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Notes on the distribution, ecology, associated vegetation and conservation status of *Gymnadenia* (Orchidaceae) in Kosovo

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Abstract

Four species of *Gymnadenia* are native to Kosovo: *G. conopsea*, *G. frivaldii*, *G. nigra*, and G. *odoratissima*. In this study, field expedition data, phytosociological relevés, herbarium specimens along with extensive literature sources were used to analyse vegetation and ecological characteristics, habitat types, distributional patterns as well as provide notes on conservation. *Gymnadenia conopsea* is distributed throughout the country, from lowlands to the alpine belt. It was recorded in various plant communities. *Gymnadenia frivaldii* grows in the alpine zone of mountains, close to streams and in wet meadows. Its relevés belong to the class *Scheuchzerio-Caricetea fuscae*. This species is classified as "Near Threatened" (NT) in Kosovo. *Gymnadenia nigra* grows in subalpine and alpine grassland on preferably calcareous substrate. It has been found in almost all mountains reaching >2000 m a.s.l., and occurs in different plant communities belonging to the class: *Elyno-Seslerietea*. *Gymnadenia odoratissima* was recorded from one locality only in Kosovo, on the massif of Maja e Zezë, Sharri Mts. It was growing in degraded beech forest and meadows on silicate bedrock. Its floristically diverse relevés associate with the class: *Mulgedio-Aconitetea*. Of the four studied species, *G. frivaldii* deserves more conservation attention because of its fragile populations.

Keywords

Flora, orchids, plant conservation, phytosociology, SE Europe

Introduction

As a result of various human activities in recent years, many plant species have become vulnerable, threatened or even extinct. In Kosovo, as in many other parts of Europe, increased agricultural and forestry activities, combined with a cessation of the traditional management of meadows, centralization of farming and expansion of urban areas has deprived many plant species from their natural habitats. The consequence of this being the confinement of once large plant populations to smaller areas, mainly in remote and fragile habitats.

Several studies have shown that habitat fragmentation and small, scattered plant populations adversely affect the genetic structure of a species (Young et al. 1996; Bateman et al. 2003). Fragmented populations are exposed to a higher risk of restricted gene flow and loss of genetic polymorphism (Franklin 1980; Frankel and Soulé 1981; Berisha et al. 2015). In the long term, probably the main adverse effect will be the inability of the species to adapt or respond to ever-changing environmental conditions (Ellstrand 1992).

The aim of different conservation programs nowadays is to preserve natural habitats and with it the existing level of genetic diversity, especially in the case of rare and endangered species (Ellstrand et al. 1993; Gray 1996). Knowing that in developing countries like Kosovo, available resources for the conservation of nature are limited, the identification of conservation priorities is of crucial importance.

Orchidaceae are a very diverse and species-rich family of plants, that represent nearly 10% of all angiosperm species. They are characterized by considerable floral diversity and a unique and often intricate pollination biology. The research focus on the biology of these plants has been mainly directed towards their pollination, adaptations to pollinators, evolution of pollination traits and the evolutionary outcomes of their unique biology (Schiestl et al. 1999; Huber et al. 2004; Cozzolino and Widmer 2005). Additionally, as the family contains numerous rare, threatened and endangered species, studies on members of the group are of fundamental importance in plant conservation efforts (Case et al. 1998; Ávila-Díaz and Oyama 2007; Tsiftsis et al. 2008, 2019).

In the present study, the genus *Gymnadenia* R.Br. (Orchidaceae) in Kosovo is studied with emphasis on its distribution, species composition, diversity, ecological and associated vegetation characteristics. *Gymnadenia* is represented in the country by four naturally occurring species: *G. conopsea* (L.) R.Br., *G. frivaldii* Hampe ex Griseb., *G. nigra* (L.) Rchb.f., and *G. odoratissima* (L.) Rich. Of these, only *G. frivaldii* is of conservation concern, being categorized as "Near Threatened" (NT) in the Kosovarian Red List of plant species. The main aims of the study were to: a) study the distribution of the four *Gymnadenia* species in Kosovo and understand their distributional patterns; b) conduct phytosociological relevés in order to provide information about the most common plant communities where these species grow and generally analyze their syntaxonomy; c) provide ecological characteristics for each species concerning EUNIS habitat type preferences; and d) assess conservation implications for *G. frivaldii*.

Materials and methods

The studied species

According to available literature sources, herbarium specimens as well as based on our own field data, in Kosovo there are four native *Gymnadenia* species. This study deals with: *G. conopsea* (L.) R. Br., *G. frivaldii* Hampe ex Griseb., *G. nigra* (L.) Rchb.f., and *G. odoratissima* (L.) Rich.

In this context, it is important to briefly discuss the taxonomic position of the species *Gymnadenia nigra* (L.) Rchb.f. Based on a study of this species by Teppner and Klein (1990), it was found that the natural distribution of *G. nigra* [homotypic synonym: *Nigritella nigra* (L.) Rchb. p.] is only in Scandinavia. Whereas in the Balkans *G. nigra* has been repeatedly erroneously reported and these specimens are in fact: *Gymnadenia rhellicani* (Teppner & E.Klein) Teppner & E.Klein [homotypic synonym: *Nigritella rhellicani* Teppner & E.Klein]. However, in the Euro+Med Plant Base (Euro + Med 2006+), on which we relied for the current study, such a conclusion is not yet supported. Consequently, we referred to the species as: *G. nigra*.

Revision of herbarium material and distribution data

In total, 179 herbarium specimens (from the Herbarium of the Faculty of Natural Sciences, University of Prishtina) as well as private herbarium collection of F. Rexhepi (41 specimens) – (see Appendix 2 for details) were studied; the majority of these samples were collected by our team during the compilation of the Red Book of the vascular flora of the Republic of Kosovo (Millaku et al. 2013) between 2009 and 2013.

Due to the fact that during the work for the Red Book, the main focus were certain natural habitats that were rich in endemic plants, some regions of the country remained poorly sampled. To compensate for this, we have conducted twenty-five additional expeditions (between 2014 and 2020) to those poorly explored areas, in order to be more confident that the presented data will allow for general conclusions about the investigated genus in Kosovo.

Plant samples were finally identified by F. Millaku, using identification keys and other relevant literature sources (Diklić 1976; Gölz and Reinhard 1986; Tutin 2010).

To establish the distribution of *Gymnadenia* species in Kosovo, the literature was extensively examined (Lakušić and Grgić 1971; Diklić 1976; Rexhepi 1986; Krivošej 1997; Ranđelović et al. 1998; Millaku 1999; Stevanović ed. 1999; Micevski 2001; Bateman et al. 2006; Millaku ed. 2013; Ponert 2014; Djordjević et al. 2017). Determining the habitat type(s) for each species was done by comparing the habitat data where the species was recorded (from herbarium, literature and relevés) and finding the corresponding EUNIS habitat(s) according to Davies et al. (2004).

Vegetation data

To gain an overview of where the studied *Gymnadenia* species grow, in terms of phytosociological plant communities and certain related ecological preferences, a total of 15 phytosociological relevés were made (Appendix 1). Based on our field experience as well as from general knowledge from plant ecology, efforts have been made to conduct appropriate and representative phytosociological relevés.

Standard principles and methods of the Zürich-Montpellier school (Braun-Blanquet 1964; Mueller-Dombois and Ellenberg 1974) were applied. Depending on the habitat type, plot sizes of 10 × 10 m and 5 × 10 m respectively were used. For each plot, a complete list of vascular plants was recorded, alongside with cover-abundance values on a five-degree scale (Braun-Blanquet 1932). For the nomenclature of plant taxa, the Euro+Med Plant Base (Euro+Med 2006+) was followed. Relevés were made at elevations ranging from 140 to 2501 m a.s.l.; four relevés were made on communities with *G. conopsea*, four relevés on communities with *G. frivaldii*, four relevés on communities with *G. nigra* and three relevés on communities with *G. odoratissima*.

Based on diagnostic species, efforts have been made to offer a syntaxonomical classification of these relevés up to the level of Alliance. For this purpose, Mucina et al. (2016), Rexhepi (1994) and Sarić and Kojić (1984) were followed.

Data analysis

Using data obtained from herbarium specimens, phytosociological relevés as well as from literature, we were able to analyze the following parameters: altitude preferences, a set of ecological values (nutrients, pH reaction, moisture, continentality, temperature and light) from accompanying species of plants, using Pignatti indicator values (Pignatti et al. 2005). EUNIS corresponding natural habitat types (Davies et al. 2004) were defined for each species. Additionally, data on the overall species richness (from relevés) and richness of endemic taxa were compared. All of the geographical distributional data were mapped to show the distribution of *Gymnadenia* species in Kosovo (Fig. 1). R software (R Core Team 2013) for statistical computing was used to do the comparative analysis and generate the graphs.

Results

Distribution of *Gymnadenia* species in Kosovo based on herbarium and field data

Based on the studied herbarium specimens, data collected from field surveys as well as literature sources, the presence of four *Gymnadenia* species is confirmed in a total of 88 different localities across Kosovo.

Within these localities, *G. conopsea* is clearly the most abundant species. Its presence has been confirmed in 60 localities and considering its ecological preferences, the species

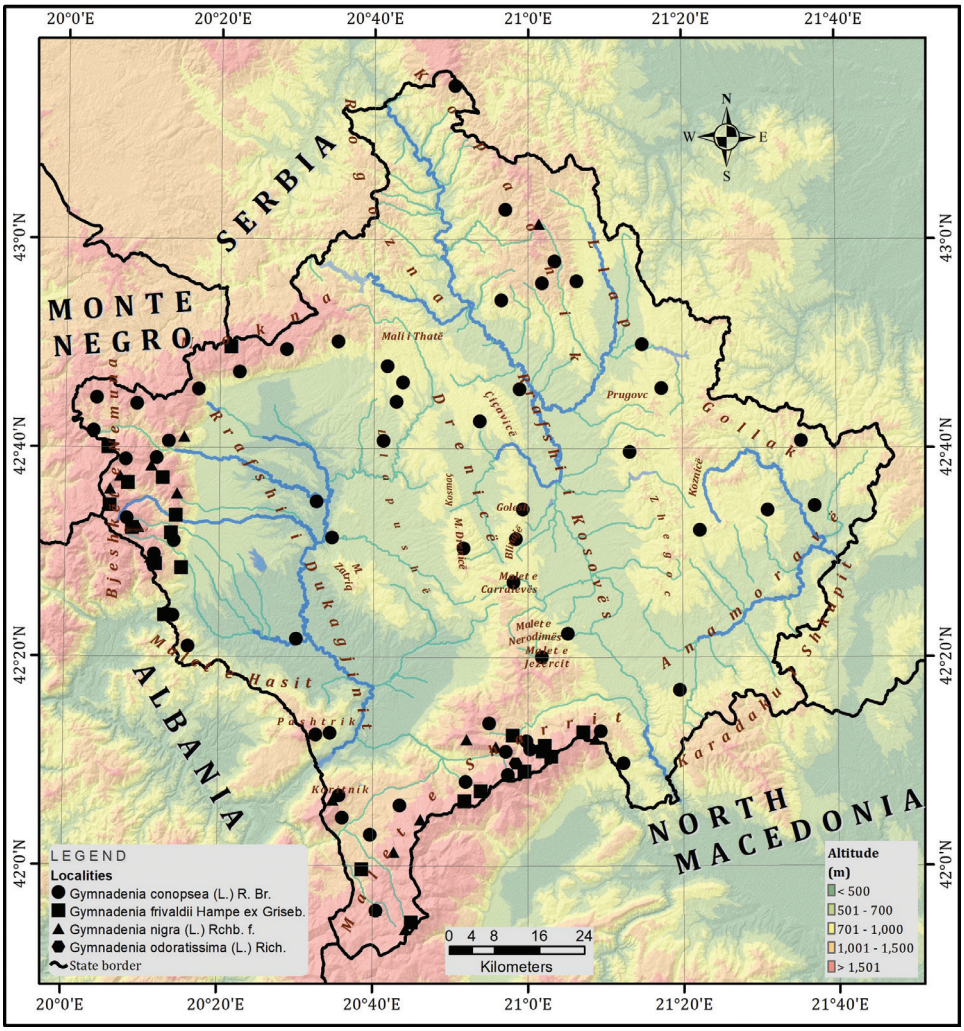

Figure 1. Known distribution of *Gymnadenia* species in Kosovo, based on herbarium specimens, as well as phytosociological relevés.

may have an even broader distribution across the country. *Gymnadenia nigra* is confirmed in 28 localities while *G. frivaldii* in 26 localities in Kosovo. *Gymnadenia odoratissima* is known so far to be present in only one locality in Kosovo (Appendix 2, Fig. 1).

Ecological characteristics and species preferences

Based on the obtained data from 15 phytosociological relevés, general data on each species preferences were evident. In terms of species distribution at different elevations, it was ascertained that the studied species show a narrow distributional preference (Fig. 2). It was established that *G. conopsea* had a preference for lower altitudes

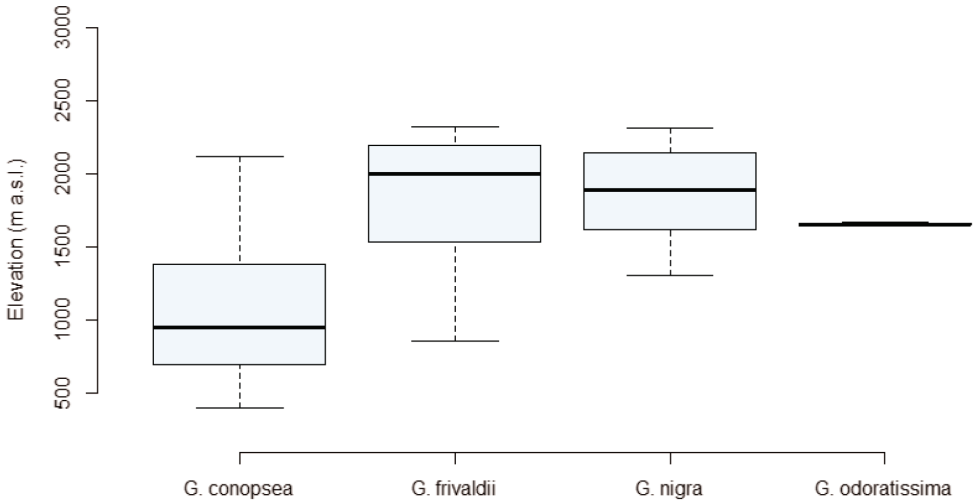

Figure 2. Elevational distribution of four *Gymnadenia* species based on the study of 179 herbarium specimens, geographically located in Kosovo.

(<1000 m a.s.l.), though it had a wider distributional range. *Gymnadenia frivaldii* showed a greater preference for higher altitudes (>2100 m a.s.l.) and had a wider distribution range compared to *G. nigra*. As for *G. odoratissima*, this parameter shows a narrow median weight due to the fact that is known from a single locality at ~1700 m a.s.l.

Concerning soil fertility and nutrients availability, it was established that all four *Gymnadenia* species communities grow on relatively poor soils, with an average value of 3. In this context, *G. nigra* communities can be distinguished with the lowest median weight compared to *G. frivaldii* and G. *odoratissima* that prefer slightly more nitrogen rich soils (Fig. 3, boxplot 1). As for soil pH reaction (Fig. 3, boxplot 2), it was observed that four studied species communities have a general preference for alkaline soils. Though six relevés were made on silicate soils, the occasionally deep, accumulated soil has a significant effect on reducing acidic influence of soil pH. In terms of soil moisture (Fig. 3, boxplot 3), it was established that plant communities of the four *Gymnadenia* species have a general preference for medium-wet soils, with *G. frivaldii* in particular, preferring wetter soils. With regard to climate-continentallity (C) values, it was clear that there are no oceanic species and the average (Fig. 3, boxplot 4) value of 5 is general for all studied species communities. The temperature (T) preferences (Fig. 3, boxplot 5) associate also with the community occurrences at respective elevations above sea level. But, additionally it relates to cold or warm habitats of Europe. In this context, apparent distinction in preferences has been observed namely between communities of *G. conopsea* (6) and *G. nigra* (3). As for light preferences (Fig. 3, boxplot 6), it was obvious that all studied communities have a clear preference for growing under full light, a preference particularly pronounced in the case of *G. nigra*.

Regarding general species richness of all plant communities of the four respective species (Fig. 4, boxplot 1), communities of *G. nigra* and *G. odoratissima* were

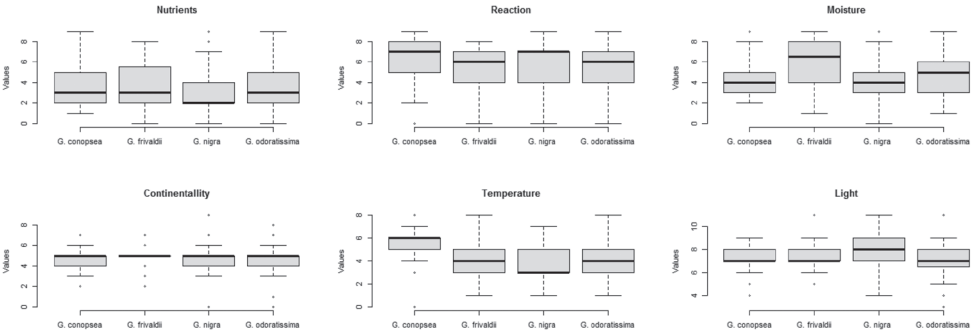

Figure 3. Boxplots representing the ecological preferences for nutrients, pH reaction, moisture, continentality, temperature and light–for four studied *Gymnadenia* species, based on their phytosociological communities.

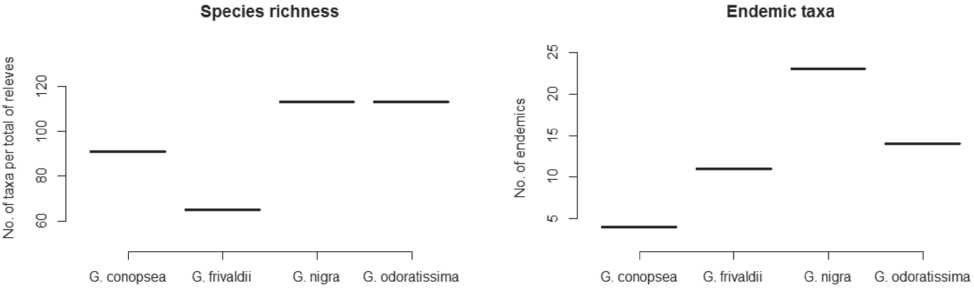

Figure 4. Comparative data on species richness and richness in endemic taxa beween the plant communities of four *Gymnadenia* species in Kosovo.

particularly distinguished by a large number of plant species. The other analyzed parameter, the number of endemic plant taxa per plant communities of a species, showed that plant communities of *G. nigra* (Fig. 4, boxplot 2) were particularly rich in endemics, while those of *G. conopsea* are notably poor. In this regard, *G. frivaldii* communities were rich in endemics too.

Vegetation characteristics

From 15 phytosociological relevés, after syntaxonomical analysis (relying on differential/characteristic species), it was noted that they belong to six different Classes (Appendix 1), with *Elyno-Seslerietea* Br.-Bl. 1948 (Class of vegetation that entails swards of Alpine and subalpine ranges of Europe) being the most abundant, in terms of relevé numbers.

Four relevés were made with *G. conopsea* communities. Three relevés fall into the alliance *Chrysopogono-Danthonion calycinae* Kojić 1959 (Dry grasslands on deep soils) and the other one into the alliance *Trifolion resupinati* Micevski 1957 (Vegetation of wet meadows).

Four relevés were made with *G. frivaldii* communities. As related with its typical natural habitats, the recorded plant communities (3) belonged to the alliance *Narthecion scardici* Horvat ex Lakušić 1968 (Relict moderately-rich fens of the Balkans) and the remaining one belongs to the alliance *Cirsion appendiculati* Horvat et al. 1937 (Tall-herb vegetation on acidic soils along mountain streams).

Additional four relevés were made with plant communities of *G. nigra*. They all fell into the alliance *Seslerio juncifoliae-Caricion firmae* Trinajstić 2005 (Alpine calcicolous sedge swards in wind-exposed habitats in the alpine belt of the Illyrian region and the Northern Dinarides). These four relevés were characterized by an exceptionally high number of associated endemic plant taxa (Fig. 4, boxplot 2).

Finally, a set of three relevés were made with plant communities where also *G. odoratissima* was growing. Due to a confined habitat (in Maja e Zezë, Sharri Mts.), it was quite difficult to record the relevé data. Two of these relevés, after analysis have shown affiliation to the alliance *Cirsion appendiculati* Horvat et al. 1937 (tall-herb vegetation on acidic soils along mountain streams and water springs at high altitudes), while the third one belonged to the alliance *Epilobion angustifolii* Oberd. 1957 (tall-herb perennial semi-natural vegetation on acidic soils of forest margins).

Discussion

Distributional patterns, habitat type and general environmental characteristics

As expected, all four species of the genus *Gymnadenia* follow different distribution patterns in Kosovo. *Gymnadenia conopsea* is a widely distributed species in Europe. In south-eastern Europe, it is common in the Carpathians, Hungary, Romania, the Balkan Peninsula and up to southern Russian and Ukrainian peninsula of Crimea. In all of these areas, it tends to occur at higher elevations, usually >800 m a.s.l. (Meekers et al. 2012). In Kosovo it represents the most common species of the genus. It was recorded in different habitats ranging from 750 m up to 1550 m a.s.l. As we had records on silicate as well as calcareous substrates, it indicates that this species can successfully grow in either one of these substrate types. Due to the wide distribution of the species, we are convinced that especially *G. conopsea* must have an even wider distribution than we depict on the map (Fig. 1). From the available data, it was confirmed in the following EUNIS Habitat types in Kosovo: E2.1 Permanent mesotrophic pastures and aftermath-grazed meadows; E3.3 Sub-mediterranean humid meadows; E1.2 Perennial calcareous grassland and basic steppes; E1.7 Non-Mediterranean dry acid and neutral closed grassland; E1.73 *Deschampsia flexuosa* grasslands; E4.31 Alpic *Nardus stricta* swards and related communities; and E4.4 Calcareous alpine and subalpine grassland.

From this genus in Kosovo, undoubtedly one of the rarest and most fragile species is the Balkan endemic *G. frivaldii*. It is worth noting that this species previously has been assigned to the genus *Pseudorchis* Ség. (syn.: *Leucorchis* E.May) and just recently, molecular based analysis has confirmed that it belongs to the genus *Gymnadenia*

(Bateman et al. 2003). *Gymnadenia frivaldii* is a species with a relatively small range in Europe limited to high-mountain belts on the Carpathians and the central and eastern Balkans (Delforge 2006). It has been reported for Albania, Kosovo, North Macedonia, Greece, Romania, Bulgaria, Montenegro, and Serbia (Diklić 1976; Bateman et al. 2006; Millaku ed. 2013, Djordjević et al. 2016, 2017; Berisha et al. 2020). This species prefers to grow on silicate substrates in fen communities and wet meadows of subalpine and alpine areas; occasionally it has also been recorded in pastures (of calcareous substrates too) as well as shrubs of the same altitudes in the mountains (Millaku ed. 2013). It has been reported (Djordjević et al. 2016) that this species is an indicator of an entire class (*Scheuchzerio palustris-Caricetea fuscae* Tx. 1937) of sedge-moss vegetation of fens in our region. The elevation distributional range was 1500–2600 m a.s.l. From the available data, mainly from the herbarium specimens, it was confirmed in the following EUNIS Habitat types in Kosovo: D2.2 Poor fens and soft-water spring mires; D2.22 *Carex nigra*, *Carex canescens*, *Carex echinata* fens; D2.26 *Eriophorum angustifolium* fens; D2.282 Balkan *Willemetia* fens; D2.3 Transition mires and quaking bogs and D2.38 *Sphagnum* and *Eriophorum* rafts.

Gymnadenia nigra was recorded on calcareous substrates of sub-alpine and alpine grasslands, or almost on all mountains exceeding 2000 m a.s.l. Based on the available data, it was confirmed in the following EUNIS Habitat types in Kosovo: E4.4 Calcareous alpine and subalpine grasslands; E1.7 Non-Mediterranean dry acid and neutral closed grassland and E1.72 *Agrostis-Festuca* grassland. All four relevés (Appendix 1) with this species, syntaxonomically fall into the Alliance: *Seslerio juncifoliae-Caricion firmae* Trinajstić 2005 (Alpine calcicolous sedge swards in wind-exposed habitats), though it has been reported also in different plant communities growing on limestone substrates (Rexhepi 1994). Also *G. nigra* previously has been assigned to *Nigritella* Rich. and *Orchis* Tourn. ex L., but phylogenetic studies (Bateman et al. 2003) have proved that it belongs to the genus *Gymnadenia*.

Gymnadenia odoratissima, a species very similar to *G. conopsea*, has an extensive distribution range in Europe. It is recorded from Spain in the west up to Ukraine in the east. Common in the mountain ranges of central Europe, up to the Sweden in the north and has been recorded also in Greece to the south. In Kosovo, Maja e Zezë massif represents the only known habitat of this species. It was recorded on silicate substrate, scattered in an area of ~1700 m^2, at an elevation ranging from 1680 m up to 1800 m a.s.l. Its corresponding habitat type was that of (EUNIS - E5.5721) Moesian Balkan thistle tall herb communities, and at the forest (rather degraded) margins of (EUNIS - G1.6933), namely the Balkan range subalpine beech forests.

Vegetation analysis

Of the four relevés with *G. conopsea* (Relevés 1–4, Appendix 1), relevés 1, 3 and 4 belonged to the Alliance: *Chrysopogono-Danthonion calycinae* Kojić 1959, with the following species being dominant: *Polygala major* Jacq., *Hypochaeris maculata* L., *Festuca nigrescens* Lam., *Danthonia alpina* Vest. and *Sanguisorba minor* Scop. The richest relevé in

terms of number of plant taxa was relevé no. 4., with 36 recorded plant taxa. Relevé no. 2 belonged to the Alliance: *Trifolion resupinati* Micevski 1957. It was recorded at a lower elevation (140 m) compared with the other ones of this group. The most dominant species in this relevé were: *Hordeum secalinum* Schreb. and *Trifolium fragiferum* L. As it is known, *G. conopsea* has a wider, more complex distributional range, though we aimed at offering its most common plant communities for comparative reasons. It was not our aim to define its syntaxonomical status, though this study can assist in that matter.

Of the four relevés with *G. frivaldii* (Relevés 5–8, Appendix 1), relevés 5, 6 and 8 belonged to the Alliance: *Narthecion scardici* Horvat ex Lakušić 1968. In these three calcareous relevés, the following plant taxa have been recorded as dominant ones: *Narthecium scardicum* Košanin, *Pinguicula balcanica* Casper, *Gymnadenia frivaldii* Hampe ex Griseb., *Eriophorum angustifolium* Honck. and *Pinguicula leptoceras* Rchb. Relevé no. 7 belonged to the Alliance: *Cirsion appendiculati* Horvat et al. (1937) based on its differential taxa. The most dominant plant taxa were: *Cirsium appendiculatum* Griseb., *Eriophorum angustifolium* Honck., *Agrostis canina* L. and *Caltha palustris* L. With a total of 31 recorded plant taxa, this was the relevé with the richest floristic diversity.

All four relevés with *G. nigra* (Relevés 9–12, Appendix 1) shared similar characteristics as they were all recorded on calcareous substrates. They all belonged to the alliance: *Seslerio juncifoliae-Caricion firmae* Trinajstić 2005, and the following taxa were recorded as dominant ones: *Helianthemum nummularium* (L.) Mill., *Dryas octopetala* L., *Oxytropis jacquinii* Bunge, *Helianthemum canum* (L.) Baumg., *Bistorta vivipara* (L.) Delarbre and *Gymnadenia nigra* (L.) Rchb.f. All relevés with *G. nigra* had high number of recorded plant taxa, with relevé no. 10 having 72 plant taxa recorded on one sampling site.

Of the three relevés with *G. odoratissima* (Relevés 13–15, Appendix 1), relevés 13 and 14 most likely should belong to the Alliance: *Cirsion appendiculati* Horvat et al. (1937), with the following taxa recorded as dominant ones: *Barbarea balcana* Pančić, *Eriophorum latifolium* Hoppe, *Caltha palustris* L., *Cirsium appendiculatum* Griseb., *Cardamine pratensis* L., and *Helianthemum nummularium* (L.) Mill. While the other remaining relevé on silicates (relevé no. 15) most likely should belong to the Alliance: *Epilobion angustifolii* Oberd. 1957. *Epilobium angustifolium* L. is the most dominant plant taxon in this relevé, as a characteristic species for the forest edges on silicate as well as a potential indicator of previously burned habitats or man-induced deforestation. High cover-abundance values on this relevé had also: *Salix caprea* L., *Pimpinella saxifraga* L. and *Avenella flexuosa* (L.) Drejer.

In the context of the vegetation diversity assessment, from only 15 relevés it was noticeable a very diverse vegetation affiliation by these four species of *Gymnadenia*. Four relevés belonged to *Elyno-Seslerietea*, three relevés to *Festuco-Brometea*, *Scheuchzerio palustris-Caricetea fuscae* and *Mulgedio-Aconitetea* respectively, and finally one releve belonged to *Epilobietea angustifolii* and *Molinio-Arrhenatheretea* respectively.

Commonly, all strategies for measuring biodiversity involve protecting a single species or several species of a given genus. Vegetation ecology contributes towards a better understanding of species indices of threats and a variety of interrelated paradigms

in ecology (Cornell and Karlson 1996; Austin 1999). Furthermore, it also gives an incomparable insight into understanding the complex relationships between plant diversity, vegetation cover and site conditions (Wiesmair et al. 2017).

Conservation aspects

Although all of the four investigated *Gymnadenia* species are included in the European Red List of Vascular Plants (Bilz et al. 2011), all categorized as "LC" [Least Concern], more attention should be paid to those species that are more vulnerable and have fragmented habitats.

From this point of view, *G. frivaldii* is characterized by limited populations and a small number of mature individuals, with the exception of those in the Mts. of Gjeravica and Dobrosh. Nonetheless, since species populations are observed to be stable, its categorization as Near Threatened [NT] (Millaku ed. 2013) in Kosovo is completely reasonable.

Due to the large number of associated endemic species and their importance, we suggest that *G. nigra* populations in Kosovo also be assessed against conservation criteria and that monitoring measures be taken. In addition, the single population of *G. odoratissima* should be carefully monitored, as it represents the only habitat of the species in Kosovo.

Conclusions

Like all members of the Orchidaceae, *Gymnadenia* species are under pressure in the wild, primarily for their ornamental merits. These species also face many other threats in the wild, but are mainly affected by the loss, degradation or increasing fragmentation of their natural habitats. This habitat fragmentation is caused by human impacts on the natural environment.

Due to the fact that populations of *G. frivaldii* grow in habitats near watercourses and wet meadows, the conservation and management of these resources is directly related to the sustainability of the habitats that host them and many accompanying species.

The data presented in this study can help in the decision-making processes of the relevant agencies to implement appropriate conservation programs as well as further research.

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Appendix 1

Phytosociological table of 15relevès. Species of *Gymnadenia* shown in bold and with grey shaded background.

