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RESEARCH ARTICLE

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Contribution to the floristic knowledge of Mount Pennino massif (Umbria-Marche Apennines, central Italy)

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Abstract

The inventory of the taxa collected in 2021 during the annual field trip of the Working Group for Floristics, Systematics, and Evolution of the Italian Botanical Society is reported. The field trip was held in the Mount Pennino massif, between Umbria and Marche administrative regions (central Italy). The flora documented for the study area amounts to 413 specific and subspecific taxa (including one hybrid), belonging to 234 genera and 64 families. Thirty-seven taxa are endemic to Italy; 42 are included in the IUCN Red List of the Italian Flora. Only three alien taxa were found. Ten taxa and one hybrid are considered floristic novelties because either new or confirmed for the regional flora of Umbria and/or Marche. Particularly, 6 taxa are new and 4 are confirmed for Umbria; one taxon and one hybrid are new for Marche.

Keywords
Central Apennines, Endemic, Floristic novelties, Herbaria, Italian vascular flora, Marche, Umbria

Introduction

In this paper, the results of the field trip of the Working Group for Floristics, Systematics, and Evolution of the Italian Botanical Society held in 2021 in the Mount Pennino massif (central Apennines), organised by the botanists of the Herbarium Universitatis Camerinensis (School of Biosciences and Veterinary Medicine, University of Camerino, Camerino) are presented. Floristic excursions of this Working Group are traditionally aimed at exploring poorly known areas from the floristic point of view and publishing the related data (e.g. Conti et al. 2007; Domina et al. 2015; Bartolucci et al. 2019; Stinca et al. 2019; Roma-Marzio et al. 2020; Barone et al. 2023).

The incentive for the organization of the field trip has come from the observation of Andrea Batelli (1854–1917), a botanist and professor at the University of Perugia (Gigante et al. 2021):

> *Mount Pennino is forgotten; it can be called a second-rate mountain in that area of famous mountains. It is the story of the bad neighbours to which it owes his misfortune. On the one hand, Mount Subasio contrasts with the celebrity of history, on the other, the Sibillini Mountains with that of altitude, the pious legend that proclaims the eternal brotherhood of men with nature, and the woolly edelweiss, a reminder of the Alps, are the enemies of the misty colossus. But mediocrity must still have its historians, even more so when it has many reasons to be rightly celebrated* (Batelli 1887a).

Batelli explored Mount Pennino and surrounding areas at the end of the 19th century, reporting 104 taxa (1886, 1887b). We must admit that more than 130 years later, the above considerations are still valid, as the knowledge about the vascular flora of this area has not increased so much. A few reports indeed have been published, most of them being single records of taxa new to the flora of Umbria (Falcinelli and Donnini 2007, 2008, 2012; Ballelli et al. 2012; Falcinelli et al. 2013; Gigante et al. 2014; Gubellini et al. 2014; Bartolucci et al. 2016).

The “Anarchive” database (http://www.anarchive.it/anArchive/index.jsp) hosts 60 specimens’ records, referred to 46 taxa, collected in Mount Pennino massif by F. Pedrotti (CAME) and V. Marchesoni (CAME, FI) in 1962, G. Zodda (AQUI) in 1967, A. Gabellini (SIENA) in 1999, F. Falcinelli (PERU) in 2006, 2011, and 2012, F. Falcinelli and D. Donnini (PERU), S. Ballelli, D. Gigante, and R. Venanzoni (PERU), and S. Ballelli, D. Gigante, L. Gubellini, F. Landucci, F. Maneli, and R. Venanzoni (PERU) in 2009.

Thus, in 2021 we decided to address the yearly floristic investigation in such area, because largely unexplored in the recent decades.

Materials and methods

Study area

The study area is localized in the Nocera Umbra (Perugia) and Fiuminata (Macerata) municipalities, in the Rivers Topino and Potenza valleys (WGS84: 43.101210°N, 12.888778°E, coordinate system WGS84; Fig. 1). The altitude ranges from 670 to 1,571 m a.s.l., at the top of Mount Pennino.

From the geological viewpoint, the Mount Pennino massif is characterized by limestone bedrock (Servizio Geologico d'Italia 1966).

Yearly average rainfall ranges between 1,200 and 1,300 mm (data drawn from Bagnara and Annifo meteorological stations, Orsomando et al. 1999) and is concentrated in the autumn–winter period, with a maximum in November–December and a minimum in summer (July). Annual average temperature ranges from about 7.5 to 11.5 °C (data drawn from Rasiglia and Pintura di Bolognola meteorological stations, Orsomando et al. 1999), with the hottest months in summer (July–August) and the coldest in winter (December–January). The investigated area shows a temperate oceanic weak semicontinental to weak euoceanic macrobioclimate, ranging from lower supratemperate to upper supratemperate thermotype and from upper humid to lower hyperhumid ombrotypes (Pesaresi et al. 2017).

The forest vegetation is dominated by coppiced *Fagus sylvatica* L. subsp. *sylvatica* woods and, secondly, by *Quercus cerris* L. and *Ostrya carpinifolia* Scop. woods. Grasslands are characterized by *Bromopsis erecta* (Huds.) Fourr., *Brachypodium rupestre* (Host) Roem. & Schult., *Sesleria nitida* Ten., and *S. juncifolia* Suffren.

Data collection

To maximize vascular flora sampling, the study area (about 700 hectares) was divided into six sectors (Fig. 1) and intensively sampled during the period 24–26 June 2021 by 20 participants (Table 1, Suppl. material 1: 1). The work, together with the floristic list, was coordinated and drawn up by the organisers (F.M. Tardella, R. Pennesi, S. Ballelli, and A. Catorci) with the contribution of all participants to the excursion. The floristic list was prepared according to the protocol already used in previous contributions (e.g. Bartolucci et al. 2019; Stinca et al. 2019; Barone et al. 2023).

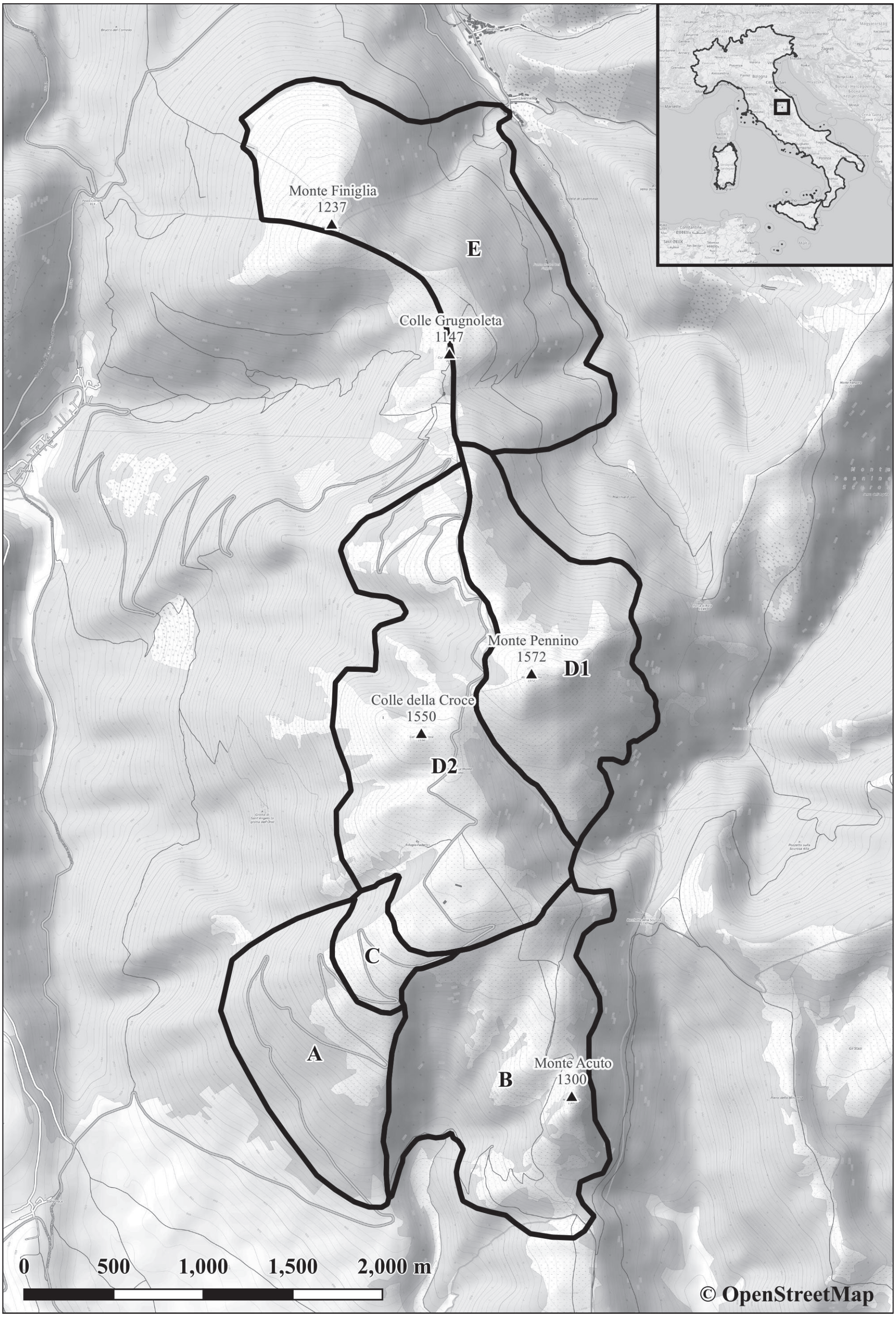

Figure 1. Study area and sampling sites. Data extracted from OpenStreetMap is licensed on terms of the Open Database License (https://www.openstreetmap.org/copyright/en).

Plant identification

The taxonomic identification was carried out at first by the single collectors using standard floras (e.g., Tutin et al. 1968, 1972, 1976, 1980, 1993; Pignatti 1982; Pignatti et al. 2017–2019) and other publications (e.g., Conti et al. 2011, 2021; Arrigoni 2014; Rešetnik et al. 2014; Peccenini and Polatschek 2016; Gargano et al. 2023). Herbarium specimens prepared are deposited in public and private herbaria (Suppl. material 1: 2). Each participant prepared a list of the identified specimens and sent it to the organisers, who merged the lists.

A revision of the critical samples collected during the fieldwork was carried out by the participants on 23–25 February 2022, at the Herbarium Universitatis Camerinensis (School of Biosciences and Veterinary Medicine, University of Camerino, Camerino). This revision was followed by specific studies and comparisons of unidentified taxa. Particularly, some herbarium specimens belonging to critical genera were sent to experts for determination: *Anthoxanthum* L., *Brachypodium* P.Beauv., and *Koeleria* Pers. (E. Banfi, Milano); *Anthyllis* L. (F. Conti, Barisciano); *Armeria* Willd. (M. Tiburtini, Pisa); *Hieracium* L. (G. Gottschlich, Tübingen); *Juniperus* L. (F. Roma-Marzio, Pisa); *Orobanche* L. (G. Domina, Palermo); *Thymus* L. (F. Bartolucci, Barisciano).

The nomenclature used to draw up the floristic list (see Suppl. material 1: 3) follows the checklists of the vascular flora native (Bartolucci et al. 2024) and alien (Galasso et al. 2024) to Italy, except for native hybrids, not considered in the above-mentioned checklists. The systematic order of the families follows Bartolucci et al. (2024) and Galasso et al. (2024). Taxa have been ordered alphabetically within each family. For each taxon, the following information is reported: accepted name, endemic or alien status, sampling site (see Table 1), Herbarium (see Suppl. material 1: 2).

Abbreviations or symbols used in the floristic list are:

E	Italian endemic (Peruzzi et al. 2014, 2015; Bartolucci et al. 2024);
A	alien taxon [CAS (Casual), NAT (Naturalized)] (Galasso et al. 2024);
*	taxon confirmed for the regional flora;
**	new record for the regional flora.

Results

During the field investigations, 1,461 samples of vascular plants were collected, belonging to 413 species and subspecies, 234 genera, and 64 families (Suppl. material 1: 3), including one hybrid (*Brachypodium retusum* (Pers.) P.Beauv. × *B. rupestre*). Thirty–seven taxa are endemic to Italy, and only three are alien (*Centaurea cyanus* All., *Hordeum vulgare* L. subsp. *vulgare*, and *Matricaria discoidea* DC. subsp. *discoidea*). Forty–two taxa are included in the IUCN Red List of the Italian Flora (Rossi et al. 2013; Orsenigo et al. 2018, 2020). Ten taxa and one hybrid are floristic novelties, because either new or confirmed for the regional flora of Umbria and/or Marche.

Table 1. List of sampling sites, with reference code, locality name, altitude, main habitats, geographic coordinates, and date of collection.

Code	Locality	Habitat	Altitude (m a.s.l.)	Coord. (WGS84)	Date
A	Umbria. Monte Pennino, south-western slope (Nocera Umbra, Perugia)	deciduous woods, grasslands and rocky slopes	1,000–1,300	43.081397°N, 12.874625°E	24 June 2021
B	Umbria. Monte Acuto (Nocera Umbra, Perugia)	deciduous woods and grasslands	1,000–1,400	43.080346°N, 12.887634°E	25 June 2021
C	Umbria. Monte Pennino, south-western slope (Nocera Umbra, Perugia)	grasslands	1,300–1,450	43.087597°N, 12.879627°E	25 June 2021
D1	Marche. Monte Pennino, top (Fiuminata, Macerata)	grasslands	1,300–1,571	43.100159°N, 12.890130°E	25 June 2021; 26 June 2021
D2	Umbria. Monte Pennino, top, and Colle della Croce (Nocera Umbra, Perugia)	deciduous woods, shrublands, grasslands	1,300–1,548	43.097945°N, 12.882154°E	25 June 2021; 26 June 2021
E	Marche. Colle Grugnoleta and Monte Finiglia, northern and north-eastern slopes (Fiuminata, Macerata)	deciduous woods, clearings, hygrophilous vegetation, humid environments	680–1,250	43.122359°N, 12.886932°E	26 June 2021

Anthoxanthum ovatum Lag. and the hybrid *Brachypodium retusum* × *B. rupestre* are new to the flora of Marche.

Six taxa are new to the flora of Umbria: *Anthoxanthum ovatum*, *Dianthus carthusianorum* L. subsp. *carthusianorum*, *Erysimum apenninum* Peccenini & Polatschek, *Hieracium pseudogrovesianum* Gottschl., *Koeleria lucana* Brullo, Giusso & Miniss., *Koeleria pyramidata* (Lam.) P.Beauv.

Four taxa are confirmed for the flora of Umbria: *Cynanchica pyrenaica* (L.) P.Caputo & Del Guacchio subsp. *neglecta* (Guss.) P.Caputo & Del Guacchio, *Polygala nicaeensis* Risso ex W.D.J.Koch subsp. *italiana* (Chodat) Arrigoni, *Stachys germanica* L. subsp. *germanica*, *Trigonella elegans* (Salzm. ex Ser.) Coulot & Rabaute.

Discussion

In the Mount Pennino massif, 413 taxa (species and subspecies) were found. Forty–two of them (10.2% of the total floristic list) are currently included in the IUCN Red List of the Italian Flora (Orsenigo et al. 2018, 2020), 36 of which are endemic (26 Least Concern, 9 Data Deficient, and one Nearly Threatened). Only the recently recognised endemic *Siler montanum* Crantz subsp. *stabianum* (Lacaita) F.Conti & Bartolucci is not included in the Red List. Conversely, only three taxa are alien.

Eleven floristic novelties were reported for the floras of Marche and/or Umbria.

Our records from Umbria and Marche extend the findings of *Anthoxanthum ovatum* in central Italy. Before this study, its Italian distribution was indeed restricted to Toscana, Campania (historical record), Calabria, Sicilia, and Sardegna (Bartolucci et al. 2024).

The records of *Dianthus carthusianorum* subsp. *carthusianorum*, *Erysimum apenninum*, and *Hieracium pseudogrovesianum* in Umbria, as well as the confirmation of *Cynanchica pyrenaica* subsp. *neglecta*, *Stachys germanica* subsp. *germanica*, and *Trigonella elegans* fill a gap in the regional distribution of these taxa in central Italy.

Polygala nicaeensis subsp. *italiana* was reported only for Emilia–Romagna and Toscana, while it is considered doubtful in Piemonte, not confirmed in San Marino (Bartolucci et al. 2024), and one historical record was known for Umbria ("In Appennino Umbro, *leg.* Piccinini 1865" (FI); see Arrigoni 2014).

Our findings of the endemic *Koeleria lucana* in Umbria are noteworthy because they are currently the northernmost records of its distribution range (Bartolucci et al. 2024).

The natural hybrid between *Brachypodium retusum* and *B. rupestre* known also in Toscana (Bonari et al. 2019), was found near the top of Mount Pennino and is reported for the first time in the Marche region.

Some of the specimens collected in the study area, preliminarily attributed to *Centaurea arrigonii* Greuter, require additional investigations aimed at clarifying their taxonomic identity.

In conclusion, the finding of eleven floristic novelties, the percentage of Italian endemic and Red List taxa in the study area, slightly higher than that of other similar mountain territories in the central Apennines (Ballelli et al. 2002, 2020), as well as their number in the Umbrian sector (30, i.e. the 25% of the Italian endemic taxa occurring in Umbria (see Bartolucci et al. 2024) suggest a high conservation value of the flora in this sector of Umbria-Marche Apennines. For this reason and considering that the floristic survey lasted only two and a half days, the study area deserves further investigation.

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Supplementary material 1

Supplementary data

Authors: Federico M. Tardella, Riccardo Pennesi, Sandro Ballelli1, Laura Cancellieri, Giuseppe Caruso, Francesco Falcinelli, Simonetta Fascetti, Gabriele Galasso, Daniela Gigante, Valentina L.A. Laface, Edda Lattanzi, Paolo Lavezzo, Carmelo M. Musarella, Lorenzo Pinzani, Giacomo Quattrini, Leonardo Rosati, Francesco Spada, Adriano Stinca, Agnese Tilia, Andrea Catorci

Data type: docx

Explanation note: 1. Participants in the field trip of the Working Group for Floristics, Systematics, and Evolution of the Italian Botanical Society (24–26 June 2021). 2. Public and private herbaria in which the collected exsiccata are stored. Codes of public herbaria follow the Index Herbariorum (available at http://sweetgum.nybg.org). 3. Inventory of the taxa collected during the field trip held in 2021 in Mount Pennino massif (Umbria-Marche, central Italy). The code of the sampling site (see Table 1 in the main text) and Herbaria (in brackets; code in Suppl. material 1: 2).

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Using drone imagery and group field activities for an in-depth investigation of the vascular flora: a case study in the Rocca di Novara Massif (NE Sicily, Italy)

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Abstract

Rocky habitats are suitable refuges for numerous rare and endemic plants. Due to their inaccessibility and the unreliability of traditional survey methods, assessing the survival status of these plants is quite a hard task. In order to address this sampling issue, we employed the drones and carried several field activities to investigate the vascular flora of Rocca di Novara massif located in Northeastern Sicily (NE Sicily hereafter). Located on the southern border of the Peloritani Mountains, Rocca di Novara, also known as Rocca Salvatesta, is a small carbonate massif and it represents one of the highest and most characteristic peaks in NE Sicily. According to our investigation, 300 taxa have been recorded, belonging to 189 genera and 51 different families. The results obtained show the presence of 13 endemic species to Italy (4.3%) and 12 strictly endemic to Sicily (4%). Some taxa of high phytogeographical value have been detected, such as *Aubrieta columnae* subsp. *sicula*, *Daphne oleoides* subsp. *oleoides*, *Edraianthus graminifolius* subsp. *siculus*, *Fritillaria messanensis*, *Odontites bocconei* subsp. *bocconei*, *Phelipanche gussoneana*, *Saxifraga callosa* subsp. *australis*. This latest species was rediscovered after 100 years on the high cliffs of Rocca di Novara thanks to the use of the drone imagery. Our results provide valuable insights into the floristic diversity of Rocca di Novara and offers a solid starting point for further phytogeographical and floristic studies on the vascular plants of the Peloritani district.

Keywords
Conservation, cliffs, drone imagery, endemic plants, Peloritani district, rare plants, red list

Introduction

This paper aims to assess the vascular plant diversity of Rocca di Novara, one of the highest and most characteristic peaks of the Peloritani Mountains (NE Sicily). The study stems from a field activity organized by the Italian Botanical Society as part of an "Intensive course in the determination of vascular plants", held on May 21, 2022. The choice to carry out a floristic survey in Rocca di Novara was prompted by limited availability of floristic literature and at the same time the presence of a rupicolous vascular flora of a significant phytogeographic interest. The Peloritani mountain range stretches for about 70 km along the north-eastern portion of the island, and is characterized by numerous reliefs with peaks that slightly exceed 1,300 m a.s.l. The morphology of the mountain range is characterized by a series of peaks, ridges, screes that go downstream into deep gorges with countless watercourses which eventually open into braided streams, often filled with debris and torrential flows (Blasi 2010; Goswami et al. 2011; Sciandrello et al. 2015). Geologically, this territory is characterized by the prevalence of crystalline and intrusive metamorphic rocks (gneisses, schists, mycaschists), issuing from different stratigraphic units and give rise to a variable and tormented morphology. In the territory sedimentary rocks such as limestones, quartz sandstones and, sandy deposits crop out in this territory, too (Appel et al. 2011; Fiannacca et al. 2013). The harsh topography of Rocca di Novara, chacterised by steep slopes, scree and the rocky cliffs acts as a physical barrier to most anthropogenic interventions; as strongly reduce accessibility also to botanists, contributing to preserve the rarest and most threatened plant species. Indeed, the floristic investigation of such inaccessible habitats has been possible thanks to drone technology. Most frequenty, we let drones flying vertically along the cliff faces while taking intermittent photographic samples for species identification (Zhou et al. 2021). This method can be used to identify most vascular plants on the cliffs and to analyze plant diversity (Zhou 2022; Zhu 2022; Kim et al. 2023; Gao et al. 2024; Tavilla et al. 2024).

From a floristic point of view, this area is characterized by a significant number of species belonging mainly to European, Eurasian and Circumboreal elements, which probably arrived during the glaciations. Furthermore, the presence of around 15 species strictly endemic to the Peloritani Mountains deserves to be mentioned, as well as the occurrence of a rather high amount of species in common with Calabria but missing in the rest of the island (Brullo et al. 1995; Sciandrello et al. 2015). Previously, Rocca di Novara has rarely been specifically investigated, except for a very short list of taxa occurring in this area published by Nicotra (1880) and Picone et al. (2000). Other sporadic data are reported by Gussone (1843), Borzì (1887), Nicotra and Campagna (1908), and Giardina (1995). This paper provides updated data on its flora and analyzes some endemic and threatened species, to enhance their conservation and management.

Materials and methods

Data collection and analysis

Field activities have been carried out in May 2022, as well as in May, June and November 2023 with the aim of deepening the research and gaining a more complete understanding of the species of seasonal flora. Observations we conducted across different seasons to capture the varied botanical landscape. Some plant specimens were collected in the field and subsequently dried and deposited at the Herbarium of the University of Catania (CAT) and in the Herbarium Mediterraneum Panormitanum (PAL). The identification of the species was mainly made by using the recent flora of Italy (Pignatti et al. 2017–2019). The nomenclature used follows mainly Bartolucci et al. (2024). Drone surveys were carried out in May and June 2022 in order to reach inaccessible rock cliffs. All drone flights were conducted using a DJI Mavic Mini.

Study area

Rocca di Novara (37°59'41.42"N, 15°08'48.21"E), also called "Rocca Salvatesta" or popularly "Cervino di Sicilia", due to its characteristic truncated-conical shape, reaches an altitude of 1340 m a.s.l. (Fig. 1). The relief is located on the southern border of the Peloritani Mountains, in the territories of Novara di Sicilia and Fondachelli Fantina. From the geological point of view, the Rocca di Novara Unity is constituted by limestone formation of Mesozoic origin overlapping on a siliceous basement (Atzori et al. 1979; Lentini and Carbone 2014). The woody vegetation is mainly represented by

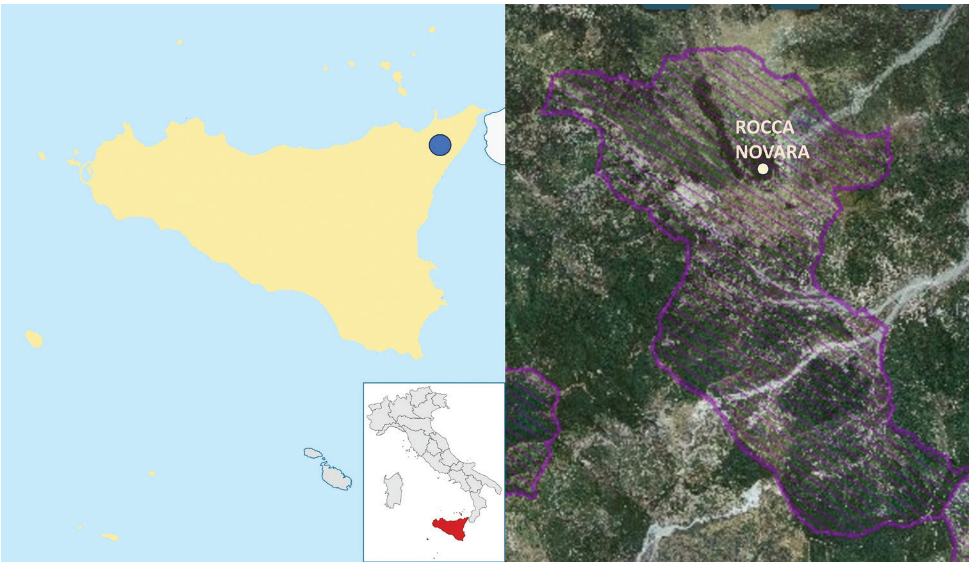

Figure 1. Map of surveyed area with the boundaries of the ZSC "Rocca di Novara" (ITA030006).

scattered nuclei of holm oak woods, where *Quercus ilex* L. is associated with isolated individuals of hop-hornbeam (*Ostrya carpinifolia* Scop.). However, the original forest vegetation is almost completely replaced by aspects of shrubby vegetation referable to the class *Rhamno catharticae-Prunetea spinosae* Rivas Goday & Borja ex Tüxen 1962 or, more frequently, by aspects that are highly degraded due to grazing and frequent fires, dominated by *Pteridium aquilinum* (L.) Kuhn subsp. *aquilinum* (*Pteridio-Tanacetetum siculi* Brullo & Marcenò, 1985), belonging to the class *Artemisietea vulgaris* Lohmeyer, Preising & Tüxen ex Von Rochow 1951. However, the ancient presence of mesophilous woods is demonstrated by the persistence of some nemoral species that find a refuge habitat near the shady cliffs or even in the shady areas under the tufts of *Pteridium aquilinum* subsp. *aquilinum*. Among these species, *Symphytum gussonei* F.W.Schultz, *Melittis melissophyllum* subsp. *albida* (Guss.) P.W.Ball (Fig. 2F), *Stachys sylvatica* L. (Fig. 2B), *Galanthus regina-olgae* subsp. *vernalis* Kamari and *Fritillaria messanensis* Raf. subsp. *messanensis* (Fig. 2G) deserve to be mentioned. The carbonate cliffs (Fig. 3A) host a very peculiar casmophilous vegetation with some species that have their only station here on the Peloritani mountains, such *Saxifraga callosa* subsp. *australis* (Moric.) Pignatti ex Tavilla & Del Guacchio, *Daphne oleoides* Schreb. subsp. *oleoides* and *Aubrieta columnae* subsp. *sicula* (Strobl) M.A.Koch, D.A.German & R.Karl. This orophilous vegetation may be referred to the class *Asplenietea trichomanis* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977. Overall, the current vegetation shows a high degree of degradation. This is due to several anthropogenic disturbance factors such as intensive grazing, frequent fires and other activities as excursions and religious pilgrimages to reach the cross at the top..

Currently, the entire surveyed area is part of the Sicilian Natura 2000 network and belongs to the SAC ITA030006 “Rocca di Novara” .

Results

A total of 300 plant taxa have been recorded, belonging to 190 genera and 51 families (Appendix 1). The pteridophytes account for 10 taxa, i.e. *Asplenium ceterach* L. subsp. *ceterach*, *A. trichomanes* L. subsp. *trichomanes*, *A. onopteris* L. (Aspleniaceae), *Polypodium cambricum* L. (Polypodiaceae), *Anogramma leptophylla* (L.) Link, *Adiantum capillus-veneris* L. (Pteridaceae), *Pteridium aquilinum* subsp. *aquilinum* (Dennstaedtiaceae), *Dryopteris pallida* subsp. *pallida* (Bory) Maire & Petitm. subsp. *pallida*, *Polystichum setiferum* (Forssk.) T.Moore ex Woyn. (Dryopteridaceae) and *Selaginella denticulata* (L.) Spring. However, Angiospermae is by far the most represented taxonomic group. In particular, 53 taxa belonging to 10 families are monocots (17.75%), while the remaining 247 taxa are eudicots (82.35%).

Nine families include more than 10 taxa each, Asteraceae with 37 taxa (12.3%), Fabaceae with 30 (10%), Lamiaceae with 24 (8%), Poaceae with 21 (7%), Caryophyllaceae with 18 (6%), Brassicaceae with 16 (5.3%), Orchidaceae with 14 (4.7%), Apiaceae with 11 (3.7%) and Plantaginaceae with 10 taxa (3.3%) (Fig. 4). The relatively

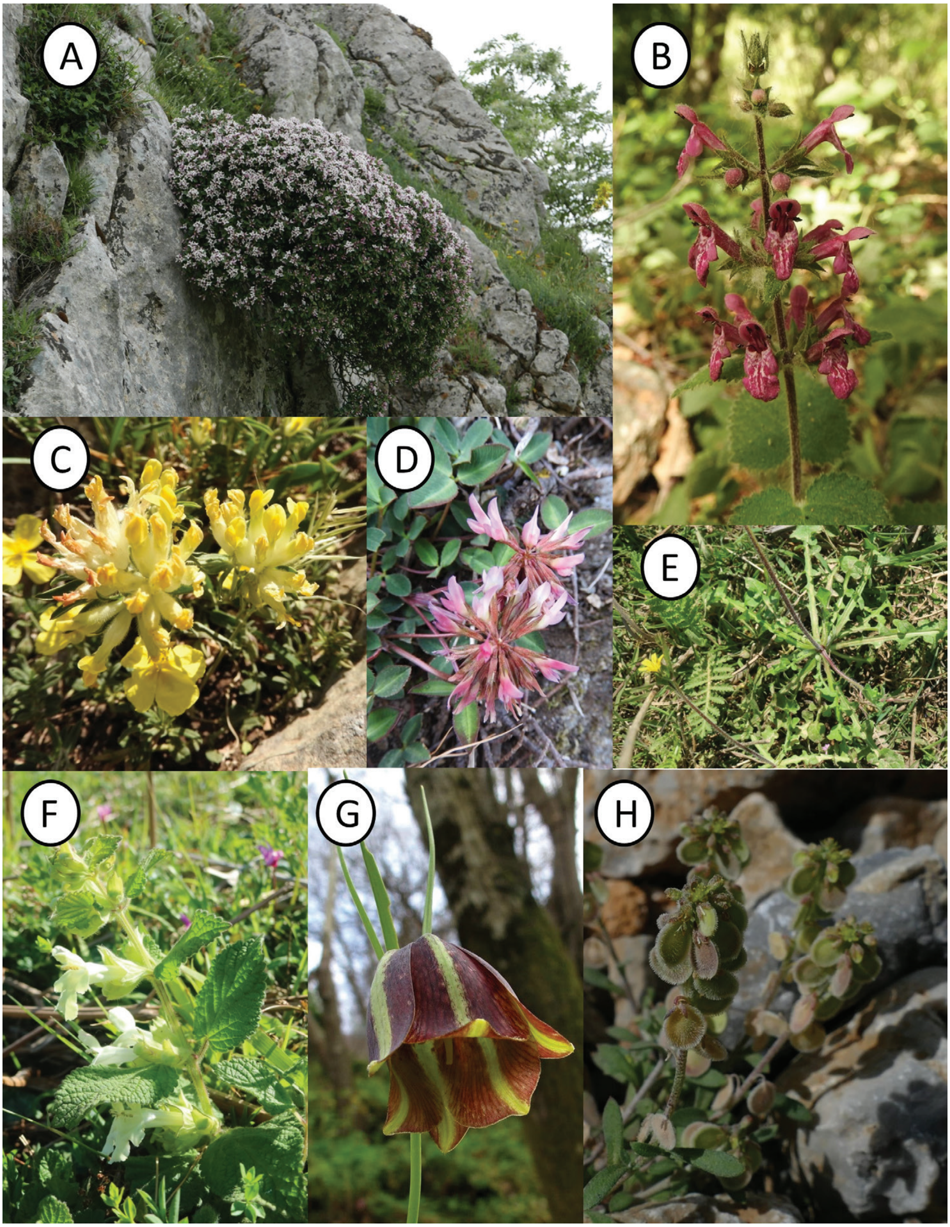

Figure 2. *Daphne oleoides* subsp. *oleoides* (**A**) *Stachys sylvatica* (**B**) *Anthyllis albiflora* (**C**) *Trifolium bivonae* (**D**) *Crepis sancta* (**E**) *Melittis melissophyllum* subsp. *albida* (**F**) *Fritillaria messanensis* subsp. *messanensis* (**G**) *Clypeola jonthlaspi* (**H**).

low number of Poaceae may be related to the lack of habitats with greater diversity of grasses, as prevailing vegetation types, such as communities with *Pteridium aquilinum* or subvertical cliffs are dominated by other families.

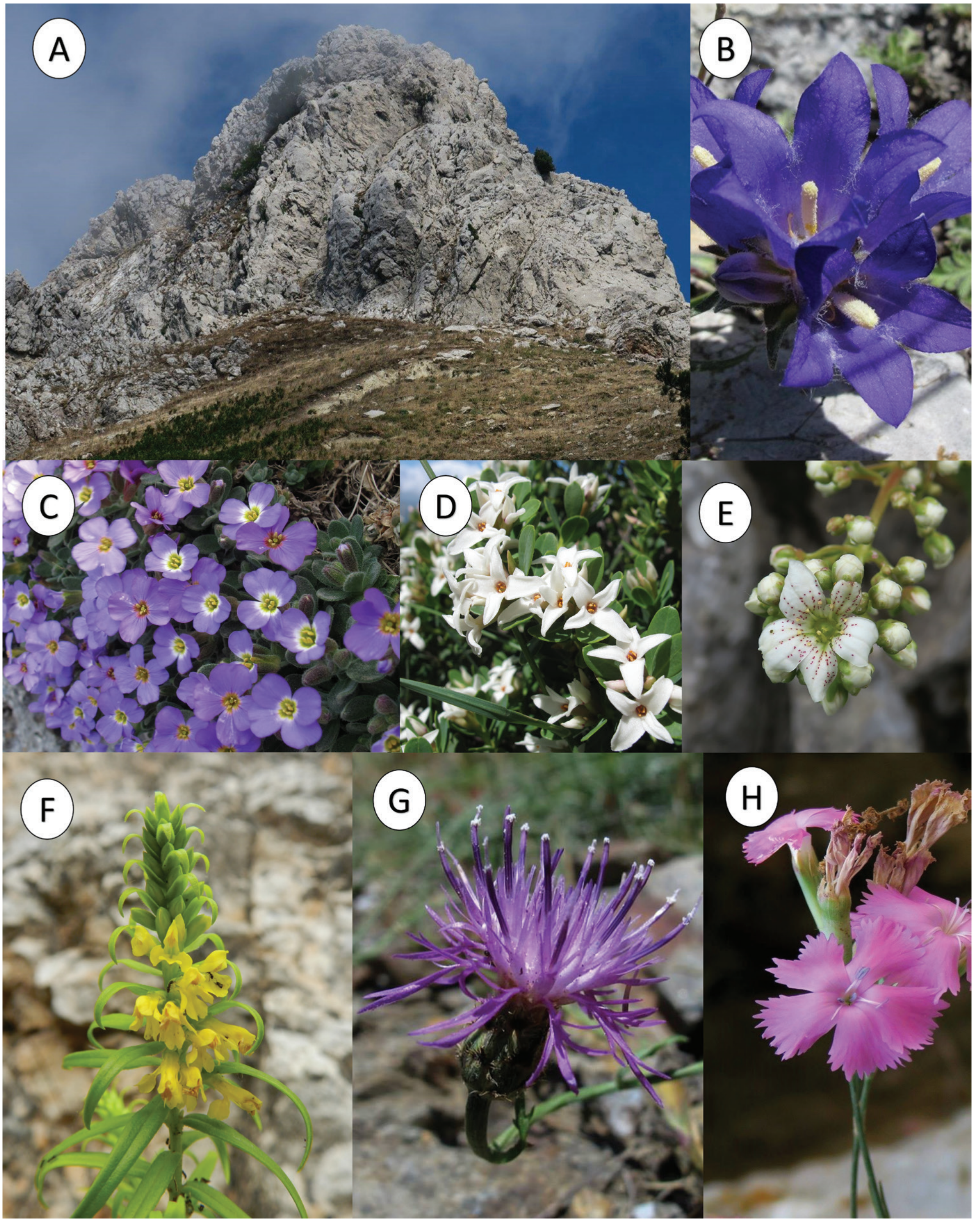

Figure 3. Panoramic view of the top of Rocca di Novara (**A**) *Edraianthus graminifolius* subsp. *siculus* (**B**) *Aubrieta columnae* subsp. *sicula* (**C**) *Daphne oleoides* subsp. *oleoides* (**D**) *Saxifraga callosa* subsp. *australis* (**E**) *Odontites bocconei* subsp. *bocconei* (**F**) *Centaurea gussonei* (**G**) *Dianthus graminifolius* (**H**).

The most abundant genus is *Trifolium* L. with 14 different taxa, followed by *Medicago* L. with 7, *Geranium* L. with 6, *Crepis* L., *Plantago* L., *Saxifraga* Tourn. ex L. and *Silene L.*each with 5 (Fig. 5).

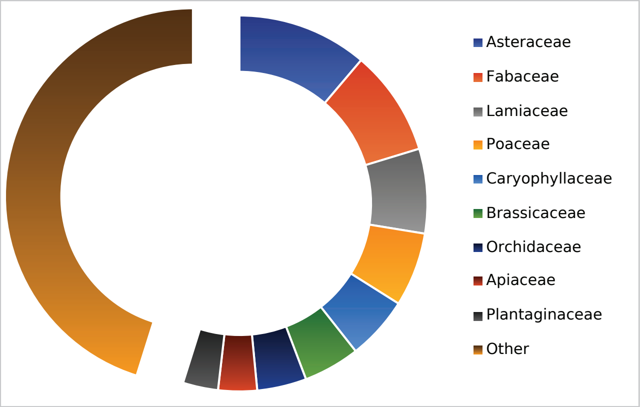

Figure 4. Areogram of the dominant families in the Rocca di Novara site (ME).

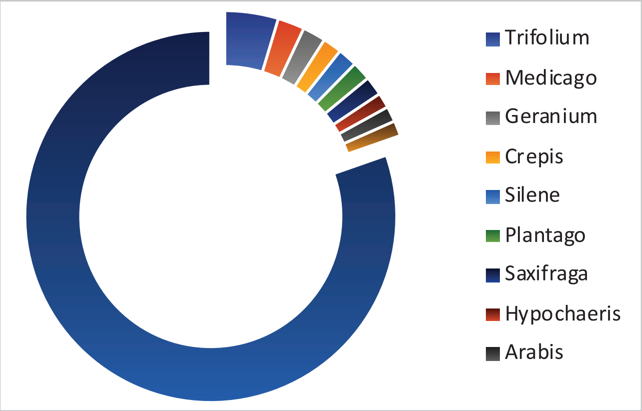

Figure 5. Distribution of predominant genera at Rocca di Novara (ME) site.

Regarding the geographic origin of the species and the infraspecific taxa, 24.7% are stenomediterranean, 21.7% eurimediterranean, 4.7% are cosmopolitan, 4.3% Italian endemics, 4% for Sicilian endemics and Eurasian, 3.7% belong to desert and subdesert areas from the Mediterranean to Central Asia (Mediterranean-Touranian) (Fig. 6).

As regards the endemic flora, it includes 12 taxa exclusive of Sicily and further 13 endemics shared with other regions of Italy.

Specifically, the Sicilian endemic taxa are *Anthyllis albiflora* (Guss.) Iamonico, Domina, Guarino & Del Guacchio (Fig. 2C), *Aubrieta columnae* subsp. *sicula*, *Centaurea gussonei* Raimondo & Spadaro, *Dianthus graminifolius* C.Presl, *Echium italicum* subsp. *siculum* (Lacaita) Greuter & Burdet, *Leontodon siculus* (Guss.) Nyman, *Odontites bocconei* (Guss.) Walp. subsp. *bocconei*, *Ophrys subfusca* subsp. *archimedea* (P.Delforge & M.Walravens) Kreutz, *O. fusca* subsp. *obaesa* (Lojac.) E.G.Camus & A.Camus, *Symphytum gussonei* F.W.Schultz, *Tanacetum vulgare* subsp. *siculum* (Guss.) Raimondo & Spadaro and *Trifolium bivonae* Guss. (Fig. 2D). Most of these species

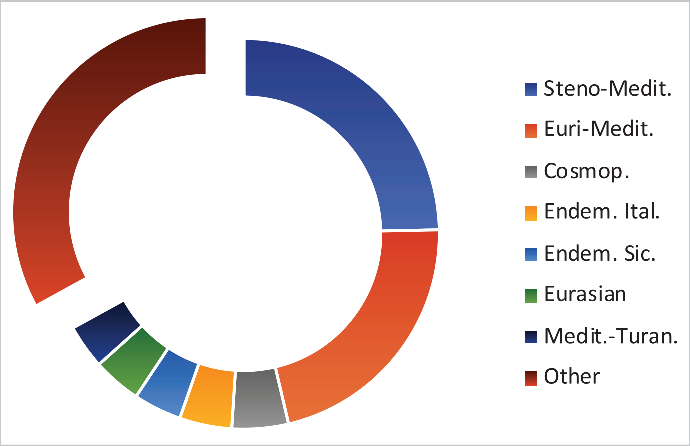

Figure 6. Proportion of major chorotype categories in the flora of Rocca di Novara (ME).

show a wide distribution across the northern mountains of Sicily, while *Centaurea gussonei*, a taxon belonging to *Centaurea parlatoris* Heldr. species complex (Domina et al. 2021), has a narrower distribution in the North-Eastern part of Sicily. Conversely, *Anthyllis albiflora* and *Dianthus graminifolius* show a distribution centered in the westerm part of the island. Regarding the latter, a taxon linked to thermophilous grasslands (Bacchetta et al. 2010), the population of Rocca di Novara is unusually linked to more orophilous cliffs stands and shows some transitional characters towards *D. busambrae* Soldano & F.Conti, deserving further taxonomic studies.

On the other hand, as far as Italian endemics are concerned, the following have been recorded: *Anthemis arvensis* subsp. *sphacelata* (C.Presl) R.Fern., *Arabis collina* subsp. *rosea* (DC.) Minuto, *Aristolochia clusii* Lojac., *Bellis margaritifolia* Huter, Porta & Rigo, *Carlina hispanica* subsp. *globosa* (Arcang.) Meusel & Kästner, *Edraianthus graminifolius* subsp. *siculus* (Strobl) Lakušić ex Greuter & Burdet, *Galium pallidum* C.Presl, *Linaria multicaulis* (L.) Mill. subsp. *multicaulis*, *Myosotis sylvatica* subsp. *elongata* (Strobl) Grau, *Orchis brancifortii* Biv., *Ranunculus pratensis* C.Presl, *Silene italica* subsp. *sicula* (Ucria) Jeanm. and *Viola aethnensis* subsp. *messanensis* (W.Becker) Merxm. & Lippert.

Besides, some rare or very rare species at a regional level, as reported in Giardina et al. (2007), have been detected, such as *Daphne oleoides* subsp. *oleoides*, *Saxifraga callosa* subsp. *australis*, *Clypeola jonthlaspi* L. (Fig. 2H), *Crepis neglecta* L. subsp. *neglecta*, *C. sancta* (L.) Bornm. subsp. *sancta* (Fig. 2E), *Tuberaria inconspicua* (Pers.) Willk., *Lomelosia crenata* (Cirillo) Greuter & Burdet subsp. *crenata*, *Melittis melissophyllum* subsp. *albida* (Guss.) P.W.Ball, *Phelipanche gussoneana* (Lojac.) Domina, Raab-Straube, Rätzel & Uhlich, and *Fritillaria messanensis* subsp. *messanensis*.From the total number of taxa, 39.3% were annual or biennial plants (therophytes), while 60.7% were perennial species. Among the perennial species, the most abundant life forms were hemicryptophytes (32.7%), geophyte (13%) and chamaephytes (10.7%). Phanerophytes and nanophanerophytes were rather few (3.7% and 0.7%, respectively) (Fig. 7).

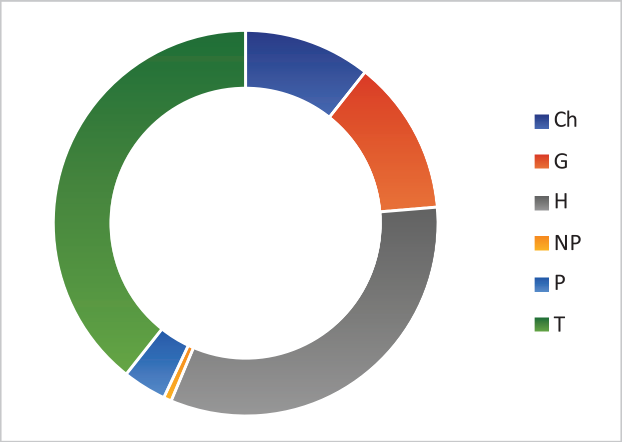

Figure 7. Breakdown of life form spectra for plant species at Rocca di Novara (ME) location.

A total of 36 taxa (12%) were found to be included in the IUCN risk categories. Among the recorded species *Aubrieta columnae* subsp. *sicula*, *Fritillaria messanensis* subsp. *messanensis*, *Linaria multicaulis* subsp. *multicaulis* are endangered, *Centaurea gussonei* and *Dianthus graminifolius* are vulnerable, *Biscutella maritima* Ten., *Neotinea lactea* (Poir.) R.M.Bateman, Pridgeon & M.W.Chase, *Plantago cupanii* Guss., *Symphytum gussonei* F.W.Schultz and *Trifolium bivonae* Guss. are classified as Near Threatened. Another 26 species are Least Concern (Orsenigo et al. 2020; Rossi et al. 2020). Some remarks regarding the presence of the taxa of greatest phytogeographical interest in the surveyed area are reported below.

Aubrieta columnae* subsp. *sicula (Strobl) M.A.Koch, D.A.German & R.Karl (Fig. 3C): this taxon shows a disjunct distribution, being represented only in the peaks of Madonie massif (W Sicily) and in the isolated stand of Rocca di Novara (Giardina et al. 2007). It is linked to mountain cliffs.

Crepis sancta (L.) Bornm.: the occurrence of this taxon in Sicily was reported for the first time by Gramuglio et al. (1959), but this report was later considered doubtful by Giardina et al. (2007) and consequently its presence in Sicily was considered uncertain in subsequent checklists of the Italian flora. Our finding confirms the presence of this species in Sicily.

Daphne oleoides* Schreb. subsp. *oleoides (Figs 2A, 3D): it is an orophilous taxon very rare in Sicily, where it is known only for the Madonie mountain and Rocca di Novara (Giardina et al. 2007). This species is linked to limestone cliffs, but sometimes it is also present on rocky slopes and screes.

***Edraianthus graminifolius* (L.) A.DC. ex Meisn. subsp. *siculus* (Strobl) Lakušić ex Greuter & Burdet** (Fig. 3B): this taxon is endemic of Southern Italy and Sicily, growing in rocky slopes and cliffs of mountain stands. In Sicily it occurs only in the Madonie massif, Rocca Busambra and Peloritani range, where it is circumscribed to Rocca di Novara and Mt. Scuderi (Sciandrello et al. 2015).

Fritillaria messanensis* Raf. subsp. *messanensis: it is an Eastern Mediterranean species, which is restricted to Calabria and Sicily in Italy. Its regional range includes only the Peloritani Mountains, where it is very sporadic, but locally abundant (Picone et al. 2003; Sciandrello et al. 2015).

Helianthemum oelandicum* (L.) Dum. Cours. subsp. *incanum (Willk.) G.Lòpez: this taxon is only report to grow on few calcareous massifs of Western Sicily (Giardina et al. 2007). It is the first report for the eastern part of the island.

***Minuartia recurva* (All.) Schinz & Thell. subsp. *condensata* (C.Presl) Greuter & Burdet**: in Sicily this species is known mainly for the Madonie massif, except for the Rocca di Novara stand, already reported by Giardina (1995).

Odontites bocconei* (Guss.) Walp. subsp. *bocconei (Fig. 3F): this endemism is found mainly in the mountains of North-Western Sicily (Madonie, Sicani, Palermo mountains and Trapani area), while is quite rare in the eastern part, where it is reported only in few localities of Peloritani range, as Pizzo Toscana, Mt. Scuderi, Rocca di Novara, Mt. Kalfa, Mt. Cavallo (Giardina et al. 2007; Sciandrello et al. 2014). In the investigated area it grows only on the calcareous cliffs of the upper part.

***Phelipanche gussoneana* (Lojac.) Domina, Raab-Straube, Rätzel & Uhlich**: this species is spread in the Central and Eastern parts of the Mediterranean area and also in the Crimean Peninsula (Rätzel et al. 2018). In Sicily its distribution is limited to a few locations such as as Rocca Busambra, Madonie massif and is here reported now for the first time in Eastern Sicily.

***Saxifraga callosa* Sm. subsp. *australis* (Moric.) Pignatti ex Tavilla & Del Guacchio** (Figs 3E, 8): The taxon is distributed across the mountains of central-southern Italy, as well as Sicily and Sardinia. In Sicily, it is an extremely rare species linked to mountain cliffs, particularly in select stations within Madonie massif (Giardina et al. 2007). The highly isolated population of Rocca di Novara was firstly discovered by Nicotra (1880) and never reconfirmed until our rediscovery confirming the presence of a small population on the summit cliffs of the mountain (see Tavilla 2024).

Conclusions

This floristic survey allowed to record the presence of 300 angiosperms in Rocca di Novara area. Ferns group counts 10 species, corresponding to 3.3% of the local vascular flora. The most numerous families were Asteraceae and Fabaceae with 12.3% and 10%, respectively, followed by Lamiaceae, Poaceae, Caryophyllaceae, Brassicaceae, Orchidaceae, Apiaceae and Plantaginaceae, with more than 10 species each one. All other families account for 49.7% of the local flora

The life-form spectrum reveals the prevalence of therophytes (39.3%). Among the perennial species, hemicryptophytes (32.7%), geophytes (13%) and camephytes (10.7%) are the most abundant life forms. Their high frequency may be related with the local ecological conditions, characterized by shallow soils, high exposure to sunlight,

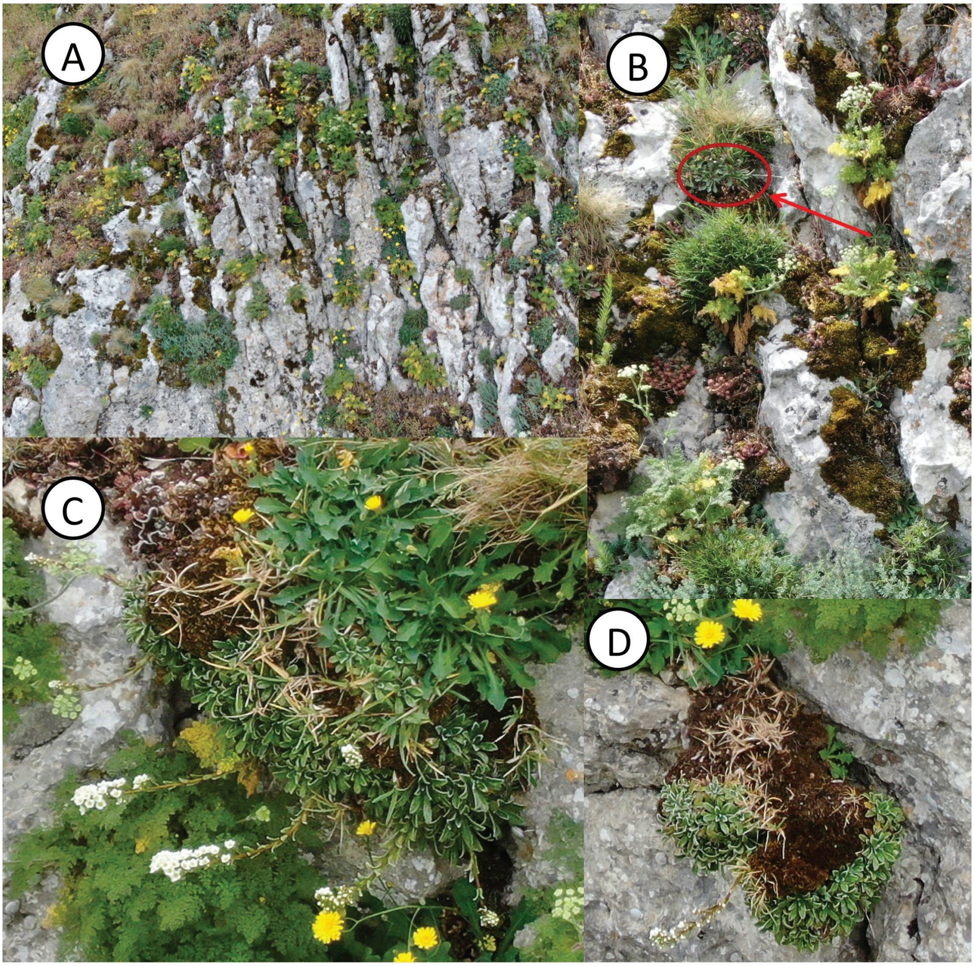

Figure 8. Photos taken with the drone: panoramic view of the cliffs (**A**) specimens of *Saxifraga callosa* subsp. *australis* (**B–D**).

seasonal drought and frequent fires. In particular, the high percentage of chamaephytes is correlated to the presence of several chasmophilous species that colonize the limestone cliffs in the upper part of Rocca di Novara. The low rate of phanerophytes and nanophanerophytes depends on the very scarce forest cover, almost completely eliminated by anthropogenic disturbances.

The occurrence of a high amount of Sicilian and Italian endemics highlights the phytogeographical interest of the local flora, probably issuing from its peculiar geomorphological and climatic characteristics.

Furthermore, the humid and cool microclimate of the top of Rocca di Novara provides conditions suitable to host some interesting disjointed stands of several rare orophilous plant taxa that in Sicily are known to occur only on the top of the Madonie massif, such as *Saxifraga callosa* subsp. *australis*, *Daphne oleoides* subsp. *oleoides*, *Aubrieta columnae* subsp. *sicula*, *Helianthemum oelandicum* (L.) Dum.Cours. subsp. *incanum* (Willk.) G.Lòpez and *Minuartia recurva* (All.) Schinz & Thell. subsp. *condensata* (C.Presl) Greuter & Burdet (Giardina et al. 2007). In the Peloritani area, only Mt. Scuderi shows such a significant presence of orophilous taxa shared with the Madonie mountain area (Sciandrello et al. 2015).

No alien species were recorded in the investigated area. This is in agreement with Guarino et al. (2021) and Barone et al. (2021), who reported for Sicily that the majority of alien species are concentrated in the coastal areas and in the most disturbed ones while the high mountain areas with little human disturbance have very low percentages of alien taxa.

The comparison of our results with the short list of species of Rocca di Novara reported by Nicotra (1880) prompts some interesting considerations. In particular, this author reported only 50 species for this area, also mentioning *Valeriana tuberosa*, previously reported by Gussone (1843), but not confirmed by him. Of these species, 13 have not beed found in our investigations. Some of them may have been confused with other related taxa and their identification remains doubtful (*Aristolochia longa* L., *Festuca rubra* L. and *F. laevigata* Gaudin), while others (*Sabulina tenuifolia* (L.) Rchb subsp. *tenuifolia*, *Anisantha diandra* (Roth) Tutin ex Tzvelev, *A. tectorum* (L.) Nevski, *Silene conica* L. and *Trigonella elegans* (Salzm. ex Ser.) Coulot & Rabaute) are therophytes widespread in Sicily and their absence could only be temporary or due to sampling biases. The absence of some species with a more pronouncedly orophilous character or linked to mesophilous forest communities (*Pimpinella tragium* Vill., *Echium vulgare* subsp. *pustulatum* (Sm.) Bonnier & Layens, *Katapsuxis silaifolia* (Jacq.) Raf. and *Silene viridiflora* L.) may be attributet to climate changes that has affected the area in past decadesand/or due to anthropogenic disturbancetriggering the reduction of wooded areas. A special mention must be made for *Silene calycina* C.Presl, a poorly known taxon probably related to *Silene bupleuroides* L. Described by Presl (1826), it was reported only for Caltavuturo (isolated mountain of the western Madonie Massif) and was later reported by Nicotra (1880) on the carbonate cliffs of Rocca di Novara. The species has never been found again and is probably extinct, as confirmed also by Sciandrello et al. (2015). Furthermore, two other species cited by Nicotra, *Thymbra capitata* (L.) Cav. and *Gypsophila arrostoi* Guss. subsp. *arrostoi*, are both linked to the lowlands subject to thermo-Mediterranean climate, which were not surveyed in this study.

Finally, this study highlights the importance of preserving the Rocca di Novara Special Area of Conservation (SAC), considering that 12% of the species inventoried are threatened and classified within the IUCN risk categories. A significant issue lies in the in the lack of conservation measures in the face of such a significant plant biodiversity and phytogeographical value of the surveyed habitat., Field investigations have highlighted numerous conservation challenges, mainly related to significant anthropogenic pressure due to intense transhumance grazing, Additionally, climate change is disrupting ecological niches, pushing plant to adapt to shifting or fading habitats. Temperature variations also generated phenological changes, such as early or delayed blooms. Protecting this ecosystem is essential to safeguard its natural heritage and ensure the survival of native species. Therefore, our results, provide an updated overview of the floristic diversity of Rocca di Novara in the Peloritani mountains and offer fundamental data for further quantitative and qualitative research on vascular plants in the Mediterranean area.

Acknowledgments

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Supplementary material 1

Inventory of the taxa collected or photographed during the field trip

Authors: Manuela Porrovecchio, Salvatore Cambria, Giulia Bacilliere, Giulio Barone, Alessandro Crisafulli, Emilio Di Gristina, Costanza Di Pasquale, Margherita Di Mauro, Gianniantonio Domina, Fabio Luchino, Claudio Marici, Giulia Miraglia, Gianmarco Tavilla, Saverio Sciandrello

Data type: xlsx

Explanation note: Vascular flora of Rocca di Novara, Peloritani, Messina. This table also shows chorotype "Choro" (Alpine-Apenn.= Alpine-Apennine, CentralEurop.= Central Europe, CentralMedit.= Central Mediterranean, Circumbor.= Circumboreal, Cosmop.= Cosmopolitan, Endem. Ital.= Endemic to Italy, Endem. Sic.= Endemic to Sicily, Eurasian= Eurasian, Euri-Medit.= EuriMediterranean, NE-Eurimedit.= Northeastern-EuriMediterranean, N-Eurimedit.= Northern-EuriMediterranean, Europ.= Europeans, Europ.-Caucas.= European-Caucasian, Eurosib.= Siberian European, Medit.= Mediterranean, Medit.-Atl.(Euri-)= Mediterranean-Atlantic (Euri-), CE-Medit.= Central Eastern Mediterranean, Medit.-Mount.= Mediterranean-mountainous, Medit.-Turan.= Mediterranean-Touranian, N-Medit.= Northern Mediterranean, NE-Medit.= Northeastern Mediterranean, NW-Medit.= Southwestern Mediterranean, Orop. S-Europ.= Orophyte Southern European, Orop. S-Medit.= Orophyte Southern Mediterranean, Orop. SE-Europ.= Orophyte South East Europe, Orop. SW-Europ.= Orophyte South West Europe, Paleosubtrop.= Paleosubtropical, Paleotemp.= Paleotemperate, S-Europ.= South Europe, S-Medit.= South Mediterranean, SE-Europ.= South East Europe, Steno-Medit.= Steno-Mediterranean, NE-Stenomedit= Northeastern-StenoMediterranean, SW-Stenomedit.= Southwestern Steno-Mediterranean, W-Stenomedit.= Western Steno-Mediterranean, Subcosmopol.= Subcosmopolitan, Subendem.= Subendemic, Submedit.= Submediterranean, Subtrop.= Subtropical, SW-Medit.= Southwestern Mediterranean, W-Europ.= Western Europe, W-Medit.= Western Mediterranean). Life form "LF" (T= therophytes, Ch= chamaephytes, P= phanerophytes, NP= nanophanerophytes, H= hemicryptophytes, G= geophyte). "Red List" were found to be in IUCN risk categories (EN= endangered, VU= vulnerable, NT= near threatened, LC= least concern).

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RESEARCH ARTICLE

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In vitro propagation of the orchid *Phalaenopsis circus via* organogenesis and somatic embryogenesis using protocorm and thin cell layer explants

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Abstract

Orchids of the genus *Phalaenopsis* have high economic value in the floriculture industry and market and high potential for breeding programs. *In vitro* propagation makes it possible to clonally mass proliferate and conserve this valuable plant. In the current research, efficient protocols, some reported for the first time, for *in vitro* propagation of *Phalaenopsis circus* through organogenesis and somatic embryogenesis (SE) are presented. We used protocorms obtained from seeds and thin cell layers (TCLs) prepared from leaves as explants. Explants were cultured on Murashige and Skoog (MS) basal medium enriched with various concentrations and combinations of plant growth regulators. Protocorms were cultured on media fortified with 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with N-phenyl-N′-1,2,3-thiadiazol-5-yl-urea (TDZ), and α-naphthalene-acetic acid (NAA) in combination with N^6-furfuryl adenine or kinetin (Kin) for organogenesis, as well 2,4-D in combination with NAA for SE. These protocorms produced either protocorm-like bodies (PLBs) or somatic embryos. Results showed that the highest number of PLBs (75.0) was obtained on medium enriched with 1.0 mg l^{-1} 2,4-D. Maximum number of somatic embryos (12.3/explant) was obtained on medium containing 0.5 mg l^{-1} 2,4-D together with 2.0 mg l^{-1} NAA. The use of transversal TCLs with 2–3 cell layers as explants cultured on medium supplemented with 0.5 mg l^{-1} IBA combined with 1.0 mg l^{-1} TDZ produced the highest number of plantlets. Plantlets were transferred to pots and acclimatized in ambient greenhouse conditions with 100% survival rate.

Keywords

Epiphytic orchids, orchid propagation, organogenesis, plant growth regulators, somatic embryogenesis, thin cell layers

Introduction

Orchids (family Orchidaceae) are among the most diverse of the flowering plant families, with more than 800 genera and 25,000 species (Chugh et al. 2009). *Phalaenopsis* as a cut and pot flowering plant is one of the most popular orchids in the world, through the development of many artificial hybrids. The genus *Phalaenopsis* comprises approximately 60 species native to tropical rainforests of South and South-East Asia, Australia and New Guinea (Winkelmann et al. 2006). They are epiphytic plants, and consist of only a few leathery leaves (Sinha et al. 2010).

Natural clonal propagation of orchids is a slow process, which results in traits segregation and is, therefore, not possible for *Phalaenopsis*. Also, sexual propagation of orchids leads to the production of heterozygous plants. Therefore, establishment of protocols for *in vitro* propagation of orchids is important as an alternative procedure for high frequency regeneration of these plants. Many orchid species are vulnerable, rare and/or threatened. One of the important approaches for conservation of these plants is *in vitro* propagation. *In vitro* propagation is an extremely useful technique for clonal propagation of many species, particularly ornamental plants like orchids (Guo et al. 2024). *In vitro* techniques may also be applied for production of a large number of healthy and disease-free plantlets in a short span of time, allowing changes in growth parameters, and generation of propagules all year round (Pati et al. 2006; Engelmann 2011). Large-scale propagation of orchids using tissue culture techniques has meant that orchids occupy a position as one of the top ten cut and pot flowers (Chugh et al. 2009). *In vitro* propagation of orchids has a few problems like low rate of shoot multiplication, high cost of production, poor rooting frequency, exudation of phenolic compounds from explants, transplantation to field, and somaclonal variation (Chugh et al. 2009; Bhattacharyya et al. 2016).

Protocorms of orchids are derived from the seed. Protocorm-like bodies (PLBs), on the other hand, are derived from vegetative organs like leaves, stems and protocorms during *in vitro* culture. Seed-derived protocorms may also be used to induce somatic embryogenesis (SE). Since the general characteristics of growth and structure are similar to those of protocorms, the regenerated structure is termed PLB (Lee et al. 2013). Therefore, PLBs are similar to protocorms in morphology and biological characteristics (Lee et al. 2013; Cardoso et al. 2020). The main difference between protocorms and PLBs is basically the origin of the tissue. PLBs are able to differentiate a shoot apical meristem and then a complete plantlet. Induction of PLBs and somatic embryo differentiation (directly or indirectly) facilitate the micropropagation of orchids (Chen et al. 2019). In orchids, the formation of protocorms and PLBs is regulated by various factors, and plant growth regulators (PGRs) are among the most important ones (Cardoso et al. 2020). N-phenyl-N´-1,2,3-thiadiazol-5-yl-urea (TDZ) and 2,4-dichlorophenoxyacetic acid (2,4-D) are the most suitable PGRs for callus and PLB induction, as reported for *Cymbidium*, *Phalaenopsis*, *Dendrobium*, and *Paphiopedilum* (Cardoso et al. 2020; Guo et al. 2024).

In vitro propagation of genera belonging to the Orchidaceae family including *Phalaenopsis* has been reported using various explants such as leaf segments (foliar explants), node sections, rhizome segments, root segments, protocorms, PLBs, tubers, shoot tips, flower buds, and inflorescence axes (Chugh et al. 2009; Sinha et al. 2010; Roy et al. 2011; Panwar et al. 2012; Zeng et al. 2012; Baker et al. 2014; Mahendran, 2014; Chen et al. 2015; Bhattacharyya et al. 2016; Kaviani et al. 2017; Asa et al. 2019; Mohammadi et al. 2019). Among all these explants, PLBs and protocorms are more efficient because of rapid multiplication on solid or liquid culture media, and maximum production in a short period of time (Luo et al. 2003; Roy et al. 2011; Zeng et al. 2012).

Many protocols for *in vitro* propagation of orchids using PLBs and protocorms as explants and various PGRs have been reported (Sinha et al. 2010; Baker et al. 2014; Kaviani et al. 2017; Mohammadi et al. 2019; Zakizadeh et al. 2019; Asa et al. 2020; Guo et al. 2024). *In vitro* propagation through PLBs obtained from somatic tissues is an important approach to obtain genetically stable plants and improvement of quality. An efficient *in vitro* propagation method for *Phalaenopsis* sp. using PLBs derived from leaf explants was introduced by Park et al. (2002b). Ishii et al. (1998), Kuo et al. (2005) and Chen and Chang (2006) proposed a protocol for regeneration of a *Phalaenopsis* cultivar by direct SE starting from leaf explant. Medium composition for tissue culture of orchids by PLBs and protocorms is species-specific and depends on several factors particularly PGRs (Luo et al. 2009; Guo et al. 2024).

PGRs such as α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), TDZ, 6-benzyladenine (BA) and 6-furfurylaminopurine or kinetin (Kin) have been used for *in vitro* propagation of *Phalaenopsis* orchids through organogenesis and SE (Tanaka 1992; Ernst 1994; Tokuhara and Mii 2001; Park et al. 2002a, b; Kuo et al. 2005; Sinha et al. 2007; Kaviani et al. 2017; Asa et al. 2019; Mohammadi et al. 2019). In *Phalaenopsis amabilis*, TDZ promoted direct SE from the epidermal cells of leaf explants (Chugh et al. 2009). Embryo formation on leaf explants was retarded by auxins like indole-3-acetic acid (IAA), IBA, NAA, and 2,4-D but promoted by cytokinins like 2-isopentyladenine (2-iP), zeatin (Zt), Kin, BAP and TDZ (Chen and Chang 2001).

Plant regeneration from thin cell layers (TCLs) is a simple and effective technique for clonal *in vitro* propagation of orchids (Teixeira da Silva 2013). It is the most important technique for *in vitro* propagation of some species. This system consists of explants of a small size (approximately 0.5–1.0 mm thick) excised from different organs (stem, leaf, flower, cotyledons, hypocotyl, epicotyl, apical meristem or embryo), either longitudinally (explants comprise epidermal, cortical, cambium, perivascular and medullary tissue, and parenchyma cells) or transversally (explants comprise all tissues present in the organ) (Chugh et al. 2009). TCLs have been successfully applied for protocorm, PLB, floral stalk-derived leaf, shoot, and callus induction in *Aranda* (Lakshmanan et al. 1995), *Coelogyne cristata* (Naing et al. 2011), *Cymbidium* spp. (Begum et al. 1994; Nayak et al. 2002; Malabadi et al. 2008; Hossain et al. 2010; Vyas et al. 2010), *Dendrobium* spp. (Zhao et al. 2007; Rangsayatorn 2009; Jaiphet and Rangsayatorn 2010), *Doritaenopsis* (Park et al. 2000), *Paphiopedilum* (Liao et al. 2011), *Renanthera* (Wu et al. 2012), *Spathoglottis* (Teng et al. 1996), and *Xenikophyton* (Mulgund et al. 2011). NAA, BAP, and TDZ are some of the PGRs used for *in vitro* propagation using TLCs through organogenesis and SE.

The purpose of the current research was to evaluate the effect of different concentrations of Kin, TDZ, NAA, IBA and 2,4-D, individually and in combination on *in vitro* propagation of *P. circus via* organogenesis and SE using two types of explants: protocorms obtained from seed and TCLs obtained from vertically cut leaves. Both organogenesis and SE can be direct and indirect (*via* callus). The difference in the type of explant and the type, concentration, and combination of PGRs play an important role in obtaining different results.

