980; Uncini Manganelli & Tomei 1996) and from herbarium specimens in: BOLO, GE, FI, PI, SIENA (Appendix). For each species the data were used to generate a list of known localities and a definition of potential areas, based on the ecological preferences of each species (habitat, substrate, aspect and altitude). Then a field inventory was planned to verify known localities and survey potential areas for new localities.

Table 1. Studied species.

Species	Biogeography	RLT listing (IUCN category)
Anagallis tenella	Atlantic relic	CR
Daphne alpine subsp. *alpina*	Microthermic relic	CR
Horminum pyrenaicum	Microthermic relic	VU
Biscutella cichoriifolia	Mediterranean relic	VU
Centaurea montis-borlae	Strictly endemic	VU
Rhododendron ferrugineum	Microthermic relic	VU
Biscutella apuana	Strictly endemic	---
Centaurea arachnoidea	Strictly endemic	---

We identified several sub-populations for each investigated species, following the IUCN criteria (IUCN 2001) and tagged each of them with an aluminum label bearing an identity code number. Herbarium vouchers collected from each site were deposited in PI. For each sub-population we counted single individuals or estimated their number based on the density in sample areas of 10x10 m (3-5 samples per sub-population). At each locality we recorded habitat type and substrate through direct observations, aspect by a compass, altitude by an altimeter. Notes on the whole flora were also made.

The geographic position was recorded in the field on 1:10000 maps produced by the technical services of “Regione Toscana” (CTR). For further elaborations, the geographic data were transferred to a digital map provided by the Park technical service (1:25000) by means of Arc-View GIS software (Esri). The same software was used to calculate the area of occupancy of the studied species.

Results and discussion

Anagallis tenella L. (*Primulaceae*)

Chromosome number: $2n=22$
RLT listing: CR
Habitat: wet cliffs at road side
Altitude/substrate: 250m/siliceous
Number of Apuan populations: 1
Number of confirmed/new/not confirmed populations: 1/0/0
Area of occupancy: 20 m^2
Number of individuals: 285
Number of other localities in Tuscany: 4

This species was only reported in a single stand in the Apuan Alps near Retignano (Lucca) at an altitude of about 250 m on siliceous soil (Ferrarini & al. 1997; see also Appendix). We were able to confirm this find, limited to just 20 m^2 and to few hundred individuals. No new sites were discovered despite an intensive search in an area covering 265 ha with similar ecological conditions (Fig. 2). The site is located above a road verge, on exposed rock kept wet by a constant dripping of water, an essential element for this plant. In Tuscany such places are linked to few wetland areas close to the sea level (Di Moisè 1958; Tomei & al. 1985). During our survey the plants did not set seed.

Considering the CR status of *A. tenella* (Conti & al. 1997), we emphasize (Vaira & al. 2005) the need for the Park to provide adequate protection for this site, particularly against mowing of the road verge immediately beneath the population, done once a year, and against any capture of the water source above.

The species is considered an Atlantic relic in Italy. The Apuan locality represents the only population significantly above the sea level and demonstrates the role played by this massif as a refugial area during cool stages of interglacial periods.

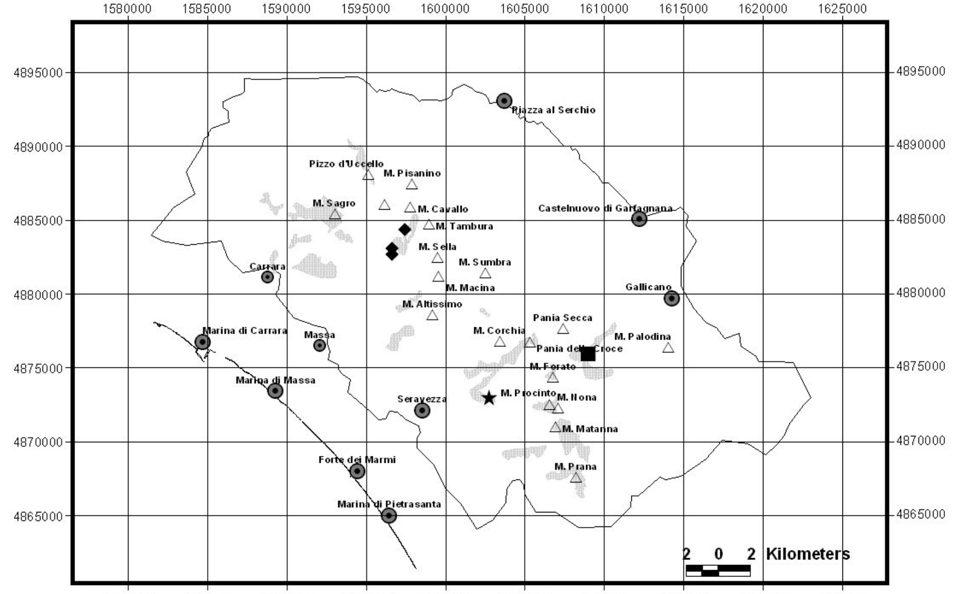

Fig. 2. Verified localities in the Apuan Alps of *Anagallis tenella* (★), *Horminum pyrenaicum* (◆), and *Rhododendron ferrugineum*(■). The shaded parts represent the studied area; the geographic grid refers to the Gauss Boaga (ROMA40) projection system.

Daphne alpina L. subsp. *alpina* (*Thymelaeaceae*)

Chromosome number: 2*n*=36
RLT listing: CR
Habitat: cliffs, screes
Altitude/substrate: 800-1300 m/calcareous
Number of Apuan populations: 33
Number of confirmed/new/not confirmed populations: 8/25/3
Area of occupancy: 437813 m^2
Number of individuals: 608
Number of other localities in Tuscany: 3

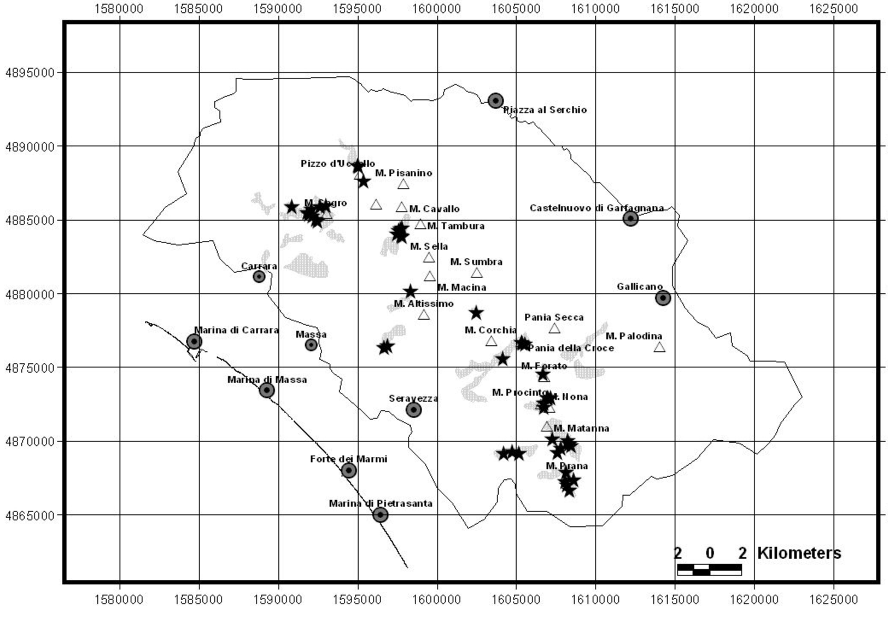

Fig. 3. Verified localities in the Apuan Alps of *Daphne alpina* subsp. *alpina* (★). The shaded parts represent the studied area; the geographic grid refers to the Gauss Boaga (ROMA40) projection system.

In Italy this taxon is mainly distributed in the Alps, with a few populations possibly reaching as far south as Central Apennine (Pignatti 1982). It has been reported in the central and southern part of the Apuan Alps, slightly below the summit area, on calcareous substrate by other authors (Caruel 1860; Pellegrini 1942; Urbani 1992; Ferrarini & al. 1997).

However, its distribution and in fact its presence in the Apuan Alps has been questioned by Pignatti (1982), who pointed out a possible confusion with closely related *D. oleoides*, in partial agreement with Ferrarini & al. (1997). We were able to confirm most reported stations, find many new ones and describe diagnostic characters that help distinguish the two taxa. In *D. alpina* subsp. *alpina* the leaves are in terminal whorls, deciduous, with rounded apex hairy below, and the bark of terminal branches is grey and fissured. On the contrary, in *D. oleoides* younger leaves are in terminal whorls, the older set arranged spirally, the leaves are persistent, shortly mucronate, glabrous on both surfaces, and the bark of terminal branches is reddish, shiny, with pronounced leaf scars.

The species occupies a wide area of its potential range. It can be found nearly everywhere where calcareous cliffs or screes occur between 800 and 1300 m asl (Fig. 3). Nevertheless, we never observed dense stands but rather single individuals or very small groups of 3 or 4 plants, resulting in a very low total number of individuals for the studied area. Fruit production is abundant, but only a few fruits reach maturity, as many are shed during ripening.

Horminum pyrenaicum L. (*Labiatae*)

Chromosome number: 2*n*=12
RLT listing: VU
Habitat: Grasslands, pastures
Altitude/substrate: 1000-1600/calcareous
Number of Apuan populations: 3
Number of confirmed/new/not confirmed populations: 3/0/1
Area of occupancy: 24177 m^2
Number of individuals: 35770
Number of other localities in Tuscany: none

In Italy this species is common in the Alps. The Apuan Alps represent its only locality in Tuscany (Pignatti 1982) and the southern border of its range. It has been reported for few localities grouped in the central part of the massif, in grasslands and pastures on calcareous substrate between 1000 and 1600 m asl (Ferrarini & al. 1997). We were able to confirm 3 of 4 reported localities (Fig. 2). At two sites the population density is around 180 individuals/100 m^2, while at the remaining site only 23 isolated plants are present in an area of 4246 m^2. The plants did not flower during the survey campaign. A CR IUCN category has recently been proposed for it (Vaira & al. 2005) in replacement of VU (Conti & al. 1997).

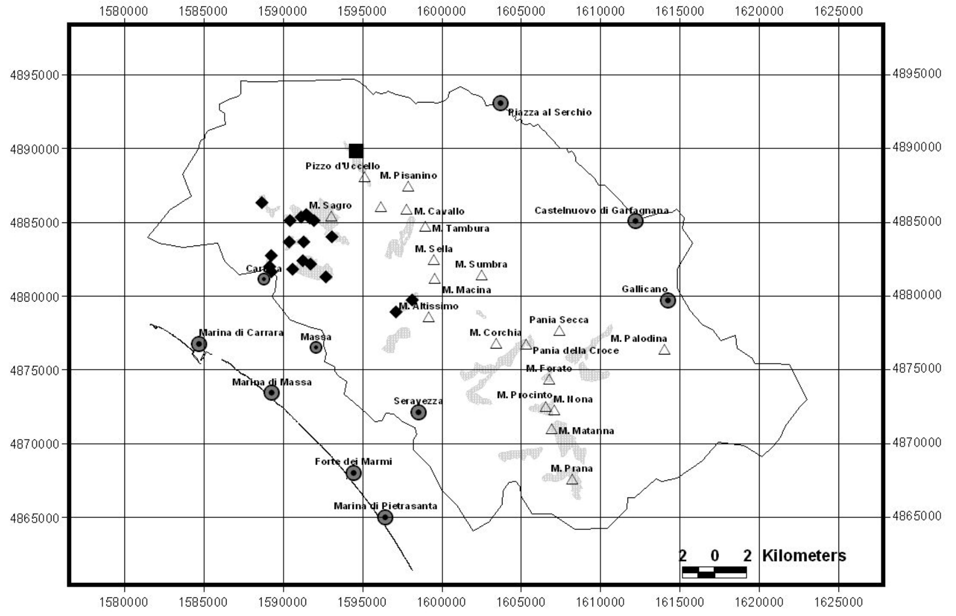

Fig. 4. Verified localities in the Apuan Alps of *Biscutella cichoriifolia* (■) and *Centaurea arachnoidea* (◆). The shaded parts represent the studied area; the geographic grid refers to the Gauss Boaga (ROMA40) projection system.

Biscutella cichoriifolia Loisel. (*Cruciferae*)

Chromosome number: 2*n*=16
RLT listing: VU
Habitat: garrigue
Altitude/substrate: 750 m
Number of Apuan populations: 1
Number of confirmed/new/not confirmed populations: 0/1/4
Area of occupancy: 3484 m^2
Number of individuals: 11
Number of other localities in Tuscany: 3

This species has a restricted distribution in Tuscany and has been reported in three localities apart from the Apuan Alps (Raffaelli 1992). Two localities have been reported in the central Apuan Alps (Ferrarini & Marchetti 1994) and two additional localities were recorded on herbarium specimens (see Appendix). We did not find the plant at any of these sites.

However, we quite unexpectedly discovered a new site with only 11 individuals in the northern part of the massif, within the extremely disturbed environment of an active marble quarry (Fig. 4).

Centaurea montis-borlae Soldano (*Compositae*)

Chromosome number: 2*n*=88
RLT listing: VU
Habitat: cliffs, platforms of Mt. Borla and adjacent places
Altitude/substrate: 1100-1400 m/marble
Number of Apuan populations: 2
Number of confirmed/new/not confirmed populations: 2/0/1
Area of occupancy: 153115 m^2
Number of individuals: 73361
Number of other localities in Tuscany: none

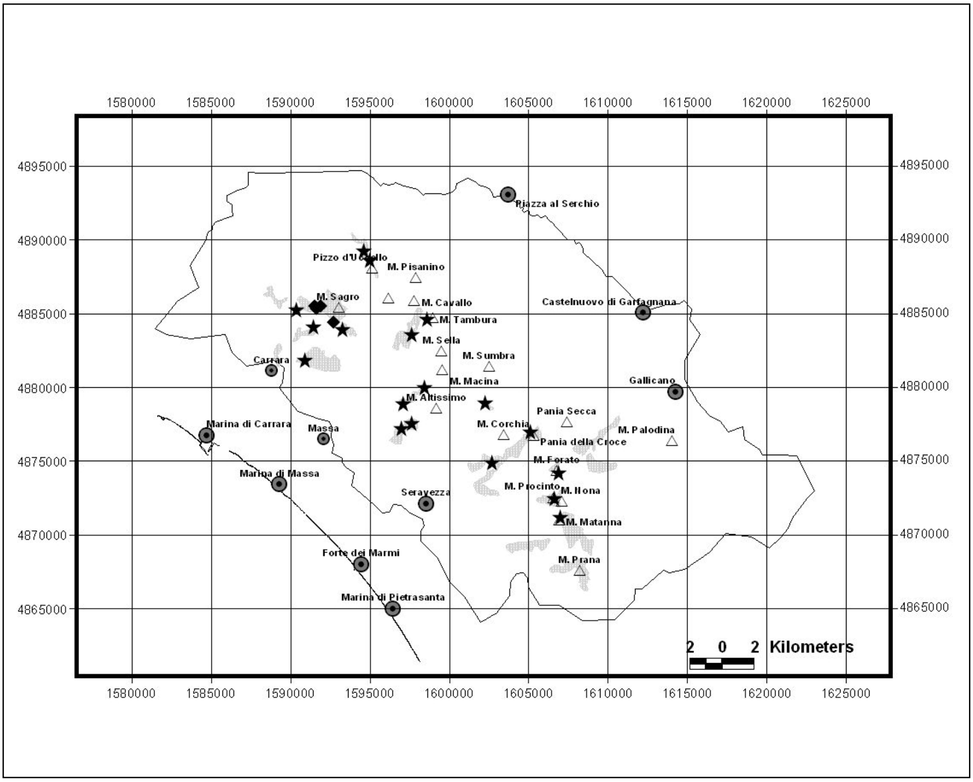

Fig. 5. Verified localities in the Apuan Alps of *Biscutella apuana* (★) and *Centaurea montis-borlae* (◆). The shaded parts represent the studied area; the geographic grid refers to the Gauss Boaga (ROMA40) projection system.

This endemic species was described by Soldano (1978), for the south-facing slopes of Mt. Borla in the northern part of the massif, where it grows on marble platforms and cliffs from 1100 to 1400 m asl. We confirmed this locality and other localities reported by Ferrarini (2001) except one ("Poggio della Signora", see Appendix). We were unable to check one located on a nearly vertical slope of friable rock ("spigolo E del Monte Sagro"). At all localities the substrate is compact marble, as defined in Carmignani & al. (2001). We did not observe the species on any other type of calcareous substrates or on marble screes, although it must be stressed that the unchecked station of Mt. Sagro is constituted by cherty limestone. We speculate that this plant might grow on the carbonate layers, very similar to marble, of this metamorphic formation. The plant grows in sparse clusters of about 50 individuals/100 m^2 and the density is rather homogeneous all over the area of occupancy except for a single individual recorded between Foce della Faggiola and Monte Maggiore. Observed seed production per individual is low and preliminary tests showed low germination rates. Cypselas badly damaged by larvae were also recorded (M. Boracchia, *pers. com.*). Further studies are in progress to clarify these aspects of reproductive biology. The habitat is heavily used for marble quarrying. As noted for other species, also *Centaurea montis-borlae* does not fully cover the potential area of marble outcrops with south-facing slopes. We checked 410 ha of such habitat on the slopes of Mt. Borla alone without finding the plant (Fig. 5). We wish to stress again that the origin of this polyploid endemic is not yet clear (Bechi & al. 1996).

Rhododendron ferrugineum L. (*Ericaceae*)

Chromosome number: $2n=26$
RLT listing: VU
Habitat: rocks
Altitude/substrate: 500 m/siliceous
Number of Apuan populations: 1
Number of confirmed/new/not confirmed populations: 0/1/0
Area of occupancy: 250 m^2
Number of individuals: 40
Number of other localities in Tuscany: 1

In Italy, this species is common on the whole Alpine chain where it grows in heaths and highland grasslands on siliceous substrates, up to about 2300 m asl (Pignatti 1982). It is rare in the Tuscan-Aemilian Apennine, where it is mainly distributed in the Aemilian slope, except for one population at Monte Vecchio – Bocca di Scala. Its presence in Tuscany is generally interpreted as a microthermic relic (Ferrarini 1973, 1974, 1979, 1982).

The species was not known for the Apuan Alps until recently (Ansaldi & al. 2004). A very small population was found in a deep gorge, at an altitude of 500 m., under conditions similar to those of prealpine zones with an oceanic climate (Fig. 2). The population lives on a steep slope of siliceous substrate, surrounded by a mesophilous wood and partially shaded by tree canopies. This habitat sharply contrasts with that of the Apennine populations, which thrive at much higher altitudes near summits in open heaths and in full sunlight. Plant habit is also different, particularly as regards the longer internodes. Another difference concerns the low density of the stand. The population is composed of about 40 individuals, with an average density of 16 individuals/100 m^2 and does not form a continuous cover like the Apennine. Further studies are in progress to ascertain the presence of *Rhododendron ferrugineum* in other places of the massif and to clarify morphological and anatomical features.

Biscutella apuana Raffaelli (*Cruciferae*)

Chromosome number: 2*n*=18
RLT listing: –
Habitat: grasslands, screes
Altitude/substrate: 300-1300 m/calcareous
Number of Apuan populations: 12
Number of confirmed/new/not confirmed populations: 7/5/6
Area of occupancy: 300360 m^2
Number of individuals: 40013
Number of other localities in Tuscany: none

The species was described by Raffaelli & Fiesoli (1993) who isolated it from the Apuan *Biscutella* populations previously identified as *B. laevigata* s. l. For this reason, we also checked literature records and herbarium specimens of *B. laevigata*.

B. apuana can be easily distinguished from *B. laevigata* of the presence of 2-4 stem leaves (lacking in *B. laevigata*) and hairy/papillose siliculas (glabrous in *B. laevigata*).

This plant has been reported for the whole territory, at altitudes ranging from 300 to 1300 m asl in grasslands and screes on calcareous substrates (Ferrarini & Marchetti 1994). In our field survey, 6 localities were not confirmed, but 5 new stations were found on the same substrate and in the same habitat type. All new localities are near active marble quarries. Four of the new localities lie in the northern part of the territory and one in the central area (Fig. 4).

Most observed populations vary in density from 1 to 47 individuals/100 m^2, but single, isolated individuals have also been observed. There are wide gaps between sub-populations where the substrate and the habitat type are suitable for the plant.

Due to its recent taxonomic recognizing from the *Biscutella laevigata* complex, *B. apuana* was not included in the Regional Red List for Tuscany (Conti & al. 1997). Its conservation status was assessed as VU in a later study (Vaira & al. 2005).

Centaurea arachnoidea Viv. (*Compositae*)

Chromosome number: 2*n*=18
RLT listing: –
Habitat: discontinuous grassland on compact rock and fine-grained debris
Altitude/substrate: 300-1300 m/calcareous
Number of Apuan populations: 16
Number of confirmed/new/not confirmed populations: 6/10/2
Area of occupancy: 192546 m^2
Number of individuals: 40423
Number of other localities in Tuscany: none

This plant is an Apuan endemic. Reports of other stations in Tuscany and in Latium (Ferrarini 2001) are not supported by herbarium specimens.

Most of the reported localities (Ferrarini 2001) were confirmed during our field survey and a good number of potential areas in the northwestern part of the territory turned out to be new localities (Fig. 5). *C. arachnoidea* grows in discontinuous grasslands on compact rock and fine-grained debris on calcareous substrate mainly of metamorphic origin. Many populations live at the edge of active quarries. Average density is 21 individuals/100 m^2, but many isolated individuals exist.

We observed many vital cypselas per individual, and preliminary tests showed a high germination rate (G. Bedini, *unpubl.*).

Judged from its extremely restricted area and small population size, the threat category of *Centaurea arachnoidea* has been assessed as VU (Vaira & al. 2005).

Conclusions

GIS treatment of the survey data provided a reliable estimate of population size, both as to the area and the number of individuals, and allowed an accurate assessment of risk categories.

The eight species studied showed different behavior regarding population size, and colonization of suitable habitats.

A first group of four species (*Anagallis tenella*, *Biscutella cichoriifolia*, *Daphne alpina* subsp. *alpina*, *Rhododendron ferrugineum*) live in the Apuan Alps with very small populations (11-608 individuals). These populations are generally considered relics of past distribution patterns. Except for *Daphne alpina* subsp. *alpina*, we observed that they occupy a tiny portion of the suitable habitat. However, we cannot currently give precise measurements, due to the lack of digital cartographic data allowing an accurate estimate of the distribution of ecological data (substrate, altitude, aspect, habitat type). For example, *Anagallis tenella* is missing from an area of 265 ha where we have directly observed, *in situ*, that conditions are similar to those of the single extant Apuan stand.

Three species have a higher number of individuals (40000-70000) and are distributed in larger areas of the territory, albeit none appears to fully cover its potential range. Again, this conclusion is supported by personal observations rather than an instrumental measurement. For example, *Centaurea montis-borlae* is missing from an area of 410 ha of Mt. Borla, where ecological conditions seem suitable for its growth. Obviously, other factors play an important role in shaping the range of a species. Low seed production and poor seed germination success might account for the inability of *Centaurea montis-borlae* to colonize adjacent areas. Thus, we have started reproductive biology studies on *C. montis-borlae* to clarify distribution patterns.

It is striking that the smallest number of individuals is associated to relic species, reinforcing the bio-geographic interpretation that small populations are the remains of a much larger past range. These small populations cannot expand their range again because they lack specific morpho-physiological adaptations to survive outside of their micro-environment. Endemic species that have evolved within the area as a result of speciation processes are fitter to the general climatic and ecological conditions of the area and appear to be able to colonize greater portions of the territory.

The case of *Horminum pyrenaicum* is slightly different because the number of individuals is relatively high. However, its area of occupancy is one order of magnitude smaller than that of endemic species, in accordance with its relic status. It seems, therefore, that the relic situation is characterized either by a low number of individuals or a restricted area of occupancy.

This study indicates that there may be differences between the potential and actual distribution of the studied species and that predictive distribution models based on habitat type, altitude, aspect and substrate should be checked in the field. A correlation can be inferred between bio-geographic type and the ratio area of occupancy/potential distribution, but with species-specific adjustments. Nevertheless, the ability to map ecological factors, leading to a definition of potential distributions for plant taxa, provides a baseline against which field data can be referred. Furthermore, a more detailed, quantitative analysis of the distribution of ecological factors in the territory would allow a stricter correlation between potential and actual distribution of plants. The discrepancy between potential and actual distribution may be due to the impact of human activity in the area, which has been going on for several centuries (marble quarries, wood cutting, sheep raising and periodical wood and pasture fires set as a misunderstood land management method by shepherds). In order to clarify this matter, we are planning further studies in the area as new digital thematic maps are available, allowing quantitative measurements of the distribution of ecological factors.

Appendix

Specimens seen: label information is given in a short form, the full information is available from the authors upon request.

Anagallis tenella: Tra Retignano e la Risvolta, 23.VII.1982, *Marchetti* (SIENA).

Biscutella apuana (many specimens are reported as *B. laevigata*): Forno,1903, *Bicknell* (GE); Alpi Apuane, Orto botanico di Pian della Fioba, 1984, *Bassani* (SIENA); A q.300 m, tra Miseglia e Ponti di Vara, 1978, *Marchetti* (SIENA); A q. 300 m, Forno, 1977, *Marchetti* (SIENA); Alpi Apuane a Fatonero, 1869, *Cocchi* (FI); Tra Levigliani e vetta del M. Pania della Croce, 800-1858 m, 1950, *Pichi Sermolli*, *Bavazzano*, *Contardo* (FI); Pania della Croce, *Raffaelli* (PI); Nei pressi della galleria Valsola, 900 m, 1988, *Raffaelli*, *Fiorini*, *Fiesoli* (FI); Tra Isola Santa e Arni, 1987, *Raffaelli* (FI); Alpi Apuane, Nei pressi della galleria del Passo del Vestito, 1987, *Raffaelli et al.* (FI); Tra Isola Santa e Arni, 1987, *Raffaelli et al.* (FI); Dintorni di Campocecina, 1969, *Moggi et al.* (FI); Da Campocecina alla vetta del M. Sagro, 1969, Moggi et al. (FI); Tra Isola Santa e Arni, 1987, *Raffaelli et al.* (FI); Canal Cerignano, Borra, nei pressi della funicolare, 1957, *Beruti* (FI); Castelnuovo Garfagnana, Tra Isola Santa e Camporgiano, 1988, *Raffaelli*. (FI); Nei pressi della galleria Valsola lungo la strada di Pian della Fioba, 1988, *Raffaelli* (FI); Tra Pian della Fioba e il Passo del Vestito, 1987, *Raffaelli* (FI); Boschi radi e rocce della valle di Arni, Substrato calcareo, 1989, *Raffaelli* (FI); Sopra la galleria del Passo del Vestito (FI); Pania, Alpi Apuane, In pascuis saxosis (prati della Pania), 1893, *Sommier* (FI); Rupi delle parti elevate della Pania della Croce Alpi Apuane,1891, *Martelli* (FI); Alpi Apuane alla Pania della Croce presso la vetta, 1891, *Martelli* (FI); Pania della Croce sopra Mosceta, 1891, *Martelli*

(FI); Toscana: Monte Matanna e M. Procinto, 1934, *Chiarugi* (FI); Lungo il Carrione sopra Carrara, 1891, *Fantozzi* (FI); Pania alla Croce, 1958, *Lanza* (FI); Prope pagum Forno, loco C. Biforco dicto, alt. 200-300 m. - 1924, *Fiori* (FI); Monte Garnerone, 1964, *Ferrarini* (FI); Legi in Apuanis, 1830, *Fiori* (FI); Legi in Apuanis, 1922, Fiori (FI); Alpi Apuane, Tambura, 1897, *Targ.-Tozz.* (FI); Alpi Apuane, Altissimo, 1897, *Targ.-Tozz.* (FI); Alpi Apuane, Tambura, 1897, *Targ.-Tozz.* (FI); Passo del Vestito, Galleria del Cipollaio, 1953, *Corradi* (FI); Altissimo, versante settentrionale, 1951, *Pichi* (FI); Carrara, *Bolzon* (FI); 1922, *Fiori* (FI); vertice della Pania, Torrente del Ponchio nell'Alpe di Terrinca, M. Altissimo, Corchia, 1869, *Simi* (FI); Colonnata (FI); Resceto, 1862, *Beccari* (FI); Cave di Ravaccione, 1913, *Savelli* (FI); Sorgenti del Frigido, 1863, *Beccari* (FI); Minucciano, Vinca (Pisanino), 1891, *Sommier* (FI); Resceto, 1862, *Beccari* (FI); Forno (MS), 1924, *Fiori* (FI); Tambura, 1857, *Grilli* (FI); Tambura, 1881, *Martelli* (FI); Valle della Turrite Secca, Grotta Giancona, 1955, *Padula* (FI); Forno, 1924, *Fiori* (FI); Sul Pisanino Alpi Apuane, 1870, *Giannini* (FI); In monte Tambura, supra Resceto (Alp. Apuan.), 1825, *Levier* (FI); M.te Tambura, Alpi Apuane, 1878, *Herbarium Della Nave* (FI); Alpi Apuane a M.te Forato, 1892, *Herbarium P. Fantozzi* (FI); Rupi di Falcovaja (?) Monte Altissimo, 1857, *Erbario M. Grilli*, (FI); Alpi Apuane, a Bertoloni, Da Parlatore in Sett. 1842, *Fiori* (FI); Tambura Alpium Apuanarum, *Savi*,1841, (FI); Tambura, 1830, *Bertoloni* (BOLO); Alpi Apuane,1844, *Puccinelli* (BOLO); In Lunentium lapidicinis ubi frequens, 1806, (BOLO); Legi in Sagro, Alpi Apuanarum, 1.VII.1808, (BOLO); Legi in Tambura, Alpi Apuanarum, 1822 (BOLO); In calcareis Tambura, Inizio luglio 1843, *Savi* (BOLO); Legi in Alpi Apuanarum sopra Fornole ad levam Frigidae. 1809 (BOLO).

Biscutella cichoriifolia: Monte Matanna e Monte Procinto. *Chiarugi* (FI); Tra Levigliani e il Passo dell'Alpino, 1862 *Savi*. (PI *as B. hispida*).

Centaurea arachnoidea: Cave di Colonnata alla "Grotta Lunga". 1841. *Meneghini* (PI, *as C. rupestris*); Lunensis lapidiciniis. *Auct. ignoto* (PI, *as C. rupestris*); Pendici del M. Uccelliera, sopra Carrara, a q. 1225 m circa. Su cipollino. 7.VII.1982. *Marchetti*. (GE); In pascosis montanis etruriae. VII.1885 *Costa Reghini* (GE *as C. rupestris*); Alpi Apuane, Miseglia sopra Carrara, alt. 300 m, erboso su calcare cavernoso. 17.VII.1984. *Bassani*, *Ferrarini* (SIENA *as C. rupestris* subsp. *arachnoidea*); In rupi di calcare cavernoso, a q. 300 m, fra Miseglia e Ponti di Vara, sopra Carrara, Alpi Apuane. 28.VI.1978 *Marchetti*. (SIENA *as C. rupestris* subsp. *arachnoidea*); Lungo la strada per Campocecina a q. 1200 m, alla curva a sud nelle pedici del M. Uccelliera, sopra Carrara, Alpi Apuane. 26.VIII.1978 *Marchetti* (SIENA *as C. rupestris* subsp. *arachnoidea*); Su rupi di calcare cavernoso, a q. 300, tra Miseglia e Ponti di Vara, sopra Carrara (MS), Alpi Apuane. 21.VI.1975. *Marchetti* (SIENA *as C. rupestris* subsp. *arachnoidea*); Lungo la strada per Campocecina a q. 1200 m, alla curva a sud nelle pendici del M. Uccelliera, sopra Carrara, Alpi Apuane. 26.VIII.1978 *Marchetti* (SIENA *as C. rupestris* subsp. *arachnoidea*); Legit in lapidicinis lunensium. VIII.1841 *Cherici* (FI *as C. rupestris*); Alpi Apuane, lungo il Carrione, fra Carrara e Colonnata. 28.VII.1891. *Fantozzi* (FI *as C. rupestris)*; Luoghi rupestri attorno Carrara. 1893. *Bolzon*. Rev. Fiori 13.XII.1904 (FI *as C. rupestris*); Alpi Apuane: nelle fessure delle rupi calcaree presso le cave di Ravaccione (Carrara). 21.V.1913. *Savelli* (FI *as C. rupestris* var. *arachnoidea*) *(*= var. *subinermis*); Sulle rupi a Torano presso Carrara. VII.1901. *Vaccari* (FI *as C. rupestris*); Torrano, Lapidicinis Lunensibus, Grotta Lunga prope Colonnata (BOLO *as C. rupestris*).

Centaurea montis-borlae: Nelle fessure delle rupi di marmo, a q. 1300 m, esp. Sud, alla Foce di Pianza nelle pendici del M. Borla, Alpi Apuane. 27.VIII.1960 *Ferrarini* (SIENA); Versante meridionale del Poggio della Signora, nelle pendici del M. Spallone, a q. 1500 m circa. Gruppo del M. Sagro, sopra Carrara. Alpi Apuane. Su calcare a liste di selce. 5.IX.1983 *Marchetti* (SIENA); Foce di Pianza (gruppo del Sagro: Alpi Apuane. Alt. 1200 m; 3.VIII.1977 (Typus) *Soldano* (FI); Toscana. Massa. Fivizzano: piedi del Monte Borla alle cave Walton. m 1250. 24.VII.1977 *Soldano* (FI).

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Cèsar Blanché & Maria Bosch

Conservation issues from research on pollination ecology - a West Mediterranean view

Abstract

Blanché, C. & Bosch, M.: Conservation issues from research on pollination ecology - a West Mediterranean view. — Bocconea 21: 45-64. 2007. — ISSN 1120-4060.

The assessment of endangered plant species is commonly performed by evaluating, among other things, the decrease in the quality and quantity of pollination services under global change parameters. This allows inferences to be made about declining pollination success, reduced genetic diversity and the low recruitment performances of descendants. Although most case studies on the pollination ecology of threatened Mediterranean species show no clear evidence of pollinator decline, negative consequences are commonly expected. In this article we review data from our own research and the literature in order to survey experimental approaches to assessing the true threat posed by pollination deficit. We report on documented cases of endangered plants – mainly from the W. Mediterranean - with limited pollination. Current conservation efforts are also outlined and further lines of research proposed.

Introduction

The conservation issues related to pollination ecology comprise a complex web of interacting factors in global change, beginning with the general context of climatic change. The trends projected by the United Nations, which have recently been endorsed by the European Environment Agency, are towards increasing average temperatures, changes in the distribution of precipitation and associated alterations (UNEP 2003; EEA 2004) (Tab. 1). These documents include projected impacts on biodiversity: mutualisms, including pollination, are under severe threat, both through risk of extinction of keystone species, and through a projected disruption of plant-animal interactions caused by differential responses to climatic changes (EEA 2004) (Tab. 2). Summaries of species and their interactions have been published by Kremen & Ricketts (2000), Thomas & al. (2004), Pounds & Puschendorf (2004), and some case studies on specific areas indicate that these changes have already begun in many parts of the world (Primack & al. 2004), including the Mediterranean area (Gavilán 2001; Peñuelas & al. 2002). Thus, the expected consequences of climatic changes on mutualistic assemblages, such as pollination, are predictable, though not yet fully confirmed.

Table 1. The summary of projected changes in Global and European climate (Sources: United Nations Environmental Program 2003: 41-42; European Environmental Agency 2004: 1-8).

PARAMETER	PROJECTION
CO_2 concentration	540-970 ppm (2100) [280 in the pre-industrial era; 368 in 2000]
Sulfate aerosol concentrations	Fall below present level by 2100
Average temperature	Globally: projected increase of 1.4-5.8ºC (1990-2100) Europe: projected increase of 2.0-6.3ºC (1990-2100) Changes in variability (daily, seasonal, inter annual, and decadal)
Average annual precipitation	Globally: projected increase. Regionally: ± 5-20% N. Europe: 10-40% wetter S. Europe: 20% drier
Glaciers and ice caps	Continued widespread retreat during the 21st century By 2050 c. 75% of Swiss Alps glaciers are likely to disappear
Global sea level	Rise by 0.09 to 0.88 mm/year (1990-2100) Around Europe by 0.8-3.0 mm/year x 2.2-4.4 (21st century)

However, in some regions, the relative impact of climatic change is likely to be lower than that of other factors. Pollination systems are under increasing threat from more direct anthropogenic sources, including habitat fragmentation, changes in land use, modern agricultural practices, use of chemicals (such as pesticides or herbicides), and invasions of non-native plants and animals, independently of (or in addition to) climatic considerations. The effects of human activities on pollination systems have lead to the so-called "pollination crisis" (Buchmann & Nabhan 1996; Kearns & al. 1998). One of the major factors in this crisis is the decline in pollinators (see Goulson & al. 2005), which some authors consider as environmental bioindicators (Kevan 1999).

Table 2. The summary of projected impact on individuals, populations, species, and ecosystems (Source: United Nations Environmental Program 2003: 45).

PARAMETER	PROJECTION
Species losses	Increase
Extinction of wildlife populations	Increase (particularly pronounced when a population is isolated by habitat loss)
Changes in phenology	Expected to continue
Habitat displacement	Move upward / poleward from current locations (accelerated by anthropogenic disturbances) *2100: 200-1200 km northward for temperate and boreal plant species. New species assemblages?
Ecosystem interactions	Disruption, species unlike to shift together
Critical / vulnerable life stages	Expected to continue to affect
Populations, species and ecosystems vulnerable to climate change 1. Species/ecosystems with limited climatic ranges and/or restricted habitat requirements 2. Species already in risk of extinction	

The loss of pollination services, besides affecting their contribution to reproduction and the maintenance of evolutionary processes of plant biodiversity, can also be evaluated in economic terms. In fact, the pollination of flowering plants by animals is a critical ecosystem service of great value to humanity (Kearns & al. 1998). World pollination services in wild ecosystems have been estimated to have a mean value per annum of 112$ billion (Costanza & al. 1997) and 200$ billion in global agriculture (Richards 1993). Marco & Monteiro (2004) demonstrated the positive effects of forest conservation to preserve native pollinators, which are increasing coffee production in Brazil. The United Nations FAO programmes also confirm the essential services provided by pollination (http://fao.org.biodiversity/pollinat_en.asp).

Claims of widespread decrease in animal pollinators and pollination decline have captured public and scientific attention in the last decade (Thomson 2001). The economic importance of pollination and its biological value makes the conservation of pollination systems a high priority.

Summary of threats to pollination systems

In our research on pollination ecology in W. Mediterranean systems, we have detected several types of endangered mutualisms or pollination disturbances that correspond to the main classes of threat to the pollination systems previously summarized by other authors (similar data are available from the E. Mediterranean region, see Petanidou & Ellis 1996; Petanidou 2004, 2005; Potts & al. 2002, among others).

Effects of agricultural practices

Several features of modern agriculture provide poor habitats for wild pollinators. Crop monocultures decrease floral diversity and the heterogeneity of pollinating agents (Pywell & al. 2005). A decrease in marginal areas (due to cultivation or transformation) results in a loss of wild vegetation to support pollinators (González 2004): fewer nesting areas for bees; fewer larval host plants for butterflies and less-varied habitats for egg laying and larval development have been the most cited effects (Kearns & al. 1998; Carvell & al. 2001; Pywell & al. 2005; Goulson & al. 2005). In some Mediterranean countries, this situation has begun to reverse, through the abandonment of agricultural fields and subsequent ecological changes, producing new successional phases leading to shrub and forest recovery (Petanidou 2004).

Delphinium bolosii, an endangered species endemic to Catalonia (Bosch & al. 1998), provides evidence of the indirect effects of shrub recovery. In the smaller of the only two existing populations, cultivation of the hazelnut tree, *Corylus avellana*, was abandoned some years ago. This could be considered as an opportunity for the recovery of the endangered population that survives in a small ravine previously surrounded by agricultural fields. However, once cultivation ceased, progressively increasing densities of the shrub *Rubus ulmifolius* began to invade the refuge of the endemic larkspur. As a result, in addition to long-term competition for habitat resources, competition in attracting pollinators was observed between *D. bolosii* and *R. ulmifolius* (Orellana & al. 2004). The study of stigmatic pollen loads showed a total heterospecific load of 8 %, a high proportion of which was from *R. ulmifolius*. The effects of purity in relation to neighboring species were assessed by comparing pure and mixed plots. Slight negative effects on reproductive traits (such as higher rates of seed abortion (50%) and declining viable seed/ovules index) were detected as a result of interspecific competition for pollinators. In short, a disturbance effect was detected and thus conservation efforts were oriented towards the removal or control of *Rubus* in this population. Similar conclusions relating to grassland management in order to prevent shrub expansion are widely reported, and in some cases the benefits for plant-animal interactions are clear (Krauss & al. 2004).

Finally, pollinator loss could affect agricultural systems. According to Matheson & al. (1996), 84 % of crop species grown in the European Union are dependent on insect pollination, and declines in bee populations are widely reported in Europe (Kearns & al. 1998; Goulson & al. 2005, and references therein), as well as in America (Kevan 1974; 1995; 1998; 2001).

Grazing

Intensive grazing threatens pollinators through the removal of food resources, the destruction of underground nests and potential nest sites, and other subtle mechanisms (Kearns & al. 1998). Direct effects on the quality and quantity of pollen after loss of foliar surfaces caused by herbivory have also been reported (Aizen & Raffaele 1996). In recent years, grazing has directly damaged threatened plants in conservation programmes in the Mediterranean region. In most cases, this has occurred through the blocking of flower and pollen production, leading to a loss of plant sexual reproduction and subsequent seed set. However, grazing has also had indirect effects on pollinators by removing their food sources. In the recent Red Book of Spanish vascular plants, overgrazing was identified as the main source of threat for endangered plant species (c. 40 %, Bañares & al. 2003).

One of our findings on endangered species comes from the Pyrenean endemic larkspur *Delphinium montanum* (Simon & al. 2001). This subalpine larkspur presents the classical bee-syndrome of a specialized spur flower. In 1994, the flowers and stems of some populations in the Cadí Natural Park (Catalonia) were extensively grazed by chamois (*Rupicapra pyrenaica*). This predation of floral stems continued for 10 years with annual losses of 92-98 % of flowers. Similar findings were reported in the reserve of Noedes and Cambredase (French Catalonia) in summer 2004 (Simon pers. observ.). The long-term effects of this predation are unknown at present and pollination limitation (>90% decline) seems buffered by demographic characteristics, such as adult longevity or seedling recruitment (Aymerich 2003).

Additional reports from Spain confirm the increasing trend in floral predation by grazing, which is particularly significant in threatened species and within protected areas (Tab. 3).

Chemicals

Pesticides pose a major threat to pollinators, not only when applied to agricultural crops, but also in grasslands, forests, urban areas and tourist resorts (Kearns & al. 1998; Carvell & al. 2001). Although environmental regulations in industrialized countries have reduced pollinator poisoning, problems are still being reported in developing countries. Pesticides, directly applied or after pollinator foraging, can also affect honey and pollen, and several

Table 3. The effect of grazing by herbivores on flowering phases of some threatened species in Spain from recent literature: CAN - Canary Islands, ARA - Aragon, C-LM - Castilla-La Mancha, MUR - Murcia, BAL - Balearic Islands, CAT - Catalonia.

Taxon	Region	Predator	Affectation	Measures	Reference
Helianthemum juliae	CAN	Rabbits	Seedlings predation	Monitoring plan	Bañares & al. (1993)
Cistus osbaeckiaefolius	CAN	Rabbits and mouflons	Fruit predation	Monitoring plan	Bañares & al. (1993)
Echium acanthocarpum	CAN	Introduced animals (rabbits, mousses, goats)	New seedlings establishment	Fencing Recovery plan	Marrero & al. (2000)
Krascheninnikovia ceratoides	ARA	Sheep	Cycle interruption and consumption of juveniles	Prohibition of grazing in a population	Domínguez & al. (2001)
Stemmantha cynaroides	CAN	Introduced rabbits and mouflons	Flowers consumption	?	Fernández & Marrero (2000)
Erodium paularense	C-LM	Livestock	Flowers and fruits	Monitoring plan	Iriondo & al. (2001)
Anthirrhinum subbeticum	MUR	Grazing (non specified)	Flowers	Monitoring plan	Sánchez & al. (2002)
Narcissus nevadensis subsp. *enemeritoi*	MUR	?	Flowers and fruits predation	Monitoring plan	Sánchez & al. (2002)
Ligusticum huteri	BAL	Goats and sheeps	Flowers / Aging of populations	Fencing Monitoring plan	Vicens (2002)
Delphinium montanum	CAT	Chamois and moles	Flowers and stems (up to 95-100%). Short-time compensation by seed bank	Fencing Monitoring plan	Simon & al. (2001) Aymerich (2003)
Peucedanum schottii	CAT	Goats and sheeps	Flowers and fruits	Any	Molero & Rovira (ined.)
Salix tarraconensis	CAT	Hispanic goats	Branches, stems and flowers	Any	Baiges & Blanché (ined.)

chemicals (organic, heavy metals and radionucleotides) have been detected in these products. Herbicides also affect pollinators by reducing the availability of nectar plants and may have greater effects than pesticides. One example leading to massive pollinator decline is herbicide spraying in alfalfa crops (Kearns & al. 1998).

Biological agents

General declines in honeybee populations have been reported in several parts of the world, including the European Union. Infection by parasitic mites, introduction of non-native pollinators and competition with other native pollinators are among the causes cited (Kearns & al. 1998; Goulson & al. 2005, and references therein).

Regarding plants, pollination disturbances caused by the introduction of invasive species have also been reported (Morales & Aizen 2002). In the Mediterranean area, the Balearics (Spain) and Hyères Islands (France), the flowers of the introduced *Carpobrotus* *edulis* compete with those of native species such as *Lotus cytisoides*, *Anthyllis cytisoides* and *Cistus monspeliensis* for pollinator services (Travesset 2004). Pollinators may also act as vectors for carrying foreign pollen of related species, and are thus possible agents of extinction through hybridization / introgression caused by genome pollution of small populations.

Fragmentation

Finally, habitat fragmentation is a major threat to pollination systems: it is one of the main topics addressed in conservation biology today, both for species and species assemblages. Studies over the last decade have produced an increasingly complex model of the impact of fragmentation on plant populations.

Hobbs & Yates (2003) note that the direct effects of fragmentation, which are generally an inevitable consequence of habitat destruction, include: the creation of small patches, the alteration of landscape processes, the isolation of patches in an altered matrix, and the reduction of population sizes. These changes produce follow–on consequences for ecosystems and species, and these in turn can have a subsequent impact on plant populations, leading to decreased abundance and risk of extinction (at least locally) for particular species. Pollination takes place at the central and most vulnerable node of a complex network of biotic interactions. The controversial discussion on the causes of bumblebee declines by Goulson & al. (2005) and Williams (2005) highlights the difficulty in tracing habitat and pollinator interactions under the pressure of global change.

If, following fragmentation, the local pollinator declines within a fragment, the location of the fragment is outside the foraging range of pollinators, or wide-ranging pollinators avoid small plant populations or isolated fragments, then pollination services and reproductive potential can be expected to decrease. This may result in plant species decline (Kearns & al. 1998; Hobbs & Yates 2003).

Results from a survey on the endangered *Seseli farrenyi* (Apiaceae) (Rovira & al. 2004) show how some of the expected effects of fragmentation can be detected. *S. farrenyi* is a species endemic to a very narrow coastal strip of Cape Creus, in Catalonia. It is a good example of the effects of fragmentation on small populations because of its limited distribution and low number of individuals, and its progressive decline due to fragmentation. It is a highly unspecific entomophilous plant, visited by at least 28 insect species. In the smallest and most fragmented population, the stigmatic pollen loads of conspecific pollen and seed set are decreasing because of a rise in the percentage of visits by ants and coleopterans (which carry less pollen shorter distances). This failure to recruit adequate pollinator services as a result of habitat fragmentation has already been reported (Aizen & Feisinger 1994; Weller 1994). The resulting pollination disruptions in the small fragment lead to an increased risk of extinction through a complementary loss of genetic diversity of 18.7%, estimated by allozyme polymorphism (López-Pujol & al. 2002). This example of how pollination is involved in the web of disturbed interactions caused by habitat fragmentation is only one among the increasing number of cases reported by the literature during the last decade.

Pollination decline and plant species conservation: controversies

However, the concept of a “pollination crisis” and its consequences for plant species conservation is far more complex than one might initially assume. Although a number of studies report partial evidence of either absolute loss of pollinators or loss of quality or quantity of pollinator services, in both widespread and endemic or endangered plant species, some controversial studies have put forward new paradigms. These address compensating strategies for pollination loss, as well as other issues.

Linked extinction of plant and animal species

Firstly, relatively few plant-pollinator interactions are absolutely obligate if the complex web of possible interactions between plant species and flower-visiting animals worldwide is considered (Kearns & al. 1998; Kawakita & Kato 2004). Most are more generalized on the part of plants and animals, and they also vary through time and space, although this generalization shows some geographic patterns (Olesen & Jordano 2002). A Mediterranean example of temporal variation is *Seseli farrenyi* (Rovira & al. 2004), whose pollen deposition varies in the course of the flowering season (Fig. 1), while an example of spatial variation can be found in the specialized flowers of larkspurs (Bosch 1999), whose flowers are similar from southern France to northern Morocco, yet receive a great variety of pollinators depending on habitat (altitude, latitude) and the available fauna of pollinators (Tab. 4).

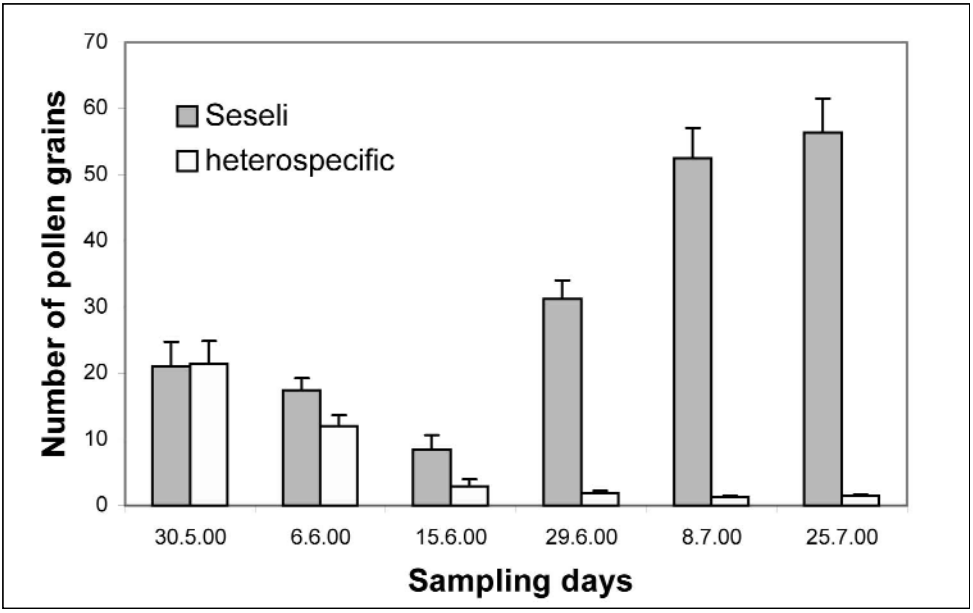

Fig. 1. Variation in pollinator activity across time in Mediterranean plants. Stigmatic pollen deposition during flowering period in *Seseli farrenyii* (from Rovira & al. 2004).

Table 4. The pollinator variation of larkspurs (*Delphinium* spp.) in the Western Mediterranean area across space from North to South (from Bosch 1999): R - robbers (<10% of visits), RR - robbers (>10% of visits); + - pollinators (<10% of visits), ++ - pollinators (>10% of visits); MON - *D. montanum* (1: Ga: W. Pyrenees; 2: Hs: Cadí range), BOL - *D. bolosii* (1: Hs: Lleida; 2: Hs: Tarragona), STA - *D. staphisagria* (1: Hs: Alacant; 3: Bl: Eivissa), PIC - *D. pictum* (2: Bl: Mallorca), VER - *D. verdunense* (1: Hs: Barcelona; 2: Hs: Girona), GRA - *D. gracile* (1: Hs: Osca), BAL - *D. balansae* (Ma: Meknès), OBC - *D. obcordatum* (1: Ma: Tétouan), FAV - *D. favargeri* (Ma: Marrakech).

Pollinators	*Delphinium*												
	MON1	MON2	BOL1	BOL2	STA1	STA3	PIC2	VER1	VER2	GRA1	BAL1	OBC1	FAV1
Hymenoptera													
Apidae													
Bombus hortorum	++	++											
Bombus wurfleini	RR												
Bombus terrestris			RR	R			++		++			++	
Bombus pasquorum			+	+			+	+	+				
Anthophoridae													
Amegilla sp.						++		++	+		++	++	++
Anthophora dispar			+										
Xylocopa violacea			++				+			R			
Megachilidae													
Oplitis sp.						++							
Megachile rotunda						++							
Halictidae													
Lassioglossum sp.			+	++	++			++	+	++	++		
Halictus sp.				+				+	++				
Eumenidae													
Alastor atropos			R	RR				+		RR			
Lepidoptera													
Macroglossum stellatarum	+		++	+	++	++	++	+	+		+	++	
Others	+		++	++				++	++	+		+	+
Diptera													
Bombylius sp.			+	+					+	+		+	
Syrphidae	++	++	+	+	++	+	++	+	+				++

Altitude (in m)	1980	2350	290	600	150	160	100	196	50	300	1930	5	1300
Flowering (months)	Jl-A	Jl-A	J	J	My-J	My-J	J	Jl-A	Jl-A	Jl-A	J-Jl	J-Jl	J-Jl

Thus, the recognition that most pollination interactions are not obligate necessarily changes our approach to their conservation. We should abandon the notion that losing one plant species implies the loss of one or more animal species via linked extinction and vice versa (except for few relevant but scarce examples and bearing in mind the recent view of possible asymmetric specialization of plant-pollinator interactions by Vázquez & Aizen 2004). If pollination "interaction webs" are relatively richly connected and shift in time and space, depending in part on the landscape context, then the work of conservation biologists is made still more complex.

Plant species extinction caused by loss of pollination

Surprisingly, for a substantial proportion of the most endangered species, significant pollination losses are not reported as the basis of the threats to their survival. Evidence of this comes, for instance, from the data given by the Red Lists from the western Mediterranean, where the nature of these threats is detailed.

Of the species listed as extinct in France (Olivier & al. 1995), Catalonia (Sáez & al. 1998; Sáez & Soriano 2000), the Balearic Islands (Sáez & Rosselló 2001) or Spain (Bañares & al. 2003), habitat degradation/destruction is responsible for most cases, followed by changes in land use and unknown causes. However, pollination or reproductive failure is not mentioned. More interestingly, the review of threats affecting the more than 2,223 populations surveyed, which belong to the 478 most endangered species in Spain (Bañares & al. 2003), reveals that the main cause of threat is overgrazing (c. 40%). Reproductive strategies are responsible for less than 10% of documented threats to endangered populations.

There is little literature on plant species that have become extinct through reproductive limitations, and specifically pollination limitation, although some cases have been described involving the absolute loss of pollinators. These include *Ixianthes*, a South African shrub belonging to Scrophulariaceae (Steiner 1993) or *Freycinetia baueriana*, a liana from New Zealand which lost its bat pollinator (Lord 1991).

The scarcity of documented extinctions through pollinator losses can be put down to a) a need for more extensive research on plant-pollinator interactions or, b) the recognition that there are no direct and immediate effects on plant reproduction, but that more subtle and slow-paced processes are taking place, whose long-term effects may be more serious than expected.

Fragmentation research does not allow generalization

Some authors (Cane 2001; Hobbs & Yates 2003) have recently questioned the view that habitat fragmentation necessarily results in a widespread collapse of plant-pollinator interactions, except in the most extreme cases. Pollinating insects may show differing responses to the same fragmentation/disturbance regime (Williams 2005).

Cane (2001), for example, proposes that the effect of habitat fragmentation on honeybees depends on the spatial distribution of resources in the new landscape and the decline of the permeability of the matrix (Fig. 2). In the first scenario (Fig. 2A), following fragmentation, the distribution of resources and nest sites forms a disrupted web broken by disturbances or discontinuities, and only a few populations are pollinated (a single population in the example). However, if the patchiness of the resources in the new landscape is within the foraging range of the bee and the matrix is permeable, fragmentation may have little impact on the pollinator (Fig. 2B). A good example can be found in the Panama Canal, where plant species import pollinators from outside each fragment (island pollinator nesting sites are disappearing but pollinators from mainland forest continue to pollinate effectively) (Murren 2002).

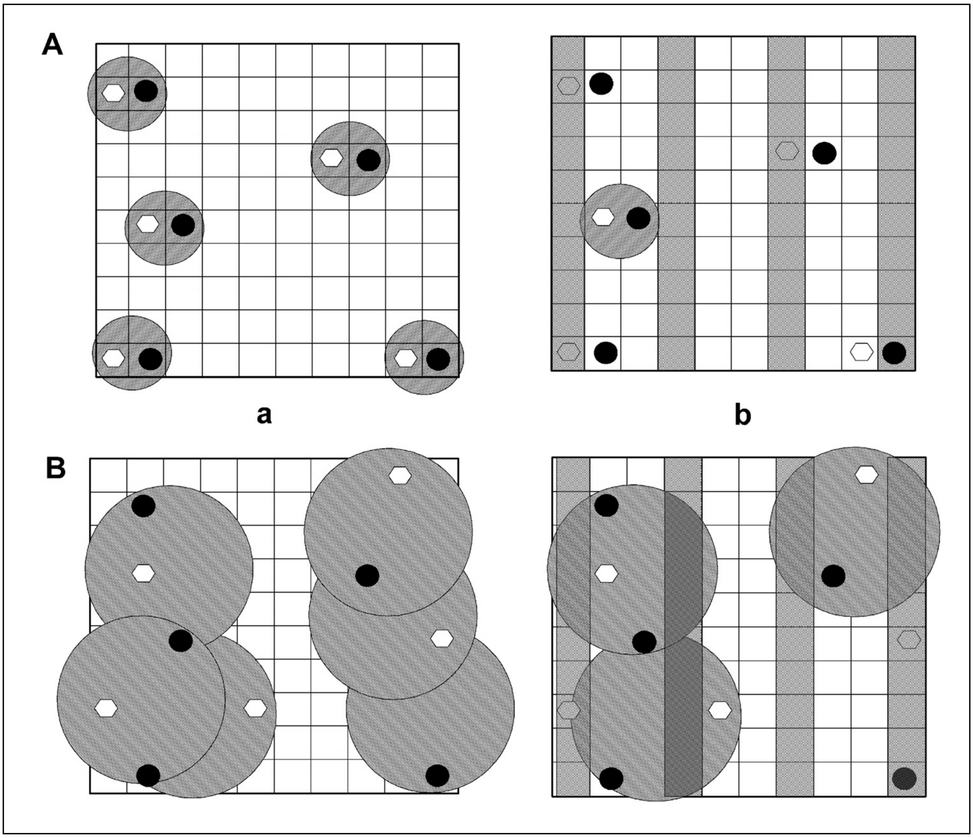

Fig. 2. Will habitat fragmentation result in a widespread collapse of plant-pollinator interactions? In a permeable matrix, the same fragmentation pattern (grey bars) produces distinct effects on 5 plant populations (black dots), depending on an interacting set of attributes (time and space distribution of resources on the fragments, pollinator search behaviour and diet breadth). Hexagons represent nesting sites and circles foraging range of pollinators. (a, before fragmentation; b, after fragmentation). A) Narrow foraging range (1 single population remain served). B) Wide foraging range (4 populations remain served).

Moreover, depending on the life history of pollinating insects, their responses to similar fragmentation patterns may also differ, with some species declining and others increasing in abundance (Davies & al. 2000; Donaldson & al. 2002) according to an interacting set of attributes. Of special interest in the Mediterranean is the effect of fire on pollination systems, recently reported by Ne'eman & al. (2000) and Potts & al. (2003) but with particular adaptive features in fire-dependent ecosystems (Hiers & al. 2000).

Empirical studies on the impact of fragmentation on insect pollinator communities have reported a wide range of results, from extremely sensitive to highly resistant / resilient responses. All these studies show that it is difficult to generalize about the effects of fragmentation on pollinator abundance.

Research on pollination and seed set declines

When no clear evidence of a direct relationship between pollination failure and plant losses is obtained from the above conservation sources, then the underlying processes of pollination disruptions may not be detected by the extensive (but not always in-depth) Red List surveys. The general hypothesis, taken from Aizen & Feisinger (1994), indicates that "As fragments and populations become smaller and more isolated, rates of visitation by pollinators and plant fecundity will decline". Several mechanisms have been postulated to explain this hypothesis, such as:

Pollinator diversity and abundance decline within fragments as they become smaller and more isolated, which results in fewer visits and lower seed set (Bosch & al. 2003).

Because pollination is a density-dependent process (Kunin 1997), small populations created by fragmentation may be less attractive and receive fewer visits, which results in a smaller seed set (Jennersten 1988).

As populations become smaller so too do genetic neighborhoods, which results in fewer mates for self-incompatible plants and increased inbreeding, both leading to a reduced seed set (Young & al. 2000).

The above hypotheses have been tested in several ways: a) by measuring the number of insect-flower visits and fecundity across fragments; b) by measuring pollen loads on stigmas, pollen tube abundance in flower styles and fecundity across fragments, and c) by comparing fruit or seed set in hand-pollinated and open-pollinated plants across fragments.

In some cases of fragmented populations of endemic species in the W. Mediterranean, our results show that all three methods are applicable and, to some degree, conclusive that a loss of pollination quality or quantity occurs, thereby decreasing genetic diversity in small fragments (Bosch & al. 2003). In a wider context, however, it is interesting to point out that from the results of all three approaches, the literature (reviewed by Hobbs & Yates 2003) reports mixed results: true and significant decline, balanced or non-significant results, and even an increase in the resulting fecundity.

Finally, some apparently contradictory results are open to discussion from the methodological point of view of the assessment of pollination declines.

How to fully document pollination declines

Some authors who examine pollinator declines (Thompson 2001, Wilcock & Neiland 2002, and the current authors), frequently discuss pollination deficits either as evidence that a decrease has occurred or as a possible negative consequence of future reductions. Because these deficits can be measured in short-term studies, these studies would be a better alternative to the documentation of insect population trends. In fact, shortages of pollinators and shortfalls of seed or fruit production are two aspects of the same problem, but for botanists, the plant perspective is to be preferred. Pollination deficits are detected mainly through pollen supplementation experiments, although pollinator supplementation can be preferable in the case of some crop plants.

Some reviews of hand-pollination experiments are available. In 62% of the natural populations studied, fruit or seed sets are limited by insufficient pollen at some times (Burd 1994). Similar percentages (59%) have also been found in crop plants (Mayfield 1998).

However, other research on threatened plants (Tepedino & al. 1999) shows much lower rates of pollination deficits (10-15%), which indicates that intact natural systems reach an evolutionary equilibrium in which reproduction is limited equally by pollination and by maternal resources. In other cases, apparent pollination limitation (only 5-10 % of fruit set) is not recovered after hand-pollination because of genetic loads or other primary causes. Furthermore, the dramatically lowered (1,000 fold) seed set after pollen supplementation in the rare *Oxyanthus pyriformis* in South Africa is directly related to the loss of the pollination services provided by the hawkmoth (Johnson & al. 2004). Indirect approaches using other evidence, such as the availability of nectar rewards (Jacquemyn & al. 2005), are also non-conclusive. Consequently, the contribution of pollination in determining plant population viability remains to be elucidated.

From our experience and from the cited reviews, several questions regarding the assessment of pollination deficits are still open to further research:

a) What is the optimum level of “natural” pollination? (other than hand-pollination. To fully demonstrate a decline we must first define the “standard” pollination level).

b) Are the populations selected for the study the most suitable? (in practice, we usually look for easily accessible and rewarding populations: results of very low visitation rates are frustrating to some extent and sometimes difficult to publish).

c) What is the smallest pollination deficit that can be detected with a given experimental design?

d) Is more pollen always better? (certain reported deficits could be reinterpreted as simply maintaining an equilibrium between a theoretical maximum of seed set and the available resources).

e) In the pollinator supplementation experimental approach, some methodological considerations - such as the non-linear effects of pollinator visitation rates – should be taken into account.

f) Pollination deficits should be placed in the context of plant life histories, so as to recognize that pollination failure can be buffered by alternative processes such as autogamy, agamospermy, wind pollination and vegetative propagation. This buffer effect is accepted in the case of long-lived perennials (Silvertown & al. 1993, 1996; Bosch & al. 2002). But conversely, there is also evidence that in annual and short-lived perennials, a reduction in seed set caused by pollinator deficits may lead to decreased population size and increased probability of extinction (Groom 1998; Lennartsson 2002).

In short, although theory and a number of studies propose that pollination disturbances or failure lead to lower profiles of sexual reproductive success, several methodological and theoretical issues call for further research. The enormous diversity of plant-animal interactions in our changing world will not make this an easy task.

Pollination systems conservation

Efforts to restore pollination systems are still at a preliminary stage, at least in Mediterranean countries. However, an increasing number of organizations are beginning to promote additional research and practical pollination restoration.

But let us conclude with Kremen & Ricketts (2000) that “pollination systems may never be restored to pristine, pre-human states” because global change will continue to: a) increase the length of the growing season, b) increase the northward movement of plant species at different rates to their pollinator webs, c) decrease and fragment both plant and pollinator populations, and d) introduce invasive weeds, exotic pollinators and non-native crops into natural ecosystems.

As Roubik (2001) suggests, the key question is whether these new systems can absorb new species and novel interactions. The challenge for conservation biology (and pollination conservation) is to understand both native and disrupted pollination systems in order to manage for pollination function over dysfunction. While we attempt to change the key factors of global change (overpopulation, over-consumption, changes in land use, disturbance regimes, climate), measures should be taken to slow the deterioration in biodiversity (Ehrlich 2003). These include:

- Scientific contributions: providing scientific information for conservation purposes and developing areas for further research, such as: the ecology of animal pollinators other than commercially important insects; the links between pollination and plant population dynamics; or the link between pollination disruption mechanisms and pollination under disrupted pollination web systems, including compensation processes for pollination failure in order to design potential management solutions.
- Pragmatic contributions: capacity to act in priority and urgent cases of threat but also to promote applied research for conservation purposes. This implies that recovery plans should include associated research on the management of threatened species among their goals, in addition to the strict recovery of demographic standards (i.e. number of individuals). Even if the biological aspects of a recovery plan fail, it still gives us the opportunity to obtain relevant biological information on endangered species if it is appropriately designed as an experiment. Basic ecological research on plant-pollinator interactions can be applied successfully to landscape management practices (Potts & al. 2001).

Some conservation activities are fine scale, as befits the requirements of locally endangered species or populations, whereas others address large-scale problems concerning widespread habitats or entire regions.

Fine scale

Some examples are given below to show conservation efforts focused on pollination at fine scale (specific plant/pollinator systems, certain agrosystems, population- or locality levels), some of them belonging to extremely endangered mutualisms, where a particular species, group of populations or single population has almost lost the ability to pollinate.

Maintenance of populations and species under complete pollination failure

Absence of pollinators. There are few examples of absolute loss of pollinators. However, in this case, at least in the short term, hand-pollination of plants may prove fruitful for conservation and a number of recovery plans have employed artificial pollination. Alternatively, exotic pollinators can be introduced, although this practice implies certain risks. Some examples have been reported in New Zealand (introduction of bumblebees to pollinate red clover) and Malaysia (introduction of weevils to pollinate oil palms) (Kearns & al. 1998).

Pre-flowering disruption. This is an extreme situation in which pollination does not take place because there are no available flowers. Some conservation activities directly address vegetative propagation, although in some cases the assisted complete rebuilding of the biological cycle requires supplemented pollinations (Shiau & al. 2002). These interventions must be limited to extreme cases of economic or symbolic importance, mainly because of the limited resources available for conservation. However, there is intense debate on the ethics of hand-pollination, as can be followed in Internet forums on wild orchids, for example. Other examples of pre-flowering disruption causes of reproductive failure are the result of genetics or difficulties in gamete production not directly related to pollination (Wilcock & Neiland 2002).

Coupled management of plant habitats and pollinators

In recent years, conservation activities have been more focused on habitat, ecosystem and regional efforts than on single-species targets (although some caution is needed in the case of multispecific recovery plans, as recently suggested by the Society for Conservation Biology [Clark & Harvey 2002], since multipack plans often pay little attention to the conservation needs of individual species). Pollination should benefit from this change of perception, which considers endangered plant populations as part of a web of interactions.

From this point of view, a good alternative is to promote, at local level: a) the maintenance of pollinators, b) habitat management for appropriate nest sites for bumblebees and for floral diversity to provide nectar and pollen (other plant species supporting services to small populations) and c) the conservation of marginal areas (in many parts of the world this may imply conservation of man-made habitats – as in the Mediterranean –, some of which are good substitutes for threatened or destroyed natural habitats).

Reintroduction of plants and pollinators

A particular case of the integrated management of the habitat of endangered plant species is the coupled reintroduction of plants and pollinators. If reintroduction of endangered plants is still relatively uncommon, few plant reintroductions to date have been stimulated by the need to support pollinators (although existing pollinators may have benefited, Kearns & al. 1998). A case of an insect-oriented action plan can be found in the small Columbrets Islands (Valencia), where the few remaining *Chrithmum maritimum* patches - on the verge of extinction - were reinforced, as they constitute the basic habitat of the endemic and endangered coleopteran *Morbidistella columbretensis* (Laguna 1998).

In plant reintroduction, the absence of native pollinators may be a serious limitation, particularly when the plant has a single pollinator species (but this obligate mutualism is infrequent, see above).

Changing agricultural practices

Some changes in agricultural practices can address both local and widespread problems. These include (briefly): a) Restrictions on the use of pesticides, herbicides and fertilizers, (though this does not automatically lead to the recovery of pollinator abundance if preceded by years of intensive land management; Fussell & Corbet 1992); b) Removal of alien pollinators and c) Domestication of wild bees and other pollinators.

Pywell & al. (2005) conclude that the promotion of marginal areas to develop natural revegetation provides habitat and resources that allow the recovery of pollinator populations. The persistence of a reticulate corridor system (as opposed to extensive open fields) of hedgerows, ditches, green lanes or tracks in semi-natural or rural areas is directly correlated with higher diversity and density of butterflies and bees (Croxton & al. 2005).

LARGE SCALE

Below we summarize four ambitious programs, including scientific- and pragmatic-oriented projects that represent large-scale approaches to the problems related to Pollination Conservation. International concern about the conservation of pollinators and pollination systems was expressed at the Third Conference of the Parties (COP 3) of the Convention on Biological Diversity, held in 1996, and in the subsequent São Paulo Declaration on Pollinators (http://www.biodiv.org/agro/pdf/pollinator/Pollinator-Report.pdf).

Migratory Pollinators Project

This project, promoted by the Arizona-Sonora Desert Museum, focuses on 4 species of pollinators (bats, hummingbirds and butterflies) that follow annual "nectar corridors" between Mexico and the US. In the first phase, the aim is to identify a model for spatial and temporal patterns of flowering phenology and pollinator migrations. Gaps in these corridors will indicate where "pollinator gardens as nectar stopovers" should be developed, and thus encourage farmers to plant nectar sources in out-of-use areas (Withgott 1999).

GPM - Global Phenological Monitoring

This program is an initiative of the International Society of Biometeorology. It aims to link phenological networks around the world in order to assess climatic change effects using a variety of tools, including specially designed GPM-gardens with selected species. The core website is at http://www.dow.wau.nl/msa/gpm/.

Introduction of pollination parameters in restoration practices

The INESP (International Network of Expertise for Sustainable Pollination) and the NAPPC (the North American Pollinator Protection Campaign) in Costa Rica have proposed that standard recovery plans should include a study of whether the restoration program has restored the pollinator community at the field site. By constructing webs describing the plant-pollinator interactions at pristine sites, a picture of a healthy pollinator system will be obtained. This can be used as a reference when studying plots in restored systems. Without the restoration of the pollination system, the restoration program is not sustainable. More information is available at http://www.nappc.org.

ALARM

ALARM (*Assessing LArge-scale environmental Risks with tested Methods*, EC Framework 6 Integrated Project 2004-2009) is a large European project which assesses changes in continental biodiversity. It includes a specific pollination module whose main objectives are to: a) quantify distribution shifts in key pollinator groups (to provide continental-scale evidence for pollinator declines); b) measure the biodiversity and economic risks associated with loss of pollination services; c) determine the relative importance of the triggers of pollination loss (land use, climate change, environmental chemicals, invasive species and socio-economic factors) and d) develop predictive models for pollinator loss and consequent risks (Potts & Roberts 2004). It is expected that ALARM will increase its scientific and technical knowledge before application of the announced European Pollinator Initiative, which is open to interested researchers (www.European PollinatorInitiative.org). The similar projects, currently being developed in Africa, are available at: www.scienceinafrica.co.za/pollinator.htm.

This non-exhaustive review highlights the extent of the endangered status of pollination mutualisms. On the basis of the data available, we conclude that further research to assess pollination declines and their related mechanisms is required and that conservation programs should be implemented at local and large scales.

Acknowledgements

This study was subsidized by grants REN2000-0829GLO and REN2003-01815GLO (MCyT, Ministerio de Ciencia y Tecnología, Spain), and Programme Ramón y Cajal to M.B. We acknowledge the comments and suggestions of two anonymous reviewers.

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I. Marques, A. Rosselló-Graell, D. Draper & J. M. Iriondo

Pollination ecology and hybridization between *Narcissus cavanillesii* A. Barra & G. Lopéz and *N. serotinus* L. in Portugal

Abstract

Marques, I. Rosselló-Graell, A., Draper, D. & Iriondo, M. J.: Pollination ecology and hybridization between *Narcissus cavanillesii* A. Barra & G. Lopéz and *N. serotinus* L. in Portugal. — Bocconea 21: 65-75. 2007. — ISSN 1120-4060.

Pollinator activity can have a major effect on the reproductive success of plant species, not only assuring their survival but also influencing gene flow and hybridization between species. *N. cavanillesii* is considered a threatened species in Portugal with only two reported populations, forming highly-fragmented, isolated patches. This autumnal flowering species develops small yellow flowers with a short perianth tube and an inconspicuous corona, allowing total exposure of the sexual structures. Although *N. cavanillesii* is self-compatible and self-pollinated, cross-pollination processes are favoured and necessary to promote higher fruit set (50%). *N. serotinus* is distributed along the Mediterranean coast and in Portugal is considered a common species. Its fragrant flowers are white and have a very short yellow corona and a long, narrow perianth tube that hides the sexual organs. Self-pollination processes are favoured, although this species can also set fruits by cross-pollination. Floral visitors are necessary to promote self-crossings. *N. serotinus* has an average fruit set of about 70%. In Portugal, these two species are sympatric and their flowering periods overlap. Artificial crossings revealed weak reproductive barriers that do not prevent interspecific crosses especially when *N. cavanillesii* is the receptor species. However, in natural conditions, hybridization events are reduced by the activity of different pollinators. *N. cavanillesii* flowers are mainly visited by the hymnoptera, *Halictus* sp., while *N. serotinus* is mostly visited by the coleoptera, *Meligethes* sp. Only the hymenoptera, *Megachile* sp. is common to both species, but less than 3% of the visits are shared between these two species. The present study shows that these two co-generic species, which are well-adapted to the same environment, have developed divergent reproductive strategies, minimizing pollinator competition and assuring survival outside the main Mediterranean flowering season.

Introduction

The genus *Narcissus* L. (*Amaryllidaceae*) is one of the most recognized insect-pollinated genera. It is geographically concentrated in the Mediterranean region, particularly in the Iberian Peninsula where it reaches its highest diversity (Barrett & al. 1996). Species taxonomy and circumscriptions vary widely and thus, there is little agreement on the number of species within the genus. Estimates range from 16-150 species (reviewed in Blanchard 1990), but most authors accept 35-70 species, divided between 2 subgenera and 10 sections

(Fernandes 1968; Barrett & al. 1996). A recent work using modern phylogenetic approaches has helped resolve systematic relationships in *Narcissus* (Graham & Barrett 2004).

Narcissus is one of the most important genus used in horticulture with more than 20000 registered names representing over a century of breeding efforts (Barrett & al. 1996). Yet, the floral biology and breeding systems of most *Narcissus* species are unknown. There is also little information concerning pollination ecology, although most species have showy flowers, some of which are highly scented (Dobson & al. 1997). Pollinators are mainly referred to as bees in *N. longispathus* (Herrera 1995), *N. triandus* and *N. pallidulus* (Barrett & al. 1997); hawkmoths in *N. assoanus* (Baker & al. 2000), *N. dubius* (Worley & al. 2000), *N. papyraceus* (Arroyo & al. 2002), *N. rupicola* (Pérez & al. 2003) and *N. tazetta* (Arroyo & Dafni 1995); and diurnal moths and pierid butterflies in *N. watieri* (Pérez & al. 2003). However, different populations of the same species can have different floral visitors, as in *N. tazetta* whose lowland marsh populations are mainly pollinated by a hawkmoth and whose hill populations are visited by a syrphid and some solitary bees (Arroyo & Dafni 1995).

Most species have white (e.g. *N. papyraceus*), pale yellow (e.g. *N. triandus*) or deep yellow flowers (e.g. *N. fernandesii*), except the deep green *N. viridiflorus*, which is thought to be pollinated by crepuscular moths (Vogel & Müller Doblies 1975). Most species flower in late winter or spring but five species flower in autumn: *N. broussonetti*, *N. cavanillesii*, *N. elegans*, *N. serotinus* and *N. viridiflorus*.

In Portugal there are seventeen recognized *Narcissus* species occurring in diverse areas which range from open sites, Mediterranean scrub communities and low elevation marshes or river banks to rocky hillsides and high mountains. Only two flower in autumn: *N. cavanillesii* and *N. serotinus*. Information on the insect pollinators of these species is scarce (for *N. serotinus* see Pérez Chiscano 1985). The present study aims to clarify the relationship between these two species with regard to flower attraction, breeding system and pollinator behavior.

Species

Narcissus cavanillesii A. Barra & G. López is a small geophyte of 4-15 cm height with one or two leaves that are not present in flowering bulbs. It normally produces one single flower and the corolla comprises six bright yellow perianth segments. The flower does not present the typical morphology of a *Narcissus* species, which normally has a long floral tube and a highly exuberant corona. *N. cavanillesii* has a very short floral tube (less than 2 mm) and an inconspicuous corona which are normally very difficult to see. This may explain why this species has only recently been accepted within the genus *Narcissus*. This morphology allows an open corolla and total exposure of sexual organs. The fruit is a small ellipsoidal capsule that releases the seeds by three longitudinal splits, when the pericarp is dry.

The species is restricted to the SE Iberian Peninsula, Algeria and Morocco and is listed under Annexes II and IV of the Habitats Directive of the European Union (CEE 92/43). In Portugal there are only two known localities (Ajuda and Montes Juntos) both reported in the Alentejo region (Malato-Beliz 1977; Rosselló-Graell & al. 2003). This species is considered Critically Endangered in Portugal according to IUCN categories due to its low area of occupancy, population size and fragmentation (Rosselló-Graell & al. 2003a). The recent construction of the Alqueva dam has also affected these two populations (Rosselló-Graell & al. 2002).

Narcissus serotinus L. presents white solitary flowers (8-25 cm height) which are highly scented even at night. Like *N. cavanillesii*, leaves are not present in flowering individuals. Flowers are erect and comprise a long, narrow floral tube that hides the internal structures, a yellow corona under 2 mm and six perianth segments. Like its congener, it has an actinomorphic structure with six stamens, located in two whorls. The upper stamens are higher or at the same height as the style but rarely exceed the floral tube. The lower stamens are shorter than the style and the nectaries are located above them. Contrary to *N. cavanillesii*, the floral tube of *N. serotinus* hides the sexual structures.

N. serotinus is considered a widespread species throughout the Mediterranean basin, distributed from Portugal to Turkey. In Portugal, this species appears mainly in the Alentejo region and is not considered a threatened species, although the construction of the Alqueva dam has led to a population decrease of 20% in this area.

Data for the present study were collected from 2001-2003 in the Ajuda population where the two species co-exist. In the studied area, *N. serotinus* is more abundant than *N. cavanillesii* (60:20 reproductive individuals, respectively) but has a lower density since *N. cavanillesii* forms denser patches due to higher vegetative reproduction.

Flowering phenology

Both species presented a very short flowering period. In 2001 *N. cavanillesii* started to flower at the end of September reaching its peak from 8 to 12 October and ending on 25 October. Flowering had a mean duration of 32 days. Floral synchrony within species (Albert & al. 2001) had a very low value of about 38%. The reproductive individuals of *N. serotinus* appeared after the flowering peak of *N. cavanillesii*. They flowered for 16 days from 12 October to 1 November. As in *N. cavanillesii*, floral synchrony within species was very low at about 48%, limiting gene flow between plants. The flowering period of the two species overlapped during 15 days allowing gene flow between them.

Floral biology

Flowering span was similar for both species (5.1 *vs* 5.5 days in *N. cavanillesii* and *N. serotinus*, respectively). In *N. cavanillesii* flowers the stigma became receptive one day before pollen was available (protogynia), thereby favoring cross-pollination. In *N. serotinus*, pollen and stigma were functional at the same time facilitating self-pollination. These results are in concordance with the flower morphology of each species. However, contrary to what was expected, the P:O value was lower in *N. cavanillesii* than in *N. serotinus* (mean ± SE respectively 211.80 ± 46.80 *vs* 417.55 ± 115.62).

Breeding system

Both species produce fruits and seeds from self- and cross-pollinations indicating the absence of self-incompatibility systems (Tab. 1). Furthermore, preliminary observations did not find wind to be a pollinator vector, as none of the emasculated seeds set fruit when insect visitation was prevented.

In *N. cavanillesii*, plants covered to prevent insect visits produced capsules with fewer seeds compared to fruits produced by cross-pollination (U=1020, *P*<0.001 and U=1350, *P*<0.01 respectively for insect exclusion and xenogamy; Tab. 1). No significant differences were found between control and xenogamy treatments (U= 1560, *P*=0.142; Tab. 1).

In *N. serotinus*, only 25% of bagged, unmanipulated flowers produced fruit (Tab. 1). However, autogamy increased fruit set to 42% (Tab. 1), which is not significantly different from that of cross-pollinations (U=1500, P=0.067). Thus, although *N. serotinus* is self-compatible, insect activity is important to self-pollination processes. No significant differences were found between control and cross-pollinated plants (U= 1740, P=0.700). Although there is considerable variation in seed production, *N. serotinus* usually produced more seeds per plant than *N. cavanillesii* (16 *vs* 6 respectively).

Floral advertisement

To examine the role of ultraviolet patterns (UV) in flower advertisement, a total of 50 plants from each species were illuminated at night with a UV lamp (365 nm wavelength) and the resulting images were captured using a video camera with a 24-bit definition. The UV photographs show strong pigmentation in *N. serotinus* flowers with all flower segments reflecting UV radiation except in the corona and around the flower tube (Fig. 1). Another important stimulus that attracts insects from a long distance is flower scent (Dobson 1994). Neutral-red staining of flowers of both species showed the location of osmophors in the corona, as observed by Vogel (1962) for *N. jonquilla*. However, in *N. cavanillesii* these structures are not relevant since the corona is inconspicuous.

Table 1. Mean fruit set and standard deviations for flowers of *Narcissus cavanillesii* and *N. serotinus* after different pollination treatments (n=60).

Pollen transfer	*N. cavanillesii*	*N. serotinus*
Control	0.50 ± 0.50	0.68 ± 0.47
Insect exclusion	0.38 ± 0.49	0.25 ± 0.43
Autogamy	0.20 ± 0.40	0.48 ± 0.50
Xenogamy	0.63 ± 0.48	0.65 ± 0.48

Fig. 1. Flowers of *Narcissus cavanillesii* (left) and *N. serotinus* (right) under human visible radiation (above) and ultraviolet radiation, 365 nm (below).

Hybridization experiments

Interspecific pollination experiments conducted between *N. cavanillesii* and *N. serotinus* resulted in fruit set, suggesting a degree of genetic compatibility between these two species. In *N. serotinus* only 12% of the flowers developed fruit when they received pollen from *N. cavanillesii as* compared to 45% when they received their own pollen (U=1200, *P*<0.001). In contrast, almost 45% of *N. cavanillesii* flowers developed fruits, either from the pollen of their own species or from their congener (U=1685, *P*=0.519). Thus, the hybridization process seems to be asymmetric causing a greater impact on endangered *N. cavanillesii*.

Pollinator activity and behavior

N. cavanillesii and *N. serotinus* were visited by a small number of insects. In 2002, most of the visits in *N. cavanillesii* were performed by two Hymenoptera, *Halictus* sp. (83% of recorded pollination visits), followed by *Megachile sp* (10.6%). The remaining visits were performed by two species of Diptera (Syrphydae and Calliphoridae) and one Hymenoptera (Apidae) (Tab. 2). These results are similar to a preliminary list of insect visitors reported in 2000, although the frequency of visits was higher (Rosselló-Graell & al. 2003a). The disturbances in this region, namely the construction of the Alqueva dam and the subsequent work of machinery, may have influenced insect activities.

In *N. serotinus* seven different insect species were attracted to their flowers. The main visitor was the Coleoptera, *Meligethes* sp. (52%), followed by the Syrphidae, *Eristalis pratorum* (21.95%) and the Hymenoptera, *Megachile* sp. (13.75%). The remaining species were mainly butterflies that can be regarded as sporadic pollinators (Table 2). However, in other nearby populations (Badajoz), butterflies like *Macroglossum stellatarum* were once recorded as the main visitors of this plant (Pérez Chiscano 1985).

Floral visitor activity started at 10 a.m., reached a peak between 12 a.m. and 2 p.m., and ended at 4-5 p.m. Visitors were normally only observed on sunny days with weak winds., No nocturnal insect activity was observed in either species. The three flower structures that could attract and be touched by pollinators were the nectaries, the anthers and the stigma.

Table 2. Pollinators observed in *Narcissus cavanillesii* and *N. serotinus*: values in bold the visitor common to both species.

Insects	*N. cavanillesii*		*N. serotinus*	
	% total visits	Time/flower	% total visits	Time/flower
Hymenoptera				
Halictus sp.(Halictidae)	83.3	1-3s	-	-
Lassioglossum sp.(Halictidae)	-	-	7.6	1-8s
Anthopora sp.(Anthophoridae)	-	-	4.8	3-4s
Mechachile sp.(Megachilidae)	**10.6**	**3-4s**	**13.7**	**2-4s**
Ceratina cucurbina (Apidae)	2.0	1-3s		
Diptera				
Episyrphus balteatus (Syrphidae)	2.1	2-4s	-	-
Stomorhina lunata (Caliphoridae)	2.0	2-4s	-	-
Eristalis pratorum (Caliphoridae)	-	-	20.8	1-4s
Coleoptera				
Meligethes sp. (Nitidulidae)	-	-	48.8	52-124s
Lepidoptera				
Pieris rapae (Pieridae)	-	-	2.2	1-3s
Aricia cramera (Lycaenidae)	-	-	2.1	1-4s

The majority of the reported insects sucked the nectar, and the main visitors can be considered effective pollinators. However, the main pollinator in each species seems to follow a different strategy. In *N. cavanillesii*, *Halictus* sp. usually landed on the flower and started to search for nectar. As the nectaries are located at the bottom, it was forced to crawl towards the flower base, rubbing itself around the stigma and the stamens. In this way, the pollen grains adhered to the insect´s head and the dorsal side of its thorax. When it landed on the next flower, the pollen grains were deposited on the stigma..This behaviour favours cross-pollination processes. In contrast, in *N. serotinus* the main visitor is a Coleoptera species, *Meligethes* sp., which behaves as a pollen thief. When gathering pollen, the insect moved between the upper and lower stamens several times. The pollen grains adhered to the thorax and were deposited on the stigma of the same plant by the upward-downward movements of the insect. However, not all the pollen was deposited and thus, when the insect visited another plant, the pollen on its body was placed on the stigma. Cross-pollinations and mainly self-pollinations are favoured by this behaviour. The activity of this pollen thief enables *N. serotinus* to rely on more "specific" and efficient pollination that may contribute to the high fruit development seen in natural conditions.

This study not only detected different insect visitors but also showed different pollination strategies for these two species. However, one insect was common for both species: *Megachile* sp. (Tab. 2). Only 2.49% of the visits were shared between the two *Narcissus* species. The remaining visits were usually constant to one of the two species, limiting natural hybridization processes between them. In fact, the few hybrid individuals in natural conditions are usually found in small clustered, isolated groups. Other nearby geophytes such as *Leucojum autumnale* or *Scilla autumnalis* also seemed to compete for these pollinators but to a lesser degree.

Discussion and Conclusions

Ecological and evolutionary implications

Geophytes are said to have an ecological advantage. The presence of a storage organ allows them to have distinct phases of growth and reproduction (Dafni & al. 1981; Burtt 1970) as well as the possibility of flowering outside the growth season (Shmida & Dafni 1989). Autumnal flowering is a peculiar feature since the autumnal and winter pollination market is scarce and also because these are harsh seasons from an environmental point of view. However, flowering outside the main season (spring) can be an advantage due to lower pollinator competition and improper pollen flow (Shmida & Dafni 1989). Can two sympatric species share this reduced market and still maintain their genetic integrity? The two species studied here occur together in the same region and in similar habitats, their flowering periods overlap and we have direct evidence that artificial cross-pollination between the two species produces viable seeds. The most effective mechanism to maintain their genetic integrity appears to be the activity of different pollinators.

Flower development, morphology and the exposure of rewards (pollen and nectar) all indicate that *N. cavanillesii* is a facultative cross-pollinated species. This is in agreement with our bagging experiment that showed partial dependence on insect pollination for fruit set. *N. cavanillesii* is mainly cross-pollinated by a hymenoptera attracted by the nectar in the bottom of the flower. In *N. serotinus*, a long and narrow tube hides the rewards. This indicates more specific flower visitors like butterflies with long proboscis or small beetles as recorded. On the other hand, more diverse insect species visited *N. serotinus* flowers possibly due to their higher advertisement capability. The flower arrangement in a symmetric, erect pattern provides a large platform where insects can land and a contrasting corona assists in locating the sexual organs. These advertisements are also supported by the presence of pigmentation patterns detected under UV filters in *N. serotinus*.

In any event, the ability to produce fruits and seeds by autonomous self-pollination may reduce the dependence of *Narcissus* on insect pollen vectors. Selfing presumably evolved because of the advantages of reproductive assurance but they are counteracted by the risk of inbreeding depression (Charlesworth & al. 1990; Lloyd 1992). Because autonomous self-pollination is facultative, the option of outcrossing, which improves seed "quality" is still preserved in both species. However, the lower fruit production in *N. cavanillesii* by spontaneous self-pollination means that protogynous mechanisms are partially effective in preventing fertilization. The role of pollinators seems particularly important for the subsistence of this species.

How is species integrity maintained?

The artificial cross-pollination experiment showed that hybridization could occur and negatively affect *N. cavanillesii*. As there are no strong geographic, ecological or compatibility barriers between the two species, the most effective mechanism for limiting pollen flow between *N. cavanillesii* and *N. serotinus* is the pollinator market and pollinator behaviour. The overlapping of their flowering periods also influences interspecific pollen competition. It is important for *N. cavanillesii* to flower earlier than *N. serotinus*, otherwise improper pollen transfer would be more prejudicial for the rare species (Murali & Sumukar 1994). In this 3-year study, the flowering periods of these two species overlapped during approximately 15 days, which seemed to limit (in addition to pollinator activity) important hybridization events. What would happen if environmental conditions changed allowing a total flowering overlap? One possibility is that interspecific gene flow would be quantitatively less efficient than within species gene flow, thus limiting the formation of hybrids. This could allow large populations of the two species to remain distinct even if they came into contact and hybridized. However, if one species is much more abundant than the other, the scarce species may be genetically displaced and absorbed (Rieseberg 1997).

Conservation needs: pollination facilitation or threat to endangered N. cavanillesii?

Several studies provide evidence of positive interactions between plants usually involving physical or resource profits from one species to the other (e.g., Bertness & Callaway 1994; Callaway & al. 2002). Most recently, the influence of shared pollinators has been suggested as an important force in community structure (reviewed in Palmer & al. 2003). Moreover, some authors have sustained the possibility of positive interactions but evidence is still limited to studies where plants with no rewards benefit from the closest rewarding species (Laverty 1992; Johnson & al. 2003). If small populations of *N. cavanillesii* have low reproductive success and coexisting *N. serotinus* facilitates reproduction, then sharing pollinators can be advantageous for these populations. However, absolute reproductive barriers between *N. cavanillesii* and *N. serotinus* do not exist and our results revealed that hybridization patterns could have a negative impact on *N. cavanillesii*. Thus, pollinators seem to play an important role in the reproductive success of *N. cavanillesii* and in preventing widespread hybridization. Furthermore, reduced pollination visits in small populations of *N. cavanillesii* where *N. serotinus* is not present (unpublished data) constitutes a warning of possible declines in pollinator services. These results indicate the importance of pollination ecology studies especially if we want to conserve and restore endangered species or communities.

Acknowledgments

The authors thank Eva Salvado and Sílvia Albano for their help and field experience in part of this work and Lori De Hond for linguistic assistance. This study is promoted by EDIA, S. A. and co-financed by EDIA, S. A. and European Regional Development Funds (ERDF).

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Adverse effects of human activities on the diversity of macrofungi in forest ecosystems

Abstract

Zervakis, G. I. & Venturella, G.: Adverse effects of human activities on the diversity of macrofungi in forest ecosystems. — Bocconea 21: 77-84. 2007. — ISSN 1120-4060.

A rapid decrease in the size and composition of macromycetes populations has been observed in various regions of Europe. Such changes have been mainly attributed (directly or indirectly) to human interference, and most notably to the degradation of natural ecosystems and to air pollution. Especially as regards ectomycorrhizal fungi, it has been demonstrated that many of them are very suitable bio-indicators of the disturbance of forest ectotrophic stability. Air pollution affects negatively not only the number of species but also the number of basidiomata produced by this category of fungi; in contrast, fungal communities seem to get enriched in lignicolous species. Studies focusing on the determination of the deterioration of forest ecosystems are practically non-existent in the Mediterranean area. Hence, monitoring of the suitable groups of macrofungi in terms of both qualitative and quantitative assessments could provide valuable pertinent data.

Introduction

The lack of knowledge on fungal biodiversity is evident by the fact that possibly only 5-10% of fungal species have been discovered and described (Hawksworth 1991). Only during the last two-three decades fungi started to receive attention as essential components of natural ecosystems. As regards the Mediterranean region in particular, pertinent data are rather scarce and fragmentary, while studies on the ecology of fungal species and the influence that environmental factors exert on their communities have been significantly delayed.

On the other hand, of significant importance to the ongoing fungal conservation initiatives in Europe is the publication of national and/or regional check-lists in many Mediterranean countries (e.g. Bernicchia & al. 2005; Dimou & al. 2002; Onofri & al. 2003; Ortega & Linares-Cuesta 2002; Venturella 1991; Zervakis & al. 1998, 1999), and the compilation of provisional-preliminary Red Lists (e.g. Ivančević 1998, Venturella & al. 1997, 2002).

Environmental factors and the diversity of macrofungi: a review of literature data

During the recent past, the intensification of human activities led to high increase of air pollution and to the degradation of ecosystems, which in turn caused significant adverse effects in fungal communities as well. Air pollution may influence fungi through the greenhouse effect producing a climate change, as well as exerting an indirect effect through modification of vegetation. Such changes might threaten climate-sensitive species and/or favor the appearance/growth of more thermopiles taxa which could in turn act as alien competitors against native species. In addition, interactions between biotrophs and their hosts are also modified. Another global problem is the destruction of habitats and the decrease of forest areas. Some other environmental or pollution-related issues with a more local or specific effect on fungi include:

- deposition of various pollutants (incl. depositions from acid rain) leading to soil modifications or contamination of water resources,
- accumulation of metals on substrates interfering with fungi,
- occurrence of residues resulting from the widespread use of pesticides and fungicides mainly in adjacent agricultural lands,
- eutrophication, i.e. contamination of water resources with nutrients draining off from agro-industrial activities,
- desertification, caused by shifts in precipitation patterns resulting from climatic change,
- fragmentation of habitats resulting from forest cutting, urban extension, alterations in land uses and change of agricultural practices.

Experimental data on the effects which the above mentioned factors exert on fungal diversity are indicative of the state of relevant research in Europe.

The most indicative examples of the adverse effects of natural or anthropogenic disturbances are provided by studies on ectomycorrhizal-forming fungi. Decline in the populations of the edible mushroom *Cantharellus cibarius* is well documented in the Netherlands, and it is likely that the decline of this species is representative for many more ectomycorrhizal species (Dahlberg 1991). Forests which are particularly rich in ectomycorrhizal fungi are often situated on hilly sites with a nutrient poor soil with a thin or absent organic layer (soil-erosion problems) and a low coverage of vascular plants. These forest communities are threatened by eutrophication and/or acidification caused by air pollution. Clearly many of the ectomycorrhizal fungi dependent on living trees and other fungi characteristic of old, undisturbed soils will disappear. However, the inoculum potential of (some) ectomycorrhizal fungi remains present for several years in clearcuts (Dahlberg 1991).

Fellner (1993) described the decline of ectomycorrhizal fungi in Central Europe in terms of the disturbance of ectotrophic forest stability as a consequence of air pollution. This process includes latent, acute and lethal stages corresponding with both specific phases of the impoverishment of ectomycorrhizal and the enrichment of lignicolous mycocoenoses. Ohtonen & Markkola (1989) estimated the effect of local air pollution on the basidiomata production by mycorrhizal fungi and on microbial activity in Scots pine forests. The species composition of the mycorrhizal fungi varied in the differently polluted areas. The biomass and number of basidiomata and biological activity in the humus seemed to decrease towards the most polluted areas. Reasons for this may lie in the pH, the amount of ammonium nitrogen, total nitrogen and total sulphur present, all of which were at a higher level in the most polluted areas. Basidiomata production and biological activity showed negative correlations with these soil parameters. The mycorrhizal fungi were in the poorest condition in the most polluted area.

Poor soil quality hinders propagation of fungi and plants, and greater distances for inoculum dispersal have to be overcame to the nearest undisturbed vegetation. Erosion, in particular, is an issue of concern in rangelands and has been documented as a cause of inoculum depletion (Hall 1979). Many less drastic disturbances occur, that also reduce inoculum, including tillage, fertilization, irrigation, pesticides and heavy grazing. Grazing has variable effects on mycorrhizal fungi depending upon the degree of grazing and the yearly variability in precipitation.

Baar & Kuyper (1993) observed that removal of litter and humus layers by management practices or by wind had a positive effect on ectomycorrhizal fungi. Field experiments have been set up in stands of *Pinus sylvestris* to study the effects of removing the ectorganic layer in a more detailed way. Sod-cutting had a positive effect on the number of ectomycorrhizal species and on the number of sporomata. Adding sods had a negative effect on the number of ectomycorrhizal species and on the number of sporomata.

Modern agriculture increasingly uses chemical treatments that can give rise to various kinds of environmental modifications. This problem is especially well documented in Europe for grassland fungi, both macromycetes such as *Hygrocybe* spp. but also soil fungi. The nitrogen content of the basidiomata of *Lactarius rufus* and *Suillus variegatus* was measured on unfertilized and fertilized plots in four forest stands of different types in Finland in 1978-1984 (Ohtonen 1986). The effects on the fungal diversity of fertilizing forests has been reviewed by Kuyper (1989). In experiments N-fertilizer was applied with the purpose to stimulate the growth of trees or to compensate for (harmful) influences of air pollution. Nitrogen fertilization has a strong inhibitory effect on ectomycorrhizal symbiosis in laboratory as well as in field experiments. Only a few species increase after fertilizing e.g. *Paxillus involutus*, *Laccaria bicolor* or are indifferent (*Lactarius rufus*). Many species decrease or even disappear completely, e.g. many *Cortinarius* and *Suillus* species. It is not yet apparent whether the mycorrhizas themselves are affected as well, yet the fructification process seems to be most sensitive. NPK fertilizer application also has a strong negative effect on most ectomycorrhizal fungi, comparable with N fertilizer alone (Hall 1978). Fertilizing causes a decline in some of the saprotrophic species and a shift in species composition towards eutraphent species. In a fertilization experiment in a Scots pine forest in the Netherlands *Mycena sanguinolenta*, *Clitocybe vibecina*, *Entoloma cetratum* decreased whereas *Mycena galopus*, *Clitocybe ditopus* and *C. metachroa* increased after application of fertilizer (Kuyper 1989).

Furthermore, according to the investigation of Newsham & al. (1992) the occurrence and the ecophysiology of fungi are affected by sulphur dioxide (SO_2), a common pollutant in the atmosphere over continental Europe and North America.

Mushrooms as bioaccumulators of heavy metals and radionuclides

Extensive research has been carried out since the 1970's on trace elements (mainly heavy metals) occurrence in mushrooms, which was mainly focusing at screening several mushrooms species as bio-indicators of environmental pollution and detecting those edible species accumulating high levels of heavy metals. The ability of macromycetes to adsorb and accumulate certain heavy metals (e.g. cadmium, mercury, lead and copper) in quantities higher than those detected in soil and plants has been demonstrated by several authors (Klan 1984; Ohtonen 1982, etc.). This is explained by the intensive contact attained between the mycelium network and the growth substrate (soil, litter, wood), and by the osmotrophic abilities of these organisms. In contrast, the proportion of metal concentration in sporomata deriving directly from atmospheric depositions seems to be of less importance due to the short lifetime of a fruiting body.

Several reviews of heavy metal concentrations in mushrooms have been published (Kalac & Svoboda 2000; Seeger 1982; Vetter 1994). Results demonstrate, among others, that some species accumulate high levels of cadmium and mercury even in unpolluted and mildly polluted areas, while the concentrations of both metals (and also of lead) increase considerably in heavily polluted sites. For example, the genus *Agaricus* seems to be-a particularly effective accumulator for both for cadmium and mercury (Schmitt & Meisch 1985; Seeger 1982), while heavily accumulating mercury and lead species are also *Calocybe gambosa*, *Lepista nuda*, *Lycoperdon perlatum*, *Macrolepiota* and *Boletus* spp. (Kalac & Svoboda 2000; Sameva & al. 1999). The content of several other metals (e.g. chromium, copper, zinc, cobalt) has been measured in mushrooms, often in quantities exceeding common levels; comparison of metal concentrations in the sporomata demonstrate that many species may accumulate selectively (Kalac & Svoboda 2000).

In regions where the anthropogenic impact is more pronounced, dry deposition of particles containing heavy metals on the relatively large surface of macromycetes also plays an important role. This issue is of special interest since consumption of edible mushrooms growing in urban and industrial regions has caused several cases of human poisoning, which was attributed to their high (toxic) accumulation of content in heavy metals.

Dimitrova & al. (1999) investigated the arsenic content in basidiomata of different fungal species. They showed that the presence of arsenic content exceeded two times the MAA (Sanitary Standards of Maximum Admissible Amount) in *Agaricus silvicola*, *A. arvensis*, *Macrolepiota procera*, *M. rhacodes*, *Lycoperdon pyriforme*, *L. perlatum*, *Clitocybe gibba* and *Calvatia utriformis*. Stijve & al. (1990) analyzed the arsenic content from seven *Laccaria* species. The arsenic accumulating ability of *L. amethystina* was amply confirmed. *L. laccata* var. *pallidifolia* and *L. purpureobadia* might also possess the ability to concentrate arsenic, but they will only do so under certain (yet unknown) conditions.

Turnau (1989) investigated the effect of different types of industrial dust on the mycorrhizal status of a *Pino-Quercetum* plant community in a mixed forest near Krakow (Poland). A marked decrease in mycorrhizal plant population was observed in all plots. Turnau & Kozlowska (1991) analyzed the influence of industrial dust on the heavy metal content in different fungal species. *Armillaria lutea*, *Auriscalpium vulgare* and *Mycena ammoniaca* demonstrated their ability to accumulate heavy metals.

It is also known that mushrooms are efficient accumulators of radionuclides, as it was particularly evidenced after nuclear-plant accidents (e.g. Chernobyl, April 1986). By this accident a wide range of radioactive nuclides were released into the environment, the most important isotope of which was ^{137}Cs because of its long half-life (30 years). Since then, the radiocesium levels detected in fruits and vegetables have decreased, while in fungi the activity of radiocesium continues to be very high. Wasser & Grodzinskaya (1996) studied radionuclide accumulation in the basidiomata of macromycetes during the vegetation season of 1990-1991 in 44 locations of the Ukraine. Content of ^{137}Cs in basidiomata was 1-2 orders of magnitude higher than in the substrata on which they were growing. Species belonging to the families *Amanitaceae*, *Boletaceae* and *Russulaceae* were mainly characterized by high contents of radiocesium and may be considered as bioindicators of the radioactive contamination of the area. Increased radiocesium accumulation in mushrooms was as follows: lignotrophs > saprotrophs > mycosymbiotrophs.

Edible mushroom harvesting

Air pollution and intensive forest management (leading to declines in forest health and forest ecosystems), together with climate change, pollution from growing urban areas, ozone depletion, introduced pathogens, and intensive timber harvesting, are thought to be major contributors to decreased mushroom diversity and productivity in Europe (Arnolds 1991). Initial studies of the impacts of edible mushroom picking have concluded that rational harvesting does not diminish subsequent fruiting (Egli & al. 1990, Norvell & al. 1995), but these small-scale studies have not adequately addressed the impacts of large-scale commercial mushroom harvesting or forest management activities over long periods of time. Ivančević (1998) stated that in the early nineties, warnings were issued about the diminishing quantities of the wild mushrooms harvested year per year, and that certain species of macromycetes were endangered. Nowadays, ECCF (European Council for the Conservation of Fungi) members from eastern and from southern Europe complain about the damage caused to their forest ecosystems by commercial harvesting of edible mushrooms that are exported to western Europe.

Pilz & Molina (1996) proposed a regional approach to edible forest mushroom monitoring and research in the USA's Pacific Northwest. Its objectives included:

Low-intensity, long-term monitoring of areas with heavy commercial harvesting to ensure harvest sustainability and evaluate reasons for potential trends.

Low-intensity, long-term monitoring of natural areas (where neither timber nor mushroom harvesting occurs) to provide control sites for interpreting trends in commercially harvested areas and to detect trends related to regional changes in the environment or forest health.

Intensive, short-term research on correlations between mushroom productivity, habitat, and stand management activities to provide forest managers with the information needed to ensure future habitat availability and mushroom collection opportunities.

Use development of this research and monitoring program as a prototype for cost-effectiveness ensuring the sustainable harvest of an array of other non-timber forest products by engaging interested public in research and monitoring activities.