Relevé syntaxonomic categories – (Class, Order and **Alliance)**	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Trifolion resupinati** Micevski 1957 [Cl.: Molinio-Arrhenatheretea / Ord.: Trifolio-Hordeetalia]	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Seslerietalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Seslerietalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Seslerietalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Seslerietalia tenuifoliae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Epilobion angustifolii** Oberd. 1957 [Cl.: Epilobietea angustifolii / Ord.: Galeopsio-Senecionetalia sylvatici]
Number of relevé	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Plot area (m^2)	100	100	100	100	50	50	50	50	100	100	100	100	50	50	50
Inclination in degree	10	2	10	15	12	5	15	10	12	10	15	12	10	12	10
Exposition	W	SE	NW	S	SW	N	SE	NW	NW	N	NE	SE	SE	NE	N
Altitude (m.a.s.l)	1400	140	800	1510	1509	2234	1847	1622	2501	1836	2324	2142	1696	1758	1710
Covering (%)	95	100	95	90	98	98	98	95	100	98	90	95	90	95	90
Substrate	Silicate	Limest.	Silicate	Limest.	Limest.	Limest.	Silicate	Limest.	Limest.	Limest.	Limest.	Limest.	Silicate	Silicate	Silicate
Locality	Kopaonik	Ferizaj	Orllan	Brezovicë	Vracë	Jazhincë	Luboten	B. e Dobroshit	Maja e *Zezë*	Luboten	Kurvallë	Zhleb	Maja e *Zezë*	Maja e *Zezë*	Maja e *Zezë*
Date	04.07.2017	25.06.2017	30.06.2018	09.07.2018	21.06.2019	30.06.2019	15.07.2019	20.07.2020	30.06.2019	08.07.2019	14.07.2019	20.07.2019	15.06.2019	15.06.2019	15.06.2019
No. of species / relevé	30	35	27	36	28	27	31	28	67	72	42	48	62	64	55
GPS Coordinates	43°14'57.30"N, 20°48'37.57"E	42°19'40.34"N, 21°10'48.39"E	42°49'17.44"N, 21°20'57.54"E	42°12'2.15"N, 21°1'53.69"E	41°54'47.34"N, 20°43'49.72"E	42°8'57.42"N, 20°59'38.81"E	42°12.905N, 021°08.234E	42°34'6.06"N, 20°14'31.25"E	42°3'49.68"N 20°45'56.99"E	42°13.115N, 21°08.002E	42°31'35.30"N, 20°8'40.73"E	42°46'7.35"N, 20°15'51.18"E	42°6'41.5"N, 20°52'46.2"E	42°6'44.9"N, 20°53'19.9"E	42°6'43.0"N, 20°52'27.1"E
Polygala major Jacq.	2	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Thymus praecox subsp. *jankae* (Čelak.) Jalas	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Pedicularis heterodonta Pančić	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hypochaeris maculata L.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dianthus cruentus Griseb.	+	.	1	.	.	.	.	.	.	.	.	1	.	.	.
Anthyllis vulneraria L.	1	.	.	4	.	.	.	.	.	.	.	.	.	.	.
Plantago media L.	+	.	+	+	.	.	.	.	.	.	.	.	.	.	.
***Gymnadenia conopsea* (L.) R.Br.**	**+**	**+**	**+**	**+**	.	.	.	.	.	.	.	.	.	.	.
Scabiosa columbaria L.	1	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Leontodon hispidus L.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anacamptis morio (L.) R.M.Bateman & al.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Festuca nigrescens Lam.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Relevé syntaxonomic categories – (Class, Order and **Alliance)**	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Trifolion resupinati** Micevski 1957 [Cl.: Molinio-Arrhenatheretea / Ord.: Trifolio-Hordeetalia]	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Chrysopogono-Danthonion calycinae** Kojić 1959 [Cl.: Festuco-Brometea / Ord.: Brachypodietalia pinnati]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Narthecion scardici** Horvat ex Lakušić 1968 [Cl.: Scheuchzerio palustris-Caricetea fuscae / Ord.: Caricetalia fuscae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Sesleretalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Sesleretalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Sesleretalia tenuifoliae]	ALL: **Seslerio juncifoliae-Caricion firmae** Trinajstić 2005 [Cl.: Elyno-Seslerietea / Ord.: Sesleretalia tenuifoliae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Cirsion appendiculati** Horvat et al. 1937 [Cl.: Mulgedio-Aconitetea / Ord.: Adenostyletalia alliariae]	ALL: **Epilobion angustifolii** Oberd. 1957 [Cl.: Epilobietea angustifolii / Ord.: Galeopsio-Senecionetalia sylvatici]
Asperula cynanchica L.	1	.	.	2	.	.	.	.	+	+	+	+	.	.	.
Trifolium pratense L.	1	.	+	1	.	.	.	.	.	.	.	.	.	.	+
Campanula patula L.	1	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Rumex acetosa L.	+	.	.	.	.	.	.	.	+	.	.	.	+	+	.
Rhinanthus minor L.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gentianella bulgarica (Velen.) Holub	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Genista sagittalis L.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphrasia rostkoviana Hayne	1	.	.	.	.	.	.	.	+	+	.	+	.	.	.
Avenella flexuosa (L.) Drejer	1	.	.	+	.	.	.	.	.	.	.	.	.	.	1
Pilosella hoppeana (Schult.) F.W.Schultz & Sch.Bip.	1	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Veratrum album L.	+	.	.	.	+	.	.	+	.	.	.	.	.	.	.
Gentiana utriculosa L.	+	.	.	.	.	.	.	.	+	+	+	+	.	.	.
Antennaria dioica (L.) Gaertn.	+	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Silene viscaria (L.) Jess.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ranunculus montanus Willd.	1	.	.	1	.	.	.	.	.	.	.	+	.	.	.
Briza media L.	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.
Lotus corniculatus L.	+	1	+	+	.	.	.	.	+	+	.	.	.	.	.
Genista tinctoria L.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Hordeum secalinum Schreb.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium fragiferum L.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa trivialis subsp. *sylvicola* (Guss.) H.Lindb.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Ranunculus sardous Crantz	.	2	.	+	.	.	.	.	.	.	.	.	.	.	.
Alopecurus utriculatus Sol.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Oenanthe silaifolia M.Bieb.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium patens Schreb.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Bromus racemosus L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla reptans L.	.	1	.	+	.	.	.	.	.	.	.	.	.	.	.
Schedonorus pratensis (Huds.) P.Beauv.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium repens L.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.
Ranunculus acris L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Lolium perenne L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.

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Carex vulpina L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Alopecurus pratensis L.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.
Verbena officinalis L.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.
Anthoxanthum odoratum L.	.	1	1	+	.	.	.	.	+	.	.	+	.	.	.
Carex melanostachya Willd.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Mentha aquatica L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Cichorium intybus L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Rorippa sylvestris (L.) Besser	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium arvense (L.) Scop.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Inula salicina L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium palustre L.	.	+	.	+	.	.	+	.	.	.	.	.	+	+	.
Carex divisa Huds.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Medicago lupulina L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa pratensis L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Prunella vulgaris L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa compressa L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Lysimachia nummularia L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex distans L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa palustris L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
Danthonia alpina Vest	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.
Inula hirta L.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.
Euphrasia stricta J.F.Lehm.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium montanum L.	.	.	2	+	.	.	.	.	.	.	.	.	.	.	.
Polygala comosa Schkuhr	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
Luzula campestris (L.) DC.	.	.	+	.	.	.	.	.	+	+	+	+	.	.	.
Filipendula vulgaris Moench	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.
Leucanthemum vulgare Lam.	.	.	2	+	.	.	.	.	+	+	+	+	.	.	+
Helianthemum nummularium subsp. *grandiflorum* (Scop.) Schinz & Thell.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
Brachypodium phoenicoides (L.) Roem. & Schult.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Eryngium campestre L.	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.

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Helictochloa pratensis (L.) Romero Zarco	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Pimpinella saxifraga L.	.	.	+	+	.	+	+	+	.	+	.	+	+	+	1
Galium verum L.	.	.	+	1	.	.	.	.	.	.	.	.	.	.	.
Helleborus odorus Willd.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Linum catharticum L.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Sanguisorba minor Scop.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.
Teucrium chamaedrys L.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Euphorbia cyparissias L.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Centaurea scabiosa L.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Arabis hirsuta (L.) Scop.	.	.	.	+	.	.	.	.	.	.	.	.	+	+	.
Hypericum perforatum L.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Dactylis glomerata L.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Ptilostemon afer (Jacq.) Greuter	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+
Knautia arvensis (L.) DC.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Viola elegantula Schott	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Primula veris L.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+
Stachys officinalis (L.) Trevis.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Carex caryophyllea Latourr.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
Narthecium scardicum Košanin	.	.	.	.	2	1	.	1	.	.	.	.	.	+	.
Pinguicula balcanica Casper	.	.	.	.	3	4	+	.	+	.	.	.	+	+	.
Carex nigra (L.) Reichard	.	.	.	.	1	1	.	1	.	.	.	.	1	+	.
***Gymnadenia frivaldii* Hampe ex Griseb.**	.	.	.	.	**1**	**+**	**+**	**2**	.	.	.	.	.	.	.
Juncus triglumis L.	.	.	.	.	1	.	.	+	.	.	.	.	.	.	.
Primula halleri J.F.Gmel.	.	.	.	.	1	+	+	.	.	.	.	.	.	.	.
Silene asterias Griseb.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Willemetia stipitata (Jacq.) Dalla Torre	.	.	.	.	+	1	+	.	.	.	.	.	.	.	.
Dactylorhiza cordigera (Fr.) Soó	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Juncus thomasii Ten.	.	.	.	.	+	.	.	.	.	.	.	.	+	+	.
Trifolium badium Schreb.	.	.	.	.	+	+	+	.	.	+	+	.	.	.	.

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Parnassia palustris L.	.	.	.	.	+	+	+	1	.	.	.	.	.	+	.
Philonotis seriata Mitt.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Eriophorum angustifolium Honck.	.	.	.	.	+	.	2	2	.	.	.	.	.	.	.
Deschampsia cespitosa (L.) P.Beauv.	.	.	.	.	+	+	.	.	.	.	.	.	+	+	.
Eleocharis acicularis (L.) Roem. & Schult.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Carex lepidocarpa Tausch	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Dianthus superbus L.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Carex flava L.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.
Juncus effusus L.	.	.	.	.	+	1	+	1	.	.	.	.	+	+	.
Sanguisorba officinalis L.	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.
Blysmus compressus (L.) Link	.	.	.	.	+	+	+	.	.	.	.	.	+	+	.
Iris pseudacorus L.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Agrostis canina L.	.	.	.	.	+	.	1	.	.	.	.	.	+	+	+
Caltha palustris L.	.	.	.	.	+	+	1	+	.	.	.	.	2	2	.
Ranunculus fontanus C.Presl	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Barbarea longirostris Velen.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Cardamine carnosa Waldst. & Kit.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Leontodon crispus Vill.	.	.	.	.	.	1	+	.	+	+	.	.	.	.	+
Carex kitaibeliana Bech.	.	.	.	.	.	+	.	+	2	1	+	.	+	+	+
Nardus stricta L.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Selaginella selaginoides (L.) Schrank & Mart.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Saxifraga rotundifolia L.	.	.	.	.	.	+	+	+	.	.	.	.	+	+	.
Silene pusilla Waldst. & Kit.	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.
Soldanella alpina L.	.	.	.	.	.	+	.	+	.	+	.	.	.	.	.
Crepis aurea subsp. *glabrescens* (Caruel) Arcang.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Dactylorhiza maculata (L.) Soó	.	.	.	.	.	+	+	.	+	+	.	.	.	.	.
Equisetum palustre L.	.	.	.	.	.	+	.	+	.	.	.	.	+	+	.
Viola aetolica Boiss. & Heldr.	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.
Plantago lanceolata L.	.	.	.	.	.	+	+	.	.	.	.	.	+	+	.

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Cirsium appendiculatum Griseb.	.	.	.	.	.	.	4	.	.	.	.	.	3	2	.
Cardamine pratensis L.	.	.	.	.	.	.	1	2	.	.	.	.	1	1	.
Helianthemum nummularium (L.) Mill.	.	.	.	.	.	.	1	.	.	+	2	4	1	+	+
Carex curvula All.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
Alchemilla hybrida (L.) L.	.	.	.	.	.	.	+	+	.	+	.	.	.	.	+
Geranium sylvaticum L.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.
Geum coccineum Sibth. & Sm.	.	.	.	.	.	.	+	+	.	.	.	.	+	1	.
Ranunculus breyninus Crantz	.	.	.	.	.	.	+	.	+	+	.	.	+	+	.
Myosotis sylvatica Hoffm.	.	.	.	.	.	.	+	+	.	.	.	.	+	+	.
Calamagrostis varia (Schrad.) Host subsp. *varia*	.	.	.	.	.	.	+	.	.	.	.	.	+	+	.
Mentha longifolia (L.) L.	.	.	.	.	.	.	+	.	.	.	.	.	+	+	.
Festuca adamovicii (St.-Yves) Markgr.-Dann.	.	.	.	.	.	.	+	.	+	+	.	.	+	+	+
Veronica chamaedrys L.	.	.	.	.	.	.	+	+	+	+	+	+	+	+	.
Pedicularis brachyodonta Schloss. & Vuk.	.	.	.	.	.	.	+	.	+	+	+	.	+	+	.
Pinguicula leptoceras Rchb.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
Ranunculus degenii Kümmerle & Jáv.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Trollius europaeus L.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Saxifraga stellaris L.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Phyteuma pseudorbiculare Pant.	.	.	.	.	.	.	.	+	.	+	.	.	+	+	.
Neotinea maculata (Desf.) Stearn	.	.	.	.	.	.	.	+	+	.	+	+	.	.	.
Ranunculus platanifolius L.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Bistorta officinalis Delarbre	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Dryas octopetala L.	.	.	.	.	.	.	.	.	1	4	.	.	.	.	+
Oxytropis jacquinii Bunge	.	.	.	.	.	.	.	.	2	3	.	+	.	.	.
Helianthemum canum (L.) Baumg.	.	.	.	.	.	.	.	.	4	2	.	+	.	.	+
Edraianthus graminifolius (L.) A.DC.	.	.	.	.	.	.	.	.	2	1	+	.	.	.	.

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Juniperus communis subsp. *nana* Syme	.	.	.	.	.	.	.	.	1	+	1	.	.	.	+
Festuca panciciana (Hack.) K.Richt.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	+
Bistorta vivipara (L.) Delarbre	.	.	.	.	.	.	.	.	+	+	+	+	+	+	.
Anemonastrum narcissiflorum (L.) Holub	.	.	.	.	.	.	.	.	+	.	+	+	+	.	.
Sesleria nitida Ten.	.	.	.	.	.	.	.	.	+	.	2	+	.	.	.
Arctostaphylos uva-ursi (L.) Spreng.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
***Gymnadenia nigra* (L.) Rchb.f.**	.	.	.	.	.	.	.	.	+	+	+	+	.	.	.
Minuartia verna (L.) Hiern	.	.	.	.	.	.	.	.	+	+	.	.	.	.	+
Dianthus integer Vis.	.	.	.	.	.	.	.	.	+	.	+	2	+	+	.
Sesleria juncifolia Suffren	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.
Achillea ageratifolia (Sm.) Benth. & Ho.f.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Phleum montanum K.Koch	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Geum montanum L.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Cerastium decalvans Schloss. & Vuk.	.	.	.	.	.	.	.	.	+	+	.	1	.	.	.
Arabis alpina L.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Saxifraga paniculata Mill.	.	.	.	.	.	.	.	.	+	+	.	+	.	.	.
Myosotis alpestris F.W.Schmidt	.	.	.	.	.	.	.	.	+	.	1	+	.	.	.
Fragaria vesca L.	.	.	.	.	.	.	.	.	+	+	.	.	+	.	.
Stachys alpina L.	.	.	.	.	.	.	.	.	+	.	1	+	.	.	.
Saxifraga scardica Griseb.	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Carduus candicans Waldst. & Kit.	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Erysimum comatum Pančić	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.
Achillea abrotanoides (Vis.) Vis.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+
Aubrieta columnae subsp. *croatica* (Schott, Nyman & Kotschy) Mattf.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Cerastium cerastoides (L.) Britton	.	.	.	.	.	.	.	.	+	.	+	.	.	.	.
Vaccinium uliginosum L.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Rubus idaeus L.	.	.	.	.	.	.	.	.	+	+	+	.	+	+	.

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Anthyllis aurea Host	.	.	.	.	.	.	.	.	+	+	.	+	.	.	+
Campanula rotundifolia L.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+
Vaccinium myrtillus L.	.	.	.	.	.	.	.	.	+	+	+	+	.	.	.
Luzula spicata (L.) DC.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Hypericum richeri subsp. *grisebachii* (Boiss.) Nym.	.	.	.	.	.	.	.	.	+	.	1	.	.	.	+
Jasione orbiculata Velen.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Saxifraga moschata Wulfen	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Salix retusa L.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	+
Crepis aurea (L.) Cass.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.
Hieracium pilosum Froel.	.	.	.	.	.	.	.	.	+	+	.	+	.	.	.
Phleum alpinum L.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.
Onobrychis montana subsp. *scardica* (Griseb.) P.W.Ball	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Carex sempervirens Vill.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.
Trinia dalechampii (Ten.) Janch.	.	.	.	.	.	.	.	.	+	+	.	+	.	.	.
Aster alpinus L.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.
Linum capitatum Schult.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.
Silene saxifraga L.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Thymus praecox subsp. *zygiformis* (Heinr. Braun ex Wettst.) Jalas	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Trifolium noricum Wulfen	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+
Poa molinerii Balb.	.	.	.	.	.	.	.	.	.	+	+	+	+	+	+
Campanula spatulata Sm.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Luzula forsteri (Sm.) DC.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	+
Oxytropis campestris (L.) DC.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Hieracium villosum Jacq.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Oenanthe peucedanifolia Pollich	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.
Dianthus sylvestris subsp. *bertisceus* Rech.f.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.

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Salix caprea L.	.	.	.	.	.	.	.	.	.	+	.	.	+	+	2
Primula elatior (L.) L.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Galium anisophyllon Vill.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	+
Achillea chrysocoma Friv.	.	.	.	.	.	.	.	.	.	+	1	.	+	+	.
Bromopsis cappadocica (Boiss. & Balansa) Holub	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.
Cyanus triumfettii (All.) Á.Löve & D.Löve	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.
Koeleria eriostachya Pančić	.	.	.	.	.	.	.	.	.	+	+	.	.	.	+
Dianthus scardicus Wettst.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Hieracium naegelianum Pančić	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.
Gentianella bulgarica (Velen.) Holub	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.
Bupleurum falcatum L.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Bupleurum karglii Vis.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.
Campanula glomerata L.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+
Saxifraga sempervivum K.Koch	.	.	.	.	.	.	.	.	.	+		+	.	.	.
Festuca koritnicensis Hayek & J.Vetter	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.
Armeria canescens (Host) Boiss.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Botrychium lunaria (L.) Sw.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Daphne cneorum L.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Dianthus microlepis Boiss.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.
Anthemis cretica L.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Scorzonera villosa Scop.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Pinus heldreichii H.Christ	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Pimpinella serbica (Vis.) Drude	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Trifolium velenovskyi Vandas	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Tephroseris papposa subsp. *wagneri* (Degen) B.Nord.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.

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Barbarea balcana Pančić	.	.	.	.	.	.	.	.	.	.	.	.	2	1	
Eriophorum latifolium Hoppe	.	.	.	.	.	.	.	.	.	.	.	.	1	2	.
Dactylorhiza cordigera subsp. *bosniaca* (Beck) Soó	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Luzula sylvatica (Huds.) Gaudin	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
***Gymnadenia odoratissima* (L.) Rich.**	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
Alchemilla viridiflora Rothm.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Athyrium filix-femina (L.) Roth	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Epilobium palustre L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	
Trifolium hybridum L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Filipendula ulmaria (L.) Maxim.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Daphne mezerum L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Stellaria alsine Grimm	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Doronicum austriacum Jacq.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
Veratrum lobelianum Bernh.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	
Viola gracilis Sm.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Thalictrum aquilegiifolium L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Ornithogalum gussonei Ten.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Veronica serpyllifolia L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Geranium macrorhizum L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Clinopodium acinos (L.) Kuntze	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
Cystopteris fragilis (L.) Bernh.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Saxifraga adscendens L.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.
Epilobium montanum L.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Rumex alpinus L.	.	.	.	.	.	.	.	.	.	.	.	.	+	2	
Veronica beccabunga L.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.

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Anacamptis coriophora (L.) R.M.Bateman & al.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Scorzoneroides autumnalis (L.) Moench	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Alchemilla velebitica (Janch.) Degen	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Epilobium angustifolium L.	.	.	.	.	.	.	.	.	.	.	.	.	.		4
Achillea atrata L.	.	.	.	.	.	.	.	.	.	.	.	.	.		1
Senecio squalidus subsp. *rupestris* (Waldst. & Kit.) Greuter	.	.	.	.	.	.	.	.	.	.	.	.	.		1
Fagus sylvatica L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Trifolium alpestre L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Achillea setacea Waldst. & Kit.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Pilosella officinarum Vaill.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Dianthus sylvestris Wulfen	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Campanula foliosa Ten.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Sedum ochroleucum Chaix	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Turritis glabra L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Campanula persicifolia L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Silene armeria L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Lathyrus pratensis L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Vicia sepium L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Galium debile Desv.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Linaria angustissima (Loisel.) Borbás	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Ligusticum mutellina (L.) Crantz	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Valeriana montana L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Stachys alopecuros (L.) Benth.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Galium mollugo L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Populus tremula L.	.	.	.	.	.	.	.	.	.	.	.	.	.		+
Betula pubescens Ehrh.	.	.	.	.	.	.	.	.	.	.	.	.	.		+

Appendix 2

List of localities based on studied herbarium specimens of *Gymnadenia conopsea* [G.c.], *Gymnadenia frivaldii* [G.f.], *Gymnadenia nigra* [G.n.] and *Gymnadenia odoratissima* [G.o.] from Kosovo.

No.	Locality	Source data
Ferizaj District		
1	Bistër	G.o. = Berisha, N. (2019), G.c. = Berisha, N. (2011), G.f. = Berisha, N. (2014), G.n. = Rexhepi, F. (2004)
2	Brezovicë	G.f. = Rexhepi, F. (1980 [P] / 1997 / 2001), Berisha, N. (2011), Millaku, F. (2012, 2014)
3	Jezerc	G.c. = Millaku, F. (2004)
4	Luboten	G.n. = Berisha, N. (2011, 2017), G.c. = Berisha, N. (2011), G.f. = Berisha, N. (2013, 2015, 2018), Rexhepi, F. (1983 [P])
5	Nerodime (E)	G.c. = Millaku, F. (2004)
6	Përrojet e Durlës	G.f. = Rexhepi, F. (2013), Millaku, F. (2013), Berisha, N. (2014)
7	Piribreg	G.f. = Rexhepi, F. (1998 [P], 2009)
8	Shtëpia e Malorëve	G.f. = Millaku, F. (2004), Berisha, N. (2014)
9	Tupan	G.c. = Berisha. N. (2011)
10	Vërtop	G.n. = Millaku, F. (2012)
Gjakova District		
11	Bishtazhin	G.c. = Rexhepi, F. (2007)
12	Bjeshka e Dobërdolit	G.c. = Millaku, F. (2006)
13	Bjeshka e Dobroshit	G.f. = Rexhepi, F. (2007)
14	Bjeshka e Junikut	G.f. = Millaku, F. (2013), G.c. = Millaku, F. (1991, 2007)
15	Bjeshka e Tropojës	G.f. = Millaku, F. (1988, 2013)
16	Gjeravicë	G.f. = Millaku, F. (2013), G.n. = Berisha, N. (2011)
17	Gryka e Lloqanit	G.f. = Berisha, N. (2014)
18	Junik	G.f. = Berisha, N. (2014)
19	Kurvallë	G.f. = Berisha, N. (2011)
20	Mirushë	G.c. = Millaku, F. (2009), Rexhepi, F. (1983 [P])
21	Morinë	G.c. = Millaku, F. (2007)
22	Pllaçicë e Vokshit	G.f. = Millaku, F. (2012)
Prizren District		
23	Bresanë	G.c. = Millaku, F. (2003), Berisha, N. (2009)
24	Brod	G.n. = Millaku, F. (2000)
25	Carralevë	G.c. = Rexhepi, F. (2004)
26	Divjakë	G.c. = Millaku, F. (2004)
27	Dragash	G.c. = Berisha, N. (2011)
28	Jazhincë	G.c. = Millaku, F. (2004), G.f. = Rexhepi, F. (2010), G.n. = Millaku, F. (2012)
29	Koritnik	G.c. = Rexhepi, F. (2002, 2009), G.n. = Berisha, N. (2012)
30	Liqenet e Durlës, Sharr	G.c. = Berisha, N. (2011), G.f. = Millaku, F. (2012)
31	Liqeni i Shutmanit, Brod	G.f. = Millaku, F. (2001)
32	Lubinjë e Ulët	G.c. = Millaku, F. (2004)
33	Maja e Zezë	G.n. = Berisha, N. (2012), G.f. = Millaku, F. (2009)
34	Oshlak, Malet e Sharrit	G.n. = Millaku, F. (2011)
35	Pashtrik	G.c. = Rexhepi, F. (2007 [P], 2010), G.n. = Rexhepi, F. (1999 [P] , 2001), Millaku, F. (2009), Berisha, N. (2011)
36	Planej	G.c. = Rexhepi, F. (1979, 2006)
37	Prevallë	G.f. = Millaku, F. (2007); G.c. = Berisha, N. (2012), G.n. = Millaku, F. (1998, 2012)
38	Rapçë	G.c. = Rexhepi, F. (1988 [P] ,1994, 2006)
39	Restelicë	G.f. = Millaku, F. (2011), G.c. = Millaku, F. (1999, 2003), G.n. = Berisha, N. (2011), Millaku, F. (2011)
40	Syrinika	G.c. = Millaku, F. (2005)
41	Vracë	G.f. = Millaku, F. (2010), G.n. = Berisha, N. (2011)
Peja District		
42	Bjeshka e Isniqit	G.n. = Berisha, N. (2017)
43	Bjeshka e Istogut	G.c. = Millaku, F. (1994, 2006)
44	Bjeshka e Lumbardhit	G.n. = Berisha, N. (2011, 2019)
45	Bjeshka e Sudenicës	G.c. = Millaku, F. (2000, 2006)
46	Bogë	G.c. = Millaku, F. (2004, 2008), G.n. = Berisha, N. (2012)
47	Hajlë	G.f. = Millaku, F. (2008); G.n. = Berisha, N. (2014)
48	Koprivnik	G.n. = Berisha, N. (2014)

No.	Locality	Source data
49	Kuqishtë	G.c. = Millaku, F. (2006, 2008, 2011); G.n. = Millaku, F. (2008),
50	Leqinat	G.c. = Millaku, F. (2006), Berisha, N. (2018), G.f. = Millaku, F. (1997), Berisha, N. (2015), G.n. = Berisha, N. (2015, 2018)
51	Lumbardh	G.c. = Millaku, F. (1989, 2001)
52	Maja e Rusolisë	G.f. = Berisha, N. (2010, 2015), G.n. = Millaku, F. (2012)
53	Mali Mokna	G.f. = Millaku, F. (2013), Berisha, N. (2015), G.c. = Berisha, N. (2012)
54	Marijash	G.n. = Millaku, F. (2009)
55	Peklen	G.n. = Berisha, N. (2019)
56	Qafë e Bogiçës	G.f. = Berisha, N. (2012, 2015)
57	Radavc	G.c. = Millaku, F. (2001)
58	Rekë e Allagës	G.c. = Rexhepi, F. (1997 [P]), Millaku, F. (2009)
59	Roshkodol	G.c. = Millaku, F. (2005)
60	Shtupeç	G.c. = Millaku, F. (2005)
61	Shushicë	G.c. = Millaku, F. (2001)
62	Zajm	G.c. = Millaku, F. (2010)
63	Zhleb	G.n. = Millaku, F. (2001)
Mitrovica District		
64	Bellobërdë	G.c. = Rexhepi, F. (2005)
65	Bërzancë	G.c. = Rexhepi, F. (1999 [P], 2004)
66	Çirez	G.c. = Millaku, F. (2007)
67	Çubrel	G.c. = Rexhepi, F. (2005)
68	Druar	G.c. = Rexhepi, F. (1981)
69	Kaçandoll	G.c. = Rexhepi, F. (1988 [P] , 2000); G.n. = Millaku, F. (2009)
70	Kopaonik	G.n. = Rexhepi, F. (1979 [P])
71	Oshtro Koplje	G.n. = Berisha, N. (2018)
72	Rakinicë	G.c. = Rexhepi, F. (1987 [P] , 2005)
73	Rashan	G.c. = Rexhepi, F. (1989)
74	Runik	G.c. = Rexhepi, F. (2000 [P], 2005)
75	Turiçec	G.c. = Rexhepi, F. (2005)
76	Vesekovc	G.c. = Millaku, F. (2009), Rexhepi, F. (1997 [P]), G.n. = Berisha, N. (2012)
Prishtina District		
77	Batllavë	G.c. = Rexhepi, F. (2001)
78	Blinajë	G.c. = Millaku, F. (2014)
79	Bradash	G.c. = Rexhepi, F. (2001)
80	Gërmi	G.c. = Rexhepi, F. (2000 [P], 2009), Berisha, N. (2017)
81	Golesh	G.c. = Berisha, N. (2016)
82	Koliç	G.c. = Millaku, F. (2000)
Gjilan District		
83	Busovatë	G.c. = Rexhepi, F. (1999)
84	Gmicë	G.c. = Millaku, F. (2004)
85	Novobërdë	G.c. = Rexhepi, F. (1977, 1982 [P], 2001)
86	Prallovë	G.c. = Rexhepi, F. (2004)
87	Qarrak	G.c. = Rexhepi, F. (2001)
88	Smirë	G.c. = Rexhepi, F. (2001)

Note: Private vouchers offered by prof. F. Rexhepi are indicated with a letter [P].

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RESEARCH ARTICLE

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Histochemical observations in *Piper malgassicum* (Piperaceae) with a special focus on the epidermis

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Abstract

This is the first contribution about the histochemistry of vegetative and reproductive aerial organs in the genus *Piper* L. *Piper malgassicum* accumulates alkaloids and terpenes in the epidermis and the underlying layers of parenchyma, both in the leaves, in the stems and in anthers. Some idioblasts appear to contain a large amount of secondary metabolites. The micro-anatomical analysis showed peculiar secretory structures both in the leaves, in the anthers and in the ovary. Several lipid aggregates, alkaloid droplets and calcium oxalate crystals were observed in leaves and stems, indicating their role in defence strategies, mechanical support, and pollinators attraction. In the anthers, we observed elaioplasts whose content suggest an alternative and indirect function in pollination and defence against micro-organisms. Besides, some lipid aggregates surrounded by microtubules, detected in the anthers, were recognized as lipotubuloids. The tapetum was of secretory type.

Alkaloids and terpenes were widely distributed in the plant confirming the important biological role of this type of biomolecules and its functional range. In the anthers, terpene and polyphenol inclusions appeared particularly abundant in the epidermal layer, whereas calcium oxalate crystals were observed close to the ovule in the ovary at maturity.

Keywords

anatomy, epidermis, histochemistry, *Piper*, *Piper malgassicum* , plant defence, secondary metabolites, terpenes

Introduction

The genus *Piper* L. belongs to the family Piperaceae and includes more than 2000 species with a pantropical distribution (Quijano-Abril et al. 2006), most of them from America (Ulloa Ulloa et al. 2017). Nowadays, the phylogenetic position of *Piper* as well as of the Piperaceae family, is among the so-called "paleoherbs", a phylogenetically complex basal group of dicots (Loconte and Stevenson 1991; Chase et al. 2016), within the order Piperales (Jaramillo et al. 2008; Palchetti et al. 2018). Piperales are herbaceous or woody plants exhibiting quite primitive morpho-anatomical features (Isnard et al. 2012) *Piper* species can be described either as shrubs, or more frequently, as creepers and lianas in the equatorial regions (Jaramillo et al. 2001). Many *Piper* species present two circles of vascular bundles in the stem, sometimes called polystelic organization, considered as a synapomorphy of the Piperaceae family, except for genus *Verhuellia* (Isnard et al. 2012). The main commercial species are *Piper nigrum* L. native to India, *P. methysticum* (L.) G. Forst. typical of West Polynesia, and *P. cubeba* L. a typical Indonesian species (Maugini et al. 2014). Due to their importance, many species are also cultivated beyond their native geographical region and, in some cases, have become naturalized (Smith et al. 2008). However, species belonging to this genus generally display an exclusive pantropical distribution. They present a high number of growth forms and biomechanical organizations (Isnard et al. 2012) with most species having a restricted area of distribution, while others are widespread (Marquis 2004; Quijano-Abril et al. 2006)

Many species of genus *Piper* have a high economic value all around the world and its trade has a long history, dating back to *ca*. 9,000 years ago. Magnoliids, including Piperaceae, are also characterized by the presence of aromatic compounds like terpenoid essential oils and other odorous volatile substances (Marinho et al. 2011) These substances are found in fruits, seeds, and leaves inside glandular pockets and trichomes, and confer the intense flavour and the characteristic aromatic fragrance in most species of the genus (Ballantini et al. 2018) These chemical components led many species of *Piper* to become an important resource from a commercial point of view. The genus is poorly represented in continental Africa. *P. guineense* Schum. & Thonn. and *P. capense* L. are the only two currently known native and endemic species. In Madagascar, the knowledge of the genus is still incomplete (Palchetti et al. 2018) According to Weil et al. (2017), the non-cultivated species of *Piper* present on the island are *P. heimii* C.DC. and *P. pachyphyllum* Baker, while the presence of *P. borbonense* is not confirmed, even though De Candolle (1923, 1869) assigned some samples from Madagascar (and Mauritius) to this species.

In this contribution, we investigated the vegetative structures and the localization of secondary metabolites of a recently described species of the genus *Piper* L. from Madagascar, *P. malgassicum* Papini, Palchetti, Gori & Rota Nodari (Palchetti et al. 2018, 2020), which is used for the production of local pepper named "voatsiperifery", probably mixed with *P. borbonense* (Miq.) C. DC. and *P. tsarasotrae* Papini, Palchetti, M. Gori & Rota Nodari. For this reason, these species are economically relevant.

