Βοτανικά Χρονικά

22

2019

Αφιέρωμα στον Δημήτριο Φοίτο
με την ευκαιρία της 90ής επετείου
των γενεθλίων του

Εκδίδεται από τους
W. Greuter, Σ. Κοκκίνη, Ι. Μανέτα, Π. Μπαρέκα & Γ. Καμάρη

ΒΟΤΑΝΙΚΑ ΧΡΟΝΙΚΑ

Εκδότες

Δημήτριος Φοίτος & Γεωργία Καμάρη

Επιμέλεια τόμου

Γεώργιος Μήτσαινας & Πέπη Μπαρέκα

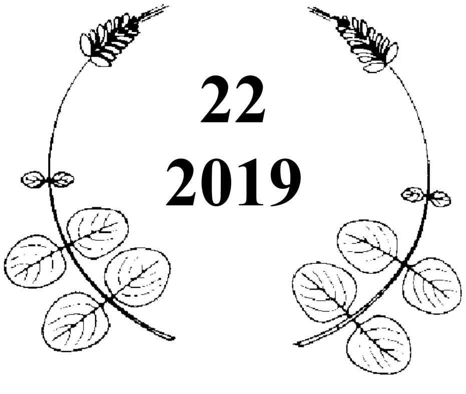
Διεύθυνση Έκδοσης

"Βοτανικά Χρονικά"
Εργαστήριο Βοτανικής
Τμήμα Βιολογίας
Πανεπιστήμιο Πατρών
265 00 Πάτρα

ISSN 0253-6064

Εκτύπωση - Βιβλιοδεσία: ΚΑΤΑΓΡΑΜΜΑ – Γραφικές Τέχνες
ΑΦΟΙ ΤΕΡΖΗ Ο.Ε., 20200 Κιάτο
Τηλ. 27420 29333
www.katagramma.gr

Botanika Chronika



22
2019

Dedicated to Dimitrios Phitos
on the occasion of his 90th birthday

Edited by
W. Greuter, S. Kokkini, Y. Manetas, P. Bareka & G. Kamari

BOTANIKA CHRONIKA

Editorial Office

"Botanika Chronika"
Botanical Institute
Department of Biology
University of Patras
GR – 265 00 Patras, Greece

ISSN 0253-6064

Printing: KATAGRAMMA Graphical Arts
Terzis Bros. G.P., GR-20200 Kiato
Tel.: +30 27420 29333
www.katagramma.gr

Dimitrios Phitos – a dedication

Werner Greuter

The tradition of publishing a festschrift, or jubilee volume, to honour a prominent scholar is of long standing. Even though in recent years the number of festschrifts to be published each year is in apparent decline, perhaps due to the shortage of time experienced by potential contributors, being honoured by such a collective testimony of the appreciation of friends, colleagues and admirers remains one of the salient highlights in a prominent scientist's career. Being honoured twice in such a way, as is the case here, is exceedingly rare to happen. The best known example concerns the Viennese botanist Karl Heinz Rechinger (1906-1998), considered as one of the founding fathers of Greek and Aegean botany and a dear old friend of today's honoree. It is particularly apt for him, as a foremost Greek botanist, to join Rechinger on this prominent pedestal.

This volume is dedicated to Professor Dimitrios Phitos. Its 384 pages include 25 papers by authors sharing their research interests with Prof. Phitos, which is tantamount to stating that they cover a very broad range of subjects. They have been written by his friends, pupils and colleagues to honour him on the occasion of his ninetieth birthday, celebrated last year. May he accept them as a token of our love and faithful admiration of his lifetime achievements throughout his rich and busy life; and, even more importantly, we trust that he will enjoy the reading and will appreciate all these valuable, original contributions to the field of learning that continues to be his own.

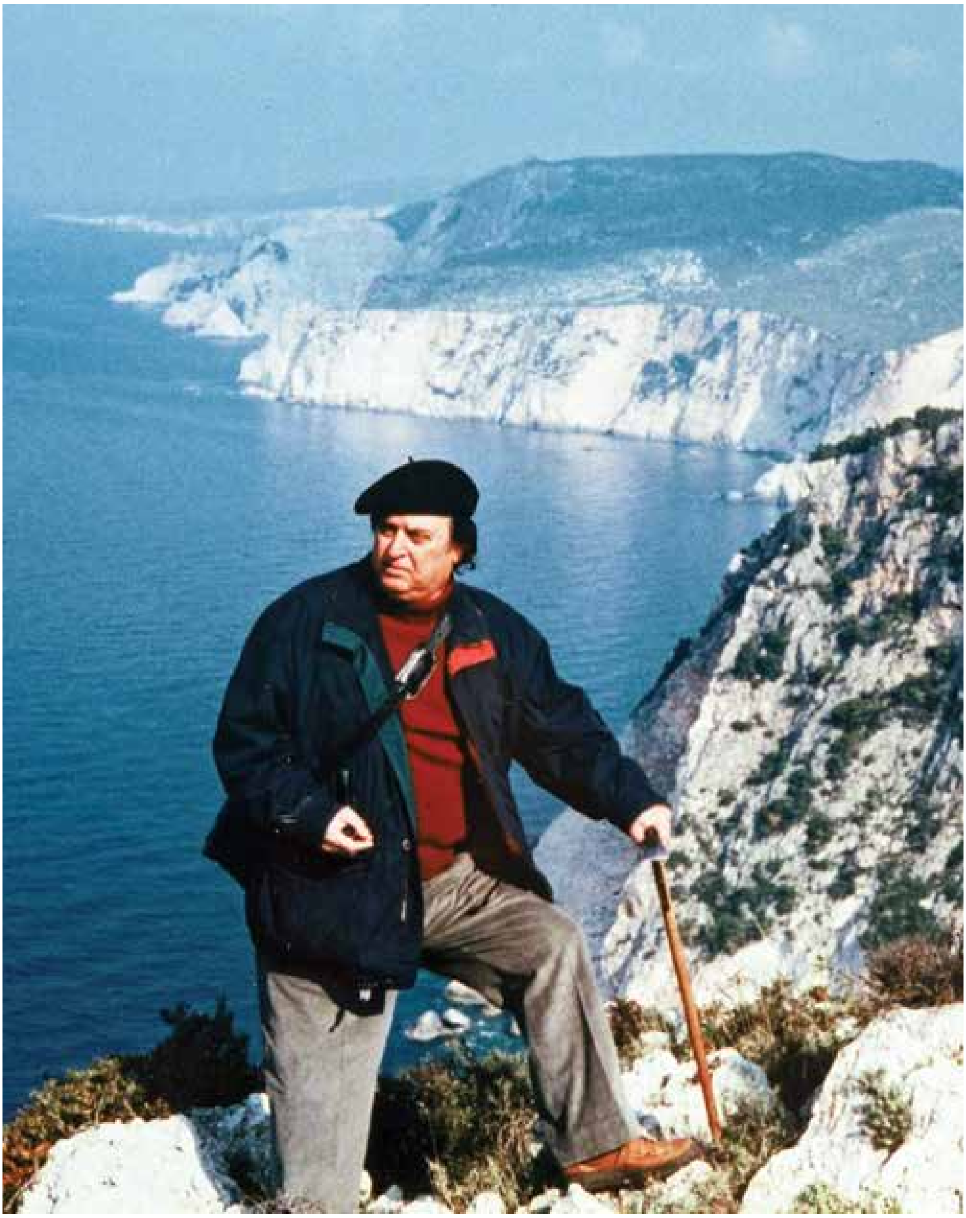
Professor Phitos is a child of Attica, born in 1928 in the Greek capital's harbor town, Piraeus. Having graduated at Athens University where he obtained his PhD in 1960 with a thesis on the phytogeography of Central Euboea. He was to devote his life and labours to the study of the plant world of his home country, Greece – which happens to be the classical cradle of botany as a science. One of his great concerns was to realize how far off from forefront botanical research Greek botany had come to be in our times. He devoted his active life and academic career to bridging this ever growing gap, and so became the first Greek scientist after Theodoros Orphanides, and the second in post-classical times, to achieve international renown in the fields of plants geography and taxonomic botany.

True to the tradition of his generation, Phitos chose the German language domain to refine his botanical training and achieve full mastery of his subject. He spent most of his six postdoctoral years in Munich then Vienna. Hermann Merxmüller became his mentor in biological thinking, model in scientific care, and teacher in modern research techniques. Munich was then (1961-1964) a leading think-tank in the domain of plant sciences, where a whole new generation of German botanists, that nation's future academic elite, was being bred. For Phitos these were fascinating years that he would never forget. Up to the present day he loves and handles skilfully the German idiom, preferring it to English . During a winter (1965/1966) spent in Vienna, Phitos perfected his knowledge of Greek plants, tutored by Karl Heinz Rechinger who, in complement to Merxmüller's more theoretically minded approach, taught Phitos the ultimate secrets of field work and sharpened his botanical flair.

Back in Greece, Phitos set out to profit the knowledge he had acquired. He was entrusted with the task of designing and organizing the Institute of Botany at the newly founded University of Patras, the development of which became the tenet of his professional life. Teaching as a reader of botany and after 1972 as full professor, he became the founding father modern Greek plant taxonomy, among other things by introducing cytotaxonomy as an entirely new research branch. He wrote the basic Greek students' textbooks on geobotany (1975; second edition, 1987) and systematic botany (1979, reedited 1984). He – and after him, his pupils – supervised well over a dozen PhD theses dealing with the taxonomy of a variety of plant groups (*Anthemis*, *Centaurea*, *Crepis*, *Limonium*, *Paeonia*, *Viola*) and the flora and phytogeography of several areas (the islands of Samos, Nisiros, Kefallinia and Crete, as well as Peloponnesus). The Institute in is now the Country's major research centre for plant taxonomy. The Patras Herbarium (UPA), which in 1966 started at zero, has become the country's major plant collection, with over 150,000 specimens.

The development in Greece, not only of taxonomic botany, but biological research and environmental awareness as a whole, owes much to Dimitrios Phitos. He was the founder and first president of the Greek Society of Biological Sciences (Ελληνική Εταιρεία Βιολογικών Επιστημών), and first vice-president of the Greek Botanical Society (Ελληνική Βοτανική Εταιρεία) of which he is now an honorary member. He founded Βοτανικά Χρονικά (Botanika Chronika), the first and only Greek journal entirely devoted to the botanical sciences, of which he was the sole editor for 16 years. He actively and enthusiastically promoted the publication of a new, modern Flora of Greece, acting as the chairman of the editorial board (or "steering committee") of *Flora Hellenica* of which the first volume was published in 1997. Two years earlier he had shown through the press the *Red Data Book of rare and threatened plants of Greece*, of which he has been the main promoter, author of many entries, and editor-in-chief; he was the driving force behind the publication, in 2009, of the second edition of that same work. His devotion to the pressing needs of nature conservation is witnessed, among others, by his many years of service in the managing board of the Greek Society for the Protection of Nature (Ελληνική Εταιρεία Προστασίας της Φύσεως) of which he was president for the term 1980-1982.

In an international context, Phitos served as regional adviser for Greece to the two major European botanical joint ventures of our time: *Flora europaea* and the *Atlas florae europaeae*. Even more importantly, he became a key-note player in Mediterranean plant taxonomy as one of the founders of OPTIMA, the Organisation for the Phyto-Taxonomic Investigation of the Mediterranean Area, member of that organisation's first



Dimitrios Phitos on a botanical excursion to Zakynthos (Ionian Islands) in 2005.

International Board (1974-1977) and of the Executive Council for three consecutive terms (1977-1995), including as its President (1983-1989). He organized the initial OPTIMA Meeting in Crete in 1975, where the foundations of international co-operation in Mediterranean botany were laid, and also the sixth OPTIMA Meeting in Delphi in 1989, for which he co-edited the proceedings volume of almost one thousand pages. For a lifetime of achievements and devoted services to Mediterranean plant taxonomy, in 2001 he was awarded the prestigious medal in gold of OPTIMA.

Phitos is the author of well over one hundred scientific papers and several books, the acknowledged specialist of, e.g., Greek *Campanula*, *Arenaria*, *Aubrieta*, *Bolanthus* & al., the floras of the Islands of Kefallinia, Chios and the Northern Sporadhes, and a leading plant systematist of our era. Yet his intimate friends will first see him as a Greek citizen. The generosity, the pride, sometimes – why not – the stubbornness that are characteristic of his nation pervade his personality. He may not always be kind to everyone, and hardly ever to those who harass him; but to his good friends, I can tell, he is kindness itself.

Despite his venerable age, Dimitrios Phitos is still in reasonably good health. His active mind and sharp wit enable him to continue as a creative botanical investigator. May he be able to carry on for the years to come his productive scientific activity. Or, as the Greeks would say: Χρόνια Πολλά, Δημήτρη, και Ευτυχισμένα!

Berlin, 9 September 2019

Professor Dimitrios Phitos:

A brief outline of his contribution in the Science of Botany

STELLA KOKKINI

Laboratory of Systematic Botany and Phytogeography, School of Biology, Aristotle University, 541 24 Thessaloniki, Greece.

Dimitrios Phitos, Emeritus Professor of the University of Patras, was born in Piraeus in 1928. He received his University degree from the School of Natural Science and Geography of the University of Athens in 1955, having previously served in his military service (1950-1953). During 1956-1962, he served as an assistant in the Systematic Botany Laboratory of the University of Athens under Professor Ch. Diapoulis. The Doctoral Diploma entitled "Phytogeographical Research in Central Euboea" was awarded to him in 1960.

For about seven years (1961-1968) he worked as a researcher at the Institut für Systematik Botanik der Universität München with Professor H. Merxmüller and in Naturhistorisches Museum Wien near Professor K. H. Rechinger.

During that period he received scholarships from:

a. Marie Kasimatis Foundation (1961-1962),
b. Alexander von Humboldt Foundation (1962-1964),
c. Austrian Government (1965-1966), and
d. N.A.T.O (Ministry of Coordination Technical Assistance Scholarship) (1968).

An invitation to Dimitrios Phitos to teach Biology in 1966, was supposed to connect him to the newly established University of Patras. Three years later in 1969, he was promoted to be an Assistant Professor of the University and in 1972 he was elected Full Professor. After that he organized the Botanical Institute of the School of Biology.

In 1973 he founded the Botanical Museum of the University and then he supervised the plant collections deposited there. For about nine years (1974-1983), he cooperated the Laboratory of Electron Microscopy of the University of Patras.

Member of the Governing Committee of the University of Patras (1974), he was elected Dean of the Faculty of School and Mathematics for the academic year 1979-1980.

In 1998, he was awarded the title of Emeritus Professor of the University of Patras.

Fig. 1. Members of the University of Patras in a meeting (1968). In the first row of seats Dimitrios Phitos.

From 1966 to 1996 (the year he retired), he taught on his own, later with his associates the courses in Biology, General Botany, Systematic Botany and Geobotany to the undergraduate students of the School of Biology of the University of Patras. Supporting his teaching, Professor Phitos wrote three educational textbooks on Systematic Botany and Geobotany.

- Phitos D. 1984, 1986, 2001, 2008: Systematic Botany,
- Phitos D. 1984, 1986, 2001, 2008: Geobotany courses,
- Phitos D. & Kamari G. 2009: Geobotany courses.

Scientific Publications

Professor Phitos has to date published or contributed in more than 150 scientific publications, which can be roughly distinguished into five groups:

A. Floristic inventories of mountainous and island areas of Greece - Phytogeography

Among these publications, to mention that for the flora of Kefallinia (Cephalonia). The study was started in 1969 by Dimitrios Phitos and Jürgen Damboldt, Professor of the University of Berlin. After Damboldt's early loss, it was to be completed and published 16 years later by Prof. Phitos [Phitos D. & $^+$Damboldt J. *Die Flora der Insel Kefallinia (Griechenland)* in Bot. Chron. 5(1-2): 1-204 + 2 Karten (1985)].

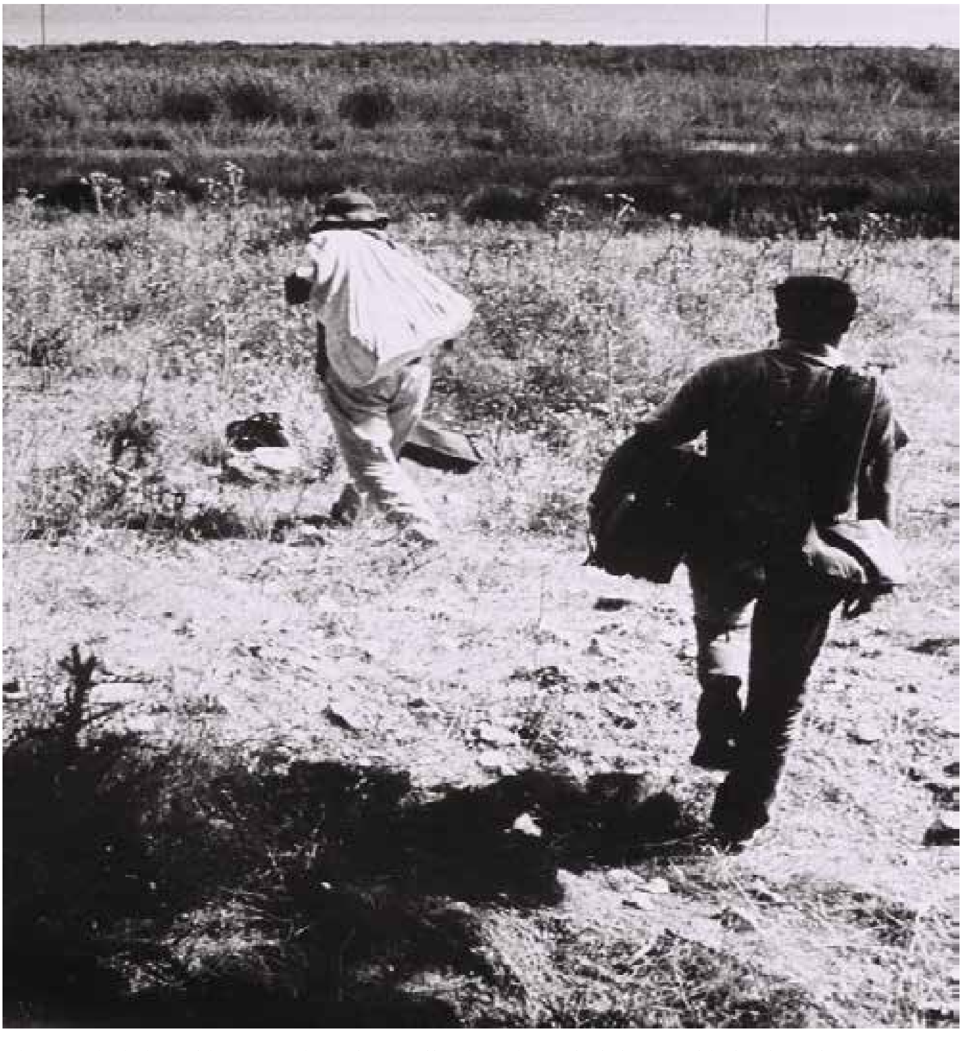


Fig. 2. Dimitrios Phitos carrying plant collections in a cloth bag on his back and Jürgen Damboldt in Kefallinia (May of 1969).

B. Taxonomic monographs of different genera and subsequent description of new taxa

His published research work includes the description of more than 50 new taxa and/or new nomenclature and taxonomic combinations, members to 11 Angiosperm families:

Alliaceae (one taxon), Amaryllidaceae (one), Asteraceae (11), Berberidaceae (one), Brassicaceae (one), Campanulaceae (17), Caryophyllaceae (14), Lamiaceae (2), Liliaceae (2), Plumbaginaceae (3) and Valerianaceae (2 taxa).

Among the newly described species by Dimitrios Phitos, in the early period of his career (1963-1965), it is worthy to mention the following seven species of the genus *Campanula*, with a narrow distribution in Greece or in W Turkey:

Campanula cymaea Phitos in Oesterr. Bot. Z. 111: 212 (1964)
C. euboica Phitos in Oesterr. Bot. Z. 112: 467 (1965)
C. icon*ia* Phitos in Oesterr. Bot. Z. 112: 481 (1965)
C. merxmuelleri Phitos in Mitt. Bot. Staatssamml. München 5:121 (1963)
C. rechingeri Phitos in Oesterr. Bot. Z. 112: 470 (1965)
C. sciathia Phitos in Oesterr. Bot. Z. 111: 215 (1964)
C. scopelia Phitos in Oesterr. Bot. Z. 111: 214 (1964)

C. Karyosystematic studies of different genera or species

The chromosome number and/or their morphology has been firstly introduced for plants of the Greek Flora by Professor Phitos.

Later on his co-workers and young Doctorial students have extensively used data from chromosomes for a better consideration of infra-generic or infra-specific taxonomy, in members of the genera *Acis, Allium, Bellevalia*, *Campanula*, *Centaurea*, *Crepis*, *Crocus*, *Fritillaria*, *Leucojum*, *Ornithogalum, Paeonia* and *Silene* as well as in taxa of local floras, especially from Kriti, Ionian Islands, East Aegean Islands and N Sporades Islands.

D. Historical – Cultural Botany

Professor Phitos scientific publications includes among others the biography of two prominent Greek botanists:

Ioannis Politis (1886-1968) in Bot. Chron. 9(1-2): 5-12 (1989), and
Panagiotis Gennadios (1847-1917) in Bot. Chron. 15: 5-16 (2002)

Besides, he wrote a comprehensive review on a plant symbol: *Lilium candidum* in Greece in Bot. Chron. 20: 99-128 (2010).

E. Editor of two Scientific Books and one Scientific Journal

Phitos D., Strid A., Snogerup S. & Greuter W. (eds) 1995: ***The Red Data Book of Rare and Threatened Plants of Greece***. – Athens: WWF for Nature.

Besides the editorial work, Dimitrios Phitos has contributed to that with 61 articles.

Phitos D., Constantinidis Th. & Kamari G. (eds) 2009: ***The Red Data Book of Rare and Threatened Plants of Greece***, Vol. 1(A-D) and Vol. 2(E-Z). – Patras, Hellenic Botanical Society.

Dimitrios Phitos has contributed to that with 29 articles (Fig. 3).

Phitos D. (ed.) 1981-2000 (Vol. 1-12, 14), Phitos D. & Kamari G. 2001 – today (eds) (Vol. 15-22): Scientific Journal ***Botanika Chronika***. – Patras, Botanical Institute, University of Patras. The Vol. 13 was dedicated to Prof. Phitos for the 70th anniversary of his birthday.

ΒΙΒΛΙΟ ΕΡΥΘΡΩΝ ΔΕΔΟΜΕΝΩΝ
ΤΩΝ ΣΠΑΝΙΩΝ & ΑΠΕΙΛΟΥΜΕΝΩΝ
ΦΥΤΩΝ ΤΗΣ ΕΛΛΑΔΑΣ

ΤΟΜΟΣ ΠΡΩΤΟΣ
A - D

Επιτροπή Έκδοσης:
Δ. Φοίτος, Θ. Κωνσταντινίδης & Γ. Καμάρη

ΠΑΤΡΑ 2009

ΒΙΒΛΙΟ ΕΡΥΘΡΩΝ ΔΕΔΟΜΕΝΩΝ
ΤΩΝ ΣΠΑΝΙΩΝ & ΑΠΕΙΛΟΥΜΕΝΩΝ
ΦΥΤΩΝ ΤΗΣ ΕΛΛΑΔΑΣ

ΤΟΜΟΣ ΔΕΥΤΕΡΟΣ
E - Z

Επιτροπή Έκδοσης:
Δ. Φοίτος, Θ. Κωνσταντινίδης & Γ. Καμάρη

ΠΑΤΡΑ 2009

Fig. 3. The covers of the two volumes of **“*Red Data Book of Rare and Threatened Plants of Greece*”,** presented in the Conference Center of the University of Patras (March 2010).

Plant taxa named after Phitos

The appreciation of his research work by plant taxonomists is reflecting to the attribution of his name to several newly described taxa of the Greek or Turkish flora.

Among them, to mention the following 10 taxa, a genus and nine species, members of eight plant families that named after Phitos:

Alliaceae
Allium phitosianum Brullo, Guglielmo, Pavone, Salmeri & Terrasi. – Pl. Biosystems 137(2): 134 (2003).

Asteraceae
The genus ***Phitosia*** Kamari & Greuter. – Bot. Chron. 13: 14 (2000), (Fig. 4, 5 and 6).
Phitosia *crocifolia* (Boiss. & Heldr.) Kamari & Greuter. – Bot. Chron. 13: 14 (2000).

Taraxacum ***phitosii*** Soest. – Proc. Kon. Ned. Akad. Wetensch., Ser. C, Biol. Med. Sci. 79: 176 (1976).

Campanulaceae
Campanula ***phitosiana*** Yıldırım & Şentürk. – Phytotaxa 399: 25-36 (2019).

Caryophyllaceae
Arenaria ***phitosiana*** Greuter & Burdet. – Willdenowia 12(1): 37 (1982).

Leguminosae
Trifolium ***phitosianum*** N.Böhling, Greuter & Raus - Bot. Chron. (Patras) 13: 39 (2000).

Fig. 4. *Phitosia* Kamari & Greuter in Bot. Chron. 13: 14 (2000) is a monotypic endemic genus of the Greek flora. Type: *Phitosia crocifolia* (Boiss. & Heldr.) Kamari & Greuter (=*Crepis crocifolia*). It was known, to occur only on the Taigetos range in S Peloponnisos, Greece (see also Figs 5 & 6).

Liliaceae
Fritillaria ***phitosia*** Kamari, Zahos & Siagou. – Phytotaxa 328(3): 227-242 (2017).

Linaceae
Linum ***phitosianum*** Christod. & Iatroú. – Phyton (Horn) 33(2): 289-290 (1994).

Plumbaginaceae
Limonium ***phitosianum*** R.Artelari. – Mitt. Bot. Staatssamml. München 20: 430 (1984).

Valerianaceae
Valeriana ***phitosiana*** Quézel & Contandr. – Taxon 16: 240 (1967).

Violaceae
Viola ***phitosiana*** Erben. – Mitt. Bot. Staatssamml. München 21(2): 396 (1985).

Fig. 5. *Phitosia crocifolia* (Boiss. & Heldr.) Kamari & Greuter *(=Crepis crocifolia)* grows on limestone substrate, on dry rocky places, in cliffs and crevices. It was known only from the Taigetos range, from Mt. Xerovouna in the north towards the summit area of Mt. Profitis Ilias and Mt. Mavrovouna to the south, at 1600-2130 m alt. (Photo by Ch. Kyriakopoulos).

Phitosia was also found in the small, but very interesting, Rindomou gorge of Mt. Taigetos, at 1350 m alt. Latter, was also found on Mt. Parnonas (Fig. 6). [Literature source: Kamari, G., Kyriakopoulos, Ch. & Kofinas, G.: New finding of *Phitosia crocifolia* (Compositae) in E Peloponnisos. – Fl. Medit. 20: 235-238 (2010)].

Fig. 6. *Phitosia crocifolia* also discovered on the main summit area (Megali Tourla or Kronion) of Mt. Parnonas, E Peloponnisos. It occurs on limestone, stony and rocky open places, at about 1810-1870 m altitude (Photo by Ch. Kyriakopoulos).

Scientific Societes αnd Honors

Professor Dimitrios Phitos is among the founders and constantly a very active member of scientific societies associated to plant taxonomy, conservation and protection of the wild flora.

Hellenic Society of Biological Sciences: Founding member (1980), first president and now Honorary Member. Chairman of the Organizing Committee of the 1st Congress of the Hellenic Society of Biological Sciences (Patras 1980).

Hellenic Botanical Society: Founding Member (1980), first Vice President and now Honorary Member. Chairman of the Organizing Committee of the 2nd Congress of the Hellenic Botanical Society held in Athens (May 8-9, 1982).

In the 8th Congress, held in the Conference Centre of the University of Patras (October 5-8, 2000), Prof. Phitos was the main speaker in the opening ceremony celebration of the 20-years of Hellenic Botanical Society (Fig. 7).

Hellenic Society for the Protection of Nature: President for the years 1980-1982 and now Honorary President.

Fig. 7. Professor Phitos was the main speaker of the opening ceremony celebration for the 20-years of Hellenic Botanical Society (8th Congress, Patras, October 5-8, 2000).

OPTIMA (Organization for the Phytotaxonomic Investigation of the Mediterranean Area): Founding member (1975) and member of the Board of Directors (International Board and Executive Council) until 1989. President of OPTIMA from 1983 to 1989.

Member of the Organizing Committee of the first OPTIMA Meeting in Iraklion Kriti, Greece, 22th-28th September 1975 (Fig. 8).

Fourteen years later, he was chairman of the Organizing Committee of the VI OPTIMA Meeting in Delphi, Greece, 10th-16th September 1989 (Fig. 9) and after 44 years he is the President of the Scientific Committee of the XVI OPTIMA Meeting in Athens, Greece (2th-5th October 2019).

Fig. 8. Participants to the first OPTIMA Meeting which took place in Iraklion (Kriti, Greece) in 22th-28th September 1975.

Fig. 9. Participants of the VI OPTIMA Meeting which took place in Delfi (Greece) in10th-16th September 1989. Professor Dimitrios Phitos chairman of the Organizing Committee, is standing on the center.

In recognition of his outstanding contribution to the phytotaxonomy of the Mediterranean area, Professor Phitos is awarded the gold medal of OPTIMA in X Meeting in Palermo, Italy, 2001 (Fig. 10).

Fig. 10. Professor Werner Greuter, President of OPTIMA and Professor Dimitrios Phitos during the celebration ceremony in X OPTIMA Meeting in Palermo (Italy), (9th-15th September 2001).

The contribution of Professor Phitos to the research of the Greek Flora has been also recognized by the **University of Crete**. During a special ceremony in November of 2010, the Rector Professor Pallikaris awards the Honorary Medal of the University of Crete to him for its valuable contribution to the study of Rare and Endemic plants of Greece (Fig. 11).

Fig. 11. Professor Ioannis Pallikaris, Rector of the University of Crete awards the Honorary Medal to Professor Dimitrios Phitos for his valuable contribution in the study of Rare and Endemic plants of Greece (Rethymnon, 17 November 2010).

Fig. 12. Celebrating 1st May Day (2011), Professor Phitos travels on a boat for the island of Kyra Panagia (N Sporades) when studied its islands flora.

Besides botany, Professor Phitos continues his multitude of interests such as reading of literature (botanical, archaeological, historical etc.), fishing (Fig. 13) and cooking.

Fig. 15. Dimitrios Phitos spent a lot of time for fishing.

Overall Professor Phitos is an exceptionally charming personality inspiring all colleagues and young researchers. His contribution to the Science of Botany is greatly appreciated and respected by all members of the Botanical scientific community.

With our warmest wishes for many returns of his birthday anniversary!

The genus *Aria* (*Sorbus* s. l., Rosaceae) in the Sicilian flora: taxonomic updating, re-evaluation, description of a new species and two new combinations for one Sicilian and one SW Asian species

FRANCESCO M. RAIMONDO, ELEONORA GABRIELJAN & WERNER GREUTER

Abstract

Raimondo, F.M., Gabrieljan, E. & Greuter, W. 2019: The genus *Aria* (*Sorbus* s. l., Rosaceae) in the Sicilian flora: taxonomic updating, re-evaluation, description of a new species and two new combinations for one Sicilian and one SW Asian species. – Bot. Chron. 22: 15-37.

The genus-level taxonomy of the *Sorbus* s. l. taxa that occur in Sicily is reviewed. The subdivision of *Sorbus* s. l. into several genera (plus hybridogenous genera) is accepted. By consequence, three genera are added to the flora of the largest island of the Mediterranean Sea, which were previously treated at subordinate levels. Apart from *Sorbus* itself, with a single species, we accept *Aria* with five species (one of them new) as well as *Cormus* and *Torminalis* with one species each. The study of a population newly found on the Madonie Mountains made it necessary to clarify the so far overlooked or misunderstood name *Sorbus meridionalis* (≡ *Pyrus meridionalis*), previously included in the synonymy of either *S. graeca* (≡ *Aria graeca*) or *Sorbus umbellata* (≡ *Aria umbellata*). This name is here lectotypified and the corresponding taxon accepted as a separate species, *Aria meridionalis* **comb. nov.**, whereas the newly found population is described as a new species, *Aria phitosiana* **sp. nov.**, a very rare and vulnerable local endemic of dolomitic areas of the Madonie Mountains in Sicily. In addition, the combination *Aria orbiculata* **comb. nov.** is published for the SW Asian *Sorbus orbiculata*.

F. M. Raimondo, PLANTA/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123-Palermo (Italy).
E. Gabrieljan, Institute of Botany after A. L. Takhtajyan, National Academy of Sciences, Department of Taxonomy and Geography of Higher Plants, Acharyana Str., 1, Yerevan 0040 (Armenia).
W. Greuter, Herbarium Mediterraneum Panormitanum, University of Palermo, Via Lincoln 2/A, 90123-Palermo (Italy).

Key words: Maloideae, taxonomy, typification, *Aria meridionalis*, *Aria phitosiana*, Mediterranean flora, Sicily, *Aria orbiculata*, SW Asia.

Introduction

The modern trend of using molecular data in plant systematics often has unexpected, unwelcome consequences for the traditionally accepted taxonomy. A particular example relates to a group of wooden maloid Rosaceae*,* treated for a long time within the genus *Sorbus*: That genus itself, and several former genera usually included in it (such as *Aria* Medik. and *Lazarolus* Medik.), together with other generally recognised genera such as *Crataegus* L. and *Mespilus* L., have been recently transferred to and included in *Pyrus* L. s. l. (Fay & Christenhusz 2018), which made "necessary" the publication of 850 new names at the specific rank alone. At the same time, new species continue to be added to *Sorbus* L. s. l., such as *Sorbus orbiculata,* recentely described from the Caucasus by the second author (GABRIELJAN 2018) many years after the publication of her monograph of *Sorbus* L. (GABRIELJAN 1978), important reference base for the revision of *Sorbus* subg. *Aria* by ALDASORO & al. (2004).

Such extreme lumping is not the obligatory consequence of recent molecular studies. SENNIKOV & KURTTO (2017) and KURTTO & al. (2018) have followed an opposite course, splitting the *Sorbus* component off the now huge and unwieldy *Pyrus* s. l. as a number of small, natural (i.e. monophyletic) segregate genera. In a recent contribution (RAIMONDO 2018), SENNIKOV & KURTTO's assessment was followed and applied to the taxa of *Sorbus* s. l. that occur in Italy (including Sicily).

Unsurprisingly, Sicily − with its high diversity of environmental systems and its central geographical position in the Mediterranean region, at the crossroads of north- to southbound and east-to-west migratory routes − houses a representative sample of *Sorbus* s. l. Several species have converged here; then, through gene exchange and geographical isolation, they differentiated into new species-level taxonomic entities.

Several authors (e.g. ALDASORO & al. 2004, SENNIKOV & KURTTO 2017), have interpreted most taxa of *Aria* (or *Sorbus* subg. *Aria*) as resulting from polyploidisation, hybridisation (intra- and interspecific as well as intergeneric), and apomixis, which, along with geographical isolation, are thought of as the main speciation mechanisms in this group. Most species are quite local and form very small populations. Examples in Sicily are *A. madoniensis* and *A. busambarensis* (RAIMONDO & al. 2012, CASTELLANO & al. 2012).

We here present an update of SENNIKOV & KURTTO'S (2017) inventory of *Sorbus* s. l. for the island of Sicily. Also, we describe a local population, newly discovered on the Madonie Mountains, as a new species, *Aria phitosiana.* Furthermore, some critical populations that had been variously referred to *S. umbellata* subsp. *meridionalis* (Guss.) Vălev, to *S. aria* subsp. *cretica* (Lindley) Holmboe, to and to *S. umbellata* are attributed to a newly circumscribed species, to be named *A. meridionalis* upon lectotypification of the basionym *Pyrus meridionalis* Guss. ex Tod.: a name, of which the correct date and authorship had so far been ignored and that had been treated by many authors (PIGNATTI 1982, 2017, GIARDINA & al. 2007, CASTELLANO 2012) as a synonym of *Aria graeca* (Spach) M. Roem., a species which also occurs in Italy and Sicily. The lectotype here designated maintains much of the traditional application of the name and, therefore, supports nomenclatural stability in the group, avoiding the displacement of one of the other recently proposed binomials (*Sorbus busambarensis* or *Sorbus madoniensis*).

The current inventory of *Sorbus* L. in Sicily

According to WARBURG & KÁRPÁTI (1968), as updated by the treatments in recent national and regional Floras and inventories (CONTI & al. 2005, GIARDINA & al. 2007, RAIMONDO & al. 2010, PIGNATTI 2017, BARTOLUCCI & al. 2018), the following taxa of *Sorbus* s. l. occur in Sicily (for full nomenclatural source references, see SENNIKOV & KURTTO 2017):

Sorbus aucuparia L. subsp. ***praemorsa*** (Guss.) Nyman
Sorbus aria (L.) Crantz
Sorbus graeca (Spach) Kotschy
Sorbus busambarensis G. Castellano & al.
Sorbus madoniensis Raimondo & al.
Sorbus umbellata subsp. ***meridionalis*** (Guss. ex Tod.) Vălev
Sorbus domestica L.
Sorbus torminalis (L.) Crantz

Sorbus umbellata (Desf.) Fritsch subsp. *umbellata* has been omitted from the above list, because there is disagreement over its possible presence in Sicily (and therefore Italy). RAIMONDO & SPADARO (2009) and RAIMONDO & al. (2010) only accept the presence of a different taxon, *Sorbus umbellata* subsp. *meridionalis* (Guss. ex Tod.) Vălev, whereas WARBURG & KÁRPÁTI (1968), PIGNATTI (2012 and 2017) and BARTOLUCCI & al. (2018) consider its occurrence as doubtful; SENNIKOV & KURTTO (2017) and KURTTO & al. (2018) implicitly dismiss it. CASTELLANO (2012) remained uncommitted, attributing some Sicilian populations to "*S. umbellata* s. l.", while others were to be described as differennt species, *S. busambarensis* and *S. madoniensis* (see CASTELLANO & al. 2012, RAIMONDO & al. 2012).

Taxonomic conspectus of Sicilian *Sorbus* s. l., according to *Atlas florae europaeae*

Following the inventory of SENNIKOV & KURTTO (2017), and the chorological treatment in *Atlas florae europaeae* (KURTTO & al. 2018) based on it, an updated digest of *Sorbus* s. l. for the Italian Flora, including Sicily, has recently been prepared (RAIMONDO 2018). Under the narrow genus concept in *Sorbus* s. l. accepted by SENNIKOV & KURTTO (2017), six genera are now present in Sicily instead of the single, broadly defined genus *Sorbus* that was previously recognised: *Sorbus* s. str., *Aria, Cormus, Torminalis, Chamaemespilus,* and *Hedlundia*. The last-named has been described to accommodate taxa of intergenetic hybrid origin, derived from crosses between *Aria* and *Sorbus* species.

Among Italian authors, BARTOLUCCI & al. (2018) dismissed SENNIKOV & KURTTO'S (2017) generic dismemberment as premature, continuing to adhere to the wide definition of *Sorbus* as a single (though paraphyletic) genus. We are therefore providing here a new digest of Sicilian taxa of *Sorbus* s. l., taking into account our own, new analysis of the taxonomy of *Aria* Spach in Sicily (for full nomenclatural source references, see SENNIKOV & KURTTO 2017):

Sorbus aucuparia subsp. ***praemorsa*** (Guss.) Nyman (≡ *Pyrus praemorsa* Guss. ≡ *Sorbus praemorsa* (Guss.) K. Koch) [Sicily (Etna and Madonie Mts: Fig. 1), Sardinia, southern Italian Peninsula].

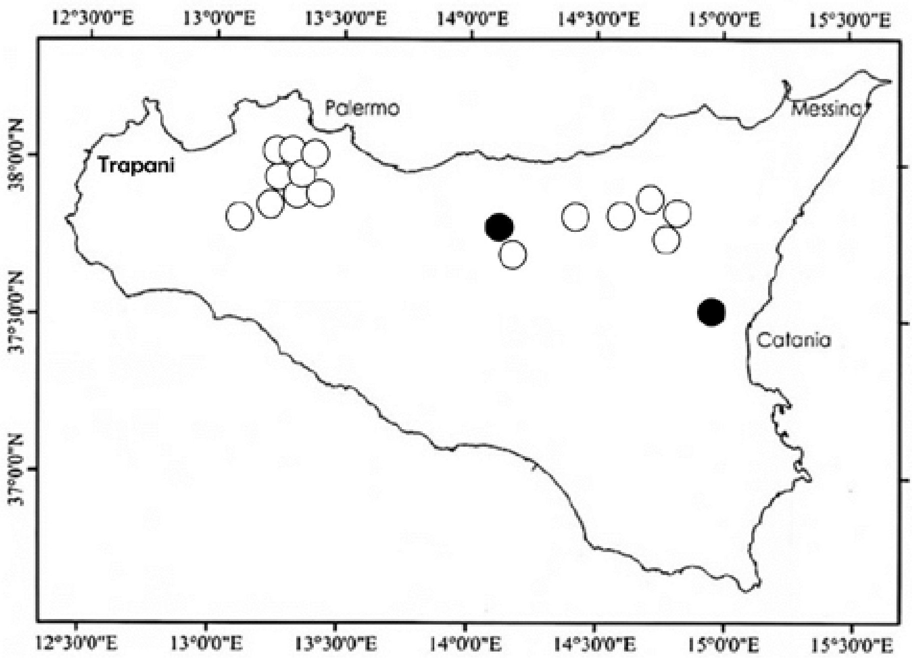


Fig. 1. Map of the Sicilian distribution of *Sorbus aucuparia* subsp. *praemorsa* (●) and of *Torminalis glaberrima* (○) (from specimens seen).

Aria edulis (Willd.) M. Roem. (≡ *Pyrus edulis* Willd. ≡ *Sorbus edulis* (Willd.) K. Koch) [= *Crataegus aria* L. (≡ *Sorbus aria* (L.) Medicus)] [Sicily (Fig. 2), Sardinia, Italian Peninsula, etc.].

Aria graeca (Spach) M. Roem. (≡ *Crataegus graeca* Spach, ≡ *Sorbus graeca* (Spach) Lodd. ex S. Schauer) [Sicily (Fig. 2), Central and southern Italian Peninsula, etc.].

Aria madoniensis (Raimondo & al.) Sennikov & Kurtto (≡ *Sorbus madoniensis* Raimondo & al.) [Sicily (Madonie Mts: Fig. 3)].

Aria busambarensis (G. Castellano & al.) Sennikov & Kurtto (≡ *Sorbus busambarensis* G. Castellano & al.) [Sicily (Rocca Busambra: Fig. 3)].

Cormus domestica (L.) Spach (≡ *Sorbus domestica* L.) [Sicily, other Italian islands, Italian Peninsula, etc.].

Torminalis glaberrima (Gand.) Sennikov & Kurtto (≡ *Sorbus glaberrima* Gand.) [= *Crataegus torminalis* L. (≡ *Sorbus torminalis* (L.) Crantz)] [Sicily (Fig. 1), Sardinia, Italian Peninsula, etc.)].

The Sicilian taxa of *Aria*

Treating the genus *Sorbus*, some Italian authors – among them, in the last 10 years, RAIMONDO & SPADARO (2009) and RAIMONDO & al. (2010) – refer to a distinct Sicilian taxon under the names *Sorbus aria* var. *meridionalis*, *Sorbus meridionalis*, or *S. umbellata* subsp. *meridionalis*. All these names derive from

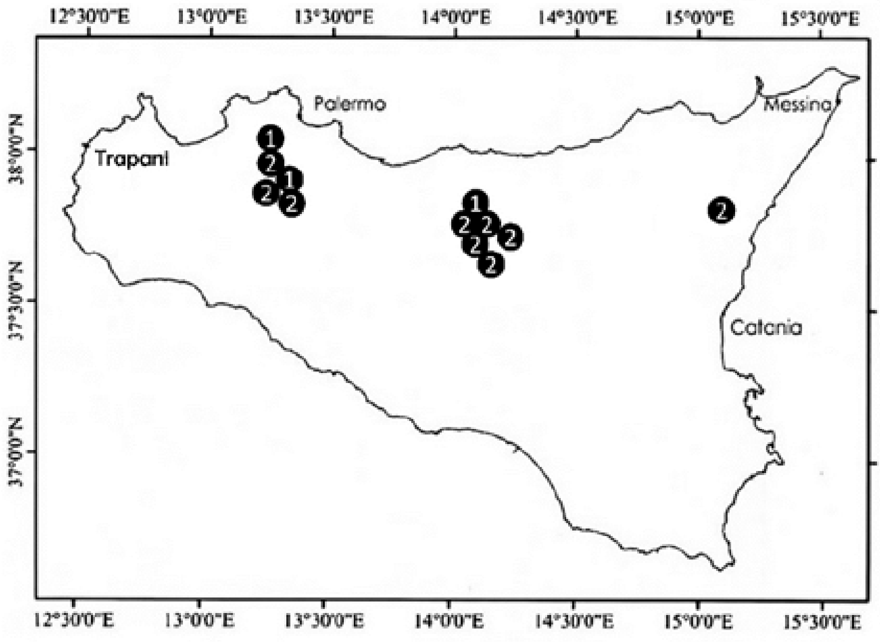


Fig. 2. Map of Sicilian locality of *Aria edulis* (**1**), and *A. graeca* (**2**) (from specimens seen).

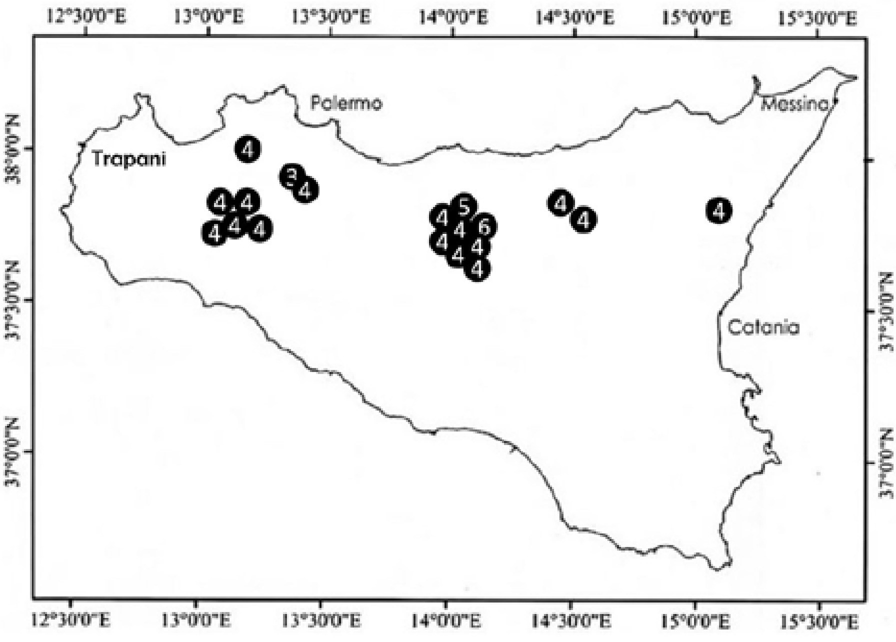


Fig. 3. Map of the Sicilian distribution of *Aria busambarensis* (**3**), *A. meridionalis* (**4**), *A. madoniensis* (**5**), and *A. phitosiana* (**6**) (from specimens seen).

GUSSONE'S (1844-1845) provisional, hence invalid name *Pyrus meridionalis* (see below).

When the first author revised the plant material he had assembled during the preparation of his doctoral thesis (RAIMONDO 1971), he came across a young and incomplete specimen he had collected in the Madonie Mountains in 1970. He dubiously assigned it to *Sorbus aria,* with the annotation "? *Sorbus meridionalis*" (*Pyrus meridionalis* Guss., nom. provis.). He felt justified, in so doing, by the fact that it is the very same taxon that *Todaro* had collected and distributed in his "Flora sicula exsiccata" as *P. meridionalis*, mixed with other taxa. TODARO did not mention the collecting locality on the label (probably because his material came from more than one locality and, judging from two different label kinds associated with it, was collected partly by *Todaro* himself but for the other part by *Citarda*): he gave its origin as "Valdemone", i.e., the Madonie Mountains (see below). When the taxonomy of Sicilian *Sorbus* sect. *Aria* had been disentangled by the description of two endemic species (by RAIMONDO & al. 2012 and CASTELLANO & al. 2012), the Monte Daino population remained unassessed, being tacitly left in *Sorbus meridionalis.* Recent visits to the Monte Daino locality allowed the in-depth study of that population, confirming its identity with part of the *Todaro 671* material. It is noteworthy that the Madonie Mountains, where 5 of the 6 Sicilian *Aria* species occur (all except *A. busambarensis*), is the unrivalled centre of diversity of *Aria* in Sicily and Italy.

On the basis of the sizeable population found on Monte Daino in the Madonie Mountains in 1970, outside of the known area of *Aria madoniensis*, and considering that it is impossible to typify the name *Pyrus meridionalis* with a specimen from that same population (see below), we are describing that population as a new species of *Aria,* that we are naming *A. phitosiana* in honour of professor Dimitrios Phitos, excellent Greek botanist and esteemed friend, on the occasion of his 90th birthday.

At the same time, the study of the Sicilian material kept in FI, along with a review of the data generated by CASTELLANO (2012), in particular those related to a population he had referred to "*Sorbus umbellata* s. l.", has led us to to confirm the occurrence, in Sicily, of a species similar to yet distinct from *Aria umbellata* (sensu SENNIKOV & KURTTO 2017). That species is now *Aria meridionalis* (≡ *Pyrus meridionalis*), here presented as a new combination (see below). A drawing by the famous Sicilian naturalist Francesco MINÀ PALUMBO for his "Iconography of Natural History of the Madonie" (MAZZOLA & RAIMONDO 2011) also belongs here, lending further support to our conclusion on the re-evaluation of *Aria meridionalis* and on its occurrence in the Madonie Mountains.

The genus *Aria* in Sicily

On the basis of literature, and after careful study of a wide range of relevant Sicilian herbarium material (FI, NAP, PAL) as well as *in situ* studies of some natural populations, *Aria* is represented in Sicily by 6 species: two are widely distributed in Europe and parts of the Mediterranean Basin (*Aria edulis* and *A. graeca*); one is Sicilian, being widespread in various mountain areas of the island (*A. meridionalis*), and 3 are stenochorous, locally endemic microspecies (*A. busambarensis*, *A. madoniensis* and our new *A. phitosiana*).

Key for species identification

1 *Leaf blade* with 8-13 pairs of secondary veins, its margin serrate or biserrate **2**

1* *Leaf blade* with 4-8 pairs of secondary veins, or less; its margin distinctly incised-lobate... **3**

2 *Leaf blade* elliptic or lanceolate, white-felted abaxially, with 10-13 pairs of secondary veins; *fruit* globular or subglobose, bright red to crimson, lenticellate proximally .... 1. ***A. edulis***

2* *Leaf blade* obovate or suborbicular, densely greyish-white-felted abaxially, with 8-11 pairs of secondary veins; *fruit* globular, crimson, with but few conspicuous lenticels all over its surface .... 2. ***A. graeca***

3 *Leaf blade* with 6-8 pairs of secondary veins, its margin biserrate or incised-serrate; *fruit* smooth or costulate, orange red or bright red **4**

3* *Leaf blade* ovate to suborbicular, with 4-7 pairs of secondary veins; *fruit* ovoid to subglobose, orange or orange red, smooth, with few or no lenticels **5**

4 *Leaf blade* orbicular or broadly elliptic, with 7-8 pairs of secondary veins, its margin biserrate with long, acute to acuminate teeth; *Inflorescence* with few (4-8) flowers; *fruit* globular, costolate, pically truncate, orange red and with many superficial lenticels .... 3. ***A. busambarensis***

4* *Leaf blade* obovate, acuminate, with 5-8 pairs of secondary veins, its margin inciso-serrato; *inflorescence* with many (9-16) flowers; *fruit* ovoid, smooth, bright red and with few superficial lenticels .... 4. ***A. meridionalis***

5 *Leaf blade* with 5-7 pairs of secondary veins, slightly, dark green adaxially, greyish-white-felted abaxially; *fruit* subglobose, orange, with no or but few superficial lenticels .... 5. ***A. madoniensis***

5* *Leaf blade* with 4-6 pairs of secondary veins, glabrous and light green adaxially, white-felted abaxially; *fruit* ± ovoid, orange red, truncate at both ends, with scarce superficial lenticels .... 6. ***A. phitosiana***

1. ***Aria edulis*** (Willd.) M. Roem., Fam. Nat. Syn. Monogr. 3: 124. 1847 ≡ *Pyrus edulis* Willd., Enum. Pl. Hort. Berol. 1: 527. 1809 ≡ *Sorbus edulis* (Willd.) K. Koch, Hort. Dendrol.: 176. 1853. − Holotype: herb. Willdenow (B-W09688-01 [photo!]).

Up to 30 m tall tree, rarely a shrub. Branches glabrous, ± smooth, with few lenticels. *Leaf blade* 7-10 cm × 4.5-7.5 cm, ovate or orbicular, with acute apex and biserrate margin, cuneate or truncate and sometimes feebly lobed at the base, glabrous adaxially, white-felted abaxially, with 8-9 pairs of straight, unbranched lateral veins; *petiole* 12-16 mm long, tomentose. *Corymb* tomentose. *Fruit* subglobose, 8.5-13 × 8- 9 mm, red at maturity and with numerous small to medium-sized, evenly distributed lenticels.

Sicilian specimens seen (Fig. 2). − [Madonie]: Passo della Botte, 17 junio 1847, [*Minà-Palumbo*] (Herb. Mus. Minà-Palumbo, Castelbuono); Palermo alla Pizzuta, *s.d*., *Parlatore* (mixed with *A. graeca*) (FI, as *Pyrus aria*); Rocca Busambra: cliffs and slopes, 37°51’N – 13°23’S, calcareous soil, 1100 m a.s.l. 02.06.1990 *Raimondo & al. 827* (PAL).

2. ***Aria graeca*** (Spach) M. Roem., Fam. Nat. Syn. Monogr. 3: 127. 1847 ≡ *Crataegus graeca* Spach, Hist. Nat. Vég. 2: 102. 1834 ≡ *Sorbus aria* var. *graeca* (Spach) Griseb., Spic. Fl. Rumel. 1: 93. 1843 ≡ *Sorbus graeca* (Spach) Schauer in Arbeiten Veränd. Schles. Ges. Vaterl. Cult. 1847: 292. 1848 ≡ *Sorbus aria* subsp. *graeca* (Spach) Nyman, Consp. Fl. Eur. Suppl. 2: 118. 1889. − Lectotype (ALDASORO & al. 2004: 106): [Crete], ex monte Ida, [*Tournefort*], Herb. Tournefort 6150 (P P00680357 [photo!]).

Shrubs or treelets. *Leaf blade* 5-9 × 4-7 cm, obovate to suborbicular, entire (not lobed), broadest in the distal half, somewhat leathery, with 8-11 pairs of lateral veins, abaxially usually with a greenish-white tomentum, biserrate with symmetrical, patent teeth. *Fruits* subglobose, usually < 12 mm in diameter, crimson red, with few large lenticels.

Flowering in May-June and ripening its fruits in October-November. Widespread in Central and SE Europe.

Sicilian specimens seen (Fig. 2). − Monte Gibilmesi, 2.X.1993, Troia (PAL [6825]); La Pizzuta, 20.V.1997, *Maniscalco* (PAL [67506]); Madonie: Quacella, 6.VI.1990, *Raimondo & al. 946* (PAL [68541]); Madonie: Monte Catarineci, 6.VI.1990, *Raimondo & al. 1494* (PAL [68542]); Sicani: Monte Rose, 1.VI.1990, *Raimondo & al. 511* (PAL [68543]); Rocca Busambra, 2.VI.1990, *Raimondo & al. 817* (PAL [68544]); Madonie, Vallone Madonna degli Angeli, 19.V.2001, *Schicchi & Certa* (PAL [72454]); Peloritani, Monte Scuderi, 13.VI.1990, *Raimondo & al. 2063* (PAL [68829], as *Sorbus aria*); Madonie, Sciara di Fiasconaro, VI.1880, *Lojacono* (FI); [Madonie]: In valle, quae discendit a Isnello supra Polizzi, ad rupes, Passo del Vadile, 500 m, 29.VII.1874, *Strobl* (FI, 2 sheets as *Sorbus meridionalis*); Valdemone, in saxosis calcareis montosis, Majo, *Todaro 671* (p. p., mixed with *A. madoniensis*) (FI, as *Pyrus aria*); Valdemone, in sylvaticis montosis, Majo, *Citarda* -Todaro Flora Sicula Exiccata 671 (mixed with *A. meridionalis*) (FI, as *Pyrus aria*); [Madonie]: Ai Monticelli [Castelbuono], *Minà-Palumbo* (mixed with *A. madoniensis*) (PAL); Palermo alla Pizzuta, s.d., *Parlatore* (mixed with *A. graeca*) (FI, as *Pyrus aria*).

3. ***Aria busambarensis*** (G. Castellano & al.) Sennikov & Kurtto in Memoranda Soc. Fauna Fl. Fenn. 93: 29. 2017 ≡ *Sorbus busambarensis* G. Castellano & al. in Pl. Biosyst. 146(Suppl.): 339. 2012. − Holotype: Sicily: Rocca Busambra (Prov. Palermo), Godrano territory, carbonatic scree above Piano della Tramontana, 1315 m, 17.10.2009, *Castellano & Raimondo* (PAL!; isotype: FI!) Fig. 3.

3.5-7 m tall treelets or shrubs, with erect habit and spreading branches in mature individuals. Trunk with smooth, shiny, brownish-gray bark; bark of young branches reddish-gray, glabrous, smooth or slightly wrinkled, with few, 0.4-0.7 mm long, strictly ovate lenticels. *Leaf petiole* gray-tomentose, (4.5-)6-9(-13.5) × (0.9-)1.2-1.4(-1.5) mm. *Leaf blade* (3.5-)6.5-7(-9) × (2.5-)5.5-6.5(-8) cm, slightly leathery, ± broadly elliptical or orbicular, rarely obovate, apex acute or sometimes acuminate, base broadly cuneate or rounded, margin biserrate with long, thin somewhat pointed teeth typically directed outward, adaxially glabrous or sparsely tomentose along the veins, abaxially light-grey felted, with (5-)7-8(-10) pairs of straight lateral veins. *Inflorescence* corymbose, tomentose, 4-8 flowered. *Fruit* (9-)11-12(-12.5) × (12.5-)13-14(-15.5) mm, subglobose to obovate in side view, apically truncate, conspicuously or sometimes slightly costulate by 10 raised lines most obvious distally, red or reddish orange, with 7-20 small lenticels, distally densely tomentose, calyx deciduous or rarely persistent.

Sicilian specimens seen (Fig. 3) – Only the holotype (PAL).

4. ***Aria meridionalis*** (Guss. ex Tod.) Raimondo & Greuter, **comb. nov.** (Fig. 4) .≡ *Pyrus meridionalis* Guss. [Fl. Sicul. Syn. 2: 831. Jul 1844-Sep 1845, nom. inval.] ex Tod., Fl. Sicul. Exs. [Centuria 7]: num. 671. 1886 ≡ *Sorbus aria* f. *meridionalis* (Guss.ex Tod.) Strobl in Oesterr. Bot. Z. 36: 238. 1886 ≡ *Sorbus meridionalis* (Guss.ex Tod.) Simonk., Enum. Fl. Transsilv.: 7. 1887 ≡ *Sorbus aria*

Fig. 4. Coloured drawing of Minà-Palumbo's *Aria meridionalis* from his unpublished XVIII Century "Iconografia della Storia Naturale delle Madonie", tome 2, 154.

var. *meridionalis* (Guss. ex Tod.) Nyman, Consp. Fl. Eur. Suppl. 2: 118. 1889 ≡ *Sorbus aria* subsp. *meridionalis* (Guss.ex Tod.) Murb. in Acta Univ. Lund., sect. 2, ser. 2, 2(1): 45. 1905 ≡ *Sorbus umbellata* subsp. *meridionalis* (Guss.ex Tod.) Vălev in Jordanov, Fl. Nar. Rep. Bălgarija 5: 365. 1973, comb. inval. (Art. 41.5). − Lectotype (designated here): *Pyrus aria,* Monte Gebbia presso Palazzo Adriano, 1824, [*Gasparrini*] in Herb. Gussone siculum (NAP [photo!]). – Fig. 5.

2.5-6 m tall treelet or shrub; bark smooth, grey. Branches glabrous, reddish, with few lenticels. *Leaves* medium-sized to large; *lamina* (9.5-)8-5 x 4.5-6 (-7) cm, generally obovate, or also ovate to elliptical, green and glabrous adaxially, greyish-white felted abaxially, with 6-8 pairs of lateral veins; base ± cuneate, margin incised-serrate or biserrate, sometimes feebly lobed, apex acute and often acuminate. *Fruit* ovoidal, 11.5-12.5 × 11-12 mm, scarlet, covered with numerous lenticels.

Flowering April to May and ripening its fruits in September to October.

Affinities. – A. meridionalis [≡ *Pyrus meridionalis*] is similar to some forms of *A. umbellata* (Desf.) Sennikov & Kurtto. It is, for now, endemic in Sicily, but further studies might well reveal its presence in other regions of eastern Europe and Caucasia. A specimen from Hungary, as mentioned by CASTELLANO (2012), is closely similar to the Sicilian material.

Nomenclatural notes. – Pyrus meridionalis is reported as a synonym of *Sorbus aria* by LOJACONO (1891), and of *P. aria* var. *graeca* by FIORI (1924). GIARDINA & al. (2007), CASTELLANO (2012), PIGNATTI (1982, 2017) and SENNIKOV & KURTTO (2017) likewise reported it among the synonyms of *S. graeca* (Spach) Kotschy.

Under the provisional, hence invalid designation *Pyrus meridionalis*, GUSSONE (1844-1845) included the entire Sicilian material of the genus *Aria* known to him at that time, which he had previously described under the name *"P. aria"* [s. l.] (GUSSONE 1843: 560). The material seen and referred to by GUSSONE (1843), from among which the nomenclatural type must be designated (TURLAND & al. 2018: Art. 7.8), is kept in the herbaria at Florence and Naples and includes at least two of the currently recognised Sicilian *Aria* species (*A. graeca* and *A. madoniensis*). Even though Gussone failed to validate *P. meridionalis,* the issue of the identity of that name is of practical nomenclatural importance, because of the so far overlooked fact that subsequently it has been validated on the printed labels of TODARO's Flora sicula exsiccata (Fig. 6 and 7). On these labels the name *P. meridionalis* is unquestionably accepted, with a full and direct reference to GUSSONE'S (1844-1845) publication, where it is made clear that the description of *P. aria* in GUSSONE (1843: 560) applies. It is that description which validates TODARO'S use of the name; therefore, the type must be chosen from its context, which means that TODARO'S own specimens are not original material for the name: only specimens used by GUSSONE prior to 1843 are eligible as a type.

The newly discovered place of validation of *Pyrus meridionalis* poses the tricky question of its date. According to Todaro (1864), his series of exsiccata was published in centuriae available for sale. Two centuriae were due to be published each year, of which the two first were distributed in 1864. Todaro Flora sicula exsiccata No. 671, where the name *Pyrus meridionalis* was validated, belongs to the seventh Centuria; it should therefore, according to schedule, have been published in 1867. No collecting date is mentioned on the specimen label of No. 671, to corroborate or contradict that assumption; but in the PAL herbarium we spotted a specimen of *Salvia triloba* L. f. (i.e., *Salvia fruticosa* Mill.), Flora sicula exsiccata No. 676*,* that belongs to the same centuria and is stated to have been collected at Mondello in April 1868. This makes us accept 1868 as the likely year of publication of Centuria 7 of the Todaro Flora Sicula Exsiccata.

Fig. 5. Lectotype specimen of *Pyrus meridionalis,* labelled in Gasparrini's handwriting (see CUCCUINI & NEPI 1999).

Fig. 6. Specimen of Todaro’s Flora sicula exiccata n° 671: “Valdemone”, *Todaro* (FI, as *Pyrus meridionalis*). There are three twigs belonging to two different species: two of *Aria graeca* and one of *A. madoniensis* (righthand, above).

—Todaro Flora Sicula Exsiccata—
Pyrus meridionalis *Guss. fl. sic. v.* 2
p. 2, *p.* 831.
In saxosis calcareis montosis—*Valdemone.*
Majo
n. 671.
Legit *Todaro*

Todaro Flora Sicula Exiccata
—
Pyrus meridionalis *Guss. fl. sic. syn.*
2. p. 2. in suppl. p. 831 in observ.
In sylvaticis montosis—*Valdemone.*
Majo.
Dal Todaro in Ott.e 1868.
n. 671.
Legit *Citarda.*
AVVELENATA
COL SUBLIMATO

Fig. 7. Detail of the labels of the specimen in fig. 6 (protologue of *Pyrus meridionalis* Guss. ex Tod.) and in Fig. 9 Todaro's Flora sicula exiccata n° 671, *Citarda*.

GUSSONE (1843: 560) records his erstwhile *"Pyrus aria"* from "Monte Gebbia presso Palazzo Adriano (*Gasparrini*), Pizzuta (*Parlatore*): Madonie, Busambra, Mistretta, Boschi di Caronia". These are the "elements from the context of the validating description" of *Pyrus meridionalis* Guss. ex Tod., from among which the type must be selected (TURLAND & al. 2018: Art. Art. 7.7). The original material, therefore, consists of two (in fact, three: see below) cited gatherings (l.c.: Art. 8.2), the specimens of which are syntypes (l.c.: Art. 9.5); plus Gussone's original but uncited specimens gathered at the other cited localities (see l.c.: Art. 40 Note 2). For lectotypification purposes, the syntypes take precedence over the uncited specimens (l.c.: Art. 9.12). Most of the original material is to be found in GUSSONE's Herbarium siculum in Naples (NAP), where 13 sheets are kept under the original denomination of *Pyrus aria*. With few exceptions representing *Aria edulis* and *A. graeca,* all of GUSSONE'S plants belong to *A. meridionalis.* All are loose and unmounted, but have apparently not been consulted and rearranged by later students. Seven of the sheets are unlabelled, and one bears two labels: one by Gussone with a composite locality ("Maio, Junio / Madonie, Busambra, Mistretta, Caronia", the second in Minà's handwriting with the locality Roccazzo del Lupo, not mentioned by Gussone (1843) and therefore not belonging to the original material. As it is impossible to associate any of the fragments on that sheet, or on the unlabelled sheets, with a given locality, they are unsuited for lectotypification purposes. There are three sheets bearing a general Gussone label, "Madonie", and one with one of the cited localities, "Boschi di Caronia". The thirteenth and last sheet is labelled "Monte Gebbia near Palazzo Adriano" in Gasparrini's hand and bears the year of collecting, 1824. It is therefore safe to conclude that it is one of the syntypes.

The other syntype, "Pizzuta (Parlatore)", is not present in GUSSONE's own herbarium but in Florence (FI 55217; Fig. 8); according to a note by an amanuensis, it was donated by PARLATORE to Florence in August 1842, together with his herbarium (Fig. 9); it is not annotated by GUSSONE. It consists of two fragments representing two different species, *Aria edulis* in fruit (to the right) and *A. graeca* in flower (to the left). We consider both as syntypes and, therefore, as eligible for lectotypification purposes, but in our opinion they are less suited than Gasparrini's specimen.

The next to use (and validate) GUSSONE's *Pyrus meridionalis,* Todaro in his Flora sicula exsiccata*,* also accepted the wide species circumscription of GUSSONE,

Fig. 8. Original specimens (syntypes) of *Pyrus meridionalis* Guss. ex Tod., collected by Parlatore from “Panormi [Palermo] alla Pizzuta” (FI 55217), with two different samples corresponding to *Aria graeca* (in flower, to the left) and to *A. edulis* (in fruit, to the right).

Fig. 9. Specimen of Todaro's Flora sicula exiccata n° 671: "Valdemone", *Citarda* [as *Pyrus aria*] (FI), with three different twigs, one corresponding to *Aria meridionalis* (to the left) and two to *A. graeca* (to the right).

as documented by the fact that one of the sheets bearing the *Todaro 671* label, at FI, bears three twigs pertaining to two different species (*A. graeca* and *A. madoniensis*) (Fig. 6). A different specimen of the "Todaro Flora sicula exsiccata", with collector *Citarda*, preserved at FI, comes from "Valdemone"; it also contains three twigs: one corresponds to *A. meridionalis* and two to *A. graeca*. As the label has it (Fig. 9), *Aria meridionalis* would occur in unspecified areas of "Valdemone" that correspond to the Nebrodi area (incl. the Madonie mountains).

Distributional notes. – Some Sicilian specimens labelled *Pyrus meridionalis* by later authors, stored in FI, only have the generic geographical indication "Valdemone". The name "Valdemone" was once used to designate the entire north-eastern part of Sicily, including – from east to west – the Peloritani, Nebrodi and Madonie mountains, whereas "Valdemone" is nowadays used in a sense that corresponds only to the Peloritani and Nebrodi mountains, in Messina province. One of Minà-Palumbo's herbarium sheets from the Madonie mountains (in PAL, identified as *Pyrus meridionalis*) contains twigs of two different *Aria* species: *A. meridionalis* and *A. madoniensis,* occurring in the same mountain massif. Another Minà-Palumbo specimen is in the Museum Minà-Palumbo in Castelbuono. It was used as the basis of MINÀ's illustration (Fig. 4) and had, before, been erroneously identified as *Sorbus aria* subsp. *cretica* (MAZZOLA & RAIMONDO 2011). From the Gussone, Parlatore, Lojacono, Strobl, Ross and Senni materials that we saw in FI it follows that *A. meridionalis* is found both in Ficuzza (near the type locality in the Sicani mountains), and in the Madonie mountains, but it also occurs in the Nebrodi mountains ("Boschi di Caronia", *Guss.* !). The recent specimens from Monte Scuderi (E Sicily), Monte Rose (W Sicily) and the photos by G. Castellano and S. Cambria (Fig. 10 *a* and *b*) from Ficuzza (*a*) and Madonie (*b*), demostrate the current presence of *A. meridionalis* in many areas of the insland.

Sicilian specimens seen. − Palermo: Ficuzza, in silvaticis montosis 5.VII.1907, *Ross 629* [as *Sorbus aria* var. *umbellata = S. flabellifolia* (*Pyrus meridionalis*)] (FI, 3 sheets); Sotto Busambra, VIII.1876, *Lojacono* [as *Pyrus meridionalis*] (FI); Madonie a Quacedda, luglio 1840, *Parlatore* (FI 55218, as *Pyrus aria*); Madonie al Piano della Battaglia …., ? 1840, *Parlatore* (FI, as *Pyrus aria*); Madonie, 22 sett. 1832, *Minà-Palumbo* (FI, as *Sorbus aria*); Madonie, Sicilia, *Parlatore* (FI, as *Pyrus aria*); Madonie, Sicilia, 1841, *Parlatore* (FI-W, as *Pyrus aria*, 2 sheets); Madonie, Busambra, Mistretta, Caronia, in saxosis montosis, Majo-Junio [*Gussone*] (NAP, as *Pyrus aria*, 3 sheets); [Nebrodi]: Boschi di Caronia, giugno 1830, [*Gussone*] (NAP); "Madonie, Busambra, Mistretta, Caronia, Majo-Junio" [*Gussone*] and "Madonie al Roccazzo del lupo, junio" [manu ignota posterior] (NAP, 2 sheets with 2 labels as *Pyrus aria* and *Sorbus aria* respectevely); no label [*Gussone*] (NAP, 7 sheets): Madonie, julio, [*Gussone*] (NAP); Madonie, s.d. [*Gussone*] (NAP); Monte Gebbia presso Palazzo Adriano [*Gasparrini*] (NAP); Valdemone, In sylvaticis montosis, Majo, *Citarda* [Todaro Flora sicula exiccata 671] (FI, p.p.); Sicilia: Valdemone, s.d., *Todaro* (p.p. mixed with *A. graeca*) (FI, as *Pyrus meridionalis*) [Madonie]: Serra di Cavallo, 17 junio 1847, [*Minà-Palumbo*] (Herb. Mus. Minà-Palumbo, Castelbuono); [Madonie]: Pomieri, Passo della Botte nel balzo, Julio 1849 [*Minà-Palumbo*] (Herb. Minà-Palumbo, Castelbuono, as *Pyrus aria*); M. Busambra, Ciacca di Bifarera, 5.6.1904, *Senni* (PAL, as *Sorbus graeca*); Peloritani: Monte Scuderi, 13.6.1990, *Raimondo & al. 2063* (PAL as *Sorbus aria*); Monti Sicani: Monte Rose, *Raimondo & al. 511* (PAL as *Sorbus graeca*).

5. *Aria madoniensis* (Raimondo & al.) Sennikov & Kurtto in Memoranda Soc. Fauna Fl. Fenn. 93: 28. 2017 ≡ *Sorbus madoniensis* Raimondo & al. in Pl. Biosyst.

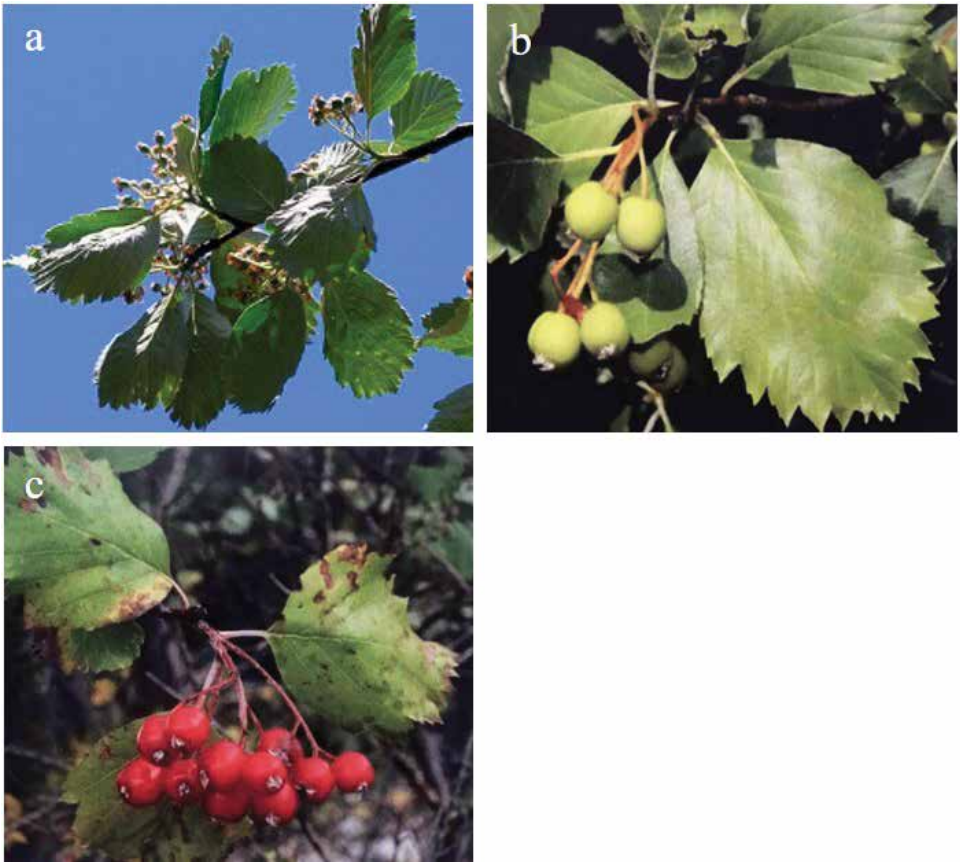


Fig. 10. *Aria meridionalis* in fruit: (**a**) from the Madonie population (photo S. Cambria); (**b**) and (**c**) from the Sicani mountains population [Monte Gebbia (*locus classicus*) and Piano delle Fontane, respectively]; photos from Castellano (2012).

146(Suppl.): 347. 2012. − Holotype: Sicily: Monti Madonie, in Località Macchia dell'Inferno sopra Castelbuono, su litosuolo calcareo, 1385 m, 5.6.2010, *Raimondo & Castellano* (PAL!; isotypes: B!, FI!, G!).

1.5-3 m tall, erect shrub with spreading branches. Trunk with smooth, glossy, greyish-red bark, young branches reddish, glabrous, smooth, with few ovate, 0.6-0.8 mm long, lenticels. *Leaf petiole* tomentose, (6-)9-13(-16.5) mm long. *Leaf blade* (2.5-)4.0-5.5(-6.4)×(1.9-)3.5-4.5(-5.5) cm, slightly longitudinally incurved, leathery, rhombic or suborbicular, acute, base broadly cuneate or rounded, margin biserrate or shallowly lobed, glabrous or thinly tomentose adaxially, with a snow-white tomentum abaxially, with 5-7 pairs of straight, unbranched lateral veins. *Inflorescence* corymbose, tomentose. *Fruit* 6.6-8.5 × 7-9 mm, orange or yellowish orange, with 0-10 small lenticels, with dense tomentum that persists to maturity distally.

Sicilian specimens seen (Fig. 3).− Monti Madonie, in località Macchia dell'Inferno sopra Castelbuono, su litosuolo calcareo, 1385 m a.s.l., 37°54'12.90''N, 14°03'40.30''E, 05 VI 2010, (fl.), *Raimondo & Castellano* (holotype: PAL; isotypes: B, FI, G). Valdemone, *Todaro 671* (FI p.p., mixed with *A. graeca*) *Todaro & Citarda* p.p.; Valdemone, *Citarda* in Todaro 671 (FI); Valdemone, *Minà-Palumbo* (PAL p.p., mixed with *A. graeca*); Madonie: Gonato, 8.VI.1847 (Herb. Mus. Minà-Palumbo, Castelbuono, as *Sorbus umbellata*, det. Dull 1990); Timpi di Monticelli, bosco serre di Quacedda, 2.VII.1846 (Survuna), [*Minà-Palumbo*] (Herb. Mus. Minà-Palumbo, Castelbuono, as *Pyrus aria*); no label [*Minà-Palumbo*] [Herb. Mus. Minà-Palumbo, Castelbuono, as *Pyrus meridionalis*).

6. *Aria phitosiana* Raimondo & Greuter, **sp. nov.** (Fig. 11).

Holotype: Sicily, Madonie, northern slope of Monte Daino, dolomitic rocky ground, c. 1550 m a.s.l., 7 VI 2018, *Raimondo* (PAL; isotypes: PAL-Gr, FI). – Figs. 12,13 & 14.

?= *Sorbus aria* var. *incisa* Lojac., Fl. Sicul. 1(2): 200. 1891, nom. illeg. [non Mutel 1834]. Holotype: Sicily, “alle serre di Quacedda” [Quacella], *Lojacono* (not extant in PAL and FI, not seen).

Treelet with upright trunk and drooping branches; bark smooth, brown, ferrugineous, in young branches with plentiful ovate lenticels. *Leaves* medium-sized; *petiole* tomentose, 3-10 mm long; *leaf lamina* flat, glabrous and dark green adaxially, white-tomentose abaxially, ovate to suborbicular or elliptic, 3.5-7(-8) × 2.5-5.5(-6) cm, with 4-6 pairs of lateral veins; base sub-cuneate, margin incised-serrate or lobulate distally; apex mucronate, lobes acute, irregularly serrate. *Leaves* of young plants similar but smaller, suborbicular to subtriangular. *Flowers* white, scented, (4-)5-16(-18) per raceme; pedicels woolly. *Fruit* subovoid to obovoid 8-10 × 4-5 mm, glabrous except distally, reddish, with with few scattered lenticels.

Flowering May to June and ripening its fruits in October-November.

Distributional notes. − A rare species, endemic to Sicily, very local and threatened. IUCN Category: Vulnerable. Lojacono (1891) reports his *Sorbus aria* var. *incisa* from the “Serre di Quacedda”, a dolomitic relief immediately adjacent to Mount Daino, the type locality of *A. phitosiana.* The last named, contrary to other *Aria* species in the montane or submontane phytoclimatic belt of the same area, prefers dolomitic ground.

Affinities. – *Aria phitosiana* is similar to *Aria umbellata*. From that polymorphic species it differs – apart from its occurrence west of the distributional range of *A. umbellata* – in the shape and colour of its fruits (reddish rather than yellowish or red, and with but rare lenticels). *Aria phitosiana* is also similar to *A. baldaccii* (C. K. Schneid.) Sennikov & Kurtto (≡ *Sorbus umbellata* var. *baldaccii* C. K. Schneid. ≡ *Sorbus meridionalis* subsp. *baldaccii* (C. K. Schneid.) Bordz.), but it is well distinct from it, and also from the Sicilian *A. madoniensis,* described from the northern slopes of the of the calcareous Mt. Carbonara massif in the same mountain system, by its arborescent rather than shrubby (pleiocormic) growth. *A. phitosana* is similar to *A. madoniensis* in leaf dimensions and shape as well as in the number of lateral veins, but differs in growth habit (Fig. 12 *a* and *b*) and in the colour of the adaxial leaf surface (Fig. 13 *a* and *b*) and of its mature fruits (reddish rather than orange) (Fig. 14 *a* and *b*). *Aria phitosiana* also differs from *A. baldaccii* (from Bosnia, Croatia, Herzegovina, Montenegro, Albania, and Greece) by its obovate leaves with acute, not obtuse lobes.

Specimens seen. – Sicily: Madonie, slopes of Mount Daino, on dolomitic grounds, 1450 m a.s.l., June 1970, *Raimondo* (PAL).

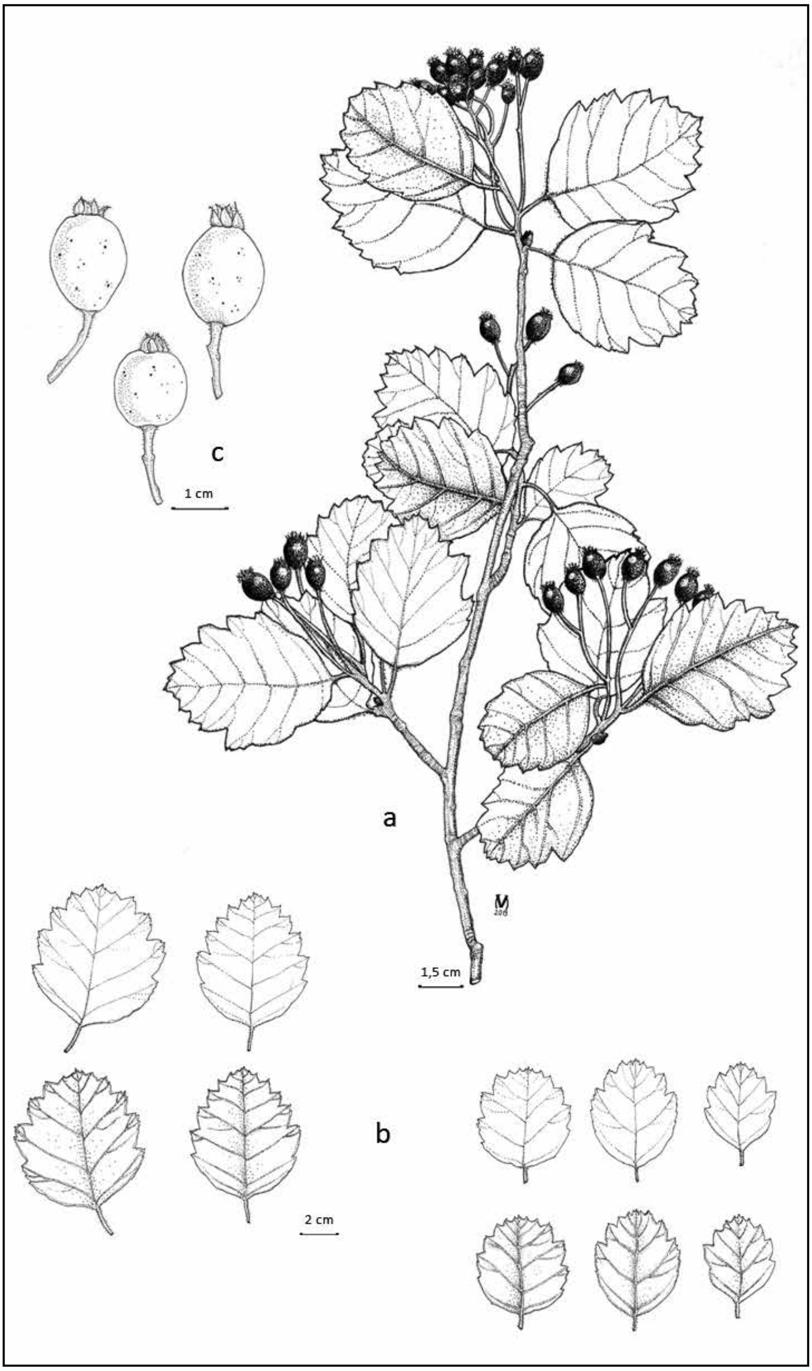


Fig. 11. Iconography of *Aria phitosiana*: (**a**) habitus (from holotype), (**b**) leaves (top row: adaxially; bottom row: abaxially), (**c**) fruits (drawing by V. Magro).

Fig. 12. Individuals of *Aria phitosiana* (**a**) and of *A. madoniensis* (**b**) in their respective natural habitat [photos F.M. Raimondo].

Fig. 13. Fruiting branches and leaves of: (**a**) *Aria phitosiana* (photo F. M. Raimondo); (**b**) *A. madoniensis* (from Raimondo & al. 2012); (**c**) leaf of a young plant of *A. phitosiana* (photo F. M. Raimondo).

Fig. 14. Fruits, from the respective locus classicus, of: (**a**) *Aria phitosiana* (photo F. M. Raimondo) and (**b**) *A. madoniensis* (photo from RAIMONDO & al. 2012).

Transfer of *Sorbus orbiculata* to *Aria*

The second author recently published the combination *Sorbus orbiculata* for a species of *Sorbus* subg. *Aria* Spach from Transcaucasia and adjacent countries (Turkey and Iran) (GABRIELJAN 1978, 2018). With the generic status of *Aria* accepted here, following SENNIKOV & KURTTO (2017), that species lacks a correct name. We take the opportunity to provide it here by publishing the following new combination.

Aria orbiculata (Gabrieljan) Gabrieljan, **comb. nov.** ≡ *Sorbus umbellata* var. *orbiculata* Gabrieljan, Ryabiny Zapadnoi Azii Gimala'ev: 175. 1978 ≡ *Sorbus orbiculata* (Gabrieljan) Gabrieljan in Novosti Sist. Vysš. Rast. 49: 88. 2018.

Conclusion

Summarizing the results of this study, the genus *Aria* is enriched by three more specific taxa. Furthermore, it is shown that Sicily is an active centre of diversity for the genus *Aria*. The Madonie Mountains represent, both in Sicily and in the whole Mediterranean area, the smallest area with a strong concentration of species. In fact almost all species present in Italian territory (5 of 7) and 5 of 6 of those present in Sicily are represented here.

In conclusion, we note that an in-depth study of the *Aria* populations related to the *Aria umbellata* group, on a geographical scale that transcends a single island, also encompassing southern Europe and Caucasia – might lead to a different assessment of the units here proposed for Sicily.

Acknowledgements

We thank Chiara Nepi (FI) and Paolo Caputo (NAP) for providing photographs of relevant herbarium material and permit their use in this paper. Thanks, also, to Alfredo Carratello, Palermo, for his assistance in consulting herbarium material in PAL. To Pietro Mazzola, Palermo, for the photo reproduced in Fig. 4. To Salvatore Cambria, Palermo, for the photo in Fig.10a. Finally, to Vincenzo Magro, Palermo, for the drawings in Fig. 11. We also thank the International Foundation pro Herbario Mediterraneo and the Foundation Herbarium Greuter for supporting this study.

References

ALDASORO, J. J., AEDO, C., GARMENDIA, F. M., HOZ, F. P. DE LA & NAVARRO, C. 2004: Revision of *Sorbus* subgenera *Aria* and *Torminaria (*Rosaceae-Maloideae). − Syst. Bot. Monogr. 69: 1-148.

BARTOLUCCI, F., PERUZZI, L., GALASSO, G., ALBANO, A., ALESSANDRINI, A., ARDENGHI, M. G., ASTUTI, G., BACCHETTA, G., BALLELLI, S., BANFI, E., BARBERIS, G., BERNARDO, L., BOUVET, D., BOVIO, M., CECCHI, L., DI PIETRO, R., DOMINA, G., FASCETTI, S., FENU, G., FESTI, F., FOGGI, B., GALLO, L., GOTTSCHLICH, G., GUBELLINI, L., IAMONICO, D., IBERITE, M., JIMÉNEZ-MEJÍAS, P., LATTANZI, E., MARCHETTI, D., MARTINETTO, E., MASIN, R. R., MEDAGLI, P., PASSALACQUA, N. G., PECCENINI, S., PENNESI, R., PIERINI, B., POLDINI, L., PROSSER, F., RAIMONDO, F. M., ROMA-MARZIO, F., ROSATI, L, SANTANGELO, A., SCOPPOLA, A., SCORTEGAGNA, S., SELVAGGI, A., SELVI, F., SOLDANO, A., STINCA, A., WAGENSOMMER, R. WILHALM, T. & CONTI, F. 2018: An updated checklist of the vascular flora native to Italy. − Pl. Biosyst. 152: 179-303.

CASTELLANO, G. 2012: Il genere *Sorbus* (Rosaceae*,* Maloideae) subg. *Aria* in Sicilia. − Università di Palermo, Dipartimento di Biologia Ambientale e Biodiversità, Pp. 238 [unpublished PhD thesis].

— G., MARINO, P., RAIMONDO, F. M. & SPADARO, V. 2012: *Sorbus busambarensis* (Rosaceae), a new endemic species of Sicily. − Pl. Biosyst. 146(suppl.1): 338-344.

CONTI, F., ABBATE, G., ALESSANDRINI, A. & BLASI C. (eds) 2005: An annotated checklist of vascular flora of Italy. – Roma.

CUCCUINI, P. & NEPI, C. 1999: Herbarium Centrale Italicum (Phanerogamic Section): the genesis and structure of a herbarium. The main collections, the collectors, the handwriting samples and the personnel in its 150-year history. – Firenze.

FAY, M. F. & CHRISTENHUSZ, M. J. M. 2018: *Pyrus* L. Pp. 94-126. In: CHRISTENHUSZ, M. J. M., FAY, M. F. & BYNG, J. W. (eds.), Plant Gateway's the global flora: a practical flora to vascular plant species of the world. Special Edition, GLOVAP Nomenclature, Part 1. − Bradford.

FIORI, A. 1923-1925: Nuova flora analitica d'Italia, 1. – Firenze.

GABRIELJAN, E. T. 1978: Rjabin'i (*Sorbus* L.) Zapadnoj Azii i Gimalaev [Rowans (*Sorbus* L.) of Western Asia and the Himalayas]. – Erevan.

— 2018: *Sorbus orbiculata* (Rosaceae)*,* a new species from Caucasus, North-East Anatolia and North-West Iran. − Novosti Sist. Vysš. Rast. 49: 87-92.

GIARDINA, G., RAIMONDO, F. M. & SPADARO, V. 2007: A catalogue of plants growing in Sicily. – Bocconea 20: 5-552.

GUSSONE, G. 1843: Florae siculae synopsis, 1. – Napoli.

— 1844-1845: Florae siculae synopsis, 2. – Napoli.

KURTTO, A., SENNIKOV, A. N. & LAMPINEN, R. (eds.) 2018: Atlas florae europaeae. Distribution of vascular plants in Europe. 17. Rosaceae (*Sorbus* s. lato). – Helsinki.

LOJACONO POJERO, M. 1891: Flora sicula, 1(2). – Palermo.

MAZZOLA, P. & RAIMONDO, F. M. 2011: Plants. Pp. 3-387. In: MINÀ PALUMBO, F., Iconography of the natural history of the Madonie, 2. – Palermo.

PIGNATTI, S. 1982: Flora d'Italia, 2. – Bologna.

— 2017: Flora d'Italia, ed. 2, 2. – Milano.

RAIMONDO, F. M. 1971: La flora forestale delle Madonie. – Università di Palermo [unpublished PhD thesis].

— 2018: Acquisizioni filogenetiche e variazioni tassonomiche in alcune rosacee legnose della flora Italiana. – Notiziario Soc. Bot. Ital. 2: 35-36.

—, CASTELLANO, G., BAZAN, G. & SCHICCHI, R., 2012: *Sorbus madoniensis* (*Rosaceae*), a new species from Sicily. – Pl. Biosyst. 146(suppl.1): 345-351.

—, DOMINA, G. & SPADARO, V. 2010: Checklist of the vascular flora of Sicily. – Quad. Bot. Ambient. Appl. 21: 189-252.

— & SPADARO, V. 2009: Addenda et emendanda to the "A catalogue of the plants growing in Sicily". – Fl. Medit. 19: 303-312.

SENNIKOV, A. N. & KURTTO, A. 2017: A phylogenetic cheklist of *Sorbus* s. l.(*Rosaceae*) in Europe. – Memoranda Soc. Fauna Fl. Fenn. 93: 1-78.

TODARO, A. 1864: Flora sicula exsiccata. − Bot. Zeitung (Berlin) 22: 54-56.

TURLAND, N., WIERSEMA, J., BARRIE, F. R., GREUTER, W., HAWKSWORTH, D., HERENDEEN, P., KNAPP, S., KUSBER, W.-H., LI, D.-Z., MARHOLD, K., MAY, T. W., MCNEILL, J., MONRO, A. M., PRADO, J., PRICE, M. J. & SMITH, G. F. 2018: International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. – Regnum Veg. 154.

WARBURG, E. F. & KÁRPÁTI, Z. E. 1968: 28. *Sorbus* L. Pp. 67-71. In: TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H. & WALTERS, S. M. (eds), Flora Europaea, 2. – Cambridge.

Ein neues Johanniskraut von den Kykladen (Agäis, Griechenland): *Hypericum* (sect. *Drosocarpium*) *perfoliatum* subsp. *phitosianum* (Hypericaceae)

WERNER GREUTER & RAINER KARL

Abstract

Greuter, W. & Karl, R. 2019: Ein neues Johanniskraut von den Kykladen (Ägäis, Griechenland): *Hypericum* (sect. *Drosocarpium*) *perfoliatum* subsp. *phitosianum (*Hypericaceae*).* – Bot. Chron. 22: 39-47.

Eine neue Unterart von *Hypericum perfoliatum* wird beschrieben und benannt. Sie ist auf einigen Inseln der Kykladen (Paros, Seriphos, Naxos) endemisch und bildet auf Andros und Naxos offensichtlich hybridogene Populationen mit *H. perfoliatum* subsp. *perfoliatum,* der auf dem griechischen Festland weit verbreiteten Nominatsippe der Art. Das Zustandekommen dieses Verbreitungsmusters wird diskutiert und, in Übereinstimmung mit früheren Ergebnissen, durch Einwanderung der letzteren während der hocheiszeitlichen Meerestiefstände und durch evolutionäre Stasis des sich ergebenden Sippengemisches während der anschließenden Phase insulärer Isolation erklärt.

Greuter W. & Karl R. 2019: A new taxon of St John's wort from the Cyclades (Aegean Islands, Greece): *Hypericum* (sect. *Drosocarpium*) *perfoliatum* subsp. *phitosianum (*Hypericaceae*).* – Bot. Chron. 22: 39-48.

A new subspecies of *Hypericum perfoliatum* is described and named. It is endemic to some Cycladean islands (Paros, Seriphos, and Naxos). On Andros and Naxos, it forms obviously hybridogenous populations with *H. perfoliatum* subsp. *perfoliatum,* the nominal taxon that is widespread on mainland Greece. The genesis of this distributional pattern is discussed. In agreement with earlier assumptions, it is interpreted as resulting from immigration of the mainland taxon in phases of lowered sea level during Pleistocene glaciation maxima, followed by evolutionary stasis during the subsequent period of insular isolation.

Werner Greuter, Herbarium Mediterraneum Panormitanum, Orto Botanico, Università di Palermo, via Abramo Lincoln 2/A, I-90133 Palermo, Italien. E-mail: w.greuter@bgbm.org; Rainer Karl, Kreuzbergweg 9, A-8580 Köflach, Österreich. E-mail: rainer.karl@gmx.at

Key words: evolutionary stasis, hybridization, introgression, isolation, migration, paleogeography.

Einleitung

Hypericum L. sensu lato, die bei weitem größte der 9 gegenwärtig anerkannten Hypericaceen-Gattungen (STEVENS 2007), zählt mit 470 Arten in 30 Sektionen zu den 100 artenreichsten Angiospermen-Familien (CARINE & CHRISTENHUSZ 2010). Sie ist weltweit verbreitet, vor allem in den Subtropen und gemäßigten Klimazonen.

Hypericum sect. *Drosocarpium* Spach, von ROBSON (2010) jüngst monographisch bearbeitet, ist im Wesentlichen eine zirkummediterrane Gruppe, die sich mit der Leitart *H. perfoliatum* L. nach Makaronesien (Madeira, Gran Canaria) und mit *H. richeri* subsp. *grisebachii* (Boiss.) Nyman bis in die Ukraine erstreckt. Das Mannigfaltigkeitszentrum dieser nach ROBSON (2010) 11 Arten umfassenden Sektion liegt in der (insbesondere südlichen) Balkanhalbinsel. Allein in Griechenland kommen 8 oder 9 Arten vor. Die Sektion ist unter anderem charakterisiert durch ihren Drüsenreichtum, wobei Verteilung und Anordnung der Drüsen in den verschiedenen Pflanzenorganen wichtige artdiagnostische Kriterien darstellen. Nach ROBSON (1981: 80) unterscheidet man “dunkle Drüsen” (schwärzliche Anhäufungen hypericingefüllter Zellen) und “helle Drüsen” (mit durchscheinendem ätherischem Öl gefüllte lysigene Hohlräume).

Die vorliegende Arbeit befasst sich mit den im Archipel der Kykladen (Ägäische Inseln, Griechenland) vorkommenden Vertretern von *Hypericum* sect. *Drosocarpium.* Nach ROBSON (2010) sind das die zwei nahe verwandten Arten *H. perfoliatum* L. und *H. trichocaulon* Boiss. & Heldr., wogegen nach HALÁCSY (1900-1901), RECHINGER (1944), DIMOPOULOS & al. (2013) und STRID (2016) einzig die erstgenannte auf den Kykladen vorkommt, *H. trichocaulon* dagegen ein Endemit Kretas ist. Die bisher rätselhafte Pflanze der Kykladen, welche von ROBSON (2010) mit Vorbehalt zu *H. trichocaulon* gestellt wird (er kartiert und bespricht sie unter dieser Art, widerspricht sich aber selbst, indem er sie im Text als innerhalb der Variationsbreite von *H. perfoliatum* liegend betrachtet), ist das zentrale Thema unserer Arbeit.

Material und Methoden

Diese Studie beruht auf der morphologischen Analyse (durch den Zweitautor) des *Hypericum perfoliatum*-Materials der folgenden Herbarien, welches leihweise an das Wiener Herbar (W) gesandt wurde: B, C, LD, M und MSB (die Abkürzungen sind genormt nach Thiers 2018). Vom Herbarium W, wo auch die Bearbeitung erfolgte, wurde das Gesamtmaterial von *Hypericum perfoliatum* und *H. trichocaulon* untersucht. Insgesamt wurden 239 Herbarbogen gesehen (zuzüglich jener in PAL-Gr und einiger nur in Form von Digitalbildern untersuchter aus WU). Davon stammen 220 aus Griechenland. Aus unserem eigentlichen Untersuchungsgebiet stammen 30 Belege von folgenden Inseln: Naxos (12), Andros (11), Paros, (3), Kea (1), Milos (1), Seriphos (1) und Siphnos (1).

Die hier benutzte beschreibende Terminologie folgt den Definitionen von ROBSON (1981).

Zur Ermittlung der Zugehörigkeit zu den hier akzeptierten Taxa wurden in erster Linie die folgenden Merkmale berücksichtigt:

Wuchs (zahlreiche ± gleichartige, zarte, fertile Stängel / wenige (2-5) robuste, fertile Stängel mit ± zahlreichen, [meist] sterilen, zarten basalen Seitentrieben);

Höhe der fertilen Stängel;

Blattmaße (Länge × Breite);

Bedrüsung der Laubblätter (dunkle Drüsen z.T. flächenständig / beinahe ausschließlich intramarginal);

Bedrüsung und Form der Kelchblätter (dunkle Drüsen intramarginal, in 2 Reihen / intramarginal und flächenständig, in 4 Reihen);

Kelchblattrand (drüsig gefranst bis tief gezähnt / drüsig gezähnelt oder mit ± sitzenden dunklen Drüsen);

Bedrüsung der Kronblätter (dunkle Drüsen auf der ganzen Fläche / nur am Rand, im distalen Drittel);

Kapselbedrüsung.

Ergebnis

Die im Folgenden beschriebene, zunächst vom Zweitautor 2008 auf der Kykladeninsel Paros gesammelte und fotografierte Pflanze der Gattung *Hypericum* stimmt mit keiner bisher beschriebenen Sippe überein. Sie unterscheidet sich sowohl habituell als auch in mehreren auffälligen strukturellen Merkmalen stark von *Hypericum perfoliatum* (Abb. 1)*,* der Art, welcher sie zweifellos am Nächsten steht. Es war zunächst unsere Absicht, sie als neue Art zu beschreiben.

Auf Grund von Hinweisen in der Literatur wurde uns indessen klar, dass der neue Fund nicht der erste dieser Sippe war. Der österreichische Entomologe Hans Malicky sammelte dieselbe schon rund 38 Jahre zuvor, ebenfalls auf Paros, und ähnliche Pflanzen sind von der benachbarten Insel Naxos bekannt geworden, wo sie eine sehr variable Population zu bilden scheinen, die sich morphologisch in unterschiedlichem Maße dem dort ebenfalls vorkommenden typischen *Hypericum perfoliatum* annähert. Wie einleitend erwähnt, waren diese Pflanzen dem Monographen ROBSON (2010) bekannt und wurden von ihm als intermediär zwischen *H. perfoliatum* und dem kretischen Endemiten *H. trichocaulon* interpretiert – eine Auffassung, welcher sich STRID (2016) anschloss. Da unsere Pflanze zu einer Sippe gehört, die sich morphologisch und arealmäßig charakterisieren lässt, und mit *H. perfoliatum* (nicht aber mit *H. trichocaulon*) durch Übergangsformen verbunden ist, beschreiben wir sie neu als Unterart der letzteren Art: ***Hypericum*** (sect. *Drosocarpium*) ***perfoliatum*** subsp. ***phitosianum*** Greuter & Rain. Karl, **subsp. nov.** – Holotypus: Griechenland, Nomos Kiklades, Paros, Stroumboulas SSW von Lefkes, S-Seite, SE-exponierter Hang, 695 m (37°02′06′′N, 25°11′26′′E), 4.6.2017, *Rainer Karl* (PAL-Gr 126167; isotypi: B, C, LD, M, MSB, W). – *Planta* herbacea e caudice lignoso multicaulis, cuncta glabra. *Caules* simplices, graciles, 10-15 cm alti, e basi diffusa, florendi tempore nuda, arcuato-ascendentes densiuscule foliati, surculis sterilibus axillaribus carentes, internodiis costa decurrente bicarinatis. *Folia* decussata, elliptica vel subovata, latitudine duplo circiter longiora, ad 25 × 6 mm metientia, sessilia, basi late cuneata vel rotundata vel imprimis superiora cordato-amplexicaulia, obtusa, margine integerrimo subrevoluto; per faciem praesertim inferiorem abunde glandulis minutissimis partim intramarginalibus nigro-punctata et insuper punctis pelludicis minusculis raris obsita; nervis lateralibus utrinque 2-3, cum tertiariis anastomosantibus inconspicuis supra aliquanto impressis. *Folia floralia* inflorescentiam fulcrantia caulinis similia sed auriculis basalibus dense glanduloso-ciliatis praedita. *Inflorescentia* pauciflora per anthesin densa, bracteis sursum descrescentibus sepalos aemulantibus obsita, cincinnis praeter florem terminalem (0-)2-4, 1-3-floris. *Flores* breviter (1-2 mm) pedicellati. *Sepala* latitudine subduplo longiora, 3-4 mm longa, e basi lata vix expansa, rotundata, dorso vittis longitudinalibus sursum ± discontinuis nec non lineolis et punctis atro-notatis, margine glandulis sessilibus nigris crebre denticulatis. *Petala* 5, cum staminibus post anthesin cito decidua, ad 12 mm longa, expansa, flava, per totam superficiem dense

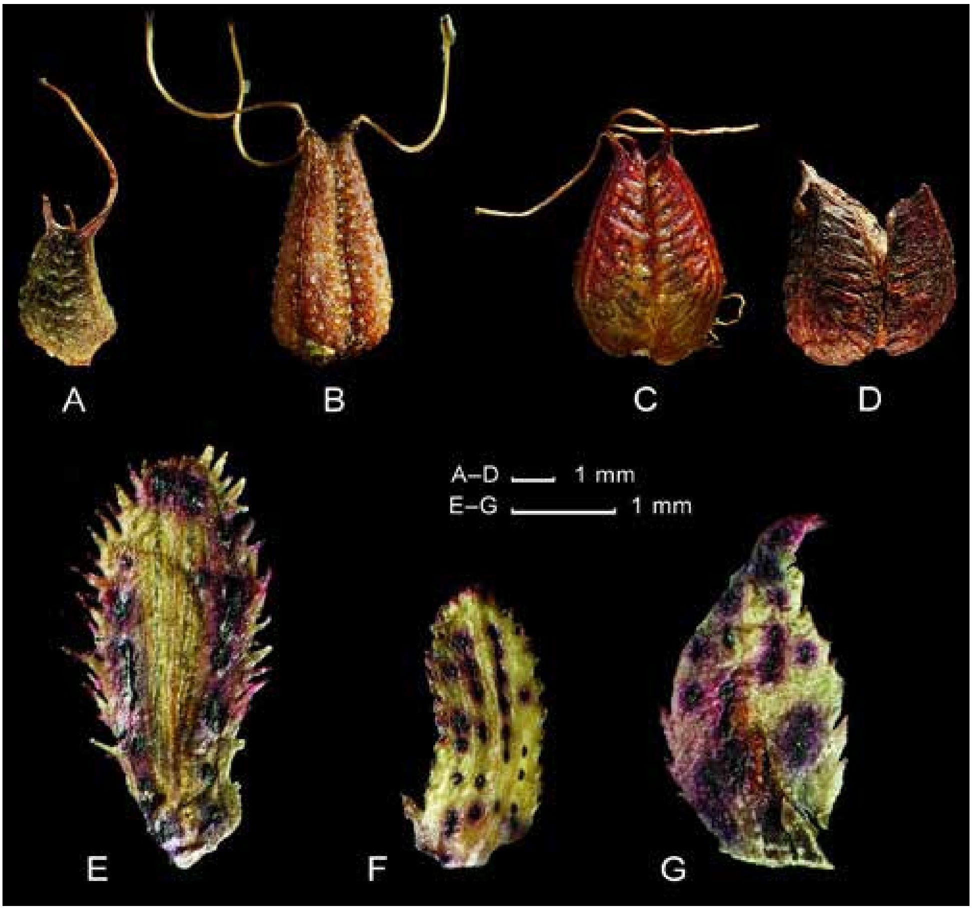


Abbildung 1. Makrofotografien von Kapseln (**A-D**) und Kelchblättern (**E-G**): **A, B** & **E**: *Hypericum perfoliatum* subsp. *perfoliatum* (**A** & **E** von Griechenland, Nomos Prevezza, NW. von Arhangelos, *Willing 35120*, B 525538; **B**, Form mit runden Kapsel-Blasen, von Griechenland, Nomos Trikala, Eparchie Kalambaka, S. Aj. Paraskevi, 8.7.1997, *Eisenblätter & Willing 58384,* B 525547). – **C** & **F**: *H. perfoliatum* subsp. *phitosianum,* (vom Holotypus, PAL-Gr 126167). **D** & **G**: *H. trichocaulon* (von Griechenland, Kreta, Eparchie Amari, Oros Kedhros, oberhalb Ano Meros, 9.9.1984, *Risse 1470,* B 525522).

nigro-lineolata et -punctata. *Stamina* numerosa, 3-fasciculata, filamentis flavis ad 10 mm longis, antheris ca. 0,5 mm longis, per anthesin curvis, flavis, connectivo glandula nigra terminato. *Carpelli* 3, stylis liberis a basi divergentibus. *Capsula* in dorso carpellorum longitudinaliter 1-3-vittata, vesiculis brevibus crassis obliquis pinnatim divergentibus aurantiaco-vesiculosis et insuper ad commissuras nonnusquam utrinque linea longitudinali glandularum atrarum ornata. *Semina* pallide brunnea, diametro subduplo longiora, ca. 1,1 mm longa, longitudinaliter minute costulata. – Die neue Sippe ist nächstverwandt mit *Hypericum perfoliatum* subsp. *perfoliatum,* mit welchem sie das Vorkommen dunkler Drüsen auf allen Organen, überdies die drüsig gefransten Öhrchen der Hochblätter und die Grundzüge des Drüsenmusters der Fruchtkapseln gemeinsam hat; sie weicht aber auffällig in mehreren Merkmalen ab, wie dem zarten, vielstängeligen Wuchs, den geringeren Maßen von Stängeln und Blättern und der wenigblütigen, kompakteren Infloreszenz. Anders als bei *H. perfoliatum* subsp. *perfoliatum* sind in subsp. *phitosianum* die blassen, durchscheinenden Drüsen der Blattspreite spärlich, die dunklen Drüsen dagegen zahlreich und über die ganze Fläche verteilt. Die apikal gerundeten Kelchblätter mit ihren dichtstehenden, beinahe sitzenden dunklen Randdrüsen und die hinfälligen, nicht nach dem Welken bis zur Fruchtreife persistierenden Kronblätter sind weitere kennzeichnende Merkmale der neuen Sippe.

Etymologie. – Dem Nestor der griechischen Botanik, Herrn Prof. Dr. Dimitrios Phitos, aus Anlass seines 90. Geburtstags zugeeignet.

Specimina visa. – **Griechenland, Ägäische Inseln, Nomos Kiklades,** Paros, Stroumboulas SSW von Lefkes, S-Seite, 695 m (37°02′05′′N, 25°11′23′′E); Kalk, felsige Phrygana; 9.5.2008, *R. Karl* (PAL-Gr, W); ibidem (37°02′06′′N, 25°11′26′′E), 4.6.2017, *R. Karl* (holotypus: PAL-Gr; isotypen: B, C, LD, M, MSB, W); Paros, Moni Thapsanon, 400 m, (37,06°N, 25,18°E), 8.5.1976, *Malicky* (W 1977-4515); Naxos, Mt. Zas [= Zeus, = Drio], am Wanderweg von Ag. Marina zum Gipfel, 650 m; (37°02′26′′N, 25°30′32′′E); Gneis, felsige Phrygana; 13.5.2008; *R. Karl* (W); Naxos, Filoti, 400-500 m; Kulturen, Weingärten, Mauern, 23.5.1988; *Burri & Krendl* (W 2007-22736); Seriphos, 5 km W Seriphos, 400-500 m, 8.4.1984, *Malicky* (W 1985-0001135).

Zwischen *Hypericum perfoliatum* subsp. *perfoliatum* und subsp. *phitosianum* intermediäre Pflanzen. – **Nomos Kiklades,** Andros, W-Seite, ca. 4 km ESE Batsi, 500-700 m; 15.6.1964; Snogerup (LD 1403176); Andros, 2 km W Apoikia, 530-550 m; Macchie; 15.5.1968; *Snogerup & Bothmer* (LD 1415232); Andros, 2 km ESE Gavrion, 35 m; Schiefer, 8.4.1971; *Snogerup & Gustafsson* (LD 1415292); Andros, 2 km W Apoikia, 430-530 m; 15.5.1968; *Snogerup & Bothmer* (LD 1415472); Andros, 2 km NE Ag. Petros), 350-400 m; Phrygana; 18.5.1968; *Snogerup & Bothmer* (LD 1414812); Andros, Inselchen Megalo W Batsi; 12.5.1968*;* *Snogerup & Bothmer* (LD 1415412); Andros, NW Apoikia, 350-450 m; 16.5.1968; *Snogerup & Bothmer* (LD 1414992); Andros, W & NW Kallivari, ca. 200 m; 12.6.1968; *Bothmer* (LD 1415052); Andros, E Sanganiari, 400-500 m; 21.5.1968; *Snogerup & Bothmer* (LD 1415112), Andros, NW Apoikia, 300-450 m; 15.5.1968; *Snogerup & Bothmer* (LD 1415172); Naxos, Keramoti, 510m; Macchie am Straßenrand; 28.5.2000; *Nielsen* (C 27/2016/3, “delicate Form”); ibidem, *Nielsen* (C 27/2016/9, “robuste Form”); Naxos, “in montibus altissimis”; Apr. 1831, *Finder unleserlich* (M 280771); Naxos, 200 m; offene Phrygana, Granit; 7.5.1992; *Böhling* (B 100525568); Naxos, Skeponi, 260 m; sandiger Gneisschutt; 22.5.1996, *Böhling* (B 100525498); Naxos, 140 m; Granodiorit, Garigue, 3.5.1987, *Juth* (B 100525502); Naxos, Koronos-Gebirge, 550 m; Gneis, Garigue, 3.6.1988; *Juth* (B 100525503); Naxos, Koronis, 670 m, Glimmerschiefer, schattig-feucht, 5.6.1992, *Böhling* (B 100525505). – Nach ROBSON (2010: 63), gehören überdies hierher die Belege *Böhling 1416, 2662* und *2675,* alle von Naxos (BM), sowie *Gathorne-Harrdy 240* von Euböa (BM, E); letztere Angabe halten wir für zweifelhaft: die Pflanze soll laut ROBSON (l.c.) “gegen typisches *Hypericum perfoliatum* neigen”, dürfte aber, auf Grund weiterer untersuchter Pflanzen von Euböa, eher eine xeromorphe Form des echten *H. perfoliatum* darstellen, wie sie als *H. heldreichii* *perfoliatum* und subsp. *phitosianum* intermediäre Form.

Gesehene Belege von *Hypericum perfoliatum* subsp. *perfoliatum* von den Kykladen. – **Nomos Kiklades**, Andros, Ammolochos, 500 m, 31.5.1992, *Snogerup & Snogerup* (B 100525501); Kea, in insula Cea (Κέως), solo schistoso, 21-24.5.1898, *Heldreich* (M 280764, WU 103667, WU-Hal-Gr 103664 & 103666); Naxos, E Koronas, ca. 500-600 m. Marmor, Phrygana. 14.5.1988. *Burri & Krendl* (W 2007-19281); Naxos, Zeus, ca. 800-900 m, Marmor, Felswände, 23.5.1988; *Burri & Krendl* (W 2007-22810); Siphnos, Weg von Kamares nach Choni im Bereich von Kata Vatos und Chlorou, 4.5.1983, *Heiselmayer & Pilsl* (herb. Pilsl 5758); Milos, Plaka, Kastro, 200 m, N-exponierte Felsen, 15.5.2003, *Runemark* (LD 1752146). – Hierher stellen wir einstweilen eine von uns nicht überprüfte Literaturangabe von *Hypericum perfoliatum* (nach HALÁCSY 1900-1901: 279): Nomos Kiklades, Tenos, *Wiedemann* (Beleg nicht auffindbar; laut Mitteilung von MARGARITA LACHMAYER & DIETER REICH nicht in WU).

Standort. – Am locus classicus auf Paros wächst *Hypericum perfoliatum* subsp. *phitosianum* in felsiger Phrygana auf Kalk; die Population am Typus-Standort ist räumlich sehr begrenzt, die beobachteten Pflanzen besiedeln einen Streifen von ca. 5 × 20 m. Als Begleitarten wurden notiert und gesammelt: *Asphodelus aestivus* L., *Carex halleriana* Asso, *Carthamus lanatus* L., *Centaurea* aff. *raphanina* Sm., *Cistus creticus* L., *Convolvulus elegantissimus* Mill., *Crucianella angustifolia* L., *Erysimum hayekii* (Jáv. & Rech. f.) Polatschek, *Helichrysum stoechas* subsp. *barrelieri* (Ten.) Nyman, *Hordeum bulbosum* L., *Opopanax hispidus* (Friv.) Griseb., *Pistacia lentiscus* L., *Rhamnus lycioides* subsp. *graeca* (Boiss. & Reut.) Tutin, *Sarcopoterium spinosum* (L.) Spach, *Stipa bromoides* (L.) Dörfl. und *Teucrium divaricatum* Heldr.

Es ist uns bekannt, dass Herr Panayiotis Trigas, Athen, unsere Pflanze ebenfalls gesammelt und unabhängig von uns als unbeschriebene neue Sippe erkannt hat. Leider hat Herr Trigas die von uns vorgeschlagene gemeinsame Veröffentlichung abgelehnt und es vorgezogen, seine Art unabhängig in einer eigenen Arbeit (TRIGAS 2018) auf die Schnelle vorab zu veröffentlichen. Eine Bezugnahme auf unsere ihm bekannte Veröffentlichungs-Absicht ist dabei bedauerlicher Weise unterblieben. Für diejenigen, die es vorziehen, die neue Sippe als eigenständige Art zu betrachten, ist der Name *Hypericum cycladicum* Trigas vermutlich korrekt, wobei allerdings zu bemerken ist, dass der Typus von der Insel Andros stammt, wo Zwischenformen zu typischem *H. perfoliatum* vorherrschen und wir das echte *H. perfoliatum* subsp. *phitosianum* bisher noch nicht gesehen haben.

Diskussion

Hypericum perfoliatum subsp. *phitosianum* ist in reiner Ausprägung bisher von den drei Kykladen-Inseln Naxos, Paros, und Seriphos bekannt geworden, überdies in annähernd typischer Ausprägung gelegentlich auf Naxos. *H. perfoliatum* subsp. *perfoliatum* wächst als einziger Vertreter der Sektion auf Kea, Milos und Siphnos, überdies in annähernd typischer Ausprägung gelegentlich auf Naxos. Auf Naxos finden sich überwiegend, und auf Andros ausschließlich, Zwischenformen der genannten Unterarten.

Auf dem griechischen Festland, woher uns reichliches Material zur Verfügung stand, ist *Hypericum perfoliatum* subsp. *perfoliatum* weit verbreitet und recht variabel. Eine eingehende Analyse dieser Variabilität, die noch aussteht, könnte durchaus ergeben, dass sich auch dort geographisch einheitliche Merkmalsmuster herausarbeiten lassen, welche die Abgrenzung infraspezifischer Sippen (ebenfalls Unterarten?) rechtfertigen würden. Dagegen ist das ursprünglich im Artrang beschriebene *H. perfoliatum* var. *heldreichii* kaum mehr als eine Modifikation trockener und sonniger Standorte. Diese Form zeichnet sich aus durch kleinere, insbesondere schmalere, armdrüsigere Blätter, die sich tendenziell aufrichten und dem Stängel anliegen, sowie durch niedrigen, zarteren, vielstängeligen Wuchs. In Wuchs und Blattgröße erinnern solche Pflanzen teilweise an *H. perfoliatum* subsp. *phitosianum*, sind aber durch die übrigen Merkmale insbesondere im Kelch- und Kronenbereich klar von diesem verschieden. Beobachtungen im Gelände zeigen, dass solche xeromorphe Pflanzen kleinräumig mit normalwüchsigem *H. perfoliatum* zusammenleben und lückenlos in dieses übergehen. Die Mehrzahl der uns vorliegenden *Hypericum perfoliatum* subsp. *phitosianum*, in welches *H. perfoliatum* subsp. *perfoliatum*, vom Festland her kommend, sekundär eingedrungen ist. Durch introgressive Hybridisierung entwickelten sich auf den größeren Inseln (Andros und Naxos) Mischpopulationen, in welchen die eine oder andere der Ursprungssippen sich nur spärlich erhalten konnten. Der Umstand, dass laut ROBSON (1981, 2010) die Differentialmerkmale der Kykladen-Sippe, insbesondere die flächenständigen dunklen Drüsen, als abgeleitet gelten, dass sich also *H. perfoliatum* subsp. *phitosianum* als das stammesgeschichtlich jüngere Glied des Sippenpaares auffassen lässt, steht dieser Interpretation nicht entgegen; denn erstens ist die Merkmalsphylogenie in der Gattung *Hypericum* eine noch wenig gesicherte Hypothese, und zweitens ist die Chronologie von Migrationsprozessen nicht zwangsläufig gleichlaufend mit der Phylogenie der Taxa.Bezüglich des Zeitpunktes, zu welchem das Eindringen von *H. perfoliatum* subsp. *perfoliatum* in den Raum der Kykladen stattgefunden haben mag, bieten sich insbesondere zwei Hypothesen an. In junger, postglazialer Vergangenheit könnte der Mensch als Transport-Agens die Einwanderung begünstigt haben. Mit Weidevieh oder vom Festland aus eingebrachtem Viehfutter könnten Verbreitungseinheiten der Festlandsippe einzelne Kykladeninseln erreicht haben und dort heimisch geworden sein. Angesichts des bedeutenden Anteils der anthropogenen Taxa an den gegenwärtigen ägäischen Inselfloren (GREUTER 1971) ist eine solche Erklärung nicht von der Hand zu weisen, auch wenn durch sie das Entstehen introgressiver Hybridschwärme bei einer Art, die kaum aggressive Ausbreitungstendenzen erkennen lässt, wenig plausibel erscheint.

Aus unserer Sicht ist es wahrscheinlicher, dass sich das heutige Verbreitungsmuster auf Prozesse der natürlichen Sippenmigration zurückführen lässt.

Die glazialeustatisch bedingte generelle Absenkung des Meeresspiegels um 100-200 m während der pleistozänen Kaltzeiten, insbesondere der zwei letzten, schuf in ägäischen Raum stark veränderte Küstenlinien, selbst wenn man von tektonisch bedingten Vorgängen absieht. Dies lässt sich kartographisch darstellen, wenn man als Küstenlinie die heutige 200 m-Isobathe annimmt (GREUTER 1979: 95). Während der Höhepunkte der Kontinentalvereisungen ergaben sich innerhalb und zu den Kykladen stark erweiterte Migrationsmöglichkeiten, welche im Zusammenspiel mit der dann vorherrschenden evolutionären Dynamik den

Abbildung 2. Verbreitung in den Kykladen von *Hypericum perfoliatum* subsp. *perfoliatum* (Kreisflächen, ●; Teilareal), *H. perfoliatum* subsp. *phitosianum* (Sterne, ✶) und ihren mutmaßlich hybridogenen Zwischenformen (Sterne mit weißem Zentrum, ✡). Die einzelnen Fundorte innerhalb der Inseln sind nicht genau georeferenziert.

beobachteten jetzt-Zustand erklärbar machen, zumal bei der von GREUTER (1979) anhand zahlreicher Beispiele postulierten, unter den Bedingungen kleinräumiger insulärer Isolation vorherrschenden Stasis. Das Beispiel von *Crepis ×cytherea* Kamari, einer hybridogenen, im ehemaligen Überlappungsbereich der Eltern-Areale entstandenen und dort stabilisierten hybridogenen Art (GREUTER 1979: 100), passt genau zum Muster der hier behandelten *Hypericum*-Sippen.

Danksagung

Dank gebührt den Leitern und Kustoden der Herbarien, die leihweise Vergleichsmaterial verfügbar gemacht haben: B, C, LD, M, MSB, PAL-Gr und W (vgl. THIERS 2018) sowie Frau CRISTINA CATTANEO, Rom. Insbesondere sind wir Herrn ERNST VITEK, Wien, für vielfache Hilfestellung bei der Organisation und Abwicklung der Ausleihen sehr verpflichtet. Frau MARGARITA LACHMAYER und Herrn DIETER REICH danken wir für Auskünfte über Belege in WU. Herrn MICHAEL RODEWALD, Berlin, verdanken wir die Reinzeichnung der Verbreitungskarte und Frau JULIA DIETRICH, Berlin, die Makroaufnahmen von Kapseln und Kelchblättern.

Literatur

CARINE, M. A. & CHRISTENHUSZ, M. J. M. 2010: Editorial. – Phytotaxa 4: 1- 4, 2010.

DIMOPOULOS, P., RAUS, T., BERGMEIER, E., CONSTANTINIDIS, T., IATROU, G., KOKKINI, S., STRID, A. & TSANOUDAKIS, D. 2013: Vascular plants of Greece. An annotated checklist. – Englera 31.

GREUTER, W. 1971: L'apport de l'homme à la flore spontanée de la Crète. – Boissiera 19: 329-337.

— 1979: The origin and evolution of island floras as exemplified by the Aegean archipelago. Pp. 87-106. In: BRAMWELL, D. (ed.), Plants and islands. –Academic Press, London & New York.

RECHINGER, K. H. 1944: Flora aegaea: Flora der Inseln und Halbinseln des Ägäischen Meeres. – Akad. Wiss. Wien, Math.-Naturwiss. Kl., Denkschr. 105(1).

HALÁCSY, E. VON 1900-1901: Conspectus florae grecae, 1. – Engelmann, Leipzig.

ROBSON, N. K. B. 1981: Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. – Bull. Brit. Mus. (Nat. Hist.), Bot. Ser. 5: 293-355.

— 1977: Studies in the genus *Hypericum* L. (Guttiferae). 2.Characters of the genus. – Bull. Brit. Mus. (Nat. Hist.), Bot. Ser. 8: 55-226.

— 2010: Studies in the genus *Hypericum* L. (Guttiferae). 5(1). Sections 10. *Olympia* to 15/16. *Crossophyllum*. – Phytotaxa 4: 5-126.

STRID, A. 2016: Atlas of the Aegean flora. – Englera 33.

THIERS, B. 2018 [laufend aktualisiert]: Index Herbariorum: A global directory of public herbaria and associated staff. (http://sweetgum.nybg.org/science/ih/) – Botanical Garden, New York.

TRIGAS, P. 2018: A new *Hypericum* (sect. *Drosocarpium,* Hypericaceae) from the Cyclades Islands (Greece). – Nordic J. Bot. 2018: e02205. (https://onlinelibrary.wiley.com/doi/epdf/10.1111/njb.02205).

Perspectives for plant conservation in the Mediterranean region

VERNON H. HEYWOOD

Abstract

Heywood, V. H. 2019: Perspectives for plant conservation in the Mediterranean region. – Bot. Chron. 22: 49-61

The many challenges facing the conservation of the rich and diverse plantlife of the Mediterranean region are reviewed, including the great diversity of political systems in the constituent countries, state of economic development, patterns of land use, and the imbalance between the European north and the south and east of the region in terms of conservation legislation, infrastructure and facilities. Area-based and species-based conservation still show significant deficits, especially as regards species recovery. Over-optimistic assumptions about the effectiveness of protected areas in conserving species biodiversity is placing many threatened species at risk. A greater emphasis is needed on ways of achieving some protection for threatened species occurring outside protected areas. More effective coordination of plant conservation at a pan-Mediterranean level is needed.

V. H. Heywood, Reading, U.K. E-mail: vhheywood@btinternet.com

Key words: Mediterranean, plant conservation, Protected Areas, species recovery, ex situ.

Introduction

The Mediterranean region with its semi-enclosed sea, elongated shape, large topographic contrasts and climate gradients is a difficult area to define as it does not coincide with any political delimitation and is not a geographical unity. It is made up of a number of territories that surround the sea but only some parts of the countries bordering it, such as Spain, France, even Egypt, can be considered Mediterranean in a true sense, while others, such as Portugal that does not even have a Mediterranean coastline, are regarded floristically as largely Mediterranean. Turkey may be included in the Mediterranean region or excluded and considered to be in Europe as in the review of plant conservation in the east Mediterranean by IUCN (VALDERRÁBANO & al. 2018). The political Mediterranean does not, therefore, coincide with the bioclimatic, biogeographical, or floristic Mediterranean. Moreover, the Mediterranean overlaps, to a considerable degree, the regions commonly referred to as the Middle or Near East and Southwest Asia.

The Mediterranean region, however defined, is one of the world's most important centres of plant diversity – both in terms of its floristic richness and its ecological communities and landscapes. Although the region covers only about 2.3 million km^2, representing some 1.6% of the planet's land surface, it houses about c. 25,000 species and numerous subspecies, representing c. 7% of the world's vascular plants (VALDERRÁBANO & al. 2018).

The ecosystems of the Mediterranean are amongst the most heavily modified in the world as the result of human action over the centuries. Successive waves of civilization have shaped the past and present landscapes through clearing of the land for crops, the use of fire, and the felling of forests to provide timber for shipbuilding, extensive terracing (now largely abandoned), large areas under irrigation, agricultural intensification, intensive cultivation of horticultural crops under glass or plastic. Agriculture and animal husbandry have been practised in the Mediterranean basin for 10 000 years (ZEDER 2008) and most of the ecosystems have been modified by human action. This has led to the characteristic landscapes of today's Mediterranean – extensive olive groves, vineyards, citrus groves, orchards, arable fields and scattered forests of oaks, pines, cedars and firs. The landscapes have also been severely affected by alien invasive species, most of which have been introduced for forestry, agriculture, ornamental horticulture or other economic or social purposes (ARIANOUTSOU & VILÀ 2012, DI CASTRI & al. 1990, BRUNEL 2016, BRUNEL & al. 2010) and have escaped from cultivation, while others have been introduced accidentally, usually by human agency.

In the last few decades, two additional factors – building for tourist development and the introduction of intensive horticulture – have combined to alter even more drastically the coastal areas. As a result, cultural landscapes now dominate the region and much of the forest has been replaced by secondary communities such as the characteristic shrubland communities (maquis, phrygana, matorral, garrigue, etc.) that form such a conspicuous part of Mediterranean landscapes.

Despite the impacts of anthropogenic change over thousands of years, and tourism which have depleted resources and led to major alterations to the vegetation and landscapes, the Mediterranean region still retains a great wealth of plantlife and is recognized as one of the major centres of plant diversity (HEYWOOD 1995, MÉDAIL & QUÉZEL 1999, MITTERMEIER & al. 2000). It is also one of the Vavilov centres of crop origin and diversification (HAWKES 1995).

The exact number of threatened plant species in the Mediterranean is not known and the threat status of only c. 7% of the 25,000 or so species has been assessed so far (DE MONTMOLLIN 2008). Of these some 572 are threatened to some degree. The total number of threatened species in the Mediterranean region is probably 2-3000, of which fewer than 10% have conservation or recovery plans. The recorded number of threatened plant species in the east and south Mediterranean is c. 200 (or 312 if Turkey is included). Very few have conservation or recovery plans.