The program design of this initiative involving (among others) integrated research and monitoring activities, voluntary participation by interested agencies, organizations or individuals, common core sampling procedures, site selection criteria and meta-data evaluation, is a good example of the type of work needed to rationalize managing of forest ecosystems and edible mushroom harvesting.

Influence of other factors on fungal diversity

Besides the adverse effect that most human activities exert on fungal diversity, appearance and abundance of mushrooms are influenced by climatic conditions. The temperature and humidity of the air and soil are among the principal factors that regulate fungal growth and reproduction. The influence of environmental factors, both climatic and edaphic, could be evidenced by the major variations observed in species distribution and yields, and also as a function of time, i.e. in variations between years and growth seasons. The evidence reported from mycodiversity studies in Greece and Sicily (Venturella & Zervakis 2000; Zervakis & al. 2002, 2002a) confirmed that fungi require a certain level of moisture; rainfalls, air humidity and soil moisture, which are all significant factors for the achievement of a good crop of mushrooms; the rainfall during the last 3-5 months preceding mushroom appearance has a marked effect on their productivity; rare species occasionally appear as a consequence of a particularly hot summer; the number of mycorrhizal species do not increase in unusually warm summers; peak temperatures exercise their influence predominantly via the soil, affecting its moisture content; particularly low temperatures have a direct (detrimental) effect on the growth of fungi. Finally, the fact that high total annual precipitation is predictive of a poor yield of ectomycorrhizal mushrooms in the autumn of the following year, may be attributed to the good yield almost certainly obtained during the autumn of the previous year, especially when the preceding summer was rainy (since this high yield would have undoubtedly reduced the potential of the mycorrhizal fungi to produce another good yield immediately after).

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Volker John

Lichenological studies in Turkey and their relevance to environmental interpretation

Abstract

John, V.: Lichenological studies in Turkey and their relevance to environmental interpretation. — Bocconea 21: 85-93. 2007. — ISSN 1120-4060.

Progress in Turkish lichenology has been made in four areas: firstly, by evaluation of material collected by non-lichenologists during historical expeditions, secondly, by analyses of random collections made by lichenologists during sightseeing trips, thirdly, by special projects concerned with floristic studies, and finally through applied studies using lichens as environmental monitors. An analysis of the 361 papers covering these topics in relation to Turkey has been undertaken. Papers by Turkish authors, which first appeared in the 1980s, relate to floristics, chemistry, bioindication, microbiology and radioactivity, with a noticeable absence of publications on systematics, taxonomy, nomenclature and physiology. Lichenological studies are relevant to environmental interpretation in Turkey in different ways: the available database of floristic information can help to calculate the influence of changing agriculture and forestry management, focussing on eutrophication, afforestation and grazing by sheep and goats. The use of lichens to demonstrate fluctuations in air pollution has been demonstrated in Izmir, Eskişehir and Ankara. Further subjects in Turkey which need to be environmentally interpreted by means of lichens include radioactivity, tourism and global climatic change; initial studies in these areas are presented, and others are considered. Fortunately the increasing interest of young Turkish scientists in lichens and their involvement in the Turkish Lichen Association (TLT) receive recognition and support for their studies by officials in government. Nevertheless, much work needs to be done.

History of lichenological studies in Turkey

Since the 19th century, extensive expeditions to Turkey have been undertaken to observe most groups of animals and plants and to determine geological features. However, since the participants of these explorations were not trained in all disciplines of natural science, the lichen samples, like numerous other organisms, had to be sent to specialists for identification. In this way, information on the different groups was scattered in several countries and in different publications. A comprehensive discussion of all material assembled from these expeditions is unfortunately lacking. Therefore, we have only floristic lists from that time, generally complemented by some ecological information. Furthermore, the samples are stored in foreign, non-Turkish herbaria. Such basic lichen floristic informations, although scattered, cover most areas of Turkey.

This first period was followed by field trips undertaken by lichenologists in the second half of the 20th century. However, most of these journeys focussed on historical sight-seeing sites, the lichens being collected more or less randomly along roadsides between historical highlights or near to them. It should be noted that collecting lichens from historical monuments themselves is still strictly forbidden. Important collections were made, for example, by Klaus Kalb, Ivan Pisut, Herbert Schindler and Antonin Vezda. Furthermore, Kalb and Vezda merit special attention for their contribution of extensive lichen materíal distributed in herbaria.

The lichenology undertaken by Turkish scientists started in the 1980s (Fig. 1). Ayşen Türk (born Özdemir) is the first 'Turkish lichenologist', as a consequence becoming the first President of the Turkish Lichenological Society (TLT, Türk Lichen Topluluğu), the first scientific association of its kind in Turkey. This group of Turkish lichenologists has developed mainly from young scientists working on floristic studies in different regions of Turkey. They merit attention since their 86 publications cover 67% of all papers produced by Turkish lichenologists (Fig. 2). In a country of 814578 km^2 it is obvious that their efforts need to be sustained in the next decades. It should be stressed that all these data need to be assembled and interpreted in order to effectively monitor many aspects of environmental change in the future.

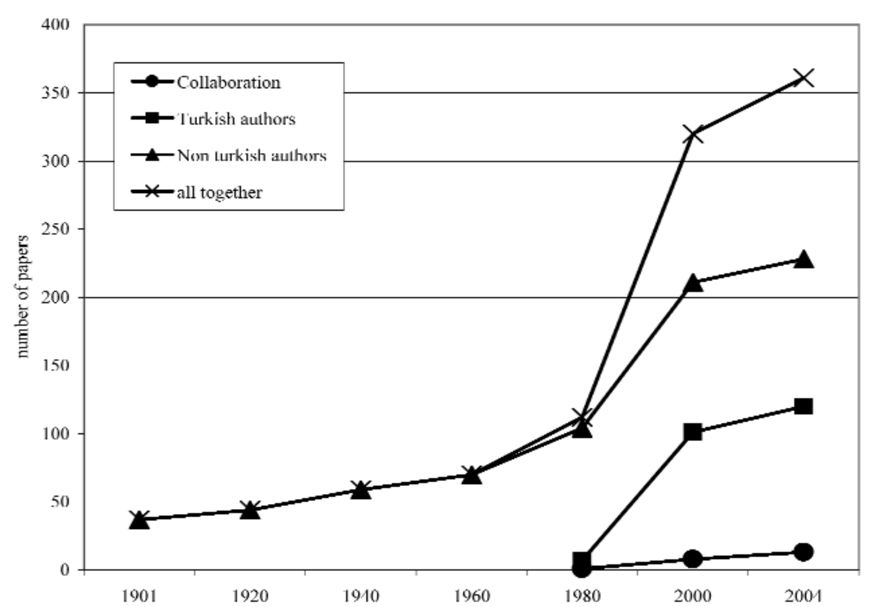

Fig. 1. Development of literature (cumulative) on Turkish lichens published by Non-Turkish authors, Turkish authors and joint papers.

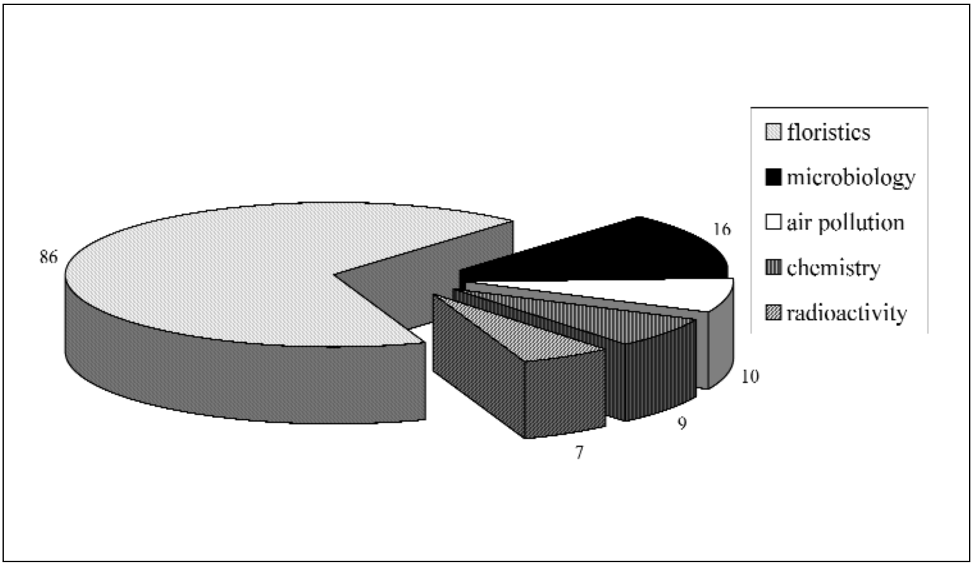

Fig. 2. Number of papers on lichens published by Turkish lichenologists and their specifications to different fields in lichenology.

Lichenological studies in Turkey focussing on applied science which employ lichens include research in microbiology (16 papers, 13%), air pollution (10 papers, 8%), chemistry (9 papers, 7%) and radioactivity (7 papers, 5%).

The remarkable result of all these activities is a number of more than 200 papers dealing with Turkish lichens in the two decades between 1980 and 2000. Note that the number of papers published by Turkish scientists in the period 2001-2004 exceeds those by non-Turkish lichenologists for the first time (Fig. 3).

Comprehensive documentation of information on Turkish lichens is in preparation, and a forthcoming publication resulting from the OPTIMA Commission initiative will include a bibliography, an up-to-date checklist, literature for the identification of Turkish lichens and information on relevant illustrations. The literature references will be ordered according to the 81 provinces of Turkey. Finally distribution maps (more precisely “known distribution”), based on the Flora Europaea grid of UTM 50 x 50 km squares, will be provided for every species (Fig. 4).

Relevance to environmental interpretation

The importance of lichen mapping based on rigorous and workable protocols has already been mentioned by Seaward (2004) in this meeting. Therefore the following second part will concentrate on showing some examples showing the relevance of lichenological studies for the interpretation of the different environmental characteristics and problems in Turkey.

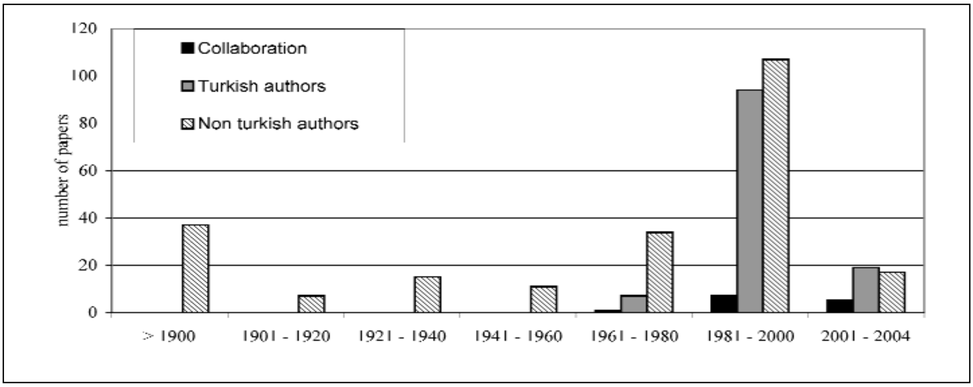

Fig. 3. Number of papers on lichens in Turkey published in periods of 20 years by Non-Turkish lichenologists, Turkish lichenologists and in collaboration of them.

Air pollution in cities and industrial agglomerations

For nearly half a century, the power of lichens as monitors was dominated by their use as indicators for air pollution, focussing on sulfur dioxide emissions. Examples of this classical use of lichens can be found for the Turkish cities of Bilecik, Bursa, Eskişehir and İzmir. Furthermore, data based on floristic observations can be used in this way for the calibration of air quality in İstanbul, Giresun and Ankara (Fig. 5).

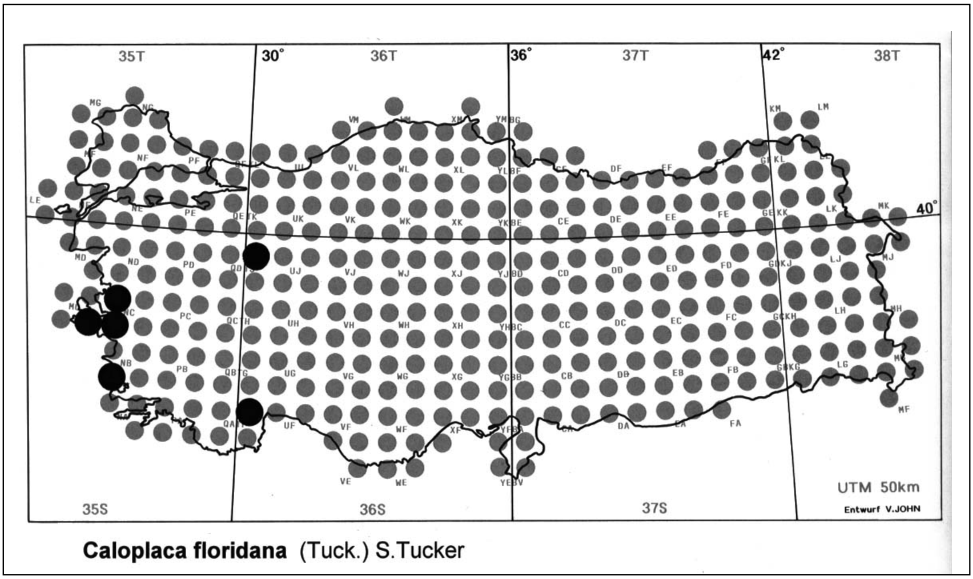

Fig. 4. UTM grid system based on 50 x 50 km squares.

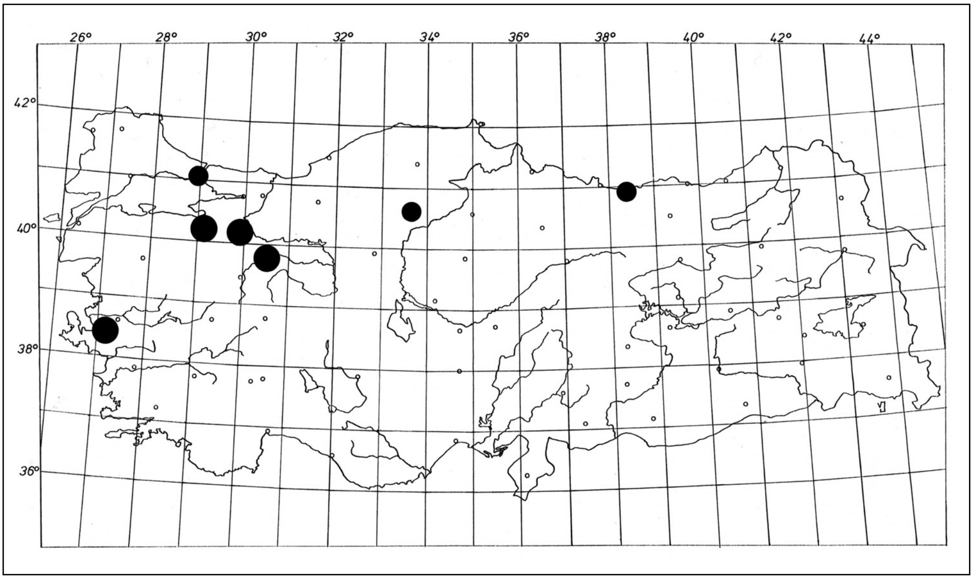

Fig. 5. Locations of the cities where an air pollution monitoring with lichens has been done (big dots) and where floristic data of the lichen vegetation can be used to calculate air pollution (small dots).

In the area of Izmir, change in air pollution could not be detected by this means during a 5-year period. However, Ankara and Eskişehir cities showed a decrease in sulphur dioxide emissions as a result of changing from coal to gas fired heating, where a clear recolonization of tree bark by lichens can be observed. The almost completed new tramway system in Eskişehir, thereby reducing pollution by motor vehicles, will undoubtedly also improves lichen recolonization, the effect as yet to be monitored. In Ankara, the recently created fountains in the city centre, by raising humidity levels, may cause a further improvement in lichen colonisation.

In the cities of Bilecik, Giresun, Izmir and Kayseri lichens have been used as bioindicators to estimate the relative input of the different heavy metals to the environment.

Monitoring radioactivity

The Chernobyl disaster resulted in a number of studies (seven papers) related to the accumulation of radiactive elements in lichens in Turkey. It should be noted that there were already relatively high background levels in some industrial areas caused by cement factories and agglomerations of certain building materials in the Aegean region which did not increase significantly as a consequence of Chernobyl (Topcuoğlu & al. 1992). In the naturally unpolluted region of the Black Sea region, the very low background level was considerably exceeded after Chernobyl. Contamination studies with lichens must accompany the planning and construction of the projected atomic power station in Turkey.

Road construction

Road construction in forestry areas, as pointed out by Degelius (1935) when he showed that enlarging a road by only 1 metre can affect the lichen flora up to a distance of 50 metres into the forest on both sides of the road. These effects can be observed in many regions of Turkey (John & Breuss 2004), the influence of road construction on the micro- and meso-climate being reinforced in some cases by the regulation of water-flow with these new road systems (Fig. 6). As the extension of all main roads in Turkey to broader highways is a governmental priority, the dry-plain areas in Central Anatolia and their associated lichen floras, as well as the forests, might be suffering under the diminution of such areas; lichen communities growing on gypsum soil clearly exemplifies these problems.

One of the saddest events of international importance resulting from roadway construction involves the “Gülekboğazı” in the Toros Mountains, made famous by Alexander the Great, which supports a unique, rich and diverse flora and fauna: wholesale landscape changes here have affected all aspects of its environment, the lichens suffering as consquence like most other organisms (Fig. 7). Coastal rocks are also suffering, or even disappearing, under road constructions; therefore, monitoring the lichens of the sea-cliffs in Turkey should be a priority.

Monitoring agricultural and forestry management practices

In Central Europe, impact of nitrogen has received more importance than sulphur dioxide in recent years. These new environmental conditions necessitate a renewal of monitoring guidelines for lichens, particularly in respect of fertilizer and pesticide applications (Verein Deutscher Ingenieure 2004). One of the gravest problems in Turkish agriculture is the grazing of sheep and goat, which cause different effects in the various climatic ecoregions of Turkey. On one hand, many lichen species are usefull as food for these animals, on the other hand they indicate overgrazing and nitrification.

There seems to be no other country undertaking so much effort in reforestation than Turkey. Keeping sheep and goats out of young plantations and protecting these areas against artificial fire allows huge areas to be recovered by forest, which will clearly change the micro-, meso- and even macro-climate (Fig. 8). Karabulut & al. (2004) give a good demonstration of this fact, the indicator values in respect of Turkish lichens aiding considerably environmental interpretation of this phenomenon.

Observance of climatic changes and Lichenometry

The global changes of climate are composed of numerous effects. Nearly all of them can be monitored by the use of lichens. Lichen physiology helps in detecting carbondioxide and even the evaluation of simple floristic inquiries demonstrate global warming (van Herk & al. 2002). As detailed and comprehensive floristic data from the past are allmost lacking in respect of the lichens in Turkey, a survey of the lichen distribution at the present time is in deep need to receive a baseline document, with the objective to monitor changes in the future. Lichenometry (Lock & al. 1979) is a tool to detect local differences

Fig. 6. Ecological features of special landscapes are well defined by the lichen vegetation. The biological balance is highly endangered by road construction and water flow changes. Black Sea mountains 1997.

Fig. 7. Toxitolerant and ubiquitous lichens characterize such destroyed habitats. Taurus Mountains 1992.

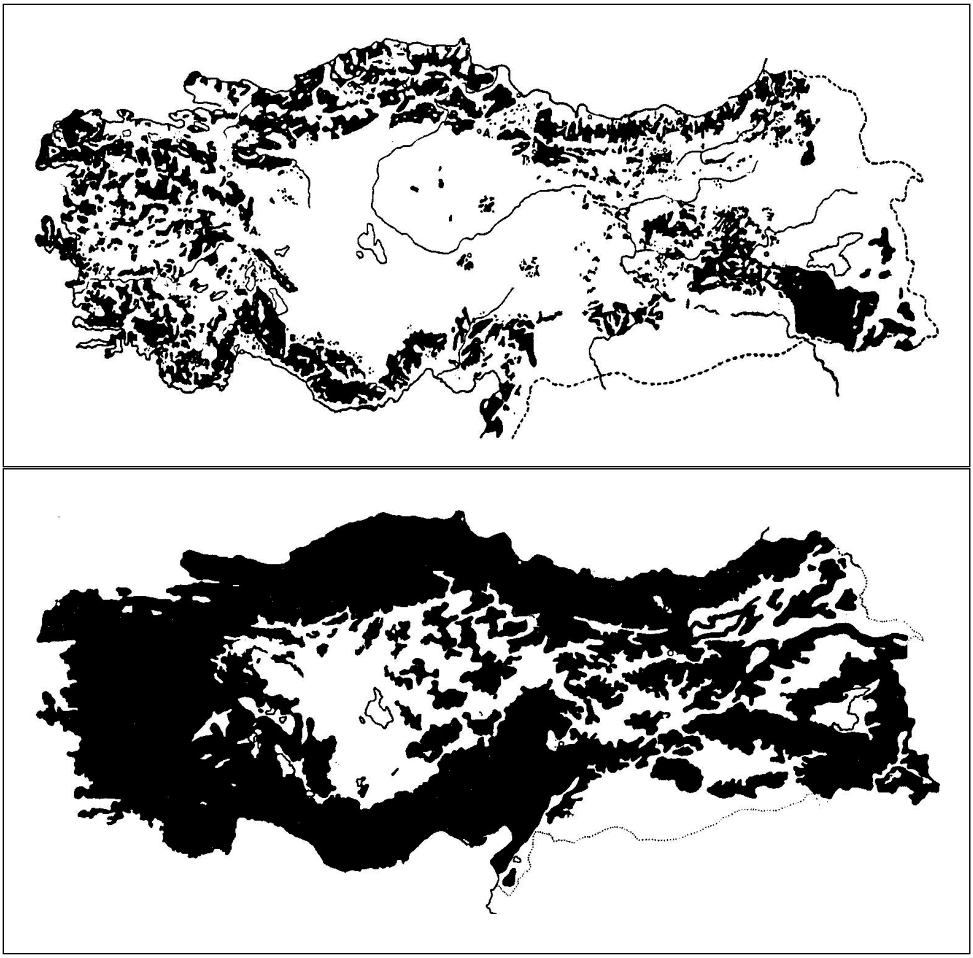

Fig. 8. The climatic effect of changing from present (above) to potential forest areas (below) can be estimated by the use of lichens in different ways.

caused by afforestation or by daming up in recent times and to describe geomorphological particularities in historical times.

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David Draper & José María Iriondo

Spatial patterns of vegetative and sexually reproducing individuals of *Marsilea strigosa* Willd.

Abstract

Draper, D. & Iriondo, J. M.: Spatial pattern of vegetative and sexually reproducing individuals of *Marsilea strigosa* Willd. — Bocconea 21: 95-104. 2007. — ISSN 1120-4060.

We analyzed the spatial distribution of *Marsilea strigosa*, an aquatic fern, considering two life stages (vegetative and sexual) and interpreted results taking into account possible facilitation or competition effects among individuals as well as existing colonization strategies. Marsilea strigosa is distributed from Spain to the Black Sea. It is considered a rare species due its high habitat specificity and small fragmented populations. We surveyed 95% of the Balearic population of *M. strigosa*, covering a surface of approximately 1000 m2. Single life stage and bivariate distribution patterns were analyzed using second-order spatial analysis based on Ripley's K-function. The relationship between vegetative and sexual node types is discussed. Aggregation of vegetative and sexual nodes was the only spatial point pattern found. The same results were obtained when both stages were analyzed together with bivariate function. We discuss the effect of life stage on the balance between competition and facilitation in dynamic environments. The importance of these two processes in colonization sites can best be understood by comparing them between different life stages.

Introduction

Marsilea strigosa is an amphibious rhizomatous geophyte (Boudrie 2004). It can produce very slender runners (2-25 cm long) that bear leaves and roots at each node. The nodes have a high capacity of vegetative reproduction, while some also produce macroscopic reproductive structures (3-5 mm) which can be easily identified during the dry season. The roots need at least a thin layer of clay.

This aquatic fern species is scattered from Spain to Crimea. Although it is widely distributed, it presents high habitat specificity, and the population sizes are frequently low. As a result, *M. strigosa* is considered a rare species belonging to Category V of the Rabinowitz (1981) classification. *Marsilea strigosa* is a hydrophyte that requires a combination of geophysical and weather conditions: small depressions in sites where a watershed appears during the rainy season followed by a dry season in which the pond dries up. This cyclic regime is probably one of the most characteristic features of the Mediterranean climate. *Marsilea strigosa* has no soil type restrictions and tolerates dramatic water level changes. The pond depth may however be a limitation. *Marsilea strigosa* can colonize pond sites up to a depth of 0.5 m. Biogeographical history is another factor inherent to this species' present distribution.

The cyclic regime of temporary ponds excludes the occurrence of plants that cannot tolerate the dry period as well as typical grassland species that cannot tolerate the flooding period. *Marsilea strigosa* shares this habitat with a few small annual or some perennial species such as *Mentha cervina* and *Isoetes setacea* (Grillas & al. 2004).

The plant spatial patterns have long been a major issue in ecology (Ashby 1935; Dale 1999; Greig-Smith 1983). The complex combinations of negative and positive interactions between plant species appear to be common in nature, and are not restricted to particular communities or biomes (Walker & Chapin 1987; Chapin & al. 1994; Callaway 1995).

The co-occurring facilitative and competitive effects in the same species occur in other ecosystems and often vary in time or space (Hay 1986; Eldridge & al. 1991; Aguiar & Sala 1994; Belsky 1994; Callaway 1994; Callaway & King 1996; Nicotra 1998), but the factors that determine the positive or negative balance between them are poorly understood. Factors that have been considered include life stage, plant density, specific physiology of the species, indirect interactions, and abiotic stress. The aim of this work was to study the spatial distribution of *M. strigosa* individuals by considering its vegetative and sexually reproductive life stages and to determine whether the production of sexual structures follows a particular pattern in its populations. The question is, do sexual nodes tend to accumulate in specific areas of the pond? Is there repulsion between sexual and vegetative nodes? Is it possible to infer information on the colonization process of the pond from the spatial distribution patterns of sexual and vegetative nodes?

Material and methods

Field sites and sampling method

The sites selected for this study were the temporary pond system of Marina de Llucmajor, Majorca (UTM 31SDD86) and two isolated ponds in Minorca (UTM 31SFE01, 31TEE93). The climate in this area is humid from autumn to mid-spring with a severe dry period in summer. The mean annual precipitation is 450 mm/yr. and mean annual temperature is 17ºC with a soothing effect from the Mediterranean Sea. The sites are almost flat, with karstic and schist soils (Majorca and Minorca respectively) with a sandy-loam texture. Some ground depressions and vegetation gaps accumulate water, allowing the formation of temporary ponds. The surrounding vegetation is mainly typical of the Mediterranean garrigue. The seasonal change in water availability is the main constringent for plant colonization in these systems, occupied by highly specialized flora composed of Mediterranean therophytic and geophytic species. The main herbivores are rabbits and goats.

Six temporary ponds were surveyed in Majorca and two temporary ponds in Minorca to cover major ecological differences. The shape and size of the sampled plots differed from pond to pond, as pond size, *M. strigosa* total population and *M. strigosa* cover varied between ponds (Tab. 1).

Table 1. Code, location in UTM and spatial features of surveyed ponds and plots.

Pond code	UTM	Island	Area surveyed (m^2)	Pond depth (m)	% of population covered	% of pond covered
B2	31SDD8136	Majorca	41	-0.19585	100	100
B3	31SDD8136	Majorca	52	-0.45307	20	30
B4	31SDD7936	Majorca	57	-0.47505	100	20
B20	31SDD8267	Majorca	20	-0.24321	60	15
B51	31SDD8359	Majorca	42	-0.39954	100	100
B61	31SDD8361	Majorca	19	-0.29735	40	30
BF	31TEE9834	Minorca	49	-0.17345	100	100
BV	31SFE0919	Minorca	803	-0.28375	50	50

The table shows the code names that were assigned to each pond. The aim was to survey the entire area of each pond due to the clonal capacity of *M. strigosa*. When this was not possible, a transect was established crossing the point with the deepest water level. Fieldwork was carried out in early summer 2003 when the ponds were totally dry and the development of reproductive structures was completed. The percent of population surveyed was determined considering the surface of the carpet of *M. strigosa* in each pond.

A grid of 1x1 m squares was established on the population using a total station transit (model TPS407, Leica Geosystems AG, CH) to position each corner of the grid. Each node was then marked with a coloured drawing pin to identify its life stage (vegetative or sexual). All grid squares were photographed and the pictures were orthogonally corrected and joined using Idrisi Kilimajaro software. The resulting composition indicated the position and life stage of each marked node (Fig. 1). A land cover layer was also obtained from the final image allowing us to identify three different categories (i.e. area covered by soil, rock and other vegetation).

Statistical analysis

The spatial distribution patterns were analysed by Ripley's *K*-function (Ripley 1976, 1981; Diggle 1983; Haase 1995; Haase & al. 1996). The edge correction was calculated by the weighted method described by Getis & Franklin (1987) and modified by Haase (1995). This approach identifies the scales above which a non-random distribution occurs, and can be used to develop hypotheses about spatial processes at specific scales with the common null model indicating complete spatial randomness (CSR). The null hypothesis was tested for all obtained points together as well as for vegetative nodes and sexual nodes.

To linearize the plot of $K(t)$ against t and stabilize variances, $L(t)$ statistic was used (Diggle 1983): $L(t) = \sqrt{[K(t)/\pi]} - t$. The expected value of $L(t)$ under a Poisson process is 0. Positive values indicate spatial clustering, whereas negative values indicate spatial segregation. When $L(t)$ and the confidence envelope are plotted on the same axes, patterns of clumping and regularity become apparent. If $L(t)$ exceeds the upper confidence interval for any distance class, the points are relatively closer together than expected, indicating clustering or clumping. If $L(t)$ falls below the lower confidence interval for a distance class,

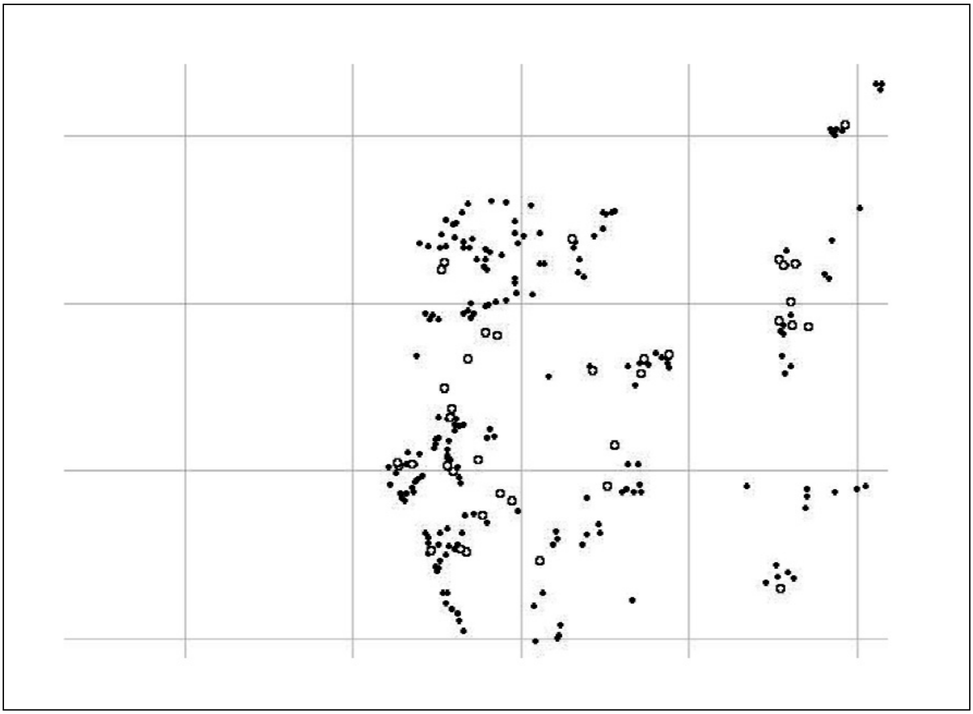


Fig. 1. Example of node distribution corresponding to part of the sample plot of pond BV from Minorca (dots: vegetative nodes; open circles: sexual nodes). Background grid is referred to 1m^2.

the points are relatively farther from one another than expected under a random distribution indicating regularity, repulsion or over-dispersal of points. The values of *L*(*t*) within the confidence envelope indicate random distribution of points at those scales (Sharpe 1991). Each study plot was analyzed for distances up to 25% the length of the shortest side.

As Ripley's *K*, and its derived *L* statistic are not bound, comparisons of spatial patterns of different species or different study plot sizes should be based on the significant spatial scale. Significance is achieved by using Monte Carlo simulation, which generates *n* simulations of a Poisson point pattern process that provide a confidence envelope (Parish & al. 1999). This confidence envelope is defined by the extreme maximum and minimum values of the simulation, where 99 simulations correspond to a 0.01 significance level (Ripley 1979). In our study we used a 99% confidence interval.

The bivariate spatial analysis, using the function $L_{12}(t)$ (a transformation of the function $K_{12}(t)$; Ripley 1977), was carried out to test the relationship between the spatial patterns of vegetative and sexual nodes. The combined patterns can suggest spatial attraction, independence, or repulsion (Parish & al. 1999).

The univariate and bivariate analyses were performed using the SPPA (v. 2.0.3. Haase) software package (http://home.t-online.de/home/haasep/sppagree.htm).

Results

The vegetative nodes were generally more common than sexual nodes, accounting for 97% of total nodes in pond BF. The proportion of sexual nodes was only higher in ponds B20 and BV (Tab. 2). The node density seems to be related to lower vegetation cover but does not seem dependent on rock cover. The Minorcan ponds (BF and BV) are characterized by an almost total absence of rocky slopes and these two ponds had the highest percentage of vegetation cover.

When the distribution patterns of vegetative nodes and sexual nodes were analysed separately, $L(t)$ exceeded the upper confidence interval for all distance classes in all ponds indicating a clustering or clumping profile at almost all distances (Fig. 2-9). Three main patterns were observed: a) a steady increase in aggregation with distance (e.g., Fig. 5), b) asymptotic profile (e.g. Fig. 7) and c) presence of a relative maximum (e.g. Fig. 9). Maximum values were obtained at a distance of c. 0.8 m (Fig. 2, 3, 7, 9). In Figure 9 an interval of maximum aggregation can be observed between 1-3 m, followed by a sharp decrease in aggregation which is, nevertheless, still significant.

The bivariate spatial pattern of the two life stages showed a significant trend to aggregation (Fig. 2-9). Lower rates of aggregation were observed in bivariate spatial patterns as compared to univariate spatial patterns (Fig. 2, 3, 5, 8, 9).

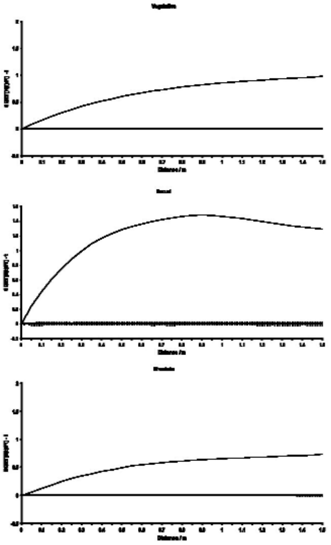
Considering all the sampled patches, exclusive vegetative clumps were common in small patches (<25 nodes), whereas reproductive nodes appeared when node size was ≥ 25.

Discussion

The sexual reproduction is considered to be more “expensive” in terms of resources than vegetative reproduction. Increased vegetative effort is generally associated with stable periods, while a major sexual strategy is considered under non-optimal environmental conditions. This is best represented in pond **BF** where *M. stigosa* covered almost all the flooded area and only 2.31% of the nodes adopted a sexual strategy. On the other hand,

Table 2. Percentage vegetative and sexual nodes, density and percentage rock, vegetation and bare soil cover.

Pond code	% vegetative	% sexual	density nodes/m^2	% rock	% vegetation	% bare soil
B2	88.53	11.47	99.51	47.13	3.84	49.03
B3	79.82	20.18	144.06	41.69	2.26	56.05
B4	59.35	40.65	19.04	26.76	9.33	63.90
B20	40.51	59.49	206.50	17.53	7.19	75.28
B51	72.88	27.12	117.21	97.18	2.75	0.07
B61	60.29	39.71	232.37	17.20	0.00	82.80
BF	97.69	2.31	39.84	1.24	81.12	17.64
BV	38.81	61.19	6.97	0.01	69.42	30.57



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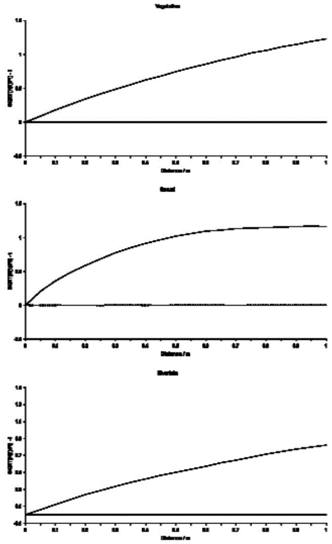

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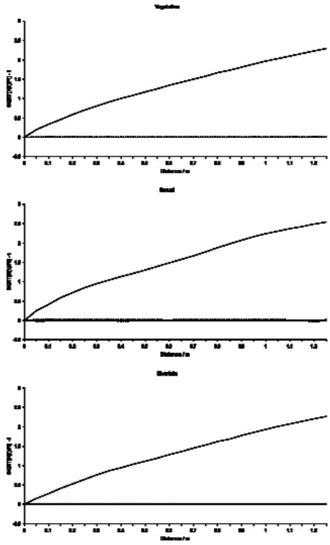

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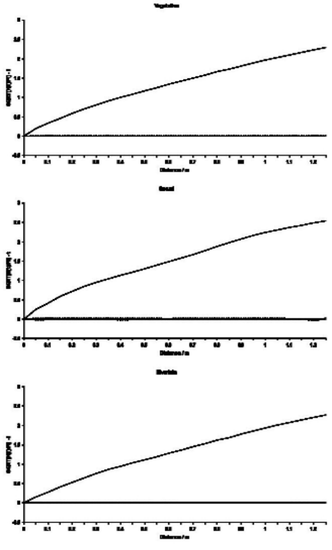

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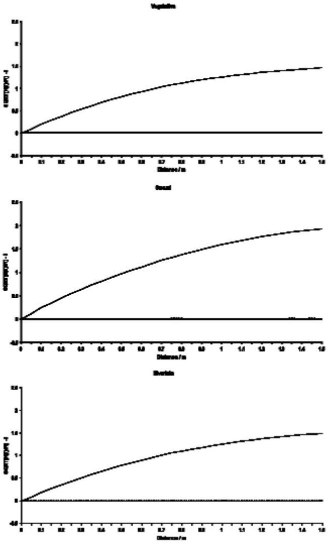
Figs. 2-5. Estimates of second-order neighborhood of ponds B2, B3, B4, B20 respectively. Each pond is analyzed considering a univariate distribution for vegetative and sexual nodes as well the interaction between both types in the bivariated graph. Thick line: observed distribution; Thin lines: 99% confidence envelope (when visible).



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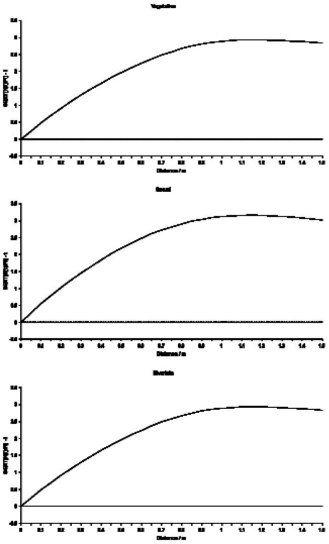

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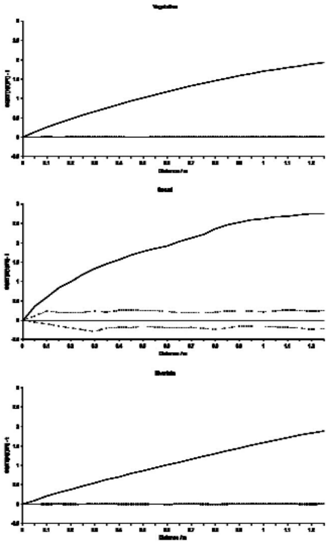

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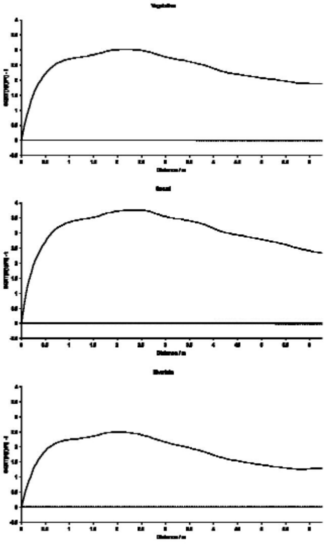

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Figs. 6-9. Estimates of second-order neighborhood of ponds B51, B61, BF, BV respectively. Each pond is analyzed considering a univariate distribution for vegetative and sexual nodes as well the interaction between both types in the bivariated graph. Thick line: observed distribution; Thin lines: 99% confidence envelope (when visible).

pond BV, which had the lowest node density, also had the highest percentage of sexual nodes. Pond BV tended to have a random point distribution pattern due to the gaps between clumps (Fig. 1, 9).

The soil availability and water level seem to be the main limitations in the constantly changing environment of temporary ponds which *M. strigosa* is well adapted to. These important resources may explain the preference for adopting one strategy over the other. Bertness & Callaway (1994) hypothesized that the importance of facilitation in plant communities increases with abiotic stress or consumer pressure, because neighbours buffer one another from extremes in the abiotic environment (e.g., temperature or salinity) and herbivory. Alternatively, they hypothesized that the importance of competition increases when abiotic stress and consumer pressure are relatively low. Although herbivory is not the main focus of this work, the two main predators, rabbits and goats, seem to affect the distribution of *M. strigosa* in quite different ways. Rabbits were observed to produce holes in *Marsilea* carpets, modifying the micro-relief and increasing landscape complexity, while goats mow the aerial parts.

The aggregation pattern observed both in vegetative and sexual nodes results from the clonal growth of *M. strigosa* through the production of runners. Considering each node individually, the vascular connection between nodes can be interpreted as an interaction of facilitation. The three distribution patterns found could be assigned to three different colonization stages: a) the presence of a relative maximum represents the first stage of colonization with isolated clumps of *M. strigosa*; b) an asymptotic pattern indicates uniform, but not saturated, surface coverage and c) surface saturation and constant dependence on distance is characterizated by a linear increase.

Marsilea strigosa generally adopts a vegetative strategy, colonizing from older shoots that also produce sexual nodes. Sexual growth, which is less frequent, seems to be affected by stochastic phenomena that permit the establishment of new clones as seen in pond BV.

The bivariate analysis showed that exclusion does not occur between vegetative and sexual nodes; in fact sexual nodes never occur in isolated clusters, but are interspersed with vegetative nodes. Furthermore, the bivariate analysis indicated aggregation between sexual and vegetative nodes, although this is most likely a result of the connectivity of nodes through runners. Vegetative clumps are common in the first stage of development, but when the number of vegetative nodes reaches a threshold, sexual nodes begin to appear. This should be interpreted as the capacity of a particular network of vegetative nodes to supply enough nutrients to produce a reproductive structure. Spatial point processes are not only able to generate point configurations, but can also reproduce biological phenomena of spatial interactions between points (nodes) with a temporal dimension (patch growth). This method is also effective in identifying various dynamic processes, which can play an important role in the functioning of temporary ponds, and places where these processes are in action. This last point is of particular interest for the concept of an ecosystem-level simulator because the functional units might be considered elementary spatial units that evolve according to specific models of fine-scale dynamics (Pelissier 1998).

Acknowledgements

We wish to thank Pere Fraga and his colleagues working for project LIFE2000NAT/E/7355 for their support with the studies performed in Minorca and Josep Lluis Gradaille and Magdalena Vicens from Soller Botanical Garden for their help and support in the work performed in Majorca. We also thank Lori De Hond for linguistic assistance and Isabel Marques, María José Albert, Maite Iriondo and Amaia Iriondo for their collaboration in data acquisition. David Draper is funded by the Portuguese FCT studentship (Contract SFRH/BD/1002/2000).

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GIS-based evaluation of the *in situ* conservation of a Crop Wild Relative: the case of Spanish lupins

Abstract

Parra-Quijano, M., Draper, D. & Iriondo, J. M.: GIS-based evaluation of the *in situ* conservation of a Crop Wild Relative: the case of Spanish lupins. — Bocconea 21: 105-116. 2007. — ISSN 1120-4060.

The six *Lupinus* species that grow naturally in Spain were used as a model of how GIS and gap analysis can be used in the assessment of the conservation status of a crop wild relative (CWR). Data on the geographic location of *Lupinus* populations was compiled along with relevant environmental data for Peninsular Spain. This information was used to generate predictive distribution models, to identify areas of high richness in *Lupinus* species and to assess whether the current network of protected areas holds sites of high richness in *Lupinus* species that might be utilized to establish genetic reserves.

Introduction

The genus *Lupinus* (*Fabaceae*) is widely distributed around the world with more than 200 species (Ainouche & al. 2004). Although there are several classifications of lupins, they are most commonly grouped into Old and New World species. New World lupins can be divided into North and Southeast American lupins, whereas Old World lupins are classified by their rough or smooth seeds. Old World lupins refer to North African and Mediterranean lupins, including a total of 12 species (Ainouche & Randall 1999). In the *Lupinus* genus there are four cultivated species of economic importance, three of which are Mediterranean species: *L. albus* L., *L. angustifolius* L. and *L. luteus* L. Wild forms of these three species and other lupin wild relatives can be found in the Mediterranean area (Gladstones 1974, 1998; Plitmann 1981). Six *Lupinus* species grow in the Iberian Peninsula - the three Mediterranean cultivated species and three wild relatives (*L. consentinii* Guss., *L. micranthus* Guss. and *L. hispanicus* Boiss. & Reut.). Spain and Portugal share *Lupinus* diversity with all six species occurring in both countries (Castroviejo & Pascual 1999).

Crop Wild Relatives (CWRs) are very important in plant breeding programs to improve agricultural quality and production. In consequence, their conservation must be a priority especially in areas where their habitats are under threat from alteration or loss. It is worthy to note that CWRs can be common or rare species, widely distributed or reduced and abundant or endangered. CWR diversity can be conserved *ex situ* (germplasm collections) and *in situ* (protected areas) (Jarvis & al. 2003). In the case of *ex situ* conservation there are several difficulties involved in collecting and storing CWRs. In fact, less than 15% of the six million PGR accessions conserved in *ex situ* collections worldwide are CWRs (FAO 1996). *In situ* conservation seems to be the most efficient and low-cost strategy for preserving CWR diversity. Protected areas conserve many species and their ecological framework at the same time (Parra-Quijano & al. 2003). Therefore, a good relationship between management and cost is achieved: species are conserved and evolution is allowed to continue. In this sense, Europe is considered an important centre for crop wild relatives and a thematic network called PGR forum (http://www.pgrforum.org) has been created to help conserve European CWRs *in situ*.

Geographical Information Systems (GIS) are a useful tool in the management and analysis of large amounts of data with a common geographical base. As a result, GIS have been used to assess the geographic distribution of individuals, populations and species in biology and ecology. Knowledge of the geographic distribution of a target species can provide additional information such as environmental conditions or human-relation aspects (political, social, economic, etc.). GIS have been used to assess the geographic distribution of many cultivated and wild species, including some CWRs. In the case of CWRs, GIS can be used not only to assess geographic distribution but also to detect species richness areas (Hijmans & Spooner 2001), to detect bias in *ex situ* collections (Hijmans & al. 2000), to collect germplasm for *ex situ* conservation (Greene & al. 1999) and to determine the coverage of protected areas for *in situ* conservation (Parra-Quijano & al. 2003). GIS is a very flexible tool that can be used jointly with other techniques like predictive distribution models (Guisan & Zimmermann 2000). These models are based on how environmental factors can determine species distribution (Johnston 1993). To predict species potential distributions, models relate known species distributions with spatial distribution of environmental variables (Guisan & Zimmermann 2000; Zaniewski & al. 2002). Predicted distributions have been used to create potential species richness maps of wild *Arachis* and to detect hotspots and suitable areas for protection between Bolivia and Brazil (Jarvis & al. 2003). In Portugal, Draper & al. (2003) used GIS-based modelling procedures to select protected areas according to habitat suitability for wild species.

The aim of this study is to compare the results from known distribution versus predicted distribution of the *Lupinus* species in Spain in terms of species richness and coverage of *in situ* conservation (all levels of Spanish protected areas). In this way, our approach improves the knowledge of the *in situ* conservation status of a CWR species in a certain region with the contribution of predictive distribution models.

Materials and methods

The area of study was peninsular Spain. Although *Lupinus* records were available for Portugal, some environmental layers (legends and projections) of Spanish and Portuguese thematic maps were not yet compatible.

Germplasm and Herbarium data were compiled from various sources. Geo-referenced data of germplasm accessions were obtained from INIA-CRF (Centro de Recursos Fitogenéticos, Instituto Nacional de Investigación y Tecnología Agraria, Spain) and ausPGRIS (Australian Plant Genetic Resources Information System). Geo-referenced herbarium data were obtained from the Real Jardín Botánico de Madrid (MA, Spain) and bibliographic data was available from the ANTHOS project database (http://www.programanthos.org.). We compiled a total of 1870 records (usually latitude/longitude points) transformed into 1870 UTM (30 grid zone) 1x1 km coordinates representing 3946 grid cells with 500x500 m resolution. The number of records for each *Lupinus* species was as follows: *L. albus* (292), *L. angustifolius* (1081), *L. consentinii* (5), *L. hispanicus* (303), *L. luteus* (159) and *L. micranthus* (30). Due to the low number of records available, *L. consentinii* was excluded from the study.

The environmental variables used to create a geo-referenced database (UTM 500x500 m resolution) may be classified into four data types: climatic, bioclimatic (indices), physical, and soil variables that were used as a mask to filter model predictions. A total of 44 environmental variables were used (Tab. 1). Details about the bioclimatic indices are explained in Tuhkanen (1980) and Draper & al. (2003).

In addition to our presence data, pseudo-absence data also had to be obtained in order to use GLM logistic models. The easiest way to obtain pseudo-absences is to choose the locations randomly over the study area (Hirzel & al. 2001; Zaniewski & al. 2002).

Table 1. Environmental variables included in the geo-referenced database.

Data Type	Variables	Units	Source
Climatic	Temperature: monthly mean, annual mean, maximum and minimum	ºC	Sánchez-Palomares & al., 1999
	Rainfall: monthly mean and annual mean	mm	Sánchez-Palomares & al., 1999
	Dry, cold and warm period	months	Tragsatec - Spanish Minist. of Agricult.
	Thermic amplitude	ºC	Sánchez-Palomares & al., 1999
Bioclimatic indices	Emberger		(Emberger 1932)
	Gorczynski		(Gorczynsky 2004)
	Dantin-Revenga		(Dantin & Revenga 1940)
	Lang		(Lang 1965)
	Angot		(Tuhkanen 1980)
	Thornwaite		(Thornwaite 1948)
Physical	Altitude	m	Draper & al. 2003
	Aspect	º	Draper & al. 2003
	Slope	º	Draper & al. 2003
	Longitude	º	Draper & al. 2003
	Latitude	º	Draper & al. 2003
Soil	Soil type (USDA classification)	8 classes	SEISnet (http://www.microleis.com), CSIC, Spain

However, this method bears the risk of generating pseudo-absences in locations that are in fact favourable to the species (Engler & al. 2004). These authors consider that choosing a wrong absence is not too relevant in common species because the numerous presence records will counteract its effect, but that in rare and threatened plant species this procedure may not be advisable due to their low number of records. Thus, in rare and threatened plant species it is better to select pseudo-absences with the help of specialized tools like ecological niche factor analysis (ENFA) models (Guisan & Zimmermann 2000). According to us the random selection of pseudo-absences is also problematic in common species because (a) presence data is not normally complete in chorological databases and therefore may not be wholly representative, and (b) being a common species increases the probability of obtaining a wrong absence through random sampling over the area. Taking into account the nature of our CWR species data, we used an intermediate procedure to obtain pseudo-absences. We first carried out a principal component analysis (Escofier & Pàges 1991) using the environmental data associated to the locations of each *Lupinus* species. The obtained first principal component (FPC) was used to create a new synthetic variable. We then obtained the mean and standard deviation of this new variable (FPC). The pseudo-absences were thus obtained by random selection from the area resulting from the following formula:

Pseudo-absence area = Total grid cells – Presence cells – (cells with FPC values within mean ± SD)

The environmental modeling procedure used multiple logistic regression (MLR) applied to UTM 500x500 m resolution layers. Only significant and non-correlated variables were used for modeling. The equation resulting from the MLR is:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + \ldots + a_nx_n$$

where y is the occurrence of the species, a_0 is the intercept, $a_1 \ldots, a_n$ are the regression coefficients and $x_1 \ldots, x_n$ are the independent variables.

The probability (P) (Hill & Domínguez 1994) of *Lupinus* species occurrence was obtained by:

$$P = \exp^y / (\exp^y + 1)$$

ROC (Relative Operating Characteristic) statistic was used to validate the models (Fielding & Bell 1997).

A value of $P = 0.8$ was considered the threshold above which the species is more likely to be present than absent. Predicted distributions were filtered by soil type variables, eliminating all categories where *Lupinus* is unlikely to be found (frequency < 5%). Thus, the soil classes eliminated were: aridisol (0.72%), histosol (0.32%), spodosol (0.16%), ultisol (0.48%) and vertisol (1.77%).

We also used the Sites of Community Importance (SCI) from the Natura 2000 Network (Spanish Ministry of Environment). The SCI layer was used to detect matches between habitat suitability levels and protected areas.

Idrisi Kilimanjaro and MapInfo 4.1 were used as GIS software while SPSS 10.0 and Statgraphics 5 were used for statistics and regressions.

Thematic maps of *Lupinus* distributions, *Lupinus* species richness, and matches with protected areas were generated with GIS software to compare known versus potential distributions.

Results

The equations resulting from the modeling process (GLM-MLR) are shown in Table 2. Dantin index was a common factor in all equations and had a negative related effect. Lang index had a high negative weight in the models for the distribution of *L. luteus* and *L. micranthus*, showing affinity for the areas with lower rainfalls. Similarly, Emberger index was also highly negative in *L. angustifolius* and *L. hispanicus* models, keeping the species between the temperate and humid Mediterranean zone of the Iberian Peninsula.

With regard to climatic variables, rainfall affected all species models. In *L. albus* annual rainfall produced a negative effect on species occurrence, whereas in *L. angustifolius* the effect was positive. Species occurrence was positively affected by January rainfall in *L. luteus* and *L. micranthus*, March rainfall in *L. hispanicus* and *L. albus*, and November rainfall in *L. albus*. These positive correlations are coherent with the annual life form of *Lupinus* species (autumn germination and flowering in spring). Temperature variables were selected in the models for *L. albus*, *L. angustifolius*, *L. hispanicus* and *L. luteus*. *L. luteus* distribution was positively related to the variable cold period, but negatively related to December mean temperature, indicating that this species tolerates a long cold period but not extremely low temperatures.

The physical variables altitude and longitude positively affected the distribution of *L. albus* and *L. angustifolius*, respectively, while *L. hispanicus* distribution was positively affected by both.

ROC statistics ranged between 0.7 and 0.9. These values fall within the expected values for adjusting models according to Fielding and Bell (1997).

Maps with known and predicted distributions are shown in Figure 1 for the five *Lupinus* species modeled. Known and predictive distributions can be compared to identify new areas with a high probability of *Lupinus* species occurrence. The models detected potential areas for exploration for all species except *L. angustifolius*.

Table 2. Equations used for modeling *Lupinus* species distributions and their ROC statistics: R - Rainfall, T - Temperature, 01, 02,…12 - January, February,…December. Max - Maximum, Min - Minimum, Dantin - Danting-Revenga index.

Lupinus	Equation	ROC
albus	Y= -0.145+1.33*[Altitude]-5.35*[Dantin]+0.657*[R03]+2.52*[R 11]-4.78*[R anual]+4.28*[T05]	0.78
angustifolius	Y= 0.82-2.94*[Dantin]-3.49*[Emberger]-2.8*[P07]+0.7*[R anual]-0.26*[T min]+1.34*[Longitude]	0.75
hispanicus	Y=1.03+1.21*[Altitude]-4.15*[Dantin]-4.84*[Emberger]+1.02*[R 03]+0.71*[Tmax]+1.1*[Longitude]	0.81
luteus	Y=0.96-2.94*[Dantin]-7.83*[Lang]+4.262*[R 01]-2.9*[T 12]+2.13*[ColdPeriod]	0.9
micranthus	Y = -1.1-1.82*[Dantin]-7.76*[Lang]+4.06*[R01]	0.7

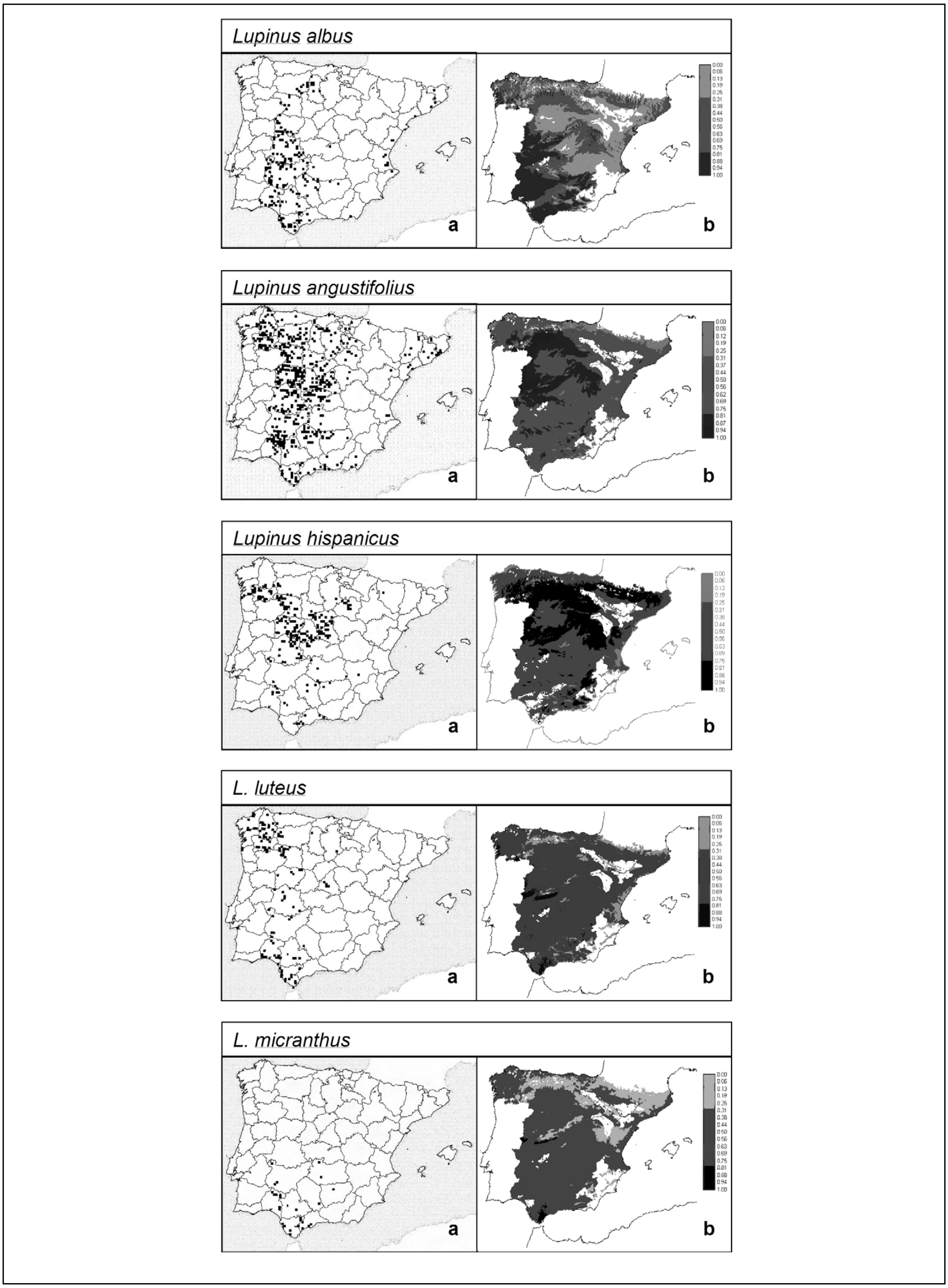

Fig. 1. Known vs. predicted distributions for five *Lupinus* species in Spain: a - known *Lupinus* populations in 10x10 km grids, b - predicted distributions generated by GLM models. The legend shows the intervals of probability according to the model: black areas correspond to a probability of 0.8 or higher, white areas have no data or have been filtered by soil type.

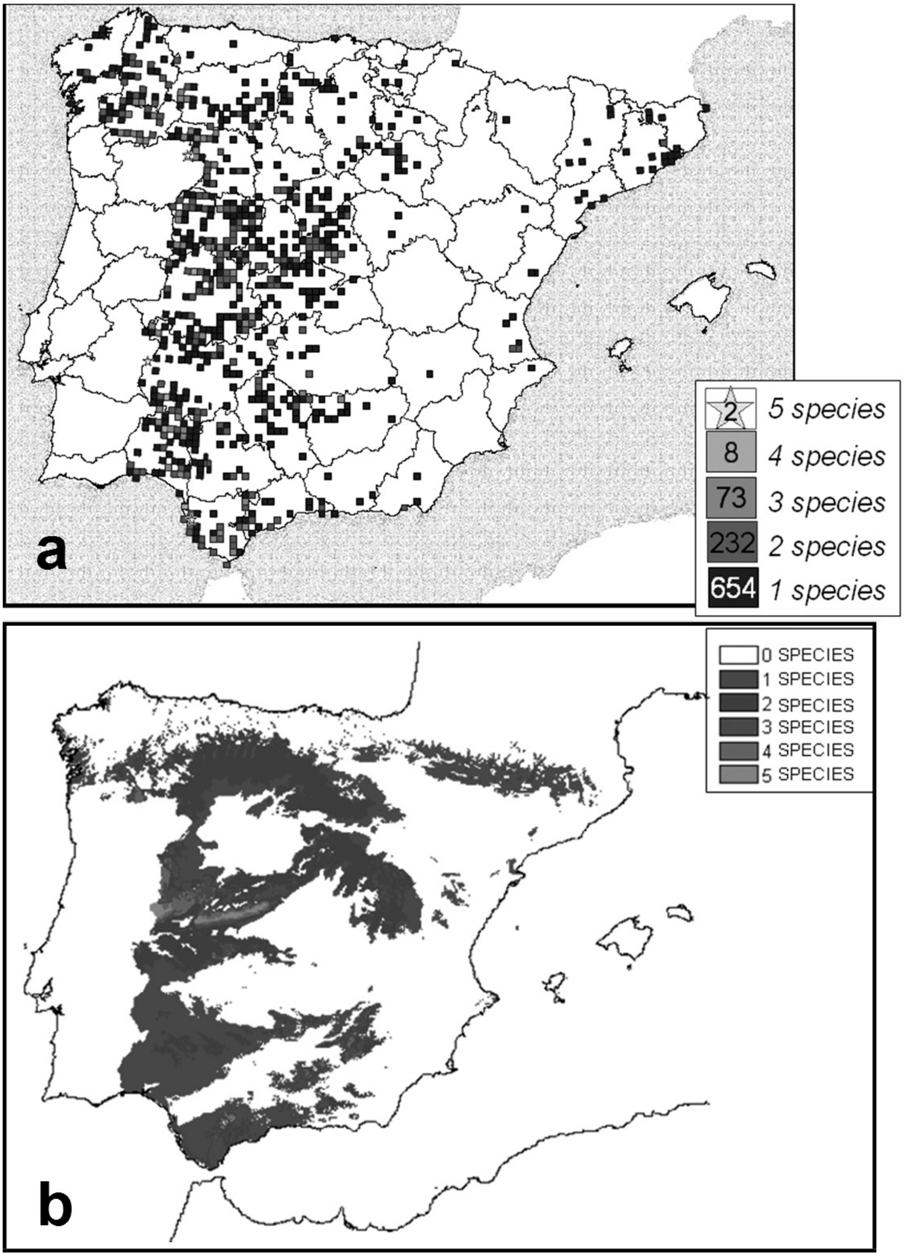

Fig. 2. Richness maps obtained from known and predicted *Lupinus* distributions: a - species richness from currently known distributions, the number in the shaded square indicates the number of 1x1 km grids that contain five, four, three, two or one species; b - species richness based on predicted distributions with p≥0.8, grid size is 500x500 m.

For *L. albus* the model detected some suitable areas to the east and north of its currently known distribution. In the case of *L. angustifolius*, the predicted distribution seems to be well represented by known populations, while for *L. hispanicus* a high probability of species occurrence was detected in several areas with no known records, especially in the Northeast. *L. luteus* and *L. micranthus* predicted distributions showed a similar pattern. In *L. luteus*, some new locations with high probability of species occurrence were found, whereas in Galicia and Andalusia some areas with known populations showed low probability of species occurrence. In *L. micranthus* a new area with high probability of occurrence far from its currently known distribution was identified in the Sierra de Gredos (Central Spain). This region showed a high probability of occurrence for all modeled species.

Known and predicted richness areas are shown in Figure 2. The richness map of currently known localities is represented by a 1x1 km grid. The richness map based on predicted localities is a product of the combination of all species predicted distributions. Shade legend is the same for both maps.

Figure 3 shows maps with the matches found between SCIs and currently known populations or predicted distributions. These maps indicate the coverage degree of Spanish protected areas over *Lupinus* populations.

The number of matches between distributions and SCI notoriously increased from 95 matches with currently known populations to 914 with predicted distribution (960%). This increase was most evident in SCIs containing 4 and 5 species.

Conclusions

The equations used by the models reflect the most influential variables affecting each *Lupinus* species distribution. A relevant relationship between the models for *L. angustifolius* and *L. hispanicus* and for *L. luteus* and *L. micranthus* is found since their most influential equation components are the same. According to equation components, *L. albus* prefers dry areas with high temperatures in April, which coincides with the period of pod filling and the end of flowering, and high rainfall in November at germination. These results are in accordance with a study on growth and yield of *L. albus* in the south of Spain (Lopez-Bellido & al. 1994). Great similarities can be found between the known and predicted distribution for *L. albus* and few new areas with a high probability of *L. albus* species occurrence were found. It is also interesting to verify how the models can also be fitted in the case of cultivated species like *L. albus*, the only *Lupinus* species cultivated on a commercial scale in Spain. *L. luteus* is cultivated in the Iberian Peninsula but mainly in Portugal. *L. angustifolius*, the third cultivated species, is found in Spain and Portugal but only in its wild form.

In the case of *L. angustifolius* and *L. hispanicus*, both species prefer semi-arid or subhumid regions according to the negative relationship with Emberger and Dantin-Revenga indices. For *L. angustifolius* the predicted distribution is very similar to the known distribution. In fact, predicted distribution does not show a high probability of finding populations in some areas where there are known populations. For this case we think that the modeling approach has been very conservative. One factor that could explain this situation is the great amount of data of known populations for *L. angustifolius* (1081 records). On

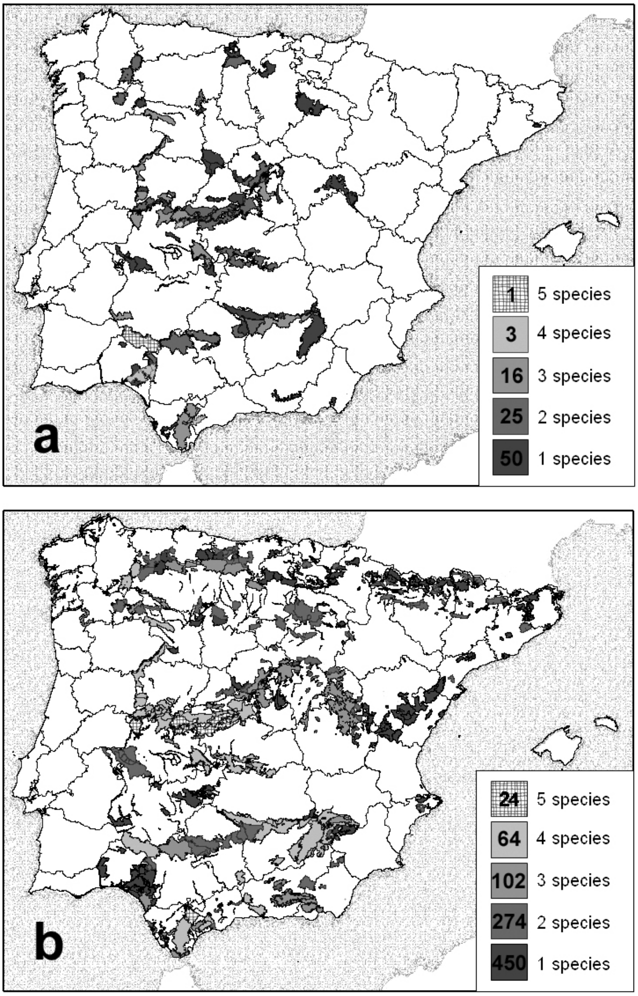

Fig. 3. Matches between SCIs and a) currently known locations or b) predicted distribution ($p \geq 0.8$) for five *Lupinus* species in Spain. The number in the shaded square indicates the number of SCI that contain five, four, three, two or one species.

the other hand, the model for *L. hispanicus* is the least conservative. *L. hispanicus* with only 303 records has a predicted distribution that shows many areas where there are no currently known populations, such as the Pyrenee Mountains. It is even possible to find areas with basic soils with a high probability of *L. hispanicus* occurrence when it is known that there is a strong relationship between *L. hispanicus* and acid soils (Castroviejo & Pascual 1999). Thus, the model for *L. hispanicus* could be improved if the final soil filter were more selective.

For *L. luteus* and *L. micranthus* equation models, the most imperative condition was the Lang rainfall index. These two species occur in arid regions, but they need rainfall in January. In the case of *L. luteus*, a cold period without extreme low temperature is important, likely for the vernalization process. For *L. micranthus* the predicted distribution is clearly oriented to the south of the Iberian Peninsula. However, the predicted distributions of both of these species detected an area with a high probability of finding new populations around the Sierra de Gredos in the center of the Iberian Peninsula where there are few *L. luteus* records and no *L. micranthus* records. The models for the other *Lupinus* species also indicated a high probability of finding new populations in this area.

It should be noted that the models applied are only based on abiotic factors. *Lupinus* species normally occur in perturbed habitats at the first stages of succession and are poor competitors. Therefore, some of the sites predicted by these models may not be suitable due to biotic factors. It is also important to take into account that *L. albus* is a cultivated species with no wild forms in Spain. Therefore, in this case, the models show potential sites for cultivation rather than natural occurrence.

On comparing predicted and known richness maps, it is evident that a larger number of SCI areas than what is currently known must contain a relevant number of different species of *Lupinus*. The predictive richness map provides relevant clues on candidate sites for a high concentration of *Lupinus* species. Thus, modeling species distribution is a tested statistical tool that allows us to identify potential high species richness areas that have not been detected previously and to select them for *in situ* conservation. In the case of *Lupinus*, efforts are currently under way in Spain to select upon the identified candidates sites that may be turned into genetic reserves for the *in situ* conservation and management of these crop wild relatives.

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C. Eichberger, M. Sigl & H. Rühfel

Trees and Shrubs on Classical Greek Vases

Abstract

Eichberger, C., Sigl, M. & Rühfel, H.: Trees and shrubs on classical Greek vases. — Bocconea 21: 117-130. 2007. — ISSN 1120-4060.

The identification of plants mentioned in classical Greek and Roman literature was performed mainly during the 19th century. Plants depicted on sculptures, vases or coins received attention much later. This paper is exclusively devoted to plants depicted on Greek vases of the classical period, mainly trees and shrubs. Some of the depicted plants can be recognised without difficulty, but for others, archaeological and mythological knowledge must support identification. The grape vine is easily recognised by its typical leaf shape and the grapes, the palm tree by its characteristic growth, whereas myrtle, olive and laurel, the leaves of which as depicted are often more or less identical, can usually be identified by their known association with a particular deity or hero. Such traditional connections, in Greek mythology, between a god or hero and a particular plant dedicated to him, especially trees and shrubs, are well known. They are illustrated here by the representations of about 18 vases.

Introduction

The identification of plants mentioned in classical Greek and Roman literature was performed mainly in the 19th century (see e.g. Sprengel 1822; Billerbeck 1834; Murr 1890, 1892; Hehn 1902, 1911). More recently, attention was paid to plant illustrations on sculptures, vases or coins (Baumann 1993, 1999, 2000; Rühfel 2003; Eichberger 2003, a.o.). Only plants depicted on classical Greek vases, mainly trees and shrubs, will be considered in this paper (the abridged version of a lecture held during the OPTIMA Meeting in Belgrade, to be published in full elsewhere). The earliest representations of trees and shrubs on Greek vases appeared between 580 and 550 BC, probably influenced by the East Ionian view of nature. Most early examples are found on black-figured vases of the late 6th and the early 5th century.

On some vases the depicted plants are easily identified; on others, however, archaeology and mythology must support botanical identification. In this paper we give examples of both kinds. The vine, for instance, with its typical leaves and grapes, or the date palm with its characteristic growth, are easy to recognize. The identification of myrtle, olive and laurel, however, is made difficult by the similar shape of their leaves and requires support from their known association with a deity or hero who is shown in the same picture. A particular plant may also be associated with certain everyday scenes, or with life to come (Elysion). From ancient times, Greek mythology connects gods and heroes with certain plants, especially trees and shrubs, but there is no strict one-to-one correlation: A given plant may be linked to several gods, and conversely, different plants may be related to one god. The most popular associations are those linking Athene with the olive tree sacred to her, and Apollo (also god of the arts and sciences) with the laurel dedicated to him.

Materials and methods

The nomenclature of the mentioned trees and shrubs follows Strid & Tan (1997, 2002), Tutin & al. (1964-1993), Davis (1965-1985) and Greuter & al. (1984-1989). All illustrations of vase pictures are reproduced by courtesy of the respective museums or institutions.

Results and discussion

Date palm *Phoenix dactylifera* L. (Gr. φοίνιξ).

On relief pictures from Babylonia and ancient Egypt, the date palm is represented more often than any other plant. There is evidence to show that cultivation of *Phoenix dactylifera* dates back around 6000 years (Zohary & Hopf 2000).

Because they yield edible fruits, mainly female plants are cultivated. The need for artificial pollen transfer to ensure fertilization was well known in antiquity (Theophrastos HP 2, 8, 4; see Amigues 1988-2003).

Fig. 1. *Phoenix dactylifera* on a late archaic black-figured lekythos of the Amasis Painter (London, British Museum, B 548) - left: Apollo holding his lyre, opposite to him a date palm, right: Artemis with her bow and quiver.

Fig. 2. *Phoenix dactylifera* – left: Calyx krater of the Kadmos Painter, around 400 BC (Saint Petersburg, Heremitage 1807), Apollo meeting his brother Dionysos in Delos, a date palm between them, right: Black-figured amphora of Exekias, ca. 530-525 BC (Boulogne-sur-Mer, Musée Communal, Inv. No. 558), Aias preparing his suicide, on the left a date palm.

In Greece the date palm was cultivated only rarely. According to Theophrastos (HP 3, 3, 5; see Amigues 1988-2003), its fruits never completely ripen there.

Phoenix theophrasti Greuter, the only *Phoenix* species native to Europe, was described in the 1960s from the river valleys and estuaries of eastern and southern Crete (Greuter 1967); it also occurs in a few places in western Turkey (Boydak 1985). This species forms several stems. The fibrous and inedible fruits turns from yellowish brown to blackish.

The representations of the date palm appear in the 2nd millennium BC in Cretan-Minoan and Mycenaean art (Marinatos 1973). The palm occupies a special place in Greek plant ornamentation and has inspired Greek art with the much used fan-shaped palmette motif. Although it is not native to mainland Greece, its rarity and unusual appearance gave the date palm a special place in art and legends (Baumann 1999). In mythology it is closely associated with Apollo and his twin sister Artemis.

In Figure 1 we see Artemis, the virgin goddess of animals and hunting, and her twin brother Apollo on a lekythos of the Amasis Painter. Apollo is holding his instrument, a seven-stringed lyre. Artemis can be recognised by her bow, her quiver and the fawn accompanying her. The artist certainly wanted to show the palm tree, sacred to Apollo, though he chose a stylised representation and did not show the petiole bases remaining on the trunk.

A vase-painting of the early 4th century BC (Fig. 2, left) shows Dionysos, the god of wine, with his retinue of satyrs (silens) and maenads (bacchantes) coming to Apollo's sanctuary. The godly brothers are shaking hands behind the Omphalos in the shade of a stately palm tree. Dionysos is holding his thyrsos wand entwined with ivy, Apollo a stick of laurel (the laurel was traditionally dedicated to Apollo). Here the representation is not of a Delphic palm, since palm trees do not grow in Delphi at the foot of Mount Parnassos, but merely of an attribute of the god Apollo.

The famous vase painter Exekias shows the Greek hero Aias near Troy preparing his suicide (Fig. 2, right). The palm tree, being rare in Greece, symbolises the fame of the hero. Exekias paints the palm with a scabrous caudex, which stands for the characteristic remainders of petiole bases covering the trunk.

Ivy *Hedera helix* subsp. *helix* L. and *Hedera helix* subsp. *poetarum* Nyman (Gr. κισσός; also κιττός).

The representations of ivy already appear in Minoan art. In Mycenaean art, ivy leaves were often used as elements of decoration, though evidence for a connection between Dionysos and the ivy in that period (1600-1100 BC) is still lacking.

Subsequently, the powerful ivy was to play an important part in the Dionysian cult. The main festivals of this god were in winter and springtime, when vines stand leafless in the vineyards and only the evergreen ivy was available to decorate the god's statue (Simon 1998: fig. 264, 265) and the wine jugs (Rühfel 1984: fig. 70, 88, 92). The wreaths crowning successful poets and actors during the main Dionysia were also made of ivy (Pauly 1905: 2838). Bacchantian revellers crowned themselves with ivy around the seasons. Drinkers appreciated the coolness of its firm leaves that stay fresh and bright for a long time. Besides, ivy was believed to be a protection against drunkenness.

Together with the widespread *Hedera helix* subsp. *helix*, a second subspecies of ivy occurs in Greece, Turkey, Cyprus and the Near East: *H. helix* subsp. *poetarum*, characterised by yellow berries and lighter green leaves, those of vegetative shoots being less lobed than in *Hedera helix* subsp. *helix*. The vase painters gave preference to *H. helix* subsp. *poetarum* with its heart-shaped leaves. *H. helix* subsp. *poetarum* probably has its origin in Transcaucasia (Rutherford & al. 1993). This subspecies was used for making poets' wreaths.

On Figure 3 (left) we see Oinopion, the founder of the famous vineyards of Chios, facing his divine father Dionysos on a neck-amphora of Exekias. He brings wine from Chios in a jug. Oinopion lifts his hand in a gesture of greeting as if to say: "With my very best wine I will fill your kantharos, my father!". The god's left hand is holding the ends of four

Fig. 3. *Hedera helix* – left: Neck amphora of Exekias, around 525 BC (London, British Museum B 210), Oinopion meeting his father Dionysos; the god is holding ivy shoots, right: Neck amphora of the Berlin Painter, around 490 BC (München, Staatliche Antikensammlungen No. 8766), Dionysos crowned and decorated with ivy, holding the thyrsos wand.

Fig. 4. *Hedera helix* – left: Attic amphora, ca. 500 BC (Baumann 1993a: 61 fig. 100, photograph: M. Hirmer), a maenad with a thyrsos wand; Maenads became frenzied by using psychedelic fungi like the fly agaric, they chew laurel leaves as well (Ranke-Graves 1960: 1, 16), right: cup of Makron, ca. 480 BC (Berlin-Charlottenburg), Dionysos, crowned with ivy and holding the thyrsos wand, accompanied by a flute-playing satyr.

ivy branches bearing heart-shaped leaves that are painted with great accuracy.

In the right-hand figure the god Dionysos appears on a neck-amphora of the so called Berlin Painter. He bears the thyrsos wand, a dried stalk of *Ferula communis*, topped by a pine cone and entwined with fresh ivy (Fig. 3, right). The thyrsos wand (Fig. 4) is another characteristic attribute of Dionysos and his attendants. The sticks were strong enough to rest upon, but too light to hurt or wound.

Myrtle *Myrtus communis* L. (Gr. μύρτος, μυρρίνη).

In classical antiquity myrtle, one of the best known shrubs of the evergreen macchia, was often planted near temples and sanctuaries. Theophrastos cites it several times. The fragrant twigs were used, and the wood as well. Pelops, a Greek hero, dedicated a cultic statue cut in myrtle wood to Aphrodite (Pausanias 5, 13, 7; Eckstein 1986-1989). The oil extracted from the leaves has astringent, deodorant and antiseptic properties and has been used to cure illnesses of the respiratory organs, as a spice and to flavour wine, especially from Chios.

The evergreen myrtle, a symbol of youth, beauty and immortality, always stands under the protection of Aphrodite, the goddess of love. In former times it was sacred to the Semitic Ištar (Blech 1982: 322). Ovidius (Fasti 4, 143; Gerlach 1960) and Plutarch (Num. 19; Feldmeier 2003) tell the tale thatAphrodite, when she rose from the sea (“born of the white sea foam”),hid her naked beauty from greedy satyrs behind a thicket of myrtle. The evergreen myrtle, frequent in Greece, was the favourite plant for making wreaths (Blech 1982: 80, 284, 318f.). *Myrtus communis* was the special attribute of the goddess of beauty (Fig 5, left). The characteristics of this plant correspond with the nature of the goddess: the delicate leaves dotted with tiny oil glands, the large white aromatic blossoms and the

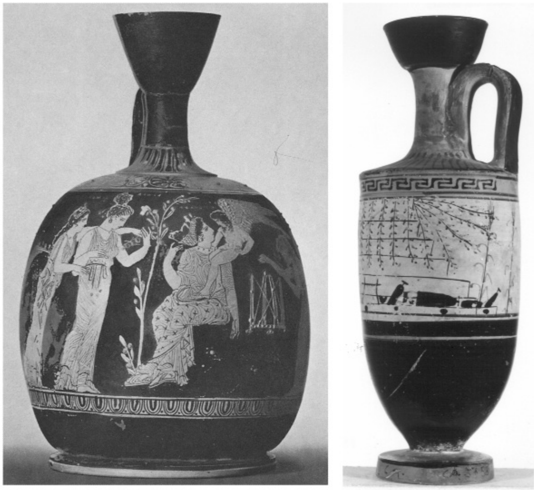


Fig. 5 *Myrtus communis* – left: ‚Lekythos of the Meidias Painter, ca. 420-410 BC (London, British Museum), Aphrodite with little Eros sitting in her garden, a branch of myrtle next to her, right: Lekythos of the Tymbos Painter, around 460 BC (Paris, Musée du Louvre CA 3758), with myrtle branches decorating a tomb.

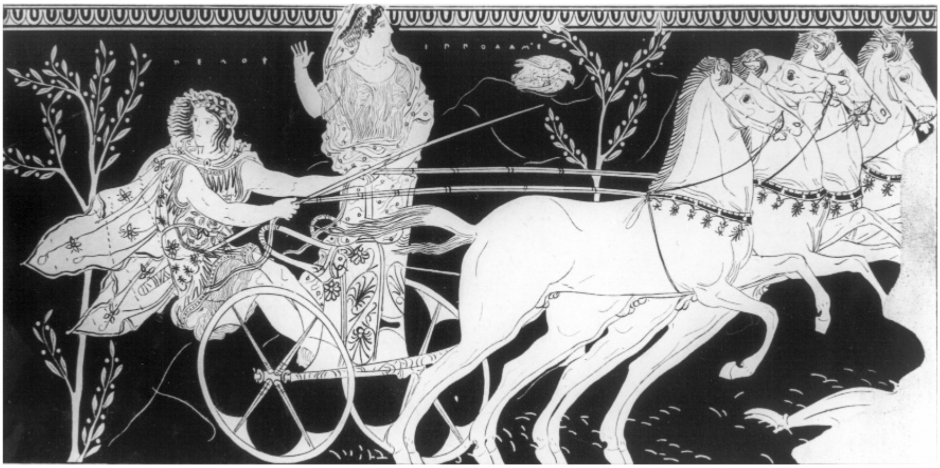
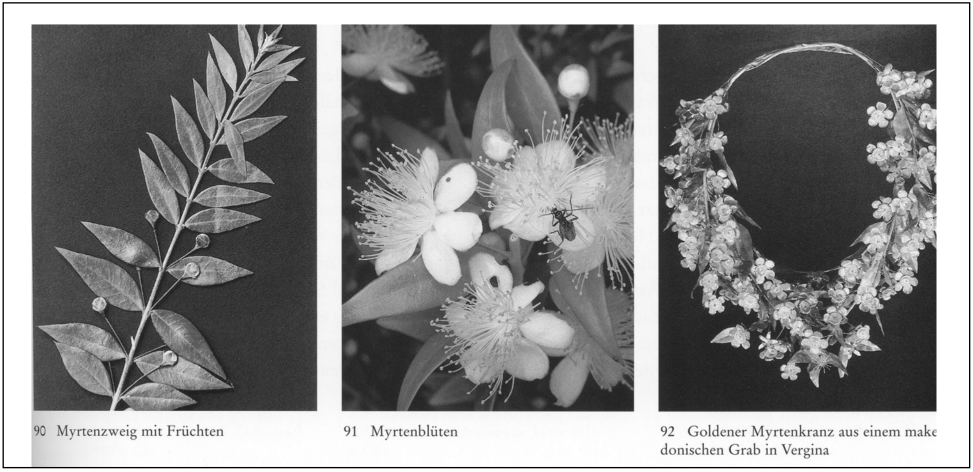


Fig. 6. *Myrtus communis* on an amphora, around 410 BC (Arezzo, Museo Civico No. 1460), Pelops and Hippodameia driving along the sea shore, myrtles on both sides.



90 Myrtenzweig mit Früchten

91 Myrtenblüten

92 Goldener Myrtenkranz aus einem make
donischen Grab in Vergina

Fig. 7. *Myrtus communis* – left: A pressed twig, with flower buds on long pedicels, centre: Fresh blossoms, right: A golden myrtle wreath from a royal tomb at Vergina (Makedonia), ca. 340 BC (Baumann 2000: fig. 90-92, photographs: H. Baumann).

little black fruits on long pedicels, sweet smelling as well. In the love-goddess's honour, brides decorated themselves with wreaths of roses and myrtles (Baumann 1999).

A vase picture of the late 5th century (Fig. 6) shows Pelops, prince of Lydia, accompanied by Hippodameia, the daughter of Oinomaos, king of Elis. Pelops had obtained Hippodameia in marriage by winning a race against her father, who had vanquished then killed previous suitors. Pelops is rushing along the seashore with Poseidon's horses and carriage, as is indicated by little waves and a dolphin. Pelops' robe and hair, decorated with a myrtle wreath, are fluttering in the air stream. Next to him, Hippodameia stands in proud bearing. The two young trees on either side of the lovers to show the presence of Aphrodite (just like the two pigeons, also sacred to her) are myrtle trees, closely connected to the goddess. The painter characterises them well with their long stalked fruits**.** He mostly ignores the opposite leaf arrangement.

The evergreen myrtle was also sacred to Persephone, carried off by Hades to be the queen of the underworld, and therefore it can also be attributed to the sphere of death (Lohmann 1979: 123ff., Blech 1982: 94f.). The dead and their tombs were crowned with myrtle, the tumuli (burial mounds) were planted with it (Euripides Elektra 512; Stoessl 1958-1968). Myrtle wreaths made of gold, as have been found in tombs (Burr Thompson & Griswold 1963: 33, Blech 1982: 426), show the exceptional popularity of that myth. The myrtle wreath from a royal tomb in Vergina (Fig. 7, right; see also Andronikos 1978: t. 154) is of perfect beauty and high fidelity. Those initiated to the Eleusinian mysteries wore myrtle wreaths during the processions because myrtle fragrance of the was considered a symbol of the Elysion to come.

On a lekythos (Fig. 5, right) branches with dainty leaves spread all over a tomb. The delicate leaves, painted in characteristically opposite pairs, indicate a tomb decoration with myrtle. On the right side, two reed canes can be discerned; also plants of death and the underworld.

Olive tree, olive *Olea europaea* L. var. *europaea* (Gr. ἐλάα) and *O. europaea* var. s*ylvestris* (Mill.) Lehr (Gr. κότινος).

Because of the long lasting cultivation of the olive tree its origin was not easy to define. Linguistic and phonetical research showed for instance that the origin could be found around the Caucasus Mountains (Genaust 1996). Until a few years ago it was thought that the oleaster (*Olea europaea* var. s*ylvestris*), a thorny and divaricate shrub with angular twigs, small leaves and very small, bitter fruits was simply a variety of the cultivated olive *Olea europaea* var. *europaea* which escaped from cultivation (Green & Wickens 1989). The oleaster is now an element of macchia and phrygana and open forests as well. It is even seen as an element of the potential natural vegetation of inshore regions ("Oleo-Ceratonion" cf. Eichberger 2001). Today it is clear that the cultivated olive derives with certainty from (non-feral) oleaster and originated within the Mediterranean area (Zohary & Hopf 2000).

The olive is one of the oldest cultivated plants of the Mediterranean region. In Palestine, for example, stone mortars and stone mills dating from that period have been found. Many presses and mills from Minoan times have also been excavated., No wonder, the tree is surrounded by rich symbolism. Minoan mural-paintings at Knossos already show olive trees (see Schäfer-Schuchardt 1993: 31 a.o.). The link between the olive tree and the worship of gods and in religious rituals is often apparent, especially in the case of Athene, who was already worshipped in the 2nd millennium BC in the Minoan-Mycenaean area (Möbius 1933, Pauly 1937: 1998ff., Ventris & Chadwick 1956, Richter 1990).

The legend of Attica's first olive tree describes a dispute between Athene, patron of Athens, and Poseidon, god of the sea. Both advanced the claim to own Attica for themselves. It was agreed that the country would belong to the god who gave the most useful gift. Poseidon caused a salt spring to gush forth out of the rocks of the Akropolis. As to Athene, she made the first olive tree grow. King Kekrops awarded the victory to the god-

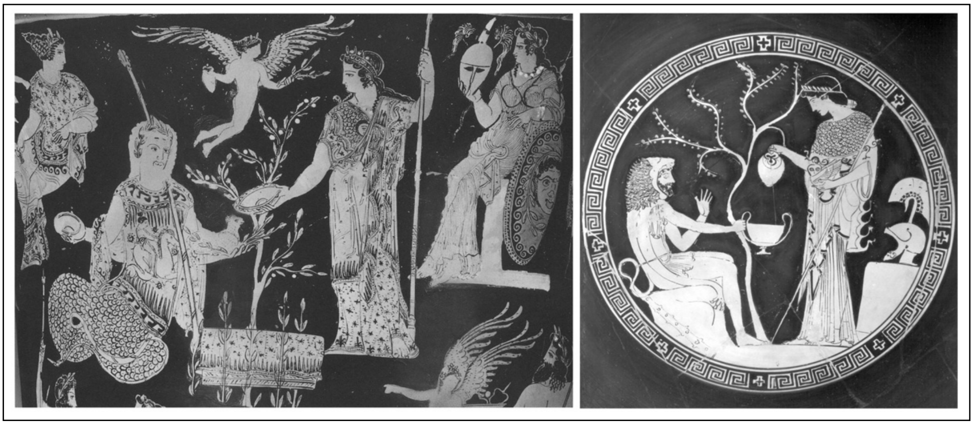


Fig. 8. *Olea europaea* – left: Calyx-krater of the Kekrops Painter, ca. 410 BC (Schloss Fasanerie, Adolphseck), Athene makes the first olive tree grow; on the left, king Kekrops, right: Red-figured cup of the Oidipus Painter, around 470/465 BC (München, Antikensammlung 2648), Athene invigorating Heracles, between them an olive tree.

Fig. 9. *Olea europaea* – left: Coin featuring Athene's owl with a fruiting olive twig (Baumann 2000: 45, fig. 100, photograph: M. Hirmer), right: Skyphos, 3rd century BC, Würzburg, Martin-von-Wagner-Museum), Athene's owl between olive twigs.

dess and made her ruler of Attica (see Fig. 8, left).

Many Attic coins show on one side (face) the head of the goddess and on the other (tale) the little owl sacred to her, framed by a wreath of olive leaves (Fig. 9, left). Olive twigs frame the sacred owl of the goddess on innumerable drinking bowls or skyphoi (Fig. 9, right; Hagenow 1982; Schäfer-Schuchardt 1993).

The amphorae were filled with the precious oil of Athens' olive trees and presented as prizes – in addition to the main prize, the olive wreath – to the victors of the Panathenea, a sports and arts competition. The Attic olive trees, which were always regarded as Athene's gift, were protected by rigorous laws (Pauly 1937: 2006-2008): Any unauthorized destruction by cutting was severely punished. In the time of Pericles, olive oil provided the foundation for the wealth of Athens and was used to pay for imported cereals. The oil was so valuable that Athens kept a monopoly in its export. Olive oil had many uses: the first pressing produced the virgin oil to be used in food; the second was used for the production of ointments and the third gave oil for lamps (Richter 1990; Baumann 1999).

The Figure 8 (right) shows a meeting between Heracles and Athene. Supporting Heracles, who sits exhausted in front of her, Athene has put away her helmet and her breast plate (aigis). She fills his kantharos with magic invigorating nectar and turns her head amiably towards the hero. The little owl (Pauly 1909: 1064ff.; Keller 1909-1913: 2, 39ff.) at Athene's hand is already stretching its wings, a good omen for Heracles. The tree between the two figures can, with some certainty, be identified as Athene's sacred olive tree.

Being an extremely popular plant in ancient Greece, we also find the olive tree as a subject of everyday life, for instance the gathering of olives on a vase of the Antimenes Painter (Fig. 10, left): A boy sits in the crown of an olive tree (with a characteristically crooked trunk) and two bearded men hold long sticks for beating off the olives. According to Pliny (NH 15, 3, 12; König 1981) reed sticks were used for this purpose. Another boy sitting on the ground is gathering the fallen olives in a basket.

Fig. 10. *Olea europaea* – left: Neck amphora of the Antimenes Painter, around 520-510 BC (London, British Museum B 226), two men and two boys harvesting olives, right: Pelike (Vatican, Museo Gregoriano Etrusco Inv. 413), oil dealer with his customer.

On the front (Fig. 10, right) and reverse of a black-figured pelike we observe a lively scene of olive oil being sold. For the precious oil, a vessel in the shape of a pelike, with a downward projecting belly and stable flat foot, was well suited. On the front of the vase a young oil dealer and his customer are sitting face to face in thze shade of a typically crooked olive tree, which stands for the dealer`s olive grove. Using a funnel, the dealer fills his customer's lekythos with oil. The painter wrote the dealer's thoughts in the middle of the picture: "Oh Father Zeus, let me become rich!". The customer, with his left hand stretched forward while speaking and swinging his stick, seems to doubt whether there is enough oil in the funnel to fill his lekythos.

Grape vine *Vitis vinifera* L. (άμπελος, also οίνας, οίνοφόρος).

We already know the cultivation of vine from Egyptian representations. Along with cereals and the olive tree, it is one of the oldest cultivated plants of mankind.

The grape vine, *Vitis vinifera*, is the single Mediterranean representative of its genus. It has its origin in the broadleaved forests of the Mediterranean and the Near East. Its sweet-smelling, paniculate flowers appear on some long shoots instead of the lowest tendrils.

The wild *Vitis vinifera* (subsp. *sylvestris* (C. C. Gmel.) Berger) does not differ much from the cultivated forms. It is found in S Europe, SW Asia, in Palestine and NW Africa.

Fig. 11. *Vitis vinifera* – left: Ionian cup around 550 BC (Paris, Musée du Louvre F 68, Cp 263), the vegetation god Dionysos amidst old grapevine plants, right: The famous cup of Exekias, around 540/535 BC (München, Staatliche Antikensammlungen No. 2044), Dionysos sailing on the sea.

Cultivated grape vines placed in *Vitis vinifera* subsp. *vinifera* are derived from this subspecies. Today we know more than 5000 different variants of grape vine (Tutin & al. 1964-1993; Hagenow 1982).

The grape vine was believed to have been created by Dionysos and was sacred to him. Numerous myths and artworks demonstrate the close links between the god of wine and this ancient cultivated plant. There are several legends about Dionysos and his gift of the first grape vine plants, to which magic power is invariably ascribed. Archaeological finds show that wine was a customary drink about 2000 BC in the Minoan culture. In Egypt, viniculture was erratic and not known before 3000 BC. Many Egyptian frescoes in tombs show the growing and pressing of wine.

Around 570 BC, the vase painters began to represent the god Dionysos together with the vine *oínas*, and twenty years later, with the ivy *kissós*, making use of much older traditions. Homer (Iliad 18, 561ff.; Rupé 1961), the first to refer to the grape vine in Greece, recounts how Achilleus received a precious shield decorated with vines. In the same Iliad (14, 325) he also praises the god, the giver of vine as a “delight of mankind“. Homer held that people who were ignorant of wine were barbarians.

On Figure 11 (left) one can see Dionysos, also the god of vegetation, amidst two flourishing stocks of grape vine; by touching those branches that still lack blossoms he creates fertility everywhere. Here the painter probably had in mind very old individuals of the wild grape vine (cf. Theophrastos HP 4, 13, 4).

Figure 11 (right) again features Dionysos, lying comfortably in his dolphin-shaped ship in the shade of its white sail. Crowned with ivy, he holds his drinking horn. The

Fig. 12. Winemaking shown on a neck-amphora, around 520/510 BC (Würzburg, Martin-von-Wagner-Museum Inv. L 208). – left: Silens (satyrs) pressing the grapes, right: Silens filling the kantharos of Dionysos with fresh wine.

miracle-working god let a vine full of grapes grow around his ship's mast like a bower. The representation of the three- to five-lobed leaves and the grapes is extremely true to nature.

One can observe the subject of wine-making on both sides of a neck-amphora of the 6th century. The front side (Fig. 12, left) shows the pressing of the grapes. A silen is treading them. He stands in a flat basket, retaining the draff (skins and pips) and letting the clear grape juice flow into a ready vat. Another silen is carrying a basket full of grapes, a third one starts climbing the vine to pick the grapes (for further details see Hagenow 1982; Richter 1990; Weeber 1993; McGovern & al. 1996).

The other side of the amphora (Fig. 12, right) shows Dionysos crowned with ivy. He is sitting on a folding chair under a vine stock. On the right, a silen carrying a large pointed amphora is ready to pour the fresh wine into a pithos that is half dug into the ground, having first filled the god's kantharos. One silen is dancing wildly on the edge of the pithos in anticipation of his beloved wine. The vine bears huge grapes; the leaves are mere dots, devoid of petioles.

The ancient Greeks, in their mythology, expressed an intense interest in natural phenomena. Since they regarded trees as sanctuaries of the gods they gave them a sacred meaning and dedicated them to certain deities. Therefore, fidelity to nature was not as important to the artists as was symbolism, as everybody knew the close links between the gods and their plants.

Acknowledgements

The authors wish to thank the curators of the indicated museums and collections holding the reproduced objects for permission to publish these reproductions. We also would like to thank two anonymous reviewers for various comments, helpful discussion and linguistic corrections to a further draft of this paper.

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V. Stevanović, K. Tan & A. Petrova

Mapping the endemic flora of the Balkans - a progress report

Abstract

Stevanović, V., Tan, K., Petrova, A.: Mapping the endemic flora of the Balkans - a progress report. — Bocconea 21: 131-137. 2007. — ISSN 1120-4060.

The distribution of Balkan endemics is mapped using similar 50×50 km UTM squares as adopted in the Atlas Florae Europaeae project. Approximately 1660 taxa at species and subspecies rank out of an estimated 2670 taxa or c. 60% of the total endemic Balkans flora, has already been mapped. The following families contribute most in terms of species number: *Caryophyllaceae* (293), *Cruciferae* (161), *Scrophulariaceae* (136), *Liliaceae* (119), *Campanulaceae* (111), *Boraginaceae* (81), *Ranunculaceae* (62), *Violaceae* (59), *Rosaceae* (49), *Dipsacaceae* (45) and *Iridaceae* (36). In addition, c. 30 smaller families as well as certain large genera that may be mentioned as the most prominent, are included: *Verbascum* (67), *Asperula* (44), *Scutellaria* and *Micromeria* (13 each), *Satureja*, *Nepeta* and *Saxifraga* (12 each) and *Pedicularis* (11).

Introduction

The project: Mapping the endemic flora of the Balkans, was initiated only two years ago. It is based on distribution data collected and mapped using similar 50×50 km UTM squares as adopted in the Atlas Florae Europaeae project coordinated at Helsinki. For the grid system applied and grid cell names see Kurtto & al. (2005). A large database has been built up and presently caters for more than 2670 taxa at species and subspecies rank. Part of this work was completed (maps and distribution data for 1100 taxa) and presented at the Third International Balkan Botanical Congress (Stevanović & al. 2003). That output includes the following families which in terms of numbers (in parentheses) contribute most to the endemic flora of the Balkans: *Caryophyllaceae* (293), *Cruciferae* (161), *Campanulaceae* (111), *Boraginaceae* (81), *Ranunculaceae* (62), *Violaceae* (59), *Rosaceae* (49), *Iridaceae* (36), c. 30 smaller families as well as certain genera of which *Verbascum* (67), *Asperula* (44), *Astragalus* (30), *Chamaecytisus* (13), *Scutellaria* and *Micromeria* (13 each), *Satureja*, *Nepeta* and *Saxifraga* (12 each) and *Pedicularis* (11), may be mentioned. Further work covers an additional 560 taxa from the following families: *Liliaceae* (119), *Plumbaginaceae* (45), *Hypericaceae* (22), *Linaceae* (24), *Euphorbiaceae* (18), *Fumariceae* (16), *Primulaceae* (13) as well as numerous small families that contribute less to the endemic flora of the Balkans. Thus both, from previous and present flora-mapping, 1660 taxa will be catered for, representing c. 60% of the endemic flora of the Balkans which is currently estimated as between 2600 and 2700 taxa. The real number would be known only after all Balkan floras are completed, because 20 years is a modest timescale for this to be achieved. Some on the completed or on-going floras and related works are: Hayek (1924-1933), Turrill (1929), Flora Europaea (1964-1980, 1993), Webb (1966), Flora na Narodna Republika Bălgarija (Jordanov 1963-1976; Jordanov & Kuzmanov 1979; Velčev & Kožuharov 1982; Velčev & Kuzmanov 1989; Kožuharov & Kuzmanov 1995), Mountain Flora of Greece (Strid 1986; Strid & Tan 1991), Flora Hellenica (Strid & Tan 1997, 2002), Flora SR Srbije (Josifović 1970-1976, 1977; Sarić & Diklić 1986), Flora Srbije (Sarić 1992), Flora Bosne i Hercegovine (Beck 1903-1921, 1927, Beck & Maly 1950, Bjelčić 1967-1983), Conspectus Florae Montenegrinae (Rohlena 1942), Flora na Republika Makedonija (Micevski 1983-2001), etc.

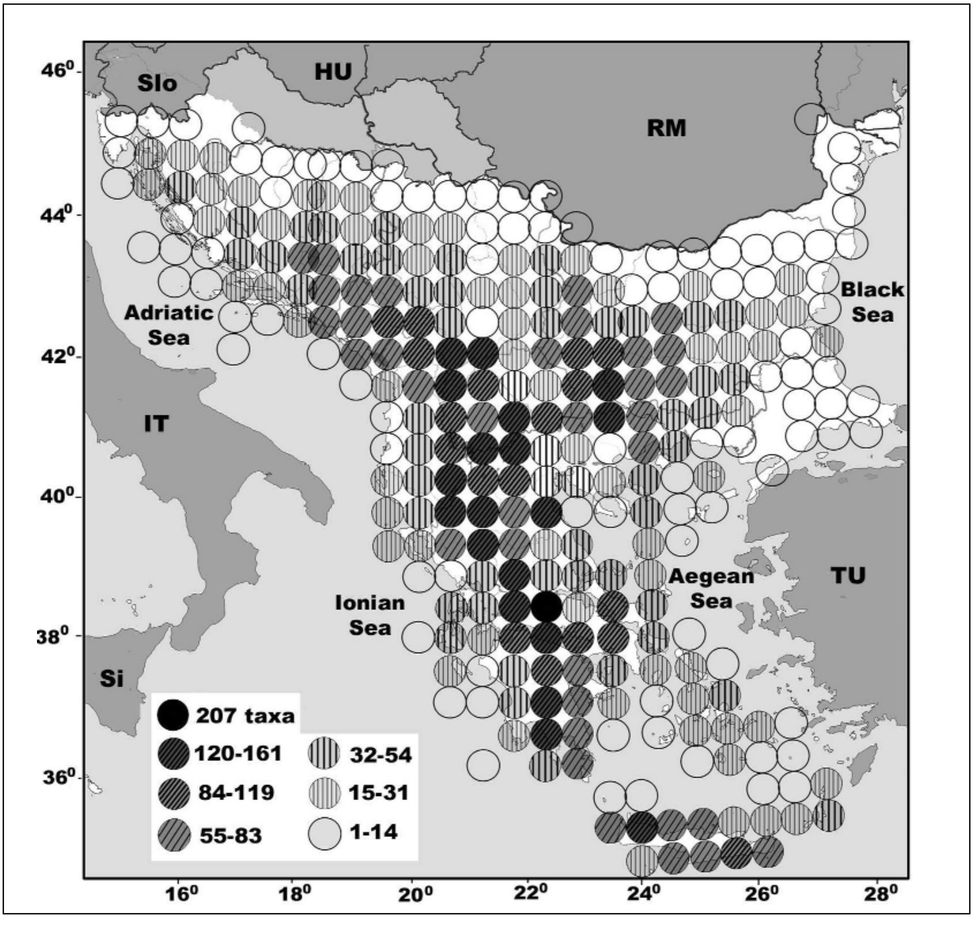

Fig. 1. Distribution of taxa per UTM square (map based on 1660 endemic taxa excl. *Compositae*, *Gramineae*, *Umbelliferae*, *Leguminosae* p.p. and *Labiatae* p.p.).

Can we determine centres of distribution or hot-spots of endemic flora in the Balkans based on a sample of 1660 endemic taxa?

Although mapping of the endemic flora is not yet complete, the data based on 1660 taxa already indicates the most important centres of endemic flora in the peninsula. From a broad global viewpoint the whole Balkan peninsula, except the very far north close to the Panonnian plain, may seem like "one great hot-spot of endemism in Europe". However, a detailed analysis of distribution of endemic taxa shows several regions distinctive by their extremely high endemism values (Fig. 1).