Despite extensive knowledge about the secondary metabolites content and a few accounts about the histochemistry of the fruit and seed of *Piper*, particularly *P. nigrum*, there is very limited evidence about the presence of secondary metabolites in the leaves and in general in the epidermis of the organs in the same genus and no data, in general, about *P. malgassicum*. The aim of this study was to correlate anatomical and histochemical features of the epidermis and underlying tissues with secondary metabolite production and defence systems of *P. malgassicum*. We show the anatomical features of the epidermis in leaves, anthers, and ovary using light microscopy (LM) and histochemical techniques. Specific histochemical staining methods were used to localize different classes of secondary metabolites in the vegetative parts of the plants. Since the presence of secondary metabolites in the anthers has been documented rarely, we also used transmission electron microscopy (TEM) to check the local ultrastructural patterns linked to the route of formation of secondary metabolites in the organelles inside anther cell wall cells.

Materials and methods

Plant material

Plants grown from seeds of *P. malgassicum* obtained from Madagascar were grown in a greenhouse at the Department of Agriculture, Food, Environment and Forestry of the University of Florence (Italy) from March to July 2019 at approximately 25 °C during the day of 14 hours and 15 °C during the night of 10 hours under artificial light in a pot (15 cm diameter) containing universal soil plus garden soil (Vigorplant, Italy), without fertilizers; humidity was kept at 70%. The plants were later transferred to the Botanical Garden of Florence (Giardino dei Semplici) Another plant was grown starting from seed directly in the laboratory at room temperature and leaves and stem of the young plant were used for further analysis (vibratome sectioning) After about one month of growth, when stems, leaves, and flowers had reached a suitable size (i.e., 2 mm diameter for the stem, 5 cm length for the leaf, and 1 cm length for the flower) for the light and TEM analyses, sections of leaf, stem, and anthers were cut with a razor blade and a vibratome.

Herbarium samples were made with material directly collected in the field (Vohiday forest, Ambositra region, Madagascar) and conserved at the Tropical Herbarium of Florence, Italy (FT) They are syntypes of the type. For the images, we used sample PS9, Voidahi (Madagascar).

Light microscopy

Leaf and stem sections for LM were cut with a vibratome (Vibratome 1000 Plus) at a thickness of 40–50 μm. Some sections were stained with selective histochemical dyes and reagents for terpenoids and lipophilic substances using Sudan Black for lipid stain-

ing (Lison et al, 1960), NADI for lipids and terpenes (Carde et al. 1964), Sudan Red III-IV for the detection of neutral lipids (Lison et al. 1960), and Fluorol Yellow 088 (FY088) fluorescent staining for lipid detection (Bundrett et al. 1991)

Other sections were stained with selective dyes for non-lipophilic molecules, like polysaccharides, polyphenols, and alkaloids. Schiff's reagent and periodic acid- Schiff (PAS) staining were used for detecting polysaccharides (Jensen 1962), staining with $FeCl_3$ for polyphenols (Strasburger E. 1923) and Wagner's reagent for alkaloids (Wagner and Bladt 1996) Mechanical tissues and other physical support structures were revealed with calcofluor for cellulose staining (Hughes and McCully 1975), phloroglucinol for lignin marking (Johansen 1940), and toluidine blue as generic staining (Beccari and Mazzi 1966) The sections were observed through a Leitz DM-RB "Fluo" light/fluorescence microscope (Wetzler, Germany) equipped with a digital camera (Nikon DS-L1, Tokyo, Japan)

Transmission electron microscopy

Anther samples (about 2 mm long) were collected and fixed in 1.25% glutaraldehyde, at 4 °C, in 0.1 M phosphate buffer (pH 6.8) for 24 h. The samples were fixed in 1% OsO4 in the same buffer for 1 h. After dehydration in an ethanol series and a propylene oxide step, the samples were embedded in Spurr's epoxy resin (Spurr 1969) Cross-sections (about 70-nm thick) were cut with a diamond knife and a Reichert-Jung ULTRACUT ultra-microtome, and then stained with uranyl acetate (Gibbons and Grimstone 1960) and lead citrate (Reynolds 1963) The sections were then examined with a Philips 201 TEM at 80 kV. Semithin sections (1–5 μm), obtained using glass knives, were stained with toluidine blue 0.1%, observed, and photographed.

Results

Macroscopic morphology

The *P. malgassicum* herbarium sample showed simple elongated ovate leaves, while the inflorescences were cylinder-shaped, about 4 cm long. Infructescences were composed of many drupes carried on peduncles inserted orthogonally to the axis (Fig. 1 A, B)

Anatomical observations

Leaf anatomy

P. malgassicum leaves are hypostomatic with dorsoventral mesophyll (Fig. 2A) The upper epidermis is composed of an outer epidermal layer of mostly square cells and an underlying hypodermal layer consisting of rectangular-shaped cells (Fig. 2B) Below, the mesophyll parenchyma was organized in one layer of palisade parenchyma

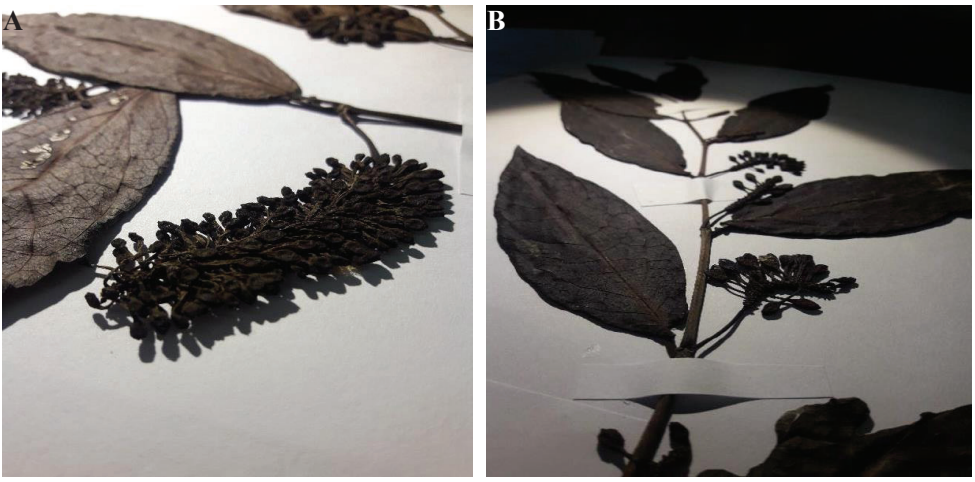

Figure 1. Herbarium specimen of *P. malgassicum* **A** inflorescence **B** leaves and infructescences (sample conserved at the Tropical Herbarium of Florence, University of Florence).

and two or three layers of spongy parenchyma. The tangentially elongated palisade cells were tightly packed and straight or slightly curved. Spongy parenchyma cells were nearly circular, loosely packed, with evident intercellular spaces (Fig. 2C) The abaxial hypodermis was composed of two regions: the epidermis and the hypodermal layer. Cells of the hypodermal layer were either rectangular or irregularly square near the stomata (Fig. 2D) The cells of the abaxial epidermis were either polygonal, circular, or squarish. Stomata (St) and glandular trichomes (Gt) were observed in this region (Fig. 2D)

LM images obtained with polarized light revealed calcium oxalate (CaOx) crystals in the leaf tissue against the dark background (Fig. 2E) CaOx deposits were detected within cells as well as embedded in or associated with the cell wall of midrib vascular bundles in the leaf lamina (Fig. 2F) Some cells of the lower hypodermis accumulated large amounts of CaOx crystals (Fig. 2G) The application of a birefringence filter confirmed that CaOx deposits were found associated with the vascular bundles in the midrib or inside the cells (Fig. 2H) CaOx accumulated in cells of the lower hypodermis immediately below the spongy tissue (Fig. 2I)

Autofluorescence of green leaves in blue light (450–490 nm) resulted in red fluorescence of the chloroplasts due to chlorophyll in all chlorenchyma cells (Fig. 3A, B), including the palisade and spongy mesophyll cells. As expected, red fluorescence was absent in the epidermis and hypodermis. Green fluorescence was attributed to the phenolic components in the cuticle of the upper (adaxial) and lower (abaxial) epidermis as well as in glandular trichomes (Fig. 3A, B) Accordingly, the cell walls of xylem elements in the mesophyll appeared green. Lipid droplets appeared as yellow fluorescence within chlorenchyma cells and in the intercellular spaces, both in the palisade and spongy mesophylls (Fig. 3B). Sudan Black staining also revealed the presence of lipids in the cuticle and inside the trichomes of the lower epidermis (Fig. 3C) Sudan Black

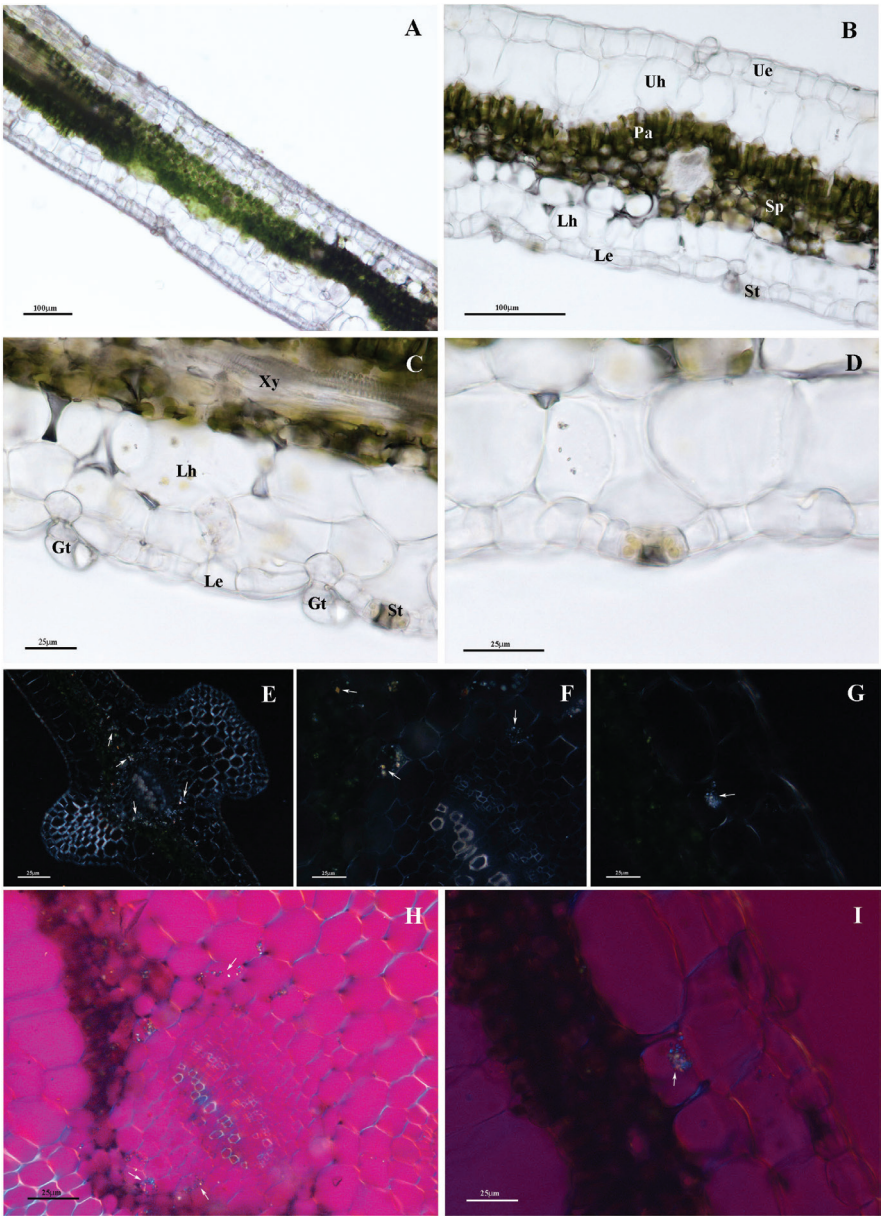

Figure 2. Cross-sections of *P. malgassicum* leaf, LM images **A–D** are cross-section of *P. malgassicum* leaf **F, G** LM observations with polarized light **H, I** LM observations using birefringence filter **A** portion of the leaf lamina **B** detail of image A **C** detail of image B **D** detail of C with stomata **E** portion of the leaf lamina. Presence of CaOx crystals (white arrows) within cells as well as embedded in vascular bundlecell walls **F** cross-section of the leaf through midrib. Abundance of CaOx crystals (white arrows) close to the bundles and embedded in the cell walls of xylemelements **G** presence of CaOx crystals (white arrows) in the lower hypodermis **H** portion of the lamina. CaOx crystals (white arrows) in the lower hypodermis **I** cross-section of the leaf through midrib showing abundant CaOx crystals (white arrow) close to the bundles and embedded in the cell walls of xylemelements. Xy: xylem, Gt: glandular trichomes; Ue: upper/adaxial epidermis, Uh: upper hypodermis, Pa: palisade tissue, Sp: spongy tissue, Lh: lower hypodermis, Le: lower/abaxial epidermis, St: stomata.

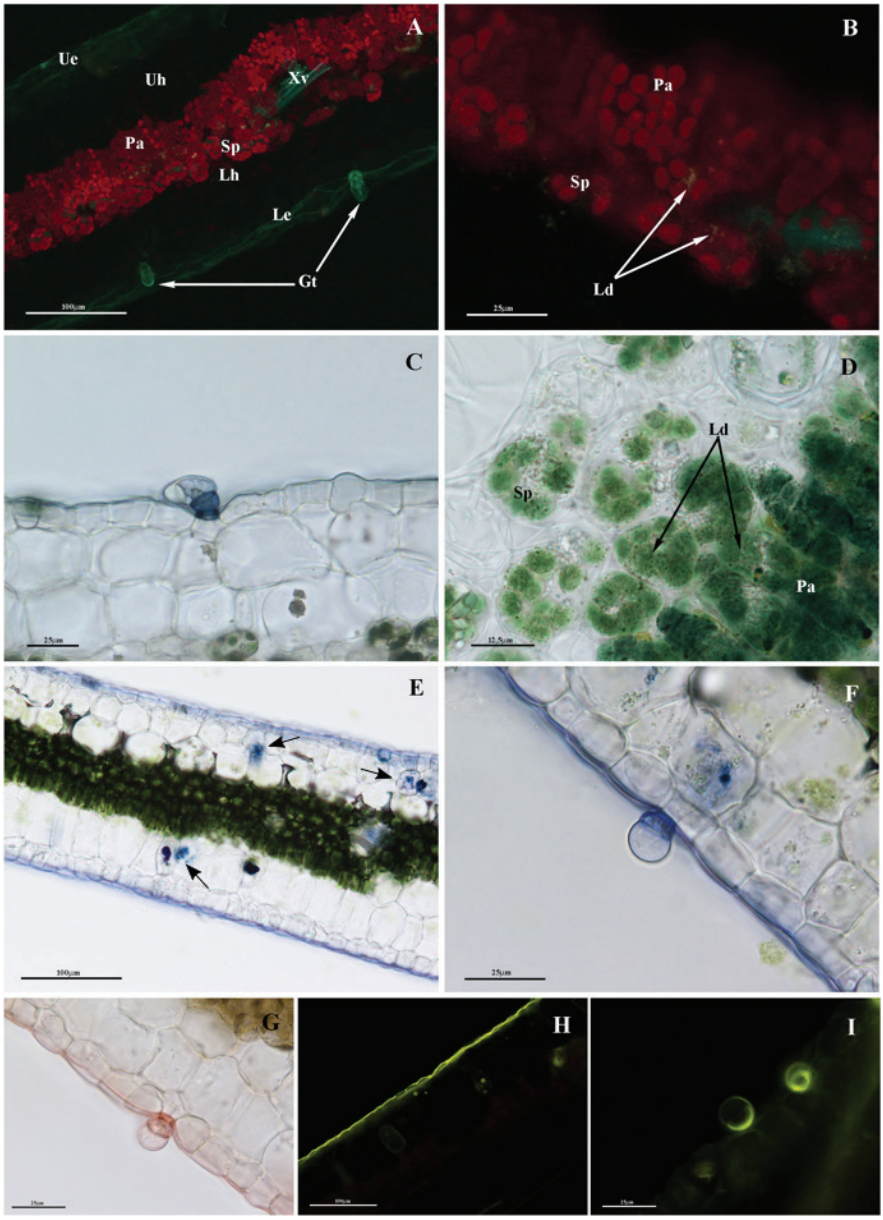

Figure 3. A portion of the leaf lamina in autofluorescence under blue-violet light showing the red fluorescence of chlorophyll and the red fluorescence of the trichome contents **B** detail of image A. Lipid droplets show green fluorescence **C** trichomes and cuticles appearing Sudan Black positive **D** sudan Black stained lipid droplets positively in the spaces between chlorenchyma cells, both in the spongy and the palisade parenchyma (black arrows) **E** leaf stained with NADI reaction. Positive cells can be observed in the mesophyll (arrows) **F** lower epidermis and trichome stained with NADI **G** LM images showing the trichome and cuticle slightly reddish while cutin and suberin resulted stainedbrownish with Sudan III–IV **H** Fluorescence images with Fluorol Yellow staining revealing the lipids in the cuticleand aggregates in the epidermis and mesophyll **I** detail of H): trichome stained with FY088. Ld: lipid droplet; Ue: upper/adaxial epidermis, Uh: upper hypodermis, Pa: palisade tissue, Sp: spongy tissue, Lh: lower hypodermis, Le: lower/abaxial epidermis, Gt: glandular trichomes.

positively stained lipid droplets also in the intercellular spaces of the mesophyll and within cells of both palisade and spongy tissue (Fig. 3D)

As shown in Fig. 3E, NADI staining revealed the presence of a thin NADI-positive layer in correspondence of the leaf cuticle and of aggregates distributed both in the epidermis and in the mesophyll where they were found close to the xylem vessels. NADI-positive trichomes could be observed mainly on the abaxial epidermis (Fig. 3F)

Sudan III-IV staining revealed the presence of neutral lipids in the leaf cuticle and in the glandular trichomes which appeared reddish (Fig. 3G) The suberin and cutin components resulted positively stained with Sudan III–IV, appearing brownish. Fluorol Yellow 088 stained the hydrophobic lipid layer (i.e., cuticle) as well as several lipid aggregates in the epidermis and mesophyll (Fig. 4H) Fluorol Yellow 088-positive material was present also in the glandular trichomes (Fig. 3I).

The PAS reaction revealed the presence of polysaccharide material in the abaxial epidermis at the level of the walls and in the glandular trichomes, which showed an intense pink colour (Fig. 4A, B) Leaf cross-sections stained with FeCl3 did not show any positive reaction, suggesting the absence of polyphenolic compounds (Fig. 4C) The Wagner reaction stained positively the epidermal cells (Fig. 4D, E) and some idioblasts in the upper hypodermis (Fig. 4F), suggesting the presence of alkaloids. A limited number of aggregates were also found in trichome basal cells (Fig. 4G)

Stem anatomy

Piper malgassicum stem showed an epidermis consisting of an outer epidermal layer and an inner hypodermal layer. The outer epidermal cells were tangentially elongated and covered with a thick cuticle (Fig. 5H) Compared to the outer epidermal cells, the hypodermal cells were prismatic and spherical. Beneath the hypodermis, the cortex formed a continuous ring. In the cortex, a 4–5 layered angular collenchyma was present (Fig. 4G) The vascular system showed isolated vascular bundles arranged in two concentric rings. Each ring of cortical bundles was formed, on average, by 7–9 vascular bundles. A smaller ring of 4–5 (on average) medullary bundles was embedded in the parenchyma of the central cylinder. The medullary bundles were larger than the cortical bundles. In general, bundles of both rings showed phloem and xylem vessels separated by fascicular cambium. Phloem cells were at the external periphery of the bundle. The central part of the central cylinder was formed by parenchyma cells with thin walls and an open space or lacuna (no pith cells) was often observed right in the middle (Fig. 4G) LM observation using a birefringence filter showed an abundance of CaOx crystals in the stem hypodermis (Fig. 5A) as well as in the leaf. In particular, CaOx accumulated abundantly in the hypodermis cells (Fig. 5B) Sudan III-IV positively stained the cuticle covering the external epidermis (Fig. 5C) and lipid droplets occurring in the lumen of the phloem within the vascular bundles (Fig. 5D) As shown in Fig. 5E, cellulose components were present in the wall of hypodermis cells. Cell walls reacting positively to calcofluor staining were observed in the cortex and the phloem of both cortical and medullar vascular bundles (Fig. 5F) Due to their lignified cell walls, xylem vessels, stained positive to phloroglucinol, but were negative to calcofluor staining (Fig. 5G, H)

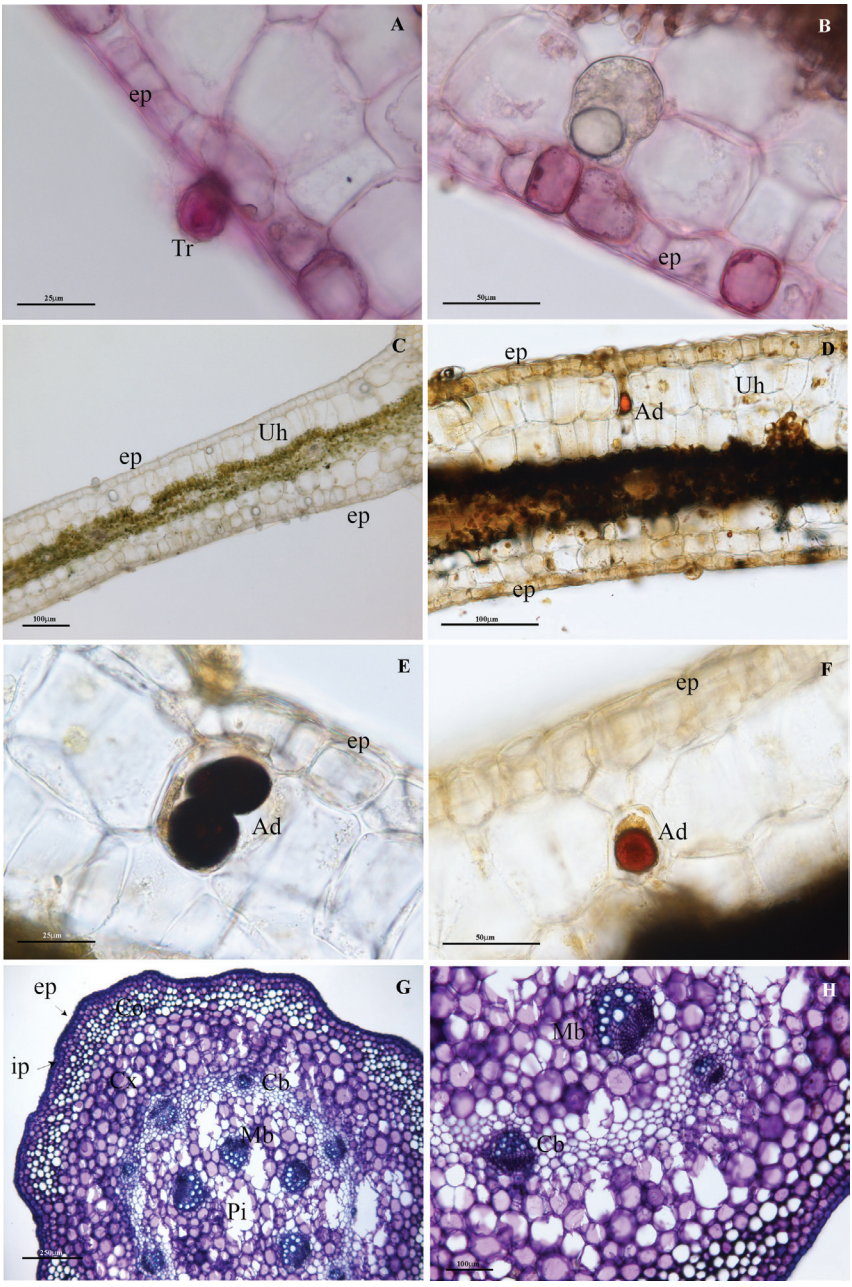

Figure 4. LM images of the cross-sections of *P. malgassicum* leaf (**A–F**) and stem (**G, H**) **A** PAS positivity of trichomes and epidermis cells **B** PAS positivity of the epidermal cells and terpenic droplet below the first layer of cells **C** staining with FeCl3 **D** leaf lamina positive to Wagner staining **E** detail of D showing alkaloid droplets in the hypodermis cell **F** detail of D showing alkaloid droplets in hypodermis idioblasts **G** stem stained with Toluidine blue showing the thick layer of collenchyma beneath the epidermis and the two concentric circles of vascular bundles **H** detail of G showing the wall thickenings of the angular collenchyma. Ad: Alkaloid droplet; Cb: cortical circle of vascular bundles; Co: angular collenchyma; cx: cortex; ep: epidermis; hp: hypodermis; Mb: medullary circle of vascular bundles; Pi: pith; Td: terpenic droplet; Tr: trichome; Uh: upper hypodermis.

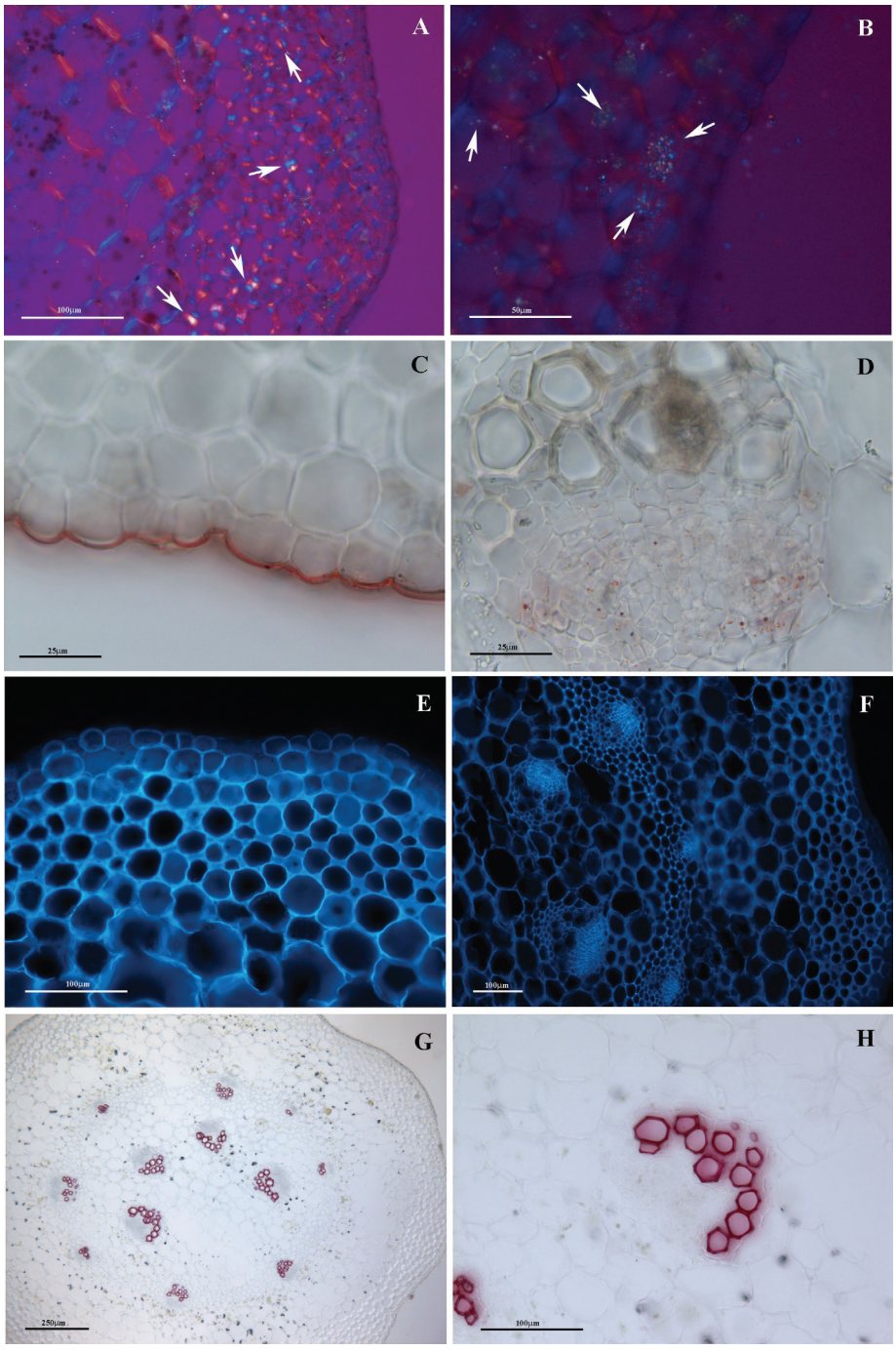

Figure 5. Stem cross-sections **A** CaOx (white arrows) in the hypodermis with birefringence filter **B** detail of A **C** sudan III–IV positive cuticle positive **D** sudan III–IV positive lipid droplets in the primary phloem **E** calcofluor positive (blue fluorescence) hypodermis cell walls **F** cortex and phloem cells walls staining positively withcalcofluor (blue fluorescence) **G** xylem vessels of both cortical and medullary bundles resulted positive to phloroglucinol **H** Detail of G.

Anther anatomy

Piper malgassicum anthers were composed of two locules, joined together by connective tissue. The pollen grains were surrounded by the tapetum and further three cell layers, the epidermis being the most external one (Fig. 6A, B) The anther wall consisted of 2–3 layers of cells, later dehiscing at maturity (Fig. 6A) The tapetum was formed by a monolayered ring of cells, even though, in some points, two cells could be observed (Fig. 6B) Two layers of cells, the middle layer inside and the endothecium outside, just beneath the epidermis, separated the tapetum from the epidermis (Fig. 6B)

The microspores in the locule were surrounded by the tapetum, some of whose cells had a higher electron density than the others (Fig. 6C) Small electron-dense particles (orbicles) were lined along the plasmalemma of tapetal cells, while some larger masses of the same electron density as the orbicles could be observed in the locule and on the microspores (Fig. 6C) Along the anther filaments some trichomes were present. Their cytoplasm contained some plastids with large electron-dense bodies (Fig. 6D) Starch grains (black arrows) were also observed inside these plastids (Fig. 6E) In the epidermis electron-dense bodies were observed in the vacuoles, while some plastids with starch grains were present in the cytoplasm (Fig. 6F) Large medium electron-dense granular bodies could be found between the vacuole and the external plasma membrane (Fig. 6F) The electron-dense masses in the vacuoles occurred as simple bodies (Fig. 6F), or could be surrounded by a more electron transparent crown (Fig. 6G) In some cells, the electron-dense bodies occupied most of the vacuole (Fig. 6H), while some plastids appeared to host bodies of lower electron density as compared with those of the vacuole (Fig. 6H) The electron-dense bodies surrounded by a less electron-dense crown apparently entered the tonoplast coming from large cisternae in the cytoplasm surrounding the vacuole (Fig. 6G) The medium electron-dense bodies appeared to be surrounded by a membrane inside some plastids that were very often observed close to mitochondria (Fig. 7)

The female inflorescence of *P. malgassicum* is a spike; in our sample, it was 3–8 cm long, with a 1–2 cm long peduncle bearing small sessile flowers on a thin axis. The fleshy ovary was surmounted by a two-branched stigma and contained a single ovule (Fig. 8A) Some resin ducts were observed in the ovary while the stigmas showed papillae in the distal portion (Figure 8B) Ovary cells located in proximity of the ovule showed CaOx crystal deposits (Fig. 8C) The spike axis showed two concentric circles of vascular bundles, the external ones were smaller in comparison to the most internal bundles and a lacuna was observed at the centre (Fig. 8D) The phloem elements contained material that stained positive with toluidine blue (Fig. 8E)

Discussion

The observations of *P. malgassicum* herbarium samples showed the typical morphological features of the species and its differences in comparison to the other phylogenetically related species, such as *P. tsarasotrae* and *P guineense*. *P. malgassicum* leaves were

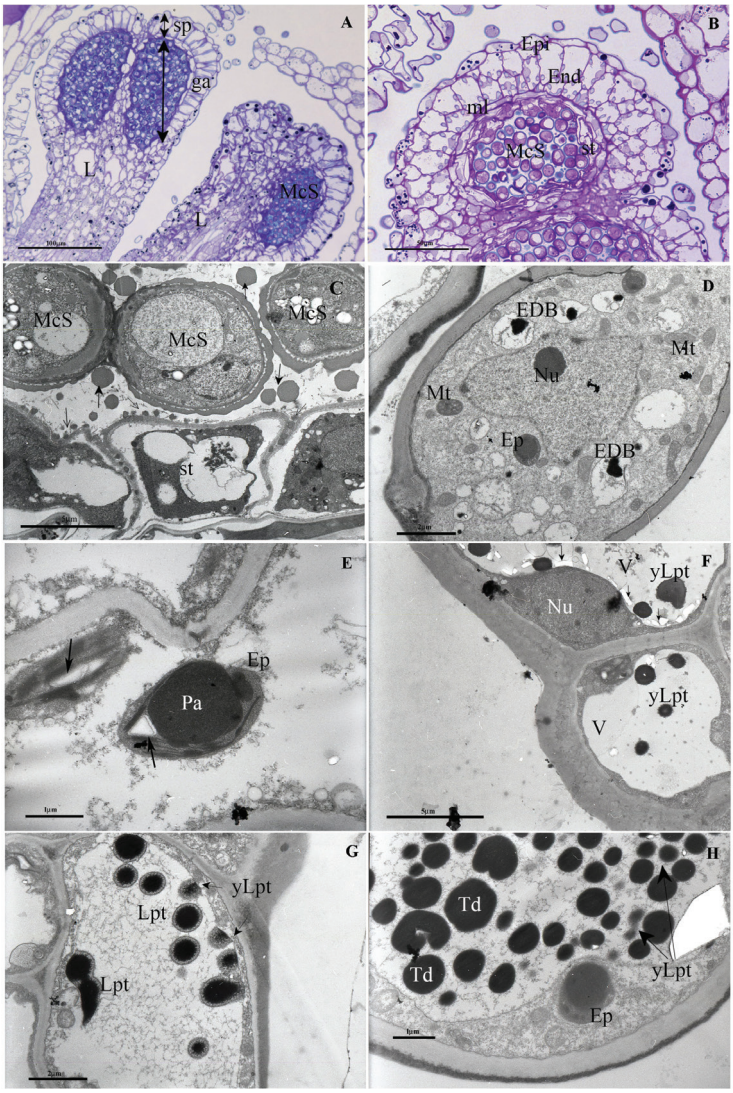

Figure 6. *P. malgassicum* anther **A** Semithin section stained with toluidine blue showing the shape and structure of the anther. Anther wall (here already dehiscent) formed by 2–3 layers of cells **B** semithin section showing the microspores surrounded by the tapetum **C** microspores are in the locule surrounded by the tapetum. Small electron-dense particles (orbicles) are lined along the tapetal cells plasma membrane, while some larger masses can be observed in the locule (black arrow) **D** anther trichome. Some plastids contain large electron-dense bodies **E** detail of D The large electron-dense bodies inside the plastid occur together with starch grains **F** anther epidermis: electron-dense bodies are present in the vacuoles. Some plastids are present with stored starch. Large medium electron dense granular bodies can be found between the vacuole and the external plasma membrane **G** in a preliminary stage, the electron-dense masses in the vacuole are surrounded by a more electron transparent layer **H** at a successive stage, the electron-dense bodies occupy a large part of the vacuolar volume. Ga: microspores/male gametophyte; L: locule; sp: tapetum/sporophytic tissue; ep: epidermis; end: endothecium, ml: middle layer; st: secretory tapetum; Mcs: microspore; EDB: electron-dense bodies; Mt: mitochondria; Pa: protein aggregate; Ep: elaioplast; yLPT: young lipotubule: Lpt: lipotubule; V: vacuole; Td: terpenic droplets.