Conservation of Mediterranean plant life

The conservation of landscapes, ecosystems and species in the Mediterranean has to be viewed in the context of the past history of the region as discussed above. A further consideration is the susceptibility of the Mediterranean to climate change (see below).

The pattern of conservation actions differs between the countries of the European northern shores and those of the North African and the east Mediterranean Levantine regions. Compared with the countries on the northern shores, the political and socio-economic conditions and the patterns of land use, and ownership, and food production and diets, notably a switch to more animal-based food production, are different.

Many of the European Mediterranean countries are parties to the European Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (the Habitats Directive and to the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and have benefitted from the various EU LIFE programmes, which have been a cornerstone of plant conservation. A considerable number of the LIFE projects have focused on Mediterranean plants and habitats (EUROPEAN COMMUNITIES 2008, HEYWOOD 2014).

Important Mediterranean initiatives are the CARE-MEDIFLORA project (www.care-mediflora.eu), which aims to improve the conservation status of threatened Mediterranean plant species through ex situ and in situ joint actions. It is implemented by seven institutions of six Mediterranean islands (Balearic Islands, Corsica, Sardinia, Sicily, Crete and Cyprus), the IUCN/SSC Mediterranean Plant Specialist Group, and ECOPLANTMED which aims to contribute to halting the loss of biodiversity and to promote a sustainable development model in the Mediterranean region by enhancing the conservation of native plants and promoting their use in habitat restoration and the plant production sector.

Protected areas and other effective area-based conservation measures

3.1 Protected Areas

Most countries today regard Protected Areas as the underpinning of their national conservation policy. They are widely regarded as the primary defence against biodiversity loss, provided they are well maintained and managed. The Mediterranean region has a wide array of protected areas although it is very difficult to assess the total coverage because of the problem of disaggregating the European data so as to include only those countries that occur within the Mediterranean biogeographic or climatic zone and then for those countries that do border the Mediterranean, calculating how many of their protected areas actually occur in the Mediterranean climatic zone. As far as can be determined, the percentage of protected land in the Mediterranean region as a whole which comes under IUCN categories I–VI is well over 10%[i].

In Europe, the two most important European networks of protected areas are the EU's Natura 2000 and the related Council of Europe's Emerald Network, together constituting the largest coordinated network of protected areas in the world (the Pan-European Network of Protected Areas), with the terrestrial component of Natura 2000 covering 17.9% of the land across all 28 EU countries, with 25,717 terrestrial sites (767,995 km^2 (European Commission Environment Nature and biodiversity Natura 2000 http://ec.europa.eu/environment/nature/natura2000/index_en.htm). Many of the areas in these networks occur within the Mediterranean region. In addition, each country has its own national and regional networks. The complex relationships between all these networks are discussed in detail in *Protected areas in Europe — an overview* (EEA Report No.5/2012). Overall, in the European Union, about 25% of the terrestrial surface in the EU is in protected areas – thereby surpassing the Convention on Biological Diversity's Aichi Target of 17% (Strategic Plan for Biodiversity 2011-2020 and the Aichi

Targets, https://www.cbd.int/doc/strategic-plan/2011-2020/Aichi-Targets-EN.pdf) – yet a substantial loss of biodiversity has been reported as discussed later.

No such regional networks occur in the southern and eastern Mediterranean regions where a diversity of protected areas exist (see the detailed review by SATTOUT 2018), some of them under national legislation, others coming under older traditional systems of protection such as the *hema* and the *Mahmeya* which had their origin in the Arab and Islamic worlds (HEYWOOD & SATTOUT 2018). In addition, the Mediterranean region as a whole has a large number of internationally designated areas such as the 79 UNESCO Biosphere Reserves, in 15 countries, and of these 20 occur in North Africa and the Levant; and 144 Ramsar sites of which 81% occur in countries of the southern Mediterranean, notably Algeria and Tunisia (SATTOUT 2018).

As HOFFMANN & al. (2018) aptly observe, 'There is … a risk of naively focusing on the amount of area, but neglecting biodiversity protection'. Indeed, it is a valid criticism that too much emphasis has been focused on the extent of protected areas rather than their ecological representativeness, quality of management and effectiveness in conserving biodiversity (KATI & al. 2015, HOFFMANN & al. 2018) and the Mediterranean region is no exception: there are still significant gaps in the ecological coverage of the region's protected areas, even in the European zone, and insufficient connectivity between them, although there have been substantial improvements in recent years. The quality and effectiveness of protected area systems in meeting their various objectives in the Mediterranean varies from region to region and even between neighbouring countries, reflecting different political systems, management structures, levels of expertise, economic circumstances and social attitudes.

It is now widely agreed that effective management of protected areas is necessary if PAs are to fulfil their objectives, although it is not the key to preventing biodiversity loss as often stated (IPBES/6/15/Add.4, 2018, p. 4). There is a commonly held assumption that well managed protected areas will conserve the biodiversity that occurs in them, but it has frequently been pointed out, that while it is true that for threatened species PAs will afford some degree of protection if they are themselves effectively protected and managed, unless the management includes actions to contain or remove the threatening processes that are putting the species at risk, the species will continue to be threatened and may well decline further or even become extinct. This is one of the main factors that explains the apparent paradox, that despite the considerable increase in the extent of PAs in recent years, biodiversity continues to be lost (HEYWOOD 2017, 2018). A recent review of the Natura 2000 network (KATI & al. 2014) revealed, as the main weaknesses of that network, 'the lack of political will from local and national governments toward effective implementation; the negative attitude of local stakeholders; the lack of background knowledge of local stakeholders, which prevented well-informed policy decisions; and the understaffing of Natura 2000 management authorities'.

3.2 Plant Micro-reserves

Although small reserves have been established in various parts of the world to afford protection to threatened species, usually in fragmented vegetation (HEYWOOD 1999), a great deal of interest has been generated by the network of plant micro-reserves (PMRs) established in the Valencia region in Spain. Micro-reserves in the Spanish sense are small protected areas of less than 20ha and often less than one or two hectares, and frequently with a high concentration of endemic, rare or threatened species (LAGUNA 2018). Since the first Valencia PMR was legally established in 1999, 300 PMRs have been created by the Generalitat Valenciana as legally protected sites, covering a total of 2,291 ha. The model has also been adopted in Bulgaria, Cyprus, Estonia, Greece (Crete), and Slovenia. In the eastern Mediterranean PMRs have been established in Lebanon and there are proposals to create a network of micro-reserves in Egypt for medicinal and aromatic plants (KADIS & al. 2013). PMRs may be considered as an option in areas where the vegetation has been subject to fragmentation and the species populations they contain are similarly reduced or fragmented. Because of the small area they occupy and their less demanding legal and management terms, it may be possible for them to be established in great numbers and to complement the larger, more conventional protected areas. On the other hand, their viability in the medium- to long-term must remain in question, especially in the light of global change and the difficulties of building in resilience to it.

3.3 Biodiversity conservation outside protected areas

Given that most biodiversity exists outside formally protected areas, increasing attention is being paid to the problems that its conservation presents poses, although the biodiversity conservation community has largely shown reluctance to engage adequately with this issue (HEYWOOD 2018). A diversity of measures is in place in some countries such as the USA and Australia outside of and complementary to the formal protected areas system, including conservation easements, covenants, trusts, partnerships, incentive-based schemes, habitat conservation planning (HCP) and mitigation banking (MACKEY & al. 2008, KAMAL & al. 2015). Most countries have paid little attention to this issue although many are increasingly engaging in community conservation practices.

The Recognition by the Convention on Biological Diversity (CBD) that some areas outside the recognised protected area networks (known as Other Effective Area-Based Conservation Measures – OECMs) contribute to the effective in situ conservation of biodiversity and act as an important complement to conventional protected areas is an important advance although no clear guidance about what these other measures are or how they may be assessed, identified or reported was provided (JONAS & MCKINNON 2018, HEYWOOD 2018). At the recently concluded CBD Conference of the Parties (November 2018) which adopted, with some modifications and additions, a draft decision by SBSTA on 'Protected Areas and Other Effective Area-Based Conservation Measures'[ii], including the following definition of an OECM: "A geographically defined area other than a Protected Area, which is governed and managed in ways that achieve positive and sustained long-term outcomes for the in situ conservation of biodiversity, with associated ecosystem functions and services and where applicable, cultural, spiritual, socioeconomic, and other locally relevant values".

An analysis by COX & UNDERWOOD (2011) of the proportional extent of land cover categories in each of the world's five Mediterranean regions – protected (IUCN categories I-VI), converted (to urban), impacted (by intensive agriculture), and unprotected, natural and semi-natural land with conservation potential –, showed that the Mediterranean Basin had 66% of unprotected land with potential for biodiversity conservation. As an example of projects that have promise for safeguarding in Mediterranean basin, they cite the 'numerous endemic taxa [that] persist only in ancient agrosilvopastoral landscapes, such as the cork oak-dominated montado of Portugal and dehesa of Spain ... These centuries-old agro-environmental systems produce economically valuable amenities such as cork, tourism and employment, while supporting numerous native wildlife and plant species, many of them endemic. In recognition of their biodiversity and cultural assets, these and other agro-environmental systems are considered high conservation priorities in Europe, and throughout the European Union governments pay farmers to voluntarily adapt their operations to benefit biodiversity and the environment (ONATE & al. 1998)'.

Cox & Underwood used the species-area relationship to estimate the number of species conserved under existing land cover status and then to illustrate the potential conservation returns that can be secured in natural and semi-natural lands that remain outside of protected areas. This part of their analysis is flawed in that it makes the common but erroneous assumption that existence of species in a protected area implies that it is effectively conserved whereas in many cases of threatened species, protected areas only afford some degree of protection unless action is taken to remove or contain the factors that cause them to be threatened (HEYWOOD 2015, 2017, HEYWOOD & al. 2018). Nonetheless, it is clear that unless a serious effort is made in the Mediterranean to increase both the quality and quantity of Protected Areas and recognize the globally important contributions of OECMs and other forms of land use and management that favour the survival of biodiversity, it will not be possible to ensure the conservation of many of the plant species and their habitats that are currently under threat, not to mention those that will be put at risk by the impacts of climate change over the coming decades. Implementing such a policy integrating the systems of conventional protected areas and OECMs with each other and into the wider landscape poses a series of problems and challenges and will require a rethinking of current strategies.

3.4 Important Plant Areas and Key Biodiversity Areas for Plants

In the Mediterranean region considerable effort has gone into the identification of Important Plant Areas (RADFORD & al. 2011) and subsequently, Key Biodiversity Areas (KBAs) – sites that contribute significantly to the persistence of globally important biodiversity (VALDERRÁBANO & GIL 2018). Detailed country information on KBAs for plants are given in chapter 3 of *Conserving wild plants in the south and east Mediterranean region* (VALDERRÁBANO & al. 2018). Although not in themselves a form of conservation but a priority determining mechanism, they are a useful tool in planning conservation strategies.

Conservation at the species level

4.1 In situ conservation

Species recovery and other forms of in situ conservation the species level have proved difficult to implement in most parts of the world (HEYWOOD & DULLOO 2005, HEYWOOD 2005, HEYWOOD 2015, 2017), although they are explicitly mandated by the Convention on Biological Diversity in Article 8 '…the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties'. In the Mediterranean region, the situation is far from satisfactory although more action has been taken in the European sector than in the south and east of the region (HEYWOOD 2014, 2018).

For those European countries that are partly or wholly within the Mediterranean region, the two key legal instruments are the EC Habitats Directive and the Council of Europe's Berne Convention. The principal and complementary components of both of these are the conservation of listed habitats and species but while progress in the first element has been substantial, implementation of species conservation has been disappointingly poor (HEYWOOD 2009, 2012). The Habitats Directive (EEC 1992 is the key policy instrument and strategy in Europe for, legally preserving Europe's characteristic, vulnerable and endangered species at the level of the EU, and it covers the Mediterranean region's richest countries in terms of plants species and endemism. Its main aim is to promote the maintenance of biodiversity by requiring Member States to take measures to maintain or restore natural habitats and wild species listed on the Annexes to the Directive at a favourable conservation status, introducing robust protection for those habitats and species of European importance. The list of species in the Annex was developed by a group of specialists but it now seriously out of date (CARDOSO 2012), yet there appears to be little appetite for its revision. Appendix 1 of the Bern Convention, which is a list of 'Strictly Protected Flora Species', has been subject to periodic amendments. It too would, however, benefit from serious revision in the light of our greatly enhanced knowledge at national level of the threatened status of plant species and the likelihood of the impacts of climate change (HEYWOOD 2009, 2012).

At a European level, the situation in terms of legal protection is summarized by OZINGA & SCHAMINÉE (2005): 'The Bern Convention lists 642 vascular plant species …, while the Habitats Directive lists 484 plant species…. Together both legal listings cover 774 species …. From the 1,939 species that are globally threatened, 79 % are not listed by the Bern Convention or the Habitats Directive. This result show that the European legal instruments provide no adequate protection for many threatened vascular plant species'.

Implementation of conservation or recovery plans for species listed in the Habitats Directive and Bern Convention Annexes has generally been poor and the same applies to species assessed as Threatened in National, Regional and Global Red Lists. A review of in situ conservation of plant species in the Mediterranean (HEYWOOD 2015) showed that of the estimated 2000–3000 threatened or endangered plant species probably less than 10% have conservation or recovery plans. In the Mediterranean, most conservation/recovery plans have been implemented by the megadiverse countries, Italy, France and Spain (HEYWOOD 2015), but even in these countries the majority of threatened species are without action plans.

It would appear that more effort and resources have been expended on preparatory studies such as assessing and listing threatened species and identifying key areas for conservation than on implementing actions to remove the threats and ensuring effective conservation. This is even more evident in the southern and eastern parts of the Mediterranean where, until recently, a biodiversity conservation culture of species recovery has been scarcely developed (HEYWOOD 2015, FOIS & al. 2018) and undue reliance has been placed on protected areas for species conservation, as is common in many other countries, especially in the tropics. It is encouraging that some recovery actions such as population augmentation of endangered species have been undertaken and more are being planned (FOIS & al. 2018). Also, a number of genetic conservation actions for Crop Wild Relatives (CWR) in the Mediterranean region have been carried out (HEYWOOD 2008) and by 2020 it is hoped to have in place a network of in situ stakeholders to promote active conservation of CWR populations across the region (MAXTED & al. 2018).

Overall, urgent action is needed to address the serious conservation deficit in terms of recovery actions in the region as a whole.

Some species reintroductions have been carried out in the Mediterranean region but there are few documented cases of where they have been successful (BOU DAGHER KHARRAT 2018).

4.2 Ex situ conservation

After a period during which ex situ conservation of wild plant material in genebanks, botanic gardens and other facilities was largely neglected by conservationists, on the mistaken grounds that it was a poor substitute for conservation in situ and risked being employed by governments as an alternative for the latter, it is now recognized that it occupies an important part of a comprehensive conservation strategy. As well as providing an insurance against the total loss of species which have become highly endangered in the wild, it is also an important resource for the provision of material that can be used for population augmentation in species recovery programmes, for reintroductions and other forms of conservation translocation, and in ecological restoration.

Europe is well placed, having a well-organized network for the ex situ conservation of seeds of plant species – ENSCONET (The European Native Seed Conservation Network32 (http://ensconet.maich.gr/)) involving 24 institutes from 17 European countries, about half of them in the Mediterranean region. In the Mediterranean region SEMCLIMED (Seeds – Climate-Mediterranean (genmeda.blogspot.com) was a project funded by the EC's INTERREG funds and developed by GENMEDOC, a network of research centres and seedbanks focused on the study and conservation of seeds of Mediterranean plants (from both European and non-European countries). In 2010 this led to the foundation of the Network of Mediterranean Plant Conservation Centres (GENMEDA: http://www.genmeda.net/).

In addition, some national genebank networks have been created, such as REDBAG (Red Española de Bancos de Germoplasma de plantas silvestres), the Spanish network of germplasm banks of wild plant species, and RIBES (Rete Italiana Banche del germoplasma per la conservazione ex situ della flora Spontanea italiana), the Italian network of seed banks for ex situ conservation of wild plant species. For details of national seedbanks in the Mediterranean see PORCEDDU & BACCHETTA (2018).

Reference must also be made to the Millennium Seedbank (MSB) of the Royal Botanic Gardens Kew at Wakehurst, which holds material of some 37,000 plant taxa. Together with its partnership, it is the largest ex situ plant conservation project in the world (RIVIÈRE & al. 2017, 2018). It contains seed samples of many Mediterranean plant species.

The European Cooperative Programme for Plant Genetic Resources (ECPGR), a collaborative Programme involving most European countries, aims at ensuring the long-term conservation and utilization of plant genetic resources in Europe. The Strategic Framework for the Implementation of a European Genebank Integrated System (AEGIS) is a platform connecting European genebanks under a common system for the long-term conservation of Plant Genetic Resources for Food and Agriculture (PGRFA), including wild species such as Crop Wild Relatives and Medicinal and Aromatic Plants (http://www.ecpgr.cgiar.org/aegis/).

Important agricultural genebanks are found in the Mediterranean region, including that of the International Center for Agricultural Research in the Dry Areas (ICARDA) (http://www.icarda.org). It is officially based in Aleppo, Syria, but after rebel action, its primary seed storage and research facilities were moved to Rabat, Morocco, and Turbol, Lebanon. The Egyptian Deserts Gene Bank at the Sheikh Zowaid Station in northern Sinai suffered damage from looting in 2011 but is now again functional.

4.3 Botanic gardens

For over 700 years, Mediterranean botanic gardens played a major role in the scientific, economic and cultural life of the region; and like botanic gardens in other parts of the world they are playing an increasing role in plant conservation (BLACKMORE & OLDFIELD 2017). The number of botanic gardens in each country of the Mediterranean region that are located in the Mediterranean climate zone, most of them in France, Italy and Spain, is approximately 170 (HEYWOOD 2015). With few exceptions, botanic gardens have not played an important role in the countries of the southern and eastern Mediterranean. The number of botanic gardens in North Africa and the Levant is relatively small, and often they have limited resources and facilities, and species-poor living collections. Currently there is no botanic garden network covering the Mediterranean region as a whole, although there are several national and regional networks such as the Asociación Ibero-macaronésica de Jardines Botánicos (AIMJB, www.jbotanicos.org/). In a recent review of the Mediterranean's botanic gardens (HEYWOOD 2015), I noted that:

'Today, many of the Mediterranean's botanic gardens are under great financial pressure and have suffered severely from the recent economic crisis with their staffing and operating budgets cut. Others have never managed to establish themselves fully as modern botanic gardens and their capacity and functions are severely restricted. If one also takes into account the imbalance in the distribution of Gardens across the region, and the large numbers of threatened endemic species in the region, it is evident that there is a lack of capacity to undertake the necessary conservation actions to maintain this unique biodiversity'.

An initiative to introduce 'ancillary botanic gardens' (ABGs) is being developed in Lebanon (TALHOUK & al. 2018). As TALHOUK & al. (2014) comment, 'For botanic gardens to be established and sustained by Lebanon and the Lebanese, there is a need to deconstruct the traditional concepts of a botanic garden and recreate institutions based on new components that are culturally acceptable'. ABGs are described as '…informal, deregulated gardens for the conservation of plant diversity and cultural plant knowledge; they are established by local communities in open sites which have existing levels of land protection owing to their primary purpose as archaeological sites, educational institutions, religious landholdings, private institutions and touristic sites'.

Conclusions and recommendations

The Mediterranean region is one of the world's major centres of plant diversity and endemism, but because of difficulties of definition, differences in physical structure and political diversity, it is difficult to make generalizations about the Mediterranean as a whole.

There is a marked contrast between the northern (European) region and the southern and eastern regions in terms of plant conservation achievements and prospects. The European part of the Mediterranean benefits from legislative structures and conservation agreements such as the Habitats Directive, the Bern Convention, and their networks of Protected Areas. No equivalent arrangements exist in the south and east Mediterranean.

The Protected Area systems of the constituent countries are generally well developed although not fully representative in terms of ecological and biodiversity coverage. Management effectiveness varies considerably throughout the region. This disparity needs to be addressed if the areas are to fulfil their various objectives, notably biodiversity conservation.

Insufficient attention has been paid to ways of protecting the large number of threatened species that occur outside formally protected areas, e.g. through various forms of agreements on land use and management that would favour the survival of biodiversity. It will be important to implement the latest CBD policy on Other Effective area-based Conservation Measures (OECMs) and the integration of such areas within existing Protected Area systems, if much of the region's biodiversity is not to be lost over time.

The conservation and recovery of the large number of threatened species in the region has generally been inadequately implemented, even in countries with appropriate facilities in place. Too many countries rely excessively on the presence of threatened species in protected areas as a means of their conservation, without taking appropriate actions to ensure their survival and persistence by removing or containing the threats to which they are subjected.

In the eastern and southern regions, there is little tradition to undertake such species-targeted conservation actions. Very few recovery programmes have been initiated, although more are being planned. Few successful species reintroductions have been reported, and virtually none in the eastern and southern regions.

Considerable progress has been made in the ex situ conservation of plant material in genebanks, botanic garden living collections, and cell and tissue cultures, although there are still substantial gaps in the representation of threatened species.

Conservation efforts in the Mediterranean often suffer from poor enforcement, lack of adequate infrastructure and specialist staff, incomplete or even conflicting policy guidance, and a failure to take into account climate change and other aspects of global change in conservation strategies.

There is a lack of pan-Mediterranean regional-scale conservation policy instruments, strategies and planning. Much greater international coordination of both area-based and species-based conservation policies is needed.

References

ARIANOUTSOU, M. & VILÀ, M. 2012: Fire and invasive plant species in the Mediterranean Basin. – Isr. J. Ecol. Evol. 58 (special volume in memory of Zeev Naveh): 195-203.

BLACKMORE, S. & OLDFIELD, S. 2017: Plant Science and Practice. The role of botanic gardens. – Cambridge University Press, Cambridge.

BOU DAGHER KHARRAT, M. 2018: Species reintroduction programmed. P. 112. In VALDERRÁBANO, M., GIL, T., HEYWOOD, V., DE MONTMOLLIN, B. (eds), Conserving Wild Plants in the South and East Mediterranean Region. – IUCN Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

BRUNEL, S. (ed.) 2006: Invasive plants in Mediterranean type ecosystems of the world. In Proceedings, Environmental Encounters Series No, 59. – Council of Europe Publishing, Strasbourg. 438p. http://archives.eppo.org/MEETINGS/2005_meetings/workshop_invasive/Proceed-ings_Meze2005.pdf

—, SCHRADER, G., BRUNDU, G. & FRIED, G. 2010: Emerging invasive alien plants for the Mediterranean Basin. – Bulletin OEPP/EPPO Bulletin 40(2): 219-238.

CARDOSO, P. 2012: Habitats Directive species lists: urgent need of revision. – Insect Conserv. Diver. 5: 169-174

COX, L. & UNDERWOOD, E. C. 2011: The Importance of conserving biodiversity outside of Protected Areas in Mediterranean ecosystems. – PLoS One 6(1): e14508.

DI CASTRI, F., HANSEN, A. J. & DEBUSSCHE, M. (eds) 1990: Biological Invasions in Europe and the Mediterranean Basin. – Kluwer Academic Publishers, Dordrecht, NL.

EUROPEAN COMMUNITIES. 2008: LIFE and endangered plants: Conserving Europe's threatened flora. – Luxembourg.

EEA 2012: Protected areas in Europe — an overview. EEA Report No 5/2012. – Publications Office of the European Union, Luxembourg. http://http://www.eea.europa.eu/publications/protected-areas-in-europe-2012

EEC. 1992: Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. – Journal L 206, 22/07/1992 P. 0007 - 0050

FOIS, M., COGONI, D., FENU, G. & BACCHETTA, G. 2018: *In situ* conservation at species/population level. Pp.108-111. In: VALDERRÁBANO, M., GIL, T., HEYWOOD, V., MONTMOLLIN, B. DE (eds). Conserving Wild Plants in the South and East Mediterranean Region. – IUCN Gland and Málaga.
https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

HAWKES, J. G. 1995: Centers of origin for agricultural diversity in the Mediterranean: from Vavilov to the present day. – Diversity 11: 108-111.

HEYWOOD, V. H. 1995: The Mediterranean flora in the context of world biodiversity. – Ecol. Mediterr. 20: 11-18.

— 2008: Challenges of in situ conservation of crop wild relatives. – Turkish J. Bot. 32: 421-432.

— 2009: The impacts of climate change on plant species in Europe. Final Version. Report prepared by Professor Vernon Heywood School of Biological Sciences, University of Reading with contributions by Dr ALASTAIR CULHAM. Convention on the conservation of European wildlife and natural habitats - 29th meeting of the Standing Committee - Bern, 23-26 November 2009. – T-PVS/Inf (2009)9E.

— 2012: Chapter III. The impacts of climate change on plant species in Europe. Pp. 95-244. In Biodiversity and climate change: Reports and guidance developed under the Bern Convention –Volume II (Nature and Environment N°160).

— 2014: An overview of *in situ* conservation of plant species in the Mediterranean. – Fl. Medit. 24: 5-24.

— 2015: Mediterranean botanic gardens and the introduction and conservation of plant diversity. – Fl. Medit. 25 (Special Issue): 103-114 doi: 10.7320/FlMedit25SI.103

— 2015a: *In situ* conservation of plant species – an unattainable goal? – Isr. J. Plant Sci. 63: 211-231.

— 2017: Plant conservation in the Anthropocene – Challenges and future Prospects. – Plant Diversity 39: 314-330.

— 2018: Conserving plants within and beyond protected areas – still problematic and future uncertain. – Plant Diversity, https://doi.org/10.1016/j.pld.2018.10.001

—, SHAW, K., HARVEY-BROWN, Y. & SMITH, P. (eds) 2018: BGCI and IABG's Species Recovery Manual. – Botanic Gardens Conservation International, Richmond.

HOFFMANN, S., BEIERKUHNLEIN, C., FIELD, R., PROVENZALE, A. & CHIARUCCI, A. 2018: Uniqueness of Protected Areas for Conservation Strategies in the European Union. Sci Rep. 8(1): 1-14.

KADIS, C., THANOS, C.A. & LAGUNA LUMBRERAS, E. (eds) 2013: Plant Micro-Reserves: From Theory to Practice. Experiences Gained from EU Life and Other Related Projects. – Utopia Publishing, Athens.

KAMAL, K., GRODZIŃSKA-JURCZAK, M. & BROWN, G. 2015: Conservation on private land: a review of global strategies with a proposed classification system – J. Environ. Plan. Manag. 58: 576-597.

KATI, V., HOVARDAS, T., DIETERICH, M., IBISCH, P. L., MIHOK, B. & SELVA, N. 2015: The challenge of implementing the European network of protected areas Natura 2000. – Conserv. Biol. 29: 260-270.

LAGUNA, E. 2018: Plant micro-reserves in the Mediterranean area. In: VALDERRÁBANO, M., GIL, T., HEYWOOD, V., DE MONTMOLLIN, B. (eds). Conserving Wild Plants in the South and East Mediterranean Region. Pp.106-107. – IUCN, Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

MACKEY, B. G. WATSON, J. E. M., HOPE, G. & GILMORE, S. 2008: Climate change, biodiversity conservation, and the role of protected areas: An Australian perspective – Biodiversity 9: 11-18.

MAXTED, N., MAGOS BREHM, J. & KELL, S. 2018: Genetic conservation of crop wild relatives. In: VALDERRÁBANO, M., GIL, T., HEYWOOD, V., DE MONTMOLLIN, B. (eds) 2018: Conserving Wild Plants in the South and East Mediterranean Region. Pp.113-114. – IUCN, Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

MÉDAIL, F. & QUÉZEL, P. 1999: Biodiversity hotspots in the Mediterranean basin: setting global conservation priorities. – Conserv. Biol. 13: 1510-1513.

MITTERMEIER, R. A., MYERS, N., GIL, P. R. & MITTERMEIER, C. G. 1999: Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions. – Cemex, Conservation International and Agrupación Sierra Madre, Monterey, Mexico.

ONATE, J. J., MALO, J.E., SUAREZ, F. & PECO, B. 1998: Regional and environmental aspects in the implementation of Spanish agri-environmental schemes. – J. Environ. Manage 52: 227-240.

OZINGA, W. A. & SCHAMINÉE, J. H. J. (eds) 2005: Target species – Species of European concern. A database driven selection of plant and animal species for the implementation of the Pan European Ecological Network. – Alterra-report 1119. Alterra, Wageningen.

PORCEDDU, M. & BACCHETTA, G. 2018: *Ex situ* conservation of Mediterranean vascular flora. Pp. 115-118. In VALDERRÁBANO, M., GIL, T., HEYWOOD, V., DE MONTMOLLIN, B. (eds).

Conserving Wild Plants in the South and East Mediterranean Region. – IUCN Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

Radford, E. A., Catullo, G., Montmollin, B. de (eds) 2011: Important Plant Areas of the south and east Mediterranean region: priority sites for conservation. – IUCN, Gland and Málaga.

Rivière, S. & Müller, J. V. 2017: Contribution of seed banks across Europe towards the 2020 Global Strategy for Plant Conservation targets, assessed through the ENSCONET database'. Oryx 52: 464-470. doi:10.1017/S0030605316001496

—, Breman, E., Kiehn, M., Carta, J. A. & Müller, J. V. 2018: How to meet the 2020 GSPC target 8 in Europe: priority-setting for seed banking of native threatened plants. – Biodivers. Conserv. 27: 1873-1890.

Talhouk, S. N., Abunnasr, Y., Hall, M., Miller, T. & Seif, A. 2014: Ancillary botanic gardens in Lebanon - Empowering local contributions to plant conservation – Sibbaldia: The Journal of Botanic Garden Horticulture 2: 111-129.

—, —, Forrest, A. & Miller, T. 2018: Ancillary botanic gardens. P. 119. In: Valderrábano, M., Gil, T., Heywood, V., de Montmollin, B. (eds) 2018. Conserving Wild Plants in the South and East Mediterranean Region. – IUCN, Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

Valderrábano, M., Gil, T., Heywood, V., Montmollin, B. de (eds) 2018: Conserving Wild Plants in the South and East Mediterranean Region. – IUCN, Gland and Málaga. https://portals.iucn.org/library/sites/library/files/documents/2018-048-En.pdf

Zeder, M. A. 2008: Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and Impact. – Proceedings of the National Academy of Sciences 105: 11597-11604.

---

[i] Chape & al. (2008) give a figure of 10.02% but there has been a substantial increase in land under protection since that figure was published.

[ii] According to the Report in the Earth Negotiations Bulletin (ENB) Volume 09 Number 725 | Sunday, 2 December 2018 Summary of the UN Biodiversity Conference 13-29 November 2018 Sharm El-Sheikh, Egypt. IISD Reporting Services iisd-rs@iisd.org (accessed 1 December 2018)

Michiel’s Codice erbario (c. 1550) and the flora of Greece

HANS WALTER LACK

Abstract

Lack, H. W. 2019: Michiel’s Codice erbario (c. 1550) and the flora of Greece. – Bot. Chron. 22: 63-72.

Michiel’s Codice erbario is a botanical manuscript written in Italian by the Venetian patrician Pietro Antonio Michiel (1510 - 1577). It is conserved in the Biblioteca Marciana in Venice and illuminated with numerous plant illustrations, of which many are by Domenico delle Greche († c. 1558). 67 of the images in this manuscript are associated with locality information referring to present day Greece, although often with more than one place name. Among the more remark-able plants reported is *Bongardia chrysogonum* from Crete, a tuberous plant which had possibly been introduced with early agriculture but never found again on this island. Four xenophytes are depicted in the manuscript, i.e. *Aeonium arboreum*, *Solanum melongena*, *Trigonella foenum-graecum* and, surprisingly, *Aeschynomene indica*. The latter is a legume occurring widely in tropical Africa, Asia, Australia and northern America. It has been first reported for Greece in 2014, and more particularly from rice fields in the north-eastern part of the country, while Mich-iel’s report refers to Crete and dates from the mid-sixteenth century. From the appended notes it seems very likely that Michiel had *Aeschynomene indica* and several other plants cultivated in his private garden on San Trovaso Island in Venice.

Prof. Dr. H. Walter Lack, Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Königin-Luise-Str. 6-8, 14195 Berlin, Germany. E-mail: h.w.lack@bgbm.org

Key words: Flora of Greece, 16th century botanical illustrations, Pietro Antonio Michiel, Marciana, *Aeschynomene indica*.

Introduction

In the mid-sixteenth century almost all of mainland Greece belonged to the Ottoman Empire and was ruled over by the sultans in Istanbul. By contrast Crete, the Ionian Islands (except Lefkas but including the fortress of Parga on the mainland) and many of the Aegean Islands formed, together with other territories like Istria and Cyprus, the Stato da Mar. It formed a part of the Serenissima Repubblica di San Marco, the Venetian Republic, and was headed by the doges based in Venice. Other names for the Stato da Mar were Domini da Mar or simply Venezia Oltramare, the latter name because all these territories were seen from Venice to be on the other side of the Adriatic, hence ‘oltramare’. In Greece the Venetian rule was and is called ‘venetokratia’ in contrast to the ‘tourkokratia’ which reigned on the mainland.

As a consequence, western knowledge on the flora of this region was largely, though not exclusively, accumulated in the centres of learning of the Serenissima, notably Padua and in the capital itself, i.e. Venice (LACK 1996, 2017). In Padua an extremely old botanical garden exists belonging to the Serenissima's only university and administered by a long and uninterrupted series of prefects though its founding charter is only dated 1545 (MINELLI 1995). By contrast, in Venice a considerable number of important botanical works were published, e. g. Prospero Alpini's posthumous De plantis exoticis, Venetiis [Venice], 1627 (TURLAND 1995), which contains the first descriptions and illustrations of many plants endemic to Crete, e.g. the very distinctive *Verbascum spinosum* L. More than half a century earlier the work on a manuscript written in Italian and illuminated with numerous plant illustrations came to an end in Venice – the so-called Codice Erbario of Pietro Antonio Michiel. It is kept in the Biblioteca Nazionale Marciana in Venice (shelf mark Marc. It., cl. II, 26-30 (= 4860-4864)) and was published with ample comments as recently as 1940 (DE TONI 1940), albeit only with a small selection of its numerous illustrations.

During a visit to the Marciana the present author has studied this manuscript and was able to confirm many of De Toni's determinations for the plant illustrations dealt with in the present paper; in case a new identifation differs, this is specifically noted as 'det. HWL'. The nomenclature used follows 'Vascular Plants of Greece. An annotated checklist' (DIMOPOULOS & al. 2013), information on the distribution of the various taxa is based on that work. All quotations from the Codice Erbario are taken verbatim from the transcript (DE TONI 1940) of Michiel's text originally written in reversed characters without diacritical signs.

Although the Codice Erbario has recently been discussed and set into context by several cultural historians (AMBROSOLI 1992, TOSI 2016, EGMOND 2017) so far only the illustrations of plants native in the Americas and the pertinent texts have been dealt with in detail (PARDO-TOMÁS 2006). The present contribution focuses on Michiel's images, descriptions and notes in the Codice Erbario that refer to plants recorded from what is now Greece. However, it does not offer a comprehensive treatment of all relevant entries, but is rather intended to attract the botanical community's attention to this remarkable manuscript.

Pietro Antonio Michiel and Domenico dalle Greche

As a member of one of Venice's twelve apostolic families, the so-called vecchie case, Pietro Antonio Michiel (1510-1575) belonged to the innermost political, cultural and social circle of the Serenissima1. In the eleventh and twelfth centuries no less than three doges had come from his family and, late in his life, Michiel wrote a letter of dedication to dogaressa Vincenza Loredana Marcello-Mocenigo († 1572), the learned wife of Alvise I Mocenigo (1507-1577), the 85th doge (DE TONI 1940). She was possibly intended by Michiel as the patroness for a planned publication of his writings which however never materialized.

According to Pietro Antonio Michiel's latest biographer (MINELLI 2011), very little is known about the life of this Venetian patrician, apart from the fact that he was

---

[1] More complete references on Michiel and on the general background of the *Codice Erbario* have recently been provided (Pardo-Tomás 2006).

asked by the Riformatori dello studio, the Serenissima's supervisory authority for Padua University, to take care of its newly founded botanical garden in the years 1551-1556 (PARDO-TOMÁS 2006). At that time Luigi Anguillara, also called Squalermo (c. 1512-1570), the first prefect (TREVISAN 1995), was in office, who later published his famous Semplici, liquali in più pareri à diversi huomini scritti appaiono, Vinegia [Venice], 1561 (on its woodcuts, see LACK 2000). To the historian of plant taxonomy, Michiel is known mainly for his Codice Erbario which is not - as the name might suggest - a herbarium in book form but an illuminated manuscript bound in five volumes. By convention, these have not been numbered but are referred to, in agreement with the colour of their binding, as libro azzurro, libro giallo, libro rosso 1, libro rosso 2 and libro verde.

Michiel, clearly a dilettante and at the same time a self-promoted patron (PARDO-TOMÁS 2006), owned a garden in Venice (not in Vicenza as given in LACK 1996) called by him 'mio giardineto' [my little garden] (DE TONI 1940: 47). It was situated in the parish of San Gervasio e Protasio, vulgo San Trovaso (DE TONI 1910, PARDO-TOMÁS 2006, BOSCARO 2016), in the Dorsoduro district, and survives today only in fragments. The largest fragment belongs to the Palazzo Brandolin Belbo at Dorsoduro 1075, now a building of Venice University, and can be visited passing through a small gate adjacent to the palace, to be reached from the Fondamenta Sangiantoffetti (ANON. 2016). The precise size of Michiel's garden is unknown, but it was described as 'vaghissimo giardino' [very large garden] (DE TONI 1910) and seems to have covered a major part of San Trovaso island. Although often called a botanical garden, this is definitely incorrect – Michiel's garden was privately owned and not open to the public, unlike the Botanical Garden of Padua University. As a consequence, Ulisse Aldrovandi (1522-1605), who was several years later the first person to supervise the newly founded Botanical Garden of Bologna University, was denied access to Michiel's garden when he wished to visit it in 1552 (MINELLI 2011). However, later the two botanists started a correspondence which continued for several years (MINELLI 2011).

While the handwriting in the five volumes is homogeneous and has been consistently attributed to Michiel, the plant illustrations are heterogeneous 2, painted on different papers (MARCON 1988), and definitely not by a single hand (DE TONI 1940: 691). However, Michiel mentioned by name only Domenico dalle Greche († c. 1558) as his plant illustrator. The latter had possibly been born in Modone [Methoni in present-day Greece] and was active as a painter, graphical artist and probably also as a publisher in Venice (DILLON 1986), known for e.g. his woodcut Sommersione del Faraone nel Mar Rosso and his work for Michiel. The names of the other illustrators involved remain unknown. One of them associated his plant images with story-telling figures, such as a representation of *Styrax officinalis* L. together with a young man holding an incense burner in his left hand and standing near the statue of a saint (DE TONI 1940: 67, EGMOND 2017: Fig. 56). This is clearly a reference to the use of the resin collected from this tree which was reported by Michiel from, among other places, Crete.

---

[2] No attempt is made here to comment on the depiction and description of the various plants regraded as fictitious, dubious or incomplete in the *Codice Erbario* (DETONI 1940).

Michiel's Codice Erbario

Michiel's Codice erbario is undated, but it is safe to assume that it was written and illustrated in the mid-sixteenth century. The dates between 1553 and c. 1570 have recently been given (PARDO-TOMÁS 2006), although it has been convincingly shown that at least the representation of *Gossypium arboreum* L. (rev. HWL; libro rosso 1: No. 203) must have been prepared in 1541 at the latest (BAUMANN & al. 2001). After the death of Michiel the manuscript remained in the family, was later bought by Giovanni Marsigli (1727-1795), prefect of the Botanic Garden of Padua University in 1760-1794 (CASADORO 1995), and subsequently acquired at an auction by Giuseppe Antonio Bonato (1753-1836), prefect of that garden in 1794-1835 (PAGANELLI 1995). The latter donated the five volumes to the Bibliotheca Pubblica di San Marco in Venice in 1796 (MARCON 1988, PLEBANI 2018).

Michiel's great work possesses a straightforward layout – the plant illustrations are painted on the recto pages, the text referring to them is written on the verso pages. In addition, the text for each entry is also clearly structured under a maximum of ten headings – (1) 'nomi', (2) 'genera', (3) 'forma', (4) luogho', (5) 'tempo', (6) 'amano' [sic], (7) 'generatione' [sic], (8) 'oppinioni' [sic], (9) 'dispareri' and (10) 'virtù'. In this respect, though not in the layout, Michiel's work is similar to the Codex Fuchs, another illuminated botanical manuscript dated c. 1550 which is kept in the Österreichische Nationalbibliothek n Vienna (shelf mark Cod. 11117-11125; BAUMANN & al. 2001, LACK 2016). Here its author, Leonhart Fuchs (1501-1566), followed the same sequence of headings called by him (1) 'nomina', (2) 'genera', (3) 'forma', (4) 'locus', (5) 'tempus', (6) temperamentum', (7) 'vires' and (8) 'appendix', but instead of in Italian, Fuchs wrote his texts in Latin. While the different entries are arranged in the Codex Fuchs alphabetically according to the Greek plant names, Michiel used life forms as his main criterion, with e.g. the trees and arborescent species mostly placed in the libro azzurro.

In our context the information provided by Michiel under the heading 'luogo' is of relevance, i.e. the provenance or presumed provenance of the plant depicted and described. Just as in the Codex Fuchs (BAUMANN & al. 2001), plants native in the four continents that were then known are represented, among them a considerable number from the Stato da Mar, and more particularly from the region that now forms Greece. For this, the following geographical terms have been used by Michiel: Arcadia, Athene [Athens], Candia [Crete], Cephalonia or Collophone Isola [Kefallinia], Chatananza [?], Chio or Scio [Chios], Ciclade [Cyclades], Corfu or Corphu, Cythnos [Kythnos], Gretia or Grecia [Greece, then a rather ill-defined area], Mesenia [Messenia], Morea [Peloponnese], Negroponte [Evvia], Peloponense [Peloponnese], Rhodi [Rhodes], Thasso [Thasos], Thesalia [Thessaly], and Zante [Zakinthos]. For localities almost certainly taken from the literature see the chapter on Michiel's garden.

Plants native in Greece depicted and described in Michiel's Codice Erbario

The Codice Erbario contains the representation of 1028 plants; of them c. 730 can be determined without doubt (PARDO-TOMÁS 2006), and only these are dealt with here. Also, all those entries are excluded from this paper in which Michiel expressed doubt on their origins. Of those remaining, 67 are associated with locality information referring to modern Greece.

It should be noted, however, that in most cases Michiel indicated more than one locality per entry, e.g. for the wide-spread *Pistacia lentiscus* L. we read 'nelle mareme de il Mare Tireno verso Napoli, Siena, et Romagnia ancora, Chio, Candia, Ponto' [in the coastal swampy area along the Tyrrhenian Sea, towards Naples, Siena, also in Romagna, Chios, Crete, the Pontus region] (DE TONI 1940: 76). Similarly, provenance of *Pallenis spinosa* (L.) Cass. is given as 'nel Bassanese se ne ritrovano, in Grecia et nel Peloponense, al Zante, et in Athene' [in the region of Bassano del Grappa we find it, in Greece and on the Peloponnese, in Zakynthos and in Athens] (DE TONI 1940: 164). Similar information, though differing in detail, is provided for several common and wide-spread Mediterranean species, among them *Anagyris foetida* L. (DE TONI 1940: 43), *Cardopathium corymbosum* (L.) Pers. (DE TONI 1940: 166), *Euphorbia apios* L. (De Toni 1940: 115), *E. dendroides* L. (DE TONI 1940: 89), *Inula candida* L. (DE TONI 1940: 372), *Quercus coccifera* L. (DE TONI 1940: 190), *Satureja thymbra* L. (DE TONI 1940: 217) and *Smyrnium perfoliatum* L. (DE TONI 1940: 553). In some cases the information provided by Michiel is relatively accurate by modern standards, e.g. for *Hypericum hircinum* L. he noted 'solamente nasce in Candia dice Dioscoride et nel Peloponeso' [grows only in Crete, as stated by Dioscorides, and in the Peloponnese] (DE TONI 1940: 61), as Michiel ignored the localities on the central and East Aegean islands.

The number of plant illustrations associated with a single provenance from what is now Greece is much smaller, i.e. *Achillea maritima* (L.) Ehrend. & Y. P. Guo (rev. HWL; DE TONI 1940: 486), *Ballota pseudodictamnus* (L.) Benth. (DE TONI 1940: 349) and *Origanum dictamnus* L. (DE TONI 1940: 348), all given for 'Candia' [Crete]; only the last of these species is endemic to that island. More remarkable is Michiel's note on the rare *Bongardia chrysogonum* (L.) Spach: 'fu portato di Candia sanza [sic] nome con delle radici di lentopetalon insieme' [imported without a name from Crete, together with the roots of *Leontice leontopetalum* L.] (DE TONI 1940: 128). In Greece both species grow as tuberous weeds of traditionally managed fields (A. STRID, pers. comm., 2018) having possibly been introduced with early agriculture (KARL & STRID 2009). Although *B. chrysogonum* has never again been recorded to occur in Crete, it is possible that it once grew there but due to the decline of traditional agriculture has disappeared (N. TURLAND, pers. comm., 2018). By contrast *L. leontopetalum* L., a species with apparently similar ecological requirements, has repeatedly been recorded from Crete (RECHINGER 1944a & b, KARL & STRID 2009). In short, Michiel's record of *B. chrysogonum* for this island is credible. By contrast, the report of *Althaea cannabina* L. from the Peloponnese is definitely erroneous (DE TONI 1940: 297), as the species is not known to occur in that region.

Other information provided by Michiel on provenances supposedly from modern Greece are definitely incorrect, such as associating an illustration showing *Calluna vulgaris* (L.) Hull, *Erica carnea* L. and *Empetrum nigrum* L. (libro azzurro 140) with the note 'in … Gretia sonno il suo proprio' [they are native … in Greece] (DE TONI 1940: 99). On the same line are several more incorrect records, among them *Betula alba* L., *Genista* cf. *aspalathoides*, given among other localities by Michiel for Rhodes, *Pulsatilla* alpina (L.) Delabre and *Myrrhis odorata* (L.) Scop., the latter said to occur on the Peloponnese. In a few cases the provenance given by Michiel had obviously been taken from the literature, like ‘in isola detta Cytiso et poi nelle Isole Ciclade’ [on the island of Kythnos and then on the Cyclades] for *Melilotus officinalis* L., a statement cited from a Dioscorides edition published in Florence in 1518 and not from observation (De Toni 1940: 266). Quite naturally the identity of a few plants depicted remains uncertain, in particular those belonging to ‘difficult’ families like Apiaceae, an example being the plant identified as *Ligusticum peloponnense* Ten. [i.e. *Molopospermum peloponnesiacum* (L.) W. D. J. Koch] (De Toni 1940: 504) said to occur ‘nelle coline del Peloponense’ [on the hills of the Peloponnese].

The plants depicted and described in the Codice Erbario are only rarely correlated with herbarium specimens, those found are now kept separate from the manuscript (Marcon 1988). In the libro azzurro a small branch identified as *Convolvolus scammonia* L. has been located, a species reported by Michiel to occur ‘in Soria, in Candia et per Levante’ [in Syria, in Crete and throughout the Levant] (De Toni 1940: 25; image of Michiel’s illustration: Cappelletti 1995: 170).

Plants introduced to Greece depicted and described in Michiel’s Codice Erbario

Considering early plant transfers in the Mediterranean area like that of *Euphorbia resinifera* Berg illustrated in the Codex Neapolitanus (c. 600 AD; p. CXLIX; kept in the Biblioteca Nazionale di Napoli) (Lawant & Winthagen 2001), it is not astonishing that at least four of the plant species associated by Michiel with what is now Greece are xenophytes in the sense of the Vascular plants of Greece (Dimopoulos & al. 2013). The first of these is *Aeonium arboreum* (L.) Webb & Berth., a native of the Canary Islands, for which Michiel gives as provenance ‘ne muri e tetti in Levante. Et io l’hebbi da Corfu’ [on the walls and roofs of the Levant. And I have it from Corfu] (De Toni 1940: 573). Michiel’s contacts to Anguillara are testified by the fact that this very species has also been described and illustrated in the latter’s Semplici (Lack 2000). The second xenophyte is *Trigonella foenum-graecum* L., the third *Solanum melongena* L. reported by Michiel to occur ‘in Candia dalli frati zoocolanti’ [in Crete from the Franciscan order] (De Toni 1940: 175); it seems possible that Michiel had this species cultivated in his garden (see below) although there is no hard evidence for this in his notes.

While the occurrence of *A. arboreum* and *T. foenum-graecum* in the Mediterranean area were already documented in the famous Codex Aniciae Julianae or Codex Vindobonensis (dated before 512 AD; f. 12 v, 335 r; kept in the Österreichische Nationalbibliothek) (Mazal 1998), the plant depicted and described in libro rosso 1, No. 199 is a great surprise. The image shows *Aeschynoneme indica* L., a legume occurring widely in tropical Africa, Asia, Australia and northern America (Gillett & al. 1967). De Toni’s comment ‘la figura precede quella che diede il Rheede … sotto il nome Neli-tali’ [the figure precedes the one given by Rheede … under the name Neli-tali] (De Toni 1940: 316) is an explicit reference to Rheede’s Hortus Malabaricus 9: t. 19, the lectotype of the name *Aeschynoneme indica* L. (Gillett & al. 1967, Jarvis 2007). Michiel notes ‘Io l’hebbi di Candia ne in altro ne ho veduta’ [I have it from Crete and have not seen it anywhere else].

Aeschynomene indica seems to have never again been recorded in Crete and is neither listed in Flora Aegaea and its supplements (Rechinger 1944a & b 1951) nor in Vascular Plants of Greece (DIMOPOULOS & al. 2013) or in the pertinent data bank (http://portal.cybertaxonomy.org/flora-greece/). However, this legume has very recently been found in and near rice fields in the Nomos & Eparchia Serron in north-eastern Greece and is regarded as 'well-established and locally naturalized [having been] observed for the past four years in cultivated fields' (TSIALTAS & TAN 2014). Nothing seems to exclude the possibility that such an introduction had already happened more than 450 years ago in Crete. The Codice Erbario also contains the illustrations and descriptions of xenophytes with a similarly wide distribution, among them *Colocasia esculenta* L., reported by Michiel from three continents – Europe (Sicily), Africa (Cyprus and Egypt) and Asia (unspecified) (DE TONI 1940: 131).

Michiel's garden in Venice

For *Aeschynomene indica* Michiel notes 'suoi fiori di Agosto ma non mi fezze [fece] silique‘ [it flowers in August but did not make me legumes‘ (DE TONI 1940: 316). This seems to indicate that Michiel had this plant in cultivation in his garden. In other examples he is more explicit, e.g. in his writing of *Platanus orientalis* L., then a rare tree in cultivation, '… in Candia ne sono in quantita, et io ne ho mantenuto uno nel mio giardineto' [in Crete there are many of them, and I have kept one in my little garden'] (DE TONI 1940: 47). The following observations could only have been made from a living specimen: 'io l'ho tenuta de molti anni ne mai mi ha multiplicato per suoe radici, ne manco ho veduto il suo seme, ma con li rami posati in terra ne ho fatto quante ho voluto' [I have kept it for many years, but it never multiplied by its roots, nor have I seen its seed, but with the branches laid on the soil, I made as many as I wished] (DE TONI 1940: 573). This note refers to *Aeonium arboreum* (see above), where Michiel even indicates that he had passed on living material to other gardens writing ''havendone date de molte piante per li giardini de semplici' [having given of it many plants for the medicinal gardens] (DE TONI 1940: 573). In a similar way Michiel noted for *Nerium oleander* L. 'In Candia ancora et de il bianco dunde io l'hebbi dal Ramusio et de una pianta io ho impito tutti li giardini de Italia' [Also in Crete, and the white [variant] which I have from Ramusio and from one specimen I have filled all gardens in Italy] (DE TONI 1940: 69). Here Michiel refers to the diplomate Giovanni Battista Ramusio (1485-1557), a collaborator of Alvise I Mocenigo, secretary of the Serenissima's Consiglio dei dieci and editor of Delle Navigationi e viaggi (DONATTINI 2016).

However, in many other cases there is no explicit indication that the respective specimen has been cultivated in Michiel's garden, which may imply that at least some of the plants depicted and described possess a different background. For *Pistia stratiotes* L., for example, we find the note 'et io hebbi questa secha' [and I have this plant as a dried specimen] (DE TONI 1940: 287). Similarly, at least some of the illustrations documenting plants native in the Americas, like *Theobroma cacao* L. and *Guaiacum officinale* L., have definitely not been based on specimens cultivated in Michiel's garden (PARDO-TOMÁS 2006).

Other records are best associated with the writings of Anguillara, Dioscorides and others and not with cultivated material. The first part of the locality information given for *Helleborus niger* L. 'in Helicone monte in Boetia iusta a Thebe … monti aspri in

Italia ' [on Mount Helicon in Boeotia near Thebes … high mountains in Italy] (DE TONI 1940: 560), for example, was almost certainly directly taken from Dioscorides, and the same applies to the provenance given for *Veratrum nigrum* L. and *V. album* L. (DE TONI 1940: 561) 'nel monte Oeta ... nel monte Parnaso … in Lombardia ne monti aspri' [on Mount Iti and Mount Parnassos … in Lombardy on the high mountains]. Since neither *Helleborus niger* (STRID 2002) nor *Veratrum album* (in the Codice Erbario surprisingly shown with yellowish tepals) have ever been recorded from what is now Greece (DIMOPOLOULOS & al. 2013) and *V. nigrum* never from such a southern Greek locality (DIMOPOLOUS & al. 2013) it is plausible to assume that in these cases the basis of Michiel's images were specimens gathered in what is now Italy.

Epilogue

Although mentioned by a few authors in the nineteenth century (e.g. BONATO 1851, VISIANI 1854), the Codice Erbario effectively became accessible to the scientific community only through the 1940 edition of the text (DE TONI 1940) which, however, reached only a tiny number of libraries outside Italy. As a consequence this source of botanical knowledge, available in Venice around 1550, remained largely unappreciated by the scientific community. For plants native in the Americas Michiel had made use of the official network of the Serenissima, notably the Venetian ambassadors at the courts of Charles V, Emperor of the Holy Roman Empire, and Philip II, King of Spain (PARDO-TOMÁS 2006). In a similar way Michiel was in contact with the Venetian ambassadors at the High Porte in Istanbul, traditionally called baili, who provided him with, e.g., specimens of *Quercus coccifera*, *Fritillaria imperialis* L., and *F. persica* L. (DE TONI 1940: 109, 156). By contrast, much less is known about Michiel's contacts in the Stato da Mar, where the Serenissima was represented by a long series of Provveditori. An additional source may have been Anguillara, who has been reported to have spent some time in Crete, possibly in Archanes (ANGUILLARA 1561, BALDACCI 1904, LACK 1996, 2017), while Pierre Bellon (1517-1564) definitely provided Michiel with seeds for his garden (DE TONI 1940: 35), although not from the area this paper is focused on.

In any case Michiel acted, together with the first prefects of the Botanic Garden of Padua University, as an important early source of botanical information, of plant illustrations and living specimens for the community of conoscenti on the Apennine peninsula and beyond. In short, he probably was at his time the most knowledgeable connoisseur of the flora of what is now Greece.

Acknowledgements

Thanks are due to J. Compton (Tilbury), A. Strid (Ørbæck) and N. Turland (Berlin), who kindly read a preliminary version of the manuscript and commented on it. Both Strid and Turland mentioned to the author the occurrence of *Bongardia chrysogonum* and *Leontice leontopetalum* in traditionally managed fields in Greece.

References

AMBROSOLI, M. 1992: Scienziati, contadini e proprietari: botanica e agricoltura nell'Europa occidentale 1350-1850. – Torino (= Biblioteca di cultura storica 190).

ANGUILLARA, L. 1561: Semplici, liquali in più pareri à diversi huomini scritti appaiono. – Vinegia.

ANON. 2016: Isola San Trovaso. – https://venezia118.wordpress.com/2016/02/11/isola-san-trovaso/ (accessed 16 July 2018).

BALDACCI, A. 1904: Le esplorazione botaniche nell'isola di Creta nei secoli XVI e XVII. – Atti Congr. Int. Sci. Storiche 10: 81-88.

BAUMANN, B., BAUMANN, H. & BAUMANN-SCHLEIHAUF, S. 2001: Die Kräuterbuchhandschrift des Leonhart Fuchs. – Stuttgart.

BONATO, G. A. 1851: Elogio dei Veneti promotori della scienza erbaria. – Padova.

BOSCARO, G. 2016: Giardini Veneziani scomparsi; l'Orto Botanico Michiel a San Trovaso. – http://theliquidpress.com/giardiniveneziani-scomparsilorto-botanico-pietro-antonio-michiel-san-trovaso/ (accessed 16 July 2018).

CAPPELLETTI, E. M. 1995: Le piante coltivate nell' orto botanico di Padova al tempo di Luigi Squalermo detto Anguillara. Pp. 163-272. In MINELLI, A. (ed.), L'orto botanico di Padova, 1545-1995. – Venezia.

CASADORO, G. 1995: Giovanni Marsigli. Pp. 93-97. In MINELLI, A. (ed.), L' orto botanico di Padova, 1545-1995. – Venezia.

DE TONI, E. 1910: Luigi Anguillara e Pietro Antonio Michiel. – Ann. Bot. (Roma) 8: 617-685.

— 1940: Pietro Antonio Michiel. I cinque libri di piante. Codice Marciano. Trascrizione e commento. – Venezia.

DILLON, G. 1986: Dalle Greche, Domenico. Pp. 99-101. In Dizionario biografico degli Italiani 32. – Roma.

DIMOPOULOS, P., RAUS, T., BERGMEIER, E., CONSTANTINIDIS, T., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. (eds) 2013: Vascular plants of Greece. An annotated checklist. – Englera 31, Berlin.

DONATTINI, M. 2016: Ramusio (Ramnusio, Ramnusius, Rhamnusius, Rhamesius), Giovanni Battista. Pp. 359-365. In Dizionario biografico degli Italiani 86. – Roma.

EGMOND, F. 2017: Eye for detail. Images of plants and animals in art and science 1500-1630. – London.

GILLETT, J. B., POLHILL, R. M. & VERDCOURT, B. 1967: Flora of tropical East Africa. Leguminosae (Part 3) Subfamily Papilionoïdeae. – London.

JARVIS, C. 2007: Order out of chaos. Linnaean plant names and their types. – London.

KARL, R. & STRID, A. 2009: *Bongardia chrysogonum* (Berberidaceae) rediscovered on the East Aegean island of Chios. – Phytol. Balcan. 15: 337-342.

LACK, H. W. 1996: Die frühe botanische Erforschung der Insel Kreta. – Ann. Naturhist. Mus. Wien 98 B: 183-236.

— 2000: An early woodcut of a plant collected in Greece. – Bot. Chron. 13: 249-254.

— 2016: A garden Eden / Ein Garten Eden / Un jardin d'Éden, ed. 3. – Köln.

— 2017: Padua und die botanische Erforschung des Stato da Mar und Ägyptens von den Anfängen bis Carl von Linné (1753). Pp. 97-108. In ENGELHARDT, D. V. & FRIGO, G. F. (eds), Padua als europäisches Wissenschaftszentrum von der Renaissance bis zur Aufklärung. – Aachen (= ENGELHARDT, D. V., KÄSTNER, I., KIEFER, J. & REICH, K. (eds), Europäische Wissenschaftsbeziehungen 12).

LAWANT, P. & WINTHAGEN, D. 2001: *Euphorbia resinifera* portrayed in a manuscript herbal nearly fifteen hundred years ago. – Bradleya 19: 3-14.

MARCON, S. 1988: 75. Pier Antonio Michiel. Erbario o Istoria generale delle piante. Pp. 157-159. In Anon., Di sana pianta. Erbari e taccuini di sanità. Le radici storiche della nuova farmacologia. – Modena.

MAZAL, O. 1998: Der Wiener Dioskurides. Codex medicus graecus 1 der Österreichischen Nationalbibliothek 1. – Graz [= Glanzlichter der Buchkunst 8(1)].

MINELLI, A. (ed.) 1995: L'orto botanico di Padova 1545-1995. – Venezia.

— 2011: Michiel, Pietro Antonio. Pp. 325-326. In Dizionario biografico degli Italiani 74. – Roma.

PAGANELLI, P. 1995: Giuseppe Antonio Bonato. Pp. 97-107. In MINELLI, A. (ed.), L'orto botanico di Padova 1545 – 1995. – Venezia.

PARDO-TOMÁS, J. 2006: Tra "oppinioni" e "dispareri": La flora americana nell'erbario di Pier' Antonio Michiel (1510-1576). – Accad. Naz. Virgiliana Sci. Misc. 16: 73-100.

PLEBANI, T. 2018: Le disavventure di un erbario. – https://marciana.venezia.sbn.it/la-biblioteca/il-patrimonio/patrimonio-librario/i-libri-raccontano/le-disavventure-di-un-erbario (accessed 14 July 2018).

RECHINGER, K. H. 1944a: Flora Aegaea. Flora der Inseln und Halbinseln des Ägäischen Meeres. – Akad. Wiss. Wien., Math.-Naturwiss. Kl., Denkschr. 105(1).

— 1944b: Neue Beiträge zur Flora von Kreta. – Akad. Wiss. Wien, Math. -Naturwiss. Kl. Denkschr. 105(2,1).

— 1951: Phytogeographia aegaea. – Oesterr. Akad. Wiss., Math.- Naturwiss. Kl., Denkschr. 105(2,2).

STRID, A. 2002: *Helleborus* L. Pp. 4-5. In PHITOS, D., STRID, A. & SNOGERUP, S. (eds), Flora Hellenica 2. – Ruggell.

TOSI, A. 2016: Botanical illustrations and the idea of the garden in the sixteenth century between imitation and imagination. Pp. 183-210. In FISCHER, H., REMMERT, V. R. & WOLSCHKE-BULMAHN, J. (eds), Gardens, knowledge and the sciences in the early modern period. – Sine loco [Basel].

TREVISAN, R. 1995: Luigi Anguillara. Pp. 37-39. In MINELLI, A. (ed.), L'orto botanico di Padova 1545-1995. – Venezia.

TSIALTAS, I. & TAN, K. 2014: Reports 240-241. P. 301. In VLADIMIROV, V., DANE, F., MATEVSKI, V. & TAN, K. (eds), New floristic records in the Balkans; 25. – Phytol. Balcan. 20: 267-310.

TURLAND, N. 1995: Linnaeus's interpretation of Prospero Alpini's De plantis exoticis. With special emphasis on the flora of Crete. – Bull. Nat. Hist. Mus. (Bot.) 25: 127-159.

VISIANI, R. de 1854: Delle benemerenze de' Veneti nella botanica. – Atti Reale Ist. Veneto Sc. Lettere Arti 2: 63-101.

A checklist of Turkish *Allium* species, with taxonomic assessments: *Allium* sect. *Allium*, sect. *Codonoprasum* and sect. *Scorodon*

NERIMAN ÖZHATAY & MINE KOÇYIĞIT

Abstract

Özhatay N. & Koçyiğit M. 2019: A checklist of Turkish *Allium* species, with taxonomic assessments: *Allium* sect. *Allium*, sect. *Codonoprasum* and sect. *Codonoprasum* and sect. *Scorodon*. – Bot. Chron. 22: 73-85.

The check-lists of three sections of *Allium* (sect. *Allium*, sect. *Codonoprasum*., sect. *Scorodon*) naturally growing in Turkey is presented: *Allium* sect. *Allium*, with 77 taxa, 34 of them endemic; *Allium* sect. *Codonoprasum*, 40 species, 12 endemic; and *Allium* sect. *Scorodon*, 36 species, 25 endemic. The taxonomic status of the following taxa is changed: *A. minus* spec./ nom. nov. and *A. tauricum* var. *pilosum*, comb. & stat. nov. Colour photographs of representatives of each section and all newly named taxa are provided. Additionally, distribution maps of the en-demic taxa are shown.

N. Ozhatay, Department of Pharmaceutical Botany, Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, Cyprus.
M. Koçyiğit, Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34116 Beyazit, Istanbul, Turkey.

Key words: Allium, Allium sect. *Allium, Allium* sect. *Codonoprasum*, *Allium* sect. *Scorodon*, names at new rank, Turkey.

Introduction

Allium L. is the largest genus of petaloid monocots, comprising ca. 1200 taxa (GOVAERTS & al. 2018). This taxonomically difficult genus is distributed throughout the northern hemisphere, with main diversity centers in Mediterranean basin, SW Asia, and Central Asia.

In Turkey the genus is represented by ca. 225 taxa assigned to 15 sections, and it is the third most speciose vascular plant genus, after *Astragalus* and *Verbascum,* and is characterized by a high rate of endemic taxa. The number of wild *Allium* species in increasing every year due to the discovery and recognition of new taxa.

Turkey is one of the most botanically diverse temperate countries on Earth. The diversity of vascular plant diversity of the country has been documented in *Flora of Turkey and the Eastern Aegean Islands,* edited by P. H. DAVIS and published in nine volumes (DAVIS 1965-1985). After completion of this monumental Flora, many new taxa have been added to the Turkish flora. Checklists of additional taxa have been published in eight papers (I-VIII) by ÖZHATAY & al. 1994, 1999, ÖZHATAY & KÜLTÜR 2006, ÖZHATAY & al. 2009, 2011, 2013, 2017. Also, two supplement volumes to *Flora* *of Turkey and the East Aegeans*: vol. 10 (1988) and vol. 11 (2000), and *Türkiye Bitkileri Listesi (Damarlı Bitkiler)* (GÜNER & al. 2012) were published, as well as many PhD and master theses on Turkish *Allium* species. Some other papers were published after the last update (DUMAN & al. 2017), viz. FIRAT (2017), FIRAT & al. (2017), and ÖZHATAY & al. (2018). Altogether, 60 new *Allium* taxa were added to the Turkish flora after 1984: either taxa new to science, or new, additional country records (Fig. 1).

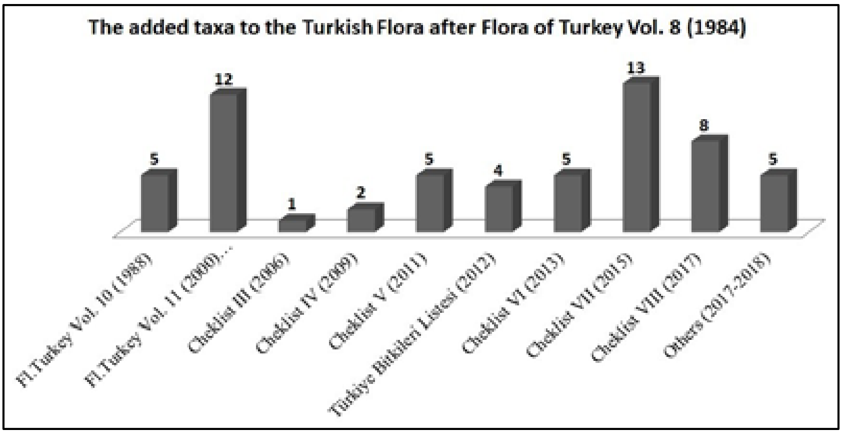


Fig. 1: Numbers of Allium taxa that were added to the Turkish Flora after the treatment by DAVIS & al. (1984).

Having surveyed all relevant literature and studied the herbarium specimens housed in ISTE, we prepared updated lists of Turkish species for 3 *Allium* sections. In the present paper, dedicated to Prof. Phitos, we present the known inventory of those three sections in Turkey. It appears that Turkey is the centre of diversity of *A.* sect. *Allium* and *A.* sect. *Scorodon*. In ISTE (the Herbarium of Faculty of Pharmacy, University Istanbul), nearly 3500 *Allium* specimens collected in Turkey are kept, including 34 type specimens.

Identification key for the Turkish taxa of *Allium* sect. *Allium*, sect. *Codonoprasum,* and sect. *Scorodon*

1.	Inner filaments 3-cuspidate (rarely 5-7 -cuspidate), proximally with a broad laminar portion, a median, anther-bearing cusp and two (to 6) usually longer, sterile lateral cusps; outer filaments simple (Fig. 2A)	**sect. *Allium***
–	All filaments simple, rarely the inner ones with two small lateral teeth at the base	**2.**
2.	One or both spathe valves excurrent into a long appendage, longer than the umbel (Fig. 2B)	**sect. *Codonoprasum***
–	Spathe valves shorter than or at most equalling the umbel, with a short apical appendage or mucro (Fig. 2C)	**sect. *Scorodon***

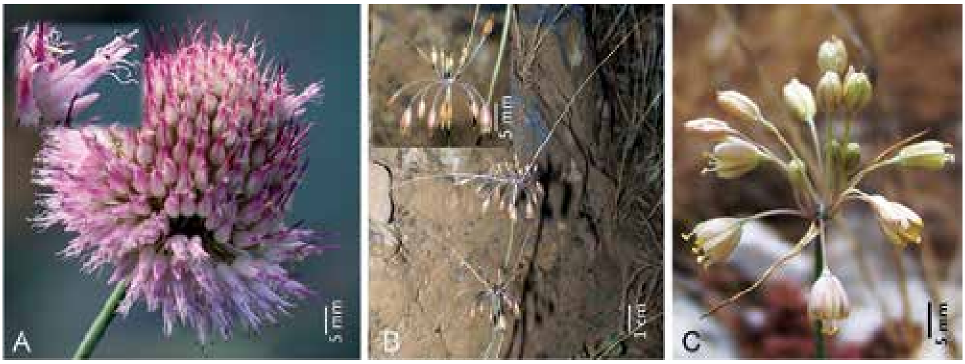


Fig. 2. *Allium* taxa, sectional characteristics. – A: Inflorescence showing markedly tricuspidates inner filaments of *A.* sect. *Allium*: *A. ilgazense.* B: Inflorescence with two long, unequal spathe valves of *A.* sect. *Codonoprasum*: *A. glumaceum*. C: Inflorescence with short, subequal spathe valves of *A.* sect. *Scorodon*: *A. opacum* (photographs by M. KOÇYIĞIT).

Checklist of wild *Allium* taxa from Turkey

In the list, taxa marked by an asterisk (*) are those already recorded in KOLLMANN (1984). Additional taxa are referenced to their first published Turkish record. Of the 77 listed taxa of *Allium* sect. *Allium,* 34 (45%) are *endemic to Turkey* (names in boldface italics).

Allium L. sect. *Allium*

Allium sect. *Allium* is the most speciose section of the genus. According to MATHEW's (1996) review the total number of taxa, globally, is 114. For Turkey, our checklist enumerates 77 taxa (65 species, 11 subspecies and one variety), 20 of which are additional to the *Flora of Turkey* account (KOLLMANN 1984). Of the 77 listed taxa of *Allium* sect. *Allium,* 34 (45%) are endemic to Turkey. Areas of high endemism are mapped in Fig. 3.

This section is characterized by having dimorphic filaments: the inner filaments are tricuspidate (rarely 5-7 cuspidate), with the median cusp carrying the anther; the outer filaments are simple.

1. **A. affine* Ledeb.
2. ***A. aksekiense*** Özhatay & al. (ÖZHATAY & al. 2014).
3. * *A. amethystinum* Tausch
4. * *A. ampeloprasum* L.
5. ***A. anatolicum*** Özhatay & B. Mathew (ÖZHATAY & MATHEW 1995)
6. ***A. antalyense*** Eren & al. (PAROLLY & EREN 2007).
7. *** A. armerioides*** Boiss.
8. * *A. artvinense* Miscz.
9. *** A. asperiflorum*** Miscz. ex Grossh.
10. * *A. atroviolaceum* Boiss.
11. * *A. aucheri* Boiss.

12. ***A. aybukeae*** H. Duman & Ekşi (DUMAN & al. 2017).
13. **** A. baytopiorum*** Kollmann & Özhatay
14. ***A. bilgili*** H. Duman & Ekşi (DUMAN & al. 2017).
15. * *A. bourgeaui* Rech. fil.
16. * *A. calyptratum* Boiss.
17. **** A. cappadocicum*** Boiss. & Balansa
18. * *A. commutatum* Guss.
19. * *A. curtum* Boiss. & Gaill. subsp. *curtum*
20. *A. cycladicum* (Bothmer) Seregin (SEREGIN 2004).
21. *A. deserti-syriaci* Feinbrun (KOYUNCU 2012)
22. * *A. dictyoprasum* C. A. Mey. ex Kunth
23. ***A. ekimianum*** Ekşi & al. (EKŞI & al. 2016).
24. ***A. eldivanense*** Özhatay (ÖZHATAY 1986).
25. ***A. enginii*** Özhatay& B. Mathew (ÖZHATAY & MATHEW 1995).
26. *A. erubescens* K. Koch (Davis 1988)
27. ***A. erzincanicum*** Özhatay & Kandemir (ÖZHATAY & KANDEMIR 2014).
28. ***A. fethiyense*** Özhatay& B. Mathew (ÖZHATAY & MATHEW 1995)
29. * *A. fuscoviolaceum* Fomin
30. ***A. goekyigitii*** Ekim & al. (EKIM & al. 1999).
31. **** A. gorumsense*** Boiss.
32. * *A. gramineum* K. Koch
33. * *A. guttatum* Steven subsp.*guttatum*
34. * *A. guttatum* subsp. *dalmaticum* (A. Kerner ex Janchen) Stearn
35. * *A. guttatum* subsp. *sardoum* (Moris) Stearn
36. * *A. hamrinense* Hand.-Mazz.
37. ***A. ilgazense*** Özhatay (ÖZHATAY 1986).
38. * *A. jubatum* Macbride
39. * *A. junceum* Sm. subsp. *junceum*
40. * *A. junceum* subsp**. *tridentatum*** Kollmann & al.
41. * *A. karyeteini* Post
42. ***A. kayae*** Özhatay & M. Koyuncu (ÖZHATAY & al. 2014).
43. ***A. koyuncui*** H. Duman & Özhatay (GÜNER & al. 2000).
44. * *A. longicuspis* Regel
45. * *A. macrochaetum* Boiss. & Hausskn
46. **** A. nevsehirense*** Koyuncu & Kollmann
47. ***** *A.* ***oltense*** Grossh.
48. ***A. pervariensis*** Fırat & Koyuncu (FIRAT & al. 2017)
49. * *A. phanerantherum* Boiss. & Hausskn. subsp. *phanerantherum*
50. ***** *A. phanerantherum* subsp**. *deciduum*** Kollmann & Koyuncu
51. *A. phanerantherum* subsp**. *involucratum*** Ekşi & al. (EKŞI & al. 2015).
52. * *A. ponticum* Miscz. ex Grossh.
53. **** A. proponticum*** Stearn & Özhatay var. ***proponticum***
54. **** A. proponticum*** Stearn var. ***parviflorum*** Kollmann
55. * *A. pseudoampeloprasum* Miscz. ex Grossh.
56. * *A. pustulosum* Boiss. & Hausskn.
57. * *A. reuterianum* Boiss.

58. * A. robertianum Kollmann
59. * *A. rollovii* Grossh.
60. **** A. sandrasicum*** Kollmann & al.
61. * *A. scabriflorum* Boiss.
62. * *A. scorodoprasum* L subsp. *scorodsoprasum*
63. * *A. scorodoprasum* subsp. *jajlae* (Vved.) Stearn
64. * *A. scorodoprasum* subsp. *rotundum* (L.) Stearn
65. * *A. scorodoprasum* subsp. *waldsteinii* (G. Don) Stearn
66. **** A. sintenisii*** Freyn
67. * *A.* s*osnovskyanum* Miscz. ex Grossh.
68. * *A. sphaerocephalon* L. subsp. *sphaerocerphalon*
69. * *A. sphaerocephalon* subsp. *arvense* (Guss.) Arc.
70. * *A. sphaerocephalon* subsp. *trachypus* (Boiss. & Spruner) K. Richter
71. **** A. stearnianum*** Koyuncu, Özhatay & Kollmann subsp**. *stearnianum***
72. **** A. stearnianum*** subsp**. *vanense*** Kollmann & Koyuncu
73. * *A.* ***stylosum*** O. Schwarz
74. * *A. trachycoleum* Wendelbo
75. ***A. tuncelianum*** (Kollmann) Özhatay & al. (Özhatay & Mathew 1995).
76. * *A. vineale* L.
77. ***A. vuralii*** Kit Tan (Tan 1987).

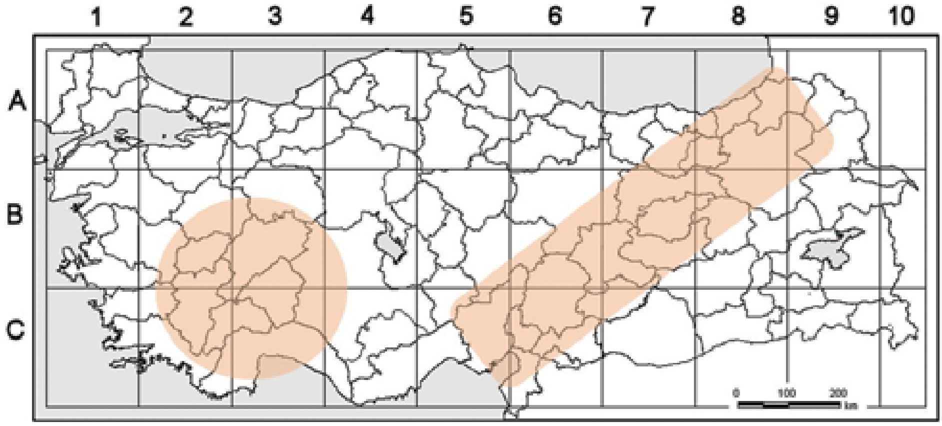


Fig. 3: High endemism areas for *Allium* sect. *Allium* in Turkey: the Anatolian Diagonal (11 taxa) and SW Turkey (10 taxa).

***Allium* sect. *Codonoprasum* (Rchb.) Endl.**

This is the taxonomically most complicated section. Recent relevant taxonomic research mainly focused on the Mediterranean basin, and resulted in the description of several new species and subspecies, increasing the number of included taxa (Brullo & al. 2014, Koçyiğit & al. 2016, Galanos & Tzanoudakis 2017, Brullo & al. 2017). The section can be readily recognised by the presence of two long, unequal spathe valves, many flowered umbels, and a long, stout scape. In Turkey, *A.* sect. *Codonoprasum* is now represented by 40 taxa (37 species, 2 subspecies and one variety) of which 12 (28%) are endemic. In this paper, the taxonomic rank of three taxa is altered. The cumulative distribution of endemic taxa in Turkey is mapped in Fig. 4.

1. *A. aeginiense* Brullo & al. (KOÇYIĞIT & al. 2014).
2. **A. albotunicatum* O. Schwarz
3. **A. bassitense* Thiéb.
4. ***A. bilgeae*** Yild. (YILDIRIMLI 2012).
5. ***A. bingoelense*** Yıld. & Kılıç (YILDIRIMLI & KILIÇ 2014).
6. *A. brussalisii* Tzanoud. & Kypr. (KOÇYIĞIT & al. 2014).
7. **A. carinatum* L. subsp. *carinatum*
8. ** A. carinatum* subsp. *pulchellum* (G. Don) Bonnier & Layens
9. **A. charaulicum* Fomin
10. **A. chloranthum* Boiss.
11. *A. convallarioides* Grossh. (BRULLO & al. 2003).
12. **A. deciduum* Özhatay & Kollmann
13. *A. dentiferum* Webb & Berthel. (KOÇYIĞIT & ÖZHATAY 2010).
14. *A. dodecanesi* Karovok. &Tzanoud. (KOÇYIĞIT & ÖZHATAY 2010).
15. **A. flavum* L.
16. *A. fuscum* Waldst. & Kit. (KOYUNCU 2012).
17. ****A. glumaceum*** Boiss. &Hausskn.
18. ***A. istanbulense*** Özhatay & al. (ÖZHATAY & al. 2018).
19. **A. karsianum* Fomin
20. ****A. kastambulense*** Kollmann
21. ***A. lazikkiyense*** Koçyiğit & al. (KOÇYIĞIT & al. 2014).
22. *A. longispathum* Redouté (KOYUNCU 2012).
23. **A. myrianthum* Boiss.
24. **A. pallens* L. subsp. *pallens*
25. **A. paniculatum* L. subsp. *paniculatum*
26. **A. paniculatum* subsp. *villosulum* (Halácsy) Stearn
27. ***A. perpendiculum*** Koçyiğit & al. (KOÇYIGIT & al. 2014).
28. ****A. phrygium*** Boiss.
29. **A. pseudoflavum* Vved.
30. ***A. rhodopeum*** subsp. ***turcicum*** Brullo & al. (BRULLO & al. 1998).
31. **A. rupestre* Steven
32. **A. rupicola* Boiss. ex Mouterde
33. **A. schergianum* Boiss.
34. **A. stamineum* Boiss.
35. **A. staticiforme* Sm.
36. *A. tauricum* (Besser ex Rchb.) Grossh. 1928, non Pall. ex D. Don var. *tauricum* Koçyiğit & Özhatay **comb. nov**.
37. *A. tauricum* (Besser ex Rchb.) Grossh. 1928, non Pall. ex D. Don var. ***pilosum*** (Kollmann & Koyuncu) Koçyiğit & Özhatay **comb. nov**.
38. ***A. turcicum*** Özhatay & Cowley (COWLEY & al. 1994).
39. ****A. variegatum*** Boiss.
40. **A. wiedemannianum* Regel

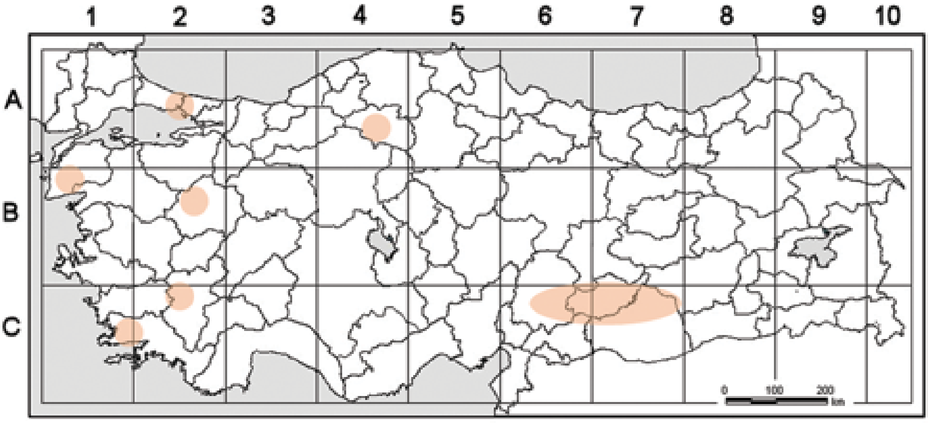


Fig 4: Cumulative distribution of endemic taxa of *Allium* sect. *Codonoprasum* in Turkey.

Allium sect. *Scorodon* K. Koch

This section has often been confused with *A.* sect. *Codonoprasum*. The distinction between *A.* sect. *Scorodon* and sect. *Codonoprasum* is based mainly on the length of spathe valves in relation to umbel size, but sometimes this characteristic cannot be ascertained in Turkish species. Also, the presence of distinct nectariferous pores on the ovary has been used as diagnostic feature for this section, but it is difficult to observe on dried specimens. By a detailed study all Turkish specimens, in this study 21 taxa, each marked by a dagger (†), are being transferred from *A.* section *Codonoprasum* to sect. *Scorodon*. The latter is now represented in Turkey by 36 species, of which 25 (69%) are endemic. Areas of high endemism are mapped in Fig. 5.

1. ***A. alpinarii*** Özhatay & Kollmann
2. **A. anacoleum* Hand.-Mazz.
3. **A. arlgirdense* Blakelock
4. †***A. armenum*** Boiss. & Kotschy
5. ***A. balansae*** Boiss.
6. †***A. brevicaule*** Boiss. & Bal.
7. †***A. djimilense*** Boiss. ex Regel
8. †***A. dumanii*** Koyuncu & Koçyiğit (Koçyigit & al. 2016).
9. †***A. ekeri*** E.Kaya & Koçyiğit (Koçyigit & al. 2014).
10. †***A. minus*** (Boiss.) Koçyiğit & Özhatay non H.J.Choi & B.U.Oh, **comb. & stat. nov.**
11. **A. frigidum* Boiss. & Heldr.
12. †**A. hirtovaginum* Cand.
13. †***A. hoshabicum*** Fırat (Fırat 2017).
14. †***A. hubermorathii*** Kollmann & al.
15. †***A. karacae*** Koyuncu (Koyuncu 1994).
16. **A. kossoricum* Fomin
17. †**A. kunthianum* Vved.

18. †*__A. kurtzianum__ Asch. & Sint. ex Kollmann
19. †__A. liliputianum__ Koçyiğit & al. (KOÇYIĞIT & al. 2014).
20. †__A. maraschicum__ Koçyiğit & Özhatay (KOÇYIĞIT & ÖZHATAY 2012).
21. *__A. microspathum__ Ekberg
22. *_A. moschatum_ L.
23. †*__A. olympicum__ Boiss.
24. †*_A. opacum_ Rech. fil.
25. †*__A. pictistamineum__ O. Schwarz
26. †_A. pilosum_ Sm. (KOÇYIĞIT & al. 2014).
27. †_A. retrorsum_ (Özhatay & Kollmann) Brullo (BRULLO & al. 2007).
28. *__A. rubellum__ M. Bieb.
29. †__A. rumelicum__ Koçyiğit & Özhatay (ÖZHATAY & al. 2010).
30. †*__A. sibthorpianum__ Schult. & Schult. fil.
31. *__A. sieheanum__ Hausskn. ex Kollmann
32. †*_A. sipyleum_ Boiss.
33. *__A. sivasicum__ Özhatay & Kollmann
34. †*_A. tauricola_ Boiss.
35. *__A. tchihatschewii__ Boiss.
36. *__A. wendelboanum__ Kollmann

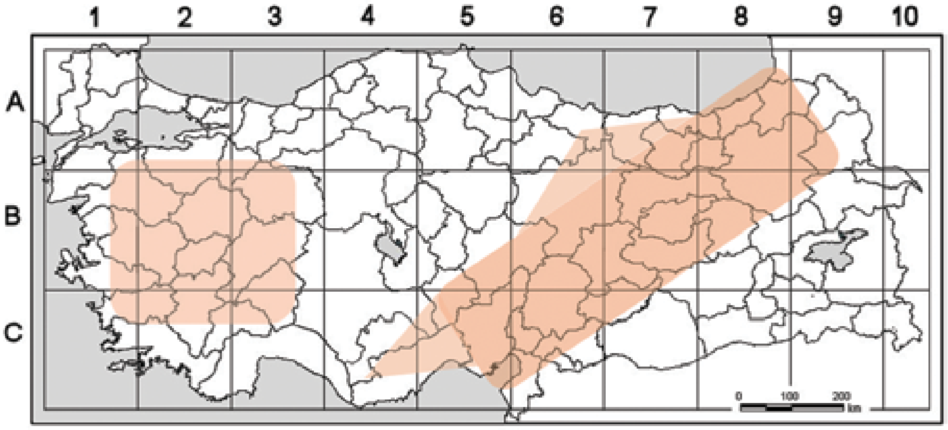


Fig. 5: High endemism areas for _Allium_ sect. _Scorodon_ in Turkey: the Anatolian Diagonal (12 taxa) and W Turkey (7 taxa).

Notes on some newly named or reevaluated taxa

Allium minus (Boiss.) Koçyiğit & Özhatay non H.J.Choi & B.U.Oh (_Allium flavum_ var. _minus_ Boiss.).

Description. – Differs from _A. flavum_ var. _flavum_ by its globose bulb, 1.5-2.5 cm in diameter, with greyish-brown outer tunics. Scape solitary or paired, 10-15(-7) cm tall, covered by leaf sheaths for ⅔ of its length. Leaves up to 5 cm long. Spathe with 2 unequal valves, the larger 2-2.5 cm long, the smaller 1-1.5 cm long. Inflorescence with subequal, 0.8-1 cm long pedicels, that are 1-1.5× as long as the perigon. Tepals bright yellow, apically rounded, 4.5 mm long. Filaments purple, 4-4.5 mm long, twice as long as the tepals. Ovary 1.5-2 × 1.2-1.8 mm, stipitate.

Specimen karyologically examined. – Turkey, Bursa, Uludağ road, rocky, 1808 m, 16 vii 2008, Koçyiğit 118 (ISTE 87644)! – Karyotype: 2*n* = 2x = 10m + 6m-SAT= 16.

Ecology. – Alpine cliffs, stony hillsides with fairly sparse vegetation, limestone & granite rocks, roadsides; at elevations of 1800-2500 m. Endemic.

Allium tauricum (Besser ex Rchb.) Grossh. [non Pall. ex D. Don]
var. *tauricum* Koçyiğit & Özhatay.

= *Allium flavum* var. *tauricum* Besser ex Rchb. ≡ *A. flavum* subsp. *tauricum* (Besser ex Rchb.) K. Richt.

Description. – Differs from *A. flavum* L., in which it has formerly been included as a subspecies or variety, by having an ovoid bulb, 1-1.5 cm in diameter, with greyish brown outer and dirty white, coriaceous inner tunics. Scapes 15-20(-30) cm tall, covered by slightly scabrid leaf sheaths for ½-⅔ of its length. Leaves equalling or exceeding the scape. Spathe with unequal 2 valves, the larger 4-5-nerved, 5-10 cm long, the smaller 3-4-nerved, 2-4 cm long. Inflorescence lax, effuse, with very unequal pedicels, 2-2.5 cm long, 4-5× as long as the perigon. Perigon cup-shaped, pink to violet, with a brown or greenish-yellow tinge; tepals and pedicels dull, covered with a layer of wax; tepals 3-3.5 × 2-2.5 mm. Filaments distally purple for 0.5-1 mm, 1.2-1.5× as long as tepals. Ovary longer than broad.

Specimen karyologically examined. – Turkey, Kahramanmaras, Imalı Dam, rocky, 645 m, 17 Jun. 2008, *Koçyiğit 85* (ISTE 87624)! – *Karyotype:* 2*n* = 2x = 7m + 9m-SAT = 16

Ecology. – *Pinus brutia, Juniperus, Cedrus* & *Abies* forests, *Quercus* scrub, phrygana, swampy soil near streams, dry, stony, grazed pasture, rocky igneous and limestone slopes, cliffs, screes, calcareous steppe, at elevations of 10-2500 m.

Allium tauricum (Besser ex Rchb.) Grossh. [non Pall. ex D. Don] ***var. pilosum*** (Kollmann & Koyuncu) Koçyiğit & Özhatay ≡ *Allium flavum* var. *pilosum* Kollmann & Koyuncu ≡ *A. villosiusculum* Seregin.

Description. – Differs from *A. tauricum* var. *tauricum* in having villous leaves that mostly exceed the scape, villous leaf sheaths, and a subglobose-oblong ovary.

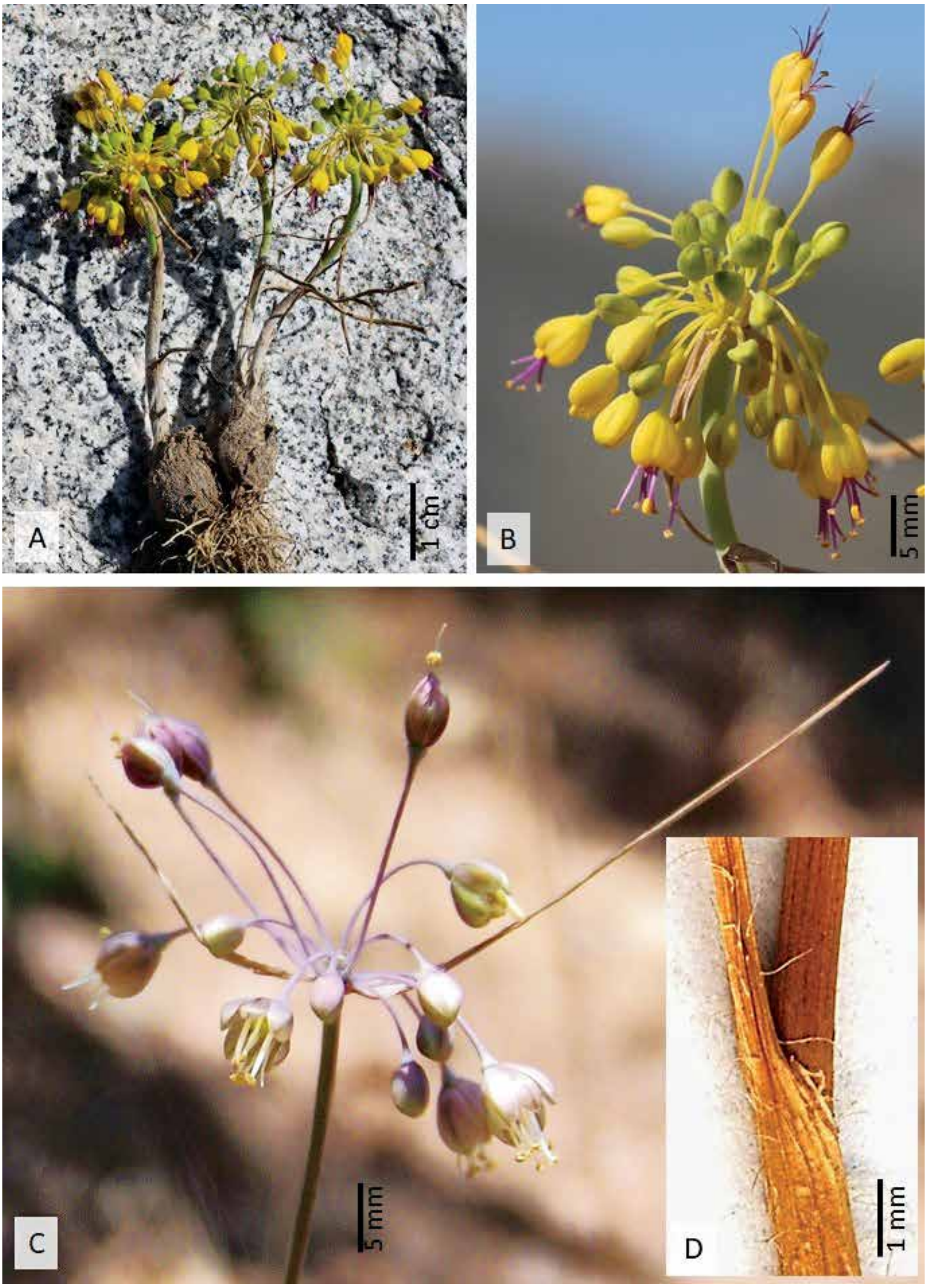


Fig. 6. *Allium* taxa. – 6A. General habit of *Allium minus* (ISTE 102899). – 6B. Umbel of *A. minus*. – 6C. Umbel of *Allium tauricum*. – 6D. Leaf sheaths of *Allium tauricum* var. *pilosum* (ISTE 87777) (Photographs: M. Koçyiğit).

Specimen karyologically examined. – Turkey, Kahramanmaras, Imalı Dam, around *Pinus brutia*, 1170 m, 17 vi 2008, Koçyiğit 82 (ISTE 87622)! – Karyotype: $2n = 2x = 12m + 4m - SAT = 16$
Ecology. – Dry hillsides, rocky hills, scrub, at elevations of 800-1200 m. Endemic.

Allium tauricum (Besser ex Rchb.) Grossh. [non Pall. ex D. Don] has been accepted as a synonym of *Allium paczoskianum* Tuzs on some systematical literatures (Czerepanov 1995, Govaerts 1995, Takhtajan 2006). However, in this study the protologue (Tuzson 1913) and herbarium specimens of *A. paczoskianum* have been examined and decided that it is a different species. The flower color (rose-violet) and structure (campanulate), leaf shape (flat) of *A. paczoskianum* is different from *A. tauricum*. Further research is necessary to resolve this confusion in nomenclature.

Acknowledgements

Thanks are due to the Directors and Curators of the Herbaria AEF, CAT, E, G, ISTE, and K. Our visits to the Herbaria G and CAT were financially supported by the Project of Illustration of the Turkish Flora. Also, we thank to Prof. Tibor Kalapos who is editor of the Botanikai Kozlemenyek for literature supports. Finally, we thank to the reviewer who checked our manuscript in detail.

References

Brullo, C., Brullo, S., Giusso del Galdo, G. & Salmeri, C. 2017: *Allium nazarenum* (Amaryllidaceae), a new species of the section *Codonoprasum* from Israel. – Phytotaxa 327: 237-251.

—, —, Fragman-Sapir, O., Giusso del Galdo, G. & Salmeri, C. 2014: *Allium therinanthum* (Amaryllidaceae), a new species from Israel. – Phytotaxa 164: 29-40.

—, Guglielmo, A. & Temssi, M. C. 1998: Notes on *Allium rhodopeum* (Alliaceae), a neglected species from the E Mediterranean area. – Pl. Biosyst. 132: 63-69.

—, —, Pavone, P. & Salmeri, C. 2003: Cytotaxonomical remarks on *Allium pallens* and its relationships with *A. convallarioides* (Alliaceae). – Bocconea 16: 557-571.

—, —, —, — 2007: Cytotaxonomic considerations on *Allium stamineum* Boiss. group (Alliaceae) – Bocconea 21: 325-343.

Cowley, J., Ozhatay, N. & Mathew, B. 1994: New species of Alliaceae & Hyacinthaceae from Turkey. – Kew Bull. 49: 481-489.

Czerepanov, S. K. 1995: Vascular Plants of Russia and Adjacent States (The Former USSR): 1-516. – Cambridge University Press.

Davis, P. H. 1965-1985: Flora of Turkey and the East Aegean Islands, 1-9. – Edinburgh.

Duman, H., Ekşi, G. & Özbek, F. 2017: Two new species of *Allium* L. sect. *Allium* (Amaryllidaceae) from Turkey. – Pl. Syst. Evol. 303: 1271-1291.

Ekim, T., Güner, A. & Duman, H. 1999: A new *Allium* species from Anatolia: *Allium goekyigitii* Ekim, Duman & Gliner. – Karaca Arbor. Mag. 5: 35-40.

Ekşi, G., Koyuncu, M. & Bona, M. 2015: *Allium phanerantherum* subsp. *involucratum* (Amaryllidaceae), a new subspecies from Turkey. – Bangladesh J. Pl. Taxon 22: 143-146.

—, —, & Gençler, Ö. A. M. 2016: *Allium ekimianum* a new species (Amaryllidaceae) from Turkey. – PhytoKeys 62: 83-93.

Firat, M. 2017: *Allium hoshabicum* a new species of *A.* sect. *Codonoprasum* (Amaryllidaceae) from Van (Turkey). – Phytotaxa 312: 129-134.

—, Koyuncu, M. & Ekşi, G. 2017: *Allium pervariensis,* sect. *Allium* (Amaryllidaceae), a new species from Siirt Turkey. – Pl. Biosyst. [DOI: 10.1080/11263504.2016.1271051].

Galanos, C. J. & Tzanoudakis, D. 2017: *Allium symiacum* (Amaryllidaceae), a new species from Symi Island (SE Aegean, Greece). – Willdenowia 47: 107-113.

Govaerts, R. 1995: World Checklist of Seed Plants 1(1, 2): 1-483, 1-529. – MIM, Deurne.

—, Kington, S., Friesen, N., Fritsch, R., Snijman, D. A., Marcucci, R., Silverstone-Sopkin, P. A. & Brullo, S. 2018: World checklist of Amaryllidaceae. – Kew [Available: http://apps.kew.org/wcsp/].

Güner, A., Özhatay, N., Ekim, T. & Başer, K. H. C. 2000: Flora of Turkey and the East Aegean Islands, 11. – Edinburgh.

Koçyiğit, M. & Özhatay, N. 2010: A contribution to the genus *Allium* L. (sect. *Codonoprasum*) in Turkey. – Turk. J. Bot. 34: 391-395.

— & — 2012: *Allium maraschicum* sp. nov. (Alliaceae) from Turkey. – Nordic J. Bot. 30: 553-559.

—, —, & Kaya, E. 2014: New species and new records for *Allium* (sect. *Codonoprasum*) from Turkey. Pp. 514-524. In Kaya, E. (ed.) Geophytes of Turkey, 3. – Yalova.

—, M., Yeşil, Y. & Koyuncu, M. 2016: *Allium dumanii* (*A.* sect. *Codonoprasum,* Amaryllidaceae), a new species from E Turkey. – Willdenowia 46: 113-119.

Kollmann, F. 1984: *Allium* L. Pp. 98-211. In Davis, P. H. (ed.), Flora of Turkey and the East Aegean Islands, 8. – Edinburgh.

Koyuncu, M. 1994: A new species of *Allium* L. (Liliaceae) from southern Anatolia. – Karaca Arbor. Mag. 2: 177-180.

—, 2012: *Allium* L. Pp. 30-44. In Güner, A., Aslan, S., Ekim ,T., Vural, M. & Babaç, M. T. (eds) Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. –Istanbul.

Mathew, B. 1996. Review of *Allium* section *Allium.* – Kew.

Özhatay, N. 1986: Two new *Allium* species from Turkey. – Notes Roy Bot. Gard. Edinburgh 44: 147-150.

—, Koçyiğit, M. & Akalin, E. 2010: *Allium rumelicum,* sect. *Codonoprasum*, a new species from European Turkey. – Phytol. Balcan. 16: 355-359.

—, —, Brullo, S. & Salmeri, C. 2018: *Allium istanbulense*, a new autumnal species of *A.* sect. *Codonoprasum* (Amaryllidaceae) from Turkey and its taxonomic position among allied species. – Phytotaxa 334: 152-166.

—, Koyuncu, M. & Kaya, E. 2014: *Allium aksekiense* & *Allium kayae,* (Sect. *Allium*) two new species from South Anatolia (Turkey). Pp. 507-513. In Kaya, E. (ed.), Geophytes of Turkey, 3. – Yalova.

— & Kültür, Ş. 2006: Check-list of additional taxa to theSupplement Flora of Turkey. III. – Turk J Bot. 30: 281-316.

—, — & Aksoy, N. 1994: Check-list of additional taxa to the supplement Flora of Turkey. – Doğa Turk. Bot. Derg. 18: 497-514.

—, —, & —1999: Check-list of additional taxa to the supplement Flora of Turkey II. – Turk. J. Bot. 23: 151-169.

—, — & Aslan, S. 2009: Check-list of additional taxa to the Supplement Flora of Turkey IV. – Turk. J. Bot. 33: 191-226.

—, — & Gürdal, M. B. 2011: Check-list of additional taxa to the Supplement Flora of Turkey V. – Turk. J. Bot. 35: 589-624.

—, — & — 2013: Check-list of additional taxa to the Supplement Flora of Turkey VI. – J Fac. Pharm. Istanbul 43(1): 33-82.

—, — & — 2015: Check-list of additional taxa to the Supplement Flora of Turkey VII. – J. Fac. Pharm. Istanbul 45: 61-86.

—, — & — 2017: Check-list of additional taxa to the Supplement Flora of Turkey VIII. – J. Fac. Pharm. Istanbul 47: 31-46.

—, & MATHEW, B. 1995: New taxa and notes on the genus *Allium* (Alliaceae) in Turkey and Arabia. – Kew Bull. 50: 723-731.

ÖZHATAY, N. & TZANOUDAKIS, D. 2000: *Allium* L. Pp. 224-232. In GÜNER, A., ÖZHATAY, N., EKIM, T. & BAŞER, K. H. C. (eds), Flora of Turkey and The East Aegean Islands, 11. – Edinburgh.

PAROLLY, G. & EREN, Ö. 2007: Contributions to the flora of Turkey, 2. – Willdenowia 37: 243-271.

SEREGIN, A. P. 2004: Additions to *Allium* sect. *Allium* (Alliaceae) from North Africa. – Komarovia 4.

TAKHTAJAN, A. L. (ed.) 2006: Conspectus Florae Caucasi 2: 1-466. – Editio Universitatis Petropolitanae.

TAN, K. 1987: *Allium vuralii* Kit Tan. – Pl. Syst. Evol. 155: 102.

TUZSON, J. 1913: Adatok a délorosz puszták összehasonlító flórájához. –Botanikai Kozlemenyek 12 (25): 181-202.

YILDIRIMLI, Ş. 2012: Nine new species from Kaz dağları, Munzur dağları, Bolkardağları and Karçal dağları, Turkey. – Ot 19(1): 1-34.

— & KILIÇ, Ö. 2014: Three new species on *Allium, Galium* and *Rubus* from Bingöl and Sivas, Turkey. – Ot 21(2): 1-14.

New estimates of nuclear DNA amount for 25 taxa from Kefallinia island

Sonja Siljak-Yakovlev, Perla Farhat, Nicolas Valentin,
Pepy Bareka & Georgia Kamari

Abstract

Siljak-Yakovlev, S., Farhat, P., Valentin, N., Bareka, P. & Kamari, G. 2019: New estimates of nuclear DNA amount for 25 taxa from Kefallinia island. – Bot. Chron. 22: 87-108.

The nuclear DNA amount has been assessed, by flow cytometry, for 25 plant taxa indigenous on Kefallinia Island. Genome size for all these taxa are here reported for the first time. Of these 25 taxa, 18 are Greek and Adriatic/Ionian endemics and 7 are more widely distributed, but of particular interest otherwise. The 2C DNA values ranged from 0.58 pg for *Briza minor* to 49.53 pg for *Paeonia mascula* subsp. *russoi.* In addition, two ploidy levels (2x & 4x) were confirmed for *Centaurea subciliaris* subsp. *subciliaris.*

S. Siljak-Yakovlev & P. Farhat, Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay, France.
P. Farhat, Laboratoire Biodiversité et Génomique Fonctionnelle, Faculté des Sciences, Université Saint-Joseph, Campus Sciences et Technologies, Beyrouth 1107 2050, Lebanon.
N. Valentin, Institute of Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, 91198, Gif-sur-Yvette, France.
P. Bareka, Laboratory of Systematic Botany, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece.
G. Kamari, Department of Biology, University of Patras, 265 00 Patras, Greece.

Key words: genome size, 2C DNA value, endemic taxa, chromosome number, ploidy level, conservation, Ionian Islands, Greece.

Introduction

The nuclear DNA amount (2C-value), or genome size, is a fundamental biological character of living organisms and it is frequently correlated with other biotic and abiotic characters (Bennett & Leitch 2005, Pustahija & al. 2013). Information on C-value can be profitably used in numerous branches of plant science, including ecology and phytogeography (Grime & Mowforth 1982, Vekemans & al. 1996, Price & Johnston 1996, Pustahija & al. 2013), systematics and evolution (Cerbah & al. 1999, 2001, Niketic & al. 2013, Lepers-Andrzejewski & al. 2011, Hajrudinovic & al. 2015) in biotechnology, and agronomy (Fyad-Lameche & al. 2016, Srisuwan & al. 2018) and also in evaluation of biodiversity (Bennett & al. 2000, Siljak-Yakovlev & al. 2010, Bou Dagher Karatt & al. 2013, Siljak-Yakovlev & al. 2017).

Flow cytometry is the widely accepted method of choice in plant genome size estimation (MARIE & BROWN 1993, KAMATÉ & al. 2001, DOLEŽEL & al. 2007, SILJAK-YAKOVLEV & al. 2008, SILJAK-YAKOVLEV & al. 2010, BAREKA & al. 2012, PELLICER & LEITCH 2014, BOURGE & al. 2018). This method is rapid, precise, involves easy sample preparation, and is accurate in detecting small differences in DNA content (BEN-MILOUD-MAHIEDDINE & al. 2011, KARRAT-SOUISSI & al. 2013, PELLICER & LEITCH 2014).

However, despite its importance, genome size has been estimated for only 3.1% of all angiosperms and 41% of gymnosperms (PELLICER & al. 2018). Evidently, there is still a wide demand for improved coverage of DNA estimates for higher plants, especially angiosperms.

The Ionian region is characterised by a high plant diversity. Kefallinia island, in particular, has attracted the interest of many botanists, and has been well studied from the floristic and phytogeographical points of view (HELDREICH 1883, PHITOS & DAM-BOLDT 1985, KARAGIANNI 2010, EFTHIMIATOU-KATSOUNI 2012).

A large part of Kefallinia is mountainous, peaking at an elevation of 1,628 m in Mt. Aenos, a mountain that hosts an *Abies cephalonica* Loudon forest that is unique for an island. The ecological, scientific and historical importance of that forest (which is the locus classicus of *Abies cephalonica*) resulted in having declared the area as a National Park.

Until now, ca. 1,100 taxa of indigenous vascular plants are known from Kefallinia island, and ca. 60 of them are Ionian and Greek endemics.Many taxa have been studied karyologically (PHITOS & DAMBOLDT 1971, 1985, KAPASA & al. 2001, SAMAROPOULOU & al. 2013a & b, BAREKA & al. 2018), but little is known about their genome size. We have measured for the first time the DNA content of 25 taxa, most of them Greek endemics or, else, of a particular taxonomic, phytogeographic or karyological interest.

The aim of this paper is to determine the genome size and ploidy level of several species growing in a geographical region with high plant diversity. Our data will be included in the genome size database of the Balkans flora (SILJAK-YAKOVLEV & al. 2010).

To check for possible pre-existing data and confirm the claimed novelty of our reports, we consulted six genome size and chromosome number databases, all of them accessed on November 25, 2018: Kew plant DNA C-values database (http://data.kew.org/cvalues), GSAD - genome size in the Asteraceae database (http://www.etnobiofic.cat/gsad), FLOWer - a plant DNA flow cytometry database (http://botany.natur.cuni.cz/flower/index.php), and Index to chromosome numbers in Asteraceae (http://www.lib.kobe-u.ac.jp/infolib/meta_pub/engG0000003asteraceae), the Chromosome Counts Database (CCDB) (RICE & al. 2015) and PhytoKaryon, the chromosome database for the Greek plants (http://www.phytokaryon.gr).

Material and methods

Collection of material

Material (leaves) of 25 wild taxa, were collected in Kefallinia (Ionian Islands, from 13 localities, mostly from Aenos National Park (Table 1, Fig. 1). The material was immediately on-site dried and conserved in silica gel until used. Locality (Fig. 1) and collection data are detailed in Table 1.

Flow cytometric analyses

The total nuclear DNA amount was assessed by flow cytometry according to MARIE & BROWN (1993) using silica dried leaves of samples and fresh leaves of one of five internal standards, in order to cover the range of DNA content: *Solanum lycopersicum* L. 'Montfavet 63-5' (2C = 1.99 pg, LEPERS-ANDRZEJEWSKI & al. 2011), *Petunia hybrida* Vilm. 'PxPc6' and *Hordeum vulgare* L. 'Sultan' (2C = 2.85 pg and 9.81 pg, MARIE & BROWN 1993 and GARNATJE & al. 2004 respectively).

The leaves (Approx. 30 mg) of both internal standard and target species were simultaneously chopped using a razor blade in a plastic Petri dish with 1 ml of Gif nuclei-isolation buffer (BOURGE & al. 2018): (45 mM MgCl2, 30 mM sodium citrate, 60 mM MOPS (4-morpholine propane sulphonate, pH 7), and 1% (w/v) polyvinylpyrrolidone 10,000, pH 7.2) containing 0.1% (w/v) Triton X–100, supplemented with 5 mM sodium metabisulphite and RNAse (2.5 U/ml). The nuclei suspension was filtered through 30 µm nylon mesh. The nuclei were stained with 100 µg/ml propidium iodide (PI), a specific DNA intercalating fluorochrome dye, and kept 5 min at 4°C

DNA content of about 3,000 stained nuclei was determined for each sample using the cytometer CytoFLEX S (Beckman Coulter- Life Science United States. Excitation 561 nm, 26 mW; emission through a 610/20 nm band-pass filter. The samples comprised from one to three individuals, measured separately. To check the reproducibility of values, two independent measurements were performed for each individual.

CytExpert software was used for histogram analyses. The total 2C DNA value (DNA contents of the diploid (2n) sets of chromosomes (irrespective of ploidy level), was calculated using the linear relationship between the fluorescent signals from stained nuclei of the species and the internal standard. The total nuclear DNA content was calculated according to the following formula:

$$\text{2C DNA content/nucleus} = \frac{[\text{Sample 2C peak mean} \times \text{Standard 2C DNA pg}]}{\text{Standard 2C peak mean}}$$

The symbol C corresponds to the holoploid nuclear genome size (the whole chromosome complement with chromosome number *n*; 1C and 2C being, respectively, the DNA content of the haploid (*n*) and diploid (2*n*) chromosome sets, irrespective of ploidy level (GREILHUBER & al. 2005).

The mean 2C-value as well as the standard deviation were calculated for measurements of samples comprising more than one individual. The monoploid genome size (1Cx) is the DNA content of a monoploid genome, with chromosome base number x, calculated by dividing the 2C value by ploidy level (GREILHUBER & al. 2005).

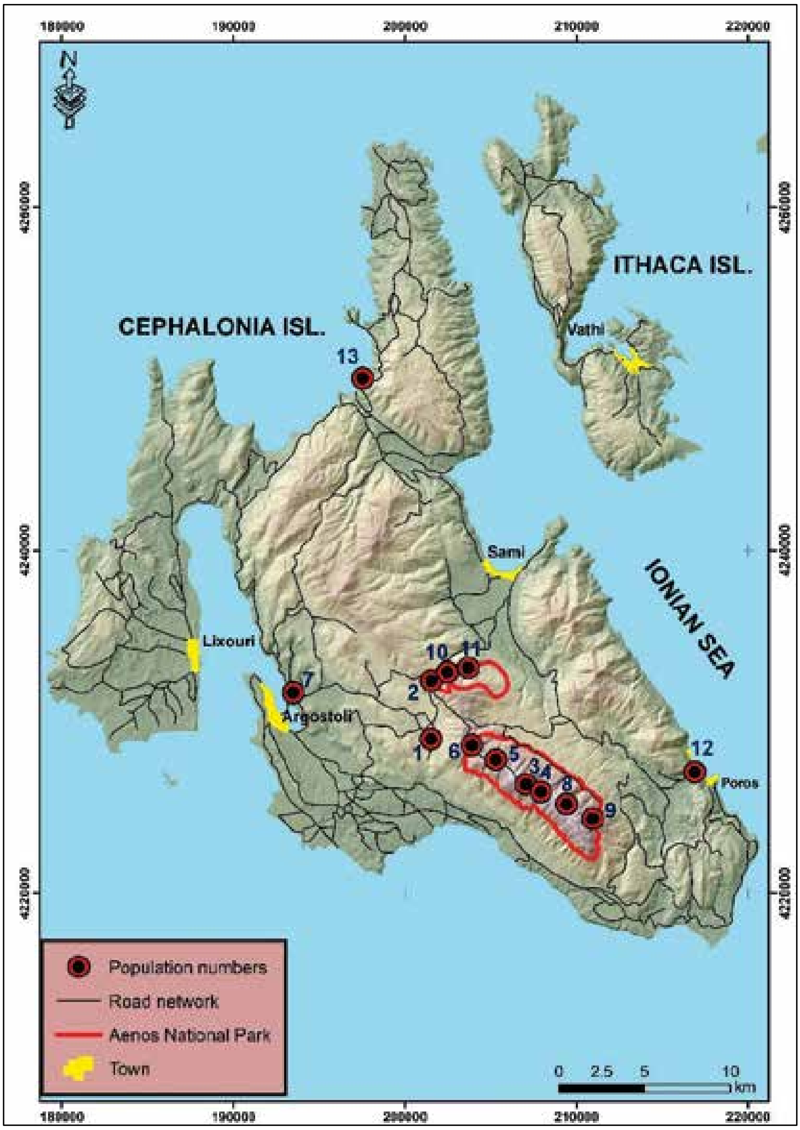


Fig. 1. Map of Kefallinia island with the collection localities of the studied 25 taxa.

Results and discussion

The nuclear DNA amounts for our 25 taxa (Figs 2-8) are presented in Table 2, and some flow cytometric histograms in Fig. 9. In our selection, 25 species of 19 genera from 12 families are represented (Table 2). Only 5 of our taxa are included in the Greek Red Data Book (PHITOS & al. 2009) according to IUCN (2019) criteria, but 5 more are legally protected in Greece (Table 2). Eighteen of the taxa are endemic, as follows:

Fig. 2. Studied taxa from Kefallinia island: A, *Astragalus sempervirens* subsp. *cephalonicus*; B, *Campanula garganica* subsp. *cephallenica*; C, *Campanula versicolor*; D, *Centaurea subciliaris* subsp. *subciliaris*; E, *Cerastium illyricum* subsp. *illyricum*; F, *Cymbalaria microcalyx* subsp. *minor*.

Table 1. Geographic origin and collection data of 25 studied taxa from Kefallinia island.

Loc. no	**Locality**	**Geographic data**		**Collection data & collectors**
		Latitude Longitude	**Altitude**	
1	Kefallinia: Aenos National Park: at the lowlands of Mt. Aenos, Omala area, above the village Epanochori, in gravels	38° 09′ 43.3″ N 20° 35′ 45.1″ E	470 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
2	Kefallinia: Aenos National Park: Mt. Roudi, between the places Agrapidies and the small church Agios Eleftherios, at the road, on calcareous rocky places	38° 11′ 33.7″ N 20° 35′ 36.8″ E	650-850 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
3	Kefallinia: Aenos National Park: Mt. Aenos, at the summit named “Chionistra”, at the pale area for the protection of *Viola cephalonica*	38° 08′ 24.5″ N 20° 39′ 32.8″ E	1610 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
4	Kefallinia: Aenos National Park: Mt. Aenos, at the place named “Thea”, on calcareous rocky places	38° 08′ 16.1″ N 20° 39′ 46.0″ E	ca. 1600 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
5	Kefallinia: Aenos National Park: Mt. Aenos, at the place named “Parko Anapsichis”, on calcareous petrosis places	38° 09′ 09.1″ N 20° 38′ 18.7″ E	1320 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
6	Kefallinia: Aenos National Park: Mt. Aenos, at the NW main enter of the Park	38° 09′ 35.0″ N 20° 37′ 20.2″ E	1070 m	21 Jul 2018, leg.: *G. Kamari, S. Moshopoulou & D. Spanou*
7	Kefallinia: Across to the town Argostoli: At the place named Agia Barbara, at the road to the town Sami, on calcareous rocks	38° 10′ 52.4″ N 20° 30′ 13.9″ E	ca. 60 m	22 Jul 2018, leg.:*G. Kamari, V. Karagianni & D. Spanou*
8	Kefallinia: Aenos National Park: Mt. Aenos, between the places named “Thea” and “Fteri”, on rocky limestone places	38° 08′ 15.0″ N 20° 40′ 02.8″ E	1560 m	22 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*
9	Kefallinia: Aenos National Park: Mt. Aenos, close to the main SE enter of the Park, above the village Arginia, on calcareous rocks	38° 07′ 24.0″ N 20° 42′ 15.2″ E	ca. 1100 m	22 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*
10	Kefallinia: Aenos National Park: Mt. Roudi, at the north-east slopes, in clearing petrosis places of *Abies cephalonica* forest	38° 11′ 50.4″ N 20° 36′ 45.4″ E	ca. 750 m	22 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*
11	Kefallinia: Aenos National Park: Mt. Roudi, at the north slopes with *Abies cephalonica* forest, at shady places	38° 11′ 48.1″ N 20° 36′ 10.5″ E	ca. 650 m	22 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*

→

12	Kefallinia: Poros gorge: Close to the village Poros, on the limestone vertical cliffs with south and south-east expose	38° 08′ 58.8′′N 20° 46′14.9′′E	20-80 m	23 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*
13	Kefallinia: Myrtos bay: At Myrtos beach, on the limestone vertical cliffs with west expose	38° 20′ 51.15′′N 20° 32′ 9.8′′ E	10-200 m	24 Jul 2018, leg.: *G. Kamari, V. Karagianni & D. Spanou*

3 are endemic to Kefallinia island: *Scutellaria rupestris* subsp. *cephalonica* (Fig. 5B), *Silene cephallenia* subsp. *cephallenia* (Fig. 6A) and *Viola cephalonica* (Fig. 8A). All have been included in the Red Data Book of Rare and Threatened Plants of Greece (PHITOS & al. 2009), the first as Endangered (EN) and the other two as Critically Endangered (CR), due to their extremely restricted area of occupancy and the limited number of individuals.

5 are endemic to the Ionian area: *Campanula garganica* subsp. *cephallenica* (Fig. 2B) is characterized as Vulnerable (Vu) in the Red Data Book (PHITOS & al. 2009). It is found on four of the Ionian Islands (Zakynthos, Kefallinia, Ithaki, Lefkada). In Kefallinia it lies within the core of the National Park of Mt. Ainos, the protection of which is governed by strict regulations (PHITOS & al. 2015).

Centaurea subciliaris subsp. *subciliaris* (Fig. 2D) is an endemic subspecies that grows on the islands of Kefallinia and Lefkada, while in mainland Greece (on Mt. Boumistos), subsp. *acarnanica* Matthäs occurs (PHITOS & DAMBOLDT 1971 & 1985, MATTHÄS 1976, SAMAROPOULOU & al. 2013a, BAREKA & al. 2018).

Cerastium illyricum is a polymorphic Greek endemic, divided into three subspecies. The typical one (Fig. 2E) grows on the islands of Zakynthos, Kefallinia, and Kerkyra; subsp. *crinitum* in the Akarnanika Mts of mainland Greece; and subsp. *brachiatum* in Peloponnisos, on Kefallinia and Zakynthos.

Stachys ionica (Fig. 7A) is endemic to the Ionian Islands of Zakynthos, Kefallinia, Ithaki, Lefkada and some of the neighbouring islets.

Thymus holosericeus (Fig. 7C) has an intense aroma and is occasionally used as a medicinal and aromatic plant.

9 are endemic to Greece (none of them is included in the Red Data Book, PHITOS & al. 2009; but 4 are protected by the Greek law (Presidential Decree 67/81).

Two, *Astragalus sempervirens* subsp. *cephalonicus* (Fig. 2A) and *Erysimum cephalonicum* (Fig. 4A) have their locus classicus in Kefallinia island.

Four, *Delphinium hellenicum* (Fig. 3B), *Dianthus fruticosus* subsp. *occidentalis* (Fig. 3C), *Scaligeria moreana* (Fig. 5C) and *Stachys parolinii* (Fig. 7B), are legally protected in Greece (Presidential Decree 67/81).

The other three, *Cymbalaria microcalyx* subsp. *minor* (Fig. 2F), *Petrorhagia graminea* (Fig. 5A) and *Silene ionica* (Fig. 6B1 & 6B2), grow on the Ionian Islands and also in neighboring areas of mainland Greece.

1 is endemic to the Adriatic-Ionian area: *Crepis neglecta* subsp. *corymbosa,* which also extends to Sicily.

Fig. 3. Studied taxa from Kefallinia island: A, *Crepis rubra*; B, *Delphinium hellenicum*; C, *Dianthus fruticosus* subsp. *occidentalis*.

Fig. 4. Studied taxa from Kefallinia island: A, *Erysimum cephalonicum*; B, *Paeonia mascula* subsp. *russoi*.

Table 2. Origin, Chorology, IUCN status, Nuclear DNA amount (2C), monoploid genome size (1Cx), chromosome number and relevant cytological references of 25 studied taxa from Kefallinia island.

Taxon (Family)	Pop. no	Chorology	IUCN status	2C DNA in pg* (± SD)	1Cx DNA in Mbp	Standard	2*n* (x)	References
Ajuga orientalis L. (Lamiaceae)	10	Me		2.35 (±0.04)	575	*Petunia*	32+ 0-4B (4x)	SAMAROPOULOU & al. (2015)
Astragalus sempervirens subsp. ***cephalonicus*** (C. Presl) Asch. & Graebn. (Fabaceae)	9	E-Gr		2.29 (±0.06)	1120	*Petunia*	16 (2x)	CARTIER (1979)
Briza minor L. (Poaceae)	6	Co		0.58	284	*Petunia*	10 (2x)	CCDB-Database (RICE & al. 2015)
Campanula garganica subsp. ***cephallenica*** (Feer) Hayek (Campanulaceae)	4	E-IoI	VU	3.52 (±0.29)	1721	*Petunia*	34 (4x)	DAMBOLDT (1965)
Campanula versicolor Sm. (Campanulaceae)	7	Balkans & Italy		3.43 (±0.1)	1677	*Solanum*	34 (4x)	DAMBOLDT & PHITOS (1971)
Centaurea subciliaris subsp. ***subciliaris*** Boiss. & Heldr. (Compositae)	3	E-IoI		2.10 (±0.01)	1027	*Petunia*	18+0-2B (2x)	MATTHÄS (1976), PHITOS & DAMBOLDT (1971 & 1985), BAREKA & al. (2018)
,, ,,	1	E-IoI		4.03 (±0.07)	985	*Petunia*	36+1-4B (4x)	PHITOS & DAMBOLDT (1971 & 1985), SAMAROPOULOU & al. (2013a), BAREKA & al. (2018)
Cerastium illyricum Ard. subsp. ***Illyricum*** (Caryophyllaceae)	3	E-IoI		3.91 (±0.07)	1912	*Petunia*	34 (4x)	DAMBOLDT & PHITOS (1971)
Crepis fraasii Sch.Bip. subsp. ***fraasii*** (Compositae)	11	EM		6.33 (±0.1)	3095	*Petunia*	12 (2x)	ANAGNOSTOPOULOS & KAMARI (1992)

Table 2 (contin.). Origin, Chorology, IUCN status, Nuclear DNA amount (2C), monoploid genome size (1Cx), chromosome number and relevant cytological references of 25 studied taxa from Kefallinia island.

Taxon (Family)	**Pop. no**	**Chorology**	**IUCN status**	**2C DNA in pg* (± SD)**	**1Cx DNA in Mbp**	**Standard**	***2n* (x)**	**References**
Crepis neglecta subsp. ***corymbosa*** (Ten.) Nym. (Compositae)	6	E-Ad/IoI		2.37	1159	*Petunia*	8 (2x)	Kamari (1976), Enke & al. (2015)
Crepis rubra L. (Compositae)	6	Me		6.49 (±0.17)	3174	*Hordeum*	10 (2x)	Kamari & Anagnostopoulos (1991)
Cymbalaria microcalyx subsp. ***minor*** (Cufod.) Greuter (Plantaginaceae)	5	E-Gr		1.80 (±0.16)	880	*Petunia*	28 (4x)	Damboldt & Phitos (1971), Speta (1986), Samaropoulou & al. (2013a)
Delphinium hellenicum Pawł. (Ranunculaceae)	1	E-Gr		9.92 (±0.02)	4851	*Petunia*	16 (2x)	Kamari & al. (2003)
Dianthus fruticosus subsp. ***occidentalis*** Runemark (Caryophyllaceae)	13	E-Gr		3.82 (±0.06)	1868	*Petunia*	30 (2x)	Bareka & Kamari (1999)
Erysimum cephalonicum Polatschek (Brassicaceae)	9	E-Gr		0.92 (±0.06)	450	*Solanum*	26 (4x)	Polatschek (1978)
Paeonia mascula subsp. ***russoi*** (Biv.) N.G.Passal. & Bernardo (Paeoniaceae)	11	Me	NT	49.53 (±3.48)	24220	*Hordeum*	10 (2*x*)	Tzanoudakis (1983)
Petrorhagia graminea (Sm.) P.W.Ball & Heywood (Caryophyllaceae)	6	E-Gr		2.01	655	*Petunia*	60 (4x)	Damboldt & Phitos (1972)
Scaligeria moreana Engstrand (Apiaceae)	8	E-Gr		2.89±0.3	1413	*Hordeum*	20+0-2B (2x)	Kyriakopoulos & al. (2014)

Table 2 (contin.). Origin, Chorology, IUCN status, Nuclear DNA amount (2C), monoploid genome size (1Cx), chromosome number and relevant cytological references of 26 studied taxa from Kefallinia island.

Taxon (Family)	Loc. no	Chorology	IUCN status	2C DNA in pg* (± SD)	1Cx DNA in Mbp	Standard	2*n* (*x*)	References
Scutellaria rupestris subsp. ***cephalonica*** (Bornm.) Greuter & Burdet (Lamiaceae)	3	E-Kef.	EN	3.45±0.19	844	*Solanum*	34 (4x)	Bothmer (1987)
Silene cephallenia Heldr. subsp. ***cephallenia*** (Caryophyllaceae)	12	E-Kef.	CR	5.45±0.14	2665	*Solanum*	24 (2x)	Damboldt & Phitos (1970)
Silene ionica Halácsy (Caryophyllaceae)	1	E-Gr		3.10±0.1	1516	*Hordeum*	24 (2x)	Samaropoulou & al. (2015)
Stachys ionica Halácsy (Lamiaceae)	13	E-IoI		2.33±0.05	570	*Petunia*	34 (4x)	Damboldt (1976), Baltisberger & Lenherr (1984)
Stachys parolinii Vis. (Lamiaceae)	2	E-Gr		1.97	482	*Petunia*	34 (4x)	Baltisberger & Lenherr (1984), Samaropoulou & al.(2013a)
Thymus holosericeus Čelak. (Lamiaceae)	10	E-IoI		1.17±0.03	286	*Solanum*	28 (4x)	Samaropoulou & al. (2013b)
Viola cephalonica Bornm. (Violaceae)	3	E-Kef.	CR	3.37±0.04	1648	*Solanum*	20 (2x)	Erben (1985), Damboldt & Phitos (1971)
Viola odorata L. (Violaceae)	5	EA		3.46±0.13	1692	*Solanum*	20 (2x)	Tiniakou (1992)

E-Keph. = Endemic to Kefallinia island
E-IoI = Endemic to Ionian area
E-Gr = Endemic to Greece
E-Ad/IoI = Endemic to Adriatic/Ionian area

Me = Mediterranean
EM = E Mediterranean
EA = European-SW Asian
Co = Cosmopolitan

CR = Critically Endangered
EN = Endangered
VU = Vulnerable
NT = Near Threatened

Fig. 5. Studied taxa from Kefallinia island: A, *Petrorhagia graminea*; B, *Scutellaria rupestris* subsp. *cephalonica*; C, *Scaligeria moreana*.