Materials and methods

Explants source and sterilization for organogenesis and SE

Capsules of *Phalaenopsis circus* were isolated from the flowers of plants grown in the greenhouse of the Hyrcan Agricultural Sciences and Biotechnology Research Institute, Amol, Iran. The approximate age of these capsules was 150 days. The capsules were first washed under running tap water along with a few drops of dishwashing liquid for 30 min and rinsed thoroughly with distilled water. Then, they were surface sterilized in 50% sodium hypochlorite solution containing 5% active chloride for 20 min with a drop of Tween 20, and washed thoroughly in distilled water and finally dipped in 70% alcohol for a minute and flamed. The sterilized capsules were cut longitudinally with the help of a sharp sterilized surgical blade for extracting seeds. Seeds were inoculated on MS (Murashige and Skoog 1962) medium for production of protocorms. Media were supplemented with 3% sucrose and solidified with 0.8% agar. Media were adjusted to pH 5.6–5.8 with 1 N HCl or NaOH before autoclaving at 121 °C, 105 kPa for 20 min. Following establishment, all the cultures were incubated at 24 ± 2 °C, 70–80% RH, and 16-h photoperiod of 50–60 μmol m^{-2} s^{-1} irradiance provided by cool–white fluorescent tubes.

Plantlet formation from protocorms and PLBs through organogenesis and SE

The explants (protocorms obtained from germinated seeds) were cultured on MS medium containing 3% sucrose and 0.8% agar. The pH of the media was adjusted to 5.6–5.8 with 0.1 N NaOH or HCl prior to autoclaving. All media contained in culture bottles were autoclaved at 105 kPa and 121 °C for 20 min. The media were enriched with different concentrations of TDZ (0.0, 0.1, 1.0, 2.0 and 5.0 mg l^{-1}) and 2,4-D (0.0, 0.01, 0.1, 1.0 and 2.0 mg l^{-1}), or with NAA (0.0, 0.1, 0.5, 1.0 and 5.0 mg l^{-1}) and Kin (0.0, 0.5, 1.0, 2.0 and 5.0 mg l^{-1}), either individually or in combination for organogenesis. For induction of somatic embryos, media were fortified with different concentrations of NAA (0.0, 0.1, 0.5, 1.0 and 5.0 mg l^{-1}) and 2,4-D (0.0, 0.01, 0.1, 0.5 and 1.0 mg l^{-1}), individually or in combination.

TCLs source and sterilization

Leaves of *P. circus* were detached from *ex vitro*-grown plantlets for preparation of TCLs. Leaves were washed for 3–4 h under running tap water and rinsed thoroughly with distilled water. Then, they were surface sterilized in 50% sodium hypochlorite solution containing 2.5% active chloride for 15 min with a drop of Tween 20, and washed thoroughly in distilled water followed by dipping in 10% silver nano-particles. Finally, explants were dipped in 70% alcohol for a minute followed by rinsing with sterilized distilled water. The sterilized leaves were cut vertically as segments in diameters of 0.5–1.0 mm and used as explants (TCLs). TCLs were cultured on MS medium supplemented with 3% sucrose and solidified with 0.8% agar. The media were enriched with different concentrations of TDZ (0.0, 0.01, 0.1, 0.5 and 1.0 mg l^{-1}) and IBA (0.0, 0.1, 0.5 and 1.0 mg l^{-1}), either individually or in combination for plantlets regeneration through organogenesis. Media were adjusted to pH 5.6–5.8 before autoclaving at 121 °C, 105 kPa for 20 min. All the cultures were incubated at 24 ± 2 °C, 70–80% RH, and a 16 h/8 h day/night photoperiod with an irradiance of 50–60 µmol m^{-2} s^{-1} provided by cool–white fluorescent tubes.

General scheme of the experiments

The present study was carried out in two sections: organogenesis and SE. First of all, seeds were sown on MS medium without PGRs for production of protocorms. The protocorms were then cultured on media containing different concentrations of TDZ and 2,4-D (for organogenesis), Kin and NAA (for organogenesis), as well NAA and 2,4-D (for SE). On the other hand, TCLs obtained from leaves were cultured on MS media containing different concentrations of IBA and TDZ (for organogenesis). Most plantlets were produced through direct organogenesis and SE (without callus production). Normally, organogenesis consists in the regeneration of shoots (stem with leaves) which are then induced to root after transfer to a new medium. Sometimes, both shoots and roots are produced on the same medium. SE, instead, always produces a complete plantlet.

Measured parameters.

After 60 days, PLB number, leaf length, leaf number, root length, and root number (produced through organogenesis) were measured. The number of somatic embryos, leaf number, root length, and root number (produced through SE) were also measured. Plantlet number, leaf length, leaf number, root length, and root number produced through direct organogenesis on TCLs were also recorded.

Plantlet development and acclimatization

For *ex vitro* establishment, *in vitro* well-rooted plantlets were taken out from culture vessels and washed thoroughly with sterile distilled water to remove adherent medium from the plantlet body and transferred to plastic pots (18 cm height × 12 cm diameter) filled with a potting mixture of LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite in the proportion of 15:10:20:5:30:20%. All the pots were then transferred to a greenhouse with temperature of 24 ± 2°C to 20 ± 2°C day/night (light intensity of 3,500 lux, RH of 80–90% and a 14 h/10 h day/

night photoperiod) for acclimatization. The pots were covered with another plastic pot (18 cm height × 12 cm diameter) to retain moisture. These pots were removed after two weeks. The number of surviving plants was recorded after three months of transfer.

Experimental design and data analysis

The experiments were established in a completely randomized design. For each treatment, three replicates and for each replicate, three specimens (explants) were taken (in total 95 treatments, 285 replicates, and 855 explants). PGR-free MS medium was used as control in the experiments. Data were subjected to analysis of variance (ANOVA) and means were compared by the LSD test at $P < 0.05$ using the SPSS ver. 17 (SPSS Inc., USA).

Results

Effect of 2,4-D and TDZ on *in vitro* propagation through organogenesis

PLBs were produced from protocorms and then developed into plantlets on most media. There were statistically significant differences ($p \leq 0.01$) in PLB number as well leaf length among different concentrations of 2,4-D in combination with TDZ (Table 1). Maximum PLB number (75.0) was obtained in explants treated with 1.0 mg l^{-1} 2,4-D without TDZ (Table 2). Increasing 2,4-D and TDZ concentrations had no positive effect on PLB number (Table 2). The minimum PLB number (14.67) was obtained in explants treated with 5.0 mg l^{-1} TDZ without 2,4-D (Table 2). All treatments without the presence of 2,4-D induced lower PLB production (<18; Table 2). The highest average leaf length (5.23 cm/explant) was obtained with 2.0 mg l^{-1} 2,4-D without TDZ (Fig. 1). The lowest average root length (2.0 cm/explant) was observed in control explants.

Effect of NAA and Kin on *in vitro* propagation through organogenesis

Differences in PLB number, leaf length and root length in samples grown under different concentrations of Kin in combination with NAA were significant ($p \leq 0.01$) (Table 1). PLB production was strongly influenced by external addition of PGRs (NAA together with Kin). Varied responses in terms of PLB number and leaf and root length were obtained using different concentrations of NAA and Kin. When the explants were grown on medium supplemented with 1.0 mg l^{-1} Kin in combination with 0.5 mg l^{-1} NAA, maximum number of PLBs (63.0) was produced. Treatment containing 0.5 mg l^{-1} Kin in combination with 0.5 mg l^{-1} NAA was found to be the next most effective PGR combination affecting the production of PLBs (51.0), followed by 0.5 mg l^{-1} NAA together with 2.0 mg l^{-1} Kin (49.67 PLBs; Table 3). When the explants were grown on control medium, minimum number of PLBs (13.67) was

Table 1. Analysis of variance of the effect of different concentrations of 2,4-D, NAA, IBA, Kin and TDZ on measured parameters of *Phalaenopsis circus* cultured under *in vitro* organogenesis and SE conditions.

		Mean squares				
Source of variations	**df**	**PLB number**	**Leaf length**	**Leaf number**	**Root length**	**Root number**
2,4-D	4	2482.51**	6.12**	1.61^{ns}	6.82**	21.49**
TDZ	4	123.95**	0.38^{ns}	27.81**	0.17^{ns}	0.45^{ns}
2,4-D × TDZ	16	186.55**	0.69**	0.36^{ns}	0.23^{ns}	0.67^{ns}
Error	48	22.91	0.24	0.66	0.15	0.9
CV (%)	-	12.65	17.08	20.67	13.21	32.85
NAA	4	921.49**	0.86**	3.19*	13.76**	16.75**
Kin	4	843.79**	5.74**	2.49^{ns}	1.01**	1.15^{ns}
NAA × Kin	16	119.58**	1.08**	1.63^{ns}	1.26**	1.01^{ns}
Error	48	37.99	0.18	0.996	0.17	0.78
CV (%)	-	19.12	16.24	31.45	12.41	24.51
		Plantlet number				
IBA	3	12.59**	1.03**	8.82**	13.59**	1.62^{ns}
TDZ	4	10.23**	9.97**	1.44^{ns}	0.48**	4.07**
IBA × TDZ	12	3.57**	0.15^{ns}	1.89*	1.02**	2.12**
Error	38	1.02	0.21	0.81	0.12	0.65
CV (%)	-	24.9	14.55	26.65	11.45	27.29
		Somatic embryo number				
2,4-D	4	58.367**	-	2.147*	4.530**	8.453**
NAA	4	2.567^{ns}	-	22.113**	4.556**	0.687^{ns}
2,4-D × NAA	16	3.892*	-	0.480^{ns}	0.91**	1.02^{ns}
Error	48	1.928	-	0.702	0.224	0.57
CV (%)	-	20.22	-	26.62	14.49	28.74

*, **: Significant at the 0.05 and 0.01 probability level, respectively, ^{ns}: Not significant at p=0.05, df: degree of freedom, CV: coefficient of variations.

produced. The highest average leaf length (5.03 cm/explant) was measured with 0.1 mg l^{-1} NAA together with 2.0 mg l^{-1} Kin (Fig. 2). The lowest average leaf length (1.6 cm) was observed in controls (without PGRs). The highest average root length (6.87 cm/explant) was obtained with 1.0 mg l^{-1} NAA together with 0.5 mg l^{-1} Kin. The lowest average root length (1.77 cm) was obtained in explants treated with 0.5 mg l^{-1} Kin without NAA.

Effect of 2,4-D and NAA on *in vitro* propagation through SE

The data clearly show that there is a significant difference among different concentrations of NAA and 2,4-D for somatic embryos number ($p \leq 0.05$) and root length ($p \leq 0.01$) (Table 1). Mean comparison of the data showed that when the explants were treated with 0.5 mg l^{-1} 2,4-D together with 2.0 mg l^{-1} NAA and 0.5 mg l^{-1} 2,4-D together with 0.1 mg l^{-1} NAA, somatic embryos number/explant (12.33 and 12.00, respectively) was maximum (Table 4, Fig. 3). The lowest somatic embryos number (4.33) was observed in explants treated with 0.1 mg l^{-1} 2,4-D together with 0.1 mg l^{-1} NAA. The highest average root length (4.97 cm/explant) was obtained with 0.1 mg l^{-1}

Table 2. Mean comparison of the effect of different concentrations of 2,4-D and TDZ on measured parameters of *Phalaenopsis circus* cultured under *in vitro* organogenesis conditions.

	Mean comparison	
2,4-D + TDZ (mg l^{-1})	PLB number	Leaf length
0.0 + 0.0	18.0h	2.0h
0.0 + 0.1	16.67h	2.4e-h
0.0 + 1.0	17.0h	2.07gh
0.0 + 2.0	16.0h	2.33fgh
0.0 + 5.0	14.67h	2.23fgh
0.01 + 0.0	33.0g	2.27fgh
0.01 + 0.1	37.0efg	2.5d-h
0.01 + 1.0	40.0d-g	2.77d-h
0.01 + 2.0	39.0d-g	2.53d-h
0.01 + 5.0	44.33cde	2.87d-g
0.1 + 0.0	45.33bcd	2.57d-h
0.1 + 0.1	42.33c-f	2.8d-h
0.1 + 1.0	52.67b	2.7d-h
0.1 + 2.0	40.0d-g	2.63d-h
0.1 + 5.0	41.67c-f	2.43d-h
1.0 + 0.0	75.0a	2.83d-g
1.0 + 0.1	52.67b	3.17cde
1.0 + 1.0	49.33bc	2.63d-h
1.0 + 2.0	39.0d-g	3.23cd
1.0 + 5.0	36.33fg	2.9def
2.0 + 0.0	39.67d-g	5.23a
2.0 + 0.1	36.0fg	4.4b
2.0 + 1.0	36.33fg	3.73bc
2.0 + 2.0	40.0d-g	3.2cde
2.0 + 5.0	44.33cde	2.9def

Means with different letters on the same column are significantly different ($p<0.05$) based on LSD test.

2,4-D together with 2.0 mg l^{-1} NAA. Root length (4.93 and 4.70 cm) was highest in explants treated with 1.0 mg l^{-1} 2,4-D together with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} 2,4-D without NAA, respectively (Table 4). The lowest average root length (2.30 cm) was measured in control explants.

Effect of IBA and TDZ on *in vitro* propagation through TCLs

Plantlets were produced from TCLs through direct organogenesis on some media. Statistically significant differences were observed between the means for plantlet number, root length and root number ($p\leq0.01$), as well leaf number ($p\leq0.05$) and applied PGRs (IBA together with TDZ) (Table 1). The highest number of plantlets (9.00) was counted in explants grown on media enriched with 0.5 mg l^{-1} IBA together with 1.0 mg l^{-1} TDZ. This number was much higher than those observed with other concentrations of IBA and TDZ, applied alone or in combination (Table 5). The lowest number of leaves (2.70) was obtained in control explants (Fig. 4). The application of 0.5 mg l^{-1} IBA along with 1.0 (Fig. 4) and 0.5 mg l^{-1} is optimum for leaves production.

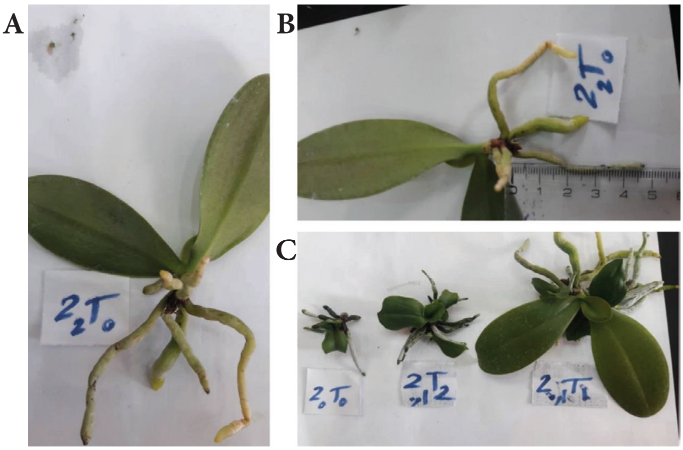

Figure 1. Micropropagation process of *Phalaenopsis circus* using different concentrations of 2,4-D and TDZ through direct organogenesis. Growth and development of leaves and roots from cultured protocorm explants obtained from germinated seeds **A, B** on medium enriched with 2.0 mg l^{-1} 2,4-D without TDZ **C** control (left), 0.1 mg l^{-1} 2,4-D and 2.0 mg l^{-1} TDZ (middle), and 0.1 mg l^{-1} 2,4-D and 1.0 mg l^{-1} TDZ (right). Scale bar: 10 mm.

Table 3. Mean comparison of the effect of different concentrations of NAA and Kin on measured parameters of *Phalaenopsis circus* grown under *in vitro* conditions for organogenesis.

	Mean comparison		
NAA + Kin (mg l^{-1})	**PLB number**	**Leaf length (cm)**	**Root length (cm)**
0.0 + 0.0	13.67j	1.6g	2.23j-l
0.0 + 0.5	22.33hij	2.13c-g	1.77l
0.0 + 1.0	33.0d-g	2.73cd	2.53h-n
0.0 + 2.0	31.0de-i	3.93b	2.2j-l
0.0 + 5.0	21.33ij	2.37c-f	2.07K-l
0.1 + 0.0	21.33ij	2.07d-g	2.4i-l
0.1 + 0.5	38.67cd	2.7cd	3.07e-i
0.1 + 1.0	48.0bc	2.57cde	2.8f-j
0.1 + 2.0	38.33cd	5.03a	3.27efg
0.1 + 5.0	23.33 g-j	2.43c-f	3.07e-i
0.5 + 0.0	25.67f-i	2.0efg	3.2e-h
0.5 + 0.5	51.0b	2.57cde	3.13e-h
0.5 + 1.0	63.0a	2.8c	3.57de
0.5 + 2.0	49.67b	3.9b	2.67g-n
0.5 + 5.0	31.0d-i	2.0efg	3.3efg
1.0 + 0.0	23.67g-j	1.67g	4.93b
1.0 + 0.5	28.0e-i	2.37c-f	6.87a
1.0 + 1.0	27.33e-i	3.6b	4.4bc
1.0 + 2.0	24.33ghi	2.17c-g	4.23cd
1.0 + 5.0	26.67f-i	1.87fg	3.47ef
5.0 + 0.0	30.33d-i	1.83fg	3.43ef
5.0 + 0.5	32.33d-h	2.47c-f	3.73cde
5.0 + 1.0	35.67def	2.67cde	3.20e-h
5.0 + 2.0	37.0de	2.5c-f	3.73cde
5.0 + 5.0	29.33d-i	2.73cd	4.20f

Means with different letters on the same column are significantly different ($p<0.05$) based on LSD test.

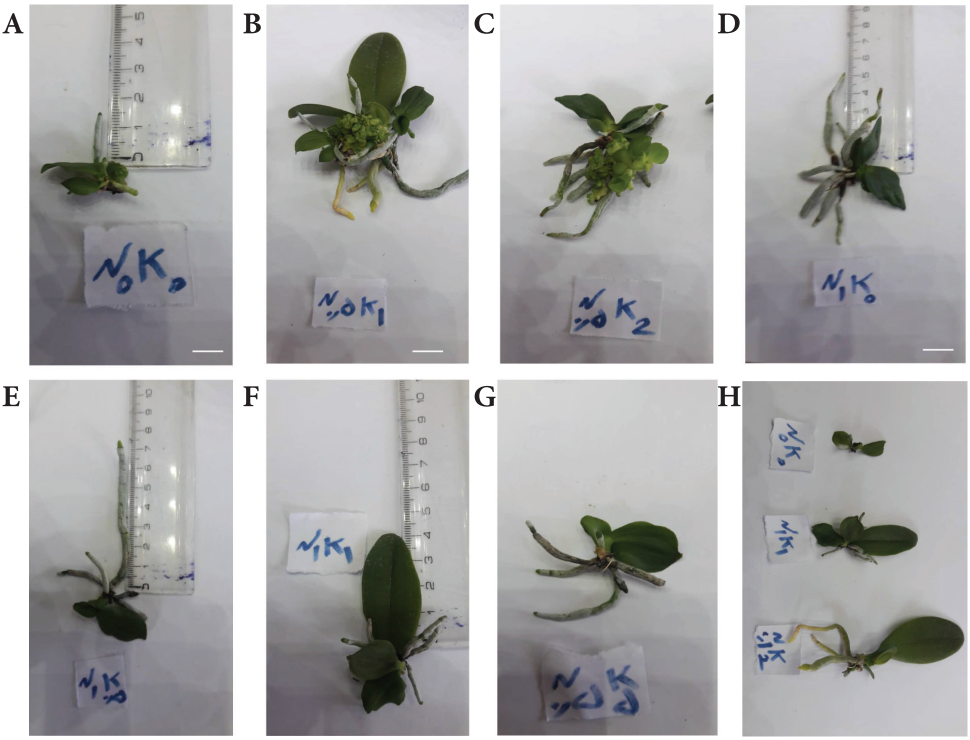

Figure 2. Micropropagation process of *Phalaenopsis circus* using different concentrations of NAA and Kin through organogenesis. Growth and development of leaves and roots from cultured protocorm explants obtained from germinated seeds **A** on control medium **B** on medium enriched with 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} Kin **C** on medium enriched with 0.5 mg l^{-1} NAA and 2.0 mg l^{-1} Kin **D** on medium enriched with 1.0 mg l^{-1} NAA without Kin **E** on medium enriched with 1 mg l^{-1} NAA and 0.5 mg l^{-1} Kin **F** on medium enriched with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} Kin **G** on medium enriched with 0.5 mg l^{-1} NAA and 5.0 mg l^{-1} Kin, and H) on control medium (up), medium enriched with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} Kin (middle) and medium enriched with 0.1 mg l^{-1} NAA and 2 mg l^{-1} Kin (down). Scale bar: 10 mm.

Applying 0.5 mg l^{-1} IBA together with 1.0 mg l^{-1} TDZ with 6.33 leaves and 0.5 mg l^{-1} IBA together with 0.5 mg l^{-1} TDZ with 5.33 leaves induced the highest number of leaves as compared to those induced with other treatments and in the absence of PGRs (2.13). Rooting efficiency was satisfactory using IBA at the concentration of 1.0 mg l^{-1} (Table 5). Both root number (5.67) and root length (5.63 cm) in the presence of 1.0 mg l^{-1} IBA were the highest. Root length and root number were lowest in explants grown on PGR-free media.

Ex vitro establishment of plantlets

Well-developed plantlets were transferred to plastic pots for *ex vitro* establishment and acclimatization (Fig. 5). A 100% establishment rate was obtained and plantlets were morphologically identical to the mother plants.

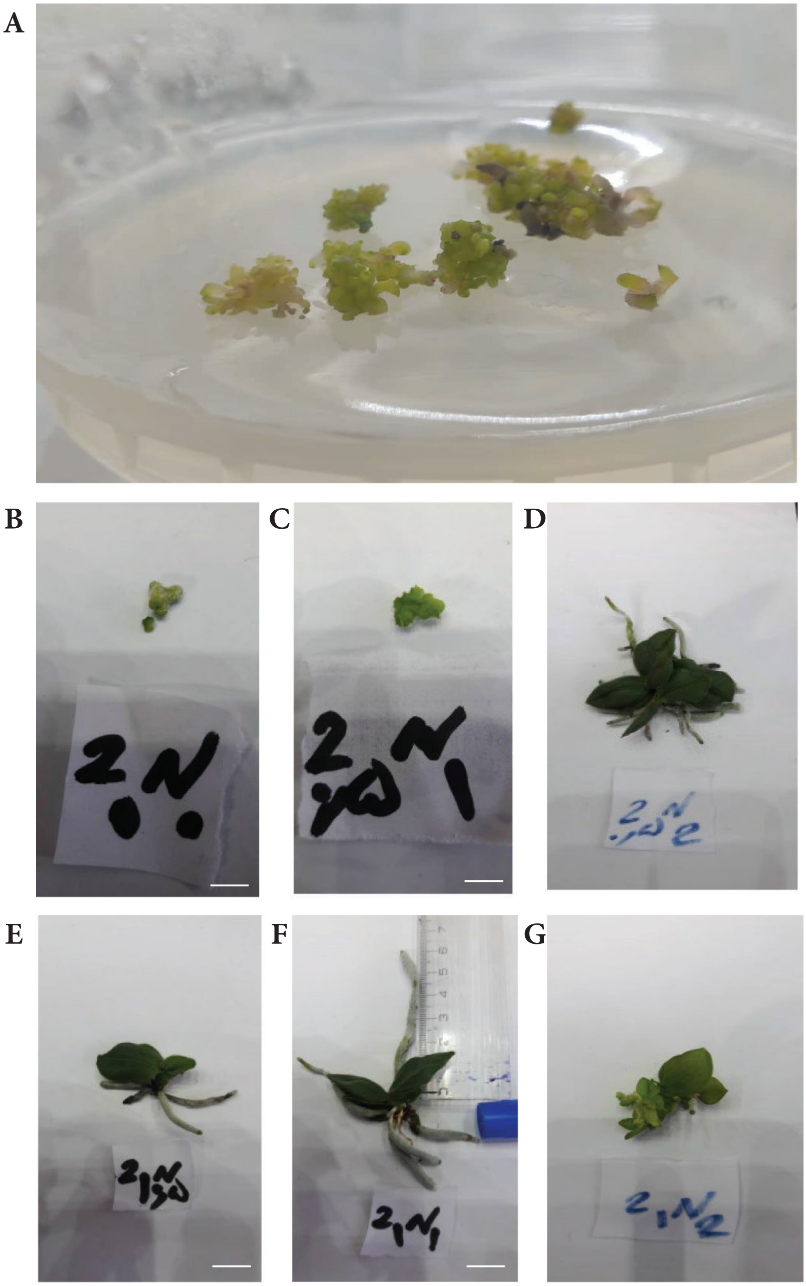

Figure 3. Micropropagation through somatic embryogenesis of *Phalaenopsis circus* using different concentrations of 2,4-D and NAA **A** Somatic embryos produced through inoculation of protocorm on medium enriched with 2,4-D and NAA **B** a somatic embryo on control medium **C** on medium enriched with 0.5 mg l^{-1} 2,4-D and 1.0 mg l^{-1} NAA **D** on medium enriched with 0.5 mg l^{-1} 2,4-D and 2.0 mg l^{-1} NAA **E** on medium enriched with 1.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} NAA **F** on medium enriched with 1.0 mg l^{-1} 2,4-D and 1.0 mg l^{-1} NAA, and **G** on medium enriched with 1.0 mg l^{-1} 2,4-D and 2.0 mg l^{-1} NAA. Scale bars: 5 mm (**A, B, C**); 10 mm (**D, E, F, G**).

Table 4. Mean comparison of the effect of different concentrations of 2,4-D and NAA on measured parameters of *Phalaenopsis circus* grown under *in vitro* conditions for somatic embryogenesis.

	Mean comparison	
2,4-D + NAA (mg l^{-1})	Somatic embryo number	Root length (cm)
0.0 + 0.0	6.33c-g	2.3j
0.0 + 0.1	5.67d-g	2.6ij
0.0 + 0.5	5.67d-g	2.77g-j
0.0 + 1.0	6.67c-f	2.87f-j
0.0 + 2.0	5.33efg	4.03bcd
0.01 + 0.0	5.67d-g	2.3j
0.01 + 0.1	4.67fg	2.77g-j
0.01 + 0.5	5.67d-g	2.43ij
0.01 + 1.0	4.67fg	3.1e-i
0.01 + 2.0	5.67d-g	4.5abc
0.1 + 0.0	4.67fg	2.77g-j
0.1 + 0.1	4.33g	3.03e-j
0.1 + 0.5	6.67c-f	2.47ij
0.1 + 1.0	5.67d-g	3.4d-h
0.1 + 2.0	6.33c-g	4.97a
0.5 + 0.0	7.67bcd	2.4j
0.5 + 0.1	12.0a	2.67hij
0.5 + 0.5	9.0b	3.03e-j
0.5 + 1.0	9.0b	2.8g-j
0.5 + 2.0	12.33a	3.53d-g
1.0 + 0.0	7.33b-e	4.7ab
1.0 + 0.1	7.0b-e	3.6def
1.0 + 0.5	8.0bc	3.93bcd
1.0 + 1.0	8.0bc	4.93a
1.0 + 2.0	7.67bcd	3.8cde

Means with different letters on the same column are significantly different ($p<0.05$) based on LSD test.

Discussion

In the present study, induction of callus formation from the protocorms and leaves of *P. circus* did not occur in any of the treatments. This is consistent with the findings for some other orchids like leaf explants of *Paphiopedilum* spp. (Guo et al. 2024). On the contrary, calluses have been successfully induced from the seeds or protocorms of some species of *Paphiopedilum* (Zeng et al. 2013; Guo et al. 2024).

Direct shoot regeneration and multiplication from PLBs and protocorms explants is a successful approach for *in vitro* propagation of orchids (Chugh et al. 2009; Lee and Yeung 2018; Guo et al. 2024), such as *Renanthera imschootiana* (Seeni and Latha 1992), *Cymbidium aloifolium* (Bujarbarua and Sharma 1997; Kaur and Sharma 1997), *Dendrobium aphyllum* (Talukdar 2001), *Habenaria marginata* (Sheelavanthmath and Murthy 2001), *Vanda coerulea* (Roy et al. 2011), *Eulophia nuda* Lindl. (Panwar et al. 2012), *Orchis catasetum* (Baker et al. 2014), *Catasetum pileatum* cv. Alba (Zakizadeh et al. 2019), and *Phalaenopsis amabilis* (Mohammadi et al. 2019).

PLBs production from protocorm culture was influenced by concentration and combination of auxins and cytokinins. In the present investigation, the highest PLB

Table 5. Mean comparison of the effect of different concentrations of IBA and TDZ on measured parameters of *Phalaenopsis circus* plantlets obtained from *in-vitro* cultured TCLs.

	Mean comparison			
IBA + TDZ (mg l^{-1})	**Plantlet number**	**Leaf number**	**Root length (cm)**	**Root number**
0.0 + 0.0	2.7e	2.13d	1.7g	1.93c
0.0 + 0.01	3.0e	2.33d	2.2fg	2.33bc
0.0 + 0.1	3.33de	2.67cd	2.2fg	2.33bc
0.0 + 0.5	3.67cde	3.0cd	2.7def	3.0bc
0.0 + 1.0	3.0e	3.0cd	2.1fg	3.0bc
0.1 + 0.0	3.33de	3.0cd	2.53def	3.0bc
0.1 + 0.01	3.0e	3.33cd	2.67def	3.0bc
0.1 + 0.1	3.0e	2.67cd	2.2fg	2.0c
0.1 + 0.5	3.67cde	3.0cd	2.8de	3.0bc
0.1 + 1.0	3.67cde	2.33d	2.27efg	3.0bc
0.5 + 0.0	3.67cde	3.33cd	2.53def	5.0a
0.5 + 0.01	3.33de	3.67cd	2.93d	3.0bc
0.5 + 0.1	4.0cde	3.0cd	3.57bc	3.33b
0.5 + 0.5	5.0c	5.33ab	4.13b	2.33bc
0.5 + 1.0	9.0a	6.33a	3.53c	2.0c
1.0 + 0.0	3.67cde	3.67cd	5.63a	5.67a
1.0 + 0.01	4.33cde	3.67cd	4.13b	2.33bc
1.0 + 0.1	4.0cde	4.0bc	3.83bc	3.0bc
1.0 + 0.5	4.67cd	3.33cd	3.93bc	3.0bc
1.0 + 1.0	6.67b	3.67cd	3.83bc	2.67bc

Means with different letters on the same column are significantly different ($p<0.05$) based on LSD test.

number in *P. circus* was obtained by 2,4-D alone and NAA together with TDZ. The combination, type, concentration and the ratio between PGRs plays a critical important role for the formation of shoots, protocorms and PLBs in many orchids (Arditti and Ernst 1993; Bhattacharyya et al. 2016; Kaviani et al. 2017). The most commonly used auxins in orchid culture media are IAA, NAA, IBA, and 2,4-D. On the other hand, Kin, BA, BAP, TDZ, and zeatin are the most commonly used cytokinins in orchid culture media (Yam and Arditi 2018).

Maximum PLB regeneration and the highest root length in *Orchis catasetum* were achieved on media containing both BA and NAA (Baker et al. 2014). The addition of exogenous PGRs in appropriate concentrations promoted shoot multiplication from PLBs (Bhattacharyya et al. 2016). A combination of 1.0 mg l^{-1} Kin and 1.0 mg l^{-1} IBA induced maximum PLB regeneration and the largest number of leaves in *Catasetum pileatum* cv. Alba. Also, the highest rooting frequency was achieved on PLBs grown in medium supplemented with 1.0 mg l^{-1} Kin together with 0.5 mg l^{-1} IBA (Zakizadeh et al. 2019). In another study, a combination of 0.5 mg l^{-1} IBA along with 0.5 mg l^{-1} Kin was suitable for rooting in *Phalaenopsis amabilis* Blume var. Grandiflora (Mohammadi et al. 2019). In the orchid *Vanda coerulea*, a combination of NAA and BAP was found to be optimal for maximum PLB regeneration (Roy et al. 2011).

The current study showed that the lowest number of PLBs was produced on media without PGRs or with low and high concentrations of PGRs. Minimum PLB number was observed on media containing different concentrations of TDZ or Kin, alone.

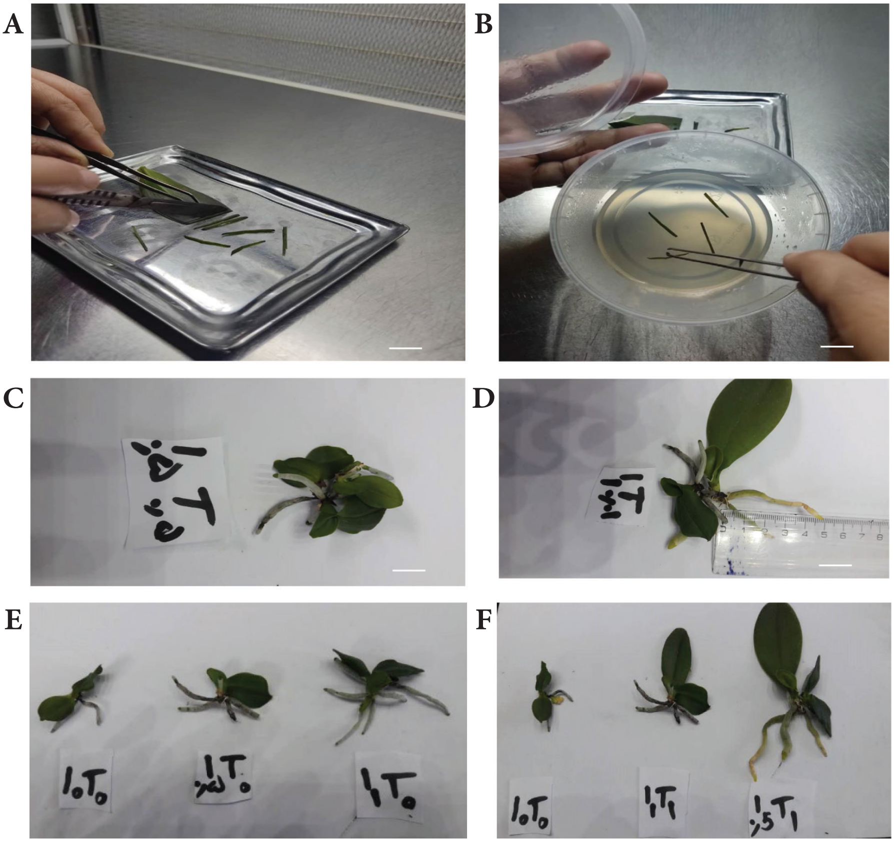

Figure 4. Micropropagation through TCLs of *Phalaenopsis circus* using different concentrations of IBA and TDZ **A** Preparing TCLs from a leaf **B** inoculation of explants on culture medium **C–F** plantlets produced **C** on medium enriched with 0.5 mg l^{-1} IBA and 0.5 mg l^{-1} TDZ **D** on medium enriched with 1.0 mg l^{-1} IBA and 0.01 mg l^{-1} TDZ **E** on media without PGRs (left), enriched with 0.5 mg l^{-1} IBA without TDZ (middle), and enriched with 1.0 mg l^{-1} IBA without TDZ, and **F** on media without PGRs (left), enriched with 1.0 mg l^{-1} IBA and 1.0 mg l^{-1} TDZ (middle), and enriched with 0.5 mg l^{-1} IBA and 1.0 mg l^{-1} TDZ. Scale bars: 10 mm (**A, B, C, E**); 15 mm (**D, F**).

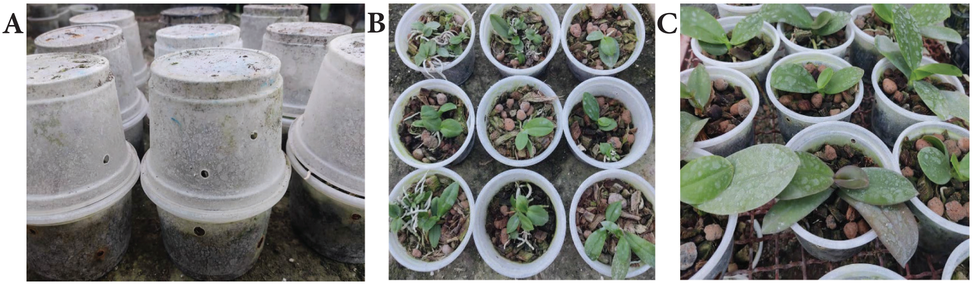

Figure 5. Acclimatization in a greenhouse of plantlets produced *in vitro* and transferred to plastic pots filled with LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite in the proportion of 15:10:20:5:30:20% **A** Cultivation of plantlets in plastic pots covered with another plastic pot having several holes **B, C** growing plantlets in pots without cover.

Similar findings were reported in other orchids (Chugh et al. 2009; Panwar et al. 2012; Lee and Yeung 2018). Appropriate formation of PLBs and shoots in *Dendrobium nobile* was performed in medium supplemented with NAA together with BAP, TDZ or meta-topolin (Bhattacharyya et al. 2016). Unlike these reports, some researchers demonstrated that the multiplication of shoots is closely related with the type and concentration of cytokinins used (Amoo et al. 2014; Bhattacharyya et al. 2016; Lee and Yeung 2018). The presence of cytokinins alone promoted optimal shoot proliferation from protocorm explants in some orchids like *Dendrobium nobile* and *C. aloifolium* (Nayak et al. 1997), *C. ensifolium* (Chang and Chang 1998), *Rhynchostylis gigantea* (Van Le et al. 1999), *D. nobile* and *C. aloifolium* (Nayak et al. 2002), and *Dendrobium* (Ferreira et al. 2006). It has been shown that TDZ is the most effective cytokinin for stimulating shoot formation in orchids (Huetteman and Preece 1993; Ket et al. 2004; Martin and Madassery 2006; Zhao et al. 2007; Mahendran and Narmatha Bai 2009). TDZ was the best cytokinin for multiple shoot induction from the protocorm of *Satyrium nepalense* D.Don. (Mahendran and Narmatha Bai 2009). TDZ was first used for *in vitro* propagation of *Phalaenopsis* and *Doritaenopsis* (Ernst 1994). Also, IBA was effective for inducing healthy roots. In *Eulophia nuda* Lindl., maximum PLB formation, shoot multiplication and elongation were obtained on MS medium containing 8.88 μM BA and 4.68 μM Kin after four weeks of culture (Panwar et al. 2012). Luo et al. (2008) showed that 0.50 mg l^{-1} Kin was appropriate for PLB formation of *Dendrobium densiflorum*. BAP alone was better than in combination with NAA for producing the highest number of PLBs in *Oncidium* (Kalimuthu et al. 2007).

The present investigation revealed that the longest roots were induced using both NAA and Kin in combination. Most studies on orchids showed the highest root length and number on media containing an auxin particularly IBA (Chugh et al. 2009; Panwar et al. 2012; Lee and Yeung 2018). Maximum root induction (86.0%) in *Dendrobium nobile* was obtained on half-strength MS medium enriched with 2.0 mg l^{-1} of IBA. The effectiveness of IBA in root induction and growth has been reported for some other orchids such as *Vanilla planifolia* (Giridhar et al. 2001) and *C. pendulum* (Nongdam et al. 2006), too. The main reasons for these differences are the orchid species and cultivars, endogenous PGR levels and type and concentration of exogenous PGRs. In the present study, addition of NAA and TDZ in appropriate concentrations induced plantlet growth and leaf formation from PLBs without callus formation. The major advantage of direct organogenesis without an intervening callus phase is reduction of somaclonal variation (Roy et al. 2011).

SE is an important method of plant regeneration, and several reports have described this technology for some orchids (Chung et al. 2005; Kuo et al. 2005; Chen and Chang 2006). The process of SE is affected by many factors particularly PGRs. In this study, combinations of 2,4-D and NAA were successfully used to induce SE in *P. circus*. There were obvious differences in the number of embryos induction between 2,4-D and NAA. Media containing low concentrations of these PGRs failed to produce embryos. The combination of 2,4-D and other PGRs during SE has been reported for many plants (Yang et al. 2018). Some studies also showed the effectiveness of NAA for SE (Yang et al. 2018). In the present study, NAA did not have a significant effect on SE, when used alone. TDZ is apparently a useful cytokinin for SE. Direct SE from young leaves of *Oncidium* 'Gower Ramsey' using half-strength MS medium containing 0.3–3.0 mg l^{-1} TDZ was reported (Chen et al. 1999). TDZ promoted direct SE from the epidermal cells and secondary SE from leaf explants of *Phalaenopsis amabilis* (Chen and Chang 2006). Auxins IAA, IBA, NAA, and 2,4-D retarded embryo formation but cytokinins 2-iP, Zt, Kin, BAP and TDZ promoted it in leaf explants of *Dendrobium* cv. Chiengmai Pink. Maximum direct embryo induction was obtained with 18.16 µM TDZ (Chung et al. 2005). Embryogenic calluses were induced from PLBs of *Cymbidium* Twilight Moon 'Day Light' on medium supplemented with NAA or 2,4-D alone or in combination with TDZ. The medium containing the combination of 0.1 mg l^{-1} NAA and 0.01 mg l^{-1} TDZ was optimal for callus formation. PLB formation from callus was obtained through transfer of callus to medium without PGRs. These callus-derived PLBs converted into normal plants on the PGR-free medium (Huan et al. 2004). Clusters of somatic embryos were formed from leaf explants of *Phalaenopsis* 'Little Steve' on half-strength MS medium enriched with BA and TDZ. Kin had no effect on direct embryo induction and 2,4-D retarded the frequency of embryogenesis that was induced by TDZ (Kuo et al. 2005). Therefore, the selection of PGR types, concentrations and combinations is an important criterion for the development of somatic embryos in orchids, as in other plants.

The TCL technique is a simple and effective approach for *in vitro* propagation of orchids. TLCs have been successfully applied for PLB and callus induction in some orchids like *Cymbidium* spp., *Dendrobium* spp., *Aranda*, *Coelogyne*, *Doritaenopsis*, *Paphiopedilum*, *Renanthera*, *Rhynchostylis*, *Spathoglottis*, and *Xenikophyton* (Teixeira da Silva 2013). In the current study, maximum plantlet regeneration was achieved from explants grown on media enriched with 0.5 mg l^{-1} IBA together with 1.0 mg l^{-1} TDZ. In *Dendrobium gratiosissimum*, MS medium containing 2.0 mg l^{-1} Kin developed the highest number of PLBs from thin protocorm sections. Also, the highest PLB production from thin stem sections was achieved from explants cultured in the presence of 5.0 mg l^{-1} Kin together with 1.0 mg l^{-1} NAA (Jaiphet and Rangsayatorn 2010). Direct induction of multiple shoot buds was obtained from thin sectioned leaf explants of *Phalaenopsis* cultured on half-strength MS medium enriched with 9.08 µM TDZ (Myint et al. 2009). In *Aranda*, *Phalaenopsis* and *Cymbidium*, the highest number of PLBs was obtained from TCLs excised from shoot tips (Teixeira da Silva et al. 2007).

Conclusions

The growing popularity of orchids around the world has encouraged propagators and breeders of these valuable plants to develop the orchid flower industry more than ever. Providing effective protocols for their *in vitro* propagation using appropriate techniques, explants, and PGRs is one way to reach this goal. The current study presents effective methods for *Phalaenopsis circus in vitro* propagation, some of which are reported for the first time for this species of orchid. Briefly, of the three auxins used, 2,4-D was found to be the best for PLB induction. Also, of the two cytokinins used, TDZ was better than Kin for plantlet production. Both organogenesis and SE were proper approaches for induction of shoots and roots and multiplication of *P. circus*. TCLs, one of the most recent techniques for the *in vitro* propagation of selected orchid species have the potential for large-scale commercial multiplication of these valuable orchids.

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SHORT COMMUNICATION

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Tetraploid European paeonies (*Paeonia*) show a homogeneous karyotype asymmetry and structure

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Abstract

Within a practical course of cytotaxonomy organized in Pisa (Italy) on February 2024 by the Group for Floristics, Systematics and Evolution of the Italian Botanical Society, we tested whether relevant differences in karyotype asymmetry and structure occur in four tetraploid European taxa from *Paeonia* sect. *Paeonia* (*P. mascula* subsp. *russoi*, *P. officinalis* subsp. *huthii*, *P. officinalis* subsp. *italica*, and *P. peregrina*). Our results point towards a homogeneous karyotype asymmetry and structure among studied tetraploid species, with no statistically significant difference among taxa and high overlap in variation highlighted by PCA.

Keywords

Cytogenetics, cytostematics, cytotaxonomy

Introduction

The Boreal genus *Paeonia* is the only member of the family Paeoniaceae (Saxifragales) and includes ca. 40 species (Tamura 2007). It consists of three monophyletic sections: *P.* sect. *Paeonia* (which includes most herbaceous species, from Eurasia), *P.* sect. *Moutan* DC. (including woody species endemic to western China), and *P.* sect. *Oanepia* Lindl. (including herbaceous species from North America) (Sang et al. 1997). The basic chromosome number of this genus is $x = 5$, which is most likely ancestral (Carta et al. 2020). Three ploidy levels have been documented: diploids ($2n = 10$, found in all sections), tetraploids ($2n = 20$, found only in *P.* sect. *Paeonia*) and (mostly artificial) hybrid triploids with $2n = 15$ chromosomes (Hong et al. 1988; Yang et al. 2017).

Due to the large chromosomes (10–15 µm long; Okada and Tamura 1979) as compared to other angiosperms (Badr and El-Shazly 2021), this genus is an ideal model to practice with karyomorphology, which is a simple, inexpensive and powerful approach to obtain useful basic comparative information in systematic studies (Astuti et al. 2017). This approach typically involves measuring chromosomes (i.e. length of long arm [L], short arm [S]) in spread metaphase plates to describe the phenotypic aspect of a chromosome complement (Levin 2002; Guerra 2012).

Metaphase plates of selected *Paeonia* taxa were utilized during a practical course on cytotaxonomy organized in Pisa (Italy) between February 6 and 9, 2024 by the Group for Floristics, Systematics and Evolution of the Italian Botanical Society. The main objective was to test whether relevant differences in karyotype asymmetry and structure could be identified in selected tetraploid European taxa from *P.* sect. *Paeonia*. Commonly used traits for this analysis included total haploid (monoploid) chromosome length (THL), the mean centromeric asymmetry (M_{CA}), the coefficient of variation of chromosome length (CV_{CL}), and the coefficient of variation of centromeric index (CV_{CI}) (Peruzzi and Altınordu 2014).

Materials and methods

One of the authors (LP) prepared a set of permanent microscope slides about twenty years ago (between 2002 and 2005), as part of a study on Italian *Paeonia* species, whose results were only partially published concerning the sole chromosome numbers (L. Peruzzi in Passalacqua and Bernardo 2004).

Squash preparations were made on root tips obtained from plants temporarily cultivated in pots at the Botanical Garden of the Calabria University, Arcavacata di Rende, Cosenza (Table 1). Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy solution for 1 hour. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

A total of 14 metaphase plates (Table 1) were analyzed by the course participants using MATO software (Altınordu et al. 2016; Liu et al. 2023). The focus was on the following quantitative traits:

- THL (total haploid [monoploid] length of chromosome complement): a proxy of genome size (Carta and Peruzzi 2016; Franzoni et al. 2024) calculated as the sum of the length of all the chromosomes in a metaphase plate, divided by the ploidy level (Peruzzi and Altınordu 2014);
- M_{CA} (mean centromeric asymmetry): intrachromosomal karyotype asymmetry (Peruzzi and Eroğlu 2013) calculated as the mean value of the difference between the two (complementary) proportions L/(L+S) and S/(L+S), multiplied by 100;
- CV_{CL} (coefficient of variation of chromosome length): interchromosomal karyotype asymmetry (Paszko 2006) calculated as the standard deviation of chromosome lengths (L+S) in a complement, divided by the mean chromosome length and multiplied by 100;
- CV_{CI} (coefficient of variation of centromeric index): degree of heterogeneity in the position of centromere in a karyotype (Zuo and Yuan 2011) calculated as the standard deviation of centromeric index S/(L+S) in a complement, divided by the mean centromeric index and multiplied by 100 (Paszko 2006).

To test for differences among taxa, univariate (non-parametric Mann-Whitney pairwise test) and multivariate (PCA, after logarithmic standardization) statistics have been conducted in PAST 4.17 (Hammer et al. 2001; Hammer 2024).

Results

All taxa studied had $2n = 4x = 20$ chromosomes. The variation of each karyomorphological trait is presented in Table 2.

According to univariate statistics, no significant differences were observed among the four studied taxa, for any of the considered karyomorphological parameters. The PCA (Fig. 1), whose first two components account for 85% of variation, show overlap among groups, although higher CV_{CI} and CV_{CL} values slightly separated the two accessions of *P. mascula* subsp. *russoi* from the others.

Table 1. Source of the tetraploid ($2n = 4x = 20$) *Paeonia* taxa studied, and the number of studied metaphase plate microphotographs.

	Locality, date, and collector(s)	**metaphase plates (*N*)**
Paeonia mascula (L.) Mill. subsp. *russoi* (Biv.) Cullen & Heywood	Italy, Sicily, Iblei (Valle Cava Grande), 10 July 2003, *L. Peruzzi et N.G. Passalacqua*	2
Paeonia officinalis L. subsp. *huthii* Soldano	Italy, Liguria (Monte Toraggio), 19 June 1995, *L. Bernardo et N.G. Passalacqua*	4
Paeonia officinalis L. subsp. *italica* N.G.Passal. & Bernardo	Italy, Abruzzo, Gran Sasso (Prati di Tivo), 13 July 2001, *L. Bernardo et N.G. Passalacqua*	5
Paeonia peregrina Mill.	Bulgaria, 1 August 2001, *D. Uzunov*	3

Table 2. Karyomorphological traits calculated by the participants for selected tetraploid European Paeonia taxa.

Metaphase plate	2*n*	*x*	THL (µm)	M_{CA}	CV_{CL}	CV_{CI}
Paeonia mascula subsp. *russoi* 1	20	5	112.38	35.02	19.26	23.26
Paeonia mascula subsp. *russoi* 2	20	5	116.63	32.44	24.54	23.04
Paeonia officinalis subsp. *huthii* 1	20	5	116.58	28.40	25.02	27.30
Paeonia officinalis subsp. *huthii* 2	20	5	103.27	22.43	14.06	20.06
Paeonia officinalis subsp. *huthii* 3	20	5	126.55	17.37	18.85	20.89
Paeonia officinalis subsp. *huthii* 4	20	5	103.73	26.71	14.79	23.87
Paeonia officinalis subsp. *italica* 1	20	5	126.36	23.12	17.24	27.39
Paeonia officinalis subsp. *italica* 2	20	5	101.14	24.55	21.25	22.24
Paeonia officinalis subsp. *italica* 3	20	5	114.59	22.18	22.34	20.18
Paeonia officinalis subsp. *italica* 4	20	5	117.54	26.35	15.01	21.22
Paeonia officinalis subsp. *italica* 5	20	5	93.41	32.02	21.47	24.04
Paeonia peregrina 1	20	5	106.36	28.25	16.14	26.01
Paeonia peregrina 2	20	5	100.33	30.50	21.29	21.74
Paeonia peregrina 3	20	5	122.11	29.48	18.06	25.27

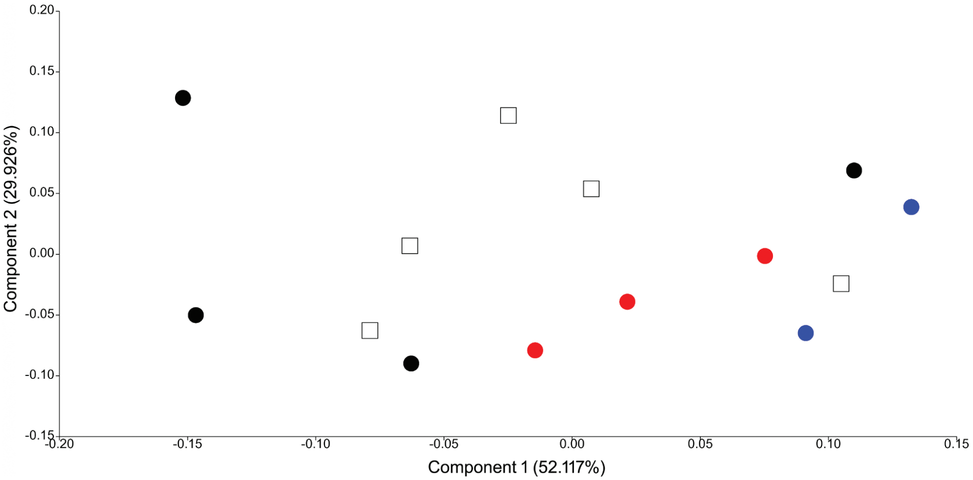

Figure 1. PCA illustrating the overall variation of karyomorphological traits in the four studied tetraploid *Paeonia* taxa. Blue dots: *P. mascula* subsp. *russoi*; black dots: *P. officinalis* subsp. *huthii*, black empty squares: *P. officinalis* subsp. *italica*; Red dots: *P. peregrina*.

Discussion

The chromosome number for Italian *P. mascula* subsp. *russoi*, *P. officinalis* subsp. *huthii*, and *P. officinalis* subsp. *italica* were already reported in Passalacqua and Bernardo (2004), while the count for *P. peregrina* from Bulgaria was not published before, but agrees with data published by Koeva and Sarkova (1997).

Regarding karyomorphological traits, our results suggest homogeneous karyotype asymmetry and structure among the studied tetraploid *Paeonia* species, with no statistically significant differences between taxa and high overlap in variation highlighted by PCA. Despite the very limited sampling, which prevents from any conclusion, the only species somehow distinct is *P. mascula*, which is also taxonomically more distinct with respect to *P. officinalis*/*P. peregrina* (Passalacqua and Bernardo 2004), based on leaf and root structure.

To our knowledge, this is the first quantitative study on this topic. Our findings are consistent with previous qualitative karyological observations of tetraploid *Paeonia* taxa from Europe (Tzanoudakis 1983; Schwarzacher-Robinson 1986; Punina 2005). This homogeneity seems even more extensive and possibly applying to large portions of *P.* sect. *Paeonia*. Indeed, for instance, CV_{CL} and CV_{CI} values calculated in the studied taxa are very similar to those reported for the tetraploid *P. mairei* H.Lév., endemic to China (Chen et al. 2023).

The similarity in karyotype structure in such tetraploids can hinder the possibility to highlight their allotetraploid status (see, e.g., Sang et al. 2004; Punina et al. 2021) using traditional Feulgen approaches. However, molecular cytogenetic techniques like GISH have been effective in cultivated hybrid varieties (Cui et al. 2022), and could be valuable in studying wild species.

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RESEARCH ARTICLE

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Notulae to the Italian flora of algae, bryophytes, fungi and lichens: 18

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Abstract

In this contribution, new data concerning algae, bryophytes, fungi and lichens of the Italian flora are presented. It includes new records, confirmations or exclusions for the algal genera *Chara* and *Tolypella*, the bryophyte genera *Pogonatum*, *Pseudephemerum*, and *Riella*, the fungal genera *Arrhenia*, *Arthonia*, *Buchwaldoboletus*, *Dacampia*, *Hebeloma*, *Inocybe*, and *Trechispora*, and the lichen genera *Aspicilia*, *Bellemerea*, *Cladonia*, *Hypotrachyna*, *Maronea*, *Parvoplaca*, *Polyozosia*, *Schismatomma*, *Solenopsora*, *Trapelia*, and *Zwackhia*.

Keywords

Ascomycota, Basidiomycota, Bryidae, Charophyceae

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

ALGAE

Chara braunii C.C.Gmel. (Characeae)

+ **LAZ**: Riserva Naturale di Decima Malafede, Roma (UTM WGS 84: 33T 287779. 4622766), permament pond, bottom between 0.2 and 1 m depth., 83 m, June 2014, *M.M. Azzella*; Piscina della Cava di Brecciolino, Tenuta presidenziale di Castelporziano, Roma (Roma) (UTM WGS 84: 33T 284805.4627879), permanent pond, bottom between 0.2 and 1 m depth, on muddy substrate, 20 m, 5 June 2024, *M.M. Azzella* (HFLA). – Species new for the flora of Lazio.

This species can be distinguished easily from other *Chara* species by the absence of a cortex and is characterized by a wide intraspecific variability (Kato et al. 2008). In Italy, it has been considered a rare species (Romanov et al. 2019), and recorded for Piemonte, Lombardia, Veneto, Emilia-Romagna, Toscana, Campania, and Sicilia (Bazzichelli and Abdelahad 2009).

M.M. Azzella, G. Filibeck, L. Rosati

Tolypella intricata **(Trentepohl ex Roth) Leonhardi (Characeae)**

+ **BAS**: Monte Santa Croce, San Fele (Potenza) (WGS 84: 33T 548210.4515459), small pond with a maximum depth of 0.7 m, 1060 m, 25 March 2024, leg. *L. Rosati, V.A. Romano, A. Stinca*, det. *M.M. Azzella, L. Rosati* (HLUC). – Species new for the flora of Basilicata.

This species was not hitherto recorded for southern Italy. The known stations of *T. intricata* in Italy are in Lombardia, Veneto, and Emilia-Romagna (Bazzichelli and Abdelahad 2009). The dense aquatic vegetation completely covering the bottom of the pond is dominated by *T. intricata* with the presence of *Groenlandia densa* (L.) Fourr. and *Ranunculus trichophyllus* Chaix. This species has a cosmopolitan distribution, with localities reported all over Europe (e.g., Becker et al. 2015), North America (Prescott 1962), and Oceania (Day et al. 1995).

L. Rosati, M.M. Azzella

BRYOPHYTES

Pogonatum nanum **(Hedw.) P.Beauv. (Polytrichaceae)**

+ **ABR**: Valico Aceretta (between Monte Ceraso and Monte delle Vitelle), south-east slope, Pescasseroli (L'Aquila) (UTM WGS84: 33T 395030.4629019), 1690 m, 4 July 2024, shoulder of a forest track in a beech forest, *F. Filippelli, L. Cancellieri* (UTV: B_000007); Rifugio Campitelli, Alfedena (L'Aquila) (UTM WGS84: 33T 414975.4616748), 1430 m, 28 July 2024, shoulder of a forest track in a beech forest, *F. Filippelli, L. Cancellieri* (UTV: B_000008). – Species new for the flora of Abruzzo.

Pogonatum nanum is a European temperate species that grows on acidic soil on banks, heaths and roadsides (Smith 2004). It is quite common in Italy, where it has been reported for many Italian administrative regions (Aleffi et al. 2023). In the new sites it was found on the ground, among the surface roots of beech trees in beech forests characterized by high levels of atmospheric humidity.

F. Filippelli, L. Cancellieri

Pseudephemerum nitidum (Hedw.) Loeske (Ditrichaceae)

+ **TOS**: Riserva Naturale Provinciale "Monte Serra di Sotto", Buti (Pisa) (UTM WGS84: 32T 625953.4844348), along stream edges, on acidic soil, 610 m, 29 May 2019, *G. Pandeli* (SIENA; Herb. Pandeli). – Species confirmed for the flora of Toscana.

A small gregarious species currently recorded in Lombardia, Umbria, and Abruzzo and, with old reports, from Emilia-Romagna and Toscana (Aleffi et al. 2023). In the new site, this species was found on quartz-rich sediments along stream edges with *Calypogeia arguta* Nees & Mont., *Cephaloziella turneri* (Hook.) Müll.Frib., *Entosthodon obtusus* (Hedw.) Lindb., *Pogonatum aloides* (Hedw.) P.Beauv., and *Polytrichum juniperinum* Hedw. The current record is a confirmation of the species for the flora of Toscana after more than 140 years (Lange 1868, 1875; De Notaris 1869; Fitzgerald and Bottini 1881). *Pseudephemerum nitidum* has been recently considered as Near Threatened in Italy (Puglisi et al. 2024).

G. Pandeli, I. Bonini

Riella notarisii (Mont.) Mont. (Riellaceae)

+ **TOS**: Area APEA, Loc. Colmata, Piombino (Livorno) (UTM WGS84: 32T 625934.4757158), in a small temporary pond near a building site, 1 m, 14 April 2023, *G. Pandeli* (SIENA; Herb. Pandeli). – Species new for the flora of Toscana.

Riella notarisii is a terricolous, hygrophilous, halophilous species, currently recorded in the southern Italian administrative regions of Sicilia and Calabria and with old reports from Sardegna (Aleffi et al. 2023). The collected specimens were found in a small temporary pond with *Ranunculus trichophyllus* Chaix, *Chara vulgaris* L., and *Nitella tenuissima* (Desv.) Kütz, and the notheworty presence of *Petalophyllum ralfsii* (Wilson) Nees & Gottsche at the edges. This rare liverwort is considered Endangered in Italy (Puglisi et al. 2023) and Near Threatened in Europe (Hodgetts et al. 2019).

G. Pandeli, I. Bonini, A. Battaglini

FUNGI

Arrhenia obscurata (D.A.Reid) Redhead, Lutzoni, Moncalvo & Vilgalys (Hygrophoraceae)

+ **CAL**: Botanical Garden, University of Calabria, Rende (Cosenza) (UTM WGS84: 33S 605863.4357365), among mosses along the stony edge of a path, 200 m, 13 March 2024, *N.G. Passalacqua, D. Puntillo, G. Sicoli* (CLU F337). – Species new to Calabria.

A completely greyish-brown and omphalinoid basidiome was found having the pileus slightly exceeding 1 cm in width, thin-fleshed, translucently striate at margin, depressed and slightly paler in the centre. The stipe was as long as pileus width, 2–3 mm width, curved and subclavate at base. Gills were rather distant, broad and decurrent. Spores were hyaline, broadly ellipsoid with a prominent lateral apiculum, and 8–10 × 5–7 µm in size. In Italy, this species has been apparently detected only in the northern regions, so far (Onofri et al. 2013).

N.G. Passalacqua, D. Puntillo, G. Sicoli

Arthonia molendoi (Frauenf.) R.Sant. (Arthoniaceae)

\+ **LOM**: Eastern Alps, Central Alps, Southern Rhaetian Alps, Stelvio (Ortler) - group, Cima di Cadì N above Passo del Tonale, NW below the summit on the ridge to M. Tonale Occidentale (Brescia) (UTM WGS84: 32T 620812.5125952), outcrops of calcareous schist on the crest in alpine vegetation, on low outcrops, on thallus and apothecia of *Rusavskia elegans* (Link) S.Y.Kondr. & Kärnefelt, 2570 m, 28 July 2006, leg. *J. Hafellner, L. Muggia*, det. *J. Hafellner* (GZU - JH77163). – Species new to Lombardia.
\+ **PIE**: Western Alps, Alpi Cozie, on the ridge W above Colle del Vallonetto (Cuneo) (UTM WGS84: 32T 381466.4888521), calcareous cliffs and boulders in alpine vegetation, on cliffs exposed to SE, on thallus and apothecia of *Calogaya biatorina* (A.Massal.) Arup, Frödén & Søchting, 2500 m, 23 July 2000, leg. *A. Hafellner, J. Hafellner*, *P.L. Nimis*, *M. Tretiach*, det. *J. Hafellner* (GZU - JH88157); Alpi Cozie, in the valley E below Colle Valcavera (Cuneo), (UTM WGS84: 32T 350630.4915542), large boulders of calcareous schist in subalpine pasture, on steep rock faces, on thallus and apothecia of *Calogaya biatorina* (A.Massal.) Arup, Frödén & Søchting, 2140 m, 23 July 2000, *J. Hafellner*, *P.L. Nimis*, *M. Tretiach* (GZU - JH87651). – Species new to Piemonte.

This species is restricted to *Rusavskia* (i.e. *Xanthoria elegans* group) and *Calogaya* (i.e. *Caloplaca biatorina-saxicola* group) and hence to preferably saxicolous teloschistalean lichens. *Arthonia molendoi* is known from all continents and is also widely distributed in Italy including the Alps, where is has so far been reported for the regions Friuli, Trentino-Alto Adige (including the type locality), Valle d'Aosta, and Liguria (Brackel 2016; Nimis and Martellos 2024). *Arthonia* populations growing on other Teloschistales refer to other species, e.g., *A. parietinaria*.

J. Hafellner

Arthonia parietinaria Hafellner & A.Fleischhacker (Arthoniaceae)

\+ **FVG**: Carnic Alps, W of Ampezzo by the road to Passo del Pura, near Albergo e Ristorante Pura (Udine) (UTM WGS84: 33T 328364.5142459), solitary *Juglans regia* L. in a meadow, on branches in the lower canopy, on thallus and apothecia of *Xanthoria parietina* (L.) Th.Fr., 715 m, 17 August 1994, *J. Hafellner* (GZU - JH87217). – Species new to Friuli Venezia Giulia.
\+ **TAA**: Val Venosta (Vinschgau), Glorenza (Glurns), near the eastern gate of the old town, (Bolzano/Südtirol) (UTM WGS84: 32T 619091.5169763), fence and hedge, on bark of *Crataegus* sp., on thallus and apothecia of *Xanthoria parietina* (L.) Th.Fr., 950 m, 3 September 2002, *J. Hafellner* (GZU - JH84423). – Species new to Trentino-Alto Adige.

Arthonia parietinaria - restricted to *Xanthoria* s.str. - is a rather conspicuous species and is fairly common in Europe (Fleischhacker et al. 2016). The earlier records of an *Arthonia* on *Xanthoria parietina* (L.) Th.Fr. in Italy, published under various other names, are relatively few and have been sorted by Brackel (2020). In Italy, most of the findings so far origin from the central and southern parts of the country (Nimis and Martellos 2024) but as demonstrated here, the species can also be expected in the valleys of the Alps.

J. Hafellner

Buchwaldoboletus sphaerocephalus (Barla) Watling & T.H.Li (Boletaceae)

+ **CAL**: Fossato, Montebello Jonico (Reggio Calabria) (UTM WGS84: 33S 567387.4209343), at the base of a partially burnt pine tree (*Pinus* sp.), 893 m, 8 September 2024, *G. Tomasello, G. Mazzacuva, V.L.A. Laface* (REGGIO); Monte Scafi, Condofuri (Reggio Calabria) (UTM WGS84: 33S 575791.4208532), on stumps of *Pinus* sp., 1060 m, 20 August 2024, *L. Torino* (REGGIO) – Species new to Calabria.

Buchwaldoboletus sphaerocephalus preferably appears in dry Mediterranean forest during autumn and winter on stumps of *Pinus* sp., mostly *P. pinea* L., *P. pinaster* Aiton, and *P. halepensis* Mill. (Galli, 1998; Munoz, 2005). Its color is yellow to yellow-fulvus and it rapidly turns blue when bruised. In Italy, this species is rather rare.

V.L.A. Laface, G. Mazzacuva, L. Torino

Dacampia engeliana (Saut.) A.Massal. (Dacampiaceae)

+ **FVG**: Southern Alps, Carnic Alps, M. Pieltinis N of Sauris, ridge on NW side of summit (Udine) (UTM WGS84: 33T 323968.5150770), Werfen slate, in mossy crevices, 2000 m, on *Solorina* sp., 28 July 1993, *J. Hafellner* (GZU - JH32662). [label text in German]. – Species new to Friuli Venezia Giulia.
+ **VEN**: Southern Alps, Venetian Pre-Alps, SE of Belluno, M. Faverghera, S above Nevegal, uppermost northern slopes at Orto Botanico (Belluno) (UTM WGS84: 33T 291249.5106850), low limestone outcrops and boulders in subalpine meadows, on soil in crevices, N-exposed, on *Solorina* sp., 1540 m, 31 August 2002, J. Hafellner (GZU - JH84434). – Species new to Veneto.
+ **VDA**: Western Alps, M. Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above Rifugio E. Soldini (Aosta), (UTM WGS84: 32T 331630.5070248), cliffs and boulders of Jurassic limestone on N-exposed slope, on soil, 2250 m, on *Solorina* sp., 30 July 2001, (GZU - JH75418). – Species new to Val d'Aosta.
+ **PIE**: Western Alps, Alpi Cozie, W ridge of Monte Nebin ca. 1 km E of Colle di Sampeyre (Cuneo) (UTM WGS84: 32T 352157 4934030), outcrops of calcareous schists, on soil, on *Solorina* sp., 2380 m, 26 July 2000, *J. Hafellner*, *P.L. Nimis*, *M. Tretiach* (GZU - JH75644); ibidem, *P.L. Nimis, M. Tretiach* (TSB32987); Alpi Cozie, on the ridge W above Colle del Vallonetto (Cuneo) (UTM WGS84: 32T 349987

4916483), cliffs and boulders in alpine vegetation, on low outcrops of N-exposed calcareous sandstone, on *Solorina* sp., 2500 m, 23 July 2000, *P.L. Nimis, M. Tretiach* (TSB34652); Western Alps, Alpi Liguri, on the pass between Cima di Pertegue (Cima di Pertegà) and Rocca del Fera, W above Úpega (Cuneo) (UTM WGS84: 32T 395587 4889053), outcrops of calcareous schists in alpine vegetation, on soil somewhat N below the ridge, on *Solorina* sp., 2200 m, 20 July 2000, *J. Hafellner*, *P.L. Nimis*, *M. Tretiach* (GZU - JH87641). – Species confirmed for Piemonte.

This rather conspicuous fungus – the black ostioles in bleaching areas of the mostly sterile host thallus are visible with the naked eye – is widely distributed in the Holarctic region, with most records from the Alps (Brackel 2014). In Italy the species is so far only known from a few regions in the north (Nimis 2024). Records for Piemonte date back to the late 19th century (Brackel 2016).

J. Hafellner, P. L. Nimis, M. Tretiach

Hebeloma populinum Romagn. (Hymenogastraceae)

+ **CAL**: Botanical Garden, University of Calabria, Rende (Cosenza) (UTM WGS84: 33S 605816. 4357354), at the edge of a riparian wood among the litter of natural *Populus canescens* trees (prevailing species), 205 m, 2 December 2022, *G. Sicoli, A.B. De Giuseppe, N.G. Passalacqua* (CLU F335). – Species new to Calabria.