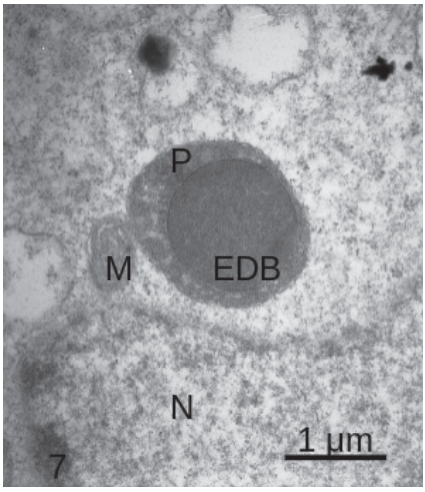

Figure 7. Epidermis cell of anther. A medium electron-dense bodies formed inside a plastid is surrounded by in a membrane and a mitochondrion is very close to the external plastid membrane. EDB: electron-dense Body; M: mitochondrion; N: nucleus; P: plastid.

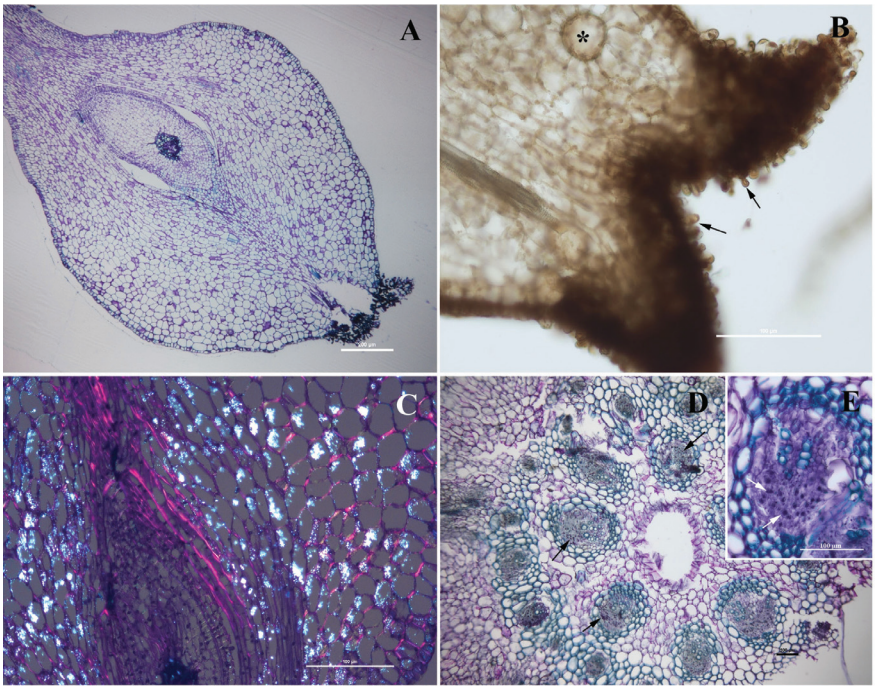

Figure 8. LM image of the longitudinal section of female inflorescence stained with toluidine blue **A** the ovary contains a single ovule and is surmounted by a 2-branched stigma **B** resin ducts as that in the image (asterisk) could be observed in the fleshy ovary. The stigmas were covered with papillae (arrows) **C** CaOx crystals deposits were present in the ovary at maturity **D** female inflorescence spike axis, cross-section. Two concentric circles of vascular bundles, with a lacuna in the middle **E** vascular bundle. Toluidine blue positive material in the phloem.

hypostomatic as in other *Piper* species, such as *P. aduncum* Vell., *P. cernuum* Vell., *P. dilatatum* Rich, *P. gaudichaudianum* Kunth, *P. glabratum* Kunth, *P. lindbergii* C. DC., *P. solmsianum* C. DC., and *P. umbellatum* Jacq. (Gogosz et al. 2012; Raman et al. 2012; Silva et al. 2017; Santos et al. 2018) However, amphihypostomatic leaves were found in *P. sarmentosum* Roxb. (Raman et al. 2012) and amphistomatic in *P. hispidinervum* C. DC (Gogosz et al. 2012) Concerning the anatomical aspects of the leaf, the microscopical analysis revealed how trichomes were not the prevailing secretory structures, but other specialized cells in the hypodermis, called idioblasts, had this function. These cells accumulated lipid-terpenoid aggregates or alkaloids. The general function of these substances and their anatomical localization lead to the hypothesis that their function in *P. malgassicum* is related to the limitation of infections by fungi and bacteria thanks to terpenoids and to the anti-herbivore activity of alkaloids. Most authors studying the genus *Piper* from a phytochemical point of view focussed their attention on the fruit, since it is the most relevant part of the plant for economical purposes. Our observations on the leaves are in agreement with other authors who showed these phytochemical features in other species of the *Piper* genus (Marinho et al. 2011; Pires Jacinto et al. 2018; Silva et al. 2017; Bertocco et al. 2017) From a phytochemical point of view, *P. malgassicum* is closely related to *P. nigrum* (Palchetti et al. 2018, 2020) and used traditionally in the same way as a spice.

Regarding the stem, *P. malgassicum* showed the typical features of Piperaceae family anatomy according to Isnard et al. (2012), but some peculiarities of this species were revealed. The stem showed an angular collenchyma under the epidermal layer organized in smaller areas as compared with other closely-related species of this genus. Probably, the low quantity of collenchyma was due to the young age of the observed *P. malgassicum* stem, which was sectioned after one month of growth from seed. In comparison to *P. malgassicum*, *P. tsarasotrae*, and *P. guineense* showed a higher number of cortical and medullary bundles (Ilodibia et al. 2016; Palchetti et al. 2018) Like *P. tsarasotrae and P. guineense*, *P. malgassicum* has larger medullary bundles than cortical vascular ones. Interestingly, *P. malgassicum*, like *P. guineense* and *P. tsarasotrae*, did not exhibit the mucilage canals observed in other *Piper* species (Ilodibia et al. 2016; Palchetti et al. 2018)

A particular anatomical aspect of *P. malgassicum* was the abundance of CaOx crystals both in the leaves and in the stem. In leaves, these aggregates prevail close to the central rib and in the cells of the spongy mesophyll, while in the stem they were found close to the hypodermis. They were abundant also inside the ovary. Such crystals were also observed in the distantly related *P. callosum* (Silva et al. 2017) and represent a defence against herbivores due to their hardness and spikiness. Moreover, they can provide further mechanical support for the plant (Maugini et al. 2014)

The anthers of *P. malgassicum* displayed the typical characteristics of Magnoliids, in particular, those of the order Piperales (Funes and Randall 2001) In accord with previous studies on others species of *Piper* (Schnarf 1931; Funes and Randall 2001; Valentin-Silva et al. 2015), the anthers of *P. malgassicum* have a secretory tapetum surrounding the microspores. Some of the tapetal cells showed signs of cytoplasmic and

nuclear condensation already at the stage in which microspores are free in the locule. This is evidence of programmed cell death (Brighigna et al. 2006; Papini 2018) and in *P. malgassicum* it appears to occur earlier than in most other angiosperms, where it occurs normally after the first pollen mitotic division (Papini et al. 1999; Varnier et al. 2005; Milocani et al. 2006) LM observations revealed dark globules in the anther epidermis. These organelles with a circular shape could be classified as elaioplasts. The presence of elaioplasts and lipid droplets in anthers is well known (Wu et al. 1999; Suzuki et al. 2013; Quilichini et al. 2014), but mainly within tapetum cells, while in this case they were located in the epidermis. Furthermore, not all the observed dark globules seem to have the typical elaioplast structure. Some of them showed a shape referable to another kind of organelle called lipotubules, according to Kwiatkowska et al. (2012)

The histochemical localization of terpenoids and alkaloids in the leaf epidermis was checked by TEM. Results suggest that the terpenoids accumulated in this tissue involved the activity of plastids, whose electron-dense contents appeared to occupy most of the plastid volume. The tentative identification of the lipid droplets as terpene-containing structures was done on the basis of images from published reports, such as Gersbach (2002, particularly Fig. 7B) and Fahn (1988) The spatial association between plastids and mitochondria may be linked to the functional connection between mitochondrial production of isoprenoids and plastids for the synthesis of terpenoids, namely the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway observed by Ahn and Pai (2008) in *Nicotiana benthamiana* plastids.

Wagner positivity indicated the presence of alkaloids that can be correlated with the TEM images showing electron-dense bodies found in the vacuole. We could exclude the possible polyphenolic nature of the bodies thanks to the negativity to FeCl3 staining. The images showed that the alkaloids enter into the vacuoles apparently from membrane cisternae probably produced by the endoplasmic reticulum, first with an electron-dense core and a less electron-dense crown. Other images of the vacuole show almost completely electron-dense bodies that are possibly to be referred to a later stage of alkaloids accumulation . Electron-dense precipitates in the vacuoles were interpreted as alkaloids also in *Cataranthus roseus* by Neumann et al (1983), even if in this case the electron-dense bodies were more irregular in shape. Also Ferreira et al. (1998) observed electron-dense bodies in the vavuoles of *Erythroxylum* leaf, interpreting the images (particularly fig. 7 in Ferreira et al. 1998) as accumulation of alkaloids around a more osmiophilic phenolic core. This interpretation may explain the more electron-dense core of the roundish bodies observed in the vacuol of P. malgassicum (our Figure 6G)

The observed NADI positivity could be linked to the production of medium electron-dense material in the plastids. We observed first organelles still containing starch grains (hence confirming their plastidial identity) together with electron-dense masses and, in other cells, possibly at a more advanced stage of development, the enlargement of the electron-dense bodies apparently formed from lipids derived from the thylakoids. Overall, the analysis of *P. malgassicum* has highlighted the presence of peculiar secretory structures in the leaf, thus confirming the aromatic nature of the species and high specialization in the biosynthesis of terpenic substances. However, the abundant

accumulation of lipid globules, alkaloids, and CaOx crystals also points to particular bio-functional strategies of defence, support, and attraction of mutualistic organisms.

In conclusion, our results show that *P. malgassicum* produces secondary metabolites (terpenoids and alkaloids) with defence functions, particularly in the leaf epidermal cells (including trichomes) and in hypodermal idioblasts. This is the first report on the histochemistry of *P. malgassicum* and the first one on the histochemistry of the epidermis in this genus.

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Notulae to the Italian flora of algae, bryophytes, fungi and lichens: 12

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Abstract

In this contribution, new data concerning bryophytes, fungi and lichens of the Italian flora are presented. It includes new records, confirmations or exclusions for the bryophyte genera *Acaulon*, *Campylopus*, *Entosthodon*, *Homomallium*, *Pseudohygrohypnum*, and *Thuidium*, the fungal genera *Entoloma*, *Cortinarius*, *Mycenella*, *Oxyporus*, and *Psathyrella* and the lichen genera *Anaptychia*, *Athallia*, *Baeomyces*, *Baglietoa*, *Calicium*, *Nephroma*, *Pectenia*, *Phaeophyscia*, *Polyblastia*, *Protoparmeliopsis*, *Pyrenula*, *Ramalina*, and *Sanguineodiscus*.

Keywords

Ascomycota, Basidiomycota, Bryidae

How to contribute

The text of the records should be submitted electronically to: Cecilia Totti (c.totti@univpm.it) for algae, Marta Puglisi (mpuglisi@unict.it) for bryophytes, Alfredo Vizzini (alfredo.vizzini@unito.it) for fungi, Sonia Ravera (sonia.ravera@unipa.it) for lichens. Each text should be within 1,000 characters (spaces included).

Floristic records

Bryophytes

Acaulon fontiquerianum Casas & Sérgio (Pottiaceae)

+ **TOS**: Parco Archeologico di Baratti e Populonia (Livorno), on sandy paths, roadsides, vineyards and arable fields near the coast, generally with a high alternation between dry and moist conditions during winter months (UTM WGS84: 32T 623678.4761494), 5 m, 20 January 2021, leg. *G. Pandeli* det. *G. Pandeli, I. Bonini* (SIENA). – Species new for the flora of Toscana.

Acaulon fontiquerianum is a gregarious and ephemeral submediteranean-suboceanic species, included as Endangered in the Red List of the Italian Flora (Rossi et al. 2013). It is quite common in the Mediterranean, and in Italy is reported only for Sicilia (Lo Giudice 1995) and Sardegna (Cogoni and Scrugli 2000), where it is considered rare. In Toscana, at the coastal site of Baratti, *A. fontiquerianum* oc-

cupies a large area of the surrounding arable fields, vineyards and paths near the pinewood, frequently accompanied by *Entosthodon fascicularis* (Hedw.) Müll.Hal., and *Riccia sorocarpa* Bisch.

G. Pandeli, I. Bonini

***Campylopus introflexus* (Hedw.) Brid. (Ditricaceae)**

+ **CAL**: Torre di Mezzapraia, Curinga (Catanzaro), on sandy dunes (UTM WGS 84: 33S 605854.4297732), 5 m, 6 January 2021, *D. Puntillo, M. Puntillo* (CLU No. 4330). – Invasive alien species new for the flora of Calabria.

Campylopus introflexus is native to the southern hemisphere, where it is known from South America, southern Africa, and southern Australasia (Hassal and Söderström 2005). In Europe, it was first discovered in the southern part of Great Britain in 1941 (Richards 1963) and in Italy in 1956 in Campania (Reimers 1956). The most recent Italian report is from Sicilia (Ellis et al. 2017). The species is recognizable for the lanceolate leaves showing a hyaline hair tip, which is 90° reflexed in dry condition (Frahm and Stech 2006), and for the capsule carried by a strongly down-curved seta.

D. Puntillo, M. Puntillo

***Entosthodon hungaricus* (Boros) Loeske (Funariaceae)**

+ **SAR**: Giara Park, Pauli Maiori, Genoni (Sud Sardegna), on soil on the borders of a temporary pond (UTM WGS84 32S 492737.4403123), 500 m, 8 March 2002, *S. Poponessi, A. Cogoni* (CAG SA2.2.2a); Giara Park, Pauli Maiori, Tuili (Sud Sardegna), on soil on the borders of a temporary pond (UTM WGS84 32S 496512.4399313), 500 m, 22 May 2002, *S. Poponessi, A. Cogoni* (CAG SA2.2.2.b). – Species new for the flora of Sardegna.

Entosthodon hungaricus was described as *Funaria hungarica* Boros and long considered a European endemic species, until, later on, it was also found in Israel, Kazakhstan, and Kirgizia (Cano et al. 1999). Because of the synonymization of *E. maroccanum* (Meyl.) Hebr. & Lo Giudice with this species, it is currently recorded also for Morocco and Sicilia (Pisarenko et al. 2001). In Europe, this species shows a peculiar distribution, ranging from the Mediterranean basin to the eastern Europe, similarly to some other xerothermic species. In Italy, this species is reported only from Sicilia (Aleffi et al. 2020).

S. Poponessi, A. De Agostini, A. Cogoni

***Homomallium incurvatum* (Schrad. ex Brid.) Loeske (Pylaisiaceae)**

+ **LIG**: Special Area of Conservation "IT1314610 Monte Saccarello – Monte Frontè", Rio Belvedere, Triora (Imperia), in riparian woods of *Alnus glutinosa* (L.) Gaertn. (UTM WGS84: 32T 400153.4876025), 940 m, 23 June 2020, *I. Briozzo, D. Dagnino* (GE B241). – Species new for the flora of Liguria.

Homomallium incurvatum is quite common; it occurs in several Mediterranean countries (Ros et al. 2013) and in most of the northern Italian administrative regions (Aleffi et al. 2020). We found this species on shady rocks within two moist riparian woods, characterized by black alder, in the ambit of the priority Habitat 91E0* "Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)", during the surveys for the Interreg ALCOTRA CoBiodiv and GeBiodiv projects.

D. Dagnino, M. Mariotti, C. Turcato

***Pseudohygrohypnum eugyrium* (Schimp.) Kanda (Pylaisiaceae)**

+ **TAA**: Along the Rio Maftina near Tione (Provincia autonoma di Trento), on a wet siliceous boulder close to the water of the stream in the woods (UTM WGS84: 32T 632918.5100377), 655 m, 25 May 2021, *F. Prosser* (Herb. Prosser No. 05252). – Species new for the flora of Trentino-Alto Adige.

Pseudohygrohypnum eugyrium was reported in Italy by Aleffi et al. (2020) only for Lombardia on the basis of a collection by G. Brusa from Lake Monate (Varese). In Europe, it is not common and is considered Near Threatened (Hodgetts et al. 2019). In the same habitat, *Dichodontium pellucidum* (Hedw.) Schimp., *Homalia trichomanoides* (Hedw.) Brid., and *Thamnobryum alopecurum* (Hedw.) Gangulee were also observed. This species was identified by comparison with the related *P. subeugyrium* (Renauld & Cardot) Ignatov & Ignatova, a recently reconsidered species (Blockeel et al. 2019).

F. Prosser

***Thuidium assimile* (Mitt.) A.Jaeger (Thuidiaceae)**

+ **LIG**: Special Area of Conservation "IT1314610 Monte Saccarello – Monte Frontè", Rio Belvedere, Triora (Imperia), chestnut forest (UTM WGS84: 32T 400506.4874932), 850 m, 23 June 2020, *I. Briozzo, D. Dagnino* (GE B321). – Species new for the flora of Liguria.

Thuidium assimile is a temperate species occurring in several Mediterranean countries (Ros et al. 2013) and in almost all the northern Italian administrative regions (Aleffi et al. 2020). It was found in a mesophilus montane chestnut forest referred to Habitat 9260 "*Castanea sativa* woods" of the 92/43/CEE Habitat Directive. Several other bryophytes were found in the same site, such as *Fissidens taxifolius* Hedw., *Plagiomnium undulatum* (Hedw.) T.J.Kop. var. *undulatum*, *Hypnum cupressiforme* Hedw. var. *cupressiforme*, *Brachythecium rutabulum* (Hedw.) Schimp. var. *rutabulum*, *B. salebrosum* (Hoffm. ex F.Weber & D.Mohr) Schimp., *Atrichum undulatum* (Hedw.) P.Beauv., and *Plagiochila porelloides* (Torr. ex Nees) Lindenb. var. *porelloides*.

D. Dagnino, G. Berta, C. Turcato

Fungi

***Entoloma rhombisporum* (Kühner & Boursier) E.Horak (Entolomataceae)**

+ **LAZ**: Accumoli (Rieti), at the edge of a mixed broad-leaved wood, on slightly acidic soil (UTM WGS84: 33T 359625.4730042), 1274 m, 10 September 1995, *M. Clericuzio* (Herb. GDOR 5092). – Species new for the flora of Lazio.
+ **TOS**: Sorano (Grosseto), in ravine vegetation, mixed broad-leaved wood, on neutral to slightly acidic soil (UTM WGS84: 33T 723444.4729709), 418 m, 10 October 2013, *M. Clericuzio* (Herb. GDOR 5093). – Species new for the flora of Toscana.
+ **LIG**: Piani di Invrea, Varazze (Savona), under *Juniperus communis* L., *Pinus halepensis* Mill., *Quercus ilex* L., and *Cistus albidus* L. (UTM WGS84: 32T 469833.4913347), elev. 66 m, 3 December 2014, *F. Boccardo* (Herb. GDOR 3546). – Species new for the flora of Liguria.

This species is characterized by its cuboid spores and lageniform cheilocystidia. Based on a morphological analysis, *E. rhombisporum* was placed in *E.* subg. *Inocephalus* Noordel., sect. *Staurospora* (Largent & Thiers) Noordel. (Noordeloos 2004). A recent phylogenetic analysis based on molecular data (He et al. 2019) places the species of the subg. *Inocephalus* with cuboid spores in a clade of its own. In Europe, *E. rhombisporum* is one of the few species provided with cuboid spores. *Entoloma rhombisporum* is a rare species, albeit reported from several European countries, e.g., Portugal, Sweden, Germany, Norway, The Netherlands, Denmark, Ireland, United Kingdom (Wales), and Slovenia (GBIF.org 2021).

M. Clericuzio, F. Boccardo, F. Dovana

***Cortinarius confirmatus* Rob.Henry (Cortinariaceae)**

+ **CAL**: Botanical Garden of the University of Calabria, Rende (Cosenza), on the ground between the edge of a riparian wood, *Populus canescens* (Aiton) Sm. as prevailing species, and a deciduous oak stand, mainly *Quercus pubescens* Willd. (UTM WGS84: 33S 605955.4357351), 200 m, 18 November 2020, *G. Sicoli, A.B. De Giuseppe, N.G. Passalacqua* (CLU No. F314). – Species new for the flora of Calabria.

A group of cespitose, medium-sized and agaricoid basidiomata referable to *C.* subg. *Telamonia* (Fr.) Wünsche (due to the dry and dull-coloured pilei) was detected in a mixed broadleaved coppice stand and identified as *Cortinarius confirmatus*, a fungus known as ectomycorrhizal in association with *Quercus* sp. pl. in the Mediterranean thermophilic area and with *Populus alba* L. along riparian woods in the continental zone. The spores, produced in a hymenophore supported by a naked silky stipe, were ovoid to ellipsoid and 7–9 × 4–5 µm in size (Liimatainen et al. 2017). In Italy, the few reports of this species are from the northern regions and Sicilia, where it has probably been described as *C. saturninus* var. *bresadolae* M.M.Moser (Moser 1980; Onofri et al. 2013).

G. Sicoli, A.B. De Giuseppe, N.G. Passalacqua

***Mycenella salicina* (Velen.) Singer (Agaricales, incertae sedis)**

+ **CAL**: Botanical Garden of the University of Calabria, Rende (Cosenza), on moss-covered woody debris in a deciduous forest (*Quercus* sp. pl. as prevailing tree species) (UTM WGS84: 33S 605954.4357296), 205 m, 2 December 2020, *N.G. Passalacqua A.B. De Giuseppe, G. Sicoli* (CLU No. F315). – Species new for the flora of Calabria.

A couple of mycenoid basidiomata belonging to *Mycenella salicina* were separately observed and identified on the ground, among woody residuals covered by moss in a clearing of a mixed broadleaved coppice stand mainly composed of *Q. cerris* L. and *Q. pubescens* Willd. The grey-brown and 2-cm-diameter campanulate and striate pilei were supported by a slender, pale, more brownish lower stipe. The spores are ventricose, whitish and almost-free gills produced sub-globose and distinctly smooth, thus differing from the verrucose spores detectable in the other species of the same genus (Knudsen 1992; Cortecuisse and Duhem 1995). *Mycenella salicina* was so far only reported from northern Italy (Onofri et al. 2013).

G. Sicoli, A.B. De Giuseppe, N.G. Passalacqua

***Oxyporus latemarginatus* (Durieu & Mont.) Donk (Oxyporaceae)**

+ **CAL**: Bosco di Mavigliano, Montalto Uffugo (Cosenza), on woody residuals laying on the ground at the edge of a deciduous woodland (UTM WGS84: 33S 604782.4360104), 205 m, 24 October 2020, *D. Puntillo, G. Maiorca, G. Sicoli* (CLU No. F414). – Species new for the flora of Calabria.

Resupinate whitish basidiomata of *Oxyporus latemarginatus* were detected and identified based on their macro-morphology and on the hyaline, inamyloid, ovoid-ellipsoid and smooth basidiospores (Stalpers 1978). Identical basidiomata had been observed among broadleaved wood debris in a courtyard of a building in the urban area of the municipality of Rende (Cosenza) in the autumn of 2019 (G. Maiorca, pers. comm.). This polyporaceous and wood-inhabiting fungus is widespread in the boreal hemisphere, mainly on deciduous trees, as a saprotroph or a weak pathogen causing white rot in the heartwood (Bernicchia 1990). Reports of *O. latemarginatus* cover almost all administrative regions in northern Italy and part of central Italy, but apparently not southern Italy, except Campania and Puglia (Sicoli et al. 2004, Onofri et al. 2013).

D. Puntillo, G. Maiorca, G. Sicoli

***Psathyrella corrugis* (Pers.) Konrad & Maubl. (Psathyrellaceae)**

+ **CAL**: Botanical Garden of the University of Calabria, Rende (Cosenza), on an old sleeper in the vicinity of a deciduous oak stand (*Quercus* sp. pl. as prevailing tree species) (UTM WGS84: 33S 605922.4357156), 215 m, 27 November 2019, *A.B. De Giuseppe, G. Sicoli, N.G. Passalacqua* (CLU No. F316). – Species new for the flora of Calabria.

Psathyrelloid basidiomata belonging to *Psathyrella corrugis* were observed sprouting from a sleeper obtained from *Quercus cerris* L. The pilei were conical-to-paraboloid, not striate, reddish-brown, but paler towards the margin, and 2–3 cm in diameter. The stipe was slender, rooting, whitish and pruinose at the apex. The gill trama was hyaline and the spores were ellipsoid, dull brown and exceeding 10 µm in length. Pleurocystidia were obclavate, lageniform-to-fusiform, and cheilocystidia were versiform and intermingled with sphaeropedunculate cells (Kits van Waveren 1985; Vesterholt and Knudsen 1992). Although reported from the majority of Italian administrative regions, *P. corrugis* has not yet been observed and described in Calabria (Onofri et al. 2013).

G. Sicoli, A.B. De Giuseppe, N.G. Passalacqua

Lichens

***Anaptychia bryorum* Poelt (Physciaceae)**

+ LOM: Dossi di Santicolo, Corteno Golgi (Brescia), along the road between Edolo and Santicolo, on a schist outcrop (Scisto di Edolo) (UTM WGS84 32T 599618.5113738), 807 m, 19 August 2019, leg. *G. Gheza*, det. *G. Gheza*, *J. Nascimbene*, *P.L. Nimis* (TSB). – Species confirmed for the flora of Lombardia.

Anaptychia bryorum is an arctic-alpine to boreal-montane species generally growing amongst mosses and dying plants on base-rich siliceous substrates in the alpine and subalpine belts, quite widespread along all the Alps (Nimis et al. 2018). The lobes, rich in marginal adventitious lobules and the upper cortex made of interwoven hyphae allow to distinguish it from the similar *Phaeophyscia constipata* (Norrl. & Nyl.) Moberg, which lacks marginal lobules and paraplectenchymatous upper cortex (Nimis 2021). The only previous record from Lombardia is referred to an *exsiccatum* by Anzi (Lich. Lang. 54 A, named "*Parmelia pulverulenta* var. *angustata*"), cited by Lynge (1935).

G. Gheza, J. Nascimbene, P.L. Nimis

***Athallia cerinelloides* (Erichsen) Arup, Frödén & Søchting**

+ VDA: Great Saint Bernard Valley, Etroubles (Aosta), on bark of *Fraxinus excelsior* L. (UTM WGS84: 32T 362853.5075438), 1281 m, 15 June 2021, *D. Isocrono, S. Ongaro* (TO 3809, Herb. Isocrono DI91). – Species new for the flora of Valle d'Aosta.

Athallia cerinelloides is a crustose epiphytic lichen, usually growing on branches and twigs of trees, quite rare in Italy (Nimis 2016). It has a more northern distribution than *Athallia cerinella* (Nyl.) Arup, Frödén & Søchting with which it can be mistaken, unless one carries out a microscopic investigation of the spores. This record is the first for Valle d'Aosta; the specimen was collected together with *Lecania cyrtella* (Ach.) Th.Fr..

D. Isocrono, S. Ongaro

***Baeomyces placophyllus* Ach. (Baeomycetaceae)**

+ LOM: Laghetti delle Valli, Schilpario (Bergamo), on soil near a small alpine lake (UTM WGS84 32T 590961.5097815), 1988 m, 10 August 2020, *G. Gheza* (PAV); trail between Pianaccio and Lago Seroti inferiore, Vezza d'Oglio (Brescia), on soil at the edge of a trail (UTM WGS84 32T 604507.5125018), 2179 m, 25 August 2020, *G. Gheza* (PAV). – Species confirmed for the flora of Lombardia.

Baeomyces placophyllus has been reported very rarely in recent times from the Italian Alps (Nimis 2016 and references therein). The only records from Lombardia were reported by Anzi (1862) from heathlands in the hills surrounding Como and high-altitude sites near Mount Gavia and the latter record was then cited by Giacomini (1937). These records widen the species range in Lombardia to the Orobic Alps. The collected specimens were sterile and had poorly developed marginal lobes.

G. Gheza

***Bagliettoa marmorea* (Scop.) Gueidan & Cl.Roux (Verrucariaceae)**

+ **PIE**: Val Pennavaira, Alto (Cuneo), growing on a limestone outcrop (UTM WGS84: 32T 420192.4884618), 725 m, 20 April 2021, leg. *M. Lonati* det. *S. Ongaro, D. Isocrono* (TO 3807, Herb. Isocrono DI104). – Species new for the flora of Piemonte.

Bagliettoa marmorea is a common crustose lichen characterized by an endolithic thallus with a typical pink lithocortex and by completely immersed perithecia. It is common in Europe, in the Mediterranean region, but it is also widespread throughout the Alps (Nimis et al. 2018). To date, it has been reported in all the Italian administrative regions except Piemonte and Valle d'Aosta. It grows on hard limestone, a substratum rarely occurring in Piemonte, mainly in slightly to moderately eutrophic environments. The absence of a radially cracked involucrellum helps to separate this species from the congeneric *Bagliettoa cazzae* (Zahlbr.) Vězda & Poelt that also shows a pink-violet lithocortex (Yuzon et al. 2014).

D. Isocrono, S. Ongaro

***Calicium quercinum* Pers. (Caliciaceae)**

+ UMB: Civitelle, Stroncone (Terni), on bark of *Castanea sativa* Mill. (UTM WGS84: 33T 310300.4706667), 850 m, 25 February 2021, leg. *R. Ciotti, S. Ravera*, det. *S. Ravera* (PAL). – Species new for the flora of Umbria.

Calicium quercinum is a pin lichen typically found on lignum and bark of deciduous trees, more rarely of conifers, especially on old oaks and *Castanea* (Nimis 2016). This specimen colonized the cracked bark of old tree trunks in a fruit chestnut grove, together with other Caliciales, e.g., *Calicium glaucellum* Ach. and *Chaenothecopsis pusilla* (Ach.) A.F.W.Schmidt.