The regions distinctive by their extremely high endemism values

Kriti, particularly the high mountain regions of Mts Ida and Psiloritis, S Peloponnisos (regions of Mts Taygetos and Parnonas), N Peloponnisos (Mts Chelmos and Killini), the mountains of Sterea Ellas (Parnassos and Giona), Mts of S and N Pindhos, Olimbos, the mountains bordering FYR Macedonia and Greece (Pelister, Kajmakčalan, Voras, Tzena), Mts Šar-Planina and Korab (in FYR Macedonia, Serbia, Albania), Slavjanka (Ali Botusch) and Pirin Mts., Mt. Rila, almost the whole Rhodopea range, Mt. Prokletije and adjacent mountains in Montenegro (Komovi, Bjelasica) and N Albania (Hekurave, Parun) and N Montenegro (Durmitor).

In these listed areas of the Balkans, and within the corresponding 50x50 km UTM squares, more than 100 endemic taxa were recorded. We presume that the total number of endemic taxa in these squares after completion of the project will exceed 200. These squares are real hot-spots of plant diversity and contribute to make the Balkans one of the most important plant areas (IPAs) in the whole Europe and also one of the World's major centres of plant diversity (Fig. 1).

Every square which contains more than 15 endemic taxa deserves special attention in plant conservation policy. Thus, most of the Balkans particularly the Aegean, Ionian and Dalmatian islands, together with coastal and mountain areas, represent significant centres of plant diversity.

Hot-spots and local endemism

A high number of endemics are restricted to only one square and may thus be termed 'local endemics'. The number of local endemics exceeds 400, representing c. 15% of the whole endemic flora. Squares with a high number of local endemics are strongly correlated to those squares which contain the greatest number of endemics (Fig. 2). The following squares have the most local endemics (numbers in parentheses): 34SFK2 – Olimbos (19); 34SGE4 – Levka Ori in Kriti (18), 34SFF1 – S & C Taygetos and NE Parnon (12); 34SFH1, 34SDK4 – Parnassos and Giona, N Pindhos – Smolikas (11 each); 34TGL1, 34SFG2, 34TEL4, 34SFF4, 35SKE4 – Slavjanka & S Pirin / N Taygetos, NW Parnon / Voras, Nidze & Kajmakčalan / SE Peloponnisos & island of Kithira / S Athos Peninisula including summit (9 each); 34TEL3, 35SLU3, 34SGH2, 34SGH1, 34TDN2 – Kozuf-Tzena / Psiloritis on Kriti / Attica / C Evvia / mt Prokletije (8 each); 34TDM3 – mts

Paštrik, Koritnik, Rudoka, Vraca, W Sara (7); 35SLE3/LE4, 34SGE2, 34TCN4, 35SKC4, 34SFJ1: island of Samothraki / W Kriti / mt Prokletije, Komovi and Bjelasica / S Evvia / Larissa (6 each).

Why the square 34SFH1 (Parnassos and Giona in S Balkans) is so extremely rich in comparison with other squares?

After mapping 1100 endemic taxa (Stevanović & al. 2003) and according to current knowledge after mapping 1660 taxa, no changes in the order of 'floristic richness' were noted. Namely, UTM square 34SFH1 (Parnassos and Giona region) with 207 recorded endemics maintains its lead. We cannot envisage significant changes would occur after

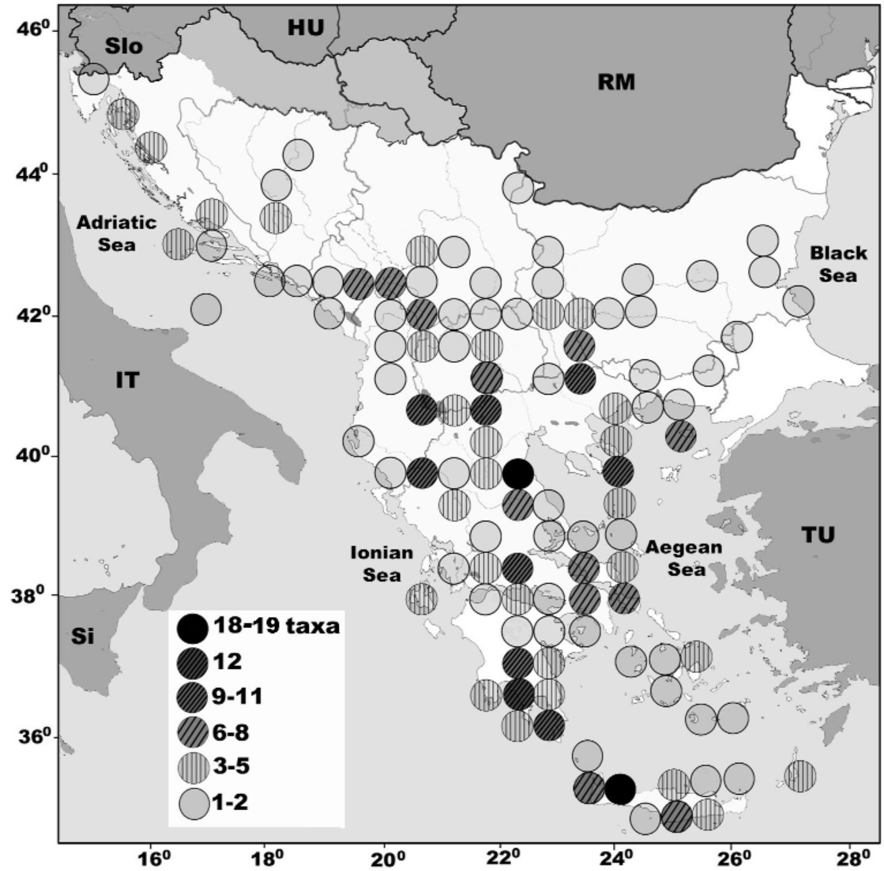

Fig. 2. Squares with the highest number of local endemics, i.e. restricted to one square only (based on sample of 1660 taxa).

mapping of 1660 taxa, even after complete mapping of an estimated 2700 taxa. The summary map based on the distribution of all taxa recorded in FH1 square perhaps provides the answer. The map (Fig. 3) shows the strong phytogeographic connection of Parnassos and Giona to two floristically rich regions in the Balkans: first to the mountain regions of N, C and S Peloponnisos reaching to Taygetos (34SEH4, 34SFH2, 34SFG1, 34SFG2, 34SFF1) and secondly, to the mountains of S and N Pindhos (34SEH3, 34SEJ1, 34SEJ4, 34SDK4).

A large number of endemic taxa from these two regions have their northern and southern distribution limits in the Parnassos and Giona region respectively. This, together with the high number of local endemics have contributed to make square 34SFH1 a ”burning” spot of endemism in the whole of Europe. By being floristically rich in itself, and by its

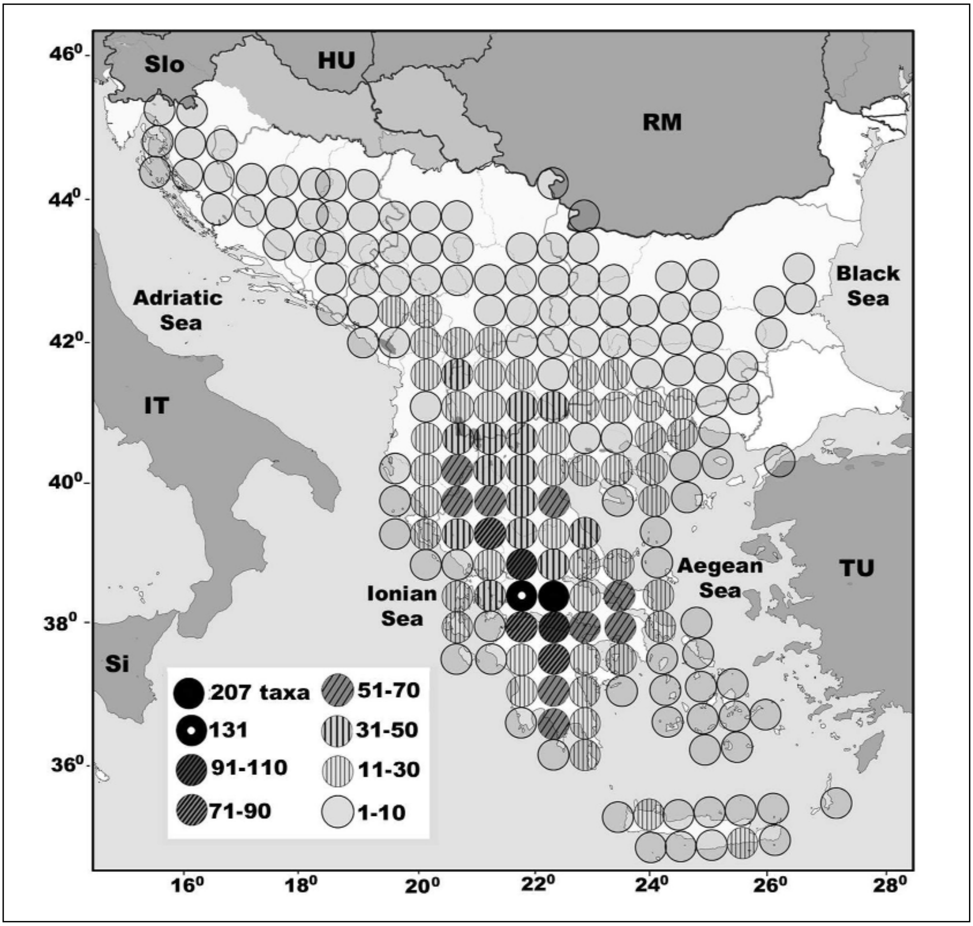

Fig. 3. Map showing strong floristic and phytogeographical connection of square FH1 (Parnassos and Giona) with other parts of the Balkans (based on sample of 1660 endemic taxa).

strategic geographical position between two extremely important centres of endemism in the Balkans (Peloponnesos and the Pindhos chain) the endemic wealth of 34SFH1 is at the same time revealed and explained.

Next step?

Despite numerous taxonomical and related chorological problems, absence of distribution data for some regions, mapping of the endemic flora of the Balkans will continue. Mapping of c. 1000 extra taxa will be our next task in the immediate future. Obviously, all this mapping of the endemic flora contributes to make taxonomical and chorological problems more apparent. Our complete database will provide floristic and phytogeographical analysis of various interesting topics, from establishing centres of diversity, the relationships including similarities and dissimilarities between floras from different squares and regions, analysis of age and area of endemics, affinities to other regions outside the Balkans, e.g., the Iberian and Ligurian peninsulas, as well as various correlations between distribution and ecology.

Acknowledgement

This work was supported by funding to V. Stevanović from the Ministry of Science and Environmental Protection, Republic of Serbia (grant No. 1505, project: Endemic flora of Serbia - distribution, ecology and conservation) and by several grants from the Carlsberg Foundation, Denmark to Kit Tan (project: Endemic plants of Greece). We thank Marjan Niketić for the electronic elaboration of our data and preparation of the maps, and Maja Tomašević and Gordana Tomović for assistance in collecting data.

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K. Tan, V. Stevanović & A. Strid

Distribution and centres of diversity for endemic geophytic Monocots in the Balkans

Abstract

Tan, K., Stevanović, V. & Strid, A.: Distribution and centres of diversity for endemic geophytic Monocots in the Balkans. — Bocconea 21: 139-146. 2007. — ISSN 1120-4060.

It is well known that the life form of a geophyte is admirably adapted for dwelling in arid and semi-arid habitats and that the ephemeral occurrence or temporal use of ecological niches in such habitats is a specific survival strategy to avoid unfavorable conditions such as drought and competition. The extremely rich endemic flora of the Balkan Peninsula is represented by several life forms and it is estimated that geophytes account for at least 10% of this flora. The majority of taxa (numbers in parentheses) belongs to the Monocot families *Liliaceae* (incl. *Alliaceae*, 124), *Iridaceae* (28), *Orchidaceae* (still unknown), *Amaryllidaceae* (5) and *Araceae* (8) which have all members geophytic. One of the most important centres of a geophytic flora is the Mediterranean region including the adjacent mountainous areas. The Balkan Peninsula is a significant centre of diversity for endemic geophytes both in terms of origin and speciation. The distribution of c. 175 endemic geophytic Monocots (excl. *Orchidaceae*, *Gramineae* & *Cyperaceae*) in the Balkans was mapped using 50x50 km UTM squares. The data permitted correlations of distribution with several interesting topics such as morphology, phenology, altitudinal range, island isolation and centres of diversity.

Introduction

As part of an on-going project: Mapping the endemic flora of the Balkans, initiated and carried out by V. Stevanović, K. Tan & A. Petrova (Stevanović & al. 2003, 2004), together with collaborators, some attention was devoted to an analysis of the life forms of endemics in the Balkans together with their distribution.

The predominant life form of the endemic Balkan flora can be assigned to the 'hemicryptophyte-chamaephyte' category (Mueller-Dombois & Ellenberg 1974) with a significantly high percentage of geophytes. We define the latter as plants with distinctly swollen, enlarged underground storage organs. The recorded number of endemic geophytes is approximately 260, representing c. 10% of the estimated endemic flora. The Monocots dominate, particularly the families *Liliaceae* (incl. *Alliaceae*) and *Iridaceae*. The present analysis caters only for those geophyte families which have been mapped. These families are the *Liliaceae*, *Iridaceae*, *Amaryllidaceae* and *Araceae*, while

Orchidaceae, *Gramineae* and *Cyperaceae* are not included at present. The total number of taxa analyzed is 170 and their distribution are mapped using 50x50 km UTM squares as adopted in the Atlas Florae Europaeae project coordinated at Helsinki.

Results and discussion

We found that the most abundant endemic geophyte is *Allium* with 42 taxa (22), a number nearly double in respect to that recorded by Turrill (1929). Several species have been recently discovered and described as local endemics especially from the Aegean and Ionian islands, Crete and the Peloponnesus. Other genera in descending number of taxa (Turrill's in parentheses) are: *Crocus* 28 (13), *Fritillaria* 2 (15), *Colchicum* 15 (16, result of a recent generic revision), *Tulipa* (10) and *Ornithogalum* 11 each, *Iris* 8 and *Muscari* (12) 8 each, *Bellevalia* 6, *Biarum* and *Scilla* 5 each, *Lilium*, *Galanthus* and *Arum* 3 each, *Hyacinthella*, *Leucojum*, *Narthecium* and *Androcymbium* 1 each.

The increase in taxa in the latter half of the previous century is the result of accurate taxonomic or floristic investigations in the Balkan countries. To mention a few, in Greece: Greuter (1967), Speta (1971, 1990), Bentzer (1973), Zahariadi (1977), Stearn (1981), Zahariadi & al. (1982), Kamari (1982, 1991, 1991a), Mathew (1982, 1983, 2000), Tzanoudakis (1983, 1986), Tzanoudakis & Vosa (1988), Persson (1988, 1991), Landström (1989), Kamari & Artelari (1990), Tzanoudakis & al. (1991), Brullo & al. (1994); in Bulgaria mainly Stefanov (1926) and Delipavlov (1976), in Turkey (European part): Kollmann (1984), Speta (1991), Ozhatay & Tzanoudakis (2000), Ozhatay (2000), etc., and in former Yugoslavia: Pulević (1976, 1978) and Randjelović & al. (1990).

The map showing the total distribution of endemic geophytes in the Balkans (Fig. 1, data obtained from the c. 170 analyzed taxa) reveals several important centres of diversity. These are the island of Crete, in particular Mt. Dikti (35SLU3) and Levka Ori (34SGE4), Taigetos in S Peloponnesus (34SFF1) and the Parnassos-Giona region in Sterea Ellas (34SFH1). Within these UTM 50x50 km squares, more than 18 to 23 endemic taxa have been recorded. Other regions in Crete (34SGE2, 35SKV4, 35SKU3, 35SLV2, 35SLU1, 35SLV4, 35SMV3, 35SMU1), N, C and SW Peloponnesus (34SFF4, 34SFF3, 34SFF2 34SFG2, 34SFG1, 34SFH2, 34SEH4, Attika (34SFH4, 34SGH2), N Evvia (34SGH1), as well as the Ionian island of Kefalonia (34SDH4) are also important centres with at least 13 to17 taxa per UTM square. As we proceed further north the number of taxa decreases and becomes restricted to the high mountains of N Pindos in Greece, mountains in FYR Macedonia and Bulgaria (Orvilos-Slavjanka, S Pirin) and a few of the SC and SW Dinaric Alps close to the Adriatic coast. The larger Aegean and Ionian islands (Kefalonia excluded) are also relatively rich in endemic geophytes despite their small altitudinal range and 6-8 taxa have been recorded from Andros, Paros, Naxos, Amorgos, Kasos, Karpathos, Levkas and Zakinthos. In comparison the Adriatic islands are extremely poor in endemic geophytes and for many of these islands no endemics have even been recorded. The inland areas of W, C and E Balkans, parts of the Black Sea coast in Bulgaria and Romania (Dobrudza) and Turkey-in-Europe have also few or no endemic geophytes. We may mention in particular, the gap in the northern border regions adjoining the Pannonian and Vlaška plains.

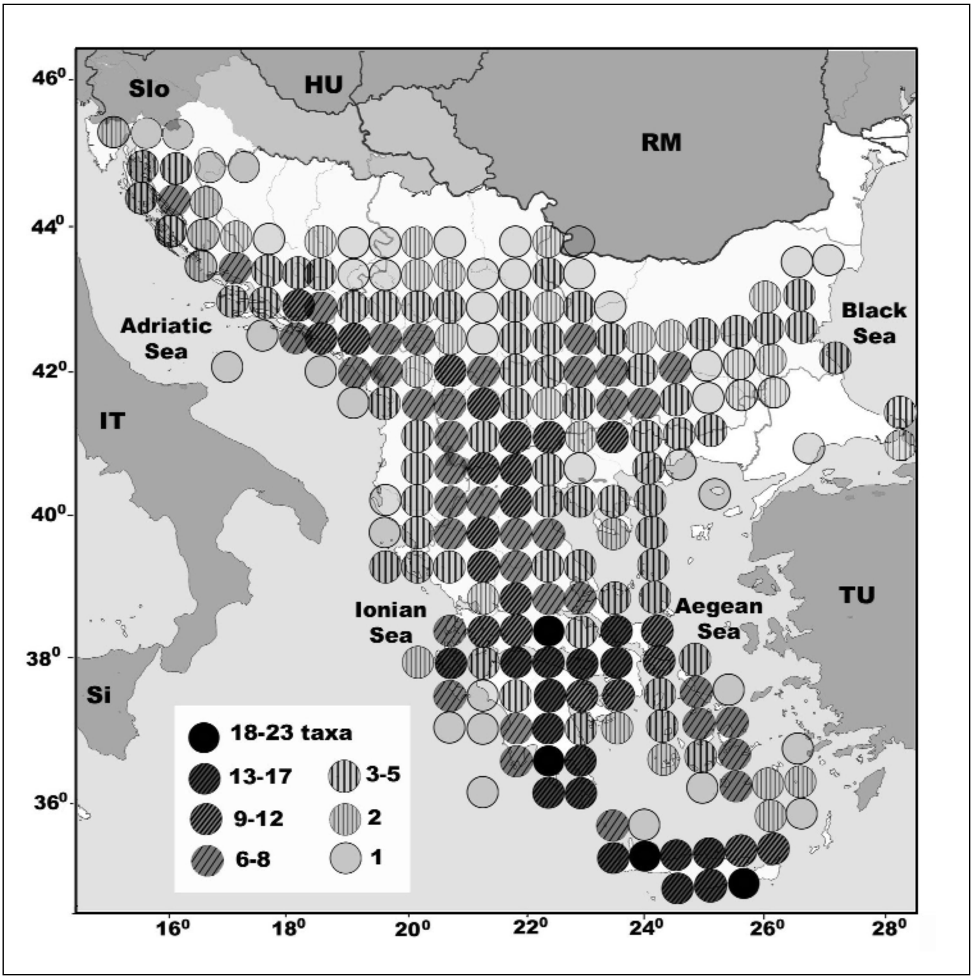

Fig. 1. Distribution of endemic geophytes based on 170 taxa (*Liliaceae*, *Iridaceae*, *Amaryllidaceae*, *Araceae*) on UTM (50x50 km) map.

As expected, the dominant life form in endemic geophytes is bulbous (or bulb-like). The entire *Liliaceae* family is a major contributor to the endemic geophyte flora, likewise the *Amaryllidaceae*. *Crocus* belongs to the transitional type of bulb to corm while the *Araceae* are entirely tuberous. Table 1 indicates the proportions of each particular geophyte life form in the analyzed Balkan flora.

If we bear in mind that the life form of a geophyte in Europe or W Asia is adapted for life in mainly arid and semi-arid habitats such as desert, steppe, Mediterranean grassland and rocky ground, as well as mountain regions of the submeridional and meridional zones, their abundance naturally decreases from the Mediterranean basin towards the north. The bulbous life form is almost absent from the flora of boreal coniferous and tundra zones and

Table 1. Geophyte life form in analyzed endemic flora.

Life form	Number of taxa	% of flora composition
bulb	130	74.3
corm-bulb	28	16.0
rhizome	9	5.1
tuber	8	4.6

replaced by the rhizomatous one. A similar distribution pattern is expressed within the Balkans, i.e., the number of endemic geophytes decreases towards the north, both in lowland and in hill regions or high alpine mountains. The obvious corollary would be that the number of endemic geophytes increases towards the Mediterranean region, reaching a maximum in the Peloponnesus and Crete; this is true for both the lowlands and mountains. The high number of endemic geophytes in the southern part of the Balkans can be partly explained by the existence of favorable habitats. Diverse topography and geological substrate as well as less dramatic changes in climate during the Ice Age have all contributed to speciation. Any significant climatic influence would presumably have supported speciation in isolated places such as mountains and islands.

Is the distribution of endemic geophytes correlated to altitude and if so, to what extent?

From the map of total distribution (Fig. 1) the centres of diversity in the Balkans are positioned in regions (as represented by squares) with wide altitudinal amplitude. Thus those regions are richer in geophytes than those regions with narrower altitudinal ranges. This conclusion is borne out by analysis of all squares in the S Balkans as occupied by islands, coastal and inland regions. We have prepared graphs presenting the relationship between the number of taxa and the average altitudinal range of each square. They are separately indicated (in terms of squares) for the island regions, coastal regions and inland areas. All clearly show the same correlation. The most important centres of diversity are situated in mountainous areas such as Parnassos and Giona (23 taxa), mountains of Peloponnesus, in particular Taygetos, Chelmos and Killini (15-19), mountains of Crete, viz., Levka Ori, Dikti and Psiloritis (15-20). The mountain regions of N and S Pindos, Thessaly, S Albania, Macedonia [FYR] and SW Bulgaria represent a second group with fewer taxa in comparison with the mountains of Crete, Peloponnesus and Sterea Ellas. Lowland and hill regions, i.e. represented by squares with narrow altitudinal ranges are generally poor in endemics as demonstrated by areas in the N and E Balkans (Vlaška plain, Black Sea coast, Peripannonian mountains). The Aegean islands, however, are an exception because they have a relatively rich endemic flora well developed at lower altitudes. In this case, it is island-isolation which is the presiding factor responsible for speciation.

How are the endemic geophytes distributed in relation to flowering time?

It is well known that the visible though ephemeral appearance of geophytes and their temporal use of ecological niches is a specific survival strategy to avoid unfavourable conditions such as drought and competition. In the Mediterranean favourable growth periods are generally in the spring and autumn while in mountainous regions it is usually late spring and summer.

Figute 2 and Table 2 show the flowering time of the four main groups of life form and as expressed in terms of the number of taxa flowering per month. Endemic geophytes flower throughout the year but the flowering peaks differ for the various life forms. The number of flowering bulbs gradually increases from January to May and gradually decrease to December. These endemic geophytes can be considered predominantly vernal. *Scilla*, *Androcymbium*, *Fritillaria*, *Tulipa*, *Muscari* and one-third of the Colchicums in the Balkans belong to this group of spring-flowering plants. Two-thirds of *Colchicum* species and a few *Allium* species are autumnal. The dominant bulbous geophytes in summer are *Allium* and *Lilium*.

Corm-bulb geophytes (*Crocus*) have two flowering peaks in early spring (February to March, reaching May to June in high mountain regions) and autumn. More taxa flower in the spring (18 taxa) than in autumn (12), thus the vernal state is predominant in this genus. The *Araceae* shows a similar flowering pattern with recognizable vernal and autumnal peaks; summer forms are completely absent. Rhizomatous geophytes as represented by *Iris* and *Narthecium* are exclusively spring-flowering plants which in the case of *N. scardicum* from the high mountains have finished anthesis by June or July.

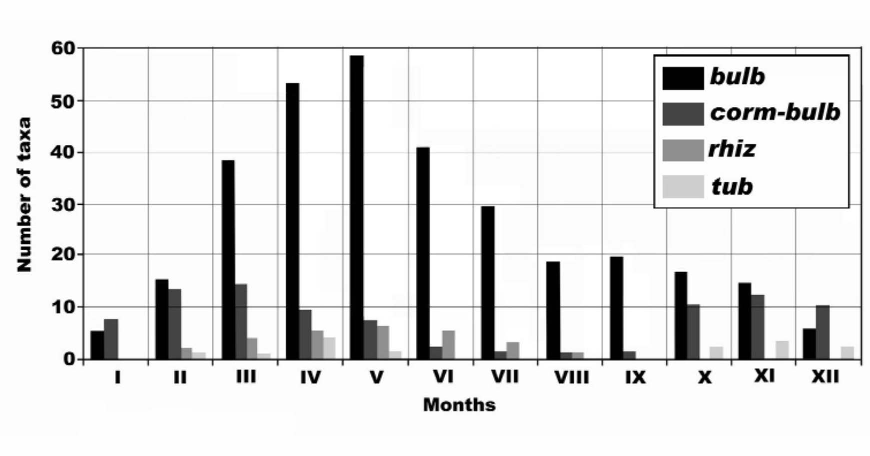

Fig. 2. Flowering time of geophyte life form (bulb, corm-bulb, rhizome = rhiz and tuber = tub) as expressed by the number of taxa per month.

Table 2. Months of flowering for the four main life forms.

Life form	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
bulb	5	15	38	53	**58**	41	29	19	20	16	14	5
corm-bulb	7	11	13	**14**	9	7	2	1	1	10	12	10
rhizome		2	4	5	**6**	5	3	1				
tuber		1	1	**4**	**4**	1			2	2	2	

Summation

Owing to space restrictions it is not possible to present here the numerous results of our mapping which clearly indicate the areas occupied by a rich and diverse endemic geophytic flora in the Balkans. Nevertheless, the results are an important contribution to aid any conservation measures aimed at protecting floristically rich sites. The paper outlines a first step in an analysis of endemic geophytes in the Balkans with respect to their distribution, centres of diversity, and their relationships with altitude and phenology. It is a basic but very necessary work in attempt to understand the phytogeographical position and evolution of the Balkan flora. Further and more detailed analysis will be presented in a second publication.

Acknowledgements

This work was supported by funding to V. Stevanović from the Ministry of Science and Environmental Protection, Republic of Serbia (grant no. 1505, project: Endemic flora of Serbia – distribution, ecology and conservation) and by several grants from the Carlsberg Foundation, Denmark to Kit Tan (project: Endemic plants of Greece). We thank Marjan Niketić for the electronic elaboration of our data and preparation of the maps, as well as Maja Tomašević and Gordana Tomović for assistance in collecting data.

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F. Conti, G. Abbate, A. Alessandrini, C. Blasi, S. Bonacquisti & E. Scassellati

An annotated checklist of the Italian vascular flora: first data

Abstract

Conti, F., Abbate, G., Alessandrini, A., Blasi, C., Bonacquisti, S. & Scassellati, E.: An annotated checklist of the Italian vascular flora: first data. — Bocconea 21: 147-153. 2007. — ISSN 1120-4060.

Between 1999 and 2002 an up-to-date check-list of the Italian Vascular Flora was achieved, following a convention funded by the Dipartimento di Biologia Vegetale, University 'La Sapienza' of Rome, and the Ministero dell'Ambiente e della Tutela del Territorio, Direzione Conservazione della Natura. The first, printed edition of this check-list (Conti & al. 2005) required a further update of data, which ended in 2004. Here we report the main national and regional data about the Italian vascular flora.

Introduction

At the end of 1999, as a result of an agreement of the Dipartimento di Biologia Vegetale of the University 'La Sapienza' of Rome with the Ministero dell'Ambiente e della Tutela del Territorio, Direzione Conservazione della Natura, an up-to-date checklist of the Italian Vascular Flora was started. The project ended in 2002. In order to draw up a first printed edition of the list, a further update of data, which ended in 2004, was necessary.The computerized database is at present lodged by the partners of the convention. For details of the frame and contents of the relational database see Abbate & al. (2001).

Briefly, for each record, the data fields reported: taxonomic and/or nomenclatural status, geographical distribution, endemicity, alien status, IUCN category, international status.

Many regional and taxonomic advisers, as well as contributors, took part in the checklist of the Italian Vascular Flora and they are all listed at the end of this paper.

Taxonomic coverage and arrangement

In this checklist all the vascular plants occurring in Italy are reported, with regard to the following taxonomic ranks: genus, species group, species, nothotaxon (only the most common ones), subspecies. The 'species group' is an unofficial rank with the actual meaning of macrospecies, concerning only a few complex genera (e.g. *Taraxacum* F. H. Wigg., *Sempervivum* L.), for which the regional data can be very inhomogeneous.

The list follows the alphabetic order of the genera respectively within *Pteridophyta*, *Gymnospermae* and *Angiospermae*, then of 'species groups' and species within the genera and, finally, of subspecies within the species.

The authors are quoted according to Brummitt & Powell (1982).

The list includes both native units and naturalized alien units, which are considered to be occurring in the 20 Italian administrative regions. The accidental alien units (ephemerals) and the cultivated ones were left out. In the check-list the word 'unit' is always to be referred to: 'species group', species, nothotaxon and subspecies.

The main sources for the list are:

- the floras that today are the most commonly used in Italy: Flora d'Italia (Pignatti 1982), Med-Checklist (Greuter & al. 1984, 1986, 1989), Flora Europaea (Tutin & al. 1968-1980, 1993),- some recent regional floras: Poldini (2001), Anzalone (1994, 1996), Bovio & al. (2000), Lucchese (1995), Conti (1998),

- the survey: Segnalazioni Floristiche Italiane (VV. AA. 1978-2004), edited by the journal Informatore Botanico Italiano,- all the floristic and taxonomic literature, so far published or currently in print.

Geographical coverage and explanatory notes

The 20 Italian administrative regions, with their present boundaries, are the reference geographical units (Fig. 1). The most important geographical novelties involved: the separation of Molise from Abruzzo and of Valle d'Aosta from Piemonte (in Flora d'Italia by Pignatti they were considered as one); the inclusion of Trieste in Friuli-Venezia Giulia, as in Poldini (2001); the exclusion of data of the territories that do not (or no longer) belong to Italy, such as Corse, Nice, partially Savoyard, Istria, Malta archipelago. Some data of borderlands between Piemonte and France will require further field investigations (Fig. 1).

Data analysis

Basing upon this survey, in Italy are present 196 families, 1267 genera, 6711 species, 7634 units (136 of which are *Pteridophyta*, 34 *Gymnospermae*, 7464 *Angiospermae*). On a national scale, the endemic units are 1021, while the alien ones are 782 (Tab. 1).

Though still today the standard of floristic knowledge varies a lot from region to region, the regions showing the maximum floristic richness are also those with the maximum environmental complexity as for orography, morphology, lithology and bioclimate. The units reported for each region can be viewed in Figure 2.

Fig. 1. The 20 Italian administrative regions.

Table 1. Main numbers of the vascular flora of Italy.

	Families	Genera	Species	Units (species + subspecies)		
				Total	Endemic	Alien
Pteridophyta	26	43	124	136	1	6
Gymnospermae	4	8	28	34	1	2
Angiospermae dicotyledones	127	910	5230	5984	882	598
Angiospermae monocotyledones	39	306	1329	1480	137	176
Total	196	1267	6711	7464	1021	782

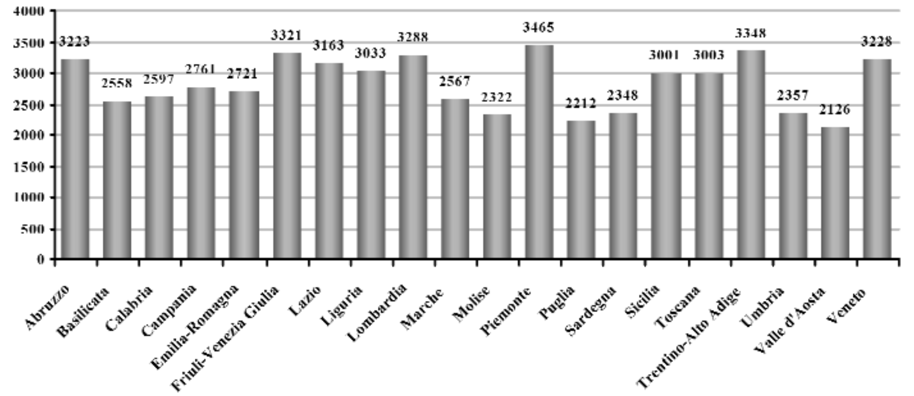

Fig. 2. Number of units (species and subspecies) occurring in each of the 20 Italian administrative regions.

Regional lists of exclusive, endemic, protected and alien units

We also compiled regional lists for some typologies of scientifically interesting units, and units useful for applicative purposes:

Exclusive units: native units occurring only in one administrative region (aliens not considered); the units in this list may have different geographic significance. This group is the distinctive mark of each region and the units here included are worth exploiting, and eventually red-listing and protecting, as suggested by version 3.1 of IUCN Categories and Criteria (IUCN 2001).

Endemic units: the endemic status is quoted only for the units endemic to the Italian territory and to the major Mediterranean islands (Corse and Malta) too; therefore the units endemic to the Alpine Chain and occurring also out of Italy are not highlighted.

Protected units: since in Italy the protection of flora is committed to each region, and only in few cases to the provinces (i.e. Piemonte, Trentino-Alto Adige), these lists, as well as useful tools, are the real subject upon which a future National Law for the Protection of Flora should be based. Some regions (i.e. Puglia, Sardegna, Sicilia) still lack such a law; at the same time other regions are protecting their whole native flora (e.g. Valle d'Aosta, Veneto, Trentino-Alto Adige) or, having a law, they still lack a specific list of units (e.g. Marche).Alien units: only naturalized alien units are considered, both at national and at regional level; the lists of alien units are strongly affected by the different level of floristic knowledge in the 20 Italian regions.

The number of units reported in these lists for each administrative region can be viewed in Table 2.

Table 2. Number of exclusive, endemic, protected and alien units (species and subspecies) for each administrative region.

Region	Units			
	Exclusive	Endemic	Protected	Alien
Abruzzo	29	177	34	230
Basilicata	6	159	31	159
Calabria	49	205	83	119
Campania	21	154	134	232
Emilia-Romagna	12	61	164	259
Friuli-Venezia Giulia	133	28	67	521
Lazio	14	164	72	317
Liguria	52	55	74	217
Lombardia	48	60	265	244
Marche	3	105	-	219
Molise	0	114	380	169
Piemonte	88	39	-	377
Puglia	39	97	-	146
Sardegna	277	254	-	199
Sicilia	344	321	-	290
Toscana	64	153	91	317
Trentino-Alto Adige	89	59	112	268
Umbria	0	94	235	193
Valle d'Aosta	21	6	56	105
Veneto	25	53	86	336

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G. Bacchetta, G. Mandis & C. Pontecorvo

Contribution to the knowledge of the endemic vascular flora of Sulcis (SW Sardinia - Italy)

Abstract

Bacchetta, G., Mandis, G. & Pontecorvo, C.: Contribution to the knowledge of the endemic vascular flora of Sulcis (SW Sardinia - Italy). — Bocconea 21: 155-166. 2007. — ISSN 1120-4060.

The results of a survey on the endemic vascular flora of Sulcis (SW Sardinia) are presented. This flora consists of 122 taxa, including 81 species, 32 subspecies, 5 varieties and 4 hybrids. The total of 80 genera, 34 families and 23 orders are represented. The analysis of biologic and chorological data highlighted the environmental peculiarities of Sulcis, given by the local evolution of a rich floristic contingent. Eight exclusive endemics, together with an unique blend of geolithologic, geomorphologic, paleogeographic, bioclimatic and vegetation features, let to attribute the rank of biogeographic sector to the Sulcis-Iglesiente territory, whose southern part can be seen as the Sulcis sub sector. The new IUCN protection categories are proposed for all taxa restricted to the Sulcis-Iglesiente sector.

Introduction

This study arose in the frame of ten years-researches about the flora and vegetation of the relief of Southern Sardinia. During these researches, particular attention was paid to the endemic entities.

Due to the isolation and the noteworthy ecosystem diversity, Sardinia is rich in endemic taxa, particularly on its mountains, as an effect of the orophytism. The mountains of Sardinia, even if lower than the Corsican ones, and without any trace of the past glaciations, are high enough to host ecological niches that are absent in the lower bioclimatic belts. The ecologic isolation, together with the insularity of Sardinia is therefore responsible for an hot spot effect (Médail & Quézel 1997) that becomes particularly strong on carbonate massifs.

From the botanical viewpoint, the relief of Sulcis is one of the most interesting and less studied of Sardinia. The relatively few floristic studies focussed on this area, and many of them date back to the last fifteen years. As concerns the endemic vascular flora, it must be mentioned the important monograph about the endemic plants of Sardinia by Arrigoni & al. (1977-1991) and, more recently, several papers on the description of new endemic taxa (Brullo 1993; Brullo & De Marco 1995; Arrigoni & Diana 1999; Bacchetta & Brullo 2000; Bacchetta & al. 2003a) or to a better definition of their chorology (Scrugli 1992; Bacchetta & al. 2003b; Bacchetta & al. 2004).

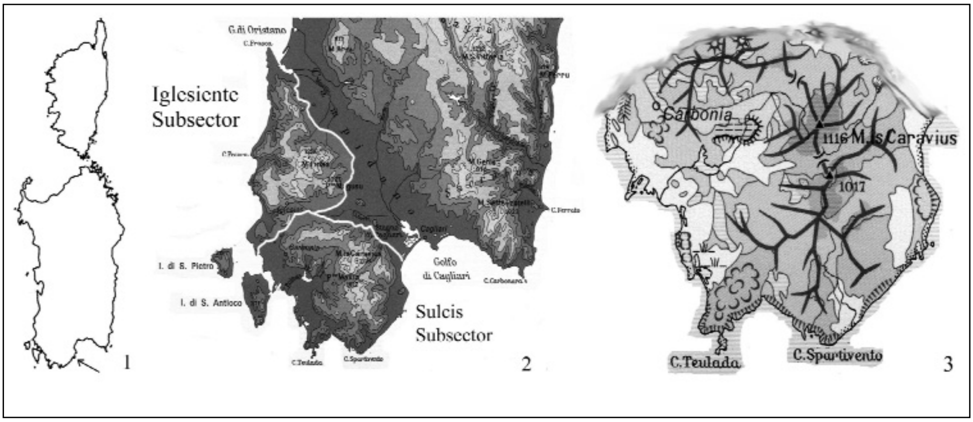
Study Area

The Sulcis-Iglesiente territory (Fig. 1-3), forming the SW part of Sardinia, is a geologic complex cut off from the rest of the island by the Graben of Campidano. The Graben of Cixerri separates on its turn the Sulcis massif, to the south, from Iglesiente, to the north. The former, having a somewhat pentagonal shape, extends approx. over 2130 km^2; the latter, having a triangular shape, covers an area of about 1225 km^2.

Sulcis-Iglesiente includes the oldest geologic units of the island: those of Bithia, Mt. Filau and Mt. Settiballas date probably back to the Precambrian (Carmignani & al. 2001). A large part of the area (about 750 km^2) is formed by the Palaeozoic units of Cabitza, Gonnesa and Nebida, consisting of sedimentary or metamorphic outcrops of carbonate rocks, aged to the Cambrian and partially emerged since the Ordovician. Further units, exclusively found in Sulcis-Iglesiente, are the metamorphites of Puddinga and Monte Argentu, in Iglesiente, and the ortogneiss forming Capo Spartivento, in Sulcis, whose formation dates back to the Ordovician. All the mentioned units rest on the Sardo-Corsican Ercinic batholite, made up by intrusive volcanic rocks of different origin and chemical composition.

The orography of Sulcis is highly tectonic and fractured, the flat areas being confined to the foothill, joint to the grabens of Campidano and Cixerri. The main range stretches from NNE to SSW. The rivers are radially oriented, rather short and torrent-like. The main rivers are Riu Palmas, Riu Gutturu Mannu and Riu Pula. The highest point of Sulcis is Punta Is Caravius (1113 m).

The climatic records, processed by the method proposed by Rivas-Martínez & al. (1999, 2002) let to classify the study area within the Mediterranean macrobioclimate and the pluviseasonal oceanic bioclimate. Thermotypes range between the lower thermo- and the upper mesomediterranean and ombrotypes between the lower dry and the lower humid (Bacchetta 2000).



Figs 1-3. **1)** The Sardo-Corsican biogeographic province, with Sulcis pointed out; **2)** The biogeographic sector of Sulcis-Iglesiente, formed by the subsectors Iglesiente (to the north) and Sulcis (to the south); **3)** Geomorphologic features of the Sulcis Subsector.

The above-mentioned lithologic, geomorphologic and bioclimatic features enriched the Sulcis-Iglesiente with peculiar vegetation-types, such as the endemic series of *Rusco aculeati-Querco calliprini* Σ (Mossa 1990), occurring on sandy soils both inland and along the coasts, within the thermomediterranean dry bioclimatic belt.

Material and methods

This paper is based on peer bibliographic and herbarium surveys, integrated by several field trips carried out in different seasons.

The bibliographic survey was focused on all reports dealing with the flora of Sulcis, as well as on the main floristic contributions referred to the whole island.

The herbarium survey was carried out at the Universities of Cagliari (CAG), Catania (CAT), Firenze (FI), Sassari (SS and SASSA), Torino (TO) and Valencia (VAL).

The field trips let to observe and collect specimens in many stands. Particular attention was paid to the collection of germoplasm and cultivation of critical taxa, in order to study their life cycle.

To classify the collected specimens, the following works were consulted: Flora Europaea (Tutin & al. 1964-80; 1993), Flora d'Italia (Pignatti 1982), Flora dels Päisos Catalans (Bolòs & Vigo 1984-2001), Flora Iberica (Castroviejo & al. 1986-2003) and the monograph Le piante endemiche della Sardegna (Arrigoni & al. op. cit.). The classification of ferns followed Ferrarini & al. (1986) and Salvo Tierra (1990), while that of orchids was based on Scrugli (1990), Grünanger (2000), Delforge (2001).

All collected specimens have been stored in CAG and CAT. The abbreviations of the author's names follow the Authors of plant names, by Brummit & Powell (1992). Taxonomical ranks accord to Judd & al. (2002). The biologic form of the surveyed taxa was checked in the field and expressed by the abbreviations reported in Pignatti (1982) based on the Raunkiaer's classification (Raunkiaer 1934). The general chorological classification follows chiefly: Le piante endemiche della Sardegna (Arrigoni & al., op. cit.) and Atlas Florae Europaeae (Jalas & Suominen 1972-1994; Jalas & al. 1996-1999; Kurtto & al. 2004).

In particular, for the chorological classification of the endemics, the units proposed by Arrigoni & Di Tommaso (1991) are adopted, with the addition of the following ones: Endemic to Sardinia and Sicily (ESS), Endemic to the Tyrrhenian Islands and N-Africa (ETI-NA) and W-Mediterranean insular endemic (EMOI).

For the individualization of the biogeographic territories, the criteria proposed by Rivas-Martínez (2002), Alcaraz-Ariza (1996) and Berastegui & al. (1997) have been followed.

The risk classes of endangered and/or protected taxa are quoted from the IUCN (IUCN 1994, 2001, 2003; Conti & al. 1997; Pignatti & al. 2001), CITES (CITES 1973; CEE 2001), Berne Convention (CEE 1982), Habitat Directive 92/43 (CEE 1992) and from the: Proposed Regional Law for the Protection of Sardinian Flora (Bacchetta & al. 1999).

Basing on the results of field investigation and consulted literature, it is here proposed to change the risk class of some of surveyed taxa, according to the IUCN procedures (IUCN 2001, 2003).

As 'endemic' we considered all taxa whose distribution range was not exceeding the limits of a biogeographic subregion, according to Ladero Alvarez & al. (1987) classification. Among the wide-ranging endemics, particular attention was paid to those mainly restricted to insular areas.

Results

The flora of Sulcis counts 122 endemic taxa (Tab. 1), belonging to 23 orders, 80 genera, 34 families and 23 orders. The surveyed taxa include 81 species, 32 subspecies, 5 varieties and 4 hybrids. For each taxon, in Table 1 are reported: family, biologic form, geographic distribution and current protection estate. In the rightmost column, the protection measures proposed in the Regional Law for the protection of Sardinian flora are reported. The law is currently submitted to the Regional Cabinet.

The biologic spectrum of the endemic flora of Sulcis highlights the dominance of hemicryptophytes (32.8%), followed by geophytes (23.8%), chamaephytes (23.0%), therophytes (10.7%), nanophanerophytes (4.9%), phanerophytes (4.1%) and hydrophytes (0.8%).

The high number of hemicryptophytes and chamaephytes can be related both to the Mediterranean climatic conditions and to the abundance of natural habitats, mainly rupestrian, of Sulcis. The richness in geophytes remarks the Mediterranean-type climate, even if the datum is strongly influenced by the orchids, representing the 38% of the total geophytes.

The low percentage of nanophanerophytes and phanerophytes can be explained by the slow speciation rate of such entities, due to their long generation time.

The few therophytes may testify the integrity of the natural conditions of Sulcis, even if no data are available for comparison with the percentage of endemic therophytes in other Sardinian sectors.

The hydrophytes are represented by *Isoëtes velata* subsp. *tegulensis*, the only endemic waterplant of Sardinia. It is well known that water acts as an homogenising factor on floras. Moreover the study area does not offer many ecological niches suitable for hydrophytes.

Almost all the vascular endemics of Sulcis are *Angiospermae*, with 97 *Dicotyledones* s. l. and 24 *Monocotyledones*. There is not any endemic *Gymnospermae* s. l. and just one *Pteridophyta* s. l. (*Lycopodiophyta*). The orders counting the highest number of endemics are *Lamiales* (17 taxa), *Asparagales* and *Asterales* (14), *Fabales* (11), *Polygonales* (10). The families counting the highest number of endemics are: *Asteraceae* (14), *Orchidaceae* and *Fabaceae* (11), *Scrophulariaceae* (10), *Apiaceae*, *Caryophyllaceae* and *Plumbaginaceae* (7). The most represented genera are: *Ophrys* (9), *Genista* (7) and *Limonium* (6).

Eight of the surveyed *taxa*, are restricted to Sulcis: *Anchusa formosa*, *Genista bocchierii*, *G. insularis* ssp. *insularis*, *Limonium carisae*, *L. malfatanicum*, *L. tigulianum*, *Ophrys* × *domus-maria*, *Silene martinolii*.

In addition to the above mentioned taxa, those endemic to Sulcis-Iglesiente are as well very important from the biogeographic viewpoint. They are: *Anchusa littorea*, *Armeria sulcitana*, *Borago morisiana*, *Dianthus mossanus*, *Helichrysum montelinasanum*, *Limonium sulcitanum*, *Ophrys* ×*maremmae* nsubsp. *woodii* and *Verbascum plantagineum*. These taxa confirm the noteworthy floristic autonomy of the Sulcis-Iglesiente massifs, isolated from the other mountains of Sardinia by the Graben of Campidano.

In addition to the taxa known from literature, the following ones are new to the endemic flora of Sulcis: *Coyncia monensis* subsp. *recurvata* (Mining of Rosas, Narcao), *Doronicum* gr. *corsicum* (Mt. Lattias, Uta), *Linaria cossoni* (Terra Sarina, Masainas), *Lavatera triloba* subsp. *pallescens* var. *minoricensis* (Chia, Domus de Maria), *Ophrys* × *laconensis* nssp. *laconensis* (Mt. Tamara,) and *Phalaris arundinacea* subsp. *rotgesii* (Mt. Lattias, Uta). In particular, *Doronicum* gr. *corsicum* was previously known only from Corsica and the record from Sulcis is new to the Italian flora. The same holds for *Linaria cossoni*, known up to now as endemic to the Tunisian coast.

The chorology of the surveyed taxa include 32.0% of Sardo-Corsican endemics, 26.2% of Sardinian endemics, altogether representing 58.2% of the total. As highlighted by Arrigoni & Di Tommaso (op. cit.) and by Mossa & Bacchetta (1998), most of the Sardinian endemics are linked to carbonate substrata, while the sardo-corsican ones to crystalline and metamorphic.

The taxa, whose distribution range is limited to insular areas, represent 84.4% of the total. Among them, 32 are endemic to Sardinia, 39 are Sardo-Corsican endemics, 17 are also occurring in the Tuscan Archipelago, 15 are in common with Sicily and further 15 are in common with the other W-Mediterranean islands (11 with Balearic Islands and 4 with Hyères Islands). Within the 15.6% taxa whose distribution range includes some continental territories, 9 are Tyrrhenian insular endemics stretching up to N-Africa; 7 are Tyrrhenian endemics *sensu strictu* and 3 are W-Mediterranean (Catalan-Provençal Province) endemics.

The above mentioned percentages confirm the biogeographic framing of the Sulcis territories within the Sardo-Corsican Province, Tyrrheno-Sicilian Superprovince and W-Mediterranean Subregion. On the other side, a strong floristic connection between Sulcis and Sicily or Northern Africa is highlighted, although the most remarkable feature still remains the floristic autonomy of the Sardo-Corsican flora, and particularly of Sulcis-Iglesiente, due to the evolution *in situ* of its original elements, descending from the Tertiary Mediterranean flora (Braun-Blanquet 1926; Contandriopoulos 1962; Faverger 1975; Arrigoni 1983). This hypothesis is not only supported by the high number of exclusive taxa and by the low number of species in common with continental areas, but also by the low number of subspecies (32) in comparison with the number of species (81).

Concerning the conservation measures (Tab. 1), our survey recorded for Sulcis 23 taxa included in the IUCN red lists, 13 of them considered at lower risk (LR), 6 vulnerable (VU), 3 endangered (EN), and 1 critically endangered (CR C).

Four *taxa* are included in the Habitat Directive CEE 92/43 and subsequent updates: one of them is prior (*Limonium insulare*) and three are not (*Linaria flava* subsp. *sardoa*, *Rouya polygama*, *Brassica insularis*).

The CITES Convention includes all *Orchidaceae* in its annex 2 and *Rouya polygama*. No taxa are quoted in the Berne Convention.

The proposed “Regional Law for the Protection of Sardinian Flora” (Bacchetta & al. 1999), currently submitted to the Regional Cabinet, includes 39 of the surveyed taxa, 10 of which are considered at a very high risk of extinction (A), 13 at high risk (B), 10 at medium risk (C) and 6 at low risk (D) (Tab. 1).

Table 1. The list of taxa endemic to Sulcis. Biogeography: WMS - W Mediterranean Subregion, ITS - Italo-Tyrrhenian Superprovince, SCP - Sardo-Corsican Province, SSP - Sardinian Subprovince, SIS - Sulcis-Iglesiente Sector, SSS - Sulcis Subsector. Extinction risk level: A - very high, B - high, C - medium, D - low.

N°	Taxonomic Units	Biol. Type	Chorology	Biogeographic Units	IUCN	Habitat	CITES	L. Reg.
1	*Allium parciflorum* Viv.	G	SA-CO	SCP.				
2	*Allium roseum* var. *insulare* Gennari	G	SA-CO	SCP				
3	*Anchusa formosa* Selvi, Bigazzi & Bacchetta	H	SA	SSS				A
4	*Anthemis arvensis* ssp. *acrochordona* Briq. & Cavill.	T	SA-SI	ITS				
5	*Apium crassipes* (Koch) Rchb. f.	H	SA-CO-SI-ITM	ITS				A
6	*Arenaria balearica* L.	H	SA-CO-AT-BL	WMS				
7	*Aristolochia navicularis* Nardi	G	SA-SI-TN-AG	WMS				
8	*Aristolochia insularis* Nardi &Arrigoni	G	SA-CO	SCP				
9	*Aristolochia tyrrhena* Nardi & Arrigoni	G	SA	SSP				A
10	*Armeria sulcitana* Arrigoni	H	SA	SIS	LR			A
11	*Arum pictum* L. f. subsp. *pictum*	G	SA-CO	SCP				
12	*Astragalus terraccianoi* Vals.	NP	SA-CO	SCP				A
13	*Barbarea rupicola* Moris	H	SA-CO	SCP				B
14	*Bellium bellidioides* L.	H	SA-CO-BL	WMS				
15	*Bellium crassifolium* Moris	Ch	SA	SSP	LR			B
16	*Biscutella morisiana* Raffaelli	T	SA-CO	SCP				
17	*Bituminaria morisiana* (Pignatti & Metlesics) Greuter	Ch	SA-TN	WMS				
18	*Borago pygmaea* (DC.) Chater & Greuter	H	SA-CO-AT	ITS	LR			B
19	*Brassica insularis* Moris	Ch	SA-CO-SI-TN	WMS		NP		D
20	*Bryonia marmorata* Petit	G	SA-CO	SCP				
21	*Bunium corydalinum* DC. subsp. *corydalinum*	G	SA-CO	SCP				
22	*Carduus sardous* DC.	H	SA-CO-AT	ITS				
23	*Carex microcarpa* Bertol. *ex* Moris	H	SA-CO-AT	ITS				
24	*Centaurium erythraea* subsp. *rhodense* (Boiss. & Reuter) Melderis	T	SA-CO-SI-ITM	ITS				
25	*Coyncia monensis* subsp. *recurvata* (All.) E. A. Leadlav	T	SA-CO	SCP				B
26	*Crepis vesicaria* subsp. *hyemalis* (Biv.) Babc.	T	SA-SI	ITS				
27	*Crocus minimus* DC. *in* Rèdouté	G	SA-CO-AT	ITS				
28	*Cymbalaria aequitriloba* (Viv.) A. Chev. subsp. *aequitriloba*	Ch	SA-CO-AT-BL	WMS				
29	*Delphinium longipes* Moris	H	SA	SS.				
30	*Delphinium pictum* Willd.	H	SA-CO-BL-H	WMS	LR			D
31	*Delphinium requienii* DC.	H	SA-CO-H	WMS				D
32	*Dianthus mossanus* Bacchetta &Brullo	Ch	SA	SIS				B

Table 1. (continued.)

33	*Dianthus arrosti* C. subsp. *sardous* Bacchetta, Brullo, Casti & Giusso	Ch	SA	SSP				B
34	*Digitalis purpurea* var. *gyspergerae* (Rouy) Fiori	H	SA-CO	SCP				D
35	*Dipsacus ferox* Loisel.	H	SA-CO	SCP				
36	*Doronicum* gr. *corsicum* (Loisel.) Poiret	H	SA-CO	SCP				
37	*Eupatorium cannabinum* subsp. *corsicum* (Loisel.) P. Fourn.	H	SA-CO-ITM	ITS				
38	*Euphorbia amygdaloides* subsp. *arbuscula* Meusel	Ch	SA-SI-ITM	ITS				
39	*Euphorbia cupanii* Guss. *ex* Bertol.	G	SA-CO-SI	ITS				
40	*Euphorbia semiperfoliata* Viv.	Ch	SA-CO	SCP				B
41	*Ferula arrigonii* Bocchieri	H	SA-CO	SCP	LR			B
42	*Galium corsicum* Sprengel	H	SA-CO	SCP				
43	*Genista aetnensis* (Biv.) DC.	P	SA-SI	ITS				D
44	*Genista bocchierii* Bacchetta, Brullo & Feoli	P	SA	SSS				
45	*Genista corsica* (Loisel.) DC. in Lam. & DC.	NP	SA-CO	SCP				
46	*Genista ferox* Poiret	NP	SA-AG-TN	WMS	VU			A
47	*Genista insularis* Bacchetta, Brullo & Feoli subsp. *insularis*	P	SA	SSS				
48	*Genista morisii* Colla	NP	SA	SSP	LR			A
49	*Genista valsecchiae* Brullo & De Marco	P	SA	SIS				A
50	*Helichrysum montelinasanum* Em. Schmid.	Ch	SA	SIS	LR			A
51	*Hordelymus caput-medusae* subsp. *crinitus* (Schreber) Pignatti	T	SA-SI-ITM	ITS				
52	*Hyoseris taurina* (Pamp.) Martinoli	Ch	SA-TN	WMS	LR			B
53	*Hypericum hircinum* L. subsp. *hircinum*	NP	SA-CO-AT	ITS				
54	*Hypochaeris sardoa* Bacchetta, Brullo & Terrasi	H	SA	SSP				A
55	*Isoëtes velata* subsp. *tegulensis* (Gennari) Batt. & Trabault	I	SA-TN	WMS	VU			A
56	*Lavatera triloba* subsp. *pallescens* (Moris) Nyman var. *minoricensis*	NP	SA-BL	WMS	CR			A
57	*Leucanthemum flosculosum* (L.) P. Giraud	Ch	SA-CO-AT	ITS				
58	*Limonium carisae* Erben	Ch	SA	SSS				A
59	*Limonium dubium* (Andr. *ex* Guss.) R. Lit.	Ch	SA-CO-SI	ITS				
60	*Limonium glomeratum* (Tausch) Erben	Ch	SA-SI	ITS				
61	*Limonium insulare* (Bég. & Landi) Arrigoni & Diana	Ch	SA	SSP	VU	P		C
62	*Limonium malfatanicum* Erben	Ch	SA	SSS				A
63	*Limonium tigulianum* Arrigoni & Diana	Ch	SA	SSS				C
64	*Linaria arcusangeli* Atzei & Camarda	Ch	SA	SSP	VU			A
65	*Linaria cossoni* Barratte	H	SA-AG	WMS				A
66	*Linaria flava* subsp. *sardoa* (Sommier) Arrigoni	T	SA-CO	SCP	LR	NP		A
67	*Lotus cytisoides* subsp. *conradiae* Gamisans	Ch	SA-CO	SCP				D
68	*Medicago intertexta* var. *tuberculata* Moris	T	SA	SSP				

Table 1. (continued.)

69	*Mentha insularis* Req. ex Gren. & Godr. subsp. *insularis*	H	SA-CO-AT-BL	WMS				
70	*Mentha requienii* Benth. subsp. *requienii*	H	SA-CO	SCP	LR			A
71	*Mercurialis corsica* Cosson	Ch	SA-CO	SCP				B
72	*Minuartia verna* subsp. *grandiflora* (C. Presl) Hayek	H	SA-SI	ITS	LR			C
73	*Nananthea perpusilla* (Loisel.) DC.	T	SA-CO	SCP	LR			A
74	*Oenanthe lisae* Moris	H	SA	SSP				C
75	*Orchis mascula* subsp. *ichnusae* Corrias	G	SA-BL	WMS			C2	C
76	*Orchis ×penzigiana* nssp. *Sardoa* Scrugli & Grasso	G	SA	SSP			C2	B
77	*Ophrys annae* Devillers-Tersch.	G	SA-CO	SCP			C2	
78	*Ophrys eleonorae* Devillers-Tersch. & Devillers	G	SA-CO-TN	WMS			C2	
79	*Ophrys conradiae* Melki & Deschâtres	G	SA-CO	SCP			C2	D
80	*Ophrys chestermanii* (Wood) Golz & Reinhard	G	SA	SSP			C2	C
81	*Ophrys morisii* (Martelli) Soo *in* Keller & al.	G	SA-CO	SCP			C2	
82	*Ophrys scolopax* subsp. *sardoa* H. Baumann, Giotta, Lorenz, Künkele & Piccitto	G	SA	SSP			C2	C
83	*Ophrys ×domus-maria* Grasso	G	SA	SSS			C2	
84	*Ophrys ×laconensis* Scrugli & Grasso nssp. *laconensis*	G	SA	SSP			C2	C
85	*Ophrys ×maremmae* nssp. *woodii* Corrias	G	SA	SIS			C2	A
86	*Ornithogalum biflorum* Jord. & Fourr.	G	SA-CO	SCP				
87	*Orobanche rigens* Loisel.	G	SA-CO	SCP				
88	*Paeonia morisii* Cesca, Passalacqua & Bernardo	G	SA-CO	SCP				B
89	*Pancratium illirycum* L.	G	SA-CO-AT	ITS				C
90	*Phalaris arundinacea* subsp. *rotgesii* (Husnot) Kerguélen	H	SA-CO	SCP				
91	*Poa balbisi* Parl.	G	SA-CO	SCP				
92	*Polygonum scoparium* Req. *ex* Loisel.	Ch	SA-CO	SCP				
93	*Ptilostemon casabonae* (L.) Greuter	Ch	SA-CO-H-AT	WMS				
94	*Ranunculus cordiger* subsp. *diffusus* (Moris) Arrigoni	H	SA-CO	SCP				
95	*Ranunculus revelieri* Boreau	T	SA-CO-GA	WMS	LR			
96	*Robertia taraxacoides* (Loisel.) DC.	H	SA-CO-SI-IT	ITS				
97	*Romulea requieni* Parl.	G	SA-CO	SCP				
98	*Rouya polygama* (Desf.) Coincy	H	SA-CO-TN	WMS	VU	NP	**X**	
99	*Rumex scutatus* subsp. *glaucescens* (Guss.) Brullo, Scelsi & Spampinato	H	SA-SI-CAL	ITS				
100	*Rumex suffocatus* Moris *&* Bertol.	H	SA	SSP	VU			C
101	*Salix arrigonii* Brullo	P	SA	SSP				C
102	*Saxifraga corsica* (Ser. ex Duby) Gren. & Godr.	H	SA-CO	SCP				
103	*Scilla autumnalis* var. *corsica* (Boullu) Briq.	G	SA-CO	SCP				

Table 1. (continued.)

104	*Scilla obtusifolia* Poiret subsp. *obtusifolia*	G	SA-AG	WMS				B
105	*Scrophularia canina* subsp. *bicolor* (Sibth. & Sm.) Greuter	H	SA-SI	ITS				
106	*Scrophularia ramosissima* Loisel.	Ch	SA-CO-BL-GA	WMS	LR			C
107	*Scrophularia trifoliata* L.	H	SA-CO-AT	ITS				
108	*Seseli praecox* (Gamisans) Gamisans	Ch	SA-CO	SCP				
109	*Silene corsica* DC.	T	SA-CO	SCP	VU			
110	*Silene martinolii* Bocchieri & Mulas	T	SA	SSS				B
111	*Silene nodulosa* Viv.	H	SA-CO	SCP				
112	*Soleirolia soleirolii* (Req.) Dandy	H	SA-CO-BL-AT	WMS	VU			A
113	*Stachys corsica* Pers.	H	SA-CO	SCP				D
114	*Stachys glutinosa* L.	Ch	SA-CO-AT	ITS				
115	*Teucrium marum* L. ssp. *marum*	Ch	SA-CO-BL-AT-H-(GA)-(HS)	WMS				
116	*Teucrium subspinosum* Pourr. ex Willd. subsp. *subspinosum*	Ch	SA-BL	WMS				B
117	*Torilis nodosa* (L.) Gaertner subsp. *nemoralaris* Brullo	T	SA-SI	ITS				
118	*Urtica atrovirens* Req. *ex* Loisel. subsp. *atrovirens*	H	SA-CO-AT	ITS				
119	*Verbascum conocarpum* Moris	H	SA-CO-AT	ITS				
120	*Verbascum plantagineum* Moris	H	SA	SIS				B
121	*Vinca sardoa* (Stearn) Pignatti	Ch	SA	SSP				
122	*Viola corsica* Nym. subsp. *limbarae* Merxm. & Lippert	H	SA	SSP				C

Discussion

Basing on the field investigation carried out in the present survey, 8 taxa have been recorded from Sulcis for the first time and 2 of them are new to the Italian flora. The data processing allows to propose some changes in the IUCN rank of the 14 taxa restricted to the Sulcis-Iglesiente biogeographic sector (Tab. 2): 3 of them are proposed as critically endangered (CR), 2 as endangered (EN), 2 as vulnerable (VU), 3 as near threatened (NT), 3 as least concern (LC) and 1 as data deficient (DD).

Due to the relatively high number of exclusive endemics of Iglesiente and Sulcis-Iglesiente, it is here proposed a new biogeographic classification for such territories. This is also justified by the palaeogeographic, geologic and geomorphologic peculiarities.

With reference to the biogeographic classifications of Sardinia proposed by Giacomini (1958), Pellettier (1960), Arrigoni (1968) and Ladero Alvarez & al. (1987), we esteem that Sardinia and Corsica should be considered an independent biogeographic province, that can be further divided into a Sardinian and a Corsican Subprovinces. In the frame of such division, by considering the already mentioned abiotic and biotic peculiarities, we propose to confer the rank of biogeographic sector to the Sulcis-Iglesiente territory and the rank of sub sector to Sulcis (Fig. 3).

Table 2. Proposed changes of the IUCN ranks of some taxa endemic to the Sulcis-Iglesiente biogeographic sector.

Table 2. Proposed changes of the IUCN ranks of some taxa endemic to the Sulcis-Iglesientebiog

No.	Taxonomic Unit	IUCN rank		Biogeographic unit
		Current	Proposed	
1	*Anchusa formosa* Selvi, Bigazzi & Bacchetta		CR B1ac(i,ii,iii,iv) + 2ac(i,ii,iii,iv)	SSS
2	*Armeria sulcitana* Arrigoni	LR	NT	SIS
3	*Dianthus mossanus* Bacchetta & Brullo		LC	SIS
4	*Genista bocchierii* Bacchetta, Brullo & Feoli		EN A2 B1ab(i,ii,iii,v) + 2ab(i,ii,iii,v) C2a (ii)	SSS
5	*Genista insularis* Bacchetta, Brullo & Feoli subsp. *insularis*		NT	SSS
6	*Genista valsecchiae* Brullo & De Marco		LC	SIS
7	*Helichrysum montelinasanum* em. Schmid	LR	VU D2	SIS
8	*Limonium carisae* Erben		CR B1ab(i,ii,iii,iv,v) + 2ab(i,ii,iii,iv,v) C2b	SSS
9	*Limonium malfatanicum* Erben		CR B1ab(i,ii,iii,iv,v) + 2ab(i,ii,iii,iv,v)	SSS
10	*Limonium tigulianum* Arrigoni & Diana		LC	SSS
11	*Ophrys* ×*domus-maria* Grasso		DD	SSS
12	*Ophrys* ×*maremmae* nsubsp. *woodii* Corrias		VU B1ac(iii, iv) + 2ac(iii, iv)	SIS
13	*Silene martinolii* Bocchieri & Mulas		EN B1a+2a	SSS
14	*Verbascum plantagineum* Moris		NT	SIS

Acnowledgements

We gratefully acknowledge financial support from the Province of Cagliari. Special thanks and appreciation go to Riccardo Guarino for the translation and checking the text in English.

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Emanuele Bocchieri & Gianluca Iiriti

Changes in land use and in the ruderal plant component as observed over the past 80 years on the Island of Molara (NE Sardinia - Italy)

Abstract

Bocchieri, E. & Iiriti, G.: Changes in land use and in the ruderal plant component as observed over the past 80 years on the Island of Molara (NE - Sardinia, Italy). — Bocconea 21: 167-173. 2007. — ISSN 1120-4060.

The authors report on the results of their floristic researches conducted on the Island of Molara (north-eastern Sardinia) as regards changes in the ruderal element linked to land use over the past 80 years. Our present-day research has identified 384 taxa, belonging to 83 families and 252 genera, whereas past floristic composition was 402. Comparison of present-day flora with that of the past showed that 86 taxa were new to the island, 285 were confirmed and 104 were no longer observed. Amongst the taxa no longer found, some 25% were ruderal. Their disappearance was due to changes in land use, in particular over the past 50 years, during which time farming activity ceased and stock rearing declined to minimal terms.

Introduction

In the framework of a research program – in the frame of INTERREG III – focusing on the changes in flora and vegetation landscape that have occurred on various Sardinian islands (Bocchieri 1990, 1992; Bocchieri & Iiriti 2000, 2002, 2003), our attention was drawn to the Island of Molara. We were able to identify the present floristic composition by numerous visits of investigated area between the end of 2001 and early in 2004.

The earliest botanical data on Molara were provided by Moris (1837-1859), who in his Flora Sardoa reported the presence of *Salix cinerea* L. Subsequent contributions were made by Béguinot (1929), Picci (1972), Lorenzoni (1970), Lorenzoni & Chiesura Lorenzoni (1973), Bocchieri (1995) and Bocchieri & Iiriti (2005). As regards endemic plants only, there are also mentions in some data sheets of the publication: Le piante endemiche della Sardegna (Arrigoni & al. 1977-1991).

Notes on the territory

The island of Molara is situated in north eastern Sardinia and belongs to the Municipality of Olbia and the Province of Sassari. The island's territory is shown on Sheet

No. 445, section III, of the topographical map of Italy and covers an area of approximately 3.5 km^2 with a coastal perimeter of 10 km (Fig. 1).

The island is made up of formations belonging to the granite pluton of Gallura which emerged during the Hercynic orogeny in the late Palaeozoic. Its coastline is rugged with very advanced erosion formations and small sandy inlets with stretches of beach attributed to the Tyrrhenian era.

The environment of the island has preserved a high degree of naturalness along the coast and in the surrounding marine ecosystems. Indeed it is one of the most interesting areas in the Marine Park of Tavolara and Capo Coda Cavallo, set up by Decree of the Ministry of the Environment of 22 September 1997. The Park extends over some 15000 hectares and includes many islets near Molara, such as Proratora, Ruja and Molarotto.

The analysis of the climate of the area under consideration is based on temperature and rainfall data for Olbia. The rainfall data are available for the period 1921-2001, while temperature data are limited to the period 1951-2001. The processing of these data shows that average annual rainfall is 600 mm, concentrated mainly in the autumn and winter. The alternation of years with high rainfall with others of drought is a phenomenon typical of Sardinia and indeed of all territories of the Western Mediterranean. Thus we find that in 1946 the Olbia station recorded no less than 1177 mm whereas in 1992 only 200 mm were recorded.

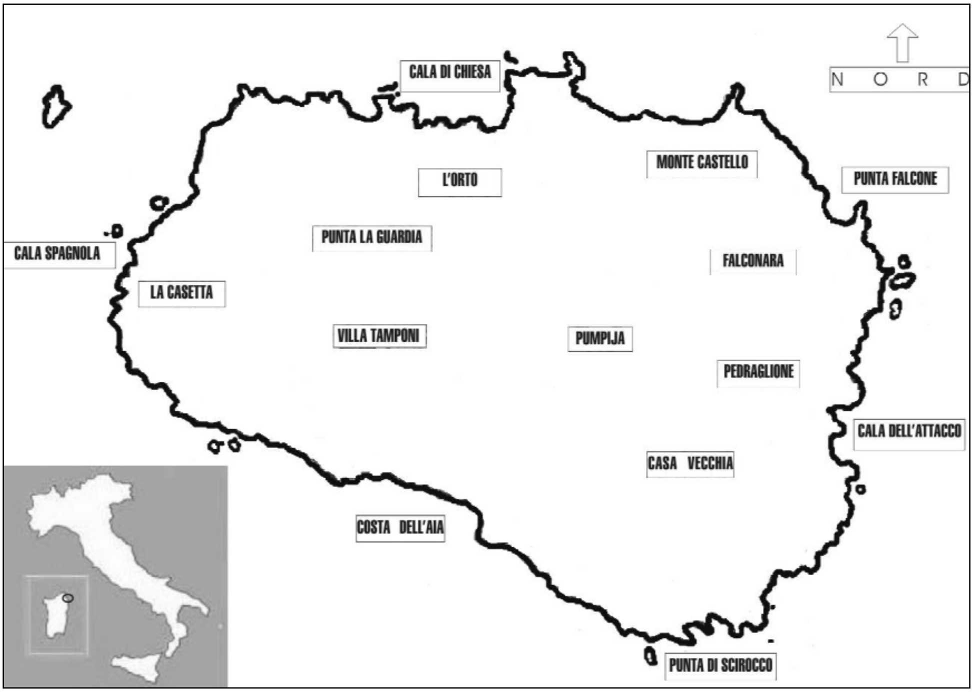

Fig. 1. The Island of Molara and its location with respect to Italy.

The mean annual temperature is 16.4°C with a mean minimum of 12.1°C and a mean maximum of 20.6°C. Dominant winds during autumn and winter blow from the north whereas during the summer we find a prevalence of southern winds, the most frequent being the Levanter (Pinna 1954).

As already was indicated in a study on the floristic component (Bocchieri & Iiriti 2005), the island of Molara has been inhabited since very ancient times as is shown by the ruins of a castle, a medieval village and an old Romanesque church. In all likelihood, one of the factors which favoured human presence on the island was the existence of a spring which made the island habitable in all seasons of the year. Moreover, there was abundance of grazing land and it was also possible to raise crops and livestock, both conditions necessary for permanent settlement in this type of place. For some fifty years now, farming activity has been abandoned, while stock rearing is limited to a few heads of cattle and goats handled by the family of Salvatore Piredda, caretaker of the island for some thirty years and who kindly gave us information – also drawn from old people from the area – dating back some 80 years.

Notes on flora and ruderal plant component

The result of our research is the identification of 384 taxa (371 spontaneous, 13 introduced) belonging to 83 families and 252 genera (Bocchieri & Iiriti 2005). The most numerous families were *Poaceae* with 42 taxa, followed by *Asteraceae* (39), *Fabaceae* (38), *Apiaceae* (15) and *Liliaceae* (15). The floristic composition identified in past years had numbered a total of 402 taxa, 360 of which were catalogued by Picci (1972) and the remainder by several botanists mentioned in the introduction to this paper. The comparison between the flora found by ourselves and that previously recorded shows that 86 taxa were new to the island, 285 were previously recorded and 104 were no longer found.

Using floristic investigations carried out by the authors mentioned above, it was discovered that of the 104 taxa not found, 24 are ruderal and weed and are the subject of this research paper. Their presence in the past was probably a result of farming and grazing activities, both widespread on the island.

The disappearance of these taxa was confirmed during recent visits aimed at ascertaining present flora and assessing its changes with respect to the situation described by Béguinot (1929) and Picci (1972), both authors of important contributions on the floristic composition of Molara. Amongst the 24 ruderal plants no longer found listed in Table 1, it should be noted that 19 are therophytes, 3 hemicryptophytes and 2 geophytes.

The differences in the floristic composition of the island can be observed by analysis of the biological spectrum and Grime's diagram (Bocchieri & Iiriti 2005). The present biological spectrum shows a clear dominance by therophytes (49.1%) followed by hemicryptophytes (19.9%), geophytes (14.8%), phanerophytes and nanophanerophytes (9.5%), camephytes (5.9%) and hydrophytes (0.8%). The comparison with the biological spectrum described by Picci (1972) shows that therophytes decreased by 2.6%, phanerophytes by 1.2% and hemicryptophytes by 0.1%, whereas camephytes increased by 0.9%, geophytes by 2.8% and hydrophytes by 0.4%. The high percentage of therophytes is a consequence of the typical climate of the Mediterranean basin, an area marked by a period of aridity dur-

Table 1. List of ruderal plants no longer found during recent visits.

Taxa	Family	Biological form	Chorological form
Spergula arvensis L.	*Caryophyllaceae*	T scap	Subcosmopol.
Fumaria agraria Lag.	*Papaveraceae*	T scap	Steno-medit.
Fumaria muralis Boiss. & Sonder	*Papaveraceae*	T scap	Subatl.
Sinapis alba L.	*Brassicaceae*	T scap	E-medit.
Sisymbrium officinale Scop.	*Brassicaceae*	T scap	Subcosmopol.
Lotus corniculatus L.	*Fabaceae*	H scap	Cosmopol.
Medicago arabica (L.) Hudson	*Fabaceae*	T scap	Euri-medit.
Medicago praecox DC.	*Fabaceae*	T scap	Steno-medit.
Trifolium incarnatum L.	*Fabaceae*	T scap	Euri-medit.
Trifolium ligusticum Balbis	*Fabaceae*	T scap	Steno-medit.
Trifolium squarrosum L.	*Fabaceae*	T scap	Euri-medit.
Vicia sativa L. subsp. a*ngustifolia* (Grufb.) Gaudin	*Fabaceae*	T scap	Subcosmopol.
Vicia hirsuta (L.) S. F. Gray	*Fabaceae*	T scap	Subcosmopol.
Erodium malachoides (L.) L'Hér.	*Geraniaceae*	T scap	Medit.-Macarones.
Erodium moschatum (L.) L'Hér.	*Geraniaceae*	T scap	Euri-medit.
Geranium pusillum L.	*Geraniaceae*	T scap	Europ.-Asiat.
Convolvulus arvensis L.	*Convolvulaceae*	G rhiz	Cosmopol.
Borago officinalis L.	*Boraginaceae*	T scap	Euri-medit.
Linaria triphylla (L.) Miller	*Scrophulariaceae*	T scap	W-medit.
Verbascum thapsus L.	*Scrophulariaceae*	H bienn	Europ.-Caucaso
Valerianella dentata (L.) Pollich	*Valerianaceae*	T scap	Submedit.-Subatl.
Conyza canadensis (L.) Cronq.	*Asteraceae*	T scap	Cosmopol.
Gladiolus segetum Ker.-Gawl.	*Iridaceae*	G bulb	Euri-medit.
Poa trivialis L.	*Poaceae*	H caesp	Eurasiat.

ing the summer months which extends from the end of May to the end of September. The high percentage of hemicryptophytes and the presence of hydrophytes are a consequence of the temperate-cool microclimate conditions and a relatively plentiful supply of water. The reduction in therophytes and increase in camephytes is evidence of the fact that over the past forty years the territory has been put to a different use, indeed during this period we find the abandonment of farming activity and reduction to minimal terms of stock breeding. The same differences are also to be found in Grime's diagram, where we find a variation of taxa marked by ruderal and competitive strategy, whereas taxa with stress-tolerant strategy display greater stability (Bocchieri & Iiriti 2005).

The land use over the past eighty years has been responsible for the variations in the island's floristic composition and vegetation landscape. In the past, this island was used almost entirely for grazing purposes and those areas with moderate slope and low rockiness could be farmed. The formerly cultivated plots were located mainly in two areas: the Orto valley with terraces used mainly for growing the vegetable, and the area between Pumpija, Pedraglione and Falconara, which was mostly used for cereal crops. The choice of the Orto valley for cultivation of vegetable was mainly due to the presence of springs providing water year round, while the interior of the island had decidedly drier soil and climate. Small plots were cultivated with vine stock and fruit-bearing trees (olives, almonds, persimmons and chestnuts), in particular in the vicinity of Villa Tamponi. At the present time they have disappeared or are present only in isolated exemplars (Fig. 2). For instance, a single surviving chestnut (*Castanea sativa* Miller) is to be found near the spring of the Orto valley, while a few almond trees (*Prunus dulcis* (Miller) D. A. Webb) grow round Villa Tamponi.

The farming activity has been abandoned for some 50 years whereas the livestock grazing on the island has by now been reduced to minimal terms. Farming activity was in all likelihood more intense in the early twentieth century, since taxa such as *Spergula arvensis* L., *Fumaria agraria* Lag., *Sisymbrium officinale* Scop., *Medicago praecox* DC., *Vicia sativa* L. subsp. *angustifolia* (Grufb.) Gaudin, *Erodium malachoides* (L.) L'Hér., *Erodium moschatum* (L.) L'Hér. were reported as being present by Béguinot (1929) but had already disappeared during the early seventies when the studies of Picci (1972) took place.

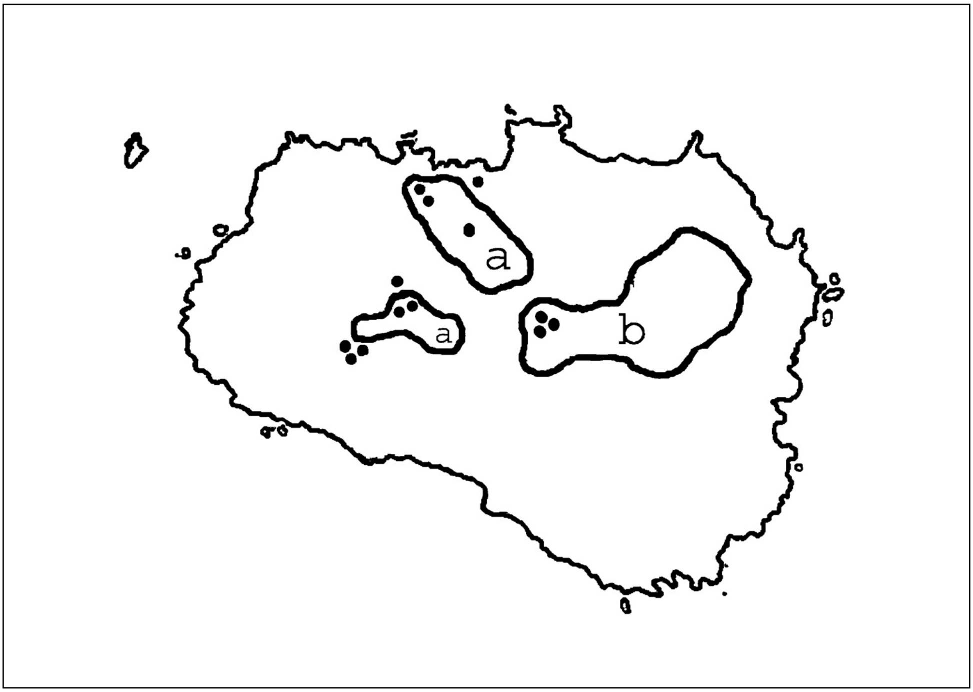

Fig. 2. Previously cultivated areas: a) vegetable crops; b) cereal crops; ●) vineyards and orchards.

Formerly cultivated areas can be recognised today by the terraces and/or heaps of stones from works to level plots of land and clear them from stones. In these areas, plant communities are mainly of the medium-low bush type, with a few scattered examples of trees which indicate vegetation evolution in the direction of more mature groupings. In these environments, the most widespread species are *Erica arborea* L., *Olea europaea* L. var. *sylvestris* Brot., *Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Cistus monspeliensis* L., *Genista corsica* (Loisel.) DC., *Lavandula stoechas* L. and we also find clearings with *Helichrysum italicum* (Royh) G. Don subsp. *microphyllum* (Willd.) Nyman, *Asphodelus aestivus* Brot., *Carlina corymbosa* L. and *Brachypodium retusum* (Pers.) Beauv. Among the rocky outcrops there are the trees of *Olea europaea* L. var. *sylvestris* Brot. which were not cut down, as is to be seen in the locality of Pedraglione where some of them are larger than average.

The progressive evolution of bush growth is also shown by the ruderal plant taxa still present today on the island but only in rare exemplars. The examples are *Papaver rhoeas* L. and *Raphanus raphanistrum* L. that highlight the disappearance of ruderal and weed communities typical of environments strongly influenced by the presence of man in favour of others having a greater degree of naturalness.

Conclusions

During the past 80 years the flora of the island of Molara has undergone variations which confirm the fact that micro-insular systems are also subject to floristic turnover.

Comparison between today's flora and that of past years showed that 86 taxa were new to the island, 285 were confirmed and 104 were not found (Bocchieri & Iiriti 2005).

Among the 104 taxa not found, 24 were ruderal and weeds (approximately 25%).

The disappearance of ruderal and weed species, which are particularly subject to the changes in the floristic component of any territory, in this case was caused by changes in land use which altered the ecological conditions that had permitted their growth and diffusion.

The dynamic relationships between different vegetation types highlighted the difference in vegetation landscape and confirmed the fact that territories at one time dedicated to productive activities are today covered in medium-low bush growth and stunted trees.

The fact that at the present time the island is privately owned has contributed to the reduction in ruderal species and, at the same time, to an increase in the typical species of local plant populations.

Notwithstanding the fact that past human use of the island altered its vegetation cover, we still find here ecosystems of significant naturalistic value whose conservation has been favoured by the fact that Molara is privately owned. Limited access to the island has prevented the arrival of large tourist flows which, if left uncontrolled, all too often are responsible for ecosystem degradation phenomena; thus Molara has been spared despite the fact that the surrounding coastal area is subjected during the summer months to considerable tourist pressure.

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M. Delcheva, S. Bancheva & S. Tzoneva

Floristic diversity and conservation values in the Tchelkov Rid area (W Rhodopi Mts), Bulgaria

Abstract

Delcheva, M., Bancheva, S. & Tzoneva, S.: Floristic diversity and conservation values in the Tchelkov Rid area (W Rhodopi Mts), Bulgaria. — Bocconea 21: 175-182. 2007. — ISSN 1120-4060.

The biodiversity of higher plants in the Tchelkov Rid (W Rhodopi Mts) has been investigated. A total of 261 species belonging to 157 genera and 50 families have been found in an area of about 500 ha. The largest families are *Poaceae, Asteraceae, Fabaceae, Caryophyllaceae, Lamiaceae, Rosaceae, Scrophulariaceae*. The highest number of Sub-Mediterranean and Euro-Mediterranean geoelements in the studied region can be explained by its geographical position. The area is enclosed between two arms of Mesta River along which Mediterranean climatic influence penetrates into Bulgaria. Eighteen taxa of conservation concern are registered: 13 endemics (12 Balkan, 1 Bulgarian endemics), 6 species from the Red Data Book of Bulgaria, 2 species protected by the Law for Protection of Biodiversity, 1 from the IUCN Red List and 1 from the CITES. A new locality of the Bulgarian endemic species *Sedum stefco* Stef. is reported. That species is of a very restricted distribution, with small populations and weak reproductive potential.

Introduction

The main aim of the current study is to evaluate the diversity of the vascular plants and their importance for conservation in the area of Tchelkov Rid (W Rhodopi Mts) by determining the composition of the contemporary flora and the potentials for its preservation.

Studied area

The studied area of Tchelkov Rid runs NE-SW in the Southwestern part of the W Rhodopi Mts between the rivers Zhizhevska and Dalboky Dol. The Rhodopi Mts are part of the Rilo-Rhodopian sub region of W Bulgaria (Stojanov 1950). They are composed by Archaic and Proterozoic metamorphic rocks: gneiss, schist, marble and some serpentinite and granite. Quartzite occurs with gneiss and amphibolite in the Tchelkov Rid area. The studied area belongs to the Continental-Mediterranean climatic zone (Nikolova 2002).

Mean winter temperature is 1.5ºC, with an absolute minimum of -14 to -15ºC. The summer is relatively cool, with mean temperature of 17.5ºC and maximum up to 22.5ºC in the lowest areas. Tchelkov Rid is in the catchments of the Mesta river. The brown mountain-forest soils are characteristic for the W Rhodopi Mts region of the Mediterranean soil area (Ninov 2002). The area belongs to the Rhodopes geobotanical region of the Illyric (Balkan) province of the European broad-leaved forest area (Bondev 1997).

Material and methods

The transect method has been applied for the investigation of the flora of the studied area of 500 ha. The maps with scale 1:25000 have been used on the field during two vegetational periods between March and October in 2002 and 2004.

The following keys were used for identification of plant material: Guide to the vascular plants in Bulgaria (Kozhuharov 1992), Flora of RP Bulgaria (Jordanov 1963-1979; Velchev & Kozhuharov 1982; Velchev & Kuzmanov 1989), Flora of R Bulgaria (Kozhuharov & Kuzmanov 1995).

The determination of the floristic elements is according Dimitrov (2002).

The abbreviations of authors are according Brummitt & Powell (1992).

Results and discussion

The vascular flora of Tchelkov Rid area comprises 261 species from 157 genera and 50 families (Tab. 1). These values for the W Rhodopi Mts are 1878 species, 594 genera, 122 families (Dimitrov 2000).

The *Magnolyophyta* group is dominant with 252 species (96.9%), and the families with highest species diversity are:

Poaceae	32	*Lamiaceae*	16	*Boraginaceae*	9
Asteraceae	31	*Rosaceae*	14	*Brassicaceae*	8
Fabacea	25	*Scrophulariaceae*	14	*Crassulaceae*	7
Caryophyllaceae	21				

The families with highest genera diversity are:

Poaceae	21	*Boraginaceae*	7
Asteraceae	19	*Rosaceae*	7
Caryophyllaceae	12	*Brassicaceae*	6
Lamiaceae	12	*Scrophulariaceae*	5
Fabaceae	8		

The genera with highest species diversity are:

Vicia	11	*Bromus*	6
Sedum	7	*Hypericum*	5
Trifolium	7	*Poa*	5

The life forms also show high diversity:

Form	Species	%
trees and shrubs	21	8
shrubs	12	57
low trees, shrubs	5	24
trees	4	19
herbs	240	92
perennial	133	55
annual	63	26
annual to biennial	21	9
biennial	13	5
biennial to perennial	5	2.5
annual to perennial	4	2
annual to semi-shrubs	1	0.5

The dominant flora elements are the Sub-Mediterranean with 43 taxa and Euro-Mediterranean with 33 species. This reflects the continental climate on the one hand and the sub-Mediterranean on the other (Stojanov 1950)

The taxa with conservation value comprise 18 species or 7% of the total flora in the studied area (Tab. 2). The endemic taxa are 12 Balkan and 1 Bulgarian species or 8% of the endemic flora of the Rhodopi Mts (Petrova 2001). In the Red Book of Bulgaria there are 6 species with category 'rare' (Velchev 1984). Two species are legally protected, 1 species is in the world list of endangered plants (Walter & Gillett 1998) and 1 species is on the list of the Convention for International Trade of Wild Plants and Animals (CITES).

About 40% of the investigated area is occupied by the habitat of *Chamaecytisus absinthioides*. It is in Anex I of Habitats Directive 92/43/EEC [4060 – Alpine and Boreal heaths; 31.4B2 – Balkano-Rhodopide Chamaecytisus absinthioides heaths] (Devillers & Devillers-Terschuren 1996). That habitat is of an European importance. In the same area there are fragments of habitat of *Juniperus oxycedrus*. It is in Directive as 5210, Arborescent matorral with *Juniperus* spp.; 32.1311, Inland *Juniperus oxycedrus* arborescent matoral. Those communities are typical for area with Sub-Mediterranean climate and they are very rare in Bulgaria.

Conclusions

The data show the high conservation value of the flora in the studied area.

The information about the diversity of flora in the Tchelkov Rid area makes possible to estimate its conservation value on the basis of the following indicators:

Taxonomic diversity:	14% of the species of the W Rhodopi Mts
	26% of the genera of the W Rhodopi Mts
	41% of the families of the W Rhodopi Mts
Endemism:	8% of the endemic taxa in the flora of the Rhodopi Mts: Balkan and Bulgarian endemics

Taxa and habitats conservation status: 6 species: national status
(Red Book of Bulgaria and/or law protected)
2 species: international status (IUCN, CITES)
2 habitats: European importance

Table 1. List of vascular plants in the area of Tchelkov Rid, W Rhodopi Mts.

Amaranthaceae
Amaranthus albus L.
Apiaceae
Anthriscus sylvestris (L.) Hoffm.
Eryngium campestre L.
Orlaya grandiflora (L.) Hoffm.
Aspleniaceae
Asplenium adiantum-nigrum L.
Asplenium septentrionale (L.) Hoffm.
Asplenium trichomanes L.
Ceterach officinarum DC.
Asteraceae
Achillea clusiana Tausch
Achillea collina Becker ex Rchb.
Achillea setacea Waldst. & Kit.
Anthemis arvensis L.
Anthemis tinctoria L.
Artemisia vulgaris L.
Carduus acicularis Bertol.
Carduus candicans Waldst. & Kit.
Carlina vulgaris L.
Centaurea cuneifolia Sibth. & Sm.
Centaurea jacea L.
Centaurea rhenana Boreau
Chondrilla juncea L.
Cichorium intybus L.
Conyza canadensis (L.) Cronquist
Crepis biennis L.
Crepis foetida L.
Eupatorium cannabinum L.
Filago eriocephala Guss.
Galinsoga parviflora Cav.
Hieracium caespitosum Dumort.
Hieracium hoppeanum Schult.
Hieracium pilosella L.
Hieracium piloselloides Vill.
Lactuca viminea (L.) J. Presl & C. Presl
Leontodon crispus Vill.
Leontodon hispidus L.
Senecio rupestris Waldst. & Kit.
Senecio vernalis Waldst. & Kit.
Tragopogon dubius Scop.
Xeranthemum annuum L.
Athyriaceae
Cystopteris fragilis (L.) Bernh.
Betulaceae
Alnus glutinosa (L.) Gaertn.
Betula pendula Roth
Carpinus orientalis Mill
Corylus avellana L.
Boraginaceae
Anchusa barrelieri (All.) Vitman
Anchusa hybrida Ten.
Anchusa officinalis L.
Borago officinalis L.
Cynoglossum hungaricum Simonk.
Echium vulgare L.
Myosotis ramosissima Rochel
Onosma heterophylla Griseb.
Symphytum ottomanum Friv.
Brassicaceae
Alyssum murale Waldst. et Kit.
Berteroa incana (L.) DC.
Berteroa obliqua (Sm.) DC.
Capsella bursa-pastoris (L.) Medik.
Erysimum diffusum Ehrh.
Rorippa pyrenaica (L.) Rchb.
Sisymbrium altissimum L.
Sisymbrium officinale (L.) Scop.
Campanulaceae
Campanula lingulata Waldst. & Kit.
Campanula sparsa Friv.
Jasione heldreichii Boiss. & Orph.
Caryophillaceae
Arenaria leptoclados (Rchb.) Guss.
Arenaria serpyllifolia L.
Cerastium pumilum Curtis
Cerastium semidecandrum L.
Cucubalus baccifer. L.
Dianthus cruentus Griseb.
Dianthus tristis Velen.
Herniaria glabra L.
Herniaria hirsuta L.
Herniaria incana Lam.
Lychnis coronaria (L.) Desr.
Minuartia bulgarica (Velen.) Graebn.

Moenchia mantica (L.) Bartl.
Scleranthus perennis L.
Silene armeria L.
Silene frivaldskyana Hampe
Silene subconica Friv.
Stellaria holostea L.
Stellaria media (L.) Vill.
Stellaria nemorum L.
Viscaria vulgaris Röhl.
Chenopodiaceae
Chenopodium album L.
Chenopodium botrys L.
Cistaceae
Xolanthes guttatus (L.) Raf.
Convolvulaceae
Convolvulus arvensis L.
Crassulaceae
Sedum acre L.
Sedum album L.
Sedum annuum L.
Sedum caespitosum (Cav.) DC.
Sedum hispanicum L.
Sedum kostovii Stef.
Sedum stefco Stef.
Cupressaceae
Juniperus communis L.
Juniperus oxycedrus L.
Cuscutaceae
Cuscuta planiflora Ten.
Cyperaceae
Carex buekii Wimm.
Carex caryophyllea Latourr.
Carex echinata Murray
Holoschoenus vulgaris Link
Dioscoreaceae
Tamus communis L.
Dipsacaceae
Knautia integrifolia (L.) Bertol.
Scabiosa ochroleuca L.
Scabiosa triniifola Friv.
Euphorbiaceae
Euphorbia agraria M. Bieb.
Euphorbia cyparissias L.
Euphorbia myrsinites L.
Fabaceae
Chamaecytisus absinthioides (Janka) Kuzm.
Genista rumelica Velen.
Lotus angustissimus L.
Lotus corniculatus L.
Medicago minima (L.) Bartal.
Melilotus alba Medik.
Ononis spinosa L.
Trifolium alpestre L.
Trifolium arvense L.
Trifolium aureum Pollich
Trifolium hybridum L.
Trifolium incarnatum L.
Trifolium medium L.
Trifolium repens L.
Vicia angustifolia L.
Vicia cassubica L.
Vicia cordata Wulfen ex Hoppe
Vicia cracca L.
Vicia dalmatica A. Kern.
Vicia grandiflora Scop.
Vicia hirsuta (L.) Gray
Vicia sativa L.
Vicia sepium L.
Vicia tetrasperma (L.) Schreb.
Vicia varia Host
Fagaceae
Quercus pubescens Willd.
Gentianaceae
Centaurium erythraea Rafn
Geraniaceae
Erodium cicutarium (L.) L'Hér.
Erodium hoefftianum C. A. Mey.
Geranium dissectum L.
Geranium sanguineum L.
Hypericaceae
Hypericum cerastoides (Spach.) N. Robson
Hypericum montbretii Spach
Hypericum olympicum L.
Hypericum perforatum L.
Hypericum rumeliacum Boiss.
Hypolepidaceae
Pteridium aquilinum (L.) Kuhn
Juncaceae
Luzula campestris (L.) DC.
Lamiaceae
Acinos arvensis (Lam.) Dandy
Acinos suaveolens (Sibth. & Sm.) G. Don
Ajuga genevensis L.
Clinopodium vulgare L.
Lamium garganicum L.
Nepeta nuda L.
Prunella laciniata (L.) L.
Salvia amplexicaulis Lam.

Salvia verticillata L.
Scutellaria galericulata L.
Stachys angustifolia M. Bieb.
Stachys recta L.
Stachys sylvatica L.
Teucrium chamaedrys L.
Thymus callieri Borbas ex Velen.
Thymus jankae Celak.
Liliaceae
Allium flavum L.
Muscari comosum (L.) Mill.
Muscari tenuiflorum Tausch
Ornithogalum pyrenaicum L.
Linaceae
Linum bienne Mill.
Linum hologynum Rchb.
Malvaceae
Malva sylvestris L.
Orchidaceae
Orchis morio L.
Orobanchaceae
Orobanche gracilis Sm.
Orobanche pubescens d'Urv.
Papaveraceae
Papaver rhoeas L.
Plantaginaceae
Plantago lanceolata L.
Plantago scabra Moench
Plantago subulata L.
Plumbaginaceae
Armeria rumelica Boiss.
Poaceae
Aegilops triuncialis L.
Aira elegantissima Schur
Anthoxanthum odoratum L.
Apera spica-venti (L.) P. Beauv.
Bromus mollis L.
Bromus racemosus L.
Bromus scoparius L.
Bromus squarrosus L.
Bromus sterilis L.
Bromus tectorum L.
Chrysopogon gryllus (L.) Trin.
Cynosurus cristatus L.
Cynosurus echinatus L.
Dactylis glomerata L.
Dichanthium ischaemum (L.) Roberty
Elymus repens (L.) Gould
Eragrostis pilosa (L.) P. Beauv.
Festuca nigrescens Lam.
Holcus lanatus L.
Hordeum murinum L.
Koeleria nitidula Velen.
Koeleria penzesii Ujhelyi
Lolium perenne L.
Phleum graecum Boiss. & Heldr.
Poa bulbosa L.
Poa compressa L.
Poa nemoralis L.
Poa pratensis L.
Poa sylvicola Guss.
Taeniatherum caput-medusae (L.) Nevski
Tragus racemosus (L.) All.
Vulpia myuros (L.) C.C. Gmel.
Polygonaceae
Polygonum aviculare L.
Rumex acetosa L.
Rumex acetosella L.
Rumex tenuifolius (Wallr.) A. Löve
Portulacaceae
Portulaca oleracea L.
Primulaceae
Anagallis arvensis L.
Lysimachia punctata L.
Ranunculaceae
Clematis vitalba L.
Nigella arvensis L.
Ranunculus polyanthemos L.
Thalictrum minus L.
Rosaceae
Agrimonia eupatoria L.
Crataegus monogyna Jacq.
Potentilla argentea L.
Potentilla neglecta Baumg.
Potentilla sulphurea Lam.
Prunus cerasifera Ehrh.
Prunus domestica L.
Prunus spinosa L.
Rosa canina L.
Rosa dumalis Bechst.
Rosa micrantha Borrer ex Sm.
Rosa myriacantha DC.
Rubus discolor Weihe & Nees
Sanguisorba minor Scop.
Rubiaceae
Asperula cynanchica L.
Cruciata laevipes Opiz
Cruciata pedemontana (Bellardi) Ehrend.

Galium verum L.
Salicaceae
Salix alba L.
Santalaceae
Thesium divaricatum Jan ex Mert. & W.D.J.Koch
Scrophulariaceae
Euphrasia liburnica Wettst.
Euphrasia pectinata Ten.
Euphrasia rostkoviana Hayne
Linaria arvensis (L.) Desf.
Linaria genistifolia (L.) Mill.
Linaria pelisseriana (L.) Mill.
Rhinanthus minor L.
Verbascum banaticum Schrad.
Verbascum rorripifolium (Halácsy) I.K. Ferguson
Veronica arvensis L.
Veronica austriaca ssp. *jacquinii* (Baumg.) Maly
Veronica beccabunga L.
Veronica cymbalaria Bodard
Veronica triphyllos L.
Selaginellaceae
Selaginella helvetica (L.) Spring
Ulmaceae
Ulmus minor Mill.
Valerianaceae
Valerianella coronata (L.) DC.
Violaceae
Viola arvensis Murray
Viola tricolor L.

Table 2. List of the taxa of conservation importance in the area of Tchelkov Rid: Bg - Bulgarian endemit, Bk - Balkan endemit, RB - Red Data Book of PR Bulgaria (R rare), LBB - Law for Bulgarian biodiversity, IUCN - List of the world-wide threatened plant species (R rare), CITES - Convention on international trade in endangered species of wild fauna and flora.

No	Taxa	Conservation value
1	*Anchusa hybrida* Ten.	RB (R)
2	*Armeria rumelica* Boiss.	Bk
3	*Campanula sparsa* Friv.	Bk
4	*Centaurea cuneifolia* Sibth. & Sm.	Bk
5	*Chamaecytisus absinthioides* (Janka) Kuzm.	Bk
6	*Dianthus cruentus* Griseb.	Bk
7	*Dianthus tristis* Velen.	Bk
8	*Erodium hoefftianum* C.A. Mey.	RB (R)
9	*Genista rumelica* Velen.	Bk
10	*Hypericum rumeliacum* Boiss.	Bk
11	*Minuartia bulgarica* (Velen.) Graebn.	Bk
12	*Onosma heterophylla* Griseb.	RB (R)
13	*Orchis morio* L.	CITES
14	*Scabiosa triniifola* Friv.	Bk; RB (R); LBB
15	*Sedum kostovii* Stef.	Bg; RB (R); LBB; IUCN(R)
16	*Sedum stefco* Stef.	Bk
17	*Silene frivaldskyana* Hampe	Bk
18	*Verbascum roripifolium* (Halacsy) I.K. Fergus.	RB (R)

Acknowledgements

The authors are grateful to the National Grassland Inventory Project, Bulgaria (PINMATRA 2002/020) for the financial support extended to this study.

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Dejan Radović

Evolving GIS at Tara National Park (Serbia)

Abstract

Radović, D.: Evolving GIS at Tara National Park (Serbia). — Bocconea 21: 183-191. 2007. — ISSN 1120-4060.

The Tara Mountain is among the most important centers of Balkan and European ecosystem and species biodiversity. It is characterized by diversified and well-maintained communities of old deciduous and mixed coniferous forests (beech/fir/spruce). They represent a unique example of well-preserved forests in SE Europe with numerous endemic and relict species of indigenous flora and fauna. Specific geomorphologic, hydrologic, geologic, soil and climatic features are also present on Tara Mountain. Tara National Park is located in the West part of the Republic of Serbia (180 km southwestward from Belgrade), and encompasses the largest part of Tara Mt. (average altitude 1000-1200 m asl). NP Tara has an area of 19175 ha, and a protected buffer zone around it of 37584 ha. The first study to declare Tara Mt. a National Park was carried out in 1951, but NP Tara was not established until 1981. During 2004 NP Tara was nominated within the UNESCO-ROSTE program as one of the next MAB Biosphere Reserves. We have developed a Geographical Information System (GIS) that has proved to be an excellent tool for evaluating and protecting the natural resources of NP Tara.

Introduction

NP Tara is one of the most researched National Parks in Serbia. During the last 60 years many scientists and managers of the Park have collected a huge amount of data dealing with the natural values of the area.

Until now the managing and directing of practically all the natural potentials of NP Tara was primarily based on the principles of forestry technology and management.

The GIS that we have created for NP Tara includes data on natural themes (ecosystems, vegetation, flora, fauna, landscapes, geology, soil, and hydrology), artificial (human) themes (roads, bridges, dam, hotels, rest camps and other visitor facilities) and management operational themes. At the same time it has proved an excellent tool in evaluating and protecting the natural resources of NP Tara, especially in preparing the indispensable documentation for nominating Tara Mt. as a Reserve of the Biosphere within the UNESCO program: Man and the Biosphere (Ashdown & Schaller 1990; Radović 2004a).

This type of research has its full scientific base in the worldwide actions of research, preservation, and protection of mountain ecosystems, because they are centers (hot spots) of high species and ecosystems diversity (Wadsworth & Treweek 1999; Convis 2001; Longley & al. 2001).

Research Methods

Study Site

NP Tara is located in the Southwest part of Serbia within 43^{o} 52’ 30” and 44^{o} 01’ North and 19^{o} 14’ 30” and 19^{o} 41’ 30” East . The highest point of NP Tara is Kozji Rid 1591 m asl, and the lowest point is at 300 m (exactly where the Perućac hydro electric power station is). Based on these characteristics, Tara Mt. is classified as a medium high mountain in Serbia.

NP Tara has an area of 19175 ha, and a protected buffer zone around it of 37584 ha. Within NP Tara we can differentiate two spatially-functional characteristic areas: High Tara (11562 ha) and Flat Tara (7613 ha).

In order to manage the NP Tara effectively it is divided into five managing totalities with a total of 751 parcels and sub parcels. The National Park is completely within the territory of the Bajina Bašta community (Fig. 1).

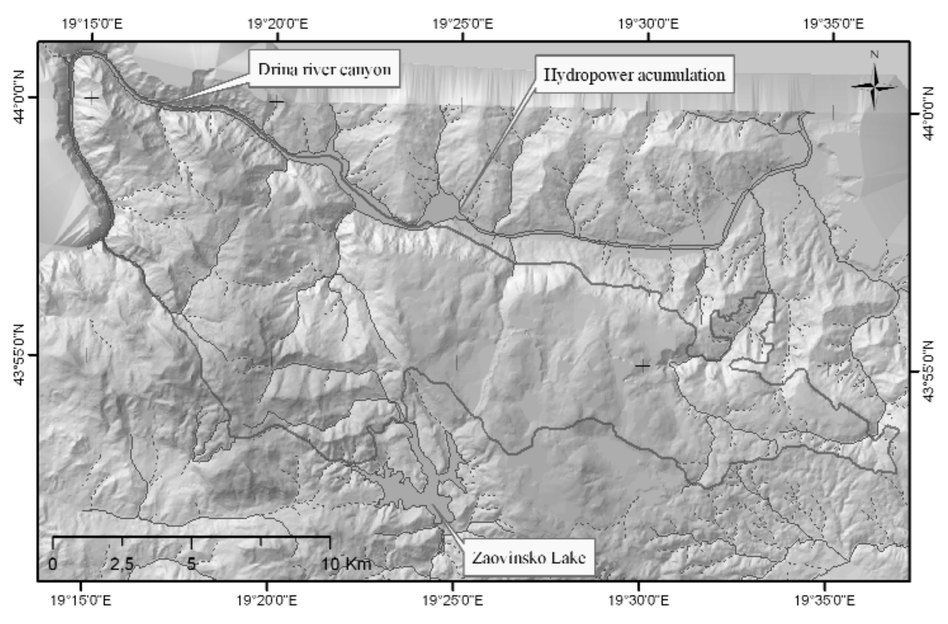

Fig. 1. Border of NP Tara on DEM.