Sparse basidiomata immediately attributable to the genus *Hebeloma* (Fr.) P.Kumm. were found in late autumn 2022 on the wet ground among litter and herbaceous plants under the crown of a poplar tree along a narrow stream. The pilei were 2.5–3.0 cm wide, smooth and clearly viscid, pale whitish-argillaceous, with a slightly inrolled margin. The gills were pale and rather dense, initially weeping then brown-spotted. The stipe was whitish to ochraceous, finely floccose. Spores were amygdaloid to citriform, verrucose to echinulate, 11.3–14.7 × 6.7–8.0 µm in size. Cheilocystidia were filiform to clavate, sometimes capitulate. The fungus had a sweetish smell and a bitter taste. So far, *H. populinum* has been reported in a few administrative regions from northern and central Italy, but not in Calabria (Onofri et al. 2013).

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

Inocybe albomarginata Velen. (Inocybaceae)

+ **LIG**: Madonna dei Partigiani, Sassello (Savona) (UTM WGS84: 32T 458180.4927530), on calcareous soil under white hornbeam (*Carpinus betulus* L.) and poplars (*Populus tremula* L.), 470 m, 8 May 2023, *F. Boccardo* (GDOR 5417). – Species new to Liguria.

Morphologically, our Ligurian collection fits well with the description of the *I. albovelutipes* Stangl epitype reported by Bandini et al. (2023). The newly obtained nrITS sequence of the GDOR5417 voucher (GB: PQ305678) shares 689/690 (99.86%) nucleotides compared with the epitype of *I. albomarginata* sequence (GB: OR102476) confirming the morphological identification. According to Onofri et al. (2013), *I. al-*

bomarginata has been reported in Italy from Lombardia and Trentino-Alto Adige, and our collection represents the first reports of this species in Liguria.

F. Boccardo, F. Dovana

Trechispora fastidiosa (Pers.) Liberta (Hydnodontaceae)

+ **CAL**: Bosco di Mavigliano, Montalto Uffugo (Cosenza) (UTM WGS84: 33S 604520.4360244), on soil 210 m, 28 November 2021, *D. Puntillo* (CLU No. 433, 434, 435). – Species new to Calabria.

The habitus of this species is similar to the juvenile stage of *Thelephora penicellata* (Pers.) Fr. and *Sebacina incrustans* (Pers.) Tul. & C.Tul. from which it differs mainly in microscopic characters and in the lack of the intense and annoying smell in the latter two species.

D. Puntillo, M. Puntillo

LICHENS

Aspicilia grisea Arnold (Megasporaceae)

+ **VDA**: Western Alps, Alpi Graie, M. Bianco (Mont Blanc) group, Val Veny W of Courmayeur, moraines E of Lago di Combal (Aosta) (UTM WGS84: 32T 334363.5071410), on inclined rock faces of gneissic boulders amongst scattered *Larix* trees, 1980 m, 30 July 2001, *J. Hafellner , P. L. Nimis, M. Tretiach* (GZU - JH 87051). – Species new to Val d'Aosta.

+ **PIE**: Western Alps, Alpi Marittime, Rocca dell'Abisso W of Colle di Tenda, E below summit, steep slopes towards uppermost Vallone dell'Abisso (Cuneo) (UTM WGS84: 32T 380673.4888905), cliffs, outcrops and dispersed boulders of gneiss exposed to the E, on inclined faces, 2630 m, 22 July 2000, leg. *A. Hafellner*, *J. Hafellner*, *M. Tretiach*, det. *J. Hafellner* (GZU - JH8736). – Species new to Piemonte.

This sorediate species, not rarely also fertile and then easier to recognize, mostly grows on horizontal to inclined faces of siliceous rock near the ground. It is widely distributed in the Alps (Nimis et al. 2018) but apparently under-collected in wide areas. In Italy it is poorly recorded, being only known from Friuli-Venezia Giulia (Tretiach and Hafellner 2000) and Lombardia (Nascimbene et al. 2021).

J. Hafellner, P. L. Nimis, M. Tretiach

Bellemerea alpina (Sommerf.) Clauzade & Cl.Roux (Lecideaceae)

+ **VEN**: Viel del Pan, Pordoi (Belluno), on siliceous volcanic rock (UTM WGS84: 32T 716833.5150861), 2492 m, 8 August 2024, *J. Nascimbene* (BOLO). – Species new to Veneto.

Bellemerea alpina is an arctic-alpine, silicicolous, crustose lichen that is quite widespread in the Italian Alps (Nimis et al. 2018; Nimis and Martellos 2024) up to the northern Apennines (Ravera et al. 2024b). The only record reported from Veneto by

Nimis (1993) is likely due to a misinterpretation by Jatta (1909–1911), since no citations of the species are reported by Massalongo (1852; Nimis, in litt.).

J. Nascimbene, G. Gheza

Cladonia ciliata Stirt. (Cladoniaceae)

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

Cladonia ciliata is an oceanic species (Litterski and Ahti 2004) commonly found on soil with moss in undisturbed maquis vegetation, particularly in humid coastal regions. In Italy, its presence is limited to a few locations, mainly along the Tyrrhenian coast (Nimis 2024). Although it is listed as Endangered (EN) on the national Red List (Nascimbene et al. 2013), recent observations indicate a positive trend for this species in Toscana (Ravera et al. 2024a). The only record of this species in Lazio is the herbarium specimen TSB 17703, collected at the Etruscan necropolis of Blera (Viterbo) (Nimis and Martellos 2024). Recent surveys (August 2024) at the same collecting site have confirmed the disappearance of the species, likely due to neglect and abandonment of the archaeological area, as well as the gradual encroachment of the Mediterranean macchia at the expense of lichen populations.

S. Ravera

Hypotrachyna sinuosa (Sm.) Hale (Parmeliaceae)

\+ **PIE**: Val Vigezzo, Craveggia (Verbano-Cusio-Ossola) (UTM WGS84: 32T 464206.5116235), on bark of *Betula* sp., 1030 m, 12 June 2024, *Ph. Clerc* (G). – Species confirmed for Piemonte.

According to Nimis (2024), *H. sinuosa* is a widespread, but rare mild-temperate species found on bark and epiphytic mosses in open, humid and cold forests. It is declining throughout Italy and presently almost extinct. *Hypotrachyna sinuosa* is easily distinguished in the field from the other Italian *Hypotrachyna* species by its small size, the farinose soralia covering apices of ± ascending lobes and its yellow-green thallus (usnic acid). It was found in an alluvial alder grove near the Swiss border. A few other thalli could be observed in a small area, all on *Alnus incana* (L.) Moench. The most recent records of this species in Piemonte date back to the late 1800s (Baglietto and Carestia 1865, 1880).

Ph. Clerc, J. Nascimbene, Ph. Blaise

Maronea constans (Nyl.) Hepp (Fuscideaceae)

\+ **PIE**: Val Vigezzo, Craveggia (Verbano-Cusio-Ossola) (UTM WGS84: 32T 464335.5116267), on bark of *Alnus incana* (L.) Moench, 1075 m, 16 April 2024, *Ph. Blaise* (G). – Species new to Piemonte.

Maronea constans is a rare mild-temperate lichen found on smooth bark, which was probably more frequent in the past (Nimis 2024). According to Scheidegger et al. (2002) it is near extinction in most Alpine countries, and it is included in the Italian

Red List of epiphytic lichens as Critically Endangered (Nascimbene et al. 2013). However, its superficial resemblance to *Rinodina* may contribute to its not being collected and recognized. The present sample was found in an alluvial alder grove near the Swiss border. Interestingly, numerous thalli could be observed (several dozens) in a small area, all on *Alnus incana* (L.) Moench.

Ph. Blaise, J. Nascimbene

Parvoplaca tiroliensis (Zahlbr.) Arup, Søchting & Frödén (Teloschistaceae)

+ **VDA**: Western Alps, Alpi Graie, M. Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini, (Aosta) (UTM WGS84: 32T 331630.5070248), cliffs and boulders of Jurassic limestone and alpine vegetation on N–NE exposed slope, on plant remnants, 2250 m, 30 July 2001, *J. Hafellner*, *P. L. Nimis*, *M. Tretiach* (GZU - JH87224). – Species new to Val d'Aosta.
+ **LIG**: Western Alps, Alpi Liguri, ridge S above the village Monesi, W above the Colle del Garezzo (Imperia) (UTM WGS84: 32T 401686.4877849), small outcrops of calcareous schist in subalpine pasture, on plant remnants and bryophytes, 1850 m, 21 July 2000, *J. Hafellner*, *P.L. Nimis*, *M. Tretiach* (GZU - JH87264). – Species new to Liguria.

The species has a wide, circumpolar arctic-alpine distribution in the northern hemisphere. In the Alps, it appears to be fairly common over calcareous substrates from the tree line to the nival belt (Nimis et al. 2018). In Italy it has so far been recorded only from the northern and central parts of the country (Nimis and Martellos 2024).

J. Hafellner, P. L. Nimis, M. Tretiach

Polyozosia reuteri (Schaer.) S.Y.Kondr., Lőkös & Farkas (Lecanoraceae)

+ **PIE**: Western Alps, Alpi Liguri, just E below Úpega, the left bank of the creek (Negrone = Corvo torrent) (Cuneo) (UTM WGS84: 32T 398664.4886844), riparian woodland, on subvertical faces of large calcareous boulders, 1280 m, 19 July 2000, *J. Hafellner , P. L. Nimis, M. Tretiach* (GZU - JH87513). – Species new to Piemonte.

This Palearctic, montane to alpine species is widely distributed in central and southern Europe including the Alps (Nimis et al. 2018). In Italy, it is known from the Alps to Calabria (Nimis and Martellos 2024) but is not common due to the narrow ecological niche, *viz.* subvertical to overhanging faces of limestone cliffs.

J. Hafellner, P. L. Nimis, M. Tretiach

Schismatomma umbrinum (Coppins & P.James) P.M.Jørg. & Tønsberg (Roccellaceae)

+ **ITA** (**CAL**): along road at border of national park 4.5 km S of village of Gambarie, near a bridge, Santo Stefano in Aspromonte (Reggio Calabria) (UTM WGS84: 33S 573190.4219551), on siliceous overhang, 1400 m, March 2023, leg. *J. Malíček, I. Frolov, J. Vondrák*, det. *J. Malíček* (PRA). – Species new to Italy (Calabria).

This species is primarily found in western parts of Europe, growing on acidic rocks beneath overhangs where it remains consistently shaded from direct sunlight. It often occurs in old, established woodlands and it has been recorded in various locations, including the Eastern Alps (Nimis et al. 2018), but its almost constant sterility suggests that it may be underreported or overlooked in other areas.

J. Malíček, S. Ravera

Solenopsora liparina (Nyl.) Zahlbr. (Leprocaulaceae)

+ **EMR**: Boschi di Bardone, Monte Prinzera Nature Reserve, Terenzo (Parma) (UTM WGS84: 32T 586002.4943894), ophiolitic mountain ridge, in rock fissures, 725 m, 21 September 2024, *Z. Fačkovcová, L. Paoli* (SAV0018514); ibidem (UTM WGS84: 32T 585743.4943499), 636 m (SAV0018515). – Species new to Emilia-Romagna.

Solenopsora liparina is a saxicolous species characterized by a crustose-placodioid thallus of olivaceous grey or grey-green colour, which can form up to 2.5-cm wide rosettes or arcs of lobes. Lobes are flat to slightly convex, 0.3–0.6 mm wide, with white pruina at the margin. It grows on ultramafic rocks with a very low silica content, such as serpentinite (Guttová and Nimis 2021). So far, scattered localities are known from the Balkan peninsulas, Turkey, Czech Republic, France, and British Isles (Guttová et al. 2019). In Italy, it was recorded only from few sites in Liguria, Lombardia, and the Apennines in Toscana (Nimis and Martellos 2024). At the locality of Monte Prinzera, it predominantly grows on west and southwest-facing rocks and in rock fissures.

L. Paoli, Z. Fačkovcová

Trapelia glebulosa (Sm.) J.R.Laundon (Trapeliaceae)

+ **VDA**: Piccolo San Bernardo pass (Aosta) (UTM WGS84: 32T 336331.5062336), on pebbles and on soil among rocks, in alpine pastures, 2065 m, 23 August 2024, *S. Ongaro, D. Isocrono* (ORO n. 334). – Species new to Val d'Aosta.

Trapelia glebulosa is distinguished by its small scattered squamules bearing apothecia at a very early stage. This species usually grows near the ground, mainly in upland areas such as the one in Val d'Aosta. Although *T. glebulosa* is common throughout the Alps (Nimis et al. 2018), it had not yet been reported for Val d'Aosta, a region that has been extensively studied. Since the species is often infertile, it may be overlooked or ignored or mistaken for small morphotypes of *T. coarctata* (Sm.) M. Choisy.

S. Ongaro, D. Isocrono

Zwackhia viridis (Ach.) Poetsch & Schied. (Lecanographaceae)

+ **PIE**: Maritime Alps, Valle Vermenagna, Robilante, Orrido delle Barme (Cuneo) (UTM WGS84: 32T 380106.490502), on oak twigs in woodlands, 820 m, 25 May 2024, *D. Isocrono* (ORO n. 395). – Species new to Piemonte.

A crustose, photophobic lichen characterized by a thin thallus with numerous, regularly distributed lirellae. The absence of soralia distinguishes this species from the similar *Z. sorediifera* (P.James) Ertz. In Italy, it has been reported more frequently in the Tyrrhenian than in the Adriatic side of the peninsula (Nimis 2024). This species is widespread throughout the Alps, although it is not very common (Nimis et al. 2018).

D. Isocrono

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RESEARCH ARTICLE

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A systematic study of some lichenized fungi from Pakistan using nrITS marker

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Abstract

In a detailed investigation of lichen diversity in different regions of Pakistan, utilizing molecular and morphological techniques, we identified and reported three species – *Dimelaena altissima*, *Lecanora chlarotera*, and *Usnea lapponica* – as new records for Pakistan. Furthermore, nrITS sequences for *Evernia prunastri*, *Lecanora horiza*, *L. hybocarpa*, *Peltigera polydactylon*, and *P. praetextata* were generated for the first time from Pakistan. Detailed morpho-anatomical descriptions, habitat information, distribution data, and results of biochemical analyses are also provided for a better understanding of the lichen diversity and ecology in this region.

Keywords

Crustose, foliose, Gilgit-Baltistan, Khyber Pakhtunkhwa, morphology, taxonomy

Introduction

Pakistan's diverse landscapes, ranging from extensive forests in the northern mountains and mineral-rich terrain in the west to fertile agricultural regions in the east, create a varied geography and climate that support extensive biodiversity (Baig and Al-Subaiee 2009; Alam et al. 2011; Malik et al. 2012). Geographically, the country lies in South Asia, between 23.5°N and 40°N latitudes and 60°E and 80°E longitudes, with a primarily tropical continental climate that exhibits distinct seasonal variations (Farooqi et al. 2005; Akhtar et al. 2022).

Pakistan likely hosts a significant diversity of lichens, yet much of it remains unexplored due to limited surveys conducted across various regions (Fayyaz et al. 2022). The taxonomic exploration of lichens in the region was initiated by Sultan Ahmad in 1965 and was followed by Hawksworth and Mehmood (1971), recording 10 species from coniferous forests and Iqbal et al. (1978), adding 17 new species through their work on macrolichens. The first comprehensive checklist of lichens of Pakistan, published by Aptroot and Iqbal (2012), includes 368 species belonging to 114 genera identified using morpho-anatomical characteristics. Since the initiation of ITS-based phylogenetic analysis of lichens in Pakistan, numerous new species have been identified across various regions and as of now, more than 440 lichen species have been documented from the country (Habib et al. 2017; Khan et al. 2018; Fatima et al. 2021; Fayyaz et al. 2022; Afshan et al. 2023; Din et al. 2024; Usman et al. 2024; Zulfiqar et al. 2024). The present study is a contribution to the lichenological exploration of Pakistan mainly based on collections from Gilgit-Baltistan and Khyber Pakhtunkhwa, Pakistan. This study not only expands the knowledge of lichen flora within the country but also provides a more comprehensive understanding of their distribution patterns and ecology.

Materials and methods

Morphological and chemical studies

Collections were made during a lichen survey of different areas of Khyber Pakhtunkhwa and Gilgit Baltistan, Pakistan, in the years 2020 and 2023, respectively. Morphological characters were observed under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). The chemistry of the thallus was determined by using standard K (5% KOH), C (commercial bleach), and KC (K followed by commercial bleach) and secondary metabolites were detected by thin-layer chromatography in solvent C as described by Orange et al. (2001). Measurements of anatomical features were made from freehand sections of apothecia mounted in water on a glass slide. The sections were observed using a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan).

DNA extraction and PCR amplification

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using a modified 2% CTAB method (Gardes and Bruns 1993). Extracted DNA was used for PCR amplification of the ITS nrDNA marker using primer pair, i.e., ITS1F forward primer (5′ CTTGGTCATTTAGAGGAAGTAA 3′) (Gardes and Bruns 1993) and ITS4 reverse primer (5′ TCCTCCGCTTATTGATATGC 3′) (White et al. 1990). PCR products were visualized with the help of a 1% agarose gel using ethidium bromide through a Gel documentation system (Sambrook and Russell 2006). PCR products were cleaned and sent for sequencing to Tsingke, China.

Phylogenetic analysis

The ITS region was amplified and sequenced for all specimens. Forward and reverse sequences were reassembled using the BioEdit Sequence Alignment Editor (Hall 2011). Initial identity verification was performed via BLAST searches at NCBI (https://www.ncbi.nlm.nih.gov/guide). ITS datasets were constructed by retrieving relevant sequences from the GenBank database. Alignment and manual editing were completed using BioEdit (Hall 2005). Phylogenetic analyses were conducted on the CIPRES Science Gateway (https://www.phylo.org/) using RAxML-HPC2 on XSEDE (version 8.2.10) with the GTR+GAMMA nucleotide substitution model. One thousand rapid bootstrap replicates were run to infer the evolutionary history of each species. The resulting Maximum Likelihood phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2014) and subsequently edited in Adobe Illustrator CC 2021. Appropriate species were selected as outgroups to root each phylogenetic tree.

Results

Taxonomy

***Dimelaena altissima* (H. Magn.) M. Ai & Xin Y. Wang, Phytotaxa 574 (4): 259 (2022)**
Fig. 1

Description. Thallus crustose, areolate-placodioid, up to 5 cm across, well-defined, lobate. Areoles: strongly convex, rounded to irregular, 0.5–1 mm in diameter, radiating towards margins, up to 1.5 mm long, 0.5 mm wide. Color: yellowish green to yellowish white when dry, no change when wet. Upper cortex: 2-layered, outer greyish, 10−20 µm thick, inner hyaline, 20−30 µm thick, cells rounded, 5−8 µm in diameter. Algal layer: continuous, even, 60−80 µm thick, photobiont chlorococcoid, cells globose, 9−13 µm in diameter. Medulla: 90−120 µm thick, hyphae hyaline, 3−4 µm wide. Apothecia: frequent, lecanorine, 1−3 per areole. Disc: round, black, pruinose, plane to concave, 0.2−0.7 mm in diameter. Margins: thick, persistent, concolorous to thallus. Epihymenium: brown, 10−15 µm tall. Hymenium: hyaline, 40−50 µm tall. Hypothecium: hyaline, 60−70 µm tall. Paraphyses: septate, branched, capitate, 1−2 µm wide apically. Asci: hyaline, clavate, 40−50 × 15−20 µm. Ascospores: brown, 1-septate, 9−12 × 5−8 µm.

Chemical study. K+ve (yellow), C-ve, KC+ve (yellow). Usnic acid and norstictic acid detected through TLC.

Phylogenetic analysis. For phylogenetic analysis, sequences of the genus *Dimelaena* were retrieved from GenBank based on their similarity to our sequences and those used in a study on the phylogeny of *Dimelaena* by Ai et al. (2022). Due to the limited availability of *Dimelaena* sequences, some sequences of the closest genus *Buellia* were also included in constructing the phylogenetic tree. The final aligned file was comprised of 525 nucleotides, among which 345 were conserved, 180 were variable, 161

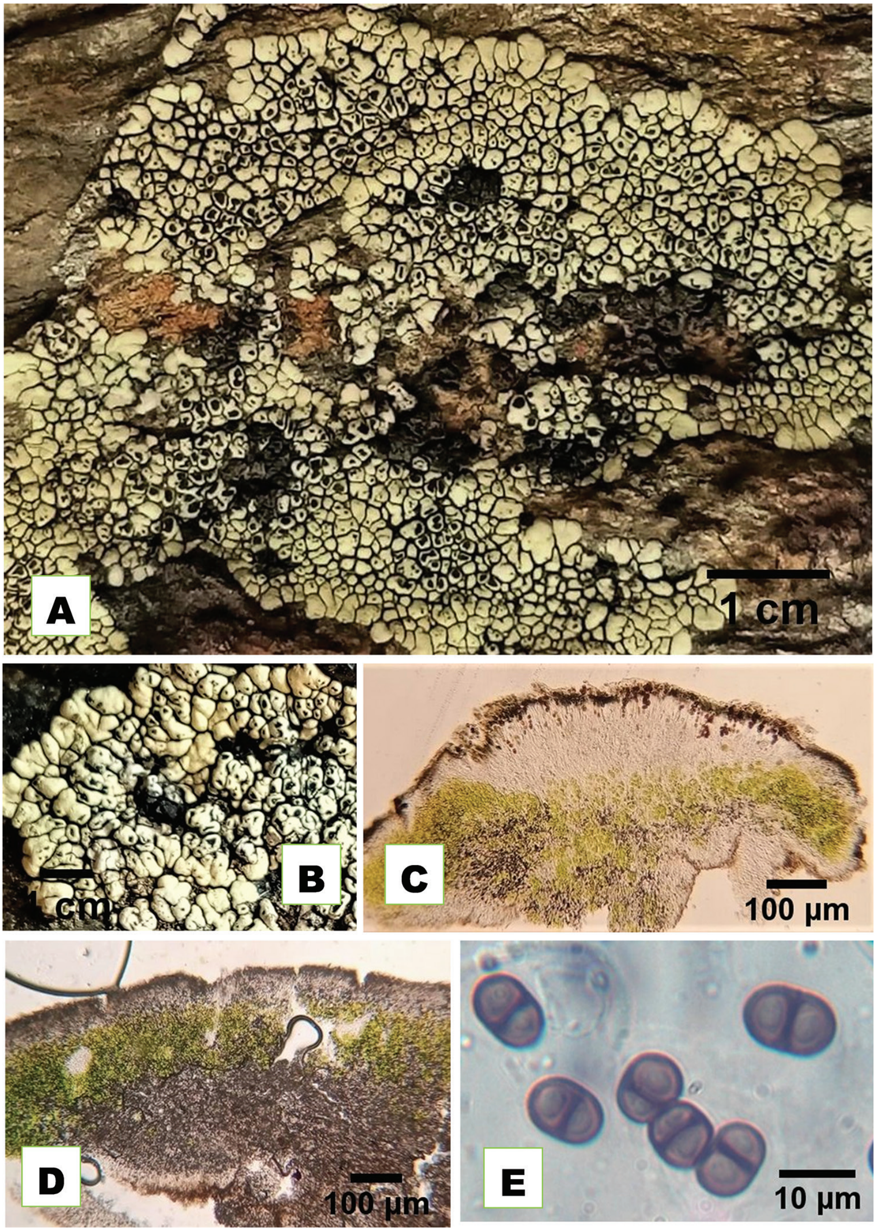

Figure 1. *Dimelaena altissima* **A** crustose-placodioid thallus **B** apothecia **C** cross-section of apothecium **D** cross-section of thallus **E** ascospores.

were parsimony-informative, and 19 were singleton variants. *Physcia aipolia* (Ehrh. ex Humb.) Fürnr. (MK811989 and MK812087) was chosen as an outgroup.

In our analysis, the newly generated sequences of *Dimelaena altissima* from Pakistan (KH-608, KH-609, and BST-03) grouped within a single clade alongside other se-

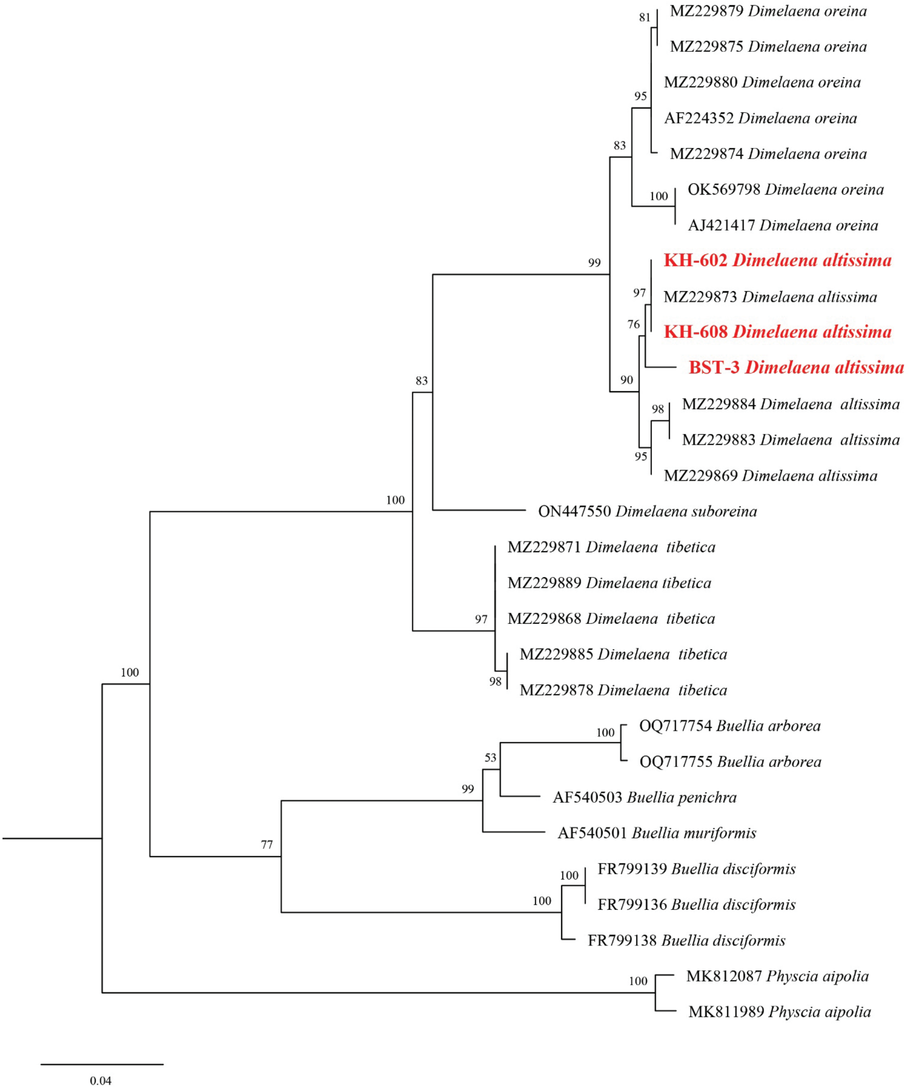

Figure 2. Molecular phylogenetic analysis of *Dimelaena* spp. by maximum likelihood (ML) method based on nrITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown above the branches and novel sequences generated during in this study are in bold.

quences of the same species (MZ229869, MZ229873, MZ229883, and MZ229884), receiving strong support (90% ML), thus confirming their identification as *D. altissima* (Fig. 2). Additionally, *D. altissima* shows a sister relationship with *D. oreina*, which also forms a distinct clade with high bootstrap support (83% ML).

Notes. *Dimelaena altissima* is distinguished by its yellowish-green, epruinose crustose thallus with flat marginal lobes, along with irregularly rounded, innate apothecia that have indistinct margins (Ai et al. 2022). The morphological comparison confirms the identity of the Pakistani taxon as *D. altissima* as it shares same morpho-anatomical characteristics with *D. altissima* described from China, such as a crustose thallus with radiate-plicate margins and lecanorine immersed apothecia (Ai et al. 2022). It is morphologically and phylogenetically closely related to *D. oreina* but differs from it by lacking well-defined apothecial margins and convex marginal lobes (Ai et al. 2022). It is the first report of *D. altissima* from Pakistan.

Habitat and ecology. The collections (KH-602 & KH-608) were found in dry temperate climate, in an open situation exposed to sun and rain, at an altitude of 841 m a.s.l., temperature typically varies from -8 °C to 28 °C, with annual rainfall varying between 700–800 mm. Whereas the other collection (BST-03) was found at an elevation above 4,173 m, on rock, in a temperate climate with minimum and maximum temperatures of -8 °C and 35 °C, respectively. The region is characterized by hilly topography, with annual rainfall varying between 90 and 150 mm and heavy snowfall during the winter months.

Distribution. This species was previously documented from China (Ai et al. 2022) and is now recorded from Pakistan for the first time.

Materials examined. Pakistan • Khyber Pakhtunkhwa Province, Kohistan, Dassu; 35°35.0'N, 73°37.0'E; alt. 841 m a.s.l.; 11 Sep. 2020; K. Habib & A. N. Khalid leg.; on rock; KH-602 & KH-608, ITS GenBank accession numbers PQ113699 and PQ113700. Pakistan • Gilgit-Baltistan, District Diamer, Babusar Top; 35°08.8'N, 74°02.9'E; alt. 4,173 m a.s.l.; 28 Aug. 2023; U. F. Ahmad, M. Usman & A. N. Khalid leg.; on rocks; in moist temperate forest; BST-03, ITS GenBank accession number PQ113698.

Evernia prunastri (L.) Ach., Lich. Univ.: 442, tab. 10, fig. 1 (1810)

Fig. 3

Description. Thallus foliose but appearing fruticose, dorsiventral, tufted, caespitose to occasionally pendent, cortex cracked at some places, palmately to dichotomously branched, up to 5.5 cm across. Branches: numerous, dorsiventral, elongated, 0.3–4 mm wide, plane, strap-shaped, pitted, twisted, tangled, blunt or pointed. Axils: right angles to V-shaped. Color: greyish-green to greenish-white when dry, pale olive green to yellow-green when wet. Soredia: marginal to rarely laminal, white, often on ridges, granular. Upper surface: matt, dull, pitted, strongly rugose, rough, ridges discontinuous**.** Lower surface: white to pale yellow, channelled, less pitted and wrinkled than upper surface. Cortex: two-layered, upper layer brown, 9–14 µm high, lower layer hyaline, 7–12 µm high, paraplectenchymatous, cell 7–12 µm in diameter. Medulla: prosoplectenchymatous, hyphae loosely arranged, white, 2–4.5 µm wide. Algal layer: discontinuous, uneven, forms small groups, algal cells present below cortex, 5–10 µm in diameter. Apothecia: not observed.

Chemical study. K +ve (yellow), C -ve, KC +ve (yellow). Usnic acid, atranorin and evernic acid detected through TLC.

Phylogenetic analysis. For phylogenetic analysis, sequences of the genus *Evernia* were retrieved from GenBank based on similarity and those used in a study by Piercey-Normore (2006). A total of 22 ITS nrDNA sequences were analyzed during this study. The final aligned file was comprised of 532 nucleotides, among which 359 were con-

Figure 3. *Evernia prunastri* **A** thallus **B** upper surface of the thallus **C** lower surface **D** granular soredia **E** cross-section of thallus **F** photobiont.

served, 158 were variable, 150 were parsimony-informative, and 8 were singleton variants. *Umbilicaria kappenii* Sancho, B. Schroet. & Vallad. (AY603130 and AY603131) was chosen as an outgroup.

In our analysis, *Evernia prunastri* forms a distinct clade and the ITS sequences of *E. prunastri* (KUM-04 and KUM-09) from Pakistan are identical to those reported from Norway (MK811838, MK811993, MK812247, and MK812058), confirming their identification as the same species (Fig. 4). Additionally, *E. divaricata* was distinctly separated from *E. mesomorpha* and *E. esorediosa* in the phylogenetic tree, yet all three species formed a clade with a high bootstrap support (92% ML). This study represents the first molecular confirmation of *E. prunastri* from Pakistan.

Notes. *Evernia prunastri*, commonly known as oakmoss, is a fruticose lichen found predominantly on exposed tree trunks, characterized by flat, isotomic-dichotomous

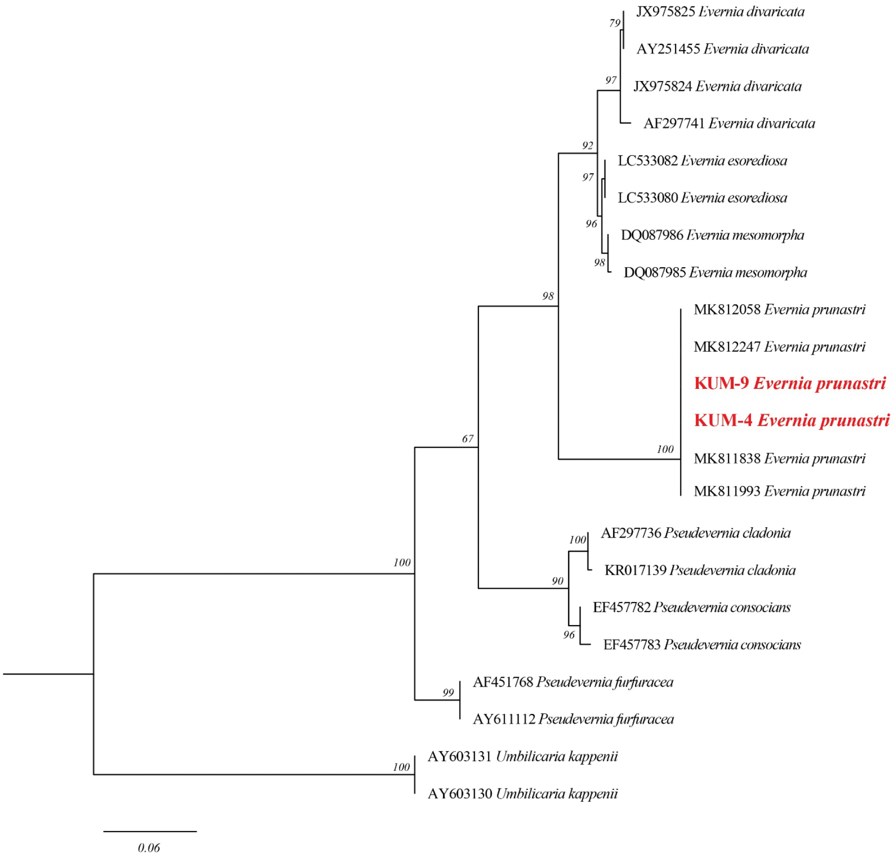

Figure 4. Molecular phylogenetic analysis of *Evernia* spp. by maximum likelihood (ML) method based on nrITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown above the branches and novel sequences generated during this study are in bold.

branches with frequent anisotomous bifurcations and occasional adventive branches or "lobuli" on the branch surface (Beltman 1978; Stone and McCune 1990). The morpho-anatomical comparison of Pakistani collections confirms their identity as *E. prunastri*, as the characteristics of Pakistani samples match with the already published description of *E. prunastri* from Britain and Ireland (Cannon et al. 2023).

Habitat and ecology. The samples were found at an elevation above 2000 m, growing on bark in the cold temperate mountainous regions. The coldest month is December, while June is the hottest month of the year, with average monthly temperatures of 3 °C and 25 °C, respectively, and rainfall varying between 600 and 1200 mm. The dominant vegetation includes Indian horse-chestnut (*Aesculus indica* (Wall. ex Cambess.) Hook.), deodar (*Cedrus deodara* (Roxb. ex D.Don) G.Don) and West-Himalayan fir (*Abies pindrow* (Royle ex D.Don) Royle).

Distribution. This species is widely distributed in Northern hemisphere including Asia, Europe and North America (Studzińska-Sroka et al. 2023; Staples et al. 2020).

Materials examined. Pakistan • Khyber Pakhtunkhwa Province, District Upper Dir, Kumrat Valley, 35°23.2'N, 74°35.0'E; alt. 2,500 m a.s.l.; 23 Sep. 2023; M. Usman & A. N. Khalid leg.; on bark; KUM-4, ITS GenBank accession number PQ113696 • same collection data as for preceding, 35°33.9'N, 72°11.4'E; alt. 2,400 m a.s.l.; 23 Sep. 2023; M. Usman & A. N. Khalid leg.; on bark; KUM-9, ITS GenBank accession number PQ113697.

Lecanora chlarotera Nyl., Flora, Regensburg 55(35): 550 (1872)

Fig. 5

Description. Thallus crustose, verrucose-areolate to rimose-areolate, up to 1 cm across, indeterminate, continuous, irregular to orbicular, esorediate. Areoles: verruculose, smooth to rough, warty. Upper surface: greenish-white to yellowish-grey, smooth to rough. Prothallus: absent. Algal layer: 100–130 µm thick, even, continuous, cells chlorococcoid, spherical to sub-spherical, 6–15 µm in diameter. Apothecia: lecanorine, frequent, scattered to aggregated, sessile, epruinose. Disc: orange to reddish-brown, smooth, concave to slightly convex, rounded, 0.3–0.8 mm in diameter, epruinose to slightly pruinose. Margin: distinct, entire, smooth, thick, concolorous to thallus, persistent, continuous. Thalline exciple: well-developed. Paraphyses: hyaline, unbranched, 1.5–2 µm wide, simple, apically swollen, apex 3–4 µm wide. Epihymenium: reddish-brown to brownish-orange, 6–18 µm tall. Hymenium: hyaline, 70–100 µm tall. Hypothecium: hyaline, 25–42 µm tall. Asci: *Lecanora*-type, hyaline, thin-walled, sub-cylindrical to clavate, 8-spored, 60–78 × 10–15 µm. Ascospores: ovoid to broadly ellipsoidal, hyaline, simple, aseptate, 12–15 × 6–9 µm.

Chemical study. K +ve (yellow), C -ve, KC -ve. Atranorin was detected through TLC.

Phylogenetic analysis. For phylogenetic analysis, sequences of the genus *Lecanora* were retrieved from GenBank based on their similarity to our sequences. A total of 48 ITS nrDNA sequences were analyzed during this study, including four newly generated sequences. The final aligned file was comprised of 559 nucleotides, among which 285 were conserved, 268 were variable, 251 were parsimony-informative, and 17 were singleton variants. *Lecanora saxigena* Lendemer & R.C.Harris (KP224466 and KP224467) was chosen as an outgroup.

In our analysis, distinct clades were formed with high bootstrap values (Fig. 6). The newly generated sequences of *Lecanora chlarotera* (KUM-18 and SN-07) and *Lecanora hybocarpa* (FM-22) form a well-supported clade (99% ML) alongside *Lecanora paramerae* I.Martínez, Aragón & Lumbsch. The Pakistani sequences of *L. chlarotera* (KUM-18 and SN-07) cluster with sequences of the same taxon from the United Kingdom (FR799201, FR799204, FR799203) and Sweden (MW375013, MW375016, MW375017, MW375018, and MW374998), indicating they represent the same species. Additionally, the sequence of *L. hybocarpa* (FM-22) groups with a sequence of the same taxon from Spain (EF105412) with strong bootstrap support (89% ML). The sequence of the Pakistani collection (K-165) forms a clade with *Lecanora horiza* sequences reported from North Macedonia (OQ717441), Switzerland (KX132998), and the USA (MG554692), indicating they represent the same species. *L. horiza* is closely related to

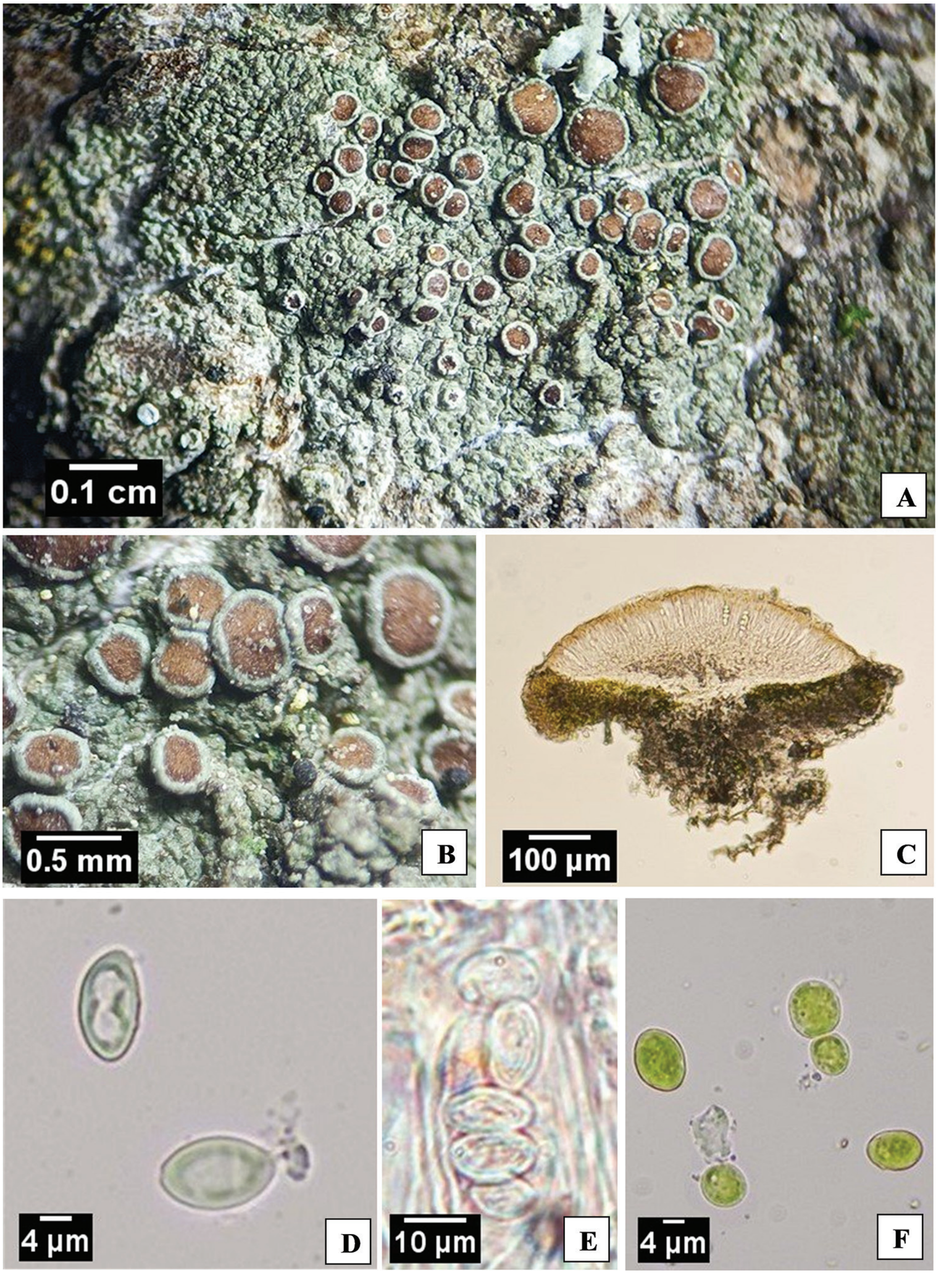

Figure 5. *Lecanora chlarotera* **A** thallus **B** apothecia **C** cross-section of apothecium **D** ascospores in water **E** asci containing ascospores **F** chlorococcoid photobionts.

L. campestris (Schaer.) Hue, *L. allophana* (Ach.) Nyl., and *L. glabrata* (Ach.) Nyl., forming a clade with high bootstrap support (82% ML).

Notes. *Lecanora chlarotera* is a common epiphytic species within the *L. subfusca* complex, widespread in Europe and North America, growing on nutrient-rich bark

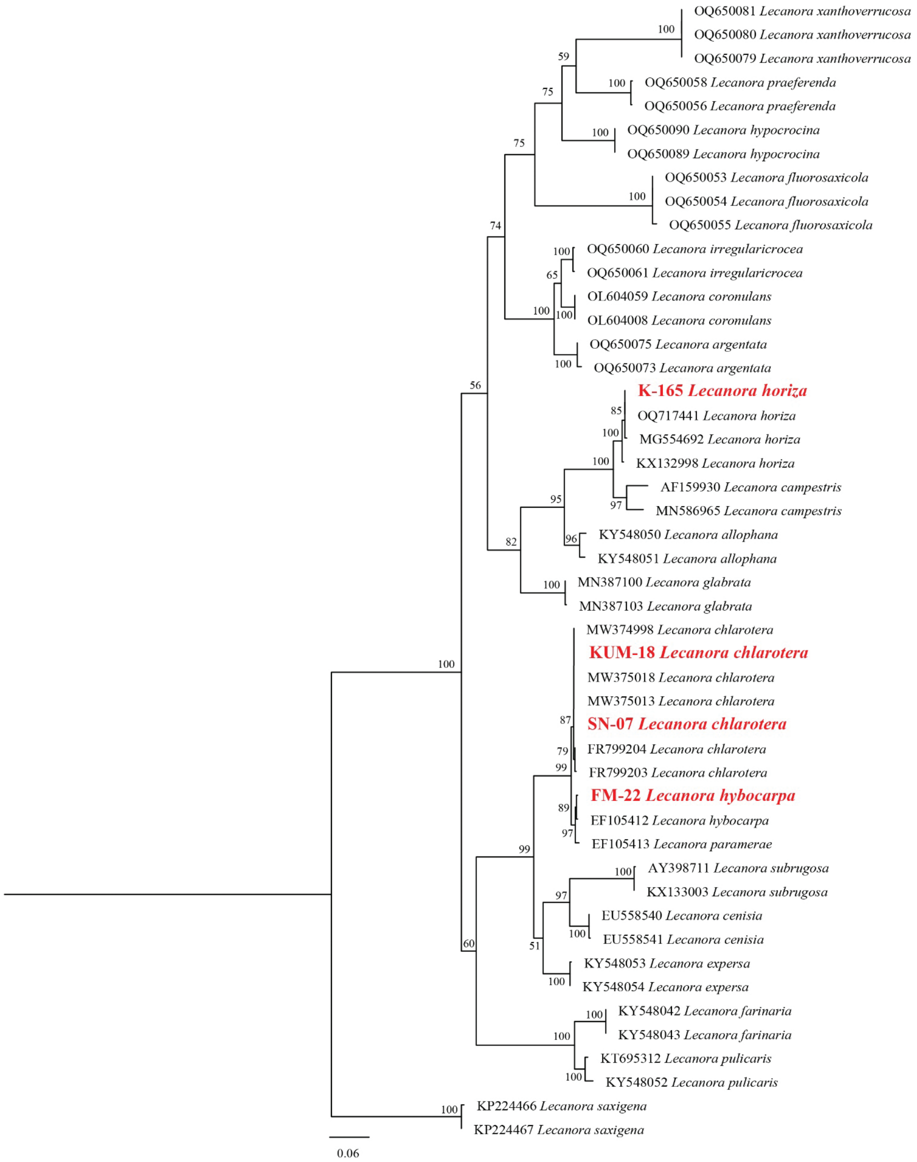

Figure 6. Molecular phylogenetic analysis of *Lecanora* spp. by maximum likelihood (ML) method based on nrITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown above the branches and novel sequences generated during this study are in bold.

from lowlands to mountainous regions (Bolognini 1992). The morpho-anatomical characteristics of the Pakistani specimen agree with the published description of *L. chlarotera* from the Czech Republic, except slightly smaller apothecia 0.3–0.8 mm (vs. 0.5–1 mm) and smoother apothecial margins (vs. smooth to crenate) (Malicek 2014). *L. chlarotera* is both morphologically and phylogenetically related to *L. hybocarpa* (Tuck.) Brodo, but the latter one has larger apothecial discs and yellow-white to grey thallus coloration (Cannon et al. 2022). Phylogenetically, *L. chlarotera* is also closely related to *L. paramerae* I. Martínez, Aragón & Lumbsch but differs in having smaller apothecia 0.3–0.8 mm (vs. 1–3 mm), pruinose apothecial disc (vs. epruinose) and taller hymenium 70–100 µm (vs. 60–70 µm) (Martínez et al. 1999).

Habitat and ecology. The sample (KUM-18) was found at an elevation of 2,500 m, growing on a bark in a cold, temperate mountainous region. The coldest month in this region is December, while June is the hottest month of the year, with average monthly temperatures of 3 °C and 25 °C, respectively, and rainfall varying between 1000 and 1200 mm. The second collection (SN-07) was made from Kaghan Valley which is drained by the River Kunhar originating from Lulusar Lake. The average rainfall for spring, summer and winter is 363, 655, and 190 mm, respectively. The maximum daily temperature of the region varies from 20 °C to 30 °C during the summer and averages 4 °C during the winter with moderate rainfall.

Distribution. This species is widespread in the Czech Republic, Europe, Japan and Turkey (Miyawaki 1988; Öztürk et al. 2010; Malicek 2014). From Pakistan, it is reported for the first time.

Materials examined. Pakistan • Khyber Pakhtunkhwa Province, District Upper Dir, Kumrat Valley, 35°33.6'N, 72°12.4'E; alt. 2,500 m a.s.l.; 23 Sep. 2023; M. Usman & A. N. Khalid leg.; on bark; KUM-18, ITS GenBank accession number PQ113695. Pakistan • Khyber Pakhtunkhwa Province, District Manshera, Kaghan Valley; 34°42.2'N, 73°26.2'E; alt. 2,454 m a.s.l.; 24 Aug. 2022; A. R. Niazi & M. Nadeem leg.; on bark; SN-07, ITS GenBank accession number PQ106505.

Lecanora horiza (Ach.) Nyl., Flora, Regensburg 66(7): 107 (1883)

Fig. 11A

Notes. *Lecanora horiza*, another species within the *L. subfusca* complex, is commonly found on deciduous trees and shrubs and occasionally on conifers, growing in sunny, nutrient-enriched conditions (Bolognini 1992; Malicek 2014). It is distributed in California, Europe, and Mexico (Bolognini 1992). Previously, it was reported from Baragali, Dungagali, Kalabagh, Murree, and Swat (Aptroot and Iqbal 2012) and is new to Kohistan, Khyber Pakhtunkhwa, Pakistan.

Material examined. Pakistan • Khyber Pakhtunkhwa Province, Kohistan, Dassu; 35°35.0'N, 73°37.0'E; alt. 841 m a.s.l.; 12 Sep. 2020; K. Habib & A. N. Khalid leg.; on rock; K-165, ITS GenBank accession number PQ469044.

Lecanora hybocarpa (Tuck.) Brodo, Beih. Nova Hedwigia 79: 134 (1984)

Fig. 11B

Notes. This species is characterized by a continuous to rimose-areolate thallus, large crystals in the thalline margin, pale brown to red-orange discs and granules in the epithecium that dissolve in K (Cannon et al. 2022). It is distributed across Europe, Spain, and the USA (https://www.gbif.org/). In Pakistan, it was previously reported from Kalam, Swat (Aptroot and Iqbal 2012) and is now recorded from Diamer, Gilgit-Baltistan.

Material examined. Pakistan • Gilgit-Baltistan, District Diamer, Raikot; 35°23.9'N, 74°04.7'E; alt. 1200 m a.s.l.; 05 Aug. 2023; N. S. Afshan & A. R. Niazi leg.; on rock; FM-22, ITS GenBank accession number PQ469043.

Peltigera polydactylon **subsp.** ***udeghe*** **Magain, Miądl. & Sérus. in Magain, Sérusiaux, Zhurbenko, Lutzoni & Miadlikowska, Herzogia 29(2/1): 518 (2016)**

Fig. 7

Description. Thallus foliose, lobate, rhizinate, up to 6 cm across, esorediate. Lobes: separate to imbricate, adnate, crisped and rounded at margin. Color: dark black to greenish when wet and dark brown when dry. Upper surface: smooth and shiny. Lower surface: pale brown with reticulate veins, rhizinate. Rhizines: abundant, dark brown to black, scattered, fasciculate or bushy. Upper cortex: hyaline to brown, 40–60 µm thick. Algal layer: dark greyish to blackish, 100–120 µm thick, Photobiont *Nostoc*, 10–15 µm. Medulla: hyphae white, 250–300 µm thick, loosely interwoven. Lower cortex: hyaline to brown, 60–70 µm thick. Apothecia: rare, saddle-shaped, 6–7 mm in length. Disc: smooth, reddish to dark brown. Margin: crenulate to smooth, creamy to pale brown. Epihymenium: dark brown, 12–18 µm. Hymenium: light brown to hyaline, 90–130 µm. Hypothecium: creamy to light brown, 30–50 µm. Asci: hyaline, clavate, 8-spored, 70–105 × 9–14 µm. Ascospores: hyaline, filiform, rarely curved, 3–5 septa, 45–60 × 3–4 µm. Paraphyses: hyaline, simple, swollen with brown pigmented apex, 5–8 µm wide at apex.

Chemical study. K-ve, C-ve, KC-ve. Zeorin was detected through TLC.

Phylogenetic analysis. For phylogenetic analysis, sequences of the genus *Peltigera* were retrieved from GenBank based on their similarity to our sequences and those used in a study on the phylogeny of *Peltigera* by Juriado et al. (2017). A total of 35 ITS nrDNA sequences were analyzed during this study, including three newly generated sequences. The final aligned file was comprised of 709 nucleotides, among which 302 were conserved, 407 were variable, 406 were parsimony-informative, and one was singleton variant. *Solorina saccata* (L.) Ach. (MK503159 and MK503160) was chosen as an outgroup.

In our analysis, the newly generated sequence of *Peltigera polydactylon* subsp. *udeghe* (FM-15) is positioned within the polydactyloid clade of section *Polydactylon*. The Pakistani collection of *Peltigera polydactylon* (FM-15) clusters with sequences of the same species from China (MH976694 and OR178744) and the USA (KX365469). Similarly, the sequence of the Pakistani collection of *Peltigera praetextata* (KUM-10) groups with sequences from Norway (MH758516) and Russia (MH758518), forming a highly supported clade alongside *Peltigera canina* (L.) Willd. within section *Peltigera* (Fig. 8).

Notes. *Peltigera polydactylon* subsp. *udeghe* is characterized by non-tomentose upper surface, crisped, undulated, and usually phyllidiated thallus margins (Magain et al. 2016). It shares morphological and chemical similarities with *Peltigera polydactylon* subsp. *polydactylon* and *P. seneca* Magain, Miądl. & Sérus., but its thallus consistently exhibits phyllidia at the margins of lobes (Magain et al. 2016). From Pakistan, it is

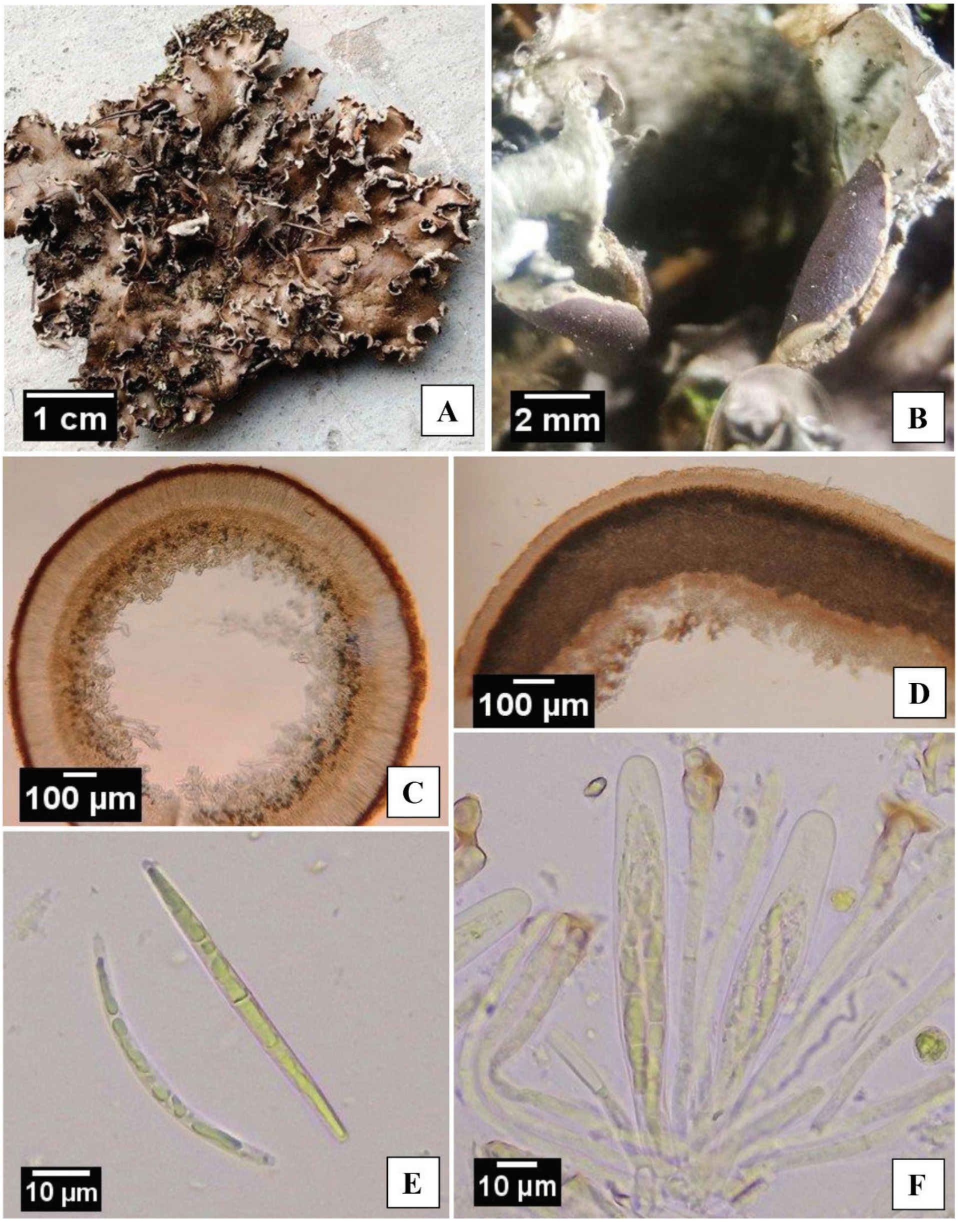

Figure 7. *Peltigera polydactylon* **A** thallus **B** apothecia **C** cross-section of apothecia **D** cross-section of thallus **E** ascospores **F** asci containing ascospores.

previously reported as *Peltigera polydactyla* (Neck). Hoffm. from Sharan (Kaghan) and Bahrain (Swat) (Aptroot and Iqbal 2012).

Habitat and ecology. Found at a high altitude on damp and detritus soil, not completely exposed to sunlight. This area, dominated by a mix of coniferous and deciduous forest trees, exhibits annual precipitation of 437 mm, and the temperature is 16 °C.

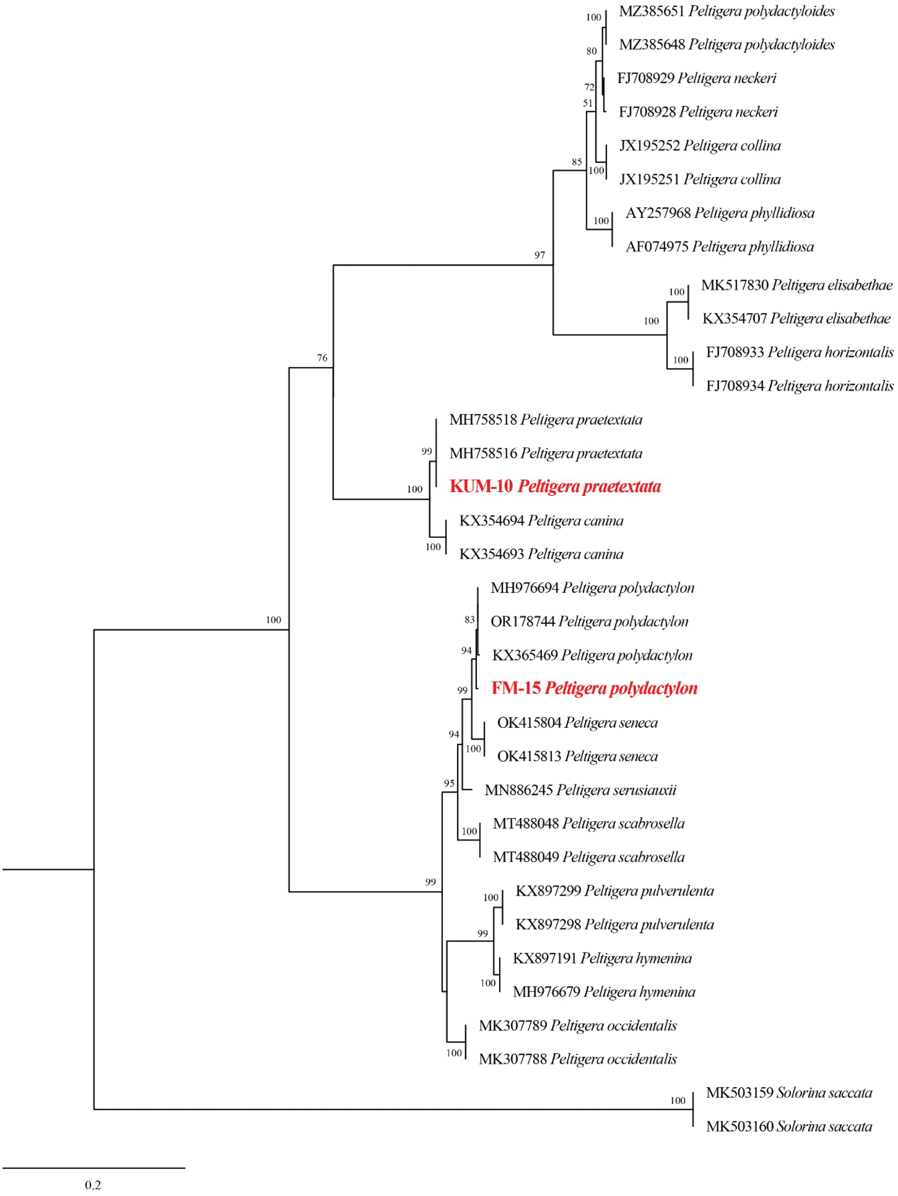

Figure 8. Molecular phylogenetic analysis of *Peltigera* spp. by maximum likelihood (ML) method based on nrITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown above the branches and novel sequences generated during this study are in bold.

Distribution. This species is widespread in Australia, Canada, New Zealand, the U.S.A and eastern Northern Asia (Magain et al. 2016).

Material examined. Pakistan • Gilgit-Baltistan, District Diamer, Fairy Meadows; 35°23'12.67"N, 74°35'02.98"E; alt. 3,300 m a.s.l.; 05 Aug. 2023; N. S. Afshan & A. R. Niazi leg.; on soil; FM-15, ITS GenBank accession number PQ469041.

Peltigera praetextata (Flörke ex Sommerf.) Zopf, Ann. Chemie 364: 299 (1909)

Fig. 11C

Notes. This species is characterized by tomentose lobe margins along with laminal and marginal phyllidia (Goward 1995). It is morphologically and phylogenetically similar to *P. canina* (L.) Willd., but the latter differs in having branched rhizines and lacking isidia (Wei et al. 2009). Its distribution spans China, Europe, India, Japan, Nepal, New Zealand, North America, South Korea, and the United States (Awasthi and Joshi 1982; Park 1990; Wei 1991; Vitikainen 1994; Galloway 2000; Brodo et al. 2001; Hur et al. 2005). In Pakistan, previous collections were made from Utror, Swat (Aptroot and Iqbal 2010), while the new specimen was collected from Kumrat Valley. This study provides the first molecular confirmation of *Peltigera praetextata* from Pakistan.

Material examined. Pakistan • Khyber Pakhtunkhwa Province, District Upper Dir, Kumrat Valley, 35°33.6'N, 72°12.4'E; alt. 2,500 m a.s.l.; 23 Sep. 2023; M. Usman & A. N. Khalid leg.; on soil; KUM-10, ITS GenBank accession number PQ469042.

Usnea lapponica Vain., Meddn Soc. Fauna Flora Fenn. 48: 173 (1925) [1924]

Fig. 9

Description. Thallus fruticose, pendent, tufted, erect, attached by a basal holdfast, up to 6 cm long. Branches: irregular, black at tips, segments terete, anisotomic dichotomous, longitudinally cylindrical, thin toward apex, up to 1 mm in diameter. Papillae: present on main branches, unevenly distributed, frequent. Color: thallus pale yellowish green to pale grey-green when dry, no change when wet. Cortex: prosoplectenchymatous, inner layer hyaline, outer greyish brown, 10–25 μm thick. Photobiont: 8–14 μm in diameter, globose to sub-globose. Medulla: loosely arranged, hyphae white. Soralia: superficial, discrete, excavated, mostly on thick branches, rounded to slightly irregular, soredia farinose and white.

Chemical study. K+ve (yellow), C-ve, KC+ve (yellow). Usnic acid and salazinic acid were detected through TLC.

Phylogenetic analysis. For phylogenetic analysis, sequences of the genus *Usnea* were retrieved from GenBank based on their similarity to our sequences. A total of 31 ITS nrDNA sequences were analyzed during this study. The final aligned file was comprised of 503 nucleotides, among which 397 were conserved, 104 were variable, 88 were parsimony-informative, and 16 were singleton variants. *Protousnea magellanica* (Mont.) Krog (DQ985192 and KR995309) was chosen as an outgroup. In the ITS phylogram, the sequence of the Pakistani *Usnea lapponica* specimen forms a clade along with sequences of the same taxon reported from Austria (KJ406267), India (KJ406266), Sweden (KJ406265), and Switzerland (KX132930) (Fig. 10).

Notes. *Usnea lapponica* is characterized by an erect to pendent fruticose thallus with deeply excavated soralia and farinose soredia (Halonen et al. 1998). For morphological comparison, the characteristics of the Pakistani sample were compared with the description of *U. lapponica* given by Halonen et al. (1999), which shows that both of

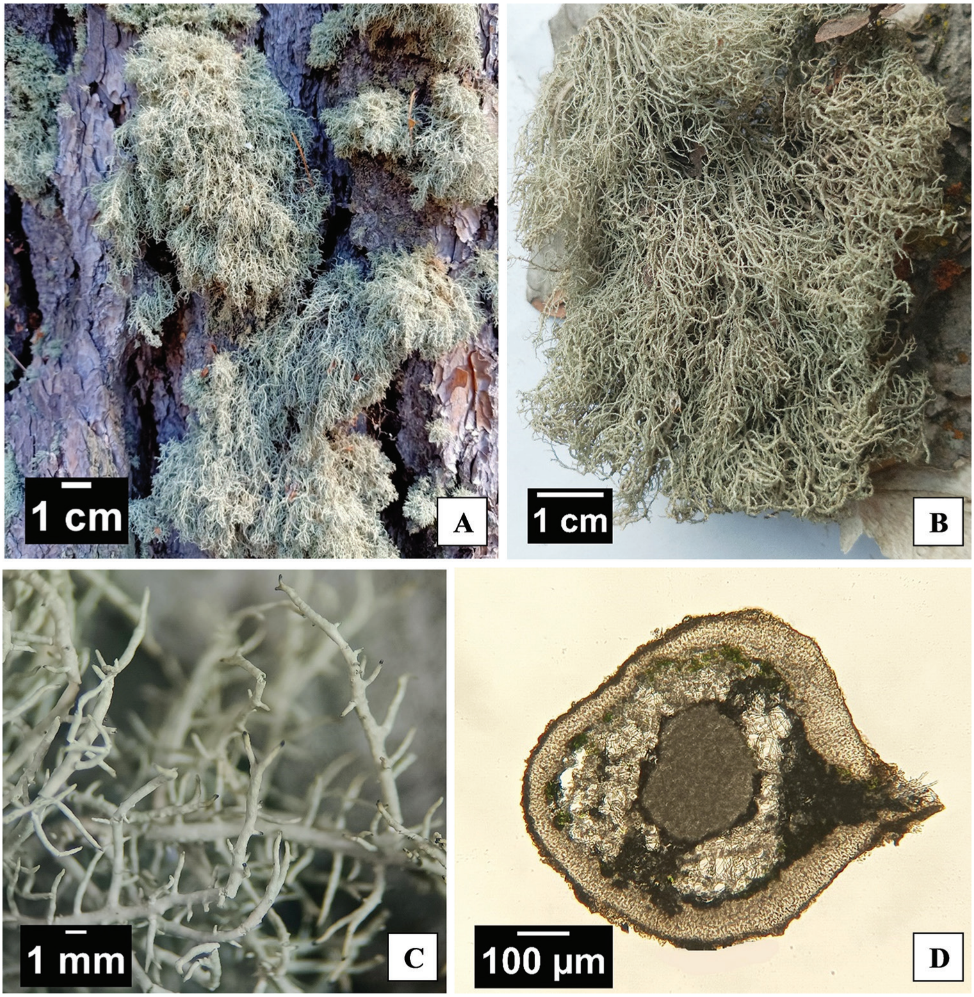

Figure 9. *Usnea lapponica* **A, B** fruticose thallus **C** branches of the thallus **D** cross-section of the branch showing central strand.

them share the same characteristics such as a highly branched thallus, slender branches, a thinner cortex, and a thicker central axis but differ from each other due to having different branching patterns (anisotomic-dichotomous vs. isotomic-dichotomous) (Halonen et al. 1999).

Habitat and ecology. Corticolous, found at an elevation above 3,228 m, growing in a cold and dry temperate mountainous region, with a mean maximum and minimum temperature of 22 °C and -18 °C, respectively, and an annual rainfall varying between 254 and 400 mm. The dominant vegetation includes *Picea smithiana* (Wall.) Boiss, *Pinus wallichiana* A.B.Jacks., and *Betula utilis* D.Don.

Distribution. This species is widespread in Europe, Finland, Iran, North America and Russia (Halonen et al. 1999; Seaward et al. 2004). From Pakistan, it is reported for the first time.

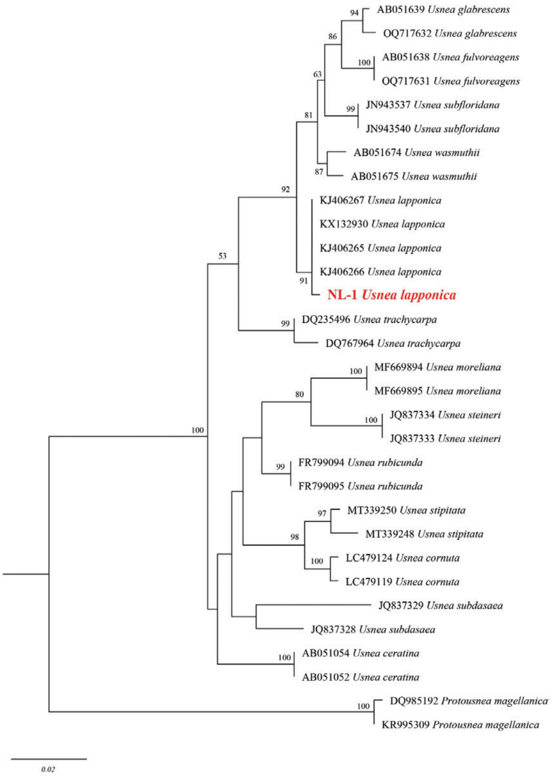

Figure 10. Molecular phylogenetic analysis of *Usnea* spp. by maximum likelihood (ML) method based on nrITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown above the branches and novel sequence generated during this study is in bold.