S. Ravera, R. Ciotti

***Nephroma resupinatum* (L.) Ach. (Nephromataceae)**

+ LOM: Mount Lesima near Rifugio Nassano, Brallo di Pregola (Pavia), on a beech stump in a beech forest (UTM WGS84: 32T 520500.4951461), 1398 m, 4 October 2017, *G. Gheza* (PAV); Bagni di Masino, Valmasino (Sondrio), at the base of an old beech in an old-growth beech forest (UTM WGS84: 32T 546265.5121129), 1149 m, 23 August 2019, *G. Gheza* (PAV). – Species confirmed for the flora of Lombardia.

Nephroma resupinatum is a broad-lobed foliose cyanolichen typically found in shaded and moist forests, in *Lobarion* communities, on bark at the base of trunks or on stumps. The last records from Lombardia date back to the second half of the 19th century (Nimis 1993). It is listed in the Red List of epiphytic lichens of Italy as Near Threatened (Nascimbene et al. 2013).

G. Gheza, M. Barcella, S. Assini

***Pectenia atlantica* (Degel.) P.M.Jørg., L.Lindblom, Wedin & S.Ekman**

– **LAZ**. – Species to be excluded from the flora of Lazio.
– **UMB**. – Species to be excluded from the flora of Umbria.

Pectenia atlantica is a a mild-temperate cyanolichen mainly found in western in Europe, doubtfully occurring in Italy where it could be restricted to humid-warm, oceanic areas (Nimis and Martellos 2021). This species and *P. plumbea* (Lightf.) P.M.Jørg., L.Lindblom, Wedin & S.Ekman, have been considered "species pairs" (i.e. closely related species that primarily differ in their reproductive modes) until a revision of the genus based on DNA analysis (Otálora et al. 2017). This made necessary to check all the specimens recorded as *P. atlantica*. All Italian samples in Herb. Ravera, on which previous records from Lazio and Umbria were based (see Nimis 2016), proved to belong to *P. plumbea* insofar as they lack the finely to strongly longitudinal stripes that characterize *P. atlantica*.

S. Ravera

***Phaeophyscia pusilloides* (Zahlbr.) Essl. (Physciaceae)**

+ **VDA**: Valpelline, Oyace (Aosta), on bark of *Fraxinus excelsior* L. (UTM WGS84: 32T 373023.5076999), 1089 m, 15 June 2021, *D. Isocrono, S. Ongaro* (TO 3808, Herb. Isocrono DI90). – Species new for the flora of Valle d'Aosta.

Phaeophyscia pusilloides is a foliose lichen with grey to grey-brown narrow epruinose lobes with distinctive capitate soralia borne, at least in the early stages, on the ends of short side lobes. Morphology and position of soralia distinguish *P. pusilloides* from *P. insignis* (Mereschk.) Moberg and *P. orbicularis* (Neck.) Moberg. The species does not tolerate highly eutrophicated conditions and often occurs, as in this case, on deciduous trees with subneutral bark.

D. Isocrono, S. Ongaro

***Polyblastia ventosa* Arnold (Verrucariaceae)**

+ VEN: Vette Feltrine, Colle Cesta, Dolomiti Bellunesi National Park (Belluno), on carbonatic rock (UTM WGS84: 32T 719837.5107983), 2010 m, 13 June 2020, *J. Nascimbene* (BOLO). – Species confirmed for the flora of Veneto.

This lichen is characterized by a crustose, endosubstratic or thinly episubstratic thallus and by black perithecia with a flattened apex and a frequently open ostiolum. Its optimum habitat is on exposed carbonatic rocks above the treeline (Nimis 2016) where it is likely common, but strongly undercollected due to its small size and endolithic habitus. The last records from Veneto date back to the second half of the 19th century (Nimis 1993).

J. Nascimbene

***Protoparmeliopsis bolcana* (Pollini) Lumbsch (Lecanoraceae)**

+ TAA: Summit of Colbricon, Paneveggio-Pale di San Martino Natural Park (Trento), on porphyric rock (UTM WGS84: 32T 712157.5128164), 2600 m, 6 August 2021, leg. *J. Nascimbene*, det. *J. Nascimbene*, *P.L. Nimis* (BOLO). – Species new for the flora of Trentino-Alto Adige.

This crustose-placodioid lichen, belonging to the *P. muralis*-complex, is characterized by flattened to weakly convex central areoles with a distinct black margin and by sparse apothecia that have an irregular shape when mature. It is mainly known from the Mediterranean belt (Nimis 2016) being very rare at high elevations, where it occurs in xeric, sun-exposed sites, as in the case of this specimen, that was collected on the horizontal surface of a porphyric block on the southern slopes of the summit area of Colbricon.

J. Nascimbene, P.L. Nimis

***Pyrenula nitidella* (Schaer.) Müll.Arg. (Pyrenulaceae)**

+ VEN: Feltre, Villabruna, Valone (Belluno), on *Carpinus betulus* L. along a gorge in the hills around Villabruna (UTM WGS84: 32T 726632.5103774), 390 m, 2 December 2017, *J. Nascimbene* (BOLO). – Species confirmed for the flora of Veneto.
+ LOM: Bosco Fontana Natural Reserve (Mantova), on *Carpinus betulus* L. (UTM WGS84: 32T 636816.5006744), 30 m, 22 November 2005, *J. Nascimbene* (BOLO). – Species confirmed for the flora of Lombardia.

In northern Italy, this crustose lichen colonizing deciduous trees in open-humid woodlands is extremely rare (Nimis 2016) and likely survives in the small, scattered remnants of lowland deciduous forests (e.g., Bosco Fontana Natural Reserve) or in microrefugia along gorges (e.g., Feltre). The last records from Veneto and Lombardia date back to the second half of the 19th century (Nimis 1993).

J. Nascimbene

***Ramalina obtusata* (Arnold) Bitter (Ramalinaceae)**

+ EMR: Malalbergo (Bologna), on bark of *Castanea sativa* Mill. in a managed chestnut orchard (UTM WGS84: 32T 654004.4898611), 900 m, 13 June 2018, leg. *S. Gambini*, det. *F. Bottegoni*, *C. Vallese* (BOLO); Ca' di Priami, Loiano (Bologna), on bark of *Castanea sativa* Mill. in a managed chestnut orchard (UTM WGS84: 32T 687763.4904825), 640 m, 4 June 2018; leg. *S. Gambini*, det. *F. Bottegoni*, *C. Vallese* (BOLO). – Species new for the flora of Emilia-Romagna.

Ramalina obtusata is a fruticose-shrubby, greenish to greenish-grey chlorolichen. The laciniae generally lack side branches and occur as fistulose, inflated and more or less pellucid. Labriform to helmet-shaped soralia are common and develop as farinose soredia mainly found on terminal or subterminal vesicles. Apothecia are extremely rare (Nimis 2016). *Ramalina obtusata* is a cool-temperate to southern boreal epiphytic lichen found on the bark of old conifers and rarely on deciduous trees, in open montane forests. This species has been reported only in north-eastern and southern Italy (Nimis and Martellos 2020). It is included in the Italian red list of epiphytic lichens as Vulnerable (Nascimbene et al. 2013).

F. Bottegoni, C. Vallese, G. Pezzi

***Sanguineodiscus aractinus* (Fr.) I.V.Frolov & Vondrák (Teloschistaceae)**

+ TOS: Fetovaia, island of Elba island, Arcipelago Toscano National Park (LI), on granite near the coast (UTM WGS84: 32T 594744.4731732), 10 m, 16 July 2021, leg. *J. Nascimbene*, det. *J. Nascimbene*, *P.L. Nimis* (BOLO). – Species new for the flora of Toscana.

In Italy, this coastal lichen of acidic siliceous rocks is currently known only from Sardegna. Previous records from inland areas are now referred to *Caloplaca viridirufa* (Ach.) Zahlbr. (Nimis 2016). This species is characterized by a dark grey to almost black thallus and brown-orange to brick red apothecia with a persistent, dark grey, thalline margin. Our specimen was collected a few meters above sea-level, together with *Physcia mediterranea* Nimis, *Xanthoparmelia glabrans* (Nyl.) O.Blanco, A.Crespo, Elix, D.Hawksw. & Lumbsch and *Xanthoria resendei* Poelt & Tav..

J. Nascimbene, P.L. Nimis

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RESEARCH ARTICLE

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The alien vascular flora of Stromboli and Vulcano (Aeolian Islands, Italy)

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Abstract

The updated censuses of the alien flora of the islands of Stromboli and Vulcano, Aeolian Islands in the Central Tyrrhenian Sea, are presented here. They currently consist of 40 and 57 specific and subspecific taxa respectively. As many as 16 and 18 taxa respectively are new in comparison with the last censuses in 2016. *Mesembryanthemum* × *vascosilvae*, commonly cultivated in both islands, is reported for the first time as naturalized alien to Italy. *Passiflora incarnata* from Stromboli and *Pelargonium graveolens* from Vulcano are reported for the first time as casual aliens to Sicily. The evolution over time of these florulas, as inferred from the main floristic works published on these islands, is commented on. Biology, ecology and possible causes of settlement of these plants are discussed briefly.

Keywords

New records, *Mesembryanthemum* × *vascosilvae*, *Passiflora incarnata*, *Pelargonium graveolens*, Xenophytes

Introduction

The key distinguishing factor between native and alien taxa is whether their presence in a region is due directly or indirectly to human activities (Pyšek and Richardson 2006).

Alien species, and in particular invasive ones, are arousing particular interest in the scientific community because they are considered one of the greatest dangers to biodiversity and an effective cost to communities (Vitousek 1990; Chornesky and Randall 2003). Much study has been conducted to adopt standardized definitions in order to

be able to easily compare data from different areas and from different contributions (Pyšek et al. 2004). The study of alien species is also interesting for the elaboration of new theoretical insights on evolutionary and ecological processes (Davis 2006), but in many cases the dates of arrival of new taxa in a territory are not known especially when it comes to territories without precise geographical limits. For small islands this verification is simpler. They have been defined as open-air laboratories for the study of biodiversity (Russell and Kueffer 2019). In islands there is a clear geographical delimitation and they are among the best known territories from the floristic point of view (e.g. Raimondo et al. 2005a; Domina and Mazzola 2011).

In this study we wanted to provide an updated account of the alien vascular floras found on the islands of Stromboli and Vulcano in the archipelago of the Aeolian Islands (Italy). These two islands have a clearly visible volcanic activity and attract every year a high tourist flow for their naturalistic and landscape interest.

The floras of the Aeolian islands have been systematically studied since the first half of the 19th century. Giovanni Gussone has visited all the circumsicilian archipelagos and reports in the *Prodromus* (Gussone 1827–1834) and in the *Synopsis* (Gussone 1842–1845) of Flora Sicula numerous plants collected or observed in all the seven main Aeolian islands. Michele Lojacono-Pojero was sent in 1878 to collect plants for the Botanical Garden of Palermo; the list of plants collected were published in a conspicuous contribution (Lojacono-Pojero 1878). Giuseppe Zodda also presents an account of the new plants observed during his visit to the archipelago (Zodda 1904) but for the alien flora of Stromboli and Vulcano he cites only *Solanum lycopersicum* L. Complete studies of the flora and vegetation of the islands of Stromboli and Vulcano date back to about 50 years ago (Ferro and Furnari 1968a, b, 1970). These studies are mainly focused on native flora and only consider the most evident elements of the alien flora. Subsequently, several contributions have been published on single alien taxa of the vascular flora of the Aeolian Islands (Troìa 1998; Pasta 2003; Domina 2005; Domina and Spallino 2007; Pasta et al. 2008; Crisafulli et al. 2011; Ardenghi and Cauzzi 2015). Domina and Mazzola (2008) presented a cultivated flora of the circumsicilian archipelagos indicating the naturalized plants. Celesti-Grapow et al. (2016) presented an elaboration on the alien flora of the main Italian islands, but without providing the floristic lists. A complete and updated study of the flora of the Aeolian Islands has been announced several times. It is well known that Professor Gioachino Ferro from Catania has dedicated a large part of his life to the study of the flora and vegetation of this archipelago and had the synthesis of his studies in an advanced stage of preparation, but his disappearance has put a halt. In recent years, the elaborations of data on the flora of this archipelago have been presented (Pasta and La Mantia 2013; Zannini et al. 2018; Pasta et al. 2019; Chiarucci et al. 2021) but the floristic lists are still unpublished.

Hence the need to prepare this summary that gives a knowledge base for the administrators and managers of these areas who need the most complete and updated data to be able to make informed choices for the conscious management of greenery in the anthropized and semi-natural environments of these territories.

Material and methods

Characteristics of the two islands

Stromboli is the northernmost island of the Aeolian Islands and the most distant from the mainland (55 km). It covers an area of 12.2 km², with an elevation of 926 m a.s.l. It hosts the homonymous volcano, considered one of the most active in the world. There are two main residential areas, San Vincenzo and Piscità on the north-eastern side, and the village of Ginostra on the western side. The main activities of its inhabitants, until the 70s of the 20th century, were agriculture and fishing. Currently the main source of livelihood is tourism. In 1971, before the tourist boom, there were 400 residents, today there are 639 (Di Chiara et al. 2015; https://italia.indettaglio.it). No noticeable increase in buildings has been recorded on Stromboli after the tourist development (Cavallaro and Famularo 1977; Moreno 1995). In August a fluctuating population of about 2,500 people is estimated (Di Chiara et al. 2015).

Vulcano is the southernmost island of the Aeolian archipelago and the closest to the mainland (20 km). It has an area of 20.87 Km² and reaches an altitude of 499 m a.s.l. The last eruption of Vulcano occurred in 1890. The volcano is currently considered quiescent and the activity of fumaroles is the most evident one. At present the island's economy is based on tourism. The island of the volcano saw intense industrial activity around the port for the extraction of volcanic products, mainly sulphur and alum until the early 20th century. The central part of the island, and the flat surroundings of the port were used for agriculture. Traces of this activity remain in the central part of the island. The surroundings of the port and the Vulcanello peninsula have been affected, since the sixties of the 20th century, by intense tourist construction that has heavily modified the landscape of the island (Lo Cascio 2012). There are 715 residents (https://italia.indettaglio.it); in August a floating population of about 4,000 people is estimated (Di Chiara et al. 2015).

Data collection and analysis

Despite the authors have twenty years of knowledge of the islands, the floristic investigation for this contribution was carried out with specific field surveys in various seasons since 2016. The presence and distribution of all alien plant species observed was recorded, assessing the invasive status.

Trivial plants were identified in situ; the problematic specimens were collected or photographed and then identified using the reference flora of the territory (Tutin et al. 1964–1980, 1993; Pignatti 1982; Pignatti et al. 2017–2019), the "European garden flora" (Walters et al. 1984, 1986, 1989; Cullen et al. 1995, 1997, 2000), and contributions on single taxa monographies (e.g. Sanders 2006, 2012; Verloove 2008; Ardenghi et al. 2014, 2015; Ardenghi and Cauzzi 2015; Gallo 2019; Smith et al. 2020). New herbarium specimens were housed in the "Herbarium Mediterraneum Panormitanum" (PAL; herbarium acronyms according to Thiers 2021+). The nomenclature follows Galasso et al. (2018), and subsequent updates summarised in the Portal to the Flora of Italy

(http://dryades.units.it/floritaly/: see Galasso et al. 2020; Martellos et al. 2020). Data concerning the identified taxa were added in a second stage: family, life form, chorology or origin according to Pignatti et al. (2017–2019), archaeophytic or neophytic status according to Galasso et al. (2018), habitat in which the plant was found, pathways of introduction, main reproductive strategy adopted, Ellenberg's indicator values according to Domina et al. (2018b), and the presences recorded in this study and in the main floristic contributions of the two islands (Gussone 1842–1845; Lojacono-Pojero 1878; Ferro and Furnari 1968a, b, 1970; Domina and Mazzola 2008; Celesti-Grapow et al. 2016) with degree of naturalization according to Galasso et al. (2018). Criptogenetic taxa recorded for the two islands (e.g. *Cyperus rotundus* L., *Digitaria sanguinalis* (L.) Scop., *Oxalis corniculata* L. in Vulcano, *Papaver dubium* L., *P. rhoeas* L. subsp. *rhoeas* in Stromboli, and *Portulaca oleracea* L.) were not included in this account.

Results

Alien species were mainly recorded in the altimetric range from sea level to 100 m a.s.l. on Stromboli, on Vulcano from sea level to 50 m a.s.l. around the town of the port and between 300 and 400 m a.s.l. in the centre of the island. More than 80% of the taxa surveyed are neophytes. Inhabited areas are the most prone to host alien species. This is justified by the fact that the large part of these plants was introduced voluntarily as ornamentals. In many cases these aliens have also colonized agricultural areas. In the small islands, where spaces are limited, it is easy to have mixed land uses, finding purely ornamental plants grown alongside vegetable gardens and seeing fruit trees used as ornamental plants.

Overall, 62 alien species and subspecies were found growing wild in the two islands, 40 in Stromboli and 57 in Vulcano. The list includes 6 archaeophytes introduced in Italy before the discovery of Americas, and 56 neophytes. In Stromboli there are 5 casual, 29 naturalized and 6 invasive taxa; in Vulcano 13, 39 and 5 taxa respectively (Table 1 and Suppl. material 1). In comparison with the unpublished data used by Celesti-Grapow et al. (2016), 16 taxa are new to Stromboli and 18 to Vulcano. *Mesembryanthemum* × *vascosilvae* (Gideon F.Sm., E.Laguna, Verloove & P.P.Ferrer) L.Sáez & Aymerich is naturalized in Stromboli and Vulcano. This is its first report from Italy. Field and herbarium studies are needed throughout the whole territory of Italy to identify the taxa belonging to this group and trace their distribution. *Passiflora incarnata* L. from Stromboli and *Pelargonium graveolens* (Thunb.) L'Hér. from Vulcano are reported for the first time as casual aliens to Sicily (Fig. 1). The former was collected near San Vincenzo in an area of about 50 m^2 with sandy soil, where it produced several young sprouts. The latter has been extensively planted in Vulcano as ornamental along the roads and in various localities (Vulcanello, near the port, at Piano, etc.), from where it has reproduced vegetatively by invading several square meters. It has been shown that the vegetative propagation of alien species in the Mediterranean islands, although does not guarantee long-distance spread, ensures a rapid expansion and maintenance within suitable habitat (Lloret et al. 2005).

Table 1. Characteristics of the alien flora of Stromboli and Vulcano: life forms, chorology, introduction time, invasiveness, habitat, pathways of introduction, reproductive strategy adopted, means of Ellenberg's indicator values.

Life form	**Stromboli**		**Vulcano**	
	no.	**%**	**no.**	**%**
T	12	30.00	16	28.07
G	4	10.00	5	8.77
H	2	5.00	2	3.51
Ch	5	12.50	6	10.53
NP	1	2.50	3	5.26
P	16	40.00	25	43.86
TOT	40	100.00	57	100.00
Chorology	**Stromboli**		**Vulcano**	
	no.	**%**	**no.**	**%**
Africa	6	15.00	9	15.79
America	21	52.50	24	42.11
Australia	3	7.50	5	8.77
Asia	2	5.00	6	10.53
Europe & Mediterranean	1	2.50	3	5.26
Wide distribution	4	10.00	8	14.04
Cultigen	3	7.50	2	3.51
TOT	40	100.00	57	100.00
Introduction time	**Stromboli**		**Vulcano**	
	no.	**%**	**no.**	**%**
Archaeophyte	2	5.00	5	8.77
Neophyte	38	95.00	52	91.23
TOT	40	100.00	57	100.00
Invasiveness	**Stromboli**		**Vulcano**	
	no.	**%**	**no.**	**%**
Casual	5	12.50	13	22.81
Naturalized	29	72.50	39	68.42
Invasive	6	15.00	5	8.77
TOT	40	100.00	57	100.00
Habitat	**Stromboli**		**Vulcano**	
agricultural	24		25	
inhabited	34		47	
reforestation	3		4	
roads	10		15	
rocky outcrops	2		2	
beaches and inland sands	6		5	
Pathway of introduction	**Stromboli**		**Vulcano**	
unintentional	10		13	
agriculture	9		9	
forestry	4		6	
industry	3		2	
ornament	24		37	
Reproductive strategy	**Stromboli**		**Vulcano**	
seed	36		51	
vegetative	15		19	
Ellenberg's Indicator Values Means	**Stromboli**		**Vulcano**	
L	8.13		8.60	
T	8.65		8.74	
K	4.76		4.69	
F	3.80		3.68	
R	5.56		5.26	
N	4.56		4.07	
S	0.15		0.16	

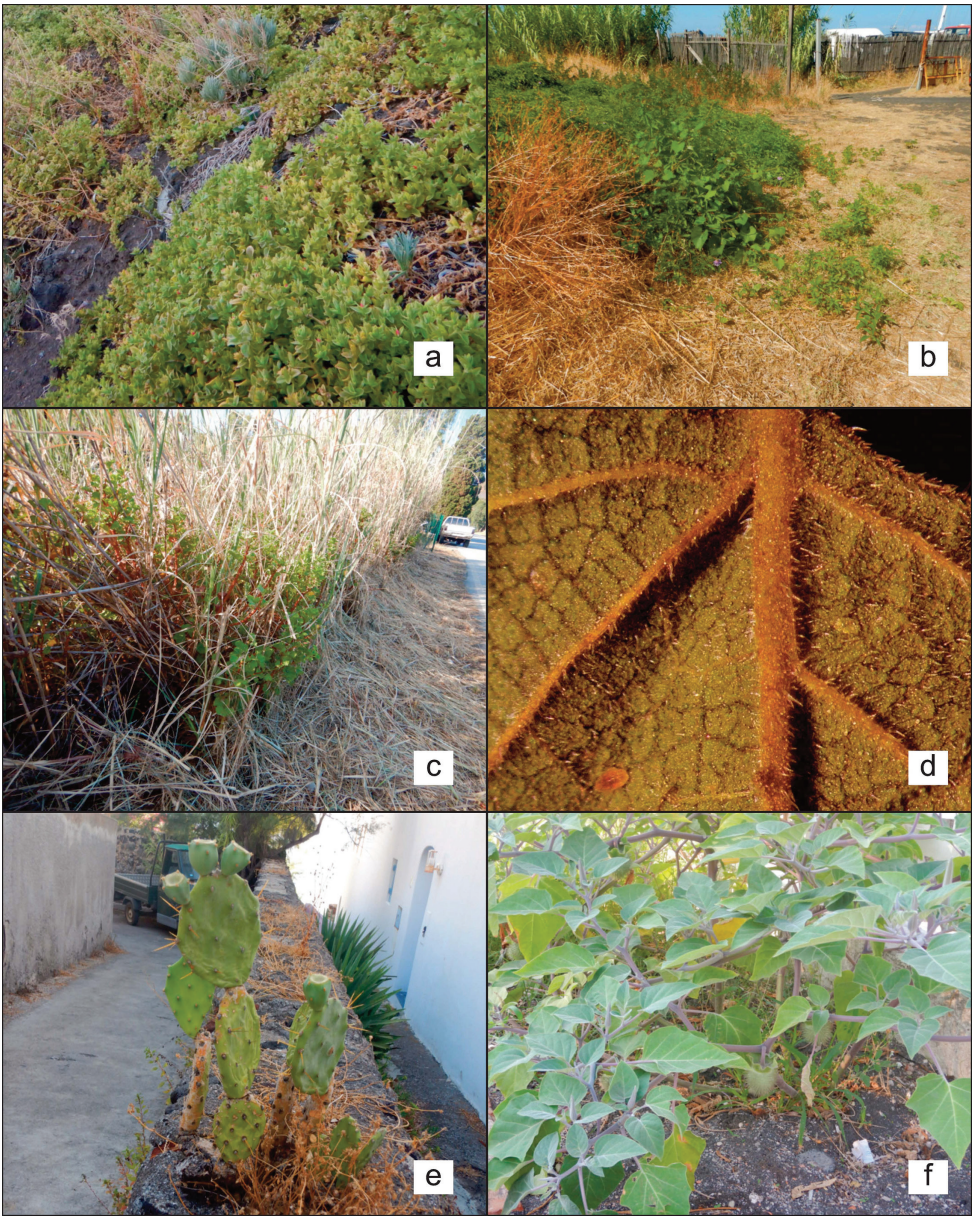

Figure 1. a *Mesembryanthemum* ×*vascosilvae* naturalized in Stromboli **b** *Passiflora incarnata* on sands in Stromboli near San Vincenzo **c** *Pelargonium graveolens* and *Saccharum biflorum* in Vulcano along the roads **d** abaxial leaf surface of *Lantana camara* subsp. *aculeata* from Stromboli **e** *Opuntia dillenii* on a wall in Stromboli at Piscità **f** *Datura wrightii* grown spontaneously in a flower bed in Vulcano and treated as an ornamental.

The species that have proved to be fearsome invasive occupying large surfaces and showing the ability to modify the environment in which they are located are: *Ailanthus altissima* (Mill.) Swingle, *Arundo donax* L., *Carpobrotus acinaciformis* (L.) L.Bolus in Stromboli, *Oxalis pes-caprae* L., and *Saccharum biflorum* Forssk. (Fig. 1). The latter,

used to build border hedges (Lojacono-Pojero 1878), has now invaded large, uncovered areas previously cultivated or made up of unfixed sand. The two florulas surveyed are similar in their floristic composition and therefore in their biological and chorological characteristics, period of introduction into the national territory, invasion status, habitat, pathways of introduction, reproductive strategy, and ecological requirements (Table 1). The two florulas are dominated by American species alike to the alien flora of the whole of Sicily (Raimondo et al. 2005b). Concerning ecological requirements, the florula of Vulcano has slightly higher light requirements (L = 8.60 vs. 813) and slightly lower preference for higher nutrient availability (N = 4.07 vs. 4.56). Comparing these florulas with the alien flora of Italy (Domina et al. 2018b) they have slightly higher light (L = 8.60 Vulcano and 8.13 Stromboli vs. 7.85), higher temperature (T = 8.74 and 8.65 vs. 7.67), and lower edaphic humidity requirements (F = 3.68 and 3.80 vs. 4.50).

Discussion

The diachronic comparison of the main floristic checklists of Stromboli and Vulcano allowed us to appreciate the exponential increasing number of alien plants (Fig. 2). This is in part due to the greater attention that scientific research has given to this biological component over time, but in part due to the increasing number of people who spend their holidays on the islands and, consequently, to the increasing number of plants introduced as ornamentals. The presence of stable nurseries and hawkers of plants, in addition to the plants transported by individual homeowners, ensures a constant supply of new taxa. The plain of Milazzo, on the mainland, opposite the Aeolian archipelago is one of the most active nursery centres in Sicily. This is also a general trend as the incidence of such species among invasive taxa is widely recognized to be

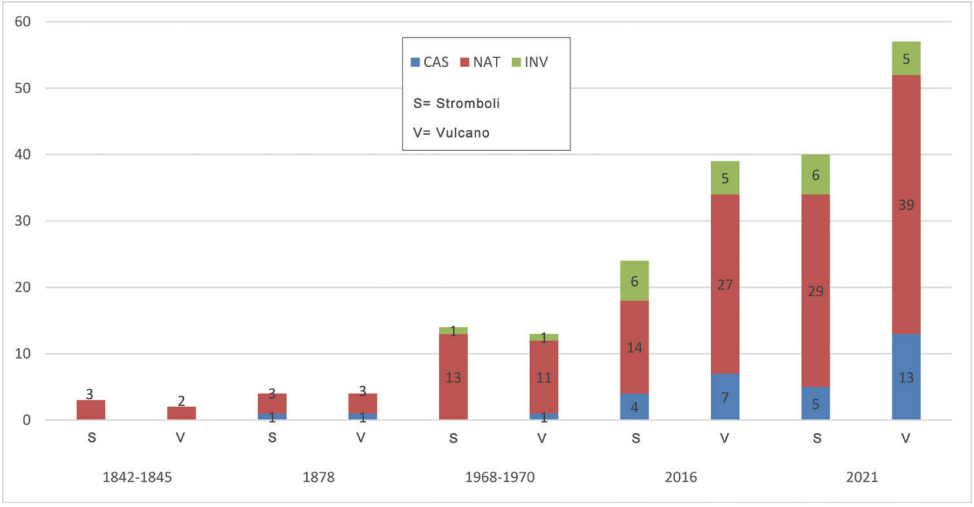

Figure 2. Alien vascular plants recorded in the main floristic works on the islands of Stromboli and Vulcano (CAS = casual; NAT = naturalized; INV = invasive).

quite high (e.g. Reichard and White 2001; Foxcroft et al. 2008). The same was also recently observed for the island of Linosa, in the Sicilian channel (Pasta et al. 2017), or for Italy as a whole (Bartolucci et al. 2021). The cessation of cultivation would lead to the disappearance of many casual species. The case of *Kleinia madraliscae* Tineo is emblematic, an alien species naturalized on the island at the beginning of the 19th century, described as native and considered endemic (Pasta 2003; Domina 2005) but within 50 years since its description, the cultivation fell into disuse and the plant almost disappeared from the island. Today it is only rarely grown in a few spots. More targeted studies on aliens trends should be conducted to verify whether the global climate change is also having appreciable effects on the alien component of the florulas of the small Mediterranean islands. The same applies to ascertaining whether the small islands are per se more subject to invasion by alien species than the mainland or whether the high rates of alien species found are mainly attributable to the strong anthropogenic pressure to which they are subjected in the summer. Besides disturbance, intense propagule pressure, defined as the rate of influx of alien propagules into the target area has been observed to be an important contributor to the establishment of new alien plants (Celesti-Grapow et al. 2010).

Conclusions

Stromboli and Vulcano, despite having similar biological, environmental and climatic conditions, so that they are classified in the same synthetic cartography subunit (Domina et al. 2018c) host a different number of endemic taxa. According to Domina et al. (2018a), Vulcano is richer than Stromboli. This despite the fact that the quality of the habitats on Vulcano has deteriorated considerably in the last century. Based on the results presented here, Stromboli and above all Vulcano appear to be particularly prone to invasion by aliens, probably due to substantial introductions for ornamental and horticultural purposes, but also to the availability of nutrient and water rich sites as orchards and surroundings of the houses. The abandonment of agriculture in large areas of the islands may be a factor that has favoured the spread of alien species (Pretto et al. 2010).

The data here presented underline the need of integrated policies for a more conscious use of the territory. This approach should promote the use of traditional agriculture, cultivation techniques and traditional cultivars of agricultural and ornamental plants rather than the embellishment of temporary vacation places with species spread throughout the globe. All this to aim at an environmental restoration, characterizing the landscape while making it pleasant and unique in the eyes of the residents and of the visitors.

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Supplementary material 1

Table with the species and subspecies of alien vascular plants present in the islands of Stromboli and Vulcano

Authors: Di Gristina E, Domina G, Barone G

Data type: occurences and biological data

Explanation note: The following data are included: family; life form (according to Pignatti et al. 2017–2019); chorology; time of introduction in Italy (cryptogenetic, archaeophyte, neophyte) ; the habitats were the plants have been recorded (agr: agricultural, inh: inhabited, ref: refforestation, roa: road, roc: rocky); means of introduction (none, agric: agricultural; forest: reafforestation, ind: industrial, orn: ornamental); reporductive strategy observed (seed, vegetative); Ellenberg's Indicator Values (according to Domina & al. 2018) and the occurrence in the two islands with the invasion status (CAS: casual, NAT: naturalized, INV: invasive).

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RESEARCH ARTICLE

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Global and regional IUCN red list assessments: 12

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Abstract

In this contribution, the conservation status assessment of two vascular plants according to IUCN categories and criteria are presented. It includes the assessment of *Genista nuragica* Bacch., Brullo & Giusso and *Jacobaea incana* (L.) Veldkamp at global level.

Keywords

Conservation, extinction risk, IUCN protocol, threats

How to contribute

The text of the global and regional assessments should be submitted electronically to Simone Orsenigo (simone.orsenigo@unipv.it) or to Giuseppe Fenu (gfenu@unica.it); the text, up to 8000 characters in length (spaces included), must include a distribution map and a picture of the assessed species.

Red List Assessments

Genista nuragica Bacch., Brullo & Giusso

Global assessment
Taxonomy and nomenclature
Order: Fabales *Family*: Fabaceae

Genista nuragica Bacch., Brullo & Giusso, Phytotaxa 449: 32 (2020).

Common name: Ginestra nuragica (It), Nuragic broom (En).

Geographic distribution range: *Genista nuragica* (Fig. 1) is an evergreen dwarf shrub that occurs only in the Barbaricino biogeographic sector (Fenu et al. 2014), on the mountain stands of Montarbu di Seui (CE Sardegna), on rocky formations locally known as "Tacchi d'Ogliastra" (Bacchetta et al. 2020; Fig. 2).

Distribution: *Countries of occurrence*: Italy (Sardegna).

Biology: *Plant growth form*: Perennial (nanophanerophyte).

Flowering and fruiting time: flowering from May to June, fruiting from June to July (Bacchetta et al. 2020).