Data sources

One of the major factors for developing a successful GIS project is the reliability of data. In this case we used a large number of data sources, but only from authorized governmental institutions:

topographic maps, maps of relief and hydrology, geological and soil maps, vegetation maps, aerial photographs, maps of five managing totalities (with 751 parcels) of NP Tara (Zvezda, Crni Vrh, Rača, Tara, Kaludjerske Bare) and tourist maps,

data consisted of 9 types of information for each parcel in each totality (name of totality, number of parcels and sub parcels, type of woodland, vegetation communities that occur, ecological belonging of vegetation to the type of soil, percentage of every vegetation species that occurs, parcel purpose, and parcel level of protection - zoning),

data on representatives of flora and fauna.

Methodology of work

Like in most GIS projects the usual procedures were applied: scanning, georeferencing, digitalizing (manual and semiautomatic), creating a database and integration of data (Radović 2004).

Content of NP Tara GIS

NP Tara GIS is divided (by type and format) into various data forms: raster data, vector data, digital elevation model and database.

Raster data include all maps and aerial photographs cited in data sources after georeferentiation.

Next entities were represented in vector shape: geology (polygon), soil (polygon), hydrology (line/polygon), vegetation communities (polygon), locations of important floristic and faunistic species (point), border of NP Tara (polygon), management totalities (polygon), departments - parcels (polygon), settlements (polygon), roads (line), mountaineer trails (line) and other objects: hotels, tourist sights, waterfalls, caves, groceries (point).

To achieve the maximum effect through project 3D analyses were anticipated. These analyses require making correlations between certain phenomenon and relief characteristics (elevation, aspect and slope). To accomplish our objectives a digital elevation model (resolution 20 m pixel) was created. DEM covered an area of about 800 km^2. DEM provided for each pixel: coordinates (in meters), elevation (in meters), aspect (in degrees) and slope (in percentages).

Material to process the aerial photographs included a series of 19 photographs of NP Tara (scale R= 1: 30000; camera Wild RC-5, 1959). By using photographs that were almost 50 years old, we were able to notice some changes in vegetation and the human influences that led to them.

Results

Geomorphologic characteristics of Tara Mt.

To interpret the geomorphology of the Park this GIS project included elevation zones, aspect and slope of relief. Analyses of elevation zones showed that most of the area in the

Park represents altitudes from 800-1200 m (12571 ha; 65,56%) (Fig. 2). Analyses of aspect showed that North (2853 ha; 14.9%), Northeast (3604 ha; 18.8%) and Northwest (2199 ha; 11.5%) take most of the Park. Analyses of slope of relief showed that: 0-9.1% encompass 4964.2 ha, 25.9%; 9.2-18.3% encompass 5363.4 ha, 28%; 18.4-27.4% encompass 3895.4 ha, 20.32%; 27.5-36.5% encompass 2768.2 ha, 14.44%.

Geological characteristics of Tara Mt.

Tara Mt. is characterized by high geological diversity including age, origin and composition. There are rocks from the Carbon, Triassic, Jurassic, Cretaceous and Quaternary periods.

The total area of 34351 ha has been managed. Authentic content was classified into 35 types of geologic cover. To be able to make adequate correlations with vegetation primarily, a generalization into six basic and seven combined types was performed. Limestone and ophiolitic bedrocks covers about 77% and 7.5% of the territory of the Park, respectively.

Soil characteristics of Tara Mt.

The soil cover was digitizaled for an area of 55024 ha, with ten types of cover. There are five types of soil cover inside the Park: chernozem on serpentines (humus silicate soil) (2413 ha, 13%); brown reddish soil on limestone (6530 ha, 34%); calcareous-skeletal soil on firm limestone (9119 ha, 47.6%); diluvium (44.55 ha, 0.23%); brown skeletoid soil on schist (1068 ha, 5.6%).

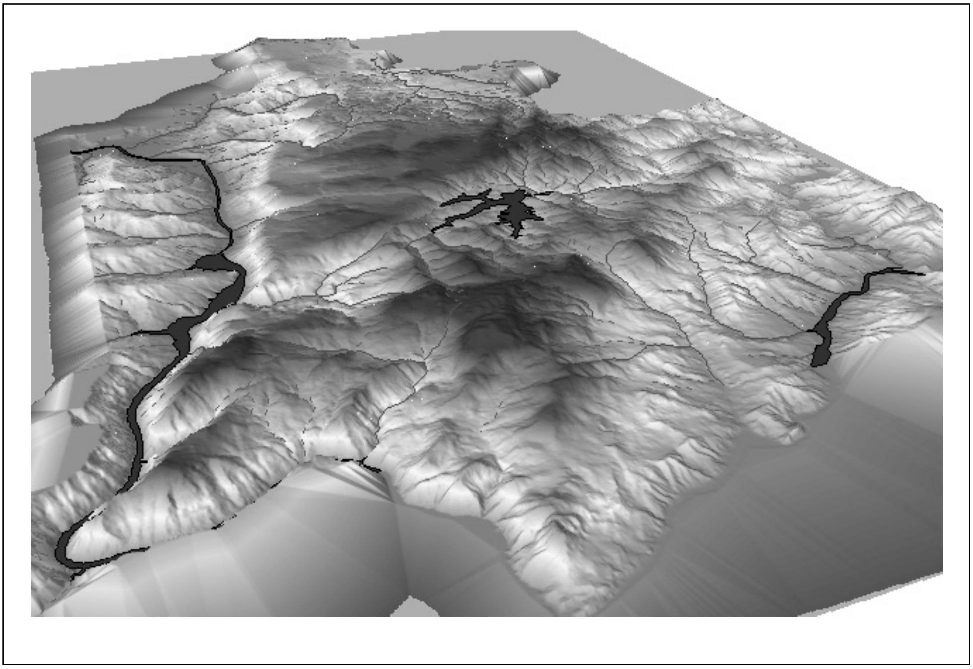

Fig. 2. Elevation zones on 3D model (NW view).

Hydrologic characteristics of Tara Mt.

The most expressed hydrologic characteristic of the Park is the Drina river (total length 346 km; 22.7 km around the Park) and the canyon. The canyon is over 1000 m high from Drina in the line of the Park. Most of the water flowing on Tara Mt. belongs to the Drina river basin. Drina represents the biggest tributary (by length, river basin area and flow of water) of the Sava river.

There are three artificial lakes in the area of the Park: Perućac hydro accumulation, Zaovinsko Jezero lake and Kruščica.

GIS theme hydrology includes all hydro elements on Tara: streams, rivers and lakes. It is possible to get information about the length of every flow or segment. The data model allows us to input information about the presence of ichtyofauna.

Climate of Tara Mt.

The climate of Tara Mt. is characterized by fresh to cool summers and quite cold winters (annual temperature variation is small). Nice weather is prolonged to the first part of autumn and October is warmer than April. Compared to other mountains in Serbia, Tara has a more expressed mountain climate. The average yearly temperature is 7.9ºC. Average yearly precipitation is 977 mm. A characteristic particular to the climate of Tara Mt. is its very high average yearly humidity (83.4%). The snow starts at the beginning of November and is present until the first part of May. Average thickness of snow cover is about 100 cm (Fig. 3).

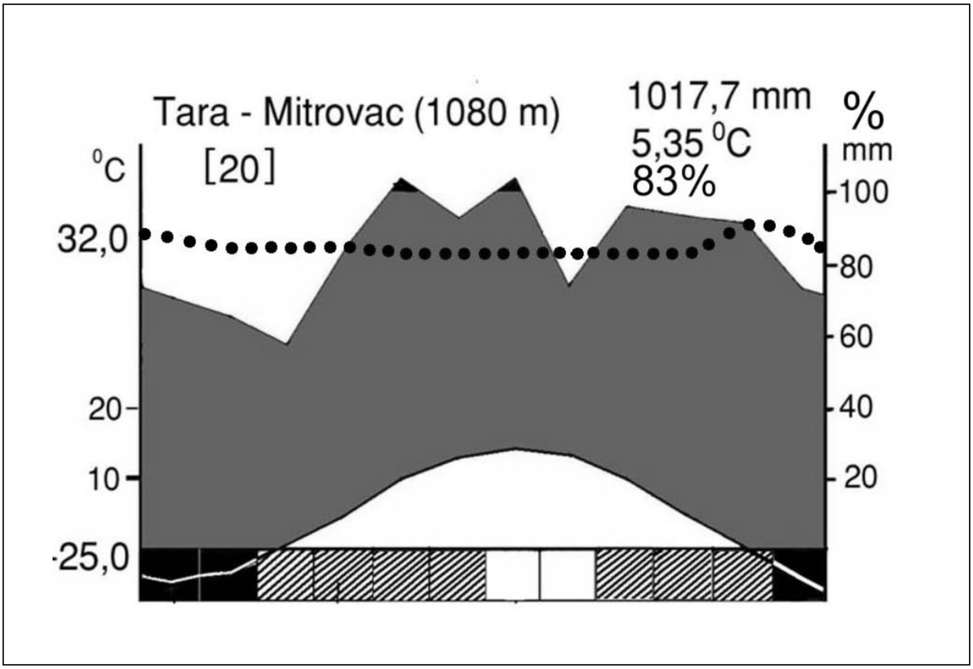

Fig. 3. Climatic diagram of Tara Mt.

Analyses of vegetation and floristic diversity of NP Tara

During more than century of floristic investigation, 1013 plant species have been recorded in Tara Mt., and that represents almost 1/3 of all flora of Serbia (Gajić 1988; Jovanović & Ostojić 2001).

The large number of relict and endemic species confirms the opinion of botanists that Tara Mt. is one of the centers of floristic and vegetation diversity in Serbia and this part of the Balkan Peninsula. Some of the most important endemic and relict species are: *Picea omorika*, *Centaurea derventana*, *Potentilla visianii*, *Aquilegia grata*, *Edraianthus graminifolius* aggr., *Gypsophila spergulifolia*, *Onosma stellulata*, *Halacsya sendtneri*, *Cephalaria pastricensis*, *Haplophyllum boissieranum*, *Daphne blagayana*, *Hieracium waldsteinii*, *Acer heldreichii* (Stevanović 2002).

In the center of floristic diversity of Tara Mt. is the Serbian (Pancic's) spurce a *Picea omorika* (Pančić) Purkyně, a unique paleoendemic species and one of the 'living fossils' of European dendroflora with a range confined to the middle course of the Drina river in western Serbia and eastern Bosnia and Hercegovina (Pančić 1887; Gajić & al. 1994; Dinić & Tatić 2006).

Within GIS analyses the theme 'vegetation' was digitizaled from vegetation map scale R=1:50000; (Mišić 1988). The vegetation map covers a total area of 28244.5 ha. The vegetation is differentiated into 35 vegetation communities in a total of 354 polygons. Attribute data are given using the Serbian and Latin name. Inquiries in the database allow us to get answers about the size of every polygon, and all other statistical indexes regarding the spread of vegetation communities. Parameters of vegetation can be easily correlated to all other themes. For instance, it can show us how the distribution of some vegetation corresponds with the type of geology, soil, elevation zones, slope and aspect of relief (Mišić 1988; Gajić & al. 1992) (Tab. 1).

Analyses of fauna diversity in NP Tara

Besides floristic and vegetation diversity, one of the most important natural potentials of Tara Mt. is the high diversity of fauna.

Until now there are records in Tara of 115 species of daily butterflies (60% of Serbian fauna), 27 species of fish (25%), 12 species of amphibians (46%), 12 species of reptiles (27%), about 170 species of birds (45%) and 51 species of mammals (48%) (Radović 2004).

Of special value to the fauna of Tara is the presence of the grasshopper *Pyrgomorphella serbica*, an endemorelict from Pliocene (Matvejev 1978). Beside this, the presence of three species of daily butterflies (*Euphydryas maturna*, *Maculinea arion*, *Parnassius apollo*) is important. They qualify Tara Mt. for one of the Prime Butterfly Areas in Europe (Jakšić 2003).

Within the ichtyofauna of NP Tara three species have a high value: *Hucho hucho* (Danubian salmon), *Salmo trutta* (brown trout) and *Thymallus thymallus* (grayling) (Simonović 2001).

Some important species of amphibians and reptiles in the area of park are: *Salamandra salamandra*, *Rana graeca*, *Zootoca* (*Lacerta*) *vivipara*, *Vipera ammodytes* (Džukić 1995).

Among important species of birds and mammals are: *Tetrao urogallus*, *Picoides tridactylus*, *Microtus multiplex*, *Ursus arctos*, *Rupicapra rupicapra* (Vasić 1977; Puzović & Grubač 2000; Savić & al. 1995).

Distribution of below listed species was mapped.

Table 1. Distribution of vegetation (absolute and proportional).

Communities	Area	
	ha	%
Carpino orientalis-Quercetum confertae cerris	61.18	0.22
Quercetum cerris carpinetosum orientalis	33.16	0.12
Quercetum cerris juglandetosum	24.85	0.09
Parietario-Juglandetum cerretosum calcicolum	16.73	0.06
Orno ostryetum carpinifoliae	2291.34	8.11
Orno ostryetum juglandetosum	85.79	0.30
Ostryo pinetum nigrae	867.94	3.07
Quercetum montanum	5.75	0.02
Alnetum glutinosae	9.85	0.03
Aceri-Ostryo-Fagetum	585.50	2.07
Fagetum submontanum	130.44	0.46
Fagetum submontanum juglandetosum	57.51	0.20
Erico-Pinetum	131.47	0.47
Fagetum montanum	352.53	1.25
Abieti-Fagetum	327.76	1.16
Piceeto-Abieto-Fagetum	2656.65	9.41
Piceeto-Abieto-Fagetum oxalidetosum	8922.71	31.59
Piceeto-Abieto-Fagetum myrtilletosum	108.74	0.38
Piceeto-Abieto-Fagetum pinetosum	1210.80	4.29
Piceeto-Abieto-Fagetum ostryetosum	21.30	0.08
Piceeto-Abieto-Fagetum ilicetosum	7192.42	25.46
Piceeto-Abieto-Fagetum taxacetosum	25.54	0.09
Piceeto-Abieto-Fagetum-Pinetum nigrae ostryetosum	53.03	0.19
Omoriko-Piceeto-Abieto-Fagetum	56.64	0.20
Omoriko-Piceeto-Abieto-Fagetum-Alnetum mixtum	574.47	2.03
Brometum erecti	47.17	0.17
Alectorolopho-Cynosuretum cristati	1831.14	6.48
Danthonietum calicinae	9.15	0.03
Nardetum strictae s. l.	148.65	0.53
Magnocaricion	43.04	0.15
Danthonietum calicinae	133.54	0.47
Poo molineri-Plantaginetum carinatae	99.37	0.35
Fagetum submontanum mixtum	24.48	0.09
Future reserve in beech forest	82.61	0.29
Reserve of *Picea omorika*	21.16	0.07
Total	28244.42	100.00

Analyses of NP Tara managing contents

GIS analyses of NP Tara contents include the area of the 5 managing units (Zvezda, Crni Vrh, Tara, Rača and Kaludjerske Bare) which include 751 parcels.

For each parcel 8 characteristics have been joined in the relational database: name of the totality, number of parcel and sub parcel, type of woodland, vegetation communities that occur, ecological belonging of vegetation to the type of soil, percentage of every vegetation species that occurs, purpose that department is used for, and level of protection.

GIS of the Park also includes Nature Reserves, settlements, roads and transportation, tourist facilities, line of visibility and aerial photos.

Conclusions

NP Tara represents an area with great natural value of worldwide importance which, to a great extent, has retained its character of an autochthon natural environment.

This work is a contribution to the crucial application of GIS technologies in the areas of: environmental ecology, biodiversity protection, biogeography, conservation geography, physical geography and spatial planning and their implementation in the protection and management of natural resources in NP Tara. This information will be accessible to a wide circle of users in the areas of natural, biotechnical, economic and social sciences, as well as in tourism, sports and recreation.

GIS of NP Tara has outstanding possibilities for adequately preparing the indispensable documentation for the nomination of Tara Mountain as a Biosphere Reserve within the UNESCO program Man and the Biosphere.

GIS of NP Tara can easily be used as a multilateral model, applicable to all other National parks, nature reserves and protected natural areas in Serbia and Montenegro.

Acknowledgements

The present work was supported by the Ministry of Science and Technology of Serbia (contract No. 401-00-402/2004-01). The author wish to thank to Vladimir Stevanović, Dragana Ostojić, Georg Džukić, Predrag Jakšić and Milan Paunović, for contribution in providing data about flora and fauna.

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Karel Sutorý

Cynoglossum montanum L. in the western part of the Mediterranean area

Abstract

Sutorý, K.: *Cynoglossum montanum* L. in the western part of the Mediterranean area. — Bocconea 21: 193-199. 2007. — ISSN 1120-4060.

Cynoglossum montanum L. (*C. haenkei* Schult., *C. hungaricum* Simk.) is reported from NW Italy (Piedmont), S France and N & C Spain. Taxonomy, nomenclature and general distribution are discussed.

Introduction

Cynoglossum montanum L. Demonstr. Pl. Horto Upsaliensi: 5 (1753) was described from material obtained in Italy and cultivated in Uppsala. The description refers to name *"Cynoglossa media altera virente folio*, *rubro flore*, *montana frigidearum regionum"* in Colonna´s (Columna´s) Ecphrasis 176 t., 175 (1606). Description and picture were designated as lectotype by Lacaita in Bull. Orto Bot. Napoli 3: 291 (1913) see Figure 1. The type material is not preserved in Linné´s herbarium (Brand 1921 or http://www.nhm.ac.uk/botany/linnaean/databasehome.html). The authorship of the whole publication is sometimes ascribed to Höjer and therefore this name appears sometimes as *C. montanum* Höjer in Linné (e.g. Cincović & Kojić 1974).

Discusion of taxonomy, nomenclature and general distribution

The above scant data have caused problems as to the identity and circumscription of the species. For example, Popov (1953) and subsequently some other Soviet botanists have used the name for *Cynoglossum germanicum* Jacq. Rather frequently, plants from the northern part of the species range have been classified under the name *C. hungaricum* Simonkai (1878) (e.g. Jávorka 1925; Dostál 1949, 1958, 1982; Soó 1968; Janchen 1958; Czerepanov 1981; Pignatti 1982; Petrova 1992; Holub & Kmeťová 1993; Fischer 1994, etc.). Should the separation of the northern taxon be justified, then in my opinion the older name *C. haenkei* Schult. 1814 has to be used. I have not found the type material of the latter, but, as follows from the concise and otherwise insufficient description, the author had in mind plants which occur in Hungary and whose leaves are rough on both sides ("auf bei-

Fig. 1. *Cynoglossa media altera virente*, *folio rubro flore*, *montana frigidearum regionum*. Colonna F. Ecphrasis p. 175, 1606.

den Seiten scharf"). Of the "Hungarian" species of the genus, only *C. montanum* can be characterized in this way. Some authors (Brand 1921; Krajina 1935; Degen 1937) have identified Schultes´ name with *C. germanicum* Jacq. However, the above quotation from the description excludes this unequivocally. The leaves of *C. germanicum* are almost glabrous on the upper side and sparsely, rather inconspicuously hairy on the lower one but never conspicuously rough. Apparently the younger name *C. hungaricum* was preferred because an unequivocal and detailed description is available for it, and plants of this species still occur at the classical locality in Budapest (Adlersberg = Sáshegy), where Simonkai even collected them for an exsiccate (No. 2636, Fl. Exs. Austro.-Hung.), which is preserved in many herbarium collections (e.g. BC, FI, G, H, K, KRAM, M, OLM, PR, PRC, Z, etc.). These facts are in sharp contrast with Schultes´ problematic description and have naturally been reflected in the frequency of usage of these two names.

Having examined herbarium specimens of this species, I have not been able to confirm any differences between plants from the southern and northern parts of its range and hence I consider the names *C. hungaricum* and *C. haenkei* to be synonymous with *C. montanum*. However, further intensive studies of plant populations and their ecological requirements might reveal certain differences. Authors who consider the two species as being different (Riedl 1962; Pignatti 1982) do not state any reliable differentiating characters.

Brand (1921: 125) in his monograph classifies *C. hungaricum* as a variety of *C. germanicum* and considers it to be a transitional form between *C. germanicum* and *C. montanum*. He describes plants from the Balkans and Italy as *C. montanum* subsp. *linnaeanum* (Brand 1921: 126/127). His concept of *C. montanum* is very broad, including a number of taxa (as subspecies, varieties and subvarieties.) which should probably be placed under other, independent species. Riedl (1962) considered *C. montanum* and. *C. hungaricum* to be two distinct species, but later (Riedl 1978) he already ranged *C. hungaricum* under the synonyms of *C. montanum*, following other authors (Hayek 1928; Krajina 1935). Recently it was used in this sense by Authier (2000) and Sutorý (2000). Kovanda (1972) does not mention *C. montanum* and includes plants from central Europe and the Balkans into *C. hungaricum*. Also Meikle (1985) believes that these two species are identical, includes another species, *C. nebrodense* Guss., and discusses furthermore the synonymy of *C. teheranicum* Riedl.

C. montanum occurs in a rather continuous range from the Czech Republic (southern Moravia) and Slovakia (see Sutorý 1978, 2000) through the easternmost part of Austria, Hungary, former Yugoslavia, Albania down to northern Greece. In Romania the species avoids higher elevations. Along the Black Sea coast it passes down to Moldavia and the southern Ukraine (Crimea). It occurs in Bulgaria and the northern part of Turkey. Somewhat isolated, the species occurs on the Apennine Peninsula, at two groups of localities in southern France and NW Italy, and at several scattered localities in the mountain ranges of central Spain.

Whereas the occurrence of the species in the Balkans, central Europe and Italy has always been rather clear even in spite of the differences in the circumscription of the species, its occurrence west of the Apennines has not yet been clearly reported. The occurrence of the species in NW Italy (Piedmont) was neither reported by Pignatti (1982), nor in earlier complex lists. A similar situation is found in France. Of all floras and checklists for the territory of France (beginning with Lamarck 1778 up to the most recent ones by

Guinochet & Vilmorin 1975; Greuter & al. 1984), only reports of *C. officinale* B *virens* (Schreb.) Rouy (Rouy 1908: 338) can be referred to this species, although with certain reservations. The description "feuilles plus étroites, lanceolées, brieve pubescentes, vertes, les sup. (*C. officinale*) large arrondies, subcordées", as well as some of the synonyms listed (*C. montanum* L., *C. haenkei* Schult.) suggest that Rouy had *C. montanum* in mind. However, the author does not state any localities or even an approximate range of the species. Schreber´s own taxon *C. virens* Schreber ex Boehmer Flora Lipsiae indigena 12, 1750 (shortly described as "A precedente /*C. officinalis*/ differt habitu duriore, viridibus foliis, hirsutis, neque mollibus") is ambiguous, being established for a plant collected near Leipzig (Germany), that is, from an area in which *C. montanum* is not known to occur. The description is short but acceptable. In the synonymy of *C. montanum* this name is given also by Richter (1835). I have not seen the type specimen. From the discussed area it was given and later omitted in local contributions by Gautier (1898) from the Pyrenees and by Gola (1909) from Piedmont.

From Spain this species was only reported by Greuter & al. (1984) but I have not seen any data on concrete localities and I suppose this record is based on the occurrence of the sometimes not distinguished *C. pustulatum* Boiss. (*C. nebrodense* auct. non Guss.). In other synoptic papers covering the territory of Spain (Willkomm & Lange 1870; Willkomm 1893; Garcia-Rollan 1981; Smithies 1984; Casas & al. 1992) the species is not mentioned. Greuter & al. (1984) also report the species as occurring in Sardinia and Sicily.

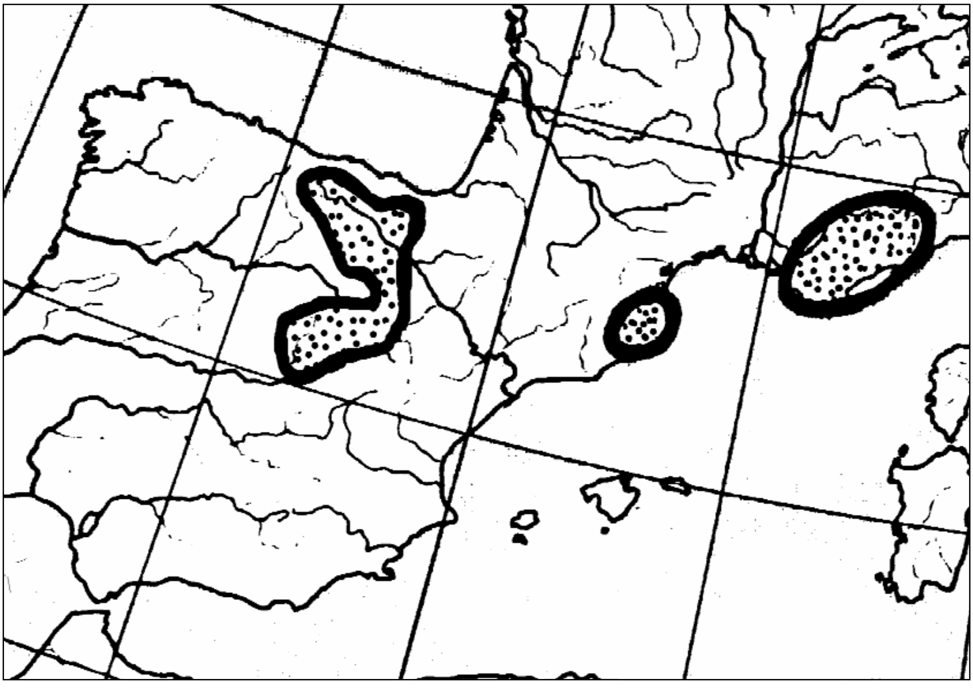

Fig. 2. Distribution of *Cynoglossum montanum* L. in the Western Mediterranean.

The former is probably based on data by Arrigoni (Greuter & Raus 1982) and the latter is likely to refer to *C. nebrodense* Guss. Its occurrence from Sicily is questionable.

Conclusion

The distribution of *C. montanum* in the western Mediterranean is satisfactorily documented by herbarium material and its area although of dispersed character reaches from Central Spain to the eastern part of the Black See region.

Synopsis of localities of *C. montanum* L. in the western Mediterranean

Italy (localities from the Apennine Peninsula are not included):

Piemont: Bagni di Valdieri (herb. Lacaita 1882 BM), Chartreuse de Pesio (Thuret 1862 P). Bad Vinadio (herb. Alioth 1856 MA). Val d´Aoste: La Léchére, Valpelline roches silicense, 1900 m (Aeschimann 1960 G). Prov. Cuneo, Alta Valle Pesio entre les Gias d´Funtena et le Pas d´Baban, 1550 m (Charpin 1993 G). Vallone del Matto a.m. 1400 (Boggiani M.O. 1901 FI). Pion de la Casa del Re nella Valletta (1800 m) Val Gesso (A. M.) (Bono P.G. 1961 FI). Vallone di Mte. Colombo presso S. Giacomo di E….. (1350 m) Val Gesso (A. M.) (Bono P. G. 1961 FI). Erbosi presco S. Anna di Valdieri (1100 m). Val Gesso (A. M.) (Bono P. G. 1962 FI).

France, *Pyrenées orientales:*

Cerdagne a Angoustrine, 1800 m (Sennen 1916 BC). Angoustrine, 1410 m, coteaux granitrique (Sennen 1926 BC, BM; 1927 BC). Capcir, a la Quillan, 1700 m (Sennen 1828 sub *C. gastonis* Sennen BC, BRNU), Cardagne (Cer..): Val de la Molina, 1600 m (Sennen 1935 READ). Coustariges (Olivier 1883, MPU). Mont-Louis, alt 1600 m (Camus 1927 P). Vernet-les-bauis, in valley leading to the Col de Jou. 2800 ft. (Ellman, Sandwith 1925 K). Vernetr (?)- Le Canigou (Müller G. 1896 Z). Barrage de Bouillouse (Galerielle 1934, MPU). Canigou, Chemin de la Gasse de Cadi (Anonymus 1891 G). Pente du Canigou (Sennen 1898 LY). Le Canigou Vallée de Taurinya (Sennen 1897 BC, MPU). Le Canigou pentes vers Taurinya, alt. 1000 m (Sennen 1898 LY).

France, *Hautes Alpes:*

Orcieres (Beauverd 1936 G). Prapic s. Orcieres (Beauverd 1932 G). Chabriéres..., Pilion oriental (Beauverd 1939 G). Biviers. Lieux arides a autonavés (Reverchon E. E. 1873 TL). Gap. Bois arides á Clarances (Reverchon E. E. 1872 TL). Gap. Lieux arides a Monteyer. (Reverchon E. 1871 TL, LY, MPU). Gap. Mont Sense. (Reverchon E. E.1871 K). Briancon, a pros Mauréle (Reverchon E. 1861 JE). Briancon. Bois de Pramaurele. (Reverchon E. 1870 K). Briancon (Anonymus 1856 G). Bords des Champs: Vari (H. Alpes) (Anonymus 1873 G).Ealus, alt 1510 m, Ailefroide (Duclos 1935 P). Galets de la Celse-Niére. (Pelvoux) (Kuhnholz-Lordat G. 1939 MPU). Massif du Pelvoux. Pré d Madame Carle (Tallon G. 1922 MPU). Beilloc prés de Prades (anonymus 1925? E). Pré de Madame Carle Vallouise (Sotiaux P.1978 BR). Isére, Mt. Rachel prés Grenoble (Verlot 1856 G). Vallon d´Ailefroide (Haffner 1932 P). Montagne de Peilasque, au dessous de ….

Des vaches (Canut 1867 FI). Mont Aurouse: de Montmaur a la maison forestiere (Rouy G. 1898 LY). Valley of Clairee, Nevache (Campbell M.S. 1937 BM). Chantemerle. Descente du Serre-Chevallier a Serre-Ratier, alt. 2000-2200 m (Retz B., Didier G. 1961 P). Vallée du Valgaudemar, entre le Refuge du Gioberney et le Lac du Lauzon, 1800-1900 m (Retz B. 1963 P).

Spain:

Prov. Avila: Hoyocasero: le”Pinar” (Rouy 1889 LY). El Nevero (Izco, Costa et Crespo 1973, MA). La Revilla: cerca del pueblo, 1000 m (Muñoz, Pons-Sorolla, Sánchez G. et S. 1979 MA). Madrid: Majaserranos Navacerrada (Anonymus 1911 MA). Escorial (herb. De Coincy 1887 P). Escorial (Lange herb.1890? C). Segovia: Aldealengua de Pedraza (puerto de Lozoya) (Romero 1982 MA). Segovia: Alto del León (Amich 1982 MA). Hinojosa de la Sierra (Soria) (Segura Zubizarreta 1962 MA). Sierra la Mata, Hinojosa de la Serra, ad septentrionem a pago versus (Sutorý K. 2001 BRNM). Sierra de Guadarrama Canencia, in declivibus (Vicioso C. 1916 MA). In locis incultis, Vaciamadrid (Madrid) (Vicioso C. 1915 MA). Palencia: Dehesa de Montejo, valle de Tosande, 1350 m (Monasterio et al. 1995 MA). P. de Guadarrama, pelouse (Bouharmont J. 1977 BR). In locis incultis Somosierra (Madrid) (Vicioso C.1918 MA). Burgos: Neila Sierra de Neila, Peňa Aguda. laderas silíceas, 1880 m (Arán V. J. 1997 MA). Burgos: Covanera, cuneta húmeda Galán Cela (Lopéz G.1986 MA). Puerto de Novacerrada, 1 km antes del Ventorillo, 1450 m (Morales, Sanz 1993 MA). In sylvis et dumosis Sierra de Guadarrama Cercedilla (Vicioso C. 1912 BC). Clairieres du bois de chatagniers a Gerta/e?/ pres Placencia (Bourgeau 1863 MA, MPU). Val...? in hispan (Boissier 1843 G). Sierra de Guadarrama Cercedilla, In ruderatis (Vicioso C.1914 MA). La Revilla: cerca del pueblo, 1000 m (Muños Garmendia, Pons-Sorolla, Sanches Garcia et Susanna MA). Guadalajara: Cantalojas, valle del Lillas, barranco de Carretas, 1460 m, (Burgos, Cardiel 1986 MA).

Acknowledgements

The following institutions have kindly provided material for this study: BC, BM, BR, BRNM, BRNU, C, E, FI, G, JE, K, LY, MA, MPU, P, READ, TL, W, Z. The work was supported by the long term research grant MK00009486201.

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Snežana Vuksanović & Danka Petrović

The occurrence of *Kickxia cirrhosa* (L.) Fritisch in Montenegro supports the earlier records of this species for the Balkan Peninsula

Abstract

Vuksanović, S. & Petrović, D.: The occurence of *Kickxia cirrhosa* (L.) Fritisch in Montenegro supports the earlier records of this species for the Balkan Peninsula. — Bocconea 21: 201-205. 2007. — ISSN 1120-4060.

During the floristic investigation of the Great Beach of Ulcinj and hinterland, *Kickxia cirrhosa* (L.) Fritsch was found for the first time in Montenegro. According to Flora Europaea the area of this species is in W Mediterranean and SW Europe, but the presence on former SFR Yugoslavia is doubtful. The record of *K. cirrhosa* (L.) Fritsch in the hinterland of Great Beach of Ulcinj confirms the presence of this taxon in the flora of Balkan Peninsula.

Introduction

Velika Plaža (Great Beach) in the vicinity of Ulcinj, the part of Ulcinjsko Polje field, is the longest (12 km) beach in the eastern Adriatic coast. It is the best preserved habitat of psamofitic vegetation in Montenegrin coastal area and on whole eastern Adriatic coast as well.

The psamophitic vegetation here is ass. *Xanthio-Cakiletum maritimae* (Beg.) Pign. that inhabits the area exposed to intensive influence of the sea and characterised by poor floristic contents, and ass. *Agropyretum mediterraneum* (Kuhn.) Br.-Bl. bordering the previous and characterized by more significant floristic diversity (Mijović 1994). Beyond the psamohalophytic area there are swamp habitats with dominant ass. *Juncetum maritime-acuti* H-ić. In the hinterland it is also very well developed the vegetation of typical xerophyts whose edificators are: *Petrorhagia saxifraga* L., *Teucrium polium* L. and *Lagurus ovatus* L.

Material and methods

The investigations were carried out from June 2003 to July 2004, and the species presented here was found in June. The specimens are identified following Fernandes (1972) and Pignatti (1982). The vouchers are entrusted to the Natural History Museum of Montenegro in Podgorica.

Results and discussion

The species *Kickxia cirrhosa* (L.) Fritisch (Fig. 1) was found for the first time in Montenegro at only one place on Velika Plaža (hinterland) growing in very small population (Fig. 2). The plant inhabits the sand substrate along the periphery of ass. *Juncetum maritime-acuti* H-ić, and regarding the other elements of the flora the dominant at this habitat are: *Petrorhagia saxifraga* (L.) Link., *Teucrium polium* L., *T. chamaedrys* L., *Sanguisorba minor* Scop., *Lagurus ovatus* L. (Fig. 3).

Fig. 1. *Kickxia cirrhosa* (L.) Fritisch.

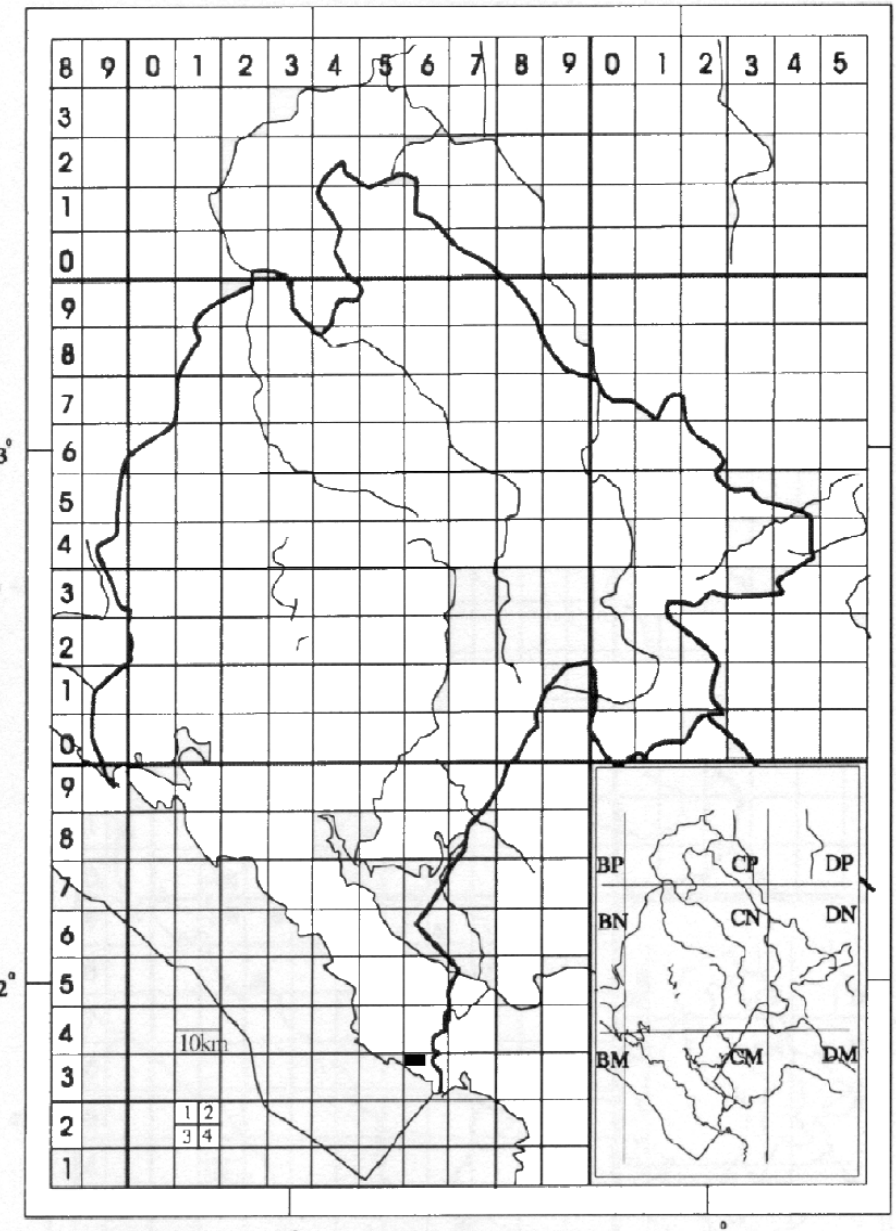

Fig. 2. *Kickxia cirrhosa* (L.) Fritisch in Montenegro (UTM: CM 63/1).

Fig. 3. Habitat of *Kickxia cirrhosa* (L.) Fritsch.

The genus *Kickxia* Dumort. is represented in Europe with 5 species whose centre of dispersion is the Mediterranean region (Fernandes 1972). According to the existing data, *K. spuria* (L.) Dumort., *K. commutata* (Bernh. ex Reichenb.) Fritsch and *K. elatine* (L.) Dumort are known in Montenegro (Rohlena 1942). *Kickxia cirrhosa* (L.) Fritsch is clearly morphologically distinct from all these species as it could be seen from Table 1.

The presence of *Kickxia cirrhosa* in the Balkan Peninsula was reported under: *Linaria cirrhosa* W. for Dalmatia (Hvar, Visiani 1847) and Greece (Crete, Halacsy 1902), and as *Kickxia cirrhosa* for Dalmatia, Albania, Greece and Crete (Hayek 1924-1933). It should be noted that in recent regional floras (Domac 1994, Qosja & al. 1996), there are no data on its presence in the respective countries. According to Flora Europaea (Fernandes 1972) the range of this species is in W Mediterranean and SW Europe, with question mark regarding its presence in the former SFR Yugoslavia. Since the taxon for the former SFR Yugoslavia is reported only on Hvar island, the question mark probably refers to this data. In addition, there are not data on the presence of *Kickxia cirrhosa* in Albania and Greece where grows *K. commutata* (Bernh. ex Reichenb.) Fritsch (*K. commutata* subsp. *graeca*) (Fernandes 1972).

On the basis of morphological characters of the collected material it is clear that the plants from Montenegro belong to *Kickxia cirrhosa* (L.) Fritisch, and not to *K. commutata* subsp. *graeca* (Bory & Chaub.) R. Fernandes.

Table 1. Morphological characters of the Montenegro *Kickxia* species.

Kickxia	leaves	corolla	capsule
cirrhosa	lanceolate-hastate to narrowly lanceolate-sagittate glabrous	4-6 mm violet to whitish tinged with violet	1.5-2mm globose
commutata	ovate-hastate to lanceolate-sagittate villous	11-15 mm whitish upper lip bluish-violet lower lip yellow	2.5-4 mm globose
elatine	ovate, sagittate or hastate	7-15 mm yellowish or bluish upper lip violet	4-4.5 mm subglobose
spuria	ovate, truncate or rounded at base	10-15 mm yellow upper lip deep purple	3-5 mm depressed globose

Conclusion

A finding of *Kickxia cirrhosa* in the hinterland of the Velika Plaža in the vicinity of Ulcinj, clearly confirm its presence in the Balkan Peninsula. In relation to Flora Europaea this record significantly extends the species range towards the south-east. It may be expected that future explorations will confirm Hayek's data on the presence of *Kickxia cirrhosa* (L.) Fritisch in Albania and Greece.

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L. Peruzzi, N. G. Passalacqua & G. Cesca

On the presence of *Doronicum plantagineum* (*Asteraceae*) in Italy

Abstract

Peruzzi, L., Passalacqua, N. G. & Cesca, G.: On the presence of *Doronicum plantagineum* (*Asteraceae*) in Italy. — Bocconea 21: 207-212. 2007. — ISSN 1120-4060.

Doronicum plantagineum is confirmed as a member of the Italian flora. Former reports of this species, or of *D. hungaricum*, from the Sila Massif (C Calabria) have been verified and are all referred to *D. plantagineum*. In addition to its confirmed presence in Sila, this species occurs in several localities of N Calabria (Pollino Massif and Verbicaro-Orsomarso mountains). *D. plantagineum* is a W Mediterranean species. Its S Italian stands are separated by more than 1000 km from its main area of distribution, the nearest localities being in SE France and NE Algeria. The presence of *D. plantagineum* in S Italy is therefore of particular phytogeographical interest. A map showing all known Italian localities of this species is provided.

Introduction

In a recent systematic revision of *Doronicum* L. (Fernández 2003) the report in Italy of plants referred in the past to the W Mediterranean *D. plantagineum* L., or to its close Balkan relative *D. hungaricum* Rchb. f, is completely ignored. Tenore (1835-1838, 1842) first recorded such plants from Italy (C Calabria) on the basis of samples collected seven years before (Tenore 1835, as "*D. pardalianches*"), now regarding them as a new variety of *D. plantagineum*: *D. plantagineum* var. *lobelii*. Subsequently, Fiori (1927) synonimised *D. plantagineum* var. *lobelii* with *D. hungaricum*, an opinion shared by Sarfatti (1965) and Pignatti (1982), although Gentile & Martini (1974) and Gentile (1979) had already reported Calabrian plants as representing *D. plantagineum*.

This note is to clarify the identity of these plants, to re-emphasise their presence in Italy (Calabria), and to add also three new localities that extend the Italian range of this taxon by c.100 km toward the north.

Material and Methods

The identity of Calabrian plants was established by using the analytical key and the descriptions in Fernández (2003).

To prepare an updated distributional map, literature data (for both *D. plantagineum* or *D. hungaricum*) and label data for the revised specimens in CLU were used. UTM coordinates of all localities were established by using topographical maps 1 : 25.000 (Ânonymous 1996).

Specimens seen

Italy, Calabria, Sila: Serra Colamauci (Sila Grande, CS), 1200 m, margine querceto, 12/V/1997, *Bernardo* (CLU, UTM 33 S XD 29.62); Camigliati, presso Camigliatello Silano (Sila Grande, CS), 1200 m, pineta, 28/V/1989, *Bernardo* (CLU, UTM 33 S XD 26.55); Sila Piccola, regione Ciricilla, ambiente paludoso, 31/V/2002, *Peruzzi* et *Passalacqua* (CLU, UTM 33 S XD 35.34); Pollino Massif: Massiccio del Pollino: versante settentrionale di Timpa del Principe, 1200 m s.l.m., pascoli ai margini di faggeta, substrato calcareo, 20/V/2004, *Peruzzi*, *Passalacqua* et *Aquaro* (CLU, UTM 33 S XE 08.14); Verbicaro-Orsomarso mountains: Piana di Campotenese, lungo la strada che porta a c.da Masistri, ca. 1000 m s.l.m., coltivi ed incolti, 19/V/2004, *Peruzzi* et *Passalacqua* (CLU, UTM 33 S WE 90.14); Complesso montuoso di Verbicaro-Orsomarso: Montagna di Masistro, ca. 1350 m s.l.m., impluvi, su suolo fortemente nitrificato, susbtrato calcareo, 19/V/2004, *Peruzzi* et *Passalacqua* (CLU, UTM 33 S WE 90.10).

Literature data

Italy, Calabria, Sila: Sarfatti (1965) quotes the following localities: Bivio Giamberga (UTM 33 S XD 27.67); Camigliatello (UTM 33 S XD 25.55); Germano (UTM 33 S XD 42.54); Santa Barbara (UTM 33 S XD 39.61); Sorgenti del Tacina (UTM 33 S XD 37.34); Timpone Morello (UTM 33 S XD 36.33); Gentile & Martini (1974) add San Nicola (UTM 33 S XD 33.52) and Quarto di Monteoliveto (UTM 33 S XD 37.52); and Gentile (1979) adds Silvana Mànsio (UTM 33 S XD 32.52).

Results and Discussion

Following the taxonomic criteria of Fernández (2003), our plants match *Doronicum plantagineum* in all features of the basal leaves and the rhizome, and in the size of disk flower corollas, while resembling *D. hungaricum* in overall plant size and in the relative length of capitula vs. phyllaries (Tab. 1). However, the very lectotype of *D. plantagineum* (designated by Jarvis & Turland 1998, picture displayed in Jarvis & al. 2004) shows capitula that are longer than the phyllaries, as Calabrian plants do, so this character does not seem reliable. The eventual absence of leaves at the base of flowering stems, in our material, is explained by ecological factors, viz., the presence of tall herbs.

The name *D. plantagineum* var. *lobelii* was proposed for Calabrian plants (Sila Piccola) by Tenore (1835-1838: 231, "*foliis angustioribus subintegerrimis*". 1842), who quoted a plate of Lobelius (1581, Fig. 1). By studying this plate, it is evident that it does not depict Calabrian *D. plantagineum*, but most probably *D. hungaricum*. For this reason, Lobelius's plate is not formally here selected as the lectotype of *D. plantagineum* var. *lobelii*, a name explicitly created for Calabrian plants, despite of its eponimy. The typification of this name needs further investigations in the Herbarium Tenore (NAP), not carried out in this paper.

Fig. 1. *Doronicum minus officinarum* (Lobelius 1581), the name *D. plantagineum* var. *lobelii* is partly based on this plate.

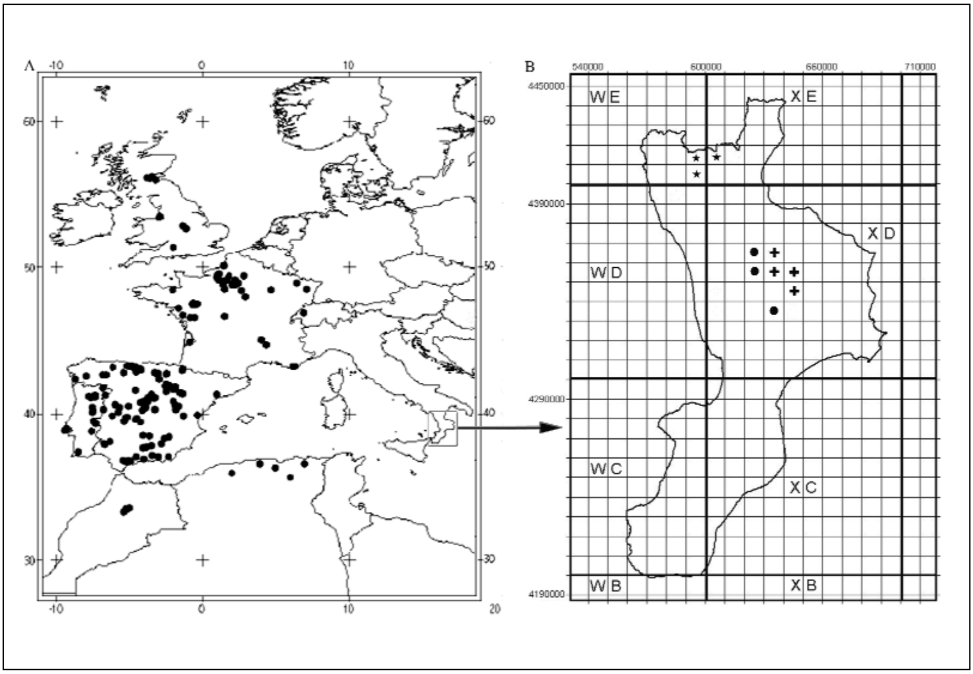

Fig. 2. *Doronicum plantagineum*: A - range modified from Fernández 2003, B - particular of Calabrian distribution, + - literature data, circles - confirmed localities, stars - new localities.

The Calabrian distribution of *D. plantagineum* is shown in Figure 2. S Italian stands are at a distance of more than 1000 km far from the main range, the nearest localities outside of Italy being in SE France and NE Algeria.

Conclusions

As far as we can tell, there is no basis for a taxonomic distinction between Calabrian plants and *Doronicum plantagineum*. This species occurs in Italy only in central and northern Calabria, where it grows in open beech and pine woods, on pastures and along field margins, from 1000 to 1500 m asl.

The presence of *Doronicum plantagineum* in S Italy is of particular biogeographical interest and represents an eastern extension of its geographical range. The distributional pattern of this species is similar to that of the *Genista anglica* L. aggregate (Brullo & al. 2001), *Adenocarpus* DC. (Brullo & al. 2001a), *Lomelosia cretica* (L.) Greuter (Verlaque & al. 1991) and several other taxa whose present distribution is presumably linked to Late Oligocene/Early Miocene palaeogeography (Peruzzi 2003). Indeed, a recent phylogenetic analysis of the genus *Doronicum* (Fernández & al. 2001) places *D. plantagineum* in a clade basal to the genus (together with *D. hungaricum*, *D. columnae* Ten. and *D. orientale* Hoffm.), supporting the assumption of a relatively old origin of this species group.

Table 1. Comparative morphological features of *Doronicum hungaricum*, *D. plantagineum* (data source: Fernández 2003) and Calabrian plants.

	D. hungaricum	Calabrian plants	*D. plantagineum*
size of plants	up to 80 cm	up to 80 cm	up to 150 cm
basal leaves size	4-9×1-3 cm	4-11×3-6 cm	4-8×2.5-6 cm
presence at flowering	yes	no/yes	no/yes
shape	oblong-elliptic	ovate to elliptic	ovate to ovate elliptic
hairs (upper surface)	short glandular and long eglandular	all glandular	all glandular
rhizome	glabrous to scarcely pubescent	pubescent to strongly pubescent	pubescent to strongly pubescent
disk flower corolla	up to 4 mm	4-5 mm	4-5.5 mm
capitula	≥ phyllaries	≥ phyllaries	≤ phyllaries
2*n*	60	?	120

Acknowledgements

Many thanks are due to Dr. I. A. Fernández, specialist in *Doronicum*, for sending us reprints of her papers and confirming the correct identification of our plants.

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S. Brullo, C. Gangale & D. Uzunov

Taxonomic remarks on the endemic flora of the Sila Massif (S Italy)

Abstract

Brullo, S., Gangale, C. & Uzunov, D.: Taxonomic remarks on the endemic flora of the Sila Mountain (S Italy). — Bocconea 21: 213-222. 2007. — ISSN 1120-4060.

Within floristic investigations on the Sila Massif (S Italy), taxonomical considerations and new contributions on the endemics of this territory are given. The flora of Sila has 13 exclusive endemics and includes further 55 endemics with a wider distribution range, 8 of which are restricted to the siliceous Calabrian massifs. In order to contribute to the knowledge of the endemics of the Silan flora, two new species (*Adenocarpus tenoreanus* and *Allium julianum*) are here described and a new combination is proposed.

Introduction

Within taxonomical investigations on the flora of Southern Italy, a contribution on the endemic flora of Sila is presented.

The Sila Massif, located in Northern Calabria and reaching 1927 m asl (Monte Botte Donato), represents a territory of remarkable phytogeographic interest because of its geology, geographical position and bioclimatic features. This area, together with other S Calabrian mountains and NE Sicily, belongs to the Calabrian Arc, an Ercinic unit dominated by siliceous substrata, mostly granites, mica-schists and kinzigitic gneiss. According to Rivas-Martinez & Loidi Arregui (1999), the bioclimate of the upper parts of Sila are to be included within the supra-temperate submediterranean thermotype and marginally also to the supramediterranean one, with subhumid or humid ombrotypes.

The mountain vegetation of Sila, between 1000-1927 m asl, includes by beechwoods, often mixed to Calabrian pine (*Pinus nigra* subsp. *calabrica*) forests, thorny cushion-like shrub communities, meadows and wetlands. In the submontane belt, ranging between 700-1000(1200) m, deciduous oakwoods dominate the landscape.

The flora of Sila has been studied by many authors (Solla 1896; Longo 1903, 1905; Trotter 1911; Grande 1913; Fiori 1919, 1922; Albo 1935; Sarfatti 1959, 1965; Gentile & Martini 1974; Cesca 1981, 1982; Ballelli & Venanzoni 1993; Cesca & Peruzzi 2002; Brullo & al. 2002, 2004; Marhold & al. 2003, etc.). On the basis of these studies, the Silan flora consists of about 1000 taxa at specific and subspecific level, 7% of which are endemics. In the present paper, taxonomic considerations on the endemic flora of the Silan Massif are given, with particular attention to some critical taxa.

Results and discussion

For the mountain belt (over 1000 m asl) of the Silan territory, 68 endemic species or subspecies are recorded. The most part (47) are, either, Apenninic/S Apenninic endemics or restricted to the mountains of Calabria and Northern Sicily. Twenty one taxa are endemic to the Calabrian siliceous massifs and thirteen of these are exclusively found on the Sila (Fig. 1). Most of them are characteristic elements of dry and open places occurring on the Silan plateau between 1200-1700 m asl, and they are: *Astragalus calabricus*, *Armeria brutia*, *Avenula praetutiana* subsp. *rigida*, *Anthemis hydruntina* subsp. *silensis*, *Centaurea sarfattiana*, *Genista silana*, *Koeleria splendens* subsp. *brutia*, *Knautia dinarica* subsp. *silana*. Other species are frequent at the fringe of pine and beech forests (*Adenocarpus tenoreanus*, *Allium julianum*, *Anthemis triumphetti* subsp. *briquetii*) or in wetlands (*Luzula calabra*, *Cardamine silana*).

Some of these taxa have been recently described (Brullo & al. 2002a, 2004; Marhold & al. 2003), and two of them new to science are presented here.

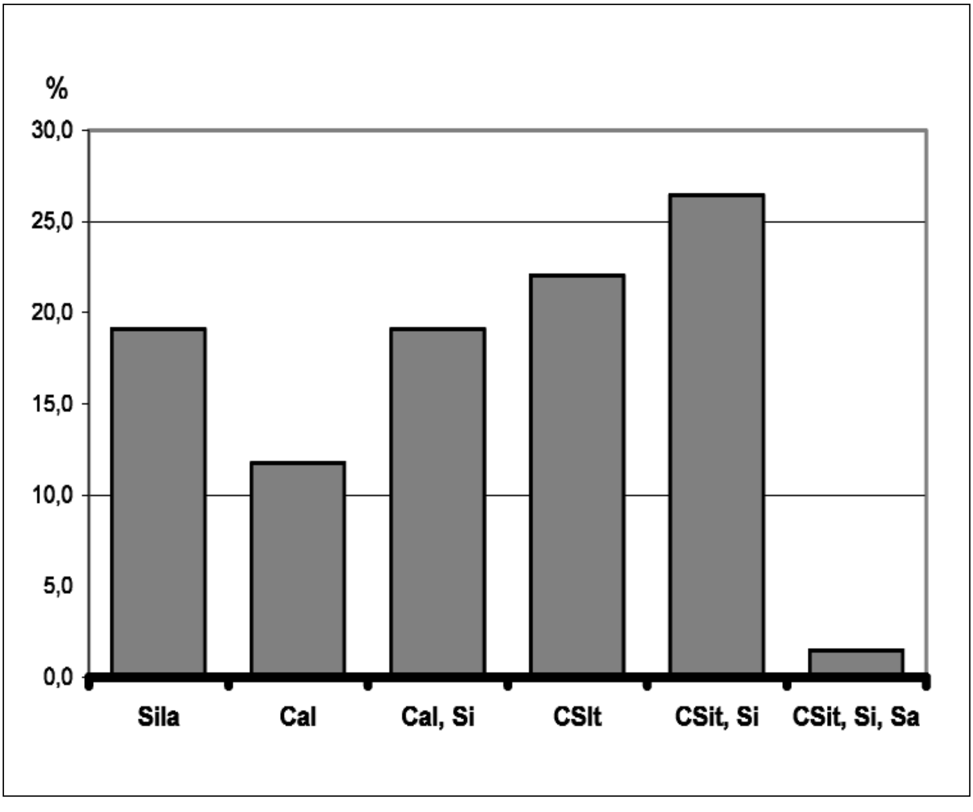

Fig. 1. Chorologic spectrum of the endemic taxa of the Silan flora: Sila - exclusive of the Sila Massif, Cal - Calabria, Si - Sicily, CSIt - C and S Italy, Sa - Sardinia.

Adenocarpus tenoreanus Brullo, Gangale & Uzunov sp. nova

Type: Calabria, Sila Piccola, Valle del Roncino, Monaco, 19/7/1998, *Bartolo*, *Brullo*, *Gangale & Giusso* (Holotype: CAT; Isotypes: CAT, CLU, FI).

Adenocarpus brutio similis sed ramulis eburnei, pedunculis 1-8 mm longis, foliolis lineari-ellipticis, stipulis 1.5-2.5 mm longis, inflorescentia 3-12 cm longa, bracteis 3-5 × 0.8-1 mm, bracteolis lineari-subulatis, 3-4 × 0.25-0.4 mm, pedicellis 2.5-4 mm longis, calice 5-7.5 mm longo, dense glanduloso sparsim piloso, labio inferiore 3.5-5 mm longo dentibus 1-1.5 mm longis, vexillo elliptico-suborbicularis 8-10 mm longo, rotundato apice, carina 9-10 mm longa.

Description**:** Shrub erect, 50-150 cm high, ivory, densely ciliate-villous, very branched. Leaves 3-foliate, with peduncle 1-8 mm long, ciliate-hairy and leaflets 7-15 × 2-5 mm, linear-elliptical, densely hairy on the lower surface, subglabrous to sparsely hairy on the upper one. Stipule 1.5-2.5 long. Inflorescence lax, 3-12 cm long. Bracts ovate-lanceolate, ciliate, 3-5 × 0.8-1 mm. Bracteoles linear-subulate, ciliate, 3-4 × 0.25-0.4 mm. Pedicels 2.5-4 mm long. Calyx 5-7.5 mm long, densely glandular with sparsely hairs; lower lip 3.5-5 mm long, with central tooth 1.2-1.5 mm long, and lateral ones 1-1.2 mm long; upper lip with two triangular teeth 2.5-3 mm long. Corolla yellow; standard elliptical-suborbicular 8-10 × 7-8 mm, rounded at apex, with appressed hairs on the back and claw 1.5-2 mm long; wings 7-10 mm long; keel 9-10 mm long. Legume brown-blackish, 20-50 x 4-5 mm, 2-9 seeded.

Iconography**:** Fig. 2.

Ecology and Distribution**:** It is localized in the Southern Sila at the margin of pine woods or in deforested areas, at 1000-1400 m altitude. It occurs on siliceous substrata, mainly granites.

This species belongs to *Adenocarpus complicatus* (L.) Gay group, a Mediterranean element represented in Italy by *A. bivonii* (C. Presl) C. Presl, *A. commutatus* Guss., *A. brutius* Brullo, De Marco & Siracusa, and *A. samniticus* Brullo, De Marco & Siracusa (Brullo & al. 2001).

Allium julianum Brullo, Gangale & Uzunov, sp. nova

Type**:** Calabria, Sila Greca, Campi, lungo la strada per il santuario S. Maria del Pathirion, 2/7/99, *Brullo*, *Giusso & Gangale* (Holotype: CAT; Isotypes: CAT, CLU, FI).

Scapus glabrus, 20-45 cm altus ad 1/2-2/3 usque vaginis foliorum tectus. Folia 5-7, usque ad 30 cm longa, pluricostata. Inflorescentia expansa, diffusa, 15-75 floribus, pedicellis inaequalibus 8-30 (40) mm longis. Spata persistens, bivalvis, valvis inequalibus 2.5-12 cm longis. Perigonium albo-viride, subcylindricum, tepalis subaequalibus, oblongis vel oblungo-obovatis, ad apicem rotundatis, 4.5-5.5 mm longis, 2-3 mm latis. Stamina simplicia, inclusa, filamentibus albis, esterioribus 1.5-2 mm longis, interioribus 2.5-3 mm longis, antheris stramineis, ellipticis ad apicem rotundatis 1-1.2 mm longis. Ovarium subcylindricum, scabri-papillosum superne. Capsula obovoidea, 4.5 × 4 mm.

Description**:** Bulb ovoid, 10-14 × 8-12 mm, with outer tunics coriaceous brown, the inner ones membranous, whitish. Scape glabrous, erect, 20 – 45 cm high, covered by leaf sheaths for 1/2 – 2/3 of its length. Leaves 5-7, green, semicylindrical, costate up to 30 cm long. Spathe persistent, with 2 unequal valves, longer then umbel, the larger 7-nerved, 6-12 cm long, the smaller 5 nerved, 2.5-6 cm long. Inflorescence expanse, diffuse 15-75

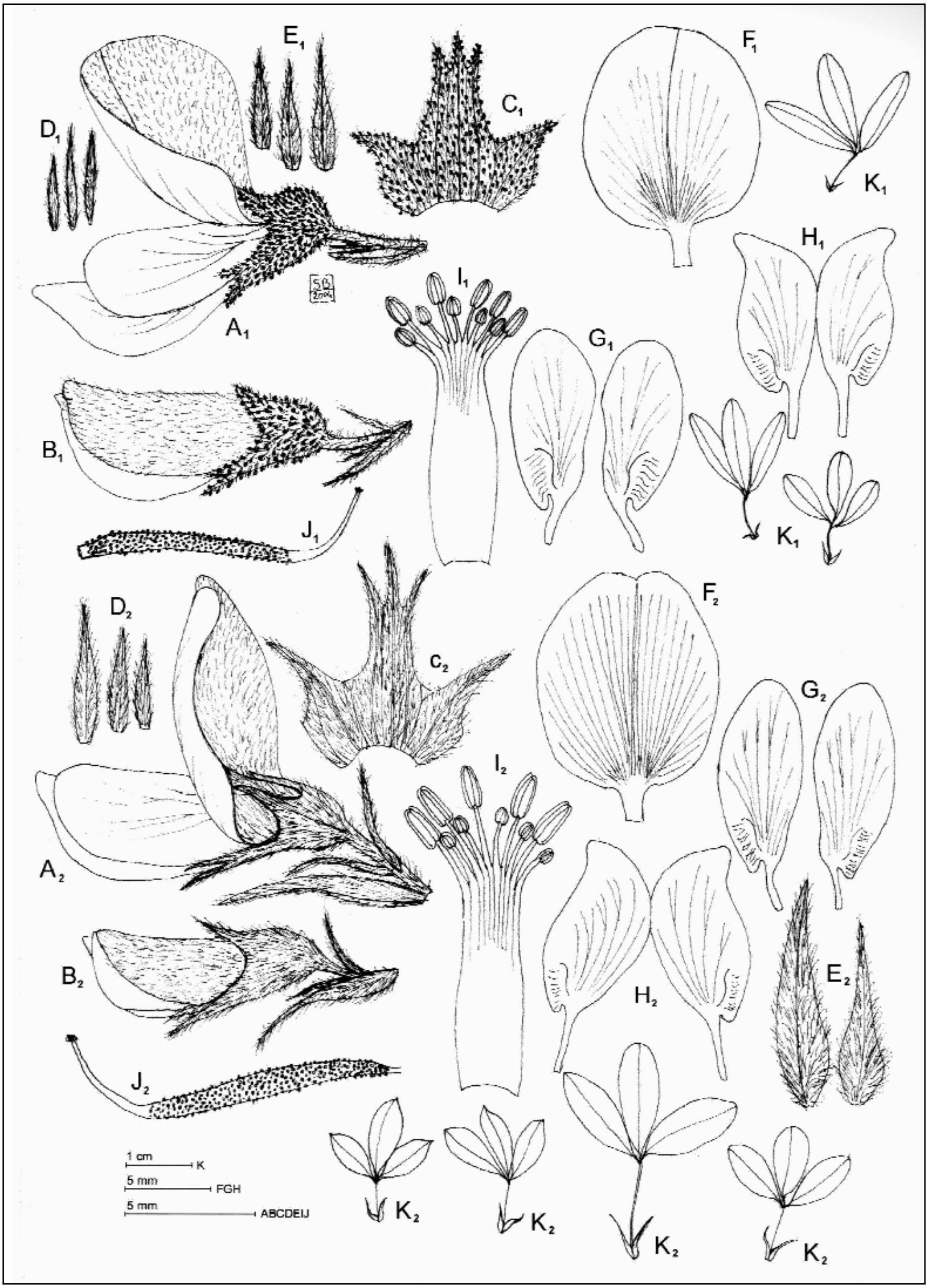

Fig. 2. *Adenocarpus tenoreanus* Brullo, Gangale & Uzunov (1), *A. brutius* Brullo, De Marco & Siracusa (2): A - flower, B - bud, C - open calyx, D - bracteoles, E - bracts, F - standard, G - wings, H - keel - I - staminal tube, J - pistil, K - leaves.

flowered; pedicels unequal, flexuous, 8-30(40) mm long. Perigon subcylindrical, with tepals subequal, white-greenish with green mibrid, oblong to oblong-obovate, rounded at apex, 4.5-5.5 mm long, 2-3 mm wide. Stamens simple, included, with filaments white, subulate, the outermost 1.5-2 mm long, the innermost 2.5-3 mm long, connate at base into an annulus 0.6-0.8 mm high; anthers elliptical, pale yellow, rounded at apex 1-1.2 × 0.7-0.9 mm. Ovary subcylindrical, greenish, scabrid-papillose above, 2.5-3 x 1.5-1.8 mm. Style white, 0.4-0.8 mm long. Capsule obovoid, green 4.5 x 4 mm.

Iconography**:** Fig. 3.

Etymology**:** The species is dedicated to Prof. Giuliano Cesca, for his significant contribution to the investigation of the Calabrian flora.

Ecology and distribution**:** It grows in the underwood of *Quercus cerris* forests at about 800 m altitude on siliceous substrata (schists). It occurs on the NE slope of Sila Massif (Sila Greca), where it is very rare and localized.

A very localized and rare endemic species, belonging to the *A*. sect. *Codonoprasum*, is well differentiated from the other known species of the *A*. *paniculatum* group and in particular it shows close relations mainly with *A*. *tenuiflorum* Ten., occurring in the Italian Peninsula (Brullo & al, 2002a).

Other species endemic to Sila territory:

Anthemis hydruntina H. Groves subsp. ***silensis*** (Fiori) Brullo, Gangale & Uzunov

A. *hydruntina* subsp. *hydruntina* is restricted to few localities of Puglia and Basilicata (S Italy), where it occurs on calcareous rocky places, while subsp. *silensis* is an orophilous *taxon*, linked to siliceous substrata and localized in a small area of the eastern slope of the Silan Massif (Brullo & al. 2004).

Cota triumfetti (L.) J. Gay subsp. ***briquetii*** (Fiori) Brullo, Gangale & Uzunov, comb. & stat. nov.

Type: Calabria Sila a S. Giovanni in Fiore, 18/6/1899, *Fiori* sub *A*. *tintoria* var. *discoidea* (Lectotype: FI!).

Basion.: *A*. *tinctoria* L. var. *briquetii* Fiori, Nuov. Fl. Anal. Ital. 2: 649 (1927).

Syn.: *A*. *tinctoria* var. *discoidea* Fiori, N. Giorn. Bot. Ital. 7: 270 (1900), non DC. (1837); *A*. *triumfetti* var. *discoidea* Fiori, in Fiori e Paoletti, Fl. Anal. Ital. 3: 260 (1903) p. p.

This taxon differs from the type, widely spread in S Europe, by the capitula discoid and achenes with a well developed coronule.

Armeria brutia Brullo, Gangale & Uzunov

Species belonging to the Tyrrhenian element, like *Armeria nebrodensis* (Guss.) Boiss. from Sicily, *A*. *aspromontana* Brullo, Scelsi & Spampinato from Aspromonte, *A*. *sardoa* Sprengel from Sardinia, *A*. *multiceps* Wallr. from Corsica and *A*. *gracilis* Ten. from Central and Southern Apennines, up to Mt. Pollino (Brullo & al. 2004).

Astragalus calabricus Fisch.

It belongs to the cycle of *Astragalus thracicus* Griseb., represented by taxa distributed in the E-Mediterranean area.

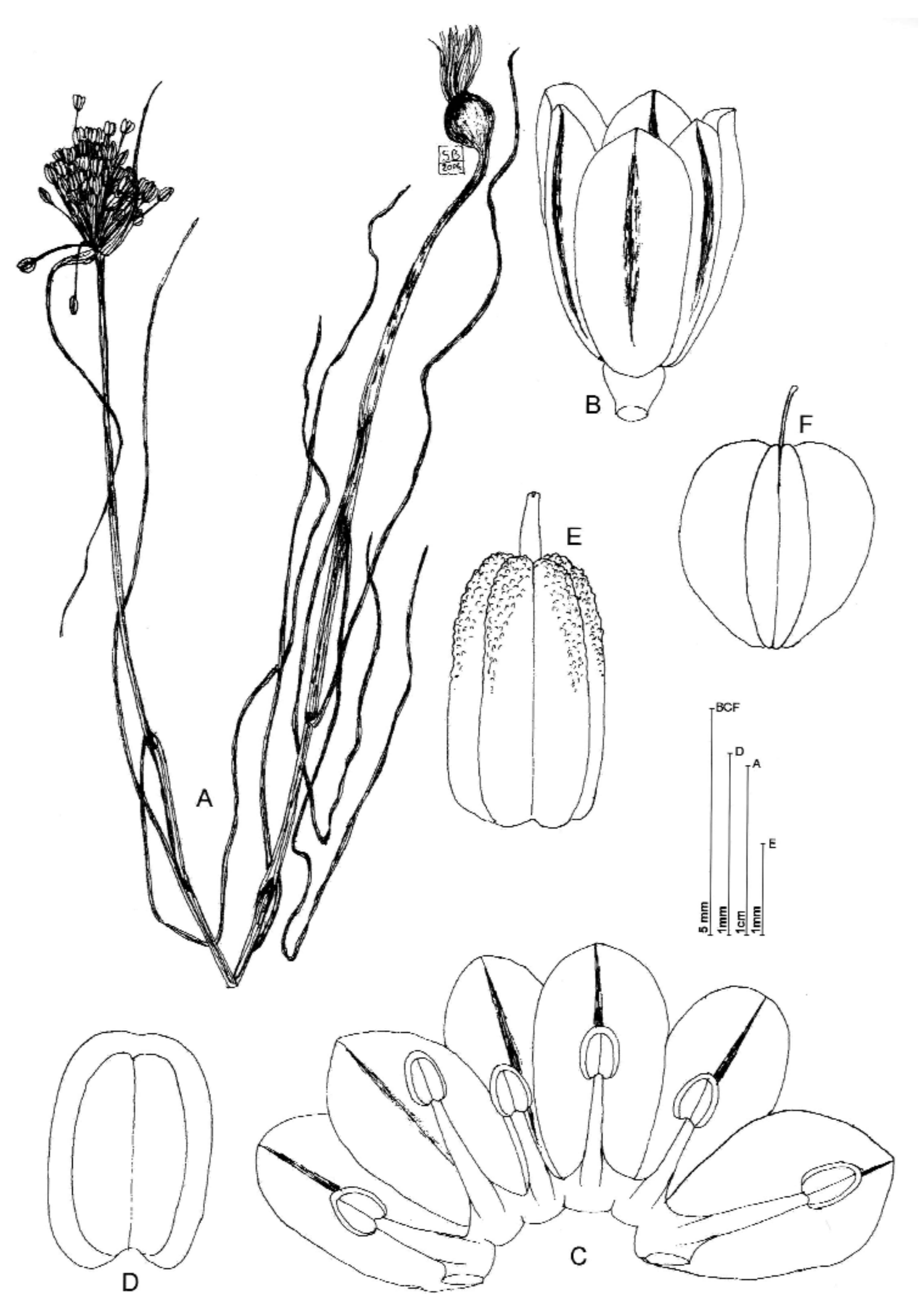

Fig. 3. *Allium julianum* Brullo, Gangale & Uzunov: A - habit, B - flower, C - open perigon and stamens, D - anther, E - ovary, F - capsule.

Avenula praetutiana (Parl. ex Arcang.) Pignatti subsp. ***rigida*** (Sarfatti) Brullo, Gangale & Uzunov

Avenula praetutiana is an Apenninic endemic, belonging to the cycle of *A. versicolor*, widely spread in C and S Europe (Brullo & al. 2004). It is represented by two subspecies: a calcicolous one, (subsp. *praetutiana*) having a discontinuous distribution on the whole Apenninic range and a silicicolous one (subsp. *rigida*), occurring on the Sila Massif.

Centaurea sarfattiana Brullo, Gangale & Uzunov

It is closely related to *C. deusta*, belonging to the sect. *Phalolepis* (Cass.) DC., which is represented in C and S Italy by manifold *taxa*, mainly chasmophilous or linked to shrub-communities (Brullo & al. 2001, 2004).

Cardamine silana Marhold & Perny

This taxon is hexaploid, like some populations of *C. raphanifolia* Pourr., but morphologically shows closer relationships with the diploid Balkan population of *C. acris* Griseb. (Marhold & al. 2003).

Genista silana Brullo, Gangale & Spampinato

It is vicariated in S Calabria (Aspromonte) by *G. brutia* Brullo, Scelsi & Spampinato, both species closely related to *G. anglica* L., a typical atlantic element distributed in Northern and Central Europe (Brullo & al. 2002).

Knautia dinarica subsp. ***silana*** (Grande) Ehrend.

According to Ehrendorfer (1975) *Knautia dinarica* is represented by subsp. *dinarica*, distributed in the Balkanic Peninsula, and subsp. *silana* exclusive of the Silan Massif, which differs from the type mainly in the pubescence of lower stem internodes and in the longer petiole of the leaves.

Koeleria splendens subsp. ***brutia*** Brullo, Gangale & Uzunov

A silicicolous taxon morphologically and ecologically well differentiated from the other known subspecies, which are normally linked to limestones; in particular *Koeleria splendens* subsp. *splendens* occurs in N Sicily, while *K. splendens* subsp. *grandiflora* (Bertol. ex Schultes) Domin is distributed in the rest of the calcareous mountains of the Apennines (Brullo & al. 2004).

Luzula calabra Ten.

This taxon, belonging to the group of Euroasiatic *Luzula campestris* Lam., was described by Tenore (1829) "*ex paludibus Silae*". According to Migliaccio (1964), *L. calabra* differs from *L. campestris* subsp. *vulgaris*, occurring in Sila territory too, in laxer anthela, longer capitula and shorter capsule, stamens, style and tepals, and in the colour of capsule and tepals. *L. calabra* occurs on wetlands of mountain belt of Sila, especially in swamps, *Nardus stricta* communities and along the border of small streams in open places, while *L. campestris* is linked to dry grassland. It is a member of igrophylous communities belonging to *Luzulo calabrae-Nardetum*.

Other endemic species occurring on Sila but ranging, as well, on other Calabrian siliceous massifs are: *Anthemis calabrica* (Arcang.) Brullo, Scelsi & Spampinato closely related to *A. cretica* L., *Hypericum calabricum* Spreng. geographic vicariant of *H. barbatum* Jacq., *Lereschia thomasii* (Ten.) Boiss. belonging to a taxonomically isolated monotypic genus, *Soldanella calabrella* Kress close to the SE European *S. hungarica* Simonkai, *Limodorum brulloi* Bartolo & Pulvirenti showing more ancestral features within the genus, *Cardamine battagliae* Cesca & Peruzzi related to SW European *C. heptaphylla* (Vill.) O. E. Schulz, *Salix brutia* Brullo & Spampinato, a Calabrian vicariant of *S. triandra* L., and *Buglossoides calabra* (Ten.) Johnston morphologically well differentiated from *B. purpurocaerulea* (L.) Johnston .

Besides, well represented is the set of Siculo-Calabrian endemics that emphasizes the closest floristic affinities of Sila with Sicilian flora rather than the rest of Apennines. Among these, there are *Carlina nebrodensis* Guss., *Silene sicula* Ucria, *Ranunculus aspromontanus* Huter, Porta & Rigo, *Cirsium vallis-demonii* Lojac., *Viola messanensis* (W. Becker) Brullo, *Pinus nigra* Arnold subsp. *calabrica* (Land.) E. Murray, *Barbarea sicula* C. Presl, *Epipactis schubertiorum* Bartolo, Pulvirenti & Robatsch, etc.

The present study is a first contribution for the characterisation of the endemic element of the Silan flora. Further research on the qualitative and quantitative features of the endemics will contribute to the interpretation of the Silan and S Calabrian flora genesis and phytogeography.

List of the endemic species to the Sila Massif

Acer lobelii Ten.; *Acer neapolitanum* Ten.; *Achillea tenorii* Grande; *Acinos granatensis* (Boiss. & Reuter) Pignatti subsp. *aetnensis* (Strobl) Pignatti; *Adenocarpus tenoreanus* Brullo, Gangale & Uzunov; *Ajuga tenorei* Presl; *Allium julianum* Brullo, Gangale & Uzunov; *Anthemis hydruntina* Groves subsp. *silensis* (Fiori) Brullo, Gangale & Uzunov; *Anthemis montana* L. subsp. *calabrica* Arcang.; *Arabis rosea* DC.; *Armeria brutia* Brullo, Gangale & Uzunov; *Artemisia variabilis* Ten.; *Astragalus calabricus* Fiori; *Avenula praetutiana* (Parl.) Pign. subsp. *rigida* (Sarfatti) Brullo, Gangale & Uzunov; *Barbarea sicula* Presl; *Buglossoides calabra* (Ten.) Johnston; *Bunium petraeum* Ten.; *Campanula tricocalycina* Ten.; *Cardamine battagliae* Cesca & Peruzzi; *Cardamine silana* Marhold & Perny; *Carduus chrysacanthus* Ten.; *Carlina nebrodensis* Guss.; *Centaurea centaurium* L.; *Centaurea sarfattiana* Brullo, Gangale & Uzunov; *Cerastium granulatum* (Huter, P. & R.) Chiov.; *Cerastium scaranii* Ten.; *Cirsium vallis-demonii* Lojac.; Cota triumfetti (L.) J. Gay subsp. *briquettii* (Fiori) Brullo, Gangale & Uzunov; *Dianthus vulturius* Guss. & Ten. subsp. *vulturius*; *Digitalis micrantha* Roth; *Echinops siculus* Strobl; *Epipactis meridionalis* H. Baumann & R. Lorenz; *Epipactis schubertiorum* Bartolo, Pulvirenti & Robatsch; *Euphorbia amygdaloides* L. subsp. *arbuscula* Meusel; *Euphorbia corallioides* L.; *Gagea chrysantha* (Jan) Schultes; *Galium aetnicum* Biv.; *Genista silana* Brullo, Gangale & Spampinato; *Helleborus bocconei* Ten. subsp. *intermedius* (Guss.) Greuter & Burdet; *Hieracium virgaurea* Coss.; *Hypericum calabricum* Sprengel; *Knautia calycina* (Presl) Guss.; *Knautia dinarica* (Murb.) Borbas subsp. *silana* (Grande) Ehrend.; *Koeleria splendens* C. Presl subsp. *brutia* Brullo, Gangale & Uzunov; *Lathyrus jordanii* (Ten.) Ces., Pass.

& Gib.; *Leontodon intermedius* Huter, P. & R.; *Lereschia thomasii* (Ten.) Boiss.; *Limodorum brulloi* Bartolo & Pulvirenti; *Linaria purpurea* (L.) Miller; *Luzula calabra* Ten.; *Luzula sieberi* Tausch. subsp. *sicula* (Parl.) Pignatti; *Paeonia mascula* (L.) Miller subsp. *russii* (Biv.) Cullen & Heyw.; *Petrorhagia saxifraga* (L.) Link subsp. *gasparrinii* (Guss.) Pignatti; *Phleum ambiguum* Ten.; *Pimpinella anisoides* Briganti; *Pinus nigra* Arnold subsp. *calabrica* (Land.) E. Murray; *Ranunculus aspromontanus* Huter, P. & R.; *Ranunculus thomasii* Ten.; *Salix brutia* Brullo & Spampinato; *Senecio samniticus* Huet; *Silene echinata* Otth; *Silene sicula* Ucria; *Soldanella calabrella* Kress; *Teucrium siculum* Rafin.; *Thalictrum calabricum* Sprengel; *Trifolium brutium* Ten.; *Vicia consentina* Sprengel; *Viola aethnensis* Parl. subsp. *messanensis* (W. Becker) Merxm & Lippert.

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Gianniantonio Domina & Pietro Mazzola

The genus *Orobanche* in Sicily. Taxa described by V. Tineo and M. Lojacono Pojero

Abstract

Domina, G. & Mazzola P.: The genus *Orobanche* in Sicily. Taxa described by V. Tineo and M. Lojacono Pojero. — Bocconea 21: 223-232. 2007. — ISSN 1120-4060.

Current knowledge of the taxonomy, distribution and ecology of *Orobanche* in Sicily is based on collections made at the beginning of the XX century or earlier. A revision of this genus, often neglected in Italy and other Mediterranean countries, has therefore been started. The taxa described as new by Vincenzo Tineo and Michele Lojacono Pojero, 2 and 13 respectively, are here examined. For each, the nomenclatural type has been established, and the synonymy, taxonomic status and known distribution are presented.

Introduction

The genus *Orobanche* has often been neglected in the floristic surveys carried out in Italy, so that our knowledge is mostly based on collections and records dating back to the first half of the last century or before.

According to recent floras, 20 to 22 species of *Orobanche* (including *Phelipaea*), i. e. two thirds of the taxa growing in Italy, occur in Sicily, and many of them were first described from here. Nevertheless, no critical evaluation of their appropriate taxonomic status, distribution, ecology and nomenclature has been made subsequent to the monographic treatment by Beck (1890, 1930) which, with respect to Sicily, is mostly based on information provided by Lojacono Pojero (1881-1883; 1887). In the frame of a programme to typify the names of taxa described by Sicilian authors, revision of the Sicilian *Orobanche* taxa has therefore been given priority, starting from the material kept in the *Herbarium Mediterraneum Panormitanum* (PAL). This herbarium, which was the Lojacono Pojero's main data source, consists primarily of collections made between 1821 and 1900 and, to a lesser extent, between 1960 and the present day. It includes the original material used by Tineo and Lojacono to describe their new taxa. Tineo (in Gussone 1843) described *O. alexandri* from Monte Catalfano and *O. nebrodensis* from the Madonie mountains. Lojacono (1881-1883; 1887) published 11 new species and 3 new varieties: *O. chironii, O. lutea, O. pubescens* var. *campuliflora, O. sabulicola, O. sanguinea* var. *maritima, O. crinita* var. *straminea, O. sicula, O. stenantha, O. tinei, O. todaroi, O. thapsoides, Phelipaea panormitana, P. elongata,* and *P. gussoneana*. Ten of these taxa were based on material collected by Lojacono himself around Palermo, on the Eolian islands, near

Balestrate, and near Terranova (now Gela). For the remainder, Lojacono used specimens collected by Tineo on the Madonie mountains. With the single exception of *O. chironii*, still in use for a species endemic to Sicily, all of Tineo's and Lojacono's new names have been relegated to synonymy by subsequent authors.

Materials and Methods

Original material was found primarily in the *Herbarium Mediterraneum Panormitanum* (PAL), where most of Tineo's and Lojacono's specimens are kept. Other visited herbaria were B, BOLO, FI, NAP, P, PRC, RO, W, and WU.

As for the transcription of the labels the following conventions have been followed:

Different labels fixed on the same sheet are referred to by small letters (a, b, c,...etc.), not implying hierarchy.

Typographical transcription symbols:
/ = line break;
Italic script corresponds to handwritten text (underlined as in original),
Roman script denotes handwriting printed by lithography,
Bold script denotes printed text, **roman** or ***italicised*** in original,
[…] = illegible word(s),
the authors' own comments are added in square brackets.

Orobanche alexandri Tin. in Guss., Fl. Sicul. Syn. 2: 845 (1843)
[***O. alba*** Steph. ex Willd., Sp. Pl. 3: 350 (1800)]
Ind. loc. In montosis; Palermo sopra S. Maria a Gesù, Montepellegrino, Catalfano; sed rara. Aprili, Majo.
Lectotype (here designated): NAP-Gussone Sicilia
(a) *Orobanche alexandri Tin. / Catalfano* [manu Tineo].
Note: Two specimens from different gatherings are mounted together on one sheet. The first (our lectotype) bears Tineo's label (unsigned). The second, belonging to *Orobanche caryophyllacea* Sm., is by G. Gasparrini (unsigned) who noted some characters but no collecting locality on the corresponding label (b). Gussone added the following label (c), in common for both: *8.b Orobanche alexandri Tin. / Aprili Majo / In apricis montosis* [manu Gussone].
Orobanche alba occurs, but is rare, on the mountainous areas of the Madonie, and also around Palermo, on Monte Cammarata, the Peloritani mountains and Mt Etna. It has been observed to parasitise *Satureja graeca, Satureja fruticulosa, Thymus spinulosus* and *Calamintha nepeta*. The plants growing around Palermo have a corolla larger than those found elsewhere.

Orobanche nebrodensis Tin. in Guss., Fl. Sicul. Syn. 2: 845 (1843)
O. sanguinea f. ***nebrodensis*** (Tin.) G. Beck, Monogr. Orob.: 207 (1890)]
Ind. loc. In montosis; sopra Isnello presso al fiume. Majo.
Holotype (illustrated in Raimondo & Mazzola 2000: t. 12), PAL 43117
16 Maggio 1830 / Orobanche nebrodensis Nob. / Sopra Isnello presso il fiume / Stylo obso-

lete villoso seu / glaberrimo ut in O. sanguinea [manu Tineo].
Note: Individuals similar to the holotype plant (i.e., with a rather lax spike and bracts much longer than the flower) occur occasionally within populations of typical *Orobanche sanguinea.* In Sicily, that species occurs occasionally inland on the Madonie mountains; both here and along the coast, where it is widespread, it parasitises *Lotus cytisoides* s. l.

Orobanche pubescens var. *campuliflora* Lojac., Naturalista Sicil. 2: 82 (1883)
≡ *O. campuliflora* (Lojac.) Lojac., Fl. Sicul. 2(2): 170 (1904)
[= *O. pubescens* d'Urv., Mem. Soc. Linn. Paris: 332 (1822)]
Ind. loc. In sylvaticis fruticetis maritimis, specimen unicum inveni in Palermo M. Gallo die 10 majo 1881.
Ic. Naturalista Sicil. 1: t. 9, f. 1.
Holotype: PAL 43240
O. pubescens / Orobanche / olim todari mihi / color sanguineis sordide / pallidissimi amarantheus / difficile definiendus / ex unico specimine / ad pedes M. sferracavallo / lect. / Leg M. Lojacono / majo 1881 [manu Lojacono].
Note: The specimen PAL 43240 was used to draw-up the iconography tab. IX, fig. 1 Lojac, 1882. *Orobanche pubescens* occurs in the coastal belt of Sicily parasitic on *Asteraceae*.

Orobanche chironii Lojac., Contr. Fl. Sic.: 12 (1878)
Ind. loc. Ad radices *Opopanax Chironii*, in nemoribus saxosis apricis, Basse rupi di Busambra. Junio.
Holotype: PAL 43117
Herbarium Horti Bot. Panormitani / *Orobanche / chironii Mihi / Contrib. Fl. Sic. / ad radices Opopanax chironii / Basse rupi di Busambra / anno 1878 / Leg. M. Lojacono P.* [manu Lojacono].
Epitype (here designated): PAL 56361 (Fig. 1).
Herbarium Mediterraneum Panormitanum / *Orobanche chironii* Lojac. / su *Anthemis cupaniana /* basse rupi di Busambra (Palermo) / 20.06.2004 / Leg.: G. Domina / Det.: G. Domina.
Note: It has been decided to designate a supporting epitype since the holotype, even if quite recognisable, has seriously deteriorated.
The species, endemic to Sicily, was known only from the bottom of the cliff at Rocca Busambra (South of Palermo). Recently it has also been found in the Madonie Mountains on *Asteraceae* (*Anthemis cupaniana* above all), not on *Opopanax chironium* as stated in the protologue. In the *locus classicus, Opopanax chironium* is very frequent and there it could play some ecological influence on seed germination.

Orobanche sabulicola Lojac., Naturalista Sicil. 2: 61 (1882)
[***O. litorea*** Guss., Fl. Sicul. Prod. 2: 184. (1828)]
Ind. loc. In dunis sabulosis maritimis ad radices Anthemidis maritimae parasitica, inveni prope Balestrate in Aprili 1881.
Lectotype (here designated): PAL 43213
Herbarium horti Bot. Panormitani / *Orob. sabulicola / mihi monogr. / Orob. Sicilia / In sabulosis / maritimis / Balestrate / Aprili 1881 / leg. M. Lojacono / Lojac. num. 20.* [manu Lojacono].

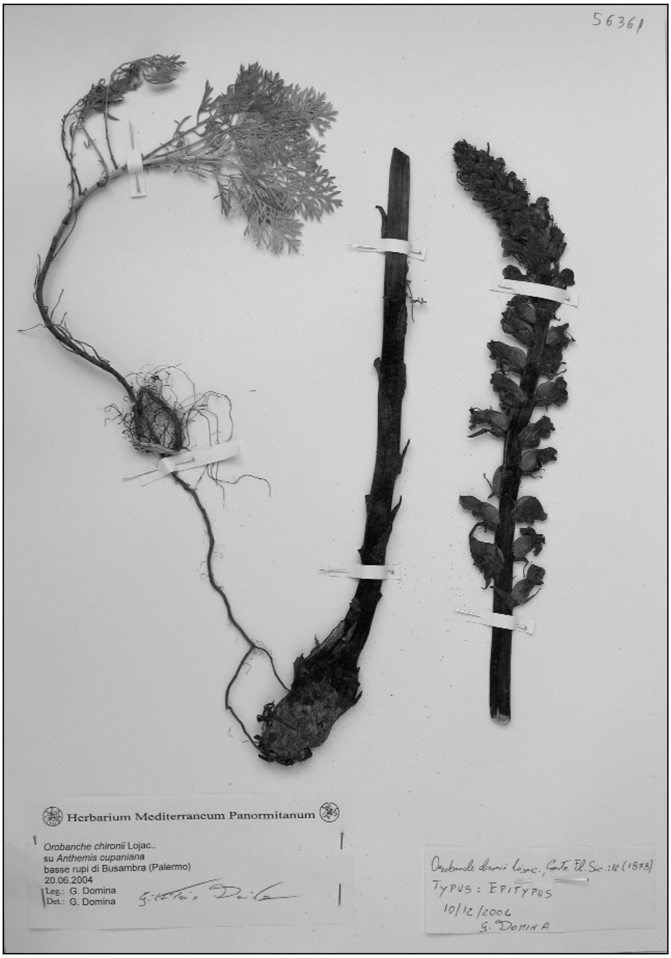

Fig. 1. Sheet PAL 56361 designated in this paper as the Epytype of *Orobanche chironii* Lojac.

Note: The type and other examined specimens are indistinguishable from *Orobanche litorea* Guss. which is spread along the sandy coasts. Other similarities between *O. sabulicola* and *O. canescens* supposed by Beck (1930) had already been excluded by Domina & Mazzola (2004).

Orobanche sanguinea var. *maritima* Lojac., Naturalista Sicil. 2:109 (1883)
[***O. sanguinea*** f. ***crinita*** (Viv.) G. Beck, Monogr. Orob.: 206 (1890)]
Ind. loc. Inveni in herbidis rupestribus umbrosis mari imminentibus. Montis Gallo al Malo Passo, a Sferracavallo ad radices *Loti cytisoidi*?
Lectotype (here designated): FI

Plantae siculae rariores / 463. Orobanche sanguinea Presl / del. Prag. p. / O. densiflora Salzm. (Auch) / var. maritima mihi monogr. / orob. ined. / In sylvaticis herbosis maritimi / mayo 1881 ... in Gallo parte di / Sferracavallo / leg. M. Lojacono.
Note: It differs from *Orobanche sanguinea* s. str. by the spike denser and the stem densely hairy. It occurs along the coast parasitic on *Lotus cytisoides.*

Orobanche crinita var. *straminea* Lojac., Naturalista Sicil. 2:107 (1883)
[***O. sanguinea*** f. ***crinita*** (Viv.) G. Beck, Monogr. Orob.: 206 (1890)]
Ind. loc. Ad radices *Loti cytisoidi* in sabulosis maritimis satis obvia simul cum varietate quae magis rara a Mondello legi et a Carini eodem tempore et supra eadem planta parasitica ! Aprili-Majo.
Lectotype (here designated): WU-herb. generale 28134
Orobanche / crinita Viv. / fl. albescente ! / bellissima var. / sferracavallo / Aprili 1877 in arenosis marit. / M. Lojacono. [manu Lojacono].
Note: Described on yellowish individuals that sometimes occur within *Orobanche sanguinea* populations.

Orobanche sicula Lojac., Naturalista Sicil. 1: 255 (1882)
[***O. variegata*** Wallr., Orob. Gen.: 40 (1825)]
Ind. loc. In Silvaticis, Valdemone (locus specialis non indicatus) Junio.
Lectotype (here designated): PAL 43216
Herbarium horti Bot. Panormitani */ n. 33 Orob. / spartii Guss. / an forma altera / Valdemone / O. sicula mihi !* [manu Lojacono].
Note: Individuals that morphologically agree with *O. sicula* Lojac. occur parasitic on woody *Leguminosae* like *Calicotome villosa, C. infesta, Spartium junceum,* etc.

Orobanche tinei Lojac., Naturalista Sicil. 6: 133 (1887)
[***O. cernua*** L. in Loefl., Iter Hispan.: 152 (1758)]
Ind. loc. In pascuis elatioribus Nebrodes. Ad radices *Artemisiae camphorata*. Serre delle Croci. Tineo.
Holotype: PAL 43119
(a) *Sopra le terre / delle Croci di / Castelbuono / amaranto colore* [manu Tineo]
(b) *Orobanche ... / O. glandulosa squamis lanceolatis adpressis, spica cylindrica, / lassiuscula, elongata, bracteis, sepalis basi ovatis, acuminatis, integri raro bifidis equalibus...tubulosis rectis costrictis...labiis denticulatis crispis, inferiore trilobo, lobis lateralibus* [manu Tineo].
(c) *Orobanche cumana / Wallr. / Questa sembra la vera Orobanche / cumana di cui c'è / pure scritto nel Reich. / sta bene a questa il detto / del Gussone habitus / fere O. ramosae* [manu Lojacono]
(d) *Orobanche cernua L. / A. Pujadas / IX 2001* [manu Pujadas]
Note: A single specimen is fixed to the sheet which bears three other labels in addition to the *indicatio loci* by Tineo (a), the first of them (b) is a provisional handwritten diagnosis by Tineo. The second (c) refers to *Orobanche cumana*, excluded from the Sicilian flora by Lojacono himself (1887). The last is the revision label by A. J. Pujadas Salvà.

Orobanche thapsoides Lojac., Naturalista Sicil. 2: 60 (1822)
Ind. loc. In arvis sabulosis (?) Siciliae meridionalis, Terranova alle case della Lupa. Aprili.
Lectotype (here designated): PAL 43238 (Fig. 2)
(a) **Herbarium horti Bot. Panormitani** */ Orobanche/ thapsoides /Mihi / 33 / Terranova Citarda / Aprile 1869* [manu Lojacono].
(b) *sub O. canescens / Sic. Merid. / Mag. '69 Cit.* [manu Lojacono].
Note: According to Beck (1930), *Orobanche thapsoides* Lojac. is to be relegated to a synonym of *O. versicolor* F. G. Schultz but the type and other original material (PAL, PRC) clearly belong to a quite distinct species by the very dense spike and the shape of the corolla. In Sicily it frequently occurs on *Galactites tomentosa* and other *Asteraceae*.

Fig. 2. Sheet PAL 43238 designated in this paper as the Lectotype of *Orobanche thapsoides* Lojac.

Orobanche todaroi Lojac., Naturalista Sicil. 1: 215 (1883)
[***O. gracilis*** var. ***todaroi*** (Lojac.) Domina & Mazzola, **comb. nov.**]
Ind. loc. In fruticetis maritimis in Insulis Aeolicis rarissima Aprili 1877.
Lectotype (here designated): PAL 43241 (Fig. 3)
(a) *Flora Eolica exsiccata / Orobanche / todari Mihi / Isole Eolie / Leg. M. Lojacono.* [manu Lojacono]
(b) **Herbarium horti Bot. Panormitani** / *Orobanche/ todari Mihi / Ins. Aeolicis 1877* [manu Lojacono].
(c) *O. Spartii Guss ?? / an O. sprunneri Reut. / cum O. spartii non cunfun / denda ! species insignis / Ins. Aeolicis / Aprile 1877 Leg. M. Lojacono* [manu Lojacono].
Note: Distinctive characters of this variety are: stem thickened; corolla very large; central lower lip lobe smaller than the external ones.

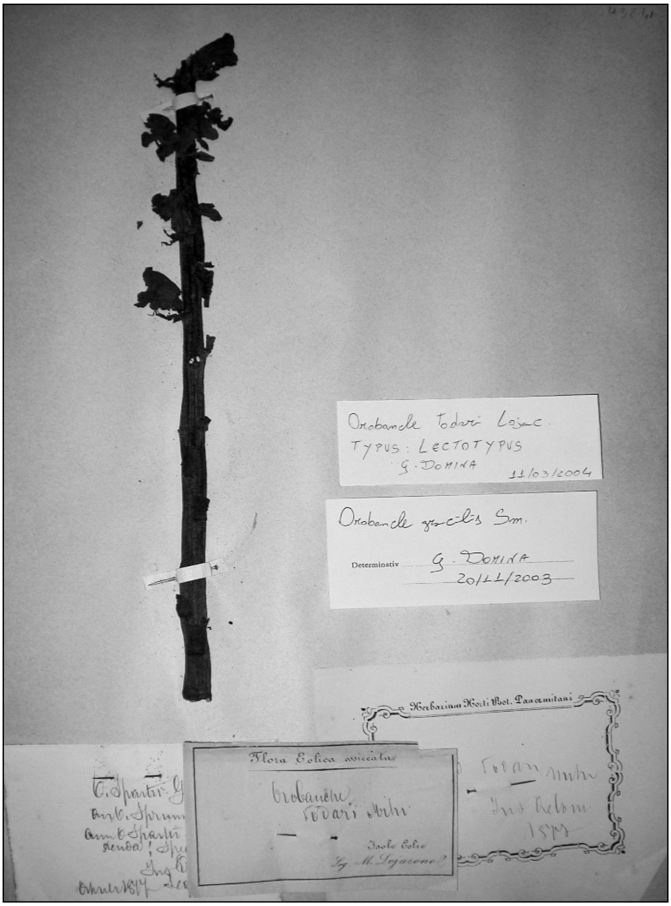

Fig. 3. Sheet PAL 43241 designated in this paper as the Lectotype of *Orobanche todaroi* Lojac.

Orobanche stenantha Lojac. Naturalista Sicil. 2: 62 (1882)
[***O. hederae*** Duby, Bot. Gall.: 350 (1828)]
Ind. loc. In fruticetis submontanis sylvaticis ad radices…in Val Demone. Junio.
Lectotype (here designated): PAL 43234 (Fig. 4)
a) *n.16 / Orobanche / stenentha / mihi ! / (forse O. glaberrima Guss.)* [manu Lojacono]
b) *Bracte floral. basim labelli attingenti sepala vix ...corolla longitudinis at brevioribus / integris basi angustis lineari* [...], *corolla angusta tubulosa circonferenza vix / 1 cent ½ lutei e odorosa ad apice anguste* [...] *valde hyalinum rubri parvi, lobi parvis / obov, cochl muro cospicue retusi omnibus irregulari anguste denticulati / plurimum ad tertium inferi* [...] */ antere parvis conspicue acutis* [...] *exsertis ! affine alla O. laurina* [manu Lojacono].

Phelipaea gussoneana Lojac., Naturalista Sicil. 1: 199-200 (1882)
[***Orobanche oxyloba*** (Reuter) G. Beck in Koch, Entwickl.-Gesch. Der Orob.: 209 (1887)]
Ind. loc. In elatioribus montosis. Ficuzza sulle basse rupi alle falde settentrionali di Busambra ad radices *Rumici triangulari* parasitica, (legi ann. 1878-81). S. M. del Bosco (Tin!) Polizzi al salto della Botte (Tin!) Toccata Grande (Cozzo della Mufera aliorum) Madonie Tin! In saxosis al piano della Battaglia di Petralia (M.L.Junio 1881).
Lectotype (here designated): PAL 43009.
Phelipaea n. sp. / rufescens Gris. / opp. caesia / ad radices Rumex / tuberosa / sotto Busambra / 13 Jun 1878 [manu Lojacono].
Note: This species was found at the basis of the cliff of Rocca Busambra and in the Madonie Mountains parasite on *Rumex intermedius*, *Arabis alpina* and several *Asteraceae.*

Phelipaea elongata Lojac., Naturalista Sicil. 2: 133 (1883)
[***Orobanche ramosa*** subsp. ***mutelii*** (F. W. Schultz) Cout., Fl. Portugal: 566 (1913)]
Ind. loc. In fruticetis sylvaticis in herbidis parum apricis ad pedes parietum calcareum versuras boreales M. Galli in consortio cum *Orob. denudata*, *sanguinea* etc. satis rara! Inveni Majo 1882.
Lectotype (here designated): FI
(a) *sec Beck forma Ph. aegyptiaca / sed non vero / Piantae siculae rariores / 557 Phelipaea elongata / n. sp. / In herbidis collinis* [...] */ sferracavallo / majo 1882 / leg M. Lojacono* [manu Lojacono]
Note: Three plants are fixed on the sheet, which was revised by G. Beck who identified the first from the left as a smaller form of *O. aegyptiaca*:
(b) *O. (trionychon) aegyptiaca Persoon. / forma floribus paulum minoribus, lobis denticulato-crenatis / = P. serotina Haussk. exsicc. / Beck* [manu Beck].
the second as *O. mutelii*:
(c) *forma O. (trionychon) muteli Schultz / VIII/84. Beck.* [manu Beck].
and the third he did not revise, probably because too injured. The lectotype specimen, however, under the definition of the term “specimen” in the ICBN (Greuter & al. 2000, Art. 8.2), is the whole sheet, because the three plants belong to one and the same taxon and were gathered at the same place and date by the same collector.
In the locality of Sferracavallo, at the foot of the northern slopes of Monte Gallo, *Orobanche ramosa* subsp. *mutelii* is rather frequent.

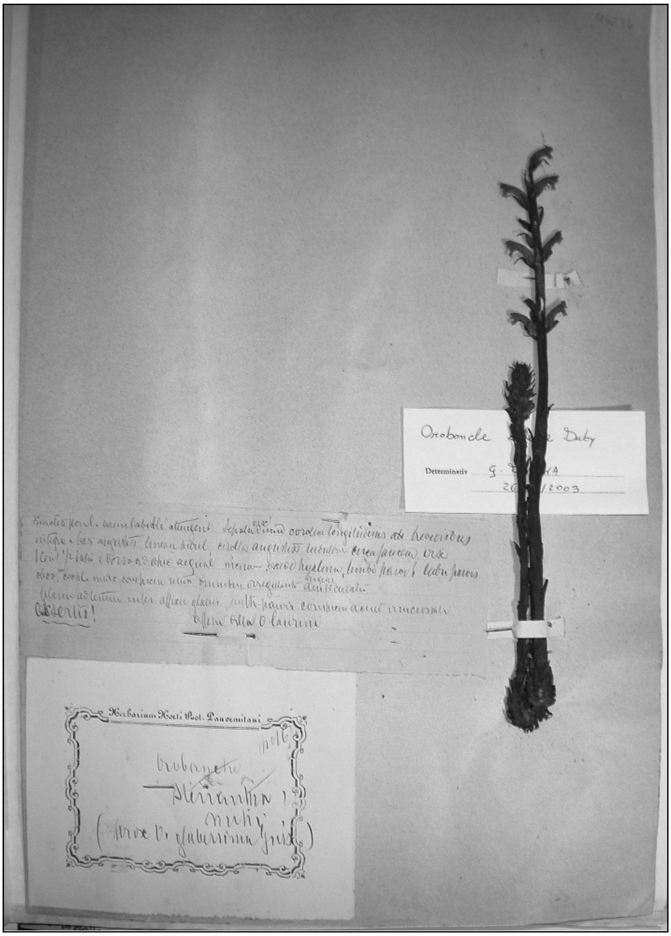

Fig. 4. Sheet PAL 43234 designated in this paper as the Lectotype of *Orobanche stenantha* Lojac.

Phelipaea panormitana Lojac., Naturalista Sicil. 1: 174 (1883)

[***Orobanche ramosa*** subsp. ***mutelii*** (F. W. Schultz) Cout., Fl. Portugal: 566 (1913)]

Ind. loc. In collibus calcareis apricis ac in herbidis maritimis copiosa ad radices *Leguminosarum* ac *Syngenesiacarum* parasitica, circa Panormum legi alli Ciaculli (aprile 1879) al Castellazzo sopra Monreale, alla Torretta ad rad. *Hypochaeridis*, et in herbidis a Capaci.