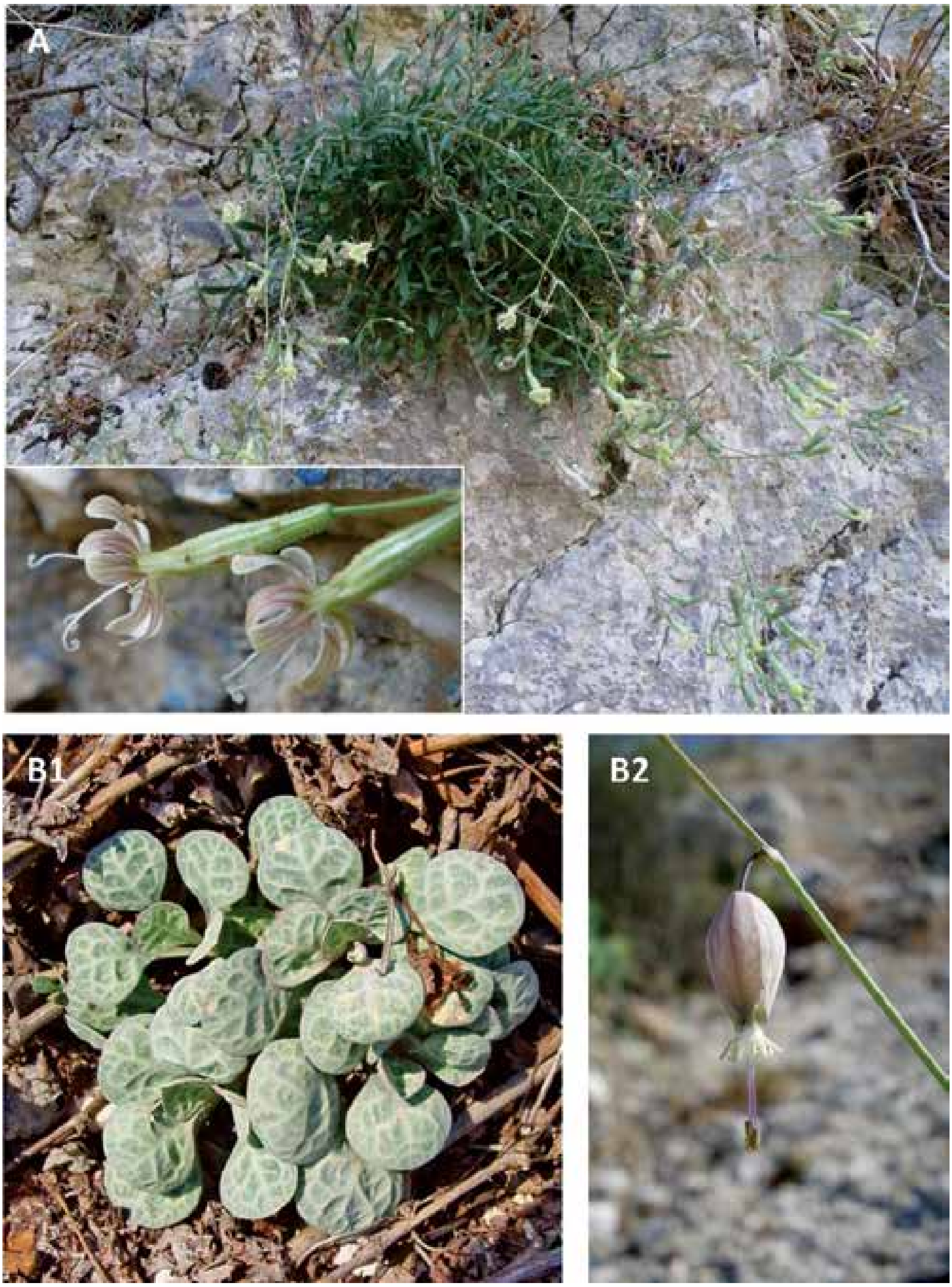


Fig. 6. Studied taxa from Kefallinia island: A, *Silene cephallenia* subsp. *cephallenia*; B1 & 2, *Silene ionica*.

Fig. 7. Studied taxa from Kefallinia island: A, *Stachys ionica*; B, *Stachys parolinii*; C, *Thymus holosericeus.*

Fig. 8. Studied taxa from Kefallinia island: A, *Viola cephalonica*; B, *Viola odorata*.

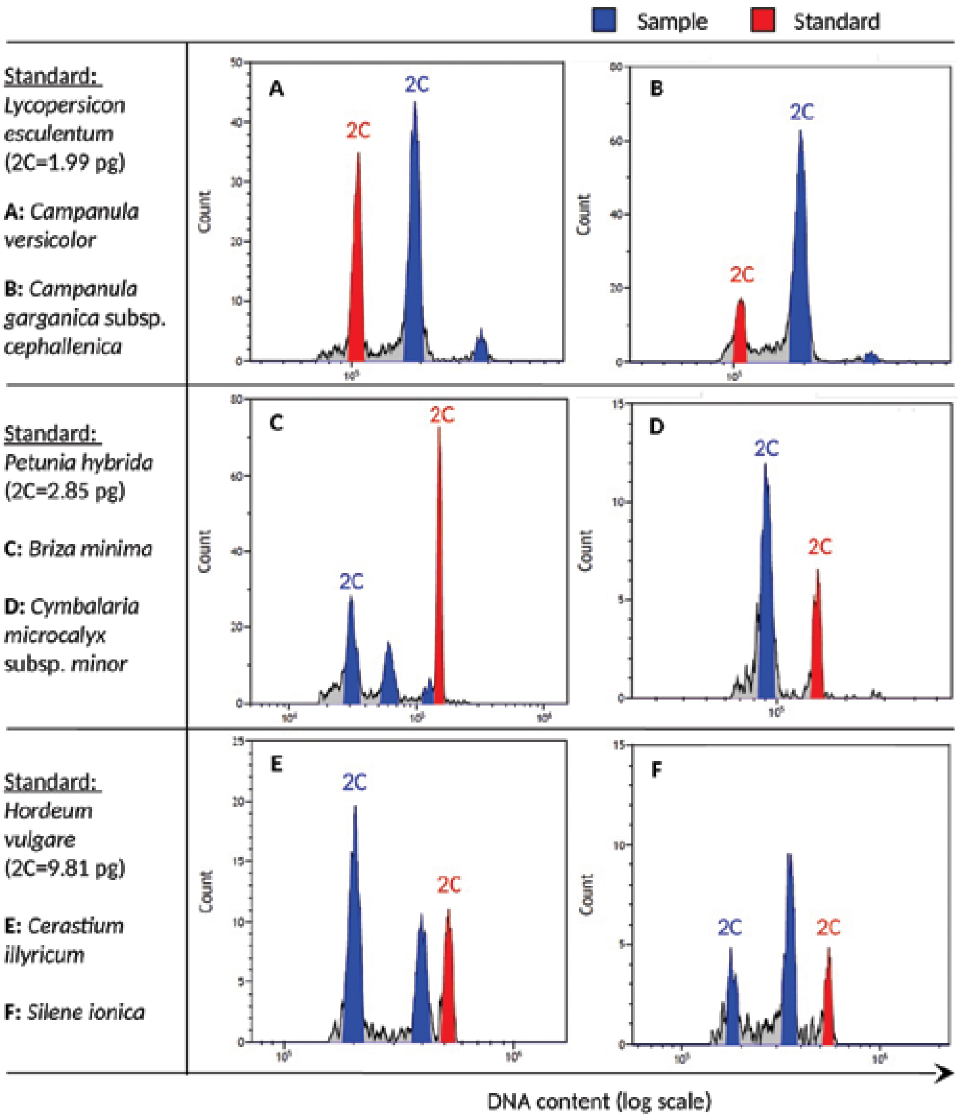


Fig. 9. Flow cytometric histograms for genome size estimation. Species (A-F) are co-chopped with different standards, as specified into the left of each boxes: (A-B) with *Lycopersicon esculentum,* (C-D) with *Petunia hybrida,* (E-F) with *Hordeum vulgare*. *After* setting up a gating strategy, initial gates (not show) helped identification of nuclei by discarding debris and doublets, results are then shown as histograms with fluorescence intensity (DNA content) on x-axis (log scale) and number of events (count) on y-axis. Highlights in blue correspond to sample peaks and ones in red to standards peaks, used to measure fluorescence geometric mean to calculate C-value.

7 taxa have a wider distribution:

3 are Mediterranean elements: *Ajuga orientalis, Crepis rubra* (Fig. 3A), and *Paeonia mascula* subsp. *russoi* (Fig. 4B), an attractive taxon, that is included as Near Threatened (NT) in the Greek Red Data Book of PHITOS & al. (2009),

1 it is E Mediterranean: *Crepis fraasii* subsp. *fraasii*,

1 occurs at the Balkan Peninsula plus Italy: *Campanula versicolor* (Fig.

2C), 1 is European-SW Asian: *Viola odorata* (Fig. 8B),

1 has a cosmopolitan distribution: *Briza minor.*

The 2C DNA values encompass a large range of values, from 0.58 pg for *Briza minor* to 49.53 pg for *Paeonia mascula* subsp. *russoi*. The values of nuclear DNA content were reported for the first time for 22 taxa. For the three remaining taxa, deviating values were obtained. We already determined the genome size of *Crepis neglecta* subsp. *corymbosa* from the same geographical region and had found 2C = 2.43 pg (ENKE & al. 2015), while in this study we report 2C = 2.37 pg, which is a very similar value. Previously WALLACE & al. (1972) reported 2C = 3.6 pg for *C. neglecta* s. l., but the technique used for that estimate was Feulgen densitometry. For *Crepis rubra* (Fig. 3A) our value (2C = 6.49 pg) was larger than those (5.7 pg) of BROWN & JONES (1975: personal communication, cited in BENNETT & SMITH 1976). For *Paeonia mascula* subsp. *russoi* we determined 2C = 49.53 pg, whereas MULRY & HANSON (1999, personal communication in BENNETT & al. 2000) had reported 35.6 pg for *P. mascula* subsp. *mascula* and BOU DAGHER-KARRAT (2013), 58.5 pg for *P. mascula* s. l. This important difference in genome size could be explained by different techniques having been used for determining the 2C DNA amount or, more likely, by taxonomic differences between the plants studied.

Thanks to our genome size assessment, two ploidy levels (2x & 4x) were confirmed for *Centaurea subciliaris* subsp. *subciliaris,* as had already been reported based on actual chromosome counts (PHITOS & DAMBOLDT 1971, 1985, MATTHÄS 1976, SAMAROPOULOU & al. 2013a, BAREKA & al. 2018).

According to the categories established by LEITCH & al. (2005), the 2C DNA values reported here are very small to rather small. Ten taxa possess a very small genome size (2C < 2.8 pg), 14 taxa belong to the category of small genome size (2.8 ≤ 2C < 7), in one taxon (*Delphinium hellenicum*) it is medium-sized (7 ≤ 2C < 28), and one (*Paeonia mascula* subsp. *russoi*) has a large genome size (28 ≤ 2C ≤ 75). In our sample of taxa, diploids dominate: only three tetraploids were detected (Table 2).

This is the first work to focus particularly on the nuclear DNA content of members of the Greek flora. We hope it is just a beginning, and that this important biological character will increasingly be taken into account in taxonomic, evolutionary and biodiversity studies.

Acknowledgements

We would like to thank the staff of the Management Body of Mt. Aenos National Park: Vasiliki Karagianni MSc, Sofia Moshopoulou MSc & Dionisia Spanou, for their company and help during field work; and Dr. Mihail Xanthakis for the production of the map. Most of the plant photographs are from the photo archive of Aenos National Park, which we sincerely thank. We also thank Dr. Mickael Bourge (Platform of Cytometry, Institute of Integrative Biology of the

Cell (I2BC), Gif-sur-Yvette) for his very useful assistance in cytometry; and the Laboratoire d'Ecologie, Systématique et Evolution (UMR CNRS 8079) for financial support of genome size measurement.

References

Alpinar, A. 1986: Some observations and findings on *Arum* L. (Araceae) species of west Turkey. – Doga Bilim Derg. A 10: 240-253.

Anagnostopoulos, A. & Kamari, G. 1992: Reports (46-50). In Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports - 2. – Fl. Medit. 2: 223-229.

Baltisberger, M. & Lenherr, A. 1984: Neue Chromosomenzahlen aus der Gruppe der *Stachys recta* L. und anderen, verwandten Artengruppen. – Ber. Geobot. Inst. ETH Stiftung Rübel Zürich 51: 39-62.

Bareka, P. & Kamari, G. 1999: Reports (1026-1030). In Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports - 9. – Fl. Medit. 9: 324-330.

—, Katopodi, E., Kamari, G. & Phitos, D. 2018: Karyosystematic study of some taxa from the Ionian floristic region (Greece). I. – Fl. Medit. 28: 85-97.

—, Siljak-Yakovlev, S. & Kamari, G. 2012: Molecular cytogenetics of *Bellevalia* (Hyacinthaceae) species occurring in Greece. – Pl. Syst. Evol. 298: 421-430.

Bennett, M. D., Bhandol, P. & Leitch I. J. 2000: Nuclear DNA amounts in angiosperms and their modern uses – 807 new estimates. – Ann. Bot. 86: 859-909.

— & Leitch, I. J. 2005: Nuclear DNA amounts in angiosperms – progress, problems and prospects. – Ann. Bot. 95: 45-90.

—, — & Hanson, L. 1998: DNA amounts in two samples of angiosperm weeds. – Ann. Bot. 82 (suppl. 1): 121-134.

— & Smith, J. B. 1976: Nuclear DNA amounts in angiosperms. – Philos. Trans. Roy. Soc. London, Ser. B, 274: 227-274.

Benmiloud-Mahieddine, R., Abirached-Darmency, M., Brown, S. C., Kaid-Harche, M. & Siljak-Yakovlev, S. 2011: Genome size and cytogenetic characterization of the Algerian *Retama* species. – Tree Genet. Genomes 7: 987-998.

Bothmer, R. von 1987: Differantiation patterns in the E. Mediterranean *Scutellaria rubicunda* group (Lamiaceae). – Pl. Syst. Evol. 155: 219-249.

Bou Dagher-Kharrat, M., Abdel-Samad, N., Douaihy, B. C., Abdel-Samad, F., Bourge, M., Siljak-Yakovlev, S. & Brown, S. 2013: Nuclear DNA C-values for biodiversity screening: Case of the Lebanese flora. – Pl. Biosyst. 147: 1228-1237.

Bourge, M., Siljak-Yakovlev, S. & Brown, S. C. 2018: Flow cytometry as tool in plant sciences, with emphasis on genome size and ploidy level assessment. – Genet. & Appl. 2: 1-12.

Cartier, D. 1979: Premières prospections caryologiques du genre *Astragalus* L. dans la flore du basin méditerraneen oriental. – Rev. Cytol. Biol. Vég., Bot. 2: 169-181.

Cerbah, M., Coulaud, J., Brown, S. C. & Siljak-Yakovlev, S. 1999: Evolutionary DNA variation in the genus *Hypochoeris*. – Heredity 80: 261-266.

—, Montreau E., Brown S., Siljak-Yakovlev S., Bertrand H. & Lambert C. 2001: Genome size variation and species relationships in the genus *Hydrangea*. – Theor. Appl. Genet. 103: 45-51.

Damboldt, J. 1965: Zytotaxonomische revision der isophyllen *Campanula* in Europa. – Bot. Jahrb. Syst. 84: 302-358.

— 1976: Beiträge zur Flora Ionica. VI. Karyologisch-systematische Bemerkungen zu einigen Labiaten. – Candollea 31: 273-281.

— & Phitos, D. 1970: Reports. In Löve, Á. (ed.), IOPB chromosome number reports XXVI. – Taxon 19: 265.

— & — 1971: Reports. In Löve, Á. (ed.), IOPB chromosome number reports XXXVI. – Taxon 20: 787.

— & — 1972: Beitrage zur Flora Ionica IV. Studien in der Gattung *Petrorhagia* (Caryophyllaceae). – Candollea 27: 27-40.

Doležel, J., Bartoš, J., Voglmayr, H. & Greilhuber, J. 2003: Nuclear DNA content and genome size of trout and human. – Cytometry A 51: 127-128.

—, Greilhuber, J. & Suda, J. 2007: Flow cytometry with plant cells. – Weinheim.

Efthymiatou-Katsouni, N. 2012: I anthropines epidrasis stin exeliktiki poria tis chloridas kai tis vlastisis tis Kefallinias apo tin proistoriki epochi mechri simera: Meleti gia efarmogi stin Perivallontiki Ekpedefsi. – PhD Thesis, University of Patras, Patras [In Greek with English summary].

Enke, N., Reinhard, K., Fatima, P., Gernot, G., Jonas, Z., Jana, O., Kamari, G. & Siljak-Yakovlev, S. 2015: Phylogeny and karyotype evolution in *Crepis* section *Neglectoides*. – Pl. Biol. 17: 775-786.

Erben, M. 1985: Cytotaxonomische Untersuchungen an südosteuropäischen *Viola*-Arten der Section *Melanium*. – Mitt. Bot. Staatssamml. München 21: 339-740.

Fyad-Lameche, F. Z., Iantcheva, A., Siljak-Yakovlev, S. & Brown, S. C. 2016: Chromosome number, genome size, seed storage protein profile and competence for direct somatic embryo formation in Algerian annual *Medicago* species. – Pl. Cell Tissue Organ Cult. 124: 531-540.

Garnatje, T., Vallès, J., Garcia, S., Hidalgo, O., Sanz, M., Canela, M. A. & Siljak-Yakovlev, S. 2004: Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. – Biol. Cell 96: 117-124.

Greilhuber, J., Doležel, J., Lysák, M. & Bennett, M. D. 2005: The origin, evolution, and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. – Ann. Bot. 95: 255-260.

Grime, J. P. & Mowforth, M. A. 1982: Variation in genome size. an ecological interpretation. – Nature (London) 299: 151-153.

Hajrudinović, A., Siljak-Yakovlev, S., Brown, S. C., Pustahija, F., Bourge, M., Ballian, D. & Bogunić, F. 2015: When sexual meets apomict – genome size, ploidy level and reproduction mode variation of *Sorbus aria* s. l. and *S. austriaca* (Rosaceae) in Bosnia and Herzegovina. – Ann. Bot. 116: 301-312.

Heldreich, Th. de 1883: Flore de l'île de Céphalonie. – Lausanne.

Iucn 2019: The IUCN Red List of Threatened Species. Version 2019-2. – http://www.iucnredlist.org. Downloaded on 12 July 2019.

Kamari, G. & Anagnostopoulos, A. 1991: Reports (1-24). In Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports - 1. – Fl. Medit. 1: 224-229.

—, Bareka, P., Constantinidis, T. & Phitos, D. 2003: Karyosystematic studies of plant taxa from the East Mediterranean region (Greece, Cyprus, Syria). – Phytol. Balc. 9: 487-502.

Kamaté, K., Brown, S. C., Durand, P., Bureau, J. M., De Nay, D. & Trinh, H. T. 2001: Nuclear DNA content and base composition in 28 taxa of *Musa*. – Genome 44: 622-627.

Kapasa, M., Nikolaidi, T., Bareka, E. P. & Kamari, G. 2001: Reports (1236-1243). In Kamari, G., Blanché, C. & Garbari, F. (eds), Mediterranean chromosome number reports - 11. – Fl. Medit. 11: 448-454.

Karagianni, B. 2010: Diachirisi ke prostasia ton endimikon, apilumenon kai spanion fiton tou Ethnikou Drimou Enou Kefalonias. – MSc Thesis, University of Patras, Patras.

Karrat-Souissi, I. A., Siljak-Yakovlev, S., Brown, S. C. & Chaib, M. 2013: Cytogeography of 28 populations of three ploidy levels of *Cenchrus ciliaris* L. in Tunisia. – Folia Geobot. 48: 95-113.

KYRIAKOPOULOS, C., BAREKA, P. & KAMARI, G. 2014: Reports (1840-1844). In KAMARI, G., BLANCHÉ, C. & SILJAK-YAKOVLEV, S. (eds), Mediterranean chromosome number reports - 24. – Fl. Medit. 24: 287-291.

LEITCH, I. J., SOLTIS, E. D., SOLTIS, P. S. & BENNETT, M. D. 2005: Evolution of DNA amounts across land plants (Embryophyta). – Ann. Bot. 95: 207-217.

LEPERS-ANDRZEJEWSKI, S., SILJAK-YAKOVLEV, S., BROWN, S. C., WONG, M. & DRON, M. 2011: Diversity and dynamics of plant genome size: an example of polysomaty from a cytogenetic study of Tahitian vanilla (*Vanilla* x *tahitensis*, Orchidaceae). – Amer. J. Bot. 98: 986-997.

MARIE, D. & BROWN, S. C. 1993: A cytometric exercise in plant DNA histograms with 2C values for 70 species. – Biol. Cell 78: 41-51.

MATTHÄS, U. 1976: Zur Cytotaxonomie von *Centaurea subciliaris* Boiss. & Heldr. (sektio *Phalolepis* (Cass.) DC.) und verwandter Sippen im europäischen Mediterrangebiet. I. – Bot. Jahrb. Syst. 95: 418-434.

NIKETIĆ, M., SILJAK-YAKOVLEV, S., FRAJMAN, B., LAZAREVIĆ, M., STEVANOVIĆ, B., TOMOVIĆ, G. & STEVANOVIĆ, V. 2013: Towards resolving the systematics of *Cerastium* subsect. *Cerastium* (Caryophyllaceae): a cytogenetic approach. – Bot. J. Linn. Soc. 172: 205-224.

PELLICER, J. & LEITCH, I. J. 2014: The Application of flow cytometry for estimating genome size and ploidy level in plants. In BESSE, P. (ed.), Molecular plant taxonomy: methods and protocols. – Meth. Molec. Biol. 1115: 279-307.

—, HIDALGO, O., DODSWORTH, S. & LEITCH, I. J. 2018: Genome size diversity and its impact on the evolution of land plants. – Genes 9: 88 [doi:10.3390/genes9020088].

PHITOS, D. & DAMBOLDT, J. 1971: Beiträge zur Flora Ionica. – Ann. Naturhist. Mus. Wien 75: 157-162.

— & — 1985: Die Flora der Insel Kefallinia (Griechenland). – Bot. Chron. 5: 182-183.

—, CONSTANTINIDIS, T. & KAMARI, G. (eds) 2009: The Red Rata Rook of Rare and Threatened Plants of Greece, 1-2. – Hellenic Botanical Society, Patras [in Greek].

—, KAMARI, G., KATSOUNI, N. & MITSAINAS, G. (eds) 2015: The Mt. Aenos of Cephalonia island: History - Physiography - Biodiversity. – Kefallinia.

POLATSCHEK, A. 1978: *Erysimum cephalonicum* (Brassicaceae), ein neuer Endemit der Insel Kefallinia (Griechenland). – Pl. Syst. Evol. 130: 219-221.

PRESIDENTIAL DECREE 67/1981: On the protection of native flora and wild fauna and the determination of the coordination and control procedure of related research. – Athens.

PRICE, H. J. & JOHNSTON, S. J. 1996: Influence of light on DNA content of *Helianthus annuus* Linnaeus. – Proc. Natl. Acad. Sci. U.S.A. 93: 11264-11267.

PUSTAHIJA, F., BROWN, S. C., BOGUNIĆ, F., BAŠIĆ, N., MURATOVIĆ, E., OLLIER, S., HIDALGO, O., BOURGE, M., STEVANOVIĆ, V. & SILJAK-YAKOVLEV, S. 2013: Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. – Pl. Soil 373: 427-453.

RICE, A., GLICK, L., ABADI, S., EINHORN, M., KOPELMAN, N. M., SALMAN-MINKOV, A., MAYZEL, J., CHAY, O. & MAYROSE, I. 2015: The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. – New Phytol. 206: 19-26.

SAMAROPOULOU, S., BAREKA, P., ARTELARI, R. & KAMARI, G. 2013a: Karyological studies on some endemic and rare species of Kephalonia, Ionian Islands, Greece. – Fl. Medit. 23: 215-221.

—, — & KAMARI, G. 2013b: Reports (1817-1823). In KAMARI, G., BLANCHÉ, C. & SILJAK-YAKOVLEV, S. (eds), Mediterranean chromosome number reports - 23. – Fl. Medit. 23: 215-221.

—, — & — 2015: Reports (1864-1867). In KAMARI, G., BLANCHÉ, C. & SILJAK-YAKOVLEV, S. (eds), Mediterranean chromosome number reports - 25. – Fl. Medit. 25: 164-167.

SILJAK-YAKOVLEV, S., STEVANOVIĆ, V., TOMASEVIĆ, M., BROWN, S. & STEVANOVIĆ, B. 2008: Genome size variation and polyploidy in the resurrection plant genus *Ramonda*: Cytogeography of living fossils. – Environ. Exp. Bot. 62: 101-112.

—, PUSTAHIJA, F., ŠOLIĆ, E. M., BOGUNIĆ, F., MURATOVIĆ, E., BAŠIĆ, N., CATRICE, O. & BROWN, S. C. 2010: Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. – Adv. Sci. Lett. 3: 190-213.

—, GODELLE, B., ZOLDOS, V., GARNATJE, T., VALLÈS, J. & HIDALGO, O. 2017: Heterochroma-tin and rDNA evolutionary implications in basic chromosome number and genome size changes during dysploidy: a case-study in *Reichardia* genus (Asteraceae, Cichorieae). – PLoS ONE, 9 Aug 2017, [10.1371/journal.pone.0182318].

SRISUWAN, S., SIHACHAKR, D., MARTIN, J., VALLÈS, J., BROWN, S. C. & SILJAK-YAKOVLEV, S. 2018: Change in nuclear DNA content and pollen size during polyploidisation of the sweet potato *Ipomoea batatas* (Convolvulaceae) complex. – Pl. Biol. [doi. org/10.1111/plb.12945].

TINIAKOU, A. 1992: Reports (56-60). In KAMARI, G., FELBER, F. & GARBARI, F. (eds), Mediterranean chromosome number reports - 2. – Fl. Medit. 2: 232-235.

VEKEMANS, X., LEFEBVRE, C., COULAUD, J., BLAISE, S. & SILJAK-YAKOVLEV, S. 1996: Variation of nuclear DNA content at the species level in *Armeria maritima* (Mill.) Willd. – Hereditas 124: 237-242.

WALLACE, H., SPARKES, C. A. & MADEN, M. 1972: Nuclear DNA content of three *Crepis* species. – Heredity 29: 367-373.

The genus *Psephellus* (Asteraceae) in the Bulgarian flora

Svetlana Bančeva & Malina Delčeva

Abstract

Bančeva, S. & Delčeva, M. 2019: The genus *Psephellus* (Asteraceae) in the Bulgarian flora. – Bot. Chron. 22: 109-116.

Psephellus is represented in the Bulgarian flora by two species, *P. marschallianus* and *P. trinervius*, both of which are very rare and have been included in the Bulgarian Red Data Book. To date, the latter species has been included in *Centaurea* in the Bulgarian literature. *P. marschallianus* is known only from several localities in two of Bulgaria's floristic regions, the (Northern) Black Sea Coast and North-East Bulgaria. Its populations, with 100-1000 individuals each, are in good state, but strongly fragmented. *P. trinervius* is known from a single population with several individuals in the North-East Bulgaria floristic region. It grows in dry, sunny places with sparse vegetation, on clayey marls. Long-term monitoring has been performed to assess the status of the populations and identify the main threatening factors. Appropriate measures have been taken to preserve the population of *P. trinervius in situ*.

S. Bančeva, & M. Delčeva, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Acad. G. Bončev Str, Bl. 23, 1113-Sofija, Bulgaria. E-mail: sbancheva@yahoo.com

Key words: Centaureinae, *in situ* conservation, monitoring, taxonomy.

Introduction

Psephellus Cass. belongs to the subtribe *Centaureinae* (Asteraceae) and includes about 100 species, mainly distributed in W Siberia, Iran, the Caucasus, Ukraine, Crimea, and Turkey. Only few species occur outside that area (Susanna & Garcia-Jacas 2007). The genus was described by Cassini in 1826 and has been accepted as such by Boissier (1875), Sosnovsky (1948), Dostál (1973), Gabrieljan (1995), Wagenitz & Hellwig 2000, Bancheva (2009), Susanna & Garcia-Jacas (2007), etc., but a number of authors of floristic works include *Psephellus* in the genus *Centaurea* (Klokov 1963, Dostál 1976, Kožuharov 1992, Petrova 2009, Asjov & al. 2002). Currently, there is plentiful morphological, anatomical, palynological, caryological and molecular evidence to support the generic rank of *Psephellus* (Wagenitz & Hellwig 2000).

The genus *Psephellus* is represented in the Bulgarian flora by two species, *P. marschallianus* (Spreng.) C. Koch and *P. trinervius* (Willd.) Wagenitz, both of which have a very limited distribution in Bulgaria and are included in the Red List of Bulgarian Vascular plants (Bančeva 2009, Petrova 2009) and in the Red Data Book of the Republic of Bulgaria (Bančeva 2015, Petrova 2015). In recent Bulgarian literature, *P. trinervius* has been included in *Centaurea* L.

This study aims at clarifying the adequate taxonomic status of Bulgarian *Psephellus* representatives, assess their population status by long-term monitoring and implement measures for supporting the populations *in situ*, if and where needed.

Material and methods

This study is based on all available literature data and specimens stored in Bulgarian herbaria, as well as the authors' own collections and observations in the field. Monitoring and assessment of population status follow the methodology for the monitoring of vascular plants and for the assessment of the state of vascular plants of the National Biodiversity Monitoring System (Executive Environment Agency, Ministry of Environment and Water: eea.government.bg/en/bio/nsmbr). Conservation measures are implemented in the single Bulgarian population of *P. trinervius* on Taušan tepe, Varna district.

Results

Psephellus Cass. in Cuvier, Dict. Sci. Nat. 43: 488. 1826.

Type: *P. calocephalus* Cassini, nom. illeg. [*Centaurea dealbata* Willd., *Psephellus dealbatus* (Willd.) C. Koch].

Herbaceous perennials or dwarf shrubs. Leaves usually pinnatisect, rarely entire, dentate, not decurrent, tomentose. Capitula long-pedunculate, heterogamous. Phyllary appendage usually not decurrent, membranous, entire, dentate or ciliate, never spiny. Outer florets sterile, with staminodes, radiant, divided into 5-10 lobes. Achenes oblong, with an elaiosome; hilum lateral. Pappus double, sometimes very short or lacking. Circa 100 species, W Siberia, Iran, Caucasus, Ukraine, Crimea, Turkey (SUSANNA & GARCIA-JACAS 2007).

Noteworthy is the absence of annuals or biennials and of spines or spinules on the phyllaries. The representatives of the genus show no weedy tendency.

Main diagnostic features on which generic subdivisions were based, concern the habit, the marginal florets, and the features of the pappus. The Bulgarian representatives of *Psephellus* have been referred to two sections:

Psephellus* sect. *Heterolophus (Cass.) Wagenitz & Hellwig (Type: *Heterolophus sibiricus* (L.) Cass. [*Centaurea sibirica* L., *Psephellus sibiricus* (L.) Wagenitz].

According to KLOKOV (1963) it comprises six species distributed from Bulgaria, the Ukraine and S Russia to W Siberia and Central Asia. In Bulgaria a single representative is present:

Psephellus marschallianus (Spreng.) C. Koch in Linnaea 24: 438. 1851 [*Centaurea marschalliana* Spreng., Syst. Veg. 3: 398. 1826].

Morphology and biology. Perennial. Stems 10-20(-30) cm tall, sigmoid, ascending. Basal leaves 6-12 cm long, pinnate, adpressed hairy, canescent, petiolate; lower and upper cauline leaves entire, the middle ones lyrate. Capitula 3-4 cm in diameter. Involucre 10-15 mm in diameter, ovate. Phyllaries triangular; phyllary appendages elongate, brown, with 3-4 up to 1 mm long fimbriae on either side. Florets pink (Fig.1).

Fig. 1. *Psephellus marschallianus* Habit.

Achenes ca. 4 mm long, with a ca. 1.5 mm long pappus. −Fl. IV-V, fr. V-VI. Insect-pollinated (BANČEVA 2015).

Distribution in Bulgaria. A dozen populations are known, in two floristic regions: the (northern) Black Sea Coast and North-East Bulgaria, at elevations up to 500 m (Fig. 2).

Habitats and populations. Occurs in dry places, as a member of subcontinental peri-Pannonic scrub and western Pontic stony steppe communities.

Conservation status. Endangered (EN) [B1ab(iii)+2ab(iii)]. Included in the Biological Diversity Act (2002, 2007).

Monitoring and assessment of the population status. Six of the Bulgarian populations have been subject to monitoring,Kabiyuška mogila (Šumen district), Probitija kamak, Taušan tepe and Tepičkite (Varna district), Kaliakra Reserve and Vidno village (Dobrič district), as described below:

Kabiyuška mogila. The population is located in a stony, dry place with eroded carbonate soils. It numbers ca. 700 individuals. The projective vegetation cover varies between 60% and 70%. *Psephellus marschallianus* grows together with *Amygdalus nana* L., *Achillea clypeolata* Sm., *Adonis vernalis* L., *Allium flavum* L., *Astragalus onobrychis* L., *Festuca valesiaca* Gaudin, *Medicago minima* (L.) L., *Melica ciliata* L., *Muscari tenuiflorum* Tausch, *Prunus spinosa* L., *Pulsatilla montana* (Hoppe) Rchb., *Satureja montana* L., *Thymus* spp., etc. In the past, the main threatening factor was the destruction of steppe vegetation, due to the ploughing of the lands. Currently, the

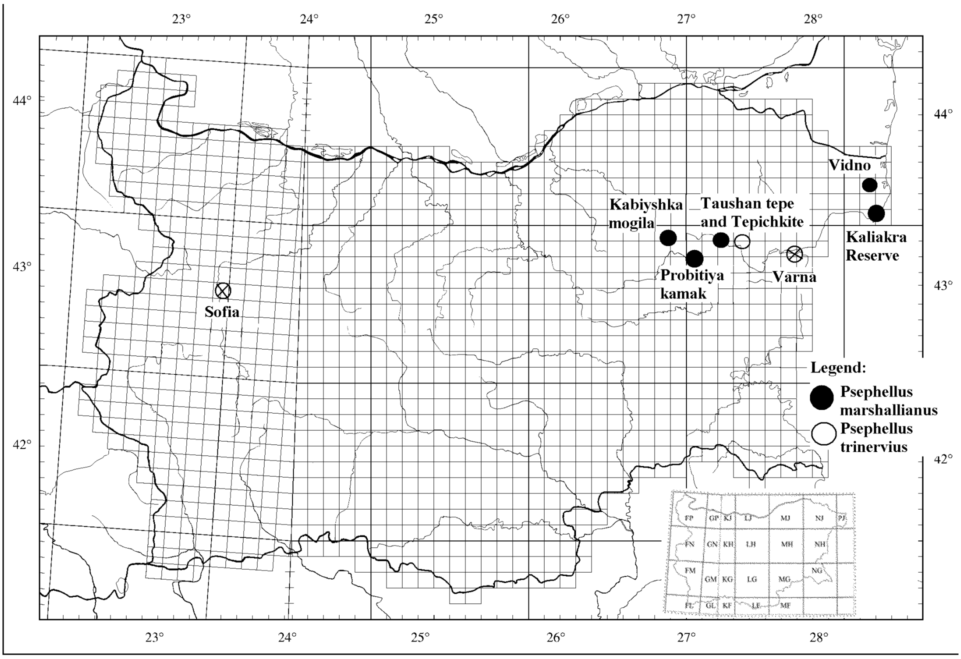


Fig. 2. Distribution of *Psephellus marschallianus* and *P. trinervius* in Bulgaria.

locality has been declared as a "Protected Site", and ploughing has ceased. According to the EUNIS Habitat classification, these two population of *P. marschallianus* belong to habitat F3.2412, subcontinental peri-Pannonic scrub, which is protected on the territory of Bulgaria and is included in the Red Data Book of Bulgaria, vol. 3 (GUSSEV & CONEV 2015). The status of the population is assessed as "Favourable", however the presence of the invasive species *Ailanthus altissima* (Mill.) Swingle could have a negative effect on it in the future.

Probitija kamak, Taušan tepe and *Tepičkite*. The bedrock is Cretaceous limestone, interspersed with marl, clayey marl, and limestone. The three populations are large, with more than 1000 individuals each. The projective vegetation cover varies between 40% and 60%. The associated plant species include *Achillea clypeolata* Sm., *Aster oleifolius* (Lam.) Wagenitz, *Astragalus vesicarius* L., *Campanula sibirica* L., *Carex humilis* Leyss., *Echinops ritro* L., *Inula ensifolia* L., *Jurinea stoechadifolia* (M. Bieb.) DC., *Paliurus spina-christi* Mill., *Salvia nutans* (L.) All., *Satureja coerulea* Janka, *Stipa lessingiana* Trin. & Rupr., and *Teucrium chamaedrys* L. As for Kabiyuška mogila, all three populations belong to habitat F3.2412, subcontinental peri-Pannonic scrub, which is protected in Bulgaria (GUSSEV & CONEV 2015). The *Taušan tepe* population is negatively affected by the spread of *Paliurus spina-christi* scrub. The status of all three populations is assessed as "Favourable".

Kaliakra Reserve and *Vidno village*. The soils are of varying depth, strongly eroded, with the Miocene calcareous bedrock emerging to the surface. *Psephellus marschallianus* grows in dry, grassy and stony places with steppe vegetation dominated by *Koeleria brevis* Steven, *Stipa lessingiana* Trin. & Rupr., *Festuca valesiaca* Gaudin, and *Paeonia tenuifolia* L. Both populations are small, each with ca. 100 individuals. The projective vegetation cover varies between 50% and 70%. The species grows together with: *Achillea clypeolata* Sm., *Adonis flammea* Jacq., *Asphodeline lutea* (L.) Rchb, *Euphorbia myrsinites* L., *E. nicaeensis* All., *Goniolimon collinum* (Griseb.) Boiss., *Iris pumila* L., *Paeonia tenuifolia* L., *Rhodax canus* (L.) Fuss, *Satureja coerulea* Janka, *Thymus zygioides* Griseb., and *Ziziphora capitata* L. According the EUNIS These two populations of *P. marschallianus* belong to habitat 08E1, Western Pontic stony steppes, which is protected on the territory of Bulgaria and is included in the Red Data Book of Bulgaria, vol. 3 (CONEV & al. 2015). A major threatening factor is the destruction of the habitat in which the species occurs. The status of the both populations is defined as "Unfavorable" to "Poor".

***Psephellus* sect. *Odontolophus* (Cass.) Wagenitz & Hellwig** (*Odontolophus* Cass.)
Type: *Odontolophus cyanoides* Cassini, nom. illeg. (*Centaurea trinervia* Stephan ex Willd., *Psephellus trinervius* (Willd.) Wagenitz)

This section comprises three species, of which *Psephellus trinervius,* distributed from Romania (Carpathians) and Bulgaria through S Russia, the Ukraine and the Crimea to Caucasia, is best known. The two other species are Caucasian (WAGENITZ & HELLWIG 2000).

Psephellus trinervius (Willd.) Wagenitz in Willdenowia 30: 39. 2000 (*Centaurea trinervia* Stephan ex Willd., Sp. Pl. 3: 2301. 1803).

Morphology and biology. Herbaceous perennial, sparsely floccose-tomentose. Stems erect or suberect, up to 30 cm tall. Leaves linear-lanceolate, entire, acute, 30-70 × 3-5 mm; the upper ones sessile, 3-veined. Upper part of flowering stems leafless. Capitula solitary. Involucre 12-15 × 6-10 mm; phyllaries pale green, with a darker, brown apex; middle phyllaries oblong-ovate. Phyllary appendages not or very shortly decurrent, creamy, pectinate-laceratae. Florets pink (Fig. 3). Fl. VI, fr. VII. (PETROVA 2015).

Distribution in Bulgaria. North-East Bulgaria (near Nevša village, Varna district); at elevations up to 300 m.

Habitats and population. Grows in dry, sunny places on clayey marl, with sparse vegetation. Participates in subcontinental peri-Pannonic scrub communities. The single known population, near Nevsha village, consists of 2 fragments – *Taušan Tepe* and *Tepičkite* (Fig. 2).

Conservation status. Critically Endangered (CR) [D]. Tertiary relict.

Monitoring and assessment of the population status. Psephellus trinervius inhabits Cretaceous limestones, interspersed with marl, clayey marl and limestone rock. Both population fragments were subject to monitoring in 2012, 2017 and 2018. In 2012 the first fragment (*Taušan Tepe*) numbered 38 individuals, whereas the second (*Tepičkite locality*), only four. The projective vegetation cover varied between 40% and 50%. Among the associated species were *Achillea clypeolata* Sm., *Aster oleifolius* (Lam.) Wagenitz, *Astragalus vesicarius* L., *Campanula sibirica* L., *Carex humilis* Leyss., *Echinops ritro* L., *Inula ensifolia* L., *Jurinea stoechadifolia* (M. Bieb.) DC., *Paliurus spina-christi* Mill., *Psephellus marschallianus* (Spreng.) C. Koch, *Salvia nutans* (L.) All., *Satureja coerulea* Janka, *Stipa lessingiana* Trin. & Rupr., and *Teucrium chamaedrys* L. The single Bulgarian population of *P. trinervius* pertains to habitat

F3.2412, subcontinental peri-Pannonic scrub, protected in Bulgaria (GUSSEV &TZONEV 2015). The most important threatening factor is the low regeneration ability of the species. At *Taushan tepe,* another negative factor was identified: the spread of *Paluirus spina-christi* scrub.

Fig. 3. *Psephellus trinervius* Habit.

In the spring of 2016, in order to support the population, branches of the competitive *P. spina-christi* were pruned (Fig. 4). During our monitoring in autumn 2017 we noted that the number of individuals of *P. trinervius* at Taušan tepe had increased to 48 (10 more than in 2013); it remained unchanged in 2018. The number of individuals at Tepičkite remained stable during the entire monitoring period. One may, thus, conclude that the activities conducted *in situ* had a favourable effect on the *P. trinervius*' population. Nevertheless, the status of the population remains assessed as "Unfavourable" to "Poor".

Conclusions

The populations of *Psephellus marschallianus* inhabiting "F3.2412, subcontinental peri-Pannonic scrub" are in a better condition than those of "08E1, western Pontic stony steppes". We believe that, in order to preserve the single Bulgarian population of *P. trinervius*, it is necessary to continue annual monitoring and correlated *in situ* activities. To this effect, a proposal to the Environmental Agency for inclusion of the species in the National Biodiversity Monitoring System has been prepared.

Fig. 4. *In-situ* conservation action (clearing of branches of *P. spina-christi*) in the population of *P. trinervius* at Taushan tepe locality.

Acknowledgements

Financial support by the National "Scientific Research" Fund of Bulgaria, under Grant DN 01/7-16.12.2016, "Flora of the Republic of Bulgaria, vol. 12: Biological Diversity in Asteraceae subfam. Carduoideae and Cichorioideae" is gratefully acknowledged.

References

Asjov, B., Dimitrov, D. Vasilev, R. & Petrova, A. (eds) 2002: Konspekt na vissata flors ns Bălgarija. Horologija i florni elementi. – Sofija.

Bancheva, S. 2009: *Psephellus marschallianus* (Spreng.) K. Koch. In Petrova, A. & Vladimirov, V. (eds), Red List of Bulgarian vascular plants. – Phytol. Balcan. 15: 79.

— 2015. P. 582: In Peev, D., Petrova, A., Ančev, M., Temniskova, D., Denčev, C., Ganeva, A., Čavdar, G. & Vladimirov, V. (eds), Red Data Book of the Republic of Bulgaria, Vol. 1. Plants and fungi. – Sofia.

Biological Diversity Act, 2002: Decree no. 283 accepted by the 39th National Assembly on 02 August 2002. – Durżaven Vestn. 77 [9.8.2002]: 9-42 [in Bulgarian].

Biological Diversity Act, 2007: Decree no. 354 accepted by the 40th National Assembly on 01 November 2007. – Durżaven Vestn. 94 [16.11.2007]: 2-44 [in Bulgarian].

Boissier, E. 1875: Flora orientalis 3, sive enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum. – Genève & Lyon.

Conev, R., Roussakova, V. & Dimitrov, M. 2015: 08E1 Western Pontic stony steppes. Pp. 148-

150. In BISERKOV, V., GUSSEV, C., POPOV, V., HIBAUM, G., ROUSSAKOVA, V., PANDURSKI, I., UZUNOV, Y., DIMITROV, M., TZONEV, R. & TZONEVA, S. (eds), Red Data Book of the Republic of Bulgaria, Vol. 3. Natural habitats. – Sofija.

DOSTÁL, J. 1973: Preliminary notes on the subtribe *Centaureinae*. – Acta Bot. Acad. Sci. Hung. 19: 73-79.

— 1976: *Centaurea* L. Pp. 254-301. In TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M. & WEBB, D. A. (eds), Flora europaea, 4. – University Press, Cambridge.

GABRIĖLJAN, Ė. 1995: *Psephellus* Cass. Pp. 336-352. In TAHTADŽJAN, A. (ed.), Flora Armenii, 9, Campanulaceae, Asteaceae. – Havlickuv Brod.

GUSSEV, H. & CONEV, R. 2015: 18F3 Subcontinental steppe scrub. Pp. 243-245. In BISERKOV, V., GUSSEV, CH., POPOV, V., HIBAUM, G., ROUSSAKOVA., PANDURSKI, I., UZUNOV, J., DIMITROV, M., TSONEV, R. & TSONEVA, S. (eds), Red Data Book of the Republic of Bulgaria, Vol. 3. Natural habitats. – Sofija.

KLOKOV, M. V. 1963: *Centaurea* podrod 6. *Heteroloplus* (Cass.) Dobrocz. Pp. 463-472. In BOBROV, E. G. & ČEREPANOV, S. K. (eds), Flora SSSR, 28. – Moskva & Leningrad.

KOŽUHAROV, S. (ed.) 1992: A field guide to the Bulgarian vascular plants. – Sofija [in Bulgarian].

PETROVA, A. 2009: *Centaurea trinervia* Stephan ex Willd. In PETROVA, A. & VLADIMIROV, V. (ed.), Red List of Bulgarian vascular plants. – Phytol. Balcan. 15: 67.

— 2015: *Centaurea trinervia* Stephan ex Willd. P. 210. In PEEV, D., PETROVA, A., ANČEV, M, TEMNISKOVA, D., DENČEV, C., GANEVA, A., ČAVDAR, G. & VLADIMIROV, V. (eds), Red Data Book of the Republic of Bulgaria, Vol. 1. Plants and fungi. – Sofia.

SOSNOVSKY, D. 1948: Obzor predstavitelej roda *Psephellus* (Cass.) D. Sosn. [Specierum caucasicarum generis *Psephelli* (Cass.) D. Sosn. emend. revisio]. – Zametki Sist. Geogr. Rast. 14: 5-22 [in Russian].

SUSANNA, A. & GARCIA-JACAS, N. 2007: Tribe Cardueae Cass. Pp. 123-147. In KUBITZKI, K. (ed.). The families and genera of vascular plants, Vol. VIII, *Asterales*. – Springer, Berlin, Heidelberg, New York.

WAGENITZ, G. & HELLWIG, F. H. 2000: The genus *Psephellus* Cass. (Compositae, Cardueae) revisited with a broadened concept. – Willdenowia 30: 29-44.

Contribution to the orchid flora of mount Simvolo (E Macedonia - NE Greece)

SPYROS TSIFTSIS

Abstract

Tsiftsis, S. 2018: Contribution to the orchid flora of mount Simvolo (E Macedonia - NE Greece). Bot. Chron. 22: 117-126.

This is the first review of the 22 orchid taxa growing on Mt. Simvolo (Σύμβολον). All of them are first records for the mountain. Compared to other mountains of E Macedonia (NE Greece), Mt. Simvolo hosts a small number of orchid species, all with a relatively restricted distribution. No endemic or but locally distributed orchids are present, But the record of Serapias parviflora, being the northernmost occurrence in Greece, is phytogeographically relevant.

S. Tsiftsis, Department of Forestry and Natural Environment, International Hellenic University, GR-66100, Drama, Greece; E-mail: stsiftsis@teiemt.gr

Key words: East Macedonia, orchids, distribution.

Introduction

Mount Simvolo is located in E Macedonia (NE Greece), between Pieria basin in the north (south of Mt. Pangeon) and the Aegean coast in the south, and administratively belongs to the prefecture of Kavala. It extends in a WSW to ENE direction and its area is circumscribed by the coordinates 40° 42' to 40° 57' N and 23° 57' to 24° 22' E. To the west, it is bordered by the plain of Strimonas river, whereas to the east it reaches the city of Kavala (Fig. 1). Contrary to other mountainous ranges of E Macedonia, Mt. Simvolo is much lower, a range of hills rather than mountains, peaking at the summit Agriada (693 m a.s.l.). Despite its low-elevation, the area's relief is pronounced, with several streams in deep valleys with steep slopes.

Geologically, the study area belongs to the crystalline mass of Rodopi (MOUNTRAKIS 1985), dominated by extensive masses of igneous rocks (granites) in the eastern part and by limestone, gypsum and marls, and gneiss in the central and western part (ANONYMOUS 1983). From a bioclimatic point of view, the area belongs in two zones. The low-altitude areas pertain to the semi-arid bioclimatic zone with mild winters, whereas the higher altitudes pertain to the subwet zone with mild winters (MAVROMMATIS 1980).

Fig. 1. Map of E Macedonia.

The only published information on the flora and vegetation of Mt. Simvolo is found in the study of ELEFTHERIADOU & al. (2005), who dealt with the woody taxa of the area. The vegetation, especially at the lower levels, is mainly dominated by formations of maquis and pseudo-maquis; and their density depends on and is affected by grazing and wildfires. Contrary to previous times, the wildfires have decreased during the last two decades, and as a result the scrub formations became denser. At higher elevations, in addition to *Quercus coccifera* L., *Carpinus orientalis* Mill., *Fraxinus ornus* L., and *Quercus pubescens* Willd. are present, the latter of which formed extensive forests in the past. In the higher parts of maquis-pseudomaquis zone, especially at elevations of above 500 m, *Q. coccifera* is replaced by *Q. frainetto* Ten., which forms pure stands in sites with favourable conditions. Along permanent water courses, a narrow wooded strip of *Platanus orientalis* L. is found, whereas in eroded or cleared areas, reafforestation with pines have occurred in the past. Natural vegetation formations mostly occur in at higher elevations and where the relief precludes agriculture. In not steeply sloping areas, especially at lower elevations in the western and southern part of the mountain, vineyards or olive groves prevail. In terms of orchid records, Simvolo is the least explored mountain area of E Macedonia (NE Greece). The present study, which presents the orchid flora of that specific area and provides information on the distribution and habitats of the recorded taxa, has been prepared to fill this gap of knowledge.

Material and methods

The data presented here are part of an ongoing project focusing on the distribution and conservation of the orchids of Greece. For that specific purpose, multiple excursions have been performed in various localities and habitats during the period 2010 to 2018. Representative specimens, kept in the author's personal herbarium, were collected exclusively from populations with a large number of individuals. Identifications were made in the field. The nomenclature follows Antonopoulos & Tsiftsis (2017) and Tsiftsis & Antonopoulos (2017). For each orchid occurrence, the UTM coordinates (World Geodetic System 1984) of 1 × 1 km grid cells are noted and mapped; and the number of cells and the corresponding vegetation types are mentioned. All taxa have been recorded in two 100 × 100 km grid cells, namely GL (34T UTM zone) and KF (35T UTM zone). For simplification, the UTM zone is not included in the 1 x 1 km grid cell designations.

Results and Discussion

List of taxa

Anacamptis morio subsp. *caucasica* (K. Koch) H. Kretzschmar & al. [14 grid cells: KF5015, KF5017, KF5217, KF5218, KF7422, KF7522, KF7126, KF6527, KF7027, KF6728, KF6730, KF7131, KF7731, KF7932]. In *Erica manipuliflora* and *E. arborea* formations, in openings of *Quercus coccifera* scrub and in grassland.

Anacamptis papilionacea (L.) R. M. Bateman & al. subsp. *papilionacea* [1 grid cell: KF5520]. In openings of heavily grazed *Quercus coccifera* scrub.

Anacamptis pyramidalis (L.) Rich. [6 grid cells: KF5713, KF5614, KF5714, KF7526, GL5017, GL5218]. In grassland and in openings of *Quercus coccifera* scrub.

Cephalanthera longifolia (L.) Fritsch [18 grid cells: KF5013, KF5413, KF5614, KF4717, KF4718, KF5018, KF5218, KF4919, KF5020, KF5120, KF5620, KF5121, KF5523, KF6224, KF6324, KF6425, GL5117, GL5218]. In *Quercus coccifera* shrub formations, in *Quercus frainetto* stands and in pine reforestations.

Dactylorhiza romana (Sebast.) Soó [5 grid cells: KF5721, KF5722, KF5822, KF7422, KF6527]. In *Quercus frainetto* stands and in *Erica manipuliflora* and *E. arborea* formations.

Himantoglossum jankae Somlyay & al. [8 grid cells: KF5017, KF5217, KF5720, KF5721, KF6425, KF6928, KF6929, GL5114]. In openings of *Quercus coccifera* scrub and in pine groves.

Limodorum abortivum (L.) Sw. [6 grid cells: KF5413, KF5815, KF4920, KF6324, KF7733, GL5117]. In *Quercus coccifera* scrub and *Quercus frainetto* stands.

Neotinea tridentata (Scop.) R. M. Bateman & al. [5 grid cells: KF5017, KF4718, KF6928, GL5017, GL5218]. In grassland and in openings of *Quercus coccifera* scrub.

Ophrys apifera Huds. [2 grid cells: KF5614, KF7932]. In grasslands with scattered shrubs of *Paliurus spina-christi* and in openings of *Quercus coccifera* scrub.

Ophrys epirotica (Renz) Devillers-Tersch. & Devillers [1 grid cell: KF5614]. In grasslands with scattered shrubs of *Paliurus spina-christi*.

Ophrys grammica (B. Willing & E. Willing) Devillers-Tersch. & Devillers [1 grid cell: KF7426]. In openings of *Quercus coccifera* scrub.

Ophrys mammosa Desf. [33 grid cells: KF5714, KF5814, KF4717, KF4817, KF5017, KF5217, KF4718, KF5018, KF5318, KF4919, KF4920, KF5020, KF6220, KF5721, KF5822, KF5523, KF5723, KF5724, KF6224, KF6425, KF6925, KF7225, KF7326, KF7426, KF7227, KF7028, KF6730, KF6831, KF7131, GL4917, GL5017, GL5117, GL5218]. In *Quercus coccifera* scrub, in grassland, in pine groves, and on roadsides.

Ophrys oestrifera M. Bieb. subsp. *oestrifera* [5 grid cells: KF5614, KF5020, KF5520, KF7426, KF7526]. In *Quercus coccifera* scrub and its openings.

Ophrys reinholdii Spruner ex Fleischm. [1 grid cell: KF6425]. In grasslands as openings in *Quercus coccifera* formations.

Orchis italica Poir. [7 grid cells: KF5013, KF5515, KF5217, KF5118, KF5020, KF5520, GL5114]. In grasslands, in openings of Quercus coccifera scrub and in pine groves.

Orchis provincialis Balb. ex Lam. & DC. [1 grid cells: KF6324]. In grasslands with *Pteridium aquilinum*.

Orchis purpurea Huds. [5 grid cells: KF5013, KF5614, KF5018, KF5020, KF7526]. In dense *Quercus coccifera* formations and in their openings.

Orchis simia Lam. [1 grid cell: KF5722]. In edges of dense *Quercus coccifera* formations.

Platanthera chlorantha (Custer) Rchb. subsp. *chlorantha* [2 grid cells: KF6324, KF6527]. In *Quercus frainetto* stands.

Serapias parviflora Parl. [1 grid cell: KF5017]. In grassland.

Serapias vomeracea (Burm. f.) Briq. [32 grid cells: KF5017, KF6719, KF680, KF6921, KF7021, KF7121, KF7122, KF7222, KF7322, KF7422, KF7522, KF6924, KF7124, KF7424, KF6925, KF7025, KF7125, KF7425, KF7126, KF7226, KF7326, KF7426, KF6527, KF7027, KF7127, KF7227, KF7529, KF6730, KF6831, KF7731, KF7832, KF7932]. In *Quercus coccifera* shrub formations and in grasslands.

Spiranthes spiralis (L.) Chevall. [26 grid cells: KF5819, KF6719, KF6820, KF5721, KF6921, KF7121, KF5822, KF7122, KF7222, KF7322, KF6924, KF6425, KF6725, KF6925, KF7025, KF7125, KF7126, KF7426, KF7526, KF6527, KF7127, KF6728, KF7128, KF7529, KF7731, KF7832]. In *Quercus coccifera* shrub formations, in grasslands and in pine reforestations.

In total, 22 different species have been recorded so far on Mt. Simvolo, which occur in 83 out of the 384 1 x 1 km grid cells of the total area (Fig. 2, Figs 3-24). As there are no other studies on the orchid flora of Mt. Simvolo, all the data presented here are new records for the area. The eastern part of Mt. Simvolo is dominated by acid igneous rocks (granite) (ANONYMOUS 1983) and by consequence the soils are poorly suited for most orchids (TSIFTSIS & al. 2008). One might have expected that the western part of the mountain would host a larger number of orchids due to the prevailing of bedrock types (e.g. marls, limestone) better suited for orchids, and to its milder climatic conditions. Due to anthropic impact (mostly wildfires and grazing), in the western part, one expects that the habitat type "semi-natural dry grasslands and scrubland facies" prevails – a habitat type which, according to the interpretation manual of European

Union Habitats (ANONYMOUS 2013), is characterised as “important orchid site”, both in terms of species number and population size. However, in the western part of the area, large surfaces have been converted to agricultural lands, and the remaining scrub formations are too dense or stock on steep slopes, where the lack of open spaces does not support orchids. Even so, the number of orchids recorded for the western part of the mountain exceeds that for the eastern part (see Figs 3-24).

The study area’s most common orchids are *Ophrys mammosa*, *Serapias vomeracea* and *Spiranthes spiralis*. *Ophrys mammosa* does not show any clear preference as regards the geological substrate or vegetation type, whereas on the contrary, both *S. vomeracea* and *S. spiralis* were recorded mostly in scrub formations of *Q. coccifera* and *Cistus creticus* L. subsp. *creticus*, on granite. The soil type that derives from granite weathering is rich in sand, and as a result it becomes very dry during spring and summer (PAPAMICHOS 1992). *S. vomeracea* in particular, which has been recorded numerous sites (within 32 grid cells), was growing in most cases in mossy micro-habitats where soil water conditions were better near the surface. In suitable micro-habitats *S. vomeracea* formed colonies consisting of hundreds of individuals.

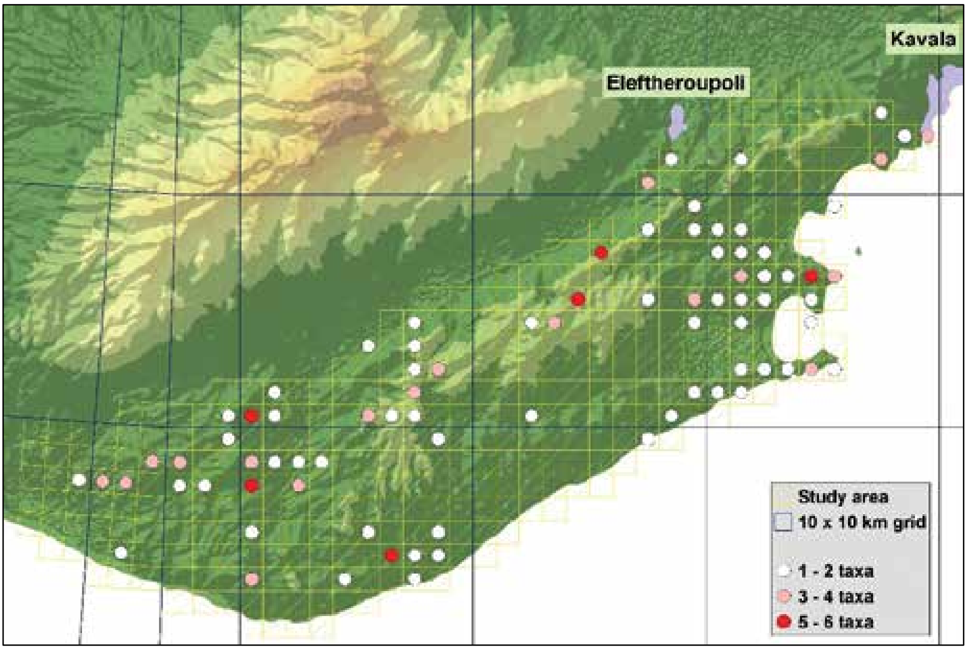
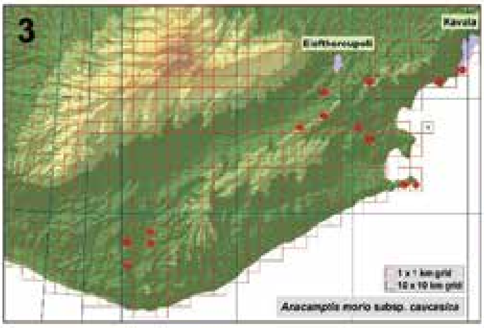
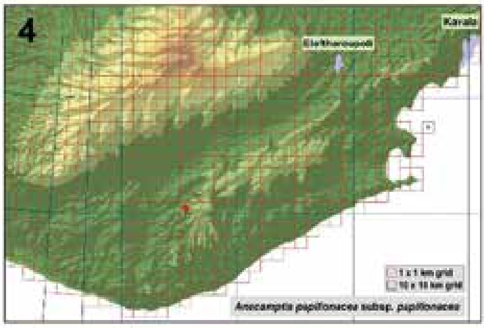
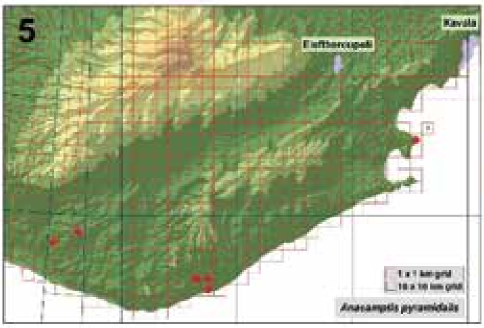


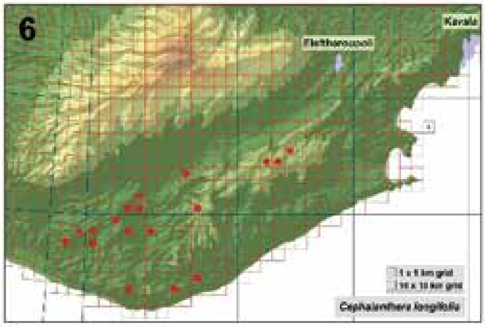
Fig. 2. Distribution of orchid species richness on Mt. Simvolo.

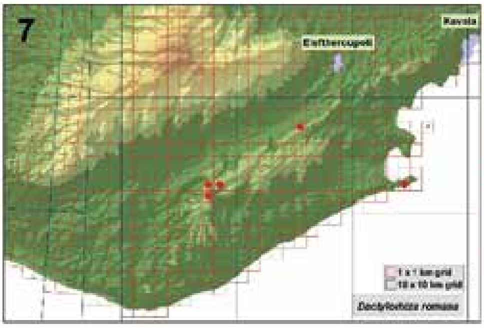
Figs 3-24. Distribution maps of the orchids of Mt. Simvolo.

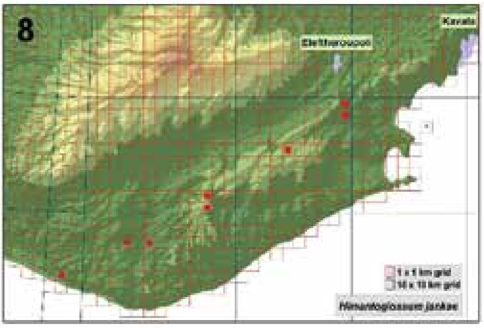


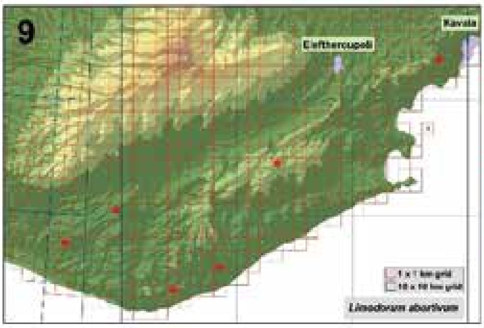


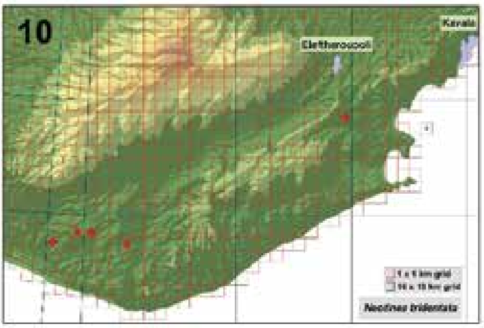


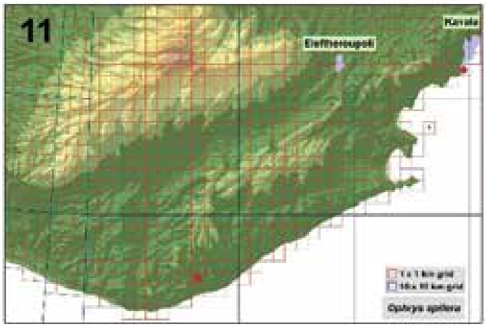


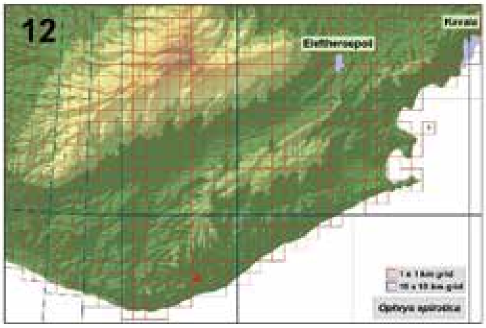


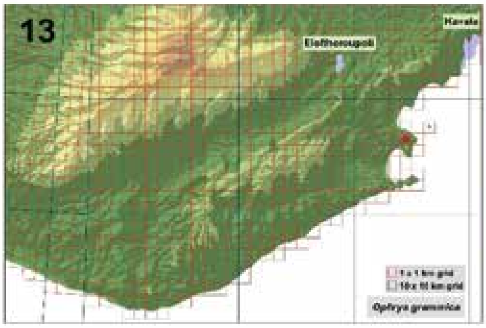


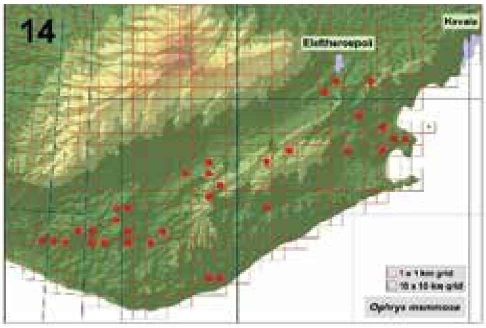


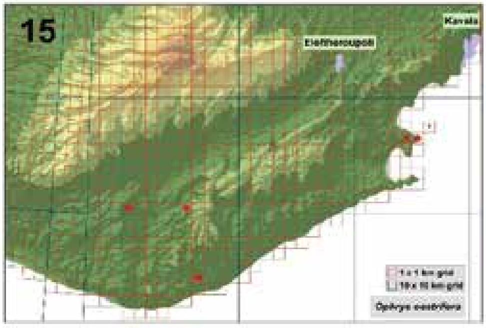


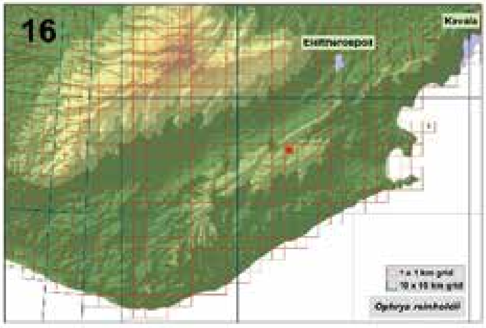

11
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys apifera

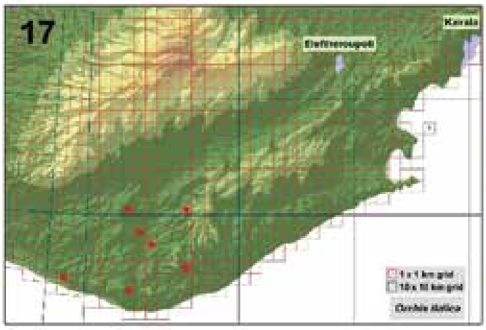

12
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys epirotica

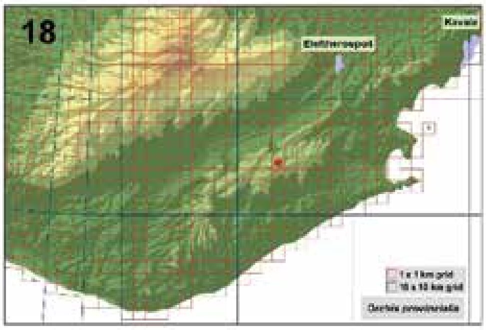

13
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys grammica

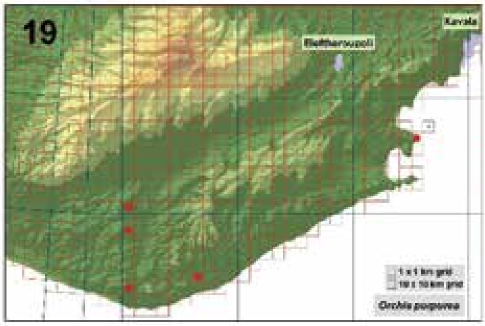

14
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys mammosa

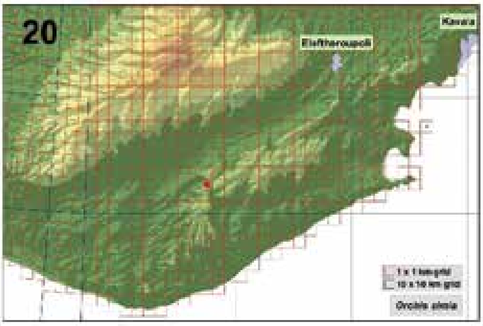

15
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys oestrifera

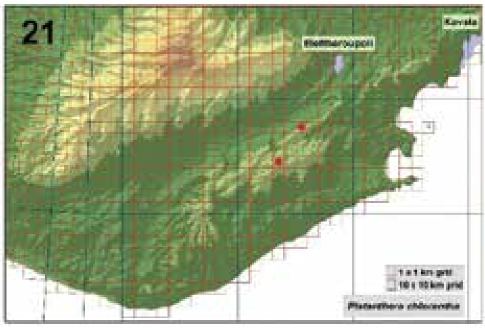

16
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Ophrys reinholdii

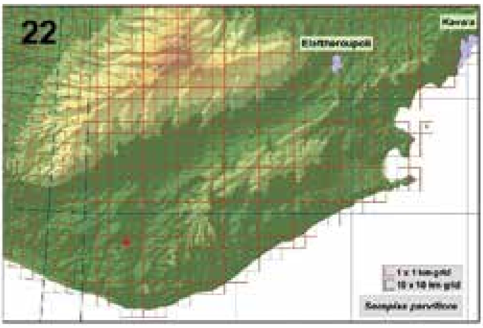

17
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Orchis italica

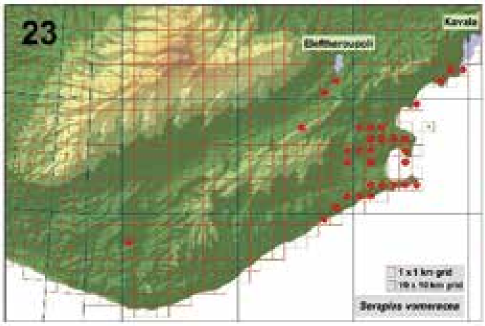

18
Kavala
Eleftheroupoli
1 x 1 km grid
10 x 10 km grid
Orchis provincialis

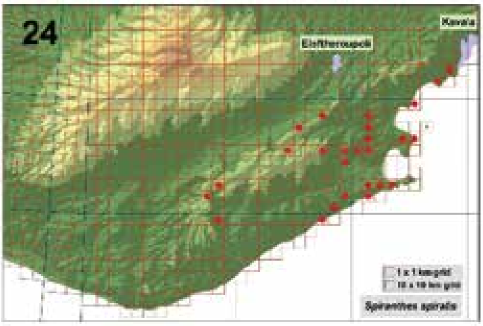

19


20


21


22


23


24

On the contrary, *Anacamptis papilionacea* subsp. *papilionacea*, *Ophrys epirotica*, *O. grammica*, *O. reinholdii*, *Orchis provincialis*, *O. simia* and *Serapias parviflora* were rare, each having been recorded in a single locality. However – except *S. parviflora* – all the others have also been recorded to occur in other mountains of E Macedonia (e.g.: *Ophrys reinholdii* and *Orchis provincialis* on Mt Pangeo; *O. epirotica* on Mts Falakro, Vrondous, and Kerdilio) (Antonopoulos & Tsiftsis 2017, Tsiftsis & Antonopoulos 2017). The find of *S. parviflora* is of phytogeographically significant. *S. parviflora* is an Atlantic-Mediterranean taxon that presents a disjunct distribution area along the Atlantic coasts of France, Spain and Portugal, a broad distribution in Italy and Greece, reaching the coastal zone of Albania and Croatia to the north. In Greece, it is widely distributed in the southern and western parts of the country, extending northward to the Corfu and Ioannina prefectures with, additionally, an isolated occurrence near Mt. Olimbos (Tsiftsis & Antonopoulos 2017). The new find of *S. parviflora* extends considerably its Greek distribution range, being the country's northernmost and most isolated known locality.

The second most interesting find is that of *Ophrys reinholdii*, seen in a single location in an opening of *Q. coccifera* scrub. Together with its localities on Mts Pangeo and Kerdilio as well as in the lowlands close to the Nestos Delta, the new locality denotes to northern borderline of that species' Greek distribution (ANTONOPOULOS & TSIFTSIS 2017). In all these locations at the edge of its distribution, only a few isolated individuals of *O. reinholdii* were seen. Such populations or colonies near to the edge of a species' range either represent the remnants of a past, larger population or result from long-distance seed dispersal (CAREY 1999, VANDEN BROECK & al. 2014, BLEHO & al. 2015).

As shown in Table 1, in the two most species-rich grid cells six orchid taxa were recorded, in four grid cells five orchids were found, and in five grid cells, four orchids. Conversely, 59 grid cells (c. 70% of the total number) host only one or two orchid taxa. The six most species-rich grid cells are concentrated in the central and western part of the study area and are characterized by the dominance of calcareous bedrock; whereas, the most species-poor grid cells lie in the eastern part of Mt. Simvolo, where the granitic mass is centred.

Table 1. Relationship between orchid taxon number and number of grid cells.

Number of recorded orchids	Number of 1×1 km grid cells
6	2
5	4
4	5
3	13
2	31
1	28

In conclusion, Mt. Simvolo is rather poor in orchid taxa, contrary to what one might expect in view of the geographical position of the area. However, both the favourable climatic conditions and the short distance of Mt. Simvolo to the other mountains of E Macedonia, which are known to be especially rich in orchids (TSIFTSIS & al. 2007), make it likely that further phytogeographically interesting records will come to light in the future.

References

ANONYMOUS, 1983: Geological map of Greece, 1:500,000. – IGME, Athens.

— 2013: Interpretation manual of European Union Habitats, EUR 28. – European Commission, DG Environment, Nature and Biodiversity.

ANTONOPOULOS, Z. & TSIFTSIS, S. 2017: Atlas of the Greek orchids, 2. – Rethymno.

BLEHO, B. I., KOPER, N., BORKOWSKY, C. L. & HAMEL, C. D. 2015: Effects of weather and land management on the Western Prairie Fringed-orchid (*Platanthera praeclara*) at the northern limit of its range in Manitoba, Canada. – Am. Midl. Nat 174: 191-203.

CAREY, P. D. 1999: Changes in the distribution and abundance of *Himantoglossum hircinum* (L.) Sprengel (Orchidaceae) over the last 100 years. – Watsonia 22: 353-364.

ELEFTHERIADOU, E., THEODOROPOULOS, K. & DIMOPOULOS, P. 2005: Simvoli sti chlorida tou Simvolou Orous: ta ksilodi fita (Contribution to the flora of Symvolo: the woody plants). Pp. 67-74. In Proceedings of the 12th Panhellenic Forestry Congress, Drama, 2-5 Oct. 2005.

MAVROMMATIS, G. 1980: To vioklima tis Elladas. Sxesis klimatos kai fysikis vlastiseos. Vioklimatiki chartes. (Le bioclimat de la Grèce, relations entre le climat et la végétation naturelle. Cartes bioclimatiques). – Dasiki Erevna 1: 1-63.

MOUNTRAKIS, M. D. 1985: Geologia tis Ellados (Geology of Greece). – Thessaloniki.

PAPAMICHOS, N. 1992: Dasika Edafi (Forest Soils), ed. 2. – Thessaloniki.

TSIFTSIS, S. & ANTONOPOULOS, Z. 2017: Atlas of the Greek orchids, 1. – Rethymno.

—, KARAGIANNAKIDOU, V. & TSIRIPIDIS, I. 2007: The orchid flora of East Macedonia (NE Greece). – J. Eur. Orch. 39(3/4): 489-526.

—, TSIRIPIDIS I., KARAGIANNAKIDOU, V. & ALIFRAGIS, D. 2008: Niche analysis and conservation of the orchids of East Macedonia (NE Greece). – Acta Oecol. 33: 27-35.

VANDEN BROECK, A., VAN LANDUYT, W., COX, K., DE BRUYN, L., GYSELINGS, R., OOSTERMEIJER, G., VALENTIN, B., BOZIĆ, G., DOLINAR, B., ILLYÉS, Z. & MERGEAY, J. 2014: High levels of effective long-distance dispersal may blur ecotypic divergence in a rare terrestrial orchid. – BMC [BioMed Central] Ecol. 14: 20.

Contribution to the knowledge of the orchid flora of Lefkas island (Ionian Islands, Greece)

ELENI KATOPODI & SPYROS TSIFTSIS

Abstract

Katopodi, E. & Tsiftsis, S. 2019: Contribution to the knowledge of the orchid flora of Lefkas island (Ionian Islands, Greece). – Bot. Chron.22: 127-143.

In the present study, up to date distribution data of the orchids of Lefkas island are presented. In total, 37 orchid taxa have been recorded, and among them the *Epipactis helleborine* subsp. *helleborine*, is new for the island, and the occurrence of *Orchis provincialis*, is being confirmed. The present work can be the basis for future and more specified studies aiming in monitoring the orchid populations occurring on Lefkas island and providing management measures that will help local authorities to prevent negative effects that cause orchids' declining.

Katopodi Eleni, Pantokratoros 14 str., GR31100, Lefkas, Greece.
E-mail: elkatopodi@hotmail.com
Tsiftsis Spyros, Department of Forestry and Natural Environment, International Hellenic University, GR-66100, Drama, Greece. E-mail: stsiftsis@teiemt.gr

Key words: Lefkas island, Ionian Islands, orchids, distribution.

Introduction

The orchid family includes a lot of endangered, vulnerable and rare species, which are under the protection of conventions (e.g. Bern convention), international and national laws [PRESIDENTIAL DEGREE 67/1981 (30-1-1981)] or are included in directives [DIRECTIVE 92/43/EEC (21-5-1992)] and Red Data Books (PHITOS & al. 1995, PHITOS & al. 2009). Moreover, all the taxa of this family are protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (HÁGSATER & DUMONT 1996).

Greece is particularly rich in orchids, having approximately 200 taxa (TSIFTSIS & ANTONOPOULOS 2017, ANTONOPOULOS & TSIFTSIS 2017), most of which are distributed in the southern part of the country, whereas a second species rich area is found in northwestern Greece and along the Ionian Islands. Ionian Islands, and especially the largest ones (Corfu, Lefkas, Kefallinia and Zakynthos) by far have been the centre of the orchidological interest of many orchid enthusiasts and researchers. Despite however the fact that Lefkas Island is a good destination for those who want to visit Greece to search and admire orchids, only three papers focusing on orchids have been published so far (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992), whereas some others refer specific species (e.g. SCHLÜTER & al. 2009, PAULUS & HIRTH 2014).

It is of great surprise that these papers were published at least 25 years ago, and no other systematic efforts have been performed. Based on this gap of knowledge, the aims of the present study are to present an up to date list of the orchids of Lefkas and to provide information on the distribution and the habitats of the taxa.

Materials and methods

Lefkas is the fourth largest island of the Ionian Archipelago, covering an area of c. 300 km^2, and is mostly considered a mountainous island with an average elevation of 500 m, while being the second highest (1158 m a.s.l.) island after Kefallinia (Fig. 1).

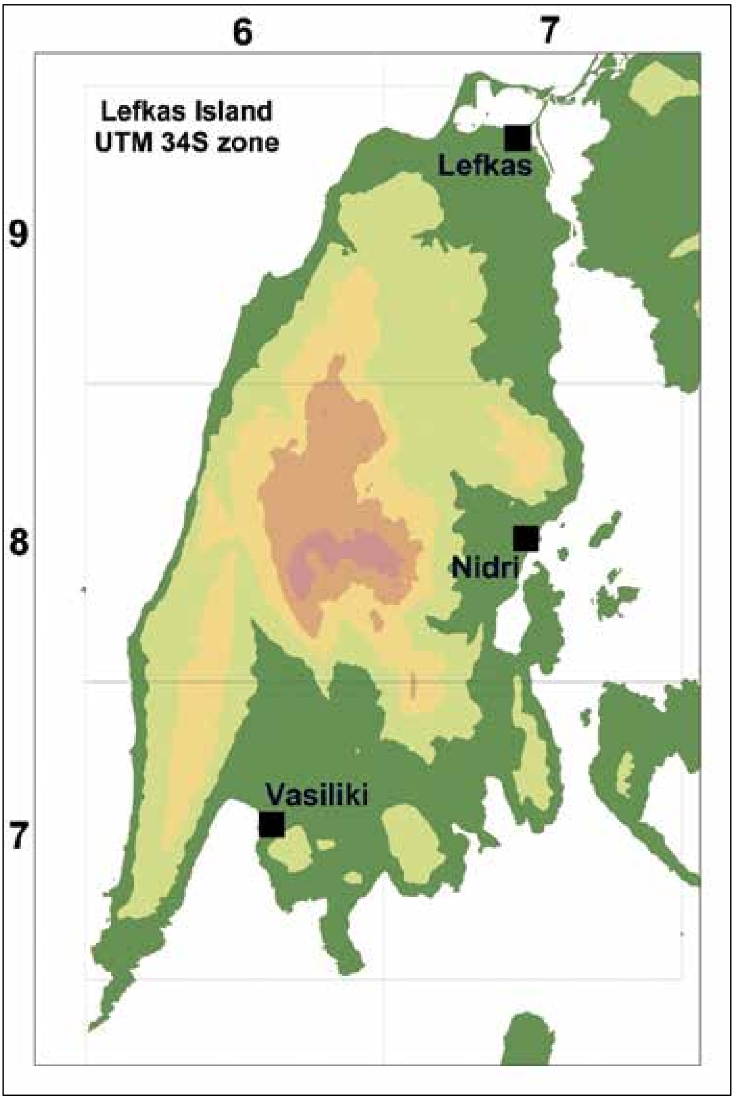


Fig. 1. Map of the study area.

Geologically, Lefkas island forms part of the External Hellenides and belongs to the Paxos (Pre-Apulian) and Ionian units (MOUNTRAKIS 1985), and in particular it consists of the carbonate sequence of the Ionian unit and the limestones of Paxos unit. The Ionian unit extends to the major part of the island, whereas the Paxos unit is restricted at the southwestern part (Lefkata peninsula) (MOUNTRAKIS 1985, TRIANTAFYLLOU 2010). The climate of the island is characterized Mediterranean. It is generally humid with mild winter, and the xerothermic period lasts approximately four months, from mid May to mid September (MAVROMMATIS 1980).

The data presented here are part of an ongoing project focusing in the distribution and conservation of the orchids of Lefkas Island. For the specific purposes, multiple excursions have been performed by the first author in various localities and habitats throughout the island. Identifications were always made during the fieldwork and nomenclature is according to TSIFTSIS & ANTONOPOULOS (2017) and ANTONOPOULOS & TSIFTSIS (2017).

For each orchid, the vegetation types, the flowering period, the number of grid cells, as well as UTM coordinates (WGS84) in 1×1 km spatial resolution where it has been recorded are presented. In cases where the finding locations are few, the nearest village (or the area) and the altitude of the site are also provided. All taxa have been recorded in two 100×100 km grid cell, namely DH and DJ (both covering a part of the 34S UTM zone). For simplification purposes, the UTM zone is not presented in the 1x1 km grid cell list of each taxon. If a taxon has been recorded in a site located in two 1×1 km grid cells (e.g. DH7075/6), both grid cells were counted. Moreover, available information about the known distribution of each orchid in Lefkas Island are also presented.

Results and Discussion

Orchid list

Anacamptis coriophora (L.) R.M. Bateman, Pridgeon & M.W. Chase **subsp. *fragrans*** (Pollini) R.M. Bateman, Pridgeon & M.W. Chase (Fig. 4A).

Habitat: temporary wet field, grasslands, sandy places.
Flowering period: May.
Distribution: [2 grid cells; Fig. 2]. DH7490, Agioi Pateres area (295 m); DJ7700, Avgerinos area (0 m).

It is also referred from the eastern part of the island (WILLING & WILLING 1983).

Anacamptis laxiflora (Lamarck) R.M. Bateman, Pridgeon & M.W. Chase **subsp. *laxiflora*** (Fig, 4B).

Habitat: wet meadows, temporary waterlogged.
Flowering period: April.
Distribution: [3 grid cells; Fig. 2]. DH6576, Vasiliki - Ponti area (0 m); DH7496, Lefkas - Megali Vrisi area (5 m); DH7190, Karia (345 m).

It is also known from several localities all around the island (WILLING & WILLING 1983).

Anacamptis morio* subsp. *caucasica (K.Koch) H.Kretzschmar, Eccarius & H.Dietr.

Habitat: phrygana, arid fields, wet meadows, full sun to semi-shaded places.

Flowering period: ends of March - April.
Distribution: [4 grid cells]. DH6776, Vasiliki (15 m); DH7293, Lazarata (400 m); DH7197, Frini (35 m); DH7095, Melissa Gorge (320 m).

So far, it has been recorded from the northern part of the island (WILLING & WILLING 1983).

Anacamptis pyramidalis (L.) Rich.

Habitat: phrygana, arid fields.
Flowering period: April - May.
Distribution: [10 grid cells]. DH6981, Agios Ilias (610 m); DH6676, Vasiliki (5 m); DH6978, Vournika - Radiki area (320 m); DH7086, Vafkeri (495 m); DH6382, Dragano (375 m); DH6886, Eglouvi (720 m); DH7192, Lazarata (400 m); DH7098, Lefkas - Agios Ioannis area (5 m); DH7095, Melissa Gorge (320 m); DH6884, Mount Elati (890 m).

It is known from several localities (WILLING & WILLING 1983, DELFORGE 1992).

Cephalanthera longifolia (L.) Fritsch (Fig. 2A).

Habitat: in the understory of maquis, on calcareous substrates (limestone).
Flowering period: April.
Distribution: [1 grid cell; Fig. 2]. DH7289, Alexandros (1.5 km NW of the school) (500 m)

So far, it has been recorded in a small number of sites (WILLING & WILLING 1983).

Dactylorhiza romana (Sebast.) Soó (Fig. 3A).

Habitat: in the understory of maquis and in other semi-shade places dominated by shrubs, limestone.
Flowering period: April.
Distribution: [2 grid cells; Fig. 2]. DH7289, Alexandros (1.5 km NW of the school) (450 m); DH7388, Kolivata (425 m).

It is known from a small number of locations towards in the centre of the island (WILLING & WILLING 1983).

Epipactis helleborine (L.) Crantz **subsp. *helleborine*** (Fig. 2C).

Habitat: shady to semi-shaded places, in the understory of *Cupressus sempervirens, Laurus nobilis* and *Arbutus unedo*.
Flowering period: June.
Distribution: [1 grid cell; Fig. 2]. DH7194, Melissa Gorge - Apetasti area (230 m).

This is the first record for the island and was made by the first author in 2018.

Himantoglossum robertianum (Loiseleur) P. Delforge

Habitat: in olive groves, abandoned fields, grasslands, phrygana and forest openings.

Flowering period: January - March.
Distribution: [61 grid cells]. It is widely distributed throughout the island. Finding localities: DH6381, DH6485, DH6585, DH6589, DH6681, DH6683, DH6684, DH6884, DH6887, DH6980, DH6982, DH6989, DH7081, DH7186, DH7282, DH7381, DH7387, DH7388, DH7483, DH7585, DH7586, DH7687, DH7688, DH6279, DH6476, DH6576, DH6579, DH6679, DH6974, DH6977, DH6980, DH7075, DH7079, DH7080, DH7275, DH7278, DH7279, DH7380, DH7377, DH7476, DH6791, DH6693, DH6793, DH6893, DH6992, DH6993, DH7093, DH7097, DH7098, DH7092, DH7192, DH7193, DH7195, DH7295, DH7296, DH7297, DH7395, DH7396, DH7492, DH7494, DH7594.

It has been recorded in several sites so far (WILLING & WILLING 1983, DELFORGE 1992).

Limodorum abortivum (L.) Swartz (Fig. 3B).

Habitat: Semi-shaded to sunny sites, in rocky places.
Flowering period: April - May.
Distribution: [3 grid cells; Fig. 2]. DH6977, Vournikas - Rodaki area (230 m); DH7389, Mount Skari - 300 m SE of Agios Georgios Monastery (520 m); DH7496, Lefkas city edges (10 m).

Rather rare in the island, recorded in a small number of sites (WILLING & WILLING 1983, DELFORGE 1992).

Neotinea lactea (Poiret.) R.M. Bateman, Pridgeon & M.W. Chase (Fig. 5A).

Habitat: phrygana, grasslands and forest openings.
Flowering period: April - May.
Distribution: [4 grid cells; Fig. 2]. DH7284, Neochori (285 m); DH7488, Mount Skari (655 m); DH7175, Marantochori - Pirgos area (145 m); DH6586, Chortata - Papaspori area (620 m).

Rarely recorded in Lefkas, known only from two sites (DELFORGE 1992).

Neotinea maculata (Desf.) Stearn

Habitat: semi-shaded and sunny places in phrygana, grasslands, scrublands and coniferous forests.
Flowering period: March - April.
Distribution: [15 grid cells]. DH7489, Agioi Pateres (295 m); DH6279, Dragano (375 m); DH7186, Vafkeri (493 m); DH6887, Eglouvi (717 m); DH6586, Chortata (625 m); DH7197, Frini (135 m); DH7074, Evgiros (238 m); DH7075/6, Marantochori (235 m); DH7097, Tsoukalades (221 m); DH7189, Alexandros area (430 m); DH7274/5, Sivota (120 m); DH6690, Exanthia (570 m); DH7094, Melissa Gorge (320 m).

So far, it has been recorded in several sites throughout Lefkas (WILLING & WILLING 1983, DELFORGE 1992).

Ophrys apifera Hudson (Fig. 3C).

Habitat: phrygana

Flowering period: May.

Distribution: [1 grid cell; Fig. 2]. DH7175, Sivota (150 m). Just a single individual has been recorded by the first author.

Only known from the northern part of the island (WILLING & WILLING 1983, HÖLZINGER & al. 1985).

Ophrys bilunulata Risso **subsp. punctulata** (Renz) Paulus

Habitat: in sunny to semi-shaded sites, in phrygana and garrigue.

Flowering period: mid February - April.

Distribution: [5 grid cells]. DH7094/5, Melissa Gorge (230m); DH6880, Sivros (230 m); DH6979, Vournikas (330 m); DH7377, Poros (280 m).

Scattered mostly in the southern part of the island (DELFORGE 1992).

Ophrys bombyliflora Link (Fig. 4C).

Habitat: in sunny or semi-shades sites, mostly in phrygana.

Flowering period: March - April.

Distribution: [2 grid cells; Fig. 2]. DH7098, Lefkas - Agios Ioannis area (0 m); DH6976, Marantochori - Agios Georgios area (160 m).

It is scattered, mostly in the eastern part of the island (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992).

Ophrys cephalonica (B. Baumann & H. Baumann) J. Devillers-Terschuren & P. Devillers (Figs 2A, D).

Habitat: phrygana and road edges.

Flowering period: April.

Distribution: [2 grid cells; Fig. 2]. DH6177, Athani (300 m); DH6484, Komilio (495 m).

These are the first published records of *O. cephalonica*, albeit the fact that it was also recorded by STEFAN HERTEL (pers. comm.).

Ophrys ferrum-equinum Desfontaines (including **var. gottfriediana** Renz)

Habitat: in semi wet meadows, grasslands, phrygana, garrigue and abandoned fields.

Flowering period: mid March - April.

Distribution: [59 grid cells]. It is widely distributed throughout the island. Finding localities: DH6381, DH6485, DH6590, DH6681, DH6682, DH6683, DH6882, DH6887, DH6980, DH6981, DH6989, DH7187, DH7282, DH7288, DH7381, DH7383, DH7385, DH7387, DH7582, DH7583, DH7586, DH7590, DH6791, DH6896, DH6992, DH6993, DH7094, DH7096, DH7097, DH7192, DH7193, DH7198, DH7294, DH7296, DH7297, DH7391, DH7395, DH7396, DH7494, DH7492, DH7592, DH7594, DH6279, DH6476, DH6579, DH6675, DH6775,

DH6875, DH6879, DH6880, DH6974, DH7074, DH7075, DH6977, DH7274, DH7275, DH7279, DH7376, DH7377.

var. *gottfriediana* Renz: [13 grid cells]. DH6679, Agios Petros; DH7189, Alexandros area; DH6476, Vasiliki; DH7187, Vafkeri; DH6887, Eglouvi; DH6589, Kalamitsi; DH7279, Katochorion; DH6875, Kontaraina; DH7198, Lefkas - Agios Ioannis area; DH7590, Nikiana; DH6681, Nikoli; DH7297, Frini; DH6585, Chortata.

It has been recorded several times in the island (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992).

Ophrys grammica (B. Willing & E. Willing) J. Devillers-Terschuren & P. Devillers

Habitat: in wet places, grasslands, phrygana and forest openings.
Flowering period: February - mid April.
Distribution: [7 grid cells]. DH6675, Vasiliki (15 m); DH6875, Kontaraina (165 m); DH7075/6, Marantochori (195 m); DH7477, Poros (285 m); DH7174, Sivota (25 m); DH6685, Chortata (620 m).

Rather rare in the island where it has been recorded several times but none of these has been ever published.

Ophrys helenae Renz (Fig. 5B).

Habitat: in abandoned fields and in rocky slopes.
Flowering period: April.
Distribution: [2 grid cells; Fig. 2]. DH7490, Nikiana (50 m); DH7477, Poros (300 m).

It has been recorded a couple of times. So far it was found in the eastern part of the island (WILLING & WILLING 1983, HÖLZINGER & al. 1985).

Ophrys leucadica Renz (Fig. 3D).

Habitat: phrygana and garrigue.
Flowering period: mid April - May.
Distribution: [1 grid cell; Fig. 2]. DH7194, Melissa George - Apetasti area (230 m).

WILLING & WILLING (1983) refer *Ophrys fusca* from Lefkas Island and they recorded it almost throughout the island. However, *O. bilunulata* subsp. *punctulata* and *Ophrys leucadica* were discriminated several years after the excursion of WILLING & WILLING (1983), so it is unclear which taxon has been recorded by these authors.

Ophrys lutea Cavaniles **subsp. *lutea*** (including **var. *phryganae***)

Habitat: In sunny to semi shaded sites, in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: mid March - April.
Distribution: [54 grid cells]. Very common throughout the island. Finding sites: DH6476, DH6478, DH6578, DH6575, DH6675, DH6579, DH6580, DH6875, DH6974, DH7075, DH7076, DH7079, DH7174, DH7175, DH7279, DH7280, DH7476, DH7477, DH6693, DH6991, DH6993, DH7091, DH7092, DH7097, DH7098, DH7198, DH7192, DH7296, DH7298, DH7397, DH7492, DH7594,

DH6381, DH6485, DH6585, DH6589, DH6681, DH6682, DH6887, DH6980, DH6982, DH7186, DH7182, DH7282, DH7281, DH7288, DH7289, DH7382, DH7383, DH7390, DH7484, DH7582, DH7586, DH7590.

var. ***phryganae*****:** [22 grid cells]. DH6693, DH6993, DH7098, DH7191, DH7192, DH7292, DH7296, DH7297, DH7492, DH7592, DH7594/5, DH7282, DH7490, DH6775/6, DH6890/1, DH6977, DH7075, DH7377, DH7477.

Rather common in the island, where it has been recorded several times (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992).

Ophrys mammosa Desfontaines **subsp.** ***mammosa***

Habitat: in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: mid March - April.
Distribution: [51 grid cells]. Widely distributed throughout the island. Finding localities: DH6693, DH6991, DH6993, DH7091, DH7092, DH7097, DH7098, DH7192, DH7296, DH7298, DH7397, DH7492, DH7594, DH6381, DH6485, DH6585, DH6589, DH6681, DH6887, DH6980, DH6982, DH7186, DH7182, DH7282, DH7288/9, DH7281, DH7382, DH7390, DH7484, DH7582, DH7586, DH7590, DH6476, DH6478, DH6578, DH6579/80, DH6575, DH6675, DH6875, DH6974, DH7075/6, DH7079, DH7174, DH7175, DH7279, DH7280, DH7476/7.

Widespread, recorded almost everywhere (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992).

Ophrys mesaritica H.F. Paulus, C. Alibertis & A. Alibertis

Habitat: in rocky places.
Flowering period: January - March.
Distribution: [5 grid cells]. DH6381, Dragano (375 m); D

H7074, Evgiros (238 m); DH7076, Marantochori (110 m); DH7275, Sivota (120 m); DH7179, Fterno (320 m).

So far, it has been recorded just a few times in the lowlands, in sites close to the sea (SCHLÜTER & al. 2009, PAULUS & HIRTH 2014). Maybe some records made by WILLING & WILLING (1983) refer to *O. mesaritica*.

Ophrys montenegrina (H. Baumann & Künkele) J. Devillers-Terschuren & P. Devillers

Habitat: in wet sites, phrygana and in erodes slopes.
Flowering period: end of January - March.
Distribution: [11 grid cells]. DH6676, Vasiliki (15 m); DH6978, Vournikas (330 m); DH6381, Dragano (354 m); DH6974, Evgiros (150 m); DH6875, Kontaraina (165 m); DH6975, Marantochori (195 m); DH7377, Poros (285 m); DH7275, Sivota (25 m); DH6879/80, Sivros (230 m); DH7278, Fterno (340 m).

Previously known from several sites, located in the central and southern part of the island (DELFORGE 1992; referred as *Ophrys herae* Hirth & Spaeth).

Ophrys minuscula (G. & W. Thiele) H. Presser & S. Hertel

Habitat: in garsslands, phrygana, garrigue and abandoned fields.

Flowering period: mid March - April.
Distribution: [21 grid cells]. Widespread throughout the island. Finding places: DH6793, Agios Nikitas (35 m); DH7189, Alexandros area (430 m); DH6675, Vasiliki (15 m); DH7186, Vafkeri (493 m); DH7079, Vournikas (330 m); DH6381, Dragano (375 m); DH6789, Exanthia (570 m); DH6975, Evgiros (238 m); DH6875, Kontaraina (185 m); DH7076, Marantochori (110 m); DH7490, Nikiana - Agioi Pateres area (295 m); DH7377, Poros (285 m); DH7274/5, SIvota (120 m); DH6880/6980, Sivros (230 m); DH6793, Sfakiotes (135 m); DH7094, Melissa Gorge (320 m); DH7197, Frini (45 m); DH7278, Fterno (340 m); DH6585, Chortata (620 m).

WILLING & WILLING (1983) and DELFORGE (1992) identified members of *Ophrys oestrifera* group as *Ophrys cornuta*, and have recorded them in c. 20 sites. Maybe some of these records refer to *O. minuscula*.

Ophrys reinholdii Spruner ex H. Fleischmann

Habitat: in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: mid March - April.
Distribution: [49 grid cells]. It forms small colonies throughout the island. Finding places: DH6693, DH6991, DH6993, DH7091/2, DH7097, DH7098, DH7192, DH7296, DH7298, DH7397, DH7492, DH7594, DH6381, DH6485, DH6585, DH6589, DH6681, DH6878, DH6980, DH6982, DH7186, DH7182, DH7282, DH7281, DH7288/9, DH7381/2, DH7390, DH7484, DH7586, DH7590, DH6476, DH6478, DH6578, DH6579/80, DH6675, DH6875, DH6974, DH7075, DH7079, DH7174, DH7175, DH7279, DH7280, DH7476/7.

It was known from less than 10 sites so far, located in the southeastern part of the island (DELFORGE 1992).

Ophrys sicula Tineo

Habitat: in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: mid March - April.
Distribution: [9 grid cells]. One of the most widespread species occurring in Lefkas island. Some finding places are: DH7282, DH7490, DH6775/6, DH6880, DH6978, DH7075, DH7377, DH7477.

It has been recorded throughout the island so far (WILLING & WILLING 1983, HÖLZINGER & al. 1985, DELFORGE 1992).

Ophrys tenthredinifera Willdenow

Under that taxon, *Ophrys ulyssea* was placed as well.
Habitat: in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: Mid March - April.
Distribution: [15 grid cells]. DH6693, Agios Nikitas (35 m); DH7189, Alexandros area (430 m); DH7186, Vafkeri (493 m); DH6977/8, Vournikas (330 m); DH6381, Dragano (375 m); DH6975, Evgiros (238 m); DH6875, Kontaraina (185 m); DH7076, Marantochori (110 m); DH7490, Nikiana - Agioi Pateres area (295 m); DH7274/5, Sivota (120 m); DH6993, Sfakiotes (135 m); DH7094 & DH7195, Melissa Gorge (320 m).

It has been recorded from both WILLING & WILLING (1983) and DELFORGE (1992) so far, but the number of the 1×1 km grid cells in which it has been recorded was limited (6 grid cells).

Orchis anthropophora (L.) Allioni

Habitat: In sunny to semi-shaded sites, in phrygana, garrigue and forest openings.
Flowering period: mid March - April.
Distribution: [5 grid cells]. DH6275, Athani (375 m); DH6381, Dragano (380 m); DH7293, Lazarata (400 m); DH7377, Poros (220 m); DH7094, Melissa Gorge (320 m).

It was recorded in a limited number of sites so far (WILLING & WILLING 1983, DELFORGE 1992).

Orchis italica Poiret

Habitat: in grasslands, phrygana, garrigue and abandoned fields.
Flowering period: March - April.
Distribution: [36 grid cells]. Very common in the island. Finding places: DH6693, DH6993, DH7191, DH7098, DH7292, DH7296, DH7297, DH7492, DH7592, DH7594/5, DH6381, DH6484, DH6590, DH6585, DH6683, DH6887, DH7186, DH7188, DH7289, DH7381, DH7387, DH7480/1, DH7485, DH7590, DH6279, DH6580, DH6675, DH6875, DH7075, DH7079, DH7278/9, DH7377, DH7477.

It has been recorded many times in the island and was considered as one of the most widespread orchids so far (WILLING & WILLING 1983, DELFORGE 1992).

Orchis pauciflora Tenore

Habitat: phrygana and garrigue in rocky sites, usually on calcareous substrates.
Flowering period: April - May (mid June).
Distribution: [6 grid cells]. DH6279, Athani (375 m); DH6886 & DH6888, Eglouvi (720 m); DH6785, DH6875 & DH6974, Mount Elati (890 m).

Known only from two sites, where it was recorded by WILLING & WILLING (1983).

Orchis provincialis Balbis ex Lamarck & De Candolle (Fig. 3D).

Habitat: in semi-shaded sites.
Flowering period: April.
Distribution: [2 grid cells; Fig. 2]. DH7487/7587, Mount Skari (520 m).

This is the first confirmed record of this species. I was referred by WILLING & WILLING (1983) but without giving any specific information.

Orchis quadripunctata Cyrillo ex Tenore

Habitat: in phrygana, garrigue, abandoned fields, in rocky slopes and on old constructions made of stone, up to 1300 m a.s.l.
Flowering period: April - May.

Distribution: [24 grid cells]. Throughout the island. Finding places: DH6793, DH7094, DH7192, DH7197, DH6381, DH6585, DH6789, DH6880/6980, DH7186, DH7189, DH7490, DH6575/6675, DH6875, DH6975, DH6880/1, DH7076, DH7079, DH7274/5, DH7278, DH7377.

It has been recorded several times so far (WILLING & WILLING 1983, DELFORGE 1992).

Serapias cordigera L. **subsp. *cordigera*** (Fig. 5C).

Habitat: in sunny sites and in the edges of maquis formations. On calcareous substrates.

Flowering period: April.

Distribution: [2 grid cells; Fig. 2]. DH7189, Alexandros area - 1.5 km NW of the local school (500 m); DH7075, Marantochori (100 m).

It was known only from a one site, located in the eastern part of the island (WILLING & WILLING 1983). Afterwards, it has been recorded in a few more sites but these findings remained unpublished [ANTONOPOULOS (pers. comm.)]

Serapias lingua L.

Habitat: phrygana, garrigue, grassland and in abandoned fields.

Flowering period: March - April.

Distribution: [60 grid cells]. One of the most widely distributed orchids, occurring throughout the island. Finding sites: DH6171, DH6279, DH6476, DH6576, DH6579, DH6580, DH6675, DH6676/6776, DH6875, DH6977, DH6980, DH7075, DH7079, DH7175, DH7274, DH7279, DH7280, DH7377, DH7476/7, DH6381, DH6590, DH6982, DH6989, DH7081, DH7087, DH7188, DH7282, DH7284, DH7289/7389, DH7381, DH7387, DH7484/5, DH7490, DH7583, DH7586, DH7588, DH6693, DH6792, DH6794, DH6891, DH6992/3, DH6993, DH7092, DH7098/9, DH7099, DH7192, DH7194/5, DH7197, DH7296, DH7297, DH7492, DH7494, DH7295.

It was recorded in several sites so far (WILLING & WILLING 1983, DELFORGE 1992).

Serapias parviflora Parl. (Fig. 2D).

Habitat: in sandy places, close to the sea.

Flowering period: May.

Distribution: [1 grid cell; Fig. 2]. DJ7700; Avgerinos-Katsi area (0.5 m).

Known only from one site, located in the eastern part of the island (WILLING & WILLING 1983). Although it has been recorded in the transitional zone between Lefkas island and mainland Greece, the location where it was found belongs to Lefkas Prefecture.

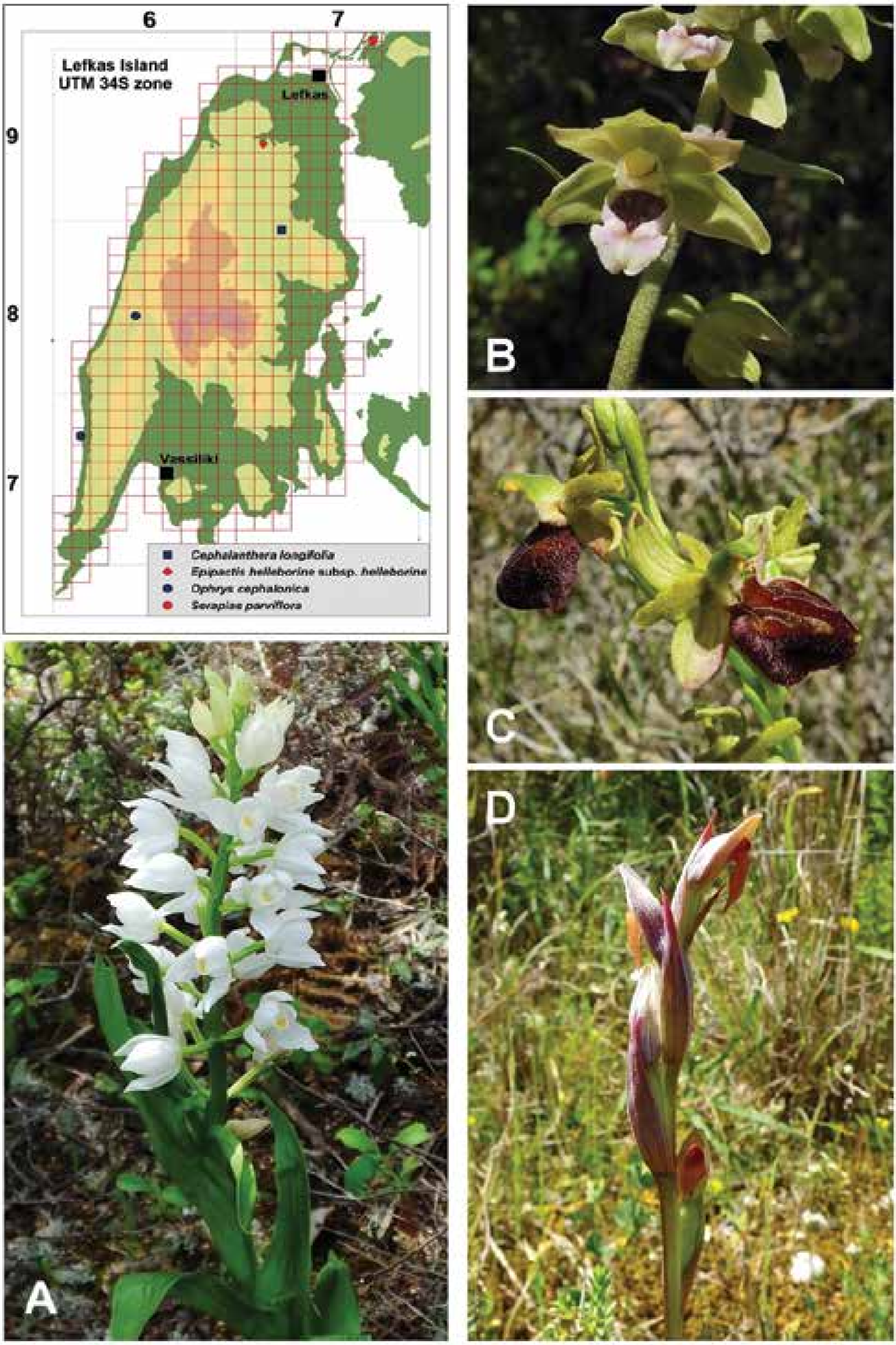


Fig. 2. Distribution map of the rarest orchids of Lefkas island. A, *Cephalanthera longifolia*; B, *Epipactis helleborine* subsp. *helleborine*; C, *Ophrys cephalonica*; D, *Serapias parviflora*.

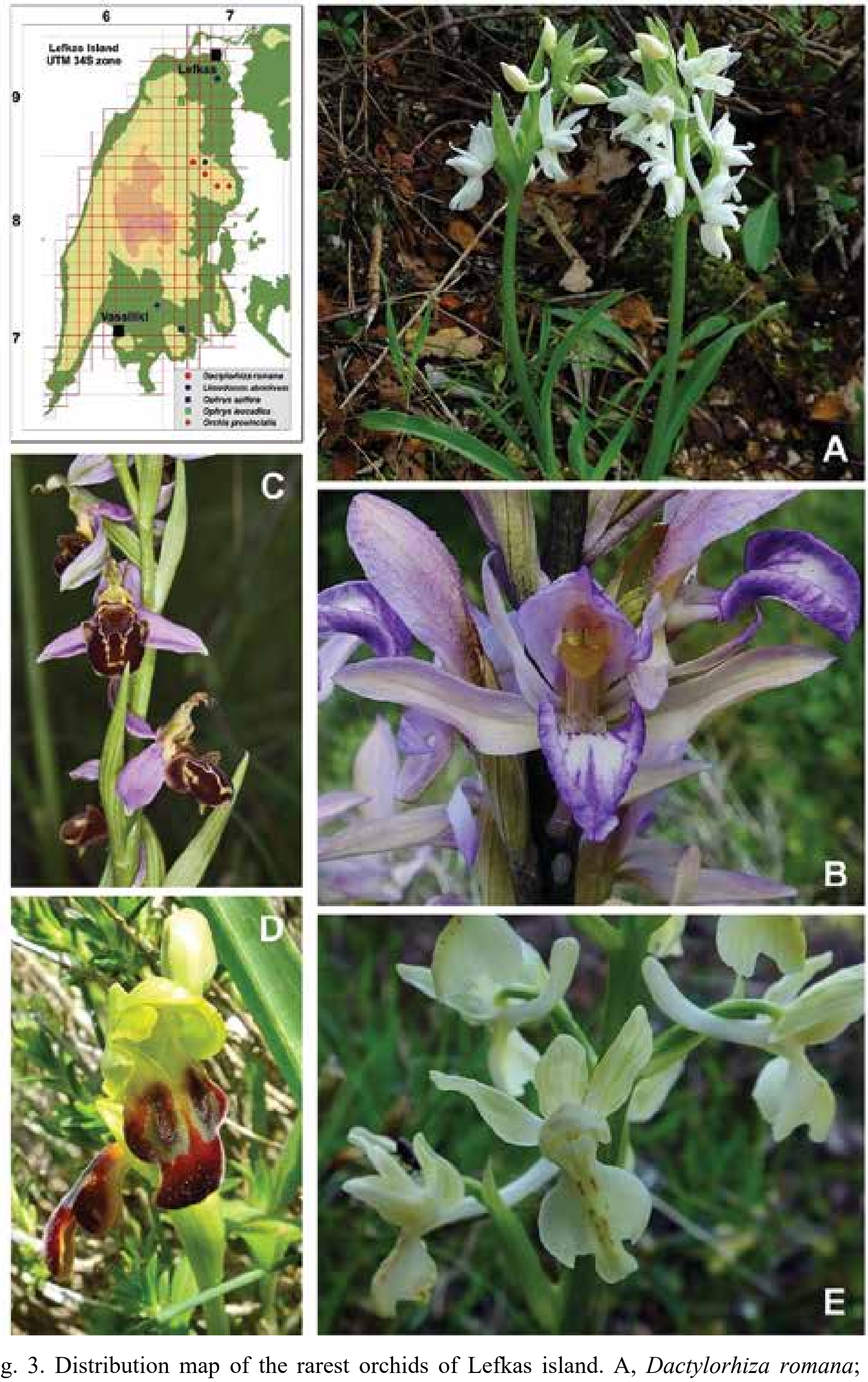


Fig. 3. Distribution map of the rarest orchids of Lefkas island. A, *Dactylorhiza romana*; B, *Limodorum abortivum*; C, *Ophrys apifera*; D, *Ophrys leucadica*; E, *Orchis provincialis*.

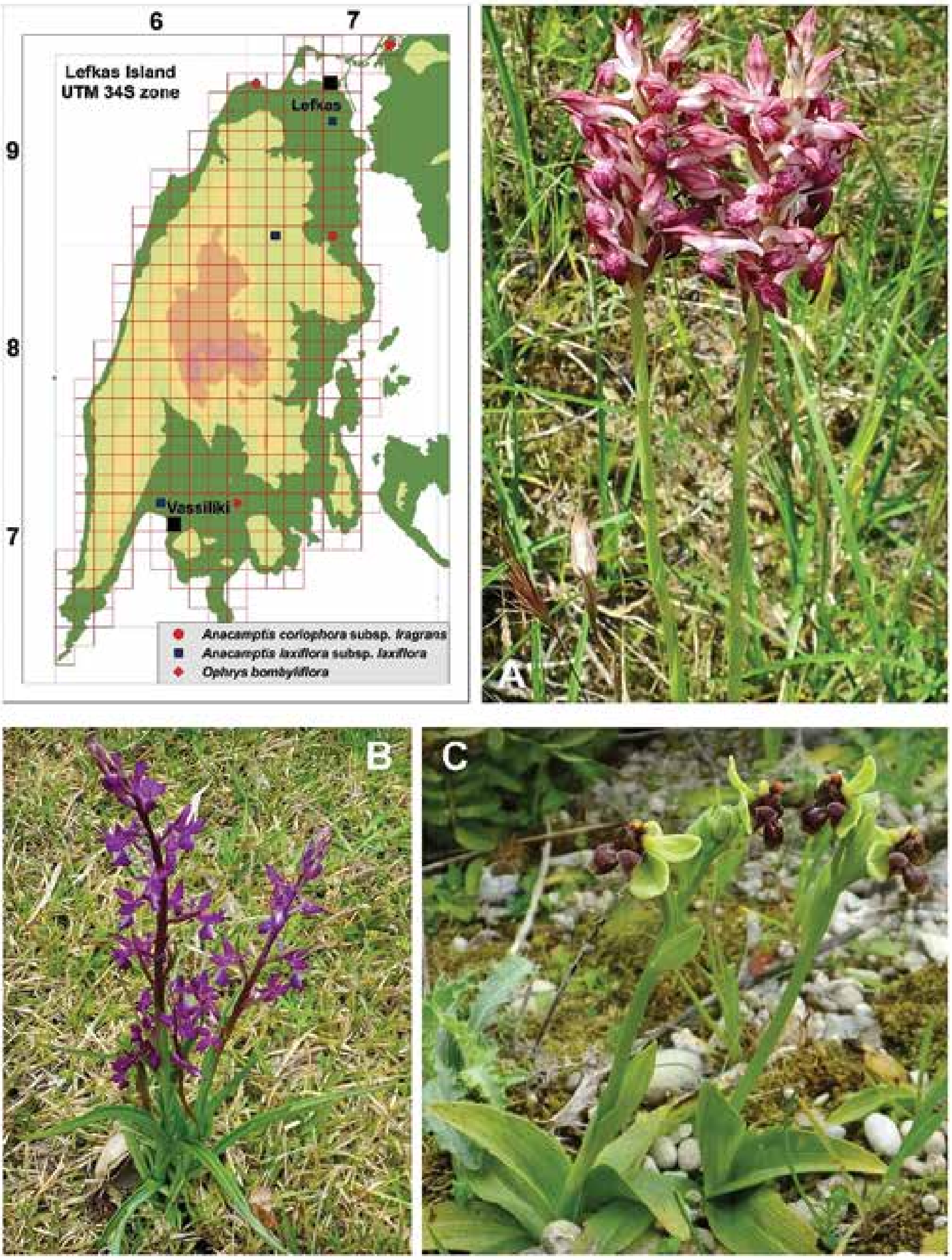


Fig. 4. Distribution map of the rarest orchids of Lefkas island. A, *Anacamptis coriophora* subsp. *fragrans*; B, *Anacamptis laxiflora* subsp. *laxiflora*; C, *Ophrys bombiliflora.*

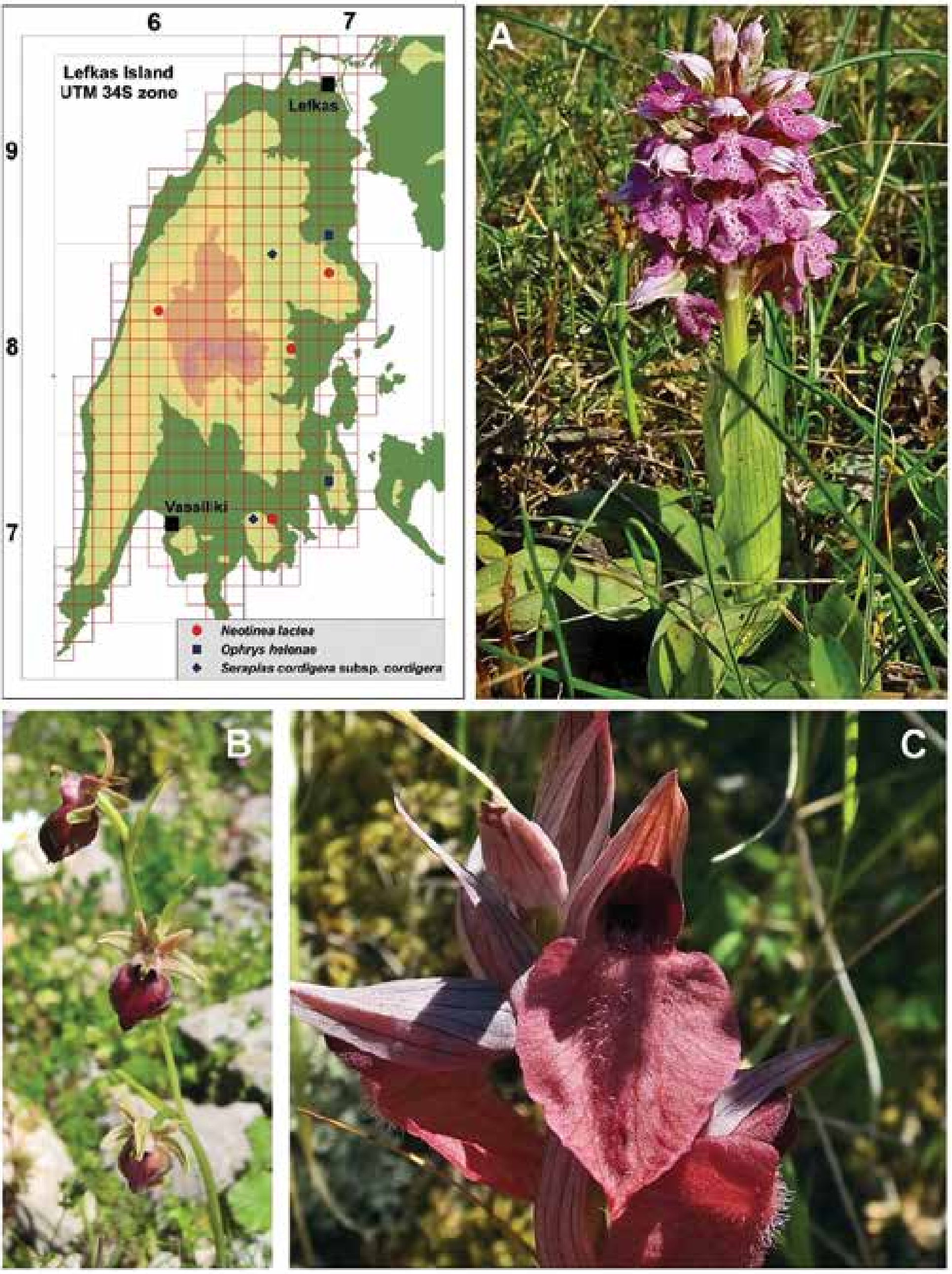


Fig. 5. Distribution map of the rarest orchids of Lefkas island. A, *Neotinea lactea*; B, *Ophrys helenae*; C, *Serapias cordigera* subsp. *cordigera.*

Spiranthes spiralis (L.) Chevall.

Habitat: phrygana, forest openings in semi-shaded and wet sites.
Flowering period: ends of September - October.
Distribution: [11 grid cells]. DH7189/7289, Alexandros area (455 m); DH7186/7, Vafkeri (495 m); DH6977, Vournika - Rodaki area (320 m); DH6775/6875, Kontaraina (115 m); DH7293, Lazarata (385 m); DH7197, Lefkas, Phaneromenis monasteri campus (195 m); DH7377, Poros (275 m); DH7094, Melissa Gorge (320 m).

It was recorded in several sites, located all around the island (WILLING & WILLING 1983).

In total, 37 orchid taxa have been recorded on the basis of the specific project so far. Out of these, *Epipactis helleborine* subsp. *helleborine* has been recorded for the first time, whereas the information presented in the literature about the occurrence of *Orchis provincialis* (WILLING & WILLING 1983) according to DELFORGE (1992) were doubtful. On the other hand, three species could not be found by the authors of the present study. Out of these, two orchids, namely *Anacamptis papilionacea* and *Ophrys spruneri*, were recorded by DELFORGE (1992), whereas *Ophrys spruneri* and *Orchis simia* were recorded by WILLING & WILLING (1983). Moreover, WILLING & WILLING (1983) and HÖLZINGER & al. (1985) refer that *Ophrys sphegodes* Mill. subsp. *sphegodes* is distributed in Lefkas island but is unclear whether the specific identified taxon refers to *O. grammica* or *O. montenegrina*.

The most common orchids are *Himantoglossum robertianum* (61 grid cells), *Serapias lingua* (60 grid cells), *Ophrys ferrum-equinum* (59 grid cells), *O. lutea* subsp. *lutea* (54 grid cells), *O. mammosa* (51 grid cells) and *O. reinholdii* (49 grid cells). Moreover, although the number of grid cells presented for *Ophrys sicula* is small (9 grid cells), this taxon is maybe the most common one, as it can be found in a great variety of habitats (e.g. abandoned fields, grasslands, phrygana, garrigue) throughout the island. On the contrary, *Cephalanthera longifolia*, *Epipactis helleborine* subsp. *helleborine, Ophrys apifera, O. leucadica, Serapias parviflora* (each recorded in 1 grid cell), as well as *Anacamptis coriophora* subsp. *fragrans, Dactylorhiza romana, Ophrys bombyliflora, O. cephalonica, O. helenae, Orchis provincialis* and *Serapias cordigera* subsp. *cordigera* (each recorded in 2 grid cells) are the rarest ones, presenting the most restricted distribution.

Among the orchids recorded on Lefkas island, *E. helleborine* subsp. *helleborine* is considered a very important floristic element as it is widely distributed in mainland Greece but is extremely rare in the islands of the Ionian Archipelago. Specifically, it was known only from Corfu and Kefallinia (TSIFTSIS & ANTONOPOULOS 2017), where a restricted number of individuals had been recorded so far. On Lefkas Island several individuals have been recorded along Melissa Gorge (230 m). It was found in the understory of *Cupressus sempervirens, Laurus nobilis* and *Arbutus unedo* trees, in semi-shaded microsites.

It is clear, that this study constitutes a significant contribution to the knowledge of the orchid flora of Lefkas, as either new species have been reported for the first time or the knowledge about the distribution area of several others has been greatly improved. Consequently, the present work can be the basis for future and more specified studies aiming in monitoring the orchid populations occurring on Lefkas island. According to the short- and long-term trends of specific orchid populations and especially those that are restricted in their geographic distribution, specific actions could be proposed to the local authorities which could help to prevent negative effects that cause orchids' declining.

References

ANTONOPOULOS, Z. & TSIFTSIS, S. 2017: Atlas of the Greek Orchids, Vol II. – Mediterraneo Editions, Rethymno, Greece.

DELFORGE, P. 1992: Les Orchidées de l'île de Leucade (Nomos Lefkada, Nissia Ioniou, Grèce). Observations et additions à la cartographie. – Natural. Belges 73 (Orchid. 5): 155-176.

HAGSATER, E. & DUMONT, V. (eds) 1996: Orchids: status, survey and conservation action plan. – IUCN, Gland.

HÖLZINGER, J., KÜNKELE, A. & KÜNKELE, S. 1985: Die Verbreitung der Gattung *Ophrys* L. auf dem griechischen Festland. – Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ. 17(1): 1-101.

MAVROMMATIS, G. 1980: To bioklima tis Ellados. Schesis klimatos kai fisikis blastiseos, bioklimatiki chartes. – Dasiki Erevna: 63 pp. + 3 maps. [in Greek].

MOUNTRAKIS, M. D. 1985: Geology of Greece. – Thessaloniki [in Greek].

PAULUS, H. F. & HIRTH, M. 2014: Zur Bestäubungsbiologie der Gattung *Ophrys* auf den Ionischen Inseln mit speziellen Bemerkungen zum Status von *Ophrys punctulata*-*O. leucadica* sowie Beschreibungen von zwei neuen Arten aus der *O. lutea*- and *O. oestrifera*-Gruppe. – J. Eur. Orch. 46(2): 233-304.

PHITOS, D., STRID, A., SNOGERUP, S. & GREUTER, W. (eds) 1995: The Red Data Book of Rare and Threatened Plants of Greece. – WWF for Nature, Athens.

—, CONSTANTINIDIS, T. & KAMARI, G. (eds) 2009: The Red Data Book of Rare and Threatened Plants of Greece, Vol. II: E–Z. – Hellenic Botanical Society, Patra [in Greek].

SCHLÜTER, P. M., RUAS, P. M., KOHL, G., RUAS, C. F., STUESSY, T. F. & PAULUS, H. F. 2009: Genetic patterns and pollination in *Ophrys iricolor* and *O. mesaritica* (Orchidaceae): sympatric evolution by pollinator shift. – Bot. J. Lin. Soc. 159: 583-598.

TRIANTAFYLLOU, M. V. 2010: Calcareous nannofossil biostratigraphy of Langhian deposits in Lefkas (Ionian Islands). – Bulletin of the Geological Society of Greece 43(2): 754-762.

TSIFTSIS, S. & ANTONOPOULOS, Z. 2017: Atlas of the Greek Orchids, Vol I. – Mediterraneo Editions, Rethymno, Greece.

WILLING, B. & WILLING, E. 1983: Beitrag zur Verbreitung der Orchideen Ätoliens und Arkananiens sowie der insel Lefkas (NW-Griechenland). – Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ. 15(3): 351-413.

Wild aromatic Bulgarian Lamiaceae and their bee pollinators

EKATERINA KOŽUHAROVA, INA ANEVA & DAVE GOULSON

Abstract

Kožuharova, E., Aneva, I. & Goulson, D. 2019: Wild aromatic Bulgarian Lamiaceae and their bee pollinators. – Bot. Chron. 22: 145-157.

Lamiaceae is famous with its numerous aromatic plant species of medicinal and culinary importance. Many species of the family reproduce primarily by seeds and therefore, the pollination process is of crucial importance. The functional flower morphology of Lamiaceae reveals a predominantly bee pollination syndrome. Due to habitat destruction, loss of flower resources, outbreaks of disease and increased use of pesticides, decline in bees' abundance and diversity has been observed over the recent decades. In relation to this, the aim of the present study was to evaluate the importance of bees as pollinators of wild aromatic Lamiaceae. The species chosen for the study are collected and used traditionally for herbal tea or for medicinal purposes in Bulgaria. The study focused on the propagation strategies, pollination ecology and conservation significance of the species. Most of them are perennials, predominantly propagating by seeds. Our survey revealed that both honey and wild bees contribute to the seed set. Therefore, the sustainable use of wild aromatic Lamiaceae requires conservation of both the natural habitats of the plant species and their pollinators.