Figure 1. *Genista nuragica* at Montarbu di Seui (CE Sardegna). Photograph by Gianluigi Bacchetta.

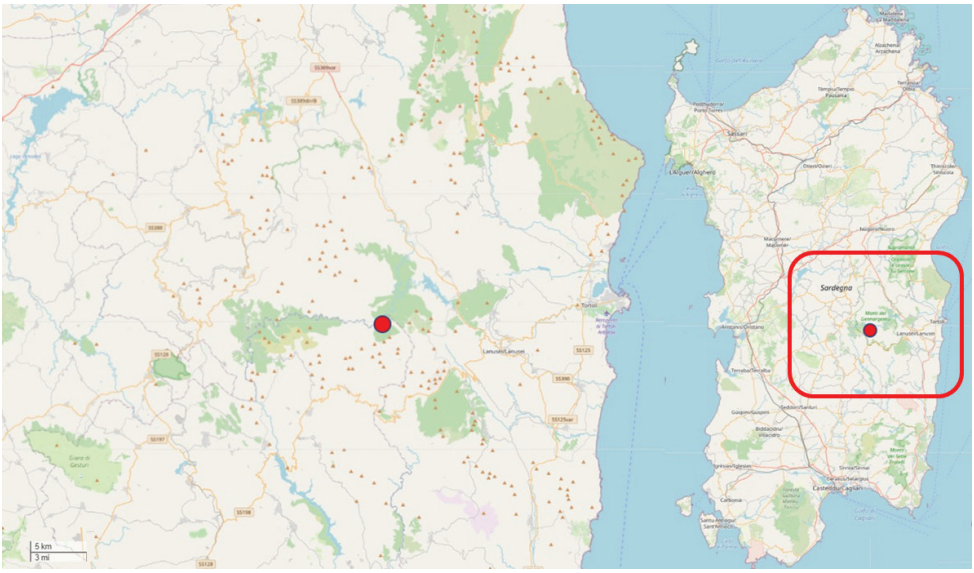

Figure 2. Geographic range and distribution map of *Genista nuragica* in Sardegna.

Reproduction: no information on pollination, dispersal strategy and seed germination is available.

Habitat and ecology: *Genista nuragica* grows on Mesozoic limestones at an elevation of 1250–1310 m a.s.l., on windy ridges characterized by lithosoils and Mediterranean pluviseasonal oceanic bioclimate with a lower supramediterranean thermotype and lower humid ombrotype according to the Rivas-Martínez classification (Bacchetta et al. 2009). It is a member of orophilous dwarf shrub plant communities characterized by the occurrence of some endemic taxa, such as *Anthyllis hermanniae* L. subsp. *ichnusae* Brullo & Giusso, *Helianthemum morisianum* Bertol., and *Sesleria barbaricina* Arrigoni (Bacchetta et al. 2020).

Population information: There is no detailed information available on population dynamics. Preliminary observations indicate a stable population.

Threats: Despite the only known population consists of a reduced number of mature individuals growing in a small area, no significant or potential threats were observed for *G. nuragica*.

CRITERIA APPLIED

Criterion B: **EOO**: 8 km^2 calculated with GeoCAT (Geospatial Conservation Assessment Tool) software (Bachman et al. 2011).

AOO: 8 km^2 calculated with GeoCAT software (Bachman et al. 2011).

b) No continuous decline observed.
c) No extreme fluctuation observed.

Criterion D: the population consists of about 100 mature plants but no signals of decline were observed.

Red List category and Criteria (Global Assessment)

NT	**Near Threatened**

Rationale for the assessment: *Genista nuragica* is a plant narrow endemic to Sardegna currently occurring only in a small area at Montarbu (Seui); the unique population consists of a low number of mature plants. Although no significant threats were observed for this species, *Genista nuragica* could be affected in the near future by the effects of the ongoing climate change, such as extreme weather events, or the monitoring of population trends could prove the presence of a decline of this species. Therefore, this species is assessed as Near Threatened (NT) at global level with the expectation that it will qualify for Critically Endangered if monitoring indicates a decline or a plausible threat.

Previous assessment: The taxon is not evaluated (NE) at the global level (IUCN 2021).

Conservation actions: *Genista nuragica* is not protected by international, national and regional laws. At present, there are no conservation measures for this species in Sardegna. The only known population grows in an area protected by a SCI ("Monti del Gennargentu" ITB021103) and a National Park "Parco Nazionale del Golfo di Orosei e del Gennargentu" established in 1998 by a Presidential Decree (D.P.R. 30 March 1998). In addition, the unique population area falls inside a site managed by the public forestry administration (Forestas Agency).

Conservation actions needed: Research activities and a monitoring programme are recommended in order to better understand the reproductive biology of the species and the population trend. Moreover, *in situ* and *ex situ* conservation actions should be designed for possible population reinforcement programmes, aimed at increasing the low number of individuals of the population.

A specific monitoring program would be important to prevent and evaluate the possible future threats (e.g., increasing grazing, human activities, or stochastic events), which could change the current conservation status of this plant.

Notes: *Genista nuragica* was described in a recent taxonomic revision of the taxa belonging to the *Genista salzmannii* DC. group of *G.* sect. *Erinacoides* Spach (Cytiseae, Fabaceae) occurring in Sardegna and Corsica. Within the species belonging to *Genista salzmannii* group, *G. nuragica* seems to be the most closely related to *G. desoleana* Vals., but several morphological features allow to distinguish these taxa. Furthermore, the two species have different ecological requirements, since *G. nuragica* is a calcicolous plant exclusively found on windy ridges, while *G. desoleana* is linked to siliceous volcanic substrates and grows on plateaux with developed soils at lower elevation never exceeding 1,000 m a.s.l.

Donatella Cogoni, Maria Silvia Pinna, Gianluigi Bacchetta

Jacobaea incana (L.) Veldkamp

Global assessment
Taxonomy and nomenclature
Order: Asterales *Family*: Asteraceae

Jacobaea incana (L.) Veldkamp, Compositae Newslett. 44: 7 (2006) ≡ *Senecio incanus* L., Sp. Pl.: 869 (1753) ≡ *Senecio leucophyllus* subsp. *incanus* (L.) Bonnier, Layens, Tabl. Syn. Pl. Vasc. France 163 (1894) ≡ *Madaractis incana* Regel, Ind. Sem. Hort. Turic. 1847: 4 (1847) = *Senecio incanus* var. *parviflorus* (All.) Rouy, Fl. Fr. 8: 332 (1903)

Common name: Senecione biancheggiante (It), Séneçon blanchâtre (Fr), Gewöhnliches Graues Greiskraut (De), Hoary Groundsel (En).

Geographic distribution range: *Jacobaea incana* (Fig. 3) is endemic to western Alps (France, Italy, Switzerland) and northern Apennines (Mount Prado, Italy; Fig. 4).

Distribution: *Countries of occurrence*: France, Italy, and Switzerland.

Biology: *Plant growth form*: perennial (scapose hemicryptophyte) (Abeli et al. 2012). *Chromosome number*: $2n = 40$ (Signorini et al. 2001).

Flowering time: From June to August.

Reproduction: Entomophilous pollination. At maturity, the seeds are carried by the wind.

Habitat and ecology: *Jacobaea incana* grows in siliceous rocky outcrops, stony alpine meadows or moraines with an altitudinal range that varies from 1800 m to 2600 m a.s.l. (Schweingruber et al. 2020).

Population information: a single study based on long-term monitoring of the southernmost population at M. Prado (N-Apennines, Italy) shows that this *J. incana* population has been stable for about 20 years and number of flowering plants is slowly increasing (Abeli et al. 2012; TA personal observation).

Figure 3. *Jacobaea incana* at Mount Prado, Northern Apennines (Italy). Photograph by Thomas Abeli.

Threats: *2.3.1 Livestock Farming & Ranching* (*nomadic grazing*): Livestock (sheep, goats) represent a threat for the southernmost population at M. Prado and probably for other population in the Alps. Livestock represents a direct threat through grazing and trampling and an indirect threat through soil nutrient enrichment due to droppings deposition.

6.1 Recreational activities: Human trampling during the tourist season involves a progressive reduction in the population viability, in fact *J. incana* cover and sexual reproduction reached the lowest values (Rossi et al. 2009).

11.2 Droughts & 11.3 Temperature extremes: Extremely dry and hot summers with a high average temperatures and low rainfall can reduce the reproductive performance of the species (Abeli et al. 2012). In particular, studies indicate an intensification of the damage in the sites where *J. incana* is affected by trampling unfavourable climatic conditions (Rossi et al. 2009).

CRITERIA APPLIED

Criterion B: **AOO** : 39,300 km^2 (calculated with a 10 × 10 km grid on occurrence data from GBIF. These data have been corrected according to the literature on the distribution of the species. The occurrences referred to other subspecies of *J. incana* have been removed; see notes).

a) Number of locations > 10
b) No decline observed
c) No extreme fluctuation observed

Red List category and Criteria (Global Assessment)

LC	Least Concern

Rationale for the assessment: This species has a wide distribution with no observed decline. The disjunct population of *J. incana* at M. Prado (N-Apennines, Italy) is stable and number of flowering plants is slowly increasing. None of the criteria are applicable.

Previous assessment: This taxon is not evaluated (NE) at the global level (IUCN 2021). At regional level, *J. incana* was assessed as LC in Switzerland (Bornand et al. 2016), while is NE in France (IUCN, France 2018).

Conservation actions: The population of M. Prado is being monitored for 20 years. Several populations are within protected areas like National Parks and Natura 2000 sites. There are twelve seed accession stored in the seed banks of the European Native Seed Conservation Network (http://ensconet.maich.gr/About.htm). These accessions are from Italy and Austria. Austrian accessions likely belong to *J. carniolica* (Willd.) Schrank, formerly considered a subspecies of *J. incana* (*J. incana* subsp. *carniolica* (Willd.) B.Nord. & Greuter). An update of the database is therefore needed. According to the Botanic Garden Conservation International database PlantSearch, there are at least four living collections of *J. incana* in botanic gardens worldwide; other collections are simply ascribed to *Senecio incanus*. As *J. incana* previously included three subspecies then split in as many species (see notes), it is not possible to understand

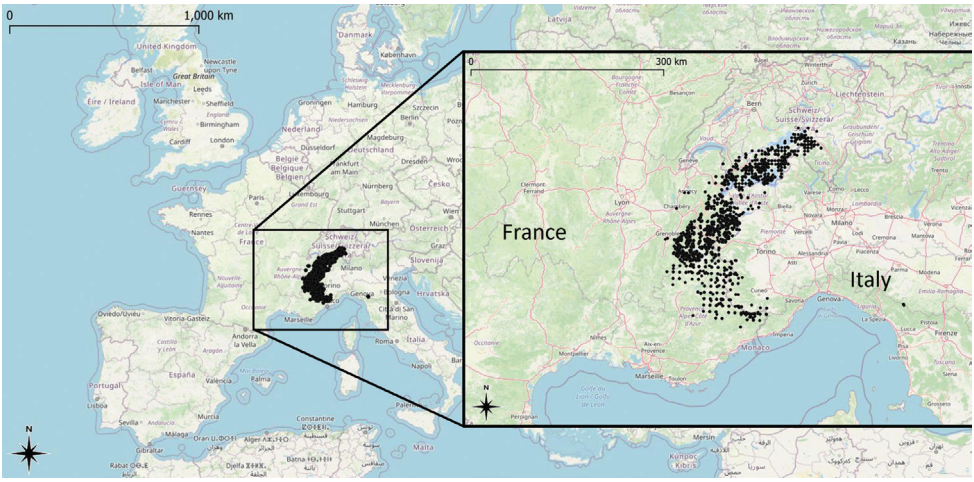

Figure 4. Global geographic range and distribution map of *Jacobaea incana*.

which of the subspecies are represented in these accessions (BGCI PlantSearch 2021; https://tools.bgci.org/plant_search.php).

Conservation actions needed: To reduce the negative effect of human and livestock trampling, fences should be used. To balance the positive and negative effects of this conservation tool, it would be better to use temporary rather than permanent fences (Lorite et al. 2021) and monitoring the demographic trends of the populations in the following years. To increase the genetic diversity immediate *ex situ* conservation activities should be carried out, through seed collection and storage in seed banks. The taxonomy of the *ex situ* accessions available should be updated according to the recent splitting of the three subspecies of *J. incana* (L.) Veldkamp into as many species (see notes). Moreover, environmental education should be conducted in order to sensitise tourists to nature conservation with particular reference to threatened species.

Notes: *Jacobaea incana* formerly included three subspecies, i.e. *J. incana* subsp. *incana* (L.) Veldkamp, *J. incana* subsp. *insubrica* (Chenevard) B.Nord. & Greuter, *J. incana* subsp. *carniolica* (Willd.) B.Nord. & Greuter. These three subspecies are now considered as distinct species. *Jacobaea incana* (L.) Veldkamp occurs in the western Alps and Apennines, *J. insubrica* (Chenevard) Galasso & Bartolucci in central Alps of Italy and Switzerland; Caccianiga, 2011), while *J. carniolica* (Willd.) Schrank, shows a more eastern distribution.

Martina D'Agostino, Thomas Abeli

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RESEARCH ARTICLE

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Notulae to the Italian native vascular flora: 12

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Abstract
In this contribution, new data concerning the distribution of native vascular flora in Italy are presented. It includes new records, confirmations, exclusions, and status changes to the Italian administrative regions. Nomenclatural and distribution updates, published elsewhere, and corrigenda are provided as Suppl. material 1.

Keywords
Endemic taxa, Floristic data, Italy

How to contribute

The text for the new records should be submitted electronically to Lorenzo Lastrucci (lorenzo.lastrucci@unifi.it). The corresponding specimen along with its scan or photograph have to be sent to FI Herbarium: Sezione di Botanica "Filippo Parlatore" del Museo di Storia Naturale, Via G. La Pira 4, 50121 Firenze (Italy). Those texts concerning nomenclatural novelties (typifications only for accepted names), status changes, exclusions, and confirmations should be submitted electronically to: Fabrizio Bartolucci (fabrizio.bartolucci@gmail.com). Each text should be within 1,000 characters (spaces included).

Floristic records of native taxa

Acanthus spinosus L. (Acanthaceae)

+ **CAL**: Cirò (Crotone), loc. Favare, 550 m a sud della centrale (WGS84: 39.37787N, 17.04018E), boscaglia mediterranea, argilloso, 280 m, 13 October 2019, *G. De Fine* (CLU). – Species new for the flora of Calabria.

N.G. Passalacqua, G. De Fine

Alchemilla exigua L. (Rosaceae)

+ **LAZ**: Settefrati (Frosinone), Santuario di Canneto (WGS84: 41.713970N, 13.902108E), pascolo in loc. Casale Bartolomucci, suolo calcareo, 1327 m, 22 July 2020, *G. Tondi*, *F. Minutillo* (FI, *Herb. Tondi*). – Species confirmed for the flora of Lazio.

This population represents the first record outside of Monti della Laga in the central Apennines (Di Pietro et al. 2015; Ballelli and Allegrezza 2016).

F. Minutillo, G. Tondi

Alchemilla fissa Günther & Schummel (Rosaceae)

+ **LAZ**: Accumoli (Rieti), Agro Nero (WGS84: 42.701884N, 13.323876E), sponde rocciose di ruscello, in radura nella faggeta di Pannicaro, presso Lago Secco, suolo marnoso-arenaceo, 1590 m, 30 May 1999, *G. Tondi* (FI). – Species new for the flora of Lazio.

F. Minutillo, G. Tondi

***Alchemilla subglobosa* C.G.Westerl. (Rosaceae)**

+ **MAR**: Monte Cavallo (Macerata), Monte Cetrognola (Riserva Statale Montagna di Torricchio) (WGS84: 42.96099269N, 13.00418242E), sotto nuclei di faggio e pascoli pingui adiacenti, substrato calcareo, 1350 m, 4 June 1987, *S. Ballelli* (FI; *Herb. Ballelli*; *Herb. Tondi*). – Species new for the flora of Marche.

This species shows its centre of distribution in northern and central Europe up to the Alps (Kurrto 2009). In Italy it is very rare and known only for few sites of the south Tyrolean and Trentino Alps (Pignatti et al. 2018; F. Festi pers. comm.).

S. Ballelli, G. Tondi

***Anemone apennina* L. (Ranunculaceae)**

– **PIE**. – Species to be excluded from the flora of Piemonte.

This species was first reported for Piemonte by Bellone et al. (2014) in two different localities in the Ligurian Alps: Limone Piemonte (Cuneo) and Frabosa Sottana (Cuneo). Recently, Bellone (2020) attributed the record from Limone Piemonte to *Anemone blanda* Schott & Kotschy, an allochthonous taxon originating from the Balkan peninsula, used for ornamental purposes. The specimen from Frabosa Sottana in TO must also be referred (revision by A. Selvaggi, 31 March 2021) to *A. blanda*, in accord with the keys proposed by Ziman et al. (2011) and Verloove (2017).

A. Selvaggi

***Apium graveolens* L. (Apiaceae)**

+ **SAR**: Status change from casual alien to native for the flora of Sardegna.

Apium graveolens is a Mediterranean species, whose presence is well documented in Sardegna (e.g., Moris 1840–1843; Milia and Mossa 1977; De Martis et al. 1984; De Martis and Loi 1989). It is common in moist places, swamps and riverbanks (Arrigoni 2013). Different horticultural varieties of this species are also widely cultivated as a vegetable . We speculate that the evaluation of its status as a casual alien may be derived from the interpretation of rarely occurring sub-spontaneous plants (Bacchetta 2006; Bacchetta et al. 2009). At any rate, its status is to be re-evaluated as a native to Sardegna.

M. Fois, L. Podda, G. Bacchetta

***Aristolochia pallida* Willd. (Aristolochiaceae)**

+ **PUG**: Gravina in Puglia (Bari), "Bosco Difesa Grande", nei pressi di Lago Splendore in bosco a *Quercus cerris* e *Q. frainetto* (WGS84: 40.765021N, 16.381718E), 462 m s.l.m., 25 April 2018, campione coltivato in Orto Botanico sino a 28 May 2021, leg. *G. Pazienza*, *L. Forte,* det. *G. Pazienza, L. Forte, V. Cavallaro* (FI; BI Nos 42445, 42448, 42449, 42450, 42451, 42452). – Species new for the flora of Puglia.

Aristolochia pallida is considered as erroneously recorded in Puglia by Bartolucci et al. (2018), where it was formerly reported by Pasquale and Licopoli (1873) for the

Gargano promontory at Sannicandro and by Palanza (1900) *"Nell'erboso sassoso del Pulicchio alle Murge di Gravina"*. The ecological conditions of this locality are similar to those reported for this species in Basilicata (Forte 2001; Pignatti et al. 2017).

G. Pazienza, L. Forte, V. Cavallaro

***Bromus chrysopogon* Viv. (Poaceae)**

+ **ITALIA** (**BAS**): Maratea (Potenza), litorale presso il porto (WGS84: ca. 39.989508N, 15.705615E), incolto litoraneo, ca. 10 m, no exp., 19 April 1990, leg. *E. Banfi*, det. *E. Banfi* 15 February 2021 (FI, MSNM sub *B. scoparius* No. 24034). – Species new for the flora of Italy (Basilicata).

+ **CAL**: Praia a Mare (Cosenza), spiaggia in faccia all'Isola di Dino (WGS84: ca. 39.873902N, 15.783107E), dune, ca. 2 m, no exp., 20 April 1990, leg. *E. Banfi*, det. *G. Osti & C. Acedo* June 2018 (FI, MSNM sub *B. scoparius* Nos. 24031, 24032, 24033, 24035). – Species new for the flora of Calabria.

Bromus chrysopogon is a southeastern-Mediterraean species described from Libya, absent in Morocco, Spain, France and the former-Yugoslavia (Clayton et al. 2006+; Valdés and Scholz 2009+), possibly native to Italy. Initially confused with *B. scoparius* L. with which it shares a compact ovoid to flabellate synflorescence, it stands out (Sherif and Siddiqi 1988) for the spikelets 20–25 mm long (vs. 8–20 mm), the lemmas 9.0–11.5 × 3.0–4.0 mm (vs. 7.0–8.0(–10.0) × 1.0–2.5 mm), the awn up to 15 mm (vs. 9(–12) mm), and the brownish-golden, never anthocyanic panicle.

E. Banfi, G. Galasso

***Bromus lepidus* Holmb. (Poaceae)**

+ C **LOM**: Magenta (Milano), Parco del Ticino, riserva "La Fagiana" (WGS84: 45.433122N, 8.829445E ± 400 m), margine di sentiero, ca. 100 m, no exp., 20 May 1993, leg. *G. Galasso*, det. *G. Osti & C. Acedo* June 2018 (MSNM sub *B. hordeaceus* No. 33852). – Cryptogenic species new for the flora of Lombardia.

Bromus lepidus is easy to identify for major lemmas shorter than 6.5 mm with a wide hyaline margin folded to an acute angle, and palea shorter than the caryopsis (Tison and de Foucault 2014). It had been reported for Italy (Piemonte and Lombardia) by Pignatti (1982), but without confirmation, and later excluded by the same author (Pignatti et al. 2017). It is impossible to evaluate if the species is native or alien to Lombardia, although in Trentino-Alto Adige it is considered a casual alien (Bartolucci et al. 2018).

E. Banfi, G. Galasso

***Carex buekii* Wimm. (Cyperaceae)**

+ **TOS**: Firenze (Firenze), Fiume Arno (WGS84: 43.765973N, 11.286693E), riva destra del fiume vicino all'Hotel "Ville sull'Arno", 10 May 2021, *L. Lastrucci* (FI). – New species for the flora of Toscana.

Carex buekii was reported as occurring only in Piemonte and doubtfully in Friuli Venezia Giulia (Bartolucci et al. 2018), where it has delimitation problems with the closely related *C. randalpina* B.Walln. (Koopman et al. 2018). This record represents the southernmost finding for Italy and the absolute southwestern limit for the range of this species (Koopman et al. 2018). In the collection site the species grows temporarily submerged.

L. Lastrucci, P. Jiménez-Mejías

***Carex strigosa* Huds. (Cyperaceae)**

+ **ABR**: Rocca S. Maria (Teramo), F.so Valle Castellana (WGS84: 42.701884N, 13.323876E), schiarita umida in faggeta mista, loc. La Piana, sotto Colle Romicito; suolo marnoso-arenaceo, 1550 m, 15 September 2017, *G. Tondi*, *F. Minutillo* (FI, *Herb. Tondi*). – Species new for the flora of Abruzzo.

F. Minutillo, G. Tondi

***Catapodium pauciflorum* (Merino) Brullo, Giusso, Miniss. & Spamp. (Poaceae)**

+ **LIG**: Bergeggi (Savona), "IT1323202 Isola Bergeggi - Punta Predani" presso Punta del Maiolo (WGS84: 44.2374N, 8.442402E), macchia mediterranea, substrato calcareo, 16 m, 15 May 2020, *D. Dagnino, L. Minuto* (GE No. 2408); Portovenere (La Spezia), "IT1345005 Portovenere - Riomaggiore - San Benedetto" Castello di Portovenere, (WGS84: 44.050324N, 9.832397E), gariga a *Euphorbia dendroides* (Habitat Natura 2000 cod.5330), 21 m, 8 May 2020, *D. Dagnino, C. Turcato* (GE No. 5055); Portovenere (La Spezia), "IT1345005 Portovenere - Riomaggiore - San Benedetto" Castello di Portovenere, (WGS84: 44.048553N, 9.833048E), tasca sabbiosa sulla scogliera calcarea, 2 m, 8 May 2020, *D. Dagnino, C. Turcato* (GE No. 5075). – Species confirmed for the flora of Liguria.

Several old records of *C. pauciflorum* for Liguria are known from Brullo et al. (2003), Ottone Penzig's handwritten notes found on a copy of De Notaris (1844) stored in the library of the University of Genova, and the GDOR herbarium (Barberis et al. 2019).

D. Dagnino, L. Minuto, C. Turcato

***Cistus albidus* L. (Cistaceae)**

+ **TOS**: Monte Argentario (Grosseto), fra Punta Avoltore e Punta di Torre Ciana, (WGS84: 42.370900N, 11.167933E), nella gariga bassa a erica multiflora e rosmarino, su breccia calcarea, 88 m, 27 December 2019 (sterile), *F. Selvi 4035* (FI); *ibidem*, 23 May 2020 (fruit), *F. Selvi* e *I. Bettarini 4036* (FI); *ibidem*, 6 April 2021 (flower), *F. Selvi* e *I. Bettarini 4044* (FI). – Species new for the flora of Toscana.

The native presence in Toscana was recently excluded (Bartolucci et al. 2016). In this new locality, *C. albidus* forms a very small population in a low garrigue with

Erica multiflora L. and *Salvia rosmarinus* Spenn., on a warm, dry slope facing to the south, on rocky-gravelly calcareous soil. This is the typical habitat of this species. It is not known as a cultivated ornamental in the area, supporting its native status in this locality. Searches in the surrounding area, however, were not successful. The population is in urgent need of protection, as plants are close to a dirt road and subject to a high risk of being collected or extirpated.

F. Selvi

***Cladanthus mixtus* (L.) Chevall. (Asteraceae)**

+ **LIG**: Vado Ligure (Savona), sulla sponda destra del Quiliano (WGS84: 44.280714N, 8.444769E), 0 m, 07 April 2021, *M. Callegari, B. Ciocia* (GE). – Species confirmed for the flora of Liguria.

Some historical herbarium specimens conserved in GDOR (Barberis et al. 2019) document the presence of the species in three localities: Voltri, Lagaccio, and Sarzana. Bibliographic data (Bertoloni 1853–1854; Ariello 1957) report the historical presence of this species in five other localities in the surroundings of La Spezia: Punta Mesco, Monterosso al Mare, Riomaggiore, Corniglia, and Canale Turì. The population consists of about ten individuals and some seedlings.

M. Callegari, B. Ciocia, G. Casazza, L. Minuto

***Convallaria majalis* L. (Asparagaceae)**

+ **UMB**: Costacciaro (Perugia), Monte Cucco, in Loc. Pignola (WGS84 43.366110N, 12.732120E), bosco misto su versante acclive con esposizione W, substrato carbonatico, c. 1050 m s.l.m., 30 May 2021, *A. Di Filippo, A. Scoppola* (UTV No. 38780). – Species confirmed for the flora of Umbria.

The species is reported as "doubtfully occurring" for Umbria (Bartolucci et al. 2018), although its presence was already reported for Monte Cucco in the sites Fosso di Musolea and Campo la Croce (Salerno and Puletti 1994, 1996), close to the site recorded here. This population is made up of clusters of a few dozen individuals, which 200–300 m upstream cover larger areas in the understory of a beech forest.

A. Di Filippo, A. Scoppola

***Crucianella angustifolia* L. (Rubiaceae)**

+ **VDA**: Quart (Aosta), tra l'oratorio Beato Emerico, Duclos e Porsod (WGS84: 45.757630N, 7.406327E), praterie xerotermiche, 950–1180 m s.l.m., 7 June 2021, *M. Lonati, A. Mainetti, S. Ravetto Enri* (FI). – Species confirmed for the flora of Valle d'Aosta.

This species was recorded by Braun-Blanquet (1961) based on specimens in MPU collected in the localities of Veynes/Sorreley (Saint-Christophe) in 1956. It has not been recently recorded for Valle d'Aosta, where it is actually considered as cryptogenic (Bovio 2014; Bartolucci et al. 2018). Dozens of individuals were sparsely distributed

over an area of about 10 hectares. Due to the species' abundance and distribution across the semi-natural xero-thermic grasslands of Quart, we believe it most appropriate to consider this species as native.

M. Lonati, A. Mainetti, S. Ravetto Enri

***Fedia graciliflora* Fisch. & C.A.Mey. (Caprifoliaceae)**

+ **LAZ**: Roma (Roma), Parco Petroselli (Talenti) (WGS84: 41.938009N, 12.557930E), pratello ai margini di un uliveto, lungo la rete di confine, suolo tufaceo, 37 m, 19 April 2019, *G. Tondi, G. Pellegrino* (FI, *Herb. Tondi*). – Species new for the flora of Lazio.

F. Minutillo, G. Tondi

***Helianthemum salicifolium* (L.) Mill. (Cistaceae)**

+ **LIG**: Diano San Pietro (Imperia), "IT1315602 Pizzo di Evigno" M. Lago (WGS84: 43.96365N, 8.06141E), prateria arida su substrato calcareo (Habitat Natura 2000 cod.6210*), facies densamente arbustata, 625 m, 25 May 2020, *D. Dagnino, M. Mariotti* (GE No. 2989). – Species confirmed for the flora of Liguria.

Some old records (De Notaris 1844; Bertoloni 1842–1844) and herbarium specimens housed in GDOR (Barberis et al. 2019) document the presence of this species in localities in the province of Savona (Albenga, Ceriale) and near Genova. According to Ottone Penzig's handwritten notes found on a copy of De Notaris (1844), stored in the library of the University of Genova, this species was also present at Capo Berta, an area close to the site of the current discovery.

D. Dagnino, M. Mariotti, I. Briozzo

***Hieracium pilosum* Schleich. ex Froel. subsp. *villosifolium* (Nägeli & Peter) Greuter (Asteraceae)**

+ **EMR**: Corniglio (Parma), tra il Lago Santo e La Sella del Marmagna (WGS84: 44.395387N, 10.000874E), prateria subalpina, 1700 m, 25 July 2020, leg. *L. Pinzani,* det. *G. Gottschlich* (FI). – Subspecies new for the flora of Emilia-Romagna.

L. Pinzani, G. Gottschlich

***Juncus ranarius* Songeon & E.P. Perrier (Juncaceae)**

+ **VEN**: Sopra Ruaz - Livinallongo (Belluno) (WSG84 46.496466N, 11.919887E), margine strada statale, 1400 m s.l.m., 4 August 2008, *S. e C. Argenti* (FI barcode FI064007, *Herb. Argenti* No. 16801). – New species for the flora of Veneto.

The distribution of *Juncus ranarius* in Italy was recently updated (Lastrucci et al. 2022), highlighting a certain ecological amplitude for this species. This record confirms the ability of *J. ranarius* to originate populations even in non-halophilic habitats.

L. Lastrucci, C. Argenti

***Lamium galeobdolon* (L.) L. subsp. *galeobdolon* (Lamiaceae)**

+ **LAZ**: Acquafondata (Frosinone) (WGS84: 41.514925N, 13.961504E), bosco di *Ostrya carpinifolia*, substrato calcareo, 990 m, 14 May 2016, *F. Minutillo* (FI, *Herb*. Minutillo, *Herb*. *Tondi*). – Subspecies new for the flora of Lazio.

F. Minutillo, G. Tondi

***Lolium arundinaceum* (Schreb.) Darbysh. subsp. *corsicum* (Hack.) J.-M.Tison (Poaceae)**

+ **SAR**: Perdu Locci, Pula (Sud Sardegna), prati umidi (WGS84: 38.995073N, 9.012160E), 1 m, 18 June 2021, *M. Fois, G. Bacchetta* (FI, CAG); Sa Giaga, Tula (Sassari), sponde sabbiose lacustri (WGS84: 40.762810N, 9.010656E), 164–165 m s.l.m., 16 June 2013, *G. Calvia* (*Herb. Calvia*). – Subspecies new for the flora of Sardegna.

Lolium arundinaceum subsp. *corsicum* is a perennial herb endemic to Corsica and Toscana (Bartolucci et al. 2018). This subspecies was doubtfully recorded for Sardegna, based on the synonymy with *Festuca arundinacea* var. *insularis* Gennari proposed by Banfi et al. (2017). We confirm its presence in several sub-halophilous wet meadows near the coast and inland plains of southern Sardegna, as well as in sandy and wet places of northern Sardegna.

M. Fois, G. Calvia, G. Bacchetta

***Ononis spinosa* L. subsp. *procurrens* (Wallr.) Briq. (Fabaceae)**

+ **LAZ**: Ostia Antica (Roma), scavi di Ostia Antica (WGS84: 41.753792N, 12.288327E), tra i ruderi, sul travertino, 5 m, 24 September 2019, *G. Tondi* (FI, *Herb*. *Tondi*). – Subspecies new for the flora of Lazio.

F. Minutillo, G. Tondi

***Pilosella fusca* (Vill.) Arv.-Touv. (Asteraceae)**

+ **LOM**: Sondalo (Sondrio), sentiero in Valle di Rezzalo, presso la chiesa di San Bernardo (WGS84: 46.35728N, 10.41861E), prateria alpina aperta di fondovalle, ca. 1850 m, 10 July 2005, leg. *S. Perego*, det. *G. Gottschlich* 2021 (MSNM No. 44104 sub *Hieracium aurantiacum*). – Species confirmed for the flora of Lombardia.

G. Gottschlich, G. Galasso

***Plantago atrata* Hoppe subsp. *fuscescens* (Jord.) Pilg. (Plantaginaceae)**

+ **TOS**: Coreglia Antelminelli (Lucca), M.te Rondinaio, sella sul crinale ad Est della vetta (WGS84: 44.114656N, 10.595474E), prateria altomontana, 1800 m, 8 July 2021, *L. Pinzani,* (FI, *Herb. Pinzani*). – Subspecies new for the flora of Toscana.

