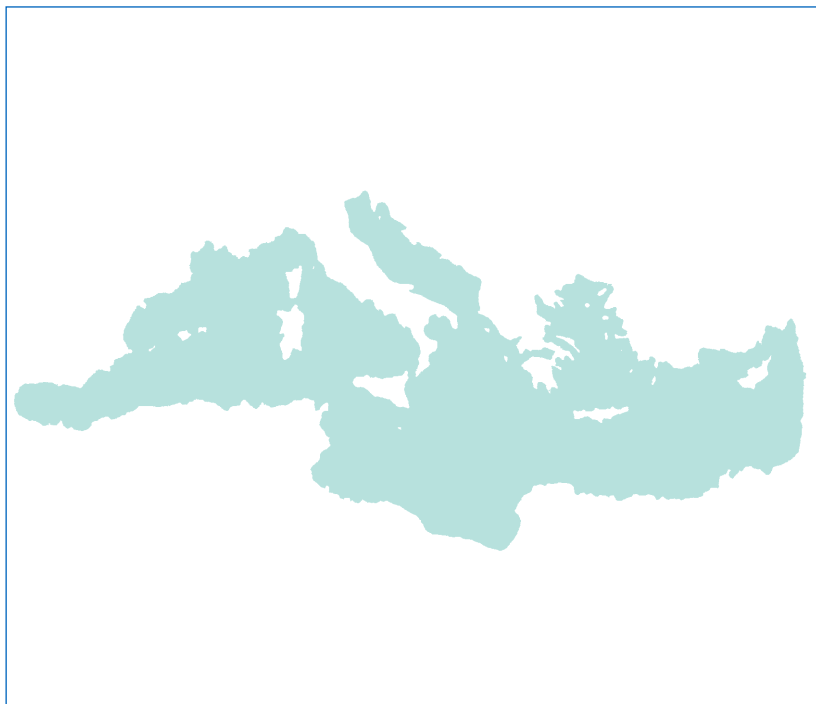


FLORA MEDITERRANEA

32 — 2022



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R. Pardi, G. Venturella, F. Cirlincione, G. Mirabile, E. Di Gristina & M. L. Gargano

Forest ecosystems in the Monti Sicani Park (Sicily)

Abstract

Pardi, R., Venturella, G., Cirlincione, F., Mirabile, G., Di Gristina, E. & Gargano, M. L.: Forest ecosystems in the Monti Sicani Park (Sicily). — Fl. Medit. 32: 5-16. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The authors analysed the forest ecosystems in the Monti Sicani Park (Sicily). In particular, the state of the natural and non-native forests in the Site of Community Interest (SCI ITA040005) - Monte Cammarata, Contrada Salaci, was evaluated in terms of distribution, evolution of forest dynamics and, silvicultural activities. The evolution of vegetation within two different forest plots are analysed and management strategies are proposed. The finding on Mount Cammarata of seedlings of *Fagus sylvatica* in the undergrowth of a conifer afforestation is noteworthy.

Key words: Reforested areas, *Cedrus atlantica*, Protected areas, Silvicultural management.

Introduction

Forests cover about 10% of the total area of the Mediterranean basin (FAO 2018). Mediterranean forest ecosystems express exceptional richness in terms of biodiversity. The most common threats that can make these ecosystems vulnerable and fragile are forest fires, overexploitation, deforestation, degradation and, incorrect silvicultural activities. These threats are accentuated today due to climate change, air pollution and, land use (Palahi & al. 2008).

In Sicily (southern Italy), even though many regional reserves and parks have been established, the interest for the Sicilian forest heritage has been mainly concretized in many reforestations. The incentive measures in the forestry sector introduced around 1970, have contributed to increase significantly the extension of the wooded areas which are, however, mainly constituted by non-native species (Domina & al. 2018b). Most of the reforested areas are in a worrying state of neglect resulting in obvious impacts on plant health, fire risk and, tree stability.

Among the different reforestations, those with *Cedrus atlantica* (Endl.) Carrière have had a better success, since the plants have found, in some environments of Sicily, ecological conditions more similar to those of their countries of origin. La Mela Veca & al. (2012) highlighted the excellent assertiveness of cedar trees and the possibility of

high wood production, equal to those found in the literature for plantations made in France (Courbet & al. 2018).

This study analyzes the state of the natural and non-native forests in the Site of Community Interest (SCI ITA040005) - Monte Cammarata, Contrada Salaci, evaluated in terms of distribution, evolution of forest dynamics and silvicultural activities.

Materials and Methods

Area of study

The Oriented Natural Reserve “Monte Cammarata” has been established with Council Decree n°86/44 (18 April 2000) by the Regional Department of Land and Environment (Sicily) on the base of the Regional Law n°98 (06 May 1981) on the protected areas, and it is registered to the n°1123 of the Official List of the Italian Protected Areas (Ministry of Ecological Transition). Subsequently, the Reserve (Council Decree n°281 19 December 2014), was named “Park of Monti Sicani”.

It is a natural environment characterized by Mediterranean forest of evergreen and deciduous oaks and conifers. The park, which is included between the territories of the municipalities of Cammarata, San Giovanni Gemini and, Santo Stefano di Quisquina, occupies a vast area of ca. 2000 hectares, and is located at the northern end of the province of Agrigento dominated by the peaks of Mount Cammarata (1578 m), Mount Gemini (1392 m) and, Pizzo della Rondine (1254 m).

The climate type is Mesomediterranean-subhumid lower (Rivas Martinez 1995), with the exception of the highest areas of Mount Cammarata that are subjected to Supramediterranean-subhumid lower climate, with abundant rainfall and snowfall in winter (Bazan & al. 2015; Domina & al. 2018).

The vegetation is mainly represented by *Quercus ilex* L. subsp. *ilex*, *Q. pubescens* Willd. s. l., *Pinus halepensis* Mill., *P. nigra* J.F. Arnold subsp. *nigra*, *Hesperocyparis arizonica* (Greene) Bartel, and *Cedrus atlantica* (Endl.) Carrière. Along the valleys and in the most sheltered areas there are small groups of *Ostrya carpinifolia* Scop., *Ulmus canescens* Melville, *Populus nigra* L. and some few *Salix* species. Moreover, it is easy to recognize other sporadic trees such as *Acer campestre* L. and *Fraxinus ornus* L. Shrubs are mainly represented by *Phillyrea latifolia* L., *Pistacia terebinthus* L., *P. lentiscus* L., *Cistus salvifolius* L., *C. creticus* L., *Ruscus aculeatus* L., *Asparagus acutifolius* L., *Smilax aspera* L., and *Hedera helix* L.

Achantis mollis L., *Paeonia mascula* subsp. *russoi* (Biv.) Cullen & Heywood and several fern species, characterize the undergrowth.

Going up along the paved road that climbs up to the top of Mount Cammarata the landscape is characterized by *Euphorbia rigida* M. Bieb. and *Sorbus domestica* L.

The need to establish the park has arisen from the need to protect ca. 150 herbaceous species of which several are rare or endemic to Sicily such as: *Anthemis cupaniana* Tod. ex Nyman, *Senecio squalidus* subsp. *microglossus* (Guss.) Arcang., and *Bivonaea lutea* (Biv.) DC. For an exhaustive list of herbaceous species see the report of the excursion of the Italian Botanical Society in the area (Domina & al. 2015).

Methods

The state of the natural and non-native forests in the Site of Community Interest (SCI ITA040005) - Monte Cammarata, Contrada Salaci, was evaluated in terms of distribution, evolution of forest dynamics, and silvicultural activities. Moreover, a GIS-based forest category map was generated, characterizing the vegetation both through field surveys, and on the basis of the Map of Forest Types, and through the evaluation of the CTR (Regional Technical Map, Fig. 1). In order to identify the habitats included within the SCI, a geographical survey of the area under examination was carried out on a GIS basis. For cartographic elaborations, apart from the CTR (scale 1: 10,000), digital orthophotos were used with interpretation of vegetation coverage through “Bing” on GIS. In particular, the surfaces related to the different land use classes of the analysed territory have been reported. The classes are referred to the Corine Land Cover (2, 3 and, 4 levels) and are accompanied by the corresponding numerical codes. This has made it possible to categorize the different digitized surfaces and to attribute to each code a different colour representing a specific land use. Photo-interpretation, supported by several field surveys using the Map of Forest Types of the Territory made by La Mela Veca & al. (2005), allowed to stratify the categories, so as to distinguish natural from artificial forests.

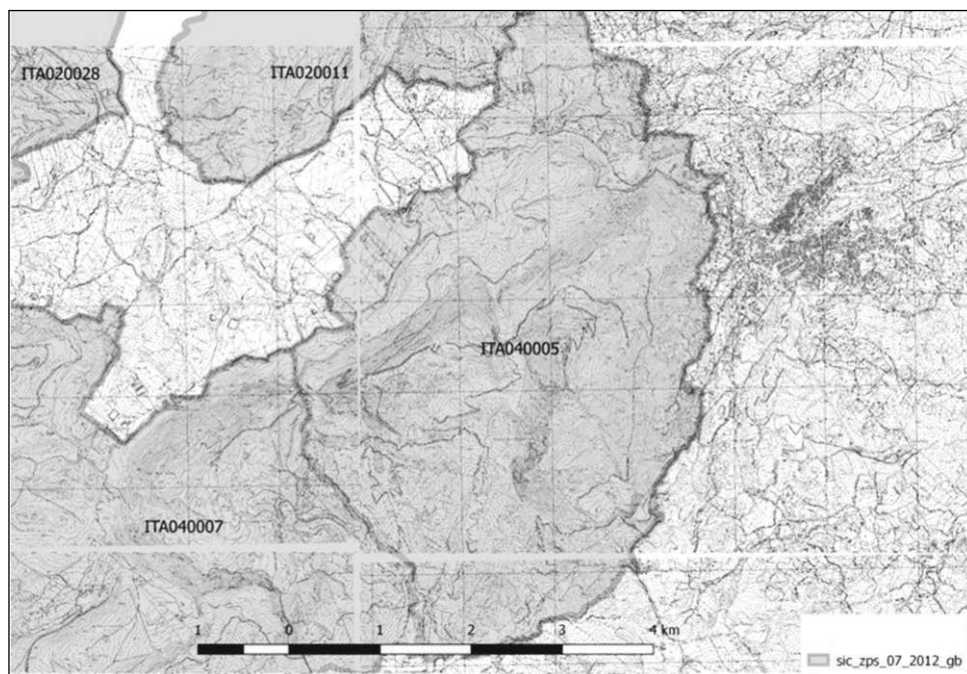


Fig. 1. Regional Technical Map (CTR) and delimitation of the Site of Community Interest (SCI ITA040005) - Monte Cammarata, Contrada Salaci.

Finally, two forest plots were bounded (Fig. 2) with the aim to analyse, over two years of observations, the evolution of trees, shrubs, and herbaceous species. The first plot, in locality Filici, located along the provincial road no. 24 “Cammarata - Santo Stefano di Quisquina”, is characterized by silvicultural practices such as thinning carried out through clear cuts. The other plot is in locality Savochello and no silvicultural practice is carried out. The delimitation of these forest plots was carried out through the use of a measuring wheel, stakes and marking tape, allowing the identification of a variable number of plant species.

Within the forest plots we proceeded to the numbering of the plants and the analysis of their distribution in the different vegetation layers (tree, shrubby and, herbaceous). In addition, we also determined the degree of cover, calculated on the basis of the projection of the crown of the plants on the ground.

Silvicultural practices

The silvicultural practices are mainly carried out on the coniferal and distributed areas into two periods of the year: a) autumnal thinnings; b) spring thinnings.

Thinning carried out by means of flush cuts, was performed mainly to stimulate the natural regeneration of broadleaf trees and reduce the density of dense stands of conifers. Trees, once felled, are left “on the fall bed” flanked by a large pile of dry plant residues. The latter practice is not recommended as the huge amount of piled up plant material

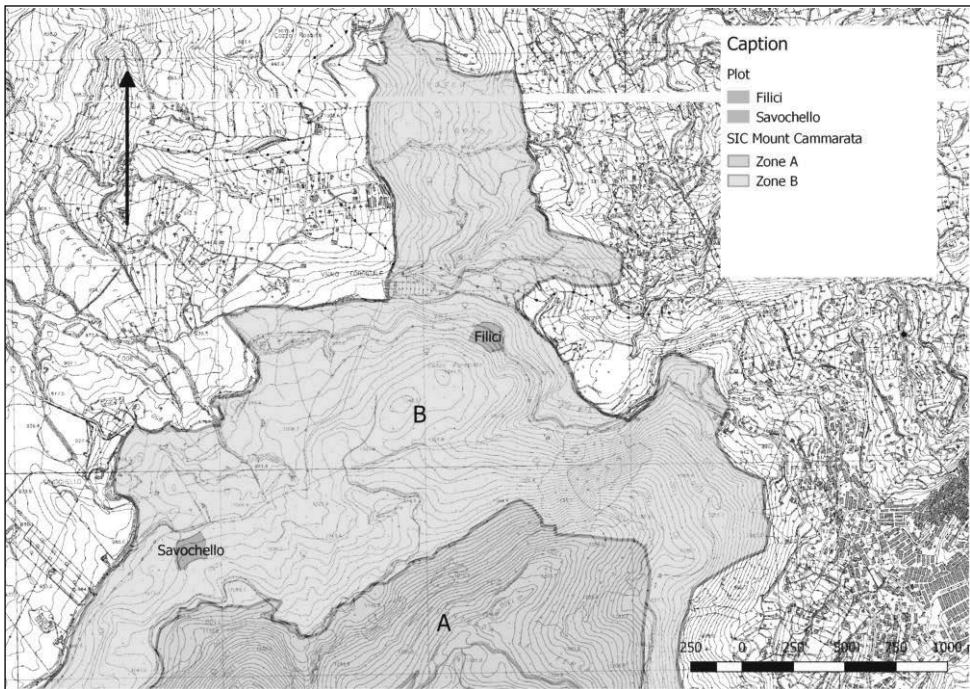


Fig. 2. Zone A and B of the Sicani Park and delimitation of analysed plots.

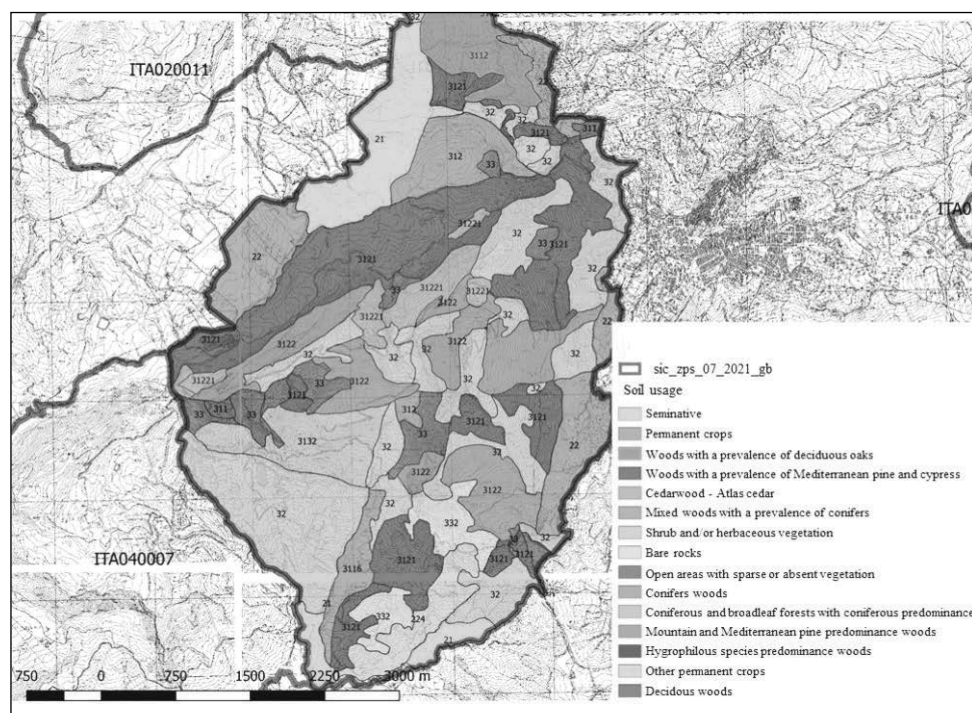


Fig. 3. Distribution map of forest types and land use in the Site of Community Interest (SCI ITA040005) - Monte Cammarata, Contrada Salaci.

increases the risk of fire within the study area. Between May and August, firebreaks are made in order to stop the advance of possible fires. Other practices consist of clearing main trails followed by cleaning firebreaks. In addition, forestry workers are assigned to the arrangement and cleaning of gutters and water-cutting transversals including the cleaning of slopes and removal of debris.

Evolution of vegetation in forest plots

The observation of the plant populations within the two forest plots, both located in “Zone B” of the Monti Sicani Park, has allowed, on the basis of the different silvicultural practices, to identify differences in evolutionary terms, in the entity and distribution of spontaneous processes of renaturalization of tree species but also the observation of shrubby and herbaceous indicator species. In forest plots with an excessive density of conifers, regeneration is scarce, as in the area of Savochello, where no thinning was carried out. On the other hand, the condition is different in areas, such as Filici, where thinning has been carried out.

The area of Filici, characterized by thinning on conifers, shows a strong renewal promoted by less competition and shading by the tall conifers introduced with reforestation (*Pinus halepensis*, *P. pinea*, *Cupressus sempervirens*, *Cedrus atlantica*) from seedlings

available in the nurseries of Sicily Region. The broadleaf trees are represented *Quercus ilex* L. subsp. *ilex*, *Q. pubescens* s. l., *Fraxinus ornus* mixed to *Prunus spinosa*, *Rosa canina*, *Cistus creticus*, *Ruscus aculeatus* and, sporadically, *Crataegus monogyna*.

The peculiarity and the most important characteristic in this area is that the thinning of conifers has led to a colonization not only of broadleaf trees but also to the natural renewal of *Hesperocyparis arizonica*, *Pinus halepensis* and, some seedlings of *Cedrus atlantica*. This is a very important aspect that allows to confirm how silvicultural practices, associated with a sustainable management based on the safeguarding of forestry coenoses, promote the development of a habitat in which there is a natural succession not only of the autochthonous oak species but also the growing, evolution and, development of conifers, which are extraneous to the natural context but which are able to establish themselves spontaneously. The presence of *Ampelodesmos mauritanicus* (Poir.) T. Durand is probably favoured in the settlement by natural reforestation.

In the Savochello plot the density is not very high and an association of different species of conifers, such as cypresses, pines and cedars can be observed. Significant natural regeneration was observed in the area where thinning is being done. The most represented species are *Quercus ilex* subsp. *ilex* and *Q. pubescens* s. l. in variable numbers according to the ecological features of the forest plot and the percentage of coverage. In these areas, besides the species mentioned above, the presence of *Acer campestre*, *Ostrya carpinifolia*, and *Fraxinus ornus* can also be observed together with *Pinus halepensis*, *P. pinea*, *Quercus pubescens* s. l., *Prunus spinosa*, *Ruscus aculeatus*, *Rosa canina*, *Rubus ulmifolius* Schott, and *Paeonia mascula* subsp. *russoi*. A different plant composition is observed in Filici forest plot such as *Pinus canariensis*, *P. halepensis*, *Cupressus sempervirens*, *Hesperocyparis arizonica*, *Quercus pubescens* s. l., *Fraxinus ornus*, *C. creticus*, *Quercus ilex* subsp. *ilex*, *R. aculeatus*, *R. canina*, and *Ampelodesmos mauritanicus*.

Due to the presence of *Quercus ilex* subsp. *ilex* regeneration, in this last area the evolution of the stand towards the evergreen Mediterranean forest is evident. Due to the shading of their canopy, pines promote the growth and development of young oak seedlings. In this area it is advisable to thin the trees in order to encourage the development of oak seedlings when an intermediate age has been reached to promote a possible rotation to the plants that already persist in the plot. The absence of silvicultural practices (with the exception of reforestation with *Pinus* species) has allowed a natural evolution of the area towards a typical Mediterranean forest, not allowing the establishment of invasive species such as *Ampelodesmos mauritanicus*.

***Cedrus atlantica* stands**

In Sicily, within the wide reforestation activity carried out since 1950, the Atlas cedar has been less used than other conifers. It has often been used as isolated plant or in small groups, within reforestations with protective purposes; only in some cases pure plants of modest extension have been realized mainly in areas of the mountain of Sicani, Madonie, Nebrodi, Peloritani and, Mount Etna in association with the other conifers already mentioned and mainly at altitudes higher than 800 m.

In the study area, pure stands of *Cedrus atlantica* occupy an area of ca. 65 ha and the altitudinal distribution ranges from 1000 to 1450 m. The cedars were planted around 1960, in the winter period from November to January, with a planting method based on holes of

40×40×40 cm, and with the realization of steps to facilitate planting in areas with high slopes. The stepping stone, or terracing, consists in the movement of a steep ground to avoid landslides, and this also allows the planting.

Most of the pure stands of cedars fall on Monte Gemini (Fig. 4) and Monte Cammarata. While along the southern slope of Monte Cammarata prevails the mixed wood of *Pinus nigra* subsp. *nigra* and *C. atlantica*. Another pure cedar wood is found near the locality of Gargiuffè, at altitudes around 1100 m. The stands consisting exclusively of mesophilic species, such as *C. atlantica* and *Pinus nigra* subsp. *nigra*, are almost all included in areas of ecological suitability.

Despite being artificial stands, pure cedar trees adapt well to the environmental context in which they were planted. This adaptation is due to the fact that these reforestations were made at altitudes suitable for their growth, since are species with a supramediterranean thermotype. Cedar is generally accompanied by *Pinus nigra* subsp. *nigra*, while native species are found in the dominated layer, with a low density. In these areas the cedar occupies the dominant layer, but it can be found a rich lower layer characterized not only by shrubby species but also by mesophilic species such as *O. carpinifolia*, *Sorbus torminalis* L., and *A. campestre*.

The first sampled area of Atlas cedar is located at the foot of Monte Cammarata, on the northeastern slope, about 200 m after the “Romeo crossroads”, which divides the road leading to Monte Cammarata from the road leading to Monte Gemini. The area observed is at an altitude of ca. 1330 m, where the stand and the evolutionary dynamics based on how the area had been managed was analyzed. Also in this area the stand of Atlas cedar is mixed with *Pinus nigra* subsp. *nigra*.

This stand rests on a calcareous soil rich in rocky outcrops, where silvicultural practices are not performed. Therefore, after the planting of the seedlings around the 60's, the silvicultural treatments (e.g., pruning and thinning) that could allow the evolution of the stand have not been carried out. The planting density should be 5 × 2 m, with a low natural regeneration, but higher than in other areas of the SCI where the stand is characterized by Mediterranean conifers. In fact, in stands with excessive density, especially the artificial stands of *P. halepensis* and *Cupressus arizonica* and *C. sempervirens*, the renewal is low or even absent, while in areas with stands of cedar with *Pinus nigra* subsp. *nigra*, the renewal is more abundant given the lower density.

Renewal in this area concerns mainly species such as *Quercus ilex* subsp. *ilex*, *A. campestre*, *F. ornus*, *O. carpinifolia*, and also shrubby and herbaceous species such as *Daphne laureola* L., *Rosa canina* L., *Hedera helix* L., and *Rubus ulmifolius*. In open areas, where the presence of cedar is rare, we also find *Prunus spinosa* L. and other herbaceous and shrubby species (Domina & al. 2015). The presence of pastures and an almost absent regeneration of *C. atlantica* should also be noted, while regeneration can be seen in more open areas of the SCI.

An unexpected finding in the area was the presence of three seedlings of *Fagus sylvatica* L. (Fig. 5): two plants 1 m tall and another of about 5-6 m, the latter with a diameter of ca. 7-8 cm. All the plants are found adjoining three individuals of *Pinus nigra* subsp. *nigra*. These seedlings, very close to each other, have been planted by the foresters ca. 24 years ago, together with other 10 individuals in the area, as reported by the Managing Authority of the Park. Of these, only the three individuals, found during the surveys, mana-

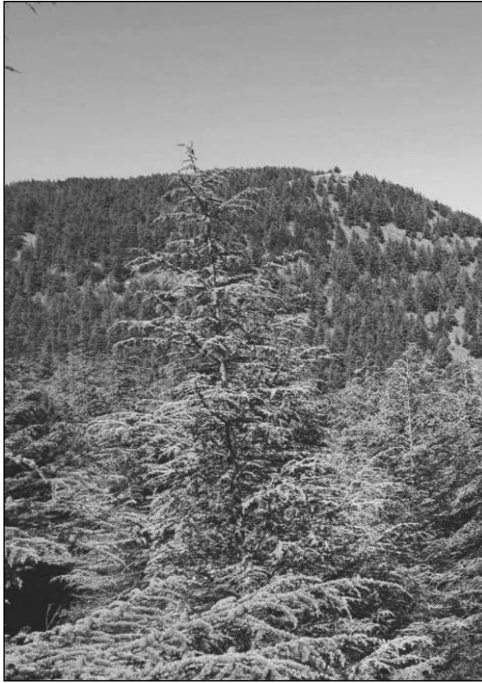


Fig. 4. *Cedrus* wood on Monte Gemini.

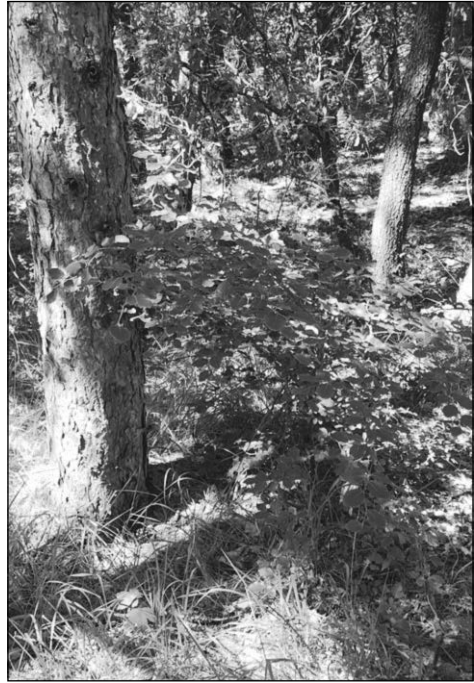


Fig. 5. *Fagus sylvatica* in the undergrowth of *Pinus nigra* subsp. *nigra* reforestation.

ged to take root and develop. This highlights that *F. sylvatica* could develop and grow if supported by careful silvicultural practices in the Monti Sicani Park.

The second sampled area is located on the northern slopes of Monte Gemini at an altitude of ca 1390 m, where the stand of *C. atlantica* and *Pinus nigra* subsp. *nigra* is characterized by trees with a greater height than those of the previous area, with a high rate of growth.

Also in this case the study area is an artificial stand on calcareous soils, where silvicultural practices are absent. The hypothesized planting pattern is 3×4 m. The stand is characterized by a precarious stability with poor natural regeneration, due to the high density of *Cedrus atlantica* and *Pinus nigra* subsp. *nigra* trees and also by the dense presence of needles that cover the soil and do not allow other species to settle. Moreover, this high presence of needles is harmful and dangerous in case of arson, but at the same time it acts as a litter, enriching the soil with necessary organic substances.

The species surveyed in addition to those already mentioned, concern species that have settled by natural regeneration, such as *Quercus ilex* subsp. *ilex*, with mild scattered individuals and with a height not exceeding 1 m; *Q. pubescens* s. l., *Ostrya carpinifolia*, *Daphne laureola*, *Rubus ulmifolius*, *Rosa canina*, *Hedera helix*, *Acer campestre*, *A. pseudoplatanus* L., and *Fraxinus ornus*.

In both areas, the absence of cedar regeneration is due to the fact that this species needs some open spaces to grow up. In fact, the regeneration of the species is noted only close to

clearings and open spaces in order to allow light penetration, whereas in areas where the density is higher, sun rays cannot penetrate and therefore this leads to a lower regeneration.

It has been noticed that in Sicily *C. atlantica* has a strong capacity of growth, confirming that this species is one of the most important species to be used in the reforestation in sub-mountainous and mountainous environment, in order to combine its productive possibilities with the ability to colonize uncultivated lands for environmental recovery practices. This species tends to renew easily under cover, because the seeds germinate normally and in abundance, but the young seedlings are not able to establish themselves because of the high density which determines conditions of scarce light.

These areas, where the planting of the species under examination has been carried out, show a remarkable evolutionary potential, especially in the sampled area near Monte Cammarata characterized by a low density of the plants. The evolutionary potential is represented by oak woodland or mixed coniferous and deciduous woodland, although the abundant foliage cover does not allow this, also hindering natural regeneration.

The best solution would be to support and stimulate the renaturalization of artificial stands, since reforestation has not always been followed by the application of adequate cultural care.

The seedlings of native species are not able to grow due to the excessive density of the tree layer as in the case of coppices at low altitudes where regeneration is almost absent. On the contrary, at higher altitudes, conditions are more favorable with the spread and development of mesophilic species.

Results

In the not thinned area, the degree of coverage of tall trees covers about 90% of the soil, while the remaining 10% is caused by the projection of the canopy of *Quercus ilex* subsp. *ilex* and *Q. pubescens* s. l. that settle in the few spaces left free from conifers. On the contrary, in the thinned area in Filici there is a degree of coverage due to the projection of the canopies of the shrubby and herbaceous layer.

This is a consequence of silvicultural practices that foresters carry out to reduce the dense presence of tall conifers and promote the establishment and development of broadleaf trees. In fact, the forest plot (200 m²) is characterized by a degree of coverage equal to 70% (projection of the canopies of the shrubs established in the spaces left free by the cuts), 20% by the young seedlings belonging to the herbaceous layer and, about 10% by the crowns of the tree layer, represented by *Hesperocyparis arizonica*, *Cupressus sempervirens* and *Pinus canariensis*.

In both areas the growth of *Quercus ilex* subsp. *ilex* was rather stunted. But while in the not thinned area this aspect is understandable because the plants are covered by tall conifers that do not allow the development of a microclimate suitable for their growth, the situation is different in the thinned area where the wide spaces left free by the silvicultural practices can allow a constant and continuous development of the plants.

Management proposals

The modern management of a forest area is based on the concept of sustainability, in order to avoid possible stresses or modifications and changes, especially due to anthropogenic causes. These could negatively affect the functionality, development, evolution, and establishment of a forest ecosystem (Ciancio & al. 2002).

As can be seen from the observations carried out in the two selected areas, the area in Filici, thanks to the silvicultural practices carried out through funding from some European projects for the improvement and enhancement of the forest stand, benefits and supports a management aimed at ensuring the preservation of forest coenosis and the evolution towards more stable structural forms.

Selective thinnings allow a renewal consistent with sustainable management, favoring the re-naturalization of the reforestations and also the opening of spaces within the area that inhibit coverage by tall conifers, and at the same time allow broadleaf trees their own establishment. The felling of the conifer allows the establishment and gradual replacement of native species without any impact on the landscape.

Alternatively, planting can be considered through localized practices and using species that belong to the native vegetation. Moreover, the cuts have determined the natural renewal of some pines, cypresses and, cedars that allow, together with the establishment of broadleaf trees, the formation of more stable structures and the evolution of the mixed forest.

For this reason, the areas of the Park where there is a high density of conifers, as in the case of the Savochello locality, should be managed by promoting selective cuts that lead to a renewal that reflects the objectives of modern sustainable management and an improvement in biodiversity levels. Felled plants are left on the fall bed and should be transported and placed in appropriate areas, to limit or prevent damage caused by any arson.

The objective of management must therefore be to increase the complexity of the ecosystems, through the conservation and monitoring of the habitats identified and the renaturalization of reforestation.

Artificial forests of conifers characterize much of the territorial mosaic of the site, with stands consisting of young forests with excessive density and degree of coverage between 80 and 90%. The excessive density of these stands does not allow the establishment of spontaneous renaturalization processes that are potentially possible.

Conclusions

This study confirms the need for policies aimed at the management of Sicily's considerable natural heritage. In the few cases in which the allocation of funds from different sources has been successful, there have been targeted practices of silvicultural and management type that have triggered virtuous processes with obvious beneficial effects on forest ecosystems, with reference to the beginning of processes of natural regeneration by native species.

The most evident problem is represented by the lack of application of the laws in force regarding the safeguarding of nature as well as the lack of controls by the Authorities in charge. All this determines a series of abuses that, in the absence of sanctions, are repeated over time with serious damage in terms of loss of plant diversity.

On the contrary, the evident enhancement of the paths in the Park allows a better use by tourists and local users and represents a fundamental basis for the enhancement of the natural heritage of the entire area.

In conclusion, compared to other Sicilian natural areas, the Monti Sicani Park, benefits from some naturalistic and silvicultural activities which, if further implemented, will guarantee an improvement in the health of the woodland over time.

The relationship between density and degree of establishment is very varied, also in relation to the characteristics of localities: almost completely absent in stands with excessive density (cypress trees), more frequent in those with a mixed composition and with a more sparse density (pine forests, cedar forests), but in both cases the degree of coverage of conifers is such as to hinder both the processes of establishment and, consequently, the establishment of the regeneration. In stands of ecological suitability, such as cedar stands, it would be necessary to act through a gradual reduction of density in order to support the spontaneous processes of renaturalization in place or to promote them, if they are not yet present, even resorting to under-planting with native shrub and/or tree species.

In some wooded areas, with low density, at altitudes higher than 1200 m, it would be necessary not to carry out any kind of practices because, in these contexts, there are points that host several Sicilian endemics in priority habitats located in ²Zone A² of the Park.

In addition, the lack of practices revealed in the two sampled areas, as well as in the stands of mixed forests of *Pinus nigra* subsp. *nigra* and *Cedrus atlantica*, could be justified by the fact that at these altitudes there are major snowfalls every year. Thanks to the abundant canopy cover and the high density of the plants, the native species and their syntaxa are safeguarded from these snow events that could lead to their death. We must also consider that the stands located on steep slopes and on the ridges are difficult to manage because of the difficult accessibility and also because of the important role they play in hydrogeological defense.

In places where cutting is recommended, these practices should be performed carefully aiming to: i) support processes of renaturalization; ii) increase the stability; iii) maintain the ecological-structural efficiency of the ecosystem. Examples of practices are selective thinnings and pruning aimed at the release of pre-settled native vegetation under cover of allochthonous.

In the SCI, the species that most hinder the development of renaturalization processes of autochthonous species are the *Cupressus*, due to the high density and the allelopathic litter. The most favorable conditions for the diffusion and development of renaturalization processes are found in pure *Pinus halepensis* woods and mixed with *Cupressus sempervirens* and *Hesperocyparis arizonica* and in mixed forests with *Cedrus atlantica* and *P. nigra* subsp. *nigra*.

Artificial forest renaturalization processes play a key role in recomposing the natural forest landscape, which is currently highly fragmented. Finally, there would also be the possibility of obtaining wood production, albeit transitory, from these reforestations. In this case, biomasses could be obtained with which to feed small plants for the production of energy for the needs of the local population.

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Invasive or threatened? The decline of the old-world *Xanthium strumarium* (Asteraceae) in Italy based on herbarium records

Abstract

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Xanthium is a taxonomically intricate genus of the sunflower family (Asteraceae). In Italy, two species of *Xanthium* sect. *Xanthium* are present: the native *X. strumarium* and then introduced *X. orientale*. In this study, we assess changes in relative frequency of these two species in Italy based on herbarium records.

Based on our analyses, we could observe a strong decline of the native *X. strumarium* in Italy, started approximately one hundred years ago. Since then, this species has become extremely rare and more effort should be spent to investigate its actual distribution range and potential extinction risk in Italy. This study also demonstrates the importance of herbaria to address questions regarding the dynamics in relative frequency and distribution of competing native and introduced species.

Key words: herbaria; neophyte; alien flora, *Xanthium orientale* subsp. *italicum*.

Introduction

Xanthium L. is a taxonomically intricate genus of tribe *Heliantheae* in the sunflower family (Asteraceae). It is a monophyletic genus placed in the core-group of subtribe *Ambrosiinae* Benth. & Hook.f., together with genera like *Ambrosia* L. and *Iva* L. (Tomasello & Heubl 2017; Tomasello & al. 2019). Like the latter genera, it is peculiar among Asteraceae for being wind-pollinated and for bearing unisexual florets placed in separated flower heads. Female heads are very peculiar structures, enclosing completely two female flowers, and are covered by hooked spines and beaks. These structures (burs) can effectively stick to animal fur and human clothing and be dispersed over hundreds of kilometres.

Burs are also very important from a taxonomic point of view, since most of its characters are diagnostic to delimit taxa in the genus. Due to their variability, taxonomic treatments in the genus have varied consistently in the past, ranging from recognising twenty

or more species (Millspaugh & Scherff 1919; Widder 1923), to lumping all taxa into two, very broadly defined species (Löve & Dansereau 1959). The taxonomic situation is particularly complicated in *Xanthium* sect. *Xanthium* (the section including species with unarmed stems). However, studies based on molecular markers have demonstrated that three polymorphic species can be unequivocally recognised in this section, i.e., *X. strumarium* L., *X. orientale* L. and *X. chinense* Mill. (Tomasello 2018). The first species is the only native to the old world. *Xanthium strumarium* is relatively easy to distinguish from the other two species, because of the small burs (up to approximately 1 cm) covered by few spines and with straight apical beaks. Its native presence in Europe is certain, as it can be inferred from the literature [it is one of the about 500 species mentioned by Dioscorides in his *De Materia Medica* (first century BC)], and from fossil records (Löve 1975). Despite the name, *X. brasiliicum* Vell. [= *X. strumarium* subsp. *brasiliicum* (Vell.) O. Bolòs & Vigo] is a morphotype of this species native to part of the Mediterranean Basin and the Middle East. In Italy, it has been collected in the past in Sicily, Sardinia, and sporadically in a few other southern Italian regions (Bartolucci & al. 2018).

Xanthium orientale has instead an American origin and in recent times has become cosmopolitan thanks to human trade and human-mediated transport (Tomasello 2018). Many different taxa have been described within the complex in Europe, including *X. italicum* Moretti, *X. riparium* Lasch, *X. saccharatum* Wallr., *X. albinum* (Widd.) Scholz & Sukopp, nowadays considered sometimes as subspecies of *X. orientale* (Greuter 2003). *Xanthium chinense* is the tropical/sub-tropical vicariant of *X. orientale* and originates from the Caribbean and south eastern North America, nowadays being found in many tropical and sub-tropical areas of the world.

In Italy the most common morphotype of the *X. orientale* complex is “*X. italicum*”, which since its first observation by Giuseppe Moretti in 1820 in the surroundings of Padua (Moretti 1822) spread all over the Mediterranean Basin. However, these plants have been treated differently in the literature of the last decades, as they are sometimes considered as independent species (e.g., Pignatti 1982; Galasso & al. 2018), treated as members of the *X. orientale* complex (Conti & al. 2005; Pignatti & al. 2017-2019) or even included in a very broad *X. strumarium* complex (e.g., Motti & Ricciardi 2005; Giardina & al. 2007). This inconsistency makes any evaluation of the distribution and conservation status of the above mentioned *Xanthium* species difficult to assess.

With the present work, we wanted to assess the changes in the relative frequencies of *X. strumarium* and *X. orientale* in Italy throughout the last 200 years, based on data retrieved from herbarium collections. For this purpose, we visited some of the most important herbaria in Italy and Central Europe, and examined all specimens collected in Italy for which information on date and place of collection was unequivocal.

Materials and Methods

Data collection. – Eight European herbaria (Table 1) were screened for specimens of *Xanthium* sect. *Xanthium* collected in Italy. A few specimens collected by the authors (ST) were also included in the study. Two additional herbaria were screened only partially: for the herbarium of the University of Pisa (PI), we considered only the vouchers uploaded in

Table 1. List of herbaria visited for this study, along with information on herbarium code (Holmgren & al. 1990) and location, and number of samples included.

Full name of the herbarium	Location and Herbarium code	No. of samples included
Herbarium Berolinense	Berlin, B	22
Herbari de l'Institut Botànic de Barcelona	Barcelona, BC	6
Università degli Studi di Bari	Bari BI	11
Università di Bologna	Bologna, BOLO	33
Università di Catania	Catania, CAT	24
Herbarium Universitatis Florentinae	Florence, FI	180
Herbarium Göttingen	Göttingen, GOET	4
Herbarium Mediterraneum Panormitanum	Palermo, PAL	7
Università di Pisa	Pisa PI	6
Herbarium of the National Museum in Prague	Prague, PR	3
Private herbarium of S.Tomasello	-	7

the JACQ database (<https://jacq.org/>); only specimens from Apulia and Abruzzo were included from those deposited at the herbarium of the University of Bari (BI). All specimens were re-identified to species level using morphological traits of the burs and following the treatment published by Tomasello (2018). Immature specimens without burs could not be unequivocally identified and were therefore excluded from the analyses. A complete list of herbarium specimens used in the study, along with complete voucher information is given as Electronic Supplementary File 1 (ESF 1).

Analyses. – Information on the collection place and date was extracted from the labels of the vouchers. Samples for which collection place (at least until the level of Italian administrative regions) and/or year of collection were not reported were excluded from the analyses. Duplicate specimens found in some of the different herbaria visited, were included only once in the analyses. To visualize the relative frequency of *X. strumarium* and *X. orientale* throughout the last two centuries we produced histograms and mosaic graphs (Hartigan & Kleiner 1984) in R (R Core Team 2018) using the packages ggplot2 (Wickham 2017) and ggmosaic (Jeppson & al. 2017). Samples were divided in 10-years age classes. Distribution maps of range expansion of *X. orientale* in Italy were produced using inkscape (Inkscape Project 2021) and the map available at: https://commons.wikimedia.org/wiki/File:Italy_map_with_provinces.svg.

Results

The oldest herbarium voucher among those examined in the present study was a specimen of *X. strumarium* found in the herbarium of the University Bologna (BOLO) and collected by A. Bertoloni in Sarzana (Liguria) in 1807. The oldest evidence of the occurrence of the new world species *X. orientale* in Italy is a specimen dated to 1820 and collected by the botanist Giuseppe Moretti. On the specimen, he annotated that the plant was “common in the surroundings of Pavia, and along the River Po”. From this, it can be assumed that the species must have arrived in Italy some years earlier, and spread until the beginning of the 20th century over most of the Italian territory (Fig. 1).

In total, we included information from 287 *Xanthium* herbarium vouchers in the analyses. The number of *Xanthium* specimens peaked in the second half of the 19th century (Fig. 2a), after which there is a change in relative frequencies of the two species (Fig. 2b). The number of specimens of *X. strumarium* drastically declines starting from 1880, and this species became rare since the second half of the 20th century. The last specimen in our dataset belonging to this species was collected in 1973 at “Gorgi Tondi” (Sicily) and it is housed in the herbarium of the University of Catania (CAT020884).

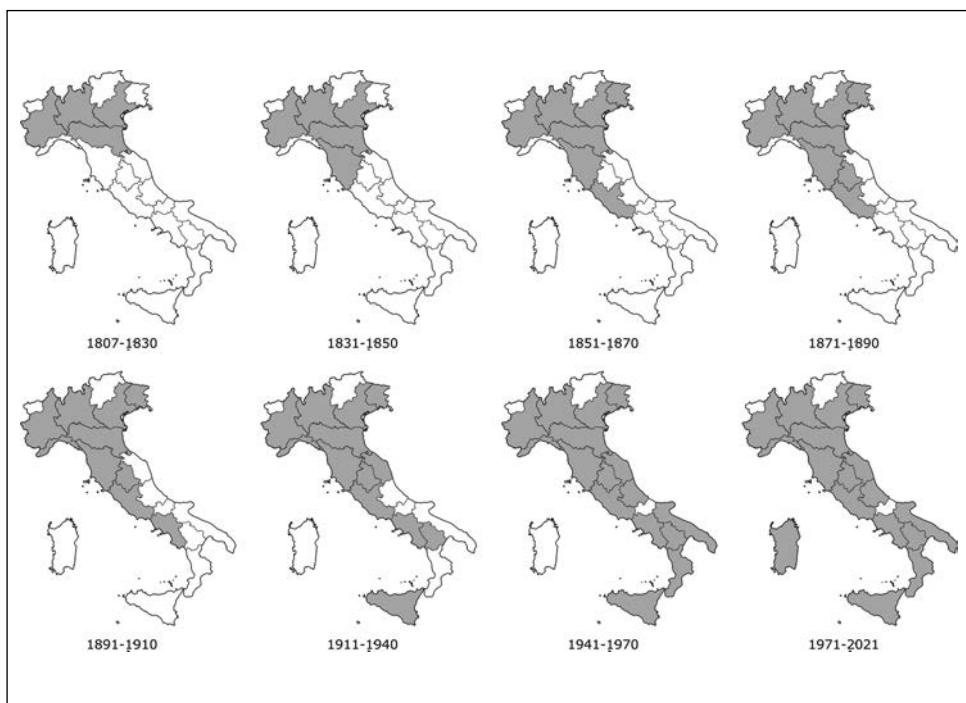


Fig. 1. Maps illustrating the expansion of *Xanthium orientale* in Italy over the last two centuries, based on the specimens housed in the herbaria visited for the present study. Administrative regions where the presence of *X. orientale* was already observed in a specific time interval are coloured in grey.

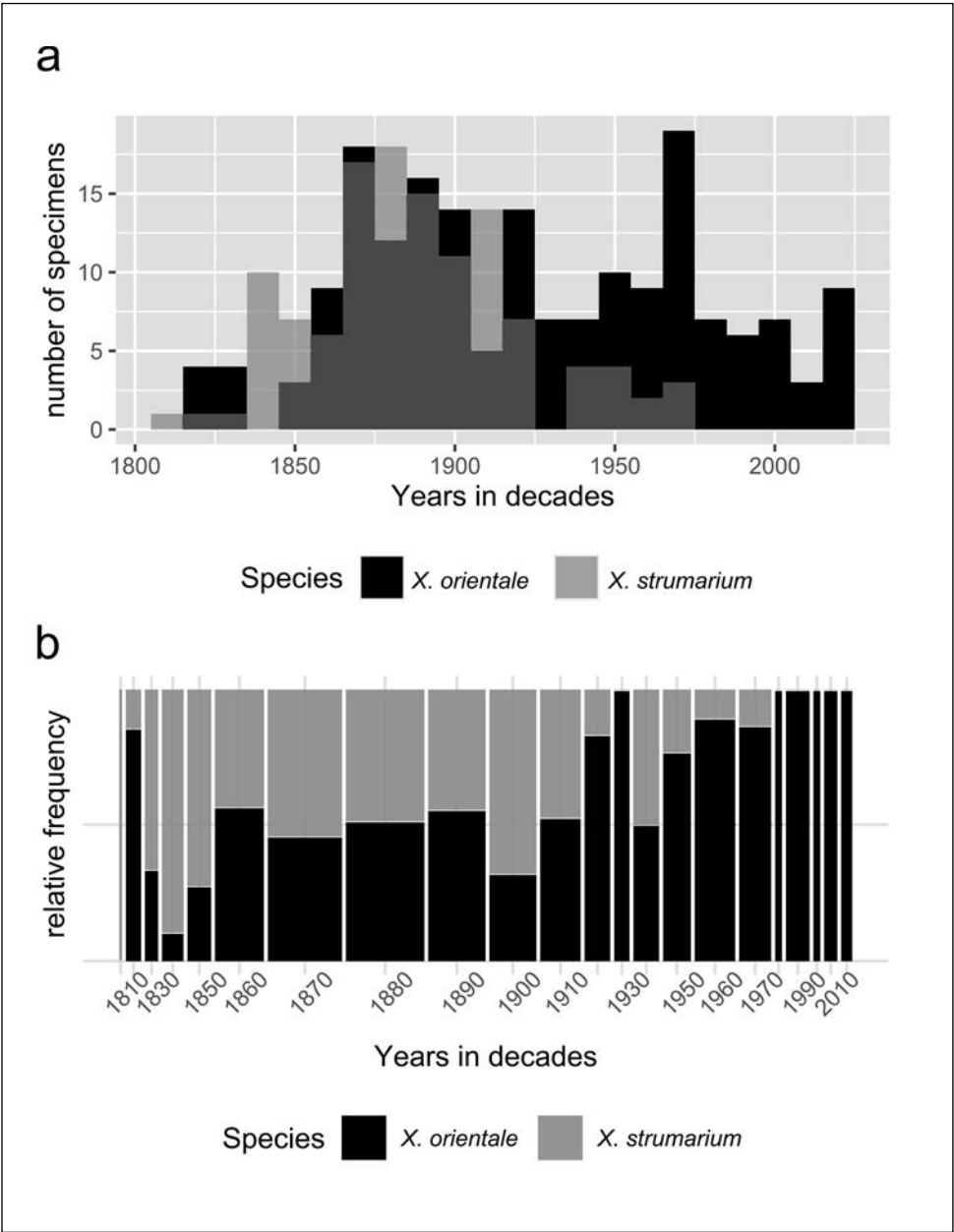


Fig. 2. Frequency of the *Xanthium orientale* (black) and *X. strumarium* (grey) specimens over the last two century. a) Histogram depicting the number of specimens found in the herbaria visited for the present study over time. Samples are subdivided in age classes of 10 years. b) Mosaic graph illustrating the relative frequency of the two species over time. Samples are subdivided in age intervals of 10 years. Bar width is proportional to the number of specimens registered in the specific age interval.

Discussion

Robust taxonomic treatments and correct identification of plant specimens are tremendously important to allow satisfactory assessments of biodiversity and species conservation status. However, there is often confusion about the identity of native and introduced *Xanthium* populations in Europe. These are sometimes correctly treated as independent species (i.e., *X. strumarium* and *X. orientale*, respectively), but often are merged into a single polymorphic species (*X. strumarium* in a very broad sense), making statements on relative frequency and distribution of these two species almost impossible. In Pignatti & al. (2017-2019), even though distinction is done between *X. strumarium* and *X. orientale*, the former is erroneously included in the *X. orientale* complex, which makes the situation even more confusing.

From the specimens examined and the analyses carried out in this study, we could observe a strong decline of *X. strumarium* in Italy, which started approximately one hundred years ago. Using the taxonomic circumscription of the most recent and accurate treatments (Greuter 2003; Tomasello 2018), *X. strumarium* in Italy has become extremely rare and its conservation status should be reconsidered. This species was certainly abundant in the whole peninsula until the 19th and beginning of the 20th centuries, and started declining after the appearance of the introduced *X. orientale* in natural environments.

We were not able to find any *X. orientale* specimen from Italy predating the first collection of “*X. italicum*” by Moretti in 1820. The author first identified the specimen as *X. echinatum* Murr., and two years later, and after correspondence with some Italian and European botanists (Löve & Dansereau 1959), he decided to describe the new species *X. italicum* (Moretti 1822). However, specimens from historical herbaria (e.g., the herbaria of Giuseppe Monti and Ferdinando Bassi in BOLO), were not included in the study. In those herbaria, voucher information is usually scarce and plants might have been cultivated and collected in botanical gardens. A specimen of *X. orientale*, referable to subspecies *orientale* (sensu Greuter 2003) was present in “Erbario Monti” (possibly collected around 1750) and another in the “Erbario Bassi” (second half of the 18th century). The vast majority of specimens belonging to the *X. orientale* complex and examined in this study were referable to *X. italicum* ($\equiv$ *X. orientale* subsp. *italicum* (Moretti) Greuter). Only four samples could be identified as *X. orientale* s.str. [$\equiv$ *X. orientale* subsp. *orientale* in Greuter (2003)], three of which from Veneto and one collected in the surrounding of Bologna (Emilia Romagna). This is in discordance with the statement of Pignatti & al. (2017-2019) that the latter is the most abundant *X. orientale* subspecies in Italy.

The most recent *X. strumarium* among the specimens examined was a voucher collected by Brullo around the “Gorgi Tondi”, in Sicily in 1973. When looking critically at other sources of data, a few more recent, sporadic discoveries might be added (e.g., plants photographed at Castiglione di Cervia (Ravenna), Frigole (Lecce) and Casale di Scodosia (Padova); https://www.actaplantarum.org/galleria_flora/galleria1.php?view=1&id=5329). However, in other cases, other *Xanthium* taxa or deviating forms of *X. orientale* subsp. *italicum* with smaller burs covered by fewer spines [e.g., variety “nigri” sensu Widder (1923)] are often misidentified as *X. strumarium*. In the most recent checklists, *X. strumarium* is reported to occur in most of the Italian territory (Conti & al. 2005; Bartolucci & al. 2018; <http://dryades.units.it/floritaly/index.php?procedure=>

taxon_page&tipo=all&id=8723). In Giardina & al. (2007) the species is reported as common in Sicily, although the same authors state that they have found it only once close to the "Bronte-Maniaci road". We believe that *X. strumarium* has become extremely rare in Italy in the last 50 years, and more effort should be devoted to investigate the actual distribution range and extinction risk of this species in Italy.

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F. M. Raimondo, G. Venturella & G. Domina

***Pyrus pedrottiana* (Rosaceae), a new species from the Nebrodi Mountains (N-E Sicily)**

Abstract

Raimondo, F. M., Venturella, G. & Domina, G.: *Pyrus pedrottiana* (Rosaceae), a new species from the Nebrodi Mountains (N-E Sicily). — Fl. Medit. 32: 25-34. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

A new species of *Pyrus* for the dendroflora of Sicily is here described. It is *Pyrus pedrottiana*, actually considered endemic to the Nebrodi Mountains, a territory which includes the largest wooded area of the island with a high forest biodiversity. In the same area, *P. vallis-demonis* and *P. ciancioi* have also been described in recent times. The authors report diagnostic characters to distinguish the new taxon from the other two sympatric species.

Key words: vascular flora, *Pomoideae*, taxonomy, endemism, Italy, Mediterranean Region.

Introduction

In Italy, *Pyrus* L. is represented by 10 species (Bartolucci & al. 2018; Arrigoni 2018; Pignatti 2019). The number of species, if compared with the first edition of Flora d'Italia (Pignatti 1982), has significantly increased in recent decades, thanks to the descriptions of five new taxa, four of which from Sicily (Marino & al. 2013). In Sicily, recent scientific papers have led to the description of *Pyrus castribonensis* Raimondo, Schicchi & Mazzola, *P. sicanorum* Raimondo Schicchi & P. Marino, *P. vallis-demonis* Raimondo & Schicchi, and *P. ciancioi* P. Marino, G. Castellano, Raimondo & Spadaro, the last two described from the Nebrodi Mountains. The exploration of the Nebrodi territory— not well explored in the past and particularly rich in taxa that are difficult to classify,— allowed the identification of new species, both woody and herbaceous. Periodic observations carried out in a large population of *Pyrus*, allowed to separate and describe a new species, probably endemic to the Nebrodi. Compared to the other *Pyrus* species already described, it has an even more significant forest value, both for its dendrological characteristics and for its widespread presence in the *Quercus cerris* woods on the northern side of the vast mountain system of eastern Sicily. In this area, the new taxon— described with the name of *Pyrus pedrottiana*— comes into contact only marginally with *P. vallis-demonis*, from which, however, it differs considerably, while it remains sufficiently isolated with respect to *P. ciancioi*, a species which is also morphologically well characterized.

Materials and method

After noticing the presence in the wild of a population not corresponding to the taxa already known in Sicily, phenological observations in the field were carried out for a three-year period on 20 very similar plants. These plants were randomly selected within an area of ca. 10 hectares in which the population studied assumes greater quantitative impact as well as a constant unmistakable connotation. The observations, carried out for comparison, also concerned other plants of the same genus present in a discontinuous and fragmentary way in the same area, north-east of Lake Maulazzo, straddling the municipalities of Militello Rosmarino and Alcara Li Fusi, at ca. 1400 m. The study was completed through comparisons with herbarium materials, with particular reference to the species recurrent in the whole Sicily [*P. pyrainus* Raf., *P. pyraster* (L.) Duroi, *P. spinosa* Forssk.] and in the Nebrodi Mountains (*P. vallis-demonis* and *P. ciancioi*) and in the nearby Madonie Mts (*P. castribonensis*). Our study was completed by chorological observations throughout the Nebrodi territory in order to circumscribe the area occupied by the critical taxon.

Results

Taxonomic analysis

The analysis of the diagnostic characters of the population studied, compared with the specific characters of the taxa mentioned above, therefore allows us to recognize the following new species:

***Pyrus pedrottiana* Raimondo, Venturella & Domina sp. nov.** (Fig. 1)

Diagnosis – Arbor modicus, assurgens, spinosus, coma sub-pyramidalis, folia glabra, lamina apiculata, plerumque ovata-extenuata, basi cuneata vel plana, viridis splendens, cum marginibus minute serratis. Inflorescentia cyma corymbosa, sepala exsertae triangulari-lesiniformia, margines ialini et vix denticulati, cum apice mucronato, reflexo, ferrugineo; petala candida, obovata-spathulata; stamina 20–25, basi connata; styli 3–5 breviori staminum et ciliati in basim. Pomus parvus plerumque globosus et cum calyx plerumque deciduus.

Type – Sicily: Monti Nebrodi, Locality Bosco Saracina, 37°56'60.0"N, 14°40'18.2 E, in the clearings of the woods with *Quercus cerris* on siliceous soil, 1425 m a.s.l., 3.09.2019, F. M. Raimondo s. n. (Holotypus PAL-Gr; Isotypi in PAL and FI).

Description – Tree up to 10–12 m tall, with an almost pyramidal crown, gray-brownish bark, cracked in longitudinal plates; lateral branches from horizontal to patent, the young with many linear lenticels, the herbaceous puberules; ovoid to conical, elongated buds with 5–7 leathery, hairless scales. Leaves glabrous, blade apiculate, from strictly ovate to orbiculate or elliptical, with base from cuneate to straight; petiole (12) 18–30 (37) mm, stipules 3.5–4.5 mm. Flowers (2) 5–9 (11) in corymbiform buds; peduncles (11) 16–20 (22) mm; 4 mm high receptacle; triangular-lesiniform sepals 5 mm long, exert, with a slightly toothed hyaline margin and mucronate, ferruginous apex, curved downwards; petals obovate-spatulate, concave, 14–15 × 7.5–8 mm; narrow nail of

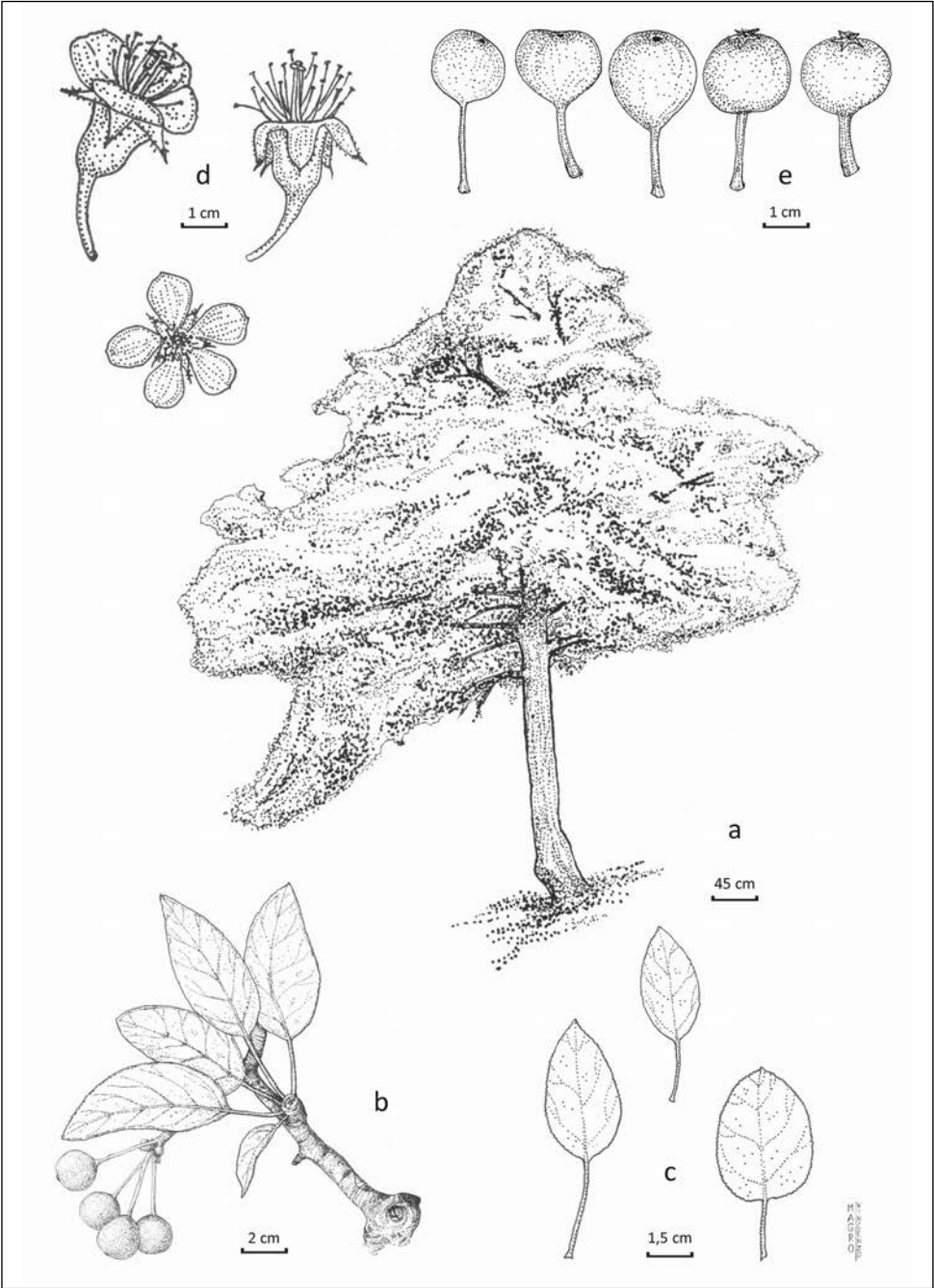


Fig. 1. Iconographic plate of *Pyrus pedrottiana*: a) tree habit; brachiblast with ripe leaves and fruits; c) more recurrent leaf forms; d) floral structures; e) various shapes of pommel recurring in this species: the least frequent in the center and on the right. (Drawings by Vincenzo Magro).

approx. 1.5 mm; stamens in number of 20-25, connected to the base; filaments more or less equal 4–7 mm; anthers 0.5–0.7 mm, purple; 3–5 styles, 8–10 mm long, bottom chiloculate. Subglobose pommel, exceptionally pyriform, 18–20 × 19–23 mm, with rough exocarp, sometimes tobacco-colored and generally with a caducous calyx when ripe. Peduncle of the pommel 12–30 mm long. Seeds 4–6 mm more or less ovate, compressed, dark brown, shiny.

Etymology – The epithet of the name of the new species recalls the surname of the illustrious active naturalist and plant ecologist Franco Pedrotti, professor emeritus of Botany at the University of Camerino (Italy), in the occasion of his 88th birthday.

Phenology – The species flowers in May along with leaf emission (Fig. 2) fruits ripen in October. The ripening of the fruits and the fall of the leaves occurs 2-3 weeks earlier than the other species of *Pyrus* occurring in the same area.

Biological form – Scapose phanerophyte (P scap) (Fig. 3).

Distribution and ecology – Mesophilous species, well represented in the area of the Nebrodi Mountains where it occupies the humid, north slopes. The distribution area covers the submontane belt characterized by extensive forests of *Quercus cerris* L. and occupies the potential space of the associations of the order *Quercetalia-pubescentipetraea*, between 1300 and 1500 m; in particular it occurs in the area around the *locus classicus* (Fig. 4). In this context, it widely colonizes clearings, exploiting the radical polloniferous capacities. It is no coincidence that some groups of individuals of this species are managed, from the silvicultural point of view, following the same treatment reserved for Turkey oak (Fig. 3f). The presence of *Quercus cerris* to which they are associated include also: *Crataegus monogyna* L., *C. laciniata* Ucria, *Pyrus spinosa* Forssk., *Malus sylvestris* L., *Daphne laureola* L., *Rosa canina* L., *Rosa sicula* Tratt., *Rubus hirtus* Waldst. & Kit., *Ruscus aculeatus* L., and, among herbaceous species: *Polygala* aff. *preslii* Spreng., *Symphytum tuberosum* subsp. *angustifolium* (A. Kern.) Nyman, *Lotus corniculatus* L. subsp. *corniculatus*, *Asphodelus ramosus* L. subsp. *ramosus*, *Clinopodium alpinum* subsp. *nebrodense* (A. Kern. & Strobl) Bartolucci & F. Conti, *Viola reichembachiana* Boreau, *Cynosurus cristatus* L., *Odontites vernus* subsp. *serotinus* Corb., *Cirsium vallis-demonii* Lojac. subsp. *vallis-demonii*, *Centaurea jacea* L. subsp. *jacea*, *Medicago cupaniana* Guss., *Plantago cupani* Guss., *Trifolium pratense* L. subsp. *pratense*, *T. repens* L., *Ajuga iva* (L.) Schreb. subsp. *iva*, *Bellis perennis* L., *Brachypodium pinnatum* (L.) P. Beauv., *Muscari commutatum* Guss., *Dactylorhiza romana* (Sebast.) Soó subsp. *markusii* (Tineo) Holub, *Sinapis pubescens* L., *Capsella rubella* Reut., *Reichardia picroides* (L.) Roth, *Hypochoeris radicata* L., *Crepis vesicaria* L., ecc.

Characters of the population – The population of the species occurs in an area of about 1000 hectares inside the Regional Park of Nebrodi. Within this area, however, the species occurs in dense and extensive groups of plants of various ages and development (Fig. 3). Overall, it is estimated the number of mature individuals exceeds 5 thousand units.



Fig. 2. *Pyrus pedrottiana*: **a)** portion of the crown in full bloom and foliation; **b-d)** corymbs and details of the flowers in various stages; **e)** final phase of fruiting with pomes partly provided with the remains of the calyx; **f)** fruits after a few days of harvesting, without calyx remains.



Fig. 3. The population of *P. pedrottiana* in its locus classicus in the Nebrodi Mountains: a) the typical plant at the edge of the forest path leading to the village of Militello Rosmarino; b) young plants cleaned of the low branches, at the edge of the wood with *Quercus cerris*; c) a large tree of *P. pedrottiana* and in the background plants of the same species of different ages, also these cleaned for grazing; d) basal part of the trunk of a mature tree of the same population; e) group of contemporary suckers at the edge of the wood, the tendentially pyramidal shape of the species is evident; f) dense nucleus of *P. pedrottiana* in the area close to the locus classicus, in which forestry treatments were carried out.

Taxonomic relationships – On the basis of the taxonomic arrangement of the genus *Pyrus* by Terpò & Amaral Franco (1968) and according to the actual systematic and taxonomic conceptions of the genus in relation to the European flora (Terpò 1984; Browicz 1993; Aldasoro & al. 1996; Aedo & Aldasoro 1997), *Pyrus pedrottiana* is to be included within the group characterized by pomes with caduche calyx that includes also *P. vallis-demonis* and *P. castribonensis* in Sicily, and *P. cordata* in Italy.

Pyrus pedrottiana has some morphological affinities with *Pyrus pyraster*, however, compared to this very variable taxon, it is well distinguished by the persistent calyx as

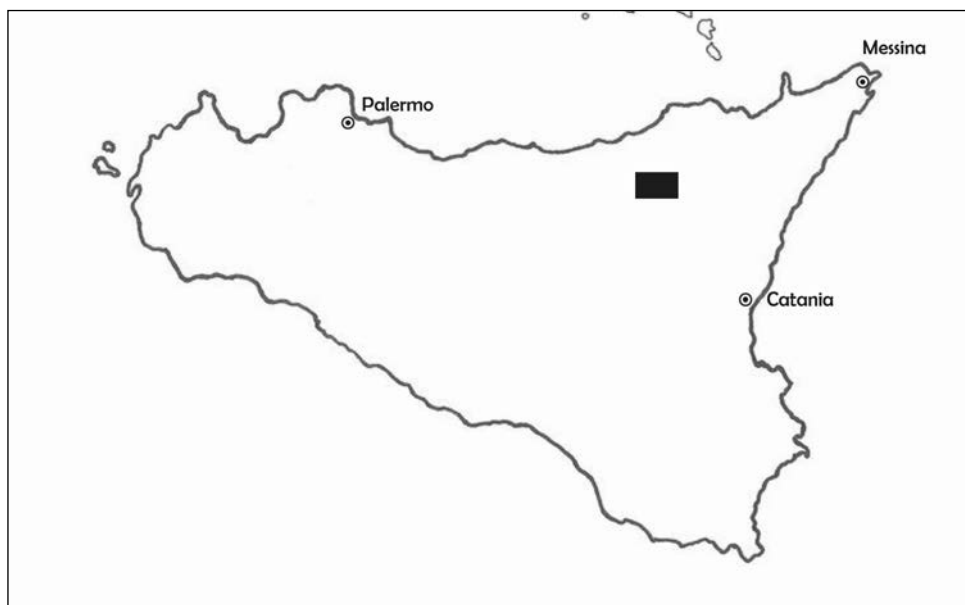


Fig. 4. The area of idtribution of *Pyrus pedrottiana*.

well as in *P. communis* and *P. spinosa*. The same with respect to the two other endemic taxa described in the same area of the Nebrodi Mts, such as *P. vallis-demonis* and *P. ciancioi* (Fig.5). From the former, *P. pedrottiana* is distinguished by the exceptionally pear-shaped fruits (with shorter peduncles (Fig. 1e), as well as by the different morphology, color and leaf consistency, not dark and rigid as in *P. vallis-demonis*. The character of the crown is also different: rising, with a tendency to pyramidal shape (Fig. 3a, b, e). Compared to *P. ciancioi*, the new taxon not only stands out for its leaf morphology and absence of tomentosity, but also for the shape and size of the fruit and peduncles, which are much shorter and stockier than in *P. ciancioi*. Furthermore, differences with the two taxa of the Nebrodi are observed in the flowers: in particular, in the sepals of the *P. pedrottiana* there is a thin marginal indentation and the mucronate, ferruginous apex, curved on the outside (Fig. 1e): this character is absent in all the other species of Sicily. *P. ciancioi*, then, is well distinguished from *P. pedrottiana* for the pommel regularly and not exceptionally with a persistent calyx.

Other specimens

Sicily: Monti Nebrodi, Locality ,Faitedda , 1350 m, on clayey soil, 18 August 2019, *F.M. Raimondo s.n.* (PAL-Gr); Monti Nebrodi, Locality Agrifoglio, 1400 m a.s.l., on siliceous soil, in the clearings of the mixed oak and holly wood, 28 May 2020, *F.M. Raimondo & E. Bajona s.n.* (PAL-Gr, FI); Monti Nebrodi, Localty Bosco Saracina, on siliceous soil, in the clearings of Turkey oak wood, 1420 m a.s.l. 7 May 2021, *F. M. Raimondo & E. Bajona s. n.* (PAL-Gr, PAL, FI).

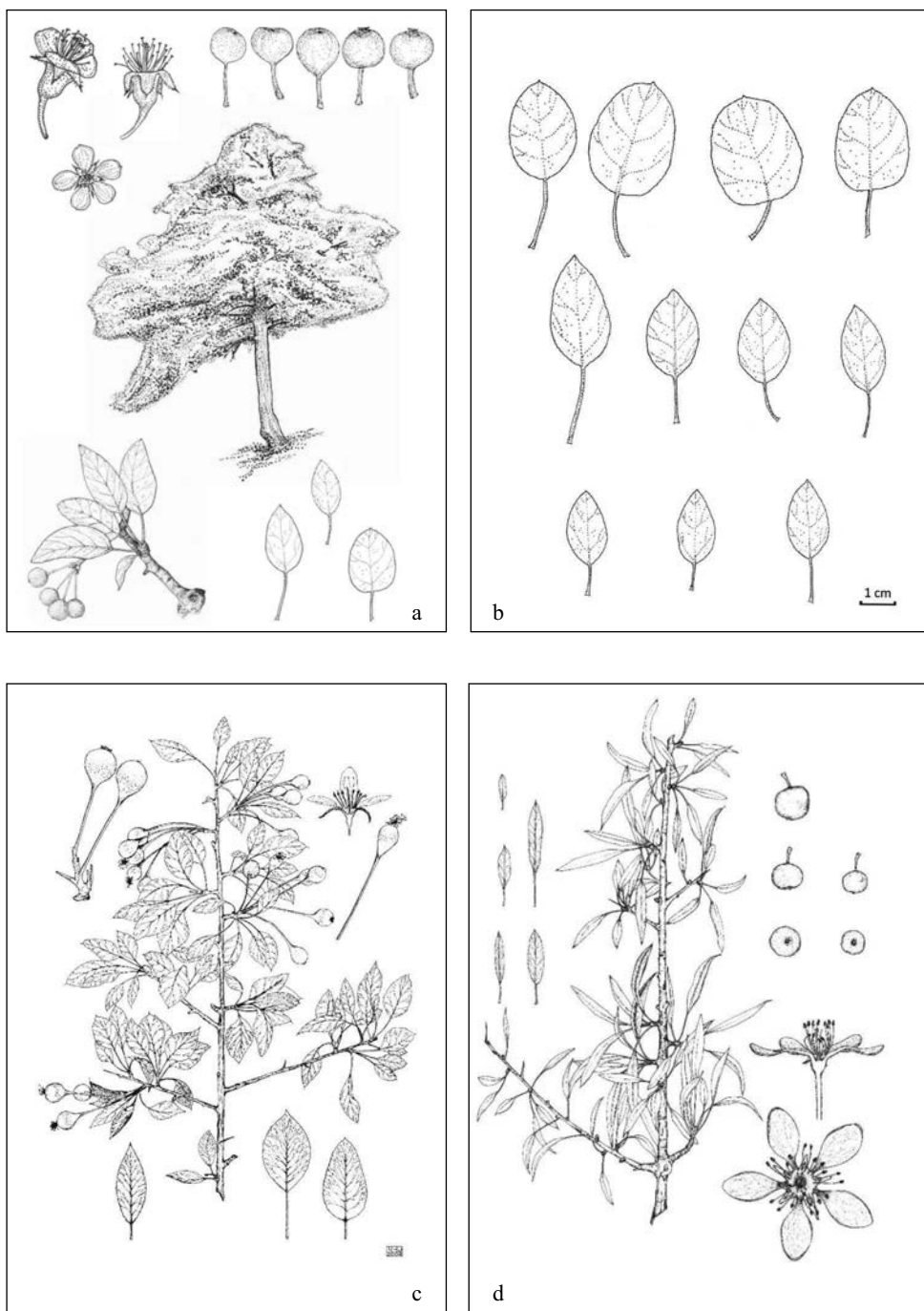


Fig. 5. Differences between the three species of pear of the Nebrodi: **a)** *P. pedrottiana*; **b)** variability of the leaf in *P. pedrottiana*; **c)** *P. vallis-demonis*; **d)** *P. ciancioi*.

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M. Erben, C. S. Christodoulou, R. Hand & K. Kefalas

***Limonium ammochoistianum*, *L. karpasiticum* and *L. paralimniticum* (*Plumbaginaceae*), three new endemic species from the eastern part of Cyprus**

Abstract

Erben, M., Christodoulou, C. S., Hand, R. & Kefalas, K.: *Limonium ammochoistianum*, *L. karpasiticum* and *L. paralimniticum* (*Plumbaginaceae*), three new endemic species from the eastern part of Cyprus. — Fl. Medit. 32: 35-45. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Limonium ammochoistianum, *L. karpasiticum* and *L. paralimniticum* (*Plumbaginaceae*) are described as new to science. The former species was long time known in Cyprus under the misapplied name *L. ocymifolium* subsp. *bellidifolium*. The three species occur in a few coastal stretches in the east of the island. The new taxa show strong morphological similarities and can be considered as segregates of a *L. ammochoistianum* species group. All hitherto known populations of the species group make up about 6000 individuals. *L. ammochoistianum* and *L. paralimniticum* should be classified as “Endangered”, whereas *L. karpasiticum* as a “Data Deficient” taxon.

Key words: micro-endemism, chromosome numbers, species group, coastal habitat.

Introduction

The *Limonium* flora of Cyprus, the third largest island of the Mediterranean, is rather poor in species compared to areas with similar size located more to the west of the basin. Currently, eight species are known to occur, two of them, *Limonium cyprium* (Meikle) Hand & Buttler and *L. mucronulatum* (H. Lindb.) Greuter & Burdet, are endemic to the island (Hand & al. 2021). Karyological research considering the majority of taxa has been dealt with recently by Kouzali & al. (2012). At the southeast tip of the island, known as Kavo Gkreko (or Cavo Greco, Cape Greco, Akrotirio Gkreko), a rare taxon was first found in 1958 by Macdonald, which was named as *L. ocymifolium* subsp. *bellidifolium* (Sm.) Meikle in the “Flora of Cyprus” (Meikle 1985). Nowadays, most authors prefer the heterotypic species name *L. aucheri* (Girard) Greuter & Burdet. Few authors, e. g. Strid (2016), advocate lumping *L. aucheri* with *L. ocymifolium* (Poir.) Kuntze. There is also a short summary about the nomenclature of this *L. aucheri* by Kouzali & al. (2012). However, both names used in Cyprus are based on material collected in the Aegean region. Furthermore, not much is

known about the phylogenetic relationship of all discussed taxa. *L. aucheri* and *L. ocymifolium* have not been included into the analysis by Malekmohammadi & al. (2017) but were integrated in the study by Koutroumpa & al. (2018). The latter study used accessions of *L. aucheri* and *L. ocymifolium* from Greece and Turkey respectively, which, according to the results, proved to be surprisingly remotely related. A recent paper by Koutroumpa & al. (2021) adds general aspects of origin and diversification of Mediterranean *Limonium* taxa.

Intensive field and herbarium studies revealed that the Cypriot plants differ considerably from plants collected in Greece as well as from all known taxa of the wider East Mediterranean region. As a result, we describe a new species group comprising three microspecies.

Material and methods

The current study is based on fieldwork at all known sites of the new taxa, as well as on studies of herbarium material in various collections (B, CYP, M).

Chromosome numbers have been counted according to the protocol described by Brullo & Erben (2011: 11). Taxonomy and nomenclature of taxa mentioned in the text follow Hand & al. (2021). Georeferenced data for all locations is stored in the database on endemic and endangered vascular plants of Cyprus kept at the Department of Forests (Lefkosia).

Results

The three new species share many morphological characters. A diagnosis for the species group is therefore first given to help distinguish it from other E Mediterranean taxa.

Limonium ammochostianum species group

Diagnosis: **Plants** perennial, glabrous, forming a sub-shrub 12–40 cm tall, with many ascending to erect stems and robust tap-root. **Caudices** 2–15 cm long, branched and densely spirally leafy in the upper half, living leaves in the upper third or in rosettes at apices of caudices. **Leaves** 6–45 × 9–10 mm, fleshy, dark green or glaucous, flat, v-shaped or caniculate in cross-section, apex rounded to obtuse or slightly emarginated, sometimes shortly mucronate, with one central nerve, gradually tapering into the petiole. **Stems** slender, 10–30 cm long, rugose, flexuous. **Sterile branches** absent or only 1–5 per stem, 5–40 mm long, straight, unbranched. **Fertile branches** 2–5 cm long, straight, obliquely directed upwards. **Spikes** 10–50 mm long, straight to slightly arched. **Spikelets** 7–10 mm long, composed of 1–8 flowers, remotely arranged with 1–6 flowers per 2 cm. **Outer bract** 1.9–3.5 × 1.6–2.6 mm, narrowly to broadly triangular-ovate. **Middle bract** 2.0–3.8 × 1.7–2.5 mm, oblong-elliptic, rounded or emarginate. **Inner bract** 5.8–7.9 × 3.0–4.3 mm, elliptic to obovate, rounded to obtuse, narrowly to broadly membranous, central part fleshy, 4.0–6.5 × 2.0–3.5 mm, elliptic to oblong-obovate, acuminate, forming a triangular tip 0.5–1.5 mm long, not reaching the upper margin. **Calyx** 5.0–6.6 mm long, calyx tube glabrous or sparsely to densely long hairy. **Corolla** pinkish to lilac-mauve.

Key to the species including diagnostic characters:

1. Leaves acute to obtuse, shortly mucronate, spikelets 8.5–10 mm long, inner bract 7.2–7.9 mm long ***L. karpasiticum***
1. Leaves emarginated to obtuse, spikelets 7.0–8.5 mm long, inner bract 5.8–6.8 mm long **2**
2. Leaves cuneate-spathulate, apice emarginated, calyx 5.0–5.8 mm long, calyx tube densely long hairy ***L. ammochostianum***
2. Leaves narrowly spathulate, apice rounded to obtuse, sometimes slightly emarginated, calyx 5.8–6.6 mm long, normally glabrous ***L. paralimniticum***

Limonium ammochostianum Erben, Christodoulou, Hand & Kefalas, **spec. nova**

– *L. aucheri* sensu auct. cypr., non (Girard) Greuter & Burdet

– *L. ocymifolium* subsp. *bellidifolium* sensu auct. cypr. non (Sm.) Meikle

Holotypus: Cyprus: Cavo Gkreko (Somera station), maritime rocks, 27.10.2017, C. S. Christodoulou (M-0244416, isotype: M-0244417).

Diagnosis: **Leaves** 20–45 × 7–10 mm, dark green, cuneate-spathulate, apex slightly emarginated to obtuse, lamina canalculated to slightly v-shaped in cross-section, living leaves more or less in rosettes at apices of caudices. **Spikes** with 2–3 spikelets per 1 cm. **Spikelets** 7.0–8.5 mm long, composed of 2–8 flowers. **Outer bract** 1.9–2.5 × 1.9–2.4 mm, triangular-ovate. **Middle bract** 2.0–2.9 × 1.9–2.2 mm, oblong-elliptic. **Inner bract** 5.8–6.8 × 3.1–4.2 mm, elliptic to obovate, rounded to obtuse. **Calyx** 5.0–5.8 mm long, calyx tube with dense, long hairs in the lower half. **Corolla** pale lilac-mauve.

Limonium karpasiticum Kefalas, Erben, Christodoulou & Hand, **spec. nova**

Holotypus: Cyprus: Rizokarpaso, Mora Psaria, maritime rocks, sea level, 3.7.2017, K. Kefalas 3 (M-0244415).

Diagnosis: **Leaves** 20–40 × 3–7 mm, glaucous, cuneate-spathulate, apex acute to obtuse, shortly mucronate, lamina flat, living leaves more or less in rosettes at the apices of caudices. **Spikes** with 0.5–1 spikelets per 1 cm. **Spikelets** 8.5–10.0 mm long, composed of 2–3 flowers. **Outer bract** 3.0–3.5 × 2.0–2.8 mm, narrowly triangular-ovate. **Middle bract** 3.1–3.8 × 2.0–2.5 mm, oblong-elliptic. **Inner bract** 7.2–7.9 × 3.6–4.3 mm, obovate-elliptic, obtuse. **Calyx** 5.5–6.5 mm long, calyx tube sparsely long hairy in the lower half. **Corolla** pinkish.

Limonium paralimniticum Christodoulou, Erben, Hand & Kefalas, **spec. nova**

Holotypus: Cyprus: Protaras, sea level, maritime rocks, 15.7.2007, C. S. Christodoulou (M-0275002, isotypes B, CYP, PAL).

Diagnosis: **Leaves** 22–40 × 3–7 mm, glaucous in maturity, narrowly spathulate to lanceolate-spathulate, apex rounded to obtuse or sometimes slightly emarginate, lamina flat or slightly v-shaped in cross-section, living leaves in the upper part of caudices; leaves usually with salt secretions. **Spikes** with 0.5–2 spikelets per 1 cm. **Spikelets** 7.0–8.5

mm, composed of 1–2 flowers. **Outer bract** 2.0–2.5 × 1.8–2.0 mm, narrowly triangular-ovate. **Middle bract** 2.2–3.2 × 1.7–2.1 mm, oblong-elliptic. **Inner bract** 5.8–6.5 × 3.0–3.9 mm, elliptic-obovate, obtuse to rounded. **Calyx** 5.8–6.6 mm long, normally glabrous, sometimes with some hairs in the lower half of the calyx. **Corolla** pale pink.

Illustrations: Fig. 1–10; for further photo documentation see Hand & al. (2021).

Phenology: *L. ammochostianum* flowers earlier than the other two species, usually from (March–) April to early June; *L. paralimniticum* and *L. karpasiticum* flower from (April–) May to July.

Etymology: The epithet of the new species *L. ammochostianum* refers to Ammochostos district in the eastern part of Cyprus. The eponymous district capital, a few kilometers away from the type locality, is also well known under its colonial name Famagusta. The species *L. paralimniticum* is named after Paralimni municipality where its distribution area is confined. The name of *L. karpasiticum* is based on the Karpasia peninsula, the characteristic “panhandle” in the northeast of Cyprus.

Chromosome numbers: Kouzali & al. (2012) published three chromosome counts based on Cypriot material, two from the type region of *L. paralimniticum* in the Protaras area (Paralimni municipality) and one from Kavö Gkrekö area, the type locality of the species *L. ammochostianum*. In all three cases they revealed the pentaploid number $2n=43$. We have not seen the corresponding specimens, so that the identity with the newly circumscribed taxa needs to be clarified, especially as regards the latter case. However, our own countings based on plants grown from seeds of the type specimens (performed by ME) confirmed the pentaploid number $2n=43$ for *L. paralimniticum*. In the other cases plants proved to be hexaploids with $2n=51$ (type material of *L. ammochostianum* and *L. karpasiticum*, also plants from Galounia near Davlos).

Additional specimens examined (all from Cyprus, divisions sensu Meikle 1985):

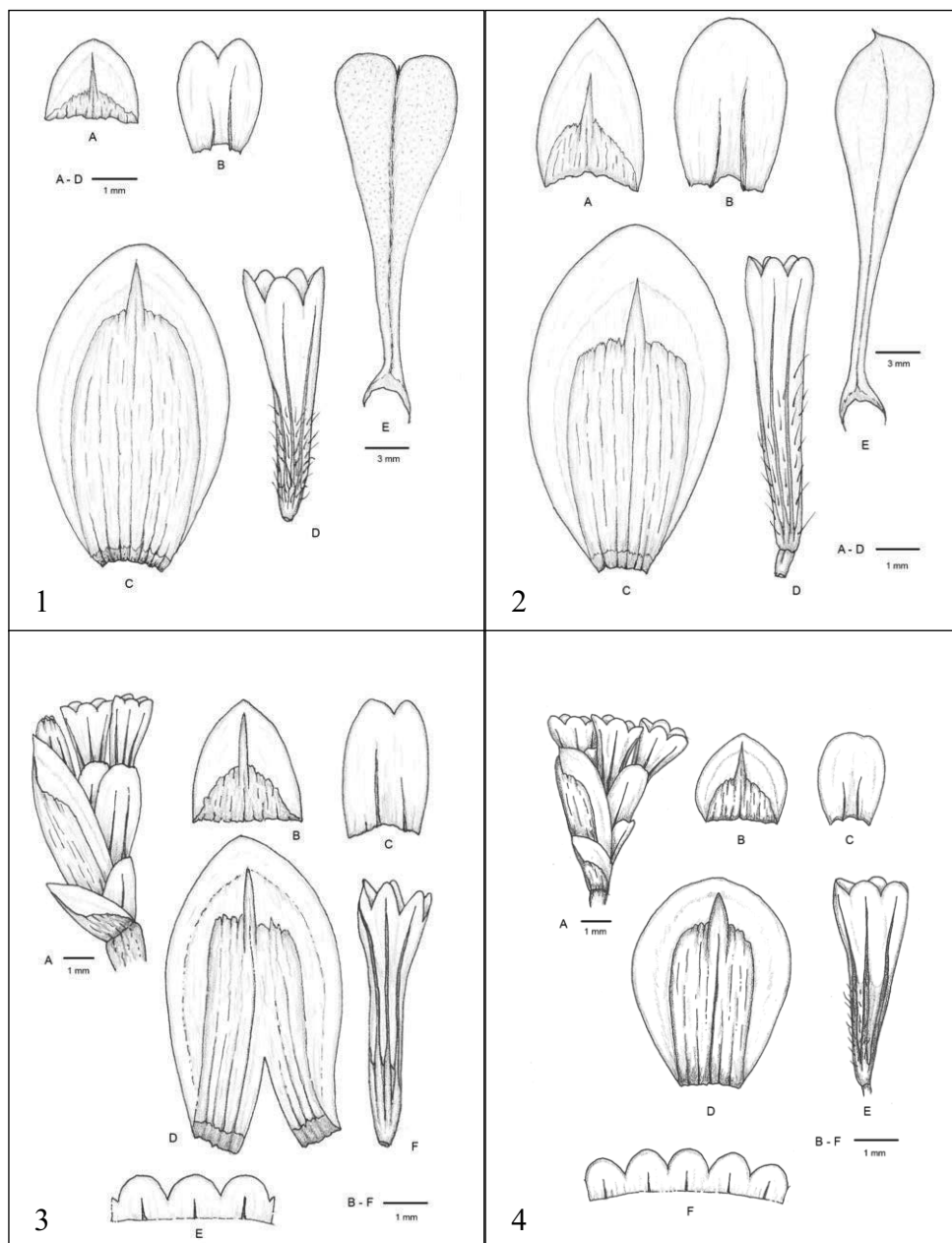
L. ammochostianum

Division 4, Cape Greco (Larnaca), calcaires coralliens du Miocène inférieur, et sables, alt. 10–20 m, 12.4.1991, G. Alziar & al., *Iter Medit.* 4, 0169 (B). – Capo Greco, 10.10.1988, Brullo & Pavone (CAT, M-0274985, M-0274986, M-0274991). – Akrotirio Gkrekö, neck of the peninsula, S of the road near to its end, rocky coastal phrygana, alt. c. 10 m, 11.5.2005, R. Hand 4925 (B). – Kavö Gkrekö, SSE Agioi Anargyroi, rocky coast, alt. 15 m, 3.4.2014, R. Hand 6361 (B, PAL).

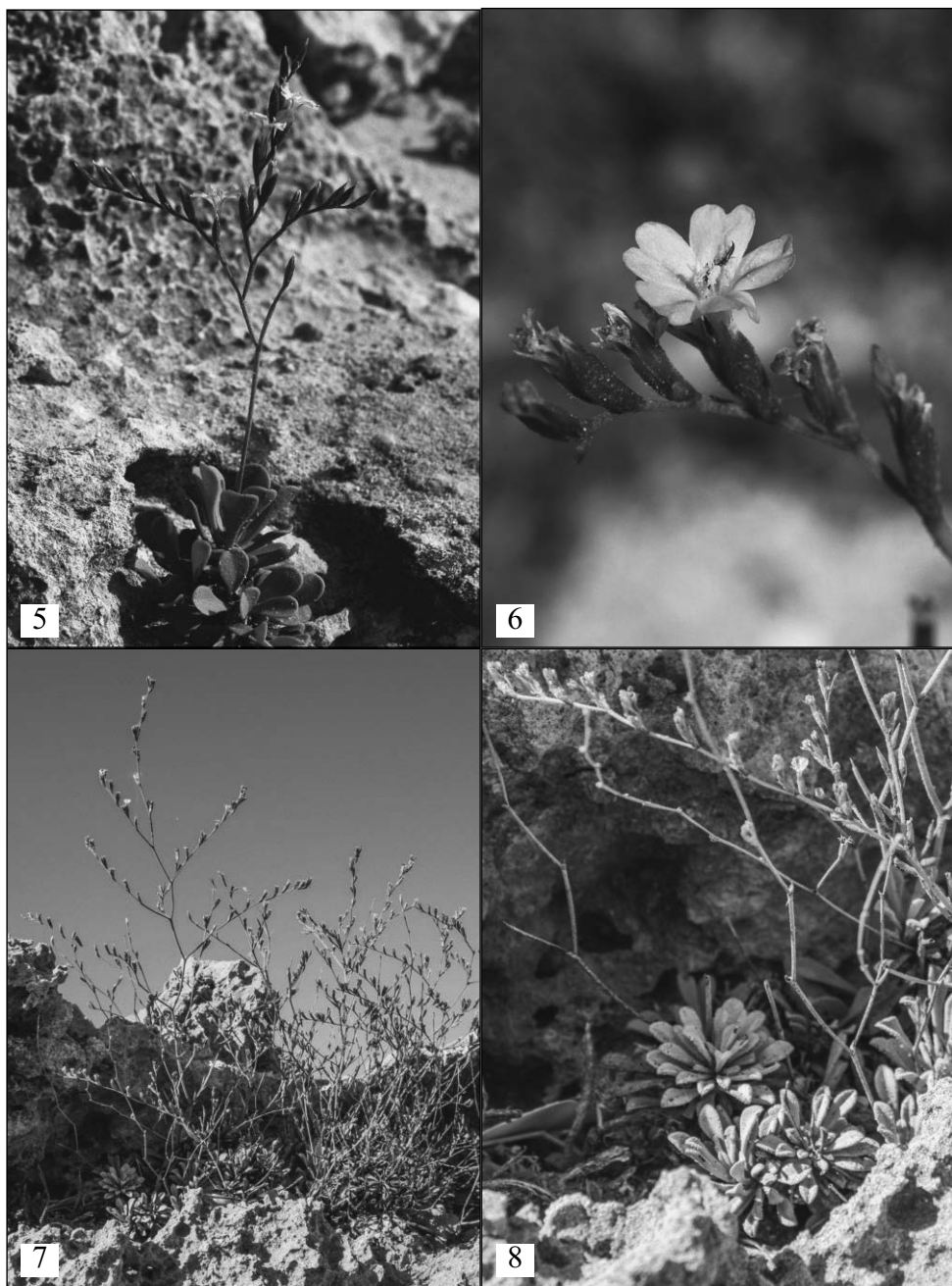
Division 8, Davlos, Galounia, rocky coast, 5 m, 11.8.2017, Kefalas 5 (M-0274993).

L. karpasiticum

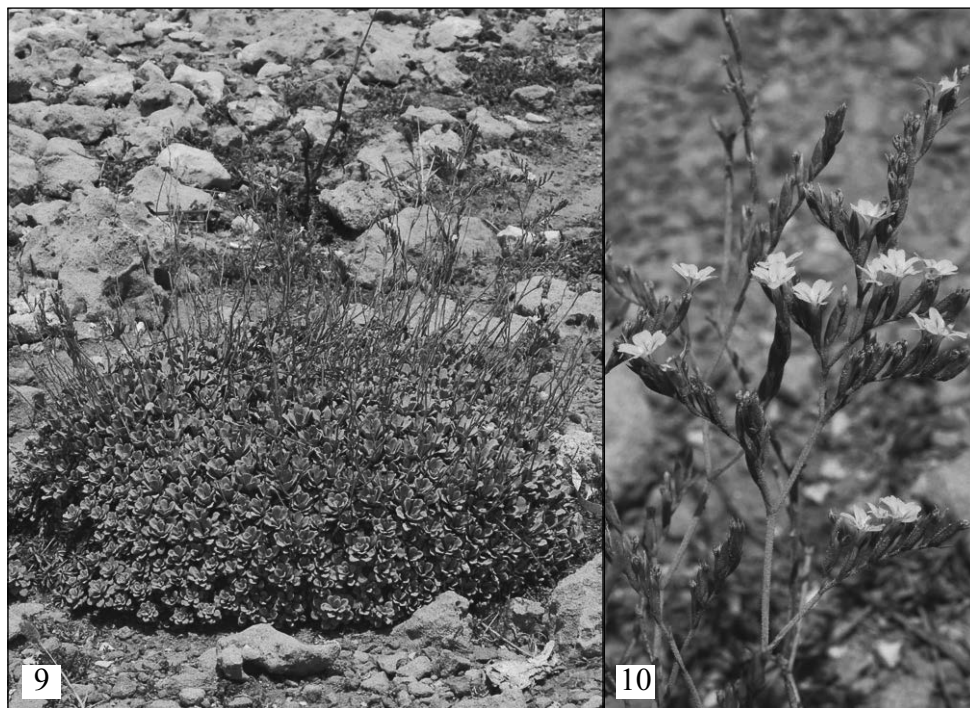
Division 4, Cape Greco, rochers calcaires, 19.4.1992, Lambinon no. 92/CY/473 & Rousselle (M-0275001).



Figs. 1-4. **1)** *Limonium ammochoastianum*: A) outer bract; B) middle bract; C) inner bract; D) calyx; E) leaf; based on the holotype (M-0244416); **2)** *L. karpasiticum*: A) outer bract; B) middle bract; C) inner bract; D) calyx; E) leaf; based on the holotype (M-0244415); **3)** *L. paralimniticum*: A) spikelet; B) outer bract; C) middle bract; D) inner bract; E) calyx lobes; F) calyx; based on the holotype (M-0275002); **4)** *L. aucheri*: A) spikelet; B) outer bract; C) middle bract; D) inner bract; E) calyx; F) calyx lobes; based on specimen Greece, Kos, Agios Stefanos, 19.9.1997, Schwarz (herb. Erben). – Drawings: M. Erben.



Figs. 5-8. **5)** *Limonium ammochoastianum*, habit, Cyprus, Kavo Gkreko, 7 May 2011; **6)** *L. ammochoastianum*, flowers, Cyprus, Kavo Gkreko, 8 June 2010. – Photos by C. S. Christodoulou; **7 & 8)** *L. karpasiticum*, habit and typical habitat, Cyprus, Rizokarpaso, Mora Psaria, 3 July 2017. – Photos by K. Kefalas.



Figs. 9-10. **9)** *Limonium paralimniticum*, habit and typical habitat, Cyprus, Protaras, near Fig Tree bay, 13 June 2010; **10)** *L. paralimniticum*, flowers, Cyprus, Protaras, near Fig Tree bay, 13 June 2010. – Photos by C. S. Christodoulou.

L. paralimniticum

Division 4, Protaras, small peninsula SE of Fig Tree Bay, coastal rocks, alt. 2 m, 21.9.2010, *R. Hand 5719* & *C. S. Christodoulou* (B, PAL), also grown from seed of this gathering, 8.5.2013, *M. Cubr 49121* (B, garden herbarium). – Protaras, east of Fig Tree, maritime rocks, 27.10.2017, *C. S. Christodoulou* (M-0274988). – Protaras, near Skoutarospilioi, maritime rocks, 27.10.2017, *C. S. Christodoulou* (M-274990). – Protaras, Kapparis, rocky coast, sea level, 10.6.2012, *K. Kefalas 492* (B).

Intermediate plants (with presumed parentage):

L. ammochostianum × *L. karpasiticum*

Division 8, Davlos, Galounia, rocky coast, 5 m, 11.8.2017, *Kefalas 6* (M-0274997).

L. ammochostianum × *L. paralimniticum*

Division 8, Davlos, Galounia, 11.08.2017, rocky coast, 5 m, *Kefalas 1* (M-0275000), 2 (M-0274998), 4 (M-0274996) and 7 (M-0274987).

Distribution

All three new taxa are rare species of Cyprus, known so far from four small coastal stretches in the eastern part of the island (Fig. 11). In the past two decades, the coastline of Cyprus has been studied relatively intensively regarding the occurrence and distribution of *Limonium*. This resulted in the mapping of many new sites of *L. cyprium*, formerly thought to be rare along the northern coast (summary of records in Hand & al. 2021). Hitherto, the southeast coast of Cyprus has been intensively studied. *L. ammochoostianum* is restricted to the coastline of Kavogkreko from “Palatia” sea caves to “Agiol Anargyroi” chapel, where the taxon has been known since more than 60 years. *L. paralimniticum* is known to occur along the Protaras coastline (Paralimni municipality). Because of the presence of extensive stretches of sandy beaches, no additional occurrences are to be expected in the Ammochoostos gulf between Protaras and Bogazi. In the Karpasia peninsula, which follows to the northeast, *L. karpasiticum* has been found on the northern coast near the tip (Mora Psaria) and another location at the base of the peninsula (Galounia, Davlos), where also *L. ammochoostianum* occurs sympatrically. In one case *L. karpasiticum* material has also been identified from Kavogkreko area.

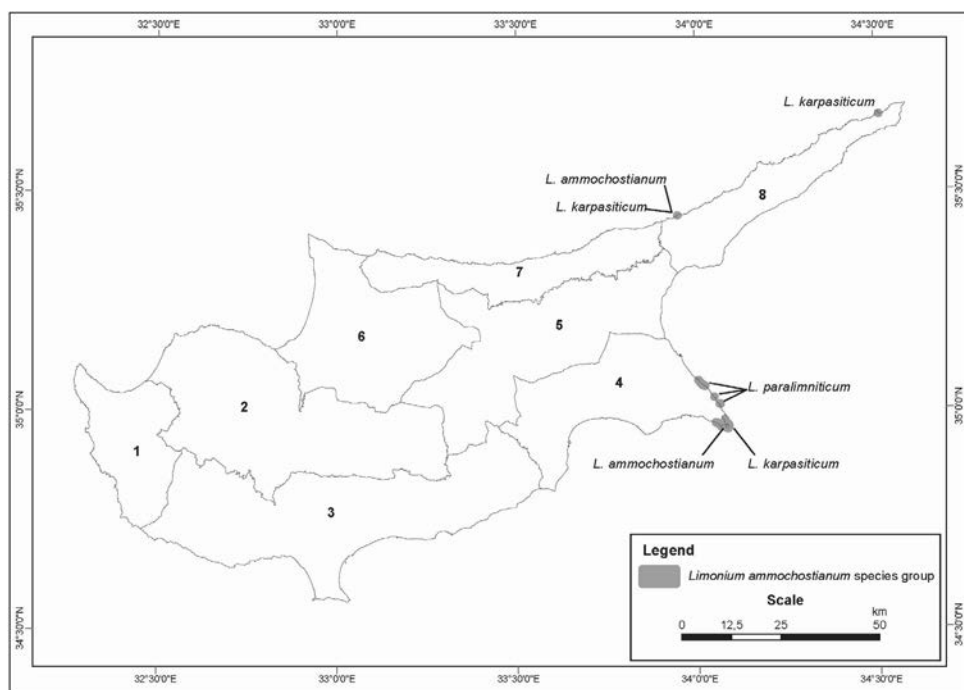


Fig. 11. *Limonium ammochoostianum* species group, distribution in Cyprus; phytogeographical divisions sensu Meikle 1985. – Map design: K. Papasavvas.

Habitat

All three species inhabit maritime calcareous rocks, which correspond to habitat type 1240: Vegetated sea cliffs of the Mediterranean coasts with endemic *Limonium* sp. pl. (sensu Annex I of the EU Directive 92/43/EEC). Other accompanying species are *Limonium virgatum* (Willd.) Fourr., *Frankenia hirsuta* L., *Crithmum maritimum* L., *Silene sedoides* Poir., *Atriplex portulacoides* L. and sporadically *Tetraena alba* (L. f.) Beier & Thulin. In the wider Kavro Gkreko area, *L. ammochostianum* is mainly found on Quaternary marine terrace calcarenite and to a lesser extent on Quaternary marine terrace calcarenite with Tera Member limestone in places; *L. paralimniticum*, along Protaras coasts, grows on Quaternary marine terrace calcarenite as well as on Pachna Formation chalk and marl, in some places topped by Quaternary marine terrace calcarenite. In the north coast of Karpasia peninsula, *L. karpasiticum* colonises Kythrea formation sandstones and Quaternary marine terrace calcarenite (Geological Survey Department 1995).

Conservation

Undoubtedly, the *L. ammochostianum* species group is a rare taxon encompassing three species with restricted and fragmented distribution, small population and threatened habitat. The estimated population of the species group is around 6000 individuals (*L. ammochostianum* 4000, *L. paralimniticum* 1700 and *L. karpasiticum* 200 individuals respectively). Considering the current knowledge, the *L. ammochostianum* species group could be assessed as Vulnerable: B1ab(iii+v)&2ab(iii+v), because the Extent of Occurrence (EOO) is 116 km², the Area of Occupancy (AOO) is 21 km² and the taxa of the species group occur at 6 locations; also, the quality of habitat has been degraded, at least at three of the locations, and as a result the population declined.

However, *L. ammochostianum* can be classified as Endangered: B1ab(iii+v)&2ab(iii+v) due to the number of locations (three), EOO (116 km²), AOO (11 km²) and the quality of habitat, which has been degraded at one location resulting in reduction of the population. Similarly, in the “Red Data Book of the Flora of Cyprus” (Tsintides & al. 2007), *L. ammochostianum* (= *L. aucheri*, including part of the population of *L. paralimniticum*) has been assessed as Endangered: B1ab(iii+v)&2ab(iii+v); this classification is still valid, although *L. ammochostianum* occurs almost entirely within a protected area, since Kavro Gkreko is a National Forest Park as well as a designated Natura 2000 site. *L. paralimniticum* could be also classified as Endangered: (B1ab(iii+v)&2ab(iii+v)) based on the EOO (13 km²), the AOO (7 km²), the number of locations (2) and the continuing decline in area/extent and quality of habitat, resulting also in population decline; habitat degradation is due to extensive touristic development along the coastal zone of Protaras (Paralimni municipality). *L. karpasiticum* is hitherto known from two locations with a population of about 200 individuals, thus it could be also characterised as Endangered under criterion D (number of mature individuals <250) (IUCN Standards and Petitions Subcommittee 2014). However, owing to its recent discovery, the insufficient survey and one collection without exact locality, we believe it is more appropriate to be classified as Data Deficient.