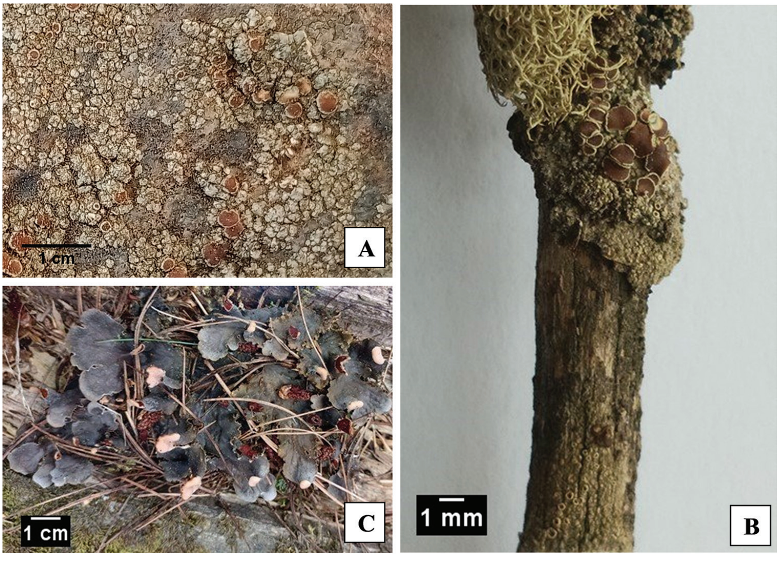

Figure 11. Thalli of selected lichen species showing distinct morphological characteristics: **A** *Lecanora horiza* **B** *Lecanora hybocarpa* **C** *Peltigera praetextata*.

Material examined. Pakistan • Gilgit-Baltistan, District Gilgit, Naltar Valley, Blue Lake; 36°14.2'N, 74°06.1'E; alt. 3,228 m a.s.l.; 30 Aug. 2023; U. F. Ahmad, M. Usman & A. N. Khalid leg.; growing on bark; NL-1, ITS GenBank accession number PQ113701.

Conclusion

In this study, we described several lichen species from Pakistan, specifically from Khyber Pakhtunkhwa and Gilgit-Baltistan. These lichen samples were analyzed using morpho-anatomical, molecular, and biochemical methods, focusing on adding to the lichen flora of the country and gaining a more comprehensive understanding of their distribution patterns and ecology relative to global lichen flora. The findings not only contribute to the knowledge of lichen biodiversity in Pakistan but also highlight the need for further extensive surveys to reveal the full extent of the country's lichenized fungi.

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RESEARCH ARTICLE

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Notulae to the Italian native vascular flora: 18

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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, and exclusions to the Italian administrative regions for taxa in the genera *Alchemilla*, *Carex*, *Chenopodium*, *Cistus*, *Convolvulus*, *Erysimum*, *Euphorbia*, *Festuca*, *Klasea*, *Lotus*, *Orobanche*, *Poa*, *Stellaria*, *Trifolium*, *Viola*. Nomenclatural and distribution updates, published elsewhere, and corrigenda are provided as Suppl. material 1.

Keywords

Endemic taxa, Floristic data, Italy, Nomenclature

How to contribute

The text for the new records, exclusions, and confirmations 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 and typifications (only for accepted names) 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

Alchemilla acutata Buser (Rosaceae)

+ **UMB**: Gualdo Tadino (Perugia) (WGS84: 43.244023°N, 12.822881°E), impluvio in pascolo su M. Serra Santa, versante W-NW; suolo calcareo, 1375 m, 13 July 2022, leg. *F. Falcinelli*, det. *G. Tondi* (FI; *Herb. Tondi*). – Species new for the flora of Umbria.

F. Falcinelli, G. Tondi

Alchemilla strigosula Buser (Rosaceae)

+ **UMB**: Monte Pozzoni (Cascia, Perugia) (WGS84: 42.659280°N, 13.162496°E), pascolo di vetta, versante N-NW; suolo calcareo, 1890 m, 22 Jun 2023, leg. *F. Falcinelli*, det. *G. Tondi* (FI; *Herb. Tondi*). – Species new for the flora of Umbria.

F. Falcinelli, G. Tondi

Alchemilla undulata Buser (Rosaceae)

+ **UMB**: Monte Pozzoni (Norcia, Perugia) (WGS84: 42.659280°N, 13.162496°E), loc. Valle della Foce, radura in faggeta, versante NE; suolo calcareo, 1480 m, 28 Jun 2022, leg. *F. Falcinelli*, det. *G. Tondi* (FI; *Herb. Tondi*). – Species new for the flora of Umbria.

F. Falcinelli, G. Tondi

Carex acutiformis Ehrh. (Cyperaceae)

+ **PUG**: Otranto (Lecce), Laghi Alimini (WGS84: 40.221778°N, 18.436083°E), Bosco igrofilo a Frassino meridionale, 5 m, 14 May 2024, leg. *N. Costanza*, det. *L. Forte, N. Costanza* (FI; BI Nos. 59076, 59077). – Species confirmed for the flora of Puglia.

This species was no longer recorded in Puglia (Bartolucci et al. 2024). It had been indicated for Gargano "*Nelle arene di spiaggia a sud di Vieste (spiaggia del Castello) fino a Torre del Ponte*" by A. Béguinot in 1902, as first record for Puglia (Béguinot 1909), and at Lago di Varano by Fenaroli (1974). At Laghi Alimini it was reported sub *Carex paludosa* Gooden. by Gehu and Biondi (1988), in the same communities where these specimens were found.

L. Forte, N. Costanza

Chenopodium pedunculare Bertol. (Chenopodiaceae)

+ **VDA**: Fénis (Aosta), prato presso il villaggio di Rovarey, Plan Clavalité (WGS84: 45.680187°N, 7.491164°E), 1518 m, 25 September 2021, leg. et det. *C. Ganz*, conf. *D. Iamonico* (AO, FI, RO). – Species new for the flora of Valle D'Aosta.

This species is native to Europe (Iamonico and Mosyakin 2018). Our finding represents the first record for north-western Italy.

D. Iamonico, C. Ganz

Convolvulus tricolor L. subsp. *cupanianus* (Tod.) Cavara & Grande (Convolvulaceae)

0 **CAM**: [Pozzuoli (Naples),] Paneta presso il Monte di Cuma (UTM WGS84: 33T 4522175.61°N, 419892.58°E ± 1000 m), May 1910, *G. Pellanda* (NAP barcode NAP0002868, sub *C. tricolor* Linn.); [Pozzuoli (Naples),] Paneta del Fusaro (4519778.94°N, 419853.38°E ± 1000 m), May 1911, *N. Terracciano* (NAP barcode NAP0002867, sub *C. meonanthus* Hoffm. et Link); [Pozzuoli (Naples),] Cuma (4522175.61°N, 419892.58°E ± 1000 m), 21 May 1910, *G. Pellanda* (PI-GUAD No. 057714, sub *C. tricolor* L.); [Bacoli (Naples),] Miliscola (4515677.90°N, 421950.97°E ± 850 m), 20 May 1913, [*G. Guadagno*] (PI-GUAD No. 057713, sub *C. tricolor* L.). – Species not recently confirmed for the flora of Campania.

E. Del Guacchio, A. De Luca

Convolvulus tricolor L. subsp. *meonanthus* (Hoffmanns. & Link) Arcang. (Convolvulaceae)

– **CAM:** Species to be excluded from the flora of Campania.

Convolvulus tricolor subsp. *meonanthus* and *C. tricolor* subsp. *tricolor* were both indicated for the Campi Flegrei at Monte di Cuma by Terracciano (1917, respectively sub *C. meonanthus* Hoffm. et Link and sub *C. tricolor* Linn.), based on two specimens housed in NAP. Motti and Ricciardi (2005, sub *C. meonanthus* and *C. tricolor*) regarded these records as very doubtful, but they did not cite any of the above-mentioned specimens, which actually are to be referred to *C. tricolor* subsp. *cupanianus*. The same taxon might have been photographed by De Santis (2007) for Castellabate (Cilento, Salerno), but no specimen is available and the population has no longer been found (De Santis, pers. comm.).

E. Del Guacchio, A. De Luca

Erysimum crassistylum C.Presl subsp. *verresianum* Peccenini & Polatschek (Brassicaceae)

+ **PIE**: Mompantero (Torino), Val di Susa, Località Seghino (WGS84: 45.144086°N, 7.066062°E), praterie xeriche mosaicate a boschi di *Quercus pubescens* e arbusteti xerofili a *Prunus spinosa* e *Ligustrum vulgare*, 706 m, 18 May 2024, *M. Lonati, G. Marengo* (FI). – Subspecies new for the flora of Piemonte.

This subspecies is considered endemic to Valle D'Aosta, where it occurs on the left side of the lower Dora Baltea valley, from Saint Pierre to Bard (Peccenini and Polatschek 2014). The samples collected in Mompantero confirm the extension of the species distribution range also in Piemonte, where it is widespread within the xerothermic grasslands of the Valle di Susa, specifically between the municipalities of Foresto and Giaglione. The record of *E. diffusum* Ehrh. (Braun-Blanquet 1961, sub *E. canescens*) for the same locality reported here is incorrect and should be referred to *E. crassistylum* subsp. *verresianum*.

M. Lonati, G. Marengo

Euphorbia cyparissias L. (Euphorbiaceae)

+ **PUG**: Lesina (Foggia), lungo la Strada Statale 693 presso loc. Masseria Nisi (WGS84: 41.851399°N, 15.384243°E), vegetazione erbacea del bordo stradale, 11 m s.l.m., 30 April 2024, leg. *G. Mei*, det. *G. Mei, A. Stinca* (FI, *Herb. G. Mei*). – Species confirmed for the flora of Puglia.

G. Mei, A. Stinca

Festuca pumila Chaix (Poaceae)

+ **LIG**: Triora (Imperia), Rifugio la Terza (WGS84: 44.059476°N, 7.728855°E), praterie rocciose calcaree, 2031 m, 10 August 2024, *G. Nota, M. Lonati* (FI). – Species new for the flora of Liguria.

In the collection area, this species is widely distributed across the southern-facing Ligurian slope, between Monte Saccarello, the Statua del Redentore, and the Rifugio la Terza, in pastures and calcareous rocky environments. The area falls within the Ligurian Alps Regional Natural Park.

G. Nota, M. Lonati

Klasea lycopifolia (Vill.) Á.Löve & D.Löve (Asteraceae)

– **EMR**. Species to be excluded from the flora of Emilia-Romagna.

The occurrence of this species in Emilia-Romagna is exclusively based on the finding of two individuals in Val Lardana and Valle del Torrente Liscato (Piacenza) by Bracchi et al. (2003): "Cimitero di Cassimoreno e Località Pian del Soppo". However, no herbarium specimen exists in MNSM or in other herbaria. One of us (SO) visited the localities indicated by Bracchi et al., but only individuals of *Centaurea scabiosa* L. have been found. Considering that Bracchi et al. (2003) stated that one of the two individuals found show a branched stem with 2–5 capitula, and that *K. lycopifolia* is characterized by a stem with a terminal solitary capitulum, we believe that the report for Emilia-Romagna should be considered erroneous. Accordingly, *K. lycopifolia* should be excluded from the regional flora.

S. Orsenigo, F. Bartolucci, F. Conti

Lotus creticus L. (Fabaceae)

+ **LAZ**: Latina (Latina), litorale di Foce Verde (WGS84: 41.408481°N, 12.865087°E), dune costiere in ricostruzione, 3 m, 12 May 2023, leg. *G. Buccomino*, det. *G. Buccomino*, *A. Tilia* (RO); *ibidem*, 19 June 2023 (FI, RO); Latina (Latina), località Capo Portiere, Parco nazionale del Circeo (41.405230°N, 12.873598°E), dune, 3 m, 19 June 2023, leg. *G. Buccomino*, det. *G. Buccomino*, *A. Tilia* (FI, RO); Isola di Ventotene (Latina), 30 May 1967, *B. Anzalone* (RO ex *Herb. Anzalone* No. 23566); Santo Stefano (Latina), 25 September 1967, *B. Anzalone* (RO ex *Herb. Anzalone* No. 23567); Anzio (Roma), località Lido di Lavinio, 27 March 1967, *B. Anzalone* (RO ex *Herb. Anzalone* No. 23568); Isola di Palmarola (Latina), 11 April 1969, *B. Anzalone* (Ro ex *Herb. Anzalone* No. 23562); Isola di Ventotene (Latina), 2–5 May 1968, *B. Anzalone* (RO ex *Herb. Anzalone* No. 23561); Circeo (Latina), 27 May 1954, *A. Cacciato* (RO ex *Herb. Cacciato*); Circeo dune di Torre Paola (Latina), 9 April 1963, *A. Cacciato* (RO ex *Herb. Cacciato*); Circeo (Latina), litorale presso Torre Paola (Gita S.B.I.), 21 April 1965, *B. Anzalone* (RO ex *Herb. Anzalone*). – Species confirmed for the flora of Lazio.

The shape of the calyx, often used to distinguish *Lotus creticus* from *L. cytisoides* L., is not really reliable, whereas the density of the hairs on the leaves is of greater importance together with the size of the rachis: leaves of *L. creticus* are very densely silky-silver and the rachis is ≤ 30% of the basal leaflets (Tison and de Foucault 2014). *Lotus creticus*, so far considered as doubtfully occurring in Lazio (Anzalone et.

al. 2010; Bartolucci et al. 2024), occurs, indeed, along the Pontine coast together with *Ononis variegata* L., *Cakile maritima* Scop. subsp. *maritima*, *Crucianella maritima* L., *Lomelosia rutifolia* (Vahl) Avino & P.Caputo, and *Thinopyrum junceum* (L.) Á.Löve.

G. Buccomino, A. Tilia

Orobanche rapum-genistae Thuill. (Orobanchaceae)

+ **VEN**: Passo Valles (Belluno), (WGS84 46.336548°N, 11.803394°E), scarpata erosa, 2050 m, 18 August 2021, leg. *L. Pinzani*, det. *G. Domina* (FI). – Species confirmed for the flora of Veneto.

L. Pinzani, G. Domina

Poa maroccana Nannf. (Poaceae)

+ **CAL**: Monasterace (Reggio Calabria) (WGS84: 38.450920°N, 16.580020°E), lungo il bordo di una strada sterrata, 4 m, 1 June 2023, *G. Tavilla*, (FI). – Species new for the flora of Calabria.

+ **PUG**: Bari (Bari), aiuola del campus universitario (WGS84: 41.108412°N, 16.883572°E), 12 m, vegetazione sinantropica degli incolti, 16 February 2024, *G. Tavilla* (FI). – Species new for the flora of Puglia.

G. Tavilla

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

+ **LIG**: Quiliano (Savona), Località Burré, al bivio tra SP29 e Via Ronco (WGS84: 44.335401°N, 8.374027°E), margine incolto della strada, 348 m, 10 April 2024, *M. Lonati*, *G. Nota* (FI). – Species new for the flora of Liguria.

M. Lonati, G. Nota

Trifolium michelianum Savi (Fabaceae)

+ **ABR**: Collarmele (L'Aquila), San Nicola (WGS84: 42.06020°N, 13.68357°E), prateria inondata, 1060 m, 6 July 2023, *G. Ciaschetti* (APP No. 72043). – Species new for the flora of Abruzzo.

This species has a northen and western Mediterranean distribution (POWO 2024). It was found in a temporary pond, together with other short-lived marsh plants (e.g., *Mentha pulegium* L. subsp. *pulegium* and *Ranunculus sardous* Crantz).

G. Ciaschetti, R. Venanzoni

Trifolium striatum L. subsp. *tenuiflorum* (Ten.) Kožuharov (Fabaceae)

+ **PUG**: Gravina in Puglia (Bari), Lago d'Olmo - Bosco Comunale Difesa Grande, (WGS84: 40.754979°N, 16.385376°E), prati umidi, 450 m, 10 May 2021, leg.

V. Tomaselli, det. *G. Pellegrino, V. Tomaselli*, (FI, BI Nos. 58997). – Subspecies confirmed for the flora of Puglia.

Trifolium striatum subsp. *tenuiflorum* forms a small population in correspondence of a wet meadow on the edge of a temporarily flooded area.

G. Pellegrino, V. Tomaselli

Viola cassinensis Strobl subsp. *pseudogracilis* (A.Terracc.) Bartolucci, Galasso & Wagens. (Violaceae)

+ **PUG**: Bovino (Foggia), Monti Dauni, Località "Confreda", (WGS84: 41.218257°N, 15.340597°E), prateria xerofila, 756 m s.l.m., 07 March 2023, campione coltivato in Orto Botanico di Bari sino a 28 March 2024, leg. *G. Pazienza*, det. *V. Tomaselli, G. Pazienza*, (FI; BI Nos. 58917, 58918, 58919). – Species new for the flora of Puglia.

The finding of this subspecies, previously considered endemic to Molise and Campania (Bartolucci et al. 2018; Perrino et al. 2018), expands its range westwards.

G. Pazienza, V. Tomaselli

Floristic records of regional alien taxa

Cistus albidus L. (Cistaceae)

+ (NAT) **LAZ**: Rome, Monte Mario Natural Reserve, Farnesina hills, path starting from via dei Colli della Farnesina and going towards French cemetery (WGS84: 41.942615°N, 12.455422°E), Mediterranean maquis, siliceous sandy soil, 70 m, 18 June 2005, *D. Iamonico, R. Lorenzetti* (FI); *ibidem*, 2 June 2024, *D. Iamonico* (RO). – Status change from casual to naturalized alien for the flora of Lazio.

Cistus albidus was firstly recorded in Lazio (as native) by Iamonico (2006). Later, this species was considered as casual alien for the region (Iamonico and Lorenzetti 2008; Anzalone et al. 2010; Lucchese 2017; Bartolucci et al. 2024). During the last 18 years, I visited the site in the city of Rome many times and verified that the population found in 2005 is able to sustain itself (several seedlings and young plants at different ages and tall were observed).

D. Iamonico

Nomenclatural and distribution updates from other literature sources

Nomenclatural and distribution updates, and corrigenda to Bartolucci et al. (2024) according to Valsecchi (1995), Pierini and Peruzzi (2014), Montanari et al. (2015), Alessandrini et al. (2016); Fridlender (2018), Lucchese (2018), Alterio et al. (2023), Conti et al. (2023), Zepigi et al. (2023), Ardenghi et al. (2024), Bacchetta et al. (2024), Bogdanović et al. (2024), Croce and Saetta (2024), Davanzo et al. (2024), Denk et al. (2024), El Mokni et al. (2024), Elvebakk and Bjerke (2024), Foelsche (2024), Franzoni et al. (2024), Giacò et al. (2024), Gottschlich et al. (2024), Iamonico (2024), Iamonico et al. (2024), Jesus et al. (2023), Lastrucci et al. (2024a, 2024b), POWO (2024), Rasti et al. (2024), Röser and Tkach (2024), Ruggero et al. (2024), Selvaggi et al. (2024), Şentürk et al. (2024), Tardella et al. (2024), Koopman et al. (2025), 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: pdf

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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New Characeae (Charophyceae, Charales) report in eastern Sicily (Italy)

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Abstract

Nowadays species belonging to the green algal family Characeae are highly endangered. For this reason, many of them are protected by European directives and included in Red Lists. In Italy, however, the Red List of Characeae does not exist and the distribution of these species is often unknown, especially in Sicily wetlands. In this study, we investigated the distribution and morphology of Characeae in fresh and brackish water wetlands, both natural and artificial, located in various areas of eastern Sicily (Italy). During the survey *Chara globularis* Thuiller, *Chara vulgaris* Linnaeus, *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster, and *Tolypella glomerata* (Desvaux) Leonhardi were found. This is the first record of these species in the investigated sites. The results improved the knowledge of freshwater algal biodiversity in Sicily and, above all, allowed to fill distribution gaps of the Characeae species, to apply adequate protection measures and create national Red Lists of Characeae.

Keywords

Aquatic flora, *Chara*, freshwater algae, *Nitella*, *Tolypella*, wetlands

Introduction

The family Characeae (Charales, Charophyceae), also called stoneworts, includes green algae with macroscopic thalli consisting of a succession of nodes and internodes of varying lengths. The nodes consist of two short central cells and 6 or more pericentral cells each of which gives origin to a ray so that a whorl of rays occurs at the level of each node; the nodes are separated from each other by internodes consisting of very long single cells (Bazzichelli and Abdelahad 2009). This family which includes different genera is arranged into two tribes: Chareae [*Chara* Linnaeus, *Lamprothamnium* J. Groves, and *Lychnothamnus* (Ruprecht) A.Braun] and Nitelleae [*Nitella* C.Agardh and *Tolypella* (A.Braun) A.Braun] (Bazzichelli and Abdelahad 2009; Guiry and Guiry 2024).

Species belonging to the Characeae live in fresh and brackish waters. They grow in a wide range of depths but mainly in shallow, still, or gently flowing waters. Characean species can live in artificial ditches, forest sumps, and temporary ponds, forming large meadows in almost all habitats where they occur (Wood and Imahori 1965; Bold and Wynne 1985; Lambert 2007).

All the Characeae species have an important ecological role in the environments in which they are present. For example, they maintain high water transparency, are able to store large quantities of carbon and nutrients, are the base of the trophic chain, and offer refuge and create habitat for many animals (Lucas 1975; Blindow et al. 2002; Schneider et al. 2015). They are considered good bioindicators (Lambert-Servien et al. 2006; Täuscher 2012; Pukacz et al. 2013): salinity, temperature, chemistry of water and light are factors that affect the distribution of these algae in the environments, and therefore, their presence or absence can provide information about several environmental factors (Gąbka et al. 2007; Boszke and Bociąg 2008).

Nowadays the species of Characeae are highly endangered. The most severe threats to their survival can be found in arid and semiarid regions due to the combination of continuous human transformation of the environment and climate changes (Romanov et al. 2022). For this reason, some species of Characeae are protected by the Habitats Directive 92/43/EC and the Water Framework Directive 2000/60/EC; moreover, in some European countries these species are protected by national legislation and included in national Red Lists (e.g. Stewart and Church 1992; Palamar-Mordvintseva and Tsarenko 2004; Blaženčić et al. 2006; Gärdenfors 2010; Auderset Joye and Schwarzer 2012; Korsch et al. 2013; Urbaniak and Gąbka 2014; Stewart and Hatton-Ellis 2020). In Italy, however, a national Red List of Characeae is not available. This is a major gap in the conservation legislation for the country, particularly because there are still many locations in Italy, especially in Sicily, where no information is available on the presence of these algae. The main information available in Sicily on the distribution of these species concerns western Sicily, particularly in the Palermo area (Formiggini 1908; Naselli-Flores and Barone 2002; Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021)

In this study, we investigated the distribution and morphology of species of Characeae in different freshwater and brackish wetlands, both natural and artificial, in eastern Sicily (Italy). The results improve our knowledge of the botanical biodiversity of these areas and fill, at least partly, gaps in the knowledge of the distribution of this species in Sicily.

Methods

Sampling areas

Sampling was conducted in the period spring/summer of 2022 and the sampling sites were chosen after a careful preliminary inspection of several areas in eastern Sicily. The selected sites hosted populations of Characeae with a visual coverage higher than 5%: Cesarò - Messina (LM), Gela - Caltanissetta (GW - A; GW - B; GW - C), Melilli - Syracuse (CU), and Troina - Enna (LA). Fig. 1 shows the sampling sites and was produced using QGIS 3.16 software; in Table 1 all the sampling sites with the coordinates and the abbreviations used are reported.

In detail, the sampling sites of Cesarò and Troina are located within the Nebrodi Regional Natural Park, in zone A and in zone B respectively. In zone A, sampling was done in one of the small natural lakes around Monte Soro at approximately 1,575 m above sea level (asl). This area is within two Natura 2000 sites: ITA030038 and ITA030043. In zone B, sampling was carried out in the artificial lake called "Ancipa – Sartori" at an altitude of approximately 947 m asl; this site falls within the Natura 2000 site ITA060005. In Gela sampling was carried out within the protected area called "Geloi Wetland" located at approximately 30 m asl. It is located in the "Piana di Gela" and falls within the Natura 2000 site ITA050012. In this area, which

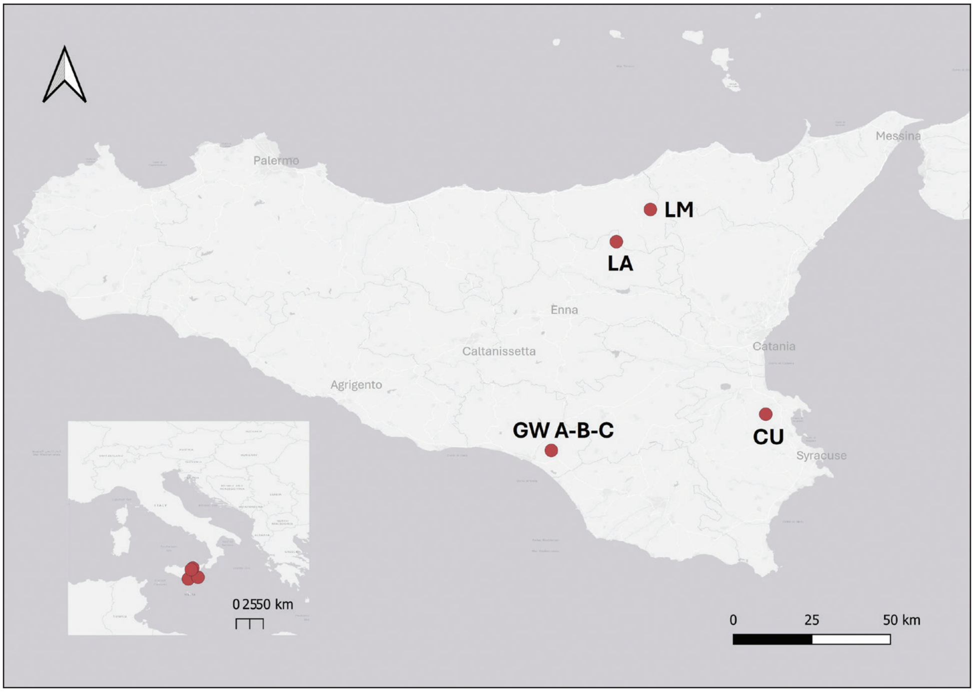

Figure 1. Map showing the sampling sites. Cesarò - Messina (LM), Gela - Caltanissetta (GW - A; GW - B; GW - C), Melilli - Syracuse (CU), and Troina - Enna (LA).

hosts many seasonal and permanent marshes, two artificial marshes were sampled; they were adjacent to each other and had brackish water because were formed by water from an underground saline spring. These marshes extend over a large area and salinity levels gradually decrease moving away from the spring. We sampled in the Acquitrino Principale del Giunco Foglioso (seasonal) and in the Pantano Pavoncelle (permanent); in the latter, we sampled in two different points, one more distant to the Acquitrino Principale del Giunco Foglioso but close to the opening of the saline aquifer, and one among the other two points (Fig. 2).

In Melilli sampling was carried out in the Curcuraggi area, an area mainly frequented by grazing cattle and characterised by the presence of temporary pools used as drinking troughs, in one of these we carried out the sampling. This temporary pool was located approximately 1 km from the Natura 2000 site ITA090024 at approximately 190 m asl.

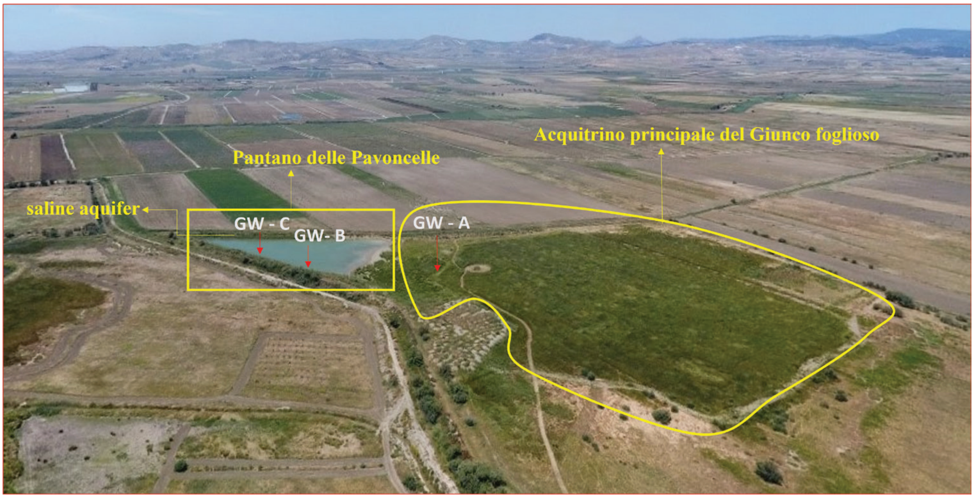

Figure 2. Map showing the sampling sites in Geloi Wetland.

Table 1. Summary of information regarding the sampling sites.

Sampling sites	Abbreviation	Coordinates	Altitude
Curcuraggi - Melilli	CU	37°13'32.9"N, 15°05'38.0"E	about 190 m asl
Geloi Wetland - Gela	GW - A Acquitrino Principale del Giunco Foglioso	37°06'14.3"N, 14°20'18.5"E	about 30 m asl
	GW - B Pantano delle Pavoncelle (between the sampling point GW - A and GW - C)	37°06'12.9"N, 14°20'13.1"E	
	GW - C Pantano delle Pavoncelle (close to the opening of the saline aquifer)	37°06'13.9"N, 14°20'15.1"E	
Lago Ancipa - Sartori - Troina	LA	37°50'11.0"N, 14°33'53.5"E	about 947 m asl
Laghetto Monte Soro - Cesarò	LM	37°57'01.7"N, 14°41'07.0"E	about 1,575 m asl

Sampling and identification of Characeae

Samples of Characeae were collected by hand, placed in plastic bags containing the same water as the sampling sites, and put in a box with ice to carry them to the Phycology Laboratory of the Department of Biological, Geological and Environmental Sciences - University of Catania. Afterwards, the thalli were washed with distilled water to remove sediment and organic matter and, through a morphological and reproductive study by observation with stereoscopes and microscopes, following the dichotomous keys provided by Bazzichelli and Abdelahad (2009) and descriptions available from AlgaeBase (Guiry and Guiry 2024), they were identified. After that, the thalli were photographed using a digital camera Panasonic DC-FZ82 and, subsequently, the photos were processed using GIMP 2.10. The most significant specimens of the species found are held in CAT Sectio Algae Herbarium exiccata of the University of Catania.

Results

Examination of the samples revealed the presence of *Chara globularis* Thuiller in CU, *Chara vulgaris* Linnaeus in LA and GW-B, *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster in LM, and *Tolypella glomerata* (Desvaux) Leonhardi in GW-A, GW-B and GW-C.

Details of the morphology, geographical distribution, conservation status, previous reports in Sicily and new reports in Sicily are provided below for the species recorded.

Chara globularis Thuiller

Fig. 3a–d

Geographical distribution. *C. globularis* is a cosmopolitan species (Urbaniak and Gabka 2014). It has been reported from United States (Smith 2010), Israel (Barinova and Romanov 2016), Iceland (Hrafnsdottir et al. 2019), and recently from Socotra Island, Yemen (Zalat et al. 2020) and India (Verma et al. 2021); it is also widespread in Europe (Guiry and Guiry 2024). In Italy, it has been reported from Trentino Alto Adige, Piedmont, Lombardy, Veneto, Friuli Venezia Giulia, Tuscany, Marche, Umbria, Latium, Sardinia and Sicily (Naselli-Flores and Barone 2002; Bazzichelli and Abdelahad 2009; Azzella 2014; Becker 2019; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021).

Conservation status. In the Scandinavian countries, Czech Republic, Balkan countries, Switzerland and many German Länder *C. globularis* is classified as least concern (LC) while, in other regions of Germany (Saarland, Saxony and Thuringia) it is considered vulnerable (VU) or endangered (EN) (Auderset Joye and Rey-Boissezon 2013). In Hungary, this species is considered vulnerable (VU) by the International Union for Conservation of Nature and Natural Resources IUCN (Azzella 2014) and in Wales, it is in the least concern (LC) threat status of Red List Assessment (Stewart and

Figure 3. *Chara globularis* Thuiller **a** habit **b** rays with 6–7 cortical articles **c** small, rudimentary, papiliform stipulodes arranged in two rows **d** detail of some oogonia and antheridia. Scale bars: 1 cm (**a, b**); 100 µm (**c, d**).

Hatton-Ellis 2020). Finally in Slovakia *C. globularis* is a near-threatened (NT) species in IUCN red-list (Hindáková et al. 2022).

Previous reports for Sicily. Palermo, Pond of Santa Rosalia (Naselli-Flores and Barone 2002); Palermo, pond of Rebuttone (Guarino et al. 2019; Romanov et al. 2019); Palermo, Coda di Riccio lake (Guarino et al. 2019); Palermo, some ponds in the Palermo district (Panzeca et al. 2021).

New reports in Sicily. Curcuraggi (CU - Melilli, Syracuse).

Morphology. Thalli are 24–30 cm tall and green in colour. The main axis has a diameter of about 400 µm. The internodes are generally much longer than the branches (Fig. 3a). The rays are slender, with 6–7 corticate articles and two small terminal bare cells (Fig. 3b). The cortex is triplostichous, isostichous. It has rudimentary spine cells and the stipulodes are in two rows and are very small, rudimentary, and papilliform (Fig. 3c). The species is monoecious: the gametangia are borne jointly at the three lowest branchlet nodes; the oogonia are solitary and have a size of about 500 × 350 µm while the antheridia are about 300 µm in diameter (Fig. 3d).

Chara vulgaris Linnaeus

Fig. 4a–d

Geographical distribution. *C. vulgaris* is a cosmopolitan species common in all continents (Korsch 2018). It occurs in Asia, America, and Europe (Guiry and Guiry 2024). In Italy, it has been reported from Trentino Alto Adige, Piedmont, Lombardy, Veneto, Liguria, Friuli Venezia Giulia, Emilia Romagna, Tuscany, Marche, Umbria, Abruzzo, Latium, Basilicata, Calabria, Apulia, Sardinia and Sicily (Bazzichelli and Abdelahad 2009; Troia and Barone 2017; Becker 2019; Guarino et al. 2019; Panzeca et al. 2021).

Conservation status. In Norway, *C. vulgaris* is classified as endangered (EN). In the Balkan countries, Sweden, and the Czech Republic it is considered at low risk of extinction, while in the German Länder, its status varies from vulnerable (VU) to least concern (LC). In Switzerland, it is vulnerable (VU) (Auderset Joye and Schwarzer 2012), in Wales in least concern (LC) threat status (Stewart and Hatton-Ellis 2020) and in Slovakia is a near-threatened (NT) species in IUCN red-list (Hindáková et al. 2022).

Previously reports from Sicily. Undefined locality in Sicily (Troia and Barone 2017); Palermo, Rocca dell'Aquila pond; Palermo, Rebuttone pond; South-east Sicily, Hyblaean Marshes; Trapani, Anguillara ponds; Palermo, portella Maganoce pond (Guarino et al. 2019); Palermo, some ponds in the Palermo district (Panzeca et al. 2021).

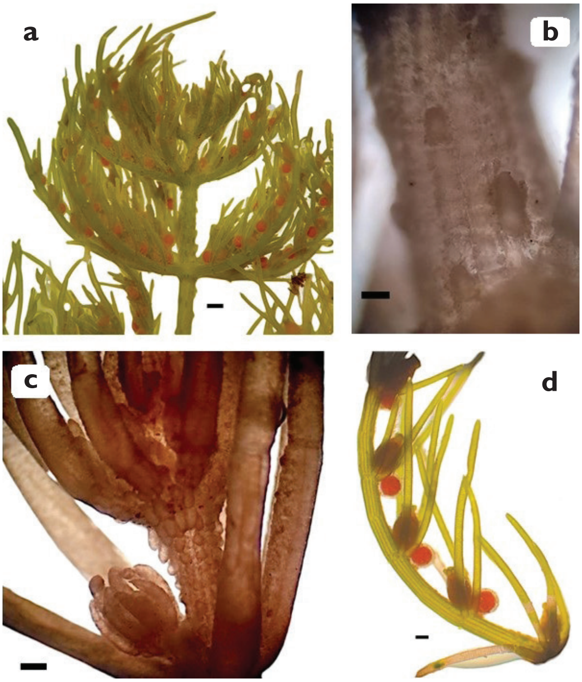

Figure 4. *Chara vulgaris* Linnaeus **a** habit of the apical part of an axis showing rays with the non-corticated terminal segments **b** diplostichous and aulacanthous cortex **c** detail of some spine cells and stipulodes arranged in two rows **d** detail of an axis with some oogonia and antheridia. Scale bars: 600 µm (**a**); 300 µm (**c**); 200 µm (**d**); 100 µm (**b**).

New reports in Sicily. Geloi Wetland (GW-B - Gela, Caltanissetta); Ancipa-Sartori lake (LA - Troina, Enna).

Morphology. Thalli are 10–30 cm tall. The main axis has a diameter of 600 µm, and the thalli are mainly green in colour but, sometimes, are also whitish/grey due to encrustations. The rays, arranged in whorls around the erect axes, are slightly curved upwards. Almost all segments are corticated, and the 2–3 terminal cells of the segments are consistently uncorticated (Fig. 4a). The cortex is diplostichous and aulacanthous (Fig. 4b). The spine cells are always solitary and papilliform. The stipulodes are clearly visible and arranged in two rows (Fig. 4c). The species is monoecious and bears both oogonia and antheridia on the same thalli (Fig. 4d). The oogonia are about 600 × 300 µm in size and the antheridia are about 350 µm in diameter.

Nitella capillaris (Krocker) J.Groves & Bullock-Webster

Fig. 5a–c

Geographical distribution. *N. capillaris* is not a widespread species. It has been mainly reported from Europe (Guiry and Guiry 2024) and Africa (Muller et al. 2017). In Italy, it was reported in Lombardy, Veneto, Emilia Romagna, Umbria, Latium, and Sicily (Bazzichelli and Abdelahad 2009; Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019).

Conservation status. In Sweden, Slovakia and Great Britain *N. capillaris* is considered extinct. In North Rhine-Westphalia, Schleswig-Holstein, Brandenburg and Thuringia is considered endangered and, following the revision of the Red List in 2010, in the Balkan countries the species is classified as vulnerable (VU) (Auderset Joye and Schwarzer 2012; Hindáková et al. 2022). In Poland, it is protected by national environmental laws and it is considered a critically endangered species (CR) by IUCN (Urbaniak and Gabka 2014).

Previous reports in Sicily. Catania, unspecified locality; Palermo, Gurgo di San Ciro (Formiggini 1908 reported as *Nitella capitata*); Trapani, Anguillara pond (Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019)

New reports in Sicily. Monte Soro Lake (LM - Cesarò, Messina).

Morphology. thalli are about 10–15 cm tall. The axes have a diameter of about 500 µm and the colour of the thalli is green or light green. The internodes are generally 2–3 times longer than the branches. In the whorls, there are generally 6 or more rays, branched once, with 2–3 second-order rays (Fig. 5a). The rays have pointed unicellular terminal segments (dactyls) (Fig. 5b). The rays of the lower whorls are usually sterile, while those of the upper whorls are fertile. *N. capillaris* is a dioecious species. The oogonia are borne in the bifurcations of the rays, generally grouped in pairs, and are about 300 × 280 µm in size, with prominent ridges (Fig. 5c). Antheridia were not observed in our specimens.

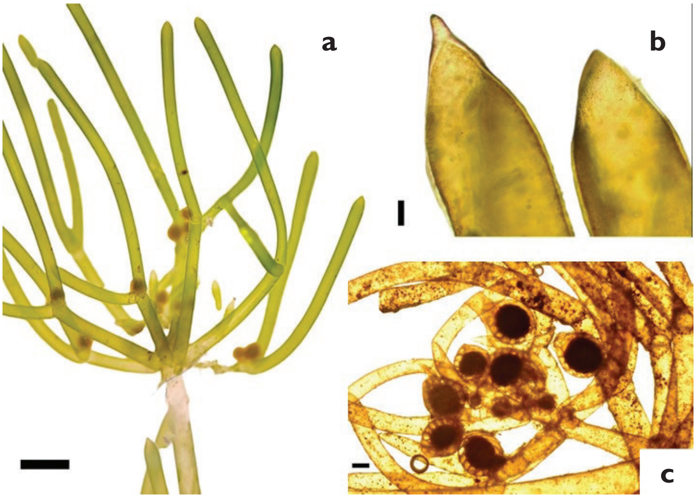

Figure 5. *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster **a** rays branched once with the formation of 2–3 second-order rays **b** dactyl **c** detail of some oogonia. Scale bars: 400 µm (**a**); 100 µm (**b, c**).

Tolypella glomerata (Desvaux) Leonhardi

Fig. 6a–c

Geographical distribution. *T. glomerata* probably has a cosmopolitan distribution (Stewart and Hatton-Ellis 2020). It was recently reported from India (Verma et al. 2021) and Israel (Barinova and Smith 2022); from Europe in Iceland (Hrafnsdottir et al. 2019), Belgium (Denys et al. 2019), Germany (Täuscher 2020; Doege et al. 2022), and Britain (Pentecost and Haworth 2021) but, generally, it is widespread throughout the European continent (Guiry and Guiry 2024). In the British Isles its populations are widely distributed but scanty and apparently in regression (Stewart and Hatton-Ellis 2020). In Italy, it has been reported from Veneto, Emilia Romagna, Tuscany, Abruzzo, Basilicata, Apulia, Sardinia and Sicily (Formiggini 1908; Sciandrello 2007; Bazzichelli and Abdelahad 2009; Becker 2019).

Conservation status. In Wales this species is in the least concern (LC) threat status of Red List Assessment (Stewart and Hatton-Ellis 2020). In the Länder of Germany, the status of *T. glomerata* varies from extinct (RE) to vulnerable (VU), whereas in the Czech Republic it is considered endangered (CR). In Sweden, after the revision of the Red List in 2010, its status dropped by one category, and it is currently classified as critically endangered (EN) (Auderset Joye and Schwarzer 2012).

Previous reports in Sicily. Undefined location in Sicily (Formiggini 1908); Caltanisetta, Gela, Piana del Signore (Sciandrello 2007).

New reports in Sicily. Geloi Wetland (GW-A, GW-B, GW-C - Gela, Caltanissetta).

Morphology. Thalli are 7–10 cm tall, with erect axes about 1 cm thick; their colour is green with white encrustations. The branches are grouped in dense clusters

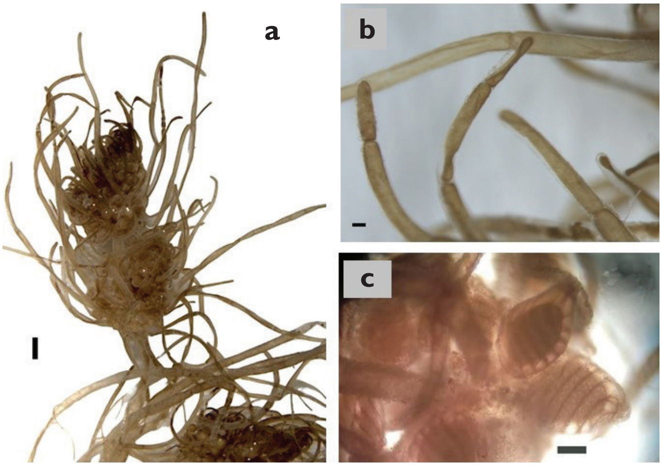

Figure 6. *Tolypella glomerata* (Desvaux) Leonhardi **a** branches agglomerated **b** segment of the rays with blunt apices **c** oogonia. Scale bars: 1 cm (**a**); 500 µm (**b**); 100 µm (**c**).

(Fig. 6a) and the whorls are composed of 6–8 rays each. The lower whorls are sterile and sparse, while the upper ones are fertile with dense rays. The terminal segments of the rays are shorter than the subterminal and their apices are blunt (Fig. 6b). *T. glomerata* is a monoecious species. The oogonia are borne in groups of 1–3 and are about 350 × 240 µm in size (Fig. 6c). The antheridia are about 250 µm in diameter.

Discussion

The results of this study improve the knowledge of the distribution of Characeae in eastern Sicily. None of the sampled species had been previously reported in the areas investigated and some scanty data were available only for nearby areas (Sciandrello 2007; Guarino et al. 2019; Romanov et al. 2019).

C. globularis has been reported from some wetlands in Palermo (Naselli-Flores and Barone 2002; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021) but, as far as we know, there are no other reports for Sicily. Curcuraggi, where we found this species, unlike the other areas that we investigated, is impacted by human activities; it is indeed known that *C. globularis* can live in environments subject to anthropogenic impacts and that it is very tolerant to eutrophication (Auderset Joye and Schwarzer 2012; Barinova and Romanov 2016).

C. vulgaris is the most widespread and most reported species of *Chara* in Italy (Bazzichelli and Abdelahad 2009) and our records allow to add new sites, Geloi Wetland (GW-B) and Ancipa-Sartori lake (LA), which had previously not been inspected for the presence of Characeae.

N. capillaris was previously reported mainly in the areas of Trapani and Palermo and in 1908 in an undefined locality in Catania (Formiggini 1908; Guarino et al. 2019; Romanov et al. 2019). We found this species at Monte Soro Lake, located in the province of Messina

(LM), an area in which the species had not previously been reported. This lake is situated approximately at 1,575 m asl and often freezes in winter. This agrees with previous studies indicating that this species is able to survive during periods of frost, since it produces a large number of oospores that are resistant to freezing stress and germinate as soon as environmental conditions become favourable again (Auderset Joye and Schwarzer 2012).

Finally, we report the presence of *T. glomerata* in Geloi Wetland (Gela) (GW-A), (GW-B), (GW-C), south-eastern Sicily. This species had already been reported for the brackish marsh system of Piana del Signore (Gela) (Sciandrello 2007), very close to our site of discovery, but had not yet been reported for the wetland examined in the present study. As reported by Bazzichelli and Abdelahad (2009), Cirujano Bracamonte et al. (2013), and Zeneli and Kashta (2016), this species is often found in slightly brackish waters; accordingly, we found this species in the only site with this type of water. We noted that the population density and its fertility decreased moving from the Acquitrino del Giunco Foglioso (sal 0.09%, Ph 8.46) to the Pantano Pavoncelle (sal 0.13%, Ph 8.48), in which the salinity is higher due to a saltwater spring. This is in agreement with Winter et al. (1996), who reported that, despite being a species that adapts well to brackish environments, salinity is a limiting factor for the fertility of this species.

Conclusions

The results of this study show that the distribution of Characeae in Sicily is not yet sufficiently known, and more attention should be given to the presence of these interesting algae in Sicilian wetlands. This would be important to fill the knowledge gaps that still exist and to create, even in Italy, a national Red List for the protection of these species and the environments in which they live.

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RESEARCH ARTICLE

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New data on the morphology, ecology and distribution of *Lupinus gredensis* (Fabaceae) in the Mediterranean region

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Abstract

Lupinus gredensis is generally treated as an endemic species to the Iberian Peninsula, with some occurrences reported for the eastern Mediterranean, considered to be the result of an introduction. The finding of five populations attributable to *L. gredensis* on the south-eastern slopes of Mt. Etna (Sicily, Italy) is here reported for the first time. *Lupinus gredensis* samples from Mt. Etna were morphologically compared with fresh and herbarium preserved specimens of the related species *L. luteus* and *L. hispanicus*. The distribution and the ecology of the surveyed populations, as well as the phytosociological characterization of the plant communities hosting *L. gredensis* are defined. Finally, the chorological status of this species in Sicily is discussed through the review of the historical occurrence of *L. luteus* in the Mt. Etna area, providing new data on the putative distribution range of *L. gredensis* in the Mediterranean region.

Keywords

Italy, microstructure of seed coat, plant community, Sicily, vascular flora

Introduction

According to Pignatti et al. (2017) and Bartolucci et al. (2024), the genus *Lupinus* L. in Italy is represented by five native taxa: *L. albus* L. subsp. *graecus* (Boiss. & Spruner) Franco & P.Silva, *L. angustifolius* L., *L. cosentinii* Guss., *L. gussoneanus* J.Agardh and

L. luteus L. Moreover, Galasso et al. (2024) reported *L. albus* subsp. *albus* and *L. polyphyllus* Lindl. as naturalized species. Except for the North American *L. polyphyllus*, all the other species mentioned for Italy are also present in Sicily (Giardina et al. 2007). The discovery of some populations on the eastern slope of Mt. Etna (E Sicily) not identifiable with any taxon known for the island, and the critical review of the herbarium specimens have made it possible to report for the first time the occurrence of *L. gredensis* Gand. in Italy. This taxon, first described by Gandoger (1901), was later treated as a variety or subspecies of the closely related *L. hispanicus* Boiss. & Reut. by some authors (Merino 1905; Gladstones 1974). More recently, it was re-evaluated to the rank of species due to the significant morphological differences between the two taxa, which mainly regard the flower color: lilac to violet in *L. hispanicus* and cream-pale yellow in *L. gredensis*, in which the lower whorls turn lilac to mauve at the end of the anthesis (Castroviejo and Pascual 1998, 2000). According to many sources (Castroviejo and Pascual 2000; Euro+Med 2024; POWO 2024), *L. gredensis* is endemic to the western and central areas of the Iberian Peninsula. However, this species has been reported as introduced into Greece, Turkey, and Bulgaria (Dimopoulos et al. 2013; Stoyanov and Apostolova-Stoyanova 2022). As concerns the Turkish (W Anatolia) and the Greek (NE Greece and east Aegean Islands) populations, the status of *L. gredensis* as an alien species is particularly controversial. Strid (2016, 2024) stated this the species occurs in natural environments and behaves like a native plant, suggesting a disjunct distribution in the Western (Spain and Portugal) and Eastern Mediterranean (NE Greece, NE Aegean area, and W Anatolia). As reported by Stoyanov and Apostolova-Stoyanova (2022), this statement could also be supported by the occurrence of a specimen (SOA 10227), which demonstrates its presence in Greece for at least a century, as well as by the record of *L. hispanicus* from Greece by Hayek (1927). The earliest specimen of *L. gredensis* (under the name *L. hispanicus*) in Turkey dates back to 1966 and is cited in Chamberlain (1970). Unfortunately, there are no data in the literature regarding the introduction of this species into cultivation, also because until its revaluation (Castroviejo and Pascual 1998) *L. gredensis* was not considered as a species distinct from *L. hispanicus*. Actually, *L. gredensis* is occasionally used, mainly in the Iberian Peninsula, as an alternative leguminous crop for marginal areas (Lema and Soengas 2023). Regarding the occurrence of *L. gredensis* in Bulgaria, Stoyanov and Apostolova-Stoyanova (2022) hypothesized that it has been imported as an ornamental plant or casually, by transportation of cereals and soil mixtures. However, they consider more probable its spreading from the adjacent territories in the Greek Rhodope. In this paper, the chorological status of *L. gredensis* in Sicily is discussed through ecological considerations and the study of historical herbarium specimens. Moreover, the description of some morphological traits of the surveyed populations, the phytosociological characterization of the Sicilian *L. gredensis* community, and its distribution in Sicily are provided.

Materials and methods

Field surveys and distribution data

We conducted field surveys in Sicily (Aeolian Islands, Nebrodi mountains, Peloritani mountains and Mt. Etna) from 2022 to 2024. Flowering and fruiting specimens of *L. gredensis* were collected in the south-eastern side of Etna volcano from five localities during spring 2024 (April to June). The collected specimens have been deposited in the CAT herbarium (the herbarium acronym follows Thiers 2024).

Additional information on the distribution of *L. gredensis* in Sicily were obtained from plant photographs published online by the members of the Facebook group "Flora spontanea Siciliana" (www.facebook.com/groups/floraspontaneasiciliana/) and from private photographic archives (R. Galesi and G. Siracusa pers. comm.).

Morphological investigations

Morphological observations were made on ten fresh individuals for each of the five populations of *L. gredensis* reported for Sicily. The specimina visa from these populations were compared with data from protologue and relevant literature (Merino 1895, Gandoger 1901, Castroviejo and Pascual 2000). In particular, the Sicilian specimens labeled as *L. luteus*, collected on Mt. Etna and stored in CAT and PAL herbaria, were examined to define their correct taxonomical attribution. Moreover, GBIF (Global Biodiversity Information Facility database, https://www.gbif.org/) records of *L. gredensis* from Portugal, Spain, Greece, and Turkey were examined. The comparison with the most similar species (*L. luteus* and *L. hispanicus*) was carried out using both fresh samples and scanned herbarium specimens preserved in ABH, CAT, GJO, PAL, PI, W, WAG. In particular, the three species have been differentiated on the basis of the following characters: stem indumentum, leaf upper surface, leaf stipules, corolla color, number of whorls in the inflorescences, and pod morphology (surface hairiness, position and beak).

Ten dry mature seeds of *L. gredensis* belonging to four different specimens from Mt. Etna, ten seeds of *L. luteus* belonging to five different specimens from Stromboli (Aeolian Islands) and two seeds obtained from the herbarium specimen CAT005493, were photographed using a scanning electron microscope (SEM) at an accelerating voltage of 10 kV. Specifically, Phenom XL G2 Desktop SEM was used. Seeds were directly mounted on to aluminium stubs with double adhesive tape and then sputter coated with gold. The images obtained where then analyzed using the software ImageJ, version 1.54 (Abramoff et al., 2004) to measure the perimeter and the area of 20 randomly selected palisade cells. The results were statistically compared with Student's t-test, using software R, version 4.2.2 (R Core Team, 2022). No cells were measured from the two seeds sampled from the specimen CAT005493.

Phytosociological survey

Vegetation relevés were sampled in the patches with high cover of *L. gredensis*, according to Braun-Blanquet's methodology (Braun-Blanquet 1928). The phytosociological nomenclature mainly refers to Mucina et al. (2016) and the bioclimatological classification follows Bazan et al. (2015).

Specimina visa

Lupinus gredensis

Italy, Sicily. Etna, s.d., *Todaro s.n.* (PAL144! sub *Lupinus luteus*); *ibid.* (PAL145!); Etna, Tarderia, 9 May 1829, *Tineo s.n.* (PAL146! sub *Lupinus luteus*); Zafferana, May 1888, *Tornabene s.n.* (CAT005482! sub *Lupinus thermis*); *ibid.* (CAT005483!); *ibid.* (CAT005484!); *ibid.* (CAT005485!); *ibid.* (CAT005486!); *ibid.* (CAT005487 Milo, April 1894, *P. Baccarini s.n.* (CAT005498! sub *Lupinus luteus*); Etna, Aironi, July 1894, *P. Baccarini s.n.* (CAT005496! sub *Lupinus luteus*); Etna, Valle di Calanna, 11 June 1903, *Cavara s.n.* (CAT005493! sub *Lupinus luteus*);, Valle di Calanna, Acqua Grande, 11 June 1903, *Cavara s.n.* (CAT005495! sub *Lupinus luteus*); Etna, Valle Calanna, 12 June 1903, *Cavara s.n.* (CAT005492! sub *Lupinus luteus*); Etna, Randazzo, 10 May 1913, *S. Tricomi s.n.* (CAT005495! sub *Lupinus luteus*); Etna, Pedemontana, 21 May 1989, *A. Cristaudo s.n.* (CAT112411! sub *Lupinus luteus*); Contrada Faro, Tarderia (Pedara), 37°39'20.3"N, 15°02'14.8"E, 990 m, 4 May 2024, *A. Cristaudo & S. Cambria s.n.* (CAT!); Contrada Monte Gorna-Grottafumata, Trecastagni (Etna), 37°39'04.7"N, 15°04'26.4"E, 750 m, 4 May 2024, *A. Cristaudo & S. Cambria s.n.* (CAT!); Nei pressi della grotta del Gatto, Zafferana Etnea (Etna), 37°40'53.67"N, 15°05'5.92"E, 930 m, 4 May 2024, *A. Cristaudo & S. Cambria s.n.* (CAT!); Monte Gervati, Nicolosi (Etna), 37°38'34"N, 15°01'18.6"E, 950 m, 4 May 2024, *A. Cristaudo & S. Cambria s.n.* (CAT!); Monte Serra Pizzuta, Nicolosi (Etna), 37°38'34.46"N, 15°1'11.34"E, 37°38'33.04"N, 15°1'8.32"E, 940 m, 4 May 2024, *A. Cristaudo & S. Cambria s.n.* (CAT!).

Lupinus luteus

Italy: Sicilia, s.d., s.c. (WAG0253257!); Italia, Calabria, Reggio, s.d., s.c. (PAL!); Italia, Sicilia, Isole Eolie, Costa del Cappero – Lipari (ME), 13 April 1877, *Lojacono Pojero s.n.* (PAL!); Sicilia. Messana (Messina), in aridis collium prope Gravitelli, alt. 200 m., suolo siliceo, April 1906, *G. Zodda s.n.* (PI044744!); Italia, Sicilia, Isole Eolie, Vulcanello (ME), 8 May 1995, *A. Carratello s.n.* (PAL!); Italia, Sicilia, Madonna di Trapani, Messina, 200 m, 16 April 2008, *R. Galesi s.n.* (CAT!); Italien, Calabria: Aspromonte Provincia Reggio di Calabria, Comune San Lorenzo; etwa 14 km N Marina di San Lorenzo und 3 km NE Bagaladi; an einem südlichen Hang vom Monte Perpoli, um den Weiler San Antonio; 38°02'24.3"N, 15°51'05.1"E ±100 m, 920 m, 3 May 2014, *K. Zernig 8773* (GJO!); Stromboli, (Aeolian Island), 256 m, 16 June 2014, *A. Cristaudo & S. Catara s.n.* (CAT!).

Lupinus hispanicus

Spain: Alájar, Sª de Aracena, subida a la ermita Virgen de los Angeles, 9 April 2004, *M. Martinez Azorin s.n.* (ABH50296!); Spain, Andalucía, prov. de Córdoba, road Adamuz to Obejo c. 11 km NW of Adamuz, bridge over río Varas, along river, 38°04'53"N, 04°37'58"W, 225 m, 23 April 2016, *P. Escobar-Garcia & E. Vitek s.n.* (W00894!); España, [Castilla y León,] Ávila, Navatalgordo, 40°24'30.69"N, 4°52'46.79"W, 22 May 2016, *P. Escobar-Garcia s.n.* (W03181!).

Results

Morphological and taxonomical considerations

Lupinus gredensis, together with *L. luteus* and *L. hispanicus*, forms a well-supported taxonomic complex from a morphological and cytological point of view within the genus *Lupinus* L. (Castroviejo and Pascual 1998, 2000; Naganowska and Ladoň 2000). According to Euro+Med (2024), these three species are widespread in the Iberian Peninsula, while their presence in other Mediterranean territories may be considered as the result of an introduction although, according to numerous other sources (Pignatti 2017; POWO 2024), at least *L. luteus* can be considered native to the Italian Peninsula, Sardinia and Sicily.

The morphological study of the five populations found in Sicily has allowed a certain attribution of the material investigated to *L. gredensis* (Fig. 1). This taxon is easily distinguishable from all the other species known for Italy by the color of the corolla. The only native species that shows some affinity with it is *L. luteus* L. that, however, always has flowers with an intense yellow corolla and densely hairy leaves on both blades. *Lupinus gredensis* clearly differs from *L. luteus* for other morphological characters, such as: leaves glabrous on the upper blade or with hairs only on the margin; inflorescence generally with less than 11 whorls; flower peduncles longer than 2 mm; ripe pod yellowish-brown (vs dark brown), almost erect before maturation, with 5–7 (vs 4–5) seeds, often adorned with a colored arch towards the hilum. Finally, the leaf stipules, in *L. luteus*, are dimorphic: linear in the basal leaves and foliaceous in the upper ones. The flowers of *L. hispanicus*, are lilac to violet throughout flowering; it has appressed-hairy stem, shorter inflorescence with 3–7 whorls and tuberculated seeds (Boissier and Reuter 1842; Castroviejo and Pascual 1998, 2000). The main diacritical characters of these three species are listed in Table 1 and illustrated in Fig. 2.

Here we give provide a description of the Sicilian plants, which agrees with Castroviejo and Pascual (2000):

Annual herb 15–80 cm tall, from villous-hairy to hairy. Stem generally branched from the base, with prostrate-ascending stems. Leaves with petiole 9.7–12.1 cm long, divided into 5–9 leaflets of 20–72.2 × 6.9–18.3 mm, mucronulate in the lower leaves, oblanceolate in the upper ones, glabrous or slightly hairy at the margin in the upper blade, appressed hairy in the lower one; stipules linear, 9.5–15.7 mm long with membra-

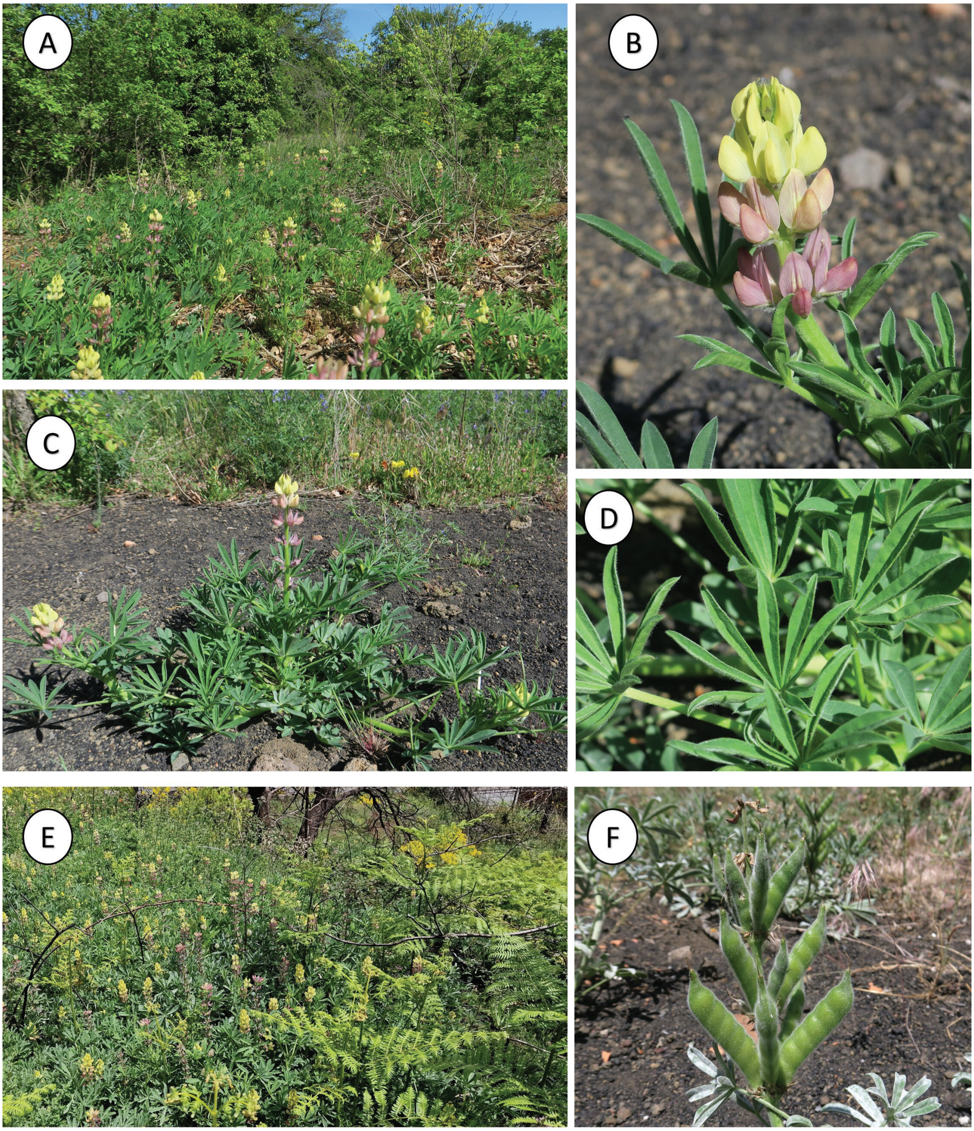

Figure 1. *Lupinus gredensis* in Sicily **A** habitat in wood clearings **B** detail of inflorescence **C** habitus **D** leaf blade **E** *L. gredensis* in the undergrowth of vegetation with *Pteridium aquilinum* **F** immature pods. In **F** the whitish colour of the leaves is due to a fungal infection.

nous margin. Inflorescence 7.5–26.1 cm long, constituted by 5–10 whorls of 5 flowers each; pedicels 1.8–2.7 mm long; bracts ovate-oblong, sharp, deciduous; bracteoles linear. Calyx bilabiate, with appressed to sub-patent sericeous hairs; upper lip fissured almost to the base, 5.9–7.3 mm long; lower lip tridentate, 7.3–9.3 mm long. Corolla pale yellow in the upper 3–4 whorls, pink or lilac in the central and lower whorls. Standard 14.2–18.4 × 8–11.1 mm; wings 15.5–17 × 7.3–9 mm; keel 12.6–14.7 × 3.8–4.8 mm. Ferruginous-brown, patent-hairy legume, up to 65 mm long and up to 11 mm wide,

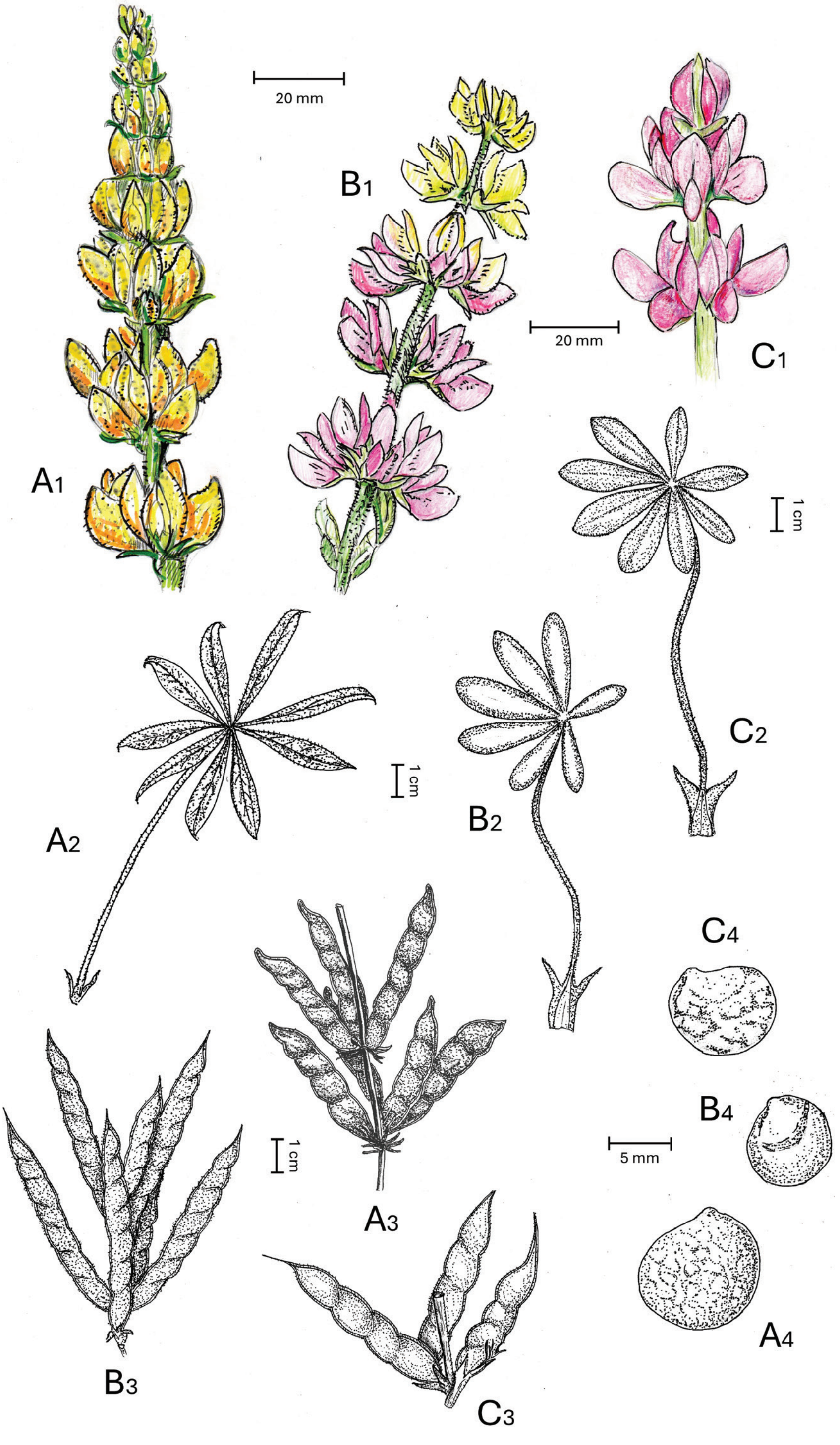

Figure 2. Illustration of *Lupinus gredensis* and of the related species *L. luteus* and *L. hispanicus*. *L. gredensis* (**A**), *L. luteus* (**B**) and *L. hispanicus* (**C**). 1. Inflorescence. 2. Leaves. 3. Pod. 4. Seed. Drawings by Rosaria Di Cicca.

Table 1. Main morphological diacritical characters of *Lupinus gredensis*, *L. hispanicus* and *L. luteus*.