*E. Kožuharova, Medical University of Sofia, Faculty of Pharmacy, Department of Pharmacognosy, Sofia, Bulgaria.
E-mail: ina_kozuharova@yahoo.co.uk (* = corresponding author).
I. Aneva, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
G. Bončev Str., Sofia, Bulgaria. E-mail: ina.aneva@abv.bg
D. Goulson, School of Life Sciences, University of Sussex BN1 9QG.
E-mail: D.Goulson@sussex.ac.uk

Key words: herbal tea, aromatic, bees, Lamiaceae.

Introduction

Most of the species of Lamiaceae are widely used as spices and many of them are collected for herbal teas from wild populations (KULIŠIĆ & al. 2006, 2007, FECKA & TUREK 2007, JARIĆ & al. 2007, MENKOVIĆ & al. 2011, MINČEVA & al. 2016). Plant reproduction is crucial for sufficient production and sustainable use of these plants as sources of essential oil and herbal tea. This is true both of aromatic plants collected in the wild populations and harvested in cultivation. The pollination process is particularly important when seed reproduction is their prevailing way of propagation.

Here, we focus on *Acinos arvensis* (Lam.) Dandy, A. *suaveolens* (Sm.) G. Don., *Calamintha nepeta* (L.) Savi., *Nepeta cataria* L., *Origanum vulgare* L. *Mentha* spp., *Thymus* spp., *Micromeria dalmatica* Benth., *Sideritis scardica* Griseb., *Satureja montana* L., *Hyssopus officinalis* L. and *Melissa officinalis* L. (Table 1, Fig. 1). We chose these species because they are commonly collected for herbal teas in Bulgaria (Hardalova & al. 1994, 1998, Evstatieva & Hardalova 2000, Medicinal Plant Act. 2000, Evstatieva & al. 2007).

Material and methods

Considering the fact that their conservation and sustainable use is related to their reproduction, we assessed these taxa for their biological type, breeding system, and pollination ecology. We used all accessible literature recorded in databases (Scholar Google, PubMed, Web of Science) with the following key words: self-pollination, vegetative propagation, apomixis, bee pollination in combination with the selected taxa, and Lamiaceae/Labiatae. Surprisingly few studies on the breeding system and pollination ecology of these aromatic plants have been published, although they are widely used and, furthermore, are appreciated as honey plants. This information deficit is particularly evident for *Melissa officinalis*, and supports our statement that further investigations in this field are necessary.

1. Propagation strategies

1.1. Biological type and breeding systems

Biological type is a good indicator of the plants' propagation strategy. The studied taxa are mostly perennials (Table 1). Only *Acinos arvensis* may, under certain conditions, have an annual life cycle. Annuals propagate exclusively by seed. In the absence of pollinators, annuals likely develop compensatory adaptations to self-pollination (Richards 1990). *Acinos arvensis* is self-compatible and self-pollinated and, both in nature and under cultivation, shows little variation except in overall size (Gill 1981).

Perennials are more likely to be xenogamous, and often show strong adaptations to vegetative propagation (Richards 1990, Table 1). For instance, *Hyssopus officinalis* can be propagated either by seeds or vegetatively (Nanova & al. 2007). It has the ability to self-pollinate spontaneously (Carlen & Simonnet 2015). The occurrence of hybrids between *Mentha* spp. demonstrates at least some degree of cross-pollination. *Mentha spicata* is capable of spontaneous self-pollination (Murray 1960, Ito & Honda 2007), and additionally it propagates almost exclusively by vegetative means (Gobert & al. 2002). *Thymus* spp. are known to have strong vegetative propagation (Japičino & al. 2006, Kožuharova 2009). Cuttings of oregano (*Origanum vulgare*), peppermint (*Mentha piperita*) and lemon balm (*Melissa officinalis*) are capable of rooting and initial establishment under controlled conditions (Kuris & al. 1980).

Despite variable ability for vegetative propagation, these plants maintain and could increase their genetic variability by seed reproduction (Table 1). *Origanum vulgare* was found not to set seed when bagged but does set seed to a normal degree under open pollination (Gill 1981). The flowers are self-incompatible and insect-pollinated

(VAN LOOY & al. 2009). Because of natural cross-pollination, oregano is a genetically heterogeneous species (FLEISHER & SNEER 1982).

Sideritis scardica Griseb. is a perennial herb that grows in the higher parts of the mountains, including in the 0subalpine belt. It is a drought-resistant and heliophilous species found on rocky slopes from 700 to 2200 m a.s.l. The species is a Balkan endemic occurring in small populations in Albania, Bulgaria, NE Greece and North Macedonia. In Bulgaria it is limited to a few localities in the Pirin Mts, Rhodope Mts and Slavyanka (Alibotuš) Mts. Its populations are threatened and extremely vulnerable (ANEVA 2016), and therefore collecting the species is prohibited by law.

Micromeria dalmatica is a Balkan endemic species occurring only in Bulgaria, NE Greece, and Montenegro. It is a perennial plant growing on dry and sunny mountain slopes. In Bulgaria its natural distribution is restricted mainly to the south.

Apomixis is rarely reported in Lamiaceae. Most apomicts remain facultative, so that they can also set sexual seed. Apomixis is a compensatory mechanism where sexuality cannot function because of hybridity, for example *Mentha* (RICHARDS 2003). *Thymus loscosii* and *Thymus vulgaris* are known to be both gynodioecious and facultatively apomictic (ASSOUAD & al. 1978, ORELLANA & al. 2005). Research on the reproductive biology of *Thymus* has focused mainly on *Thymus vulgaris*, which was found to be a self-compatible species but at the level of each flower, protandry avoids self pollination. Bees are required to transfer the pollen from one flower to another (ASSOUAD & al. 1978, BRABANT & al. 1980, VERNET & al. 1996). Apomixis is reported for *Hyssopus officinalis* (DASKALOVA & YURUKOVA-GRANČAROVA 1996). Gynodioecious plants are common, occurring for instance in *Thymus* spp. and *Origanum vulgare* (ORELLANA & al. 2005, ASSOUAD & al. 1978, GODIN & EVDOKIMOVA 2017). Additionally, there is male sterility in *Origanum vulgare* (LEWIS & CROWE 1956) and *Thymus vulgaris* (BELHASSEN & al. 1991), and nuclear genes are involved in its determination.

2. Pollination ecology

2. 1. Mutualistic networks

According to contemporary environmental perceptions, living organisms are not in simple linear relationships but participate in complex, interconnected networks. Knowing the mutualistic relationships in these networks and their principles is important for better understanding of evolutionary processes. This has also a substantial practical aspect – conservation of a single taxon often is not effective because it does not exist in isolation from other taxa. Conservation strategies should be based on conservation of the completely mutualistic network. Survival of the network and the ecosystems in which the organisms are situated depends on maintaining and preserving the aggregate of interacting taxa (KEARNS & al. 1998, BRONSTEIN & al. 2004, 2006, MEMMOTT & al. 2007, VÁZQUEZ & al. 2004, THÉBAULT & FONTAINE 2010, FORTUNA & al. 2010, HEGLAND & TOTLAND 2012). Mutualistic networks are of key importance to entomophilous plants that depend on insects for pollination and on seeds for propagation.

Table 1. Biological types, vegetative propagation, self-pollination.

	Biological type	**Vegetative propagation**	**Ability to spontaneous self-pollination**	**Lack of spontaneous self-pollination**
Acinos arvensis (Lam.) Dandy (*Calamintha acinos* Clairv.)	Annual to perennial		Gill (1981)	
Acinos suaveolens (Sm.) G. Don.	Perennial			
Calamintha nepeta (L.) Savi.	Perennial			
Hyssopus officinalis subsp. *aristatus* (Godr.) Briq.	Shrub	Nanova & al. (2007)	Carlen & Simonnet (2015)	
Melissa officinalis L.	Perennial	Kuris & al. (1980)		
Mentha aquatica L.	Perennial			
Mentha arvensis L.	Perennial			
Mentha longifolia (L.) Huds.	Perennial			
Mentha pulegium L.	Perennial			
Mentha spicata L.	Perennial	Gobert & al. (2002)	Murray (1960), Ito & Honda (2007)	
Mentha suaveolens Ehrh. (*M. rotundifolia* (L.) Huds.)	Perennial			
Nepeta cataria L.	Perennial			
Origanum vulgare L.	Perennial	Kuris & al. (1980)		Gill (1981)
Origanum vulgare subsp. *hirtum* (Link.) Jetswaart	Perennial			
Satureja montana subsp. *kitaibelii* (Wierzb.) P. W. Ball	Perennial to shrub			
Sideritis scardica Griseb.	Perennial			
Sideritis syriaca L. (*S. taurica* Steph.)	Perennial			
Thymus spp.	Perennial	Japičino & al. (2006), Kožuharova (2009)		
Thymus callieri Borb.	Perennial			
Thymus comptus Friv. (*T. glaucus* Friv.)	Perennial			
Thymus glabrescens Willd.	Perennial			
Thymus longicaulis C. Presl	Perennial			
Thymus longidentatus (Deg. & Urum.) Ronn.	Perennial			
Thymus pulegioides L. (*T. montanus* Waldst. & Kit.)	Perennial			
Thymus sibthorpii Benth.	Perennial			
Thymus striatus Vahl.	Perennial			

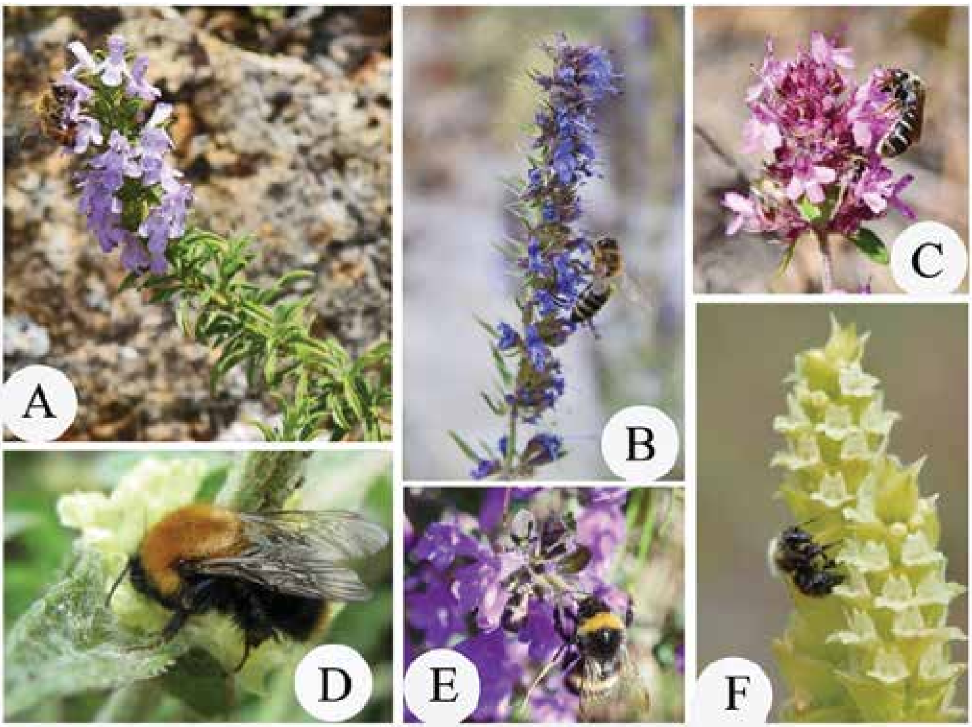


Fig. 1. Some labiates and their pollinators: A, *Satureja montana* L.; B, *Hyssopus officinalis* L., C, *Thymus moesiacus* Velen.; D, *Sideritis scardica* Griseb.; E, *Acinos alpinus* (L.) Moench; F, *S. scardica* Griseb.

Mutualistic networks of plants and their pollinators are among the most important forces maintaining biodiversity in terrestrial ecosystems (OLESEN & JORDANO 2002, VÁZQUEZ & AIZEN 2004, FORUP & al. 2008, ALARCÓN & al. 2008, VALDOVINOS & al. 2009, RAMOS-JILIBERTO & al. 2009, BOSCH & al. 2009). The components are flexible and adaptive, which makes them stable (VALDOVINOS & al. 2010, OLLERTON & al. 2015). The richer in components they are, the better are they sustainable and resilient (VALDOVINOS & al. 2009, SENAPATHI & al. 2016). Wild bees and other pollinators are crucial for the optimal stability of the mutualistic networks (SENAPATHI & al. 2016). These networks are threatened by human impact, global pollinator decline, invasive alien plants proliferation and concomitant decline of the local autochthonous ones, etc. (MEMMOTT & al. 2007, BARTOMEUS & al. 2008, HEGLAND & al. 2009, VILÀ & al. 2009, KAISER BUNBURY & al. 2009, 2011, BREEZE & al. 2011).

2.2. The "gullet" pollination syndrome

Functional flower morphology indicates what the pollen vectors are likely to be. Pollination syndromes are described by FAEGRI &VAN DER PIJL (1971). These authors use the term "blossom" to denote the functional pollination unit – usually a flower or compact inflorescence. Anemophilous plants are pollinated by wind and have typical blossoms with reduced petals, produce an excess of small, smooth pollen grains and have exactly correlated flowering times. Entomophilous pollination syndromes occur across several angiosperm classes, to judge from the functional morphology and ways of access to nectar and pollen in the blossoms. These classes are characterized as follows, with regard to the blossoms: "dish/bowl" shape – granting free access to nectar and pollen, radial symmetry (including compound blossoms); or "bell" shape – ± hidden nectar, wide tube, radial symmetry or slight zygomorphy; or "funnel" shape – with hidden nectar, narrow but short tube, radial symmetry to slight zygomorphy; or "flag" type – the sexual organs are found in the lower part, zygomorphy, the pollen is deposited on the pollinator's abdomen (ventral side, sternotribic pollination); or "gullet" type – the sexual organs are restricted to the functionally upper side, zygomorphy, pollen is deposited on the pollinator's dorsal side and head, ± hidden nectar (nototribic pollination). Lamiaceae have blossoms of the "gullet" type (FAEGRI & VAN DER PIJL 1971, DAFNI & NEAL 1997). In most cases, the "gullet" syndrome indiates predominant bee pollination (SHMIDA & DUKAS 1990, PROCTOR & al. 1996, ORELLANA & al. 2005 CLAßEN-BOCKHOFF 2007, WESTERKAMP & CLAßEN-BOCKHOFF 2007, KURIYA & al. 2015).

2.3. Bees as pollinators

There is a long-term debate on the importance of honeybees as pollinators, compared to wild. The conclusion is that the wild bees should not be neglected (AEBI & al. 2012, OLLERTON & al. 2012). Despite the fact that apiarists often lose a high proportion of their hives due to *Varroa* mite, diseases and pesticides the number of honey bee colonies worldwide has risen by 45 % during the last 50 years (AIZEN & HARDER 2009). Therefore, honeybees are not threatened by extinction. HATFIELD & al. (2012) demonstrated that honeybee and commercial bumblebee farming could be responsible for wild pollinator decline. The high density of bees often results in spread of diseases that put at risk other native and managed pollinators (GOKA & al. 2001, GRAYSTOCK & al. 2013, 2015). Managed bees may contaminate wild bees with newly introduced diseases, or they may reinforce diseases already present in wild populations (GOULSON & HUGHES 2015). Commercial bumblebees such as *Bombus terrestris* may themselves turn into a dangerous invasive pest (DAFNI & SHMIDA 1996, DAFNI 1998, GOULSON 2003, DAFNI & al. 2010, SCHMID-HEMPEL & al. 2015).

Both honeybees and wild bees contribute to the optimal seed set of *Origanum vulgare* (GILL 1981, FLEISHER & SNEER 1982, VAN LOOY & al. 2009, KOŽUHAROVA & al., unpubl. data), *Sideritis scardica*, (KOŽUHAROVA & al., unpubl. data), *Sideritis syriaca* (UZUNOV, unpubl. data), *Nepeta cataria* and to a lesser extent to *Mentha* spp. Even though *Thymus vulgaris* is capable of spontaneous self-pollination (see above), it still depends on bees as pollen vectors. Pollination is mainly by bees (BRABANT & al. 1980), whereas self-pollination is assumed to vary from 0% to 80% in natural conditions (VALDEYRON & al. 1977), depending on both the genotype and the immediate environment (DOMMÉE 1981).

The most important visitors of *Nepeta cataria* flowers are honeybees (*Apis mellifera*), solitary bees (Halictidae), and bumblebees (*Bombus* spp.) (SIH & BALTUS 1987), followed by flies and butterflies (AIZEN & al. 2002). In North American habitats (pairies, deserts and pine forests), native bees, *Apis mellifera* and *Bombus* spp., even when they are introduced, visit *Nepeta cataria* blossoms (CRUDEN & al. 1981). Although it is a self-compatible species with full capacity to produce seed autonomously, it exhibits a mixed mating system, and fruit set is highest in hand-pollinated flowers, followed by open-pollinated flowers, and lowest in bagged flowers (SIH & BALTUS 1987).

Even in *Hyssopus officinalis,* which is listed as a self-pollinated plant (see above), insect pollination dominates (SOWMYA 2008).

2.4. Hazards and risks

Pollinators are fundamental for maintaining both biodiversity and agricultural productivity. However, several factors are causing decline in their abundance and diversity (POTTS & al. 2010, 2015, GOULSON & al. 2015). Habitat destruction, loss of floral resources, and increased prophylactic use of systemic pesticides are causing such decline (POTTS & al. 2010, 2015, GOULSON & al. 2015). Decline of pollinators in NW Europe is well documented (BIESMEIJER & al. 2006, CARVALHEIRO & al. 2013). OLLERTON & al. (2014) noted that 23 species of bees and flower-visiting wasps have gone extinct in the UK. They also found that the rate of extinction is highly variable, and raised the question whether these extinctions have stopped or whether they will continue in the future.

The study demonstrates the importance of maintaining the year-on-year effort of recording natural history data to accumulate long-term data sets (OLLERTON & al. 2014). The relationship between use of pesticides and the decline of pollinators is an important and controversial topic that is unlikely to go away any time soon. Neonicotinoids are often applied as systemic seed treatments to crops and have reported negative impact on pollinators when they appear in floral nectar and pollen (WRIGHT & al. 2015, STANLEY & al. 2015a & b). There is strong evidence that this scales up to cause losses of bee biodiversity and declines in abundance (GOULSON & HUGHES 2015, GOULSON & al. 2015, STANLEY & al. 2015a & b, WRIGHT & al. 2015, WOODCOCK & al. 2016, WOOD & GOULSON 2017).

What about pollination if the pollinators vanish?

Recently, pollinating robots have begun being developed, with a view to replacing bees in crop pollination. There are a number of questions over how efficient they will be and at what financial and environmental costs. Even the constructors admit that plants and pollinating insects have undergone millions of years of evolution to develop sophisticated systems for collecting and transporting pollen, and that is will be hard to replicate this (AMADOR & HU 2017). It is rather dubious how the man made machines can respond to the huge diversity of plant blossoms so that efficient pollination is accomplished? Additionally, pollinating robots will presumably not be deployed for wild plants.

Conclusion

Most of the Lamiaceae aromatic plants depend on bees for pollination at least to a certain extent. Therefore, the sustainable use of wild aromatic labiates requires preservation of both components - the plants and their pollinators.

Acknowledgments

Ina Aneva expresses her thanks for the support provided by the project DN 16/3 (National Science Fund of Bulgaria) and by the Bulgarian Ministry of Education and Science under the National Research Programme "Protection of environment and reducing the risk of hazards and natural disasters" approved by DO № 230/06.12.2018..

References

AEBI, A., VAISSIÈRE, B. E., DELAPLANE, K. S., ROUBIK, D. W. & NEUMANN, P. 2012: Back to the future: Apis versus non-Apis pollination - a response to OLLERTON & al. – Trends Ecol. Evol. 27:142-143. Doi: 10.1016/j.tree.2011.11.017.

AIZEN, M. A. & HARDER, L. D. 2009: The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. – Curr. Biol. 19: 915-918. Doi: 10.1016/j.cub.2009.03.071.

—, ASHWORTH, L. & GALETTO, L. 2002: Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? – J. Veg. Sci. 13(6): 885-892.

ANEVA, I. 2016: Biological and phytochemical in situ and ex situ study of the species of genus *Sideritis* L. with conservation status in Bulgaria. PhD Thesis, Pp. 305. – Sofia, Bulgaria.

ASSOUAD, M. W., DOMMÉE, B., LUMARET, R., & VALDEYRON, G. 1978: Reproductive capacities in the sexual forms of the gynodioecious species *Thymus vulgaris* L. – Bot. J. Linn. Soc. 77(1): 29-39.

AMADOR, G. J. & HU, D. L. 2017: Sticky solution provides grip for the first robotic pollinator. – Chem 2(2): 162-164.

BELHASSEN, E., DOMMÉE, B., ATLAN, A., GOUYON, P. H., POMENTE, D., ASSOUAD, M. W. & COUVET, D. 1991: Complex determination of male sterility in *Thymus vulgaris* L.: genetic and molecular analysis. – Theor. Appl. Genet. 82(2): 137-143.

BIESMEIJER, J. C., ROBERTS, S. P. M., REEMER, M., OHLEMÜLLER, R., EDWARDS, M., PEETERS, T., SCHAFFERS, S. G., POTTS, R., KLEUKERS, C., THOMAS, I., SETTELE, J. & KUNIN, WE. 2006: Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. – Science 313: 351-354. doi: 10.1126/science.1127863

BRABANT, PH., GOUYON, PH., LEFORT, G., VALDEYRON, G. & VERNET, PH. 1980: Pollination studies in *Thymus vulgaris* L. (Labiatae). – Acta Oecol. - Oecol. Plant 1: 37-44.

BRONSTEIN, J. L., ALARCÓN, R. & GEBER, M. 2006: The evolution of plant–insect mutualisms. – New Phytol. 172(3): 412-428.

—, DIECKMANN, U. & FERRIÈRE, R. 2004: Coevolutionary dynamics and the conservation of mutualisms. – Evolutionary Conservation Biology: 305-326. http://www.iiasa.ac.at/~dieckman/reprints/BronsteinEtal2004.pdf

CARLEN, C., & SIMONNET, X. 2015: Breeding and Germplasm Preservation. Pp. 113-130. In MÉTHÉ, A. (ed.), Medicinal and Aromatic Plants of the World. – Springer, Dordrecht.

CARVALHEIRO, L. G., KUNIN, W. E., KEIL, P., AGUIRRE-GUTIÉRREZ, J., ELLIS, W. N., FOX, R., GROOM, Q., HENNEKENS, S., VAN LANDUYT, W., MAES, D., VAN DE MEUTTER, F., MICHEZ, D., RASMONT, P., ODE, B., POTTS, S. G., REEMER, M., ROBERTS, S. P., SCHAMINÉE, J., WALLISDEVRIES, M. & BIESMEIJER, J. C. 2013: Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. – Ecol. Lett. 16: 870-878. Doi: 10.1111/ele.12121

CLAßEN-BOCKHOFF, R. 2007: Floral construction and pollination biology in the Lamiaceae. – Ann. Bot. 100(2): 359-360.

CRUDEN, R. W. & MILLER-WARD, S. 1981: Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: an hypothesis. – Evolution 35: 964-974.

DAFNI, A. 1998: The threat of *Bombus terrestris* spread. – Bee World 79: 113-114. Doi: 10.1080/0005772X.1998.11099392

—, KEVAN, P., GROSS, C. L. & GOKA, K. 2010: *Bombus terrestris,* pollinator, invasive and pest: An assessment of problems associated with its widespread introductions for commercial purposes. – Appl. Entomol. Zool. 45: 101-113. Doi: 10.1303/aez.2010.101

— & SHMIDA, A. 1996: The possible ecological implications of the invasion of Bombus terrestris. Pp. 183-200. In MATHESON, A., BUCHMANN, S. L., O'TOOLE, C., WESTRICH, P. & WILLIAMS, I. H. (eds), The Conservation of Bees. – Academic Press.

— & NEAL, P. 1997: Size and shape in floral advertisement: measurement, concept and implications. Pp. 121-140. In RICHARDS, K. W. (ed.), Proc. Int. Symp. Pollination. – Acta Hort. 437.

DASKALOVA, T. & YURUKOVA GRANCHAROVA, P. 1996: Macrosporogenesis and development of female gametophyte in *Hyssopus officinalis* L. ssp. *officinalis* (Lamiaceae). – Phytol. Balcan. 2: 96-98.

DOMMEE, B. 1981: Role du milieu et du genotype dans la region de la reproduction de *Thymus vulgaris* L. – Acta Oecol. - Oecol. Plant 2(16): 137-147.

EVSTATIEVA, L. & HARDALOVA, R. 2000: Diversity and resources of medicinal plants. Pp. 437-466. In POPOV, A. & MESHINEV, T. (eds), High mountain treeless zone of the Central Balkan National Park, Biological diversity and problems of its conservation. – Sofia.

EVSTATIEVA, L., HARDALOVA, R. & STOYANOVA, K. 2007: Medicinal plants in Bulgaria: diversity, legislation, conservation and trade. – Phytol. Balcan. 13(3): 415-427.

FAEGRI, K. & VAN DER PIJL, L. 1971: The principles of pollination ecology. – Oxford.

FECKA, I. & TUREK, S. 2007: Determination of water-soluble polyphenolic compounds in commercial herbal teas from Lamiaceae: peppermint, melissa, and sage. – J. Agric. Food Chemistry 55: 10908-10917.

— & TUREK, S. 2008: Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques. – Food Chem. 108: 1039-1053.

FLEISHER, A. & SNEER, N. 1982: Oregano spices and *Origanum* chemotypes. – J. Sci. Food Agric. 33: 441-446.

FORTUNA, M. A., STOUFFER, D. B., OLESEN, J. M., JORDANO, P., MOUILLOT, D., KRASNOV, B. R. & BASCOMPTE, J. 2010: Nestedness versus modularity in ecological networks: two sides of the same coin? – J. Animal Ecol. 79(4): 811-817.

GILL, L. S. 1981: Biosystematics of the tribe *Saturejinae* (Labiatae) in Canada. II. – Cytologia 46(1/2): 45-55.

GOBERT, V., MOJA, S., COLSON, M., & TABERLET, P. 2002: Hybridization in the section Mentha (Lamiaceae) inferred from AFLP markers. – Am. J. Bot. 89(12): 2017-2023.

GODIN, B. H. & EVDOKIMOVA, T. A. 2017: Morphologija obepolih i pestichnih tzvetkov Origanum vulgare. Izvestija visshih uchebnih zavedenii. Povolzhskii rajon. – Estestvenie nauki 1: 3-17.

GOKA, K., OKABE, K., YONEDA, M. & NIWA, S. 2001: Bumblebee commercialization will cause worldwide migration of parasitic mites. – Mol. Ecol. 10: 2095-2099. Doi: 10.1046/j.0962-1083.2001.01323.x

GOULSON, D. 2003: Effects of introduced bees on native ecosystems. – Annu. Rev. Ecol. Evol. Syst. 34: 1-26.

— & HUGHES, W. O. 2015: Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. – Biol. Conserv. 191: 10-19. Doi: 10.1016/j.biocon.2015.06.023

—, NICHOLLS, E., BOTÍAS, C., ROTHERAY, E.L. 2015: Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. – Science 347: 1255957. Doi: 10.1126/science.1255957

GOUYON, P. H., VERNET, P., GUILLERM, J. L. & VALDEYRON, G. 1986: Polymorphisms and environment: the adaptive value of the oil polymorphisms in *Thymus vulgaris* L. – Heredity 57: 59.

GRAYSTOCK, P., GOULSON, D. & HUGHES, W. O. 2015: Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. – Proc. R. Soc. 282(1813): 20151371. Doi: 10.1098/rspb.2015.1371

GRAYSTOCK, P., YATES, K., EVISON, S. E., DARVILL, B., GOULSON, D. & HUGHES, W. O. 2013: The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. – J. Appl. Ecol. 50:1207-1215. Doi: 10.1111/1365-2664.12134

HARDALOVA, R., EVSTATIEVA, L. & GUSSEV, C. 1994: Characterization of wild medicinal plant resources in Bulgaria and recommendations for their long term stable development. Pp. 41-72. In SAKALIAN, M.(ed.), National Biological Diversity Conservation Strategy. Main reports. Vol. 2. – The Biodiversity Support Program. Bilvest - 2000, Sofia [in Bulgarian].

—, — & — 1998: Wild medicinal plant resources in Bulgaria and recommendations for their long-term development. – Bulgarias's biological diversity: conservation status and needs assessment 1: 527-561.

HATFIELD, R., JEPSEN, S., MADER, E., BLACK, S. H. & SHEPHERD, M. 2012: Conserving bumble bees. – Guidelines for Creating and Managing Habitat for America's Declining Pollinators. 32. Portlans OR: The Xerress Society for Invertebrate Conservation.

HEGLAND, S. J. & TOTLAND, Ø. 2012: Interactions for pollinator visitation and their consequences for reproduction in a plant community. – Acta Oecol. 43: 95-103.

IAPICHINO, G., ARNONE, C., BERTOLINI, M. & AMICO ROXAS, U. 2006: Propagation of three Thymus species by stem cuttings. – International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation 723: 411-414.

Ito, M. & Honda, G. 2007: Geraniol synthases from perilla and their taxonomical significance. – Phytochemistry 68: 446-453.

Jarić, S., Popović, Z., Mačukanović-Jocić, M., Djurdjević, L., Mijatović, M., Karadžić, B. & Pavlović, P. 2007: An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). – J. Ethnopharmacol. 111: 160-175.

Kearns, C. A., Inouye, D. W. & Waser, N. M. 1998: Endangered mutualisms: the conservation of plant-pollinator interactions. – Annual Review Ecol. Syst. 1998: 83-112.

Kožuharova, E. 2009: New ex situ collection of rare and threatened medicinal plants in the Pirin Mts (Bulgaria). – Ekoloji 18, 72: 32-44.

Kulišć, T., Dragović-Uzelac, V. & Miloš, M. 2006: Antioxidant Activity of Aqueous Tea Infusions Prepared from Oregano, Thyme and Wild Thyme. – Food Technol. Biotech. 44(4): 485-492.

—, Kriško, A., Dragović-Uzelac, V., Miloš, M. & Pifat, G. 2007: The effects of essential oils and aqueous tea infusions of oregano (*Origanum vulgare* L. spp. *hirtum*), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) on the copper-induced oxidation of human low-density lipoproteins. – Int. J. Food Sci. Nutr. 58(2): 87-93.

Kuris, A., Altman, A. & Putievsky, E. 1980: Rooting and initial establishment of stem cuttings of oregano, peppermint and balm. – Sci. Hortic.-Amsterdam 13(1): 53-59.

Kuriya, S., Hattori, M., Nagano, Y. & Itino, T. 2015: Altitudinal flower size variation correlates with local pollinator size in a bumblebee-pollinated herb, *Prunella vulgaris* L. (Lamiaceae). – Int. J. Evol. Biol. 28(10): 1761-1769.

Medicinal Plant Act. 2000: State Gazzete 29, 27 April 2000.

Memmott, J., Craze, P. G., Waser, N. M. & Price, M. V. 2007: Global warming and the disruption of plant–pollinator interactions. – Ecol. Lett. 10(8): 710-717

Menković, N., Šavikin, K., Tasić, S., Zdunić, G., Stešević, D., Milosavljević, S. & Vincek, D. 2011: Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). – J. Ethnopharmacol. 133(1): 97-107.

Mincheva, I., Kožuharova, E., & Rastrelli, L. 2016: Ethnobotany and exploitation potential of *Oreganum vulgare* L. in the Rhodopes, Bulgaria. – Pharmacology OnLine 3: 168-173.

Murray, M. J. 1960: The genetic basis for a third ketone group in Mentha spicata L. – Genetics 45(7): 931-937.

Nanova, Z., Slavova, Y., Nenkova, D. & Ivanova, I. 2007: Microclonal Propagation of Hyssop (*Hyssopus officinalis* L.). – Bulg. J. Agric. Sci. 13(2): 213.

Ollerton, J., Price, V., Armbruster, W. S., Memmott, J., Watts, S., Waser, N. M., Totland, Ø., Goulson, D., Alarcon, R., Stout, J. & Tarrant, S. 2012: Overplaying the role of honey bees as pollinators: a comment on Aebi and Neumann (2011). – Trends Ecol. Evol. 27: 141-142. Doi: 10.1016/j.tree.2011.12.001

—, Erenler, H., Edwards, M., Crockett, R. 2014: Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. – Science 6215: 1360-1362. https://doi.org/10.1126/science.1257259

Orellana, M. R., Rovira, A. M., Blanché, C. & Bosch, M. 2005 : Pollination and reproductive success in the gynodioecious endemic *Thymus loscosii* (Lamiaceae). – Can. J. Bot. 83(2): 183-193.

Potts, S., Biesmeijer, K., Bommarco, R., Breeze, T., Carvalheiro, L., Franzén, M. González-Varo, J. P., Holzschuh, A., Kleijn, D., Klein, A.-M., Kunin, B., Lecocq, T.,

LUNDIN, O., MICHEZ, D., NEUMANN, P., NIETO, A., PENEV, L., RASMONT, P., RATAMÄKI, O., RIEDINGER, V., ROBERTS, S. P. M., RUNDLÖF, M., SCHEPER, J., SØRENSEN, P., STEFFAN-DEWENTER, I., STOEV, P., VILÀ, M. & SCHWEIGER, O. 2015: Status and trends of European pollinators. Key findings of the STEP project. – Pensoft Publishers, Sofia, pp. 72.

—, —, KREMEN, C., NEUMANN, P., SCHWEIGER, O. & KUNIN, W. E. 2010: Global pollinator declines: trends, impacts and drivers. – Trends Ecol. Evol. 25: 345-353. doi: 10.1016/j.tree.2010.01.007

PROCTOR, M., YEO, P. & LACK, A. 1996: The natural history of pollination. – HarperCollins Publishers, pp. 479.

RICHARDS, A. J. 1990: Plant breeding systems. – Unwin Human London, University Press, Cambridge, pp. 529

— 2003: Apomixis in flowering plants: an overview. – Philos. Trans. R. Soc. Lond. *B* Biol. Sci. 358(1434): 1085-1093.

SCHMID-HEMPEL, R., ECKHARDT, M., GOULSON, D., HEINZMANN, D., LANGE, C., PLISCHUK, S., RUZ, E. L., SALATHÉ, R., SCRIVEN, J. J. & SCHMID-HEMPEL, P. 2014: The invasion of southern South America by imported bumblebees and associated parasites. – J. Anim. Ecol. 83: 823-837. doi: 10.1111/1365-2656.12185

SHMIDA, A. & DUKAS, R. 1990: Progressive reduction in the mean body sizes of solitary bees active during the flowering season and its correlation with the sizes of bee flowers of the mint family (Lamiaceae). – Isr. J. Plant Sci. 39(1-2): 133-141.

SIH, A. & BALTUS ,M.-S. 1987: Patch size, pollinator behavior, and pollinator limitation in catnip. – Ecology 68(6): 1679-1690.

SOWMYA, K. S. 2008: Insect pollinators of selected medicinal crops with special reference to the role of honeybees in pollination of honey plant, *Ammi majus* L. – Doctoral dissertation, University of Agricultural Sciences GKVK, Bangalore.

STANLEY, D. A., GARRATT, M. P., WICKENS, J. B., WICKENS, V. J., POTTS, S. G. & RAINE, N. E. 2015b: Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. – Nature 7583: 548-550.

STANLEY, D. A., SMITH, K. E. & RAINE, N. E. 2015a: Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. – Sci. Rep 5: 16508. Doi: 10.1038/nature16167

THÉBAULT, E. & FONTAINE, C. 2010: Stability of ecological communities and the architecture of mutualistic and trophic networks. – Science 329(5993): 853-856.

VALDEYRON, O., DOMMEE, B. & VERNET, PH. 1977: Self- fertilization in male fertile plants of a gynodioecious species: *Thymus vulgaris* L. – Heredity 2: 243-249.

VAN LOOY, K., JACQUEMYN, H., BREYNE, P. & HONNAY, O. 2009: Effects of flood events on the genetic structure of riparian populations of the grassland plant *Origanum vulgare*. – Biol. Conserv. 142(4): 870-878.

VÁZQUEZ, D. P. & AIZEN, M. A. 2004: Asymmetric specialization: a pervasive feature of plant-pollinator interactions. – Ecology 85(5): 1251-1257.

VERNET, P., GOUYON, R. H. & VALDEYRON, G. 1986: Genetic control of the oil content in *Thymus vulgaris* L: a case of polymorphism in a biosynthetic chain. – Genetica 69(3): 227-231.

WESTERKAMP, C. & CLAßEN-BOCKHOFF, R. 2007: Bilabiate flowers: the ultimate response to bees? – Ann. Bot. 100: 361-374.

WOOD, T. & GOULSON, D. 2017: The Environmental Risks of neonicotinoid pesticides: a review of the evidence post-2013. – Environ. Sci. Pollut. Res. 24: 17285-17325.

WOODCOCK, B. A., ISAAC, N. J., BULLOCK, J. M., ROY, D. B., GARTHWAITE, D. G., CROWE, A. & PYWELL, R. F. 2016: Impacts of neonicotinoid use on long-term population changes in wild bees in England. – Nat. Commun. 7: 12459. Doi: 10.1038/ncomms12459

WRIGHT, G. A., SOFTLEY, S. & EARNSHAW, H. 2015: Low doses of neonicotinoid pesticides in food rewards impair short-term olfactory memory in foraging-age honeybees. – Sci. Rep. 5: 15322. Doi: 10.1038/srep15322

Karyological study of eight *Trifolium* L. taxa from Greece

SOFIA SAMAROPOULOU, EVAGELOS BALIOUSIS & PEPY BAREKA

Abstract

Samaropoulou, S., Baliousis, E. & Bareka, P. 2019: Karyological study of eight *Trifolium* L. taxa from Greece. – Bot. Chron. 22: 159-170.

The karyotype morphology and a karyomorphometric study for 8 *Trifolium* taxa (*T. arvense*, *T. boissieri*, *T. fragiferum*, *T. glomeratum*, *T. physodes*, *T. subterraneum*, *T. resupinatum and T. suffocatum*) is carried out for the first time in Greek material and mitotic metaphase plates are provided. Karyotype features and new cytological findings (B-chromosomes) from eleven populations are discussed.

S. Samaropoulou & P. Bareka, Laboratory of Systematic Botany, Department of Crop Science, Agricultural University of Athens, GR-11855 Athens, Greece. E-mails: s.samarop@aua.gr; bareka@aua.gr

E. Baliousis, Gortinias 2, Dionisos Attiki, GR-14569, Greece. E-mail: baliousisv@biol.uoa.gr

Key words: cytology, distribution, karyotype morphology, Greek flora.

Introduction

Trifolium L. (clovers) is one of the largest genera of the Fabaceae family comprising ca. 255 species worldwide (SMÝKAL & al. 2015, SCOPPOLA & al. 2018). Its main centers of diversity are located in the Mediterranean basin (with highest concentration in Anatolia and Greece), western North America (with highest concentration in California) and the highlands of eastern Africa (with highest concentration in Ethiopia). Its distribution range extends throughout the temperate and subtropical regions of the world. It also occurs, thought with lower frequence, in the tropics of West Africa and South America, where it is restricted to the montane and alpine zones (ZOHARY & HELLER 1984). The results of molecular phylogenetic studies are consistent with a Mediterranean origin of the genus, probably in the Early Miocene. Several species are widely grown as livestock forage and green manure crops (ELLISON & al. 2006). In addition, clovers are widely used in bee-keeping and rarely also as pot herbs and medicinal plants (ZOHARY & HELLER 1984). At least ten species of *Trifolium* are of agricultural significance (SMÝKAL & al. 2015).

In Greece the genus is represented by 95 taxa (species and subspecies), eight of which are Greek endemics. It is the fifth taxon-rich genus of the Greek flora (see in DIMOPOULOS & al. 2013). Classification and number of taxa frequently vary among the authors depending on the taxonomical approach (mainly related to the broader or narrower concept of infraspecific taxa), the progress of botanical exploration and the increase of systematic and evolutionary studies.

The taxa of the genus in Greece occur in various types of natural, semi-natural and anthropogenic habitats. Frequently, they thrive in open man-made habitats such as roadsides, path margins, archaeological sites and other disturbed places. They often contribute significantly in the floristic diversity of the agricultural land (cultivated, uncultivated and fallow fields). They also constitute important floristic elements of several post-fire communities. According to ELLISON & al. (2006) a common feature of the diverse habitats that *Trifolium* species occupy worldwide is high solar radiation; few clover species tolerate shade. This might be one of the factors that explain their habitat preferences in Greece too.

Triflolium alexandrinum L. is the only taxon which is registered as alien in the Greek flora. It is a fodder crop of SW Asian origin which is locally naturalized (DIMOPOULOS & al. 2013). Naturalization and invasion process of some *Trifolium* taxa have been studied in Australia (EMMS & al. 2005) and New Zealand (GRAVUER 2004), areas where the genus was initially absent. At the moment there is no such concern in Greece.

Aspects concerning species richness and distribution in South Europe are discussed by SCOPPOLA & al. (2018), while ecological and chorological information about *Trifolium* taxa of Greece is provided by VRAHNAKIS & al. (2006) and FOTIADIS & al. (2010).

Karyological studies are considered a useful tool in taxonomic and evolutionary studies. The most common and ancestral basic chromosome number in the genus *Trifolium* is x = 8, found in about 80% of the species belonging to all of the genus' sections, while aneuploidy with x = 5, 6 & 7 has also been reported (ZOHARY & HELLER 1984, ELLISON & al. 2006, VIŽINTIN & al. 2006) mostly in annual species (FALISTOCCO & al. 2013). Moreover, several ploidy levels (4x, 6x, 12x) have been referred in about 40 species of the genus (MAJUMDAR & al. 2004, ELLISON & al. 2006, USLU 2012).

Chromosome numbers from Greek populations of some *Trifolium* taxa have already been given by RUNEMARK (2006), however and to our knowledge, no karyotype morphology has been described or a microphotograph of a mitotic metaphase plate has been given.

In the present study a karyological study of 8 *Trifolium* species is carried out. The chromosome number, karyotype morphology, as well as 8 karyomorphometric indices are given and discussed.

It is a great pleasure and honor for the opportunity given to us to publish this work as a tribute to Prof. Emer. Dimitrios Phitos as a recognition to his achievements and guidance.

Material and methods

Seeds of the studied taxa were collected from several locations of Greece and remained in wet conditions at 4 °C until they germinated. Vouchers are deposited in the private collection of the second author and in the Herbarium of the Agricultural University of Athens (ACA).

Root tips were pretreated in 8-hydroxyquinoline (0.002% w/v) for 6 hrs in Room Temperature, then fixed in 3:1 (v/v) absolute ethanol:glacial acetic acid for 24 hours at 4°C and stored in 75% ethanol at -20 °C.

Hydrolysis in 1N HCl 60°C lasted for 15 min and staining was achieved after 2.5 hrs in Feulgen (DARLINGTON & LA COUR 1969). The stained root tips were put on a slide with a drop of 2% aceto-orcein solution and then squashed according to ÖSTERGREN & HENEEN (1962) and KAMARI (1976).

At least five plates per taxon were observed using AXIOLAB Zeiss microscope. Chromosome terminology follows LEVAN & al. (1965), STEBBINS (1971) and KAMARI (1976), taking into consideration comments and suggestions by SYBENGA (1959), BENTZER & al. (1971) and FAVARGER (1978). The karyomorphometric study includes chromosome counts, a presentation of the karyotype formula, maximum (max-L), minimum (min-L), total (TCL), average (ACL) and total haploid (THL) chromosome length (Table 1). Concerning the asymmetry of the karyotype, the interchromosomal asymmetry (CV_{CL}) according to PASZKO (2006) and the intrachromosomal asymmetry (M_{CA}) according to PERUZZI & EROĞLU (2013) are estimated, as well as the coefficient of variation of centromeric index (CV_{CI}), measuring the centromere position heterogeneity (PASZKO 2006).

Results and Discussion

***Trifolium arvense* L. — 2*n* = 14 + 2B** (Fig. 1A).

— Attiki, Mt. Pendelikon, near the summit Pirgari, sandy road margins, 1020 m, 22 Jun 2007, *Bal. 3897*.

— Attiki, municipality of Thrakomakedones, ca. 1 km ESE of the monastery of Kimiseos Theotokou, recently burnt *Pinus halepensis* Mill. forest, coarse-grained fluviolacustrine formations on Parnitha piedmont, 350 m, 4 May 2018, *Bal. 12908*.

Trifolium arvense is a therophyte with a paleotemperate distribution found usually at the edges of meadows and fields, road sides and sand dunes. The chromosome number 2*n* = 14 has been counted numerous times from almost all of the distribution range of the species (CCDB Database - RICE & al. 2014 see for references). Recently, a karyomorphometric study of a population from Turkey was carried out (KIRAN & al. 2015), revealing a symmetrical karyotype consisting of 2*n* = 4M + 10m chromosomes. Additionally, the somatic number of 2*n* = 12M + 4sm = 16 was also reported from Iranian populations (SALIMPOUR & al. 2008) with two satellite chromosome pairs.

Chromosome counts (2*n* = 14) of populations from Serifos (Kiklades) and Ikaria (E Aegean) islands were given by RUNEMARK (2006). In the present study two populations of Attiki are studied, revealing the same somatic number 2*n* = 14. Moreover, two metacentric B-chromosomes are found, much smaller than the rest of the complement's chromosomes.

The karyotype is diploid, symmetrical, with 2*n* = 4m + 10sm = 14 chromosomes, whose size ranges from 0.63 to 1.25 μm, little differentiated from the one given by KIRAN & al. (2015). The total chromosome length (TCL) is 13.12 μm and the average (ACL) is 0.94 μm. The presence of B chromosomes in the taxon's karyotype is reported here for the first time.

Table 1. Studied species with karyomorphometric indices: basic chromosome number (x), chromosome number (2*n*), karyotype formula, minimum (min l+s) and maximum (max l+s) chromosome length, total (TCL) and average (ACL) chromosome length, total haploid chromosome length (THL), karyotype asymmetry indices (CV_{CL} and M_{CA}) and variation of centromeric position (CV_{CI}).

Taxa	**x**	**2*n***	**Karyotype formula**	**min**	**max**	**TCL**	**ACL**	**THL**	**CV_{CL}**	**CV_{CI}**	**M_{CA}**
T. arvense	7	14	4m + 10sm	0.63	1.25	13.12	0.94	6.56	20.9	22.5	37.38
T. boissieri	8	16	8m + 8sm	1.04	2.29	25.83	1.61	12.92	22.35	14.05	25.66
T. fragiferum	8	16	12m + 4sm	1.04	2.08	26.25	1.64	13.13	20.14	10.55	20.75
T. glomeratum	8	16	12m + 4sm	0.83	1.87	20.21	1.26	10.1	25.88	14.23	17.76
T. physodes	8	16	14m + 2sm	1.45	2.29	30.42	1.9	15.21	9.7	10.29	15.5
T. resupinatum	8	16	16m	1.04	1.67	19.17	1.2	9.58	17.39	9.94	17.57
T. subterraneum	8	16	12m + 4sm	0.83	2.08	22.29	1.39	11.15	25.45	11.67	21.28
T. suffocatum	8	16	6m + 10sm	0.42	1.04	11.04	0.69	5.52	26.36	21.03	24.36

***Trifolium boissieri* Guss. — 2*n* = 16 + 2B** (Fig. 1B).

— Kiklades, the island of Kea, path margins on the way to Karthaia archaeological site, 50 m, 27 May 2018, *Bal. 12680.*

Trifolium boissieri is an East Mediterranean therophyte that grows mostly on olive groves, shrublands and rocky places.

The chromosome number (2*n* = 16), as well as a mitotic metaphase plate was already given from a Turkish population by TAYLOR & al. (1983). Moreover, RUNEMARK (2006) reported the same chromosome number from Serifos and Amorgos islands. The karyotype morphology of *T. boissieri* is in accordance with the one given by TAYLOR & al. (l.c.), consists of 2*n* = 8m + 8sm = 16 chromosomes, ranging in size between 1.04 and 2.29 µm and it is characterized by the presence of two metacentric chromosomes, much longer than all the others of the complement. The total chromosome length (TCL) is 25.83 µm, while the average length (ACL) is 1.61 µm. Two B-chromosomes are also observed, both of them metacentric, for the first time for this species.

***Trifolium fragiferum* L. — 2*n* = 16 + 1B** (Fig. 1C).

— Attiki, Stamata village, near the chapel of Agia Paraskevi, damp places by *Platanus orientalis* L. stream, 350 m. 12 Jul 2007, *Bal. 4000.*

Tifolium fragiferum is a perennial species native to Europe and SW Asia. The chromosome number 2*n* = 16 found here agrees with previous references from several countries (BLEIER 1925, KARPECHENKO 1925, PÓLYA 1948, TARNAVSCHI 1948, GADELLA & KLIPHUIS 1968, 1971, 1972, KAZIMIERSKI & KAZIMEIRSKA 1970, KUZMANOV & STANCEV 1972, KOZUHAROV & al. 1974, 1975, NIELSEN 1975, KLIPHUIS 1977, NATARAJAN 1977, 1978, KUZMANOV 1978, STRID 1980, PETROVA & KOZUHAROV 1982a & b, MAGULAEV 1987, POGAN & al. 1987, SEMERENKO & SHVETS 1989, MUÑOZ RODRÍGUEZ 1993a & b, DEMPSEY & al. 1994, BADR 1995, ISSOLAH & ABDELGUERFI 1999, LÖVKVIST & HULTGÅRD 1999, VIŽINTIN & al. 2006).

From Greece, the chromosome number has been given from Kiklades Islands (Paros) by RUNEMARK (2006), however, no karyotype was provided. The karyotype formula is given as 2*n* = 12m + 4sm = 16 + 1B. The TCL is equal to 26.25 µm and the ACL is 1.64 µm. The size of the chromosomes is measured from 1.04 µm to 2.08 µm. Additionally, one B-chromosome is observed and reported here for the first time.

***Trifolium glomeratum* L. — 2*n* = 16 + 2B** (Fig. 1D).

— Attiki, Stamata village, in the locality called Koukounaries, vineyards, 350 m, 7 May 2007, *Bal. 3618.*

— Peloponnisos, Mt. Aphrodisio, ca. 1 Km S of Nasia village, *Quercus frainetto* Ten. forest and roadsides, radiolarites, 700-800 m, 26 May 2013, *Bal. 8749.*

Trifolium glomeratum is an annual taxon native to Europe and SW Asia.

The taxon is characterized by a diploid, symmetrical karyotype with $2n = 12\text{m} + 4\text{sm} = 16$ chromosomes. Their size ranges from 0.83 to 1.87 μm. The total chromosome length is 20.21 μm and the average is 1.26 μm. RUNEMARK (2006) also reported $2n = 16$ from Kiklades Islands, while there are numerous reports from other countries too (WEXELSEN 1928, LORENZO ANDREU 1951, PRITCHARD 1969, CHEN & GIBSON 1971, DAHLGREN & al. 1971, FERNANDES & SANTOS 1971, GONZÁLEZ BERNÁLDEZ & al. 1973, KOZUHAROV & al. 1974, 1975, FERNANDES & al. 1977, FERNANDES & QUEIRÓS 1978, ANGULO & FIGUERAS 1979, COLOMBO & al. 1980, ANGULO & al. 1981, PETROVA & KOZUHAROV 1982a, DALGAARD 1986, DEMPSEY & al. 1994, MUÑOZ RODRIGUEZ 1996, ISSOLAH & ABDELGUERFI 1999, VIŽINTIN & al. 2006, SALIMPOUR & al. 2008). Moreover, the chromosome numbers $2n = 14$ and $2n =$ ca. 28 have also been given by BLEIER (1925) and CASTROVIEJO & al. (2003) respectively.

***Trifolium physodes* M. Bieb. — $2n = 16 + 1\text{B}$** (Fig. 1E).

— Attiki, Mt. Pendelikon, near the water tanks of Dionisos municipality, path margins in densely regenerated *Pinus halepensis* Mill. forest, 550 m, 3 Jun 2007, *Bal. 3773*.

Trifolium physodes is a Mediterranean hemicryptophyte growing in forests and shrublands. Its karyotype is diploid consisting of $2n = 14\text{m} + 2\text{sm} = 16$ chromosomes. Their minimum and maximum lengths are 1.45 μm and 2.29 μm respectively. The total chromosome length (TCL) is 30.42 μm and the average length is 1.9 μm. One B-chromosome is observed which is metacentric. The chromosome number found here is in agreement with previous references: FERNANDES & SANTOS (1971), FERNANDES & al. (1977), FERNANDES & QUEIRÓS (1978) and MUÑOZ RODRÍGUEZ (1993b) in material from Portugal, DE LEONARDIS & al. (1981) in material from Italy, PETROVA & KOZUHAROV (1982a) in material from Bulgaria and RUNEMARK 2006 in material from Greece (Kriti and Naxos isl.). Additionally, a karyotype analysis along with a relevant photo was given from a Turkish population by GIRI & al. (1981) showing the same chromosome morphology with the material studied here.

***Trifolium resupinatum* L. — $2n = 16$** (Fig. 1F).

— Ionian Islands, Kefallinia, margins of Xi seashore, 10 Apr 2005, *Bal. 1703*.
— Peloponnisos, Mt. Aphrodisio, between Dafni and the crossroad to Nasia village, roadsides, 550-600 m, 26 May 2013, *Bal. 8673*.

Trifolium resupinatum is an annual species found in Europe and the Mediterranean area. The karyotype of the populations studied consists of $2n = 16$ chromosomes, confirming previous studies for material covering almost all of its distribution range (KARPECHENKO 1925, ALMEIDA 1957, FERNANDES & SANTOS 1971, 1975, FERNANDES & al. 1977, COLOMBO & al. 1978, FERNANDES & QUEIRÓS 1978, POGAN & al. 1985, KUMARI & BIR 1990, KHATOON & ALI 1993, JAHAN & al.1994, BADR 1995, MUÑOZ RODRIGUEZ 1996, ISSOLAH & ABDELGUERFI 1999, VIŽINTIN & al. 2006, RUNEMARK 2006, SALIMPOUR & al. 2008, USLU 2012, KIRAN & al. 2015). In

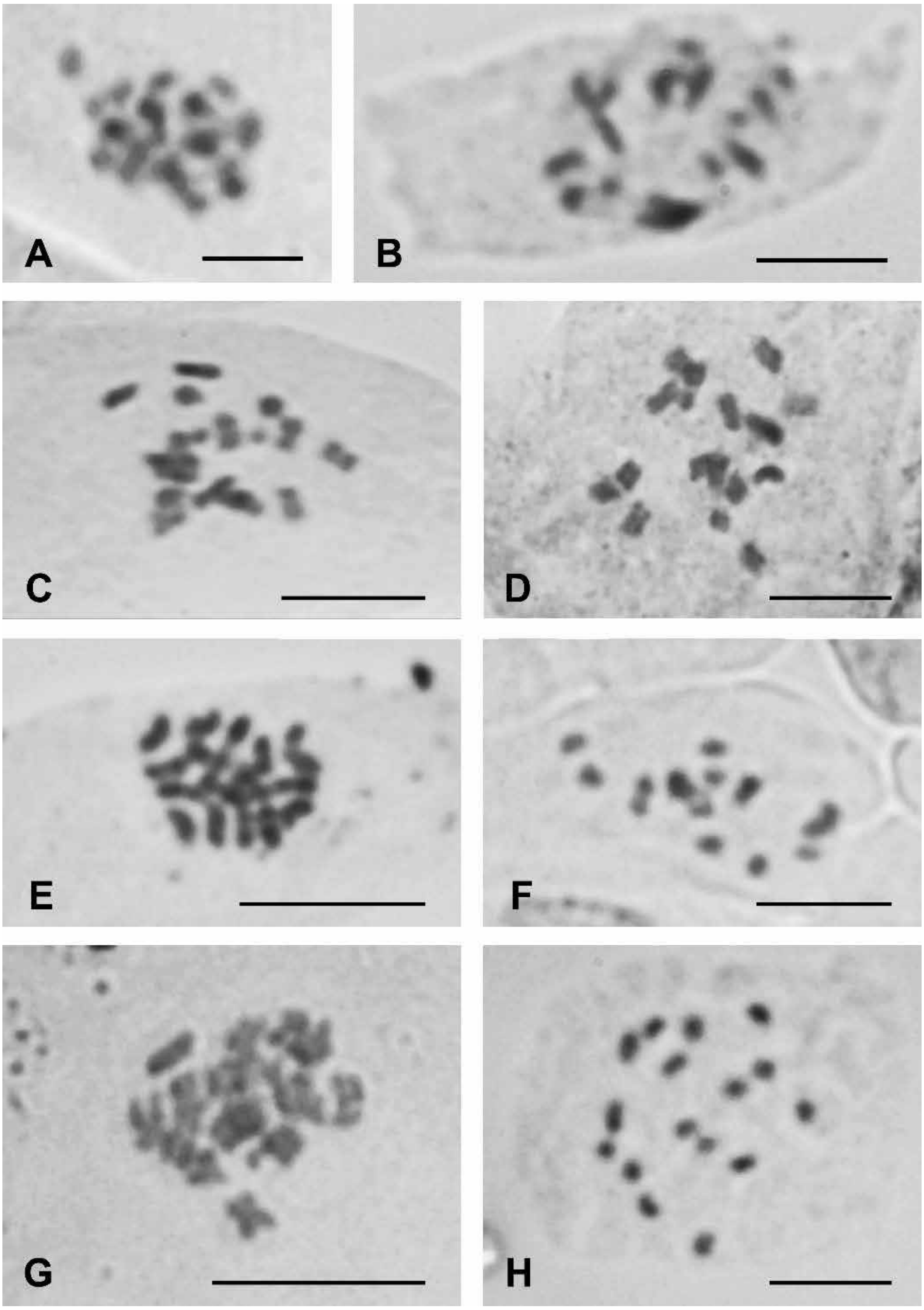


Fig. 1: Microphotographs of mitotic metaphase plates of *Trifolium* species: A, *T. arvense*, 2*n* = 14; B, *T. boissieri*, 2*n* = 16; C, *T. fragiferum*, 2*n* = 16; D, *T. glomeratum*, 2*n* = 16; E, *T. physodes*, 2*n* = 16; F, *T. resupinatum*, 2*n* = 16; G, *T. subterraneum*, 2*n* = 16; H, *T. suffocatum*, 2*n* = 16. – Scale bars = 5 µm.

addition, cases of tetraploidy ($2n = 4x = 32$) have been published by ALMEIDA (1957) in material from Portugal and by PETROVA & KOZUHAROV (1982a) in material from Bulgaria. Finally, the chromosome number $2n = 14$ has also been given by BLEIER (1925) and WIPF (1939).

According to our findings, all chromosomes are metacentric (m). The TCL is given as 19.17 μm while the ACL is 1.2 μm. The chromosomes range in size between 1.04 and 1.67 μm.

***Trifolium subterraneum* L. — $2n = 16$** (Fig. 1G).

— Peloponnisos, Mt. Aphrodisio, ca. 1 Km S of Nasia village, forest roadsides, 700-800 m, 26 May 2013, *Bal. 8755*.

Trifolium subterraneum is a therophyte with a wide geographical distribution covering Europe, Mediterranean area and temperate Asian countries. The chromosome number $2n = 16$, found also in the material studied here, has already been given (CCDB Database - RICE & al. 2014 see for references therein). Moreover, YATES & BRITTAN (1952) and BROCK (1953) referred the somatic number $2n = 16$, while a case of tetraploidy has been recorded in material from Iran by SALIMPOUR & al. (2008).

The karyotype is symmetrical, consisting of $2n = 16$ chromosomes, 12 of them metacentric and 4 submetacentric. Their total chromosome length is 22.29 μm. The maximum length is 2.08 μm, while the minimum is 0.83 μm and the average size is 1.39 μm.

***Trifolium suffocatum* L. — $2n = 16$** (Fig. 1H).

— Attiki, Mt. Pendelikon, northern slopes of Megali Mavrinora, sparse *Quercus coccifera* L. scrub, marbles, 550 m, 14 May 2008, *Bal. 4195*.

Trifolium suffocatum is a Mediterranean and European therophyte growing mostly in open cultivated fields, grasslands and phrygana.

The chromosome number $2n = 16$ of the taxon has been reported previously by several authors (LORENZO ANDREU 1951, PRITCHARD 1969, CHEN & GIBSON 1971, FERNANDES & SANTOS 1971, FERNANDES & al. 1977, GONZÁLEZ BERNÁLDEZ & al. 1973, FERNANDES & QUEIRÓS 1978, ANGULO & al. 1981, PETROVA & KOZUHAROV 1982a & b, MUÑOZ RODRIGUEZ 1996, MONTGOMERY & al. 1997, RUNEMARK 2006). Its karyotype is symmetrical, with $2n = 6m + 10sm = 16$ chromosomes, whose length varies from 0.42 μm to 1.04 μm. The TCL and ACL are 11.04 μm and 0.69 μm respectively.

References

ALMEIDA, J. L., de 1957: Trevos autotetraploides I. O caso de *Trifolium resupinatum* L. ssp. *suaveolens* (Willd.) Dinsm. – Agron. Lusit. 19: 127-144.

ANGULO, M. D. & FIGUERAS, M. C. 1979: Números cromosómicos y sacos polínicos en especies del género *Trifolium*. – Genét. Ibér. 31: 129-160.

—, — & SANCHEZ DE RIVERA, A. M. 1981: Estudios cariohistológicos en el género *Trifolium*. – Bol. Soc. Brot. 53: 877-885.

BADR, A. 1995: Electrophoretic studies of seeds proteins in relation to chromososmal criteria and the relationships of some taxa of *Trifolium*. – Taxon 44(2): 183-191.

BENTZER, B., BOTHMER, R. VON, ENGSTRAND, L., GUSTAFSSON, M. & SNOGERUP, S. 1971: Some sources of error in the determination of arm ratios of chromosomes. – Bot. Notiser 124: 65-74.

BLEIER, H. 1925: Chromosomenstudien bei der gattung *Trifolium*. – Jahrb. Wiss. Bot. 64: 604-636.

BROCK, R. D. 1953: Species formation in *Trifolium subterraneum*. – Nature 171: 939.

CASTROVIEJO, S., AEDO, C., ALDASORO, J. J., BENEDÍ, C., CIRUJANO, S., GÓMEZ CAMPO, C., HEDGE, I. C., HERRERO, A., JURY, S., LAÍNZ, M., LÓPEZ GONZÁLEZ, G., MONTSERRAT, P., MORALES, R., MUÑOZ GARMENDIA, F., NAVARRO, C., NIETO FELINER, G., PAIVA, J., RICO, E., ROMERO ZARCO, C., SÁEZ, J., SALES, F., SALGUEIRO, F. J., SORIANO, C., TALAVERA, S., VELAYOS, M., VILLAR, L. (eds) 2003: Flora Iberica. Plantas vasculares de la Peninsula Iberica e Islas Baleares. Vols. I-VIII, X, XIV. – Real Jardín Botánico-CSIC, Madrid.

CHEN, C. C. & GIBSON, P. B. 1971: Karyotypes of fifteen *Trifolium* species in section *Amoria*. – Crop Sci. 11: 441-445.

COLOMBO, P., COLOMBO, R., MARCENÒ, C. & PAVONE, P. 1978: Numeri cromosomici per la flora italiana: 517-525. – Inf. Bot. Ital. 10(3): 406-413.

—, MARCENÓ, C. & PRINCIOTTA, R. 1980: Numeri cromosomici per la flora italiana: 794-805. – Inf. Bot. Ital. 12: 333-340.

DAHLGREN, R., KARLSSON, Th. & LASSEN, P. 1971: Studies on the Flora of the Balearic Islands, I. Chromosome numbers in Balearic Angiosperms. – Bot. Notiser 124(1): 249-269.

DALGAARD, V. 1986: Chromosome numbers in flowering plants from Madeira. – Willdenowia 16: 221-240.

DARLINGTON, C. D. & LA COUR, L. F. 1969: The Handling of Chromosomes. – London, U.K.

DE LEONARDIS, W., PAVONE, P., TERRASI, M. C. & ZIZZA, A. 1983 (1981): Numeri cromosomici per la flora italiana 814-830. – Inform. Bot. Ital. 13(2-3): 158-167.

DEMPSEY, R. E., GORNALL, R. J. & BAILEY, J. P. 1994: Contributions to a cytological catalogue of the British and Irish flora, 4. – Watsonia 20: 63-66.

DIMOPOULOS, P., RAUS, TH., BERGMEIER, E., CONSTANTINIDIS, TH., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. (eds) 2013: Vascular plants of Greece: An annotated checklist. – Berlin & Athens.

ELLISON, N. W., LISTON, A., STEINER, J. J., WILLIAMS, W. M. & TAYLOR, N. L. 2006: Molecular phylogenetics of the clover genus (*Trifolium*-Leguminosae). – Molec. Phylogen. Evol. 39: 688-705.

EMMS, J., VIRTUE, J. G., PRESTON, C. & BELLOTTI, W. D. 2005: Legumes in temperate Australia: A survey of naturalisation and impact in natural ecosystems. – Biol. Conserv. 125(3): 323-333.

FALISTOCCO, E., MARCONI, G. & FALCINELLI, M. 2013: Comparative cytogenetic study on *Trifolium subterraneum* (2*n* = 16) and *Trifolium israeliticum* (2*n* = 12). – Genome 56: 307-313.

FAVARGER, C. 1978: Philosophie des comptages de chromosome. – Taxon 27: 441-448.

FERNANDES, A. & QUEIROS, M. 1978: Contribution à la connaissance cytotaxinomique des Spermatophyta du Portugal. IV. Leguminosae (Suppl. 3). – Bol. Soc. Brot. (sér. 2) 52: 79-164.

— & SANTOS, M. F. 1971: Contribution a la connaissance cytotaxinomique des Spermatophyta du Portugal IV. Leguminosae. – Bol. Soc. Brot. (sér. 2) 45: 177-226.

— & SANTOS, M. F. 1975: Contribution à la connaissance cytotaxinomique des spermatophyta du Portugal. IV. Leguminosae, suppl. 1. – Bol. Soc. Brot. (sér. 2) 49: 173-196.

—, SANTOS, M. F. & QUEIROS, M. 1977: Contribution à la connaissance cytotaxonomique des Spermatophyta de Portugal. Leguminosae. – Bol. Soc. Brot. (sér. 2) 51: 137-186.

FOTIADIS, G., VRAHNAKIS, M. S., MEROU, TH. & VIDAKIS, K. 2010: Ecology, chorology and commonness of the *Trifolium* taxa in Greece. – Feddes Repert. 121(1-2): 66-80.

GADELLA, TH. W. J. & KLIPHUIS, E. 1968: Chromosome numbers of flowering plants in the Netherlands IV. – Proc. Roy. Netherlands Acad. Sci. ser. C. 71: 168-183.

— & — 1971: Chromosome numbers of flowering plants in the Netherlands V. – Proc. Kon. Ned. Akad. Wetensch. Ser. C 74: 335-343.

— & — 1972: studies in chromosome numbers of Yugoslavian angiosperms. – Acta Bot. Croatica 31: 91-103.

GIRI, N., TAYLOR, N. L. & COLLINS, G. B. 1981: Chromosome numbers in some *Trifolium* species with a karyotype for *T. physodes*. – Canad. J. Genet. Cytol. 23: 621-626.

GONZÁLEZ BERNÁLDEZ, F., SÁNCHEZ DE RIVERA, A. M. & ANGULO, M. D. 1973: Estudios cromosómicos en el género *Trifolium* IV. – Lagascalia 3(2): 195-203.

GRAVUER, K. 2004: Determinants of the introduction, naturalisation, and spread of *Trifolium* species in New Zealand. – MSc Thesis, Lincoln Univ., New Zealand.

ISSOLAH, R. & ABDELGUERFI, A. 1999: Chromosome numbers within some spontaneous populations of 10 *Trifolium* species in Algeria. – Caryologia 52: 151-154.

JAHAN, B., VAHIDY, A. A. & ALI, S. I. 1994: Chromosome numbers in some taxa Fabaceae mostly native to Pakistan. – Ann. Missouri Bot. Gar. 81: 792-799.

KAMARI, G. 1976: Cytotaxonomic study of the *Crepis neglecta* L. complex in Greece. – Ph.D. Thesis, University of Patras, Patras, Greece [in Greek].

KARPECHENKO, G. D. 1925: Karyologische studien über dir gattung *Trifolium* L. – Bull. Appl. Bot., Genet. & Plant Breeding 14: 271-279.

KAZIMIERSKI, T. & KAZIMIERSKA, E. M. 1970: Badania miezancow rodzaju *Trifolium* L. II. Morfologia i cytogenetika miezancow *Trifolium neglectum* C. A. M. x *Trifolium fragiferum* L. – Acta Soc. Bot. Poloniae 39: 297-320.

KHATOON, S. & ALI, S. I. 1993: Chromosome Atlas of the Angiosperms of Pakistan. – Department of Botany, University of Karachi, Karachi.

KIRAN, Y., ŞAHIN, A., TÜRKOĞLU, I., EMRE, I. & KURŞAT, M. 2015: Karyological notes on seven *Trifolium* L. taxa from Turkey. – Turk. J. Bot. 10(2): 11-16.

KLIPHUIS, E. 1977: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports LVI. – Taxon 26(2/3): 267-268.

KOZUHAROV, S., PETROVA, A. & MARKOVA, T. 1974: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports XLIV. – Taxon 23(2/3): 377-378.

—, — & — 11975: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports XLVII. – Taxon 24(1): 145-146.

KUMARI, S. & BIR, S. S. 1990: Karyomorphological evolution in Papilionaceae. – J. Cytol. Genet. 25: 173-219.

KUZMANOV, B. 1978: Citotaxonomicno izsledvane na balgarski bobovi rastcnija. – Evoljuclja na cvetnite rastenija i florogenez 1: 11-71.

— & STANCEV, G. 1972: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports XXXVIII. – Taxon 21(5/6): 681.

LEVAN, A., FREDGA, K. & SANDBERG, A. 1965: Nomenclature for centromeric position on chromosomes. – Hereditas 52: 201-220.

LORENZO ANDREU, A. 1951: Cromosomas de plantas de la estepa de Aragón, III. – Anales Estac. Exp. Aula Dei 2: 195-203.

LÖVKVIST, B. & HULTGÅRD, U. M. 1999: Chromosome numbers in south Swedish vascular plants. – Opera Bot. 137: 1-42.

MAGULAEV, A. Y. 1987: A cytotaxonomical study of species belonging to the subgenus Lotoidea of the genus *Trifolium* (Fabaceae) from the flora of the Crimea and the Caucasus. – Bot. Žurn. 72: 1356-1363.

MAJUMDAR, S., BANERJEE, S., KUMAR, D. E. K. 2004: Meiotic behavior of chromosome in PMCs and karyotype of *Trifolium repens* L. from Darjeeling Himalaya. – Acta Biol. Cracov. Bot. 46(1): 217-220.

MONTGOMERY, L., KHALAF, M., BAILEY, J. P. & GORNAL, K. J. 1997: Contributions to a cytological catalogue of the British and Irish flora, 5. – Watsonia 21: 365-368.

MUÑOZ RODRÍGUEZ, A. F. 1993a: *Trifolium* sect. *Trifolium*. II. Estudio cariológico. – Acta Bot. Malac. 18: 89-118.

— 1993b: Estudio biosistemático de *Trifolium* sect. *Vesicastrum* en la Península Ibérica. – Stud. Bot. Univ. Salamanca 11: 259-295.

— 1996: *Trifolium* sect. *Paramesus* y sect. *Trifoliastrum* en la Península Ibérica. II. Estudio cariológico. – Stud. Bot. Univ. Salamanca 14: 103-128.

NATARAJAN, G. 1977: Contribution à l'etude caryosystematique des especes de la garrigue languedocienne. – These, Academie de Montpellier, France.

— 1978: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports LXII. – Taxon 27(5/6): 526-531.

NIELSEN, I. 1975: Chromosome counts in the genus *Trifolium*. – Bot. Tidsskr. 70: 180-183.

ÖSTERGREN, G. & HENEEN, W. K. 1962: A squash technique for chromosome morphological studies. – Hereditas 48: 332-341.

PASZKO, A. 2006: A critical review and a new proposal of karyotype asymmetry indices. – Plant Syst. Evol. 258: 39-48.

PERUZZI, L. & EROĞLU, H. E. 2013: Karyotype asymmetry: again, how to measure and what to measure? – Comp. Cytogenet. 7(1): 1-9.

PETROVA, A. & KOZUHAROV, S. 1982a: Cytotaxonomic study of genus *Trifolium* L. in Bulgaria. I. – Phytology 19: 3-23.

— & — 1982b: Cytotaxonomic study of genus *Trifolium* L. in Bulgaria. II. – Phytology 20: 20-41.

POGAN, E., CZAPIK, R. & JANKUN, A. 1985: Further studies in chromosome numbers of Polish Angiosperms. Part XVIII. – Acta Biol. Cracov., Ser. Bot. 27: 57-74.

—, JANKUN, A. & TURASLA-SZYBOWSKA, K. 1987: Further studies in chromosome numbers of Polish angiosperms. Part XX. – Acta Biol. Cracov., Ser. Bot. 29: 1-17.

PÓLYA, L. 1948: Chromosome numbers of certain alkali plants. – Arch. Biol. Hung. 18: 145-148.

PRITCHARD, A. J. 1969: Chromosome numbers in some species of *Trifolium*. – Austral. J. Agric. Res. 20: 883-887.

RICE, A., GLICK, L., ABADI, S., EINHORN, M., KOPELMAN, N. M., SALMAN-MINKOV, A., MAYZEL, J., CHAY, O. & MAYROSE, I. 2014: The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. – New Phytol. 206(1): 19-21. Doi: 10.1111/nph.13191

RUNEMARK, H. 2006: Reports (1473-1571). In KAMARI, G. BLANCHÉ, C. & GARBARI, F. (eds): Mediterranean chromosome number reports-16. – Fl. Medit. 16: 408-425.

SALIMPOUR, F., SHARIFNIA, F., MOSTAFAVI, G., HAJRASOLIHA, M. SH. and UKHNEH, E. 2008: Chromosome counts and determination of ploid levels in Iranian species of *Trifolium*. – Chromosome Botany 3: 53-63.

SCOPPOLA, A., LÓPEZ TIRADO, J., MANZANO GUTIÉRREZ, F. & MAGRINI, S. 2018: The genus *Trifolium* (Fabaceae) in south Europe: a critical review on species richness and distribution. – Nord. J. Bot. 36: njb-01723. Doi:10.1111/njb.01723

SEMERENKO, L. V. & SHVETS, I. V. 1989: Karyologicheskoe isuchenie bobovykh (Fabaceae) Belorussii. Pp. 66-68. In Tesizy II Symp. – Plant Karyology.

SMÝKAL, P., COYNE, C. J., AMBROSE, M. J., MAXTED, N., SCHAEFER, H., BLAIR, M. W., BERGER, J., GREENE, S. L., NELSON, M. N., BESHARAT, N., VYMYSLICKÝ, T., TOKER, C., SAXENA, R. K., ROORKIWAL, M., PANDEY, M. K., HU, J., LI, Y. H., WANG, L. X., GUO, Y., QIU, L. J., REDDEN, R. J. & VARSHNEY, R. K. 2015: Legume crops phylogeny and genetic diversity for science and breeding. – CRC Crit. Rev. Plant Sci. 34: 43-104. Doi: 10.1080/07352689.2014.897904

STEBBINS, G. L. 1971: Chromosomal evolution in higher plants. – Edward Arnold (Publishers) Ltd, London.

STRID, A. 1980: Reports. In LÖVE, A. (ed.), IOPB chromosome numbers reports LXIX. – Taxon 29(5): 709-710.

SYBENGA, J. 1959: Some sources of error in the determination of chromosome length. – Chromosoma 10: 355-364.

TARNAVSCHI, I. T. 1948: Die chromosomenzahlen der Anthophyten flora von Rumänien mit einem ausblick auf das Polyploidie problem. – Bull. Jard. Mus. Bot. Univ. Cluj. 28: 1-130.

TAYLOR, N. L., GILLETT, J. M. & GIRI, N. 1983: Morphological observations and chromosome numbers in *Trifolium* L. section *Chronosemium* Ser. – Cytologia 48: 671-677.

USLU, E. 2012: Karyology of nine *Trifolium* L. taxa from Turkey. – Caryologia 65(4): 304-310. Doi: 10.1080/00087114.2012.752925

VIŽINTIN, L., JAVORNIK, B. & BOHANEC, B. 2006: Genetic characterization of selected *Trifolium* species as revealed by nuclear DNA content and ITS rDNA region analysis. – Plant Sci. 170(4): 859-866.

VRAHNAKIS, M. S., FOTIADIS, G., MEROU, TH. & PLASTARGIA, S. 2006: Genus *Trifolium* in Greece: distributional aspects and life traits. In LLOVERAS, J., GONZALEZ-RODRIGUEZ, A., VAZQUEZ-YANEZ, O., PINEIRO, J., SANTAMARIA, O., OLEA, L. & POBLACIONES, M. J. (eds): Sustainable Grassland Productivity. – Proc. 21st General Meeting of the European Grassland Federation: 321-323.

WEXELSEN, H. 1928: Chromosome numbers and morphology in *Trifolium*. – Univ. Calif. Publ. Agric. Sci. 12: 355-376.

WIPF, L. 1939: Chromosome numbers in root nodules and root-tips of certain Leguminosae. – Bot. Gaz. 101: 51-67.

YATES, J. J. & BRITTAN, N. H. L. 1952: Cytological studies of subterranean clover (*Trifolium subterraneum* L.). – Austral. J. Agric. Res. 3: 300-304.

ZOHARY, M. & HELLER, D. 1984: The genus *Trifolium*. – Jerusalem: Israel Academy of Sciences and Humanities, x + 606 pp. + 1 plate.

The taxa of *Crocus* ser. *Flavi* (Iridaceae) in Greece: a taxonomic and karyomorphometric study

THEOPHANIS KARAMPLIANIS & THEOPHANIS CONSTANTINIDIS

Abstract

Karamplianis, Th. & Constantinidis Th. 2019: The taxa of *Crocus* ser. *Flavi* (Iridaceae) in Greece: a taxonomic and karyomorphometric study. – Bot. Chron.22: 171-194.

This study focuses on the morphology and karyology of Greek members of *Crocus* ser. *Flavi* and their consequences for taxonomy. The series, in Greece, consists of *Crocus balansae*, *C. olivieri, C. flavus* subsp. *flavus,* and *C. flavus* subsp. *dissectus*. The former two have $2n = 6$, whereas the latter two, $2n = 8$ chromosomes. Their karyotypes are basically similar, but differ in relative length, presence of B-chromosomes, and presumed chromosomal translocations. Gross morphology varies within the group with respect to perigon coloration, style dissection, as well as number and width of leaves. In the case of *C. flavus*, intra-population variation of morphological features supports a conservative taxonomic approach, with *C. flavus* subsp. *atticus* reduced to synonymy under *C. flavus* subsp. *flavus,* because morphology and karyomorphology do not support subspecific distinctness. On the other hand, *C. olivieri*, *C. balansae* and their Turkish endemic ally, *C. istanbulensis,* that exhibit additional differences in corm tunic features, are best regarded as distinct species.

Th. Karamplianis & * Th. Constantinidis, Department of Ecology & Systematics, Faculty of Biology, National & Kapodistrian University of Athens, Panepistimiopolis, GR-157 03 Athens, Greece. E-mails: theo_karamp@biol.uoa.gr; constgr@biol.uoa.gr (* = corresponding author).

Key words: Crocus ser. *Flavi*, karyotype asymmetry, morphology, somatic chromosomes, statistics, taxonomy.

Introduction

The genus *Crocus* L. (Iridaceae) comprises c. 200 taxa (HARPKE & al. 2016), its total distribution area extending from the Iberian Peninsula and Morocco to west China longitudinally, and from Poland and the Caucasus region to the northern parts of the Arabian Peninsula and Cyrenaica, latitudinally (MATHEW 1982, RUKŠĀNS 2010). According to DIMOPOULOS & al. (2013), 29 taxa (species and subspecies) are found in Greece, a number that recent taxonomic amendments and re-circumscriptions (RUKŠĀNS 2013, 2014, HARPKE & al. 2015, TAN & al. 2014) have raised to 35. According to current understanding, 4 taxa of *Crocus* ser. *Flavi* B. Mathew are growing in Greece, viz., *C. olivieri* J. Gay, *C. balansae* J. Gay ex Maw, *C. flavus* Weston subsp. *flavus* and *C. flavus* subsp. *dissectus* Baytop & B. Mathew. The first species has a wide distribution and altitudinal range on both mainland and insular Greece (GAY 1831, HERBERT 1847, ORPHANIDES 1869, BAKER 1873, HOOKER 1873, BOISSIER 1882, HALÁCSY 1896, ZAGANIARIS 1938, PAPANICOLAOU & ZACHAROF 1980, VOLIOTIS 1986, MATHEW 2000, SNOGERUP & al. 2001), and karyological data are plentiful (MATHEW &

BRIGHTON 1972, BRIGHTON & al. 1973, BRIGHTON 1976, MATHEW 1976, 1982, PAPANICOLAOU & ZACHAROF (1980), USLU & al. 2012, YETIŞEN & al. 2013, and YÜZBAŞIOĞLU & al. 2013. The second species is distributed from the East Aegean Islands (Chios and Samos, Greece) to Anatolia (Turkey), and its karyomorphology is reported here for the first time. Both species have a karyotype of $2n = 6$ somatic but no supernumerary B-chromosomes. *C. flavus* subsp. *flavus* is mainly found in the northern parts of the Greek mainland: its northern Greek populations, together with an outlying one in Central Greece, form the southernmost part of its total distribution area (MATHEW & BAYTOP 1976, PAPANICOLAOU & ZACHAROF 1980). It has also been reported from the island of Kerkyra (Corfu) by MAZZIARI (1834), but this report lacks recent confirmation. Its karyology was studied by MATHEW (1976, 1982) and PAPANICOLAOU & ZACHAROF (1980). *C. flavus* subsp. *dissectus* was considered a Turkish endemic until recently, when PAPANICOLAOU & ZACHAROF (1980) reported a population from Mt. Gramos (NW Greece) and found its chromosome number to be $2n = 8$. The southernmost Greek population of *C. flavus* subsp. *flavus* is located in the Tatoi area in Attiki, and was already known to *Heldreich*, who collected it in 1878, followed by *S. Atchley* and *E. A. Bowles* in 1926, *E. A. Bowles* in 1938, *K. Goulimis* in 1956, and *E. Stamatiadou* in 1969 (see HALÁCSY 1904, BOWLES 1924, GOULIMIS 1956 and MATHEW 1982).

Chromosome numbers and karyotype morphology, within *Crocus,* are important to assess species relationship and taxonomy, as pointed by out PERUZZI & ALTINORDU (2014) in their study of *Crocus* ser. *Verni*. Karyological data often correlate well with taxonomy and may support phylogenetic hypotheses (e.g. HARPKE & al. 2015). Karyotype studies may therefore be valuable in tracing evolutionary lineages within the genus. In *Crocus* ser. *Flavi*, the evolutionary advanced taxa show a clear tendency toward more asymmetrical karyotypes (HARPKE & al. 2013). Supernumerary B-chromosomes also may have played a role in genome evolution (HARPKE & al. 2013: 626), underpinning hybridization and phylogenetic trends within the genus.

Materials and methods

The material investigated in this study consists of dried specimens collected in Greece and deposited at the Herbarium of the National and Kapodistrian University of Athens (ATHU), including voucher specimens for our karyological data. Material from the herbaria P, K, WU, B, IPK (see THIERS, continuously updated) was consulted in addition. Living plants collected in the field and cultivated in an experimental garden of School of Biology at National & Kapodistrian University of Athens were used for karyological studies, a topic to be included in the first author's PhD thesis, currently in progress. Their origin, chromosome number and karyomorphometric data are presented in Table 1.

For our morphometric study, quantitative morphological features of 5 taxa were measured, and qualitative features assessed, on 184 individuals from 24 populations distributed in Greece, the Balkans and NW Turkey. Measurements were taken on dried specimens, using a Zeiss Stemi 2000 stereomicroscope, whereas specimen photographs were processed by the Image J v.1.50f software (SCHNEIDER & al. 2012).

For compiling the morphological data matrix we used both quantitative and qualitative characters. The most informative characters in terms of univariate analysis were used (Table 2 and 3). At first, variables of 71 vegetative and floral characters with 2 (binary) to 10 states (multistate characters) and quantitative characters (Table 3) were analysed for the whole genus, in

Table 1. Origin, karyomorphometry and chromosome relative length of *Crocus olivieri* and *Cr. flavus* aggregates according to literature and our own data. For details of the headings see the text (material and methods). The following references were used, particularly for the last column: [1]STEBBINS (1971), [2]PLUMMER & al. (2003), [3]MATHEW & BRIGHTON (1972), [4]BRIGHTON (1976), [5]YÜZBAŞIOĞLU & al.(2013).

***Crocus* taxa**	**Lat.**	**Long.**	**x**	**2n**	**THL**	**CV$_{CI}$**	**CV$_{CL}$**	**M$_{CA}$**	**1-4 A-C**	**B -**	**SAT-**			PLUMMER & al. [2]					**Ref.**
									STEBBINS [1]	**chrom**	**L**	**S**	**int**	**VS**	**S**	**M**	**L**	**VL**	
Cr. antalyensis	s.d.	s.d	4	8	19.94	11.99	26.94	41.22	2A	0	0	0	0	0	0	0.88	0.12	0	3
Cr. balansae	38.04	26.1167	3	6	29.92	15.07	11.03	51.16	4A	0	0	0	2	0	0	1	0	0	new
	s.d.	s.d.	3	6	26.74	6.84	4.04	20.80	4A	0	0	0	2	0	0	1	0	0	4
Cr. candidus	s.d.	s.d	3	6	29.12	9.64	6.29	22.97	4A	0	0	0	2	0	0.33	0.33	0.33	0	4
Cr. flavus* subsp. *flavus	40.0184	21.5146	4	8	29.83	18.92	32.02	42.04	3A	1	0	2	0	0.11	0	0.22	0.67	0	new
	40.0184	21.5146	4	8	34.1	38.18	41.12	45.54	3A	2	0	2	0	0	0.2	0.4	0.4	0	new
	40.0184	21.5146	4	8	29.48	26.74	24.6	40.54	3A	0	0	2	0	0	0	1	0	0	new
	38.1977	23.7846	4	8	34.93	27.68	19.72	42.62	3A	1	0	2	0	0.11	0	0.67	0.22	0	new
	s.d.	s.d	4	8	32.49	6.38	19.53	25.53	3A	0	0	2	0	0	0	1	0	0	4
	s.d.	s.d	4	8	25.13	8.41	23.40	25.95	3A	11	0	2	0	0.58	0	0.42	0	0	4
Cr. flavus* subsp. *dissectus	s.d.	s.d	4	8	28.18	7.00	16.64	29.10	3A	0	0	2	0	0	0	1	0	0	4
Cr. graveolens	s.d.	s.d	3	6	28.09	19.06	24.58	34.76	2A	0	0	0	2	0	0	1	0	0	4
Cr. hyemalis	s.d.	s.d	3	6	33.02	19.45	17.94	28.80	3B	2	0	2	0	0.4	0	0.2	0.2	0.2	4
Cr. istanbulensis	s.d	s.d	3	6	24.68	55.07	7.55	53.76	3A	0	0	0	2	0	0	1	0	0	5
Cr. olivieri	38.4019	26.0200	3	6	24.02	7.66	8.36	57.48	4A	1	0	0	2	0	0	1	0	0	new
	41.2214	23.7703	3	6	27.47	26.67	8.739	59.4	4A	0, 2	0	0	2	0	0	1	0	0	new
	38.5664	26.011	3	6	24.86	12.73	6.86	53.05	4A	1	0	0	2	0	0	1	0	0	new
	37.7727	22.5222	3	6	25.48	10.12	13.27	22.52	4A	0-3	0	0	2	0	0	1	0	0	new
	s.d.	s.d	3	6	22.27	3.49	6.90	21.81	3A	0	0	0	2	0	0	1	0	0	4
	s.d.	s.d	3	6	23.74	8.40	5.93	19.42	3A	2	0	0	2	0.25	0	0.5	0.25	0	4
Cr. vitellinus	s.d.	s.d	4	8	25.30	8.81	23.95	20.14	A	0	0	1	0	0	0	1	0	0	4
	s.d.	s.d	4	8	18.51	8.43	20.37	22.87	A	0	0	0	0	0	0	1	0	0	4

s.d.: without geodata

Table 2. Most informative morphological characters (both qualitative and quantitative), with their loadings as implemented by the Principal Coordinate Analysis.

	Character code	Character abbreviation	Character description	Axis 1	Axis 2	Axis 3	Axis 4
1	**51**	**leaf_l**	Leaf length	1.7725	-28.082	12.966	-21.615
2	**54**	**tub_l**	Perigone tube length	1.1155	-9.0739	18.603	-24.091
3	**49**	**cor_n**	Length of corm cup	1.0871	-0.37562	-1.2609	-13.48
4	**58**	**L_in**	Length of inner perigone segments	0.17725	-2.1809	3.4142	-1.298
5	**55**	**L_out**	Length of outer perigone segments	0.17598	-3.0064	3.665	-0.65309
6	**70**	**Anth_l**	Anthers length	0.16	-0.49034	2.2991	0.58583
7	**1**	**cor_shp**	Corm shape	0.087736	0.004749	-0.003291	0.005106
8	**2**	**cor_tun**	Corm tunic type	1.29E-29	8.50E-30	4.06E-30	-5.09E-30
9	**15**	**bract_col**	Bract coloration	7.73E-30	2.90E-30	1.63E-30	-2.23E-30
10	**28**	**Phar_pub**	Pharynx pubescence	5.04E-30	2.11E-30	1.09E-30	-1.63E-30
11	**22**	**Out_sgm_homo**	Outer perigone segments homogeneity	1.66E-30	7.55E-31	4.75E-31	-5.08E-31
12	**20**	**In_sgm_homo**	Inner perigone segments homogeneity	1.64E-30	3.53E-31	2.07E-31	-3.85E-31
13	**26**	**tub_mot**	Perigone tube motif	3.65E-31	1.27E-31	8.55E-32	-9.30E-32
14	**24**	**sgm_mot**	Perigone segments motif	1.82E-31	6.34E-32	4.28E-32	-4.65E-32
15	**27**	**Phar_col**	Pharynx coloration	-4.39E-31	-2.12E-31	-1.11E-31	1.02E-31
16	**13**	**Cataph_col**	Cataphylls coloration	-1.16E-30	-3.51E-31	-1.80E-31	3.32E-31
17	**43**	**Elaiosome**	Elaiosome	-5.10E-30	-3.17E-30	-1.62E-30	1.99E-30
18	**44**	**Raphe_typ**	Raphe type	-5.10E-30	-3.17E-30	-1.62E-30	1.99E-30
19	**9**	**Leaf_ind**	Leaf indumentum	-7.73E-30	-2.90E-30	-1.63E-30	2.23E-30

Table 3. Dataset of the most informative qualitative morphological characters of *Crocus* ser. *Flavi* (abbreviations as indicated below).

Taxa	**Accession numbers**	**Characters**										
		0	**0**	**0**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**
		1	**2**	**9**	**3**	**5**	**0**	**2**	**4**	**6**	**7**	**8**
***Crocus olivieri* J. Gay**	1286_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1286_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1286_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1286_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1760_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1761_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1761_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	1761_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2081_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_10	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_11	1	0	4	3	0	0	1	0	0	1	2

***Crocus olivieri* J. Gay**	2097_12	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_13	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_14	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_15	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_9	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2102_b	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_10	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_11	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_12	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_13	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_9	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	72_16	1	0	4	3	0	0	1	0	0	1	2
Crocus flavus* Weston subsp. *flavus	2113_1	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp.** ***flavus***	2113_10	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_16	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_17	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_18	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_19	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_20	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_21	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_22	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_23	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_24	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_9	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_10	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_16	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_17	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus* Weston subsp. *flavus	2113_cult_18	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_19	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_20	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_21	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_22	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_23	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_24	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_25	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_26	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_27	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_28	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_29	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_30	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_31	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_32	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_33	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_34	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_35	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_36	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_37	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_38	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_39	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_40	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_10	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus* Weston subsp. *flavus	72_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_9	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_9	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	2124_1	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	2124_2	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	2124_3	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	2124_4	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_1	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_2	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_3	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_4	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_5	3	4	1	2	3	1	0	0	0	1	0

***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	23990_6	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	23990_7	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	23990_8	3	4	1	2	3	1	0	0	0	1	0
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_1	1	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_2	1	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_3	1	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_4	1	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_5	1	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_6	2	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_7	2	6	4	3	0	0	1	0	0	1	2
***Crocus istabulensis* (B.Mathew) Ruksans**	46030_8	1	6	4	3	0	0	1	0	0	1	2
***Crocus balansae* J. Gay**	B34_1	1	0	4	3	0	0	0	1	2	2	3
***Crocus balansae* J. Gay**	B34_2	1	0	4	3	0	0	0	1	2	2	3
***Crocus balansae* J. Gay**	B34_3	1	0	4	3	0	0	0	1	2	2	3
***Crocus balansae* J. Gay**	B34_4	1	0	4	3	0	0	0	1	2	2	3
***Crocus balansae* J. Gay**	B34_5	1	0	4	3	0	0	0	1	2	2	3
Crocus flavus* Weston subsp. *flavus	P2107918_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	P2107918_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	P2107918_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	P2107918_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	P2107918_5	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp.** ***flavus***	P2107918_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107918_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107918_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107924_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_3	3	4	1	2	3	1	0	0	0	1	0
Crocus balansae **J. Gay**	s.n._1	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	s.n._2	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	s.n._3	1	0	4	3	0	0	0	1	2	2	3
Crocus flavus **Weston subsp.** ***dissectus*** **Baytop & Mathew**	ZE544	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp.** ***dissectus*** **Baytop & Mathew**	ZE544_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***dissectus*** **Baytop & Mathew**	ZE544_2	3	4	1	2	3	1	0	0	0	1	0

Trait	**State**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**corm shape**	flattened-globose	subglobose	ovate	globose	subovate						
2	**corm tunic**	smooth with acute dentate basal appendages	membranous or papery	papery or stiff with basal rings	coarsely – parallel fibrous reaching the cap	membranous with parallel fibres	fine reticulate fibrous	coarsely reticulate fibrous	hard reticulate fibrous	parrallel fibrous lower, reticulate upper	papery with vertical bands	interwoven
9	**leaf indumentum**	glabrous	pubescent at margins	pubescent at upper surface	pubescent underneath	pubescent	other					
13	**cataphyl colour**	white	greenish	brownish	maculate							
15	**bract colour**	white	greenish	brownish	maculate							
20	**inner segments colour homogenity**	no	yes									
22	**outer segments colour homogenity**	no	yes									
24	**segments motif**	lacking	vertical striped	feathered	tinged basally	tinged apically	uncolored margins					
26	**tube motif**	lacking	vertical striped	tinged								
27	**pharynx colour**	white	yellow	violet	deep violet							
28	**pharynx pubescence**	glabrous	pubescent	ciliate	papillose							

order to estimate which characters are most informative for taxon discrimination, especially for closely related taxa. A dissimilarity matrix was calculated using the Gower distance measure in three clustering modes (Single Linkage, Complete Linkage and Unweighted Pair-Group Method with Arithmetic Mean, UPGMA) to render phenograms. We selected, as best suited, those phenograms with Cophenetic Correlation values above 75%, an indication that these phenograms exhibit the most reliable classification (Sokal 1986). The analysis was carried out via Past version 3.15 (Hammer & al. 2001) and the derived morphological data matrix was compared with the karyomorphology correlations within the *Crocus flavus* group.

Karyology and karyomorphometric analysis. −Root tips (see Table 1) were pretreated in 1 : 1 solution of cycloheximide (0.006 % w/v) and 8-hydroxyquinoline (0.006 % w/v) for 2–3 h. They were fixed in Carnoy solution (3 : 1 v/v absolute ethanol : glacial acetic acid) for at least 24 h at 4°C and stored in a solution of 70 % ethanol at −20 °C until use. The tips were hydrolysed in 1n HCl for 11 min at 60°C, and stained with Feulgen's stain. After 3-4 h of staining the tips showed a uniform rose appearance, and their chopped tips were macerated in 45% (v/v) glacial acetic acid for the squash procedure. Metaphases were observed using a Zeiss Axio Imager A1 microscope and documented with an AxioCam MR3. Selected photographs (at least five mitotic plates per population) were measured using KaryoType version 2 (Altinordu & al. 2016) software. Chromosome karyotype formula follows the classification of Levan & al. (1964). The formation of the karyotype is crucial in taxonomic treatment either for species with different centromere positions or different relative size. The former case might reflects chromosome translocations whereas the latter segmental interchange. Furthermore, the known karyotype formula was compared with their New Relative Length (NRL; Plummer & al. 2003) in order to match the homologous chromosomes.

Karyograms were constructed by organizing the chromosomes into groups, ordering them according to their arm length ratio and overall length. The data are presented in relative lengths over physical ones to avoid metaphase biases. The Total Haploid Length (THL), in µm, reported in this study is an estimate of the length of the haploid chromosome complement. Ideograms are based on the mean centromeric index per population and arranged in decreasing size.

Karyotype asymmetry was estimated according to the asymmetry classification of Stebbins (1971) and analysed according to the most informative indices, as proposed by Peruzzi & Altinordu (2014) for *Crocus* ser. *Verni*. Chromosome Length of Chromosome Index (CV_{CI}; CV_{CL}; Paszko 2006), and the mean centromeric asymmetry (M_{CA}; Peruzzi & Altinordu 2014) were calculated.

Furthermore, a multivariate analysis was carried out using Past v. 3.15 (Hammer & al. 2001, Hammer 2013). A dataset of 20 OTU's (operational taxonomic units) × 16 variables (8 quantitative and 8 qualitative) was used in order to calculate the average taxonomic distance and construct a dendrogram. The clustering analysis was performed using UPGMA and Gower general Dissimilarity Coefficient, as described by Gower (1971). The proposed method follows Podani & Schmera (2006). Gower's algorithm is best suited for a mixture of scale types, and is tolerant of missing values.

The results of the karyomorphometric study were presented both in Discriminant Analysis plots and phenograms, after Cluster Analysis.

Results and Discussion

Taxonomic treatment

The results of our study indicate that the members of *Crocus olivieri* aggregate sensu Mathew (1982) are best treated as distinct species. Hence, we accept specific status for *C. olivieri*, *C. balansae* and the Turkish endemic *C. istanbulensis* (B.

Mathew) Rukšāns (included here so as to consider the aggregate as a whole), because they differ in morphology and show minor karyological differences as well. Conversely, the members of the *C. flavus* group are morphologically variable. The characters used to circumscribe its members are the number of stigmatic branches, the width and length of the leaves, and the shape and cup length of the corm. An evaluation of these and other characters shows that the corm tunics are important for taxonomic delimitation in the group, but leaf dimensions and stigma dissection are of lesser value. This is consistent with the idea of a but recent divergence of taxa in this group (HARPKE & al. 2013).

Crocus flavus subsp. *atticus* Kit Tan & al. was claimed to differ from *C. flavus* subsp. *flavus* in style dissection (TAN & al. 2014: 123): in the former, the style would be divided into 6 distinct branches, whereas in the latter the style is shortly trifid. On a visit to the type locality of *C. flavus* subsp. *atticus,* in mid-February 2016, we found that style division is variable: Almost half of the studied flowers had trifid styles. Also, multifid styles were but apically divided (for up to 2 mm) and deeply frilled. Style division number, in half of the individuals, ranged from 4 to 7, and was not correlated with any other morphological feature. Stigma maturity in the material collected in the Tatoi district was evaluated using their hydrogen peroxide response (MCINNIS & al. 2006). We found that several stigmata were unreceptive and therefore immature. The degree of style dissection in the studied population may indeed be correlated with the maturity stage of the stigmas.

Several *Crocus flavus* plants from Mt. Parnitha near Athens, which is the locus classicus of *C. flavus* subsp. *atticus*, show non-bracteolate flowers, described as characteristic for the latter (TAN & al. 2014). This morphological character was thoroughly studied by CHOOB (2000) for *Crocus* as a whole. It was found that, anatomically, the presence or absence of a bracteole is correlated with the developmental stage of the inflorescence. CHOOB (2000) linked the "anomaly" of lacking bracteoles with the rank of inflorescence axis. He writes (CHOOB 2000:103): "all Crocus species have prophylls at the bases of paracladia"; and "the bract and bracteole in *C. flavus* are prophylls. The flower is on the 3rd (4th) axis". Therefore, the peculiarity of *C. flavus* subsp. *atticus* (and *C. flavus* in general) is due to organ reduction (CHOOB 2000:104): "organ reduction occurs by three processes (a) abortion, (b) ablasty and (c) fusion", and is an ontogenetic developmental process. This may explain why the ratio of bracteolate against non-bracteolate flowers depends on inflorescence development and, hence, collecting date. The type material of *C. flavus* subsp. *atticus* was collected early in its flowering period (8th of February), whereas the material studied by TAN & al. (2014) was gathered later (2nd of March), is in a more advanced developmental stage and, therefore, its secondary axial inflorescence lacks bracteoles.

Crocus flavus Weston, Bot. Univ. 2: 237. 1771, **subsp. *flavus***

= *C. flavus* subsp. *atticus* Kit Tan & al. in Phytol. Balcan. 20: 123. 2014.

Morphometric study. – The UPGMA phenogram constructed on the basis of morphological data shows four major branches (Fig. 1). The first, uppermost branch consists of *Crocus balansae* individuals only; the next branches are more complex, with

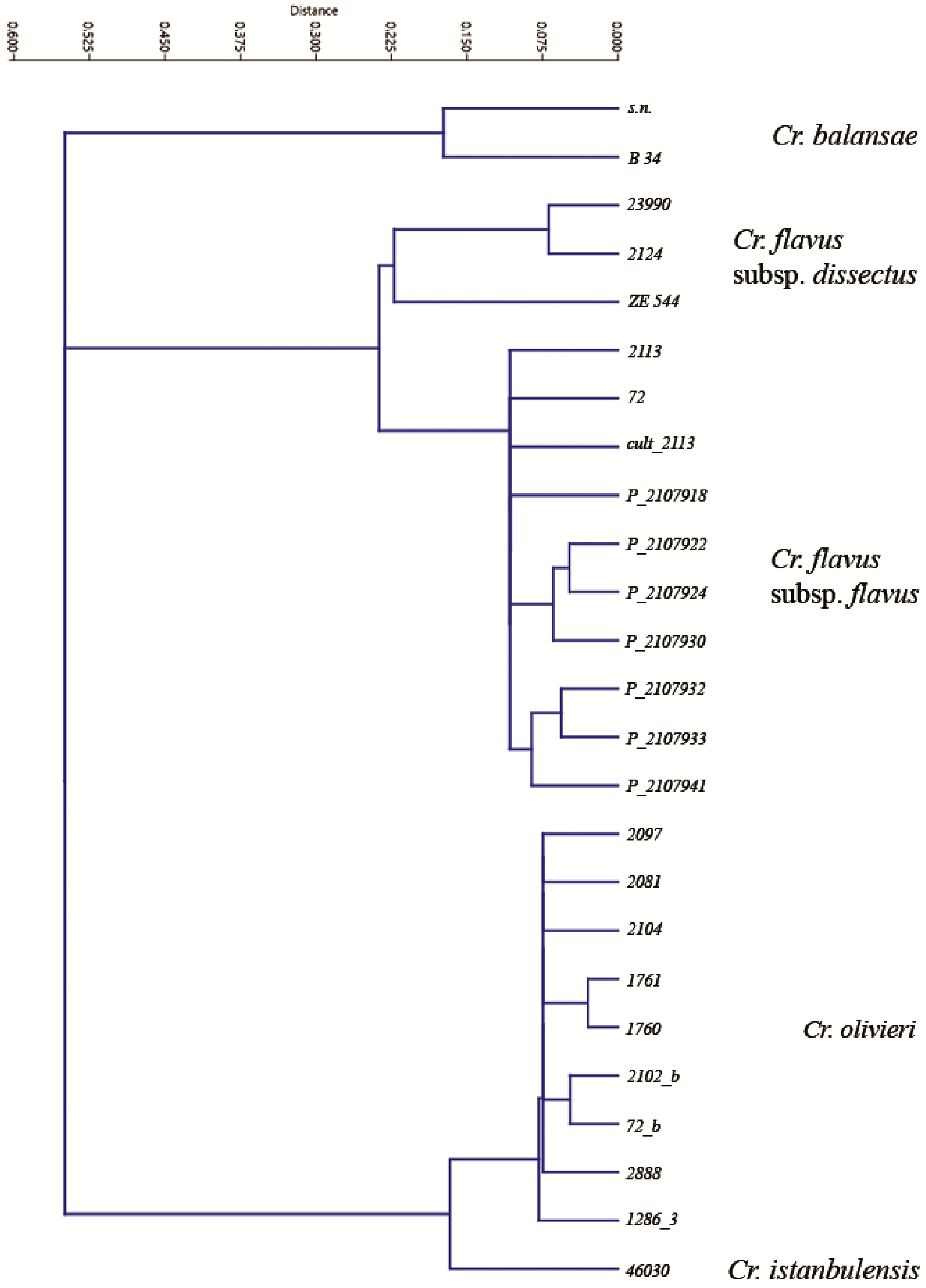


Fig. 1. Dendrogram of 25 *Crocus* ser. *Flavi* populations from Greece and adjacent areas (Balkans and Turkey) using Gower's distance clustering analysis. Cophenetic correlation *r* = 0.9284.

C. istanbulensis and *C. olivieri* at the bottom and *C. flavus* subsp. *flavus* (incl. subsp. *atticus*) and *C. flavus* subsp. *dissectus* forming another branch at the middle. This phenogram sorts out characters that are major factors in segregating taxa within *C.* ser. *Flavi,* and supports our taxonomic approach. The first two characters, namely, leaf length (measured at flowering time and discriminating between synanthous and hysteranthous species) and perigon tube length account for 79.2 % of variation in the group, followed by leaf width, perigon segment length, corm cup length, anther length, corm shape, and pistil per stamen ratio.

The Principal Coordinate Analysis (PCoA) and Discriminant Analysis (DA) of the morphometric data show that the *Crocus flavus* cluster overlap (Fig. 2a). On the other hand, PCoA and DA of the whole taxonomic group indicate the three taxa of the *C. olivieri* aggregate as distinct (Fig. 2b). As a consequence of the morphometric study, the two members of the *C. flavus* aggregate remain at subspecific rank, whereas members of the *C. olivieri* group are better be treated as distinct species.

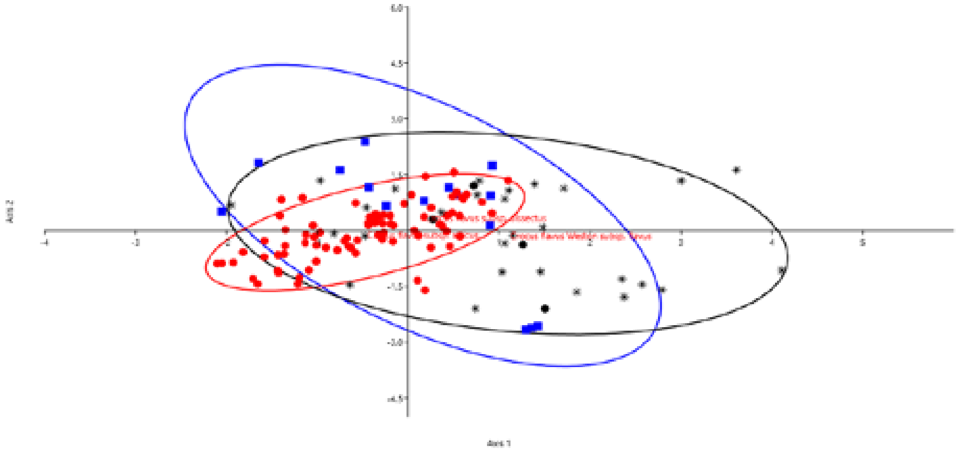


Fig. 2a. Discriminant Analysis of the Greek members of *Crocus flavus* aggregate.

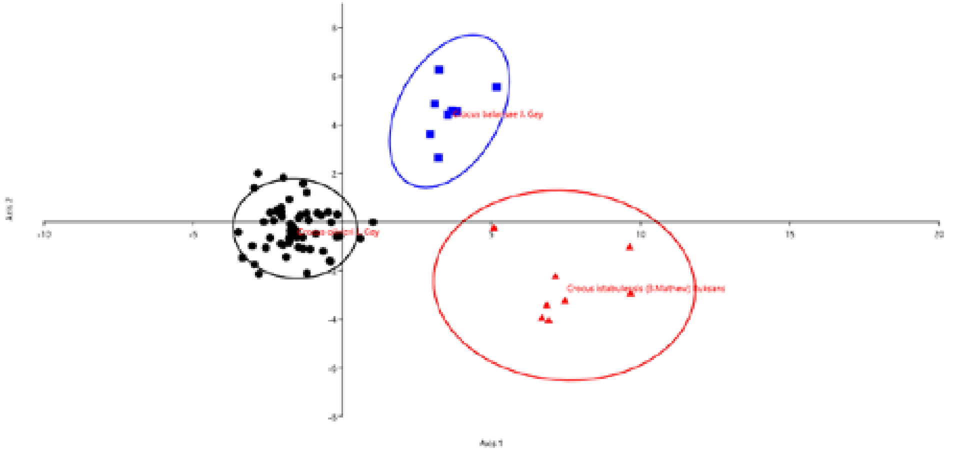


Fig. 2b. Discriminant Analysis of the Greek members of *Crocus olivieri* aggregate.

Karyology, karyomorphometry and chromosome evolution. – The karyology and karyomorphology of the *Crocus flavus* subsp. *flavus* population from Attiki ("subsp. *atticus*") are first reported in this study (Fig. 3g-h). The karyotype consists of eight chromosomes coupled with a B-chromosome ($2n = 8 = 2m + 2sm + 2sm\text{-}SAT + 2st + 0\text{-}1B$) and is compared with *C. flavus* subsp. *flavus* from a Macedonian population (Grevena, Figs 3e-f). The two populations present a similar, if not identical, karyotype with respect to chromosome number and asymmetry, which does not deviate significantly from the general, stable karyotypic pattern of *C. flavus* subsp. *flavus*.

Crocus flavus subsp. *flavus* shows remarkable morphological variation, hence its large synonymy. Yet, its karyological variability turns out to be the lowest of any *Crocus* taxon. The karyotype, in *C. flavus* subsp. *flavus,* usually varies in the presence and number of supernumerary B-chromosomes. Up to 11 B-chromosomes have been observed in *C. flavus* subsp. *flavus* populations from Turkey (BRIGHTON 1976). The presence of supernumerary B-chromosomes does not seem to correlate with major phylogenetic discontinuities, as HARPKE & al. (2013: 626) have shown in their phylogenetic analysis of the genus. B-chromosomes are present in many other *Crocus* taxa, not included in this study. We may conclude that, in the case of *C. flavus,* morphological variation is not reflected in karyotype differentiation. In terms of cytogeography, plants from the Pindos range as well as those from Mt. Vermion in Macedonia (BRIGHTON 1976) and those from Mt. Parnitha have a similar karyotype and karyotype asymmetry (Table 1). Their karyomorphometric indices are also very similar, if not identical, and form the closest tree branches in the clustering analysis to follow.

We take it that the basic chromosome number in *Crocus* ser. *Flavi* is $x = 4$, one of the most widespread basic numbers in the whole genus (see FEINBRUN 1958:186), being present in all generic subdivisions; the vast majority of taxa with a different chromosome numbers have derived through polyploidy and dysploidy. HARPKE & al. (2013: 625) postulate that the haploid series in *Crocus* has $n = 4/5$, 8/10 and 16 chromosomes. Their conclusion, that "reconstruction assumes fewer polyploidization events but at deeper nodes of the tree, and a high frequency of chromosome losses towards the tips", was corroborated by means of the CHROMEVOL chromosome evolution software (GLICK & MAYROSE, 2014).

The somatic chromosome number in Greek *Crocus* ser. *Flavi* is either 6 or 8. The clearest difference amongst the taxa is intra-chromosomal asymmetry. The derived species of this series (*Cr. olivieri* aggregate; HARPKE & al. 2013) reported with karyotype dominated by acrocentric (ST, T) chromosomes and it seems more asymmetric than its most primitive allies consisted of M and SM chromosomes (*Cr. flavus* aggregate). Secondly, the inter-chromosomal asymmetry discriminates the taxa taxonomically as L/S index ranges between 1.28 - 1.49 and 1.5 - 1.79 for *Cr. olivieri* and *Cr. flavus* groups, respectively. Additionally, the presence of B-chromosomes in some populations as well as satellites or secondary constricted areas, either intercalary as in *C. olivieri* or on the short arm as in *C. flavus*.

Chromosome number seems to have evolved by descending dysploidy, as the most basal species of the series, *C. paschei* Kernd. has $2n = 14$ chromosomes (HARPKE & al. 2013), whereas our species, which are most derived, have $2n = 6$, 8 chromosomes. This indicates that chromosome rearrangements took place, involving possible changes of centromeric position and a satellited chromosome pair with a secondary constriction, and a loss of a chromosome pair as it is depicted in Fig. 4.

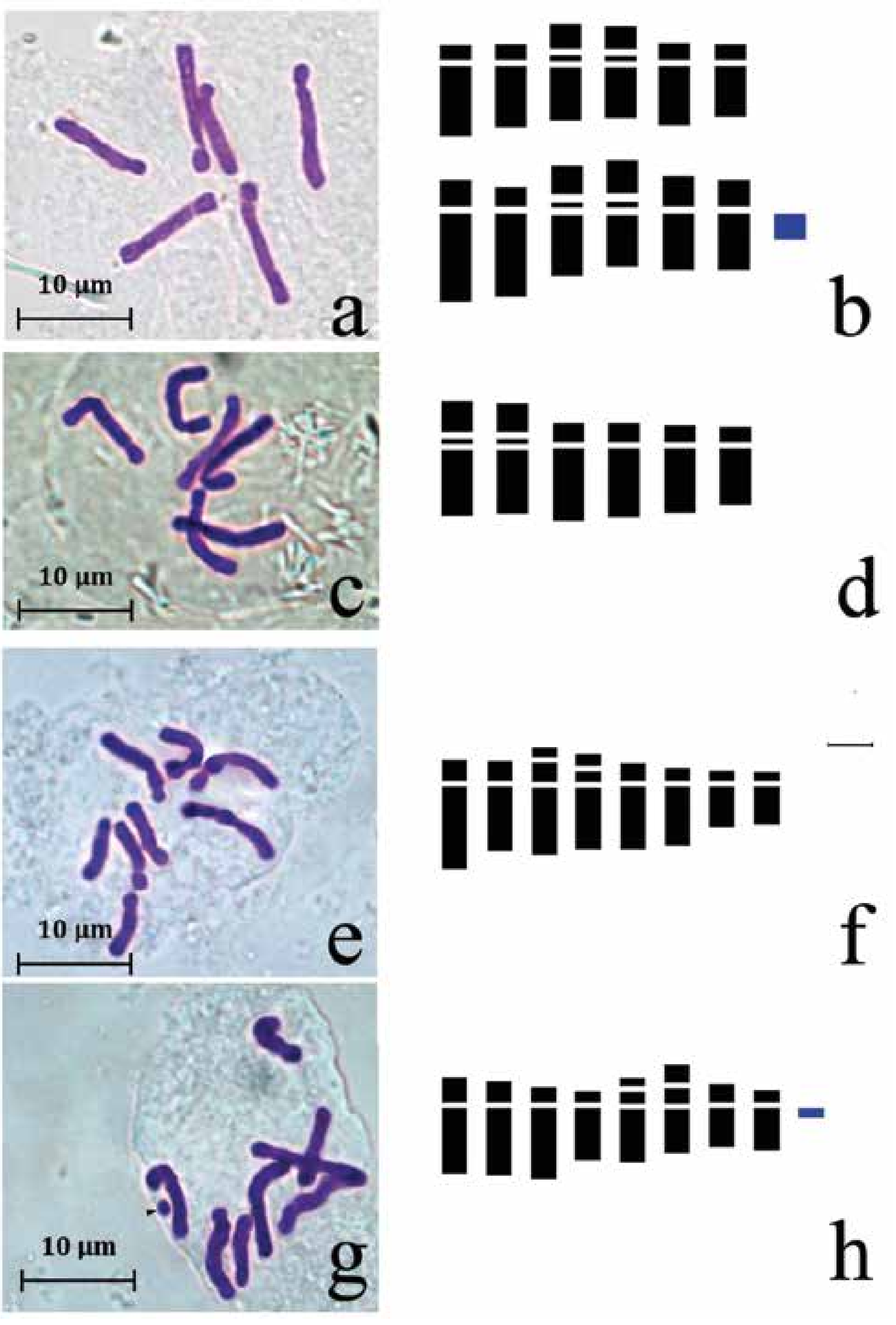


Fig. 3. Karyotypes and their corresponding idiograms of Greek *Crocus* ser. *Flavi:* a, b, *Cr. olivieri*; c, d, *Cr. balansae* and e-h, *Cr. flavus* subsp. *flavus*.

FIG. 4. The average idiograms of *Crocus olivieri* (2*n* = 6; pink idiogram) and *Cr. flavus* (2*n* = 8; black idiogram) superimposed for comparison in the evolutionary scenario of descended dysploidy – Scale bar = 10 µm.

Our findings indicate that THL differs significantly between the *Crocus flavus* and *C. olivieri* aggregates, with 34.969 ± 3.94 µm against 24.24 ± 0.39 µm, respectively. It is significant that the THL decreases from the more ancestral *C. flavus* to the most derived *C. olivieri*, lending support to our evolutionary scenario of descending dysploidy.

In the PCoA analysis of the karyomorphometrical data (Fig. 5), the first two coordinates account for 83.26 % of the total variation. The first axis of the plot discriminates the taxa in accordance with Stebbins class and L/S ratio, followed by the chromosome number, THL, MCA and CVCI. It is clear that chromosome types according to STEBBINS's (1971) classification have major impact, as they describe karyotype asymmetry. Also, the ratio between the longest and the shortest chromosomes of a karyotype (L/S), which is partially an index of inter-chromosomal asymmetry (also included in the Stebbins classification), correlates well with the taxon similarities depicted in a phenogram based on the most informative karyomorphological data (Fig. 6), where *C. olivieri* samples both from the Greek mainland and islands cluster in the same branch. This indicates that the species is distinct, lacking infraspecific differentiation due to topographic isolation barriers. *Crocus flavus* subsp. *flavus* from Attiki and Macedonia have a similar karyotype and belong to the same cluster, which supports their conspecificity.

The CV_{CL} karyotype asymmetry index is the most variable parameter, especially when B-chromosomes are present. As JONES & HOUBEN (2003) propose, the B-chromosomes seem to be "escapees" from the A chromosomes (see also MARTIS & al. 2012), being found in both *Crocus flavus* and *C. olivieri*. Chromosome length measurements suggests that the B-chromosomes are derived from the third longest chromosome pair in the case of *C. olivieri* and from the second longest in the case of *C. flavus*.

The M_{CA} asymmetry index discriminates well between the *Crocus flavus* and *C. olivieri* group of taxa. The index is well correlated with the presumed phylogeny of the

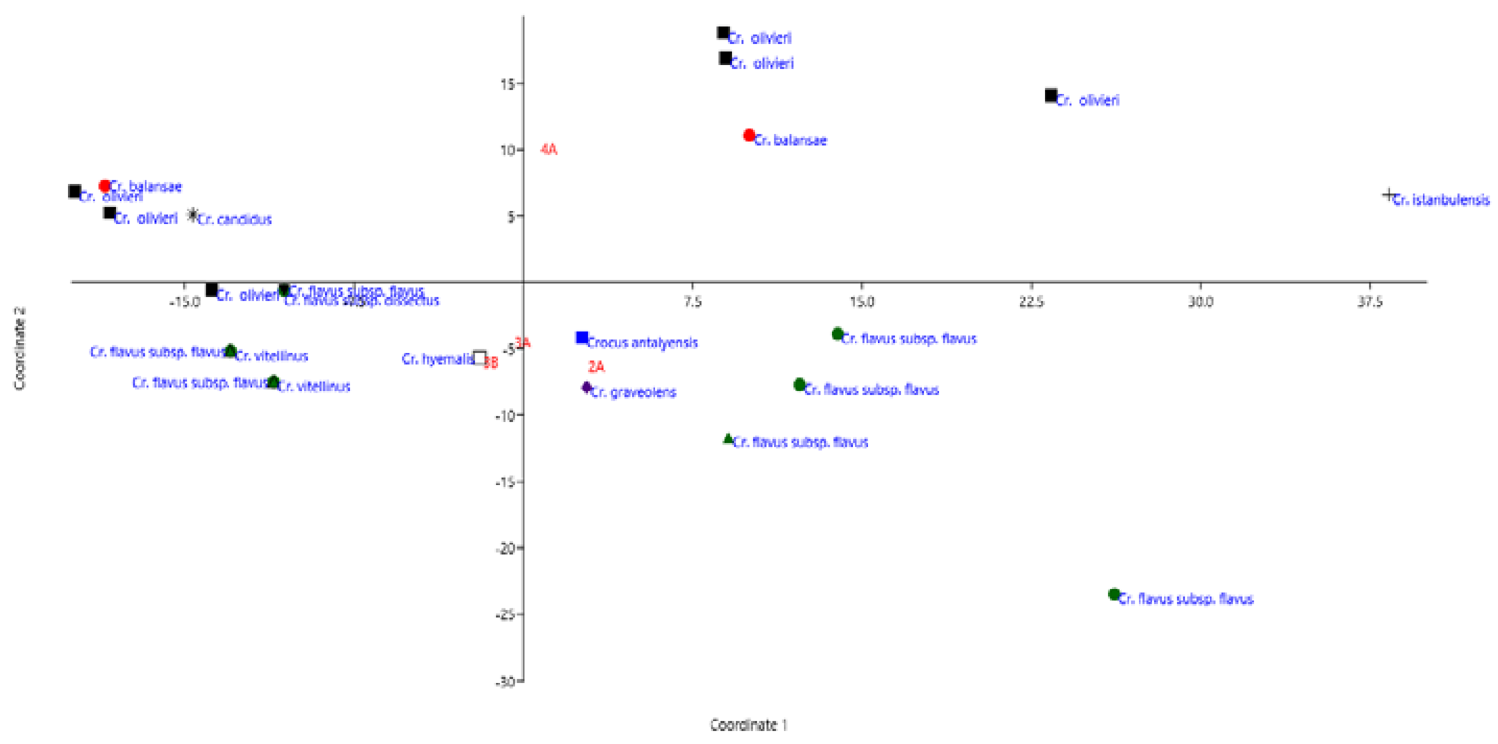


Fig. 5. Principal Coordinate Analysis (PCoA) of the most informative karyomorphometric indices within *Crocus* ser. *Flavi*. Variation correlates well with the Stebbins classification. The *Crocus flavus* subsp. *flavus* population from Attiki is indicated by a green triangle.

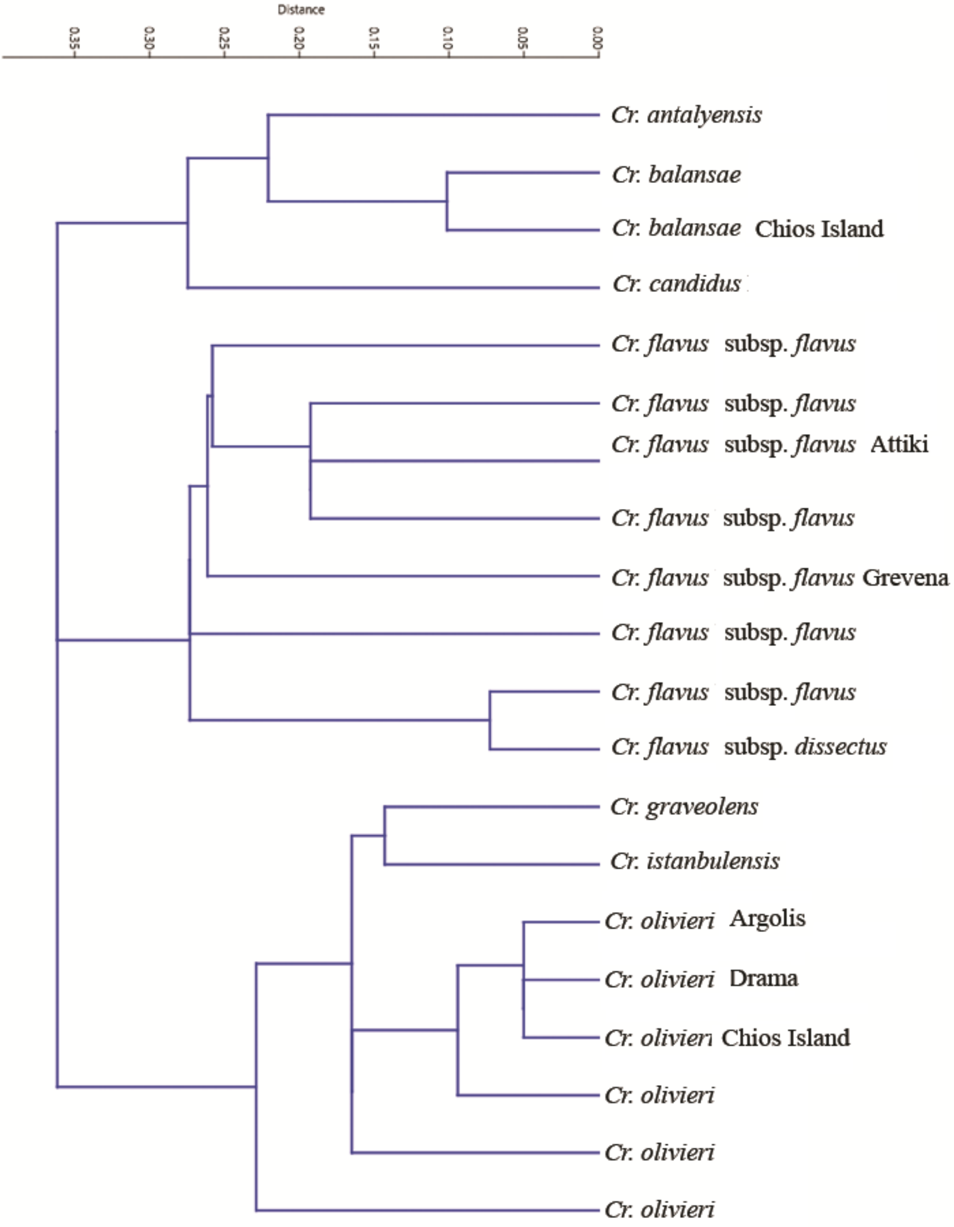


FIG. 6. Dendrogram of the Gower Distance of the karyomorphometric data of the *Crocus* ser. *Flavi*. Cophenetic correlation $r = 0.789$.

group, as its values are higher in the most derived species. M_{CA} also supports a descending dysploidy, from $n = 8$ to $n = 6$, within this group. This hypothesis is also consistent with a scenario of Robertsonian translocations, especially considering that the L/S index decreases from a range of 1.79-1.76 in the more ancestral *C. flavus* to a range of 1.57-1.28 in the derived *C. olivieri*.

Acknowledgements

This paper is dedicated to Dimitrios Phitos, Professor Emeritus of the Biology Department, University of Patras, in appreciation of his many achievements. The authors sincerely thank Assoc. Prof. E. Kapsanaki, Ass. Prof. Z. Gonou, Dr I. Pyrri and Dr A. Sergentani (Department of Ecology & Systematics, Faculty of Biology, National & Kapodistrian University of Athens) for their help and support, in particular for granting access to the Zeiss Axio Imager A1.

References

ALTINORDU, F., PERUZZI, L., YU, Y. & HE, X. 2016: A tool for the analysis of chromosomes: KaryoType. – Taxon 65: 586-592.

BAKER, J. G. 1873: A review of the known species of *Crocus* VI. – Gard. Chron. 1873: 609-609.

BOISSIER, E. 1882: Flora Orientalis, sive Enumeratio Plantarum in Oriente a Graecia et Aegypto ad Indie Fines Hucusque Observatatum, 5,1. – Lyon, Genève & Basel.

BOWLES, E. A. 1924: A handbook of *Crocus* and *Colchicum*. – London.

BRIGHTON, C. A. 1976: Cytology of *Crocus olivieri* and its allies. – Kew Bull. 31: 209-217.

—, MATHEW, B. & MARCHANT, C. J. 1973: Chromosome counts in the Genus *Crocus* (Iridaceae). – Kew Bull. 28: 451-464.

CHOOB, V. V. 2000: Patterns of flower and inflorescence architecture in *Crocus* L. (Iridaceae). – Ann. Bot. (Roma), ser. 2, 58: 91-104.

DIMOPOULOS, P., RAUS, T., BERGMEIER, E., CONSTANTINIDIS, T., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. 2013: Vascular plants of Greece: an annotated checklist. – Englera 31: 1-372.

FEINBRUN, N. 1958: Chromosome numbers in *Crocus*. – Genetica 29: 172-192.

GAY, J. 1831: Nouvelles espèces de *Crocus*. – Bull. Sci. Nat. Géol. 25: 319-321.

GLICK, L. & MAYROSE, I. 2014: ChromEvol: Assessing the pattern of chromosome number evolution and the inference of polyploidy along a phylogeny. – Molec. Biol. Evol. 31: 1941-1922.

GOULIMIS, C. N. 1956: New additions to the Greek flora. – Athens.

GOWER, J. C. 1971: A general coefficient of similarity and some of its properties. – Biometrics 27: 857-874.

HALÁCSY, E. V. 1896: Beitrag zur Flora von Griechenland. – Oesterr. Bot. Z. 46: 11-19.

— 1904: Conspectus Florae Graecae, 3. – Leipzig.

HAMMER, Ø. 2013: PAST v. 3.15. http://folk.uio.no/ohammer/past [accessed May 2017].