Discussion

The new species are similar in habit, leaves and inflorescences to *L. aucheri*, a species which is frequent on many islands of the central and southern Aegean region and rare in Attika and Peloponnese (Greece; see also fig. 4). They differ from *L. aucheri* first of all by having fleshy, canaliculate in cross-section leaves (vs. flat leaves), larger (vs. 5.5–6.3 mm), more remotely arranged spikelets, greater bracts (vs. inner bract 4.3–5.2 × 3.2–3.9 mm) and often longer calyces (vs. 4.3–5.4 mm).

The known populations of the 3 new species differ slightly in morphological characters from each other but also in their flowering periods. Furthermore, in one of the four known coastal stretches, where the species occur, transitional plants have been identified. Most probably, the new taxa represent cases of rather recent speciation events. The differences between the three taxa are small, but they are easily diagnosable. Also, because of the almost parapatric distribution, classification as subspecies would be a viable solution, but in view of the current preferred practice in the Mediterranean area, we have opted for microspecies. Additional future fieldwork in some remote coastal areas of the Karpasia peninsula may improve the chorological knowledge as will do additional karyological work. The somewhat complicated known distribution patterns, e. g. in *L. ammochostianum* which “brackets” the other species occurrences as well as the occurrence of transitional plants with or without the postulated parental taxa may be explained by relictual sites or long-distance dispersal by coastal currents in the past.

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L. Serra Laliga, J. A. Rodríguez & M. Ferreira Romero

***Tulipa gesneriana* (Liliaceae), new casual alien species in Spain**

Abstract

Serra Laliga, L., Rodríguez, J. A. & Ferreira Romero, M.: *Tulipa gesneriana* (Liliaceae), new casual alien species in Spain. — Fl. Medit. 32: 47-51. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Tulipa gesneriana (Liliaceae) has recently been found as casual alien in the Alicante province (E of Spain). It is the first locality of this species which grows wild in Spain in semi-natural, disturbed grasslands, similarly to other geophytes such as *Hyacinthus orientalis* or *Sternbergia lutea*.

Key words: *Tulipa*, xenophytes, Iberian Peninsula.

Introduction

During one of our field studies on the flora of Alicante (SE of Spain), we located a small population of *Tulipa* with large red flowers of clear artificial origin. Before this discovery, there was only one autochthonous species in Spain of *Tulipa*, with yellow flowers, called *T. sylvestris* subsp. *australis* (Link) Pamp., with North-Western Mediterranean distribution (Serra 2007).

Material and methods

For the georeferencing of this population, the GPS of a smartphone was used. The information was embedded in the ©OruxMaps program and the coordinates were contrasted with the orthophoto of the Generalitat Valenciana viewer (<https://visor.gva.es>) and offered in 1 km² UTM grid cell and coordinates x, y in the ETRS89 Datum.

The nomenclature of the mentioned taxa follows that adopted in Güemes (2013), while the biogeographic and bioclimatic data follow Rivas Martínez & al. (2007) and Serra (2007). The cited material is found in the herbaria MA (Thiers 2021+) and LSH (private herbarium of one of the authors).

Results and discussion

A bibliographic review has been carried out to find out which non-native species of the genus *Tulipa* have been mentioned previously in Spain. In Flora Iberica (Güemes 2013) mentions some ornamental species, although Güemes considers that they have always been present in gardens. Of the three species he cites, *T. clusiana* DC. has white tepals with a pink central line and linear or linear-lanceolate leaves 1-2 cm wide, while the other two (*T. gesneriana* L. and *T. agenensis* DC.) have reddish tepals (sometimes of other colors) and lanceolate or oblong-lanceolate leaves up to 8 cm (Grey-Wilson & Mathew 1980, 1982; Pignatti 2017).

This plant is a bulbous geophyte that has red tepals, the interior and exterior of similar size, $5.5 \times 4\text{--}4.5$ cm, obovate, with a black spot at the base bordered with yellow, and oblanceolate leaves up to 30×7 cm, glabrous. With these characters we must assign the Alicante population to *T. gesneriana* since all the tepals are of a similar length while in *T. agenensis* they are unequal, the internal ones shorter and narrower (Grey-Wilson & Mathew 1982).

***Tulipa gesneriana* L., Sp. Pl.: 306. 1753**

= *T. planifolia* Jord. in Ann. Soc. Linn. Lyon sér. 2, 5: 13. 1858

= *T. didieri* Jord., Observ. Pl. Nouv. 1: 34. 1846

= *T. hortensis* Gaertn., Fruct. Sem. Pl. 1: 64. 1788

= *T. strangulata* Reboul, Nonnul. Sp. Tulip. Not.: 6. 1822

The first reference to the presence of tulips grown in Spain is probably due to Abu I-Jayr, who in his work *Umdat al-tabib*, from the 11th or 12th centuries, would reflect their use in the gardens of Al-Andalus, S from Spain (Hernández Bermejo & García Sánchez 2009). Other later authors mention them but never growing spontaneously (Clusius 1576; Willkomm & Lange 1862; Lázaro Ibiza 1920; Bolòs & Vigo 1994; Güemes 2013).

Numerous forms and cultivars of this taxon have been described in Europe as independent species but are currently considered synonymous with *T. gesneriana* (Raamsdonk & Vries 1995; Zonneveld 2009; Veldekamp & Zonneveld 2012; Govaerts 2021). This is the case of *T. didieri* or *T. planifolia*, binomen under which these plants have recently been mentioned in France (Tison & De Foucault 2014), while in Italy the presence of *T. gesneriana* is recognized (Pignatti 2017; Galasso & al. 2018; Domina & al. 2018).

Observation: SPAIN, ALICANTE: (Benilloba), Barranc d'Agustí, 30SYH2687, (726672, 4287257), 500 m, 5 April 2021, L. Serra 12754 & A. Bort, LSH 14385, MA.

It appears in grasslands of *Festuco-Brometea erecti* Br.-Bl. & Tüxen ex Br.-Bl. 1949 highly nitrified on a slope of a small ravine, 420 m from the nearest houses, under subhumid ombrotype and mesomediterranean thermotype in the Alcoyan-Dianic subsector (Setabensean Sector, Balearic-Catalan-Provençal Province), together with *Brachypodium phoenicoides* (L.) Roem. & Schult., *Brassica fruticulosa* Cirillo, *Bryonia dioica* Jacq., *Galium aparine* L., *Geranium purpureum* Vill., *Mercurialis tomentosa* L., and *Rubus ulmifolius* Schott.



Fig. 1. *Tulipa gesneriana*. Benilloba, Barrac d'Agustí, 5, April 2021: **a)** the studied population, **b)** detail of flower, photos by L. Serra.

The found population consists of 6 well-developed and flowered specimens (Fig. 1). It does not seem that it has a significant colonization capacity, but it will be necessary to observe in the future if the population remains stable or ends up disappearing. It must have its origin from plants grown in the vicinity, since it is very close to the Frainós river and the town of Benilloba. It is a similar case to that of other geophytes that we have recently located naturalized in nearby areas such as *Sternbergia lutea* (L.) Spreng. or *Hyacinthus orientalis* L. (Serra & al. 2018).

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***Bolboschoenus* (Cyperaceae) in the Maltese Islands – an enigma of taxonomic interest**

Abstract

Mifsud, S.: *Bolboschoenus* (Cyperaceae) in the Maltese Islands – an enigma of taxonomic interest. — Fl. Medit. 32: 53-71. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This work aimed for a taxonomic investigation conducted on populations of *Bolboschoenus* (Cyperaceae) growing in wetlands throughout the Maltese Islands. A detailed account of the ecology and morphometric analysis of eleven flower-bearing populations is provided and supplemented by images. Emphasis was made on the inflorescence and the achenes, whereas palynological observations were also included. No significant taxonomic difference has been found between ray-bearing and rayless plants and all examined material did not correspond to *B. maritimus*, the only species reported in previous floristic literature. Findings instead showed two distinct morphotypes that are undocumented. The one prevailing in mainland Malta was completely sterile (a unique finding for the genus) except for one small population, which harboured fragment pockets of fertile plants corresponding best to *B. glaucus* s. l. The other morphotype occurred only on the Island of Gozo and consisted of achene-producing plants that did not match any of the described *Bolboschoenus* species. Morphometrics indicated an intermediate position between *B. glaucus* and *B. maritimus*. Further investigations that are more diagnostic from morphometrics are advocated for reliable taxonomic conclusions of both populations, possibly leading to the first records of the hybrid *B. glaucus* × *maritimus*, as indicated in this morphological study.

Key words: *Bolboschoenus maritimus*, *Bolboschoenus glaucus*, wetland species, halophytes, glycophytes, Malta, Gozo.

Introduction

The genus *Bolboschoenus* (Asch.) Palla was erected after being segregated from its sectional ranking of *Scirpus* L. and accommodated the single species *B. maritimus* (L.) Palla (Palla 1905). In mainland Europe, the genus only gained a widespread taxonomic recognition by the late 20th century after the pioneer works of Van der Veken (1965); Oteng-Yeboah (1974); Browning & al. (1993, 1996, 1997b); Hroudová & al. (1998b, 1999a, 1999b) and Browning & Gordon-Gray (2000). Disagreement on this segregation initially persisted for a while, until phylogenetic sequencing provided better support for the delimitation of *Bolboschoenus* and other genera previously nested in *Scirpus* s. l. (Muasya & al.

2009; Jung & Choi 2011). A distinct morphological differentiation was already well documented for these segregated genera, where *Bolboschoenus* was characterised with spikelets longer than 1 cm, noded culms and long leaf-like bracts (Browning & al. 1988; Pignotti 2003). *Bolboschoenus* has a worldwide distribution with three to five species representing each continent, where the Southeast Asian, Amazonian and Russian regions are the least studied (Browning & Gordon-Gray 2000).

B. maritimus represented the only species in Europe (e.g. Pereira Coutinho 1976; DeFilipps 1980; Pignatti 1982; Rothmaler 1982; Jeanmonod & Gamisans 2009; etc.) and so is the case in the Maltese Islands (Haslam & al. 1979; Lanfranco 1989; Weber & Kendzior 2006, Casha 2017, Brullo & al. 2020). Inevitably, the genus has been later questioned and investigated by more dedicated *Cyperaceae* authorities in central Europe. Several infraspecific rankings have been used in literature and attributed for some well-defined morphotypes of *B. maritimus* (De Filipps 1980; Hroudová & al. 1998a; Pignotti 2003; Marhold & al. 2004), initially based on the inflorescence's architecture such as the length and number of ray-borne spikelets (Casper & Krausch 1978; Defillips 1980;) and then on the morphology and anatomy of the achenes (Browning & al. 1993, 1995, 1997a, 1997b; Hroudová & al. 1998a, 1998b; Browning & Gordon-Gray 2000).

The diversification of the genus shaped up when new characters were being considered, the most important being the morphology of the achenes (Browning & Gordon-Gray 1993; Hroudová & al. 1998b), and within a few years, five taxa were reported from Europe: *B. maritimus*, *B. glaucus* (Lam.) S.G.Sm., *B. laticarpus* Marhold & al., *B. planiculmis* T.V. Egorova and *B. yagara* (Ohwi) Y.C.Yang & M.Zhan (e.g. Hroudová & al. 1999b; Hroudová & al. 2001; Marhold & al. 2004). The morphology, distribution and ecology of these five European taxa were documented in great detail in a monograph by Hroudová & al. (2007b), which led to further taxonomic revisions of *Bolboschoenus* in some European countries, the latest being in the Netherlands (Simons & al. 2016), Serbia (Nikolić & al. 2019) and Italy (Di Natale & al. 2020). Most of these works corrected numerous herbarium accessions labelled as the "*B. maritimus*" to any of the five species mentioned above.

Bolboschoenus has never been investigated in the Maltese Islands, and the aim of this study was primarily to investigate species diversity occurring in Malta, based on morphological analysis. The study commenced in early January 2021 on all the populations known by the author, using the available literature for character selection and final determination.

Records in Malta

A literature review on the Maltese vegetation spanned on the last 150 years conveyed that only *Bolboschoenus maritimus* (= *Scirpus maritimus*) was recorded in the Maltese Islands. Only in a very recent work by Brullo & al. (2020) was the species assigned to *B. maritimus* subsp. *compactus* (Hoffm.) Hejný.

The first record from the Maltese Islands dates back to Grech Delicata (1853) from Marsa and (Duthie 1874) from Wied tal-Gnejna. New records appeared shortly after, namely from Wied Bingemma-San Blas valley system in Gozo (Sommier & Caruana Gatto 1915) and from the Imsellit-Għajn Mula-Rihana valley system (Borg 1927). During this time, the population in Marsa was already considered locally extinct (Sommier & Caruana Gatto 1915). Marsa used to

be a large marshland wetland area featuring brackish vegetation during *Delicata*'s period but it was drained and dried completely due to public health issues by 1869, and gradually the area became developed into shipbuilding and industrial estates (Haslam & Borg 1998).

More than half a century later, the same records were mentioned in Maltese literature (Lanfranco 1969; Haslam & al. 1977; Haslam & Borg 1998) and in the Red Data Book (Lanfranco 1989). However, in the early eighties, Michael Briffa (pers. comm., Apr-2016) has already found some new populations at Wied il-Fiddien, Wied tas-Salini and Wied Gerżuma in mainland Malta and at Wied Sara and Wied Marsalforn in Gozo, and recently reconfirmed by Tabone (2007, 2008), Casha (2017) and the present author (e.g. Mifsud 2011). The populations from Marsa, Wied Bingemma, Wied Gerżuma, and Salini, were so far not substantiated, including surveys conducted in this study. However, new records have been reported during the last 20 years and includes Wied il-Bahrija and Ramla valley (Tabone 2007), whereas Mistra valley (Wied tal-Kalkara) and Ta' Cianti valley are first published in this study. Overall, the species is locally frequent in some valleys, but these wetland habitats are continuously declining through water extraction for agriculture, climate change, and pollution.

Methodology

The fourteen examined *Bolboschoenus* populations known by the author from the Maltese Islands are given in Table 1 and displayed in map Fig. 1. For each population, the pH, conductivity (μ S) and total dissolved solids (ppm) level of the water the plants were found in was recorded during January 2021 using a portable water tester Hanna HI98130. During visits, the population size was estimated, photographs of plants and inflorescences were taken, and the ecological and environmental data were recorded. Seven representative plants were sampled and studied ex-situ. In four populations, plants with both rayless and ray-bearing spikelets were treated as different samples (as indicated in Table 1) in case each form corresponds to a different species. Samples of inflorescences were collected and examined during March and April, whereas the achenes were harvested at the end of May and June. This study also incorporated a trial study on the pollen to increase the knowledge of the genus since palynology in *Cyperaceae* is lacking (pers. comm. Jane Browning, Dec. 2021) and maybe useful to indicate sterility. The pollen of rayless and ray-bearing samples was also compared in three populations.

Most of the morphological characteristics taken into account are given in Table 2, which explains how the measurements or characters were assessed. Most of these characters are considered as diagnostic in recent taxonomic investigations (e.g. Hroudová & al. 2007a, 2007b; Simons & al. 2016; Pisová & al. 2017; Nikolić & al. 2019; Di Natale & al. 2020). Styles of old spikelets were rehydrated in 3% KOH, which provided an easier assessment. Information from the works of Hroudová & al. (1999a, 2007b) and Marhold & al. (2004) was used for general morphology, ecology and distribution; Nikolić & al. (2019) for statistical measurements of the inflorescences and achenes; and Browning & al. (1995), Hroudová & al. (2007b) and Nikolić & al. (2019) for detailed data on the achenes of *B. glaucus*.

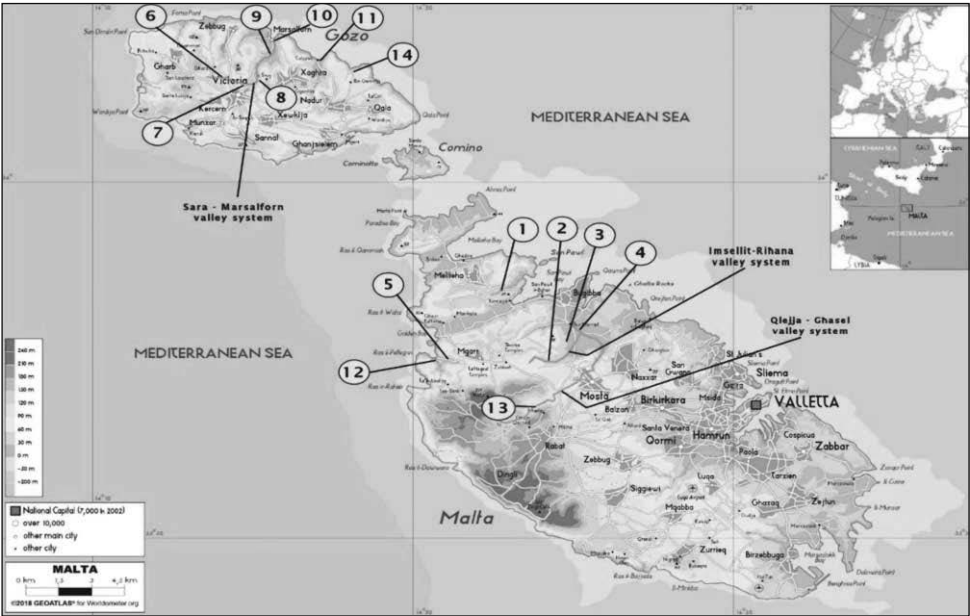


Fig. 1. Map of locations of the examined populations of *Bolboschoenus* in the Maltese Islands (BLB01-14) some of which are found along common valley systems, namely, Wied Imsellit-Mula-Rihana in mainland Malta and Wied Sara-Cianti-Marsalforn in Gozo. (map provided online by Worldometer.info, 01-May-2021).

Table 1. Locations of examined populations, including population size and date of sample collections and water analyses. Populations marked by * denote that rayless plants were present and were examined and compared separately with ray-bearing plants (in case they represented a different taxon).

Sample code	Island	Locality	Toponym (valley name)	Valley system	Population size in terms of length along the valley (metres) [f = fragmented]	Sample collection date	Date of water analyses
BLB01*	MALTA	Mellieha	Wied tal-Mistra	A	50–100 (f)	26/04/2021	25/01/2021
BLB02*	MALTA	San Pawl il-Baħar	Wied tal-Hżejjen	B	50–100	05/05/2021	25/01/2021
BLB03	MALTA	San Pawl il-Baħar	Wied tal-Arkata	B	300–400	01/04/2021	25/01/2021
BLB04	MALTA	San Pawl il-Baħar	Wied Riħana	B	80–100	01/04/2021	25/01/2021
BLB05	MALTA	Mġarr	Wied tal-Ġnejna	C	400 – 500 (f)	01/04/2021	07/02/2021
BLB06	GOZO	Għasri	Wied Sara	D	> 500	29/03/2021	01/02/2021
BLB07	GOZO	Victoria	Wied tal-Kapuċċini	D	20–30	10/04/2021	01/02/2021
BLB08*	GOZO	Victoria	Wied ta' Ċianti	D	300–400	29/03/2021	01/02/2021
BLB09*	GOZO	Żebbuġ	Wied ta' Marsalforn	D	20–30	10/04/2021	01/02/2021
BLB10	GOZO	Żebbuġ	Marsalforn (shore)	D	50–100	10/04/2021	01/02/2021
BLB11	GOZO	Nadur	Wied tar-Ramla	E	50–100	26/04/2021	09/02/2021
BLB12	MALTA	Rabat	Wied tal-Baħrija	F	20–30	No flowering	07/02/2021
BLB13	MALTA	Rabat	Wied tal-Qlejgha	G	10–20	No flowering	25/01/2021
BLB14	GOZO	Xagħra	Wied Ta' San Blas	H	5–10	No flowering	09/02/2021

Table 2. List of characters examined for the taxonomic assessment of *Bolboschoenus* occurring in the Maltese Islands, including brief explanation of how they are evaluated or measured during inflorescence.

Character	Remarks
Number of sessile spikelets	Sessile spikelets are defined as those borne on rays (=peduncles) that are shorter than their own length. A ray is hence defined as those being longer from the longest subtending spikelet in a given inflorescence.
Number of ray-borne spikelets	Total sum of spikelets born on rays.
Length of sessile and ray-borne spikelets	Measurement in mm from the insertion of the peduncle at the base of the spikelet to the summit, excluding bristles and anthers.
Width of sessile and rayed spikelets	Measurement in mm of the widest part of the spikelet, excluding bristles and anthers.
No. of rays	Total number of rays in an inflorescence.
Length of rays	Average length of rays in mm (the total length of all rays divided by the number of rays).
No. of spikelets per ray	The total number of all ray-borne spikelets divided by the number of rays.
Length of achene	Measurement in mm from the base to the tip of the achene, including the beak.
Width of achene	Measurement in mm along the widest part of the achene.
Length of beak	Measurement in mm from the curvature of achene's summit to the tip of beak. Under the stereomicroscope, it has a slightly different colour tone from the achene.
Mesocarp/exocarp ratio	Ratio of the thickness of the exocarp and the underlying mesocarp.
Cross section shape	Cross-sectional shape is assessed along the widest part of the achene.

Pollen examination

Pollen from three plant samples from each population were studied. Fresh inflorescences (or up to 48 hours after collection) were shaken or tapped over a 10 × 10 cm clean aluminium foil on which pollen was released and deposited over. A small amount of pollen was transferred on a drop of water on a microscope slide and examined with the × 40 and × 100 magnification of a Carl-Zeiss AxioLab RE compound microscope. Microphotographs of the pollen using an eyepiece reticule as a scale bar were taken at × 100 magnification. Using the image software Piximètre V5.10 by A. Henriot and J.-L. Cheype, the length and width of at least 31 pollen grains per sample were measured. Statistical data, including a X-Y scatter plot, were used to compare and detect any variation between presumed different taxa, such as the ray-bearing and rayless specimens.

Achenes examination

Achenes from various spikelets of a single sampling plant were examined when fully ripe and hence when they have hardened and darkened to a brown colour. Five sample plants per population were examined, and results were averaged down. When a population comprised of rayless and ray-bearing plants, five samples from both of each morphotype were studied. The morphology and sizes of eleven achenes per sample were examined and photographed with a binocular microscope, using a calibrated eyepiece reticule as a scale

at the $\times 6$ magnification. The length and width of the achene and the length of the beak was carried out on the microphotographs using the software Piximètre V5.10 (*ibid.*). The thickness of the exocarp and mesocarp is highly diagnostic and was measured using agar to hold the transversally-sectioned achenes in place while being examined and photographed under the microscope. The thickness of the exocarp:mesocarp layers and the anatomy of the cells of the exocarp of five achenes per sample were examined under a Carl-Zeiss AxioLab RE light microscope under the $\times 40$ and $\times 100$ magnification, with auxiliary top-light illumination. The imaging software Piximètre (*ibid.*) was again employed to measure the mesocarp and exocarp thickness and calculate their ratio. Usually, this was carried out from three places along the pericarp wall and averaged out.

Results

Ecology and other observations on the examined populations

The field surveys allowed the collection of inflorescences and pollen from 11 out of the 14 populations that were surveyed. The small populations of BLB12 (Wied tal-Qlejgha), BLB13 (Wied tal-Bahrija) and BLB14 (Wied San Blas) did not produce inflorescences despite several staggered visits and hence were not considered in the results. The ecology and vegetative parts were found to be similar to the other populations except that they were heavily shaded by strands of *Arundo donax* L. and which may inhibit flowering as it had already been previously observed (Hroudová & al. 1997b, 2007b).

The populations in the Maltese Islands seemed to share the same habitat of fluvatile or/and stagnant parts of valleys, preferring exposed areas to sunlight. The conductivity and total dissolved solids (see Table 3) were more or less in the same range between 1800 to 5900 μS , with the majority having values of approximately 2500 μS . Slightly polluted waters explain this elevated value, generally originating from leaching of artificial fertilizer seeping from fields lining the valley sides were examined material resided. Population BLB01, had a relatively low conductivity value of 410 μS since it was situated where the valley was not lined by cultivated fields. Moreover, the valley was rather dry, and the small population was in an isolated water pond formed from surrounding, uncontaminated, run-off water. Only the shore-dwelling population at Marsalforn (BLB10) had an elevated conductivity value of 7180 μS , but however, the population did not progress further towards the sea, where water ponds had values of 13,000 μS and above. Hence, all *Bolboschoenus* populations in the Maltese Islands occurred in freshwater wetlands.

Inflorescence and spikelets

Populations were predominantly composed of specimens with three to five distinctly long rays that were on average, between 25 and 52 mm long with 5 or more spikelets each. However, there were smaller individuals within most populations without or at most three short rays (< 18 mm long) with only 1–3 spikelets each. In addition, several populations included intermediate forms, exhibiting a gradual variation from the dominant umbellate inflorescences to the rayless and compact morphotype, most remarkable in BLB01. The morphometric results of each flowering population are given in the upper half of Table S1 in the Electronic Supplementari File 1 (ESF1) and summarised in Table 4.

Table 3. Conductivity (μS), pH and TDS (ppm) readings of water sampled from waterbodies of each examined *Bolboschoenus* population.

Sample code	Locality	Toponym	Conductivity (μS)	pH	TDS (ppm)
BLB01	Mellieħa	Wied tal-Mistra	410	8.01	0.25
BLB02	San Pawl il-Baħar	Wied tal-Hżejjen	4300	7.88	2.16
BLB03	San Pawl il-Baħar	Wied tal-Arkata	3570	8.02	1.78
BLB04	San Pawl il-Baħar	Wied Rihana	5900	7.72	3.01
BLB05	Mġarr	Wied tal-Ġnejna	3320	7.33	1.66
BLB06	Għasri	Wied Sara	2420	7.83	1.21
BLB07	Victoria	Wied tal-Kapuċċini	2450	7.94	1.23
BLB08	Victoria	Wied ta' Ċianti	1780	8.09	0.89
BLB09	Żebbuġ	Wied ta' Marsalforn	3500	7.05	1.72
BLB10	Żebbuġ	Marsalforn (shore)	7180	7.81	6.26
BLB11	Nadur	Wied tar-Ramla	2970	7.51	1.49
BLB12	Rabat	Wied tal-Baħrija	2830	8.1	1.41
BLB13	Rabat	Wied tal-Qlejgħa	1840	8.22	0.92
BLB14	Xagħra	Wied Ta' San Blas	3590	7.73	1.79

Most of the plants exhibited an umbellate inflorescence, consisting of 6–13 sessile spikelets overtopped with rays bearing about 3–6(–8) spikelets each, these slightly shorter than the sessile spikelets (Fig. 2a). However, the length differs upon the maturity of the spikelets, where spikelets are always longer during the infructescence. The variation of spikelet length has also been documented (Browning & al. 1998; Hroudová & al. 2005; Nikolić & al. 2019), where some examples with long catkin-like spikelets have been described under the epithet *macrostachys*. When some spikelets were measured during the fruiting period, they were about 40% longer and up to 35 mm long, but maintained more or less the same width (Fig. 2b). The length of the rays varied from about 10 – 50 mm and on average, they were 25 mm long. Secondary branching has been observed in only one individual at Ta' Ċianti valley (Fig. 2d). The total number of spikelets borne on peduncles was about 2 to 3 times as much as the number of sessile spikelets. However, other plants that were smaller in size and formed compact, capitate, rayless inflorescences or with only a few short rays were consistently encountered within most populations (Fig. 2e). Since these inflorescence characters were considered diagnostic (Hroudová & al. 2007b; Pisová & al. 2017; Nikolić & al. 2019), this 'rayless' morphotype has been preliminary investigated. Intermediates between the two forms have also been observed (Figs. 2c, 2g).



Fig. 2. Variation in the inflorescences from different populations of *Bolboschoenus* in the Maltese Islands (year 2021): [a] typical form with 4–5 long rays each bearing 3–7 spikelets (BLB05, Wied tal-Ġnejna, 28-Mar); [b] robust form with large and multi-clustered spikelets (BLB08, Wied ta' Cianti, 1-Jun); [c] long-rayed (left), compact and rayless (right) and intermediate forms with short rays (middle) (BLB01, Wied tal-Mistra, 2-Apr) ; [d] specimen with secondary branches (BLB08, Wied ta' Cianti, 1-Jun); [e] semicompact form with short rays and many small dark spikelets (BLB04, Wied Rihana, 1-Apr) [f] head-like inflorescence without rays similar to *B. maritimus* (BLB09, Wied ta' Marsalforn, 15-Apr); [g] lax inflorescences with long rays (left) and compact form with short rays (BLB02, Wied tal-Hżejjen, 4-May)

Pollen morphology

The pollen mass in fresh flowers of all populations was found to be fragrant - described best as a farinose and rose-flower scent. In addition, when examining population BLB11 (30-May-2021), several bees, namely, *Apis mellifera* s. l. and bee species from the Halictidae family (pers. comm. Thomas Cassar, June 2021) were attracted to the spikelets and harvested pollen from them (see ESF1, Fig. S1). The fragrance and insect-relationship were unexpected from a wind-pollinated genus (De Fillips 1980) which seems that it evolved to gain an extra advantage in territories where insect pollination is predominant.

The pollen shape is generally conical-ovate, with one pole rounded to subtruncate and wide, tapering gently to the other pole, which is obtuse to subacute, as shown in Fig. 4. The surface seems to be unevenly verrucose, and sometimes, there is a shallow, plug-shaped, protrusion at the wide pole (Fig. 4). The germinative cell is large and granular, either central and free in many of the pollen examined or less frequently as a stout and T-shaped body, where its central limb adheres at the wide pole, and the other two perpendicular limbs are in contact with the lateral sides just above the other pole. As a result, three empty vacuoles, two laterally at the wide pole and a larger one below the germinative cell are formed (Fig. 4). This pattern was found homogeneously within all examined samples.

The average size was found to vary between $51.8 \times 30.4 \mu\text{m}$ (BLB05) to $62.4 \times 32.9 \mu\text{m}$ (BLB09), with further statistical details given in ESF1, Table S1 and illustrated in ESF1, Fig. S2. The pollen sizes between the compact/rayless forms and the ray-bearing morphotypes were compared in three populations. The results, displayed as scatter-plot diagrams and quartile statistical data (see ESF1, Fig. S3), show a major overlap between the two types. Consequently, no diagnostic differentiation could be interpreted from a palynological point of view.

Since very limited literature dealing specifically with the palynology of *Bolboschoenus* was found, interpretations on these ‘vacuoles’ cannot be made. For example, they were not shown or discussed in pollen studies of *B. maritimus* by Sosam & Al-mayyahi (2018). The resulting measurements cannot be critically compared and discussed at present, but this data can be used for future comparisons and supply data in what seems to be a knowledge gap on the palynology not only in *Bolboschoenus*, but within many genera of the *Cyperaceae* (pers. comm., Jane Browning, Dec-2021).

Achene morphology

The size and morphology of the achenes were reported in the lower half of ESF1, Table S1, based on a sample of 55 achenes per population (eleven achenes/five specimens). There was a remarkable difference in fertility between the populations in Gozo and mainland Malta. Most plants within the Gozitan populations (BLB6–BLB11) were fertile, with a few plants (mostly the rayless forms) having poor or no production of achenes. Regarding the Maltese populations, BLB01 was slightly fertile, where a small pocket of plants had a few achenes at the base of some spikelets, no achenes could be found when examining some 50 to 100 plants within populations BLB02–BLB05.

Upon studying the morphology of the achenes, two defined morphotypes are distinguished based on the features described below:

Achenes of the populations in Gozo (Fig. 3a) were distinct for their larger size and thicker exocarp layer. Achenes measured $2.74 - 3.80 \times 1.66 - 2.21$ (mean 3.14×1.94) in size with a distinct nipple to mucro shaped beak about 0.3 mm long (Fig. 3a.1). The meso-

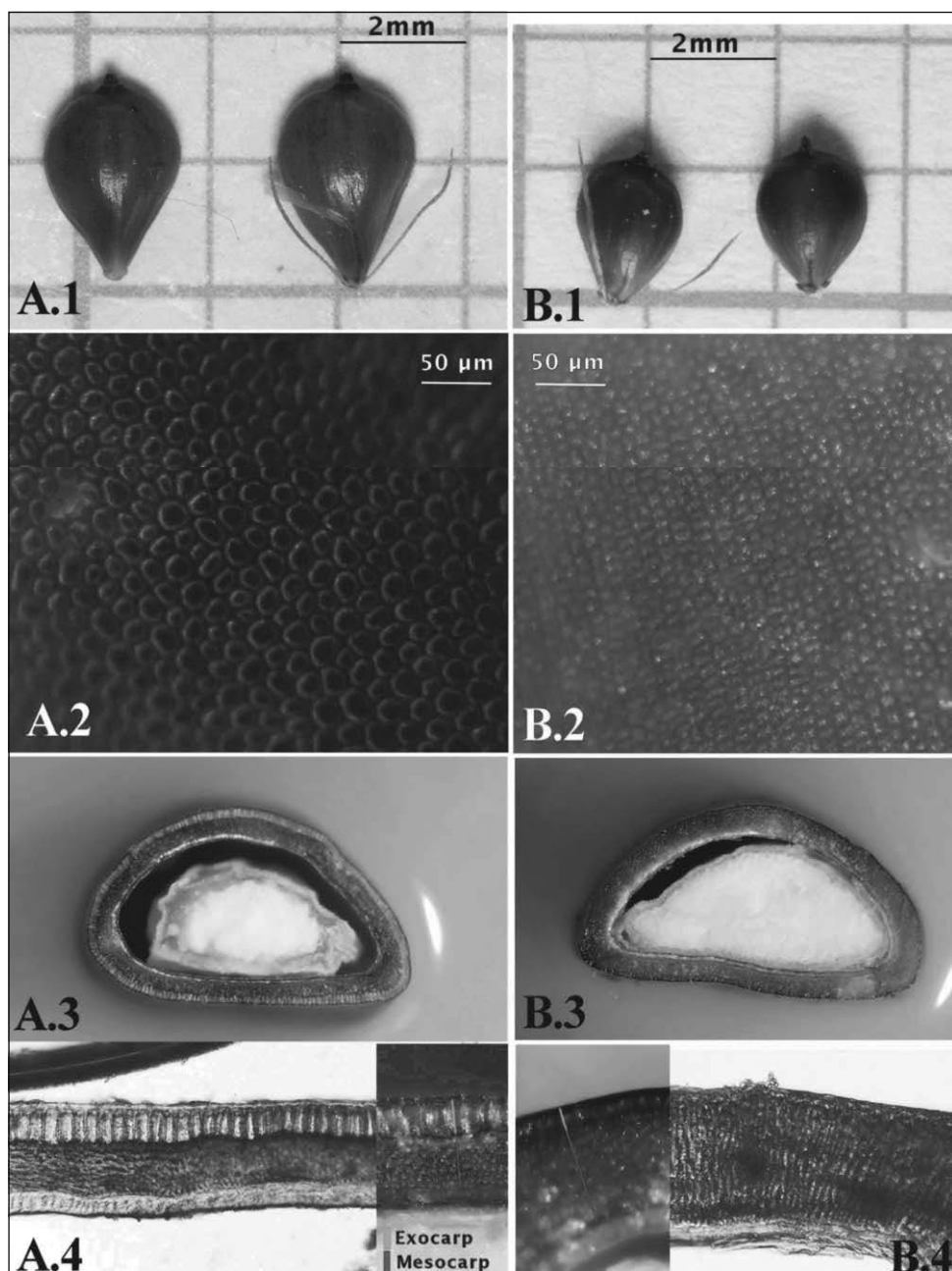


Fig. 3. Comparison of achenes from population BLB01 (Wied tal-Mistra, mainland Malta) [A1—4] and population BLB08 (Wied ta' Cianti, Gozo) [B1—4]: A,B.1: Shape and size of achene; A, B.2: pattern on the coat of the achene; A,B.3: cross-sectional shape and layers of the pericarp; A,B.4: Section of pericarp coat at $\times 100$ magnification showing the three layers of the exocarp (green), mesocarp (purple) and thin layer of endocarp (unmarked).

carp was 1.5 to 2.1 times the thickness of the exocarp layer (Fig. 3a.4), the latter composed of rectangular cells, two to three times longer as broad (Fig. 3a.3). The arrangement of the exocarp cells formed a circular to elliptic pattern measuring 20–30 μm across and grouped in a regular grape-like pattern (Fig. 3a.2). Small air bubbles were formed in the exocarp cells shortly after being immersed in water – a character given importance by Browning & al. (1998) and Browning & Gray (2000).

Population BLB01 in Mistra (Fig. 3b) had smaller achenes, measuring $2.12 - 2.58 \times 1.66 - 2.21$ mm (mean 2.34×1.68 mm), including a beak of about 0.24 mm long (Fig. 3b.1). The exocarp was very thin and hardly visible, approximately one-fifth of the thickness of the mesocarp layer (Fig. 3b.4). The exocarp cells were squarish, with the breadth equal to their length (Fig. 3b.3). The surface of the exocarp consisted of rectangular to irregular polygon-shaped cells, 10–12 μm long, and arranged in short rows resembling ladders (Fig. 3b.2). No air bubbles were seen in the cells when immersed in water.

The outline shape of the nut was similar in both morphotypes, obovate with a gently curved constriction at the lower third and attenuating and tapering to an acute base. The apex is broadly rounded to subtruncate and ends with a distinct, dark-coloured beak at the summit. The cross-section is generally lenticular to plano-convex, sometimes compressed sub-trigonal, usually with the abaxial side flat or gently convex and the adaxial side broadly convex but never distinctly trigonal or triangulate. The perigon bristles were equal in length in both morphotypes (approx. 2.0–3.0 mm). There are 4–6 perigon bristles in both morphotypes, and at least three are persistent when the achene falls out. All specimens examined had a trifid style.

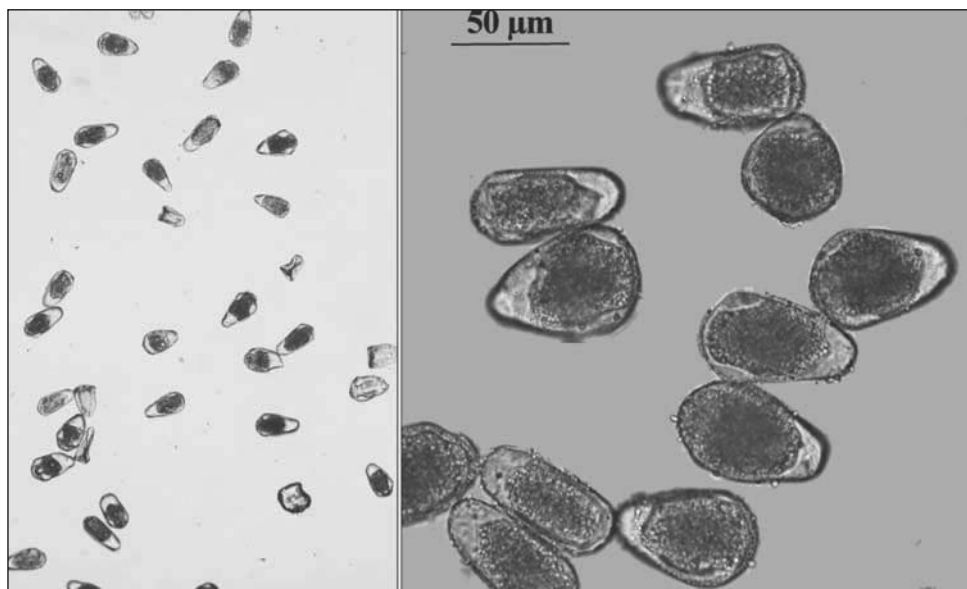


Fig. 4. Pollen grains at $\times 40$ (left) and $\times 100$ magnifications (right) from population BLB09 (Wied ta' Marsalforn). All populations had a similar pollen morphology regarding to the outline shape and size.

Discussion

The morphological analysis of the Maltese populations identified two distinct morphological differences: one in the architecture of the inflorescences and a more important ones in the achenes.

Most plants had inflorescences with long rays in all populations, but a small number of plants had compact, head-like inflorescences, hence either without rays or a few short ones. As repeatedly advocated by recent taxonomical studies, achene morphology is much more diagnostic and reliable for *Bolboschoenus*; primarily postulated by Browning & Gordon-Grey (1993; 2000) and Hroudová & al. (1998b, 1999b), reconfirmed in subsequent works (e.g. Hroudová & al. 2007b) and later supported by genetic (Pisová & al. 2017) and statistical analysis (Pisová & al. 2017; Nikolić & al. 2019). Indeed, when the achene of the rayless plants was examined, as discussed below, these forms did not correspond with *B. maritimus*, which have a different achene morphology (refer to Table 4).

The pooled morphometric data gained from the Maltese material (two morphotypes) was compared with *B. maritimus*, *B. laticarpus* and *B. glaucus* (Fig 4.) in which morphological data was retrieved from Marhold & al. 2004; Hroudová & al. 2007b and Nikolić & al. 2019). The morphotype ‘astericum’ of *B. glaucus* has been selected from the four presented by Nikolić & al. (2019) as it had the best overall resemblance with the Maltese material. The species *B. yagara* and *B. planiculmis* have been excluded from a critical comparison. Firstly, Malta is far away from the distributional range of these two species, with the nearest records reported from northern regions of Italy bordering Austria (Di Natale & al. 2020). Secondly, they are morphologically the most different amongst the *Bolboschoenus* taxa recorded in Europe – *B. planiculmis* achenes have the lateral sides distinctly concave and possess a very thick exocarp layer, whereas *B. yagara* has narrow achenes that are strongly trigonous (Browning & Gordon-Gray 2000; Hroudová & al. 2007b; Wollstonecroft & al. 2011; Pisová & al. 2017).

The morpho-anatomical characteristics of achenes of the rayless forms were identical to those of the umbellate forms within each population. In fact, all examined material (including the rayless forms) the exocarp layer of the achenes was consistently narrower than the mesocarp, and hence, none of the populations (including the rayless forms) corresponds to *B. maritimus*, which instead has a characteristic thicker exocarp. Likewise, pollen morphology showed no differentiation between rayless and pedunculate plants within three tested populations (ESF1 Figs. S2 and S3). Therefore, it can be concluded that *B. maritimus* does not presently occur on the Maltese Islands.

These rayless individuals likely correspond to ecotypes, either formed by younger, first-flowering plants, or plants growing in drier parts or deprived ground where they do not reach the full vigour of the adult plants, a condition already observed by Browning & al. (1998) in some *Bolboschoenus* species in Africa.

Once concluded that the rayless individuals are not *B. maritimus*, the *Bolboschoenus* populations in the Maltese Islands are considered to be one wide-sense taxon split in two distinct morphotypes based on the different characters of the achenes. However, a second and more puzzling question had emerged from the present investigation - why is there a high degree of female-sterility (plants failing to produce achenes), particularly within the populations of mainland Malta? Since *Bolboschoenus*

Table 4. Comparison of the two morphotypes, one in Malta, and the other in Gozo, with the respective characters of the inflorescences and achenes of *Bolboschoenus glaucus*, *B. laticarpus* and *B. maritimus*.

Plant material or taxon	Maltese population (excl. rayless ecotypes) Morphotype 1: mainland Malta	Maltese population (excl. rayless ecotypes) Morphotype 2: Island of Gozo	<i>B. glaucus</i>	<i>B. laticarpus</i>	<i>B. maritimus</i>
Ecological preference	Glycophyte		Glycophyte	Glycophyte	Halophyte
Habitat	Valley beds with freshwater streams that dry completely in summer. Not present in shores with high salinity.		Freshwater wetlands that dry completely in summer, such as river banks, small streams and flood plains.	Wide range of wetland habitats preferring river banks, floodplains and temporarily flooded land	Seashore and marshland areas with high salinity, much less frequently in freshwater wetlands
Distribution	Malta (Central Med. Region)		South Europe and the Med. Region	Central Europe	Throughout Europe
INFLORESCENCES					
$\bar{x}$ =mean; r=range					
No. of sessile spikelets	6–12(–13)	5–13(–16)	(5)–6–10(–15)	(4)–5–8(–11)	(1)–3–7(–10)
Length of sessile spikelets (range, mm)	7–18(–20.5)	9–22(–26)	(7)–9–13(–17)	(9)–11–16(–23)	(8.5)–11–25(–37.5)
Length of sessile spikelets (average, mm)	14.2	15.3	11.74	13.7	18.4
No. of rays	(3)–4–6(–7)	(3)–4–7(–8)	(3)–6–8(–11)	(1)–3–5(–6)	0–2(–5)
No. of rays ($\bar{x}$)	5.3	4.8	7	3	1.32
Length of rays (r, mm)	(10)–18–36(–50)	(9)–17–39(–58)	(12)–16–24(–32)	(10)–17–34(–41)	0–23(–40)
Length of rays ($\bar{x}$, mm)	27.5	28.7	20.18	25.6	12.3
Total no. of ray spikelets	(8)–11–42	(9)–12–45	(16)–23–48(–77)	(3)–6–18(–32)	0–7(–18)
Total no. of ray spikelets ($\bar{x}$)	23.2	23.0	36	12.6	3.4
Number of spikelets per ray	4.4 (2 – 8)	4.9 (3 – 9)	(2)–3–7(–10)	(1)–2–4(–6)	0–3(4)
Ray spikelet length (r, mm)	8.7–15.8(–18.4)	10.3–18.8(–20.3)	(7)–9–13(–17)	(8)–10–14(–19)	11–21(–34)
Ray spikelet length ($\bar{x}$, mm)	10.7	12.3	11.2	12.35	11.1
ACHENES					
$\bar{x}$ =mean; r=range					
Achene production	Mostly sterile	Mostly fertile	Fertile	Fertile	Fertile
Achene length ($\bar{x}$, mm)	2.34	3.10	2.59	2.81	2.91
Achene width ($\bar{x}$, mm)	1.68	1.97	1.81	1.91	2.01
Achene length (r, mm)	2.12 – 2.58	2.84 – 3.37	2.12 – 3.05	2.55 – 3.17	2.22 – 3.83
Achene width (r, mm)	1.52 – 1.84	1.74 – 2.11	1.53 – 2.24	1.67 – 2.22	1.41 – 2.5
Beak length ($\bar{x}$, mm)	0.25	0.28	0.18	0.31	0.33
Mesocarp/Exocarp ratio	× 4.5 – 6.0	× 1.7 – 2.4	× (2)–4–5 (–10)	× 3.0	× 0.5
Bristles	4–5 persistent bristles	3–4 persistent bristles	4–6 persistent	2–3 persistent	Caducous, 0 (–1)
Length of bristles	Reaching the top of the achene (1.8–2.9 mm long)	Reaching between half and almost the top of the achene (1.7–3.0 mm long)	Half to two-thirds of achene length	half to two-thirds of achene length	Half of the achene length
Outline shape	Obovate with a broadly rounded to sub-truncate summit and a slight constriction at the basal third.	Obovate with a rounded to a broadly rounded summit and a slight constriction at the basal third.	Elliptic-obovate with rounded and almost truncate summit	Broadly obovate	Elliptic-obovate to broadly obovate
Surface of achenes (x40 magnification)	Glossy, smooth, cellular pattern made up of irregular polygonal units arranged in short rows, 12–18 µm across	Glossy, smooth to minutely pitted, cellular pattern made up of distinct rounded units in clusters, 20–32 µm across	Glossy, cellular	Smooth, forming a fine network structure	Glossy, cellular, highly visible polygonal network
Achene colour	Varies with maturity, initially light olive-brown, finally reaching a cinnamon brown colour when old	Varies with maturity initially medium olive-brown, finally reaching a dark brown to almost black	Golden to dark brown	Medium to dark brown, sometimes blackish	Medium to rust-brown, rarely dark brown
Exocarp cells L/W ratio	Squarish (isodiametric), as long as wide when, slightly air-filled or not.	Rectangular, longer side about twice as much the shorter, distinctly air-filled.	Rectangular to about twice to three times long as wide; rarely isodiametric	Shortly rectangular, about twice long as wide	Rectangular, three times long as wide
Cross-section	Variable – Planoconvex to compressed subtrigonal, abaxial edge flattened, corners rounded or not developed	Variable - lenticular to compressed subtrigonal, abaxial edge flattened, corners rounded to vaguely developed	Lenticular, planoconvex or compressed to faintly trigonous	Obtusely trigonous, well-defined rounded edges, the abaxial sometimes flattened.	Lenticular or less often compressed obtusely trigonous
Number of styles	Always trifid		Trifid	Bifid and trifid	Bifid and trifid

is primarily a wind-pollinated genus, failed fertilisation due to deficiency by pollinators is not among the plausible reasons.

Owing to their shorter rays and less vigorous production of ray-born spikelets, the inflorescences of the Maltese populations are grossly more similar to *B. laticarpus* than that of *B. glaucus*; however, when compared with *B. glaucus* morphotype “astericus” sensu Nikolić & al. (2019), there is a closer resemblance in the inflorescences. At least two morphological characters are judged crucial to exclude *B. laticarpus* from the examined material. Firstly, the cross-sectional shape of the achene of *B. laticarpus* is too much trigonous when compared with that of the Maltese plants (Marhold & al. 2004; Hroudová & al. 2007b; Simons & al. 2016; Rumsey & al. 2019; Nikolić & al. 2019; Di Natale & al. 2020). Secondly, the mesocarp/exocarp ratio of about $\times 3$ reported (e.g. Hroudová & al. 2007b; Nikolić & al. 2019) is considerably different from that of approximately $\times 4$ – 5 found in one morphotype and of $\times 1.5$ – 2 in the other.

Hence, as compared in Table 4, the best match for the examined material in Malta is *Bolboschoenus glaucus*. This is a thermophilic species originating from the Old World (described from Senegal) and widely distributed in Africa (Browning & Gordon-Gray 2000), south Europe (Browning & Gordon-Gray 2000; Hroudová & al. 2007b) and the Near East (Wollstonecroft & al. 2011), hence its presence in Malta is within the current distribution. The plano-convex, non-trigonous cross-sectional shape of the achene, the mesocarp:exocarp ratio of $\times 1.7$ – 2.3 ($\times 4$ – 5 in one population) of the pericarp and the consistent 3-fid style found in Maltese material are strong characteristics of *B. glaucus* (Browning & Gordon-Gray 2000; Hroudová & al. 2007a, 2007b; Nikolić & al. 2019). The few fertile plants (achenes available) in BLB01 match well with *B. glaucus* however, the sterile populations in mainland Malta (approx. 99.5% of the plants) could not be assessed, since they do not produce achenes.

Regarding the Gozitan populations, the morphological and biological findings are closest to *B. glaucus* amongst the five European taxa but are not fully congruent with the characteristics of *B. glaucus* as reported in the literature (*op. cit.*). First of all, the achenes with an average size of 3.2×2.0 mm are significantly larger when compared to *B. glaucus*, which on average are 2.5×1.7 mm. In addition, and more importantly, the mesocarp is only 1.6–2.3 times thicker than the exocarp and remarkably different from the reported ratios of three times as much (Browning & al. 1995; Hroudová & al. 2007b & Nikolić & al. 2019) to five times or more (Hroudová & al. 2007b; Di Natale & al. 2020). Nevertheless, collection from North America (specimen Smith & Taylor 3134) was found to have the mesocarp is 1.5 times thicker than the exocarp (Browning & al. 1995), and hence similar to those from Gozo. The high variability of *B. glaucus* was well reported by Nikolić & al. (2019), who assigned four morphotypes and hinted that *B. glaucus* s. l. might consist of an aggregate of unnamed taxa. The present study similarly confirms the variability of *B. glaucus* in locally-distinct morphotypes that require further investigations employing better diagnostic tools from morphometrics to determine any significant taxonomic units.

Finally, the high degree of female sterility, specifically, pollen-producing florets that fail to develop into a fruit was generally observed in all populations in mainland Malta. In exhaustive field examination, some 200 plants were checked in populations BLB02, BLB03, BLB04 and BLB05 and all spikelets resulted being fruitless. Only a few plants

(0.5 % on a rough estimation) had 2–8 achenes in a few spikelets in population BLB01. Some sterile plants have been found in the Gozitan populations (especially the rayless forms), but these populations were generally fertile. It is noteworthy to mention that extensive sterile populations of *Bolboschoenus* (>99% of plants) have not been reported in the literature reviewed in this study or witnessed by some experts that has been consulted (pers. comm. Richard Lansdown, Mar-2021; Jane Browning, May-2021; Danijela Nikolić, Jun-2021; Zdenka Hroudová, Dec-2022).

Summing up, at least two taxonomic interpretations can be postulated for the *Bolboschoenus* populations in the Maltese Islands. The simplest one would be to attribute all the Maltese populations to *B. glaucus* s. l. with the one in mainland Malta assigned to the nominate form (it resembles the ‘asteriscus’ morphotypes defined by Nikolić & al. 2019) but unexplainably sterile, and the Gozitan population assigned to a new morphotype here provisionally labelled as ‘macrocarpus’ for its large achenes compared to the other morphotypes of *B. glaucus*.

However, a more interesting morphological relationship exists when comparing carefully the achenes of the Gozitan material with that of *B. maritimus* and *B. glaucus*. The mesocarp/exocarp ratio of about $\times 2$ in the Gozitan populations is intermediate between a thin exocarp (ratio ca. $\times 5$) in *B. glaucus* to a thicker one in *B. maritimus* (ca. $\times 0.5$). The achene size (average 3.1 mm) is larger than that reported for *B. glaucus* (2.6 mm), and smaller than *B. maritimus* (3.5 mm). The beak is well developed like *B. maritimus*, but the inflorescence architecture is rayed and similar to that of *B. glaucus*, yet less vigorous in terms of the number of rays and the number of spikelets per rays. Rayless forms (like *B. maritimus*) have been observed within the larger populations.

The second interpretation is hence the formation of complex hybrid patterns between long-existing putative parent species: *B. glaucus* in freshwater valleys and *B. maritimus* in marshlands. This can, at least in part, be an explanation of the elevated sterility in the Maltese populations. From a distributional range point of view, both species co-exist in the southern Mediterranean region (Browning & Gordon-Gray 2000: Fig.8; Hroudová & al. 2007b; Di Natale & al. 2020); with *B. glaucus* extending further south into Africa (Browning & Gordon-Gray 2000) and *B. maritimus* north into mainland Europe (Hroudová & al. 2007b). The high degree of variation in the inflorescences’ architecture (Fig. 2a-g) and the occurrence of pockets *B. glaucus* s. str. detected in few fertile specimens in population BLB01 (thus flagging the presence of one of the putative parents in Malta) are further arguments supporting hybridisation.

Although *B. maritimus* has not been retrieved in this study, its absence does not necessarily imply that it never existed in the Maltese Islands. *B. maritimus* might have been present in brackish and marshland wetlands that once existed in Marsa but were destroyed in the mid-19th century for public health concerns. As a matter of fact, Grech Delicata (1853) recorded ‘*Bolboschoenus maritimus*’ from Marsa, which used to be a brackish marshland at that time. There is currently no evidence to confirm whether Grech Delicata’s record is *B. maritimus* or not. Still, ecological findings on *Bolboschoenus* throughout Europe are consistent: *B. maritimus* prefer (or grow) on brackish water, while the other species are glycophytes and prefer freshwater habitats (Hroudová & al. 1999a, 2007b).

No herbarium material from Marsa has been found to confirm Grech Delicata’s record. The only voucher specimen at Argotti Botanic Gardens [ARG] was from Wied tal-Ġnejna

collected on the 6th of April 1914 and was recorded as *B. maritimus* and was reported as rare. The specimen consisted of leaves and blossomed inflorescences and did not bear any achenes. This locality corresponds to population BLB05 and was covered in this study.

Morphometric analyses alone are not enough for the formal description of a *B. glaucus* × *maritimus* hybrid or to explain the predominant sterility in one of the morphotypes which is a new observation of *Bolboschoenus*. Further molecular and perhaps karyological investigations are hence advocated to understand in greater detail the taxonomy and biology (including sterility) of the Maltese populations of *Bolboschoenus*.

Conclusion

Morphological investigation of eleven populations of *Bolboschoenus* in the Maltese Islands resulted that *B. maritimus* reported in floristic literature does not occur. Two distinct populations are ascribed to the thermophilic *B. glaucus* one exhibiting polymorphism (possibly by introgression of another species forming a hybrid complex) and the other with a high degree of sterility. This study could not determine whether this phenomenon is driven by external ecological influences or intrinsic genetic factors. Deeper investigations using better diagnostic tools from morphometrics are required to establish exactly what these forms genetically represent. The present author is looking forward in contributing and working with other authorities studying this genus, particularly *B. glaucus* s. l., while concurring with Nikolić & al. (2019) that the variability of *B. glaucus* should be investigated more carefully for cryptic taxa. In conclusion, local conservation of *Bolboschoenus* and its wetland habitats are strongly advocated because the *Bolboschoenus* population/s in the Maltese Islands might correspond to a taxon novelty not yet reported anywhere in Europe. Maltese valleys and wetlands are increasingly facing threats and pressures, including habitat loss, water abstraction, nitrogen pollution, introduction of alien species and increased drought by climate change.

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S. Cambria, G. Giusso del Galdo, P. Minissale, S. Sciandrello & G. Tavilla

***Lablab purpureus* (Fabaceae), a new alien species for Sicily**

Abstract

Cambria, S., Giusso del Galdo, G., Minissale, P., Sciandrello, S. & Tavilla, G.: *Lablab purpureus* (Fabaceae), a new alien species for Sicily. — Fl. Medit. 32: 73-78. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Lablab purpureus (Fabaceae) is a species native to tropical Africa, but widely cultivated and naturalized in many tropical and sub-tropical countries. We report the occurrence of this species for the first time in Sicily, close to the town of Palermo. The population seems to be well established and takes part to a sub-nitrophilous vegetation dominated by *Oloptum miliaceum*, representing the first case of naturalization of this species in Italy.

Key words: biological invasion, naturalization, invasive species, Italy, vascular flora, xenophytes.

Introduction

Lablab Adans. is a monotypic genus that includes only one species, i.e., *Lablab purpureus* (L.) Sweet (Moteetee & Van Wyk 2012). Previously, it was included within the genus *Dolichos* L., but morphological, nomenclatural, and molecular surveys showed that the two genera should be separated (Verdcourt 1968, 1970; Wojciechowski & al. 2004). Actually, *Lablab* can be distinguished from *Dolichos* and related genera by several characters including a blade-like style, a non-penicillate stigma and verrucose pod margins (Verdcourt 1978). Some authors (Verdcourt 1970; Maass & al. 2005) and databases (POWO 2021; PROTA 2022) recognized the occurrence of three different subspecies within this taxon, although Amkul & al. (2021) deny their existence on the basis of genetic investigations.

During floristic investigation focused on the alien flora in Sicily, we unexpectedly found a huge population of *L. purpureus*. This species is probably indigenous to tropical Africa, but is widely cultivated in tropical and subtropical regions of the world (CABI 2021; POWO 2021). It is a crop whose use as food has been reported in India prior to 1,500 BC and in Egyptian Nubia from the 4th century AD (Maass & al. 2010). In fact, it is well known the food use of its unripe beans and seeds, which have also an important medicinal value for dissolving kidney stones (Bobos 2016; Bobos & Ivanitskaya 2018). Besides, *L. purpureus* is characterized by several qualities that make it an excellent forage also for dry areas. It is drought resistant and adaptable to different environmental conditions, staying green during the dry season (Murphy & Colucci 1999). According to the literature, the

species has been introduced and is often a weed in many areas, such as India, China, Central and South America, Australia, Indonesia, Thailand, Pacific Islands, Cape Verde, etc. (Sánchez-Pinto & al. 2005; Celesti-Grawpow & al. 2009; POWO 2021). In some areas it is of considerable economic interest, representing for instance the third most significant vegetable in central and south-western regions of Bangladesh (Rashid & al. 2007).

As concerns the European countries, this species is generally cultivated only as an ornamental plant and was reported as naturalized in the Canary Islands and Madeira (Borges 2008; Verloove 2013). In the Italian territory, *L. purpureus* is hitherto recorded only in Lombardy (N Italy) as casual alien (Banfi & Galasso 2010; Galasso & al. 2018), while previously Fiori (1925) reported it only as a cultivated plant. Therefore, our finding is the first record for Sicily and the earliest naturalized population in Italy.

Material and Methods

We carried out the study of the new wild population on living material. The specimens are stored in the Herbarium of the University of Catania (CAT, herbarium acronyms follow Thiers 2021). The taxonomical identification was made following several papers and floras regarding this taxon (Verdcourt 1970, 1978; Laguna-Lumbreras 2001; Wu & Thulin 2010; Hyde & al. 2021). We mapped the distribution range of the species and its area of occupancy (AOO) in Sicily by using QGIS tools (QGIS.org 2021). According to the phytosociological method (Braun-Blanquet 1964), the vegetation in which the species occurs was investigated. Furthermore, the risk assessment protocol for invasive alien species was used to identify the risk class (Weber & Gut 2004).

Results and Discussion

Lablab purpureus (L.) Sweet in Hort. Brit., ed 1, pt. 2: 481, (1826).

Synonyms: *Dolichos lablab* L., Sp. Pl.: 725 (1753); *Dolichos purpureus* L., Sp. Pl., ed. 2, 2: 1021 (1763); *Lablab niger* Medik. in Vorles. Churpf. Phys. Ges. 2: 354 (1787); *Lablab vulgaris* Savi in Nuov. Giorn. Lett. (Pisa) 8: 116, fig. 8/a-c (1824); *Vigna aristata* Piper, Contr. U.S. Natl. Herb. 22: 665 (1926).

Lablab purpureus is an annual or short-living perennial plant, with leaves alternate, trifoliate, flowers in axillary inflorescences, whitish-purple to purple, style not winged along the margins and legumes oblong-falcate, usually verrucose along the margins, seeds 3–5, oblong, with hilum linear (Verdcourt 1970; Wu and Thulin 2010). The occurrence of *L. purpureus* in Italy is known only for a locality in Lombardy region. In particular, just a single plant was observed in Milan city along the roadsides, probably arising from seeds accidentally dropped by a plant cultivated in the nearby ornamental gardens (Banfi & Galasso 2010). During fieldwork in north-western Sicily, we found a consistent population of *L. purpureus* constituted by almost one hundred plants, growing along a roadside near Altavilla Milicia (Palermo, 38° 2'36.41"N 13°33'25.51"E; see Figs. 1, 2).



Fig. 1. Distribution map of *L. purpureus* in Sicily.



Fig. 2. *Lablab purpureus* from Sicily: **A)** details of flower; **B)** habitus; **C)** leaves.

In particular, the species colonizes a clayey slope at an altitude of 18 m a.s.l., covering a surface of about 250 m². It is linked to a plant community characterized by other allochthonous taxa, such as *Ricinus communis* L., *Boerhavia coccinea* Mill., and *Opuntia ficus-indica* (L.) Mill. (Table 1). It is a typical alien-dominated phytocoenosis (Vicini & al. 2020) with a mainly summer-autumn blooming behavior, linked to really disturbed stands with soils particularly rich in nitrates. This community can be ascribed to *Boerhavia-Oryzopsisietum miliaceae* Brullo 1984, included in the *Bromo-Oryzopsisietum miliaceae* O. Bolòs 1970 alliance (Brullo 1984). This association was recorded almost 40 years ago in the Palermo area and today, as we observed, it is widely spread in many sites all around Sicily, especially along coastal roadsides. *L. purpureus* increases the alien biodiversity of this plant community. In our research, we applied one weed risk assessment approach. Based on the methodology described by (Weber & Gut 2004), sum of points for the *L. purpureus* in Italy amounted 22/39, and the species can be categorized as intermediate risk, therefore, it requires further observation. According to the classification by Pyšek & al. (2004), the population of *L. purpureus* in Sicily should be considered as naturalized. The data in our possession suggest that the species has been present for at least three years in this locality, probably escaped from some nearby gardens where it could have been used as an ornamental. The species bears fruit regularly and can be assumed that its local spread is linked mainly to seeds dispersion. In conclusion, the occurrence and the spread of *L. purpureus* in Sicily need to be adequately monitored, since it may represent a future risk to natural and semi-natural ecosystems, and it could invade the nearby areas.

Table 1. Phytosociological relevés of plant community with *L. purpureus* in Sicily.

Relevés	1	2
Area (mq)	50	50
Altitude (m a.s.l.m)	18	18
Slope (%)	40	40
<i>Lablab purpureus</i> (L.) Sweet	2	2
<i>Oloptum miliaceum</i> (L.) Röser & H.R. Hamasha	2	1
<i>Opuntia ficus-indica</i> (L.) Mill.	.	3
<i>Boerhavia coccinea</i> Mill.	1	1
<i>Ricinus communis</i> L.	1	.
<i>Erigeron bonariensis</i> L.	.	+

Specimina visa

ITALY: Sicily, Altavilla Milicia (Palermo), roadsides near the tollbooth of the motorway, 38° 2'36.41"N 13°33'25.51"E, 18 m a.s.l., 14 July 2021, *S. Cambria s. n.* (CAT).

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C. Charalampidou, E. Eleftheriadou & K. Theodoropoulos

The vascular flora of damp meadows and mires in W Rhodopi (NE Greece)

Abstract

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The plant diversity of damp meadows and mires occurring in the Greek part of Rhodopi Mountain Range, at an elevation of above 1000 m, was investigated. The vascular flora of the investigated area consists of 340 taxa, which belong to 175 genera and 58 families. Seven taxa of vascular plants were recorded for the first time for the floristic region of North-East Greece. The most species-rich families are *Poaceae*, *Rosaceae*, *Asteraceae*, *Cyperaceae*, *Lamiaceae*, *Fabaceae* and *Juncaceae*, whereas 20 families contribute with only one taxon. The most species-rich genera are *Carex*, *Juncus* and *Ranunculus*, and 103 genera have only one taxon. Most of them are hemicryptophytes (203 taxa), followed by therophytes (51 taxa), geophytes (45 taxa), phanerophytes (27 taxa) and chamaephytes (14 taxa). The high percentage of hemicryptophytes reflects the continental character of the flora. The majority of the taxa (228 taxa) are Widespread (76 taxa European-SW Asian, 53 taxa Euro-Siberian, 37 taxa European etc.), followed by the Mediterranean unit with 57 taxa (24 taxa Mediterranean, 22 taxa Mediterranean-European, etc.) and the Balkan unit with 53 taxa (31 taxa Balkan, 17 taxa Balkan-Anatolian, etc.).

Key words: floristic inventory, phytodiversity, Balkans, chorology, protected areas.

Introduction

In Europe, peat bogs have a tendency of becoming less and less frequent from north to south and they occur in areas where forests generally represent the natural plant community, where humans haven't interfered (Minelli 2004).

In Greece, peatlands are infrequent attributable to special characteristics, such as the warm/dry climate, the limestone bedrocks' predominance, which are combined with anthropogenic factors over numerous thousand years (Bouzinou & al. 1994, 1997; Papazisimou & al. 2002). Peatlands, primarily topogeneous fens are extant in various regions, specifically in country's north sectors (Botis & al. 1993; Bouzinou & al. 1997, 2000). Ombrotrophic peatlands are not known in Greek territory, although more oligotrophic mires are present in the uplands of northern Macedonia and Epirus (Payne & Mitchell 2007).

According to Tanneberger & al. (2017), the extent of mires in Greece amounts to 0.035% of the total area of the country.

The mountain range of Rhodopi sustains a rich variety of flora and vegetation. In Rhodopi, some habitat types meet their southernmost distribution limits, such as the “Transition mires and quaking bogs (7140)” habitat type that occurs in the National Park of Rhodopi Mountain range. This habitat type code replaced that of “*Sphagnum* acid bogs (7130, Blanket bogs)”. Here we can find some species with their main distribution in N and high-montane Eurasia (extending occasionally to North America), which reach the Northeast or Northern part of the country, without extending southwards; e.g. *Drosera rotundifolia* (Athanasiadis & Gerasimidis 1978; Theodoropoulos & Eleftheriadou 2012).

Similar surveys in which floristic lists of this particular habitat type are presented, have been conducted in neighboring Bulgaria by Atanassova & Marinova (2005) and Dimitrov (2016). In Greece, knowledge of the vascular plants of these areas is provided by Sarika-Hatzinikolaou & al. (1996) and Eleftheriadou & Theodoropoulos (2015), with the latest study being the only one in the present research area. Thus, this study aims to fill the gap in knowledge of this habitat type by presenting a complete catalogue of all vascular plants.

Material and methods

Study area – The research area is situated in the west and central parts of Greek Rhodopi, where four sites [GR1140001 – Dasos Fraktou, GR1140002 – Rodopi (Simida), GR1140003 – Periochi Elatia, Pyramis Koutra and GR1120003 – Oros Chaintou-Koula kai gyro koryfes] were included in the “Natura 2000” Network. The research area is located in the last three sites mentioned above (Fig 1). The surrounding vegetation consists of pure or mixed *Pinus sylvestris*, *Picea abies* and *Fagus sylvatica* stands.

Geologically, the area is part of the Rhodopi massif (Mountrakis 1985). The substrate in most of the study area consists of acid igneous (granites, granodiorites, monzonites) and acid to intermediate volcanic rocks (pyroclastics), whereas a small part has metamorphic rocks (amphibolites, gneisses, schists with marble intercalations and marble or crystalline limestones) (I.G.M.E. 1983).

The climate can be characterized as humid continental with a harsh winter, a short hot summer and a rather uniform distribution of rainfall during the year. The bioclimate can be characterized as axeric, montane and hyperhumid with strong winter (Mavrommatis 1980).

Data collection and analysis – The research was based on our own field collections, carried out during spring and summer of 2018-2020, as well as on herbarium specimens collected by Eleftheriadou, Theodoropoulos and Athanasiadis during summer of 1990 and 1992. In addition, unpublished material from the investigation region was used (leg. Eleftheriadou, Theodoropoulos, Athanasiadis, August 2000). Voucher specimens are deposited in the herbarium of the Laboratory of Forest Botany-Geobotany, Department of Forestry and Natural Environment, in the Aristotle University of Thessaloniki, Greece (TAUF). The collected plant specimens were determined following the main national and European floras (Jordanov & al. 1963-1995; Tutin & al. 1968-1980, 1993; Pignatti 1982, 2017-2019; Strid 1986; Strid & Tan 1991, 1997, 2002) as well as selected taxonomic lit-

erature (Erben 1985; Krendl 1986-1987). The nomenclature follows Dimopoulos & al. (2013, 2016). Families, genera, species and subspecies are arranged alphabetically within the major groups of vascular plants, viz. *Pteridophyta*, *Gymnospermae*, *Angiospermae* (*Dicotyledones* and *Monocotyledones*). Life-form categories are assorted in accordance with Raunkiaer's classification system as was modified by Dimopoulos & al. (2013). Chorological types of taxa derive from Dimopoulos & al. (2013).

The collection sites (Fig.1) and dates are coded as follows:

Collecting localities

- 1a: Dasiko chorio Elatias, 24.32714 E, 41.47914 N, 1535 m, 25.7.2019.
- 1b: idem, 24.32616 E, 41.47900 N, 1541 m, 5.8.2000.
- 1c: idem, 24.32600 E, 41.47883 N, 1540 m, 5.8.2000.
- 1d: idem, 24.32783 E, 41.47833 N, 1533 m, 5.8.2000.
- 1e: idem, 24.32816 E, 41.47800 N, 1530 m, 5.8.2000.
- 1f: idem, 24.32816 E, 41.47816 N, 1531 m, 5.8.2000.
- 2: Stravorema, 24.34388 E, 41.49295 N, 1310 m, 26.7.2019.
- 3: Aletras, 24.14254 E, 41.51633 N, 1320 m, 19.5.2020.
- 4a: Baklavas, 24.13769 E, 41.50552 N, 1300 m, 19.5.2020.
- 4b: idem, 24.13766 E, 41.50433 N, 1280 m, 6.8.2000.
- 5: Stamna, 24.57509 E, 41.42819 N, 1375 m, 8.6.2020.
- 6: Agkathoto, 24.66564 E, 41.29086 N, 1190 m, 7.6.2020.
- 7: Kalyvia Lepida, 24.68948 E, 41.40150 N, 1475 m, 14.6.2020.
- 8a: Klivanos, 24.69686 E, 41.41026 N, 1495 m, 14.6.2020.
- 8b: idem, 24.69833 E, 41.41166 N, 1510 m, 4.8.2000.
- 8c: idem, 24.69800 E, 41.4115 N, 1505 m, 4.8.2000.
- 8d: idem, 24.69783 E, 41.41133 N, 1505 m, 4.8.2000.
- 8e: idem, 24.69750 E, 41.4110 N, 1500 m, 4.8.2000.
- 8f: idem, 24.69716 E, 41.41083 N, 1495 m, 4.8.2000.
- 9a: Loxada river, 24.69649 E, 41.41029 N, 1500 m, 10.7.2020.
- 9b: idem, 24.67966 E, 41.41333 N, 1487 m, 4.8.2000.
- 9c: idem, 24.67866 E, 41.41283 N, 1483 m, 4.8.2000.
- 10a: Kreminari mandra, 24.63483 E, 41.42633 N, 1510 m, 4.8.2000.
- 10b: idem, 24.63450 E, 41.4285 N, 1515 m, 4.8.2000.
- 10c: idem, 24.6565 E, 41.43016 N, 1520 m, 4.8.2000.
- 11a: Southeast of the village Livaditis, 24.6945 E, 41.29783 N, 1365 m, 3.8.2000.
- 11b: idem, 24.69433 E, 41.29733 N, 1362 m, 3.8.2000.
- 11c: idem, 24.68466 E, 41.29550 N, 1340 m, 3.8.2000.
- 11d: idem, 24.68400 E, 41.29483 N, 1435 m, 3.8.2000.
- 11e: idem, 24.68800 E, 41.29500 N, 1345 m, 3.8.2000.
- 11f: idem, 24.68416 E, 41.29666 N, 1340 m, 3.8.2000.
- 11g: idem, 24.68383 E, 41.29866 N, 1335 m, 3.8.2000.
- 11h: idem, 1350 m, 6.8.1992.
- 12a: Kalyvia Kontogianni, 24.73933 E, 41.33300 N, 1450 m, 2.8.2000.
- 12b: idem, 24.73916 E, 41.33283 N, 1450 m, 2.8.2000.
- 12c: idem, 24.73983 E, 41.33350 N, 1460 m, 2.8.2000.
- 12d: idem, 24.73984 E, 41.33351 N, 1460 m, 2.8.2000.
- 13a: Dasiko chorio Erymanthou, 24.71153 E, 41.33129 N, 1320 m, 8.7.2018.
- 13b: idem, 1300 m, 2.7.1990.

13c: idem, 1320 m, 5.8.1992.

14: Livadotopos, 1140 m, 5.7.1990.

15: Kalyvia Kontolia, 1350 m, 5.7.1990.

16: Erymanthos, 1450-1560 m, 4.7.1990.

17: Kioungkia, 1280 m, 4.10.1990.

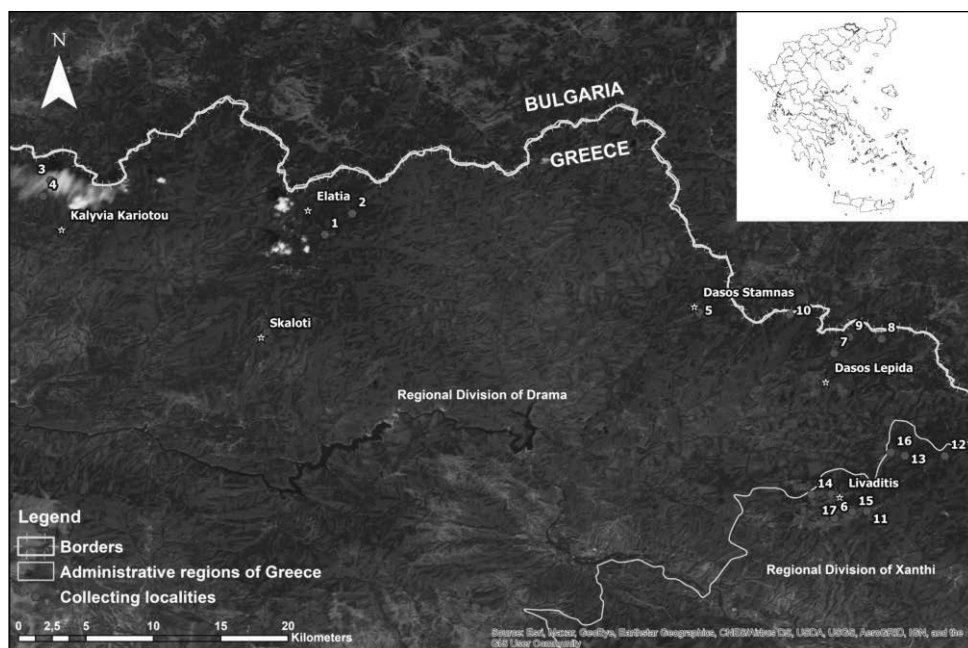


Fig. 1. Map of the study area and collection sites.

Results and discussion

Floristic catalogue – The following abbreviations and symbols are used in the floristic catalogue:

Char.: leg. Ch. Charalampidou (TAUF), followed by collection number and locality code (in brackets)

ETA: leg. E. Eleftheriadou, K. Theodoropoulos, N. Athanasiadis (TAUF), followed by locality code (in brackets)

TE: Theodoropoulos & Eleftheriadou 2012

CET: Charalampidou, Eleftheriadou & Theodoropoulos 2021

!: specimen seen

E: East of longitude (World Geodetic System 1984 in decimal degrees)

N: North of latitude (World Geodetic System 1984 in decimal degrees)

s.n.: sine numero, specimen without collection number

PTERIDOPHYTA**Aspleniaceae**

Asplenium trichomanes L. subsp. *trichomanes* - H, Co, – ETA s.n. ! (13b).

Athyriaceae

Athyrium filix-femina (L.) Roth - G, Co – Char. 1198 (1a).

Cystopteridaceae

Cystopteris fragilis (L.) Bernh. - G, Co – ETA s.n. ! (13b).

Dennstaedtiaceae

Pteridium aquilinum (L.) Kuhn subsp. *aquilinum* - G, Co – ETA s.n. ! (13c).

Equisetaceae

Equisetum arvense L. – G, Ct – Char 1227 (2).

Equisetum fluviatile L. – G, Ct – ETA s.n. ! (11a), ETA s.n. ! (15).

Equisetum palustre L. – G, Ct – Char. 1226 (2), Char. 1606 (4a), ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

GYMNOSPERMAE**Cupressaceae**

Juniperus communis L. subsp. *communis* - P, Ct, – ETA s.n. ! (11h), TE 2012: 1409

Juniperus oxycedrus subsp. *deltoides* (R.P. Adams) N.G. Passal. - P, EM – ETA s.n. ! (14).

ANGIOSPERMAE - DICOTYLEDONES**Aceraceae**

Acer opalus subsp. *obtusatum* (Willd.) Gams - P, MA – ETA s.n. ! (13c).

Apiaceae

Angelica sylvestris L. - H, ES – ETA s.n. ! (12a).

Chaerophyllum aureum L. - H, ME – ETA s.n. ! (13b).

Chaerophyllum hirsutum L. - H, Eu – ETA s.n. ! (9b), ETA s.n. ! (9c).

Oenanthe peucedanifolia Pollich - H, MA – ETA s.n. ! (9b), ETA s.n. ! (9c), ETA s.n. ! (11a).

Oenanthe pimpinelloides L. subsp. *pimpinelloides* - H, MS – ETA s.n. ! (15).

Oenanthe silaifolia M. Bieb. - H, MS – Char. 1208 (1a), Char. 1268 (2), Char. 1269 (2).

Pastinaca hirsuta Pančić - H, Bk – Char. 1213 (1a), Char. 1229 (2), Char. 1900 (8a).

Seseli peucedanoides (M. Bieb.) Koso-Pol. - H, ES – ETA s.n. ! (16).

Asclepiadaceae

Vincetoxicum hirundinaria subsp. *nivale* (Boiss. & Heldr.) Markgr. - H, BA – Char. 407 (13a), Char. 411 (13b).

Asteraceae

Achillea millefolium L. - H, ES – Char. 1270 (2).

Achillea setacea Waldst. & Kit. - H, MS – ETA s.n. ! (14).

Antennaria dioica (L.) Gaertn. - C, ES – ETA s.n. ! (11h).

- Anthemis austriaca* Jacq. - T, MS – Char. 1615 (3), Char. 1616 (3).
Anthemis chia L. - T, Me – Char. 1899 (8a).
Anthemis cretica L. - H, ME – Char. 1820 (5).
Anthemis macedonica Boiss. & Orph. subsp. *macedonica* - T, Bk – Char. 2062 (9a).
Centaurea affinis Friv. subsp. *affinis* - H, Bk – ETA s.n. ! (14), ETA s.n. ! (16).
Centaurea grisebachii subsp. *confusa* (Hayek) Dostál - H, • – ETA s.n. ! (14).
Centaurea phrygia subsp. *stenolepis* (A. Kern.) Gugler - H, MS – ETA s.n. ! (16).
Cichorium intybus L. - H, MS – ETA s.n. ! (14).
Cirsium appendiculatum Griseb. - H, Bk – Char. 1244 (2).
Doronicum austriacum Jacq. - H, ME – Char. 1228 (2).
Gnaphalium sylvaticum L. - H, ES – Char. 1230 (2).
Hieracium olympicum Boiss. - H, BA – ETA s.n. ! (11b), ETA s.n. ! (13c).
Hieracium pannosum subsp. *friwaldii* (Rchb. f.) Freyn - H, Bk – ETA s.n. ! (16).
Hypochaeris maculata L. - H, ES – Char. 1219 (2), Char. 1231 (2), Char. 408 (13a), Char. 412 (13a).
Inula hirta L. - H, ES – ETA s.n. ! (14), ETA s.n. ! (16).
Pilosella cymosa (L.) F.W. Schultz & Sch. Bip. subsp. *cymosa* - H, Eu – ETA s.n. ! (15).
Pilosella cymosa subsp. *sabina* (Sebast.) H. P. Fuchs - H, MS – ETA s.n. ! (16).
Pilosella leucopsilon (Arv.-Touv.) Gottschl. - H, MS – ETA s.n. ! (11h).
Pilosella officinarum Vaill. - H, ES – ETA s.n. ! (16).
Pilosella onegensis Norrl. - H, ES – Char. 391 (13a), Char. 395 (13a), Char. 1207 (1a), Char. 1242 (2), Char. 1279 (2), ETA s.n. ! (4b), ETA s.n. ! (9b), ETA s.n. ! (9c), ETA s.n. ! (12c), ETA s.n. ! (12d).
Scorzoneroideis autumnalis (L.) Moench - H, ES – Char. 1245 (2), Char. 1249 (2), Char. 1276 (2).
Senecio viscosus L. - T, MS – ETA s.n. ! (14).
Tanacetum corymbosum subsp. *cinereum* (Griseb.) Grierson - H, Bk – ETA s.n. ! (14).
Tragopogon porrifolius L. - T, Me – Char. 1630 (3).
Tripleurospermum tenuifolium (Kit.) Freyn - H, MS – Char. 415 (13a).

Betulaceae

- Betula pendula* Roth - P, ES – Char. 1649 (3).
Carpinus orientalis Mill. subsp. *orientalis* - P, MS – ETA s.n. ! (16).
Ostrya carpinifolia Scop. - P, MS – ETA s.n. ! (11h).

Boraginaceae

- Anchusa thessala* Boiss. & Spruner - T, MS – ETA s.n. ! (15).
Buglossoides arvensis subsp. *sibthorpiana* (Griseb.) R. Fern. - T, MS – Char. 1576 (3), Char. 1623 (3).
Myosotis nemorosa Besser - H, ES – Char. 1187 (1a), Char. 1890 (8a), ETA s.n. ! (8c), ETA s.n. ! (11b), ETA s.n. ! (12b), TE 2012: 1409.
Myosotis sylvatica subsp. *subarvensis* Grau - T, Me – Char. 1581 (3).
Pulmonaria officinalis L. - H, Eu – Char. 1573 (3), Char. 1603 (4a).
Symphytum ottomanum Friv. - H, BA – Char. 1237 (2).

Brassicaceae

- Alyssum alyssoides* (L.) L. - T, Eu – Char. 1627 (3).
Alyssum murale Waldst. & Kit. - H, ME – ETA s.n. ! (14).
Arabis glabra (L.) Bernh. - H, Eu – Char. 1619 (3).
Arabis hirsuta (L.) Scop. - H, ME – Char. 1631 (3).
Barbarea sicula C. Presl - H, Me – Char. 1824 (5).
Capsella bursa-pastoris (L.) Medik. - T, Co – Char. 1618 (3), Char. 1624 (3).
Descurainia sophia (L.) Prantl - T, ES – Char. 1574 (3), Char. 1636 (3).

Raphanus raphanistrum L. subsp. *raphanistrum* - T, MS – ETA s.n. ! (15).

Rorippa pyrenaica (All.) Rechb. - H, Eu – Char. 1640 (3).

Rorippa thracica (Griseb.) Fritsch - H, BA – ETA s.n. ! (13b).

Thlaspi arvense L. - T, Co – Char. 1582 (3).

Campanulaceae

Campanula lingulata Waldst. & Kit. - H, BA – ETA s.n. ! (14).

Campanula macrostachya Willd. - H, BC – ETA s.n. ! (16).

Campanula patula subsp. *epigaea* (Degen) Hayek - H, Bk – ETA s.n. ! (13b), ETA s.n. ! (8c).

Campanula persicifolia L. - H, ES – ETA s.n. ! (16).

Campanula sparsa subsp. *sphaerotherix* (Griseb.) Hayek - T, Bk – Char. 1195 (1a).

Campanula spatulata subsp. *spruneriana* (Hampe) Hayek - G, Bk – ETA s.n. ! (14).

Jasione heldreichii Boiss. & Orph. - H, BA – ETA s.n. ! (14).

Caryophyllaceae

Cerastium holosteoides Fr. - T, Eu – ETA s.n. ! (13b).

Dianthus deltoides L. subsp. *deltoides* - H, ES – Char. 1223 (2).

Dianthus gracilis subsp. *drenowskianus* (Rech. f.) Strid - H, Bk – ETA s.n. ! (16).

Dianthus stenopetalus Griseb. - C, Bk – ETA s.n. ! (14).

Gypsophila muralis L. - H, ES – ETA s.n. ! (14).

Lychnis coronaria (L.) Desr. - H, MS – ETA s.n. ! (14).

Sagina procumbens L. - H, Ct – ETA s.n. ! (14).

Scleranthus annuus subsp. *polycarpus* (L.) Thell. - T, Eu – Char. 1590 (4a).

Scleranthus perennis subsp. *marginatus* (Guss.) Nyman - H, Me – ETA s.n. ! (14).

Spergula arvensis L. - T, Co – ETA s.n. ! (15).

Stellaria alsine Grimm - H, ES – Char. 1197b (1a).

Stellaria graminea L. - H, ES – ETA s.n. ! (13b).

Stellaria media (L.) Vill. - T, Co – Char. 1907 (7).

Chenopodiaceae

Chenopodiastrum hybridum (L.) S. Fuentes & al. - T, Ct – Char. 1905 (7).

Chenopodium album L. - T, Co – ETA s.n. ! (11h).

Cistaceae

Helianthemum nummularium (L.) Mill. subsp. *nummularium* - C, Me – Char. 393 (13a), Char. 400 (13a), Char. 420 (13a), ETA s.n. ! (14).

Helianthemum nummularium subsp. *tomentosum* (Scop.) Schinz & Thell. - C, Me – ETA s.n. ! (16).

Dipsacaceae

Knautia ambigua Boiss. & Orph. - H, Bk – ETA s.n. ! (11h).

Scabiosa columbaria L. subsp. *columbaria* - H, MS – ETA s.n. ! (13b).

Scabiosa webbiana D. Don - H, MS – ETA s.n. ! (14).

Succisa pratensis Moench - H, ES – ETA s.n. ! (11b), ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

Droseraceae

Drosera rotundifolia L. - H, Bo – ETA s.n. ! (11h), ETA s.n. ! (14), ETA s.n. ! (15), – ETA s.n. ! (16), ETA s.n. ! (17), TE 2012: 1409.

Ericaceae

Bruckenthalia spiculifolia (Salisb.) Rchb. – C, BA – TE 2012: 1409.

Vaccinium vitis-idaea L. subsp. *vitis-idaea* - C, Bo – ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

Euphorbiaceae

Euphorbia amygdaloides L. subsp. *amygdaloides* - H, MS – Char. 1902 (8a).

Euphorbia cyparissias L. - H, Eu – Char. 1579 (3), Char. 1596 (4a).

Euphorbia seguieriana subsp. *niciciana* (Novák) Rech. f. - H, BA – ETA s.n. ! (14).

Euphorbia stricta L. - T, MS – ETA s.n. ! (11e).

Fabaceae

Dorycnium herbaceum Vill. - H, ME – ETA s.n. ! (14).

Genista carinalis Griseb. - C, BA – Char. 394 (13a), Char. 403 (13a), Char. 410 (13a), Char. 1224 (2).

Genista januensis subsp. *lydia* (Boiss.) Kit Tan & Ziel. - C, BA – ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

Lathyrus niger (L.) Bernh. - H, ME – Char. 1280 (2).

Lathyrus pratensis L. - H, Pt – Char. 1260 (2), Char. 1597 (4), ETA s.n. ! (11b).

Melilotus neapolitanus Ten. - T, MS – Char. 1621 (3).

Securigera varia (L.) Lassen - H, MS – ETA s.n. ! (16).

Trifolium alpestre L. - G, MS – Char. 402 (13a), ETA s.n. ! (13b), ETA s.n. ! (14).

Trifolium hybridum subsp. *elegans* (Savi) Asch. & Graebn. - H, Me – Char. 1202 (1a), Char. 1222 (2), ETA s.n. ! (9b), ETA s.n. ! (9c), ETA s.n. ! (11e), ETA s.n. ! (12a), ETA s.n. ! (12d).

Trifolium ochroleucon Huds. - H, ME – ETA s.n. ! (8c).

Trifolium patens Schreb. - T, ME – ETA s.n. ! (4b), ETA s.n. ! (15).

Trifolium spadiceum L. - H, ES – Char. 1215 (1a), Char. 1257 (2).

Vicia angustifolia L. - T, Pt – ETA s.n. ! (15).

Vicia grandiflora Scop. - T, ME – Char. 1639 (3).

Vicia incana Gouan - H, ME – ETA s.n. ! (16).

Vicia sepium L. - H, ES – Char. 1602 (4a).

Fumariaceae

Fumaria vaillantii Loisel. - T, MS – Char. 1626 (3).

Gentianaceae

Centaurium erythraea Rafn subsp. *erythraea* - H, MS – ETA s.n. ! (14).

Geraniaceae

Erodium ciconium (L.) L'Hér. - T, MS – Char. 1628 (3).

Geranium dissectum L. - T, MS – Char. 1642 (3).

Geranium lanuginosum Lam. - T, Me – Char. 1637 (3).

Geranium pusillum Burm. f. - T, MS – Char. 1201 (1a).

Geranium sanguineum L. - G, MS – ETA s.n. ! (14).

Globulariaceae

Globularia bisnagarica L. - H, Eu – ETA s.n. ! (17).

Hippocastanaceae

Aesculus hippocastanum L. - P, Bk – ETA s.n. ! (13c).

Hypericaceae

Hypericum maculatum subsp. *immaculatum* (Murb.) A. Fröhl. - H, Bk – Char. 1203 (1a), ETA s.n. ! (11b), ETA s.n. ! (11e), ETA s.n. ! (12a).

Hypericum perforatum subsp. *veronense* (Schrank) Ces. - H, Pt – ETA s.n. ! (11e).

Lamiaceae

Acinos alpinus subsp. *hungaricus* (Simonk.) Soják - H, BA – ETA s.n. ! (16).

Ajuga genevensis L. - G, MS – Char. 1647 (3).

Ajuga reptans L. - G, MS – ETA s.n. ! (10a), ETA s.n. ! (10b).

Betonica officinalis L. - H, MS – Char. 398 (13a), ETA s.n. ! (11h), ETA s.n. ! (14).

Galeopsis bifida Boenn. - T, ES – Char. 1267 (2), ETA s.n. ! (15).

Galeopsis speciosa Mill. - T, MS – ETA s.n. ! (1b), ETA s.n. ! (1c).

Lamium garganicum subsp. *laevigatum* Arcang. - H, Me – ETA s.n. ! (13b).

Mentha longifolia (L.) Huds. - H, Pt – Char. 385 (13a), ETA s.n. ! (13c).

Mentha spicata L. - H, MS – Char. 1210 (1a), ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (4b), ETA s.n. ! (11a).

Mentha spicata subsp. *condensata* (Briq.) Greuter & Burdet - H, Me – Char. 1580 (3).

Prunella laciniata (L.) L. - H, Me – ETA s.n. ! (14).

Prunella vulgaris L. - H, MS – ETA s.n. ! (14), ETA s.n. ! (1e).

Sideritis scardica Griseb. - H, Bk – ETA s.n. ! (13c).

Stachys alpina L. - H, Eu – ETA s.n. ! (16).

Stachys angustifolia M. Bieb. - H, Eu – ETA s.n. ! (14).

Stachys recta L. subsp. *recta* - H, ME – ETA s.n. ! (16).

Stachys sylvatica L. - H, ES – Char. 1255 (2).

Teucrium chamaedrys L. - C, Me – ETA s.n. ! (14).

Thymus degenii Heinr. Braun - C, Bk – Char. 406 (13a), Char. 1238 (2).

Thymus praecox subsp. *polytrichus* (Borbás) Jalas - C, Eu – ETA s.n. ! (14).

Thymus sibthorpii Benth. - C, BA – ETA s.n. ! (14).

Linaceae

Linum catharticum L. - T, Me – ETA s.n. ! (14).

Linum hologynum Rchb. - H, Bk – ETA s.n. ! (14).

Malvaceae

Malva neglecta Wallr. - T, MS – ETA s.n. ! (14).

Oleaceae

Fraxinus ornus L. - P, ME – ETA s.n. ! (14).

Onagraceae

Epilobium lamyi F. W. Schultz - H, Pt – ETA s.n. ! (4b).

Epilobium lanceolatum Sebast. & Mauri - H, MS – ETA s.n. ! (11h).

Epilobium montanum L. - H, MS – Char. 1266 (2).

Epilobium palustre L. - H, Bo – Char. 1211 (1a), ETA s.n. ! (8b), ETA s.n. ! (11a), ETA s.n. ! (12a), ETA s.n. ! (12b), ETA s.n. ! (12d).

Epilobium parviflorum Schreb. - H, Pt – ETA s.n. ! (11a).

Epilobium tournefortii Michalet - H, Bk – ETA s.n. ! (15).

Orobanchaceae

Euphrasia hirtella Reut. - T, ES – Char. 1251 (2).

Euphrasia liburnica Wettst. - T, BI – ETA s.n. ! (12d).

Euphrasia pectinata Ten. - T, ES – ETA s.n. ! (13c).

Rhinanthus rumelicus Velen. - T, BC – ETA s.n. ! (15).

Parnassiaceae

Parnassia palustris L. - H, ES – Char. 1234 (2), ETA s.n. ! (12d), TE 2012: 1409.

Plantaginaceae

Plantago atrata Hoppe - H, Eu – Char. 1595 (4a).

Plantago holosteum Scop. - H, Eu – ETA s.n. ! (14).

Plantago lanceolata L. - H, Co – Char. 392 (13a), Char. 399 (13a), ETA s.n. ! (14).

Plantago major subsp. *intermedia* (Gilib.) Lange - H, MS – Char. 2057 (9a).

Plantago major L. subsp. *major* - H, MS – Char. 389 (13a).

Plantago media L. subsp. *media* - H, ES – Char. 1252 (2), Char. 1599 (4a).

Polygalaceae

Polygala comosa Schkuhr - H, Eu – Char. 1604 (4a), Char. 1648 (3).

Polygonaceae

Persicaria bistorta (L.) Samp. - G, ES – Char. 1895 (8a), Char. 2055 (9a).

Persicaria maculosa Gray - T, [Co] EA – ETA s.n. ! (15).

Rumex acetosella subsp. *acetoselloides* (Balansa) Nijs - H, MS – ETA s.n. ! (13b).

Rumex acetosella subsp. *multifidus* (L.) Schübl. & G. Martens - H, BI – Char. 1191 (1a).

Rumex arifolius All. - H, Eu – Char. 1271 (2).

Rumex conglomeratus Murray - H, MS – ETA s.n. ! (4b).

Rumex obtusifolius subsp. *subalpinus* (Schur) Rech. f. - H, MS – Char. 1578 (3).

Primulaceae

Lysimachia nummularia L. - H, MS – ETA s.n. ! (14).

Lysimachia punctata L. - H, Eu – ETA s.n. ! (14).

Lysimachia vulgaris L. - H, MS – ETA s.n. ! (4b).

Ranunculaceae

Clematis vitalba L. - P, MS – ETA s.n. ! (14).

Ranunculus acris L. subsp. *acris* - H, ES – Char. 1204 (1a), Char. 1205 (1a).

Ranunculus fontanus C. Presl – T, Me – Char. 1189 (1a), ETA s.n. ! (12b), ETA s.n. ! (12c).

Ranunculus illyricus L. - H, MS – ETA s.n. ! (14).

Ranunculus polyanthemos L. subsp. *polyanthemos* - H, MS – ETA s.n. ! (15).

Ranunculus polyanthemos subsp. *polyanthemoides* (Boreau) Ahlfr. - H, Eu – Char. 1607 (4a), Char. 1646 (3).

Ranunculus repens L. - H, Pt – Char. 1893 (8), ETA s.n. ! (1d), ETA s.n. ! (9b) ETA s.n. ! (9c), ETA s.n. ! (11a), ETA s.n. ! (12c).

Ranunculus serbicus Vis. - G, BI – ETA s.n. ! (1b), ETA s.n. ! (1c).

Thalictrum aquilegifolium L. - G, Eu – Char. 1777 (6).

Rosaceae

Alchemilla glabra Neygenf. - H, Eu – ETA s.n. ! (1f), ETA s.n. ! (8c).

- Alchemilla lanuginosa* Rothm. - H, Bk – ETA s.n. ! (16).
Alchemilla mollis (Buser) Rothm. - H, BA – ETA s.n. ! (14).
Alchemilla monticola Opiz - H, ES – Char. 1235 (2), ETA s.n. ! (8c), ETA s.n. ! (9b), ETA s.n. ! (9c), ETA s.n. ! (11e).
Alchemilla straminea Buser - H, Eu – ETA s.n. ! (15).
Cotoneaster integerrimus Medik. - P, MS – ETA s.n. ! (11h).
Cotoneaster tomentosus (Aiton) Lindl. - P, ME – ETA s.n. ! (16).
Crataegus monogyna Jacq. - P, Pt – Char. 1591 (4a).
Crataegus rhipidophylla Gand. - P, MS – Char. 1632 (3), Char. 1773 (6).
Filipendula ulmaria (L.) Maxim. - H, ES – Char. 1259 (2).
Filipendula vulgaris Moench - H, ES – ETA s.n. ! (14).
Fragaria vesca L. - H, MS – ETA s.n. ! (13b).
Geum coccineum Sm. - H, BA – Char. 1776 (6), Char. 1822 (5), TE 2012: 1409.
Geum rhodopeum Stoj. & Stef. - H, Bk – Char. 1233 (2).
Malus sylvestris (L.) Mill. - P, Eu – ETA s.n. ! (14).
Potentilla argentea L. - H, ES – ETA s.n. ! (13b), ETA s.n. ! (14).
Potentilla erecta (L.) Raeusch. - H, ES – Char. 1190 (1a), Char. 1897 (8a), ETA s.n. ! (12a), TE 2012: 1409.
Potentilla reptans L. - H, Pt – ETA s.n. ! (14).
Prunus cerasifera Ehrh. - P, MS – ETA s.n. ! (13c).
Prunus cocomilia Ten. - P, EM – Char. 1593 (4a).
Prunus domestica L. subsp. *domestica* - P, MS – ETA s.n. ! (14).
Prunus domestica subsp. *insititia* (L.) Bonnier & Layens - P, MS – ETA s.n. ! (13c).
Pyrus communis L. - P, MS – ETA s.n. ! (14).
Rosa arvensis Huds. - P, Me – ETA s.n. ! (14).
Rosa spinosissima L. - P, Pt – ETA s.n. ! (16).
Sanguisorba officinalis L. - H, Bo – ETA s.n. ! (11b), TE 2012: 1409
Sanguisorba minor subsp. *balearica* (Nyman) Muños Garm. & C. Navarro - H, MS – ETA s.n. ! (15).
Sorbus aucuparia L. subsp. *aucuparia* - P, MS – ETA s.n. ! (13c).
Sorbus umbellata (Desf.) Fritsch subsp. *umbellata* - P, Bk – ETA s.n. ! (11h).

Rubiaceae

- Asperula aristata* subsp. *nestia* (Rech. f.) Ehrend. & Krendl - H, Bk – Char. 2058 (9a).
Asperula purpurea subsp. *apiculata* (Sm.) Ehrend. - C, BA – ETA s.n. ! (14).
Asperula purpurea (L.) Ehrend. subsp. *purpurea* - C, ME – ETA s.n. ! (16).
Cruciata laevipes Opiz - H, MS – Char. 1232 (2), Char. 1644 (3), ETA s.n. ! (8c).
Cruciata verna (Scop.) Gutermann & Ehrend. - H, MS – ETA s.n. ! (8c), ETA s.n. ! (9b), ETA s.n. ! (9c), ETA s.n. ! (10a), ETA s.n. ! (10b), ETA s.n. ! (10c), ETA s.n. ! (12a).
Galium macedonicum Krendl - H, Bk – ETA s.n. ! (14).
Galium palustre L. - H, MS – Char. 1194 (1a), Char. 1243 (2), ETA s.n. ! (11a), ETA s.n. ! (12b).
Galium rivale (Sm.) Griseb. - H, ES – ETA s.n. ! (13c).
Galium uliginosum L. - H, ES – ETA s.n. ! (4b), CET 2021: 364.
Galium verum L. subsp. *verum* - H, Pt – Char. 409 (13a), Char. 1248 (2), ETA s.n. ! (8c).

Salicaceae

- Populus tremula* L. - P, Ct – ETA s.n. ! (16).
Salix amplexicaulis Bory & Chaub. - P, Me – ETA s.n. ! (13c).
Salix fragilis L. - P, MS – ETA s.n. ! (15).

Santalaceae

Arceuthobium oxycedri (DC.) M. Bieb. - P, MS – ETA s.n. ! (14).

Thesium alpinum L. - H, AA – ETA s.n. ! (14).

Thesium linophyllon subsp. *montanum* (Schrad.) Čelak. - H, Eu – ETA s.n. ! (16).

Saxifragaceae

Saxifraga bulbifera L. - H, ME – Char. 1575 (3).

Saxifraga rotundifolia L. subsp. *rotundifolia* - H, ME – Char. 1250 (2).

Scrophulariaceae

Verbascum banaticum Schrad. - H, Bk – ETA s.n. ! (14).

Verbascum phlomoides L. - H, MS – ETA s.n. ! (13c).

Valerianaceae

Valeriana officinalis L. subsp. *officinalis* - H, MS – Char. 1239 (2), Char. 2063 (9a).

Valerianella carinata Loisel. - T, MS – Char. 1586 (4a).

Veronicaceae

Digitalis lanata Ehrh. subsp. *lanata* - H, BA – ETA s.n. ! (13c).

Digitalis viridiflora Lindl. - H, Bk – Char. 1199 (1a).

Veronica acinifolia L. - T, ME – Char. 1258 (2).

Veronica beccabunga L. - H, MS – ETA s.n. ! (4b).

Veronica chamaedrys L. subsp. *chamaedrys* - H, ES – ETA s.n. ! (8c).

Veronica serpyllifolia L. subsp. *serpyllifolia* - H, Ct – Char. 1594 (4a).

Veronica urticifolia Jacq. - H, Eu – Char. 1625 (3).

Violaceae

Viola macedonica Boiss. & Heldr. - H, Bk – Char. 1823 (5).

ANGIOSPERMAE – MONOCOTYLEDONES**Alliaceae**

Allium flavum L. subsp. *flavum* - G, Me – ETA s.n. ! (14).

Anthericaceae

Anthericum liliago L. - G, ME – ETA s.n. ! (14).

Cyperaceae

Blysmus compressus (L.) Link - G, ES – ETA s.n. ! (4b).

Carex buekii Wimm. - H, MS – ETA s.n. ! (15).

Carex canescens L. - H, Co – Char. 1225 (2).

Carex caryophylllea Latourr. - H, ES – Char. 1588 (4a), Char. 1770 (6).

Carex echinata Murray - H, Ct, – Char. 1214 (1a), ETA s.n. ! (10a), ETA s.n. ! (10b), ETA s.n. ! (11c), ETA s.n. ! (12b), TE 2012: 1409.