Character	*L. gredensis*	*L. hispanicus*	*L. luteus*
Stem indumentum	sparsely hairy	appressed-hairy	sericeous
Leaves upper blade	glabrous with hairs on the margin	glabrous or sparsely hairy	hairy
Leaf stipules	linear	linear	linear or foliaceous
Corolla color	cream, lilac to mauve in the lower whorls	lilac to violet	yellow
Number of whorls	5–10	3–7	6–12
Pod surface	sericeous, yellowish-brown	sericeous, yellowish-brown	densely hairy, dark brown
Pod shape	almost erect before maturation	slightly divaricate	horizontal or divaricate
Pod beak	erect-patent	erecto-patent	curved
Seeds per pod	5–7	(3)4–5	4–5
Seed surface	smooth with a colored arch near the hilum	tubercolated	smooth with a darker layer above a white bottom

almost erect before maturation, ending with a sharp, erect-patent beak of 8–10 mm, with 5–7 seeds. Seeds 4.6–6.2 mm in diameter, lenticular to globose-lenticular, generally white with a colored arch near the hilum and a smooth head.

According to various authors (Heyn and Herrnstadt 1977; Aïnouche and Bayer 2000; Aïnouche et al. 2004; Marzouk 2006), the microstructure of the seed coat and the unicellular pattern of microsculptures represent significant taxonomical characters in the genus *Lupinus*, which allow distinguishing the different taxa. The micromorphological investigation of seed coat (Fig. 3) of *L. gredensis* revealed a smooth surface with very cohesive and prominent isodiametric palisade cells (macrosclereids). The outer periclinal wall of these cells is characterized by irregular microsculptures with curved tips, while the anticlinal walls look from slightly curved to straight. As concerns *L. luteus*, previously examined by Mahé (2011), the cells are significantly ($p < 0.005$) greater (area = 143.13 μm^2 ± 6.29 SE; perimeter = 47.43 μm ± 1.06 SE) and with a surface more prominently sculptured if compared to *L. gredensis* (area = 32.00 μm^2 ± 1.72 SE; perimeter = 22.13 μm ± 0.69 SE). Besides, *L. luteus* shows a different micromorphological pattern, as well as less marked and quite obscure anticlinal walls. Although it was not possible to take precise measurements on the seeds sampled from the herbarium specimen CAT005493, the microstructure patterns of the periclinal walls and the cell size are more similar to those of *L. gredensis* rather than *L. luteus*. However, both *L. luteus* and *L. gredensis* fully belong to the group of smooth seeded lupins for the cohesive and isodiametric external palisade cells.

Analitycal key to species of the genus *Lupinus* in Italy

An analytical key for recognizing the various taxa belonging to the *Lupinus* genus in Italy is shown below.

1 Corolla yellow or yellowish; flowers clearly arranged in regular whorls........**2**
– Corolla white or blue; flowers arranged in raceme or irregular whorls.........**3**

2 Flowers always with bright yellow corolla; leaves hairy on both blades; pod always horizontal or divaricate .... ***L. luteus***

– Flowers pale yellow at the beginning, progressively turning pink or mauve with ripening; leaves hairy only on the lower blade; pod almost erect before maturation........ ***L. gredensis***

3 Flowers clustered in false whorls or irregular whorls; leaves hairy on both blades........ **4**

– Flowers scattered along the axis of the inflorescence; leaves hairy only in the lower blade **5**

4 Lower flowers not whorled, corolla 10–14 mm long; calyx with trifid lower lip; seeds smooth........ ***L. gussoneanus***

– Lower flowers whorled, corolla 15–17 mm long; calyx with bifid lower lip; seeds rough .... ***L. cosentinii***

5 Upper lip of calyx subentire or with slightly marked teeth; leaves with wide oblong-obovate to oblanceolate leaflets **6**

– Upper lip of calyx deeply bifid; leaves with narrow and linear leaflets........ ***L. angustifolius***

6 Perennial plant; leaves with 11–15 leaflets of (4)7–15 × 1.5–3 cm .... ***L. polyphyllus***

– Annual plant; leaves with 5–9 leaflets of 2–8 × 0.5–2 cm........7

7 Flowers with white corolla .... ***L. albus* subsp. *albus***

– Flowers with blue corolla .... ***L. albus* subsp. *graecus***

Distribution and ecology of *L. gredensis* in Sicily

Field investigations allowed to ascertain the occurrence of at least five populations, all located on the south-eastern side of Mt. Etna and precisely in the territories of Trecastagni, Nicolosi, Pedara, and Zafferana Etnea. However, based on the analysis of photographic material published online (www.facebook.com/groups/floraspontanea-siciliana/) or stored in private photographic archives (R. Galesi and G. Siracusa pers. comm.) and kindly made available, it can be assumed that this species is also present in other areas, often in localities adjacent to those known to us (Fig. 4). Further data on the distribution of this species were provided by the review of herbarium specimens labeled as *L. luteus* which, as far as the Mt. Etna area is concerned, are often referable to *L. gredensis*. The locations reported in the oldest specimens, although often not very precise, are largely comparable with those detected by field investigations. The only locality quite distant from the other populations is located in the surroundings of Randazzo (northern side of Etna), as recorded by a specimen collected by S. Tricomi in 1913 (CAT005495) and another in 1989 (CAT112411).

In Sicily, *L. gredensis* grows only on volcanic sands at altitudes between 700 and 1,100 m a.s.l., colonizing clearings of deciduous or holm oak forests, roadsides and uncultivated lands. As highlighted by Milla et al. (2011), *L. gredensis* shows lower fitness and a competitive disadvantage compared to *L. angustifolius*, widespread in the same environments, being much less abundant than the latter. *Lupinus gredensis* is able

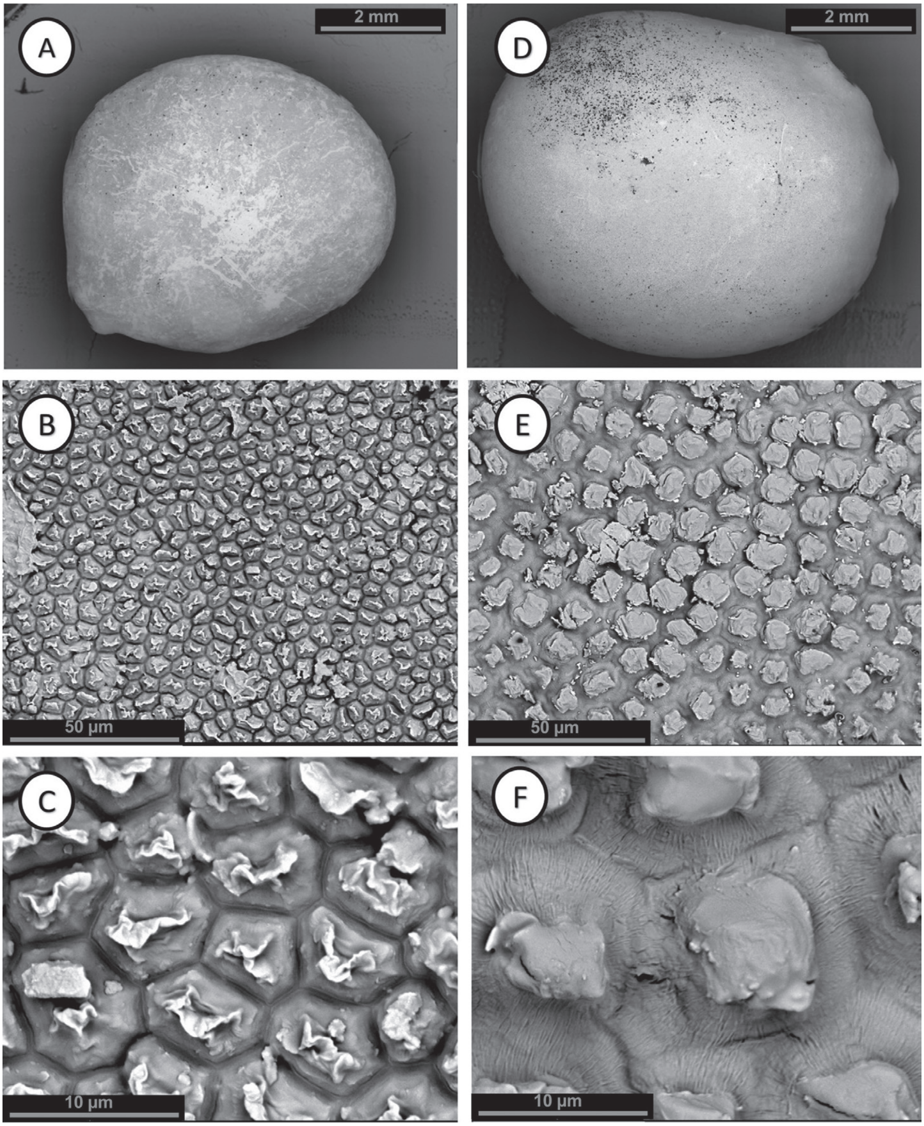

Figure 3. SEM micrographs of seed. *Lupinus gredensis*: **A** whole seed view (× 37) **B** seed coat view (× 2900) **C** detail of seed coat (× 14000). *Lupinus luteus*: **D** whole seed view (× 37) **E** seed coat view (× 2900) **F** detail of seed coat (× 14000).

to form denser populations only in the presence of substrates characterized by highly incoherent and poorly developed soils with a very fine texture, where competition with *L. angustifolius* and other ruderal species is less marked. Overall, the observed ecological preferences coincide with those reported by Castroviejo and Pascual (2000) and by Costa et al. (2012) in the Iberian Peninsula. In fact, according to these authors, *L. gredensis* colonizes abandoned crops, grasslands and degraded scrubs in siliceous or de-

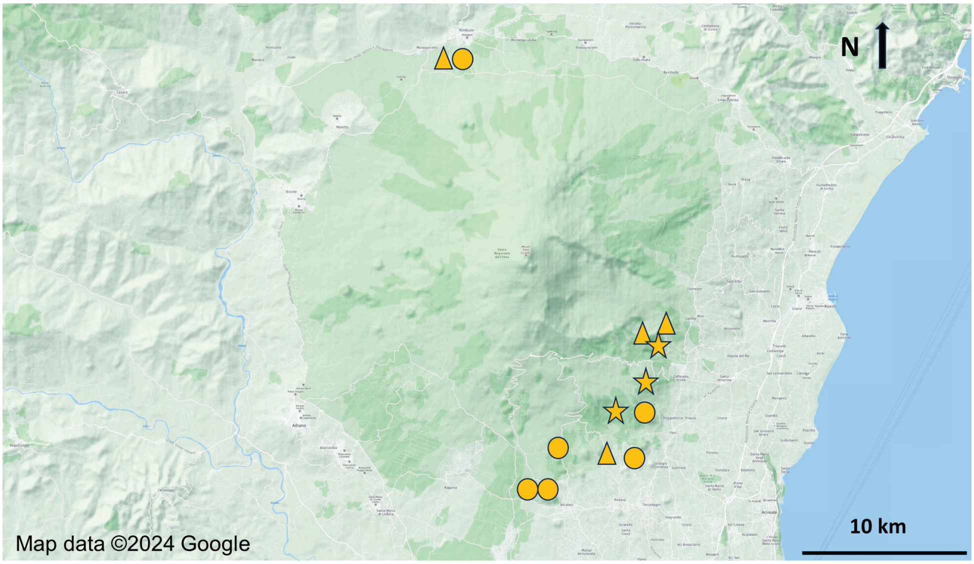

Figure 4. Distribution of *Lupinus gredensis* on Mt. Etna (Sicily). Data obtained from field investigations (circles); herbarium specimens (triangles); photographic documentations (stars).

carbonated soils up to 1,500 m a.s.l. As regards the Bulgarian populations, they occur mainly on ruderal habitats on siliceous substrates up to 700 m, along roadsides and uncultivated lands (Stoyanov and Apostolova-Stoyonova 2022).

Distribution and ecology of *L. luteus* in Sicily

According to field and herbarium investigations, the occurrence of *L. luteus* in Sicily is confirmed in numerous other areas of the island, such as on the Peloritani and Nebrodi mountains, western Sicily (Ficuzza and Alcamo), the Aeolian islands, and Pantelleria (Giardina et al. 2007). On Mt. Etna, its occurrence can be confirmed only in the basal thermo-Mediterranean belt of the eastern side between 100 and 350 m, as resulting from our observations and from the literature (Musmeci 2024). From the ecological point of view, this species is linked to dry meadows on siliceous soils (mainly quartzarenites, metamorphites and vulcanites) from sea level to 600 m a.s.l.

Phytosociological remarks

This species has been found in various plant communities, although it is mainly linked to aspects of ruderal vegetation attributable to the class Chenopodietea Br.-Bl. in Br.-Bl. et al. 1952. In particular, *L. gredensis* sometimes colonizes the clearings and edges of mesophilous woods belonging to the class *Quercetea ilicis* Br.-Bl. ex Molinier 1934 or is represented in sub-nitrophilous communities characterized by *Pteridium aquilinum* (L.) Kuhn, linked to stations with deep and fertile soils (Brullo and Marcenò

1985). However, the most characteristic populations of *L. gredensis* can be referred to a ruderal community linked to siliceous sandy soils (Table 2), belonging to the order *Brometalia rubenti-tectorum* (Rivas Goday & Rivas-Mart., 1973) Rivas-Mart. & Izco, 1977. Due to its ecological and floristic peculiarities, we consider appropriate to describe a new association, namely *Linario aetnensis-Lupinetum gredensis* Cristaudo & Cambria, ass. nov. (holotypus: Table 2, rel. 2, hoc loco). This association, fairly poor from a floristic point of view, is clearly dominated by *L. gredensis* accompanied by few other species with low coverage, such as *Linaria multicaulis* (L.) Mill. subsp. *aetnensis* Giardina & Zizza, *Lupinus angustifolius*, *Anisantha tectorum* (L.) Nevski, *Isatis tinctoria* L. subsp. *canescens* (DC.) Arcang. Overall, this community shows a pioneer character in the colonization of coarse sandy soils and is found mainly in uncultivated and large woodland clearings. From a bioclimatic point of view, this vegetation belongs to the meso-Mediterranean belt with a humid ombrotype.

Discussion and conclusions

Based on herbarium investigations, the occurrence of *L. gredensis* in Sicily and Italy, although reported here for the first time, cannot be considered as the result of a recent introduction. In fact, our investigations reveal that it had previously been confused by numerous authors with *L. luteus*, a rather sporadic taxon on the island, found mainly in NE Sicily and on some small islands, but which seems to be missing from the meso-Mediterranean belt of Mt. Etna. In fact, all the examined samples of *L. luteus* coming from altitudes above 500–600 m in the Mt. Etna area, must be attributed to *L. gredensis* based on the morphology of their leaves, inflorescences, and pods. The SEM observations of the seed coat coming from the herbarium specimen CAT005493, collected by Cavara (1904) and attributed to *L. luteus*, provides further support for its correct attribution to *L. gredensis*.

Furthermore, all the historical localities of the examined Etnean specimens, tentatively referred to *L. luteus*, coincide with those in which *L. gredensis* was found in the present study. However, *L. luteus* was not found on Mt. Etna during field surveys, except for a few localities at very low altitudes according to its thermophilous requirements. The first mention of *L. luteus* on Mt. Etna dates to Rafinesque-Schmaltz (1815), subsequently also reported by Strobl (1880), who however did not collect any samples. On the contrary, Tornabene (1889–1892) does not report this taxon for Mt. Etna, citing instead *Lupinus termis* Forssk. Later, Cavara (1904) claims that the samples labeled as *L. termis*, from the Tornabene herbarium (CAT) and collected in Zafferana Etnea (Fig. 5), should be referred instead to *L. luteus*, while in our opinion they should be attributed to *L. gredensis*. The presence of *L. luteus* on Mt. Etna is also reported generically by Lojacono (1891) and, more recently, by Sciandrello et al. (2020). Further information was provided by Cavara (1904) who considered *L. luteus* to be an infrequent but fairly widespread species on the eastern side of the volcano, even in natural environments (such as Valle Calanna, at an altitude of 900–1000 m), denying the possibility of its introduction by man.

Table 2. Phytosociological relevés of the plant community with *Lupinus gredensis* on Etna. Relevés 1 and 2 were made in Monte Gorna-Grottafumata, Trecastagni (CT, Italy), on 4th May 2024; relevés 3 and 4 were made in Monte Gervati, Nicolosi (CT, Italy) on 4th May 2024.

***Linario aetnensis-Lupinetum gredensis* ass. nov.**				
Releves	1	2	3	4
Surface (mq)	90	90	90	90
Altitude	750	750	950	950
Char. Ass.				
Lupinus gredensis	4	4	3	4
Linaria multicaulis subsp. *aetnensis*	1	+	1	1
Char. *Echio Plantaginei-Galactition tomentosae*				
Isatis tinctoria subsp. *canescens*	+	+	+	+
Lotus ornithopodioides	1	.	1	1
Hypochaeris achyrophorus	+	.	.	.
Medicago murex	.	+	.	.
Char. *Brometalia rubenti-tectorum* and Chenopodietea				
Lupinus angustifolius	2	1	+	+
Vicia pseudocracca	+	+	1	+
Anisantha tectorum	1	1	.	+
Anisantha diandra	+	+	.	+
Avena barbata	+	+	.	+
Vicia villosa	+	.	.	1
Erodium cicutarium	+	+	.	.
Lathyrus sphaericus	+	+	.	.
Fumaria officinalis	+	.	.	.
Brassica fruticulosa	.	.	+	.
Geranium molle	+	.	.	.
Other species				
Ornithopus compressus	1	1	+	+
Hypochoeris radicata	+	+	+	+
Festuca myuros	+	+	+	.
Anchusella cretica	+	+	.	+
Oenothera odorata	1	+	.	.
Scleranthus annuus	+	+	.	.
Thapsia garganica	.	.	1	1
Scrophularia canina	.	.	2	1
Silene conica	+	+	.	.
Rumex multifidus	.	.	+	+
Trifolium incarnatum	+	+	.	.
Centaurea giardinae	.	.	+	+
Aira cupaniana	.	.	+	+
Petrorhagia dubia	.	.	.	+
Rumex bucephalophorus	.	+	.	.
Dactylis glomerata	.	+	.	.
Jacobaea ambigua subsp. *ambigua*	.	.	.	+
Trifolium stellatum	+	.	.	.

The oldest herbarium specimen we have traced dates back to 1825 (PAL146) and was probably collected from Tineo in the Tarderia woodland, a location where *L. gredensis* can currently be found. Therefore, considering the historical data on the presence of *L. gredensis* on Mt. Etna, which clearly deny its recent introduction in

Figure 5. Specimen of *Lupinus gredensis* from the Tornabene herbarium (CAT), dated May 1888 and collected in Zafferana Etnea (Catania, Italy).

Sicily, and the presence of native populations even in natural or semi-natural contexts such as clearings and woodland edges, the native status of this species cannot be completely excluded. However, it may be also the result of an ancient introduction. Given the present state of knowledge, it may be, therefore, correct to define *L. gredensis* as a cryptogenic species for the Sicilian flora. Besides, the presence of this species only above 500 m of altitude highlights its markedly mesophilous character, as emphasized also by Castroviejo and Pascual (1998), who reported its occurrence in the Iberian Peninsula at altitudes up to 1,500 m. From the phytogeographical viewpoint, the presence of *L. gredensis* in Sicily should not be surprising given that, according to Raimondo et al. (2010), the western Mediterranean element represents the 9.75% of all Sicilian flora. In fact, except for *L. hispanicus*, all the native species of the genus *Lupinus* listed in Flora Iberica (Castroviejo and Pascual 2000) are present also in Sicily. If the status of *L. gredensis* as a native species in Sicily is confirmed, its distribution range should be expanded to include the Iberian Peninsula and Sicily (Fig. 6). Further field and herbarium investigations may clarify the possible occurrence of the species in other areas of Sicily and Italy, thus providing more information for defining the distribution range of this taxon. At the same time, additional analyses, including in-depth population genetic studies using SNPs or ribotyping, could disentangle the uncertainties about the native status of *L. gredensis* in Italy.

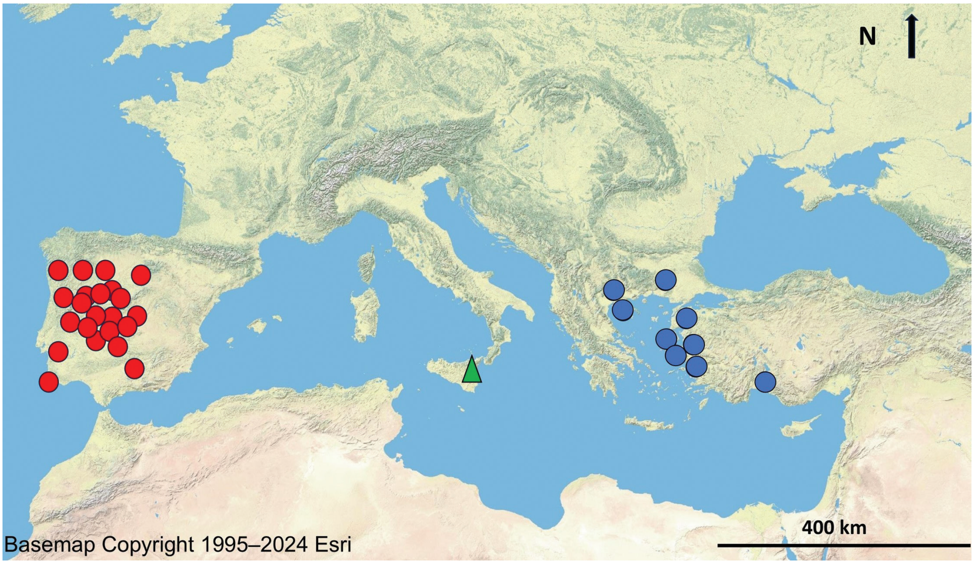

Figure 6. Distribution range of *Lupinus gredensis*. The dots indicate the verified data collected from literature, herbarium specimens and GBIF records (https//:www.gbif.org), the green triangle refers to the specimens collected by the authors on Mt. Etna (Sicily). Presently, is considered as cryptogenic in Sicily. Red dots indicate sites in which the species is considered native, blue dots sites in which the species is considered introduced.

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RESEARCH ARTICLE

Italian Botanist
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Global and Regional IUCN Red List Assessments: 17

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Abstract

In this contribution, the conservation status assessment of three vascular plants according to IUCN categories and criteria are presented. It includes the assessment of *Campanula bergomensis* F.Mangili & L.Mangili, *Iris hippolyti* (Vved.) Kamelin and *Stipa aktauensis* Roshev. 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

Campanula bergomensis F.Mangili & L.Mangili

Global assessment

Taxonomy and nomenclature

Order: Asterales *Family*: Campanulaceae

Campanula bergomensis F.Mangili & L.Mangili, Phytotaxa 637(2): 120–132 (2024)

Common name: Campanula bergamasca (It).

Geographic distribution range: *Campanula bergomensis* (Fig. 1) is a perennial species occurring only in the eastern Bergamo Prealps (sensu Marazzi 2005; Italy, Lombardy, district of Bergamo), in a few areas (Fig. 2). Most populations are located in small valleys close to the village of Clusone (BG), mainly in the Borlezza hydrogeographic basin (e.g., Val di Tede, Val di Frucc, Val dei Dadi, Val Romentareck; Valle et al. 2024). Other isolated records are reported from Valcanale, Val Nossana (Serio basin), and Val Conchetta (Dezzo basin; Valle et al. 2024).

Distribution: *Countries of occurrence*: Italy.

Biology: *Plant growth form*: perennial (hemicryptophyte).

Figure 1. *Campanula bergomensis* from Valle dei Dadi (BG). Photograph by Luca Mangili.

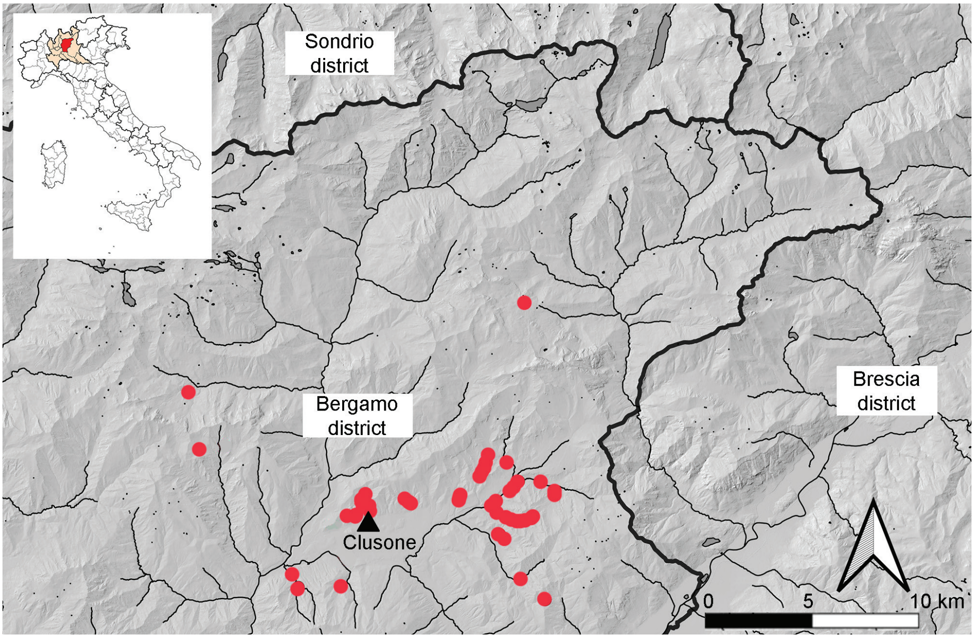

Figure 2. Distribution map of *Campanula bergomensis* in the Bergamo Prealps. In the box, position of Bergamo district with respect to the administrative region of Lombardia and to Italy.

Flowering and fruiting time: From mid-July to mid-August.

Reproduction: No information on dispersal strategy, pollination and seed germination is available. Preliminary experiments performed at Città Studi Botanical Garden (Milan) seem to indicate low germination rates (Valle et al. 2024).

Habitat and ecology: *Campanula bergomensis* grows on dolomitic debris cones, generally on poorly developed and well-drained soils, from 450 to 1.250 m a.s.l. The species is particularly associated with wide and flat debris cones, an unusual geomorphological feature in the Lombardy Prealps. *Campanula bergomensis* can be found on the edge of sparsely vegetated slopes with *Pinus mugo* Turra-dominated vegetation (with *Amelanchier ovalis* Medik., *Sesleria coerulea* (L.) Ard., *Calamagrostis varia* (Schrad.) Host, *Hieracium* sp., *Dryas octopetala* L., *Globularia cordifolia* L.). *Campanula bergomensis* shows a S-R strategy *sensu* Grime (Valle et al. 2024).

Population information: There is no detailed information available on population dynamics. Field observations indicate some stable subpopulations. Given the recent description of the species, the search for new populations is still ongoing. All observations known to date are reported in the digital atlas of Gruppo Flora Alpina Bergamasca (app.floralpinabergamasca.net), which covers the districts of Bergamo, Brescia, Sondrio, and Lecco. With the exception of one observation in Val d'Ancogno in 1999 (Luca Mangili, ex verbis), where the species has not been observed recently, the species is known with several subpopulations at all stations indicated in the digital atlas.

Threats:

6.1. Recreational activities: some populations include individuals growing on trails used by motocross and mountain bike and are currently threatened by human trampling.

7.3 Natural system modifications. (*Other ecosystem modifications*): debris cones where the species grows, when stabilized, are subject to colonization by shrub and tree species, resulting in habitat modification that leads to the disappearance of the species.

10.3 Geological events. (*Avalanches/landslides*): the debris cones where the species grows are located at the base of dolomitic cliffs, which are often unstable and subject to continuous erosion. In the event of landslides or collapse due to erosive processes, the disappearance of populations located nearby may occur.

11. Climate change & severe weather (*11.1 Habitat shifting & alteration*; *11.2 Droughts*; *11.3 Temperature extremes*): a potential threat in the future is constituted by the increasing number of drought periods and extreme meteorological events.

CRITERIA APPLIED

Criterion B: **EOO**: 100 km^2 calculated with sRedList (2023) with minimum convex polygon.
AOO: 64 km^2 calculated with a 2×2 km cell fixed grid.

a) No severely fragmented.
b) No continuous decline observed or estimated.

Red List category and Criteria (Global Assessment)

NT	**Near Threatened**	

Rationale for the assessment: *Campanula bergomensis* is an endemic species of a small portion of the Bergamo Prealps, whose populations are concentrated in the side valleys of Val Borlezza, on a few debris cones. In most of the known stations, it occurs, however, with a good number of individuals. Isolated individuals, on the other hand, have been found in Val Seriana (Val Nossana, Val Flex), and on the northern slope of Presolana (Val Conchetta). If not adequately monitored, the main populations in Val Borlezza, given the particularity of the debris cones on which they grow, can be severely damaged by the presence of bikers and motorbikers, which is very high in the area, and by erosive processes during heavy rains, which are accentuated in this phase of climatic warming. Despite the reduced AOO and the presence of some threats in some populations, continuous decline has not been observed. However, considering the peculiar ecology and the fact that the abovementioned threats, especially those related to the natural evolution of the debris cones vegetation and to recreational activities, could increase in the near future, and adopting the precautionary approach suggested by the IUCN guidelines, this species could be assessed as NT at the global level, on the understanding that if the predicted threats will actually affect populations, it would qualify as EN due to the small AOO.

Previous assessment: The taxon has never been evaluated (IUCN 2024).

Conservation actions: *Campanula bergomensis* is currently not protected by international, national or regional laws and no conservation measures for this species are applied. Some populations of *C. bergomensis* grow within a protected area of the Natura 2000 Network. In particular, the Val Nossana population is included in the Special Area of Conservation (SAC) "Val Nossana—Cima di Grem" (IT2060009); populations of Val Bielone, Romentareck, Val dei Mulini (Colle di Passeraia), and Monte Simer fall within the SAC "Val Sedornia, Valzurio, e Pizzo della Presolana" (IT2060005).

Conservation actions needed: Given the very recent description of the species, it is essential to acquire more information on its range, reproductive biology, and ecology in order to plan appropriate *in situ* and *ex situ* conservation actions. Essential, given the uniqueness of the species, is also the adjustment of the current legislation, with the inclusion of the species in the protection lists of the Lombardy Region (LR 31/2008), to prevent with greater force human activities in the area in which the species occurs.

Barbara Valle, Federico Mangili, Marco Caccianiga

Iris hippolyti (Vved.) Kamelin

Global assessment

Taxonomy and nomenclature

Order: Asparagales *Family*: Iridaceae

Iris hippolyti (Vved.) Kamelin in A.L.Takhtadzhyan (ed.), Redkie Izchez. Vidy Fl. SSSR, ed. 2: 102 (1981)

Common name: Hippolyti's Iris (En).

Geographic distribution range: *Iris hippolyti* (Fig. 3) is a rare endemic plant of Uzbekistan. It grows on gray-brown desert soils of the remnant low mountains, Kokchatau and Karatau, situated in the eastern region of the Kyzylkum Desert, one of the largest deserts in Central Asia. The geographic extent of these isolated hills covers approximately 2.5 km^2, with a maximum elevation of 447 m a.s.l. (Shomurodov 2018; Shomurodov et al. 2021; Sennikov et al. 2023). The species is distributed in the northeastern area of Bukhara region and in the eastern area of Navoi region. The site in Kokchatau is located near the village of Zafarabad, the site in Karatau is located in the Kara-Karga mountain pass area (Fig. 4).

Distribution: *Countries of occurrence*: Uzbekistan.

Biology: *Plant growth form*: perennial (geophyte).

Flowering and fruiting time: flowering in April and fruiting in May (Vvedensky 1941; Sennikov et al. 2023).

Reproduction: Literature sources do not provide enough information about reproduction.

Habitat and Ecology: *Iris hippolyti* is a perennial, early-growing, grass-like bulbous ephemeroid characterized by rosette-shaped vertical shoots. In its natural environment,

Figure 3. *Iris hippolyti* (Vved.) Kamelin from Kokchatau low mountain (Uzbekistan, Bukhara region). Photograph by Khabibullo F. Shomurodov.

vegetative growth typically starts from mid-February to early March (Shomurodov 2018; Shomurodov et al. 2021). Its habitat is characterized by sparse vegetation and rugged terrain, consisting mainly of scattered rocks and gravel with limited soil cover. Vegetation, where present, is highly adapted to the harsh, dry conditions, showcasing drought-resistant features. This type of environment is challenging for flora due to the minimal water availability and extreme temperature fluctuations between day and night, typical of desert ecosystems.

Population information: Until 2018, *I. hippolyti* was known to have only one local population in Kokchatau. This population occupies an area of approximately 2–3 hectares, forming part of a mixed herb-*Artemisia-Zygophyllum* dominated plant community. In 2015, an estimated 300 individuals were recorded, in the 2020, the population had declined to 138 individuals (Tojibaev et al. 2020). In 2022, during field research in the Karatau Mountains of eastern Kyzylkum, K. Tojibaev identified a new subpopulation of the species. The herbarium specimen collected by him from this area is stored in the TASH herbarium. This local population is confined to a very narrow range, with fewer than 40 individuals. *I. hippolyti* is not dominant in any of the phytocoenoses where it occurs.

Threats:

1.3 Residential & commercial development (*Tourism & recreation areas*): the sites where the plants are scattered correspond to the area where local people gather for hik-

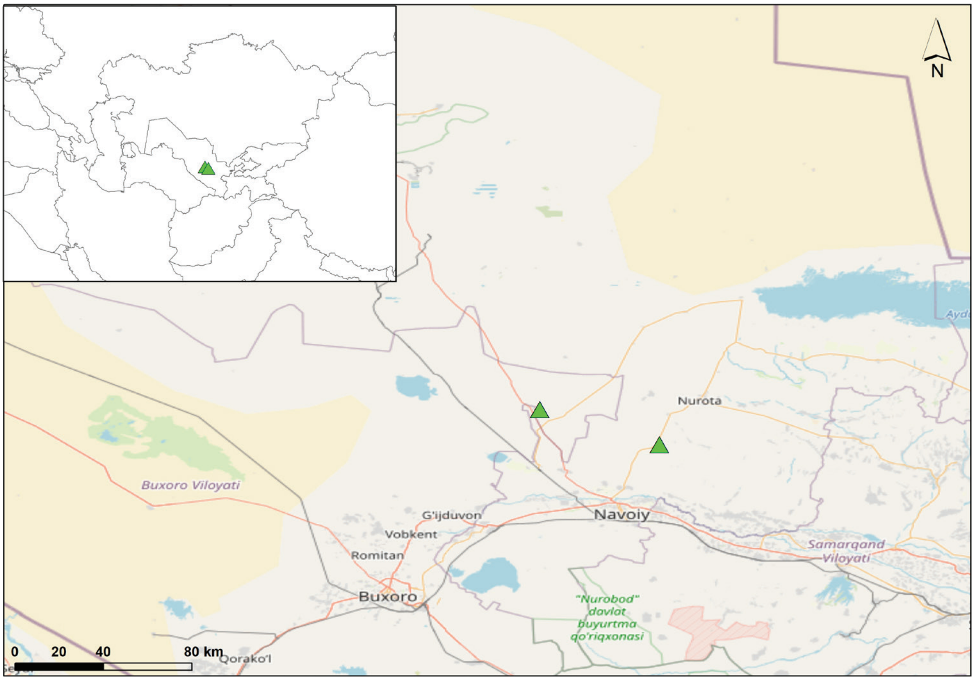

Figure 4. Geographic range and distribution map of *Iris hippolyti* in Kyzylkum remnant low mountains (Uzbekistan).

ing and outdoor activities (Shomurodov 2018; Shomurodov et al. 2021). Satellite images show that the mine workers living in the settlement of Zafarabad destroyed more than 2000 m^2 of *I. hippolyti*'s habitat for recreational activities.

2.3 Livestock farming & ranching (*2.3.1 Nomadic grazing* and *2.3.2 Small-holder grazing, ranching or farming*): Livestock farming is the main source of income for the residents of Kyzylkum. The main risk factor for almost all species from these territories listed in the National Red Book of the Republic of Uzbekistan is grazing. Persistent livestock grazing exerts one of the most harmful impacts on the population, contributing to habitat degradation and population decline (Shomurodov and Khabibullaev 2022).

11 Climate change & severe weather (*11.2 Drought*, *11.3 Temperature extremes*): *I. hippolyti* grows in the regions (Bukhara and Navoi) most affected by global climate change in Uzbekistan (Khabibullaev et al. 2022). A threat for the species is represented by climatic anomalies, namely ground freezing at the end of February. The vegetative phase of *I. hippolyti* begins relatively early, typically in late February or early March. However, approximately once every 5–6 years, the region experiences severe frost events, which result in significant mortality among the juvenile plants (Khaitov 2024).

CRITERIA APPLIED:

Criterion A: the population experienced an observed decline of 54% in the number of individuals over the last 10 years.

Criterion B: **EOO**: 8 km^2 calculated with a GeoCAT (Geospatial Conservation Assessment Tool) software (Bachman et al. 2011).
AOO: 8 km^2 calculated with GeoCAT software and based on user defined cell width (2 km) (Bachman et al. 2011).

a) Two locations based on the main threats (*Nomadic grazing* and *Tourism & recreation areas*).
b) Observed continuing decline in extent and quality of habitat (iii) and number of mature individuals (v).

Criterion D: The global population, consisting of a total of 175 individuals, is currently experiencing a continuous decline in number of mature individuals.

Red List category and Criteria (Global Assessment)

EN	**Endangered**	**B1b(iii, v)+2b(iii, v) + D**

Rationale for the assessment: *Iris hippolyti* is a species endemic to the remnant low mountains of the Kyzylkum Desert, with an extremely limited distribution, comprising only two known subpopulations. Despite supporting the largest number of individuals, the Kokchatau population has been experiencing a gradual decline. Anthropogenic pressures, both direct and indirect, continue to pose the most significant threat to the survival and stability of this species. The current status of the species' subpopulations is critical, with dangerously low numbers of individuals. The small EOO and AOO, the reduced population size and the observed decline in the number of mature individuals, according to criteria B and D, support the risk category of Endangered (EN) at a global scale for this species.

Previous assessment: The taxon is not evaluated at the global level (IUCN 2024).

Conservation actions: *Iris hippolyti*, as a rare and endemic species, has been classified as Category 1 (Critically Endangered) in the National Red Book of the Republic of Uzbekistan, indicating its status as being on the brink of extinction (Khasanov et al. 2019). The species has been introduced into cultivation at the Tashkent Botanical Garden, and the reintroduction of individuals propagated under *ex-situ* conditions is deemed essential for its conservation. Furthermore, it is imperative to implement targeted conservation actions to ensure the survival of this species in its natural habitat.

Conservation actions needed: The effects of climate change are increasingly affecting biodiversity in Uzbekistan (Korolyuk at al. 2024). The *in-situ* conservation of *I. hippolyti* requires reinforcement. Additionally, *ex situ* conservation within protected areas presents a viable strategy. To support this approach, bioclimatic modeling of the species' potential distribution and the identification of regions that offer optimal ecological conditions are essential steps for effective conservation planning.

Khabibullo F. Shomurodov, Bekhruz Sh. Khabibullaev, Giuseppe Fenu

Stipa aktauensis Roshev.

Global assessment

Taxonomy and nomenclature

Order: Poales *Family*: Poaceae

Stipa aktauensis Roshev., Izv. Bot. Sada Akad. Nauk S.S.S.R. 30: 302 (1932)

Common name: Aktau's stipa (En).

Geographic distribution range: *Stipa aktauensis* (Fig. 5) is a rare endemic of the Kyzylkum remnant mountains, from the Poaceae family (Shomurodov 2018). It grows on dry, gravelly slopes and rock cracks of the remnant low mountains – Kuldzhuktau, Auminzatau, Bukantau, Tamditau, Muruntau, Aktau, and Sultan-Uizdag of the Kyzylkum Desert. Plant distributed mainly on the southern, western, and eastern slopes of the remnant mountains in sparse and scattered populations at altitudes from 400 to 700 m a.s.l. (Rakhimova et al. 2017; Fig. 6).

Distribution: *Countries of occurrence*: Uzbekistan.

Biology: *Plant growth form*: perennial (hemicryptophyte).

Flowering and fruiting time: flowering and fruiting in April-May (Drobov 1941), fruiting sometimes lasts until June (Rakhimova et al. 2017; Shomurodov 2018).

Reproduction: Existing literature lacks sufficient information on reproduction.

Habitat and ecology: *Stipa aktauensis* is a perennial herbaceous plant with a dense tussock form, reaching a height of 25–30 cm and lives up to 30 years. The remnant mountain's terrain is often barren and dry, with coarse gravel and rock-strewn surfaces shaped by erosion over centuries. In this harsh environment, where rain is infrequent, the rocks of the mountains are home to unique microhabitats, providing shelter for endemic plants like *S. aktauensis*. This plant thrives on the shallow soils that collect in cracks and crevices of the mountain rocks, surviving the extreme temperatures and arid conditions of the desert. The surrounding landscape is typically desolate, with a mix of low shrubs, sparse grasses, and wide, open spaces.

Population information: Historical literature lacks data regarding the local populations size or the total number of individuals of the species (Rodzhevits 1934; Drobov 1941; Paziy 1968). Recent field studies have documented over 2,000 individuals of *S. aktauensis*. The population density varies across different regions, with higher concentrations in Kuldzhuktau, Tamditau, and especially Aktau, compared to Auminzatau, Bukantau, and Sultan-Uizdag. Specifically, approximately 600 individuals were recorded in Kuldzhuktau, more than 400 in Aktau, 400 in Tamditau, around 200 in Auminzatau, 150 in Muruntau, and 300 in Bukantau. Sultan-Uizdag exhibited the smallest population, with only about 80 individuals identified. In all populations, livestock grazing is causing a decline in the number of individuals. The population status of this species has been evaluated through comprehensive analysis of its communities (Rakhimova et al. 2017; Shomurodov 2018).

Figure 5. *Stipa aktauensis* Roshev from Kuldzhuktau (Uzbekistan, Navoi region). Photograph by Khabibullo F. Shomurodov.

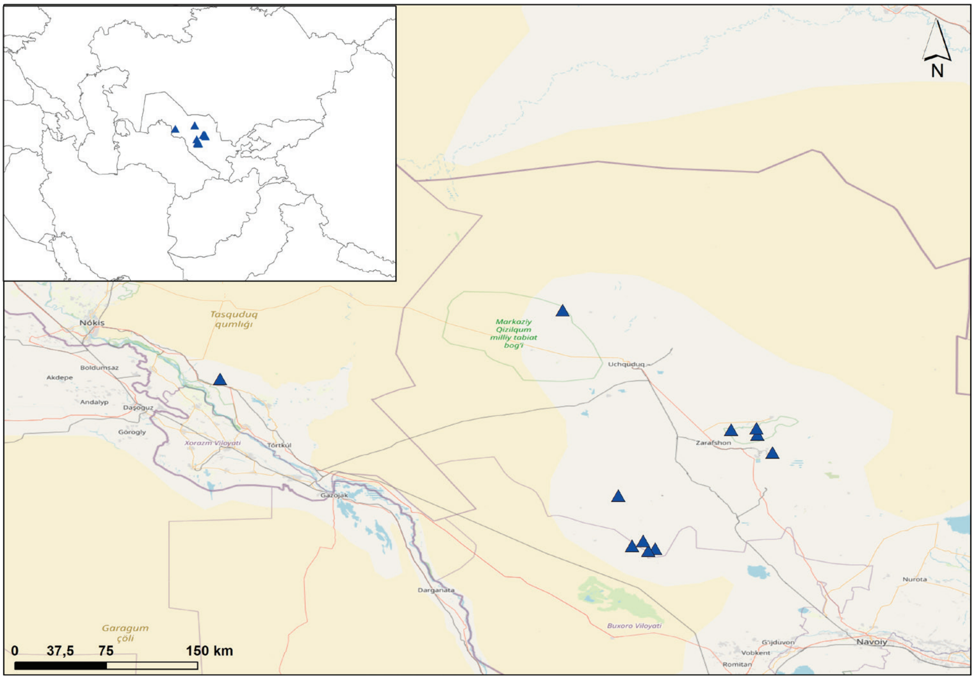

Figure 6. Geographic range and distribution map of *Stipa aktauensis* in Kyzylkum remnant low mountains (Uzbekistan).

Threats:

2.3 Livestock farming & ranching (*2.3.1 Nomadic grazing* and *2.3.2 Small-holder grazing, ranching or farming*): Constant livestock grazing in the habitat of *S. aktauensis* is a threat by causing habitat degradation, reducing population density through direct consumption, and disrupting the species' ability to regenerate. Overgrazing also leads to soil erosion and shifts in plant community composition, making it harder for *S. aktauensis* to compete and thrive.

3 Energy production & mining (*3.1 Oil & gas drilling, 3.2 Mining & quarrying, 3.3 Renewable energy*): Several mines and energy-related activities are present at sites where *S. aktauensis* grows, leading to a progressive loss of habitat quality and individuals. The most active point of geological exploration in Kyzylkum is Kuldzhuktau, where the search for light metals (including gold), and marble is especially active today. In addition, the habitat of the species is being destroyed to build wind power stations there and in Tamditau. In Sultan-Uizdag, the proliferation of stone crushing equipment and the substantial increase in gravel production are severely impacting the local population, resulting in the lowest population size in this region.

CRITERIA APPLIED:

Criterion B: **EOO**: 32,725 km^2 calculated with a GeoCAT (Geospatial Conservation Assessment Tool) software (Bachman et al. 2011).
AOO: 44 km^2 calculated with GeoCAT software and based on user defined cell width (2 km; Bachman et al. 2011).

a) Five locations based on the main threat (*Nomadic grazing*).
b) Continuing decline, observed in extent of occurrence (i), area of occupancy (ii), extent and quality of habitat (iii), number of locations or subpopulations (iv), number of mature individuals (v).

EN	**Endangered**	**B2ab(i, ii, iii, iv, v)**

Red List category and Criteria (Global Assessment)

Rationale for the assessment: *Stipa aktauensis* is an endemic species that lacks territorial protection and is restricted to the remnant low mountain ranges of Kyzylkum. The vitality of the subpopulations was assessed as critical (Shomurodov 2018). Alongside other endemic species of the remnant mountains, *S. aktauensis* is experiencing a rapid decline in populations year after year. Its restricted range to specific relict mountains further exacerbates its vulnerability. The species has an EOO of approximately 32,725 km² and an AOO of 44 km². Considering the small AOO, the continuous decline and the persistence of identified threats, which remain unmitigated, the condition of the populations is projected to deteriorate in the future. Therefore, according to criterion B, this species can be classified as Endangered (EN) at a global level.

Previous assessment: This species was not previously evaluated (IUCN 2024).

Conservation actions: *Stipa aktauensis* has been included in the National Red Book of the Republic of Uzbekistan in several categories from 1975 to the present (Khasanov et al. 2019). Attempts to cultivate it in the territory of the desert station in Kyzylkum were unsuccessful. At present, seeds of *S. aktauensis* are not conserved in any germplasm bank. It is crucial to raise international awareness of the critical status of rare species in Uzbekistan through collaborative projects or publications (e.g., Fenu et al. 2022, 2023) and to explore alternative solutions to address this pressing issue.

Conservation actions needed: Research and monitoring activities are recommended to improve understanding of the reproductive biology, ecology, threats, and population trends of the species, as well as to facilitate *ex-situ* conservation measures, including the establishment of a seed bank.

Giuseppe Fenu, Khabibullo F. Shomurodov, Bekhruz Sh. Khabibullaev

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RESEARCH ARTICLE

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Notulae to the Italian alien vascular flora: 18

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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, exclusions, and status changes from casual to naturalized or invasive for Italy or for Italian administrative regions for taxa in the genera *Acacia*, *Akebia*, *Allocasuarina*, *Aloë*, *Brachychiton*, *Convolvulus*, *Freesia*, *Gleditsia*, *Hesperocyparis*, *Kalanchoë*, *Liriope*, *Mazus*, *Melia*, *Phyla*, *Platycladus*, *Prunus*, and *Retama*. Nomenclatural and distribution updates, published elsewhere, and corrections are provided as Suppl. material 1.

Keywords

Alien species, floristic data, Italy, nomenclature

How to contribute

The text for the new records, status changes from casual to naturalized or invasive, exclusions, and confirmations 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 and typifications (only for accepted names) should be submitted electronically to Gabriele Galasso (gabriele.galasso@comune.milano.it). Each text should be within 1,000 characters (spaces included).

Floristic records

Acacia melanoxylon R.Br. (Fabaceae)

+ (INV) **ITALIA** (**SAR**): San Teodoro (Gallura Nord-Est Sardegna), Monte Nieddu (WGS84: 40.750272°N, 9.589148°E), invasiva in ambiente ripariale, 370 m, 15 July 2024, *M. Fois, A. Cuena-Lombraña* (FI, CAG). – Status change from naturalized to invasive alien for the flora of Italy; status change from casual to invasive alien for the flora of Sardegna.

Despite being recently reported as a casual alien (Galasso et al. 2024), this species has to be considered invasive in Sardegna. Several individuals, in all life stages, have been observed spreading in some natural environments, especially under humid and shady conditions, in Rio Leni (Villacidro, Sud Sardegna province), Monte Arci (Pau, Oristano province), Monte Nieddu (San Teodoro, Gallura Nord-Est Sardegna province), and Berchida (Siniscola, Nuoro province).

M. Fois, A. Cuena-Lombraña

Akebia quinata (Thunm. ex Houtt.) Decne. (Lardizabalaceae)

+ (NAT) **ITALIA** (**TAA**): Castelnuovo (Trento), scarpata incolta al margine settentrionale della strada che collega la loc. Spagolle (Castelnuovo) con Via G. Gozzer (Borgo Valsugana), a E del ponte che attraversa il Torrente Moggio (WGS84: 46.05056°N, 11.47202°E), scarpata incolta, 380 m, SSE, 14 April 2024, leg. *F. Valentini, D. Boker, L.M. Boker*, det. *F. Valentini* (FI, TR). – Status change from casual to naturalized alien for the flora of Italy (Trentino-Alto Adige).

Akebia quinata was previously reported as a casual alien in Trentino-Alto Adige by Wilhalm (2011). This new population was first seen in 2023. It consists of a large population, with individuals growing from the soil to the upper part of the trees. In 2024 numerous flowering individuals were detected.

F. Valentini, D. Boker

Allocasuarina verticillata (Lam.) L.A.S.Johnson (Casuarinaceae)

+ (NAT) **ITALIA** (**SAR**): Domus de Maria (Sud Sardegna), antica strada romana (WGS84: 38.909013°N, 8.899185°E), naturalizzata nella macchia, 81 m, 5 May 2024, *A. Lallai, M. Sarigu, L. Podda* (FI, CAG). – Status change from casual to naturalized alien for the flora of Italy (Sardegna).

This species was introduced in Sardegna in the second half of the 19th century (Gennari 1874) and has been used as an ornamental tree in urban greenery and for reforestation in degraded contexts. The area where the species was found has been subject to reforestation by the Regional Forestry Agency since the end of the 1950s to the 1970s with several non-native species, such as *Acacia saligna* (Labill.) H.L.Wendl., *A. pycnantha* Benth., and *Brachychiton populneus* (Schott & Endl.) R.Br. The population recorded here covers a surface of about 100 m^2, each with at least fifty individuals, including adults, juveniles, and seedlings, arranged in two groups of individuals. Accordingly, *Allocasuarina verticillata* is to be considered naturalized.

A. Lallai, M. Sarigu

Aloë microstigma Salm-Dyck subsp. *microstigma* (Asphodelaceae)

+ (NAT) **ITALIA** (**SIC**): Lampedusa e Linosa (Agrigento), Isola di Lampedusa, Cala Francese (WGS84: 35.496217°N, 12.626316°E), rocky uncultivated land, 10 m, 20 March 2024, *G. Domina* (FI, SAF). – Naturalized alien species new for the flora of Italy (Sicilia).

This species is native to Cape Province (South Africa), and is used in horticulture as low maintenance garden plant. The new population probably comes from maintenance pruning remains from nearby houses. An additional population of a few individuals has been observed in Favignana (Trapani province) at Cala del Passo, 30 m a.s.l. on a cliff overhanging the sea. Plant samples were identified according to Klopper et al. (2023).

G. Barone, G. Domina

Brachychiton populneus (Schott & Endl.) R.Br. (Malvaceae)

+ (NAT) **ITALIA** (**SAR**): Domus de Maria (Sud Sardegna), antica strada romana (WGS84: 38.913738°N, 8.907798°E), naturalizzata nella macchia, 30 m, 5 May 2024, *L. Podda, G. Bacchetta* (FI, CAG). – Status change from casual to naturalized alien for the flora of Italy (Sardegna).

Since the first report in 2018 (Ruggero et al. 2024), several individuals of various ages have been observed naturalized within the coastal scrub vegetation together with other non-native species (*Allocasuarina verticillata* [Lam.] L.A.S.Johnson, *Acacia saligna* [Labill.] H.L.Wendl., and *A. pycnantha* Benth.) in an area subjected to reforestation by the Regional Forestry Agency until the 1970s.

L. Podda, G. Bacchetta

Convolvulus tricolor L. subsp. *tricolor* (Convolvulaceae)

– **CAM**. – Alien species to be excluded from the flora of Campania.

The individuals reported as *Convolvulus tricolor* s.l. from the Phlegrean Fields (Napoli province) (Terracciano 1917) are to be referred to *C. tricolor* subsp. *cupanianus* (Tod.) Cavara & Grande (Bartolucci et al. 2024). The plants from the island of Capri (Cerio 1939) were described as intermediate between the same native subspecies and the autonym, non-native, subspecies. As Cerio (1939) was not able to assess if the plant was escaped or indigenous, and no specimen collected by him is available (*Herbarium I. Cerio*, pers. obs.), *C. tricolor* subsp. *tricolor* is to be excluded from the flora of Campania.

E. Del Guacchio, A. De Luca

Freesia leichtlinii Klatt subsp. *alba* (G.L.Mey.) J.C.Manning & Goldblatt (Iridaceae)

+ (NAT) **SIC**: Castellammare del Golfo (Trapani), Monte Inici, Contrada Belvedere (WGS84: 38.030211°N, 12.873714°E), side road, 190 m, 16 March 2024, *R. Rocca* (FI). – Status change from casual to naturalized alien for the flora of Sicilia.

In Sicilia, this species was previously reported as a casual alien. The report of *Freesia refracta* (Jacq.) Eckl. ex Klatt on the Island of Pantelleria (Domina and Mazzola 2008) refers to this taxon. In Castellammare del Golfo we observed an abundant population, comprising numerous individuals of various ages, many seeding, extending along the road, spanning approximately 50 m^2. Not far from the main population two smaller nuclei were observed, less than 1 km away. Similarly, another population consisting of several dozens of individuals on a road embankment on the road SS514, in southeastern Sicilia (Chiaramonte Gulfi, Ragusa province, WGS84: 37.057117°N, 14.649669°E) was observed.

R. Rocca, G. Barone

Gleditsia triacanthos L. (Fabaceae)

+ (NAT) **MOL**: Campobasso (Campobasso), poco a S di Via Campania (WGS84: 41.557252°N, 14.677393°E), lungo un sentiero, 657 m, 8 May 2024, *P. Fortini, L. Quaranta* (FI, IS). – Naturalized alien species new for the flora of Molise.

Around 10 individuals approximately 10–12 m tall were found along the path in a peripheral area of Campobasso. At least five individuals are mature and abundantly producing fruit and a notable renewal of young plants has been observed all around.

P. Fortini, L. Quaranta

Hesperocyparis arizonica (Greene) Bartel (Cupressaceae)

+ (NAT) **UMB**: Orvieto (Terni), nei pressi del Pozzo di San Patrizio (WGS84: 42.722664°N, 12.120481°E), pareti rocciose verticali che dalla città digradano verso il basso, 260 m, 11 February 2024, *G. Marengo, M. Lonati* (FI). – Status change from casual to naturalized alien for the flora of Umbria.

This species grows in vertical cliffs near the urban area of Orvieto, and probably has originated from individuals cultivated in the nearby urban parks. The population is stable, consisting of several shrubby and fruit-bearing individuals.

G. Marengo, M. Lonati

Kalanchoë ×houghtonii D.B.Ward (Crassulaceae)

+ (NAT) **SIC**: Palermo (Palermo), Monte Pellegrino, W slope (WGS84: 38.158301°N, 13.345287°E), pine grove, 70 m, 14 April 2024, *R. Rocca* (FI). – Status change from casual to naturalized alien for the flora of Sicilia.

This nothotaxon was first reported as a casual alien in Sicilia in 2017 (Di Gregorio et al. 2017). We observed an expanding population in Monte Pellegrino, reproducing both sexually and through leaf propagules. Two additional populations were recorded: one on a cliff in the harbour of Terrasini (Palermo province, WGS84: 38.15599°N, 13.08188°E), the other in Palermo along the motorway spur route connecting to Tommaso Natale/Sferracavallo (WGS84: 38.191133°N, 13.283020°E). In 2017, numerous individuals of *Kalanchoë ×houghtonii* were observed on the roofs of the historic center of Palermo (G. Galasso pers. comm).

R. Rocca, G. Barone

Liriope spicata (Thunb.) Lour. (Asparagaceae)

+ (NAT) **SAR**: Senorbì (Sud Sardegna), loc. Funtana ‘e Canna (WGS84: 39.541880°N, 9.134429°E), area ruderale, 202 m, July 2024, *I. Orrù, F. Mascia* (FI). – Naturalized alien species new for the flora of Sardegna.

Individuals of the species have been found in ruderal areas, along the edges of the railroad, and originated from dispersed propagules of plants that were cultivated over 10 years ago in the garden of a cantonment house and no longer present today.

I. Orrù, F. Mascia

Mazus pumilus (Burm.f.) Steenis (Mazaceae)

+ (NAT) **EMR**: Parma (Parma), Basilica di Santa Maria della Steccata (WGS84: 44.802658°N, 10.327379°E), tra le fessure del selciato, 53 m, 1 July 2024, *M. Adorni, A. Alessandrini* (FI). – Naturalized alien species new for the flora of Emilia-Romagna.

M. Adorni, A. Alessandrini

Melia azedarach L. (Meliaceae)

+ (NAT) **SAR**: Oristano (Oristano), strada SP56 (WGS84: 39.92388°N, 8.58032°E), incolto, substrato sabbioso, presente lungo i bordi stradali, 9 m, 29 July 2024, leg. *V. Lozano, G. Brundu*, det. *G. Brundu* (FI, *Herb. Uniss Agraria*). – Status change from casual to naturalized alien for the flora of Sardegna.

The species was observed also in: Selargius (Cagliari) (WGS84: 39.253887°N, 9.173048°E), uncultivated land, 10 m, 9 June 2024, *L. Podda*; San Vito (Sud Sardegna), road SS125 'Orientale Sarda' (WGS84: 39.520588°N, 9.595178°E), roadside, 8 m, 15 June 2024, *L. Podda*; Cagliari (Cagliari), Viale G. Marconi (WGS84: 39.243090°N, 9.152730°E), roadside, 6 m, 5 July 2024, *L. Podda*; Oristano (Oristano), road SP81, Viale Repubblica 23 (WGS84: 39.90775°N, 8.56968°E), roadside, 9 m, 29 July 2024, *V. Lozano, G. Brundu*. The observed individuals probably originated from plants cultivated as ornamentals in adjacent gardens. In recent years, we have observed several naturalized adult individuals and seedlings in ecosystems subject to anthropogenic disturbance including the southern (particularly Campidano), central eastern (Sarrabus and Ogliastra) and central western parts (Oristano) of Sardegna.

V. Lozano, L. Podda

Phyla canescens (Kunth) Greene (Verbenaceae)

+ (NAT) **LOM**: Gerenzano (Varese), Via R. Lepetit 34 (WGS84: 45.632838°N, 8.966914°E), verde curato all'interno dell'Insubrias BioPark, 227 m, 28 September 2016, *E. Meneguzzo* (FI, *Herb. E. Meneguzzo*). – Status change from casual to naturalized alien for the flora of Lombardia.

In recent years this population of *Phyla canescens* has expanded to occupy an area of approximately 200 m^2.

Enzo Meneguzzo, Nicola M.G. Ardenghi

Platycladus orientalis (L.) Franco (Cupressaceae)

+ (NAT) **TAA**: Merano (Bolzano), loc. Quarazze (WGS84: 46.684801°N, 11.149760°E), vegetazione sinantropica di recupero delle scarpate e delle aree più disturbate della vegetazione forestale, 365 m, 8 March 2024, leg. *G. Mei, E. Grande*, det. *G. Mei, A. Stinca* (FI, *Herb. G. Mei*). – Status change from casual to naturalized alien for the flora of Trentino-Alto Adige.

The discovery of a population characterized by individuals of different ages, renewal and seedlings in the municipality of Merano, highlights how the species has now become naturalized in Trentino-Alto Adige.

G. Mei, A. Stinca

Prunus amygdalus **Batsch (Rosaceae)**

+ (NAT) **SAR**: Quartu Sant'Elena (Cagliari), loc. Simbirizzi, incolti del lago (WGS84: 39.264670°N, 9.197378°E), naturalizzata negli incolti, 47 m, 25 July 2024, *G. Calvia, F. Mascia, G. Bacchetta* (FI, CAG). – Status change from casual to naturalized alien for the flora of Sardegna.

This species has been cultivated throughout the island since a long time, exhibiting little to no tendency to become wild. At most, it would often spread around the planted trees through vegetative propagation. However, in recent years we have observed adult and subadult specimens in areas far from old plantations, with regeneration occurring through seed propagation as well. This naturalization has been noted in several areas of the island, including both the southern (particularly Campidano and Marmilla) and northern (Logudoro and Mejlogu) parts.

G. Calvia, F. Mascia

Retama monosperma **(L.) Boiss. subsp.** ***monosperma*** **(Fabaceae)**

+ (NAT) **ITALIA** (**LIG**): Ventimiglia (Imperia), fraz. Latte, lungo la strada SS1 Aurelia (WGS84: 43.795199°N, 7.571592°E), margine della strada, negli interstizi del muro di contenimento, 37 m, 20 February 2024, *M. Lonati, G. Nota* (FI). – Status change from casual to naturalized alien for the flora of Italy (Liguria).

The species colonizes uncultivated fields and abandoned terraces in the area between Latte and Bordighera (Imperia province), also settling in cracks of walls and sidewalks of urban infrastructures.

M. Lonati, G. Nota

Nomenclatural and distribution updates from other literature sources

Nomenclatural, status, and distribution updates according to Negri (1943), Schroeder (1972), Roma-Marzio et al. (2016), Paton et al. (2019), Moroni and O'Leary (2020), Knapp and Naczi (2021), Alterio et al. (2023), Jesus et al. (2023), Andreone and Guazzone (2024), Antonietti and Dellavedova (2024a, 2024b), Antonietti et al. (2024a, 2024b, 2024c), Bajona et al. (2024), Bonivento et al. (2024), Borghesan et al. (2024), Brusa (2024), Chepinoga et al. (2024), Đurović et al. (2024), Elvebakk and Bjerke

(2024), Fiaschi et al. (2024), Freire et al. (2024), Missouri Botanical Garden (2024 [onwards]), Pascale (2024), Pasta and Badalamenti (2024), Picco and Deandrea (2024), POWO (2024a [onwards], 2024b [onwards], 2024c [onwards], 2024d [onwards], 2024e [onwards], 2024f [onwards], 2024g [onwards], 2024h [onwards]), 2024i [onwards]), Roma-Marzio et al. (2024), Röser and Tkach (2024), Rota and Olivero (2024), Selvaggi and Dellavedova (2024), Sukhorukov et al. (2024), Verloove et al. (2024), Pasta et al. (2025), and corrections to Galasso et al. (2024), available at the Portal to the Flora of Italy (2024), are provided in Suppl. material 1.

G. Galasso, F. Bartolucci

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Antonietti A, Dellavedova R (2024b) Nota n. 1290. *Hosta ventricosa* Stearn (Asparagaceae). In: Selvaggi A, Soldano A, Pascale M, Dellavedova R (Eds) Note floristiche piemontesi n. 1243–1314. Rivista Piemontese di Storia Naturale 45: 214–215.

Antonietti A, Ardenghi NMG, Dellavedova R (2024a) Nota n. 1308. *Vitis ×novae-angliae* Fernald (Vitaceae). In: Selvaggi A, Soldano A, Pascale M, Dellavedova R (Eds) Note floristiche piemontesi n. 1243–1314. Rivista Piemontese di Storia Naturale 45: 218.

Antonietti A, Clemente F, Dellavedova R (2024b) Nota n. 1284. *Thladiantha dubia* Bunge (Cucurbitaceae). In: Selvaggi A, Soldano A, Pascale M, Dellavedova R (Eds) Note floristiche piemontesi n. 1243–1314. Rivista Piemontese di Storia Naturale 45: 212.

Antonietti A, Dellavedova R, Selvaggi A (2024c) Nota n. 1283. *Parthenocissus inserta* (A.Kern.) Fritsch (Vitaceae). In: Selvaggi A, Soldano A, Pascale M, Dellavedova R (Eds) Note floristiche piemontesi n. 1243–1314. Rivista Piemontese di Storia Naturale 45: 211–212.

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Supplementary material 1

Supplementary data

Authors: Gabriele Galasso, Fabrizio Bartolucci

Data type: pdf

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: 15

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Abstract

The chromosome numbers for seven Italian endemic taxa of *Armeria* (Plumbaginaceae) are here presented, including three species (*Armeria aspromontana*, *A. garganica*, and *A. macropoda*) for which chromosome data are reported for the first time. Overall, this study confirms the diploid status and the chromosomal stability of *Armeria*.

Keywords

Armeria, cytotaxonomy, endemism, Plumbaginaceae

How to contribute

Texts concerning new chromosome data should be submitted electronically to Antonio Giacò (antonio.giaco@biologia.unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

Armeria aspromontana Brullo, Scelsi & Spamp. (Plumbaginaceae)

Chromosome number. 2*n* = 18 (Fig. 1).

Voucher specimen. Italy. **Calabria**. Italia, Bocca del Lupo, Aspromonte, Contrada Nardello (WGS84: 38.156663°N, 15.871213°E), 23 June 2019, *L. Bernardo* (PI066769).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Armeria aspromontana* is endemic to the Aspromonte Massif (Brullo et al. 1997). The chromosome number of this species is reported here for the first time, and is in line with that observed in all other *Armeria* taxa (Rice et al. 2015; Peruzzi and Bedini 2024).

Armeria brutia Brullo, Gangale & Uzunov (Plumbaginaceae)

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

Voucher specimen. Italy. **Calabria**. Italia, Sila, Silvana Mansio, close to Monte Scuro (WGS84: 39.334742°N, 16.339223°E), 18 June 2019, *L. Bernardo* (PI066749).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Armeria brutia* is endemic to the Sila Massif (Brullo et al. 2004). The observed chromosome count is consistent with that reported by Brullo et al. (1995, under the name *A. canescens* (Host) Ebel) for Monte Scuro.

M. Tiburtini, L. Bernardo, S. Fruzzetti, L. Peruzzi

Armeria garganica Arrigoni (Plumbaginaceae)

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

Voucher specimen. Italy. Apulia, Gargano, San Marco in Lamis, Foggia (WGS84 41.751227°N, 15.690502°E), 1 June 2022, *D. Iamonico* (PI66708).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Armeria garganica* is endemic to Puglia, southern Italy (Arrigoni 2015). The chromosome number of this species is reported here for the first time, and is in line with that observed in all other *Armeria* taxa (Rice et al. 2015; Peruzzi and Bedini 2024).

M. Tiburtini, D. Iamonico, L. Baluganti, L. Peruzzi

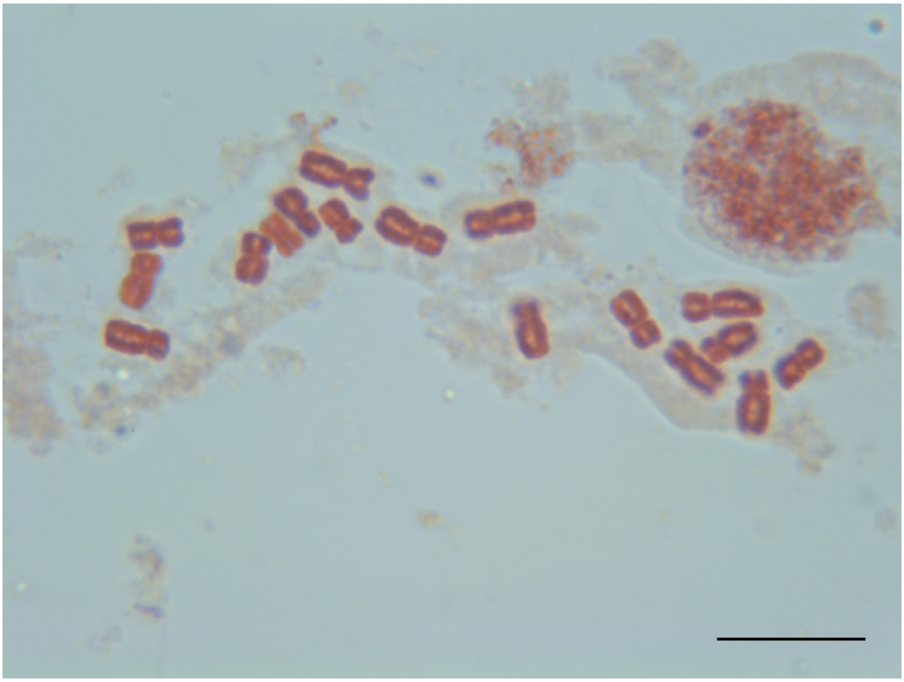

Figure 1. *Armeria aspromontana* from Contrada Nardello (Santo Stefano in Aspromonte, Reggio Calabria), 2*n* = 18. Scale bar = 10 µm.