L. Pinzani

***Poa palustris* L. subsp. *palustris* (Poaceae)**

+ **LAZ**: Alvito (Frosinone), Valle di Rio, Font.na Lepore (WGS84: 41.745481N, 13.729308E), canale di scolo del fontanile, 847 m, 13 May 2021, *L. Cancellieri, G. Filibeck* (FI, *Herb. Cancellieri*); Vallerotonda (Frosinone), Valle Verrecchia, sotto Sorgente di Collelungo (WGS84: 41.624519N, 13.980796E), canale di scolo di fontanile, 1340 m, 4 June 2021, *G. Filibeck, G. Cangelmi* (*Herb. Cancellieri*); San Biagio Saracinisco (Frosinone), Valle Monacesca, Le Fontane (WGS84: 41.642979N, 13.960188E), torbiera, 1775 m, 20 July 2021, *L. Cancellieri, G. Cangelmi* (*Herb. Cancellieri*); Vallerotonda (Frosinone), ecotoni sopra loc. La Selva - Lago di Cardito (WGS84: 41.607930N, 13.989928E), boscaglia igrofila, suolo alluvionale a matrice calcarea, 1030 m, 22 June 2013, *G. Tondi, F. Minutillo* (FI, *Herb*. *Tondi*). – Species new for the flora of Lazio.

This species, previously overlooked, is quite common in wet habitats across a wide elevational range in SE-Lazio (Val Comino, Meta and Mainarde districts).

L. Cancellieri, G. Cangelmi, G. Filibeck, F. Minutillo, G. Tondi

+ **MOL**: Scapoli (Isernia), Colle Rotondo (WGS84: 41.617687N, 14.013347E), canale di scolo di fontanile, 1160 m, 8 July 2021, *L. Cancellieri, G. Cangelmi, G. Filibeck* (UTV). – Species confirmed for the flora of Molise.

L. Cancellieri, G. Cangelmi, G. Filibeck

***Polypogon monspeliensis* (L.) Desf. (Poaceae)**

+ **UMB**: Magione (Perugia), loc. Sant'Arcangelo sul Trasimeno (WGS84: 43.087983N, 12.156851E), vegetazione sinantropica delle canaline di scolo a bordo strada, 258 m, 16 June 2021, leg. *G. Mei*, det. *G. Mei*, *A. Stinca* (FI). – Species confirmed for the flora of Umbria.

G. Mei, A. Stinca

***Potamogeton coloratus* Hornem. (Potamogetonaceae)**

+ **LIG**: Genova (Genova), laghetti di Nervi (WGS84: 44.394861N, 9.045050E), pozza di torrente, 67 m, 26 January 2017, leg. *M. Calbi*, det. *G. Barberis, M. Calbi, D. Dagnino* (GE No. 5136); *ibidem*, 17 September 2020, leg. *V. Del Nero*, det. *G. Barberis, V. Del Nero, D. Dagnino* (GE No. 5185). – Species confirmed for the flora of Liguria.

De Notaris (1844, under the name *P. plantagineus* Du Croz ex Roem. & Schult.) quoted this species "in paludibus et in fossis secus litora Liguriae totius frequentissime", but in the 20th century almost all coastal wetlands disappeared.

G. Barberis, M. Calbi, V. Del Nero

***Pyrus nivalis* Jacq. (Rosaceae)**

+ **EMR**: Canossa (Reggio Emilia), ZSC "IT4030014 Rupe di Capotrera, Rossena", strada SP54 tra Ciano d'Enza e Rossena, dopo la curva al Km 2 (WGS84: 44.591183N,

10.417198E), boscaglia con *Quercus pubescens*, *Fraxinus ornus*, *Ulmus minor* e *Spartium junceum*, 347 m, NNE, 10 October 2020, leg. *G. Riva*, det. *E. Banfi* (FI, MSNM Nos. 50930, 50931, 50932). – Species new for the flora of Emilia-Romagna.

We found numerous 2–3 m tall shrubs of this species.

G. Riva, E. Banfi, G. Galasso

***Ranunculus baldensis* Dunkel (Ranunculaceae)**

+ **VEN**: Lusiana-Conco (Vicenza), loc. sopra Piazza Campana (WGS84: 45.793816N, 11.552451E), compluvio a margine boscaglia, 915 m, 4 May 2021, *leg. F. Fratolin*, *det. F.G. Dunkel* (FI, *Herb. Dunkel*, *Herb. Argenti*); *idem*, loc. presso Buso della Sperlonga (WGS84: 45.799687N, 11.550342E), margine ceduo, 1105 m, 4 Mai 2021, *leg. D. Campoccia, C. Argenti*, *det. F.G. Dunkel* (FI, *Herb. Dunkel*, *Herb. Argenti*); *idem*, loc. Ristoro (WGS84: 45.797986N, 11.545593E), compluvio a margine bosco, 1100 m, 4 Mai 2021, *leg. D. Campoccia, C. Argenti*, det. *F.G. Dunkel* (FI, *Herb. Dunkel*, *Herb. Argenti*). – Species confirmed for the flora of Veneto.

Ranunculus baldensis belongs to the *R. auricomus* L. complex and was described from Mt. Baldo in Trentino-Alto Adige. It was reported as probably extinct for Veneto (Dunkel 2010) based on a historical specimen kept at FI collected on M. Grappa by L. Vaccari in "Collalti, selve umide cedue di San Michele".

F.G. Dunkel, D. Campoccia, C. Argenti, F. Fratolin

***Ranunculus pedrottii* Spinosi ex Dunkel (Ranunculaceae)**

+ **LAZ:** Amatrice (Rieti), Fosso Cerruglia (WGS84: 42.588509N, 13.336007E), prati umidi, 1290 m, 8 May 2013, *F. Bartolucci, F. Conti* (APP Nos 52604, 52605, 52621–52624, 57186, 57195–57200); *ibidem*, 15 June 2012, *F. Bartolucci, F. Conti* (APP Nos 52645, 52648, 52649, 52651–52653); *ibidem*, 18 June 2016, *F. Conti* (APP Nos 57188–57190, 60271–60282); *ibidem*, 23 July 1997, *G. Tondi* (APP); *ibidem*, 14 June 1998, *G. Tondi* (APP). – Species new for the flora of Lazio.

The species was considered so far endemic to the high karstic plateaux around Castelluccio (Pian Grande di Castelluccio di Norcia and Pian Perduto) between Marche and Umbria (Dunkel 2011).

F. Conti, F. Bartolucci, F.G. Dunkel, G. Tondi

***Ranunculus rionii* Lagger (Ranunculaceae)**

+ EMR: Collecchio (Parma), quarry lake close to the Chiesuole Naturalistic Area (WGS84: 44.787751N, 10.208318E), 22 June 2021, leg. *R. Bolpagni, A. Dalla Vecchia,* det. *L. Lastrucci et R. Bolpagni* n. FI064048 (FI barcode FI064048). – Species new for the flora of Emilia-Romagna.

Ranunculus rionii grows along the shores of an abandoned quarry lake.

R. Bolpagni, A. Dalla Vecchia, L. Lastrucci

***Romulea columnae* Sebast. & Mauri (Iridaceae)**

+ **EMR**: San Leo (Rimini), propaggini occidentali di Monte Gregorio (WGS84: 43.912870N, 12.354773E), prato arido su substrato calcareo affiorante, 505 m s.l.m., 25 February 2021, *T. Bruschi, F. Santi* (FI); Cervia (Ravenna), radura prativa nella pineta (punto soggetto a calpestio), 27 March 1983, *F. Semprini* (*Herb. Semprini*). – Species new for the flora of Emilia-Romagna.

About 15 flowered individuals were observed in San Leo. This species was not recorded by Bruschi and Polverelli (2020), that studied the flora of this area.

T. Bruschi, F. Santi

***Rorippa sylvestris* (L.) Besser subsp. *sylvestris* (Brassicaceae)**

+ **CAL**: Sant'Eufemia d'Aspromonte (Reggio Calabria), Contrada San Bartolo (WGS84: 38.260484N, 15.843146E), lungo un'antica "saja" usata per irrigare i campi, 498 m, 8 July 2020, leg. *A. Crisafulli*; det. *A. Crisafulli, V.L.A. Laface, C.M. Musarella, G. Spampinato* (FI, REGGIO). – Species and subspecies confirmed for the flora of Calabria.

Rorippa sylvestris subsp. *sylvestris* is reported throughout Italy, with the exception of Calabria where, according to Bartolucci et al. (2018), the taxon is of doubtful occurrence. The collected individuals grow in large numbers along an old "saja" used to irrigate the nearby fields.

C.M. Musarella, V.L.A. Laface, G. Spampinato, A. Crisafulli

***Rosa rubiginosa* L. (Rosaceae)**

+ **MAR**: Bolognola (Macerata), siepi e radure nel vers. N-NW del M. Castel Manardo (WGS84: 42.979389N, 13.242039E), ca. 1430 m., 26 June 2021, *L. Gubellini* (FI, PESA). – Species new for the flora of Marche.

The species grows in hedges, clearings and wood edges at mountain altitudes.

L. Gubellini, N. Hofmann

***Sedum caeruleum* L. (Crassulaceae)**

+ **CAL**: Montebello Jonico (Reggio Calabria), Rocche di Prastarà (WGS84: 37.952121N, 15.745786E), rocce, 325 m, 2 May 2021, leg. *V.L.A. Laface*; det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (FI, REGGIO); Montebello Jonico (Reggio Calabria), Prastarà (WGS84: 37.951119N, 15.744581E), muro a secco, 292 m, 16 May 2021, leg. *V.L.A. Laface*; det. V.L.A. *Laface, C.M. Musarella, G. Spampinato* (REGGIO); Montebello Jonico (Reggio Calabria), Borgata Caracciolino (WGS84: 37.945481N, 15.749657E), bordo strada, 182 m, 16 May 2021, leg. *V.L.A. Laface*; det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); Montebello Joni-

co (Reggio Calabria), SS 106 (WGS84: 37.933378N, 15.735161E), bordo strada, 31 m, 16 May 2021, leg. *V.L.A. Laface*; det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); Montebello Jonico (Reggio Calabria), Contrada Tigani (WGS84: 37.934654N, 15.739465E), bordo strada, 63 m, 16 May 2021, leg. *V.L.A. Laface*; det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO). – Species new for the flora of Calabria and for peninsular Italy.

The species grows abundantly on dry stone walls and along roadsides, and can also be found with numerous individuals in rocky plant communities occupied by *Lomelosia cretica* (L.) Greuter & Burdet. In Italy it was recorded so far only for Sicilia and Sardegna (Bartolucci et al. 2018).

C.M. Musarella, V.L.A. Laface, G. Spampinato

***Sedum dasyphyllum* L. subsp. *glanduliferum* (Guss.) Nyman (Crassulaceae)**

+ **UMB**: Spoleto (Perugia), Colle Sant'Elia, pareti rocciose sottostanti la Rocca Albornoziana (WGS84: 42.733744N, 12.742815E), affioramenti di natura carbonatica, 418 m s.l.m. , 7 May 2021, leg. et det. *F. Bonini*. – Subspecies new for the flora of Umbria.

Unlike other Italian administrative Regions, no information was available until now on the occurrence of *Sedum dasyphyllum* subspecies in Umbria (Bartolucci et al. 2018).

F. Bonini, D. Gigante

***Stellaria ruderalis* M.Lepší, P.Lepší, Z.Kaplan & P.Koutecký (Caryophyllaceae)**

+ **PIE**: San Pietro Val Lemina (Torino), lungo il greto del Torrente Lemina (WGS84: 44.897584N, 7.315345E), suolo prevalentemente sabbioso, 411 m, 16 July 2021, *S. Carfagno, M. Lonati* (FI). – Species new for the flora of Piemonte.

This recently described species has been already reported for Sardegna, Toscana, and Veneto (Lepší et al. 2019), and recently recorded also in Lombardia (Bartolucci et al. 2021).

S. Carfagno, M. Lonati

+ **TAA**: Tenno (Trento), alla base di un muro presso Ville del Monte, parcheggio all'inizio della strada per Canale di Tenno (WGS84: 45.930205N, 10.824805E), 560 m a.s.l., 23 March 2021, leg. *F. Prosser, G. Tomasi* (FI; ROV No. 76947); Via Dós Trento (Trento), a bordo strada (WGS84: 46.0728136N, 11.1096564E), 300 m a.s.l., 24 March 2021, leg. *G. Bonari, L. Sottovia* (ROV No. 80104); Bolzano (Bolzano), centro storico, Via A. Hofer, cortile del Museo di Scienze dell'Alto Adige (WGS84: 46.50114N, 11.357272E), 270 m a.s.l., 12 April 2021, leg. *T. Wilhalm* (BOZ). – Species new for the flora of Trentino-Alto Adige.

G. Tomasi, F. Prosser, T. Wilhalm, G. Bonari

***Trifolium michelianum* Savi (Fabaceae)**

+ **PUG**: Gravina in Puglia (Bari), Bosco Difesa Grande, Lago d'Olmo (WGS84: 40.755172N, 16.385009E), prati umidi su suoli periodicamente inondati, 450 m, 14 June 2019, *V. Tomaselli* (FI, BI Nos 42443, 42444, 42446 e 42447). – Species new for the flora of Puglia.

Trifolium michelianum is a Mediterranean-Atlantic species with isolated locations in North Africa, the Balkan Peninsula, and Asia Minor (Caldarella et al. 2013). In the localities of Puglia, it forms small populations, growing in wet and periodically flooded meadows.

V. Tomaselli, F. Carruggio, L. Forte

***Veronica cymbalaria* Bodard subsp. *cymbalaria* (Plantaginaceae)**

+ **PIE**: Alto (Cuneo), Valle Pennavaire (WGS84: 44.112460N, 7.996134E), ambiente ruderale nei pressi di una costruzione, 770 m, 23 April 2021, *M. Pittarello, G. Nota, D. Barberis* (FI). – Species confirmed for the flora of Piemonte.

Veronica cymbalaria was reported by Bertolani Marchetti (1955) for Valsesia, but recently was indicated as reported by mistake in Bartolucci et al. (2018).

M. Pittarello, G. Nota, D. Barberis

Floristic records of regional alien taxa

***Antirrhinum tortuosum* Bosc. ex Lam. (Plantaginaceae)**

+ (NAT) **LOM**: Bergamo (Bergamo), Via San Vigilio (WGS84: 45.710450N, 9.648429E), muro in pietra, 456 m, SW, 16 July 2021, *E. Banfi* (FI, MSNM No. 51025). – Naturalized regional alien species new for the flora of Lombardia.

This species was confused so far with *A. majus* L., also occasionally present on the walls of "Città Alta".

E. Banfi, G. Galasso

***Centranthus calcitrapae* (L.) Dufr. subsp. *calcitrapae* (Valerianaceae)**

+ (NAT) **VEN**: Domegliara (Verona), nello scalo merci della stazione ferroviaria (WGS84: 45.525713N, 10.821137E), massicciata, 117 m, 21 April 2021, *A. Bertolli, F. Prosser* (FI, ROV No. 80106); *ibidem*, 9 June 2021, *F. Prosser, G. Tomasi* (ROV No. 80108); Verona, nello scalo merci della stazione ferroviaria di Verona Porta Nuova (WGS84: 45.4251109N, 10.9813051E), massicciata, 63 m, 9 June 2021, *F. Prosser, G. Tomasi* (ROV No. 80109). – Naturalized regional alien species new for the flora of Veneto.

As far as northern Italy is concerned, this taxon was, so far, known only for Liguria and Lombardia (Bartolucci et al. 2020).

A. Bertolli, F. Festi, F. Prosser, G. Tomasi

***Crepis bursifolia* L. (Asteraceae)**

+ (NAT) **SAR**: Sassari (Sassari), Viale Italia, lungo la via principale e nelle vie traverse, in aiuole e bordi stradali (WGS84: 40.716621N, 8.554115E) 205 m, 7 June 2021, *G. Brundu, V. Lozano, S. Cherchi* (FI, *Herb. UniSS Agraria*). – Naturalized regional alien species new for the flora of Sardegna.

Crepis bursifolia is a perennial herb and a ruderal plant. In 2021, it was found to be abundant along the streets of the city center of Sassari. The record for Limbara by Veri and Bruno (1974) has been questioned by Calvia and Ruggero (2020).

V. Lozano, G. Brundu, S. Cherchi

***Euphorbia taurinensis* All. (Euphorbiaceae)**

+ (NAT) **TAA**: Mezzocorona (Trento), nella stazione ferroviaria (WGS84: 46.208705N, 11.129098E), massicciata, 210 m, 31 October 1999, leg. *F. Prosser,* det. *F. Prosser 11 May 2021* (ROV No. 32604, sterile plants); *ibidem*, 4 May 2021, leg. *F. Festi, M. Tognetti*, det. *F. Prosser 10 May 2021* (FI, ROV No. 80105, fertile plants). – Naturalized regional alien species new for the flora of Trentino-Alto Adige.

Euphorbia taurinensis was indicated as native in Piemonte and Liguria by Bartolucci et al. (2018). The species was also reported as alien for Veneto (Argenti and Lasen 2001), where it was observed in Feltre railway station between the 1970s and 1996.

A. Bertolli, F. Festi, F. Prosser, G. Tomasi

***Phalaris aquatica* L. (Poaceae)**

+ (NAT) **PIE**: Tortona (Alessandria), scarpata stradale della nuova tangenziale di Tortona (WGS84: 44.918263N, 8.886254E), 100 m, no exp., 22 June 2020, *S. Orsenigo* (PAV); *ibidem*, 21 May 2021, leg. *S. Orsenigo*, det. *S. Orsenigo* e *M. Pallanza* (FI, PAV). – Naturalized regional alien species new for the flora of Piemonte.

We have found about ten individuals, observed for four years, forming big tufts along the road margin, close to the railway.

S. Orsenigo, M. Pallanza

***Sedum caespitosum* (Cav.) DC. (Crassulaceae)**

+ (NAT) **VEN**: Sommacampagna (Verona), nell'Aeroporto Catullo (WGS84: 45.400643N, 10.891048E]), sede di stradina asfaltata dismessa, 75 m, 14 April 2021, *A. Bertolli, F. Prosser, G. Tomasi* (FI, ROV No. 80107). – Naturalized regional alien species new for the flora of Veneto.

This is a stenomediterranean species, known for southern and central Italy (Bartolucci et al. 2018), recently recorded for Emilia-Romagna (Alessandrini and Montanari 2020).

A. Bertolli, F. Festi, F. Prosser, G. Tomasi

Nomenclatural and distribution updates from other literature sources, and corrigenda

Nomenclatural and distribution updates, and corrigenda to Bartolucci et al. (2018) are provided in Suppl. material 1.

F. Bartolucci, G. Galasso

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Supplementary material 1

Supplementary data

Authors: Fabrizio Bartolucci, Gabriele Galasso

Data type: Species data

Explanation note: 1. Nomenclatural updates; 2. Note updates; 3. Distribution updates; 4. Synonyms, misapplied or included names.

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Notulae to the Italian alien vascular flora: 12

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Abstract

In this contribution, new data concerning the distribution of vascular flora alien to Italy are presented. It includes new records, confirmations, exclusions, and status changes for Italy or for Italian administrative regions. Nomenclatural and distribution updates published elsewhere are provided as Suppl. material 1.

Keywords
Alien species, floristic data, Italy, nomenclature

How to contribute

The text for the new records should be submitted electronically to Lorenzo Lastrucci (lorenzo.lastrucci@unifi.it). The corresponding specimen along with its scan or photograph has to be sent to FI Herbarium: Museo di Storia Naturale (Botanica), Sistema Museale di Ateneo, Via G. La Pira 4, 50121 Firenze (Italy). Those texts concerning nomenclatural novelties (typifications only for accepted names), status changes, exclusions, and confirmations should be submitted electronically to Gabriele Galasso (gabriele.galasso@comune.milano.it). Each text should be within 1,000 characters (spaces included).

Floristic records

***Albizia julibrissin* Durazz. (Fabaceae)**

+ (CAS) **MAR**: Pesaro (Pesaro e Urbino), Via S. Pertini ai margini del Ponte Giovanni XXIII (WGS84: 43.900625N, 12.875721E), margine stradale erboso, ca. 10 m, 18 July 2021, *L. Gubellini* (FI, PESA). – Casual alien species new for the flora of Marche.

A single individual has been observed, flowering in a roadside verge.

L. Gubellini, N. Hofmann

***Aubrieta deltoidea* (L.) DC. (Brassicaceae)**

+ (CAS) **PIE**: Salbertrand (Torino), fraz. San Romano, presso il Camping Gran Bosco (WGS84: 45.062215N, 6.867815E), interstizi tra un muretto e la strada asfaltata in prossimità di una aiuola coltivata, 1023 m, 3 April 2021, *M. Probo, M. Lonati* (FI). – Casual alien species new for the flora of Piemonte.

Some individuals occur spontaneously near a private garden where the species is cultivated as ornamental.

M. Probo, M. Lonati

***Bothriochloa pertusa* (L.) A.Camus (Poaceae)**

+ (NAT) **ITALIA** (**LIG**): Sestri Levante (Genova), ZSC "IT1333308 Punta Manara", loc. Ginestra (WGS84: 44.2617N, 9.4158E), bordo del sentiero, a margine di oliveto in coltura, ca. 110 m, SE, 23 July 2020, leg. *D. Dagnino, C. Turcato*, det. *E. Banfi* (FI, GE No. 4891, MSNM Nos. 50854, 50855). – Naturalized alien species new for the flora of Italy (Liguria).

This species is similar to *Bothriochloa insculpta* (Hochst. ex A.Rich.) A.Camus, represented in Italy by subsp. *panormitana* (Parl.) Giardina & Raimondo, from which is distinguished by the number and color of the racemes (3–8 brown-straw *vs* of 10–15 blackish) and by the lower glume bearing long woolly whitish hairs grouped in the distal half (Vega 2000). It is native to warm temperate and tropical Old World from where it has been introduced in Mesomerica, South America (Colombia) and southern North America (Alabama, Florida, Louisiana, Mississippi), while in Europe is reported from Sweden and considered doubtfully alien in Greece (Valdés and Scholz 2009+; POWO 2019+). It prefers uncultivated and grassy places, but at present it is impossible to evaluate the potential aggressiveness of the species.

E. Banfi, D. Dagnino, C. Turcato, G. Galasso

***Brugmansia aurea* Lagerh. (Solanaceae)**

+ (CAS) **CAM**: San Giorgio a Cremano (Napoli), al confine con Napoli, Via Cupa Rubinacci (WGS84: 40.834822N, 14.328418E), bordo strada, 32 m, 7 April 2021, leg. *R. Motti*, det. *R. Motti, A. Cozzolino, A. Stinca* (FI, PORUN-Motti); *ibidem*, 9 July 2021, *A. Stinca* (PORUN-Stinca). – Casual alien species new for the flora of Campania.

In Italy, two species belonging to the South American genus *Brugmansia* Pers. have been recorded: *B. aurea* in Calabria (Laface et al. 2020) and *B. suaveolens* (Humb. & Bonpl. ex Willd.) Sweet in Campania (Stinca 2020). The new finding refers to an individual probably originated vegetatively from plants cultivated nearby.

R. Motti, A. Cozzolino, A. Stinca

***Centaurea diluta* Aiton (Asteraceae)**

+ (NAT) **BAS**: Matera (Matera), confine tra Puglia e Basilicata, in prossimità del vulcanello di Gravina di Puglia (WGS84: 40.700063N, 16.428955E), incolti in prossimità di colture di orzo, 208 m, 3 June 2021, *M. Terzi, F.S. D'Amico* (FI). – Naturalized alien species new for the flora of Basilicata.

A population of *Centaurea diluta* was found on clayey soils along the edge of a path close to the border between Basilicata and Puglia.

M. Terzi, F.S. D'Amico

***Chasmanthe bicolor* (Gasp.) N.E.Br. (Iridaceae)**

+ (NAT) **SAR**: La Maddalena (Sassari), Parco Nazionale dell'Arcipelago di La Maddalena, Isola La Maddalena (WGS84: 41.236234N, 9.410872E), in prossimità delle strade e nelle radure della macchia, 67 m, 27 March 2021, *G. Brundu, V. Lozano, V. Lazzeri* (FI, *Herb. Uniss Agraria*). – Naturalized alien species new for the flora of Sardegna.

Chasmanthe bicolor is a perennial herb native to the Western Cape Province in South Africa (de Vos 1985; Duncan 2001).

V. Lozano, G. Brundu, V. Lazzeri

***Cupressus sempervirens* L. (Cupressaceae)**

+ (NAT) **CAL**: Reggio Calabria (Reggio Calabria), Via V. Florio, nei pressi del porto (WGS84: 38.119704N, 15.649376E), dentro il tronco cavo di una pianta di *Melia azedarach*, 23 m, 13 April 2021, leg. *G. Spampinato*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (FI, REGGIO); *ibidem*, Piani di San Nicola, fraz. Ortì (WGS84: 38.151801N, 15.695425E), bordo strada, 559 m, 18 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); *ibidem*, Diminniti di Sambatello, nei pressi del cimitero (WGS84: 38.174036N, 15.702576E), terreno abbandonato, 425 m, 18 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); Condofuri (Reggio Calabria), fraz. Straci (WGS84: 37.924573N, 15.844811E), nel bosco e a bordo strada, 33 m, 11 May 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); *ibidem*, Trombia (WGS84: 37.937525N, 15.866372E), eucalipteto e bordo strada, 106 m, 18 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); *ibidem*, fraz. Pietra (WGS84: 37.947734N, 15.865268E), pineta e bordo strada, 151 m, 18 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO); Montebello Jonico (Reggio Calabria), Canale (WGS84: 37.958997N, 15.695899E), eucalipteto e bordo strada, 148 m, 18 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (REGGIO). – Naturalized alien species new for the flora of Calabria.

Cupressus sempervirens reproduces easily by seed and at most of the reported localities, the species has formed small populations, with some mature and reproductive adult individuals.

G. Spampinato, V.L.A. Laface, C.M. Musarella

***Cuscuta campestris* Yunck. (Convolvulaceae)**

+ (NAT) **LIG**. – Status change from casual to naturalized alien for the flora of Liguria.

We found several populations of *Cuscuta campestris* along the Magra Valley (La Spezia), showing a high number of individuals growing on different host plants (e.g., *Convolvulus arvensis* L., *Euphorbia maculata* L., *Xanthium italicum* Moretti, etc.) in ruderal environments linked to the riverside terraces or the anthropized areas of the Magra plain. We collected several herbarium specimens of these populations, currently housed in GE.

D. Dagnino, I. Briozzo, C. Turcato

***Cyrtomium falcatum* (L.f.) C.Presl (Dryopteridaceae)**

+ (CAS) **SAR**: Cagliari (Cagliari), Via Vittorio Veneto (WGS84: 39.225230N, 9.105523E), fessure di un muro di contenimento in pietra calcarea, 41 m, 25 February 2021, *L. Rosati, M. Marignani* (FI). – Casual alien species new for the flora of Sardegna.

We observed, growing on a wall colonized also by *Adiantum capillus-veneris* L. and *Cymbalaria muralis* G.Gaertn., B.Mey. & Scherb. subsp. *muralis*, a single individual.

L. Rosati, M. Marignani

***Delosperma cooperi* (Hook.f.) L.Bolus (Aizoaceae)**

+ (CAS) **ABR**: Teramo (Teramo), Via Conte Contin (WGS84: 42.656388N, 13.696388E), bordo stradale, ca. 254 m, 15 May 2021, *N. Olivieri* (FI). – Casual alien species new for the flora of Abruzzo.

Some individuals of the species are settled on the concrete roadside, sometimes covered with moss, together with *Sedum dasyphyllum* L. subsp. *dasyphyllum* and sporadically *S. palmeri* S.Watson, in an area not subject to foot traffic.

N. Olivieri

***Dimorphotheca fruticosa* (L.) Less. (Asteraceae)**

+ (CAS) **SAR**: Carloforte (Sud Sardegna), Isola di San Pietro, loc. Nasca (WGS84: 39.157107N, 8.264874E), bordo strada, dietro ad un muretto a secco nella macchia, 96 m, 6 March 2021, leg. *L. Rosati, M. Marignani, G. Cocco, S. Bardi*, det. *L. Rosati* (HLUC); *ibidem*, loc. Tacca Rossa (WGS84: 39.161726N, 8.302175E), scarpata stradale, 48 m, 7 March 2021, leg. *L. Rosati, M. Marignani, G. Cocco, S. Bardi*, det. *L. Rosati* (FI). – Casual alien species new for the flora of Sardegna.

Some flowering individuals were observed growing in garrigues dominated by *Cistus monspeliensis* L.

G. Sotgiu Cocco, L. Rosati, S. Bardi, M. Marignani

***Dracaena draco* (L.) L. subsp. *draco* (Asparagaceae)**

+ (CAS) **SAR**: Cagliari (Cagliari), Viale Buon Cammino (WGS84: 39.222723N, 9.115725E), epifita su *Celtis australis*, 94 m, 15 April 2021, *A. Lallai, G. Bacchetta, L. Podda* (FI, CAG). – Casual alien species new for the flora of Sardegna.

This species is native to Macaronesia and Morocco, where it grows on slopes in association with other xerophilous taxa (Almeida Pérez 2003). The juvenile specimen found in Sardegna grows epiphytic in the bifurcation of the branches on a decayed *Celtis australis* L. subsp. *australis*, approximately 20 m away from a mature individual.

A. Lallai, G. Bacchetta, L. Podda

***Erigeron philadelphicus* L. (Asteraceae)**

+ (CAS) **EMR**: Ferrara (Ferrara), Via Dosso Dossi (WGS84: 44.842018N, 11.619414E), vegetazione sinantropica ruderale, 10 m, 7 May 2021, leg. *M. Curuzzi, G. Mei*, det. *G. Mei* (FI, *Herb. G. Mei*). – Casual alien species new for the flora of Emilia-Romagna.

G. Mei, M. Curuzzi

***Euthamia graminifolia* (L.) Nutt. (Asteraceae)**

– **ITALIA** (**PIE**). – Alien species to be excluded from the flora of Italy (Piemonte).

The report of *Euthamia graminifolia* in Piemonte (Soldano and Sella 1988) is to be referred to *E. lanceolata* (L.) G.L.Nesom (see below).

G. Galasso, A. Soldano

***Euthamia lanceolata* (L.) G.L.Nesom (Asteraceae)**

+ (NAT) **ITALIA** (**PIE**): Castelletto Cervo (Biella), baraggia sul lato W della strada provinciale verso San Giacomo di Masserano (WGS84: 45.53388N, 8.24138E), brughiera, 229 m, no exp., 20 August 1990, *A. Soldano 8801* (*Herb. A. Soldano* sub. *E. graminifolia*); *ibidem*, baraggia lungo la strada SP315 San Giacomo–Buronzo (WGS84: 45.536587N, 8.240707E ± 10 m), brughiera, ca. 221 m, no exp., 12 September 2009, *G. Galasso* (MSNM Nos. 43684, 43685, 43686 sub. *E. graminifolia*); Masserano (Biella), lato W della Baraggia delle Quattro Madame (WGS84: 45.54800N, 8.25416E), brughiera, 235 m, no exp., 4 September 1993, *A. Soldano 9436* (*Herb. A. Soldano* sub. *E. graminifolia*). – Naturalized alien species new for the flora of Italy (Piemonte).

According to the recent revision of the genus *Euthamia* (Nesom 2021a, b), our specimens previously attributed to *E. graminifolia* (L.) Nutt. belong to *E. lanceolata*. Although the height of the involucres (4.0–4.2 mm) is more or less intermediate between the two species (3.6–4.2 mm in *E. lanceolata vs* 4.0–5.0 mm in *E. graminifolia*), stem and leaf surfaces are clearly hirtellous (*vs* glabrous).

G. Galasso, A. Soldano

***Gazania rigens* (L.) Gaertn. (Asteraceae)**

+ (NAT) **SAR**. – Status change from casual to naturalized alien for the flora of Sardegna.

Gazania rigens was detected as naturalized in the sand dunes of Torre dei Corsari (Arbus, Sud Sardegna) and in the small peninsula of Capo Coda Cavallo (San Teodoro, Sassari). These self-sustaining populations are fast-spreading, after a recent introduction as ornamentals in gardens.

M. Fois, A. Cuena-Lombraña, G. Brundu

***Honorius boucheanus* (Kunth) Holub (Asparagaceae)**

+ (CAS) **LOM**: Mantova (Mantova), Corte Cherubine, Via Legnago 17 (WGS84: 45.165820N, 10.832494E), all'ombra di un filare d'alberi, 19 m, no exp., 24 April 2021, leg. *E. Guidotti, P. Lanfredini, M. Marchini*, det. *E. Guidotti, M. Pallanza, S. Orsenigo* (FI). – Casual alien species new for the flora of Lombardia.