Carex flacca Schreb. subsp. *flacca* - G, Eu – Char. 1577 (3), Char. 1600 (4a), Char. 1605 (4a), Char. 1883 (8a), Char. 1885 (8a).

Carex flava L. - H, Ct – ETA s.n. ! (1e).

Carex hirta L. - G, MS – Char. 1192 (1a), Char. 1273 (2), ETA s.n. ! (4b), ETA s.n. ! (10a), ETA s.n. ! (10b), ETA s.n. ! (12a).

- Carex leporina* L. - H, ES – Char. 1274 (2), ETA s.n. ! (8c), ETA s.n. ! (11a), ETA s.n. ! (12a), ETA s.n. ! (12d).
- Carex nigra* (L.) Reichard - G, Ct – Char. 1884 (8a), Char. 1886 (8a), ETA s.n. ! (8b), ETA s.n. ! (8d), ETA s.n. ! (8e), ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.
- Carex pallescens* L. - H, Ct – ETA s.n. ! (8c), ETA s.n. ! (10a), ETA s.n. ! (10b), ETA s.n. ! (11b), ETA s.n. ! (11f), ETA s.n. ! (11g).
- Carex punctata* Gaudin - H, ME, – Char. 1240 (2), Char. 1901 (8a), ETA s.n. ! (15).
- Carex remota* L. - H, MS – Char. 1247 (2).
- Carex rostrata* Stokes - G, Bo – ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (1d), ETA s.n. ! (1e), ETA s.n. ! (8b), ETA s.n. ! (8d), ETA s.n. ! (8e), ETA s.n. ! (4b), ETA s.n. ! (10a), ETA s.n. ! (11a), ETA s.n. ! (11c), TE 2012: 1409.
- Carex spicata* Huds. - H, ES – Char. 1888 (8a).
- Carex viridula* Michx. - H, MS – ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.
- Eleocharis palustris* (L.) Roem. & Schult. subsp. *palustris* - G, Co, – Char. 1236 (2), ETA s.n. ! (15).
- Eleocharis quinqueflora* (Hartmann) O. Schwarz - G, Bo – ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.
- Eriophorum angustifolium* Honck. - G, Bo – Char. 2054 (9a), ETA s.n. ! (8b), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (12b), TE 2012: 1409.
- Eriophorum latifolium* Hoppe - H, MS – Char. 1212 (1a), Char. 1592 (4a), Char. 1610 (4a).
- Isolepis cernua* (Vahl) Roem. & Schult. - T, Co – ETA s.n. ! (14).
- Scirpus sylvaticus* L. - G, MS – Char. 1264 (2), Char. 1611 (4a), Char. 1894 (8a), ETA s.n. ! (11b).

Hyacinthaceae

- Muscari comosum* (L.) Mill. - G, ME – ETA s.n. ! (14).
- Ornithogalum collinum* Guss. subsp. *collinum* - G, Me – Char. 1772 (6), Char. 1880 (8a).
- Ornithogalum kochii* Parl. - G, Me – Char. 1629 (3).
- Ornithogalum pyrenaicum* subsp. *sphaerocarpum* (A. Kern.) Hegi - G, MS – ETA s.n. ! (14).

Iridaceae

- Iris reichenbachii* Heuffel - G, Bk – ETA s.n. ! (16).

Juncaceae

- Juncus acutiflorus* Hoffm. - G, Eu – Char. 1253 (2).
- Juncus articulatus* L. - G, Bo – ETA s.n. ! (8c), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (12b), ETA s.n. ! (12d), TE 2012: 1409.
- Juncus bufonius* L. - T, Co – ETA s.n. ! (14), ETA s.n. ! (15).
- Juncus conglomeratus* L. - H, Eu – ETA s.n. ! (14).
- Juncus effusus* L. subsp. *effusus* - H, Eu – ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (11a), ETA s.n. ! (12a).
- Juncus sphaerocarpus* Nees - H, Pt – ETA s.n. ! (14).
- Juncus tenuis* Willd. - H, [N-Am.] – Char. 1892 (8a).
- Juncus thomasi* Ten. - G, Eu – Char. 1200 (1a), Char. 1265 (2), ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (10c), ETA s.n. ! (12d).
- Luzula campestris* (L.) DC. subsp. *campestris* - H, MS – ETA s.n. ! (14).
- Luzula forsteri* (Sm.) DC. - H, Me – ETA s.n. ! (8c).
- Luzula luzulina* (Vill.) Dalla Torre & Sarnth. - H, Eu – Char. 1197a (1a), Char. 1585 (4a).
- Luzula luzuloides* (Lam.) Dandy & Wilm. subsp. *luzuloides* - H, Eu – Char. 1587 (4a), Char. 1601 (4a), Char. 1612 (4a), Char. 1896 (8a), ETA s.n. ! (12a).

Luzula multiflora (Ehrh.) Lej. subsp. *multiflora* - H, Ct – Char. 1241 (2), Char. 1584 (4a), Char. 1609 (4a), Char. 1774 (6), Char. 1881 (8a).

Luzula sudetica (Willd.) DC. - H, AA – ETA s.n. ! (10a), ETA s.n. ! (10b), ETA s.n. ! (10c), ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

Melanthiaceae

Veratrum lobelianum Bernh. - G, MS, – Char. 387 (13a), ETA s.n. ! (15), TE 2012: 1409.

Orchidaceae

Anacamptis morio subsp. *caucasica* (K. Koch) H. Kretzschmar & al. - G, MS – Char. 1613 (4a).

Cephalanthera longifolia (L.) Fritsch - G, MS – ETA s.n. ! (1e).

Dactylorhiza cordigera (Fr.) Soó subsp. *cordigera* - G, Bk – Char. 1206 (1a), Char. 1256 (2), Char. 1614 (4a), Char. 1898 (8a), ETA s.n. ! (1e), ETA s.n. ! (4b), ETA s.n. ! (10c), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (12d), TE 2012: 1409.

Epipactis palustris W. Rossi - G, MS – Char. 1188 (1a).

Gymnadenia frivaldii Griseb. - G, Bk – ETA s.n. ! (1e).

Neotinea ustulata (L.) R.M. Bateman & al. - G, Eu – ETA s.n. ! (14).

Poaceae

Agrostis canina L. subsp. *canina* - H, ES – Char. 1209 (1a), Char. 1275 (2), Char. 1278 (2), Char. 382 (13a), ETA s.n. ! (8c), ETA s.n. ! (8d), ETA s.n. ! (8e), ETA s.n. ! (11b), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (11e), ETA s.n. ! (12a), TE 2012: 1409.

Agrostis capillaris L. - H, ES – ETA s.n. ! (8c).

Agrostis stolonifera L. - H, ES – ETA s.n. ! (4b).

Agrostis vinealis Schreb. - H, ES – Char. 1262 (2), ETA s.n. ! (15).

Aira elegans Roem. & Schult. - T, MS – ETA s.n. ! (14).

Anthoxanthum odoratum L. - H, Co – Char. 1589 (4a), ETA s.n. ! (8f).

Avenella flexuosa (L.) Drejer - H, Co – ETA s.n. ! (14).

Brachypodium pinnatum (L.) P. Beauv. - H, ES – ETA s.n. ! (14).

Briza media L. subsp. *media* - H, ES – ETA s.n. ! (14).

Bromus hordeaceus L. subsp. *hordeaceus* - T, Co – Char. 1620 (3), Char. 1634 (3), Char. 1635 (3).

Bromus squarrosus L. subsp. *squarrosus* - T, Pt – ETA s.n. ! (15).

Bromus tectorum L. - T, Pt – Char. 1633 (3), Char. 1645 (3).

Calamagrostis epigejos (L.) Roth - H, ES – ETA s.n. ! (11a), ETA s.n. ! (11b).

Chrysopogon gryllus (L.) Trin. - H, MS – ETA s.n. ! (14).

Cynosurus cristatus L. - H, MS – ETA s.n. ! (4b), ETA s.n. ! (15).

Danthonia alpina Vest - H, Eu – ETA s.n. ! (14).

Danthonia decumbens (L.) DC. - H, Eu, – ETA s.n. ! (11c), ETA s.n. ! (11d), TE 2012: 1409.

Deschampsia cespitosa (L.) P. Beauv. subsp. *cespitosa* - H, Co, – ETA s.n. ! (11a), ETA s.n. ! (12a), TE 2012: 1409.

Elytrigia campestris (Godr. & Gren.) Kerguélen - H, Eu – ETA s.n. ! (14).

Elytrigia repens (L.) Nevski - G, ES – ETA s.n. ! (11h).

Festuca arundinacea subsp. *interrupta* (Desf.) Tzvelev - H, Me – ETA s.n. ! (15).

Festuca cyllenica Boiss. & Heldr. - H, BA – ETA s.n. ! (14).

Festuca drymeja Mert. & W.D.J. Koch - G, Me – ETA s.n. ! (14).

Festuca macedonica J. Vetter - H, Bk – ETA s.n. ! (14).

Festuca peristerea (J. Vetter) Markgr.-Dann. - H, Bk – Char. 1771 (6), Char. 1882 (8a), Char. 1887 (8a), Char. 1889 (8a).

Festuca rubra L. subsp. *rubra* - H, Ct – ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (1d), ETA s.n. ! (1e), ETA s.n. ! (8c), ETA s.n. ! (8f), ETA s.n. ! (12a), ETA s.n. ! (12b), ETA s.n. ! (12c), TE 2012: 1409.

Glyceria notata Chevall. - G, Co – ETA s.n. ! (13c), ETA s.n. ! (15).

Holcus lanatus L. - H, ES – Char. 383 (13a), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (12a), TE 2012: 1409.

Holcus mollis L. subsp. *mollis* - G, Eu – ETA s.n. ! (16).

Hordeum murinum L. subsp. *murinum* - T, MS – Char. 1572 (3), Char. 1622 (3), Char. 1638 (3).

Koeleria macrantha (Ledeb.) Schult. - H, Bo – ETA s.n. ! (14).

Molinia caerulea (L.) Moench - H, ES – ETA s.n. ! (8c), ETA s.n. ! (11c), ETA s.n. ! (11d), ETA s.n. ! (11e), TE 2012: 1409.

Nardus stricta L. - H, ES – ETA s.n. ! (8f), ETA s.n. ! (12b), ETA s.n. ! (12c), ETA s.n. ! (12d), TE 2012: 1409.

Phleum nodosum L. - H, ME – ETA s.n. ! (4b).

Poa annua L. subsp. *annua* - T, Co – Char. 1641 (3), Char. 1903 (7).

Poa nemoralis L. subsp. *nemoralis* - H, ES – Char. 1571 (3), Char. 1583 (4a).

Poa palustris L. subsp. *palustris* - H, Ct – ETA s.n. ! (15).

Poa pratensis L. subsp. *pratensis* - G, Ct – Char. 1643 (3), Char. 1906 (7), ETA s.n. ! (1b), ETA s.n. ! (1c), ETA s.n. ! (12a).

Poa timoleonis Boiss. - H, EM – Char. 1598 (4a), Char. 1617 (3).

Poa trivialis subsp. *sylvicola* (Guss.) H. Lindb. - H, MS – ETA s.n. ! (1b), ETA s.n. ! (1c).

Floristic analysis – As a result of the investigations, 340 plant species from 58 families and 175 genera have been distinguished in the study area (Table 1).

The most species-rich family of the vascular flora of the study area is *Poaceae*, which accounts 40 taxa (11.76% of all species). Next most species-rich families are *Rosaceae* (29 taxa), *Asteraceae* (28), *Cyperaceae* (22), *Lamiaceae* (21), *Fabaceae* (16), *Juncaceae* (14), *Caryophyllaceae*, *Brassicaceae* and *Rubiaceae*. The ten most taxa-rich families together include 204 species representing the 60% of the total vascular flora of the study area. Most of these families are consistently the richest in number of taxa in several similar surveys (Sarika-Hatzinikolaou & al. 1996; Atanassova & Marinova 2005; Eleftheriadou & Theodoropoulos 2015; Dimitrov 2016). At the other end of the scale, 21 families contribute with only one taxon. The most species-rich genera are *Carex* (15 taxa), *Juncus* (8 taxa), *Ranunculus* (7 taxa) and *Luzula*, *Festuca*, *Poa*, *Campanula*, *Epilobium*, *Plantago*

Table 1. Numbers of plant families and genera in the four main taxonomic groups of the vascular flora of the study area.

	Families	Genera	Species ¹	Subspecies	Taxa
Pteridophytes	5	5	7	2	7
Gymnosperms	1	1	2	2	2
Angiosperms - Dicotyledons	43	127	233	62	241
Angiosperms - Monocotyledons	9	42	90	25	90
Total	58	175	332	91	340

1. are comprised of (a) species that have no subspecies and (b) species that have one or more subspecies

(with 6 taxa each), which account for 19.41% of the total species of the study area, whereas 103 genera have only one taxon. The first three genera appear as prevalent in Eleftheriadou & Theodoropoulos (2015) too and specifically *Carex* in the same ranking.

Six taxa of vascular plants were identified for the first time for the floristic region of North-East Greece. The presence of an additional species (*Barbarea sicula*), previously considered doubtful in the area, is confirmed by us. All those mentioned above are presented alphabetically in Table 2. Also important is the presence of *Galium uliginosum* that was recorded for the first time in Greece by Charalampidou & al. (2021).

Spectra analysis – The life-form spectrum shows that hemicryptophytes prevail (59.71%) followed by therophytes (15%). Geophytes, phanerophytes and chamaephytes follow with 13.24%, 7.94% and 4.12% respectively (Table 3). Hemicryptophytes-usually prevail in similar habitats according to Sarika-Hatzinikolaou & al. (1996) and Eleftheriadou & Theodoropoulos (2015). The prevalence of hemicryptophytes, which is attributed to temperate zone and mountainous Mediterranean areas (Emberger 1966), reflects the intensely mountainous nature of the research area.

The analysis of the chorological spectrum shows that the most prevalent chorological group of plants are the Widespread taxa (228 taxa – 66.06%), followed by the Mediterranean (57 taxa – 16.76%) and the Balkan taxa (53 taxa – 15.59%). Although the percentage of Greek endemics (0.29%) seems very low at first, this is reasonably justified, according to Dimopoulos & al. (2013), by the fact that taxa occurring in small areas on both sides of the border with neighbouring countries have been classified simply as Balkan endemics rather than Greek endemics. Finally, the low rate of 0.29% is recorded also for the Alien taxa (Table 4). Widespread taxa also prevail in similar habitats according to Eleftheriadou & Theodoropoulos (2015), namely, the European-SW Asian & Euro-Siberian floristic elements are first in ranking. Although a different classification system was followed in similar studies, the European – SW Asian floristic element also prevailed according to Atanassova & Marinova (2005).

Table 2. Taxa reported for the first time or confirmed for the floristic region of North-East Greece are indicated with “!” under the NE column.

Taxon	Family	Chorology	Floristic regions*					
			NE	NC	NPi	SPi	EC	StE
<i>Alchemilla mollis</i>	<i>Rosaceae</i>	BA	!	+		+		
<i>Barbarea sicula</i>	<i>Brassicaceae</i>	Me	!	+	+	+	+	+
<i>Blysmus compressus</i>	<i>Cyperaceae</i>	ES	!	+	+	+	+	+
<i>Eleocharis quinqueflora</i>	<i>Cyperaceae</i>	Bo	!	+	+			+
<i>Equisetum fluviatile</i>	<i>Equisetaceae</i>	Ct	!	+	+			
<i>Oenanthe peucedanifolia</i>	<i>Apiaceae</i>	MA	!	+		?		?
<i>Tanacetum corymbosum</i> subsp. <i>cinereum</i>	<i>Astearaceae</i>	Bk	!			+		

*Floristic regions of Greece according to Strid & Tan (1997)

Table 3. Life-form spectrum of the vascular flora of the study area.

Life-forms	Number of taxa	Percentage (%)
Chamaephytes (C)	14	4.12
Geophytes (Cryptophytes) (G)	45	13.23
Hemicryptophytes (H)	203	59.71
Phanerophytes (P)	27	7.94
Therophytes (T)	51	15.00
Total	340	100.00

Table 4. Chorological spectrum of the vascular flora of the study area. The chorotypes are classified into 5 wide chorological groups.

Chorological group/category	NUMBER OF TAXA	%
1. Widespread taxa	228	66.06
European (Eu)	37	10.88
European-SW Asian (EA)	76	22.35
Euro-Siberian (ES)	53	15.59
Paleotemperate (Pt)	14	4.12
Circumtemperate (Ct)	16	4.71
(Circum-)Boreal (Bo)	9	2.65
Arctic-Alpine (AA)	2	0.59
Cosmopolitan (Co)	20	5.88
[Cosmopolitan], European-SW Asian	1	0.29
2. Mediterranean taxa	57	16.76
East Mediterranean (EM)	3	0.88
Mediterranean (Me)	24	7.06
Mediterranean-Atlantic (MA)	1	0.29
Mediterranean-European (ME)	22	6.47
Mediterranean-SW Asian (MS)	7	2.06
3. Balkan taxa	53	15.59
Balkan (Bk)	31	9.12
Balkan-Anatolia (BA)	17	5.00
Balkan-Central Europe (BC)	2	0.59
Balkan-Italy (BI)	3	0.88
4. Endemic taxa	1	0.29
Greek endemic (•)	1	0.29
5. Alien taxa	1	0.29
[North American] (N-Am.)	1	0.29
Total	340	100.00

Conclusion

The damp meadows and mires in the Greek part of Rhodopi Mountain Range, despite their limited extent, present high plant diversity. The vascular flora of the investigated areas consists of 340 taxa, which belong to 175 genera and 58 families. Seven taxa were identified for the first time for the floristic region of North-East Greece. The most species-rich family of the vascular flora is *Poaceae*, which accounts for 40 taxa (11.76% of all species). The most species-rich genus is *Carex* with 15 taxa. Hemicryptophytes and therophytes are the most abundant plant life forms with 59.71% and 15% respectively, while the most abundant chorological category consists of the Widespread plants with 228 taxa belonging to this category.

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G. Caruso, S. Casavecchia, E. Biondi

The vascular flora of Uria basin (Catanzaro, S-Italy) and its conservation relevance

Abstract

Caruso, G., Casavecchia, S. & Biondi, E.: The vascular flora of Uria basin (Catanzaro, S-Italy) and its conservation relevance. — Fl. Medit. 32: 99-116. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The inventory of the taxa collected during a survey held in 2011-2013 in Uria Basin, Catanzaro Province (Calabria, S-Italy) is here reported. The investigated territory, part of an area locally known as Presila Catanzarese, is located between the Ionian Sea and the Sila Massif, along the Ionian coast of Calabria region. The vascular flora of the area is scarcely known. The floristic samplings were performed in 318 sites selected as representative of the local environmental diversity as regards to climate, litho-morphology and land-use. The research led to the identification of 871 species and subspecies of vascular plants (652 of which never recorded before in the area), belonging to 100 families and 452 genera. Among these taxa, 8 are new [the most relevant being *Erodium salzmännii*, *Helianthemum jonium*, *Hypericum hircinum* subsp. *hircinum*, *Leontodon siculus*, *Populus nigra* subsp. *neapolitana*] and 3 confirmed for Calabrian flora, 51 are endemic to Italy, 32 included in IUCN Red Lists of Italian flora. In the area have been found 54 alien species, 2 of which new to Italy [*Eucalyptus astringens* and *E. propinqua*], 2 formerly considered native (or cryptogenic) to Italy (*Oxalis corniculata* and *Juglans regia*). Data confirmed the great relevance of this territory for plant diversity and the need of long-term protection that seems to justify the recent establishment of two specific protected areas (Valli Cupe Regional Natural Reserve and ZSC Valle Uria - IT9330185).

Key words: Calabria, conservation, endemism, flora, Presila catanzarese, Valli Cupe Regional Natural Reserve.

Introduction

The floristic knowledge of a territory is of considerable importance for scientific and conservation (Carli & al. 2018) purposes. In particular, the information concerning the endemic plants, such as their distribution and threats, are key elements to drive national conservation strategies (Brundu & al. 2017; Orsenigo & al. 2018) and legislation addressing nature conservation (European Commission 2019a, 2019b; European Union 2014; Brundu & al. 2020). Wild vascular plants were recently used in some areas of Italy to evaluate the long-term changes in the floristic composition of vegetation of mountain ecosys-

tems (Calabrese & al. 2018; Frate & al. 2018) and for risk assessment and management of soils polluted by industrial processes (Visconti & al. 2018).

The hydrographic basin of the Uria River, as well as likely the rest of the Presila Catanzarese, due to the richness of habitats of this territory supported by a heterogeneous morphology, geology, altitude, water availability is expected to host a rich vascular flora. In addition to traditional land uses (e.g. forestry, pasture, agriculture, etc.) this territory has been characterized, during the last two decades, by an increasing eco-touristic interest. Around twenty, maybe thirty thousand tourists now rambles yearly the area, representing a huge opportunity for the economy of the place, but also a potential threat for the long term conservation of the natural resources of the area. This make it urgent a systematic research addressed to increase the floristic knowledge of this territory.

Materials & methods

Study area

Calabria is the southernmost continental administrative region of Italian peninsula. Itself a peninsula, with an area of about 15,223 sq km, it results biologically quite isolated by the rest of Apennine range, because separated northward by the Pollino Massif and surrounded by about 740 km of sea coasts (Ionian Sea eastward and southward, Tyrrhenian Sea westward). Because of its geographical position, right at the center of the Mediterranean basin, of its complex geological and biological history, and because of its habitat richness (due mostly to the wide variation of geomorphology, geology and climate) the region shows a very rich and complex native vascular flora (Bartolucci & al. 2018) counting 2,627 taxa of specific and subspecific rank. According to Peruzzi & al. (2014), 60 (2%) of the Calabrian plants are strictly regional endemic, and 210 (8%) are Italian endemic (total 10% endemics), while, according to Celesti-Grapow & al. (2010), 179 are the alien plant taxa occurring in the region (totalizing 2,806 plants, both native and alien).

The regional territory doesn't result homogeneously well known from a botanical point of view. According to Scoppola & Blasi (2005) many poorly studied areas still occur in Calabria (Fig. 1). Among the less investigated territories of Calabria, at the NE corner of the Catanzaro Province (interesting the municipalities of Magisano, Sellia Marina, Sersale, Soveria Simeri, Zagarise), a hilly belt commonly known as Presila Catanzarese exists, and part of the study area (Uria basin) overlaps this area, just between the Sila Massif and the Ionian Sea (Fig. 2).

The study area also overlaps some protected areas: the Sila National Park (NW corner of the study area) and the most recent site of community importance established in the Italian Republic (Valle Uria - IT9330185) as part of the European environmental network Rete Natura 2000. The establishment of a new SCI has been possible because inside the regional territory one of the habitats listed in the Habitat Directive 43/92 (European Community 1992; Biondi 2007; Biondi & al. 2005), the 92C0 *Platanus orientalis* and *Liquidambar orientalis* woods (*Platanion orientalis*) wasn't represented (Gianguzzi & al. 2016). Recently in this territory has also been established, through the Regional Law 41/2016, a regional natural reserve named "Valli Cupe" (Consiglio Regionale Calabria 2016a, 2016b). The reserve encompasses three different areas, one of which totally, and

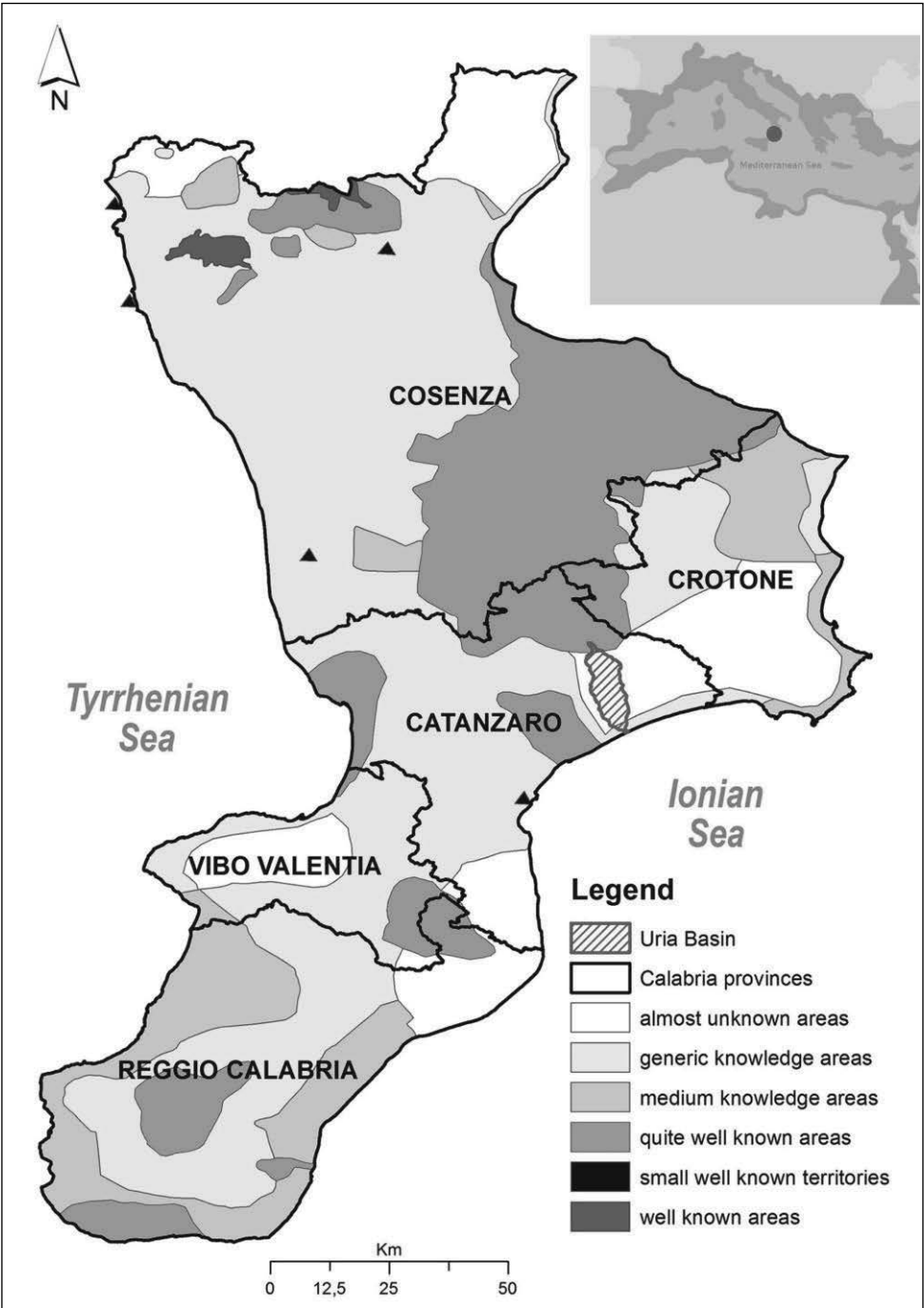


Fig. 1. Different levels of floristic knowledge recorded inside the territory of Calabria according to Bernardo & al. (2005, modified), provinces administrative borders and location of the study area.

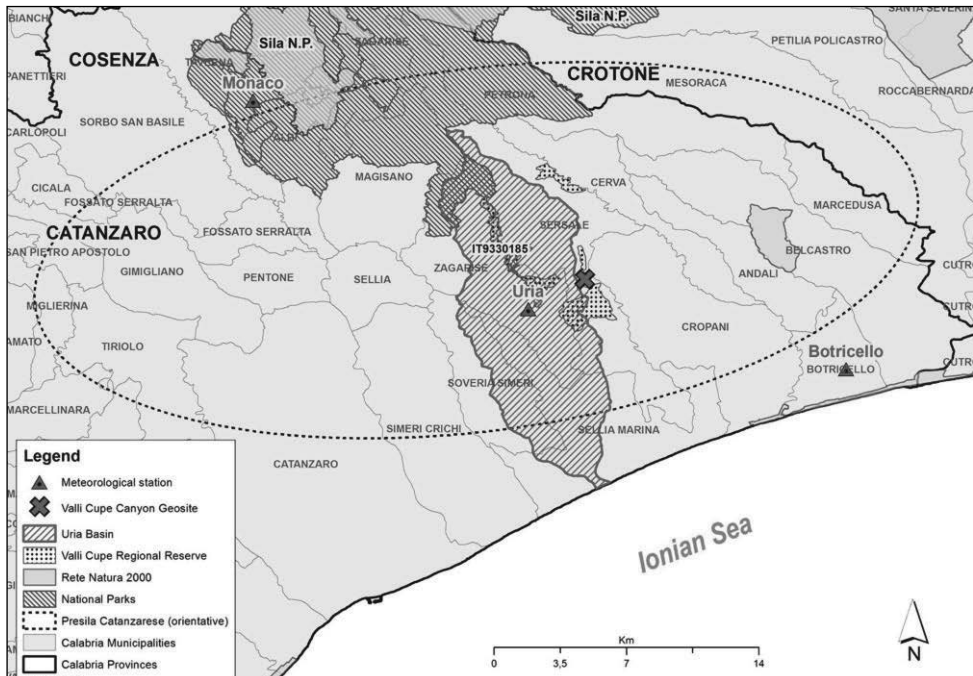


Fig. 2. The NE corner of Catanzaro Province, including Presila Catanzarese (no precise administrative border exists), Uria basin (study area), municipalities, protected areas located close to the study area or overlapping it.

another partially overlapping the study area. A few years ago has been also identified a site (Valli Cupe Canyon Geosite) of geological interest (Procopio 2006) just outside of the study area perimeter (Fig. 2).

The study area is located by the Ionian coast of Calabria from the coastline towards inland approximately for 19.8 km, while it is not wider than 5.5 km. The area, so resulting longer than wider, it is almost N-S oriented. It occupies a 72.8 sq km area (0.48% of the total Calabrian surface), and has a perimeter about 48.5 km long. Its centroid has the following coordinates: 16°42'20"E - 38°57'50"N (Fig. 3A).

The geomorphology is characterized by the alternation of hills and watersheds, except in the lower part, southward, which is roughly flat. Slopes steepness is higher inland (in some cases almost vertical and even jutting), mainly where the contour lines are close each other. Watersheds are mostly oriented in N-S direction and all of them converge towards the central valley. The altitude range is around 0-1,300 m a.s.l., maximum in the north, minimum in the south, close to the sea coast (Fig. 3B). According to Marchetti & al. (1970a; 1970b; 1968; 1971), the northern part of the study area is dominated by sedimentary rocks (conglomerate and sandstone) alternate with crystalline substrata (granite, schist). The southern part is dominated by clay, sand and alluvial formations (Fig. 3C). Hydrography is concentrated on the central axis, with changing names from wellspring

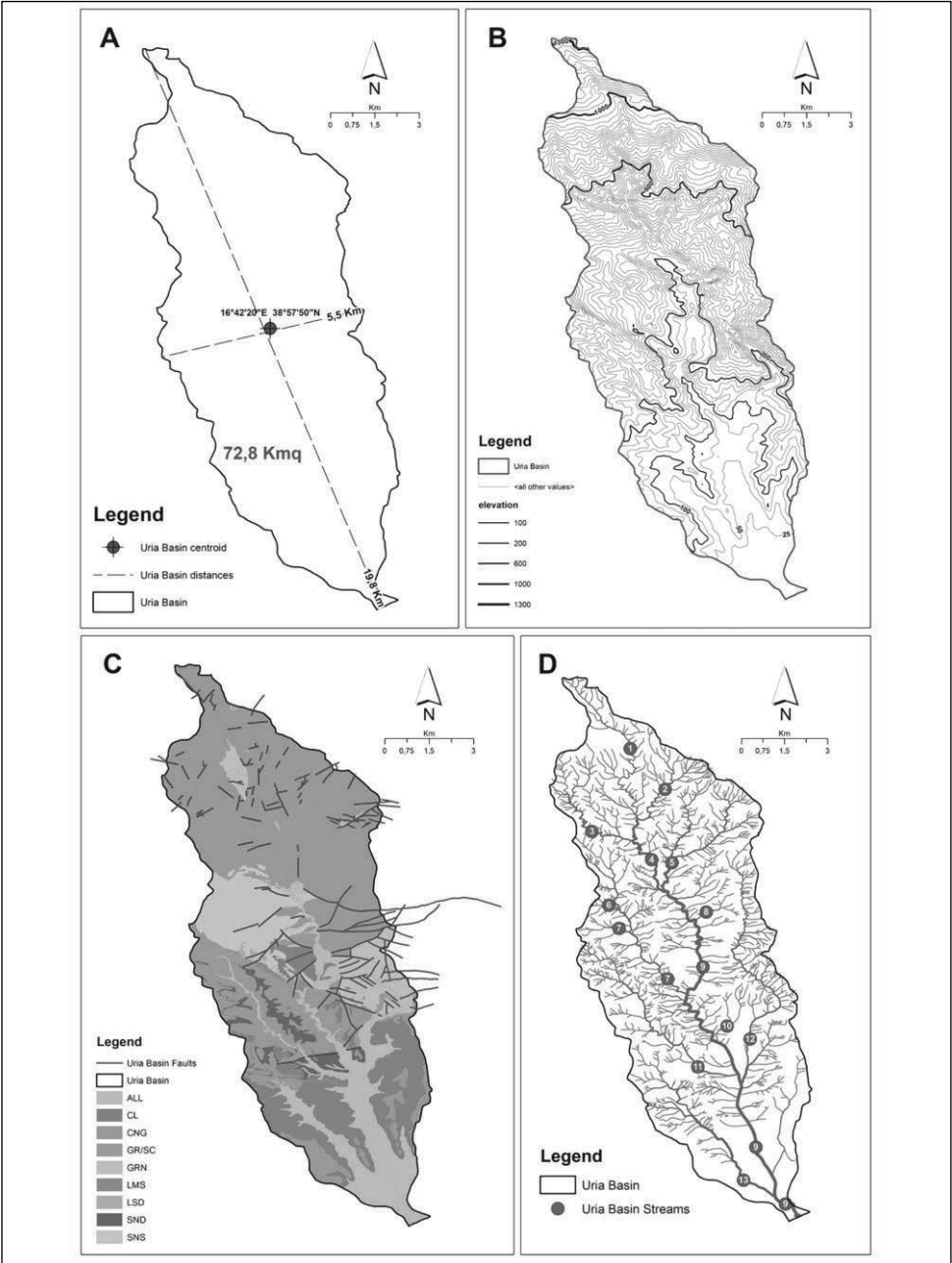


Fig. 3. Location and general geographic features of the Uria basin, the study area (A), geomorphology (contour lines) (B), geology (with faults) (C) and hydrography of Uria valley with main tributaries: 1 - Torrente Campanaro; 2 - Fosso di Rupa; 3 - Fosso di Cona; 4 - Torrente Ervarulo; 5 - Fosso Rovetto; 6 - Fosso Triarie; 7 - Fosso Valle; 8 - Fosso Catoia; 9 - Torrente Uria; 10 - Fosso Gufo; 11 - Torrente Scilotraco; 12 - Torrente Raga; 13 - Fosso Umbro (D).

(Campanaro), along the stream (Ervarulo) down to the mouth (Uria). Uria branch, the only one in the basin with permanent water, is where many smaller streams converge to, both from the right (Fosso Valle, Torrente Scilotraco, Fosso Umbro) and the left (Fosso di Rupa, Fosso Rovetto, Fosso Catoia, Fosso Gufo, Torrente Raga) sides of the basin (Fig. 3C).

The climatic data provided by the available (Monaco, Uria, Botricello) meteorological stations closer to the study area (Electronic Supplementary File 1, Fig. 1) applied to Rivas-Martinez & Rivas-Saenz (1996-2019) bioclimatic classification system produced pluvio-thermometric diagram shown in the ESF1, Fig. 2.

According to the bioclimatic classification of Italian territory proposed by GIS Natura 2.0 (DEI-MATT-DPN 2002) the macrobioclimate of the study area is mainly mediterranean, except in the northern part where is temperate. The thermotype is thermomediterranean in the southern part, mesomediterranean in the middle and supratemperate in the north. The ombrotype is mainly subhumid in the central part, while it is dry southward and hyperhumid northward (ESF1 Fig. 2).

According to Bernardo & al. (2005) the level of botanical information available for the study area is quite low. Nevertheless, later published papers on the vascular flora of the Presila Catanzarese, demonstrates the presence of native species of conservation interest. A few of these are *Carpinus betulus* L., *Paragymnopteris marantae* (L.) K.H.Shing subsp. *marantae*, *Osmunda regalis* L., *Platanus orientalis* L., *Pteris cretica* L., *Tilia platyphyllos* Scop. subsp. *platyphyllos*, *Woodwardia radicans* (L.) Sm. (Caruso & al. 2004; Lupia 2004; Caruso & al. 2008; Caruso & al. 2012; Caruso & al. 2013), the South Italian endemics *Lomelosia crenata* subsp. *pseudisetensis* (Lacaita) Greuter & Burdet and *Stipa oligotricha* Moraldo subsp. *oligotricha* (Caruso 2007a; Caruso & Uzunov 2011; Banfi & al. 2015), the strictly endemic *Centaurea calabra* G. Caruso, S.A. Giardina, Raimondo & Spadaro (Caruso & al. 2013d). In the area also exotic species have been found, such as *Crassula lycopodioides* Lam., *Vicia faba* L., *Eucalyptus globulus* Labill., *Callitropsis arizonica* (Greene) D.P. Little (Caruso 2007b; Caruso & al. 2007c; Caruso & al. 2008b). A first fragmentary floristic account on the flora of the study area has been provided by Bernardo & al. (2012), albeit including only a few stands, while the first partial report of the local flora has been presented by Caruso & al. (2013a) as part of a PhD work. A list of the floristic information and visited locations inside the study area before this survey is available in the ESF1, Table 4.

Floristic survey

The floristic investigation of the study area has been performed applying the classic collection based floristic methodology. A systematic botanical collection plan, counting 318 stands (ESF1, Table 5), to be sampled along a 3-year survey period (2011-2013), has been established in order to represent the habitat diversity occurring in the study area. In some cases, the sampled stands are very close due to the complex mosaic of habitats and the variable ecology of the basin. Each stand has been sampled and the collected specimens dried and stored. Afterwards, the taxonomic identification has been carried out using standard floras such as Tutin & al. (1968-1980, 1993), Pignatti (1982) and Pignatti & al. (2017-2019). Part of the collected material has been provided for identification to specialists such as Banfi E. for Poaceae and Gangale C. for *Carex*. Sets of the plant material are stored in the following herbaria: CLU, ANC, MSMN (*Poaceae*) and in one of the authors' personal

collection (Herbarium Giuseppe Caruso). The names applied to native plant species and subspecies follow Bartolucci & al. (2018) and Pignatti & al. (2017-2019); alien species have been named according to Celesti-Grapow & al. (2009; 2010) and Galasso & al. (2018); names of families and genera follow Peruzzi (2010).

Life form following Raunkiaer (1934) and Ellenberg & Mueller-Dombois (1967), along with chorological type according to Pignatti & al. (2017-2019) have been used to calculate the biological and chorological spectra.

The floristic richness expected for the area was calculated according to Cristofolini (1998), by means of the extrapolation of a locally valid linear regression between the logarithm of the surface of some reference areas (in km²) and the logarithm of their respective taxa densities (nr taxa/km²) (Table 1). For this purpose, the subregional Calabrian floras published in the last 40 years have been considered (La Valva & Ricciardi 1978; La Valva 1984; Maiorca & Spampinato 1994; Bernardo & Maiorca 1997; Maiorca & al. 2002; Musarella & Tripodi 2004; Maiorca & al. 2007; Maiorca & Puntillo 2009; Di Marco & al. 2012). The regression line represents the expected taxa density logarithm, according to the area extent logarithm. By applying this methodology it is possible to obtain an objective estimation of floristic diversity, irrespective of the size of the area.

Results

The vascular flora of the study area counts 871 species and subspecies. The observed floristic richness for the Uria Valley was higher than expected (745 taxa, according to the linear regression formula), with an observed/expected ratio of 1.17, a value that places the Uria Valley up the regression line (Table 1; Fig. 5). This result could be explained by the high diversity of habitats found along the valley and the high density of sampling areas.

The found taxa belong to 100 families and 452 genera. The most represented taxa belong to the *Asteraceae* with 113 taxa, *Fabaceae* with 93, *Poaceae* 91, *Apiaceae* 44, *Lamiaceae* 39, *Brassicaceae* 37, *Caryophyllaceae* 31, *Rosaceae* 27, *Ranunculaceae* 16, *Plantaginaceae* 16, *Cyperaceae* 16, *Polypodiaceae* 15, *Boraginaceae* 14, *Geraniaceae* 13, *Rubiaceae* 12, *Orchidaceae* 12, *Amaranthaceae* 12, *Caprifoliaceae* 11. The remaining families do contribute to this flora with less than 10 species each (Fig. 6). The most represented genera are *Trifolium* with 22 species, *Carex* with 10, *Geranium*, *Medicago* and *Vicia* with 10, *Silene* with 9, *Lathyrus* 8, *Festuca*, *Galium*, *Hypericum*, *Plantago*, *Rumex* with 7, *Allium*, *Euphorbia*, *Juncus*, *Lotus*, *Rosa*, *Verbascum* with 6, while the rest of genera (434) contributes with less than 6 species. The majority of the genera (91) occurring in the study area counts just 1 or 2 species.

The biological spectrum of the vascular flora of the study area, according to Raunkiaer (1934) and Ellenberg & Mueller-Dombois (1967) is dominated by therophytes (36.3%) and hemicryptophytes (33.7%), while helophytes (0.2%) and hydrophytes (0.6%) are the less represented (Fig. 7).

The chorological spectrum of the Uria basin shows a prevalence of stenomediterranean (21%) and eurimediterranean (20.6%) taxa. The contingent of endemic (and subendemic) species counts 51 entities (5.9%) while the alien (and cultivated) species are 54 units (6.2%) (Fig. 8).

Table 1. Expected and observed floristic diversity in subregional floras published in the last 40 years (including the present). The values of observed and expected nr. taxa/km² ratio higher than 1 are marked in bold font.

Subregional Calabrian floras	area (km ²)	nr. taxa	log area (x)	log taxa/area (y)	logarithmic regression (y = -0,8315x + 2,5584)	observed taxa/area	expected taxa/area (y=10 ^x)	obs./exp.
Pentedattilo Cliff	0.01	251	-2	4.34	4.2214	25100	16649.45	1.51
Argentino Valley	39.8	846	1.599883072	1.33	1.228097226	21.26	16.91	1.26
Mavigliano Wood	6.5	602	0.812913357	1.97	1.882462544	92.62	76.29	1.21
Cassano High	1	421	0	2.62	2.5584	421	361.74	1.16
Crati Outlet	3	482	0.477121255	2.21	2.161673677	160.67	145.10	1.11
Dino Island	0.5	295	- 0.301029996	2.77	2.808706441	590	643.73	0.92
La Vota Lakes	1.85	243	0.267171728	2.12	2.336246708	131.35	216.89	0.61
Cirella Island	0.12	161	- 0.920818754	3.13	3.324060794	1341.67	2108.92	0.64
Papasidero	54.65	580	1.737590166	1.03	1.113593777	10.61	12.99	0.82
Uria Valley	72.8	871	1.862131379	1.08	1.010037758	11.96	10.23	1.17

The Uria basin vascular flora survey revealed a pool of 8 taxa new to Calabria flora: *Carduus collinus* Waldst. & Kit. subsp. *collinus*, *Erodium salzmannii* Delile, *Helianthemum jonium* Lacaita & Grosser, *Hypericum hircinum* L. subsp. *hircinum*, *Leontodon siculus* (Guss.) Nyman, *Populus nigra* subsp. *neapolitana* (Ten.) Asch. & Graebn., *Tommasinia altissima* (Mill.) Reduron, *Torilis leptophylla* (L.) Rchb.f.. Additional 3 taxa are confirmed to Calabria flora: *Chamaemelum fuscum* (Brot.) Vasc., *Foeniculum vulgare* Mill. subsp. *vulgare*, *Geranium brutium* Gasparr.

Among the 88 native taxa listed in the ESF1, Table 1, the most threatened endemics are *Acer cappadocicum* subsp. *lobelii* (Ten.) A.E. Murray, *Centaurea calabra* G. Caruso, S.A. Giardina, Raimondo & Spadaro, *C. sarfattiana* Brullo, Gangale & Uzunov, *Chaerophyllum hirsutum* L., *Crepis apula* (Fiori) Babç., *Dianthus vulturius* Guss. & Ten. subsp. *vulturius*, *D. longicaulis* Ten., *Euphorbia meuselii* Geltman, *Hypericum barbatum* subsp. *calabricum* (Spreng.) Peruzzi & N.G. Passal., *Lomelosia crenata* subsp. *pseudisetensis* (Lacaita) Greuter & Burdet, *Pimpinella anisoides* V.Brìg., *Salix apennina* A.K. Skvortsov, *S. brutia* Brullo & Spamp., *Silene echinata* Otth, *Stipa oligotricha* Moraldo subsp. *oligotricha*. Other threatened taxa are the native *Barlia robertiana* (Loisel.) Greuter, *Clematis cirrhosa* L., *Dracunculus vulgaris* Schott, *Ephedra distachya* L. subsp. *distachya*, *Equisetum palustre* L., *Genista monspessulana* (L.) L.A.S.Johnson, *Limodorum abortivum* (L.) Sw., *Lotus biflorus* Desr., *Ophrys bertolonii* Moretti subsp. *bertolonii*, *Platanus orientalis* L., *Pteris cretica* L., *Saponaria calabrica* Guss., *Serapias parviflora* Parl., *Spiranthes spiralis* (L.) Chevall., *Tilia platyphyllos* Scop. subsp. *pseudorubra* C.K. Schneid., *Ulmus glabra* Hudson.

Discussion

According to the available literature (Caruso & al. 2004, 2008, 2012, 2013; Lupia 2004; Caruso 2007a, 2007b; Bernardo & al. 2012; Banfi & al. 2015; Roma-Marzio & al. 2017) (Fig. 4) 219 vascular plant taxa were formerly recorded for the Uria basin. This survey added 652 unrecorded taxa to the local vascular flora, which now counts 871 units, significantly improving the floristic knowledge of the area.

A high level of phytodiversity occurs in the Uria basin, especially considering it in relation to the whole flora of Calabria, both counting or not alien species. In the first case (aliens included) the Uria flora represents the 31.0% ($X_{n+a} = 870 \times 100 / 2,806 = 31.0\%$)

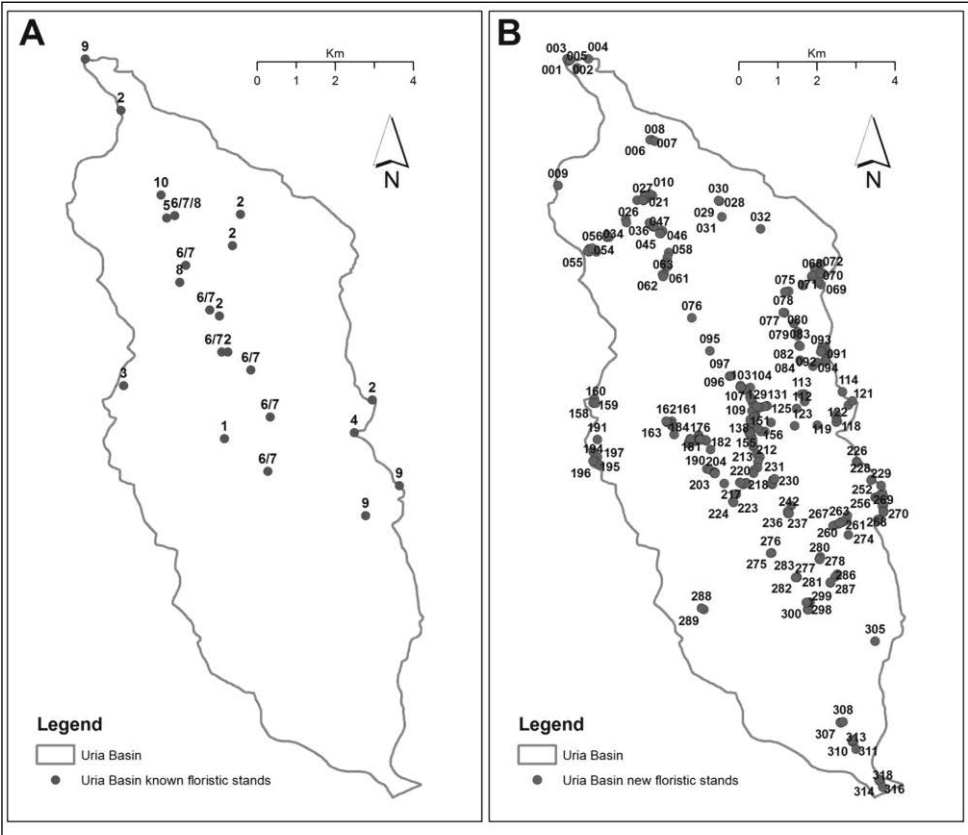


Fig. 4. Distribution of formerly available floristic information in the study area by literature (1. Banfi & al. 2015; 2. Bernardo & al. 2012; 3. Caruso & Uzunov 2011; 4. Caruso 2007; 5. Caruso & al. 2007; 6. Caruso & al. 2008; 7. Caruso & al. 2012; 8. Lupia 2004; 9. Caruso & al. 2013; 10. Roma-Marzio & al. 2017) (A) and distribution of the floristic sampling stands across the study area (B). A few of these stands, at the scale of this map, overlap because very close each other. This close sampling strategy has been necessary to better investigate the complex habitat mosaic of the study area.

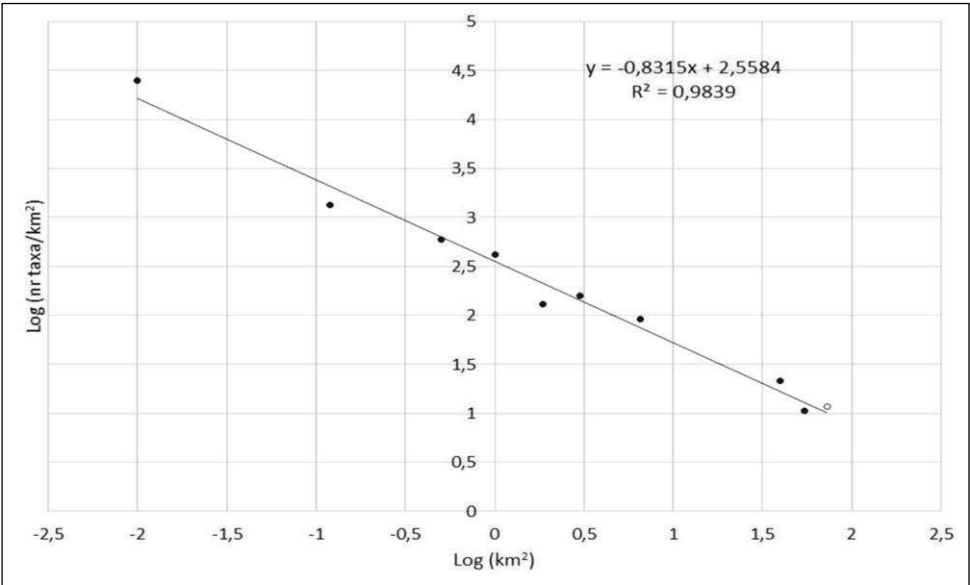


Fig. 5. Subregional Calabrian floras. Linear regression, with relative formula, between the logarithm of areas (km²) and the logarithm of the ratio nr. taxa/km². The value obtained for the Uria Valley is the empty dot.

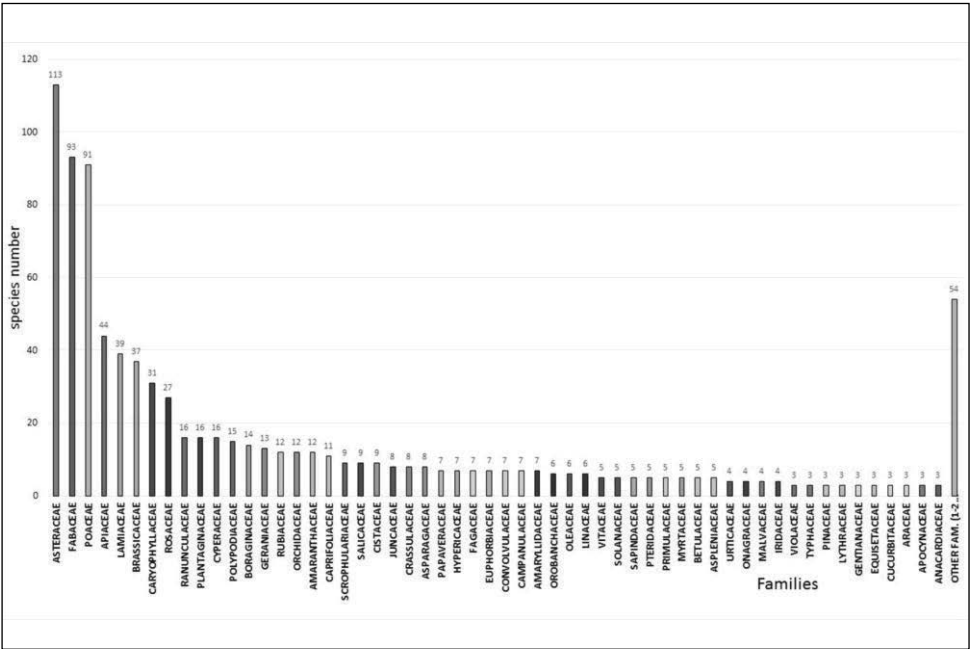


Fig. 6. Number of species per family in the vascular flora of Uria basin.

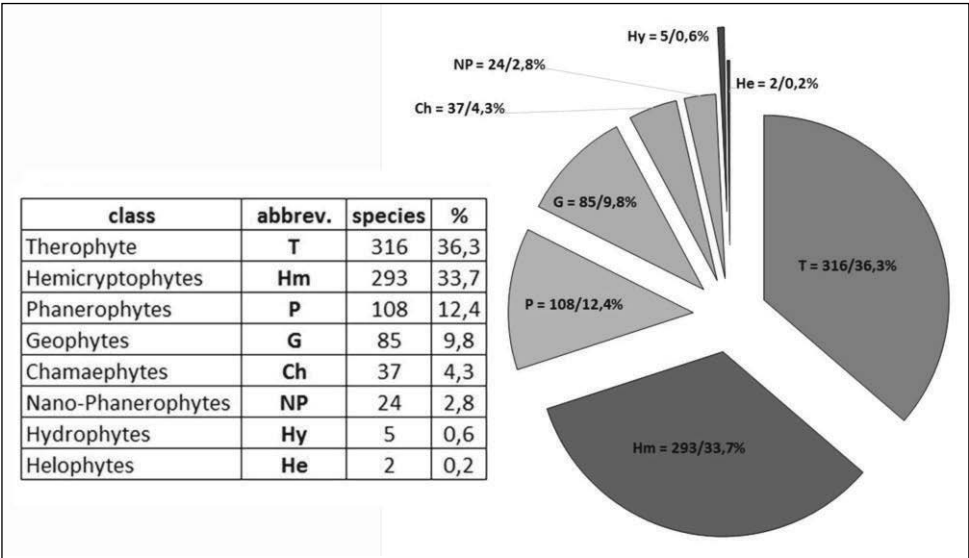


Fig. 7. Biological spectrum of the vascular flora of Uria basin. The growth forms are grouped by main categories.

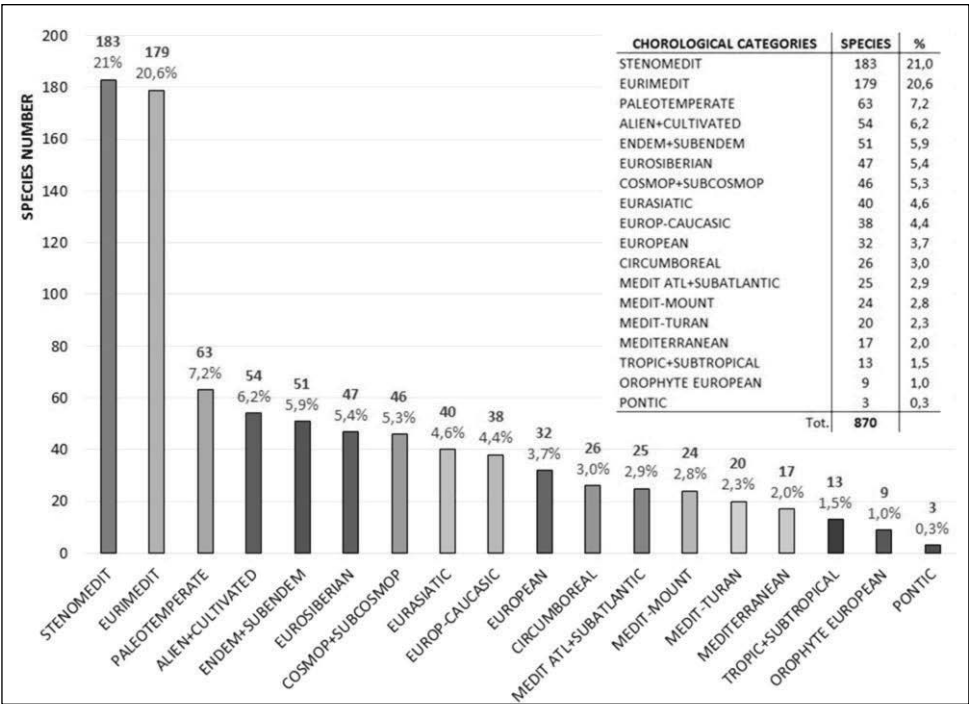


Fig. 8. Chorological spectrum of the Uria basin vascular flora.

of Calabria flora, while in the second (just native plants) Uria flora represents the 31.4% ($X_n = 824 \times 100 / 2,627 = 31.4\%$) of the regional flora. By relating this already startling information to the percentage of the surface of Calabria region occupied by Uria basin ($X_U = 72.8 \times 100 / 15,223 = 0.48\%$) it is possible to appreciate how much of Calabrian phyto-diversity is concentrated in such a small territory: almost one third of the regional flora inside less than 0.5% of the whole regional surface. But, even more important, applying a methodology useful to measure floristic diversity irrespective of the size of the area the flora of the Uria Valley confirms its high level of phytodiversity. Data confirm the high quantitative interest of the vascular flora hosted in the Uria valley, but also its qualitative importance, how demonstrate 3 taxa confirmed and 8 firstly recorded for Calabrian native flora (ESF1, Tables 1 and 5). Among these, considerable, from a phytogeographic point of view, are *Erodium salzmannii* Delile formerly occurring only in Sardinia on the Italian territory, *Carduus collinus* Waldst. & Kit. subsp. *collinus* and *Helianthemum jonium* Lacaita & Grosser, both here at southernmost known location, the Tyrrhenian *Hypericum hircinum* L. subsp. *hircinum*, the Sicilian endemic *Leontodon siculus* (Guss.) Nyman, *Populus nigra* subsp. *neapolitana* (Ten.) Asch. & Graebn., *Tommasinia altissima* (Mill.) Reduron and *Torilis leptophylla* (L.) Rchb.f.

Equally relevant is the high frequency of endemic (and subendemic) taxa (51 units), 32 of which included in IUCN Red Lists of Italian flora, 19 never assessed. Relevant, is also the occurrence of a rich contingent (37 taxa) of native plants whose extinction risk has been assessed, because of their conservation significance, according to IUCN rules (ESF1, Table 2).

Not less important, for opposite reasons, is the occurrence in the Uria basin of 54 alien species, 2 of which new to Italy [*Eucalyptus astringens* (Maiden) Maiden and *E. propinqua* H.Deane & Maiden] and 2 formerly considered native (or at least cryptogenic) to Italy (*Oxalis corniculata* L. and *Juglans regia* L.). For all alien taxa whose degree of invasiveness wasn't formerly known, an evaluation is here proposed at regional (Calabria) or national (Italy) level, ranging from casual to invasive (ESF1, Table 3).

Conclusions

The rich vascular flora of the Uria basin would deserve this territory to be included, as soon as possible, among Important Plant Areas and/or Biodiversity Hot-Spots. Because of this, it would seem that recently established protected areas (SCI "Valle Uria" and Regional Natural Reserve "Valli Cupe") could certainly play an active role in the conservation of this rich floristic diversity, and maybe even in the management of the important amount of alien species found, a threat for ecosystems and one of the main drivers of worldwide biodiversity loss (Bellard & al. 2016).

Some concern do emerge, mostly about the future interpretation, and destiny itself, of the habitat 92C0 [*Platanus orientalis* and *Liquidambar orientalis* woods (*Platanion orientalis*)] in Italy. Many Italian Rete Natura 2000 sites in fact, SCI "Valle Uria" included, do owe their inclusion in the European environmental network mainly to the presence of *Platanus orientalis*, which was, at the time, considered native to Italy. The changed status of this species, native to archeophyte, proposed by Rosati & al. (2015) could affect the recognizability of the habitat in the country. Recently, Rinaldi & al. (2019) surveying the

genetic structure and variation of *Platanus orientalis* in populations from Albania, Bulgaria, Greece, S-Italy (Uria included), Sicily and Turkey, found an overall decrease in estimated genetic diversity towards western populations, with those from S-Italy and Sicily showing the lowest values, resulting, according to the authors, from range fragmentation experienced by the species during the Tertiary at its westernmost limit. This would be confirmed by the high level of genetic isolation of these populations. In our opinion, this issue, considering the phytogeographic, ecological, phytosociological and conservation role played by this taxon, deserves further investigation, perhaps extending the genetic study to the whole range of the species, including many other populations eastward.

Further floristic investigation is also desirable for the hydrographic basins adjoining Uria valley, and in general for Presila catanzarese. Information doubtlessly will improve national floristic dataset and will support the conservation policies even in facing the growing touristic interest in the area.

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Une première investigation sur la flore horticole de la région de Annaba (Nord-Est algérien): Biodiversité et intérêt socio-écologique

Abstract

Bellili, A. M., Meddad-Hamza, M. Babali, B., Belabed-Zediri, H., Belabed, A. I. & Hamel, T.: Une première investigation sur la flore horticole de la région de Annaba (Nord-Est algérien): Biodiversité et intérêt socio-écologique. — Fl. Medit. 32: 117-129. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

A first investigation on the horticultural flora of the Annaba region (North-East Algeria): Biodiversity and socio-ecological interest. — The present study was undertaken with the aim of improving knowledge and proposing a first checklist of horticultural plants present in public and private green spaces in the region of Annaba (northeastern Algeria). During the period from 2018 to 2021, a total of 164 taxa recorded. The results obtained are given in terms of floristic composition, forms of life, type of nativity and socio-ecological interest of the plants observed. However, the real impact of potentially invasive species on plant communities and ecosystems deserves urgent assessment with specific studies.

Key words: Horticultural flora, floristic composition, life forms, socio-ecological interest..

Introduction

Deux composantes importantes participent à la création de la biodiversité végétale, à savoir: la végétation indigène vivant à l'état naturel et la végétation cultivée, cette dernière comprend des espèces alimentaires concentrées le plus souvent dans les champs et les vergers et des espèces ornementales essentiellement retrouvées dans les pépinières, les jardins et les autres types d'espaces verts (Östberg & al. 2018; Meghan & al. 2020). Cette flore horticole est considérée une composante importante du paysage urbain, avec de multiples rôles écologiques et fonctions socio-économiques (Muzafar & al. 2018; Caneva & al. 2020). Ces espaces verts peuvent être exploités pour la préservation *ex-situ* des espèces végétales rares ou menacées d'extinction et constituent ainsi des lieux propices pour la sauvegarde de la biodiversité (Sakhraoui & al. 2019).

Les études ont montré que les écosystèmes urbains contribuent à l'amélioration de la qualité de l'air en limitant la concentration des différents gaz ou particules fines résultant essentiellement du trafic routier (Nowak & al. 2006). Ils participent également au bien-être des habitants et peuvent être porteurs de véritables valeurs culturelles et esthétiques (Savard & al. 2000).

Tous les pays du monde possèdent dans leurs parcs et leurs jardins une flore ornementale le plus souvent introduite. Au moyen âge, le matériel végétal était sujet de grandes opérations d'échange, après la découverte des Amériques, plusieurs plantes ont été apportées par les premiers navigateurs en Europe, en Asie, en Afrique et vice versa (Gritti & al. 2006).

En Algérie, d'importantes collections de plantes exotiques furent introduites lors de la période coloniale par le jardin d'essai du Hamma. En 1867, Hardy, le directeur du jardin, dénombre 8214 espèces et variétés (Carra & Gueit 1952), parmi lesquelles nous retrouvons des arbres à intérêts industriels, des arbres fruitiers, des plantes ornementales, des plantes médicinales et des plantes aromatiques (Reichard & White 2001; Sakhraoui & al. 2019).

Vu le développement que connaît l'Algérie dans le secteur de l'horticulture et l'importance accordée ces dernières années à l'horticulture ornementale et les aspects paysagers, l'établissement d'un inventaire relatif à la flore horticole ne peut que remplir une grande lacune dans ce secteur et mettre le point sur la biodiversité végétale existante dans le pays.

Dans la littérature, la région de Annaba (Nord-Est algérien) qui couvre une grande partie de la péninsule de l'Edough est connue pour être riche en espèces végétales endémiques et rares (cf. Quézel & Santa 1962-1963; Hamel & al. 2013). Aujourd'hui, sa flore spontanée demeure étudiée (cf. Hamel 2013; Hamel & al. 2013, 2017; Allem & al. 2017b; Hamel & Boulemtafes 2017a, 2017b; Boulemtafes & al. 2018; Fetnaci & al. 2019; Martin & al. 2020). Toutefois, la flore horticole notamment celle à intérêt ornementale ont été par contre, négligée et complètement délaissées. C'est dans ce contexte, que s'inscrit notre travail qui consiste à combler ce manque par la réalisation d'un inventaire des espèces horticoles existantes dans la région de Annaba ciblant plusieurs sites.

Zone d'étude

L'étude a été menée au niveau de douze sites urbains dont deux sites périurbains situés dans la région de Annaba (Nord-Est de l'Algérie) qui selon la subdivision biogéographique proposée par Quézel & Santa (1962) appartiennent à la Numidie (K_3) (Fig. 1). Les cimetières, jardins publics et universitaires ont été ciblés en suivant la plantation ancienne de plus de 50 ans. D'autres sites des jardins privés de maisons ont été visités en raison de leur potentiel horticole (Fig. 2 et Tab. 1). Faute de données climatologiques spécifiques aux sites étudiés, nous avons eu recours à la méthode des extrapolations (Seltzer & Auberty 1946) pour déterminer le type de climat de chacun des sites. Les précipitations moyennes sont de l'ordre de 689 mm par an à Bône Marché (incluant: Cours de la révolution, Square El Houria, Campus universitaire de Sidi Amar, Campus universitaire d'El Bouni, Square Edough Est, Square Edough Ouest, Cimetière Chrétien et Parc d'attraction de Sidi Achour), 787mm à Bône Port (incluant: Jardins du Majestic et Cimetière Zeghoune), 967 mm à Herbillon (incluant: Jardins de Chétaibi) et 1169 mm à l'Edough (incluant: l'Arboretum de l'Edough).

Méthodologie

L'inventaire de la flore horticole de la région de Annaba a été réalisé durant quatre années consécutives (2018-2021). L'ensemble des sites d'étude ont été parcourus

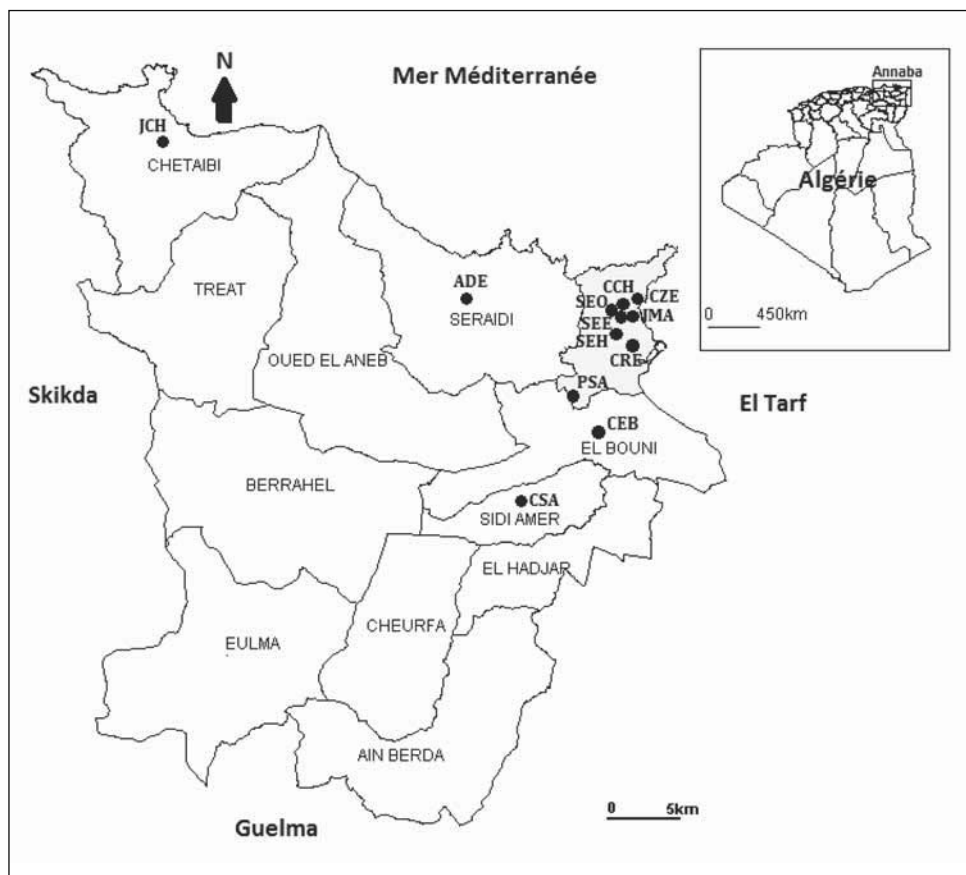


Fig. 1. Localisation des sites d'étude.

autant que possible, avec une prospection de chaque habitat durant les quatre saisons, de chaque année de la période d'étude. Les espèces, qu'elles soient en fleurs, en fruits ou en graines, sont photographiées et des échantillons sont récoltés de manière provisoire pour identification.

L'identification s'est faite en plusieurs étapes: tout d'abord en se référant à plusieurs ouvrages spécialisés en espèces horticoles comme Bruni & al. (2006); Odenwald & Turner (2006); Cullen & al. (2011); Vêla & al. (2013) et à la flore d'Algérie de Quézel & Santa (1963-1963), complétée par la flore d'Afrique du Nord de René Maire (1952-1987) pour les familles qui sont parues. Ensuite la plupart des identifications sont vérifiées, complétées et actualisées en consultant la flore d'Andalousie orientale (Blanca & al. 2009).

La nomenclature est ajustée selon l'index synonymique de Dobignard & Chatelain (2010-2013) et sa version actualisée en ligne (APD 2022). Le statut d'indigénat ou d'introduction des taxons recensés se réfère à la base de données de l'Afrique du Nord (APD 2022).

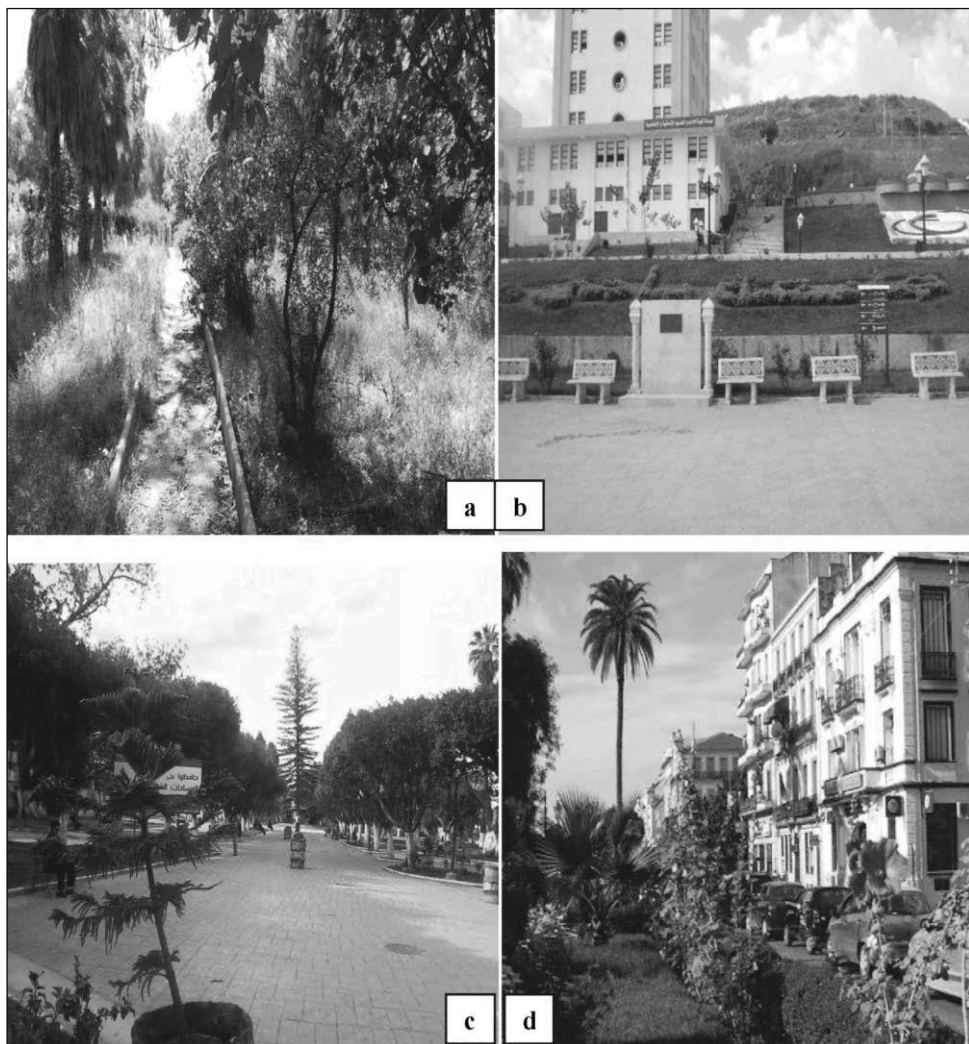


Fig. 2. a. Photos du jardin public du campus universitaire de Sidi Amar, b. campus universitaire d'El Bouni, c. square El Houria, d. cours de la révolution (cliché Hamel T., mars 2021).

Les formes de vie (ou types biologiques) des taxons observés sont données selon Raunkiaer (1934); Ellenberg & Müller-Dombois (1967); Blanca & al. (2009) et Tison & al. (2014).

De même, un recensement des plantes butinées par les abeilles (*Apis mellifera* L.) a été réalisés lors de nos sorties périodiques dans les sites étudiés. Les plantes dont les fleurs ont été butinées au moins deux minutes ont été considérées comme des plantes mellifères (Hamel & Boulemtafes 2017a).

Tableau 1. Les coordonnées des sites échantillonnés.

Code	Site	Coordonnées (Google Earth 2021)	Altitude (m)	Superficie (m ²)	Précipitation (mm)
CRE	Cours de la révolution	36°53'54"N ; 7°45'37"E	4	1028	689
SEH	Square El Houria	36°54'5"N ; 7°45'21"E	7	623	689
CSA	Campus universitaire de Sidi Amar	36°48'52"N ; 7°42'50"E	37	3828	689
CEB	Campus universitaire d'El Bouni	36°51'37"N ; 7°43'47"E	68	2942	689
JMA	Jardins du Majestic	36°54'34"N ; 7°45'52"E	10	50-100	787
SEE	Square Edough Est	36°54'20"N ; 7°45'30"E	8	390	689
SEO	Square Edough Ouest	36°54'35"N ; 7°45'24"E	10	671	689
CCH	Cimetière Chrétien	36°54'42"N ; 7°45'26"E	15	1088	689
CZE	Cimetière Zeghoune	36°54'54"N ; 7°45'55"E	18	795	787
ADE	Arboretum de l'Edough	36°54'25"N ; 7°39'53"E	735	325	1169
JCH	Jardins de Chétaibi	37° 3'50"N ; 7°22'38"E	27	50-100	967
PSA	Parc d'attraction de Sidi Achour	36°52'24"N ; 7°42'55"E	33	1150	689

Résultat

Richesse floristique

Un total de 164 espèces de plantes horticoles appartenant à 133 genres et 71 familles ont été identifiées dans les cimetières, jardins publics et privés de la région de Annaba (Annexe 1, ESF 1). La famille des *Asteraceae* était la plus importante en termes de nombre d'espèces et constitue 7,31% des plantes identifiées (12 espèces), suivie par les *Lamiaceae* avec 6,70% (11 espèces), les *Fabaceae* et les *Rosaceae* avec 6,09 % soit 10 espèces pour chacune de ces familles. Ces quatre familles représentent à elles seules plus d'un quart de la flore étudiée. S'ajoutant à ces dernières, les *Moraceae* (6 espèces soit 3,65%), les *Oleaceae* et les *Pinaceae* (5 espèces soit 3,04 % pour chacun), les *Rutaceae* et les *Iridaceae* (4 espèces soit 2,43 % pour chacun) étaient moyennement représentées. Le reste des familles étaient le plus souvent monospécifique ou bien bispécifique.

Le grand nombre des plantes recensées se concentraient dans le campus universitaire de Sidi Amar et les jardins privés du Majestic avec respectivement 71 et 61 espèces. En revanche, certaines espèces ont une répartition très large dans la région d'étude à savoir par voie de plantation (*Euonymus tobira* Thunb., *Lantana camara* L., *Lonicera japonica* Thunb., *Myoporum laetum* G. Forst., *Parthenocissus quinquefolia* (L.) Planch., *Rosa ×hybrida* Vill., *R. canina* L., *Schinus molle* L. et *Tecoma capensis* (Thunb.) Lindl.) ou bien par voie d'envahissement (*Ailanthus altissima* (Mill.) Swingle, *Eucalyptus camaldulensis* Dehnh. et *E. globulus* Labill.). Tandis que d'autres espèces sont notées dans un seul site d'étude, c'est le cas de: *Oenothera rosea* Aiton, *Sequoia sempervirens* (D.Don) Endl., *Taxus baccata* L., *Cercis siliquastrum* L. et *Pyracantha coccinea* M. Roem.

Forme biologique et écologique de la flore recensée

La répartition des types biologiques dans les sites étudiés suit, dans l'ensemble, le schéma suivant: phanérophytes (118 taxons, soit 71,95%) > chamaephytes (17 taxons, soit 10,36%) > hémicryptophytes et géophytes (12 taxons, soit 7,31% pour chacun) > thérophytes (4 taxons, soit 2,43%).

De plus, la composition floristique a permis de distinguer plusieurs groupements végétaux à savoir:

- groupement des taxons forestiers, il est le plus dominant et représenté essentiellement par: *Quercus suber* L., *Arbutus unedo* L., *Tilia europaea* L., *Cupressus sempervirens* L., *Taxus baccata* L., *Ilex aquifolium* L., *Myrtus communis* L., *Rhamnus alaternus* L., *Olea europaea* L. s. l., *Pistacia lentiscus* L., *Platanus × hispanica* Münchh., *Populus alba* L., *Prasium majus* L., *Schinus molle* L. et *Viburnum tinus* L.
- groupements des arbres fruitiers, il est largement représenté dans les vergers privés du Majestic et Chétaibi. Parmi ces espèces, on peut citer: *Eriobotrya japonica* (Thunb.) Lindl., *Citrus limon* (L.) Burm. f., *Citrus × aurantium* L., *Ficus carica* L., *Juglans regia* L., *Morus alba* L., *Musa × paradisiaca* L., *Prunus cerasifera* Ehrh., *Prunus dulcis*, (Mill.) D. A. Webb *Punica granatum* L., *Vitis vinifera* L. subsp. *vinifera* et *Ziziphus jujuba* Mill., ce dernier a donné le nom arabe de la ville de Annaba.
- groupement des plantes pionnières des pelouses artificielles perturbées; il comprend des herbacées annuelles ou vivaces, comme *Argyranthemum frutescens* (L.) Sch. Bip., *Calendula officinalis* L., *Cenchrus clandestinus* (Chiov.) Morrone, *Helianthus annuus* L. et *Heliotropium amplexicaule* Vahl.
- groupement rupicole, il est caractérisé par des taxons qui se développent essentiellement sur les anciens murs comme *Cenchrus setaceus* subsp. *morronei* Ibn Tattou, *Erigeron karvinskianus* DC., *Hedera algeriensis* Hibberd, *H. helix* L. et *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier.
- groupement des milieux humides, il est caractérisé par *Acanthus mollis* L., *Adiantum capillus-veneris* L., *Ailanthus altissima* (Mill.) Swingle, *Alnus glutinosa* (L.) Gaertn., *Bidens aurea* (Aiton.) Sherff., *Cyperus alternifolius* L., *Mentha × piperita* L., *M. suaveolens* Ehrh., *Nerium oleander* L., *Ruscus hypophyllum* L., *Sambucus ebulus* L., *S. nigra* L., *Tamarix gallica* L., *Ulmus* cfr. *minor* Mill. et *Zantedeschia aethiopica* (L.) Spreng.

Statut d'indigénat

Sur le nombre de taxons horticoles recensés, les taxons non indigènes prédominent nettement dans la flore étudiée (109 espèces soit 66,46 %); parmi ces taxons, les cultivés sont légèrement plus nombreux que les naturalisés/introduits. Les taxons dont la présence est problématique sont représentés par 6 espèces et les échappés de culture sont au nombre de trois (*Bidens frondosa* L., *Cenchrus clandestinus* (Chiov.) Morrone et *C. setaceus* subsp. *morronei* Ibn Tattou). Les taxons d'origine spontanés cultivés dans nos sites d'étude sont assez importants avec 48 espèces dont six sont endémiques (*Abies numidica* Carrière, *Cedrus atlantica* (Endl.) Carrière, *Hedera algeriensis* Hibberd, *Iris unguicularis* Poir., *Origanum vulgare* subsp. *glandulosum* (Desf.) Ietsw. et *Thymus munbyanus* subsp. *coloratus* (Boiss. & Reut.) Greuter & Burdet).

Intérêt de plantation

Les résultats montrent que 61 espèces et 51 genres appartenant à 35 familles sont cultivés dans les différents sites d'études pour leur port décoratif. 44 espèces sont cultivées pour la beauté de leurs fleurs. Néanmoins, certaines espèces recensées offrent une combinaison entre des fleurs et un port décoratif telles que *Bougainvillea glabra* Choisy, *Euonymus tobira* Thunb., *Gazania rigens* (L.) Gaertn., *Jasminum officinale* L., *Mirabilis jalapa* L., *Lantana camara* L., *Lonicera japonica* Thunb., *Strelitzia reginae* Banks et *Zantedeschia aethiopica* Spreng.

25 taxons sont cultivés pour leurs intérêts alimentaires et 24 taxons sont cultivés pour leurs intérêts de feuillage. 17 espèces à essences sont cultivées dans les exploitations horticoles de la région. Ces espèces possèdent des vertus médicinales pour le traitement de plusieurs maladies.

Flore horticole alimentaire

Les familles botaniques sont 22 et celles avec le plus grand nombre d'espèces alimentaires utilisées sont les *Rosaceae* (19,51%), les *Lamiaceae* (14,63%), les *Rutaceae* (7,31%) et à suivre les autres. Ce sont surtout les phanérophytes (85,36%) les plus communes utilisées, suivi par les chamaephytes (12,19%). Les géophytes sont représentées par un seul taxon (*Asparagus acutifolius* L.) dont on mange les tiges fragiles. Les fruits des arbres et des arbustes spontanés tels que *Arbutus unedo* L., *Ficus carica* L., *Myrtus communis* L., *Olea europaea* L. s. l., *Quercus ilex* L., *Crataegus azarolus* L., *Vitis vinifera* L. subsp. *sylvestris* et *Castanea sativa* Mill. sont recherchés pour être consommés directement ou transformés de plusieurs manières.

Parmi les *Rosaceae* cultivées, celles utilisées sont particulièrement: *Cydonia oblonga* Mill., *Eriobotrya japonica* (Thunb.) Lindl., *Malus pumila* Mill., *Prunus cerasifera* Ehrh., *P. dulcis* (Mill.) D. A. Webb, *P. insititia* L. et *Pyracantha coccinea* M. Roem., dont on mange les fruits. Une large consommation de fruits du genre *Citrus* est à noter dans la région d'étude.

Les *Lamiaceae* ont surtout une utilisation condimentale et aromatique (*Mentha × piperita* L., *M. suaveolens* Ehrh., *Salvia officinalis* L., *S. rosmarinus* Spenn., *Ocimum basilicum* L. et *Origanum vulgare* subsp. *glandulosum* (Desf.) Ietsw.).

Une mention spéciale est attribuée au figuier de Barbarie (*Opuntia ficus-indica* (L.) Mill.), qui est devenu naturalisé en Algérie, utilisé comme clôture des champs dans les formes très épineuses, et dans tous les cas il est très recherché pour son fruit, et qui est également utilisé pour la préparation d'une mélasse.

Flore horticole mellifère

L'inventaire floristique des espèces mellifères dans nos sites d'étude a permis d'identifier 121 espèces horticoles. Elles ont été réparties en 96 genres et 49 familles avec la dominance de sept familles: *Asteraceae* (12 taxons), *Lamiaceae* (11 taxons), *Fabaceae*, *Lamiaceae* et *Rosaceae* (10 taxons pour chacun), *Oleaceae* et *Moraceae* (5 taxons chacune).

En revanche, les espèces les plus butinées et à haute valeur apicole étaient au nombre de 23 représentant 14,02% de toutes les espèces inventoriées à savoir: *Acacia dealbata* Link, *Acacia mearnsii* De Wild., *Acacia melanoxylon* R. Br., *A. saligna* (Labill.) H.L. Wendl., *Ceratonia siliqua* L., *Cercis siliquastrum* L., *Citrus × aurantium* L., *C. limon* (L.) Burm. f., *C. reticulata* Blanco, *Crataegus azarolus* L., *Eriobotrya japonica* (Thunb.)

Lindl., *Eucalyptus camaldulensis* Dehnh., *E. globulus* Labill., *Euonymus tobira* Thunb., *Hedera algeriensis* Hibberd, *Lavandula angustifolia* Mill., *L. dentata* L., *L. stoechas* L., *Prunus cerasifera* Ehrh, *P. dulcis* (Mill.) D. A. Webb, *Salvia officinalis* L., *Tilia europaea* L. et *Ziziphus jujuba* Mill.

Profil des taxons envahissants

Les prospections réalisées dans les différents espaces verts de la région d'étude, nous ont permis de recenser un total de 18 espèces potentiellement envahissantes (Tableau 2). Toutes ces espèces semblent avoir une concurrence relativement forte vis-à-vis des espèces indigènes et elles peuvent avoir un impact modéré sur les communautés naturelles.

Tableau 2. Liste des taxons envahissants de la région d'étude.

Taxon	Sites naturels voisins envahies
<i>Acacia mearnsii</i> De Wild.	Subéraie, maquis, plage maritime, pelouse, zone lacustre
<i>Acacia melanoxylon</i> R. Br.	Subéraie, maquis, plage maritime, pelouse, zone lacustre
<i>Acacia saligna</i> (Labill.) H.L. Wendl.	Subéraie, maquis, plage maritime, pelouse, zone lacustre
<i>Ailanthus altissima</i> (Mill.) Swingle	Ripisylve, zone lacustre
<i>Anredera cordifolia</i> (Ten.) Steenis	Maquis, ripisylve
<i>Carpobrotus edulis</i> (L.) N.E. Br.	Plage maritime
<i>Cenchrus clandestinus</i> (Chiov.) Morrone	Pelouse
<i>Cenchrus setaceus</i> subsp. <i>morronei</i> Ibn Tattou	Plage maritime
<i>Clerodendrum chinense</i> (Osbeck) Mabb.	Maquis, pelouse
<i>Eucalyptus camaldulensis</i> Dehnh.	Ripisylve, zone lacustre
<i>Eucalyptus globulus</i> Labill.	Ripisylve, zone lacustre
<i>Ipomoea indica</i> (Burm.) Merr.	Pelouse
<i>Nicotiana glauca</i> Graham	Plage maritime, pelouse, maquis
<i>Oenothera rosea</i> Aiton	Pelouse
<i>Salpichroa organifolia</i> (Lam.) Baill.	Maquis, pelouse
<i>Senecio angulatus</i> L. f.	Maquis
<i>Tropaeolum majus</i> L.	Plage maritime
<i>Vinca major</i> L.	Ripisylve

Discussion

La connaissance de la flore horticole de la région de Annaba reste toujours partielle. Les résultats du présent travail ont permis de dénombrer 164 espèces. Cette richesse spécifique horticole est nettement supérieure au total des 50 espèces recensées dans les pépinières et les jardins de la région de Skikda (Nord-Est algérien) (Sakhraoui & al. 2019). Quatre familles

sont particulièrement bien représentées dans la zone inventoriée: *Asteraceae*, *Lamiaceae*, *Fabaceae* et *Rosaceae*. Ces quatre familles, qui jouent un rôle de premier plan à l'échelle de la planète (Craven 2009). Quant à la richesse floristique du jardin botanique du Hamma, le plus grand et le mieux connu à l'échelle nationale, elle est plus importante que celle enregistrée dans l'ensemble de nos jardins ou encore l'ensemble des localités prospectées. Ce jardin recèle 8214 espèces, parmi lesquelles certaines sont des espèces indigènes (Carra & Gueit 1952). Cela indique clairement que la filière d'horticulture est l'une des principales voies d'introduction d'espèces exotiques dans différentes régions du monde.

Toutes les plantes inventoriées dans le présent travail sont déjà signalées sur les listes d'espèces exotiques envahissantes établies dans la région méditerranéenne, ou encore dans d'autres régions du monde (Brunel & Tison 2005; Lambdon & al. 2008; Brunel & al. 2010; Pyšek & al. 2017). Vu le manque de données chorologiques de nombreuses espèces naturalisées en Algérie, il est impossible de préciser l'état de leur invasion et de leur dynamique actuelle.

D'après cette étude, il ressort que les ressources des plantes horticoles sont très diversifiées. Cette diversité des communautés végétales offre à de multiples espèces (naturalisées, cultivées et parfois d'origine spontanées) une niche écologique d'une part; et d'autre part, par leurs interactions avec les communautés animales. Cette diversité assure à l'ensemble des zones vertes urbaines, qui sont des écosystèmes bien délimités, leur fonctionnement puisque ces espèces végétales se situent à l'extrémité de la chaîne alimentaire urbaine (Belabed & al. 2014; Aouissi & al. 2017; Belabed-Zediri & al. 2021; Belabed & al. 2021).

Toutefois les espaces verts urbains jouent un rôle clé dans l'atténuation du changement climatique et sont responsables de 80 % des émissions de gaz à effet de serre et de 50 % de la production totale de déchets (Gargano & al. 2021).

En revanche, la région d'étude est dominée par des phanérophytes (71,95%). La forte diversité spécifique est couplée avec un fort recouvrement dans les sites d'étude. Ces espèces jouent de ce fait un rôle déterminant dans la mise en place d'un cortège floristique spécifique aux milieux forestiers (Le Compte-Barbet 1975) et urbains (Meddour & al. 2020).

La flore médicinale de la zone d'étude compte 22 taxons soit 27,5% de la flore médicinale spontanée et cultivé de la péninsule de l'Edough évaluée à 80 espèces (Hamel & al. 2018) et soit 6,28% de la flore médicinale de l'Algérie estimée à 320 espèces (Beloued 1998). La comparaison de la flore comestible italienne (Camangi & al. 2013) avec celle de notre région d'étude montre une homogénéité substantielle du point de vue des familles et des espèces les plus représentatives qui devient encore plus prononcé par rapport à celui de Sardaigne (Camarda & al. 2017).

Une base de données pourra être réalisée avec l'apport des résultats des relevés d'autres campagnes d'échantillonnage qui seront probablement effectuées sur l'ensemble de la zone afin de rendre accessible les indications thérapeutiques des plantes cultivées de la région de Annaba.

Les observations de butinage d'*Apis mellifera* L. dans les sites étudiés ont permis de recenser 121 espèces mellifères horticoles. Cette haute valeur mellifère est expliquée par la forte diversité des *Asteraceae* et des *Fabaceae* (Hamel & Boulemtafes 2017a). Une part importante de cette flore est dominée par des arbres fruitiers. Ces espèces sont considérées comme étant une source alimentaire importante pour les abeilles (Hamel & al. 2019).

Les espèces potentiellement envahissantes cultivées sont essentiellement des plantes ligneuses, le maintien des individus sur une longue période permettrait l'adaptation aux

différentes conditions environnementales et faciliterait la naturalisation (Gaertner & al. 2009). De nombreuses espèces non naturalisées observées dans nos sites d'étude ayant une multiplication végétative naturelle sont considérées comme envahissantes avérées en Méditerranée (Arianoutsou & al. 2010; Podda & al. 2011; Meddour & al. 2020). Dans la zone d'étude, leur présence est limitée pour l'instant aux périmètres de culture, mais elles ont de fortes chances d'échapper des lieux de plantations pour coloniser les milieux voisins possédant des caractéristiques édaphiques similaires. Cependant la biologie de plusieurs espèces observées laisse présager dans un avenir plus ou moins proche une possibilité d'installation dans les milieux naturels (cf. Hamel 2016; Hamel & Azzouz 2018; Hamel & al. 2020; Dechir & Hamel 2021). La mise en place d'un plan de surveillance au sein des jardins botaniques et des arboretums permettrait d'identifier les espèces invasives émergentes susceptibles de se répandre dans les milieux naturels (Branquart 2012). Il est nécessaire donc de suivre de près l'évolution de l'aire de chaque espèce envahissante.

Conclusion

Ce premier inventaire de la flore horticole de la région de Annaba fourni une base de données pour l'amélioration et la gestion de la verdure urbaine de la zone d'étude. Ainsi, l'application de bonnes pratiques vertes est un véritable investissement pour l'avenir de cette ville. De même, un changement de mentalité s'impose chez les citoyens pour qu'ils comprennent que planter des plantes en ville n'est pas seulement une question d'esthétique et de décoration mais aussi une source de vie (alimentaire et mellifère).

A l'heure actuelle, il est souhaitable et il convient d'approfondir les connaissances floristiques de la région par une augmentation des prospections de terrains en développant des guides afin d'aider les gens et de lever une grande partie des ambiguïtés concernant les espèces introduites insuffisamment documentées et non évaluées.

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V. Matevski, M. Kostadinovski, R. Čušterevska, C. Cvetkoska, S. Cvetanoska & A. Ivanova

New records of some vascular plants for the flora of North Macedonia

Abstract

Matevski, V., Kostadinovski, M., Čušterevska, R., Cvetkoska, C., Cvetanoska, S. & Ivanova, A.: New records of some vascular plants for the flora of North Macedonia. — Fl. Medit. 32: 131-141. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This paper presents new data on the distribution of 10 vascular plant species from the territory of North Macedonia. Three species (*Erysimum cheiri*, *Iberis umbellata* and *Silene squamigera*) are listed for the first time for the flora of North Macedonia, while the other 7 rare species (*Chaenorhinum rubrifolium*, *Crepis zacintha*, *Galatella linosyris*, *Geranium versicolor*, *Glinus lotoides*, *Limosella aquatica* and *Polygonatum verticillatum*) are registered at several new localities on its territory.

Key words: biodiversity, floristics, chorology, higher plants.

Introduction

Floristic research on the territory of North Macedonia (Macedonia, The Socialist Republic of Macedonia, The Republic of Macedonia) has a long, almost 180-year tradition, starting from the middle of the 19th century (Grisebach 1843-1844), intensively carried out until today. The results of the research have been published in numerous scientific papers, which have been analytically processed in the eight books of the edition Flora of the Republic of Macedonia (Flora of North Macedonia) (Micevski 1985, 1993, 1995, 1998, 2001, 2005; Matevski 2010, 2021), within the project activity of the Macedonian Academy of Sciences and Arts. However, serious field research constantly leads to the discovery of new, hitherto unknown species for the territory of North Macedonia as well as new localities of rare plant species which, according to current knowledge, have a very limited distribution on its territory. This is the case with the latest data in the last 10 to 15 years presented in the works of Matevski (2007, 2016, 2017, 2018a, 2018b; 2019); Matevski & Kostadinovski (2009); Matevski & Teofilovski (2011); Matevski & Čarni (2019); Kostadinovski (2007); Kostadinovski & al. (2019); Stevanović, & al. (2009, 2010); Vladimirov & al. (2011, 2012, 2014, 2016a, 2016b, 2019a, 2019b); Niketić & al. (2014, 2017); Đurović & al. (2014); Melovski (2016); Melovski & Hristovski (2015);

Teofilovski (2017, 2018, 2020); Nikolov (2017, 2020); Čušterevska & al. (2020) and others. Thus, knowledge about the flora of North Macedonia is continuously enriched.

The latest field research realized by the authors of this paper in different parts of the territory of North Macedonia in the past period, led to the discovery of 3 new species (*Erysimum cheiri* (L.) Crantz., *Iberis umbellata* L. and *Silene squamigera* Boiss.), coupled with new data on 7 other rare and insufficiently known species (*Chaenorhinum rubrifolium* (DC.) Fourr., *Crepis zacintha* (L.) Babč., *Galatella linosyris* (L.) Rechb. f., *Geranium versicolor* L., *Glinus lotoides* L., *Limosella aquatica* L. and *Polygonatum verticillatum* (L.) All.).

Materials and Methods

The new data are based on the plant material collected during field research conducted by the authors, followed by study of the processed herbarium material stored in the Herbarium of the Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje (MKNH). In addition, for each of the taxa, information on geographic distribution, locality and habitat, GPS coordinates, along with collection date are provided. Appropriate floristic literature is cited in the comments section for each species. Species habitat data are provided by the authors' personal observations. The nomenclature and taxonomy of the plant are consistent with the Euro + Med (2006-) database. Voucher specimens of all taxa are preserved in the Herbarium (MKNH).

Results and discussion

Crepis zacintha (L.) Babč. (Asteraceae)

North Macedonia:

Localities from literature: Zelenikovo, Ostri (Bornmüller 1926); Bregalnica - v. Karaorman, Negorci (Jurišić 1923); Ohrid, Lukovo (Šmarda 1968).

New localities: Gevgelija - v. Dolno Konjsko, 41.183477° N; 22.324043° E; 516 m a.s.l., 7.6.2005, *M. Kostadinovski s.n.*; Štip: Penuš, dry grassland, 41.706382° N, 22.108975° E, 248 m a.s.l., 6.6.2015, *V. Matevski s. n.* (MKNH).

A Southern European – Euro-Mediterranean species with a very limited distribution on the territory of North Macedonia. Two new localities have been discovered (the vicinity of Štip, along the river Bregalnica, near the abandoned village Penuš, as well as in the vicinity of Gevgelija - Dolno Konjsko v.).

Galatella linosyris (L.) Rechb. f. [Syn.: *Aster linosyris* (L.) Bernh.] (Asteraceae)

North Macedonia:

Localities from literature: Kapina (Soška 1939); Star Dojran, Pčinja - Katlanovska Banja; Žedenska Gorge - v. Rašče (Micevski 1970); Jažince (Teofilovski 2015).

New localities: Dojran - between Star and Nov Dojran, Kaldhma, dry grassland in the belt of *Quercus coccifera*, 41.205107° N; 22.708445° E; 165 m a.s.l., 22.9.1999, *V. Matevski s. n.*; Skopje - v. Vučidol, dry grasslands, 42.042924° N; 21.336155° E; 365 m a.s.l., 13.11.2001, *V. Matevski s. n.*; Štip - v. Testemelci, dry grasslands, 41.73167191° N; 22.11229993° E; 270 m a.s.l., 24.6.2018, *V. Matevski, R. Čušterevska, S. Cvetanoska & C. Cvetkoska s. n.*; Galičica - Dva Javora, 40.98260° N; 20.85833° E; 1566 m a.s.l., 3.9.2020, *V. Matevski, M. Kostadinovski, R. Čušterevska, S. Cvetanoska & C. Cvetkoska s.n.* (MKNH).

The distribution area of *Galatela linosyris* extends to the territories of Central, Eastern and Southern Europe, Asia Minor and North Africa. With the newly discovered localities, the distribution area of this rare plant species on the territory of North Macedonia is gradually determined.

***Erysimum cheiri* (L.) Crantz.** (Basionym: *Cheiranthus cheiri* L.) (Fig. 1a, 1d)
(*Brassicaceae*)

North Macedonia:

New locality: Ohrid-Old City, on the limestone rocks, just above Ohrid Lake, 41.111020° N; 20.792737° E; 709 m a.s.l., 17.6.2010, 1.5.2016, *V. Matevski s. n.* (MKNH)

Erysimum cheiri is an old cultivated plant, the origin of which has not been determined with certainty. Mosleh & al. (2019) states that this ornamental species originates from Europe, especially from the Mediterranean region, so that today it is cultivated all over the world. According to Ball (1964), it grows naturally in southern Greece (S. Greece and the islands of the S. Aegean Sea), while in other countries of Central, Western and Southern Europe, it is an introduced or cultivated plant. Polatschek & Snogerup (2002) note that it is a naturalized plant of disputed origin that has been cultivated in Greece since ancient times and it has become increasingly rare in recent times, with some of its oldest forms being able to trace their origins to some Greek taxa. It grows on walls, cliffs or rocks, but sometimes it grows spontaneously; hence, it happens to escape into the wild. According to the Euro + Med plant base, in natural conditions it is found only on the territory of Croatia (Marhold 2011). As maintained by Nikolić (2021), on the territory of Croatia, the plant is present only as cultivated and naturalized in the coastal parts of central and southern Dalmatia, in the Zadar-Šibenik archipelago, in the Kvarner-Velibit area, Istria, North Dalmatia, and a minor number of localities in the continental parts of Croatia. Barina & al. (2013) cite this species as naturalized in Albania, which has been confirmed by the discovery of a sustainable natural population along the sea rocks in the vicinity of Vlora.

This species has been discovered on the territory of North Macedonia, in the narrower urban area of the city of Ohrid. It develops along the vertical limestone rocks rising above Lake Ohrid, in the old part of Ohrid, on the stretch between the Church of St. Sophia and the Monastery of St. John at Kaneo. The population of *Erysimum cheiri* has sustained in the area for many years, together with *Aurinia saxatilis* subsp. *orientalis* (Ard.) T. R. Dudley, *Urtica pilulifera* L., *Parietaria judaica* L., *Sedum album* L., *Silene vulgaris* (Moench) Garcke subsp. *vulgaris*, and others. In Ohrid, it is definitely a naturalized species, which has encountered suitable ecological conditions in the area, and it flourishes in that ambient space. This area had not been accessible from the mainland for a long time,

but in the past two decades, a wooden bridge has been constructed to shorten the distance between St. Sophia Church and the Monastery of St. John at Kaneo; thus, during the flowering period, numerous passers-by enjoy the beauty of this ornamental species.

***Iberis umbellata* L. (Fig. 1b, 1d)**
(*Brassicaceae*)

North Macedonia:

New locality: Pehčevo - Trebomirska River, beech forest clearings, 41.747331° N, 22.983864° E; 1320 m a.s.l., 26.10.2014, *V. Matevski s.n.* (MKNH).

As indicated by Pinto de Silva & al. (1964), in natural conditions this Mediterranean species occurs on the territories of Albania, France, Greece, Italy and former Yugoslavia while in other parts of Europe, it develops as a cultivated plant or a plant with an unknown status. In the distribution map of *Iberis umbellata* in Euro + Med PlantBase (Marthold, 2011), the presence of this species is more accurately mapped in only three countries of former SFR Yugoslavia - Montenegro, Bosnia and Herzegovina and Croatia.

Micevski (1993) notes that *Iberis umbellata* is an ornamental plant, but no natural populations of this species are known from the territory of Macedonia. With a small number of specimens, this plant has been registered in the eastern parts of North Macedonia, in the Maleševo Mountains, at the Trebomirska River locality, near Pehčevo. It grows in clearings in beech forest; nonetheless, the locality and habitat where the species has been recorded (in the beech forest belt) is distant from urban or rural areas where this plant could be grown. This is probably a spontaneous occurrence of the species in North Macedonia.

***Silene squamigera* Boiss. (Fig. 1c, 1d)**
(*Caryophyllaceae*)

North Macedonia:

New locality: Galičica - Prespa side, 40.976222° N, 20.883362° E, 1238 m a.s.l., 24.6.2009, *R. Čušterevska s.n.*; Galičica - Prespa side, on burned places, 40.97714°N, 20.882492°E, 1296 m a.s.l., 28.6.2009, *R. Čušterevska s. n.* (MKNH).