—, HARPER, D. A. T. & RYAN, P. D. 2001: PAST: paleontological statistics software package for education and data analysis. – Palaeontol. Electronica 4(1): 1-9.

HARPKE, D., MENG, S., RUTTEN, T., KERNDORFF, H. & BLATTNER, F. R. 2013: Phylogeny of *Crocus* (Iridaceae) based on one chloroplast and two nuclear loci: ancient hybridization and chromosome number evolution. – Molec. Phylogen. Evol. 66: 617-627.

—, CARTA, A., TOMOVIĆ, G., RANĐELOVIĆ, V., RANĐELOVIĆ, N., BLATTNER, F. & PERUZZI, L. 2015: Phylogeny, karyotype evolution and taxonomy of *Crocus* series *Verni* (Iridaceae). – Pl. Syst. Evol. 301: 309-325.

—, KERNDORFF, H., PASCHE, E. & PERUZZI, L. 2016: Neotypification of the name *Crocus biflorus* Mill. (Iridaceae) and its consequences in the taxonomy of the genus. – Phytotaxa 260: 131-143.

HERBERT, W. 1847: A history of the species of *Crocus*. – London.

HOOKER, J. D. 1873: Plate 6031. *Crocus olivieri*. – Curtis's Bot. Mag. 99: t. 6031.

JONES, N. & HOUBEN, A. 2003: B chromosomes in plants: escapees from the A chromosome genome? – Trends Pl. Sci. 8: 417-423.

LEVAN, A., FREDGA, K. & SANDBERG, A. A. 1964: Nomenclature for centromeric position on chromosomes. – Hereditas 52: 201-220.

McInnis, M. S., Emery, C. D., Porter, R., Deshikan, R., Hancock, T. J. & Hitchock, J. S. 2006: The role of stigma peroxidases in flowering plants: insights from further characterization of a stigma-specific peroxidase (SSP) from *Senecio squalidus* (Asteraceae). – J. Exp. Bot. 57: 1835-1846.

Martis, M. M, Klemme, S., Banaei-Moghaddam, A. M., Blattner, F. R., Macas, J., Schmutzer, T., Scholz, U., Gundlach, H., Wicker, T. & Šimková, H. 2012: Selfish supernumerary chromosome reveals its origin as a mosaic of host genome and organellar sequences. – Proc. Natl. Acad. Sci. U.S.A. 109: 13343-13346.

Mathew, B. 1976: *Crocus olivieri* and its allies (Iridaceae). – Kew Bull. 31: 201-208.

— 1982: The *Crocus*. – London.

— 2000: Peloponnisos (Greece) - A *Crocus* paradise. – Bot. Chron. 13: 255-265.

— & Baytop, T. 1976: Some observations on Turkish *Crocus*. – Notes Roy. Bot. Gard. Edinburgh 35: 61-67.

— & Brighton, C. A. 1972: A new *Crocus* from Turkey. – Kew Bull. 27: 327-329.

Mazziari, D. 1834: Flora Corcirese. – Ionios Anthologia 2(1): 424-469.

Orphanides, T. 1869: Sur le profit que l'horticulture européenne peut retirer de la flore grecque. − Bull. Congrès Internatl. Bot. Hort. St.-Pétersbourg 1869: 112-129.

Papanicolaou, K. & Zacharof, E. 1980: *Crocus* in Greece: new taxa and chromosome numbers. – Bot. Not. 133: 155-163.

Paszko, B. 2006: A critical review and a new proposal of karyotype asymmetry indices. – Pl. Syst. Evol. 258: 39-48.

Peruzzi, L. & Altinordu, F. 2014: A proposal for a multivariate quantitative approach to infer karyological relationships among taxa. – Comp. Cytogenet. 8: 337-349.

Plummer, J. A., Shan, F., Galwey, N. & Yan, G. 2003: New methods for comparison of chromosomes within and between species. – Caryologia 56: 227-231.

Podani, J. & Schmera, D. 2006: On dendrogram-based measures of functional diversity. – Oikos 115: 179-185.

Rukšāns, J. 2010: Crocuses. A complete guide to the genus. – Portland & London.

— 2013: Seven new Crocuses from the Balkans and Turkey. – Alpine Gardener 81: 1-28; 188-193.

— 2014: Crocuses from "The Holy Mountain" of Athos, Greece. – Int. Rock Gard. 59: 4-16.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012: NIH Image to ImageJ: 25 years of image analysis. – Nat. Meth. 9: 671.

Snogerup, S., Snogerup, B., Phitos, D. & Kamari, G. 2001: The flora of Chios island (Greece). – Bot. Chron. 14: 5-199.

Sokal, R. R. 1986: Phenetic taxonomy: theory and methods. – Annual Rev. Ecol. Syst. 17: 423-442.

Stebbins, G. L. 1971: Evolution in higher plants. – London.

Tan, K., Zografidis, A. & Mermygkas, D. 2014: Report 131. In Vladimirov, V. & Tan, K. (eds), New floristic records in the Balkans: 24 – Phytol. Balcan. 20: 123-125.

Thiers, B. [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. – New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.

Uslu, E., Babaç, T. & Yilmaz, A. 2012: Karyological studies on some *Crocus* L. taxa from Turkey. – Caryologia 65(1): 7-10.

Voliotis, D. 1986: Verbreitung einiger seltenen und subendemischen Arten der griechischen Flora mit taxonomischen und chorologischen Betrachtungen. – Folia Geobot. Phytotax. 21: 91-104.

Yetişen, K., Şen, U., Yildirim, T. & Özdemir, C. 2013: Morphological and anatomical study on endemic *Crocus olivieri* Gay subsp. *istanbulensis* Mathew subspecies (Iridaceae). – Anadolu University Journal of Science and Technology - C 3(1): 31-37.

YÜZBAŞIOĞLU, S. DEMIRCI, S. & KAYA, E. 2013: Report 1809. In KAMARI, G., BLANCHÉ, C. & SILJAK-YAKOVLEV, S. (eds), Mediterranean chromosome number reports - 23. – Fl. Medit. 23: 255-291.

ZAGANIARIS, D. N. 1938: Herbarium macedonicum, primum mille. – Sci. Ann. Fac. Phys. Math. Aristot. Univ. Thessalonika 4: 97-131.

Monitoring six local endemic taxa of the Mt. Athos and assessment according to the IUCN Red List categories and criteria

Antony Karydas & Georgia Kamari

Abstract

Karydas, A. & Kamari, G. 2019: Monitoring six local endemic taxa of the Mt. Athos and assessment according to the IUCN Red List categories and criteria. – Bot. Chron. 22: 195-208.

Six local endemic taxa of the Athos peninsula (Northern Greece) were examined thoroughly, which are already included in The Red Data Book of Rare and Threatened Plants of Greece. More fieldwork for these taxa was undertaken in order to outline their habitats, evaluate their population size and the factors that affect their distribution. Maps with their occurrence in their habitats were created and their potential threats they face *in situ* were projected. In order to assess their conservation status, the IUCN Red List Categories were applied and accordingly, a threat category was granted for each taxon. Consequently, two species (*Helichrysum sibthorpii*, *Silene orphanidis*) are proposed as Endangered (EN), three (*Aethionema orbiculatum*, *Aubrieta erubescens*, *Crepis athoa*) as Vulnerable (VU) and one (*Viola athois*) as Near Threatened (NT). Additionally, potential threats that these taxa face are being presented and possible conservation measures are proposed.

A. Karydas & G. Kamari, Plant Biology Division, Department of Biology, University of Patras, 265 00 Patras, Greece. E-mails: euripidis_1999@hotmail.com; kamari@upatras.gr

Key words: Plant Conservation, Threats, Distribution, “The Holy Mountain”, Greece.

Introduction

The Athos peninsula of Chalkidikis or “The Holy Mountain”, is situated in Central Macedonia, Northern Greece. It covers an area of about 350 Km^2 and extents from north to south to approximately 50 km, concluding to mountain Athos with an altitude of 2033 m. The peninsula is more than 90% covered with forests, making it one of the most forested areas in Greece. Throughout the centuries a large number of monasteries have been established and the peninsula has been historically dedicated solely to the monk community. This particular historical background left the peninsula relatively intact from intensive urban and agricultural development. This element, coupled with various other factors such as the relative isolation of the peninsula, the geographical position, the altitudinal span, the geological, landscape and climatic diversity, resulted to a rich mosaic of vegetation (Dafis 1998). Hence, five vegetation zones can be encountered, namely the coastal, the evergreen broadleaved and Mediterranean pine forests, the mixed broadleaved deciduous forests, the Mediterranean montane coniferous forests, and finally the subalpine zone (Athanasiadis & al. 1998). The sequence of these zones is primarily governed by altitude and secondarily according to aspect, slope, and geological substrates. This is the background of an impressively rich flora (Ganiatsas

1963), with about 1453 taxa belonging to 539 genera and 109 families. Hence the peninsula also reveals a high degree of endemism, with 14 local, 41 Greek and 59 Balkan endemics (BABALONAS & al. 1998).

As far as the conservation status, only six taxa of the Athos peninsula were included in The Red Data Book of Rare and Threatened Plants of Greece in 1995. These were the following: *Anthemis cretica* L. subsp. *sibthorpii* (Griseb) Govaerts, *Aubrieta erubescens* Griseb., *Helichrysum sibthorpii* Rouy, *Silene orphanidis* Boiss., *Fritillaria euboeica* Rix and *Galanthus nivalis* L. (PHITOS & al. 1995). The first four taxa are local endemics, the fifth is a Greek endemic and the latter is a central European taxon, with the Athos peninsula being the southern most extreme point of distribution. It is noteworthy that *Fritillaria euboeica* has never been rediscovered on Athos peninsula, despite the goodwill of the first author.

In 2009, the new edition of The Red Data Book of Rare and Threatened Plants of Greece (in Greek) another 6 local endemic taxa were added to the taxa already included in 1995 (PHITOS & al. 1995 & 2009). These were *Aethionema orbiculatum* (Boiss.) Hayek, *Centaurea athoa* DC. subsp. a*thoa, Centaurea peucedanifolia* Bois. & Orph., *Crepis athoa* Boiss., *Isatis tinctoria* L. subsp. *athoa* (Boiss.) Papan. and *Viola athois* W. Becker.

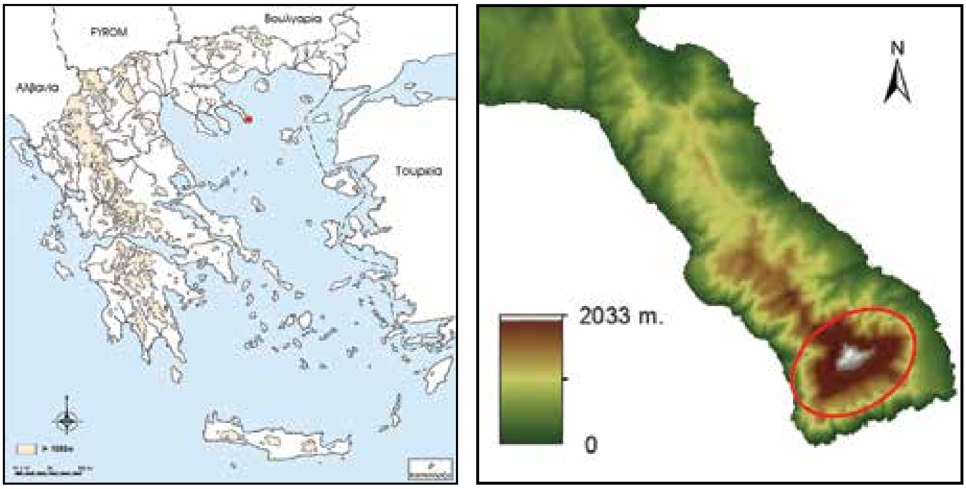
Moreover, *Helichrysum sibthorpii, Silene orphanidis and Viola athois* are included in the annexes II-V of the DIRECTIVE 92/43/EEC (DAFIS & al. 1997) and additionally *Aethionema orbiculatum*, *Aubrieta erubescens* and *Crepis athoa* in the PRESIDENTIAL DECREE 67/1981.

In this study, a comprehensive field assessment is made for six local endemic taxa of Athos peninsula: *Aethionema orbiculatum*, *Aubrieta erubescens*, *Crepis athoa*, *Helichrysum sibthorpii*, *Silene orphanidis* and *Viola athois*. The above six taxa are listed under an IUCN Red List category, based on field data. Additionally, potential threats that these taxa face are being presented and possible conservation measures are proposed.

Methods

Twelve site visits by the first author were conducted during the years 2006 and 2018 at Mt. Athos of Agion Oros peninsula (Fig. 1) in order to locate and study these six local endemic taxa. Information regarding flowering period and site location for these species was also based on literature sources indicated in Table 1.

The selection of the specific time of the visits was determined by the flowering season, a time when the taxa are more conspicuous phaenologically. During those visits, the habitats of these six taxa were examined in order to determine threats and/or conservation status and field data were collected regarding their biotic and abiotic environment. Such information included the number of individuals, number of populations and subpopulations, time of flowering, dominant and companion species, geological substrate, elevation, slope and aspect. The points where the species under study were encountered were recorded using a portable Global Positioning System (G.P.S.) apparatus. The coordinates of these points were incorporated in an EXCELL table, converted from WGS 84 to HGRS87 coordinate system (Hellenic Geodetic Reference System 1987), and incorporated to QGIS in order to construct distribution maps and calculate the extent of occurrence. Furthermore, the distribution maps and the population data allowed the application



of the IUCN (2018) criteria in order to classify each taxon into an IUCN Red List category.

Fig. 1. Location of the studied taxa on Mt. Athos, in the Athos peninsula or "The Holy Mountain", of the Northern Greece.

Table 1. Summary of the literature sources of the studied taxa.

Taxon	Literature source
Aethionema orbiculatum (Boiss.) Hayek	TAN & SUDA 2002; KARYDAS & KAMARI 2009a, as Vulnerable (VU)
Aubrieta erubescens Griseb.	PHITOS 1970; GUSTAVSSON 1986; PHITOS 1995, as Rare (R); KARYDAS & PHITOS 2009, as Vulnerable (VU)
Crepis athoa Boiss.	KAMARI 1991; KAMARI & KARYDAS 2009, as Vulnerable (VU)
Helichrysum sibthorpii Rouy	STRID & PAPANICOLAOU 1981; Strid 1995, as Vulnerable VU); KARYDAS 2009, as Endangered (EN)
Silene orphanidis Boiss.	STRID & PAPANICOLAOU 1985; CONSTANTINIDIS 1995, as Vulnerable (VU); KARYDAS & KAMARI 2009b, as Endangered (EN)
Viola athois W. Becker	RAUS 1986; KARYDAS & TINIAKOU 2009, as Near Threatened (NT)

Results

The taxa of this study are local endemics of mountain Athos. *Helichrysum sibthorpii, Silene orphanidis* and *Aethionema orbiculatum* are found exclusively at the rocky summit. *Crepis athoa* and *Viola athois* are scattered through the subalpine zone, extending from the stony meadows near the treeline, to the rocky Athos peak. *Aubrieta erubescens* has a wider range of distribution, occurring in the subalpine zone as well as in two mid altitude sites (KARYDAS 2007).

Habitat description

Aubrieta erubescens (Fig. 2) is found on limestone substrates, growing on screes, stony or gravelly slopes and rock crevices in the subalpine zone of Mt. Athos (1580-

2020 m), together with *Alyssoides utriculata* (L.) Medicus, *Crepis athoa, Berberis cretica* L., *Daphne oleoides* Schreber, *Isatis tinctoria*. subsp. *athoa, Prunus prostrata* Labill., *Pterocephalus perennis* Coulter subsp. *perennis, Sedum ochroleucum* Chaix, *Sempervivum marmoreum* Griseb., *Thlaspi perfoliatum* L., etc. It is also found in mixed woodland and maquis openings at lower altitudes (800-1000 m), with common woody species such as *Fraxinus* sp., *Juniperus oxycedrus* L., *Quercus coccifera* L., *Q. ilex* L.etc.

It forms three subpopulations (Fig. 2), usually scattered in groups of 4-8. The largest sub population consists of about 145 individuals, spread in at least 9 different sites in mixed forest openings at an altitude of 800-990 m, in the east foothills of Mt. Athos, above the Monastery of Megistis Lavras. Another subpopulation, consisting of about 90 individuals occurs in the rocky alpine zone, scattered at 13 different sites. Lastly, a smaller subpopulation of about 25 individuals, is found in 2 distinct sites in the south facing foothills of Mt. Athos, above Kerasia, at an altitude of about 900-990 m, in rocky openings of maquis vegetation (KARYDAS & PHITOS 2009).

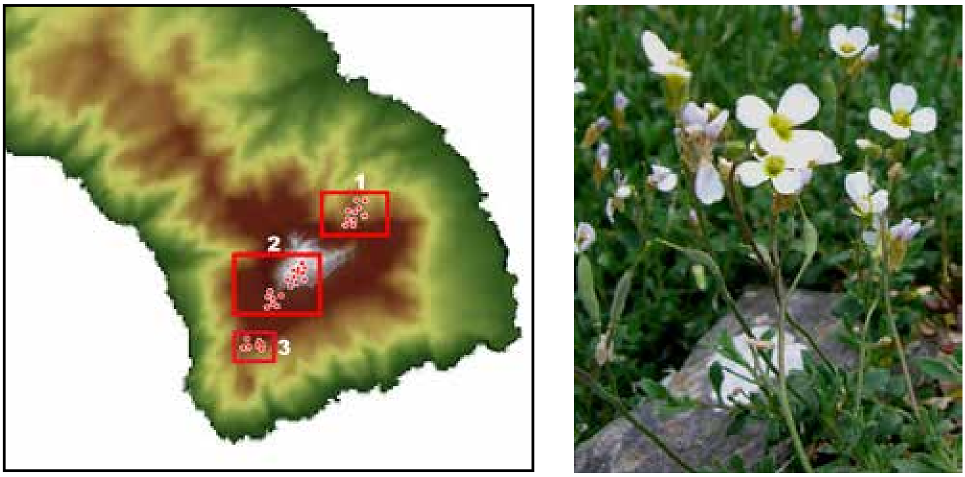


Fig. 2. Location of the *Aubrieta erubescens* subpopulations and an individual on Mt. Athos.

Aethionema orbiculatum (Fig. 3), ***Helichrysum sibthorpii*** (Fig. 4) and ***Silene orphanidis*** (Fig. 5) are found exclusively on the rocky summit of Mt. Athos (Fig. 1). These three chasmophytic taxa, are commonly encountered together with other rare and endemic chasmophytes, such as *Asperula lutea* Sibth. & Sm., *A. suberosa* Sibth. & Sm, *Arabis bryoides* Boiss., *Draba athoa* (Griseb.) Boiss. and *Potentilla speciosa* Willd. (CONSTANTINIDIS 1995).

Aethionema orbiculatum (Fig. 3) is found exclusively on cliffs and rock crevices, at an altitude of around 1800 to 2000 m, but with no preferences with respect to the aspect. It grows in small groups of 2-6 individuals or solitary.

Ae. orbiculatum is a relatively more abundant compared to the previous two taxa. Several hundred individuals have been observed, scattered at the upper part of the rocky summit, comprising one population (KARYDAS & KAMARI 2009). The estimated

Fig. 3. The area of occurrence and an individual of *Aethionema orbiculatum* on Mt. Athos.

Helichrysum sibthorpii is observed between 1838 and 2000 m altitude, in crevices of limestone rocks (Fig. 4). It forms a single population of around 102 individuals, scattered in at least 32 distinct locations (KARYDAS 2009). Usually *H. sibthorpii* is found solitary, or in small groups of 2 to 4 individuals, rarely in groups up to 12 individuals. It is met more frequently in sites with south-southwest and north-northwest aspects. Its estimated area of occurrence is about 0.121416 sq. km.

Silene orphanidis also grows on limestone crevices, from an altitude of 1800 to 1960 m (Fig. 5). It is encountered solitary or in small groups of 2 to 6 individuals. It prefers mainly south and southwest aspects, but occasionally plants can be found in all but the north and northeast facing slopes of the mountain summit. In total, less than 100 individuals were counted. These were scattered at least 19 different locations in the Mt. Athos summit and consist of a single population that is divided into two groups (KARYDAS & KAMARI 2009). The first includes individuals at the west and northwest aspect and the second mainly south and east. The area of occurrence is estimated to be near 0.136419 sq.km.

Crepis athoa occurs on stony meadows and rock crevices of limestone substrate, from about 1500 to 1980 m altitude (Fig. 6). It has a relative wide occurrence, with an estimated area of about 0.428215 sq. m. in the subalpine zone of Mt. Athos, more commonly found at the south facing slopes (KARYDAS 2007).

It grows next to shrub species such as *Astragalus angustifolius* Lam. subsp. *pungens* (Willd.) Hayek, *Prunus prostrata* Labill. and *Pterocephalus perennis* Cult. subsp. *perennis*. Some rare and endemic herbaceous taxa can be found near its vicinity, such as the Balkan endemics *Dianthus petraeus* Waldst. & Kit. subsp. *orbelicus* (Valen.) Greuter & Burdet, *Erysimum pusillum* Bory & Chaub., *Linum elegans* Spruner ex Boiss., *Silene waldsteinii* Griseb., the Greek endemics *Centranthus longiflorus* Steven subsp. *junceus* (Boiss. & Heldr.) I. Richardson and *Euphorbia deflexa* Sm., as well as the local endemics *Aubrieta erubescens*, *Centaurea athoa* subsp. *athoa* and *Satureja athoa* K. Malý (KARYDAS 2007). However, *C. athoa* can also be found less frequently in forest openings near the treeline, at an altitude of 1350-1500 m, near tree species such as *Abies borisii-regis* Mattf., *Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe, *Populus tremula* L., and *Juniperus foetidissima* Willd. and *J. communis* subsp. *hemisphaerica* (C. Presl) Nyman. It forms a single and undisturbed population, at the uppermost parts of Mt. Athos (KAMARI & KARYDAS 2009). More than 1000 individuals in total have been observed scattered throughout its area of occurrence.

Viola athois likewise, grows in rocky meadows and rock crevices of limestone substrate, from about 1500 to 2000 m altitude. It has a relative wider range of extent in the subalpine zone of Mt. Athos, compared with other endemic taxa (Fig. 7). Its area of occurrence is about 0.506053 sq. m., which is analogous to that of *Crepis athoa*. Similarly, it can be rarely encountered in forest gaps near the treeline, at an altitude a just below 1500 m, accompanied by species mentioned above (KARYDAS & TINIAKOU 2009). *V. athois* forms a single population that includes several thousand individuals,

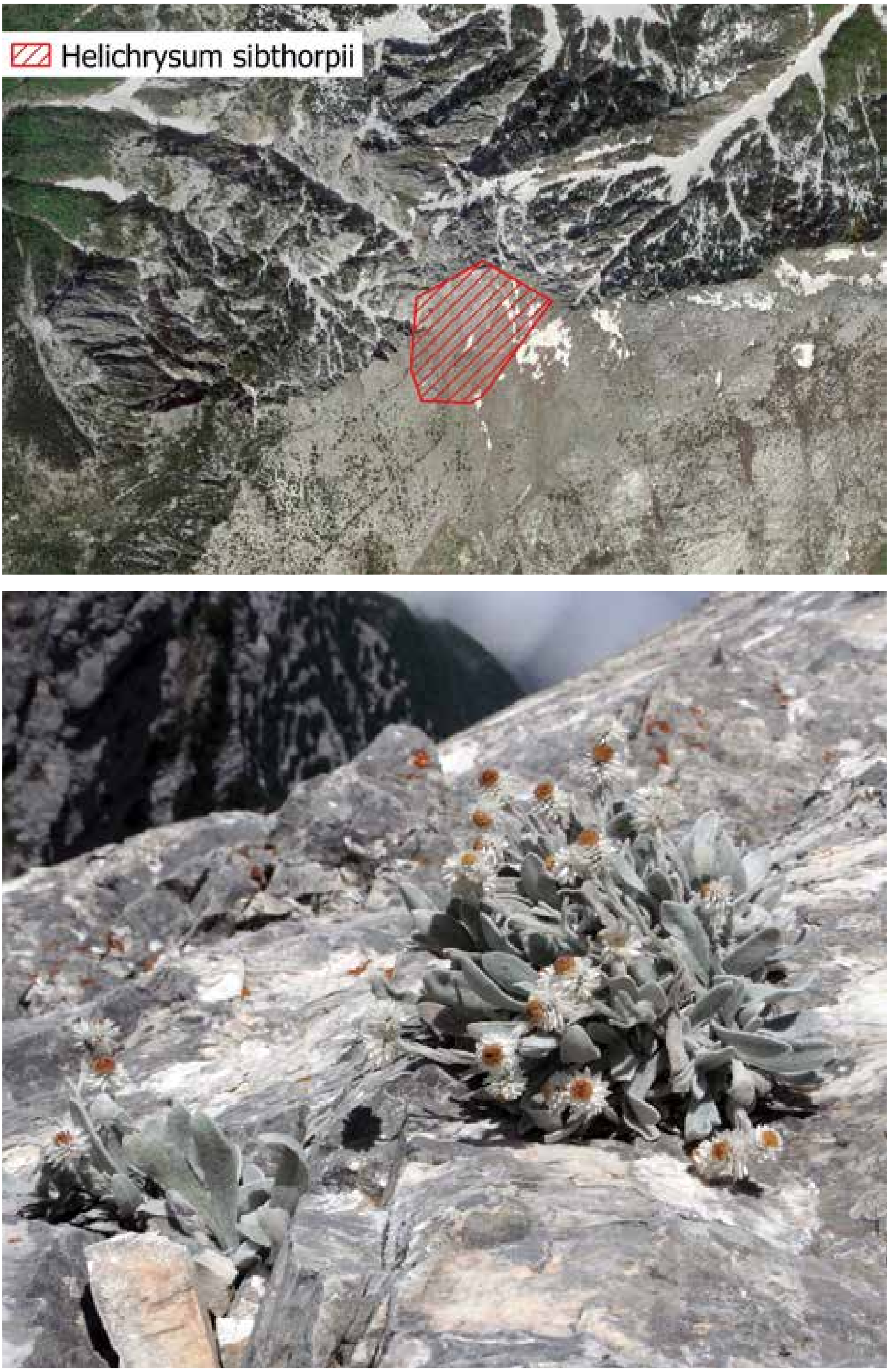


Fig. 4. The area of occurrence and an individual of *Helichrysum sibthorpii* on Mt. Athos.

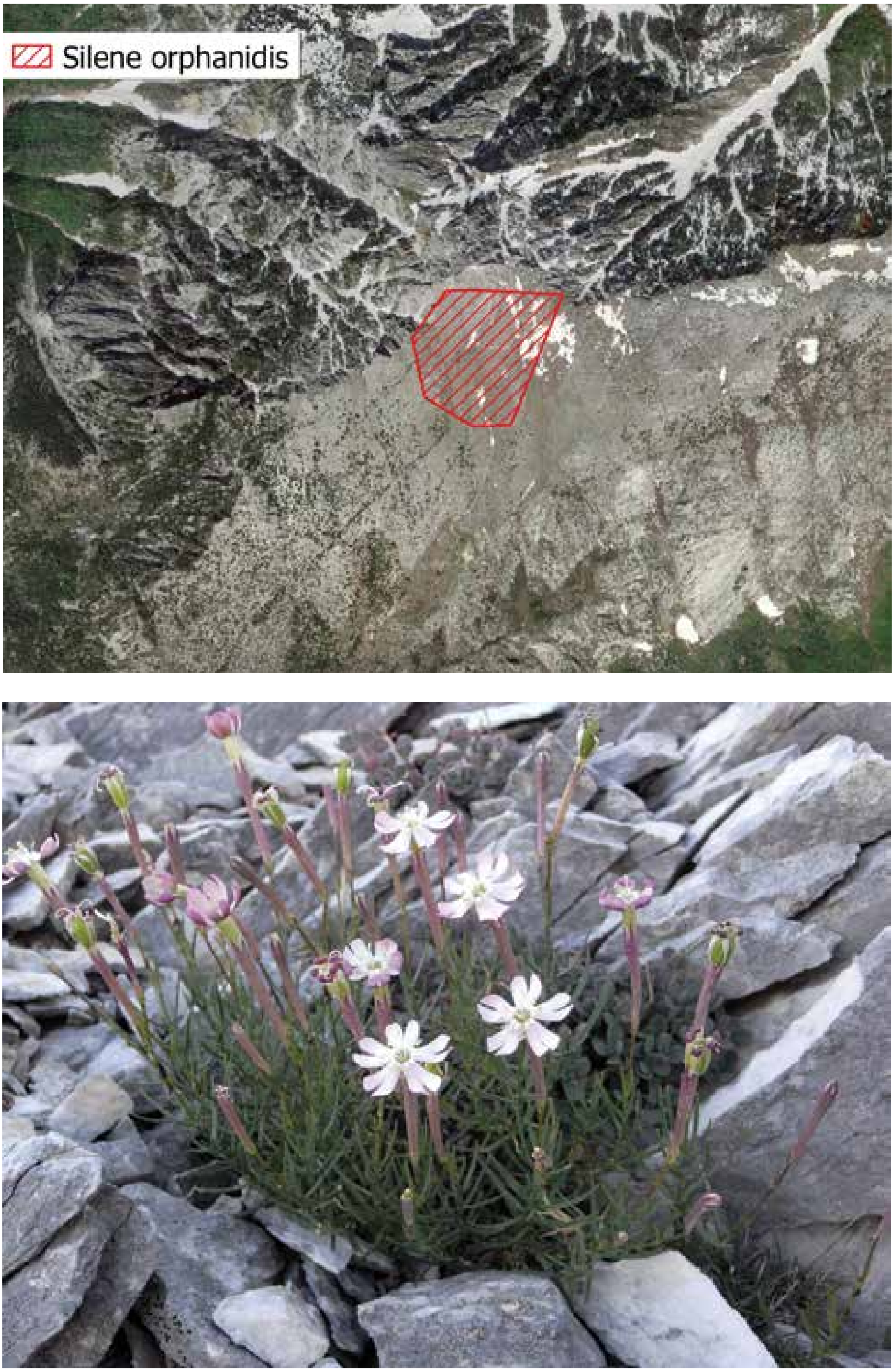


Fig. 5. The area of occurrence and an individual of *Silene orphanidis* on Mt. Athos.

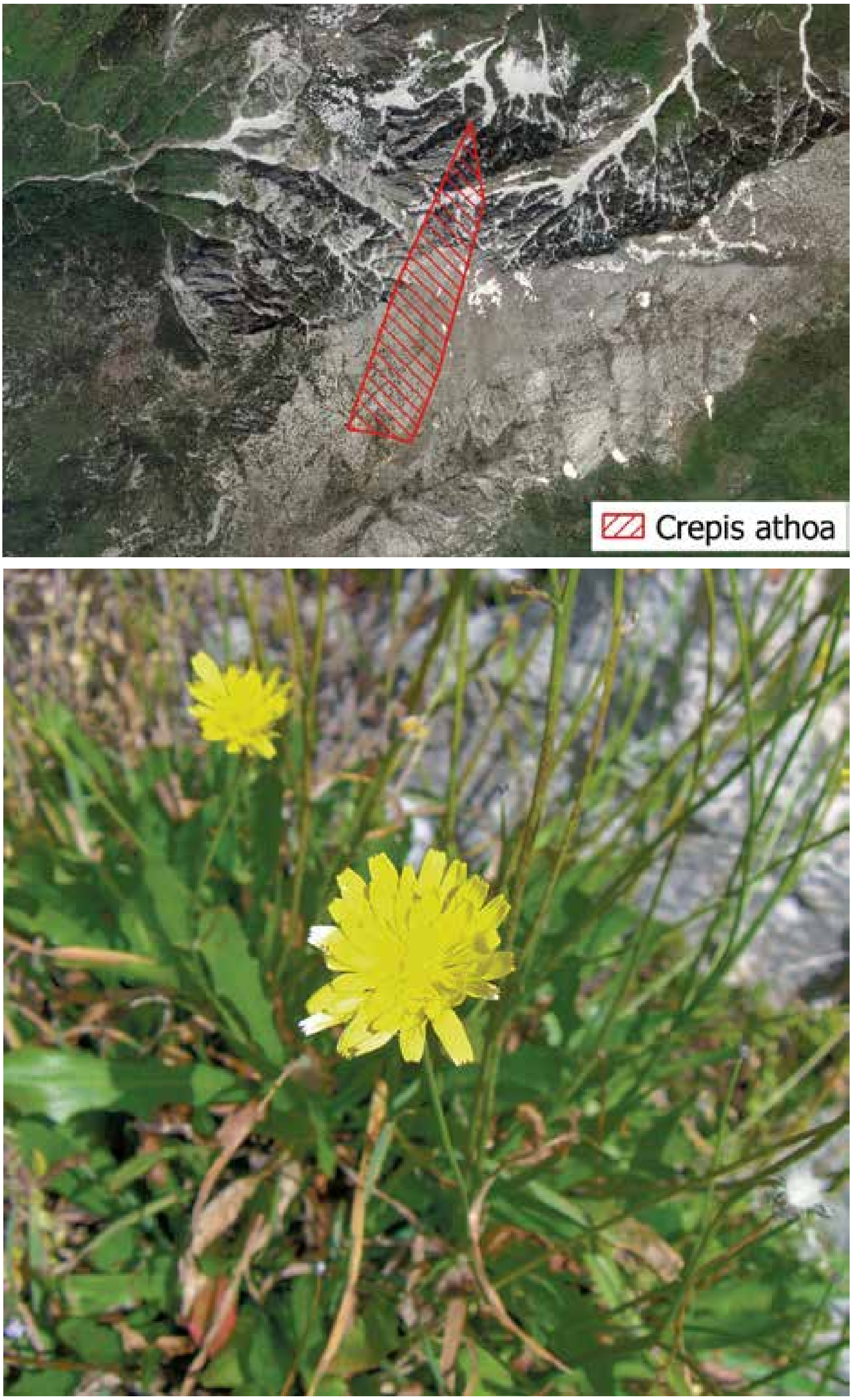


Fig. 6. The area of occurrence and an individual of *Crepis athoa* on Mt. Athos.

scattered throughout its area of occurrence, particularly at the south facing slopes of the mountain.

Discussion

The IUCN categories and criteria were formed as an universally accepted, objective and explicit outline for the classification of species that are potentially at high risk of extinction. The field data that were obtained for the taxa under investigation, were sufficient to apply the IUCN 2018 Red List Category criteria. The results are summarized in Table 2.

Aethionema orbiculatum can be characterized as Vulnerable (VU) according to IUCN (2018) Red List criteria, because of its restricted area of occupancy (criterion D2) and the total number of nature individuals that does not exceed 1000 (criterion D1).

Aubrieta erubescens is proposed to be classified as Vulnerable (VU) IUCN Red List Category status, because of its limited total area of occurrence and the fragmented nature of its less than 5 subpopulations. Furthermore, the total number of mature individuals is estimated at less than 1000. Hence, it complies with the Vulnerable (VU) category, fulfilling the D1 and D2 criteria. The three sub populations of *A. erubescens* are found scattered in the rocky summit of Mt. Athos, as well as in mid altitude mixed forest and scrub vegetation, away from human activities. Grazing animals are not allowed in the peninsula, so apart from some low intensity mule grazing, this activity does not constitute a threat. However, the population size is small, so collection of living plants or other plant material will be detrimental to the species. Forest fires may also be a potential threat for the lower altitude subpopulations.

Crepis athoa can be characterized as Vulnerable (VU), primarily due to its small number (barely exceeding 1000) of mature individuals (criterion C2) and also due to its limited area of occurrence (criterion B1).

Helichrysum sibthorpii occupies a very limited total area of occurrence, and because of the fragmented nature of its single population and the fact that the total number of mature individuals is estimated to be less than 250, the species is proposed to be given the Endangered (EN) IUCN Red List category status, as it fulfills the D criterion.

Silene orphanidis also can be granted as Endangered (EN). It forms a single population, which in any case does not reach 100 mature individuals, and has a restricted area of occurence.

For these two threatened taxa, it can be alleged that their single populations are found scattered in the rocky summit of Mt. Athos, in very inaccessible sites, away from human activities. However, their population size is so small, that any collection of living plants or other plant material is harmful to the species.

For these two threatened taxa, it can be alleged that their single populations are found scattered in the rocky summit of Mt. Athos, in very inaccessible sites, away from human activities. However, their population size is so small, that any collection of living plants or other plant material is harmful to the species.

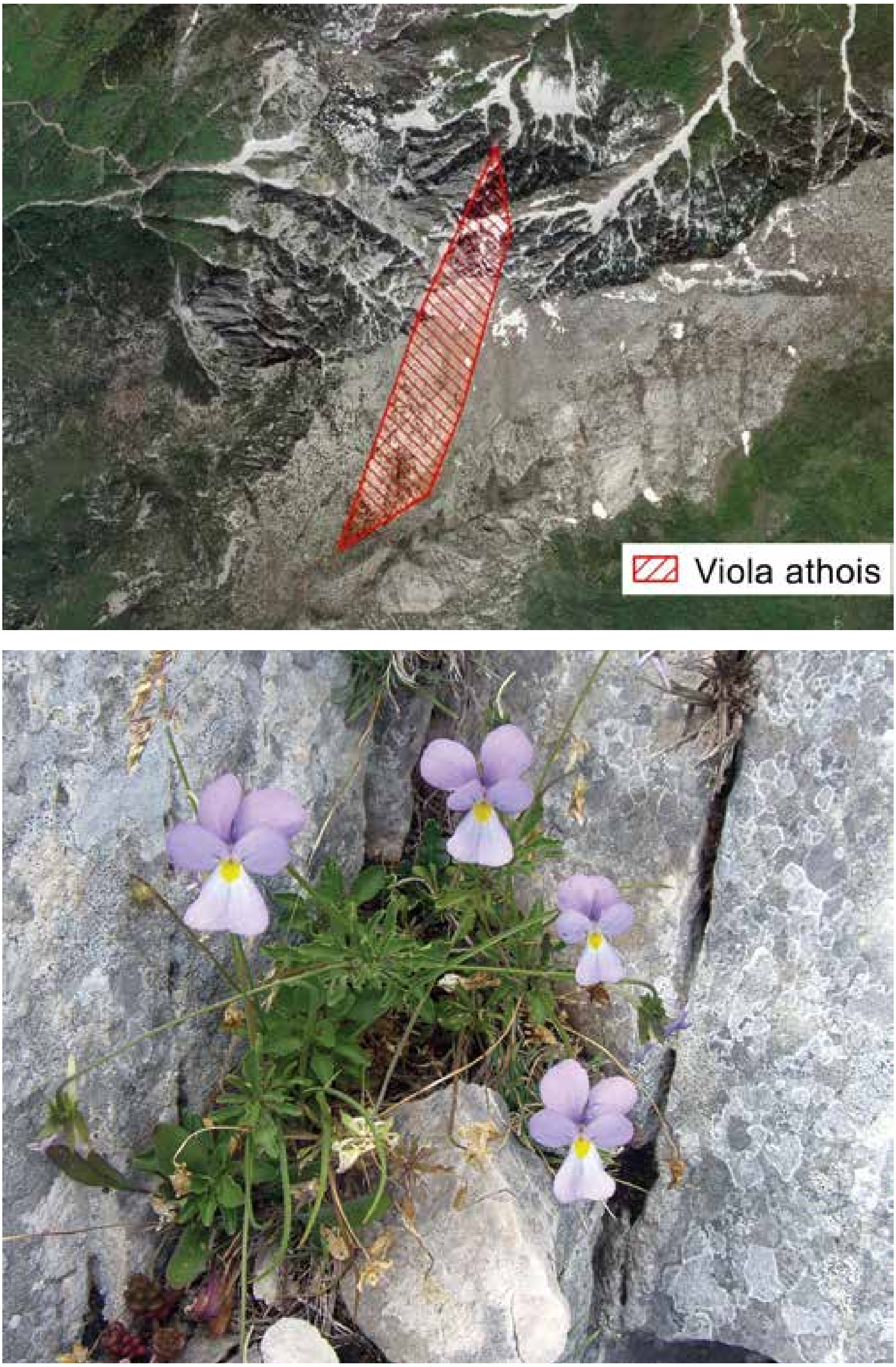


Fig. 7. The area of occurrence and individuls of *Viola athois* on Mt. Athos.

Table 2. Summary of taxa information and IUCN Red List categories (Endangered: EN, Vulnerable: VU, Near Threatened: NT).

Taxon	**Habitat (altitude)**	**Individuals (subpopulations)**	**Main threats**	**IUCN Red List category**
Aethionema orbiculatum	Crevices of limestone rocks (1800-2000 m)	‹1000 (1)	Population size	**VU**
Aubrieta erubescens	Mixed woodland openings, screes, stony slopes and rock crevices (800-2020 m)	‹1000 (3)	Population size - Fire	**VU**
Crepis athoa	Stony meadows and limestone rock crevices (1350-1980 m)	≥1000 (1)	Building activities - Mule grazing	**VU**
Helichrysum sibthorpii	Crevices of limestone rocks (1838-2000 m)	‹250 (1)	Population size	**EN**
Silene orphanidis	Crevices of limestone rocks (1800-1960 m)	‹250 (1)	Population size	**EN**
Viola athois	Stony meadows, limestone rock crevices and forest openings near the timberline (1350-2000 m)	›1000 (1)	Building activities - Mule grazing	**NT**

Finally, ***Viola athois*** can be declared as Near Threatened (NT), mainly due to its relatively small number of mature individuals, (its population exceeds 1000 mature individuals). For both *Crepis athoα* and *Viola athois,* it can be argued that they are not facing any immediate pressures that may lead to their extinction or severe population decline at present or in the near future, as they are found away from most human activities. On the other hand, what could influence negatively their population include the small scale building activities right at the heart of their habitat. In the area of Panagia chapel, at 1500 m altitude, a new guest house was under construction since 2007 and the current one is being expanded, in conjunction with the build of a large water reservoir that was completed in 2014.

Additionally, the old chapel of "Metamorphsis tou Sotiros" on the summit of Mt. Athos, has been demolished and a new larger one has been built in its place, from 2013 until 2017. The loading and transportation of building materials (not the construction process itself), may have some negative consequences to their habitat. Visits during 2014 and 2015 however, have shown that the populations of these taxa are thriving. Moreover, Mt. Athos, like the rest of the peninsula, is not subjected to livestock grazing, as this is forbidden by the monastic community. On the other hand, few dozen mules, used for the transportation of building materials have been observed to graze at the forest openings near the timberline and in the subalpine area during the summer months. This coincides with the flowering and fruiting period of those taxa and could potentially hinder their dispersal. Nevertheless, this low intensity grazing does not pose a high risk for the population.

No practical measures have been applied to date, regarding the protection of the seven species of this study. Thus, any actions that may potentially be detrimental to their habitats or populations must be prohibited. For instance, it is recommended to avoid over-collection of living plant material. Furthermore, plans for building guest houses in the subalpine zone must be stopped. Mules should be kept confined in certain areas and not allowed to wonder and graze in the habitat of these species.

Furthermore, labelling in their habitat is thought to be useful, as it would make the public more aware and vigilant regarding their presence and importance. In addition, long term monitoring of is recommended, which would allow a more thorough view of their population dynamics. Lastly, *ex situ* conservation is suggested, notably by focused research, propagation efforts and subsequently development of a seed bank for these species in Universities, Botanic Gardens and Research Institutes.

References

Athanasiadis, N., Theodoropoulos K., Gerasimidis, A., Eleftheriadou, E., Tsiripidis, I. & Korakis G. 1998: Vegetation units of the evergreen broadleaved zone in Agio Oros. – Cultural Capital of Europe Organisation, Thessaloniki 1997. OCCET ’97 Publications.

Babalonas, D., Konstantinou, M. & Haralambidis, S. 1998: The Flora of Agio Oros. – Cultural Capital of Europe Organisation, Thessaloniki 1997. OCCET ’97 Publications.

Constantinidis, Th. 1995: *Silene orphanidis* Boiss. Pp. 470-471. In Phitos, D., Strid, A., Snogerup, S. & Greuter, W. (eds), The Red Data Book of Rare and Threatened Plants of Greece. – World Wide Fund for Nature, Athens.

Council Directive 92/43/EEC 1992: On the conservation of natural habitats and wild fauna and flora, Annexes, II, IV & V. – European Union.

Dafis, S. 1998: Nature and Natural environment of Agio Oros. – Cultural Capital of Europe Organisation, Thessaloniki 1997. OCCET ’97 Publications.

—, Papastergiadou, E., Georgiou, K., Babalonas, D., Georgiadis, T., Papageorgiou, M., Lazaridou, T. & Tsiaousi, B. 1997: Directive 92/43/EEC. – Habitat Directive in Greece: Natura 2000 Network. EKBY.

Ganiatsas, K. 1963: The vegetation and flora of Agio Oros peninsula. – Mont Athos Library, Agio Oros, 2003.

Gustavsson, L.-Å. 1986: *Aubrieta* Adanson. Pp. 268-274. In Strid, A. (ed.), Mountain flora of Greece, 1. – Cambridge University Press, Cambridge.

IUCN Standards and Petitions Subcommittee 2018: Guidelines for Using the IUCN Red List Categories and Criteria. Version 12. Prepared by the Standards and Petitions Subcommittee. Available from: http://www.iucnredlist.org/documents/RedListGuidelines. pdf (accessed 1 May 2018).

Kamari, G. 1991: *Crepis* L. Pp. 576-595. In Strid, A. & Tan, K. (eds), Mountain flora of Greece, 2. – Edinburgh University Press, Edinburgh.

— & Karydas, A. 2009: *Crepis athoa* Boiss. Pp. 322-323. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 1(A-D). – Hellenic Botanical Society, Patras, Greece [in Greek].

Karydas, A. 2007: Monitoring and ex situ conservation of some endemic taxa of Agio Oros. – Master Postgraduate dissertation, University of Ioannina.

— 2009: *Helichrysum sibthorpii* Rouy. Pp. 89-90. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 2(E-Z). – Hellenic Botanical Society, Patras, Greece [in Greek].

— & Kamari G. 2009a: *Aethionema orbiculatum* (Boiss.) Hayek. Pp. 51-52. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 1(A-D). – Hellenic Botanical Society, Patras, Greece [in Greek].

— & — 2009b: *Silene orphanidis* Boiss. Pp. 326-327. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 2(E-Z). – Hellenic Botanical Society, Patras, Greece [in Greek].

— & Phitos, D. 2009: *Aubrieta erubescens* Griseb. Pp. 144-145. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 1(A-D). – Hellenic Botanical Society, Patras, Greece [in Greek].

— & Tiniakou A., 2009: *Viola athois* W. Becker. Pp. 383-384. In Phitos, D., Constantinidis, Th. & Kamari, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, 2(E-Z). – Hellenic Botanical Society, Patras, Greece [in Greek].

Phitos, D. 1970: Die Gattung *Aubrieta* in Griechenland. – Candollea 25(1): 69-87.

— 1995: *Aubrieta erubescens* Griseb. Pp. 78-79. In Phitos, D., Strid, A. Snogerup, P. & Greuter, W. (eds), The Red Data Book of Rare and Threatened Plants of Greece. – World Wide Fund for Nature, Athens.

—, Constantinidis, Th. & Kamari, G. (eds) 2009: The Red Data Book of Rare and Threatened Plants of Greece, 1(A-D): 1-405 & 2 (E-Z): 1-413. – Hellenic Botanical Society, Patras, Greece.

—, Strid, A., Snogerup, S. & Greuter, W. (eds) 1995: The Red Data Book of Rare and Threatened Plants of Greece. – World Wide Fund for Nature, Athens, pp. 527.

Presidential Decree 67/1981: On the protection of native flora and wild fauna and the determination of the coordination and control procedure of related research. – Athens.

Raus, Th. 1986: *Viola* L. Pp. 608-640. In Strid, A. (ed.), Mountain Flora of Greece, 1. – Cambridge University Press, Cambridge.

Strid, A. 1995: *Helichrysum sibthorpii* Rouy. Pp. 308-309. In Phitos, D., Strid, A., Snogerup, S. & Greuter, W. (eds) 1995: The Red Data Book of Rare and Threatened Plants of Greece. – World Wide Fund for Nature, Athens.

— & Papanicolaou, K. 1981: Floristic notes from the mountains of Northern Greece. –Nord. J. Bot. 1(1): 66-82.

— & — 1985: The Greek mountains. Pp. 89-111. In Cómez-Cambo C. (ed.), Plant conservation in the Mediterranean area. – Dr. W. Junk Publichers, Dordrecht, etc.

Tan, K. & Suda, J. 2002: *Aethionema* R. Br. Pp. 261-265. In Strid, A. & Tan, K. (eds), Flora Hellenica, 2. – A.R.G. Gantner Verlag, K.G.

Past, present, and future of *Campanula* (Campanulaceae) systematics – a review

ELENI LIVERI, ANDREW A. CROWL & NICO CELLINESE

Abstract

Liveri, E., Crowl, A. A. & Cellinese, N. 2019: Past, present, and future of *Campanula* (Campanulaceae) systematics-a review. – Bot. Chron. 22: 209-222.

Campanula L. (Campanulaceae, Campanuloideae*)* is a large genus with a long and complex taxonomic history. Traditional classifications were based on morphological characters that often resulted in artificial grouping. The last two decades, however, have seen extensive phylogenetic studies of the broader Campanuloideae utilizing molecular data and modern analytical methods. These studies have revealed many interesting patterns and insights into the evolution of this group and have provided compelling evidence for the polyphyly of *Campanula*. Mediterranean hotspots such as the Middle East, Greece, and the Balkan Peninsula are particularly species-rich and have attracted much attention from botanists. In this paper, Professor Dimitrios Phitos's contribution to the systematics of Greek *Campanula* is highlighted and aspects of his work are discussed. We conclude that both broad- and narrow-scale analyses to date do not provide a sufficient taxonomic framework for a modern *Campanula* classification, but emphasise the need for more focused studies with increased genomic data and complete taxon sampling in order to resolve the taxonomic complexity and elucidate the historical evolution of *Campanula* in the Mediterranean region and beyond.

E. Liveri, Botanical Institute, Division of Plant Biology, Department of Biology, University of Patras, GR-26500 Patras, Greece. E-mail: eleniliveri@upatras.gr
A. A. Crowl, Department of Biology, Duke University, Durham, NC 27708, USA. E-mail: andy.crowl@gmail.com
N. Cellinese, Florida Museum of Natural History, University of Florida, FL 32611 Gainesville, U.S.A. E-mail: ncellinese@flmnh.ufl.edu

Key words: Campanula, Campanulaceae, taxonomy, morphology, phylogeny.

Introduction

The Campanulaceae Juss. represent a diverse, worldwide distributed angiosperm clade that has received considerable attention by taxonomists (LAMMERS 2007a). In its current delimitation, Campanulaceae comprise approximately 84 traditionally defined genera and ca. 2400 species that show remarkable morphological and habitat diversity, being distributed on all continents, from tropical rainforests to arctic tundra (LAMMERS 2007a). Five major lineages, traditionally treated as subfamilies (or sometimes families), have been distinguished within Campanulaceae: Campanuloideae Burnett (ca. 1054 species), primarily distributed in temperate regions of the Old World; Lobelioideae Burnett (ca. 1192 species), mainly distributed in tropical and sub-tropical regions of the New World; Cyphioideae Walp. (ca. 64 species), restricted to Africa; Nemacladoideae M. H. G. Gust (ca. 15 species), restricted to southwestern North America; and Cyphocarpoideae Miers (3 species), endemic to Chile (LAMMERS 2007a, b). Despite recent phylogenetic studies, relationships among these remain somewhat unclear (COSNER & al. 1994, 2004, LUNDBERG & BREMER 2003, TANK & DONOGUE 2010, BEAULIEU & al. 2013, CROWL & al. 2016).

The Campanuloideae (ca. 50 genera and 1054 species) represent the second largest and most widespread lineage, and the only one with radial floral symmetry (Fig. 1). The majority of species are distributed in the Old World, with diversity hotspots in Eurasia, especially the Mediterranean Basin (LAMMERS 2007a, b). Species distributions range from entire continents to single islands (e.g., CROWL & al. 2015). Three main subclades have been defined within Campanuloideae: platycodonoids-Platycodoninae R. Schönland, wahlenbergioids-Wahlenbergiinae Sond., and campanuloids-Campanulinae R. Schönland (Fig. 2; EDDIE & al. 2003, ROQUET & al. 2008, CELLINESE & al. 2009, HABERLE & al. 2009, ROQUET & al. 2009, YOO & al. 2018). Most species (ca. 420) are included in the controversial genus *Campanula* L. (LAMMERS 2007a, b).

Campanula is distributed across the circumboreal region, showing great diversity in habitat preferences and morphology. Most species are herbaceous with pentamerous flowers. The corolla can be characterised as campanulate, tubular, infundibuliform (funnel-shaped), cupuliform (bowl-shaped) or rotate, mostly fused and varying in color from blue to violet or white (rarely yellow, red, or pink). The ovary is usually obconical or oblong-obconical with (2-)3-5 locules. Calyx teeth are often longer than the ovary and they may alernate with appendages. Capsules are pendent or erect, dehiscing by pores or valves or, rarely, indehiscent (FEDOROV & KOVANDA 1976, LAMMERS 2007a). Calyx morphology and capsule dehiscence have been used widely in traditional classifications.

Protandry and secondary pollen presentation are present in *Campanula* (and in Campanulaceae), showing a great variety of mechanisms (ERBAR & LEINS 1989, 1995, LEINS & ERBAR 1990, YEO 1993). Secondary pollen presentation involves the relocation of pollen from anthers to disparate floral organs (HOWELL & al. 1993, YEO 1993). In all cases, pollen presentation occurs via introrse anthers and, especially in Campanuloideae, a ring is formed by connivent anthers (LEINS & ERBAR 1990). Until now, two mechanisms of secondary pollen presentation have been described within Campanuloideae: the simple deposition and the brushing mechanism. The simple deposition is found in many *Campanula* species in which pollen is released onto stylar hairs within the anther ring before anthesis (LEINS & ERBAR 1990). The stamens then quickly wither while the style elongates, exposing the pollen to the outside environment. After that, the collecting hairs of the style invaginate into their own large base, causing pollen grains to be released and the style to become characteristically pitted. The brushing mechanism has been presented in *Phyteuma* and is more complex than the simple deposition. The deposition of the pollen takes place onto stylar hairs with the aid of the corolla, which remains fused at the apical tip while the style elongates. The growing style eventually ruptures through the corolla hood, presenting the pollen grains to pollinators (LEINS & ERBAR 2006). The evolution of secondary

Fig. 1. Representative Mediterranean species of Campanuloideae: A, *Campanula andrewsii* A. DC.; B, *C. carpatha* Halácsy; C, *C. cretica* D. Dietr.; D, *C. creutzburgii* Greuter; E, *C. garganica* Ten.; F, *C. hawkinsiana* Hausskn. & Heldr.; G. *C. laciniata* L.; H, *C. pelviformis* Lam.; I, *C. saxatilis* L. – Photos A, B, G, I, by V. Ketsilis-Rinis; C, E, F, by G. Kofinas and D, H, by A. A. Crowl.

pollen presentation mechanisms has been hypothesised to be functionally related to the evolution of bilateral symmetry and anther dehiscence (CROWL & al. 2016).

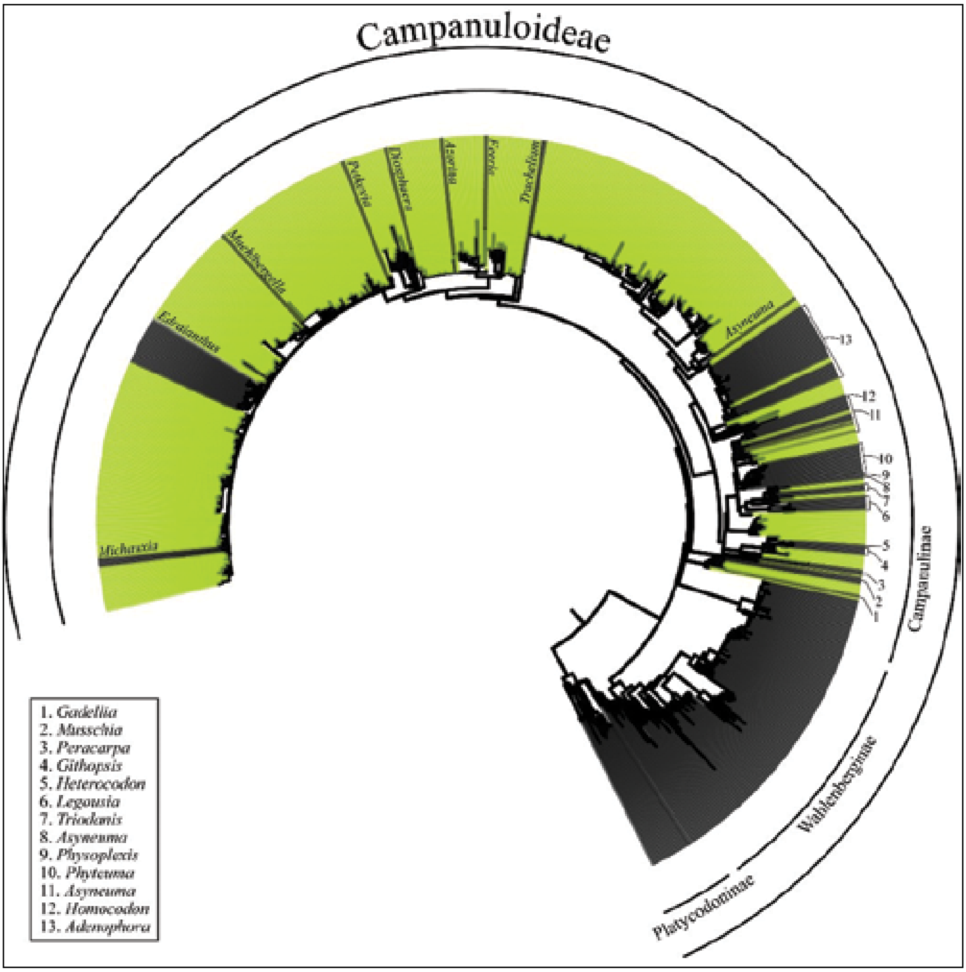


Fig. 2. Phylogeny of the Campanuloideae clade based on combined nuclear and plastid data. Modified from CROWL & al. (2016). The highly polyphyletic *Campanula* is highlighted in green, all other genera are shown in black.

Campanula has a long taxonomic history since LINNAEUS'S (1753) first naming of the genus. CANDOLLE (1830), in his classic monograph of Campanulaceae, divided *Campanula* into two main sections based mainly on calyx morphology (presence/absence of appendages between the teeth): *C.* sect. *Medium* (with appendages present between the calyx teeth) and *C.* sect. *Eucodon* (lacking calyx appendages). This classification was followed by most nineteenth-century authors (e.g. ENDLICHER 1838, WILLKOMM 1868, BENTHAM & HOOKER 1876, ROUY 1908). BOISSIER (1875) recognized the same sections but added capsule dehiscence to further define them:

capsules opening by basal pores or valves in *C.* sect. *Medium* and apical or medial position pores or valves in *C.* sect. *Rapunculus*. Subsequent authors accepted this classification in the following years (FIORI 1927, FEDOROV 1957, FEDOROV & KOVANDA 1976). DAMBODLT (1976, 1978), in the framework of the *Flora of Turkey and the East Aegean Islands*, proposed a different classification system, dividing *Campanula* into six subgenera: *C.* subg. *Campanula*, *C.* subg. *Megalocalyx* Damboldt, *C.* subg. *Sicyocodon* (Feer) Damboldt, *C.* subg. *Roucela* (Dumort.) Damboldt, *C.* subg. *Brachycodonia* (Fedorov) Damboldt and *C.* subg. *Rapunculus* (Boiss.) Kharadze. He broadly followed FEDOROV (1957) with some adjustments, based primarily on life form, mode of capsule dehiscence, seed morphology and cytology (DAMBOLDT 1976, 1978). *C.* subg. *Campanula* was further divided into15 sections defined by calyx morphology and the shape of basal and cauline leaves. Other important regional taxonomic studies on *Campanula* were carried out by HAYEK (1925, 1931), QUÉZEL (1953), SHETLER (1963), PHITOS (1965) and OGANESIAN (1995). The high morphological variability, even within species, and the lack of taxonomic clarity, still causing much confusion among taxonomists, have resulted in the description of a large number of species, subspecies, varieties and even formae.

The evolutionary history of *Campanula*

Campanula is one of the most speciose flowering plant lineages of the Holarctic (JONES & al. 2017). Most of the species range from the Arctic and north temperate zones to eastern Africa, southern Asia, and northern Mexico, with poor representation in North America and Africa (LAMMERS 2007b). Eurasia hosts most of the diversity, particularly in the mountain ranges to the north of the Mediterranean Sea, from the Pyrenees across the Alps and Balkans as far as the eastern Caucasus (TUTIN & al. 1976, OGANESIAN 2008, CASTROVIEJO & al. 2010). According to *Flora Europaea* (FEDOROV & KOVANDA 1976), 144 *Campanula* species are found in Europe, but presumably the real number is much higher, as many new species have been described since. Based on more recent data from *Euro+Med PlantBase* (CASTROVIEJO & al. 2010; see Fig.1 for examples), about 278 species (plus many subspecies) have been recorded in the Mediterranean region, with more than 80% endemic species. Diversity of endemic *Campanula* species is especially high in the Eastern Mediterranean region, the Balkan Peninsula, the Caucasus and Turkey, each of these recognized as an endemism hotspot (DAMBOLDT 1978, GAGNIDZE 2005, BORSCH & al. 2009).

Early genetic works attempted to improve the classification of *Campanula* with karyological and statistical analyses, but these data were often insufficient for unambiguous taxon delimitation (e.g. GADELLA 1962, 1963, 1964, 1966, 1967, PHITOS 1963a, b, 1964a, b, 1965, CONTANDRIOPOULOS 1964, 1966, 1970, 1972, 1976, 1980a, b, EDDIE & INGROUILLE 1999). These studies suggest that the most common basic chromosome number in *Campanula* (also generally in Campanulaceae) is x = 17. The same chromosome number is also present in relatively unrelated genera such as *Nesocodon* Thulin, *Canarina* L., and *Ostrowskia* Regel (EDDIE & al. 2003). Statistical analyses of morphological characters corroborated the extensive polymorphism of *Campanula* (e.g. EDDIE & INGROUILLE 1999) but partly failed to reflect true evolutionary relationships. In many cases the morphology-based studies resulted in assemblages of heterogeneous taxa (KOVAČIĆ 2004).

Before phylogenetic approaches in Campanulaceae became common-place, traditional studies based mainly on morphological characters led to the recognition of at least two major *Campanula* sections (*C.* sect. *Rapunculus* and *C.* sect. *Medium*) (KOVAČIĆ 2004). From the first molecular phylogenetic study on Campanuloideae however, interesting results emerged. Based on ITS sequence data, *Campanula* was recovered as polyphyletic due to the nested placement of several other genera, such as *Edraianthus* A. DC. and *Phyteuma* L. (EDDIE & al. 2003), and the high degree of polyphyly has been confirmed in all subsequent studies (Fig. 2, ROQUET & al. 2008, BORSCH & al. 2009, CELLINESE & al. 2009, HABERLE & al. 2009, MANSION & al. 2012, CROWL & al. 2014, JONES & al. 2017, YOO & al. 2018).

Campanula seems to be divided into two main clades, the *Rapunculus* clade (or rapunculoid group) and the *Campanula* s. str. clade (EDDIE & al. 2003, ROQUET & al. 2008, CELLINESE & al. 2009, HABERLE & al. 2009), with a few taxa falling outside. The rapunculoid group and *Campanula* s. str. very broadly correspond to the main two sections that CANDOLLE (1830) and BOISSIER (1875) had recognized, based on fruit dehiscence and calyx morphology. Although all molecular studies confirmed the polyphyletic origin of *Campanula* (Fig. 2), two main challenges prevented researchers from providing a robust phylogenetic hypothesis for this group: incomplete taxon sampling and insufficient molecular markers, which fail to capture the putatively recent diversification of these taxa.

The most comprehensive phylogenetic inference for *Campanula* was generated by MANSION & al. (2012), including more than 70% of extant described species. The results revealed that the clade including *Campanula* and its closely related genera (referred to as *Campanula* s. l. in MANSION & al. 2012 and hereafter) is divided into 17 lineages encompassing ca. 600 species. However, relationships among and within clades were not fully resolved nor well supported, especially in taxa found in Mediterranean hotspots such as the Middle East, Greece, and the Balkan Peninsula (MÉDAIL & QUÉZEL 1997, MYERS & al. 2000, GRIFFITHS & al. 2004, ALEKSIĆ & al. 2018).

In the Mediterranean region, the following recent studies have focused on spe-cific groups within *Campanula* s. l., testing the monophyly of traditional sections, subsections or even species complexes, and attempting to disentangle phylogenetic relationships and evolutionary processes occurring in this hotspot of biological diversity:

- Isophyllous *Campanula* species group, Mediterranean (PARK & al. 2006).
- *Edraianthus*, Balkan Peninsula (STEFANOVIĆ & al. 2008, SURINA & al. 2011).
- *Campanula alpina* s. l., Balkans (RONIKIER & al. 2008, RONIKIER & ZALEWSKA-GALOSZ 2014).
- *Campanula lusitanica* complex, Western Mediterranean (CANO-MAQUEDA & TALAVERA 2011).
- *Campanula pyramidalis* complex, Balkans (LAKUŠIĆ & al. 2013, RADOSAVLJEVIĆ & al. 2015, JANKOVIĆ & al. 2016).
- *Campanula* subg. *Roucela* or *Roucela* complex, Mediterranean (CROWL & al. 2015).
- *Campanula erinus* complex, Mediterranean (CROWL & al. 2017).
- Capitulate *Campanula* species, Balkans (ALEKSIĆ & al. 2018).

These studies have contributed significantly to our knowledge of *Campanula* s. l. but reinforced the notion that, as traditionally defined, *Campanula* is not a natural entity and its complex historical evolution is hard to reconstruct even with molecular tools. Evolutionary processes, such as interspecific hybridization and introgression, genome duplication, and incomplete lineage sorting have proven to represent common obstacles for phylogenetic reconstructions. Multiple whole genome duplications during the evolution of Campanuloideae have been associated with the evolution of nov-el traits (insular woodiness) and shifts to novel niches (island endemics and montane species) (CROWL & al. 2016). Additionally, allopolyploidy appears to have resulted in cryptic diversity in widespread taxa (e.g. *Campanula erinus*: CROWL & al. 2017).

Recent phylogenetic studies have aimed also to estimate the divergence time in Campanuloideae and *Campanula* s. l. Campanuloideae were inferred to have originated during the late Palaeocene to early Eocene and for *Campanula* s. l., the divergence time was estimated during the late Eocene to early Oligocene (MANSION & al. 2012, CROWL & al. 2016, JONES & al. 2017). However, divergence time estimations for the clades within *Campanula* s. l. are not exactly congruent among the studies, likely due to sampling strategy, the type of analysis conducted, and the calibration points used (JONES & al. 2017). Unfortunately, the fossil record for Campanulaceae is extremely poor, with arguably only a single fossil – the seeds of *Campanula paleopyramidalis* Łańc.- Środ. (ŁAŃCUCKA-ŚRODONIOWA 1977, 1979) – suitable for calibrating divergence-time estimations. This fossil represents a Tertiary relative of the extant *C. pyramidalis* L. (ŁAŃCUCKA-ŚRODONIOWA 1977). The seeds of *C. paleopyramidalis* were described from the Miocene of the Nowy Sacz Basin, in the West Carpathians (southern Poland), dating from the Miocene (16.5-17.5 Ma BP) (ŁAŃCUCKA-ŚRODONIOWA 1977, 1979, NEMCOCK & al. 1998). This age range is most appropriately applied as an internal calibration point, representing the most recent ancestor of *C. pyramidalis* and *C. carpatica* Jacq. (CELLINESE & al. 2009, MANSION & al. 2012, CROWL & al. 2016, JONES & al. 2017).

The geological and climatic history of the Mediterranean area has also played an important role in the diversification of *Campanula* (CROWL & al. 2015). The desiccation of the Mediterranean Basin during the Messinian Salinity Crisis (5.96-5.33 Ma) may have forced many *Campanula* species into mountain refugia, such as the Alpide belt, which resulted in a reduction of diversification rates in this region (JONES & al. 2017). In contrast, the diversification rates accelerated substantially in the *Roucela* complex during the Messinian Salinity Crisis and the mid-Aegean trench formation, likely due to allopatric speciation (CROWL & al. 2015). Therefore, shifts in diversification rates may be driven by various factors (biotic and abiotic) and are taxon specific (JONES & al. 2017).

Phitos's contribution to the taxonomy of Greek *Campanula*

Greece represents one of the plant diversity hotspots in the Mediterranean basin counting approximately 5828 species and 1982 subspecies of vascular plants (native and naturalised), belonging to 1083 genera and 185 families (DIMOPOULOS & al. 2013, 2018). Over 750 species are endemic, making Greece an important center of endemism in the Mediterranean. This diversity is especially impressive when taking into account the relatively small area of the country (131.957 km^2). The geographical position of Greece at the intersection of three continents, the complex geological history, and the anthropogenic pressure since ancient times, have all been suggested as contributing to this great diversity (PHITOS & al. 2009).

The Campanuloideae are represented in Greece by eight traditionally defined genera. *Campanula* is the most speciose with 75 recognized species, and if we include *Asyneuma*, *Edraianthus*, *Halacsyella* Janch., *Jasione* L., *Legousia* Durande, *Petromarula* Vent. ex R. Hedw. and *Phyteuma* L., the number rises to 91.

Greek campanulas have been extensively studied by Dimitrios Phitos. Phitos graduated from the University of Athens (Greece) where he also obtained his PhD. Afterwards, he moved to the University of Munich (Germany) to study under the guidance of Professor Hermann MERXMÜLER, with whom he started his research on *Campanula* sect. *Quinqueloculares* (Boiss.) Phitos. Later, he returned to Greece to become the first Professor of Botany at the University of Patras, where he established the Botanical Institute and the Herbarium (UPA). PHITOS was also the first researcher in Greece who used cytotaxonomy in his studies and trained his students in these techniques. His main scientific interests remain in systematics, phytogeography and cytotaxonomy. PHITOS's editorial work includes some of the most important books for the Greek flora such as the *Red Data Books of Rare and Threatened Plants of Greece* (PHITOS & al. 1995, PHITOS & al. 2009) and *Flora Hellenica* (PHITOS & al. 1997). In addition to *Campanula* sect. *Quinqueloculares*, Phitos contributed considerable knowledge on many other *Campanula* species and related taxa, such as *Symphyandra* A. DC. (e.g. PHITOS 1966, DAMBOLDT & PHITOS 1971, PHITOS & KAMARI 1984, 1988). Since 1998 he serves as Emeritus Professor at the University of Patras.

PHITOS overall described 20 new *Campanula* species and subspecies, many of them belonging to *C.* sect. *Quinqueloculares*, now recognised as one of the most diverse taxa in this group. His research was based on morphological, phytogeographical and karyological data (PHITOS 1963a, b, 1964a, b, 1965, 1969). Additional studies followed (PAPATSOU & PHITOS 1975, RUNEMARK & PHITOS 1996, PHITOS & KAMARI 2009a, b, TURLAND & PHITOS 2009, PHITOS 2016, KYRIAKOPOULOS & al. 2017) with further species described from Turkey (e.g. *Campanula sorgerae* Phitos in DAMBOLDT 1976).

Campanula sect. *Quinqueloculares* includes ca. 39 species mostly distributed in Greece and coastal Turkey. Over half are Greek endemics, and 25% are found only in Turkey. The rest of the species occur in other European countries (e.g. France and Italy), the Caucasus and the Middle East (CASTROVIEJO & al. 2010). They are biennial (monocarpic) or, more rarely, perennial chasmophytes characterised by a 5-locular ovary, style with 5 stigmas, capsule opening with 5 pores, and calyx appendages more or less covering the ovary (PHITOS 1965, 1969).

Despite the epithet of the section (*Quinqueloculares* = five locules), locule number varies between 3 and 5 in several species. One sub-group with a consistently trilocular ovary has been recognised within the section. Other morphological features that are considered diagnostic for the delimitation of species are: shape and pubescence of rosette leaves, shape and size of calyx appendages, and corolla shape and length (PHITOS 1969). However, these characters, as many others, show extreme variation, even within the same species. An excellent example is provided by the *C. celsii* group occurring from Attiki to the adjacent Evvia island and the Kiklades in the Aegean Sea. Within this group, four subspecies have been recognised based on the variation of rosette leaf shape (from spathulate to sublyrate) and pubescence (from softly appressed to scabrous), petiole crenation (from strong to slight), and sepal shape (from lanceolate to triangular). The combination of morphological and cytogenetic studies (PHITOS 1963a, b, 1964a, b, 1965) provided a first attempt to generate hypotheses on the evolutionary relationships within *C.* sect. *Quinqueloculares* and highlighted the extreme polymorphism of the constituent taxa. This was further emphasized in the morphological analyses by EDDIE & INGROUILLE (1999), who found considerable overlap among species and failed to identify distinct species clusters. A detailed, comprehensive phylogenetic study of all species traditionally included in *C.* sect. *Quinqueloculares* is necessary to fully understand the historical evolution of this complex group (LIVERI & al., in prep). Given the significant species polymorphism in the context of a polyphyletic *Campanula*, it is important to establish whether the *C.* sect. *Quinqueloculares* group has a single origin and, if so, to better understand the processes of its diversification and endemism in the eastern Mediterranean Basin.

Overall, Phitos's contribution to Mediterranean *Campanula* and related taxa had a significant impact on our knowledge of the diversity of this group; it has laid the foundation necessary for a modern classification and understanding of Campanuloideae, as is evident by his input in recent molecular studies (MANSION & al. 2012, CROWL & al. 2014, 2015).

Conclusions

Despite extensive studies, the taxonomy and classification of Campanuloideae and *Campanula* remain unclear, ambiguous, incomplete, and controversial (MANSION & al. 2012, ALEKSIĆ & al. 2018). As traditionally defined, *Campanula* clearly does not represent a natural entity (Fig. 2). Morphological synapomorphies are lacking, and characters are too variable across and within species to provide a stable framework for unambiguously recognising distinct taxa. Moreover, morphological and molecular analyses are often incongruent (e.g. ALEKSIĆ & al. 2018), further hindering our ability to understand the natural history of this diverse group. Every attempt to date has failed to generate clear hypotheses on the evolution of *Campanula,* and it has become obvious that the molecular tools used so far (a few plastid and nuclear genes) are insufficient to unravel the complexity of this group.

Recent broad-scale analyses (MANSION & al. 2012, CROWL & al. 2014, 2016, YOO & al. 2018) have identified many unresolved, species-rich clades and highlighted a need for more focused studies in which a more inclusive taxon sampling is analysed. To this end, CROWL & al. (2017) demonstrated that using a phylogenomic approach may be the most promising path to gain insight into the evolution of complex *Campanula* lineages in the Mediterranean and beyond.

After decades of attempts to clarify the evolutionary history of *Campanula* at broad and narrow scales, it seems that we are still far from a comprehensive understanding of this enigmatic group. However, as our ability to sample herbarium specimens and obtain large, genomic datasets increases, this goal is becoming more attainable.

Acknowledgements

We are deeply grateful to Prof. Dimitrios Phitos for his invaluable mentorship in the past several years. We are at very different stages in our academic careers and yet have all greatly benefitted from our interactions with Prof. Phitos as a colleague, mentor, and wonderful friend. His guidance has undoubtedly shaped our minds and fostered our enthusiasm for *Campanula* research. We would like to express our thanks to Prof. Georgia Kamari for her mentorship and for providing input on this manuscript. We would also like to thank Prof. Werner Greuter for helpful comments that greatly improved this manuscript. Lastly, many thanks to Vlasis Ketsilis-Rinis and Giannis Kofinas for the photographs provided in Fig.1.

References

Aleksić, J. M., Škondrić, S. & Lakušić, D. 2018: Comparative phylogeography of capitulate *Campanula* species from the Balkans, with description of a new species, *C. daucoides*. – Pl. Syst. Evol. 304: 549-575.

Beaulieu, J. M., Tank, D. C. & Donoghue, M. J. 2013: A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. – BioMed. Centr. Evol. Biol. 13: 80.

Bentham, G. & Hooker, J. D. 1876: *Genera plantarum*, 2. – London.

Boissier, E. 1875: Campanulaceae. Pp. 884-962. In Flora orientalis, 3. – Genève, Basel.

Borsch, T., Korotkova, N., Raus, T., Lobin, W. & Löhne, C. 2009: The petD group II intron as a species level marker: utility for tree inference and species identification in the diverse genus *Campanula* (Campanulaceae). – Willdenowia 39: 7-33.

Candolle, A.-P. de 1830: Monographie des Campanulées. – Paris.

Cano-Maqueda, J. & Talavera, S. 2011: A taxonomic revision of the *Campanula lusitanica* complex (Campanulaceae) in the Western Mediterranean region. – Anales Jard. Bot. Madrid 68: 15-47.

Castroviejo, S., Aldasoro, J. J., Alarcón, M. & Hand, R. 2010: Campanulaceae. Euro+Med Plantbase – the information resource for Euro-Mediterranean plant diversity. Published on the Internet http://ww2. bgbm. org/EuroPlusMed/ [accessed October 2018].

Cellinese, N., Smith, S. A., Edwards, E. J., Kim, S.-T., Haberle, R. C., Avramakis, M. & Donoghue, M. J. 2009: Historical biogeography of the endemic Campanulaceae of Crete. – J. Biogeogr. 36: 1253-1269.

Contandriopoulos, J. 1964: Contribution à l'étude caryologique des Campanulées de Grèce. – Bull. Soc. Bot. France 111: 222-235.

— 1966: Contribution à l' étude cytotaxonomique des Campanulacées de Grèce. II. – Bull. Soc. Bot. France 113: 453-493.

— 1970: Contribution à l' étude cytotaxonomique des Campanulacées du Proche-Orient. – Bull. Soc. Bot. France 117: 55-70.

— 1972: Contribution à l' etude cytotaxonomique des Campanulacées du Proche-Orient: 3. – Bull. Soc. Bot. France 119: 75-94.

— 1976: Contribution à l’ etude cytotaxonomique des Campanulacées du Proche-Orient: 4. – Bull. Soc. Bot. France 123: 33-46.

— 1980a: Contribution à l’ etude cytotaxonomique des Campanulacées d’ Iran. – Rev. Biol. Ecol. Médit. 7: 27-36.

— 1980b: Contribution à l’étude cytotaxonomique du genre *Campanula* L. en Afrique du Nord et centrale. – Bol. Soc. Brot. 53: 887-906.

COSNER, M. E., JANSEN, R. K. & LAMMERS, T. G. 1994: Phylogenetic relationships in the *Campanulales* based on *rbcL* sequences. – Pl. Syst. Evol. 190: 79-95.

—, RAUBESON, L. A. & JANSEN, R. K. 2004: Chloroplast DNA rearrangements in Campanulaceae: phylogenetic utility of highly rearranged genomes. – BioMed. Centr. Evol. Biol. 4:27

CROWL, A. A., MAVRODIEV, E., MANSION, G., HABERLE, R., PISTARINO, A., KAMARI, G., PHITOS, D., BORSCH, T. & CELLINESE, N. 2014: Phylogeny of Campanuloideae (Campanulaceae) with emphasis on the utility of nuclear pentatricopeptide repeat (PPR) Genes. – PLoS ONE 9(4): e94199.

—, VISGER, C. J., MANSION, G., HAnd, R., WU, H.-H., KAMARI, G., PHITOS, D. & CELLINESE, N. 2015: Evolution and biogeography of the endemic *Roucela* complex (Campanulaceae*: Campanula*) in the Eastern Mediterranean. – Ecol. Evol. 5: 5329-5343.

—, MILES, N. W., VISGER, C. J., HANSEN, K., AYERS, T., HABERLE, R. & CELLINESE, N. 2016: A global perspective on Campanulaceae: biogeographic, genomic, and floral evolution. – Amer. J. Bot. 103: 233-245.

—, MYERS, C. & CELLINESE, N. 2017: Embracing discordance: phylogenomic analyses provide evidence for allopolyploidy leading to cryptic diversity in a Mediterranean *Campanula* (Campanulaceae) clade. – Evolution 71: 913-922.

DAMBOLDT, J. 1976: Materials for a flora of Turkey 32: Campanulaceae. – Notes Roy. Bot. Gard. Edinburgh 35: 39-52.

— 1978: *Campanula* L. Pp. 2-64. In Davis, P. H. (ed.), Flora of Turkey and the East Aegean Islands, 6. – Edinburgh.

DAMBOLDT, J. & PHITOS, D. 1971: Reports. In Löve, Á. (ed.), IOPB Chromosome Number Reports XXXVI. – Taxon 20: 787.

DIMOPOULOS, P., RAUS, T., BERGMEIER, E. CONSTANTINIDIS, T., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. 2013: Vascular plants of Greece: An annotated checklist. – Englera 31.

—, RAUS, T. & STRID, A. 2018: Flora of Greece Web. Vascular plants of Greece: An annotated checklist. – Published on the Internet: http://portal. cybertaxonomy. org/flora-greece/content [accessed October 2018].

EDDIE, W. M. M. & INGROUILLE, W. J. 1999: Polymorphism in the Aegean “five-loculed” species of the genus *Campanula*, Section *Quinqueloculares* (Campanulaceae). – Nordic J. Bot. 19: 153-169.

—, SHULKINA, T., GASKIN, J., HABERLE, R. C. & JANSEN, R. K. 2003: Phylogeny of Campanulaceae s. str. Inferred from ITS sequences of nuclear ribosomal DNA. – Ann. Missouri Bot. Gard. 90: 554-575.

ENDLICHER, S. 1838: Genera plantarum. – Vindobonae.

ERBAR, C. & LEINS, P. 1989: On the early floral development and the mechanisms of secondary pollen presentation in *Campanula*, *Jasione* and *Lobelia*. – Bot. Jahrb. Syst. 111: 29-55.

— & — 1995: Portioned pollen release and the syndromes of secondary pollen presentation in the *Campanulales-Asterales*-complex. – Flora 190: 323-338.

FEDOROV, A. A. 1957: *Campanula* L. Pp. 126-450. In KOMAROV, A. L. (ed.), Flora SSSR*,* 24. – Moskva, Leningrad.

— & KOVANDA, M. 1976: *Campanula* L. Pp. 74-93. In TUTIN, T. G., BURGES, N. A., CHATER, A. O., EDMONDSON, J. R., HEYWOOD, V. H., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M. & WEBB, D. A. (eds), Flora Europaea, 4. – Cambridge.

FIORI, A. 1927: Nuova flora analitica d' Italia. – Firenze.

GADELLA, T. W. J. 1962: Some cytological observations in the genus *Campanula.* – Proc. Kon. Ned. Akad. Wetensch., C. 65: 269-278.

— 1963: Some cytological observations in the genus *Campanula.* II. – Proc. Kon. Ned. Akad. Wetensch., C. 66: 270-283.

— 1964: Cytotaxonomic studies in the genus *Campanula.* – Wentia 11: 1-104.

— 1966: Some notes on the delimitation of genera in the Campanulaceae. I, II. – Proc. Kon. Ned. Akad. Wetensch., C. 69: 502-521.

— 1967: The taxonomic significance of two artificially produced hybrids in the genus *Campanula.* – Acta Bot. Neerl. 15: 624-629.

GAGNIDZE, R. 2005: Vascular plants of Georgia. A nomenclatural checklist. – Tbilisi.

GRIFFITHS, H. I., KRYŠTUFEK, B. & REED, J. M. 2004: Balkan biodiversity: Pattern and process in the European hotspot. – Dordrecht.

HABERLE, R. C., DANG, A., LEE, T., PEÑAFLOR, C., CORTES-BURN, H., OESTREICH, A., RAUBENSON, L., CELLINESE, N., EDWARDS, E. J., KIM, S.-T., EDDIE, W. M. M. & JANSEN, R. K. 2009: Taxonomic and biogeographic implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. – Taxon 58: 715-734.

HAYEK, A. 1925: *Campanula* L. Pp. 328-391. In HEGI G. (ed.), Illustrierte flora von Mittel-Europa, 6. – Berlin.

— 1931: *Campanula* L. In Prodromus florae peninsulae Balcanicae, 2. – Repert. Spec. Nov. Regni Veg., Beih. 30(2): 522-548.

HOWELL, G. J., SLATER, A. T. & KNOX, R. B. 1993: Secondary pollen presentation in angiosperms and its biological significance. – Austral. J. Bot. 41: 417-438.

JANKOVIĆ, I., ŠATOVIĆ, Z., LIBER, Z., KUZMANOVIĆ, N., RADOSAVLJEVIĆ, I. & LAKUŠIĆ, D. 2016: Genetic diversity and morphological variability in the Balkan endemic *Campanula secundiflora* s. l. (Campanulaceae). – Bot. J. Linn. Soc. 180: 64-88.

JONES, K. E., KOROKTOVA, N., PETERSEN, J., HENNING, T., BORSCH, T. & KILIAN, N. 2017: Dynamic diversification history with rate upshifts in Holarctic bell-flowers (*Campanula* and allies). – Cladistics 33: 637-666.

KOVAČIĆ, S. 2004: The genus *Campanula* L. (Campanulaceae) in Croatia, circum-Adriatic and west Balkan region. – Acta Bot. Croat. 63: 171-202.

KYRIAKOPOULOS, C., LIVERI, E. & PHITOS, D. 2017: *Campanula kamariana* (section *Quinqueloculares*), a new species from S Peloponnisos, Greece. – Fl. Medit. 27: 131-135.

LAKUŠIĆ, D., LIBER, Z., NIKOLIĆ, T., SURINA, B., KOVAČIĆ, S., BOGDANOVIĆ, S. & STEFANOVIĆ, S. 2013: Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. – Taxon 62: 505-524.

LAMMERS, T. G. 2007a: Campanulaceae. Pp. 26-56. In KUBITZKI, K. J., KADEREIT, W. & JEFFREY, C. (eds), The families and genera of vascular plants. – Berlin.

— 2007b: World checklist and bibliography of Campanulaceae. – Kew.

ŁAŃCUCKA-ŚRODONIOWA, M. 1977: New herbs described from the Tertiary of Poland. – Acta Palaeobot. 18: 37-44.

— 1979: Macroscopic plant remains from the freshwater Miocene of the Nowy Sacz Basin (West Carpathians, Poland). – Acta Palaeobot. 20: 3-117.

LEINS, P. & ERBAR, C. 1990: On the mechanisms of secondary pollen presentation in the *Campanulales-Asterales* complex. – Bot. Acta 103: 87-92.

— & — 2006: Secondary pollen presentation syndromes of the *Asterales* - A phylogenetic perspective. – Bot. Jahrb. Syst. 127: 83-103.

LINNAEUS, C. 1753: Species plantarum. – Stockholm.

LUNDBERG, J. & BREMER, K. 2003: A phylogenetic study of the order *Asterales* using one morphological and three molecular data sets. – Int. J. Pl. Sci. 164: 553-578.

MANSION, G., PAROLLY, G., CROWL, A. A., MAVRODIEV, E., CELLINESE, N., OGANESIAN, M., FRAUNHOFER, K., KAMARI, G., PHITOS, D., HABERLE, R., AKAYDIN, G., IKINCI, N., RAUS, T. & BORSCH, T. 2012: How to handle speciose clades? mass taxon-sampling as a strategy towards illuminating the natural history of *Campanula* (Campanuloideae). – PLoS ONE 7(11): e50076.

MÉDAIL, F. & QUÉZEL, P. 1997: Hotspots analysis for conservation of plant biodiversity in the Mediterranean basin. – Ann. Missouri Bot. Gard. 84:112-127.

MYERS, N., MITTERMEIER, R. A., MITTERMEIER, C. G., FONSECA, G. A. DA & KENT, J. 2000: Biodiversity hotspots for conservation priorities. – Nature 403:853–858.

NEMCOK, M., POSPISIL, L., LEXA, J. & DONELICK, R. A. 1998: Tertiary subduction and slab break-off model of the Carpathian-Pannonian region. – Tectonophysics 295: 307-340.

OGANESIAN, M. 1995: Synopsis of Caucasian Campanulaceae. – Candollea 50: 275-308.

— 2008: Campanulaceae. Pp. 139-162. In TAKHTAJAN, A. L. (ed.), Caucasian Flora Conspectus. – S. Peterburg, Moskva.

PAPATSOU, S. & PHITOS, D. 1975: Two new taxa from eastern Aegean. – Notes Roy. Bot. Gard. Edinburgh 34: 203-204.

PARK, J.-M., KOVAČIĆ, S., LIBER, Z., EDDIE, W. M. & SCHNEEWEISS, G. M. 2006: Phylogeny and biogeography of isophyllous species of *Campanula* (Campanulaceae) in the Mediterranean area. – Syst. Bot. 31: 862-880.

PHITOS, D. 1963a: Beiträge zur Kenntnis der *Campanula rupestris*-Gruppe. – Phyton (Horn) 10: 124-127.

— 1963b: Eine neue art der gattung *Campanula* aus der Ägäis. – Mitt. Bot. Staatssamml. München 5: 121-124.

— 1964a: Trilokuläre *Campanula*-Arten der Ägäis. – Oesterr. Bot. *Z.* 111: 208-230.

— 1964b: Beiträge zur Kenntnis der südägäischen *Campanula*-Arten. – Ber. Deutsch. Bot. Ges. 77: 49-54.

— 1965: Die quinquelokulären *Campanula*-Arten. – Oesterr. Bot. Z. 112: 449-498.

— 1966: Die Gattung *Symphyandra* in der Ägäis. – Ber. Deutsch. Bot. Ges. 79: 246-249.

— 1969: Cytotaxonomical study of the *Campanula* species (subsection *Quinqueloculares*) in Greece. – Athens [in Greek].

— 2016: The section *Quinqueloculares* of the genus *Campanula* (Campanulaceae) and its polymorphism. Taxonomic review of the last decades. – Bot. Chron. 21: 91-102.

—, CONSTANTINIDIS, TH. & KAMARI, G. (eds) 2009: The Red Data Book of Rare and Threatened Plants of Greece, Vol. 1: A-D. – Hellenic Botanical Society, Patra [in Greek].

—, STRID, A. & SNOGERUP, S. 1997: Flora Hellenica, 1. – Königstein.

—, STRID, A., SNOGERUP, S. & GREUTER, W. (eds) 1995: The Red Data Book of Rare and Threatened Plants of Greece. – WWF for Nature, Athens.

— & KAMARI, G. 1984: Cytotaxonomical contributions on the Flora of Crete. II. – Candollea 39: 447-451.

— & — 1988: Contribution to the cytotaxonomy of the genus *Campanula* (Campanulaceae) in Greece. – Willdenowia 17: 103-105.

— & — 2009a: *Campanula merxmuelleri* Phitos. Pp. 185-186. In PHITOS, D., CONSTANTINIDIS, TH. & KAMARI, G. 2009: Rare and Threatened Plants of Greece, Vol. 1: A-D. – Hellenic Botanical Society, Patra [in Greek].

— & — 2009b: *Campanula reiseri* Halácsy. Pp. 192-194. In PHITOS, D., CONSTANTINIDIS, TH. & KAMARI, G. 2009: The Red Data Book of Rare and Threatened Plants of Greece, Vol. I: A-D. – Hellenic Botanical Society, Patra [in Greek].

QUEZEL, P. 1953: Les Campanulacées d'Afrique du Nord. – Feddes Repert. Spec. Nov. Regni Veg. 56: 1-65.

RADOSAVLJEVIĆ, I., JAKSE, J., SATOVIĆ, Z., JAVORNIK, B., JANKOVIĆ, I. & LIBER, Z. 2015: New microsatellite markers for *Campanula pyramidalis* (Campanulaceae) and cross-amplification in closely related species. – Appl. Pl. Sci. 3: 1400117.

RONIKIER, M., CIEŚLAK, E. & KORBECKA, G. 2008: High genetic differentiation in the alpine plant *Campanula alpine* Jacq. (Campanulaceae): Evidence for glacial survival in several Carpathian regions and long-term isolation between the Carpathians and the Alps. – Molec. Ecol. 17: 1763-1775.

— & ZALEWSKA-GALOSZ, J. 2014: Independent evolutionary history between the Balkan ranges and more northerly mountains in *Campanula alpina* s. l. (Campanulaceae): Genetic divergence and morphological segregation of taxa. – Taxon 63: 116-131.

ROQUET, C., SÁEZ, L., ALDASORO, J. J., SUSANNA, A., ALARCÓN, M. L. & GARCIA-JACAS, N. 2008: Natural delineation, molecular phylogeny and floral evolution in *Campanula* L. – Syst. Bot. 33: 203-217.

—, SANMARTÍN, I., GARCIA-JACAS, N., SÁEZ, L., SUSANNA, A., WIKSTRÖM, N. & ALDASORO, J. J. 2009: Reconstructing the history of Campanulaceae with a Bayesian approach to molecular dating and dispersal-vicariance analyses. – Molec. Phylogen. Evol. 52: 575-587.

ROUY, G. 1908: Flore de France, 10. – Paris.

RUNEMARK, H. & PHITOS, D. 1996: A contribution to the distribution of the genus *Campanula* section *Quinqueloculares* in Greece. – Bot. Chron. 12: 5-8.

SHETLER, S. G. 1963: A checklist and key to the species of *Campanula* native or commonly naturalized in North America. – Rhodora 65: 319-337.

STEFANOVIĆ, S., LAKUŠIĆ, D., KUZMINA, M., MEDEDOVIĆ, S., TAN, K. & STEFANOVIĆ, V. 2008: Molecular phylogeny of *Edraianthus* (Grassy Bells; Campanulaceae) based on non-coding plastid DNA sequences. – Taxon 57: 452-475.

SURINA, B., SCHÖNSWETTER, P. & SCHNEEWEISS, G. M. 2011: Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius,* Campanulaceae) within the Balkan refugium. – J. Biogeogr. 38: 1381-1393.

TANK, D. C. & DONOGHUE, M. J. 2010: Phylogeny and phylogenetic nomenclature of the *Campanulidae* based on an expanded sample of genes and taxa. – Syst. Bot. 35: 425-441.

TURLAND, N. & PHITOS, D. 2009: *Campanula saxatilis* L. subsp. *saxatilis*. Pp. 197-199. In PHITOS, D., CONSTANTINIDIS, TH. & KAMARI, G. (eds) 2009: The Red Data Book of Rare and Threatened Plants of Greece, Vol. I: A-D. – Hellenic Botanical Society, Patra [in Greek].

TUTIN, T. G., BURGES, N. A., CHATER, A. O., EDMONDSON, J. R., HEYWOOD, V. H., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M. & WEBB, D. A. (eds) 1976: Flora Europaea, 4. – University Press, Cambridge.

WILLKOMM, H. M. 1868: Campanulaceae. Pp. 279-298. In WILLKOMM, H. M. & LANGE, J., (eds), Prodromus florae hispanicae, 2. – Stuttgart.

YEO, P. F. 1993: Secondary pollen presentation. Form, function and evolution. – Wien.

YOO, K.-O., CROWL, A. A., KIM, K.-A., CHEON, K.-S. & Cellinese, N. 2018: Origins of East Asian Campanuloideae (Campanulaceae) diversity. – Molec. Phylogen. Evol. 127: 468-474.

Development of a flora database of Kefallinia island, Greece

MICHAIL XANTHAKIS

Abstract

Xanthakis, M. 2019: Development of a flora database of Kefallinia island, Greece. – Bot. Chron. 22: 223-231.

The flora of Kefallinia island has been well studied by scientists since the 1800s. In order to have a comprehensive database of the island's floral diversity, all the pertinent species records were collected, organized and analyzed through a database management software. The spatial distribution of each plant species on the island was mapped using record coordinates and GIS. A total of 12856 records were archived; they belong to 137 families, 636 genera and 1613 species from 315 locations on the island. This database could be utilized to protect Kefallinia's biodiversity and establish a thematic and informative ecotourism program in the island.

M. Xanthakis, Management Body of Mt. Aenos National Park, Koutavos Environmental Center, P.O. Box 222, GR-28100, Argostoli, Kefallinia island, Greece.
E-mail: ainosnationalpark@gmail.com

Key words: Ionian Islands, plant diversity, spatial distribution, vegetation data.

Introduction

Local and foreign botanists including HELDREICH (1882), BORNMULLER (1928), KNAPP (1964), PHITOS & DAMBOLDT (1985), PHITOS & al. (2004), EFTHIMIATOU-KATSOUNI (2006) and the FLORA IONICA WORKING GROUP (2016) have documented and studied the plants of Kefallinia. The island is known to have the highest diversity of plants among the Ionian Islands. In fact, about 1200 species of plants, many of which are rare and threatened, were mentioned by PHITOS & al. (2015) in Kefallinia. The island is indeed a prolific area for scientists to study floral diversity and for enthusiasts to enjoy nature.

The diversity of plants on the island may have been influenced by its geomorphology and variety of habitats. Successive cracks and earthquakes have shaped its geomorphology. The habitat types that harbor an abundance of plant species include fir forest, oakwood forest, artificial lakes and limestone rocky slopes, and the Mediterranean stony cliffs and gorges (KNAPP 1964).

Despite the numerous species records that had already been collected from Kefallinia, no inclusive database of all those records had been established yet. The purposes of this research were to collect, organize and analyze all those species records through a database management software and to study the spatial distribution of the species across the island through GIS.

Material and Methods

The bibliographic data containing the observations on Kefallinia's plants were extensively collected. These documentations were processed in the Microsoft Access Database Management program to create the appropriate queries. The records were categorized and systematized using the following information: the taxonomic family, genus, species and subspecies of each plant; its variety, junction and sex; and the serial number, observation site, projected site coordinates in EGSA87, observer's name, source and other details associated with each record. The scientific names of the plants were verified and updated through an annotated checklist of the vascular plants of Greece (Dimopoulos & al. 2013). The coordinates associated with the records were imported into the ESRI Geographic Information System (GIS) through the ArcGIS 10.2 program to map the spatial distribution of each plant species recorded on the island.

Results and Discussion

The database contains a total of 12856 records of plants comprising 137 families, 636 genera and 1613 species obtained from 315 different locations on the island. Among all these records, 12548 (97.60%) are angiosperms, whereas 203 (1.58%) are pteridophytes, and 105 (0.81%) are gymnosperms. The recorded angiosperms comprise 124 families (90.50%), 616 genera with 1,584 species and subspecies. The pteridophytes consist of 10 families (7.29%), 15 genera with 21 species and subspecies. The gymnosperms include 3 families (2.19%), 5 genera with 7 species and subspecies.

The angiosperm families having the most numerous records in the database are provided in Figure 1. From Figure 1 above, we note that the Fabaceae family has 1621 records, followed by the family, Poaceae with 1112, Orchidaceae with 691, Apocynaceae with 640, Asteraceae with 637, Caryophyllaceae with 586, Lamiaceae with 566, Apiaceae with 533 records, and so on.

The families with the greatest numbers of genera per family and species per family are illustrated in Figs 2 and 3, respectively. Next, the following Figure shows 15 plant families in descending order showing the largest number of different genera. From Fig. 2, we note that the Poaceae family has 62 different genera, the Acanthaceae and Apocynaceae families have 53, Brassicaceae 41, Apiaceae 40, Fabaceae 38, and so on. Fig. 3 shows the 15 families in descending order showing the largest number of different plant species. From the graph of Fig. 3, we note that the Fabaceae family includes 168 different species followed by the Poaceae family with 134, Apocynaceae with 83, Acanthaceae with 79, Apiaceae with 67, Asteraceae with 66, Orchidaceae with 62 and so on. The different genera and species having the largest aggregates of records per genus and records per species are specified in Figs 4 and 5. The following are presented in a chart, 15 genes with the largest number of records in the database.

From Fig. 4, we observe that the genus *Ophrys* L. has 383 records in the database, followed by *Trifolium* L. with 341, *Medicago* L. with 212, *Vicia* L. with 197, *Silene* L. with 186, *Euphorbia* L. with 179, *Lotus* L. with 138 and so on. We also give a graph with the first 15 species of plants in a number of records. From Fig. 5 we observe that *Ophrys lutea* Cav. has 78 records, *Ophrys fusca* Ten. and *Ophrys tenthredinifera* Willd. 44, *Crepis neglecta* M.Bieb. 41, *Anacamptis papilionacea* (L.) R.M.Bateman & Pridgeon & M.W.Chase, 40, *Colchicum cupanii* Guss. 37, *Ophrys ferrum-equinum* Desf. has also 37 records in the Kefallinia flora database, followed by *Quercus coccifera* L. with 37 and so on.

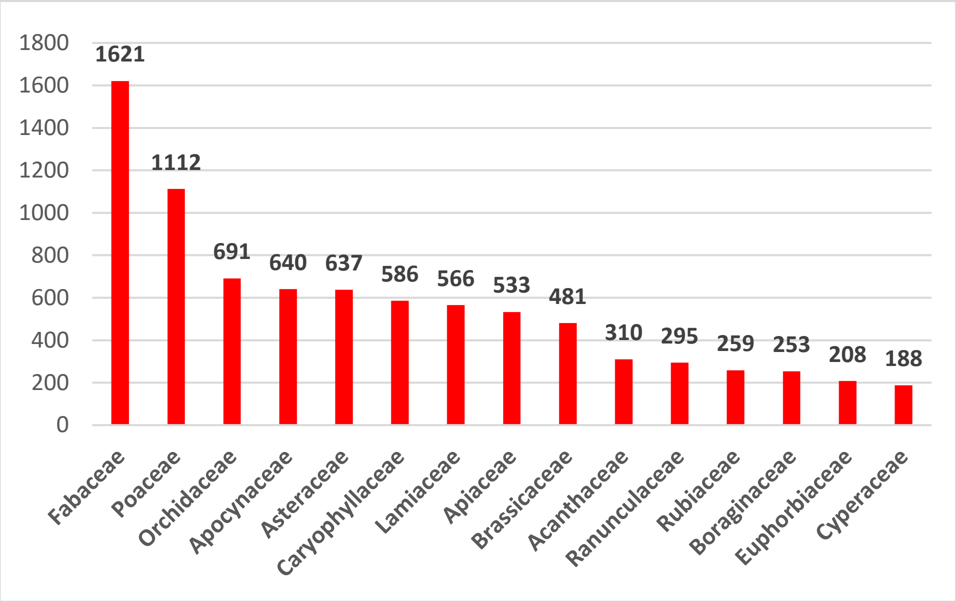


Fig. 1. The number of records per angiosperm family in the database.

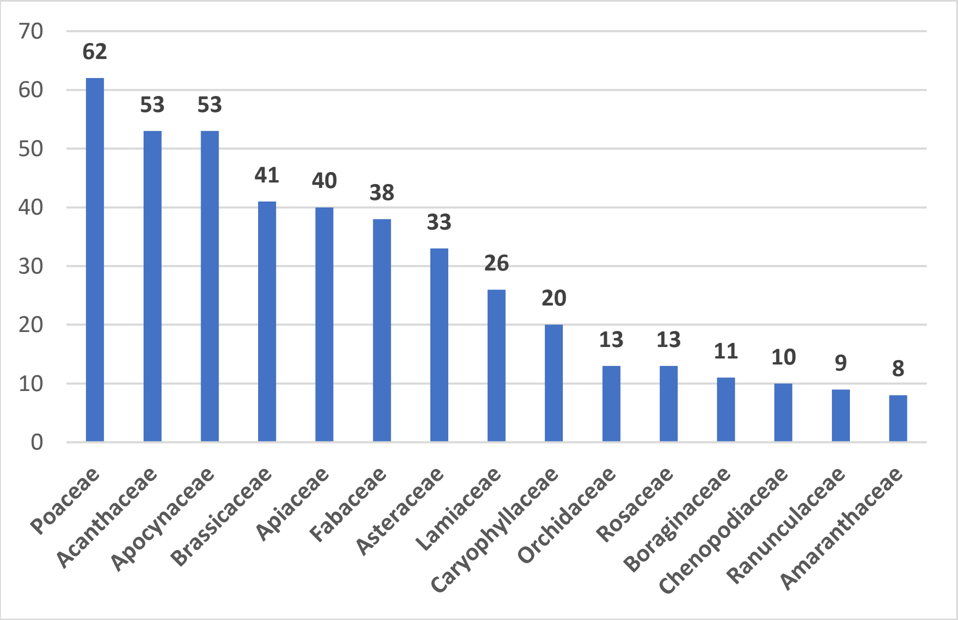


Fig. 2. The number of genera recorded per family in the database.

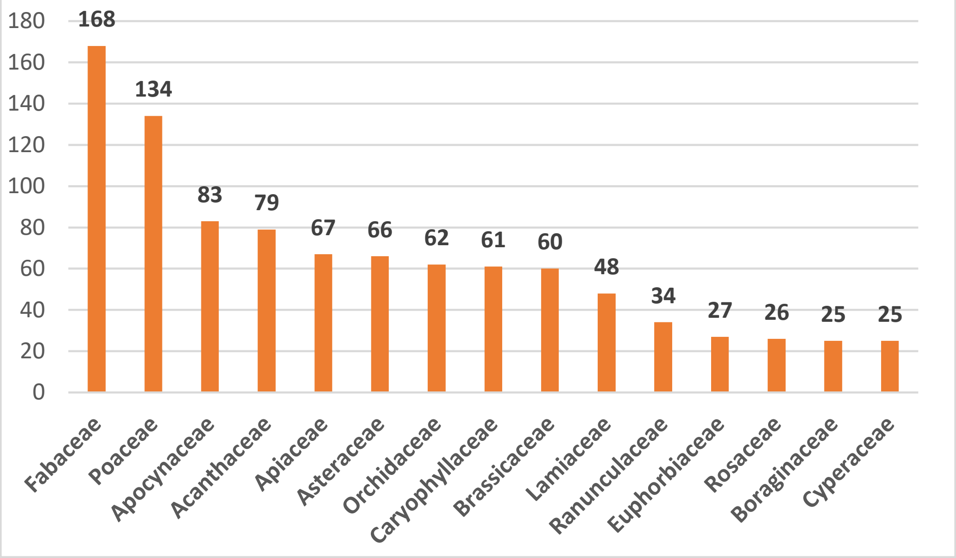


Fig. 3. The number of species recorded per family in the database.

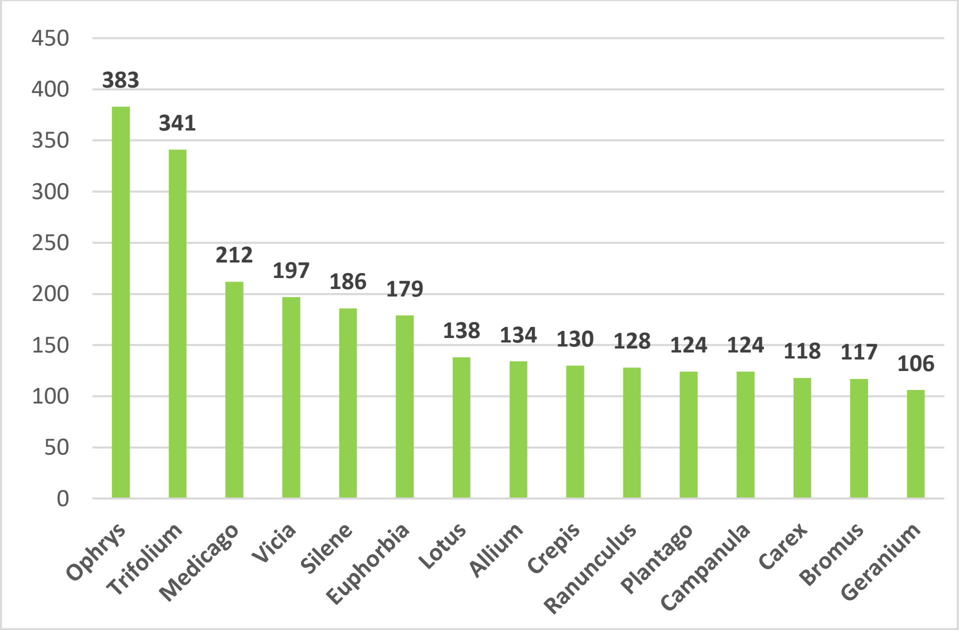


Fig. 4. The number of records obtained per genus in the database.

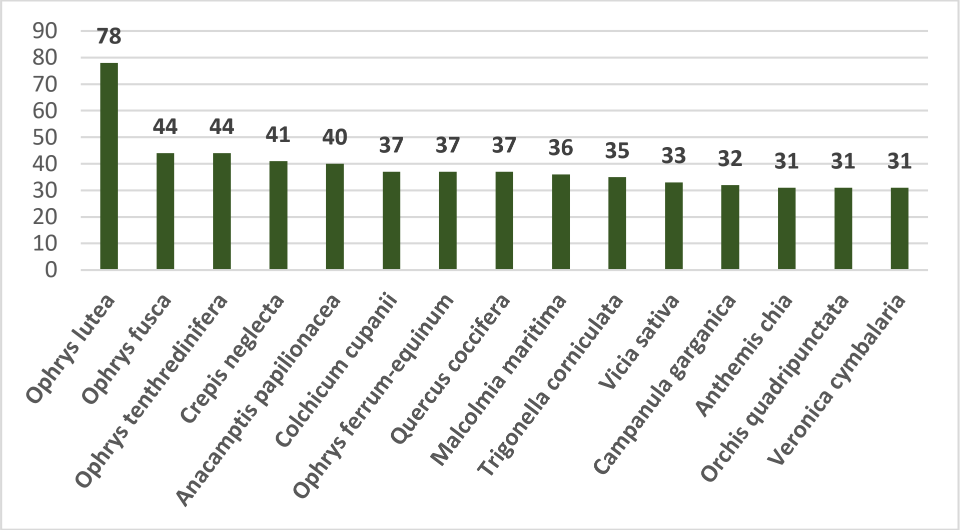


Fig. 5. The number of records obtained per species in the database.

The contributions of the individual and groups of scientists and researchers to the database in terms of their respective records are described in Fig. 6. The analysis of the flora species in the database (Fig. 6) showed that the Flora Ionica group (FI) had the most records with 8519 observations, Heldreich (H) had 1201, followed by the records of Phitos (Ph) with 928, Damboldt (D) with 413, Bornmüller (B) with 409, Baumann (Ba) with 241, Katsouni (Kats) with 229, Schmidt (Schm) with 118, Fischer (F) with 117, Unger (U) with 109, Snogerup (Sn) and Spreitzenhofer (Spr) with 88, Tzanoudakis with 50 records, Krendl (Kr) and Demut (D) with 38 and 30 records respectively.

The numbers of records obtained from the various study areas on the island are quantified per location in Fig. 7. Important in terms of the analysis of the observations is the site in Kefallinia, where the records of the flora species were carried out. Regarding the number of records per place is given the graph below. Based on Figure 7, we note that 1243 recordings were made in Argostoli, Mt. Aenos was 1181, in Sami 864, in Agios Nikolaos (Pyrgi) 653, in Fiskardo 609, in Kateleios 547 and so on.

The different areas on the island that harbor the most abundant families and species of plants are identified in Figs 8 and 9. Here is the graph with the number of plant families appearing at the observation collection sites. In the chart appear for supervisory reasons, the first 15 places with the largest number of plant families. From Fig. 8, we note that 106 families were recorded in Argostoli, followed by Agios Nikolaos (Pyrgi) with 97, Sami with 92, Kateleios with 90, Mt. Aenos and Fiskardo with 88, Lourdas with 87, Skala with 80 plant families and so on. Here is the graph with the number of species recorded in various places in Kefallinia, where for surveillance reasons they have been limited to the 15 most populated in number of species. From the results of Fig. 9, we conclude that 824 different plant species were recorded in Argostoli, followed by Mt. Aenos, with 699, Sami with 684, Agios Nikolaos (Pyrgi) with 631 species, Fiskardo with 572 species, Gerogobos with 537 species, Kateleios with 517 and so on

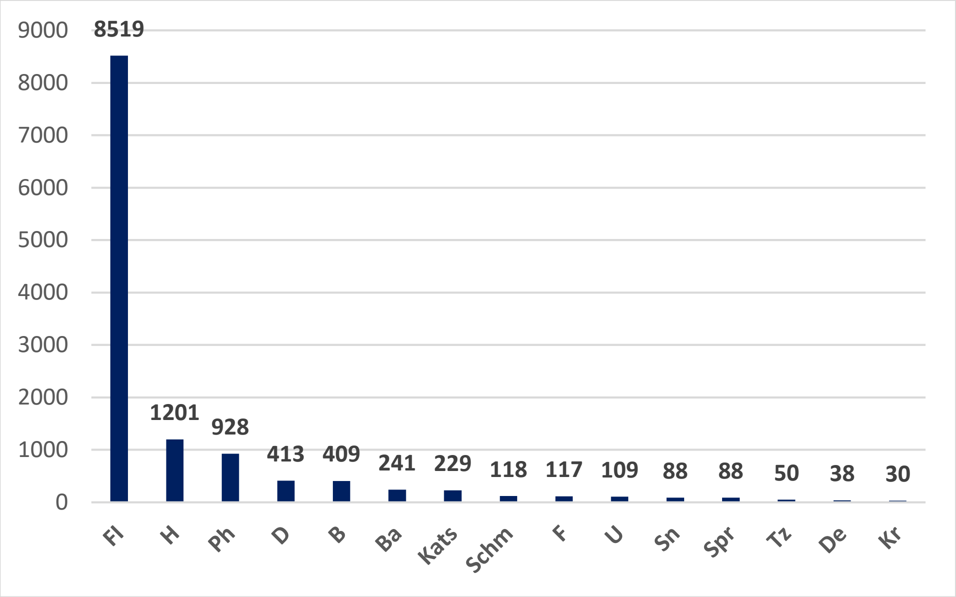


Fig. 6. The number of records obtained per scientist who contributed to the database.

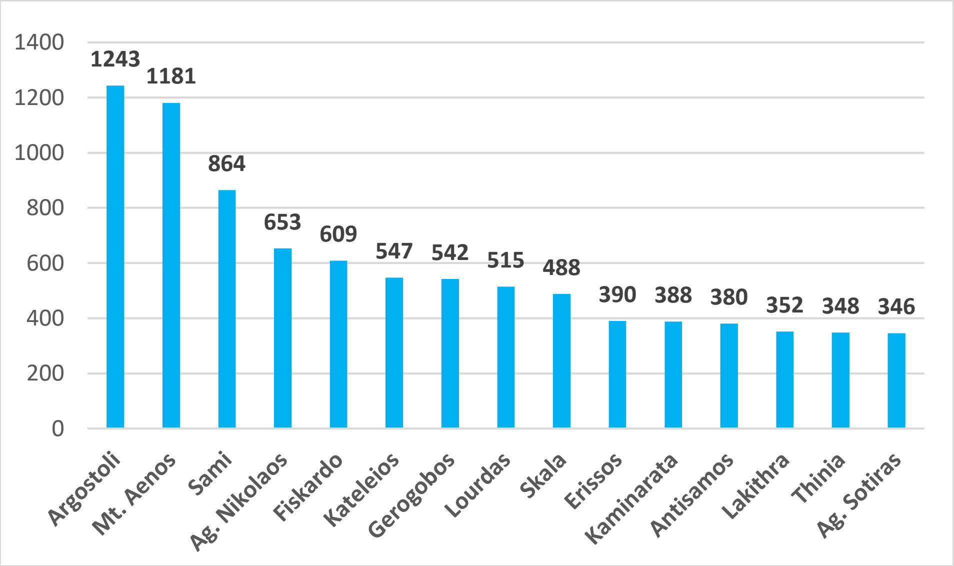


Fig. 7. The number of records obtained per area surveyed on the island Kefallinia.

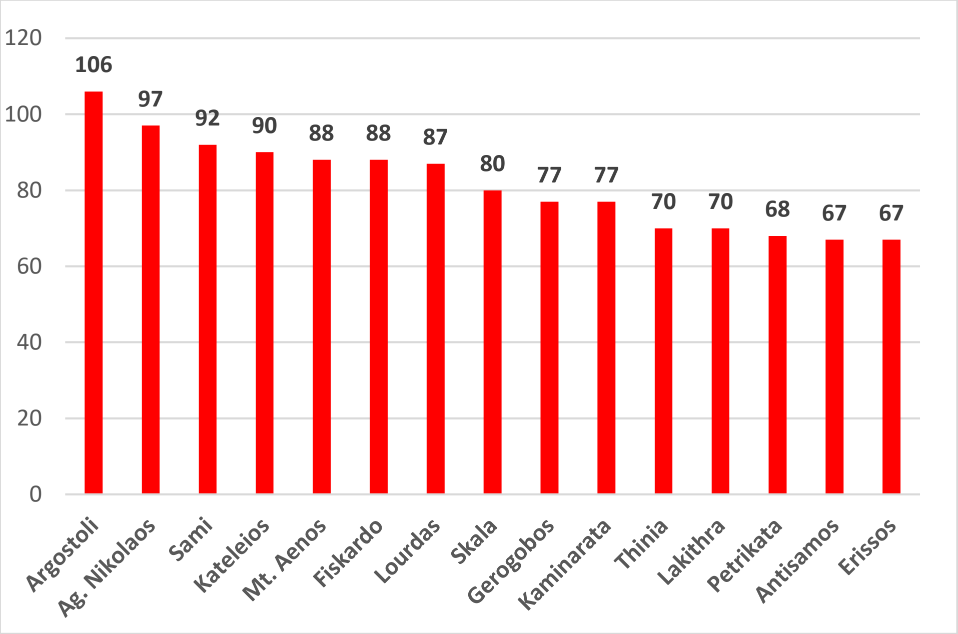


Fig. 8. The number of families per area surveyed on the island Kefallinia.

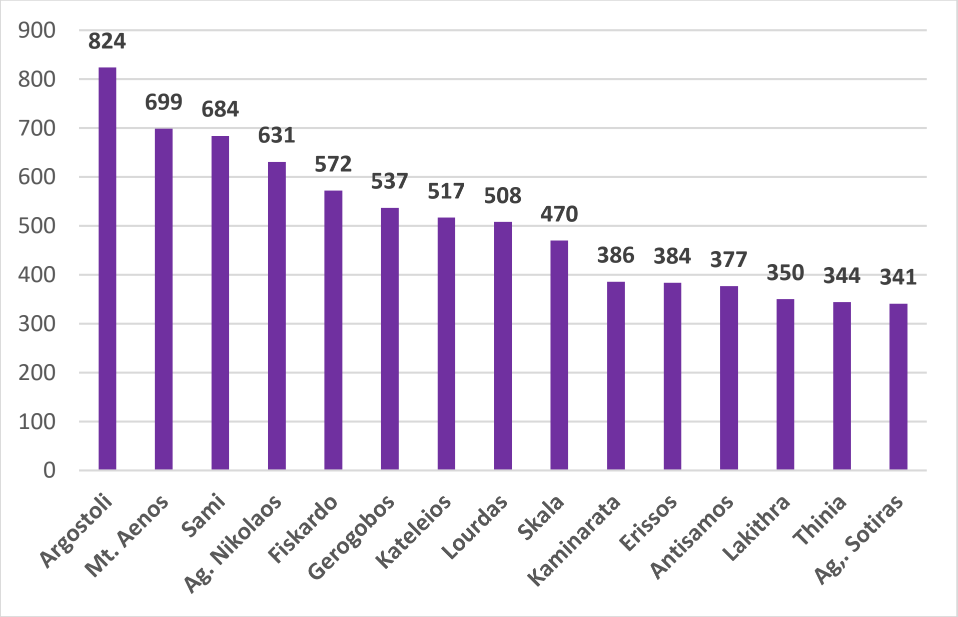


Fig. 9. The number of plant species per survey area on the island Kefallinia.

After the analysis of the records of the Flora species in Kefallinia island, the geographic information processing software (GIS) was introduced, so that for each species the spatial distribution in the island was mapped. The maps of the spatial distribution of two plant species, namely *Campanula garganica* subsp. *cephallenica* (Feer) Hayek and Fritillaria messanensis subsp. gracilis (Ebel) Rix are illustrated in Fig. 10.

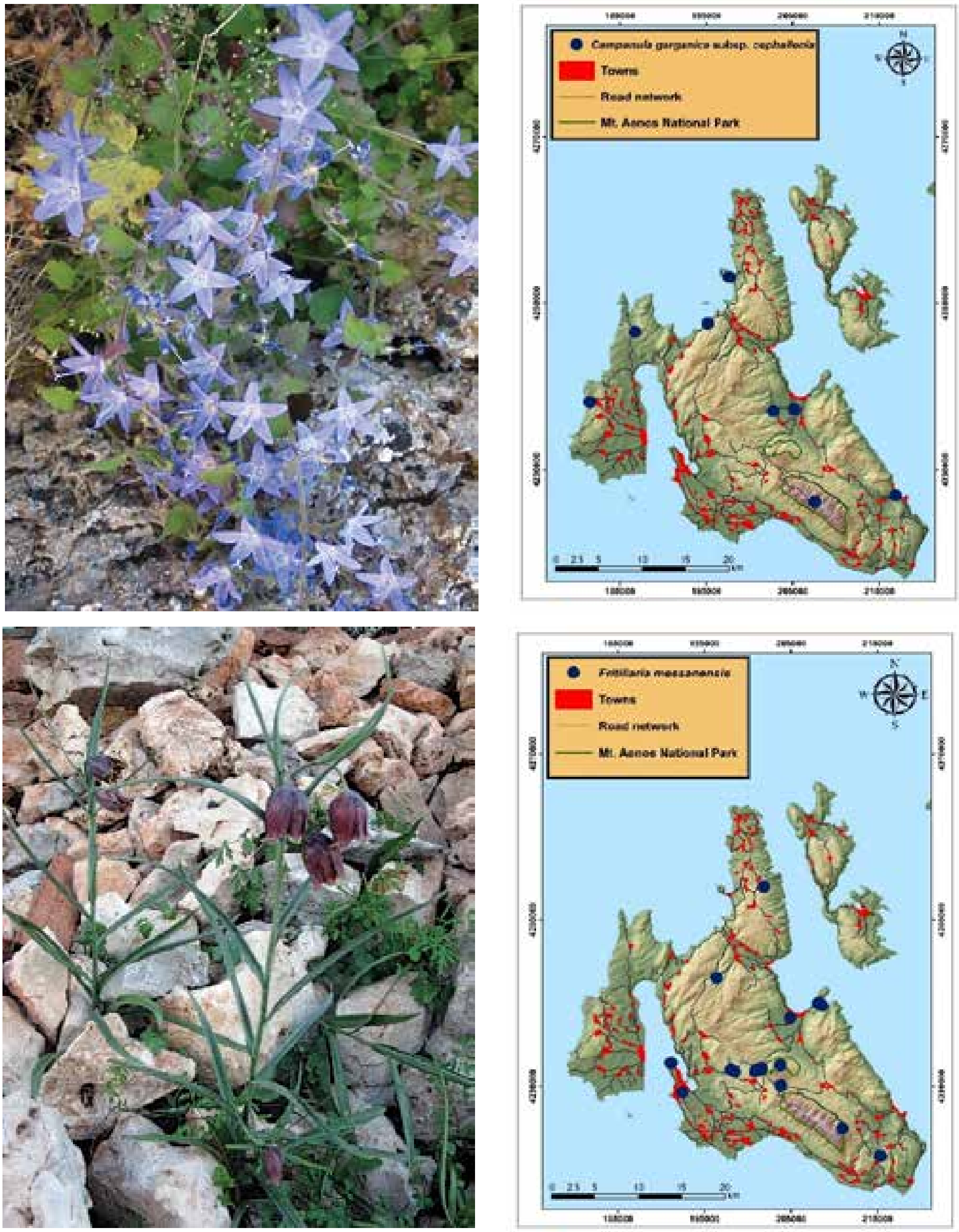


Fig. 10. Specimen photo and map of *Campanula garganica* subsp. *cephallenica* on Kefallinia island (above) and the same of *Fritillaria messanensis* subsp. *gracilis* (bottom).

Recommendations

The flora database established by this research for Kefallinia substantially contributes information to further protect and conserve the biodiversity of the island. Hence, it is recommended to utilize the database in the planning or enhancement of conservation strategies for the island. Moreover, the database could also be used to educate locals and tourists about the floral diversity of the island. Thus, it is also suggested that the database be considered in the formulation of protocols for a thematic and informative ecotourism program in the island.

Acknowledgements

This work was made possible with the help of Management Authority of Mt. Aenos National Park and funding from the Green Fund through its program titled: "Natural Environment-FDPP 2019". Special thanks are also due to the Emerita Professors of the University of Patras, Dimitrios Phitos and Georgia Kamari for their valuable advice and guidance.

References

BORNMÜLLER, J. 1928: Ergebnis einer botanischen Reise nach Griechenland im Jahre 1926 (Zante, Cephalonia, Achaia, Phokis, Aetolien). – Repert. Spec. Nov. Regni Veg. 25: 161- 203 & 270-350.

DIMOPOULOS, P., RAUS, TH., BERGMEIER, E., CONSTANTINIDIS, TH., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. (eds) 2013: Vascular plants of Greece: An annotated checklist. – Botanischer Garten und Botanisches Museum Berlin-Dahlem, Berlin & Hellenic Botanical Society, Athens. Accessed at http://portal.cybertaxonomy.org/flora-greece/intro on 14/03/2019.

EFTHIMIATOU-KATSOUNI, E. N. 2006: Contribution to the research of biodiversity of Cephalonia-Ithaca (Ionian Islands). – Master thesis. University of Ioannina, pp. 323 [in Greek with English summary].

FLORA IONICA WORKING GROUP, 2016: Flora Ionica – An inventory of ferns and flowering plants of the Ionian Islands (Greece). – Accessed at https://floraionica.univie.ac.at on 10/03/2019.

HELDREICH, TH. 1882: Flore de l'île de Céphalonie ou Catalogue des plantes qui croissent naturellement et se cultivent le plus fréquemment dans cette île, redigé – G. Bridel, Lausanne, pp. 90.

KNAPP, R. 1964: Die Vegetation von Kephallinia, Griechenland. Geobotanische Untersuchung eines mediterranen Gebietes und einige ihrer Anwendungs-Möglichkeiten in Wirtschaft und Landesplanung. – Geobot. Mitt. (Giessen) 29: 1-206.

PHITOS, D. & DAMBOLDT J. 1985: The flora of Cephalonia island. – Bot. Chron. 5(1-2): 1-204 + 2 maps [in Greek].

PHITOS, D., KATSOUNI, N. & KAMARI, G. 2004: Some interesting species from the flora of Kefallinia and Ithaki (Ionian Islands, Greece). – Bot. Chron. (Patras) 16: 97-101.

PHITOS, D., KAMARI, G., KATSOUNI, N. & MITSAINAS, G. (eds) 2015: Mt. Aenos of Cephalonia: History, Physiography, Biodiversity. – Katagramma - Graphic Arts, Patra, pp. 318.

Chorological additions for some noteworthy taxa of the Greek flora

ELEFTHERIOS KALPOUTZAKIS, THEOPHANIS CONSTANTINIDIS & PANAYIOTIS TRIGAS

Abstract

Kalpoutzakis, E., Constantinidis, Th. & Trigas, P. 2019: Chorological additions for some noteworthy taxa of the Greek flora. – Bot. Chron.22: 233-249

This contribution brings new distribution data for some rare, endemic or phytogeographically interesting members of the Greek flora, together with occasional taxonomic comments. The taxa include *Alyssum densistellatum*, *Chaerophyllum aureum*, *Conium maculatum, Consolida regalis* subsp. *paniculata*, *Coronilla valentina* subsp. *glauca, Euphorbia hypericifolia, Minuartia hamata, Myosurus minimus, Orobanche hederae, Quercus petraea* subsp. *polycarpa, Ranunculus bullatus* subsp. *cytheraeus,* and *Thlaspi arvense,* all of them newly found in, or confirmed for, given phytogeographical regions of Greece. Some finds (of *Anthyllis splendens, Maillea crypsoides*, *Malcolmia nana*, *Convolvulus pentapetaloides*, and *Vicia pubescens*) are new island records. New localities are reported for rare or local taxa: *Allium brussalisii, A. optimae*, *Asarum europaeum* subsp. *europaeum, Gymnospermiun peloponnesiacum, Helichrysum taenari*, *Nepeta orphanidea* var. *orphanidea, Paeonia mascula* subsp. *hellenica* and *Sibthorpia europaea*.

E. Kalpoutzakis, Division of Pharmacognocy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, 157 71 Athens, Greece.
E-mail: elkalp@pharm.uoa.gr
*Th. Constantinidis, Faculty of Biology, Department of Ecology and Systematics, National and Kapodistrian University of Athens, 157 03 Athens, Greece.
E-mail: constgr@biol.uoa.gr (*corresponding author).
P. Trigas, Laboratory of Systematic Botany, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece. E-mail: trigas@aua.gr

Key words: distribution, Evvia, floristics, Kiklades, Kriti, Peloponnisos, phytogeography, plant diversity, plant taxonomy.

Introduction

According to recent summarized data, the Greek territory presents a high floristic richness encompassing at least 6620 vascular plant taxa (species and subspecies) with an endemism rate of c. 22% (DIMOPOULOS & al. 2013, 2016). Apart from the overall taxonomic diversity in vascular plants, the distribution of taxa in different Greek regions is also of relevance, as it provides the basis for any phytogeographical analysis within Greece or including Greece. In this paper, we present new distribution data for some noteworthy members of the Greek flora, including several new regional records and recent confirmations of historical records. Moreover, new localities are provided for rare, endemic or threatened taxa, particularly those previously known from scant material. When necessary, taxonomic comments are added to certain species, to facilitate unambiguous identification.

Material and methods

Plant families (with the exception of Veronicaceae, here included in Plantaginaceae), genera and species names are according to Dimopoulos & al. (2013) and arranged in alphabetical order. Voucher specimens cited are kept in ATHU, unless a different herbarium is specified. The majority of new records come from south and central mainland or insular Greece. Notes are provided under each taxon.

Results and discussion

Alliaceae

Allium brussalisii Tzanoud. & Kypriot.