Ic.: Naturalista Sicil. 1: t. 6, f. 1.

Lectotype (here designated): PAL 43001

Herbarium Horti Bot. Panormitani / *n. 9 / Phelipaea / albiflora Gren. Godr. ? / cum Ph. emarginata mea / et Ph. ramosa non / confundenda. / flor .... albescen / tubus vix color sed differis / circulatim infer ... / leg. M. Lojacono*. [manu Lojacono].

Orobanche lutea Lojac., Naturalista Sicil. 1: 256 (1882) [nom. Illeg. non Baumg.]
[***O. variegata*** Wallr., Orob. Gen.: 40 (1825)].
Ind. loc. In sylvaticis, in dumetis umbrosis nemorum. Ficuzza alla macchia di Zingaro sulla via delle Quattro Finaite a sinistra salendo, rarissima (Jun. 1878).
Neotype (here designated): PAL 56360
Herbarium Mediterraneum Panormitanum / *Orobanche variegata* Wallr. / su *Calycotome infesta* / Ficuzza alla macchia di Zingaro (Palermo) / 26.05.2004 / leg. G. Domina.
Note: This name has been attributed to yellowish individuals that occasionally occur within the *Orobanche variegata* Wallr. population.

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P. Colombo, V. Spadaro & F. M. Raimondo

Morpho-anatomical analysis of *Viola tineorum* and *V. ucriana* (*Violaceae*) endemic to the mountains around Palermo (NW-Sicily)

Abstract

Colombo, P., Spadaro, V. & Raimondo, F. M.: Morpho-anatomical analysis of *Viola tineorum* and *V. ucriana* (*Violaceae*) endemic to the mountains around Palermo (NW-Sicily). — Bocconea 21: 233-247. 2007. — ISSN 1120-4060.

Viola sect. *Melanium* is represented in Sicily by six species. Two of them, originally described as varieties of *Viola nebrodensis,* were later transferred at the species rank and named *V. tineorum* and *V. ucriana*. The study of micromorphological characteristics gives additional evidence agreeing with the independent specific status of *V. tineourum* and *V. ucriana*, that are confined to two very restricted areas south of Palermo: Rocca Busambra and Monte Pizzuta respectively.

Introduction

Taxa of the *Viola nebrodensis* group occur isolated on the calcareous mountains of the Madonie (Central N-Sicily), Rocca Busambra, and Monte Pizzuta (South of Palermo) (Pignatti 1982). Among these, *V. nebrodensis* C. Presl. since a long time had been considered as an independent species, including the Busambra and Pizzuta populations as varieties named *V. nebrodensis* var. *grandiflora* and *V. nebrodensis* var. *lutea*. This status was rather problematic from the taxonomical point of view. In fact, although morphologically mutual very close, and also with *V. nebrodensis* s. str., the Rocca Busambra and Monte Pizzuta populations have different anatomical characteristics, distribution ranges, and karyological numbers, consequently they have been treated at specific rank by Erben & Raimondo (1995). In this paper the results of a study on the leaf micromorphology and architecture, stem and petiole anatomy and the S.E.M. surface fine structure of leaves and seeds are presented in order to give additional data that agree with the specific rank of the taxa in question.

Materials and metods

Viola tineorum Erben & Raimondo is a dense caespitose hemicryptophyte, quite glabrous, with root slightly tickened, stems prostrate or ascending, leaves herbaceous, dark green, glossy; blades, ovate obtuse or rounded; lower leaves, smaller and with shorter petioles than the upper ones.

Viola ucriana Erben & Raimondo is a dense caespitose hemicryptophyte hairy, with root slightly tickened, stems glabrous or scarcely hairy; prostrate or ascending; leaves herbaceous, green-greyish, blades rounded or broadly ovate, apex obtuse, basis emerginate or abruptly attenuate, midrib glabrous or hairy.

The study material was collected in the following localities:

Viola tineorum - Rocca Busambra, basis of the North facing slope, 900 m a.s.l.

Viola ucriana - Fratantoni slopes, near the Pizzuta top, 800 m a.s.l.

Soon after collection, fresh material, previously sectioned and coloured for the exact identification of tissues and their location, was fixed in F.A.A., then dehydrated and coloured with saphranine and light green, and finally, included in paraffine. The cross sections of 10-15 µm, obtained using a rotative microtome were mounted in the Canadian balsam. The number and size of epidermal cells, stomata, hairs, mean cross section of leaves, palisade and lacunose tissues and other structures on the leaf surfaces were measured on the epidermal replications. The xilematic pattern was studied on blades diaphanized according to Fuchs (1963), following the Hickey (1973) terminology. Concerning general terminology, Esaù (1965) is followed. The study material, pre-treated at the critical point was finally observed at the S.E.M.

Results

Viola tineorum – the leaves in the middle part of the stem have an average surface of 400 mm² and are amphystomatic with anisocytic stomata. From the morphological point of view, in the cross section, the leaf margin is linear, rounded (Fig. 1A-B); marginal cells are generally larger than epidermal on both adassial and abassial blades. The cross section of the leaf midrib is 355 µm (Fig. 1C), I order veins are generally 340 µm. 2-stratified palisade parenchyma (131 µm); the spongy parenchyma (123 µm), reveals many small intercellular spaces. Epidermis, including cuticle, is thicker than the lower one on the upper blade (Tab. 1).

The phytoderma (Vignal & Cherel 1983), examined in detail, shows several interesting epidermal details that, in the lack of marked morphological characteristics, are suitable for the taxonomic delimitation of *Viola tineorum* from *V. ucriana*. The most strongly differ-

Table 1. Leaf thickness (µm).

	Epidermis + upper cuticle	*Epidermis + lower cuticle*	*Whole leaf*	*Midrib*	*Upper palisade*	*Lacunose tissue*
V. tineorum	56	37	340	355	131	123
V. ucriana	55	35	284	352	125	136

entiated characteristic in both species is epidermis of medial and basal leaves. Here adassial cells are isodiametric; density is 173 per mm², the single cell being 116, 58, 56. Abassial cells are 261 per mm², 99, 51, 37. The most remarkable characteristic in the leaves of *V. tineorum* is represented by 5 large and holding out epidermal cells that are in linear succession on the upper surface along the midrib. Among these, the central cell is the largest (Fig. 1C). In the lower blade epidermal cells are more numerous, homogeneous in size, and larger than in the abassial. Epidermis is frequently mucilaginous (Metcalfe 1957) and single or groups calcium oxalate crystals are usually present in the mesophyll (Fig. 1D). In the lacunose tissue and near the vascular bundles, there are secretory channels, in some of which brown bodies of unknown nature are included (Skottsberg 1940). Mucilaginous cells are very frequent in the leaf surface (Fig. 1E). In diaphanized leaves (Fuchs 1963) these mucilages are similar to Hydropotes, that are epidermal multicellular structures active for water and mineral salt assumption (Metcalfe 1957; Watson & Dallwitz 1992). Hydropoten are evident in *Brasenia peltata* Pursh, *Cabomba acquatica* Aubl., *Nymhaea alba* L., *Nuphar luteua* Sibth. & Smith., *Ranunculus fluitans* Lam., *Caltha palustris* L., and, in many monocotiledons, and probably in a large number of plants (Riede 1920-1921). Observed at the S.E.M the upper blade appears consisting of isodiametric cells with slightly sinuose margins; cuticle is slightly sculptured except for some sporadic striatures located on the epidermal cells surrounding some stomata (Fig. 2A-B). Stomata, 48 per mm², on epidermis have a long front pore variable in diameter. The lower blade (Fig. 2C-D) consists of more numerous isodiametric cells with very sinuose margins and usually smaller than those in the upper blade. The stomatic patterns are of the same type (Fig. 3A-B), i.e. anisocytic, but more numerous (Tab. 2).

Viola ucriana – the leaves in the middle and basal parts of the stem are mostly larger, having 500 mm² surface; they are amphystomatic and have anisocytic stomata (Fig. 4A-B). Epidermal cells have marked sinuose margins, are more convex and bear a few cuticle

Table 2. Epidermal parameters.

	Upper blade		Lower blade	
	Viola ucriana	*V. tineorum*	*V. ucriana*	*V. tineorum*
N° epidermal cells × mm²	162	173	331	261
N° stomata × mm²	43	48	89	69
Epidermal cells length (µm)	138	116	112	99
Epidermal cells width (µm)	79	58	66	51
Epidermal cells thickness (µm)	55	56	35	37,0
Stomata length (µm)	42	45	38.5	39
Stomata width (µm)	27	33	28.5	31

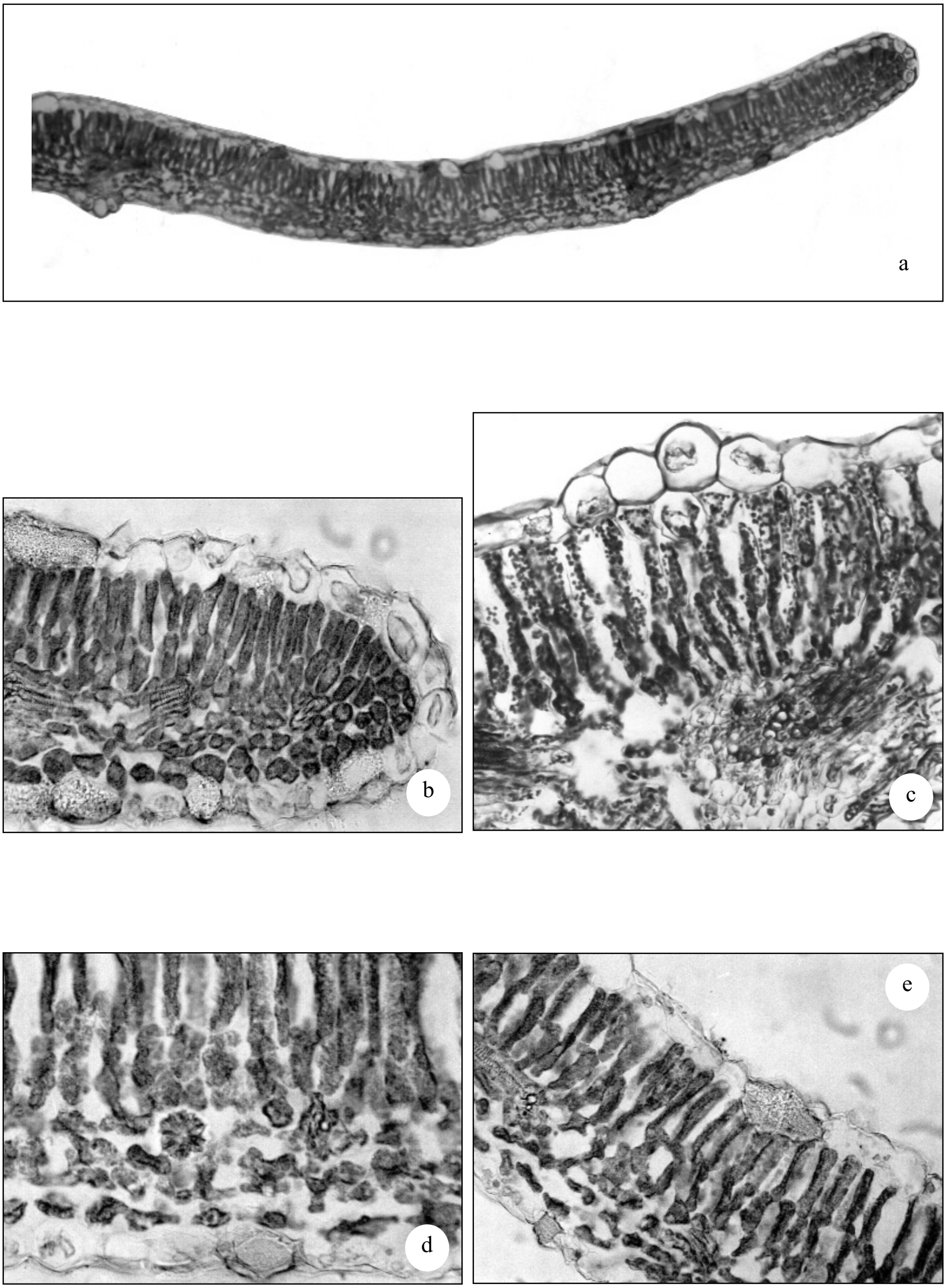

Fig. 1. *Viola tineorum*: a) cross section of the leaf (×100); b) detail of the cross section of the margin (×200); c) cross section near the midrib (×200); d) cross section of the leaf: detail of the palisade with mineral cristals; e) cross section of the leaf: detail of the palisade and of the muciparous glands.

ornamentations with the exception of the stomata in which dense cuticular strips perpendicular to the rime are found (Fig. 5A-B). Numeric data are included in Table 2.

In cross section the leaf margins are linear, rounded; cells are larger than those in both upper and lower blades (Fig. 6A). The leaves are on average 284 µm thick between the veinlets and reach 352 µm at the midrib level; the palisade parenchyma is two-layered in the area between veins; it is one-layered, 125 µm thick and provided with collecting cells above the midrib; the lacunose tissue is 136 µm, thickness of the adassial cuticle and epidermis include is about 55 µm, 35 µm the abassial (Tab. 1).

The peculiar structure of the leaves at the midrib level (Fig. 6B) represents a noteworthy difference from *V. tineorum.* Two superficial protrusions are visible on the foliar surfaces; in fact, 3 very large cells, among which the inner is smaller than the lateral ones, are found on the adassial surface. The protrusion on the abassial surface consists 6 or 7 large cells that are almost isodiametric, connected to one or two angular collenchyma centripetal layers.

Crystals

Calcium oxalate crystals, isolated or less frequently grouped, occur between the palisade and the lacunose tissues (Melchior 1925; Solereder 1940) (Fig. 1D).

Secretory elements

Secretory elements, including brown mucilaginous bodies, have been observed in the mesophyll of some Hawaian species (Skottsberg 1940). Similar bodies variable in size have been found in the epidermal tissues (Fig. 1E, 6B) of both *Viola ucriana* and *V. tineorum*: secretory elements between epidermal cells on the adassial blade of *V. tineorum* are included between 85 and 115 µm, those on the abassial blade between 91 and 27 µm. In *V. ucriana* these secretory elements are smaller, ranging between 75 and 100 µm in the adassial bladed and between 63.5 and 38.5 µm in the abassial.

Architecture

Leaves, that are symmetric, obovate (Stearn 1966), rounded or slightly retuse, rounded at the basis, have been diaphanised according to Fuchs (1963). Terminology for the xylematic pattern follows Hickey (1979).

Viola tineorum midrib is 1.25-2 µm in diameter. It runs along the lamina up to the rounded apex (Fig. 7A); upper veins form an acute angle of divergence, while lower veinlets have less acute angle. Veinlets are generally thick, straight, ramified at some distance from the margin which bears 8-10 small incisions as many as spaced crenations. Parallel intermarginal veins at the leaf margin are also evident. They are resulting from the fusion and straightening of the lesser arched brachydodromous exmedian segments that appear independent venations. Irregular intercostal areas; III order veinlets reticulate and orthogonal form an angle toward the apex. Thin orthogonal IV e V order veinlets are partly distinguishable. At the margin of the leaf small free veinlets form terminal dichotomic ramifications (Fig. 7B). Areoles are well developed, large irregularly; quadrangular; branches are usually uniramified and bear evident terminal spiralate tracheids (Fig. 7C).

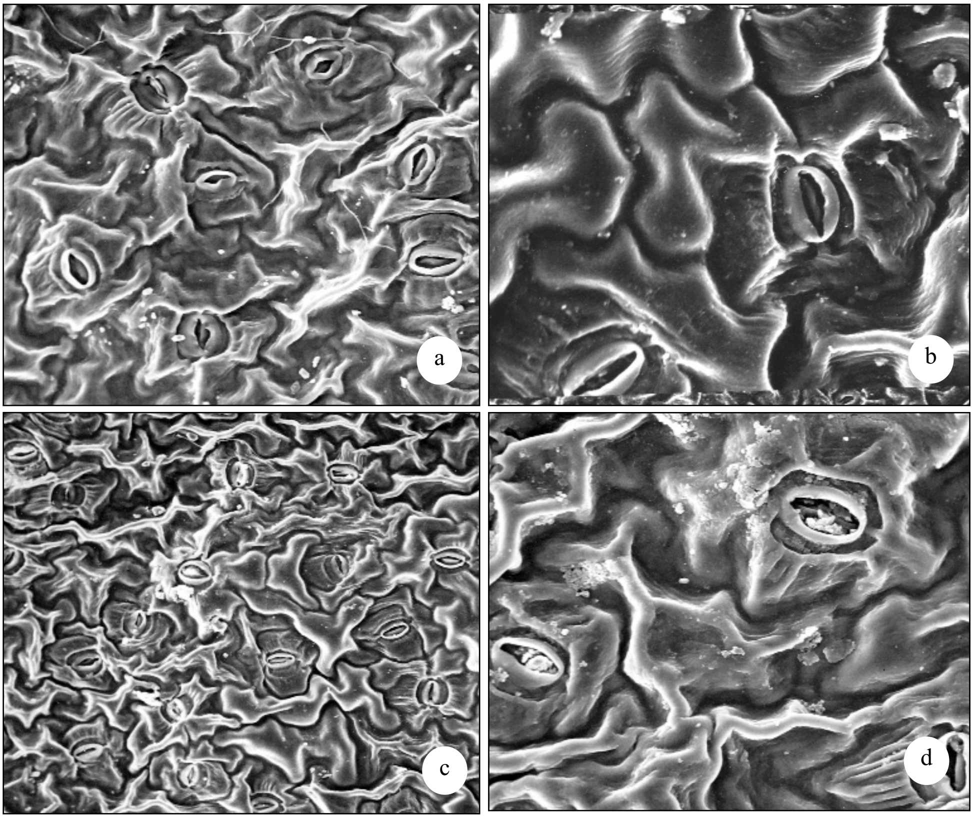

Fig. 2. *Viola tineorum*: a) adassial leaf surface at the S.E.M.; b) detail of the adassial surface at the S.E.M. with stomata; c) abassial leaf surface at the S.E.M.; d) detail at the S.E.M.of the abassial surface with stomata.

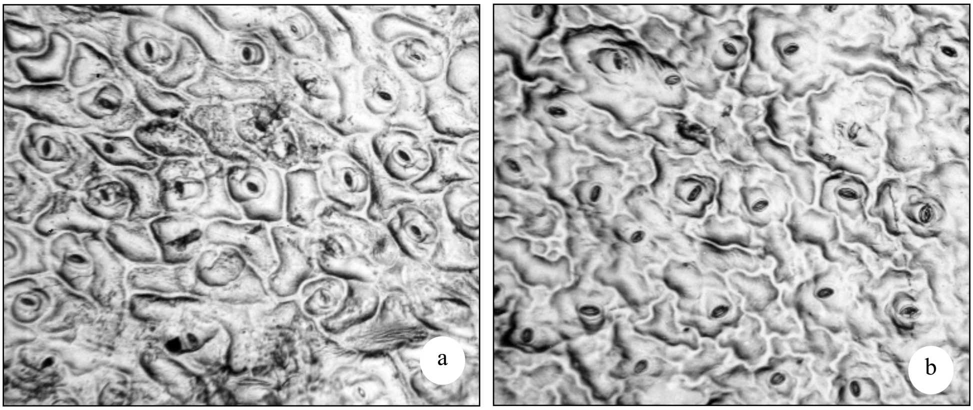

Fig. 3. *Viola tineorum*: a) epidermal replication of the leaf adassial surface (×200); b) epidermal replication of the leaf abassial surface (×200).

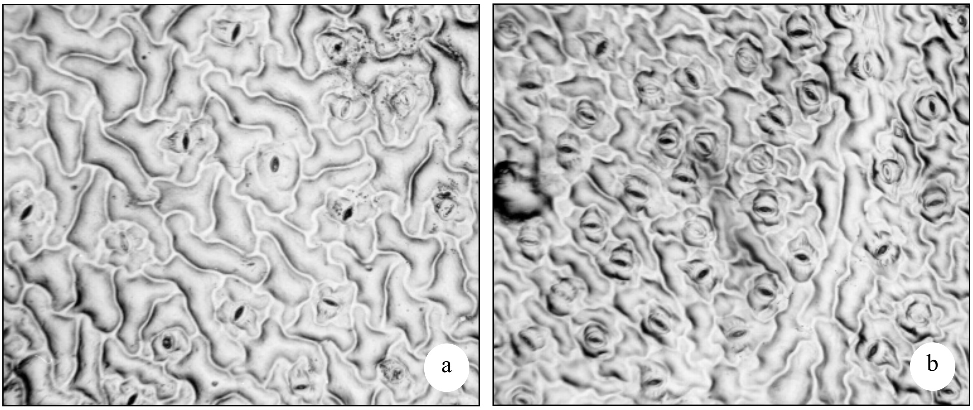

Fig. 4. *Viola ucriana*: a-b epidermal replication with anisocytic stomata (×200).

Viola ucriana - all leaves are provided with camptodrome or cladodrome veins; more or less large midrib runs straigt between the petiole and the rounded an sligthly retuse apex (Fig. 8A). The angle of divergence between the veins and the midrib is acute. II order veins are generally slight, straight and ramified near the margin crenations. Intermarginal veins provided with terminal branches running into crenations are also evident. Among these, the most developed is connected to the next crenation. Intercostal are quite regular. III order veins originate from the lower side of veins. The angle of divergence is acute, ramified reticle is irregular. Thin and almost undistinguishable IV and V order veinlets are also present. Short terminal veinlets are also ramified at the leaf margin. Areoles are well developed, irregular, polygonal, small.

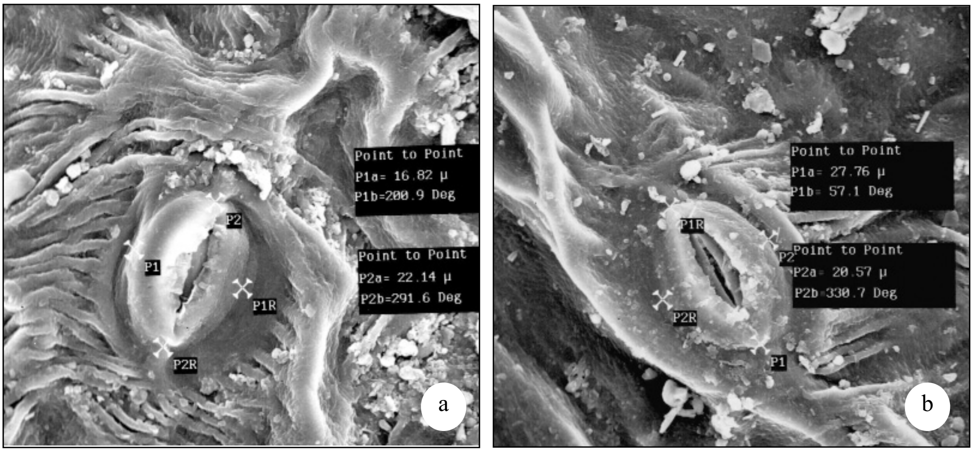

Fig. 5. *Viola ucriana*: a-b cuticular stripes at the S.E.M.

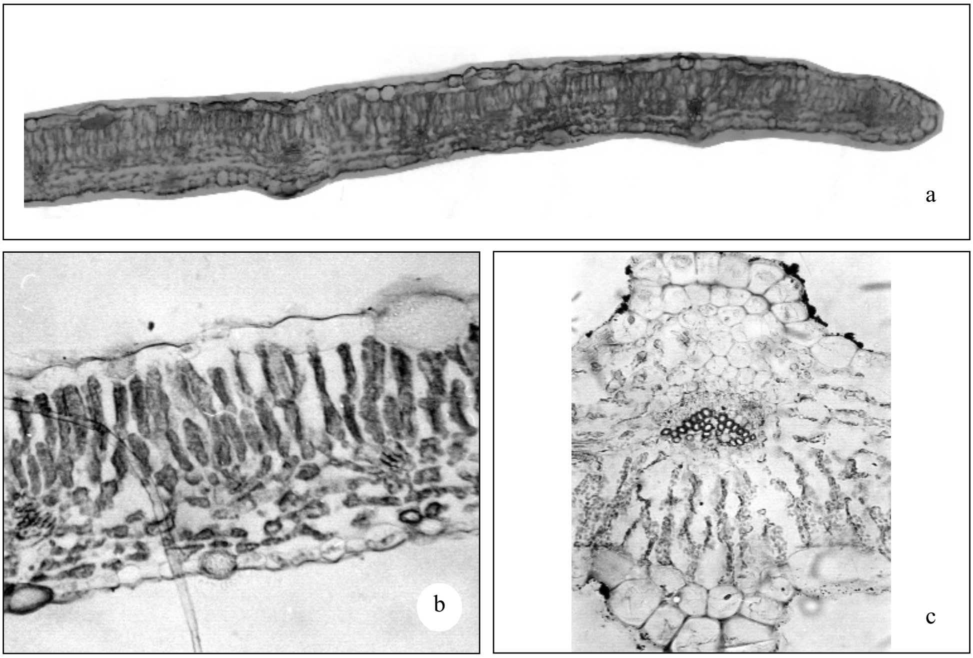

Fig. 6. *Viola ucriana*:a-b) cross section of the leaf and detail of the mesophyllous (×200); c) detail of the midrib in cross section (×200).

Viola ucriana - all leaves are provided with camptodrome or cladodrome veins; more or less large midrib runs straigt between the petiole and the rounded an sligthly retuse apex (Fig. 8A). The angle of divergence between the veins and the midrib is acute. II order veins are generally slight, straight and ramified near the margin crenations. Intermarginal veins provided with terminal branches running into crenations are also evident. Among these, the most developed is connected to the next crenation. Intercostal are quite regular. III order veins originate from the lower side of veins. The angle of divergence is acute, ramified reticle is irregular. Thin and almost undistinguishable IV and V order veinlets are also present. Short terminal veinlets are also ramified at the leaf margin. Areoles are well developed, irregular, polygonal, small.

Petiole

In *Viola tineorum* the petiole cross section shows that it is provided with a main central semicircular bundle and smaller lateral ones. The vascular cylinder is flottered and concave on the adassial side; on the convexe abassial side and in the pith there are many secretory channels. Chlorophyllous parenchima, 2-3 stratified, is covered with a single epidermal strate. Large oxalate crystals are formed between the pith and chlorophyllous parenchima. In *Viola ucriana* the petiole cross section shows a large central bundle in which V-shaped xylem is open to words the adassial surface. Phloem is in front to the abassial surface. Here,

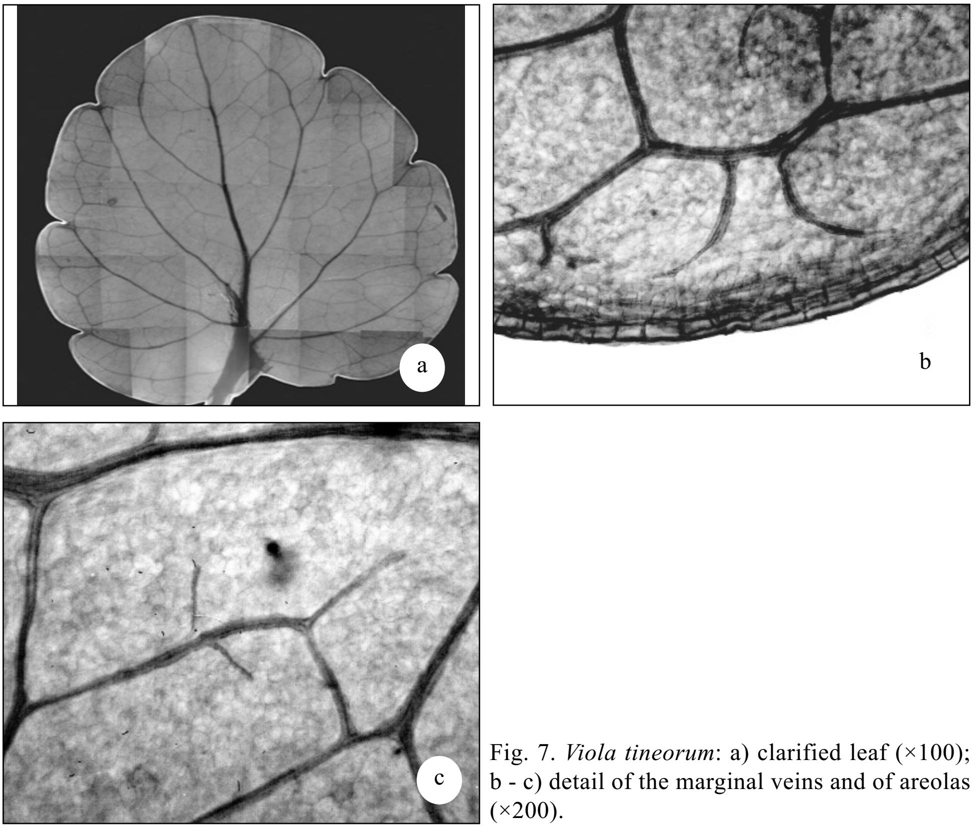

Fig. 7. *Viola tineorum*: a) clarified leaf (×100); b - c) detail of the marginal veins and of areolas (×200).

few cribrosous elements are found togheter with many secretory channels in contact with the endodermal, petiole therefore has three nodes (Metcalfe 1979). Epidermis is mucilaginous and chlorophyllous parenchyma is less developed. In a section out next to the lamina, petiola is quite different, revealing an proximately pentogonous shape epidermal layer with a dense trichome covering 2 or 3 lamellar collenchyima layers thickening around bundles where flexible collenchyma keels (Erben & Raimondo 1995). Two more marked parenchyma prominences are by the sides of the adassial surface. In the middle of petiole are 5 bundles, of wich the main, I order, is central, surrounded by the II and III order ones that are displayed in a semicircle. Petiole is multilacunose nodule since from it stipular venations originate. Each bundle is collateral with 9 secretory channels near the phloem: among these, 5 are surrounding the main bundle, 3 and 1 are displayed next to the III and II order bundles, respectively. Two collenchima sheets include both large and small bundles like a sandwich. Other secretory structure and sporadic, small oxalate crystals are found sparse in the pith.

Stem

In both species prostate and ascending stems are found in the same individual.

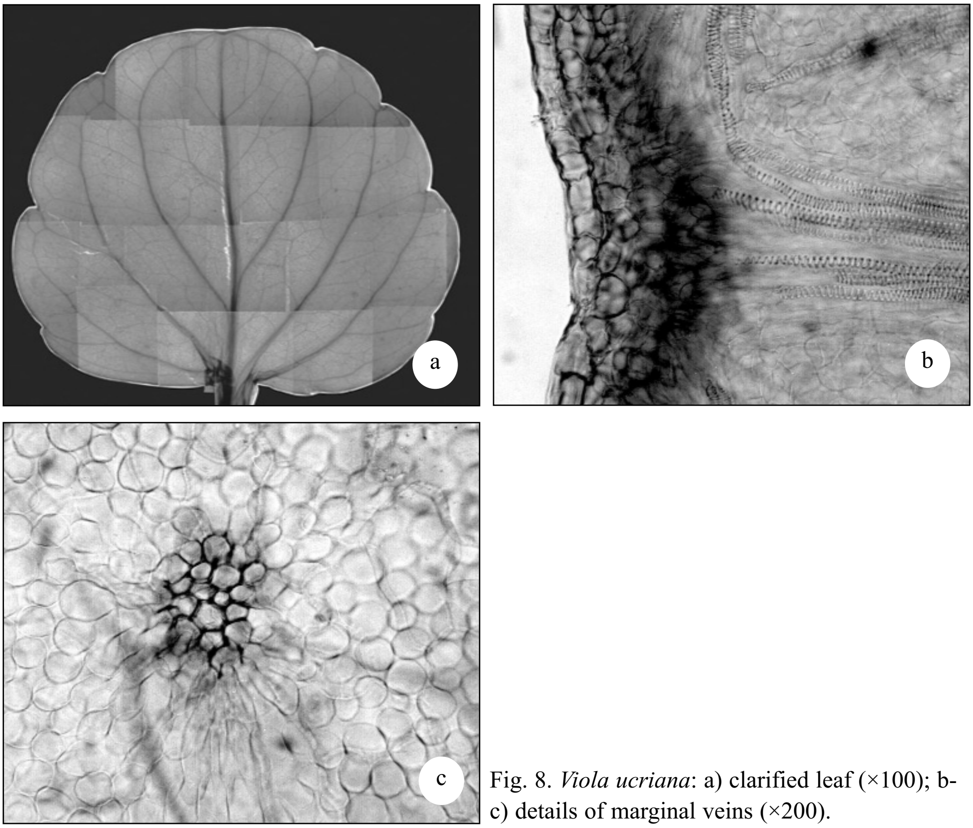

Fig. 8. *Viola ucriana*: a) clarified leaf (×100); b-c) details of marginal veins (×200).

In the apical cross section of stem, epidermis is layered with cell walls more tickened outside. Beside collenchyma on the epidermal side is located especially near the veins and cuttings (Fig. 12A). In the parenchymatous bark frequent large oxalate crystals are included. Secretory channels are found next to endodermis or are bordering on phloem. Pericycle is parenchymatous. Each open collateral bundle is distinct, separated by a parenchymatous band in which thin xylem segments reveal some starting cambium activity. Vessel are simple or sometimes provided with scalar plates. Pith, usually allow, includes abundant isolated or grouped crystals. In prostrate stems, epidermis is more thickened and rather suberized. Cortex is always parenchymatous; but cells gradually decrease while crystals are increasing in size.

Endoderma is rich in starch with storage function. Phloem forms a continuous ring rich in cells including brown bodies. Xylem also forms a continuous ring.

Vessels more or less small in size (30-50 µm), are displayed in rays separated by uniseriate rays. Sclerenchymatous fibers are septate and strongly lignified. Pith cells are roundish, smaller and with thinner wall than the cortex ones. Numerous large crystals are included in the pith. In *Viola ucriana* (Fig. 12B) outer parts of the stem rare earlier suberified: cortex is thinner, parenchymatous, and cells are larger and more thin-

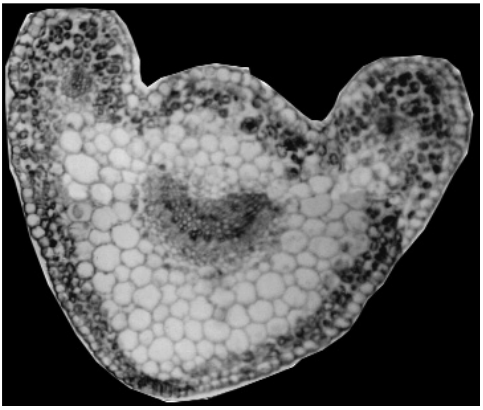

Fig. 9. *Viola tineorum*: cross section of the petiole (×100).

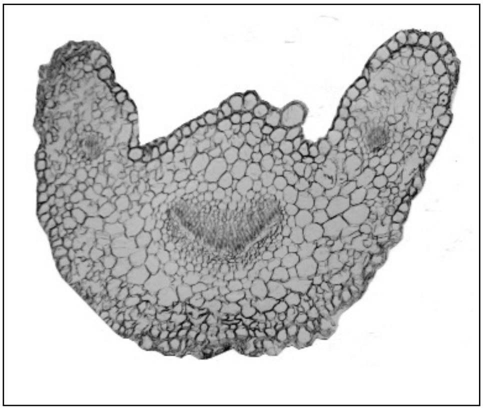

Fig. 10. *Viola ucriana*: cross section of the petiole (×100).

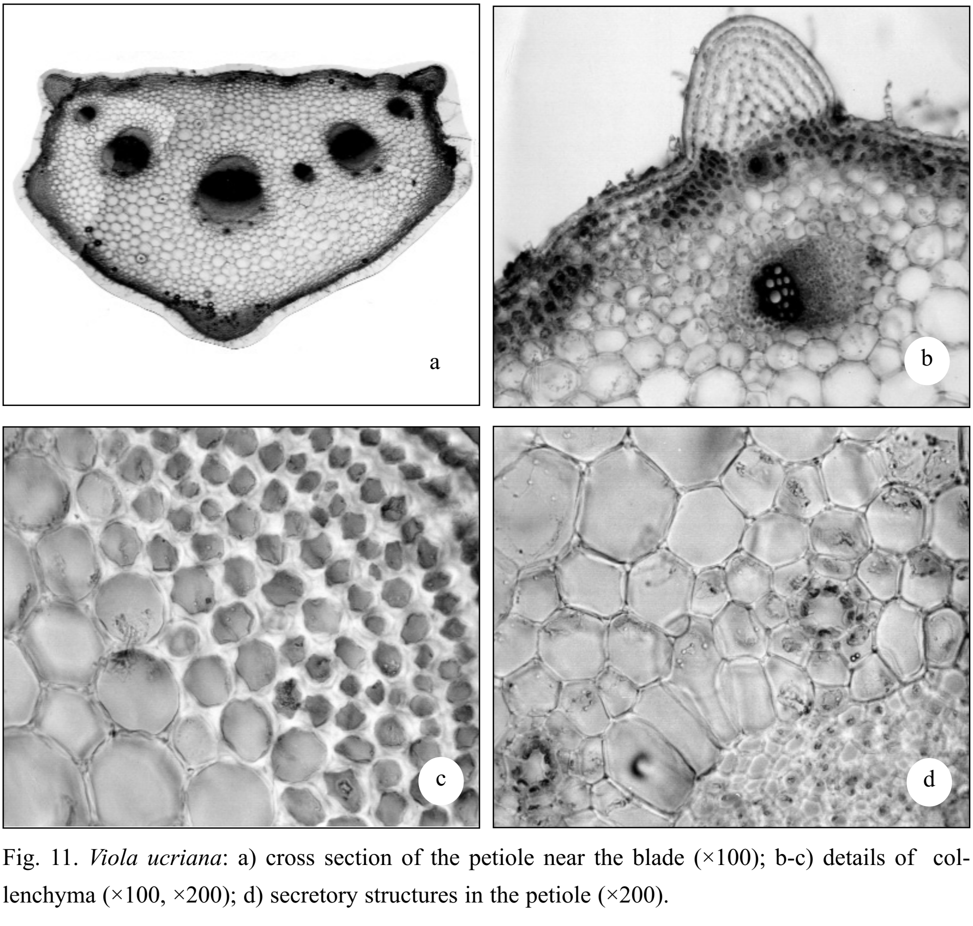

Fig. 11. *Viola ucriana*: a) cross section of the petiole near the blade (×100); b-c) details of collenchyma (×100, ×200); d) secretory structures in the petiole (×200).

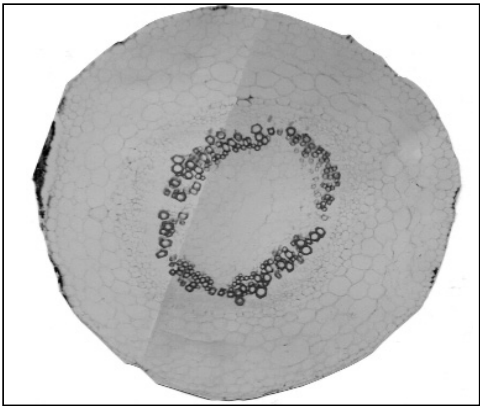

Fig. 12. *Viola ucriana*: stem cross section (×100).

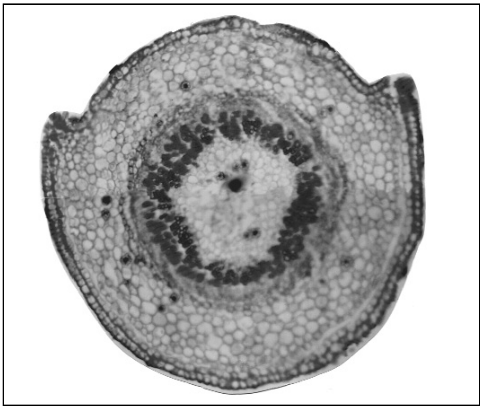

Fig. 13. *Viola ucriana*: stem cross section (×100).

walled than *V. tineorum*. Besides, oxalate crystals are rare or absent. Very small secretory channels are contiguous with phloem. Periclycle is parenchymatous. Vessels bear simple piths, small protoxylem and more or less large meta-xylem elements, markedly lignified. Parenchima is paratracheal. Thin medullary rays are present. Pith becomes empty in the inner part.

Root

Viola tineorum and *V. ucriana* are very similar as far as roots are concerned. Indeed both species grow on calcareous substrates, and roots are deeply wedged in the rock. Owing to this condition and a protective thickened periderm wich appears rather worm-out in the suberified parts is formed. Cortex is more thickened and more abundant siliceous druses

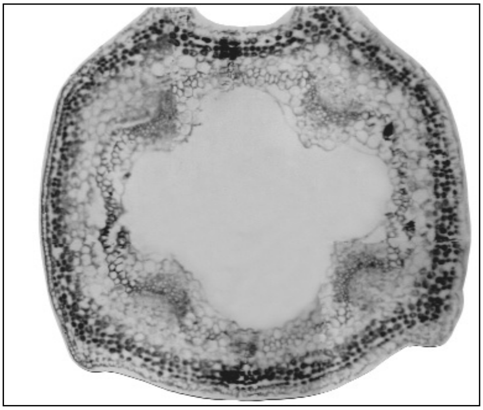

Fig. 14. *Viola tineorum*: cross section of the petiole (×100).

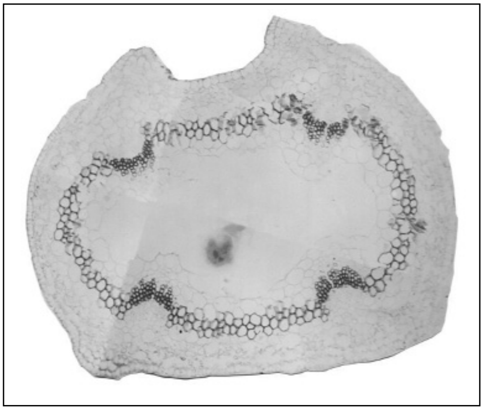

Fig. 15. *Viola ucriana*: cross section of the petiole (×100).

and minute crystals, in comparison with *V. ucriana*. The vascular cylinder is dense with larger and more lignified tracheae. The pith is lacking.

Viola ucriana: moderately thickened and divided in the upper part, the root periderm is suberified, displayed in several thin layers rather inconsistent. Cortex partly consists of a storage parenchyma and partly of other parenchymatous cells provided with large intercellular spaces. Structure is secondary, with a solid stele consisting of exarch protoxylem surrounded by a very dense xylem provided with very large vessels that are 25-30 µm, mixed with lignified fibers. Traces of numerous lateral roots starting from the central cylinder are also evident.

Flower stalk

Viola tineorum pedicels are very light. The cross section cut at the medium height appears somewhat quadrangular bearing two collenchima ribs on the abassial surface and two more prominent ones on the adassial surface (Fig. 14). Epidermis, 1-layered, is composed of small, thin-walled cells. Sub-epidermal gelatinous tissue is surrounding the 3-4 layered chlorophyllous parenchyma which is formed by small roundish shaped cells; secretory channels are found in the inner cortex around the central cylinder. The cylinder, sinusoid outlined, consists of 4 large vascular bundles separated by pith rays that include a cambium layer forming a sketch of secondary structure. This structure is incomplete since the stalk degenerates soon after anthesis; small minor bundles are found among the pith rays. Pith is large and empty.

In *Viola ucriana* stalk is quite similar to *V. tineorum* (Fig. 15), but it is more flattened; pith rays are broader and curved. Finally it is more markedly lignified.

Seeds

Ovule is bi-tegmented, crassi-nucellate in both species. Testa in the seed consists of a dense reticle with sclerotic cells and stomata near the calaza. In *V. ucriana* tegmen observed at the S.E.M. looks like a drop (Fig. 16A), almost smooth on the surface convex. Highly magnificated (660 ×) its structure is still smooth but some sculptures polyhedric delimiting numerous areoles 40 µm long (Fig. 16 B) are evident.

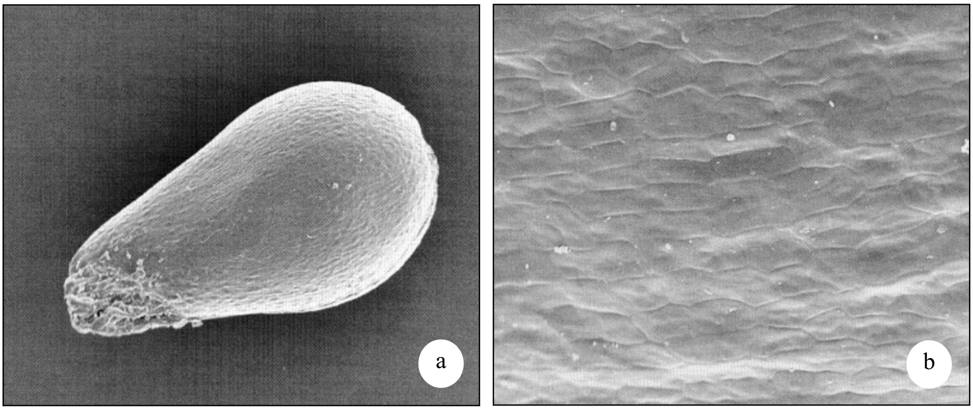

Fig. 16. *Viola ucriana:* a) a seed at the S.E.M.; b) tegmen detail.

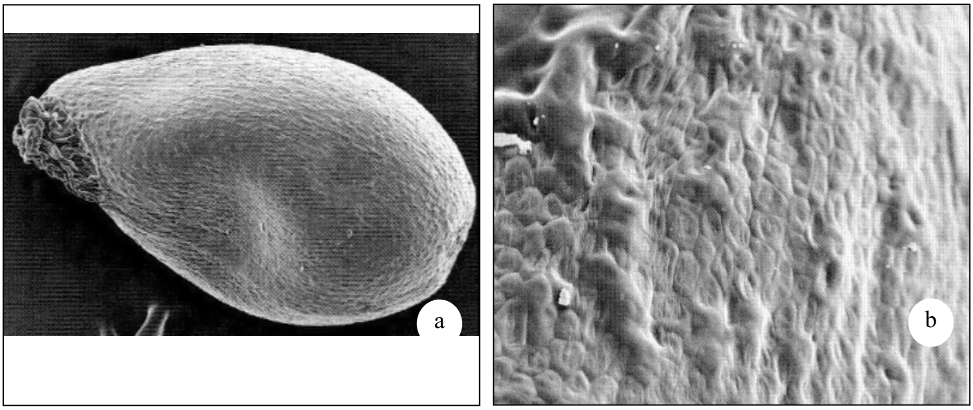

Fig. 17. *Viola tineorum*: a) seed at the S.E.M.; b) tegmen detail.

Viola tineorum seeds are somewhat larger, ovate, convex, with the eso-tegmen almost smooth (Fig. 17A); at high magnification (660 ×) numerous small (20 µm) slightly centrally depressed polyhedric sculptures are evident (Fig. 17B). On the abassial side a light concavity is evident (Fig. 17C).

Discussion and conclusion

Anatomic comparison points out several structural characteristics suitable for significantly distinguishing *Viola tineorum* from *V. ucriana*. In particular, significant differences concern the leaf architecture, the dermatologic pattern of both the adassial and abassial sides near the midrib. Differences are also evident in the leaf epidermal parameters, in the venation arrangement and the relevant angle of divergence, in the size and shape of the areoles and free ramifications; in the fine leaf epidermal structure at the S.E.M., especially of stomata; in the anatomy of petiole; in the composition and size of secretory elements in the lignification degree and diameter of vessels in both stems and roots. Further remarks concern environmental adaptation of both studied species. Edaphic and environmental conditions are rigorous, especially referring to altitude, that in the study case is above 1000 m and the winter temperature which frequently reaches 0 °C. Another factor is represented by the violent north wind especially when it is associated with low temperature. Both species under study are herbaceous, therefore they are sensitive to the above mentioned factors. Their adaptations typically concern the slopy habitats: minimal habitus, short stem internodes, deepened into the calcareous substrate roots, reduced foliar surface on long, flexible petioles, epidermis provided with sub-epidermal mucilage or rich in muciparous cells and channels active in the direct water assumption in winter or when supplies are scarce (Francini Corti 1967).

In *Viola tineorum* which occurs between 1300-1400 m a.s.l., owing to the high altitude rigours, all venations of the leaves are considerably lignified; the xylematic pattern is scarcely reticulated since leaves can absorb H_2O through the mucilage and the number of mucous cells.

Viola ucriana, which occurs at lower altitudes, smaller leaves that are rather independent, from rigours, show xylematic pattern less lignified, uniramified terminal, thinner than in *V. tineorum* tracheids; a higher number of cells and muciparous channels per mm², that are larger on average. Mesophilous adaptations can be observed in the leaves of both species.

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K. Uzunova, S. Bancheva & F. M. Raimondo

Studies on the leaf epidermal structure of genus *Cyanus*, sect. *Napuliferae* (*Compositae*)

Abstract

Uzunova, K., Bancheva, S. & Raimondo, F. M.: Studies on the leaf epidermal structure of genus *Cyanus*, sect. *Napuliferae* (*Compositae*). — Bocconea 21: 249-256. 2007. — ISSN 1120-4060.

In order to assess the taxonomic significance of the epidermal structure, seven taxa from genus *Cyanus* Mill. (sect. *Napuliferae*) have been studied: *Cyanus pseudaxillaris*, *C. orbelicus*, *C. velenovskyi*, *C. napulifer*, *C. nyssanus*, *C. tuberosus* and *C. thirkei*. The investigated taxa possess thick covering of trichomes. The hairs consist of two or more basal cells (stalk) and a long whip cell. Five types of trichomes, very well distinguished by the number, form, size and the wall thickening of the stalk, were established. The cuticle is thin and easily could be distorted under gentle maceration. Some taxa possess cuticle corrugations, somewhere parallel to long cell axis. There are striations on the whole cuticle or only around the stomata and the trichome bases. The main stomatal type is anomocytic, but there are some stomata from anisocytic type, frequently untypical: the three cells do not encircle the stoma. There are stomata with only one subsidiary cell, which is rounded, smaller and well distinguishable by the other epidermal cells. The comparative study shows the epidermal features possess taxonomic significance and could be used as additional characters to differentiate the species into sect. *Napuliferae* of genus *Cyanus*.

Introduction

The genus *Cyanus* Mill. belongs to the subtribe *Centaureinae* and is among the most taxonomically complicated genera of *Compositae*. Many taxonomists consider *Cyanus* as a subgenus or section in the genus *Centaurea* (Gajić 1975; Wagenitz 1975; 1975a; Dostàl 1976; Strid & Tan 1991; Kožuharov 1992). In the last 10 years they have accumulated a lot of molecular data confirming the split of the genus *Centaurea* s. l. into several smaller genera (Susanna & al. 1995; Häfner 2000; Garcia Jacas & al. 2000, 2001). As a basis of the new taxonomic scheme of the *Centaurea* s. l. and *Centaureinae* (Greuter & al. 2001; Greuter 2003) serve the Wagenitz's pollen type classification, the base chromosome numbers (Wagenitz & Hellwig 1996), and the newly accumulated molecular data. In this scheme *Cyanus* has a generic status.

The representatives of the sect. *Napuliferae* are well distinguished from the other *Cyanus* species by its fusiform or napiform roots. All *Napuliferae* taxa, except *C. thirkei* are Balkan endemics. The origin center of this group is the Balkan Peninsula. The main evolutionary trend is the adaptation of the more xerophytic habitats. Regard to this, the species possesses more or less developed white-lanate hair cover.

During the biosystematic studies on sect. *Napuliferae* (Bancheva & Raimondo 2003) have been detected some differences between the leaf epidermal structures of the investigated taxa. The taxonomic significance of epidermal structure, especially the type of the trichomes, has been emphasized by many authors (Metcalfe & Chalk 1965; Stace 1965, 1968; Uzunova & al. 1997).

The aims of the present comparative study are: (1) to examine the leaf epidermal structure of seven *Cyanus* species: *Cyanus pseudaxillaris* (Stef. & Georgiev) Holub, *C. orbelicus* (Velen.) Soják, *C. velenovskyi* (Adamović) Wagenitz & Greuter, *C. napulifer* (Rochel) Soják, *C. tuberosus* (Vis.) Soják, *C. thirkei* (Sch. Bip.) Holub and *C. nyssanus* (Petrović) Soják, and (2) to estimate the taxonomic value of the leaf epidermal features.

There are not much informations about the epidermal characteristics of this family so rich of taxa. Metcalfe & Chalk (1965) point out anomocytic and anisocytic stomatal types and nine types of non-glandular trichomes. They describe for *Centaurea* multicellular trichomes with uniseriate "pedestal" and whip like terminal cells. Hellwig (1992) determines different types of hairs of tribe *Astereae*. Sahu (1984) determines 13 types of non-glandular and 7 types of glandular trichomes of genus *Vernonia* Schreb., and uses the trichome complement to establish intra-generic relationships. Ogundipe & Adegbite (1991) examine the leaf epidermis of genus *Aspilia* Thouars, and elaborate a key for identification of the species on the basis of epidermal features.

Material and methods

The study is based on material collected from 11 natural populations, 10 from Bulgaria and 1 from Serbia (Tab. 1). The preparation of the leaves for observation in SEM has been accomplished according to Huttunen & Laine's protocol (1983). Observations were carried out with a SEM Leica S420 at 15 kV in the Department of Botany, Palermo University (Italy). For investigations of the stomatal type semi-permanent glycerin-jelly mounts have been prepared after clearing in perhydrol and washing in water. For obtaining better results the trichomes were removed. The terminology used is after Dilcher (1974) and Payne (1978). The vouchers are kept in the Herbarium of Institute of Botany, Sofia (SOM).

Results and discussion

The investigated species from genus *Cyanus* have some common epidermal features: amphystomatal leaves, sunken stomata from anomocytic, anisocytic and hemiparacytic type, thick covering of trichomes. All observed trichomes could be described as multicellular, uniseriate flageliforme. They consist of two or more basal cells (stalk) ending with one very long slightly twisted upper cell (whip) (Fig. 2, 3, 8, 10). The thick lanate covering on the leaves is due to these cells. We consider that the various structures of the foot cells could be used as a base for differentiation of 5 trichome types in sect. *Napulifera* of genus *Cyanus*:

Table 1. Origin of the populations examined: BU - Bulgaria, SR - Serbia, Banch. - Bancheva, M. N. - M. Niketić

Cyanus	Origin	Coll. date	Coll.
pseudaxillaris	BU: Thracian plain: Besaparski Ridove near Sinitevo, 490 m asl	26.05.1998	Banch. Sh9801
orbelicus	BU: Pirin Mt. above Hiža Banderica, 1850 m asl	22.07.1995	Banch. Sh9562
	BU: Rila Mt. above Hiža Grančer, 2350 m asl	11.07.1996	Banch. Sh9627
napulifer	BU: C Stara Planina Mt.: near Hiža Planinski Izvori, 1700 m asl	04.08.1995	Banch. Sh9605
	BU: C Stara Planina Mt.: near Hiža Vasil Levski, 1600 m asl	03.08.1997	Banch. Sh9724
velenovskyi	BU: C Stara Planina Mt.: Djuzata	24.08.1997	Banch. Sh9733
tuberosus	BU: Slavjanka Mt.: Konjarite, open grassy places, 1700 m asl	28.08.1996	Banch. Sh9660
thirkei	BU: E Rhodope Mts: Mandrica - Likan Češma, c. 700 m asl	28.04.2003	Banch. Sh03011
	BU: E Stara Planina Mt.: Kačula, c. 900 m asl	18.06.1996	Banch. Sh9616
nyssanus	SR: Suva Planina Mt.: Golaš: Divna Gorica, 1120-1300 m asl		M. N.

Type I: 2-3 basal cells, 10.0-25.0 µm in diameter, sometimes with fine striations (*C.orbelicus*, *C. velenovskyi*, *C. napulifer*, *C. tuberosus*) (Fig. 1, 8, 9, 11).

Type II: 12-15 basal cells, 30.0-35.0 µm in diameter, frequently by one sunken and one swollen cells (*C. pseudaxillaris*) (Fig. 10).

Type III: 7 cells 20.0- 22.0 µm in diameter, thin-walled, filiform (*C. thirkei*).

Type IV: 5-8 large basal cells, 55.0-68.0 µm in diameter, thin- walled. The first one likes goblet (*C. thirkei)* (Fig. 3).

Type V: 5-7 large, thick-walled, rounded cells, 40.0- 65.0 µm in diameter (*C. pseudaxillaris*) (Fig. 2, 4).

The presence of various trichome types and peculiarities of the cuticle, different forms of epidermal cells and stomata types allow the investigated species to be distinguished by their epidermal structure.

Cyanus pseudaxillaris (Stef. & Georgiev) Holub is characterized by cuticle with almost parallel corrugations, isodiametric to elongate epidermal cells of the upper epidermis (Fig. 2, 4). The epidermal cells of the lower epidermis have wavy anticlinal walls (Fig. 10). Stomatal type is mixed - anomocytic, anisocytic and hemiparacytic. The trichomes belong to the types II and V.

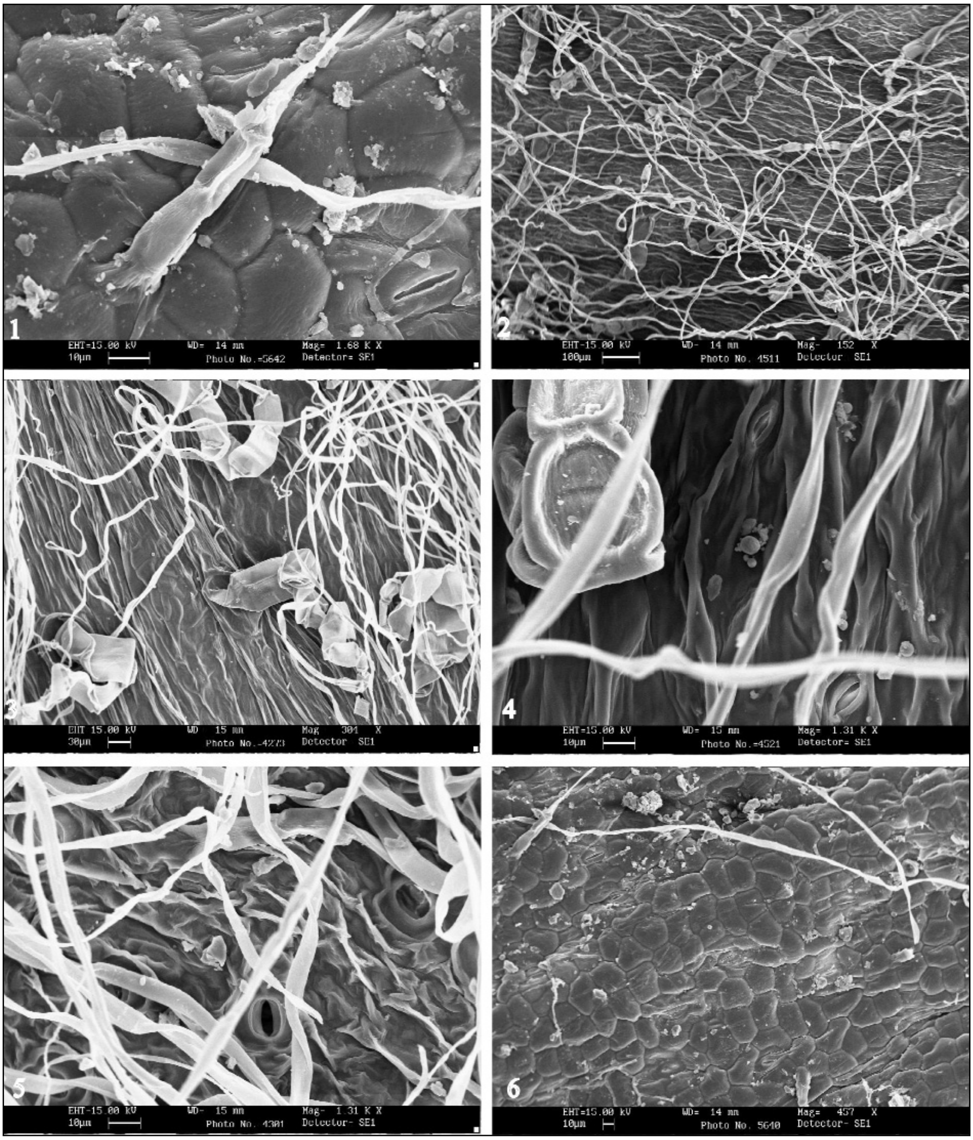

Fig. 1-6. Epidermal structure: 1 & 6 *Cyanus napulifera*, 2 & 4 *C. pseudaxillaris*, 3 *C. thirkei*, 5 *C. tuberosus*.

C*. *orbelicus (Velen.) Soják is very thick hairy. The cuticle has corrugations and fine striations somewhere. Stomata are rotund, anomocytic type. The trichomes are from type I (Fig. 9).

C*. *velenovskyi (Adamović) Wagenitz & Greuter – the upper and lower cuticles have corrugations and rough striations. The stomata are rotund, slightly sunken, anomocytic

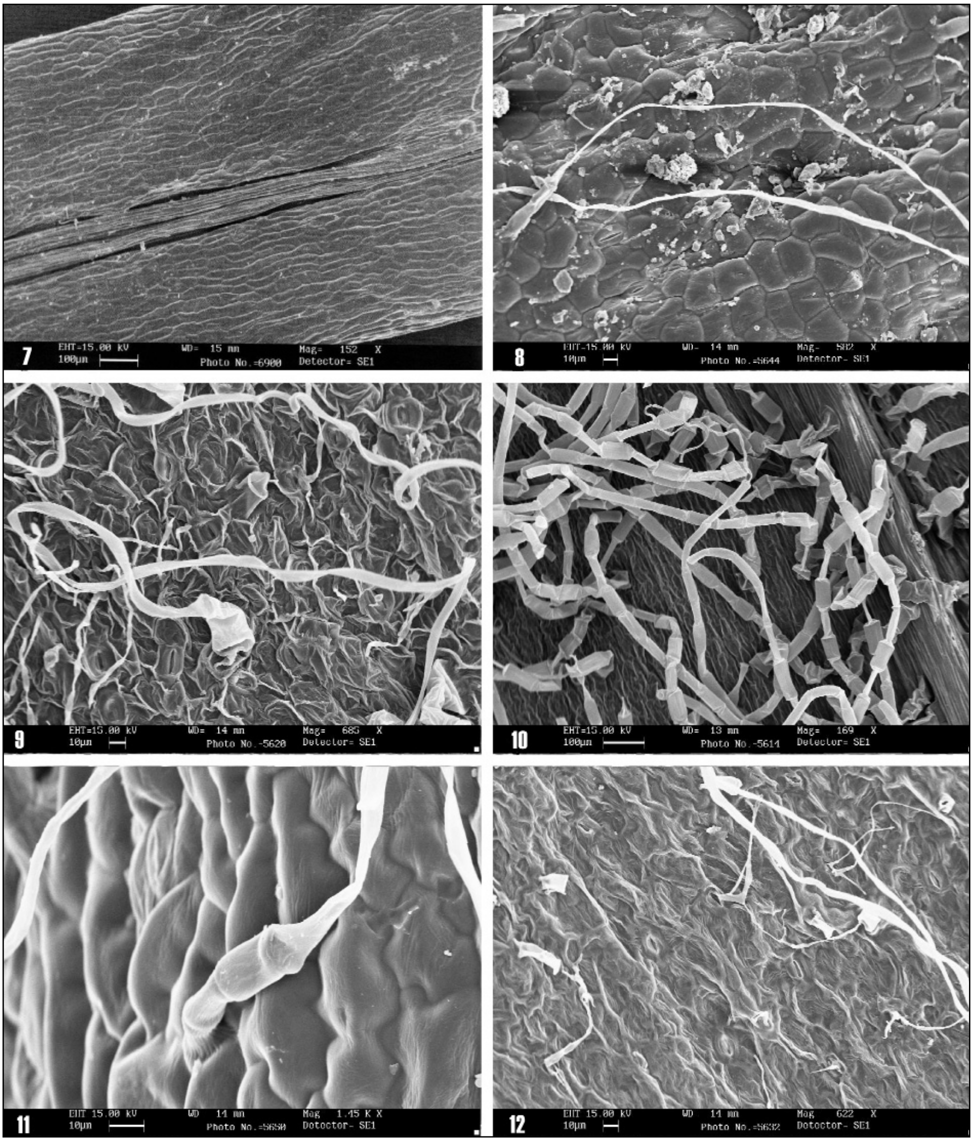

Fig. 7-12. Epidermal structure: 7 *Cyanus nyssanus*, 8 *C. napulifera*, 9 *C. orbelicus*, 10 *C. pseudaxillaris*, 11 *C. tuberosus*, 12 *C. velenovskyi*.

type. The trichomes, which basal cells have striations, belong to the type I (Fig. 12).

C. napulifer (Rochel) Soják – the upper epidermis consists of polygonal epidermal cells and sunken ellipsoidal stomata from anomocytic type (Fig. 1, 8). The epidermal cells of lower epidermis posses low wavy anticlinal walls, arranged like “puzzle” (Fig. 6). The trichomes are from type I. The basal cells have fine striations, including around the stomata.

C. tuberosus (Vis.) Soják – the upper epidermis consists of isodiametric, slightly swollen cells. The stomata are situated almost on the same level as the epidermal cells. The cuticle has rough striations (Fig. 5, 11). The trichomes are from type I.

C. thirkei (Sch. Bip.) Holub – the cuticle posses fine corrugations and striations. Epidermal cells have low undulate anticlinal walls. The stomata belong to the anomocytic type and are situated almost on the same level as the epidermal cells. The trichomes are from types III and IV (Fig. 3).

C. nyssanus (Petrović) Soják – the cuticle on the upper and lower epidermis is completely smooth (Fig. 7). The epidermal cells and stomata are arranged in almost parallel rows. The trichomes, from the type I, are distributed only on the midvein on the lower surface. The upper surface is entirely without trichomes.

The comparison between epidermal structures of the investigated *Cyanus* taxa shows the tendency of developing of xeromorphic features. As a rule, the trichomes are distributed on the lower epidermis. All *Cyanus* taxa (excluding *C. nyssanus*) posses thick trichome covering, on both surfaces. Moreover the specific trichome morphology helps the reduction of the evaporation. The stalks, which are formed of several cells, erect the flagelliforme long cells thus between the surface and trichome cover it remains a space, which protects the epidermis from extreme evaporation. Furthermore the cuticle corrugations and striations, and the sunken stomata restrict the cuticle transpiration.

The established epidermal features: the trichome types, the cuticle peculiarities and the different stomatal types, allow the identification of the species by their epidermal structure. According Cutler & al. (1978) the leaf surface is under strong genetic control and consequently the trichome type and the indumentum features are of taxonomical value (Stace 1965; Uzunova & al. 1997).

The current investigation shows that *C. nyssanus* can be easily differentiated from the other taxa by its glabrous upper surface (Fig. 7). The epidermal structures of *C. orbelicus* and *C. velenovskyi* are very similar (Fig. 9, 12). Both species, being close relatives, possess trichomes from type I, as well striations and corrugations, which bring them together. *C. napulifer* and *C. tuberosus* have trichomes from type I also, but the epidermal cells of the lower surface are wavy and arranged like “puzzle” (Fig. 1, 6, 8, 11). The last taxon could be distinguished by “comb like” upper epidermis (Fig. 11). *C. pseudaxillaris* has combination of trichomes from types II and V (Fig. 2, 10). So it could be very well differentiated by the other species. The specific combination of trichomes from types III and IV, and the cuticle structure (Fig. 3), allow us to distinguish *C. thirkei* from all other species.

The study establishes that the epidermal structure has an important role for the adaptation of the *Cyanus* species from sect. Napuliferae to the environmental xerophytic conditions and could be very useful to reveal their inter-generic relationships.

Acknowledgements

We are grateful to Anna Mannino for the technical assistance in the making of the photos.

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L. Peruzzi, K. F. Caparelli & G. Cesca

Contribution to the systematic knowledge of the genus *Ornithogalum* L. (*Hyacinthaceae*): morpho-anatomical variability of the leaves among different taxa

Abstract

Peruzzi, L., Caparelli, K. F. & Cesca, G.: Contribution to the systematic knowledge of the genus *Ornithogalum* L. (*Hyacinthaceae*): morpho-anatomical variability of the leaves among different taxa. — Bocconea 21: 257-265. 2007. — ISSN 1120-4060.

Comparative anatomy of leaf cross sections of fourteen *Ornithogalum* taxa is carried out: *O. orthophyllum* subsp. *baeticum*, *O. collinum*, *O. comosum*, *O. divergens*, *O. exscapum* var. *exscapum*, *O. exscapum* var. *ambiguum, O. exscapum* var. *parlatorei, O. gussonei*, *O. montanum, O. kochii* subsp. *monticola, O. refractum*, *O. televrinum*, *O. umbellatum* 3*x* (= *O. angustifolium*), *O. umbellatum* 4*x* (= *O. vulgare*). Analytical drawings are presented. Thirteen noteworthy leaf characters were quantified, organized in a data matrix (two more *Ornithogalum* species from literature were added) and finally analyzed through Neighbour Joining (NJ) and UPGMA methods. Leaf features in *Ornithogalum* result useful in order to group species, while often are not sufficient to characterize each single taxon.

Introduction

According to Speta (1998), the genus *Ornithogalum* L. comprises at least fifty species, distributed from the Mediterranean to W Afghanistan, showing the greatest diversity in the E Mediterranean (Zahariadi 1965). Since several decades, this taxonomically extremely difficult genus was the object of several studies on bulb structure and germination-type (Zahariadi 1962, 1965; Speta, 1990, 1990a, 1990b), classical cytotaxonomy (Peruzzi & Passalacqua 2002; Garbari & al. 2003; Tornadore & al. 2003; Aquaro & Peruzzi 2006 and literature cited therein), karyotype evolution (Raamsdonk 1986), chemotaxonomy (Øvstedal 1991), morphometry (Moret & al. 1991; Øvstedal 1991; Raamsdonk & Heringa 1987; Moret 1992; Moret & Galland 1992; Coskuncelebi & al. 2002), seed micromorphology (Coskuncelebi & al. 2000). At our best knowledge, there was no attempt to carry out an analytical and comparative study on leaf features, in order to test their possible taxonomic use. With this aim, we studied leaf cross-sections of fourteen *Ornithogalum* taxa from Central Mediterranean area.

Material and methods

The fourteen studied taxa are summarized in Table 1. Segments, 2-3 cm long, were taken from the basal third of leaves in springtime and fixed in a 50% ethylic alcohol 50% glycerol solution; afterwards, 40-50 µm wide cross sections were cut. Finally, camera lucida drawings were effected for each studied plant. We added also two more units (*O. kochii* subsp. *kochii*, *O. sibthorpii*) whose data were available in literature (Tab. 1).

Thirteen leaf features were organized in a data matrix (Tab. 2), subsequently analyzed through Neighbour Joining (NJ) and UPGMA methods under distance criterion defaults of PAUP 4.0b10 (Swofford 2002).

We selected as out-group one species of *Loncomelos* Raf. (*L. narbonensis*), following the results of Pfosser & Speta (1999), which clearly indicate the genera *Ornithogalum*, *Loncomelos* and *Honorius* S. F. Gray as monophyletic.

Table 1. Sources of the studied leaves and previous literature available on this matter: 1 - Tornadore (1986), 2 - Garbari & Giordani (1984), 3 - Speta (1990), 4 - Giordani & Garbari (1989), 5 - Speta (1990a), 6 - Zahariadi (1962), 7 - Speta (1990b).

Taxon	Label	Living plants	Lit.
Loncomelos narbonensis (L.) Raf.	LONAR	Italy: Rende (Cosenza): spontaneous in the Botanic Garden of Calabria University	1
O. orthophyllum subsp. *baeticum* (Boiss.) C. Zahariadi	BAETI	France: Les Vignes (Herault, Causse du Larzac), 2003 (cult. Hort. Bot. Calabria University, acc. n. 475)	\
O. collinum Guss.	COLLI	Sicily: Nebrodi, Cesarò, 2004 (cult. Hort. Bot. Calabria University, acc. n. 620)	2, 3
O. comosum L.	COMOS	Croatia: between Aržano and Kamensko, 2003 (cult. Hort. Bot. Calabria University, acc. n. 138)	\
O. divergens Boreau	DIVER	Italy: Calabria, Sila Piccola, loc. Ciricilla, 2002 (cult. Hort. Bot. Calabria University, acc. n. 749)	\
O. exscapum Ten. var. *exscapum*	EXSCA	Italy: Calabria, Sila Greca, loc. Sferracavallo, 2001 (cult. Hort. Bot. Calabria University, acc. n. 38, 264)	3
O. exscapum Ten. var. *ambiguum* (N. Terracc.) Fiori	EXAMB	Italy: Calabria, Sila Grande, banks of lake Cecita, 2001 (cult. Hort. Bot. Calabria University, acc. n. 271)	3
O. exscapum Ten. var. *parlatorei* Peruzzi et N. G. Passal.	EXPAR	Italy: Calabria, Mount Cocuzzo, 2001 (cult. Hort. Bot. Calabria University, acc. n. 110)	\

Table 1. (continued.)

O. gussonei Ten. (≡ *O. tenuifolium* Guss.)	GUSS	Sicily: Iblei, between Sortino and Buccheri, 2001 (cult. Hort. Bot. Calabria University, acc. n. 14)	3
O. kochii Parl. subsp. *kochii*	KOCHI	\	4
O. kochii Parl. subsp. *monticola* (Jord. & Fourr.) Peruzzi	MONTI	France: Alpes Maritimes, Saint Martin d'Entraunes, 2003 (cult. Hort. Bot. Calabria University, acc. n. 667)	5
O. montanum Ten.	MOSIR	Italy: Basilicata, Mount Sirino, 2002 (cult. Hort. Bot. Calabria University, acc. n. 19)	\
	MORUG	Italy: Basilicata, Pollino, Piano di Ruggio, 1997 (cult. Hort. Bot. Calabria University, acc. n. 315)	
	MOPAL	Italy: Calabria, Sila Greca, Paludi, 2002 (cult. Hort. Bot. Calabria University, acc. n. 603)	
	MOFIC	Sicily, Bosco della Ficuzza, 2002 (cult. Hort. Bot. Calabria University, acc. n. 497)	
O. refractum Kit. ex Willd.	REFCT	Italy: Calabria, Pollino, Castrovillari, 2001 (cult. Hort. Bot. Calabria University, acc. n. 248)	\
	REFCA	Italy: Calabria, Pollino, Alto di Cassano, 2002 (cult. Hort. Bot. Calabria University, acc. n. 161)	
	REFSIC	Sicily, Nebrodi, Tre Arie, 2001 (cult. Hort. Bot. Calabria University, acc. n. 629)	
O. sibthorpii Greuter (≡ *O. nanum* Sibth. & Smith)	SIBTH	\	6, 7
O. televrinum Speta	TELAR	Croatia: between Aržano and Kamensko, 2003 (cult. Hort. Bot. Calabria University, acc. n. 142)	3
	TELBI	Croatia: Biokovo, 2003 (cult. Hort. Bot. Calabria University, acc. n. 335)	
O. umbellatum L. 3*x* (= *O. angustifolium* Boreau)	UMBEL	France: Heyrieux (Isere), 2003 (cult. Hort. Bot. Calabria University, acc. n. 641)	\
O. umbellatum L. 4*x* (= *O. vulgare* Sailer)	VULGA	Italy, Tuscany, Empoli (Florence), 2004 (cult. Hort. Bot. Calabria University, acc. n. 180)	\

Results and Discussion

Cross sections of the leaves are reported in Figures 1-3. NJ and UPGMA trees are shown in Fig. 4. Both trees show very similar topologies, but for the placing of the two *O. televrinum* samplings, alternatively basal to *O. exscapum* group (under NJ) or to *O. umbellatum* group (under UPGMA).

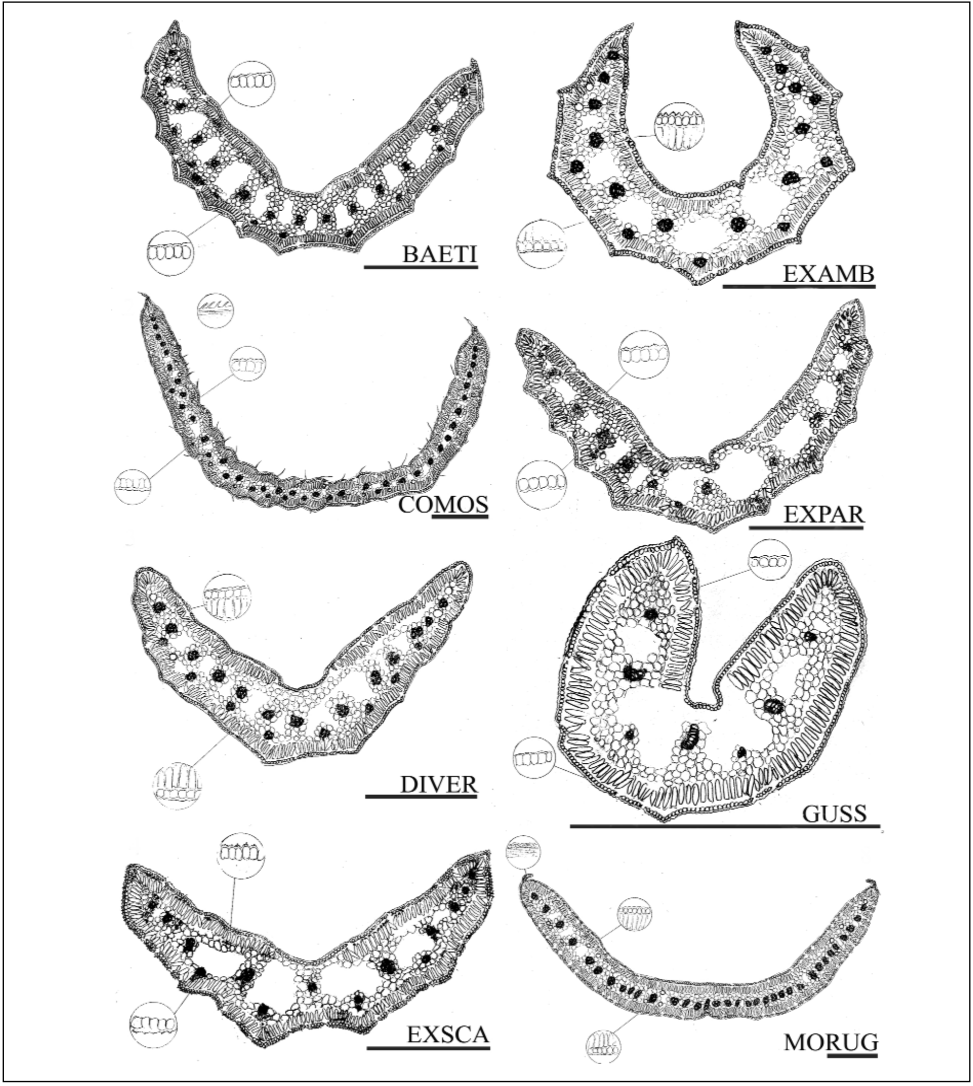

Fig. 1. Leaf cross sections: *O. orthophyllum* subsp. *baeticum* (BAETI), *O. comosum* (COMOS), *O. divergens* (DIVER), *O. exscapum* var. *exscapum* (EXSCA), *O. exscapum* var. *ambiguum* (EXAMB), *O. exscapum* var. *parlatorei* (EXPAR), *O. gussonei* (GUSS), *O. montanum* (MORUB); scale bars = 1 mm.

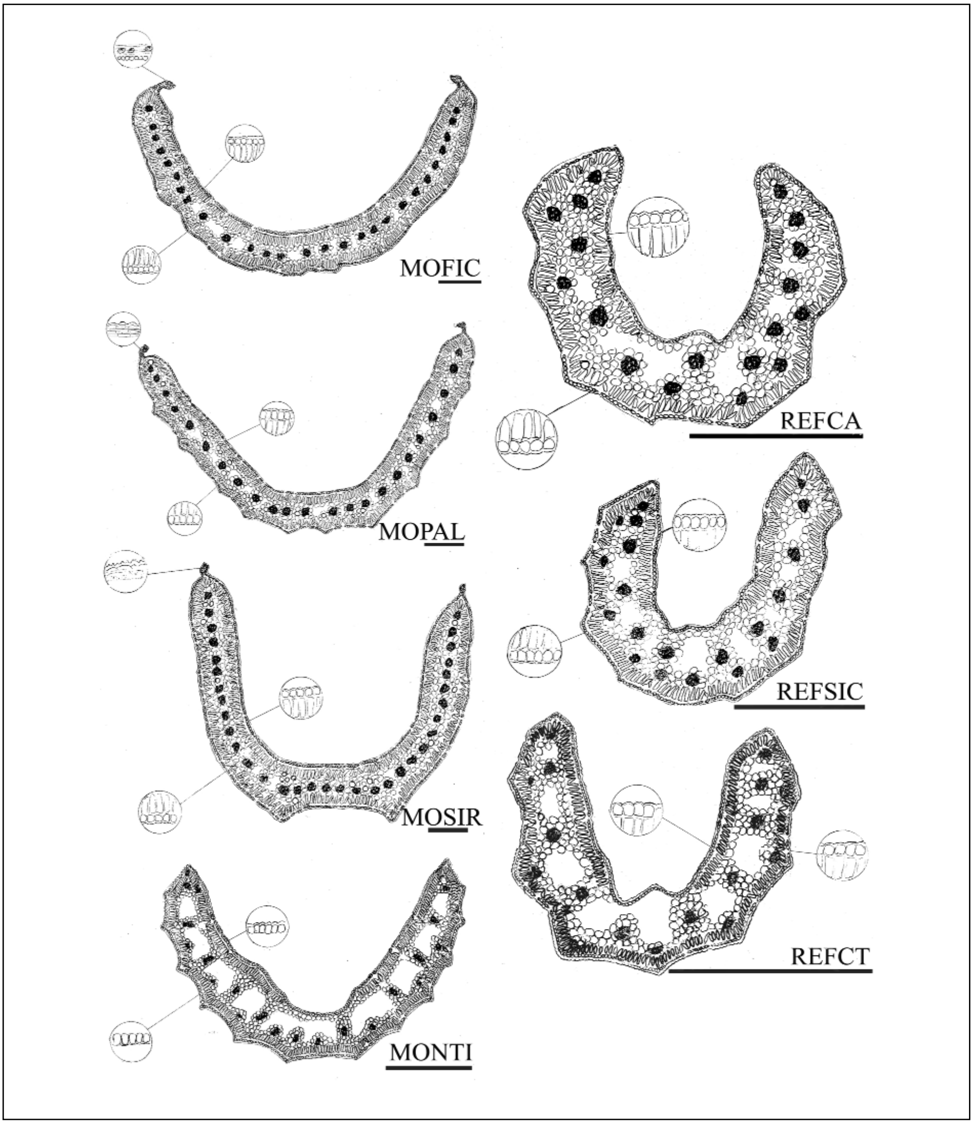

Fig. 2. Leaf cross sections: *O. montanum* (MOFIC, MOPAL, MOSIR), *O. kochii* subsp. *monticola* (MONTI), *O. refractum* (REFCA, REFSIC, REFCT); scale bars = 1mm.

By considering only leaf features, it results that *Ornithogalum montanum* (subgen. *Oreogalum* Zahar.) and *O. comosum* (sect. *Obtusangula* Zahar.) are the most distinct species, maintaining some ancestral character close to *Loncomelos*. *O. gussonei* and *O. collinum* (both belonging to subgen. *Hypogaeum* Zahar.) are also rather isolated. *O. umbellatum* cycle (subgen. *Ornithogalum sensu strictissimo*) is fully recognised, as *O. exscapum*

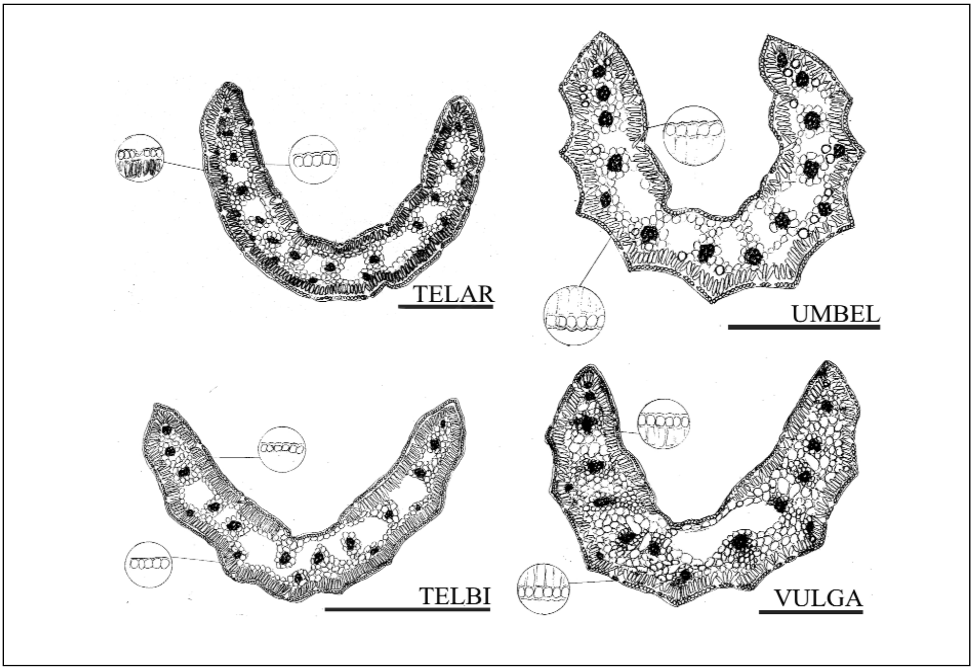

Fig. 3. Leaf cross sections: *O. televrinum* (TELAR, TELBI), *O. umbellatum* 3x (UMBEL), *O. umbellatum 4x* (VULGA); scale bars = 1mm.

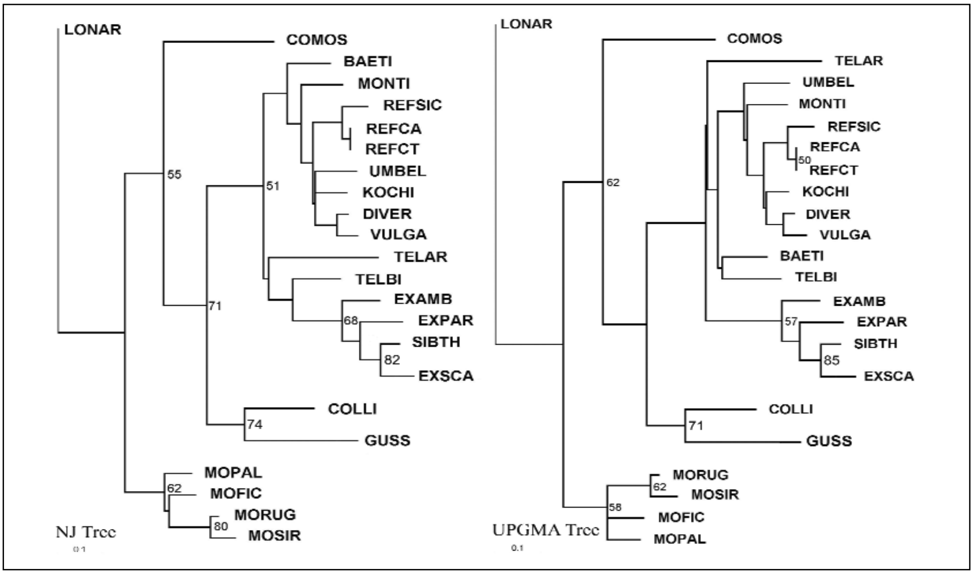

Fig. 4. NJ and UPGMA trees of the considered taxa. Bootstrap values above 50% are reported on the right of the nodes.

Table 2. Data Matrix. Character: **1** leaf cross section margins: 0 hairy-appendiculate, 1 appendiculate, 2 acute-appendiculate, 3 acute, 4 obtuse, **2** abaxial surface keels: 0 no, 1 low, 2 normal, 3 pronouced, **3** palisade in the middle of adaxial surface: 0 continuous, 1 reduced, 2 interrupted, **4** "channel-like" structures: 0 many, 1 few, 2 no, **5** adaxial cuticle in cross section: 0 almost linear, 1 undulate, 2 acute, 3 "curly-bracket"-like, **6** abaxial cuticle in cross section: 0 almost linear, 1 undulate, 2 acute, 3 "curly-bracket"-like, **7** leaves number: 0: 0-6, 1: 6-9, 2: 9-12, **8** leaves erect before flowering: 0 no, 1 yes, **9** leaf colour: 0 green, 1 glaucous-green, 2 hardly glaucous, **10** leaves present at flowering: 0 always, 1 often no, 2 no, **11** vascular bundles number: 0: many in 1 series, 1: >30 in 2/3 series, 2: 10-30 in 2/3 series, 3: <9 in 2 series, **12** leaf width: 0: >5 mm, 1: 2-5 mm, 2: <2 mm, **13** 30-Germination: 0 epigeal, 1 hypogeal.

		Character												
No	Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	LONAR	1	1	0	0	0	2	0	0	0	0	1	0	0
2	BAETI	2	2	2	1	1	0	0	0	0	0	2	1	0
3	COLLI	0	3	2	2	0	0	2	0	0	1	2	1	1
4	COMOS	0	0	0	0	0	0	1	0	2	0	1	0	0
5	DIVER	4	2	2	1	0	0	0	1	0	0	2	1	0
6	EXSCA	3	3	2	2	3	3	1	0	1	0	2	1	0
7	EXAMB	3	3	2	1	2	0	1	0	1	0	2	1	0
8	EXPAR	3	2	2	2	2	1	1	0	1	0	2	1	0
9	GUSS	4	1	2	2	0	0	2	0	0	2	3	2	1
10	KOCHI	3	2	2	?	0	0	0	1	0	0	2	1	0
11	MORUG	1	1	0	0	1	0	0	0	0	1	0	0	1
12	MOFIC	1	0	0	0	1	0	0	0	0	0	0	0	1
13	MOPAL	1	1	0	0	0	0	0	0	0	0	0	0	1
14	MOSIR	1	1	0	0	1	1	0	0	0	1	0	0	1
15	MONTI	3	3	2	1	1	0	0	1	0	0	2	1	0
16	REFCA	4	2	2	1	1	0	0	1	0	0	2	1	0
17	REFSIC	4	2	2	1	0	0	0	1	0	0	2	1	0
18	REFCT	4	2	2	1	1	0	0	1	0	0	2	1	0
19	SIBTH	3	3	2	2	1	3	1	0	1	0	2	1	0
20	TELAR	4	0	1	1	1	1	0	0	2	0	2	1	0
21	TELBI	3	2	2	2	1	0	0	0	2	0	2	1	0
22	UMBEL	4	3	2	0	0	0	0	1	0	0	2	1	0
23	VULGA	4	2	2	1	0	0	1	1	0	0	2	1	0

s.l. (subgen. *Amphigalum* Zahar.). Finally, *O. televrinum* seems to represent the connection between *O. umbellatum* and *O. exscapum* groups.

Leaf features in *Ornithogalum* result useful in order to group similar species, while often are not sufficient to characterize each single taxon, being so probably reliable for phylogenetic inference.

Acknowledgemtens

Kind thanks are due to Dr. Jean-Marc Tison (L'Isle D'Abeau, France) which provided bulbs of *O. orthophyllum* subsp. *baeticum*, *O. kochii* subsp. *monticola* and *O. umbellatum* 3x from France.

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M. Urbani, G. Becca & M. G. Ledda

Notes on systematics and chorology of *Asparagus* L. (*Asparagaceae*) in Sardinia (Italy)

Abstract

Urbani, M., Becca, G. & Ledda, M. G.: Notes on systematics and chorology of *Asparagus* L. (*Asparagaceae*) in Sardinia (Italy). — Bocconea 21: 267-271. 2007. — ISSN 1120-4060.

The occurrence in Sardinia (Italy) of *Asparagus acutifolius* L., *A. albus* L. and *A. horridus* L. is here confirmed. The three taxa were studied on material collected from natural populations and specimen from several herbaria (SS, SASSA, CAG, FI and Ancona). Notes on chorology, systematics, ecology and fenology of the mentioned species are given. The exclusion of *A. aphyllus* L. and the uncertain presence of other taxa related to the *A. officinalis* L. group, are discussed.

Introduction

The first idea of this study started few years ago from a project dealing with the cultivation of wild *Asparagus* as a possible crop for marginal areas in Sardinia (Fiori & al. 2001) in which one of us was involved. The intriguing opportunity for all of us was to find a possible way of communication between two areas usually, at least in our experience, quite distant: agriculture and systematic botany. This contribution deals with the study of some natural populations and herbarium material of the taxa of the genus *Asparagus* in Sardinia. In this note the presence of some species is not confirmed, and possible reasons for some of the most common misleading are given, using different sources of information.

The genus *Asparagus*, described by Linnaeus (1753), actually includes a quite variable number of taxa: from 160 to 290 species (Kubitzki & Rudall 1998) or about 300 species, together with the close genus *Protasparagus* (Dahlgren & al. 1985). *Asparagus* is spread in the Old Word mostly in the arid, semi-arid or Mediterranean climatic regions of Africa, Mediterranean, Eastern Asia and only one species native in Australia. Sardinia can be considered crucial in defining some of the Mediterranean taxa. For the island *Asparagus albus* L., *A. officinalis* L., *A. maritimus* Mill. (= *A. scaber* Brign.), *A. acutifolius* L., *A. aphyllus* L. and *A. horridus* L. (= *A. stipularis* Forssk.) had been reported by Barbey (1884), Fiori (1923), Bozzini (1959), Pignatti (1982) and Valdés (1980).

Material and methods

Morphological and anatomical studies were carried out on dry and fresh material collected from 15 different sites (Tab. 1). The Sardinian material conserved in the SS, SASSA, CAG, FI and Ancona herbaria was checked and reviewed. The distribution in Sardinia was reported utilizing a UTM geographic grid of 5×5 km squares (Fig. 1-3). The abbreviations of countries or specific territories names in the distribution of the taxa are as in Valdés (1980) or Greuter & al. (1989). The citotaxonomic study was carried out on germinating seeds collected from the sites in Table 1. *Specimina visa* for reference are conserved in SS. The Feulgen tecnique was carried out and material was stained with fuxine.

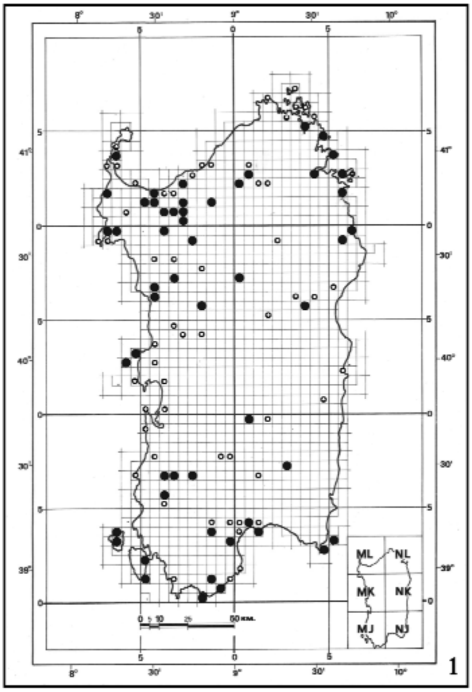
Results

Asparagus acutifolius L. Sp. Pl.: 314 (1753) typus in LINN: 434/9(!)

A. acutifolius is a rhizomatous Geophyte common in shrubs communities, *Quercus ilex* woods, wood glades, edges, marginal areas, from sea level to 1000-1100 m asl. The shoot architecture of *Asparagus* is highly complicated. In *A. acutifolius* the young shoots (turions) rise from March/April when the shoots of the previous year begin to dry. Flowering period is comprised from the end of August to October, flowers on one-year-old shoots. Fruiting from October to Dicember. *A. acutifolius* is a Mediterranean taxon, its distribution includes: Lu, Hs Bl, Ga, Co, It, Sa, Si, Croatia, Slovenia, Al, Gr, Cr, Bu, Tu, An, Cy, LS, IJ, Eg, Li, Tn, Ag and Ma (Bozzini 1959; El-Gadi 1978; Pignatti 1982; Sibthorp & Smith 1823; Valdés 1980). Distribution in Sardinia in Figure 1. The chromosome number, $2n = 20$, is the first report from Sardinia and it is confirmed in all the investigated populations.

Table 1. The sites of populations sampling in Sardinia.

Asparagus acutifolius **L.**	***A. albus*** **L.**	***A. horridus*** **L.**
La Crucca 85 m., 40° 45’ N, 8° 29’ E	Tramariglio 50-75 m., 40° 36’ N, 8° 10’ E	Bosa Marina 5-10 m., 40° 17’ N, 8° 29’ E
Porticciolo 27-30 m., 40° 38’ N, 8° 12’ E	Capo Caccia 100-120 m., 40° 34’ N, 8° 10’ E	S. Giovanni in Sinis 12-40 m., 39° 53’ N, 8° 26’ E
Platamona 15 m., 40° 49’ N, 8° 31’ E	Piano Multas 125-150 m., 40° 17’ N, 8° 30’ E	Cala Regina 50 m., 39° 11’ N, 9° 22’ E
Monte Doglia 40-50 m., 40° 39’ N, 8° 16’ E	Cala Regina 50 m., 39° 11’ N, 9° 22’ E	Capo Carbonara 35-50 m., 39° 06’ N, 9° 31’ E
Osilo 542 m., 40° 46’ N, 8° 40’ E	Capo Carbonara 35-50 m., 39° 06’ N, 9° 31’ E	
Ittiri 218 m., 40° 38’ N, 8° 32’ E		
Macomer 560-640 m., 40° 17’ N, 8° 47’ E		
Tempio 100-120 m., 40° 51’ N, 9° 01’ E		



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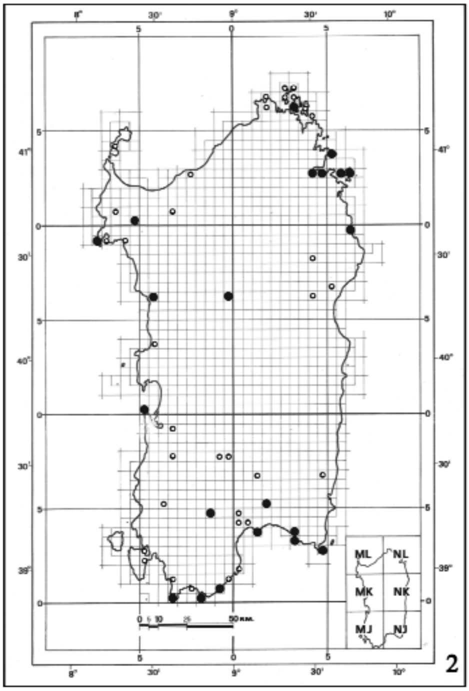

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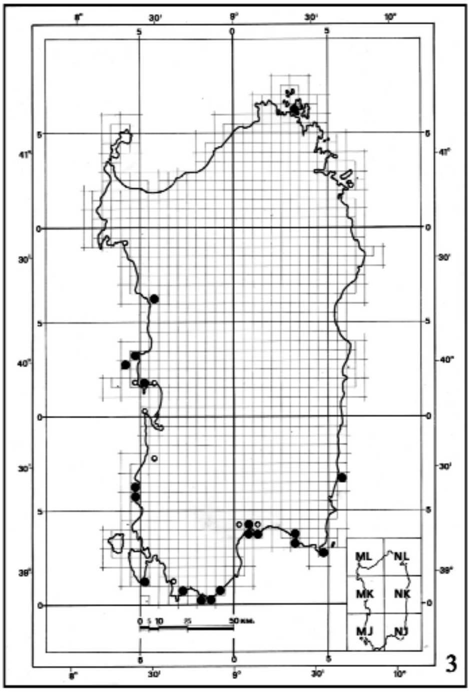

3

Fig.1-3. Distribution of: **1)** *Asparagus acutifolius* L., **2)** *A. albus* L., **3)** *A. horridus* L. in Sardinia (white dots: based only on literature data).

Asparagus albus L. Sp. Pl.: 313 (1753) typus LINN: 434/7(!)

A. albus is a rhizomatous Geophyte or fruticous Chamephyte characteristic of arid and xeric sites especially, but not exclusively, close to the sea. This taxon occurs on arid slopes, low shrubs communities, cliffs, walls, edges, from sea level to 1000 m asl. Aerial shoots pluriennial, but phylloclades generally fall at the end of the first year's vegetative season (in summer). At the axil of the leaves (reduced to thorns) vegetative and floral buds are present, that generate new phylloclades in spring and flowers in summer. By the activation of these meristems, a plant, can flower and form new phylloclades, in a very short time, even after a summer rain. New shoots grow at the beginning of spring, they differentiate and lignificate quickly. In the Sardinian populations of *A. albus* observed, flowers come out on shoots of the year at the end of the summer when, most of phylloclades are already fallen down. Later on, during autumn and winter new cladodes are formed. Fruiting from October to December. This species is W Mediterranean, its distribution includes: Lu, Hs, Bl, Co, It (Calabria), Sa, Si, Li, Tu, Ag, Ma (Bozzini 1959; El-Gadi 1978; Pignatti 1982; Valdés 1980). In Figure 2 the distribution in Sardinia. The chromosome number, $2n = 20$, is the first report for Sardinia and it is confirmed in all the populations investigated.

Asparagus horridus L. in J. A. Murray, Syst. Veg., ed. 13: 274 (1774) typus in LINN: 434/10(!)

Synonyms: *A. stipularis* Forssk.; *A. aphyllus* L. b *stipularis* (Forssk.) Fiori; *A. horridus* L. fil. (1781); *A. aphyllus* var. *stipularis* Baker (1875).

A. horridus is a rhizomatous Geophyte or fruticous Chamephyte, mainly present in the coastal rocky places and in open and low shrubs communities from 0 to 500 m asl. New shoots rose numerous at the beginning of spring. Flowering from the second half of May to June; Bozzini (1959) reports an earlier flowering period (April-May). Flowers come out on the shoots of the year. Fruiting at the beginning of summer with a second flowering period in Autumn, as reported in Fiori (1923) and Bozzini (1959). *A. horridus* is Mediterranean taxon, its distribution includes: Lu, Hs, Bl, Canary Islands, Sa, Si, Me, Cr, Gr, LS, IJ, Sn, Eg, Li, Tn, Ag, Ma (Bozzini 1959; El-Gadi 1978; Pignatti 1982; Sibthorp & Smith 1823; Valdés 1979, 1980). Distribution in Sardinia in Figure 3. Chromosome number is $2n = 20$, it confirms Bozzini (1959) report from Capo S. Elia (Cagliari).

Species excludendae or incertae

In Bozzini (1959) *A. aphyllus* L. is a taxon somehow intermediate between *A. acutifolius* and *A. horridus* (sub *A. stipularis* Forssk.), but morphologically closer to the first. The main differences from *A. acutifolius* are due to the diverse cladode's length present at each node in *A. aphyllus* as in the Linnaeus (1753) description: "*Asparagus aphyllus, spinis fasciculatis inaequalibus divergentibus*". In Fiori (1923), presumably following Baker (1875), *A. aphyllus* L. includes the taxon *A. horridus* (sub *A. horridus* × *stipularis*). Following this interpretation the two names, *A stipularis/A. aphyllus*, were used, at least by Italian authors, to indicate the same taxon, at least at specific level. More studies are needed to clarify the circumscription of *A. aphyllus* in the entire Mediterranean region. In this work *A. aphyllus* is excluded from the Sardinian flora on the basis of different characters that include: chromosome number, cladode's number and length at each node, number of vascular bundles per cladode. The presence of taxa belonging to the *Asparagus* *officinalis* L. group is supported by only two specimens in Ancona's and CAG herbaria (!). In both cases they could be collected from cultivated or escaped isolated plants. Since natural populations couldn't be found, the presence of *A. officinalis*, or that one of any other of its allied taxa, i.e. *A. maritimus* Mill., in Sardinia is still to be confirmed.

Conclusions

The presence in Sardinia of three taxa is here confirmed: *Asparagus albus* L., *A. acutifolius* L. and *A. horridus* L. The same chromosome number, $2n = 20$, was assessed from the three taxa studied. This is confirm for *A. horridus* L. in Sardinia, but it is a new report for *A. albus* L. and *A. acutifolius* L. In the latter this diploid number, is the second report from the W Mediterranean region. Sardinian populations of *A. acutifolius* are differentiated from the Italian and Sicilian ones studied by Bozzini (1959) and related to the NW Mediterranean ones by their diploid chromosome number. The extensive distribution of the diploid type (all the investigated populations) can be interesting for a possible horticultural exploitation of the Sardinian *A. acutifolius*. The occurrence in Sardinia of *Asparagus officinalis* L. or of any other related taxa, i.e. *A. maritimus* Mill., is still to be confirmed. On the contrary *A. aphyllus* L. is to be excluded from the flora of the island, especially on the basis of cladodes' anatomy and of chromosome investigations.

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B. Valdés, V. Girón & I. Carmona

Regressive psammophilous plant formations at “Monte Algaida” pine forest, Doñana Natural Park, SW Spain

Abstract

Valdés, B., Girón, V. & Carmona, I.: Regressive psammophilous plant formations at “Monte Algaida” pine forest, Doñana Natural Park, SW Spain. — Bocconea 21: 273-281. 2007. — ISSN 1120-4060.

While many scientific studies have been developed at the wide territory of Doñana area (SW Spain) which extends in Huelva and Sevilla provinces, especially at the Doñana Biological Reserve, Doñana National Park and parts of Doñana Natural Park, very little attention has been paid to the area covered by the Natural Park in Cádiz province (“Monte Algaida”), at the other side of Guadalquivir river. Monte Algaida covers a band of aeolian sands developed over a sedimentary clay substrate. A study of this area has shown that it is covered by seven woody vegetation units: juniper woodlands; “monte blanco”, a scrubland dominated by *Halimium halimifolium*; “Lentiscar”, a scrubland dominated by *Pistacia lentiscus*; scrublands dominated by *Corema album; Tamarix formations*, and in more reduced proportion some formations of *Populus alba* and herbaceous communities dominated by *Juncaceae*. An interesting herbaceous community has been detected and analyzed. Floristic composition of the woody vegetation units of “Monte Algaida” is analyzed in comparison with the same units in Huelva province. This shows that plant communities at “Monte Algaida” are rather degraded and are in clear regression, owed, most probably, to the lack of new sand input by wind from the coast, and mainly to antropic pressure.