Silene squamigera is a southern Balkan endemic species (Sect. *Lasiocalycinae*), present in Central and Southern Greece (Chater & Walters, 1964) while in Euro + Med PlantBase, its areal also covers the territory of Turkey. Greuter (1997) distinguishes two subspecies - *S. squamigera* subsp. *squamigera* (Greece) and *S. squamigera* subsp. *vesiculifera* (endemic to SE Anatolia). It is remarkable that there is available data about the incidence of the current species on the territory of Serbia - in the vicinity of Niš (Slavnić 1970), which was later revised; consequently, it refers to the species *S. echinata* (Niketić & Stevanović 2012).

Until now, there were no data whatsoever on the presence of this species in North Macedonia. It has been observed in two localities in Galičica Mountain, that extend its range to the territory of North Macedonia. According to current knowledge, it is the northernmost location of the species within the boundaries of its distribution area. In Galičica Mt, it grows on limestone background (1,200-1,300 m), in a transitional zone, between the belt of dry grasslands and subalpine pastures.

The most significant morphological characteristics of plants originating from Galičica Mt as well as ecological features completely correspond to those emphasized in the description by Greuter (1997) ("*Calyx costae, especially in the upper half, covered with swollen, white, bladder-like, shortly apical bases of the hair; teeth wide rounded, not with fringes with bars, but small papillomas on the margins ...* ").

***Geranium versicolor* L.**

(*Geraniaceae*)

North Macedonia:

Localities from literature: Bitola - Bukovo, Djavato (Vandas 1909; Micevski 2005); Galičica (Vandas 1909); Mariovo: Satoka (Soška 1939).

New locality: Struga - Crn Drim gorge, v. Modrič, 41.367752° N; 20.585151° E; 682 m a.s.l., 21.7.1997, *V. Matevski s. n.* (MKNH).

This Mediterranean species is known from the southern part of the Balkan Peninsula and C. & S. Italy (Webb & Ferguson 1968). In North Macedonia, so far it has been known only from Galičica Mt, Prespa and Mariovo. A new locality has been registered in the gorge of the Crn Drim River, near the village of Modrič. The gorge of the Crn Drim is an important refuge where relict species (*Aesculus hippocastanum* L., *Ramonda serbica* Pančić, *Dioscorea balcanica* Košanin and others) are found. Simultaneously, it is an important phytogeographical corridor where many Mediterranean and sub-Mediterranean plants occur, such as *Salvia officinalis* L., *Vincetoxicum huteri* Vis. & Asch., *Moltkia petraea* (Tratt.) Griseb., *Thymus rohlena* Velen. and others.

***Glinus lotoides* L.**

(*Molluginaceae*)

North Macedonia:

Localities from literature: Strumica: Monospitovo marsh (Micevski 1995).

New locality: Prilep - Mariovo: Rasim Bej Most, along the Crna Reka river, 41.194107° N; 21.716121° E; 421 m a.s.l., 6.10.2006, *V. Matevski s. n.* (MKNH).

On the territory of North Macedonia, *Glinus lotoides* had only been known from the surroundings of Strumica - in the belt of the well-known swamp near the Bansko spa, at the foot of Belasica Mountain. This species has been recorded in the area of Mariovo, in the middle course of the Crna Reka river, at the locality Rasim Bej Most. It is the species second locality within North Macedonia. *Glinus lotoides* has been sighted in a sandy habitat near the Crna Reka river in spots where the river water level raises during the spring months where new loads of sand are accumulated every year.

***Chaenorhinum rubrifolium* (DC.) Fourr.**

(*Plantaginaceae*)

North Macedonia:

Localities from literature: Kavadarci - v. Dradnja, on limestone rock, 650 m a.s.l., 20.6.2001 (MKNH) (Matevski 2002).

New locality: Prilep: v. Debrešte - Debreška Krasta, limestone, 41.485527° N; 21.340256° E; 823 m a.s.l., 23.6.2004, *V. Matevski & M. Kostadinovski s. n.* (MKNH).

Chaenorhinum rubrifolium is a Mediterranean species with a predominantly central-western Mediterranean range whereas in the Eastern Mediterranean it is currently known only from Greece (Fernandes 1972). It is represented by three subspecies - *C. rubrifolium* (DC.) Fourr. subsp. *rubrifolium*, *C. rubrifolium* subsp. *formenterae* (Gand.) R. Fern. and *C. rubrifolium* subsp. *ravey* (Boiss.) R. Fern., whereof only the typical subspecies occurs in the Eastern Mediterranean.

According to the available floristic data, to date this species has been observed only at a single site in the vicinity of Kavadarci - near Dradnja village, on a carbonate substrate in the belt of hilly pastures (Matevski 2002). A new locality of the species was recorded in the central part of North Macedonia, in the vicinity of Prilep - Debreška Krasta. It develops in the belt of hilly pastures that phytocenologically belong to the ass. *Sileno-Thymetum ciliatopubescentis* (all. *Diantho haematocalycis-Festucion hirtovaginatae* Matevski & al. 2018).

***Limosella aquatica* L.**

(*Scrophulariaceae*)

North Macedonia:

Localities from literature: Bistra: Toni Voda (Micevski & Matevski 1986); Galičica: between Asan Djura and Dzafa (Matevski & Kostadinovski 2009) (MKNH).

New localities: Pelister - Golemo Ezero, temporary ponds, 40.966620° N; 21.205585° E; 2214 m a.s.l., 3.10.2018, *V. Matevski & R. Čusterevska s. n.*; Šar Mt. - Karanikola-Šeremetica, 42.068580° N, 20.792368° E; 2298 m a.s.l., 4.08.2017, *Lj. Melovski, S. Hristovski, V. Matevski & R. Čusterevska s. n.* (MKNH).

Limosella aquatica is a therophyte with a very interesting ecological adaptation in aquatic biotopes with periodic variations of the water level. During summer months, these biotopes become dry, and they are favourable habitats for development of populations of the current species. In the meantime, on the territory of North Macedonia, two new additional localities have been discovered of this remarkable and rare plant species: in Pelister Mt (Golemo ezero – the Great Lake) and Šar Mountain (Karanikola).

***Polygonatum verticillatum* (L.) All.**

(*Asparagaceae*)

North Macedonia:

Localities from literature: Bistra Planina (Leute, 1978); Belasica: Semer - Kaja, in beach forest, 1700 m (Stojanov 1921).

New localities: Bitola:Mariovo, Nidže - Lubnica, in the foothills of Dobro Pole, in a mixed fir-beech forest, 41.051444° N, 21.858928° E; 1474 m a.s.l., 19.6.1995, *V. Matevski s. n.*; Bitola:Mariovo, v. Gradešnica, Pette Češmi, in the foothills of Zmejca Mt., 41.059030° N, 21.840758° E; 1200 m a.s.l., 10.6.1996, *V. Matevski s. n.*; Galičica - up the Lipona Livada, in a mixed fir-beech forest, 40.948780° N, 20.830966° E; 1700 m a.s.l., 23.7.2020, *V. Matevski, M. Kostadinovski & R. Čusterevska s. n.* (MKNH).

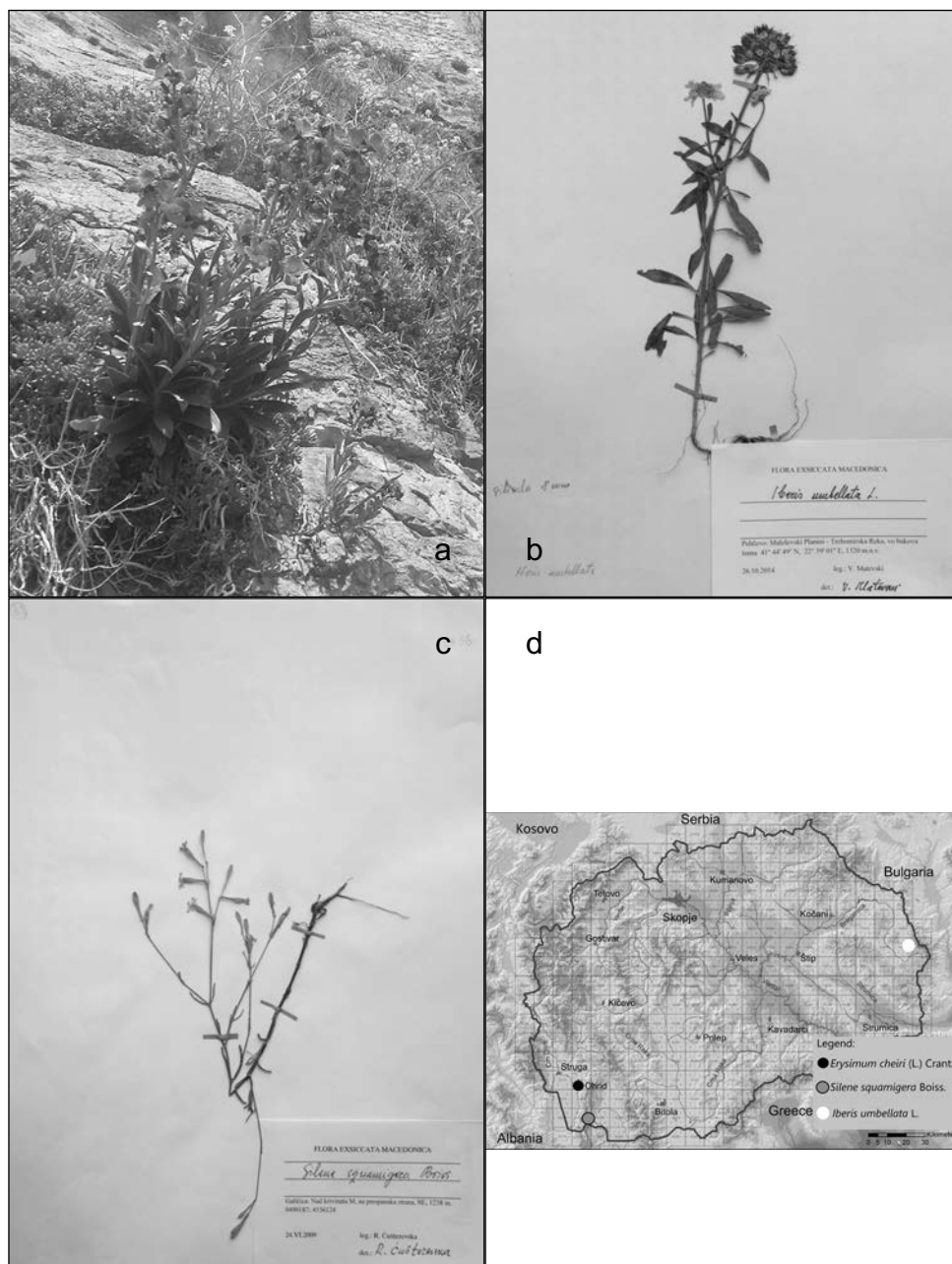


Fig.1. **a)** *Erysimum cheiri* - Ohrid: Old City, on the limestone rocks, above Ohrid Lake, 1.5.2016 (V. Matevski) (MKNH); **b)** *Iberis umbellata* - Pehčevo: Trebomirska River, beech forest clearing, 26.10.2014, V. Matevski s. n. (MKNH); **c)** *Silene squamigera* - Galičica – Prespa side, 1238 m a.s.l., 24.6.2009, R. Čušterevska s. n. (MKNH); **d)** Distribution map of *Erysimum cheiri*, *Iberis umbellata* and *Silene squamigera* in North Macedonia.

Polygonatum verticillatum has worldwide distribution, from montane to alpine Himalaya, Kashmir, Southeast Tibet, Russia (W. Siberia, Caucasia), West Asia to Europe (except for the Mediterranean region) (Saboon & al. 2016). According to De Filips (1980), in Europe it spreads from Arctic Norway, southwards to N. Spain, C. Italy and S.W. Bulgaria, and eastwards to c. 26° E, in Latvia and Romania. Two localities have been cited for Macedonia - Belasica: Semer - Kaja (Stojanov 1921, but it is disputable whether the site is in the Greek or the Macedonian part of Belasica Mt) and Bistra Mt (Leute 1978).

This very rare and insufficiently known species has been observed at three localities in Macedonia (two in the Mariovo region - Lubnica and Zmejca, and one locality in Galičica Mt (up the Lipova Livada), always in mixed fir-beech forest.

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K. Bertsouklis, R. W. Martin & M. Papafotiou

First contribution to the comparison of flower morphology of six *Arbutus* species (*Ericaceae*), one hybrid and one undefined origin cultivar of the Mediterranean Basin and North America

Abstract

Bertsouklis, K., Martin R. W. & Papafotiou, M.: First contribution to the comparison of flower morphology of six *Arbutus* species (*Ericaceae*), one hybrid and one undefined origin cultivar of the Mediterranean Basin and North America. — Fl. Medit. 32: 143-148. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Three Mediterranean *Arbutus* species and one natural hybrid, as well as three North American species and one Californian hybrid of unknown origin were studied microscopically; a comparison of the internal anatomy of the flowers of these species is reported for the first time. Anatomical features were found to be similar in general but unique to the *Arbutus*. However, the length of the anther appendages, the spurs, seems to provide a heritage trait linking the Californian hybrid *Arbutus* ‘Marina’ to the *A. canariensis* and support other morphological findings between the East Mediterranean hybrid, *A. ×andrachnoides*, and its parents *A. andrachne* and *A. unedo*.

Key words: strawberry tree, anther appendages, blossom, spur.

Introduction

Fossil findings of plants from the upper Cretaceous period in a clay pit in New Jersey have shown the presence of spurs in the anthers of flowers having some characteristics similar to modern *Ericales* and even *Ericaceae* (Crepet 2009). Present day *Ericaceae* have been studied including the development of the stamens and their appendages (Hermann & Palser 2000). Many of these were found to have “awns” and “spurs”. The prevalence of appendages in *Ericaceae* have suggested a different mechanism involving the pollination process.

Arbutus (*Ericaceae*) includes many popular shrubs or tree species in both hemispheres. *A. unedo* L. is prominent in the Mediterranean region (“Strawberry Tree”). It is being utilized in the northwest coast of the western hemisphere and in the Mediterranean region in landscaping as an attractive and simply maintained bush. Its bell-shaped blossoms in the fall are generally white with a rare variety originated in Ireland with a pink to ruby color called *Arbutus unedo* var. *rubra* W.T. Aiton. *A. andrachne* L. of the central and eastern Mediterranean commonly (Greek or Eastern Strawberry tree), has also potential in land-

scape use, reforestation, honey production and pharmaceutical industry (Bertsouklis & Papafotiou 2013; Bertsouklis & Papafotiou 2016; Yilmaz 2016). *A. ×andrachnoides* Link is believed to be a natural hybrid between *A. unedo* and *A. andrachne* and it is native to Greece and the eastern Mediterranean region (Arabatzis 2001; Torres & al. 2002). It is challenging however, to differentiate from *A. andrachne* (Bačić & al. 1992; Cullen & al. 1997). A significant difference has been found in the length/width ratio of the leaves (Bertsouklis & Papafotiou 2016) and in a different pattern of the stomata and the surrounding tissue when studied with electron scanned microscopy (Bačić & al. 1992). *A. canariensis* Duhamel is an indigenous tree of the Canary Islands. It has most recently been assessed for The IUCN Red List of Threatened Species in 2017 and is listed as Near Threatened under criteria B1a+2a (Beech 2017) portraying many of the same traits of the Mediterranean *Arbutus* but offering pink to ruby blossoms instead of white. In the natural habitat, *A. canariensis*, like *A. unedo*, is a fall bloomer whereas *A. andrachne* is a spring bloomer.

In the western hemisphere, *A. peninsularis* Rose & Goldman is indigenous to the Sierra de la Laguna mountains in the southern region of Baja California in Mexico. *Arbutus menziesii* Pursh (Pacific madrone) is prevalent for much of the Pacific Northwest extending from central Vancouver Island south to mid California. *A. menziesii* extends from British Columbia to Baja California along the West Coast of North America (Hileman & al. 2001). It also can grow to immense size; a circumference of 10 m has been reported (Coe 1983); the species is found along the seashore, often in rocky regions, bending and twisting out to open space to obtain the sunshine. *A. xalapensis* Kunth (Texas madrone) is found in Texas and northeastern Mexico. The bark of *A. xalapensis* is red but the color turns to a white appearance, depending on the brightness and harshness of its sun exposure during the year.

A mysterious and popular cultivar *Arbutus* ‘Marina’ has been propagated in California particularly in the regions with a Mediterranean climate (Fig. 2). The tree is known to have originated in California, at the San Francisco 1915 Panama American Exposition. It is thought to have been brought to the exposition from somewhere in the Mediterranean region. Details of its history after the exposition are described (Baldwin 2020). However, of pertinence here is that in the early 1990’ Briggs Nursery Inc. near Olympia WA, USA, contracted to produce plantlets by micro propagation techniques. This action fueled the starting of wide distribution of it in California and apparently elsewhere. Demoly (2004) named and described two hybrids: *Arbutus* ×*thuretiana* Demoly and *Arbutus* ×*reyorum* Demoly. The first is a cross between *A. canariensis* and the *A. andrachne* and the latter a second cross between the *A. ×andrachnoides* and the *A. canariensis*. Further, he suggested that the latter hybrid was displayed in the 1915 Panama American Exposition but doesn’t indicate how it could have arrived there (Demoly 2004). World War I was erupting at that time, which added to confusion of normal record keeping. However, Demoly (2004) also writes that a plant called *Arbutus* ‘Marina’ was introduced in France in 1993 from the Madrona Nursery located in England. Liam Mackenzie the 1986 founder of that nursery revealed he used to travel in the early 1990’s to the Courson Flower Show, held south of Paris, to sell plants and probably sold *A. ‘Marina’* there. However, he had himself acquired *A. ‘Marina’* plants from Peter Catt of Liss Forest Nursery in Hampshire, UK (Mackenzie 2018 pers. comm.). Mr. Catt, formerly of the Liss Forest Nursery, though unsure of dates, said he had bought many plants from Briggs Nursery in that early time frame (Catt 2018 pers. comm.). Demoly compared the morphology of the *A. ‘Marina’* to the *A. ×reyorum*

and feels justifying maintaining the name of the cultivar as *Arbutus* × *reyorum* ‘Marina’ (Demoly 2018 pers. comm.). Nevertheless, there is no other direct evidence that they are the same. This investigation was prompted finding the link between the spurs of the *A. ×reyorum* ‘Marina’ and the *A. canariensis* compared to other *Arbutus* trees.

Materials and Methods

Flowers of the *Arbutus* species were obtained from various regions for examination. *A. unedo*, *A. andrachne* and *A. × andrachnoides* were obtained in the region of Tatoi Former Royal Palace (Varympompi, Athens, Greece, N 38°08'14.0", E 23°47'59.0". *A. menziesii* and *A. unedo* were acquired near Anacortes, WA, USA (N 48°30'54.011 W 122°37'10.169); *A. peninsularis* blossoms were obtained from the Sierra de la Laguna mountains (Baja California, Mexico, N 23°32'44.489" W 110°00'37.68"); *A. xalapensis* flowers were picked and sent from Dripping Springs TX, USA (N 30°11'31" W 98°05'07") near Austin; *A. canariensis* and *A. ×reyorum* ‘Marina’ were obtained, with permission, from the Golden Gate Botanical Garden in San Francisco.

The significance of the results was tested by one-way analysis of variance (ANOVA). The spur length means were compared by Student’s *t* test at $P \leq 0.05$ (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The blossoms of all studied species of *Arbutus* had a similar form (Fig. 1). The corolla of the studied species was urceolate similar to most genera of the *Arbutoidae* subfamily (Matthews & Knox 1927) (Fig. 1A). Stamens furnished with awn (bristle-like appendages) were enclosed in the corolla. It required cutting and removing sections of it with dissecting scissors to expose the inner parts of the blossom (Fig 1B). Ten anthers with each having two pollen sacs and two spurs were found in all species. The filaments of the stamens were well developed and broadened at increasing distance from the anther until it suddenly reduced in diameter just before it attached to the corolla. White dense hairs, almost luminous in color, encompassed the lower one third of each stamen. Its purpose is not known. The spurs tapered from their attachment point to the anther to their tip. The internal parts of the blossoms of *A. ×reyorum* ‘Marina’ and *A. canariensis* have similar appearances (Fig 3, 4). The spurs seem to be shorter and thicker where they attach to the anther, and with less complicated curvature compared to *A. unedo* (Fig. 1) and the other *Arbutus* species (Fig. 5, 6). *A. unedo* had the longest spur length (Table 1). *A. × andrachnoides* had a value closer to *A. andrachne*, along with other traits found in a previous study (Bertsouklis & Papafiotiou 2016). The length of all three of these species were found to be different and longer than both *A. canariensis* and *A. ×reyorum* ‘Marina’. According to Pascual & al. (1993) *A. canariensis* in the Canary Islands had thick and short spurs like what we found in the blossoms from San Francisco.

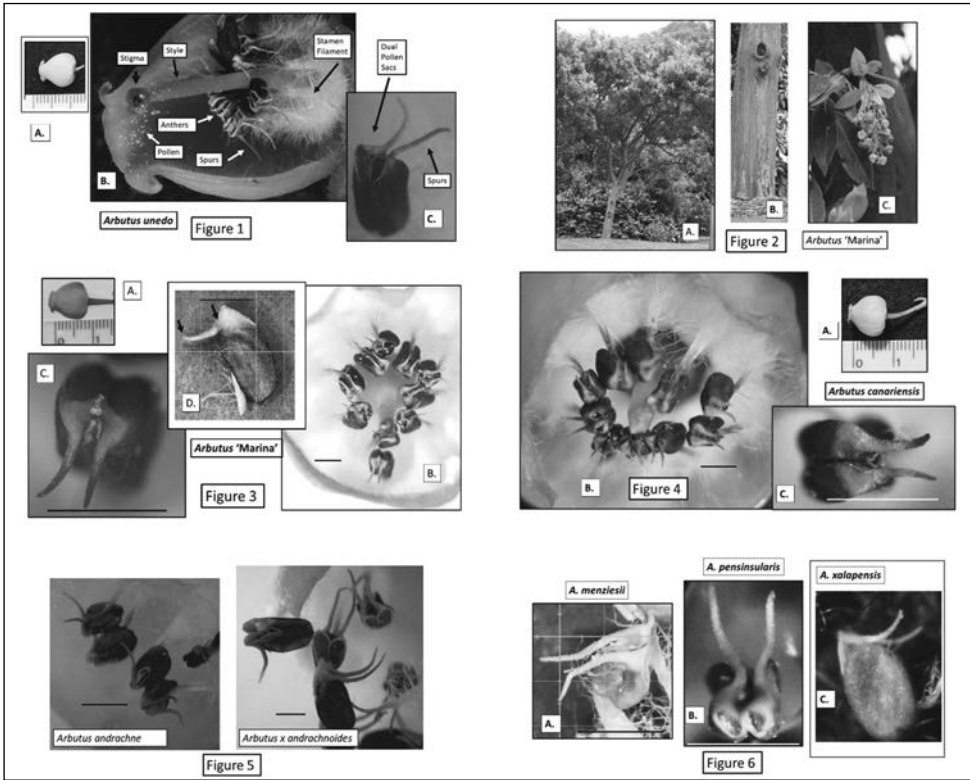


Fig. 1. *Arbutus unedo* A) Blossom; B) Dissected blossom; C) Single anther with dual pollen sacs and the dual spurs. Fig. 2. *Arbutus* $\times$ *reyorum* 'Marina' tree: A) Tree; B) expanded insert of the trunk; C) cluster of blossoms, picture taken April 25, 2019 at Golden Gate Botanical Garden San Francisco. Fig. 3. *A. x reyorum* 'Marina': A) Blossom; B) View into the blossom with the opening end of the corolla removed exposing the anthers and their spurs; C) A magnified view similar orientation as B. Single anther; D) Single anther. Fig. 4. *A. canariensis* A) blossom; B) Stamens; C) Single anther with its dual spurs like the view of Figure 3C for the *A. 'Marina'*. Fig. 5. Blossoms of *A. andrachne* and *A. x andrachnoides*. Bars represent 1mm. Fig. 6. Anthers and spurs of three western hemisphere *Arbutus* trees. A) *A. menziesii*, an oblique view of two spurs; B) *A. pensinsularis*, apical view of single anther; C) *A. xalapensis*, a lateral view of an anther showing the curved spurs.

Table 1. Comparing the spur length measure (mm) of the spurs of various species.

Species	Mean Length (mm)
<i>A. unedo</i>	1.27 $\pm$ 0.18 a
<i>A</i> $\times$ <i>andrachnoides</i>	1.19 $\pm$ 0.18 b
<i>A. andrachne</i>	1.10 $\pm$ 0.13 b
<i>A. x reyorum</i> 'Marina'	0.69 $\pm$ 0.10 c
<i>A. canariensis</i>	0.69 $\pm$ 0.14 c

Mean separation in columns by Student's *t*-test, $P \leq 0.001$. Means followed by the same letter are not significantly different, $n=15-66$.

Conclusions

The dominate morphology of the dual spurs in the *Arbutus* species studied and their definitive structure found implies that the hybrid and cultivar *A. ×reyorum* ‘Marina’ have the *A. canariensis* in their heritage line. The color of the blossoms and the peeling bark further supports this indication. Additionally, this finding confirms the premise of Demoly (2004) that it is the hybrid *Arbutus ×reyorum* Dem. However, by itself it does not rule out that it could be *Arbutus ×thuretiana* Demoly that he also has identified as a hybrid of the *A. canariensis*. The spur length of the Greek hybrid, *A. ×andrachnoides*, agrees with other reported morphology findings that place *A. ×andrachnoides* closer to *A. andrachne*.

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N. Lachashvili, K. Kereselidze & M. Kikvidze

The checklist of trees of Georgia (Caucasus) and their provisional regional assessment according to IUCN categories and criteria

Abstract

Lachashvili, N., Kereselidze, K. & Kikvidze, M.: The checklist of trees of Georgia (Caucasus) and their provisional regional assessment according to IUCN categories and criteria. — Fl. Medit. 32: 149-189. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

A checklist of trees distributed in Georgia is presented for the first time. The checklist includes 129 species of vascular plants. They belong to 52 genera and 28 families. Of 129 species 6 are represented by two subspecies and 1 by two varieties. On the whole, the checklist includes 136 taxa. For each taxon (species, subspecies and varieties) a regional, expert based, assessment, according to IUCN criteria, are provided. Regionally (in Georgia) 9 taxa are critically endangered (CR), 4 endangered (EN), 9 vulnerable (VU), and 15 are near threatened (NT). Due to the lack of data, 33 taxa could not be assessed and fell under the category of data deficient. 66 taxa resulted not endangered so far and have been assigned to the category least concern (LC). References to taxonomy, key synonyms, assessment argumentation and references used in provisional species assessment are given for each taxon.

Key words: conservation, native flora, floristic composition.

Introduction

Georgia, despite its small size, is characterized by both floristic and ecosystem diversity. More than 4000 species of vascular plants are distributed here (Davlianidze & al. 2018). In terms of habitat creation, trees occupy a prominent place among them.

In Georgia, trees are distributed in different climate zones from the lowlands to the subalpine belt. They grow on humid, as well as on arid and semi-arid ecotopes, also in wetlands. Accordingly, the trees common in Georgia are represented by species of different bioecology. Due to the above, their floristic composition is rich.

Trees are especially important for maintaining the Earth's ecological balance. In addition, their use is versatile. Therefore, they are under constant anthropogenic pressure (felling, grazing, etc.). Thus, their recording, condition evaluation and assessment, according to the international standards, are an urgent issue. Despite the above, the composition of trees common in Georgia is still unknown and no checklist exists for them either.

The aim of our research was to compile a checklist of trees of Georgia and to make their regional provisional assessments according to IUCN categories and criteria, which will promote the protection and conservation for this group of plants.

Materials and methods

In addition to our own research, the second edition of “Flora of Georgia” (Ketskhoveli 1971-1983; Gagnidze 1985-2011) and the “Nomenclatural checklist of flora of Georgia” (Davlianidze & al. 2018) laid the foundation for the creation of the checklist.

Names and authors of taxa are checked with the international databases: Euro+Med (2006-), The Plant List (2013), GBIF.org (2022), IPNI (2022), POWO (2022), Tropicos.org (2022), WFO (2022). As a result, some of the “narrow” species included in the “Flora of Georgia” (Ketskhoveli 1971-1983; Gagnidze 1985-2011) and the “Nomenclatural checklist of flora of Georgia” (Davlianidze & al. 2018) could not be found in this checklist. These species are given as subspecies or as synonyms.

Provisional taxa assessment and categorization were carried out in accordance with IUCN Red List (2021) criteria. Our assessment of taxa is based on our own research, literary data, results of various concluded and ongoing projects and materials preserved in various herbariums of Georgia (TBI, TGI, BATU). Some taxa are assessed based on the data from the “Red list of the endemic plants of the Caucasus” (Solomon & al. 2013). The verbal information of various researchers is also used.

The calculation of the extent of occurrence (EOO) and the area of occupancy (AOO) of the taxa, the exact distribution coordinates of which we did not have, was carried out on the basis of literature and herbarium data.

Description of study area

Georgia is located in the central part of Caucasus Ecoregion between latitudes 41°07' and 43°35'N, and longitudes 40°04' and 46°44' (Fig. 1). Its area is 69,700 km² and hypsometric amplitude ranges from -1 m a.s.l. (Kolkheti lowland) to 5203 m a.s.l. (Mt. Shkhara) (Gobejishvili 2012; Bolashvili & al. 2018).

From a geotectonic point of view, the territory of Georgia belongs to the central part of the Alpine-Himalayan folds (Gobejishvili 2012; Bolashvili & al. 2018).

Georgia is a mountainous country with a diverse and difficult relief. There are three main morphological units: the middle and high mountainous relief of the Caucasus ridge, the intermountain bar and the mountains of southern Georgia (Gobejishvili 2012; Bolashvili & al. 2018).

Georgia is situated on the northern edge of the subtropical climate zone and is under the influence of subtropical, temperate and high-altitude circulatory processes. Despite the small area, almost all types of subtropical climate zones are established on the territory of Georgia, in particular, 23 types of climate are distinguished. Such diversity of climate is due to the location of Georgia and its difficult physical-geographical conditions (Kordzakhia 1961; Gobejishvili 2012; Bolashvili & al. 2018).



Fig. 1. Location of Georgia in the Caucasus Ecoregion.

The territory of Georgia is characterized by diversity of soils. Forty-eight types of soil are recorded there. Both moist and dry soils are presented. Moist and wet soils of acidic reaction mostly predominate in Western Georgia, while dry alkaline and neutral soils prevail in Eastern Georgia (Urushadze 1999, 2016; Gobejishvili 2012; Bolashvili & al. 2018).

Results

129 species of trees, which belong to 52 genera and 28 families of vascular plants, are recorded in Georgia. 6 species are represented by two subspecies and one species by two varieties.

This data does not include non-native trees, which have spread in Georgia for various reasons and in different forms.

It is worth noting that the presented list also includes the species of woody plants, which mainly are shrubs and are rarely found in tree forms. The checklist does not include *Swida armasica* (Sanadze) Gvin., which is not universally taxonomically recognized (Euro+Med 2006-; The Plant List 2013; GBIF.org 2022; IPNI 2022; POWO 2022; Tropicos 2022; WFO 2022).

According to the regional (Georgia) IUCN Red List assessments, 9 taxa are critically endangered (CR), 4 are endangered (EN), 9 are vulnerable (VU), and 15 are close to vulnerable (NT). The endangered taxa are distributed according to IUCN categories as follows:

Critically Endangered (CR) – *Arbutus andrachne* L., *Buxus sempervirens* L., *Populus euphratica* Oliver, *Pyrus demetris* Kuth., *P. ketzkhovelii* Kuth., *P. sachokiana* Kuth., *P. takhtadzhianii* Fed., *Sorbus caucasica* Zinserl., *S. hajastana* Gabrieljan;

Endangered (EN) – *Pinus brutia* Ten. var. *pityusa* (Steven), *Betula megrellica* Sosn., *Pyrus fedorovii* Kuth., *Celtis planchoniana* K.I.Chr.;

Vulnerable (VU) – *Acer ibericum* M. Bieb., *A. sosnowskyi* Doluch., *Betula medwediewii* Regel, *B. raddeana* Trautv., *Pistacia atlantica* Desf., *Quercus pontica* K. Koch, *Q. robur* subsp. *imeretina* (Steven ex Woronow) Menitsky, *Q. robur* subsp. *pedunculiflora* (K. Koch), *Zelkova carpinifolia* (Pall.) K. Koch;

Near Threatend (NT) – *Celtis australis* subsp. *caucasica* (Willd.) C.C. Towns., *Crataegus pontica* (K. Koch) Menitsky, *Juglans regia* L., *Populus canescens* (Aiton) Sm., *P. nigra* L., *Pterocarya pterocarpa* (Michx.) Kunth ex I. Iljinsk., *Pyrus oxyprion* Woronow, *Quercus macranthera* Fisch. & C.A. Mey. ex Hohen., *Salix excelsa* S.G. Gmel., *S. wilhelmsiana* M. Bieb., *Sorbus buschiana* Zinserl., *S. velutina* (Albov) Schneid., *Ulmus elliptica* K. Koch, *U. glabra* Huds., *U. minor* Mill.

Due to data deficiency, 33 taxa could not be evaluated and were assigned the category **Data Deficient (DD)**. They are: *Abies nordmanniana* (Steven) Spach, *Acer cappadociicum* subsp. *divergens* (K. Koch ex Pax) A.E. Murray, *A. hyrcanum* Fisch. & C.A. Mey., *A. pseudoplatanus* L., *A. tataricum* L., *A. velutinum* Boiss., *Castanea sativa* Mill., *Celtis australis* L. subsp. *australis*, *Corylus avellana* var. *pontica* (K. Koch) H.J.P.Winkl., *C. colurna* L., *Crataegus caucasica* K. Koch, *C. pseudoheterophylla* Pojark., *Cydonia oblonga* Mill., *Fraxinus angustifolia* subsp. *oxycarpa* (Willd.) Franco & Rocha Afonso, *Fraxinus excelsior* subsp. *coriariifolia* (Scheele) A.E.Murray, *Laurus nobilis* L., *Ostrya carpinifolia* Scop., *Populus alba* L., *P. hyrcana* Grossh., *Prunus padus* L., *Pyrus communis* L. subsp. *communis* (*P. balansae* Decne.), *P. georgica* Kuth., *Quercus hartwissiana* Steven, *Salix armeno-rossica* A.K. Skvortsov, *S. elbursensis* Boiss., *Sorbus colchica* Zinserl., *S. fedorovii* Zaik., *S. turcica* Zinserl., *Tamarix tetrandra* Pall. ex M. Bieb., *Taxus baccata* L., *Tilia cordata* Mill., *Vitex agnus-castus* L., *Ziziphus jujuba* Mill.

Although most of the trees are under anthropogenic pressure and a significant proportion of them grow in close to extreme environments, most of them (66 taxa) are not endangered and have been assessed as **Least Concern (LC)**.

Below is a checklist of trees distributed in Georgia. Taxa are sorted by families. For each taxon (species and subspecies) the reference to taxonomy, the IUCN Red List category and criteria, the assessment argumentation and the references used in species assessment are given.

GYMNOSPERMAE

CUPRESSACEAE

Juniperus communis var. *saxatilis* Pall. (*J. oblonga* M. Bieb.; *J. communis* subsp. *oblonga* (M. Bieb.) Galushko]

References to taxonomy: The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili (1971); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Lachashvili & al. (2017); Lachashvili & Eradze (2017); Shetekauri (2017).

Juniperus excelsa M. Bieb. (*J. isophyllos* K. Koch.)

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili (1971).

Juniperus foetidissima Willd.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskaveli (1960); Lachashvili (1971); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Juniperus oxycedrus L. (*J. rufescens* Link)

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & Eradze (2017); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili (1971); Lachashvili & al. (2004), (2014); Lachashvili & Eradze (2017); Shetekauri (2017).

Juniperus polycarpus K. Koch [*J. excelsa* subsp. *polycarpus* (K. Koch) Takht.]

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskaveli (1960); Lachashvili (1971); Lachashvili & al. (2004, 2014); Lachashvili & Eradze (2017).

*PINACEAE****Abies nordmanniana*** (Steven) Spach

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Ketskhoveli (1960); Lachashvili (1971); Gagnidze & Kemularia-Natadze (1985); Kvachakidze (1985), (2001); Dolukhanov (2010); Shetekauri & Chelidze (2016).

Picea orientalis (L.) Peterm.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Lachashvili (1971); Gagnidze & Kemularia-Natadze (1985); Kvachakidze (1985), (2001); Dolukhanov (2010); Shetekauri & Chelidze (2016).

Pinus brutia* var. *pityusa (Steven) Silba [*P. pityusa* Steven; *P. brutia* subsp. *pityusa* (Steven) Nahal.]

References to taxonomy: The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: EN;

Assessment argumentation: the taxon is assessed based on Solomon & al. (2013) [12];

References used in species assessment: Ketskhoveli (1960); Lachashvili (1971); Kvachakidze (2001); Dolukhanov (2010); Solomon & al. (2013).

Pinus sylvestris* var. *hamata Steven [*P. kochiana* K. Klotzsch ex K. Koch; *P. sylvestris* subsp. *hamata* (Steven) Fomin; *P. sosnowskyi* Nakai]

References to taxonomy: The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Lachashvili (1971); Gagnidze & Kemularia-Natadze (1985); Kvachakidze (1985), (2001); Lachashvili & Mamukelashvili (1986); Dolukhanov (2010); Shetekauri & Chelidze (2016); Shetekauri (2017).

TAXACEAE***Taxus baccata* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Ketskhoveri (1960); Lachashvili (1971); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Kvachakidze (2001); Shetekauri & Chelidze (2016); Goginashvili & Tvauri (2021).

ANGIOSPERMAE**DICOTYLEDIONEAE****ANACARDIACEAE*****Cotinus coggygria* Scop.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveri (1960); Lachashvili 1983a; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili, (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Pistacia atlantica* Desf. [*P. mutica* Fisch. & C.A.Mey.; *P. atlantica* subsp. *mutica* (Fisch. & C. A. Mey.) Rech. f.]**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. 2020; GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B1 ab (iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², area – fragmented, number of area fragments – 7, decline in habitat quality caused by cutting and grazing;

References used in species assessment: Ketskhoveri (1960); Lachashvili 1983a; Lachashvili & al. 2020.

***Rhus coriaria* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili 1983a; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & Eradze (2017); Shetekauri (2017).

AQUIFOLIACEAE

Ilex colchica Pojark.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983a; Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

BETULACEAE

Alnus glutinosa subsp. *barbata* (C.A. Mey.) Yalt. (*A. barbata* C.A. Mey.)

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveri (1960); Dolokhanov (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

Alnus incana (L.) Moench

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Dolokhanov (1975); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Shetekauri (2017).

Betula litwinowii Doluch. [*B. pubescens* var. *litwinowii* (Doluch.) Ashburner & McAll.]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected

threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Dolokhanov (1975); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017); I. Akobia's field data (2012-2020) was used to assess the species.

***Betula medwediewii* Regel**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B1ab (iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Dolokhanov (1975); I. Akobia's field data (2012-2020) was used to assess the species.

***Betula megrelica* Sosn.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: EN B1ab (iii, v) + 2ab (iii, v);

Assessment argumentation: extent of occurrence - <5000 km², area of occupancy - <500 km², decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Dolokhanov (1975); IUCN (2021);

***Betula pendula* Roth**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Dolokhanov (1975); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Lachahsvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017); I. Akobia's field data (2012-2020) was used to assess the species.

***Betula raddeana* Trautv.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B2ab (iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Dolokhanov (1975); Sakhokia & Khutzishvili

(1975); Shetekauri (2017); I. Akobia's field data (2012-2020) was used to assess the species.

BUXACEAE

***Buxus sempervirens* L. (*B. colchica* Pojark.)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR A2acde;

Assessment argumentation: Population reduction – 95%;

References used in species assessment: Ketskhoveli (1960); Gagnidze (1983b); Dmitrieva (1990a); Matchutadze & al. (2013); Gagnidze & Kemularia-Natadze (1985); Mitchell & al. (2018); Supatashvili & al. 2019; B. Berdzenishvili's field data (2018)-2020) was used to assess the species.

CELASTRACEAE

***Euonymus europaeus* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983c; Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

***Euonymus latifolius* (L.) Mill.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983c; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Euonymus leiophloeus* Steven**

References to taxonomy: Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983c; Dmitrieva (1990a); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

CORNACEAE

Cornus iberica Woronow [*Swida iberica* (Woronow) Pojark. ex Grossh.]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ghviniashvili 1984; Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Cornus mas L.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ghviniashvili 1984; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Cornus sanguinea subsp. *australis* (C. A. Mey.) Jav. [*C. australis* C. A. Mey.; *Swida australis* (C.A. Mey.) Pojark. ex Grossh.; *S. koenigii* (C. K. Schneid.) Pojark. ex Grossh.]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ghviniashvili 1984; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

CORYLACEAE

Carpinus betulus L. (*C. caucasica* Grossh.)

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Carpinus orientalis* Mill.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Corylus avellana* L. var. *avellana

References to taxonomy: Güner (2012); GBIF.org (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Corylus avellana* var. *pontica* (K. Koch) H.J.P.Winkl. (*C. imeretica* Kem.-Nath.; *C. pontica* K. Koch)**

References to taxonomy: Güner (2012); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985).

***Corylus colurna* L. (*C. iberica* Wittm. ex Kem.-Nath.; *C. kachetica* Kem.-Nath.)**

References to taxonomy: Euro+Med (2006-); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Ostrya carpinifolia* Scop.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: kemularia-Natadze (1975)a; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Shetekauri & Chelidze (2016).

EBENACEAE

***Diospyros lotus* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1985)a; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986).

ELAEAGNACEAE

***Elaeagnus angustifolia* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili 1984; Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Hippophae rhamnoides* L. [*Elaeagnus rhamnoides* (L.) A.Nelson]**

References to taxonomy: Euro+Med (2006-); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected

threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzhishvili (1975); Lachashvili 1984; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

ERICACEAE

***Arbutus andrachne* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR B2 ab (iii, v);

Assessment argumentation: area – fragmented, number of area fragments – 2, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Gagnidze (1985)a; Dmitrieva (1990b); Goginashvili & al. (2018); Aliev & al. 2020.

Note: the authors of the article (N. Lachashvili, K. Kereselidze and M. Kikvidze) categorically distances from mentioning the integral part of Georgia - Abkhazia - as an independent state in the certified article (Aliev & al. 2020) and protest against the mentioned scientific journal.

***Vaccinium arctostaphylos* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze (1985)b; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990b); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

FAGACEAE

***Castanea sativa* Mill.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Ketskhoveli (1960); Gagnidze (1975); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Dolukhanov (2010); Tavadze & al. (2013).

***Fagus orientalis* Lipsky**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveri (1960); Gagnidze (1975); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Quercus hartwissiana* Steven**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Gagnidze (1975); Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990a); Dolukhanov (2010); Matchutadze & al. (2013).

***Quercus macranthera* Fisch. & C.A. Mey. ex Hohen.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Ketskhoveri (1960); Gagnidze (1975); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Kvachakidze (2001); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Quercus petraea* subsp. *iberica* (Steven ex M.Bieb.) Krassiln. (*Q. iberica* Steven ex M. Bieb.; *Q. dshorochensis* K. Koch)**

References to taxonomy: Euro+Med (2006-); Güner, 2012; The Plant List (2013); Lachashvili & al. (2021); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveri (1960); Gagnidze (1975); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001);

Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Quercus pontica* K. Koch**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B2ab (iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², the species is assessed based on Solomon & al. (2013);

References used in species assessment: Gagnidze (1975); Dmitrieva (1990a); Solomon & al. (2013).

***Quercus robur* subsp. *imeretina* (Woronow) Menitsky (*Q. imeretina* Woronow)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B2ab (iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², the taxon is assessed based on Solomon & al. (2013);

References used in species assessment: Gagnidze (1975); Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990a); Dolukhanov (2010); Solomon & al. (2013); Goginashvili & Tvauri (2021).

***Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky (*Q. pedunculiflora* K. Koch)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B1ab (I, ii, iii);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Gagnidze (1975); Sakhokia & Khutzhishvili (1975); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Dolukhanov (2010); Lachashvili & Eradze (2017); Goginashvili & Tvauri (2011).

JUGLANDACEAE

***Juglans regia* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Ketskhoveli (1960); kemularia-Natadze (1975)b; Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007).

***Pterocarya pterocarpa* (Michx.) Kunth ex I. Iljinsk. [*Juglans pterocarpa* Michx.]**

References to taxonomy: Güner (2012); The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Ketskhoveli (1960); Kemularia-Natadze (1975)b; Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Dolukhanov (2010).

LAMIACEAE

Vitex agnus-castus L.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1985).

LAURACEAE

Laurus nobilis L.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: kemularia-Natadze (1973); Dmitrieva (1990a).

MALVACEAE (TILIACEAE)

Tilia begoniifolia Steven [*T. caucasica* Rupr.; *T. rubra* subsp. *caucasica* (Rupr.) V. Engl.; *Tilia dasystyla* subsp. *caucasica* (V. Engl.) Pigott]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); Lachashvili & al. (2021); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Cholokashvili 1983; Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

Tilia cordata Mill.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of

occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Cholokashvili 1983; Shetekauri (2017); Goginashvili & Tvauri (2021).

MORACEAE

***Ficus carica* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1975)a; Lachashvili & al. (2007); Lachashvili & Eradze (2017).

OLEACEAE

***Fraxinus angustifolia* subsp. *oxycarpa* (Willd.) Franco & Rocha Afonso (*F. oxycarpa* Willd.)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze (1985)b; Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

***Fraxinus excelsior* subsp. *coriariifolia* (Scheele) A.E.Murray (*F. coriariifolia* Scheele)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze (1985)b; Lachashvili & al. (2007).

Fraxinus excelsior* L. subsp. *excelsior

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Sakhokia & Khutzishvili (1975); Kutateladze (1985)b; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al.

(1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

RHAMNACEAE

***Frangula alnus* Mill.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Mukbaniani 1983; Gagnidze & Kemularia-Natadze, (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Lachashvili & Eradze (2017); Shetekauri (2017).

***Rhamnus cathartica* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Mukbaniani 1983; Lachashvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Ziziphus jujuba* Mill.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Mukbaniani 1983.

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***Crataegus caucasica* K. Koch**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Mukbaniani 1980; Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

Crataegus kyrtostyla Fingerh. (*C. monogyna* Jacq.)

References to taxonomy: The Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Mukbaniani 1980; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

Crataegus meyeri Pojark.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Mukbaniani 1980; Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Crataegus orientalis Pall. ex M. Bieb.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Mukbaniani 1980; Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Crataegus pentagyna Waldst. & Kit. ex Willd.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Mukbaniani 1980; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili &

Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

Crataegus pontica K. Koch [*C. azarolus* var. *pontica* (K.Koh) K.I.Chr.]

References to taxonomy: Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Mukbaniani 1980; Mardaleishvili & Tskhadadze 2003; Lachashvili & al. (2017); Lachashvili & Eradze (2017).

Crataegus pseudoheterophylla Pojark.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Mukbaniani 1980; Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Cydonia oblonga Mill.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze 1980a; Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Malus orientalis Uglitzk. [*M. sylvestris* subsp. *orientalis* (Uglitzk.) Browicz]

References to taxonomy: Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Kutateladze & 1980b; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Mespilus germanica* L. [*Crataegus germanica* (L.) Kintze]**

References to taxonomy: The Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze 1980c; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Prunus avium* (L.) L. [*Cerasus avium* (L.) Moench; *C. silvestris* Garsault]**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & Eradze (2017); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Ghvinianidze 1980a; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Prunus divaricata* Ledeb. (*P. cerasifera* Ehrh.)**

References to taxonomy: Güner (2012); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Ghvinianidze 1980b; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Prunus laurocerasus* L. (*Laurocerasus officinalis* M. Roem.)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ghvinianidze 1980c; Gagnidze & Kemularia-

Natadze (1985); Gagnidze & al. (1985); Lachahsvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

Prunus mahaleb L. subsp. ***mahaleb*** [*Cerasus mahaleb* (L.) Mill.]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Lachashvili & Eradze (2017); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ghvinianidze 1980a; Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Prunus padus L. (*Padus avium* Mill.)

References to taxonomy: Euro+Med (2006-); The Plant list (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Sakhokia & Khutzishvili (1975); Ghvinianidze 1980d; Gagnidze & al. (1985); Lachahsvili & Mamukelashvili (1986); Shetekauri & Chelidze (2016); Shetekauri (2017).

Pyrus communis subsp. ***caucasica*** (Fed.) Browicz

References to taxonomy: Euro+Med (2006-); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Sakhokia & Khutzishvili (1975); Kutateladze 1980d; Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

Pyrus communis L. subsp. ***communis*** (*Pyrus balansae* Decne.)

References to taxonomy: Euro+Med (2006-); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze 1980d; Dmitrieva (1990a).

***Pyrus demetrii* Kuth.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR D;

Assessment argumentation: extent of occurrence – 309 km², area of occupancy – 28 km², area – fragmented, number of area fragments – 7, decline in habitat quality caused by land use and grazing;

References used in species assessment: Kutateladze 1980d; Goginashvili & Tvauri (2011); Lachashvili & Eradze (2017); Lachashvili & al. (2017); K. Kereselidze's field data (2019-2020) was used to assess the species.

***Pyrus fedorovii* Kuth.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: EN B1ab (iii, v);

Assessment argumentation: extent of occurrence – 529 km², area of occupancy - < 500 km², area – fragmented, number of area fragments – 4, decline in habitat quality caused by land use and grazing;

References used in species assessment: Makashvili 1952; Kutateladze 1980d; Lachashvili & Eradze (2017); Lachashvili & al. (2017).

***Pyrus georgica* Kuth.**

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze 1980d; Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Pyrus ketzkhovelii* Kuth.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR B2ab (iii);

Assessment argumentation: extent of occurrence – <100 km², area of occupancy - <10 km², number of area fragments – 1, decline in habitat quality, caused by land use and grazing;

References used in species assessment: Kutateladze 1980d; Lachashvili & Eradze (2017); Lachashvili & al. (2017); K. Kereselidze's field data (2019-2020) was used to assess the species.

***Pyrus oxyprion* Woronow**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and / or likely to be close to it in the future;

References used in species assessment: Kutateladze 1980d; Lachashvili & al. (2007).

***Pyrus sachokiana* Kuth.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR D;

Assessment argumentation: extent of occurrence – 6778 km², area of occupancy – 20 km², area – fragmented, number of area fragments – 5, decline in habitat quality caused by land use and grazing; Number of mature individuals – 29;

References used in species assessment: Kutateladze 1980d; Lachashvili & al. (2007); Goginashvili & Tvauro (2011); Lachashvili & Eradze (2017); K. Kereselidze's field data (2019-2020) was used to assess the species.

***Pyrus salicifolia* Pall.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1980d); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Pyrus takhtadzhianii* Fed.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR B1ab (iii) + 2ab (iii);

Assessment argumentation: extent of occurrence - < 100 km², area of occupancy - <10 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Kutateladze 1980d.

***Sorbus aucuparia* L. (*S. boissieri* Schneid.; *S. caucasigena* Kom. ex Gatsch.)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1980e); Sakhokia & Khutzhishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

Sorbus buschiana Zinserl.

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Sakhokia & Khutzishvili (1975); Kutateladze (1980e); Shetekauri (2017).

Sorbus caucasica Zinserl.

References to taxonomy: Güner (2012); The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: CR B2 ab (iv);

Assessment argumentation: extent of occurrence - <100 km², area of occupancy - <10 km², number of area fragments – 1; poor regeneration ability, continuing decline in mature individuals;

References used in species assessment: Kutateladze (1980e); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Shetekauri & Chelidze (2016); K. Kereselidze's field data (2019-2020) was used to assess the species.

Sorbus colchica Zinserl. [*Aira colchica* (Zinserl.) Mezhen'skyj]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); Tropicos.org (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze (1980e); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Dmitrieva (1990a).

Sorbus fedorovii Zaik.

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze (1980e); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a).

Sorbus graeca (Spach) Lodd. ex Schauer [*Aira graeca* (Spach) M. Roem.]

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); Tropicos.org (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1980e); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

Sorbus hajastana Gabrieljan

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR B1ab (iii) + 2ab (iii); CR D;

Assessment argumentation: extent of occurrence - <100 km², area of occupancy - 4 km², number of area fragments – 1, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Shetekauri (2017).

Sorbus subfusca (Ledeb. ex Nordm.) Boiss.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1980e); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri (2017).

Sorbus torminalis (L.) Crantz. [*Torminalis glaberrima* (Gand.) Sennikov & Kurtto]

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); Tropicos.org (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Kutateladze (1980e); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

Sorbus turcica Zinserl. [*Aria umbellata* (Desf.) Sennikov & Kurtto]

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021); Tropicos.org (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Kutateladze (1980e).

Sorbus velutina (Albov) C.K. Schneid.

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Kutateladze (1980e).

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Populus alba L. [*P. nivea* (Aiton) Willd.; *P. pseudonivea* Grossh.]

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Shkhian (1973); Makashvili 1952.

Populus canescens (Aiton) Sm.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Ketskhoveli (1960); Shkhian (1973); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Populus euphratica Oliver

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: CR B1ab (v) + B2ab (v);

Assessment argumentation: extent of occurrence - <100 km², area of occupancy - <10 km², number of area fragments – 1;

References used in species assessment: Shkhian (1973); Lachashvili & al. (2007); Goginashvili & Tvauri (2011), (2021).

Populus hyrcana Grossh.

References to taxonomy: Solomon & al. (2013); The Plants list (2013); Davlianidze & al. (2018); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Shkhian (1973); Solomon & al. (2013).

***Populus nigra* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Ketskhoveli (1960); Shkhian (1973); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

***Populus tremula* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990b); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

Salix alba* L. subsp. *alba

References to taxonomy: Güner (2012); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Salix alba* subsp. *micans* (Andersson) Rech. f.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Makashvili 1952; Gagnidze & al. (1985); Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990b); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

***Salix armeno-rossica* A.K. Skvortsov**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Shkhian (1973); Shetekauri & Chelidze (2016).

***Salix caprea* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Sakhokia & Khutzishvili (1975); Gagnidze & Kemularia-Natadze (1985); Gagnidze & al. (1985); Dmitrieva (1990b); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Salix elbursensis* Boiss.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, number of mature individuals, tendency of their decline in numbers, quality of habitat, etc.);

References used in species assessment: Shkhian (1973); Sakhokia & Khutzishvili (1975); Gagnidze & al. (1985); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Salix excelsa* S.G. Gmel.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1973); Lachashvili & al. (2007); Lachashvili & Eradze (2017); Shetekauri (2017).

***Salix pentandroides* A.K. Skvortsov**

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Sakhokia & Khutzishvili (1975); Gagnidze & al. (1985); Shetekauri (2017).

***Salix triandra* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Shkhian (1973); Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Salix wilhelmsiana* M. Bieb.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1973); Gagnidze & Kemularia-Natadze (1985); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

SAPINDACEAE (ACERACEAE)***Acer campestre* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Shetekauri (2017).

***Acer cappadocicum* Gled. subsp. *cappadocicum* (*A. laetum* C.A. Mey.)**

References to taxonomy: Güner (2012); Lachashvili & Eradze (2017); Lachashvili & al. (2021); GBIF.org (2022); IPNI (2022); POWO (2022);

IUCN red list category and criteria: LC

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable.

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Shetekauri (2017).

Acer cappadocicum* subsp. *divergens (K. Koch ex Pax) A.E.Murray (*A. divergens* K. Koch & Pax)

References to taxonomy: Güner (2012); The Plant list (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1983b).

Acer hyrcanum Fisch. & C.A. Mey

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1983b); Shetekauri & Chelidze (2016).

Acer ibericum M. Bieb. [*A. monspessulanum* subsp. *ibericum* (M. Bieb. ex Willd.) Yalt.]

References to taxonomy: Euro+Med (2006-); Davlianidze & al. (2018); Lachashvili & Eradze (2017); Lachashvili & al. (2021);

IUCN red list category and criteria: VU B1 ab (iii, v);

Assessment argumentation: extent of occurrence - <20000 km², area of occupancy - <2000 km², area – fragmented, decline in habitat quality caused by cutting and grazing;

References used in species assessment: Lachashvili (1983b); Lachashvili & al. (2007); Goginashvili & Tvauri (2011), (2021); Lachashvili & Eradze (2017).

Acer platanoides L.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Shetekauri & Chelidze (2016); Shetekauri (2017).

Acer pseudoplatanus L.

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of

occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Lachahsvili & Mamukelashvili (1986); Dmitrieva (1990a).

***Acer sosnowskyi* Doluch.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU D2;

Assessment argumentation: the species is assessed based on Solomon & al. (2013);

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Solomon & al. (2013).

***Acer tataricum* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985).

***Acer trautvetteri* Medw. [*A. heldreichii* subsp. *trautvetteri* (Medw.) A.E.Murray]**

References to taxonomy: Euro+Med (2006-); Lachashvili & Eradze (2017); Davlianidze & al. (2018); Lachashvili & al. (2021);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Ketskhoveli (1960); Lachashvili (1983b); Gagnidze & Kemularia-Natadze (1985); Lachahsvili & Mamukelashvili (1986); Dmitrieva (1990a); Kvachakidze (2001); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Acer velutinum* Boiss.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Lachashvili (1983b); Lachahsvili & Mamukelashvili (1986).

*STAPHYLEACEAE****Staphylea colchica* Steven**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983d; Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990a).

***Staphylea pinnata* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze 1983d; Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

*TAMARICACEAE****Tamarix ramosissima* Ledeb.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Khintibidze 1983; Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & al. (2017).

***Tamarix smyrnensis* Bunge (*T. hohenackeri* Bunge)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Khintibidze 1983; Gagnidze & Kemularia-Natadze (1985); Lachashvili & al. (2007); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017).

***Tamarix tetrandra* Pall. ex M. Bieb.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Khintibidze 1983.

ULMACEAE

Celtis australis L. subsp. ***australis***

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); GBIF.org (2022);

IUCN red list category and criteria: DD;

Assessment argumentation: data deficient (unknown extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, continuing decline in quality of habitat etc.);

References used in species assessment: Shkhian (1975)b; Dmitrieva (1990b).

Celtis australis subsp. ***caucasica*** (Willd.) C.C. Towns. (*C. caucasica* Willd.)

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); Lachashvili & al. (2021); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1975)b; Lachashvili & Mamukelashvili (1986); Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Celtis planchoniana K.I.Chr. (*C. glabrata* Stev. ex Planch.)

References to taxonomy: Euro+Med (2006-); Güner (2012); The Plant List (2013); Lachashvili & Eradze (2017); Lachashvili & al. (2021); GBIF.org (2022); WFO (2022);

IUCN red list category and criteria: EN B1ab (iii);

Assessment argumentation: extent of occurrence - <5000 km², area of occupancy - <5000 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Shkhian (1975)b; Lachashvili & al. (2007); Lachashvili & Eradze (2017).

Ulmus elliptica K. Koch

References to taxonomy: The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1975)c; Gagnidze & Kemularia-Natadze (1985); Dmitrieva (1990b); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

***Ulmus glabra* Huds.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1975)c; Lachashvili & Mamukelashvili (1986); Dmitrieva (1990b); Goginashvili & Tvauri (2011), (2021).

***Ulmus minor* Mill. (*U. foliacea* Gilib., *U. suberosa* Moench, *U. georgica* Schchian)**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: NT;

Assessment argumentation: the species assessment data are approximate to VU category parameters and/or likely to be close to it in the future;

References used in species assessment: Shkhian (1975)c; Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990b); Lachashvili & al. (2007); Dolukhanov (2010); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Goginashvili & Tvauri (2011).

***Zelkova carpinifolia* (Pall.) K. Koch**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: VU B1ab (iii);

Assessment argumentation: extent of occurrence - 7000 km², area of occupancy - <2000 km², area – fragmented, decline in habitat quality, caused by cutting and grazing;

References used in species assessment: Ketskhoveli (1960); Shkhian (1975)c; Kvachakidze (2021); Lomidze & al. 2020.

VIBURNACEAE***Sambucus nigra* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kutateladze (2001); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017).

***Viburnum opulus* L.**

References to taxonomy: Euro+Med (2006-); The Plant List (2013); Davlianidze & al. (2018); GBIF.org (2022); IPNI (2022); POWO (2022); WFO (2022);

IUCN red list category and criteria: LC;

Assessment argumentation: at the current stage, neither the existing nor the expected threats to the species (extent of occurrence, area of occupancy, the number of mature individuals and the tendency of their decline in numbers, etc.) are noticeable;

References used in species assessment: Gagnidze & Kemularia-Natadze (1985); Lachashvili & Mamukelashvili (1986); Dmitrieva (1990a); Kutateladze (2001); Shetekauri & Chelidze (2016); Lachashvili & Eradze (2017); Lachashvili & al. (2017); Shetekauri (2017).

General conclusion

Trees spread in Georgia are presented by 129 species. They belong to 52 genera and 28 families of vascular plants. Six species are presented by two subspecies and 1 species by two varieties.

According to the regional assessment (Georgia) 9 species are critically endangered (CR), 4 taxa - endangered (EN), 9 - vulnerable (VU), and 15 are near threatened (NT). Due to the lack of data, 33 taxa could not be assessed and fell under the category of data deficient. 66 taxa are not endangered at present and have been assigned the category least concern.

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Mediterranean plant germination reports – 4

edited by Sara Magrini & Cristina Salmeri

Abstract

Magrini, S. & Salmeri, C. S. (eds): Mediterranean plant germination reports – 4. — Fl. Medit. 32: 191-232. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This is the fourth issue of the series of germination reports from Mediterranean areas (sensu Med-Checklist). It comprises germination protocols for 17 taxa: *Centaurea* from central Italy by V. Di Cecco & L. Di Martino (No. 86); *Limonium* from Sardinia by M. E. Boi & al. (No. 87); *Glaucium*, *Helichrysum*, and *Plantago* from Central Italy by S. Magrini & al. (Nos. 88-90); *Anthemis* from Sicily by C. Blandino & al. (Nos. 91-97); *Silene* from Sicily and Calabria by C. Brullo & C. Salmeri (Nos. 98-102); lastly, *Armeria* from North Italy by Vuerich & al. (2022).

Editorial

This fourth issue of the series of germination reports from Mediterranean areas (sensu Med-Checklist) comprises six contributions reporting germination protocols for 18 taxa from Italy, Sardinia, and Sicily.

Six contributions are focused on 11 endemic species.

Di Cecco & Di Martino (2022, No. 86) reported germination data for *Centaurea tenoreana* Willk. (*Asteraceae*), endemic to Abruzzo (central Italy), and Boi & al. (2022, No. 87) for *Limonium merxmuelleri* Erben subsp. *merxmuelleri* (*Plumbaginaceae*), a Sardinian endemic.

Magrini & al. (2022, Nos. 88-90) reported germination protocols for three species from coastal dunes of central Italy: *Glaucium flavum* Crantz (*Papaveraceae*) and *Helichrysum stoechas* (L.) Moench (*Asteraceae*) from Tuscany, and *Plantago coronopus* L. (*Plantaginaceae*) from Latium.

In Blandino & al. (2022, Nos. 91-97), germination protocols for seven *Anthemis* (*Asteraceae*) taxa from Sicily are reported: among them, the dune species, *A. maritima* L. subsp. *maritima*, and six endemic species, *A. aeolica* Lojac., *A. aetnensis* Spreng., *A. cretica* L. subsp. *messanensis* (Brullo) Giardina & Raimondo, *A. cupaniana* Tod. ex Nyman., *A. parlatoreana* Raimondo, Bajona, Spadaro & Di Gristina, and *A. pignattiorum* Guarino, Raimondo & Domina.

Brullo & Salmeri (2022, Nos. 98-102) provided germination protocols for five *Silene* (*Caryophyllaceae*) species from Sicily and Calabria, including the first germination reports for the two endemic species, *S. calabra* Brullo, Scelsi & Spamp. from SW Calabria and *S. crassiuscola* Brullo, C.Brullo, Cambria, Bacch., Giusso & Ilardi. from NW Sicily, together with *S. colorata* Poir., *S. fruticosa* L., and *S. nicaeensis* All. from the Sicilian territories.

Lastly, Vuerich & al. (2022) reported the first germination data for *Armeria helodes* F.Martini & Poldini (*Plumbaginaceae*), endemic to Italy.

Available seed germination data for Mediterranean plants

During the first four years, over 60 authors from Italy, Malta, and Greece sent their contributions for publication in the “*Mediterranean plant germination reports*” column, for a total of 29 contributions mainly referred to species/populations from Sicily (51 reports) and peninsular Italy (41), besides seven reports from Greece and Malta.

Today, 102 reports of germination protocols have been published over four issues, for a total of 95 species, mainly belonging to the family *Asteraceae* (37), especially to the genera *Helichrysum* (11) and *Anthemis* (7), but also *Caryophyllaceae* (10), *Brassicaceae*, *Euphorbiaceae*, and *Fabaceae* (5). Among them, reports for 44 endemics have been published, especially for the family *Asteraceae* (25).

Moreover, germination protocols for 10 species which are threatened with extinction in Italy (Orsenigo & al. 2018; Rossi & al. 2013, 2020) have been published: four Critically Endangered species, *Abies nebrodensis* (Lojac.) Mattei (*Pinaceae*), *Euphorbia gasparrinii* Boiss. subsp. *gasparrinii* (*Euphorbiaceae*), *Anthemis aeolica* and *A. cretica* subsp. *messanensis* (*Asteraceae*), six Endangered ones, *A. pignattiorum* (*Asteraceae*), *Armeria helodes* (*Plumbaginaceae*), *Astragalus aquilanus* Anzal. (*Fabaceae*), *Muscari gussonei* (Parl.) Nyman (*Asparagaceae*), *Spiranthes aestivalis* (Poir.) Rich. (*Orchidaceae*), and *Viola kitaibeliana* Schult. (*Violaceae*), besides other 10 species which are Near Threatened.

This column can be a useful tool for researchers, conservationists, botanists, and other specialists, providing species-specific information on the pre-treatments and key factors to improve seed germination of native plants. Moreover, these germination data will early flow into an online, open-access database managed by the RIBES association to make them available to a wider audience.

For manuscript editing and submission, please follow the general instructions for the authors of *Flora Mediterranea* (<http://www.herbmedit.org/guide.html>) and the specific instructions for the *Mediterranean plant germination reports* (Magrini & Salmeri 2019; https://www.herbmedit.org/flora/FL29_269-271.pdf). Authors are invited to submit by e-mail their manuscripts in English to one of the editors.

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*RIBES, Rete Italiana Banche del germoplasma per la conservazione Ex Situ della flora italiana, www.reteribes.it

Valter Di Cecco & Luciano Di Martino

Seed germination reports for *Centaurea tenoreana* (Asteraceae)

Abstract

Di Cecco, V. & Di Martino, L.: Seed germination reports for *Centaurea tenoreana* (Asteraceae) [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 194-196. 2022. <http://dx.doi.org/0.7320/FlMedit32.194>

This work aims to study the seed germination capacity for *Centaurea tenoreana* (Asteraceae). This species is narrowly endemic to the territory of the Maiella National Park, in Abruzzo (Italy). The species did not show dormancy, and excellent germination results were obtained at constant temperatures both in the dark and in the light. The highest germination value (98.95%) was obtained by sowing the cypsels on 1% agar and then incubating at a constant temperature of 15°C with a light-dark photoperiod of 12/12 hours. The records of the first germination are provided for the taxon investigated.

Key words: Asteraceae, narrowly endemics, Michele Tenore, Maiella National Park.

Introduction

Centaurea tenoreana Willk. is a white-tomentose cornflower with deeply engraved leaves and a purple-pink flower. It grows in arid and stony meadows from 500 to 2,000 m a.s.l., where it blooms between July and August. The species is endemic to the Central Apennines and exclusive to the Maiella National Park and was chosen as the symbol of the “Michele Tenore” Botanical Garden in Lama dei Peligni (Chieti - Italy).

The work was carried out in the Maiella Seed Bank – Lama dei Peligni. The experimental design was set up starting from preliminary bibliographic analysis and from the consultation of germination protocols already tested for similar taxonomic units (Ellis & al. 1985; Royal Botanic Gardens Kew 2022), in order to identify the optimal temperature ranges and the need to apply any pre-treatments (Baskin & Baskin 2014; ISTA 2012).

The seed ecology of *C. tenoreana* had never been investigated before and there were no literature data on the germination and propagation of these species.

86. *Centaurea tenoreana* Willk. (Asteraceae)

Accession data:

It: Abruzzo. Fara San Martino (Chieti), loc. Milazzo (WGS84: 42.07747°N 14.14180°E), pietraia, 1,705 m a.s.l., 07 Aug 2022, *V. Di Cecco* (MSB_N258, Maiella Seed Bank).

Germination data

Pre-treatments: Soaking in water for 24h, sterilization with a solution of 3% sodium hypochlorite plus Tween 20 for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar, pH 5.75

Sample size: 80 seeds (20 × 4 replicates)

Germination	Thermoperiod	Photoperiod [light/dark]	T _i [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
98.95%	constant 15°C	12/12h	1.5	2.5	5.0	3.9
93.62%	constant 20°C	0/24	-	-	-	-
93.61%	constant 20°C	12/12h	2.0	2.4	7.8	5.3
83.09%	constant 5°C	0/24	-	-	-	-

Observations

Centaurea tenoreana is a species that lives on stony ground and limestone cliffs. It is present only on the Maiella massif, Monte Morrone and Monti Pizzi. Growth chambers with thermoperiod and adjustable photoperiod (Panasonic MLR-352 and Panasonic MIR-154) were used. The viability of the non-germinated seeds at the end of the tests is estimated by carrying out the cutting test. The Petri dishes incubated in the dark were opened after 30 days to evaluate the final germination.

By increasing the temperature up to 25°C, the percentage of final germination drops considerably (64.21%) even if germination takes place in the first days of the test. The tests show an absence of dormancy and an excellent germination capacity of freshly harvested seeds.

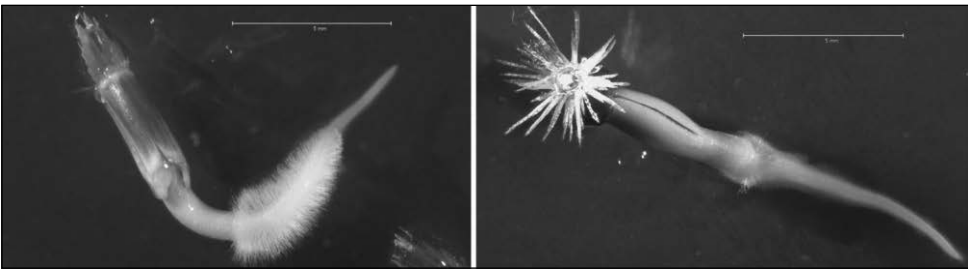


Fig. 1. Germinated seeds of *Centaurea tenoreana*.

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M. E. Boi, L. Podda, M. Porceddu & G. Bacchetta

Seed germination report for *Limonium merxmuel- leri* subsp. *merxmuel- leri* (*Plumbaginaceae*)

Abstract

Boi, M. E., Podda, L., Porceddu, M. & Bacchetta, G.: Seed germination report for *Limonium merxmuel-
leri* subsp. *merxmuel-
leri* (*Plumbaginaceae*) [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 197-200. 2022. <https://doi.org/10.7320/FIMedit32.197>

The present report illustrates the first germination data of *Limonium merxmuel-
leri* subsp. *merxmuel-
leri*, a pioneer species of mine waste materials of Iglesiente region (South-West Sardinia). After harvesting, seeds were subjected to germination tests at the Sardinian Germplasm Bank (BG-SAR). The results show a high germination capability and germination rate at each tested temperature, with a slight germination reduction at the highest temperature (25°C) that simulates the aridity of the summer period.

Key words: endemics, metallophyte, mine environments, Sardinia, Mediterranean vascular flora.

Introduction

*Limonium merxmuel-
leri* Erben subsp. *merxmuel-
leri* is a metallophyte and a pioneer species of mine wastes in Sardinian abandoned mine sites. It grows in a few square kilometres in the Iglesiente biogeographic subsector (Sardinia, Italy) and it is considered of medium/high conservation priority at the regional scale (Bacchetta & al. 2012). Taking into account its metallophyte character and its natural distribution on mine waste surfaces, the knowledge of the germination behaviour of this species can help in the design of effective phytoremediation action.

This work reports the first germination data concerning this taxon. The germination tests were carried out at the Sardinian Germplasm Bank (BG-SAR; Porceddu & al. 2017). The results of these experiments showed a high germination capability together with a high germination rate.

**87. *Limonium merxmuel-
leri* Erben subsp. *merxmuel-
leri* (*Plumbaginaceae*)**

Accession data

Sa: Iglesias (South-West Sardinia), Monte Agruxiau (WGS84: 39.302329°N, 8.488891°E), garrigue, 117 m a.s.l., 23 Sept 2021, *M. E. Boi, L. Podda & L. Murgia* (BG-SAR 108/21, Sardinian Germplasm Bank).

Germination data

Pre-treatments: no treatment.

Germination medium: 1% agar.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100.0%	constant 15°C	12/12h	3.0	3.5	7.0	4.1
100.0%	constant 20°C	12/12h	2.0	2.8	7.0	3.8
96.0%	constant 25°C	12/12h	2.0	3.0	7.0	4.3

Observations

Limonium merxmuelleri subsp. *merxmuelleri* is an endemic dwarf frutex of Sardinia (Fois & al. 2022), that occurs exclusively in the Iglesiente subsector and, in particular, in a limited area in the southernmost portion of the Metalliferous ring, between the municipalities of Iglesias and Gonnese. It is a chamaephyte with spatulate-lanceolate leaves and violet flowers that blooms from the end of July to October (Fig. 1), giving mature fruits from September to December. It occurs together with other endemic vascular species, for instance, *Iberis integerrima* Moris, *Lysimachia monelli* (L.) U.Manns & Anderb. subsp. *monelli*, and *Reseda luteola* L. subsp. *dimerocarpa* (Müll.Arg.) Abdallah & de Wit, leading several plant assemblages exclusive of these environments, for instance, *Reseda luteolae*-*Limonium merxmuelleri* and its subass. *Iberidetosum integerrimae* (Angiolini & al. 2005).

L. merxmuelleri subsp. *merxmuelleri* germinated with a high percentage at all tested temperatures (15°C, 20°C and 25°C; 100% or close; Fig. 2) with faster germination that finished approximately in a week. This behaviour is common in other *Limonium* species and has already been observed in *Limonium avei* (De Not.) Brullo & Erben (Santo & al. 2017). Only a slight reduction in terms of germination (96%) occurs at the highest temperature (25°C) which corresponds to the mean values of summertime as well as to the high drought conditions of this period.

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This research was financially supported by “Comune di Iglesias” in the frame of the “Progetto di conservazione e traslocazione di *Limonium merxmuelleri* nella Valle del Rio San Giorgio”. We gratefully thank also IGEA S.p.A. Moreover, we want to thank Francesca Meloni, Mauro Fois, Luca Murgia and Ludovica Dessì for the constant support.



Fig.1. Blooming specimen of *Limonium merxmuellerei* subsp. *merxmuellerei* and an example of mine environments.



Fig. 2. Details of germination tests of *Limonium merxmuellerei* subsp. *merxmuellerei*

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S. Magrini, A. Alonso Simón, E. Fiorucci, E. Pennuzzi, S. Superchi & L. Zucconi

Seed germination protocols for three coastal dune plants in central Italy

Abstract

Magrini, S., Alonso Simón, A., Fiorucci, E., Pennuzzi, E., Superchi, S. & Zucconi, L.: Seed germination protocols for three coastal dune plants in central Italy [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 201-206. 2022. <https://doi.org/10.7320/FIMedit32.201>

Here, successful germination protocols for the following three coastal dune species are presented: *Glaucium flavum*, *Helichrysum stoechas* and *Plantago coronopus*. It is the first report of germination data for these species in the Italian peninsula. Seeds were collected in coastal dunes along the Tyrrhenian coasts in south Tuscany and north Latium (central Italy). The germination ability was tested at the Tuscia Germplasm Bank (BGT) at constant temperatures (from 5 to 30°C), under both light (with a 12/12h photoperiod) and total darkness. Our results show a high germination ability and germination rate for all the tested species.

Key words: beach, Latium, psammophytes, Tuscany.

Introduction

Mediterranean coastal dunes are dynamic environments, particularly vulnerable due to the strong impact of human activities and in need of conservation actions. The research focused on the assessment of germination requirements of dune species is carried out at the Tuscia Germplasm Bank where over 150 seed accessions of 35 species collected in the beaches and dunes along the Tyrrhenian coasts of central Italy are long-term preserved. Here we present successful germination protocols for some populations of the following species: *Glaucium flavum* Crantz, *Helichrysum stoechas* (L.) Moench, and *Plantago coronopus* L.

88. *Glaucium flavum* Crantz (*Papaveraceae*)

Accession data

It: Tuscany. Orbetello (Grosseto), Ansedonia (42.408195°N, 11.302421°E), beach, 1 m a.s.l., 21 Jul 2020, *S. Magrini* (BGT-A-67820, Tuscia Germplasm Bank).

Germination data

Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T _i [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
98.3%	constant 10°C	0/24h	21.0	31.1	37.0	29.8
90.0%	constant 10°C	12/12h	30.0	39.4	40.0	39.2
80.0%	constant 15°C	12/12h	8.0	16.6	70.7	21.9

Observations

Glaucium flavum is a short-lived perennial native to Europe, Northern Africa, Macaronesia and temperate zones in Western Asia, growing mainly on the seashore along the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species in the Italian peninsula. Germination tests were carried out using six constant temperature regimes (5–30°C) under both light and dark conditions.

The results showed high germination percentages ($\geq 80\%$) in the range between 10°C and 15°C, with the maximal response at 10°C in both full darkness and light (98.3% and 90.0%, respectively), showing the characteristic Mediterranean-type germination (Thanos 1989).

The germination decreases with increasing temperature, with very low germination percentages recorded at 25–30°C (2% in the light and 5% in darkness) and no germination at 5°C. This species showed a very strict temperature-dependent germination, as highlighted also for Sicilian populations (Walter & al. 2020). The germination was quite slow, the fastest at 15°C in the light ($T_i = 8$ days and $T_{50} = 16.6$ days).

Seed germination under light conditions was just a little lower than in full darkness, indicating that the seeds of this population are not affected by the strong photoinhibition reported for this and other sand beach species, like *Cakile maritima* Scop., *Matthiola sinuata* (L.) W.T.Aiton, and *Malcolmia littorea* (L.) R.Br. (Thanos & al. 1989, 1991; De Vitis & al. 2014, 2018; Carta & al. 2017; Magrini & al. 2019).

89. *Helichrysum stoechas* (L.) Moench (*Asteraceae*)

Accession data

It: Tuscany. Grosseto (Grosseto), Principina a Mare (WGS84: 42.690539°N, 10.997792°E), back dune, 3 m a.s.l., 28 Jun 2020, *S. Magrini* (BGT-A-64720, Tuscia Germplasm Bank).

Germination data

Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
90.1%	constant 15°C	0/24h	2.0	4.9	16.0	6.3
87.9%	constant 20°C	0/24h	2.0	2.1	13.8	4.2
87.8%	constant 25°C	0/24h	2.0	2.0	12.6	4.0
85.0%	constant 10°C	0/24h	5.4	10.3	24.8	11.3
84.9%	constant 25°C	12/12h	2.0	4.5	27.6	7.9
84.5%	constant 15°C	12/12h	2.0	2.7	30.0	10.9

Observations

Helichrysum stoechas is widely spread along the Mediterranean coasts, common in the dune sclerophyllous scrubs along the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species in central Italy. Germination tests were carried out using eight constant temperature regimes (5–40°C) under both light and dark conditions.

High germination percentages (≥ 85%) were recorded at 10–25°C in the dark, with slightly lower values at 15°C and 25°C under a 12/12h photoperiod. Lower percentages (< 63%) were recorded at T ≥ 30°C, especially at 35°C in darkness (33.3%) and at the lower temperatures, 5°C under both light and dark conditions (3.3 and 12.9%, respectively) and 10°C with a light/dark photoperiod (28.8%). No germination was recorded exclusively at 40°C. Seeds germinated within two days at 15–25°C, with the fastest germination recorded at 20°C and 25°C in darkness (T₅₀ = 2.1 and 2.0 days; Mean Germination Rate - MGR = 0.216 and 0.203 day⁻¹, respectively). The slowest germinations were recorded at 5°C under both light and total darkness (T₅₀ = 28.2 and 25.9 days, MGR = 0.035 and 0.037 day⁻¹, respectively).

Our results obtained at 15°C with a 12/12h photoperiod are in accordance with Royal Botanic Gardens Kew (2022) and with Salmeri & Brullo (2021) which reported a similar germination percentage (G = 83%, even if after a longer period, 63 days instead of 30, and 81%, respectively) for Sicilian populations. On the other hand, the germination percentage reported by Salmeri & Brullo (2021), at the same temperature but with a 0/24h photoperiod, is lower than our results (G = 82% vs. 90.1%, respectively).

Strong photoinhibition was recorded exclusively at 5–10°C (PISG index = 0.68 and 0.53; Carta & al. 2017), while germination percentages under light conditions were slightly lower than in full darkness at the other temperatures.

90. *Plantago coronopus* L. (Plantaginaceae)

Accession data

It: Latium. Tarquinia (Viterbo), Sant'Agostino (WGS84: 42.173181°N, 11.738444°E), back dune, 3 m a.s.l., 6 Sep 2021, *L. Zucconi* (BGT-A-87321, Tuscia Germplasm Bank).

Germination data

Pre-treatments: sterilization with a solution of 5% sodium hypochlorite for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100.0%	constant 20°C	12/12h	1.0	1.0	6.4	2.9
99.1%	constant 10°C	0/24h	4.0	3.9	14.8	5.5
99.0%	constant 15°C	12/12h	1.0	3.3	4.2	3.3
97.9%	constant 15°C	0/24h	1.6	3.4	5.6	3.6
96.9%	constant 20°C	0/24h	1.0	1.0	4.6	1.4
96.9%	constant 25°C	12/12h	1.0	1.0	2.2	1.1
96.9%	constant 25°C	0/24h	1.0	1.0	2.0	1.4
94.4%	constant 10°C	12/12h	3.4	4.8	16.2	6.4
92.4%	constant 30°C	12/12h	1.0	1.0	4.0	1.6
91.8%	constant 30°C	0/24h	1.0	1.0	16.0	2.2

Observations

Plantago coronopus is widely spread along the Mediterranean coasts. It is common in the back dunes along the Italian shorelines (Acosta & Ercole 2015). Here we report the first germination data for this species in Italy. Germination tests were carried out using six constant temperature regimes (5–30°C) under both light and dark conditions.

P. coronopus seeds exhibit a dormancy period but dormancy is gradually broken as post-maturation time increases (Luciani & al. 2001). Following the results in Luciani & al. (2001), seeds with a long after-ripening time (402 days) were used, obtaining good results. Our results highlighted the ability of *P. coronopus* seeds to germinate in a wide range of temperatures (5–30°C) both in the dark (29–99%) and in the light (25–100%). High germination percentages (≥ 91.8%) were recorded at all the tested temperatures under both light and dark conditions, except at 5°C where germination percentages lower than 30% were recorded. Our results are higher than those reported by Royal Botanic Gardens Kew (2022) at 15°C and 20°C in the light (G = 99% vs. 90% and G = 100% vs. 90%, respectively). We are not in accordance with the statement by Luciani & al. (2001) that at higher temperatures the germination of *P. coronopus* tends to decrease, becoming rather scarce at 30°C, since higher percentages (> 90%) were recorded in our test even at such temperature.

P. coronopus showed fast germination in the range of 15–30°C (T₁ = 1 day and T₅₀ = 1–3.4 days) with total germination in 2 days at 25°C in the light. The fastest germination was recorded in the 20–30°C range under both light and dark conditions (T₅₀ = 1 day) and the slowest at 5°C under light and dark conditions (T₅₀ = 21–22 days). Species like these are

defined as “very fast germinating species” (Parsons 2012), because seeds germinate in a very short period after the substrate is wetted, allowing a rapid response to favourable environmental conditions that increase their colonization ability, particularly important for species growing in stressful habitats like beaches and coastal dunes (Luciani & al. 2001; Liu & al. 2013).

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C. Blandino, F. Carruggio, C. Impelluso, M. Castrogiovanni & A. Cristaudo

Germination protocols for seven Mediterranean taxa of the genus *Anthemis* (Asteraceae) from Sicily

Abstract

Blandino, C., Carruggio, F., Impelluso, C., Castrogiovanni, M. & Cristaudo, A.: Germination protocols for seven Mediterranean taxa of the genus *Anthemis* (Asteraceae) from Sicily [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 207-219. 2022. <http://dx.doi.org/10.7320/FIMedit32.207>

In this study, we characterize the germination behaviour of seven closely related taxa within the genus *Anthemis*, with respect to temperature, light, and cypsela morph. The study taxa are *Anthemis aeolica*, *A. aetnensis*, *A. cretica* subsp. *mesanensis*, *A. cupaniana*, *A. maritima* subsp. *maritima*, *A. parlatoresana*, and *A. pignattiorum*, all native or endemic to Sicily. The species selected are representative of both lowland and mountain habitats.

Seeds were collected from wild populations, at the time of natural dispersal. All of the species possess heteromorphic cypselas with different size, shape, surface ornamentation, and colour. For all of the taxa, two cypselas morphs were identified and tested separately: cypselas with thin and light-coloured pericarp and cypselas with thick and dark-coloured pericarp. Germination was tested in fresh and after-ripened cypselas. Seven constant and, only for *A. aeolica*, four alternating temperatures were applied both in light and total darkness. The best germination protocols for each seed accession are provided.

Fresh light-coloured cypselas reached optimal germination at temperatures ranging from 10 to 20°C. This seed morph both widened its thermal range for germination and increased the final germination percentage following one to three months of after-ripening, showing to possess non-deep physiological dormancy. Dark cypselas showed low germination under any temperature and light conditions, suggesting the presence of a deeper degree of physiological dormancy. However, the role of the pericarp has to be better assessed.

Key words: after-ripening, temperature, light, seed dormancy, cypselas heteromorphy.

Introduction

Anthemis is one of the largest genera within the tribe *Anthemideae*, including approximately 175 species worldwide (Oberprieler & al. 2009, 2022a). It has a circum-Mediterranean distribution, with a geographical range that encompasses most of Europe, the Mediterranean Basin, and part of South-West Asia, as well as North and East Africa

(Oberprieler & al. 2009). The genus *Anthemis* is characterized by a high degree of endemism. It separated from its closest relatives about 10-15 Ma (Oberprieler 2005), becoming a species-rich group. The high phenotypic plasticity of *Anthemis* species allowed these plants to colonize many habitat types. Indeed, the ancestors of this genus were adapted to a montane-humid climate, whereas younger lineages became more adapted to a dry and Mediterranean-like climate (Lo Presti & Oberprieler 2009).

In Sicily, the genus *Anthemis* is represented by 13 species (Oberprieler & al. 2022b), seven of which are the subject of this study. *Anthemis aeolica* Lojac. and *A. maritima* L. subsp. *maritima* are coastal species; *A. aetnensis* Spreng., *A. cupaniana* Tod. ex Nyman, and *A. cretica* L. subsp. *messanensis* (Brullo) Giardina & Raimondo are mountain species; *A. parlatoresana* Raimondo, Bajona, Spadaro & Di Gristina and *A. pignattiorum* Guarino, Raimondo & Domina instead grow at hilly elevations (300-700 m a.s.l.). All of them are endemic to Sicily except for *A. maritima* subsp. *maritima*, which has a Mediterranean distribution.

Differences in the biogeographical history, isolation, and adaptation to peculiar habitats could have influenced many of the life history traits in the present *Anthemis* species. The timing for seed germination and subsequent seedling establishment is a crucial trait for plant reproduction success and has to be finely tuned to the environmental conditions. This given, the germination behaviour of the study species may differ between montane and lowland taxa. Indeed, the establishment of seedlings should have to occur when sufficient water is available in the soil and temperature is favourable for their growth, avoiding the onset of summer drought in Mediterranean environments and winter frosts, especially for high mountain species.

Physiological dormancy has been reported for the seeds of many *Anthemis* species, such as *A. arvensis*, *A. cotula*, *A. chrysanthra*, and *A. ruthenica* (Ellis & Ilnicki 1968; Martinkova & al. 1997; Aguado & al. 2011). Moreover, the presence of heteromorphic cypselas has been highlighted, with central diaspores (i.e., disc cypselas) and peripheral ones (i.e., ray cypselas) differing in their morphology, anatomy, dispersal, and germination behaviour. Cypselas heteromorphy has been found in the genus *Anthemis* itself (*A. ruthenica* and *A. chrysanthra*), as well as in other *Asteraceae* taxa from highly seasonal environments within the genera *Bidens*, *Senecio*, *Picris*, and *Crepis* (McEvoy 1984; Corkidi & al. 1991, Baskin & Baskin 2014). Central cypselas are often less dormant and germinate over a wider range of temperatures, to higher percentages and at faster rates than peripheral ones (Brändel 2007). The pericarp is the structure that protects the true seed within cypselas, appearing to be primarily responsible for the heteromorphy and, in part, the cause of the different germination responses among diaspores (Brändel 2007; Aguado & al. 2011). For instance, a thick pericarp may slow down or, eventually, prevent the imbibition of embryos or impose on them a mechanical compulsion, so conditioning germination occurrence and/or its parameters.

All the species considered in this report possess heteromorphic cypselas: with thin and light-coloured pericarp and with thick and dark-coloured pericarp (Fig. 1). Entire cypselas, hereafter seeds, were used as germination units. Seven constant temperatures (3, 5, 10, 15, 20, 25, 30, and 35°C) were tested for all the species and further four alternating temperatures (15/10, 20/10, 20/15, and 25/20°C) only for *A. aeolica*. All of the temperature treatments were tested both in light/dark (12/12 h photoperiod) and darkness conditions (0/24 h). Under alternating temperatures, the light period was associated with the higher temperature. Both fresh and after-ripened seeds ($T = 22 \pm 2^\circ\text{C}$; RH = 50%; 1- to 12-month duration) were compared. Finally, tests

were repeated separately for the two identified cypsela morphs to account for possible differences in the germination behaviour due to the pericarp structure.

Our results identified and described the best germination protocols for each seed accession.

91. *Anthemis aeolica* Lojac. (*Asteraceae*) (Fig. 2a)

Accession data

- Si:** Lipari (Messina), Lisca Bianca - Panarea (WGS84: 38.63906°N, 15.114147°E), volcanic tuff, 7 m a.s.l., 16 Jul 2017, *S. Catara & A. Cristaudo* (SO.PRO.ME/CT/17/707, BGS-CT).
- Si:** Lipari (Messina), Lisca Bianca - Panarea (WGS84: 38.63906°N, 15.114147°E), volcanic tuff, 7 m a.s.l., 16 Jul 2020, *M. Castrogiovanni & C. Impelluso* (SiMaSeed/CT/20/669, BGS-CT).

Germination data

Pre-treatments: no treatment; *seeds submitted to a 3-months after-ripening period

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
99%	constant 5°C	12/12 h	9.0	11.7	19.0	12.6
99%	constant 15°C	12/12 h	4.0	4.8	8.0	5.5
98%	**alternating 15/10°C	12/12 h	5.0	5.0	8.0	5.6
97%	**alternating 20/10°C	12/12 h	4.0	4.4	8.0	4.9
97%	**alternating 20/15°C	12/12 h	3.0	3.9	11.0	4.6
93%	constant 10°C	12/12 h	5.0	6.6	9.0	7.0
93%	constant 25°C	12/12 h	2.0	5.2	16.0	6.8
93%	**alternating 25/20°C	12/12 h	3.0	4.0	10.0	5.0
91%	constant 20°C	12/12 h	4.0	4.5	10.0	5.1
88%	constant 3°C	12/12 h	20.0	23.4	30.0	24.4
84%	*constant 30°C	12/12 h	2.0	3.3	29.0	7.1

**Alternating temperatures were tested only in fresh seeds from the SO.PRO.ME/CT/17/707 accession

Observations

Anthemis aeolica is a narrow endemic species whose only known population occurs on the islet of Lisca Bianca, in the Aeolian Islands. Therefore, it is a species of great biogeographical and conservation concern. According to the IUCN (2012), this species must be considered Critically Endangered (CR) (Orsenigo & al. 2018). It grows on volcanic tuff and shares its habitat with many rupicolous and halophytic taxa, such as *Dianthus rupicola* subsp. *aeolicus*, *Hyoseris taurina*, *Limonium minutiflorum*, *Limbarda crithmoides*, and *Lotus cytisoides*.

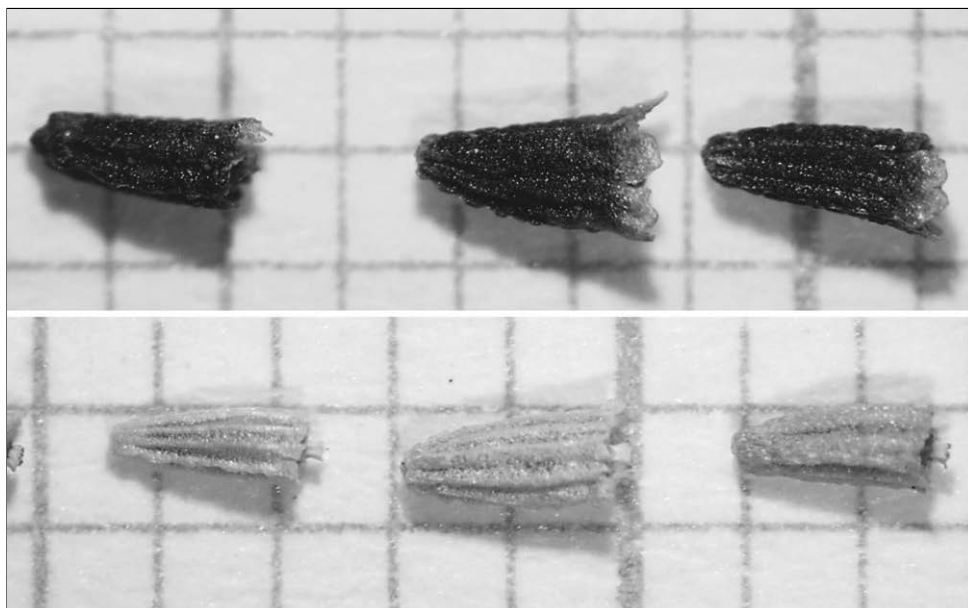


Fig. 1. Examples of a) dark and b) light-coloured cypselas from *Anthemis aeolica*.

A. aeolica produces dimorphic cypselas (Fig. 1), which showed opposite germination behaviour. Fresh light-coloured ones reached high germination percentages (> 90%) within a wide range of constant temperatures (5-25°C) and under alternating regimes in the presence of light. High germination values (> 80%) were also reached at 30°C, under light conditions, but only in 3-month aged seeds, indicating a positive physiological response to after-ripening. Conversely, dark cypselas displayed a deep dormancy level with low or no germination (3-8%) under all the conditions tested, favouring their inclusion in the soil seed bank. The fastest germination was obtained for light cypselas at 20 and 25°C constant regimes, under which almost all of the seeds germinated within three days. The lack of germination at very high temperatures in fresh light-coloured seeds suggests a protection strategy (i.e., thermo-dormancy or thermo-inhibition) against casual rainfalls during the summer (Hills & van Staden 2003; Cristaudo & al. 2019; Carruggio & al. 2021).

A generalized negative effect of darkness on seed germination was detected, making useful further experiments on more specific gap detection factors such as light quality and nitrate concentration (Fenner & Thompson 2005), which could be greatly important under high competition conditions like those experienced by *A. aeolica*.

92. *Anthemis aetnensis* Spreng. (Asteraceae) (Fig. 2b)

Accession data

Si: Nicolosi (Catania), Crateri Silvestri (WGS84: 37.69714°N, 15.01239°E), volcanic ash and lapilli fields, 1,787 m a.s.l., 20 Jun 2020, *A. Cristaudo & F. Carruggio* (SiMaSeed/CT/20/646, BGS-CT).

Germination data

Pre-treatments: no treatment.

Germination medium 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T _i [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	3.0	4.9	15.0	6.1
97%	constant 15°C	12/12 h	3.0	5.7	18.0	6.6
97%	constant 25°C	12/12 h	3.0	5.9	18.0	6.9
96%	constant 10°C	12/12 h	5.0	7.4	18.0	8.7
87%	constant 30°C	12/12 h	3.0	9.2	22.0	10.2

Observations

A. aetnensis is an endemic species to Mount Etna. The plant forms small cushions on the high slopes of the volcano, sometimes together with *Astragalus siculus* Biv. According to the IUCN criteria (2012), *A. aetnensis* has been considered as Near Threatened (NT) (Orsenigo & al. 2018).

The germination of fresh light-coloured cypselas was promoted in the presence of light, in a wide temperature range (10-30°C). Fresh dark-coloured cypselas reached germination values of 50-70% at temperatures ranging from 20 to 30°C, only if exposed to light, confirming the presence of positive photoblastic seeds even in this species.

Germination range partially broadened towards higher temperature values in light-coloured cypselas following one year of after-ripening (60% of germination at 35°C in the light). Moreover, seeds from this morph were equally able to germinate in both the presence and absence of light after this period. Changes in germination requirements following an after-ripening treatment suggest that a physiological change occurred. These results show that the species is adapted to Mediterranean mountain ecosystems, avoiding germination at low temperatures when the risk of winter frost could affect seedling establishment (Picciau & al. 2019; Carruggio & al. 2020).

93. *Anthemis cretica* subsp. *messanensis* (Brullo) Giardina & Raimondo (*Asteraceae*) (Fig. 2c)

Accession data

Si: Messina (Messina), Antennamare (WGS84: 38.15373°N, 15.462297°E), semi-rupestrian habitats, 1,062 m a.s.l., 26 Jun 2019, *A. Cristaudo* (SiMaSeed/CT/19/379, BGS-CT).

Germination data

Pre-treatments: 12-month dry after-ripening at $22 \pm 2^\circ\text{C}$ and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
93%	constant 15°C	12/12 h	5.0	5.8	21.0	7.2
90%	constant 10°C	12/12 h	8.0	10.4	20.0	11.3
89%	constant 20°C	12/12 h	3.0	4.2	26.0	5.5
88%	constant 25°C	12/12 h	2.0	4.0	25.0	5.8
87%	constant 10°C	0/24 h	-	-	-	-
82%	constant 15°C	0/24 h	-	-	-	-

Observations

Anthemis cretica subsp. *messanensis* is a rare endemic species to Peloritani Mountains (NE Sicily) that grows in rocky habitats. A single population was known so far for Mt. Antennamare, whereas a new population was recently found close to Mt. Poverello (Fiumedinisi - Messina) (Tavilla & al. 2021), 12 km apart from the first one. The IUCN conservation status for *A. cretica* subsp. *messanensis* is Critically Endangered (CR) (Orsenigo & al. 2018).

Our tests highlighted a germination behaviour that deviates from that of the other investigated taxa. Indeed, fresh light-coloured cypselas showed a pronounced dormancy under all the temperatures tested, both in the presence and absence of light. Some germination was achieved at 5 and 10°C in the light but without exceeding 50% final germination. After a 3-month after-ripening period, dormancy began to be released, only in seeds exposed to light whereas it was maintained in seeds incubated in total darkness. Final germination values above 80% occurred one year after seed collection, at temperatures ranging from 10 to 25°C in the light and at 10 and 15°C in darkness. Germination slightly decreased at 20 and 25°C (approx. 70%) and was completely inhibited by higher temperatures in the dark. Seeds with dark cypselas had very low germination under all the temperatures and light treatments.

In conclusion, seeds of *A. cretica* subsp. *messanensis* possess physiological dormancy. Moreover, the small proportion of seeds able to germinate soon after natural dispersal only responds to low temperatures, such as in late autumn/winter plants. The preference for low-temperature germination, which is a more typical trait of Mediterranean coastal species rather than mountain species (Picciau & al. 2019), ensures that an adequate supply of water in the soil is available for seedlings at the time of their growth and establishment.

94. *Anthemis cupaniana* Tod. ex Nyman. (*Asteraceae*) (Fig. 2d)

Accession data

Si: Monreale (Palermo), Serra del Frassino (WGS84: 37.99833°N, 13.25041°E), calcareous rocky slopes, 1,060 m a.s.l., 22 Jul 2020, *F. Carruggio*, *M. Castrogiovanni*, *R. Galesi* & *C. Impelluso* (SiMaSeed/CT/20/684, BGS-CT).

Germination data

Pre-treatments: 2-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T _i [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 10°C	12/12 h	5.0	6.6	11.0	7.5
95%	constant 15°C	12/12 h	4.0	5.7	17.0	6.9
90%	constant 20°C	12/12 h	3.0	4.8	16.0	6.5
84%	constant 25°C	12/12 h	4.0	6.0	14.0	7.0

Observations

Anthemis cupaniana is a Sicilian endemic species, which occurs on the calcareous and dolomitic mountains of Central-Western Sicily from 500 to 1,600 m of altitude (Domina & al. 2015; Oberprieler & al. 2022a). It is a tetraploid species, probably originated by the union of two diploid species within the *A. pedunculata* group, from Northern Africa, and the *A. cretica* group, from Southern Europe and Southern-Western Asia (Oberprieler & al. 2022a). According to the IUCN criteria, *A. cupaniana* has been recognized as Near Threatened (NT) (Orsenigo & al. 2018).

The highest and fastest germination for seeds exposed to after-ripening for two months was recorded at temperatures ranging from 10 to 25°C, in the presence of light. The optimal conditions for germination were similar to those detected for *A. aetnensis*, a species close to *A. cupaniana* for both ecology and phylogeny.

Germination in darkness is lower under all the tested conditions (although 70% germination percentage was reached in the 10-20°C range). No germination was recorded in seeds from dark cypselas under all the conditions tested.

95. *Anthemis maritima* L. subsp. *maritima* (Asteraceae) (Fig. 2e)

Accession data

Si: Balestrate (Palermo), spiaggia Balestrate (WGS84: 38.04406°N, 12.988917°E), coastal dune, 3 m a.s.l., 21 Jul 2020, *F. Carruggio*, *M. Castrogiovanni* & *R. Galesi* (SiMaSeed/CT/20/673, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T _i [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	2.0	4.0	15.0	5.0
98%	constant 20°C	0/24h	-	-	-	-
97%	constant 15°C	0/24h	-	-	-	-
94%	constant 10°C	0/24h	-	-	-	-
94%	constant 25°C	0/24h	-	-	-	-
94%	constant 15°C	12/12 h	3.0	4.7	15.0	5.8
93%	constant 10°C	12/12 h	5.0	7.3	17.0	8.6
93%	constant 25°C	12/12 h	2.0	3.3	12.0	4.0
91%	constant 5°C	0/24h	-	-	-	-
80%	constant 30°C	12/12 h	2.0	3.2	22.0	5.0

Observations

Anthemis maritima is a perennial species, mainly distributed in the Western Mediterranean region and growing on sand dunes, which it contributes to build up (De Lillis & al. 2004).

Germination data for *A. maritima* subsp. *maritima* were already published by Porceddu & al. (2019), who reported a final germination percentage above 80% only for seeds incubated at 20°C in the presence of light.

The best germination for our accession was achieved after a three-month after-ripening period at temperatures ranging from 10 to 30°C in the presence of light and from 5 to 25°C in darkness. After a further six months, seeds incubated in the absence of light were able to germinate to high percentages even at 30°C. Seeds from dark cypsels did not exceed 50% germination under any temperature and light conditions after a two-month after-ripening period.

The lack of photoinhibition in this psammophilous species contradicts the general pattern known for plants with similar habitat preferences (Carta & al. 2017). Photoinhibition in sandy coastal habitats avoids seedling establishment in the proximity of soil surface where the risk of desiccation and mechanical damage from shifting sand is higher. However, *A. maritima* subsp. *maritima* is not the only psammophilous species indifferent to light. A similar germination behaviour has been reported also for *Dianthus morisianus* from Sardinia (Cogoni & al. 2012) and for other sand dune species occurring in Sicily (Salmeri & Trubia 2019).