E. Guidotti, P. Lanfredini, M. Marchini, M. Pallanza

***Hyacinthoides non-scripta* (L.) Chouard ex Rothm. (Asparagaceae)**

+ (CAS) **UMB**: Gualdo Tadino (Perugia), Appennino di Gualdo Tadino, Valle del Fònno, versante SW (WGS84: 43.226585N, 12.815555E), margine erboso delimitato da rocce calcaree rivestite da una fitta lecceta, 790 m, 2 April 2021, *M. Loreti, P. Salerno* (FI). – Casual alien species new for the flora of Umbria.

Only a group of five plants was found in an impervious Appennine area with a high level of wilderness, included into the ZSC "IT5210014 Monti Maggio-Nero (sommità)".

M. Loreti, P. Salerno

***Ipheion uniflorum* (Lindl.) Raf. (Amaryllidaceae)**

+ (CAS) **LOM**: Concorezzo (Monza e Brianza), lungo Via G. Mazzini (WGS84: 45.585790N, 9.341909E), incolto, 172 m, 2 April 2021, *G. Patera* (FI). – Casual alien species new for the flora of Lombardia.

A few individuals of this species originated from seeds produced by some adults growing in a nearby garden.

G. Patera

***Koelreuteria paniculata* Laxm. (Sapindaceae)**

+ (NAT) **VDA**: Arnad (Aosta), presso la Chiesa di San Martino, dietro il giardino pubblico, lungo l'argine del Torrente d'Arnad (WGS84: 45.6446009N, 7.7206899E), greto e argini del torrente, molti esemplari in fruttificazione, 361 m, 2 August 2021, *A. Selvaggi* (FI); *ibidem*, più in basso, scendendo lungo il Torrente d'Arnad, nel greto (WGS84: 45.64437562N, 7.71966776E), greto fluviale, 358 m, 2 August 2021, *A. Selvaggi* (AO). – Naturalized alien species new for the flora of Valle d'Aosta.

The naturalized population extends more than 100 metres along the river Torrente d'Arnad and includes numerous individuals at different stages of growth. All the individuals originate from few cultivated plants grown in the public garden situated behind the San Martino church.

A. Selvaggi, P. Ferraris

***Melinis repens* (Willd.) Zizka subsp. *repens* (Poaceae)**

+ (INV) **SAR**. – Status change from naturalized to invasive alien for the flora of Italy (Sardegna).

Since the first report in 2018 (Galasso et al. 2019a) we have observed a rapid expansion of this taxon, which is spreading from roadsides of coastal areas to inland fields, to the extent that we believe it is becoming a problematic weed for the nearby crops.

G. Bacchetta, G. Calvia, L. Podda

***Nassella tenuissima* (Trin.) Barkworth (Poaceae)**

+ (CAS) **LOM**: Costa Volpino (Bergamo), nei pressi della ex cava di gesso lungo il margine destro della strada di recente costruzione che sale alla fraz. Volpino (WGS84: 45.836317N, 10.102783E), margine stradale, 202 m, SE, 28 May 2021, leg. *A. Federici*, det. *G. Galasso, E. Banfi* (FI, MSNM No. 50996); Milano (Milano), passaggio pedonale parallelo a Via E. Fieramosca, presso la Residenza Arcimboldi, recinzione al di sopra della trincea della linea tramviaria (WGS84: 45.515361N, 9.214027E), fughe nella pavimentazione stradale a mattonelle, 134 m, no exp., 2 June 2021, *G. Galasso* (FI, MSNM Nos. 50984, 50985). – Casual alien species new for the flora of Lombardia.

In both cases, the collected specimens were growing very far from the mother plants.

G. Galasso, E. Banfi, A. Federici

***Nelumbo nucifera* Gaertn. (Nelumbonaceae)**

+ (NAT) **TOS**. – Status change from casual to naturalized alien for the flora of Toscana.

The first records of this species for Toscana date back to Montelucci (1964, 1970) from Massarosa (Lucca province), where it has been recorded again in 2017 (Peruzzi and Bedini 2021+). Here, new records from Parco di Fonte Santa (Bagno a Ripoli, Firenze, WGS84: 43.710670N, 11.372633E, 578 m, 29 July 2021, *L. Lazzaro, L. Lastrucci*, FI) and from Piazza delle Gondole in Pisa (WGS84: 43.716768N, 10.409048E, obs. *B. Pierini, L. Peruzzi*) are added. In these sites, the populations are in an evident naturalized status, with the presence of many flowers and fruits. We therefore propose the change of status for this species in Toscana from casual to naturalized.

L. Lazzaro, L. Lastrucci

+ (NAT) **MAR**: Potenza Picena (Macerata), c.da Castelletta, stagno artificiale (WGS84: 43.346644N, 13.657330E), stagno, 24 m, 10 July 2021, *D. Fiacchini* (FI). – Naturalized alien species new for the flora of Marche.

Nelumbo nucifera grows inside the artificial pond located less than 30 metres from the right bank of the Asola stream. The species occupies a total area of *c.* 1,500–2,000 m^2.

D. Fiacchini, L. Gubellini

***Parasenegalia visco* (Lorentz ex Griseb.) Seigler & Ebinger (Fabaceae)**

– **SAR**. – Alien species to be excluded from the flora of Sardegna.

Parasenegalia visco was first reported by Podda et al. (2010) from Rio Santa Lucia in Capoterra (Cagliari) as a new casual alien species for Sardegna. Later, it was indicated by the same authors as a naturalized alien (Podda et al. 2012; Puddu et al. 2016). Field insights and a review of specimens in CAG have shown that these records are due to a misidentification of *Paraserianthes lophantha* (Willd.) I.C.Nielsen subsp. *lophantha*.

L. Podda, A. Lallai, G. Bacchetta

***Parkinsonia aculeata* L. (Fabaceae)**

+ (INV) **SAR**. – Status change from casual to invasive alien for the flora of Sardegna.

Recently, rapid colonization from seeds in dense thickets have been observed in several areas from Cagliari to the road SS131 and to Santa Margherita (Pula, Cagliari), or wetlands near Cagliari such "Stagno di Santa Gilla", and "Stagno di Molentargius" (Cagliari province).

G. Bacchetta, M. Fois, L. Podda

***Passiflora caerulea* L. (Passifloraceae)**

+ (NAT) **LAZ**. – Status change from casual to naturalized alien for the flora of Lazio.

During the last four years, investigations revealed that this species is widespread in northern Lazio with well-established populations in the provinces of Viterbo (e.g., Viterbo, Montalto di Castro, Oriolo Romano) and Roma, where it also grows on the beaches of Lake Bracciano. These populations regularly produce fruits and propagate both vegetatively and by seed.

S. Magrini, S. Buono

***Platanus hispanica* Mill. ex Münchh. (Platanaceae)**

+ (INV) **ITALIA** (**LAZ**). – Status change from naturalized to invasive alien for the flora of Italy (Lazio).

Recent investigations in the province of Roma revealed that this species has largely colonized the shores of the river Tiber and Aniene, all along the urban and suburban area and beyond, as well as other riparian habitats. From there, it is currently spreading also along roadsides and in archaeological sites (Celesti-Grapow and Ricotta 2020).

L. Celesti-Grapow

***Platycladus orientalis* (L.) Franco (Cupressaceae)**

+ (NAT) **VDA**: Saint-Vincent (Aosta), sulla collina tra Cillian e Felliey (WGS84: 45.739636N, 7.663596E), un esemplare inselvatichito in fessura delle rupi di serpentino, 660 m, 26 May 2012, leg. *M. Bovio, G.P. Mondino*, det. *G.P. Mondino* (AO); Bard (Aosta), presso il sentiero che costeggia le rocce alla base del Forte di Bard (WGS84: 45.60614630N, 7.74672678E), fessure delle rocce "montonate", 400 m, S, 27 July 2021, *A. Selvaggi* (FI); *ibidem* (WGS84: 45.6066190N, 7.7471075E), fessure delle rocce, 420 m, 2 August 2021, *A. Selvaggi* (AO). – Naturalized alien species confirmed for the flora of Valle d'Aosta.

Many individuals of this species have colonized the rock cracks at different points around the base of Forte di Bard; some of these plants are already fertile or ready to disperse the seeds. The presence of the species in Valle d'Aosta was previously an-

notated by Bovio (2014) as casual, but not by Galasso et al. (2018a). Other findings were recorded also in Collina di Châtillon (WGS84: 45.739636N, 7.663596E and 45.753752N, 7.615628E, 17 April 2019, obs. *M. Bovio, C. Ganz, M. Broglio, G. Trompetto*), Pont-Saint-Martin (WGS84: 45.598159N, 7.801948E, 1 May 2019, obs. *M. Bovio*), and between Plan de Brun and Perloz (WGS84: 45.606255N, 7.802994E, 21 May 2021, obs. *M. Bovio, C. Ganz, O. Deval, G. Jacquemet*).

A. Selvaggi, M. Bovio, G.P. Mondino

***Populus* ×*canadensis* Moench (Salicaceae)**

+ (INV) **ITALIA** (**LAZ**). – Status change from naturalized to invasive alien for the flora of Italy (Lazio).

+ (INV) **SAR**. – Status change from casual to invasive alien for the flora of Sardegna.

Recent investigations in Lazio revealed that this nothospecies has largely spread in riparian habitats along the shores of the river Tevere and its affluents, in the urban and suburban area of Roma and beyond. It is also currently spreading in archaeological sites and on the top of buildings, particularly in roof gutters. Similarly, in Sardegna, it is spreading in suburban areas and in riparian sites from planted stands (e.g., along the river Tirso at Fordongianus, Oristano, WGS84: 39.997N, 8.807E, obs. *G. Brundu*).

G. Brundu, L. Celesti-Grapow

***Pteris multifida* Poir. (Pteridaceae)**

+ (CAS) **TOS**: Pisa (Pisa), Via Roma 56 (WGS84: 43.719187N, 10.395475E), in tombino, 4 m, 10 June 2021, *L. Pinzani* (FI, *Herb. L. Pinzani*). – Casual alien species new for the flora of Toscana.

L. Pinzani

***Quercus palustris* Münchh. (Fagaceae)**

+ (CAS) **ITALIA** (**LOM**): Milano (Milano), piazza d'Armi, tra Via delle Forze Armate, Via della Rovere e Via G. Mazzarino (WGS84: 45.464368N, 9.122097E), boscaglia umida, 123 m, no exp., 7 July 2021, leg. *G. Galasso*, det. *G. Galasso, E. Banfi* (FI, MSNM Nos. 51005, 51006, 51007, 51008); Besnate (Varese), stagno Lagozzetta (WGS84: 45.703333N, 8.744722E), spontaneizzata sulle rive boschive dell'area umida, 276 m, no exp., 18 July 2021, leg. *M. Martignoni*, det. *G. Galasso, E. Banfi* (FI, MSNM No. 51038). – Casual alien species new for the flora of Italy (Lombardia).

In Milano, some individuals of the species, identified according to Jensen (1997), grow in a former semi-natural military area, with temporary pools rich in particularly interesting species.

G. Galasso, E. Banfi, M. Martignoni, N. Pilon

***Rhus typhina* L. (Anacardiaceae)**

+ (CAS) **ABR**: Bellante (Teramo), loc. Bellante Stazione, Via del Commercio (WGS84: 42.706944N, 13.852777E), vegetazione lungo il bordo stradale, ca. 98 m, 19 July 2021, *N. Olivieri* (FI). – Casual alien species new for the flora of Abruzzo.

Several young shrubby individuals occur in the record site, some of which bearing fruit, on both sides of a road.

N. Olivieri

***Robinia neomexicana* A.Gray (Fabaceae)**

+ (CAS) **MAR**: Pietrarubbia (Pesaro e Urbino), lungo la strada SP1 (WGS84: 43.790709N, 12.361800E), siepi, ca. 715 m, 10 June 2021, *L. Gubellini* (FI, PESA); *ibidem*, 13 August 2021, *L. Gubellini* (FI, PESA). – Casual alien species new for the flora of Marche.

The species was identified according to Peabody (1984) and Isely and Peabody (1984).

L. Gubellini, N. Hofmann

***Salvia hispanica* L. (Lamiaceae)**

+ (CAS) **CAL**: Cirò (Crotone), nei pressi dell'abitato, poco a N di Via Madonna delle Grazie (strada SP7) (WGS84: 39.382263N, 17.063990E), incolto, 290 m, 12 September 2019, *G. De Fine* (CLU). – Casual alien species new for the flora of Calabria.

N.G. Passalacqua, G. De Fine

***Scirpus atrovirens* Willd. subsp. *hattorianus* (Makino) Verloove & Lambinon (Cyperaceae)**

+ (CAS) **LAZ**: Latina (Latina), fraz. Borgo Piave (WGS84: 41.486944N, 12.846667E), sponde di un piccolo pantano, suolo sabbioso e argilloso, 28 m, 5 July 1999, leg. *F. Minutillo*, det. *L. Lastrucci*, conf. *F. Verloove* (FI). – Casual alien subspecies new for the flora of Lazio.

The specimen was identified according to Whittemore and Schuyler (2002).

F. Minutillo, G. Tondi

***Sedum rubrotinctum* R.T.Clausen (Crassulaceae)**

+ (CAS) **MAR**: Cupra Marittima (Ascoli Piceno), margine della strada SS16 Adriatica (WGS84: 43.0291619N, 13.8575740E), margine stradale, ca. 6 m, 3 August 2020, *N. Olivieri* (FI). – Casual alien species new for the flora of Marche.

This Mexican species, identified according to Groendijk-Wilders and Springate (2011) and Horwath (2014), was recently reported as a casual alien in Campania by Del Guacchio et al. (2020). In Cupra Marittima this culton has colonized the edge of the sidewalk in an area of limited extension within the town. The plant grows on a thin and not very developed substrate.

N. Olivieri

***Semiarundinaria fastuosa* (Lat.-Marl. ex Mitford) Makino (Poaceae)**

+ (CAS) **LOM**: Bergamo (Bergamo), Parco Regionale dei Colli di Bergamo, inizio del sentiero di Via dei Vasi partendo da Via Castagneta, lato a valle (WGS84: 45.71567N, 9.65165E), ex ceduo di castagno in evoluzione verso un bosco misto di farina, carpino bianco e acero campestre, ca. 380 m, NE, 13 July 2021, *E. Banfi* (FI); *ibidem*, 24 July 2021, *E. Banfi* (MSNM Nos. 51032, 51033, 51034, 51035, 51036, 51037, 51352, 51353). – Casual alien species new for the flora of Lombardia.

Semiarundinaria fastuosa is a bamboo native to central-southern Japan, cultivated as ornamental in Italy and escaped in Piemonte (Galasso et al. 2019b), to whose report we refer for the diagnostic characters of the species. Other individuals have been observed in the semi-abandoned garden of a property in Via F. Cavagnis, about 1 km away, testifying to a previous historical use of the species in Bergamo.

E. Banfi, G. Galasso

***Sesbania punicea* (Cav.) Benth. (Fabaceae)**

+ (INV) **SAR**. – Status change from naturalized to invasive alien for the flora of Sardegna.

The species is currently considered as naturalized, especially along freshwater courses (Galasso et al. 2018b). However, a large and spreading population has been observed along 'Bau sa Teula' (Villagrande Strisaili, Nuoro), where it is rapidly colonizing the coastal sector of the same river basin (Tortolì and Lotzorai, Nuoro). Accordingly, its status needs to be changed to invasive.

M. Fois, A. Cuena-Lombraña, G. Bacchetta

***Sorbaria tomentosa* (Lindl.) Rehder (Rosaceae)**

+ (CAS) **EMR**: Castell'Arquato (Piacenza), muro a secco di Via Sforza Caolzio (WGS84: 44.851962N, 9.868245E), muro a secco, 198 m, 1 September 2020, *D. Barberis, A. Mainetti, G. Nota* (FI). – Casual alien species new for the flora of Emilia-Romagna.

A single specimen, about 1 m tall and bearing fruits, has settled in the interstices of a dry-stone wall near private gardens and vegetable gardens. The identification was done according to Rahn (1989) and Tomaszewski (2001) who reported the hairiness of the follicles as diagnostic. This is in contrast with the identification key by Pignatti et al. (2019), which uses the characters number of nerves per leaflet and direction of the follicles in the infructescence, thus giving a false determination in favour of the similar *Sorbaria sorbifolia* (L.) A.Braun.

D. Barberis, A. Mainetti, G. Nota

***Stenotaphrum secundatum* (Walter) Kuntze (Poaceae)**

+ (CAS) **CAL**: Motta San Giovanni (Reggio Calabria), fraz. Lazzaro (WGS84: 37.973402N, 15.661162E), bordo strada, 4 m, 9 July 2021, *G. Mei* (REGGIO, *Herb. G. Mei*); Reggio Calabria (Reggio Calabria), Catona (WGS84: 38.190620N,

15.646328E), bordo strada, 20 m, 11 July 2021, leg. *V.L.A. Laface*, det. *V.L.A. Laface, C.M. Musarella, G. Spampinato* (FI, REGGIO). – Casual alien species new for the flora of Calabria.

V.L.A. Laface, G. Mei, C.M. Musarella

***Tecomaria capensis* (Thunb.) Spach (Bignoniaceae)**

+ (CAS) **LAZ**: Gaeta (Latina), pendice W di Monte Orlando, Via Santa Maria Ausiliatrice (WGS84: 41.208235N, 13.581049E), boscaglie incolte sul bordo strada, 88 m, 9 January 2020, *S. Ravetto Enri, M. Pittarello, M. Lonati* (FI). – Casual alien species new for the flora of Lazio.

A small population of this species, occupying an area of approximately 5×5 m, has been found on the edge of the road between Gaeta and Monte Orlando. Several seedlings occur near an adult individual.

S. Ravetto Enri, M. Pittarello, M. Lonati

***Ulmus pumila* L. (Ulmaceae)**

+ (NAT) **TOS**: Siena (Siena), Viale Sardegna (strada SR2) (WGS84: 43.327430N, 11.331531E), margini stradali e ruderi, 281 m, 14 June 2020, leg. *E. Fanfarillo*, det. *E. Fanfarillo, T. Fiaschi, C. Angiolini* (FI, SIENA); *ibidem*, 22 February 2021, leg. *E. Fanfarillo*, det. *E. Fanfarillo, T. Fiaschi, C. Angiolini* (FI, SIENA); *ibidem*, 13 April 2021, leg. *T. Fiaschi*, det. *T. Fiaschi, E. Fanfarillo, C. Angiolini* (FI, SIENA). – Naturalized alien species new for the flora of Toscana.

Ulmus pumila is present with several individuals, sometimes of medium-large size, colonizing roadsides, sidewalks, and wasteland.

E. Fanfarillo, T. Fiaschi, C. Angiolini

***Yucca aloifolia* L. (Asparagaceae)**

+ (CAS) **TAA**: Naturno (Bolzano), Val Venosta (WGS84: 46.6506277N, 10.9882332E), radura boschiva di un bosco termofilo a roverella, 590 m, 1 August 2020, *G. Bonari* (FI). – Casual alien species new for the flora of Trentino-Alto Adige.

Sporadic individuals of this species were observed.

G. Bonari, E. Kindermann, C. Wellstein

Nomenclatural and distribution updates from other literature sources

Nomenclatural, status, distribution updates, and corrections to Galasso et al. (2018a) are provided in Suppl. material 1.

G. Galasso, F. Bartolucci

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Supplementary material 1

Supplementary data

Authors: Gabriele Galasso, Fabrizio Bartolucci (Eds)

Data type: species data

Explanation note: 1. Nomenclatural updates; 2. Note updates; 3. Distribution updates; 4. Synonyms, misapplied or included names.

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RESEARCH ARTICLE

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Chromosome numbers for the Italian flora: 12

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Abstract

In this contribution, new chromosome data obtained on material collected in Italy are presented. It includes the first counts for *Hieracium glanduliferum* s.str. and *H. tenuiflorum*, counts for two *Armeria* species endemic to Italy, and for *Onopordum illyricum* subsp. *illyricum*. We also present here the first chromosome count of *Allium permixtum* for Italy, where this species is known for few localities.

Keywords

Amaryllidaceae, Asteraceae, cytotaxonomy, endemics, Plumbaginaceae, polyploidy

How to contribute

Texts concerning new chromosome data should be submitted electronically to Giovanni Astuti (giovanni.astuti@unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

***Allium permixtum* Guss. (Amaryllidaceae)**

Chromosome number. $2n = 24$ (Fig. 1).

Voucher specimen. Italy. Abruzzo. Rocca di Cambio (L'Aquila), Monte Ocre, vallone Canavine conca di Settacque, prati umidi, 1570 m (WGS84: 42.2471477N, 13.442932E), 22 June 2005, *F. Conti, F. Bartolucci, L. Bernardo, D. Iamonico, M. Latini, I. Londrillo, R. Lorenzetti, N. Ranalli, E. Pellegrini, L. Peruzzi, N. Ranalli, E. Scassellati, D. Tinti, V. Viscosi* (APP15719, APP15721).

Method. Squash preparations were made on root tips obtained from cultivated plants. Root tips were pre-treated with 0.4% colchicine for 4 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C, the tips were stained with leuco-basic fuchsine.

Observations. *Allium permixtum* occurs in Italy and the Balkan Peninsula (Anderson 1991; Conti 1995; Cheshmedzhiev and Marinov 2009; Brullo et al. 2010; Hallaçi and Shuka 2013; Bartolucci et al. 2018). In Italy, this species was not recently confirmed in Sicily (Brullo et al. 2010) and is very rare in Abruzzo, where it has been recorded only for some protected areas of the central Apennines, such as the National Park of Gran Sasso and Monti della Laga (Conti 1998; Conti and Bartolucci 2016) and the National Park of Abruzzo, Lazio and Molise (Conti 1995; Conti and Bartolucci 2015). The population studied here represents the first record for the Regional Park of Sirente-Velino (Abruzzo, central Italy). This is also the first chromosome count for the species in Italy (Bedini and Peruzzi 2021 onwards), and it agrees with previous counts made for Greece and Bulgaria where both diploid and triploid cytotypes have been reported ($2n = 2x = 16$, $2n = 3x = 24$) (Tzanoudakis 1982, 1986; Cheshmedzhiev and Marinov 2009, in both sources under the name *A. phthioticum*).

F. Bartolucci, F. Conti

Figure 1. *Allium permixtum* Guss. from Monte Ocre (Rocca di Cambio, L'Aquila), $2n = 24$. Scale bar: 10 µm.

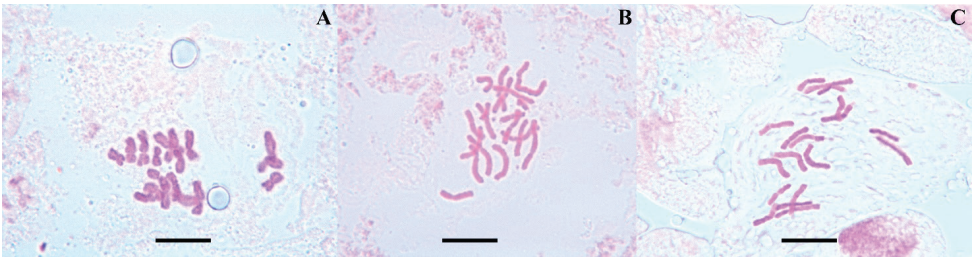

Figure 2. *Armeria denticulata* (Bertol.) DC. from (**a**) Castello della Brina (Sarzana, La Spezia), (**b**) Poggio Pelato (Rosignano, Livorno), and (**c**) Monteferrato (Prato), $2n = 18$. Scale bar: 10 µm.

Armeria denticulata (Bertol.) DC. (Plumbaginaceae)

Chromosome number. $2n = 18$ (Fig. 2).

Voucher specimens. Italy. Liguria. Sarzana (La Spezia), nei pressi del castello della Brina (WGS84: 44.14732N, 9.95068E), 11 June 2021, *L. Sandroni* (PI049856–PI049876). **Toscana.** Rosignano Marittimo (Livorno), Poggio Pelato (WGS84: 43.43525N, 10.43103E), 25 May 2021, *L. Sandroni & G. Astuti* (PI049924–PI049948); Prato, Monteferrato (WGS84: 43.92042N, 11.07572E), 9 June 2021, *L. Sandroni* (PI049902–PI049923).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. This species is endemic to ophiolitic substrates of western Liguria and Toscana (Arrigoni 2015). The type locality of this species is in Liguria, but very close to the border with Toscana, where the vast majority of populations is found. We report here three counts, one obtained from plants collected in the type locality and two from elsewhere in Toscana. These data confirm the $2n = 18$ chromosome number reported for Impruneta, Firenze (Arrigoni et al. 1976, 1980). This is also the only known number for the entire genus (Rice et al. 2014+).

Armeria saviana Selvi (Plumbaginaceae)

Chromosome number. $2n = 18$ (Fig. 3).

Voucher specimen. Italy. Toscana. Arcidosso (Grosseto), Stribugliano, lungo la Via del Campo Sportivo (WGS84: 42.85868N, 11.47165E), 28 May 2021, *L. Sandroni & G. Astuti* (PI049882–PI049886).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

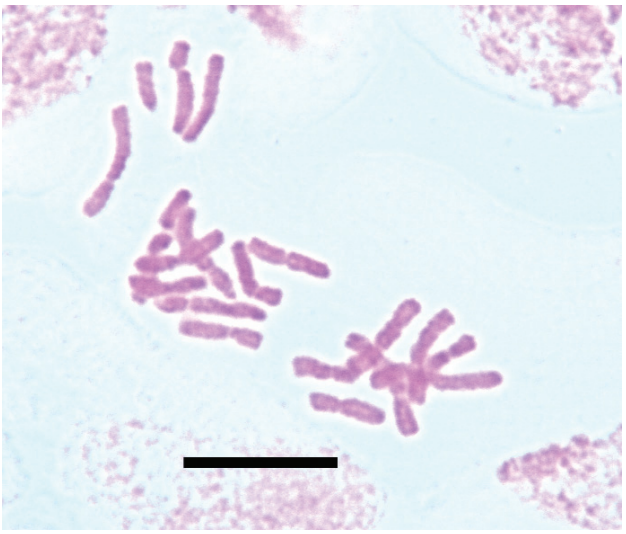

Figure 3. *Armeria saviana* Selvi from Stribugliano (Arcidosso, Grosseto), 2*n* = 18. Scale bar: 10 μm.

Observations. This species is endemic to southern Toscana, and has been only recently described (Selvi 2009). It shows a very narrow distribution range on the surroundings of Monte Labbro (Grosseto). *Armeria saviana* is morphologically very similar to *A. denticulata*, from which it can be distinguished for a few leaf and inflorescence features. The chromosome count reported here confirms the previous count published by Selvi (2009).

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Hieracium glanduliferum Hoppe subsp. *glanduliferum* (Asteraceae)

Chromosome number. 2*n* = 27 (Fig. 4).

Voucher specimen. Italy. Emilia-Romagna. Ventasso (Reggio Emilia), Monte Prado (WGS84 44.252477N, 10.400372E), tra la sella e la vetta del Monte Prado, 1980 m, 25 July 2019, *S. Orsenigo* (PAV).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. *Hieracium glanduliferum* subsp. *glanduliferum* is a south European alpine species with isolated populations that occur in the northern Apennines on Monte Prado, Monte Cusna, and Monte Cimone (Foggi and Ricceri 1989; Gottschlich 2018). This is the first count for this taxon for Italy, whereas other counts have been published for *Hieracium glanduliferum* Hoppe subsp. *piliferum* Hoppe ex Nägeli & Peter, for which two different chromosome numbers have been reported so far, 2*n* = 3*x* = 27 from Italy, Switzerland, and Austria (Scholte 1977; Chrtek et al. 2009) and 2*n* =

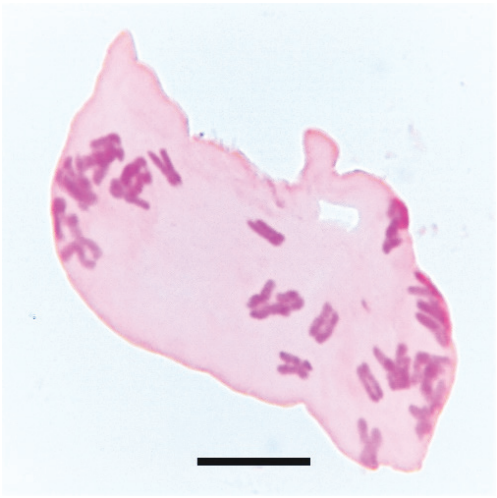

Figure 4. *Hieracium glanduliferum* Hoppe subsp. *glanduliferum* from Monte Prado (Ventasso, Reggio Emilia), 2*n* = 27. Scale bar: 10 µm.

4*x* = 36 from Austria and Slovakia (Mráz 2003; Chrtek et al. 2009, in all sources under the name *Hieracium piliferum* Hoppe).

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***Hieracium tenuiflorum* Arv.-Touv. (Asteraceae)**

Chromosome number. 2*n* = 27 (Fig. 5).

Voucher specimen. Italy. Liguria. Sanremo (Imperia), San Romolo (WGS84 43.84670N, 7.740880E), pendici del Monte Bignone, margine della strada verso Sanremo, 570 m, 28 May 2021, *M. Ottonello, B. Cera, S. Orsenigo* (PAV).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. *Hieracium tenuiflorum* is a south European perennial species (Gottschlich 2018). It was originally described for Monte Bignone, Monte Ceppo, and Monte Arpetta (Bicknell 1896), in the Ligurian Alps. In Italy, this species is widespread throughout the Alps and pre-Alps, but it occurs also in the northern and central Apennines south up to Abruzzo (Bartolucci et al. 2018).

The chromosome number 2*n* = 3*x* = 27, reported here for the first time, is consistent with observations made in other taxa belonging to *H.* sect. *Hieracium*, such as those of the *H. murorum* aggregate in Italy (Selvi and Fiorini 1996; Geraci et al. 2007) and Europe (Chrtek et al. 2009).

S. Orsenigo, B. Cera, A. Giacò

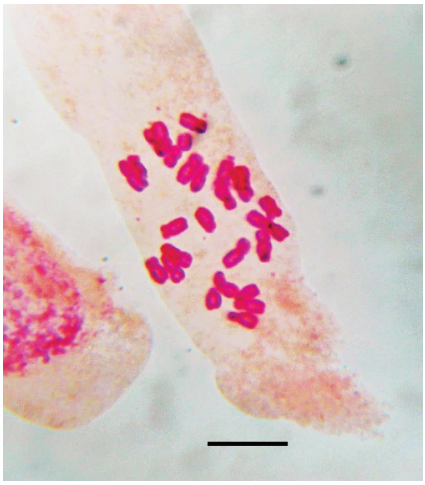

Figure 5. *Hieracium tenuiflorum* Arv.-Touv. from San Romolo (Sanremo, Imperia), 2*n* = 27. Scale bar: 10 μm.

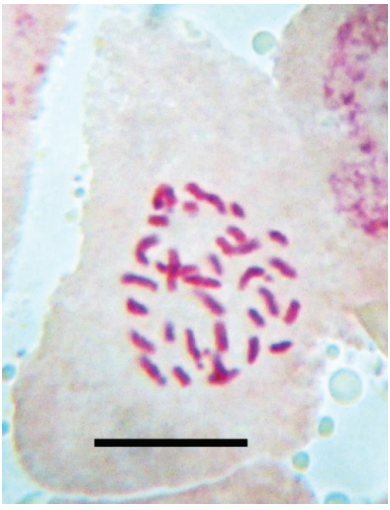

Figure 6. *Onopordum illyricum* L. subsp. *illyricum* from c.da Rossillo (San Marco Argentano, Cosenza), 2*n* = 34. Scale bar: 10 μm.

Onopordum illyricum L. subsp. *illyricum* (Asteraceae)

Chromosome number. 2*n* = 34 (Fig. 6).

Voucher specimen. Italy. Calabria. San Marco Argentano (Cosenza), c.da Rossillo (WGS84: 39.61343N, 16.22801E), 16 August 2021, *L. Peruzzi* (seeds collected and deposited at the germplasm bank of the Department of Biology, University of Pisa; IPEN: IT-0-PI-2021-0419).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in

Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. This species occurs in southern Europe, from the Iberian Peninsula to the Balkans, in Turkey, and in Syria (Greuter 2006+). Although several chromosome counts are available in the literature (Moore and Frankton 1962; Chuksanova et al. 1968; Valdes 1970; Kuzmanova et al. 1979; Snogerup 1995), no data have been published so far for Italy, where two subspecies can be found, i.e., *O. illyricum* subsp. *illyricum*, distributed throughout south-central Italy (including Sardegna and Sicilia), and *O. illyricum* subsp. *carduncul*us (Boiss.) Arènes, which is known only for Sicilia (Bartolucci et al. 2018). We confirm here the chromosome number $2n = 34$ previously reported for the species.

G. Astuti, A. Giacò, L. Peruzzi

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