Introduction

To preserve wild fauna in one of the most important regions used by many migrating bird species, the Spanish Council of Scientific Research (C.S.I.C.) and the World Wildlife Fund bought, in 1964, 7000 ha of a territory located at the right side of the Guadalquivir river which had been a hunting area for centuries. The C.S.I.C. formed with these 7000 ha a reserve: Doñana Biological Reserve. But this relatively small area was not enough to guaranty protection to the reach fauna which permanently or temporary inhabits this area. This was the reason why the Spanish Government declared in 1969 as National Park a wider area of 37000 ha which was later declared as a Biosphere Reserve by the UNESCO. But even this area was not enough to preserve the biological richness of the Guadalquivir marshes and surrounding sands, owed mainly to the increasing anthropic activity (agriculture, urban expansion, hunting, etc.), and in 1989 the Andalusian Government declared as

Natural Park an area of 54000 ha surrounding the National Park. As a result, the Doñana area, traditionally known as “Coto de Doñana” (coto meaning “hunting area”) forms one of the widest Spanish protected areas with more than 100000 ha.

Doñana area is located on the Atlantic coast of SW Spain bordering the estuary of Guadalquivir river (Fig. 1). Most Doñana area extends at the right side of the Guadalquivir, covering a wide area of SE Huelva and SW Sevilla provinces, but a part of the area, which belongs to Doñana Natural Park, is located in Cádiz province, at the left side of Guadalquivir river, close to the river mouth. This area is known as “Monte Algaida”.

Doñana area extends over three large land units: the stabilized aeolian sands, the mobile sand dunes and the Guadalquivir marshes, which include both salty and fresh-water marshes. Vegetation is very reach, with over 75 different plant communities covering the three land units.

The “Monte Algaida” includes only two of these land units: an area of stabilized aeolian sands, the “Monte Algaida pine forest”, and a wide area of salty marshes, which limit

Fig. 1. The study area.

the sands to the North and West. The stabilized aeolian sands form a band SW-NE oriented at the Doñana Natural Park, Cádiz province. The sands were formed at the coast and blown inland during the Quaternary over a sedimentary clay substrate on which the surrounding salty marshes develop. Isolated from the coast for centuries, the "Monte Algaida" pine forest does not receive any new sand input.

The salty marshes extends from the stabilized sands to the Guadalquivir river on the sedimentary clay platform. They hold a characteristic plant community dominated by *Arthrocnemum macrostachyum* (Moric) Moris, with a wide band of *Spartina densiflora* Brongn. community which extends along the river side and at the edges of several artificial chanels. Include four salt-works (Monte Algaida, Santa Teresa, San Carlos and Nuestra Señora del Rocío) two currently abandoned.

To the East, the stabilized sands are limited by arable fields resulting from drainage and reclamation of the salty marshes. To the South, the limits are formed by a small artificial lagoon (Laguna de Tarelo) and a series of orchards which extends towards the village of Bonanza and the city of Sanlúcar de Barrameda, and completely separate the stabilized sands from the Guadalquivir river mouth, c. 7 km away, and from the coast.

While many scientific studies have been developed at the wide territory which extends in Huelva province, especially at the Doñana Biological Reserve, Doñana National Park and parts of Doñana Natural Park, very little attention has been paid to the area covered by the Natural Park in Cádiz province ("Monte Algaida"), at the other side of Guadalquivir river.

As a part of a wide study of the flora and vegetation of Doñana area, this paper includes some observations on the main woody plant formations of the stabilized sands of "Monte Algaida" in comparison to what are considered the best preserved and representative plant communities of the same type of the main territory of Doñana area in Huelva province.

Material and methods

The study covers the area known as "Monte Algaida pine forest", this is, the stabilized aeolian sands, which form a band SW-NE oriented at the Doñana Natural Park, Cádiz province (Fig. 2). Neither the surrounding fresh-water communities, which form a very narrow and discontinuous band around the sands, nor the surrounding salty marshes vegetation are including.

The area has been regularly visited from January to the end of July 2004. Plant communities have been identified, and the species listed. When wanted, plant material has been collected and identified in laboratory.

For the recognized woody plant communities only woody species, including climbers, are listed, with the exception of the "Monte blanco" and the riparian *Populus alba* dominated community, where also perennial herbs are mentioned, as they are important components of these two communities.

Plant formations have been mapped at 1:10000 scale by using GPS and aerial photographs.

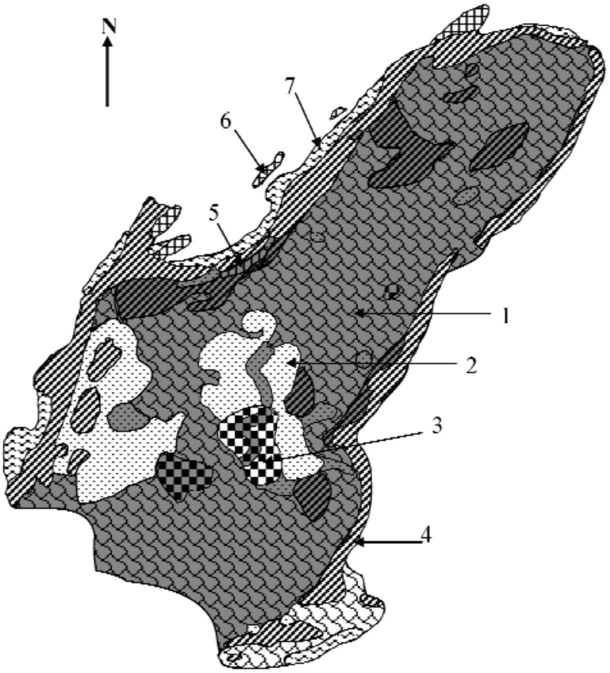

Fig. 2. Vegetation map of “Monte Algaida” pine forest: 1 - the juniper woodland, 2 - the “Monte Blanco”, 3 - the “Camarinal”, 4 - the “Lentiscar”, 5 - the *Tamarix* formations, 6 - riparian formations, 7 - herbaceous communities (gray: area planted with *Pinus pinea*).

Results

Plants in “Monte Algaida pine forest” exclusively depend from rain water. Water drains through the sands and accumulates at the bottom on the impermeable clay substrate. The slow flowing away of this water at the ridges of the sands during Autumm, Winter and Spring allows the presence of some fresh-water depending communities, such as some *Populus alba* L. communities, and herbaceous communities dominated by *Juncaceae*, mainly *Juncus effusus* L. and *J. acutus* L.

On the stabilized aeolian sands, as it is the general rule in Doñana area (see Allier & al. 1974), plant formations distribute according to water availability. Three plant formations cover the central higher parts of the stabilized sands, where water-table is deeper: the “sabinar”, a juniper woodland dominated by *Juniperus phoenicea* subsp. *turbinata* (Guss.) Nyman; the “Monte blanco”, a scrubland dominated by *Halimium halimifolium* (L.)

Willk., and the “camarinal” a scrubland dominated by *Corema album* (L.) D. Don.; they distribute according to the topography of the area and the distance of the surface to the water-table. A fourth community, non existing today in this area, is the coastal juniper plant community dominated by *Juniperus oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball. Most probably this formation covered a certain area in Monte Algaida pine forest, as the only specimen still growing in the SE part of these sands was burned in 1994. Most of the characteristic species of this juniper forest are coincident with the *Juniperus phoenicea* woodland; and in any case its potential area has been covered by the later formation.

Two other plant formations occupy a more reduced marginal area, more or less in direct contact with the sedimentary clay substrate, where water is available most of the year: *Tamarix* plant formations and some communities dominated by *Populus alba*.

Another rather interesting floristically rich formation occupies a wide band between those two groups of communities (see Fig. 2): the “lentiscar”, a scrubland dominated by *Pistacia lentiscus* L.

Most of the stabilized sands are covered by a planted forest of *Pinus pinea* L. It is not too dense, though, and allows the presence of the above indicated native plant formations.

The Juniper woodland

This low forest, which rarely exceedes 5 m in height, represents the most mature stage of succession in the xeric zones of the stabilized sands (Villar & al. 1997).

In Monte Algaida, the dominant species are: *Juniperus phoenicea* subsp. *turbinata* (Guss.) Nyman, *Rhamnus lycioides* subsp. *oleoides* (L.) Jahandiez & Maire, *Phyllirea angustifolia* L., *Cistus salvifolius* L., *Halimium calycinum* (L.) K. Koch, *Halimium halimifolium* (L.) Willk. and *Asparagus acutifolius*, and in a lower proportion, *Osyris quadripartita* Decne, *Rosmarinus officinalis* L., *Myrtus communis* L. and *Daphne gnidium* L.

All characteristic components of this juniper forest are present in Monte Algaida, but the main difference with the juniper forest of the main nucleus of Doñana area is the constant presence of *Pancratium maritimum* L., a species which characterizes coastal dunes, but which is very frequent in Monte Algaida, more than 7 km away from the coast.

The “Monte Blanco”

This is a scrubland dominated by *Halimium halimifolium* (L.) Willk., which substitutes the juniper forest in many areas and covers a wide area of the stabilized sands in Doñana area. The floristic composition in “Monte Algaida” is very poor, and in many zones is only represented by *H. halimifolium*, a species which also occurs in all plant formations on sandy soils, as a consequence of its plastic character and its ecophysiological control of water potential (Zunzunegui & al. 1997).

In “Monte Algaida” the Monte Blanco is formed by only two species: *H. halimifolium* and *H. calycinum* (L.) K. Koch, with a single specimen observed of *Stauracanthus genistoides* (Brot.) Samp. In Doñana area, Huelva province, the Monte Blanco is a very rich community formed mainly by *Cistaceae*, *Labiatae* and *Leguminosae*. Apart from the former three species, the following are characteristic components of this plant formation in the areas where it is better preserved: *Lavandula stoechas* L. subsp. *luisieri* (Rozeira) Rozeira, *Thymus mastichina* subsp. *donyanae* Morales, *Rosmarinus officinalis* L., *Helianthemum hirtum* (L.) Miller, *Cistus libanotis* L., *Ulex australis* Clemente, *Ulex erio-*

cladus C. Vicioso, *Cytisus grandiflorus* DC., *Scrophularia frutescens* L., *Helichrysum picardii* Boiss. & Reut., *Thymus mastichina* subsp. *donyanae* Morales, and the perennial herbs *Dianthus hinoxianus* Gallego, *Iberis contracta* subsp. *welwitschii* (Boiss.) Moreno and *Euphorbia baetica* Boiss.

The “Camarinal”

This is a scrubland dominated by the *Empetraceae Corema album* (L.) Don, endemic of the atlantic coast of the Iberian Peninsula (from La Coruña to Cádiz provinces) and the Azores Islands. In Doñana area this plant formation is related to the forests of *Juniperus phoenicea* subsp. *turbinata* and *J. oxycedrus* subsp. *macrocarpa* of which *Corema album* is one of the component species.

In Huelva province the “Camarinal” is formed by *Corema album* (L.) D. Don, *Halimium calycinum* (L.) K. Koch, *Rubia peregrina* subsp. *longifolia* (Poiret) O. Bolós, *Helichrysum picardii* Boiss. & Reut., *Asparagus aphyllus* L. and *Daphne gnidium* L. The “Camarinal” of “Monte Algaida” is represented only by *Corema album*. Its recognition is not clear and it could be consider as a mere component of the juniper forest.

The “Lentiscar”

This is a dense shrubby formation up to 4 m height dominated by *Pistacia lentiscus* L., which in Doñana area constitutes a phase of degradation of *Quercus suber* L. forests.

In “Monte Algaida” the “lentiscar” forms a band around the stabilized sands, which extends between the juniper woodland and the scrubland of *Halimium halimifolium* and the *Tamarix*, *Populus alba* and herbaceous communities which occupy the marginal area, in contact with the clay substrate. It is a rich community formed by *Pistacia lentiscus* L., *Phillyrea angustifolia* L., *Rhamnus lycioides* subsp. *oleoides* (L.) Jahandiez & Maire, *Rhamnus alaternus* L., *Myrtus communis* L., *Ruscus aculeatus* L., *Cistus salvifolius* L., *Chamaerops humilis* L., *Aristolochia baetica* L., *Clematis cirrhosa* L., *C. flammula* L., *Daphne gnidium* L., *Rubia peregrina* subsp. *longifolia* (Poiret) O. Bolòs, *Smilax aspera* L., *Tamus communis* L., and in some spots *Cytisus grandiflorus* L. Then, all woody taxa which characterize the “Lentiscar” of Doñana area except *Quercus coccifera* are present in “Monte Algaida”, where its plant composition is closer to those of “Coto del Rey” at the North of the Guadalquivir marshes, than to those at other parts of Doñana area (see Rivas Martínez & al. 1980).

Riparian formations

Riparian forest dominated by *Populus alba* L. are not widespread in Doñana area, where they occupy wet fresh-water area which are rarely over-flowed. Their floristic composition is rather rich and includes trees, shrubs, climber and herbaceous species.

In “Monte Algaida”, this plant formation is reduced to a short and narrow band between the “lentiscar” and the salty marshes and periferal wet areas covered by *Tamarix* formations and herbaceous communities dominated by *Juncaceae*. Consequently, *Populus alba* is accompanied by some species which are characteristic of riparian formations, together with species from the surrounding plant communities.

The main components, including perennial herbs are: *Populus alba*, *Tamarix africana* Poiret, *Rubus ulmifolius* Schott, *Pistacia lentiscus* L., *Myrtus communis* L., *Phillyrea* *angustifolia* L., *Clematis cirrhosa* L., *Smilax aspera* L., *Aristolochia baetica*, *Lonicera periclymenum* L., *Arum italicum* Miller, *Iris pseudacorus* L. and *Bryonia cretica* subsp. *dioica* (Jacq.) Tutin. Four species which characterise these riparian forest in Doñana area are missing at "Monte Algaida": *Fraxinus angustifolia* Vahl, *Ranunculus ficaria*, *Crataegus monogyna* Jacq. and *Tamus communis* L. In a way, it seems as if the "Lentiscar" had invaded the area which should have been occupied by a riparian now most degraded forest.

The Tamarix formations

In Doñana area, *Tamarix* grow on temporary overflowed soils with high clay content and some salinity. In "Monte Algaida" this formation is much degraded, often overgrazed and formed in some places almost exclusively by old and depauperate specimens of *Tamarix africana* Poiret. It covers some marginal places mostly within the area covered by salty marshes. The most constant accompanying species is *Asparagus acutifolius* L., and close to the fresh-water herbaceous formations, *Phragmites australis* (Cav.) Trin.

A Cerinthe gymnandra community

A peculiarity of "Monte Algaida" pine forest is the widespread presence of a plant community dominated by the annual *Cerinthe gymnandra* Gasp. and the geophytes *Arum italicum* Miller and *Narcissus papyraceus* Ker-Gawler. While *Arum italicum* is a common component of riparian vegetation at Donaña area and is also present in the *Quercus suber* forests and often in the wet areas of the "lentiscar" (see Rivas Martínez & al. 1980), *Narcissus papyraceus* is very rare and had only been punctually indicated for some riparian areas, while *Cerinthe gymnandra* had not even been recorded for Doñana area.

At "Monte Algaida" *C. gymnandra* and *Narcissus papyraceus* occur in all open areas of the wide band of "lentiscar" with independence of the edaphic humidity. The associate populations of both species are, however, particularly dense when soil humidity increases, where the presence of *Arum italicum* is more frequent.

The position of this community, which is also formed by other herbaceous species, on soils with a higher or lower proportion of clay may explain the abundance of these three species, particularly *N. papyraceus*.

Phytosociologically, this community should be included in the class *Stellaretea mediae* R. Tx, Lohmeger & Preising in R. Tx. 1950, em. Rivas Martínez 1977 (Synonym: *Rudereto-Secalinetea* Br.-Bl. 1936), in spite of the fact that two of the characteristic species are perennial.

Discussion

Centuries of longstanding human intervention in Doñana area to exploit natural resources, has altered the plant communities to their present composition. Reduction of the original woodlands to profit timber and coal, periodical local clearing by fire to increase pastures, intense agriculture transformation of marshes and stabilized sands and planting of pines and eucalyptus, especially during part of the 20th century are the main factors which have modify native vegetation (Granados & al. 1987, 1988; García Novo 1997; García Murillo & Sousa 1999).

However, from 1964 the protective measures have been implemented in the area with the formation of the Doñana Biological Reserve first, followed by the declarations of Doñana National Park and Doñana Natural Park. Land management has favored natural vegetation recovering. Certainly, the resulting actual plant formations may greatly differ from the original in much of the area. But there are many, sometime extensive, spots where vegetation must represent more closely the original situation, on account of their floristic richness and the singularity of its components.

All woody plant formations of “Monte Algaida” pine forest but the “lentiscar”, when compared with those more representative examples of the same formations at the main part of Doñana area, in Huelva province, show a poor and degraded floristic composition. Many of the most characteristic species are missing, which seems to indicate that plant communities at “Monte Algaida” pine forest are in clear regression owed, most probably, to the lack of new sand input by wind from the coast, as the stabilized sands are now completely separate from the coast by the orchards, farms and building which extend from the forest to the city of Sanlúcar de Barrameda, to the limited extend of the aeolian sands which do not allow a long term retention of rain water, and mainly to anthropic pressure.

Three anthropic actions have had higher incidence in the dynamics and actual composition of “Monte Algaida” woody plant communities: pine plantation, fire and nitrification. Most of the area was planted with *Pinus pinea* by previous clearing of the vegetation. The vegetation has recovered, but the floristic composition of resulting communities greatly differ from the original, if as original are understood the better preserve equivalent formations at the main part of Doñana area at the other side of Guadalquivir river (Huelva province). This may be the reason why the “lentiscar” is the best preserved plant formation, as most of its area is outside the pine forest.

The second factor must have been the fire. Last fire occurred in 1994, when a small area where the last old specimen of *Juniperus oxycedrus* subsp. *macrocarpa* still persisted, was burned. The fact that in this and other spots the “Monte blanco” is formed by only *Halimium halimifolium* indicates fire action, as this is the species of this community better adapted to fire (and also to cutting) by sprouting.

Besides, some spots of “Monte Algaida” are used as recreative areas by people from the neighboring city of Sanlúcar de Barrameda. The progressive nitrification, which is patent in many herbaceous communities not included in this note, must be the response to this land use, and may also be responsible for the wide presence in the area, more than 7 km away from the coast, of *Pancratium maritimum*, which at the coast of Doñana area is especially frequent in nitrified and ruderalized coastal areas, as near Matalascañas tourist resort and wide areas of the beach between Matalascañas and Mazagón intensely used by tourists. Maybe that the widespread presence of the plant community dominated by *Cerinthe gymnandra*, *Narcissus papyraceus* and *Arum italicum* is also a response to the nitrification.

Acknowledgements

This study is a part of the project “Study of the flora and vegetation of Doñana area, Andévalo and Sierra de Huelva”, of the Consejería de Medio Ambiente, Andalusian Government (INTERREG, E.C.).

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A. Zeddam, M. Meurer & Ch. Neff

Impact des activités humaines sur la végétation de la forêt des Senhadja-Gherbès, Skikda, Algérie

Abstract

Zeddam, A., Meurer, M. & Neff, Ch.: Impact des activités humaines sur la végétation de la forêt des Senhadja-Gherbès, Skikda, Algérie. — Bocconea 21: 283-289. 2007. — ISSN 1120-4060.

The authors aimed to highlight the combined effects of fire, grazing and reafforestation on the vegetation of a coastal cork oak forest located in the north-eastern of Algeria. The results show that fire and grazing are the main factors of disturbance in this cork oak forest even fire seems to stimulate the very good and fast recolonization by shrubby species of the maquis such as *Cistus* and *Calicotome*. Intensive bovine grazing characterizes the high ligneous formations with clear or absent underlayer on weak slopes while the plantations with *Eucalyptus* sp.or with *Pinus pinaster* seem to favour the soil erosion as testifiy by an increase of the sand's rates in the upper soil's layers. Moreover, the reafforestation would disturb the re-establishment of the original vegetation especially the shrubby species under pines and *Eucalyptus* although the high percentage of regenerations of *Quercus suber* out of the plantations testifie to the high potentialities in the studied area allowing the return of the oaks.

Introduction

En Algérie septentrionale, la régression de la subéraie est essentiellement due au feu, au pâturage et à la surexploitation du liège (Boudy 1955; Zeraia 1981). Bien que le feu, un phénomène fréquent en région méditerranéenne (Trabaud & al. 1997), soit reconnu comme étant un facteur nécessaire inclus dans le fonctionnement des écosystèmes (Arianoutsou & Margaris 1982) les activités humaines ont altéré les cycles naturels des incendies (Ferrandis & al. 1999). Le pâturage est reconnu pour contrer les régénérations. En 30 ans, près de 50% (Boudy 1955; Bneder 1984) des forêts de *Quercus suber* ont disparu remplacées par des matorrals plus ou moins dégradés, la dégradation atteignant parfois son stade ultime, le sol nu.

Dans le cadre de la lutte contre la déforestation et l'érosion des sols, de nombreux projets de reboisements ont été élaborés. Malheureusement, dans les forêts du Nord-est algérien, les plus arrosées, les tentatives de reboisements par *Eucalyptus* sp. ont échoué, l'échec étant attribué par Thomas (1978) au feu et aux parasites. Les résultats avec *Pinus pinaster* ne sont pas meilleurs. Au contraire, les reboisements, principalement par *Eucalyptus*, semblent perturber le processus naturel de réinstallation de la végétation pré - incendie (Bergmeier 1997).

Réalisée dans le cadre d'un projet de reboisement, cette étude a pour objectif l'analyse des effets conjugués du feu, du pâturage et des reboisements sur la végétation de la forêt des Senhadja-Gherbès, une subéraie littorale localisée dans l'aire phytogéographique et bioclimatique optimale du chêne-liège en Algérie (Maire 1926; Barry & al. 1974; Quézel & Médail 2003). La carte (Zeddam 1989), document plus synthétique que le texte et plus facilement exploitable (Ozenda 1963), établie sur la base des résultats, permettra une intervention plus rapide et plus efficace des aménagistes en ciblant les zones à risques et en limitant ainsi les actions d'intervention.

Description de la zone d'étude

Localisée à l'Est de Skikda (36°50'33" et 36°54'49" Lat. N–7°8'53" et 7°14' Long. E) la zone étudiée (Fig. 1) s'étend sur environ 2500 ha entre 40 m et 560 m d'altitude. La forêt couvre trois communes: Filfila, Djendel et Ben Azzouz.

Le substrat géologique est constitué de grès numidiens. Les expositions dominantes sont NE, E et N. P moy. ann.: 900-1000 mm. Température moy. ann.: 17,20°C. Période estivale sèche: 3 à 4 mois. Bioclimat: subhumide et humide, hiver à variante chaude et tempérée. Etage de végétation: Thermo-méditerranéen.

La végétation est constituée de forêts de *Quercus suber*, de forêts de *Quercus suber* dominant et de *Quercus canariensis**, de forêts de *Quercus canariensis** de faible étendue

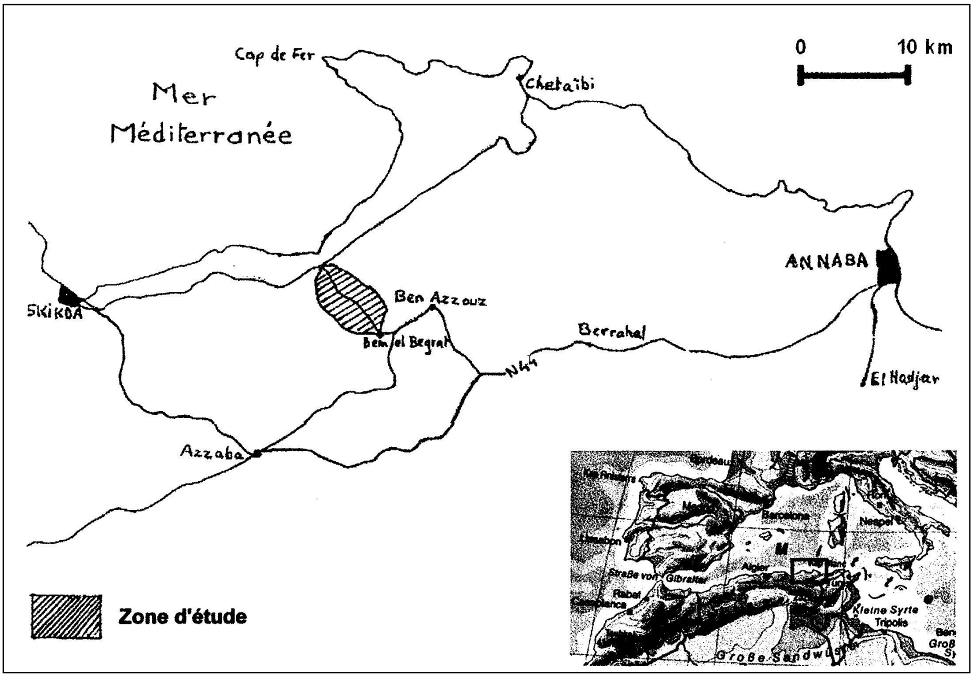

Fig. 1. Localisation de la zone d'étude (encadrée du trait gras).

réfugiées dans les ravins frais et humides. Cependant, plus abondants sont les maquis, avec ou sans *Quercus suber*, à *Phillyrea angustifolia* subsp. *media*, *Olea europaea* var. *oleaster*, *Pistacia lentiscus*, *Erica arborea*, *Arbutus unedo*, *Quercus coccifera*, *Myrtus communis*, *Lavandula stoechas* etc. A côté de la végétation naturelle, des plantations anciennes abandonnées d'*Eucalyptus* sp. ou de *Pinus pinaster* sont envahies par les espèces de maquis. Très affectée par les incendies, la zone en a connu un en 1983 qui a causé de grands dommages.

Spectre biologique (établi à partir des 50 relevés de végétation)

Phanérophytes: 25,5%, Chaméphytes: 6%, Hémicryptophytes: 24,5%, Géophytes: 4,5%, Thérophytes: 39,5%.

Le pourcentage relativement élevé de thérophytes caractérise le climat méditerranéen (Daget & al. 1977) alors que le faible taux de chaméphytes peut s'expliquer par l'impact du couvert des phanérophytes (Danin & Orshan 1990) ou peut-être une humidité élevée car selon ces auteurs les chaméphytes sont liés à la sécheresse et à la lumière. Le taux élevé d'hémicryptophytes serait d'une part lié aux précipitations annuelles élevées (Danin & Orshan 1990) mais également à un milieu perturbé par le pâturage (Debussche & al. 2001) ce qui expliquerait le taux des géophytes que Médail (1996) associe à un environnement forestier relativement peu ou pas perturbé.

Méthodes

La carte des groupements végétaux, unités synthétiques homogènes, fait ressortir les relations spatiales et temporelles entre:

- les types de végétation définis par des unités physionomiques et floristiques,
- les types d'environnement définis par les facteurs écologiques prépondérants (Long 1974),
- 35 photographies aériennes (au 1/10 000^{e}) ont été analysées pour définir les différents types de végétation, lesquels sont identifiés par des critères physionomiques, floristiques et d'environnement (Gagnon, 1974).

Sur le terrain, lors de la réalisation des relevés, une limite entre deux strates est maintenue lorsqu'elle coïncide avec une discontinuité physionomique et/ou floristique. Ainsi, les limites théoriques des unités délimitées sur le plan d'échantillonnage sont maintenues, effacées ou corrigées. Réalisé sur une aire minimale de 100 m2, chaque relevé comporte:

- une liste exhaustive des espèces (la nomenclature utilisée ici est celle de la flore de Quézel et Santa, 1962 – 1963 actualisée pour certaines espèces),
- un relevé de sol,
- les caractères de la végétation et du milieu: pente, altitude, exposition, recouvrement des différentes strates, les trois espèces dominantes, le type de végétation (*sensu* Ionesco & Sauvage, 1962) et le nombre de régénérations de *Quercus suber*,

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[*] *Quercus canariensis* a été reconnu comme le chêne caducifolié le plus répandu en Afrique du Nord en situations littorale et sublittoral et il est considéré comme une espèce à part différente de *Quercus faginea* qui se retrouve en petits îlots isolés dans des stations éloignées de la mer. (Quézel & Médail 2003, p. 153). La confusion entre les deux est due à l'extrême polymorphie des deux espèces.

- une estimation de l'intensité des incendies et du pâturage (troncs d'arbres ou de buissons brûlés, excréments de bovins ou d'ovins).

Le traitement des données floristiques (50 relevés – 123 espèces en présence - absence de fréquence supérieure à 2) a été réalisé par l'analyse factorielle des correspondances (AFC).

Compte tenu des exigences édaphiques du chêne-liège, les analyses de sol ont porté sur la granulométrie et la matière organique. La texture étant "un facteur écologique essentiel dans la répartition des groupements à chêne-liège" (Sauvage 1960) alors que la matière organique "augmente la résistance mécanique des agrégats" (Tricard & Kilian 1979).

Les résultats de l'AFC montrent des ensembles de points - relevés et de points - espèces distribués le long des axes factoriels et correspondant aux groupements végétaux qui sont "des unités concrètes identifiables et cartographiables sur le terrain" (Long 1974).

La signification écologique des axes factoriels est basée sur les plus fortes valeurs de contribution des points - relevés. Elle permet de suivre les gradients de facteurs réellement actifs le long de chaque axe. Les trois premiers axes sont retenus pour leur forte contribution et leur signification écologique.

La caractérisation floristique des groupements est basée sur les espèces à forte contribution et complétée par un tableau synoptique.

La représentation cartographique des unités phytoécologiques ou groupements végétaux déterminés par l'AFC est basée sur:

- les limites des unités physionomiques représentées sur la carte de l'occupation des terres,
- la localisation des relevés,
- sur les seuils de variations des facteurs écologiques réellement actifs sous-tendus par les axes factoriels,
- les caractéristiques de milieu accompagnant chaque relevé et donc chaque groupement végétal.

Résultats

L'AFC a permis de déterminer sept groupements végétaux. Leur répartition sur les deux plans factoriels (Fig. 2) met en évidence les gradients écologiques sous-tendus par les 3 premiers axes factoriels.

Ainsi, l'axe 1, le plus informatif, exprime un gradient d'humidité inverse d'un gradient d'intensité des incendies. Au côté négatif sont localisées les stations les plus sèches ou les moins humides (reboisements anciens d'*Eucalyptus* sp. et *Pinus pinaster*, à *Quercus coccifera* et *Genista ulicina* ainsi que des matorrals à *Cistus monspeliensis* et *Calicotome spinosa*: groupements I et II) à flore héliophile, thermoxérophile et indicatrice d'incendies et/ou de pâturage et/ou de milieu ouvert (Benabid 1976; Aimé 1976; Arianoutsou & Margaris 1982; Meurer & al. 1986; Fennane 1987; Meurer 1988; Neff 1995; Bergmeir 1997; Ferrandis & al. 1999) avec des espèces telles que *Cistus salviifolius*, *Genista tricuspidata*, *Calicotome spinosa*, *Lavandula stoechas*, *Ampelodesmos mauritanica*, *Genista ulicina*, *Quercus coccifera*, *Cistus monspeliensis*, *Trifolium campestre*, *Aira caryophyllea*, *Asphodelus microcarpus*.

Au côté positif on trouve les stations les plus humides (groupements V, VI et VII) caractérisées par des sols profonds et une flore forestière, humifère (Quézel et Santa 1962;

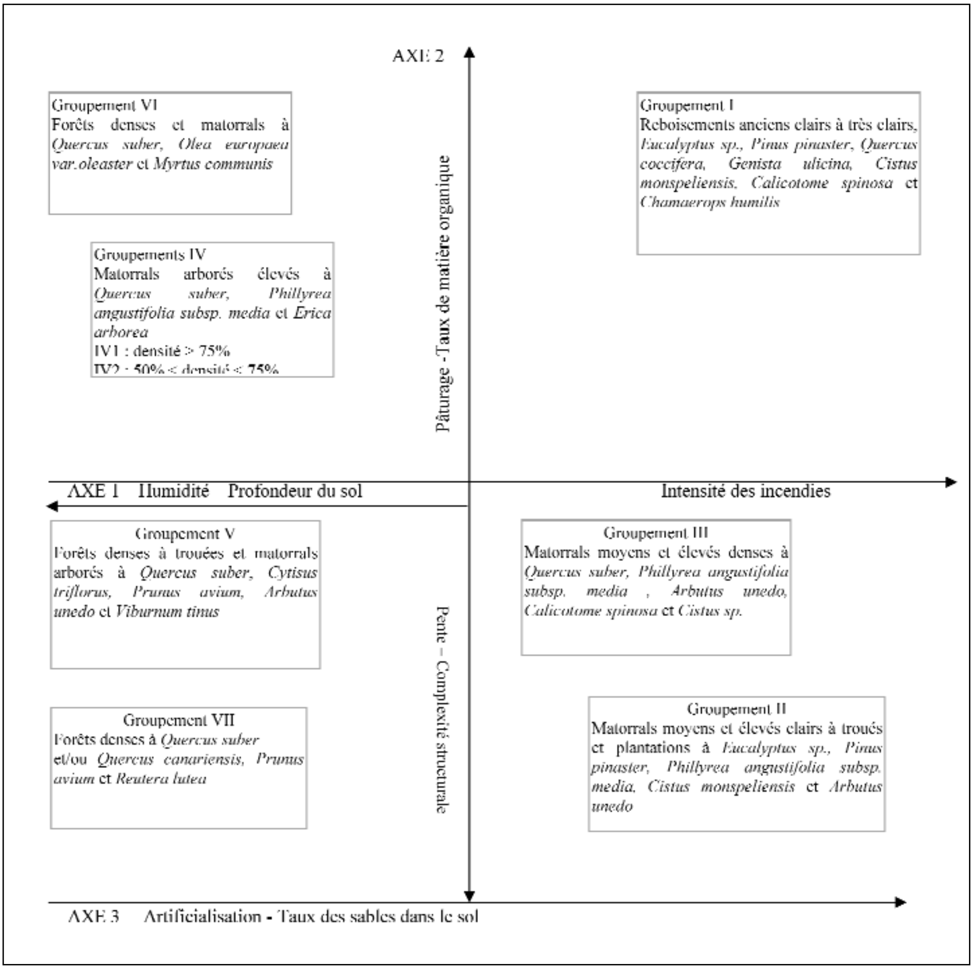

Fig. 2. Organisation des groupements végétaux le long des gradients de facteurs écologiques.

Zeraia 1981; Aimé 1976; Fennane 1987; Médail 1996) voire hygrophile avec C*ytisus triflorus*, *Prunus avium*, *Viburnum tinus*, *Arbutus unedo*, *Tamus communis*, *Hedera helix*, *Reutera lutea*, *Laurus nobilis*, *Carex halleriana*, *Ruscus hypophyllum*, *Quercus canariensis*, *Pteridium aquilinum*.

Cependant, la présence ou l'abondance de ces espèces n'excluent pas la présence de quelques espèces indicatrices de milieu perturbé par le feu ou le pâturage. Les groupements de transition III et IV confirment les gradients inverses d'humidité et d'intensité des incendies.

Au côté négatif de l'axe 2 se localisent les formations à strates ligneuses basses et denses sur pente forte alors qu'au côté positif se localisent les formations ligneuses hautes à sous-bois clair ou absent, sur pente faible et caractérisée par une strate herbacée riche en nitrophiles car soumises à un pâturage intense, essentiellement bovin.

L’axe 3 exprime un gradient d’artificialisation (reboisements) conjugué à la richesse en sables du sol, conséquence probable de la réduction voire l’absence de la strate arbustive sous *Eucalyptus* ou *Pinus pinaster*. La végétation herbacée de substitution ne peut protéger le sol lequel est soumis dans cette région à une érosion hydrique intense.

Discussion et Perspectives

Dans cette subéraie de basse altitude il apparaît que les incendies et le pâturage sont les facteurs prépondérants dans la dégradation des forêts de chêne-liège progressivement remplacées par les formations arbustives basses caractéristiques des paysages méditerranéens (Bond 2000). Par ailleurs, dans ces régions très arrosées du Nord-est algérien, les reboisements avec *Eucalyptus* ou *Pinus pinaster*, ou encore *Pinus halepensis* en exposition sud, semblent représenter un facteur d’érosion du sol et perturber le processus de réinstallation de la végétation originelle. Ces résultats suggèrent que pour un reboisement efficace, il est conseillé un choix d’espèces autochtones s’intégrant à, et/ou assurant la transition vers la végétation potentielle, laquelle présente des capacités de régénération évidentes bien que contrées par le pâturage et les incendies. Meurer (1988) met en évidence la considérable capacité de régénération des espèces autochtones particulièrement dans les surfaces non exposées au pâturage.

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G. Venturella, A. Bernicchia & A. Saitta

Contribution to the knowledge of diversity and distribution of lignicolous fungi from Sicily (southern Italy)

Abstract

Venturella, G., Bernicchia, A. & Saitta, A.: Contribution to the knowledge of diversity and distribution of lignicolous fungi from Sicily (southern Italy). — Bocconea 21: 291-295. 2007. — ISSN 1120-4060.

Ecological and distributional data on eleven lignicolous fungi, such as *Antrodia ramentacea, Ceriporiopsis mucida, Ceriporia viridans, Eichleriella deglubens, Kavinia himantia, Oxyporus latemarginatus, Phanerochaete sordida, P. velutina, Phellinus erectus, P. rimosus* and *Phylloporia ribis* are here reported.

Introduction

The knowledge on diversity and distribution of lignicolous fungi in the Mediterranean area are still inadequate if compared with data arising from other European countries. The literature data on habitats, substrata and host plants for lignicolous fungi are mainly referred to northern and central European countries. Few data arises from Mediterranean ecosystems, particularly as the plants and small shrubs of Mediterranean maquis are concerned. In order to fill the gap of knowledge on such interesting group of fungi, a specific part of the project for the assessment of fungal biodiversity in Sicily (southern Italy) has been devoted to the census of lignicolous fungi and the acquirement of ecological data.

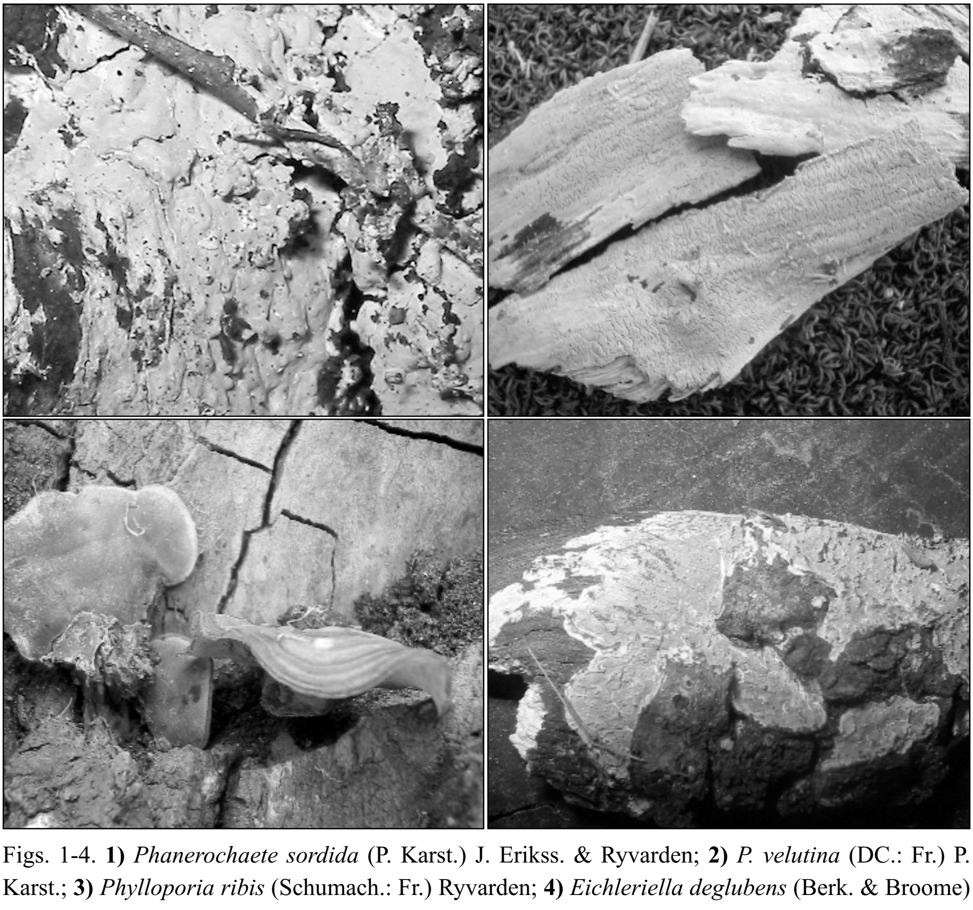
Materials and methods

Periodical observation on the occurrence of lignicolous fungi in the Sicilian territory were carried out in the last ten years. Basidiomata of lignicolous fungi were collected in broadleaved and conifer forest ecosystems of Sicily. The collected fungi were identified in laboratory on fresh and dried materials using a Leica microscope. The scientific binomials were mostly obtained using Bernicchia (1990) for *Polyporaceae*; Eriksson & Ryvarden (1976) and Eriksson & al. (1978) for species belonging to the family *Corticiaceae*. The herbarium samples are kept in the *Herbarium Mediterraneum Panormitanum* (PAL) in Palermo.

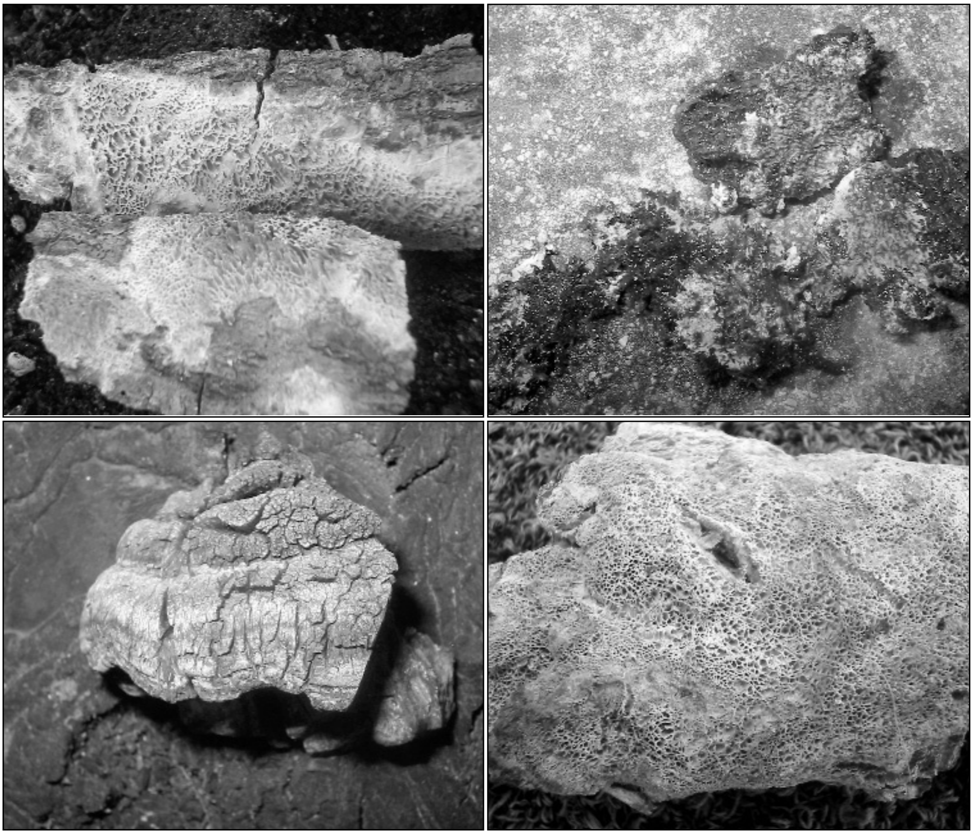
Results

Following up the survey of Saitta & al. (2004) that reported from the Sicilian territory 209 woody taxa (181 *Basidiomycetes* and 28 *Ascomycetes*), additional data on the distribution and ecology of 11 *Aphyllophorales* are here pointed out as new records for Sicily.

The saprotrophs *Phanerochaete sordida* (P. Karst.) J. Erikss. & Ryvarden (Fig. 1) and *P. velutina* (DC.: Fr.) P. Karst. (Fig. 2) are currently reported in literature on deciduous and conifer woods. *P. sordida* is very common in Northern Europe (Bernicchia 1990) and it is considered as one of the most widespread species of the family *Corticiaceae*. *P. velutina* is included in the list of common species in Scandinavia, Finland and Norway while it is reported as infrequent in the rest of Europe (Bernicchia 1990). The distribution of *P. sordida* and *P. velutina* in Italy, previously limited to scattered localities of some Italian regions, is now widened to Sicily where the two species were collected on small branches of *Quercus ilex* L.



Figs. 1-4. **1)** *Phanerochaete sordida* (P. Karst.) J. Erikss. & Ryvarden; **2)** *P. velutina* (DC.: Fr.) P. Karst.; **3)** *Phylloporia ribis* (Schumach.: Fr.) Ryvarden; **4)** *Eichleriella deglubens* (Berk. & Broome) Lloyd.

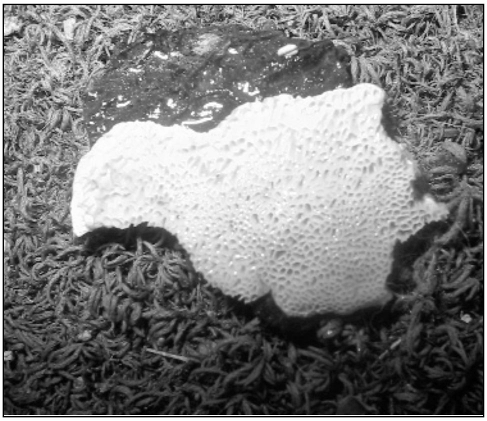


Figs. 5-8. **5)** *Ceriporiopsis mucida* (Pers.: Fr.) Ryvarden & Gilbertson; **6)** *Kavinia himantia* (Schwein) J. Eriksson; **7)** *Phellinus rimosus* (Berk.) Pilát; **8)** *Oxyporus latemarginatus* (Durieu & Mont.) Donk.

Phylloporia ribis (Schumach.: Fr.) Ryvarden (Fig. 3) is an infrequent necrotroph parasitic species collected in Sicily on stumps or at the base of plants of *Quercus ilex*. It is frequently misidentified as *Phellinus torulosus* (Pers.) Bourd. & Galz., but it differs from the latter for the absence of setae in the hymenial layer.

Eichleriella deglubens (Berk. & Broome) Lloyd (Fig. 4) is a very rare saprotroph recorded on trunks of *Quercus ilex*. Its distribution in Italy is limited to scattered localities of few regions, i.e. Sardinia, Trentino Alto Adige and Veneto (Bernicchia 1990; Onofri & al. 2003). The new finding from the Sicilian territory widens southwards the distributive area of such interesting species.

Ceriporiopsis mucida (Pers.: Fr.) Ryvarden & Gilbertson (Fig. 5), is a widespread lignicolous saprotroph growing on rotten woods of conifers and broad-leaved plants. In Sicily it was collected on *Q. ilex* logs, but also on strobiles of *Pinus halepensis* Miller, an unusual substratum not previously reported in literature.



Figs. 9-11. **9)** *Antrodia ramentacea* (Berk. & Broome) Donk; **10)** *Ceriporia viridans* (Berk. & Broome) Donk.; **11)** *Phellinus erectus* A. David, Dequatre & Fiasson.

Kavinia himantia (Schwein) J. Eriksson (Fig. 6) is a saprotroph of the *Corticiaceae* family, infrequent in Europe and rare in the Sicilian territory where it was collected on stumps of *Q. ilex* and *Fagus sylvatica* L.

Phellinus rimosus (Berk.) Pilát (Fig. 7) is widely distributed in the temperate zones but very rare in Italy. It was previously collected only in Sardinia (Bernicchia 1990) at the base of living plants of *Pistacia lentiscus* L. In Sicily it was recently collected at the base of the trunk of some plants of *Quercus pubescens* Willd.

Oxyporus latemarginatus (Durieu & Mont.) Donk (Fig. 8) is an infrequent saprophytic species in Europe. In many Italian regions it was collected on different substrata and plants (Bernicchia 1990). In Sicily it was recorded for the first time on branches of *Quercus suber* L. and *P. halepensis*, two new substrata never reported in literature.

Antrodia ramentacea (Berk. & Broome) Donk (Fig. 9) is a saprophyte which grows preferentially on fallen trunks of conifers. It is widely distributed in Italy but infrequent in Sicily where it was collected in a mixed conifer wood of *Pinus halepensis*, *P. pinea* L., *Cupressus sempervirens* L. and *C. arizonica* Greene.

Ceriporia viridans (Berk. & Broome) Donk (Fig. 10) is an infrequent species not easy to identify since the spore shapes are very variable. It was collected for the first time in

Sicily, on fallen trunks of *Eucalyptus camaldulensis* Dehnh. In the same vegetational type also *Phellinus erectus* A. David, Dequatre & Fiasson (Fig. 11), a very rare species in Europe and previously reported in Italy only from Sardinia (Bernicchia 1990), was collected at the base of living plants of *E. camaldulensis*.

Conclusions

In Sicily the assessment of lignicolous fungi diversity is still in progress and new distributive and ecological data are frequently available with the finding of unrecorded taxa. A huge number of species were collected in *Quercus ilex* woods, from 0 to 1550 m asl, while others were found on different substrata in broad-leaved or conifer woods. The use of plants for timber and charcoal by the local populations, the high level of exploitation of woods and the type of management by the Regional Forestry Administration produced a large amount of logs, branches and stumps which remains for a long time in the underwood as ideal substratum for lignicolous fungi. The eleven taxa reported in this survey are infrequent or rare at regional, national and European level and are suitable for further investigation on their ecology, chorology and role in forest ecosystems of the Mediterranean area.

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A. Mijović, Z. Popović, D. Mišić & B. Karadžić

Estimation on photosynthetic efficiency in three populations of *Nepeta rtanjensis* Diklić & Milojević

Abstract

Mijović, A., Popović, Z., Mišić, D. & Karadžić, B.: Estimation on photosynthetic efficiency in three populations of *Nepeta rtanjensis* Diklić & Milojević. — Bocconea 21: 297-301. 2007. — ISSN 1120-4060.

The causes of limited number and distribution of critically endangered *Nepeta rtanjensis* were investigated in the sense of ecophysiological status of this species. Three populations of *N. rtanjensis* were subjected to analysis of photosynthetic efficiency: natural population (growing at Rtanj Mt.), re-introduced population (the population established by planting of seedlings near the natural site) and the cultivated population (growing in controlled conditions). The seedlings for planting were obtained by the method of in vitro micropropagation and pre-adapted for ex vitro planting. The aim of this study was to achieve data on ecophysiological adaptation of in vitro propagated seedlings. Results showed that the favorable photosynthetic efficiency of these plants was related to their successful adaptation at natural site. Such adaptive response is in favor of further propagation and re-introduction of this rare species.

Introduction

Nepeta rtanjensis Diklić & Milojević (fam. *Lamiaceae*) is a rare endemorelict species with limited distribution at Rtanj Mt. (SE Serbia). Significant distance and distinctness of this species' areal related to the distributional center of Mediterranean complex of *Nepeta sibthorpii* points out to its relict character. It is preserved only on few places, on the open calcareous stony grounds in the zone of the oak forest ass. *Quercetum frainetto-cerridis* Rudski (Tomić 2006), at 650-850 m asl. According to IUCN categorization *N. rtanjensis* is critically endangered (CR B_{2c}) species in Serbia (Diklić 1999). The number of individuals of *N. rtanjensis* at Rtanj Mt. is about 500-700. It has been postulated that the fungal infection is the main cause that affects adversely upon the seed viability (Rančić & al. 2002; Ljaljević-Grbić 2006). *N. rtanjensis*, like the majority of *Nepeta* species, is a plant with some medical properties. Nepetalactone is the main component of essential oil of this species (Chalchar & al. 2000; Mišić 2003), so the individuals of this species may be produced in pharmacological purposes. The cultivation of *N. rtanjensis* could be a way to prevent its further disappearing because of uncontrolled gathering in natural sites. The ecophysiological investigations presented in this study were the part of the greater experiment performed in order to evaluate the establishment of planted individuals of *N. rtanjensis*.

Material and methods

The estimation of photosynthetic efficiency was performed on three populations of *N. rtanjensis*. The natural population growing at limited area at Rtanj Mt. was observed to investigate the ecophysiological status of this plant in natural habitat (with extreme high temperatures and irradiation, and low humidity). A number of individuals of *N. rtanjensis* that were obtained in the micropropagation process (Mišić 2003) represented an initial material establishing other two populations. These plants were growing in laboratory conditions for 6 to 12 months, pre-adapted to *ex vitro* conditions in glass house and then they were planted on the garden plots in March 2004. A thousand individuals from garden plots were planted at Rtanj Mt. in April 2004. The site for planting was open, calcareous and similar to original species' site. This group of individuals was considered as 're-introduced' and observed in order to evaluate its adaptive response. The third population was planted at experimental plots at the Institute for Biological Research in Belgrade, where they were subjected to regular watering. This 'cultivated' population was considered as control. Photosynthetic efficiency of reintroduced and cultivated plants has been evaluated based on the chlorophyll fluorescence, using an induction fluorometer (Plant Stress Meter, Umea, Sweden). Photosynthetic efficiency is known to be one of the most sensitive processes and it can be inhibited by high temperature and intensive irradiation, before other symptoms of stress are detected (Krause & Weis 1984; Öquist & Wass 1988; Scheriber & al. 1994).

All measurements in natural site were performed on a sunny summer day, July 1st, 2004 (at 32ºC). Individuals from garden plots were measured on July 4th (at 30ºC). All plants were dark adapted for at least 20 min before measuring. The actinic photon flux density was 200 μmol $m^{-2}s^{-1}$. Data analysis was carried out using the analytical tool of the Statistica for Windows program package. All correlation coefficients r are Pearson coefficients for all correlations. Relationships are called significant if $p < 0.05$. Means of F_v/F_m and $t_{1/2}$ were compared using t-test.

Results and discussion

In the present study, we monitored the photosynthetic efficiency of planted individuals of *N. rtanjensis*, in order to evaluate their establishment through the ecophysiological aspect. Measuring of chlorophyll fluorescence during the actual exposure to direct sunlight in the summer period allowed a direct determination of the degree of environmental stress experienced by individuals of this sun-adapted species. A high percentage of light absorbed in PS II antennae that is utilized in PS II photochemistry corresponds to a high level of excess light, and the ratio of variable fluorescence and maximal fluorescence (F_v/F_m) in photoinhibited, intact leaves correlates very well with the quantum yield of net photosynthesis (Ögren 1988). It was documented that decrease in fluorescence parameters (F_v/F_m) from optimal values (0.849, Björkman & Demming 1987) implies decreased photochemical conversion efficiency of PS II, which could be considered as an indicator of the stress conditions.

On the basis of differences in environmental conditions that planted individuals have experienced on garden plots (‘control’) and on the natural site (‘re-introduced’), it was possible to expect differences between them related to functioning of PS II. Contrary to the expectation, we haven’t found a significant difference between all investigated groups according to values of F_v/F_m, as shown of Figure 1. However, values for half rise time were significantly higher for plants from natural population, in comparison with reintroduced individuals on the same site ($t = 2.12$, $p = 0.049$), and in comparison with individuals that were cultivated on garden plots ($t = 2.77$, $p = 0.011$). In natural population $t_{1/2}$ had the highest mean value (437.78±187.77 ms), and only in this group we found a significant relation ($r = -0.75$) between the suppression of photosynthetic efficiency and prolonged half rise time (Fig. 2). Mean value for $t_{1/2}$ in reintroduced individuals was 301.10±75.78 ms, and the lowest values were registered in cultivated group of plants 280.50±81.99 ms.

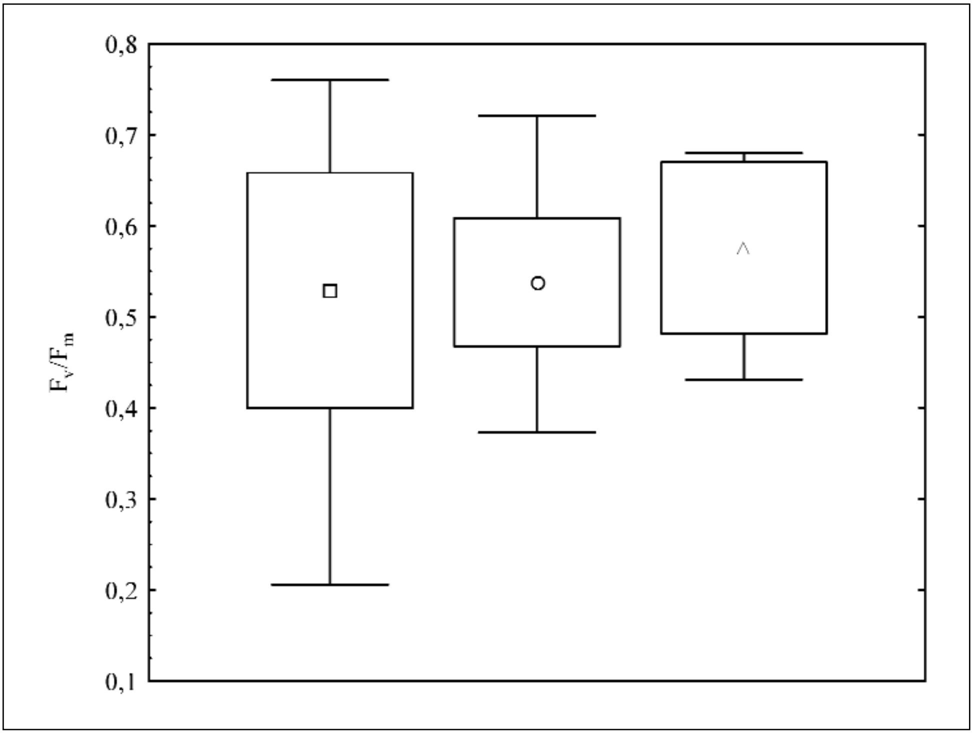

Fig. 1: The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) for individuals from natural population (square symbol), reintroduced (circle) and cultivated individuals (triangle). Means±S.D.

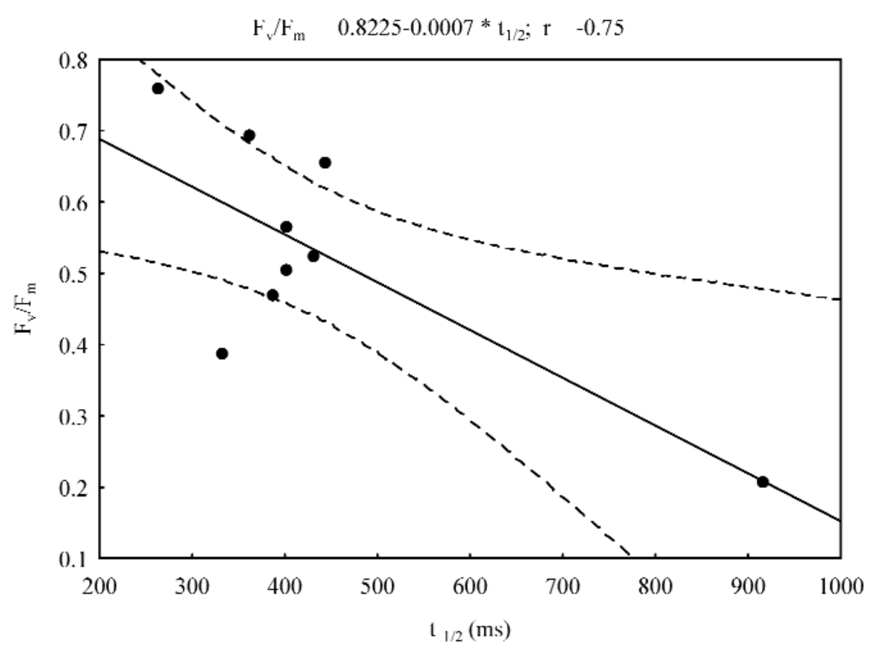

Fig. 2: The relationship between the photosynthetic efficiency (F_v/F_m) and half-rise time ($t_{1/2}$) of individuals from natural population (F_m/F_v = 0.225-0.0007* $t_{1/2}$; r = -0.75).

On the basis of measured parameters of photosynthetic efficiency we could conclude that plant material obtained in micropropagation process is a good source for planting with the purpose of reintroduction. In addition, such similarity in values for F_v/F_m could be a good predictor for successful adaptation of planted individuals, which was proved with a remarkable percent of established individuals in reintroduction experiment (> 99%, Mijović & al. 2005).

Conclusion

An analysis of photosynthetic efficiency showed that the individuals of *N. rtanjensis* obtained in the micropropagation process appear as suitable for planting at the natural habitat and for the cultivation purposes as well. Moreover, we haven't found a significant difference between all investigated groups according to the ratio of variable and maximum fluorescence (F_v/F_m). According to our estimating, an endangered status of this species is not a consequent of decreased photosynthetic efficiency, i.e. low photosynthetic capacity in the means of plant productivity.

Acknowledgments

This work was supported by Ministry of Science and Environmental Protection of Serbia, Grant No #143025.

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G. Aquaro, L. Peruzzi & G. Cesca

Chromosome numbers of 20 flowering plants from ex-Yugoslav countries

Abstract

Aquaro, G., Peruzzi, L. & Cesca, G.: Chromosome numbers of 20 flowering plants from ex-Yugoslav countries. — Bocconea 21: 303-312. 2007. — ISSN 1120-4060.

Twenty angiosperms were collected from several Croatian and Bosnian localities, aiming to make karyological studies. The results are reported in the present work: *Allium commutatum* Guss., $2n = 16$; *A. roseum* L., $2n = 32+0\text{-}2B$; A*quilegia kitaibelii* Schott, $2n = 34$; *Artemisia alba* Turra s. l., $2n = 18$; *Colchicum autumnale* L., $2n = 36$; *Doronicum columnae* Ten., $2n = 60$; *Euphorbia gregersenii* K. Maly ex G. Beck, $2n = 14$; *E. montenegrina* (Bald.) Rohlena, $2n = 22$; *Fritillaria montana* Hoppe, $2n = 18+2B$; *Galanthus nivalis* L., 2n = 24; *Moltkia petraea* (Tratt.) Griseb., $2n = 16$; *Narcissus tazetta* L. subsp. *tazetta*, $2n = 20$; *Ornithogalum comosum* L., $2n = 18$, *O. televrinum* Speta, $2n = 72$; *Plantago media* L. s. l., $2n = 12, 24$; *P. reniformis* G. Beck, $2n = 12$; *P. serpentina* All., $2n = 12$; *Potentilla erecta* (L.) Räuschel, $2n = 28$; *Ranunculus fontanus* J. et C. Presl, $2n = 40$; *Silene armeria* L., $2n = 24$. For *Euphorbia montenegrina* is reported the first counting, while data for *Allium roseum, Aquilegia kitaibelii* and *Ranunculus fontanus* do not agree with those resulting in literature from other provenances.

Introduction

In July 1990 one of the authors (Cesca) collected in Dalmatia several bulbs of *A. commutatum* and *Narcissus tazetta* subsp. *tazetta*; subsequently, during the Third International Balkan Botanical Congress (May 2003, Sarajevo, Bosnia & Hercegovina), Peruzzi collected other live plants from several Croatian and Bosnian localities, aiming to make karyological studies. The results are reported in the present work.

Material and methods

The living plants (Tab. 1), herbarium vouchers and/or photographic documentation of the studied plants are conserved at CLU.

Chromosome analysis

The squash preparations were made from root tips or young ovules, according to the following schedule: pretreatment in 0,5% colchicine solution for 4 hours; Carnoy fixing for at least 1 hour; hydrolysis in HCl 1 N for 7 minutes at 60°C; staining with leuco-basic

Fig. 1. The collection localities. Bosnia and Hercegovina: 1 Zavidovići, 2 Vranduk (serpentines), 3 Zvijezda Mt., 4 Trebević (hill near Sarajevo), 5 Igman Mt., 6 Jahorina Mt. (top), 8 Neretva r.: between Jablanica and Grabovica; Croatia (Dalmatia): 7 Aržano, 9 Omiš, 10 Biokovo, 11 Korčula island: Lukovac islet, Obljak islet.

fuchsine for 3 hours. Chromosomes were observed often under Phase Contrast. Karyotype formulas and chromosome terminology are used according to Levan & al. (1964). At least five plates were measured in order to build the idiograms.

Results and Discussion

Allium commutatum (*Alliaceae*) – 2*n* = 16 (Fig. 2).

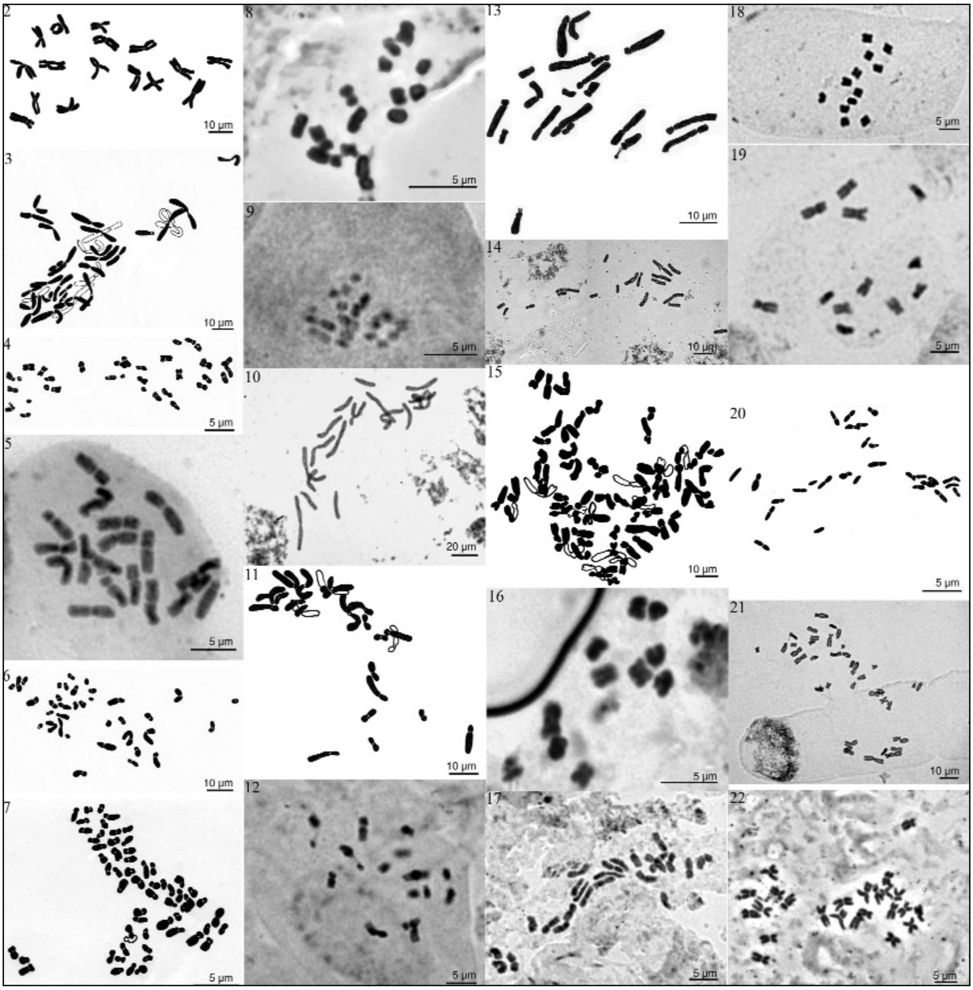
Several samplings, collected at different distances from the sea, did not revealed any relevant karyotype variation.

Previous data: 2*n* = 16 from Italy (Garbari & Cela Renzoni 1974; Brullo & al. 1997); 2*n* = 16, 24 from France (Guern & al. 1991); 2*n* = 16, 24, 32 (Bothmer 1982);

$2n$ = 24+1B from Italy (Marcucci & Tornadore 1997); $2n$ = 32 from Greece (Karavokyrou & Tzanoudakis 1991).

Table 1. Source of the studied plant materials and their chromosome numbers.

Taxon	Provenance	Hort. Bot. Calabria University Accession numbers	Chromosome number
Allium commutatum Guss.	Lukovac	18-m, 127-m, 152-m, 160-m, 240-m, 345-m, 361-m, 379-m, 473-m, 571-m, 642-m; Fig. 1, star 11	$2n = 16$
A. roseum L.	Omiš	132-1; Fig. 1, star 9	$2n = 32+0\text{-}2B$
Aquilegia kitaibelii Schott	Zavidovići	109-1; Fig. 1, star 1	$2n = 34$
Artemisia alba Turra s. l. (incl. *A. alba* subsp. *chitachensis* Maire)	Jablanica - Grabovica	687-m; Fig. 1, star 8	$2n = 18$
Colchicum autumnale L.	Igman	105-1; Fig. 1, star 5	$2n = 36$
Doronicum columnae Ten.	Jahorina	n. 1-s; Fig. 1, star 6	$2n = 60$
Euphorbia gregersenii K. Maly ex G. Beck	Zavidovići	531-m; Fig. 1, star 1	$2n = 14$
E. montenegrina (Bald.) Rohlena	Zavidovići	5-m; Fig. 1, star 1	$2n = 22$
Fritillaria montana Hoppe	Aržano	146-2; Fig. 1, star 7	$2n = 18+2B$
Galanthus nivalis L.	Igman	129-1; Fig. 1, star 5	$2n = 24$
Moltkia petraea (Tratt.) Griseb.	Jablanica - Grabovica	560-m; Fig. 1, star 8	$2n = 16$
Narcissus tazetta L. subsp. *tazetta*	Obljak	559-1; Fig. 1, star 11	$2n = 20$
Ornithogalum comosum L.	Aržano	138-s; Fig. 1 – star 7	$2n = 18$
O. televrinum Speta	Aržano	142-s; Fig. 1, star 7	$2n = 72$
	Biokovo	335-s; Fig. 1, star 10	
Plantago media L. s. l.	Trebević	583-3; Fig. 1, star 4	$2n = 24$
	Aržano	358-3; Fig. 1, star 7	$2n = 12$
P. reniformis G. Beck	Jahorina	n. 35-3, 495-3; Fig. 1, star 6	$2n = 12$
P. serpentina All.	Aržano	104-3; Fig. 1 – star 7	$2n = 12$
Potentilla erecta (L.) Räuschel	Zvijezda	135-f; Fig. 1, star 3	$2n = 28$
Ranunculus fontanus J. et C. Presl	Zvijezda	39-f; Fig. 1, star 3	$2n = 40$
Silene armeria L.	Vranduk	n. 530-3; Fig. 1, star 2	$2n = 24$



Figs. 2-22. Drawings: *Allium commutatum* 2*n* = 16 (2), *A. roseum* 2*n* = 32+2B (3), *Aquilegia kitaibelii* 2*n* = 34 (4), *Colchicum autumnale* 2*n* = 36 (6), *Doronicum columnae* 2*n* = 60 (7), *Galanthus nivalis* 2*n* = 24 (11), *Narcissus tazetta* subsp. *tazetta* 2*n* = 20 (13), *Ornithogalum televrinum* 2*n* = 72 (15), *Potentilla erecta* 2*n* = 28 (20). Microphotographs: *Artemisia alba* s. l. 2*n* = 18 (5), *Euphorbia gregersenii* 2*n* = 14 (8), *E. montenegrina* 2*n* = 22 (9), *Fritillaria montana* 2*n* = 18+2B (10), *Moltkia petraea* 2*n* = 16 (12), *Ornithogalum comosum* 2*n* = 18 (14), *Plantago media* from Croatia 2*n* = 12 (16), *P. media* from Bosnia & Hercegovina 2*n* = 24 (17), *P. reniformis* 2*n* = 12 (18), *P. serpentina* 2*n* = 12 (19), *Ranunculus fontanus* 2*n* = 40 (21), *Silene armeria* 2*n* = 24 (22).

A*. *roseum (*Alliaceae*) – 2*n* = 32 + 0-2B (Fig. 3).

Previous data: 2*n* = 16 from Italy (Marcucci & al. 1992); 2*n* = 16, 24, 32, 40, 48 also from Italy (Marcucci & Tornadore 1997a); 2*n* = 16 (from Montenegro), 32, 48 (from Croatia) and 40 (from Slovenia) (Lovka 1995); 2*n* = 16, 24 from Egypt (Hamoud & al.

1990); 2*n* = 24 from Italy (Messeri 1930, 1931); 2*n* = 32 from Spain (Castroviejo & Feliner 1986) and Greece (Tzanoudakis & Vosa 1988); 2*n* = 40 from Turkey (Özhatay 1990).

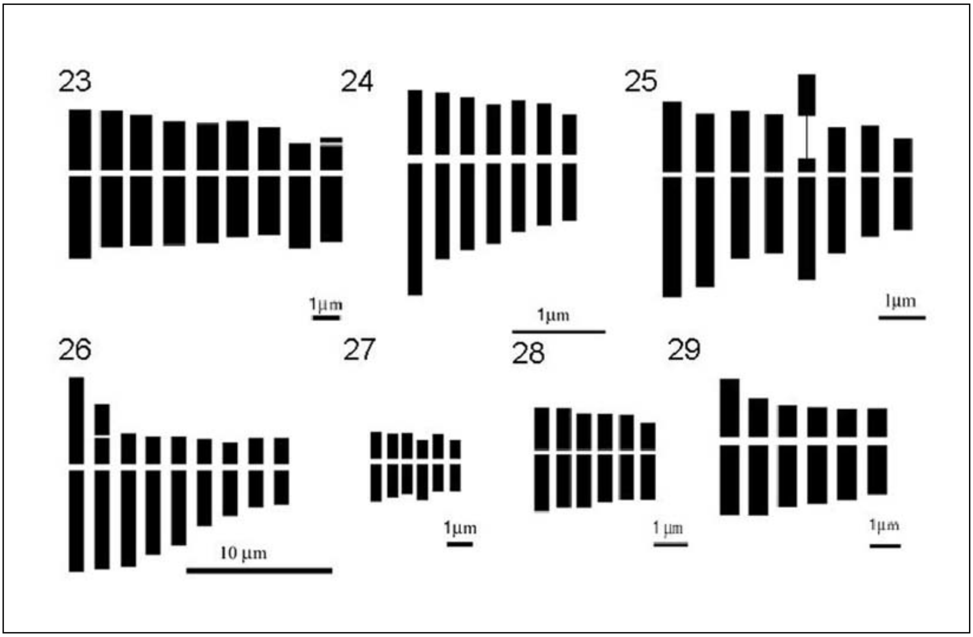
Aquilegia kitaibelii (*Ranunculaceae*) – 2*n* = 34 (Fig. 4).
Previous data: 2*n* = 14 from Croatia (Skalińska 1964).

Artemisia alba s. l. (*Asteraceae*) – 2*n* = 18 (Fig. 5, 23).
Previous data: 2*n* = 18+0-1B, 36+0-4B, 54 (Peruzzi & al. 2005 and literature cited therein).

Colchicum autumnale (*Colchicaceae*) – 2*n* = 36 (Fig. 6).
Previous data: 2*n* = 36 (Sveshnikova & Krichphalushij 1985; Krichphalushi 1989; Dobeš & Hahn 1997); 2*n* = 38 from Italy (D'Amato 1955).

Doronicum columnae (*Asteraceae*) – 2*n* = 60 (Fig. 7).
Previous data: 2*n* = 60 from Italy (Garbari & al. 1980; Baltisberger 1990), from Alps (Lippert & Heubl 1988) and from Greece (Baltisberger 1991).

Euphorbia gregersenii (*Euphorbiaceae*) – 2*n* = 14 (Fig. 8, 24).
Previous data: 2*n* = 14 (Hurusawa & Shimoyama 1976).



Figs. 23-29. Haploid idiograms of *Artemisia alba* s. l. $x = 9$ (23), *Euphorbia gregersenii* $x = 7$ (24), *Moltkia petraea* $x = 8$ (25), *Ornithogalum comosum* $x = 9$ (26), *Plantago media* from Croatia $x = 6$ (27), *P. reniformis* $x = 6$ (28), *P. serpentina* $x = 6$ (29).

E. montenegrina (*Euphorbiaceae*) – 2*n* = 22 (Fig. 9).
No previous data available.

Fritillaria montana (*Liliaceae*) – 2*n* = 18+2B (Fig. 10).
Previous data: 2*n* = 18 from Italy (Honsell 1961; Altamura & al. 1984; Cesca 1984) and Balkans (Šopova & Sekovski 1989; Lovka 1995); 2*n* = 27 from Italy (Cesca 1984).

Galanthus nivalis (*Amaryllidaceae*) – 2*n* = 24 (Fig. 11).
Previous data: 2*n* = 18 from Serbia (Sušnik & Lovka 1973); 2*n* = 24 from Czech Republic (Javurkova 1980), Serbia (Drušković & Lovka 1995) and Italy (Miceli & Garbari 1976; D'Amato & Bianchi 1999).

Moltkia petraea (*Boraginaceae*) – 2*n* = 16 (Fig. 12, 25).
Previous data: 2*n* = 16 (Strey 1931; Britton 1951; Grau 1966; Drušković & Lovka 1995; Baltisberger & Baltisberger 1995).

Narcissus tazetta subsp. ***tazetta*** (*Amaryllidaceae*) – 2*n* = 20 (Fig. 13).
Previous data: 2*n* = 14, 20, 21, 22, 24, 28, 30, 32 (Fedorov 1969); 2*n* = 14 (Tseng & Chen 1984); 2*n* = 20 (Baldini 1990), 2*n* = 20+1B (Baldini 1995) from Italy; 2*n* = 20, 22 (Brandham & Kirton 1987; Lü 1990).

Ornithogalum comosum (*Hyacinthaceae*) – 2*n* = 18 (Fig. 14, 26).
Previous data: 2*n* = 18 from Italy (Garbari & Tornadore 1972; Tornadore & Garbari 1979; Tornadore & Marcucci 1988), Serbia (Raamsdonk 1986; Lovka 1995), Greece (Moret & Couderc 1986) and Turkey (Özhatay & Johnson 1996; 2*n* = 14, 16 also; Dalgic & Özhatay, 1997; 2*n* = 14, 20 also), Kieft & Loon (1978). The polyploid plants from Morocco quoted by Moret & Couderc (1986) are very likely to refer to *O. algeriense* Jord. & Fourr.

O. televrinum (*Hyacinthaceae*) – 2*n* = 72 (Fig. 15).
Previous data: 2*n* = 72 (Speta 1990), 2*n* = 70+2B, 72, 73, 76 (Lovka 1995).

Plantago media s. l. (*Plantaginaceae*) – 2*n* = 12, 24 (Fig. 16-17, 27).
Previous data: 2*n* = 12, 24 (Peruzzi & Gargano 2006 and literature cited therein).

P. reniformis (*Plantaginaceae*) – 2*n* = 12 (Fig. 18, 28).
Previous data: 2*n* = 12 from Bosnia: Mount Treskavica (Šiljak-Yakovlev 1981), Mount Treskavica, Jahorina and Maglić (Šiljak-Yakovlev & al. 1992).

P. serpentina (*Plantaginaceae*) – 2*n* = 12 (Fig. 19, 29).
Previous data: 2*n* = 12 from Serbia (Baltisberger 1992) and Italy (Capineri & al. 1978).

Potentilla erecta (*Rosaceae*) – 2*n* = 28 (Fig. 20).
Previous data: 2*n* = 18 (Davlianidze, 1985); 2*n* = 28 (Popoff 1935; Dmitrieva & Parfenov 1985; Parfenov & Dmitrieva 1987).

Ranunculus fontanus (*Ranunculaceae*) – 2*n* = 40 (Fig. 21).
Previous data: 2*n* = 48 from Italy (Peruzzi & Cesca 2004 and literature cited therein).

Silene armeria (*Caryophyllaceae*) – 2*n* = 24 (Fig. 22).
Previous data: 2*n* = 24 (Degraeve 1980; Loon & Setten 1982; Strid 1983).

Conclusions

Most of the studied species show chromosome complements agreeing with data reported in literature (*Allium commutatum*, *Artemisia alba*, *Colchicum autumnale*, *Doronicum columnae*, *Euphorbia gregersenii*, *Fritillaria montana*, *Galanthus nivalis*, *Moltkia petraea*, *Narcissus tazetta* subsp. *tazetta*, *Ornithogalum comosum*, *O. televrinum*, *Plantago media* s. l., *P. reniformis*, *P. serpentina*, *Potentilla erecta*, *Silene armeria*). Among these species, it is noteworthy to evidence the first documentation of a diploid cytotype of *Artemisia alba* for Balkan Peninsula, previously known only for Iberian Peninsula, N Africa and S Italy. *Allium roseum* shows the presence of up to two B-chromosomes in tetraploids, never quoted before in literature. *Aquilegia kitaibelii* shows a new, polyploid/aneuploid chromosome complement. The first counting for *Euphorbia montenegrina* is reported. Finally, *Ranunculus fontanus* shows a singular 2*n* = 40 complement, which perhaps can derive from hybridizations phenomena between *R. flammula* subsp. *flammula* (2*n* = 32) and the typical *R. fontanus* (2*n* = 48). Further investigation on this matter is required.

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C. Burgarella, S. Fici & Á. Lora-González

Effect of number of seed parents on the genetic diversity of artificially regenerated stands of holm oak

Abstract

Burgarella, C., Fici, S. & Lora-González, Á.: Effect of number of seed parents on the genetic diversity of artificially regenerated stands of holm oak. — Bocconea 21: 313-317. 2007. — ISSN 1120-4060.

Artificial populations of holm oak (*Quercus ilex* L. s. l.) and their natural seed origin populations selected from Andalusia (Southern Spain) and Sicily (Southern Italy) were analysed with six nuclear microsatellite loci to explore the effect of seed collection methods. Low effective allele numbers (3.07-6.67) and extremely low effective population sizes (35.4-102.9) were detected in both reforestation stands, probably due to an inappropriate forester seed collection strategy limited to a few mother trees. Analysis of simulated progenies showed that optimal sampling size to obtain seed collections genetically similar to the origin population should range from 20 to 50 individuals, but could be different between the two regions, being higher in the more genetically diverse Andalusia.

Introduction

The reforestation with autochthonous species should preserve genetic diversity to maintain fitness and adaptability potential of forest ecosystems. The sampling strategy, stand structure and mating system of a tree population determine the genetic structure of the harvested material and, consequently, the similarity of the seedlots to the origin population (Degen & al. 1996).

Within the silvicultural framework, the relationship between level of genetic diversity and population size has frequently been analysed with reference to adult trees (see Young & Boyle 2000). Another approach proposes expressing the diversity of seedlots in terms of number of seed parents included in the harvest (eg. Lexer & al. 1999), shifting the point of view to the progeny (i.e. seeds as forest reproductive material). Recently, Blakesley & al. (2004) addressed the question of the minimum number of mother trees required to maintain the original genetic variability in the seedlots collected for artificial regeneration of an endangered tree species.

In the present contribution, the effects of the seed sampling strategy on the genetic diversity of simulated seedlot samples have been investigated, and the results have been compared with two empirical cases of artificial regeneration with *Quercus ilex* L. s. l., located in Sicily (Southern Italy) and in Andalusia (Southern Spain).

Materials and methods

The natural and artificial populations for sampling were selected taking into account the informations supplied by forestry agents and nursery workers to meet the following criteria: ‘natural seed origin population’, a population usually used as source of seed for forestry nurseries; ‘nursery’, a stock of seedlings (sampled only in Sicily) whose seed-source is the above mentioned natural population; ‘artificially regenerated population’, a reforested stand whose seed-source is the above-mentioned seed origin population. The Sicilian origin population, Piano Zucchi forest, and nursery, Piano Noce, are located in the Madonie mountain chain (east of the province of Palermo), while the regenerated stand is situated in the Palmeto mountain (west of the same province). In Andalusia, the seed origin population is an open formation (*dehesa*), El Cazador, in the Sierra Morena (Sevilla), while the artificial stand is located in the Sierra de Gador (Almería). The leaves from 30-40 individuals were collected per sample. The individuals were sampled in natural populations maintaining an average distance of 50 m between them.

Six nuclear microsatellite loci were used for genetic analysis: MSQ4, QpAG15, QpAG36, QpAG46 transferred to *Q. ilex* by Soto & al. (2003); QrZAG11 and QrZAG20 modified from Kampfer & al. (1998).

To understand the effect of the number of seed parents on the genetic diversity of seedlot samples, computer simulations were run to reproduce the biological conditions of the natural systems studied. Two stands, of 1000 differently aged individuals each, were simulated on the basis of the allelic frequencies of the natural seed origin populations. The *in silica* Sicilian stand had a density of 30 trees/ha, while the Andalusian stand had 60 trees/ha. Fictitious seed collections (1000 seeds) from different numbers of randomly selected trees (2, 10, 20, 30, 50, 100) were produced.

The computer simulations were performed with ECO-GENE (Degen & al. 1996). The effective number of alleles of simulated seedlots was calculated as $Ae = 1/\sum p^2$ (where p = allele frequency) with POPGENE 1.31 (Yeh & al. 1999). Effective population size of sampled populations (*Ne*) was estimated with a likelihood procedure which takes into account the changes in allelic frequencies between two generations and the possibility of migration with MLNE (Wang & Whitlock 2003).

Results and discussion

For the Sicilian populations 120 individuals were analyzed and 65 alleles were found, while the Andalusian populations comprised 157 individuals with a total allele number of 90.

As expected, the effective number of alleles (*Ae*) of fictitious seedlots increased with the number of seed parents, because of progressively higher effective population size (Fig. 1a, b). The regression curve reached a *plateau*, corresponding approximately to the *Ae* value of the fictitious seed origin population. In the Sicilian case (Fig. 1a) *Ae* of simulated progenies reached that of the origin population with 10 mother trees, while 20 mother trees were needed in the Andalusian simulation. *Ae* absolute values in the Andalusian case were also notably higher when compared with Sicilian *Ae* values (Fig. 1b). This difference is explained by the higher genetic richness found in the Andalusian sampled populations

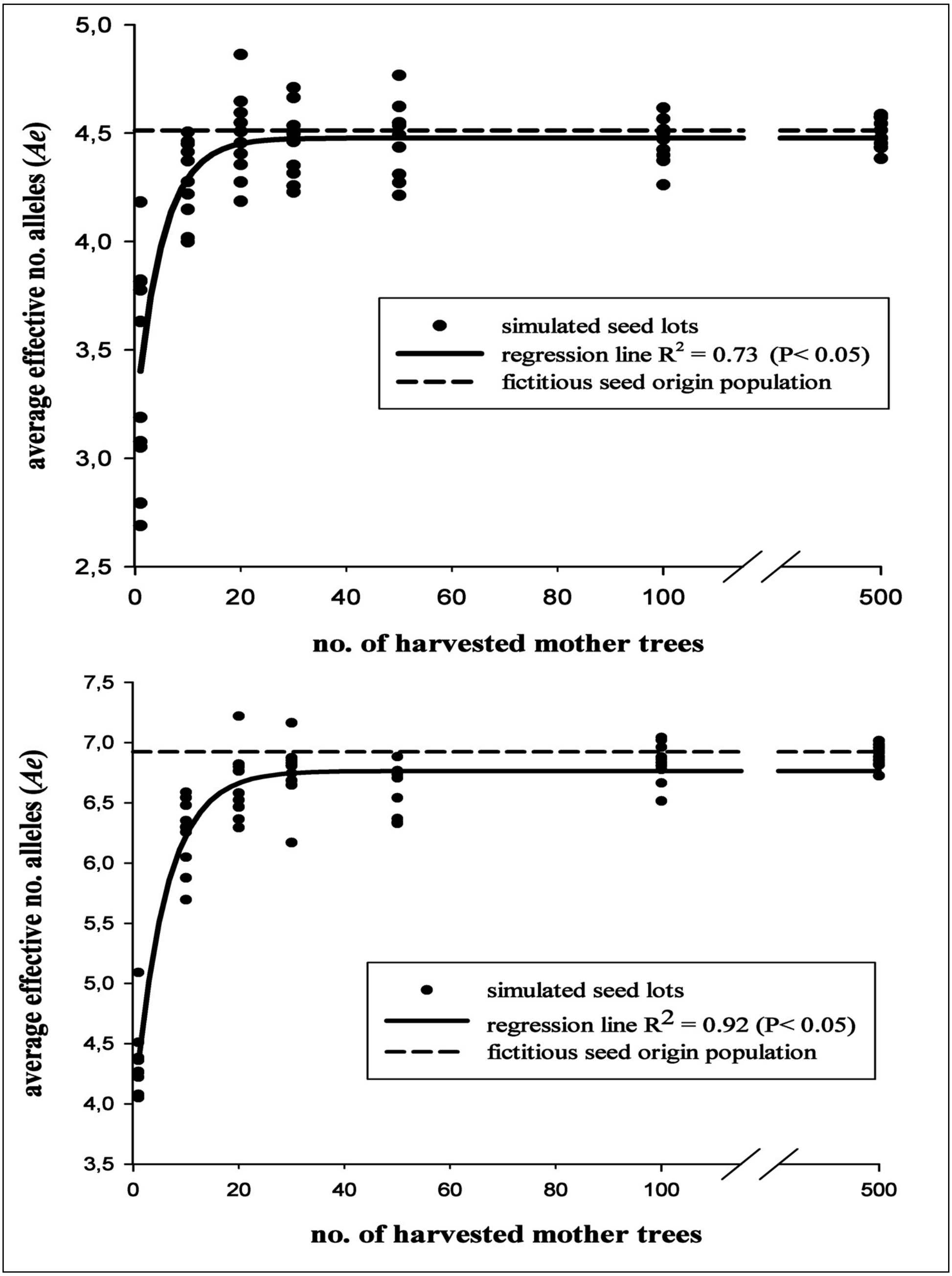

Fig. 1. Effect of number of seed parents on the effective allele number (mean value) of simulated seedlot samples (dots) harvested from a fictitious seed origin population (dashed line). For each number of seed parents (2, 10, 20, 30, 50, 100, 500) 10 simulations were performed. *Ae* of fictitious seedlots increases with the number of seed parents because of progressively higher effective population size, until reaching a *plateau*. a) Sicily, b) Andalusia.

and suggests that the number of mother trees required to maintain the most common alleles of the origin population in a harvested seedlot is smaller in Sicily than in Andalusia.

Comparing the results of the theoretical model with the empirical estimates of Ae corresponding to the sampled artificially regenerated stands, the value of Monte Palmeto (Ae = 3.07) might indicate a number of mother trees lower than 10 (the same could be concluded for the nursery Ae value, Ae = 3.65), while the Andalusian artificial stand (Ae = 6.67) might indicate a number of mothers between at least 10 and 20. Estimates of effective population sizes (Ne) are congruent with those results, with Ne = 52.5 for the nursery and Ne = 35.4 for the reforestation in Sicily; and Ne = 102.9 for the reforestation in Andalusia. These values are very low considering that Ne generations would reduce the heterozygosity due to genetic drift by 50%. In both cases examined, the results observed can be explained by a seed harvest from a limited number of trees, whose consequences on the diversity of the progenies are similar to those due to a bottleneck or founder effect.

Conclusions

Although the studied populations of Sicily and Andalusia share the same trend of variation for the effective number of alleles of seedlots in dependence on the number of harvested trees, optimal sampling size to obtain seed collections genetically similar to the origin population could be different for the two regions, being higher in Andalusia. As a guideline, this study suggests that the number of sampled trees should range from 20 to 50 individuals. The only reference on forest management available at the moment is the Austrian forest seed law, which fixes the minimum number of seed parents per stand at 20 in the case of the oaks *Quercus petraea* and *Q. robur* (Lexer & al. 1999).

Since extremely low female and total effective population sizes were detected, the studied reforestation stands seem to have been subject to a genetic process whose natural equivalent is a bottleneck or founder effect. This is probably the effect of an inappropriate forester seed collection strategy limited to a few trees that might be accentuated by mating system properties (differences in flowering phenology, annual variation in fertility, stand structure and density), causing some genetic differences among harvested seedlots.

The results of this study highlight the importance of taking into account differences in forest genetic resources among regions which might require specific management practices according to the characteristics of each particular species in each area.

Finally, nuclear microsatellites have proven to be useful tools for investigating genetic diversity of nursery seedlots and artificially regenerated stands of holm oak, including cases when genotype information of parent identity is lacking.

Acknowledgments

This research was carried out with the support of the Plant Genetics Laboratory of the Centre for Agricultural Formation and Research of Córdoba (CIFA) and the Forest Genetics Centre of the Spanish National Institute of Agriculture and Food Research (CIFOR-INIA). We also thank M. Navascués for helpful support and comments on the manuscript, and two anonymous referees for their suggestions on the text.

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Genetic and geographical relationships of manna ash populations from Serbia

Abstract

Bojović, S., Heizmann, P., Pavlović, P., Mitrović, M., Djurdjević, L. & Barbero, M.: Genetic and geographical relationships of manna ash populations from Serbia. — Bocconea 21: 319-324. 2007. — ISSN 1120-4060.

The objectives of this study were to reveal DNAs (RAPD) individual variation and to analyze the genetic and geographical diversity of *Fraxinus ornus* populations from Serbia. PCR-RAPD variation was examined in three natural populations (90 trees) and the data analysed with genetic softwares (POPGENE, Arlequin and TFPGA) for obtaining parameters of genetic diversity and structure. PCR-RAPD markers reveal individual variation in *F. ornus*. A total of 122 fragments were amplified using 7 primers and of these 77 (63%) fragments were polymorphic. The mean gene diversity for all populations was 0.27 and the Shannon's index was between 0.38 and 0.42. The partition of total genetic variance indicates a relatively restricted population differentiation as expected in outcrossing species. Present and future information on genetic structure and variability in *F. ornus* needs to be incorporated into strategies for the preservation of genetic resources of tree species.

Introduction

RAPD markers are already accepted in the assessment of genetic and geographical relationships natural populations of forest trees (Khasa & Dancik 1996; Schierenbeck & al. 1997). *Fraxinus ornus* L. (manna ash) is a mainly a Mediterranean forest tree species of wide ecological amplitude. Its wide tolerance of various soils makes it a species of choice for the afforestation of limestone barrens and for soil conservation. In the region of Serbia large-scale afforestation with *F. ornus* was performed during the fifties of the last century for preventing erosion and for regulating river courses. Because of the deforestation in this region during the past decade, the intensified reforestation with *F. ornus* has again become a necessity. The genetic analyses are rare or non-existing for the majority of European deciduous tree species (i.e. *F. ornus*).

The objectives of the present study were to analyze the genetic structure of *F. ornus* by evaluating the degree of intra- and inter-population genetic variation.

Material and methods

Plant material

Altogether 90 adult trees of *F. ornus* L. from natural populations of biogeographically contrasted zones in Serbia were analyzed: Košutnjak (44° 46'N, 20° 27'E): 210 m asl, calcareous and sandy soil, continental-Danubian type of climate, ass. *Fraxino orni-Quercetum virgilianae* Gajić (Tomić 2006); Užice (43° 53'N, 19° 47'E): 700 m asl, calcareous soil, continental climate, ass. *Quercetum frainetto-cerridis* Rudski (Tomić 2006); Pirot (43° 13'N, 22° 33'E): 500 m asl, calcareous soil, continental climate, ass. *Syringo-Carpinetum orientalis* (Rudski) Mišić (Tomić 2006) (Fig. 1).

Fig. 1. The natural range of *F. ornus* and location of sampled populations from Serbia: **A** Košutnjak, **B** Užice, **C** Pirot.

DNA extraction

DNA was extracted from the leaves of adult trees. Approximately 5 g of leaf tissue were ground to fine powder in liquid nitrogen and quartz sand in a mortar and pestle. The powdered tissue was suspended in 21 ml of extraction buffer (0.1 M Tris-HCl pH 8; 0.05 M EDTA pH 8; 0.1 M NaCl; 1% SDS). The homogenate was then incubated at 65°C for 20 min with occasional mixing. Seven ml of potassium 5 M acetate (pH 9.25) was added and the tube was placed on ice for 20 min. After centrifugation (3500 rpm, 0°C, 20 min), 1 ml of ammonium acetate 10 M pH 7.5 and 1 volume of isopropanol was added and the sample was placed on ice for 20 min. The pellet obtained after centrifugation (3500 rpm, 0°C, 10 min) was rinsed with 70% ethanol, dried and then resuspended in 1 ml of 50 mM Tris-HCl, pH 7.5/EDTA 10 mM buffer. The sample was then incubated for 30 min at 37°C with RNase A (100 µg ml^{-1}) then Tris 10 mM, pH 7.5/EDTA 1 mM was added to give a final volume of 6 ml and this was extracted with a phenol/chloroform mix (1:1 phenol : chloroform). The aqueous layer was removed and the DNA was precipitated by addition of 0.1 volume of 7 M ammonium acetate (pH 7.4) and 1.5 volume of isopropanol. To eliminate the traces of phenol, the DNA pellet was rinsed with 70% ethanol. The final pellet was vacuum dried and dissolved in 1 ml of TE buffer and stored at 4°C.

DNA amplification conditions

The amplification was carried out with 100 ng of DNA in a 25 µl total volume, with 2.5 µl of reaction buffer PC2 10X (Ab Peptides), 5 nM of dNTP (200 µM), 50 pM of each primer (2 µM) and 3.75 units of Klen Taq DNA polymerase (Ab peptides). Forty one RAPD decamer oligodeoxynucleotide primers were used for screening: 40 (kits G, H with 20 primers in each kit) purchased from Operon Technologies (Alameda, California, USA) and 1 primer (OPO-08) purchased from GIBCO BRL (Custom Primers, Life Technologies, Gaithersburg). DNA amplification was repeated at least twice, and only reproducible and unambiguous fragments were scored. The thermocycler program was as follows: preliminary denaturation (3 min at 94°C) followed by 40 cycles consisting of denaturation (30 sec at 94°C), annealing (1 min at 35°C), extension (2 min at 72°C) and a final extension (5 min at 72°C). The DNA fragments were separated in 1.5% agarose gels in buffer TAE (Tris-base 40 mM, sodium acetate 20 mM, EDTA 1mM, with pH 7.2 adjusted with glacial acetic acid).

Data analysis

DNA profiles were scored as present (1) or absent (0). A fragment (loci) was considered to be polymorphic if the presence and absence of the band were observed and monomorphic if the band was present among all individuals. The genetic diversity parameters: Nei's (Nei 1973) gene diversity (h), Shannon's information index (Lewontin 1972) (I), the percentage of polymorphic RAPD loci (p) and Nei's unbiased measures of genetic identity and genetic distance (Nei 1978) were calculated by the software POPGENE 1.31 (Schneider & al. 1997). The partition of total genetic variance, AMOVA by ARLEQUIN 1.1. (Yeh & al. 1999) and Mantel test by TFPGA 1.3 (Miller 2000).

Results

A total of 122 fragments were amplified using 7 primers and of these 77 (63%) fragments were polymorphic. The mean gene diversity for all populations was 0.27 and the Shannon's index was between 0.38 and 0.42. The percentage of polymorphic loci was relatively similar across populations, ranging between 53.3% (A) and 59.8% (C), with an average of 63.1% (Tab. 1). The range of variation in gene diversity (h) between the *F. ornus* populations was from 0.25 to 0.28. The mean gene diversity for all populations was 0.27. The relative degree of diversity in each population as measured by Shannon's index varied from 0.38 (C) to 0.42 (A). The mean Shannon's index for five populations was 0.40.

Our results indicate that 12% of the total genetic diversity is attributable to the differences among populations and 88% is within-population variation (FST in the molecular variance analysis = 0.12, $P<0.05$, tested using 1023 random permutations). All pair wise FST values derived from AMOVA were significant ($P<0.05$) when individual pairs of populations were compared. The geographic structuration of the diversity can be analyzed by the Nei's unbiased genetic distances (Nei 1978) among populations (Tab. 2). The highest genetic distance was found between the A and C (0.08) and the lowest between the B and C (0.01). The dendogram based on Nei's (1978) unbiased genetic distance matrix reveals a distinct grouping structure among populations (Fig. 2). The Mantel test revealed significant correlation between genetic and geographical distance matrice ($r=0.7$, $P<0.05$).

Table 1. Five *F. ornus* populations: h - gene diversity (Nei 1973), I - Shannon's diversity measure, % p - polymorphic RAPD loci, (s. d.) - standard deviations, MV - meanean values.

Populations	h (s. d.)		I (s. d.)		% p
Košutnjak	0.280	(0.182)	0.418	(0.254)	53.28
Užice	0.271	(0.200)	0.399	(0.283)	54.10
Pirot	0.254	(0.194)	0.381	(0.269)	59.84
MV	0.268	(0.192)	0.399	(0.269)	55.74

Table 2. Five *F. ornus* populations: coefficients of genetic identity (above diagonal) and genetic distances (below diagonal) (Nei 1978).

Population	Košutnjak	Užice	Pirot
Košutnjak	0	0.9700	0.9176
Užice	0.0304	0	0.9881
Pirot	0.0860	0.0120	0

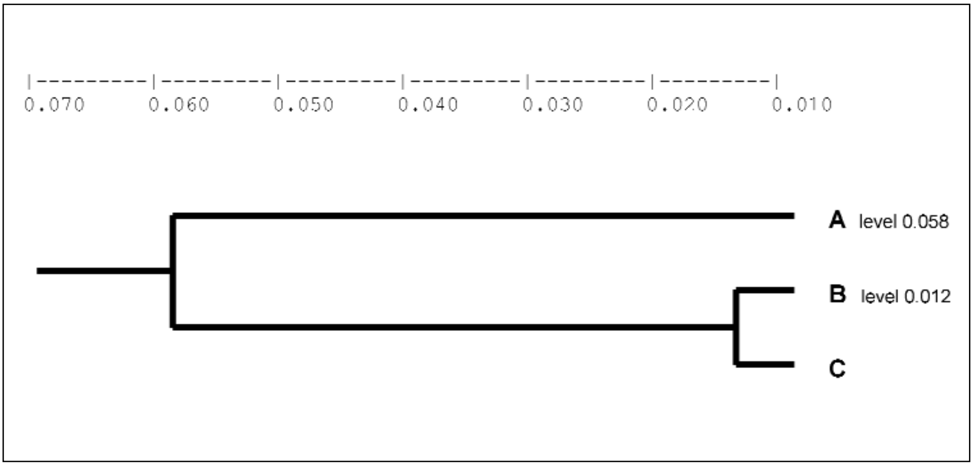

Fig. 2. UPGMA dndrogram based on Nei’s genetic distance (Nei 1978): **A** Košutnjak, **B** Užice, **C** Pirot.

Discussion and conclusion

Our results indicate that PCR-RAPD markers are sufficiently informative and powerful to assess genetic variability in *F. ornus*. RAPDs were shown to be sensitive for detecting individual variation of *F. ornus*. A total of 122 fragments were amplified using 7 primers and of these 77(63%) fragments were polymorphic. The mean gene diversity for all populations was 0.27 and the Shannon’s index was between 0.38 and 0.42. The partition of total genetic variance indicates a relatively restricted population differentiation as expected in outcrossing species.

Müller-Starck & al. (1992) in his review of results of isoenzymes studies on genetic variation in various coniferous and angiosperm tree species, observed that species with large geographic ranges (such as *Picea abies*, *Pinus sylvestris*, *Fagus sylvatica*) tend to show little genetic differentiation among populations within regions, but greater differentiation among populations derived from different glacial refuge. Our data seems to confirm a similar pattern holds in *F. ornus* with RAPD markers in the continuous distribution in the region of Serbia. Of the total genetic variation, 87% was within population and 13% was among population variation.

The significant relationship between geographical and genetic distance among populations is an indication that isolation by distance is the process accounting for the distribution of genetic variation among populations within the region.

In conclusion, our results indicate that RAPDs are sufficiently informative and powerful to assess genetic variability in *F. ornus*. RAPD markers can open the ‘research runways’ to other more informative markers. These more informative, but more difficult to use and more expensive markers, can be used in more specific and deeper analyses. However, in the first phase of the assessment of genetic variation in forest tree populations, the use of RAPD markers is quite satisfactory.

Acknowledgements

The authors would like to thank the Ministry of France (Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche - MENESR) for funding the post-doctoral research for S.B for one year. This work forms part of the research project (Project Code 143049) sponsored by Ministry of Science, Technology and Development of Republic Serbia.

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Cytotaxonomic considerations on *Allium stamineum* Boiss. group (*Alliaceae*)

Abstract

Brullo, S., Guglielmo, A., Pavone, P. & Salmeri, C.: Cytotaxonomic considerations on *Allium stamineum* Boiss. group (*Alliaceae*). — Bocconea 21: 325-343. 2007. — ISSN 1120-4060.

The species of the *Allium stamineum* group are examined in their morphological, karyological and taxonomic aspects, on the base of literature data as well as herbarium and field surveys. This group is widely distributed in the eastern Mediterranean area, where it is represented by many species well differentiated as regards their morphology, ecology and distribution. Most of the species are diploid with $2n=16$ and occur in natural habitats, while some triploid ($2n=24$) and tetraploid ($2n=32$) are linked to synanthropic places. Nomenclature, morphology, karyology, ecology and distribution are provided for each species, as well as the iconography of their main distinctive features. The identification keys of these species are provided too.

Introduction

The *Allium stamineum* group comprises many critical taxa belonging to *Allium* sect. *Codonoprasum* Reichenb. A marked heterogeneity and morphological diversity, in fact, characterize a lot of populations whose taxonomic treatment is rather difficult. It is differentiated by a pool of characters partly shared with the other taxa of the section and other ones distinctive of this group. They are divaricate spathe valves ending with a very long appendix, lax and diffuse umbel, conical-campanulate perigon, with mean size 4-7 mm (rarely smaller) and colour varying from pinkish white to greenish yellow or to green-blue or purplish, stamens long exserted from perigon and ovary with inconspicuous nectaries.

The group is widely distributed in the Eastern Mediterranean area, mainly in the Balkan and Aegean regions, Anatolian peninsula, extending into the Middle East, in Iranian and Palestinian territories. Its western boundary in the Mediterranean area corresponds to Mt. Gargano in S Italy.

Many distinct species are recognizable into this group, taxonomically well defined and validly described, as confirmed by literature data (Rechinger 1943; Wendelbo 1971; Kollmann 1984, 1986; Stearn 1984; Meikle 1985; Brullo & al. 1993, 1996a; Karavokyrou & Tzanoudakis 1994) and by our detailed herbarium and field surveys. Some of these species are known for a long time, such as *A. stamineum* Boiss., *A. decaisnei* C. Presl, *A. hymettium* Boiss. & Heldr., *A. guicciardii* Heldr. and *A. phrygium* Boiss.; others have been more recently described, as *A. deciduum* Ozhatay & Kollmann, *A. cyprium* Brullo, Pavone

& Salmeri, *A. dodecanesi* Karavokyrou & Tzanoudakis, *A. daninianum* Brullo, Pavone & Salmeri, etc. Further studies are in progress about many other critical populations collected in various localities of E-Mediterranean area, resulting significantly distinct from all up to known species.

In this contribution the taxa presently known are examined to better emphasize the inter- and intraspecific diversity and the taxonomic relationships within the group inquiring into their morphology, karyology and nomenclature.