96. *Anthemis parlatoresana* Raimondo, Bajona, Spadaro & Di Gristina (*Asteraceae*) (Fig. 2f)

Accession data

Si: Castellammare del Golfo (Trapani), M.te Inici (WGS84: 38.01929°N, 12.870433°E), rocky outcrops, 277 m a.s.l., 21 Jul 2020, *F. Carruggio*, *M. Castrogiovanni* & *R. Galesi* (SiMaSeed/CT/20/672, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	2.0	1.71	5.0	2.3
99%	constant 3°C	12/12 h	12.0	15.4	21.0	16.0
99%	constant 5°C	12/12 h	9.0	9.7	14.0	10.4
99%	constant 10°C	12/12 h	4.0	4.0	8.0	4.6
99%	constant 15°C	12/12 h	2.0	2.53	5.0	3.1
98%	constant 25°C	12/12 h	1.0	1.62	6.0	2.2
98%	constant 20°C	0/24h	-	-	-	-
94%	constant 10°C	0/24h	-	-	-	-
94%	constant 15°C	0/24h	-	-	-	-
88%	constant 5°C	0/24h	-	-	-	-

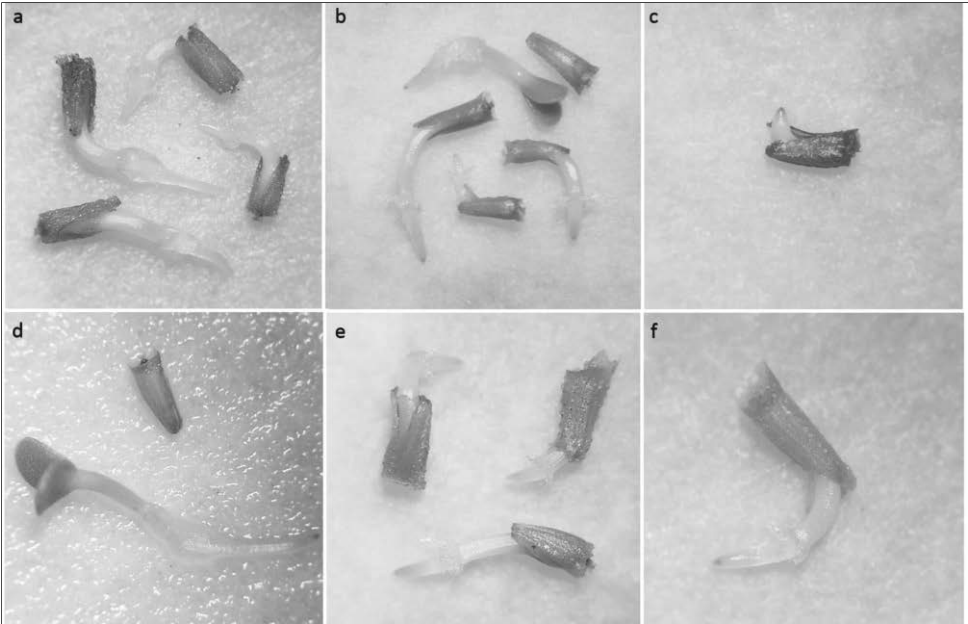


Fig. 2. Germinated seeds and seedlings: a) *Anthemis aeolica*; b) *A. aetnensis*; c) *A. cretica* subsp. *messanensis*; d) *A. cupaniana*; e) *A. maritima* subsp. *maritima*; f) *A. parlatoreana*.

Observations

Anthemis parlatoresana has been recently described as a new plant species by Raimondo & al. (2021). It is related to *A. pignattiorum* and *A. ismelia*, with which it shares similar ecological requirements, and to *A. cupaniana*, from which differs in having more thermophilous adaptive traits. *A. parlatoresana* is currently known only for Mount Inici, a limestone coastal relief in the province of Trapani (NW Sicily). It grows on rocky outcrops and screes, on N-NE facing slopes, at an altitude between 250 and 750 m. This report presents the first germination data for this species.

After 50 days of collection, seeds of *A. parlatoresana* achieved high germination percentages at temperatures ranging from 3 to 15°C, in the presence of light, and within a slightly narrower range in darkness (5-15°C). Seeds tested after three months of after-ripening expanded the thermal range for germination, reaching almost 100% even at 20 and 25°C in the presence of light and at 20°C in darkness.

Our results demonstrate that seeds of *A. parlatoresana* exhibit a non-deep physiological dormancy, with germination being regulated by conditional dormancy, quite similarly to *A. messanensis* and *A. pignattiorum* (Soltani & al. 2017; Carruggio & al. 2021). The ability to germinate at low-temperature values and the partial or full inhibition at higher ones (> 25°C) confirm a pattern that has been highlighted in other lowland Mediterranean species (Picciau & al. 2019).

As in the other study species, the dark cypselas are almost unable to germinate under all the conditions tested.

97. *Anthemis pignattiorum* Guarino, Raimondo & Domina (*Asteraceae*)

Accession data

Si: Avola (Siracusa), Cava Grande del Cassibile (WGS84: 36.96803°N, 15.093833°E), calcarenite cliffs, 461 m a.s.l., 09 Jun 2020, *F. Carruggio, M. Castrogiovanni & R. Galesi* (SiMaSeed/CT/20/641, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at $22 \pm 2^\circ\text{C}$ and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 40 seeds (20 × 2 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 10°C	12/12 h	5.0	6.1	9.0	6.9
100%	constant 15°C	0/24h	-	-	-	-
90%	constant 20°C	12/12 h	4.0	5.8	15.0	7.0
89%	constant 5°C	12/12 h	8.0	9.9	24.0	11.8
89%	constant 10°C	0/24h	-	-	-	-

85%	constant 15°C	12/12 h	3.0	4.9	9.0	5.6
85%	constant 30°C	12/12 h	6.0	14.9	30.0	14.9
85%	constant 20°C	0/24h	-	-	-	-
80%	constant 25°C	12/12 h	4.0	14.5	29.0	16.5

Observations

Anthemis pignattiorum is an endemic species to Hyblaean Mountains (SE Sicily). It has been recorded only at Cava Grande del Cassibile (Siracusa), where it grows on vertical calcarenite cliffs with northern exposure (Guarino & al. 2013). *A. pignattiorum* has been assessed as Endangered (EN), according to the IUCN criteria (Orsenigo & al. 2018). The germination trials were performed with only two replicates of 20 seeds each, due to the poor availability of viable seeds. Indeed, *A. pignattiorum* is a very rare species and grows on vertical and unreachable sites. In addition, the high relevance of pre-dispersal seed loss due to the incidence of larval infestation limits the number of available seeds. However, the data reported are the first germination records for this species.

Fresh seeds of *A. pignattiorum* (20 days) proved high germination capability over a narrow range of temperatures (5–15°C) in light conditions. The thermal range for germination enlarged with after-ripening, reaching an upper limit of 30°C after 90 days from collection. Such as in *A. parlatoreana* and *A. messanensis*, germination appeared regulated by conditional dormancy (Soltani & al. 2017).

Dark cypselas failed to achieve high germination percentages under all the conditions tested.

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Cristian Brullo & Cristina Salmeri

Seed germination reports for five species of the genus *Silene* (*Caryophyllaceae*)

Abstract

Brullo, C. & Salmeri, C.: Seed germination reports for five species of the genus *Silene* (*Caryophyllaceae*) [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 220-226. 2022. <https://doi.org/10.7320/FlMedit32.220>

In this contribution, new germination data for five *Silene* taxa coming from the natural habitats of Sicily and Calabria are given. The investigated taxa are: the threatened endemic *S. calabra* and *S. crassiuscula*, together with *S. colorata*, *S. fruticosa*, and *S. nicaeensis* from Sicilian sites. Seed germinations were tested at the Catania Germplasm Bank (BGS-CT), at constant temperatures (from 5 to 30°C), under both light (12/12h photoperiod) and total darkness conditions. The results emphasized a high germination ability and rate in all examined species.

Key words: Calabria, endemic, Italian flora, threatened plants, Sicily.

Introduction

Silene L. is a genus of *Caryophyllaceae* including about 700 species and may be considered one of the largest genera of this family worldwide (Morton 2005; Iamónico 2018). The Mediterranean basin and SW-Asia are considered its main centres of species diversity (Greuter 1995; Oxelman & Lidén 1995). According to Pignatti (2017), the total number of *Silene* taxa occurring in Italy is 86, many of which are endemic (ca. 35%).

In this contribution, the germination protocols of five different species of *Silene* were investigated, two of which are Italian endemics, both included in the Italian Red List of threatened vascular plants (Rossi & al. 2020). These are *S. calabra* Brullo, Scelsi & Spamp., endemic to SW Calabria, and *S. crassiuscula* Brullo, C. Brullo, Cambria, Bacch., Giusso & Ilardi, endemic to NW Sicily, while the other studied taxa are represented by Sicilian populations of *S. colorata* Poir., a widespread Mediterranean therophyte, *S. fruticosa* L., growing in rupestrian sites of the Central and Eastern Mediterranean area, and *S. nicaeensis* All., a quite rare CW Mediterranean psammophyte.

The germination tests were carried out at the Catania Germplasm Bank (BGS-CT), using different constant temperatures (from 5 to 30°C), under both light (12/12h photoperiod) and full darkness conditions. First germination reports are provided for the two endemic species, as well as for the remaining taxa in the Sicilian territory.

98. *Silene calabra* Brullo, Scelsi & Spamp. (*Caryophyllaceae*)

Accession data

It: Calabria, Pentidattilo, (WGS84: 37.946472°N, 15.758747°E), conglomerate rocks, ca. 130 m a.s.l., 10 Sep 2004, *A. Crisafulli & F. Rossello* (123AC/FR/04, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: no treatment

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water

Sample size: 60 seeds for each test (15 × 4 replicates)

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
98%	constant 15°C	0/24h	-	-	-	-
97%	constant 15°C	12/12h	2	5.2	7	4.4

Observations

Silene calabra is a rupestrian species endemic to the Ionian slope of Mt. Aspromonte (SW Calabria), where it grows in conglomerate, calcarenite and limestone cliff habitats at 100-800 m a.s.l. (Brullo & al. 1997). Seeds of *S. calabra* showed successful germination at the constant temperature of 15°C, both in light and full dark conditions.

Due to the poor number of available seeds, no other germination conditions could be tested so far, though other optimal thermal ranges (mainly 10-25°C) are expectable also for this species, similarly to other taxa of *Silene* characteristics of dry climates (GENMEDOC 2006; Murru & al. 2017; Zani & Müller 2017; Magrini & al. 2019; Carruggio & al. 2021).

99. *Silene colorata* Poir. (*Caryophyllaceae*)

Accession data

Si: Siracusa, Priolo Gargallo, Saline di Priolo (WGS84: 37.142500°N, 15.219444°E), maritime sand dunes, 4 m a.s.l., 27 Mar 2017, *G. Giusso & S. Sciandrello* (UNICT_340, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: no treatment

Germination medium: 1% agar

Sample size: 60 seeds for each test (20 × 3 replicates)

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
90%	constant 15°C	12/12h	2	2.3	5	3.7
88%	constant 10°C	0/24h	-	-	-	-
85%	constant 25°C	12/12h	1	0.6	7	1.2
85%	constant 25°C	0/24h	-	-	-	-
83%	constant 20°C	0/24h	-	-	-	-
80%	constant 10°C	12/12h	5	3.5	7	5.0

Observations

Silene colorata is a psammophilous therophyte that grows on the sandy coast of the Mediterranean basin. This is the first germination report for the Sicilian territory. Seeds of *S. colorata* showed the best germination rate (90%) at 15°C under light conditions; germination values $\geq 80\%$ also occurred at 10°C and 25°C both in light-dark photoperiod (80% and 85%, respectively) and full darkness (88% and 85%, respectively). Seed germination was somehow negatively affected by light at 10°C (80% at 12/12h vs 88% at 0/24h) and 20°C (72% at 12/12h vs 83% at 0/24h) or light insensitive at 25°C. Conversely, light exposure showed positive effects at 15°C, allowing the highest germination value, while just 72% of germinated seeds were obtained under full darkness.

Reports from Royal Botanic Gardens Kew (2022) partly agreed with our results (90% at 15°C and 69% at 20°C with 12/12h photoperiod), though seed germination at 10°C in light conditions was lower (56%); on the other hand, 100% of germinated seeds were reported at 16°C and 21°C (12/12 photoperiod) and 95-100% under 8/16 photoperiod at 15°C and 20-25°C, respectively. Fully contrasting results were provided by Del Vecchio & al. (2021) for one Venetian population, which achieved very low germination values, ranging from 0% at 5°C under light to max. 36% at 20°C in full darkness, with light negatively affecting seed germination at all temperatures, except 25°C.

100. *Silene crassiuscula* Brullo, C.Brullo, Cambria, Bacch., Giusso & Ilardi (*Caryophyllaceae*)

Accession data

Si: Palermo, Sferracavallo (WGS84: 38.211111°N, 13.281388°E), rocky coasts, 3 m a.s.l., 11 May 2012, *V. Ilardi* (UNICT_113, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: no treatment

Germination medium: 1% agar

Sample size: 60 seeds for each test (20 × 3 replicates)

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 10°C	0/24h	-	-	-	-
98%	constant 25°C	12/12h	1	0.6	4	1.2
97%	constant 10°C	12/12h	2	2.7	12	3.6
92%	constant 15°C	12/12h	2	1.9	3	2.4
90%	constant 20°C	12/12h	1	0.6	3	1.7
90%	constant 30°C	12/12h	1	0.7	4	1.5
88%	constant 5°C	0/24h	-	-	-	-
85%	constant 15°C	0/24h	-	-	-	-
85%	constant 25°C	0/24h	-	-	-	-

Observations

Silene crassiuscula is a succulent therophyte endemic to the rocky coasts of NW Sicily, included in the Italian Red List under the NT IUCN category (Rossi & al. 2020). This is the first germination report for the species. The maximum germination rate (100%) was obtained at 10°C in full dark conditions. However, seeds of *S. crassiuscula* showed high germination values (100-85%) in a wide thermal range, from 5°C to 30°C. Light exposure did not particularly affect seed germination, which overall was > 80% both in the light and total dark conditions; some photoinhibition effect occurred at 5°C with a germination decline to 67%.

101. *Silene fruticosa* L. (Caryophyllaceae)

Accession data

Si: Messina, Taormina (WGS84: 37.853888°N, 15.283888°E), limestone cliffs, 337 m a.s.l., 21 June 2012, C. Brullo, S. Brullo, G. Giusso & S. Sciandrello (UNICT_023, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: no treatment

Germination medium: 1% agar

Sample size: 60 seeds for each test (20 × 3 replicates)

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
100%	constant 10°C	12/12h	6	6.4	10	6.9
100%	constant 15°C	12/12h	2	2,9	5	3.4

100%	constant 20°C	12/12h	2	2.1	5	2.8
100%	constant 25°C	0/24h	-	-	-	-
98%	constant 25°C	12/12h	2	2.0	10	3.5
95%	constant 15°C	0/24h	-	-	-	-
93%	constant 10°C	0/24h	-	-	-	-
90%	constant 20°C	0/24h	-	-	-	-

Observations

Silene fruticosa is a casmophilous chamaephyte that grows in the calcareous cliffs of Sicily, Malta, Greece, Cyprus, Libya, and Egypt. Seeds of *S. fruticosa* showed high germination rates ($\geq 90\%$) in a wide thermal range (10-25°C). Full germination was achieved at 10°C, 15°C and 20°C under light conditions, while it slightly decreased (90-95%) in total darkness. Seed germination was affected by low temperature, since at 5°C it decreased up to 62%, with 12/12h light-dark photoperiod, while only 5% of germination was reached under total dark conditions, thus revealing a major light stimulation in cold conditions. Conversely, at 25°C full dark conditions allowed 100% of seed germination, which remained slightly lower (98%) under light photoperiod. Such effects of light and temperature on the germination behaviour of *S. fruticosa* somehow agree with the results of Walter & al. (2020). Very similar germination rates were also reported by Royal Botanic Gardens Kew (2022), which indicated 100% and 96% of germination at 15°C and 25°C, respectively, under a 12/12h light-dark photoperiod.

102. *Silene nicaeensis* All. (Caryophyllaceae)

Accession data

Si: Caltanissetta, Gela, Manfria (WGS84: 37.098055°N, 14.142500°E), maritime sandy dunes, 0 m a.s.l., 24 May 2012, *C. Brullo, S. Brullo, M. Patanè & D. Torrìsi* (UNICT_005, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: no treatment.

Germination medium: 1% agar.

Sample size: 60 seeds for each test (20 × 3 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T1 [d]	T50 [d]	Tmax [d]	MTG [d]
100%	constant 25°C	12/12h	1	0.6	3	1.1
100%	constant 25°C	0/24h	-	-	-	-
92%	constant 10°C	0/24h	-	-	-	-

90%	constant 15°C	12/12h	2	3.5	10	4.1
90%	constant 20°C	12/12h	1	1.0	10	1.8
88%	constant 15°C	0/24h	-	-	-	-

Observations

Silene nicaeensis is a psammophilous hemicryptophyte that grows on the sandy coasts mainly of the Central-Western Mediterranean area. Plants of the investigated southmost Sicilian population show a perennial life cycle, which is quite common in the North African plants (*S. nicaeensis* var. *perennis* Maire). Seeds revealed high germination values (88-100%) in the thermal range from 15°C to 25°C, both in the light (12/12h photoperiod) and total darkness, except for 20°C in dark conditions which provided a germination percentage < 80%. The maximum germination rate (100%) resulted at 25°C. Seeds showed marked photosensitivity at 10°C, as germination reached 92% in total darkness, while it dropped down to just 8%, under a 12/12h photoperiod. Moreover, a marked decline in germination was recorded at 30°C, both in light and dark conditions, revealing the occurrence of thermo-inhibition in this population.

Royal Botanic Gardens Kew (2022) reported for this species (not specified provenances) very fluctuating germination data, ranging from 100% to 56%, with the highest responses at 16°C and 26°C (with 12/12h photoperiod), and at 20°C and 25°C (with 8/16h photoperiod), while, the lowest germination rate occurred at 10°C, similarly to our results.

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Seed germination of the endemic *Armeria helodes* (*Plumbaginaceae*) in Italy

Abstract

Vuerich, M., Pasquini, S., Casolo, V., Boscutti, F. & Petrusa, E.: Seed germination of the endemic *Armeria helodes* (*Plumbaginaceae*) in Italy [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 4]. Fl. Medit. 32: 227-232. 2022. <https://doi.org/10.7320/FIMedit32.227>

Armeria helodes is a narrow endemic species of Friuli Venezia Giulia (Italy), with a restricted and fragmented range of small populations in remnants of lowland alkaline fens (habitat present in Annex I of the EU Habitats Directive). It is listed as Critically Endangered in the IUCN Red List, nevertheless, best practices for germplasm conservation/germination are still not studied. At maturity, achenes are held inside chaffy bracts (calyx) and their effects on germination are unknown. Therefore, we performed a germination test comparison between fruits embedded in the calyx or free from it, also applying two dormancy-breaking pretreatments (i.e. KNO₃, GA3). Approximately 26% of the inspected fruits enclosed inside the calyx were empty or had a small size and coat darkening. The optimal germination rate (60%) was reached when chaffy calyx was removed either before the test or during storage. Dormancy-breaking pretreatments did not increase the germination capacity. Our results represent the first report on the Italian *A. helodes* germination ability.

Key words: endangered species, alkaline fens, germplasm conservation, Friuli Venezia Giulia.

Introduction

Armeria helodes F.Martini & Poldini (*Plumbaginaceae*) is a geographically and ecologically isolated neo-endemic entity of the Italian peninsula, as it is exclusively found in a small fragmented range, in few populations located in the low plain of Friuli Venezia Giulia, growing in wetland habitats characterized by fresh marshy soil (Martini & Poldini 1987; Martini 1987; Conti & al. 2005; Arrigoni 2015; Bartolucci & al. 2018). Although all populations are located in protected areas, the number of mature individuals is declining because of soil drainage and nutrients enrichments due to surrounding agricultural activities. Therefore, *A. helodes* was recently listed as Critically Endangered on the national Red List (Foggi & Abeli 2010; Rossi & al. 2015). It is also included in Annex II of the Habitats

Directive (Council Directive 92/43/EEC) as a plant of community interest whose conservation requires the designation of Special Areas of Conservation (SAC). For successful *ex situ* conservation and restoration actions, detailed knowledge about the seed germination ability of this endangered fen species is of utmost importance. An appropriate germplasm collection in *Armeria* species is limited by the low seed production (from 3 to 50% of seeds collected from the calyxes analyzed), due to non-fertilized ovaries because of poor pollen provision (Baker & al. 1994; Tauleigne-Gomes & Lefèvre 2005; Mouga & al. 2021). Seed germination of *A. helodes* is also expected to undertake a “warm germination strategy” (Thompson & Grime 1983) and to possess non-deep physiological dormancy (Baskin & Baskin 2014), as reported for other species from base-rich fens (Fernández-Pascual 2016).

Materials and methods

Accession data

Armeria helodes F. Martini & Poldini (*Plumbaginaceae*)

It: Friuli Venezia Giulia. Talmassons (Udine), Risorgive di Flambro (WGS84: 45.917902N°, 13.080120°E) in the protected area Risorgive dello Stella (SAC: IT 3320026), alkaline fens, 21-22 m a.s.l., 30 Jun 2021, *M. Tomasella* (accession id AE_F_2021_1, ITA368 Banca del Germoplasma Autoctono Vegetale del F.V.G. (BaGAV) Germplasm Bank).

Fruits were collected in June 2021 from natural populations in the protected regional area of Risorgive di Flambro. The collected mature dry fruits of *A. helodes* (achenes held inside their papery chaff) were separated into two different sub-groups, one left inside and the other removed from the calyx. The test aimed at understanding whether germination in *A. helodes* could be influenced by the presence of the covering structure, as reported in the Seed Information Database (Royal Botanic Gardens Kew 2022) for *A. alpina* (DC.) Willd. During calyx removal operation, visual inspection has allowed us to determine a large proportion of dead diaspores (50% on average), as most of the calyxes were empty or presented achenes with distinguishable morphological alterations as small size or a dark-brown coat (Fig. 1). The abnormal fruits in this sub-group were then discarded since they could affect germination results. The two distinct sub-groups were then stored at 2°C at the BaGAV Germplasm Bank of Region Friuli Venezia Giulia for 5 months until the beginning of the germination test.

Germination test

Seven different trials on the accession germination parameters were performed:

- 1) achenes stored for 5 months within the calyx,
- 2) achenes as the latter, with the calyx removed just before the germination test,
- 3) achenes stored for 5 months without calyx,
- 4) achenes stored for 5 months without calyx, pre-treated with 1,000 ppm GA3 for 24h,

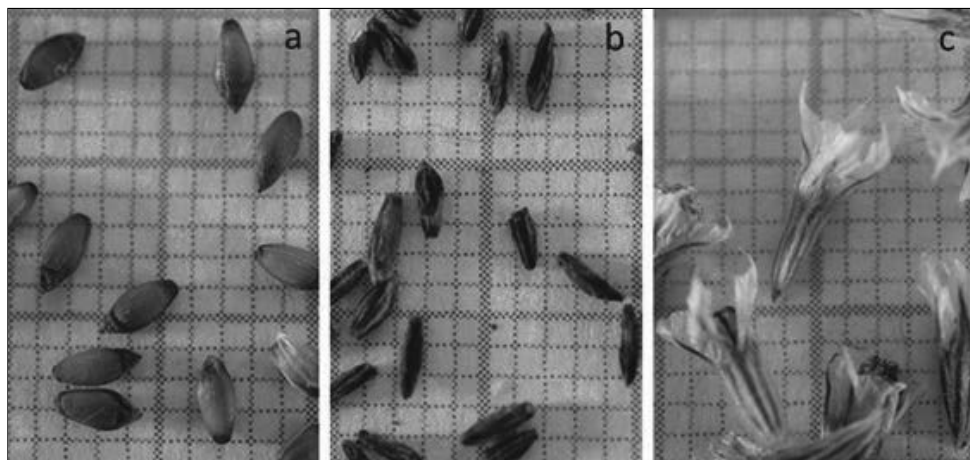


Fig. 1. Fruits of *Armeria helodes*: **a)** normal indehiscent single-seeded achenes; **b)** brown small-sized abnormal achenes not included in the germination test; **c)** chaffy calyx holding single-seeded achene. Pictures on 1 mm graph paper (M. Vuerich).

- 5) achenes stored for 5 months without calyx, pre-treated with 0.2% KNO₃ for 24 h,
- 6) calyxes,
- 7) achenes stored for 5 months without calyx, both pre-treated with 2% KNO₃ for 24 h.

Pre-treatment: sterilization with a solution of 5% sodium hypochlorite for 10 minutes followed by 3 rinses in sterile ultrapure water.

Germination data: germination tests were carried out in parallel at the Department of Agricultural Food, Animal and Environmental Sciences, University of Udine and at the Centro Nazionale Carabinieri Biodiversità (CNCB – Peri, Verona) with an alternating temperature regime of 25/15°C and 12/12h of light/dark photoperiod for the first five trials and 20/30°C and 8/16h light/dark for the latter.

Germination medium: 0.8% agar (University of Udine) and sterile Whatman paper (CNCB – Peri) imbibed with 5 ml of sterile ultrapure water.

Sample size: 50 achenes or calyxes for each test (10 × 5 replicates) (University of Udine) and 100 achenes or calyxes (25 × 4 replicates) (CNCB – Peri).

Results

Here we report the first published germination reports for *A. helodes* in Italy (Table 1). Despite this endemic species has been already the object of conservation and some effective restoration actions by the LIFE project “FRIULI FENS” (LIFE06

NAT/IT/000060) carried out in the Special Areas of Conservation (SAC) present in the lower plan of Friuli Venezia Giulia (Mosanghini & al. 2022), no published reports on optimal germination potential of *A. helodes* are available to the best of our knowledge.

The results showed that the removal of the calyx was successful to increase the germination percentage in *A. helodes*, reaching 60 and 58% in fruits removed from the calyx before sowing or storage, respectively. Regarding the germination speed, the elimination of calyx before fruit storage or just before the germination test did not lead to major differences ($T_1 = 1.4$ days for both, $T_{50} = 2.50$ and 2.58 days, and $T_{max} = 5.40$ and 4 days, respectively). In contrast, the covering of chaffy structure negatively affected the final germination percentage (8.0%), with $T_1 = 12.5$ days, $T_{50} = 13$ days and $T_{max} = 14$ days, also due to a limited number of normal achenes found, suitable for reproducibility of germination test and restoration action occurring in the whole accession. It was observed that 26.0 ± 13.4 % of non-germinating fruits contained in the calyx were empty or non-fertilized ovaries and that the percentage of the viability of the total analyzed calyxes (germinated and alive non-germinated fruits inside calyxes) reached only 34.0 ± 13.4 %.

Table 1. Results of the germination tests of *Armeria helodes* with calyx removal and different pre-treatments.

University of Udine								
Germination	Chaffy calyx removal	Pre-treatment	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
60.0%	yes, before test	no	15/25°C	12/12h	1.4	2.50	5.4	3.07
58.0%	yes, before storage	no	15/25°C	12/12h	1.4	2.58	4.0	2.95
42.0%	yes, before storage	1000 ppm GA3	15/25°C	12/12h	2.0	2.73	5.2	3.40
36.0%	yes, before storage	0.2% KNO ₃	15/25°C	12/12h	1.8	2.34	3.8	2.88
8.0%	no	no	15/25°C	12/12h	12.5	13.00	14.0	13.33
CNCB – Peri								
Germination	Chaffy calyx removal	Pre-treatment	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
60.0%	yes, before storage	2% KNO ₃	20/30°C	8/16h	1.5	2.4	-	3.1
3.0%	no	2% KNO ₃	20/30°C	8/16h	14.0	-	14.0	14.0

Discussion

Regarding the influence of temperature regimes, *A. helodes* exhibited comparable seed germination traits under the two conditions tested in the different laboratories. This feature might suggest for *A. helodes* a “warm germination strategy” (Thompson & Grime 1983) already described for species of lowland alkaline fen grasslands, which germinate at warmer temperatures in autumn and subsequently acquire the ability to germinate towards colder temperatures in the short or long term (Fernández-Pascual & al. 2013; Fernández-Pascual 2016).

When comparing germination ability in the more closely related species, such as *A. alpina* (Martini & Poldini 1987; Pignatti & al. 2017), it could be observed that percentages ranged from 59 to 80% in light 8/16 at 15°C for *A. alpina*, where the removal of the covering structure from fruits increased the germination rate (Royal Botanic Gardens Kew 2022). For *A. maritima* and *A. alpina*, a dormancy-breaking pre-treatment with 2,000 ppm KNO₃ (Ellis & al. 1985) or 250 ppm GA3 (Mondoni & al. 2011) were also recommended. However, in our trial, fruit or calyx priming in 2% KNO₃ did not change the germination rate, whereas 1,000 ppm of GA3 or 0.2% KNO₃ even lowered the germination rate to 42 and 36%, respectively, suggesting that *A. helodes* seeds are not physiologically dormant.

The large proportion of dead or aborted seeds per total fruits could be ascribed to a specific reproductive vulnerability of *A. helodes* to unfavourable climatic (drought episodes or heat waves during the second half of June, Meteo FVG Report 2021) or habitat conditions during either pollination or seed ripening. This feature, associated with an intermediate level of germination capacity of seeds, could restrain a successful restoration program for this species.

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Mediterranean plant karyological data – 32

edited by G. Kamari, C. Blanché & S. Siljak-Yakovlev

Abstract

Kamari, G., Blanché, C. & Siljak-Yakovlev, S. (eds): Mediterranean plant karyological data – 32. — Fl. Medit. 32: 233-246, 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This is the thirty-two of a series of karyological data from Mediterranean area, peri-Alpine communities and the Atlantic Islands, in English or French language. It comprises contributions on 6 taxa: *Iris* and *Delphinium* from Greece by Kriemadi, E. & Bareka, P. (Nos 2008-2009); *Centaurea* from Kosovo and Albania by S. Siljak-Yakovlev (Nos 2010-2011); *Centaurea* from Sicily by Barone, G., Gianguzzi, V., Cottone, C. & Di Gristina, E. (Nos 2012-2013).

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Eleni Kriemadi & Pepy Bareka

Karyological data of some Greek taxa

Abstract

Kriemadi, E. & Bareka, P. 2022: Karyological data of some Greek taxa [In Kamari, G., Blanché, C. & Siljak-Yakovlev, S. (eds), Mediterranean plant karyological data-32]. – Fl. Medit. 32: 234-237. <http://dx.doi.org/10.7320/FIMedit32.234>

Chromosome numbers and karyotypes are given for 2 taxa occurring in Greece. Karyotype microphotographs are provided, and their karyotype morphology is discussed. For both studied taxa, *Iris attica* and *Delphinium hellenicum*, the somatic chromosome number $2n = 16$ has been found for all the populations examined.

Keywords: Chromosome number, distribution, endemism, Greece, karyomorphology.

Introduction

In the context of updating the PhytoKaryon database (Kamari & al. 2017-onwards), which aims to record the chromosomal diversity of the plants of Greece, populations of two native species were studied. For both *Iris attica* (a Balcan endemic) and *Delphinium hellenicum* (a Greek endemic) $2n = 16$ chromosomes are reported, while the karyotype morphology is in accordance with previous references.

2008. *Iris attica* Boiss. & Heldr. — $2n = 2x = 16$ (Figs 1A, 1B & 1C).

- Gr:** Sterea Hellas, Nomos Attikis, Mt. Parnitha, Aeras summit, near the Sannatorium, alt. 1019 m., 38° 08' 57" N, 23° 43' 16" E, 3 Apr 2021, leg. E. Kriemadi E15 (AUA). – Purple form, $2n = 16$. – Fig. 1A.
- Sterea Hellas, Nomos Attikis, Mt. Parnitha, Aeras summit, near the Sannatorium, alt. 1019 m., 38° 08' 57" N, 23° 43' 16" E, 3 Apr 2021, leg. E. Kriemadi E16 (AUA). – Yellow form, $2n = 16$. – Fig. 1B.
- Sterea Hellas, Nomos Attikis, Mt. Beletsi, alt. 580 m., 38° 13' 08" N, 23° 49' 32" E, 3 Apr 2021, leg. E. Kriemadi E17 (AUA). – Yellow form, $2n = 16$. – Fig. 1C.

Iris attica is a Balkan endemic species growing in rocky limestone substrate at low and moderate altitude. Until recently the synonym *Iris pumila* subsp. *attica* (Boiss. & Heldr.) K.

Richt. was used, however according to the latest typification of some species of the genus (Boltenkov & al. 2021) the name *Iris attica* was re-established.

The chromosome number of $2n = 16$, found here for both yellow and purple forms, is in accordance with previous studies by Simonet (1932), Mitra (1956), Randolph & Mitra (1959), Lovka & al. (1971), Sauer & Stegmeier (1979), Sauer & Leep (1979) and van Loon & Oudemans (1981). The karyotype consists of one metacentric to sub-metacentric chromosome pair (m-sm), while the rest are acrocentric to subtelocentric (st-t). At least 2 chromosome pairs bear small spherical satellites at their short arms. Chromosome size ranges from 8.67 to 4.67 μm .

2009. *Delphinium hellenicum* Pawł. — $2n = 2x = 16$ (Fig. 1D).

Gr: Sterea Hellas, Nomos Attikis, Mt. Imittos, SE slopes, alt. 239 m., 37° 59' 39" N, 23° 50' 37" E, 15 Jul 2021, leg. E. Kriemadi E22 (AUA).

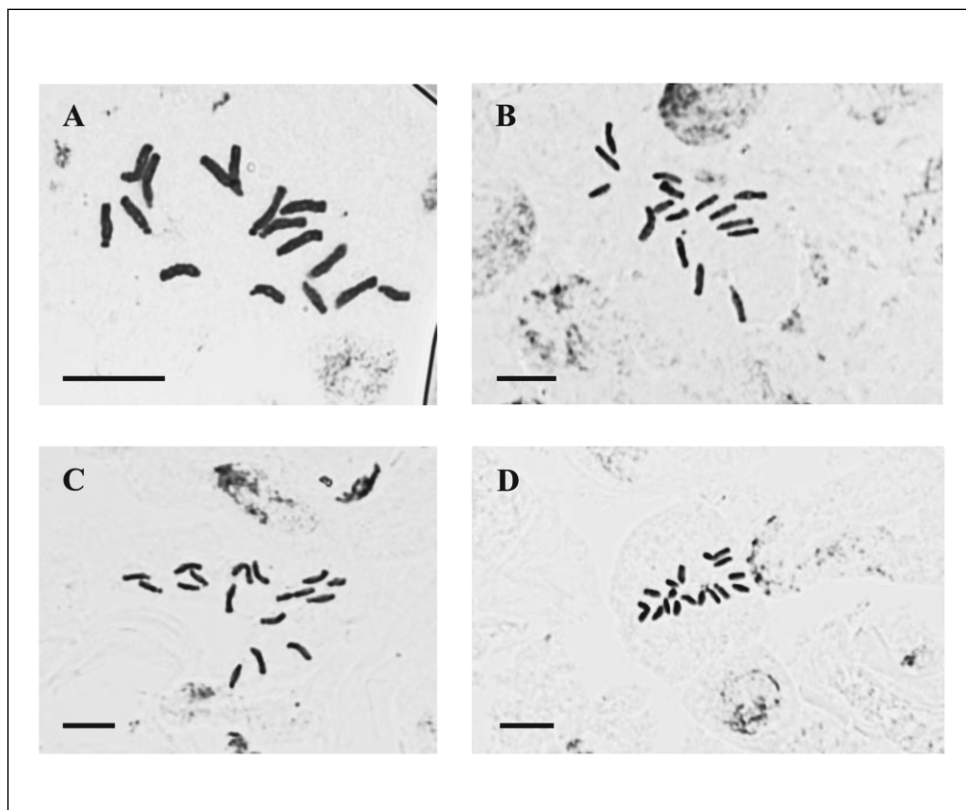


Fig. 1. Microphotographs of mitotic metaphase plates of: **A**, *Iris attica*, $2n = 16$ (E15); **B**, *I. attica*, $2n = 16$ (E16); **C**, *I. attica*, $2n = 16$ (E17); **D**, *Delphinium hellenicum*, $2n = 16$. – Scale bars = 10 μm .

Delphinium hellenicum is a Greek endemic species distributed in Peloponnisos, Sterea Hellas and the Ionian Islands. It is legally protected in Greece by a Presidential Decree (Pr. Decree 67/1981).

The chromosome number $2n = 2x = 16$ and the karyotype morphology in the population studied here from Sterea Hellas, agrees with that given by Kamari & al. (2003) in material from Peloponnisos. Additionally, the nuclear DNA amount has been estimated in a population from Kefallinia (Ionian Islands) (Siljak-Yakovlev & al. 2019). The 2C DNA value of the studied material was 9.92 pg (± 0.02) and according to the categories established by Leitch & al. (2005) it is characterized as medium-sized ($7 \leq 2C < 28$).

The karyotype consists of one pair of submetacentric chromosomes (sm), which is the largest in size, and 7 pairs of acrocentric to subtelocentric chromosomes (st-t). At least, two visible small, spherical satellites on the short arms of an acrocentric chromosome pair (st-SAT) are counted. Chromosome size varies from 5.72 to 2.15 μm .

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Sonja Siljak-Yakovlev

Karyological investigation of two Balkan endemic *Centaurea* species

Abstract

Siljak-Yakovlev, S. 2022: Karyological investigation of two Balkan endemic *Centaurea* species [In Kamari, G., Blanché, C. & Siljak-Yakovlev, S. (eds), Mediterranean plant karyological data-32]. – Fl. Medit. 32: 238-243. <http://dx.doi.org/10.7320/FIMedit32.238>

Chromosome numbers, karyotype feature and its numerical data are presented for two Balkan endemic *Centaurea* species, *C. kosaninii* and *C. melanocephala*. The somatic chromosomes number $2n = 22$ was found for both *Centaurea* species. However, the karyotypes showed the significant differences in size and morphology of chromosomes and in number of satellite chromosome pairs, two in *C. melanocephala* and three in *C. kosaninii*.

Keywords: *Centaurea kosaninii*, *Centaurea melanocephala*, chromosome number, karyotype feature, numerical data on karyotype.

Introduction

Genus *Centaurea* L. is one of the largest in the *Asteraceae* [subtribe *Centaureinae* (Cass.) Dumort., tribe *Cardueae* Cass.], and comprises about 250 annual, biannual and perennial species according to the most recent studies (Sussana & Garcia-Jacas 2007). It is widely distributed, mostly in the Eastern Hemisphere, with the diversification center in the Middle East. The genus is well represented in all the Mediterranean region and many species are knowing from the Balkan Peninsula.

Centaurea is the most complex genus with basic chromosome number ranging from $x = 7$ to $x = 16$ (Siljak-Yakovlev 1986; Garcia-Jacas & al. 1996) and at least four different pollen types (Wagenitz 1955). As in many plant groups, descending dysploidy and polyploidy were found to be the most important evolutionary mechanisms occurring in this genus (Siljak-Yakovlev 1986).

Two investigated species belong to *Acrocentron* (Cass.) DC. section for which only two basic chromosome numbers, $x = 10$ (more often occurring) and $x = 11$, are recorded.

The aim of this study was to verify and eventually confirm the chromosome number, and to characterize, for the first time, the karyotype of two *Centaurea* endemic species from the Balkans.

2010. *Centaurea kosaninii* Hayek — $2n = 2x = 22$; 2C DNA = 3.13 pg (Figs 1, 1a, 1b; Table 1).

Syn. = *Colymbada kosaninii* (Hayek) Holub in Folia Geobot. Phytotax. 7: 315 (1972)

Ko: Kosovo, Brezovica, ultramafite substrate, 22.08.1980, leg. Dr Čedomil Šilić.

The native range of *Centaurea kosaninii* is Western Balkan Peninsula (NE Albania and Kosovo). It grows mostly on serpentine, but also rarely, on limestone substrate and is considered as relict species (Stevanovic & al. 2003). The chromosome number of $2n = 22$ reported already by Siljak-Yakovlev (1981 & 1986) is now confirmed.

The karyotype of *C. kosaninii* is composed of seven metacentric (pairs 1, 2, 3, 4, 5, 8 & 11), three submetacentric (pairs 6, 7 & 10; two of which, pairs 6 & 10, bearing satellites on their short arms) and one subtelocentric chromosome pairs (9) also with satellites (Fig. 1, 1a & 1b). The karyotype formula was as follows: $2n = 14m + 6sm + 2st = 22$.

Numerical data concerning this karyotype are presented in the Table 1. Chromosome types were determined according to the usual recommendation and nomenclature of Levan & al. (1964). Karyotype features were evaluated using the following morphometric parameters: length of the long (l) and short (s) arm, arm ratio (r), total chromosome length (TL), the relative length of each chromosome pair (RL) following Levan & al. (1964), the ratio (R) between the longest and the shortest chromosome pair (Siljak-Yakovlev 1986) and the index of asymmetry, AsI %, following Arano and Saito (1980). Idiogram was constructed from the mean values obtained from at least five metaphase plates corresponding to five different individuals.

Table 1. Numerical data concerning the chromosome set of *Centaurea kosaninii*.

Chrom. pair	Long arm (LA) in μm	Short arm (SA) in μm	Total length (TL) in μm	Relative length	Rapport LA/SA	Centromeric index	Chromosome type
1	1.62 (± 0.02)*	1.48 (± 0.03)*	3.10	120.08	1.10	47.69	m
2	1.62 (± 0.04)	1.43 (± 0.02)	3.05	118.05	1.14	46.79	m
3	1.45 (± 0.05)	1.25 (± 0.03)	2.70	104.43	1.16	46.38	m
4	1.49 (± 0.03)	1.13 (± 0.02)	2.63	101.70	1.32	43.15	m
5	1.25 (± 0.06)	1.25 (± 0.06)	2.50	96.86	1.00	50.00	m
6	1.35 (± 0.03)	0.78 (± 0.03)	2.13	81.73	1.75	37.04	sm-SAT
7	1.33 (± 0.03)	0.75 (± 0.02)	2.08	80.52	1.77	36.09	sm
8	1.09 (± 0.03)	0.94 (± 0.08)	2.03	78.70	1.17	46.15	m
9	1.58 (± 0.02)	0.41 (± 0.12)	1.99	77.19	3.88	20.59	st-SAT
10	1.23 (± 0.02)	0.70 (± 0.09)	1.93	74.92	1.75	36.36	sm-SAT
11	0.94 (± 0.03)	0.76 (± 0.02)	1.70	65.84	1.23	44.83	m
* standard error		$\Sigma\text{TL}=51.68 \mu\text{m}$	R=1.82	IAs%=57.86			

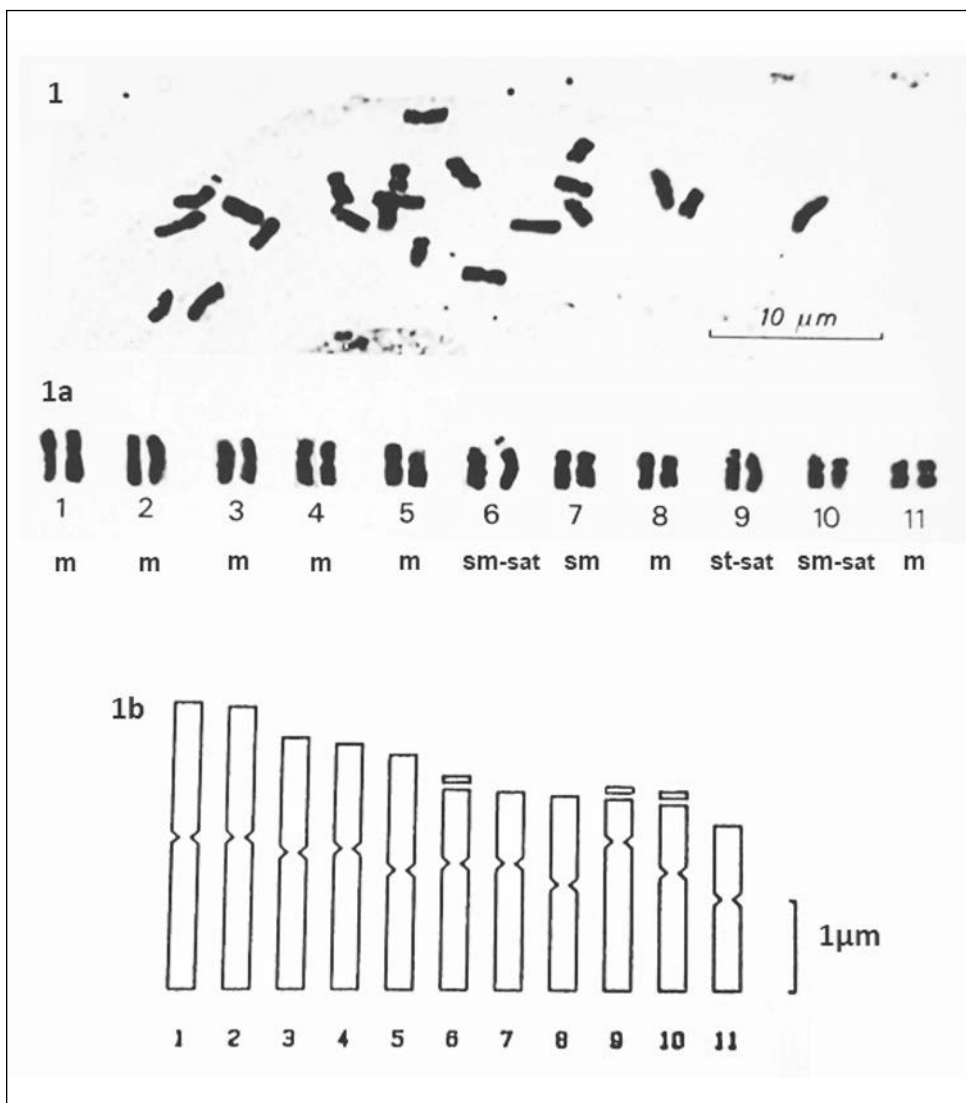


Fig. 1. Mitotic metaphase plate of *Centaurea kosaninii* (1), corresponding karyogram (1a) and idiogram (1b).

The chromosomes are small and total karyotype length (ΣTL) was 51.68 μm. The index of asymmetry was $IAS\% = 57.86$ and ratio (R) between the longest and the shortest chromosome pair was $R = 1.82$.

In addition, the genome size or nuclear DNA amount has been estimated for this species from the same population and 2C value was 3.13 pg or 3061 Mbp (Siljak-Yakovlev & al. 2010).

2011. *Centaurea melanocephala* Pančić — $2n = 2x = 22$ (Figs. 2, 2a, 3, 3a & 4; Table 2).

Al: Prov. Kukës: Kolsh, 24.07.2002, leg. *Tone Wraber*).

Centaurea melanocephala Pančić, Fl. Serbiae 443 (1874). This species presents two synonyms:

= *Centaurea candelabrum* Hayek & Košanin in Repert. Spec. Nov. Regni Veg. Beih. 30(2): 746 (1931)

= *Colymbada candelabrum* (Hayek & Košanin) Holub in Folia Geobot. Phytotax. 7: 315 (1972)

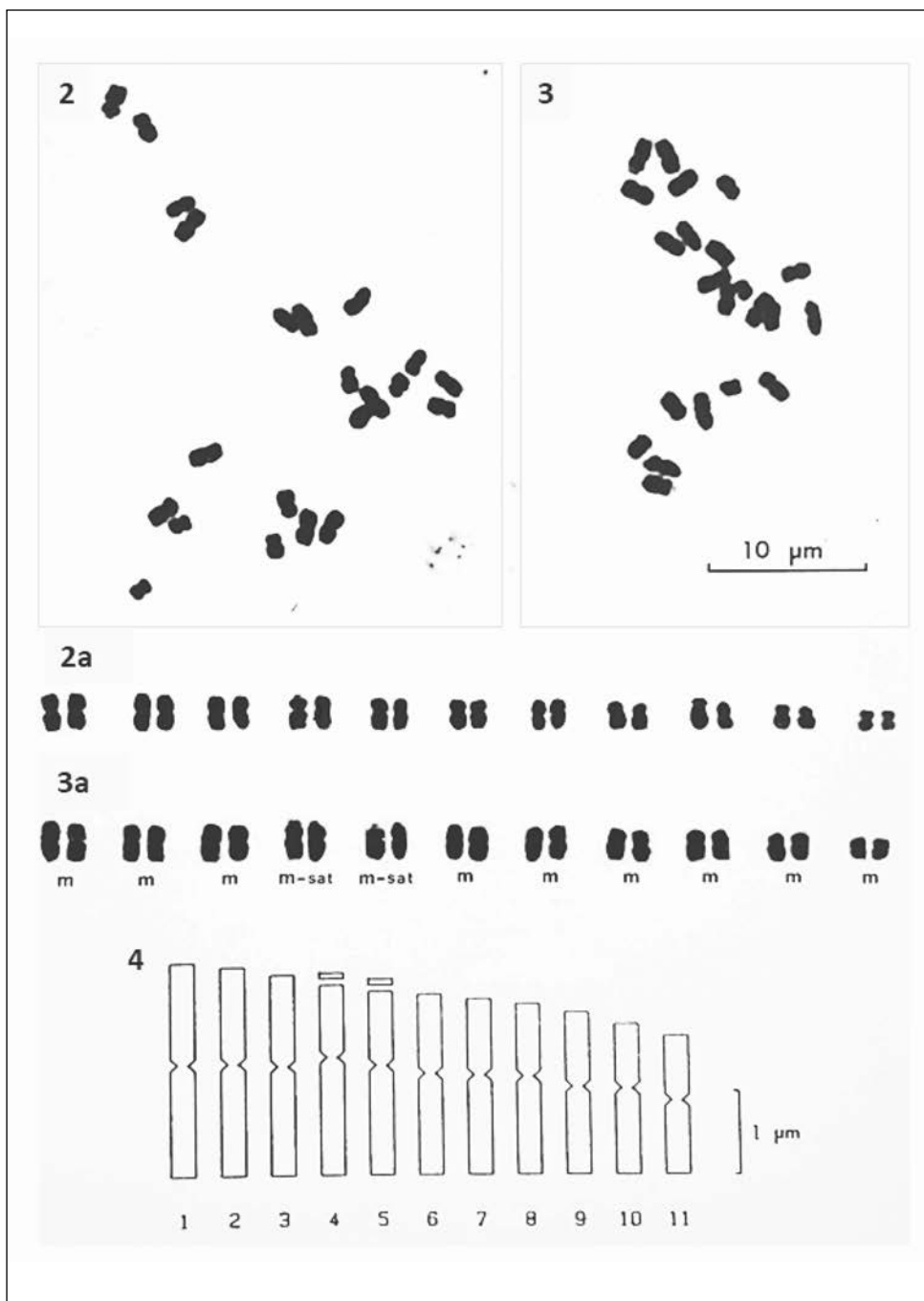
Centaurea melanocephala is also an endemic species from West Balkan Peninsula (Albania, Serbia). It grows in the mountain rocky grasslands on ultramafite substrate at an altitude from 800 to 1000 m.a.s.l.

The chromosome number of this species has been indicated for the first time from Kosovo in 1990 (Siljak-Yakovlev & Wraber) and then in 2010 (Siljak-Yakovlev & al). Recently, Novaković & al. (2022) reported the same chromosome number $2n = 22$ from Serbia.

The karyotype of *C. melanocephala* is composed of ten metacentric (pairs 1, 2, 3, 5, 6, 7, 8, 9, 10 & 11) and one metacentric-submetacentric pair (pairs 4 bearing satellites on their short arms). Pair 5 also carries the satellites (Figs. 2, 2a, 3, 3a & 4). The karyotype formula was as follows: $2n = 20m + 2m-sm = 22$, revealing a very symmetrical karyotype

Table 2. Numerical data concerning the chromosome set of *Centaurea melanocephala*.

Chrom. pair	Long arm LA (µm)	Short arm (SA) in µm	Total length (TL) in µm	Relative length	Rapport LA/SA	Centromeric index	Chromosome type
1	1.29 (±0.04)*	1.17 (±0.03)*	2.46	110.01	1.11	47.42	m
2	1.29 (±0.04)	1.10 (±0.02)	2.40	107.29	1.17	46.09	m
3	1.25 (±0.03)	1.04 (±0.03)	2.29	102.29	1.20	45.45	m
4	1.35 (±0.05)	0.81 (±0.02)	2.17	97.03	1.67	37.50	m-sm-SAT
5	1.25 (±0.05)	0.83 (±0.06)	2.08	93.29	1.50	40.00	m-SAT
6	1.15 (±0.03)	0.90 (±0.03)	2.04	91.43	1.29	43.88	m
7	1.13 (±0.04)	0.85 (±0.02)	1.98	88.63	1.32	43.16	m
8	1.10 (±0.03)	0.81 (±0.08)	1.92	85.83	1.36	42.39	m
9	0.98 (±0.05)	0.83 (±0.12)	1.81	81.13	1.18	45.95	m
10	0.96 (±0.04)	0.71 (±0.09)	1.67	74.64	1.36	42.50	m
11	0.81 (±0.02)	0.71 (±0.02)	1.52	68.10	1.15	46.58	m
* standard error		ΣTL=44.68 µm	R=1.62	IAs%=56.22			



Figs. 2-4. Mitotic metaphase plates of *Centaurea melanocephala* (2 & 3), corresponding karyograms (2a & 3a) and idiogram (4).

(IAS% = 56.22). Numerical data concerning this karyotype are presented in the Table 2. Total karyotype length (Σ TL) was 44.68 μ m indicating that the chromosomes of *C. melanocephala* are smaller than those of *C. kosaninii*. The ratio (R) between the longest and the shortest chromosome pair was $R = 1.62$.

Despite the same chromosome number, karyotype analyses show important differences between the two investigated species, suggesting their ancient differentiation.

Acknowledgment

Our colleague Dr. Tone Wraber passed away in 2010, we started this work on *Centaurea melanocephala* with him, and we now want to dedicate it to his memory.

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G. Barone, V. Gianguzzi, C. Cottone & E. Di Gristina

Karyological data of two Sicilian endemic *Centaurea* species

Abstract

Barone, G., Gianguzzi, V., Cottone, C. & Di Gristina, E. 2022: Karyological data of two Sicilian endemic *Centaurea* species [In Kamari, G., Blanché, C. & Siljak-Yakovlev, S. (eds), Mediterranean plant karyological data-32]. – Fl. Medit. 32: 244-246. <http://dx.doi.org/10.7320/FIMedit32.244>

Chromosome numbers are given for 2 *Centaurea* species endemic to Sicily. For both studied taxa, *Centaurea valdemonensis* and *C. virescens*, the somatic chromosome number, found on material from their *loci classici*, is diploid ($2n = 18$).

Keywords: Chromosome number, endemism, Sicily.

Introduction

The taxonomy of the genus *Centaurea* in the Mediterranean is still much debated, so much so that the treatment in collective groups of species has been proposed instead of the classic subdivision into sections (Hilpold & al. 2011, 2014). In Sicily *Centaurea* includes 37 species (Bartolucci & al. 2018; Domina & al. 2021; Domina & al. 2022). Among these the largest groups are that of *Centaurea busambarensis* Guss. and that of *C. parlatoris* Heldr. These groups have been recently reviewed (Domina & al. 2016, 2017 and 2021) and some taxa have been better taxonomically circumscribed. Recently on statistical analysis of morphometric characters a new species, *C. valdemonensis* Domina, Di Grist. & Barone, has been described in the group of *C. busambarensis* (Domina & al. 2022) and *C. virescens* (Guss.) Domina & Raimondo has been proposed at the specific rank in the group of *C. parlatoris* (Domina & al. 2021).

The aim of this study was to assess the chromosome number of the Sicilian endemics *Centaurea virescens* and *C. valdemonensis* whose value was still unknown.

2012. *Centaurea valdemonensis* Domina, Di Grist. & Barone — $2n = 2x = 18$.

Si: Nebrodi Mountains, Rocche del Crasto, crevices of limestone, rocks, 38.013182° N 14.737629° E (WGS84), 1280 m a.s.l., 24 June 2022, G. Domina & E. Di Gristina s.n. (PAL109753, SAF100085).

Centaurea valdemonensis occurs in a single population northeast Sicily, on the Nebrodi Mountains between 1200 and 1300 m a.s.l.

It is distinguished from the most related species, *C. busambarensis* Guss. by the 1-2 pinnatisect rosette leaves, and by the shorter appendage of the median capitula bracts with shorter fimbriae.

The chromosome number of $2n = 18$ found here from the locus classicus of the species corresponds with the one reported for the other species of the *C. busambarensis* group (Tornadore & al. 1974; Cela Renzoni & Viegi 1982; Raimondo & Bancheva 2004; Astuti & al. 2021).

2013. *Centaurea virescens* (Guss.) Domina & Raimondo — $2n = 2x = 18$.

Si: Palermo Mts, Monte Occhio, N slope, rocky pastures, 38.095853°N 13.191859°E (WGS84), 500 m a.s.l., 10 Jul 2020, leg. G. Domina & G. Barone s.n. (PAL, SAF100105).

Centaurea virescens is a Sicilian endemic species distributed in the mountains around Palermo (Domina & al. 2021) between 500 and 1200 m a.s.l.

This taxon differs from *C. parlatoris* s. s. by a different shape of the rosette and lower cauline leaves, and a longer pappus.

The chromosome number of $2n = 18$ found here from the locus classicus of the species corresponds with the one reported for the other species of the *C. parlatoris* group (De Santis & al. 1976; Colombo & Trapani 1989; Raimondo & Spadaro 2006, 2008).

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FLORA MEDITERRANEA

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3rd Mediterranean Plant Conservation Week

Plant Conservation Strategies:
from Science to Practice

Chania, Crete, Greece

27 September – 1 October 2021

Proceedings

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Presentation

The Mediterranean Basin hosts a flora of around 25,000 flowering plants and ferns, and c. 50% of them are endemic to the area, with main centres of diversity being found in relatively isolated territories of the basin, such as islands, islets and mountains. However, this plant richness is severely threatened and its conservation deserves particular attention.

The ‘*Mediterranean Plant Conservation Week*’ (MPCW) event, first time initiated by the CEPF (Critical Ecosystem Partnership Fund), the IUCN Center for Mediterranean cooperation and the IUCN/SSC/MPSG (Mediterranean Plant Specialist Group) in 2016, represents an opportunity to exchange successful examples of plant conservation initiatives and to enhance capacity building. It aims at becoming a gathering point for botanists, civil society institutions, community members and all those interested in Mediterranean plant conservation programs. The 1st MPCW took place in Ulcinj, Montenegro, 2016, and the 2nd MPCW in La Valetta, Malta, 2018. The 3rd one was initially planned to take place in 2020 but it was postponed for a year due to the Covid-19 pandemic.

Finally, the 3rd Mediterranean Plant Conservation Week (3MPCW), entitled ‘*Plant Conservation Strategies: from Science to Practice*’, was held in Chania, Greece, from 27 September to 1 October 2021, organized by IUCN/SSC/MPSG, CIHEAM Mediterranean Agronomic Institute of Chania (CIHEAM-MAICh), CEPF and the Network of Mediterranean Plant Conservation Centres (GENMEDA) (http://www.medplantsweek.uicnmed.org/public_html/medplantsweek/en/3rd-mpcw/about-3rd-mpcv/).

In this section of *Flora Mediterranea*, we have assembled a number of selected articles presented during the 3MPCW.

The main theme of the 3MPCW was training and experience sharing in the field of Mediterranean plant conservation: participants learnt about current techniques in different fields of plant conservation and created partnerships that would help them to carry out their own projects; in addition, the 3MPCW included a series of presentations, discussions, workshops, under its three main themes: 1) Taxonomy, inventories, Conservation Status assessment, 2) Conservation planning and *ex situ* / *in situ* techniques 3) Networking, learning and communication.

The selected articles of the first theme here included present works about inventories and assessments of endemic plants of Sicily, Lesvos island of Greece, Cyprus, Lebanon, but also of a prioritized checklist of crop wild relatives (CWRs) from Italy, as well as floristic inventories from Mediterranean island wetlands. Selected articles under the second theme above present works about various conservation techniques applied for Mediterranean plant species and habitats, which are expected to help in the design and implementation of conservation projects.

Among the co-organizers and sponsors of the event, special acknowledgements are due to the MAVA Foundation and to CIHEAM for always supporting initiatives of cooperation for biodiversity studies and conservation actions in the Mediterranean area, as well as to the *International Foundation pro Herbario Mediterraneo* and OPTIMA for offering the hospitality of the journal *Flora Mediterranea*, thus contributing in spreading experiences and knowledge!

Gland, Cagliari, 18 November 2022

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G. Barone, S. Ciancaleoni, L. Raggi, D. Donnini, D. Gigante, V. Negri & G. Domina

Drafting a prioritized checklist of Crop Wild Relatives and Wild Harvested Plants of Italy: problems and solutions

Abstract

Barone, G., Ciancaleoni, S., Raggi, L., Donnini, D., Gigante, D., Negri, V. & Domina, G.: Drafting a prioritized checklist of Crop Wild Relatives and Wild Harvested Plants of Italy: problems and solutions. — Fl. Medit. 32: 251-260. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The National checklists of Crop Wild Relatives (CWR) and Wild Harvested Plants (WHP) are the basic tools for the development of in situ and ex situ conservation strategies of plant genetic resources. Here we discuss the methodologies and the prioritization process we previously used in the preparation of the prioritized checklist of CWR and WHP for Italy.

The starting point were the most up-to-date Italian checklists of native and alien flora with their updates used as a nomenclatural and distributive source of data. Sardinia and Sicily were kept separate from peninsular Italy to perform detailed analyses focused on the taxa of the two major islands. The origin, the endemic status, cultivation, economic importance, uses, gene pool or taxon group, and the Red List status information were added. The WHP status was attributed to all the taxa with known direct uses. A qualitative approach was adopted in the prioritization process, the main criteria used were: 1) the inclusion of wild relative taxa of crops listed in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITP-GRFA) and/or by the Italian Institute of Statistics (ISTAT) for cultivated areas and yield in the last 5 years; 2) the threatened taxa occurring in national or global Red lists; 3) the endemism. This prioritization process generated 102 taxa as most in need of specific protection and /or monitoring measures, 57 taxa requiring monitoring because of their restricted distribution although not requiring specific protection measures and 735 taxa not requiring any specific protection. However, different prioritization processes could have been applied to the Italian checklists yielding different results. Here we discuss the matter.

Key words: conservation, useful plants, Mediterranean flora.

Introduction

Crop Wild Relatives (CWR) are plants closely related to socio-economically important crops used as food, fodder, ornamentals, materials or other uses. According to Maxted & al. (2006), a broad definition of CWR would include any wild taxon belonging to the same genus as a crop.

Thanks to their genetic closeness to crops and their high diversity, which was not eroded during domestication and successive selection process, CWR are a source of beneficial alleles that can be relevant for crop improvement and to attain food security (Harlan & de Wet 1971; Maxted & al. 2006, 2010). Indeed, CWR have been used for long time and are still used nowadays to breed new crop varieties (Hajjar & Hodgkin 2007; Maxted & Kell 2009; Vincent & al. 2013; Dempewolf & al. 2014; Zhang & al. 2017). However, CWR genetic diversity still needs to be fully explored often representing a relevant and ‘untapped source of genetic material’ (Okie 2006).

Wild Harvested Plants (WHP) are those plants that are part of the local traditional knowledge because collected in the wild and used as additional source of food, medicine, combustible, and materials. When locally used as a food source or additional familiar income, they have the potential to increase food security and nutrition of those populations living in harsh environments and developing countries (Asprilla-Perea & Díaz-Puente 2019; Ulian & al. 2020). Overexploitation, habitat degradation and climate change are among the main factors determining biodiversity loss, in general, and of CWR and WHP, in particular. For this reason, the compilation of inventories emerges as one of the first steps to be carried out in the development of monitoring plans and more comprehensive *in situ* and *ex situ* protection policies (Corlett 2016). In fact, diversity of crop wild relatives is poorly represented in gene banks; at this regard, over 70% of wild relatives has been identified as high priority for further collations according to Castañeda-Álvarez & al. (2016). Finally, CWR and WHP play an essential role in the ecosystems providing several services both to natural and semi-natural environments (FAO 2019).

According to Maxted & al. (2007), several key topics need to be addressed when attempting to formulate an effective national conservation strategy for CWR diversity including i) creating a national CWR inventory, ii) analysing the CWR inventory content, national patterns of CWR distribution and threat status for CWR diversity, iii) assessing current conservation actions, iv) identifying priority sites for conservation, and v) creating CWR conservation action plans.

In 2021, considering the recently updated checklist of the vascular native and exotic flora of Italy (Bartolucci & al. 2018; Galasso & al. 2018) and the last Italian Red Lists (Rossi & al. 2016; Orsenigo & al. 2018, 2020), we decided to update the previous inventory of the CWR and WHP of Italy of Landucci & al. (2014) whose taxonomy, taxa distribution, threat status, use categories and genetic data for CWR and WHP needed to be reviewed and integrated. A national CWR checklist is a list of the taxa found among the national flora and related to crops; however, being the number of CWR taxa in their broad concept too high to actively conserve all of them, it is often necessary to establish a methodology to select those most in need of conservation. This is done by the integration of these checklists with additional data which allows the construction of prioritised inventories (Maxted & al. 2013).

Starting from the previous work and methodologies of Landucci & al. (2014), and based upon the considerations above, a CWR and WHP priority list has been drafted for Italy. Based on our experience in the preparation of this updated Italian checklist and inventory of CWR and WHP we will focus our discussion on some of the methodological aspects and their related issues.

Materials and methods

In order to properly discuss the problems related to the development of a CWR/WHP prioritized list for an entire country and the relative solutions, a summary of the method we applied in Ciancaleoni & al. (2021) - whose results are the starting point of this new investigation - follows.

In Ciancaleoni & al. (2021) we applied a floristic approach to draft the CWR and WHP checklist of Italy; giving a wide point of view on a country's diversity, this approach provides the best foundation for developing a National Strategic Action Plan for the conservation and sustainable use of CWR and WHP. The checklist was then annotated following the same approach of Landucci & al. (2014), instead of the suggested layout produced by Thormann & al. (2017), to allow an easier comparison of the new with the old one. Information related to origin (i.e., native or introduced, archaeophyte or neophyte status), the indication of the national or infra-national endemic status, cultivation, economic importance, ethnobotanical, medicinal or other uses, gene pool or taxon group for CWR (Euro+Med 2006-; Vincent & al. 2013; Pignatti & al. 2017; IPNI 2021; USDA 2021) were included for each taxon in the checklist. Finally, the need for monitoring or protection was integrated following the most recent Red Lists available (Rossi & al. 2016; Orsenigo & al. 2018, 2020; IUCN 2020). In the end, three distinct annotated checklists were produced for the Italian Peninsula, Sardinia, and Sicily, respectively.

The CWR status was then attributed to all those taxa related to a cultivated species (i.e., same genus following Maxted & al. 2006) according to the data used as source of information (Vincent & al. 2013; ISTAT 2019; USDA 2021) while the WHP to those (taxa) with one or more known direct uses regardless of the actual commercialization of their products (Magos Brehm & al. 2008). Furthermore, due to the observation that some CWR are found both in cultivation and in the wild, cultivated plants have been included in the inventory, as well alien species, considering the possibility that the latter could have adapted to new environments developing interesting traits.

The prioritization methodology we adopted in Ciancaleoni & al. (2021) consisted of a qualitative approach based on the combination of the following criteria (Khoury & al. 2013; Landucci & al., 2014): i) the socioeconomic value, based on the presence of the related crop in the Annex I of the ITPGRFA (FAO 2001) and/or in the list of the Italian Institute of Statistics for cultivated areas and yield in the last 5 years (ISTAT 2019); ii) the need of protection, based on the inclusion of the taxa in a threatened category of national and global Red lists. Endemism was used as an additional indicator to determine threat.

The combination of the above mentioned criteria was used to define three priority categories, namely "A", "B" and "C" from the highest to the lowest need of protection: "A" category, includes native and alien taxa related to an important crop for food and agriculture that needs specific protection and/or monitoring measures; taxa in this group are present in at least one of the most recent National Red Lists (Rossi & al. 2016; Orsenigo & al. 2018, 2020) or in the IUCN Red List (IUCN, 2021) with a pertaining threat category; "B", includes national endemic or sub endemic taxa that require monitoring because of their restricted distribution, although they do not necessarily require specific protection measures; "C", all the remaining native taxa that, on the grounds of current knowledge, do not need any specific protection measure.

With the present paper we aim at developing a critical view of the list we proposed in Ciancaleoni & al. (2021), starting from figures resulting from the process, and taking into account strengths/weaknesses of the available sources and the entire decision-making structure, also based on the comparison with similar lists produced in other countries.

Results and Discussion

The described approach we adopted in Ciancaleoni & al. (2021), which identifies CWR in a broad sense including all taxa belonging to the same genus of a crop, resulted in the identification of 8,766 CWR/WHP taxa for Italy belonging to 7,334 species (<https://www.optima-bot.org/index.php/es/8-category-en-gb/217-the-italian-cwr-whp-database>). In particular: 6,839 taxa (5,516 species) are CWR only, 108 (108) WHP only, and 1,821 (1,710) CWR and WHP at the same time. Taxa and species resulted distributed as follows: 7,916 (6,641), 2,745 (2,600), and 2,952 (2,738) for the Italian Peninsula, Sardinia, and Sicily, respectively.

Priority taxa identified through the above-described qualitative process - whose number is here reported followed by number of species (in brackets) - were 102 (82) for the “A” category and 57 (50) for the “B” category, while the remaining 735 taxa (648) were designated to category “C”, thus considered as not deserving any immediate specific protection or monitoring measures. The identified taxa to be protected with the highest priority in Italy (“A” category) belong to 36 different genera. Among them, *Allium* L., *Asparagus* L., *Avena* L., *Brassica* L., *Cichorium* L., *Citrullus* Schrad., *Daucus* L., *Diploaxis* DC., *Festuca* L., *Lactuca* L., *Lathyrus* L., *Malus* Mill., *Prunus* L., *Trifolium* L., and *Vicia* L. have already been reported to be of highest conservation priority, both for Italy (Landucci & al. 2014) and globally (Castañeda-Álvarez & al. 2016).

The new produced inventory provided a list of those CWR and/or WHP taxa deserving priority conservation actions in Italy. The available results can be useful not only for further scientific insights to the issue but also for shedding light on the importance of Plant Genetic Resources for Food and Agriculture conservation according to their relevance and threat status. However, in developing this inventory we had to deal with several aspects and problems which deserved special attention. We are referring to the evaluation methodology for the priority level assessment for each taxon. In fact, when we tried to compare our results to others coming out from other geographic contexts it emerged the difficulty related to the different approaches and methodologies adopted from time to time. Sometimes a quantitative approach is adopted (evaluation in parallel with scores for each criterion) and other times a qualitative one (evaluation based on serial systems where the scores are given in sequence, one criterion at a time). As a result, most of the time, different methodologies make the results difficult to compare.

During the prioritization process, one of the main criteria applied is the social-economic importance of the related crop. Socio-economic importance can be estimated using data regarding trading values and country’s food supply which can be extracted from FAO-STAT, a food and agriculture free database for over 245 countries and territories administered by FAO Statistics Division (FAO 2021). The main issue here is that only major crops or crop groups are usually listed in this database, and some may be missing or grouped

with others according to the FAO commodity list codes. Furthermore, crop groupings in FAO food supply statistics differ from those in production statistics (Kell & al. 2015). This makes problematic the scoring process of those combined crop groups because it is not possible to obtain a crop-specific economic value. Possible solutions are to use the value as it is for every crop of that commodity group or use additional data and sources as indicators to estimate it. The disadvantage here is that it could bring bias in the evaluation and different approaches to solve this issue. Furthermore, Annex 1 of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO 2001) has often been used as a list of socio-economic important plants globally. However, it has been recently pointed out that this list is not comprehensive enough, so it needs to be integrated with other data at national (i.e., national agricultural statistic services) and global scale (i.e., FAOSTAT). Sometimes it may be considered sufficient to pair the ITPGRFA Annex I with a national trading value statistic, whereas in other cases it has emerged the need to also use food supply data expressed in Kcal/capita/day to have a more comprehensive point of view on the crop importance for the local population (FAO 1997).

Socio-economic importance is one of the necessary criteria for a taxon to be included in the highest priority category (“A”) used in our paper (Ciancaleoni & al. 2021). Here we decided to estimate the social-economic importance by using the crops listed in Annex I of the ITPGRFA (FAO 2001) and/or by the Italian Institute of Statistics for cultivated areas and yield in the last 5 years (ISTAT 2019). The two groups together include the most socioeconomically important crops for food and agriculture for Italy and the rest of the World. Consequently, because all the food crop genera resulted already included in our inventory by the combination of the two above mentioned sources, we evaluated as not necessary to include data from the FAOSTAT and this with the aim also of allowing an easier comparison with the previous work from Landucci & al. (2014). In our database, more than 1,200 taxa resulted mentioned in at least one of the two sources used. In other regions, according also to the available information and the different level of development, the approaches registered have been different like in Tunisia (El Mokni & al. 2022) and China (Kell & al. 2015).

Also, the choice of considering only taxa native to Italy is questionable, taxa like *Juglans regia* L., widely cultivated and reported as established in the wild in several regions as a cryptogenic in Lombardia, Veneto and Sardinia, and as casual or naturalized from many Italian regions could have been considered; the same could be true for *Opuntia ficus-indica* (L.) Mill. due to their importance as crops. Another widely used criteria to prioritize CWR taxa is their threat status. The major problem with it is due to the lack of national Red Lists and assessments for many taxa. Alternatively, it is possible to use other indicators such as endemism or restricted distribution for those taxa not assessed yet. Furthermore, there are cases when a taxon is assessed at different geographical scales (i.e., regional, national), so that in a scoring process, this could be misleading. Again, this becomes time-consuming when the evaluation needs to be done on a wide checklist.

In Ciancaleoni & al. (2021) we considered the threatened taxa assessed in the most recent Italian and IUCN Red Lists (Rossi & al. 2016; Orsenigo & al. 2018, 2020; IUCN 2021). We decided to use the IUCN Red List for those alien species that occur outside of our target region and that were not assessed for Italy. We considered the following categories: Critically Endangered or Possibly Extinct, (CR(PE)), Critically Endangered (CR),

Endangered (EN), Vulnerable (VU), and Near Threatened (NT). Taxa characterized by a low-risk level (LC) or by inadequate information (DD) indicating a lack of knowledge about these species were not considered. Furthermore, when the assessment was referred to the species only, we transferred the assessment at least to the nominate subspecies. As mentioned above, one option to face the incompleteness of the Red Lists is to integrate them with other factors which could function as indicators of a potential threat. Because, in general, species with restricted distribution should be given higher priority, we decided to use endemism as an additional indicator and we included it among the criteria of the “B” priority category. Other approaches used in the literature include the use of a neighbouring nation’s red list as a proxy of the taxa threat status (Mponya & al. 2020), the collection and combination of all the taxa assessments available in the literature and online (El Mokni & al. 2022) or, lastly, the decision to avoid the use of this criterion due to the insufficient data available (Rahman & al. 2019). Finally, it should be noted that using the threatened taxa approach often results in excluding from the priority list CWR taxa that indeed are of importance for future breeding and for guaranteeing food safety and food risk management. For example, *Beta vulgaris* L. subsp. *maritima* (L.) Arcang., the direct CWR of *B. vulgaris* L., an important crop worldwide, is not a taxon under threat due to its wide distribution, should its populations be excluded a priori from a list aimed at protecting important genetic resources?

The degree of relatedness of a CWR to the crop is another among the most common factors used as criteria in the priority evaluation. It is well known that it is originally based on the gene pool concept by Harlan & de Wet (1971) which, in turn, is not available for all crops yet. Therefore, in 2006, Maxted & al. developed the taxon group concept that, being based on the taxonomical hierarchy, could help fill the gap when the gene pool information is missing because it can approximate the genetic distance. Nowadays there are two freely available databases with data regarding gene pool and taxon group for a wide range of CWR (Vincent & al., 2013; USDA 2021). Unfortunately, these data are not available for every taxon, bringing researchers to different approaches to compensate for the missing information (e.g., using the Taxon Group 4 for all the CWR taxa in the same genus of the related crop). When dealing with the degrees of relatedness, the evaluation of taxa related to more than one crop (e.g., brassicas) needs to be discussed. Should we consider the higher gene pool (or taxon group) value and evaluate it accordingly or consider the fact that this plant is related to more crops as an added value? Here again, there are different approaches and points of view.

In our evaluation, we decided to add this information to the database, but not to use it in the prioritization process. This is because otherwise we would have not been able to compare our results with the previous work from Landucci & al. (2014) and because it was not possible to obtain enough data regarding the degree of relatedness from the available databases (only 700 taxa got a gene pool or taxon group higher than TG4).

Conclusions

Despite possible methodological problems and the difficulty that is easily encountered when trying to compare results obtained in different countries and geographic contexts,

there is a consensus on the relevance of assessing CWR and WHP species and taxa for prioritized inventories. As recently highlighted by FAO (2019), Plant Genetic Resources for Food and Agriculture (PGRFA), part of the broader concept of Biodiversity for Food and Agriculture (BFA), are in constant decline and crop diversity is following the same trend (FAO 2010). Furthermore, lack of funding, human resources, knowledge, and coordination are some of the weak points in the global strategy for the protection of these important resources. Among the priority actions to counteract this situation, addressing the knowledge and data gaps and establishing monitoring plans are considered essential. Specifically, the need to preserve CWR and WHP should pass through efficient conservation planning actions based on the selection of those taxa more in need of protection through the help of comprehensive prioritised inventories like the one for Italy discussed here. As highlighted also in other contexts, the need to better coordinate the protection efforts both at research level, with similar methodologies, and policy level remains essential and needs to be better developed to preserve this part of biodiversity and its contributions to food, agriculture, and ecosystems, especially considering climate change and growing anthropic pressure on the environment.

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The genus *Limonium* (*Plumbaginaceae*) in Tunisia: taxonomy, biogeography and conservation

Abstract

Ben Haj Jilani, I., Ben Othman, W., Sefi, O., Ben Khalifa, S., Meddeb, S., Daoud-Bouattour, A., Ghrabi-Gammar, Z., Bottollier-Curtet, M. & Muller, S. D.: The genus *Limonium* (*Plumbaginaceae*) in Tunisia: taxonomy, biogeography and conservation. — Fl. Medit. 32: 261-277. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The recent increase in the known richness of *Limonium* in Tunisia, mainly due to the description of 25 new endemic species in the last decades, has made the only existing identification key for the country obsolete. Based on an exhaustive review of the literature dealing with *Limonium* from Tunisia, consultation of herbarium specimens preserved in Tunisia and Sicily, and a number of new collections, we propose here a new complete key, accompanied by an updated mapping for all taxa. The obtained results reveal that most Tunisian *Limonium* are perennial herbs (81 %), halophytes (79 %), endemic to the country (61 %), and mainly distributed along coasts and around chotts. A significant number of taxa (51 %) present important conservation issues, mainly related to microendemism (especially on islands and peninsulas) and to the alarming acceleration of habitat degradation. Djerba Island is currently the subject of a project involving local authorities, residents and environmental protection associations aimed at conserving the microendemic *Limonium formosum*, whose only location is threatened in the short term by a project to turn it into a tourist attraction.

Key words: North Africa, halophilous vegetation, endemism.

Introduction

The genus *Limonium* Mill. (*Plumbaginaceae*) is extremely variable, diversified and complex, as a result of its high degree of agamospermy, polyploidy, hybridization and polymorphism (Cowan & al. 1998; Palop-Esteban & al. 2007). Its phylogeny is consequently still partially resolved (Lledo & al. 2005). The first classification attempts were conducted by Boissier (1848, 1859) who considered it under the name *Statice* L. Rechinger (1936, 1938, 1943), in his work on the Aegean region, was one of the first authors to transfer *Statice* species into the genus *Limonium* (Brullo & Erben 2016). Subsequently, the name *Statice* was definitively considered invalid by Greuter & al. (2000). Thanks to molecular and phylogenetic investigations, the conception of the genus *Limonium* has

evolved during the last decades, resulting in the transfer of some species into various small genera, such as *Acantholimon*, *Goniolimon* or *Myriolimon* (Lledo & al. 2005). To date, *ca.* 600 taxa have been described (Koutroumpa & al. 2018), most of which being concentrated in the Mediterranean Basin (Greuter & al. 1989). This region, which houses about 70 % of the known species and most of the stenoendemics, appears as the biodiversity center of the genus (Koutroumpa & al. 2021).

In North Africa, including Macaronesia, Dobignard & Chatelain (2013) reported 107 taxa, among which 69 are endemic to a single country. However, these assessments are highly dependent on field survey efforts and taxonomical studies conducted over the past decades. Indeed, *Limonium* richness of Greece, which was once estimated at 15 taxa by Pignatti (1972), was recently increased to 85 species by Brullo & Erben (2016)! The same comparison made in the three Maghreb countries (Table 1) reveals that only Tunisia has been recently investigated, and that the small changes reported for Algeria and Morocco were a result of taxonomical modifications. In Tunisia, Brullo & Erben (1989) distinguished 36 species, among which 21 were new for science. Other taxa were discovered or described later, such as *L. comosum* Erben, *L. formosum* Bartolo, Brullo & Giusso, *L. elfahsianum* Brullo & Giusso del Galdo and *L. steppicum* Sefi, Ghrabi-Gammar & Brullo (Erben 2001, Bartolo & al. 2003, Brullo & al. 2006, Sefi & al. 2020). One species, formerly recognized as *Limonium ferulaceum*, has been transferred to the genus *Myriolimon*. Accordingly, these recent changes radically alter the knowledge of the genus *Limonium* in Tunisia, and seriously complicate the identification of species in the field.

Moreover, as the variability and diversity of *Limonium* are strongly linked to geographical and ecological isolation, most species of this genus occur in rather peculiar habitats, such as rocky coasts and salt marshes (Brullo & Erben 2016). The recent field surveys conducted by the authors (Ghrabi-Gammar & al. 2020; Ben Charfi & Numa 2021) have highlighted several types of threats, which currently weigh on fragile biotopes with high conservation challenges. Most regions do not benefit from any protection status and are subject to an accelerated loss of habitats, likely to lead to the extinction of several *Limonium* species in the near future.

The aims of the current study are: (1) to give an updated identification key based on the state-of-art regarding taxonomy, nomenclature and chorology of Tunisian *Limonium*, (2) to provide distribution maps for all *Limonium* taxa of Tunisia, and (3) to assess the threat sta-

Table 1. Richness in taxa and strict endemics of *Limonium* in the three Maghrebi countries, from Quézel & Santa (1963), Aimé & Roiron (1985), Pottier-Alapetite (1981), Fennane (1999) and Dobignard & Chatelain (2013).

		Taxa	Strict endemics
Algeria	1963-1985	27	9
	2013	28	5
Morocco	1999	34	10
	2013	36	12
Tunisia	1981	18	1
	2013	42	26

tus of *Limonium* taxa at the national scale, and present an example of conservation actions for *L. formosum*, a critically endangered microendemic from Djerba Island.

Material and methods

Identification key

Apart from the partial key of *Limonium steppicum* and its allied species present in Djerba (Sefi & al. 2020), no recent key including the newly described species has been established yet. Thus, we propose in the current work a complete identification key of *Limonium* taxa occurring in Tunisia and so far, recognized. In so doing, and in order to fully grasp the diacritic characters of the different *Limonium* species, we extracted all available data from literature. Moreover, we ascertained and completed missing measurements by careful examination of 110 samples collected in different regions of Tunisia (Kroumiria, Northeast, Center and South, including the island of Djerba) and kept at the herbarium of the *Institut National Agronomique de Tunisie* (INAT), as well as those of the herbarium specimens of 41 *Limonium* species, consulted at the herbarium of the University of Catania, Sicily (CAT). Insofar, as the geographic isolation is a major cause of speciation in the genus, geographic criteria were also used for taxa identification (Tison & De Foucault 2014; Brullo & Erben 2016). Endemism and distribution areas are consequently specified for stenoendemic taxa. Endemism status was determined from available literature as well as online databases, such as *Plants of the World Online*, *African Plant database*, *eflora Maghreb* and *Euro+Med Plantbase*. Finally, according to the biogeographical distribution of *Limonium* species, four levels of endemism were identified: exclusive to Tunisia (T), shared between two countries, in our case Algeria-Tunisia (AT), related to at least three North African countries (NA) and on a larger scale, *i.e.* Mediterranean and beyond (Med). The nomenclature of the taxa was updated according to Dobignard & Chatelain (2013), Le Floch & al. (2010) and Domina & El Mokni (2019).

Status assessment

In Tunisia, the *Code forestier* (Anonyme 2012) is the main legislative text promulgated for the protection of rare and threatened taxa of wild flora and fauna, as well as those covered by international treaties (Khabbach & al. 2021). Nevertheless, this code refers more specifically to forest-dependent taxa and therefore could not ensure adequate protection of those that thrive in other habitats or ecosystems. Furthermore, there is the so-called *Registre National des Espèces Sauvages* (REGNES; Anonyme 2010), established in 2010 thanks to the support of IUCN, but including only *ca.* 50 species of birds, emblematic for their number or conservation status, and *ca.* 100 plant taxa.

Likewise, the authors were actively involved, under the aegis of IUCN, in the threat status assessment, at the Mediterranean scale, of some Tunisian Monocotyledonous taxa and others occurring more particularly in freshwater wetlands (e.g. García & al. 2010). Nonetheless, it is worth mentioning that so far, no taxa of the genus *Limonium* occurring in Tunisia, have been assessed in the IUCN Red List of threatened species. Even the National Red List of Tunisia,

which is being devised, is dedicated only to the avifauna and to some groups of the flora, namely *Lycopodiophytes*, *Polypodiophytes*, Gymnosperms and Monocotyledons.

Data were collated as exhaustively as possible from the available literature (e.g., Pottier-Alapetite 1981; Brullo & Erben 1989, 2016; Erben 2001; Bartolo & al. 2003; Brullo & Giusso del Galdo 2006; Pavon & Vêla 2011; Tison & De Foucault 2014; Greuter & Domina 2015; Médail & al. 2015; Erben & al. 2018; Gamoun 2020; Sefi & al. 2020), from herbarium samples preserved at INAT, including those from P. Leboulenger's personal collection, from harvesting samples mentioned in the *eFlora Maghreb* database and from field surveys conducted by the authors. Historical stations were considered for the calculation of the area of occupancy (AOO) and the extent of occurrence (EOO). These areas were computed using the GeoCAT tool developed by RBG Kew. The locations were grouped according to the physiographic subdivisions of Tunisia, defined by Cuénod & al. (1954).

This thorough literature analysis, underpinned by field surveys, has allowed us to build a sufficiently complete dataset to calculate the values of AOO and EOO, and to determine the categories and criteria justifying the assessment of the conservation status of *Limonium* taxa. The geolocation data allowed us to establish biogeographical distribution maps of the different taxa, but more particularly of the stenoendemic ones. These maps were created using QGIS 3.16.

Phylogeny

Based on the phylogenetical studies by Lledo & al. (2005) and Koutroumpa & al. (2018), 10 of the 43 taxa occurring in Tunisia can be assigned to the infrageneric subdivisions recognized by Koutroumpa & al. (2018). They belong to the following clades: Subgen. *Pteroclados* (Boiss.) Pignatti *s.l.* Generally perennial herbs, with entire or sinuated basal leaves; stems often bearing wings; spikelets distichous; calyx infundibuliform; corolla often white, sometimes yellow or light pink; fruit with circumscissile dehiscence (Karis 2004; Koutroumpa & al. 2018).

Sect. *Pteroclados*. Species of the sect. *Pteroclados* differ chemically from all other *Limonium* by producing glycine betaine but not balanine betaine (Hanson & al. 1994).

Subsect. *Odontolepideae* (Boiss.) Koutroumpa. This subsection, mostly distributed in the Mediterranean region, is opposed to the sect. *nobile* endemic of Macaronesia, and is characterized by cuspidate inner bracts and usually conspicuously winged stems (Karis 2004). In Tunisia, it comprises *L. bonduellei*, *L. lobatum* and *L. sinuatum*.

Subgen. *Limonium*

Sect. *Limonium*. Perennial herbs reaching 15 to 150 cm; leaves large, pinnately veined, organized in basal rosette; sterile branches few or absent; inflorescence with more or less dense spikes; spikelets small, usually with 1-4 flowers; calyx obconical or very narrowly funnel-form; calyx limb short, undulate, bearing 5-10 distinct lobes, usually with short lobes placed between larger lobes; corolla bluish-violet, rarely lilaceous. This section, understood here in the restricted conception of Koutroumpa & al. (2018), comprises *L. boitardii* and *L. narbonense*.

Sect. *Nephrophyllum* Rech.f. *s.l.* (including species of the sect. *Limonium* subsect. *Hyalolepideae*, i.e. part of the '*L. bellidifolium* complex'; Koutroumpa & al. 2018).

This section comprises only Irano-Turanian elements, with the notable exception of *L. bellidifolium*, which is present in Tunisia. It is characterized by rosette leaves disappearing during flowering, amplexicaule or semi-amplexicaule stem leaves (sometimes absent), generally present sterile branches, and numerous small spikelets with broadly membranous (hyaline) outer and inner bracts (Boissier 1848; Akhani & al. 2013; Erdal 2015; Malekmohammadi & al. 2017).

Sect. *Pruinosum* (Batt.) Koutroumpa. This section is characterized by stems and branches covered by calcariferous tubercles with a central punctate depression, numerous sterile branches, one-flowered spikelets, calyces with membranous limbs, and deciduous leaves (Koutroumpa & al. 2018). In Tunisia, it comprises *L. allezettei* and *L. pruinsum*.

Sect. *Schizhymenium* (Boiss.) Bokhari. This section encompasses annual species bearing characteristic subtubular calyces with limbs lacerating at maturity and ribs forming more or less hooked barbs (Bokhari 1972). In Tunisia, it comprises *L. awei* and *L. echioides*.

Key of Tunisian *Limonium*

The key of Tunisian *Limonium* integrates classic criteria concerning longevity (annual vs perennial), stems (winged versus not winged, with or without tubercles), occurrence of sterile stems, leaves (color, shape, margin, apex), inflorescences (size, shape, spikes, spikelets, bracts) and flowers (size and color of calyx and corolla) (e.g. Brullo & Erben 2016). The values and modalities of the used criteria were extracted from Pottier-Alapetite (1981), Brullo & Erben (1989), Erben (2001), Bartolo & al. (2003), Brullo & Giusso del Galdo (2006), Tison & De Foucault (2014), Erben & al. (2018) and Sefi & al. (2020). Endemism and distribution areas are specified for stenoendemic taxa. The used nomenclature follows Brullo & Erben (1989), Erben (2001), Le Floe'h & al. (2010) and Sefi & al. (2020). Endemism is indicated between square brackets after species names: A, Algeria; L, Libya; M, Maghreb; T, Tunisia.

- | | | |
|---|---|-------------------------|
| 1 | Stems winged; leaves sinuate (subgen. <i>Pteroclados</i>) | 2 |
| 1 | Stems not winged; leaves entire or absent (subgen. <i>Limonium</i>) | 4 |
| 2 | Calyx divided in 5 acute lobes alternating with 5 ridges; leaves mucronate; spikelet < 10 mm; annual | <i>L. lobatum</i> |
| 2 | Calyx nearly undivided, truncated serrated; leaves rounded; spikelet > 10 mm; perennial or annual | 3 |
| 3 | Perennial; corolla and calyx blue or purplish | <i>L. sinuatum</i> |
| 3 | Annual; corolla and calyx golden yellow | <i>L. bonduellei</i> |
| 4 | Annual; calyx with 5 long, narrow teeth and decurrent nerves (sect. <i>Schizhymenium</i>) | 5 |
| 4 | Perennial; calyx teeth short with no decurrent nerves | 6 |
| 5 | Corolla purplish; calyx teeth without a hooked tip | <i>L. awei</i> |
| 5 | Corolla pink; calyx teeth extended by hooked tip | <i>L. echioides</i> |
| 6 | No rosette of radical leaves at flowering | 7 |
| 6 | Rosette of radical leaves present at flowering | 9 |
| 7 | Leaves spatulate-oblongate; panicle dichotomous; spikelets grouped at branch extremity; outer bract entirely scarious (sect. <i>Nephrophyllum</i> s.l.) | <i>L. bellidifolium</i> |
| 7 | Leaves ovate-rounded; panicle non-dichotomous; calcariferous tubercles; one-flowered spikelets (sect. <i>Pruinosum</i>) | 8 |

8 Inner bract twice as long as the outer bract; flattened tubercles without papillae ...	<i>L. alleizettei</i> [ML]
8 Inner bract 3-4 times longer than the outer bract; tubercles spiked with papillae.....	<i>L. pruinsum</i>
9 Numerous sterile stems	10
9 No or few sterile stems	14
10 Inflorescences < 22 cm	<i>L. intricatum</i> [T]
10 Inflorescences reaching 40-45 cm	11
11 Leaves green; inflorescences branched in upper part	<i>L. hipponense</i> [T]
11 Leaves glaucous; inflorescences branched in basal part	12
12 Spikes spreading with uniseriate or distichous spikelets; inner and outer bracts with narrow margins	<i>L. virgatum</i>
12 Spikes slightly curved with $\pm$ lax spikelets; inner and outer bracts with wide margins.....	13
13 Leaves with 1 mm-long mucron; angle of inflorescence branches > 40°	<i>L. xerophilum</i> [T]
13 Leaves mucronulate; angle of inflorescence branches < 40°	<i>L. zeugitanum</i> [T]
14 Leaves < 2 cm	15
14 Leaves > 2 cm	16
15 Inflorescences reaching 40 cm; leaf width > 5 mm, without nerve	<i>L. pescadense</i> [AT]
15 Inflorescences < 20 cm; leaf width < 5 mm, with 1 nerve	<i>L. zembrae</i> [T]
16 Leaves with pinnate nerves (sect. <i>Limonium</i>)	17
16 Leaves with palmate or indistinct nerves	18
17 Large corymbiform panicle with erect branches; corolla purplish	<i>L. narbonense</i>
17 Spreading branches bearing sessile glomerules; corolla blue	<i>L. boitardii</i> [T]
18 Inflorescences distichous (sect. <i>Limonium</i> subsect. <i>Densiflorae</i>)	19
18 Inflorescences non-distichous	20
19 Inflorescences > 20 cm; a few sterile stems; corolla violet.....	<i>L. densiflorum</i>
19 Inflorescences generally < 20 cm; no sterile stems; corolla purplish	<i>L. gougetianum</i> [AT]
20 Leaves without mucron	21
20 Leaves mucronate or with an apical tip	25
21 Inflorescences in dense panicle > 70 cm; leaves with 3-7 nerves	<i>L. thaenicum</i> [T]
21 Inflorescences in lax or narrow panicle < 70 cm; leaves with < 3 nerves	22
22 Leaves green, flat; inflorescence branched from the base	<i>L. elfahsianum</i> [T]
22 Leaves glaucous, coriaceous; inflorescence branched at the upper part	23
23 Leaves glaucous on only one face reaching 12 cm; inner bract with acute apex and 3 times longer than outer bract	<i>L. spathulatum</i> [M]
23 Leaves entirely glaucous; inner bract with obtuse apex and 2 times longer than outer bract ...	24
24 Inflorescences in lax panicle; leaves oblanceolate with apex acute to obtuse; fertile branches < 8 cm; calyx < 5.4 mm; corolla diameter < 6.5 mm	<i>L. punicum</i> [T]
24 Inflorescences in narrow panicle; leaves cuneate-spatulate with apex obtuse to rounded; fertile branches < 20 cm; calyx > 5.4 mm; corolla diameter > 7.0 mm	<i>L. serratum</i> [T]
25 Leaves < 7 $\times$ 1.5 cm	26
25 Leaves > 7 $\times$ 1.5 cm	31
26 No or rare sterile stems; leaves mucronate or ended by an apical tip	27
26 Few sterile stems; leaves mucronulate	28
27 Leaves coriaceous, reddish glaucous, ended by an apical tip > 1 mm, with 1-5 nerves; inflorescences reaching 60 cm, branched in the upper part	<i>L. cercinense</i> [T]
27 Leaves flat, glaucous, mucronate, with 1-3(5) nerves; inflorescences < 30 cm, branched in the lower part	<i>L. formosum</i> [T]
28 Leaves reddish glaucous, with apex acute	29
28 Leaves glaucous, with apex obtuse to rounded	30

29 Inflorescences < 25 cm; fertile branches reaching 4 cm; outer bract with obtuse apex; calyx > 5 mm	<i>L. clupearum</i> [T]
29 Inflorescences reaching 55 cm; fertile branches reaching 10 cm; outer bract with acute apex; calyx < 5 mm	<i>L. oblongeolatum</i> [T]
30 Calyx < 5.3 mm; corolla pale reddish violet, diameter < 6 mm	<i>L. lacertosum</i> [T]
30 Calyx > 5.3 mm; corolla reddish violet, diameter > 6 mm	<i>L. pyramidatum</i> [T]
31 Leaves with indistinct nerves; corolla whitish	<i>L. tunetanum</i> [ATL]
31 Leaves with 1-10 nerves; corolla violet	32
32 Leaves ended by an apical tip > 1 mm	33
32 Leaves only mucronate or mucronulate	37
33 Spikes lax > 3 cm, with < 5 spikelets/cm	<i>L. menigense</i> [T]
33 Spikes dense < 3 cm, with > 5 spikelets/cm	34
34 Leaf length < 10 x 2.2 cm, with apex acute	<i>L. steppicum</i> [T]
34 Leaf length > 10 cm and/or leaf width > 2.2 cm, with apex obtuse to round	35
35 Few short, branched sterile stems; leaves < 10 cm	<i>L. neapolense</i> [T]
35 Generally no sterile stems; leaves reaching 15-20 cm	36
36 Spikes rather lax reaching 3-4 cm, with < 6 spikelets/cm	<i>L. delicatulum</i> subsp. <i>orientale</i> [AT]
36 Spikes dense < 1.5 cm, with > 6 spikelets/cm	<i>L. byzantium</i> [T]
37 Leaves with < 3 nerves	38
37 Leaves with > 3 nerves	40
38 Spikes > 2 cm; spikelets > 7 mm; calyx > 5.5 mm; corolla diameter > 7.5 mm	<i>L. korbousense</i> [T]
38 Spikes < 2 cm; spikelets < 7 mm; calyx < 5.5 mm; corolla diameter < 7.5 mm	39
39 Spikelets > 5 mm; corolla diameter > 6 mm	<i>L. confertum</i> [T]
39' Spikelets < 5 mm; corolla diameter < 5 mm	<i>L. tritonianum</i> [T]
40 Inflorescences < 50 cm, branched in the lower part; leaves mucronate; spikes with > 8 spikelets/cm	<i>L. comosum</i> [T]
40 Inflorescences > 50 cm, branched in the upper part; leaves mucronulate; spikes with < 8 spikelets/cm	41
41 Leaves reddish glaucous; spikelets < 5 mm	<i>L. rubescens</i> [ATL]
41 Leaves glaucous; spikelets > 5 mm	42
42 Leaves with apex acute, mucronulate; spikes with 4-7 spikelets/cm	<i>L. kairouanum</i> [T]
42 Leaves with apex obtuse, mucronate; spikes with 2-5 spikelets/cm	<i>L. tacapense</i> [T]

Biogeography and endemism

Notwithstanding its small surface area, Tunisia hosts on its own 43 taxa of *Limonium*. This is the highest diversity recorded in North Africa, compared to Morocco (36), Algeria (28), Libya (17) and Egypt (13) (Dobignard & Chatelain 2010). The distribution of *Limonium* in Tunisia (Electronic Supplementay File1, Fig. S1) reveals that this great diversity results mainly both from the length of the Tunisian coasts in relation to the country's surface area and the wide range of bioclimates they cover, from the arid Saharan bioclimate to the humid Meso-Mediterranean one.

In addition, the genus *Limonium* is strongly distinguished from the other genera of the flora of Tunisia, being the richest in endemics. Indeed, the overwhelming majority of its taxa are strictly endemic to the country (Domina & El Mokni 2019) (T, 26 taxa, ca. 62 %; Fig. 1). This exceptional endemism rate, ranks the country second in North Africa after the Canary Islands (82,6%) (Dobignard & Chatelain 2010). Except for *L. boitardii*, described

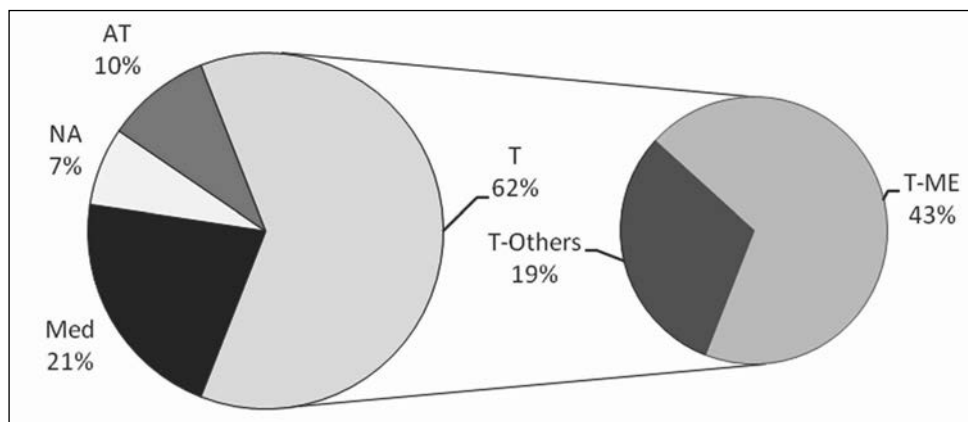


Fig. 1. Chorological spectrum of Tunisian *Limonium* endemic taxa: Tunisian endemics (T), including micro-endemics (T-ME) and other Tunisian endemics (T-Others), Algero-Tunisian endemics (AT), North African endemics (NA) and Mediterranean endemics (Med).

in the 1930s (Maire 1937), all endemic species have been described recently (Pignatti 1982; Brullo & Erben 1989; Erben 2001; Bartolo & al. 2003; Brullo & Giusso del Galdo 2006; Sefi & al. 2020). Among these 26 taxa, 18 are restricted to small geographical areas and present significant conservation issues (ESF1, Fig. S2). Such microendemics (T-ME, ca. 43 %) probably result from long-term apomixis and hybridization events (Palop-Esteban & al. 2007; Brullo & Erben 2016). Considering exclusive endemics and those shared with conterminous countries, the total (T and AT endemics) represents ca. 71% of all *Limonium* taxa occurring in Tunisia.

The remaining taxa include 3 North African endemics (including Maghrebi, Algero-Tuniso-Libyan and Maghrebo-Libyan; NA, ca. 9 %) and 9 more widely distributed taxa (Med, ca. 21 %). The latter include 5 species with a wide Mediterranean range (*L. awei*, *L. echioides*, *L. narbonense*, *L. sinuatum* and *L. virgatum*), one Ibero-North-African species (*L. bonduellei*) and 3 species with particularly interesting distributions: *L. lobatum*, a Mediterranean-Irano-Turanian species, has the largest distribution of the *Limonium* taxa present in Tunisia; *L. bellidifolium* occurs exclusively in southern Europe with the exception of Tunisia (where it is located only in the NE, near Radès and Hammam-Lif; Pottier-Alapetite 1981), suggesting past immigration from Sardinia; and finally, *L. pruinatum* occurs from Syria and Lebanon to Libya and Tunisia, the latter being the western limit of its range.

Insular and peninsular areas are particularly noteworthy, due to the increased speciation process that leads to high levels of endemism (Whittaker & Fernández-Palacios 2007; Kier & al. 2009). Their role as a long-term climatic refuge area has also promoted speciation in the genus *Limonium* within the Mediterranean (Brullo & Erben 2016). Among the Tunisian endemic species of *Limonium*, 7 are strictly endemic to the islands and peninsulas: *L. cercinense* (Kerkennah archipelago), *L. formosum* (Djerba island), *L. lacertosum* (Cap Serrat and Cap Negro peninsulas), *L. steppicum* (Djerba island and Zarzis peninsula), and *L. chupeanum*, *L. korbousense* and *L. zeugitanum* (Cap Bon peninsula). Moreover, 7 others are mainly distributed

on islands and peninsulas, but reach the nearby continental coasts: *L. confertum*, *L. gouge-tianum*, *L. intricatum*, *L. menigense*, *L. pescadense*, *L. pyramidatum* and *L. zembrae*.

Chorology and ecology

The distribution of *Limonium* taxa according to the Tunisian physiographic subdivisions (ESF1, Fig. S1) highlights their mainly coastal ecology, as well as the richness of the northern coasts. While the Northeast (NE), the Center (C) and the South (S) each harbor 16-17 taxa (*i.e.* 40 % of all *Limonium* taxa in Tunisia), the Cap Bon (CB) and Mogods (Mog) appear to be the richest regions if their relative areas are considered (ESF1, Fig. S1). Besides, it is interesting to emphasize the frequency of occurrences of Mediterranean taxa, characterized in Tunisia by wide distributions, spanning over 4 to 5 geographical regions. These are particularly *L. avei*, *L. echioides* and *L. virgatum*, as well as the Mediterranean-Irano-Turanian species *L. lobatum* (ESF1, Fig. S3). On the other hand, the analysis of the ecological requirements of Tunisian *Limonium* taxa (Fig. 2) shows a significant preponderance of halophytes (Hal, 79 %) *versus* indifferent taxa (Indif, 5 %) and non-halophytes (NHal, 16 %). Commonly referred to as ‘sea lavenders’, *Limonium* species indeed occur more frequently in salty habitats, such as salt marshes and sea coasts.