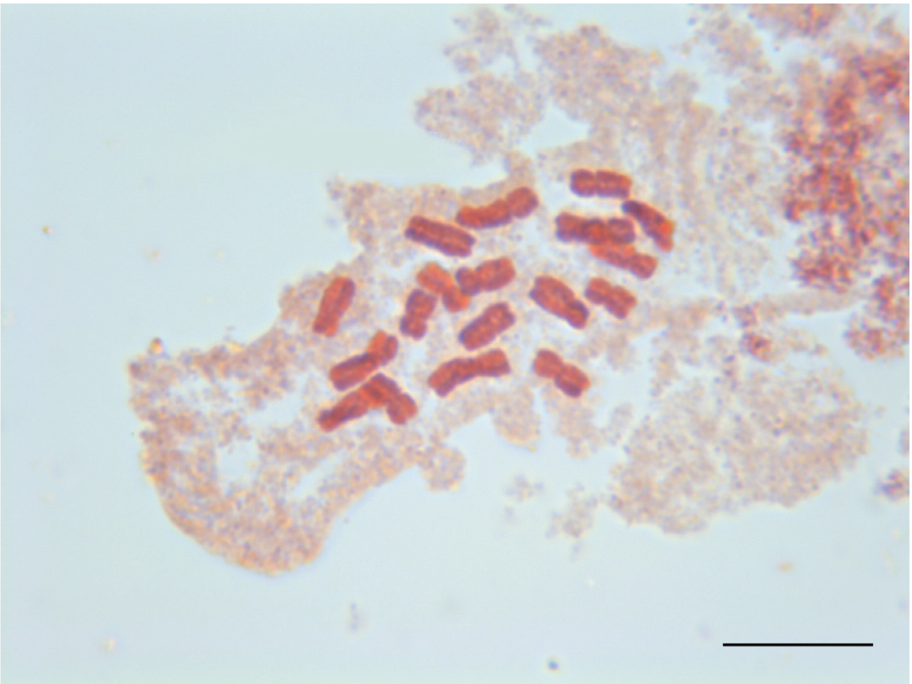

Figure 2. *Armeria brutia* from Silvana Mansio (Casali del Manco, Cosenza), 2*n* = 18. Scale bar: 10 µm.

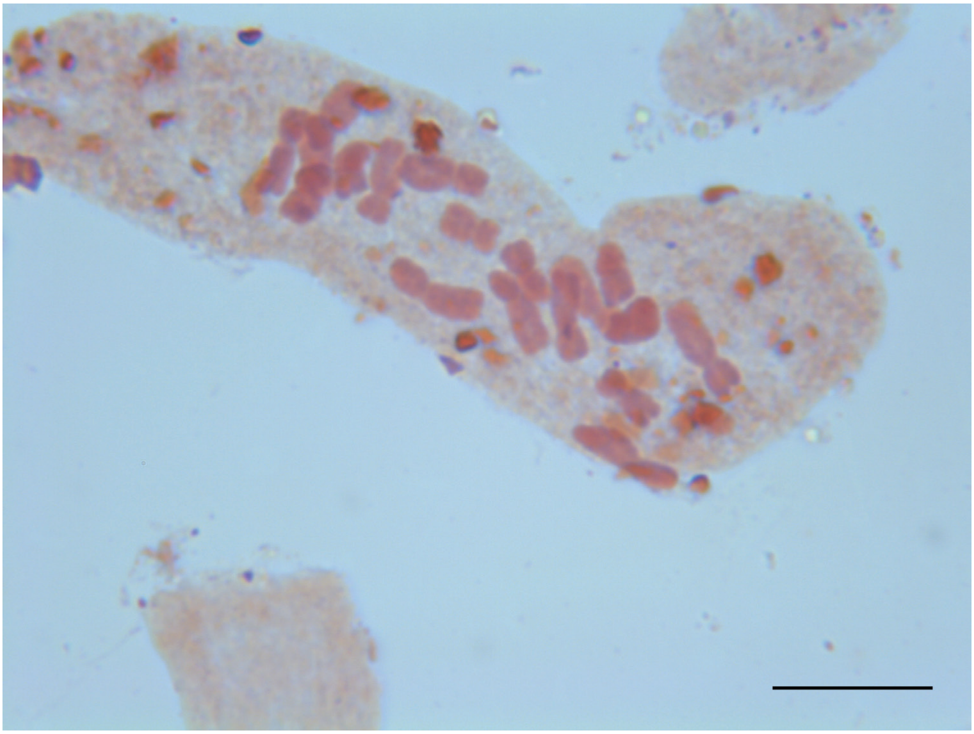

Figure 3. *Armeria garganica* from San Marco in Lamis (Gargano, Apulia), $2n = 18$. Scale bar: 10 µm.

Armeria gracilis Ten. (Plumbaginaceae)

Chromosome number. $2n = 18$ (Figs 4–9).

Voucher specimen. **Italia**. **Abruzzo**, Monte Morrone, Passo San Leonardo (WGS84 42.053020°N, 14.053311°E), 19 June 2020, *F. Bartolucci & F. Conti* (PI65462).

Voucher specimen. **Italia**. **Marche**, Monte Cucco, Sigillo (WGS84 43.359190°N, 12.748779°E), 15 June 2021, *D. Iamonico* (PI066729).

Voucher specimen. **Italy**. **Abruzzo**, Monte Corno, Vado di Corno (WGS84 42.447867°N, 13.588635°E), 26 June 2020, *F. Bartolucci & F. Conti* (PI65400).

Voucher specimen. **Italy**. **Abruzzo**, Abruzzo, Majella al Blockhaus (42.148431°N, 12.748938°E), 17 June 2020, *F. Bartolucci & F. Conti* (PI65420).

Voucher specimen. **Italy**. **Abruzzo**, Monti della Meta, loc. Campitelli, Alfedena, L'Aquila, (WGS84 41.699966°N, 13.982889°E), 24 June 2020, *F. Bartolucci & F. Conti* (PI066182).

Voucher specimen. **Italy**. **Basilicata**, Serra del Prete, Pollino Massif, (WGS84 39.911380°N, 16.132727°E), 23 June 2019, *L. Bernardo* (PI55795).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

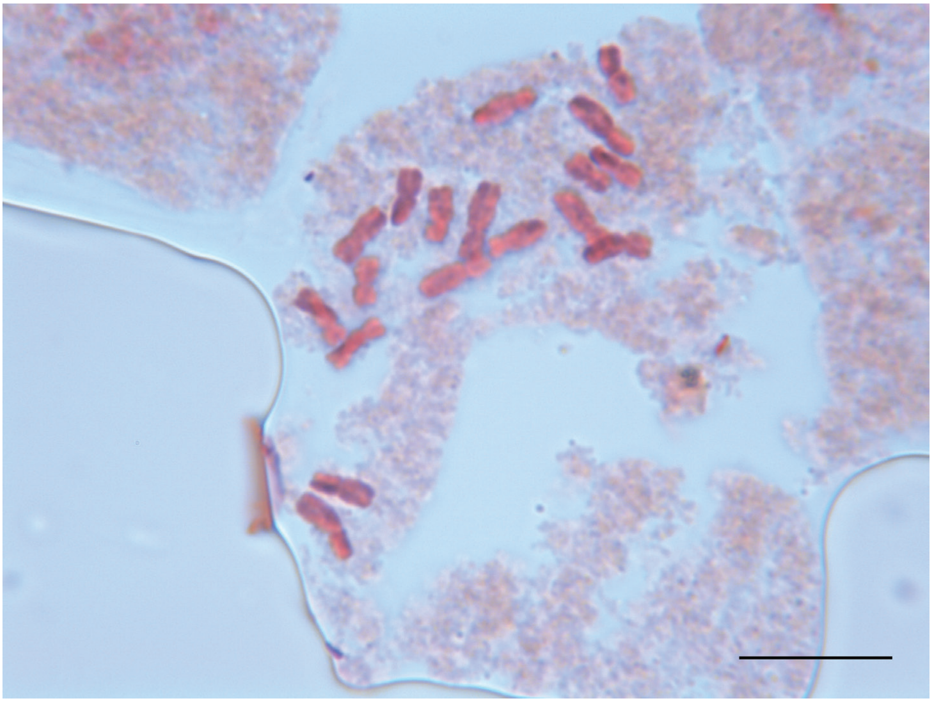

Figure 4. *Armeria gracilis* from Monte Morrone (Aquila, Abruzzo), 2*n* = 18. Scale bar: 10 µm.

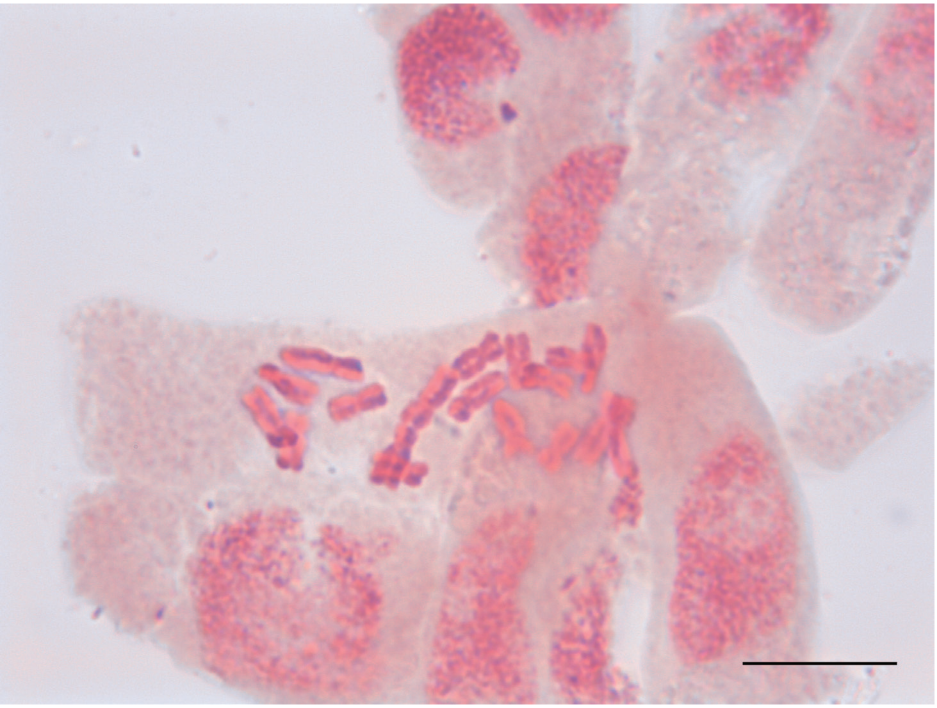

Figure 5. *Armeria gracilis* from Monte Cucco (Sigillo, Abruzzo), 2*n* = 18. Scale bar: 10 µm.

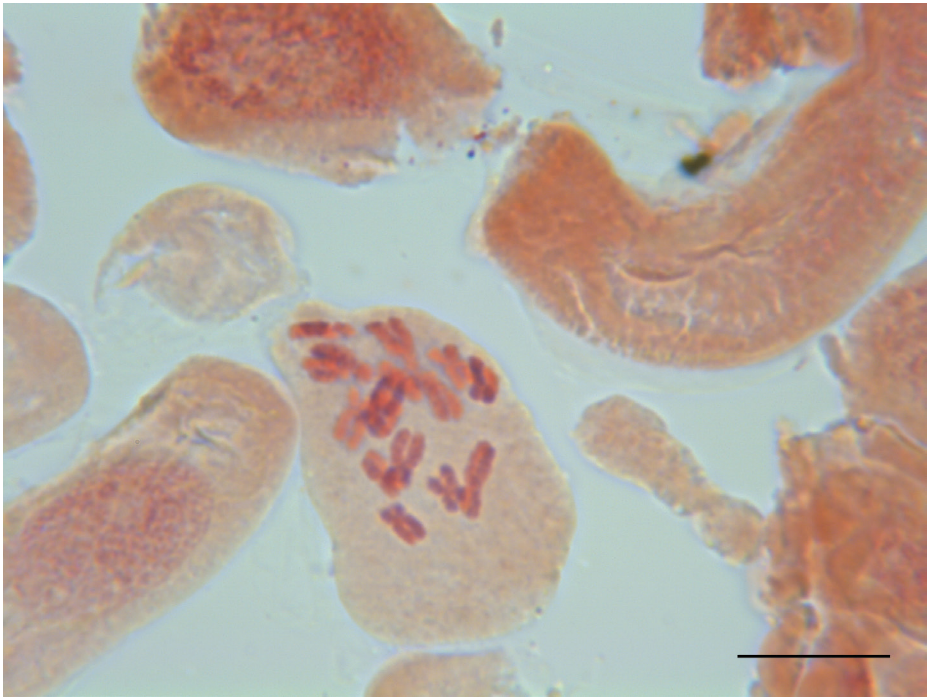

Figure 6. *Armeria gracilis* from Monte Corno (L'Aquila, Abruzzo), 2*n* = 18. Scale bar: 10 µm.

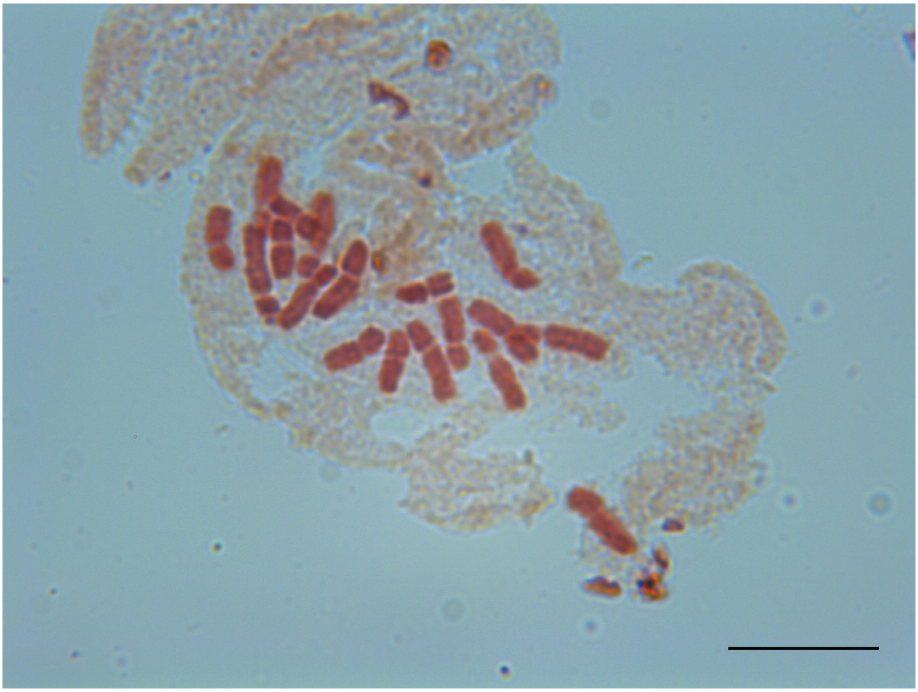

Figure 7. *Armeria gracilis* from Blockhaus mountain, Majella (Chieti, Abruzzo), 2*n* = 18. Scale bar: 10 µm.

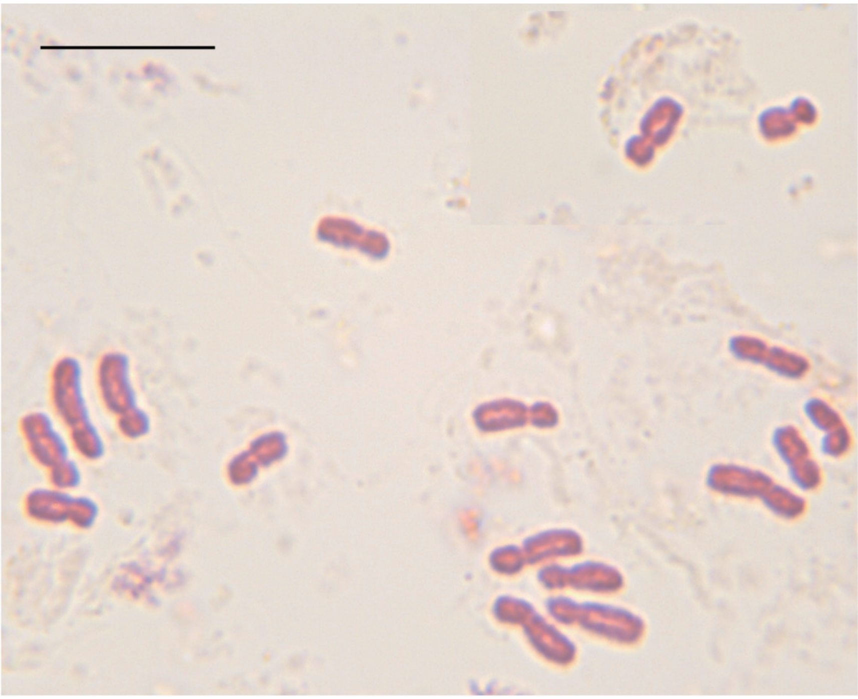

Figure 8. *Armeria gracilis* from Monti della Meta, Alfedena (L'Aquila, Abruzzo), $2n = 18$. Scale bar: 10 μm.

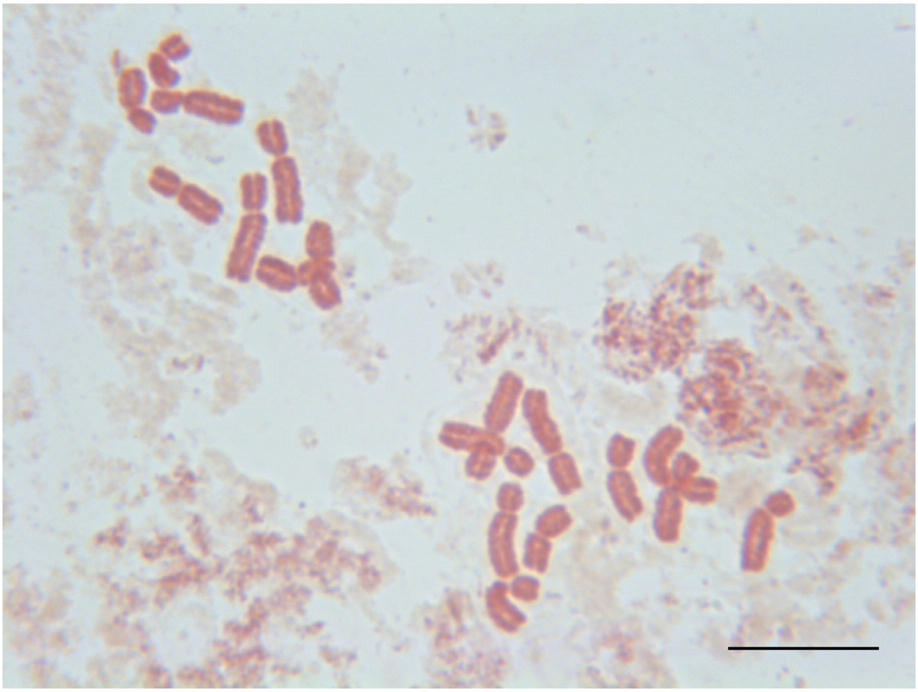

Figure 9. *Armeria gracilis* from Serra del Prete, Pollino Massif (Potenza, Basilicata), $2n = 18$. Scale bar: 10 μm.

Observations. According to Arrigoni (2015), the taxa studied here should be referred to *A. gracilis* subsp. *gracilis* for populations from Gran Sasso, Monte Morrone, and Pollino Massif (central and southern Italy) or to *A. gracilis* subsp. *majellensis* (Boiss.) Arrigoni for populations from the Majella Massif and Monti della Meta (central Italy). However, as already highlighted by Bartolucci et al. (2024) and Iamonico et al. (2024), the distinction of these two taxa is doubtful and need further studies. The count from Vado di Corno agrees with that made by Kovanda (1983, under the name *A. majellensis* Boiss.) on plants from Gran Sasso. The count from Pollino Massif agrees with that made by Brullo et al. (1995, under the name *A. majellensis* Boiss. subsp. *ausonia* Bianchini) on plants from the same area. Plants from the latter area, originally described as *A. gracilis* var. *pollinensis* N.Terracc. (Terracciano 1891), show several morphological differences and need further studies.

M. Tiburtini, F. Bartolucci, F. Conti, L. Bernardo,
L. Baluganti, V. Conti, V. Cardella, D. Iamonico, L. Peruzzi

Armeria gussonei Boiss. (Plumbaginaceae)

Chromosome number. 2*n* = 18 (Fig. 10).

Voucher specimen Italy. **Sicily**, Rocca Busambra, Palermo, (WGS84 37.856170°N, 13.402200°E), 5 July 2023, *G. Domina & G. Barone* (PI066439).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. The chromosome count agrees with that made by Colombo et al. (1980) for the same area.

M. Tiburtini, G. Barone, S. Fruzzetti, L. Peruzzi

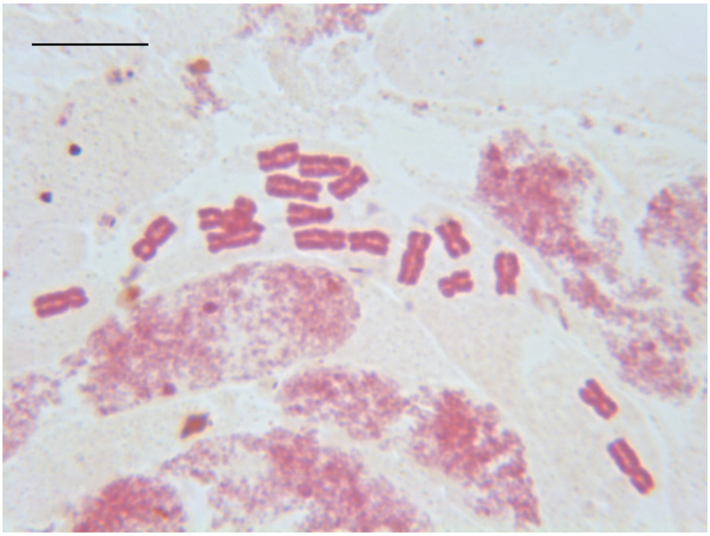

Figure 10. *Armeria gussonei* from Rocca Busambra (Palermo, Sicily), 2*n* = 18. Scale bar: 10 µm.

Armeria macropoda Boiss. (Plumbaginaceae)

Chromosome number. 2*n* = 18 (Fig. 11, 12).

Voucher specimen. Italy. Campania, Ripa della Falconara, pendici del Monte Terminio (WGS84 40.818083°N, 14.941297°E), 20 June 2019, *L. Bernardo* (PI065461).

Voucher specimen. Italy. Basilicata, Pignola, Serranetta, Potenza, (WGS84 40.564865°N, 15.815888°E), 1 June 2022, *D. Iamonico* (PI66709).

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. *Armeria macropoda* is endemic to southern Italy. Although both the studied localities are referred by Arrigoni (2015) to this species, the population from Basilicata shows several morphological differences, resembling more *A. garganica* than *A. macropoda* from Monte Terminio, that likely corresponds to the type locality of this species (Iamonico et al. 2024). The chromosome number of this species is reported here for the first time.

M. Tiburtini, L. Bernardo, D. Iamonico, L. Baluganti, L. Peruzzi

Armeria nebrodensis (Guss.) Boiss. (Plumbaginaceae)

Chromosome number. 2*n* = 18 (Fig. 13).

Voucher specimen. Italy. Sicily. Monte San Salvatore, Madonie, Piano Grande (WGS84 37.842780°N, 14.031291°E), 4 July 2023, *G. Domina* (PI66440).

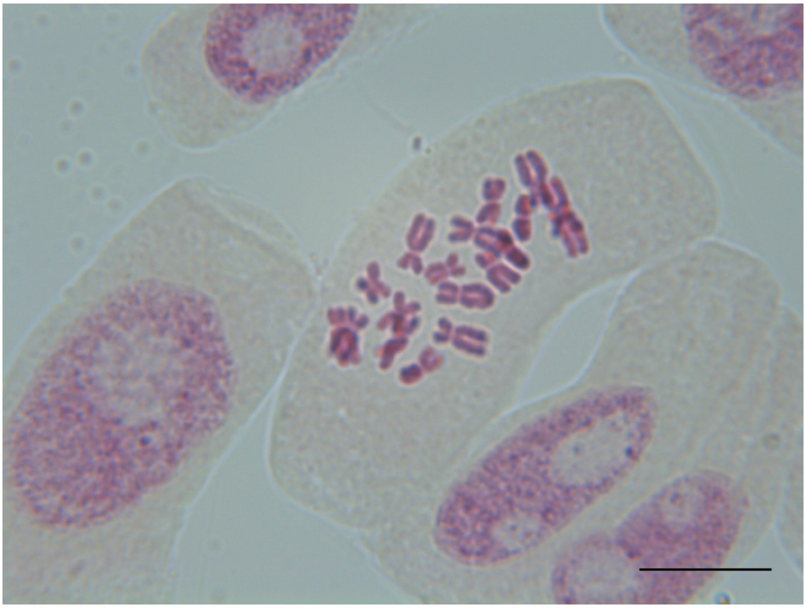

Figure 11. *Armeria macropoda* from Ripa della Falconara, Monte Terminio, (Avellino, Campania), 2*n* = 18. Scale bar: 10 μm.

Method. Squash preparations were made on root tips obtained from germinating seeds. Root tips were pre-treated with 0.4% colchicine for 3 h and then fixed in Carnoy solution for 1 h. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

Observations. This chromosome count agrees with that made by Bartolo et al. (1981) for the same area.

M. Tiburtini, S. Fruzzetti, G. Barone, L. Peruzzi

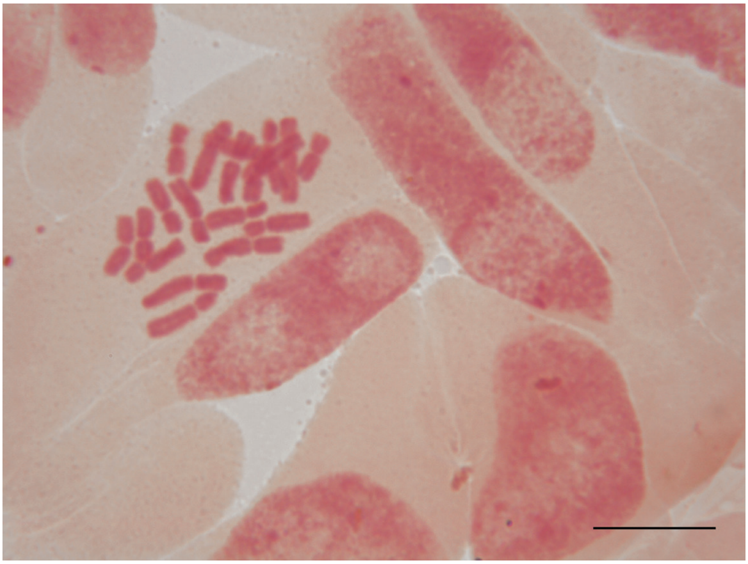

Figure 12. *Armeria macropoda* from Mt. Serranetta near Pignola (Potenza, Basilicata), $2n = 18$. Scale bar: 10 µm.

Figure 13. *Armeria nebrodensis* from San Salvatore (Madonie, Sicily), $2n = 18$. Scale bar: 10 µm.

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RESEARCH ARTICLE

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Three new records of genus *Hohenbuehelia* (Pleurotaceae, Agaricales) in Pakistan

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Abstract

Morphological and molecular phylogenetic analyses were conducted to identify *Hohenbuehelia* species collected during 2020–2023 in Lahore, Margalla Hills, and Abbottabad in the Khyber Pakhtunkhwa region of Pakistan. The phylogenetic analysis was based on the internal transcribed spacer region (ITS) of nuclear ribosomal DNA. Among collected taxa, we identified *H. canadensis*, *H. portegna*, and *H.tristis*, which represent new records for the country. This research contributes to our knowledge of fungal diversity and the distribution of this genus in Pakistan. A key to the known species of *Hohenbuehelia* in Pakistan is also provided.

Keywords

Basidiomycota, nrITS, Phylogeny, Pleurotoid mushroom, Taxonomy

Introduction

Hohenbuehelia Schulzer (Anamorph–*Nematoctonus* Drechsler) is a pleurotoid genus of agaric fungi belonging to the family Pleurotaceae, with *H. petaloides* (Bull.) Schulzer as type species. It is characterized by spathulate, reniform, or flabelliform pileus, usually having a gelatinous layer under the pileus surface, with decurrent lamellae, reduced or no stipe, ellipsoid basidiospores, lecythiform cheilocystidia (if present), thick-walled metuloid pleurocystidia and hyphae with clamp-connections (Singer 1986; Corner 1994; Silva-Filho and Cortez 2017; Holec and Zehnálek 2020; Xu et al. 2023).

One hundred and forty taxa are listed under *Hohenbuehelia* in Index Fungorum (https://www.indexfungorum.org/names/Names.asp, accessed on 24 April 2024). Many species of this genus are decomposers, widely distributed in tropical and temperate areas (Laessøe and Peterson 2019). These species grow on decayed wood, dead wood, logs, and sometimes on the bark of living trees, or herbaceous stems (Holec and Zehnálek 2020). Only four *Hohenbuehelia* species have been previously recorded from Pakistan on a morpho-anatomical basis, i.e. *H. atrocaerulea* (Fr.) Singer, *H. petaloides*, *H. reniformis* (G. Mey.) Singer, and *H. testudo* (Berk.) Pegler (Ahmad et al. 1997; Khalid et al. 2022). Here, we present three new records of *Hohenbuehelia* for Pakistan: *H. canadensis* from Abbottabad, *H. portegna* from Lahore, and *H.tristis* from Margalla Hills, based on both morphology and phylogenetic results. The present study aims to uncover the distribution of these species, as well as contribute to the mycological knowledge and biodiversity records of the region.

Materials and methods

Sampling sites

During mycological surveys conducted in 2020–2023, specimens were gathered from three different locations (Fig. 1). One of these sites was Abbottabad, located in the northern part of Khyber Pakhtunkhwa (Pakistan) at an elevation of 1,256 m a.s.l. This area is spread over 1,967 km^2 and surrounded on the north by Mansehra District, east by Muzaffarabad District, south by Rawalpindi District, and west by Haripur District. Abbottabad is mostly a rugged mountainous region with a mean maximum temperature of 23 °C and minimum temperature of 11 °C; monsoon rainfall occurs from the start of July till September (Waseem et al. 2021).

The second collection site was Lahore bound on the north and west by the Sheikupura District, east by Wagah, and south by Kasur District (Coordinates: 31°15'N to 31°45'N and 74°01'E to 74°39'E). It covers an area of 404 km^2 and is 217 m a.s.l. (Government of the Punjab 2007). It features a hot semi-arid climate with rainy, long, and extremely hot summers, dry winters, annual monsoons, and dust storms. This area experiences monsoon rainfall from July to September, these rains increase the humidity. This high temperature and humidity favor the growth of a huge variety of mushrooms in lawns, gardens, wastelands, and under trees.

Our third sampling site was Margalla Hills National Park (MHNP), Islamabad (33°43'N, 73°55'E) at an elevation ranging from 450 to 1,580 m a.s.l. (Jabeen et al. 2009). The park covers an area of 15,883 ha with rugged terrain. The climate is humid subtropical, with hot summers followed by the monsoon season, and mild and wet winter seasons. The average minimum and maximum temperatures are 19.5 °C and 33.3 °C, respectively (Khalid et al. 2015), and the mean annual rainfall is about 940 mm. There are two rainy seasons each year; the summer monsoon goes from July to September with heavy rainfall and winter rainfall from January to March. Average relative humidity during the monsoon season varies between 59 to 67% (Masroor 2011).

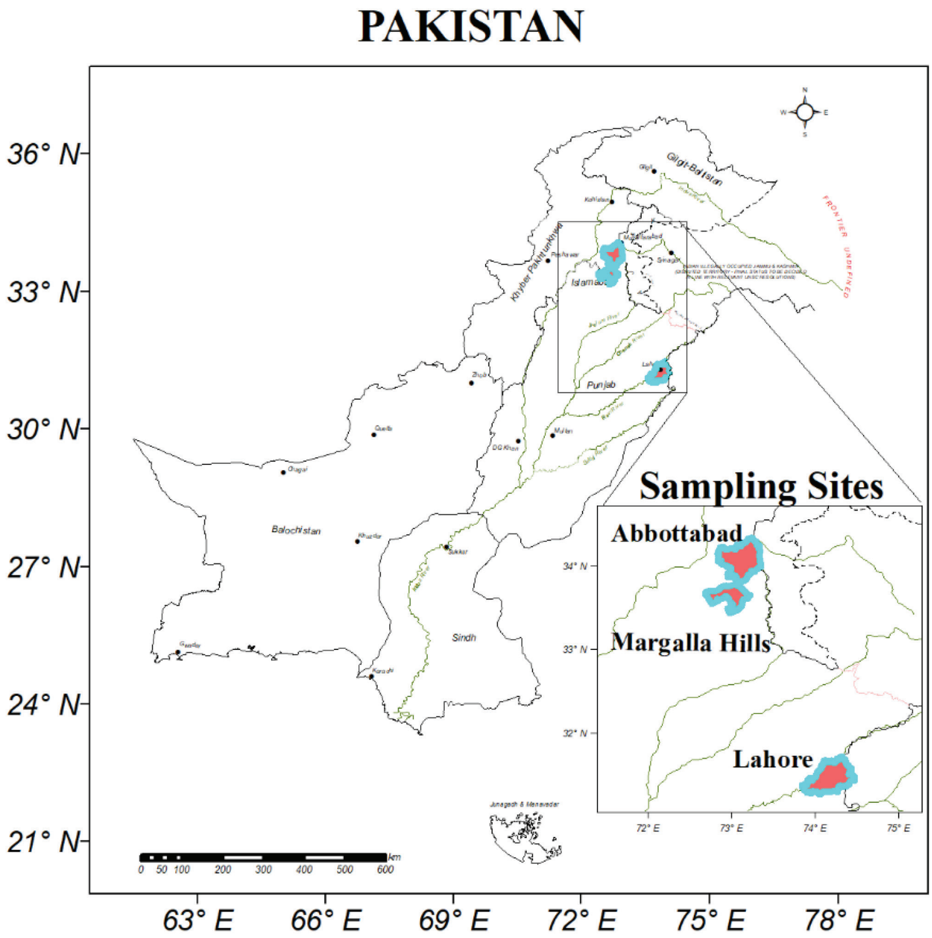

Figure 1. Map showing the geographical location of sampling sites.

Morphological study

Fresh specimens were collected and labelled, photographed in their natural habitat, and dried at 40–50 °C using a fan heater to preserve them. The collected material was carried to the Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Lahore, and analyzed micro-morphologically, then deposited at the LAH Herbarium. The specimen's color was referred to according to Munsell Soil Color Charts (Munsell 1975), and terminologies followed Vellinga (2001).

For microscopic characterization, dried specimens were subjected to free-hand sections, mounted in 5% KOH, stained with Congo red, and observed under a compound microscope (LABOMED, Labo America, Inc., USA) at 40× and 100× magnification. At least 30–40 measurements of each feature (shape, color, and size of basidiospores, basidia, cystidia, hyphae of the stipe, and pileus covering) were taken using Scope Image 9.0(X5). The following abbreviations are used: 'l' for length, 'w' for width, 'avl' for average length, 'avw' for average width, 'Q' for the quotient of length and width, and 'Q_{av}' for average quotient.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from dried material using the CTAB method following Bruns (1995). ITS1F and ITS4 primer pairs were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (White et al. 1990; Gardes and Bruns 1993). After PCR amplification, the PCR products were sequenced at TsingKe Biotech Co. (China) and newly generated sequences were deposited in GenBank under accession numbers PP594062, PP594063, PP594061, and PP702161.

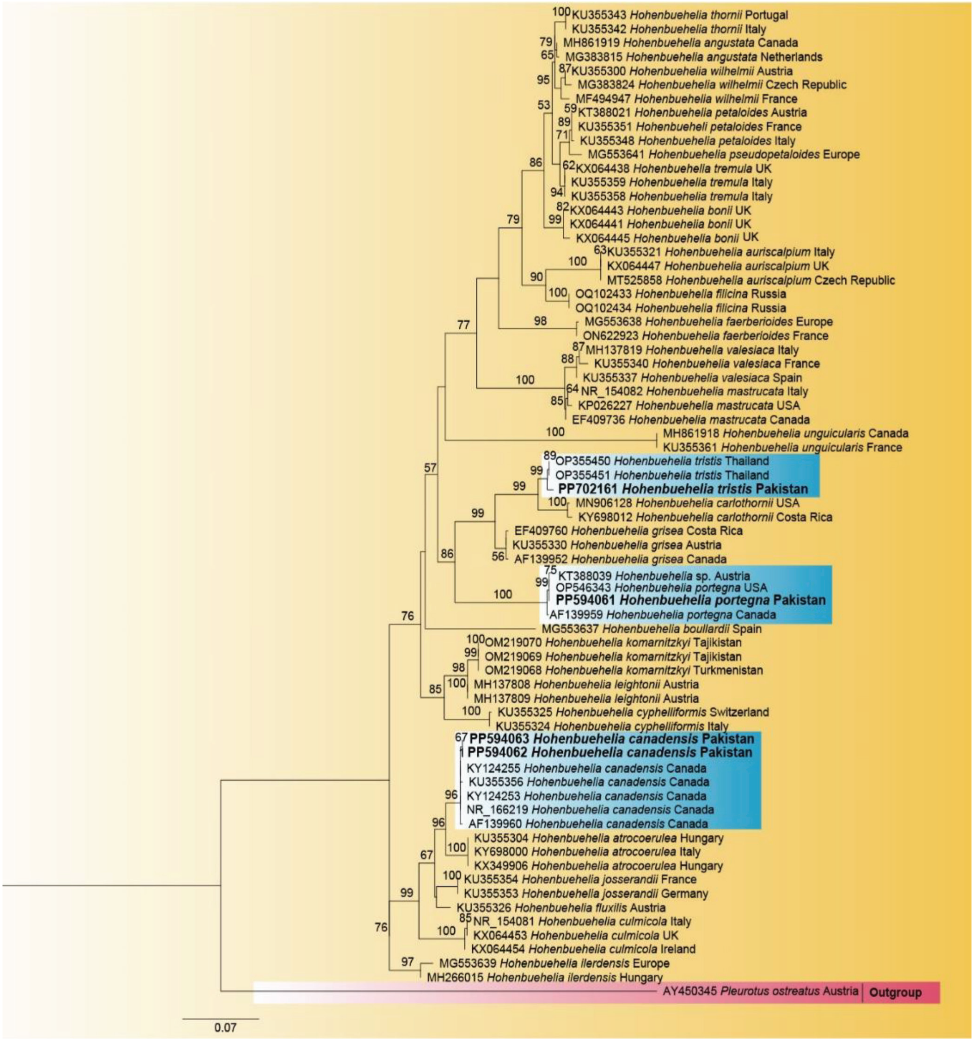

Figure 2. Phylogenetic relationships within the genus *Hohenbuehelia* as inferred by using ML analysis of ITS sequences. Values for ML BS ≥ 50% are appended to nodes. Sequences determined in the present study appear in bold. The phylogram is rooted with *Pleurotus ostreatus*.

Sequence alignment and phylogenetic analysis

The newly generated sequences, and additional sequences, were accessed from NCBI GenBank, and sequences from published records were used for phylogenetic analysis following Xu et al. (2023) and Kalinina et al. (2023). MUSCLE Alignment tool v.3.8. was used to align the DNA sequences (Edgar 2004), and BioEdit sequence alignment editor v.7.2.5.0 (Hall 1999) was used to trim the sequences. The Maximum Likelihood (ML) method for the ITS dataset was carried out via RAXL-HPC2 v. 8.1.11 (Stamatakis 2014) on CIPRES Science Gateway Portal v.3.1. (Miller et al. 2010). In ML analysis, 1000 bootstrap repetitions were acquired as statistical supports with rapid bootstrapping. Significant support was considered to be ≥50%. FigTree software v. 1.4.3. was used for displaying the phylogram (Rambaut et al. 2014) and then exported to Adobe Illustrator for final editing.

Results

Phylogenetic results

Our ITS-based dataset consists of 70 DNA sequences of *Hohenbuehelia* including *Pleurotus ostreatus* (Jacq.) P. Kumm. as outgroup. The final alignment is composed of 707 characters: 408 conserved, 287 variable, and 221 parsimony-informative. All newly generated sequences are grouped in the genus *Hohenbuehelia*. Sequences of *H. canadensis* (E-89, M-17) and *H. portegna* (MMK-02) grouped with *H. canadensis* and *H. portegna* sequences, respectively downloaded from GenBank, with strong bootstrap support. The other Pakistani species *H. tristis* (AN-203) clustered with the sequences of the same species retrieved from GenBank, with strong bootstrap value (Fig. 1). All these three species are recorded for Pakistan for the first time.

Taxonomy

Hohenbuehelia canadensis Consiglio, Setti & Thorn, Persoonia 41: 208 (2018)

Fig. 3

Description. Basidiomata small-sized, solitary, on fallen branches, soft. **Pileus** 1.5–2.0 cm wide, fan-shaped, margin incurved, undulating; cream to light brown to camel brown (2.5Y8/1) from center becomes dark to grayish brown (7.5YR5/2) towards margins, surface flexuous, smooth, sticky, scales absent; context moderately thick, soft to firm, unchanging when bruised or cut; margins without striation. **Lamellae** radiating at point of attachment, moderately close, striate, entire margins, pale cream (10YR8/4). **Context** gelatinous. **Odor and Taste** not recorded.

Basidiospores (6.4–) 7.6–9 (−11) × (3.2–) 3.9–5 (−5.8) µm, avl × avw = 8.85 × 8.12 µm, hyaline, inamyloid, smooth, ellipsoid, slightly granular content or oily drops.

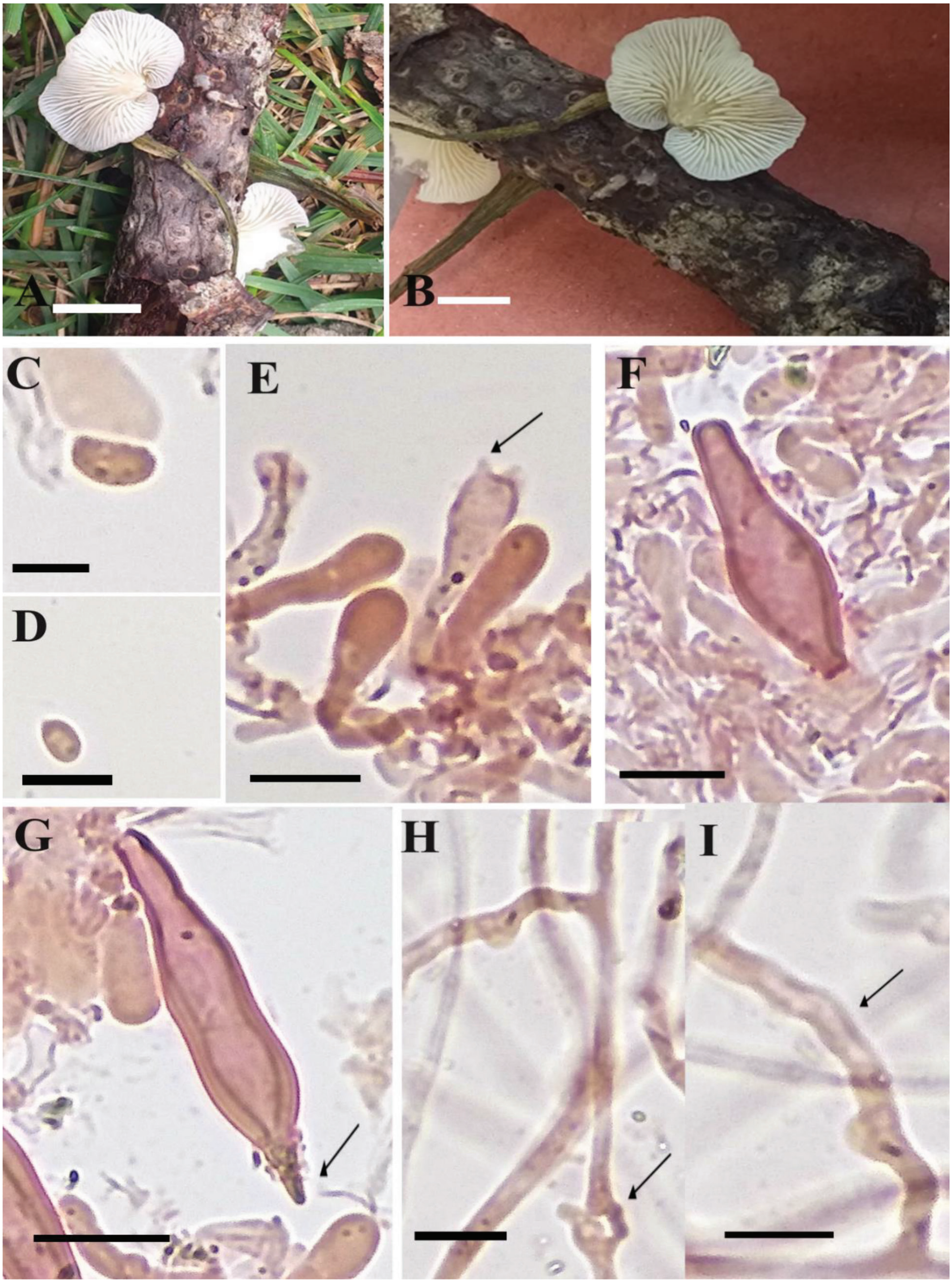

Figure 3. Micro-morphological features of *Hohenbuehelia canadensis* (M-17): **A, B** basidiomata on fallen branch **C, D** basidiospores **E** basidia (sterigmata pointed by the arrow) **F** cheilocystidia **G** cheilocystidia (cap pointed by the arrow) **H** hymenophoral hyphae (arrow showing clamp connections) **I** pileipellis with terminal elements (arrow pointed). Scale bars: 1 cm (**A, B**); 5 µm (**C**); 10 µm (**D**); 10 µm (**E–G**); 5 µm (**H, I**).

Basidia, 22–25 × 6.7–7.3 µm, clavate, 4–spored. **Hymenophoral trama** irregular, hyaline; hyphae 2.2–4.1 µm diam. **Cheilocystidia** present along the lamellar edge, lanceolate, dextrinoid, and metachromatic, partly covered with refringent, yellow or whitish. **Pleurocystidia** clavate-lecythiform, the base 14–18 × 4.2–5.9 µm. **Pileipellis** thin, approximately 11–13 µm, 2–4 µm in diam., brown, clamp-connections present in all pseudotissues.

Habit and habitat. Singly or occasionally in small imbricate clusters on a log of *Abies pindrow* (Royle ex D. Don.) Royle.

Material examined. Pakistan. Pakistan, Khyber Pakhtunkhwa province, Hazara division, Abbotabad district, Mushkpuri top, 33°40'43"N, 72°52'32"E, at 1300 m.a.s.l., on a log of *Abies pindrow*, 30 July 30, 2020, Urooj Ashraf, Najam-ul-Sehar Afshan & Abdul Nasir Khalid (M-17: GenBank PP594062, E-89: GenBank PP594063, LAH38208).

Hohenbuehelia portegna (Speg.) Singer, Lilloa 22: 256 (1951) [1949]

Fig. 4

Description. Basidiomata small, pleurotoid, soft. **Pileus** laterally attached, 1–2 cm wide, convex from aside, highly striate, brown (10YR7/6) when young, brownish yellow (10YR6/6) when mature, shiny, margins incurved. **Lamellae** very crowded, narrow, adnate, white to creamy white, entire margins. **Stipe** strongly reduced and lateral, present up to 1–3 mm pseudo–stipe appears when young, disappearing at maturity. **Odour and Taste** not recorded.

Basidiospores (8.0–) 8.10–9.80 (–9.90) × (4.01–) 4.33–5.88 (–5.92) µm, Q = 1.2–2.0, Qav = 1.6, ellipsoid, thin-walled, appearing smooth, hyaline, no germ pore, apiculate. **Basidia** clavate, hyaline, (23.0–) 23.3–40.80 (–41.90) × (4.01–) 4.2–4.5 (–4.7) µm, sterigmata 2, 3–4 µm long, clavate. **Cheilocystidia** (16.50–) 16.80–20.90 (–21.45) × (4.15–) 4.30–6.95 (–7.01) µm, avl × avw = 17.40 × 6.68 µm, lecythiform, capitate apex, thin to slightly thick-walled, hyaline in 5% KOH. **Pleurocystidia** (34.80–) 36.10–79.85 (–82.05) × (9.0–) 9.65–17.95 (–19.60) µm, avl × avw = 59.90 × 13.63 µm, metuloid, fusiform, lanceolate, apex covered with a layer of crystals, very abundant, brownish in 5% KOH. **Hymenophoral trama** regular, made up of narrow, cylindrical, 3–6 µm wide hyphae**. Lamella edge** fertile, cystidia absent. **Pileipellis** an entangled trichoderm of semi-erect cylindrical, cutis, 2.0–4.0 µm wide. Clamp-connections abundant.

Habit and habitat. Gregarious on a decaying wooden door.

Specimen examined. Pakistan. Punjab, Lahore District, Quid e Azam Campus, University of the Punjab, 31°30'0.20"N, 74°18'32.27"E at 217 m.a.s.l., University of the Punjab, Quaid e Azam Campus, Spring, 13 July 2023, Abdul Nasir Khalid & Muhammadah Khalid, (MMK-02: GenBank PP594061, LAH38209).

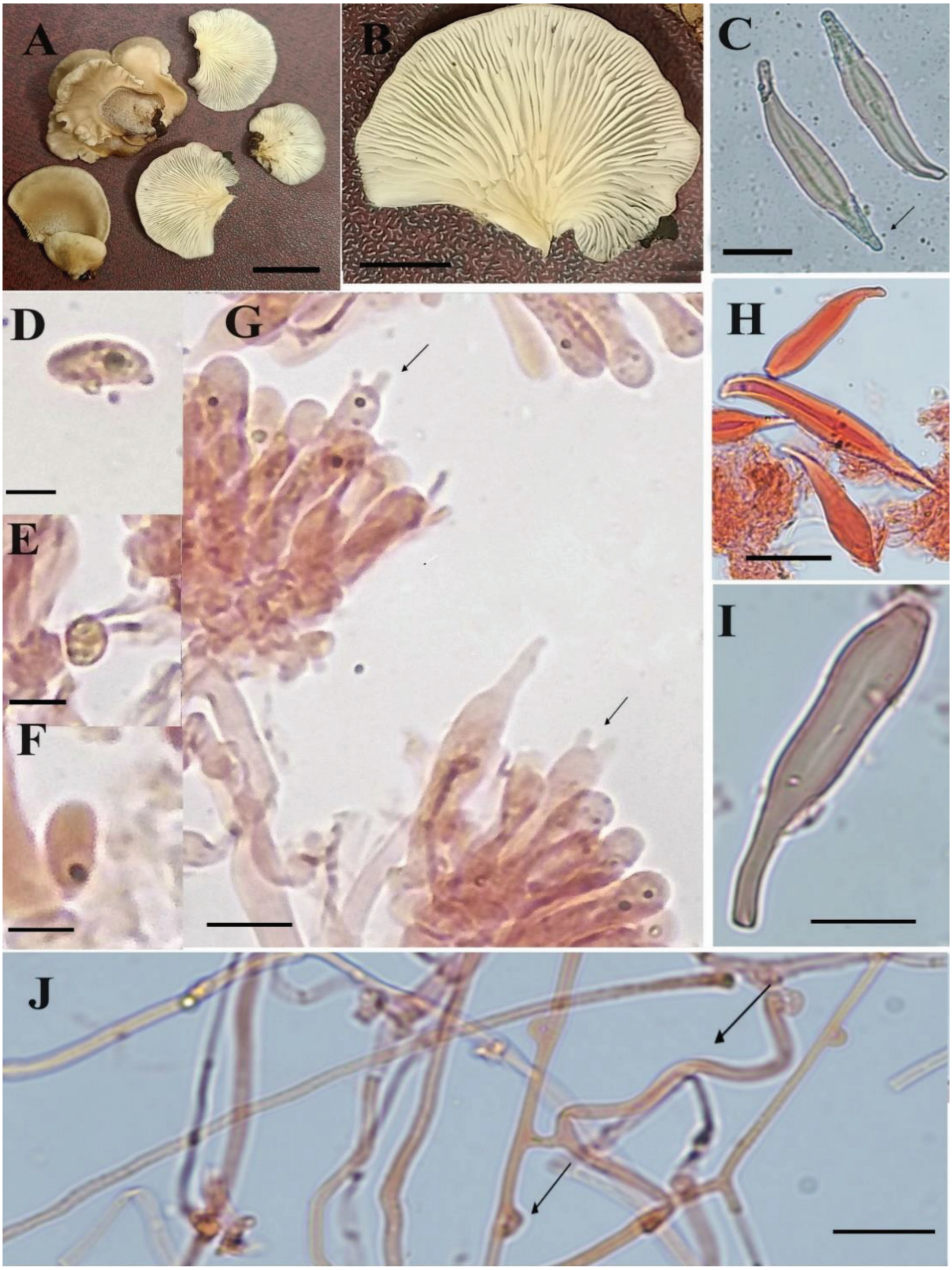

Figure 4. Micro-morphological features of *Hohenbuehelia portegna* (MMK-02): **A, B** basidiomata **C** cheilocystidia (cap pointed by arrow) **D–F** basidiospores **G** basidia (sterigmata pointed by the arrow) **H, I** pleurocystidia (cap pointed by arrow) **J** hymenophoral hyphae (arrow showing clamp connections), Pileipellis with clamp connections (arrow pointed). Scale bars: 1 cm (**A**); 0.5 cm (**B**); 5 µm (**C**); 5 µm (**D–F**); 10 µm (**G**); 10 µm (**H–J**).

Hohenbuehelia tristis **G. Stev., Kew Bull. 19(1): 26 (1964)**
Fig. 5

Description. Basidiomata small-sized, solitary to gregarious, overlap on fallen small branches, soft. **Pileus** 0.8–1 cm in width, 0.4–0.7 cm in length, dimidiate to flabelliform, spathulate, petaloid when immature, light grey (10 YR 8/1-2) to whitish towards margins, shiny, slightly translucent, surface sparsely pubescent, margins incurved. **Lamellae** decurrent, radiating from the attachment point, white to creamy white, broad, sub-distant, lamellulae present in 1–4 tiers. **Stipe** absent, sometimes dorsally attached pseudo-stipe present, 0.3 cm long when young, disappear at maturity. **Odour and Taste** not recorded.

Basidiospores (6.07–) 6.20–7.60 (–7.70) × (4.10–) 4.15–4.50 (–4.55) µm, avl × avw = 7.0–4.40 µm, Q = 1.40–1.80 µm, Qav = 1.58 µm, sub-ellipsoid to oblong, smooth, thin-walled, guttulate, inamyloid, hyaline in 5% KOH. **Basidia** (20.0–) 22.30–26.90 (–22.66) µm, avl × avw = 24.40 × 6.90 µm, narrowly clavate to sub-cylindrical, mostly with 4 sterigmata, some with 2 sterigmata, 2–3.5 µm long, smooth, thin-walled, basidioles numerously present, hyaline in 5% KOH. **Cheilocystidia** (15.50–) 15.80–16.90 (–17.45) × (6.15–) 6.30–6.95 (–7.05) µm, avl × avw = 16.40 × 6.68 µm, lecythiform, mucronate, sub–lageniform, with capitate apex, thin to slightly thick-walled, hyaline in 5% KOH. **Pleurocystidia** (34.80–) 36.10–79.85 (–82.05) × (9.0–) 9.65–17.95 (–19.60) µm, avl × avw = 59.90 × 13.63 µm, metuloid, fusiform, conical, narrowly utriform, encrusted with crystals towards base, extremely thick–walled with narrow lumen, very abundant, brownish in 5% KOH. **Hymenophoral trama**–sub–regular, hyphae 2.60–4.70 µm in diam., thin-walled, septate, clamp–connections present, hyaline. **Pileipellis** ixotrichoderm, with cylindrical terminal elements, with brown intracellular pigments, pileocystidia absent, pileus trama composed of two types of hyphae; 1) horizontally arranged thin hyphae, 1.85–2.30 µm wide, septate, thin-walled, clamped, hyaline, 2) interwoven hyphae, 3.10–4.70 µm wide, septate, smooth, slightly thick-walled, hyaline, clamp–connections frequently present.

Habit and habitat. Gregarious to imbricate, on small dead fallen branches.

Specimen examined. PAKISTAN, Islamabad, Margalla Hills National Park, Trail 5, 33°40'43"N, 72°52'32"E, at 1580 m a.s.l, 27 August, 2023, Fauzia Razzaq, Fatima Rehmat, (AN-203: GenBank no. PP702161, LAH38210).

Discussion

Based on micro-morphological and phylogenetic analyses, we identified three species of *Hohenbuehelia* that contributes to the taxonomic knowledge of fungi in Pakistan. Fungal surveys were conducted from geographically diverse states including Abbot-

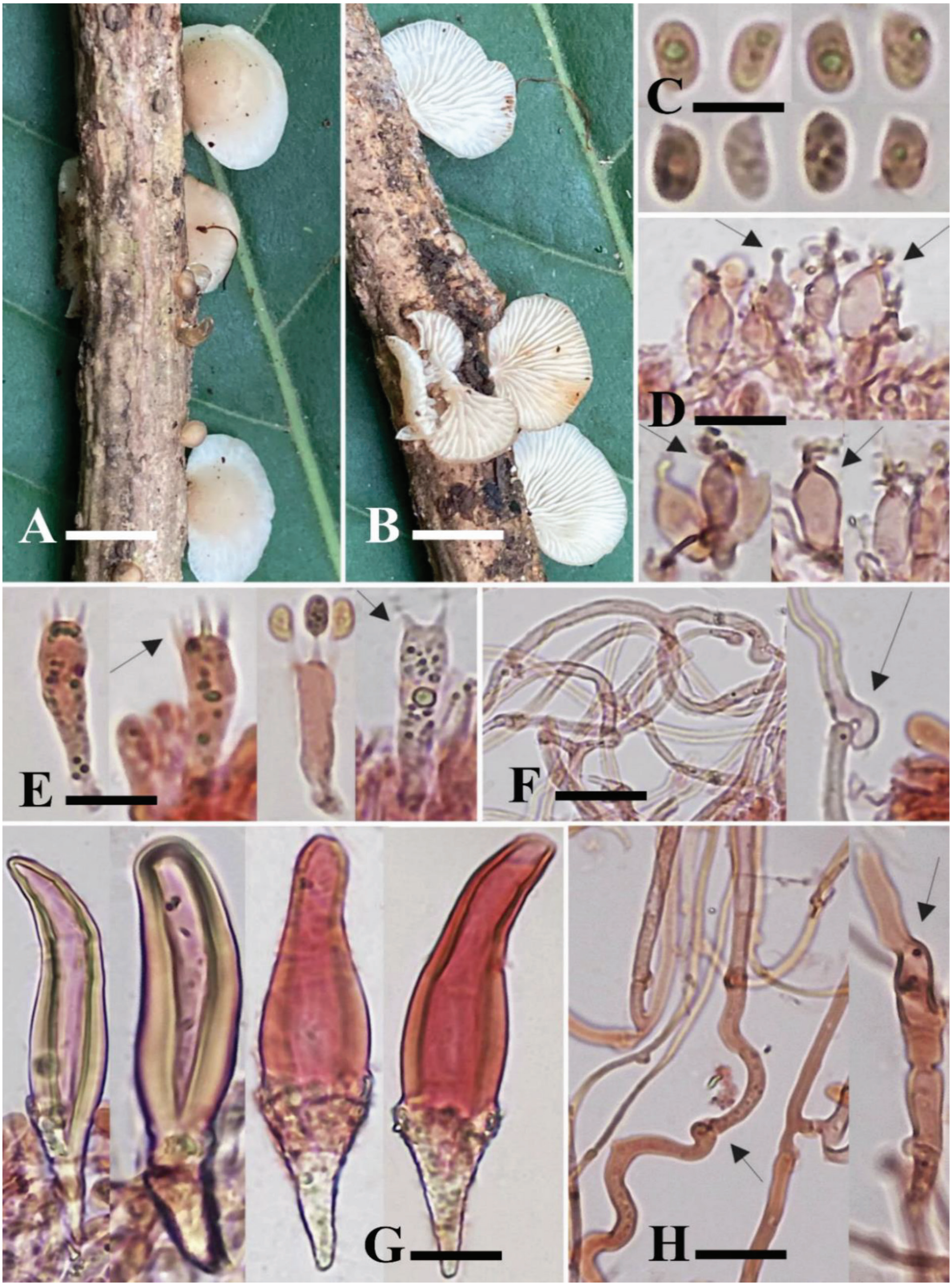

Figure 5. Micro-morphological features of *Hohenbuehelia tritis* (AN-203): **A, B** basidiomata on fallen branch **C** basidiospores **D** cheilocystidia (cap pointed by the arrow) **E** basidia (sterigmata pointed by the arrow) **F** hymenophoral hyphae **G** pleurocystidia **H** pileipellis hyphae with clamped-connections and terminal elements (arrow pointed). Scale bars: 0.5 cm (**A, B**); 5 µm (**C**); 10 µm (**D**); 10 µm (**E**); 5 µm (**F**); 10 µm (**G**); 5 µm (**H**).

tabad, Lahore, and Margalla Hills, which led to the discovery of previously unrecorded species in this country.

Our Pakistani collections of *Hohenbuehelia canadensis* Consiglio, Setti & Thorn, agree phylogenetically and morpho-anatomically with the specimen *H. canadensis* originally described from Canada. This species is characterized by its small blackish dimidiate fruiting bodies, less than 2 cm broad, cream to pale grayish lamellae, and long ellipsoidal to cylindrical basidiospores (Consiglio et al. 2018). Our collection of *H. canadensis* mostly agrees with the type specimen, but the latter has black and small-sized basidiospores (6.9–8 × 3.6–4.2 µm) (Consiglio et al. 2018), while in the Pakistani species they are larger (6.4−11 × 3.2−5.8 µm), creamy to light brown or camel brown from the center, become dark to greyish brown towards the margins pileus.This might be due to the type specimen description based on the herbarium specimen and may depend on the developmental stage.

Our second specimen, *Hohenbuehelia portegna* (Speg.) Singer matched very well with the macro- and micro-morphological features described for *H. portegna* from Brazil and Mexico. The Brazilian collection differs from our specimen by slightly smaller pileus (1.8–2.2 cm in diam.) and larger (7–11.5 µm in diam.) basidiospores (Silva-Filho and Cortez 2017). The Maxican species differs by having small-sized pileus (0.5–0.8 cm in diam.) and larger (8–9.6 (–11.0) µm in length) basidiospores (Ramírez-Cruz et al. 2022) than our specimen of *H. portegna*.

The third Pakistani species matched with *Hohenbuehelia tristis* G. Stev., described in New Zealand and Thailand (Stevenson 1964; Phonemany et al. 2023). Both the Pakistani and New Zealand collections share similarities in having flabelliform pileus, lamellae creamy–white decurrent to radiating from attachment point, and thick-walled metuloid pleurocystidia, encrusted with crystals. The New Zealand collection differs from ours by having buff to sordid colored pileus, larger metuloid (80–90 × 15–20 µm), and pileipellis as tufts of parallel larger hyphae (3–8 µm in diam.) (Stevenson 1964). Both micro–morphologically and phylogenetically our species (AN-203) agreed with Thailand's collection of *H. tristis*, but the Thai species differs by its larger yellowish-white basidiomata (1.5–2.0 × 2.0–3.0 cm), and slightly larger (5.1–9 × 3.5–5.2 µm) basidiospores (Phonemany et al. 2023). These differences might be due to different developmental stages and environmental factors. This study expands the known fungal diversity in Pakistan by documenting three species of *Hohenbuehelia* for the first time from different geographical regions in the country. Additionally, it provides information through a combination of molecular phylogenetic analysis and morpho-anatomical characterization.

Key to the *Hohenbuehelia* taxa known from Pakistan

1 Pileus medium-sized, up to 9 cm in diam **2**
– Pileus small-sized, up to 2 cm in diam .... ***H. canadensis***

2	Pileus white, yellowish white or with pale gray shades	***H. tristis***
–	Pileus dark brown to black or light brown shades	**3**
3	Basidiomata flabelliform to spathulate	**4**
–	Basidiomata reniform, ungulate to dimidiate	***H. reniformis***
4	Basidiospores elongate to cylindrical, up to 8–11 µm long	***H. portegna***
–	Basidiospores broadly ellipsoid, up to 7 µm long	**5**
5	Cheilocystidia fusoid, lecythiform or Ventricose	***H. testudo***
–	Cheilocystidia clavate to lageniform with apical modification	**6**
6	Thick-walled pilocystidia, basidiospores 5–10 × 3–4.5 µm	***H. petaloides***
–	Thin-walled pilocystidia, basidiospores 5.5–9.5 × 3–5 µm	***H. atrocaerulea***

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RESEARCH ARTICLE

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Good practices for the industrial cultivation of the cyanobacterium *Arthrospira platensis* under a greenhouse in a temperate zone (northern Italy)

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Abstract

Arthrospira, *Spirulina*, and *Limnospira* are cyanobacteria widely known as food supplements or additives and cultivated worldwide under the commercial name of spirulina. Many studies have been focused on the improvement of operational conditions for optimizing cell growth and harvesting. At present, greater attention is paid to obtaining a good-quality, possibly food-grade, product that can be added to different food formulations and to reducing the environmental impact by saving water and avoiding or minimizing the release of mineral salts in the environment. A few studies have addressed these aspects although, in most cases, through laboratory experiments or pilot plants. This study focused on the effects of medium recycling, monitored at each harvesting step, and nutrient replenishment on *Arthrospira platensis* growth, biomass, biochemical composition, and extracellular polysaccharide (EPS) production, in a production plant located in a temperate zone (northeastern Italy). Four out of the seven largest ponds (11,000 L each) of the plant were followed for a three-month semi-continuous cultivation, which included the period with the highest biomass productivity. Recycling the culture medium after biomass harvest effectively allowed the best nutritional status of the cells. Biomass productivity increased from June to August 2023 (mean values: 0.029 and 0.038 g L^{-1} d^{-1} at 25–26 °C and 27–29 °C, respectively). Protein, polysaccharides, and C-phycocyanin contents were 62, 22, and 12%, respectively, while EPS was < 7 mg L^{-1}. The biochemical composition did not vary during the cultivation period, differently from previous studies performed with small culture volumes and for a short time.

Keywords

Arthrospira platensis, nutrient recycling, biochemical composition, extracellular polysaccharides, biomass quality, waste prevention

Introduction

Among the cyanobacteria, some genera (e.g., *Arthrospira*, *Spirulina*, and *Limnospira*) possess helically-coiled trichomes as a prominent diacritical feature. They are widely used as food supplements or food additives under the commercial name of spirulina, which is one of the most widely consumed microalga in large-scale production since the 1970s. Spirulina biomass contains a high quantity of proteins (60–70% on a dry weight basis) with all essential amino acids and is rich in vitamins, dietary minerals, and many bioactive molecules including, among others, essential fatty acids, and pigments (e.g., C-phycocyanin) (Soni et al. 2017).

Spirulina thrives naturally in alkaline waters, rich in minerals, at temperatures ranging from 35 to 40 °C, and thus its cultivation was initially carried out in outdoor open ponds located in tropical geographic areas. The most widespread cultivations concern the species *Arthrospira platensis* Gomont, and the successful commercial cultivation of this cyanobacterium, compared to other microalgae, is ascribable to its extremophilic characteristics allowing fast and strong growth both under high pH values (i.e., pH 9–11), which minimize the risk of biological contamination, for example by protozoa, bacteria, fungi, other microalgae (AlFadhly et al. 2022a; Bumandalai et al. 2024), and under high temperature (optimal cell growth temperature: 35–38 °C; Belay 2013). In the early 1990s, China became the biggest producer of Spirulina biomass, however, concerns arose for the safety of cultures exposed to weather variations and contamination, especially in the absence of appropriate controls and certifications (Belay 2008; Bumandalai et al. 2024), due to dust, microorganisms (microalgae, bacteria, fungi, viruses), protozoa, small animals, and other material (insect fragments, rodent hair, feather fragments).

In the last decade, many studies on spirulina bioactive molecules, secondary metabolites and their recognized therapeutic properties (e.g., antioxidant, anti-inflammatory, immunomodulating, anti-viral, anti-bacterial activities) have renewed interest in its extensive exploitation for application in the nutraceutical, animal feed, cosmetics, and pharmaceutical fields worldwide (AlFadhly et al. 2022b; Dinicolantonio et al. 2020; Ragusa et al. 2021), with an estimated market value of $629.63 million by 2025 (Priyanka et al. 2023). Concurrently, the European Commission's strategic policies have focused on the key role of algal biomass production for producing high-quality food-grade proteins with a low carbon footprint, according to the approach of ecological sustainability concerning business development. This renewed interest has extended spirulina cultivation to wider geographical areas, including subtropical and temperate ones.

The most common systems used for *A. platensis* culturing are raceway ponds, and the pilot plants set up for production in subtropical and temperate zones are mainly open ponds placed inside greenhouses, which prevent external contamination, rain intrusion, and mitigate temperature excursion allowing cell growth all year round. Several studies have already reported the optimization of spirulina cultivation in subtropical and temperate areas such as Spain (Jimenez et al. 2003a, b; Guidi et al. 2021), Mongolia (Lu et al. 2011), South Africa (Grobbelaar 2009), South Korea (Kim et al. 2018) and northern Italy (Kurpan et al. 2024). These studies were performed in a real environment, i.e., in open ponds (southern Spain and South Africa) or ponds under a greenhouse (in the Canary Islands and in temperate areas with low winter temperatures, such as Mongolia and northern Italy) having volumes from 2,000 to 125,000 L. The reported trials were mainly aimed at testing the feasibility of the cultivation, by evaluating the responses of the cultures in terms of growth to light and temperature conditions and by verifying biomass productivity and, in some cases, the seasonality. Generally, the results obtained indicate that the cultivation of spirulina in temperate areas is possible, despite the interruption of plant operativity in winter months (Lu et al. 2011) or the addition of a heating system to the greenhouse (Kurpan et al. 2024). Data on real operative plants is scarce and, as indicated by Kurpan et al. (2024), "more pilot- and commercial-scale studies are needed to stimulate the production of spirulina and other microalgae in Europe" and other temperate areas.

In recent years, most studies on spirulina cultivation have focused on: i) obtaining a high-quality food-grade product, suitable for increasing the nutritional value of food formulations while maintaining the economic sustainability of the production process; ii) reducing the impact on the environment due to intensive cultivation, by saving water and avoiding or minimizing the release of mineral salts in the environment (Salehipour-Bavarsad et al. 2024).