Material and methods

This work is based on literature data and herbarium investigations as well as on field collections in type localities and other sites of E-Mediterranean area to verify the variability among natural populations. Specimens from the following Herbarium collections were examined: B, BM, C, CAT, FI, G, HUJ, ISTE, M, OXF, P, PAL and UPA. The karyological analyses were made on mitotic plates from root tip cells of cultivated bulbs, pre-treated with 0.3% colchicine water solution, fixed in ethanol-acetic (3:1) and stained according to the Feulgen technique. Metaphases handling and chromosome measures were made by two image analysis systems, IKAROS 4.6 (Metasystem) and KS300 (Zeiss). Karyotyping was worked out by a specific software (Cromolab© 1.1) for the recognition of homologues, couple ordering, chromosome classification and karyotype formula based on the centromere position (Levan & al. 1964; Tzanoudakis 1983). The karyotype symmetry degree was tested on account of different parameters, such as the categories of Stebbins (1971), the TF% index (Huziwara 1962) and the REC and SYi indices (D'Ovidio & Marchi 1990).

Results

This investigation allowed to individuate in the *A. stamineum* group a lot of species taxonomically well distinct. Their nomenclature, morphology, karyology, ecology and distribution are here provided, together with the iconography of their main distinctive features.

Allium stamineum Boiss., Diagn. ser. 2(4): 119 (1859).

Typus: *Lectotypus*: Caria, 1843, Pinard (G-BOISS!). *Isotypi*: BM!, FI!, P!

Iconography: Fig. 1.1 - 2.1 a, b, c.

Description: Bulb ovoid, 12-17 × 6-8 mm, with outer tunics coriaceous, dark brown, the inner ones membranous, whitish yellow. Scape glabrous, erect, 10-50 cm high, covered by leaf sheaths for 1/2-2/3 of its length. Leaves 3-4, green, semicylindrical, costate, up to 25 cm long. Spathe persistent, with 2 unequal valves, longer than umbel, the larger 7-nerved, 2.5-10(-12) cm long, the smaller 5-nerved, 2.5-7 cm long. Inflorescence lax, diffuse, 10-25-flowered; pedicels unequal, flexuous, 7-25 mm long. Perigon conical-campanulate, with tepals equal, greenish yellow tinged with purple, oblong-elliptical, rounded at apex, 4.5-5.5 mm long, the outers 2.4-2.5 mm wide, the inners 2-2.2 mm wide. Stamens simple, exserted, with filaments subulate, 4-6.5 mm long, white below and purplish above, connate at base into an annulus 0.6-1 mm high; anthers ellipsoid, yellow, rounded at apex, 1.5 × 0.8-1 mm. Ovary subglobose, yellow-greenish, rugose-

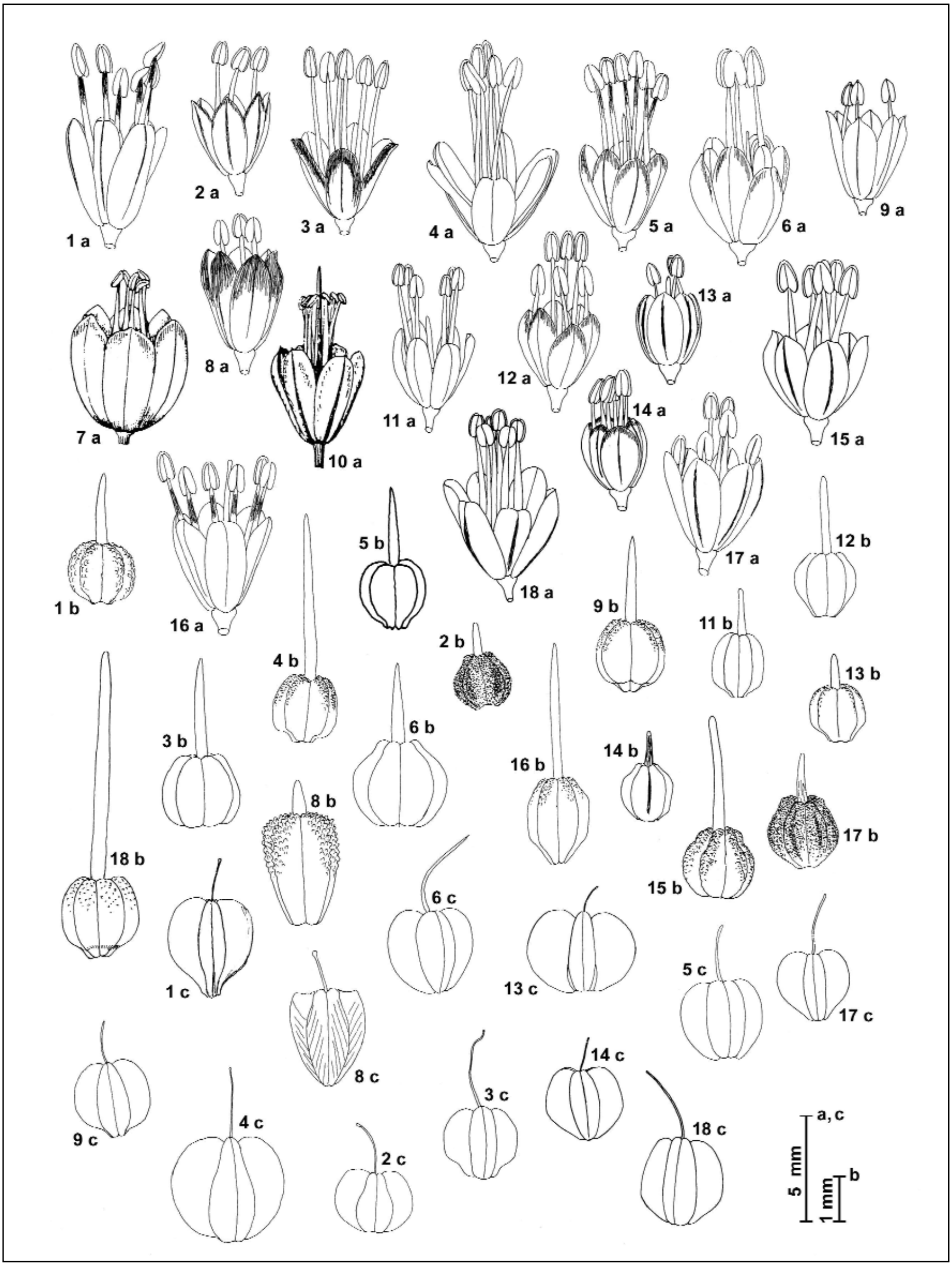

Fig. 1. Flower (a), ovary (b) and capsule (C) of the investigated species of *Allium stamineum* group: 1 - *A. stamineum*, 2 - *A. decaisnei*, 3 - *A hymettium*, 4 - *A. guicciardi*i, 5 - *A. phrygium*, 6 - *A. albotunicatum*, 7 - *A. hermoneum*, 8 - *A. pictistamineum*, 9 - *A pseudoflavum*, 10 - *A. pseudostamineum*, 11 - *A. deciduum*, 12 - *A. retrorsum*, 13 - *A. cyprium*, 14 - *A. lefkarense*, 15 - *A. marathasicum,* 16 - *A. dodecanesi*, 17 - *A. daninianum*, 18 - *A. garganicum*.

papillose above, 1.6-1.7 × 1.8-1.9 mm. Style white, 1.5-2.5 mm long. Capsule widely obovoid, stipitate at base, green, 4.5 × 4 mm.

Karyology: Populations from two localities of *locus classicus*, the Caria region in SW Turkey, show a diploid chromosome complement 2*n*=16. The karyotype arrangement (Fig. 3.1) can be resumed by the following formula: 2*n*=2x=16: 8 m + 2 $\mathrm{m}^{\mathrm{sat}}$ + 4 msm + 2 sm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 74.83, SYi 79.57, TF% 44.31.

Diploid specimens from Cape Sounion (Greece) uncertainly identified by Miceli & Garbari (1979) as *A. stamineum* should be referred to other specific unit.

Specimen karyologically examined: Turkey, Alikurt (Denizli), 27.VI.1987, *Brullo*, *Pavone & Signorello* (CAT); Ortaklar (Aydin), 24.VI.1988, *Brullo*, *Pavone & Signorello* (CAT).

Ecology: Meadows and shrub communities.

Distribution: Southern and Western Anatolia.

Allium decaisnei C. Presl, Bot. Bemerk. 114 (1844).

Typus: *Lectotypus*: Sinai, Arabice, Betel Nagiel in fissuris rupium montis St. Caterinae, 20.VII.1835, Schimper 250 (PRG!). *Isotypi*: BM!, OXF!

Iconography: Fig. 1.2 - 2.2 a, b, c.

Description: It differs from *A. stamineum* in having bulb 12-20 × 10-15 mm, with outer tunics pale brown, the inner ones whitish. Scape 15-25 cm high. Leaves up to 12 cm long. Spathe with larger valve 2.5-5 cm long, the smaller one 1.5-2.5 cm long. Inflorescence with pedicels unequal, 10-20 mm long. Tepals subequal, tinged with brown, sublanceolate, acute at apex, 3-4 mm long, 1.7-2 mm wide. Stamens with filaments 1.5-4 mm long, white, connate at base into an annulus 0.7 mm high; anthers straw-yellow, 1.1-1.2 × 0.7-0.8 mm. Ovary ovoid-pyriform, greenish, entirely papillose, 1.4-1.5 × 1.5 mm. Style 1 mm long. Capsule subglobose, flattened above, 3 × 3.8 mm.

Karyology: Specimens from Negev desert show a diploid chromosome complement 2*n*=16. This count was already quoted by Kollmann (1985), sometimes with 1 B chromosome. The karyotype arrangement (Fig. 3.2) can be resumed by the following formula: 2*n*=2x=16: 10 m + 4 msm + 2 sm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 84.06, SYi 79.31, TF% 44.23.

Specimen karyologically examined: Israel, Northern Negev, 15 Km a Nord di Beer Sheva, 24.V.1989, *Brullo 15* (CAT); Judean desert, 5 Km SO di Gerico presso il Monastero di S. John, 24.V.1989, *Brullo 11* (CAT).

Ecology: Rocky places of arid territories within subdesertic shrub vegetation.

Distribution: Palestine, Sinai.

Allium hymettium Boiss. & Heldr., in Boiss. Diagn. Pl. Or. Nov. 3(4): 120 (1859).

Typus: *Lectotypus*: Hymettos, 1841, Spruner (G-BOISS!).

Iconography: Fig. 1.3 - 2.3 a, b, c.

Description: It differs from *A. stamineum* in having bulb 8-14 × 5-8 mm, with outer tunics pale brown, the inner ones whitish. Scape 8-15 cm high. Leaves 4, up to 10 cm long. Spathe with larger valve 2-4 cm long, the smaller one 1-2 cm long. Inflorescence contracted; pedicels 5-10 mm long. Tepals unequal, 4-4.5 mm long, pinkish yellow with striae dark brown purplish, the outers elliptic, 2 mm wide, the inners oblanceolate, 1.6-1.8 mm wide. Stamens 3.5-6 mm long, white, connate at base into an annulus 0.8-0.9 mm high provided with interstaminal teeth, anthers ellipsoid, straw-yellow, slightly apiculate, 1.2-1.3 × 0.8 mm. Ovary subglobose-ovoid, greenish, smooth, 1.8 × 1.8 mm. Style 2 mm long. Capsule subglobose, 3.5 × 3.5 mm.

Karyology:The plants from the type locality show a diploid chromosome complement $2n$=16, as already reported for this species by Johnson (1982). The karyotype arrangement (Fig. 3.3) can be resumed by the following formula: $2n$=2x=16: 8 m + 6 msm + 2 sm; some plates showed microsatellites on the short arms of some chromosomes. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 70.87, SYi 77.36, TF% 42.80.

Specimen karyologically examined: Greece, Monte Hymettos, colline scistose, 4.VI.1992, *Brullo & Pavone* (CAT).

Ecology: Ephemeral communities occurring in rocky places.

Distribution: Central Greece (Attica, Evvia).

Allium guicciardii Heldr., Sched. Herb. Graec. Norm. (1876).

Typus: *Lectotypus*: in m. Parnassi reg. alpina, VIII.1855, *Guicciardi 2985* (G-BOISS!).

Iconography: Fig. 1.4 - 2.4 a, b, c.

Description: It differs from *A. stamineum* in having bulb 10-18 × 8-12 mm. Scape covered by leaf sheaths up to 1/2 of its length. Leaves 4-6. Spathe with larger valve 6-15 cm long, the smaller one 4-9 cm long. Inflorescence 50-70-flowered; pedicels 10-50 mm long. Perigon with tepals unequal, yellowish green, pruinose, elliptical, 4.5-5 mm long, the outers 2.2-2.3 mm wide, the inners 2-2.1 mm wide. Stamens with filaments 6.5-7 mm long, connate at base into an annulus 0.4-0.6 mm high. Ovary subglobose to obovoid, greenish, rugose above, 1.5-2 × 1.8-2 mm. Style 4-5 mm long. Capsule subglobose, 3.5-5 × 3.5-5 mm.

Karyology: All specimens collected in Greece and Romania resulted tetraploid with a somatic chromosome number $2n$=32. However, Alden (1976) found in some Pindhos mountains (N Greece) populations with $2n$=16, besides other ones with $2n$=32. The karyotype shows a diploid arrangement (Fig. 3.15) of chromosomes since it was not possible to put them in four. Its structure can be represented by the following formula $2n$=4x=32: 22 m + 6 msm + 4 sm. Some difference among populations regards the presence of microsatellites on short chromosome arms. The index values expressing the karyotype symmetry degree are: Stebbins' categories 2A, REC 71.22, SYi 79.33, TF% 44.24.

Specimen karyologically examined: Greece, Melissi, 20.VI.1987, *Brullo*, *Pavone & Signorello* (CAT); Larisa, 21.VI.1987, *Brullo*, *Pavone & Signorello* (CAT); Metsoia, 20.VI.1987, *Brullo*, *Pavone & Signorello* (CAT); Romania, Macin, VIII 1993, *Brullo & Scelsi* (CAT).

Ecology: Rocky crevices and phrygane mainly disturbed by human activities.

Distribution: Central and Northern Greece, Romania.

Allium phrygium Boiss., Fl. Or. 5:256 (1882).

Typus: *Lectotypus*: Ouchak Phrygiae, 910 m, fin August 1857, *Balansa 89* (G!).

Iconography: Fig. 1.5 - 2.5 a, b, c.

Description: It differs from *A. stamineum* in having bulb 8-16 × 6-10 mm, with outer tunics striate, pale brown, the inner ones pale brown. Scape 12-30 cm high, covered by leaf sheaths up to 1/2 of its length. Leaves up to 16 cm long. Spathe with smaller valve 2-5 cm long. Inflorescence 10-15-flowered; pedicels 10-30 mm long. Tepals unequal, yellow-greenish with brown striae, generally rounded at apex, 4-4.5 mm long, the outers 2-2.2 mm wide, the inners 1.5-1.8 mm wide. Stamen filaments 3.5-6 mm long, connate at base into an annulus 0.3-0.5 mm high; anthers 1 × 0.6 mm. Ovary subglobose to ovoid, greenish, smooth, 1.5-2 × 1.5 mm. Style 1-1.5 mm long. Capsule globose, 3.5 × 3.5 mm.

Karyology: Specimens from Usak show a diploid chromosome complement $2n=16$, the same count reported by Özhatay (1985, 1993). The karyotype arrangement (Fig. 3.4) can be resumed by the following formula: $2n=2x=16$: 10 m + 6 msm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 74.17, SYi 80.79, TF% 44.49.

Specimen karyologically examined: Turkey, Civril, Kizilkadag (Usak), 22.VI.1998, *Brullo & Pavone* (CAT).

Ecology: Phrygane and steppe meadows.

Distribution: Central and Western Anatolia.

Allium albotunicatum O. Schwarz, Feddes Repert. 36: 73 (1934).

Typus: *Lectotypus*: Turkey, in fruticetis sempervirentibus prope Burnova, solo calcareo, *O. Schwarz* 890 (B!); *Syntypus*: Turkey, Smyrna, in vallibus montis Coracis prope Lidia, c. 200 m, *O. Schwarz* 278 (B!).

Iconography: Fig. 1.6 - 2.6 a, b, c.

Description: It differs from *A. stamineum* in having bulb ovoid subglobose, 9-15 × 7-10 mm, with outer tunics white to greyish, split from base. Scape 15-60(-85) cm high. Leaves 4-5, up to 20 cm long. Spathe with longer valve 3-6 cm long, the smaller 2.5-4.5 cm long. Inflorescence 15-50-flowered; pedicels 10-40 mm long. Perigon obovoid with tepals unequal, pale green to yellow green tinged with purple, the outers 4 × 2.3-2.5 mm, the inners 4.5 × 2 mm. Stamens simple, unequal, with outer filaments 1.2-1.5 mm long, inner ones 5-6 mm long, connate at base into an annulus 0.6-0.8 mm high; anthers 1.7-1.9 × 0.9-1 mm. Ovary ovoid-pyriform, smooth, 2 × 2.2 mm. Style 2 mm long. Capsule obovoid, not stipitate at base, 4-5.5 × 4-5.5 mm.

Karyology: Shmida & Kollmann (1977) and Kollmann (1985) reported a triploid chromosome count $2n=24$ for Israel populations.

Ecology: Xeric maquis at low altitude.

Distribution: W Anatolia, Lebanon, Palestine.

Allium hermoneum (Kollmann & Shmida) Brullo, Guglielmo, Pavone & Salmeri *stat. nov.*

Bas.: *Allium albo-tunicatum* O. Schwarz subsp. *hermoneum* Kollmann & Shmida, Israel J. Bot. 26: 141 (1977).

Typus: *Holotypus*: Mt. Hermon, 2360 m, 14.VI.1974 *Shmida* (HUJ!).

Iconography: Fig. 1.7 - 2.7 a.

Description: It differs from *A. albo-tunicatum* in having bulbils along the lower part of stem. Scape 15-30 cm high. Leaves 3-4, up to 15 cm long. Spathe with longer valve up to 3

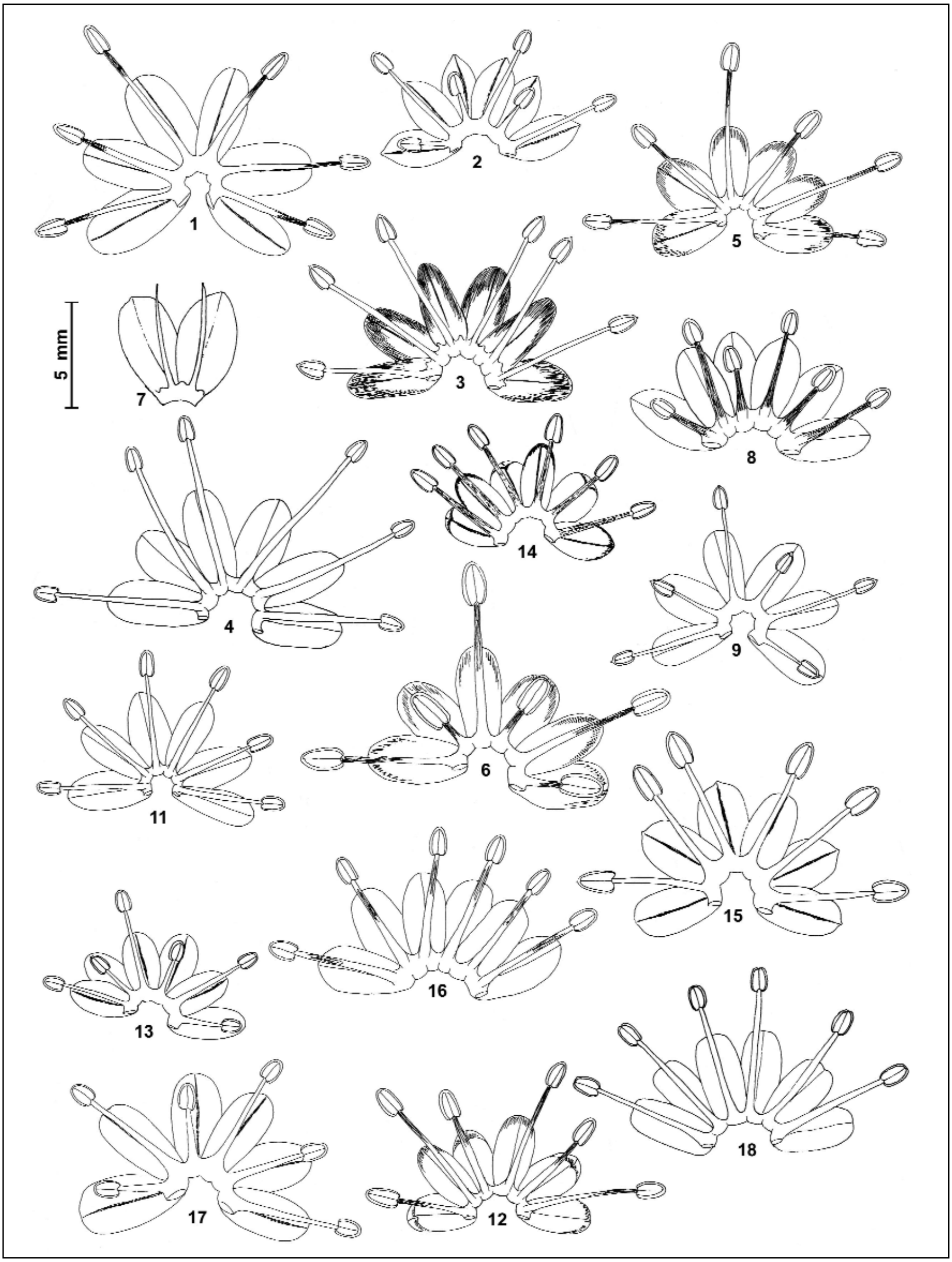

Fig. 2. Perigon and stamens of the investigated species of *Allium stamineum* group: 1 - *A. stamineum*, 2 - *A. decaisnei*, 3 - *A hymettium*, 4 - *A. guicciardii*, 5 - *A. phrygium,* 6 - *A. albotunicatum,* 7 - *A. hermoneum*, 8 - *A. pictistamineum,* 9 - *A pseudoflavum,* 10 - *A. pseudostamineum*, 11 - *A. deciduum*, 12 - *A. retrorsum,* 13 - *A. cyprium,* 14 - *A. lefkarense,* 15 - *A. marathasicum*, 16 - *A. dodecanesi,* 17 - *A. daninianum,* 18 - *A. garganicum*.

cm long, the smaller up to 2 cm long. Inflorescence up to 15-flowered; pedicels 10-20 mm long. Perigon with tepals subequal, widely obtuse, 5-5.2 × 2.7-2.8 mm. Stamens simple, equal, 5 mm long, connate at base into an annulus 0.6 mm high with interstaminal lobes.

Karyology: Shmida & Kollmann (1977) and Kollmann (1985) reported both diploid and tetraploid chromosome counts, 2*n*=16 and 2*n*=32 respectively, for specimens of *locus classicus*.

Ecology: Alpine belt at an altitude of 2000-2750 m.

Distribution: Syria and Lebanon (Mt. Hermon).

Allium pictistamineum O. Schwarz, Feddes Repert. 36: 72 (1934).

Typus: *Lectotypus*: Magnesia Sipyli, in rupibus calcareis supra Akpunar, Sipyli pedis, c. 200 m, VI.1933, *Schwarz 696* (B!).

Iconography: Fig. 1.8 - 2.8 a, b, c.

Description: It differs from *A. stamineum* in having bulb 12-16 × 5-12 mm, with inner tunics purple. Scape flexuous, 8-45 cm high, covered by leaf sheaths for 1/2 of its length. Leaves up to 30 cm long. Spathe with larger valve 2-4 cm long, the smaller one 2-3 cm long. Inflorescence with pedicels 10-20 mm long. Perigon subcylindrical-campanulate, with tepals equal, greenish yellow below and green pruinose tinged with lilac above, slightly obtuse at apex, 4-4.5 mm long and 2 mm wide. Stamen filaments 2.5-4 mm long, violet, connate at base into an annulus 1 mm high; anthers straw-yellow, 1 × 0.6 mm. Ovary obovoid, greenish, tuberculate above, 2.5 × 2 mm. Style 0.5-1 mm long. Capsule obovoid, 4.5 × 3.5 mm.

Karyology: The plants studied have a diploid chromosome complement 2*n*=16. The karyotype arrangement (Fig. 3.5) can be resumed by the following formula: 2*n*=2x=16: 8 m + 6 msm + 2 sm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 81,48, SYi 72,19, TF% 41,92.

Specimen karyologically examined: Turkey, tra Emiralem e Menemen, Izmir, 19.VI.1998, *Brullo & Pavone* (CAT).

Ecology: Rocky crevices at low altitude.

Distribution: Western Anatolia.

Allium pseudoflavum Vved., Byull. Sredneaz. Gosud. Univ. 19:123 (1934).

Typus: *Lectotypus*: Transcaucasia, Armenia, distr. Nor-Bajazat, in montibus propen pag. Subbotan, 7.VIII.1928, *Zedelmeier & Gejdeman* (LE).

Iconography: Fig. 1.9 - 2.9 a, b, c.

Description: It differs from *A. stamineum* in having bulb 10-13 × 8-10 mm, with outer tunics pale brown striate, the inner ones pale brown. Scape 10-25 cm high. Leaves 4-5, up to 15 cm long. Spathe with larger valve 3-5(-8) cm long, the smaller one 1.5-3.5 cm long. Inflorescence with pedicels 8-15 mm long. Tepals unequal, greenish yellow tinged with brown, oblong, 4-4.5 mm long, the outers 2 mm wide, the inners 1.7 mm wide. Stamens unequal with filaments white, the outers generally included, 1.8-2 mm long, the inners exserted, 4-4.5 mm long, connate at base into an annulus 0.5-0.7 mm high; anthers apiculate at apex, 1.2 × 0.5 mm. Ovary greenish, slightly rugose above, 1.5 × 1.4-1.5 mm. Style 2 mm long. Capsule subglobose, 3.5 × 3.5 mm.

Karyology: Populations from three different localities of C Anatolia show a diploid chromosome complement 2*n*=16; this count agrees with that one quoted by Özhatay

(1993). The karyotype arrangement (Fig. 3.6) can be resumed by the following formula: 2*n*=2x=16: 10 m + 6 msm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 77.71, SYi 84.31, TF% 45.17.

Specimen karyologically examined: Turkey, pantani di Develi (Kayseri), 8.VII.1989, *Brullo & Signorello* (CAT); monti calcari a Nord di Hinis, 6.VII.1989, *Brullo & Signorello* (CAT); Zara, 3.VII.1989, *Brullo & Signorello* (CAT).

Ecology: Steppe meadows and salty marshes.

Distribution: C, N and E Anatolia and N Iran.

Allium pseudostamineum Kollmann & Shmida, Israel J. Bot. 26(3): 138 (1977).

Typus: *Holotypus*: S Hermon, above Majdal Shams, 2100 m, 30.VII.1969, *Shmida* (HUJ!).

Iconography: Fig 2.10 a.

Description: It differs from *A. stamineum* in having bulb oblong-ovoid, 15-20 × 8-15 mm, with outer tunics grey brown, the inner ones membranous, purplish. Scape 10-15(-20) cm high. Leaves up to 8 cm long. Spathe slightly longer than umbel, the larger 3-4 cm long, the smaller 1.5-2 cm long. Inflorescence compact, spherical to hemispherical, up to 50-flowered; pedicels subequal, 15-20 mm long. Perigon with tepals greenish brown, rounded and apiculate or retuse at apex, 5 mm long. Stamens with filaments 5-7.5 mm long. Capsule globose, 4 × 4 mm.

Karyology: A diploid chromosome number 2*n*=16 is reported by Shmida & Kollmann (1977) on specimens from the *locus classicus*.

Ecology: Rocky places of mountain and alpine belts, mainly among gravels in windy habitats.

Distribution: Syria and Lebanon (Mt. Hermon).

Allium deciduum Özhatay & Kollmann, Notes R.B.G Edinb. 41:246 (1983).

Typus: *Holotypus*: Mugla, d. Koycegiz, Sandras dagi, nr Boceli, 1670 m, 24.VII.1977, *E. Özhatay 1219* (ISTE 43971!).

Iconography: Fig. 1.11 - 2.11 a, b.

Description: It differs from *A. stamineum* in having bulb 10-16 × 8-10 mm, with outer tunics fibrous-membranous, greyish brown. Scape 15-30 cm high, covered by leaf sheaths for 1/2 of its length. Leaves 3, up to 18 cm long. Spathe with 2 valves deciduous, unilateral, the larger 7-8-nerved, 2.5-6 cm long, the smaller 4-5-nerved, 2.5-3.5 cm long. Inflorescence with pedicels 10-25 mm long. Perigon greenish yellow tinged with lilac, with tepals unequal, 4 mm long, the outers 1.8-2 mm wide, the inners 1.4-1.5 mm wide. Stamen filaments 4-4.5 mm long, white, connate at base into an annulus 0.5 mm high; anthers 1.1 × 0.6 mm. Ovary obovoid, smooth, 1.7-2 × 1.5-1.7 mm. Style 1-2 mm long. Capsule obovoid, 3-4 × 3-4 mm.

Karyology: The investigated plants from *locus classicus* have a diploid chromosome complement 2*n*=16, the same count quoted by Kollmann (1984) and Özhatay (1993). The karyotype arrangement (Fig. 3.7) can be resumed by the following formula: 2*n*=2x=16: 12 m + 4 msm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 68.56, SYi 86.78, TF% 46.46.

Specimen karyologically examined: Turkey, Sandras Dag, 26.VI.1988, *Brullo*, *Pavone & Signorello* (CAT).

Ecology: Shady rocky places in the under wood of Coniferous forests, at 500-2000 m of altitude.

Distribution: SW Anatolia.

Allium retrorsum (Özhatay & Kollmann) Brullo, Guglielmo, Pavone & Salmeri *st. nov.*

Bas.: *Allium deciduum* Özhatay & Kollmann subsp. *retrorsum* Özhatay & Kollmann, Notes R.B.G Edinb. 41:247 (1983).

Typus: *Holotypus*: Icel, Bolkar Daglari, Arslankoy, Bogazagzi etekleri, 1970 m, 6.VIII.1976, K. Alpinar ISTE 35799 (ISTE!).

Iconography: Fig. 1.12 - 2.12 a, b.

Description: It differs from *A. stamineum* in having bulb 15-20 × 10-14 mm, with outer tunics greyish brown. Scape solitary or paired, 15-25 cm high, covered by leaf sheaths for 1/2 of its length. Leaves up to 20 cm long. Spathe with 2 valves deciduous, unilateral, the larger 5-7-nerved, 4.5-13 cm long, the smaller 3-4-nerved, 2-6 cm long. Inflorescence with pedicels unequal, 10-25 mm long. Tepals greenish yellow tinged with pink and purple striate at margins, rounded at apex, 4.5 mm long, the outers 2 mm wide, the inners 1.5-1.8 mm wide. Stamen filaments 2-5.5 mm long, connate at base into an annulus 0.4-0.5 mm high; anthers 1.8 × 0.8 mm. Ovary ellipsoid, greenish, 1.5-2 × 1.2-1.8 mm. Style 1-2 mm long. Capsule subglobose, 3.5 × 3.5 mm.

Karyology: Specimens from *locus classicus* are diploid with a chromosome complement $2n=16$. The karyotype arrangement (Fig. 3.8) is very different from that one of *A. deciduum* and it can be represented by the formula: $2n=2x=16$: 8 m + 4 msm + 2 sm + 2 st. The index values expressing the karyotype symmetry degree are: Stebbins' categories 2A, REC 79.22, SYi 63.12, TF% 38.30.

Specimen karyologically examined: Turkey, Arslankoy (Mersin), 2.VII.1988, *Brullo, Pavone & Signorello* (CAT).

Ecology: Shrub communities of the mountain belt.

Distribution: SE Anatolia.

Allium cyprium Brullo, Pavone & Salmeri, Candollea 48: 280 (1993).

Typus: *Holotypus*: cultivated plant originating from Cyprus, versante occidentale di monte Olympus, ca. 1600 m, 20.VI.1989, *Brullo & Pavone* s.n. (CAT!).

Iconography: Fig. 1.13 - 2.13 a, b, c.

Description: It differs from *A. stamineum* in having bulb 15 × 8 mm, with outer tunics decaying, the inner ones hyaline. Scape green violet, solitary or paired, 10-13 cm high, covered by leaf sheaths for 1/2 of its length. Leaves 8-20 cm long. Spathe sometimes shorter than umbel, with larger valve 6-nerved, 2.5-3.5 cm long, the smaller one 3-nerved, 1-2 cm long. Inflorescence few-flowered with pedicels 5-15 mm long. Perigon ovoid-subglobose, with tepals equal, dark violet, 3-3.5 mm long and 1.5-1.7 mm wide. Stamens with filaments black violet, the outers included, 2 mm long, the inners exserted, 4.5 mm long, connate at base into an annulus 0.5 mm high; anthers ovoid-ellipsoid, straw-yellow, 1 mm long. Ovary subglobose narrowed at base, rugose above, 1.5 × 1.5 mm. Style 1 mm long. Capsule subglobose, flattened above, 3.5 × 5 mm.

Karyology: The plants from *locus classicus* have a diploid chromosome complement $2n=16$. The karyotype arrangement (Fig. 3. 9) can be resumed by the following formula:

2*n*=2x=16: 12 m + 4 msm; sometimes one metacentric pair shows microsatellites on the short chromosome arms. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 82.78, SYi 86.73, TF% 46.28.

Specimen karyologically examined: see type specimen.

Ecology: In *Pinus brutia* woods and dwarf shrub communities, 1400-1700 m asl.

Distribution: Cyprus (Mt. Troodos).

Allium lefkarense Brullo, Pavone & Salmeri, Candollea 48: 280 (1993).

Typus: *Holotypus*: Cipro: colline presso Lefkara, in ambienti rocciosi, esemplare coltivato, 2.VI.1989, *Brullo & Pavone* s.n. (CAT!).

Iconography: Fig. 1.14 - 2.14 a, b, c.

Description: It differs from *A. stamineum* in having bulb 7-12 × 12-20 mm, with outer tunics dark brown, decaying, the inner ones membranous orange or brown. Scape 20-40 cm high, covered by leaf sheaths for 1/3-1/2 of its length. Leaves 8-27 cm long. Spathe shorter than umbel or subequal, with larger valve 5-nerved, 2-4 cm long, the smaller one 3-nerved, 1-2 cm long. Inflorescence with pedicels 10-30 mm long. Perigon obovoid-subglobose to slightly campanulate, with tepals unequal, greenish with purple striae at apex and purplish green midvein, 3-3.5 mm long, the outers elliptical, slightly hooded at apex, 2 mm wide, the inners oblong, 1.5 mm wide. Stamen filaments dark purple, up to 4 mm long, sometimes the outers included and 1.5-2 mm long, connate at base into an annulus 0.5 mm high; anthers 1.3 mm long. Ovary ovoid-pyriform, smooth, 1.7-1.8 × 1.5-1.7 mm. Style purple, 1 mm long. Capsule globose, 3.5 × 3.5 mm.

Karyology: The plants from *locus classicus* are diploid with a chromosome complement 2*n*=16. The karyotype arrangement (Fig. 3.10) is represented by the formula: 2*n*=2x=16: 14 m + 2 msm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 80,58, SYi 89,26, TF% 47,03.

Specimen caryologically examined: see type specimen.

Ecology: Xeric garigues, 100-200 m asl.

Distribution: Cyprus.

Allium marathasicum Brullo, Pavone & Salmeri, Candollea 48: 283 (1993).

Typus: *Holotypus*: cultivated plant originating from Cyprus, Marathasa presso Prodhromos, negli incolti, 7.VI.1989, Brullo & Pavone s. n. (CAT!).

Iconography: Fig. 1.15 - 2.15 a, b.

Description: It differs from *A. stamineum* in having bulbiliferous bulb, 8-10 × 12-13 mm, with outer tunics decaying, the inner ones hyaline. Scape 28-45 cm high, covered by leaf sheaths for 1/2 of its length. Leaves 15-25 cm long. Spathe with larger valve 5-nerved, 7-10 cm long, the smaller one 3-nerved, 3-4.5 cm long. Inflorescence with pedicels up to 20 mm long, erect at fruiting. Perigon campanulate-urceolate, with tepals unequal, elliptical, apiculate at apex, greenish brown with brown midvein, 4-4.5 mm long, the outers 2.2-2.3 mm wide, the inners 2 mm wide. Stamen filaments white, 5 mm long, connate at base into an annulus 0.8 mm high; anthers 1.7 mm long. Ovary subglobose-pyriform, tuberculate above, 2.2 × 2 mm. Style 3.5 mm long. Capsule subglobose, 4 × 3.5 mm.

Karyology: This is the only taxon found to be triploid with a somatic chromosome number 2*n*=24. Its karyotype structure (Fig. 3.11) is well represented by the chromosome formula: 2*n*=3x=24: 15 m + 6 msm + 3 sm; sometimes one metacentric triplet showed microsatellites on the short chromosome arms. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 86.47, SYi 77.83, TF% 43.62.

Specimen karyologically examined: see type specimen.

Ecology: Synanthropic habitats along the road.

Distribution: Cyprus.

Allium dodecanesi Karavokyrou & Tzanoudakis, Ann. Musei Goulandris 9: 141 (1994).

Typus: *Holotypus*: cultivated plant, originated from Greece, prov. Dodecanissos, Island Lipsos, 1 April 1991, Panitsa 2466 (UPA).

Iconography: Fig. 1.16 - 2.16 a, b.

Description: It differs from *A. stamineum* in having bulb globose-ovoid, 10-15 × 8-12 mm, with outer tunics blackish, the inner ones whitish. Scape 20-40 cm high, covered by leaf sheaths for 1/2 of its length. Leaves 3-5, up to 16 cm long. Spathe with larger valve 3.5-8 cm long, the smaller one 1.5-2 cm long. Inflorescence 20-40-flowered, with pedicels 10-25 mm long. Perigon with tepals equal, elliptical, greenish white tinged with pink-purple, rounded at apex, 4-5 mm long and 1.8-2 mm wide. Stamen filaments 4.5-5 mm long, connate at base into an annulus 0.8-1 mm high; anthers 1.3 × 0.7 mm. Ovary obovoid or obovoid-ellipsoid, slightly rugose above, 2-2.5 × 1.8 mm. Style up to 5 mm long. Capsule globose, 4 × 4 mm.

Karyology: As already recorded by Karavokyrou & Tzanoudakis (1994), specimens from Kalimnos are diploid with a somatic chromosome number 2*n*=16. The karyotype arrangement (Fig. 3.12) can be represented by the formula: 2*n*=2x=16: 10 m + 2 msm + 4 sm, rather similar to that one reported in the protologue. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 84.33, SYi 77.63, TF% 43.70.

Specimen karyologically examined: Greece, Kalimnos, La Passi (calcari), 31.VIII.1994, *Brullo C18* (CAT).

Ecology: Phrygane near the coast and *Pinus* woods.

Distribution: Dodecanese archipelago (Lipsos and Kalimnos).

Allium daninianum Brullo, Pavone & Salmeri, Willdenowia 26: 239 (1996).

Typus: *Holotypus*: Israel, Esdraelon Valley, near Yoqneam, 15.V.1990, Brullo A56, cult. in the Botanical Garden of Catania (CAT!).

Iconography: Fig. 1.17 - 2.17 a, b, c.

Description: It differs from *A. stamineum* in having bulb 7-12 × 6-10 mm, with outer tunics black or dark violet, decaying, the inner ones hyaline. Scape 5-25 cm high. Leaves 4-5 with sheaths tinged with violet. Spathe with larger valve 8-20 cm long, the smaller one 3.5-10 cm long. Inflorescence up to 80-flowered; pedicels 15-40 mm long. Perigon conical-campanulate, with tepals subequal, purplish pink to purple with dark purple midvein, 4-5 mm long and 2-2.3 mm wide. Stamen filaments unequal, purple above, the outers 2-4 mm long, the inners 4-5 mm long, connate at base into an annulus 1 mm high; anthers 1.2 mm long. Ovary subglobose-ovoid, entirely papillose, 2 × 2.2 mm. Style purplish below, 1.5 mm long. Capsule globose, 3-3.5 × 3.2-3.5 mm.

Karyology: As already quoted by Brullo & al. (1996), this species is diploid with a somatic chromosome number 2*n*=16, the same count reported by Kollmann (1985) for Israel pop-

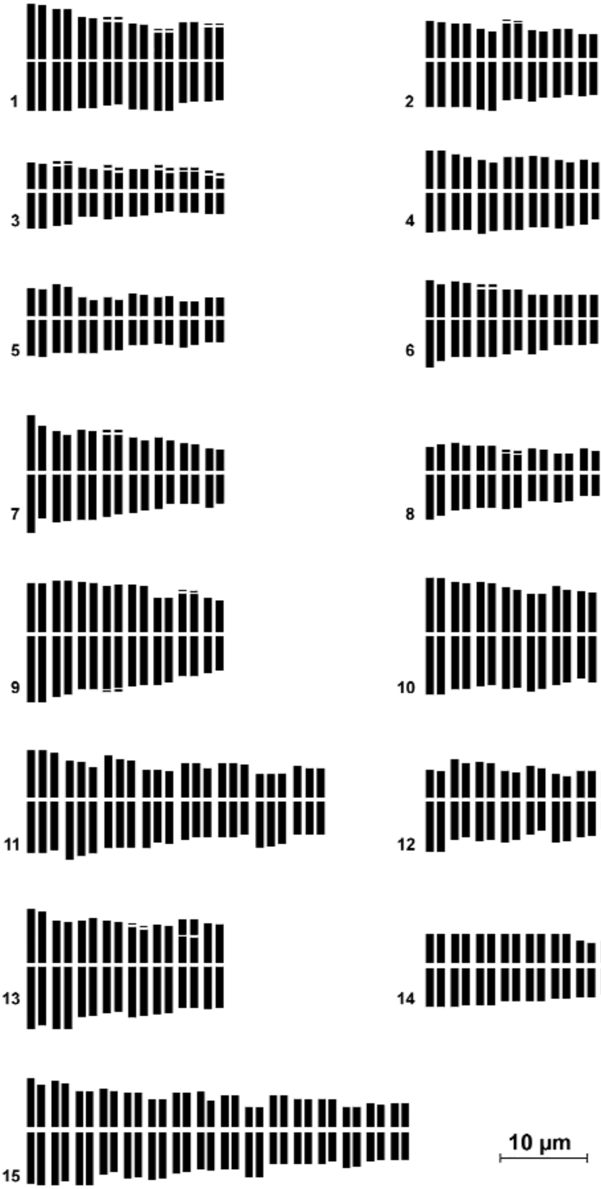

Fig. 3. Karyotypes of the examined species of the *Aliium stamineum* group. 1 - *A. stamineum*, 2 - *A. decaisnei*, 3 - *A hymettium*, 4 - *A. phrygium*, 5 - *A. pictistamineum*, 6 - *A pseudoflavum*, 7 - *A. deciduum*, 8 - *A. retrorsum*, 9 - *A. cyprium*, 10 - *A. lefkarense*, 11 - *A. marathasicum*, 12 - *A. dodecanesi*, 13 - *A. daninianum*, 14 - *A garganicum*, 15 - *A. guicciardii*.

ulations of *A. stamineum*. The karyotype arrangement (Fig. 3.13) can be represented by the formula: $2n=2x=16$: 10 m + 4 msm + 2 $\mathrm{sm}^{\mathrm{sat}}$. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 87.83, SYi 81.53, TF% 44.13.

Specimen karyologically examined: Israel, Esdraelon Valley, near Yoqneam, 15.V.1990, cult., *Brullo A56* (CAT); Judean Foothills, near Netiv Halamed-hei, 10.V.1989, cult., *Brullo A1* (CAT); Judean, Mt. Kiryat Anavim, 5.V.1989, cult. *Brullo A14* (CAT).

Ecology: Maquis, dwarf shrub communities and rocky places, 0-900 m asl.

Distribution: Middle East (Israel, Lebanon, Jordan, Transjordan, Syria).

Allium garganicum nom. provv.

Iconography: Fig. 1.18 - 2.18 a, b, c.

Description: It differs from *A. stamineum* in having scape up to 40 cm high, covered by leaf sheaths up to 1/2 of its length. Leaves 4-5, green, semicylindrical, costate, up to 20 cm long. Spathe with larger valve 5-6-nerved, 7-11 cm long, the smaller 4-5-nerved, 4-6 cm long. Inflorescence 12-35-flowered; pedicels unequal, flexuous, 20-25 mm long. Perigon conical-campanulate, with tepals yellowish to greenish yellow, slightly pruinose, 4-4.5 mm long, 1.8-2 mm wide. Stamens with filaments 5-6 mm long, white, connate at base into an annulus 0.4-0.5 mm high; anthers 1-1.1 × 0.5-0.6 mm. Ovary slightly papillose above, 2 × 1.9-2 mm. Style 3.5-5 mm long. Capsule ellipsoid, 3.2-3.5 × 2.8-3 mm.

Karyology: Plants from the *locus classicus*, the only station presently known, show a diploid chromosome complement $2n=16$. The karyotype (Fig. 3.14), quite regular with rather small chromosomes, can be resumed by the formula: $2n=2x=16$: 14 m + 2 msm. The index values expressing the karyotype symmetry degree are: Stebbins' categories 1A, REC 79.79, SYi 88.80, TF% 47.03.

Ecology: Rocky meadows near the coast.

Distribution: SE Italy (Gargano peninsula).

Specimen karyologically examined: Puglia, Gargano, stazioni calcaree semirupestri presso Peschici, 6.7.2002, *Brullo & Giusso* (CAT).

Discussion

The marked morphological and karyological diversity among the populations of the *A. stamineum* group should be the result of many chorological, environmental and biological factors, as paleogeographic events, ecological and edaphic requirements, reproductive strategies. They surely played an important role in the evolutionary processes within this group, the same as we already observed in other sister groups of *A.* sect. *Codonoprasum*, such as *A. paniculatum* L., *A. staticiforme* Sm. and *A. hirtovaginum* Cand. (Brullo & al. 1991, 1994, 1995, 1996, 1997, 1997a, 1997b, 2001, 2003, 2004).

The group has its speciation centre in the Anatolian peninsula, where it counts the most number of different taxa and widespread populations. Then, its geographic distribution extended both towards West in the Balkan peninsula and to East in the Iranian territories (Fig. 4), where a lot of taxa with a punctiform distribution are known (Shmida & Kollman 1977a; Brullo & al. 1992, 1993, 1996; Karavokyrou & Tzanoudakis 1994). Based on publishing data, a new species from Puglia (S Italy), here provisionally designed as *A. garganicum*, represents a very significant record that further widens the geographic distribution of *A. stamineum* group towards West Mediterranean area and improves the Italian floristic set in eastern elements.

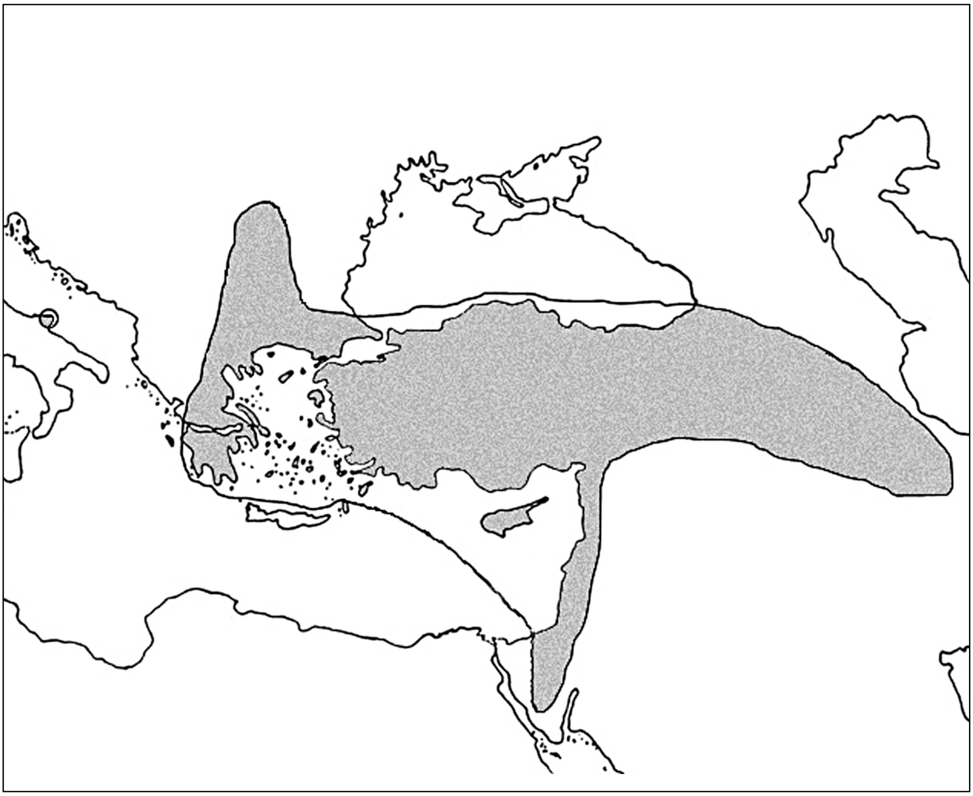

Fig. 4. Geographic distribution of the *Allium stamineum* group in the Mediterranean area.

Most species are linked to conservative habitats, as rupestrian and rocky places, typically cliffs, garigues and meadows, or sometimes open pinewoods and salt marshes. Populations growing in these habitats are prevalently diploid with a somatic chromosome number 2*n*=16 and probably the marked speciation processes in this group were favored by geographic isolation or adjustment to different ecological conditions. Colonization of secondary habitats as synanthropic ones, instead, seems to be favoured by a polyploid arrangement of chromosome complement, as we can observe in *A. marathasicum* from Cyprus, a triploid species (2*n*=24) localized in ruderal places, and *A. guicciardii* from Balkan Peninsula, tetraploid (2*n*=32) and often growing in seminatural habitats disturbed by human activities.

The following analytical keys were realized for comparing and better distinguishing the different taxa within the *A. stamineum* group.

1 Perigon 3-4 mm long.......... **2**
1 Perigon 4-5.5 mm long.......... **4**
2 Stem 10-13 cm tall; tepals dark violet; capsule 5 mm wide.......... ***A. cyprium***
2 Stem 15-40 cm tall; tepals greenish or greenish yellow; capsule 3.5-3.8 mm wide.......... **3**
3 Perigon campanulate; tepals sublanceolate, acute at apex; stamen filaments and style white; ovary entirely papillose.......... ***A. decaisnei***
3 Perigon obovoid-subglobose; tepals oblanceolate-elliptical, rounded at apex; stamen filments and style purple; ovary smooth.......... ***A. lefkarense***
4 Leaves flat; tepals purplish pink; ovary entirely papillose; style purplish below.......... ***A. daninianum***
4 Leaves semicylindrical; tepals greenish to yellow; ovary smooth to slightly papillose above; style white.......... **5**
5 Outer bulb tunics white-grey; perigon obovoid.......... **6**
5 Outer bulb tunics pale brown to black; perigon campanulate to campanulate-urceolate.......... **7**
6 Larger spathe valve 3-6 cm long; smaller one 2.5-4.5 cm; umbel 15-50-flowered; perigon 4-4.5 mm long; interstaminal teeth absent.......... ***A. albotunicatum***
6 Larger spathe valve 3 cm long; smaller one 2 cm; umbel max. 15-flowered; perigon 5-5.2 mm long; interstaminal teeth present.......... ***A. hermoneum***
7 Spathe valves deciduous, unilateral.......... **8**
7 Spathe valves persistent, opposed.......... **9**
8 Outer bulb tunics fibrous-membranaceous; larger spathe valve 7-8-nerved, 2.5-6 cm long; umbel 7-20-flowered; perigon 4 mm long; stamen filaments entirely white; anther 1.1 mm long; ovary obovoid; capsule obovoid.......... ***A. deciduum***
8 Outer bulb tunics coriaceous; larger spathe valve 5-7-nerved, 4.5-13 cm long; umbel 20-30-flowered; perigon 4.5 mm long; stamen filaments purple above; anther 1.8 mm long; ovary ellipsoid; capsule subglobose.......... ***A. retrorsum***
9 Larger spathe valve 5-6-nerved.......... **10**
9 Larger spathe valve 7-nerved.......... **11**
10 Smaller spathe valve 3-nerved; perigon campanulate-urceolate; tepals greenish brown, 2-2.3 mm wide, apiculate at apex; anther 1.7 mm long; capsule subglobose, 4 mm long.......... ***A. marathasicum***
10 Smaller spathe valve 4-5-nerved; perigon conical-campanulate; tepals greenish yellow, 1.8-2 mm wide, rounded at apex; anther 1-1.1 mm long; capsule ellipsoid, 3.2-3.5 mm long.......... ***A. garganicum***
11 Outer bulb tunics black; tepals greenish white.......... ***A. dodecanesi***
11 Outer bulb tunics brown; tepals greenish yellow to greenish brown.......... **12**
12 Perigon 4-4.5 mm long.......... **13**
12 Perigon 4.5-5.5 mm long.......... **16**
13 Outer bulb tunics dark brown; stem up to 45 cm tall; leaves up to 30 cm long; tepals obtuse at apex; stamen filaments entirely violet; ovary obovoid, above tuberculate, 2.5 mm long; capsule obovoid, 4.5 mm long.......... ***A. pictistamineum***

13 Outer bulb tunics pale brown; stem max. 30 cm tall; leaves up to 16 cm long; tepals rounded at apex; stamen filaments entirely white or purple above; ovary subglobose or subglobose-ovoid, smooth or rugose above, 1.5-2 mm long; capsule globose-subglobose, 3.5 mm long..**14**

14 Outer bulb tunics coriaceous; leaves up to 10 cm long; tepals pinkish yellow; interstaminal teeth present...***A. hymettium***

14 Outer bulb tunics striate-coriaceous; leaves up to 16 cm long; tepals greenish yellow; interstaminal teeth absent...**15**

15 Stamen filaments white and purple above, subequal, 3.5-6 mm long, annulus 0.3-0.5 mm high; anthers rounded at apex; ovary smooth...***A. phrygium***

15 Stamen filaments white, unequal, the outers 1.8-2 mm long, the inners 4-4.5 mm long; annulus 05-0.7 mm high; anthers apiculate at apex; ovary rugose................***A. pseudoflavum***

16 Inner bulb tunics purplish; stem 10-15 (-20) cm tall; leaves to 8 cm long; larger spathe valve 3-4 cm long; smaller spathe valve 1.5-2 cm long; umbel compact spherical to hemispherical; tepals greenish brown, apiculate or retuse at apex.................................***A. pseudostamineum***

16 Inner bulb tunics whitish; stem (10)-15 -50 cm tall; leaves to 25 cm long; larger spathe valve to 15 cm long; smaller spathe valve 2.5-9 cm long; umbel lax, diffuse; tepals greenish yellow, rounded at apex...**17**

17 Leaves 3-4; perigon conic campanulate; tepals tinged with purple; stamen filaments 4-6.5 mm long; annulus 0.6-1 mm high; ovary subglobose; style 1.5-2.5 mm long; capsule obovoid stipitate...***A. stamineum***

17 Leaves 4-6; perigon campanulate; tepals pruinose; stamen filaments 6.5-7 mm long; annulus 04-06 mm high; ovary subglobose-ovoid; style 4-5 mm long; capsule subglobose.................
....***A. guicciardii***

Acknowledgements

This work was supported by research funds granted by the University of Catania. Special thanks to the Herbarium Curators of B, BM, C, CAT, FI, G, HUJ, ISTE, M, OXF, P, PAL and UPA.

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Martin Bobinac & Mirjana Ocokoljić

Growth variability of one-year-old beech (*Fagus moesiac*a Cz.) seedlings and their coppice vigour

Abstract

Bobinac, M. & Ocokoljić, M.: Growth variability of one-year-old beech (*Fagus moesiaca* Cz.) seedlings and their coppice vigour. — Bocconea 21: 345-348. 2007. — ISSN 1120-4060.

Height growth of one-year old beech (*Fagus moesiaca* Cz.) seedlings was studied in different environmental conditions: in a closed stand and in a felled site. In a closed stand, the seedlings form a phenotype of a sciomorphic structural form, whereas the seedlings in the felled site show significant divergences compared to the unique and complex biological properties of the seedlings formed in the conditions of the closed canopy. A greater significance of differences between the identical biological properties depends on the sequence of their formation, and the most expressive differences occur in the development of the “above-epicotyl-axis”. The significantly different length of the “above-epicotyl-axis” in the seedlings formed in different environmental conditions also conditions a significantly different number of the leaves and axillary buds. In this way, in the same seedling growth stage, the potential of the coppicing base of the dormant buds in the seedlings of sciomorphic structural form (two opposite buds in cotyledon axil, two opposite buds in primary leaf axils) is significantly different from the potential of the coppicing base from the dormant buds in the seedlings with a higher growth intensity of the “above-epicotyl-axis”.

Introduction

In the earliest phases of ontogenesis, the environmental conditions are decisive for the biological characteristics of Moesian beech (Bobinac & Vilotić 1995, 1996; Bobinac 1999, 2002, 2003). In this way, environmental conditions also affect the coppice vigour of beech dormant buds occurring after several decades of direct anthropogenic influence. After Pjatnickij & al. (1963), the application of a particular stand cultivation system can also affect the coppicing base. Taking into account that the coppicing base of a stump is defined by stump diameter and height, and that under a particular silvicultural system stump diameter depends mostly on tree age, while stump height is a constant magnitude, the coppicing base potential of the preventive buds of adult beech trees is primarily conditioned by the growth characteristics of seedlings in the initial phases of development.

This paper deals with the coppicing base potential of the preventive buds in one-year old beech seedlings from different site condiztions.

Material and methods

Morphological characteristics of beech seedlings during the first vegetation growth period were studied on two types of regeneration areas at the site of montane beech forest on deep eutric brown soil. Fifty dominant seedlings with undisturbed growth were analysed from each the closed canopy part of the stand and the central part of the circular felled site, by the random sample principle. The following quantitative parameters were measured:

1. length of hypocotyl, from the base of cotyledons to the widest part of root collar zone (H_y),
2. length of epicotyl, from the base of cotyledons to the base of primary leaves (E_p),
3. length of "above-epicotyl-axis", from the axil of primary leaves to the base of terminal bud (I_h),
4. thickness of the widest part of root collar zone (d_{kv}),
5. thickness of the hypocotyl, about 5 mm below the base of cotyledons (d_{Hy}),
6. thickness of the middle of epicotyl part (d_{Ep}),
7. number of leaves (B.L.),
8. number of large vegetative buds, terminal and large lateral buds (B.P.).

All linear parameters are expressed in mm. The testing of differences between mean values was performed by t-test.

Results

In beech germination, cotyledons emerge above the ground surface (epigeal germination – intermediary type according to Schütt & Werner 1978). The mean values of the study seedling parameters and the results of t-test are presented in Table 1. In the conditions of the closed canopy, the length of "above-epicotyl-axis" ranged from 0.4 mm to 3.0 mm, mean length was 2.01 mm. In the conditions of the felled site, the length of syleptically formed "above-epicotyl-axis" ranged from 15.0 mm to 112.0 mm, mean length was 56.28 mm. The seedling height during the first year ranged between 81.6 mm and 144.0 mm, with mean height 113.19 mm in the conditions of the closed canopy and 145.0 mm to 314.0 mm, with mean height 188.06 mm, in the conditions of the felled site. The seedlings in the conditions of the closed canopy form two opposite primary leaves, and the seedlings in the felled site, in addition to the two opposite leaves on the "above-epicotyl-axis", also form 4-15 alternate leaves.

In the same seedling growth stage, the total potential of the coppicing base of the dormant buds in the seedlings of sciomorphic structural form (two opposite buds in the cotyledon axil, two opposite buds in primary leaf axils) is lower than the potential of the coppicing base of seedlings with a higher growth intensity of the "above-epicotyl-axis". Also, on the averagely shorter length of the internode between the two opposite buds in the cotyledon axil and in primary leaf axils, the potential coppicing base of the dormant buds is higher in the seedlings growing on the felled site. However, the total potential coppicing base of the preventive buds at the constant stump height is higher, per stump height unit, in adult beech trees originating from the seedlings of sciomorphic structural form than in beech trees originating from the seedlings with a more intensive growth in the first and in the following years.

Table 1: Mean values of biological parameters of Moesian beech seedlings in the first year in different stand conditions and the results of t-test.

Parameter	Stand conditions		Probability	
	Closed canopy	Felled site	*p*	significance
H_y (*mm*)	75.40	70.22	0.049485	*
E_p (*mm*)	35.77	31.36	0.001972	**
I_h (*mm*)	2.01	56.28	0.000000	***
H_t (*mm*)	113.19	188.06	0.000000	***
d_{kv} (*mm*)	1.81	3.18	0.000000	***
d_{Hy} (*mm*)	1.22	2.18	0.000000	***
d_{Ep} (*mm*)	0.82	1.89	0.000000	***
B.L. (pcs)	2.00	8.14	0.000000	***
B.P. (pcs)	1.00	4.40	0.000000	***

Conclusions

Seedlings formed in the closed canopy have the phenotype of sciomorphic structural form, with a poorly expressed (regularly leafless) syleptically formed “above-epicotyl-axis”.

Seedlings in the felled site regularly have a more intensive growth of “above-epicotyl-axis” than the seedlings in the completely closed canopy and thus the higher seedlings at the end of the first vegetation period, more numerous leaves, i.e. a higher number of vegetative buds in their axils.

In the same seedling growth stage, the potential of the coppicing base of the dormant buds in the seedlings of sciomorphic structural form is lower than the potential coppicing base of the seedlings with a higher growth intensity of the “above-epicotyl-axis”.

The total potential of the coppicing base of dormant buds at the constant stump height, which is under the direct anthropogenic influence, is higher in adult beech trees originating from the seedlings of sciomorphic structural form.

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D. Gargano, L. Peruzzi, K. F. Caparelli & G. Cesca

Preliminary observations on the reproductive strategies in five early-flowering species of *Gagea* Salisb. (*Liliaceae*)

Abstract

Gargano, D., Peruzzi L., Caparelli, K. F. & Cesca, G.: Preliminary observations on the reprodutctive strategies in five early-flowering species of *Gagea* Salisb. (*Liliaceae*). — Bocconea 21: 349-358. 2007. — ISSN 1120-4060.

The field and laboratory studies about *Gagea bohemica* (Zauschn.) Schult. & Schult. f., *G. chrysantha* Schult. & Schult. f. s. l., *G. granatellii* (Parl.) Parl., G. lutea (L.) Ker-Gawl., *G. fragifera* (Vill.) Ehr. Bayer & G. Lòpez are carried on community structure, phenology and reproductive biology. The studied species show differences in terms of community characteristics (species composition, structure), time and duration of flowering, and seed production. The species of rocky environment (*G. granatellii* and *G. bohemica*) show the highest number of vegetative individuals with respect to the flowering ones, the shortest duration of flowering and the lowest seed-set. It appears as a consequence of the low resource availability. The species of meadows (*G. chrysantha*, *G. fragifera* and *G. lutea*) appear instead to suffer overall the biological competition for resources and pollinators. Gross morphological analysis of the pollen grains revealed that deformed pollen is more abundant in triploid (*G. chrysantha, G. granatellii*) and heptaploid (*G. fragifera*) species, compared to the tetraploid (*G. bohemica*) and the hexaploid (*G. lutea*) ones. Seed production was very low. There is a positive correlation between amount of morphologically regular pollen and number of produced seeds. In most of the studied species we observed a trend to increase the seed production in individuals with many flowers; probably this is the result of a strategy aimed diminishing the effects of pollen- and pollinator-limitation.

Introduction

The species of the genus *Gagea* Salisb. appear typically insect-pollinated plants, which offer nectar (Orueta & Viejo 1996) and/or pollen (Petanidou & Vokou 1990) as reward. In terms of sexual reproduction, the performance of these plants is affected by their early flowering and probably by intrinsic factors (i.e. ploidy levels), which can reduce the sexual efficiency and favor the vegetative propagation. Early flowering plants have a short suitable period for their reproductive efforts. Indeed, in woodland areas, the extent of this period goes from the first prolonged phase of weather suitable for pollinators activity to the time of the canopy closure (Schemske & al. 1978). The rigidly preset time suitable for reproduction can produce in the flowering of these plants the strong seasonality observed in Mediterranean communities (Petanidou & al. 1995). Moreover, early flowering plants are often subject to unpredictable pollinator activity and to problems of pollen limitation. For these reasons, they adopted strategies for the reproduction enhancement, as for instance the increase of the number of flowers which sequentially occur for each inflorescence (Harder & Thomson 1989; Nishikawa & Kudo 1995; Nishikawa 1998). Another aspect influencing the reproduction success in early flowering plants is the resource availability, overall in order to promote or suppress flowering. Consequently, these plants balance the resource allocation among the different phases of their life-cycle (Dafni & al. 1981), eventually varying from year to year their flowering rates.

Considering the special scientific interest of early flowering plants, we studied the behavior of five *Gagea* species in Calabria (S Italy), which in that region typically occur in small and isolated populations (Peruzzi & Gargano 2005). We attempted to understand the possible link between ecological conditions and population dynamics. In this way, we used also a community level approach to estimate the effects of pollen and resource competition on the reproductive strategies of the studied species.

Here are presented the preliminary results on community structure, phenology and reproductive biology research of *G. bohemica*, *G. chrysantha*, *G. fragifera*, *G. granatellii* and *G. lutea*.

Material and Methods

This study was carried in four localities, whose data were recorded using a G.P.S. The population of *G. bohemica* occurs on calcareous rocky grounds at 1010 m asl (loc. Ospedaletto, Campotenese, province of Cosenza); *G. chrysantha* population is in a *Quercus pubescens* open maquis at 225 m asl (loc. Salerno, Montalto Uffugo, province of Cosenza); *G. granatellii* population occurs along the rocky slope of a river valley at 603 m asl (loc. Giancorella, Rose, province of Cosenza), and finally, at 1283 m asl, in meadows surrounded by beech woods, there are the populations of *G. fragifera* and *G. lutea* (loc. Piano di Mezzo, Masistri, province of Cosenza).

We counted the number of flowering and vegetative individuals of *Gagea* species in plots of 1 m^2, and also identified all the other species recorded within the sample areas and counted their individuals. These data were used to characterize communities and to evaluate their structure by means of Shannon's Index H, a widely used diversity index which takes into account both the number of species and their relative abundances (Shannon 1948; Pielou 1966, 1966a). We checked the flowering of *Gagea* species and other species weekly by counting the buds, flowers and fruits. The plants size and the diameter of open flowers were measured by digital caliper.

The evaluation of fitness in experimental individuals of *Gagea* (9 for each species) was maid by ovule/seed ratio. We related data on reproductive fitness to some morphological aspect (plant size, number and diameter of flowers), analyzed pollen grains and calculated a morphologically regular/deformed pollen ratio, and this parameter was also related to reproductive efficiency and plant morphology. The evaluation of relationships among the different parameters was maid by Pearson's correlation test.

We performed flower manipulations on some individuals for each species, aimed to test the effects of induced cross- and self- pollination. For individual isolation, in order to exclude pollinators and to verify spontaneous self-pollination, we used the cages with fine nylon mesh. Finally, we emasculated and isolated some individuals to test the occurrence of agamospermy.

Results and discussion

Community characterization

G. chrysantha community occurs in Mediterranean belt and it is dominated by early-flowering herbs, i.e. *Scleranthus annuus* L., *Cerastium glomeratum* Thuill., *Sherardia arvensis* L., *Trifolium pratense* L. Data regarding the three plots with *G. chrysantha* are reported in Table 1. There is a substantial variation in the ratio between reproductive and vegetative individuals of *Gagea* among the three plots. Also the *H* value is variable among the sample areas and this indicates differences in terms of number of species and contribution of each species to the total number of community individuals.

G. granatellii occurs also under Mediterranean condition, but on rocky and temporarily wet ground, with many bryophytes and few vascular plants, such as *Theligonum cynocrambe* L., *Sedum hispanicum* L. and *Euphorbia helioscopia* L. The structural characteristics of these communities are summarized in Table 1, which shows also the increasing young *Gagea* individuals percentage at the lowest values of *H*.

G. bohemica grows on rocky substrate. Its community is characterized by other geophytes and some species typical of rocky grasslands, i.e. *Muscari neglectum* Guss., *Allium* sp., *Orchis* sp., *Sesleria calabrica* (Deyl) Di Pietro. We recorded the highest rate of flowering *Gagea* individuals in the plot c, where the low *H* value results from the few species occurring and from the *Gagea* dominance, whose individuals represent more than 79% of the total (Tab. 1).

G. fragifera and *Gagea lutea* occur nearly together in mesophylous grasslands of mountain belt. Their communities are characterized by nitrophylous species, such as *Stellaria media* (L.) Vill., *Urtica urens* L., *Cruciata laevipes* Opiz, *Capsella rubella* Reuter and some grasses (*Poa annua* L., *Milium effusum* L.). Table 1 shows the data related to the plots placed within these communities. The low *H* values appear due to the grasses dominance and we recorded few *Gagea* individuals in the sample areas.

Table 1. Main characters recorded from the plots placed within the communities including *Gagea* populations.

Gagea	*bohemica*			*chrysantha*			*fragifera / lutea*			*granatelli*	
Plot	a	b	c	a	b	c	a	b	c	a	b
N° of species	10	28	6	12	21	32	12	12	15	17	13
Total of individuals	96	194	63	169	233	381	296	437	307	618	374
H	1.88	2.77	0.78	2.03	1.99	2.86	1.40	1.90	1.46	1.27	1.70
N° repr. *Gagea*	0	5	20	1	6	15	3	9	1	8	3
N° veg. *Gagea*	17	45	30	9	6	7	2	24	1	128	46

Table 2. The communities including *Gagea* populations: a) mean *H* value, b) number of co-flowering species.

Community with	a	b
G. bohemica	1.81	3
G. chrysantha	2.29	9
G. fragifera/G. lutea	1.59	8
G. granatelli	1.49	6

Two species groups can be identified, according to their habitats: a first one which live in meadow communities (type A: *G. chrysantha*, *G. fragifera* and *G. lutea*) and a second one living in rocky environments (type B: *G. granatellii* and *G. bohemica*). In both types it is possible to trace a further distinction. Indeed, within type A, *G. chrysantha* occurs – as early element – in secondary open spaces of Mediterranean woodland, i.e. shrubbylands and garigues. On the contrary, both *G. fragifera* and *G. lutea* are included within mesophylous meadows which, according to Maiorca & Spampinato (1999), belong to the *Molinio – Arrhenatheretea*. The latter community probably originates from beech woods fragmentation. As far B type species are concerned, *G. granatellii* appears related to ruderal communities typical of shaded and seasonally wet soils, that Brullo & al. (2001) include in the *Stellarietea mediae*; while *G. bohemica* prefers sites strongly exposed like mountain ridges within *Sesleria calabrica* communities, referable to the *Festuco – Brometea* pastures (Biondi & al. 1988; Abbate & al. 1994; Maiorca & Spampinato 1999).

Comparative phenology

The large overlapping between the phenology of *G. chrysantha* and *G. granatellii* (species occurring in the same climatic belt, but in different habitats) could be seen on Figure 1. The flowering peaks are from the beginning to the half of March. *G. bohemica*, shows the maximum flowering at the beginning of April. Both *G. fragifera* and *G. lutea*, instead, show their flowering peaks at the end of April.

The flowering period is between twenty (*G. granatellii*) and thirthy-five (*G. chrysantha*) days. The large overlapping between flowering times of the species of Mediterranean belt – *G. chrysantha* and *G. granatellii* – can be explained by the necessity to bloom as soon as suitable temperatures permit and before canopy closure (Schemske & al. 1978).

The results of comparison of the flowering phenologies in five species of *Gagea* respect the other flowering species recorded in their communities is shown on Figure 2.

G. chrysantha shows an intermediate flowering pattern comparing with those of other species in its own community. In fact, its blooming is placed between the earliest species (*Romulea bulbocodium* Sebast. & Mauri, *Senecio vulgaris* L., *Cardamine hirsuta* L., *Erophila verna* (L.) Chevall.) and others (*Erica arborea* L., *Cerastium glomeratum* Thuill., *Sherardia arvensis* L., *Geranium molle* L.). As a consequence when *G. chrysantha* is at the blooming

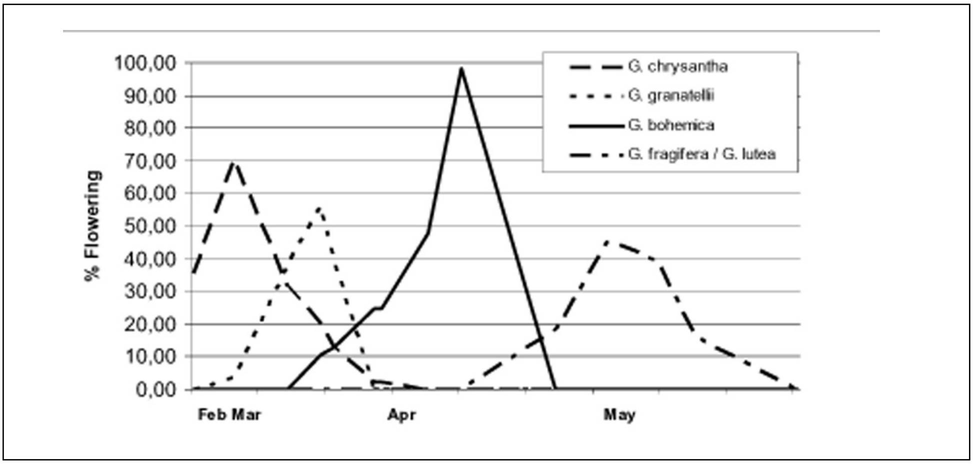

Fig. 1. Comparative flowering phenology of the studied *Gagea* species.

peak its flowers make 31.5% of total flowring, while of *Senecio vulgaris* represent 45%.

The flowering of *G. granatellii* follows *Romulea bulbocodium* and *Cardamine hirsuta*; at maximum flowering, *Gagea* represents 48.4% of the total amount of open flowers. Co-flowering species are *Euphorbia helioscopia* (29%), *Veronica cymbalaria* Bodard (12.9%) and *Vicia sativa* L. var. *angustifolia* (L.) H. T. Ho (6.5%).

G. bohemica is the earliest flowering species within its own community; indeed, other plants (*Senecio vulgaris*, *Saxifraga tridactylites* L., *Muscari neglectum*) start to flower only after the peak of *G. bohemica*.

Finally, *G. fragifera* and *G. lutea* flower together with other species such as *Ranunculus ficaria* L. and *Scilla bifolia* L.; when *G. fragifera* and *G. lutea* reach the blooming peak, their flowers are 81.3% of the total. The decrease of *Gagea* blooming is instead accompanied by the beginning of flowering for *Capsella rubella*, *Cruciata laevipes*, *Stellaria media* and *Taraxacum officinale* Weber.

Two different patterns resulted from the phenology of the studied species. The first one includes *G. chrysantha* and *G. fragifera* / *G. lutea*; it is characterized by a flowering time of thirty-four/thirty-five days and by similar times of blooming increase and decrease. These species occur in sites with good moisture availability but possibly high competition for resources and pollinators, as shown by *H* values and by the high number of co-flowering species (Tab. 2). This is true even if *G. fragifera* and *G. lutea* communities have lower *H* values than those with *G. chrysantha*, because this is a consequence of grasses dominance which reduces the diversity but not the resource competition. Two species with a very similar floral model (both with yellow, stellate flowers/inflorescences) are blooming together with *G. fragifera* and *G. lutea*: *Ranunculus ficaria* and *Taraxacum officinale* group (sect. *Ruderalia*)

The second one includes *G. granatellii* and *G. bohemica*; it shows a flowering time of twenty/twenty-four days and a faster blooming decrease phase. The habitat of these species has low and quickly decreasing resources (because of the drying), so producing a shorter

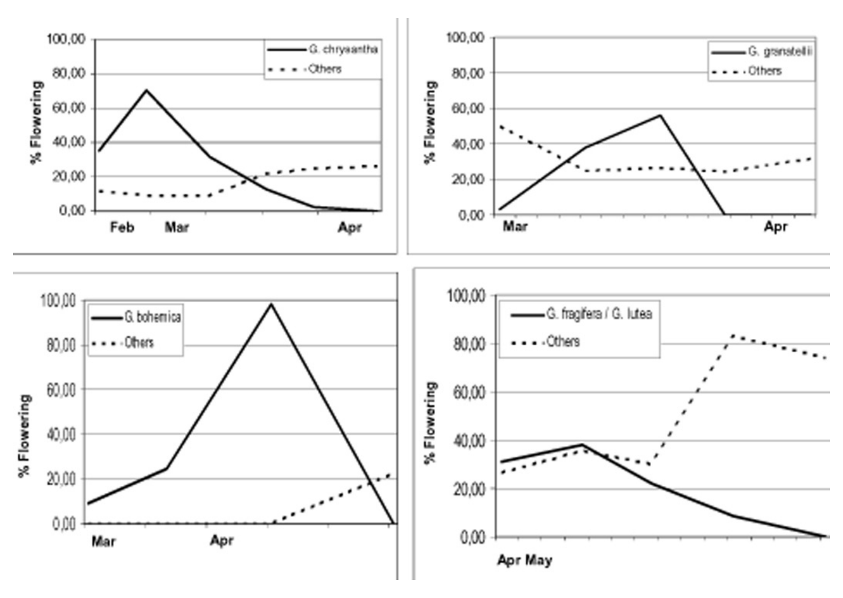

Fig. 2. Comparative flowering phenology among the species of *Gagea* and the other flowering species occurring in their communities.

suitable time for flowering, low *H* values and low number of co-flowering species (Tab. 2). Moreover, in this case, all the co-flowering species show different floral models respect to *Gagea*, i.e. different flower color, shape and size.

We believe that the two patterns result from ecological characteristics of the biotopes and communities in which the species occur. Indeed, it is proved as moisture availability (Dafni & al. 1981; Bell & Stephens 1984; Zimmerman & al 1989; Friedel & al. 1993) and interspecific competition for resources and pollinators (Ratchke 1983; Bawa 1983; Waser 1983) can affect the blooming patterns.

Reproductive performance

Considering morphological aspects, Table 3 shows the results of correlation tests among size of plants, number of flowers and diameter of flowers. All species show a number of flowers increasing with plant size (in *G. lutea* and *G. bohemica* this trend is less evident than in the others). The flower size is negatively related to plant size in *G. lutea* and *G. chrysantha*, while this relation is positive in *G. fragifera*, *G. bohemica* and, less evidently, also in *G. granatellii*. As far the correlation between flower size and number is concerned, Table 3 shows a clear positive relation in *G. lutea* and *G. granatellii*, in *G. fragifera* this relation is not evident, while the trend becomes negative in *G. bohemica* and *G. chrysantha*.

Table 4 shows the results from pollen observation and seed-set. The ratio between morphologically regular and deformed pollen is the lowest in *G. granatellii*, while it reaches

Table 3. Values of Pearson’s coefficient related to the following correlation tests: a) plant size - number of flowers, b) plant size - flowers diameter, c) flowers diameter - number of flowers.

Species	a	b	c
G. bohemica	0.16	0.58	-0.09
G. chrysantha	0.43	-0.21	-0.10
G. fragifera	0.67	0.24	0.18
G. granatelli	0.51	0.12	0.49
G. lutea	0.16	-0.30	0.73

Table 4. Pollen features and seed production: a) morphologically regular/deformed pollen ratio, b) seed/ovule ratio.

Species	a	b
G. bohemica	3.98	0.003
G. chrysantha	2.46	0.019
G. fragifera	1.51	0.009
G. granatelli	0.21	0.000
G. lutea	8.38	0.028

the maximum value in *G. lutea*. The amount of deformed pollen seems to be related to the ploidy level: 3*x* (*G. chrysantha*, *G. granatellii*) and 7*x* (*G. fragifera*) species show pollen more deformed than 4*x* (*G. bohemica*) and 6*x* (*G. lutea*) ones (for information on the ploidy levels of the studied populations cfr. Peruzzi, 2003; Peruzzi & Aquaro, 2005). Seed set is very low in all the species and totally absent in *G. granatellii* (Tab. 4). Although the low seed set, there is a clear positive correlation (P = 0.76) between the amount of morphologically regular pollen and the number of seeds produced. Table 5 summarizes the results originated from correlations among seed production and morphological parameters in each species; for *G. chrysantha* seed-set is negatively related to all the considered morphological traits. In other species, the amount of produced seeds is positively linked to the number of flowers and (weakly) to the size of flowers.

The flower manipulations, aimed to test effects of spontaneous or provocated self- and cross-pollination and occurrence of agamospermy, did not produce seed-set. This occur-

Table 5. Percentage of plants producing seeds - a, Pearson's coefficients for correlations between seed production and: b) plant size, c) number of flowers, d) diameter of flowers.

Species	a	b	c	d
G. chrysantha	55.56	-0.60	-0.17	-0.22
G. granatelli	0.00	//	//	//
G. bohemica	25.00	-0.03	0.85	0.16
G. lutea	37.50	0.19	0.75	0.40
G. fragifera	35.71	0.45	0.44	0.24

rence may be due to the low sexual fitness of the studied populations, at least at the time of our study. Indeed, it is well known as sexual efficiency may vary among different *Gagea* species and/or populations either for weather conditions at flowering time (Orueta & Viejo 1996; Kudo & al. 2004) or the ploidy level (Guerlesquin 1965; Heyn & Dafni 1971; Zarrei & Zarre 2005).

The seed production in the studied populations seems to be affected by a strong pollen limitation which can be related to the severe condition which limit pollinators activity in the early flowering plants (Schemske & al. 1978). Considering the absence of seed production also in the hand-pollinated plants, we think that the pollen limitation was also accompanied by a strong resource limitation. This was more evident in the two species of rocky environment, in which the lowest seed-production can be referred to the more unstable habitat (Dafni & al. 1981). Regarding to the increase of seed production observed in the plants with many flowers, overall in the species of meadows (but for *G. chrysantha*), this occurrence agrees with Harder & Thomson (1989), which consider the extension of anthesis by sequential flowering as a strategy for promote pollination success under pollinator-limited condition, although in the later flowers the seed production can decrease with respect to the early ones of the same plant (Nishikawa 1998).

Conclusions

A single year of observation is not sufficient to generalize the discussed results, because climatic variation from year to year can produce differences in flowering times (Schemske & al. 1978) and these variations in the springtime can also strongly affect the efficiency of sexual reproduction (Kudo & al. 2004). However, our preliminary data suggest the effects of extrinsic ecological factors (i.e. climatic condition, stand characteristics and community features) and intrinsic ones (morphology, ploidy level) as affecting the results of the reproductive efforts in *Gagea* species.

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A. Rosselló-Graell, I. Marques, D. Draper & J. M. Iriondo

The role of breeding system in the reproductive success of *Narcissus cavanillesii* A. Barra & G. López (*Amaryllidaceae*)

Abstract

Rosselló-Graell, A., Marques, I., Draper, D. & Iriondo, J. M.: The role of breeding system in the reproductive success of *Narcissus cavanillesii* A. Barra & G. López (*Amaryllidaceae*). — Bocconea 21: 359-365. 2007. — ISSN 1120-4060.

The main purpose of this study was to evaluate the influence of the breeding system in the reproductive success of *Narcissus cavanillesii* A. Barra & G. López (*Amaryllidaceae*), a species listed under Annexes II and IV of European Community Habitat and Species Directive. This taxon, which occurs in only two populations in Portugal, is of conservation interest in this country due to its low area of occupancy and population size, and fragmentation. Hand-pollinations were carried out to evaluate the performance of self- and cross-pollination offspring. Results show that xenogamous pollinations produce more fruits and seeds than autogamous ones. However, no significant differences were detected neither in seed weight nor in seed germination percentage between treatments. Germination rate (T_{50}) was significantly higher in autogamous crosses whereas radicle vigour as well as the number of days until the appearance of the first leaf were lower. A fitness coefficient was estimated for each of these parameters by comparing the results obtained from selfing and outcrossing treatments. These results are important for the formulation of effective management strategies in the conservation of this scarce geophyte in Portugal. This study was promoted by EDIA, S. A. and co-financed by EDIA, S. A. and European Regional Development Funds (ERDF).

Introduction

There is a general concern about the conservation of rare and threatened species. In many cases these species have small size or fragmented populations and are endangered by habitat destruction or fragmentation. These populations often have reduction in the performance of progeny due to inbreeding levels (Barret & Kohn 1991; Menges 1991; Given 1994) or pollinator limitation that negatively affects reproductive success (Ellstrand 1992; Kearns & Inouye 1993; Gómez 2000; Lau & Galloway 2004). In this context, breeding system is an important factor that conditions the species reproductive success. Thus, information about reproductive biology is useful in assessing management strategies for rare or endangered species (Albert & Iriondo 1997; Osunkoya 1999; Navarro & Guitián 2002).

N. cavanillesii is an autumnal flowering geophyte included in Annexes II and IV of the European Community Habitat and Species Directive (Council Directive – 92/43/EEC).

The geographical distribution range of this autumnal flowering species extends from Algeria and Morocco (Maire 1959; Valdés & al. 1987) to Portugal and Spain. Populations are mainly distributed in SW Spain, essentially in Andalusia where it is considered a common taxon (Valdés & al. 1987). In Spain this species is also reported from Extremadura (Rivas-Goday & Ladero-Álvarez 1973; Devesa 1995) where it is classified as a taxon of special interest (Decreto 37/2001 del 6 de marzo, D. O. Extremadura). In Portugal, *N. cavanillesii* is restricted to two localities, Ajuda and Montes Juntos, in the Alto Alentejo region (Malato-Beliz 1977; Rosselló-Graell & al. 2004) corresponding to the species' western range limit in the Iberian Peninsula. This taxon presents in this country a low area of occupancy and population size, and fragmentation. Moreover, both *N. cavanillesii* Portuguese populations are affected by the establishment of the Alqueva dam in the Guadiana basin. Thus, *N. cavanillesii* is a species of conservation interest in Portugal.

In this study the role of breeding system on reproductive success of *N. cavanillesii* was evaluated. The specific aims are reflected in the following questions:

what is the breeding system of the species,

is there a pollinator limitation for fruit set,

is there any evidence of inbreeding depression in the studied population,

if not, does target species show any evidence for a decline in fitness after outcrossing between the two Portuguese populations?

Methods

The field work was carried out in Ajuda population during the flowering period of 2003. Controlled pollinations were performed to determine the effect of pollen source on fruit set, seed set, seed weight, final seed germination percentage, germination rate (T_{50}), radicle vigour and the number of days until the appearance of first leaf.

Hand pollination experiments

The flowers were randomly selected before anthesis and five pollination treatments were performed using 60 flowers in each one: 1) natural pollination, unbagged and untouched flowers; 2) spontaneous autogamy, bagged and untouched flowers; 3) induced autogamy, bagged flowers pollinated with pollen from the same flower; 4) within-population xenogamy, bagged flowers cross-pollinated with a mixture of pollen from at least ten arbitrarily selected flowers from the same population, and 5) between-population xenogamy, bagged flowers cross-pollinated with a mixture of pollen from at least ten randomly flowers from Montes Juntos population.

For treatments 3, 4 and 5, flowers were emasculated before anther dehiscence using a fine forceps to avoid pollen contamination and then hand-pollinated. Flowers producing fruits were recorded for each treatment. Mature fruits were collected and the seeds per fruit (seed set) were counted and weighted with a high precision balance (accuracy ± 0.0001 g). Mean fruit set, mean number of seeds per capsule and mean seed weight as well as standard deviations were calculated for each treatment.

Seed germination tests

The seeds from each treatment were placed in Petri dishes (25 seeds per dish) on wet filter paper with distilled water and maintained at 15ºC in growth chambers with a 16 h:8 h photoperiod. Previous experiments have shown that *N. cavanillesii* seeds reveal optimal germination rates under these conditions (Rosselló-Graell & al. 2002). Depending on the cases, four to eight replicates were used per treatment. Seeds from both spontaneous autogamy and induced autogamy were used together for the autogamous treatment germination assay. Germination parameters recorded were final seed germination percentage, T_{50}, defined by the time needed to reach 50% of the final germination value, radicle vigour, measured by root length on the seventh day after germination, and the days until the appearance of first leaf. All parameters were recorded each two days.

Data analysis

To evaluate the effect of pollen source on studied parameters different statistical analysis were carried out. One-way ANOVA ($P < 0.05$) was used when no significant deviations from normality were found in the variables. Scheffe test ($P < 0.05$) was applied for comparison of means. Germination percentage was analysed after arc-sine transformation. Non-parametric Kruskall-Wallis and Mann-Whitney tests were applied to compare treatments when variables were not normal. All statistical analyses were performed with SPSS package 10.0.

Fitness

The fitness reduction due to lowered genetic variation could be exposed early in the life of plants (Menges 1991; Waser & Price 1994). Thus, several authors had used fruit set, seed set, seed weight germination and even percentage of offspring survival or growth rate to calculate inbreeding depression for target species (Charlesworth 1988; Stevens & Bougourd 1988; Waser & Price 1994; Fischer & Matthies 1997; Quilichini & al. 2001; Navarro & Guitián 2002). In that sense and in order to estimate offspring fitness (W) we used the following expression for each pollination experiment:

$$W = w1 \ x \ w2 \ x \ w3 \ x \ w4 \ x \ w5 \ x \ w6 \ x \ w7$$

where: $w1$ = fruit set; $w2$ = seed set; $w3$ = seed weight; $w4$ = final seed germination percentage; $w5 = 1/T_{50}$; $w6$ = radicle vigour and $w7$ = 1/number of days until the appearance of first leaf. As with the values of T_{50} and number of days until the appearance of first leaf, the higher the value the lower the fitness is, we used the inverse of these variables for the calculation of the fitness coefficients. All variables were previously transformed by dividing the variable by the highest value obtained in each treatment. W ranges between 0-1.

Results

Hand pollination experiments

N. cavanillesii produced fruits and seeds from both self- and cross-pollination treatments (Tab. 1). Significant differences in fruit set were found among treatments (H = 29.982, P <0.001). Flowers that were cross-pollinated set more fruits than self- or induced autogamy (U = 1020, $P < 0.001$ and U = 1350, $P < 0.01$, respectively). No significant differences were found between control and cross-pollinated plants ($U = 1560$, $P = 0.142$).

The seed set significantly differed among treatments ($H = 31.050$, $P < 0.001$) and xenogamous pollinations set more seeds than induced autogamous crosses ($U = 249.500$, $P < 0.05$) (Tab. 1). No statistically significant differences were detected in seed weight among treatments ($H = 8.794$, $P = 0.66$).

Seed germination

No significant differences in final germination percentage were detected among treatments ($F = 2.094$, d.f. = 3; $P = 0.132$) (Tab. 2). Germination rate differed significantly when treatments were compared ($H = 7.929$, $P < 0.05$). T_{50} was higher in between-populations xenogamy than in within-population xenogamy ($U = 7.500$, $P < 0.01$). Radicle vigour and the number of days until the appearance of first leaf did not differed significantly among treatments ($U = 27.302$, $P = 0.203$ and $U = 5.546$, $P = 0.134$, respectively) (Tab. 2).

Fitness

Within-population xenogamous crosses provided the highest reproductive fitness coefficient value (0.72), whereas autogamous crosses had the lowest reproductive fitness coefficient (0.18) (Tab. 3). The results show too a better performance of offspring from within-population than from between-population crosses (0.72 and 0.58, respectively).

Table 1. Mean and standard deviations for fruit set, seed set and seed weight after different pollination treatments (n = 60) of *Narcissus cavanillesii*.

Pollen transfer	Fruit set	Seed set	Seed weight (mg)
Control	0.50±0.50	4.5±2.7	1.54±0.43
Spontaneous autogamy	0.20±0.40	3.0±1.4	1.57±0.38
Induced autogamy	0.38±0.49	2.5±1.3	1.76±0.28
Within-population xenogamy	0.63±0.48	5.6±3.5	1.63±0.45
Between-population xenogamy	0.60±0.49	5.7±1.8	1.44±0.22

Table 2. Mean and standard deviations for final germination percentage, germination rate, radicle vigour and number of days until appearance of the first leaf after different pollination treatments of *Narcissus cavanillesii*.

Pollen transfer	Germination (%)	T_{50} (days)	Radicle vigour (mm)	Days until first leaf
Control	94±8	12±1.6	5.2±1.1	10.2±3.2
Autogamy	87±8	12.5±1.9	5.1±2.0	10.0±2.71
Within-population xenogamy	79±18	10.7±1.5	5.9±1.6	9.9±3.10
Between-population xenogamy	87±7	13.1±1.3	6.1±1.8	9.7±3.4

Table 3. Reproductive fitness coefficients (*W*) for each pollen transfer experiment in *Narcissus cavanillesii*. *w1* = fruit set; *w2* = seed set; *w3* = seed weight; *w4* = final seed germination percentage; *w5* = $1/T_{50}$; *w6* = radicle vigour and *w7* = 1/number of days until first leaf. The maximum reproductive fitness coefficient value is highlighted in bold. As with the values of T_{50} and number of days until the appearance of first leaf, the higher the value the lower the fitness is, we used the inverse of these variables for the calculation of the fitness coefficients. All fitness components were transformed to obtain a coefficient that might range from 0 to 1, being 1 the value with the highest fitness.

	w1	***w2***	***w3***	***w4***	***w5***	***w6***	***w7***	***W***
Control	0.79	0.78	0.94	0.94	0.90	0.87	0.95	0.41
Autogamy	0.60	0.52	0.96	0.87	0.86	0.82	0.97	0.18
Within-population xenogamy	1	0.98	1	0.79	1	0.95	0.98	**0.72**
Between-population xenogamy	0.95	1	0.85	0.87	0.82	1	1	0.58

Discussion

Results show that this species has a mixed mating system allowing for seed production from both xenogamous and autogamous crosses. However, the results of the control treatment suggest that there is a significant predominance of fruits set from xenogamous crosses than from autogamous ones. This can be explained by the species' floral structure, protogyny and pollinator assemblage that all together facilitate outcrossing processes in the studied population. Although most *Narcissus* species are protandrous (Barret & al. 1996), *N. cavanillesii* presents protogyny. Protogyny facilitates cross-pollination and thus prevents self-fertilization (Dafni 1992; Snow & al. 1996; Richards 1997). Moreover, *N. cavanillesii* has an open corolla exposing sexual structures that improves cross-pollination chance. A previous study in the same population revealed that *N. cavanillesii* flowers were mainly visited by Hymenoptera, like *Halictus* sp. and *Megachile* sp. Less frequent visits were made by some Diptera and some honeybees (Marques & al. 2007). The absence of significant differences in fruit set between control and xenogamous crosses suggests that pollinator activity is not a limiting factor for fruit production (Albert & al. 2001).

A comprehensive and realistic estimate of the effects of inbreeding in nature should include measurements of the fitnesses of cross and self offspring (Stevens & Bougourd 1988) but has seldom been examined in sensitive plant species (Ellstrand & Elam 1993). In many plant species, inbreeding is associated with reduced performance of selfed progeny relative to those produced by outcrossing (Menges 1991; Quilichini & al. 2001). The level of inbreeding depression may vary with the mating system and may also be a function of population size (Ellstrand & Elam 1993).

The results show a significant reduction in seed set after selfing and reproductive fitness coefficients show a better performance of progeny from within-population crosses when compared with the progeny from the other treatments. The differences in reproductive fitness coefficient between xenogamous and autogamous crosses indicate that inbreeding depression may occur in the studied population after autogamous crossings. Nevertheless, because in our study progeny mainly proceeds from xenogamous crosses we can expect that the population presents low levels of inbreeding depression.

Despite these results, the capability of clonal reproduction of *N. cavanillesii* should be considered due to the implications on population genetic variation (increased crossings between close relatives) in order to accurate determine the levels of inbreeding depression of the studied population.

Results show the importance of xenogamous crosses for offspring performance and to maintain population genetic variation. In that sense, management strategies involving the conservation of *N. cavanillesii* Portuguese populations should focus on the preservation of pollinators community and preventing habitat fragmentation in order to minimize inbreeding depression processes.

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I. Marques, D. Draper & M. A. Martins-Loução

Influence of temperature on seed germination in seven Mediterranean grassland species from SE Portugal

Abstract

Marques, I., Draper, D. & Martins-Loução, M. A.: Influence of temperature on seed germination in seven Mediterranean grassland species from SE Portugal. — Bocconea 21: 367-372. 2007. — ISSN 1120-4060.

The aim of this work was to analyze seed germination characteristics between species that share the same habitat. We studied the influence of temperature on the germination of seven species that grow in grassland areas of SE Portugal: *Bellardia trixago* (L.) All. (*Scrophulariaceae*), *Daucus carota* L. (*Apiaceae*), *Petrorhagia nanteuilii* (Burnat) P.W. Ball & Heywood (*Caryophyllaceae*), *Scabiosa atropurpurea* L., *S. simplex* Desf. (*Dipsacaceae*), *Silene colorata* Poiret and *S. gallica* L. (*Caryophyllaceae*). Seeds were incubated under 16/8 hours light/dark photoperiod, constant 15ºC, 20ºC or 25ºC and alternating 25/15ºC temperature conditions. Final germination percentage, germination rate (T50), uniformity (T75-T25) and skewness (T50/Mean Germination Time) were obtained. Seed germinability varied among the tested temperatures. *B. trixago, D. carota, P. nanteuilii, Scabiosa atropurpurea* and *S. simplex* presented the highest results at the lowest temperatures although *D. carota* seemed indifferent to the tested temperatures. *S. colorata* and *S. gallica* only germinated at 25ºC and 15/25ºC but the very low results suggest that seeds may present dormancy. All the species presented a rapid and constant germination with most seeds germinating in the first days. In general, the seven species showed different behavior among the assayed temperatures, which allow these species to explore different ecological conditions in the same habitat.

Introduction

Grassland communities occurring in meso- and thermo-Mediterranean xerophile habitats that are mostly open are mainly dominated by therophyte species. They often grow in oligotrophic soils with calcareous substrates. In more continental areas, these xerothermic communities are mainly dominated by tussock-grasses, chamaephytes and perennials species. These communities are partially of natural and anthropogenic origin. Germination may be considered the major and the first constraint to plant establishment in these communities. Comparative studies of species sharing the same habitat (Smith-Ramírez 1998; Albert & al. 2002; Valbuena & Vera 2002) and analyses of species functional traits can help to understand biological systems in ecological terms.

In this study, we selected seven herbaceous species: *Bellardia trixago* (L.) All. (*Scrophulariaceae*), *Daucus carota* L. (*Apiaceae*), *Petrorhagia nanteuilii* (Burnat) P. W. Ball & Heywood (*Caryophyllaceae*), *Scabiosa atropurpurea* L., *S. simplex* Desf. (*Dipsacaceae*), *Silene colorata* Poiret and *S. gallica* L. (*Caryophyllaceae*). All of these species are annual except *S. atropurpurea* that may be biennial. These taxa are common in open grasslands of the Western Mediterranean region where they frequently coexist. The flowering period is mainly in spring and seeds are released during summer at the end of their life cycle.

The main objectives of this work were to identify the optimal seed germination temperature for each species and to compare their germination physiological behavior. Germination profile, timing and pace will help us to recognize whether the species sharing the same habitat present the same degree of fitness and, thus, belong to similar functional groups.

Material and Methods

Plant material

Seeds of *B. trixago*, *D. carota*, *P. nanteuilli*, *S. atropurpurea*, *S. simplex*, *S. colorata* and *S. gallica* were collected in July-August 2003 from open grasslands in Evora District (Alentejo, Portugal). All the seeds presented the same degree of ripeness. Following the classification of Rivas-Martínez & al. (1990), climate is Mediterranean subhumid with an annual mean temperature of 16ºC and a 3-4 month dry period. Seeds were kept in paper bags under laboratory conditions (darkness, 21 ± 1ºC) until they were used.

Germination experiments

One-month-old stored seeds were placed in Petri dishes (7 cm in diameter) lined with two filter paper discs and moistened with 3 ml of distilled water. Seeds were incubated at 15ºC, 20ºC, 25ºC or 25/15 ºC under a 16-h light/ 8-h dark photoperiod. Light was provided by cool white fluorescent tubes with an irradiance of $35 \mu mol m^{-2} s^{-1}$ (OSRAM LUMILUX Cool white). When the alternating temperature was used, the lower temperature coincided with the dark period. The criterion of germination was radicle emergence. Seeds were monitored every two days for a period of 30 days and germinated seeds were removed. Each value is the mean of four replicates of 25 seeds ± standard deviation (sd). Non-germinated seeds were checked for viability using the tetrazolium test.

Germination parameters

The following parameters were used to compare germination behaviour among treatments: 1) final germination percentage; 2) germination rate defined by T_{50}, time needed to reach 50% final germination value; 3) uniformity (T_{75}- T_{25}) defined as the time between 25% and 75% final germination value and 4) skewness (considered as T_{50}/Mean Germination Time) a value measuring the asymmetry of germination distribution. A value of T_{50}/MGT near 1 means that the germination before T_{50} is faster than germination after T_{50}, which is normally the case. However, in some cases values greater than 1 can occur, when germination before T_{50} is lower than after T_{50}. Mean Germination Time was calculated as follows: Σ ni x di / N, where Ni is the number of seeds germinated in each day, Di is the duration of the study and N the total number of seeds germinated. The parameters T_{50}, uniformity and skewness were calculated using the mathematic expression that expressed each germination curve. Expressions were determined using CurveExpert 1.3 considering a sigmoid model. Correlation coefficients were always superior to 0.95.

Statistical analysis

For each species the selected parameters were submitted to a one-way ANOVA with temperature as factor. For comparison of means, Scheffe post hoc multiple comparison test was used ($p < 0.05$). Germination percentages were arcsin transformed. All statistical analyses were performed with SPSS package 11.0.

Results

Tables 1 and 2 show the effect of temperature on the selected germination parameters of the seven studied species. Seed germinability varied among the tested temperatures but 25°C was generally too high to promote germination except in *D. carota*, which attained high germination percentages at all, temperatures tested (Tab. 1). When alternating temperatures were assayed, some of the species (*P. nanteuilli* and *S. simplex*) showed a similar germination response to that at low incubation temperatures (Tab. 1). *P. nanteuilli* presented high results at 15-20ºC and 25/15ºC temperatures followed by *B. trixago* and the *Scabiosa* species. These five species generally achieved higher germination results at lower temperatures (15ºC and 20ºC). On the contrary, in *Silene colorata* and *S. gallica* no germination occurred at 15ºC or 20ºC and at the remaining temperatures germination percentages were always very low (Tab. 1). For each species, T_{50} also varied among temperatures but the values obtained were always very low, indicating rapid germination usually reaching 50% of the final germination percentage within one week (Tab. 2). These results are also supported by the low values of uniformity, which are inferior to four days (Tab. 2). Skewness values indicate that germination was usually faster before rather than after T_{50}, with some exceptions at higher temperatures (Tab. 2).

Discussion and Conclusion

Interspecific variation in germination behaviour has been commonly found in seeds of species from the same habitat (Herranz & al. 1998; Doussi & Thanos 2002). Relatively low temperatures (between 15ºC and 20ºC) are usually recorded as optimal germination temperatures among Mediterranean species (Thanos & Doussi 1995; Baskin & Baskin 1998; Doussi & Thanos 2002). This is in agreement with the results obtained for *B. trixago*, *D. carota*, *P. nanteuilli*, *S. atropurpurea* and *S. simplex*. Temperature seems to play an important ecological role in controlling the germination of these species. In this sense and considering that these species disperse their seeds during summer, soil conditions would be favorable for germination in early autumn or early spring when temperatures and rainfall would meet germination requirements. The positive influence of alternating temperatures on germination has been observed by several authors (Baskin & Baskin 1998; Albert & al. 2002) and has been interpreted as an adaptive mechanism of seeds in upper soil layers.

Table 1. Effect of incubation temperature on final germination percentage (mean values ± standard deviation). F: Anova F ratio value (** $p < 0.01$; *** $p < 0.001$ n. s.) for each species. Superscripts with different letters indicate significant differences ($p < 0.05$) within each species.

Species	15°C	20°C	25°C	25/15 °C	F
B. trixago	55 ± 6^{b}	53 ± 5^{b}	0	14 ± 13^{a}	14.52**
D. carota	88 ± 5^{a}	91 ± 6^{b}	70 ± 7^{a}	68 ± 7^{a}	18.56***
P. nanteuilii	100 ± 0^{b}	99 ± 2^{b}	2 ± 4^{a}	100 ± 0^{b}	286.64***
S. atropurpurea	$54 \pm 8^{a,b}$	64 ± 11^{b}	37 ± 7^{a}	31 ± 3^{a}	5.35**
S. simplex	58 ± 10^{b}	54 ± 10^{b}	11 ± 5^{a}	38 ± 6^{b}	26.81***
S. colorata	0	0	28 ± 23	15 ± 6	5.77 (n.s.)
S. gallica	0	0	14 ± 1	20 ± 5	1.11 (n.s.)

Table 2. Effect of incubation temperature on germination rate (T_{50}), uniformity (T_{75}-T_{25}) and skewness (T_{50}/MGT). Mean values ± standard deviation. F: Anova F ratio value (** $p < 0.01$; *** $p < 0.001$n. s.) for each species. Superscripts with different letters indicate significant differences ($p < 0.05$) within each species. Blanks correspond to absence of germination in a species at that particular temperature.

	Species	15°C	20°C	25°C	25/15°C	F
T_{50}	*B. trixago*	$5 \pm 0^{a,b}$	7 ± 1^{b}	-	4 ± 3^{a}	7.35**
	D. carota	7 ± 1^{b}	8 ± 0^{b}	7 ± 1^{b}	5 ± 0^{a}	11.69***
	P. nanteuilli	3±0	3±1	3±7	4±2	0.09 (n.s.)
	S. atropurpurea	6±1	6±0	5±0	5±0	2.68 (n.s.)
	S. simplex	3±0	4±1	3±1	2±0	2.29 (n.s.)
	S. colorata	-	-	5±1	13±1	45.50 (n.s.)
	S. gallica	-	-	6±1	5±1	0.17 (n.s.)
T_{75}-T_{25}	*B. trixago*	4 ± 1^{b}	4 ± 1^{b}	-	1 ± 1^{a}	13.45**
	D. carota	4±0	4±0	3±1	4±0	3.23 (n.s.)
	P. nanteuilli	1±0	1±0	1±2	1±0	0.24 (n.s.)
	S. atropurpurea	3 ± 1^{b}	4 ± 0^{b}	3 ± 1^{b}	1 ± 1^{a}	14.93***
	S. simplex	$2 \pm 1^{a,b}$	3 ± 1^{b}	0 ± 0^{a}	1 ± 0^{a}	12.54***
	S. colorata	-	-	3±1	4±1	2.18 (n.s.)
	S. gallica	-	-	1±0	4±3	3.40 (n.s.)
T_{50}/MGT	*B. trixago*	0.88±0.01	0.95±0.03	-	0.88±0.05	3.99**
	D. carota	0.95 ± 0.02^{b}	0.97 ± 0.02^{b}	0.95 ± 0.06^{b}	0.78 ± 0.03^{a}	18.66***
	P. nanteuilli	0.91±0.03	0.83±0.08	0.27±0.55	0.92±0.04	4.78 (n.s.)
	S. atropurpurea	0.84±0.08	0.86±0.02	0.82±0.05	0.92±0.02	2.57 (n.s.)
	S. simplex	0.77 ± 0.05^{a}	0.74 ± 0.05^{a}	0.90 ± 0.07^{b}	$0.86 \pm 0.01^{a,b}$	7.84**
	S. colorata	-	-	0.80±0.04	1.17±0.05	7.68 (n.s.)
	S. gallica	-	-	0.96±0.01	0.86±0.12	2.52 (n.s.)