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: the NE slopes of Zipougias summit, SW of Sitena (37°17΄N, 22°38΄E), stony openings in *Abies cephalonica-Pinus nigra* forest, limestone, c.1000 m, 10.08.2009, *Kalpoutzakis & Trigas 2475* (ACA). Ibid.: W slope of Repa summit c. 5-5.5 km from Prastos towards Agios Vasilios village (37°14΄N, 22°40΄E), stony area in open *Abies cephalonica-Pinus nigra* forest with *Juniperus communis*, limestone, c.1100-1150 m, 23.08.2009, *Kalpoutzakis 2503.* Ibid.: NNE slope of Tripa summit, c. 0.7 km along a dead-end forest road (37°18΄N, 22°40΄E), *Abies cephalonica-Pinus nigra* forest, limestone, c.1260-1280 m, 23.08.2009, *Kalpoutzakis 2506*. Ibid.: NW slope of Tourla summit (east of Profitis Ilias summit), c. 1.5-2 km from the locality known as Kastanies Koukou towards Agios Vasilios village (37°18΄N, 22°39΄E), roadsides, *Abies cephalonica-Pinus nigra* forest, limestone, c.1250-1300 m, 23.08.2009, *Kalpoutzakis 2514*. Ibid.: c. 1.0 km from Agios Vasilios towards Prastos village (37° 11΄N, 22°41΄E), scrub with *Quercus coccifera*, *Genista acanthoclada* and scattered *Juniperus drupacea*, limestone, c. 885 m, 30.08.2009, *Kalpoutzakis 2567*. Ibid.: Mt. Madara, c. 3.4-4.1 km from Peleta towards Kremasti (37°02΄N, 22°53΄E), macchia with *Quercus coccifera*, *Phillyrea latifolia*, *Cotinus coggygria*, *Arbutus* sp., *Juniperus* sp., *Genista acanthoclada*, limestone, c. 800-900 m, 6.08.2010, *Kalpoutzakis 3674.* Ibid.: c. 4.2 km from Agios Andreas village towards Agios Georgios monastery, SE of Agios Andreas (37°19΄N, 22°47΄E), stony area, scrub with *Quercus coccifera*, *Phillyrea latifolia*, *Genista acanthoclada*, *Globularia alypum*, *Cistus salviifolius*, *Erica manipuliflora*, *Thymbra capitata*, limestone, c. 330 m, 17.09.2010, *Kalpoutzakis 3696* (with capsules and a few flowers). Ibid.: c. 11.7-11.8 km from Leonidion towards Vaskina (37°11΄N, 22°49΄E), stony places, macchia with *Quercus coccifera*, *Phillyrea latifolia*, *Cotinus coggygria*, *Arbutus unedo*, *A. adrachne*, *Pistacia terebinthus*, *Hypericum empetrifolium* subsp. *empetrifolium,* c. 857 m, 21.08.2011, *Kalpoutzakis 4083*. Ibid.: S slope of Fouskounia summit (37°14΄N, 22°39΄E), stony area, *Abies cephalonica-Pinus nigra* forest with *Quercus coccifera*, *Juniperus oxycedrus*, *Phillyrea latifolia*, limestone, c.1220-1250 m, 17.08.2012, *Kalpoutzakis 4265*. Ibid.: c. 6.4-8.4 km from Prastos towards Vaskina village (37°14΄N, 22°44΄E), stony places, scrub with *Quercus coccifera*, *Phillyrea latifolia*, *Acer sempervirens, Cotinus coggygria*, *Arbutus unedo*, limestone, c. 652 m, 18.08.2012, *Kalpoutzakis 4267* (at various places). Ibid.: NE slope of Tourkos summit (37°13΄N, 22°42΄E), stony and gravelly place, *Abies cephalonica forest* with scattered *Juniperus drupacea, J. oxycedrus, Quercus coccifera*, *Phillyrea latifolia,* limestone, c. 993 m, 27.08.2013, *Kalpoutzakis 4396*. Ibid.: SW slope of Mavrila summit (37°11΄N, 22°42΄E), stony and gravelly slope, openings in *Abies cephalonica-Juniperus drupacea* forest with scattered *Quercus coccifera and Phillyrea latifolia*, limestone, 1087 m, 29.08.2013, *Kalpoutzakis 4403*. Ibid.: c. 5.2 km from Kosmas towards Chouni village (37°05΄N, 22°45΄E), stony places, openings in *Abies cephalonica* forest, limestone, c. 850 m, 29.08.2013, *Kalpoutzakis 4409*. Ibid.: the NE slope of Kouniali summit NW of Prastos village (37°15΄N, 22°40΄E), stony slope, macchia with *Quercus coccifera, Acer sempervirens, Genista acanthoclada, Thymbra capitata* and scattered *Pinus nigra*, *Juniperus drupacea*, *J. oxycedrus*, limestone, c. 820-840 m, 21.08.2014, *Kalpoutzakis 4587*. Ibid.: W slope of Igoumenos summit (37°14΄N, 22°45΄E), stony slope, scrub with *Quercus coccifera, Cotinus coggygria, Genista acanthoclada, Thymbra capitata*, limestone, c. 1008 m, 28.08.2014, *Kalpoutzakis 4606*. Ibid.: the N-NW slope of Pefkovounia summit (37°18΄N, 22°37΄E), stony area, *Abies cephalonica-Pinus nigra* forest, limestone, c.1184 m, 19.08.2015, *Kalpoutzakis 4788.*

- Nomos Arkadias/Lakonias, Eparchia Kinourias/Epidavrou-Limiras, Mt. Madara, NW slopes of Troumbas summit (37°01΄N, 22°53΄E), stony places with *Abies cephalonica*, *Quercus coccifera*, *Phillyrea latifolia*, *Juniperus* sp., limestone, c.1000-1100 m, 10.08.2005, *Kalpoutzakis 1746.*

- Nomos Lakonias, Eparchia Lakedemonos, Mt. Parnonas, c. 5.8 km from Kallithea towards Agriani village (37°06΄N, 22°36΄E), remnants of burnt *Pinus nigra* forest with *Anthyllis hermanniae*, *Sarcopoterium spinosum*, flysh?, c. 930 m, 18.09.2010, *Kalpoutzakis 3716* (with capsules and a few flowers).

- Nomos Lakonias, Eparchia Epidavrou-Limiras, Mt. Kourkoula, c. 150 m SE and above of the chapel of Panagia Kourkoula (36°49΄N, 22°48΄E), stony area, macchia with *Quercus coccifera*, *Ceratonia siliqua*, *Arbutus unedo*, *Olea europaea* var. *oleaster*, *Salvia pomifera*, *Calicotome villosa*, *Genista acanthoclada*, *Anthyllis hermanniae*, *Cistus creticus*, *Thymbra capitata*, *Satureja thymbra*, marl and limestone, c. 470 m, 17.08.2012, *Kalpoutzakis* 4266 (cultivated material). Ibid.: Mt. Chionovouni, N slopes (36°58΄N, 22°54΄E), stony area, *Abies cephalonica* forest with a few *Juniperus oxycedrus*, *Acer sempervirens*, *Quercus coccifera* scrub, limestone, c. 900-1000 m, 19.08.2012 *Kalpoutzakis 4268*. Ibid.: Mt. Koulochera, the NE foothills, c. 2.3 km from Richea to Metamorphosis village (36°50΄N, 22°59΄E), stony slope, macchia with *Quercus coccifera, Phillyrea latifolia, Cotinus coggygria, Pistacia terebinthus, Globularia alypum, Thymbra capitata*, limestone, c. 625 m, 11.08.2015, *Kalpoutzakis 4766.*

Allium brussalisii was described from low to moderate altitudes of Mt. Parnitha near Athens (TZANOUDAKIS & KYPRIOTAKIS 2008) and has also been reported from Peloponnisos (DIMOPOULOS & al. 2013), apparently based on an unpublished collection from the Vouraikos gorge (N Peloponnisos) by D. Tzanoudakis. The species is characterized, among others, by its unilateral, entire (one-valved) or partially divided spathe not enclosing the base of the pedicels. Our collections, all from SE Peloponnisos, do not show significant differences from the plants of Mt. Parnitha. They usually (but not always) have more slender stems and occasionally fewer flowers per inflorescence. The plants of *Kalpoutzakis 2506* are very short but otherwise typical. The outer bulb tunics, in plants of *Kalpoutzakis 3716,* do not extend upwards to form a neck around the stem base; they may have been affected by fire in the area, three years prior to being collected. The single plant of *Kalpoutzakis 1746* has a spathe completely divided into two opposite valves.

The above cited collections (16 on Mt. Parnonas, 2 on Mt. Madara, 1 on Mt. Chionovouni, 1 on Mt. Kourkoula and 1 on Mt. Koulochera) indicate that the species is fairly common in the area and more populations presumably exist. Its flowering season is the same as on Parnitha, i.e., mainly August, rarely to mid-September. Population sizes vary from a few up to 200 plants, but on the north-east slopes of Tourla summit *(Kalpoutzakis 2506)* at least 500 plants were counted in August 2012. Our records at c. 1200-1300 m a.s.l. almost double the previously known altitudinal range of the species.

Allium optimae Greuter

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: c. 6-6.1 km from Agios Andreas towards Orino Korakovouni village (37°18΄N, 22°44΄E), macchia with *Quercus coccifera*, *Q. ilex, Arbutus unedo*, *Pistacia terebinthus, Acer sempervirens*, limestone, c. 238 m, 16.05.2014, *Kalpoutzakis 4455* (ACA). Ibid.: the ravine of Leonidio, place known as Makria Plevra, c. 3.2-3.3 km from Leonidio towards Vaskina village (37°10΄N, 22°50΄E), macchia with *Quercus coccifera*, *Pistacia lentiscus, P. terebinthus, Cercis siliquastrum, Calicotome villosa*, stony slope, limestone, c. 338 m, 24.05.2014, *Kalpoutzakis 4459*. Ibid.: the edges of the plateau west of the Mavrila summit (37°12΄N, 22°42΄E), sparse *Abies cephalonica* and *Juniperus drupacea* trees, limestone, c. 945 m, 12.06.2014, *Kalpoutzakis 4471* (cultivated). Ibid.: c. 7.8 km from Leonidio towards Tsitalia village (37°08΄N, 22°51΄E), macchia with *Quercus coccifera*, *Pistacia lentiscus, Phillyrea latifolia*, stony place, limestone, c. 468 m, 03.05.2016 & 18.06.2016, *Kalpoutzakis* (photos!).

This recently described species is a local endemic of the eastern coast of the Maleas Peninsula, previously known only from two neighbouring localities: the bay of Palea Monemvasia (*locus classicus*) and the bay of Limin Jerakas, at 0-10 m a.s.l. (GREUTER 2012). It is closely related to *Allium pallens* L., a species widely distributed throughout the Mediterranean region, from Spain and Morocco eastwards to the Middle East (BRULLO & al. 2003). *Allium optimae* seems to be taxonomically distinct from *A. pallens*, although the morphological features provided in its original description (stamens slightly exerted from the perigon or subequal to the tepals, and spathe valves abruptly contracted into a slender apical appendage) are rather variable and are shared by both species. *Allium optimae*, however, has shorter tepals and a different leaf shape: the lamina is flat, with three prominent, acute ribs along the abaxial surface, whereas in *A. pallens* it is semicylindrical and lacks prominent ribs. Our collections extend the spatial and altitudinal distribution range of *A. optimae*. About 20-100 individuals were counted in each of the Parnonas localities.

Apiaceae

Chaerophyllum aureum L.

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: c. 9 km from Kastanitsa on the way to Stravorachi-Agios Petros (37°13΄N, 22°36΄E), moist place close to a spring, opening in *Abies cephalonica-Pinus nigra* forest, schist, c. 1440 m, 15.06.2014, *Constantinidis & Kalpoutzakis s.n.* (in flower). Ibid:. 14.07.2014, *Kalpoutzakis 4488* (in fruit).

- Nomos Lakonias, Eparchia Lakedemonos, Mt. Parnonas: c. 0.5 km NE of Polidroso (Tzitzina) (37°10΄N, 22°36΄E), on a stream bank, *Abies cephalonica* forest, limestone, c. 1050 m, 12.08.2013, *Kalpoutzakis 4370* (in fruit); c. 3.8-4.2 km from Stravorachi locality, on the way to Vamvakou (37°12΄N, 22°35΄E), roadside, *Abies cephalonica-Pinus nigra* forest, limestone, c. 1265 m, 12.08.2013, *Kalpoutzakis 4376* (in fruit); ibid. 21.07.2014, *Kalpoutzakis 4541* (in fruit).

A new record for the flora of Peloponnisos. All new localities are in rather damp places of forest clearings and on shaded roadsides. The species is local, but occasionally predominates due to its tall habit.

Conium maculatum L.

- Nomos Evvias, Eparchia Chalkidos, Evvia island: c. 9 km from Nea Artaki towards Katheni (38°32΄N, 23°43΄E, c. 120 m, edges of cultivated fields and ditches, 24.05.2015, *Kalpoutzakis* (photos!).

New for Evvia island and the WAe phytogeographical region. This widespread Eurasian species has a scattered distribution in mainland Greece, but is quite rare in the Aegean region, hitherto only known from Kriti, Kos, Lesvos and Samothraki islands (STRID 2016). The species is distinct and difficult to confuse with any other Greek umbel, the only exception being *C. divaricatum* Boiss. & Orph., which usually lacks purple spots on the stem and has 0-1 involucral bracts rather than 3-5, as in *C. maculatum* (LEUTE 1971). More than 30 flowering individuals were observed in the single known Evvia locality.

Aristolochiaceae

Asarum europaeum L. subsp. ***europaeum***

- Nomos Pellis, Eparchia Almopias: c. 27.5 km N of Vorinon village along the forest road, place known as Sfagion (41°07′N, 22°04′E), a stream in *Fagus* forest, apparently granite, c. 1350 m, 13.08.2002, *Constantinidis & Evergetis 10422*.

Very rare in Greece. To our knowledge, there is only one confirmed record of the species from the Greek territory, that on Mt. Varnous, the summit of Peneriga, at 2090-2110 m (Greuter *& Zimmer 23727,* see SNOGERUP 1997). Our new collection, between the mountains of Voras and Pinovon, represents a second Greek population, discovered within the same phytogeographical area. Older literature records (e.g. Mt. Athos, ZAGANIARIS 1940; Attiki, SARLIS 1994) are dubious or definitely erroneous. A recent report from *Castanea* forests on Mt. Vermio (KONSTANTINIDIS & al. 2008) should be considered with care in the absence of a herbarium voucher, particularly since *Asarum* does not appear in the detailed floristic study of the mountain by CHOCHLIOUROS (2005).

Our specimens were found in a habitat typical for *Asarum europaeum*, viz., shady and damp stream banks in deciduous beech forest, and were not in flower when collected. However, their identity is unquestionable and was further confirmed by the distinct, pungent, ginger-like smell of the fresh rhizome. The habitat of the previously known population is completely different: grazed slopes above the timberline, where the plants grow hidden in the shelter of prostrate shrubs of *Juniperus communis* subsp. *nana* (W. GREUTER, pers. comm.).

Asteraceae

Helichrysum taenari Rothm. — Fig. 1B.

- Nomos Lakonias, Eparchia Epidavrou-Limiras: the promontory of Maleas, SE of Agios Mironas settlement, rocks, cliffs and stony places of the Likogremos, Chounares and Vardia hills (36°27′N, 23°10′E), limestone, c. 350-420 m, 08.06.2008, *Constantinidis & Kalpoutzakis 12061*. - Ibid.: the south-easternmost parts of Maleas promontory, around and to the NW of the lighthouse (36°27′N, 23°11′E), phrygana along the path, limestone rocks and cliffs, c. 120-160 m, 12.06.2011, *Constantinidis & Kalpoutzakis 12745*. - Ibid.: Maleas promontory, southernmost parts, a ravine c. 1.2 km WNW of the Agia Irini chapel (36°26′N, 23°10′E), rocky places and cliffs close to locality known as Varoulko, c. 10-30 m, 13.06.2011, *Constantinidis & Kalpoutzakis* obs.

Fig. 1. A, *Ranunculus bullatus* subsp. *cytheraeus* from Milos island; B, *Helichrysum taenari* from the Maleas promontory; C, *Orobanche hederae* from Platanenhof (Evvia island) and D, *Cuscuta monogyna* from Mt. Gaidourovouni.

These are three populations of a very local Greek endemic, previously thought to be restricted to the Tenaron promontory. The Maleas populations were mentioned for the first time by KOKMOTOS & al. (2009) but no exact localities were provided. The populations sampled or observed in 2011 were unknown at that time.

Berberidaceae

Gymnospermium peloponnesiacum (Phitos) Strid — Fig. 2A.
- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: Koromilia summit SSW of Kosmas village (37°03΄N, 24°45΄E), stony slope with scattered *Juniperus drupacea* trees, thick fertile substrate and gravel on limestone, c. 1480 m, 26.03.2016, *Kalpoutzakis 4840.*

This new locality forms the southernmost distribution border of the rare *Gymnospermiun peloponnesiacum*, endemic to Peloponnisos. The species was reported from Parnonas in CONSTANTINIDIS & KALPOUTZAKIS (2015), but no locality details were provided and no flowering material was available at that time. The population is small and may not exceed 100 mature individuals. For details of the species' total distribution, see KARL & STRID (2009).

Brassicaceae

Alyssum densistellatum T. R. Dudley
- Nomos Viotias, Eparchia Thivon: c. 2.6 km NNE of the village of Pavlos, along a secondary road that leads to Tragana (38°32΄N, 23°06΄E), open hills with sparse vegetation composed mainly of scrub (*Quercus coccifera*, *Genista acanthoclada*, etc.) on serpentine, 200-230 m, 04.05.2003, *Constantinidis & Saitanis 10573*.

New for the Greek mainland. This is an interesting record of an otherwise local insular serpentine endemic, previously thought to be restricted to central and northern Evvia Island (HARTVIG 2002, TRIGAS 2003, STRID 2016). The species is related to the variable *Alyssum montanum* L. and presumably comes closest to subsp. *montanum*, from which it differs in a number of fine but apparently constant morphological features (see STRID 2016). The discovered population on the serpentine outcrops between Pavlos and Tragana is the only one known so far from the Greek mainland. *A. montanum* itself also grows on the Tragana serpentine; yet, the two species remain distinct. We presume that additional populations of *A. densistellatum* will be found in the serpentine localities of Nomos Viotias and Nomos Fthiotidos, by a careful search.

Malcolmia nana (DC.) Boiss.
- Nomos Kikladon, Eparchia Milou, Milos island: Chivadolimni (36°41΄N, 24°26΄E), sandy beach by the lake, c. 5 m, 18.04.2015, *Trigas & Kougioumoutzis 5868* (ACA).

New for Milos island. *Malcolmia nana* has a wide but scattered distribution in the Mediterranean area. It has been recorded in a few localities in the Aegean area. There is only one previously known locality in the Kiklades, on the island of Naxos (STRID 2016).

Thlaspi arvense L.
- Nomos Lakonias, Eparchia Lakedemonos, Mt. Parnonas: the S slope of Profitis Ilias summit, c. 5.5 km along the dead-end forest road that leads to the summit (37°07΄N, 22°38΄E), stony slope

Fig. 2. A, *Gymnospermium peloponnesiacum* from Koromilia peak of Mt. Parnonas; B, *Myosurus minimus* from Mikrothives; C, *Nepeta orphanidea* var. *orphanidea* from Megali Tourla summit of Mt. Parnonas; D, *Nepeta orphanidea* var. *parnidea* from the same mountain.

with scattered *Abies cephalonica*, *Juniperus drupacea,* limestone, c. 1590 m, 17.06.2012, *Kalpoutzakis 4197*.

New for Peloponnisos and S Greece. *Thlaspi arvense* is a rather rare species in Greece, known from a few localities mostly in the northernmost part of the country (ARTELARI 2002). However, the species sometimes grows in ruderal habitats or arable fields, and therefore our collection on Parnonas may represent a recent introduction, presumably following transport of animal feed from the north.

Caryophyllaceae

Minuartia hamata (Hausskn. & Bornm.) Mattf.

- Nomos Rethimnis, Eparchia Milopotamou, Mt. Psiloritis (Idi), Kriti island: S slope of Skinakas summit, close to the observatory (35°12΄N, 24°53΄E), stony area with scattered *Juniperus oxycedrus* subsp. *deltoides, Berberis cretica*, *Prunus prostrata* and *Astragalus angustifolius* subsp. *echinoides,* 1650-1730 m, 05.06.2009, *Kalpoutzakis 2337*.

- Nomos Magnisias, Eparchia Almirou: c. 6.5 km NNE of Anavra village (38°07΄N, 22°34΄E), quarry surrounded by *Quercus* woodland, 980 m, 11.05.2011, *Trigas & Karetsos 4907* (ACA).

New for Kriti and the Kriti-Karpathos phytogeographical region (KK), as well as for the East-Central region (EC) of Greece. The distribution of *Minuartia hamata* is widely disjunct, in the Iberian and the Balkan Peninsulas eastwards to Iran (KAMARI 1997). It is widely distributed in the mountains of mainland Greece, but is almost absent from the Aegean Islands, where it has been recorded only from Mt. Oros on Chios (SNOGERUP & al. 2001). The record from Mt. Psiloritis is the first for the south Aegean area. About 30 individuals were observed; the species seems to be particularly rare in this area.

Convolvulaceae

Convolvulus pentapetaloides L.

- Nomos Attikis, Eparchia Idras, Idra island: the monastery of Theotokou Zourvas in the easternmost part of the island (37°21΄N, 23°32΄E), abandoned fields with phrygana, c. 170 m, 19.03.2014, *Trigas & Valli 5767* (ACA).

New for Idra and the Myrtoon Sea islands. This widespread Mediterranean annual has a scattered distribution in Greece, mainly confined to the Aegean (STRID 2016). Only a few individuals were observed on Idra. The species is probably more common in Greece than is known, but it is underrepresented in the floristic record, due to its small size and early flowering period.

Crassulaceae

Rosularia serrata (L.) A. Berger

- Nomos Lakonias, Eparchia Epidavrou-Limiras: the south-easternmost parts of Maleas promontory, around and to the NW of the lighthouse (36°27΄N, 23°11΄E), phrygana along the path, limestone rocks and cliffs, c. 120-160 m, 12.06.2011, *Constantinidis & Kalpoutzakis 12749*.

This record is the first for mainland Peloponnisos (Pe). Previous records from Pe concern the islands of Kithira (GREUTER & RECHINGER 1967) and Antikithira (STRID

2016); the species was thought to be absent from the mainland. About 10 small plant clusters were seen growing in rock fissures and ledges, c. 0.5 km NW of the Maleas lighthouse. It may well be more widespread in the area.

Cuscutaceae

Cuscuta monogyna Vahl – Fig. 1D.

- Nomos Lakonias, Eparchia Epidavrou-Limiras, NE slopes of Mt. Gaidourovouni (36°55΄N, 22°54΄E), rather open macchia vegetation with *Arbutus andrachne*, *Cotinus coggygria*, *Quercus coccifera*, *Phillyrea latifolia*, *Fraxinus ornus*, *Globularia alypum*, *Phlomis cretica*, *Asperula elonea*, *Teucrium capitatum* and *Hypericum empetrifolium* subsp. *empetrifolium*, limestone, c. 800-860 m, 30.07.2011, *Constantinidis & Kalpoutzakis 12757*.

A widespread species that is rare in Greece, predominantly parasitic on trees and shrubs. Already collected on Mt. Malevo (Mt. Parnonas, Peloponnisos) by Orphanides (HALÁCSY 1902), but later reported as absent (STRID & STRID 2010), doubtfully present (DIMOPOULOS & al. 2013) and present (STRID 2016) in Peloponnisos. At the Gaidourovouni locality it was parasitising *Cotinus coggygria, Arbutus andrachne*, and also *Asperula elonea* and *Hypericum empetrifolium* subsp. *empetrifolium*. The species, readily recognised by its elongate inflorescences, develops mainly in summertime.

Euphorbiaceae

Euphorbia hypericifolia L.

- Nomos Attikis, Eparchia Attikis: Kallithea, at the intersection of Sokratous and Agion Pandon streets (37°57΄N, 23°42΄E), roadsides, 35 m, 06.08. 2016, *Kalpoutzakis 4928*.

- Nomos Attikis, Eparchia Pireos: small port of Keratsini, close to the abandoned power plant (37°57΄N, 23°36΄E), flower beds by the port, 3 m, 29.11.2013, *Kalpoutzakis* (*photos*!).

The collection and documentation of the allochthonous *Euphorbia hypericifolia* (*Chamaesyce hypericifolia* (L.) Millsp.) in Athens and the suburbs of Pireas constitute a first record for Sterea Ellas. The species is a widespread xenophyte outside of its native area, the tropical and subtropical regions of the New World, currently expanding its range to areas of Africa, Asia and Europe (see SCIANDRELLO & al. 2016). It belongs to a group of similar-looking and taxonomically related species of *Euphorbia* subsect. *Hypericifoliae* Boiss. (YANG & al. 2012) that comprises *E. hypericifolia, E. hyssopifolia* L., *E. nutans* Lag., *E. glomerifera* (Millsp.) L. C. Wheeler, *E. parviflora* L., and a few other New World species (see BURCH 1965, HÜGIN 1998, MARQUES DA SILVA & al. 2014). Misidentifications occur occasionally within this group. Our plants are distinguished from their relatives by a combination of characters that include erect or erect-ascending habit, glabrous stems and leaves, triangular, 1-1.5 mm long stipules, a ± compact, glomerulate dichasial inflorescence with a few basal leaves, stipule-like bracts at the junction of the involucre with the pedicels, glabrous subspherical, 1- 1.3 mm long and 1.2-1.5 mm wide capsules, and short seeds (< 1 mm) with a few, faint transverse ridges.

There are some recent records of *E. hypericifolia* for Greece, based on specimens from Kriti (GREGOR & MEIEROTT 2013) and N Peloponnisos (SUTORÝ & VERLOOVE 2015). Few plants were seen in Athens and Piraeas (< 20 individuals per locality), growing either on roadsides or in irrigated flower beds together with other synanthropic species. It is not clear yet if they represent casual introductions or established and perhaps expanding populations.

Fabaceaee

Anthyllis splendens Willd.

- Nomos Kikladon, Eparchia Milou, SW part of Sikinos island, around the chapel of Agia Marina, SSW of the ancient Episkopi monument (36°39′N, 25°05′E), rocky slopes and cliffs, scree and stony places with low vegetation (*Pistacia lentiscus*, *Sarcopoterium spinosum*) on limestone, 380-420 m, 13.04.2014, *Constantinidis 13355*.

Sikinos is a new island record for this rare Aegean endemic, previously known from Kithnos, Amorgos, Folegandros, Anafi and from a single locality in NE Kriti (STRID 2016). The species usually is a chasmophyte of steep rocks or inaccessible cliffs. The Sikinos population inhabits a cliff facing NW and is associated with two other Aegean endemics: *Campanula heterophylla* L. and *Pimpinella pretenderis* (Heldr.) Halácsy.

Coronilla valentina subsp. ***glauca*** (L.) Batt.

- Nomos Arkadias, Eparchia Kinourias, E slopes of Mt. Parnonas, c. 4.0-4.6 km from Leonidio on the way to Tiros (37°10′N, 22°53′E), macchia with *Pistacia lentiscus* and *Calicotome villosa*, stony places, limestone, c. 35 m, 06.06.2017, *Kalpoutzakis 4951,* and 25.01.2018, *Kalpoutzakis 4970*. Ibid.: c. 0.85 km ENE of Pragmateftis village (37°11′N, 22°54′E), macchia with *Pistacia lentiscus* and *Calicotome villosa* in a stony area, limestone, c. 60 m, 25.01.2018, *Kalpoutzakis 4971*.

The above specimens confirm the occurrence of this taxon in Peloponnisos. The plant is not reported by DIMOPOULOS & al. (2013, 2016) and not given for Pe in the electronic maps of The Flora of Greece Web (http://portal.cybertaxonomy.org/flora-greece/cdm_dataportal/taxon/646e4e78-bb03-40e5-aa03-c2e47525dc41). STRASSER (1997) includes this subspecies but without further details.

Vicia pubescens (DC.) Link

- Nomos Kikladon, Eparchia Milou, Milos Island: Ageria area in the NE part of the island (36°44′N, 24°31′E), grassy places by a small stream, c. 75 m, 17.04.2015, *Trigas & Kougioumoutzis 5847* (ACA).

New for Milos island and the West Kiklades. This widespread Mediterranean species, quite common in the Aegean, is absent from several central Aegean Islands, according to STRID (2016).

Fagaceae

Quercus petraea subsp. ***polycarpa*** (Schur) Soó,

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: NE slope of Kouniali peak (37°15′N, 22°40′E), sparse *Abies cephalonica* woodland with *Pinus nigra* subsp. *nigra*, *Juniperus oxycedrus, J. drupacea*, *Acer sempervirens*, etc., c. 825 m, 26.08.2014, *Kalpoutzakis 4597* (ACA). Ibid.: between Igoumenos and Oriontas peaks (37°14′N, 22°45′E), sparse macchia on limestone, c. 1100 m, 28.08.2014, *Kalpoutzakis 4604* (ACA). Ibid.: E slope of Oriontas peak (37°15′N, 22°45′E), limestone rocks, c. 1130 m, 28.08.2014, *Kalpoutzakis 4605* (ACA).

New for Peloponnisos. *Quercus petraea* is distributed in Europe and eastwards to N Iran (Christensen 1997). *Quercus petraea* subsp. *polycarpa* is found in the eastern part of the species range, from the Balkan Peninsula to Anatolia, Caucasia and N Iran. It is rather common on the northern Greek mainland, and is known from a few localities in central Greece, southwards to Mt. Timfristos in Sterea Ellas and Mt. Dirfis on Evvia Island. The disjunct localities of Mt. Parnonas expand the distribution range of the taxon southwards on the Greek mainland by ca. 200 km. The subspecies is fairly rare on Mt. Parnonas; in all, only a few trees, up to 6 m tall, have been observed in the three localities.

Hyacinthaceae

Bellevalia trifoliata (Ten.) Kunth

- Nomos Irakliou, Eparchia Kenourgiou, Kriti island: c. 1.6 km SW of Zaros village, locality known as Paridia (35°07΄N, 24°53΄E), damp cereal field, c. 300 m, 05.04.2015, *Kalpoutzakis 4663*.

This collection confirms the presence of *Bellevalia trifoliata* in Kriti and expands its known range by c. 70 km toward the east. The species was first recorded from Kriti in 1993, south of the village of Vrises on the way to Hora Sfakion (W Kriti), but has not been found again in that area (Turland & Chilton 1994, Bareka & al. 2015). About 15 individuals were observed in the new locality; further populations may be present in the surrounding areas.

Lamiaceae

Nepeta orphanidea Boiss. var. ***orphanidea*** — Fig. 2C.

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: ESE slopes of Megali Tourla summit, locality known as Liouta (37°16΄N, 22°37΄E), steep rocky slope and the bottom of two neighbouring ravines close to the tree line, limestone, c.1600-1700 m, 19.07.2010, *Kalpoutzakis 3606*. Ibid.: 21.07.2014, *Kalpoutzakis 4534, 4536*. Ibid.: 01.08.2015, *Kalpoutzakis 4748*.

Nepeta orphanidea is restricted to Mt. Parnonas. The above-cited specimens belong to the rare var. *orphanidea*, otherwise known only from Orphanides's collection made in 1850 (Baden 1987) and not collected since. It has remote, pedunculate verticillasters forming a lax synflorescence, and somewhat longer bracteoles and calyces, as compared to the more common var. *parnidea* Baden (Fig. 2D), in which all verticillasters except the lowermost 1-2 are crowded. Kalpoutzakis, on Mt. Parnonas, observed three distinct, small groups of var. *orphanidea* plants, growing together with var. *parnidea*.

Orobanchaceae

Orobanche hederae Duby — Fig. 1C.

- Nomos Evvias, Eparchia Karistias, Evvia island: between the villages of Konistres and Androniani, along the path from Androniani to the place known as Platanenhof (38°36΄N, 24° 04΄E), *Platanus orientalis* forest, on *Hedera helix*, c. 90-150 m, 23.05.2015, *Kalpoutzakis s.n.*

New for Evvia island and the West Aegean phytogeographical region (WAe). The species has a wide but scattered distribution on the Greek mainland reported from all phytogeographical regions (DIMOPOULOS & al. 2013) and some Aegean Islands (STRID 2016). No previous records exist from the NAe region either. The species was locally common in the *Platanus* forest close to Platanenhof.

Plantaginaceae

Sibthorpia europaea L.

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: c. 9 km from Kastanitsa on the way to Stravorachi-Agios Petros (37°13΄N, 22°36΄E), moist place close to a spring on the bank of the main road, clearing in *Abies cephalonica-Pinus nigra* forest, schist, c. 1440 m, 25.08.2009, *Kalpoutzakis 2525*. Ibid.: NW slopes of Gaitanorachi summit, c. 1.4 km from the place known as Stravorachi on the way to Vamvakou village (37°13΄N, 22°36΄E), moist substrate around small springs close to the road, clearing in the *Abies cephalonica-Pinus nigra* forest, schist, c. 1430 m, 27.08.2009, *Kalpoutzakis* 2545. Ibid.: chestnut forest of Agios Petros, c. 1.1 km SE of the village, very close to the chapel of Agios Georgios (37°19΄N, 22°33΄E), moist place alongside a stream, clearings in *Castanea sativa-Populus tremmula* forest, siliceous substrate, c. 925 m, 19.08.2014, *Kalpoutzakis 4581*. Ibid.: Tanos river, c. 2.5 km from Agios Petros to the Malevis monastery, on the banks of the river beneath the Tanos bridge (37°19΄N, 22°33΄E), *Platanus orientalis* forest with *Pteridium aquilinum*, moist place, siliceous substrate, c. 825 m, 04.08.2017, *Kalpoutzakis 4964*. Ibid.: c. 1.6-1.7 km along the forest road from Ano Koutroufa to Vourvoura village (37°21΄N, 22°29΄E), moist places around springs on the bank of the road, *Platanus orientalis* forest with *Pteridium aquilinum*, siliceous substrate, c. 1080 m, 12.08.2018, *Kalpoutzakis 5030.*

According to TURLAND (2009), *Sibthorpia europaea* is a rare species in Greece. Only a few populations are known, located on Mt. Pilion, in W and central Kriti and in the Mt. Parnonas area (see also STRID 2016). From the latter, an old record from close to the Agios Petros village was known (BOISSIER 1879), but no recent collections. CONSTANTINIDIS & KALPOUTZAKIS (2015) confirmed the occurrence of the species on Mt. Parnonas, without adding further details. All the populations reported here grow on moist, non-calcareous substrate near springs, streams and water reservoirs, in shady places. Their habitat is threatened, and the populations are at risk. At the *Kalpoutzakis 2525* locality, the plants grow close to a forest road and are threatened by landslides (as happened 4 years ago) and road widening. The *Kalpoutzakis 2545* locality is privately owned, and the population's survival depends on the land-owner's goodwill. The *Kalpoutzakis 4581* locality is often crowded in summertime.

Paeoniaceae

Paeonia mascula subsp. ***hellenica*** Tzanoud.

- Nomos Arkadias, Eparchia Kinourias, Mt. Parnonas: the summit of Ano Pournaros between Kosmas and the small town of Leonidio (37°07΄N, 22°45΄E), slope facing NE, close to the crest line and a small ravine in mixed *Abies cephalonica-Juniperus drupacea* forest, deep fertile soil on limestone, c. 1000-1175 m, 17.04.2010, *Kalpoutzakis 2966*. Ibid.: southern parts of the Pigonia summit, c. 8.1 km from the mountain refuge towards the village of Platanaki (37°10΄N, 22°38΄E), *Abies cephalonica-Pinus nigra* forest with some *Juniperus drupacea*, stony places, limestone, c. 1240-1250 m, 19.07.2012, *Kalpoutzakis 4329*.

These new populations, together with those of the Taigetos and Sangias mountains, form the southernmost border of the taxon's range. According to information provided by the locals, some *Paeonia mascula* subsp. *hellenica* populations on Mt. Parnonas apparently became extinct between 1950 and 1980 (CONSTANTINIDIS & KALPOUTZAKIS 2015). The estimated number of plants of various ages, in the Ano Pournaros locality, is c. 2000. About 90% of the flowers seen have pure white petals; within the remaining 10% the petals are white tinged pink at the base and edges, or pink-veined. This population extends over a c. 1 km long area. On the Pigonia peak only 3 plants were seen, but several suitable habitats that are not easily accessible were not visited.

Poaceae

Maillea crypsoides (d'Urv.) Boiss.

- Nomos Kikladon, Eparchia Androu, east-central part of the Andros island, Vori beach (37° 54′N, 24°52′E), sand dunes, c. 3 m, 29.04.2018, *Trigas 6337* (ACA).

New for Andros island. This small sand dune specialist grows in Greece and Cyprus. It has a rather wide distribution in the Kiklades and the Kriti-Karpathos area in the Aegean (see STRID 2016), but it is fairly rare across its entire distributional range.

Ranunculaceae

Consolida regalis subsp. ***paniculata*** (Host) Soó

- Nomos Lakonias, Eparchia Lakedemonos, Mt. Parnonas: c. 1.6 km from Kallithea village towards Geraki (37°04.′N, 22°39′E), oat fields, limestone, c. 730 m, 26.06.2011, *Kalpoutzakis 4051*. Ibid.: S slope of the Profitis Ilias summit, c. 5.5 km along the dead-end forest road that leads to the summit (37°07′N, 22°38′E), stony slope with scattered *Abies cephalonica* and *Juniperus drupacea* trees, limestone, c. 1590 m, 29.06.2014, *Constantinidis & Kalpoutzakis s.n.*

New for Peloponnisos. Often growing as a weed on arable land. The above-cited collections may represent a recent introduction, as has been noted for *Thlaspi arvense*.

Myosurus minimus L. — Fig. 2B.

- Nomos Magnisias, Eparchia Almirou: the area of Mikrothives, c. 4.2 km N of the village, (39° 17′N, 22°45′E), cereal fields and cabbage (*Brassica*) plantations; also at a small vernal pool by a field edge, igneous substrate (mostly lava), c. 210-225 m, 01.05.2017, *Constantinidis & Papadrosou 14124.*

New for the East-Central (EC) floristic region. All our plants have slender scapes, usually longer than the fruiting receptacle, that become only slightly swollen during maturation. Thus, they differ significantly from the related *Myosurus sessilis* S. Watson. They were found in the mud of a shallow, winter-flooded depression that totally dries up during late spring. They were collected at the unusually low altitude of c. 220 m (normally 1200-1900 m, see STRID 2002) and are seriously threatened by possible expansion of the nearby cultivated land. The species is known from similar habitats in other European countries (e.g. HULINA 2005, LUKÁCS & al. 2013), with declining populations.

Ranunculus bullatus subsp. ***cytheraeus*** (Halácsy) Vierh. — Fig. 1A.

- Nomos Lakonias, Eparchia Epidavrou-Limiras: Maleas promontory, c. 2 km NW of the chapel of Agia Marina on the way to the chapel of Profitis Ilias (36°26′N, 23°07′E), low vegetation consisting of scrub and phrygana (*Pistacia lentiscus*, *Teucrium brevifolium*, *T. capitatum*, *Thymbra capitata, Salvia pomifera*, *Phlomis cretica*, *Erica manipuliflora*, *Genista acanthoclada*), sandy substrate, c. 30 m, 28.11.2009, *Kalpoutzakis & Karamplianis 2702*.

- Nomos Kikladon, Eparchia Milou, Milos Island: Ageria area at the NE part of the island (36°44′N, 24°31′E), phrygana, c. 150 m, 08.11.2015, *Trigas 6049* (ACA).

New for mainland Peloponnisos (Pe) and the Kiklades area (Kik). Previous records from the Elafonisos, Kithira and Antikithira islands, all considered part of the Peloponnisos phytogeographical region, apparently refer to this subspecies, but there was no confirmed record from the mainland itself. A previous record from the Kiklades (Naxos), according to DIMOPOULOS & al. (2013), refers to subsp. *bullatus.* Plants from Milos have short (up to 6 cm), branched scapes with 1–4 flowers and a ciliate leaf lamina with sparsely hirsute adaxial surface, or with both surfaces glabrous. A recent collection from central Kriti (*Kalpoutzakis 5041*) includes plants with a single scape and some with branched scapes bearing up to 5 flowers. *Ranunculus bullatus* subsp. *cytheraeus* has recently been erected to species level (BALDINI & JARVIS 2002), but the morphological differences from the typical subspecies need evaluation, at least within Greece (see GREUTER & RECHINGER 1967).

Acknowledgments

The authors thank W. Greuter for reviewing and improving an earlier version of the manuscript.

References

ARTELARI, R. 2002: *Thlaspi* L. Pp. 253-261. In STRID A. & TAN K. (eds), Flora Hellenica, 2. – Ruggell.

BADEN, C. 1987: Biosystematic studies in the *Nepeta sibthorpii* group (Lamiaceae) in Greece. – Opera Bot. 93: 1-54.

BALDINI, R. M. & JARVIS, C. E. 2002: Typification of *Ranunculus bullatus* L. (Ranunculaceae). – Bull. Nat. Hist. Mus. London (Bot.) 32(1): 7-11.

BAREKA, P., TURLAND, N. J. & KAMARI, G. 2015: *Bellevalia juliana* (Asparagaceae), a new hexaploid species from E Kriti (Greece). – Plant Biosyst. 149: 703-709.

BOISSIER, E. 1879: Flora Orientalis, 4. – Genevae & Basiliae.

BRULLO, S., GUGLIELMO, A., PAVONE, P. & SALMERI, C. 2003: Cytotaxonomical remarks on *Allium pallens* and its relationships with *A. convallarioides* (Alliaceae). – Bocconea 16(2): 557-571.

BURCH, D. G. 1965: A taxonomic revision of the genus *Chamaesyce* (Euphorbiaceae) in the Caribbean. – Ph.D. thesis, University of Florida. – Gainesville.

CHOCHLIOUROS, S. P. 2005: Floristic and phytosociological research of Mount Vermion - an ecological approach. Ph.D. thesis, University of Patras. – Patras [in Greek with English summary].

CHRISTENSEN, K. I. 1997: *Quercus* L. Pp. 42-50. In STRID, A. & TAN, K. (eds), Flora Hellenica, 1. – Königstein.

CONSTANTINIDIS, TH. & KALPOUTZAKIS, E. 2015: Plant Guide to Mount Parnon and Moustos Wetland Protected Area. Endemic, Rare and Threatened species. – Astros.

DIMOPOULOS, P., RAUS, TH., BERGMEIER, E., CONSTANTINIDIS, TH., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. 2013: Vascular plants of Greece: an annotated checklist. Englera 31. – Berlin & Athens.

—, —, —, —, —, —, — & — 2016: Vascular plants of Greece: an annotated checklist. Supplement. – Willdenowia 46: 301-347.

GREGOR, T. & MEIEROTT, L. 2013: Reports 68-76. P. 277. In VLADIMIROV, V., DANE F., STEVANOVIČ V. & TAN, K. (eds), New floristic records in the Balkans: 22. – Phytol. Balcan. 19: 267-303.

GREUTER, W. 2012: Results of the seventh ''Iter Mediterraneum'' in Peloponnese, Greece, May to June 1995. – Bocconea 25: 5-127.

— & RECHINGER, K. H. 1967. Χλωρίς Κυθέρεια, simul purgatorium nomenclaturae florae graecae ichoatum. – Bossiera 13.

HALÁCSY, E. VON 1902: Conspectus Florae Graecae, 2. – Lipsiae.

HARTVIG, P. 2002: *Alyssum* L. Pp. 199-224. In STRID A. & TAN K. (eds), Flora Hellenica, 2. – Ruggell.

HÜGIN, G. 1998: Die Gattung *Chamaesyce* in Europa. Bestimmungsschlüssel mit taxonomisch-nomenklatorischen Anmerkungen. – Feddes Repert. 109(3-4): 189-223.

HULINA, N. 2005: List of threatened weeds in the continental part of Croatia and their possible conservation. – Agric. Conspec. Sci. 70(2): 37-42.

KAMARI, G. 1997: *Minuartia* L. Pp. 170-191. In STRID, A. & TAN, K. (eds), Flora Hellenica, 1. – Königstein.

KARL, R. & STRID, A. 2009: *Bongardia chrysogonum* (Berberidaceae) rediscovered on the East Aegean island of Chios. – Phytol. Balcan. 15(3): 337-342.

KOKMOTOS, E., KALPOUTZAKIS E. & CONSTANTINIDIS, TH. 2009: *Helichrysum taenari* Roth. Pp. 91-93. In PHITOS, D., CONSTANTINIDIS, TH. & KAMARI, G. (eds). The Red Data Book of Rare and Threatened Plants of Greece, 2(D-Z). – Patras [in Greek].

KONSTANTINIDIS, P., TSIOURLIS, G., XOFIS, P. & BUCKLEY, G. P. 2008: Taxonomy and ecology of *Castanea sativa* Mill. forests in Greece. – Pl. Ecology 195 (2): 235-256.

LEUTE, G.-H. 1971: Die Arten der Gattung *Conium* L. (Umbelliferae). – Ann. Naturhist. Mus. Wien 75: 91-98.

LUKÁCS, B. A., SRAMKÓ, G., MOLNÁR, A. V. 2013: Plant diversity and conservation value of continental temporary pools. – Biol. Cons. 158: 393-400.

MARQUES DA SILVA, O. L., CORDEIRO, I. & ROSSI CARUZO, M. B. 2014: Synopsis of *Euphorbia* (Euphorbiaceae) in the state of São Paulo, Brazil. – Phytotaxa 181:193-215.

SARLIS, G. P. 1994: Contribution to the study of the flora of Attica (Greece). – Lagascalia 17(2): 229-256.

SCIANDRELLO, S., GIUSSO DEL GALDO, G. & MINISSALE, P. 2016: *Euphorbia hypericifolia* L. (Euphorbiaceae), a new alien species for Italy. – Webbia 71(1): 163-168.

SNOGERUP, S. 1997: *Asarum* L. P. 69. In STRID, A. & TAN, K. (eds), Flora Hellenica, 1. – Königstein.

— , SNOGERUP, B., PHITOS, D. & KAMARI, G. 2001: The flora of Chios island (Greece). – Bot. Chron. 14: 5-199.

STRASSER, W. 1997: Pflanzen des Peloponnes (Süd-Griechenland). – Vaduz/Lichtenstein.

STRID, A. 2002: *Myosurus* L. Pp. 69-71. In STRID, A. & TAN, K. (eds), Flora Hellenica, 2. – Ruggell.

— 2016: Atlas of the Aegean flora. Part 1: Text & Plates. Part 2: Maps. – Englera 33(1 & 2).

— & STRID, B. 2010: Flora Greaca Sibthorpiana. An annotated re-issue, volumes 3-4. – Ruggell.

SUTORÝ, K. & VERLOOVE, P. 2015: *Euphorbia hypericifolia* L. P. 122. In RAAB-STRAUBE, E. VON & RAUS TH. (eds), Euro+Med-Checklist Notulae, 4. – Willdenowia 45(1):119-129.

TRIGAS, P. 2003: Contribution to the study of the endemism of the flora of the Island of Evvia (W Aegean, Greece). – Ph.D. thesis, University of Patras, Patra [in Greek with English summary].

TURLAND, N. J. 2009: *Sibthorpia europaea* L. Pp. 302-304. In PHITOS, D., CONSTANTINIDIS, TH. & KAMARI, G. (eds), The Red Data Book of Rare and Threatened Plants of Greece, Volume 2 (E-Z). Hellenic Botanical Society, Patras [in Greek].

— & CHILTON, L. 1994: Studies on the Cretan flora 3. Additions to the flora of Karpathos. – Bull. Nat. Hist. Mus. London (Bot.) 24(1): 91-99.

TZANOUDAKIS, D. & KYPRIOTAKIS, Z. 2008: *Allium brussalisii* (Alliaceae), a new species from Greece. – Bot. J. Linn. Soc. 158(1): 140-146.

YANG, Y., RIINA, R., MORAWETZ, J. J., HAEVERMANS, T., AUBRIOT, X. & BERRY, P. E. 2012: Molecular phylogenetics and classification of *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). – Taxon 61: 764-789.

ZAGANIARIS, D. N. 1940: Herbarium macedonicum. Tertium et quartum mille. – Sci. Ann. Fac. Phys. Math. Aristotelian Univ. Thessaloniki 6: 38-141.

Plant iconography in the ancient Western Mediterranean Greek colonies (*Rhode* and *Emporion*, Catalonia)

CÈSAR BLANCHÉ

Abstract

Blanché, C. 2019: Plant iconography in the ancient Western Mediterranean Greek colonies (*Rhode* and *Emporion*, Catalonia). – Bot. Chron. 22: 251-289.

Iconographic representations of ten plant species found in archaeological discoveries from the ancient Greek colonies in the Western Mediterranean (Rhode and Emporion, Catalonia) are reported. They belong to *Hedera helix, Hordeum vulgare, Iris* sp., *Laurus nobilis, Myrtus communis, Nymphaea coerulea, Olea europaea, Phoenix dactylifera, Rosa* sp. and *Vitis vinifera.*

Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona. Av. Joan XXIII s/n, E-08028- Barcelona, Catalonia.

Key words: Plant Iconography, Flora Catalana Antiga, Ancient flora, 6th-2nd centuries BC, Iberian Peninsula.

Introduction

The Bay of Roses (Catalonia, Iberian Peninsula, Fig. 1) is a c. 15 km wide gulf, near the easternmost edge of the Pyrenees, which became a significant site of Greek colonisation in the Western Mediterranean during ancient times (7th-3rd centuries BC).

A selection of plant iconography found among archaeological relicts recovered from these Greek sites has been recorded within the framework of the project *Flora Catalana Antiga* (Ancient Catalan Flora). On the occasion of the 90th birthday of Prof. Dimitrios Phitos, founding member and past President of OPTIMA, we are happy to offer him this collection of plant illustrations in the classical Greek tradition found in (far) Western Mediterranean harbours and settlements. It reveals sound cultural (and botanical!) links between the coasts of our shared Mediterranean, as imagined in "*Pont de Mar Blava";* the symbolic and virtual "Bridge over the blue sea", coined nearly a century ago in the book of the same title by the Catalan Hellenist and intellectual Lluís NICOLAU D'OLWER (1928).

Historical context

After preliminary commercial contacts during the Iron Age with Phoenicians, Greeks and Etruscans, the local inhabitants of the NE Iberian Peninsula saw the establishment of Phocaean Greeks (from *Phokaia,* Fig. 1) in the Bay of Roses in the mid-sixth century BC (PUIG & MARTÍN 2006, AQUILUÉ & CABRERA 2012). The landscape was somewhat different then: coastal marshes and low hills were scattered among the estuaries of three rivers (Muga, Fluvià, Ter), which have since shifted to different locations.

Fig. 1 - Location of the studied sites: a) Study area and most relevant ancient localities cited in the text; b) Studied area (dots: Greek colonies; square: Iberian city). Free cartography from Dmaps.com and ICGC - Institut Cartogràfic i Geològic de Catalunya.

The initial settlement, to the south of the bay, gave rise to the city of *Emporion* (Εμποριον). Some time later (5th century BC), Emporion became an autonomous city (*polis*), independent of Phocaean centres (such as *Massalia*, today Marseille in France), maintaining direct commercial relationships with Athens (AQUILUÉ & al. 2007, AQUILUÉ & CABRERA 2012) and freely exporting local cereals as well as wine and fish preserves from the SW and S of the Iberian Peninsula. Probably to counteract this development, a new settlement was founded by Phocaean colonists from *Massalia,* in the northern part of the bay, close to the La Muga river estuary, in the first half of the 4th century BC. Rhode, likewise, gradually became an independent *polis*, producing characteristic black varnished pottery to substitute imports from Attica and starting their own coin mint (PUIG & MARTIN 2006), not far (15 km away) from Emporion. They established a new harbour, called *Rhode* (Ροδη), better protected from the northerly winds, with a very favourable strategic position close to communication routes (Fig. 1).

Contemporaneously, in this same area, there was a third large city, Ullastret, the capital of the local Iberian populations, which was inhabited from the 7th century BC to the 2nd AD (MARTIN & PLANA 2012). Located some 15 km south of Emporion, among lagoons and between the estuaries of the rivers Ter and Daró, Ullastret was an urban structure of notable size, with an important agricultural development (BUXÓ 2001, BURJACHS & al. 2005). It was strongly influenced by the culture and commerce of neighbouring Emporion, which, in turn, became its gateway for the exportation of Iberian products.

It should be noted that navigation routes were also established from the Bay of Roses: a) towards the Balearic Islands, en route to the Bonifacio strait and through *Ebusus* (Eivissa) to the Cap de la Nau and the southern Punic markets in Andalusia and N Africa; and b) to the northern coast (up to the river estuaries in the present-day departments of Hérault and Aude, in France) (IZQUIERDO 1996).

This period ended in 218 BC, when the Roman army took control, in the context of the Punic Wars.

Material and Methods

The repertory of plant iconography presented here is a selection from the ongoing project, FCA-*Flora Catalana Antiga* (Ancient Catalan Flora), started in 2017, to prepare a botanical catalogue of the known flora from the classical era in this region of the Western Mediterranean, as a collaborative initiative with students of the University of Barcelona (BLANCHÉ 2016, 2019). The territorial coverage and botanical descriptions are taken from *Flora dels Països Catalans* (BOLÒS & al. 2005). Chronological coverage ranges from Protohistory to late Antiquity, giving contextual consideration to both indigenous populations and colonisations. For each taxon, information is collected from the following sources: **(i)** archaeobotanical data; **(ii)** biological material (mainly pollen) from palaeovegetation and palaeoclimatic studies; **(iii)** archaeological artefacts displaying plant information (representations of plants on ceramics, in paintings, sculptures, on coins and any other objects), and **(iv)** epigraphical and written (i.e., from classical texts) records. The information obtained from fieldwork, published sources, databases and document repositories as well as from excavation sites and museums is stored in a database in Microsoft Access (more details in BLANCHÉ 2016, 2019). At present, the database includes nearly 1500 documented citations obtained from 221 sources (roughly 1/3 of the expected total volume). The inventory consists, up to now, of 257 taxa (obviously with a degree of identification reliability much lower than for current data). The plants are mainly spermatophytes, although examples of fungi, algae, bryophytes and pteridophytes have also been recorded.

The present contribution deals with the tentatively identified species represented on artefacts from the Greek settlements in the Bay of Roses. The results are preliminary, aimed at illustrating the current state of the cataloguing and database-building progress. Some excellent open-access databases of complementary scope, such as: *Flora Ibérica,* launched by the University of València (MATA 2010-2019), the *Beazley Archive* (ANONYMOUS 1997-2019), owned by Oxford University, and, in particular, the document database built by the project *Iberia Graeca* (CABRERA & AQUILUÉ 2008-2019), have proved a significant help in locating archaeological artefacts with plant representations.

Botanical identifications by the author have been attempted initially through morphology, but in only a few cases can such identifications purport to be wholly accurate. In any case, this method has only been applied to those exceptional artefacts that were decorated with high quality art. In the majority of cases, low cost productions coming from the distant extreme W colonies show very schematic and simplified drawings. The likeness of the images to plant species is remote in many cases, so that plant identities are obtained through symbolism, established through whole series of pictorial "ontogenies" or by attributes extracted from classical literature, rather than by a comparison of the illustrations themselves to actual plants. This system of species attribution is exemplified in many references (BAUMANN 1991, MIRÓ 2006, KUNZE-GÖTTE 2006, MATA & al. 2010, AVRAMIDOU 2011, JASHEMSKI & al. 2018, among others).

Results

A total of 136 plant representations of Greek colonial origin (6th-3rd centuries BC) from the archaeological sites of Emporion and Rhode had been registered in the *Flora Catalana Antiga* database, up to November 1st, 2018. Most (67.6 %) of these plant illustrations are from pottery (of very diverse typology), followed by coins (25 %), epigraphic marks (5.8 %) and sculptures (only 2 records, less than 1.5 %).

The recorded artefacts are partly of local production (coins from the mints of Emporion or Rhode), but mainly of foreign manufacture (from Attica, Euboea, Rhodes or the Italian Peninsula). They were shipped to these W colonies both for local use (by Greek colonists and indigenous Iberian elites) and redistribution through trade routes along the Iberian coast (ROUILLARD 2009, ADROHER & ROUILLARD 2017, AQUILUÉ & al. 2017).

So far, ten plant taxa have been botanically identified (with varying degrees of certainty; see discussion). They are presented here in alphabetical order, with detailed records given in Tables 1 to 10. Eighteen further records of plant illustrations belong to thus far unidentified species (see Tables 11-13).

Hedera helix L. (Araliaceae)

Ivy is the most frequently represented plant species of our data set. It accounts for 27.2 % of the registered findings from Rhode and Emporion. The detailed records of *H. helix* are listed in Table 1.

Two records are from relatively old (6th-5th centuries BC), tridimensional plastic ceramic receptacles: an *askos* representing an animal (ram, goat or ox, depending on the author) with a painted ivy leaf garland around the neck, of Syracusan origin and an *oenochoe* representing a female head with a decorative garland, also depicting ivy leaves, of Attic origin. However, *H. helix* is mainly represented on Attic pottery (most pieces are fragments), belonging to two main groups: older vessels with black figures (6th-5th centuries BC) and more recently, with red figures (5th-4th century BC).

Ivy is frequently represented in black-figure ceramics as a radial complex of climbing branches around characters in Dionysiac scenes (e.g., satyrs, maenads, donkeys, Dionysos; Fig. 2A); some with well depicted cordate and/or acuminate upper *Hedera* leaves and some with leaf outlines, simplified until they become merely points along a voluble axis. Another common representation of ivy is as a horizontal vine canopy, over the scenes or as vertically climbing plants on vertical columns or supports, sometimes used as a scene divider or scene frame margin, also ranging from cordiform to point-shaped leaves in the most simplified decorations (see Fig. 4I). Typical archaic Mannerist artists (475-450 BC), among others, characteristically feature Dionysiac or feasting scenes inside a framework of black ivy (MIRÓ 2006:180). In some cases, it is not easy to distinguish *Hedera* from *Vitis* vines, as both are associated with Dionysos (AVRAMIDOU 2011). In Dionysiac scenes, grapevine leaves (sacred to the god) were usually replaced by ivy leaves to give the idea of eternity, difficult to express with deciduous *Vitis* leaves (BAUMANN 1991).

A second group of Attic pottery includes red-figure receptacles, of which the Emporiton representatives have been reviewed in the monographic survey by MIRÓ (2006). Many of them are kraters or cups, durable and well conserved. Ivy is represented: (a) in general decorations of receptacles (borders, ribbons, necks); (b) in frames delimiting scenes, and (c) as significative within scenes (mainly of a dionysiac theme; followed by conversation, school, palestra or combat scenes: Fig. 2B-C).

Fig. 2. *Hedera helix* (A-E)– A: Dyonisiac scene with ivy climbing vine and *Myrtus communis* (head crown) (Tab. 1, HE he 176). B: Fragment of Dyonisiac scene with ivy branch (centre) and thyrsus (extreme left) (Tab.1, HE he 159). C: Maenad with thyrsus (of cordate ivy leaves) and satyr (Tab. 1, HE he 154). D: Decorative ivy leaf in sandal strips of monumental marble feet attributed to godess Isis (Tab. 1, HE he 145). E: Ivy wreath with fruiting umbels and cordate acute leafs.(Tab. 1, HE he 186).

Especially abundant are crowns or hair garlands of ivy leaves, as well as thyrsus sticks with symbolic heads of *Hedera* (sometimes, in other iconographic representations, *Vitis* leaves or *Pinus* cones), according to the expected attributes (of evidently phallic inspiration). Whereas, in borders or frame columns, ivy leaf outlines range from cordate to point-shaped, true ivy leaves are represented with more detail and fidelity in hair garlands or thyrsus heads (Fig. 2C).

Finally, probably as a part of the statuary of a 2nd century BC Emporiton temple, a decorative ivy leaf has been found on the sandal straps at the front of two monumental white marble feet (Fig. 2D), interpreted as belonging to the goddess Isis (RUIZ DE ARBULO & VIVÓ 2008).

Decorative bands and borders on tableware sometimes faithfully depict *Hedera* vines (voluble stems, leaves and fruiting umbels, Fig. 2E).

***Hordeum vulgare* L. (Poaceae)**

Spikes of an undetermined *Poaceae* species are represented in the hair decoration of a female character on coins minted in Emporion and Rhode, listed in Table 2. Because of the small size, figure simplification and erosion due to use and time effects, it is not possible to botanically identify such spikes, so we must turn to context or symbolic meaning. The drachmas coined during the end of the 4th- to the 3rd century BC in Emporion (with the inscription ΕΜΠΟΡΙΤΩΝ: *Emporiton*, corresponding to the inhabitants of the city) inspired on other Mediterranean mintages (Sicilian, Punic, *cf.* RIPOLLÈS 2010). A long controversy has continued, between several authors, for more than a century (discussed in PUJOL 1989 and VILLARONGA 2000), about the identity (Ceres-Persephone, Arethusa or Diana) of the female head represented on the obverse of the Emporiton coins.

Following VILLARONGA (2000), the female head on the oldest coins (with a standing horse on the reverse, interpreted as a Punic pattern, see also RIPOLLÈS 2012) must correspond to a Persephone/Kore character, with curls, some of them turned into spikes (Fig. 3A), showing stems and flowering/fruiting spikes of a *Poaceae* species that form a diadem fastening the hair. According to the expected attributes of this goddess, the spikes should belong to barley (*Hordeum vulgare* L.), well known and consumed in Greece and in the Catalan countries, and also cultivated, stored and used in the surroundings of ancient Emporion, as documented by archaeobotanical findings: hulled barley remains (seed, starch, carbons, phytoliths) have been reported from the same chronological period no farther away than 10-15 km, by CASTRO & HOPF (1982), PONS & al. (1998) and ASENSIO & al. (2014).

Some years later, on the reverse of these Emporiton coins, the standing horse was changed for a winged Pegasus (VILLARONGA 2000, 2003). In the very first issue, the female head on the obverse still shows two beautiful spikes in the hairstyle; in the second issue, the head size and number of curls have been reduced; in the third, some mintages still bear spikes but in successive issues, the spikes are lost, transformed into a diadem and giving rise to a new pattern (called *sicul* or Sicilian), where the female character must be Arethusa (the Syracusan nymph, accompanied by three dolphins). This evolution happened during the 3rd century BC. The most recent, also without cereal spikes in the hair, belong to a third pattern where the female character represents Diana. Examples of each issue with *Hordeum* spikes are listed in Table 2.

Coming from the nearby town of Rhode, and also during the 3rd century BC, in addition to the iconography of roses (see below), some coins of Syracusan inspiration

(Fig. 3B) also show a female head with spikes (one, or more commonly, two; RIPOLLÈS 2010). Table 2 reports the silver drachma of Rhode with a female head, with a trident on the back, and two spikes, as well as the mint legend (ΡΟΔΕΤΩΝ, *Rodeton* = the Rhode inhabitants) with a rose underneath on the reverse (see Fig. 14. 11, 12 & 13 in CAMPO 2006). By contextual analogy, we also provisionally attribute these spikes to *H. vulgare*.

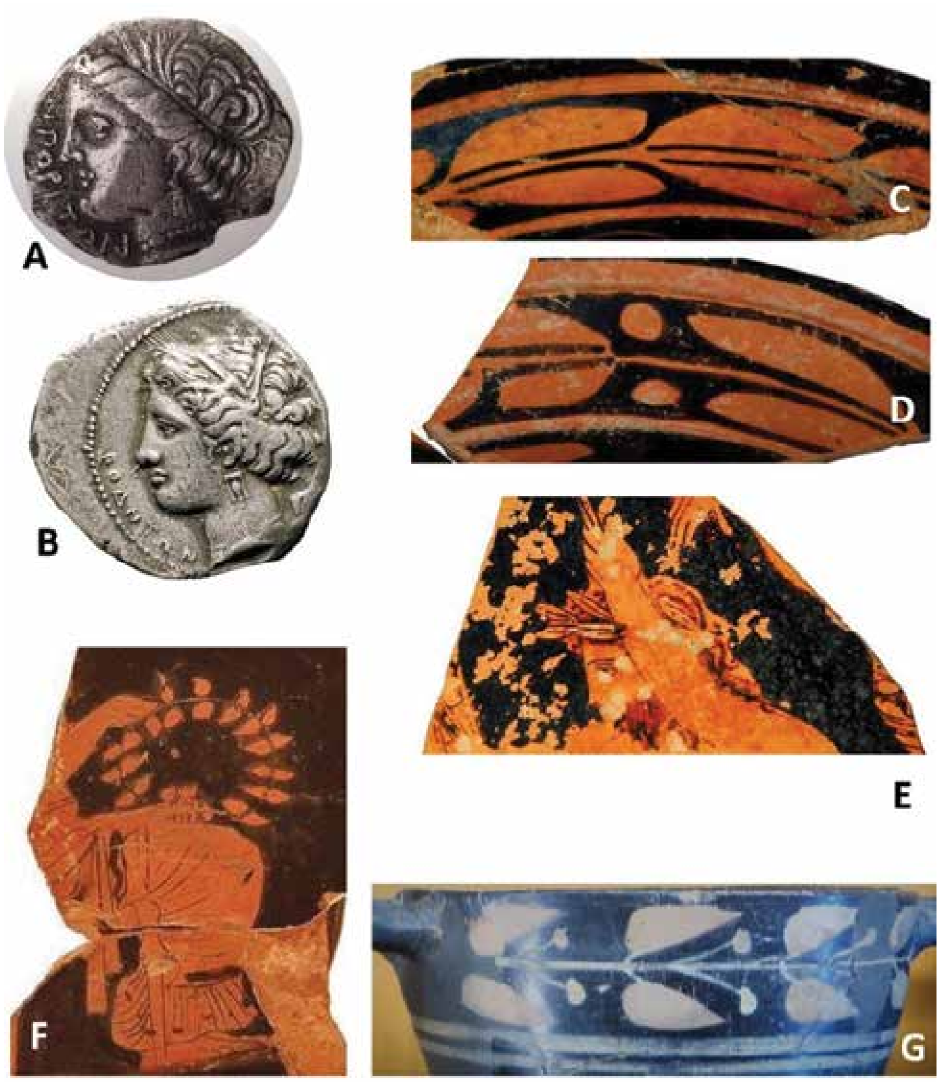


Fig. 3. *Hordeum vulgare* (A, B). *Laurus nobilis* (C-E). *Myrtus communis* (F, G). – A, Silver drachma featuring a woman's head with spikes in the hair (Tab. 2: HO vu 23). B, Same motif (Tab. 2: HO vu 25). C, D, Attic kraters with laurel wreaths under the neck , C (Table 4: LA no 35), oriented right. D (Table 4: LA no 36), oriented left. E, Red-figure krater, Dionysiac character with laurel crown (Table 4: LA no 44) F, Red-figure Attic cup, presumed gynaeceum scene, myrtle crown in woman's hand (Table 5: MY co 3). I, Red-figure Attic skyphos, band of leafy fruiting myrtle (Table 5: MY co 7).

Iris sp. (Iridaceae)

A single record from a fragment of a Corinthian aryballos of black figures (dated 575-500 BC) found in Emporion (Table 3) depicts the lower half of a male character approaching a plant that could be provisionally attributed to a member of the genus *Iris* s.l. (CABRERA & AQUILUÉ 2008-2019). This is a taxon relatively rarely depicted on ancient Greek pottery as a figurative element (although in sculpture it is represented, for instance, in the akroteria of temples or on coins, see NALIMOVA 2017), whereas similar flower outlines inspired more common floral lotoid-iridoid compositions as merely decorative elements or fleurs-de-lys (sometimes named lilies or lotuses in fine arts or archaeological terminology).

In any case, it is not a flower easily rendered by ancient artists due to its complex blossom structure. Three species of irises could be the best model candidates: (a) *Moraea sisyrinchium* (L.) Ker Gawl., of which the subterraneous corm is reported as edible by Theophrastus (AMIGUES 2010); (b) *Iris pallida* Lam. or *I. germanica* var. *florentina* (L.) Dykes, both rhizomes being used as a perfume source since ancient times, depicted in ancient Egypt, from the 18th dynasty ("Botanische Kammer" in Karnak; see GERMER, 1985), and also known by Theophrastus (FAURE 1987, AMIGUES 2010) and (c) *Limniris pseudacorus* (L.) Fuss, well known helophyte growing in wet areas in Greece (CRESPO et al., 2013), known as medicinal by Pliny and Dioscorides, who called its root ακόρου (JASHEMSKI & al. 2002, SEGURA & TORRES 2009). Further research is needed on symbolic iconography of parallels, including Etrusco-Italic ones.

Laurus nobilis L. (Lauraceae)

The autochthonous character of laurel in NE Catalonia is difficult to demonstrate (VILLAR 1986, BOLÒS & al. 2005), even more so if we refer to its occurrence in classical times (ARROYO & al. 2001). In any case, the recorded representations of *Laurus nobilis* from Emporion nearly all belong to artefacts imported from Greece (Table 4), thus reflecting a foreign, E Mediterranean origin.

With the exception of a Euboean krater (with a laurel border), all such items belong to the classical 5th to 4th centuries BC red-figure Attic pottery, generally big containers with relatively detailed and narrative scenes, as well as general decorative laurel motifs. They have been studied in the ceramological monograph by MIRÓ (2006).

Figurative records depict laurel leaf crowns as winners' attributes (Trojan War heroes, mythological scenes, theatre triumphs, Fig. 3E), with leaves oblongo-lanceolate, subacuminate and shortly petiolate, as is botanically correct. Sometimes leaves [incorrectly] appear nearly (or fully) opposite, likely due to the space being too reduced for a proper detailed representation of several layers of leaves in a painting of a few millimetres. This is also the case with a crown painted on plastic oenochoes featuring a female head (Table 4, LA no 34, as well as the drawing in plate 254, no. 3246 of MIRÓ, 2006, corresponding to another analogous vase, now lost). *L. nobilis* generally appears fruitless, perhaps due to its simplified representation, perhaps – because dioecy was known – indicating male gender, as recognised and reported by Theophrastus under the name "male laurel" for non-fruiting individuals (AMIGUES 2010: 77). However, in some artefacts, some detailed crowns or garlands show small dots among the leaves, which can be interpreted as true berries (if ovoid and black) or better, as floral buds (when round and white). This is the case in some quality fragments of feast/symposion scenes. Apollo also bears a laurel crown, accompanied by Artemis holding a laurel branch, in a panathenaic amphora of the first half of the 5th century BC (Fig. 730 in MIRÓ 2006). As an attribute of Apollo, the laurel symbol is still, by extension, indicative of Greek origin: in representations of Orpheus, to show that he is dressed in Greek style, he wears a laurel crown; when dressed in Thracian costume, he wears a Thracian hat, surely following cartoon models (DEL BARRIO 2017: Fig. 3).

Decorative bands around the border or below the lip of kraters in Emporiton Attic pottery used to have a band of lauroid leaves, attributed by archaeologists to *L. nobilis* (Fig. 3 C-D). Generally fruitless (although some have white dot-shaped fruits or buds), they show an imperfectly opposite leaf arrangement (even if decurrent and short petiolate or sessile limb), usually lacking midrib (always present in *Olea* leaf illustrations). Some experts point out that opposite leaves are not, in fact, an unusual feature in laurel representations. JASHEMSKI & al. (2002) report Pompeian graffiti with laurel wreaths in which leaves are shown as distinctly opposite, but, as plausible explanations, they argue that "we should not expect a hasty drawing to be botanically correct" and "it is quite possible that such crowns were made by stitching leaves on a ribbon". In any case, ours are provisional attributions, awaiting further in-depth comparative iconographic research with the help of Attic production databases.

An additional finding comes from numismatics. The laurel crown can be seen in portraits, presumably of Apollo, on Emporiton coins from the 4th century BC, as a three-quarter portrait (CNH 5/15, RIPOLLÈS 2010). Although morphologically imprecise, we know that laurel was sacred to Apollo (BAUMANN 1991), and many representations show the god crowned with laurel and holding a laurel branch, or sometimes only his attributes, such as laurel branches (JASHEMSKI & al. 2002: 120).

Myrtus communis L. (Myrtaceae)

Five pottery records retrieved from the *Iberia Greca* database have been considered as depicting myrtle (Table 5). Although *M. communis* is a wild species in the Emporiton area (BOLÒS & al. 2005), and was well known and locally used aeons ago (pre-Iberian anthracological remains of myrtle wood dating from the 10th century BC have been recovered by PIQUÉ (2002) from the Ter river plain area, very close to Emporion and Ullastret), all iconographic records are of eastern origin.

A fragment of a red-figure Attic cup (dated 5th century BC) features a sitting female person, with a myrtle crown in her hand (also reported by MIRÓ (2006), number 672), in a presumed gynaeceum scene (Fig. 3F), for which floral crowns and wreaths are typical (MIRÓ 2006). The woman's wreath, removed from the head and held by hand, connotates the semantic potential of myrtle sprigs, broken or bent branches and wreaths to imply – a tentative hypothesis – the fragility or precariousness of an amorous liaison (KUNZE-GÖTTE 2006).

A second possible *Myrtus* image (Fig. 2A, Table 6, MY co 6) comes from an old (550-500 BC) fragment of Attic black-figure kylix (ophthalmic type) with a Dionysiac scene, where a male character holding a kantharos bears a head crown and is surrounded by a climbing vine. The god's head crown can be identified as being of myrtle by the opposite, acute leaves and long axillary fruiting pedicels, whereas the climbing and radiating ivy branches fill out the surrounding space.

Some artists even endeavoured to outline myrtle accurately, in decorative bands, right down to the fruit's apical calyx remains (see the red-figure skyphos coming from near Ullastret – Fig. 3G – and the myrtle wreath with berries in an Athenian oinochoe – Beazley Archive VN 9033423). However, other database records attributed to myrtle are less clear, as those represented in two Emporiton 5th century BC alabaster receptacles with black figures: (a) a scene with a siren, aulos and tomb, with a myrtle branch that needs further study as the piece is worn out and the identification is insecure (Beazley Archive); and (b) an alleged myrtle branch with schematic linear leaves, beside an owl (Iberia Greca), for which the Athenian context casts doubt on the attribution to *M. communis* (rather than to *Olea europaea*, see below).

The iconographic function of myrtle as attribute, symbol, and ornament on Attic vases of the Archaic and Classical periods is reviewed by KUNZE-GÖTTE (2006), who points out that myrtle is often misidentified, mainly because ancient artists never really sought naturalistic likeness. She opines that it is of prime importance to look carefully at features such as leaf shape (often leading to confusion with laurel) and berries (bearing in mind the vase painters' endeavour to characterise myrtle with rudimentary brushstrokes). Identification must be consistent with the semantic role of myrtle in a given iconographic context. Following JASHEMSKI & al. (2018) and KUNZE-GÖTTE (2006), myrtle was the most widely used material for wreaths, and its usage prevailed in a variety of public or private rites, civic ceremonies, sacrifices, etc. In matrimonial or funerary contexts, myrtle was closely associated with Aphrodite and her cult. Myrtle wreaths are also worn by various divinities on different occasions. Most commonly, they symbolise the explicit or implicit power of Aphrodite. KUNZE-GÖTTE (2006) specifically reports the ornamental use of myrtle on vases that represent Dionysos' wedding with Ariadne, thus providing a possible explanation for the myrtle wreath on our Emporiton ophthalmic cup.

In a general way, myrtle was considered to promote and reinforce love and therefore was the symbol of lovers and their poets; it was grown in gardens (cultivation details are given by Theophrastus) and used as a medicinal plant (SEGURA & TORRES 2009, AMIGUES 2010).

The study of the symbolism of a particular plant species' iconography should also consider semiotic evolution over time. For instance, the predilection for featuring Apollo with myrtle wreaths and crowns reflects Athens' pro-Delphic political penchant in certain periods. An anti-Delphic political strife favoured visual narratives in which the god was deprived not only of the tripod (the indispensable instrument of divination), but also of the laurel (his standard Delphic attribute). Once laurel crowns had disappeared, myrtle wreaths were still used as a visual symbol of the god's Delphic character (KUNZE-GÖTTE 2006).

Nymphaea caerulea Savigny (Nymphaeaceae) = *Nymphaea nouchalii* var. *caerulea* (Savigny) Verdc.

A few decorative iconographic motifs evoking the "lotus" flower, all originating from Emporion, are listed in Table 6, as given by primary sources. They include simplified lotoid shapes on the necks of Attic craters (similar to those found in the Segura river area from the 4th-5th centuries BC; see BLANCHÉ 2019) and a set of Attic lekythos of black varnish, with palmettes framed below with schematic outlines of "lotus" flowers (a pattern repeated many times in parallel in the same period – 5th century BC – in objects found in Emporion: MAC- EMP RG SN 45, 46, 48, 50; MAC –B 380; MUPHV 28; MAC-G 547, MAG 5303, 5307, 5310, etc.; not exhaustively listed). This floral motif was widely established in the eastern Mediterranean, and it was incorporated in Greek artefacts without significant figurative intent (BAUMANN 1991), and in the indigenous western, Iberian material culture as well (MATA & al. 2010).

There exists, however, a distinctive and remarkable sculptural representation, tentatively similar to the image of *Nymphaea coerulea*. It is an element of the acroterion belonging to the roof of a 5th century BC temple of Emporion, with antefix ornaments consisting of palmettes and a flower approaching the blue lotus (Fig. 4A). In this piece, over a basal cord finished by hooked scrolls, a 2-sepaloid whorl contains a polypetalous corolla, represented by 5 acute, diverging petals. This sculpted element recalls the Western Mediterranean representations of *N. coerulea* found in the decoration of ostrich eggs used as funerary containers, characteristic of Punic deposits from Ebusus (Eivissa) and other sites in the SE Iberian Peninsula (BLANCHÉ 2019) or in the Iberian jar from Carmona (Sevilla), both with criss-crossed flowers and buds (Fig. 116 in MATA & al. 2010). Such *N. coerulea* evocations belong to the so-called "orientalising period" of Iberian archaeologists (BLANCHÉ 2019).

Olea europea L. (Oleaceae)

Up to seven records refer to the olive tree iconography, all found in Emporion (Table 7). Six of them are clearly figurative olive trees, being an attribute of Athena, represented both as a goddess and in her transmutation, an owl.

In classical Antiquity, in coins, the figure of the olive tree was not used by many cities, but the influence of Athens led to that image becoming widespread all over the Mediterranean (RIPOLLÈS 2010). In the Iberian Peninsula, the presence of the olive tree can be identified with certainty only in the edition of silver coins minted in Emporion during the 4th century BC. They are divisors with the head of Athena on the obverse, and on the reverse, very schematically, an owl in frontal view surrounded by two *Olea* branches with opposite leaves, also very simplified and attributable confidently to the olive tree only by the context. They faithfully reproduce the design of 5th-4th centuries BC hemidrachms from Athens (Fig. 9 in RIPOLLÈS 2010).

The combination of owl and olive tree in Emporion is also repeatedly present in red-figure pottery (5th-4th centuries BC) imported from Attica (skyphos) and Campania (hydria) (MIRÓ 2006). The conserved fragments of these receptacles show some crude drawings of both owls and olive trees. Probably because of the modest artistic quality and cheap manufacture, they were widely produced in great number as small bowls

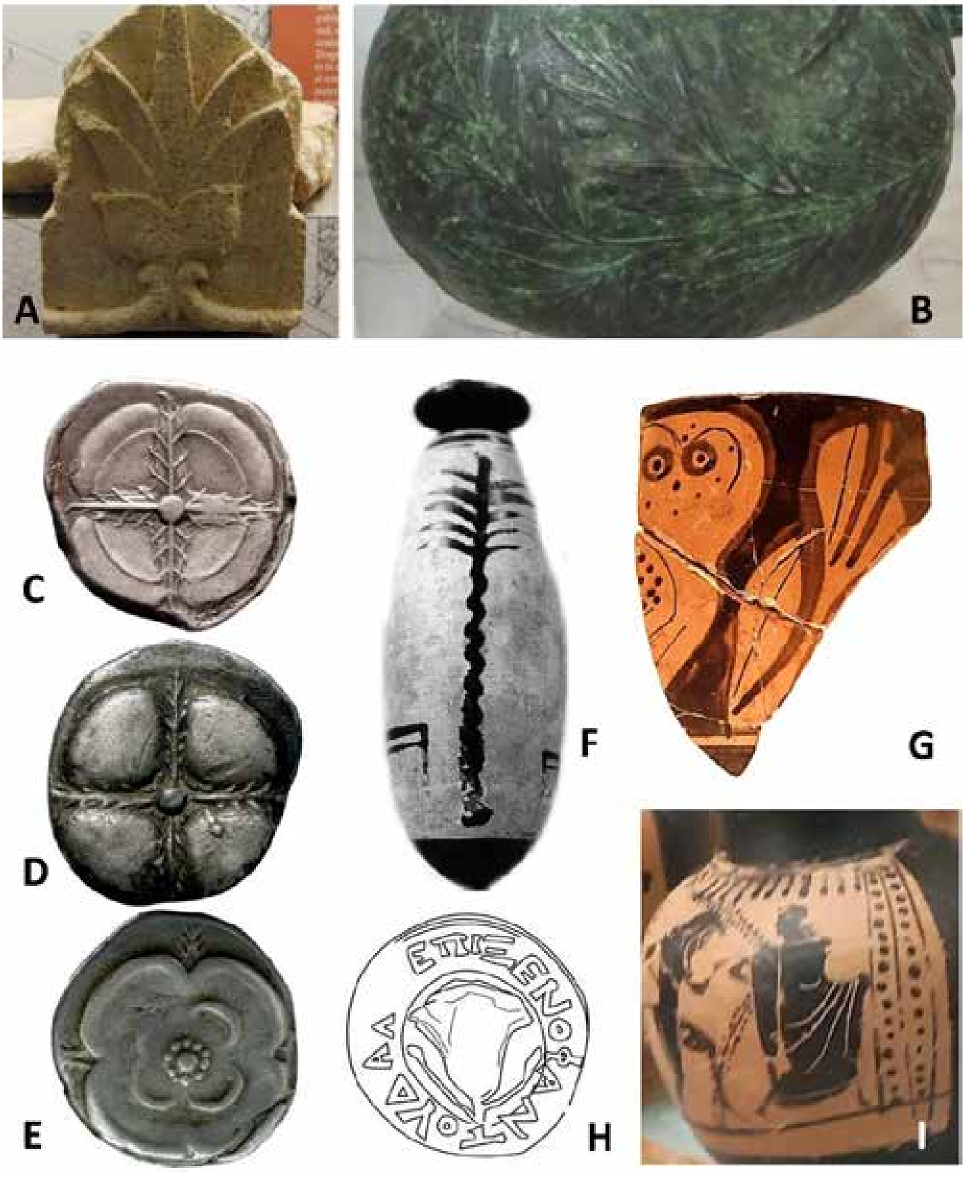


Fig. 4. *Nymphaea coerulea (*A). *Olea europaea* (B, G). *Rosa sp.* (C, D, E, H). *Phoenix dactylifera* (F). *Vitis vinifera* (I). A, Antefix of acrotherion (Tab. 6 , NY co 24). B, Roman bronze, detailed olive branche, fruiting (Table 2, OL eu 11). C: Drachma from Rhode, rose from below, 2-whorled corolla and medium sized sepals (Tab.9, RO sp 27); D, same motif, with 1-whorled corolla and narrow sepals (Tab. 9, RO sp 36). E, rose viewed from above, (Tab. 9, RO sp 33). F, Alabastron of white ground (Tab. 8, PH da 52) G, Red-figure Attic skyphos, schematic olive branch with Athenea owl (Table 2, OL eu 31). H, rose, lateral view. Rodian epigraphic stamp in amphoric handle (Tab. 9, RO sp 16). I, Black-figure oenochoe with Dyonisiac scene, with very schematic pending grapes and frame border of dotted schematic vine leaves (Table 10, VI vi 54).

(many parallels of Attic origin can be found in the Beazley Oxford database).

According to MIRÓ (2006: 199), the production of artefacts showing the owl surrounded by olive tree branches started around 480 BC and peaked in 450-425 BC. The Emporiton *Olea* records always present short, erect, fruitless branches with opposite, entire leaves, longer toward the base, with obtuse to subacute tip, widest in the distal half, basally decurrent and very shortly, if at all, petiolate, and with a single, conspicuous midrib; they follow a very consistent pattern, compatible with the essential botanical morphology and, although they are decidedly non-naturalistic, the artists truly (and successfully) tried to faithfully represent the plant, of course far from the quality of Roman bronzes (Fig. 4B and Table 2, OL eu 11).

Decorative borders and garlands sometimes attributed to olive foliage are of uncertain attribution.

Phoenix dactylifera L. (Arecaceae)

Illustrations of the date palm tree are not frequent on Emporiton artefacts. A single item, a red-figure Attic alabastron of white background with black varnish (dated 480-475 BC) shows a funerary scene with a female character on one side and a palm tree on the reverse (Table 8). The *P. dactylifera* silhouette shows a 10-leaved crown, fruitless, in the upper quarter of the single, undulate trunk. The figure is rather naturalistic, considering the simplicity of the drawing (Fig 4F); in some parallel representations (VN 3070 of Beazley Archives, among many others), the palm trees are even simpler and more schematic. A glimpse of a second possible palm tree appears on the breastplate of a male character with a beam of spears, on a fragment of a red-figure (350-325 BC) krater (MIRÓ 2006; numbers 3487 and 3145, respectively).

As *Phoenix dactylifera* is not autochthonous in NE Catalonia, this representation, imported from Attica to our territory, again fits the concept of an orientalising element (BLANCHÉ 2019). The graphic message might evoke the origin of the product contained, its excellence, or the place where the funerary scene was situated (the very similar palm tree from VN 3070 illustrates a youngster in oriental costume, thus suggesting the flavour of a certain eastern Mediterranean environment). Exotic evocation is also claimed for, e.g., the Amazon scene represented on a red-figure lecit (475-450 BC, Louvre object 5250 LIMC).

The basis of the mythic symbolism is the myth that a date palm, on the island of Delos, was the sacred tree under which Apollo was born (RUIZ DE ARBULO 2009). The use of the palm as a motif in ancient Greek art indicates "*aretē*" (excellence), becoming a pictorial symbol for a complex of attributes and virtues cherished by the Greeks, both as victory wreath and palm frond (MILLER 1979, KEFALIDOU 2009).

Rosa sp. (Rosaceae)

Flowers as the main image on coins were rare in ancient Greek cities, and even rarer in Iberian colonies. The city that most often used flowers as principal motif was Rhodes, in the S. Aegean, which used a rose in lateral view (RIPOLLÈS 2010). Possibly chosen as ideal to commemorate a founding myth associated with this easterly city of Rhodes (Ρόδος, Rhódos), the mint of the western city of Rhode (Roses), principally during the 3rd century BC, also adopted a flower, interpreted as a rose, as its emblem. The flower was mainly viewed from below (although exceptions of lateral views also exist).

The evolution of the rose emblem on the Rhode coin reverse has been studied by VILLARONGA (2000) and CAMPO (2006). The main representation consists of a rose in a very topical presentation: a tetramerous (!) flower viewed from below, in the first editions showing high quality, neat detail, and in the later ones, nearly a century later, progressively simplified. The flower pattern consists of a central knob representing the pedicel, adjoined by four long, subtriangular, alternipetalous sepals forming a cross, each with a prominent midrib and a dentate-lobulate margin. In a peripheral position, there are four more, rounded petals (with an external margin giving the impression of relief or, alternatively, of four additional, superposed petals, suggesting two petal-whorls). Some minor variations include: (a) narrower (to near-linear) sepals with a larger number of lobules, approaching a fish-bone pattern; and (b) different relative sizes of each of the 2 petal "whorls", resulting in their merging into one simple whorl (Figs 4C-D).

Of course, tetramerous flowers are not found in Western Mediterranean species of the genus *Rosa* L. (although some species of a group of Asian taxa of *R.* sect. *Pimpinellifoliae* have 4 petals; ANONYMOUS 2008). Thus, an (erroneous) simplification of the pentamerous pattern must be assumed. However, some *Rosa* species growing in the vicinity of the site of Emporion do exhibit pinnately lobed (or pinnatifid) sepals: the most abundant are *R. agrestis* Savi and *R. canina* L., as well as *R. micrantha* Borrer ex Sm. and *R. pouzinii* Tratt. (BOLÒS & al. 2005).

More significative is a series of roses (belonging to the so-called "group 6c" -- see CAMPO (2006) and her Fig. 14: 13-15) viewed from above (Fig. 4E). The coins show two tetramerous petaloid whorls, superposed, the tips of the pinnatifid sepals emerging between the outer petals, a central crown of nine stamens (standing for polyandry), and a knob corresponding to a simplified stigmatic assemblage (as considered by MONTSERRAT & SILVESTRE 1998). Over time, this pattern is progressively simplified, the coins become lighter and revert to the upward view of the flower, a variant known from a unique coin. CAMPO (2006: Fig. 14.6) also shows a flower from above that is pentapetalous, thereby resembling a true *Rosa* (but bearing only five antipetalous stamens, thus losing polyandry).

Finally, some issues (CAMPO 2006: Fig. 14.7-10) show the rose in lateral view. This is a modification that brings the (westerly) Rhode coins closer to those of (easterly) Rhodes (*Rhodos)*, as the Aegean city adopted the rose (ρόδον / *rhódon*) in lateral position (as borne on the amphoric stamps, see below). The flower is represented to look tridimensional (three petals seen in the foreground, giving a sense of depth to the remaining, hidden two) and it shows botanically coherent characters (inflated receptacle, pinnatifid sepals); this pattern was lost in later issues and reverted to the more usual view from below.

Eight records from Table 9 correspond to epigraphic roses recovered in Emporion from amphoric fragments belonging to the 2nd century BC amphoras-of-transport of Rhodian style, with a circular stamp of a central rose on the handle, plus the inscription of the trader or owner's name (Fig. 4H). All of these undoubtedly represent a rose in lateral view, the typical and well known mark of origin (Rhodes in the Aegean) of eastern Greek wines, added to authenticate export packs sent by sea from *Dicaearchia* (later

Puteoli, near today's Naples) to Massalia or directly to Emporion (PASCUAL & PÉREZ BALLESTER 2017).

Vitis vinifera L. (Vitaceae)

Vitis vinifera was known by the indigenous pre-colonial population (certainly grapes would form part of the food supply), as seeds have been recovered from 7th-6th century BC Iberian sites in the zone of influence of Emporion (Sant Martí d'Empúries, Ullastret or Pontós, see CASTRO & HOPF 1982, CANAL 2001, PONS 2016), although the expansion of grapevine culture is related to Phoenician and Greek colonisations (BUXÓ, 2001). Iconography of *V. vinifera* is documented in the FCA database, up to now, from seven pottery artefacts, unearthed in Emporion, of Attic (both red- and black-figures) and Campanian origin (Table 10), dating from the 6th-4th centuries BC.

These records include grape representations, some in isolation or pending from vines or on climbing branches. The artistic quality of such small receptacles (of cup, lekythos and oenochoe type) is relatively simple compared to expensive tableware showing magnificent vine shoots and fruiting grapes (remember: these objects were exported to small towns in far-off western colonies) and, as many depicted scenes are of a Dionysian theme, the very schematic leaf outline (Fig. 4I) makes it difficult to ascribe them to ivy or grapevine (also as both are considered attributes of Dionysos and his thiasos/processional characters: see comments under *Hedera helix*). As a provisionally adopted criterion, attribution to *V. vinifera* is more convincing when grapes are represented. The grapes are also very schematic and barely suggested by a remotely conical brush stroke surrounded by a grape outline, or even, simply the outline alone (whereas ivy fruit umbels are distinctly represented, BLANCHÉ 2016). However, that technique seems effective enough to represent the ambience where such Dionysiac or palestra scenes are developing.

A relatively more luxurious artefact is a red-figure Campanian 400-350 BC pelike (amphora-like) with several plant elements (palmettes, rosettes, fern-like scrolls, fruiting tree, etc.) and a winged figure interpreted as Eros (CABRERA & AQUILUÉ 2008-2018). At his right, a raceme-like T-shaped figure could also symbolise a grape, by analogy (VI vi 57, Table 10).

Non-identified plant elements

Three additional groups (for flower, fruit and tree/shrub representations) include a total of 18 records with representations that cannot yet be taxonomically associated with a genus or species with a requisite minimum degree of confidence (see Tables 11-13), representing 13,2 % of the total record. They need further research, mainly through an approach using comparison with analogues of authenticated Greek pottery in databases.

In addition, a very large number of pottery objects (cups, bowls, dishes, lamps) from Rhode attained a wide distribution in the W (and C) Mediterranean, the called “black varnish pottery from the Roses workshops”. They were sold for domestic purposes, produced in local ovens, consuming important quantities of combustible from local vegetation. Their decoration, consisting of impressed rosettes, palmettes, anthemions and cordate leaves, is not discussed here, having been well studied and classified by Puig (2006). These images do not represent specific taxa but are but loosely inspired by plant forms and patterns, without the intention to represent single species but for decorative purposes only. They were engraved by semi-industrialised systems of small impressed wheels and cartridges producing, in combination, an infinite variety of figures (Puig 2006).

Discussion

The iconographic findings from Rhode and Emporion are mainly the result of eastern Mediterranean importation, and thus colonists (and locals) act as mere consumers for both shapes and subjects. Concerning the Attic repertory (the vast majority of records, dating from the 6th-5th centuries BC), potters from the Keramicos quartier in Athens produced vases, shaped to meet western tastes, for consumers all along the Iberian coast. Although distribution/redistribution routes are poorly known, one is to assume that these were correlated with shapes that could be easily packed to fit into the ships' holds (Adroher & Rouillard 2017). A similar pattern is observed in later imports from Campania (around the 3rd century BC).

Decorations or illustrations in our records are Greek in their origin, taste, images and format. Local consumers did not participate in the design, they chose from the selection provided by merchants or intermediaries, not directly from producers (Adro-her & Rouillard 2017). Moreover, some vases appear to have been produced almost exclusively for export to markets outside of Athens and Greece, with illustrations of mythology, religion, arts, athletics, and daily life of Greece depicted on them (Avramidou 2011).

The catalogue of the plant species represented is thus very distinct and divergent from the contemporary Iberian indigenous population's mainstream graphics, overall imagery and known flora (Mata & al. 2010), except in a few cases.

It was the destination market for such imported artefacts (their connection with death, burial and incineration practices; or, for luxury vases and tableware, with feasts and wine drinking in the domestic sphere) that basically determined the depicted scenes, subject areas and represented plant species, as well as the artistic quality (and the price). The most repeated subjects, in pottery for local customers of Emporion and its surroundings (Ullastret), are clearly those of palestra and the Dionysiac scenes, followed at a distance by banqueting and mythological subjects. Libation, farewell or gynaeceum scenes are in a minority (Aquilué & al. 2017). Indirect references to ceremonies related to plant use can also be found in pottery images. Celebration of the Dionysiac flower festival (*anthestḕria*, from ῾*ánthos* = flower) is interpreted by Miró (2006:247 and Fig. 618) from a set of fragments of a vase, together with other scenes with children, suggesting the adoption of such an Athenian feast in Emporion. Scenic variety diminished in the 4th century BC, following some degree of industrialisation of vase production (Miró 2006:245)

The reverse is also true: the arrival of the Greek red-figure ceramic productions, from the last quarter of the 5th century bc, in Ullastret, made the local workshop practices change greatly, through both technical and aesthetic innovations such as the incorporation of plant motifs: volutes, palmettes, borders of ivy or Olea leaves, among others (Codina & al. 2017). The rose figures of the eastern Rhodes coins inspired those coined by the Rhode mint (Campo 2006). The latter is, perhaps, the best example of a plant motif that originated in situ in the western colonies: the Rhode flowers, viewed from below, provided the city with an important pictorial identity symbol, just as the rose in lateral view gave to Rhodos/Rhodes or celery to Selinous/ Selinunte (Thonemann 2016).

Only a small number of plant species was used in classical ornaments in ancient Greek finds from the Western Mediterranean colonies. Mediterranean evergreen trees or shrubs were preferred. Naturalistic figurative plant representations are mainly restricted to scenic illustration (as character attributes or for environmental context) on red- or black-figure pottery or on coin mintages.

Decorative bands show stylised plant depictions. During the 5th-4th centuries BC, the Attic workshops also produced artefacts with decorative ornamentation, usually classified as "Saint Valentin" or reserved-overpainted. Numerous skyphos or kantharos (around 100 were uncovered in Emporion) show bands, wreaths and garlands of ivy, laurel, myrtle, or olive, alone or combined, sometimes with relatively detailed morphology, sometimes very schematic or of uncertain identity. The detailed study by Miró (2006: 234) will be very useful for increased botanical precision.

Merely ornamental plant patterns have not been included here. Following on ancient (Minoan) naturalistic illustration, plant representations evolved in some cases in the subsequent classical period into floral ornaments, conserving basic features of the inspiration species, but in which representation of the plant itself is no longer the main purpose: it aims only to maintain and evoke the basic shape and motion (thus, palms became palmettes, roses became rosettes, or grapevine shoots became spirals) (Baumann 1991). Although vegetative ornamentation is widely known as an important part of Greek artistic language, its function in the Western Mediterranean, as well as in Greece can, with rare exceptions, be considered as subordinate, framing or complementary, rather than self-sufficient. (On the contrary, in N Macedonia or Apulia, plant motifs frequently acquire exceptional independent value: see Nalimova 2017).

References

Adroher Auroux, A. M. & Rouillard, P. 2017: Cerámica griega en contextos ibéricos del cuadrante sureste peninsular: contextos diversos, problemas diversos, respuestas diversas. Pp. 73-85. In: X. Aquilué, P. Cabrera & M. Orfila (eds), Homenaje a Glòria Trias Rubiés. Cerámicas griegas de la Península Ibérica: cincuenta años después (1967-2017). – Centro Iberia Greca, Empúries.

Amigues, S. 2010: Théophraste. Recherches sur les plantes. À l'origine de la botanique. – Belin, Paris.

Anonymous [Beazley Archive] 1997-2018: Classical Art Research Centre - Beazley Archive Databases [online]. – University of Oxford. Available at http://www.beazley.ox.ac.uk/index.htm [Acceded 30/10/2018].

Anonymous [e-Floras] (2008): Flora of China (9:354) [online]. – Missouri Botanical Garden, St. Louis & Harvard University Herbaria, Cambridge. Available at http://www.efloras.org [Accessed 15 Nov 2018].

Anonymous [Arxiu CBV] 2016: Museu d'Arqueologia de Catalunya (Girona) / Visita 2016. – Foto CBV-0049/0050.

Aquilué, X. & Cabrera, P. (coord.). 2012: Iberia Graeca. El legado arqueológico griego en la Península Ibérica. – Centro Iberia Graeca, Empúries.

Aquilué, X., Castanyer, P., Santos, M. & Tremoleda, J. 2007: Empúries. Guies del Museu d'Arqueologia de Catalunya. – Generalitat de Catalunya, Empúries.

—, Carreras, P. & Miró, M. T. 2017: Presencia de los talleres de cerámica ática en los poblados ibéricos indiketes y su relación con Emporion (Empúries). Pp. 97-110. In Aquilué, X., Cabrera, P. & Orfila, M. (eds), Homenaje a Glòria Trias Rubiés. Cerámicas griegas de la Península Ibérica: cincuenta años después (1967-2017). – Centro Iberia Greca, Empúries.

Arroyo-García, R., Martínez-Zapater, J. M., Fernández Prieto, J. A. & Álvarez-Arbesú, R. 2001: AFLP evaluation of genetic similarity among laurel populations (*Laurus* L.). – Euphytica 122: 155-164.

Asensio, D., Jornet, R., Morer, J. & Pons, E. 2014: El complex arqueològic del jaciment ibèric del Mas Castellar de Pontós (Alt Empordà). Les campanyes 2012-2013. Pp. 127-236. In Frigola, J. (ed.), Dotzenes Jornades d'Arqueologia de les Comarques Gironines, Besalú. Llibre d'Actes. – Generalitat de Catalunya, Girona.

Avramidou, A. 2011: *The Codrus painter*. – University of Wisconsin Press, Madison.

Baumann, H. 1991: La Botanique dans l'antiquité grecque. – Bot. Chron. 10: 481-494.

Blanché, C. 2016: Aportacions a Flora Catalana Antiga, 1. *Hedera helix* L. (Araliaceae). – Rev. Soc. Cat. Hist. Farm. 31: 21-28.

— 2019: Aportacions a Flora Catalana Antiga, 2. Elements orientalitzants. – Rev. Soc. Cat. Hist. Farm. 34: 91-113.

Bolòs, O. de., Vigo, J., Masalles, R. M. & Ninot, J. M. 2005: Flora Manual dels Països Catalans, ed. 3. – Pòrtic, Barcelona.

Burjachs, F., Bach, J., Buxó, R., Llàcer, P., McGlade, J., Picazo, M., Piqué, R. & Ros, M. T. 2005: El territori d'Emporion i les seves dades paleoambientals. – Empúries 54: 25-32.

Buxó, R. 2001: L'origen i l'expansió de l'agricultura a l'Empordà. – CCG Edicions, Girona.

Cabrera, P. & Aquilué, X. (coord.). 2008-2018: Base documental *Iberia Graeca* [online]. Ministerio de Educación y Cultura, Museo Arqueológico Nacional, Generalitat de Catalunya, Museu d'Arqueologia de Catalunya. – Available at www.iberiagreca.com [Accessed 01/11/2018].

Campo, M. 2006: La moneda a Rhode: producció i circulació. Pp. 575-58. In Puig A. M. & Martin A. (coord.), La colònia grega de Rhode (Roses, Alt Empordà). – Museu d'Arqueologia de Catalunya, Girona.

Canal, D. 2001: Anàlisi carpològica de la concentració de llavors de la fossa Fs6 del Mas Castellar de Pontós: un repte interpretatiu. – Cypsela 13: 219-230.

Castanyer, P., Santos, M., Aquilué, X., Tremoleda, J., Pons, E., Martin, A., Rovira, M. C. & Mata, J. M. 2008: Elaboración y comercio de plata y plomo en la Emporion griega y en los hábitats de su entorno. – Rev. Arqueol. Ponent 18: 270-291.

Castro, Z. & Hopf, M. 1982: Estudio de restos vegetales en el poblado protohistórico Illa d'en Reixach (Ullastret, Gerona). – Cypsela 4: 103-111.

Codina, F., Martín, A. & de Prado, G. 2017: La influencia de la cerámica ática en las producciones del período ibérico pleno en Ullastret. Pp. 150-162. In: Aquilué, X., Cabrera, P. & Orfila, M. (eds.), Homenaje a Glòria Trias Rubiés. Cerámicas griegas de la Península Ibérica: cincuenta años después (1967-2017). – Centro Iberia Greca, Empúries.

Crespo, M. B., Herrero, A. & Quintanar, A. (eds) 2013: Iridaceae. Pp. 400-491. In Castroviejo S. & al. (eds), Flora Iberica, vol. 20. – Real Jardín Botánico-CSIC, Madrid.

Del Barrio, C., 2017: Orfeo entre los tracios en una cratera de Villaricos. Pp. 163-169. In Aquilué, X., Cabrera, P. & Orfila, M. (eds), Homenaje a Glòria Trias Rubiés. Cerámicas griegas de la Península Ibérica: cincuenta años después (1967-2017). – Centro Iberia Greca, Empúries.

Faure, P. 1987: Parfums et aromates de l'Antiquité. – Fayard, Paris.

GERMER, R. 1985: Flora der pharaonischen Ägypten. – Verlag Philipp Von Zabern, Main am Rhein.

IZQUIERDO, P. 1996: Los condicionantes de la navegación en la antigüedad: una aproximación al caso de la *Provincia Hispania Citerior* mediterránea. Pp. 299-306. In I Simposio de Historia de las técnicas 1995. La construcción naval y la navegación. – Santander.

JASHEMSKI, W. F., MEYER, F. G. & RICCIARDI, M. 2002: Plants. Evidence from wall paintings, mosaics, sculpture, plant remains, graffiti, inscriptions and ancient authors. Pp. 80-180. In JASHEMSKI, W. F. & MEYER, F. G. (eds), The Natural History of Pompeii. – Cambridge University Press, Cambridge.

JASHEMSKI, W. F., GLEASON, K. L., HARTSWICK, K. J., MALE, A.-A. (eds) 2018: Gardens of the Roman Empire. – Cambridge University Press, Cambrige (UK).

KEFALIDOU, E. 2009: The Plants of Victory in ancient Greece and Rome. Pp. 39-44. In MOREL, J. P. & MERCURI, A. M. (eds), Plants and Culture: Seeds of the Cultural Heritage of Europe. – Edipuglia, Santo Spirito.

KUNZE-GÖTTE, E. 2006: Myrte als Attribut und Ornament auf attischen Vasen. Akanthiskos, 1. – Akanthus, Kilchberg.

MARTIN, A. & PLANA R. 2012: Formació i desenvolupament de l'ocupació ibèrica d'Ullastret (Baix Empordà): un centre indígena en una zona de contacte de cultures. – Cypsela 19: 179-192.

MATA, C., BADAL, E., COLLADO, E. & RIPOLLÈS, P. P. (eds) 2010: Flora Ibérica. De lo real a lo imaginario. – Museu de Prehistòria de València. Diputació de València, València.

MATA, C. (co-ord.) 2010-2018: Flora Ibérica [online]. - Universitat de València. – Available at http://www.florayfaunaiberica.org [Accessed 30/10/2018].

MILLER, H. F. 1979: The Iconography of the Palm in Greek Art. – PhD Thesis, University of California, Berkeley.

MIRÓ, M. 2006: La ceràmica àtica de figures roges de la ciutat grega d'Emporion. Monografies Emporitanes 14. – Museu d'Arqueologia de Catalunya, Barcelona.

MONTSERRAT, P. & SILVESTRE, S. 1998: *Rosa* L. Pp. 143-195. In CASTROVIEJO, S. & al. (eds), Flora Iberica, 6. – Real Jardín Botánico-CSIC, Madrid.

NALIMOVA, N. 2017: The Origin and Meaning of Floral imagery in the Monumental Art of Macedonia (4th–3rd centuries BC) [on line]. Bookonlime. – Available at https://bookonlime.ru/lecture/1-origin-and-meaning-floral-imagery-monumental-art-macedonia-4th-3rd-centuries-bc [Downloaded 30/11/2018]

NICOLAU D'OLWER, L. 1928: El pont de la mar blava [Reedition 2017]. – Ed. Adesiara, Martorell.

PASCUAL, G. & PÉREZ BALLESTER, J. 2017: El comercio de vino griego en la península Ibérica (siglos III aC- I dC). Pp. 294-307. In AQUILUÉ, X. CABRERA, P. & ORFILA, M. (eds), Homenaje a Glòria Trias Rubiés. Cerámicas griegas de la Península Ibérica: cincuenta años después (1967-2017). – Centro Iberia Greca, Empúries.

PIQUÉ, R. 2002: Paisatge i explotació forestal durant el I milleni A.N.E. a la plana empordanesa. – Cypsela 14: 211-228.

PONS, E. 2016: El vi a l'època ibèrica a Pontós. Pp. 14-18. In BOIX, L. & TREMOLEDA, J. (eds), Un mar de vinyes. – Museu d'Arqueologia de Catalunya, Empúries.

—, BOUSO, M., GAGO, N. & FERNANDEZ, M. J. 1998: Significació funcional de les sitges amortitzades de Mas Castellar de Pontós: una aproximació metodològica. – Cypsela 12: 63-79.

PUIG, A. M. 2006: Les ceràmiques de vernís negre. Pp. 303-471. In PUIG, A. M. & MARTIN, A. (coord.), La colònia grega de Rhode (Roses, Alt Empordà). – Museu d'Arqueologia de Catalunya, Girona.

— & MARTIN, A. (coord.). 2006: La colònia grega de Rhode (Roses, Alt Empordà). Sèrie Monogràfica, 29. – Museu d'Arqueologia de Catalunya, Girona.

PUJOL, A.: 1989: La población prerromana del extremo nordeste peninsular. Génesis y desarrollo de la cultura ibérica en las comarcas gerundenses, Vol. 2. – CSIC-Universitat Autònoma de Barcelona, Bellaterra.

RIPOLLÈS, P. P. 2010: La flora en las monedas antiguas de la Península ibérica. – Gaceta numismática 177: 3- 28.

— 2012: La numismàtica grega a Ibèria: seques i circulació monetària. Pp. 74-81. In AQUILUÉ, X. & CABRERA, P. (eds), Iberia Graeca. El llegat arqueològic grec a la península Ibèrica. – Ministerio de Educación y Cultura- Generalitat de Catalunya, Empúries.

ROUILLARD, P. 2009: El vaso griego y la Contestania. Pp. 42-50. In OLCINA, M. & SÁNCHEZ J. J. (eds), Huellas Griegas en la Contestania Ibérica. – Museu Arqueològic d'Alacant, Alacant.

RUIZ DE ARBULO, J. & VIVÓ, D. 2008: Serapis, Isis y los dioses acompañantes en Emporion: una nueva interpretación para el conjunto de esculturas aparecido en el supuesto *Asklepeion* emporitano, – Rev. Arqueol. Ponent 18: 71-140.

—, J. 2009: El Altar y el Templo de Augusto en la *Colonia Tarraco*. Estado de la cuestión. In NOGUERA, J. M. (ed.): *Fora Hispaniae*. Actas del Seminario de Lorca (Murcia) celebrado del 23 al 27 de septiembre de 2002. – Monogr. Mus. Arq. Murcia 3: 155-189.

SEGURA, S. & TORRES, J. 2009: Historia de las Plantas en el mundo antiguo. – CSIC-Universidad de Deusto, Deusto.

THONEMANN, P. 2016: The Hellenistic World: Using Coins as Sources. – Cambridge University Press, Cambridge.

VILLAR, L. 1986: *Laurus* L. Pp. 199-201. In CASTROVIEJO, S. & al. (eds), Flora Iberica, 1. – Real Jardín Botánico-CSIC, Madrid.

VILLARONGA, L. 2000: Les monedes de plata d'Empòrion, Rhode i les seves imitacions: de principi del segle III a.C. fins a l'arribada dels romans, el 218 a.C. – Institut d'Estudis Catalans, Barcelona.

— 2003: La plata emporitana de la segona guerra púnica, final del segle III a.C. – Institut d'Estudis Catalans, Barcelona

Common legend explanation / abbreviations for all tables:

REF: FCA Reference number; **LOC**: Location/Site (E= *Emporion;* P= *Palamós;* R= *Rhode;* U=*Ullastret*); [**CENT:** Century BC]; **[B]:** Bronze; **[P]:** Pottery; **[S]:** Sculpture; **[N]:** Numismatics; **[E]:** Epigraphy. Reference (bibliographic record & image source: [F] (ficha) = Card Number; CBV =Author's photographic archive. Objects represented in Fig. 2-4 are highlighted (**bold type**).

Table 1. Iconographic records for ***Hedera helix*** L. (Araliaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	**LOC [CENT]**	**Description**	**Reference**
HE he 151	E [V BC]	**[P]:** Attic cup with tall pedestal foot , restored. Red figures, with scenes of persecution and a tree with fruits of white paint (ZZarbr10), palmettes and ivy leaves, big and loose. MAC-Barcelona Inv. Num. 582	Cabrera & Aquilué, 2008-ss [F 13]
HE he 152	E [V BC]	**[P]:** Attic cup with tall pedestal foot bottom. Red figures, with a Dionysian scene, the god wearing a crown of ivy leaves. MAC-Barcelona Inv. Num. 467. Identified by context.	Cabrera & Aquilué, l.c. [F 19]
HE he 153	E [IV BC]	**[P]:** Attic cup with tall pedestal foot. Red figures, with a conversation scene in the palestra and garland of ivy leaves, overpainted on the inside face.MAC-Empúries Inv. Num. 1443	Cabrera & Aquilué, l.c. [F 31]
HE he 154	**E [V BC]**	**[P]: Edge of Attic *kantharos*. Red figures, with Dionysian scenes. Satyr and maenad with thyrsus of cordiform ivy leaves and border in the form of ornamental garland of "laurel" leaves , opposite and with a prominent midrib (LA no 31). MAC-Barcelona Inv. Num. 579. Identifications sure (Fig. 2C)**	Cabrera & Aquilué, l.c. [F 47]
HE he 155	E [V BC]	**[P]:** Attic cup. Red figures, with Dionysian scenes. Border with naturalistic ivy border: voluble stems, 3-lobed cordiform leaves and globose umbelliform inflorescences. MAC-Barcelona Inv. Num. MAC-B SN RG 3	Cabrera & Aquilué, l.c. [F 2491]
HE he 156	E [IV BC]	**[P]:** Attic *skyphos* cup. Red figures, with Dionysian scenes. Under the edge, scene of young person with end of thyrsus with head of cordiform and acute ivy leaves; decoration of loose ivy leaves. MAC-Barcelona Inv. Num. 546 4429	Cabrera & Aquilué,l.c. [F 2597]

HE he 157	E [V BC]	**[P]:** Attic *skyphos* cup. Red figures, with Dionysian scene (fragment). Under the ovals border at the edge, detail of the end of a thyrsus with head of cordiform and acute ivy leaves. MAC-Empúries Inv. Num. EMP RG SN 7	CABRERA & AQUILUÉ, l.c. [F 2966]
HE he 158	E [V BC]	**[P]:** Fragment of Attic krater. Red figures, with a Dionysian scene (fragment). Detail of gripped thyrsus and head of a character with a crown of cordate and acute ivy leaves. MAC-Empúries Inv. Num. 1424	CABRERA & AQUILUÉ, l.c. [F 2971]
HE he 159	**E [V BC]**	**[P]: Attic *skyphos* cup. Red figures, with Dionysian scene (fragment). Thyrsus and crown of ivy leaves, dressed in celebration. MAC-Barcelona Inv. Num. 535 / Exposed at MAC Empúries [CBV 0049] (Fig. 2B)**	**CABRERA & AQUILUÉ, l.c. [F 2979]; CBV 0049**
HE he 160	E [IV BC]	**[P]:** Attic bell-krater. Red figures, with a Dionysian scene (fragment). Thyrsus doubtfully attributed to ivy and diadem MAC-Barcelona Inv. Num. 554	CABRERA & AQUILUÉ, l.c. [F 2982]
HE he 161	E [IV BC]	**[P]:** Attic calyx-krater. Red figures, with Dionysian scene (fragment). Musician (tambourine = *tympanon*) with ivy crown, with no-lobed leaves, little or not cordate. MAC-Barcelona Inv. Num. 545 & MMM E3	CABRERA & AQUILUÉ, l.c. [F 2983]
HE he 162	E [IV BC]	**[P]:** Attic calyx-krater. Red figures, with Dionysian scene (fragment). Head of character with garland / crown of ivy + head of thyrsus with ivy leaves. MAC-Barcelona Inv. Num. 4502	CABRERA & AQUILUÉ, l.c. [F 3013]
HE he 163	E [V BC]	**[P]:** Attic krater. Red figures, with a Dionysian scene (fragment). Head of character (satyr, with horns) with garland / crown of ivy + head of thyrsus with ivy leaves. MAC-Empúries Inv. Num. 86 N1 7033-69	CABRERA & AQUILUÉ, l.c. [F 3017]
HE he 164	E [IV BC]	**[P]:** Attic krater. Red figures, with a Dionysian scene (fragment). Head of a bearded character with horns (satyr) with ivy garland / crown (the leaves would be confused with the horns) = detail of white paint for the umbel flowers), with golden ribbon / diadem. Bigger and cordate ivy leaves, in red, in the surroundings to fit the scene. MAC-Empúries Inv. Num. 85 MN 5016 1	CABRERA & AQUILUÉ, l.c. [F 3018]
HE he 165	E [IV BC]	**[P]:** Attic pelike. Red figures, with a Dionysian scene (fragment). Head of female character, with earrings, and the top of a thyrsus with leaves attributable (with doubts) to ivy MAC-Barcelona Inv. Num. 555	CABRERA & AQUILUÉ, l.c. [F 3027]

HE he 166	E [V BC]	**[P]:** Attic column-krater. Red figures, with a conversation scene between several figures. Punctiform lines attributable (with doubts) to ivy and lotoid decoration in rib and neck MAC-Empúries Inv. Num. 265 (607 i 17). He he 2 is a parallel.	Cabrera & Aquilué, l.c. [F 3059]
HE he 167	E [V BC]	**[P]:** Attic plastic *oenochoe* jug. Red figures, in the form of a female head; painted garland of schematic ivy in the head. MAC-Girona Inv. Num. 801/14624	Cabrera & Aquilué, l.c. [F 3133]
HE he 168	E [V BC]	**[P]:** Attic column-krater. Red figures, with battle scene with hoplite; scene boundary with double band of ivy leaves. MAC-Barcelona Inv. Num. 477	Cabrera & Aquilué, l.c. [F 3266]
HE he 169	E [VI-V BC]	**[P]:** Attic oenochoe of trilobate mouth. Black figures, with Dionysiac scene (maenad, satyr), framed by arbor and stakes where leaves with possible ivy / vine ceiling function grow. Very simplified and punctured rendering. MAC-Empúries Inv. Num. 2827	Cabrera & Aquilué, l.c. [F 3922]
HE he 170	E [V-IV BC]	**[P]:** Attic lecanis cover (fragment). Red figures, with gynaeceum scene. Neck surrounded by garland of cordiform leaves, probably ivy. MAC-Barcelona Inv. Num. 507	Cabrera & Aquilué, l.c. [F 4302]
HE he 171	E [IV BC]	**[P]:** Euboean bell-krater. Red figures. Fragment of the edge, with border of bay leaves (fruitless) and Dionysian scene with satyr and Dionysus: ivy crown and thyrsus of an indeterminate plant species.) MAC-Barcelona Inv. Num. 539	Cabrera & Aquilué, l.c. [F 4326]
HE he 172	E [IV BC]	**[P]:** Attic alabastron. Black figures, restored. Dionysiac scenes framed by a chesswalk and surrounded by columns and covered with a climb that can be a simplified ivy (or a *Vitis* vine) MAC-Girona- Inv. Num. 14614	Cabrera & Aquilué, l.c. [F 4497]; CBV -0050
HE he 173	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Fragment, with Nike, surrounded by simplified ivy branches.MAC -Barcelona- Inv. Num. 436	Cabrera & Aquilué, l.c. [F 4518]
HE he 174	E [VI-V BC]	**[P]:** Attic oenochoe (without neck). Black figures. Fragment of the body, with Dionysian scene, with maenad surrounded by simplified schematic ivy branches. MAC -Barcelona- Inv. Num. 409	Cabrera & Aquilué, l.c. [F 4554]
HE he 175	E [V BC]	**[P]:** Attic skyphos. Black figures. Fragment, with Dionysian scene, of satyr, surrounded by ivy branches. MAC -Barcelona- Inv. Num. 4506	Cabrera & Aquilué, l.c. [F 4556]

HE he 176	**E [VI-V BC]**	**[P]: Attic ophthalmic cup. Black figures. Fragment of the edge, with an apotropaic eye and with a Dionysian scene, described as with "kantharos, vine branch and myrtle crown". Provisional identification: ivy and myrtle [to be checked]. MAC -Barcelona- Inv. Num. 422 (Fig. 2A)**	**CABRERA & AQUILUÉ, l.c. [F 4559]**
HE he 177	E [VI-V BC]	**[P]:** Body of Attic hydria. Black figures. Fragments with Hermes, possible horse and garland / frame of cordate leaves, similar to ivy, loose. MAC -Barcelona- Inv. Num. 489 i 490	CABRERA & AQUILUÉ, l.c. [F 4645]
HE he 178	E [VI-V BC]	**[P]:** Attic cup. Black figures (fragment, edge). Dionysian scene, with donkey and possibly ivy arbor (or decorative patterns of ivy plant cover).MAC -Barcelona- Inv. Num. 421	CABRERA & AQUILUÉ, l.c. [F 4648]
HE he 179	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Dionysian scene, with the god, characters, dog and hare, covered, possibly, by ivy and / or grape arbor.MAC -Girona- Inv. Num. 816. Other parallels exist.	CABRERA & AQUILUÉ, l.c. [F 4654]
HE he 180	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Dionysian scene, with characters, itiphallic donkey and a tall arched plant cover (below which a thiassos is marching), possibly of ivy / grapevine (unclear). MAC -Empúries- Inv. Num. EMP RG SN 54. Other parallels exist.	CABRERA & AQUILUÉ, l.c. [F 4662]
HE he 181	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Scene with Athena, giant and young characthers. Border with pointed decoration and character entangled by- or from which branches spread in several directions, similar to ivy. MEV- Museu Episcopal de Vic- Inv. Num. 289. Restored. *Emporion* origin discussed.	CABRERA & AQUILUÉ, l.c. [F 4672]
HE he 182	E [V BC]	**[P]:** Attic cup fragment (edge). Black figures. Dionysian scene: Dionysus under creeping branches similar to ivy. MAC - Barcelona- Inv. Num. 438. [Possibly part of the same piece as HE he 183]	CABRERA & AQUILUÉ, l.c. [F 4678]
HE he 183	E [V BC]	**[P]:** Attic cup fragment (edge). Black figures. Dionysian scene with satyr and donkey that seems to want to eat from the upper plant cover, of ivy. MAC - Barcelona- Inv. Num. 450. [Possibly part of the same piece as HE he 182]	CABRERA & AQUILUÉ, l.c. [F 4682]
HE he 184	E [VI BC]	**[P]:** Plastic Siracusan askos, varnished and painted, representing a ram [according to authors, according to others, goat, or ox], with necklace made of a garland of painted ivy leaves. MAC - Barcelona- Inv. Num. 2333.	CABRERA & AQUILUÉ, l.c. [F 5032]

HE he 185	E [V BC]	**[P]:** Tall foot Attic cup. Red figures. Fragment (border). Child and school scene. Ivy leaves. MAC - Barcelona- Inv. Num. 529.	CABRERA & AQUILUÉ, l.c. [F 7037]
HE he 186	**U[V BC]**	**[P]: *H. helix* wreath with fruiting umbels and cordate acute leafs. Red-figure Attic kylix. Ullastret, V BC. MAC-U. (Fig. 2E)**	**CBV 089-091**
HE he 145	**E [II BC]**	**[S]: Decorative *hedera* (schematic leaf remembering ivy) on the sandals wearing the front of a white marble feet (found in Temple M) attributed by RUIZ DE ARBULO & VIVÓ 2008 to Isis (Fig. 2D)**	**RUIZ DE ARBULO & VIVÓ 2008 [Fig. 41, 42 & 43]**

Table 2. Iconographic records for ***Hordeum vulgare*** L. (Poaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	**LOC [CENT]**	**Description**	**Reference**
HO vu 18	E [III BC]	**[N]:** Emporitan drachma with a female head of Persephone wearing ears in the hairstyle (Identified by context as *Hordeum vulgare* L., hulled barley, but could be other gramineae). The ears take the position as if they were a headband on the bottom of the curls band and the bracts divide the rest of the hairstyle. Reverse: standing horse. Prototype I, Lam. I	VILLARONGA, 2000 [Lam. 1]
HO vu 19	E [III BC]	**[N]:** Emporitan drachma with Pegasus, in the reverse, and, in the anverse, with female head that still retains spikes. There are few curls in the band, a little irregular, and finished in small hooks. First emission (Prototype II, Lam. I), beautiful and in style, with a big head and the earring of three pendants, supported by a base formed by two horizontal lines. (Identified by context as *Hordeum vulgare* L., hulled barley, but they could be other gramineae)	VILLARONGA, l.c. [Lam. 1]
HO vu 20	E [III BC]	**[N]:** Emporitan drachma with Pegasus, in the reverse, and, in the anverse, with female head that still retains spikes. Second emission, group 2.1. (Prototype III, Lam. I, called *Punic* type by VILLARONGA, 2000), with the smallest head and spikes in the	VILLARONGA, l.c. [Lam. 1]

		hairstyle of less curls, (Identified by context as *Hordeum vulgare* L., hulled barley, but they could be other gramineae)	
HO vu 21	E [III BC]	**[N]:** Emporitan drachma with Pegasus, in the reverse, and, in the anverse, with female head that still retains spikes. Third emission, group 2.2. , with particular symbols, the crescent and the star, still shows the spikes in the hairstyle in some mintages. Prototype IV, Lam I. (Identified by context as *Hordeum vulgare* L., hulled barley, but they could be of other gramineae).	Villaronga, l.c. [Lam. 1]
HO vu 22	E [III BC]	**[N]:** Emporitan drachma with Pegasus, in the reverse, and, in the anverse, with female head only with traces of spikes. Hairstyling with a single ruler of curls, some ending hooked, some others represented simply by parallel lines. The ear and the bracts of the first emissions have become lines arranged in the form of a diadem that supports the hairstyle, which is very simplified. (Identified by context as *Hordeum vulgare* L., hulled barley, by analogy to HO vu 18-21, but they could be other gramineae). Prototype 1C, coin 204	Villaronga, l.c. [Lam. 1]
HO vu 23	**E [III BC]**	**[N]: Emporitan drachma with female head with spikes and mint legend, on the anverse, and standing horse crowned by a flying Nike on the reverse (Identified by context as *Hordeum vulgare* L., hulled barley, by analogy to HO vu 18-21, but they could be other gramineae). MAN - Museo Arqueológico Nacional. (Fig. 3A)**	Ripollès, 2012 [Fig. 7.5]
HO vu 24	R [III BC]	**[N]:** Silver drachma from Roses with a female head with spike and mint legend on the anverse (ΡΟΔΕΤΩΝ = *Rodeton*, 'those of Roses'), with a rose underneath, on the reverse. (Identified by context as *Hordeum vulgare* L., hulled barley, by analogy to HO vu 18-21, but could be other gramineae)	Campo, 2006 [Fig. 14]
HO vu 25	**R [III BC]**	**[N]: Silver drachma from Roses with a female head with a trident and two spikes, and the mint legend (ΡΟΔΕΤΩΝ = *Rodeton*, 'those of Roses') on the anverse, with a rose underneath, on the reverse side. (Identified by context as *Hordeum vulgare* L., hulled barley, by analogy to HO vu 18-24, but could be of other gramineae) (Fig. 3B)**	**Campo, 2006 [Fig. 14. 11, 12 & 13]**

Table 3. Iconographic records for ***Iris sp***. (Iridaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	**LOC [CENT]**	**Description**	**Reference**
IR sp 1	E [VI BC]	**[P]:** Corinthian aryballos. Black figures. Fragment with nude male figure, with iridoid flower (lotoid?) at his feet.- MAC-Empúries Inv. Num. EMP RG SN 78	CABRERA & AQUILUÉ, 2008-ss [F 5287]

Table 4. Iconographic records for ***Laurus nobilis*** L. (Lauraceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	**LOC [CENT]**	**Description**	**Reference**
LA no 1	E [IV BC]	**[N]:** Portrait of Apollo (three-quarter), with face crowned by laurel	RIPOLLÈS 2010
LA no 30	E [V BC]	**[P]:** – Attic stamnos. Red figures, with scenes from the Trojan war. Character with (pressumed) laurel wreath. MAC-Barcelona Inv. Num. 589– N.v. / To be checked	CABRERA & AQUILUÉ, 2008-ss [F 18]
LA no 31	E [V BC]	**[P]:** Attic kantharos (edge). Red figures, with Dionysian scenes: satyr and maenad with thyrsus of cordate ivy leaves [HE he 154] and border in the form of ornamental garland of "laurel" leaves, drawn as opposite and with a prominent midrib. MAC-Barcelona Inv. Num. 579- Clear identifications.	CABRERA & AQUILUÉ, l.c. [F 47]
LA no 32	E [IV BC]	**[P]:** Attic bell-krater. Red figures, with banquet and palestra scenes, with a branch of hanging grapes (very schematic) [VI vi 51]. Border under the edge, in the form of ornamental garland of "laurel" leaves, drawn as opposite, also of poor quality. MAC-Empúries Inv. Num. 1495	CABRERA & AQUILUÉ, l.c. [F 53]
LA no 33	E [V BC]	**[P]:** Attic amphora of panathenaic type (restored). Red figures, with mythological scenes, with laurel wreath and laurel branch. MAC-Barcelona Inv. Num. 540 (coming from 'Necrópolis del Portixol, tumba del Guerrero, (Colección Alfarás)')	CABRERA & AQUILUÉ, l.c. [F 57]

LA no 34	E [V BC]	**[P]:** Attic plastic oenochoe, representing a female head with "laurel" crown. Botanical identity provisional, to be confirmed. MAC-Empúries Inv. Num. 2362 (coming from 'Necrópolis Martí, inhumació 19')	Cabrera & Aquilué, l.c. [F 61]
LA no 35	**E [IV BC]**	**[P]: Attic bell-krater. Red figures, with Dionysian scenes. Border under the edge, in the form of an ornamental garland of "bay leaves", drawn opposite, of medium quality. MAC-Empúries Inv. Num. EMP RG SN 8 (Fig. 3C)**	**Cabrera & Aquilué, l.c.** **[F 2978]**
LA no 36	**E [IV BC]**	**[P]: Attic bell-krater. Red figures, fragment. Border under the edge, in the form of an ornamental garland of "laurel" leaves, drawn opposite, of medium quality, bearing fruits. MAC-Empúries Inv. Num. EMP RG SN 13 (Fig. 3D)**	**Cabrera & Aquilué, l.c.** **[F 3012]**
LA no 37	E [V BC]	**[P]:** Attic bell-krater. Red figures, fragment. Male character with a thyrsus on the hand (only trunk) and crown of bay leaves (well differentiated from those of ivy), fruitless, overlaid over the hair. MAC-Empúries Inv. Num. 86 N1 7033-73	Cabrera & Aquilué, l.c. [F 3016]
LA no 38	E [V BC]	**[P]:** Attic pelike (with handles). Red figures. Centauromachy complex with many characters. One of them, with crown of bay leaves (well differentiated from those of ivy), fruitless. MAC-Empúries Inv. Num. 1494	Cabrera & Aquilué, l.c. [F 3061]
LA no 39	E [V BC]	**[P]:** Neck fragment of Attic bell-krater. Red figures. Mythological scene of Melanippos; on the neck, border of bay leaves, without visible fruits. MAC-Barcelona Inv. Num. 537	Cabrera & Aquilué, l.c. [F 3265]
LA no 40	E [V BC]	**[P]:** Neck fragment of Attic bell-krater. Red figures. Young characters scene in banquet; on the neck, border of bay leaves, without visible fruits. Text of the card indicates *hojas de olivo, hojas de laurel* [sic] MAC-Barcelona Inv. Num. 553/566	Cabrera & Aquilué, l.c. [F 4295]
LA no 41	E [IV BC]	**[P]:** Attic bell-krater. Red figures. Fragment with character (Apollo?, Dionysius?) with crown of pressumed bay leaves (but they could be a different species, distinct pattern). MAC-Girona Inv. Num. 98602	Cabrera & Aquilué, l.c. [F 4307]
LA no 42	E [IV BC]	**[P]:** Euboaean bell-krater. Red figures. Edge fragment, with border of bay leaves (fruitless) and Dionysian scene with satyr and Dionysus: ivy crown and thyrsus of an indeterminate plant speciesMAC-Barcelona Inv. Num. 539	Cabrera & Aquilué, l.c. [F 4326]
LA no 43	E [IV BC]	**[P]:** Attic bell-krater. Red figures. Fragment with tripod + crown possibly (doubtful identification) of laurel. Scene of theatrical triumph. MAC-Barcelona Inv. Num. 4302	Cabrera & Aquilué, l.c. [F 7167]