Furthermore, reducing the operativity costs is an important issue, especially in geographical areas having suboptimal environmental conditions that do not allow to achieve the maximum productivity. For decreasing operativity costs, one of the most widely applied actions, also in line with the need to decrease the environmental footprint of microalgae cultivation, is the recycling of water and nutrients contained in the medium after removing the cells at each harvesting stage. This is a commonly applied procedure for semi-continuous cultivations ever since the big Earthrise farm was established in California (Belay 2013). In this work, it was reported that this practice can have deleterious effects on the quality of the biomass as the organic matter that remains in the filtered medium may stimulate the growth of opportunistic mixotrophic algae, as well as of bacteria and fungi, and can also have autoinhibitory effects on algal growth (Belay 2013).

Studies addressing the problem of water and nutrient recycling with experiments aimed at evaluating how much this practice may affect biomass quality have been performed mainly with small culture volumes (Depraetere et al. 2015; Li et al. 2022) and in one study also in a large pond (Yuan et al. 2019). The reported results highlighted a consistent reduction in growth rate and protein production, an increase in extracellular polysaccharides (EPS), and culture contamination. However, when growth trials are performed in a real productive environment, the recycling of water and nutrients is strictly necessary. It was calculated that, by re-adding the nitrogen (N) and phosphorus (P) consumed based on their content in the harvested biomass, a 15-fold savings of money compared to the use of fresh medium was obtained (Guidi et al. 2021). In other cases, nutrients were completely re-added (Lu et al. 2011) or either recycled or replaced with fresh medium based on the accumulation of organic matter in the medium (Kurpan et al. 2024).

Suggested solutions, such as subjecting the spent medium to ferric chloride flocculation plus carbon adsorption (Morocho-Jácome et al. 2015) or applying an ultrafiltration step using a 5-µm filter (Han et al. 2025), although promising, were tested only with a 2,000 or 600 mL cultures, respectively and did not appear to be applicable to thousands of liters of medium.

In our opinion, and also as suggested by Kurpan et al. (2024), this subject is not sufficiently covered; in fact, despite the high number of studies performed on spirulina cultivation optimization, there is a lack of information on the temporal dynamics of nutrient consumption in large-scale cultures and, consequently, on the management of their reintroduction when the medium is recycled to prevent a nutrient shortage. This study, therefore, focused on an in-depth monitoring of nutrient (nitrate and phosphate) levels in the medium over three months of semi-continuous cultivation, where medium recycling was performed concurrently with monitoring of cell biomass composition. The cultivation system investigated in the present study has a year-long operativity and is already based on medium recycling, thus allowing to obtain further data for a year-based production. The overall aim of the study was to optimize the nutrient inputs to potentially reduce the use of chemicals and the operativity costs, together with the environmental footprint of the cultivation, without affecting the quality of the biomass.

Methods

Arthrospira platensis strain and culture conditions

The strain used for this study was of Moroccan origin and identified as *Arthrospira platensis* Gomont according to the 16S gene sequencing performed by an external laboratory. For mass cultivation a modified Zarrouk Medium (1966), kept at pH values between 9.7 and 10.3, was used.

Production plant operational conditions

The production plant belongs to the company Micoperi Blue Growth srl (MBG) and is located in Ravenna (NE Italy, 44°42.82'N, 12°23.28'E); it consists of a greenhouse containing 11 raceways, seven of which have a volume of 11,000 L, while four are smaller (2 × 5,000 and 2 × 1,000 L) and utilized to scale up the cultures (total pond area = 458 m^2) (Fig. 1a, b). Twice a year, each pond is filled with medium and inoculated with the *A. platensis* culture growing in one of the backup ponds; the depth of the culture is kept at 150–200 mm depending on the season and mixing is provided by a paddle wheel. The cultures are exposed to natural sunlight; however, the greenhouse has shading systems that are activated, according to the season, when the light intensity, measured on the roof, reaches high values. The only temperature regulation consists of bringing it to lower values in summer through a ventilation opening in the roof.

Figure 1. The greenhouse of the Micoperi Blue Growth company where *Arthrospira platensis* is cultivated **a** panoramic view of all the ponds **b** close-up view of *A. platensis* culture.

Annual temperature (min, max, and average) and irradiance values measured inside the greenhouse and on the roof are reported in Figs 2, 3. The ponds operate according to a semi-continuous regime performed by harvesting variable volumes of the culture, based on the biomass concentration measured in each pond. The biomass harvest is performed using a pumping system connected to a vibro-sifter which separates the algal biomass from the liquid medium. The biomass is dehydrated by freeze-drying before its commercialization. Filtered medium is reintroduced into the pond and then reinstated with the main nutrients (N and P) according to the amount of the harvested biomass and to the N and P content of the biomass (around 10 and 0.8%, respectively, as shown below). At the end of the six-month cultivation period, the entire biomass is harvested, followed by the complete elimination of the culture medium. Subsequently, the empty ponds are washed and sanitized and then the production, which is carried out throughout the year, is resumed.

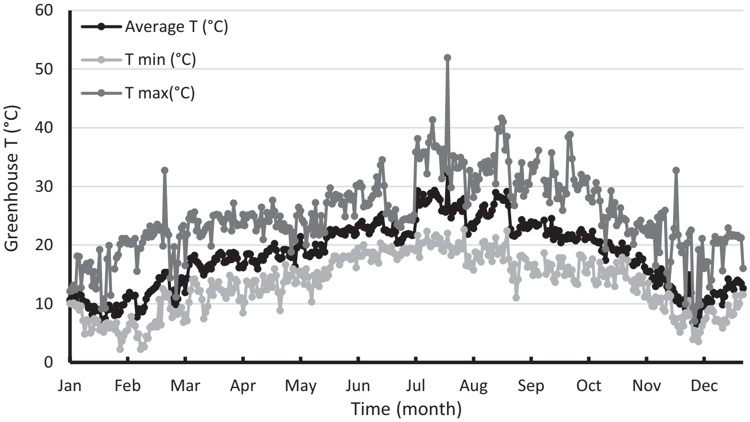

Figure 2. Minimum, maximum, and average temperatures measured inside the greenhouse in the year 2023.

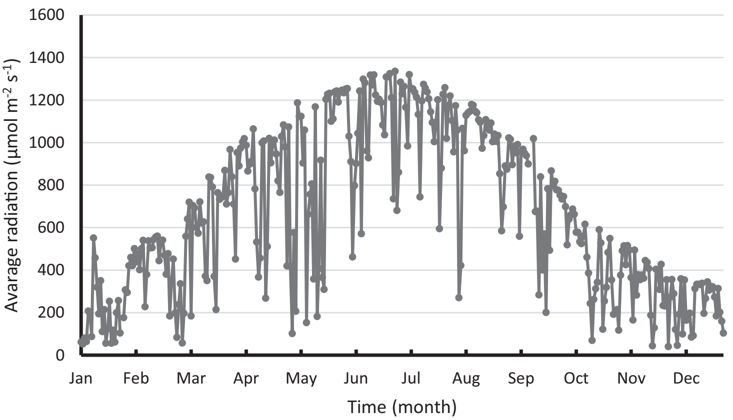

Figure 3. Irradiance measured on the greenhouse roof in the year 2023.

Study procedure

For this study the ponds monitored consisted of four out of the seven larger raceways included in the production plant of MGB. The 4 ponds were monitored between the 10th of May and the 2nd of August 2023, corresponding to the beginning of a new culturing period, and the harvest was performed weekly or bi-weekly at all ponds (Table 1).

Dry weight determination

In each pond, starting from the inoculation day (day 0) the biomass dry weight (DW) was determined every day, in the morning, measuring the absorbance at 750 nm using a spectrophotometer (HACH Lange DR1900) to assess the productivity and the optimal biomass harvesting time. The data were then converted to g DW L^{-1} based on a comparison with DW measurement, which is periodically performed as follows: culture sub-samples (0.02 L) were filtered through pre-weighed glass fiber filters (Whatman GF/C, diameter 47 mm, 1.2-µm pore size); then the filters were washed with a double volume (40 mL) of ammonium formate (0.5 M) according to the Zhu and Lee (1997). Finally, the filters were dried at 105 °C for at least 1 h or until constant weight. Algal biomass dry weight was calculated following the equation:

$$DW(g\,L^{-1}) = \frac{W_t(g) - W_0(g)}{V(L)}$$

where Wt and W_0 are the weight (g) of the filter after drying and before sample filtration, respectively, while V is the volume of filtered culture (L).

Table 1. *Arthrospira platensis* cultivation period followed in the present study. All the days in which the nutrient concentrations were analyzed, as well as harvesting days, are indicated.

Date	Days	Harvesting days
10/05/2023	0	
30/05/2023	20	Yes
01/06/2023	22	
06/06/2023	27	Yes
08/06/2023	29	
13/06/2023	34	Yes
15/06/2023	36	
21/06/2023	42	Yes
23/06/2023	44	
28/06/2023	49	Yes
29/06/2023	50	
05/07/2023	56	Yes
07/07/2023	58	
19/07/2023	70	Yes
21/07/2023	72	
02/08/2023	84	Yes

Algal biomass productivity

Volumetric productivity (VP, g L^{-1} day^{-1}) of algal biomass was determined using the following equation:

$$VP = (DW_2 - DW_1) / (t_2 - t_1),$$

where DW_2 and DW_1 are the values of algal biomass (g L^{-1}), t_2 and t_1 is the corresponding period (day).

Areal productivity was determined according to Kim et al. (2018):

$$\text{Areal productivity (AP, g m}^{-2}\text{ d}^{-1}\text{)} = \text{Volumetric productivity (VP)} \times (\text{Odepth} \times 1000),$$

where Odepth is the pond operating depth.

Nutrient analysis

During the three-month experimentation, inorganic nutrients were measured in the medium of the four ponds on each harvest day, and two days after the nutrient recycling/replenishment step to allow a correct mixing of the medium. For the analyses, subsamples of the culture medium were pre-filtered through Whatman GF/C filters (1.2-µm pore size); aliquots of 1 mL were then filtered through a 0.22-µm syringe filter and injected into an ion chromatograph (883 Basic IC plus, Metrohm) equipped with an anionic column (Metrosep A Supp 4—250/4.0) for N and P.

The nutrient uptake rates (U) were calculated according to Lim et al. (2006). Nitrate and phosphate uptake rates (mmol g $biomass^{-1}$ day^{-1}) were calculated from the nutrient concentration in the medium (C) and the change in algal biomass (γ) over a period (Δt), when the depletion of nutrients was linear, and no replenishment was performed. Δt, therefore, differs depending on nutrient sources and algal conditions. The following equations were used:

$$U = -\frac{C_t - C_o}{\gamma\ \Delta\tau}$$

$$\gamma = \frac{B_t - B_0}{\ln B_t - \ln B_0},$$

where B_t and B_0 are *A. platensis* dry weight biomasses (g L^{-1}) at time t and 0, respectively.

Protein extraction and analysis

For this and successive intracellular analyses, *A. platensis* biomass was harvested by filtration (11-µm pore size). Protein extraction was performed on freeze-dried algal biomass (about 10 mg). The samples were extracted with 3.0 mL of NaOH (0.5 M) and incubated at 90 °C for 15 min, then transferred on ice for 2 min and subsequently centrifuged (2,250 × g, 10 min). The resulting supernatant was collected, and the extraction process was repeated three times. Protein content was determined on the collected supernatant with the Folin phenol reagent according to Lowry's method (1951).

Intracellular polysaccharide extraction and analysis

Intracellular polysaccharides were extracted from freeze-dried samples (5–10 mg), digestion was carried out using sulfuric acid 80% at 37 °C for 60 min, then they were quantified spectrophotometrically (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) through the Phenol Sulfuric Method (DuBois et al. 1956; Kochert 1973) using glucose as standard.

C-phycocyanin analysis

To extract C-phycocyanin (C-PC), freeze-dried aliquots of *A. platensis* biomass were weighed (30 mg), then 2 mL of 0.01 M sodium phosphate buffer (pH 7.4) was added. The samples were incubated at 25 °C for 24 hours with agitation. At the end of the incubation period, tubes were centrifuged for 20 min at 2,500 × g. Subsequently, the supernatant was collected and diluted 1:20 with the previously used extraction buffer and centrifuged. The absorbance of the supernatant was measured with a spectrophotometer (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) at 615 nm, and 652 nm. The concentration of C-phycocyanin was calculated according to the equation of Bennett and Bogorad (1973) reported in Pan-uai et al. (2018).

Cell elemental composition

Carbon (C) and N cell contents were determined using 2–4 mg of freeze-dried algal biomass. The analysis was conducted using a ThermoFisher organic elemental analyzer (Flash 2000) set for CHNS–O determination with a copper/copper oxide column. The standard 2,5-bis-(5-tert-bu-tyl-2-benzooxazol-2-yl) thiophene (BBOT) was used for calibration.

(P cell content was determined following Menzel and Corwin (1965): 10 mg of freeze-dried algal biomass was digested with a solution of 5% potassium persulfate in an autoclave at 121 °C for 30 min. Total P was then determined spectrophotometrically (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) by using the ascorbic acid method at 885 nm.

Contaminants control

The amounts of bacteria, molds, and yeasts were evaluated by an external laboratory through colony-forming unit (CFU) counts, as part of the quality control of the company which is performed on each fresh batch of harvested biomass. The presence of other microorganisms (cyanobacteria, microalgae, zooplankton) was checked weekly by observing culture aliquots under an optical inverted microscope (Zeiss Axiovert 100).

Extracellular polysaccharides analysis

Extracellular polysaccharides (EPS) were extracted from culture medium filtered through Whatman GF/C filters, following the Myklestad and Haug protocol (1972). Two volumes of absolute ethanol were added to one volume of culture and stored at -20 °C for 24 h. The solution was centrifuged for 25 min at 14,700 × g at 4 °C and the pellet was used to measure polysaccharide content. Carbohydrate digestion was carried out using 80% sulfuric acid at room temperature for 20 h; total carbohydrate content of the medium was determined by the phenol sulfuric method (DuBois et al. 1956; Hellebust and Craigie 1973) using glucose as standard.

Statistical analysis

Differences among samples in nutrient (N and P) concentrations (and cellular composition over time were tested by multivariate analysis of variance (ANOVA) using PAST 4.11 software (Hammer and Harper 2001). Additionally, Pearson's correlation coefficient between productivity and temperature was calculated. A significance threshold of $P < 0.05$ was applied to all the analyses.

Results

Arthrospira platensis growth and productivity

Growth patterns of *A. platensis* biomass and of the temperature average measured in the pond medium for the entire period considered (in total 84 days) are reported in Fig. 4 which highlights that during the study period, three sub-periods (i.e., temporal phases) were observed in terms of temperature variation and its increasing values. The first sub-period, from day 0 to the first biomass harvest performed on day 20, was characterized by the lowest recorded temperature values (i.e., 23–24.5 °C) and by a steady growth of *A. platensis* cultures with a biomass concentration increasing from 0.190 ± 0.004 g L^{-1} to 0.400 ± 0.036 g L^{-1}. In the second period, from day 20 to day 40, temperature slightly increased with average values never exceeding 26 °C, still resulting in a slow growth of the cultures so that harvesting was performed weekly and it consisted of the collection of culture volumes equal to 1/6 of the total pond volumes, after which constant biomass values of 0.395 ± 0.011 g L^{-1} were reached. In the third period (from day 42 to day 84), concomitant to the temperature increase (between 27 and 29 °C), a further increase in biomass concentration was observed (0.461 ± 0.003 g L^{-1}) allowing the harvest of higher culture volumes (1/4 of the total pond volume) either weekly (on days 44 and 56) or biweekly (on days 70 and 84). Similarly to the second period, after each harvest *A. platensis* cultures resumed biomass values comparable to the preceding ones (0.457 ± 0.021 g L^{-1}).

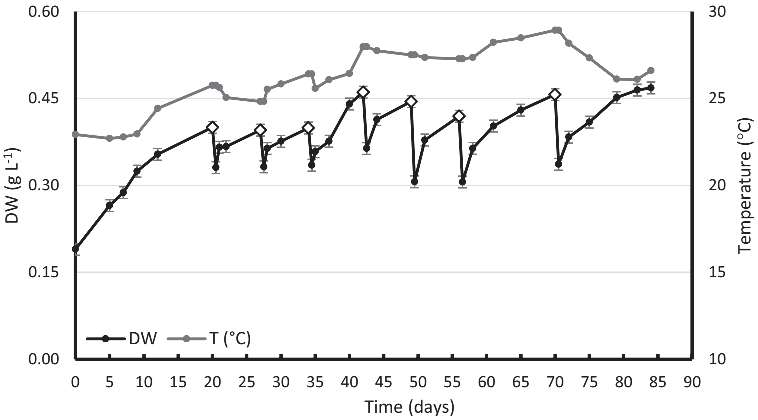

Figure 4. Growth pattern of *Arthrospira platensis* cultivation from day 0 to day 84. Biomass is expressed in terms of mean dry weight (DW, g L^{-1}) for the four ponds. Temperatures represent the average values measured in the culture medium (°C). Harvesting days are indicated with white symbols.

During the first 20 days, biomass productivity was 0.011 g L^{-1} d^{-1}; in the second and third temporal phases, it increased by 2–3-fold (0.029 ± 0.005 and 0.038 ± 0.008 g L^{-1} d^{-1}, respectively), showing a significant positive correlation with temperature ($P < 0.05$). The maximum volumetric productivity value of 0.038 g $L^{-1}d^{-1}$, measured during the study period, corresponded to an areal productivity of 7.6 g m^{-2} d^{-1}.

Nutrient concentrations in the culture medium

Nitrate and phosphate concentrations in the culture medium measured during the cultivation time are shown in Fig. 5, where values registered immediately after the harvesting day indicate the nutrient levels following the medium recycling/replenishment process. Nitrate concentrations showed a decrease during the initial period of biomass growth (uptake rate: 0.219 mmol g^{-1} day^{-1}). Thereafter, for four weeks, during the semi-continuous operativity of the ponds, concentrations remained at values very similar to those initially added; subsequently, a small, but not statistically significant (ANOVA, $P > 0.05$), increase was observed from the initial value of 860.51 to the final one of 937.74 mg L^{-1}. Phosphate concentrations showed a decreasing trend until day 20 (uptake rate: 0.010 mmol g^{-1} day^{-1}); starting from day 44, they displayed an opposite trend compared to nitrates with a significant decrease from the initial value of 79.21 to the final one of 55.90 mg L^{-1} (ANOVA, $P < 0.05$). As a consequence, the N/P molar ratio in the medium remained at a constant value near the Redfield value of 16 until the end of June (day 49); subsequently, it increased up to a maximum of 25.8 at the end of the study period (Fig. 6).

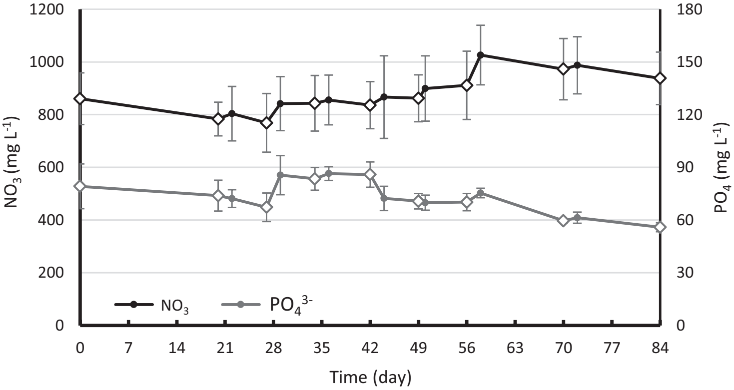

Figure 5. Nitrate and phosphate concentrations (mg L^{-1}) measured in the culture medium during the growth of *Arthrospira platensis*. White lozenge symbols indicate the nutrients measured before the harvesting, the round symbols (black for nitrate, grey for phosphate) represent the nutrients measured two days after the reintegration procedure.

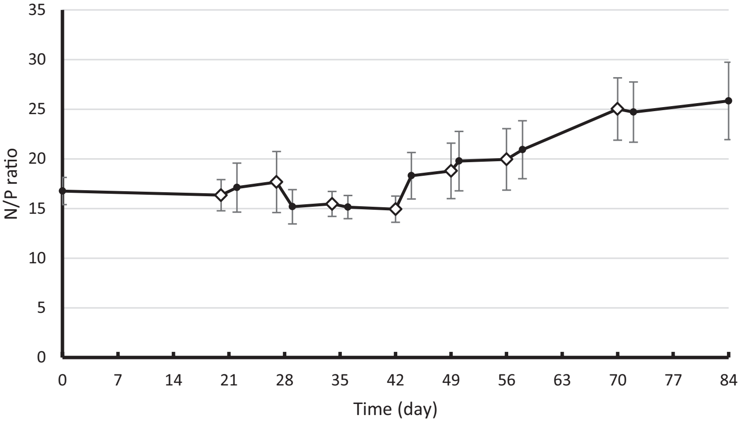

Figure 6. Molar N/P ratio in *Arthrospira platensis* culture medium.

Cellular carbon, nitrogen, and phosphorus

Carbon, nitrogen, and phosphate contents in *A. platensis* cells were assessed in all harvested biomass lots. Like for proteins, polysaccharides, and C-phycocyanin (see below), values at day 0 were not recorded as the biomass analysed on that day was derived from ponds with different characteristics. Fig. 7 shows the constancy of the three elements throughout the entire study period, with no significant differences among ponds and harvesting days (ANOVA, $P > 0.05$). The average cell contents were 46.10 ± 1.92, 10.30 ± 0.64, and 0.85 ± 0.05% for C, N, and P, respectively. The constant cellular C and N contribution led to a stable trend over time of both C/N and N/P molar ratios (average values: 5.2 ± 0.2 and 27.7 ± 1.2, respectively).

Biochemical composition of the biomass

Fig. 8 shows the average percentage of proteins and polysaccharides in lyophilized spirulina biomass. Total proteins displayed no significant differences among ponds and harvesting days (ANOVA, P > 0.05), remaining constant over time (average value: 61.6 ± 0.4%). Differently, the contribution of polysaccharides decreased significantly (ANOVA, P < 0.05) over time, ranging from a maximum of 26.44 ± 3.67% to a minimum of 18.25 ± 1.16%. C-phycocyanin (C-PC) content in *A. platensis* biomass was also monitored (Fig. 9) due to its important commercial value. Like proteins, no significant changes were found during the study period (ANOVA, P > 0.05), with an average contribution of 12.24 ± 0.17%.

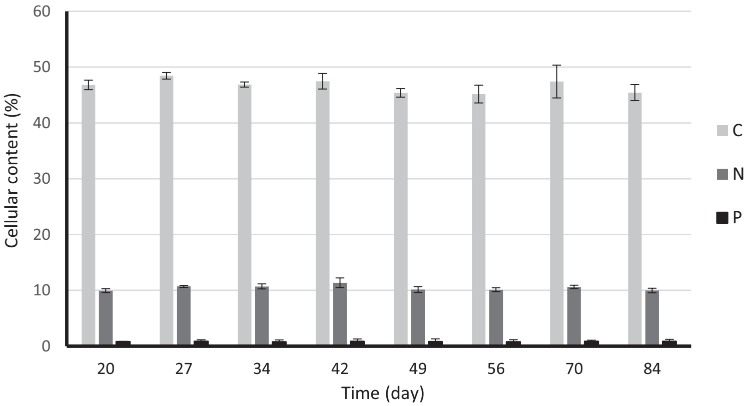

Figure 7. Cellular carbon, nitrogen and phosphorous content in the harvested *Arthrospira platensis* biomass.

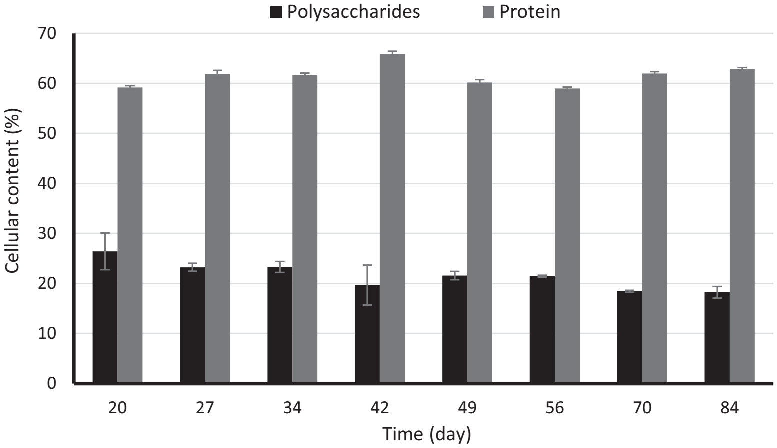

Figure 8. Cellular content (%, on dry weight) of polysaccharides and proteins in the harvested *Arthrospira platensis* biomass.

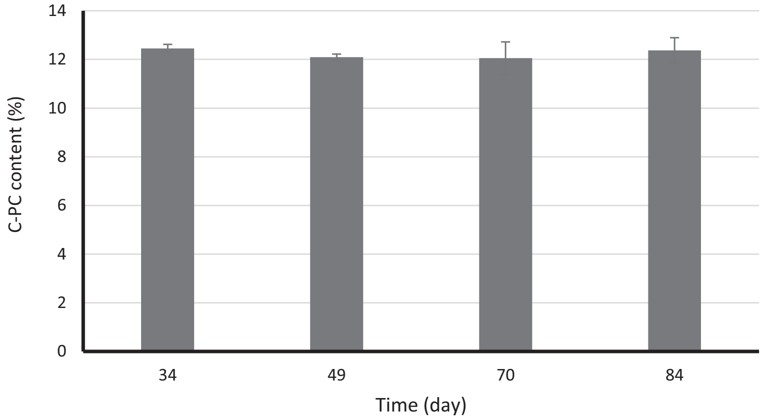

Figure 9. Cellular content (%, on dry weight) of C-phycocyanin in the harvested *Arthrospira platensis* biomass.

Extracellular polysaccharides and contaminants

As shown in Fig. 10, EPS concentration in the ponds at the first harvest was low (ca. 2 mg L^{-1}); subsequently, a slight increase was observed, after which EPS levels remained within a constant range of 5–7 mg L^{-1} for the entire period.

In order to assess food-grade quality of the cultivated *A. platensis*, each fresh biomass batch harvested was analysed for the presence of bacteria, molds, and yeasts, and their abundances were very low (Table 2). Moreover, opportunistic microalgae or zooplankton were rarely found in the biomass during this study (Fig. 11).

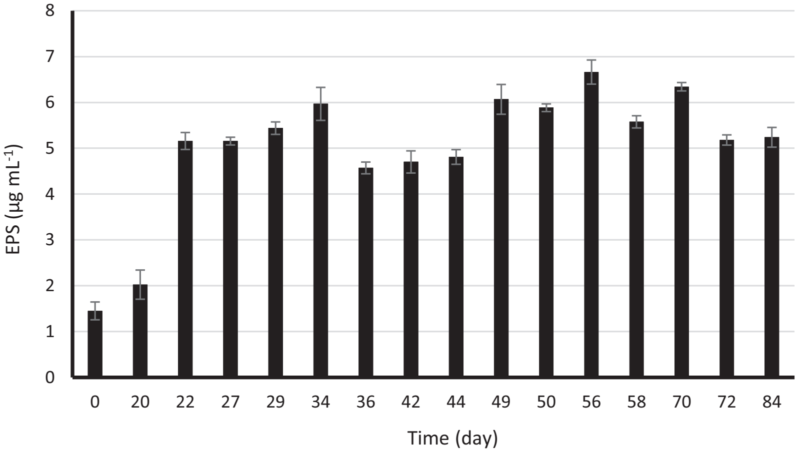

Figure 10. Concentration of extracellular polysaccharides (EPS, mg L^{-1}) in the culture medium of *Arthrospira platensis* for all the growth period considered.

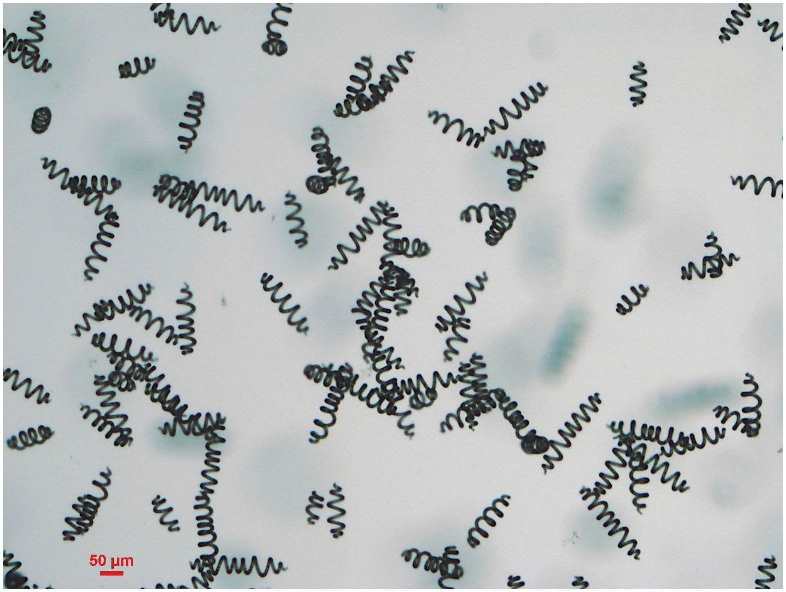

Figure 11. *Arthrospira platensis* culture under the microscope (320×).

Table 2. Contaminants in the fresh biomass batches harvested and analysed in the present study. The biomass from the different ponds was combined in a single batch and analysed.

DAY	BACTERIA	MOLDS	YEASTS
		(CFU g FW biomass^{-1})	
20	7.8*10^3	<40	<10
27	4.8*10^3	<40	<10
34	2.5*10^3	<150	<10
42	8.0*10^3	<40	<10
49	1.7*10^3	<10	<10
56	1.2*10^3	<10	<10
70	2.7*10^3	<10	25

Discussion

Over the study period, the combination of high irradiance and temperature values of 27–29 °C led to maximum productivity from mid-June till the beginning of August. Although *Arthrospira platensis* has an optimal temperature growth range of 35–38 °C, with variability among strains (Belay 2013; AlFahdly et al. 2022a), it can grow under a wider temperature range (20–37 °C, Soni et al. 2017) and values around 30 °C or lower have been reported to promote spirulina growth (e.g., Trabelsi et al. 2009; AlFadhly et al. 2022a).

The maximum volumetric productivity reached a value of 0.038 g $L^{-1}d^{-1}$. It corresponded to an areal productivity of 7.6 g m^{-2} d^{-1}, in line with results reported for various productive plants located in Mongolia (range 5.29–9.09 g m^{-2} d^{-1}; Lu et al. 2011), while it was higher than the one observed in the Canary Islands in ponds of 8000 L

(6 g m^{-2} day^{-1}, Guidi et al. 2021) and lower than those obtained in southern Spain and Italy in very large ponds during summer (450 m^2: 14 g m^{-2} d^{-1}, Jimenez et al. 2003a; 300 m^2: 16 g m^{-2} d^{-1}, Kurpan et al. 2024). This production variability indicates that, besides the geographical location, the size of the culture system and/or its operativity highly influence the performance of the plant, as previously reported by Guieysse and Plouviez (2024), and that the industrial production in temperate zones is feasible with a year-long operativity, even without a heating system, as in northern Italy (Kurpan et al. 2024). The cultivation system of this study, in fact, has a year-long operativity, and the company's historical data attest that the highest productivity values span over a wider period (i.e., from June to October), while they decrease by 25% from February to May (T range: 16–24 °C), reaching the lowest values in December and January, (T range: 8.7–11.2 °C), which correspond to a 65% decrease (data not shown). At this stage, cells still appear healthy and, as soon as the photoperiod and temperature increase, cell growth resumes shortly. While this implies low productivity for a few months, conversely it avoids downtime due to pond emptying and, presumably, ensuring a well-timed turnover of the cultures.

The harvesting approach applied in this study, consisting of the collection of variable and low culture volumes with respect to the pond volume (from 1/6 to 1/4 of the total volume) and based on the biomass concentration in the ponds, ensured that the biomass in the ponds remained in the range 0.300–0.460 g L^{-1}, preventing its exposition to unsuitable irradiance levels (either too high or low). This is consistent with a previous study on repeated batch cultivation (Randmann et al. 2007) showing an optimal growth rate with a blend biomass concentration of 0.400 g L^{-1}, and also with cultivation strategies in larger ponds (Grobbelaar 2008).

Nitrate and phosphate concentrations were strictly monitored to evaluate the accuracy of the nutrient recycling strategy. Replenishment only of the presumably consumed nutrients after each harvest would be expected to maintain stable nutrient concentrations for the entire duration of the cultivation period. In fact, nitrates did not significantly change, whereas the decrease in phosphate concentration in the culture medium could be due to salt precipitation with the progression of biomass growth, as previously reported (Lodi et al. 2003). In addition, deviations of the C/N and N/P cellular ratios from the Redfield ratio (i.e., 6.6 and 16, respectively) could be ascribed to the high N content commonly reported for this species (Guo et al. 2022). The slow nutrient uptake observed, together with the slow consumption of the added carbonate (data not shown), clearly indicate that the recycling of the residual medium from the harvested biomass is an important issue both from an economic point of view and for avoiding the release of nitrogenous compounds in the environment (Depraetere et al. 2015).

A. platensis has important nutritional properties largely due to its high protein content (e.g., Soni et al. 2017), reported in various studies as being between 55 and 70% DW. Repeated reuse of the culture medium was reported to cause a significant protein content decrease from 35.8 to 26.7% (Depraetere et al. 2015) or from 38.64 to 14.25% (Andrade et al. 2019); in these studies, C-PC, which has important commercial applications (Lafarga et al. 2020) and also plays a secondary role as intracellular N storage compounds, also decreased like other pigments, while cellular sugars increased as a response to nutrient depletion. The percentages of proteins and C-PC reported for our cultures remained constant over time; proteins showed an average value of 61.6% which is similar to the average value measured for one year by MBG (65,9%, data not shown) and it is consistent with the protein content reported for most of the commercialized spirulina products (Priyanka et al. 2023). C-PC also had an average value (12.24%) consistent with that found by Xie et al. (2015), i.e., 11% of the total biomass, in spirulina grown under fed-batch conditions with a nitrate concentration of 10 mM. Starting values of storage polysaccharides in the studied cultures are in accord with the range of 13–25% DW previously reported (Priyanka et al. 2023), although this amount is variable depending on the culture medium used and the irradiance available for the cells (Habib et al. 2008). The decrease observed in the last period of monitoring could be linked to the presence of nitrates as the only N source for the cultures; in the absence of ammonium, as in this case (data not shown), the highest consumption of reducing equivalents was observed (Flores and Herrero 2005), in which stored polysaccharides might be involved and utilized. This is also the reason why the presence of C in the medium is one of the most important factors for the optimum growth of this organism and its enzyme activities (Chauhan et al. 2012).

Results of this study clearly highlighted that the biochemical composition of the biomass remained constant despite recycling of the medium after each harvesting step. Previous studies reported that the protein content of the biomass was not affected only through the application of an ultrafiltration method to the medium before its reintroduction in the cultivation system (Han et al. 2025). Most of the studies on the effect of medium recycling on spirulina growth and quality, however, were performed using small vessels and running few cultivation cycles; only a few similar studies were performed in industrial systems, where the recycling of the medium was applied for short periods: one month in the Canary Islands (Guidi et al. 2021) or for periods defined by the increase in extracellular organic matter (Kurpan et al. 2024). This study is the first one that applied this operativity for a long time in a commercial plant.

EPS released from *A. platensis* cells can also provide important information on culture health; in addition, they can severely affect harvesting efficiency by reducing medium filterability. Although this parameter is not often monitored in cultivation systems, the values in our cultures were by far lower than those reported for fresh medium (range: 14–40 mg L^{-1}) (Depraetere et al. 2015; Li et al. 2022). Several factors can influence the release of polysaccharides in the culture medium as a means to protect the cyanobacteria; a study by Phélippé et al. (2019) emphasizes the impact of high and low temperatures, nutrient deficiency, high salinity, and particularly high light intensity. Trabelsi et al. (2009) found that the interaction between temperature and light intensity had a strong influence on EPS concentration in spirulina batch cultures, while when growth conditions were favourable EPS production was low. A further stress factor that could influence EPS synthesis is the recycling of the culture medium. To the best of our knowledge, there are no reports on EPS monitoring in large-scale systems. For small volumes, Li et al. (2022) reported 1.75 times increase in EPS concentration from the first to the third recycling, and, as reported above, concentrations were higher (range: 14 to 22.5 mg L^{-1}) than the ones observed in this study. In Depraetere et al. (2015) extracellular sugars were ca. 40 mg L^{-1} when only fresh medium was used and gradually increased to 200 mg L^{-1} during 45 days of recycled medium addition. In both studies, the recycled medium was applied to a culture volume of about 1 L, indicating that the number of stressors triggering EPS in a small volume is higher than in large ponds where spirulina is in a more favourable environment, similar to that in which spirulina blooms under natural conditions. The low EPS values observed throughout the analyzed period were consistent with the observation that the ponds were regularly free of foam and cells retained their healthy shape (i.e., normal length and spiral form). This, together with the low amounts of contaminants recorded, which were in line with measurements that have been made over the last seven years by the MBG company (data not shown), attest to the good practices used throughout the cultivation process and are in agreement with previously published data (Jimenez et al. 2022a; Guidi et al. 2021). Only on one occasion, following mechanical rupture of the sieving machine, an increase in EPS levels (20–25 mg L^{-1}) and the presence of organic matter flocs in the medium with an enhancement in bacteria numbers (Zerlotin 2021) were observed. During such an event, the presence of opportunistic microalgae or zooplankton consequently increased. Given the lack of a directive for algal biomass, documentary research on similar food products and the analysis of the company's historical data allowed to fine-tune a microbiological values guide to be followed for monitoring the quality of the harvested product. For the administrative region of Emilia-Romagna (where MBG is located), the limit for ready-to-eat vegetables is 1.0×10^6 CFU for total mesophilic bacteria and 1.0×10^2 CFU g^{-1} for molds and yeasts (Regione Emilia-Romagna 2009–2011), which are an order of magnitude higher than those reported here for *A. platensis* biomass.

Conclusions

Weekly monitoring of the medium and *Arthrospira platensis* biomass, collected from four ponds in the productive industrial plant (MBG) located in a temperate zone proved that it is possible to recycle the medium for some months without affecting the quality of the microalga. This was attested by the good biochemical composition of the biomass and the low presence of organic substances and contaminants in the medium. This can be attributed to an appropriate management of the system, considering both operation mode and operational conditions, including a moderate mixing of the ponds, a stringent monitoring of the environment parameters, and a gentle harvesting procedure. These good practices adopted are also testified by the fact that the four ponds showed similar values for each measured parameter, and it is noteworthy that this occurred in a temperate zone with high climate variability. The results of this study allow to conclude that a better operativity in terms of nutrient recycling could be adopted to decrease the level of nutrients in the medium and facilitate its disposal as a waste at the end of the six-month cultivation period. It is thus possible to envisage a solution consisting of gradually decreasing the re-addition of nutrients during the last harvesting steps (e.g., in the last month of the culturing period). Further studies performed at an industrial plant could be aimed at testing if a decrease of nutrient replenishment could be performed without affecting the quality of the biomass leading to economic savings and an improvement in the environmental footprint of the cultivation, thus achieving a more sustainable process.

Author contributions:

Rossella Pistocchi: Conceptualization, Project administration, Resources, Validation, Visualization, Writing – original draft. Franca Guerrini: Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review and editing. Silvana Vanucci: Methodology, Validation, Visualization, Writing – review and editing. Lorenzo Montalbetti: Data curation, Formal analysis, Visualization, Methodology, Investigation, Writing – review and editing. Mara Simonazzi: Data curation, Formal analysis, Visualization, Methodology, Investigation, Writing – review and editing. Matilde Mazzotti: Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – review and editing. Federica Abbondanzi: Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – review and editing. Guido Emiliani: Data curation, Funding acquisition, Project administration, Writing – review and editing. Laura Pezzolesi: Conceptualization, Resources, Validation, Visualization, Writing – review and editing.

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RESEARCH ARTICLE

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Typification of names allied to *Centaurea ambigua* (Asteraceae) from the Apennine Peninsula (Italy)

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Abstract

A contribution to the nomenclature of the Italian endemic *Centaurea ambigua* is presented. A survey of original material for the names applied to the three currently accepted subspecies (*C. ambigua* subsp. *ambigua*, *C. ambigua* subsp. *laciniata*, *C. ambigua* subsp. *nigra*) was performed. Lectotypes for five names based on specimens preserved at FI, G and NAP are designated: *C. ambigua*, *C. dissecta* var. *macrocephala*, *C. dissecta* var. *melanocephala*, *C. dissecta* var. *nigra*, and *C. laciniata*. Finally, *C. ambigua* subsp. *laciniata*, actually considered a subspecies narrowly endemic to Abruzzo (central Italy), is proposed here as a heterotypic synonym of *C. ambigua* subsp. *ambigua*.

Keywords

Endemism, Lectotype, Nomenclature, Taxonomy

Introduction

The genus *Centaurea* L. with more than 700 currently accepted species (POWO 2024+) and with 1342 accepted names in the world (WFO 2024+) is one of the largest genera of the Asteraceae Berchtold & J.Presl. It is traditionally cosidered as taxonomically critical genus, due to hybridization and polyploidy, and has been widely investigated from a systematic and taxonomic point of view in the last two decades (e.g., Conti et al. 2011; Pisanu et al. 2011; Hilpold et al. 2014a, 2014b; López-Alvarado et al. 2014;

Domina et al. 2017; Garcia-Jacas et al. 2019; Stinca et al. 2019; Del Guacchio et al. 2020; Novaković et al. 2022; De Luca et al. 2023; Giacò and Peruzzi 2024).

Centaurea ambigua Guss. is a polymorphic species endemic to the Apennine Peninsula distributed from Emilia-Romagna to Campania (Peruzzi et al. 2014; Pignatti et al. 2018; Bartolucci et al. 2024), in which three subspecies are currently recognized (Bartolucci et al. 2024): *C. ambigua* subsp. *ambigua* endemic to the Apennines, *C. ambigua* subsp. *laciniata* (Guss. ex DC.) Arcang. endemic to Abruzzo, and *C. ambigua* Guss. subsp. *nigra* (Fiori) Pignatti endemic to central Italy. This latter taxon was regarded by Guarino and Rampone (2006) as a separate species named *C. delucae* C.Guarino & Rampone. Furthermore, within the distribution range of the three cited subspecies several distinct morphological varieties or forms have been described, usually linked to single localities. To date, the names of the three accepted taxa mentioned above are not yet typified (Peruzzi et al. 2015).

Here we present a first nomenclatural contribution that is part of an ongoing study of the systematics and taxonomy of *C. ambigua*, and we designate the nomenclatural types for five names.

Material and methods

This study is based on an extensive literature analysis and detailed examination of herbarium specimens (including the original material) kept in APP, FI, G, and NAP (codes according to Thiers 2024+) and primarily available through online resources (i.e. FI: http://parlatore.msn.unifi.it/types/search.php; G: https://www.ville-ge.ch/musinfo/bd/cjb/chg/index.php?lang=en). Type designations follow the Shenzhen Code (hereafter "ICN"; Turland et al. 2018).

For each name, we list the type specimen citation and relevant data indicated in the protologue ("protologue citation"). Further nomenclatural and/or taxonomical notes are provided. Names (accepted names are given in bold italics) are listed in alphabetical order of their basionyms. The images of the designated type specimens are provided in Suppl. material 1.

Typification of the names

Centaurea ambigua **Guss., Pl. Rar.: 360(–362, pl. 61). 1826. [after 4 June 1826] subsp.** ***ambigua***

≡ *Centaurea dissecta* var. *ambigua* (Guss.) Fiori in Fiori & Bég., Fl. Italia 3(2): 336. 1904. [April 1904]

Protologue citation. "In collibus aridis, et in cultis Aprutii; Caramanico, Roccamorice".

Lectotype (designated here). [Italy, Abruzzo] tra Roccamorice e S. Spirito alla Majella nei siti calcarei, 20 August 1824, *Gussone s.n.* (NAP barcode NAP0005395 [digital photo!], Suppl. material 1: fig. S1).

Nomenclatural note. Gussone (1826) described *C. ambigua* providing an extended diagnosis and a detailed description, both in Latin, and a precise locality. Furthermore, the author cited in the protologue the illustration "Ic. nostra t. 61". We performed a survey of the original material at NAP, where Gussone's main collection is housed (Stafleu and Cowan 1976) and maintained as two separate collections, named "Gussone Generale" and "Gussone Sicilia" (La Valva 1993; Santangelo and Strumia 2014). We traced three specimens collected by Gussone before the description of the species in the collection "Gussone Generale": 1) the herbarium specimen NAP0005395 bears a label added by L. Grande "*Centaurea ambigua* Guss! | tra Roccamorice e Santo Spirito | 20 agosto 1824 legit Gussone!"; 2) the herbarium specimen NAP0005395 bears two original labels *manu* Gussone not linked with the two individuals on the sheet (a whole individual on the left and a rosette of basal leaves on the right) "*Centaurea ambigua* [Guss.! added by L. Grande]| 20 Agosto 1824 | tra Roccamorice e S. Spirito | alla Majella nei siti calcarei" and "(a) 20 Ago 1824 | tra Roccamorice e S. | Spirito nei luoghi | collinari"; 3) the herbarium specimen NAP0005396 bears a label *manu* Gussone "(b) *Centaurea* | *ambigua* [Guss.! added by L. Grande] | 1824 Roccamorice | […]". The morphology of the three herbarium specimens is congruent with the protologue and with the current application of the name (Pignatti et al 2018; Bartolucci et al. 2024). Along with the illustration cited in the protologue, they can be safely considered as original material (Art. 9.4 of the ICN). We select here the herbarium specimen NAP0005395 as the lectotype for the name *C. ambigua*.

Taxonomic note. Based on a preliminary analysis of several herbarium specimens in APP collected within the putative native range (Apennines, from Emilia-Romagna to Campania), the typical *C. ambigua* (small capitula and pale involucral bracts), seems restricted to the low mountainous, calcareous sectors of the northern Maiella (Abruzzo, central Italy). Records from other areas in Abruzzo or other Italian administrative regions should be verified, and a more in-depth study is be needed to delineate the exact distribution range of *C. ambigua* subsp. *ambigua*.

Centaurea dissecta var. *macrocephala* Groves ex Fiori in Fiori & Bég., Fl. Italia 3(2): 335. 1904. [April 1904]

Protologue citation. "Abr. a Macchia Lunga (Groves!)".

Lectotype (designated here). [Italy, Abruzzo] Macchia lunga, Abruzzi, 1888, *Profeta s.n. ex Herb. Groves* (FI barcode FI058574 [digital photo!], Suppl. material 1: fig. S2).

Nomenclatural note. The name *Centaurea dissecta* var. *macrocephala* is legitimate even though it was published under the illegitimate *C. dissecta* Ten. (1812: LI), a later homonym of *C. dissecta* Hill (1762) (Art. 55.2 of the ICN). Fiori (1904) published this name providing a short description and quoting only a single locality. We traced in FI the specimen barcoded FI058574 bearing the label "*Centaurea dissecta* Ten. forma *macrocephala*, Macchia lunga, Abruzzi, 1888, *Profeta ex Herb. Groves*". This specimen is complete, well preserved and agree with the protologue and can be safely considered as original material (Art. 9.4 of the ICN). The herbarium specimen FI058574 is selected here as lectotype for the name *C. dissecta* var. *macrocephala*.

The type locality "Macchia lunga" is probably a toponym close to Roccapia (L'Aquila), where other botanists at that time have made some gatherings (e.g., E. Levier). Furthermore, there are other samples collected by Profeta (see nomenclatural notes of *C. dissecta* var. *melanocephala*) around that area (i.e. Mt. Genzana, L'Aquila). In addition, the morphological features of the type specimen of *C. dissecta* var. *macrocephala* clearly correspond with those observed by us in the specimens collected in the area of Mt. Genzana.

Taxonomic note. This variety, based on the studied type specimen and on field observations, is morphologically different from the subspecies currently recognized under *C. ambigua*. Pending further in-depth systematic and taxonomic studies, we provisionally include this variety in the variability of *C. ambigua* subsp. *nigra*, primarily for the black involucral bracts.

***Centaurea dissecta* var. *melanocephala* Fiori in Fiori & Bég., Fl. Italia 3(2): 335. 1904. [April 1904]**

Protologue citation. "Abr. al Sirente e M. Lengiana"—*Centaurea dissecta* var. *humilis* Groves, in schedis.

Lectotype (designated here). [Italy, Abruzzo] in glareosis Montis Sirente in Aprutio (sub Canale), July 1877, *Profeta s.n. ex Herb. Groves* (FI barcode FI058575 [digital photo!], Suppl. material 1: fig. S3).

Nomenclatural note. The name *Centaurea dissecta* var. *melanocephala* is legitimate even though it was published under the illegitimate *C. dissecta* Ten. (1812: LI), a later homonym of *C. dissecta* Hill (1762) (Art. 55.2 of the ICN). Fiori (1904) described *C. dissecta* var. *melanocephala* providing a short description and quoting two localities. We traced in FI several relevant specimens labeled by Fiori as *C. dissecta* var. *melanocephala*. The specimen FI058575 is labeled "*Centaurea dissecta* var. D *humilis* Ten. (fide Levieri), in glareosis Montis Sirente in Aprutio (sub Canale), 7/77, *Profeta ex Herb. Groves*"; the specimen FI058576 is labeled "*Centaurea dissecta* var. D *humilis* Ten. (Ten. errore sub *C. cinerea*), ad rupes Montis Sirente versus Canale, 5300, 7/75, *Groves*"; the specimen FI058577 is labeled "*Centaurea dissecta* var. D *humilis* Ten. (fide Levieri), ad rupes Montis Sirente in Aprutio, 5000, 7/77, *Profeta ex Herb. Groves*"; the specimen FI058578 is labeled "*Centaurea dissecta* var. D *humilis* Ten. (Ten. errore sub *Cent. cinerea*) […] ad Rupes montis Canale, Sirente, 5300, 7/76, *Profeta ex Herb. Groves*"; the specimen FI058579 is labeled "*Centaurea dissecta* Ten., Monte Genziana [Genzana], Abruzzi, 1889, *Profeta ex Herb. Groves*". For the latter specimen, Fiori reports the locality "M. Lengiana", but certainly due to an incorrect interpretation of the spelling, which clearly shows "M. Genziana", now Mt. Genzana. These specimens can be safely considered as original material, and their morphology is congruent with the protologue (Art. 9.4 of the ICN). We select here the herbarium specimen FI058575 as the lectotype for the name *C. dissecta* var. *melanocephala*.

Taxonomic note. Pending further in-depth systematic and taxonomic studies, we provisionally include this variety in the variability of *C. ambigua* subsp. *nigra*.

Centaurea dissecta **var.** ***nigra*** **Fiori in Fiori & Bég., Fl. Italia 3(2): 335. 1904. [April 1904]**

≡ *Centaurea parlatoris* subsp. *nigra* (Fiori) Dostál, Bot. J. Linn. Soc. 71: 197. 1976.
≡ *Centaurea ambigua* subsp. *nigra* (Fiori) Pignatti, Giorn. Bot. Ital. 113(5–6): 367. 1980.
≡ *Centaurea delucae* C.Guarino & Rampone, Bocconea 19: 85. 2006.

Protologue citation.“Abr. al M. Velino (Levier!), Gr. Sasso al Corno Grande (Levier!) ed a Pizzo Cefalone (Martelli!) e Camp. al M. Meta (Levier hb.!); reg. alp.”.

Lectotype (designated here).—[Italy, Abruzzo] Gran Sasso d’Italia. Rupi della Traversa di Pizzo Cefalone, 16 August 1893, *U. Martelli s.n.* (FI barcode FI058572 [digital photo!], Suppl. material 1: fig. S4).

Note. The name *Centaurea dissecta* var. *nigra* is legitimate even though it was published under the illegitimate *C. dissecta* Ten. (1812: LI), a later homonym of *C. dissecta* Hill (1762) (Art. 55.2 of the ICN). Fiori (1904) described *C. dissecta* var. *nigra* providing a short description, and quoting several herbarium samples and their collectors. We traced in FI some of the herbarium specimens cited by Fiori in the protologue: the duplicate specimens barcoded FI058572 and FI058582 are labeled “Gran Sasso d’Italia, rupi della Traversa del Pizzo Cefalone, 16 agosto 1893, *U. Martelli*”; the specimen FI058573 is labeled “Gran Sasso d’Italia (Corno Grande) ad 26-2700 metri circa, 21 agosto 1879, *E. Levier*”. The morphology of these specimens is congruent with the protologue and with the current application of the name (Pignatti et al. 2018; Bartolucci et al. 2024), and they can be safely considered as original material (Art. 9.4 of the ICN). We select here the herbarium specimen FI058572, bearing the label “*Centaurea dissecta* Ten. var. *nigra* Nob.” made by Fiori, as the lectotype for the name *C. dissecta* var. *nigra*.

Centaurea laciniata **Guss. ex DC., Prodr. 6: 582. 1838. [early January 1838]**

≡ *Centaurea ambigua* subsp. *laciniata* (Guss. ex DC.) Arcang., Comp. Fl. Ital.: 392. 1882.
≡ *Centaurea dissecta* var. *ambigua* f. *laciniata* (Guss. ex DC.) Fiori in Fiori & Bég., Fl. Italia 3(2): 336. 1904. [April 1904]

Protologue citation. “In asperis regni Neapolitani ad Aprutium. Cent. ambigua Ten. itin. ex Guss.”.

Lectotype (designated here). [Italy, Abruzzo] Napoli/Abruzzo, *1831*, *Gussone s.n.* (G barcode G00487426; the image of the lectotype is available at https://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=339609&lang=en).

Nomenclatural note. de Candolle described *Centaurea laciniata* (1838) providing a description and a generic locality. In G-DC (barcode G00487426) we traced one specimen under the name "*C. laciniata* Nob. ined." This is a specimen sent to de Candolle by Gussone under the name "*C. laciniata* Guss. ined.". This sample was collected in Abruzzo (without indication of a specific locality) and on the label de Candolle reports "*De hoc loquitur Tenore in itinere a Monte Corno sub Cent. ambigua nomine* [Tenore speaks of this on his journey from Monte Corno under the name Cent. ambigua]", information that does not help to identify the collection locality. The herbarium specimen is complete, well preserved, agrees with the protologue, and can be considered as original material (Art. 9.4 of the ICN). Although it might potentially be a holotype, at the same time there is a possibility of further original material in other herbaria. Accordingly, the specimen G00487426 is prudentially selected here as lectotype for the name *C. laciniata.*

Taxonomic note. The name *Centaurea laciniata*, currently accepted as *C. ambigua* subsp. *laciniata*, applies to plants narrowly endemic to Abruzzo, central Italy (Bartolucci et al. 2024; POWO 2024+). This subspecies is traditionally distinguished only by its undivided lower leaves and pinnatifid upper leaves (Arcangeli 1882; Fiori 1904). These characters are inconsistent and without taxonomic value, also occuring in *C. ambigua* s.str. Thus, *C. laciniata* should be considered as a heterotypic synonym of *C. ambigua* subsp. *ambigua*.

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Supplementary material 1

Digital images of the designated type specimens

Authors: Fabrizio Bartolucci, Fabio Conti

Data type: pdf

Explanation note: **figure S1.** Lectotype (NAP0005395) of the name *Centaurea ambigua* Guss. kept at NAP (reproduced with permission of the Herbarium, University of Naples, Italy). **figure S2.** Lectotype (FI058574) of the name *Centaurea dissecta* var. *macrocephala* Groves ex Fiori kept at FI (reproduced with permission of the Herbarium, Museum of Natural History, University of Florence, Italy). **figure S3.** Lectotype (FI058575) of the name *Centaurea dissecta* var. *melanocephala* Fiori kept at FI (reproduced with permission of the Herbarium, Museum of Natural History, University of Florence, Italy). **figure S4.** Lectotype (FI058572) of the name *Centaurea dissecta* var. *nigra* Fiori kept at FI (reproduced with permission of the Herbarium, Museum of Natural History, University of Florence, Italy).

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RESEARCH ARTICLE

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Contribution to the knowledge of the bryophyte flora of the Lucanian side of the Pollino National Park

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Abstract

The findings of the Bryological Excursion conducted by the Working Group for Bryology of the Italian Botanical Society from 5 to 8 July 2012 on the Lucanian side of the Pollino National Park are presented herein. The objective of the excursion was to address the paucity of knowledge regarding bryological diversity in understudied regions of Italy. This endeavour led to the discovery of 150 species of bryophytes, 41 of which are novel to the Basilicata region. For each species, the collection location and growth substrate are indicated. Some of the species are of floristic and phytogeographic interest. This is a group of boreal and arctic-alpine species, previously documented only in the Alpine arc and at the highest peaks of the Apennine chain. The Pollino massif represents the southernmost limit of their range. It is worth noting the presence of *Brachydontium trichodes*, *Schistidium papillosum*, *Tortula laureri* and *Herzogiella striatella*. Another noteworthy assemblage of species is found on the ophiolitic rocks of Timpa delle Murge. The distinctive ecological characteristics of this substrate result in the evolution of species that are exclusive to these environments. The ecological characteristics and distribution of each species within these two groups are described in detail.

Keywords

Biodiversity, bryophytes, chorology, floristic novelties, Pollino massif

Introduction

The Pollino massif represents the highest mountain range of the Southern Apennines, situated in Italy between the Basilicata and Calabria regions. This mountain range constitutes, along with the Verbicaro-Orsomarso mountains, part of the Pollino National Park, which, with an area of 1,930 km², is the largest national park in Italy. The protected area was established primarily to protect the rare *Pinus leucodermis* Antoine species (Fig. 1), which is characteristic of the mountain peaks and has been documented to exceed one hundred years of age and reach heights of up to 40 meters (Avolio 1996).

In addition to this species, the Pollino National Park exhibits several other distinctive floristic features as a result of its geographical location, which allows for the formation of a range of different biogeographical links. The biogeographical relation with the southern Balkan district is undoubtedly the most evident, as evidenced by the occurrence of a considerable number of amphi-adriatic species, including *Pinus leucodermis* Antoine and *Festuca bosniaca* Kumm. Furthermore, the following species have been identified: *Carex kitaibeliana* Degen ex Beck, *Edrajanthus graminifolius* (L.) A.DC. ex Meisn., *Sesleria autumnalis* (Scop.) F.W. Schultz, *Gentianella crispata* (Vis.) Holub, *Cytisus spinescens* Presl, and other species frequently exert a significant influence on the vegetational pattern (Bonin 1969, 1972). Concurrently, the Pollino massif represents the southernmost limit for various boreal or arctic-alpine species, including *Orthilia secunda* (L.) House, *Pyrola minor* L., *Chrysosplenium dubium* J.Gay ex Ser., *Saxifraga aizoides* L., and *Carex pallescens* L. Additionally, species such as *Carex vesicaria* L., *Senecio alpinus* (L.) Scop.,

Figure 1. *Pinus leucodermis* Antoine in the Serra di Crispo (2050 m a.s.l.).

and others, which were distributed across the Italian Peninsula during the ice ages, persisted as isolated relicts in the postglacial period (Bernardo 1995; Bernardo et al. 2000).

Regarding the vegetation, it can be observed that above 800 m a.s.l. and up to 1100 m a.s.l. in the supramediterranean belt, forests of deciduous trees are present, with the most common species being *Quercus pubescens* Willd., *Q. cerris* L., *Q. frainetto* Ten., often mixed with *Carpinus orientalis* Mill., *Castanea sativa* Mill., and *Alnus cordata* (Loisel.) Duby. It is noteworthy that *Fagus sylvatica* L. can grow alongside *Abies alba* Mill. in the Pollino massif. Up to approximately 1500 m a.s.l., the Anemono apenninae-Fagetum (Gentile 1970) Brullo 1983 (Di Pietro 2009) association can be observed. Above the tree limit, the dry grasslands of the alliance Seslerion apenninae Furnari in Bruno and Furnari (1966) (Di Pietro 2010) are found. The presence of *Pinus leucodermis* serves to distinguish and define the unique mountain and high-mountain vegetation of the park. This species is found in isolated or small populations, climbing rough rock walls and exhibiting resilience to strong winds.

From a geological perspective, the Pollino massif is predominantly composed of Meso-Cenozoic limestone rocks of sedimentary origin, with outcrops of ophiolitic rocks (basalt vulcanites) characterising the Timpa della Murge and the Timpa di Pietrassasso. The Orsomarso mountains exhibit a geology that is analogous to that of the Pollino massif. The pervasive occurrence of limestone gives rise to the prevalence of karst phenomena, including sinkholes, caves, subterranean rivers, and striking geological features such as canyons (Bonardi et al. 1988).

In terms of the bioclimate, the mountainous region is characterized by a temperate-oceanic bioclimate with an orotemperate thermotype and hyperhumid umbrotype at elevations above 1500 m a.s.l. Additionally, a temperate-Mediterranean transition climate is observed at elevations between 1000 m and 1500 m a.s.l., characterised by a supratemperate thermotype and humid umbrotype (Blasi 2006).

Despite the notable botanical interest of the massif, the bryophyte flora of this mountain system in particular and of the Basilicata Region in general remains relatively understudied. In fact, most of the reports are derived from a limited number of incomplete studies dating from the late 19th and early 20th centuries (Pasquale 1850; Giordano 1871, 1879, 1892; Bottini et al. 1883; Brizi 1890; Bottini 1894; Micheletti 1895; Bottini 1909; Zodda 1909, 1910, 1913; Cavara and Grande 1913; Gavioli 1934; Giacomini 1938); the most recent contributions to the knowledge of the bryoflora of the Pollino massif were made by: Puglisi et al. (2009) with the report of *Calliergon cordifolium* (Hedw.) Kindb., *Marchantia polymorpha* subsp. *montivagans* Bischl. & Boisselier and *Marchantia polymorpha* L. subsp. *polymorpha*; Puglisi et al. (2014) with the report of *Orthotrichum cupulatum* Hoffm. ex Brid. var. *fuscum* (Venturi) Boulay, *Schistidium dupretii* (Thér.) W.A.Weber, *Distichium inclinatum* (Hedw.) Bruch & Schimp., *Grimmia longirostris* Hook and *Orthotrichum acuminatum* H.Philib.; Puglisi et al. (2016a) (2016b) that they report *Mnium spinosum* (Voit) Schwägr., *Brachythecium tommasinii* (Sendtn. ex Boulay) Ignatov & Huttunen, *Campylidium sommerfeltii* (Myrin) Ochyra, *Grimmia laevigata* (Brid.) Brid., *G. montana* Bruch & Schimp., *G. tergestina* Tomm. ex Bruch & Schimp., *G. trichophylla* Grev. and *Orthotrichum pumilum* Sw.; and finally Puglisi and Privitera (2018) with the report of *Anomodon rugelii* (Müll.Hal.) Keissl.

The most complete research carried out recently for the entire region is that by Colacino et al. (2013) which reports 55 new taxa (47 mosses and 8 liverworts) for Basilicata Region and 90 confirmations of taxa (85 mosses and 5 liverworts) reported before 1950. Nevertheless, the most recent checklist by Aleffi et al. (2023) documents for the Basilicata Region only 272 taxa (232 mosses and 40 liverworts), which represents, together with Molise, the Italian region with the lowest number of reported taxa.

The annual excursion of the Working Group for Bryology of the Italian Botanical Society was conducted precisely on the Lucanian side of the Pollino massif. The aim was to fill in the gaps existing in some areas of the Lucanian territory that are little explored and little known from a bryological point of view. The excursion highlighted the presence in the explored area of some remarkable mosses, which merit special consideration both from a floristic and a biogeographical point of view.

Study area

The study area is situated within the Basilicata region, at an altitude ranging between 950 and approximately 2000 meters above sea level. The excursion entailed an exploration of the beech forests situated in the Acqua Tremola locality (Terranova del Pollino, loc. Case del Conte), after which the route continued towards Serra di Crispo-Grande Porta del Pollino. This area is included in the SCI IT9210245, which encompasses Serra di Crispo, Grande Porta del Pollino, and Pietra Castello. From the Grande Porta (1957 m a.s.l.), the route continues in a northerly direction towards Serra di Crispo (2053 m a.s.l.). This area is notable for its so-called ‘Garden of the Gods’, which contains majestic, centuries-old specimens of *Pinus leucodermis*. In this area, the vegetation, which is strongly influenced by the wind, consists of high-altitude grasslands and scattered shrub vegetation, characterised by krummholz of *Juniperus nana* Willd. and *J. hemisphaerica* J. & C. Presl. (Fig. 2).

From Grande Porta we descended towards Piani di Pollino and then towards Lago Fondo (1508 m) and Lago Duglia (1375 m), small lakes that vary in size depending on the season, passing Pietra Castello (1671 m) and crossing beech-abetine woods with *Abies alba*. The second part of the excursion focused on the Fosso Jannace gorge, a canyon characterised by several areas of beech, fir and monumental maple trees, as well as the presence of several wetlands and waterfalls, and then climbed towards the La Catusa spring (Fig. 3) and the Frido stream to study the riparian vegetation. Finally, at Timpa delle Murge, several areas with outcrops of ophiolitic rocks (basalt volcanites) and Mediterranean vegetation with *Ilex aquifolium* L. were explored.

Materials and methods

Identification of bryophytes was based on Cortini Pedrotti (2001, 2005), Smith (2004) and Casas et al. (2006) for mosses; Smith (1991), Paton (1999) and Casas et al. (2009) for liverworts. The nomenclature follows Hodgetts et al. (2020). Species

Figure 2. In the foreground krummholz of *Juniperus nana* Willd. and *J. hemisphaerica* J. & C.Presl.

Figure 3. Beech forest and rochy outcrops in the La Catusa spring area.

distributions follow Aleffi et al. (2023). Taxa are listed in alphabetical order with the locality, habitat of collection and the Italian IUCN Threat Category (Puglisi et al. 2023, 2024). The specimens are kept in the herbaria of the participants of the excursion. Taxa new to Basilicata are indicated with an asterisk (*), while a cross (+) marks taxa reported before 1968. The bryophyte flora was studied in the following areas (Fig. 4):

1. Acqua Tremola (Loc. Case del Conte) (1474 m a.s.l.) 39°58'02.2"N, 16°13'01.1"E.
2. Acqua Tremola trail towards Serra di Crispo (1700 m a.s.l.) 39°57'41.3"N, 16°12'40.9"E.
3. *Pitt'accurc'* source, under Serra di Crispo (1846 m a.s.l.) 39°55'58"N, 16°12'20"E.
4. Under Piano Iannace (1650 m a.s.l.) 39°56'41.55"N, 16°11'43.87"E.
5. Piano Jannace (1926 m a.s.l.) 39°55'52"N, 16°12'36.3"E.
6. Serra di Crispo (1985 m a.s.l.) 39°55'55.5"N, 16°12'43.3"E.
7. Serra di Crispo (2050 m a.s.l.) 39°56'06"N, 16°12'40"E.
8. Pietra Castello trail towards Lago Fondo (1876 m a.s.l.) 39°56'40"N, 16°12'57"E.
9. Piano S. Francesco (1500 m a.s.l.) 39°57'15"N, 16°12'18"E.
10. Lago Fondo (1500 m a.s.l.) 39°56'46"N, 16°13'38"E.
11. Serretta della Porticella (1985 m a.s.l.) 39°56'17"N, 16°12'28"E.
12. Fontana Rummo (1720 m a.s.l.) 39°55'12"N, 16°11'24"E.
13. Piano di Vacquarro (1480 m a.s.l.) 39°55'29"N, 16°10'34"E.
14. Lago Duglia (1375 m a.s.l.) 39°56'58"N, 16°13'58"E.
15. Lago Duglia (old sawmill) (1387 m a.s.l.) 39°56'54"N, 16°14'42"E.
16. Frido torrent, Mezzana Salice Conocchielle (947 m a.s.l.) 39°58'45"N, 16°10'23"E.
17. Municipal road of San Severino Lucano 39°57'55"N, 16°11'48"E.
18. Timpa delle Murge (between Case del Conte and La Catusa source) (1216 m a.s.l.) 39°58'47"N, 16°15'10"E.
19. La Catusa source, San Giorgio Lucano (1300 m a.s.l.) 39°59'16"N, 16°14'26"E.

Results

The research carried out in the Lucanian part of the Pollino National Park led to the identification of 150 bryophyte taxa (14 liverworts and 136 mosses). According to the Checklist of Italian Bryophytes (Aleffi et al. 2023), 2 liverworts and 39 mosses are new to Basilicata, while 10 other moss taxa recorded before 1968 were confirmed for the region. The list of bryophytes collected during this research is presented below, with an indication of the location and collection environment.

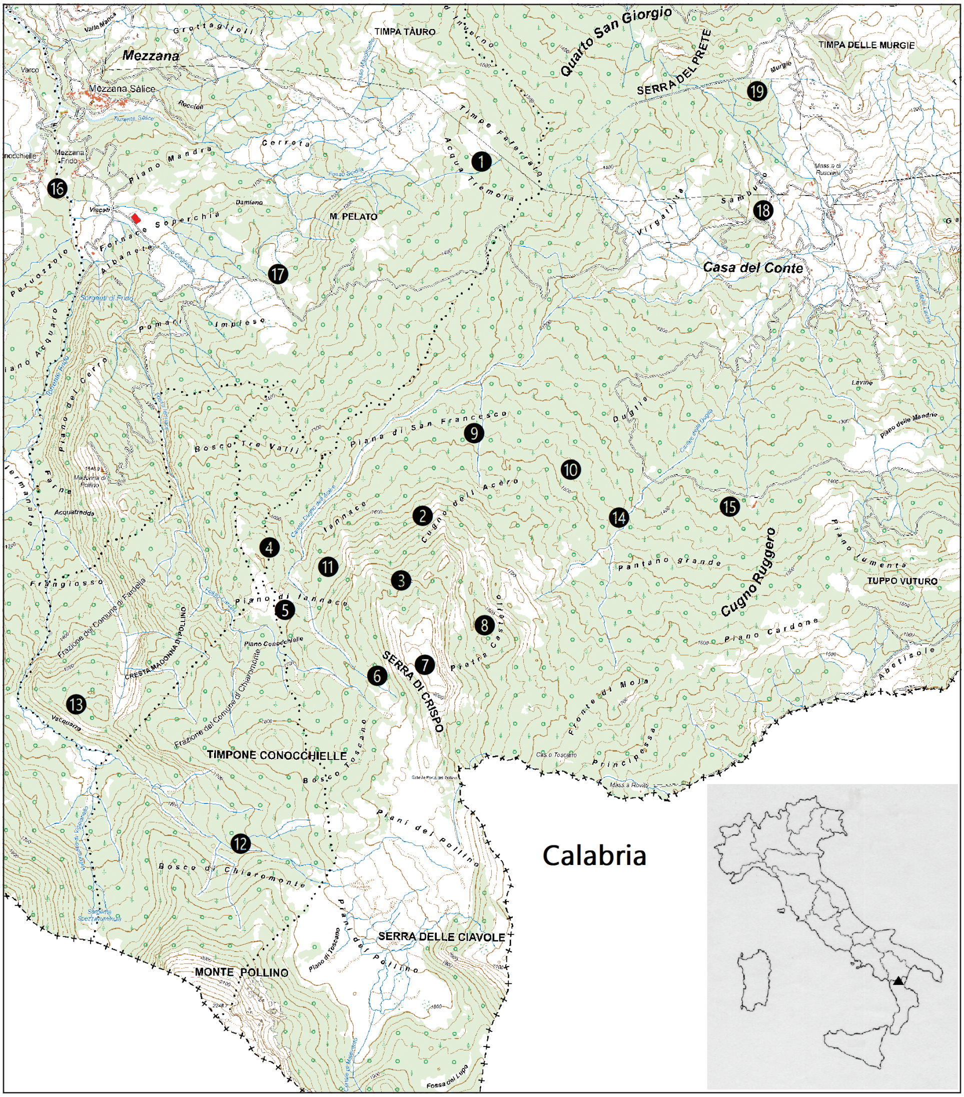

Figure 4. Map of the study area showing the collection sites.