Nonetheless, some taxa stand out markedly, being more or less indifferent to salinity. Firstly, *L. alleizettei* and *L. pruinsum* can develop in both salty and non-salty steppe environments, with however a predilection for gypsum outcrops (Pottier-Alapetite 1981). Secondly, *L. avei*, *L. bonduellei*, *L. echioides*, *L. elfahsanium* and *L. kairouanum* are non-halophilous taxa of arid environments. The two latter are microendemics restricted to a single area, namely the regions of El Fahs (TD) and Kairouan (C) respectively, in matorrals on silty-sandy substrates for the first taxon, and around dry depressions on arid soils for the second one (Brullo & Erben 1989; Brullo & Giusso del Galdo 2006). Finally, two taxa (*L. lobatum* and *L. tacapense*) develop along the banks of wadis in more or less freshwater habitats.

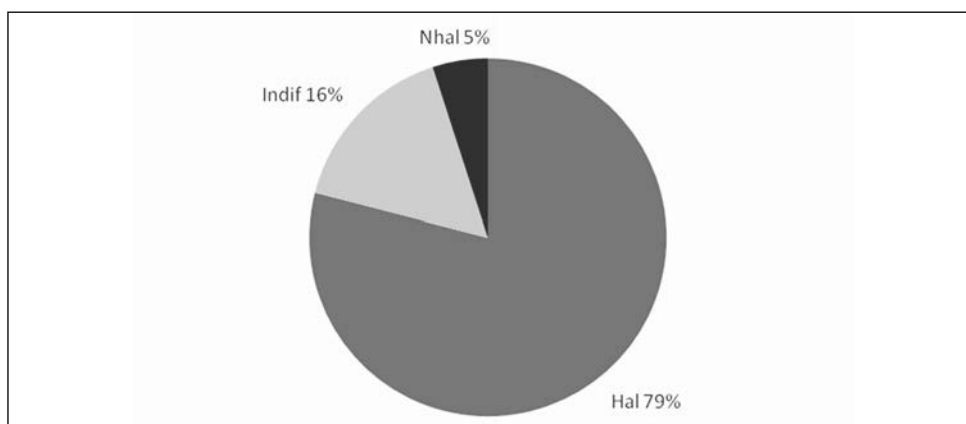


Fig. 2. Ecological spectrum of Tunisian *Limonium*: Halophytes (Hal), Indifferent (Indif), Non-halophytes (Nhal).

The *Limonium* taxa occurring in Tunisia are mainly perennial herbs (hemicryptophytes, 81 %) or dwarf shrubs (chamaephytes, 9 %). The other four species (*L. avei*, *L. bonduellei*, *L. echioides* and *L. lobatum*) are annuals (therophytes, 9 %). The prevalence of perennial taxa (ca. 90 %) can be explained by the salinity and aridity that characterize the coastal habitats of most *Limonium* taxa, namely saltmarshes and seashores (Brullo & Erben 2016; Sefi & al. 2020).

Conservation

Assessment

The current state of the art on the Tunisian *Limonium*, supplemented by recent field surveys, has allowed to assess at the regional level 21 % as Least Concern (LC), 5 % as Near Threatened (NT) and 50 % as Threatened, the remaining 24% being classified as Data Deficient (DD) (Fig. 3; ESF1, Table S1). Threatened taxa encompass the following categories: 33 % Vulnerable (VU), 10 % Endangered (EN) and 7 % Critically endangered (CR). During this assessment, the IUCN criteria and thresholds most applied were B1 and B2, related to the geographical distribution as indicators for either extent of occurrence (EOO) and/or area of occupancy (AOO). These criteria led to assess 7 taxa as CR (3) and EN (4). Criterion D was also used to appraise mainly microendemics (13 taxa) with a restricted population, such as *L. kairouanum*, *L. korbousense*, *L. menigense*, *L. neapolense*, *L. punicum*, etc.

Threats

Preferentially growing in coastal regions, the *Limonium* taxa assessed as Threatened (e.g. *L. cercinense*, *L. gougetianum*, *L. hipponense*, *L. intricatum*, etc.), are potentially imperiled by the deterioration of their habitat quality. These habitats, generally with easy

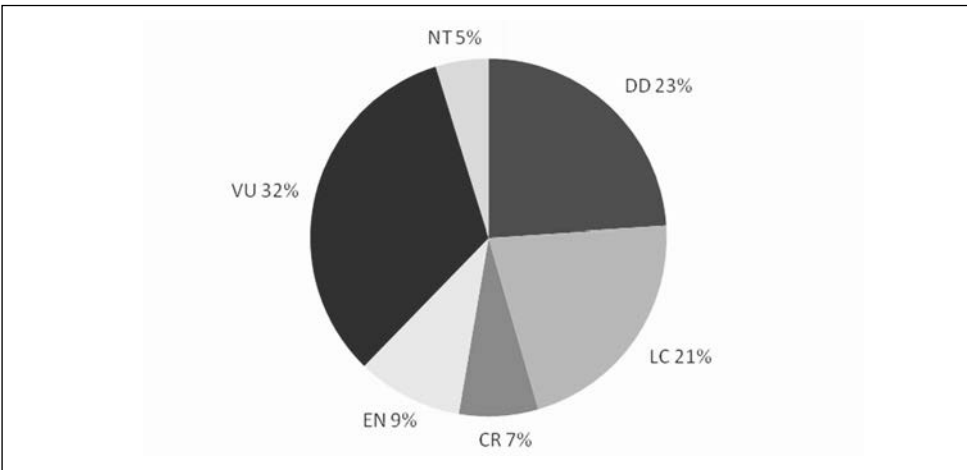


Fig. 3. IUCN Red List categories of *Limonium* occurring in Tunisia: Critically Endangered (CR), Data Deficient (DD), Endangered (EN), Least Concern (LC), Near Threatened (NT) and Vulnerable (VU).

access (coastal fringes, roadsides, urban areas), are severely affected by anthropic disturbances, mainly due to anarchic and illegal urbanization, uncontrolled tourism (recreational activities) and the extension of touristic facilities (Threats 1.1, 1.3, 6.1), leading to a decrease, even a fragmentation and loss of the habitat. The negligence of the inhabitants and users of the sites make them generally polluted and altered by waste of all kinds (Threat 9.4). Overgrazing (Threat 2.3), drought (Threat 11.2) and desertification (Threat 11.1) of steppes (Le Houérou 2001) also threaten some taxa, such as *L. formosum*, *L. kairouanum*, *L. korbousense* and *L. steppicum*. Such threats, evidenced in Tunisia (Ghrabi-Gammar & al. 2020; Ben Charfi & Numa 2021), have also been reported elsewhere, for instance concerning *L. duriaei* in Algeria and Morocco (Rhazi & Grillas 2010) or *L. strictissimum* in Corsica and Sardinia (de Montmollin & Strahm 2005).

Eleven taxa are included in Key Biodiversity Areas (KBA) and Important Plant Areas (IPA), and of these, 7 are enclosed in protected areas (National Parks and/or Nature Reserves) (Table 2). Some of them, namely *L. gougetianum*, *L. intricatum*, *L. pescadense*, *L. pyramidatum* and *L. zembrae*, grow in rocky habitats, generally difficult to access. These habitats are little threatened (except by drought) and are therefore relatively well preserved, constituting a refuge for these Tunisian microendemics (Pavon & Véla 2011; Véla & Pavon 2012; Greuter & Domina 2015).

Finally, 6 taxa develop in sites included in the Ramsar convention. Nevertheless, it should be brought to mind that the designation of a Ramsar site does not guarantee any particular protection, this being the prerogative of national legislation (Matthews 2013). These 6 taxa together with the remaining 29 ones do not benefit from any conservation action and continue to be heavily impacted by human activities. It is therefore, increasingly urgent to undertake conservation actions in order to abate these threats and to protect fragile habitats, especially when they host microendemic species such as *L. formosum* growing on the island of Djerba.

Djerba 'la douce', the island of Limonium

Djerba, the largest island (30 km long and 20 km wide) on the North African coast, is located in Southeastern Tunisia. It is a mythic island, known as the 'lotophagi island' in Homer's *Odyssey* (late 8th century BC). The lôtós, which according to Homer is a delicacy so sweet that it made Ulysses' companions forget everything, would be the fruit of the native *Ziziphus lotus* (Ballabriga 1968). This legend could explain the name '*Djerba la douce*' (i.e. Djerba the sweet), which also evokes the mildness of the island's climate.

From a scientific point of view, Djerba is a remarkable island area, constituting a 'climatic refuge' favorable to the process of speciation, hence its richness today in microendemics such as *Limonium*. It belongs to the arid Thermo-Mediterranean bioclimatic belt and is characterized by halophilous and gypso-halophilous coastal habitats. It is already endowed with three Ramsar sites, and soon a fourth is being included. Most of the island is a sandy plain rarely exceeding 20 m above sea level, edged by mobile and fixed dunes, lagoons flooded with saltwater, and some outcrops of sandstone and clay-limestone rocks. In addition to its three Ramsar sites, Djerba is classified as a *Key Biodiversity Area* (KBA), *Important Plant Area* (IPA). (Valderrabano & al. 2018). It is home to a rich and diversified vascular flora, estimated to 408 taxa (Vanden Berghen 1981), with 7 Tunisian endemic taxa including 5 *Limonium* species. Recent botanical investigations allowed us to update the

data on the 10 *Limonium* inhabiting the island and to describe an eleventh one, also present on the conterminous Zarzis peninsula (*L. steppicum*; Sefi & al. 2020). Two species (*L. formosum* and *L. menigense*) are strictly endemic to Djerba. According to the available data, *L. menigense* is considered as endangered (EN). Nevertheless, it was not found again during our recent fieldworks, and is therefore possibly extinct. Historical locations need to be tracked accurately to verify if the species still exists.

The microendemic species of *Limonium* are mostly enfeoffed to coastal wetlands and dunes. The inclusion of these habitats in the Ramsar list and their belonging to the Maritime Public Domain provides them with some theoretical protection (prohibited construction), but illegal urbanism, uncontrolled touristic activities and the resulting pollution represent high threats, especially for the fragile habitat of *L. formosum*. This species is restricted to a small sandy and rocky coastal area confined between two hotels and threatened by a project for the construction of a seaside tourist attraction. An *ex-situ* multiplication trial was set up, in order to reintroduce it into other similar habitats on the island; training sessions were conducted for associations active in the protection of biodiversity to monitor population and habitat trends of this taxon.

An initiative to create a natural micro-reserve for the *in-situ* protection of the island's microendemic was developed. It has only resulted in the elaboration and adoption of a participatory action plan for its protection by the representatives of different structures (*Agence de Protection et d'Aménagement du Littoral*, *Direction Générale des Forêts*, municipality, tourism office, local associations), which intervene on the site at regional and national levels. Nevertheless, this is not enough to guarantee the protection of the species. For a concrete and effective protection, this participatory plan must necessarily be imple-

Table 2. Tunisian *Limonium* taxa occurring in Ramsar sites (RS), National Parks (NP), National Reserves (NR), Key Biodiversity Areas (KBA) and Important Plant Areas (IPA).

Taxon	RS	Protected Areas	KBA	IPA
<i>L. boitardii</i>		NP - NR	x	x
<i>L. cercinense</i>	x		x	x
<i>L. confertum</i>	x			
<i>L. formosum</i>			x	x
<i>L. gougetianum</i>		NR	x	x
<i>L. intricatum</i>		NR	x	x
<i>L. menigense</i>	x		x	x
<i>L. pescadense</i>		NP - NR	x	x
<i>L. pyramidatum</i>		NR	x	x
<i>L. sinuatum</i>		NP	x	x
<i>L. steppicum</i>	x		x	x
<i>L. thaenicum</i>	x			
<i>L. zembrae</i>		NP - NR	x	x
<i>L. zeugitanum</i>	x			

mented and followed by the creation in the near future of a micro-reserve. The active involvement of all stakeholders, including the local community, is a guarantee of sustainable protection of this microendemic of the island, especially from the urbanization project that threatens its only habitat.

Conclusion

While being the smallest country in North Africa, Tunisia hosts the highest diversity in *Limonium* taxa. This may be related to the north-south orientation of the coasts that span from the arid Saharan to the humid Meso-Mediterranean bioclimate. The only other country with a comparable geography is Morocco, which has almost as many taxa as Tunisia (Dobignard & Chatelain 2013). The main differences between the two countries that could explain the greater richness of Tunisia are probably the physiography of their southern steppe regions, which are occupied by vast sandy and rocky plains dotted with salt lakes (chotts) and only small reliefs (jbels) in Tunisia (Le Houérou 1959), while they are much more mountainous and devoid of chotts in Morocco, as well as the location of Tunisia, at the crossroads between the biogeographical influences of the Sahara, the Tyrrhenian region and the western and eastern basins of the Mediterranean. Nevertheless, the number of recent discoveries in Tunisia suggests that meticulous survey work in other North African countries would lead to a probable increase in the number of *Limonium* species.

This second synthesis for Tunisia after that of Brullo & Erben (1989), is therefore only a first step towards the knowledge of the genus *Limonium* in North Africa. Our analysis of the different features related to biogeography, endemism, chorology and ecology corroborates previous studies carried out in the Mediterranean (Pignatti 1962; Brullo 1978, 1980; Artelari 1984; Brullo & Erben 1989, 2016; Arrigoni & Diana Corrias 1993, 1999), as to the complexity of the genus *Limonium* and to the preponderant role that geographical and ecological isolation plays in its diversity and speciation.

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J. A. Buhagiar, M. Iannaccone & A. Lamoliere

A vulnerability index for successful eradication of IAPs targets interventions at their most vulnerable stages

Abstract

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Invasive alien plant species (IAPs) are non-native plant species whose introduction and spread outside their natural range poses a threat to biodiversity. Their removal is often financially and environmentally costly from repeated interventions. Tackling IAPs at their most vulnerable stages can be a cost-reducing and effective strategy. A vulnerability index has been devised to plan an IAPs removal strategy based on attacking the most vulnerable stages of a species and focuses on the ease and effectiveness of removing aerial and root biomass as well as propagules. The need for repeated interventions is also factored in the vulnerability index since this forms part of the weakening strategy.

Key words: alien species, invasiveness, plant management.

Introduction

Anthropogenic impacts on natural habitats including Natura 2000 (N2K) sites, are a major concern for the Maltese Islands. A huge economic burden arises from the intentional or unintentional introduction of invasive alien plant species (IAPs) (EC 2011, 2019; Egoh & al. 2014). IAPs are establishing and spreading in many parts of the world, including the Mediterranean Basin which hosts some 13000 endemic plant species, causing the decline of indigenous biodiversity, and having negative impacts on ecosystems, the economy and human health (Fritts & Rodda 1998; Myers 2000; Miller & Hobbs 2007; Pimentel & al. 2011; Lockwood & al. 2013). IAPs are invasive to different degrees, and an eradication strategy depends in part on propagule pressure comprising propagule size and frequency of introductions, as well as difficulty to permanently remove the biomass of the established IAPs including their rooting apparatus. For certain species, eradication is straightforward and a one-time exercise but for others, it becomes a daunting task that needs several months if not years of repeated interventions (Kohli & al. 2004; Hulme 2007; Hulme & al. 2009). Ziller & al., (2020) proposed a priority-setting scheme for the management of IAPs in protected areas based on three criteria – species risk of invasion, invasion stage and

species frequency. But this only goes so far as to decide which IAP takes priority for intervention. Though much has been written on strategies for eradication of IAPs, it appears that not much importance has been given to tackling effective and cost-cutting eradication of IAPs at their most vulnerable life cycle stages. The importance of choosing the correct strategy for eradication of IAPs at their most vulnerable stages varies by family and species knowing fully that IAPs are vulnerable to different degrees. It is therefore imperative to identify their strengths and weaknesses and much akin to warfare, attack the IAPs at their most vulnerable life cycle stages.

Vulnerable stages may relate to both life cycle stages and choosing the right time of the year when to tackle certain actions focused on weakening the plant to reduce growth, as well as vegetative and propagule spread, is crucial. IAPs tissue composition is a prime vulnerability consideration aimed at reducing growth and biomass. Thus, the initially weak herbaceous growth in invasive climbers such as *Cardiospermum* sp. pl. is vulnerable to wilting when the main stem is severed (Gildenhuis & al. 2013; Chapman & al. 2017) whereas leaf and stem succulence in species of the genus *Aptenia*, *Carpobrotus*, *Aloe* and *Opuntia* render them vulnerable to rotting when cut and rolled or piled in a heap without need of removing biomass off-site thus helping to reduce effort and cost. Reduced propagule spread by cutting out inflorescences and harvesting seeds before maturation and dispersal, the prompt removal of tillers and suckering or creeping offshoots, as well as eradication of seedlings before establishment, are all strategies that target vulnerable stages. Conversely, IAPs with a dormant soil seed bank, root suckering, bulbil formation and sub-soil rhizomes are harder to eradicate and require multiple strategies and interventions for complete eradication. Tackling these vulnerable life cycle stages at the appropriate time helps to reduce chances of reinvasion, and speed up the eradication process.

IAPs have been recorded for the Maltese Islands from at least 19 families with the number of species constantly growing due to the unrestricted importation of exotic plant species for the horticultural trade and use as landscaping ornamentals (MEPA 2013; Roy & al. 2020; EPPO Global Database). Additional invasions have resulted from the past use of exotic species for a rapid greening strategy of the Maltese Islands in the 1970s. The aim of the project was to investigate the use of different IAPs removal strategies targeting the most vulnerable stages for the eradication of a selection of IAPs. Experience gathered from different eradication strategies helped to establish a vulnerability index for a range of IAP species, including the three species selected for intervention sites, as part of the SiMaSeed INTERREG V-funded seed bank and habitat restoration project.

Materials and Methods

Our project focused on the removal of IAPs in eight different plant families shown in Table 1, using a multidisciplinary approach mainly divided into three phases: Assessment, Intervention and Monitoring. The assessment started with species identification and a search in the European and Mediterranean Plant Protection Organisation (EPPO) database to establish the recorded invasiveness of the IAPs across Europe. This was followed by a preliminary assessment of the extent of invasiveness in the chosen N2Ks intervention site. One such site was Wied Babu, an incised valley and associated garrigue in the limits of

Zurrieq Malta which is shown in Fig. 1. It is good to note that we focused on four IAPs in three families occurring on this site, though other IAPs were present as well. The extent and rate of IAPs invasion in N2K sites were next studied using historical and more recent aerial survey photographs, the oldest dating back to 1957, satellite images and drone surveys followed by walk through ground-truthing and digital imaging. Information was additionally gathered from interviews with stakeholders, all of which contributed precious information on the rate of spread. A study of the IAPs’ growth characteristics and reproductive cycle including its phenology, helped to identify growth, anatomical and tissue vulnerabilities as well as to calculate propagule load in order to assess the extent of a potential invasion by these IAPS if left unchecked. Crucially it allowed us to identify, assess and prioritise the most vulnerable stages. These studies served as a preparation for the intervention phase by assigning a vulnerability index as to how easy or difficult the eradication process will be. Their eradication vulnerability index was calculated using a 10-point scale, subdivided into five vulnerability categories. The least vulnerable and hence most difficult to eradicate was assigned a 1-2 value, whereas the most vulnerable and hence easiest to eradicate being assigned a 9-10 value as shown in Fig. 2. The intervention stage involved applying the best strategy for each IAPs removal using informed management decisions based on the vulnerability index followed by monitoring over a period of several months to assess the success of our intervention strategy.

In order to assign an IAP to one of the vulnerability categories, five critical contributors to the vulnerability index shown in Fig. 3, were selected namely:

- A. ease of weakening the plant to reduce its above-ground growth;
- B. ease of weakening the plant to reduce its below-ground growth;
- C. ease for collection of propagules;
- D. ease of eradication of soil seed bank and/or uprooting of seedlings or rhizomes;
- E. ease of suppression of regrowth and re-invasion from residual propagules.

Each contributor was ranked with a value of 2, 1 or 0 with the critical contributor value of 2 signifying a high vulnerability and therefore an easy IAP removal task for that contribution, 1 signifies a moderate vulnerability contributor and 0 signifies a low vulnerability contributor and therefore a difficult IAP removal task. Based on the contributor assessment, the established index allowed different strategies to target the most vulnerable stages.

Table 1. List of invasive alien plant species (IAPs) studied with families and common names.

Plant Family	Invasive Alien Plant Species	Common Name
Aizoaceae	<i>Carpobrotus acinaciformis</i> (L.) L. Bolus	Hottentot Fig
Asparagaceae	<i>Agave Americana</i> L.	Century Plant
	<i>Agave sisalana</i> Perrine	Sisal
Euphorbiaceae	<i>Ricinus communis</i> L.	Castor Oil Plant
Fabaceae	<i>Acacia saligna</i> (Labill.) Wendland	Blue-leaved Wattle
	<i>Leucaena leucocephala</i> (Lam.) de Wit	Lead Tree
Oxalidaceae	<i>Oxalis pes-caprae</i> L.	Cape Sorrel
Poaceae	<i>Arundo donax</i> L.	Giant Reed
Sapindaceae	<i>Cardiospermum grandiflorum</i> Swartz	Balloon Vine
Simaroubaceae	<i>Ailanthus altissima</i> (Mill.) Swingle	Tree of Heaven

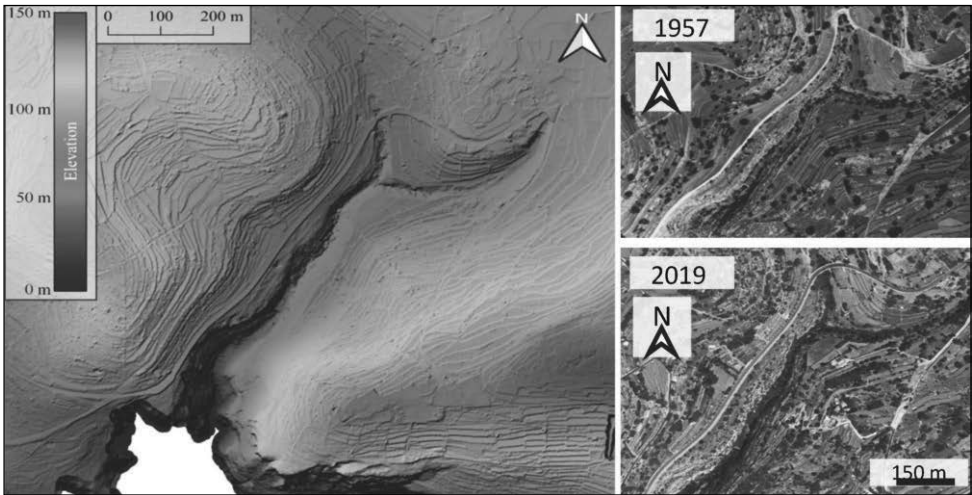


Fig. 1. QGIS elevation map of the Wied Babu, N2K site (left). Cloudisle elevation ranges from 0 m (purple) to 150 m (red). Historical aerial photos of Wied Babu from 1957(top right) when still cultivated and from 2019 (bottom right) when valley bed had become almost completely covered with a canopy of the IAP climber species *Cardiospermum grandiflorum*. Retrieved from PA Mapping unit and Google Earth (2021) respectively.

Results and Discussion

A comparison of the extent of invasion of four IAPs and the different strategies employed for their removal from N2K sites is given in detail below. Other IAPs are also included though these apply for very small trials and associated observations. All interventions related to removal of IAPs were carried out during the period June 2020 to June

1-2	3-4	5-6	7-8	9-10
Very low vulnerability VERY DIFFICULT TO ERADICATE	Low vulnerability	Medium vulnerability	High vulnerability	Very high vulnerability VERY EASY TO ERADICATE
Soil Bulbils & Rhizomes	Root fragmentation and suckering	Floating seeds and Soil seed banks	Bulbous plants	Leaf, stem and root Succulents

Fig. 2. Vulnerability index for eradication of IAPs subdivided into five vulnerability categories with 1-2 showing least vulnerability and therefore highest difficulty to eradicate and 9-10 showing the highest vulnerability and therefore the greatest ease for eradication. Examples are given for each vulnerability category.

	Contributors to Vulnerability Index	2 = High	1 = Moderate	0 = Low	TOTAL
A	Removal of above ground biomass				
B	Removal of below-ground biomass				
C	Collection of propagules				
D	Uprooting of seedlings/Rhizomes				
E	Suppression of regrowth/reinvasion				

Fig. 3. Five contributors to the vulnerability index of an IAPs marked A to E in table. A vulnerability contributor value of 2 indicates the species has high vulnerability in that contributor category, 1 a moderate vulnerability contributor whilst 0 indicates very low vulnerability for that contributor.

2021. The intervention stage initially focused on simple management decisions such as if seeds or other propagules could be collected before they are dispersed, at what stage would such a collection be made efficiently and with the minimum of labour input? Wherever possible, if seeds or other propagules were available for collection, this was the first practical intervention made followed by removal of above-ground biomass whilst minimizing labour and negative intervention impacts such as trampling on site. Our interventions were mostly dedicated to individuals of *Agave sisalana* Perrine, *A. americana* L., *Acacia saligna* (Labill.) H. L. Wendl. and *Cardiospermum grandiflorum* Sw. on several test plots in the Wied Babu and other N2K disturbed habitat sites. A description of eradication actions on these three species studied in detail follows together with how the vulnerability index of these three species was arrived at. The value of the different contributors to the vulnerability index is indicated in Table 2. Further data are given in Fig. 4. The vulnerability index of six other species has also been included though data for this is limited to field observations for limited trials.

Agave sisalana and *A. americana* growing over much of the upper reaches of the Wied Babu valley, limits of Zurrieq and an N2K site, were planted as an ornamental plant or possibly as a preventive barrier against accidental falls into the valley some six to five decades ago. The original mother plants have formed large clonal stands through extensive suckering each covering areas of several square meters. Furthermore, the mature mother plants produce tall inflorescences (totem poles) which when mature are covered with seed capsules and aerial bulbils that arise as a modification of the flower bud somatic tissue through vivipary (Frankel & Galun 1977). The monocarpic mother plant will eventually die but its suckers as well as the bulbils shed before or after the tall inflorescence collapses, will persist and contribute to further colony expansion. The seeds, though dispersed, are not known to germinate in our environment. The strategies employed for the local eradication of this species involved attacking its most vulnerable stages namely the removal of the developing inflorescence before the bulbils are formed and systematically cutting the fibrous blade-like leaves at their bases to reveal a cylindrical trunk. The cutting of the leaves at their bases may sound like a formidable task but in effect, this is difficult only in so far as removing the first few succulent leaves using a hand saw followed by severing the rest around the trunk as illustrated in Fig. 3a. The removal of the developing inflorescence at an early stage of its formation when it is some 1 to 2m tall will save a lot of work in the eradication strategy since postponement will lead to the development and dispersal

Table 2. Table showing the value of the different contributors to the vulnerability index of nine IAPs. Five vulnerability categories are shown with the most vulnerable to eradication in green and the least vulnerable to eradication in red with additional notes on vulnerable stages.

IAP Species & Contributor Value	A	B	C	D	E	Index	Ease of Eradication	Vulnerable stage of intervention
<i>Carpobrotus acinaciformis</i>	2	2	2	2	2	10	Very Easy	Severe plant from roots, roll up and allow to rot
<i>Agave sisalana</i>	2	2	2	2	1	9	Easy	Remove totem, propagules, leaf bases and offsets
<i>Leucaena leucocephala</i>	2	1	2	2	1	8	Moderately Easy	Collect pods when still green, severe tree at base
<i>Cardiospermum grandiflorum</i>	1	1	2	2	1	7	Needs persistence	Collect capsules, severe & uproot stem, seedlings
<i>Ricinus communis</i>	1	1	1	1	1	5	Difficult	Collect semi ripe inflorescence and seed capsules
<i>Acacia saligna</i>	2	0	0	1	1	4	Difficult	Lop off main trunk, remove tillers & seedlings
<i>Ailanthus altissima</i>	1	0	1	1	0	3	Very Difficult	Lop off trunk and suckers before leaf emergence
<i>Oxalis pes-caprae</i>	1	0	0	1	0	2	Extremely difficult	Use herbivores and light exclusion to weaken plants
<i>Arundo donax</i>	1	0	0	0	0	1	Extremely difficult	Cut off canes & remove new shoots

of some 600 bulbil propagules per mother plant, all sufficiently developed and ready to establish since they have fully formed roots and leaves. Collection of these propagules is easy if they have just fallen but will be time-consuming if they root. Mature totem poles may also have capsules with seeds but locally at least, the seeds are not known to germinate. The next task is the removal of the rooted stump of the mother plant and all clonal suckers. This is a time-consuming task but not hard to perform with the right tools. Removal of the voluminous biomass offsite may be a challenge. Since it is easy to overlook suckers, periodic site visits to uproot overlooked plantlets need to be carried out. The vulnerability index contributors for this species have been assigned a value of 2 for vulnerability categories A to D and 1 for category E giving a total vulnerability index of 9 and thus classifying the eradication of this species as easy.

Cardiospermum grandiflorum Swartz growing in the dry river valley section of Wied Babu is by far the most invasive. Its climbing habit drapes a continuous cover, thus blotting out light and weakening the underlying indigenous arborescent species including *Ceratonia siliqua*, *Pistacia lentiscus* and *Ficus carica* as well as threatening native assemblages of smaller indigenous perennials. Its vine-like stem allows it to spread, rooting wherever this touches the ground further fuelling the invasion but the species also produce a huge seed load most of which germinates readily. Eradication strategies involved two, time-consuming but not labour-intensive strategies, one of which again centres on the reduction of propagule dispersal as a vulnerable stage in the life cycle of this species. Mature seed capsules were collected when still wet from rain or dew (early in the morning) thus reducing seed dispersal from an otherwise fragile capsule that dehisces easily when dry. This helped to reduce the further shedding of seeds in the intervention area. The collection of seedlings about three to four weeks after periods of heavy rain and concurrently severing main stems of mother plants or rooted suckers some 30 cm above the ground not only kills the climbing vines but allows an easy handle for uprooting the remaining poorly-anchored root apparatus. All remaining aerial parts were left to wither and decay on the supporting shrubs. Based on the time-consuming but not labour-intensive work involved in the eradication of *C. grandiflorum*, vulnerability contributors A, B and E were assigned a value of 1 each whilst C and D were assigned a value of 2. This species, which has therefore been

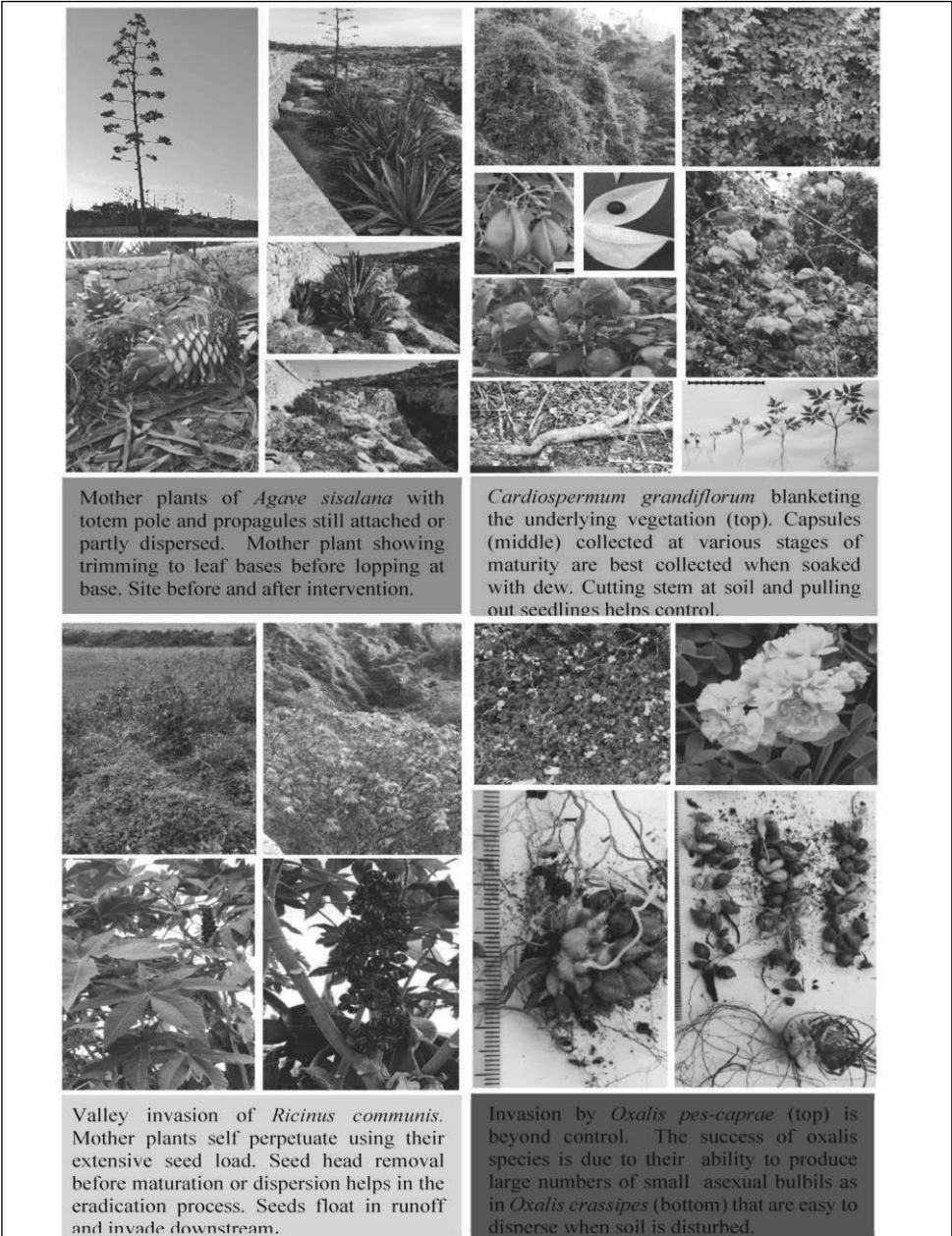


Fig. 4. Morphological and life cycle stages for four different IAPs on which the invasiveness vulnerability index was based. From left to right, *Agave sisalana*, *Cardiospermum grandiflorum*, *Ricinus communis* and *Oxalis pes-caprae*. Different types of propagules produced by the species were key considerations to arrive at an informed management decision followed by morphological and tissue characteristics of above and below ground biomass. The colours correspond to four out of five vulnerability categories with green showing the most vulnerable to red showing the least vulnerable to eradication.

assigned a total vulnerability index of 7, is placed between moderately easy and difficult to eradicate since eradication requires frequent, repeated, and persistent interventions for seed collection, seedling uprooting, severing of the main and removal of the remaining rooted stump.

Acacia saligna (Labill.) Wendland was introduced in the 1970s as part of a fast greening project for the Maltese Islands and though the original scheme called for the use of these fast-growing and resistant species as nurse trees for the indigenous but slower-growing trees, this part of the scheme was never put into practice. The *Acacia* trees have persisted contributing to invasions, especially after the mother tree dies out naturally or after burning. The reason for this is the presence of an extensive soil seed bank under the mother tree which is stimulated to germinate only after the mother tree ceases to produce ethylene gas that acts as a seed germination inhibitor. Whereas for the previous two species, eradication focused on the vulnerability of the propagule production and dispersal stages, this does not apply here for even if the seed pods are collected before they dehisce, the soil seed bank will not be eliminated. As indicated earlier, once the main trunk of the mother plant is sawed off at ground level, ethylene production is suppressed and the seed bank is activated. Thus, since seed germination is triggered by a decline in ethylene production, it renders the species vulnerable to eradication if timed close to the start of the rainy season in September, where germinated seedlings can subsequently be easily uprooted. If the cutting down operation is timed before the onset of the dry season, then the stage is set to subsequently eradicate the plant using two fronts. Several tillers may be produced from the remaining stump but these can be easily pulled off when still young. This process will help to remove the source of sugars needed to nourish the roots which will eventually die. The summer drought will also help to desiccate the exposed soil further weakening the remaining root apparatus. On the other front, the seeds forming the soil seed bank will now readily germinate with the first rains in September being a warm and humid period of the year. These seedlings will grow rapidly and need to be uprooted in their entirety when about 20 to 30 cm high. One may try to uproot earlier but these may break off and if left to establish beyond 30 cm they will be hard to uproot or incompletely so. It is good to note that the seeds germinate in waves and the seed bank may persist for several years after the removal of the mother tree. The tiller removal and seedling uprooting process need to be repeated at frequent intervals over many months if not years. This species has been assigned a total vulnerability index of four comprised of a 2 for contributor A, two 0s for contributors B and C and two 1s for contributors D and E. This makes the species difficult to eradicate compared to the previous two. It is good to note that different environmental situations may call for different eradication strategies. Bachetta & al. (2018) refer to the use of germination-inhibiting sheeting (and indirectly light-excluding) to suppress regrowth, as well as sieving to reduce the seed bank but this may well apply for *Acacia* species growing on sandy beaches which would be difficult to apply on soil.

Amongst the other species included in Table 1, it is good to comment on the vulnerability index of two species that are extremely difficult to eradicate namely *Oxalis pes-caprae* and *Arundo donax*. The latter has become a highly invasive plant in valleys whilst the former which has overrun the entire Mediterranean basin and beyond, is now considered an impossibility to eradicate. And for both, the low vulnerability of their propagule stage is key. Though *Oxalis*

suffers from repeated disturbance as when the soil is tilled in cultivation or when grazed upon by goats and sheep, it will thrive when left undisturbed and the production of a huge number of clonal bulblets renders it impossible to eradicate. Likewise, *Arundo donax*, whose subsurface rhizomes are very hard to uproot and even have the capacity to survive burning. With a vulnerability index of 2 and 1 respectively, the lack of appropriate vulnerable stages renders the two species extremely difficult to eradicate.

Conclusion

The use of a vulnerability index for IAPs removal helps to provide an informed management decision to plan a strategy for eradication. Key conclusions from this exercise are:

- A species may have multiple or a limited number of vulnerable stages;
- IAPs with a limited number of vulnerable stages are most difficult to eradicate;
- A good knowledge of the biology of IAPs especially their phenology helps successful eradication;
- Planning interventions to coincide with vulnerable stages can reduce work effort and expenses;
- Plants with a vulnerability index of 1-2 will need repeated eradication procedures and possibly mechanical and chemical intervention, though the latter is the least desirable.
- Finally, it is good to stress that the vulnerability to eradication for different IAPs must be considered in the context of the specific habitat as well as the prevailing environmental and climatic conditions in which that IAP is found. Therefore, these results/conclusions cannot be automatically generalised to a wider context but refer only to the conditions observed in Malta and only to species considered in this study.

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Contribution to the study of the plant diversity in communities with *Quercus alnifolia* in Cyprus

Abstract

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The Cyprus endemic species *Quercus alnifolia* grows at an altitudinal range of 600–1525 m, mainly as a shrub. It is one of the most ecologically important endemic species of Cyprus. This species forms communities that extend throughout the entire Troodos Mountain range, and it appears either in pure stands, or in mixture with other taxa or even, as an understorey in pine stands. The priority habitat type of Annex I of the Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora "Scrub and low forest vegetation with *Quercus alnifolia* - 9390", is characterized by arborescent *Q. alnifolia*-dominated formations (pure stands). This habitat type occurs in the central Troodos range, where it has a relatively wide distribution. In the present study, a detailed inventorying of the plant communities with *Q. alnifolia* has been realised in three different Natura 2000 sites of Cyprus: National Forest Park of Troodos, "Koilada Kedron - Kampos", and Machairas Forest. Surveys to sample the plant composition and communities at field scale and at elevations ranging from 1070 m to 1380 m, were performed during 2020 and 2021. An average number of 19 different plant taxa have been registered on each one of the 36 sampling plots. 107 plant taxa have been recorded on the sampling plots and an analysis of the floristic composition has been conducted. Low intensity pressures have been recorded on *Q. alnifolia* communities, across the sampling areas. The conservation status of the communities with *Q. alnifolia* is favourable and significant regeneration of the species has been recorded. The need to monitor these forests and their intrinsic floristic diversity, is underlined since there seems to be a gap in the Mediterranean vegetation survey recent scenario.

Key words: endemism, endemic habitat type, Troodos Mountains, vegetation.

Introduction

Quercus alnifolia Poech (Poech 1842: 12) is a Cyprus endemic plant species (Anagiotos & al. 2012), which grows on volcanic geological substrate and its presence is copious in the central hilly area of Troodos (Neophytou & al. 2011), on altitudes of 400–1.800 m (Loizides 2011). Concerning its taxonomy, *Q. alnifolia* was traditionally placed in Sect. *Cerris* (Camus 1938; Schwarz 1936), but it groups with the closely related species *Q.*

aucheri and *Q. coccifera* and belongs to ilicoid oaks (Sect. *Ilex*) (Menickij, 1984; Denk & Grimm 2010); as a putative ancestor of the kermes oaks (*Q. coccifera* s. l.) (Hipp & al 2020). Sclerophyllous oaks of *Quercus* Group *Ilex* (Denk & Grimm 2010) are an emblematic feature of many relevant Mediterranean forest habitats (Blondel & Aronson 1999) and currently include the two narrow Eastern Mediterranean endemics, *Q. alnifolia* Poech. and *Q. aucheri* Jaub. et Spach. and the widespread *Q. ilex* L. and *Q. coccifera* L., four sympatric species that share ecological preferences (Vitelli & al. 2017). This new taxonomical position of *Q. alnifolia* is very important, since its ecological positioning served as a major driver for evolution and this is mandatory to understand the entire communities with *Q. alnifolia*, with major outputs for biodiversity conservation.

Quercus alnifolia is an endemic high evergreen shrub (usually multi-branched) or small tree that can reach up to 10 m (EUR27–European Commission. 2007; Loizides 2011; Anagiotos & al.2012). It has been appointed as the national tree of Cyprus and it has been listed in the IUCN Red List of Threatened Species as Least Concern in 2015 (Gorener & Beech 2017). *Q. alnifolia* is also protected by the national forest law and by the Habitat directive 92/43/EEC (Anagiotos & al. 2012). This species is of great ecological importance to Cyprus, as it is one of the most essential components of the forest landscape in the greater Troodos Mountain range; it also prevents soil erosion and its acorns are a major source of nutrition for fauna and micro-fauna (EUR27–European Commission. 2007; Petrou & al. 2015). *Q. alnifolia* forms pure stands (habitat type 9390*) or it is established as understorey to stands of *Pinus nigra*, *Pinus brutia*, *Cedrus brevifolia* and other Mediterranean shrubs (Loizides 2011; Petrou & al. 2015; Milios & al. 2021).

The habitat type “Scrub and low forest vegetation of *Quercus alnifolia* (9390)” is an endemic and a priority habitat type of Annex I, Directive 92/43/EEC (EUR27–European Commission 2007). This habitat type occurs in the central Troodos range, where it has a relatively wide distribution (PLANT-NET CY Project, n.d.), it can be found on rocky, steep, and smooth mountain slopes, mainly on diabase as a substrate (Andreou & al. 2010). Through the project “Establishment of a Plant Micro-Reserve Network in Cyprus”, a plant micro-reserve that hosts a representative stand of this habitat type has been established within the Natura 2000 site of “Koilada Kedron-Kampos” (PLANT-NET CY Project, n.d.; Kadis & al. 2010). Among the main pressures affecting this habitat type are recreational activities, and disturbance of natural habitats (PLANT-NET CY Project, n.d.).

A review of the available literature focusing on the plant communities of Cyprus and on those with *Quercus alnifolia*, shows that more phytosociological research is needed since even studies that are focused on mountainous ecosystems are limited (Barbero & Quézel 1979, 1989; Costa & al. 1984; Géhu & al. 1990; Brullo & al. 1997; Brullo & al. 2001, 2005; Sotiriou & Gerasimidis 2009; Sotiriou 2010; Wagensommer 2017; Şekerciler & Ketenoğlu 2019). Concerning communities with *Quercus alnifolia*, Barbero & Quézel (1979) described the alliance *Quercion alnifoliae* and the associations *Querco alnifoliae - Pinetum brutiae* Barbéro & Quézel 1979 and *Querco alnifoliae-Crepidetum fraasii* Barbéro & Quézel 1979. Preislerová & al. (2022) mention the alliance *Quercion alnifoliae* Barbero et Quézel ex Bergmeier, Mucina & Theurillat in Willner & al. (2015), that is an alliance endemic to the island of Cyprus, but shares many species with woody formations of *Quercus*, *Pinus*, *Pistacia* and *Styrax* elsewhere in the Eastern Mediterranean (Willner & al. 2015). Mesomediterranean evergreen endemic golden oak forests of Cyprus (Mucina &

al. 2016) belong to this alliance and are present on dry, alkaline oligotrophic-mesotrophic substrate and present late spring-summer phenological optimum and late-successional status (FloraVeg.EU 2022).

Sotiriou (2010) and Sotiriou & Gerasimidis (2009) showed that *Quercus alnifolia* participates in the following plant associations: i) *Quercus alnifoliae-Pinetum brutiae*, ii) *Quercus alnifoliae-Pinetum pallasianae*, iii) *Quercus alnifoliae-Crepidetum fraasii*, iv) *Paenion masculae-Pinetum pallasianae*, v) *Sorbo creticae-Pinetum pallasianae* and v) *Alno orientalis - Platanetum orientalis*. In the first three associations, *Q. alnifolia* participates as a characteristic species and those associations, belong to the class *Quercetea ilicis* Br. - Bl. ex de Bolos y Vayreda 1950 and to the order *Quercetalia ilicis* Br. - Bl. 1936 em. Rivas – Martinez 1975.

This study has been conducted in the framework of a thesis concerning to the comparison of plant and invertebrates communities in formations with *Quercus alnifolia* and it aims to contribute to the available knowledge on plant diversity in plant communities with *Quercus alnifolia* in Cyprus, in three Natura 2000 protected areas. This study aims at contributing to a detailed inventory of the plant taxa that participate in the plant communities of *Q. alnifolia* on the “Chrysovrysi” linear trail at the National Forest Park of Troodos, on the part of the European E4 trail that traverses the site “Koilada Kedron - Kampos” and on Machairas Forest.

Materials and Methods

Study Areas

Three study areas (Fig. 1) were selected for the study of the composition of *Quercus alnifolia* communities. They belong to the Mediterranean Biogeographical Sub-Region (Rivas Martinez & al. 2014; Hadjipanayiotou 2017a, 2019a, 2019b) and to the Natura 2000 network of protected areas. The main criterion consisted of the fact that this species is common almost throughout the entire Troodos Mountain Range (TMR). Consequently, the following three sites were selected as they cover the central, the western and the eastern parts of the distribution of the species respectively. The National Forest Park of Troodos (site code: CY5000004) is located in the center of the TMR, the site “Koilada Kedron - Kampos” (site code: CY2000008) is in the central part of Pafos forest (Dasos Pafou), that is located towards the western part of the TMR and Machairas Forest (site code: CY2000004) at the eastern part of the TMR. In the following sections, each study area will be addressed separately.

National Forest Park of Troodos

The National Forest Park of Troodos (NFP of Troodos) (Fig. 1b) has an area of about 9,009 ha (0.97% of Cyprus), it consists of plutonic rocks, and it extends from an altitude of 700 m (Saitas area), to an altitude of 1951 m (Chionistra peak) (Hadjipanayiotou, 2019b). The vegetation of this area is dominated by *Pinus brutia* forests at the lower altitudes and *Pinus nigra* forests at the higher altitudes (> 1200 m) (Game and Fauna Service & Ministry of Interior 2016). Precipitation at the NFP of Troodos spans from 660 mm at the lower parts and surpasses 1100 mm at its highest altitude (Xofis & al. 2020).

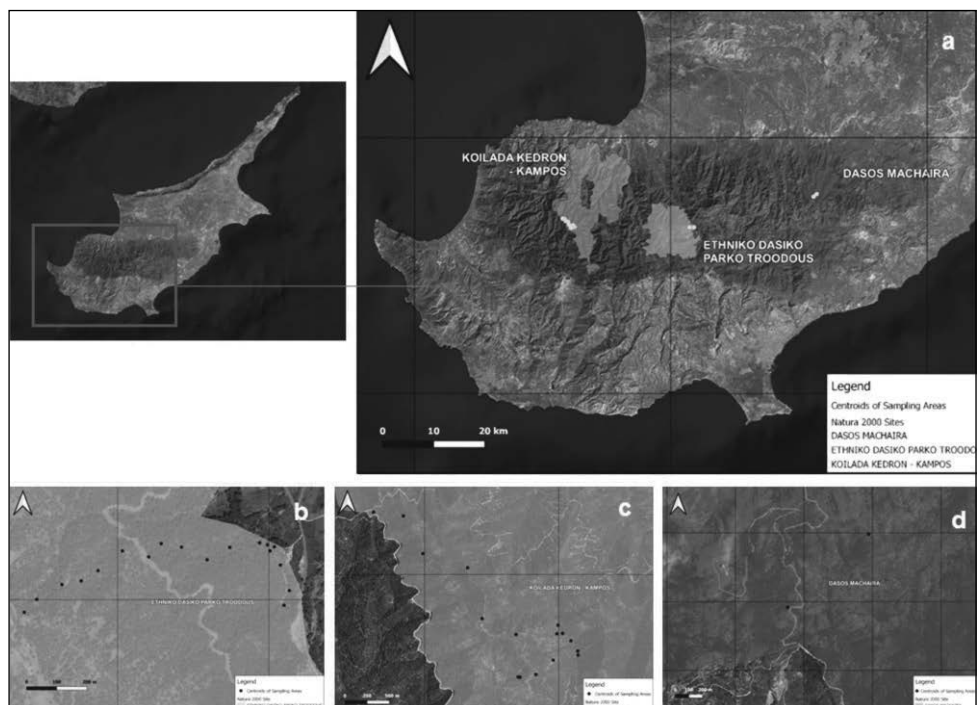


Fig. 1. **a.** The three study areas, on the island of Cyprus and the centroids of the sampling plots. **b.** at the “Chrysovrysi” trail of the National Forest Park of Troodos. **c.** at part of the European E4 trail of the site “Koilada Kedron - Kampos”. **d.** at the site “Machairas Forest”.

Additionally, the Saitas weather station, the hottest month is July and the coldest is February, with average daily temperatures of 26.5 °C and 8.4 °C respectively (Xofis & al. 2020).

The ecological importance of the NFP of Troodos, can be deduced, inter alia, from the fact that it hosts 11 Annex 1 habitat types, of which four, are priority habitat types with good to excellent conservation status (Mazaraki & al. 2013). Also, the habitat type 6460*: Peat grasslands of Troodos can only be found in this specific area and nowhere else in the world (Mazaraki & al. 2013). Moreover, the NFP is the most important forest ecosystem of the island, and it hosts more than 750 plant taxa (40% of the total flora of Cyprus) and 70 of those are Cypriot endemics (50% of the total Cypriot endemics) (Kounnamas & Andreou 2022). Lastly, it has been classified as one of the 13 “Plant Diversity Hot Spots” in the Mediterranean (Hadjipanayiotou 2019b).

Sampling surveys took place at the “Chrysovrysi” linear trail, which extends for ca. 1.5 km, with its starting point just outside of the Amiantos village and ending point approaching the Amiantos Mines. These sampling surveys were realized at specific spatial intervals of 100 m, while alternating left and right, from the previous recording to the next one and they were 18 in number.

Koilada Kedron - Kampos

The area of “Koilada Kedron - Kampos” (Fig. 1.c) is located in the central part of Pafos forest, it occupies an area of 18258 ha, on the diabase rocks of the Troodos ophiolite (Hadjipanayiotou 2017a). Pafos Forest, extends from small altitudes near sea level, to altitudes reaching 1352 m (Tripylos peak). In the greater area of the Pafos forest, the mean yearly rainfall is 1060 mm, the mean daily maximum temperature (August) and the mean daily minimum temperature (December) are 33.6 °C and 3.9 °C respectively (PLANT-NET CY Project, n.d.). *Pinus brutia* forests constitute the dominant vegetation of “Koilada Kedron - Kampos”, as they cover ca. 86% of the area (PLANT-NET CY Project, n.d.), however 12 other habitat types can be found there as well, like the endemic habitat type 9590* (*Cedrus brevifolia* forests) (Christodoulou & al. 2017).

The site of “Koilada ton Kedron-Kampos” has an extensive natural habitat that hosts a large number of species of plants and animals (Hadjipanayiotou 2017a). The endemic species *Cedrus brevifolia* forms the endemic priority habitat type 9590*, which only grows in the Forest of Paphos, on the Troodos Mountain range (Christodoulou & al. 2017). The site hosts 36 plants that are endemic to Cyprus, three of which are listed in Annex II Dir. 92/43/EEC: *Ranunculus kykkoensis*, *Phlomis cypria* and *Arabis kennedyae* (PLANT-NET CY Project, n.d.; Hadjipanayiotou 2017a).

Sampling surveys took place at the part of the European E4 trail that traverses the site “Koilada Kedron - Kampos”. This portion of the trail extends for ca. 6 km. These sampling surveys were realized at specific spatial intervals of 600 m, while alternating left and right, from the previous recording to the next one and they were 16 in number.

Machairas Forest

Machairas Forest (Figure 1d) occupies an area of 4428 ha, it extends from an altitude of 400 m to an altitude of 1423 m (Kionia peak) (Hadjipanayiotou 2019a), and it consists of igneous rocks of the Troodos ophiolite and in particular, of the sheeted dyke complex (diabase) (Tsintides & al. 2012). Pure *Pinus brutia* stands, dominate starting from the lowest altitudes up to the peak of Kionia. Precipitation at Machairas Forest spans from 446 mm to 707 mm, whereas the mean daily temperatures are 30.5 °C and 3.6 °C, for August (hottest month) and December (coldest month) respectively (Hadjipanayiotou 2019a).

The continuous, well-preserved, and natural forest ecosystems and the great variety of habitats within them, highlight the site’s ecological importance (Hadjipanayiotou 2019a). This site hosts 280 plant taxa, out of which 226 are indigenous (13% of the total indigenous flora of the island) and out of which 42 are endemics (29% of the total endemic flora of the island) (Tsintides & al. 2012). Lastly, it hosts almost the entirety of the population of the threatened plant species *Crocus hartmaniannus* (Hadjipanayiotou 2019a). Field surveys at the Machairas Forest took place at the most accessible spots, due to the accessibility restriction caused by very steep slopes.

Figures 1a, 1b, 1c and 1d were produced using QGIS software and shapefiles with all NATURA 2000 sites which were obtained from the European Environment Agency and the Greek Biotope/Wetland Centre (EKBY).

Plant Surveying Methodology

Field surveys to sample the plant species composition of communities with *Quercus alnifolia* at field scale were performed during May to September of 2020 and April to June of 2021, adapted from the Braun-Blanquet approach (Braun-Blanquet 1964, 1979; Rivas-Martínez 2005; Biondi 2011; Pott 2011). Thirty-six sampling plots were surveyed at the trails while alternating left and right, from the previous recording to the next one. In fact, recordings were made at specific spatial intervals different for each study area, as described in the above subsections. Recordings didn't only take place at these equidistant positions within the trails, but also at places where differentiations in plant composition (or coverage) of the communities were observed. At every place of recording, four corners were set, so as to have the exact coordinates of the sampling area and each sampling area had a surface of 100 m².

In order to collect easy-to-use data from the field concerning to the regeneration of *Quercus alnifolia* and the existence of individuals of different age, the vertical structure of the vegetation, was also investigated and partitioned as follows. The tree layer was divided by height into the classes T₁: 20-35 m, T₂: 10-20 m, T₃: 5-10 m and T₄: 0.5-5 m. The shrub layer was divided by height into the classes S₁: 2-5 m, S₂: 1-2 m and S₃: 0.1-1 m. The herb layer was not divided by height, and it includes plant taxa without woody parts, up to a height of one meter. Data recorded in situ at the sampling areas include plant cover, exposure, altitude, incline, chance of encountering stones and gravel, the degree of anthropogenic intervention, the height of vegetation layers, the cover of individual vegetation floors and finally, the degree to which the soil consists of fine grains.

Two hundred ninety-one specimens of plant taxa were collected, dried, and identified. The identification process was mostly carried out using the dichotomous key for plant genera provided by the Flora of Cyprus website (<https://flora-of-cyprus.eu/>), together with Flora of Cyprus (Meikle 1977, 1985). Data concerning vascular plants nomenclature and life-forms (phanerophytes (P), chamaephytes (Ch), hemicryptophytes (H), geophytes (G) and therophytes (T)) follow: Dimopoulos & al. (2013, 2016, <https://portal.cybertaxonomy.org/flora-greece/intro>), Stavrou & al. (2008), Flora of Israel Online (<https://flora.org.il/en/en/>) and Crivellaro (2012).

Results

All data concerning to the plant species composition of communities with *Quercus alnifolia* and the abiotic information for each sampling plot are included in the Electronic Supplementary File 1: Tables S1 and S2. Table S1 presents the vertical structure of the vegetation and reveals the dynamic conservation status of *Q. alnifolia* since very young and young individuals of *Quercus alnifolia* were present on almost all the plots (on the shrub and herb layers) and ESF1: Table S2 presents a global table with all plant taxa registered on each sampling plot and their coverage. The number of taxa registered on each sampling plot is between 8 and 33 (mean number 19.4). The dominant trees height was 10m-35m (mean height 24.7m) on the sampling plots of the “Chrysovrysi” trail of the National Forest Park of Troodos, 5m-30m (mean height 14.6m) on the sampling plots of the European E4 trail of the site “Koilada Kedron - Kampos” and 3m-6m (mean height 4.5m) on the sampling plots of “Machairas Forest”.

Quercus alnifolia communities, recorded across the 36 sampling plots include 107 plant taxa, belonging to 57 families and 91 genera. Among these taxa there are 14 endemic (13.3%) plants, belonging to 11 families and 13 genera. The richest families for all taxa recorded, are *Asteraceae*, *Fabaceae*, *Lamiaceae*, *Poaceae*, *Brassicaceae* and *Rubiaceae* (Fig. 2). These families represent 10.5% of the families and 42% of the taxa registered on all the sampling plots (Fig. 2). For the endemic taxa, the richest is the family of *Lamiaceae* (29%).

Concerning to the life forms of all the taxa registered, therophytes predominate with 35% followed by phanerophytes (26%) and hemicryptophytes (22%) (Fig. 3). There are differences on these proportions regarding the three areas of the sampling plots since phanerophytes are represented by 35% on “Chrysovrysi” trail and 24% on the other two areas, hemicryptophytes by 27% on “Chrysovrysi” trail and 16-19% on the other two areas, as also therophytes are represented by 18% on the sampling plots of “Chrysovrysi” trail, but 44% and 39% on “Koilada Kedron - Kampos” and on “Machairas Forest”, respectively.

The frequency of appearance of the different taxa on the sampling plots of Table S2, is presented on ESF1: Fig. S1. *Quercus alnifolia* may dominate or coexist on the sampling areas together with the tree species *Pinus brutia* or the Cyprus endemic *Cedrus brevifolia*. Among the taxa recorded with high to medium frequency of appearance on the sampling plots are also *Rubia tenuifolia*, *Arbutus andrachne*, *Cistus creticus*, *C. salviifolius*, the endemics *Pterocephalus multiflorus*, *Thymus integer*, *Rubia laurae*, *Scutellaria cypria*, *Arenaria rhodia* subsp. *cypria*, etc. Additionally, Table 1 presents the 14 endemic taxa recorded on the sampling plots and their frequency of appearance on them.

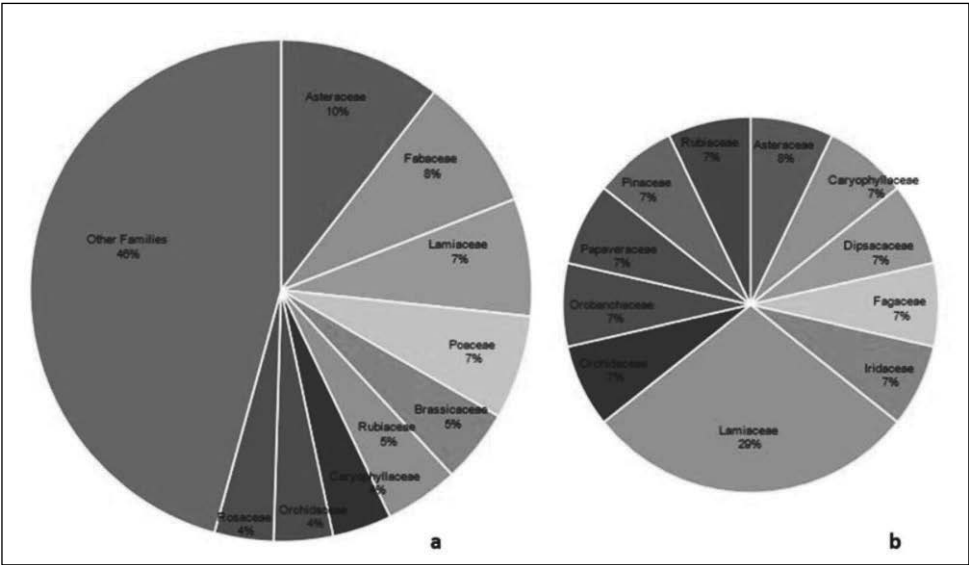


Fig. 2. Taxonomic richness by Family, including all taxa (a) and endemic taxa (b).

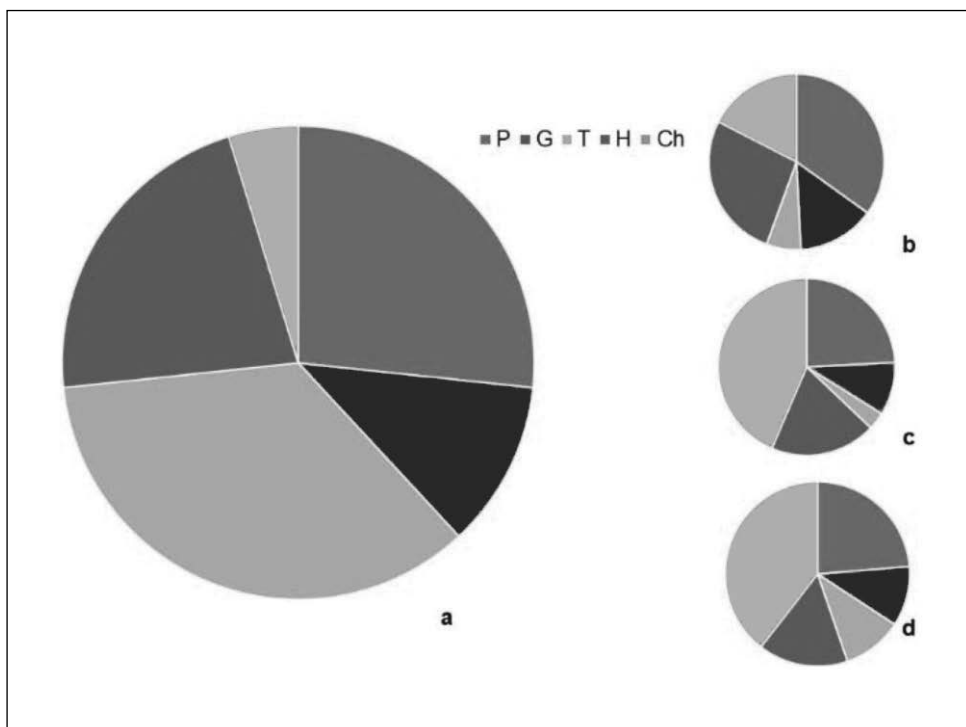


Fig. 3. Life form spectrum of all taxa recorded across **a**, all three study areas, **b**, the National Forest Park of Troodos, **c**, the site “Koilada Kedron - Kampos” and **d**, Machairas Forest. Abbreviations: P = phanerophytes, Ch = chamaephytes, H = hemicryptophytes, G = geophytes and T = therophytes.

Discussion

The island of Cyprus is a part of the Mediterranean Basin Biodiversity Hotspot (Myers & al. 2000), and it is characterized by great biodiversity, as well as a high endemism rate (Şekerciler & Ketenoğlu 2019). This study focused on the Cyprus national tree, the emblematic Cyprus endemic *Quercus alnifolia* and the composition of the plant communities with *Q. alnifolia* at elevations ranging from 1070 m to 1380 m, in three NATURA 2000 areas. The inventory of the composition and structure, together with the existing knowledge, is very important for the monitoring of the conservation status of its populations and of the *Q. alnifolia* dominated communities that form the endemic and priority habitat type 9390 included in Annex I of the Directive 92/43/EEC.

The conservation status of the plant communities with *Quercus alnifolia* on the studied areas, roughly examined in this study, seems to be favourable. Exceptional natural regeneration was observed for the species, where individuals of different age and seedlings were detected across almost all sampling plots that this species was recorded in. Pressures affecting the plant communities of *Q. alnifolia* on the areas studied, are of low intensity and are mainly related to visitors' recreational activities and garbage dumping. It is also

Table 1. Cyprus endemic taxa recorded on the sampling plots, their life-form, the sampling area they were found in (National Forest Park of Troodos, “Koilada Kedron - Kampos” and/or Machairas Forest) and their frequency of appearance on the sampling plots (%).

Family	Taxon	Life-form	Recorded in present study	
			Sampling Area	Frequency (%)
Asteraceae	<i>Lactuca cyprica</i> (Rech. f.) N. Kilian & Greuter	H	KKK	11.1
Caryophyllaceae	<i>Arenaria rhodia</i> subsp. <i>cyprica</i> (Holmboe) McNeill	T	All sampling areas	19.4
Dipsacaceae	<i>Pteroccephalus multiflorus</i> Poech	T	NFP & MF	50
Fagaceae	<i>Quercus alnifolia</i> Poech	P	All sampling areas	100
Iridaceae	<i>Gladiolus triphyllus</i> (Sm.) Ker-Gawl.	G	NFP & KKK	8.3
Lamiaceae	<i>Thymus integer</i> Griseb.	P	NFP & KKK	25
Lamiaceae	<i>Scutellaria cyprica</i> Rech.f.	H	All sampling areas	22.2
Lamiaceae	<i>Scutellaria cyprica</i> subsp. <i>elatior</i> (Meikle) Hand	H	All sampling areas	13.9
Lamiaceae	<i>Mentha longifolia</i> subsp. <i>cyprica</i> (Heinr. Braun) Harley	H	KKK	2.8
Orchidaceae	<i>Epipactis troodi</i> H. Lindb.	G	KKK	2.8
Orobanchaceae	<i>Odontites cypricus</i> Boiss.	C	NFP	5.5
Papaveraceae	<i>Papaver paphium</i> M. V. Agab. & al.	T	KKK	5.5
Pinaceae	<i>Cedrus brevifolia</i> (Hook. f.) A. Henry	P	KKK	8
Rubiaceae	<i>Rubia lauræ</i> (Holmboe) Airy Shaw	H	NFP	8

noteworthy that about 9.2% of Cyprus’ endemics plants can be found on the area studied taking part on the structure of the communities with *Q. alnifolia*.

Quercus alnifolia can colonize remote rocky slopes with high inclination and *Q. alnifolia* communities contribute to soil stabilization and erosion prevention and establish extensive life-friendly environments in areas where other tree species can’t survive (Rivas-Martinez 2005; Sotiriou 2010; Loizides 2011). The number of plant taxa registered on each sampling plot differs, with a mean number of 19 taxa, decreasing when the inclination of the plot and tree/shrub coverage are higher and augmenting when the inclination and the tree/shrub coverage are lower as was expected according *Q. alnifolia* communities’ ecology.

Plant diversity is considerable at the family, genera, species level and in terms of life forms. Sotiriou & Gerasimidis (2009) mentioned reduced proportions of therophytes in sampling plots from their phytosociological study on the National Forest Park of Troodos, a result similar to the results of this study, where therophytes have a percentage of 18% since phanerophytes and hemicryptophytes predominate with 62%. Stavrou & al. (2008) in their research on the site “Koilada – Kedron Kampos” found that therophytes was the dominant life-form (49.76%), which is the case for this study as well (44%), however the proportions of phanerophytes and hemicryptophytes together are lower than those of the National Forest Park of Troodos and represent 43% of the taxa recorded. It’s important to state that therophytes are quite abundant in these forests and this fact enhances the probability of forest areas being more interventioned or perturbed, and that the lower frequency of nanophanerophytes reflects a problematic around land use changes, that drive vegetation from the climaxic stages.

The presence of pre-forest mantles with *Arbutus andrachne*, *Crataegus monogyna* and some climbing taxa like *Asparagus acutifolius*, *Rubia* sp.pl., *Rubus sanctus*, *Smilax aspera*, *Clematis cirrhosa* etc. enhances the forest typology and near-climax dynamics. This is incremented also by the presence of trees like *Acer obtusifolium*, *Cedrus brevifolia*, *Juniperus oxycedrus* subsp. *oxycedrus*, *Prunus* sp. pl., etc. The debate around being an edaphoxerophile vegetation series is also notorious by the presence of taxa like *Pistacia terebinthus*, *Quercus calliprinos* and the frequency of low shrubs like *Cistus* sp. pl. It is fundamental to mention that communities with *Quercus alnifolia* follow the edaphoxerophile concept of vegetation series brought by Rivas-Martinez (2005) since forest association and vegetation series of *Quercus alnifoliae* - *Crepidetum fraasii* are related with the ultrabasic soils habitat and the climax of an edaphoxerophile series inside the geoseries complex. This complex includes the forest of *Q. boissieri* (*Anagyro foetidae*- *Quercetum boissieri*) in the bottom slope where there are well developed soils with no ecological limitations, while the *Quercus alnifoliae* - *Crepidetum fraasii* is positioned alongside the also edaphoxerophile *Cedrus brevifolia* and *Pinus brutia* forests where they get intermixed in substrate permutations. Milios & al. (2021) mentioned the rare for Europe and the mediterranean, climax communities of the two Cyprus endemics *C. brevifolia* and *Q. alnifolia*. They proposed the development of silvicultural treatments for the conservation of the narrow Cyprus endemic *C. brevifolia* natural formations, also to areas where groups of *Q. alnifolia* sprouts (multi-stemmed plants) and seedlings – saplings of *Cedrus brevifolia* growing in most cases under the above and side shade of *Q. alnifolia*, with respect to both species' ecology. The development of site index curves for classification of *Q. alnifolia* trees to site qualities could be useful for the rational management and monitoring of natural habitats of the species (Petrou & al. 2015).

Detailed studies of plant communities' composition and structure contribute to the conservation planning of shrublands and forest ecosystems (Stavrou & al. 2008). The most ecologically important endemic species of Cyprus and a rare endemic relict with low genetic diversity (Vitelli & al. 2017), *Quercus alnifolia*, and its habitat type are unique in Europe and the Mediterranean. The need to monitor these forests and their intrinsic floristic diversity, seems to be a gap in the Mediterranean vegetation survey recent scenario and for this reason further and continuous studies, concerning monitoring and assessment of the conservation status of the species populations and plant communities, for their sustainable conservation, are crucial.

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Population characteristics of *Juniperus drupacea* (Cupressaceae) at the westernmost marginal area of its world distribution (Mt. Parnon, Greece)

Abstract

Daskalakou, E. N., Oikonomidis, S., Boutsios, S., Ioannidis, K. & Thanos, C. A.: Population characteristics of *Juniperus drupacea* (Cupressaceae) at the westernmost marginal area of its world distribution (Mt. Parnon, Greece). — Fl. Medit. 32: 305-316. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Juniperus drupacea Labill. is a relict, dioecious tree/shrub found in Turkey, Syria, Lebanon and Israel, while in Europe it is native only in Greece (on Mt. Parnon and in a restricted area of Mt. Taygetos). According to the IUCN Red List criteria, *J. drupacea* has been assessed as endangered (EN) for Europe, with major threats being grazing of saplings, overexploitation of its wood and climate change. For the first time to our knowledge, the structure of the diverse stands of *J. drupacea* across its entire population range on Mt. Parnon is being studied. Thirteen monitoring plots (20 × 25 m) have been selected and established to cover fully both the species' geographic distribution on Mt. Parnon as well as the heterogeneity of its habitats. The morphometric traits (gender, canopy height, canopy base height, diameter at breast height and number of trunks) for each individual within the plots were recorded during the monitoring periods of 2020 and 2021. The Female / Male individual ratios, in both the northern and the southern area of the species' range deviate significantly from the expected ratio 1:1 of the evolutionary stable strategy, it should be noted that from the 729 individuals recorded 254 were juveniles. Canopy height may reach 11.5 m with a DBH up to 132 cm whereas individuals are usually multi-trunked (1-9 trunks were recorded). The average tree density was 1121.5 ± 139.8 individuals per hectare for the entire mountainous population range.

Key words: biodiversity, conservation, Syrian juniper, sustainable management, population structure.

Introduction

Junipers are slow-growing and long-lived evergreen small trees or shrubs, well adapted to dry environments and found in a variety of habitats, from sea level to above the timberline (Adams 2014). *Juniperus* (Cupressaceae) is one of the most diverse genera of conifers and includes ca. 75 species (Adams 2014; Dörken 2019), which are widely grown in temperate and subtropical regions of the Northern Hemisphere south to tropical Africa (Bonner 2008), with ca. 20 taxa distributed in Europe, Canary Islands, Azores, Asia Minor and Africa (Adams 2014).

Caryocedrus is one of the three, putatively monophyletic *Juniperus* sections and it is represented exclusively by *Juniperus drupacea* Labill. This species is well separated from its congeners on the basis of both molecular data and its unique morphological features, i.e. the unusually large seed cones [(up to 3 cm in diameter), with three ovules fused and forming a hazelnut-like “stone” at maturity], in addition to the inflorescence-like structure of male reproductive units (Dörken 2019).

Juniperus drupacea is a relict tree/shrub species, with distinct male and female individuals (dioecious). Its global geographical distribution is relatively limited and fragmented, mostly in SE Turkey, Western Syria, Israel and Lebanon (Boratynski & al. 1992). In Europe, it is found exclusively in SE Peloponnese, Greece, in Mt. Parnon and in a restricted area of Mt. Taygetos, where it forms small patches (Tan & Iatrou 2001; Bergmeier 2002; Dimopoulos & al. 2013). Greece is a well known hotspot for plant diversity in Europe and the Mediterranean area, showing impressively high endemism (Georghiou & Delipetrou 2010; Dimopoulos & al. 2013); in particular, the Peloponnese area is considered one of the 10 Mediterranean Basin hotspots, on the basis of plant endemism and richness (Médail & Quézel 1999). All over the country, junipers are represented by 8 taxa: *Juniperus communis* L., *J. drupacea*, *J. excelsa* M. Bieb., *J. foetidissima* Willd., *J. macrocarpa* Sm., *J. oxycedrus* L., *J. phoenicea* L. and *J. sabina* L. (Arabatzis 1998; Dimopoulos & al. 2013).

The *Juniperus drupacea* populations in Mt. Parnon are of particular ecological interest as they represent more than 95% of the species distribution in Greece (Constantinidis & Kalpoutzakis 2015). Mt. Parnon juniper forests grow on limestone soils, at an altitude of 700-1600 m a.s.l., and form either pure or mixed stands with black pine (*Pinus nigra* JF Arnold subsp. *nigra*) and the endemic Greek fir (*Abies cephalonica* Loudon) (Maerki & Frankis 2015).

According to the International Union for the Conservation of Nature (IUCN), *J. drupacea* is globally classified as ‘Least Concern’ (LC) (Gardner 2013); however, in Europe, it is considered Endangered (EN) (Gardner 2017; Rivers & al. 2019), as it is represented only by the Greek populations; at national level, the species is protected by the Presidential Decree 67/1981. Moreover, the species is the main structural plant of the subtype 42.A5 (Syrian juniper woods) of the priority habitat “9560* - Endemic forests with *Juniperus* spp.”, according to the Directive 92/43/EEC (Anonymous 2007). The greater area of Mt. Parnon has been included (GR2520006) in the European Natura 2000 Network of protected areas, a testimony of the importance of these natural juniper stands and their protection. Furthermore, the species seems to be threatened by climate change and the adoption of specific strategies is suggested for the conservation of its genetic and morphological diversity in the future (Walas & al. 2019).

The discontinuous geographical distribution of the species and the long isolation between the mountainous populations of SE Asia areas and the European ones, separated by the Aegean Sea and an estimated distance of almost 800 km (Tan & al. 1999), is also reflected to the genetic and morphological differentiation among the disjunct populations of the species (Sobierajska & al. 2016).

Therefore, taking into consideration the fragmented natural distribution and the predicted reduction of the species worldwide distribution (Walas & al. 2019), as well as the limited knowledge for the Greek populations, this study aims to describe the current *J. drupacea* population and its structure on Mt Parnon.

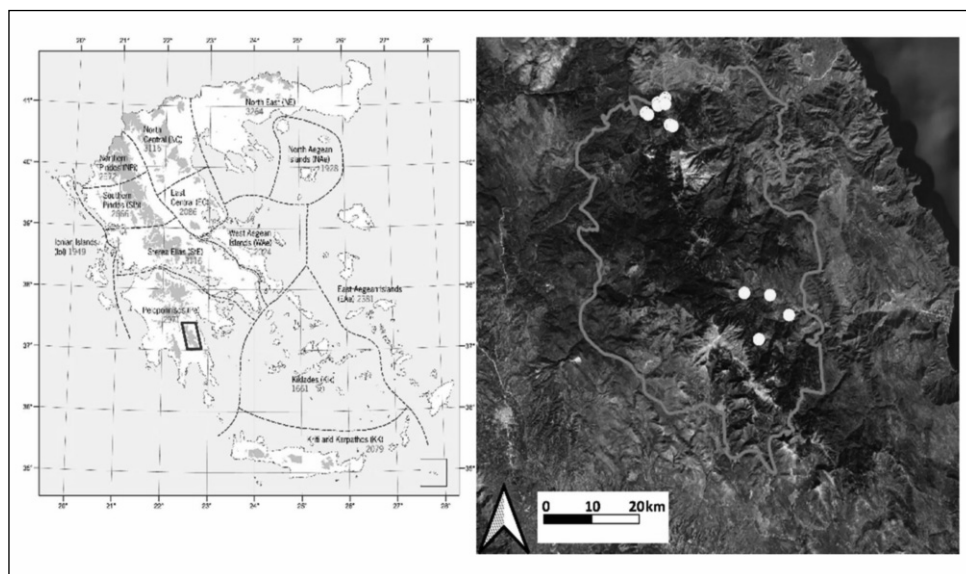


Fig. 1. Map of Greece (with the 13 phytogeographical regions, Dimopoulos & al. 2013) with the study area highlighted (left). Map with *Juniperus drupacea* monitoring plots in the northern and southern areas of Mt. Parnon (right) within the boundaries of the NATURA 2000 site.

Materials & Methods

Study area

Monitoring was carried out on Mt Parnon (Arcadia prefecture, southeast Peloponnese), Greece (Fig. 1). The majority of the monitoring plots were established on the protected area of the Natura 2000 European Network “Mount Parnon (and Malevi area)” - code GR2520006. In the particular area around the Malevi Monastery, juniper trees appear as the dominant species (Maerki & Frankis 2015). The above forest is a unique and extremely sensitive ecosystem at national level and has been declared as a Preserved Monument of Nature (Government Gazette 121/D/21-2-1980) while the wider area has been designated as an Area of Absolute Nature Protection [(Government Gazette 353/6-9-2010) and amendments, according to Government Gazette 60/A/31-3-2011].

Monitoring

Thirteen (13) monitoring plots, of 500 m² area each (20 × 25 m), were established in October 2020 (Table 1): nine (9) plots in the northern and four (4) in the southern area of Mt. Parnon. The plots were located at varying altitudes, exposures and slopes in order to cover the entire geographic range of the species as well as the heterogeneity of its habitats. A total of 729 *J. drupacea* individuals were recorded in all 13 monitoring plots; 550 and 179 trees were tagged in the northern and southern areas of Mt. Parnon, respectively. The morphometric traits, i.e. tree gender, individual height, diameter at breast height (DBH) and the number of trunks for each individual were recorded during the years 2020-2021.

Table 1. Summary table of the individual characteristics of *Juniperus drupacea* for each monitoring plot, including mature and juvenile individuals. Mean values are drawn from the total number of individuals of each monitoring plot and are accompanied by the standard error of the mean. For the calculation of the mean DBH of each site, firstly, we calculated the mean DBH for all the trunks of each individual and then the DBH of the site was calculated.

Location	Site code	DBH, cm	Canopy Height, m	Canopy base height, m	Trunks /individual	Individual s /plot	Density (individuals/ha)
N. Parnon	A1	4.7 ± 0.7	4.1 ± 0.3	0.0 ± 0.0	1.4 ± 0.1	49	980
N. Parnon	A2	7.1 ± 0.4	6.2 ± 0.3	0.0 ± 0.0	1.8 ± 0.1	87	1740
N. Parnon	A3	4.8 ± 0.3	3.5 ± 0.1	0.0 ± 0.0	1.8 ± 0.1	89	1780
N. Parnon	A4	4.7 ± 0.4	3.3 ± 0.2	0.0 ± 0.0	1.4 ± 0.1	76	1520
N. Parnon	A5	5.9 ± 1.0	4.0 ± 0.3	0.0 ± 0.0	1.3 ± 0.1	34	680
N. Parnon	A6	4.7 ± 0.7	4.1 ± 0.2	0.1 ± 0.0	3.8 ± 0.3	55	1100
N. Parnon	A7	5.0 ± 0.8	4.4 ± 0.5	0.0 ± 0.0	1.3 ± 0.2	20	400
N. Parnon	A8	3.7 ± 0.4	2.7 ± 0.1	0.0 ± 0.0	1.7 ± 0.2	69	1380
N. Parnon	A9	5.5 ± 0.5	3.3 ± 0.2	0.0 ± 0.0	1.6 ± 0.1	71	1420
S. Parnon	J1	65.8 ± 8.4	6.6 ± 0.4	1.7 ± 0.1	1.0 ± 0.0	12	240
S. Parnon	J2	20.9 ± 3.4	3.0 ± 0.3	0.1 ± 0.0	1.0 ± 0.0	56	1120
S. Parnon	J3	18.1 ± 1.8	3.4 ± 0.2	0.0 ± 0.0	1.0 ± 0.0	33	660
S. Parnon	J4	28.1 ± 3.2	5.1 ± 0.4	0.9 ± 0.2	1.1 ± 0.1	78	1560

The datasets, corresponding to the northern and southern parts of the mountain, were compared and the observed differences of individual growth traits and gender distribution between the two study areas are presented and discussed.

Statistical analysis

The Shapiro – Wilk normality test revealed, for all the continuous variables, a deviation from the normal distribution and henceforth we used non-parametric tests for assessing the significance of our hypothesis. The Mann-Whitney test was used for the relationship between the continuous variables, while for the gender structure a chi-square test was performed to test the deviation from the value 1:1 (Female : Male), the evolutionary stable strategy (Fisher 1930). Pearson's correlation index was calculated for the continuous variables in order to discover any potential relationships among the biotic variables and two additional abiotic ones, altitude and shadiness of the habitat, where the values for each of the abiotic variables were extracted from raster data of forest cover (Hansen & al. 2013) and from the Copernicus DEM (2016).

Finally, a factor analysis for mixed data (FAMD) was performed with gender, canopy height, mean DBH and number of trunks as its parameters. For the comparison of the DBH data in multi-trunked trees, the mean DBH of all trunks for each individual was used.

For the handling of the entire dataset, the statistical tests and the drawing of diagrams, we used R version 3.6.3 (R Development Core Team 2005).

Results

The number of juniper trees i.e. all individuals including saplings in each monitoring plot ranged from 12 to 89, with a mean value of 56 ± 7 individuals. Therefore, tree density ranged from 240 to 1780 individuals per hectare and by combining all data we obtain an average value of 1121.5 ± 139.8 individuals per hectare, for the entire mountainous population range (Table 1).

A significant proportion (30%) of the individuals examined were found to have multiple trunks (Electronic Supplementary File 1: Fig. S1) and the number of trunks observed ranged up to 9 (only 1 individual with 9 trunks was recorded). The number of trunks per tree was found to differ statistically significantly (Mann-Whitney test: p-value = 0) between the southern and the northern part of the Mt. Parnon. Individuals in the southern part are usually single-trunked (<10% multi-trunked) compared with the population of the northern part where a significant proportion of trees (40%) are multi-trunked.

The Female/Male individual ratios (Fig. 2), in both the northern (F/M = 0.67 ± 0.11 - chi-square test: p-value < 0.001) and the southern (F/M = 1.84 ± 0.81 - chi-square test: p-value < 0.05) plots, deviate significantly from the evolutionary stable strategy ratio 1:1. Male individuals outnumber significantly the female ones in the north and vice versa in the south. It should be also noted that in the northern population, where the individuals are fre-

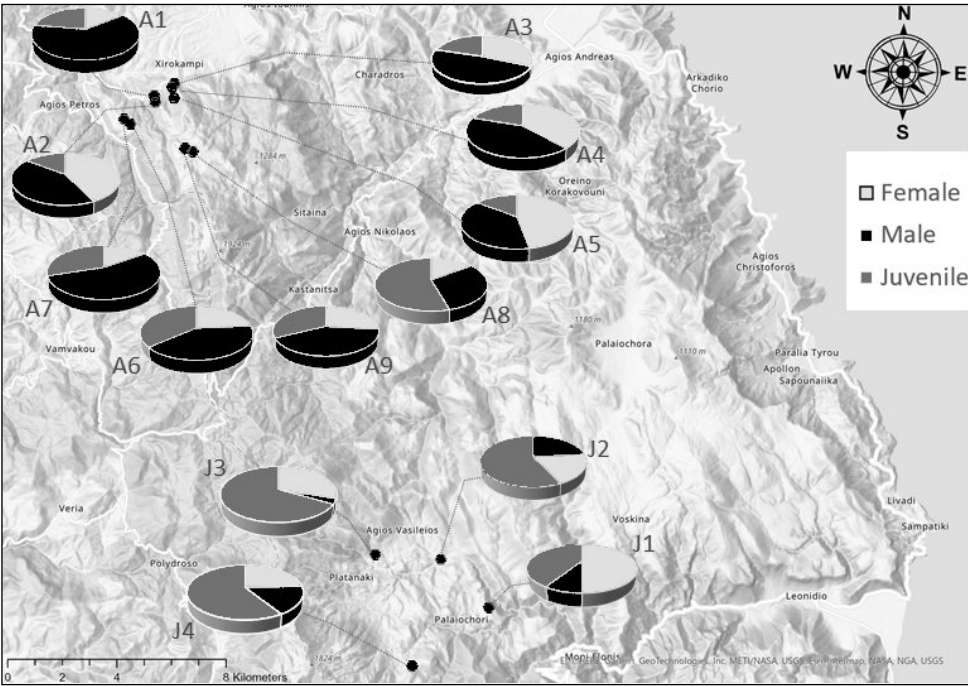


Fig. 2. *Juniperus drupacea* gender contribution (%) amongst the monitoring plots established in the northern and southern areas of Mt. Parnon.

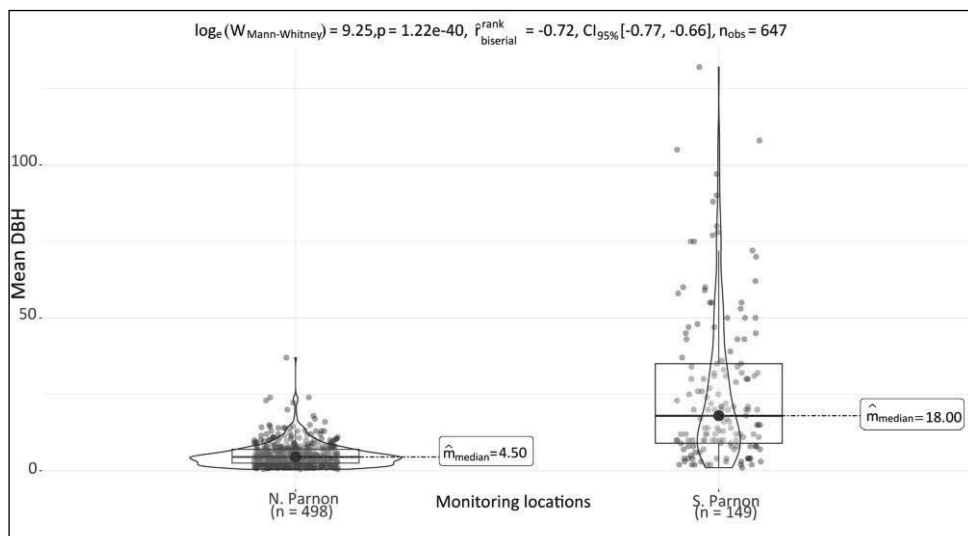


Fig. 3. Statistic plot representing the mean DBH between North and South Parnon along with the results of the non-parametric statistical test.

quently multi-trunked, there are no differences regarding the number of trunks for male and female individuals, while the juveniles are mostly single-trunked (Kruskal-Wallis p -value < 0.001 , Dunn-test p -values < 0.001 for both male-juvenile and female-juvenile).

In regard to DBH (Fig. 3), by comparing the values between the northern and the southern monitoring plots a statistically significant difference (Mann-Whitney test: p -value ≈ 0) was observed. Moreover, the DBH of the individuals of the southern plots was notably about twice as large as that of the northern part of the mountain. On the other hand, the canopy height comparison between the two areas returned a much lower statistical difference (Mann-Whitney test: p -value = 0.044).

The starting lowest level of the canopy (foliage) was also found to differ statistically between southern and northern *J. drupacea* populations (Mann-Whitney test: p -value ≈ 0). Specifically, in most individuals recorded in the northern plots the canopy was starting from the very basis of the trunk. The same phenomenon was rarely seen in the southern monitoring plots, where the canopy was observed to start from a much higher point of the trunk.

In regard to the possible relationships (Fig. 4) between the continuous variables and the two abiotic factors (altitude, canopy shadiness), we observed a significant positive correlation between the shadiness of the monitoring plots and the height of the individuals (Pearson's $R = 0.37$, p -value < 0.001). Negative correlations were observed between the altitude of the plots on the one hand and the canopy height and the DBH on the other (Pearson's $R = -0.15$ and -0.26 , p -value < 0.001 , respectively). Interestingly, positive correlations were also observed between the number of trunks on the one hand and the canopy height (Pearson's $R = 0.27$, p -value < 0.001) and the canopy base height (Pearson's $R = 0.30$, p -value < 0.001) on the other.

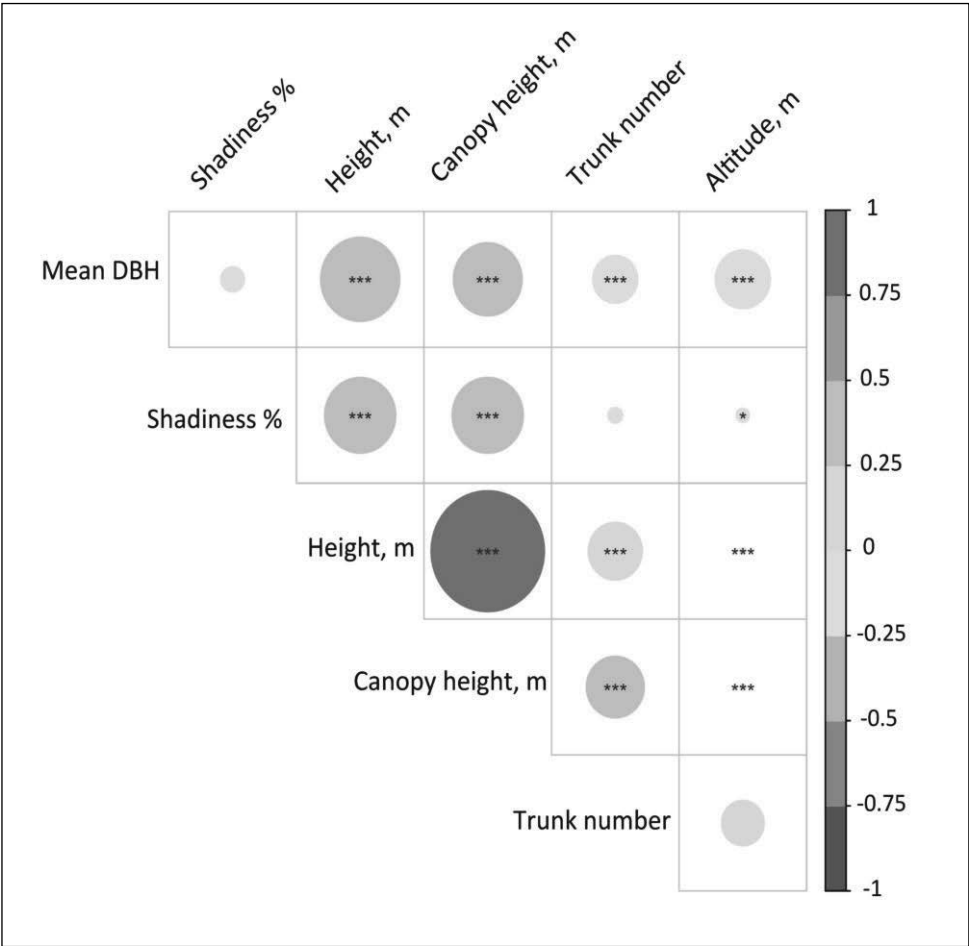


Fig. 4. Correlation plot for the individual characteristics of *Juniperus drupacea* and for two abiotic parameters (altitude and shadiness). The stars inside the circles represent the significance level, while the size of the circles represents the absolute value of the Pearson index.

Lastly, the applied FAMD (Fig. 5), using the variables: number of trunks, DBH, canopy height and gender revealed a significant difference between the northern and the southern part of the population studied. 59.3% of variance is explained by the two first components and 92.2% by the four factors used.

Discussion

Only limited ecological studies have been implemented on *Juniperus drupacea* in the Mediterranean region (Douaihy & al. 2017) and still remain open questions about phenol-

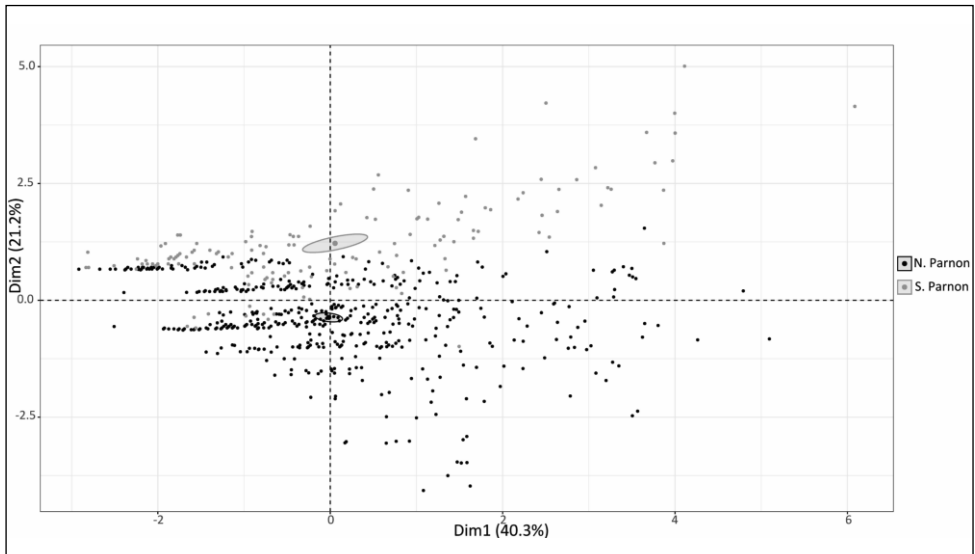


Fig. 5. FAMD plot with the variables used: 1) canopy height 2) mean DBH 3) gender and 4) number of trunks. The grey dots represent the individuals on the south part of Mt. Parnon and the black dots the individuals on the north part. The 95% confidence ellipses are also presented.

ogy and regeneration that have to be answered (Maerki & Frankis 2015). In this study, for the first time to our knowledge, tree growth traits (DBH, canopy height, canopy base height, number of trunks and gender ratio) are investigated in the *J. drupacea* European populations, at the westernmost part of the species distribution range, in Mt. Parnon (Peloponnese, Greece).

On the basis of extensive field surveys, the structure of *J. drupacea* populations was revealed to follow two distinct patterns in the northern and the southern part of Mt Parnon, respectively. This clustering was confirmed by Factor Analysis of Mixed Data and shows a clear differentiation in number of trunks per individual, trunk diameter at breast height (DBH), canopy height and tree gender.

According to our analysis, *J. drupacea* individuals from the southern monitoring plots were larger in diameter (thicker) measured at breast height, when compared to the northern-recorded ones. In the former area, the majority of Syrian juniper individuals are carrying just one and single trunk, indicating that the above juniper trees are undoubtedly of older age, at least 75 years old. Based on the preliminary interpretation of the existing, old aerial photographs (1945) of the study area, we conclude that the majority of the northern monitoring plots were agricultural land in the past and have been recently forested.

The overall mean canopy height was less than 7.2 m, always a lower value compared to the upper canopy height limits, e.g. 18-25 m, reported elsewhere (Boratynski & al. 1992; Yucedag & al. 2021); taller trees were isolated and located on the underside of the Malevi Monastery. Absolute canopy height values were in agreement with earlier surveys (Boratynski & Browicz 1982; Bergmeier 2002) and quite similar to those reported for the

eastern Mediterranean Syrian juniper populations (Douaihy & al. 2017).

Furthermore, the number of measured trunks per individual varied between the two populations, with the multi-trunked individuals dominating the northern populations. Multiple trunks is apparently a trait inherent to *J. drupacea* (and other juniper species) and this is corroborated by the fact that the northern area has been under strict protection for over 40 years now while the southern one has been probably affected by land management history, particularly grazing (and wildfires, to a lesser extent). In addition, the number of trunks per individual affected negatively the mean diameter at breast height of the Syrian juniper trees, which probably can be explained by the allocation of individual tree growth resources into several trunks (Bui & Leoncini 2021). Plot altitude seems to affect the individual tree growth conditions; higher plot altitudes show a negative effect on both canopy height and DBH, probably due to the particular site quality, competition among plants, limited soil depth and local microclimatic conditions as well (Coomes & Allen 2007; Wu & al. 2015; Zheng & al. 2021).

The statistically significant positive correlation between the trunk number and the canopy height, as well as the canopy base height probably indicates the strong competition between the multiple trunks for light or nutrients, e.g. as proved in various understorey trees (Koop 1987; Del Tredici 2001; Tanentzap & al. 2012), without any dominant or secondary trunk clearly still observed in our surveys.

Regarding the gender ratio, more female individuals were observed in the southern monitoring plots with males being dominant in the northern part of the mountain. A deviation from the theoretical gender ratio of 1:1 was recorded in both European populations and indicated, to a lesser extent, in the Lebanese populations as well (Douaihy & al. 2017). Biased sex ratio, e.g. in other junipers, might be associated both to the greater reproductive costs by females and the past land management history as well (Ward 1982), or strongly affected by elevation, with males favored in higher altitudes (Ortiz & al. 2002). Patterns of natural selection on sex ratio may be affected by the quality and stability of the immediate habitat, as well as by life-history traits, competition and dispersal; environmental effects, both temporal and spatial, can also create a biased sex ratio (Sapir & al. 2008). The contribution of juvenile (young, non-reproductive trees without cones) or unidentified individuals varies greatly between the northern and southern plots of Mt. Parnon (27% and 59%, respectively) thus being dominant in the latter case. When all junipers recorded, i.e. both juvenile and mature individuals, the overall tree density corresponds to quite dense thickets, accounting for more than 850 trees/ha in the old, mature juniper forest, with even higher values (> 1000 trees/ha) in the abandoned but forested old fields, with slight density variations observed from north to south distribution of the species. Finally, the most part of the northern section of Mt. Parnon has been designated as a Preserved Monument of Nature (Government Gazette 121/D/21-2-1980) more than forty years ago, which probably influenced juniper tree density and regeneration. On the other hand, the strongly affected by overgrazing southern part, was much more sparsely colonized, and quite recently (since 2012) protected by the Greek Law and Authorities.

In conclusion, further scientific knowledge is necessary, enhanced by field surveys and laboratory studies, which is expected to contribute to the conservation of biodiversity on the fragile and endangered, by a number of threats, mountainous forest ecosystems of Mt. Parnon. In addition, introducing forest management plans on the Syrian juniper conservation in the Mediterranean Basin (e.g. Tahlouk & al. 2001) are of particular interest for the

protected areas of the European Natura 2000 network, thus offering new knowledge for the Greek forests in the future.

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Conservation status of the endemic vascular flora of Sicily

Abstract

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The results of a comparative study of the quantitative data of the IUCN risk categories attributed to the endemic taxa of the Sicilian vascular flora are reported. 430 Sicilian strictly endemic taxa has been evaluated by comparing the data of the risk categories attributed by published sources. 400 taxa have received at least one assessment of their conservation status, 30 taxa have not yet been considered. 278 received an assessment of their conservation status in accordance with the IUCN criteria, while the remaining 152 have not yet been evaluated according to these criteria.

Key words: assessment, IUCN criteria, Mediterranean flora, native flora, red list, risk categories.

Introduction

How it is well known, among the large islands of the Mediterranean basin, Sicily is the one with the richest and most diversified plant heritage. Over 3.250 specific and infraspecific taxa of native, naturalized or traditionally grown exotic plants are reported for this island (Giardina & al. 2007; Raimondo & al. 2010; Bartolucci & al. 2018a; Galasso & al. 2018). In this contingent, the most represented families are *Asteraceae*, *Poaceae*, *Fabaceae*, *Brassicaceae*, *Apiaceae*, *Caryophyllaceae*, *Lamiaceae*, *Rosaceae*, etc. According to Bartolucci & al. (2018a), the native flora of Sicily is made up of 2.736 specific and subspecific taxa.

The endemic contingent of the island accounts for just over 15% and the most represented families are: *Asteraceae*, *Fabaceae*, *Plumbaginaceae*, *Brassicaceae*, *Poaceae*, *Caryophyllaceae*, etc. The Sicilian endemic contingent includes taxa often with a punctual distribution. The number of endemics at risk of extinction has increased year by year. Several taxa, attributed to lower risk categories, even if they fall within protected areas, do not enjoy any particular protection, others have seen reduced or altered their elective habitat, suffering a strong demographic contraction. It is the case, for instance, of *Adenostyles alpina* subsp. *nebrodensis* (Wagenitz & I. Müll.) Greuter, an endemic of the Madonie Regional Park (NC-Sicily). In the past, probably, its single population was well represent-

ed, as suggested by the consistent number of historical specimens stored in the *Herbarium Mediterraneum Panormitanum* (PAL). At present, however, the taxon survives with only one single individual. The strong contraction of its population was caused probably by the alteration of its habitat. In fact, during the twentieth century, before the establishment of the Madonie Regional Park, the watercourse along which the population grew, was interrupted and channeled causing the loss of the necessary humidity for the growth of the plants. Therefore, this taxon is currently at risk of extinction. Another case that deserves mention is that of *Ptilostemon greuteri* Raimondo & Domina. It is a shrub known only from the northern slopes of Monte Inici (Castellammare del Golfo, TP, NW-Sicily). The entire population, located in two different slopes (Cappellone Valley and the valley between Pizzo Branco and Cozzo Monaco) of Monte Inici, is represented of a few thousand individuals. The repeated and close wildfires represent the main threat for the conservation of its population. Although the species is capable of rapid renewal after fires, in the long run, repeated and nearby fires are causing its population reduction. In fact, *P. greuteri* was classified as Endangered (EN) at the time of the species description (Raimondo & Domina 2006), currently, due to a better knowledge of the size of the population and the threats, it is listed as Critically Endangered (CR).

There are several contributions, including expert-based evaluations, on the endangered flora of Sicily. The first censuses and evaluations (Raimondo & al. 1992; Raimondo & al. 2011) were drawn up on an expert-based approach rather than detailed methodical evaluations. Instead, the following ones Red list (Rossi & al. 2013, 2020; Orsenigo & al. 2018) made within the Italian Botanical Society on behalf of the Ministry of the Environment of the national government, follow the scientific criteria proposed by the IUCN (2019). Since not all endemic Sicilian taxa at risk of extinction have received an assessment of their conservation status, in this paper we report the comparison of the quantitative data of the IUCN risk categories attributed to the endemic taxa of the Sicilian vascular flora.

Material and Methods

Comparison of the quantitative data of the IUCN risk categories attributed to the endemic taxa of the Sicilian vascular flora was done integrating the data taken from the previous published sources (Raimondo & al. 1992, 2011; Rossi & al. 2013, 2020; Brullo & Brullo 2020, Brullo et al. 2011). Since different taxa have a different taxonomic delimitation in the different contributions, in this paper we follow the taxonomic and nomenclatural data from Bartolucci & al. (2018a, 2018b, 2018c, 2019a, 2019b, 2020) and Galasso & al. (2018a, 2018b, 2018c, 2019a, 2019b, 2020a, 2020b, 2021). For *Brassica* was followed Malfa & al. (2020) and for *Aria* and *Sorbus* Raimondo & al. (2019).