LA no 44	**E [V BC]**	**[P]: Attic bell-krater. Red figures. Fragment with male figure with (pressumed) laurel crown (*corona de laurel* [sic]). MAC-Barcelona Inv. Num. 4508 (Fig. 3E)**	**Cabrera & Aquilué, l.c. [F 7173]**
LA no 45	E [IV BC]	**[P]:** Attic bell-krater. Red figures. Fragment with border of bay leaves (or olive tree garland, doubtful). MAC-Barcelona Inv. Num. 271 (571)	Cabrera & Aquilué, l.c. [F 7176]

Table 5. Iconographic records for ***Myrtus communis*** L. (Myrtaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
MY co 3	**E [V BC]**	**[P]: Attic cup low footed. Red figures. Fragment with a gynaeceum scene: female character, with a crown of "myrtle" leaves in hand. MAC-Barcelona Inv. Num. 4420 (not morphologically sure, identified by context) (Fig. 3F)**	**Cabrera & Aquilué, 2008-ss [F 33]**
MY co 4	E [V BC]	**[P]:** Attic alabastron. Black figures. Scene with siren, aulos and tomb; bottom left, possible branch of myrtle (insecure, all the piece very worn)MAC-Girona Inv. Num. 827	Cabrera & Aquilué, l.c. [F 4552]
MY co 5	E [V BC]	[P]: Attic alabastron. Black figures. Scene with owl, and possible branch of olive tree (by the context), or of linear leaves although identified by the source as myrtle (*rama de mirto* [sic]), insecure. MAC-Girona Inv. Num. 808	Cabrera & Aquilué, l.c. [F 4553]
MY co 6	**E [V BC]**	**[P]: Attic ophthalmic cup. Black figures. Fragment of the edge, with an apotropaic eye and with a Dionysian scene, described as with "kantharos, vine branch and myrtle crown". Provisional identification: ivy and myrtle/laurel [to be checked] MAC -Barcelona- Inv. Num. 422 (Fig. 2A)**	**Cabrera & Aquilué, l.c. [F 4559]**
MY co 7	**U [V BC]**	**[P]: Skyphos with wreath of myrtle leaves, opposite, acute, wider at base, shortly petiolate (with some distal excrescence). MAC-Ullastret (Fig: 3G)**	**CBV - 0092**

Table 6. Iconographic records for ***Nymphaea coerulea*** Savigny (Nymphaeaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
NY co 32	E [V BC]	**[P]:** Attic column-krater. Red figures. Conversation scene between several figures. Punctiform lines attributable (with doubts) to ivy + edge and neck with lotoid decoration neck. MAC-Empúries Inv. Num. 265 (607 i 17). Parallel to NY co 7	Cabrera & Aquilué, 2008-ss [F 3059]
NY co 33	E [V BC]	**[P]:** Black varnish Attic lekythos. Palmettes framed below by an schematic "lotus" flower. MAC-Empúries Inv. Num. 4422. It has several parallels.	Cabrera & Aquilué, l.c. [F 3938]
NY co 34	E [V BC]	**[P]:** Attic lekythos. Black figures. Palmettes framed below by an schematic "lotus" flower - MAC-Empúries Inv. Num. EMP RG SN 47	Cabrera & Aquilué, l.c. [F 4533]
NY co 24	**E [V BC]**	**[S]: Element of the acrotherion of the Temple roof: antefixes with palmettes and schematic lotus flowers with pattern similar to Ebusitan decorated eggs. (Fig. 4A)**	**CBV-041**

Table 7. Iconographic records for ***Olea europaea*** L. (Oleaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
OL eu 5	E [IV BC]	**[N]:** Silver coins minted in *Emporion* (Villaronga 1994: 4, no. 6 and Fig 9). They reproduce the design of V-IV BC Athens hemidrachmas. Anverse: galeate Athenian head. Reverse: frontal owl, surrounded by two olive branches, very simplified (identified by context).	Ripollés 2010 [Fig. 9]
OL eu 11	**P [I-II AD]**	**[B]: Bronze Jar with Dionysiac motifs, including olive branch. Subaquatic finding (Palamós). MAC-Girona (Fig. 4B)**	**CBV – 0093 & 0094**

OL eu 31	**E [V BC]**	**[P]: Edge of Attic skyphos. Red figures. Fragment with owl and olive branch with leaves. MAC-Barcelona Inv. Num. 410. (Identified by context, not by morphology) (Fig.4G)**	**Cabrera & Aquilué, 2008-ss [F 44]**
OL eu 32	E [V BC]	**[P]:** Edge of Attic skyphos. Red figures. Fragment with owl and olive branch with leaves. MAC-Barcelona Inv. Num. 506. (Identified by context, not by morphology)	Cabrera & Aquilué, l.c. [F 4291]
OL eu 33	E [V BC]	**[P]:** Edge of Attic skyphos. Red figures. Fragment with owl and olive branch with a leaf fragment, to the right of the bird. MAC-Barcelona Inv. Num. 550. (Identified by context)	Cabrera & Aquilué, l.c. [F 4292]
OL eu 34	E [IV BC]	**[P]:** Campanian hydra. Red figures. With a) owl and olive branch (by context; morphology not very different from laurels represented in other pieces) with several leaves, to the right of the bird and b) pteridophytic roll [PT ss 5] . MAC-Girona Inv. Num. 790.	Cabrera & Aquilué, l.c. [F 5062]
OL eu 35	E [V BC]	**[P]:** Edge of Attic skyphos. Red figures. Owl and olive branch (by context) with several leaves, to the right of the bird. MAC-Barcelona Inv. Num. 508-1	Cabrera & Aquilué, l.c. [F 7160]
OL eu 36	E [IV BC]	**[P]:** Attic bell-krater. Red figures. Fragment with border and garland of laurel/olive leaves (to be cheched). MAC-Barcelona Inv. Num. 271 (571)	Cabrera & Aquilué, l.c. [F 7176]

Table 8. Iconographic records for ***Phoenix dactylifera*** L. (Arecaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
PH da 52	**E [V BC]**	**[P]: Attic alabastron. Red figures. Funerary scene with female figure on one side and palm tree on the reverse. Museu d'Art de Girona Inv. Num. MD 788 (Fig. 4F)**	**Cabrera & Aquilué, 2008-ss [F 3134]**

Table 9. Iconographic records for ***Rosa sp.*** (Rosaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
RO sp 1	R [III BC]	**[N]:** Reverse: rose seen from below (main type)	Ripollès, 2010 [Fig. 24]
RO sp 2	R [III BC]	**[N]:** Reverse: rose seen from below (main type)	Ripollès, l.c. [Fig. 25]
RO sp 4	R [IV-III BC]	**[N]:** Drachma from *Rhode*. Reverse: rose seen from below (main type)	Mata, 2010-ss [F 001 027 028]
RO sp 5	R [IV-III BC]	**[N]:** Drachma from *Rhode*. Reverse: flower of 4 double petals with reproductive verticils. Sepals emerging at petals junction. Overhead view.	Mata, l.c. [F 5]
RO sp 6	R [IV-III BC]	**[N]:** *Rhode* bronze divider. Reverse: multipetal flower of 8 petals with well marked reproductive organs. Overhead view.	Mata, l.c. [F 312]
RO sp 7	R [IV-III BC]	**[N]:** Tritetartemorion from *Rhode*. Reverse: solitary flower of 5 petals, marked sepals. View from below	Mata, l.c. [F 359]
RO sp 8	R [IV-III BC]	**[N]:** Trihemitetartemorion from *Rhode*. Reverse: rose in longitudinal view	Mata, l.c. [F 360]
RO sp 9	R [IV-III BC]	**[N]:** *Rhode* bronze divider. Reverse: rose in longitudinal view	Mata, l.c. [F 361]
RO sp 14	U [III BC]	**[N]:** Rhode silver drachma found at Ullastret. MAC-Ullastret Num. Inv. 4237	Castanyer *et al.,* 2008 [Fig. 32]
RO sp 23	R [III BC]	**[N]:** Group 1 (1a) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') with monogram AT / ANT; Rev: rose view from below, good style. 23 copies documented. Fig.14.1	Campo, 2006 [Fig. 14.1]

RO sp 24	R [III BC]	**[N]:** Group 1 (1b) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') with monogram AT / ANT; Rev: rose view from below, similar to 1a, with bigger inner circle of the largest petals. 5 copies documented. Fig. 14.2	CAMPO, l.c. [Fig. 14.2]
RO sp 25	R [III BC]	**[N]:** Group 1 (1c) – Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') with monogram AT / ANT; Rev: rose view from below, similar to 1b, with schematic style. bigger inner circle of the largest petals. 7 copies documented. Fig. 14.3	CAMPO, l.c. [Fig. 14.3]
RO sp 26	R [III BC]	**[N]:** Group 2 (2a) – Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') without mark; Rev: rose view from below, good style. 14 copies documented. Fig. 14.4	CAMPO, l.c. [Fig. 14.4]
RO sp 27	**R [III BC]**	**[N]: Group 2 (2b) – Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') without mark; Rev: rose view from below, good style, similar to group 2a. 6 copies documented. Fig. 14.5 (Fig. 4C)**	**CAMPO, l.c. [Fig. 14.5]**
RO sp 28	R [III BC]	**[N]:** Group 3 - Silver tritetartemorion. Anv: female head to the right, unmarked; Rev: "globule and radius" according to the author, interpretation as *Rosa sp*. (or other pentapetal flower) seen above, with radial expansions (which are deficient as polyandry representation, as there are few) 6 copies documented. Fig. 14.6	CAMPO, l.c. [Fig. 14.6]
RO sp 29	R [III BC]	**[N]:** Group 4 - Silver trihemitetartemorion. Anv: female head to the right, unmarked; Rev: rose, side view, Rodian type. 1 copy documented. Fig. 14.7	CAMPO, l.c. [Fig. 14.7]
RO sp 30	R [III BC]	**[N]:** Group 5 - Bronze. Anv: female head to the right, unmarked; Rev: rose, side view, Rodian type. 45 copies documented. Fig. 14.8 to 10	CAMPO, l.c. [Fig. 14.8-10]
RO sp 31	R [III BC]	**[N]:** Group 6 (6a) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') behind: trident; Rev: rose view from below, good style. 2 copies documented. Fig. 14.11	CAMPO, l.c. [Fig. 14.11]
RO sp 32	R [III BC]	**[N]:** Group 6 (6b) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') behind: trident (similar to group 6a); Rev: rose view from below, simplified style. 2 copies documented. Fig. 14.12	CAMPO, l.c. [Fig. 14.12]
RO sp 33	**R [III BC]**	**[N]: Group 6 (6c) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') behind: trident (similar to group 6a);**	**CAMPO, l.c. [Fig. 14.13]**

		Rev: rose view from above, with polyandric button (9 stamens). 3 copies docu-mented. Fig. 14.13 (Fig. 4E)	
RO sp 34	R [III BC]	**[N]:** Group 7 - Bronze. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') behind: trident (similar to group 6c); Rev: rose view from above, with diffuse polyandric button; simplified design, good style. 12 copies documented. Fig. 14.14 to 14.17	Campo, l.c. [Fig. 14.14-17]
RO sp 35	R [III BC]	**[N]:** Group 8 (8a) - Silver drachma. Anv: female head to the left, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') without mark. Style similar to that of drachmas with standing horse from *Emporion*. Rev: rose view from below, simplified design, good style. 12 copies documented. Fig. 14.18	Campo, l.c. [Fig. 14.18]
RO sp 36	**R [III BC]**	**[N]: Group 8 (8b) - Silver drachma. Anv: female head to the left, small, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') without mark. Rev: rose view from below, simplified design, good style (similar to group 8a). 3 copies documented. Fig. 14.19 (Fig. 4D)**	**Campo, l.c. [Fig. 14.19]. Mata & al, 2010 [Fig. 11]**
RO sp 37	R [III BC]	**[N]:** Group 8 (8c) - Silver drachma. Anv: female head to the left, small, external legend ΡΟΔΕΤΩΝ (*Rodeton*, 'those from Roses') without mark (similar to group 8b). Rev: rose view from below, simplified design, schematic style. 3 copies documented. Fig. 14.20	Campo, l.c. [Fig. 14.20]
RO sp 15	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal of a rose, printed on the handle, with the inscription <EPI [---] AR [---] KOL>. MAC-Empúries Inv. Num. EMP 1084	Cabrera & Aquilué, 2008-ss [F 9789
RO sp 16	**E [II BC]**	**[E]: Transport amphora of Rodian type (Origin: Rodhes), with circular seal of a central rose, printed on the handle, with the inscription < EPI XENOFANTOY DAL[---] >. MAC-Empúries Inv. Num. EMP RG SN 131 (Fig. 4H)**	**Cabrera & Aquilué, l.c. [F 9790]**
RO sp 17	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal of a central rose, printed on the handle, with the inscription < [---] KSENOFANTO[---] [---]L[---]>. MAC-Empúries Inv. Num. EMP 11568	Cabrera & Aquilué, l.c. [F 9791]
RO sp 18	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal of a central rose, printed on the handle, with the inscription < EPI[-]E[---]E[---]INZI [--] 7> MAC-Girona Inv. Num. 2682	Cabrera & Aquilué, l.c. [F 9800]

RO sp 19	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal with double card, and a rose inscribed in the interior, printed on the handle, with the inscription < IPPOKRATEYS> and a secondary seal with Helios head (or indeterminate flower). MAC-Empúries Inv. Num. EMP 1080	CABRERA & AQUILUÉ, l.c. [F 9898]
RO sp 20	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal with double card, and a rose inscribed in the interior, printed on the handle, with the inscription < IPPOKRATEYS> and a secondary seal with not readable symbol. MAC-Empúries Inv. Num. EMP 1081	CABRERA & AQUILUÉ, l.c. [F 9899]
RO sp 21	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal with double card, and a rose inscribed in the interior, printed on the handle, with the inscription < [----]?[----]U[-]>, with possible secondary seal. MAC-Empúries Inv. Num. EMP 1082	CABRERA & AQUILUÉ, l.c. [F 9900]
RO sp 22	E [II BC]	**[E]:** Transport amphora of Rodian type (Origin: Rodhes), with circular seal with double card, and a rose inscribed in the interior, printed on the handle, with the inscription < ?[-------]EUS>. MAC-Empúries Inv. Num. EMP 1083	CABRERA & AQUILUÉ, l.c. [F 9901]

Table 10. Iconographic records for ***Vitis vinifera*** L. (Vitaceae) from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga data-base (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
VI vi 51	E [IV BC]	**[P]:** Attic bell-krater. Red figures. Banquet and palestra scenes, with a branch of hanging grapes (very schematic). Wreath under the edge, in the form of an ornamental garland of laurel leaves (LA no 32), opposite, also of low quality. MAC-Empúries Inv. Num. 1495	CABRERA & AQUILUÉ, 2008-ss [F 53]
VI vi 52	E [V BC]	**[P]:** Tall foot Attic cup. Red figures. Background, with Dionysian scenes with vine branch [proposed, it could also be ivy]. MAC-Barcelona Inv. Num. 4344	CABRERA & AQUILUÉ, l.c. [F 2898]

VI vi 53	E [IV BC]	**[P]:** Tall foot Attic cup. Edge, with Dionysian scene with thyrsus [doubtful identification: pine cone?, vine? it could also be ivy] and schematic vine grapes hanging (arbor?). MAC-Empúries Inv. Num. EMP RG SN 10	Cabrera & Aquilué, l.c. [F 2987]
VI vi 54	**E [VI-V BC]**	**[P]: Attic oenochoe with trilobate mouth. Black figures. Dionysian scene with satyr, maenad and several schematic vine grapes hanging (arbor?).MAC-Empúries Inv. Num. 2826 (Fig. 4I)**	**Cabrera & Aquilué, l.c. [F 3923]; CBV - 0095**
VI vi 55	E [IV BC]	**[P]:** Attic bell-krater (edge). Red figures. Banquet scene with schematic vine grapes (hanging vine?). MAC-Empúries Inv. Num. 4568	Cabrera & Aquilué, l.c. [F 4327]
VI vi 56	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Dionysian scene with maenads and vine with undefined leaves (Hedera? Vitis shoots?) from which hang it looks like grapes (*racimo de uva* [sic] in Cabrera & Aquilué, *l.c.*). MAC-Girona Inv. Num. 817	Cabrera & Aquilué, l.c. [F 4519]
VI vi 57	E [IV BC]	**[P]:** Campanian pelix. Red figures. Scene with Eros, female figure and several elements of a possible gynaeceum with 5 plant representations: a) palmettes (not listed); b) octopetal rosettes or capitula [ZZ flower 116]; c) rolls of pteridophytic/circinate inspiration; d) branch or stick with pointed acute leaves, on the right side of a female figure with a box in her left hand [ZZ arbr 29]; e) tree or branch of lanceolate leaves and spherical fruit, below the wing of Eros [ZZ arbr 30] .f) possible schematic grapes, under Eros [VI vi 57] [MAC-Girona Inv. Num. 832	Cabrera & Aquilué, l.c. [F 5061]

Table 11. Iconographic records for **undetermined flower** from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
ZZ flor 114	E [V BC]	**[P]:** Attic hydria (fragment). Red figures. Female character (pregnant?) with a diadem of lotoid / iridoid flowers, under a horse with the same ornament, over an unidentified tree branch of simple leaves. MAC-Empúries Inv. Num. 4480	Cabrera & Aquilué, 2008-ss [F 3028]
ZZ flor 115	E [VI-V BC]	**[P]:** N. Aegean oenochoe. Black figures. Indeterminate fragment; swan and unidentified rosette or capitulum. MAC-Barcelona Inv. Num. 4494	Cabrera & Aquilué, l.c. [F 4916]

ZZ flor 116	E [IV BC]	**[P]:** Campanian pelix. Red figures. Scene with Eros, female figure and several elements of a possible gynaeceum with 5 plant representations: a) palmettes (not listed); b) octopetal rosettes or capitula [ZZ flower 116]; c) rolls of pteridophytic/circinate inspiration; d) branch or stick with pointed acute leaves, on the right side of a female figure with a box in her left hand [ZZ arbr 29]; e) tree or branch of lanceolate leaves and spherical fruit, below the wing of Eros [ZZ arbr 30] .f) possible schematic grapes, under Eros [VI vi 57]. MAC-Girona Inv. Num. 832	Cabrera & Aquilué, l.c. [F 5061]
ZZ flor 117	E [V BC]	**[P]:** Attic bridal lebes (fragment). Red figures. Mythology, athlete with helmet. Indeterminate flower or capitulum, with six petals or tube flowers, acute. MAC-Barcelona Inv. Num. 509	Cabrera & Aquilué, l.c. [F 7195]

Table 12. Iconographic records for **undetermined fruit** from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	**LOC [CENT]**	**Description**	**Reference**
ZZ fruit 7	E [V BC]	**[P]:** Attic bell-krater (fragment). Red figures. Female character, with a plate (three-story) with "*frutas y dulces*" [sic] (Cabrera & Aquilué, 2008-ss) in hand. MAC-Barcelona Inv. Num. 444- Unidentifiable.	Cabrera & Aquilué, 2008-ss [F 50]
ZZ fruit 8	E [V BC]	**[P]:** Attic closed vase-krater (fragment). Red figures. Banquet scene. Table with objects, on the left, tray with indeterminate fruit. MAC-Barcelona Inv. Num. 4479	Cabrera & Aquilué, l.c. [F 7204]

Table 13. Iconographic records for **undetermined tree/shrub/plant** from *Emporion* and *Rhode* (Girona, Catalonia) stored at the Flora Catalana Antiga database (FCA, updated 1/11/2018).

REF	LOC [CENT]	Description	Reference
ZZ arbr 20	E [V BC]	**[P]:** Tall foot Attic cup (restored). Red figures. Scenes of persecution and an unidentified tree with white painted fruits, palmettes and ivy leaves. MAC-Barcelona Inv. Num. 582	Cabrera & Aquilué, 2008-ss [F 13]
ZZ arbr 21	E [V BC]	**[P]:** Attic cup (fragment). Red figures. Unidentified tree branch with linear/linear-lanceolate leaves. MAC-Empúries Inv. Num. EMP RG SN 5	Cabrera & Aquilué, l.c. [F 2945]
ZZ arbr 22	E [V BC]	**[P]:** Attic hydria (fragment). Red figures. Female character (pregnant?) with a diadem of lotoid / iridoid flowers, under a horse with the same ornament, over an unidentified tree branch of simple leaves. MAC-Empúries Inv. Num. 4480	Cabrera & Aquilué, l.c. [F 3028]
ZZ arbr 23	E [V BC]	**[P]:** Edge of Attic skyphos. Red figures. Male character, in a farewell scene with a crown of simple leaves not easily identifiable [pressumibly a laurel crown] and, in the hand, an unidentified plant branch, with small simple leaves. MAC-Barcelona Inv. Num. 4303	Cabrera & Aquilué, l.c. [F 4324]
ZZ arbr 24	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Unidentifiable petal decoration (strokes). Mythological scene with bull and undetermined branch, at the foot of Heracles according to a line scheme of punctiform leaves - MAC-Barcelona Inv. Num. 411	Cabrera & Aquilué, l.c. [F 4528]
ZZ arbr 25	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Neck with petaloid strokes. Conversation scene between male and female characters. Indeterminate branch, behind the characters, similar to a vine or scroll, according to a line scheme with punctiform leaves - MAC-Empúries Inv. Num. EMP RG SN 44	Cabrera & Aquilué, l.c. [F 4530]
ZZ arbr 26	E [VI-V BC]	**[P]:** Attic lekythos. Black figures. Neck with petaloid strokes. Dionysiac scene with maenads, donkey and indeterminate tree, with main trunk and schematic punctiform leaves. MAC-Barcelona Inv. Num. 376	Cabrera & Aquilué, l.c. [F 4579]
ZZ arbr 27	E [V BC]	**[P]:** Attic lekythos. Black figures. Scene with siren and unidentified tree with linear trunk, punctiform leaves, incomplete and unclear. MAC-Girona Inv. Num. 809	Cabrera & Aquilué, l.c. [F 4776]
ZZ arbr 28	E [IV-III BC]	**[P]:** Campanian piece with cover. Black varnish. Garland making the whole turn and representing several combined species: four petals or umbel, profile of a capsule or capitulum with duster, fusiform leaf profile or spike. To be identified. MAC-Girona Inv. Num. 832	Cabrera & Aquilué, l.c. [F 5059]
ZZ arbr 29	E [IV BC]	**[P]:** Campanian pelix. Red figures. Scene with Eros, female figure and several elements of a possible gynaeceum with 5 plant representations: a) palmettes (not	Cabrera & Aquilué, l.c. [F 5061]

		listed); b) octopetal rosettes or capitula [ZZ flower 116]; c) rolls of pteridophytic/circinate inspiration; d) branch or stick with pointed acute leaves, on the right side of a female figure with a box in her left hand [ZZ arbr 29]; e) tree or branch of lanceolate leaves and spherical fruit, below the wing of Eros [ZZ arbr 30] .f) possible schematic grapes, under Eros [VI vi 57]. MAC-Girona Inv. Num. 832	
ZZ arbr 30	E [IV BC]	**[P]:** Campanian pelix. Red figures. Scene with Eros, female figure and several elements of a possible gynaeceum with 5 plant representations: a) palmettes (not listed); b) octopetal rosettes or capitula [ZZ flower 116]; c) rolls of pteridophytic/circinate inspiration; d) branch or stick with pointed acute leaves, on the right side of a female figure with a box in her left hand [ZZ arbr 29]; e) tree or branch of lanceolate leaves and spherical fruit, below the wing of Eros [ZZ arbr 30] .f) possible schematic grapes, under Eros [VI vi 57]. MAC-Girona Inv. Num. 832	Cabrera & Aquilué, l.c. [F 5061]
ZZ arbr 31	E [IV BC]	**[P]:** Magna Graecia / Sicilian calyx-krater (various fragments). Red figures. Dionysian scene, with several characters. Tree branch or indeterminate bush (leaves lanceolate, fruits) merging from a rock. Similar to laurel / olive from other samples. MAC-Barcelona Inv. Num. 564,1901,4202,4502,4526,4527	Cabrera & Aquilué, l.c. [F 7210]

The Maamora forest (Morocco): A common heritage of humanity in danger

Mohamed Fennane & Mohamed Rejdali

Abstract

Fennane, M. & Rejdali, M. 2019: The Maamora forest (Morocco): A common heritage of humanity in danger. – Bot. Chron. 22: 291-306.

The Maamora forest (Morocco) is by far the world's largest extant cork oak (*Quercus suber*) forest. During centuries, this forest has played major ecological, economic and social roles, stabilising a wide expanse of sandy soils prone to wind erosion and sand spillage, conveyed by sand storms as far off as Europe. For this reason, and also in view of the forest's many beneficial effects on the stabilisation of local human population and improvement of the region's economy, it becomes imperative to launch an effective recovery and reforestation action, to be funded and supervised by both national and international agencies. Morocco's Maamora cork oak forest stocks on the Atlantic plain between the cities of Rabat and Kenitra. Its total potential area of ca. 300,000 ha has by today been reduced to a mere 50,000 ha of low-density forest, with a very degraded or even absent climax vegetation corollary. Here, research programs and publications relevant to this forest are reviewed, dealing with all its various aspects such as ecology, floristics, faunistics, human culture and socio-economy. Despite the many initiatives and endeavours to protect, conserve and redeem its ecosystems, the future of the Maamora forest remains a subject of grave concern. So far, attempts by decision-makers and managers both failed to contain the forest's rapid regression and decline, mainly due to subsisting heavy, inordinate human pressure. The authors try to devise a way out of the current impasse. They presume that nature itself, assisted by fencing and natural regeneration, might well be able to meet the current challenge. This paper presents a synthetic review of studies devoted to the Maamora forest and initiatives aiming at the safeguard of this unique, mega-diverse forest ecosystem.

M. Fennane, Mohammed V University in Rabat, Institut scientifique, Avenue Ibn Batouta, B.P. 703, Agdal, Rabat, Morocco. E-mail: mohamed.fennane@um5.ac.ma
M. Rejdali, Institut Agronomique et Vétérinaire Hassan II, Madinat Al-Irfane, B.P. 6202, Rabat, Morocco. E-mail: m_rejdali@hotmail.com

Key words: Atlantic plain, ecology, oak forest, forest ecosystem, diversity.

Introduction

Maamora is, undoubtedly, the most famous forest in Morocco: overexploited, well studied, and heavily threatened. This forest is well known for its biogeographical, ecological, floristic, faunistic and socio-economic aspects. Being situated in a region with high human population density, its multiple economic products and services make it an area where man-nature relationships – or perhaps conflicts – are very complex.

For decades, man, regrettably driven by his insatiable needs and armed with increasingly powerful mechanical tools, has kept imposing his interests and has caused a regressive dynamic process which, today, threatens the very survival of the cork oak stands.

However, for nearly a century, the Maamora forest has been at the heart of concern of forestry services, administrative and political authorities. Many studies, reports, seminars, projects, and management plans have been undertaken, but have so far failed to yield concrete, satisfactory results in stopping the hemorrhagic process or, indeed, reverse the negative trend (FENNANE & REJDALI 2015).

At the level of Maamora, the exploitation or conservation issue remains more than ever a "headache" for politicians, managers and, hence, for human society.

The present paper is a modest contribution toward answering the three following questions and envisioning the way ahead.

How and why did we get into this alarming situation?

What can be done to save the cork oak stands and avoid their irrevocable loss?

What are the chances of success of new management actions and visions?

Maamora area: geography, ecology and diversity

According to EMBERGER (1928), the potential area of Maamora is ca. 300,000 ha (Fig. 1), bounded by the Atlantic Ocean to the north-west, the Bou Regreg valley to the south-west, the Beht valley to the east and the latitude parallels of Sidi Yahya Gharb to the north and of Tiddas and Khémisset to the south. The general bioclimate is subhumid to semi-arid, with aridity increasing along a west-to-east and north-to-south gradient.

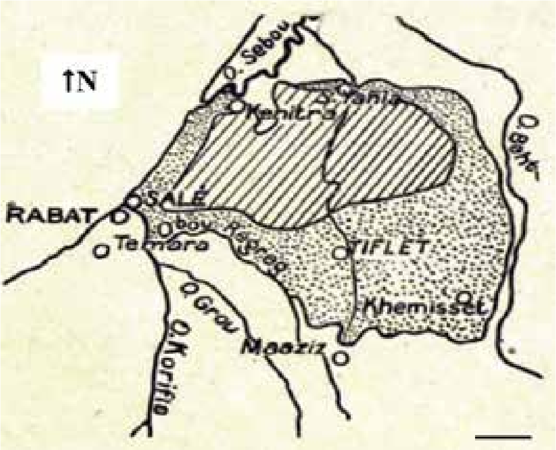


Fig. 1. Maamora area according to Emberger 1928 (scale bar = 10 km). Forest area at the beginning of the 20th Century (hatched) and potential forest area (stippled).

The mean annual rainfall varies from 500 to 300 mm, restricted to ca. 60 days, mainly in the winter period. The dry period lasts at least 5 months (May to September) and coincides with high temperatures; the average maximum temperature, in July and August, is ca. 35°C. Occult precipitation (by mist condensation and dewfall) is frequent and constitutes an important supplementary water supply for the cork oak, which also benefits from the high water table on top of the impermeable clay substrate.

The landscape is a low-lying plateau (at < 300 m of elevation), slightly sloping from east to west, divided into five sectors by the hydrographic network of Sebou and Beht river tributaries running from south-west to north-east. The almost consistently sandy soils are of varying depth, overlying a ± thick layer of clay sandstones or clayey sands, locally rich in pisoliths (reddish or blackish concretions of iron oxide and manganese). Underneath, the substratum consists of marls and gray clays of Tortonian (Miocene) age. The presence of hydromorphic-type soils, in dayas where the cork oak (and any other woody species) are patently absent, is also of note.

The cork oak is the overarching element of this vast, apparently homogeneous climax ecosystem, which nevertheless shows significant diversity. Thus, at least four subsets of unequal importance can be distinguished: cork oak woods, mixed matorral, dayas and a littoral dune belt of Red juniper stands (Fig. 2).

The cork oak woods

Cork oak woods proper are mainly present in the western part of the Mamoora, where the physical environment is most suitable: sufficient moisture and sandy soil. These conditions cater for a good resilience of the ecosystem. The tree stratum is fairly uniform, dominated by *Quercus suber* L., in addition to isolated individual trees of a local endemic, *Pyrus mamorensis* Trab. The undergrowth shows a specific composition, being normally very rich in woody and herbaceous species, with (Fig. 3): *Teline linifolia* (L.) Webb & Berth., *Thymelaea lythroides* Barratte & Murb[1]*., Phillyrea latifolia* L., *Halimium halimifolium* (L.) Willk., *Cistus salviifolius* L., *Lavandula stoechas* L., *Stauracanthus boivinii* (Webb) Samp., *Stauracanthus genistoides* (Brot.) Samp.[2], *Daphne gnidium* L., *Solanum sodomeum* L., *Chamaerops humilis* L., *Ferula communis* L., *Tuberaria guttata* (L.) Fourr., *Cladanthus mixtus* (L.) Chevall., *Asphodelus ramosus* L., *Asphodelus gracilis* Braun-Blanq. & Maire[1].

The cork oak woods show several facies, expressed by a variation in total tree coverage, especially by cork oak, and by the nature of the floristic composition of the undergrowth. Facies differences are the result of natural and/or artificial factors acting separately or in concert. The main natural factor is related to the soil, in particular the depth of the sand (LEPOUTRE 1965); artificial factors relate to degradation by humans and domestic ani mals. In this paper, we will deal with cork oak woods in particular.

---

[1] Endemic to Morocco and the Iberian Peninsula
[2] Endemic to Morocco

Fig. 2. The main Maamora vegetation formations: a) Cork oak woods; b) Mixed matorral; c) Daya; d) Red juniper stands.

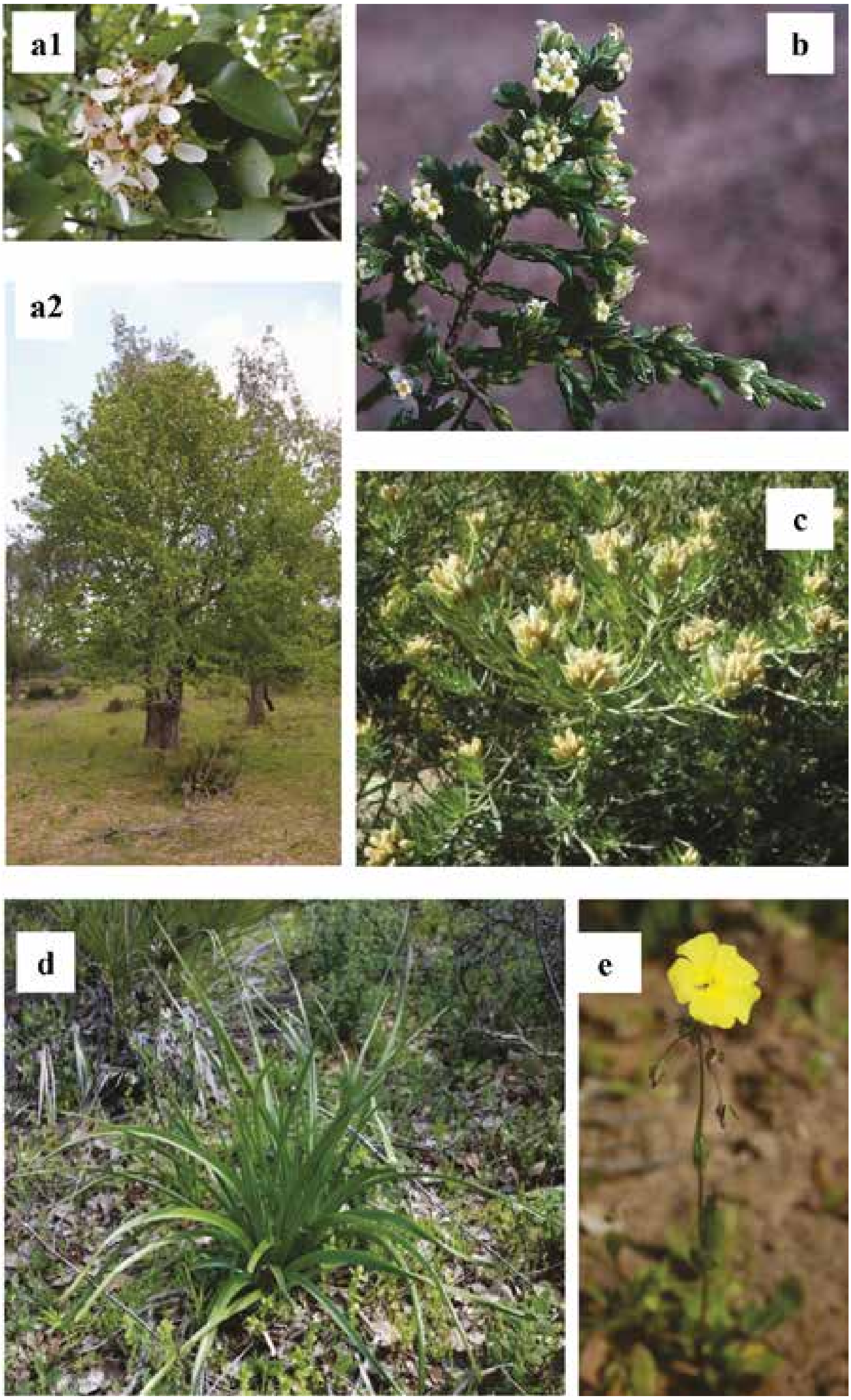


Fig. 3. Some interesting plant species of Maamora: a1 & a2) *Pyrus mamorensis*; b) *Thymelaea lythroides* ; c) *Teline linifolia*; d) *Asphodelus gracilis*; e) *Tuberaria guttata.*

The mixed matorral

This formation, very degraded nowadays, occupies the relatively dry eastern part of the Maamora, growing on hard substrates. It became much fragmented and, anyway, has completely lost its cork oak component. Its current remains are bunches of matorral of *Olea europaea* subsp. *oleaster* (Hoffmanns. & Link) Negodi, *Rhus pentaphylla* (Jacq.) Desf., *Phillyrea latifolia* L., etc.

The dayas

Dayas are ± widespread. They are wet depressions, temporarily covered with shallow water after rainfalls. Such areas are unsuited for the growth of trees and shrubs, but instead are covered with a rich, remarkably diverse and original herbaceous flora of therophytes and hemi-cryptophytes, with *Ranunculus aquatilis* L., *Panicum repens* L., *Illecebrum verticillatum* L., *Lemna gibba L.*, *Lemna minor* L., etc.

The red juniper

Red Juniper scrub covers the coastal dune belt, where red juniper, being is resistant to salt spray (salted sea water carried away by winds) and, unlike the cork oak, indifferent to the calcareous nature of the soils is at an advantage. The biological reserve of Mehdia includes a nice example of this juniper. It has remained in good condition for decades, thanks to the protection of which it has benefited.

Ecological history and recent evolution of Maamora

Until the beginning of the 20th century, the Maamora cork oak was in relatively good shape and ecologically well balanced. Like the argan tree in the Souss area, here the cork oak formed the largest plain forests in Morocco. BOUDY (1958) estimates its surface at 135,000 ha, and speaks of a forest of fair appearance, regular and sufficiently vigorous, without any alarming symptom concerning the permanence of the cork oak. A “happy” situation that could to a large extent be explained by the then prevailing hostility between the different tribes of the region and the climate of insecurity prevailing in the forest.

From the 1920s onward, the situation was to deteriorate radically, for three main reasons:

- The anthropogenic pressure on the forests increased dramatically owing to the needs of a rapidly growing population; by 2011, the population exceeded 340,000 inhabitants (HCEFLCD 2015) spread over 300 slum settlements (AAFI 2007);
- The equipment and implements used for the exploitation of natural resources are increasingly fearful;
- The forest management policies applied are incongruent with ecological standards and the tenets of sustainable development.

Products and goods extracted from cork oak woods:

The socio-economic role of the Maamora forest has always been of great importance. It offers employment for more than 300,000 working days to the local population, thus providing significant financial resources to the human communities (Fig. 4).

As an indication: the cumulative revenue of the Maamora forestry services (natural and artificial forests: *Quercus suber*, *Eucalyptus* spp., *Pinus* spp., *Acacia* spp.) between 1992 and 2009 attained 1,517,274,832 MAD [ca. 158,000,000 US$] (HCEFLCD 2015), corresponding to an annual average of 84,293,046 MAD (ca. 7,800,000 € / year).

The forest the subject of regular exploitation by the forestry administration and by the locals who have users' rights. Illegal exploitation practices, may sometimes reach unreasonable levels, especially in times of scarcity. Poaching (illegal harvesting, overgrazing, harvesting of non-timber products, etc.) is a serious permanent problem.

Finally, and for many years now, the forest has been damaged and mistreated by unauthorised picnic habits of the neighboring urban population. The number of visitors seeking recreational space and during their leisure time reaches daily peaks of up to 30,000 people and 5000 cars (FENNANE & REJDALI 2015) (Fig. 5).

Recent studies commissioned by the forest administration have dealt in considerable detail with all these aspects (HCEFLCD 2014, 2015 and 2016). Some examples follow:

- Firewood: c. 200,000 m^3 / year, compared with 97,000 m^3 between 1950-1955 (BOUDY 1958).
- Industrial wood: 350,000 m^3 of Eucalyptus and Pines, i.e. 50% of the national production.
- Cork: 7,000 tons / year, representing 70% of the national production
- Pasture: 24 million forage units / year; 336,518 sheep and 90,553 bovines in 2011, which is more than three times the equilibrium load capacity.
- Non-timber products (per year): 1,000 tons of honey; 3,000 tons of acorns; 30 tons of mushrooms, truffles and lichens; 5,000 tons of tannin.
- Other products: snails; aromatic and medicinal plants.

Counterpart of man to cork oak woods

Ever since the management of the Maamora was taken over by the national administration, particular attention is being paid to the forest in view of its high ecological and economic importance, socio-economic and political impact. Numerous projects, programmes and actions have been undertaken with the aim of conserving the forest as much as possible while exploiting its products and services for economic purposes and for the improvement of the standard of living of the local population.

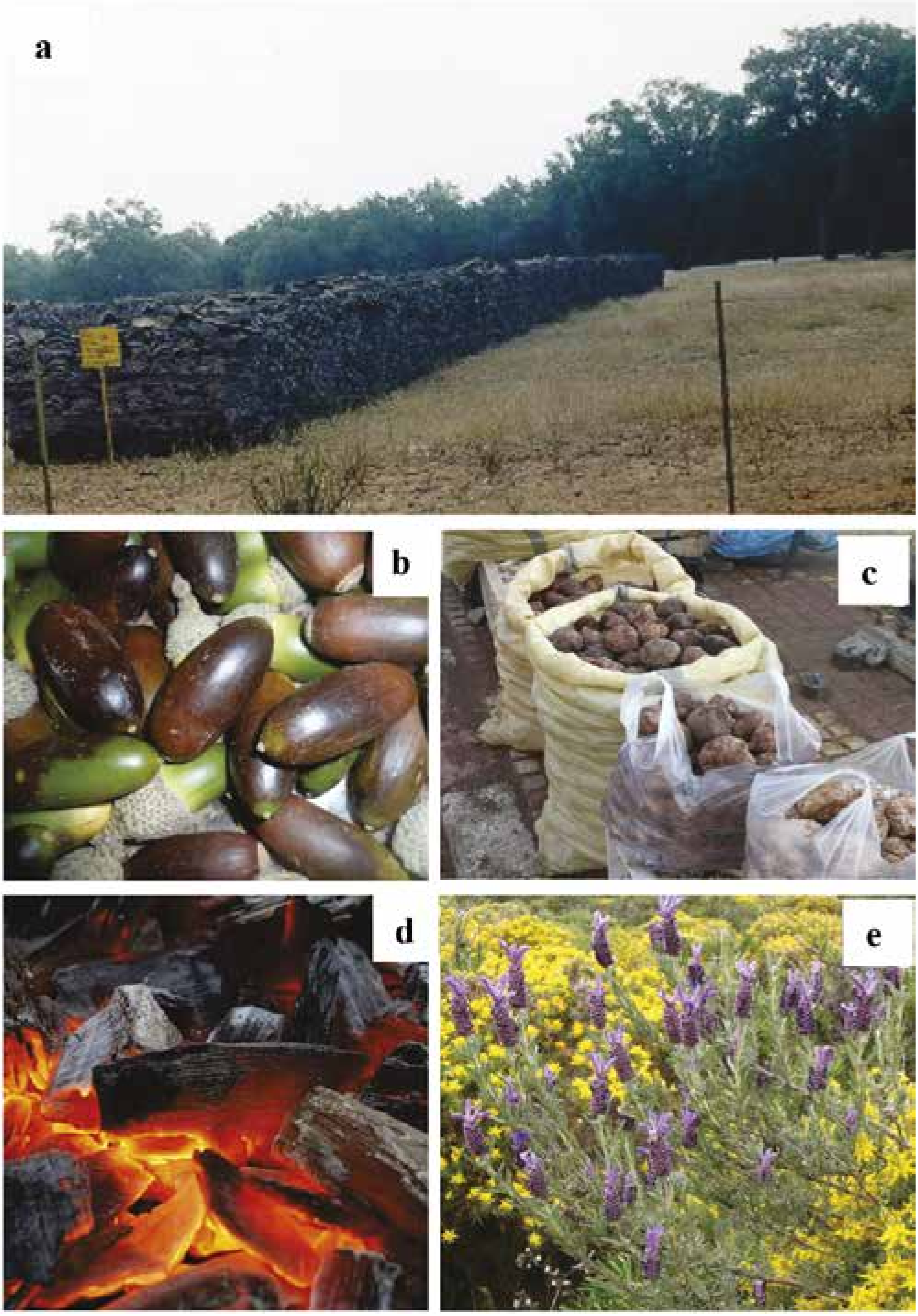


Fig. 4. Some products of Maamora cork oak: a) cork; b) *Quercus suber* acorns; c) *Terfezia leonis*; d) charcoal butning; e) medicinal plants (*Lavandula*, *Stoechas*, *Genista*)

Rescue program 1918-1950

As mentioned above, at the dawn of the 20th century, the state of the Maamora cork oak was satisfactory in general terms. The main threats, at the time, were charcoal and tannin production for handicrafts and the use of cork cannons for making apiaries. The unfortunate and unorganised procedures used killed the trees. Boudy (1958) estimates that, around 1915-1920, at least 60,000 trees were killed each year for the extraction of tannin. The forest authorities of the time gave priority to supervising the activities of craftsmen (barkers and charcoal burners) and to restoring the damaged stands. A rescue program was set up in a timely fashion, which lasted over a 30-years period. The main results were:

- The obligation, for charcoal burners, to use mutilated trees only, under the supervision of forest managers.
- The creation of a corporation of tanners who were to refrain from foot barking.
- The harvesting of 3.5 million m^3 of wood, much of which was to be carbonized and exported.
- Cutting of trees from spare stands and those in very bad ecological conditions are carried out in the central and eastern parts of the forest; nearly 60,000 hectares regenerated.
- A significant loss of stubs and, consequently, a decrease in tree density.

Fig. 5 (high and below): Wild picnics in the Maamora cork oak.

Main management projects 1951-2012

Vidal management, 1951-1972

Aims:

- Preserve the cork oak woods.
- Reafforest treeless areas and clearings by introducing exotic species.
- Produce and exploit the maximum quantity of cork.

Results:

- Cork oak woods regression (loss of 13,000 hectares) due to lack of regeneration.
- Increased areas reafforested with eucalyptus, pines and acacias.
- First regeneration experiments by seedlings and acorns.

Danish management, 1973-1992

Aims:

- Preserve the most beautiful cork oak woods (with a density of > 100 feet / ha) while maintaining an economically acceptable level of income.
- Replace oaks, where they were unhealthy, by fast-growing exotic species.
- Meet local demand for tannin from acacia bark.
- Develop the recreational function of urban and sub-urban forests.
- Respect the interests and traditional users' rights (of wood and pasture) of the local population.

Results:

- Catastrophic regression of cork oak woods (loss of 27,000 hectares).
- Increased areas reafforested with eucalypts (+16,000 hectares) and pines (+ 6,500 hectares).
- Poor results of regeneration efforts.

Sylvo-pastoral management (FAO), 1993-2012

Aims:

- Conservation and restoration of cork oak woods by regeneration and plantation of cork oak.
- Promoting the involvement of local people in the forest development process.
- Improvement of pasture-land and good use of forage resources.
- Development of auxiliary activities, such as hunting, tourism, and leisure.

Results:

- Successful regeneration of cork oak woods on more than 8,300 hectares.
- Increase of the surface area of cork oak woods (+ 5,600 hectares) at the expense of exotic reafforested species.
- Creation of forest cooperatives and associations to serve as local partner organisations.
- Management of urban and sub-urban forests in partnership with NGOs.

Overall assessment of the period 1951-2012

Forest areas

The forest management undertaken in the Maamora forest during 60 years have led to a 30 % reduction of the cork oak woods in favour of exotic species, notably eucalypts, pines and acacias (Table 1). Fortunately, successful regeneration efforts, started in 1992, managed to reverse the trend and somewhat improve the situation. Over a period of 20 years, more than 18,000 ha of cork oak have been regenerated.

Table 1. Evolution of Maamora forest area (hectares) between 1951 and 2011 (HCEFLCD 2015).

	1951	1972	1992	2011
Cork oak	100,000	87,000	60,000	70,400
Eucalyptus	31,000	38,000	54,000	42,672
Pines	1,000	6,000	12,000	9,851
Acacias	0	1,000	5,000	2,816
Other tree genera	1,000	1,000	1,500	5,827
Total	133,000	133,000	133,500	131,566

State of the ecosystem

During the last six decades, the cork oak has suffered quantitatively (reduction of its surface), but also qualitatively (Fig. 6). Stands with a reasonable density (> 100 feet / ha) do not cover more than 30,000 ha (Aafi 2007). In a general way, the trees are struggling for survival, lack vigour and resistance to climatic stress and parasite attacks.

Other ecosystem components, such as soil and groundwater, underwent increasing damage, a trend that continues to date. Overgrazing, the uprooting of trees and scrub, bad agricultural practices, and soil erosion by water and wind have greatly deteriorated soil quality.

Exploitation of groundwater for crop irrigation has lowered the water table; in addition, the groundwater begins to be polluted by pesticides.

Finally, after 60 years of programs and projects, the future of the Maamora forest is more dire than ever. The forest suffers from unrelented pressure. For the record, between 1998 and 2008, 31,706 offences were recorded; 50% concerned illegal wood harvesting and 14% related to abusive grazing (Table 2).

However, the main threats are unchanged:

- Uncontrolled exploitation of firewood, coal ...
- Irregular grazing, branch cutting
- Extension of settlements and agricultural land
- Recurrent droughts
- Low natural (and artificial) regeneration
- Acorn collecting

- Attacks by parasites
- Unauthorised picnics and unregulated recreational activities
- Fire

Table 2. Number of offenses recorded in Maamora, period 1998-2008 (HCEFLCD 2015).

Nature of the offense	**Number**	**Percentage**
Adjudicator abuse	1,106	3,5
Pasture	4,413	13,9
Wood cutting	15,791	49,8
Barking and bark yielding	761	2,4
Cork oak removal	325	1,0
Destruction or displacement of boundaries (boundary marks)	26	0,1
Agriculture or tree and scrub eradication	1,592	5,0
Extraction of adjunct products	909	2,9
Fire	462	1,5
Fire inside or within the protected area of the forest	1,147	3,6
Non-performance of fire-fighting measures	289	0,9
Construction or encampment in protected areas of the forest	2,094	6,6
Clearing of special trees	191	0,6
Contraventions to the decree of 14/09/1918 (peddling ...)	498	1,6
Hunting	775	2,4
Various	1,327	4,2
Total	**31,706**	**100 %**

The Maamora in the era of sustainable development

Current context

The current context of the Maamora forest is quite different from past contexts. The problems are, of course, always very complex, but there are some new data that leave room for a little hope and optimism. New-generation leaders are aware of the world's current awareness of the importance of biodiversity and sustainable development.

Fig. 6. Images showing the regression of cork oak between 2006 and 2016 in the region of Larache.

There is also the will to learn from past errors to better manage the future. In this context, a critical study of the period 1951-2012 was carried out, with detailed diagnoses of successes and failures (HCEFLCD 2014). Finally, there is the positive attitude toward working together with the local population and with civil society organizations. Naggar (1914) mentioned 19 pastoral associations and 46 forestry cooperatives that cooperate or work in partnership with the forest services.

As a part of this encouraging scenario, a new vision is emerging for the Maamora with the 2014-2035 management project (HCEFLCD 2016), which is an answer to the unending question of how to exploit the forest without compromising sustainability? This question is the concern of politicians, foresters, elected officials, NGOs, human communities. And there is no other choice but to meet the ends and save the area from an ecological and economic catastrophe. The unpalatable option of finding a sand desert extending to the very gates of the big cities Rabat, Salé and Kenitra is not Utopia.

Management project 2014-2035

Aims

- Accelerating the regeneration processes of cork oak.
- Valorising cork produced from the stumps of cut-back trees.
- Ensuring the ongoing yield of wood in sufficient quantity from eucalypts.
- Producing pine wood in sufficient quantity.
- Producing acacia tannin in sufficient quantity.
- Increase fodder production to reduce pressure on forest.
- Improve infrastructure provided for recreation and rest.

Discussion

The aim of the 2014-2035 project launched by the forestry department is reinforcing previous achievements, focusing on the renewal of cork oak and on alleviating anthropic pressures. The current concern for attaining an ecological balance is makes it desirable to reinforce spontaneous and enhanced regeneration by all the management techniques that are now properly controlled by foresters (Fig. 7). The improvement of the understory pastoral resources through the introduction of more yielding grazing species, if successful, will likely help improve the cork oak state. Finally, the successful control of recreational activities will do the forest a lot of good.

This new vision gains strength from two aspects that it encompasses: it takes into account the ecological dimension, and it increases the involvement of the local population and bodies that represent it (elected functionaries, associations, cooperatives, etc.).

Is the 2014-2035 project the last chance to save the Maamora? A positive answer depends on human behaviour. Fortunately, for a large part of the forest, the ecological damage has not yet crossed the point of no return. A mild climate, with sufficient rainfall, will certainly help contain the damage greatly and will assist the project in reaching its goals. Financial resources are obviously necessary, but if competence and good governance are absent, they will be wasted.

Conclusion

The Maamora, a secular forest, dying under the preoccupied, worried or indifferent eye of one and the other according to the person. For a long time, it was feared and fought for its insecure climate, exploited and overexploited for its wealth, studied and managed for its management. Today, its state, its ills and its threats are known; better still we know what must be done to avoid the irrevocable.

Fig. 7. Successful cork oak planting in the Maamora (NAGGAR 2014).

The current vision for the future of the Maamora, takes into account the past mistakes. Henceforth, everyone knows that sustainability of the forest depends on respecting the basic rules of nature and the total and massive adherence of the local populations.

Supporting natural regeneration and easing human pressure are indeed key solutions for sustaining the Maamora.

In the present socio-economic context of the country and of the region in particular, it is utopian to think of Maamora as an integral reserve, but we wish and strongly recommend that it be possible for a part of the forest. Wherever full protection can be achieved, the positive effects on the recovery of cork oak woods will soon come, with direct ecological and economic benefits! A "climax" cork oak stands, close to Casablanca, Rabat and Kénitra will not leave indifferent the inhabitants of these cities, but also national and international tourists. It is a matter of time, but here nature is master, and we must let it do, better yet help it, but above all do not upset it.

References

AAFI, A. 2007: Etude de la diversité floristique de l'écosystème de chêne-liège de la forêt de la Mamora. – Thèse Institut Agron. et Vétér. Hassan II, Rabat, pp. 190.

—, BADOUZI, B., BELGHAZI, T. & MOUJJANI, S., 2011: Semis et plantations dans la forêt de chêne-liège de la Maâmora (Maroc). – Forêt Méditerranéenne, t. XXXII, 3: 301-314.

BELGHAZI, B., EZZAHIRI, M., AMHAJAR, M. & BENZIANE, M., 2001: Régénération artificielle du chêne-liège dans la forêt de la Maâmora (Maroc). – Forêt Méditerranéenne, t. XXII, 3:

253-261.

BOUDY, P. 1958: Economie forestière nord-africaine. – Description forestière du Maroc ($2^{ème}$ édition), Tome 3: 375. – Larose Edit., Paris.

EMBERGER, L. 1928: Les limites naturelles de la forêt de la Maâmora. – Bull. Soc. Sci. Nat. Phys. Maroc 8: 220-222.

FENNANE, M. & REJDALI, M. 2015: The world largest cork oak Maamora forest: challenges and the way ahead. – Fl. Medit. 25(special issue): 277-285.

HCEFLCD (Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification), 2014: Révision de l'étude d'aménagement de la forêt de la Maamora (Volume 8). Marché n°30 / 2009 / DREFLCD-NO. – Rabat.

— 2015: Projet FFEM: Optimiser la production de biens et services par les écosystèmes boisés méditer-ranéens dans un contexte de changements globaux. – Rapport final. http://www.fao.org/forestry/44813-0e6be9d792c5e725847cb773bfbd11ae2.pdf

— 2016: Développement durable des écosystèmes forestiers de la région – Rabat-Salé-Kénitra sous la contrainte des changements climatiques (en arabe). – Doc. interne, Direction régionale des Eaux et Forêts.

LEPOUTRE, B. 1965: Régénération artificielle du chêne-liège et équilibre climatique de la subéraie en forêt de la Maâmora. – Ann. Rech. Forest. Maroc, n° 9, Rabat, pp. 188.

NAGGAR, M. 2014: La Gestion durable des subéraies marocaines. Doc. Interne. HCEFLCD. – Division de l'Amènagement Forestier, Rabat.

Type specimens in the Herbarium of Aristotle University of Thessaloniki (TAU)

DIMITRIOS RAPTIS, REGINA KAROUSOU & EFFIE HANLIDOU

Abstract

Raptis, D., Karousou, R. & Hanlidou, E. 2019: Type specimens in the Herbarium of Aristotle University of Thessaloniki (TAU). – Bot. Chron. 307-318

The historical collections of TAU (Thessaloniki Aristotle University Hebarium) hold a total of 44 specimens collected in the 19th century, which are nomenclatural types (one lectotype, 30 iso-(lecto)types, 10 syntypes) or probable types (4). Twenty of them are types of names which are currently accepted. Twelve are types of names currently considered as homotypic synonyms of accepted names, while the rest are heterotypic synonyms. An annotated list of all type specimens is provided.

D. Raptis, R. Karousou & E. Hanlidou*, Laboratory of Systematic Botany & Phytogeography, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, GR-541 24, Greece. *Corresponding author: E-mail: chanlidu@bio.auth.gr

Key words: TAU Herbarium, historical collections, nomenclatural types, Theodor von Heldreich, Theodoros Orphanides.

Introduction

The Thessaloniki Aristotle University herbarium (TAU) is a fairly small Herbarium holding around 60000 specimens, mainly collected from Greece. It is located in the School of Biology, Laboratory of Systematic Botany and Phytogeography. TAU is divided in two sections, the New collections and the Historical collections. The New collections are constantly enriched with specimens deposited by the scientific staff, collected after 1950. The Historical collections hold specimens dating back from the early 19th century up to the late thirties and they count more than 6000 specimens. The majority is collected from Greece, but there are also several specimens from European countries and quite a few from other continents.

The main collectors of this section are Theodor Heinrich Herman von Heldreich, Theodoros Orphanides, Joseph Sartori, Vassilios Tuntas, Giacomo Guicciardi, and Dimitrios Zaganiaris.

Not much is known about the beginning of TAU; our knowledge is mainly based on narrative snippets from former members of our Lab. However, part of the TAU's story is also told by its specimens. It seems that it all began in the mid-thirties, when, in 1936, Dimitrios Zaganiaris (1901-1941) was elected Assistant Professor of Systematic Botany and Phytogeography in the University of Thessaloniki. Zaganiaris was an enthusiastic botanist and a tireless collector and besides his own collections, he enriched the herbarium, by exchanging his duplicata with specimens of other herbaria. Thus, around 2500 specimens of the above-mentioned collectors were deposited in TAU.

The present work is part of a wider project intending to the classification, documentation and digitisation of TAU's collections. The project aims to the mobilization and sharing of the specimens, since – in light of modern developments in the field of museum collections – it is now evident that linking data virtually enhances their utility in answering scientific questions. In the frame of the pre-digitisation process, in this study we present an annotated list of the 44 nomenclatural types and possible types, collected during the 19th c. (up to 1901), all kept in TAU's Historical collections.

Material and methods

The type specimens were identified by comparing the label data (collector, collection date and number, locality) of the TAU specimens to those of the original specimens cited in the protologues. Moreover, the data were compared to those of digitized specimens, already identified as nomenclatural types, which are found in several herbaria (B, BM, C, CAS, E, FR, G, G-BOISS, GOET, GZU, HAL, HBG, JE, K, L, LD, M, MICH, MPU, MSB, P, S, U, UPS, W, WAG, WU) and are available via internet. Main bibliographic sources referring to type specimens (STRID 1986, STRID & TAN 1991, 1997, 2002) as well as other relevant published information were also checked.

In the following account families/genera/species are in alphabetical order. All entries provide:

The name (basionym) of the taxon, author and the citiation of the protologue.

The TAU specimen code, the verbatim transcription of the original label (in quotation marks) and the status of the type (in capital letters).

The currently accepted name (homotypic synonyms are marked with ≡, heterotypic with =). When not given, the first name is currently accepted.

Additional remarks, if necessary.

The herbaria where other type specimens are found. The lectotype, when known, and the corresponding reference, are given. An exclamation mark (!) indicates that a photograph of the specimen, available via internet, was seen by the authors.

The protologues are found in Euro+Med PlantBase (Euro+Med 2006-) and for a few taxa not included there, in the International Plant Names Index (IPNI 2012). The currently accepted names are according to DIMOPOULOS & al. (2013) and for a few taxa not included there, according to The Plant List (2013). The Herbaria designations are according to the Index Herbariorum (THIERS 2019).

Type Specimen List

A total of 44 nomenclatural types are found in TAU, which were collected in the 19th century, the early era of the botanical exploration of Greece. They were collected from the phytogeographic regions of Peloponnese (17), Sterea Hellas (15), Cyclades (3), North Central Greece (3), Crete (2), North Pindos (1) and West Aegean islands (1).

Thirty of the specimens derive from the collections of Theodor von Heldreich, one of the most prominent botanists and explorers of the Greek flora. Most of them were collected by him, while a few were collected by Karl Hermann Zahn, Giacomo

Guicciardii and Giovanni Battista Samaritani. Ten come from the collections of Theodoros Orphanides, the first modern Greek plant scientist. The rest derive from the collections of Joseph Sartori (2), Antonio Baldacci (1) and Carl Baenitz (1).

Twenty are type specimens of names which are currently accepted. Twelve are types of names currently considered as homotypic synonyms of accepted names. Most of the above taxa are Greek (18) and Balkan (9) endemics. The rest are types of heterotypic synonyms.

Alliaceae

Allium achaium* var. *parnassicum Boiss., Fl. Orient. 5: 259. 1882
≡*Allium parnassicum* (Boiss.) Halácsy
TAU060867 “HERBARIUM ORPHANIDEUM. 3577. *Allium achaium* Boiss. & Orph. var. *parnassicum*. In cacumine m. Malevo. Aug 1855. legit Agraniotis”. SYNTYPE.
The specimen is identified with one of the two collections cited in the protologue. A specimen of *A. frigidum* Boiss. & Heldr. is found on the same herbarium sheet.
Other type material: G-Boiss, JE!, K!, WU!.

Apiaceae

Athamanta arachnoidea Boiss. & Orph., in Boissier, Fl. Orient., Suppl.: 262. 1888
≡*Bubon arachnoideum* (Boiss. & Orph.) Hand.
TAU060870 “HERBARIUM ORPHANIDEUM. *Athamanta arachnoidea* Boiss. & Orph. Legit in monte Taygeto Laconiae prope Aspra-Spilia circa Pigadia. 4-16 Jul 1868. E. Psarides, THEODORUS G. ORPHANIDES”. Typus probabiliter.
The collectors and collection site are identical to those cited in the protologue, however the collection date is different (1870). TAN & IATROU (2001) mentioned that *A. arachnoidea* was first collected from Aspra Spilaea in August 1868 by E. Psarides and Th. Orphanides, and the specimen was distributed as Heldreich Graec. Norm. 1032 (G-Boiss, LD). However, it seems that the specimen of TAU was collected by the same collectors a few days earlier. A similar specimen, collected in 9 July, is found in WU.

Ferulago sartorii Boiss., Fl. Orient. vol. 2: 999. 1872
TAU060869 “3619 *Ferulago sartorii* Boiss. sp. nov. in petrosis ad viam infer Βραχνού & coenobium Παναγίας πανάχραντος Sartori. 22 Aug 1857”. ISOLECTOTYPE.
Other type material: G-Boiss [Lectotype (BERNARDI 1979)]. Specimens collected later (July 1868) from the *locus classicus* were distributed as Heldreich Graec. Norm. 931 and are found in B! and LD!.

Asteraceae

Achillea ambrosiaca (Boiss. & Heldr.) Boiss., Fl. Orient. vol. 3: 276. 1875
TAU060873 “Herb. De Heldreich 2469. *Achillea ambrosiaca* (*Ptarmica*!). In m. Olympi Thessaliae rupestibus in reg. alpina ad nives. 25-31 Jul 1851 Th. De Heldreich”. ISOLECTOTYPE.
Other type material: G-BOISS [Lectotype (FRANZÉN 1986)], GOET!, JE!, K!, LD!, W, WU!

Brassicaceae

Alyssum taygeteum Heldr., Sched. Herb. Graec. Norm.: 1405. 1897
TAU060874 "DE HELDREICH HERBARIUM GRAECUM NORMALE. 1405. *Alyssum Taygeteum* Heldr. spec. nova. Affine *A. suffrutescenti* β. *Olympico* Boiss. fl. or. I, 276 (i.e. *A. erosulo* Clem. et Pestal. in Clem. Sert. or. p. 12), a quo foliis anguste lineari-oblongis longioribus et siliculae stylo abbreviato differt. Flores ignoti., M. Taygetus: in summo cacumine Hagios Elias, alt 7000'. Aug. 1897". ISOLECTOTYPE.
Other type material: G [Lectotype (HARTVIG 2002)], GZU! JE!, K!, LD!, PRC!, S!, WU!.

Barbarea conferta Boiss. & Heldr., in Boissier, Fl. Orient. Suppl.: 36. 1888
=*Barbarea vulgaris* subsp. *arcuata* (Opiz) Hayek
TAU060876 "De Heldreich plantae exsicc. Florae Hellenicae. 3646. *Barbarea conferta* Boiss. & Heldr. suppl. In montis Kyllenes Achaiae regione media, prope pagum Trikala, alt. 3500'-4000'. 'Apano-Machala' 1 Aug 1871". ISOLECTOTYPE.
Other type material: B [Lectotype (TAN 2002)], WU!

Caryophyllaceae

Alsine baldaccii Halácsy, Consp. Fl. Graec. 1: 237. 1900
≡*Minuartia baldaccii* (Halácsy) Mattf.
TAU060877 "1896. ITER ALBANICUM (EPIROTICUM) QUARTUM 223. Gen. et spec: *Alsine Baldacii*, Hab. In reg. abiet., Leg. et Flor: m. Smolika supra Paleoseli distr. Konitza, 19 Jul. Dott. Antonio Baldacci". ISOLECTOTYPE.
Other type material: WU-Hal [Lectotype (KAMARI 1997)], G!, K.

Convolvulaceae

Cuscuta macranthera Boiss., Diagn. Pl. Orient. ser. 2, 3: 126. 1856
≡*Cuscuta approximata* Bab. subsp. *macranthera* (Boiss.) Feinbrun & Greuter
TAU060879 "De Heldreich Herbarium Graecum normale. No 443. *Cuscuta macranthera* Heldr. & Sart.! in De Heldr. Fl. Gr. exsicc., De Heldr. Pl. exsicc. Taygeti ann. 1844. No 395. In *Daphni buxifolia* Sibth. freq. in reg superior. m. Parnassi, alt. 5-6000'. legit J. Guicciardi. Jul 1855". SYNTYPE.
The specimen is identified with one of the two collections cited in the protologue.

Dipsacaceae

Scabiosa taygetea Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 1, 6: 73. 1846
TAU060687 "*S. taygetea* Boiss. & Heldr., Taygetus super. Aug 1844". Typus probabiliter.
The collector's name is not written on the label, however the handwriting is identical to that on other Heldreich's labels. The lectotype of *S. taygetea* is a specimen in G-Boiss labeled "Mt Taygetos an Kako Chroni, pres de la Varvara, leg. Heldreich 292, Jul 1844" (KOKKINI 1991). A specimen in WU-Halácsy-Graecum also has July 1844 as the collection date. However, the collection date of other specimens, found in BM, W, WAG, which are labeled as types (typus probabiliter or isotypes), is July and August 1844.

Euphorbiaceae

Euphorbia zahnii Halácsy, Consp. Fl. Graec. 3: 100. 1904
=*Euphorbia valerianifolia* Lam.
TAU060883 “DE HELDREICH HERBARIUM GRAECUM NORMALE. 1580. *Euphorbia zahnii* Heldr. nova species. E. sect. *V. Tithymalus*. §. 6 *Galarrhaei* sec. Boiss. Fl. or. IV. Species ex affinitale *E. akenocarpae* Guss. et *E. Cybirensis* Boiss. (l. c. p. 1098) distinctissima foliis lanceolatis acutissimis rigidiusculis glaucescentibus Ilypericorum instar pellucido-glandulosis, nervo medio pallido, seminibus laevibus ovoto-globosis brunneis aliisque notis. Laconia borealis: in faucibus m. Selitza. ad confines Messeniae pr. Kalamata, inter Lupinos cultos. Detexit et legit am. Henricus Zahn. 24 Mart. et fruct. 7 April. 1899”. ISOTYPE.
Other type material: CAS!, JE!, WU!

Fabaceae

Alhagi tournefortii Heldr., Sched. Herb. Graec. Norm.: 1624. 1901.
=*Alhagi graecorum* Boiss.
TAU060887-1 “DE HELDREICH HERBARIUM GRAECUM NORMALE. 1624. *Alhagi Tournefortii* Heldr., Alhagi Tourn. Voy. Lev. II 4. *Hedysarum Pseudoalhagi* Chaub. et Bory. Flor. Pelop. Non MB. *Alhagi graecorum* Bois. Fl. or. II. 559. Hal. Consp. I, 461 p.p. (quoad pl. ex ins Syros). Differt ab. *A. Graecorum* Boiss. ex Attica glabritie omnium partium, vexillo minus amplo carina subbreviori. Flora Aegaea: Cycladum insula Syros in halipedo juxta urbem. 31 Jul. 1901”. ISOTYPE.
There are at least 5 duplicates in TAU.
Other type material: JE!, WU!

Astragalus drupaceus Boiss., Diagn. Pl. Orient. ser. 2, 2: 32. 1856
TAU060884 “FLORA GRAECA EXSICCATA. No 131. fruct. In m. Malevo Laconiae pro. Hajanni, alt 3000'. 17 Jun 1852. THEODORUS G. ORPHANIDES.” ISOLECTOTYPE.
Other type material: G-Boiss, [Lectotype (AGERER-KIRCHHOFF 1976)], BM!, C, E, FR!, K, LD!, M, MSB!, P, S!, W, WU!, Z.

Astragalus lacteus Boiss., Diagn. Pl. Orient. ser. 2, 2: 31. 1856
TAU060888 “Herb: Sartorianum., Reliquiae cura Heldreichii emissae. Laconia mt. Malevo prope Spilia Legit Sartoris”. Typus probabiliter.
Collector’s number and date are missing from the TAU specimen. A specimen found in G-Boiss (“In summis m. Malevo Peloponnesi [Parnon]. Sartori no 1488, 20.5.1837”) is reported as type (STRID 1986). A specimen found in P is characterized as an isotype and has almost similar label data to the G-Boiss specimen.

Genista halacsyi Heldr., Sched. Herb. Graec. Norm. no. 1526. 1899
TAU060885 “DE HELDREICH HERBARIUM GRAECUM NORMALE. 1526. *Genista Halacsyi* Heldr. nova spec., Cl. Halacsy in Consp. Fl. Gr. I, 331 plantam nostram ad *G. sericeam* Wulf. ducit cum observatione: "planta Graeca a Dalmatica foliis angustioribus parum differt; specimina a capris mutilata ob ramulos brevissimos et folia diminuta faciem valde alienam habent" - Plane dissentiens propono speciem propriam nomine cl. et am. Halacsy ornatam. Heldr., Laconia: in faucibus Taygeti

Langada dictis inter Spartam et Kalamata, Legit am. H. Zahn.”. ISOTYPE.
Other type material: BM!, G!, JE!, M!, WU!

Lathyrus neurolobus Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 1, 9: 125. 1849
TAU060726 “1585, diagn. IX. 125., *Lathyrus neurolobus* Boiss. & Heldr., nov. spec. ad rivulus Castanetorum Cretae occidentalis. Jun 1846, de Heldreich”. ISOTYPE.
Other type material: BM!, E!, GOET!, WAG!, WU!

Lotus lamprocarpus Boiss., Diagn. Pl. Orient. ser. 1, 9: 33. 1849
=*Lotus palustris* Willd.
TAU060889 “De Heldreich Herbarium Graecum normale. No 361, ad falsus humides Phaleri Atticae vulgaris. Jul 185-”. SYNTYPE.
The collector and collection locality are identical to those of one of the two original collections cited in the protologue. A specimen of Heldreich found in B, with no collector’s number and date, is labeled as type.

Onobrychis pulchella Boiss., Diagn. Pl. Orient. ser. 2, 6: 64. 1856
=*Onobrychis alba* subsp. *pentelica* (Hausskn.) Nyman
TAU060886-1 “De Heldreich Graecum normale. No 664. *Onobrychis pulchella* Boiss., in pascuis reg. abietinae m. Tymphresti (Veluchi hod.). Eurytaniae, l. d. Rovia, alt 4000' supra Carpenisi. 8 Aug 1857. Detexerunt am. J. B. Samaritani & J. Guicciardi.”. ISOTYPE.
Two duplicates are found in TAU.
Other type material: G, G-Boiss, JE!, MPU!

Fumariaceae
Corydalis parnassica Orph. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 6: 9. 1856
≡*Corydalis blanda* subsp. *parnassica* (Orph. & Heldr.) Lidén
TAU060890 “*Corydalis parnassica* Orph. & Heldr., 325. Orph. fl. gr. exs. sub *C. tuberosa*. In reg. super. m. Parnassi pr. Trypios Vrachos 6000'. 24 Jun 1854”. ISOLECTOTYPE.
Other type material: G-Boiss [Lectotype (LIDÉN 1996)], BM!, L!, S, WU!.

Iridaceae
Crocus peloponnesiacus Orph., in Boissier, Diagn. Pl. Orient. ser. 2, 4: 95. 1859
=*Crocus hadriaticus* Herb.
TAU060894 “FLORA GRAECA EXSICCATA. 68. *Crocus peloponnesiacus* Orph in Boiss. Habit. In reg infer. m. Malevo Laconiae pr. Ajanni alt. 3000'. 27 Oct 1851. THEODORUS G. ORPHANIDES Universitatis Athenarum Botan. Professor”. ISOTYPE.
Other type material: MB!, HAL!, JE!, WU!.

Gladiolus glaucus Halácsy, Consp. Fl. Graec. 3: 186. 1904
=*Gladiolus illyricus* W.D.J. Koch
TAU060893 “DE HELDREICH HERBARIUM GRAECUM NORMALE. 1379. *Gladiolus glaucus* Heldr. nova species in sched. 1895. Laconia borealis: in districta

Alagonia prope Megali Anastasova, alt. 3000'. 1-15 Jun 1896. Legit am. H. Zahn". ISOTYPE.
Other type material: BM!, MPU!, WU!

Lamiaceae
Nepeta camphorata Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 1, 7: 49. 1846
"*Nepeta camphorata* Boiss. & Heldr. 331. Taygetus (Πενταπλούς) Heldr". ISOLECTOTYPE.
Although the collection data are incomplete, the comparison to the label data found in other herbaria (e.g. in WU "DE HELDREICH HERBARIUM GRAECUM NORMALE 1470. *Nepeta camphorata* Boiss. & Heldr. in Boiss. Diagn. Ser. I, 7, 49. Boiss. Fl. Or. IV, 653. Heldr. exs. ann. 1844 e Taygeto No 331. M. Taygetus: in regione abietina superiori (l. classico et unico) Jul 1897") shows that the specimen of TAU was collected in 1844 by Heldreich.
Other type material: G-Boiss [Lectotype, "Reg. abietinae Taygeti, 1844" (BADEN 1987)], BM!, E, K, OXF.

Sideritis attica Heldr., Delt. Fusiogr. Tmem. Sull. Parn.: 46. 1900
≡*Sideritis raeseri* subsp. *attica* (Heldr.) Papan. & Kokkini
TAU060898-1 "DE HELDREICH HERBARIUM GRAECUM NORMALE. 1677. *Sideritis attica* Heldr. Περί των φυτών των παρεχόντων το Ελληνικόν τσάι εν Δελτίω (Bulletin) Φυσιογνωστικού Τμήματος Συλλ. Παρνασσού. Dec. 1900 p. 46. Flora Attica: in saxosis regionis abietinae mont. Parnethis, prope cacumen rar., Aug 1901". LECTOTYPE.
The specimen was selected as lectotype by PAPANIKOLAOU & KOKKINI (1982). There are 11 duplicates in TAU (TAU060898-1 to TAU060898-11).
Other type material: W

Liliaceae
Tulipa crocata Orph., Bull. Congr. Bot. St. Petersb. 1869 (1870) 116.
=*Tulipa orphanidea* Heldr., Gartenflora 11: 309. 1862
TAU060900 "HERBARIUM ORPHANIDEUM. *Tulipa crocata* Orph. *Tulipa Orphanidea* Heldr. in Flora, *T. Bithynica* dec Boissier. Legi prope Malevo Laconiae prope Hajos Petros. Apr 1857. THEODORUS G. ORPHANIDES". ISOTYPE and SYNTYPE.
The specimen is an isotype of *T. crocata* described by Orphanides. It is also a syntype of *T. orphanidea,* described by Heldreich, since it is included among the several original collections cited in the protologue.
Other type material: WU! JE!

Tulipa euanthiae Orph., in Boissier, Diagn. Pl. Orient. ser. 2, 4: 100. 1859
=*Tulipa undulatifolia* Boiss.
TAU060899 "HERBARIUM ORPHANIDEUM. *Tulipa Euanthiae* Orphan in Boiss-Diagn. Legi in monte Malevo Laconiae prope Xirocampi supra Hajos Joannis 23 Apr/ 5 Mai 1857, THEODORUS G. ORPHANIDES". ISOTYPE.
Other type material: WU! JE!

Linaceae

Linum pycnophyllum Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 1: 97. 1854
≡*Linum punctatum* subsp. *pycnophyllum* (Boiss. & Heldr.) Gustavsson
TAU060705 "*Linum pycnophyllum* Boiss. Heldr. In reg. alpina mt. Cyllenes, No 2134. 8 Jul 1848"
TAU060743 "2134. *Linum pycnophyllum* Boiss. & Heldr., *Linum punctatum* Presl? In reg. alpina m. Kyllenes, raro. 8 Jul 1848, de Heldreich". ISOLECTOTYPE.
Other type material: G-BOISS [Lectotype (HARTVIG 1986)], BM!.

Poaceae

Agropyron flaccidifolium (Boiss. & Heldr.) P. Candargy, Etude Monogr. Hordees (Archiv. Biol. Veg., Athenes, Fasc. 1) 29, in clavi, 51. 1901
≡*Elymus flaccidifolius* (Boiss. & Heldr.) Melderis
TAU060905 "De Heldreich Herbarium Graecum normale. No 501. *Agropyrum flaccidifolium* Boiss & Heldr.! Mss. De Heldr. pl. exsicc. (ann. 1855!) no 3107. In planitia maritima Atticae ad monumentum Karaiskaky inter Juncos 11 Jun 1856". ISOTYPE.
Other type material: GOET! JE! W!

Lolium compressum Boiss. & Orph. ex Nyman in Nyman, Consp. Fl. Eur. 4: 845. 1882, nom. nud.
=*Lolium perenne* L.
TAU060904 "De Heldreich Herbarium Graecum normale. No 548. *Lolium compressum* Boiss. & Orph. in arenosis planitiei Atticae prope Psychico ad viam Cephissia, 14 Mai 1856". SYNTYPE.
The specimen is identified with one of the two collections cited in the protologue.

Melica rectiflora Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 1, 13: 56. 1854
TAU060719 "De Heldreich pl. Creticae, ad rupes, Sphakia: Gorge de Hagia Rumeli, 6 Apr 1846". ISOLECTOTYPE.
Other type material: G-Boiss [Lectotype (STRID 1991)], BM! G! GOET! JE! W!

Phleum parnassicum Boiss. & Heldr. ex Nyman, in Nyman Consp. Fl. Eur. 4: 792. 1882, nom. nud.
=*Phleum pratense* L.
TAU060903 "De Heldreich Herbarium Graecum normale. No 267. *Phleum parnassicum* Boiss. & Heldr. in reg. abietina m. Parnassi in oropedio Livadi dicto supra Rachova, alt 4000'. Jun 1855, Legit J. Guicciardi". Typus probabiliter.
A duplicate in WU is annotated as an authentic specimen.

Poa parnassica Boiss. & Heldr. in Boissier, Diagn. Pl. Orient. ser. 2, 4: 137, in obs. 1859
=*Poa thessala* Boiss. & Orph. [syn. *Poa alpina* subsp. *parnassica* (Boiss.) K. Richt.]
TAU060926 "De Heldreich Herbarium Graecum normale. No 564. *Poa Parnassi* Boiss & Heldr. Diagn. Pl. Or. Inedit. De Heldr. Pl. exsicc. (Parnassi! ann 1852) No 1986. In lapidosis reg. alpinae m. Parnassi, alt. 6-7000' (Tripios-vrachos & εις το Ξες)". SYNTYPE.

In the protologue of *P. parnassica* there is no reference to any specimens. However, the specimen of TAU, labelled as Type by Scholz in 1984, is a syntype of *P. alpina* var. *parnassica* Boiss. (SCHOLZ 1991), which is currently treated as a synonym of *P. thessala*. Duplicates are found in G-Boiss and WU.

Poa thessala Boiss. & Orph., in Boissier, Diagn. Pl. Orient. ser. 2, 4: 135. 1859
TAU060906 "HERB. ORPHANIDEUM. No 3125. *Poa Thessala* Boiss & Orph. In reg infer. M. Olympi Thessal. Pr. Kryo-vrysi. 28 Jul 1857. Orphanides". SYNTYPE.
The label data are identical to those of one of the two original collections cited in the protologue. The specimen of TAU, labelled as typus by Scholz in 1984, is a syntype (SCHOLZ 1991).

Poa timoleontis Boiss., Fl. Orient. vol. 5: 607. 1884
TAU060902 "De Heldreich Herbarium Florae Hellenicae No 104. *Poa Timoleontis* Heldr. spec. nov. Flora Attica: in pascuis aridis regionis inferioris & mediae m. Hymetti, alt. 500'-2500' 2 Mai 1878, Th. De Heldreich". SYNTYPE.
The specimen is identified with one of the four collections cited in the protologue. It is labeled as type by Scholz in 1984 and characterized as syntype (SCHOLZ 1991).
Other type material: B!, G!, G-BOISS!, GOET!, HBG!, LD!, MICH!, MPU! S!

Trisetum laconicum Boiss. & Orph., in Boissier, Diagn. Pl. Orient. ser. 2, 4: 129. 1859
TAU060780 "HERB. ORPHANIDEUM, No 2890, Legi in monte Malevo Laconiae prope Vromopigadon, alt 4000', 7-19 Jul 1850. Th. G. Orphanides". ISOTYPE.
Other type material: B!, G!, JE!, UPS!, W!, WU!

Rubiaceae
Asperula baenitzii Boiss., Fl. Orient. Suppl.: 280. 1888
TAU060911-1 "Dr. C. Baenitz, herbarium Europaeum. De Heldreich, herbarium Florae hellenicae. No 75. n. sp. (Ex affinitae Asp. Mucosae Boiss & Heldr.) In rupium fissuris regionis abietinae montis "Pateras" Atticae occidentalis, alt. 3500'-4000'. 8 Jun 1876. Legit Constant. Bartholomatos". ISOTYPE.
There are at least two duplicates in TAU.
Other type material: FR!, HBG!, JE!, M!, MPU!, W!

Asperula chlorantha Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 6: 90-91. 1859
TAU060908 "Herb. De Heldreich. 3300. *Asperula chlorantha* Boiss. & Heldr., nov. spec., ad rupes m. Arapokephalo pr. Prustova Aetoliae, Samaritani & Guicciardi. 26 Jul 1857". ISOTYPE.
Other type material: WU!

Asperula longiflora Waldst. & Kit. **var.** ***condensata*** Boiss., Fl. Orient., Suppl.: 281. 1888
≡*Asperula aristata* subsp. *condensata* (Boiss.) Ehrend. & Krendl
TAU060912 "De Heldreich plantae exsiccatae e Graecia. 3299. *Asperula longiflora* Waldst. & Kit. var. *condensata* Boiss. in reg. superiori m. Veluchi Euritaniae legerunt am. J. B. Samaritani et J. Guicciardi. 30 Jul 1847". ISOLECTOTYPE.

Other type material: G-BOISS [Lectotype (SCHÖNBECK-TEMESY & EHRENDORFER 1991)], WU.

Asperula lutea Sm. **var.** ***pinifolia*** Boiss., Fl. Orient., Suppl.: 280. 1888
≡*Asperula pinifolia* (Boiss.) Ehrend. & Schönb.-Tem.
TAU060913 "Herb. De Heldreich. 3301. *Asperula lutea* Sm. var. *pinifolia* Boiss., in m. Veluchi reg. super. l. d. Megarheuma, Samaritani & Guicciardi". ISOLECTOTYPE.
Other type material: G-BOISS [Lectotype (SCHÖNBECK-TEMESY & EHRENDORFER 1991)], JE!

Asperula muscosa Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 2: 109. 1856
TAU060909 "Herb. De Heldreich. 562. Orph. No 1969. Olymp. Thessal. 38, 2458, Heldr. 21 Jul 1851". ISOLECTOTYPE.
Other type material: G-BOISS [Lectotype (SCHÖNBECK-TEMESY & EHRENDORFER 1991)], GOET!, JE!, LD, MPU!, WU.

Asperula suffruticosa Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 2: 111. 1856
TAU060910 "De Heldreich Herbarium Graecum normale. No 759. *Asperula suffruticosa* Boiss. & Heldr., In Dirphys & Xirovuni Euboeae montibus ad rupes, alt 4500'-5000'. 7 Aug 1858". ISOTYPE.
Other type material: G-Boiss, UPS!, W!.

Scrophulariaceae
Celsia boissieri Boiss., Fl. Orient. vol. 4: 353. 1879
≡*Verbascum* boissieri (Boiss.) Kuntze
TAU060916-1 "De Heldreich Herbarium Graecum normale. No 317. *Celsia boissieri* Boiss., in reg. media m. Parnethos Atticae alt. 2000' circ. 08 Mai 1855". SYNTYPE.
The specimen is identified with one of the four collections cited in the protologue.
Other type material: MPU!

Verbascum foetidum Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 3: 141, 1856
TAU060914 "HERB. DE HELDREICH. 2737. *Verbascum foetidum* Boiss. & Heldr., in monte Parnasso. in reg. abietina Aug 1852". ISOTYPE.
Other type material: G-BOISS.

Verbascum graecum Boiss., Diagn. Pl. Orient. ser. 2, 3: 148. 1856
TAU060917 "1744, *Verbascum graecum* Heldr. et Sart. Parnes Παναγία Κλισθών Heldr. 20 Jun 1847". ISOLECTOTYPE.
Other type material: G-BOISS [Lectotype (RAUS 1991)].

Verbascum myconium Heldr., Sched. Herb. Graec. Norm.: 1669. 1901.
=*Verbascum lasianthum* Benth.
TAU060915-1 "DE HELDREICH HERBARIUM GRAECUM NORMALE. 1669, nov. spec. Ab affini *V. mucronato* specifice differt indumento lutescenti-pannoso in caulc ramisque rariori tamen persistente, racemis inaequalibus (nec virgatis) paniculam

pyramidatam formantibus, glomerulis minus dissitis, floribus pedicello calycem subaequante suffultis, calycis indumento eo ceterarum partium consimili obsiti (nec lana bombycina occultati) laciniis parum longioribus. Flora Aegaea: Cycladum ins. Mykonos, in collibus graniticis, cultis derelictis frequens. 1 Jul 1901". ISOTYPE.
There are 5 duplicates in TAU.
Other type material: JE!, P!, WU!

Valerianaceae
Valeriana olenaea Boiss. & Heldr., in Boissier, Diagn. Pl. Orient. ser. 2, 2: 118. 1856
TAU60750 "2088! *Valeriana olenaea* Boiss. & Heldr. nov. spec. m. Olenos: in rupibus reg. superior. rar. ad Κολόπανο. 21 Jul 1848. de Heldreich". ISOLECTOTYPE.
The specimen is identified with one of the two collections cited in the protologue (the other, by Orphanides, is from Mt Kyllini).
Other type material: G-BOISS [Lectotype (FRANZÉN 1991)], BM!, E!, G, GOET!, K!
Type specimens from Kyllini are found in LD!, WAG!, WU!.

Acknowledgements

This work is part of the Master Thesis of the first author (D. Raptis), carried out in Aristotle University of Thessaloniki, School of Biology, Postgraduate Studies Program "Conservation of Biodiversity and Sustainable Exploitation of Native Plants".

References

AGERER-KIRCHOFF, C. 1976: Revision von *Astragalus* L. sect. *Astragalus* (Leguminosae). – Boissiera 25: 1-197.

BADEN, C. 1987: Biosystematic Studies in the *Nepeta sibthorpii* Group (Lamiaceae) in Greece. – Opera Bot. 93: 1-54.

BERNARDI, L. 1979: Tentamen revisionis generis *Ferulago*. – Boissiera 30: 1-182.

DIMOPOULOS, P., RAUS, T., BERGMEIER, E., CONSTANTINIDIS, T., IATROU, G., KOKKINI, S., STRID, A. & TZANOUDAKIS, D. (eds) 2013: Vascular plants of Greece: An annotated checklist. – Berlin & Athens.

EURO+MED. 2006: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. – Published on the Internet http://ww2.bgbm.org/EuroPlusMed/ [accessed 20 May 2019].

FRANZÉN, R. 1986: Taxonomy of the *Achillea clavennae* Group and the *A. ageratifolia* Group (Asteraceae, Anthemideae) on the Balkan Peninsula. – Willdenowia 16: 13-33.

—, 1991: *Valeriana* L. Pp. 342-348. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

HARTVIG, P. 1986: *Linum* L. Pp. 553-565. In STRID, A. (eds), Mountain Flora of Greece, 1. – Edinburgh.

—, 2002: *Alyssum* L. Pp. 199-224. In STRID, A. & TAN, K. (eds), Flora Hellenica, 2. – Ruggell.

THIERS, B. 2019: Index Herbariorum: A global directory of public herbaria and associated staff. http://sweetgum.nybg.org/science/ih/. – Botanical Garden, New York [accessed 20 May 2019].

IPNI 2012: The International Plant Names Index. – Published on the Internet http://www.ipni.org [accessed 20 May 2019].

KAMARI, G. 1997: *Minuartia* L. Pp. 170-191. In STRID, A. & TAN, K. (eds), Flora Hellenica, 1. – Köningstein.

KOKKINI, S. 1991: *Scabiosa* L. Pp. 359-368. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

LIDÉN, M. 1996: New taxa of tuberous *Corydalis* (Fumariaceae). – Willdenowia 26: 23-35

PAPANIKOLAOU, K. & KOKKINI, S. 1982: A taxonomic revision of *Sideritis* L. section *Empedoclia* (Rafin.) Bentham (Labiatae) in Greece. Pp. 101-128. In MARGARIS, N., KOEDAM, A. & VOKOU, D. (eds), Aromatic Plants: Basic and Applied Aspects. – The Hague.

RAUS, T. 1991: *Verbascum* L. Pp. 170-188. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

SCHOLZ, H. 1991: *Poa* L. Pp. 762-774. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

SCHÖNBECK-TEMESY, E. & EHRENDORFER, F. 1991: *Asperula* L. Pp. 281-300. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

STRID, A. (ed.) 1986. Mountain Flora of Greece, 1. – Cambridge.

— 1986: *Astragalus* L. Pp. 460-478. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 1. – Cambridge.

— 1991: *Melica* L. Pp. 783-786. In STRID, A. & TAN, K. (eds), Mountain Flora of Greece, 2. – Edinburgh.

— & TAN, K. (eds) 1991: Mountain Flora of Greece, 2. – Edinburgh.

— & — (eds) 1997: Flora Hellenica, 1. – Köningstein.

— & — (eds) 2002: Flora Hellenica, 2. – Ruggell.

TAN, K. 2002: *Barbarea* R. Br. Pp. 170-173. In STRID, A. & TAN, K. (eds), Flora Hellenica, 2. – Ruggell.

— & IATROU, G. (2001): Endemic plants of Greece. The Peloponnese. – Copenhagen.

THE PLANT LIST. 2013: Version 1. – Published on the Internet http://www.theplantlist.org/ (accessed 20 May 2019).

Impact and management of herbicide resistant weeds

ILIAS ELEFTHEROHORINOS

Abstract

Eleftherohorinos, I. 2018: Impact and management of herbicide resistant weeds. – Bot. Chron. 22: 319-328.

Weed resistance to herbicides is a serious agricultural issue that threatens the sustainability of world food production, as its widespread appearance has reduced the diversity of weed control tactics and posed economic and environmental risks. The mechanism of herbicide resistance is either target-site (structural alteration of the target protein or overproduction of the target enzyme) or non-target site (herbicide metabolism by the resistant weed plants or reduced herbicide translocation at the site of action). Weed resistance is developed either to one (single herbicide resistance) or more herbicides with same (cross-resistance) or different mechanism of action (multiple resistance). Resistance evolution is affected by interacting factors related to weed traits, physicochemical herbicide properties and agricultural practices, while its impact is linked to reduced crop yield, increased production cost and negative effects to environment. Recent data on weed resistance are very worrying, as populations of 495 weed species worldwide and 23 in Greece are found resistant to one herbicide. These results strongly suggest that actions should be taken to control the already prevalent weed resistant populations and to prevent the evolution of new ones.

Eleftherohorinos, I., Emeritus Professor of Weed Science, Aristotle University of Thessaloniki, School of Agriculture, Thessaloniki, Greece. E-mail: eleftero@agro.auth.gr

Key words: herbicide metabolism; multiple resistance; resistance evolution; target-site resistane; weed management.

Introduction

Weed resistance to herbicides, also known as herbicide resistance, is considered a very important agricultural problem worldwide, since the existence of resistant weeds limits the choice of herbicides for weed control while the alternative or complementary methods used often result in economic and environmental impacts. In addition, herbicide resistance has altered public regulatory approval processes and scientific research for development of new weed management programs. Since most of the herbicide terms such as herbicide mechanism of action, herbicide mode of action, herbicide tolerance, herbicide resistance, herbicide cross-resistance, and herbicide multiple resistance are often inappropriately used, these terms will be redefined according to widely accepted definitions by weed scientists. This is appropriate as this clarification may contribute to a better understanding of weed resistance to herbicides and its management.

Herbicide mechanism of action (alternatively herbicide site of action), is the biochemical site within a plant that is directly affected by a herbicide (WSSA 2011), or the initially affected target protein (enzyme, structural protein) by a herbicide that ultimately inhibits the biochemical pathway where the protein is involved.

Herbicide mode of action has a broader meaning and includes a sequence of biological events [penetration of the herbicide into the plant, translocation and accumulation at its site of action, binding to its target protein (mechanism of action), inhibition of protein involvement in a biochemical pathway, generation of reactive oxygen species in some cases, disruption of a vital metabolic pathway or cell structure, reduction of plant growth and development, and ultimately plant death] occurring within the plant due to the presence of a herbicide (Eleftherohorinos 2014, Wssa 2011). According to the mechanism or mode of action, herbicides are classified into three classes (Heap 2018). The first class includes herbicides that inhibit light processes or biosynthesis of essential photosynthetic pigments [1) electron flow in photosystem II, 2) electron flow in photosystem I, (3) biosynthesis of chlorophyll, 4) biosynthesis of carotenoids]. The herbicides of the second class inhibit cell metabolism processes [1) biosynthesis of fatty acids with 16 or 18 carbon atoms, 2) biosynthesis of the branched chain amino acids valine, leucine and isoleucine, 3) biosynthesis of the aromatic amino acids tryptophan, phenylalanine and tyrosine, 4) biosynthesis of the amino acid glutamine, 5) biosynthesis of folic acid, 6) biosynthesis of ATP and, 7) biosynthesis of fatty acids with more than 18 carbon atoms]. Regarding the herbicides of the third class, they influence growth/cell division processes [1) mitosis-cell division, 2) biosynthesis of fatty acids with more than 18 carbon atoms, 3) cellulose biosynthesis, 4) translocation of endogenous auxin, 5) auxin activity]. It is worth mentioning that two thirds of the above-mentioned herbicides have their target-site of action within the chloroplast.

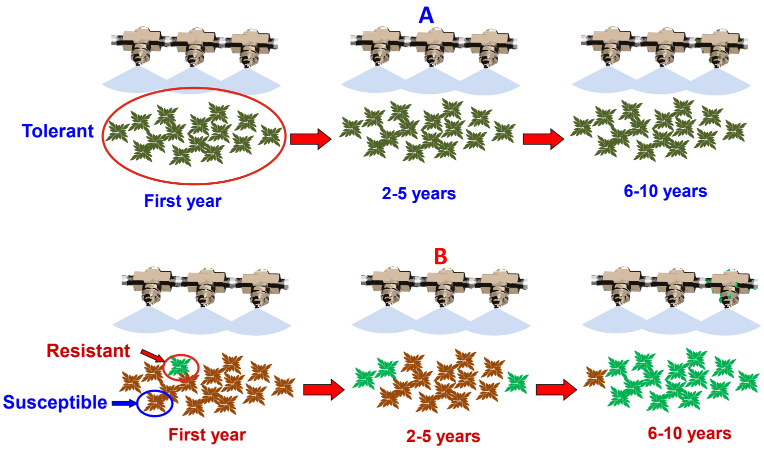


Fig. 1. A, Weed tolerance to herbicide as indicated by a non-population change over time (weed plants were and remain tolerant-dark green); B, Weed resistance to herbicide as indicated by a population change over time (plants were susceptible-brown color and are changing to resistant-light green) (Wssa 2011).

Herbicide tolerance is the inherited ability of a weed species to survive and reproduce after herbicide treatment at recommended dose. This implies that any selective pressure to render a weed species tolerant is non-existent, as the weed is naturally tolerant (Fig. 1A). For this reason, the tolerant weed species is classified as non-sensitive on the label of the herbicide. Since weed tolerance cannot be handled through herbicide application, it is not going to be further discussed.

Weed resistance to herbicide or **herbicide resistance** is the inherited ability of some weed plants to survive and reproduce following exposure to a herbicide treatment normally lethal to wild type (WSSA 2011). This means that herbicide resistance is normally present at very low frequencies in a weed population before the first herbicide application, and it is selected as a consequence of the selective pressure from the repeated use of a herbicide (Fig. 1B). This implies that herbicide resistance is a weed trait changing over time from susceptible to resistant, and for this reason the weed species with a resistant population is classified as susceptible on the label of the herbicide.

Cross-resistance of a weed population is the coexistent resistance into two or more herbicides with same mechanism of action, while **multiple resistance** is the coexistent weed resistance to herbicides with different mechanism of action. These two aspects of herbicide resistance are of great importance for agriculture since they limit the use of a large number of herbicides for weed control.

Within a weed population, herbicide resistance may arise through pre-existing natural resistance, imported resistance, and natural dispersal. The pre-existing natural resistance is evolved from some weed plants that contain an altered gene (or genes), which enables them to survive a herbicide application that would normally kill this weed species. This genetic variation may alter physiological weed traits leading then to reduce herbicide uptake and translocation at the site of action, enhance the ability to detoxify herbicides, or enable weeds to transport the herbicide to a site within the plant (e.g. vacuole or cell wall) where it is not lethal. Herbicide resistance can be introduced in a field crop as a weed contaminant in the seed used for sowing or via animals. Also resistance can be evolved through natural dispersal of resistant weed seeds by wind and water or via pollen transferred from a herbicide-resistant crop or weed plants to sensitive weed plants (KALOUMENOS & al. 2013a).

Mechanisms of herbicide resistance

The mechanism involved in herbicide resistance is either target-site (TSR) or non-target-site (NTSR). The TSR is endowed by alterations in the gene encoding the herbicide target protein (structural alteration of the target protein or overproduction of the target enzyme), while the potential mechanisms involved in NTSR are reduced herbicide uptake and translocation, reduced herbicide activation, enhanced herbicide detoxification (herbicide metabolism), changes in intra or inter-cellular compartmentalisation and enhanced repair of herbicide-induced damage (DELYE 2013). Among the NTSR mechanisms, the most commonly reported ones are herbicide metabolism and reduced herbicide translocation at the site of action.

The **structural alteration** of **target protein** (enzyme, stractural protein), which reduces the ability of a herbicide to bind to its target protein, is the most commonly reported TSR mechanism. This is shown in Fig. 2, where the binding of the herbicide to its target-site enzyme, like a key (herbicide) in a lock (enzyme) analogy, prevents the enzyme to bind the substrate (Fig. 2A) and therefore inhibits its catalytic activity to produce essential substances needed for weed survival. In contrast, in the case of herbicide inability to bind to the altered resistant enzyme (different lock) (Fig. 2B), the

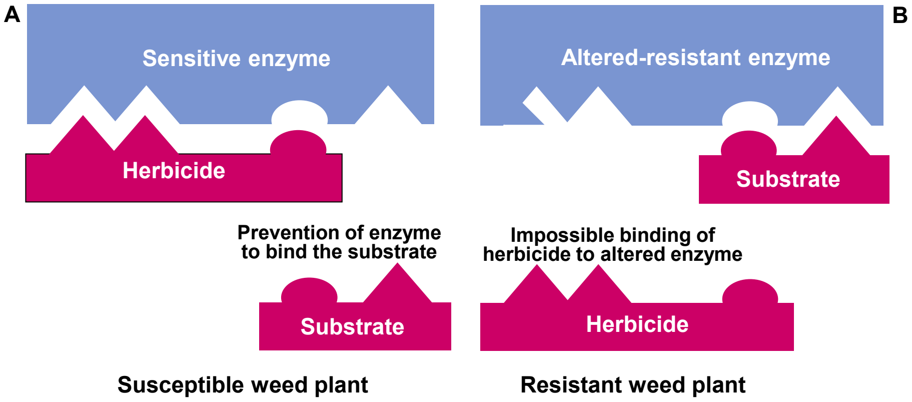


Fig. 2. Resistance due to structural alteration of the target enzyme.

enzyme binds the substrate and catalyzes the production of substances required for weed survival.

Weed resistance to one (single resistance) or more herbicides (cross-resistance) with same mechanism of action is controlled by one gene only that encodes the altered protein. Regarding cross-resistance, it is resulted from the same or closely overlapping binding sites by particular herbicides on the target protein, which implies that there are a number of different functional mutations on the gene encoding the target protein.

(A) The binding of the herbicide to its sensitive target enzyme (like key-herbicide in lock-enzyme) prevents enzyme to bind the substrate and therefore inhibits the production of substances required for survival of the susceptible weeds.

(B) The inability of herbicide to bind to its altered target enzyme (different lock) allows the enzyme to bind substrate and to catalyze the production of substances needed for survival of the resistant weeds.

The second TSR mechanism due to **overproduction of target enzyme** results from the overexpression of a gene (s) due to an altered gene promoter or to more existing copies of the same gene (gene amplification) (GAINES & al. 2013). The overproduction of the target enzyme enables the resistant weeds to survive because the penetrated herbicide at its site of action is not adequate to inhibit the activity of the overproduced target enzyme.

The most important NTSR mechanism is **herbicide metabolism** by a non-target-site enzymes that catalyze the structure alteration of a herbicide (e.g. hydroxylation, hydrolysis) or the formation of complexes between the herbicide and glutathione, glucose or an amino acid (Fig. 3). The enzymes involved in the alteration of the herbicide structure are monooxygenases, dioxygenases, dehydrogenases and hydrolases (esterases, phosphatases, amidases, nitrilases, deaminases), whereas the respective enzymes for formation of complexes between the herbicide and glutathione, glucose or amino acid are glutathione-S-transferases, glycosyltransferases or aminotransferases. Althoug this type of NTSR can confer unpredictable cross- or multiple-resistance, the genetic determinants remain unknown and for this reason it can be considered as the dark side of resistance compared with TSR (DELYE 2013).

The NTSR mechanism due to **reduced herbicide translocation** at its site of action is attributed to: 1) reduced herbicide absorption-penetration through cell membrane, 2) reduced herbicide translocation from cytosol to its site of action (e.g. chloroplast), 3) herbicide deposition from cytosol into vacuoles (where it is not active), 4) herbicide deposition from cytosol to cell wall (SAMMONS & GAINES 2014). According to SHANER (2009), these four processes are facilitated or prevented through the involvement of ATP binding cassette (ABC) transporters. The alleles involved in this type of resistance have not been identified so far.

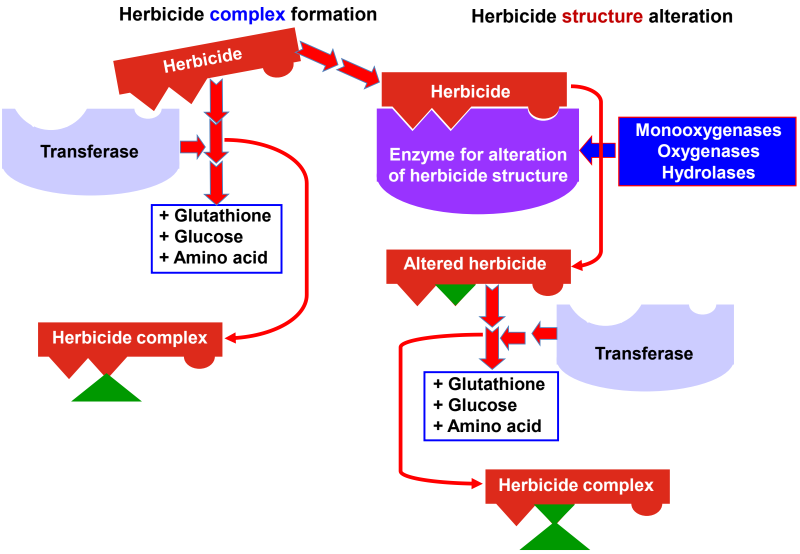


Fig. 3. Weed resistance due to herbicide metabolism (structure alteration and/or formation of complexes with glutathione, glucose or an amino acid) catalyzed by enzymes.

Factors affecting herbicide resistance

Weed resistance is the result of weed evolutionary adaptation to herbicide application and is affected by many interacting factors such as herbicide chemistry and rate applied, mechanism of action, agricultural practices applied, particular ecological and biological traits of weed species, genetic, biochemical and physiological mechanisms capable of conferring weed resistance to herbicides, and environmental conditions (DELYE 2013, RENTON & al. 2014). Concerning weed traits, the ability of a weed to evolve herbicide resistance is favored when: 1) the frequency of resistance gene in an original population is high, 2) the resistance mechanism is controlled by a dominant gene of nucleus, 3) the weed species is cross-pollinated (potential flow of the dominant resistant gene via pollen), 4) the seed production and germination ability of the resistant weed plants are similar or greater than that of the susceptible ones, 5) the seed dispersal of the resistant weed plants by machinery, wind, water, and animals is similar or greater than that of the susceptible ones, 6) the competitive ability of the resistant plants is similar or higher than that of susceptible ones, 7) the adaptability of resistant plants is wider than that of sensitive, 8) the altered-resistant gene has a positive pleiotropic effect on adaptability, 9) the nutrient and water requirements of resistant plants are similar or lower than that of susceptible weed plants and, 10) the tolerance of resistant weed plants to insect, fungi, viruses, bacteria and nematodes is similar or greater than that of the susceptible plants.

The evolution rate of herbicide resistance is accelerated when the **herbicide** used: 1) is very active and therefore exerts a high selective pressure on weed plants, 2) has a long residual activity resulting in a long-lasting selective pressure, and 3) has a mechanism of action controlled by a single dominant gene of the nucleus, which encodes the altered target-site protein or the herbicide metabolism enzyme.

The number of herbicide resistant populations decreases when **farmers**: 1) take preventive measures to reduce spread of resistant weed seeds, 2) apply deep plowing every 3-5 years, 3) apply appropriate crop rotation systems, 4) take measures to improve the competitive ability of a crop against weeds, 5) record germination time, composition, density (abundance), and distribution of different weed species in a field crop before and after application of herbicides, 6) apply herbicides at the recommended doses and at the appropriate weed growth stage, 7) rotate herbicides with different mode of action, 8) use mixtures of herbicides with different mode of action, 9) combine soil cultivation between crop rows and application of selective herbicides on the crop row, 10) apply integrated weed management systems and, 11) maintain a database with distribution of weed species in field crops and their management problems (BARRES & al. 2016, BECKIE & HARKER 2017, LAMICHHANE & al. 2017).

Weed resistance is also affected by environmental factors such as drought, flood, heat, frost, intensity and duration of light, soil fertility, water pollution, as well as air and soil pollution. However, the effect of these factors on weed resistance is not clear as it is not adequately and in depth studied.

Global status of herbicide resistance in weeds

Recent data on herbicide resistance is very worrying as populations of **502 weed species** are already resistant to one herbicide, whereas the respecive weed species with single resistance (to one herbicide), coexistent cross-resistance, and coexistent multiple resistance are **258** (150 dicots and 108 monocots) (HEAP 2019). It is worth mentioning that weed resistance has recorded to 167 out of 300 registered herbicides, whereas the most important ones of these weed species are resistant to the most widely used herbicides (e.g. sulfonylureas, imidazolinones, glycines, aryloxyphenoxypropionates, cyclohexanediones).

In Greece, **23 weed species** have developed single resistance or cross-resistance to herbicides that inhibit electron flow in photosystem II, or ALS enzyme involved in biosynthesis of the amino acids valine, leucine, and isoleucine (KALOUMENOS & al. 2013a,b, NTOANIDOU & al. 2017), or the activity of the ACCase enzyme needed for biosynthesis of fatty acids with 16-18 carbon atoms (PAPAPANAGIOTOU & al. 2015), or the EPSPS enzyme needed in biosynthesis of the aromatic amino acids tyrosine, tryptophan, and phenylalanine (MYLONAS & al. 2015). In addition, four weed species (*Avena sterilis*, *Echinochloa oryzicola*, *Apera spica-venti, Millium vernale*) have evolved multiple resistance to ACCase- and ALS-inhibiting herbicides (Table 1).

Table 1. Weed species in Greece with single, cross- and multiple resistance to herbicides (ELEFTHEROHORINOS in press).

Weed species	**Herbicide resistance**
Echinochloa crus-galli (L.) Beauv.	PS II-inhibitor (propanil)
Amaranthus retroflexus L.	PS II-inhibitor (metribuzin)
Chenopodium album L.	PS II-inhibitor (metribuzin)
Lolium rigidum Gaud.	ALS-inhibitors (chlorsulfuron, mesosulfuron + iodosulfuron) ACCase-inhibitors (clodinafop, diclofop, tralkoxydim)
Solanum nigrum L.	PS II-inhibitor (prometryn)
Papaver rhoeas L.	ALS-inhibitors (chlorsulfuron, tribenuron, triasulfuron, mesosulfuron + iodosulfuron, florasulam, pyrithiobac, imazamox)
Sorghum halepense L.	ACCase-inhibitors (fluazifop, quizalofop, propaquizafop)
Avena sterilis L.	ACCase-inhibitors (diclofop, clodinafop, fenoxaprop, tralkoxydim, pinoxaden) ALS-inhibitors (mesosulfuron+iodosulfuron, pyroxsulam)
Echinochloa oryzicola Vasing.	ALS-inhibitors (penoxsulam, bispyribac, imazamox, foramsulfuron, nicosulfuron, rimsulfuron) ACCase-inhibitor (cyhalofop)
Oryza sativa L.	ALS-inhibitors (imazamox, imazethapyr)
Conyza albida L., *C. bonariensis* L., *C. canadensis* L.	EPSPS-inhibitor (glyphosate)
Phalaris minor Retz.	ACCase-inhibitors (diclofop, clodinafop, fenoxaprop)
Sinapis arvensis L.	ALS-inhibitors (tribenuron, imazamox)
Cyperus difformis L.	ALS-inhibitors (azimsulfuron, halosulfuron, imazosulfuron)
Bifora radians M.Bieb.	ALS-inhibitors (chlorsulfuron, tribenuron, florasulam, tritosulfuron)
Apera spica-venti (L.) P.Beauv.	ALS-inhibitors (chlorsulfuron, mesosulfuron+iodosulfuron, pyroxsulam) ACCase-inhibitors (clodinafop, pinoxaden, clethodim, cycloxydim)
Millium vernale M. Bieb.	ACCase-inhibitors (clodinafop, diclofop) ALS-inhibitors (mesosulfuron+iodosulfuron, pyroxsulam)
Camelina microcarpa Andrz. ex DC.	ALS-inhibitors (mesosulfuron+iodosulfuron, tribenuron, pyroxsulam+florasulam, imazamox)
Galium spurium L., *G. aparine* L.	ALS-inhibitors (chlorsulfuron, tribenuron, florasulam)
Rapistrum rugosum L.	ALS-inhibitors (tribenuron, imazamox)

Impact of herbicide resistance in agriculture and environment

The economic impact of herbicide resistance is related to reduction in crop yield and product quality as a result of reduced weed control. Regarding production cost, it is increased from the use of more expensive treatments to control resistant weeds. The environmental risks are resulted from the alternative herbicide and non-chemical treatments used to control resistant weeds, which usually have greater potential of environmental pollution and negative effects to non-target organisms (weed species, soil microflora, and fauna). Also, the use of soil cultivation (tillage), as a complementary method to control resistant weeds, poses risks of soil erosion and compaction.

Change of weed species over time from the continuous use of herbicides is reflected as increased frequency and density of resistant weed species over sensitive ones, and, in some cases, as a reduction of weed species number. This is confirmed from the results reported by Potts & al. (2010), who found that the use of herbicides for 38 years (1968-2005) in a winter cereal area of 6,200 hectares, resulted in the loss of five out of 214 existing weed species and the appearance of nine new ones. The reduced ability of herbicides to eradicate weed species is related to very effective weed survival mechanisms such as great reproductive ability, seed longevity and dormancy, natural dispersal ability, wide adaptability, great competive or allelopathic ability (fitness advantage) against crops, and genetic variation in some weed species (resistant populations) caused from the continuous use of herbicides.

Herbicide resistance in several economically important weeds has prompted a reevaluation of its indirect effect on soil microflora (soil microbial community structure and activity). This was made as different weeds release various plant metabolites from roots that alter soil environment by selecting for specific rhizosphere microbial communities (Kremer 2014). This is confirmed by the herbicide-resistant weeds Amaranthus and Chenopodium that do not support arbuscular mycorrhizal fungi (AMF) symbioses, potentially reducing AMF propagule density and establishment with crop plants.

Soil microflora, except from the indirect effect of the weed presence, is affected directly from the toxic herbicides used for weed control. These effects are reflected as reduced abundance of microorganisms and, by extension, rate reduction of biological processes involved such as: (1) nitrogen fixation (Rhizobium spp.), 2) nitrogen transformation [conversion of ammonium (NH4+) to nitrite (NO2-)-Nitrosomonas or conversion of nitrite (NO2-) to nitrates (NO3-)-Nitrobacter], 3) plant nutrient absorption through mycorrhiza (VA), 4) decomposition of organic-plant residues.

Fauna is directly affected by the herbicide acute toxicity and indirectly from the reduction of the survived weeds used either as hosts or to feed various animal organisms. This is confirmed by the experiments conducted in United Kingdom, which showed that weed control differences result in different effects on populations of soil saprophytic organisms (Collembola, fed from dead organic matter), soil coleoptera (Carabidae, fed by weed seeds), herbivores, pollinators and butterflies (Brooks & al. 2003, Edwards 1993).

The herbicides used to control resistant and susceptible weeds affect indirectly soil physicochemical properties. These changes result from the reduction of the organic matter caused by the decreased incorporation of plant residues due to the weed control and, also, by the reduced involvement of certain microorganisms and other non-target soil organisms in the decomposition of crop plant residues. Furthermore, the increased mobility (leaching) of some herbicide in soils increases the potential for surface and ground water pollution.

Management of weed resistance to herbicides

The above-mentioned economic and environmental impacts of herbicide resistance strongly suggest to undertake measures and implement methods that: 1) contribute to effective management of already existing resistant weed populations, 2) reduce the spread of the already selected resistance, 3) reduce the possibility for prevalence of new resistant weed species and finally, 4) extend the sustainable use of the effective herbicides.

The management of the already existing resistant populations along with the reduction of the possibility for avoiding evolution of new resistant weed populations require knowledge about species identification, biology, and ecology of weeds, herbicide mode of action, herbicide resistance mechanisms, factors affecting herbicide resistance, and methods used for herbicide resistance management (NORSWORTHY & al. 2012). In addition to these, a change in attitudes among farmers, scientists and institutions is needed in order to accept the principle 'change the herbicide when is effective' or 'do not use an effective herbicide continuously'. This is necessary since it is the only way to reduce the possibility of evolving new resistant weeds and, therefore, to increase the longevity (sustainable use) of the effective and less dangerous herbicides.

References

BARRÈS, B., MICOUD, A., CORIO-COSTET, M. F., DEBIEU, D., FILLINGER, S., WALKER, A-S., DÉLYE, C., GROSMAN, J., & SIEGWART, M. 2016: Trends and challenges in pesticide resistance detection. – Trends in Plant Sci. 21: 834-853.

BECKIE, H. J. & HARKER, K. N. 2017: Our top 10 herbicide-resistant weed management practices. – Pest Manag. Sci. 73: 1045-1052.

BROOKS, D. R., BOHAN, D.A., CHAMPION, G.T., & al. 2003: Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. – Philosophical Transactions of the Royal Society 358: 1847-1862.

DELYE, C. 2013: Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. – Pest Manag. Sci. 69: 176-187.

EDWARDS, C. A. 1993: Effects of herbicides on soil and surface-inhabiting invertebrates. – Brighton Crop Prot. Conf.–Weeds 1:133-138.

ELEFTHEROHORINOS, I. G. 2014: In AGROTYPOS (ed.), Weed Science: Weeds-Herbicides-Environment-Principles and Methods of Weed Management. – Athens, [in Greek].

GAINES, T. A., WRIGHT, A. A., MOLIN, W. T., LORENTZ, L., RIGGINS, C. W., TRANEL, P. J., BEFFA, R., WESTRA, P. & POWLES, S. B. 2013: Identification of genetic elements associated with EPSPS gene amplification. – PLOS ONE 8:e65819.

HEAP, I. 2019: International survey of herbicide resistant weeds. – Available at web site http://www.weedresearch.com/in.asp

KALOUMENOS, N. S., CAPOTE, N., AGUADO, A. & ELEFTHEROHORINOS, I. G. 2013a: Red rice (*Oryza sativa*) cross-resistance to imidazolinone herbicides used in resistant rice cultivars grown in northern Greece. – Pest. Bioch. Phys. 105:177-183.

—, Chatzilazaridou, S. L., Mylona, P. V., Polidoros, A. N. & Eleftherohorinos, I. G. 2013b: Target-site mutation associated with cross-resistance to ALS-inhibiting herbicides in late watergrass *(Echinochloa oryzicola* Vasing). – Pest Manag. Sci. 69:865-873.

Kremer, R. J. 2014: Environmental Implications of Herbicide Resistance: Soil Biology and Ecology. – Weed Sci. 62(2): 415-426.

Lamichhane, J. R., Devos, Y., Beckie, H. J., Owen, M. D. K., Tillie, P., Messéan, A. & Kudsk, P. 2017: Integrated weed management systems with herbicide-tolerant crops in the European Union: lessons learnt from home and abroad. – Critical Rev. Biot. 37: 459-475.

Mylonas, P. N., Giannopolitis, C. N., Efthimiadis, P. G., Menexes, G., Madesis, P. B. & Eleftherohorinos, I. G. 2014: Glyphosate resistance of molecularly identified *Conyza albida* and *Conyza bonariensis* populations. – Crop Prot. 65: 207-215.

Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., Bradley, K. W., Frisvold, G., Powles, S. B., Burgos, N. R., Witt, W. W. & Barrett, M. 2012: Reducing the risks of herbicide resistance: Best management practices and recommendations. – Weed Sci. Special Issue: 31-62.

Ntoanidou, S., Madesis, P., Diamantidis, G. & Eleftherohorinos, I. 2017: Trp574 substitution in the acetolactate synthase of *Sinapis arvensis* confers cross-resistance to tribenuron and imazamox. – Pest. Bioch. Phys. 142: 9-14.

Papapanagiotou, A. P., Paresidou, M. I., Kaloumenos, N. S. & Eleftherohorinos, I. G. 2015: ACCase mutations in *Avena sterilis* populations and their impact on plant fitness. – Pest. Bioch. Phys. 123: 40-48.

Potts, G. R., Ewald, J. A. & Aebischer, N. J. 2010: Long-term changes in the flora of the cereal ecosystem on the Sussex Downs, England, focusing on the years 1968-2005. – J. Appl. Ecol. 47: 215-226.

Renton, M., Busi, R., Neve, P., Thornby, D. & Vila-Aiub, M. 2014: Herbicide resistance modelling: past, present and future. – Pest Manag. Sci. 70: 1394-1404.

Sammons, R. D. & Gaines, T. A. 2014: Glyphosate resistance: state of knowledge. – Pest Manag. Sci. 70: 1367-1377.

Shaner, D. L. 2009. Role of translocation as a mechanism of resistance to glyphosate. Weed Science 57: 118-123.

Wssa. 2011: WSSA lesson module: Herbicide resistant weeds. Available at http://wssa.net/LessonModules/herbicide-resistant-weeds/index.htm

Conformations of endoplasmic reticulum in untreated and Cr(VI)-treated dividing root tip cells of *Lens culinaris* L. visualized by confocal microscopy

ELEFTHERIOS P. ELEFTHERIOU & IOANNIS-DIMOSTHENIS S. ADAMAKIS

Abstract

Eleftheriou, E. P. & Adamakis I.-D. S. 2019: Conformations of endoplasmic reticulum in untreated and Cr(VI)-treated dividing root tip cells of *Lens culinaris* L. visualized by confocal microscopy. – Bot. Chron. 22: 329-344.

The effects of hexavalent chromium [Cr(VI)] on the endoplasmic reticulum (ER) during all mitotic phases in root tip cells of *Lens culinaris* (lentil) were studied by confocal laser scanning microscopy (CLSM) after immunofluorescence labelling of ER, and were compared with ER distribution and typical microtubule (MT) arrays in the corresponding phases of untreated cells. In interphase cells MTs and ER did not coincide, while after Cr(VI) exposure ER became granular and spread throughout the cytoplasm. In prophase ER formed a well-defined preprophase band (ER-PPB) coinciding with the preprophase band of MTs (MT-PPB). After Cr(VI) application ER-PPB broadened considerably as was previously shown for MT-PPBs, supporting the view that MTs provide a scaffold for ER. Unusual for higher plants, in metaphase ER was arranged in a spindle-like structure with strands projecting inside the spindle and terminating on kinetochores, similarly to MTs, retained even after Cr(VI) exposure. Anaphase ER was primarily gathered in the polar regions but also in the peripheral cytoplasm ensheathing the chromosomes. In telophase ER signal was localized on the reorganizing nuclear envelope, the phragmoplast margins and on the cell plate, but in Cr(VI)-treated cells ER distribution was severely disrupted reflecting the deranged phragmoplasts. It is concluded that ER distribution both in untreated and Cr(VI)-treated cells reflects MT changes and organization. Its morphology during mitotic phases and cytokinesis is consistent with the view that in dividing plant cells ER organization is supported by MTs rather than actin filaments (AFs) as is the case for interphase and differentiated plant cells.

E. P. Eleftheriou, Department of Botany, School of Biology, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece. E-mail: eelefth@bio.auth.gr (=Corresponding author)
I.-D. S. Adamakis, Department of Botany, School of Biology, National and Kapodistrian University of Athens, 157 84 Athens, Greece. E-mail: iadamaki@biol.uoa.gr

Key words: Endoplasmic Reticulum, Hexavalent chromium, Microtubules, *Lens culinaris,* MT-ER correlations.

Abbreviations: AF, actin filament; CLSM, confocal laser scanning microscopy; Cr, chromium; Cr(III), trivalent chromium; Cr(VI), hexavalent chromium; ER, endoplasmic reticulum; ER-PPB, preprophase band of ER; MT, microtubule; MT-PPB, preprophase band of MTs.

This article is dedicated to Professor Emeritus Dimitrios Phitos on the occasion of his 90th birthday.

Introduction

Chromium (Cr) is the 21st most abundant element in earth's crust with an average concentration of 100 ppm. Naturally, Cr occurs in several oxidation states, the most common and stable being the trivalent [Cr(III)] and hexavalent [Cr(VI)] ones, which exhibit very different chemical and biological features but both at high concentrations are toxic to living organisms. Cr(III) is less water soluble, slightly mobile, very stable in soil and less toxic to living organisms, while Cr(VI) is readily soluble, very unstable in soil, easily mobilized and highly toxic (SHANKER & al. 2005, MEGREMI 2010, SHAHID & al. 2017). Under reducing conditions, Cr(VI) converts to Cr(III), which is insoluble and strongly absorbed onto the surface of soil particles. Consequently, the form that is most available to plants is Cr(VI), which in soil dissociates to CrO_4^{2-} and $HCrO_4^{2-}$.

High levels of total Cr in the soil may occur naturally by the erosion of metal rich rocks such as ophiolites and Ni-laterites (MEGREMI 2010, ECONOMOU-ELIOPOULOS & al. 2011). However, in most situations the primary causes for high Cr concentrations locally are anthropic activities aiming to exploit its unique physical properties, such as lustre, colourful compounds, hardness, high resistance to corrosion and discoloration, in a broad range of industrial applications including heavy metallurgy, chrome plating, wide tanning, manufacturing of paints, pigments and textile dyes, wood preservation and paper production (NRIAGU 1987). The consequent extensive soluble Cr emissions into the environment have frequently caused local contamination worldwide (SHAHID & al. 2017), raising concerns for human health risks by its presumed uptake and accumulation by plants and animals and thus entering the food chain (BROADWAY & al. 2010, AHMED & al. 2016). The most highly Cr-contaminated sites worldwide were reported to be India, Eastern Africa, South America and China (ERICSON 2011), with effluents of leather tanneries being the most serious anthropogenic Cr pollutants (USEPA 1992). Of local concern is the case of Asopos River in Central Greece. The increased amounts of Cr - and other metals - in the soil of the area were attributed both to natural sources due to occurrence of ultrabasic rocks (ANTIBACHI & al. 2012) and to the intense industrialization of the drainage basin of Asopos River over the last decades (VASILATOS & al. 2008, BOTSOU & al. 2011, ECONOMOU-ELIOPOULOS & al. 2011). As a result, elevated amounts of Cr, and in particular of Cr(VI), were measured in the river's water, in groundwater, in surface sediment samples along the river, and in the seabed mud at the river’s estuarine (VASILATOS & al. 2008, BOTSOU & al. 2011, TZIRITIS & al. 2012).

The toxicity of Cr to mammals, including humans, has extensively been investigated (e.g. SORAHAN & al. 1998, KIM & al. 2004, POLISAK & al. 2011), where it was shown that it could be even carcinogenic (STERN 2010, LINOS & al. 2011). Though less than animals, in plants numerous studies have indicated that none of Cr species is an essential trace element for plant metabolism and that at high concentrations all forms of Cr proved to be harmful for plant development and growth. Most studies refer mainly to Cr toxicity to morphological, biochemical and physiological parameters such as seed germination, root, stem and leaf growth, photosynthesis, mineral nutrition, biomass production, yield, genotoxicity, and induction of metabolic modifications and reactive oxygen species (reviews by SHANKER & al. 2005, OLIVEIRA

2012, SHAHID & al. 2017). Fewer studies have been conducted on the detrimental effects of Cr to plant cell cycle and structure.

Dividing plant cells are dynamic and sensitive systems that constitute an excellent material for the study of the detrimental effects caused by biotic/abiotic stresses, including toxic heavy metals. The readily visible chromosomal changes during plant cell division cycle are under strict spatial control, underlain by the two major cytoskeletal elements, microtubules (MTs) and actin filaments (AFs) (GUNNING & STEER 1996, MÜLLER 2012). In particular, MTs untergo significant dynamic changes forming successive arrays corresponding to particular cell phases such as interphase MTs, preprophase band of MTs (MT-PPB), the metaphase and anaphase spindle, the phragmoplast and the perinuclear metatelophase array of MTs (SMIRNOVA 2012). MT arrays are useful markers of cell phases if chromatin/chromosomes are not visualized, and vice versa. Recent studies have shown that MTs constitute a universal target of Cr(VI) toxicity in dividing plant cells (ELEFTHERIOU & al. 2012, 2013, 2015a,b, 2016). However, other subcellular structures such as endoplasmic reticulum (ER) are linked to cell division cycle (HEPLER 1980, 1981, QUADER & ZACHARIADIS 2006), but their behaviour under Cr(VI) stress has been poorly investigated. Knowledge of ER response under biotic/abiotic stresses is vital (MATSUSHIMA & al. 2002, CHO & KANEHARA 2917, GRIFFING & al. 2017). Given that Cr(VI) induced an extensive alteration of the MT arrays in *L. culinaris* (ELEFTHERIOU & al. 2013), the objective of the present study was to clarify its detrimental effects on the morphology and distribution of ER during all mitotic phases of root tip cells and correlate ER changes with altering MT arrays.

Materials and methods

Plant material and preliminary experiments

In the present study seedlings of *Lens culinaris* Moench. (lentil), raised from seeds purchased from a local market, were used. This plant was selected due to its abundance as an experimental material, quick germination, easy handling, its nutritional importance, local cultivation, and because the Cr(VI) effects on MTs and chromosomes have been previously studied (ELEFTHERIOU & al. 2013).

A preliminary germination test of lentil seeds was carried out in Petri dishes on filter paper soaked with distilled or tap water, which showed a germination index of almost 100%. In a second preliminary experiment, seeds of lentil were directly placed on aqueous solutions of Cr(VI), supplied as potassium dichromate ($K_2Cr_2O_7$), at concentrations 0 (control), 50, 100, 500 and 1000 µM for 4 days to check the effect of Cr(VI) on germination. In a third preliminary experiment new seeds were pre-germinated for 24 h in distilled water. Then, sets of 30 selected seedlings of about equal size were exposed to aqueous solutions of 0 (control), 10, 50, 100, 250, 500 and 1000 µM Cr(VI) (0, 2.9, 14.7, 29.4, 73.5, 147 and 294 mg/L, respectively). The length of the developing roots was measured every 24 h and results were used to draw graphs expressing the rate of root lengthening. Data were presented as mean ± standard error (SE) of three independent experiments. Statistical analyses (ANOVA with Dunnett's multiple comparison test) were performed using Graph Pad software (San Diego, CA, USA), with significance at $P < 0.05$.