This strategy seems particularly important for these five species that might be able to germinate when daily temperatures have already risen but night temperatures are still low. *D. carota* with high germination percentages at all tested temperatures (Tab. 1) seems to present an opportunistic behaviour, independent of its longer germination time (Tab. 2).

The first five selected species produce a substantial fraction of soft-coated seeds, which germinated without the necessity of any particular pre-treatment. This seed pool is responsible for the maintenance of population levels in a variety of conditions and explains their colonizing role in disturbed areas. Uniformity and skewness values indicate that almost all of these soft-coated seeds can germinate at the same time and usually in the first few days with optimum temperatures. This strategy can be useful for the rapid colonization of new areas but it also implies that they must have high reproductive fitness and low intraspecific competition. If not, the soil seed pool would quickly diminish, and although most of the seeds germinate well, they would not be able to complete the life cycle.

Contrary to this previous group of five species, *Silene colorata* and *S. gallica* only germinated at 25ºC and 25/15ºC. These species seem to produce a major fraction of hard-coated seeds. The hardness and impermeability of the seed coat might be the cause of dormancy in these species as in other *Silene* species (Thompson 1970). The results of the tetrazolium test show that all non-germinated seeds were viable, thus supporting the hypothesis of seed dormancy. It would be interesting to observe the response of these species after pretreatments like soaking in a gibberelic acid solution or dry heat. The non-dormant fraction of these seeds can germinate just after dispersion if soil and temperature conditions are favorable whereas dormancy allows the rest of seeds to stay dormant during the hot and dry summer. The obtained results seem to indicate that these *Silene* species are able to control their population effectives under natural conditions through dormancy, thus maintaining a permanent soil seed bank in the field.

In conclusion, the seven grassland species do not present similar germination behaviours, which should be expected since they belong to different families. Even though they share the same type of habitat, they seem to explore different ecological niches dependent on seasonal characteristics, allowing their coexistence. Three different patterns were observed: 1) species with an opportunistic behaviour, *D. carota*, 2) species adapted for early spring or early autumn germination in Mediterranean climate, *B. trixago*, *P. nanteuilli*, *Scabiosa atropurpurea* and *S. simplex* and 3) species with seed dormancy, *Silene colorata* and *S. gallica*. Thus, with the exception of *D. carota*, temperature is an important driving force for germination to occur. This different behaviour can be interpreted as an important ecological strategy since the species could explore environmental conditions and available resources in different ways.

Acknowledgements

This study was promoted by EDIA S. A. and co-financed by EDIA S. A and ERDF.

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Mirjana Ocokoljić & Nebojša Anastasijević

Effect of low temperatures at the beginning of the vegetation growth period on beech yield in Belgrade

Abstract

Ocokoljić, M. & Anastasijević, N.: Effect of low temperatures at the beginning of the vegetation growth period on beech yield in Belgrade. — Bocconea 21: 373-377. 2007. — ISSN 1120-4060.

This paper points out the effect of temperatures at the beginning of the vegetation period on beech yield in Belgrade. The reconnaissance in the field study and the comparative analysis were performed during years 2001, 2002 and 2003. The reasons of the reduced yield are related to the variability of temperatures during the study period, but also with the conditions of cultivation outside the ecological optimum.

Introduction

The beech forests in Serbia are characterized by ecological and taxonomic diversity, which distinguishes them from beech forests of Europe. However, general devastation and intensive felling decreased the area of the best stands and deteriorated the ecological conditions of their development. Still, in Serbia, Moesian beech is the most widely distributed species on 25% of the territory covered with forests, and it is the national wealth (Mišić 1997; Janković & Nikolić 1998; Jovanović 2000).

In addition to Moesian beech significance for forestry practice, there is its great significance (and also of European beech) in urban coenoses (Vukićević 1996). Their cultivation in parks enriches the diversity of dendroflora, and simultaneously represents a specific experiment in adaptation to ecological circumstances in Belgrade (Ocokoljić & Anastasijević 2004).

The seed yield and quality of Moesian beech and European beech in the green spaces in Belgrade were studied aiming at the investigation of the effect of low temperatures at the beginning of the vegetation period on fructification. The study is based on the fact that the selection in using beech trees as seed sources enables the provision of good quality seed and planting material for the needs of green spaces in settlements, as well as for the regeneration of beech forests.

Material and methods

Altogether 20 trees were selected in Belgrade green spaces, 10 trees of each species. All the analysed trees are in the central part of Belgrade, which is, from the thermal aspect, the transition between the climate of the Adriatic Littoral to continental climate. This position of Belgrade makes it possible to assess the reactions of Moesian beech and European beech to the effect of low and extremely low temperatures at the beginning of the vegetation period.

For this reason, Table 1 presents the relevant data of the Republican Hydro-Meteorological Institute in Belgrade on air temperatures in April, which are especially significant, because it is the period when beech flowers most frequently (Popović 1953; Ocokoljić & Ninić-Todorović 2003). In all three study years 2001, 2002 and 2003 the flowering phenophases occurred in mid April.

The effect of low temperatures on the yield of Moesian beech and European beech during three successive years was studied, taking into account that there was no yield in 2002. Closed fruits picked in the fall from trees recently felled in logging operations, at the same time when a completely brown fruit indicates seed maturity. After the fruits were stripped from the branches, they were spread to dry in a thin layer until they open and the nuts were shacked out. The seeds were categorized based on the morphometric analysis and the analysis of germination percentage (Tab. 2, 3). Seed fullness was measured by the method of cross cutting on the sample of 100 seeds in four repetitions. Seed germination percentage was assessed on the same number of samples and repetitions by standard methods of germination tests according to Suszka (1975). The prescribed testing method for bout beeches was to germinate stratified seeds on the top of moist blotters at 3 to 5ºC. Test duration ruined up to 24 weeks, which includes 140 days of stratification at the same 3 to 5ºC. Because of the lengthy tests, viability estimation by tetrazolium staining is recommended as an alternate method (ISTA 1993). In this study tetrazolium staining do not included. The Excel statistically processed the data, with the computation of the basic statistical parameters.

Results

The study data on seed characteristics show the effect of low temperatures at the beginning of the vegetation period on seed quality of both Moesian and European beech. The low temperatures caused the lower quality of seeds in all trees. Namely, although 2001 was a year with the maximum yield of all 20 beech trees, in both species the percentages of full seed and germination percentages were lower in 2001 than in 2003 (Tab. 2, 3).

Table 1. The temperature in April 2001 and 2003.

Temperature (ºC)	2001	2003
Mean	11.9	12.0
Mean absolute	5.7	7.9
Absolute minimum	- 0.9 (day 14)	1.1 (day 8)

Table 2. The biometric parameters and germination percentage of seeds collected from 10 Moesian beech trees in 2001 and 2003.

2001

Tree	Length (cm)	Width (cm)	100 seeds (g)	Full seed (%)	Germination (%)
1	1.98	1.13	12.0	25.6	15
2	1.90	1.10	17.1	21.5	10
3	2.00	1.00	22.1	18.4	14
4	1.65	0.99	16.6	12.3	12
5	1.74	0.90	14.5	18.2	11
6	1.68	0.92	10.1	15.4	14
7	2.01	0.97	24.2	19.5	18
8	1.12	0.93	11.5	19.2	11
9	1.54	0.98	16.2	21.4	12
10	1.69	0.99	24.5	18.3	17
Mean value	1.73	0.99	16.9	19.0	13

2003

Tree	Length (cm)	Width (cm)	100 seeds (g)	Full seed (%)	Germination (%)
1	2.28	1.24	34.0	82.2	62
2	2.18	1.14	36.1	72.3	43
3	2.11	1.15	41.4	65.4	50
4	1.56	0.90	30.0	75.2	36
5	1.50	0.92	32.3	78.3	49
6	1.56	0.97	29.2	66.3	35
7	1.63	0.94	33.0	71.3	49
8	1.47	0.92	30.2	71.1	47
9	1.35	0.94	25.0	92.5	54
10	1.47	0.95	24.4	81.2	36
Mean value	1.71	1.01	31.6	75.6	46

Conclusions

The analysis of several morpho-physiological characteristics of the seeds of 10 trees Moesian beech and 10 trees European beech in Belgrade, shows the adverse effects of low temperatures at the beginning of the vegetation growth period on seed quality, percentage of full seed and germination percentage. This fact should be taken into account in the plans of cultivation of these two beech species for the requirements of practice.

Table 3. Biometric parameters and germination percentage of seeds collected of 10 European beech trees in 2001 and 2003.

2001

Tree	Length (cm)	Width (cm)	100 seeds (g)	Full seed (%)	Germination (%)
1	1.56	0.45	15.0	22.7	12
2	1.64	0.51	16.4	20.4	15
3	2.10	0.62	19.1	17.1	18
4	1.57	0.74	17.6	12.0	20
5	1.87	0.87	18.9	14.2	21
6	1.54	0.92	15.5	16.4	17
7	1.87	0.94	21.3	20.8	18
8	1.54	0.95	17.5	15.4	15
9	1.54	0.54	18.6	22.7	17
10	1.57	0.87	19.7	17.0	17
Mean values	1.68	0.74	18.0	17.9	17

2003

Tree	Length (cm)	Width (cm)	100 seeds (g)	Full seed (%)	Germination (%)
1	1.28	0.57	36.5	65.2	58
2	1.54	0.58	31.4	78.4	49
3	1.78	1.00	39.4	68.6	55
4	1.95	0.94	38.2	76.4	44
5	1.99	0.57	35.7	82.3	47
6	1.88	0.59	31.2	65.8	54
7	1.74	0.85	30.0	73.4	68
8	1.58	0.54	30.7	72.5	57
9	1.54	0.78	28.9	89.6	52
10	1.62	0.88	26.8	80.1	44
Mean values	1.69	0.73	32.9	75.2	53

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S. Rossini Oliva, B. Valdés &. M. D. Mingorance

Nerium oleander as a means to monitor and minimize the effects of pollution

Abstract

Rossini Oliva, S., Valdés, B. &. Mingorance, M. D: *Nerium oleander* as a means to monitor and minimize the effects of pollution. — Bocconea 21: 379-384. 2007. — ISSN 1120-4060.

Vegetation is very important to stabilize and minimize the effects of pollution. Industrialized areas near the city of Huelva, southwest Spain, have both soil and atmospheric pollution. Contaminants move off-site following wind direction, spreading the contamination beyond its original centre. This study checks the possibility of using *Nerium oleander* L. to minimize the effects of soil and atmospheric pollution. Samples were collected along three transects running along the prevailing wind directions (NE, NW and SE), and a fourth against wind direction (SW); a control site (Bkg) was chosen for comparison. Leaves and roots, together with soil samples were collected and the concentration of Cu, Fe, Mn, Pb and Zn was determined. This species is useful to fix Fe and Pb from soil through roots. Leaf Cu, Fe and Mn concentration are directly correlated with those in roots. No correlation between metal content in soil and root and/or soil-and leaf were found except for Pb, for which there was a positive correlation between soil and root concentration; when the contamination level is high this species is able to reflect the environmental quality.

Introduction

Industrial activities generate large amounts of pollutants. The direct effects include air, water and soil pollution. The use of vegetation can be a cheap means for pollution control when the appropriate species is chosen. Phytostabilization is a common phytoremediation technique that uses plants to clean up metal-contaminated soils. Phytoremediation includes a lot of techniques that use plants to clean up polluted sites or render harmless environmental contaminants (Wong 2003). They differ in the way plants can remove, immobilize or degrade pollutants, as well as the type of contaminants that plant species can target; it has been proposed as a cost-effective, environmentally friendly alternative technology (Lasat 2002).

The phytostabilization is based on the immobilization of trace elements through the plant root system reducing metal mobility. In this remediation technique, the choice of appropriate plant species is very important to ensure a self-sustainable vegetation cover (Pichtel & al. 2000; Wong 2003).

The possibility of using herbaceous plants to remove and fix metals from soils has received considerable attention but not many studies have been made using trees and shrubs (Punshon & Dickinson 1997). Plants can also intercept atmospheric contaminants directly from the air by leaf accumulation or absorption and, therefore, they are used in biomonitoring studies (Bargagli 1995). It is known that *Nerium oleander* L. is a species suitable to use as a biomonitor (Sawidis & al. 1995; Aksoy & Öztürk 1997; Rossini Oliva & Valdés 2003; Rossini Oliva & Mingorance 2004). This evergreen species is widespread in Huelva province and survives close to the industrial complexes, where few other plant species grow.

The objective of this study was to investigate the use of *Nerium oleander* L. to minimize soil and atmospheric pollution.

Material and methods

Sample collection

The samples of *Nerium oleander* leaves, roots and soil were collected in different contaminated sites at different distances (0.5, 1, 2, 4, 6, 8 km) along four transects from the industrial complex of Huelva city, Southwest of Spain. Three transects running to the dominant wind direction (SE, NW and NE) from the industrial complex and another against wind direction (SW) were selected. A control site (Bkg) 40 km away from the industrial focus was sampled to establish the normal element contents in *N. oleander*.

The sample collection, including plants and soils, were carried out according to Rossini Oliva & Mingorance (2004). No root samples were collected along the NE transect or at the control site.

Sample preparation and analysis

Aliquots of the samples were digested in a closed microwave digestion system (Milestone MLS 1200) using HNO_3 and H_20_2 mixture (Mingorance & al. 1993) for plants and *aqua regia* for soil (Bettinelli & al. 1989). The total concentrations of elements (Cu, Fe, Mn, Pb and Zn) were determined by simultaneous inductively coupled plasma mass spectrometry (ICP/MS). All metal concentration values reported are based on soil or plant dry weight.

The accuracy of the soil and plant analysis was determined by the analysis of *Citrus* leaves (NIST 1572), pine needles (NIST 1575), sewage sludge amended soil (BCR 143) and Montana soil (NIST 2711). Regression analysis between the reference and obtained metal concentration shows good recoveries (slope of the regression line is 1.05±0.020). The precision of the analysis was determined by RSD giving values between 2-8%.

Statistical evaluation of the data was done by using SPSS v. 11.5 (Illinois, USA)

Results and discussion

Leaf, root and soil mean concentration along the transects is shown in Figure 1. Concentration is expressed as the mean value between the different sampling points of each transect. Leaf mean concentration of Cu was significantly higher ($p < 0.05$) than those in the control site (Fig. 1). This indicates an accumulation of Cu by this species. In the case of Fe, no differences were found between the concentration at the control site and the other sampling sites; Mn concentration in the SW transect was significantly lower than in the control; concentration was significantly higher than at the control for Pb in the NE transect and for Zn in the NW transect. The Pb values are very dispersed and this may be the reason why differences with respect to the control site were not very significant.

A significant positive correlation ($p<0.05$) was found between leaf and root content for Cu (R= 0.87), Mn (R= 0.90) and Fe (R= 0.73), indicating that leaf concentration has a direct relationship to the root contents. No correlation was found between the soil and either root or leaf contents, except for Pb between the soil and root (R= 0.63). This seems

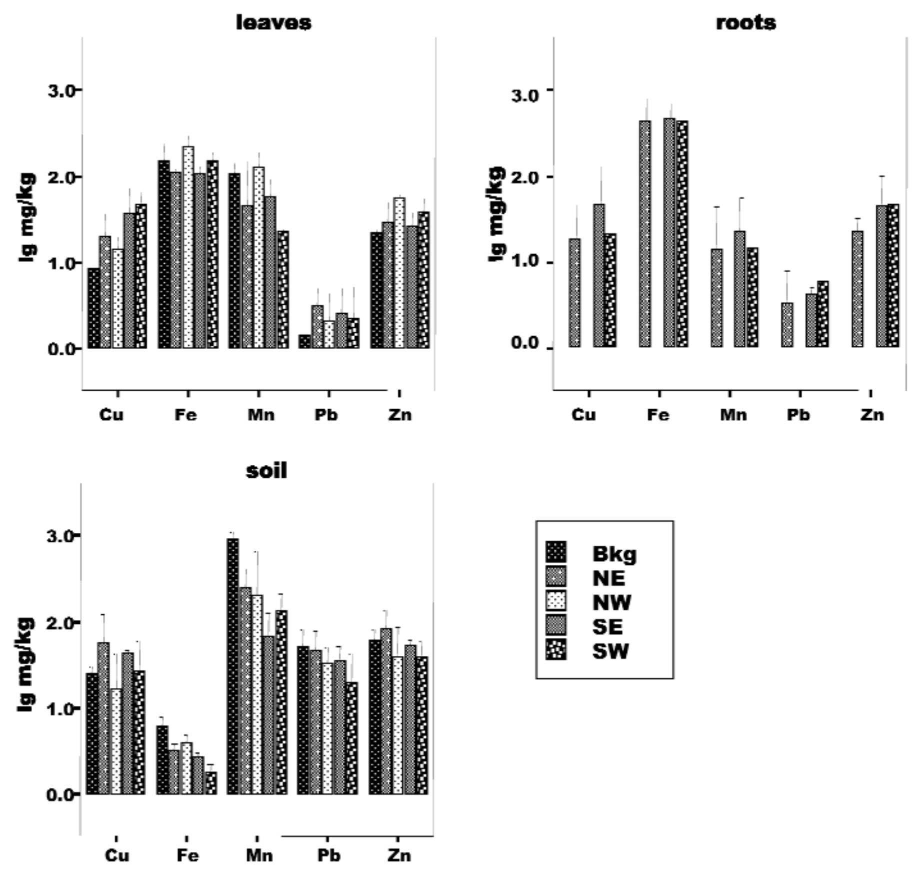

Fig. 1 Mean metal concentrations in leaves, roots and soil along transects (NE, SE, SW, NW) and at the control site (Bkg).

to indicate that metal content in plant tissues is not just a function of the total metal content of the soil, but also of the form in which it occurs, physiological transport barriers, and climatic factors.

The transfer factor (TF), defined as the ratio of root metal concentration in relation to that in leaves is presented in Table 1. Table 1 shows that the element Mn has a TF value lower than 1, which indicates that it is easily translocated within plants. The same result was obtained in southern Spain by using *Cynodon dactylon* (Madejón 2004). For Cu, it was not possible to establish a common pattern: plants growing in the SW transect easily translocate Cu (TF<1) and those growing in NE and SE transects are able to maintain a balance between leaf and root concentrations (TF close to 1). For Zn, TF values for SE and NE transects are close to 1 and for SW it is >1. The last case indicates a low transfer mobility of Zn from roots to leaves. On the other hand, the TF values higher than 1 for Fe and Pb indicate higher accumulation in the roots. This suggests that *N. oleander* is useful in removing Fe and Pb from soil.

The mobility ratio (MR) is defined as the leaf/soil metal concentration. According to Baker (1981) this species can be classified as an excluder of Cu, Mn, Fe and Pb, since the MR is lower than 1 (Tab. 1), except for Mn in NW transect and Cu in SW, NW and SE transects indicating that this species is able to maintain a low metal concentration in the leaf and that translocation is minimal.

This species can be considered as an accumulator, for only 20% of the cases, both Mn and Cu, which indicated a higher accumulation in leaf than in soil. It can also be considered as an indicator for Cu in another 40% of the cases, which means that the internal content reflects the external concentration. Mingorance & al. (2007) found that oleander acts as Cu and Zn indicator and as excluder for Al, Ba, Cr, Fe and Pb. In relation to Zn, this species is excluder in 40% of the cases and can be considered as a bioindicator for 60% of the cases.

Table 1. The mobility ratio (MR) and transfer factor (TF, >1 in bold) in *Nerium oleander* along transects (NE, SE, SW, NW) and at the control site (Bkg).

	MR					TF		
Elements	NE	SE	SW	NW	Bkg	NE	SE	SW
Cu	0.36	1.0	2.5	0.97	0.30	1.0	1.3	0.54
Fe	0.008	0.008	0.006	0.021	0.003	3.9	4.5	3.3
Mn	0.11	0.98	0.16	2.3	0.11	0.36	0.27	0.59
Pb	0.047	0.056	0.15	0.063	0.09	1.8	1.7	23
Zn	0.38	1.0	1.1	1.6	0.35	0.90	1.3	1.6

For all elements studied, the species has an excluder behavior at the control site, suggesting that leaves of *N. oleander* are able to reflect quantitative aspects of environment quality when pollution levels are high.

Conclusions

Nerium oleander L. can fix Fe and Pb from the soil by root accumulation. In addition, the leaves uptake atmospheric pollution by direct absorption from the air and by translocation from roots. The amount of Cu, Fe and Mn in the leaves is directly related to the root content but is independent of soil content. In relation to the resistance mechanisms of this species to the studied elements, it can be considered as an excluder for Cu, Fe Mn, and Pb. It can also be considered to be a partial accumulator (20%) for Cu and Mn, and as a bioindicator of Cu (40%). In addition, it is a partial excluder (40%) and a partial bioindicator (60%) for Zn. The study demonstrates that in the absence of high contamination, the species acts as an excluder which means that it needs a high pollution level to supply information on the quantitative aspects of changes in environment quality.

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D. Draper, I. Marques & M. A. Martins-Loução

The role of a gene bank as an impact mitigation tool in the Alqueva dam (Portugal)

Abstract

Draper, D., Marques, I. & Martins-Loução, M. A.: The role of a gene bank as an impact mitigation tool in the Alqueva dam (Portugal). — Bocconea 21: 385-390. 2007. — ISSN 1120-4060.

The construction of the Alqueva dam in Portugal will result in a large-scale habitat fragmentation and ecosystem alterations that adversely affect both terrestrial and aquatic biodiversity. As an impact-minimization action and help for future restoration programs 1200 plant specimens (over 300 species) are now preserved in the "Antonio Luis Belo Correia Gene Bank" of Lisbon University Botanical Garden. After the flooding, several actions of restoration and impact compensation of the area will be implemented, some of which are illustrated in this paper.

Introduction

The construction of the Alqueva dam in Portugal will result in the loss of 25000 ha of Mediterranean river ecosystems as well as surrounding rocky areas. The habitat fragmentations will involve either reduction/loss of habitat or a decrease in habitat connectivity that will adversely affect both terrestrial and aquatic biodiversity. Due to the direct impact on plant populations and future changes in land use, the "Antonio Luis Belo Correia Gene Bank" of Lisbon University Botanical Garden has developed a conservation project (2001-2004) focused on the collection, storage and long-term conservation of germplasm from the area affected by the Alqueva dam. In order to achieve the aims of the project it was necessary to reorganize the existing gene bank facilities. New laboratories were designed to assure (1) the plannification of seed sampling, (2) the characterization of the germplasm collected and (3) the long-term preservation of seeds. During the first two years, the gene bank staff was trained in collection protocols and seed preservation, while in the last two years the massive efforts were focused to the collection of the target species. The long-term conservation method adopted was that proposed by Gómez-Campo (2001).

The conservation project consists of a mitigation action aiming at: i) minimising the loss of genetic resources that will cause the construction of the dam, and ii) becoming a tool for further conservation actions (habitat re-establishment, re-introductions, re-inforcements...) in the area. The fact that a global action plan was defined previously allowed an adequate collecting effort before the flooding. This remarkable particularity ensures the use of autochthonous germplasm in the future mitigation actions that are expected to facilitate its success (Falk & al.1996).

Seed preservation of the plants affected by the Alqueva dam

Considering the deep transformation of the affected area and its surroundings, the impact of the Alqueva dam would have consequences at all levels of the trophic web. The main alteration of vegetation involves changes in primary biological productivity of ecosystems including effects on riparian plant-life and on down-stream habitats. Another feature of the same problem is the repercussion on fauna due to changes on herbivorous behavior and population dynamics that may also affect the carnivorous composition.

Due to the size of the area to be flooded, and the short time (2001-2002) available to collect, a collecting strategy was defined based on a previous eco-geographical survey of the flooded area (Draper & al. 2003). The main goal of this survey was to detect different ecological conditions that may affect the genetic adaptation on these particular ecological profiles since collecting in completely different profiles may ensure a wide genetic diversity. The characterization of the original ecological profile will also be used in the future actions to select the most accurately seed samples to be used in each place. This collecting strategy was followed during the project resulting in a total number of 1200 specimens from over 300 species, covering most ecological niches which would be destroyed or modified by the dam construction (Fig. 1). These species represent 58 plant families and 60% of the plant diversity present in the area (Fig. 2).

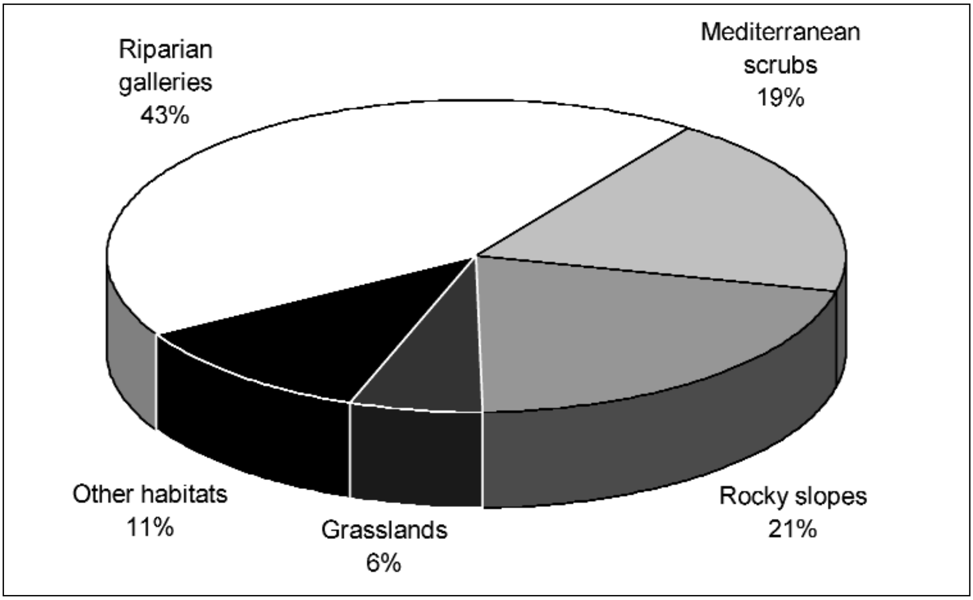

Fig. 1. Distribution of collected samples by ecological niches (n=1200 samples).

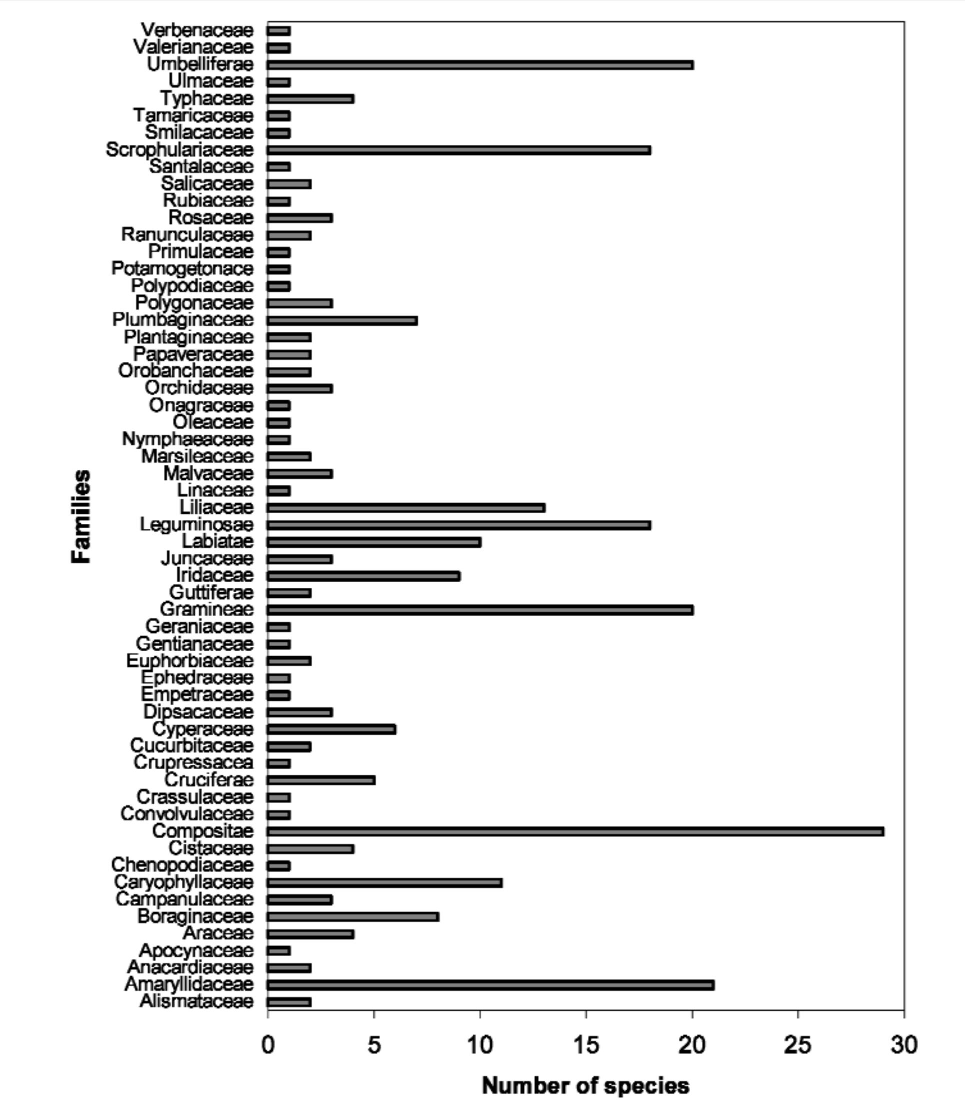

Fig. 2. Number of species (by plant family) preserved in the Alqueva gene bank.

The future utilization of the preserved material is conditioned by the knowledge of its characteristics and its viability (Jaramillo & Baena 2000). Thus, in order to determine the germplasm potential use, it was important to characterize all the material held in the gene bank. The characterization of the Alqueva germplasm includes herbarium vouchers, field information, germination profiles, patterns of drying behavior, viability status, relation between the number of seeds, its weight and volume, and cytogenetic data.

Main restoration actions

In the context of this project, the *ex situ* conservation of native species acts as a 'back-up' providing the possibility to undertake future actions to minimize the lost of the existent genetic diversity. The restoration actions may be summarized as follows.

Re-inforcement of remaining riparian populations and habitats

This action will consider the creation of new riparian galleries segments as well as the recovery of the remaining ones. The aim of this restoration is to ensure plant population dynamics but also to feed, hide, and provide nesting conditions to several groups of animals. The target plant species for this action were:

Agrostis stolonifera
Alisma plantago-aquatica
Amaranthus albus
Asparagus aphyllus
Aster squamatus
Bryonia craetica subsp. *dioica*
Carthamus lanatus subsp. *lanatus*
Crataegus monogyna
Cynodon dactylon
Cynosurus echinatus
Cyperus longus
Cyperus longus subsp. *badius*
Cytisus scoparius
Dittrichia viscosa subsp. *viscosa*
Echinochloa crus-galli
Epilobium hirsutum
Epilobium tetragonum
Flueggea tinctoria
Foeniculum vulgare
Foeniculum vulgare subsp. *piperitum*
Fraxinus angustifolia
Juncus acutus
Lactuca serriola
Lythrum salicaria
Marsilea batardae
Mentha suaveolens
Nerium oleander
Olea europaea var. sylvestris
Osyris alba
Panicum repens
Paspalum paspalodes
Phragmites australis
Picris echioides
Piptatherum miliaceum
Pistacia lentiscus
Polygonum amphibium
Polygonum equisetiforme
Polygonum monspeliensis
Populus deltoides
Pulicaria paludosa
Rubus ulmifolius
Salix salvifolia
Schoenoplectus mucronatus
Scirpoides holoschoenus
Silybum marianum
Smilax aspera
Tamarix africana
Typha latifolia

Increase of connectivity between riparian galleries and sclerophyllous pasturelands

The creation of an artificial new shoreline has generated a sharp break in communities and landscapes. Thus, this action aims to increase the ecotone area suitable for the establishment and migration of animal populations. Specific habitat restoration will be done focusing some priority mammals such as *Microtus cabrerae* (Habitat Directive 92/43/EEC) that lives in open rush meadows or close to temporary ponds. The use of different plants will create areas suitable not only for their nesting but also for refuge and feeding. The considered species were:

Aristolochia baetica
Aristolochia paucinervis
Asparagus acutifolius
Asparagus albus
Calamintha baetica
Centranthus calcitrapae
Cistus albidus
Cistus monspeliensis
Coronilla glauca
Crataegus brevispina
Cynodon dactylon
Daphne gnidium
Elaeoselinum foetidum
Euphorbia nicaensis
Hyacinthoides hispanica
Hypericum perfoliatum
Jasminum fruticans
Lavandula sampaiana
Lonicera implexa
Olea europaea var. sylvestris
Osyris alba
Phillyrea media
Pistacia lentiscus
Quercus coccifera
Retama sphaerocarpa
Rhamnus alaternus
Rhamnus oleoides
Rubia peregrina
Rubus ulmifolius
Ruscus aculeatus
Scirpus holoschoenus
Smilax aspera
Smyrnium olusatrum
Teucrium fruticans
Teucrium lusitanicum
Urginea maritima
Viburnum tinus

Adaptation of artificial structures for aquatic fauna

Several plant species will be used taking into consideration the importance of this structure in the colonization of riverbanks as well as their role as bridges between shorelines, refuge and protection of aquatic birds, amphibians and fishes. The proposed species were:

Alisma lanceolatum
Alisma plantago-aquatica
Baldellia ranunculoides
Carex hispida
Ceratophyllum demersum
Cynodon dactylon
Cyperus eragrostis
Cyperus longus
Cyperus longus subsp. *badus*
Elatine macropoda
Eleocharis palustris
Epilobium hirsutum
Epilobium tetragonum
Equisetum ramosissimum
Equisetum telmateia
Eryngium corniculatum
Eryngium galioides
Glinus lotoides
Hypericum undulatum
Iris pseudacorus
Isoetes histrix
Isoetes velatum
Juncus acutiflorus
Juncus acutus
Juncus fontanesii
Juncus inflexus
Juncus maritimus
Juncus pygmaeus
Lythrum hyssopifolia
Lythrum junceum
Lythrum salicaria
Marsilea batardae
Mentha cervina
Mentha pulegium
Mentha suaveolens
Narcissus jonquilla
Nuphar luteum
Phragmites australis
Potamogeton nodosus
Ranunculus bullatus
Ranunculus ololeucos
Ranunculus peltatus
Rubus ulmifolius
Scirpoides holoschoenus
Typha domingensis
Typha latifolia
Veronica anagallis-aquatica

Re-inforcement of existing plant populations and establishment of new patches of endangered species

Specific actions will be performed for the endangered taxa *Marsilea batardae*, *Narcissus cavanillesii* and *Digitalis purpurea* subsp. *heywoodii*. New populations of some aquatic macrophytes becoming extinct in the area will be re-established, e.g., *Nuphar luteum*, *Potamogeton nodosus*.

A multidisciplinary approach including zoologists, botanists and foresters is expected to ensure the success of these actions.

Acknowledgements

Authors are indebted to Antónia Rosselló-Graell and Joana Brehm for their foremost contribution during the project. This study was promoted by EDIA S. A. and co-financed by EDIA S. A and ERDF.

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Stella Mérola & Francesco M. Raimondo

European and Mediterranean plants in the wild flora of Uruguay

Abstract

Mérola, S. & Raimondo, F. M.: European and Mediterranean plants in the wild flora of Uruguay. — Bocconea 21: 391-404. 2007. — ISSN 1120-4060.

In this research are analysed the European and Mediterranean floristic representatives established in the most humanized regions of Uruguay as a consequence of the continuous exchange of plant entities occurred since the last five centuries between Europe and South America. As far as Uruguay is concerned, the taxa adventitious and the ones that became spontaneous, have been listed and analyzed according to their occurrence and territorial significance (k). There have been selected the regions from the middle West to South, Centre and East according to the most humanized areas in terms of labored soils and population density. Herbarium samples for all the species found have been collected. In total 174 taxa have been taken into account, 7 of the most represented families includes the 73% of the total species found. According to the chorological scheme, the Mediterranean types and the South European amount 52% of the European contingent surveyed for this work in Uruguay. In relation to the introduction process, the majority of taxa is adventitious (72% naturalized and casuals) in contrast to the ones escaped from cultivation that amount to 28%. The gradient in the species occurrence with the correspondence analysis taxa/sites, shows that there is no defined trend of correlation between territories and colonizing species variability. The gradient on the axis 1 is more related to higher human land use and to the proximity to the more important points connecting with foreign countries. The gradient on the axis 2, instead is likely more related to a specific culture type like rice. Regarding to the species with higher influence (k) in the colonized area, the k value shows that only 5% of the total species number, have value higher than 40. On the other hand, whilst k values lower than 10 represent 54% of the total species number.

Introduction

Between the Old and the New World, a continuous exchange of plant entities of economic and environmental interest has occurred since the last five centuries. The main causes of this exchange, have been cultivation for cropping or for ornamental purposes as well as dispersion caused by other human activities of the European conquest in South America.

Regarding the climate features in the new colonized areas, Uruguay is included in the temperate zone (Bidegain & al. 1977) and climate parameters don't have variations of importance. The annual media temperature is 17ºC; the annual medium rain records have a minimum value of 1000 mm in the South and a maximum of 1500 mm in the North East.

The atmospheric annual medium relative humidity has a variation between 70% and 75% in the whole territory, the most humid month is July (80%) and the driest is January (65%). According to Koeppen scale, the whole territory may be characterized as: temperate climate, moderate and rainy (the temperature of the coldest month is between 3ºC and 18ºC): "C type". Climate of humid atmospheric conditions (irregular rain, intermediate conditions between class w and s in Koeppen scale): "f type". Specific variation of temperature (the temperature of the hottest month is higher than 22ºC): "a type". In conclusion, the Uruguayan territory has a "Cfa" climate type in the Koeppen scale.

In this research the European and Mediterranean floristic representatives established in the most humanized regions of Uruguay are analysed.

Methods

The taxa adventitious and the ones that became spontaneous in Uruguay have been listed and analyzed according to their occurrence and territorial significance (k). The species list and its analysis is done based on the chorological scheme according to Pignatti (1982) and, regarding the aliens, the classification based on the introduction process by Viegi & al. (1974). The taxa were identified according to Tutin & al. (1968-1980; 1993).

The main categories that represent the introduction process of the analyzed taxa are defined as follows:

plants escaped from cultivation becoming spontaneous (S): the exotic cultivated plants that have escaped from the culture, established in a territory and persist on their own reproductive system;

casual plants (C): the exotic adventitious ones with temporary occurrence in one or a few localities;

naturalized plants (N): the exotic adventitious plants with constant occurrence in zones where they persist on their own reproductive system.

To define the studying areas, there has been used the geographic map of Uruguay (S.G.M.), (Fig. 1: regions 1-12) and the map of regions by labored soils percentage (Evia & Gudynas 2000). There has been selected the regions from the middle West to South, Centre and East (Fig. 1) according to the most humanized areas in terms of labored soils and population density. The total of 57 sites were localized on uncultivated ground, in cities and ruins, road edges, agriculture farms, cattle and dairy farms in intensive and extensive production system.

Herbarium samples for all the species found have been collected. With the purpose of analyzing possible relations between localities and colonizing species, were selected 57 random sites in the twelve regions and there have been carried out surveys and a presence/absence matrix. The obtained data was analyzed with an indirect ordination method, the taxa/sites correspondence analysis (CA) (Hill 1973; Kent & Crocker 1994) with PC-ORD informatics program. Finally, the influence on the territory (or territorial significance) was evaluated by the constant (k), which is expressed for each species as the percentage of records in relation to the total surveys.

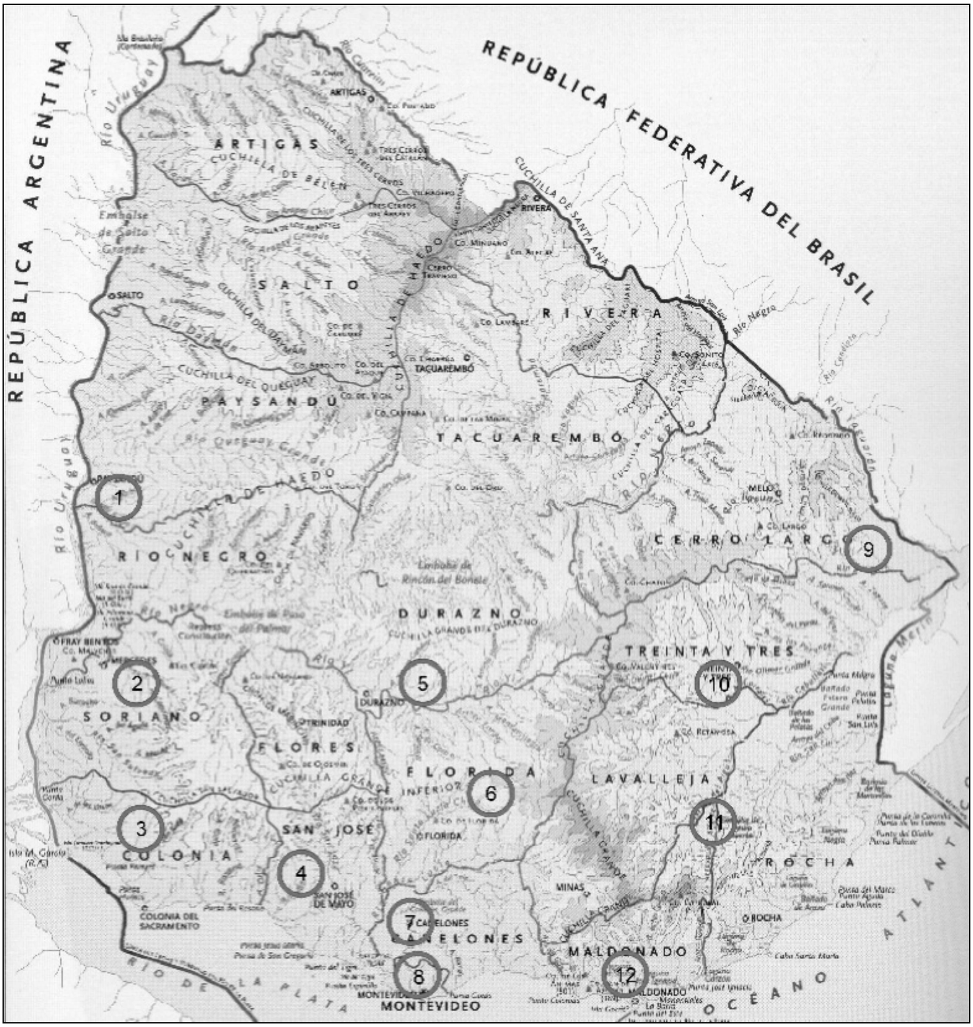

Fig. 1. Geographic map of Uruguay (S.G.M.) with surveyed regions marked.

Results

The total of 174 taxa were taken into account (Tab. 1). The most represented families by the higher number of taxa are: *Poaceae* (35), *Asteraceaeae* (31), *Papilionaceae* (25), *Schrophulariaceae* (10), *Apiaceae* (10), *Labiatae* (8), *Crucifereae* (7).

Remarkable is that 50 species are Euri-Mediterranean (29.59%) and 5 of them are Euri-Mediterranean-Turanian (2.96%). The strictly Mediterranean chorological type and its West, South, Atlantic and Macaronesian variants are represented by 16 taxa (9.47%); the Steno-Mediterranean and its variants includes 14 species (8.28%), the South European and

Table 1. List of taxa with chorological type: escaped from cultivation becoming spontaneous (S), casual (C), naturalized (N); introduction process (I. P), incidence (k).

Taxa	Chorological type	I. P.	k
PIACEAE			
Ammi majus L.	Euri-Medit.	N	52.63
Ammi visnaga (L.) Lam.	Euri-Medit.	N	10.53
Apium nodiflorum (L.) Lag.	Euri-Medit.	N	7.02
Conium maculatum L.	Paleotemp. div. Subcosmop.	N	1.75
Daucus carota L.	Paleotemp. div. Subcosmop.	S	28.07
*Foeniculum vulgare*subsp. *vulgare* Mill.	S-Medit.	S	1.54
Oenanthe globulosa subsp. *globulosa* L.	Steno-Medit.-Occid.	N	1.75
Pastinaca sativa subsp. *sativa* L.	Eurosib. div. Subcosmop.	N	8.77
Torilis arvensis (Hudson) Link	Subcosmop.	C	3.51
Torilis nodosa (L.) Gaertn.	Euri-Medit.-Turan.	N	5.26
APOCYNACEAE			
Vinca major L.	Euri-Medit.	S	3.51
ARALIACEAE			
Hedera helix L.	Submedit. Subatl.	S	3.51
ASTEREAE			
Achillea millefolium subsp. *millefolium* L.	Eurosib.	S	1.75
Anthemis cotula L.	Euri-Medit.	N	38.6
Arctium minus Bernh.	Europeo (Euri-Medit.)	N	8.77
Artemisia vulgaris L.	Circumbor.	S	–
Carduus acanthoides L.	Europ.-Caucas.	N	–
Carduus pycnocephalus L.	Euri- Medit.-Turan.	N	18.54
Carduus tenuiflorus Curtis	W- Europ.-Subatl.	N	38.6
Carthamus lanatus subsp. *lanatus* L.	Euri-Medit.	N	12.28
Centaurea calcitrapa L.	Euri-Medit.div. Subcosmop.	N	15.79
Centaurea debeauxii Gren. & Gordon	Non determined	C	1.75
Centaurea melitensis L.	Steno- Medit. div Pantrop e Subtrop.	N	1.75
Centaurea solstitialis L.	Steno-Medit. div. Subcosmop.	N	5.26
Chamomilla recutita (L.) Rauschert	S.E. Asiat (?) div. Sucosmop.	S	–
Cichorium intybus L.	Cosmopol.	N	36.84
Cirsium vulgare subsp. *vulgare* (Savi) Ten.	Paleotemp. div. Subcosmop.	N	47.37
Coleostephus myconis (L.) Rchb.	Steno-Medit.	N	35.09
Crepis capillaris (L.) Wallr.	Centro-Europ. (Subatl.)	C	1.75
Cynara cardunculus L.	Steno-Medit.	S	29.82
Dittrichia viscosa subsp. *viscosa* (L.) W. Greuter	Euri-Medit.	N	1.75
Hedynopsis cretica (L.) Dum	Steno-Medit.	C	–
Hypochoeris radicata L.	Europ.-caucas.	N	28.07
Lactuca saligna L.	Euri-Medit.-Turan.	N	1.75
Lactuca serriola L.	Euri-Medit.-S-Siber.	N	24.56

Table 1. (continued.)

Leucanthemum vulgare Lam.	Euro-Sib.	S	–
Picris echioides L.	Euri-Medit. (baricentro oriental)	N	26.32
Senecio vulgaris L.	Euri-Medit. div. Cosmop.	N	5.26
Silybum marianum (L.) Gaertn.	Medit.-Turan.	C	1.75
Sonchus asper (L.) Hill	Eurasiat.div. Subcosmop.o Cosmop.	N	19.3
Sonchus oleraceus L.	Eurasiat. div. Subcosmop.	N	38.6
Taraxacum officinale Weber	Circumbor.	N	7.02
Urospermum picroides (L.) Scop.	Euri-Medit.	C	–
BORAGINACEAE			
Borago officinalis L.	Euri-Medit.	N	8.77
Echium plantagineum L.	Euri-Medit.	N	40.35
Myosotis sylvatica Hoffm.	Paleotemp.	C	–
CARYOPHYLLACEAE			
Cerastium glomeratum Thuill.	Euri-Medit.div. Subcosmop.	N	3.51
Polycarpon tetraphyllum subsp. *tetraphyllum* (L.)	Euri-Medit.	N	8.77
Sillene gallica L.	Euri-Medit. div. Cosmop.	N	14.04
Stellaria media subsp. *media* (L.) Fern.-Vill.	Cosmopol.	N	1.75
CHENOPODIACEAE			
Chenopodium album subsp. *album* L.	Subcosmop.	N	8.77
Chenopodium murale L.	Subcosmop.	C	–
CONVOLVULACEAE			
Calystegia silvatica (Kit.) Griseb.	SE- Europ.	C	1.75
Convolvulus arvensis L.	Paleotemp. div. cosmop.	N	12.28
CRASSULACEAE			
Sedum acre L.	Steno-Medit.	C	–
CRUCIFERAE			
Brassica rapa subsp. *sylvestris* L.	Medit?	S	2
Capsella brusa-pastoris (L.) Medicus	Cosmopol.(sinantr.)	N	–
Hirschfeldia incana (L.) Lagr.-Foss.	Medit.-Macarones.	C	2
Lepidium graminifolium L.	Euri-Medit.	C	–
Raphanus raphanistrum subsp. *raphanistrum* L.	Euri-Medit. div. Circumbor.	N	18
Rapistrum rugosum subsp. *rugosum* (L.) All.	Euri-Medit.	N	35
Sisymbrium officinale (L.) Scop.	Paleotemp. div. Subcosmop.	C	4
CYPERACEAE			
Cyperus difformis L.	Paleotemp. div. Subcosmop.	N	2
Cyperus rotundus L.	Subcosmop. Trop. e subtrop.	N	2
DIPSACACEAE			
Dipsacus fullonum L.	Euri-Medit.	N	28
Scabiosa artropurpurea L.	Steno-Medit.	N	14
EUPHORBIACEAE			
Euphorbia peplus L.	Eurosib. div. Cosmop. (Archeofita)	N	4

Table 1. (continued.)

FUMARIACEAE			
Fumaria agraria Lag.	Steno-Medit.	N	–
Fumaria capreolata L.	Euri-Medit.	N	1.75
Fumaria officinalis L.	Paleotemp. div. Subcosmop.	N	10.53
GENTIANACEAE			
Blackstonia perfoliata (L.) Huds.	Euri-Medit.	N	12.28
Centaurium pulchellum (Sw.) Druce	Paleotemp.	N	57.89
GERANIACEAE			
Erodium malacoides (L.) L'Hér. ex Aiton	Medit.-Macarones.	N	8.77
Geranium dissectum L.	Eurasiat. div. Subcosmop.	N	8.77
IRIDACEAE			
Iris pseudacorus L.	Eurasiat.	S	3.51
Gladiolus italicus Miller	Euri-Medit.	S	–
LABIATAE			
Marrubium vulgare L.	Euri-Medit.-Sudsiber. div. Subcosmop.	N	1.75
Mentha Pullegium L.	Euri-Medit. div. Subcosmop.	N	28.07
Mentha spicata L.	Euri-Medit.	S	3.51
Mentha suaveolens subsp. *suaveolens* Ehrh.	Euri-Medit.	S	10.53
Mentha x piperita L.	Non determined	S	3.51
Prunella vulgaris L.	Circumbor.	N	7.02
Salvia verbenaca L.	Medit.-Atl.	C	–
Stachys arvensis L.	Europ.(Subatl.) div. Subcosmop.	N	7.02
LILIACEAE			
Asparagus officinalis L.	Euri-Medit.	S	1.75
MALVACEAE			
Malva nicaensis All.	Steno-Medit.	N	3.51
OLEACEAE			
Nerium oleander L.	S-Medit.	S	–
OXALIDACEAE			
Oxalis corniculata L.	Euri-Medit. div. Cosmop.	N	15.79
PAPAVERACEAE			
Papaver somniferum L.	Euri-Medit. div. Subcosmop.	S	–
PAPILIONACEAE			
Lathyrus hirsutus L.	Euri-Medit.	C	5.26
Lotus angustissimus L.	Euri-Medit.	C	1.75
Lotus corniculatus L.	Paleotemp. div. Cosmopol	S	49.12
Lotus pedunculatus Cav.	Non determined	S	–
Lotus subbiflorus subsp. *subbiflorus* Lag.	W-Medit.	S	10.53
Lotus tenuis Waldst. & Kit.	Paleotemp.	S	7.02
Medicago arabica (L.) Huds.	Euri-Medit.	S	5.26
Medicago lupulina L.	Paleotemp.	S	56.14
Medicago polymorpha L.	Euri-Medit. div. Subcosmop.	S	7.02
Medicago sativa subsp. *sativa* L.	Eurasiat.	S	10.53

Table 1. (continued.)

Melilotus alba Medicus	Eurasiat. div. Subcosmop.	S	22.81
Melilotus indica (L.) All.	Medit.-Turan. div. Subcosmop.	N	19.3
Spartium junceum L.	Euri-Medit.	S	12.28
Genista monspessulana L.A.S.Johnson	Steno-Medit.-Macarones.	S	–
Trifolium alexandrinum L.	E-Medit.	S	1.75
Trifolium angustifolium subsp. *gibellianum* Pign.	N-medit	C	1.75
Trifolium campestre Schreb.	W-Paleotemp.	S	1.75
Trifolium pratense L.	Eurosib. div. Subcosmop.	S	28.07
Trifolium repens subsp. *repens* L.	Paleotemp. div. Subcosmop.	S	50.88
Ulex europaeus L.	Subatlant.	N	3.51
Vicia benghalensis L.	Steno-Medit.	N	10.53
Vicia sativa subsp. *nigra* (L.) Ehrh.	Medit.-Turan. div. Subcosm.	N	17.54
Vicia sativa subsp. *sativa* L.	Medit.-Turan. div. Subcosm.	C	7.02
Vicia villosa subsp. *eriocarpa* (Hauskn.) P. W. Ball	Euri-Medit.	N	–
Vicia villosa subsp *varia* (Host) Corb	Euri-Medit.	N	–
PINNACEAE			
Pinus pinaster Aiton	W-Medit. (steno)	S	3.51
PLANTAGINACEAE			
Plantago lanceolata L.	Eurasiat. div. Cosmopol.	N	28.07
Plantago major L.	Eurasiat. div. Subcosmop.	N	3.51
POACEAE			
Aira caryophyllea subsp. *caryophyllea* L.	R- Paleo-subtrop..	N	1.75
Aira elegantissima Schur	Euri-Medit.	N	–
Avena barbata Potter	Euri-Medit.-Turan.	N	3.51
Avena fatua L.	Eurasiat.	N	1.75
Avena sativa L.	Eurasiat.	S	12.28
Avena sterilis L.	Euri-Medit.-Turan.	N	21.05
Brachypodium sylvaticum (Hudson) P. Beauv.	Paleotemp.	C	5.26
Briza maxima L.	Paleosubtrop.	N	14.04
Briza minor L.	Subcosmop. (Regioni calde)	N	24.56
Bromus hordaceus subsp. *hodaceus* L.	Subcosmop.	N	5.26
Bromus inermis Leyss.	Eurasiat.	N	1.75
Cynodon dactylon (L.) Pers.	Termocosmop.	N	92.98
Cynosurus echinatus L.	Euri-Medit.	C	1.75
Dactylis glomerata L.	Paleotemp	S	7.02
Desmazeria rigida subsp. *rigida* (L.) Tutin	Euri-Medit.	C	1.75
Digitaria sanguinalis L. Scop.	Cosmopol.	N	12.28
Echinocloa colonum (L.) Link	Paleotrop. e Subtrop.	N	7.02
Echinocloa crus-galli (L.) Beauv.	Subcosmop.	N	7.02
Eragrostis cilianensis (All.) F.T. Hubbard.	Termocosmop.	N	3.51
Festuca arundinacea subsp. *arundinacea* Schreb.	Paleotemp.	S	7.02
Gaudinia fragilis (L.) P. Beauv.	Euri-Medit.	N	8.77

Table 1. (continued.)

Melilotus alba Medicus	Eurasiat. div. Subcosmop.	S	22.81
Melilotus indica (L.) All.	Medit.-Turan. div. Subcosmop.	N	19.3
Spartium junceum L.	Euri-Medit.	S	12.28
Genista monspessulana L.A.S.Johnson	Steno-Medit.-Macarones.	S	–
Trifolium alexandrinum L.	E-Medit.	S	1.75
Trifolium angustifolium subsp. *gibellianum* Pign.	N-medit	C	1.75
Trifolium campestre Schreb.	W-Paleotemp.	S	1.75
Trifolium pratense L.	Eurosib. div. Subcosmop.	S	28.07
Trifolium repens subsp. *repens* L.	Paleotemp. div. Subcosmop.	S	50.88
Ulex europaeus L.	Subatlant.	N	3.51
Vicia benghalensis L.	Steno-Medit.	N	10.53
Vicia sativa subsp. *nigra* (L.) Ehrh.	Medit.-Turan. div. Subcosm.	N	17.54
Vicia sativa subsp. *sativa* L.	Medit.-Turan. div. Subcosm.	C	7.02
Vicia villosa subsp. *eriocarpa* (Hauskn.) P. W. Ball	Euri-Medit.	N	–
Vicia villosa subsp *varia* (Host) Corb	Euri-Medit.	N	–
PINNACEAE			
Pinus pinaster Aiton	W-Medit. (steno)	S	3.51
PLANTAGINACEAE			
Plantago lanceolata L.	Eurasiat. div. Cosmopol.	N	28.07
Plantago major L.	Eurasiat. div. Subcosmop.	N	3.51
POACEAE			
Aira caryophyllea subsp. *caryophyllea* L.	R- Paleo-subtrop..	N	1.75
Aira elegantissima Schur	Euri-Medit.	N	–
Avena barbata Potter	Euri-Medit.-Turan.	N	3.51
Avena fatua L.	Eurasiat.	N	1.75
Avena sativa L.	Eurasiat.	S	12.28
Avena sterilis L.	Euri-Medit.-Turan.	N	21.05
Brachypodium sylvaticum (Hudson) P. Beauv.	Paleotemp.	C	5.26
Briza maxima L.	Paleosubtrop.	N	14.04
Briza minor L.	Subcosmop. (Regioni calde)	N	24.56
Bromus hordaceus subsp. *hodaceus* L.	Subcosmop.	N	5.26
Bromus inermis Leyss.	Eurasiat.	N	1.75
Cynodon dactylon (L.) Pers.	Termocosmop.	N	92.98
Cynosurus echinatus L.	Euri-Medit.	C	1.75
Dactylis glomerata L.	Paleotemp	S	7.02
Desmazeria rigida subsp. *rigida* (L.) Tutin	Euri-Medit.	C	1.75
Digitaria sanguinalis L. Scop.	Cosmopol.	N	12.28
Echinocloa colonum (L.) Link	Paleotrop. e Subtrop.	N	7.02
Echinocloa crus-galli (L.) Beauv.	Subcosmop.	N	7.02
Eragrostis cilianensis (All.) F.T. Hubbard.	Termocosmop.	N	3.51
Festuca arundinacea subsp. *arundinacea* Schreb.	Paleotemp.	S	7.02
Gaudinia fragilis (L.) P. Beauv.	Euri-Medit.	N	8.77

Table 1. (continued.)

Hainardia cylindrica (Willd.) Greuter	Euri-Medit.	C	1.75
Holcus lanatus L.	Circumbor.	S	19.3
Hordeum murinum subsp. *leporinum* (Link) Arcangeli	Circumbor.	N	3.51
Koeleria pyramidata (Lam.) P. Beauv.	Nord- e Centro-Europ.	N	7.02
Lolium multiflorum Lam.	Euri-Medit.	S	54.39
Phalaris aquatica L.	Steno-Medit.-Macarones.	S	14.04
Phalaris canariensis L.	Macarones.	S	1.75
Poa annua L.	Cosmopol.	N	3.51
Polypogon maritimus subsp. *maritimus* Willd.	Steno-Medit.-Macarones.	N	3.51
Polypogon monspeliensis (L.) Desf.	Paleosubtrop.	N	1.75
Setaria italica (L.) Beauv.	Asia Tropic. ?	S	5.26
Sorghum halepense (L.) Pers.	Termocosmop.	N	33.33
Vulpia bromoides S. F. Gray	Paleotemp.	N	8.77
Vulpia myuros (L.) C.C.Gmel.	Subcosmop.	N	1.75
POLYGONACEAE			
Rumex conglomeratus Murray	Eurasiat. Centro-occid.	N	17.54
Rumex crispus L.	Subcosmop.	N	35.09
Rumex pulcher subsp. *pulcher* L.	Euri-Medit.	N	21.05
PRIMULACEAE			
Anagallis arvensis L.	Euri-Medit. div. Subcosmop.	N	31.58
RANUNCULACEAE			
Ranunculus muricatus L.	Euri-Medit.	N	7.02
ROSACEAE			
Duchesnea indica Focke	Asia Tropic. div. Subcosmop.	S	1.75
Rubus idaeus L.	Circumbor.	S	–
Rubus ulmifolius Schott	Euri-Medit.	S	3.51
RUBIACEAE			
Galium aparine L.	Eurasiat.	N	3.51
SALICACEAE			
Populus alba L.	Paleotemp.	S	3.51
SCROPHULARIACEAE			
Antirrhinum majus L.	W-Medit.	S	–
Bellardia trixago (L.) All.	Euri-Medit.	N	12.28
Cymbalaria muralis Gaertn. Mey & Scherb.	S-Europ. div. Subcosmop.	N	1.75
Parentucellia viscosa (L.) Caruel	Medit.-Atl.	C	3.51
Verbascum virgatum Stokes	W- Europ. Subatl.	N	12.28
Veronica anagallis-aquatica L.	Cosmopol.	N	3.51
Veronica arvensis L.	Subcosmop.	N	1.75
Veronica persica Poir.	Subcosmop. (Neofita)	N	1.75
Veronica polita Fries	Subcosmop. (Arqueofita)	N	–
Veronica serpyllifolia L.	Circumbor. div. subcosmop.	N	–

Table 1. (continued.)

URTICACEAE			
Urtica dioica L.	Subcosmop.	N	–
VIOLOCEAE			
Viola odorata L.	Paleotemp.	S	1.75

its variants 2 species (1.18%). Notoriously, the Mediterranean types and the South European amount to 88 taxa which represent 52% of the European contingent surveyed for this work in Uruguay.

In relation to the introduction process (Tab. 1), the main role reached by the taxa in the new environment is adventitious, among which the naturalized ones reach 59% and casuals 13%, in contrast to the ones escaped from cultivation that amount to 28%.

The correspondence analysis taxa/sites (Fig. 2), about the gradient in the species occurrence, shows that there is no defined trend between territories and colonizing species. Nevertheless, at least two groups showing some variability can be distinguished. The gradient that expresses the higher variability on the first axis is followed by: *Brachypodium sylvaticum*, *Ranunculus muricatus*, *Festuca arundinacea*, *Geranium dis-*

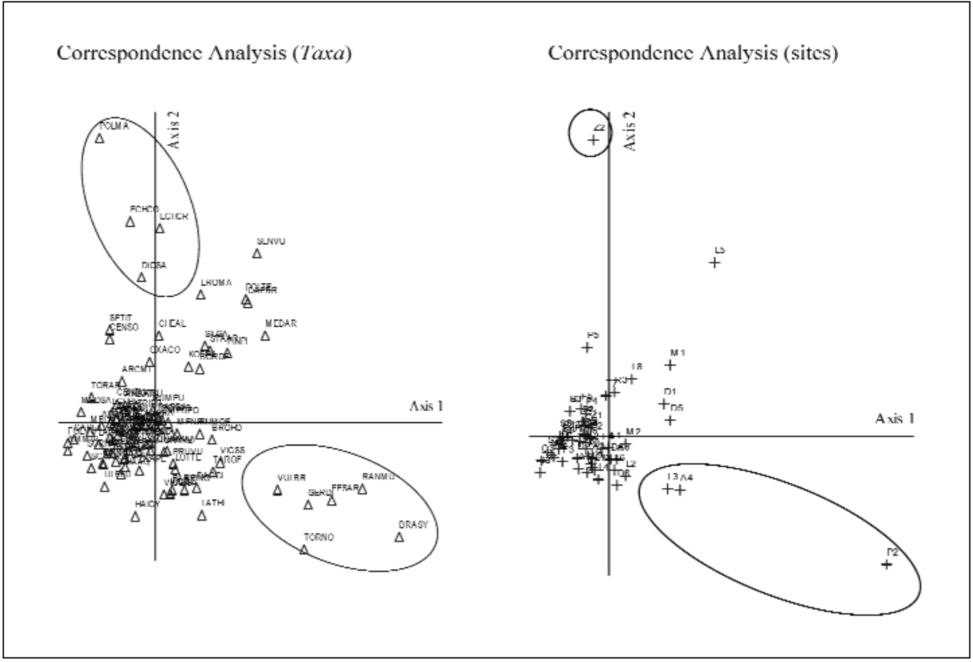

Fig. 2. The gradient in the taxa occurrence in correspondence to territories (sites) where surveys were done (site Z2 belongs to region 9, L3 to region 7, A4 to region 8, P2 to region 1, M1 to region 12, D1 to region 5).

Table 2. Range of k values for the studied taxa.

k	numb. of species	%
-	24	15
1-10	94	54
10-20	25	14
20-30	13	7
30-40	9	5
40-93	9	5

sectum, *Torilis nodosa*, *Vulpia bromoides*, this have correspondence mainly with regions 1, 7 and 8 (Fig. 1: Paysandú Montevideo and Canelones: sites L_3, A_4 and P_2). The first is located in a zone that has 70 to 100% of disturbed soils and the other two are in mostly urbanized zones with 95 to 100% of disturbed soils, both characterized by lime-argillaceous sedimentary soils with tendency to high PH. The second gradient on the axis 2, is followed by *Polypogon maritimus*, *Echinochloa colonum*, *Echinochloa crus-galli*, and have correspondence mainly with region 9 (Fig. 1: site Z_2). Both regions are in zones with 30 to 70% and 70 to 95% of disturbed soils, these are in the east area of the territory characterized by plane soils (Duran 1985). This region is directly related with the rice culture; at the same time the last two named species grow as invasive of summer cultures (Mazocca 1976), in particular rice and *P. maritimus* is invasive of wet areas (Rosengurt & al. 1970). These results have the limitation of a very low eigen value, 0.3, this indicates a very little correspondence or low correlation between the parameters and lack of hardness of the indicated tendency.

Regarding to the species with higher influence in the colonized area (Tab. 2), the *k* value shows that only 9 taxa (5% of the total species number) have a *k* value higher than 40, instead *k* values lower than 10 concern 94 taxa (54% of the total species number); 24 taxa (15%) belonging to the total contingent found did not appear in the 57 systematic relieves.

In the group with a higher value of k = 40-93 are included: *Cynodon dactylon* (93), *Centaurium pulchellum* (58), *Medicago lupulina* (56), *Lolium multiflorum* (54), *Ammi majus* (53), *Trifolium repens* (51), *Lotus corniculatus* (49), *Cirsium vulgare* (47) and *Echium plantagineum* (40). In the next group, k = 30-40 are: *Anthemis cotula* (39), *Carduus tenuiflorus*, (39), *Sonchus oleraceus* (39), *Cichorium intybus* (37), *Rumex crispus* (35), *Coleostephus myconis* (35), *Rapistrum rugosum* (35), *Sorghum halepense* (33), *Anagallis arvensis* (32). Most of the species are invasive of crops, pastures, uncultivated ground and road edges (Tab. 1, Fig. 3-8) (Mazocca 1976). On the other hand, *Lolium multiflorum*, *Lotus corniculatus*, *Lotus subbiflorus*, *Lotus tenuis*, *Medicago lupulina*, *Trifolium repens* are remarkable since their diffusion as forage cultivation, until today they play an important role in cattle nutrition (Izaguirre & al. 1998; Rosengurt & al. 1970).

Figs. 3-8. Common species invasive of crops, pastures, uncultivated ground and road edges: 3 *Lolium multiflorum*, 4 *Coleostephus myconis*, 5 *Cynara cardunculus*, 6 *Lactuca serriola*, 7 *Vicia sativa* subsp. *nigra*, 8 *Ammi visnaga*.

Conclusions

The 174 taxa that were found, belong to 36 families, conversely the 7 more represented families include the 73% of the total species found.

Regarding the chorological classification, among the six more represented groups the most important is the Euro-Mediterranean with 29.59% of the species; the following chorological types in decreasing importance are the Paleotemperated, Mediterranean, steno-Mediterranean, sub-cosmopolitan and Eurasian. All Mediterranean types and the South European are about 52% of the European contingent.

In relation with the introduction process, the majority is naturalized (59%). At the same time, inside the group of 9 taxa with major acquired importance in the colonized area (with *k* values between 40 and 93) belong to the more represented families: *Papilionaceae*, *Poaceae*, *Apiaceae* and *Asteraceae*; four of them are escaped from cultivation and five are naturalized; three of them are euri-Mediterranean, five are Paleo-temperate and one is thermo-cosmopolitan. In the group of 9 *taxa* with k values between 30 and 40, most are *Asteraceae* and all of them are naturalized; three are euri-Mediterranean, one of them is steno-Mediterranean, and the rest are thermo-cosmopolitan, sub-cosmopolitan, cosmopolitan, West Euro - sub-Atlantic, and Eurasian chorological types (Tab. 1). From that point of view, the majority of naturalized taxa inside the European contingent shows that the introduction process is mainly involuntary.

The gradient in the species occurrence with the correspondence analysis taxa/sites (Fig. 2), shows that there is no defined trend between territory and colonizing species, except for a few species that show a tendency with a low correspondence and its specifically related to human activity in different aspects. The gradient on the axis 1 is more related to higher population density, higher agriculture intensity and the proximity with the more important points connecting to foreign countries; at the same time is related to one of the sites of higher number of taxa presence (Montevideo). The gradient on the axis 2, instead, is more likely related to a specific culture type like rice.

Acknowledgements

This study is part of the project of research by S. Merola done under the coordination of F. M. Raimondo. CORI project of Palermo University is gratefully acknowledged.

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Ch. Gussev, Y. Bosseva, B. Pandova, S. Yanev & M. Stanilova

Resource assessment of *Leucojum aestivum* L. (*Amaryllidaceae*) populations in Bulgaria

Abstract

Gussev, Ch., Bosseva, Y., Pandova, B., Yanev, S. & Stanilova, M.: Resource assessment of *Leucojum aestivum* L. (*Amaryllidaceae*) populations in Bulgaria. — Bocconea 21: 405-411. 2007. — ISSN 1120-4060.

Monitoring of 22 populations of *Leucojum aestivum* L. (*Amaryllidaceae*) was performed in the period 2001-2004. Analysis of management practices and resource assessment of 16 economically significant localities were made in 2003 and 2004. Population density varied from 7.38±4.10 to 46.37±2.67 ind/m^2. Exploitation reserves (from 483 to 52 903 kg) and possible annual harvest were estimated by means of model individuals. Comparison between populations of fresh-mass productivity of individual (7.47±0.40 – 25.43±2.08 g) and galanthamine content (0.9 – 2.6 mg/g average) showed great variability.

Introduction

Leucojum aestivum L. (*Amaryllidaceae*) is a resource species for the pharmaceutical industry in Bulgaria, as Galanthamine-containing natural populations have been of economic importance since 1960. We published an earlier detailed account of the problems related to the protection and sustainable use of this species with regard to the great demand in crude drug in the last 3 years (Gussev & al. 2003). Several assessments have been carried out to quantitatively and qualitatively characterize the resources of *L. aestivum* in Bulgaria (Stoyanov & Savchev 1964; Astadjov 1969; Stefanov 1990; Mitrev 1995). However, contemporary understanding of the sustainable use of medicinal plants requires the analysis of management practices, annual monitoring of populations and quantitative assessment of reserves, and the monitoring of Galanthamine content and requirements for sustainable management.

Commonly known as summer snowflake, *Leucojum aestivum* is categorized as endangered in the Red Data Book of Bulgaria (Velchev 1984). A regime of protection and regulated use was prescribed according to the Protection of Biological Diversity Act (2002) in Bulgaria and Order # RD-521 of the Ministry of the Environment and Waters, pursuant to which:

Access to economically important localities is controlled, and only 16 Galanthamine populations are permitted for use (Tab. 1).

Table 1. Localities of *L. aestivum* in Bulgaria permitted for use.

No	Locality	District	Coordinates	Area (ha)
1	Gradina	Plovdiv	42°08’N 25 12’E	20.0
2	Vinitza	Plovdiv	42°08’N 25°08’E	18.6
3	Debelata Koria	Plovdiv	42°22’N 24°47’E	0.4
4	Ormana	Yambol	42°32’N 26°31’E	30.0
5	Palauzovo	Yambol	42°32’N 26°44’E	16.0
6	Blatetz	Sliven	42°38’N 26°32’E	15.0
7	Blatoto	Burgas	42°24’N 27°40’E	29.0
8	Kalinata	Burgas	42°42’N 27°40’E	20.0
9	Chairite	Burgas	42°48’N 27°32’E	2.0
10	Karaagach	Burgas	42°13’N 27°45’E	0.5
11	Zidarovo	Burgas	42°20’N 27°24’E	30.0
12	Lozenski Pat	Haskovo	41°46’N 26°10’E	48.0
13	Biser	Haskovo	41°52’N 25°60’E	35.8
14	Dolnata ova	Haskovo	41°49’N 26°08’E	20.0
15	Kotchovo	Shumen	43°14’N 26°48’E	12.0
16	Osmar	Shumen	43°13’N 26°51’E	19.0

The defined terms, methods and regulations of use include plant-friendly exploitation methods, capacity of the ecological reserves of individuals, and floating herbage yields.

The regulations and exploitation quotas were set. The amount of herbage to be collected annually is regulated by an explicit Order of the Minister of the Environment and Waters after assessment of population status. The owners of the localities then issue permits to buyers and herb-gatherers and control use.

Methods

The main methodologies were based on the guidelines established in the design of plant conservation or recovery plans (Jakson & Akeroyd 1994), The Handbook of Ecological Monitoring (Clarke ed. 1986), and Methodology for determination of medicinal plant resources (Anonymus 1986).

The population monitoring and assessment from 2001-2004 were carried out according to a standard protocol which included the following basic parameters: total and effective productive area, density (total density as well as that of generative and vegetative individuals), anthropogenic influence (habitat destruction, grazing, irregular harvesting), yield, exploitation reserves and possible annual harvest.

The method of concrete localities was applied to measure quantitative reserves, as yield was calculated by the following parameters: area of locality (ha) and yield (g/m^2). The yield was calculated as a product of the average fresh mass of model individuals (g) and density (ind/m^2) (M – average, m – standard error). The model individuals (30-40 from locality) were harvested at the beginning of flowering and their fresh mass was measured by electronic balances. The density was measured by plot units of 0.025 m^2, representative of the locality ($P<0.1$). The exploitation reserves (kg) were calculated as the product of yield (its lower limit M-2m) and effective productive area. The possible annual harvest (kg) was calculated by dividing the number of exploitation reserves by 4 (herbage yield, i.e. one year of harvesting plus a 3-year recovery period).

Galanthamine content of the model individuals was presented as an average for the population. The standard protocol of analyses included the following main procedures: model individuals were dried at 60ºC; methanol extraction (3 times 30 min sonification for 24 h), centrifugation, filtration, evaporation, dissolution, filtration) and HPLC analysis (Waters quaternary system, PDA detector) (Pandova & al. in press). ANOVA were applied to prove the significance of variations between the populations according to Gal-content ($P<0.05$).

A Bulgarian *Leucojum* Data Base (BLDB) was developed for the purposes of preservation and management of data relating to the monitoring of populations and resource assessment (Georgiev & al. 2003).

Results and Discussion

The analysis of management practices showed that existing legislation is adequate for the sustainable management of *L. aestivum* populations as a source of *Herba Leucoji aestivi* (Galanthamine source). The management of most localities was facilitated by their proclamation as protected areas, where the regime ensures ecologically-friendly utilization.

The assessment of population status and exploitation reserves has been carried out using an unsuitable protocol and the control of herb-gathering is poor and ineffective. As there is no audit of the permitted quotas, these quotas are often exceeded. Illegal gathering also occurs and business is not integrated in the protection of populations or efforts for the sustainable use of resources.

Twenty-two populations were subjected to monitoring (density assessment of the 16 localities permitted for gathering were presented in Table 2, data concerning the rest of populations is contained in BLDB). In four localities (Vinitza, Debelata Koria, Chairite, Lozenski Pat) we observed permanent habitat degradation (forestation with poplar cultivars, drainage and intensive grazing), as well as the destruction of populations (low density, lack of flowering and fruit-bearing). These populations could not be used for industrial purposes and urgent restoration measures need to be undertaken.

The remaining 12 localities are of interest for use under the legal regime prescribed. The total area of these localities is 247.3 ha (varying from 0.5 to 35.8 ha) representing 80% of the total national population of Galanthamine-containing plant species. The effective production area where *Leucojum* populations occur is 74.7 ha. It was found that herb-gatherers cut all individuals regardless of their development (young and generative).

Population density (ind/m^2) varied within a wide range from 7.38±4.10 to 46.37±2.67 ind/m^2. The annual fluctuations in density are mainly due to soil moisture content (flooding in spring and autumn) and gathering methods (height above ground at which herbage is cut). Cutting close-to-the ground negatively influences the preparation of the bulb for

Table 2. Density (ind/m^2) assessment of the *Leucojum aestivum* localities permitted for use. M - average, m - standard error.

Locality	2001						2002					
	vegetative		generative		total		vegetative		generative		total	
	M	m	M	m	M	m	M	m	M	m	M	m
Gradina	29.60	5.72	8.09	4.47	37.69	6.37	39.53	2.51	6.84	0.67	46.37	2.67
Ormana	15.38	4.25	5.78	4.00	21.17	4.44						
Palauzovo	3.52	4.00	3.86	3.97	7.38	4.10						
Blatez	32.00	5.90	3.16	4.01	35.16	5.93						
Blatoto							27.63	2.16	8.16	0.96	35.79	2.27
Kalinata	13.40	4.30	3.93	4.04	17.33	4.57						
Karaagach							19.15	1.58	5.28	0.53	24.43	1.70
Zidarovo												
Lozenski Pat	20.63	4.54	1.88	3.96	22.51	4.50						
Biser	8.34	4.24	1.24	3.97	9.58	4.31						
Dolnata Ova	9.52	5.10	6.29	4.09	15.81	5.32						
Kotchovo												
Osmar												

Locality	2003						2004					
	vegetative		generative		total		vegetative		generative		total	
	M	m	M	m	M	m	M	m	M	m	M	m
Gradina	27.20	4.99	16.19	4.01	43.39	5.13						
Ormana	11.79	4.05	10.45	4.05	22.24	4.20	19.36	1.90	12.00	1.46	29.98	2.57
Palauzovo	9.18	4.02	4.58	4.00	13.77	4.03	8.29	0.89	2.92	0.36	11.21	1.06
Blatez					27.15	4.59	17.58	1.37	5.23	0.35	17.58	1.37
Blatoto	15.94	4.12	8.16	4.01	24.03	4.15	13.17	1.42	6.00	0.60	17.58	1.37
Kalinata	16.45	4.14	2.86	4.00	19.31	4.16	9.09	1.04	5.52	0.84	14.61	1.45
Karaagach	14.34	4.03	9.04	3.98	23.39	4.06						
Zidarovo	20.36	4.29	11.37	4.09	31.74	4.48						
Lozenski Pat												
Biser												
Dolnata Ova												
Kotchovo	5.00	4.00	7.15	4.00	12.15	4.04	4.17	0.61	3.83	0.44	8.00	0.85
Osmar	10.23	4.15	5.66	4.01	15.89	4.26	3.82	0.70	6.62	0.82	10.44	1.37

the next vegetation period and often leads to temporary dormancy or poor vegetation. *L. aestivum* maintains a high soil reserve of conserved bulbs and seeds. Density could be increased through artificial flooding. For example, total density at the Gradina locality increased from 37.69±6.37 ind/m^2 in 2001 to 46.37±2.67 in 2002 and 43.39±5.13 for 2004. Population density is an initial parameter for yield calculation, which is why reserve assessment must be carried out annually at the beginning of flowering.

Mean fresh mass of model individuals varied between populations (7.47±0.40 – 25.43±2.08 g) due to differences in ecological conditions and hydrological regime. In case of low variability of soil moisture in 2003 and 2004 (Gradina, Ormana, Dolnata Ova), the

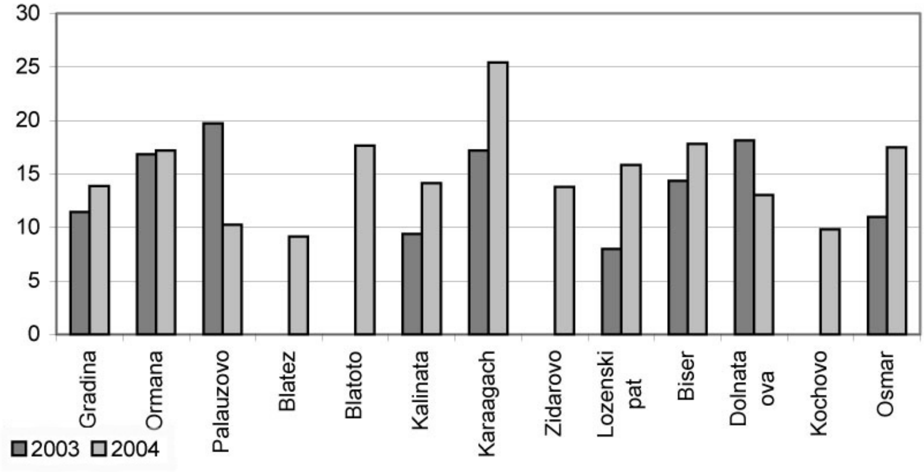

Fig. 1. Average fresh mass of model individuals (g).

differences in the average mass were insignificant. Excessive drought and accidental flooding drastically influence herbage mass and that is why annual assessment of the mass is obligatory for the resource assessment (Fig. 1).

The exploitation reserves and possible annual harvest were calculated for localities with a good status at the time of assessment (well-moisturized, normal plant phenology and development without extreme influences): 6 localities in 2003 and 9 in 2004 (Tab. 3).

Galanthamine content in 2003 varied between and within populations (Fig. 2, Tab. 4). All populations were profitable according to Galanthamine content (0.9-2.6 mg/g average). The same regularity in Galanthamine content by populations was detected by Stefanov in 1990, which confirms the stability of this parameter.

Conclusions and Recommendations

Only 12 of the monitored populations of *L. aestivum* are economically important. The developed methodology in this study was effective and comparatively easy to apply.

An Action Plan should be developed for the localities permitted for usage, with emphasis on habitat reclamation. Management Plans need to be developed for all populations.

The basic condition for the effectiveness of adaptive management for sustainable use are: precise and well-timed monitoring of population status according to preset population parameters; annual assessment of the reserves immediately prior to harvesting time of the locality; control of gathering and observing permitted quotas.

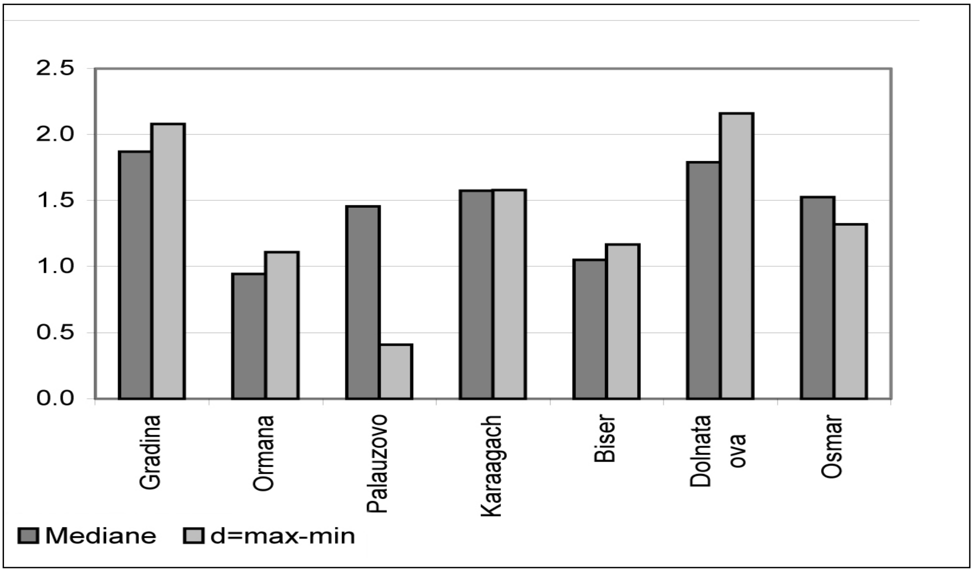

Fig. 2. Galanthamine content (mg/g) – 2003.

Table 3. Assessment of exploitation reserves and possible annual harvest of *Leucojum aestivum* in Bulgaria in 2003 and 2004.

Locality	Density (ind/m^2)				Fresh mass (g)				Yield (g/m^2)				Effec. area (ha)	Exploit. Reserv.(kg)		Possib. Ann. Harvest (kg)	
	2003		2004		2003		2004		2003		2004						
	M	m	M	m	M	m	M	m	M	m	M	m		2003	2004	2003	2004
Gradina	43.39	5.13			11.46	0.97	13.86	0.95	497.29	72.30			15	52903		13226	
Ormana	22.24	4.20	29.98	2.57	16.84	0.73	17.18	0.92	374.47	72.57	515.01	52.05	5	11467	20545	2867	5136
Palauzovo	13.77	4.03	11.21	1.06	19.71	2.35	10.25	0.52	271.31	85.77	114.90	12.30	4	3991	3612	998	903
Blatetz			17.58	1.37			9.14	0.35			160.64	13.93	4		5311		1328
Blatoto			17.58	1.37			17.67	1.00			310.56	29.87	10		25081		6270
Kalinata	19.31	4.16	14.61	1.45	9.38	0.44	14.13	0.87	181.13	39.94	206.45	24.05	4	4050	6334	1013	1583
Karaagach	23.39	4.06			17.18	1.69	25.43	2.08	401.84	80.17			0.2	483		121	
Kotchovo			8.00	0.85			9.81	0.52			78.48	9.33	1.5		897		224
Osmar	15.89	4.26	10.44	1.37	12.00	1.03	17.5	0.91	190.68	53.68	182.72	25.83	2	1667	2621	417	655

Table 4. ANOVA analysis of Galanthamine content of model individuals (mg/g) – 2003.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Populations	62.436378	9	6.9373753	36.940379	5.304E-42	1.9153106
Within Population	49.766801	265	0.1877992			
Total	112.203180	274				

Acknowledgements

This research was sponsored by NATO's Scientific Affairs Division in the framework of the Science for Peace Programme. The authors express their gratitude to Dr. Vladimir Valchev for his help in resource assessment as well as to all regional representatives of the Ministry of Environment and Waters.

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S. P. Kell, S. L. Jury, H. Knüpffer, B. V. Ford-Lloyd & N. Maxted

PGR Forum: serving the crop wild relative user community

Abstract

Kell, S. P., Jury, S. L., Knüpffer, H., Ford-Lloyd, B. V. & Maxted, N.: PGR Forum: serving the crop wild relative user community. — Bocconea 21: 413-421. 2007. — ISSN 1120-4060.

The Euro-Mediterranean region is an important centre for the diversity of crop wild relatives – species closely related to crops that can provide useful traits for crop improvement. The European Community funded project, PGR Forum (www.pgrforum.org) created a web-based Crop Wild Relative Information System (CWRIS) to provide access to crop wild relative data to a broad user community, including plant breeders, protected area managers, policy-makers, conservationists, taxonomists and the wider public. The system includes data on uses, geographical distribution, biology, population and habitat information, threats (including IUCN Red List assessments) and conservation actions. This information is vital for the continued sustainable utilization and conservation of crop wild relatives. Accessible via the CWRIS is a catalogue of CWR derived from two major databases: Euro+Med PlantBase and Mansfeld's World Database of Agricultural and Horticultural Crops; with the addition of data sources for forestry, ornamental, medicinal and aromatic groups. Results show that approximately 80% of the Euro-Mediterranean flora consists of crop wild relatives and other utilized species, as well as the crops themselves; in other words, at least three-quarters of the plant species in the region have a current or potential direct use to humankind. This paper presents a summary of the methodology used to create a Euro-Mediterranean catalogue of CWR, and some preliminary results from analysis of the data.

Introduction

The combined European and Mediterranean region (here referred to as Euro-Mediterranean region) is an important area for the taxonomic and genetic diversity of crop wild relatives (CWR). Major crops, such as oats *(Avena sativa* L.), sugar beet (*Beta vulgaris* L.), apple (*Malus domestica* Borkh.), annual meadow grass (*Festuca pratensis* Huds.) and white clover (*Trifolium repens* L.), have wild relatives in Europe and the Mediterranean. Many minor crops have also been developed and domesticated in the region, such as arnica *(Arnica montana* L.), asparagus *(Asparagus officinalis* L.), lettuce (*Lactuca sativa* L.), and sage (*Salvia officinalis* L.). Other groups of socio-economic importance in the region are forestry species such as *Abies alba* Mill., *Populus nigra* L. and *Quercus ilex* L., ornamentals such as species of *Dianthus*, *Euphorbia*, *Geranium* and

Primula, and medicinal and aromatic plants such as species of *Anemone*, *Campanula*, *Helianthemum*, *Orchis* and *Verbascum*.

The term ‘crop wild relative’, as well as specifically relating to the wild relatives of agricultural and horticultural crops, may also be applied in a more general sense to taxa related to other species of direct socio-economic importance, and thus include food, fodder and forage crops, medicinal and aromatic plants, condiments, ornamental and forestry species as well as plants used for industrial purposes, such as oils and fibers. CWR taxa provide a wealth of useful traits transferable to socio-economically important species. They are essential components of natural and semi-natural habitats, as well as of agricultural systems, and are critical for maintaining ecosystem health. Their conservation and sustainable use is also vital for improving agricultural production, increasing food security, and maintaining the environment. CWR germplasm (genetic material) has been utilized by humankind for thousands of years to improve the quality and yield of crops. Farmers have used traditional farming methods involving selection for millennia, and more recently plant breeders have utilized genes of CWR to improve crops for resistance to pests and diseases and tolerance to abiotic stresses, such as drought and salinity. Other uses can lead to increased protein and vitamin content, the improvement of medicinal plants and development of pharmaceuticals.

Threats to crop wild relatives

Major threats to CWR are habitat alteration, fragmentation and loss, changes in land management practices and genetic pollution. Many CWR have limited distributions and habitat niches. For example, in the UK, *Asparagus officinalis* subsp. *prostratus* (Dumort.) Corb. is confined to free draining sea cliffs and sand dunes, where it is threatened by changes in land use and agricultural practices, tourism and soil erosion (UK Biodiversity Steering Group 1998; T. C. G. Rich pers. comm. 2003). CWR are commonly weeds of traditional farming practices and are often associated with the cultivation of local varieties (landraces). With an increase in industrial farming and cultivation of high-yielding varieties, the taxonomic and genetic diversity of the associated CWR has decreased, resulting in genetic erosion and local extinction. A further hidden potential threat to CWR is that many of the less common species that are not immediately threatened are overlooked in conservation planning. Without active management, these taxa are also likely to be at risk.

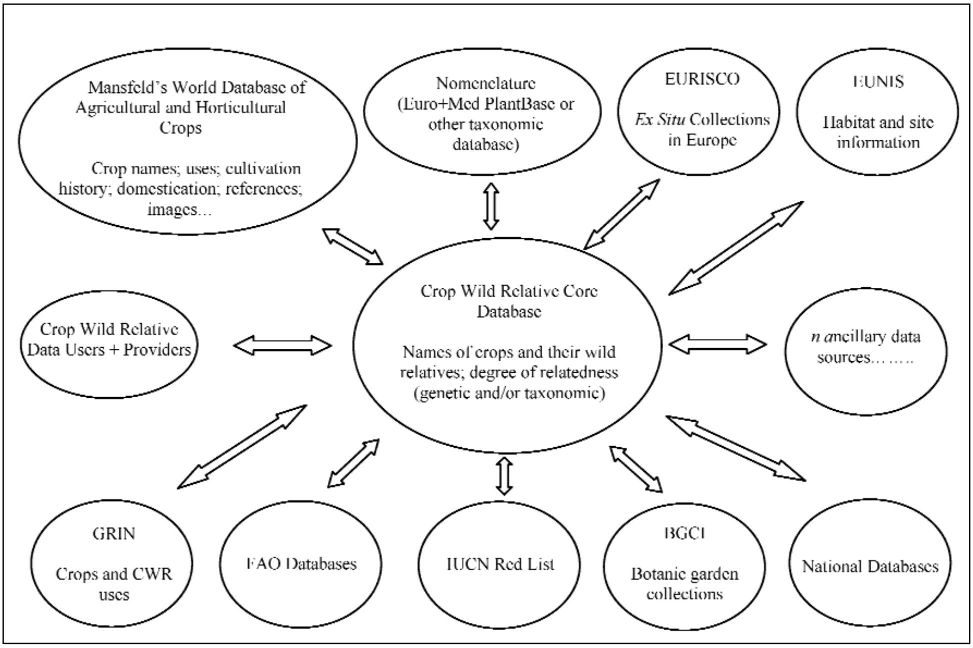
Although it is acknowledged that populations of CWR throughout the Euro-Mediterranean region are under threat from habitat alteration and loss, their conservation, both *in situ* and *ex situ* has received relatively little systematic attention (Maxted 2003). There have been a number of initiatives to inventory CWR taxa at country level (notably Schlosser & al. (1991) and Hammer & Schlosser (1995) for the former German Democratic Republic, Mitteau & Soupizet (2000) for France, and for the European part of Russia (Smekalova pers. comm. 2003)), and at regional level for Europe, (notably those proposed by Zeven & Zhukovsky (1975), Heywood & Zohary (1995) and Hammer & Spahillari (1999, 2000)). However, prior to the PGR Forum project, there had not been a coordinated effort focussing on the production of a comprehensive Euro-Mediterranean catalogue.

A gateway for crop wild relative information

PGR Forum (www.pgrforum.org) has created an online information system to make CWR data available to a broad user community, including plant breeders, protected area managers, policy-makers, conservationists, taxonomists and the wider public (Fig. 1). The web-enabled Crop Wild Relative Information System (CWRIS) (PGR Forum 2005; Kell & al. 2007) includes data on CWR taxonomy, uses, geographical distribution, biology, population and habitat information, threats (including IUCN Red List assessments) and conservation actions. This information is vital for the continued sustainable utilization and conservation of crop wild relatives.

Accessible via the CWRIS is the Catalogue of Crop Wild Relatives for Europe and the Mediterranean (Kell & al. 2005). The Catalogue was generated by firstly matching the genus names found in Mansfeld's World Database of Agricultural and Horticultural Crops, which enumerates agricultural and horticultural cultivated plant species (but with the exception of ornamental and forestry species) (Hanelt & IPK Gatersleben 2001; IPK Gatersleben 2003), with the genus names present in Euro+Med PlantBase (Euro+Med PlantBase 2005, Version September 2005). This was followed by abstracting the taxa within those matching genera from Euro+Med PlantBase, thus creating the first catalogue of crop wild relatives for Europe and the Mediterranean (Fig. 2). This list contains 23513 of

Fig. 1. Crop Wild Relative Information System (CWRIS). Accessible via the information system is the crop wild relative taxon database. This is linked to *n* external data sources. Some existing data sources are shown. The two-way arrows indicate the reciprocal nature of the system.



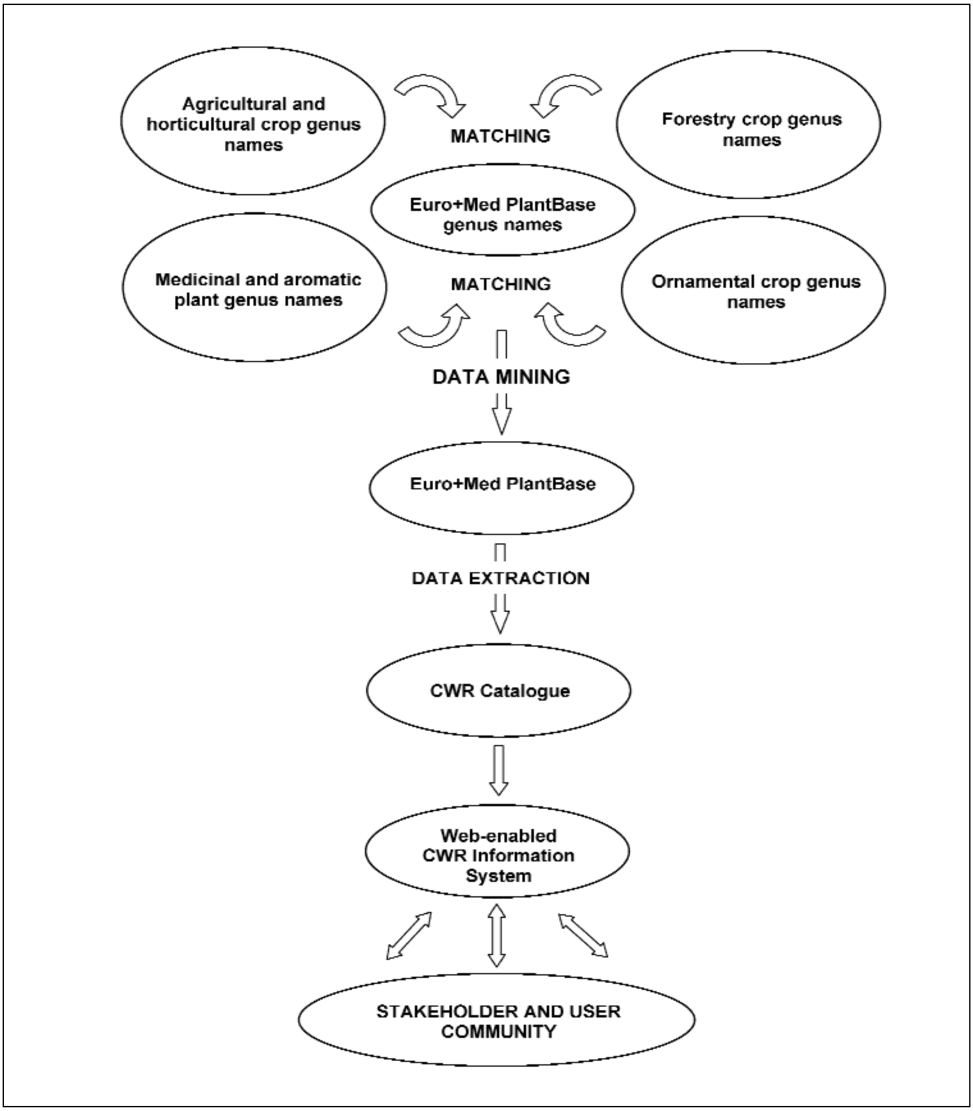


Fig. 2. Methodology for the creation of the CWR Catalogue for Europe and the Mediterranean (Kell & al. 2005). The method can be adapted for any country or region by replacing Euro+Med PlantBase with the country or regional flora.

the 30983 species recorded by Euro+Med PlantBase as present in the region; thus, around three quarters of the species can be considered crops and CWR, i.e. those species found within the same genus as an agricultural or horticultural crop.

The same procedure was followed to include forestry, ornamental, medicinal and aromatic plants in the E+M CWR Catalogue. The genus name lists for these groups were extracted from the following sources: for forestry taxa, Schultze-Motel's (1966) 'Enumeration of cultivated forest plant species', for ornamental taxa, a list provided by the Community Plant Variety Office (see CPVO 2001) (this list contains those taxa for which the title had been granted and all active applications as of July 18 2003) (Kwakkenbos pers. comm. 2003), and for medicinal and aromatic plants, the database 'Medicinal and Aromatic Plant Resources of the World' (MAPROW) (Schippmann pers. comm. 2004) (in addition to those listed in the Mansfeld Database).

The addition of the forestry and ornamental groups to the agricultural and horticultural crop and CWR list, and the extension of the medicinal and aromatic groups, resulted in a catalogue of 25687 crop and CWR species for Europe and the Mediterranean, which is 83% of the 30983 species recorded by Euro+Med PlantBase as present in the region. This suggests that more than three-quarters of the plant species in the region have a current or potential direct use to humankind. Of these, 90% (23216 species) are considered native. The 25687 species listed in the catalogue are spread across 1239 genera; this represents 49% of crop genera worldwide.

Further analysis indicates that approximately 68% (17495) of crop and CWR species found across the Euro-Mediterranean region are found in Europe alone; 89% (15656) of these species are considered native. The country with the highest crop and CWR species richness is Turkey, with 7235 species; that is 28% of the crops and CWR of the Euro-Mediterranean region, or 41% of the crops and CWR of Europe. Of the Euro-Mediterranean crop and CWR species, 58% (14994) are endemic to the region, and 49% of European crop and CWR species (8624) are endemic to Europe.

Table 1 shows the number of species in each of the four socio-economic groups: agricultural and horticultural crops, forestry species, ornamentals and medicinal and aromatic plants. The percentage of the total number of Euro-Mediterranean crop and CWR species (25687) attributable to each group is given.

Table 2 is a matrix showing the percentage of species common to all four groups. The bottom left side of the matrix shows species shared by each group in the left column as a percentage of the species in each group given across the top row. The top right side of the matrix expresses the percentages in reverse. For example, 13% of species in the agricultural and horticultural crop and CWR list are also found in the forestry list; and conversely, 92% of forestry crop and CWR species are found in the agricultural and horticultural list.

It is interesting to note that very high percentages of crop and CWR species extracted from the genus list derived from Mansfeld's World Database of Agricultural and Horticultural Crops are common to the other three socio-economic groups; that is, 95% of the species in the forestry list, 90% in the ornamental list and 92% in the medicinal and aromatic plant list. This can be explained by the fact that many crop species have several uses, as do ornamental plants (e.g. medicinal, vegetable), and that cultivated medicinal and aromatic plants are also included in the Mansfeld Database. Moreover, there are many species within the same genera as the agricultural and horticultural crop genera that have uses classified within one of the other three socio-economic groups, and thus these groups will share many of the same CWR. Also of note are the high percentages of medicinal and aromatic plant species common to the other three groups: that is, 77% of agricultural and horticultural crops (though as noted above, the Mansfeld Database also includes cultivated medicinal and aromatic plants), 95% of forestry species and 88% of ornamental species.

Table 1. Total number of crop and CWR species in the Euro-Mediterranean region and the numbers and percentages of species in each group: 1 - agricultural and horticultural, 2 - forestry, 3 - ornamental, 4 - medicinal and aromatic.

	E+M crop and CWR species	1	2	3	4
Total n° of E+M crop and CWR species	25687	-	-	-	-
Total species per crop group	-	23513	2843	7499	19784
Species per group as % of total	-	92	11	29	77

Table 2. Matrix showing the percentage of crop and CWR species shared by each of the four groups: 1 - agricultural and horticultural, 2 - forestry, 3 - ornamental, 4 - medicinal and aromatic.

%	1	2	3	4
Agricultural and Horticultural	-	95	90	92
Forestry	11	-	17	14
Ornamental	29	45	-	33
Medicinal and Aromatic	77	95	88	-

This illustrates the extremely broad use of plants for medicinal and aromatic uses, many of which are species harvested from the wild. Perhaps not surprisingly, the group with the least percentage of species common to the other three groups is the forestry species, with 11% of species common to the agricultural and horticultural crops, 17% common to the ornamental species and 14% common to medicinal and aromatic plants.

It should be noted that Euro+Med PlantBase is currently undergoing taxonomic editing. The results presented in this paper are those based on the September 2005 version of the database. Further and more detailed analyses of the data can be found in Kell & al. (2007a).

Using the CWRIS to aid conservation planning

The aim of PGR Forum was to provide tools and techniques for the conservation and sustainable use of CWR. The CWR Catalogue, accessed via the CWRIS, provides the vital information resource that is needed for conservation planning and management. Decisions must be made, whether at national, regional or international level, as to which are the priority taxa in most need of conservation action. One of the fundamental aspects of this process is for CWR users and conservation practitioners to know which species are most closely related to the crop of interest, i.e., the species that are likely to be of greatest use in breeding for crop improvement using conventional methods.

In order to indicate the degree of relatedness of CWR to the associated crop, a method has been proposed by Maxted & al. (2006). The Gene Pool concept (Harlan & de Wet 1971) permits taxa to be grouped according to their relative closeness to the crop, into the primary (GP1A and 1B), secondary (GP2) or tertiary genepool (GP3). However, to assess whether a taxon should be placed in GP1A, GP1B, GP2 or GP3 requires detailed knowledge of genetic diversity and breeding systems. In the many cases where this information is not available, the authors have proposed a Taxon Group (TG) concept as follows: TG1 = same species, TG2 = same section or series, TG3 = same subgenus, TG4 = same genus, TG5 = same tribe, but different genus, thus permitting a 'proxy' assessment of the degree of genetic relatedness of a CWR to the crop using the existing taxonomic classification. The authors propose a formal definition of a CWR as being a taxon that occurs in GP1 and GP2, or TG1-TG4. Although the Taxon Group concept does not give as accurate an indication of genetic relatedness as the Gene Pool concept, it serves as an alternative indicator in the absence of detailed genetic information. Furthermore, the implementation of the Taxon Group ranking system is straightforward, since this information is inherent in the taxon's classification.

Information on uses, both of the crops and their wild relatives, is available via the CWRIS, drawing on major data sources such as Mansfeld's World Database of Agricultural and Horticultural Crops (Hanelt & IPK Gatersleben 2001; IPK Gatersleben 2003) and GRIN Taxonomy (USDA, ARS, National Genetic Resources Programme 2006). In cases where new information on use needs to be entered into the system, PGR Forum used and adapted existing data standards, such as those of the Taxonomic Databases Working Group (TDWG) (Cook 1995) and IUCN – The World Conservation Union (IUCN 1995-2006).

The CWRIS also aims to provide information on geographical distribution, biology, population and habitat information, threats (including IUCN Red List assessments) and conservation actions. This information can be drawn from a wide range of existing sources, many of which are actively managed, thus creating a dynamic CWRIS providing the most up-to-date information, combined with the option to link to any number of data sources in the future. An XML schema (Extensible Markup Language) acts as a means of linking the database to other data sources.

PGR Forum has created a unique system to provide access to crop and CWR information for use by a broad range of stakeholders, either at regional or national level. The CWR Catalogue, accessible via the system, provides the vital baseline upon which the detailed taxon and population level data can be built. The means of bringing together multiple data sources, both for the creation of the Euro-Mediterranean CWR Catalogue and for the CWRIS itself, illustrates how existing information resources can be harnessed and utilized to produce a dynamic system that is vital to make informed decisions about the conservation of plant genetic resources.

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Stampato nel mese di settembre 2007
presso la Tipolitografia Luxograph s.r.l.
Piazza Bartolomeo da Messina, 2/e
Palermo