The 430 Sicilian strictly endemic taxa have been evaluated by comparing the data of the IUCN risk categories attributed by the mentioned published sources (Raimondo & al. 1992, 2011; Rossi & al. 2013, 2020; Brullo & Brullo 2020) and are listed in the Electronic Supplementary File (ESF 1).

Results

From the analysis of the data, it emerges that 400 taxa (93%) have received the assessment of their conservation status; only 30 taxa (7%) have not yet been considered (Fig. 1a).

Because only the two Red Lists drawn up by the Italian Botanical Society follow the IUCN criteria, we have also calculated the number of taxa whose conservation status has been assessed in accordance with these criteria. It was found that 278 (65%) Sicilian endemic taxa received the evaluation of their conservation status in accordance with the IUCN criteria, while the risk category of a considerable part of the Sicilian endemic taxa, 152 (35%), is not still been evaluated (Fig. 1b).

In addition, we also investigated whether the conservation status of the taxa has improved or worsened. A substantial number of taxa (29.5%) have improved its conservation status. For example, the conservation status of taxa belonging to the genus *Limonium* and *Ophrys* has significantly improved. In the case of the *Limonium* taxa, the lower current risk assessment can be explained on the basis that while in the past the risk was overestimated when the distribution of the species was narrow, now if the habitat of the species is conserved and there are no threats, the risk is considered lower. In the case of the *Ophrys* taxa, the lower risk can be interpreted with the increase of the knowledge on their distribution. In fact, the ever increasing number of lovers for these species has made it possible to discover new populations and this has allowed the improvement of their conservation status. *Calendula suffruticosa* subsp. *maritima* (Guss.) Meikle (Fig. 2) has been assigned to a lower risk category both as a result of studies aimed at the knowledge of its punctual distribution (Grammatico 2011) which have increased its Area of Occupancy but also because it has proved to be a plant that takes advantage of anthropogenic disturbance.

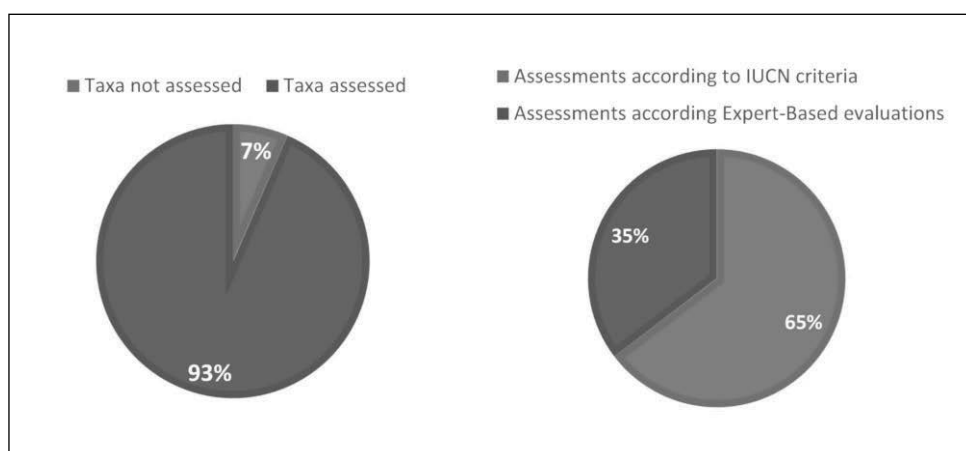


Fig.1. **a.** Percentage of Sicilian endemic taxa with an assessment of their conservation status and taxa without any assessment; **b.** Assessments of the Sicilian endemic taxa done according to the IUCN criteria and assessment done on Expert-Based evaluations.



Fig. 2. *Calendula suffruticosa* subsp. *maritima* along the coast of Trapani (NW Sicily) growing on construction waste.

On the contrary, only a small number of taxa (3.9%) have, instead, shown a worsening of their risk categories and this is to be related to the deterioration of their growth habitat, this is true above all for wetlands and coastal habitats (Domina & al. 2018, 2020; Sciandrello 2020). For example, in the case of *Oncostema dimartinoi* (Brullo & Pavone) F. Conti & Soldano, geophyte endemic to Lampedusa (Pelagie Islands) (Fig. 3), the increase in tourism over the years is a threat to the expansion of the species.

Discussion and conclusion

Considering that a part of the Sicilian endemic contingent has not yet received any evaluation of its status according to IUCN criteria, therefore, there is a need to further invest in research aimed at highlighting the real conditions of threat or conservation of the Sicilian endemic contingent which, due to its uniqueness, is not only of regional or national interest.

The increase in knowledge on the distribution and dynamics of populations in many taxa in the last years testifies that much remains to be explored even in a well-known geographical area such as Sicily where a large number of professional botanists work. Field research is a need for constant monitoring that many endemic taxa deserve. Although several actions have been launched at national and local level to protect the most sensitive environments, much still needs to be done to ensure the survival of the species that grow in these environments.



Fig. 3. Example of a direct threat from tourism on the natural population of *Oncostema dimartinoi* in Lampedusa Island.

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Hicham El Zein & Rhéa Kahale

First comprehensive IUCN Red List assessment of 100 endemic species of the flora of Lebanon

Abstract

El Zein, H. & Rhéa Kahale, R.: First comprehensive IUCN Red List assessment of 100 endemic species of the flora of Lebanon. — Fl. Medit. 32: 327-338. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This review presents the first IUCN Red List of 100 vascular plant species endemic to Lebanon. The taxa were assessed using the IUCN guidelines and criteria to evaluate their conservation status. Assessed taxa are either endemic to the coast of Lebanon, the Beqaa, the mountain ranges of Mount Lebanon, Anti-Lebanon and Mount Hermon. Most of the species assessed were in the Endangered category (54), followed by the Vulnerable (20), Critically Endangered (16), Least Concern (7), Near Threatened (2) and Data deficient (1). The rarity of these endemic taxa was illustrated by the fact that 29% had an AOO inferior or equal to 20 km². Unregulated urbanisation, road construction, quarries, forest fires and overgrazing constituted the main causes for the decrease of many endemic species. Most of the threatened taxa occurred in rocky habitats, woodlands and shrublands of the supra (20), montane (36), oro-Mediterranean (28) belts. Around 81% and 70% of the taxa respectively listed as CR and EN were located in the montane and oro-Mediterranean belts. Only 2 taxa occurred in coastal habitats and only 8 were related to wetlands. Thirty-five of the threatened taxa were included in at least one protected area, while 17 were present in two protected areas. Mount Hermon hosted 17 threatened taxa, followed by Horsh Ehden (9), Jabal Moussa (8), Chouf Biosphere (7), Tannourine (6), Ehmej el-Dichar (3), Ras Chaqaa (1) and Yammoune (1). The distribution of the threatened taxa within protected areas highlighted the fact that some habitats are underrepresented in the network of natural reserves in Lebanon. High plateaus of the oro-Mediterranean belt over 2000 m were the less protected habitats. The regions of Mounts Makmel, Mneitre, Sannine and Kneisse particularly lacked protection, as well Anti-Lebanon range. These areas should be targeted in priority for conservation actions such as the creation of protected areas. Due to the limited size of the country and its high density in human population, most of the threats are widespread but vary depending on the altitudinal belt.

Key words: Levant, Mediterranean Basin, flora, threats, conservation, species.

Introduction

There is currently a global crisis on biodiversity with the sixth mass extinction threatening an important number of species due to the direct or indirect impacts of human activities (Ceballos & al. 2015; Torres-Romero & al. 2020). Red Lists have become an essential

reference prior to the establishment of conservation strategies (Brummit & al. 2008; Betts & al. 2020) and the role of the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species has become fundamental as it brought the scientific community to use comparable standardised system to estimate the vulnerability of species and to quantify their risks of extinction (Possingham & al. 2002). It also encourages the compilation of important amount of data about each species (Rodrigues & al. 2006).

The Mediterranean Basin, a region that simultaneously shelters exceptional concentrations of endemic species and experiences a high rate of loss of habitat, was considered as one of the global hotspots (Myers & al. 2000; Cuttelod & al. 2008). Located in the eastern Mediterranean, Lebanon consists of a local hotspot for biodiversity (Médail & Quézel 1997; Verlaque & al. 1997). The flora of Lebanon consists of 2612 specific and subspecific taxa. Lebanese exclusive taxa (strictly endemics) are 154 (Tohmé & Tohmé 2014). The efforts to catalogue the floral diversity of Lebanon to identify the areas of high floristic diversity and endemism where the conservation effort should concentrate (Bou Dagher-Kharat & al. 2018; El Zein & al. 2018), the construction of a Red List of threatened plant species for the country constitute another essential tool for practitioners. This paper focuses on the conservation status of the vascular endemic species of Lebanon.

Materials and Methods

Data

In addition to the knowledge of the authors and local experts involved in conservation, data about the endemic species was collected actively between 2012 and 2020: The herbarium samples collected during the 19th and 20th centuries gathered in bibliographic references (Mouterde 1966, 1970, 1984) gave precious indications about the occurrences of endemic species in the country. The historical localities reported from the samples were visited in priority to verify if the populations were still extant. The field observations allowed the update of most of the historical information. When the plants were not observed during exploration, the most recent reference for species occurrences in Lebanon was used (Tohmé & Tohmé 2014). Taxonomy was updated in accordance with the International Plant Names Index (INPI 2022) and the Euro+Med Plantbase (2022). The maps were prepared with QGIS software (QGIS Development Team 2022) using layers available on the DIVA-GIS website (2022).

The targeted species are endemic to Mount Lebanon and/or Anti-Lebanon and/or Mount Hermon and/or the Beqaa or to the coastal Mediterranean strip. Altitudinal belts were used to differentiate the zonation of the vegetation in Lebanon. They were defined based on the work of Abi-Saleh (1982): coastal-Mediterranean is the belt influenced by the Mediterranean Sea, thermo-Mediterranean is located between the coast and 500 m, meso-Mediterranean between 500 to 1000, supra-Mediterranean between 1000 to 1600 m, montane-Mediterranean between 1600 and 2000 and oro-Mediterranean above 2000 m. The protected areas include the nature reserves and the nature sites under the protection of the Ministry of Environment of Lebanon (MoE-UNEP-GEF 2016).

Red Listing

The International Union for Conservation of Nature (IUCN) Red List categories and guidelines (2022) were the reference used to evaluate the conservation status of the endemic species. Seven categories were used to define the species: Extinct (EX), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) and Data Deficient (DD). Among the five criteria, B (geographic range) and D (very small or restricted population) were used as they were based on data related to geographic distribution, which was available for most of the taxa. The number of subpopulations, number of locations, the extent of occurrence (EOO) and the area of occupancy (AOO) were calculated for each taxon. The calculation of the EOO and AOO, two key parameters for the evaluation of the geographic range of the species, was carried out using the GeoCAT software (Bachman & al. 2011). The EOO is estimated by using the minimum convex polygon method which is the area that encompasses all the occurrences of a taxon in the shortest boundary. The AOO is calculated by summing the number of cells occupied by individuals in a grid of 1×1 km. The use of criteria A and C was not possible because data about temporal trends of the species' populations was not available.

In addition to the collection of occurrences of the taxa, the different threats affecting them and their habitats were identified. During several explorations in the same sites, the impacts on the vegetation were observed. For instance, some species had disappeared after the repeated passages of the herds. Depending on the intensity of the grazing and the palatability of the plants, some species would be eaten before having been able to flower or make seeds and others would have been extirpated from the site by the degradation of their habitats due to heavy trampling and soil destruction. Another example is the total eradication of the natural habitats which was observed frequently in many localities due to the construction of buildings, the creation of quarries or the levelling works for the establishment of agricultural terraces. The threats result in the continuing decline of area extent, quality of habitats and number of individuals. Some species are more fragile than others and don't recover properly after being disturbed. Others have a limited capacity of dissemination with reduced seed production. This information was noted attentively to determine the severity of the impacts of the threats on each species as defined by the guidelines of IUCN. Based on the mapping of the species to understand their distribution and the ability of their pollen to be disseminated, the fragmentation of the population was estimated. The data collected about the threats also allowed the calculation of the number of locations for each species. These data are necessary to fulfil the conditions a, b and c of criterion B and to determine the IUCN status.

Threats by altitudinal belts

The presence of the threats affecting the endemic plants was represented by altitudinal belt. For each taxon, the threats were ordered by severity (Electronic Supplementary File 1: Table S1). For example, some species are primarily affected by urbanisation and secondarily by overgrazing. The three first threats were considered for each species. Using the altitudinal belt on which each species occur, the extend of the threats per altitudinal belt was represent in a histogram. The complete references to these assessments published online are included in the ESF1.

Results

Over the 100 endemic taxa assessed, 90 were listed under a threatened category, namely 20 as VU, 54 as EN and 16 as CR. Two species were listed as NT, seven as LC and one as DD. The most used criteria to assess their conservation status were B1 and B2 as the most reliable information available about the taxa was their distribution. The fieldwork yielded a total of 961 observations that could be directly used to map the distribution of the taxa and calculate their AOO and EOO. Twenty-nine species had an AOO inferior or equal to 20 km² and therefore considered as narrow endemic. Thirty-nine taxa had an EOO inferior to 200 km².

Odontarrhena libanotica (Nyár.) Španiel, Al-Shehbaz, D.A. German & Marhold was the only species that was listed as DD as it could not be observed during fieldwork and was not even reported from recent bibliographic references (ESF 1: Table S1). The recent fieldwork allowed the rediscovery of one plant species that was considered possibly extinct, *Hieracium kneissaeum* Mouterde listed as CR. The population was estimated of 436 individuals in Mount Kneisse where it is threatened by the intensification of grazing.

Most of the threatened endemic taxa (83) occurred over 1200 m asl in rocky habitats, woodlands and shrublands of the supra (20)/montane (36)/oro-Mediterranean (27) belts, while two species, *Matthiola crassifolia* Boiss. & Gaill. and *Gagea dayana* Chodat & Beauv. were found in coastal habitats. Only 8 species were related to wet habitats, such as *Alchemilla diademata* Rothm., *Eleocharis macrantha* Boeckeler and *Stachys hydrophila* Boiss.

Threats

The most common threats were urbanisation, overgrazing, quarrying and agriculture as they destroy the species and their habitats, affecting respectively 48%, 40%, 40% and 26% of the taxa. Logging, forest fire and dam construction constituted threats that directly impacted particular taxa or/and their habitats. Collection of plants and reforestation are threats that target particular species. The importance of the threats varies depending on where species occur across the altitudinal belt (Fig. 1). For instance, lower altitude habitats are intensely impacted by urbanisation which includes the construction of houses, commercial buildings and roads, while high plateaus located above 2000 m of altitude are heavily impacted by overgrazing.

The two significant past threats that have affected several locations at high altitude were the construction of ski resorts in Mount Lebanon (Bcharre, Laqlouq, Faraya, Zaarour) and Mount Hermon (on the Israeli-occupied slopes) and their associated touristic housing, as well as the construction of dams (Janne on Nahr Ibrahim river, Bqaatouta on Nahr el-Kalb river, Hamat and Chatin on Nahr el-Joz river). However, these threats could occur again in the future. Climate change is a general threat impacting all taxa at all altitudinal belts. It was considered as a future threat to the taxa occurring below 1600 m of altitude. The taxa occurring over 1600 m of altitude and those occurring in wetlands were considered more vulnerable to warming and drought events. Although primarily affected by quarrying, urbanisation and agriculture, wetland plants like *Alchemilla diademata* might disappear with the intensification of drought events as they totally depend on water.

Presence in protected areas

Only 35 of the threatened taxa were present in at least one protected area, which included one species listed as CR, 21 as EN and 13 as VU. Seventeen taxa were present in 2 pro-

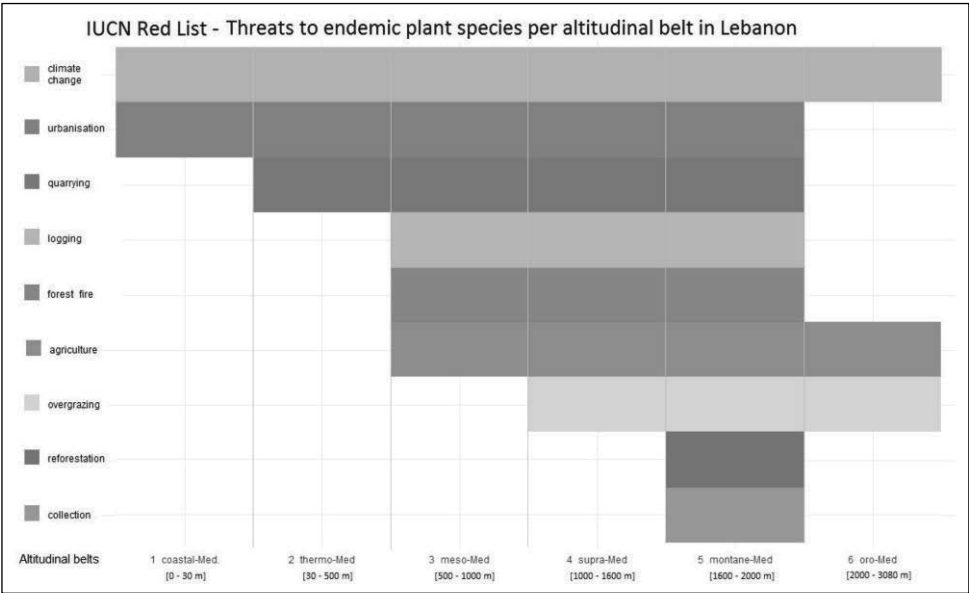


Fig. 1. Threats affecting the 100 assessed endemic taxa in Lebanon according to the altitudinal belt.

tected areas, including 3 species that have their entire distribution included in protected areas, namely *Allium feinbergii* Oppenh., *Cephalaria cedrorum* Mouterde and *Salvia peyronii* Boiss. *Allium feinbergii* was assessed as LC and occurs at the oro-Mediterranean in the nature reserves of Shouf Biosphere and Mount Hermon. Climate change constitutes the most important threat in this context. *Cephalaria cedrorum* was assessed as VU and occurs uniquely at the montane-Mediterranean in Shouf Biosphere. *Salvia peyronii* was assessed as CR and occurs in the nature site of Jabal Moussa at the supra-Mediterranean where it is threatened by over-grazing and forest fires (Fig. 2). Mount Hermon is the protected area that hosted the highest number of threatened taxa with 10 taxa listed as EN and 7 as VU. This is due to the fact that it is the only protected area located in the oro-Mediterranean belt. The other protected areas sheltering threatened taxa in descending order are Horsh Ehden with 9 threatened taxa, followed by Jabal Moussa (8), Shouf Biosphere (7), Tannourine (6), Ehmej el-Dichar (3), Ras Chaqaa (1) and Yammoune (1). Many regions lacking protection are located on the coast, in the east of the country in Anti-Lebanon, and in the north of Mount Lebanon, such as Makmel, Mneitre, Sannine and Kneisse (Fig. 3). Other examples of unprotected taxa are *Astragalus kurnet-es-saudae* Eig, *Matthiola crassifolia* Boiss. & Gaill and *Senecio bertramii* Post. *Astragalus kurnet-es-saudae* occurs at the oro-Mediterranean in North Lebanon and is CR. *Matthiola crassifolia* is a coastal species assessed as EN and threatened by intense urbanisation. *Senecio bertramii* was assessed as CR and occurs at the montane-Mediterranean in North Lebanon.

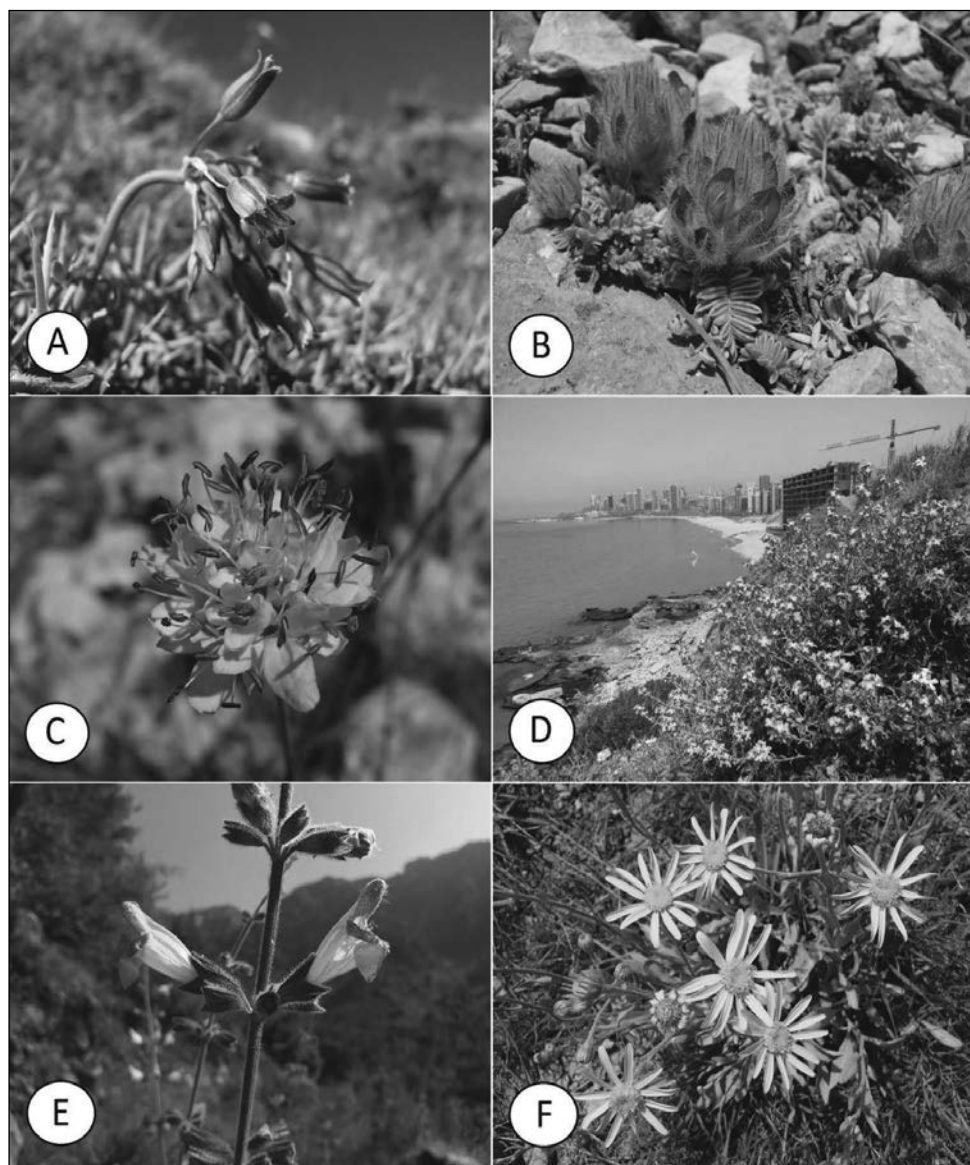


Fig. 2. Illustration of protected and unprotected Lebanese endemic plant species. A : *Allium feinbergii* Oppenh. is Least concerned (LC) and occurs at the oro-Mediterranean in the nature reserves of Shouf Biosphere and Mount Hermon; B: *Astragalus kurnet-es-saudae* Eig is Critically Endangered (CR), occurs at the oro-Mediterranean in North Lebanon and is currently unprotected; C: *Cephalaria cedrorum* Mouterde Vulnerable (VU) occurs at the montane-Mediterranean in Shouf Biosphere; D: *Matthiola crassifolia* Boiss. & Gaill is Endangered (EN), occurs at the coast and is unprotected; E: *Salvia peyronii* Boiss. is CR and occurs in the nature site of Jabal Moussa at the supra-Mediterranean; F: *Senecio bertramii* Post is CR, occurs at the montane-Mediterranean in North Lebanon and is unprotected. Photos by H. El Zein.

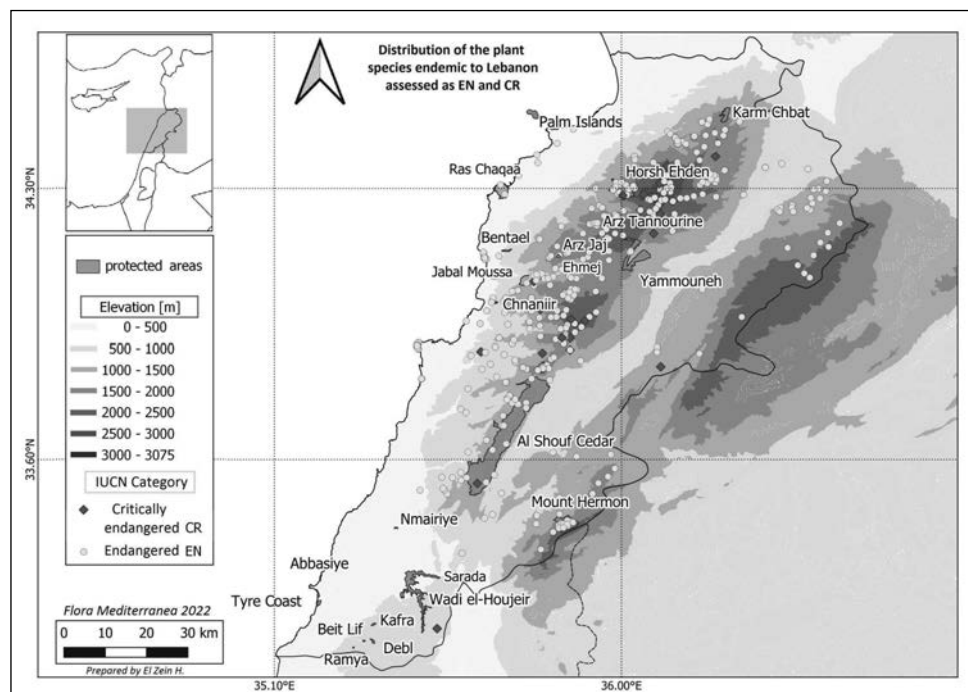


Fig. 3. Map showing the distribution of the taxa assessed as Endangered and Critically Endangered in Lebanon as well as the protected areas.

Discussion

Threats are often specific to specific altitudinal belts and habitat and do not affect the taxa equally. For example, urbanisation has massively been affecting natural habitats from the coastal to the supra-Mediterranean belts. The construction of roads and buildings and the creation of quarries irreversibly destroy the vegetation and constitute the most important threat at the national level. Lebanon was classified as the ninth most populated country in the world (World Population Review 2022) explaining the intensity of the anthropic activities and their impacts even in remote areas. Meanwhile, the lack of national coordinated spatial planning for urbanisation is considered as another parameter responsible for the loss of biodiversity (Masri 1997). The destruction of ecosystems by quarries is widespread in the country (Darwish & al. 2011) and durably threatens numerous species such as *Atocion reuterianum* Boiss. & Blanche and *Rhododendron ponticum* var. *brachycarpum* Boiss.

Overgrazing is a widespread threat that has slow but significant impacts over time. Due to the increase of urbanisation, the rangelands have been reduced and restricted to the remaining non-urbanised areas. In summer, this activity is essentially concentrated in the montane and oro-Mediterranean belts and the size of the flocks are larger, resulting in an intensification of pressure on the natural vegetation to feed the goats. The vegetation is heavily affected and the reproduction cycles, such as the production of flowers and seeds,

are directly impacted. Seedlings and young trees are systematically eaten, preventing the regeneration of the forests. Meanwhile the soils are trampled by repeated passages, leaving the soil bare of vegetation and increasing the erosion phenomenon due to rainfall runoffs.

Logging directly impacts the two endemic oak tree species *Quercus kotschyana* O. Schwarz and *Q. look* Kotschy. Forest species are inescapably the victims of fire, such as *Cyclamen libanoticum* Hildebr. that has been severely threatened by the fires that occurred in 2017 in the area of Jabal Moussa. Collection of plants mainly targets bulbous species with ornamental value, especially irises, such as *Iris cedreti* Dinsm. ex Chaudhary and *I. sofarana* Foster. Reforestation affects species for which other threats were minimised thanks to their presence in protected areas. *Cephalaria cedrorum* is an interesting case as it grows at the cedar altitudinal level in open areas and is threatened by the thick shade resulting from tree plantation.

Climate change manifesting through drought events was here considered as a future threat that will affect all the species. However, many changes have already occurred over the past 40 years and impacted the Mediterranean terrestrial biodiversity and ecosystems faster than other areas of the world (Hassoun & al. 2020; Guiot & al. 2021). Around 48% of the Mediterranean wetlands were lost between 1970 and 2013 which also impacted on the animals living in these habitats threatening 36% of them with extinction. Moreover, terrestrial biodiversity, forest productivity, freshwater ecosystems and agrosystems will be negatively impacted by decreased precipitations, changes in biological communities as a result of harsher environmental conditions, and a decrease of biological processes such as nutrient uptake, primary production, or decomposition. Another response to climate change will be the extension of drylands (Hassoun & al. 2020; Guiot & al. 2021). The ecosystems of the southern Mediterranean are particularly at risk of fragmentation or disappearance due to anthropic activities such as urbanisation, agriculture, overexploitation of firewood and overgrazing. For the taxa occurring above 2000 m of altitude, the drought and warming events were considered as current threats as there already have been changes in the average temperature and amount of precipitation. Mediterranean mountains are fragile ecosystems that are currently facing climate change more intensely than other non-Mediterranean ranges (Bravo & al. 2008), especially the vegetation of the oro-Mediterranean belt that is more vulnerable (Rosbakh & al. 2017). It is happening through reduction of snow cover, soil fertility and water availability. The changes are fast and the species located at the highest level of the mountains cannot migrate to a higher altitudes. Even coastal species and habitats will be impacted by the imminent sea-level rise (Snoussi & al. 2008; Castillo & al. 2022).

The threats to plant diversity in Lebanon are similar to those dominating globally, which are habitat loss, overexploitation and climate change (Corlett 2016). Habitat loss includes conversion of natural or semi-natural habitats into agriculture, pastures, quarries or urbanised areas. Invasive plant and animal species, causing competitive exclusion of native species or destroying native vegetation, are widespread threats at the global scale that seem relatively of minor importance in Lebanon.

These assessments constitute the first step towards the evaluation of the conservation status of the Lebanese flora. The list of 100 taxa reflects the national scientific efforts carried out for years to explore the country and identify the threats and their impact on the flora. Emphasis was made on endemic species as they represent relevant natural patrimony

and the most fragile taxa due to their restricted distribution (Callmänder & al. 2005). As presented by other studies of Ethiopia, Eritrea, Cape Verde and Italy (Vivero & al. 2006; Romeiras & al. 2016; Orsenigo & al. 2018; Fois & al. 2022), the focus on the endemic flora gives an interesting perspective for conservation. Although this red list is quite representative of the trend of the anthropogenic disturbances on the endemic taxa, not all the species were yet assessed and other Levantine endemic taxa are currently being evaluated. Many other species, although not endemic, are rare at the national scale or have ecological significance. Their status need to be assessed to better evaluate the threats affecting them. A national red list is of high importance to understand the patterns of threats and their regional variations (Venter 2006). The next assessments will include more endemic and non-endemic taxa and will allow a more accurate definition of the priority areas for conservation in the country. The red listing process needs to be constantly updated considering the current pace of the anthropic disturbances and the climatic changes. Protected areas in Lebanon should include the most threatened taxa and their related habitats to reverse the erosion of biodiversity. In the future and following long term conservation actions, we might hopefully be able to use the IUCN Green List of species, a new tool that measures species recovery and conservation success across their indigenous range (IUCN 2021).

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The Mediterranean Island Wetlands (MedIsWet) inventory: strengths and shortfalls of the currently available floristic data

Abstract

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MedIsWet (Conservation of the island wetlands of the Mediterranean Basin) is a MAVA funded project which aims at investigating all seasonal or permanent island wetlands both natural and artificial, with a minimum extent of 0.1 hectares. More than 16,000 wetlands from almost all the Mediterranean, including islands from France, Italy, Malta, Croatia, Cyprus, Tunisia, Turkey, Greece and Spain were mapped. Over 2,500 of them were inventoried in the field and more than 500 scientific contributions catalogued. In total, more than 35,000 plant occurrences were uploaded, in a standardised and comparable way, on the national open-source web portals. These can be related to the recorded threats, uses and other spatially retrievable information. Here, we show strengths and shortfalls of the already available information about the floristic records. Although further improvements are needed, we discuss how these data can be used for research and policy actions and to develop conservation projects.

Key words: databases, floristic records, field surveys, rapid assessment, hydro-hygrophilous vegetation.

Introduction

The Mediterranean is a crossroads for both humans and wildlife, where nearly 30,000 plant species (~20% of the world total) cohabit with five hundred million people (~10% of

the world total) in less around 5% of the Earth's surface (Cowling & al. 1996; Blondel & al. 2010). It is the second largest biodiversity mega-hotspot in the world, and 60% of its total flora is constituted by Mediterranean endemics, with 4,500 of them considered to be endangered by the increasing human population and their related activities (Greuter 1994).

A significant component of the Mediterranean plant biodiversity is harboured in about 11,000 islands and islets, of which 250 are inhabited by humans, representing one of the largest 'archipelagos' in the world (Fois & al. 2020; Médail 2013; 2022). Being mostly 'continental' or 'chersogenous' islands, with some exceptions such as Cyprus, they are generally conservative systems, also known as 'refugia', remarkably well buffered against the effects of climatic and evolutionary changes and rich in old relic elements (Greuter 2001; Médail & Diadema 2009). Moreover, recent diversifications of species or still diversifying lineages are favoured by the isolation and environmental heterogeneity of most Mediterranean islands (Fois & al. 2022; Médail 2022). The phytogeographic interest of Mediterranean islands has been more recently accompanied by their conservation concerns, with high rates of their floras endangered due to both intrinsic (e.g., low genetic diversity, narrow and/or fragmented populations) and extrinsic causes (e.g., human-induced pressures). Consequently, wetlands, which are generally not particularly rich in plants of phytogeographic interest, are instead increasingly investigated by botanists because they are recognised as one of the most fragile Mediterranean ecosystems (Almond & al. 2020). It is not surprising, for instance, that several plants living in wet habitats (henceforth: wetland plants) are listed in the Annexes of the 'Habitats' Directive (92/43/EEC) and in the 'Top 50 Mediterranean Island Plants' to be urgently conserved (Pasta & al. 2017).

Recent literature reviews from Sardinia have summarised research trends in wetlands and identified some patterns which are shared with most Mediterranean islands (Cuenca-Lombrana & al. 2021; Fois & al. 2021): plants (especially vascular plants) and vertebrates (especially birds) were the most investigated 'species groups' in Sardinian wetlands. An exponentially increasing number of papers was detected from the beginning of this century, with topics principally related to ecology and conservation. These were possibly favoured by the increasing attention given to wetlands by several associations and supported by policy initiatives. Amongst wetland types, large coastal wetlands were the most investigated, with important knowledge and conservation gaps found for small wetlands, especially if ephemeral. In Italy as a whole, instead, most papers are mainly focused on lakes and rivers (Barone & al. 2022). Even basic floristic inventories in irrigation ponds and other similar artificial wetlands are very scarce, despite their high representativeness, especially in Sicily (Panzeca & al. 2021). A similar situation can be identified by exploring Ramsar sites (<https://rsis.ramsar.org>). Of the 24 Ramsar sites found in Mediterranean islands (none in Greek, Croatian and Turkish islands), the Tourbière de Moltifao and Mares temporaires de Tre Padule de Suartone (Corsica) are the only two inland freshwater wetlands, despite their demonstrated conservation importance, especially for plants, amphibians and invertebrates (e.g. Grillas & al. 2004; Minissale & Sciandrello 2016; Bagella & al. 2016).

In this context, the "Conservation of the island wetlands of the Mediterranean Basin" (MedIsWet) project, funded by MAVA Foundation, has been conceived to fill knowledge and conservation gaps in the relevant set of Mediterranean wetlands in islands, giving particular attention to the hitherto less investigated ones, such as small wetlands and artificial ones. Accordingly, the main project objectives were to: i) document the existence and significance of the Mediterranean island wetlands (both natural and artificial, above 0.1 ha,

no linear systems included) and disseminate knowledge to the public and scientific community; ii) mature and implement restoration projects in at least nine wetlands across the Mediterranean islands; and iii) promote their protection through laws, concrete legislative measures and activities at local, national and Mediterranean scale. MedIsWet has evolved as a natural progression of WWF Greece's project "Conservation of the island wetlands of Greece" that launched in 2004 and which was completed in 2016, also in response to the Ramsar resolution XII.14 "Conservation of the Mediterranean basin island wetlands" that was adopted in 2015 and provided for the documentation and protection of the Mediterranean island wetlands. Among the abiotic and biotic information available on the online national databases, a species list of plants was also reported for each inventoried wetland. Here, we present these floristic datasets of potential interest for all Mediterranean botanists and wetland managers.

The MedIsWet Databases

All nine national databases (data from the Croatian database were not included in this publication) were identically structured and they are compatible to allow for the merging of all databases under one host in the future. The interdependent and combined search options allow the user to select a set of wetlands of interest by combining different filters such as location (e.g., island, municipality), protection status, wetland type, and many more. All data from each database can be exported and can be freely used under the Attribution-Share Alike 4.0 International licensing (CC BY-SA 4.0).

For this paper, we retrieved floristic data from the following databases:

Greece: <https://www.ygrotopio.gr>; Cyprus: <https://www.cypruswetlands.org>, Spain: <https://www.humedalesdebaleares.es>; Turkey: <https://adasulakalanlari.org>, France: <https://franceiswet.fr>; Malta: <https://www.maltawetlands.org>; Tunisia: <https://zoneshumides-tunisie.tn>; Italy: <https://italiaiswet.it>.

In most cases, data are available both in English and in the national languages and include both data from cited literature and from original field surveys. The information in the field was gathered following the "Rapid Assessment" protocol, defined by MedWet (significant modifications in the protocol were made for the Greek project) in order to provide a general overview of a maximum number of sites with limited resources (Tomàs-Vives 2008). The plant species checklist is only one of the 17 parameters recorded by desktop and field surveys. We inventoried all plants found within wetland boundaries, including several terrestrial ones, which are nevertheless also common along wetland banks and saturated soils. Each record represents a species which is reported with the respective taxonomical binomial name (or genus, when a specific identification was not possible) and family, wetland site (toponym and code), vegetation type, and approximated coverage (dominant, co-dominant, present). Nomenclature was standardised as far as possible according to global accepted taxonomy (e.g., <http://www.theplantlist.org>), although local adaptations were accepted. For summarising results here, possible synonymy was revised only for the most common taxa.

Results

Descriptive statistics

A total of 35,659 records of plant presence, including both data collected on the field and on existing literature, were uploaded in the above listed databases, around 92% of them are reported at species and subspecies levels. Data were gathered in 2,645 sites from 107 Mediterranean islands belonging to eight different countries (Table 1). It should be noted that the databases may include more sites, but without plant species information.

With some case-by-case exceptions, floristic inventories were almost equally covering the different wetland types (Fig. 1). Considering the databases together, floristic data were reported for 1,022 artificial wetlands (39% of the total), 1,148 small wetlands with a surface area < 1 ha and 1,146 coastal wetlands (each accounting for nearly 43% of the total).

Plant families and species

Among the 224 families present in the databases, including around 4,080 specific and subspecific taxa, the most representative was *Poaceae* (460 specific and subspecific taxa), followed by *Asteraceae* (377) and *Fabaceae* (366) (Fig. 2). In terms of number of records, the richest family is *Poaceae* (4,909 records), followed by *Asteraceae* (2,922), and *Juncaceae* (1,864) (Fig. 3). The most recorded were *Phragmites australis* (Cav.) Steud. (1,288 records) and the introduced *Arundo donax* L. (723), followed by *Pistacia lentiscus* L., *Dittrichia viscosa* (L.) Greuter and *Tamarix africana* Poir. (around 550 each) (Fig. 3).

Table 1. Information on number of sites with plant information, islands and records per country or Italian administrative region (Sardinia and Sicily). The total number of plant presence records and respective percentages at species level are also reported in the last column.

Country/Region	N. sites	N. islands	N. records (% sp. level)
Greece	698	61	4563 (63)
Cyprus	297	1	2519 (78)
Spain	135	5	1702 (94)
Turkey	58	22	528 (86)
France	382	3	9329 (97)
Malta	54	2	365 (94)
Tunisia	5	3	135 (100)
Sicily (Italy)	514	4	2558 (100)
Sardinia (Italy)	502	6	13960 (99)

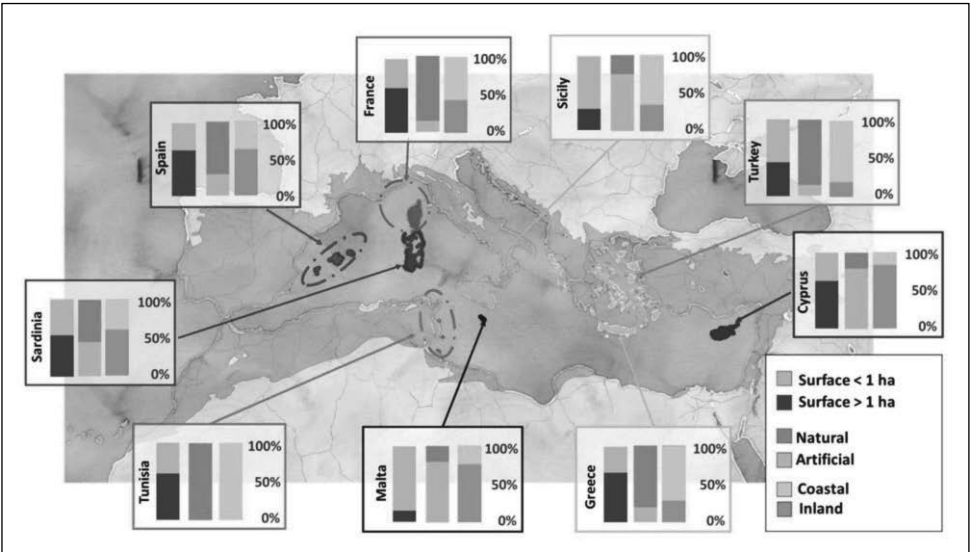


Fig. 1. Distribution of wetlands included in the MedIsWet databases per country/region with floristic data and relative percentages of natural vs. artificial, coastal vs. inland, and wetlands with a surface area < vs. > 1 hectare.

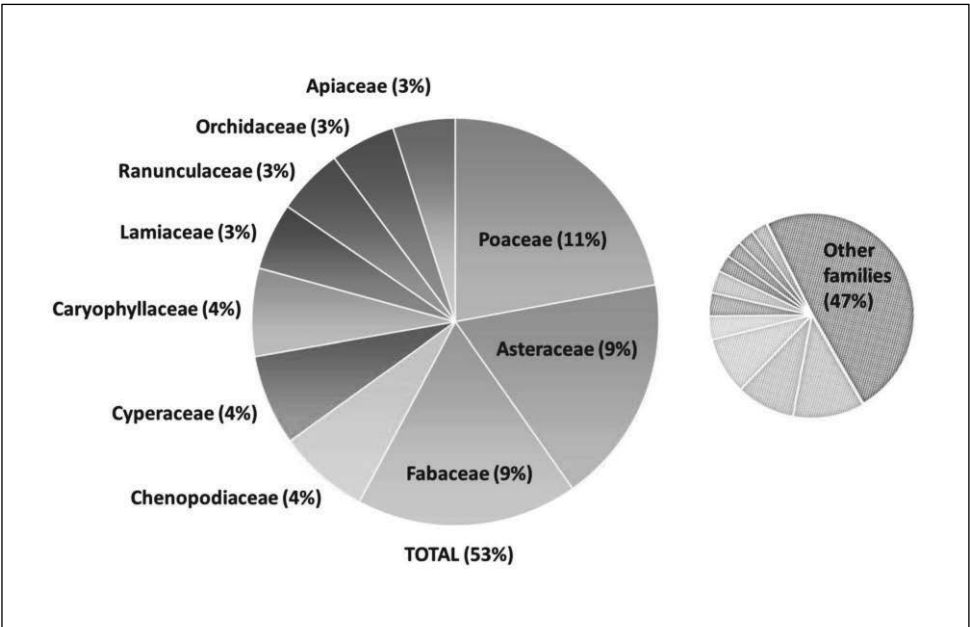


Fig. 2. The most species-rich families spectrum of the flora of Mediterranean islands wetlands.

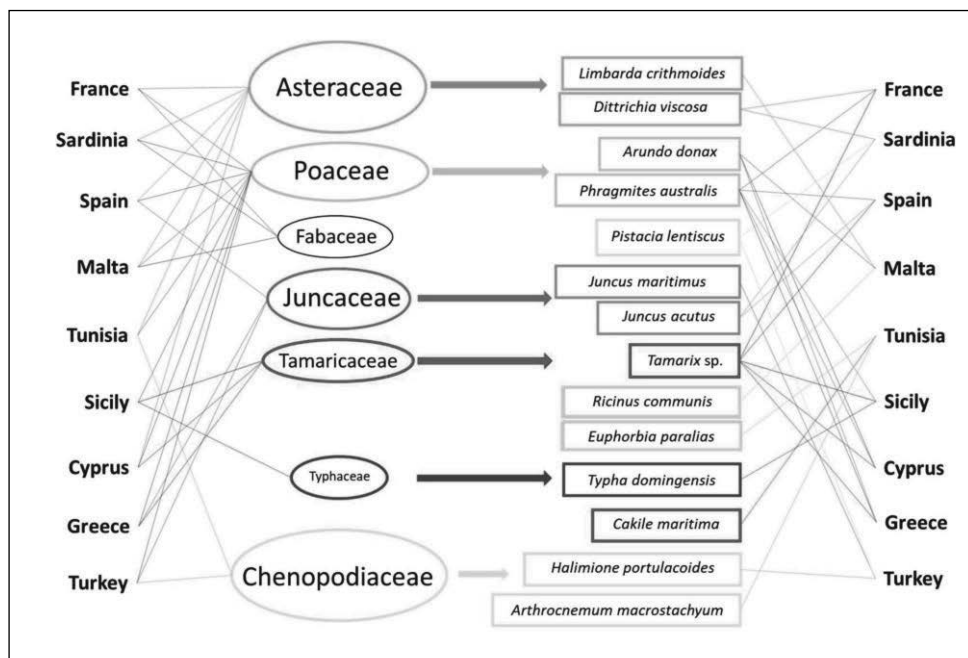


Fig. 3. Synthetic scheme of most recorded families and taxa. Connectors are referred to the top three families and taxa in each country/region. Arrows indicate the corresponding families of the taxa. In the cases of *Pistacia lentiscus* (*Anacardiaceae*), *Cakile maritima* Scop. (*Brassicaceae*), *Ricinus communis* L. and *Euphorbia paralias* L. (*Euphorbiaceae*), the respective families were not reported because these did not result as 'top recorded' ones, despite the high number of records of such taxa belonging to them.

Discussion

This paper briefly summarises the floristic data collected to date and that is currently available from the above-mentioned databases. It is important to highlight that the project has been designed with a multidisciplinary approach, which also includes faunistic inventories, human activities, threats, ecosystem services provided by wetlands, etc. Therefore, incomplete floristic datasets were compensated for a large variety of information. Moreover, the database was conceived as a baseline to be continually updated and implemented by the same providers and also by possible external support from managers or visitors, who are always invited to inform the authors about possible missing relevant information.

MedIsWet datasets are as exhaustive as possible in compiling records already published and mostly already reported in other databases. However, particular efforts were invested in inventorying all those sites that were never recorded before, in order to fill gaps in knowledge and enlarge the representativeness of the current status, including the negative aspects. The numbers of the most common taxa are incomparable – although complementary – to global datasets, such as GBIF (<https://www.gbif.org>). However, floristic information for several understudied

sites were for the first time documented, providing useful information on the presence of both native rare and alien invasive taxa. For instance, one unknown population of the rare *Thelypteris palustris* Schott was recently found during one field survey in the Sicilian Nebrodi mountains (wetland code ITG13SIC11403; Sciandrello & al. 2021), and *Ludwigia palustris* (L.) Elliott and *Halopeplis amplexicaulis* (Vahl) Ces., Pass. & Gibelli were for the first time recorded in one small dam in Sardinia (wetland code ITG27SAR2050) and for the island of San Pietro (wetland code ITG27PIE0005), respectively. Some alien taxa were also recorded for the first time, as in the case of *Tetragonia tetragonoides* (Pall.) Kuntze that was found in one natural coastal wetland (ITG26SAR2741; Galasso & al. 2021), while others such as *Carpobrotus edulis* (L.) N.E. Br., included in the “100 worst” invasive species of Europe (Nentwig & al. 2018), have been recorded almost everywhere exerting its ground cover effect on natural vegetation in degraded environments.

Similar to the MedIsWet dataset, *Poaceae*, *Asteraceae*, and *Fabaceae* are generally the most represented families in general floras of several Mediterranean islands, such as Sardinia and Balears (Mayoral & al. 2018) or Sicily (Raimondo & al. 2010). This spectrum sensibly differ to those obtained from checklists of macrophytes or aquatic plants (e.g., Murphy & al. 2019), as we also inventoried several terrestrial plants found along wetland banks and saturated soils, such as *Pistacia lentiscus* or *Dittrichia viscosa* or invasive taxa like *Ricinus communis*. Since wetlands are generally associated with salt marsh, dune and sandy coastal habitats, plant species richness increases with the inclusion of halophytic and psammophytic vegetation due to variable site conditions (Yılmaz & al. 2020).

As regards the record frequencies per family and species, the third most common family in Mediterranean floras, *Fabaceae*, is in the case of Mediterranean wetlands, replaced by the *Juncaceae*, due to the large presence of species of the genus *Juncus* living in wet habitats. The 27 species of *Juncus* reported in our datasets are present in a large array of wetland types, from coastal salt marshes to inland freshwater places; some taxa, such as *Juncus acutus* L., are also common in degraded (e.g., overgrazed) and artificial environments but it frequently occurs as the only or almost only marsh vegetation representative in wetlands with few flora species.

Without claiming our information is complete, the inventories presented here constitute a basic tool with geographic and field data in support of research and conservation plans, which has to be continuously updated and implemented. Like most shared data, this is a complementary tool in support of diverse other initiatives, such as satellite-based wetland monitoring programs (Weise & al. 2020), concrete policy and conservation actions (Emmanouilidou 2019) or research programmes on wetland plants ecology and distribution. Plant inventories, and biological data more in general, are fundamental to individuate the areas of conservation irreplaceability as well as potential risks related, for instance, to the presence of some harmful or invasive species. Otherwise, conservation projects have to include further information related, among others, to economic interests or the general potential feasibility of a project. The MedIsWet project itself was conceived with a view to having a second phase of concrete restoration actions, subsequent or simultaneous to the initial inventory stage. As presented during the 12th European Conference on Ecological Restoration (SERE 2021), all project partners are currently implementing at least one restoration action in sites identified in the initial phase. The data can be replicated or used for implementing similar strategies by any organisation interested in wetlands conservation.

Conclusions

The wetland inventories presented herewith are important complementary contributions to plant knowledge, which is still recognised as a crucial gap to be filled across the Mediterranean. However, without the improvement and regular update of such information, the protection of wetlands would risk being insufficient. Acknowledging that human and economic resources are as crucial as they are limited, enlarging collaborations to develop and fund new and more integrated projects is one of, if not the only, possibility to ensure the long-term maintenance of what has been done so far (Taylor & al. 2021). Accordingly, the data presented here, and all the rest of the information reported in the databases is freely available, with the aim of laying the foundations of an even more inclusive and constructive network of wetland conservationists.

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Management actions to achieve Favourable Conservation Status for the priority plant species of the Habitats Directive *Androcymbium rechingeri* (Colchicaceae) in Greece

Abstract

Fournaraki, C., Grigorakis, E., Gotsiou, P., Markaki, E., Kokkinaki, A., Choreftakis, M., Kontakos, D., Lymberakis, P., Nousia, P. & Sklavaki, P.: Management actions to achieve Favourable Conservation Status for the priority plant species of the Habitats Directive *Androcymbium rechingeri* (Colchicaceae) in Greece. — Fl. Medit. 32: 351-360. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Androcymbium rechingeri Greuter is a range restricted geophyte. It occurs only on sandy beaches and coastal areas in West Crete, Greece: Elafonisi islet, Elafonisi coast, Falasarna and Imeri Gramvousa islet and it has also been reported in coastal areas of Libya. *A. rechingeri* has been categorized as Endangered according to the IUCN Red List and the Red Data Book of Greece (1995). It is listed as priority species in Annex II of the Habitats Directive and is protected by the Greek Presidential Decree 67/81 and the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). Management actions are currently in progress with the aim to achieve Favourable Conservation Status for the species. The actions include fencing and designation of pathways in the highly touristic areas of Elafonisi and Falasarna, re-establishment of the plant population at part of Elafonisi, seed collection from all subpopulations for *ex situ* conservation, planning for long-term monitoring of the *in situ* conservation actions, and raising awareness of the residents and visitors. This work presents the results from population reinforcement of *A. rechingeri* started during 2016-2019, in the framework of the CARE-MEDIFLORA project at the Elafonisi coast, and continued to this day with the monitoring of the results. Moreover, the results from the germination and viability tests of the seeds samples collected from the various subpopulations of the species are presented. Finally, the various management actions that have been implemented for species conservation are described.

Key words: threatened plant species, West Crete, conservation actions, *in situ*, reinforcement protocol, *ex situ*, germination.

Introduction

Life form & distribution - *Androcymbium rechingeri* Greuter (Colchicaceae) is a geophyte which is range restricted in Europe, occurring only on sandy beaches and coastal areas

in West Crete, Greece (Elafonisi islet, Elafonisi, Falasarna and Imeri Gramvousa islet) (Kypriotakis & al. 1998; Fournaraki & al. 2005). Elsewhere, it has been reported in N Libya (WCSP 2022) and in coastal areas of N Africa from Tunisia to NW Egypt (Strid 2016).

Legal status - *A. rechingeri* is listed as priority species in Annex II of the Habitats Directive (Council Directive 92/43/EEC). It is included in Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and it is protected by the Greek Presidential Decree 67/81. Most of the subpopulations in West Crete are included in NATURA 2000 sites (GR4340002, GR4340001 and GR4340015).

Conservation status - The conservation status of *A. rechingeri* has been assessed as Endangered under the IUCN Red List criteria B1ab(iii)+2ab(iii) (Delipetrou 2011) and it is included in the Red Data Book of Greece as Endangered (Phitos & Kypriotakis 1995).

According to the last national report under Article 17 of the Habitats Directive (Council Directive 92/43 EEC), for the 6-year period 2013-2018 (EIONET 2022), the Conservation Status of the range and of the total population of the species *A. rechingeri* are assessed as Favourable, due to the recent discovery of some new, small subpopulations along the west coast of Crete. However, the Conservation Status of its habitat and its future prospects are assessed as Unfavourable-Inadequate due to severe pressures and threats. Consequently, the overall assessment of its Conservation Status is found Unfavourable-Inadequate.

Pressures and Threats - In Greece, the habitat of *A. rechingeri* is adequately protected at the locations on the islets. However, there is continuous degradation at Elafonisi coast and Falasarna due to recreational activities and tourism, overgrazing, agricultural activities, and construction of roads, parking areas and other facilities. In particular, both Elafonisi coast and Falasarna are highly touristic, attracting thousands of visitors every year, and there is extensive use of vehicles and trampling all over the areas due to recreational activities such as swimming, sunbathing, water sports and hiking. Umbrellas and sunbeds are established on the beach, the surroundings are extensively used as parking lots and camping grounds, and toilets, snack bars and boats are found all around. In addition, both areas and their surroundings are subjected to seasonal grazing by the local shepherds and in the vicinity of Falasarna there is a considerable number of agricultural installations (i.e. greenhouses) occupying and polluting the habitat.

Conservation actions - A first assessment of the whole population of *A. rechingeri* in West Crete was conducted by the CIHEAM Mediterranean Agronomic Institute of Chania (MAICH) during 1995-1998 with the project LIFE Western Crete 95/GR/A22/GR/01143/KRI (Kypriotakis & al. 1998). Conservation actions were initiated in 2004 with the project "CRETAPLANT: A Pilot Network of Plant Micro-Reserves in Western Crete" of the EU LIFE-NATURE-2004 Programme (Thanos 2007). The outputs of CRETAPLANT included a detailed inventory of the *A. rechingeri* populations (Fournaraki & al. 2005), *ex situ* conservation of all the subpopulations in W Crete, development of seed storage, germination, growth and outplanting protocols (Fournaraki & al. 2007), and the establishment of a Plant Micro-Reserve for *A. rechingeri* at Elafonisi islet.

In the past the local municipalities had placed fencing at Elafonisi coast to protect the priority habitat "Coastal dunes with *Juniperus* spp." (Code: 2250 *). The purpose of this action was the limitation of trampling and parking of cars beneath the *Juniperus macrocarpa* trees. This was very effective in restoring dunes and generally the natural vegetation of the area and among others for the conservation of the natural population of *A. rechingeri*

in this particular area. Moreover, other endemic and endangered species of flora that are recorded within the habitat of *A. rechingeri* are also protected. Specifically on the beach of Elafonisi the following species are found: *Bellevia brevipedicellata* Turrill, *Limonium elafonisicum* Alf. Mayer, *Viola scorpiuroides* Coss., *Centaurea pumilio* L. etc. (Kypriotakis & al. 1998). In the same area, part of the subpopulation is located within the priority habitat '2250* Coastal dunes with juniper species (*Juniperus* sp. pl.)'.

During the implementation of the CARE-MEDIFLORA international project (<http://care-mediflora.eu/>) (2016-2019), this species fulfilled the criteria for selection as 'target species' for conservation (Fenu & al. 2019a, Fenu & al. 2019b) and the following actions have been carried out: *ex situ* conservation at the seed bank of MAICH with five seed collections in the wild from respective natural subpopulations, germination experiments for plant production purposes, and *in situ* conservation at the Elafonisi area (setting up fences and information signs, and experimentation with different methods for the reinforcement of the species population); but so far only preliminary results have been published (Fenu & al. 2020).

In the work here described, results of conservation actions are presented, which are implemented in the framework of a national project for the conservation of *A. rechingeri* (2020-2022), funded by the Green Fund of the Greek Ministry of Environment & Energy, with the aim to contribute to the achievement of an overall Favourable Conservation Status for *A. rechingeri*, particularly with regard to its habitat and Future Prospects in the areas of Elafonisi and Falasarna. In addition, the results of the past reinforcement of the Elafonisi coast population, which was initiated with CARE-MEDIFLORA project, are given after a monitoring period of five years.

Main threats addressed - The threats that are mainly addressed involve the impact of tourism and recreation areas, the severe recreational activities and the nomadic grazing activities on the populations of Elafonisi and Falasarna.

Materials and methods

All field works and plant material collections of *Androcymbium rechingeri* have been implemented in the areas of Elafonisi, Falasarna and Imeri Gramvousa islet in West Crete (Fig. 1). The actions have been carried out by the Mediterranean Plant Conservation Unit of MAICH in close collaboration with the Decentralised Administration of Crete - Forest Directorate of Chania and the Management Unit of Samaria National Park and the Protected Areas of Western Crete of the Natural Environment and Climate Change Agency (N.E.C.C.A.). Research permits were obtained from the Greek Ministry of Environment & Energy.

Ex situ conservation

For *ex situ* conservation, seed collections were made from all known populations of *A. rechingeri* in West Crete. The collections were implemented in the periods May or June from 2020 until 2021 (Table 1). The seed collections were curated and stored using the GENMEDA (Bacchetta & al. 2006) and ENSCONET protocols (ENSCONET 2009a & 2009b). After cleaning, the seeds were placed in open containers in the Dry Room (temperature: 15-20 °C, relative humidity: 15-20%). The seeds remained in the Dry Room until packaged and stored in the Cold

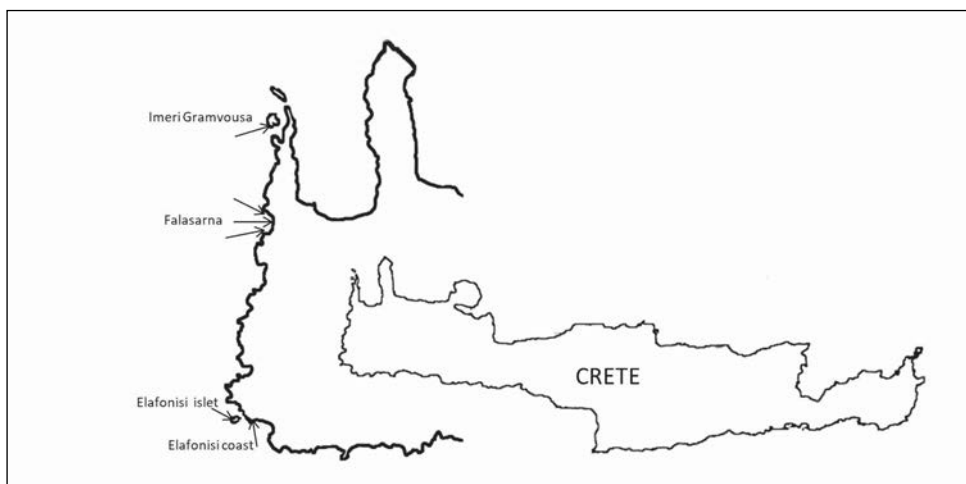


Fig. 1. Map of Crete showing the locations of various subpopulations of *Androcymbium rechingeri*.

Room (temperature: minus 18-20 °C). For safety reasons, part of the collections was duplicated at the Seed Bank of the National & Kapodistrian University of Athens.

Germination and viability tests were implemented for all the 2020-2021 accessions 4-12 months after their collection and during the Dry Room phase. The germination protocol of *A. rechingeri* was determined in previous experiments (Pretreatment: Seed scarification, Temperature: 15 °C, Photoperiod: 12 h light/12 h dark, Substrate: 2% agar) (Fournaraki 2010). The seed viability was estimated from the results of the germination tests and the cut

Table 1. *Androcymbium rechingeri* seed collections 2020-2021.

Sub-population locality	Date of seed collection	No of seeds stored in MAICH Seed Bank	No of seeds duplicated
Falasarna, mid	19/5/2020	7960	2000
Falasarna, south	20/5/2020	8620	2000
Falasarna, north	20/5/2020	6448	2000
Elafonisi coast	15/6/2021	5009	1000
Elafonisi islet	15/6/2021	2773	500
Imeri Gramvousa islet	24/6/2021	3270	500

tests which were performed on the non-germinated seeds after the end of the germination tests. To determine the germination rate, the parameter T50 was used (GENMEDA 2006).

In situ conservation

Population reinforcement. Experimentation on population reinforcement of *A. rechingeri* started in the framework of the CARE-MEDIFLORA project, during 2016-2019, at the Elafonisi coast (Fenu & al. 2019a, Fenu & al. 2019b, Fenu & al. 2020) and continues up to date with the monitoring of the results. During the national conservation project, further population reinforcement of *A. rechingeri* was implemented at the Elafonisi coast on 12 January 2021: a total of 1400 seeds were directly sown in the soil of a fenced area, within 14 grids fixed to the ground. In each metal grid, 100 seeds were sown, in 50 separate cells. The seeds used were from the seed collection from Elafonisi on 19/5/2016 which was stored in the Seed Bank of MAICH. All planting locations were recorded with GPS and mapped in order to be easily identified for the long-term monitoring. The place selected for population reinforcement was a fenced area at Elafonisi coast from where the species had almost disappeared due to the severe and continuous degradation of the habitat due to trampling by the thousands of summer visitors, parking of cars and other activities.

Fence erection. After relevant studies and permissions, two fences were established on the coast of Elafonisi and walking pathways were marked in the highly touristic areas of Falasarna beaches. The above interventions were carried out in collaboration with the competent management authorities (Decentralised Administration of Crete/Forest Directorate of Chania and the Management Unit of Samaria National Park and the Protected Areas of Western Crete) and after consultation with the Municipalities and land-owners.

Results & Discussion

Ex situ conservation

A total of 6 seed collections, one from each of the known sub-populations of *Androcymbium rechingeri*, were made in May 2020 and June 2021 (Table 1). In the June collections most of the capsules had opened and shed their seeds and therefore the collections were smaller in number of seeds.

In Figures 2 & 3, results of germination experiments and seed viability tests are presented. Final germination rates for the various seed samples range between 66-97%. Seed germination was faster (T_{50} = 17-19 days) for the sub-populations in the SW (Elafonisi area) compared to the sub-populations in the NW (Falasarna and Imeri Gramvousa) (T_{50} = 23-26 days) (Fig. 2). Various factors that affect the seed germination behavior of a population have been identified, among which are: 1) the time of collection, 2) storage of seeds in the dry room and in the laboratory (Fournaraki 2010). The small differences observed in the germination behavior of the different seed collections may be due to the above factors. Moreover, these differences have been observed in the same subpopulation of *A. rechingeri* in collections of different years (Unpublished data from the Seed Bank of MAICH).

Seed viability was over 70% for all sub-populations except from the area of 'Falasarna mid' which was slightly under 50% (Fig. 3). The low seed viability of this subpopulation (Falasarna mid) requires further investigation.

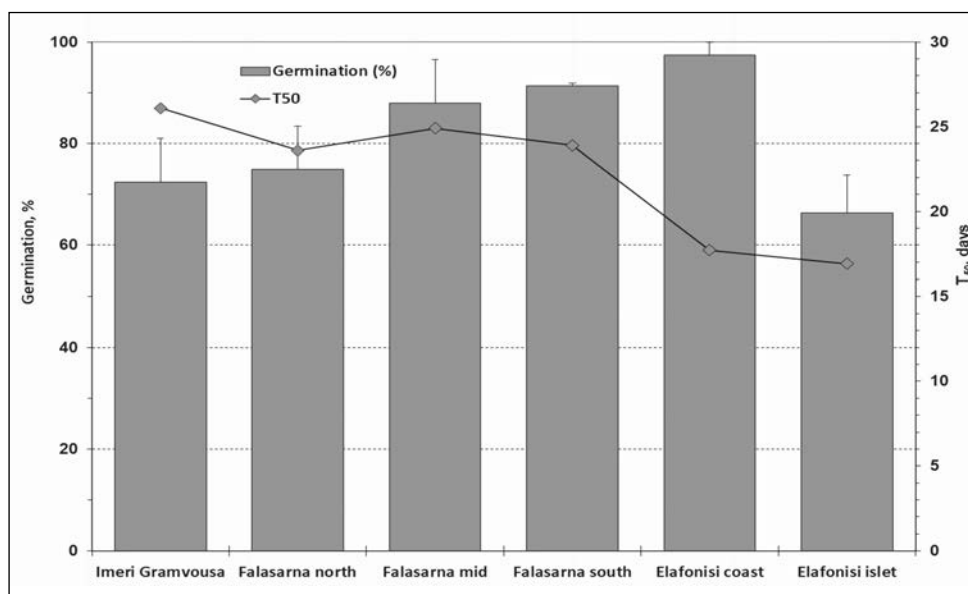


Fig. 2. Final germination percentage and germination rate (T_{50}) of the different sub-populations of *Androcymbium rechingeri* at a constant temperature of 15 °C and photoperiod 12 h light/12 h dark. Pretreatment: Seeds scarified. The vertical lines above the columns represent the standard error.

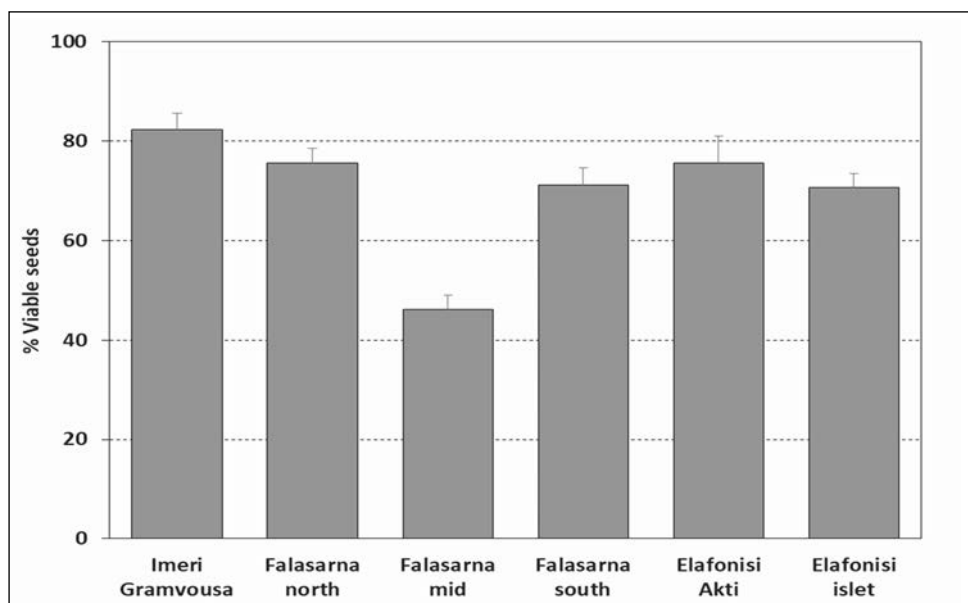


Fig. 3. Seed viability of the different sub-populations of *Androcymbium rechingeri* collected in 2020-2021. The vertical lines above the columns represent the standard error.

In situ conservation

Elaboration of optimum protocol for population reinforcement – Results from 5 years of monitoring of population reinforcement of *A. rechingeri* are presented in Fig. 4. Overall, the results can be summarised as follows:

- There is no significant difference in the successful establishment of *A. rechingeri* when sowing seeds directly in the field or planting seedlings during the winter period (December). In both cases the survival rate in fenced areas after 5 years is approximately 40% (Fig. 4).
- The establishment of new plants is successful when the locations are fenced in order to prevent grazing and trampling. After 5 years, the survival rate outside fenced areas is only about 10%.

Monitoring of plant establishment in the 1st year may show incorrect low survival rate (Fig. 4), due to non-distinguishable seedlings in the field because of their tiny size.

In addition, it was noted that plants established from seeds started to flower after 4 years from sowing and plants established from seedlings started to flower 3 years after planting.

Although there is no significant difference in the successful establishment of *A. rechingeri* plants when sowing seeds directly in the field or planting seedlings, sowing seeds is preferred as possible losses during plant production in the nursery are avoided and there is no risk of accidentally introducing alien species or other harmful organisms in the wild by transferring them with the plant substrate. Overall, sowing seeds directly in a fenced area is also a faster and less expensive method in terms of resources needed.

Therefore, the optimum reinforcement protocol for the *A. rechingeri* population is the direct sowing of seeds in the early winter period (December or January) provided that the pressure on the habitat of the species by tourism activities has been reduced.

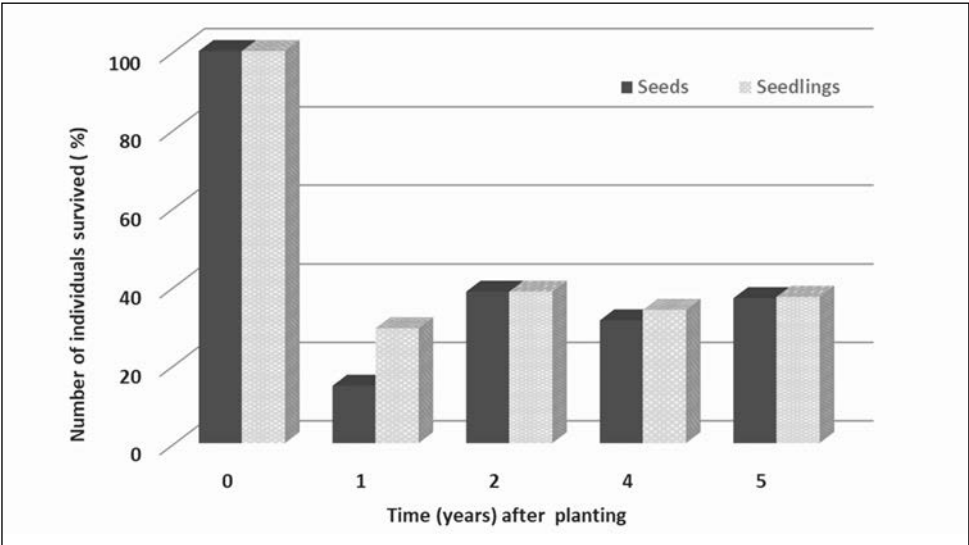


Fig. 4. Monitoring of *Androcymbium rechingeri* population reinforcement. Success rate when sowing seeds directly in the field (seeds) or planting seedlings (seedlings) inside a fenced area.

With regard to the last reinforcement action, the first monitoring of results, after 1 year, in January 2022, has shown that only 29 plants were established from the 1400 seeds sown. However, as mentioned above, the 1st year monitoring results are not indicative of the success of the action.

Fencing: Two fenced areas were constructed in Elafonisi coast (7,700 m² and 9,600 m²) and two pathways were demarcated in Falasarna (550 m in total). The locations specified for the fenced areas and pathways are those where the highest degradation of the sub-populations and the habitat was observed mainly due to the construction of roads, parking of vehicles and trampling by the thousands of tourists visiting Elafonisi and Falasarna.

Information signs providing information about the project and indicating the threatened species were placed in prominent positions at the entrance of the demarcated pathways and the fenced areas. Leaflets about *A. rechingeri* and the current project were prepared in English and Greek in order to be distributed to visitors. Until the end of the national project (2020-2022) it is anticipated to hold information days at the Municipalities of Kantanos-Selino and Kissamos. T-shirts with drawings of *A. rechingeri* have been prepared for distribution to students and residents participating in the information campaign. In addition, it is anticipated to hold a training seminar in order to inform the staff of the competent authorities (Forest Directorate, Management Unit, Municipalities, etc.) about issues related to the long term monitoring of the *in situ* conservation actions and the maintenance needs of the infrastructure of the project.

Conclusions

Ex situ conservation of all the subpopulations of *Androcymbium rechingeri* in Crete has been secured at the Seed Bank of MAICH. The observed variation in seed viability among subpopulations of *A. rechingeri* needs further investigation. The optimum reinforcement protocol for the *A. rechingeri* population is the direct sowing of seeds in the early winter period (December or January) provided that the pressure on the habitat of the species by tourism activities has been reduced. The survival rate in the fenced areas after 5 years was approximately 40%, while in unprotected areas, the survival rate was only about 10%.

The long-term development of scientific knowledge on the biology of the species, the population distribution and estimation, the conservation status of the subpopulations and the long-term monitoring of reinforcement actions have contributed to the implementation of an effective management plan for improving the species conservation status. Also, the above measures can improve the conservation status of other endangered species and habitats. The management of protected areas where there are intensive tourism activities requires multifaceted actions and approaches to be effective. It requires the close cooperation of all management bodies as well as great awareness of the inhabitants and visitors of Crete.

Finally, both areas, Elafonisi and Falasarna are popular tourist destinations and receive a large number of visitors annually. Therefore, the interventions can be an opportunity for demonstration projects aimed at informing and raising awareness on issues of sustainable tourism development in ecologically sensitive areas.

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S. González-Orenga, P. P. Ferrer, M. Boscaiu, O. Vicente & E. Laguna

Studies on salt and drought tolerance of endemic and rare species in Valencian salt marshes as a tool for reintroduction programmes

Abstract

González-Orenga, S., Ferrer, P. P., Boscaiu, M., Vicente, O. & Laguna, E.: Studies on salt and drought tolerance of endemic and rare species in Valencian salt marshes as a tool for reintroduction programmes. — Fl. Medit. 32: 361-365. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

Plant growth under stress conditions depends on the effectiveness of tolerance mechanisms in each species. The strategy of conducting comparative studies on genetically related taxa with different degrees of tolerance is a valuable approach, especially for distinguishing tolerance-relevant responses. The results may help design and implement conservation, reinforcement or reintroduction programmes and manage threatened populations of such rare and endemic species in the area of interest, the Albufera Natural Park.

Key words: endemism, conservation, Mediterranean, climate change, abiotic stresses.

Introduction

Drought and salinity are the environmental factors that affect the plants the most, although Mediterranean plants are generally well adapted to these adverse conditions. However, predictions estimate that, due to climate change, environmental conditions will become more stressful, especially in dry, semi-arid and arid areas. These changes could affect the occurrence of many wild species, particularly those that are already threatened, rare or endemic. Both drought and salt stress cause the activation of a series of defence or response mechanisms in plants, including control of ionic transport, accumulation of compatible solutes or osmolytes, and activation of antioxidant systems.

The study was performed in the Albufera Natural Park, a coastal area holding saline ecosystems of great ecological value and biodiversity. It is the most relevant protected area of Valencia Community (Spain) (Ballester & al. 2003). The study focused on two *Limonium* species (*Plumbaginaceae*) (*L. albuferae* P.P. Ferrer, Roselló, Roselló & E. Laguna and *L. dufourii* (Girard) Kuntze), one *Thalictrum* species (*Ranunculaceae*) (*T. maritimum* Dufour) and two *Bupleurum* species (*Apiaceae*) (*B. tenuissimum* L. and *B. fruticosum* L.) (Fig.1).

The unique population of *L. albuferae*, a recently described endemic species, is found in the salt marsh complex of the Albufera Natural Park (Ferrer-Gallego & al. 2016), where-

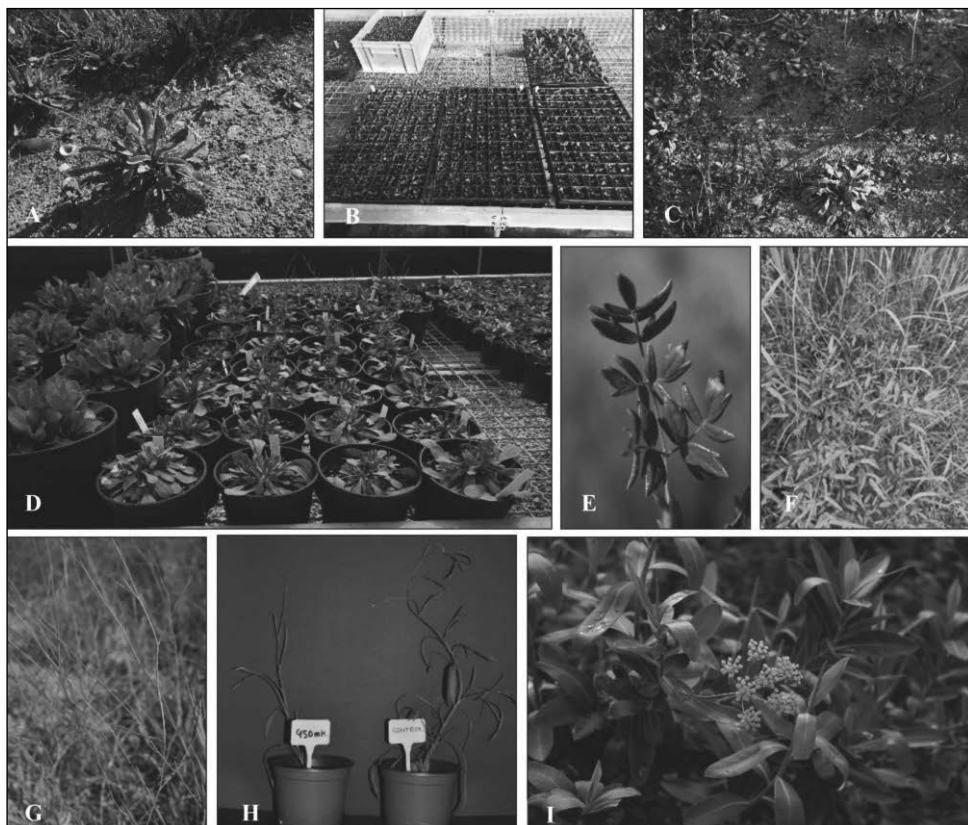


Fig. 1. *Limonium albuferae* in ‘Raco de l’Olla’ (El Saler, Valencia) (A) and plant production at the Centre for Forestry Research and Experimentation (CIEF) (Valencia) (B); *Limonium dufourii* in El Saler (Valencia) (C) and plant production at the CIEF (D); *Thalictum maritimum* in Torreblanca (E) and Almenara (F) (Castellón, Spain); *B. tenuissimum* in Marjal dels Moros (Sagunt, Valencia) (G), and at the end of treatments with 450 mM of NaCl and control conditions in the greenhouse of the Polytechnic University of Valencia (H); *B. fruticosum* in the Botanical Garden of Valencia (I).

as *L. dufourii* is present in a few other salt marshes outside this natural park (Navarro & al. 2006; Aguilera & al. 2010). Nevertheless, *L. dufourii* is one of the most threatened endemic species of *Limonium* in the Valencian territory (Aguilera & al. 2010). In the past, this species was more widely distributed along the coast and salt marshes of the provinces of Valencia and Castellón (Crespo & Lledó 1998). Nowadays, it is represented by only five separate natural populations restricted to small coastal areas.

On the other hand, *T. maritimum* is an eastern Iberian endemic, growing only in a few coastal areas (Ferrer-Gallego & al. 2015). Furthermore, the species occurs worldwide in only four sites, all of which are located in the Valencian Community, with its southern limit within the Albufera Natural Park (Ballester & al. 2003).

Finally, *B. tenuissimum* is a rare species in Spain, which has become extinct in recent decades in many localities, including the Albufera Natural Park. *B. fruticosum*, a congeneric species, was included as comparative material. At present, all these species – except *B. fruticosum* – are included in conservation programmes aimed at their reintroduction in this territory.

This multidisciplinary research included the analysis of the selected species and the parameters affecting their distribution and growth in their natural habitats, as well as the evaluation of their tolerance limits to salt and water stress under controlled greenhouse conditions. The main physiological and biochemical mechanisms of tolerance to drought, salinity and other abiotic stresses are known for some of the structural species in this habitat type (Katschnig & al. 2013; Pardo-Domenech & al. 2015; Al Hassan & al. 2016). Nevertheless, little information is available on the stress responses of differential, often endemic, rare, and/or endangered species such as those included in this study.

The results obtained allowed us to establish the relative tolerance to water deficit and salt stress of the analysed species and their relationships with environmental factors such as climate, vegetation and soil. Therefore, the optimal areas for the establishment of the most interesting species can be determined based on this information. Furthermore, the relevant tolerance mechanisms to both types of stress were identified in each species analysed. All plants activate the same defence mechanisms in response to abiotic stress, but their contribution to tolerance differs by genus and even species. In general, an intensification of ion transport to aerial parts and the activation of enzymatic antioxidant systems to maintain redox balance were noticed. As a result, most of the species studied did not show oxidative stress, except for the two species of the genus *Bupleurum*.

The species of the genus *Limonium* showed the slightest growth inhibition under stress, especially under conditions of high salinity. Therefore, this genus is clearly the most tolerant of those studied; its defence against stress is mainly based on the activation of the transport of toxic ions to the aerial part, the accumulation of compatible solutes and the activation of enzymatic antioxidant systems. The data obtained indicate that salinity is not a limiting factor for reintroducing the two *Limonium* species of interest in the Albufera Natural Park (Valencia, Spain), as both tolerate much higher salinities under controlled conditions than those present in their natural habitats. However, water scarcity could be a problem for *L. albuferae*, whereas *L. dufourii* should not be introduced in areas prone to prolonged flooding (González-Orenga & al. 2019a, 2019b; González-Orenga & al. 2021a).

Thalictrum maritimum was more tolerant than expected to saline stress but less resistant to water stress, which caused greater growth inhibition. Its defence mechanisms seem to be more related to active ion transport to the aerial part and maintenance of foliar K⁺ homeostasis, as well as activation of enzymatic antioxidant systems. Consequently, this species behaved as a moderate halophyte, with optimal growth in the absence of salt, but tolerating much higher concentrations than its natural habitats; however, it was sensitive to water deficit (González-Orenga & al. 2020).

The two species of the genus *Bupleurum* showed a clear difference; the relevant endangered species, *B. tenuissimum*, is more tolerant of salinity, whereas the one used as comparative material, *B. fruticosum*, is more drought tolerant but susceptible to salinity even at low concentrations. Their primary defence mechanisms are related to immobilisation of toxic ions in the roots, accumulation of proline and activation of enzymatic and non-enzymatic antioxidant systems (González-Orenga & al. 2021b).

The results obtained in this study provide critical information for the reintroduction and conservation or maintenance of the species of interest, and allowing the understanding of the population reduction in recent years. On the other hand, the results may also contribute to a better knowledge of water and stress tolerance mechanisms in the studied genera, providing information on stress responses in species that have not been previously analysed.

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M. Iannaccone, A. Lamoliere & J. A. Buhagiar

Use of *Suillus collinitus* to increase survival rate and resilience of transplanted *Pinus halepensis* seedlings in habitat restoration practices

Abstract

Iannaccone, M., Lamoliere, A. & Buhagiar, J. A: Use of *Suillus collinitus* to increase survival rate and resilience of transplanted *Pinus halepensis* seedlings in habitat restoration practices. — Fl. Medit. 32: 367-374. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

In-situ conservation is considered the best way to preserve biodiversity where multiple species can persist with all their natural ecological interactions. The latter element is often ignored. Criticalities can arise when *ex-situ* grown plants are translocated *in-situ* without their natural soil microbial associations, especially where abiotic stresses can be severe. Mycorrhizal Fungi (MF) provide several benefits to plants and ecosystem in which are present. The aim of the project was to investigate the use of indigenous MF to enhance acclimatization and survival of *ex-situ* grown plants translocated for habitat restoration. The seeds of *Pinus halepensis* Mill. as well as sporocarps of naturally associated indigenous MF *Suillus collinitus* (Fr.) Kuntze (1898), were collected from Natura 2000 sites in the Maltese Islands. *P. halepensis* seeds were germinated under greenhouse conditions. *S. collinitus* was inoculated at sowing and seedling stage by placing fragments of the ripe sporocarps in the *P. halepensis* seedling trays. All the mycorrhized plantlets produced have been reintroduced into selected area of Natura 2000 in the Maltese Islands as part of SiMaSeed restoration project. Such MF inoculation actions can help to enhance restoration and resilience of habitats to reduce biodiversity loss.

Key words: ectomycorrhizae, Mediterranean flora, habitat restoration.

Introduction

The UN's 2030 Agenda for Sustainable Development calls for the preservation of biodiversity, which is threatened due to many factors, among them habitat degradation (www.sdgs.un.org/goals). Actions to enhance restoration and resilience of habitats to reduce biodiversity loss are needed.

Though *in-situ* conservation is considered the best way to preserve biodiversity where species can persist with all their natural ecological interactions, the latter element is often ignored (Godefroid & al. 2011; Fenu & al. 2019). Criticalities can arise when *ex-situ* grown indigenous plants are translocated *in-situ*, without their natural soil microbial associations, especially in a Mediterranean climate where abiotic stresses are severe (Doyle & al. 2021).

Factors which affect plant acclimatization as well as possible solutions for a better establishment *in-situ* are summarised in Doyle & al. (2021). Among possible solutions, Haskins & Pence (2012) found that plant translocation is more successful if mutualistic relationship with soil microbiota have been established. Mutualistic relationship between mycorrhizal fungi and plant, promote plant productivity, alleviate abiotic and oxidative stresses, enhance access to micro- and macro- nutrients as well as water (Smith & Read 2008).

The thermophilous vegetation of the Maltese Islands, located in the middle of the Mediterranean Basin, has been extensively described in Sommer and Caruana Gatto (1915), Borg (1927), Haslam (1969), Haslam & al. (1977), Lanfranco (1984, 1995a), Schembri (1994, 1997), Lanfranco & Schembri (1986), Anderson & Schembri (1989), Savona-Ventura (2001), Casha (2015), Brullo & al. (2020).

At present, woody vegetation in the Maltese Island is restricted to localized areas, though, presumably in the past it was much more abundant as many toponyms might confirm (Brullo & al. 2020). For instance, Wied Żnuber which in Maltese translates to Aleppo pine valley, suggests that long ago it was populated by stands. Wied Żnuber is part of the Special Protected Area of Conservation of International Importance of Rđumijiet ta' Malta: Ir-Ramla ta-Cirkewwa sa Il-Ponta ta' Benghisa (MT 0000024). The above-mentioned valley currently records few individuals of *P.halepensis*.

In the Maltese Island the *P. halepensis* is only cultivated in gardens or street edges, as well as it is used for reforestation and has been found associated with the ectomycorrhizal fungus *Suillus collinitus* (Fr.) Kuntze (1898), by several authors including Lanfranco (1995b), Mifsud (2011), Iannaccone & Buhagiar (2020).

The aim of the project was to restore part of the population of *P. halepensis* present in Wied Żnuber with indigenous ectomycorrhizal fungus *S. collinitus*. This was intended to enhance acclimatization and survival of seedlings that had prior inoculation of *ex-situ* grown plants, post transplantation.

Material and Methods

Plant material

One-year-old *Pinus halepensis* cones were collected in August 2019 from an Aleppo pine stand in Ghajn Tuffieħa and then stored at the Seed Bank of the Biology Department at the University of Malta. In November 2020 cones were opened at 80°C in a dry oven for 3 hours. Seeds were cleaned from bracts, then sown at the beginning of January 2021 in a mix of premium compost (Mikskaar), white peat (Mikskaar) and perlite (Kauf) (2:3:1) in square ribbed nursery pots (ARCA™, 8 × 8 × 12 cm). Seeds were sown 1 cm below the surface. A total of 10 replicates were prepared. Each replicate consisted of 6 pots, with 5 seeds per pot (N=30), for a total of 300 seeds. Pots were then watered by capillarity and exposed to Maltese natural environmental conditions in a semi-shaded area. After germination, seedlings were watered as required.

The seed were considered germinated after the full emergence of the cotyledons in erect position, since it was not possible to perform any observation of the radicle as commonly agreed for germination experiments (Come, 1970). Seedling emergence was recorded after a total period of 4 months under local natural conditions of temperature and humidity, after ambi-

ent temperatures rose above 15°C, as shown in Figure 1. After a period of 6 months, site was surveyed and the survival rate of non-mycorrhized plants as well as mycorrhized was assessed.

Fungal material

Four medium sized sporocarps of *S. collinitus* were collected from Il-Buskett, limits of Dingli, Malta in December 2020, cleaned under running water, chopped, and blended in 2 litres of sterile distilled water and stored at 4°C. Two inoculations were performed, the first a week after sowing *P. halepensis* seeds, the second on 8 weeks-old *P. halepensis* seedlings. The inoculation was performed by diluting 1 litre of the mother solution into 3 litres of sterile distilled water, then pots were soaked in such solution until the substrate was saturated (Chen & al. 2006). Mycorrhization was visually assessed by removing the seedlings from the pot and examining the root morphology as well as the presence of the mycelium (Chen & al. 2006).

Before and after being granted approval from the Environment & Resource Authority (ERA) as the responsible body, several surveys were carried out before proceeding with the planting intervention in order to identify the most suitable area for transplantation,

Results and Discussion

Pinus halepensis Mill.

Malta: Ghajn Tuffieha, Mellicha, Malta, (WGS84: 35.931195°N, 14.344549°E), J.A. Buhagiar, M. Iannaccone and A. Lamoliere (SiMaSeed/BDUM/19/93, Seedbank of the Department of Biology, University of Malta).

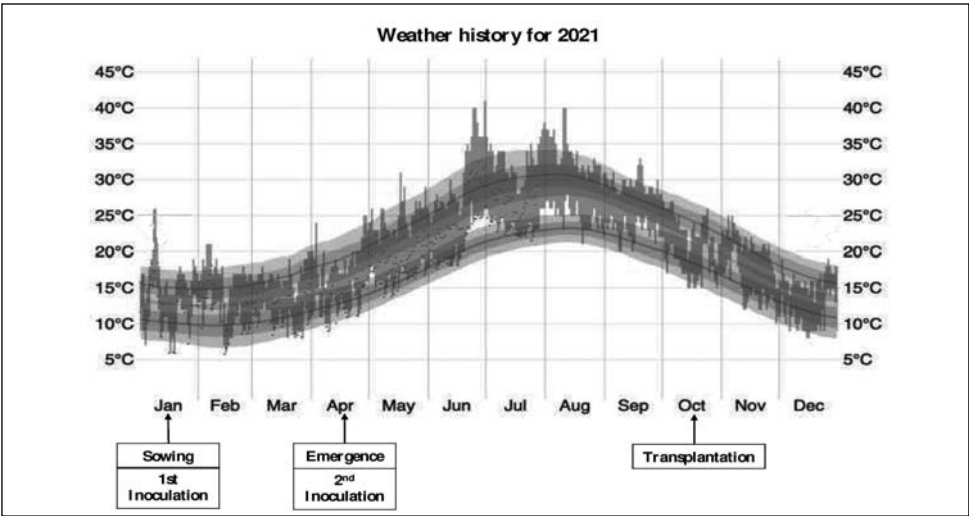


Fig. 1. The daily range of reported temperatures (grey bars) and 24-hour highs and lows, placed over the daily average high and low temperature, with 25th to 75th and 10th to 90th percentile bands (Wheaterparks modified by M.Iannaccone).

Sample size: 300 seeds

The total germination under seminatural condition was of 52%. The present results agreed with Thanos (2000). As expected, due to 19 months of storage in a semi-controlled environment, seed viability has generally decreased compared to other results mentioned in Nunes & Calvo (1999), Thanos (2000), Daskalakou & Thanos (2004) as well as references within.

Suillus collinitus (Fr.) Kuntze (1898)

Malta: Il-Buskett, Had-Dingli, Malta, (WGS84: 35.859490°N, 14.397619°E), J.A. Buhagiar, M. Iannaccone and A. Lamoliere (MYCOBDUM-MI/2020/1), Mycobank of the Department of Biology, University of Malta).

The ectomycorrhizal fungus *Suillus collinitus* has been commonly found associated with *P.halepensis* (Galli 2013). Collection date November 2020. As the source of inoculum spores, as well as mycelium, have been widely used by Cordell & al. (1987), Torres & Honrubia (1994), Honrubia & al. (1997), and González-Ochoa & al. (2002) to achieve mycorrhization. The use of mycorrhized plants for afforestation has been studied by Le Tacon & al., 1988 and in the specific case of *P. halepensis* and *Pisolithus arhizus* (Scop.: Pers.) Rauschert 1959 by Querejeta & al. (1998).

In our project, a total of 59 seedlings of *P. halepensis* were translocated *in-situ*, of which 11 were successfully mycorrhized with *S. collinitus*, as shown in Fig. 2. The remaining 48 did not shown any evidence of mycorrhization. It was observed that the growth performance of mycorrhized *P. halepensis* in the nursery, survived periods of summer heatwaves better

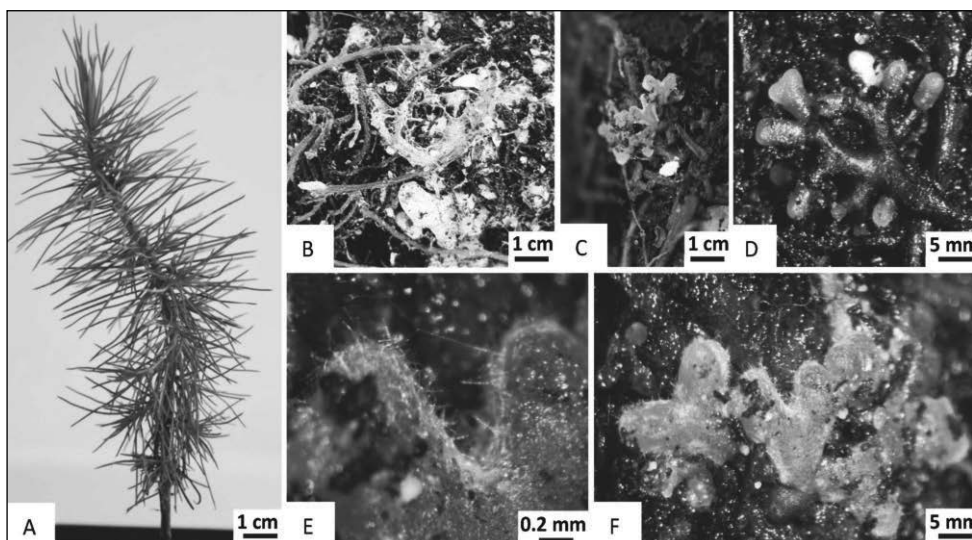


Fig. 2. **A.** *Pinus halepensis* seedling. **B.** *P. halepensis* root system embedded in *Suillus collinitus* mycelium. **C-D.** *P. halepensis* modified roots associated with *S. collinitus*. **E-F.** Close up.

than non-mycorrhized seedlings (Smith & Read, 2008). The same observation is valid for *in-situ* transplanted seedlings after a period of 6 month, as shown in Fig. 3.

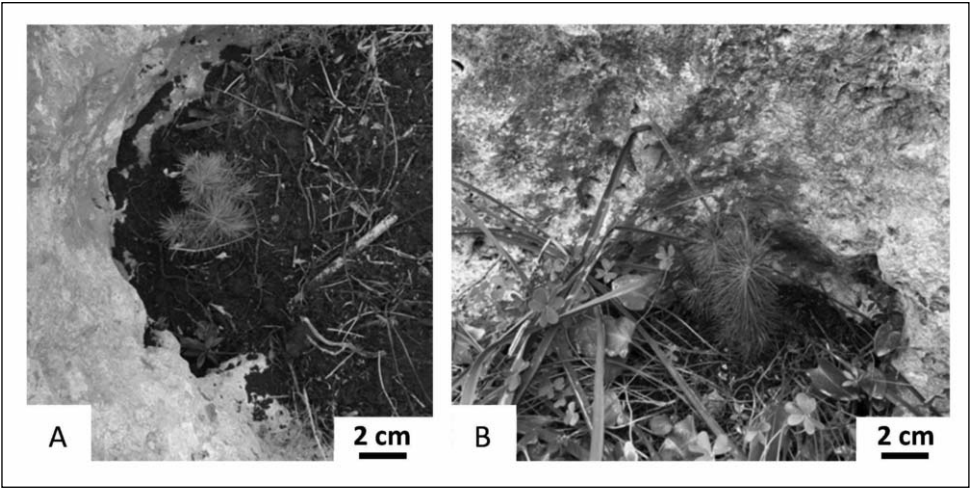


Fig. 3. *Pinus halepensis* associated with *S. collinitus* **A.** Translocated. **B.** 6-months after translocation.

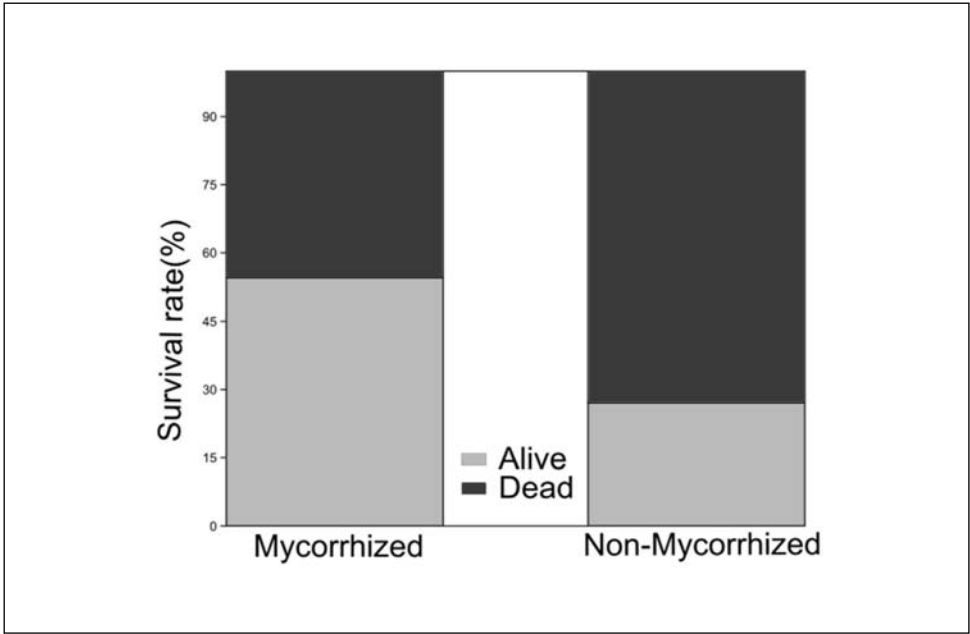


Fig. 4. Difference in survival rate (%) between Mycorrhized and Non-Mycorrhized plants.

As regards survival rates of transplanted plants after a period of six months, it was observed that the non-mycorrhized seedlings have higher mortality compared with the mycorrhized seedlings. Though no further examination on dead and surviving seedlings were carried out, the mycorrhized seedlings probably benefited from the advantages provided by the ectomycorrhizae association of *S. collinitus* and also other biotic and abiotic factors.

For further studies, a larger experimental sample should be taken into consideration, whilst the subjects from the present study will continue to be monitored. Furthermore, it is suggested that the use of indigenous arbuscular mycorrhizal fungi as well as other soil-born beneficial fungi such as *Trichoderma* spp. be given more importance in habitat restoration practices.

Conclusions

Through the present research, it was possible to confirm that cones of *P. halepensis* can be preserved in semi-controlled conditions for at least 19 months, whilst still maintaining a good degree of viability at 52% seed germination.

It was also possible to successfully associate ectomycorrhizal fungi *S. collinitus* to *P. halepensis* simply by introducing liquid inoculum containing spores and mycelium. Furthermore, such association appear to enhance plant acclimatization in *in-situ* translocation.

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Basiliki Kakampoura & Maria Panitsa

Plant diversity of olive groves under different management practices: a case study on Lesbos Island (East Aegean area, Greece)

Abstract

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The Mediterranean Basin is evolving due to human - nature interactions. The management of agroecosystems strongly affects plant diversity. Olive groves cover 19% of the cultivated area in Greece of which 8% is organic, cultivated using environment-friendly farming practices, while the rest includes conventional (intensive) or abandoned olive groves. Lesbos, the third-largest island of Greece, is characterized by high plant diversity. The current study aims to investigate plant species diversity of olive groves under different management practices. Sampling activities have been carried out in 65 plots. Field surveys to sample the vascular flora and vegetation were performed between April and May of 2020 and 2021. More than 210 plant taxa have been recorded in the studied olive groves. The results showed that organic olive groves are characterized by a rich and diverse flora, mainly dominated by therophytes and especially of annual leguminous species indicating moderate long-term human interference. Intensive olive groves have a poor and rather common vascular flora also dominated by therophytes. Abandoned olive groves present a rather poor but diverse flora, mainly dominated by chamaephytes, hemicryptophytes and phanerophytes and by a lower proportion by annual plants, depending also on the time lapse after abandonment.

Key words: Mediterranean ecosystems, biodiversity, intensive olive cultivation, α -diversity, β -diversity.

Introduction

The occurrence of olive trees is a reliable index of Mediterranean-type ecosystems (Blondel & al. 2010) and *Olea europaea* L. is possibly the most emblematic of all Mediterranean plants (Thompson 2020) since ancient times; that is why, according to the Greek Mythology, ancient gods such as Zeus and Athena were protectors of the olive tree and its cultivation (Loumou & Giourga 2003).

The cultivation of olive groves in the Mediterranean Basin has a long history, probably dating back to more than 6.000 years, when it started to spread from Eastern Mediterranean to the West (Thompson 2020) and slowly after 4000 years reached countries such as

Tunisia and Spain, when the humanity was starting to understand the benefits of the olive fruits (Loumou & Giourga 2003). Centuries ago, it is said that the first Levantine traders and then the Roman Empire spread the olive groves even at the North African and Southwest European countries (Ladisa & al. 2012). It should be mentioned that there is a debate concerning the migration history of olives, olive domestication and cultivar identity as several data suggest the repeated trails to cultivate wild olives in NW Africa, Spain and elsewhere (Meyer & al. 2012; Diez & al. 2015; Kaniewski & al. 2012). Concerning to Greece, Phoenicians are said to be the first merchants, who brought *Olea europaea* to the country, while the trade at the Aegean archipelago begun around 1000 BC (Terral & al. 2004).

Olea europaea var. *sylvestris* (Mill.) Lehr., the wild olive tree, is one of the most common trees occurring on the slopes and on the manmade cultivated terraces throughout of the Mediterranean (Loumou & Giourga 2003). Through the time, the domestication of wild olive tree has led to different cultivated varieties of olive trees and their huge expansion in many Mediterranean countries along with human activities (Besnard & al. 2001).

Olive groves represent a core feature of the Mediterranean environment and culture; human activities had a marked and long-standing impact on the biodiversity of olive groves (Dretakis & al. 2012). The significance of traditional farming practices on Mediterranean areas and among them on continental Greece and Greek islands for the conservation of plant diversity has been highlighted in different studies (Turland & al. 2004; Bergmeier & Strid 2014; Bergmeier & Meyer 2018). The recent intensification of the cultivation's management, namely the increased use of fertilizers and pesticides as well as the abandonment of cultivated areas induce a sharp decrease in biodiversity, leading to the dominance of a few ruderal plant taxa (Storkey & al. 2012; Solé-Senan & al. 2014; Storkey & Neve, 2018; Panitsa & al. 2020; Georgiadis & al. 2022,).