These preliminary results were directive for the main experiments, which were carried out in 24 h pre-germinated seedlings exposed to 50, 100 and 250 µM Cr(VI) for 24 or 48 h that induced sub-toxic and toxic effects. Always, seedlings placed in distilled water were used as controls.

Visualization of endoplasmic reticulum

For ER and chromatin/chromosomes dual visualization in meristematic root tip cells of control and Cr(VI)-treated seedlings of *L. culinaris*, a specific immunostaining protocol of ER was applied and DNA was fluorescently stained (Zachariadis & al. 2001, 2003, Eleftheriou & al. 2012). In particular, excised root tips of 24 h pre-germinated seedlings that were then placed in 0 (control), 50, 100 and 250 μM Cr(VI) for 24 and 48 h, were fixed for 60 min in 8% (w/v) paraformaldehyde in PEM buffer (50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4.7H_2O$), pH 6.8. After washing with fresh PEM, the cell walls were digested for 40 min in 2% cellulase (Onozuka R-10, Serva), 2% macerozyme-R10 (Serva) and 0.4% β-glucuronidase in PEM. Following a quick wash in PEM, the root tips were gently squashed onto polylysine-coated coverslips. The separated cells were left to dry, then extracted with 5% DMSO + 5% Triton-X 100 for 1 h and incubated overnight at room temperature with a mouse monoclonal anti-HDEL (the tetrapeptide histidine, aspartic acid, glutamic acid, leucine) antibody (2E7, Santa Cruz) in dilution 1:40 (Napier & al. 1992). After washing with PEM, the cells were incubated with 1:80 FITC-anti-mouse antibody for 3 h at room temperature, followed by 1 h at 37°C. In order to correlate the ER phases with mitosis, DNA was counterstained with 3 $\mu g.ml^{-1}$ propidium iodide in PEM and the coverslips were finally mounted in an anti-fade solution.

The specimens were examined with a Nikon D-Eclipse C1 confocal laser scanning microscope (CLSM), with an optical sectioning step of 0.20 or 0.30 μm. An exciter at 488 nm and a barrier at 515/30 nm, and an exciter at 543 nm and a barrier at 570 nm were used for ER and DNA, respectively. Special care was taken in order to retain the laser beam gain equal among the different treatments. Image recording was done with proper software (EZ-C1 3.20) according to the manufacturer's instructions. Selected photographs were processed with Adobe Photoshop CS6 and plates were constructed with CorelDraw X7 software.

Immunolocalization of MTs

Because MTs display dynamic changes during cell cycle underlying mitotic phases and their sensitivity to Cr(VI) toxicity has been reported (Eleftheriou & al. 2013), they constitute a dependable marker of a given mitotic phase to which the conformation of ER can be compared. Thus, in the present study they were used as controls to recognize specific mitotic phases and the respective structure of ER. For MT immunolocalization, the same procedure as described above was applied, except that a mouse anti-α-tubulin antibody (DM1A, Santa Cruz) or a rat anti-α-tubulin antibody (YOL 1/34, Serotec), diluted 1:80 in PEM were used (Eleftheriou & al. 2012, 2013). After washing with PEM, the cells were incubated with 1:80 FITC-anti-mouse or FITC-anti-rat in the same buffer for 3 h at room temperature, followed by 1 h at 37°C. DNA was also counterstained with 3 $\mu g.ml^{-1}$ propidium iodide in PEM and the coverslips were finally mounted in an anti-fade solution.

Results

Seeds placed on Cr(VI) solutions of increasing concentration showed an escalating hindrance of germination in a concentration dependent manner (Fig. 1A). At the highest concentrations of 500 and 1000 μM no seeds could germinate at all. When 24 h pre-germinated seedlings were transferred to graded Cr(VI) concentrations of 10, 50, 100, 250, 500 and 1000 μM for 4 days, primary root growth displayed a concentration dependent decline (Fig. 1B).

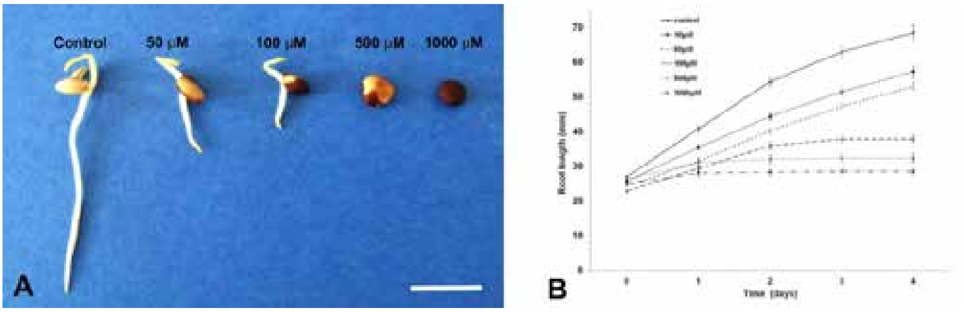


Fig. 1. A, Representative seedlings of *Lens culinaris* germinated directly on graded Cr(VI) solutions for four days as depicted. At the higher concentrations (500, 1000 µM) seeds did not germinate at all; B, Kinetic of root growth of pre-germinated seedlings in water for 24 h and then transferred to graded Cr(VI) concentrations (0, 10, 50, 100, 500, 1000 µM) for four days. Each point represents the average ±SE of a sample n = 90. Note the decline of root lengthening upon increasing Cr(VI) concentration. Scale bar = 10 mm.

All figures. Single CLSM sections or projections of serial CLSM sections were selected as explained in the particular legends, depending on which provide the best information. To explore integrated information, CLSM sections illustrating three or four aspects of the same cell were used. In that case, different images of a cell were identified with the same letter and numbered consecutively. The interrelation of different cell components such as MTs or ER and nuclei, is conveyed by merging them. Green colour corresponds to either MTs or to ER, as indicated, and red colour to DNA (chromatin or chromosomes). All cells in the plates are positioned with their longitudinal axis vertical in accordance with the normal orientation of the root.

Control interphase cells contained cortical MTs parallel to each other and perpendicularly oriented relative to the longitudinal axis (Fig. 2A). ER in the same cells formed thick aggregations in the cytoplasm and around the nucleus (Fig. 2B). Comparing to MTs, it is evident that the organization and distribution of the two components do not coincide (*cf.* Fig. 2A and 2B). In the Cr(VI)-treated interphase cells ER formed a granular network scattered throughout the cytoplasm and at the perinuclear space, revealing morphological derangement, even at the lower concentration of 100 µM (Fig. 2C). The nuclear envelope was also brightly labelled (Fig. 2B1, B3, C1, C3).

Prophase cells in untreated roots displayed a typical MT-PPB encircling symmetrically the nuclei (Fig. 3A1) and contained fully condensed chromosomes (Fig. 3A2). Towards late prophase the MT-PPB diminished – and finally disintegrated – at the gain of the organizing MT-prophase spindle (Fig. 3A1, A3), which will further develop to the metaphase spindle (see Fig. 4A). In control prophase cells ER formed a well-defined narrow preprophase bundle of ER (ER-PPB) in the cortical cytoplasm, symmetrically girdling the nucleus (Fig. 3B), morphologically and spatially closely similar to that of the MT-PPB (cf. Fig. 3A). In Cr(VI)-treated cells the ER-PPB became broad and irregular, with diffused perinuclear and cytoplasmic signal (Fig. 3C). The nuclear envelope labelling was very weak or disappeared (Fig. 3C2).

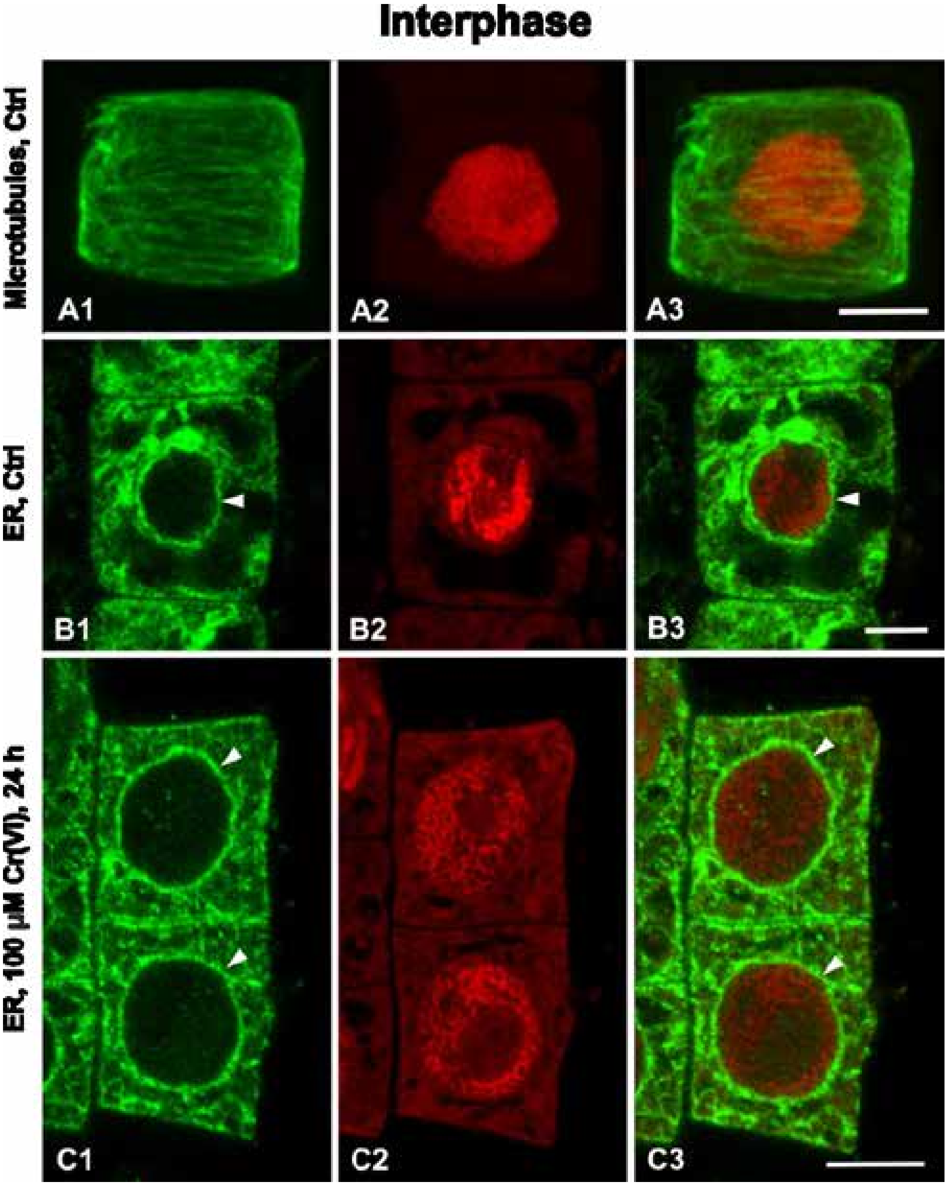


Fig. 2. Interphase: Comparison of MTs and ER in control and 100 µM Cr(VI) for 24 h. A, Projections of serial CLSM sections. Transversely aligned MTs (A1), the nucleus (A2) and overlay of the two images (A3); B, Single CLSM sections, control. Dense local aggregations of ER (B1), its nucleus (B2) and merge (B3); C, Single CLSM sections. Dispersed morphology of ER in 100 µM Cr(VI)-treated cells for 24 h. – Arrowheads point to nuclear envelope. – Scale bars = 10 µM.

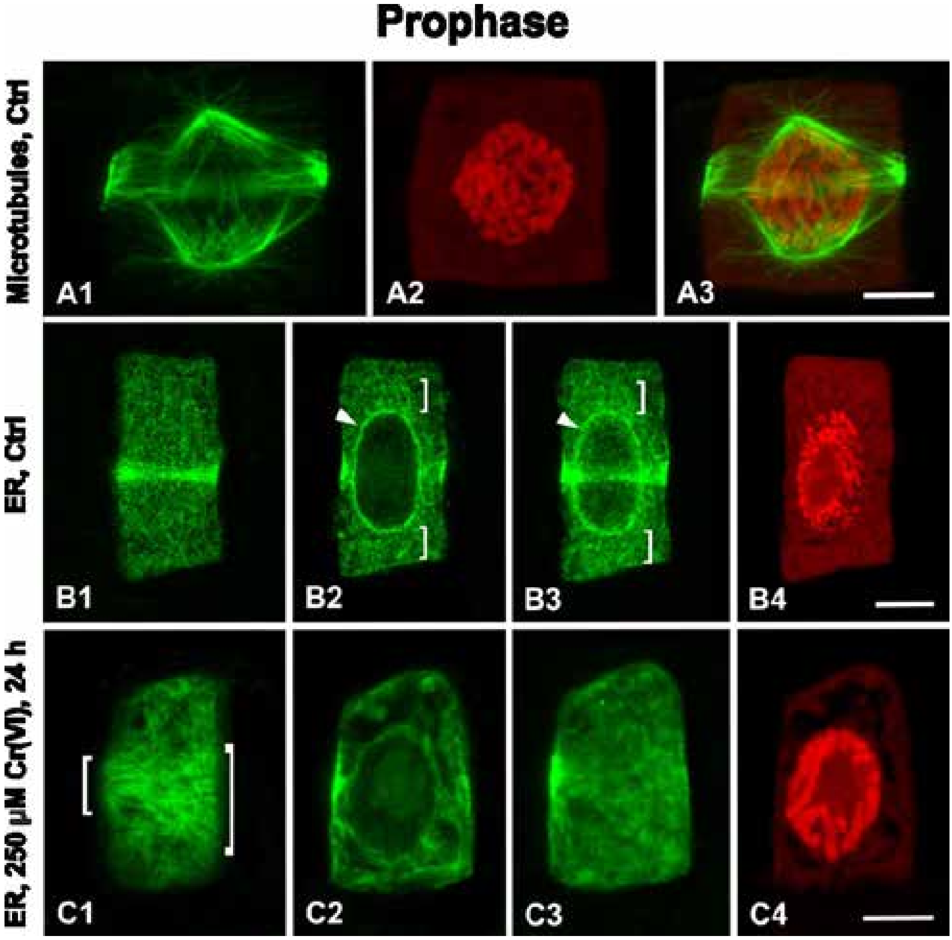


Fig. 3. Prophase: A, Control. Projection of serial CLSM sections. Transition from the disorganizing MT-PPB to the organizing prophase spindle (A1) of a typical late prophase cell (A2), surrounding the chromosomes (A3); B, Control, ER, early prophase. Single CLSM sections in cortical (B1) and mid-plane (B2), projection of serial CLSM sections (B3) and the nucleus (B4), showing a narrow ER-PPB, a strong labelling of the nuclear envelope (arrowheads) and weak accumulation of ER at the polar regions (brackets); C, 250 μM Cr(VI)-treated cell for 24 h. Highly and irregularly broadened ER-PPB (brackets) and absence of nuclear envelope signal in single cortical (C1) and mid-plane (C2) CLSM sections, and projection (C3). Single CLSM section of the nucleus (C4) revealing its late prophase status. – Scale bars = 10 μM.

Metaphase cells contained symmetrical bipolar spindles of MTs (Fig. 4A1) and fully condensed chromosomes, each distinguished in two sister chromatids bound at their kinetochores positioned on the equatorial plane of the metaphase plate, with chromatid arms projecting on either side (Fig. 4A2). Bundles of kinetochore MTs were attached to the chromatid kinetochores and converged to the poles (Fig. 4A3). ER of metaphase control cells accumulated primarily in the polar regions and ER

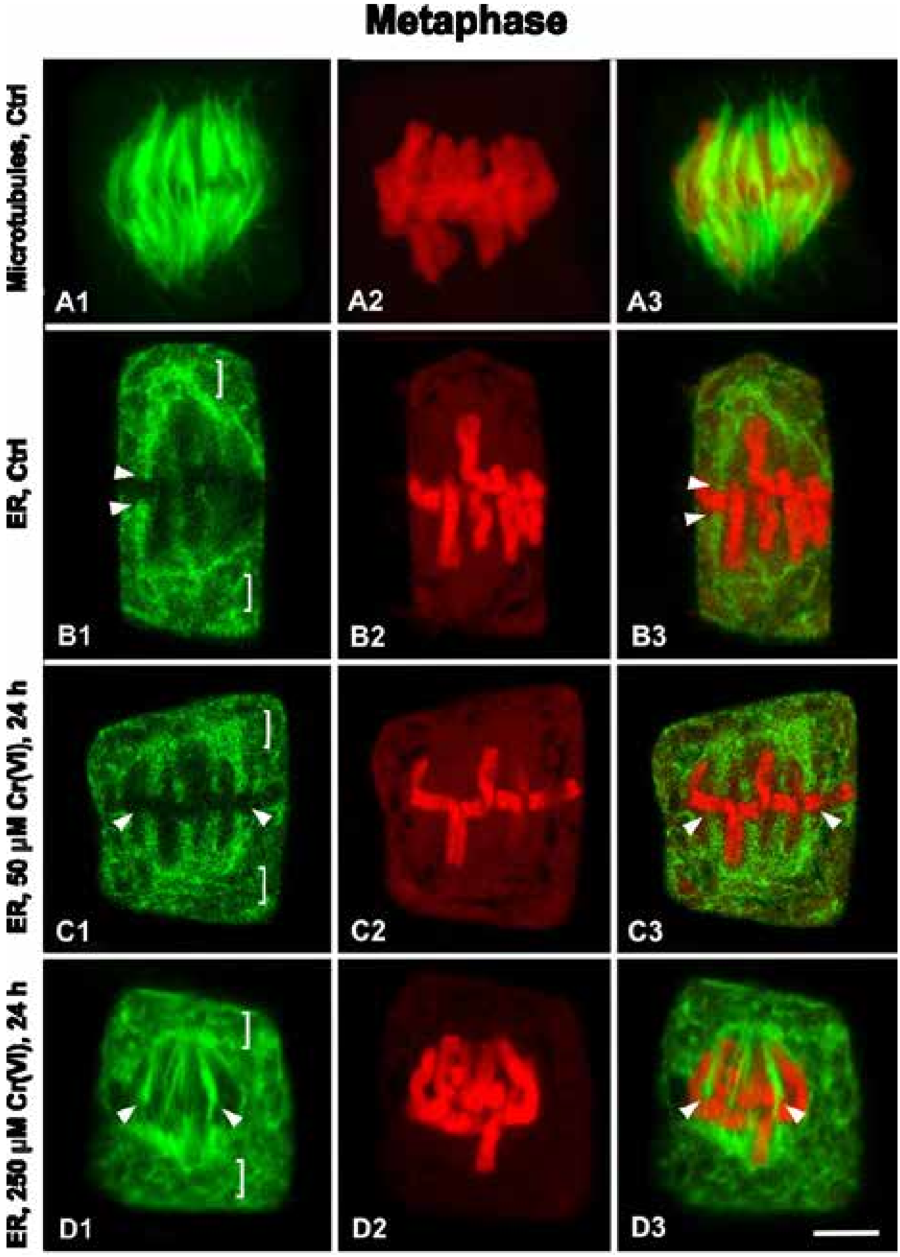


Fig. 4. Metaphase, projection of serial CLSM sections: A, Symmetric metaphase spindle of MTs (A1), chromosomes aggregated on the metaphase plate (A2) and overlay (A3); B, Control. ER is aggregated primarily at the polar regions (brackets), having strands projecting toward the chromosome plate, occasionally terminating on kinetochores (arrowheads); C, D, 50 µM (C) and 250 µM (D) Cr(VI)-treated metaphase cells for 24 h. ER is localized at the polar regions (brackets) and strands extend among chromosomes reaching kinetochores (arrowheads). In D2 chromosomes appear as an immobilized mass rather than aligned on the metaphase plate. – Scale bars = 10 µM.

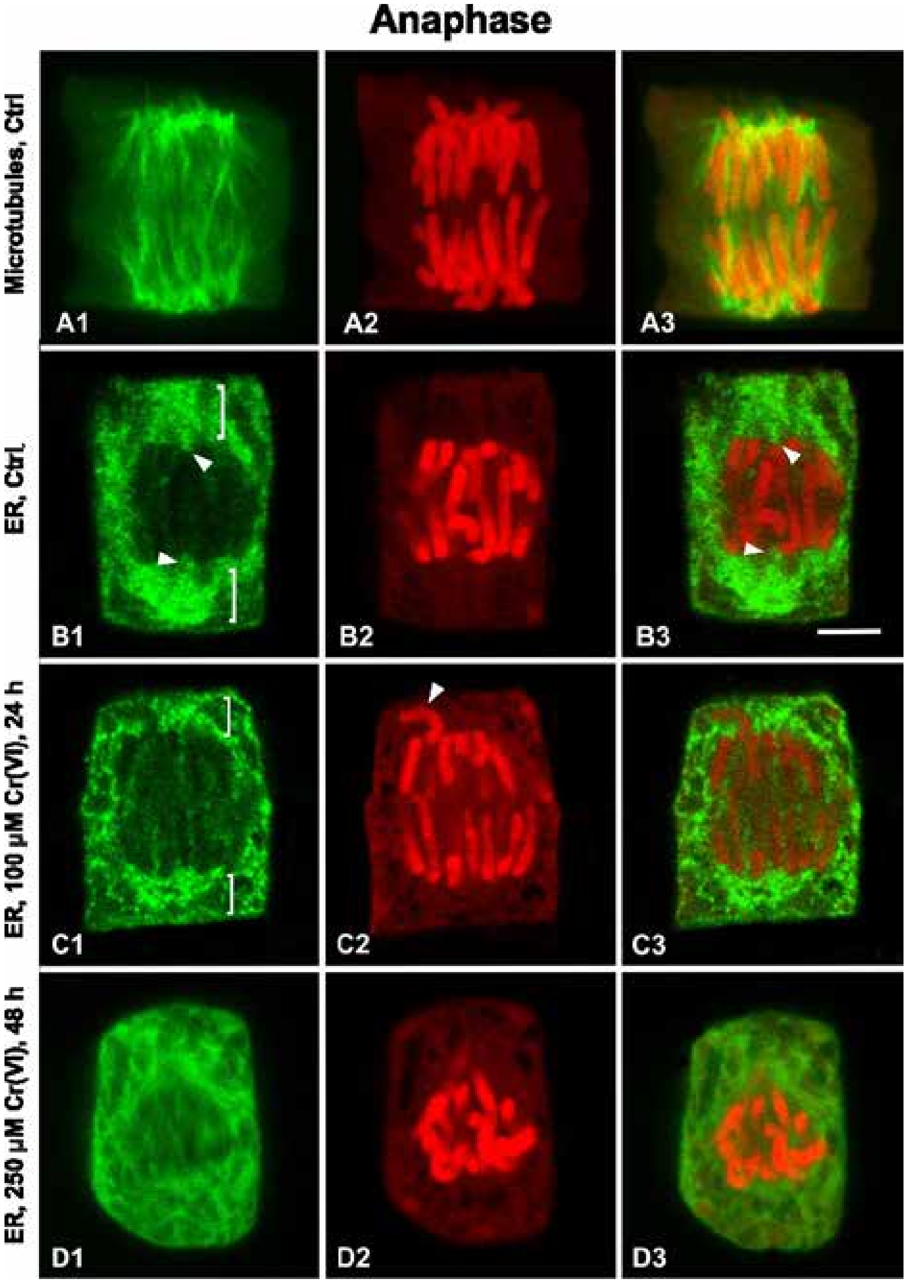


Fig. 5. Anaphase, projection of serial CLSM sections: A, Typical anaphase spindle (A1), segregating daughter chromosomes (A2) and their interrelation (A3); B, Control. ER is massively aggregated at the polar regions and around separating chromatids, but scarcely among them; C, 100 μM Cr(VI)-treated cell for 24 h. ER pervades the cortex and endoplasm, while daughter chromosomes have been segregated. – Arrowhead points to a projecting chromatid arm; D, 250 μM Cr(VI)-treated cell for 48 h. ER occurs around the chromosomes (D1, D3), which remained immobilized in an unorganized mass (D2). – Scale bar = 10 μM.

strands penetrated among the aggregated chromosomes terminating at the kinetochores (Fig. 4B), largely reflecting the MT-spindle (*cf.* Fig. 4A). In Cr(VI)-treated metaphase cells ER appeared unaffected forming ER spindles both in low (Fig. 4C) and high (Fig. 4D) Cr(VI) concentrations. Invading strands of ER continued to be extending to the kinetochores. At high Cr(VI) concentrations chromosomes formed a mass in the central region, not positioned on the metaphase plate (Fig. 4D2; *cf.* Fig. 4A2, B2, C2).

Anaphase consisted of shortening spindle MTs pulling away daughter chromatids to opposing directions (Fig. 5A), where they will reorganize the new nuclei. In control cells ER occurred profusely primarily in the polar and perinuclear region encasing the mitotic apparatus, but scarcely invaded among the chromosomes (Fig. 5B). In Cr(VI)-treated anaphase ER was almost undisturbed, encountered mostly around the chromosomes and faintly among them (Fig. 5C). Occasionally, ER had a granular appearance (Fig. 5C1, C3), while some atypical projections of chromatid arms could be seen (Fig. 5C2). In higher Cr(VI) concentrations and longer exposures ER did not change considerably (Fig. 5D), while chromatids remained immobilized in the central area of the cell without been separated (Fig. 5D2).

Control telophase featured the daughter chromosomes arrived at the polar regions where they organize the new nuclei, and the organization and centrifugal expansion of phragmoplast, at the margins of which MTs abounded (Fig. 6A). In telophase control cells ER signal was localized at the phragmoplast periphery, on the expanding cell plate and at the outline of the new nuclei (Fig. 6B). Upon progression, ER was transposed centrifugally towards the parental walls preceding the expansion of phragmoplast and cell plate, similarly to MTs (Fig. 6B; *cf.* Fig. 6A). In low Cr(VI) concentrations, ER distribution in telophase cells was similar to the control (data not shown), but in higher concentrations ER was labelled at the margins of the disrupted phragmoplasts (Fig. 6C, D). Frequently, lagging or bridging chromosomes connected the main chromosome masses (Fig. 6C2, D2).

Discussion

ER has significant biosynthetic and regulatory functions in plant cells. During cell division cycle, it occupies strategic locations and undergoes dramatic changes in spatial distribution, thus disclosure of its presumed disturbance under stressful factors is of prime importance. Its controlled arrangement throughout the cytoplasm is strictly guided by cytoskeleton elements, MTs and AFs (GUNNING & STEER 1996, GRIFFING & al. 2017). It is generally accepted that in animal cells ER network is distributed along MTs tracks (LEE & CHEN 1988, TERASAKI 2000), while in plant cells, although MT-ER associations have been observed in many instances by electron microscopy (review by QUADER & ZACHARIADIS 2006), there is accumulating evidence that, in most cases, AFs are involved in ER organization rather than MTs (STAEHELIN 1997, BOEVINK & al. 1998). In meristematic cells of untreated roots of *L. culinaris* distinct changes in the distribution of ER were observed in parallel with the successive chromosomal alterations and the dynamic reorganization of MT arrays. In accordance, our results show that the conformation of ER does not coincide always with that of MTs,

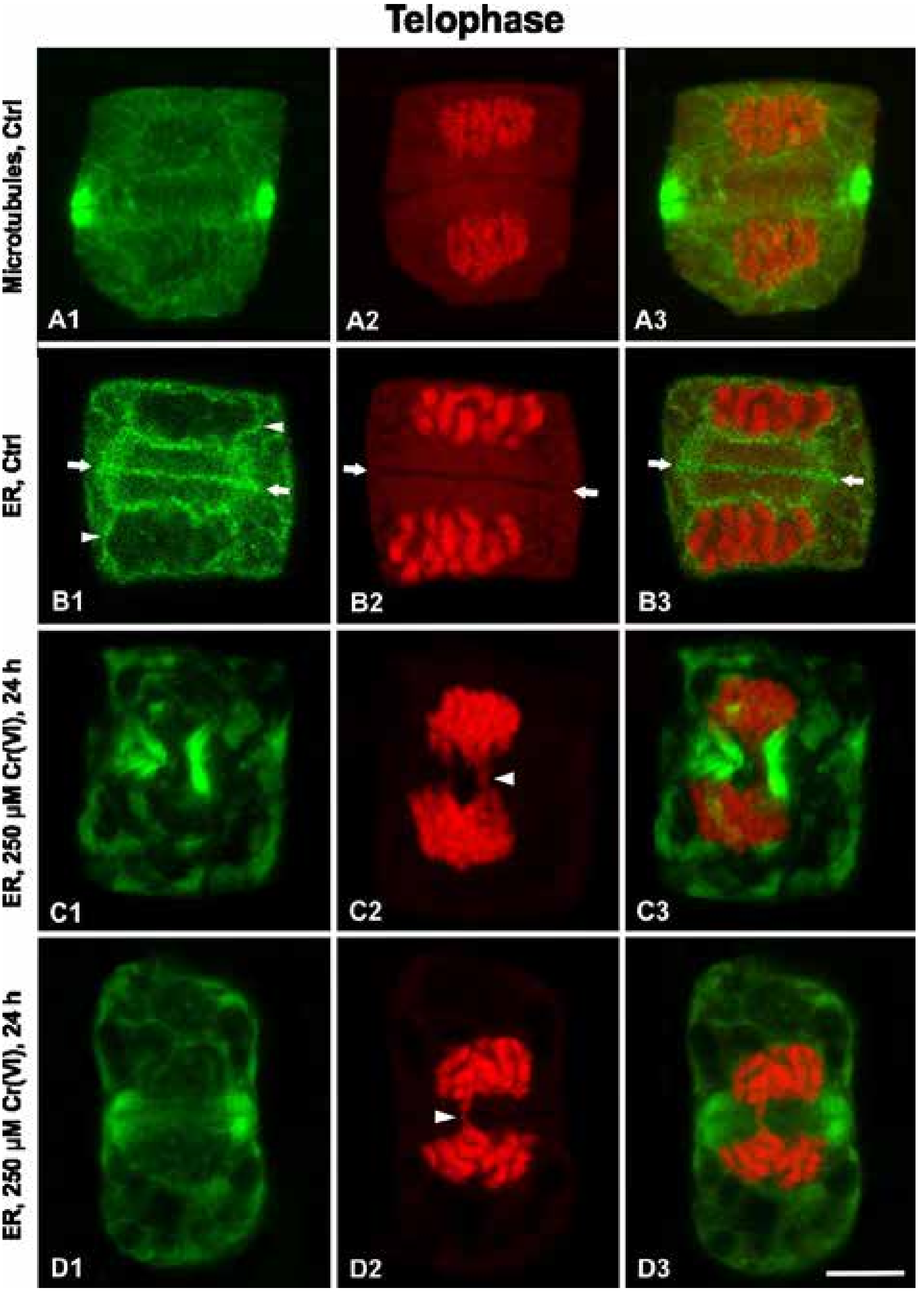


Fig. 6. Telophase, projections of CLSM serial sections: A, Late telophase showing the arrival of phragmoplast MTs to the lateral walls (A1), the new nuclei (A2), and overlay (C3); B, Control, midtelophase cell. ER is labelled at the phragmoplast margins, on the growing cell plate (pairs of opposing arrows) and on the developing nuclear envelopes (arrowheads); C, D, 250 µM Cr(VI)-treated cells for 24 h. Late telophase cells with ER localized at the abnormal phragmoplast margins (C1, D1), bridged daughter chromosomes groups (C2, D2; arrowheads), and their relationship after merging (C3, D3). – Scale bars = 10 µM.

being in good agreement with data for other higher plants such as wheat (GUNNING & STEER 1996, QUADER & ZACHARIADIS 2006) and the pterophyte *Asplenium nidus* (ZACHARIADIS & al. 2003), confirming the view that ER is not always scaffolded by MTs.

In interphase MTs and ER were quite dissimilarly arranged since MTs occurred in the cell periphery, ran parallel to each other and were transversely oriented to the longitudinal cell axis, while ER ramified in the cytoplasm and the perinuclear regions, occasionally in dense accumulations (Fig. 2), consistent with the view of ER guidance by AFs. This discrepancy was observed both in control and Cr(VI)-treated seedlings. Moreover, the nuclear envelope was strongly labelled with the same anti-HDEL antibody that recognizes luminar ER resident proteins both in the control and Cr(VI)-treated roots, not only in interphase (Fig. 2) but also in mitotic phases that contain organized nuclear envelope (Fig. 3, 6), in the context of the view that it is a special form of ER (HEPLER 1981, GUNNING & STEER 1996, STAEHELIN 1997).

On the other hand, in prophase MTs and ER network seemed to be largely coinciding. They organized the MT-PPB and ER-PPB, respectively, which concurred in the central zone of the cell encircling symmetrically the nucleus. Furthermore, following MTs reorganization to form the prophase spindle at the perinuclear area at the expense of the MT-PPB which gradually disintegrated, ER started to accumulate in the polar regions (Fig. 3B), but a clearly organized ER-prophase spindle was not detected. The organization of a well-defined ER-PPB in control pre-prophase and prophase cells of *L. culinaris* is unusual as it was not reported for other higher plants such as wheat (GUNNING & STEER 1996, QUADER & ZACHARIADIS 2006) nor for the pterophyte *Asplenium nidus* (ZACHARIADIS & al. 2003). Instead, in the gymnosperms *Pinus brutia* and *P. nigra* a well-defined ER-PPB and a bipolar prophase ER spindle were organized (ZACHARIADIS & al. 2001, 2003). In *L. culinaris* the coincidence of MTs and ER within the PPB denotes that MTs may constitute a scaffold for ER in the PPB. This notion is further supported by the fact that in Cr(VI)-treated cells ER-PPB was severely disturbed and became highly widened (Fig. 3C), as this was reported for MT-PPBs in Cr(VI)-treated cells of *Allium cepa* (ELEFTHERIOU & al. 2012) and *L. culinaris* (ELEFTHERIOU & al. 2013). Moreover, inhibitor experiments in *Pinus* roots showed that the control of ER organization may switch from AF dependence during interphase to MTs during mitosis and cytokinesis (ZACHARIADIS & al. 2003).

The close association of metaphase MTs and ER conformations indicates that direct contacts may occur, as was initially shown in barley epidermal cells by HEPLER (1980). MTs formed the well-defined spindle of the mitotic apparatus, while ER displayed strong accumulations at the polar regions, but also formed spindle-like interzonal strands projecting among the chromosomes, in parallel with the kinetochore bundles of MTs, frequently terminating at the kinetochores themselves (Fig. 4B). Similar images were published for wheat roots and were considered to indicate a role for ER in MT assembly/disassembly, presumably through regulation of local Ca^{2+} concentration (GUNNING & STEER 1996). After Cr(VI) exposure ER arrangement did not change much compared to control and continued to be connected with kinetochores both in low (Fig. 4C) and high (Fig. 4D) Cr(VI) concentrations. In counterpart Cr(VI)-treated cells spindle MT arrays did not disorganize but appeared compact due to presumed stabilization as evidenced by gradual increase of acetylated *a*-tubulin, an indicator of MT stabilization (ELEFTHERIOU & al. 2013). Should MT stabilization retain the MT-ER connections, it may explain the unaffected morphology of the MT-ER system in metaphase cells of Cr(VI)-treated roots.

In control anaphase ER lost the spindle-like morphology and displayed the strongest accumulation in the polar regions and the cytoplasm (Fig. 5B), encasing and isolating the mitotic apparatus (HEPLER 1980, HAWES & al. 1981). Moreover, it displayed hardly discernible interzonal ER arrays, as it was shown for wheat at the same phase (GUNNING & STEER 1996, QUADER & ZACHARIADIS 2006) and the pterophyte *Asplenium nidus* (ZACHARIADIS & al. 2003). On the other hand, in *Pinus brutia* and *P. nigra* well defined anaphase ER-spindles were reported, having closely similar arrangement with the MT arrays, favouring the view that the pattern of ER organization in these gymnosperm species is controlled during mitosis and cytokinesis by MTs (ZACHARIADIS & al. 2003). Again, anaphase MT spindles after Cr(VI) exposure appeared compact (ELEFTHERIOU & al. 2013), indicating that immobilization of MTs may lead to stabilization of ER network, an assumption that deserves further investigation.

During development of the cell plate at telophase/cytokinesis, ER is considered to play crucial roles as it (1) provides a structural framework that holds vesicles in position, (2) directs vesicle fusion within the plane of the plate, (3) regulates the local homeostasis of Ca^{2+} ions required for vesicle fusion, and (4) is directly involved in the formation of plasmodesmata (HEPLER 1982). MT-dependent ER reorganization during cytokinesis correlates with the fact that vesicles forwarded toward growing cell plate are steered along phragmoplast MTs (VERMA & GU 1996). After exposure to Cr(VI), MTs persisted but phragmoplasts were deformed and disoriented, while daughter chromosomes were unsegregated, bridged or lagging (ELEFTHERIOU & al. 2013). In the present study ER was abnormally arranged reflecting the disrupted phragmoplasts, denoting again a MT-ER interrelationship.

To sum up, data obtained here indicate that ER arrangement during mitosis of *L. culinaris* root tip cells is guided by MTs rather than AFs as evidenced by their co-localization and persistence under adverse conditions. This is consistent with the emerging trend that "organelles can no longer be seen as discrete membrane-bound compartments, since they can physically interact and ‘communicate’ with one another" and "the ER may form a connecting central role in this process" (GRIFFING & al. 2017). It may then be concluded that the low derangement of ER during most mitotic phases after exposure to toxic doses of Cr(VI) might be attributed to its association with and support by mitotic MT arrays, which were previously shown to be stabilized under Cr(VI) stressful conditions (ELEFTHERIOU & al. 2013). Should additional experimentation is available, this view might be reinforced.

References

AHMED, F., HOSSAIN, M., ABDULLAH, A. T., AKBOR, M. & AHSAN, M. 2016: Public health risk assessment of chromium intake from vegetable grown in the wastewater irrigated site in Bangladesh. – Pollution 2: 425-432.

ANTIBACHI, D., KELEPERTZIS, E. & KELEPERTSIS, A. 2012: Heavy metals in agricultural soils of the Mouriki-Thiva area (central Greece) and environmental impact implications. – Soil Sediment. Contam. Int. J. 21: 434-450.

BOEVINK, P., OPARKA K., CRUZ, S. S., MARTIN, B., BETTERIDGE, A. & HAWES, C. 1998: Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. – Plant J. 15: 441-447.

BOTSOU, F., KARAGEORGIS, A. P., DASSENAKIS, E. & SCOULLOS, M. 2011: Assessment of heavy metal contamination and mineral magnetic characterization of the Asopos River sediments (Central Greece). – Marine Poll. Bull. 62: 547-563. Doi:10.1016/j.marpolbul.2010.11.029

BROADWAY, A., CAVE, M. R., WRAGG, J., FORDYCE, F. M., BEWLEY, R. J., GRAHAM, M. C., NGWENYA, B. T. & FARMER, J. G. 2010: Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment. – Sci. Total Environ. 409: 267-277.

CHO, Y. & KANEHARA, K. 2017: Endoplasmic reticulum stress response in *Arabidopsis* roots. – Frontiers Plant Sci. Doi: 10.3389/fpls.2017.00144.

ECONOMOU-ELIOPOULOS, M., MEGREMI, I. & VASILATOS, C. 2011: Factors controlling the heterogeneous distribution of Cr(VI) in soil, plants and groundwater: evidence from the Assopos basin, Greece. – Chemie der Erde 71: 39-52. Doi:10.1016/j.chemer.2011.01.001.

ELEFTHERIOU, E. P., ADAMAKIS, I.-D. S. & MELISSA, P. 2012: Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of *Allium cepa* L. – Protoplasma 249: 401-416. Doi:10.1007/s00709-011-0292-3.

—, —, FATSIOU, M. & PANTERIS, E. 2013: Hexavalent chromium disrupts mitosis by stabilizing microtubules in *Lens culinaris* Moench. root tip cells. – Physiol. Plant. 147: 169-180. Doi:10.1111/j.1399-3054.2012.01652.x.

—, MICHALOPOULOU, V. A. & ADAMAKIS I.-D. S. 2015a: Aberration of mitosis by hexavalent chromium in some Fabaceae members is mediated by species-specific microtubule disruption. – Environ. Sci. Pollution Res. 22: 7590-7599. Doi: 10.1007/s11356-014-3880-x

—, ADAMAKIS, I.-D. S., PANTERIS, E. & FATSIOU, M. 2015b: Chromium-induced ultrastructural changes and oxidative stress in roots of *Arabidopsis thaliana*. – Int. J. Mol. Sci. 16, 15852-15871. Doi:10.3390/ijms160715852.

—, —, & MICHALOPOULOU, V. A. 2016: Hexavalent chromium-induced differential disruption of cortical microtubules in some Fabaceae species is correlated with acetylation of α-tubulin. – Protoplasma 253: 531-542. Doi 10.1007/s00709-015-0831-4.

ERICSON, B. 2011: Common global pollution issues: Blacksmith Institute's experience. In Presentation to the 10th Meeting of the International Committee on Contaminated Land. – Washington, DC.

GRIFFING, L. R., LIN, C., PERICO, C., WHITE R. R. & SPARKES, I. 2017: Plant ER geometry and dynamics: biophysical and cytoskeletal control during growth and biotic response. – Protoplasma 254: 43-56. Doi 10.1007/s00709-016-0945-3.

GUNNING, B. E. S. & STEER, M. W. 1996: Plant Cell Biology, Structure and Function. – Jones and Bartlett Publishers, Sudbury, Massachusetts, Boston.

HAWES, C. R., JUNIPER, B. E. & HORNE, J. C. 1981: Low and high voltage electron microscopy of mitosis and cytokinesis in maize roots. – Planta 152: 397-407.

HEPLER, P. K. 1980: Membranes in the mitotic apparatus of barley cells. – J. Cell Biol. 86: 490-499.

— 1981: The structure of the endoplasmic reticulum revealed by osmium tetroxide-potassium ferricyanide staining. – Eur. J. Cell Biol. 26: 102-110.

— 1982: Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. – Protoplasma 111: 121-133.

KIM, H. Y., LEE, S. B. & JANG, B. C. 2004: Subchronic inhalation toxicity of soluble hexavalent chromium trioxide in rats. – Arch. Toxicol. 78: 363-368. Doi: 10.1007/s00204-004-0553-4.

LEE, C. & CHEN, L. B. 1988: Dynamic behavior of endoplasmic reticulum in living cells. – Cell 54: 37-46.

LINOS, A., PETRALIAS, A., CHRISTOPHI, C. A., CHRISTOFORIDOU, E., KOUROUTOU, P., STOLTIDIS, M., VELOUDAKI, A., TZALA, E., MAKRIS, K. C. & KARAGAS, M. R. 2011: Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece – An ecological study. – Environ. Health 10: 50. Doi:10.1186/1476-069X-10-50.

MATSUSHIMA, R., HAYASHI, Y., KONDO, M., SHIMADA, T., NISHIMURA, M. & HARA-NISHIMURA, I. 2002: An endoplasmic reticulum-derived structure that is induced under stress conditions in Arabidopsis. – Plant Physiol. 130: 1807-1814. Doi: 10.1104/pp.009464.

MEGREMI, I. 2010: Distribution and bioavailability of Cr in central Euboea, Greece. – Central Eur. J. Geosci. 2: 103-123. Doi: 10.2478/v10085-009-0042-3.

MÜLLER, S. 2012. Plant Cell Division. – eLS. John Wiley & Sons, LTd: Chichester. Doi: 10.1002/9780470015902.a0023760.

NAPIER, R. M., FOWKE, L. C., HAWES, C., LEWIS, M. & PELHAM, H. R. 1992: Immunological evidence that plants use both HDEL and KDEL for targeting proteins to the endoplasmic reticulum. – J. Cell Sci. 102: 261-271.

NRIAGU, J.O. 1987: Production and uses of chromium. – Adv. Environ. Sci. Technol. 20: 81-103.

OLIVEIRA, H. 2012: Chromium as an environmental pollutant: insights on induced plant toxicity. – J. Bot. ID375843, 8p. Doi:10.1155/2012/375843.

POLJSAK, B., PÓCSI, I. & PESTI, M. 2011: Interference of chromium with cellular functions. Pp. 59-86. In GASPAR, B. (ed.), Cellular Effects of Heavy Metals. – Springer.

QUADER, H. & ZACHARIADIS, M. 2006: The Morphology and Dynamics of the ER. Pp. 1-23. In ROBINSON, D. G. (ed.), The Plant Endoplasmic Reticulum. – Plant Cell Monographs, vol 4. Springer, Berlin, Heidelberg. Doi: 10.1007/7089_063.

SHAHID, M., SHAMSHAD, S., RAFIQ, M., KHALID, S., BIBI, I., NIAZI, N. K., DUMAT, C. & RASHID, M. I. 2017: Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. – Chemosphere 178: 513-533. http://dx.doi.org/10.1016/j.chemosphere.2017.03.074.

SHANKER, A. K., CERVANTES, C., LOZA-TAVERA, H. & AVUDAINAYAGAM, S. 2005: Chromium toxicity in plants. – Environ. Int. 31: 739-753. Doi:10.1016/j.envint.2005.02.003.

SMIRNOVA, E. 2012: Microtubules during the cell cycle of higher plant cells. In Najman S. (ed.), Current Frontiers and Perspectives in Cell Biology, ISBN: 978-953-51-0544-2, InTech. http://www.intechopen.com/books/current-frontiers-and-perspectives-in-cell-biology/microtubules-during-thecell-cycle-of-higher-plant-cells.

SORAHAN, T., BURGES, D. C. & HAMILTON, L. 1998: Lung cancer mortality in nickel/chromium platers. – Occup. Environ. Med. 55: 236-242. Doi:10.1136/oem.55.4.236.

STAEHELIN, L. A. 1997: The plant ER: a dynamic organelle composed of a large number of discrete functional domains. – Plant J. 11: 1151-1165.

STERN, A. H. 2010: A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure. – Environ. Res. 110: 798-807. Doi: 10.1016/j.envres.2010.08.002.

TERASAKI, M. 2000: Dynamics of the endoplasmic reticulum and Golgi apparatus during early sea urchin development. – Mol. Biol. Cell 11: 897-914.

TZIRITIS, E., KELEPERTZIS, E., KORRES, G., PERIVOLARIS, D. & REPANI, S. 2012: Hexavalent chromium contamination in groundwaters of Thiva Basin, Central Greece. – Bull. Environ. Contam. Toxicol. 89: 1073-1077. Doi 10.1007/s00128-012-0831-4.

USEPA (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY), 1992: Superfund Record Decision: Cannelton Industries. MI. EPA/ROD/RO5e92/220. http://nepis.epa.gov.

VASILATOS, C., MEGREMI, I., ECONOMOU-ELIOPOULOS, M. & MITSIS, I. 2008: Hexavalent chromium and other toxic elements in natural waters in the Thiva – Tanagra – Malakasa Basin, Greece. – Hellenic J. Geosci. 43: 57-66.

VERMA, D. P. S. & GU, X. 1996: Vesicle dynamics during cell plate formation in plants. – Trends Plant Sci. 1: 145-149.

ZACHARIADIS, M., QUADER, H., GALATIS, B. & APOSTOLAKOS, P. 2001: Endoplasmic reticulum preprophase band in dividing root-tip cells of *Pinus brutia*. – Planta 213: 824-827. Doi: 10.1007/s004250100563.

—, —, —, & — 2003. Organization of the endoplasmic reticulum in dividing cells of the gymnosperms *Pinus brutia* and *Pinus nigra* and of the pterophyte *Asplenium nidus*. – Cell Biol. Int. 27: 31-40. Doi:10.1016/S1065-6995(02)00254-8.

Comparison of the photosynthetic traits between red and green leaves of *Cercis siliquastrum* L.: physiological differences during their development

ANDRI IASONOS, ALEXANDRA KYZERIDOU & YIOLA PETROPOULOU

Abstract

Iasonos, A., Kyzeridou, A. & Petropoulou, Y. 2019: Comparison of the photosynthetic traits of *Cercis siliquastrum* L.: physiological differences during their development. – Bot. Chron. 22: 345-354

Young leaves in some plants are transiently red due to the presence of anthocyanins, which disappear upon maturation. Among the many hypotheses concerning foliar anthocyanins, in the present study we have focused in the photoprotective function against excess visible light and the induction of shade characteristics on otherwise exposed leaves. We took advantage of the intra-species variation in leaf redness displayed by *Cercis siliquastrum* individuals, growing under the same environmental conditions. Pigment levels and certain photosynthetic parameters were compared in young green and red leaves of the same age during their development with the aid of spectral reflectance and in vivo chlorophyll fluorescence techniques. Compared to greens, red leaves contained less total chlorophyll while chlorophyll a/b ratio did not differ between the two phenotypes. Quantum yields of energy capture and electron transfer up to final PSI electron acceptors were lower in red leaves, indicating an enhanced number of inactive PSII centers and increasing limitations along the linear electron flow. The specific energy fluxes for absorption (ABS/RC) and dissipation (DI_0/RC) per active PSII reaction center were higher in reds and the difference between the two phenotypes diminished with age, following the pattern of anthocyanin decline. In addition, total photosynthetic performance (PI_{total}) was significantly lower in red leaves, indicating a photosynthetic inferiority for the red phenotype, which was reflected in a reduced relative leaf growth rate. In conclusion, our results do not confirm a shade acclimation in red leaves of *C. siliquastrum* and weaken the alleged photoprotective function of foliar anthocyanins. Alternatively, if anthocyanins afford some photoprotection, this is not adequate to fully compensate for the higher vulnerability of red leaves.

A. Iasonos, A. Kyzeridou & Y. Petropoulou*, Laboratory of Plant Physiology, Department of Biology, University of Patras, 26504 Patras, Greece.

*Corresponding author: E-mail: petropo@upatras.gr.

Key words: anthocyanins, photoprotection, shade acclimation, chlorophyll fluorescence, *Cercis siliquastrum.*

Introduction

Leaves of some plants appear red because of anthocyanin accumulation at concentrations that mask the green color of chlorophyll. Anthocyanins are water-soluble, flavonoid pigments whose absorbance maxima are shifted into the visible part of the spectrum. In general, they abound in flowers and fruits, being responsible (in combination with other pigments) for the plethora of their colors and tints.

Foliar anthocyanins are mostly red, absorbing strongly in the green and less in the yellow/blue part of the spectrum (HARBORNE 1976). They may be found in adaxial and/or abaxial epidermis, palisade and/or spongy mesophyll and trichomes. They are localized exclusively in the vacuole (HRAZDINA & al. 1978, LEE & COLLINS 2001, KYTRIDIS & MANETAS 2006). In a few species leaf redness is permanent, yet the transient anthocyanin accumulation is more common and may be developmentally or environmentally induced. In the former case, young or senescing leaves appear red while being green at the mature phase. In the latter, mature (green) leaves turn temporarily red as a response to biotic or abiotic stress factors like pathogen attack, drought, nutrient deficiency, UV-B radiation or low temperatures accompanied by excess visible light (CHALKER-SCOTT 1999, MANETAS & al. 2003, MANETAS 2006, ZELIOU & al. 2009, GOULD & al. 2018).

Although the adaptive significance of anthocyanins in flowers and fruits is rather obvious, i.e. to facilitate pollination and seed dispersal providing optical signals to animals, their possible function(s) in leaves is still under debate. Thus, a variety of functional explanations have been proposed concerning anthocyanin accumulation in leaves, among them photoprotection against excess light energy and defense against potential herbivores and/or pathogens being the more popular. According to the photoprotective hypothesis, anthocyanins may reduce the excitation pressure in the underlying chloroplasts by acting as sunscreens and/or detoxify ROS produced under photoinhibitory conditions through their antioxidant activity (MANETAS & al. 2002, NEILL & al. 2002, CLOSE & BEADLE 2003, GOULD 2004, MANETAS 2006, KARAGEORGOU & MANETAS 2006, KYTRIDIS & MANETAS 2006, ARCHETTI & al. 2009, HUGHES 2011).

In addition, given that foliar anthocyanins absorb selectively in the visible spectrum, they modify quantitatively and qualitatively the light interior of a red leaf, imposing a particular shade on mesophyll chloroplasts, which is red-enriched and green depleted. Thus, regardless of the ascribed function(s), anthocyanic leaves display an array of photosynthetic adjustments compatible to the so called “shade acclimation syndrome” (KARABOURNIOTIS & al. 1999, GOULD & al. 2002, MANETAS & al. 2003, HUGHES & SMITH 2007, KYPARISSIS & al. 2007).

Based on the above, in this investigation we asked whether the presence of an anthocyanic screen causes changes in selected photosynthetic traits in the corresponding leaves. To this aim, *Cercis siliquastrum* L. a plant displaying intra-species variation in leaf anthocyanin content was used for experimentation. Selected photosynthetic parameters were compared in young, green and red leaves during their development, sampled from the corresponding phenotypes occupying the same habitat.

Materials and Methods

Plant material and sampling

Cercis siliquastrum L. (Fabaceae) is a small deciduous tree producing bright pink flowers in early spring, before leaf appearance. New leaves are produced from late March to mid-May, shortly after flowering commencement. This plant displays an intra-species variation in the colour of young, developing leaves (ranging from dark red to green), which become invariably green upon maturation. Thus, individuals within the species, growing under apparently similar environmental conditions, can be grouped in different phenotypes (i.e. fully red, fully green or of intermediate redness), based on the anthocyanin content of their young leaves. In red leaves, anthocyanins reside in the vacuoles of epidermal cells (both adaxial and abaxial). 16 trees (8 red and 8 green) growing as ornamentals side by side in the Patras University Campus were tagged and used throughout the study. For all measurements, leaves of five successive age classes according to their position from shoot apex (nodes 1-5) were used. All leaves were fully exposed to direct solar radiation. On each sampling date, one branch was tagged and removed from each individual (i.e. 8 branches per phenotype) late in the afternoon, put in air-tight plastic bags containing a moist filter paper to avoid desiccation, transferred to the laboratory and left in the dark at room temperature overnight. In the next morning, chlorophyll fluorescence, spectral reflectance and leaf area measurements were performed on five leaves of each branch (i.e. a total of 40 leaves from each phenotype, 8 leaves per age class).

Chlorophyll a fluorescence measurements

Fast chlorophyll a fluorescence transients were monitored with a Hansatech fluorimeter (Handy-PEA; Hansatech Instruments Ltd, King's Lynn, Norfolk, UK). Fluorescence was recorded from 10 μs to 2 s, for time intervals of 10–300 μs, 0.3–3 ms, 3–30 ms, 30–300 ms, and 0.3–2 s and a data acquisition rate of 10^5, 10^4, 10^3, 10^2, and 10 readings s^{-1}, respectively. For excitation, an array of six red LEDs (with peak at 650 nm) providing 3000 μmoles photons m^{-2} s^{-1} at leaf level was used. From the cardinal points of the fluorescence vs time curve, several biophysical parameters were numerically calculated according to the JIP-test (STRASSER & al. 2004, JIANG & al. 2008, TSIMILLI-MICHAEL & STRASSER 2008, YORDANOV & al. 2008) and the following were used in the present study:

Quantum yields per absorbed (ABS) excitation energy: $\varphi_{Po} = TR_0/ABS = F_V/F_M$, the maximum quantum yield of primary photochemistry; $\varphi_{Eo} = ET_0/ABS$, the quantum yield of electron transfer to the intermediate electron carriers; $\varphi_{Ro} = RE_o/ABS$, the quantum yield of the reduction of PSI end electron acceptors, where TR, ET and RE stand for trapping, electron transfer and reduction, respectively.

Specific fluxes per active (i.e. Q_A-reducing) reaction center (RC): ABS/RC for absorption and DI_0/RC for dissipation.

Photosynthetic performance index: PI_{total}, expresses the relative performance up to the PSI final electron acceptors.

Spectral reflectance measurements

Spectral reflectance was recorded on the adaxial (upper) leaf surface with a diode array spectrometer (Unispec, PP Systems, Haverhill, MA), equipped with an internal halogen source, a bifurcated fiber optic cable and an appropriate leaf clip. A spectralon (reflectance > 97%) standard was used as the reference and the spectra were dark corrected for stray light with the internal source off. From the raw reflectance data the following indices were computed, where R denotes reflectance and the subscripts refer to a specific wavelength or spectral band:

Normalized difference vegetation index: $NDVI = (R_{750} - R_{705}) / (R_{750} + R_{705})$, is positively correlated to leaf chlorophyll content and independent of anthocyanin levels (GITELSON & MERZLYAK 1994).

Red/green index = R_{RED}/R_{GREEN}, where RED and GREEN refer to the corresponding 600-699 nm and 500-599 nm broad bands, respectively. This index is positively correlated to leaf anthocyanin content (GAMON & SURFUS 1999).

Chl a/b index = $3.50 - 122.42 * D_{680.5}$, where $D_{680.5} = (R_{680.5} - R_{677.3}) / (680.5 - 677.3)$, according to RICHARDSON & al. (2002). This index is positively correlated to leaf Chl a/b ratio.

Leaf area and relative growth rate

On each measuring date, leaf area and dimensions (maximum length and width) were computed with the Image-Pro Plus program after scanning the corresponding leaves with a Canon (MP 460) scanner. From regression lines of the actual leaf area to the product of leaf length × width, a linear equation was established.

For growth rate assessment, 8 juvenile leaves/phenotype were tagged (i.e. one leaf per individual) and their dimensions were measured with a ruler at frequent intervals. The area of each leaf was calculated from the above mentioned linear equation. Relative leaf growth rate (Fig. 3) was computed from area differences between the successive measuring dates, normalized over initial leaf area and divided by the number of intervening days.

Statistics

Significance of differences in the measured parameters between red and green leaves, were assessed by one-way ANOVA or paired t-test (for relative leaf growth rate) using the SPSS statistical package. The number of independent measurements in each case is given in the legends of the figures.

Results and Discussion

As it was mentioned in the Introduction, the adaptive significance of leaf anthocyanins remains obscure. Thus, more comparative physiological studies are needed in order to elucidate their possible function(s). The use of plants displaying an intra-species variation of leaf redness (as is the case of *C. siliquastrum*) provides the advantage to by-pass species-dependent differences in the evaluated parameters. In addition, the comparative study of the photosynthetic traits in young green and red leaves during their development, gives the opportunity to monitor the effect of anthocyanins in parallel to their decline in the red phenotype.

Fig. 1 presents the pigment profile (assessed through the corresponding reflectance indices) of green and red leaves in relation to their age. Compared to greens, red leaves contained significantly more anthocyanins (red/green index), which declined substantially with age. A smaller trend of decrease was observed also in green leaves. Total chlorophylls displayed the opposite pattern as expected, i.e. they increased with leaf age in both leaf types, yet total Chl content (NDVI) was on average 20% lower in red leaves. Concerning Chl a/b ratio, this was fairly constant during leaf development and independent of their anthocyanic content. In contrast to previous studies conducted with various anthocyanic species (GOULD & al. 2002, MANETAS & al. 2003, HUGHES & al. 2005, HUGHES & SMITH 2007, ZELIOU & al. 2009), the above results do not support a shade acclimation of the red leaves. It has been shown, for example, that young or mature red leaves of several species display a lower Chl a/b ratio, when compared to their green counterparts. A lower Chl a/b ratio is considered as a typical "shade" character, reflecting a higher investment in light harvesting (containing both Chl a and b) relative to core complexes (containing only Chl a), as a result of the enhanced needs for light capture in shaded leaves (ANDERSON 1986). Please note that all leaves in our study were fully exposed to solar radiation, in order to eliminate possible differences arising from their light history. In a comparative study with anthocyanic and acyanic species, it was shown that Chl a/b ratio increased

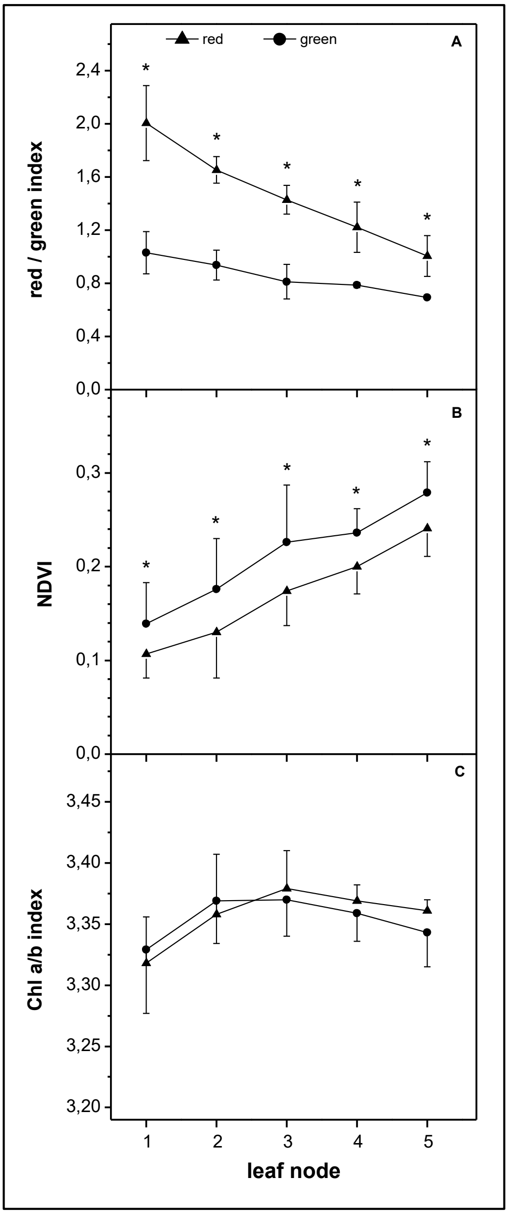


Fig. 1. Anthocyanin content (as red/green index, A), total chlorophyll content (as NDVI, B) and Chl a/b ratio (as Chl a/b index, C) in green (●) and red (▲) leaves of C. siliquastrum versus leaf age. Values are means±SD from 32 measurements per each age class (8 plants per phenotype). Asterisks denote statistically significant differences between the two phenotypes (P <0.05).

gradually with leaf age in the juvenile red leaves (in parallel to anthocyanin decline), while in the corresponding green leaves remained relatively constant at much higher values (HUGHES & al. 2007). In the same study, however, Chl a/b ratio of *Cercis canadensis* young leaves (containing significantly less anthocyanin than the other red species) was as high as in the green ones, presumably because of less shading, and this finding is in agreement with our results. Shaded leaves, compared to fully exposed, usually have higher total chlorophyll concentrations on a dry-mass basis, but similar on a leaf-area basis (LARCHER 1995). This has been also reported for the red leaves of several species, when compared to their green counterparts (MANETAS & al. 2003, HUGHES & al. 2005, HUGHES & SMITH 2007). However, in our case, NDVI (which correlates to the area-based Chl content) was constantly lower in red leaves. The same was observed in the winter red phenotype of two Mediterranean shrubs, as well as in the permanently red leaves of *Prunus cerasifera* (KYPARISSIS & al. 2007, ZELIOU & al. 2009, NIKIFOROU & MANETAS 2010). We have to note at this point, that the shade imposed by anthocyanins in a red leaf is not canopy-like or neutral, due to their selective absorption in the green/yellow part of the spectrum (KARABOURNIOTIS & al. 1999, GOULD & al. 2002).

This spectral selectivity may affect the photosynthetic behavior of red leaves thus leading to some deviations from the classical shade acclimation syndrome (KYPARISSIS & al. 2007).

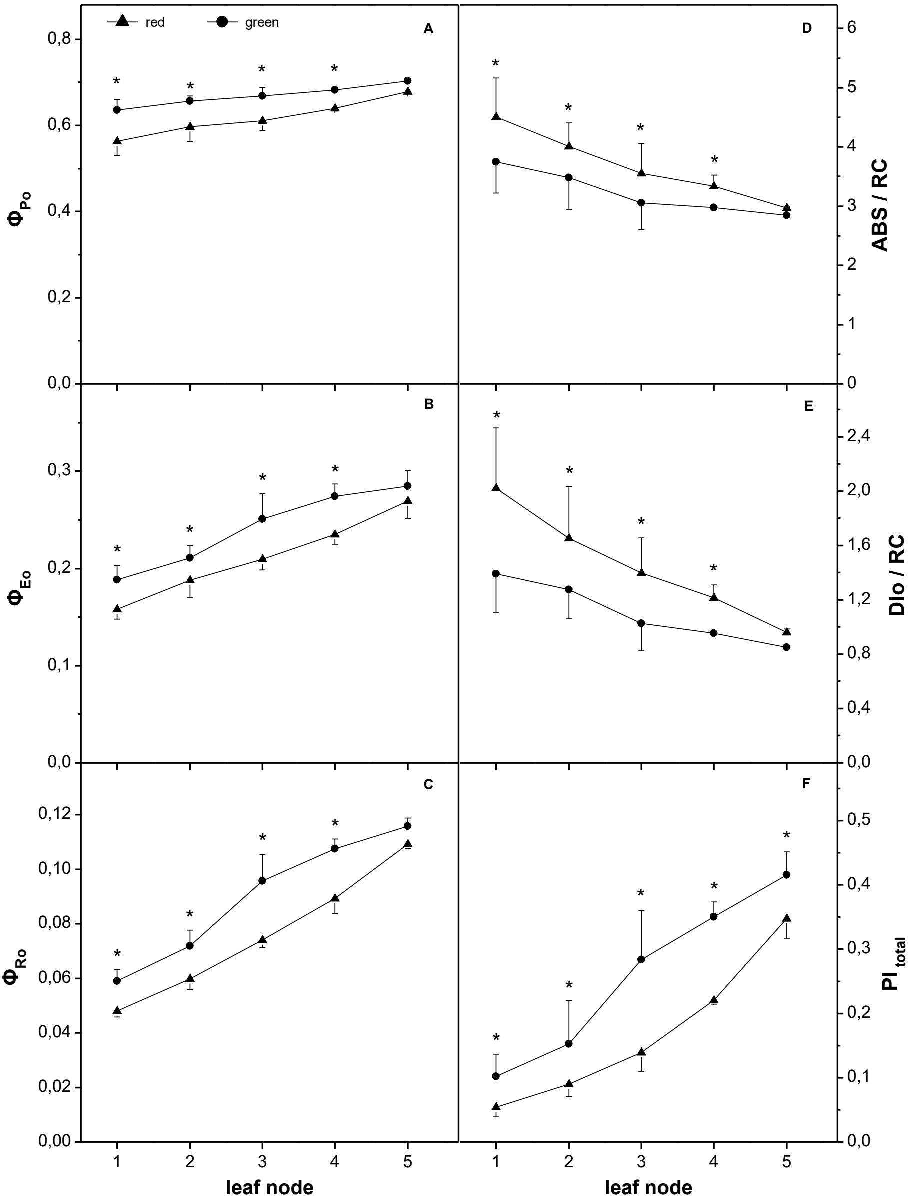


Fig. 2. Photosynthetic parameters derived from OJIP analysis of in vivo chlorophyll fluorescence in green (●) and red (▲) leaves of *C. siliquastrum* versus leaf age. A, φ_{P0}, maximum quantum yield of primary photochemistry (equivalent to F_V/F_M); B, φ_{E0}, quantum yield of electron transfer to intermediate carriers; C, φ_{R0}, quantum yield of reduction of PSI final electron acceptors; D, ABS/RC, absorption per reaction center; E, DI_0/RC, dissipated energy flux per reaction center; F, PI_{total}, photosynthetic performance index. Values are means±SD from 32 measurements per each age class (8 plants per phenotype). Asterisks denote statistically significant differences between the two phenotypes ($P < 0.05$).

One may argue that the lower chlorophyll content combined with the accumulation of light attenuating anthocyanins could afford some photoprotection in red leaves by reducing the excitation energy and/or increasing the antioxidant potential in the mesophyll. According to the predictions of the photoprotective hypothesis, anthocyanic leaves would be less vulnerable to photoinhibitory risk. Traditionally, photoihibition is detected by a decrease of the maximal PS II photochemical efficiency (given as F_V/F_M or φ_{P0}). Consequently, compared to greens, equal or even higher φ_{P0} values would be expected in red leaves. Although this parameter is considered a less sensitive indicator of photoinhibition (STRASSER & al. 2004), in the present study red leaves displayed slightly (~ 10%), yet significantly, lower φ_{P0} values from the corresponding greens (Fig. 2). Moreover, the differences between the two phenotypes in the other quantum yield parameters were higher, i.e. ~17% for the electron transfer to the intermediate e- carriers (φ_{E0}) and ~25% for the reduction of PSI final electron acceptors (φ_{R0}), indicating an enhanced number of inactive PSII centers and increasing limitations along the linear electron flow in red leaves (ZELIOU & al. 2009).

Both parameters related to the specific energy fluxes per PSII reaction center (ABS/RC and DI_0/RC) were higher in red leaves (Fig. 2D, E) and the difference between the two phenotypes diminished with age, following the pattern of anthocyanin decline. ABS/RC is a relative measure of antenna size per PSII reaction center (STRASSER & al. 2004). At first, the enhanced antenna size of red leaves is an indication of shade acclimation (ANDERSON 1986). According to the assumptions of the JIP-test, however, the antenna size is expressed per active reaction center (STRASSER & al. 2004). Thus, this parameter could increase either due an enhanced antenna size and/or due the decreased number of active PSII centers. Given, however, that no difference was observed in the Chl a/b index between the two phenotypes (Fig. 1C), this increase is rather indicative of PSII inactivation. One should expect a lower DI_0/RC in red leaves, due to the actually lower PAR levels reaching the mesophyll. The increased number of inactive, yet still absorbing, PSII centers, however, along with the limitations in electron flow displayed by red leaves would lead to a higher dissipation of absorbed energy as heat.

Based on the above, we may conclude that our results weaken the alleged photoprotective function of leaf anthocyanins, implying a photosynthetic inferiority for the red phenotype, as it has been proposed for the winter-red Mediterranean shrubs *Cistus creticus* and *Pistacia lentiscus*. Alternatively, if anthocyanins afford some photoprotection this is not adequate to fully compensate for the higher vulnerability of red leaves (KYTRIDIS & al. 2008, ZELIOU & al. 2009, NIKIFOROU & MANETAS 2010).

The inferiority of red leaves is manifested in their considerably reduced photosynthetic capacity (Fig. 2F). PI_{total} is a sensitive measure of photosynthetic performance, which combines the amount of active PSII centers and the partial quantum yields and efficiencies along the electron transport chain. It is positively correlated to CO_2 assimilation rates and, accordingly, to productivity (STRASSER & al. 2004). In addition, it is expected that the photosynthetic inferiority of the red phenotype would lead in a reduced growth, as it was observed in the red phenotype of *Cistus creticus*, when vegetative growth was assessed as the rate of new leaf production (NIKIFOROU & al. 2010). As it is shown in Figure 3, this was also confirmed in the present study. Compared to greens, the relative growth rate of juvenile red leaves was indeed significantly lower.

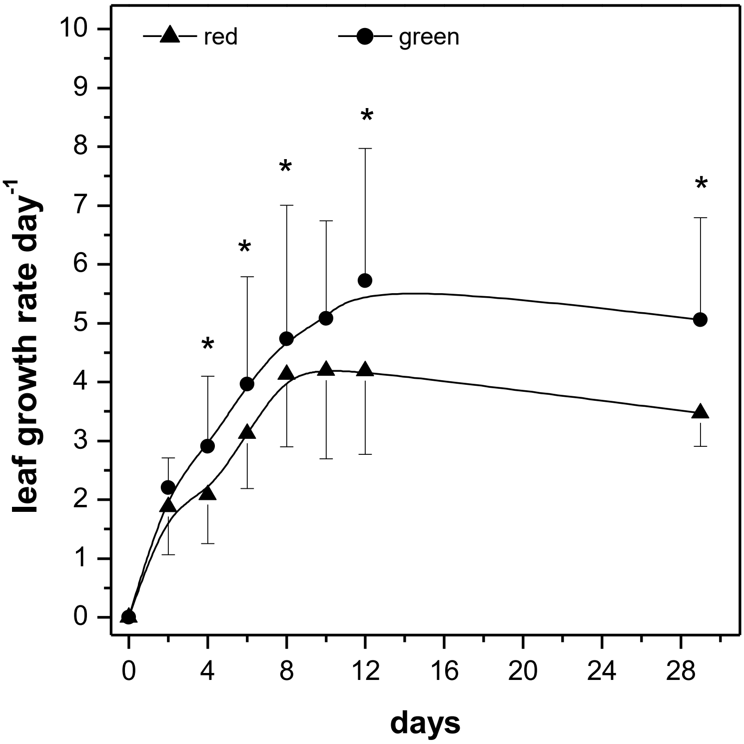


Fig. 3. Relative leaf growth rate in the green (●) and red (▲) phenotype of *C. siliquastrum*. Values are means±SD from 8 leaves (1 leaf per individual). Asterisks denote statistically significant differences between the two phenotypes (P <0.05).

References

Anderson, J. M. 1986: Photoregulation of the composition, function, and structure of thylakoid membranes. – Ann. Rev. Plant Physiol. Plant Mol. Biol. 37: 93-136.

Archetti, M., Döring, T. F., Hagen, S. B., Hughes, N. M., Leather, S. R., Lee, D. W., Lev-Yadun, S., Manetas, Y., Ougham, H. J., Schaberg, P. G. & Thomas, H. 2009: Unravelling the evolution of autumn colours: an interdisciplinary approach. – Trends Ecol. Evol. 24: 166-173.

Chalker-Scott, L. 1999: Environmental significance of anthocyanins in plant stress responses. – Photochem. Photobiol. 70: 1-9.

Close, D. C. & Beadle, C. L. 2003: The ecophysiology of foliar anthocyanins. – Bot. Rev. 69: 149-161.

Gamon, J. A. & Surfus, J. S. 1999: Assessing leaf pigment content and activity with a reflectometer. – New Phytol. 143: 105-117.

Gitelson, A. A. & Merzlyak, M. N., 1994: Spectral reflectance changes associate with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. – J. Plant Physiol. 143: 286-292.

Gould, K. S. 2004: Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. – J. Biomed. Biotechnol. 4: 314-320.

—, VOGELMANN, T. C., HAN, T. & CLEARWATER, M. J. 2002: Profiles of photosynthesis within red and green leaves of *Quintinia serrata* A. Cunn. – Physiol. Plantarum 116: 127-133.

—, JAY-ALLEMANDB, C., LOGAN, B. A., BAISSAC, Y. & BIDEL, L. P. R. 2018: When are foliar anthocyanins useful to plants? Re-evaluation of the photoprotection hypothesis using *Arabidopsis thaliana* mutants that differ in anthocyanin accumulation. – Environ. Exp. Bot. 154: 11-22.

HARBORNE, J. B. 1976: The anthocyanin pigments. Pp. 1-36. In HARBORNE, J.B. (ed.), Comparative Biochemistry of the Flavonoids. – Academic Press, London.

HRAZDINA, G., WAGNER, G. J. & SIEGELMAN, H. W., 1978: Subcellular localization of enzymes of anthocyanin biosynthesis in protoplasts. – Phytochemistry 17: 53-56.

HUGHES, N. M. 2011: Winter leaf reddening in 'evergreen' species. – New Phytol.t 190: 573-581.

— & SMITH, W. K. 2007: Seasonal photosynthesis and anthocyanin production in 10 broadleaf evergreen species. – Func. Plant Biol. 34: 1072-1079.

—, NEUFELD, H. S. & BURKEY, K. O. 2005: Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. – New Phytol. 168: 575-587.

—, MORLEY C. B. & SMITH, W. K. 2007: Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. – New Phytol. 175: 675-685.

JIANG, H. X., CHEN, L. S., ZHENG, J. G., HAN, S., TANG, N. & SMITH, B. R. 2008: Aluminum-induced effects on Photosystem II photochemistry in Citrus leaves assessed by the chlorophyll a fluorescence transient. – Tree Physiol. 28: 1863-1871.

KARABOURNIOTIS, G., BORNMAN, J. F. & LIAKOURA, V. 1999: Different leaf surface characteristics of three grape cultivars affect leaf optical properties as measured with fibre optics. Possible implication in stress tolerance. – Aust. J. Plant Physiol. 26: 47-53.

KARAGEORGOU, P. & MANETAS, Y. 2006: The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. – Tree Physiol. 26: 613-621.

KYPARISSIS, A., GRAMMATIKOPOULOS, G. & MANETAS, Y. 2007: Leaf morphological and physiological adjustments to the spectrally selective shade imposed by anthocyanins in *Prunus cerasifera*. – Tree Physiol. 27: 849-857.

KYTRIDIS, V.-P. & MANETAS, Y. 2006: Mesophyll versus epidermal anthocyanins as potential in vivo antioxidants: evidence linking the putative antioxidant role to the proximity of oxy-radical source. – J. Exp. Bot. 57: 2203-2210.

—, KARAGEORGOU, P., LEVIZOU, E. & MANETAS, Y. 2008: Intraspecies variation in transient accumulation of leaf anthocyanins in *Cistus creticus* during winter: evidence that anthocyanins may compensate for an inherent photosynthetic and photoprotective inferiority of the red-leaf phenotype. – J. Plant Physiol. 165: 952-959.

LARCHER, W. 1995: 'Physiological plant ecology: ecophysiology and stress physiology of functional groups'. 3rd edition. – Springer-Verlag: Berlin, Germany.

LEE, D. W. & COLLINS, T. M., 2001: Phylogenetic and ontogenetic influences on the distribution of anthocyanins and betacyanins in leaves of tropical plants. – Int. J. Plant. Sci. 162: 1141-1153.

MANETAS, Y. 2006: Why some leaves are anthocyanic and why most anthocyanic leaves are red? – Flora 201: 163-177.

—, DRINIA, A. & PETROPOULOU, Y. 2002: High contents of anthocyanins in young leaves are correlated with low pools of xanthophyll cycle components and low risk of photoinhibition. – Photosynthetica 40: 349-354.

—, PETROPOULOU, Y., PSARAS, G. K. & DRINIA, A. 2003: Exposed red (anthocyanic) leaves of *Quercus coccifera* display shade characteristics. – Func. Plant Biol. 30: 265-270.

NEILL, S. O., GOULD, K. S., KILMARTIN, P. A., MITCHELL, K. A. & MARKHAM, K. R. 2002: Antioxidant capacities of green and cyanic leaves in the sun species *Quintinia serrata*. –Funct.

Plant Biol. 29: 1437-1443.

NIKIFOROU, C. & MANETAS, Y. 2010: Strength of winter-leaf redness as an indicator of stress vulnerable individuals in *Pistacia lentiscus*. – Flora 205: 424-427.

—, ZELIOU, K., KYTRIDIS, V.-P., KYZERIDOU, A. & MANETAS, Y. 2010: Are red leaf phenotypes more or less fit? The case of winter leaf reddening in *Cistus creticus*. – Environ. Exp. Bot. 67: 509-514.

RICHARDSON, A. D., DUIGAN, S. P. & BERLYN, G. P. 2002: An evaluation of noninvasive methods to estimate foliar chlorophyll content. – New Phytol. 153: 185-194.

STRASSER, R. J., TSIMILLI-MICHAEL, M. & SRIVASTAVA, A. 2004: Analysis of the chlorophyll a fluorescence transient. Pp. 321-362. In PAPAGEORGIOU, G. C. & GOVINDJEE (eds), Chlorophyll fluorescence: a signature of photosynthesis. – Advances in photosynthesis and respiration series, 19. Rotterdam: Kluwer.

TSIMILLI-MICHAEL, M. & STRASSER, R. J. 2008: In vivo assessment of stress impact on plant's vitality: applications in detecting and evaluating the beneficial role of mycorrhization on host plants. Pp. 679-703. In VARMA, A. (ed.), Mycorrhiza, 3. – Springer, Berlin Heidelberg.

YORDANOV, I., GOLTSEV, V., STEFANOV, D., CHERNEV, P., ZAHARIEVA, I., KIROVA, M., GECHEVA, V. & STRASSER, R. J. 2008: Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. – J. Plant Physiol. 165: 1954-1963.

ZELIOU, K., MANETAS, Y. & PETROPOULOU, Y. 2009: Transient winter leaf reddening characterizes weak (stress sensitive) individuals, yet anthocyanins cannot alleviate the adverse effects on photosynthesis. – J. Exp. Bot. 60: 3031-3042.

Phenotypic adaptation of *Phlomis fruticosa* L. to environments with different precipitation. A common garden experiment

CHRISTOS CHONDROGIANNIS, AGGELIKI KALISPERI & GEORGE GRAMMATIKOPOULOS

Abstract

Chondrogiannis, Ch., Kalisperi, A. & Grammatikopoulos, G. 2019: Phenotypic adaptation of *Phlomis fruticosa* L. to environments with different precipitation. A common garden experiment. – Bot. Chron. 22: 355-364.

Variable phenotypic response to environmental gradients among populations is either attributed to phenotypic or to genetic intraspecific variation of the corresponding traits. In the latter case, the populations are characterized as ecotypes. Common garden experiments, in which, individuals originated from distinct environments grow under similar conditions, are usually employed to gain insight into the possibility of genetic differentiation, without necessarily using genetic analysis. Phlomis fruticosa is a Mediterranean shrub with great phenotypic plasticity regarding leaf demography, morphology and physiology. In order to address the role of precipitation amount in photosynthetic plasticity of the summer leaves of P. fruticosa, individuals originated from two regions with distinct summer aridity, were germinated and grew under identical conditions. The common garden experiment was carried out in the region with the higher aridity and the seasonally measured photosynthetic parameters were compared a) between the two populations and b) against field measurements of the local natural population. Yields of primary photochemistry, electron transport up to PSI, dissipation of energy, chlorophyll content and chlorophyll to carotenoid ratio were identical among the populations. However, stomatal resistance (RS) to water vapor was higher in Patra population and the difference increased during the driest months of the year. In the natural population, stomata were practically closed at the same period. We conclude that RS is definitely up regulated in Patras population. However, more experimentation is needed to characterize the two populations.

Ch. Chondrogiannis, A. Kalisperi & G. Grammarikopoulos*, Laboratory of Plant Physiology, Department of Biology, University of Patras, Patras, Greece

*Corresponding author: E-mail: grammati@upatras.gr

Key words: phenotypic plasticity, photosynthesis, common garden, Mediterranean shrub.

Introduction

Phlomis fruticosa L. is a species with a rich repertoire of adaptations against the hot and dry summer of Mediterranean climate. Anatomical, morphological, biochemical and physiological properties contribute to survival under the prolonged drought (GRAMMATIKOPOULOS & al. 1995, KYPARISSIS & al. 1995, PETSAS & GRAMMATIKOPOULOS 2009). Seasonal leaf dimorphism should be characterized as the dominant adaptation, providing the plant with an especially constructed "summer leaf" (KYPARISSIS & MANETAS 1993, KYPARISSIS & al. 1997). This leaf experiences extremely low water contents for periods of unpredictable length, during July, August and September. Increased trichome density, leaf curling, intensive stomatal regulation, dew absorption, chlorophyll loss and down regulation of light reactions of photosynthesis are some of the traits which facilitate drought resistance of the summer leaf (GRAMMATIKOPOULOS & MANETAS 1994, GRAMMATIKOPOULOS & al. 1995, KYPARISSIS & al. 1995, PETSAS & GRAMMATIKOPOULOS 2009). Also, *P. fruticosa* is widely distributed in the northern part of Mediterranean region, occurring in habitats that differ strongly in total annual or summer precipitation. These regions include southern Italy, Albania, almost all of the Greece, as well as Cyprus and southern Anatolia (FANELLI & al. 2015). Consequently, each local population has to cope with varying aridity and precipitation frequency. Therefore, enhanced intraspecific physiological plasticity of the already specialized ‘summer’ leaf could be expected.

Phenotypic plasticity is the prerequisite for phenotype acclimation to environmental gradients. Sustained environmental pressures along these gradients, usually lead to evolutionary adaptations which need to be fixed over generations. However, short-term morphological and/or physiological changes of a phenotype usually reflect re-versible modifications of individuals or populations, to seasonal, temporal or even un-predictable changes of local environmental conditions. As a result of genotypic response to a particular habitat environmental pressure, populations can be characterized as 'ecotypes' (DUDDLEY 1996, ARNTZ & DELPH 2001). Yet, a genetic basis is not always necessary for traits variation along an environmental gradient. One of the most popular protocols, used to disentangle the effects of genetic and environmental variation on the phenotype is the "common garden" one (CG). In CG experiments, genetically distinct strains, families or populations are compared under identical environmental conditions (VILLEMEREUIL & al. 2016, HIRANO & al. 2017).

Research questions about plant adaptation to diverse environments bring together fields of plant population biology and plant ecophysiology (MOONEY 1976, PEARCY & al. 1987). In the present study, we examined variation of selected physiological parameters, related to photosynthesis, under a simple CG experiment, using seeds of two distinct populations of *P. fruticosa*, which naturally grow in areas with significant difference of precipitation amount and minor differences in mean summer temperature. A comparison of the CG experiment with measurements of naturally growing plants in the vicinity of the experimental site is also presented.

Material and methods

Plant material and growth conditions

Mature seeds of *P. fruticosa* L. were collected during summer, from several plants growing in undisturbed field locations, either around the Patra University (38.29°N, 21.79°E, 48 m a.s.l.) or around the Ioannina University (39.37°N, 20.50°E, 490 m a.s.l.). Seeds were stored for three months under dry conditions in the lab. At mid-November seeds were thoroughly washed and germinated in petri-dishes (20 seeds/petri-dish) in a growth chamber (12h photoperiod, T=15°C, light intensity 25 μmol.m-2.s-1), adding 1ml of sterile water each second day. Three weeks later, seedlings of 1-2 cm in height were transplanted in small pots and grew in a glasshouse during the winter and watered each second day. In April, seedlings of uniform size were selected, transplanted in larger pots (25 cm depth, 20 cm in diameter) with potting soil, and placed under natural environmental conditions in the vicinity of the glasshouse. Apart from natural precipitation, potted plants, received once or twice a week, tiny amounts of water (100 ml) in order to survive the dry period. Young plants were properly arranged in two groups (representing populations), in order to receive full sunlight. The acclimation period lasted 40 days.

Climatological information

Both Patra and Ioannina are characterized by the general Mediterranean type of climate (dry, hot summer and mild cold winters). However, local differences of climate exist, due to different altitude, geomorphology and vicinity to the sea. Representative climatic information of 30 years data for the two regions is presented in Fig.1 A, B, C, D. In Fig.1E, F the amount of precipitation and the maxima, mean and minima of temperature during the experimental period are presented.

Chlorophyll fluorescence measurements

Recordings of chla fluorescence induction kinetics were captured by a portable fluorimeter (Handy-PEA, Hansatech Instruments Ltd. King's Lynn Norfolk, UK). For the measurements, dark adapted samples were used (30 min adaptation time). A bank of three red LEDs (peak at 650 nm) providing 3000 µmol m-2 s-1, was used for excitation. Fluorescence was recorded from 10 µs to 2s with intervals of 10 µs, 100 µs, 1 ms, 10 ms and 100 ms between the readings, for time periods of 10-300 µs, 0.3-3 ms, 3-30 ms, 30-300 ms, and 0.3-2 s, respectively. Fluorescence data were then analyzed according to the JIP-test (Strasser & al. 2000) as extended to analyze events around PSI (Stirbet & Govindjee 2011). More than 30 parameters were extracted, related to different steps of energy flow around and between the two photosystems. The parameters used in the present study, are: a) Quantum yield and efficiencies: φP0 = FV/FM, maximum quantum yield of primary PSII photochemistry; φR0, quantum yield for reduction of end electron acceptors at the PSI acceptor side and b) DI0/RC, dissipated energy flux per reaction center.

Stomatal diffusion of water vapor

Stomatal resistance to water vapor (RS, sec/cm) was measured with a dynamic diffusion porometer (AP4, Delta devices, Cambridge, UK). One mature and exposed leaf from each plant was sampled ($n = 10$). For corresponding field measurements, an open gas analyzer (Li-6400, Licor, Lincoln, Ne, USA) was used. Measurements were performed around 10:00 a.m. in order to achieve maximum stomatal opening and to avoid mid-day closure occurring in summer (Grammatikopoulos & al. 1995).

Reflectance spectrum measurements

Leaf spectral reflectance was measured on the upper surface of the leaves (one mature and exposed leaf from each plant, $n = 10$), with a portable spectrometer (Unispec; PP Systems, Haverhill, MA, USA) equipped with an internal halogen source and a bifurcated fiber optic cable, directly attached on the leaf surface with the help of a leaf clip. The indexes calculated from raw spectra data, were: a) normalized difference index, NDI = (R750 − R705)/(R750 + R705) (Gitelson & Merzlyak 1994), related to chl content, and b) structure-independent pigment index, SIPI = (R800 − R445)/(R800 + R445), a measure of carotenoid to chlorophyll ratio (Peñuelas & al. 1995).

Data analysis

All statistical analyses were conducted using the SPSS (IBM-SPSS Statistics). Differences of the measured parameters between populations were assessed by t-test. The significance level was set to $P<0.05$.

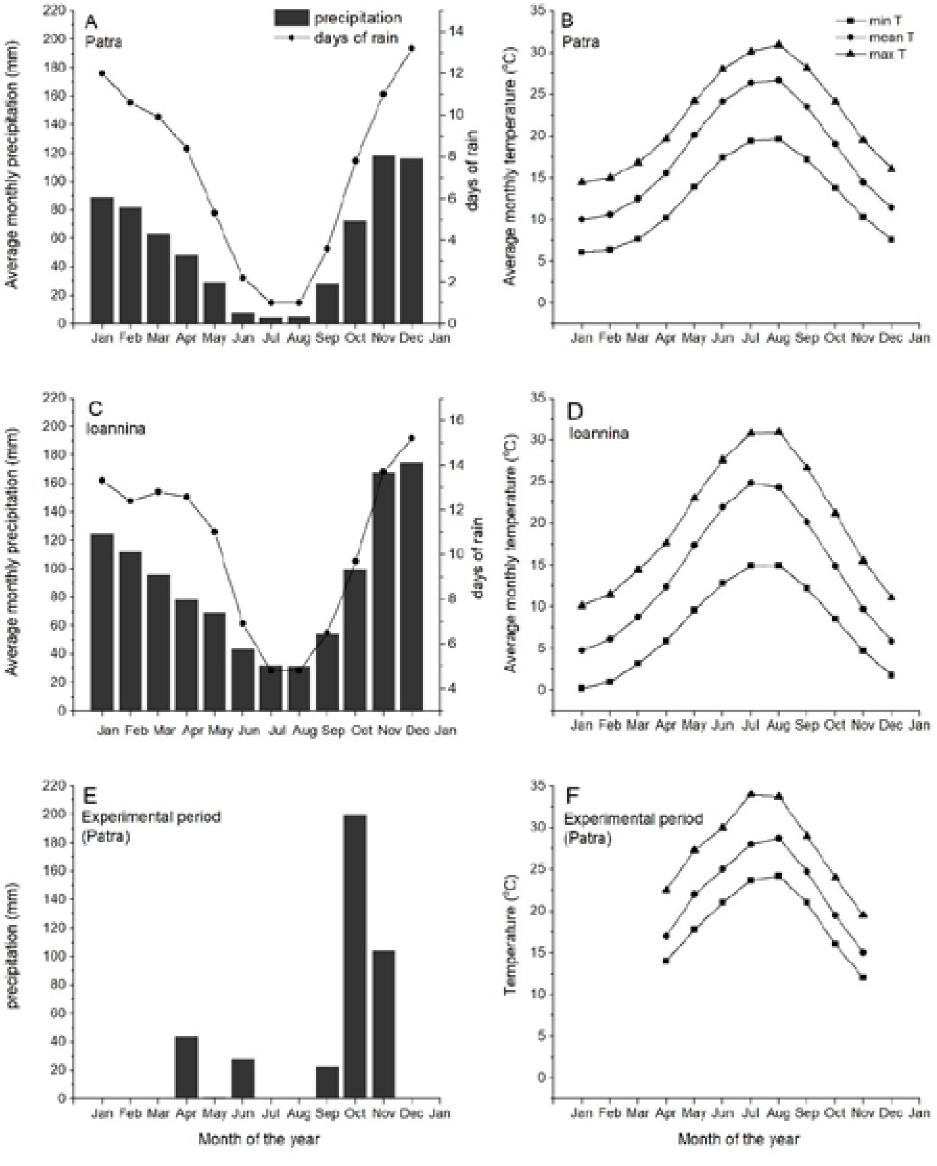


Fig. 1. Climatological data for the habitats from which the two ecotypes originated and additional data for the CG experimental period. Average monthly precipitation (columns) and days of rain (circles) for Patra (A), Ioannina (C) and CG site (E). Average monthly high (▲), mean (●) and low (■) temperature, for Patra (B), Ioannina (D) and CG site (F).

Results and Discussion

Although CG experiments are very important in order to evaluate the contribution of ecophysiological plasticity to site-specific responses and except the necessity for manipulation of key environmental variables of the experimental protocols, lately, a further suggestion for outdoor measurements at different times of the year is stated by many authors, as an alternative approach (CARLSON & al. 2011, WEST & al. 2012, CARLSON & al. 2016).

The average monthly precipitation and the total precipitation during summer are the main environmental variables that differ between the two regions from where seeds of our CG experiment had been collected. Monthly precipitation is 40-100% higher in Ioannina during the year, while in summer, the amount of precipitation in Ioannina becomes 5-6 times higher than that of Patra (Fig. 1A, C). In fact, total summer precipitation in Patra does not exceed 18 mm, which is 2.5% of the annual precipitation. Corresponding values for Ioannina are 107 mm and 10%. Such a difference can be critical for a species survival and induce substantial phenotypic modifications (WARREN & al. 2005, ADAMS & al. 2016). During the experimental period, the summer precipitation only slightly surpassed the average value (28 mm, Fig. 1E). However, October was wetter than usual (73 mm) reaching 200mm of precipitation (Fig. 1A, E). Mean monthly temperature during summer is 2-2.5°C higher in Patra, while during the experimental period was further increased only by 0.5-1.5°C (Fig.1B, D, F). The temperature difference that could be substantial in order to induce phenotypic differences refers to winter months and is of minor importance for the summer leaves examined herein.

As shown in Table 1, seed germination was twice more successful for Patra seeds. The young plants originated from the germinated seeds of both populations were robust, reaching similar plant height, while the number of leaves of Ioannina population, at the commencement of the experiment (May 9), was significantly higher than that of Patra. However, at the end of June, the period of partial shedding of leaves, the remaining leaves, destined to live until next summer (KYPARISSIS & al. 1997), were equal in the two populations.

Table 1. Seed germination, plant height, and number of leaves of the young plants grown from the seeds collected from the two different populations of *P. fruticosa*. Values are means ± SD of 15 plants per population. Significance of difference between populations was estimated according to t-test ($P < 0.05$, ns: not significant). * denotes significant difference at different date inside populations.

		Patra	Ioannina	P
Seed germination (%)		58 ± 10	28 ± 9	<0.001
Plant height (cm)	May 9	13.25 ± 4.81	13.95 ± 2.86*	ns
	June 23	17.18 ± 4.00	18.41 ± 4.41	ns
Number of leaves	May 9	77 ± 6*	108 ± 15*	<0.001
	June 23	50 ± 11	64 ± 13	ns

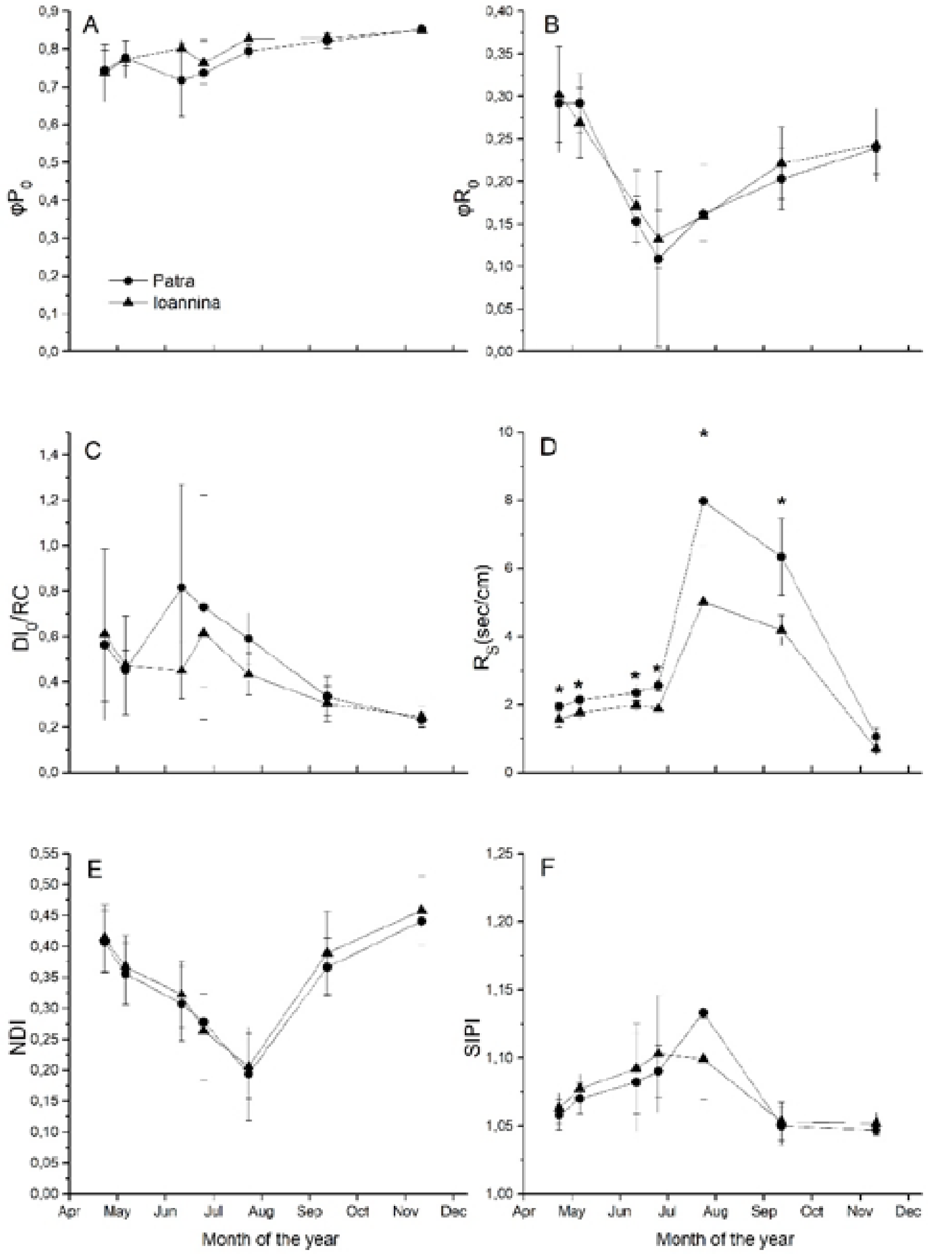


Fig. 2. Seasonal fluctuation (May-November) of the photosynthetic parameters in Patra (●) and Ioannina population (▲). Maximum quantum yield of primary PSII photochemistry φP_0 (A), quantum yield for reduction of end electron acceptors at the PSI acceptor side φR_0 (B), dissipated energy flux per reaction center DI_0/RC (C), stomatal resistance to water vapor R_S, (D), normalized difference index, NDI (E) and structure-independent pigment index, SIPI (F).Each value represents the mean ± SD of 10 measurements. * denotes statistical significant differences between populations ($P<0.05$).

Representative parameters of the JIP-test analysis, related to energy flux through the two photosystems, such as φP0, φR0, and DI0/RC were almost identical between the two populations and irrespectively of the expected seasonal fluctuation (Fig. 2A, B, C). The rest of the large set of parameters, extracted from JIP-test, showed exactly the same profile (data not shown). A trend, though statistically not significant, for higher energy dissipation in Patra population during the hottest summer months was found. Analogous profiles were detected for reflectance spectral indexes NDI, and SIPI (Fig. 2E, F), indicating similar fluctuations of chl content and carotenoids to chl ratio respectively in both populations. In other species such as *Querqus rubra* and *Fagus sylvatica*, positive or negative correlations of photosynthetic parameters with annual precipitation and drought intensity has been found in CG experiments (KUBISKE & ABRAMS 1992, NGUYEN & al. 2017).

Patra population showed increased values of stomatal diffusion resistance R_S in comparison with Ioannina population. Difference in R_S was statistically significant during the whole experimental period, but was more intense during the driest months. Precipitation in July and August was null in Patra as depicted in Fig. 1E. Therefore, plants of Patra population could be characterized as more sensitive to drought stress. Their ability to close stomata more efficiently than plants originated from Ioannina region may prevent excessive water loss and maintain water content above critical wilting threshold. Patra habitat is drier than that of Ioannina and benefit of saving even the smallest amount of water could be very important. For example, it has been shown that dew deposition on hairy leaves of *P. fruticosa* for just a couple of hours early in the morning could ameliorate the adverse effects of prolonged aridity during summer (GRAMMATIKOPOULOS & MANETAS 1994). Recently, it was found that stomatal density and conductance were positively correlated to drier habitat as a mechanism for increased transpiration and leaf cooling, due to co-existing high temperature (CARLSON & al. 2016). However, in *P. fruticosa*, other summer leaf adaptations such as dense epidermal trichome and leaf curling may reduce light absorption and confine leaf temperature increase, in addition to transpiration cooling (KYPARISSIS & MANETAS 1993, GRAMMATIKOPOULOS & al. 1995).

Different R_S in *P. fruticosa* could be due to less open stomata or smaller density of stomata on the epidermis. In related CG experiments with the C4 prairie grass *Andropogon gerardii* of Kansas, (distributed in areas with great precipitation gradients), ecotypes from the driest environment showed higher photosynthetic rate compared to mesic ecotypes, when the experiment was carried out in mesic sites (MARICLE & al. 2017). The more drought-tolerant ecotypes had fewer stomata compared to more mesic ecotypes (VARVEL & al. 2018). On the contrary, most of the leaf morphology and physiology traits, including photosynthesis, of *Eucalyptus sideroxylon* were unrelated to seed-source rainfall (WARREN & al. 2005), but stomata and R_S were not studied. Concerning our results, if higher R_S in Patra population was the result of fewer stomata, this could be safely considered as a genetically modified trait due to rainfall difference between the two regions. Then, populations could be characterized as ecotypes. Unfortunately, estimation of the stomatal density on leaf surface of *P. fruticosa* is a challenge, even with the use of scanning electron microscope, due to the presence of the dense trichome. On the other hand, higher R_S may reflect different suites of traits, which selectively increase population drought tolerance throughout increased RS sensitivity,

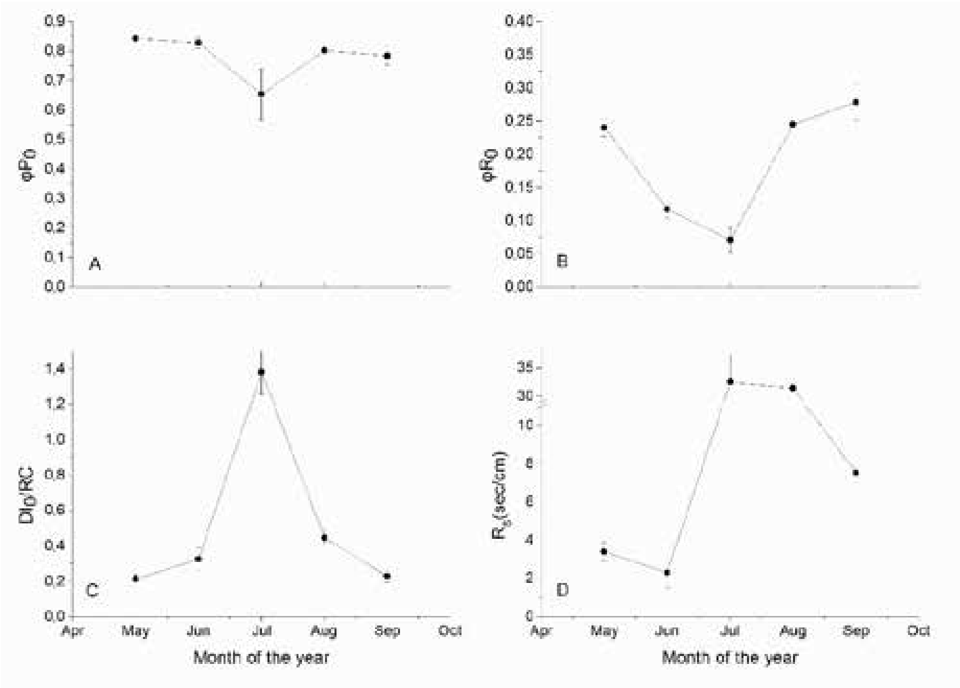


Fig. 3. Seasonal fluctuation (May-September) of the photosynthetic parameters in natural occurring young plants around the CG experiment site. Maximum quantum yield of primary PSII photochemistry φP0 (A), quantum yield for reduction of end electron acceptors at the PSI acceptor side φR0 (B), dissipated energy flux per reaction center DI0 /RC (C) and stomatal resistance to water vapor RS, (D).Each value represents the mean ± SD of 10 measurements. * denotes statistical significant differences between populations (P<0.05).

lacking any genetic basis (Sandquist & Ehleringer 1997, Nicotra & al. 2007).

The photosynthetic parameters and stomatal diffusion to water vapor were recorded during the dry period of the year, in young plants, naturally occurring in the hills region around the Patra university campus. As shown in Fig. 3, the trends of seasonal changes were similar to those of the CG experiment. However, the naturally occurring plants showed values similar or slightly higher to those of Patra population of the CG experiment. The R_S values above 10 sec/cm during July and August indicate practically closed stomata. It should be noted that the only difference of the field growing young plants was their growth in natural soil. Therefore, differences of the belowground part of plants between field grown and potted individuals cannot be excluded.

Acknowledgments

We thank Dr. Aris Kyparissis, who generously provided seeds of *P. fruticosa* from Ioannina region. The LiCor 6400 gas analyzer used in this study was donated to the Laboratory of Plant Physiology by the Bodossaki Foundation.

References

ADAMS, W., STEWART, J., COHU, C., MULLER, O. & DEMMIG-ADAMS, B. 2016: Habitat temperature and precipitation of *Arabidopsis thaliana* ecotypes determine the response of foliar vasculature, photosynthesis, and transpiration to growth temperature. – Front Plant Sci. 7: 1-18.

ARNTZ, A. & DELPH, L. 2001: Patterns and process: evidence for the evolution of photosynthetic traits in natural populations. – Oecologia 127: 455-467.

CARLSON, J., HOLSINGER, K. & PRUNIER, R. 2011: Plant responses to climate in the cape floristic region of South Africa: evidence for adaptive differentiation in the proteace. – Evolution 65: 108-124.

—, ADAMS, C. & HOLSINGER, K. 2016: Intraspecific variation in stomatal traits, leaf traits and physiology reflects adaptation along aridity gradients in a South African shrub. – Ann. Bot. 117: 195-207.

DUDLEY, S. 1996: The response to differing selection on plant physiological traits: evidence for local adaptation. – Evolution 50: 103-110.

FANELLI, G., ATTORRE, F., GIUDICE, M. DEL, GJETA, E. & SANCTIS, M. DE 2015: *Phlomis fruticosa* scrublands in the central Mediterranean region: syntaxonomy and ecology. – Phytocoenologia 45: 49-68.

GITELSON, A. & MERZLYAK, M. 1994: Spectral reflectance changes associate with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. – J. Plant Physiol. 143: 286-292.

GRAMMATIKOPOULOS, G. & MANETAS, Y. 1994: Direct absorption of water by hairy leaves of *Phlomis fruticosa* and its contribution to drought avoidance. – Can. J. Bot. 72: 1805-1811.

—, KYPARISSIS, A. & MANETAS, Y. 1995: Seasonal and diurnal gas exchange characteristics and water relations of the drought semi-deciduous shrub *Phlomis fruticosa* L. under Mediterranean field conditions. – Flora 190: 71-78.

HIRANO, M., SAKAGUCHI, S. & TAKAHASHI, K. 2017: Phenotypic differentiation of the *Solidago virgaurea* complex along an elevational gradient: Insights from a common garden experiment and population genetics. – Eco. Evo. 7: 6949-6962.

KUBISKE, M. & ABRAMS, M. 1992: Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. – Can. J. For. Res. 22: 1402-1407.

KYPARISSIS, A. & MANETAS, Y. 1993: Seasonal leaf dimorphism in a semi-deciduous Mediterranean shrub: ecophysiological comparisons between winter and summer leaves. – Acta OEcol. 14: 23-32.

—, PETROPOULOU, Y. & MANETAS, Y. 1995: Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L. Labiatae) under Mediterranean field conditions: avoidance of photoinhibit ory damage through decreased chlorophyll contents. – J. Exp. Bot. 46: 1825-1831.

—, GRAMMATIKOPOULOS, G. & MANETAS, Y. 1997: Leaf demography and photosynthesis as affected by the environment in the drought semi-deciduous Mediterranean shrub *Phlomis fruticosa* L. – Acta Oecol. 18: 543-555.

MARICLE, B., CAUDLE, K., LINDSEY, K., BAER, S. & JOHNSON, L. 2017: Effects of extreme drought on photosynthesis and water potential of *Andropogon gerardii* (Big Bluestem) ecotypes in common gardens across Kansas. – Trans. Kans. Acad. Sci. 120: 1-16.

MOONEY, H. 1976: Some contributions of physiological ecology to plant population biology. – Syst. Bot. 1: 269-283.

NGYEN, Q., POLLE, A. & PENA, R. 2017: Intraspecific variations in drought response and fitness traits of beech (*Fagus sylvatica* L.) seedlings from three provenances differing in annual precipitation. – Trees 31: 1215-1225.

NICOTRA, A., HERMES, J., JONES, C. & SCHLICHTING, C. 2007: Geographic variation and plasticity to water and nutrients in *Pelargonium australe*. – New Phytol. 176: 136-149.

PEÑUELAS, J., FILELLA, I. & BARET, F. 1995: Semiempirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. – Photosynthetica 31: 221-230.

PETSAS, A. & GRAMMATIKOPOULOS, G. 2009: Drought resistance and recovery of photosystem II activity in a Mediterranean semi-deciduous shrub at the seedling stage. – Photosynthetica 47: 284-292.

SANDQUIST, D. & EHLERINGER, J. 1997: Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. – New Phytol. 135: 635-644.

STRASSER, R., SRIVASTAVA, A. & TSIMILLI-MICHAEL, M. 2000: The fluorescence transient as a tool to characterize and screen photosynthetic samples. Pp. 445-483. In YUNUS, M., PATHRE, U & MOHANTY, P. (eds), Probing Photosynthesis: Mechanisms, Regulation and Adaptation. – Taylor and Francis.

STIRBET, A. & GOVINDJEE, G. 2011: On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. – J. Photochem. Photobiol. B 104: 236-257.

VARVEL, N., HILT, C., JOHNSON, L., GALLIART, M., BAER, S. & MARICLE, B. 2018: Genetic and environmental influences on stomates of big bluestem (*Andropogon gerardii*). – Environ. Exp. Bot. 155: 477-487.

VILLEMEREUIL, P. DE, GAGGIOTTI, O. E. & MOUTERDE, M. 2016: Common garden experiments in the genomic era: new perspectives and opportunities. – Heredity 116: 249-254.

WARREN, C., TAUSZ, M. & ADAMS, M. 2005: Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (*Eucalyptus sideroxylon* subsp. *tricarpa*) grown in a common garden? – Tree Physiol. 25: 1369-1378.

Effects of prolonged darkening on the photosynthetic characteristics of olive tree (*Olea europaea* L.) bark

MANOLIS FILIPPOU, COSTAS FASSEAS & GEORGE KARABOURNIOTIS

Abstract

Filipou, M., Fasseas, C. & Karabourniotis, G. 2019: Effects of prolonged darkening on the pho-tosynthetic characteristics of olive tree (*Olea europaea* L.) bark. – Bot. Chron. 365-370

We investigated the effects of prolonged darkening on selected structural and functional characteristics of olive tree bark. The entire length of 15-years-old olive tree branches was covered for more than 14 months with successive layers of aluminum foil fixed with self-adhesive aluminum tape. South-facing neighboring branches were used as controls. Prolonged darkening caused a dramatic decrease in the photosynthetic capacity, total chlorophyll and carotenoid concentration, Fv/Fm, dark respiration rates, as well as in the number of mitochondria and peroxysomes in comparison to control branches. The prolonged darkening affected the fine structure of the bark chloroplasts (since the grana were disorganized and the number of lamellae was reduced), but not their number, size, or the presence of starch grains and plastoglobuli. The darkening of the stems did not cause apparent symptoms to the bearing leaves. The persistence of a minimum chloroplast organization and activity under such conditions provide some evidence that bark chloroplasts take active part to carbon metabolism irrespective of the light regime.

M. Filipou & C. Faseas, Laboratory of Electron Microscopy, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855 Botanikos Athens, Greece

G. Karabourniotis*, Laboratory of Plant Physiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855 Botanikos Athens, Greece

*Corresponding author: E-mail: karab@aua.gr

Key words: corticular photosynthesis, chloroplasts, intrinsic fluorescence, photosynthetic pigments, stem darkening, *Olea europaea.*

Introduction

Despite that leaves are the major plant organs responsible for photosynthetic CO_2 assimilation, petioles, green flowers, fruits, stems, calyces and even roots are able to photosynthesize. Among alternative photosynthetic organs, stems may have a significant contribution to the whole plant carbon gain. Based on the source of assimilated CO_2, two main categories of photosynthesis within plant stems can be distinguished; stem photosynthesis and corticular (or bark) photosynthesis (NILSEN 1995, ASCHAN & PFANZ 2003). Carbon dioxide in stem photosynthesis is derived from the atmosphere and enters the stem trough the abundant epidermal stomata. In this case positive net assimilation rates are observed (NILSEN 1995). Corticular photosynthesis occurs in stems which lack epidermal stomata and are not capable to assimilate carbon from the atmosphere (FOOTE & SCHAEDLE 1976). It is generally accepted that the main role of corticular photosynthesis is the recapture of the respiratory CO_2, which significantly contributes to the stem-internal CO_2 recycling and the overall annual tree carbon balance, giving adaptive advantages (PFANZ & ASCHAN 2001, ASCHAN & PFANZ 2003, CERNUSAK & CHEESMAN 2015, BLOEMEN & al. 2016). It is also a mechanism to actively raise the cortical O_2 concentration and counteract temporal/spatial hypoxia inside plant stems (WITTMANN & PFANZ 2015 & 2018). Corticular photosynthesis is significantly limited by the low periderm light transmittance (SCHAEDLE 1975, WITTMANN & al. 2001), as well as by the high internal CO_2 concentrations (MANETAS 2004, PFANZ & ASCHAN 2001) which lower the activity of bark chloroplasts (PFANZ & ASCHAN 2001). Due to the limited peridermal light transmittance, corticular chloroplasts show many aspects of the shade-acclimated behavior (PILARSKI 1999, WITTMANN & al. 2001, MANETAS 2004). The bark of *Olea europaea*, a major crop with a significant economical value for the Mediterranean region, is equipped with photosynthetic tissue that can efficiently contribute not only to the reduction of the inner respiratory CO_2 levels, but also to the whole tree carbon balance (FILIPPOU & al. 2007). Corticular photosynthesis in olive tree bark is comparable and even higher than that of deciduous trees, although the evergreen character of olive tree may be a limiting factor for the illumination of the bark surface. Due to the fact that bark chloroplasts operate under extremely low light conditions, we aimed to assess the state of the photosynthetic machinery of olive bark under the absence of light. We studied the fine structure, photosynthetic capacity and photochemical efficiency of bark chloroplasts after 14 months of artificial darkening.

Materials and Methods

For experiments, 40-50 years-old olive (*O. europaea*) trees were selected from the experimental olive grove of the Agricultural University of Athens, Greece. The trees were watered according to the usual agricultural practice and were exposed to natural sunlight conditions. Six 15-years-old branches from six different trees were covered with five successive layers of aluminum foil fixed with self-adhesive aluminum tape. The branches remained in dark for more than 14 months. The entire length of each branch, including young stems of the current growth period, was darkened. Darkened branches were periodically checked for the intactness of the coverings and for any apparent symptoms of the leaves. South-facing neighboring branches exposed to full sunlight (2000 μmol m^{-2} s^{-1}) were used as controls. For transmission electron microscopy (TEM), samples several mm deep were taken with a razor blade from the surface of south-facing bark of stems and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4 °C for 24h. The solution contained caffeine (1%) in order to retain phenolic substances within the vacuoles (MÜLLER & GREENWOOD 1978). Samples were post-fixed in 0.1% OsO_4 for 24 h, washed in buffer, dehydrated in a series of ethanol followed by propylene oxide, embedded in Spurr epoxy resin and polymerised at 70 °C for 36 h. Ultra-thin sections were cut with a Reichert OMU-3 ultramicrotome with glass knives and stained with uranyl acetate and lead citrate. The samples were examined and photographed with a ZEISS 9S TEM.

For chlorophyll and photosynthesis measurements, discs with a total area of 2355 cm^2 were cut from branches with the use of a cork borer. All non-chlorophylous woody tissues from stem discs were removed. Chlorophyll (total chlorophyll, chl a and chl b) and carotenoid concentrations, were determined in 80% acetone extract by the method of GRAAN & ORT (1984).

The intrinsic photochemical efficiency of PS II was evaluated from the Fv / Fm ratio which was measured using a portable chlorophyll fluorescence instrument (FIM 1500, ADC Ltd, UK).

Samples were taken into the laboratory as described above. The phellem remained intact and followed dark adaptation for 30 min in a standard leaf clip before measurement. Excitation light was adjusted to 1500 μmol m^{-2} s^{-1} for bark and 1150 μmol m^{-2} s^{-1} for leaves, from red light emitting diodes. Fluorescence induction curves of 5 s duration were recorded.

Photosynthetic capacity and dark respiration were measured as oxygen gas exchange with a leaf disk electrode (Model LD2, Hansatech, Norfolk UK) in thermostatically controlled oxygen electrode at 27 °C and about 5% CO_2. For every sample, three stem disks (r=0.5 cm) of total area of 2.355 cm^2 were placed in the cuvette and O_2 evolution was measured with increasing photon fluence rates in the range of 0-1900 μmol m^{-2} s^{-1} using light emitting diodes (LH36U, Hansatech). Before the actual measurement the tissues were not pre-illuminated. Six samples from bark were measured.

Student's t-test was used to identify significant differences between means.

Results and Discussion

The prolonged darkening of the stems affected the fine structure of the chloroplasts (Fig. 1), but not their number and size (data not shown). Large starch grains and plastoglobuli were abundant in both darkened and control stems. In darkened chloroplasts elimination of grana and reduction in the number of lamellae were observed, compared to the control stems (Fig. 1). A reduction in the number of mitochondria and peroxysomes was apparent in the darkened samples. Prolonged darkening caused a dramatic decrease in the photosynthetic capacity, dark respiration rates, total chlorophyll, carotenoid concentration, and Fv/Fm, in relation to control branches (Table 1). Chl a/b ratio increased compared to the controls (Table 1). The darkening of the stems did not cause apparent symptoms or damage to the bearing leaves.

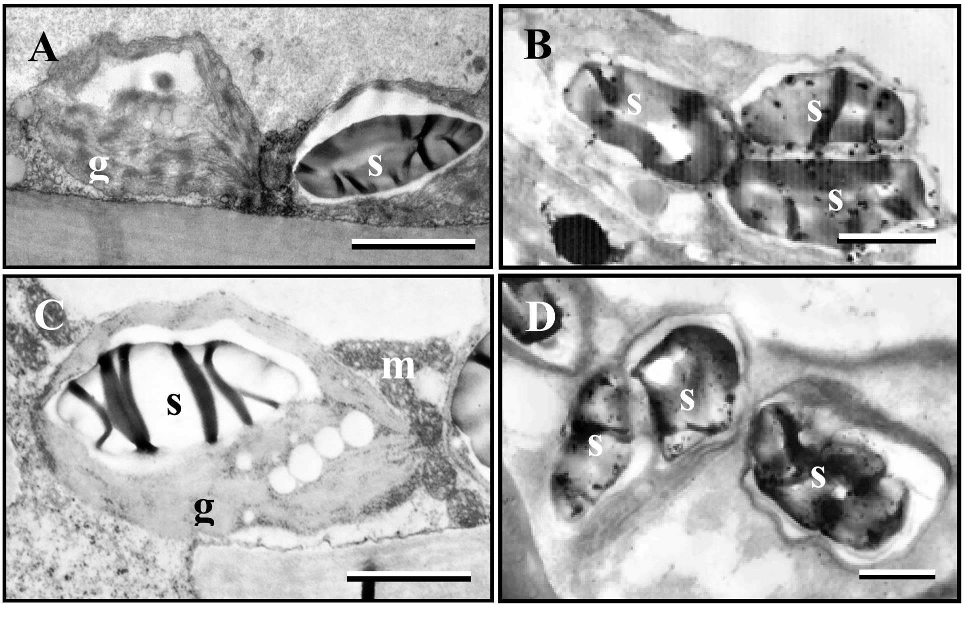


Fig. 1. Microphotographs of darkened (B, D) and fully exposed (A, C) chloroplasts of one (A, B) and twenty (C, D) year old, olive tree bark. Abbreviations: g = grana; m = mitochondria; s = starch. – Scale bars = 1 μm.

In the absence of light, vital activities of the stem were acclimated to the new regime: Respiration rates (Table 1) as well as the number of mitochondria (data not shown) were reduced, probably to keep the internal CO_2 and O_2 concentrations in permissible levels. Prolonged exclusion of natural light (four months) from Pinus monticola branches by the use of aluminium foil produced marked changes in the carbon stable isotope ratios (CERNUSAK & al. 2001). SCHMITZ & al. (2012) used paired, attached branches to study the effects of covering branches with aluminium foil (for a 5-6 days period) on the gas exchange characteristics of leaves and the hydraulic conductivity of branches. They found that covering stems altered the gas exchange characteristics of leaves, such that water-use efficiency was greater in exposed leaves of covered than of uncovered branches. Leaf-specific hydraulic conductivity of stems was lower in covered than in uncovered branches, implicating stem photosynthesis in the maintenance of hydraulic function. TRANG TRAN (2018) found that prolonged darkening of the stems of *Fraxinus latifolia* (for a 4 week period) altered the concentration of photosynthetic pigments. For many plant species, light deprivation in the form of severe shading or darkening of leaves leads to rapid senescence, especially when only a part of the plant is affected. Leaf senescence may be caused either due to a reduction in photosynthesis and subsequent carbon starvation or to the absence of a photosynthesis-independent light signal suppressing senescence (LIEBSCH & KEECH 2016). The degeneration processes are characterized by the degradation of macromolecules and the disassembly of cellular components, including chloroplasts which are accompanied by the mobilization and translocation of valuable resources from the leaves to growing organs or reserve organs.

Table 1. The effect of prolonged darkening on certain physiological parameters of 15 years old stems of O. europaea. Data are means ± se, n=6. Asterisks denote significant differences: ** = P<0,01.

Parameter	Darkened stems		Fully exposed stems
Photosynthetic capacity measured at 1800 μmol $m^{-2}s^{-1}$ PAR (μmol m^{-2} s^{-1})	1.43 ± 0.30	**	5.22 ± 0.39
Dark respiration rate (μmol m^{-2} s^{-1})	1.44 ± 0.19	**	5.85 ± 0.29
Fv/Fm	0.06 ± 0.01	**	0.78 ± 0.01
Chl*a* concentration (μg cm^{-2})	5.48 ± 1.00	**	18.55 ± 0.43
Chl*b* concentration (μg cm^{-2})	1.95 ± 0.27	**	8.87 ± 0.18
Chl*a*/*b* ratio	2.75 ± 0.12	**	2.10 ± 0.05
Total chl concentration (μg cm^{-2})	7.43 ± 1.27	**	27.39 ± 0.55
Total carotenoids concentration (μg cm^{-2})	10.54 ± 0.34	**	25.03 ± 0.78

The absence of senescence symptoms and the maintenance of a minimal operational capacity of the chloroplasts machinery even under prolonged darkening of the stems could be attributed to light signals derived from the bearing leaves. Certain structures, such as vascular tissues (SUN & al. 2003) or sclereids (KARABOURNIOTIS & al. 1994, KARABOURNIOTIS 1998) can transfer light deep into the organs, contributing to photomorphogenic activities in target tissues. However, no evidence for this function within olive tree bark exists while the intensity of the transferred light is unlikely to be able to support corticular photosynthesis rates.

The dramatic reduction in Fv/Fm in darkened stems (Table 1) shows that a high risk for photoinhibition exists in the chlorenchyma layers. However, even under these conditions, positive photosynthetic rates were measured (Table 1), probably because breakdown in the fine structure of the chloroplasts was not observed (Fig. 1B & D). The observed extremely low values of the Fv/Fm values could be attributed to the partial degradation of the photoprotective mechanism of the PS II (due to the reduction in the levels of the carotenoids pool) and/or to the extremely high CO_2 levels created, hypoxia or extremely low pH values. High internal CO_2 levels in stems may impede photosynthesis possibly through acidification of the protoplast and impairment of the pH-dependent high energy state quenching followed by reduction in the capability of heat dissipation (MANETAS 2004, see also LEVIZOU & al. 2004).

In conclusion, prolonged darkening caused significant changes on functional and structural parameters of olive tree bark. In the absence of light, all measured parameters were affected and the photoselective, photosynthetic and respiratory metabolism was severely down regulated. The persistence, however, of a minimum chloroplast organization and activity under such conditions provide some evidence that bark chloroplasts take active part to carbon metabolism irrespective of the light regime.

References

ASCHAN, G. & PFANZ, H. 2003: Non-foliar photosynthesis-a strategy of additional carbon acquisition. – Flora 198: 81-97.

BLOEMEN, J., VERGEYNST, L., OVERLAET-MICHIELS, L. & STEPPE, K. 2016: How important is woody tissue photosynthesis in poplar during drought stress? – Tees-Struct. Funct. 30: 63-72.

CERNUSAK, L., MARSHALL, A. J., COMSTOCK, J. & BALSTER, N. 2001: Carbon isotope discrimination in photosynthetic bark. – Oecologia 128: 24-35.

CERNUSAK, L. A. & CHEESMAN A. W. 2015: The benefits of recycling: how photosynthetic bark can increase drought tolerance. – New Phytol. 208: 995-997.

FILIPPOU, M., FASSEAS, C. & KARABOURNIOTIS, G. 2007 : Photosynthetic characteristics of olive tree (*Olea europaea*) bark. – Tree Physiol. 27: 977-984.

FOOTE, K. C. & SCHEADLE, M. 1978: Physiological characteristics of photosynthesis and respiration in stems of *Populus tremuloides* Michx. – Plant Physiol. 58:91-94.

GRAAN, T. & ORT, D. R. 1984: Quantitation of the rapid electron donors to P700, the functional plastoquinone pool, and the ratio of the photosystems in spinach chloroplasts. – J. Biol. Chem. 259: 14003-14010.

KARABOURNIOTIS, G. 1998: Light-guiding function of foliar sclereids in the evergreen sclerophyll *Phillyrea latifolia*: A quantitative approach. – J. Exp. Bot. 49: 739-746.

—, PAPASTERGIOU, N., KABANOPOULOU, E. & FASSEAS, C. 1994: Foliar sclereids of *Olea europaea* L. may function as optical fibers. – Can. J. Bot. 72: 330-336.

LEVIZOU, E., PETROPOULOU, Y. & MANETAS, Y. 2004 : Carotenoid composition of peridermal twigs does not fully conform to a shade acclimation hypothesis. – Photosynthetica 42: 591-596.

LIEBSCH, D. & KEECH O. 2016: Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. – New Phytol. 212: 563-570.

MANETAS, Y. 2004: Probing corticular photosynthesis through the in vivo chlorophyll fluorescence measurements: Evidence that high internal CO_2 levels suppress electron flow and increase the risk of photoinhibition. – Physiol. Plantarum 120: 509-517.

MÜLLER, W. & GREENWOOD, A. 1978: The ultrastructure of phenolic-storing cells fixed with caffeine. – J. Exp. Bot. 29: 757-764.

NILSEN, E. T. 1995: Stem photosynthesis: Extent, patterns, and role in plant carbon economy. Pp. 223-240. In GARTNER, B.L. (ed.), Plant Stems. – Academic Press, New York.

PFANZ, H. & ASCHAN, G. 2001. The existence of bark and stem photosynthesis in woody plants and its significance for the overall carbon gain. An ecophysiological and ecological approach. – Progr. Bot. 62: 477-510.

PILARSKI, J. 1999: Gradient of photosynthetic pigments in the bark and leaves of lilac (*Syringa vulgaris* L.). – Acta Physiol. Plant. 21: 365-373.

SCHEADLE, M. 1975: Tree photosynthesis. – Annu. Rev. Plant Physiol. 26: 101-115.

SCHMITZ, N., EGERTON, J. J. G., LOVELOCK, C. E. & BALL M. C. 2012: Light dependent maintenance of hydraulic function in mangrove branches: do xylary chloroplasts play a role in embolism repair? – New Phytol. 195: 40-46.

SUN, Q., YODA, K., SUZUKI, M. & SUZUKI, H. 2003: Vascular tissue in the stem and roots of woody plants can conduct light. – J. Exp. Bot. 54: 1627-1635.

TRAN TRANG, T. 2018: The effect of light exposure on the total chlorophyll content, Chl a/b ratio, and Car/chl ratio in the barks of *Fraxinus latifolia* seedlings. – University Honors Theses. Paper 575. Portland State University.

WITTMANN, C. & PFANZ H. 2015: Bark and woody tissue photosynthesis: a means to avoid hypoxia or anoxia in developing stem tissues. – Funct. Plant Biol. 41: 940-953.

— & — 2018: More than just CO_2 recycling: corticular photosynthesis as a mechanism to reduce the risk of an energy crisis induced by low oxygen. – New Phytol. 219: 551-564.

—, ASCHAN, G. & PFANZ, H. 2001: Leaf and twig photosynthesis of young beech (*Fagus sylvatica*) and aspen (*Populus tremula*) trees grown under different light intensities regimes. – Basic Appl. Ecol. 2: 145-154.

BOTANIKA CHRONIKA 22, 2019

CONTENTS

⟶