During the 20th century, intensified farming practices (e.g. heavy use of agrochemicals and fertilizers, mechanized cultivation techniques) induced the sudden and deliberate removal of local wild flora, replacing the traditional agricultural practices (Beaufoy 2001). Thus, the intensification of agriculture from the one side increases olive production, but at the same time it is included among the major threats of global biodiversity (Solomou & Sfougaris 2011).

Lesbos (Lesvos), one of the biggest islands of the Aegean area, was named the Olive Island (Elaion Nisos) due to the cultivation of olive groves on hilly and terraced surfaces (Loumou & Giourga 2003). Local olive groves cover an area of about 40,215.8 ha., i.e., nearly 41% of the island's cultivated land (Taxidis & al. 2015). The different management of olive groves includes organic olive groves, mostly occurring on terraced areas, as well as the conventional (= intensive) and abandoned ones, which are found mostly at hilly areas (Kizos & Koulouri 2005, 2006). On Lesbos, as elsewhere, most farmers use chemical plant control against alien species such as *Oxalis pes-caprae* L. (Petsikos & al. 2007).

In the framework of our project focused on assessing the plant diversity of Greek olive groves, the aim of this study was to investigate the impact of different management practices on the vascular plant diversity of olive groves of the island of Lesbos. With this aim, the authors investigated the differences in terms of plant diversity among organic, conventional, and abandoned olive groves and illustrate the plant traits revealing these differences.

Material and Methods

Study Area

Lesbos, the third largest island of Greece with an area of 1969 km², is located at the Northeastern Aegean area. The climate of Lesbos is typical Mediterranean and suitable for cultivation of olive trees. According to the data provided by the Hellenic Meteorological Agency for the time 1955-2010, it is characterized by short winters and dry hot summers with, about five to six drought months, maximum temperature in July (31°C) and minimum in December (6.1°C) while average monthly humidity is 56% for July and 73% for December. Local vascular flora counts more than 1550 plant taxa (Bazos 2005; Strid 2016). The dominant natural vegetation is dominated by phrygana, olive groves, pinewoods, and rather localised oak forests. The olive groves are located mainly in the southern and eastern part of the island. The olive groves examined in this study are close to the Natura 2000 site GR4110005, of Mount Olympus (<https://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=GR4110005>), (Fig. 1).

Most of the olive groves in the studied area are conventional (intensive), few are organic and even fewer and covering smaller surfaces are the abandoned ones. This pattern is due

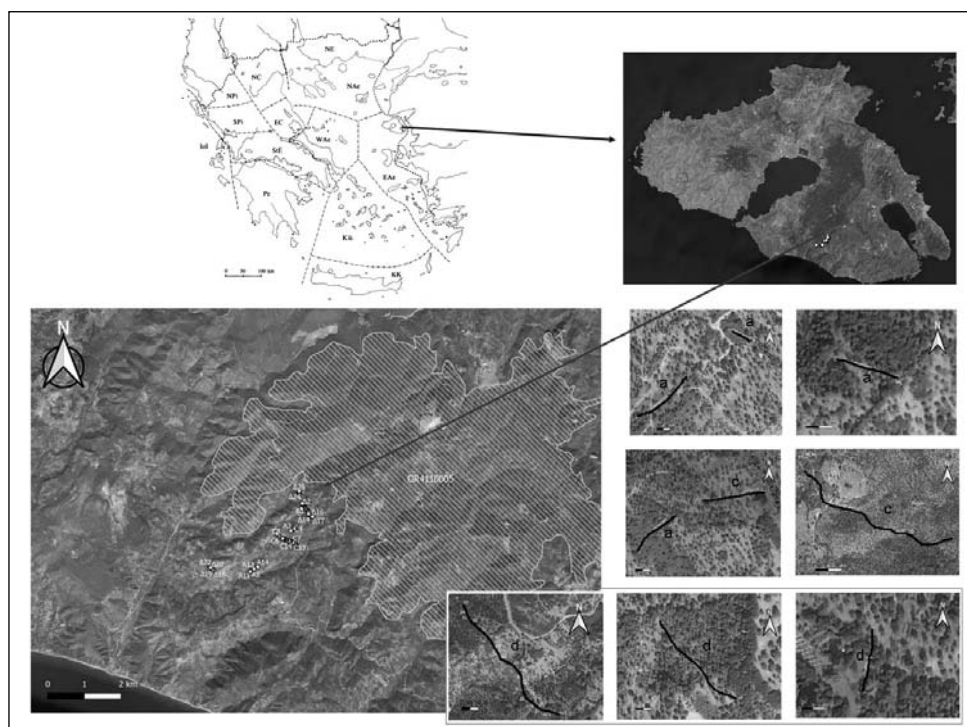


Fig. 1. Sampling area on Lesbos Island. A: Plots on abandoned olive groves (a), C: Plots on conventional olive groves (c) and D: Plots on organic olive groves (d).

to economic reasons since the local population is almost exclusively depended on olive grove cultivation and year after year this activity becomes more and more intense due to state subsidies for agricultural crops.

Sampling Methodology

Field work was carried out in the olive groves of the district of Ampeliko during spring 2020 (April and May) and repeated visits were carried out in all seasons of the year until December 2021 to record all the vascular flora. The sampling areas were selected in order to have a similar number of plots for each management typology, i.e., organic, conventional (intensive), and abandoned olive groves.

The floristic inventory has been carried out via transects of SW-NE direction at various elevations, and square sampling plots of 0.5 x 0.5 m and 25 m distance between them, all mapped using QGIS. The transects were not perfectly straight because of the irregular topography of the investigated (mostly terraced) areas, and the occurrence of fences which sometimes limited the accessibility to the groves. Plant communities' composition and structure of 65 sampling plots has been recorded using Braun-Blanquet method (1964). Of them, 25 were carried out on organic olive groves, 25 on conventional and 15 on abandoned olive groves. The mean elevation of the sampling areas was 373.2 m a.s.l. Data concerning vascular plants nomenclature and life forms (phanerophytes (P), chamaephytes (C), hemicryptophytes (H), geophytes (G) and therophytes (T)) follow Dimopoulos & al. (2013, 2016).

The free access statistical program Past (version 4.09, 2021) has been used to study α - and β - diversity as well as the species richness of the sampling plots. Shannon, Menhinick and Margalef diversity indices are simple species richness indices, characterized by the great advantage of ease calculation and Chao 1 is a simple estimator of the absolute number of species in an assemblage (Magurran 2004). Concerning β - diversity, Whittaker, Cody as also Wilson & Schmida indices have been used since Whittaker's index is one of the simplest and the most effective measures of species richness, Cody's index provides a good measure on species spatial turnover and Wilson & Schmida's index includes the elements of species loss and gain. Chao's 2 and Jackknife 2 estimators use presence/absence data by taking account of the distribution of species among samples, while Jackknife 1 estimator uses the number of species occurring only in a single sample (Magurran 2004).

Results

A total of more than 210 plant taxa belonging to 39 families and 135 genera occurs on the 65 sampling areas. More in detail, 145 taxa, belonging to 32 families and 104 genera, have been found in organic olive groves, 106 taxa (26 families, 78 genera) in conventional ones and 62 taxa (23 families, 53 genera) in abandoned groves (Table 1). Almost one third of the taxa have been observed growing only on organic olive groves, 19.5% only on conventional and 9.5% only on abandoned ones while only 10% of the taxa are present on all three types of olive groves, 28% are common among the organic and conventional olive groves, 17.7% among the organic and abandoned olive groves, and 12.4% among conventional and abandoned ones.

The species-richest families are: *Asteraceae*, *Poaceae*, *Fabaceae*, *Caryophyllaceae* and

Apiaceae. These families represent 15.6% of the families and count as much as 57.0% of the taxa found on organic olive groves, 19.2% of the families and 61.0% of the taxa of conventional ones and 21.7% of the families and 61.0% of the taxa on abandoned olive groves (Electronic Supplementary File 1: Table S1). Figure 2 shows the rate of taxa belonging to these families in the flora of organic, conventional, and abandoned olive groves. With 15 taxa, the genus *Trifolium* resulted to be the species-richest; interestingly enough, 73% of these taxa were present on organic, 60% on conventional and 33% on abandoned olive groves.

Concerning life-forms, therophytes predominate on all different managed olive groves and the percentage of therophytes and hemicryptophytes is higher in organic and conventional than in abandoned ones. Moreover, the percentage of phanerophytes and chamaephytes is higher on abandoned olive groves than in organic and conventional ones. Fig. 3 shows the different life-form spectra of the vascular flora of organic, conventional, and abandoned olive groves. Fig. 4 shows that the proportions of the families, genera, taxa, taxa of the richest families and life-forms recorded on organic olive groves are much higher than those on conventional and abandoned ones.

The maximum number of plant taxa per sampling plot for organic olive groves was 27,

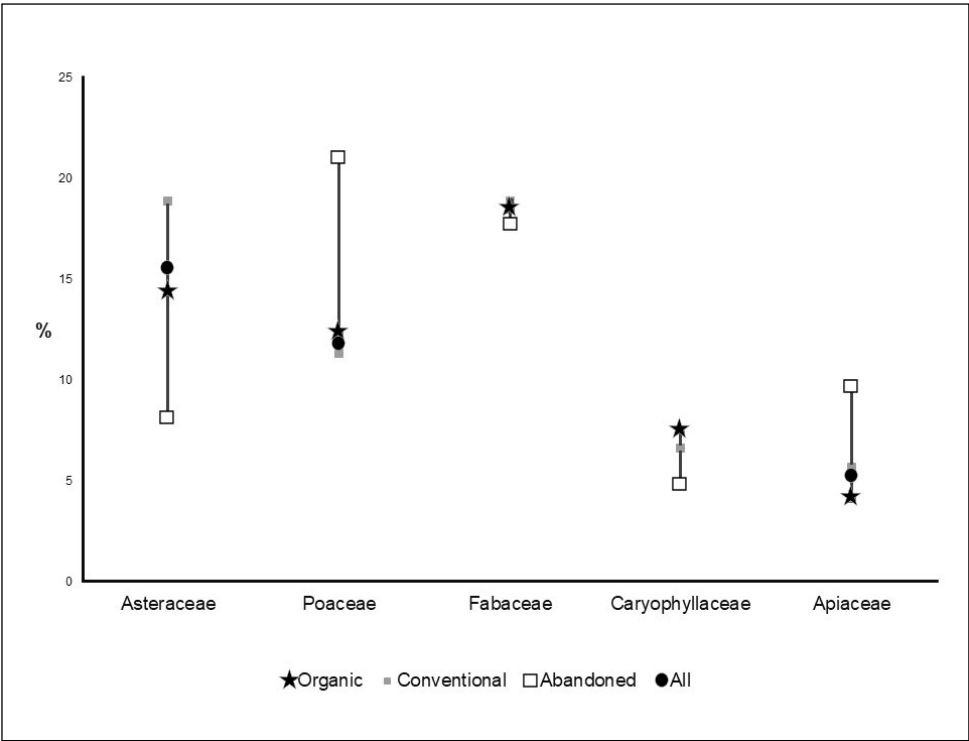


Fig. 2. Proportions of the taxa registered in organic, conventional, and abandoned olive groves, separately, belonging to the richest in species families of *Asteraceae*, *Poaceae*, *Fabaceae*, *Caryophyllaceae* and *Apiaceae*.

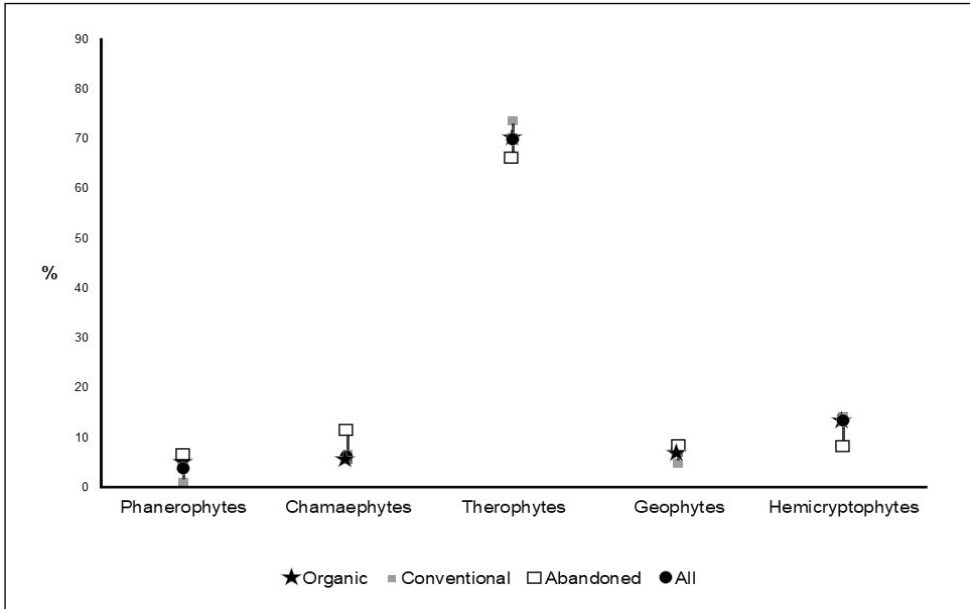


Fig. 3. Life-forms spectra concerning the vascular flora of organic, conventional, and abandoned olive groves.

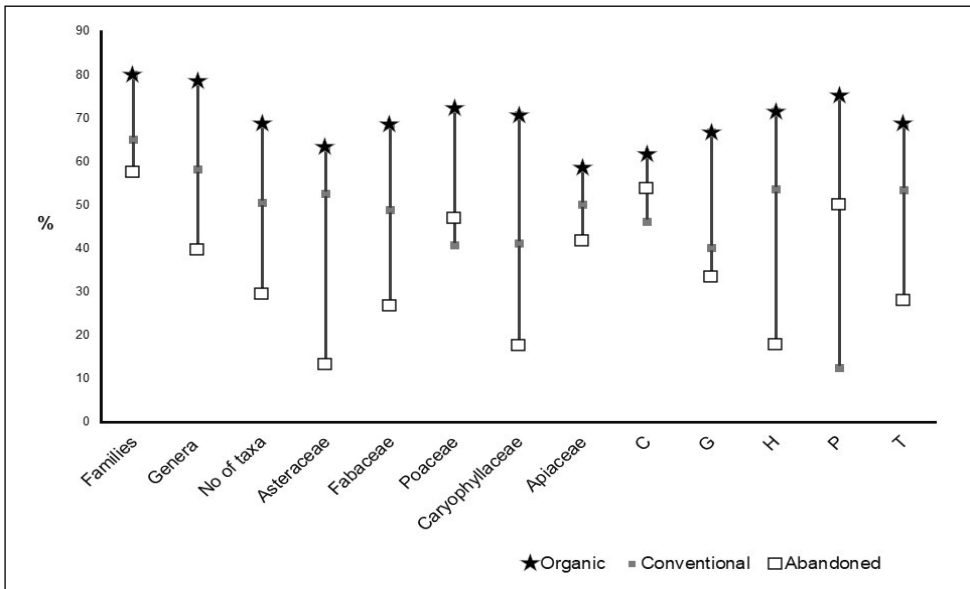


Fig. 4. Organic, conventional, and abandoned olive groves' proportions of the families, genera, number of taxa, richest families and life-forms on the total numbers recorded on all sampling areas.

with an average of 15 plant taxa, while for the conventional ones the maximum number of taxa was 17 with an average of 11 plant taxa; as for the abandoned olive groves, the highest number of plant taxa was 15 with an average value of 8 taxa.

The most common herbaceous species are: *Anthemis chia* L., *Trifolium campestre* Schreb., *Lagoecia cuminoides* L., *Crepis commutata* (Spreng.) Greuter, *Aegilops biuncialis* Vis., *Petrorhagia dubia* (Raf.) G. López & Romo, *Medicago orbicularis* (L.) Bartal., *Filago germanica* (L.) Huds. and *Dactylis glomerata* L., which were recorded on all three types of olive groves. The plants with the highest vegetation cover, mainly recorded on abandoned olive groves, are: *Cistus parviflorus* Lam., *Cistus criticus* L., *Phillyrea latifolia* L., *Sarcopoterium spinosum* (L.) Spach and *Lavandula stoechas* L.

Concerning α -diversity values, using the Shannon, Menhinick, Margalef, Fischer-alpha and Chao-1 indices, it was derived that α -diversity is much higher in the sampled organic olive groves than in the conventional and abandoned ones (Table 1). Additionally, values of all β -diversity indices among sampling plots of organic olive groves, are higher than those among plots of conventional and plots of abandoned ones. Values of β -diversity are much higher among plots of organic and abandoned olive groves, than among organic and conventional ones (Table 1). Quadrat richness calculations using four non-parametric species richness estimators and their standard deviations (St. dev.) also showed much higher values for organic olive groves than for conventional and abandoned ones (Table 2).

Discussion - Conclusions

Agricultural landscape dynamics in the Mediterranean have changed significantly the last 150 years and this is also the case for the Aegean Island of Lesbos (Kizos & Koulouri 2006). Local economic driving forces significantly changed Lesbos' olive agricultural landscape, mainly decreasing diversity of cultivated areas (Kizos & Koulouri 2005). The most common land use change is by far abandonment, which represents 96% of the olive land use changes (Kizos & Spilanis 2004) and significantly affects biodiversity (Myers &

Table 1. Values of α -diversity (α) and β -diversity (β , light grey cells) indices for sampling areas on organic, conventional, and abandoned olive groves and β -diversity (β , dark grey cells) indices between the pairs of Organic-Conventional, Organic -Abandoned and Conventional -Abandoned olive groves (grey cells).

	Index	Organic	Conventional	Abandoned	Organic- Conventional	Organic - Abandoned	Conventional -Abandoned
α	Shannon_H	4.916	4.412	3.954			
	Menhinick	9.265	6.732	6.498			
	Margalef	28.59	19.51	13.65			
	Chao-1	283.3	172.2	188.3			
β	Whittaker	11.888	9.223	9	13.106	17.947	15.658
	Wilson-Schmida	19.837	18.355	12.422	25.477	33.589	31.288
	Cody	255.5	197.5	79.5	298	351	285.5

Table 2. Quadrat richness: values of non-parametric species richness estimators and their standard deviations (St.dev.) for organic, conventional, and abandoned olive groves.

Organic			Conventional		Abandoned	
		St. dev		St. dev		St. dev
Chao 2:	278.082	32.448	165.12	20.252	187.956	53.202
Jackknife 1:	251.4	12.699	160.88	7.754	111.733	11.138
Jackknife 2:	305.105	NA	189.362	NA	147.762	NA

al. 2000; Haines-Young 2009).

The role and importance of traditional agriculture in the establishment and maintenance of biological diversity is variable and contentious (Siebert 2004). Our results showed that traditionally managed, organic olive groves are characterized by a high α -diversity, a rich and diverse flora, mainly dominated by therophytes and especially by annual leguminous species that are indicators of long-term but moderate human interference (Arianoutsou & Margaritis 1981; Barbero & al. 1990; Panitsa & al. 2003). Local extensive olive groves managed by ploughing show a higher abundance and diversity of ruderal species and host many annual flowers supporting key plant-insect interactions which in turn provide important pollination services to the adjacent agricultural areas, playing a more important role with respect to the abandoned groves, where many perennial species, characteristic of the phrygana plant communities, dominate (Potts & al. 2006).

Conventional olive groves are also dominated by therophytes and have a poorer and rather ubiquitous vascular flora, while abandoned olive groves present a rather poor but diverse flora, mainly dominated by chamaephytes, hemicryptophytes and phanerophytes and to a lower proportion by annual plants, depending also on the time lapse after abandonment. The best adapted plant taxa, that are also found in most sampling areas are assigned to the families *Asteraceae*, *Poaceae* and *Fabaceae* as the members of these families occur in most of the sampling areas, and this was the case of other similar studies (Solomou & Sfougaris 2011). In fact, other research focused on the olive groves of Messenia (south Greece) and Magnesia (inner central Greece), revealed the same pattern, with the organic olive groves showing the highest diversity followed by the conventional and the abandoned groves with the therophytes as predominating life-form (Solomou & al. 2013; Kjellström 2014). Our results showed that most of the “characteristic therophytic and generally herbaceous indicator species” defined for the organic olive groves by Solomou & Sfougaris (2021) namely *Medicago lupulina* L., *Hordeum bulbosum* L., *Trifolium arvense* L., *T. campestre* Schreb., and *Anagallis arvensis* L., as well as *Sonchus oleraceus* L. for conventional olive groves, and additionally, the eurytrophic herbaceous taxa they mentioned for olive groves, have also been recorded in the sampling areas of Lesbos. Due to ongoing secondary succession processes, the vegetation of abandoned olive groves counts a higher number of woody species (mostly subshrubs and shrubs) which prevent the establishment and growth of perennial herbs under the low density of sunlight (Koulouri & Giourga 2007; Rühl & Pasta 2007; La Mantia & al. 2008; Maccherini

& al. 2013). The abandonment of traditional olive groves has a significant environmental impact inducing a fast and sharp shrinkage of plant diversity, an increase of erosion and fire risk, leading to major changes in the traditional (semi-natural + cultural) Mediterranean landscapes (Duarte & al. 2008).

As for α -diversity indices, our results show that all the indices are positively correlated with plant species richness, as is the case for Shannon diversity index (Petsikos & al. 2007; Solomou & Sfougaris 2011, 2021). This is also the case of β -diversity, where all indices used have higher values at the organic olive groves revealing the floristic differences among them and the much higher species richness and dissimilarity of the floristic composition at the organic sampling plots. These conclusions are corroborated by the values of non-parametric species richness estimators (quadrat richness) that are all much higher for sampling plots of organic olive groves if compared with the conventional and abandoned ones. Plant species diversity, total and mean number of species and consequently α - and β -diversity are usually higher in organic than in conventional farming systems, as was also shown by Solomou & Sfougaris (2011, 2021).

Concluding, the authors confirmed the results of studies carried out on the Greek mainland (Solomou & Sfougaris 2011, 2021; Kjellström 2014), showing that herbaceous plant species diversity could be the best indicator for environmental monitoring in olive groves. The indices of α - and β -diversity used in this study revealed significant differences among the different management systems of olive groves when considering these different aspects of diversity and could also be used for providing useful information about the impact of different farming practices in olive groves. Olive groves' landscapes have the potential for biodiversity conservation in the Mediterranean region, when they are adequately managed (Rey & al. 2019); since their vascular flora presents an exceptional resemblance to the flora of Mediterranean type ecosystems (Margaris & Mooney 1981), even in terms of functioning, cost-efficient condition, and ecosystem services (Loumou & Giourga 2003).

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A. Lamoliere, M. Iannaccone & J. A. Buhagiar

Use of morphocolorimetric analysis to monitor germination success of hydroprimed seeds of *Coronilla valentina* subsp. *glauca* (Fabaceae)

Abstract

Lamoliere, A., Iannaccone, M. & Buhagiar, J. A.: Use of morphocolorimetric analysis to monitor germination success of hydroprimed seeds of *Coronilla valentina* subsp. *glauca* (Fabaceae). — Fl. Medit. 32: 387-393. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This research aimed to investigate if the germination rate of *Coronilla valentina* seeds (Fabaceae) increased when subjected to extended hydro-priming. Analysis of hydroprimed seeds was carried out by using morphocolorimetric analysis to characterise the imbibition at different hydration time intervals. Seeds that had not been hydro-primed represented the untreated control. Seed swelling is a reliable and time-efficient method to measure seed imbibition, yet a poor predictor of seed germination success. Timing and temperature of the hydropriming treatment influence the germination rate of *C. valentina* subsp. *glauca*, with the optimum hydropriming treatment, was found to occur within a 72h period following the 75°C to 25°C treatment.

Key words: germination protocols, Mediterranean flora, morphometrics, hydropriming.

Introduction

The Mediterranean Basin is a biodiversity hotspot where many habitats are under threat and actions for habitat restoration are needed (Canals Ventin & Lazaro 2019). *Coronilla valentina* subsp. *glauca* (L.) Batt, the shrubby scorpion-vetch, is a perennial shrub with a patchy distribution in the Maltese Islands, where it forms an *Erico multiflorae*-*Coronilletum glaucae*, a typical high sea cliff garrigue phytosocial association with woody vegetation (Brullo & al. 2020). This shrub belongs to the Fabaceae family and its habitus stays low and wide, with the typical flower structure from the family, and glaucous green foliage. The characterisation of this subspecies is based on the low segment number of a dehiscent legume with a membranous stipule, less than 2 cm (Pignatti 1987). A critical stage of the plant life cycle is germination, and, as for many Fabaceae, *C. valentina* subsp. *glauca* seeds have a hard-coated testa which can prevent germination because their low permeability limits water uptake (Lambers & al. 2008). In real conditions, this results in limited population recruitment due to the low germination percentage, especially in its natural habitat characterised by calcareous soils (Escudero & al. 1996).

Hydropriming is the controlled hydration of seeds in water or a solution of low osmotic potential to initiate the germination metabolism before sowing (Singh & al. 2015). This simple and cheap technique improves seed water-uptake efficiency and is useful in areas with unpredictable abiotic stresses such as high temperature and drought (Sarfraz & al. 2019). During hydropriming, the seeds swell over a period of time as reflected in their change of volume and seed coat colour. The imbibition pressure will eventually lead to the rupture of the seed coat, causing a chain reaction of metabolism activation: starting with hydration of respiratory enzymes, renewed protein synthesis, the release of hydrolytic enzymes mobilizing stored reserves, cell enlargement and renewal of cell division (Lambers & al. 2008; Singh & al. 2015). However, excessive swelling may be an indication of over-imbibition which could lead to seed storage reserves deterioration and fermentation, eventually resulting in embryo death. Although seed swelling and color change are easy to characterise by visual observation, the use of morphocolorimetric methodology based on image analysis provides an accurate and quantitative approach, therefore providing objective and repeatable data (Vale & al. 2020).

This study aimed to investigate the efficiency of hydropriming treatment on seeds of *C. valentina* subsp. *glauca*. to increase the final germination percentage. The morphocolorimetric analysis is used to characterize the seeds of this subspecies and quantify the imbibition process over time.

Materials and methods

Accession data

Coronilla valentina subsp. *glauca* was identified according to Pignatti (1982) and circa 5000 seeds were collected in June 2021 in the Maltese Islands (WGS84: 35°49'18.4"N 14°27'36.4"E, 106 m a.s.l), in the locality of Wied Babu, part of the Rdumijiet ta' Malta S.A.C. (Natura 2000 site).

Treatment

Seeds were dried, manually extracted from their pod, cleaned and examined for infestation and macrocontaminants at the Seedbank facilities of the Department of Biology, University of Malta. Different conditions of the hydropriming treatment were tested using 2 temperatures and 4 time interval variables, plus a control group with no hydropriming and no temperature treatment (Table 1).

Each treatment was applied to 4 replicates of twenty-five seeds. Seeds were afterward sown in a compost: perlite (2:1) substrate and exposed to optimum germination conditions (fluctuating temperature 15 to 23.5°C, fluctuating relative humidity from 50 to 85%). Pots were watered by capillarity.

Data collection

The success of the treatment was recorded in daily germination emergence. Swelling of the seed due to imbibition was characterized by morphocolorimetric data using image analysis (Fig. 1) carried out with ImageJ 1.53 (Shneider & al. 2012) and the Particles8 plu-

Table 1. Variables of the Hydropriming treatment

Conditions (unit)	Value
Priming Time (hours)	0
	24
	48
	72
	96
Temperature (°C)	40°C constant
	75°C, then left to cool to 25°C

gin (Landini 2008). The image capture and analysis were based on the methodology detailed by Vale & al. (2020), with Hue, Saturation, and Brightness threshold value adjusted for the chosen blue background and seed color to increase contrast and therefore image segmentation accuracy. The settings used are described in the ImageJ macro, detailed in the Electronic Supplementary Material 1 (ESF1).

Data Analysis

Automatic classification of the seeds was carried out using k-means cluster analysis to identify 2 groups (Swollen and Not swollen, see Fig. 2). Explanatory variables were integrated into a PCA plot. Data analysis, including Kendall’s τ correlation, was carried out using Past 4.07 (Hammer 2001).

Results

Field observations of specimens during seed collection match the description of *Coronilla valentina* subsp. *glauca* according to Pignatti (1982). This is the first recorded morphocolorimetric characterisation for seeds of this subspecies. A summary of the main morphocolorimetric variables is given in Table 2.

Germination dynamics of the replicates subjected to different treatments indicate a clear influence of timing and temperature parameters on germination success. Exposure to a constant 40°C decreased germination success over time, while the 75°C to 25°C treatment show an optimum reached at 72h hydropriming (Fig. 3).

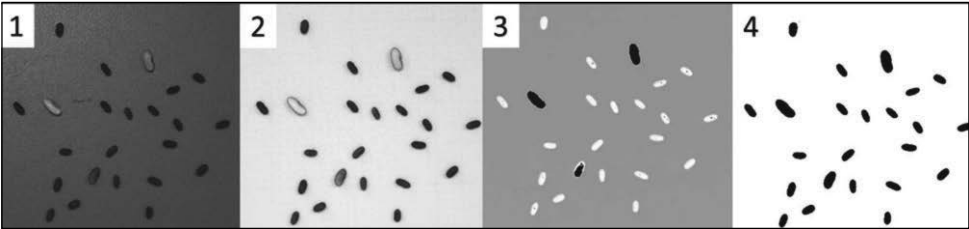


Fig. 1. Example of image segmentation using ImageJ: Original (1) Brightness (2) Hue (3) Mask (4).

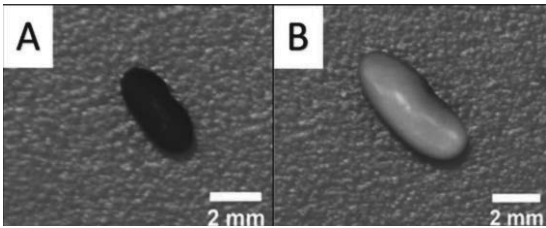


Fig. 2. Seed imbibition characterisation Not Swollen (A) and Swollen (B)

Table 2. Selection of morphocolorimetric variables extracted from N=500 seeds of *Coronilla valentina* subsp. *glauca*.

Variables	Measures				Shape index							Colorimetrics parameters		
	Perim	Area	Feret	Breadth	Circ	Roundness	Compactness	Solidity	Concavity	Convexity	Shape	RedAverage	GreenAverage	BlueAverage
unit	mm	mm ²	mm	mm	N/A	N/A	N/A	N/A	N/A	N/A	N/A	None	None	None
Min	7.28	2.87	2.55	1.16	0.42	0.37	0.61	0.93	160.50	0.74	16.24	41.63	28.76	45.15
Max	11.12	5.47	3.75	2.06	0.77	0.70	0.84	0.99	807.00	0.95	30.20	93.89	52.82	69.64
Mean	8.87	4.09	3.27	1.57	0.65	0.49	0.70	0.98	321.25	0.90	19.34	55.60	37.19	57.39
Stand. dev	0.56	0.44	0.20	0.13	0.05	0.05	0.03	0.01	83.31	0.03	1.78	7.92	2.50	4.04

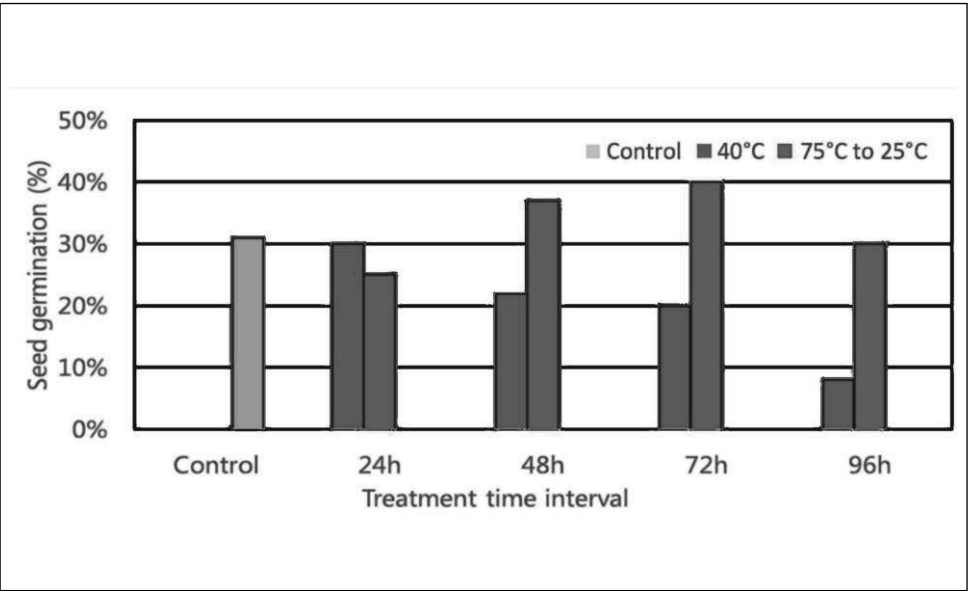


Fig. 3. Germination success according to variation in hydropriming pre-treatments conditions.

A Kendall's τ correlation was performed and show concordance between the count of swollen seeds obtained by a human observer and computer classification, $r(\tau) = .969$, $p < .0001$. Furthermore, the PCA plot displays a clear dichotomy of the 2 groups (swollen and non-swollen seeds) classified by the k-means clustering process. A Kendall's τ correlation shows a negative correlation between the count of swollen seeds and germinated seeds, at the end of the treatments. ($r(34) = -.347$, $p < .05$). The 2 first Principal Components of the analysis (PC1 and PC2) are plotted in Fig. 4 and account for 95.34 % and 4.11 % of the variability observed, respectively. The 3 most important loadings of PC1 are solely colorimetric indexes based on pixel value integrated density (RedIntDen, 0.82043; GreenIntDen, 0.50214; BlueIntDen, 0.27337) while the most important morphometric only parameter is the number of pixels forming the endocarp image (Pixels, 0.004012).

Discussion

Seed swelling characterisation using morphocolorimetric parameters is found to be a reliable and time-efficient method to measure seed imbibition, yet cannot be used as a pre-

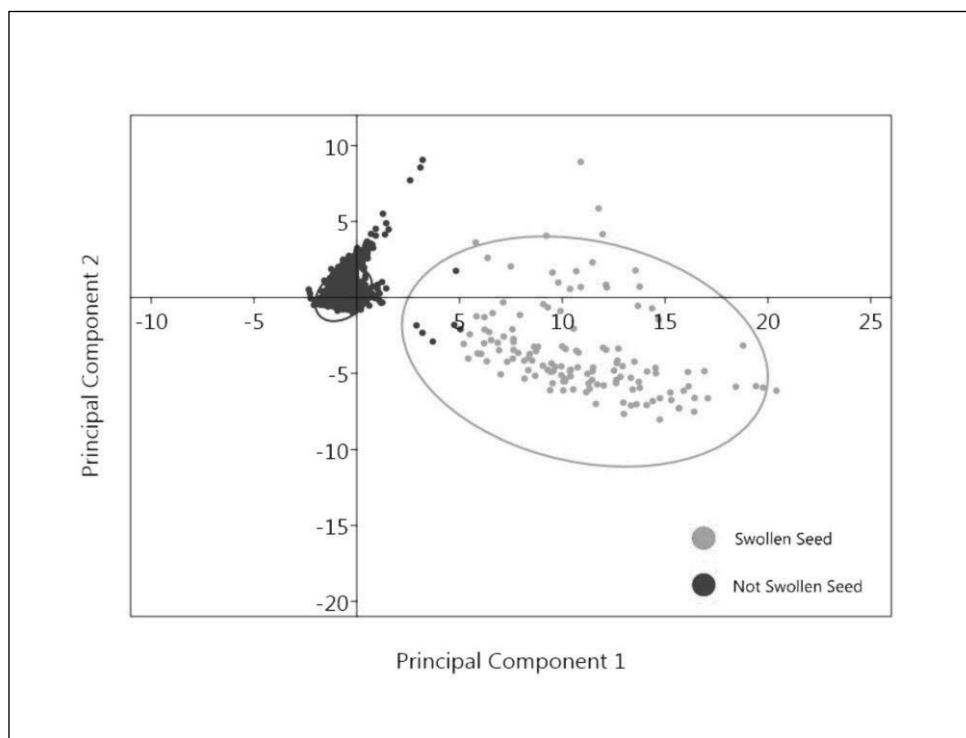


Fig. 4. PCA plot of the morphocolorimetric variations between swollen and non-swollen seeds, based on within-group variance-covariance matrix, with 95% ellipses.

dictor of seed germination success. The timing and temperature of the hydropriming treatment are found to influence the germination rate of *C. valentina* subsp. *glauca*, with the optimum hydropriming treatment, was found to occur within a 72h period following the 75°C to 25°C temperature treatment. This is the highest final germinate rate published to date for this subspecies, considering the results from Escudero & al. (1997).

The results presented in Fig. 3 show that appropriate temperature and timing help prevent over imbibition of the seed and ensure a higher germination rate. As expected due to imbibition, increased treatment time in both treatment conditions (40°C and gradual decrease from 75°C to 25°C) leads to an increased count of over-swollen seeds showing a decrease in germination success. Morphocolorimetric analysis of the seeds before treatment provides further characterisation of *C. valentina* subsp. *glauca* in the Maltese Islands and the methodology used for the Image analysis can be used in further studies of other species, including most of the Fabaceae (Lo Bianco & al. 2015; Vale & al. 2020). The results of the morphocolorimetric classification indicate that hydropriming treatment principally influences the color and the surface area of the endocarp while maintaining a consistent shape and external structure. All the seedlings produced were used for reintroduction into selected areas of disturbed Natura 2000 habitats in the Maltese Islands as part of the SiMaSeed habitat restoration project.

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S. Magrini, C. Bonomi, G. Bacchetta, G. Bedini, A. Borzatti, F. Boscutti, V. Carasso, A. Carta, S. Casavecchia, V. Casolo, R. Ceriani, A. Cristaudo, V. Di Cecco, L. Di Martino, I. Digangi, G. Fabrini, F. Guglielmo, M. Mariotti, V. Negri, M. Porceddu, M. Villani, E. Zappa & C. Salmeri

The RIBES strategy for *ex situ* conservation: conventional and modern techniques for seed conservation

Abstract

Magrini, S. Bonomi, C., Bacchetta, G., Bedini, G., Borzatti, A., Boscutti, F., Carasso, V., Carta, A., Casavecchia, S., Casolo, V., Ceriani, R., Cristaudo, A., Di Cecco, V., Di Martino, L., Digangi, I., Fabrini, G., Guglielmo, F., Mariotti, M., Negri, V., Porceddu, M., Villani, M., Zappa, E. & Salmeri, C.: The RIBES strategy for *ex situ* conservation: conventional and modern techniques for seed conservation. — Fl. Medit. 32: 395-401. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The Italian seed bank network (RIBES) aims to improve the quality and safety of the germplasm reserves of native plant species in Italy to ensure the long-term conservation of endangered and/or endemic flora. The strategy includes traditional methods to secure seed conservation. A comprehensive priority list for seed collection is being defined, it was prepared by crossing data of various checklists (red lists, endemics) and will soon be cross-referenced with an updated list of accessions of the whole network. A safety-backup program of duplicates will quickly be implemented to secure the conservation of the most threatened species in at least two seed banks of the network. On the other hand, the RIBES strategy also includes research by applying modern techniques. In collaboration with the Millennium Seed Bank, research on the storage behaviour of seeds and spores through thermal analysis is ongoing to inform conservation. Using the Differential Scanning Calorimetry (DSC), we could evaluate seed lipid properties such as glass transition temperature, melting, crystallization, oxidation behaviour, and thermal stability.

Finally, RIBES participates as a co-funder in the LIFE Nature project SEEDFORCE, coordinating 11 seed banks of the network for collecting seeds/spores of 29 threatened species of EU interest.

Key words: conservation strategy, Italian flora, priority lists, seed banks, seed/spore research.

Introduction

The Convention on Biological Diversity (1992) recognised the importance of *ex situ* conservation and the relevance of networks for conservation (CBD Article 18).

In 2002, the Global Strategy for Plant Conservation (GSPC) has set out specific targets to be met by 2010, among them: Target 8: “60% of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10% of them included in recovery and restoration programmes” and Target 16: “Networks for plant conservation activities established or strengthened at national, regional and international levels” (CBD 2003). These GSPC targets stimulated the establishment of several networks in Europe for *ex situ* conservation: e.g. REDBAG - *Red Española de Bancos de Germoplasma de Plantas Silvestres* in Spain (2002); the network of the *Conservatoires botaniques nationaux* in France; GENMEDA - *Network of Mediterranean plant conservation centres* (whose members implemented the development of GENMEDOC and SEMCLIMED projects); ENSCONET - *European Native Seed CONservation NETwork* (2004) in Europe.

Considering this proliferation of initiatives at the international level and the lack of institutional coordination at the national level in Italy, in December 2005, 18 groups involved in the field of *ex situ* conservation of native plants (mostly belonging to public institutions, but also private or non-profit organizations) founded the association RIBES – *Rete Italiana Banche del germoplasma per la conservazione Ex Situ della flora minacciata* (Italian network of germplasm banks for *ex situ* conservation of threatened flora) (Rossi & al. 2012). RIBES was founded to promote and, as far as possible, ensure a) the *ex situ* conservation of Italian native flora at risk of extinction, also for reintroduction programs, and b) the *ex situ* conservation of native species of biogeographical and ecological significance and potential interest for renaturalization actions, such as environmental restoration and requalification (art. 2 of the Statute). The activities were organized into four working groups governed in a participatory manner and dedicated to very specific areas of action: (1) the collection of germplasm, (2) its treatment in seed banks, (3) data management, (4) promotion and dissemination (Rossi & al. 2012).

Today, RIBES includes 18 members representing 14 Italian regions and it is an active member/partner of other wider networks so providing an active connection with the international context: ENSCONET – *European Native Seed Conservation Network* (<http://ensconet.maich.gr/>), GENMEDA – *Network of Mediterranean Plant Conservation Centres* (<http://www.genmeda.net/>), *Millennium Seed Bank Partnership*, Royal Botanic Gardens, Kew (<http://brahmsonline.kew.org/msbp>); INSR – *International Network for Seed-based Restoration* (<https://ser-insr.org/>).

The ex situ conservation

According to the last surveys (2015, 2018), more than 25,000 accessions of seeds and spores are conserved *ex situ* in the RIBES seed banks, with about 3,200 taxa representing approx. 40% of the Italian native vascular flora.

Cross-checking the list of seed/spore accessions with the Italian Red Lists (Rossi & al. 2013, 2016, 2020; Orsenigo & al. 2018) and the list of endemics (Peruzzi & al. 2014) provided us with a detailed picture of the consistency of *ex situ* conservation in Italy. It showed that 303 threatened and near-threatened taxa are long-term conserved in seed banks, in particular, 32% of the Critically Endangered, 41% of the Endangered and 27% of the Vulnerable species. Moreover, 90 taxa, 41% of the so-called Policy Species (species listed in Annexes of the Habitat Directive or Bern Convention; Rossi & al. 2013), are secured in the Italian seed banks.

In 2018, a specific survey focused on endemic species was carried out, based on the list of Italian endemics (Peruzzi & al. 2014) and the new red list of species endemic to Italy (Orsenigo & al. 2018). The survey revealed that the RIBES network conserves seed/spore accessions of 554 taxa, 41% of the Italian endemics, stored in 16 seed banks, mainly in Sicily (300 taxa), Sardinia and Tuscany (>110). These taxa belong to 48 families, among them, *Asteraceae* with ca. 120 taxa, *Brassicaceae*, *Fabaceae*, and *Caryophyllaceae* with more than 40 taxa, are the most represented families. It is noteworthy that 48% of the threatened endemic taxa are stored in 15 seed banks, mainly in Sicily and Sardinia, including 52% of the Critically Endangered, 46% of the Endangered, and 42% of the Vulnerable taxa.

Another specific survey carried out in 2015 and focused on Crop Wild Relatives (CWRs), wild plant species that share a common ancestor with cultivated crop plants, showed that 37% of the Italian CWRs listed in the FAO Treaty (FAO 2001) are stored in 14 seed banks, mainly in the seed bank of Perugia, for a total of 6029 seed-lots of 229 taxa belonging to 11 families, mainly *Fabaceae* (75 taxa), *Poaceae* (68) and *Brassicaceae* (49). Among them, 294 accessions of 14 CWRs endemic to Italy (50%), mainly in 5 seed banks: Catania, Sardinia, Perugia, Padova, and Palermo (Magrini & al. 2016).

The strategy for an effective ex situ conservation

The ultimate goal of the RIBES network is the general improvement of the quality and safety of the germplasm reserves of native plant species in Italy to ensure effective long-term conservation and protection of the endangered and/or endemic flora. To achieve this goal, the RIBES strategy includes mainly traditional methods, such as the definition of priority lists to optimize the collection of seeds and spores, and the implementation of a safety-backup program of duplicates of the accessions, together with the use of modern techniques to define seed behaviour during storage and secure seed conservation.

Toward a comprehensive priority list for seed collection for the Italian flora

Prioritization of endemic and threatened species is a crucial point of conservation actions, particularly in species-rich areas like Italy. Priority lists are a fundamental and useful tool to identify the target species for *ex situ* conservation, optimizing collection efforts and conservation costs (Gauthier & al. 2010; Bacchetta & al. 2012a, 2012b).

A priority list of 43 Italian CWRs listed in the FAO Treaty (FAO 2001) was drawn up in 2016 to identify the target species for seed collection and *ex situ* conservation measures, selecting all the endemic taxa (28), the policy species (4), the threatened (CR, EN, VU) and near threatened (NT) taxa at both Italian and European level (19) (Rossi & al. 2013; Peruzzi & al. 2014; Bilz & al. 2011), and investigating their occurrence in *ex situ* collections. In the priority list, 20 taxa were considered of highest priority (HP) for collection as not preserved at all in seed banks and other 5 taxa of priority (P) for further collections being stored in seed banks as a single accession (Magrini & al. 2016). After the recent publication of the first comprehensive national inventory of CWRs (Ciancaleoni & al. 2021), such list and the list of the accessions stored in the RIBES seed banks have already been cross-referenced with the existing checklists mentioned above (Rossi & al. 2013, 2016, 2020; Peruzzi & al., 2014; Orsenigo & al. 2018) to have a complete set of data to update the previous priority list (Villani & al. 2021).

A preliminary priority list for the threatened species and the endemics is in progress. We populated a database which is continuously updated with all the available data on the endemics, threatened species, and all the relevant published papers. The analysis of the database of the seed bank collections (obtained with the last surveys in 2015-2018) highlighted many collection gaps concerning the coverage of threatened taxa, endemics and their geographic representativeness. Then, a further survey across the network is required soon to update the accession database and to be able to draw up a comprehensive updated priority list for the Italian flora.

Safety-backup program for duplicates

A duplicate security backup program will soon be implemented to ensure the conservation of the most endangered species. A safety duplicate is a sub-sample of an accession made to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes. The first step will foresee the selection of the priority species to duplicate, according to updated priority lists. Safety duplicates, including both the duplication of material and its related information, will be deposited in at least another seed bank in the network. Moreover, one duplicate for each species will be sent to the Millennium Seed Bank, Royal Botanic Gardens, Kew (UK). This activity will bring benefits in terms of the safety of the collections of the most important species and will also stimulate research collaboration between seed banks.

Thermal analysis: a new tool to inform seed conservation

Under conventional storage conditions, dry orthodox seeds stored at low temperatures (-20°C) can be expected to live for tens or hundreds of years. However, longevity is known to be extremely variable, depending on the species and seed traits (Liu & al. 2018). Seed lipids have long been thought to be a determinant of seed ageing, with lipid composition impacting different susceptibility to oxidation and variation in thermal behaviour (Priestley 1985; Walters 1998; Mira & al. 2019).

Research has been ongoing since 2019 in collaboration with the Millennium Seed Bank, Royal Botanic Gardens, Kew (UK), to better understand the storage performance of seeds through Differential Scanning Calorimetry (DSC). DSC is a powerful technique that can be used to characterize the physical properties of seeds and has the potential as a rapid, non-invasive tool to understand variability in the storage performance of seeds stored in seed banks by observing lipid phase transitions (Hamilton & al. 2009).

Understanding the balance of seed lipid composition and the thermal characteristics of their melting and crystallisation behaviours could prove to be a useful tool for identifying long-term cold storage problems in seeds and potentially predicting the optimal storage temperatures, likely by avoiding freezing damage promoted by lipid crystallization and melting (Mira & al. 2019). The first DSC analyses of seeds of 15 Mediterranean terrestrial orchids showed useful results suggesting different optimal storage temperature ranges for distinct genera (Magrini & al. 2022).

The “Mediterranean plant germination reports” column

Since 2019, a new series of germination accounts for the Mediterranean flora (*sensu* Med-Checklist), proposed by RIBES, has been published annually in *Flora Mediterranea*

journal (Magrini & Salmeri 2019). In the initial three issues (2019-2021) of the “Mediterranean plant germination reports” edited by Sara Magrini & Cristina Salmeri, 23 papers were published for a total of 85 germination reports for 80 taxa belonging to 25 families. Seed germination is an important step for an effective conservation activity, thus, making these and other published germination data available to a wider audience, we are building an online, open-access database within the RIBES website, that typically includes sections on taxonomy, location data, storage behaviour, besides germination results, as part of the RIBES conservation strategy.

The LIFE SEEDFORCE project

In 2021, the LIFE20 NAT/IT/001468 “Using SEED banks to restore and reinFORCE the endangered native plants of Italy” – SEEDFORCE was funded by the European Commission. The project goal is to improve the conservation status of 29 Annex II species with an Unfavourable-Inadequate or Unfavourable-Bad conservation status, according to the last Report ex Art.17, occurring in 76 SCI/SACs mainly in Italy, and in France, Slovenia and Malta. RIBES participates in the project as a co-financier ensuring: 1) the long-term germplasm conservation according to international standards, 2) the implementation of a duplicate security system of the accessions which will be kept in at least 2 banks of the network.

Conclusions

The development of a national plan aiming at the safe and successful conservation of seeds of the Italian plants comprises the following steps:

- to optimize the long-term conservation of seeds by using traditional and modern analytical methods;
- to assess the conservation of the threatened/endemic species stored in seed banks to update the national inventory;
- to define an updated national priority list for seed collection, filling gaps in *ex situ* conservation and optimizing collection efforts;
- to implement a safety-backup program of duplicates to secure the conservation of the most threatened species in at least two seed banks of the network;
- to share information on germination behaviour and any other requirements for long-term seed storage.

We expect the conservation strategy planned by the RIBES network will be able to ensure the rapid achievement of the conservation targets for the Italian native flora by optimizing seed storage efforts at a national scale, fostering collaboration between research bodies and local authorities, and increasing public awareness on the risks of biodiversity loss.

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Salvatore Pasta

Never underestimate Sicilians: some case histories dealing with narrow native ranges, deliberate introduction, claimed extinction and predictable plant invasion worldwide

Abstract

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First described from Sicily or originally occurring only on this island, after being introduced worldwide, several vascular plants proved to be able to adapt to the environmental conditions encountered in different biomes, and in some cases became a threat for the biodiversity of the newly invaded habitats.

These paradigmatic and somewhat paradoxical cases shed light on the complexity of invasion forecasting procedures. They provide insightful examples on the global effect of voluntary or involuntary introductions of potentially invasive species and the importance of better studying the ecology and demography of rare plants within their native home range.

Key words: horticulture, international plant trade, invasion biology, Mediterranean-type biomes, niche shift, population genetics, risk assessment.

Introduction

Since 16th-17th centuries AD not only exotic plants from Americas, Asia and Africa reached Europe, but also Mediterranean countries became the target of extensive plant gathering and trade for medicinal and ornamental purposes (Tongiorgi Tomasi 1984; Lack 1996). On this purpose, Sicily played a key role long before the colonisation of Americas not only as connection hub for the trade of many vascular plants coming from the eastern and southern Mediterranean countries, but also as homeland of other plants which have been spread across temperate and Mediterranean Europe and beyond. For instance, the cosmopolitan *Verbascum thapsus* L., apparently corresponding to the medicinal (but toxic) plant called ‘thapsos’ by Theophrastus, probably takes its name from an ancient Helladic colony where the common mullein was gathered during Antiquity located on the islet of Thapsos in southeastern Sicily (Muenscher 1935), while the edible spiny “kaktos” that Theophrastus claimed to be growing only in Sicily (Hist. Pl. 6.4.10) probably corresponds to the wild artichoke, *Cynara scolymus* L. (Rottenberg & Zohary 1996).

Moreover, many scholars have mentioned Sicily as the first European site of introduction of many palaeotropical Asian and African plant species of high economic importance (e.g., citrus trees, mulberry trees, sumac, sugar cane, etc.) during the Middle Ages (e.g., Metcalfe 2017).

During the 16th and 17th centuries, the curators of the botanical gardens created by the universities and mecenates of the island actively participated in the dense network of seed exchanges between the institutions and private collectors and passionates that were beginning to populate the whole of Europe. For instance, Francesco Cupani was in contact with many of his Italian and European colleagues (Pulvirenti & al. 2015). Similarly, Boccone (1674) reported to have communicated the seeds of several good-looking hardy Mediterranean plants to several botanical gardens such as those of Paris, Amsterdam, Padua and London and to have recommended to their curators to cultivate them for ornamental purposes. Indeed, some of them have been successful, as testified by the use of *Convolvulus cneorum* L. and *Iberis semperflorens* L. - two of Boccone's beloved plants - in the front garden of many private houses in today's London (S. Pasta, *pers. obs.*).

During the 19th century, the straw used as fodder for the horses of the French Army came not only from Algeria but also from Italian and Sicilian hay fields (Gaudefroy & Mouillefarine 1871). The seeds germinated in the military camps of Paris gave rise to the ephemeral occurrence of several dozens of Mediterranean species, like *Vicia sicula* (Raf.) Guss. This wild vetch, endemic to Maghreb, Sicily and Tyrrhenian Italy (Roskov & al. 2014), has been reported as a casual alien also for Belgium, where it was recorded only once in 1920 (Verloove 2006), as testified by a single herbarium specimen (BR0000012011728) (F. Verloove *pers. comm.*).

Deliberate or accidental introductions have probably happened much more frequently than expected, and most of them may have passed unnoticed just because they experienced only local success. This seems to have happened to several other Sicilian plant taxa introduced abroad. For instance, the occurrence of *Centaurea solstitialis* L. in California was reported by the end of the 1870s, and this plant was already considered a noxious weed in the 1890s (Robbins 1940). More recent investigations (Widmer & al. 2007) pointed out that the Californian populations of yellow star thistle belong not only to subsp. *solstitialis* but also to subsp. *schouwii* (DC.) Gugler, described from the Madonie Mountains (Peruzzi & al. 2019) and currently living in NW-Africa, Sardinia, Sicily and southern Italy (Greuter 2006).

Thellung (1919) coined the term "Sicilian colony" to emphasize the sudden arrival of as many as 42 species new to the Canton of Zurich (19 of them being new to the entire Swiss territory) as a direct consequence of the steep increase of fruit import at the beginning of the First World War. In fact, most of those newcomers were weeds typical to southern European orchards and colonized the commercial railway station of Zurich when Switzerland became the main buyer of the Italian citrus products.

As for *Desmazeria sicula* (Jacq.) Dum., this rare plant living along the rocky shores of N Africa, Malta, Sicily, Calabria, Sardinia and Southern Spain (Lansdown & Vela 2019) may have been introduced in Belgium (Auquier 1978) by the mine workers migrated from southern Sicily after the World War II (Cumoli 2009).

This paper aims at presenting several emblematic cases of plants described from Sicily and thought to be rare or even extinct, which instead have been traded far away and became invasive elsewhere on the planet. These paradigmatic and somewhat paradoxical examples shed light on the complexity of invasion mechanisms and the need of improving our understanding about plant invasions to prevent and forecast them.

An amazing case of global success: the “Sicilian” sweet pea

The sweet pea *Lathyrus odoratus* L. was once probably endemic to central and east Mediterranean (Sicily, S-Italy, Crete, Cyprus and several Aegean islands). Due to its enormous success as an ornamental plant, it has been introduced worldwide and became naturalized in 32 countries across all continents, and currently occurs even on very distant islands like Galapagos archipelago, Juan Fernández (c. 700 km west off Chile), Chatham Islands (c. 800 km east off New Zealand) and Hawai'i (GBIF Secretariat 2021; KBD 2022).

The sweet pea variety called “Cupani” is thought to be the closest to the wild ancestor of all present-day breeds (Inoue & al. 2000). Francesco Cupani was the curator of the botanical garden of Misilmeri, a town some 15 km southeast of Palermo. The sweet pea was mentioned for the first time by Cupani (1694) among the plants newly discovered in Sicily; two years later Cupani (1696) described it as “*Lathyrus distoplatyphyllus, hirsutus, mollis, magno, & peramæno flore odoro*”. In 1699, Cupani sent the seeds of the wild sweet pea to several correspondents all over Europe. One of them, Caspar Commelijn, director of the botanical garden of Leiden, provided the earliest botanical illustration of the plant, named “*Lathyrus distoplatyphyllus hirsutus mollis et odorus*” in his catalogue (Commelijn 1701).

In the following decades an authentic “sweet pea mania” gripped English and Dutch botanists, plant collectors and gardeners, who shared the seeds sent from Sicily with many private passionates, who started to breed the plant to modify the flower traits for ornamental purposes, so that the species experienced a very fast spread and success and was already released commercially in the 1720s. The economic success of this plant lasted for centuries: for instance, a National Sweet Pea Society was founded in England in 1901, and in the following decades regular competitions among amateur gardeners were organized to promote the selection of countless varieties (Unwin 1952). Not surprisingly, the huge bulk of knowledge on *L. odoratus* breeding has stimulated several basic studies on inheritance mechanisms worldwide (e.g., Punnett 1923). During last century the main centres of production and experimentation moved first to Japan and then to New Zealand, which still leads the world production (Rice 2002).

Born to go wild? *Genista etnensis* from Sardinia to Sicily and beyond

The giant Etna broom *Genista etnensis* (Raf.) DC. is considered to be native to Sicily (Mt. Etna), Eastern and Southern Sardinia and Corsica. Based on several morphological traits, some authors (e.g., Bacchetta & al. 2016; Fridlender 2018) have recently suggested to refer the plants growing on these islands to three different taxa.

Sardinia represents the main pool of the species in terms of extent of occurrence, although local populations show low values both in terms of genetic variability and number of haplotypes (De Castro & al. *submitted*). Here *G. etnensis* mostly occurs in the gaps of the evergreen woodlands and is common in the shrubland communities of the hill and mountain belts. In Corsica as well the species prefers the natural gaps within *Q. ilex*-and/or *Pinus pinaster*-dominated forest communities and disappears once forest cover becomes too dense (Delage & Hugot 2020). Disputed for long time, the native status of *G. etnensis* in Corsica has been reported in the most recent and influential monograph on local vascular flora (Jeanmonod & Gamisans 2013). Indeed, according to the most recent genetic investigations, the populations of Corsica are represented by an exclusive haplotype and they may even represent the “core” population of the species (De Castro & al. *submitted*).

In Sicily, *Genista etnensis* behaves as a pioneer colonizer of recent lava flows and volcanic ashes on the slopes of Mt. Etna, where it occurs in the gaps of wood communities between 1200 and 1900(2000) m a.s.l., as a pioneer species on volcanic debris and even above the timberline (Sciandrello & al. 2020). The giant broom plays an important role in creating nitrogen-rich islands (Fernández Sanjurjo & al. 2003). Since the beginning of the 19th century, and even more intensively after the World War II, *G. etnensis* has been introduced out of its original range for nature restoration purposes; due to these massive plantation efforts, on Mt. Etna the giant broom also occurs from sea level up to 1200 m a.s.l. (Sciandrello & al. 2020). *G. etnensis* was also planted in the Peloritani Mts. and Nebrodi Mts. (NE-Sicily) but it did not spread there (A. Crisafulli, *pers. comm.*). The extremely low genetic diversity of the populations of Mt. Etna (De Castro & al. *submitted*) induces to suspect that *G. etnensis* might have been introduced in Sicily from Sardinia, where the giant broom occurs in much less disturbed habitats.

Soon after its discovery on Mt. Etna, seeds of *G. etnensis* were sent by the Sicilian botanists to the British gardeners. This species is still a popular ornamental plant in England, where it was first recorded as escaped in the wild in 1977 (Surrey: OABIF 2022a) started to spread in the 1980s (NBN Atlas 2022) and it currently occurs not only in southern England but also in North Wales and near Sheffield (BSBI 2022a).

As for Campania, individuals from Mt. Etna were introduced on the slopes of Mt. Vesuvius at the beginnings of 20th century (Agostini 1959). After 60-70 years of apparently harmless acclimatisation, during last 40 years local population was able to modify local soil properties and to perform a very fast spread, turning so invasive to pose a serious threat to local flora and vegetation (Stinca & al. 2015).

No precise information is available on the regional origin and the date of introduction of *G. etnensis* in many other Italian and Mediterranean localities, but most of (if not all) the propagation material (seeds or plugs) probably comes from Mt. Etna. The species behaves as casual in Molise (Bartolucci & al. 2016), while it has escaped and is spreading in Emilia-Romagna (Alessandrini & al. 2012), Tuscany (Bartolucci & al. 2020), Greece (Polymenakos & Tan 2012), south-western France (Leblond & al. 2020) and Anatolia (O. Şentürk, *pers. comm.*). *G. etnensis* has been introduced for ornamental purposes in all continents. In California, where it grows in many private and public gardens since more than one century (Hrusa & al. 2002), it did not turn invasive so far (Grotkopp & Rejmánek 2007). No data are available about its behaviour in Argentina (Burkart 1952), south-eastern India (Sharma & al. 1977), South Africa (Glen 2002), south-eastern Australia (Spencer 2002) and Zimbabwe (Maroyi 2006).

Plant trade in the web age

The voluntary spread of useful plants is old as mankind. For this reason, it is just impossible to assess the original home range of many archaeophytes, as their introduction worldwide started by early seafarers (Fuller & al. 2011). Since the early 1500s, European merchants and scientists increased their collecting, cultivating and breeding activities across the globe, mostly focusing on ornamental and medicinal plants, and begun to transfer many alien plants across continents. As a consequence of this imperial-colonial phase of botanical collections, the description of many plants native to the Americas, Asia and Africa is based on plants cultivated in the European botanical gardens (e.g., Kew, Paris,

Madrid, St. Petersburg, Palermo) and their original home range is unknown. This process did not slow down in the following centuries, and today both species introduction and establishment appear relentless and unstoppable (Seebens & al. 2017). If compared with past times, the international e-commerce of potentially dangerous weeds has dramatically increased in terms of speed, intensity and species number. Hence, internet plant market appears one of the most effective allies for invasive plants (Humair & al. 2015).

A formidable example of the potential of internet trade in ‘reviving’ even very rare plants is provided by *Cistus ×skanbergi* Lojac.. Despite having completely disappeared in its *locus classicus*, Lampedusa Island (Strait of Sicily), and its extreme rarity throughout the whole Mediterranean Basin, the so-called dwarf pink rock rose is far from being extinct worldwide. On the contrary, a quick automatic query made on the internet using the words “*Cistus*” or “rockrose” + “*skanbergi*” (or “*skanbergii*”) gave c. 9200 pages referring to this handsome plant, currently produced by dozens of nurseries and used for private greening purposes in Europe and abroad (e.g., USA, Australia and New Zealand); more recently, *C. ×skanbergi* started to escape from cultivation in southern Europe and California (Pasta *submitted-a*).

The Hyblaeon sea lavender, a narrow endemic turned subcosmopolitan

Limonium hyblaeum Brullo is a salt-tolerant hemicryptophyte considered strictly endemic to SE-Sicily (Brullo & Brullo 2021). It dominates a species-poor plant community adapted to grow on the salt-sprayed calcareous rocky shores. Within its native range, *L. hyblaeum* is currently threatened with urban sprawl along the coast, so that during last decades its populations experienced increasing fragmentation and even local extinction (Pasta *submitted-b*).

Erben (1986) identified several specimens of *Limonium hyblaeum* collected in three countries very far from Sicily, i.e., in England, in Chile and in Australia (Fig. 1). As for UK, the species was first observed in 1979 (i.e. even before being described by Brullo 1980) growing as a garden escape on the sea cliffs of East Sussex (Ingrouille 1981). An updated map (BSBI 2022b) points out the remarkable spread of *L. hyblaeum* along the coasts of southern England during the last 30 years, probably due to seagull dispersal (OABIF 2022b). As for Chile, the plants observed in the 1970s in a private green area near Pichidanguí were unable to escape from cultivation, and the species does not feature among the casual and naturalized vascular plants of Chile (Fuentes & al. 2020).

The fast spread of *Limonium hyblaeum* in Australia between 1970s and 2000s is well documented. The so-called “Sicilian sea-lavender” currently occurs in South Australia, Western Australia, Victoria and New South Wales, and its invasive behaviour is a major concern for local conservation biologists and managers (Walsh 1996, Parsons 2013).

***Senecio squalidus* L. & “Sons”: when colonization and hybridization trigger evolution**

The history of the establishment of *Senecio squalidus* L., once probably strictly endemic to Sicily and introduced in England at the end of the 17th century (Harris 2002), is just extraordinary. Very soon after its introduction, it had already escaped from cultivation and started to grow on the walls of Oxford college. During 18th the so-called “Oxford Ragwort” exploited the railway lines to disperse across the country; this species is currently the most frequent neophyte in urban and suburban habitats of central Britain (Hill & al. 2002), being fairly ubiquitous in most urban areas of UK and still spreading (Botham & al. 2009). During its establishment, and along with its spread across Europe (IUCN/SSC-



Fig. 1. *Limonium hyblaenum*: native distribution range in Sicily (a) and secondary range in UK (b), Chile (c) and Australia (d), from Parsons (2013) modified.

ISSG 2010), the original niche of *S. squalidus* apparently expanded and shifted, as testified by the different assessment of its Ecological Indicator Values given in several parts of its native and secondary distribution range (Table 1). During its spread across Great Britain, *S. squalidus* encountered several native congeneric species, giving rise to several new hybrid species, reproductively isolated from their parents (Abbott & Lowe 2004).

Senecio squalidus shows such an invasive attitude to require *ad hoc* measures for its management and control (Heywood & Brunel 2008); during the last decades it was able to colonize even the Falkland/Malvinas Islands (Upson & Lewis 2014), USA and Canada (USDA-ARS 2010, USDA-NRCS 2010).

Senecio squalidus grows together with its two parent species *S. aethnensis* and *S. siculus* All. (= *S. chrysanthemifolius* Poir.), on the slopes of a highly dynamic and active volcano, Mount Etna (Sicily), forming a hybrid zone at intermediate altitudes between 2000 and 2400 m a.s.l. (James & Abbott 2005). The exceptional morphological plasticity of the aforementioned *Senecio* species has been noticed since the 17th century (Cupani 1696) and has been studied by generations of botanists, who gave plenty of different names to different ecomorphotypes (Barone & al. 2022), measured their traits and tried to interpret their clinal variation as a response to temperature and altitude gradients (Pasta *submitted-c*). Such plasticity remained unexplained until quite recently, when a survey of randomly amplified polymorphic DNA marker variation confirmed that *S. squalidus* is a diploid hybrid derivative of *S. aethnensis* and *S. siculus* All. (Abbott & al. 2002).

After 30 years of dedicated research and dozens of publications focused on *S. squalidus* (Pasta *submitted-c*), this species has become a target model to study many aspects of the ecology, genetics, and evolution of invasive diploid plants (Abbott 1992, Lowe & Abbott 2003, Abbott & al. 2009, Brennan & al. 2014, Chapman & al. 2016, Nevado & al. 2020).

Table 1. Ellenberg Indicator Values (EIVs) assigned to *Senecio aethnensis* and *S. chrysanthemifolius* within their native range and to *S. squalidus* throughout its native and European secondary range according to different sources. Standard abbreviations of EIVs: L (= Light), F (= Soil moisture), R (= Soil reaction, pH), N (= Nitrogen soil content), T (= Temperature), K (= Continentality) and S (= Salinity); n.a. = not assessed.

Taxon, geographical area and source	L	F	R	N	T	K	S
<i>S. chrysanthemifolius</i>							
Italy (Pignatti & al. 2017-2019)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
<i>S. aethnensis</i>							
Italy (Pignatti & al. 2017-2019)	11	2	1	1	n.a.	2	0
<i>S. squalidus</i>							
Italy (Pignatti & al. 2017-2019)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Central Europe (Ellenberg & al. 1991)	8	5	7	8	n.a.	n.a.	n.a.
England (Hill & al. 1999)	8	4	7	7	n.a.	n.a.	n.a.
S-Aegean islands, Greece (Böhling & al. 2002)	6	4	8	6	7	5	0
Sweden (Torbjörn & al. 2021)	7	4	6	7	n.a.	5	2

Discussion

And the winner is? Looking for general proxies to predict biological invasions

Taking in mind the intrinsic stochastic nature of invasions, invasion biologists are still looking for general patterns and reliable predictors (e.g., Hayes & Barry 2008, Pyšek & al. 2020) to improve our ability to forecast biological invasions. On this purpose, three hypotheses have been invoked, i.e. the Enemy Release (Keane & Crawley 2002, Colautti & al. 2004), the Evolution of Increased Competitive Ability (Blossey & Notzold 1995), and the Novel Weapons (Callaway & Aschehoug 2000). Indeed, these hypotheses do not conflict, as two or more of these favouring factors may synergically allow a species to expand into a broader or somehow different ecological niche (Lau & Schultheis 2015). Moreover, the strong selection forces encountered by potential invaders might speed up their evolution and induce major niche shifts (Lee 2002; Parker & al. 2003; Colautti & Barrett 2013; Early & Sax 2014, Bock & al. 2015; Dlugosch & al. 2015; Braasch & al. 2019).

With no doubt, invasion biologists and plant conservation practitioners should investigate the ecology of potentially invasive plants starting from studying their behaviour within their native range (Hierro & al. 2005); moreover, they should focus on the traits which may favour the establishment and the fast spread of alien organisms. For instance, comparing the seedling performance of 30 woody species, Grotkopp & Rejmánek (2007) pointed out that *G. etnensis* shows the highest percentage of root biomass 30 days after seed germination, testifying the high investment of the giant broom to ensure establishment after emergence.

Plant invasions performed by naturally rare organisms

Narrow-ranged does not mean harmless. The case of *Limonium hyblaicum* provides a new striking example of a still poorly understood phenomenon, concerning originally narrow-ranged plants which were rare or even at the brink of extinction within their native range but turned inva-

sive once they colonized new habitats and biomes (Rocha & Bergallo 2012; Colautti & al. 2017). To our knowledge, just a handful similar cases concerning vascular plants have been reported and investigated worldwide so far, i. e. *Pinus radiata* D. Don, *Rhododendron ponticum* subsp. *baeticum* (Boiss. & Reut.) Hand.-Mazz., *Hypericum canariense* L. and *Lotus maculatus* Breitf. (Pasta *submitted-b*).

Natura facit saltus: *Genetics, niche-shifts and invasions*

Although many introduced species have to face the consequences of bottlenecks in population size, like the reduction of fitness and evolutionary potential, many of them succeed to become invasive. This apparent paradox may often be explained by rapid evolution along with establishment (Colautti & Lau 2015), while in other cases the newcomers maintain high genetic variation in ecologically relevant traits (Estoup & al. 2016).

In this context, polyploidy and hybridization represent favourable traits for many invasive plants, as already pointed out by Stebbins (1985) and recently confirmed for many evolutionarily distant plants (Pandit & al. 2011; te Beest & al. 2012; Yakimowski & Rieseberg 2014; Rosche & al. 2017).

Recent investigations pointed out the importance of hybridisation in plant invasions. It has been suggested that hybridisation may promote the evolution of invasiveness by increasing genetic variability or introducing novel genes that allow invasion of new habitats (Abbott & al. 2003). Many invasive species are the result of hybridisation events (Schierenbeck & Ellstrand 2009), and hybridisation has also been shown to allow range expansion in native species (Rieseberg & al. 2007). On this purpose, the hybrid nature of *S. squalidus* probably provided enough genetic variation to expand its range in Britain. In fact, several experiments have shown that the British populations of *S. squalidus* managed to adapt to the different climatic patterns encountered whilst spreading across their new range, so that the northern populations can survive under cooler and wetter conditions, whilst the southern ones are better able to cope with drought stress (Allan & Pannell 2009).

Repetita non juvat: *the dangerous effects of propagule pressure and admixture*

Until the end of 18th century *Ailanthus altissima* (Mill.) Swingle grew only in China and Taiwan before being introduced in Europe and north America, where it had disappeared during the last glacial events. Nowadays the tree-of-Heaven, favoured by massive introduction of material coming from different populations, is an almost ubiquitous and “iconic” invasive plant worldwide, especially in forest fringes, urban and suburban areas and in archaeological sites (Kowarik & Säumel 2007; Raimondo 2012; Badalamenti & al. 2012; Campagnaro & al. 2018).

Increasing experimental evidence (e.g., Dlugosch & Parker 2008; Albright & al. 2010; Smith & al. 2020) shows how repeated introductions from different interfertile provenances may benefit the genetic diversity of alien species and enhance their ecological plasticity and fitness (Ferrero & al. 2015). In other cases, admixture may lead to reproductive incompatibility between populations originating from different introduction events, and this in turn may cause the rapid differentiation of new species in the invaded areas (Irimia & al. 2021).

Similarly, the recent success of the population of *Genista etnensis* on Mt. Vesuvius may issue from the admixture of living material from different populations of Mt. Etna (De Castro & al. *submitted*), and this mechanism could also explain the fast spread recently experienced by the giant Etna broom across the European and Mediterranean countries.

Conclusions

Take-home messages...

IUCN specialist groups should better communicate with each other. On this purpose, *L. hyblaeum* provides a paradoxical example of ‘double-blind’ listing. In fact, on the one hand this species was subjected to risk assessment and considered “LR” (Low Risk) on the regional level by Conti & al. (1997), while Orsenigo & al. (2018) assigned to it the IUCN category “LC” (Least Concern) on the national level. On the other hand, this sea lavender is featured among the invasive plants of Europe listed by the DAISIE working group (Roy & al. 2020). Similarly, due to the ongoing shrinkage of its small coastal populations (Piazza & Paradis 2000), *G. etnensis* features among the Critically Endangered vascular plants of Corsica (Delage & Hugot 2020), while it has been indicated as “LC” on the global level (Rivers & Beech 2017).

The increasing amount of information provided by blacklists available worldwide must be adequately consulted to avoid the introduction of plants that have already shown a clearly invasive behaviour under similar climatic conditions and/or in geographically close countries.

... and “lessons” to unlearn

Being among the earliest and most active actors and “teachers” of plant breeding and introduction worldwide, the British gardeners have had a very important role (and responsibility) in favouring the invasion of tens of vascular plant species in the Mediterranean-type and temperate biomes worldwide (Hulme 2011).

Indeed, the worlds’ scientific community of alien plant specialists should go far beyond the simple academic ‘sport’ of recording new occurrences, trying instead to get more engaged, for instance supporting the adoption of sustainable greening options, the exclusive use of certified native plants, publicly speaking out against the use of aliens known to be adapted to invade new suitable biomes. In fact, in my opinion it is time to change direction and to fight against insane projects which foresee the deliberate introduction of plenty of exotic woody plants in the Mediterranean Basin, a territory with a long story and a recent worrying increase of naturalisation and invasion cases performed by species coming from other Mediterranean-type or subtropical regions (Lambdon & al. 2008; Arianoutsou & al. 2013). Finally conscious of their past and recent responsibilities and their current duties (Hulme & al. 2008, Dehnen-Schmutz 2011), the European horticultural societies and the botanic gardens are working together to reduce and stop the use of invasive alien ornamental plants (Heywood & Brunel 2011).

Moreover, botanists should support local and national decision-makers by sharing continuously updated black-lists to be used outside the academic congresses as guidelines for all activities dealing with plant use (e.g., forestry, habitat restoration, landscaping, gardening) and by providing first-hand information about any sudden shift of casual and fully-naturalized alien plants towards invasiveness in order to promptly detect and eradicate the most dangerous and dynamic newcomers (Stasny & Sargent 2017; Stasny & al. 2020).

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Effect of storage conditions on seed germination of eight Tyrrhenian endemic vascular plant species of conservation interest

Abstract

Porceddu, M., Cuena-Lombrana, A., Boi, M. E., Podda, L., Sarigu, M., Dessi, L., Meloni, F., Atzeri, P. & Bacchetta, G.: Effect of storage conditions on seed germination of eight Tyrrhenian endemic vascular plant species of conservation interest. — Fl. Medit. 32: 421-433. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The conservation of endemic and endangered plant species is of great interest to the scientific and research community. In this frame, seed banks play a crucial role when biodiversity preservation and climate change are considered. The study of seed viability and germination during storage conditions provides basic and useful information to ensure successful *ex situ* conservation. The aim of this study was to evaluate whether storage time and conditions (i.e., base collection at -25°C and active collection at +5°C) affect seed germination in the long term. For these purposes, eight Tyrrhenian endemic vascular plant species (mostly endangered) with orthodox seeds were studied: *Brassica insularis*, *Centranthus amazonum*, *Dianthus morisianus*, *Digitalis purpurea* var. *gyspergerae*, *Ferula arrigonii*, *Helicodiceros muscivorus*, *Iberis integerrima* and *Verbascum plantagineum*. These species were stored in the Sardinian Germplasm Bank (BG-SAR) at -25°C and at +5°C for a time ranging from 2 to 12 years. Germination tests were carried out following the optimal conditions reported in the literature for each species. The results showed, in general terms, the high seed germination capacity of all species stored at both conditions; regarding the time of seed storage, germination in some tested species (such as *B. insularis* and *C. amazonum*) slightly decreased over time. We argued that seed dehydration, low seed moisture content during storage and the use of hermetic glass containers can be considered key factors for long-term conservation of these orthodox seeds. In conclusion, this study showed that the conservation of these endemic species is ensured by seed bank storage, according to the general assumption that seed longevity depends on seed lot quality, on well-sealed storage containers and conditions before and during storage.

Key words: *ex situ* conservation, endangered species, mediterranean flora, seed banking, seed longevity.

Introduction

Sardinia is considered a priority region for biodiversity conservation due to its high number of endemic species (Cañadas & al. 2014). According to Bartolucci & al. (2018)

and Galasso & al. (2018), the Sardinian flora consists of 2922 taxa, of which 2441 are autochthonous and 481 alien. Recently, Fois & al. (2022) reported that the Sardinian vascular native flora consists of ca. 2300 taxa, of which 341 are considered Sardinian endemics. Most of these species are facing several threats, e.g., land-use and land-cover change, habitat fragmentation, climate warming and impact of invasive plant species (Fenu & al. 2015). In this context, seed banks in general and, in particular, the Sardinian Germplasm Bank (BG-SAR) play a central role in the conservation of Sardinian plant diversity and protection of the most sensitive species of the island (Porceddu & al. 2017a). Accordingly, BG-SAR contributes to guaranteeing the long-term conservation of the genetic diversity of Sardinian threatened taxa on the basis of the regional responsibility criterion by also investigating and providing conservation strategies for these species at the local level (Bacchetta & al. 2012a; Mattana & al. 2012; Fenu & al. 2015).

It is an unquestionable fact that the study of seed germination of endemic and/or endangered taxa has a pivotal role in their preservation. Indeed, seed germination is a crucial stage in the life cycle of a plant wherein the metabolic mechanisms that lead to the growth and emergence of the radicle and plumule are reactivated (Baskin & Baskin 2014).

Seed harvest, seed dehydration, storage conditions and storage periods play a key role in seed viability, germination and growth (Hay & Probert 2013). Seed harvest is the first step in acquiring and ensuring a high-quality seed lot, and germplasm collection of threatened endemic plants is often difficult (Porceddu & al. 2017b). Another key step is the evaluation of the tolerance to drying and the low temperatures of a specific taxon. Indeed, seeds, depending on their tolerance to drying and low temperatures, are commonly classified as orthodox (i.e., dehydration tolerant; include seeds of most native Mediterranean vascular plant species), recalcitrant (i.e., dehydration sensitive) or intermediate between them (Bacchetta & al. 2006b, 2008a).

All seed samples should be dried to equilibrium in a controlled environment of 5–20°C and 10–25% relative humidity (RH), depending on the species (Bacchetta & al. 2006b, 2008a). After hermetic sealing, the seed batches must be stored in a range between -25 and -18°C (base collection) and/or at 5–10°C (active collection) to guarantee their conservation with an estimated viability of decades (Bacchetta & al. 2006b, 2008a). In general, the combination of 3–5% moisture content and storage temperature below 8°C permits long-term seed preservation; however, viability may decrease because of deterioration processes (FAO/IPGRI 1994). Consequently, studies about long-term viability are needed to determine the storability of seed materials in seed banks and to provide conditions that will maintain the viability of each accession above a minimum value (FAO/IPGRI 1994).

Several studies have shown how the storage times, seed traits, environmental conditions at the site of collection and methods of preservation can affect the viability and longevity of accessions stored in seed banks (Probert & al. 2009; Godefroid & al. 2010; Mondoni & al. 2011; Royal Botanic Gardens Kew 2022). For instance, Pérez-García & al. (2007, 2009) showed that seeds of Brassicaceae stored for 40 years at sub-zero temperatures (-5 to -10°C) and low moisture content (ca. 3%) can provide a successful technology for *ex situ* plant conservation. Furthermore, these authors highlighted that the seeds stored at room temperature for 34–39 years showed germination percentages comparable to those of a cold room. These results underlined that a temperature under zero might not be as important a factor in seed conservation, as expected, at least for medium-term preservation, and

suggested the possibility of using ultra-dry methods as valid tools to effectively preserve germplasm in seed banks (Singh & al. 2003; Pérez-García & al. 2007). Moreover, currently, very few studies concerning the times and methods for seed preservation on Mediterranean endangered vascular plant species are available. *Hypericum scruglii* Bacch., Brullo & Salmeri and *Senecio morisii* J. Calvo & Bacch., two exclusive and endangered plant species from Sardinia, had been recently evaluated after long-term conservation in the seed bank (Cuenca-Lombraña & al. 2020; Porceddu & al. 2020). These studies showed the high viability of long-term stored seeds and the possible use of germplasm collections when fresh seeds are not available or limited.

The main aims of this research were to determine how storage times and conditions (i.e., base collection at -25°C and active collection at +5°C) can affect seed germination of eight endemic and endangered vascular plant species of Tyrrhenian islands: *Brassica insularis* Moris, *Centranthus amazonum* Fridl. & A. Raynal, *Dianthus morisianus* Vals., *Digitalis purpurea* var. *gyspergerae* (Rouy) Rouy, *Ferula arrigonii* Bocchieri, *Helicodictyon muscivorus* (L.f.) Engl., *Iberis integerrima* Moris and *Verbascum plantagineum* Moris. Seed collections of these taxa were stored at BG-SAR for a time ranging from a minimum of two to a maximum of 12 years, and seed germination requirements for each taxon were already known.

Materials and Methods

Study species

Among the eight selected Tyrrhenian endemic plant species under study (Fig. 1), four are exclusive to Sardinia, two to Sardo-Corsican, one to Sardo-Corsican-Balearic and one to Sardo-Corsican-Sicily-Tunisian (Table 1). Information on the taxa selected for this study is shown in Table 1. Each accession for each taxon tested for seed germination came from the same collection site, as reported in Table 1. To evaluate the differences in germination percentages of the different accessions for each taxon tested in this study, we used the data reported in Table 1 (see the “Germination percentage; optimal temperature and photoperiod in hours” column) based on the assumption that the initial germination percentages (i.e., before storage) for each species collected in the same locality remained constant or very similar.

Sardinian Germplasm Bank (BG-SAR)

BG-SAR, active since 1997, preserves, studies and manages the germplasm of Mediterranean plant species with particular focus on Sardinian endemic, threatened and policy species, as well as Crop Wild Relatives (CWR), landraces, useful plants and archaeological plant remains. In detail, BG-SAR preserves approximately 3500 seed lots from different Mediterranean areas, many of which are endemic to the Tyrrhenian islands, and ensures the preservation of several Sardinian taxa listed in the Italian national Red Lists (Porceddu & al. 2017a), especially those considered threatened according to the criteria of the IUCN (Orsenigo & al. 2018, 2021) or inserted in the attention list as the Top 50 species of the Mediterranean islands (Pasta & al. 2017). As of 2015, BG-SAR stored the germplasm of ca. 47% of policy species and ca. 42% of the strict Sardinian endemics (Fenu



Fig. 1. Taxa preserved at BG-SAR and selected for this study.

Table 1. Study species details and information about the best seed germination protocols reported in the literature. References for IUCN category: ¹Dulloo & al. (2011); ²Orsenigo & al. (2018); ³Orsenigo & al. (2016); ⁴García Murillo (2018). References for germination protocols: ¹Bacchetta & al. (2007); ²Mattana & al. (2010); ³Cogoni & al. (2012); ⁴Bacchetta & al. (2008b); ⁵Bacchetta & al. (2006a); ⁶BG-SAR unpublished data.

Taxon	Family	Collection site	N° of years of storage	Distribution	IUCN category	Germination percentage; optimal temperature and photoperiod in hours
<i>Brassica insularis</i>	Brassicaceae	Isola dei Cavoli-Serpentara (Villasimius)	11, 10, 8, 5	SA-CO-SI-TN	NT ¹	97 %; 20°C, 12/12. ¹
<i>Centranthus amazonum</i>	Caprifoliaceae	Monte Corrasi (Oliena)	9, 8, 6, 5	SA	CR ²	85 %; 10°C, 12/12. ²
<i>Dianthus morisianus</i>	Caryophyllaceae	Portixeddu (Buggerru)	10, 8, 7, 6, 2	SA	CR ²	97 %; 15°C, 12/12. ³
<i>Digitalis purpurea</i> var. <i>gyspergerae</i>	Plantaginaceae	Monte Lattias (Uta)	12, 11, 4	SA-CO	NE	89 %; 20°C, 12/12. ⁴
<i>Ferula arrigonii</i>	Apiaceae	Isola dei Cavoli-Serpentara (Villasimius)	11, 10, 8	SA-CO	LC ³	57 %; 10°C, 12/12. ¹
<i>Helicodiceros muscivorus</i>	Araceae	Isola dei Cavoli-Serpentara (Villasimius)	11, 10, 8, 6, 3	SA-CO-BL	NT ⁴	84 %; 15°C, 0/24. ⁵
<i>Iberis integerrima</i>	Brassicaceae	Valle Rio San Giorgio (Iglesias)	12, 11, 9, 8, 3	SA	NT ²	99 %; 20°C, 12/12. ⁶
<i>Verbascum plantagineum</i>	Scrophulariaceae	Zona diga Riu Monte Nieddu (Pula)	11, 4	SA	VU ²	89 %; 15°C, 12/12. ⁴

& al. 2015). Currently, the seed bank preserves more than 50% of policy species and Tyrrhenian Island endemics (unpublished data; work in progress). Among them, most are taxa listed in the Habitats Directive (such as *Astragalus maritimus* Moris, *A. verrucosus* Moris, *B. insularis*, *Gentiana lutea* L. subsp. *lutea*, *Helianthemum caput-felis* Boiss., *Lamyropsis microcephala* (Moris) Dittrich & Greuter, *Linum muelleri* Moris, *Ribes sardoum* Martelli, *Daucus rouyi* Spalik & Reduron and *Silene velutina* Pourr. ex Loisel.), and several accessions of the 10 most threatened exclusive endemic vascular plant species (*sensu* Bacchetta & al. 2012b) of Sardinia, such as *R. sardoum*, *D. morisianus*, *A. maritimus* and *A. verrucosus* (Porceddu & al. 2017a).

Seed processing carried out at BG-SAR

The seed conservation processes carried out at BG-SAR follow internationally recognised protocols and guidelines for seed bank standards (Bacchetta & al. 2006b, 2008a). The collected germplasm was added to the seed bank after a quarantine period in an isolated room set at controlled environmental parameters (20°C, 40% of RH). This procedure allows slow and gradual post-ripening and permits the evaluation of the phytosanitary state (identification of fungal infections, phytophagous insects, or other harmful parasites). After this period, the germplasm is cleaned, quantified, selected, weighed, prepared for morpho-colorimetric characterisation by image analysis and processed for preservation.

Concerning long-term conservation, all seed lots are dried at 15°C and 15% RH to reduce the internal seed moisture content to ca. 3–5% (according to the oil content) and stored at -25°C (as base collections under long-term conservation) and/or at +5°C (as active collections under medium-term conservation) in hermetic glass vials and Bormioli glass containers.

Germination test

Prior to germination tests, the hermetic Bormioli glass containers were stabilised within the dehydration room for six hours, and then the glass vials were opened, while the right seed amounts for testing were collected and stored for 24 hours at 15°C and 15% RH. For each species, four replicates of 25 seeds were sown on the surface of 1% water agar in 90 mm diameter plastic Petri dishes and incubated in a 12 h light/12 h dark photoperiod (except *Helicodiceros muscivorus* in full darkness) for a maximum of three months at constant temperatures, following the optimal conditions for each species from the literature (Table 1).

The experiments were carried out using growth chambers (SANYO MLR-351, SANYO Electric Co., Ltd.), equipped with white fluorescent lamps (FL40SS.W/37 70–10 $\mu\text{mol m}^{-2} \text{s}^{-1}$). At the end of the germination tests, when no additional germination occurred for two consecutive weeks, non-germinated seeds were cut with a scalpel to determine the number of filled, viable and empty seeds (Bacchetta & al. 2008a).

Data analysis

For each germination trial, the final germination percentage (FGP) and the germination rate (T_{50}) were calculated. The FGP was calculated as the mean of four replicates ($\pm$ SD) based on the total number of filled seeds (empty seeds were excluded). The T_{50} was determined as the time (expressed in days) required to reach 50% of the germination percentage; this value was only calculated when 50% germination was reached (Cuenca-Lombrana & al. 2016).

Statistical analysis

Generalised linear models (GLMs) were used to evaluate the effect of the storage conditions (i.e., stored at -25° and $+5^{\circ}\text{C}$) and the years of storage (see Table 1) on FGP. A quasibinomial error structure with logit link function and F-tests with an empirical scale parameter instead of chi-squared on the subsequent ANOVA were used to overcome residual overdispersion. To test the correlation between FGPs and years of storage, a linear regression analysis was performed. All statistical analyses were carried out using R v. 3.0.3 (R Development Core Team 2014).

Results

Seed germination

The GLM analysis highlighted, for the “Storage condition” (SC) factor, no statistically significant differences ($P > 0.05$) on seed germination, whereas statistically significant differences ($P < 0.05$) were found for the “Years of storage” (YS) factor, as well for the two-way interaction SC $\times$ YS (Table 2).

In general, all seeds of the tested species stored from 2 to 12 years, both belonging to $+5^{\circ}\text{C}$ and -25°C , germinated at percentages $> 50\%$ (Fig. 2; Electronic Supplementary File 1: Table S1).

In detail, *Brassica insularis* showed different germination responses, in particular when seed storage time was considered; in fact, seeds stored for 10 years highlighted germination percentages of $\sim 2\%$ and $\sim 29\%$ at $+5^{\circ}\text{C}$ and -25°C , respectively; among the ungerminated seeds, $\sim 88\%$ and $\sim 68\%$ were viable/imbibed, and $\sim 10\%$ and $\sim 3\%$ were dead at $+5^{\circ}\text{C}$ and -25°C , respectively (Fig. 2). *C. amazonum* showed an FGP $> 80\%$ with a percentage of dead seeds $< 14\%$ and a percentage of viable/imbibed seeds of $\sim 4\%$. *D. morisianus* had an FGP $> 90\%$ with a percentage of dead seeds $< 4\%$, and no viable/imbibed seeds (Fig. 2). *D. purpurea* var. *gyspergerae* had an FGP $> 90\%$ with a percentage of dead seeds $< 13\%$ (Fig. 2). *F. arrigonii* showed an FGP $> 60\%$ with a percentage of dead seeds $< 30\%$ and a percentage of viable/imbibed seeds $< 6\%$ (Fig. 2). *H. muscivorus* had an FGP $> 70\%$ with a percentage of dead seeds of $\sim 0\%$ and a percentage of

Table 2. GLM results for the effect on seed germination of the following factors: “Storage condition” (SC, $+5^{\circ}\text{C}$ and -25°C) and “Years of storage” (YS, from 2 to 12) and their interaction.

	d.f.	Deviance	Residual d.f.	Residual deviance	F	P (> F)
Null			359	9822.8		
Storage condition (SC)	1	23.2	358	9799.6	2.2001	0.3189
Years of storage (YS)	11	1655.9	347	8143.6	14.2501	$< 2.2 \times 10^{-16}$ ***
SC $\times$ YS	9	290.6	329	324.3	3.0569	0.0015 **

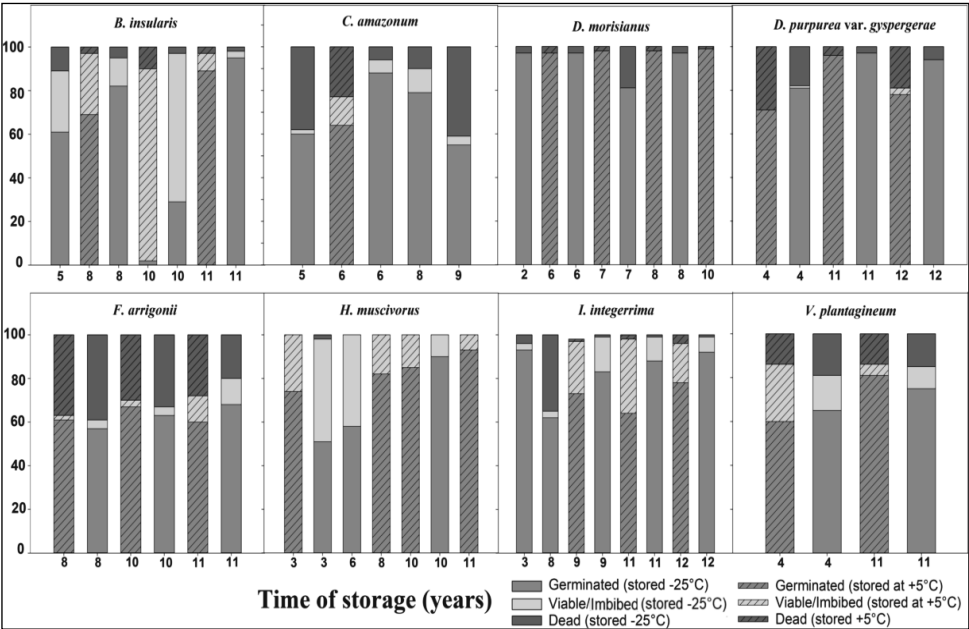


Fig. 2. Effects of time of storage (in years) and storage conditions (+5°C and -25°C) on final germination percentage for the selected Tyrrhenian plant species seeds stored at Sardinian Germplasm Bank (BG-SAR) and their percentage of viable/imbibed and dead seeds.

viable/imbibed seeds < 16% (Fig. 2). *I. integerrima* showed an FGP > 90% with a percentage of dead seeds < 4%. A seed lot storage time for 9, 11 and 12 years highlighted FGPs of 83%, 88% and 92%, respectively, at -25°C, showing higher percentages of germination than seeds stored at +5°C for 9, 11 and 12 years (73%, 64% and 88%, respectively) (Fig. 2). *V. plantagineum* had an FGP > 80% with a percentage of dead seeds < 15% and a percentage of viable/imbibed of ~14% (Fig. 2).

Effects of storage time

Linear regression analysis was carried out to verify if the years of conservation were correlated with the FGP. Figure 3 highlights a general positive linear trend over the years for most of the species tested. *B. insularis* showed a decreasing trend in both storage conditions, probably due to the low germination percentage recorded in seeds stored for 10 years (Fig. 3). *C. amazonum* showed a decreasing trend with years of storage at -25°C. In contrast, *D. morisianus* showed a positive linear trend over the years of storage at +5°C, while a decreasing trend was observed for the storage conditions at -25°C. In the case of *D. purpurea var. gyspergerae*, *F. arrigonii* and *H. muscivorus*, the FGPs increased with the years of storage in both conditions (+5°C and -25°C). *V. plantagineum* showed a growing trend for the storage conditions at +5°C (Fig. 3).

In general, as indicated by the R² and P values, there were no linear correlations between FPG and years of storage (Fig. 3) for any tested species. In addition, given the

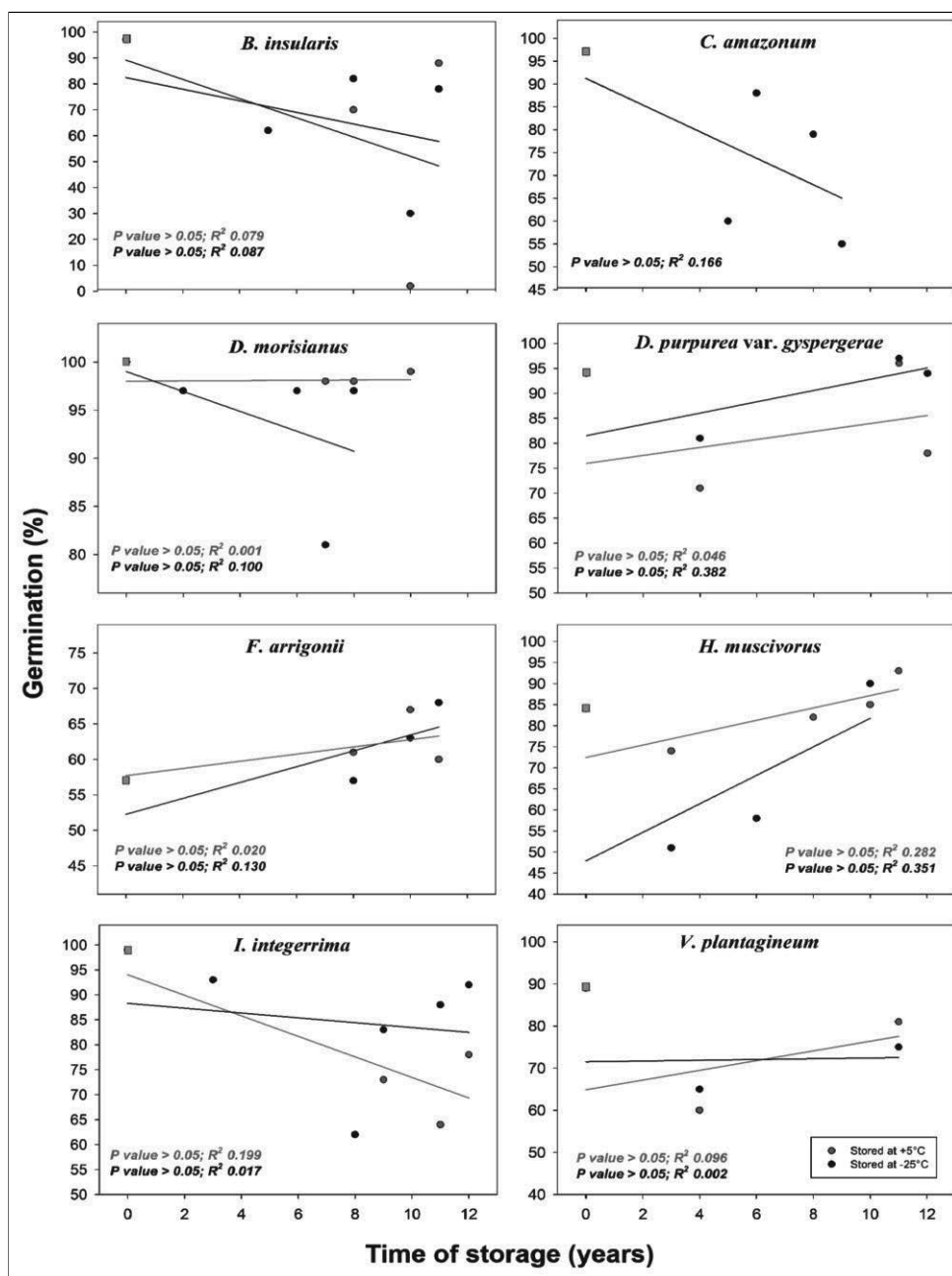


Fig. 3. Correlation between final germination percentages and years of storage represented by linear regression for all selected Tyrrhenian plant species stored at +5°C and at -25°C. Green squares correspond to the optimal germination percentage reported in the literature (see Table 1). Red dots and straight lines represent the storage conditions at +5°C and black dots and straight lines represent storage conditions at -25°C.

lack of statistical significance obtained for storage conditions highlighted by GLM (Table 2), the analyses were repeated by joining the dataset of final germination percentages obtained at +5°C and at -25°C. The R^2 and P values obtained for the unified/combined dataset showed that no linear correlations between FPG and years of storage were present.

Discussion

The Tyrrhenian endemic vascular plant species analysed in this study, in accordance with the optimal germination protocols from the literature, showed high percentages of germination, both for accessions stored at +5°C and for those at -25°C. *Brassica insularis* overall showed a germination capacity slightly lower than the attested one from the literature equal to 97% (Bacchetta & al. 2006a); in particular, low percentages of germination were obtained only at 10 years of storage, for causes that were not determined in this study. Godefroid & al. (2010), working on the species stored in the seed bank of the National Botanic Garden of Belgium, indicated that the loss of viability during storage and low germination percentage could be seed lot-specific.

Centranthus amazonum showed a decline in germination percentage compared with the 97% reported in the literature (Mattana & al. 2010). *Digitalis purpurea* var. *gyspergerae*, *Ferula arrigonii* and *Helicodicerus muscivorus* showed an increment in the germination response compared with their values reported in the literature at 89% (Bacchetta & al. 2008b), 57% and 84% (Bacchetta & al. 2008b), respectively, whereas *Iberis integerrima* and *Verbascum plantagineum* had a slight drop in the germination percentage compared to 99% and 89%, respectively (Table 1), known from the literature (BG-SAR unpublished data; Bacchetta & al. 2008b).

As shown in Fig. 2, the analyses carried out on each species confirm, in general terms, the high germination capacity of seeds stored at both temperatures (+5°C and -25°C). The high germination percentages recorded after several years of storage demonstrated how the preservation methods implemented in BG-SAR are effective for long-term storage. However, the statistical analysis (see Table 2) showed that both storage temperatures (+5°C and -25°C) seemed to be effective since there were no statistically significant differences. Based on these results, it was not possible to determine which storage temperature between +5°C and -25°C was the most advantageous for preserving the eight analysed species, at least after 12 years of conservation.

BG-SAR stores the dried seeds in transparent and hermetic glass containers, which fully prevent water uptake and allow the use of silica gel with moisture indicators as an excellent monitoring system of internal moisture conditions. In accordance with Gómez-Campo (2007), when containers are not perfectly tight, seeds tend to become balanced with the environmental air humidity and any potential benefit of low temperatures will be offset by the increased moisture content, which adversely affects seed longevity. As far as the time of storage is concerned, the germination percentage seems to decrease with the years of conservation in some tested species (e.g., *B. insularis* and *C. amazonum*). Such behaviour has also been detected in other species; for example, out of 124 accessions of 72 native Australian species, only 12 accessions of 10 species showed a significant decline after 5–12 years of seed bank storage at -20°C (Crawford & al. 2007). However, based on our

analyses (see Fig. 3), no correlation between the final germination percentage and the time of storage was proven. Hence, the percentage decrease in seed germination may depend on other factors, such as harvest time and/or climatic conditions during seed ripening and may differ among families (Godefroid & al. 2010).

The seed quality also plays an important role in conservation, and the Food and Agriculture Organization of the United Nations (FAO/IPGRI 1994) has set several standards that aim at ensuring maximum seed quality; focal features are, for instance, the harvest time (as close as possible to fruit/seed ripening and prior to dispersal), the time between harvest and storage under controlled dry conditions (3–5 days or as short as possible), the prompt cleaning and pest check (FAO/IPGRI 1994).

Particularly important to ensure long-term storage is the state of dehydration, and specifically the ultra-drying condition. In fact, low humidity is certainly the key factor for the long-term storage of orthodox seeds (Singh & al. 2003). This was remarked by Pérez-García & al. (2007), who proved high seed viability, with germination percentages close to 100%, in 37 species of the Brassicaceae family after 40 years of ultra-dry storage (moisture content ranged from 0.3 to 3% of fresh weight basis after storage). Notwithstanding, seed desiccation to less than 3% of moisture content (ultra-drying of orthodox seeds) has rarely been used in seed banking. Furthermore, according to Pérez-García & al. (2007), the results of our study suggest that temperature might not be a unique factor for seed preservation, at least within 12 years of storage.

Conclusions

Our study revealed that seed bank storage ensures the conservation of these Tyrrhenian endemic vascular plant species. Stored seeds at germplasm banks can be useful for translocation actions when there is little or no fresh material available. To ensure the *ex situ* conservation of these species, it is important to test seed viability every 5–10 years (FAO/IPGRI 1994) for monitoring seed longevity over time and to continue with the collection and preservation of the species of high conservation value. Similar efforts should be addressed in other Mediterranean endemic/endangered vascular plant species from the perspective of climate change and related issues on biodiversity conservation.

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