List of taxa

Liverworts

**Cephaloziella baumgartneri* Schiffn.: on stump of *Fagus sylvatica* (8) (LC).

Frullania dilatata (L.) Dumort.: on bark of *Fagus sylvatica* (14, 15) and *Populus alba* (16) (LC).

Lophocolea bidentata (L.) Dumort.: on soil along the river (15) (LC).

Marchantia polymorpha L. subsp. *polymorpha*: on damp soil near the fountain (13) (LC).

Metzgeria furcata (L.) Corda: on bark of *Fagus sylvatica* (1, 15) (LC).
**Pellia epiphylla* (L.) Corda subsp. *epiphylla*: on ophiolitic rocks (18) (LC).
Plagiochila asplenioides (L.) Dumort.: on rocks (8, 12) (LC).
Plagiochila porelloides (Torr. ex Nees) Lindenb. var. *porelloides*: on rocks below *Pinus leucodermis* (7); on rocks (19) (LC).
Porella cordeana (Huebener) Moore: on rocks (11) (LC).
Porella platyphylla (L.) Pfeiff.: on bark of *Fagus sylvatica* (1); on rocks (5, 15); on soil (14); on stump of *Fagus sylvatica* (15) (LC).
Radula complanata (L.) Dumort.: on bark of *Fagus sylvatica* (1, 14, 15) and *Populus alba* (16); on rocks (5, 19); on stump of *Fagus sylvatica* (15) (LC).
Radula lindenbergiana Gottsche ex C.Hartm.: on rocks (8) (LC).
Reboulia hemispherica (L.) Raddi subsp. *hemisphaerica*: on damp soil (16) (LC).
Solenostoma gracillimum (Sm.) R.M.Schust.: on rocks below *Pinus leucodermis* (7) (LC).

Mosses

**Bartramia ithyphylla* Brid.: on rocks (11) (LC).
**Brachydontium trichodes* (F.Weber) Milde: on soil (14) (EN).
Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen: on calcareous rocks (2, 8); on soil near the river (8); on stump of *Fagus sylvatica* (8, 15) (LC).
+*Brachythecium albicans* (Hedw.) Schimp.: on soil and on rocks (5) (LC).
**Brachythecium cirrosum* (Schwägr.) Schimp.: on soil (29) (LC).
**Brachythecium mildeanum* (Schimp.) Schimp.: on soil near the river (8) (LC).
Brachythecium rivulare Schimp.: on damp soil and rocks near the river (8, 13, 15, 19) (LC).
Brachythecium rutabulum (Hedw.) Schimp.: on soil near the river (15, 16, 19) (LC).
Brachythecium salebrosum (Hoffm. ex F.Weber & D.Mohr) Schimp.: on stump of *Fagus sylvatica* (8); on bark of *Populus alba* (16) (LC).
Brachythecium tommasinii (Sendtn. ex Boulay) Ignatov & Huttunen: on damp rocks (4, 5, 19) (LC).
+*Bryoerythrophyllum recurvirostrum* (Hedw.) P.C.Chen: on soil (2, 6) (LC).
Bryum canariense Brid.: on bark of *Fagus sylvatica* (1); on ophiolitic rocks (18) (LC).
Bryum dichotomum Hedw.: on calcareous (3) and ophiolitic rocks (18) (LC).
Calliergonella cuspidata (Hedw.) Loeske: on damp soil near the fountain (13) (LC).
Ceratodon purpureus (Hedw.) Brid. subsp. *purpureus*: on soil (6, 17); on ophiolitic rocks (18) (LC).
Cirriphyllum crassinervium (Taylor) Loeske & M. Fleisch.: on soil (14) (LC).
Cratoneuron filicinum (Hedw.) Spruce: on rocks (8, 19) and on soil near the river (8, 16) (LC).
Ctenidium molluscum (Hedw.) Mitt.: on rocks (5) (LC).
**Dichodontium pellucidum* (Hedw.) Schimp.: on rocks (5) (LC).
Dicranella heteromalla (Hedw.) Schimp.: on bark of *Pinus leucodermis* (7); on stump of *Fagus sylvatica* (15); on soil (14, 15) (LC).
+*Dicranum scoparium* Hedw.: on soil (2, 13, 15); on rocks (11) (LC).

**Dicranum tauricum* Sapjegin: on stump of *Fagus sylvatica* (8); on rocks (12); on soil (15) (LC).
Distichium capillaceum (Hedw.) Bruch & Schimp.; on rocks (5, 7); on soil (6) (LC).
Ditrichum heteromallum (Hedw.) E.Britton: on soil (14) (LC).
**Ditrichum pusillum* (Hedw.) Hampe: on rocks (8) (LC).
**Drepanocladus aduncus* (Hedw.) Warnst.: on damp soil near the fountain (13) (LC).
Encalypta streptocarpa Hedw.: on soil (2, 6); on rocks (7, 10, 12) (LC).
Encalypta vulgaris Hedw.: on rocks (3); on soil (6) (LC).
**Enthostodon obtusum* (Hedw.) Lindb.: on soil (14) (LC).
Eucladium verticillatum (With.) Bruch & Schimp.: on damp soil (16) (LC).
Eurhynchiastrum pulchellum (Hedw.) Ignatov & Huttunen: on bark of *Fagus sylvatica* (15) and *Populus alba* (16) (LC).
Exsertotheca crispa (Hedw.) S.Olsson, Enroth & D.Quandt: on rocks (5) (LC).
Fissidens dubius P.Beauv. var. *dubius*: on rocks (2) (LC).
**Fissidens serrulatus* Brid.: on rocks (2) (LC).
Fissidens taxifolius Hedw.: on rocks (12); on damp soil (15) (LC).
Flexitrichum flexicaule (Schwägr.) Ignatov & Fedosov: on rocks (11, 12) (LC).
Geheebia lurida (Hornsch.) J.A. Jiménez & M.J.Cano: on soil (6) (LC).
**Grimmia alpestris* (F.Weber & D.Mohr) Schleich.: on ophiolitic rocks (18) (VU).
Grimmia anodon Bruch & Schimp.: on ophiolitic rocks (18) (LC).
+*Grimmia decipiens* (Schultz) Lindb.: on ophiolitic rocks (18) (LC).
**Grimmia funalis* (Schwägr.) Bruch & Schimp.: on ophiolitic rocks (18) (LC).
Grimmia laevigata (Brid.) Brid.: on rocks (5) (LC).
Grimmia longirostris Hook.: on ophiolitic rocks (18) (LC).
Grimmia montana Bruch & Schimp.: on rocks (5); on ophiolitic rocks (18) (LC).
Grimmia ovalis (Hedw.) Lindb.: on rocks (5); on ophiolitic rocks (18) (LC).
Grimmia pulvinata (Hedw.) Sm.: on rocks (3); on ophiolitic rocks (18) (LC).
Grimmia tergestina Tomm. ex Bruch & Schimp.: on ophiolitic rocks (18) (NT).
Grimmia trichophylla Grev.: on rocks (5); on ophiolitic rocks (18); on damp soil (16) (LC).
Habrodon perpusillus (De Not.) Lindb.: on rocks (7); on bark of *Fagus sylvatica* (14) (LC).
Hedwigia ciliata (Hedw.) P.Beauv.: on ophiolitic rocks (18) (LC).
**Hedwigia emodica* Hampe *ex* Müll.Hal.: on ophiolitic rocks (18) (LC).
**Herzogiella striatella* (Brid.) Z.Iwats.: on stump of *Fagus sylvatica* (8) (DD).
**Heterocladium heteropterum* (Brid.) Schimp.: on rocks (11) (LC).
Homalothecium lutescens (Hedw.) H.Rob. var. *lutescens*: on damp soil (16) (LC).
Homalothecium philippeanum (Spruce) Schimp.: on rocks (4, 5 19); on soil (17) (LC).
Homalothecium sericeum (Hedw.) Schimp.: on bark of *Fagus sylvatica* (1, 14) and *Populus alba* (16); on stump of *Fagus sylvatica* (15); on soil (2); on rocks (2, 5, 7, 10, 12, 19); on ophiolitic rocks (18) (LC).
**Homomallium incurvatum* (Schrad. ex Brid.) Loeske: on soil near the river (15) (LC).
Husnotiella sinuosa (Mitt.) J.A.Jiménez & M.J.Cano: on rocks (19) (LC).
+*Hygroamblystegium tenax* (Hedw.) Jenn.: on damp soil (8, 16) (LC).
**Hypnum andoi* A.J.E.Sm.: on soil (6) (LC).

Hypnum cupressiforme Hedw. var. *cupressiforme*: on bark of *Fagus sylvatica* (2, 14) and *Populus alba* (16); on soil (15) and rocks (19) near the river; on ophiolitic rocks (18) (LC).

Hypnum cupressiforme Hedw. var. *lacunosum* Brid.: on bark of *Pinus leucodermis* (7) (LC).

**Isopterygiopsis pulchella* (Hedw.) Z.Iwats.: on stump of *Fagus sylvatica* (15) (LC).

Isothecium alopecuroides (Lam. ex Dubois) Isov.: on soil (2) and rocks (15, 19) (LC).

Lescuraea incurvata (Hedw.) E.Lawton: on soil (2) and rocks (2, 3, 4, 5, 9, 12, 19); on bark of *Fagus sylvatica* (2, 5, 15), *Pinus leucodermis* (7) and *Abies alba* (9) (LC).

**Lescuraea plicata* (Schleich. *ex* F.Weber & D.Mohr) Broth.: on soil near the river (8) (LC).

**Lescuraea saviana* (De Not.) E.Lawton: on bark of *Pinus leucodermis* (7) (DD).

Leucodon sciuroides (Hedw.) Schwägr.: on bark of *Fagus sylvatica* (1, 5, 9, 10, 14), *Abies alba* (9) and *Populus alba* (16); on rocks (5); on ophiolitic rocks (18) (LC).

Lewinskya affinis (Schrad. ex Brid.) F.Lara, Garilleti & Goffinet: on bark of *Fagus sylvatica* (5, 14, 15) and *Populus alba* (16); on rocks (15, 19) (LC).

Lewinskya rupestris (Schleich. ex Schwägr.) Lara, Garilleti & Goffinet: on bark of *Fagus sylvatica* (1); on rocks (3, 4, 5, 12, 19); on ophiolitic rocks (18) (LC).

Lewinskya shawii (Wilson) F.Lara, Garilleti & Goffinet: on bark of *Fagus sylvatica* (9, 15) and *Abies alba* (9); on rocks (11) (NT).

Lewinskya speciosa (Nees) F.Lara, Garilleti & Goffinet: on rocks (2, 15); on bark of *Fagus sylvatica* (14, 15) (LC).

Lewinskya striata (Hedw.) F.Lara, Garilleti & Goffinet: on bark of *Fagus sylvatica* (1, 2, 5, 14) (LC).

**Microeurhynchium pumilum* (Wilson) Ignatov & Vanderp.: on rocks (19) (LC).

Mnium stellare Hedw.: on rocks (19) (LC).

Neckera menziesii Drumm.: on rocks (4, 19) (LC).

**Neckera pumila* Hedw.: on rocks (19) (LC).

Nogopterium gracile (Hedw.) Crosby & W.R.Buck: on bark of *Fagus sylvatica* (14); on rocks (19) (LC).

Orthotrichum cupulatum Brid. var. *cupulatum*: on rocks (2, 3, 5); on bark of *Fagus sylvatica* (9), *Abies alba* (9) and *Populus alba* (16) (LC).

Orthotrichum pallens Bruch ex Brid.: on bark of *Fagus sylvatica* (14) (LC).

Orthotrichum pumilum Sw. ex anon.: on bark of *Fagus sylvatica* (5) (LC).

Orthotrichum stramineum Hornsch. ex Brid.: on bark of *Fagus sylvatica* (1, 5, 14, 15); on rocks (15) (LC).

+*Palustriella commutata* (Hedw.) Ochyra: on damp soil (8, 17); on rocks (11, 19) (LC).

Palustriella falcata (Brid.) Hedenäs: on damp soil (8) and rocks (19) (LC).

**Pohlia cruda* (Hedw.) Lindb.: on rocs (12) (LC).

**Pohlia lescuriana* (Sull.) Ochi: on soil near the river (8) (LC).

Pohlia wahlenbergii (F.Weber & D.Mohr) A.L.Andrews var. *wahlenbergii*: on soil near the river (8, 13) (LC).

**Polytrichastrum alpinum* (Hedw.) G.L.Sm.: on rocks (7) (LC).

Polytrichum juniperinum Hedw.: on soil (5, 13); on ophiolitic rocks (18) (LC).

**Polytrichum piliferum* Hedw.: on ophiolitic rocks (18) (LC).

**Pseudoleskeella catenulata* (Brid. ex Schrad.) Kindb.: on rocks (5) (LC).

Pseudoleskeella nervosa (Brid.) Nyholm: on bark (2) and stump (8) of *Fagus sylvatica*; on rocks (4, 7, 8, 19) (LC).

Pterigynandrum filiforme Hedw.: on bark of *Fagus sylvatica* (1, 2, 9, 14, 15), *Pinus leucodermis* (7) and *Abies alba* (9); on rocks (12, 19) (LC).

Ptychostomum capillare (Hedw.) Holyoak & N.Pedersen: on rocks (4, 5, 8); on damp soil along the river (15, 16, 19); on ophiolitic rocks (18) (LC).

Ptychostomum elegans (Nees) D.Bell & Holyoak: on rocks (8) (LC).

Ptychostomum imbricatulum (Müll.Hal.) Holyoak & N.Pedersen: on soil (6) (LC).

**Ptychostomum inclinatum* (Sw. *ex* Brid.) J.R.Spence: on ophiolitic rocks (18) (LC).

Ptychostomum pallens (Sw. *ex* anon.) J.R.Spence: on bark of *Fagus sylvatica* (14, 15); on rocks (4) and soil (14); on ophiolitic rocks (18) (LC).

Ptychostomum schleicheri (DC.) J.R.Spence ex D.Bell & Holyoak var. *schleicheri*: on damp soil (2, 8) and rocks (5) (LC).

+*Ptychostomum torquescens* (Bruch & Schimp.) Ros & Mazimpaka: on damp soil near the river (8, 15, 16); on ophiolitic rocks (18) (LC).

**Ptychostomum turbinatum* (Hedw.) J.R.Spence: on rocks (5) (LC).

Pulvigera lyellii (Hook. & Taylor) Plášek, Sawicki & Ochyra: on bark of *Fagus sylvatica* (10, 14) and *Populus alba* (16) (LC).

+*Racomitrium canescens* (Hedw.) Brid. subsp. *canescens*: on soil (17); on ophiolitic rocks (18) (LC).

**Rhizomnium magnifolium* (Horik.) T.J.Kop.: on soil along the river (15) (LC).

+*Rhizomnium punctatum* (Hedw.) T.J.Kop. var. *punctatum*: on soil along the river and on stump of *Fagus sylvatica* (15) (LC).

**Rhynchostegiella litorea* (De Not.) Limpr.: on soil (14) (LC).

Rhynchostegium megapolitanum (Blandow ex F.Weber & D.Mohr) Schimp.: on soil (14) (LC).

Rhynchostegium murale (Hedw.) Schimp.: on soil (17) (LC).

Rhynchostegium riparioides (Hedw.) Cardot: on damp soil along the river (14, 16, 17, 19) (LC).

**Sanionia uncinata* (Hedw.) Loeske: on rocks (7, 8) (LC).

Schistidium apocarpum (Hedw.) Bruch. & Schimp.: on rocks (5, 12); on ophiolitic rocks (18) (LC).

Schistidium atrofuscum (Schimp.) Limpr.: on rocks (3, 7) (LC).

**Schistidium confertum* (Funk) Bruch & Schimp.: on rocks (2, 8, 10, 15); on soil (6, 16) (LC).

Schistidium crassipilum H.H.Blom.: on soil along the river (16); on ophiolitic rocks (18) (LC).

**Schistidium papillosum* Culm.: on ophiolitic rocks (18) (VU).

Schistidium rivulare (Brid.) Podp.: on rocks (5) (LC).

**Sciuro-hypnum starkei* (Brid.) Ignatov & Huttunen: on rocks (5) (LC).

Syntrichia laevipila Brid.: on bark of *Populus alba* (16) (LC).

Syntrichia montana Nees var. *montana*: on soil (7, 16); on ophiolitic rocks (18) (LC).

Syntrichia norvegica F.Weber: on rocks (3) (LC).

Syntrichia ruraliformis (Besch.) Mans.: on rocks (2); on ophiolitic rocks (18) (LC).

Syntrichia ruralis (Hedw.) F.Weber & D.Mohr var. *ruralis*: on soil (2, 6, 15); on rocks (2, 3, 4, 5, 7, 12, 19); on bark of *Fagus sylvatica* (5, 15) and *Populus alba* (16); on ophiolitic rocks (18) (LC).

Thamnobryum alopecurum (Hedw.) Gangulee: on rocks (19); on ophiolitic rocks (18) (LC).

+*Timmia austriaca* Hedw.: on rocks (8) (LC).

**Timmia norvegica* J.E.Zetterst.: on rocks (7) (LC).

**Tortella fragilis* (Drumm.) Limpr.: on soil (6); on ophiolitic rocks (18) (LC).

Tortella nitida (Lindb.) Broth.: on ophiolitic rocks (18) (LC).

Tortella squarrosa (Brid.) Limpr.: on rocks (4); on ophiolitic rocks (18) (LC).

Tortella tortuosa (Hedw.) Limpr.: on rocks (2, 4, 5, 7, 8, 10, 12); on ophiolitic rocks (18) (LC).

Tortula inermis (Brid.) Mont.: on stump of *Fagus sylvatica* (15) (LC).

**Tortula laureri* (Schultz) Lindb.: on rocks (5) (DD).

Tortula muralis Hedw. subsp. *muralis* var. *muralis*: on ophiolitic rocks (18) (LC).

Tortula subulata Hedw.: on soil (2, 14); on rocks (2, 3, 12); on ophiolitic rocks (18) (LC).

**Trichostomopsis australasiae* (Hook. & Grev.) H.Rob.: on soil (6) (LC).

Vinealobryum insulanum (De Not.) R.H.Zander: on rocks (19); on ophiolitic rocks (18) (LC).

Zygodon rupestris Schimp. ex Lorentz: on bark of *Populus alba* (16) (LC).

Discussion

In most cases, these species are widely distributed in central-southern Italy, and their presence in Basilicata was not known due to a simple lack of observation. However, of particular interest among the species collected is the discovery of *Brachydontium trichodes*, a Mediterranean mountain species found in the mountain belt below the tree line, where deciduous hardwoods predominate. In Pollino Park the species was found on rocks near Lago Duglia (1375 m a.s.l.); in Italy it has only been reported from Lombardy in Valganna (Garovaglio 1843) and from Emilia-Romagna in the Tuscan-Emilian Apennines (Velluti 1993) (Fig. 5A). *B. trichodes* grows mainly on shady rocks by streams in mountainous areas, although it can also grow on other soft and friable rocks. It usually grows on vertical or overhanging rock faces but is sometimes found on small pebbles.

Another interesting discovery is *Grimmia alpestris*. This species has been reported in Italy from many localities in the regions of the Alpine Arc, in Sardinia from M.te Gennargentu (Massari 1897) and in Sicily from Puglisi (2009) from M.te Etna. It is a boreo-montane species collected on ophiolitic rocks of Timpa delle Murge (between Case del Conte and La Catusa spring, 1216 m a.s.l.).

The ophiolitic rocks of Timpa delle Murge are also the preferred substrate for other species that are very rare from a chorological point of view and that find in this habitat

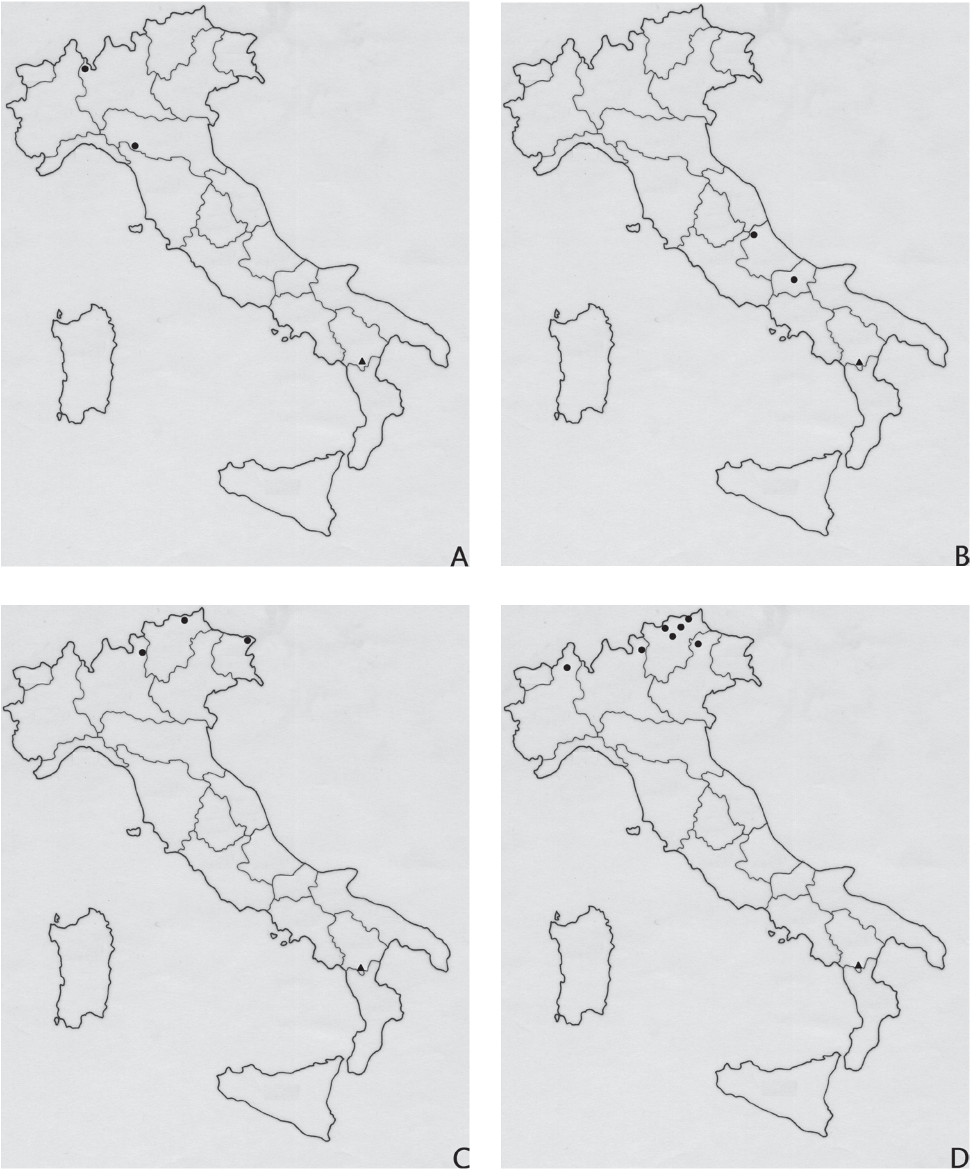

Figure 5. Distribution maps in Italy of **A** *Brachydontium trichodes* (F.Weber) Milde **B** *Lescuraea saviana* (De Not.) E.Lawton; C. *Tortula laureri* (Schultz) Lindb.; D: *Herzogiella striatella* (Brid.) Z.Iwats.

the southernmost refuge of their distribution area (Fig. 6). Among these we can mention *Grimmia funalis* and *Schistidium papillosum*.

Grimmia funalis is a subarctic-subalpine species distributed in the Alpine and Apennine regions of Italy and Sardinia (Herzog 1909); it grows on a variety of rocks, but usually showing some degree of basal richness, on dry to periodically wet rocks, ledges and scree, usually above 300 m.

Figure 6. Ophiolitic rocks outcrops with *Ilex aquifolium* shrubs.

Schistidium papillosum is an arctic alpine species distributed in Val d'Aosta (Schumacker et al. 1999), Piedmont (Blom 1996), Trentino-Alto Adige (Blom 1996; Düll-Wunder 2008; Hofbauer and Dickson 2020), Veneto (Scortegagna 2021), Liguria (Poponessi et al. 2013) and Tuscany (Blom 1996). The species grows on rocks, rarely on bark, in mesic habitats at low to high altitudes (0–2000 m).

The highest peaks of the Pollino Massif are a refuge for some species that have a very sporadic distribution in Italy, being mainly found on the highest peaks of the Alps and the Apennines. Some species are found in the Serra di Crispo and Piano Jannace, at altitudes between 1950 and 2050 metres above sea level. Among these, we remember *Lescuraea saviana*, a subalpine species collected on the bark of *Pinus leucodermis* in Serra di Crispo (2050 m a.s.l.). This species grows mainly at the base of trunks, boulders, outcrops and on soil between 600 and 2500 m a.s.l. In Italy it is only reported in Molise (Giacomini 1938) and in Abruzzo (Mastracci and Düll 1991) in the Laga mountains (Fig. 5B).

Timmia norvegica grows on the rocks of the Serra di Crispo (2050 m a.s.l.). It is a subarctic-subalpine species reported from the regions of the Alpine Arc and from Abruzzo (Mastracci and Düll 1991). *T. norvegica* grows in a fine turf of other mosses and herbs on dry or damp, base-rich rock ledges at high altitudes. It prefers calcareous schist rocks to limestone and basalt.

Another subarctic-subalpine species is *Tortella fragilis*, collected on the Serra di Crispo soil and reported from many locations of the regions of the Alpine arc, from Emilia-Romagna (Fitzgerald and Bottini 1881) and Abruzzo (Aleffi et al. 1997). Rarely fruiting, capsules ripen in summer. Grows on substrates that are probably drained for part of the year, acid or calcareous rocks, dry meadows, logs or peaty humus in springs, fens, open sands on lake shores, snow-covered edges; low to high altitudes (0–3600 m).

Tortula laureri was collected on the rocks of Piano Jannace (1926 m a.s.l.). It is an arctic alpine species that has only been reported in Italy in the second half of the 19th century from Lombardy at M.te Tonale (De Notaris 1869), Trentino-Alto Adige at M.te Gries (Juratzka 1882) and Friuli-Venezia Giulia at Mangart (Wallnofer 1888) (Fig. 5C). The species prefers to grow on rocky outcrops, on soil or soil over rocks, on mineral soils and on infertile peat.

It is worthy of note that the bryoflora of Lucania is home to a plethora of species that are new to science and extremely rare in Italy. One such species is *Herzogiella striatella*, which was collected on a stump of *Fagus sylvatica* along the Pietra Castello path towards Lago Fondo (1876 m a.s.l.). It is an arctic-montane species that has been documented in various locations within Italy. These include Piedmont at Campello-Monti (Levier 1905), Lombardy at Passo del Tonale (De Notaris 1869), Trentino-Alto-Adige at Forcella Sarentina (Heufler 1851), Cima Paganella and Paneveggio (Venturi 1879) and Val Passiria (Schäfer-Verwimp et al. 2021), as well as from Veneto at Valle Ornella (Lorentz and Molendo 1867) (Fig. 5D). It typically inhabits montane districts, growing on peaty soil in crevices amongst boulders at high altitudes. However, it can also be found in woodlands at lower altitudes.

Rhizomnium magnifolium is another arctic-montane species that has been observed growing on soil along the river of Lago Duglia (1387 m a.s.l.). It has also been reported in the Alpine regions and in Emilia-Romagna. (Gerdol and Tomaselli 1988). *Rhizomnium magnifolium* is a species that is typical of wet, base-poor, montane habitats, including sheltered gullies and snowbeds. However, it is also known to occur in mildly base-rich flushes down to approximately 400 metres altitude in the hills.

Regarding the conservation status of these taxa, all observed species are classified as LC (Least Concern) in the Red List of Italian Bryophytes (Puglisi et al. 2023, 2024) with the exception of *Brachydontium trichodes* considered Endangered (EN) in Italy, *Grimmia alpestris* and *Schistidium papillosum* considered Vulnerable (VU), and *Grimmia tergestina* and *Lewinskya shawii* considered Near Threatened (NT). Some of these species have been found on ophiolitic rocks demonstrating the importance of this particular type of environment.

Conclusion

The discovery of 41 species hitherto unknown to the Basilicata region, gathered during an excursion by the Italian Botanical Society's Working Group for Bryology in the Lucania sector of the Pollino National Park, serves to reinforce the notion that vast areas of Italian territory remain entirely uncharted or only partially documented from a bryological perspective. Consequently, there is a pressing need to intensify research efforts in these regions with a view to more accurately quantifying the Italian bryophyte heritage and investigating the biogeographical, chorological and ecological attributes of each species. Furthermore, these findings reinforce the significance of protected areas for the conservation of biodiversity, even in the context of less-studied taxonomic groups such as bryophytes.

The research has identified a group of boreal and arctic-alpine species that have their southern biogeographical limit in the Pollino Massif. These include *Grimmia alpestris*, *G. funalis*, *Schistidium papillosum*, *Lescuraea saviana*, *Timmia norvegica*, *Tortella fragilis*, *Tortula laureri*, *Herzogiella striatella* and *Rhizomnium magnifolium*. These species covered the Italian Peninsula during the ice ages and remained isolated as relics in the postglacial period. This is corroborated by the presence of a notable assemblage of boreal and arctic-alpine species within the phanerogamic flora, which have established refugia on the highest peaks of the Apennine chain.

The presence of habitats with highly diverse ecological characteristics also results in a notable increase in biodiversity. One illustrative example is the occurrence of ophiolitic rock outcrops, which host a distinctive bryophytic flora comprising numerous rare saxicolous species belonging to the genera *Grimmia*, *Hedwigia*, *Schistidium* and *Ptychostomum*. These species demonstrate a high degree of adaptation to the arid and particularly exposed nature of this habitat.

The floristic richness of the Pollino massif, despite remaining relatively unknown and understudied, is of considerable importance. This is due to the notable variety of environments and their altitudinal differences. It is therefore crucial to extend the exploration of the Pollino massif to the Calabrian side of the Park. This will facilitate a more detailed and comprehensive understanding of the bryophytic flora of the region within the broader context of the national territory and the Mediterranean basin.

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RESEARCH ARTICLE

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Islands of lichen diversity in urban environments: a hidden richness in botanical gardens

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Abstract

Botanical gardens in city centres are green islands, offering a sanctuary for diverse plant and fungal species amidst urban landscapes. Although lichens are not usually a primary focus in botanical gardens, these spaces can serve as important refuges. In this work, the epiphytic lichen biota has been investigated in the Botanic Garden of Pisa, to uncover the hidden elements of biodiversity of botanical gardens in terms of non-target organisms. A total of 57 epiphytic lichen taxa and one non-lichenized fungus were recorded, including several threatened species. Two species, *Arthopyrenia platypyrenia* and *Coenogonium tavaresianum*, are new records for Tuscany, while *Lecania cyrtellina* is so far known in the region as occurring only in the Botanic Garden of Pisa. Lichen colonization reflects the richness of tree species, while the diffused presence of nitrogen-tolerant lichens could be associated with urban environmental conditions. Comments on *Arthopyrenia platypyrenia*, *Coenogonium tavaresianum*, *Gyalecta liguriensis*, *Lecania cyrtellina*, *Lecanographa amylacea*, *Micarea misella*, *Porina borreri*, and *Waynea stoechadiana* are given.

Keywords

Biodiversity, Botanic Garden of Pisa, Epiphytic lichens, Species conservation, Tuscany

Introduction

Botanical gardens are exceptional reservoirs of biodiversity, hosting not only plants intentionally cultivated and conserved but also a wide range of other organisms (e.g., lichens, fungi, bryophytes), which complement to the ecological complexity and richness of these specific environments (Ravera et al. 1999; Aptroot and Honegger 2006). Often located in town centres, they play a vital role as cultural and ecological hubs, serving as green refuges that mitigate urban heat and provide crucial climate-regulating benefits (Blackmore and Oldfield 2017; Cannon and Kua 2017; Primack et al. 2021; D'Antraccoli et al. 2023a). Botanical gardens support a variety of trees, including rare species (Jackson et al. 2001; Stevens 2007), providing a wide range of physical and chemical properties, which at once, create habitats that encourage the growth of epiphytic lichens.

Italy played a pivotal role in the establishment of botanical gardens, with its rich tradition of medicinal plant cultivation in monasteries and medical schools evolving into more systematic physic gardens during the 16th Century. According to Chiarugi (1953), Pisa (1543), Padua (1545, July), and Florence (1545, December) pioneered this movement, soon followed by significant botanical gardens in Bologna, Palermo, Naples, Rome, and other cities. These early academic institutions became essential centres for scientific study and the cultivation of medicinal plants, advancing medicine, botany, and global plant exchange, and reflecting Italy's leadership in botanical innovation during the Renaissance (Spencer and Cross 2017). While lichens are not typically a central focus in botanical gardens, historical records reveal occasional documentation of their presence. For example, notable specimens from the Botanic Garden of Pisa, currently preserved in the Herbarium of the University of Trieste (Martellos et al. 2023) and the Herbarium of the University of Pisa (see also Roma-Marzio et al. 2020, 2023 for details on digitized specimens) date back to 1862 (*Arthonia galactites* (DC.) Dufour collected by P. Savi and O. Beccari [PI067228, TSB25884]), 1882 (*Alyxoria varia* (Pers.) Ertz & Tehler [PI067229, TSB25514] and *Lecanora horiza* (Ach.) Linds. [TSB25512, TSB25513] collected by G. Arcangeli) and 1883 (*Alyxoria varia* [TSB25535, TSB25536] collected by A. Mori), offer valuable insights into the diversity that was present there in the past. Another herbarium specimen collected in that period in 1882 by G. Arcangeli refers to *Lecanora subfusca* (L.) Ach. var. *diffusa* Arcang. [PI067226, PI067227], a variety described from the Botanic Garden of Pisa, which grew on a ca. 100-year-old *Ginkgo biloba* L., and was also included in a series of exsiccata (Baglietto, Cesati & De Notaris, Erb. Critt. Ital. Ser. II, no. 1267). Early noteworthy observation of lichens was also made in the Botanical Garden of Florence by Cengia-Sambo in 1928, who reported the presence of an unidentified *Lecanora* species growing on the leaves of a 14-year-old *Syagrus coronata* (Mart.) Becc. cultivated in a warm and humid greenhouse (Cengia-Sambo 1928). In recent years, the diversity of lichens in botanical gardens across Italy has received increasing attention. Some studies have explored urban environments, such as the Botanical Garden of Rome, Lazio (Bartoli 1990; Ravera et al. 1999, 2003), as well as more natural sites as the Botanical Garden of the Apuan Alps "Pellegrini-Ansaldi" in Tuscany (Loppi and Putortì 2001) and the Garden of the Apennine Flora in Capracotta, Molise (Genovesi and Ravera 2014).

The Botanic Garden and Museum of the University of Pisa (hereafter Botanic Garden of Pisa) currently hosts more than 2,000 species in cultivation, grouped in about 30 collections (D'Antraccoli et al. 2024). The localization and the metadata associated with each plant specimen are accessible through the public version of the botanic garden's plant documentation system, U-plant DISCOVER (https://uplantdiscover.sma.unipi.it/). One of the most representative collections of the botanic garden is the Arboretum, consisting of about 200 species in cultivation (D'Antraccoli et al. 2023a). The oldest trees are a specimen of *Ginkgo biloba* L. and one of *Magnolia grandiflora* L., both planted in 1787 by Giorgio Santi (Savi 1828). The garden also hosts other noteworthy trees, including specimens of *Myrtus communis* L. and *Platanus hispanica* Mill. ex Münchh. (both over 200 years old), as well as specimens of *Araucaria bidwillii* Hook., *Chamaerops humilis* L., *Cinnamomum camphora* (L.) J.Presl, *Jubaea chilensis* (Molina) Baill., *Quercus virginiana* Mill., *Taxus baccata* L., *Tilia platyphyllos* Scop., *Washingtonia filifera* (Gloner ex Kerch., Burv., Pynaert, Rodigas & Hull) de Bary, and *Wisteria sinensis* (Sims) DC. These trees, introduced during the 19th century, range from 100 to 200 years (D'Antraccoli et al. 2022).

The aim of this study is to explore the epiphytic lichen diversity in the Botanic Garden of Pisa, to uncover the hidden biodiversity of botanical gardens in terms of non-target organisms. No published studies have specifically addressed the lichen biota in the Botanic Garden of Pisa, aside from the historical herbarium specimens mentioned earlier. By investigating this overlooked aspect, we also aim to contribute new and interesting data on the distribution of lichens in Tuscany.

Materials and methods

Study area

The Botanic Garden of Pisa extends within the urban city centre of Pisa, Italy (43.719966°N, 10.396161°E, alt. 5 m a.s.l.) for about 25,000 m^2, which are divided into seven sectors (Fig. 1), including a complex of greenhouses. The climate of the area

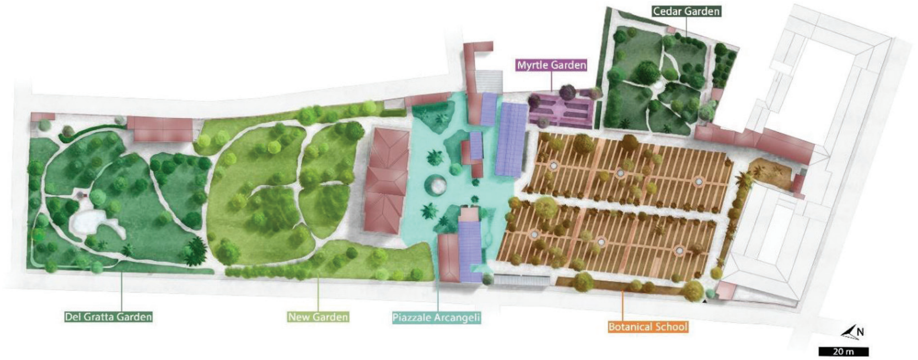

Figure 1. Planimetry of the Botanic Garden and Museum of the University of Pisa and its subdivision in sectors (from D'Antraccoli et al. 2023a, modified).

is characterized by mean annual temperature 15.2 °C, monthly mean 7.2 °C in the coldest month (January) and 24.4 °C in the hottest month (August). Rainfall totals 880 mm per year, with a minimum in summer (July: 29 mm) and a maximum in autumn (November: 144 mm) (LaMMA Consortium, Pisa climatology 1991–2020). According to the Köppen-Geiger climate classification, the region features a warm temperate climate with hot, dry summers (Kottek et al. 2006).

Data collection

Epiphytic lichens were surveyed during 2023 in the study area across more than 100 trees representing 61 different taxa. The trees were carefully selected to capture the full range of substrate variability, enabling comprehensive data collection on epiphytic lichen diversity. Lichens were identified based on their morphological, anatomical, and chemical characteristics by standard methods used in lichenology (Wirth et al. 2013). Relevant samples collected are deposited in the herbarium of the University of Pisa (PI) and in the herbarium of the Plant Science and Biodiversity Centre, Slovak Academy of Sciences (SAV), Slovakia. The distribution of new or confirmed species for Tuscany was discussed according to the distribution data available from Nimis (1993, 2024) and recently published papers.

Results

A total of 57 epiphytic lichen taxa and one non-lichenized fungus (Table 1), belonging to 53 species and subspecies within 40 genera were recorded.

Table 1. The list of lichens and non-lichenized fungi recorded on the trees of the Botanic Garden of Pisa, and their IUCN Red List category in Italy (Nascimbene et al. 2013). Species recorded only from Botanic Garden of Pisa and/or new for Tuscany are marked with asterisk (*). Abbreviations: DD = Data Deficient; LC = Least Concern; NT = Near Threatened; VU = Vulnerable. ^{n} = species tolerant to eutrophication (Nimis 2024).

Recorded taxon	Substrate	IUCN
Agonimia opuntiella (Buschardt & Poelt) Vězda	*Liriodendron tulipifera* L.	LC
Alyxoria varia (Pers.) Ertz & Tehler	*Acer oblongum* Wall. ex DC., *Ginkgo biloba* L., *Phillyrea angustifolia* L., *Pittosporum tobira* (Thunb.) W.T.Aiton	
^{n}*Amandinea punctata* (Hoffm.) Coppins & Scheid.	*Gleditsia triacanthos* L., *Jubaea chilensis* (Molina) Baill., *Melia azedarach* L., *Pinus halepensis* Mill.	
Arthonia radiata (Pers.) Ach.	*Sorbus aria* (L.) Crantz.	
**Arthopyrenia platypyrenia* (Nyl.) Arnold	*Pittosporum tobira* (Thunb.) W.T.Aiton	
^{n}*Candelaria concolor* (Dicks.) Stein	*Aesculus pavia* L., ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Cornus sanguinea* L., *Cotinus coggygria* Scop., *Diospyros lotus* L., *Gymnocladus dioicus* (L.) K.Koch., *Lagerstroemia indica* L., *Larix kaempferi* (Lamb.) Carrière, *Melia azedarach* L., *Phillyrea angustifolia* L., *Pittosporum tobira* (Thunb.) W.T.Aiton, *Pseudocydonia sinensis* (Dum.Cours.) C.K.Schneid., *Sequoia sempervirens* (D.Don) Endl., *Taxus baccata* L., *Vitex agnus-castus* L.	

Recorded taxon	**Substrate**	**IUCN**
Catapyrenium psoromoides (Borrer) R.Sant.	*Pinus halepensis* Mill.	NT
^{n}*Catillaria nigroclavata* (Nyl.) J.Steiner	*Laburnum alpinum* (Mill.) Brecht. & J.Presl	
^{n}*Cerothallia luteoalba* (Turner) Arup, Frödén & Søchting	*Catalpa bungei* C.A.Mey.	DD
Chrysothrix candelaris (L.) J.R.Laundon	*Arbutus unedo* L., *Gleditsia triacanthos* L., *Pyrus communis* L.	
Cladonia sp.	*Melia azedarach* L.	
**Coenogonium tavaresianum* (Vězda) Lücking, Aptroot & Sipman	*Calocedrus decurrens* (Torr.) Florin	VU
Dendrographa decolorans (Sm.) Ertz & Tehler	*Acer oblongum* Wall. ex DC., *Aesculus carnea* J.Zeyh., *A. pavia* L., *Butia capitata* (Mart.) Becc., *Calocedrus decurrens* (Torr.) Florin, *Camellia* sp., *Carya illinoinensis* (Wangenh.) K.Koch, *Citrus ×aurantium* L., *Cornus sanguinea* L., *Diospyros lotus* L., *Fraxinus excelsior* L., *Ginkgo biloba* L., *Gleditsia triacanthos* L., *Liriodendron tulipifera* L., *Magnolia grandiflora* L., *Phillyrea angustifolia* L., *Pyrus communis* L., *Quercus castaneifolia* C.A.Mey, *Q. virginiana* Mill., *Vitex agnus-castus* L.	
^{n}*Diploicia canescens* (Dicks.) A.Massal.	*Carya illinoinensis* (Wangenh.) K.Koch, *Ginkgo biloba* L., *Pinus halepensis* Mill., *P. halepensis* Mill. subsp. *brutia* (Ten.) Holmboe, *Quercus virginiana* Mill.	
Evernia prunastri (L.) Ach.	×*Chitalpa tashkentensis* T.S.Elias & Wisura	
Flavoparmelia caperata (L.) Hale	*Arbutus unedo* L., ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Cotinus coggygria* Scop., *Gleditsia triacanthos* L., *Jubaea chilensis* (Molina) Baill., *Lagerstroemia indica* L., *Larix kaempferi* (Lamb.) Carrière, *Liriodendron tulipifera* L., *Melia azedarach* L., *Prunus avium* L., *Pyrus communis* L., *Vitex agnus-castus* L.	
Flavoparmelia soredians (Nyl.) Hale	*Diospyros lotus* L., *Melia azedarach* L.	
Gyalecta liguriensis (Vězda) Vězda	*Magnolia grandiflora* L., *Salix eleagnos* Scop.	LC
^{n}*Hyperphyscia adglutinata* (Flörke) H.Mayrhofer & Poelt	*Abies alba* Mill., *Acer campestre* L., *A. oblongum* Wall. ex DC., *Aesculus carnea* J.Zeyh., *A. pavia* L., *Butia capitata* (Mart.) Becc., *Calocedrus decurrens* (Torr.) Florin, *Camellia japonica* L. 'Eloisa Borrini', *C. japonica* L. 'Pomponia semiplena', *C. japonica* L. 'Tedeum', *Carya illinoinensis* (Wangenh.) K.Koch, *Catalpa* sp., *C. bungei* C.A.Mey., ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Cinnamomum camphora* (L.) J.Presl, *Cornus sanguinea* L., *Corylus avellana* L., *Cotinus coggygria* Scop., *Crataegus rhipidophylla* Gand., *Cydonia oblonga* Mill., *Diospyros lotus* L., *Fraxinus excelsior* L., *Ginkgo biloba* L., *Gymnocladus dioicus* (L.) K.Koch., *Lagerstroemia indica* L., *Larix kaempferi* (Lamb.) Carrière, *Melia azedarach* L., *Olea europaea* L. subsp. *europaea*, *Phillyrea angustifolia* L., *Pinus halepensis* Mill. subsp. *brutia* (Ten.) Holmboe, *P. nigra* J.F.Arnold subsp. *nigra*, *P. pinea* L., *Pseudocydonia sinensis* (Dum.Cours.) C.K.Schneid., *Pyrus communis* L., *Quercus castaneifolia* C.A.Mey, *Taxus baccata* L., *Ulmus laevis* Pall., *Viburnum tinus* L., *Vitex agnus-castus* L.	
Lecania cyrtella (Ach.) Th.Fr.	*Pinus nigra* J.F.Arnold subsp. *nigra*	
**Lecania cyrtellina* (Nyl.) Sandst.	*Jubaea chilensis* (Molina) Baill.	
Lecania naegelii (Hepp.) Diederich & van den Boom	×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Phillyrea angustifolia* L., *Vitex agnus-castus* L.	
Lecanographa amylacea (Pers.) Egea & Torrente	*Acer oblongum* Wall. ex DC., *Aesculus pavia* L., *Catalpa bungei* C.A.Mey., *Cedrus brevifolia* (Hook.f.) Elwes & A.Henry, *Cinnamomum camphora* (L.) J.Presl, *Diospyros lotus* L., *Ginkgo biloba* L., *Phillyrea angustifolia* L., *Quercus virginiana* Mill.	
Lecanora argentata (Ach.) Malme	*Vitex agnus-castus* L.	

Recorded taxon	Substrate	IUCN
[n]*Lecanora chlarotera* Nyl. subsp. *chlarotera*	*Camellia japonica* L. 'Pomponia semiplena', ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Vitex agnus-castus* L.	
Lecanora expallens Ach.	*Aesculus pavia* L., ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Melia azedarach* L., *Quercus virginiana* Mill.	
Lecanora strobilina (Spreng.) Kieff.	*Melia azedarach* L.	
[n]*Lecidella elaeochroma* (Ach.) M.Choisy var. *elaeochroma* f. *elaeochroma*	*Aesculus carnea* J.Zeyh., *A. pavia* L., ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Melia azedarach* L., *Phillyrea angustifolia* L., *Pinus halepensis* Mill., *Pyrus communis* L., *Sorbus aria* (L.) Crantz., *Vitex agnus-castus* L.	
Lepraria sp.	*Acer campestre* L., *A. monspessulanum* L., *Aesculus carnea* J.Zeyh., *Arbutus unedo* L., *Carya illinoinensis* (Wangenh.) K.Koch, *Cycas revoluta* Thunb., *Diospyros lotus* L., *Fraxinus excelsior* L., *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Olea europaea* L. subsp. *europaea*, *Pyrus communis* L.	
Leprocaulon quisquiliare (Leers) M.Choisy	*Acer campestre* L., *A. monspessulanum* L., *Arbutus unedo* L., *Calocedrus decurrens* (Torr.) Florin, *Camellia japonica* L. 'Tedeum', *Carya illinoinensis* (Wangenh.) K.Koch, *Catalpa* sp., *Cinnamomum camphora* (L.) J.Presl, *Cycas revoluta* Thunb., *Diospyros lotus* L., *Fraxinus excelsior* L., *Ginkgo biloba* L., *Gymnocladus* dioicus (L.) K.Koch., *Liriodendron tulipifera* L., *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Olea europaea* L. subsp. *europaea*, *Phytolacca dioica* L., *Prunus avium* L., *Pyrus communis* L., *Quercus virginiana* Mill., *Styphnolobium japonicum* (L.) Schott	
Melanelixia subaurifera (Nyl.) O.Blanco, A.Crespo, Divakar, Essl., D.Hawksw. & Lumbsch	×*Chitalpa tashkentensis* T.S.Elias & Wisura	
Micarea adnata Coppins	*Melia azedarach* L.	DD
Micarea misella (Nyl.) Hedl.	*Jubaea chilensis* (Molina) Baill.	
Micarea prasina Fr.	*Lagerstroemia indica* L., *Pinus nigra* J.F.Arnold subsp. *nigra*	
Normandina pulchella (Borrer) Nyl.	*Phillyrea angustifolia* L.	
Opegrapha sp.	*Pittosporum tobira* (Thunb.) W.T.Aiton	
Opegrapha niveoatra (Borrer) J.R.Laundon	*Ginkgo biloba* L.	
Opegrapha vulgata (Ach.) Ach.	*Ulmus laevis* Pall.	
Parmotrema perlatum (Huds.) M.Choisy	*Melia azedarach* L., *Prunus avium* L., *Pyrus communis* L.	
Pertusaria heterochroa (Müll.Arg.) Erichsen	*Melia azedarach* L.	
Pertusaria hymenea (Ach.) Schaer.	*Vitex agnus-castus* L.	
Phaeophyscia sp.	*Pittosporum tobira* (Thunb.) W.T.Aiton	
[n]*Phaeophyscia hirsuta* (Mereschk.) Essl.	*Acer monspessulanum* L., *Cornus sanguinea* L.	
[n]*Phaeophyscia orbicularis* (Neck.) Moberg	*Acer campestre* L., *Camellia japonica* L. 'Pomponia semiplena', *Cornus sanguinea* L., *Cotinus coggygria* Scop., *Metasequoia glyptostroboides* Hu & W.C.Cheng	
[n]*Physcia adscendens* H.Olivier	*Acer campestre* L., *Aesculus carnea* J.Zeyh., *A. pavia* L., *Arbutus unedo* L., *Carya illinoinensis* (Wangenh.) K.Koch, ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Cinnamomum camphora* (L.) J.Presl, *Cornus sanguinea* L., *Cotinus coggygria* Scop., *Crataegus rhipidophylla* Gand., *Cydonia oblonga* Mill., *Diospyros lotus* L., *Gymnocladus dioicus* (L.) K.Koch., *Lagerstroemia indica* L., *Larix kaempferi* (Lamb.) Carrière, *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Olea europaea* L. subsp. *europaea*, *Phillyrea angustifolia* L., *Pinus nigra* J.F.Arnold subsp. *nigra*, *P. pinea* L., *Pseudocydonia sinensis* (Dum.Cours.) C.K.Schneid., *Pyrus communis* L., *Sorbus aria* (L.) Crantz., *Taxus baccata* L., *Ulmus laevis* Pall., *Vitex agnus-castus* L., *Wisteria sinensis* (Sims) DC.	
Physcia clementei (Turner) Lynge	×*Chitalpa tashkentensis* T.S.Elias & Wisura	
[n]*Physcia tenella* (Scop.) DC.	*Catalpa* sp., *Cornus sanguinea* L., *Phillyrea angustifolia* L., *Pinus halepensis* Mill.	

Recorded taxon	Substrate	IUCN
^{n}*Physciella chloantha* (Ach.) Essl.	*Camellia japonica* L. 'Tedeum', *Carya illinoinensis* (Wangenh.) K.Koch, *Cotinus coggygria* Scop., *Diospyros lotus* L., *Ginkgo biloba* L., *Liriodendron tulipifera* L., *Phytolacca dioica* L.	
Physconia sp.	*Liriodendron tulipifera* L.	
^{n}*Physconia grisea* (Lam.) Poelt subsp. *grisea*	*Carya illinoinensis* (Wangenh.) K.Koch, *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Pyrus communis* L.	
Porina aenea (Wallr.) Zahlbr.	*Styphnolobium japonicum* (L.) Schott, *Ulmus laevis* Pall.	
Porina borreri (Trevis.) D.Hawksw. & P.James	*Aesculus pavia* L., *Magnolia grandiflora* L., *Vitex agnus-castus* L.	
Punctelia borreri (Sm.) Krog	*Liriodendron tulipifera* L.	
Punctelia subrudecta (Nyl.) Krog	*Aesculus pavia* L., *Aesculus carnea* J.Zeyh., *Carya illinoinensis* (Wangenh.) K.Koch, ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Liriodendron tulipifera* L., *Olea europaea* L. subsp. *europaea*, *Phillyrea angustifolia* L., *Phytolacca dioica* L., *Pseudocydonia sinensis* (Dum.Cours.) C.K.Schneid., *Pyrus communis* L., *Ulmus laevis* Pall.	
Pyrenula chlorospila Arnold	*Aesculus carnea* J.Zeyh., *A. pavia* L., *Corylus avellana* L., *Phillyrea angustifolia* L., *Vitex agnus-castus* L.	
Strangospora pinicola (A.Massal.) Körb.	*Jubaea chilensis* (Molina) Baill.	VU
Waynea stoechadiana (Abbassi Maaf & Cl.Roux) Cl.Roux & P.Clerc	*Acer campestre* L., *Pinus nigra* J.F.Arnold subsp. *nigra*	VU
^{n}*Xanthoria parietina* (L.) Th.Fr.	*Aesculus carnea* J.Zeyh., *A. pavia* L., *Camellia japonica* L. '*Pomponia semiplena*', *Carya illinoinensis* (Wangenh.) K.Koch, ×*Chitalpa tashkentensis* T.S.Elias & Wisura, *Crataegus rhipidophylla* Gand., *Cydonia oblonga* Mill., *Fraxinus excelsior* L., *Laburnum alpinum* (Mill.) Brecht. & J.Presl, *Larix kaempferi* (Lamb.) Carrière, *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Phytolacca dioica* L., *Pyrus communis* L., *Ulmus laevis* Pall., *Vitex agnus-castus* L.	

Discussion

Epiphytic lichens were surveyed in both indoor and outdoor environments of the Botanic Garden. However, so far, no lichens were found on trees cultivated in greenhouses, despite several of them being examined carefully (namely *Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell., *B. discolor* F.Muell., *B. rupestris* (T.Mitch. ex Lindl) K.Schum., *Cycas taiwaniana* Carruth., *C. circinalis* L., *Ficus elastica* Roxb. ex Hornem., *Strelizia nicolai* Regel & K.Koch, *Sterculia balanghas* L.). Only a colonization by green algae was noticed on some of the barks (especially on *Cycas taiwaniana* and *Ficus elastica*). All recorded lichens were found in outdoor conditions with varying levels of colonization across different trees. Sparse lichen growth, with thalli appearing sporadically or in minimal coverage, was observed on trees of the genera *Camellia*, *Cedrus*, *Citrus*, *Sequoia*, *Viburnum*, and *Wisteria*. No lichen colonization was observed on the bark of *Araucaria bidwillii*, a tree that has stood in the garden for 152 years. In contrast, species like *Aesculus pavia*, ×*Chitalpa tashkentensis*, *Melia azedarach*, *Phillyrea angustifolia*, *Pyrus communis*, and *Vitex agnus-castus* exhibited a high degree of colonization, particularly in terms of diversity, with more than ten lichen species identified from the individual trees.

According to the red list of epiphytic lichens of Italy (Nascimbene et al. 2013), three lichen species are considered Vulnerable, one is included in the category Near Threatened, two in Least Concern and other two in Data Deficient IUCN categories (Table 1). Two species, *Arthopyrenia platypyrenia* (Nyl.) Arnold and *Coenogonium tavaresianum* (Vězda) Lücking, Aptroot & Sipman, are newly recorded for the lichen biota of Tuscany, and *Lecania cyrtellina* (Nyl.) Sandst. is so far known only from the study area (Ravera et al. 2024). Additionally, the record of *Arthopyrenia platypyrenia* is the second for Italy.

The lichen biota in the Botanic Garden is mainly composed by acidophilous/oligotrophic species (about 70%), overall reflecting the availability of acidic (bark) substrates. Nevertheless, the wide distribution of species tolerant to eutrophication, or even enhanced by the deposition of dust and nitrogen compounds (such as *Hyperphyscia adglutinata*, *Physcia adscendens*, *Candelaria concolor*, and *Xanthoria parietina*, early colonizers of eutrophicated substrates), well reflect the influence of urban atmospheric conditions, especially in more open conditions. This phenomenon is particularly evident on the bark of conifers, which in their natural environment are generally colonized by acidophilous epiphytes, but in the Botanic Garden of Pisa are more often colonized by nitrophilous lichens. Lichen colonization is influenced by various interacting factors, such as light availability, relative humidity, bark chemistry, water-holding capacity, health status of the substrate and environmental pollution (Hauck 2005; Loppi and Baragatti 2011; Ellis 2012). Some of them are directly shaped by the characteristics of the individual tree hosting the lichen, as well as the composition of the surrounding vegetation (Petersson et al. 2022 and references therein). Consequently, a condition with a greater diversity of tree species and structural features (such as in even aged forests) often supports higher lichen species richness (Petersson et al. 2022). The same could apply also to botanical gardens. In general, the diverse range of trees, with their physical and chemical properties, creates various habitats that support the growth of epiphytic lichens thriving on their surfaces (e.g. Käffer et al. 2016). In fact, there is evidence that the lichen biota in a botanical garden is proportionally richer than that of the surrounding environment, especially in urban contexts (Aptroot and Honegger 2006; Wilkie 2020).

On the other hand, along with nitrophilous species commonly found in urban areas, the Botanic Garden of Pisa hosts several species, whose presence is typically associated with more natural environments. Among them, *Coenogonium tavaresianum*, *Gyalecta liguriensis*, *Lecanographa amylacea*, and *Porina borreri* are also recognized as indicators of undisturbed forests and ancient woodlands (Nimis 2024) and were observed on some of the garden's monumental trees, including *Ginkgo biloba*, *Magnolia grandiflora*, *Cinnamomum camphora*, and *Quercus virginiana.* Additional species like *Catapyrenium psoromoides*, *Micarea adnata*, *M. misella*, *M. prasina*, *Opegrapha niveoatra*, *Porina aenea*, *Pyrenula chlorospila*, and *Waynea stoechadiana* exhibit affinities for natural and semi-natural habitats (Nimis 2024). Notably, some of them, including *C. tavaresianum*, *G. liguriensis*, *L. amylacea*, *M. prasina*, *O. niveoatra*, and *W. stoechadiana*, have also been recorded in the Botanical Garden of Rome (Ravera et al. 1999), highlighting the role of botanical gardens as alternative habitats for species with specific ecological demands. Botanical gardens in city centres serve as vital green islands, offering a sanctuary for diverse plant and fungal species amidst urban landscapes. Their importance as refugia for biodiversity can be also exemplified by the New Botanical Garden of the University Zürich: despite a relatively small area (53,000 m^2), it hosts approximately 10% of all lichen species recorded in Switzerland (Aptroot and Honegger 2006).

In the following section there are specific notes on several species of concern found within the Botanic Garden of Pisa.

Arthopyrenia platypyrenia

This species is an inconspicuous non-lichenised fungus with endosubstratic thallus (Nimis 2024). On the studied trees, it appears only as a discoloration of the bark. It has rounded black perithecia with characteristic pluriseptate ascospores (in collected samples mostly 5–6-septate in size 26.2–28.7 × 8.7–10 μm), with clear constriction at each septum and a 2.5 μm thick gelatinous sheath. The distribution and ecology of this species is poorly understood, with only a few reported occurrences across Europe. It was found especially on the bark of *Hedera* sp. (Coppins and Orange 2009) and *Fraxinus ornus* L. (Consortium of Lichen Herbaria 2024). The only previous Italian record refers to the samples found in Calabria on *Hedera helix* L. climbing on the base of *Populus nigra* L. in humid conditions (Puntillo 1996). In the Botanic Garden of Pisa, it colonizes the bark of *Pittosporum tobira*. This species is recorded here for the first time in Tuscany and for the second time in Italy.

Coenogonium tavaresianum

This species is recorded here for the first time in Tuscany. The olive-green, finely granulose to verrucose thalli colonize the bark of *Calocedrus decurrens* quite abundantly. The species features small biatorine apothecia with pale discs and dark margins, which are constricted at the base. Another distinguishing feature is the presence of small, 1-septate ascospores, mostly measuring 12.5 × 2.5–3 μm in collected samples. The distribution of species in Italy is primarily confined to humid woodlands along the Tyrrhenian coast (Nimis 2024), but it has also been reported from more urban environments such as the Botanical Garden of Rome (Ravera et al. 1999). It is included in the red list of epiphytic lichens of Italy as vulnerable and likely experiencing a declining trend (Nascimbene et al. 2013).

Gyalecta liguriensis

This crustose species is characterized by muriform and distinctively lemon shaped ascospores with pointed apices. It usually grows on ancient trees in coastal areas (Alvarez Andrés and López de Silanes 2002; Nimis 2024). In the study area, a few thalli were recorded on more than 230-year-old tree *Magnolia grandiflora* and younger tree *Salix eleagnos*. However, the latter is no longer cultivated in the garden, as the tree died in 2024.

Lecania cyrtellina

This is a crustose epiphytic lichen, that primarily grows on the basic bark of deciduous trees such as *Acer*, *Fraxinus*, *Ulmus*, and *Alnus* (Reese Næsborg 2008). In the Botanic Garden of Pisa, it grows on more than 130 years old Chilean wine palm *Jubaea chilensis*. The lichen features a pale thallus and clustered small lecanorine apothecia (0.1–0.3 mm in diam.) with thin and disappearing thalline margin. The ascospores are mostly simple, narrow and short (9 × 2.5 μm in collected samples), unlike the other related species *L. cyrtella* (Reese Næsborg 2008). Most Italian records of *L. cyrtellina* are from recent years, as the species was not consistently distinguished from *L. cyrtella* in the past. The Botanic Garden of Pisa is currently the only known locality for this species in Tuscany, as reported by Ravera et al. (2024).

Lecanographa amylacea

This species shows strong morphological dimorphism depending on the photobiont type present in the lichen. The sorediate morphotype is characterized by the presence of *Trebouxia* photobionts, while the non-sorediate fertile morphotype contains *Trentepohlia* as a photobiont (Ertz et al. 2018). In the Botanic Garden of Pisa, a photomorph with *Trentepohlia* sp. was observed on the bark of various trees (e.g., *Acer*, *Aesculus*, *Catalpa*, *Cedrus*, *Cinnamomum*, *Diospyros*, *Ginkgo*, *Phillyrea*, and *Quercus*), exhibiting white farinose thallus with black, densely white-pruinose apothecia. In Italy, it is considered as a rare species (Nimis 2024) linked mostly to old trees in ancient woodlands and urban parks or old olive groves (Ravera et al. 2021). This is the second locality reported in Tuscany, as it had previously been found in another location between Pietrapiana and Saltino (Nimis 1993).

Micarea misella

This species shows a thallus of small, pale green-grey granules and black apothecia (proper exciple K+ violet, small simple ascospores). It was found on old tree *Jubaea chilensis*. Its occurrence in Italy is more confined to the area of Alps, but it is also distributed along the Apennines (Nimis 2024). In Tuscany, it was found for the first time in 2016 in Abetone (Ravera et al. 2016). Here we present the second record for Tuscany.

Porina borreri

This is a crustose lichen producing black prominent perithecia with multiseptate ascospores. The apex of asci is mostly truncate with a refractive ring structure. This species is mostly recorded in old woodlands with higher humidity (Nimis 2024). Only one previous record has been published from Tuscany, referring to a locality close to Volterra (Nimis 1993).

Waynea stoechadiana

This is a sorediate squamulose lichen, which initially may resemble *Hypocenomyce scalaris* (Ach.) M. Choisy, but differs in several key characteristics, such as the chemical reactions of thallus (C-, KC-) and the type of ascospores (mostly 1-septate, longer and narrower). The species belongs to Mediterranean elements. It is considered as a rare species of conservation concern, with IUCN status Vulnerable (Nascimbene et al. 2013; Nimis 2024). It is mainly confined to warm, humid conditions along the Tyrrhenian coast (Nimis 2024). In Tuscany, the occurrence records are quite scarce (see Nimis 1993, 2024), however, other localities with rich populations of this species have been documented by recently collected material between Punta Ala and Follonica (Italy, Tuscany, Punta Ala: the beach Cala Violina (42.857027°N, 10.774941°E), *Quercus ilex* wood around picnic zone, on *Q. ilex*, 20 m, 28 April 2023, *L. Paoli, Z. Fačkovcová, A. Bérešová* SAV0017540, SAV0018119, SAV0018120).

Conclusion

Botanical gardens can contribute to biodiversity conservation not only for taxa in cultivation (see for instance D'Antraccoli et al. 2023b), but also in the case of non-target spontaneously growing organisms such as lichens, which can benefit indirectly from habitats and conditions provided by botanical gardens, such as in urban environments. Although lichens are not usually a primary focus in botanical gardens, these spaces can serve as important refuges for their diversity. Indeed, the lichen biota of the Botanic Garden of Pisa includes also rare species according to the red list of epiphytic lichens of Italy (Nascimbene et al. 2013), and for three taxa, this is currently the only known locality in Tuscany. Lichen colonization (especially by acidophilous/oligotrophic species) well reflects the richness of tree substrates, while the distribution of nitrogen-tolerant lichens is likely associated with urban environmental conditions.

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RESEARCH ARTICLE

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Contribution to the knowledge of the bryological flora of Molise (Southern Italy)

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Abstract

Molise is one of the least known Italian administrative regions from a bryological point of view. The data collected during some bryological excursions in Molise are here reported. The list includes 88 species, of which 26 are new or confirmed for the regional flora: *Antitrichia curtipendula*, *Cryphaea heteromalla*, *Dialytrichia mucronata*, *Eurhynchium striatum*, *Fabronia pusilla*, *Frullania tamarisci*, *Fontinalis antipyretica*, *Gymnostomum aeruginosum*, *Isothecium myosuroides*, *Leptodon smithii*, *Lewinskya speciosa*, *Marchantia quadrata*, *Neckera pumila*, *Nogopterium gracile*, *Oxyrrhinchium hians*, *Plagiomnium rostratum*, *Plagiothecium nemorale*, *Plasteurhynchium striatulum*, *Porella arboris-vitae*, *Schistidium crassipilum*, *Scleropodium touretii*, *Scorpiurium circinatum*, *Syntrichia papillosa*, *Syntrichia virescens*, *Thuidium tamariscinum*, *Tortella flavovirens* var. *flavovirens*.

Keywords

Bryophytes, Molise, southern Italy, Biodiversity, Floristic data

Introduction

According to the most recent checklist of the Italian Bryological Flora (Aleffi et al. 2023), Molise is, together with Basilicata, the least known Italian administrative region with regard to this systematic group. At the time of publication of the checklist, in fact, only 157 species of mosses and 25 of liverworts and hornworts were known, with a negligible increase in knowledge compared to the checklists of the end of the last century (Cortini Pedrotti 1992; Aleffi and Schumacker 1995). For comparison, the Italian bryological flora consists of 1220 species in total, of which 301 are liverworts and hornworts and 919 moss species, excluding subspecies and varieties.

This contribution collects miscellaneous data from some excursions in Molise, with the aim of filling, at least in part, this gap.

Materials and methods

The records of bryophytes collected during some excursions in Molise are listed. All specimens were collected and identified by the author and are preserved in his personal herbarium. Nomenclature and regional distribution are consistent with the checklist of the Italian Bryological Flora by Aleffi et al. (2023). The collection sites and the main ecological data for all specimens are reported in the floristic inventory. Species new or confirmed for Molise are marked by an asterisk.

The samples were collected in the following sites. Geological notes are taken from the Geological Map of Italy at a scale of 1:100,000, sheets 148 – Vasto, 153 – Agnone, 161 – Isernia, and 162 – Campobasso.

- Collemeluccio (Pescolanciano – Isernia): forest with a prevalence of silver fir with turkey oak and beech in the cooler exposures. Schistose sandstones, limestones, marls and clays (Miocene).
- Surroundings of Carpinone (Isernia): hills, ravines and waterfalls along the Carpino stream and its tributaries. Scaly limestones and marls (Cretaceous), whitish calcarenites and breccias alternating with marls and clays (Eocene-Oligocene), marls, flint limestones and calcarenites (Eocene-Miocene).
- M. Capraro, surroundings of the Garden of the Apennine Flora of Capracotta (Isernia): beech forests, shrubs, pastures and rocks. Organogenic limestones and calcareous marls (Miocene).
- Montedimezzo (Vastogirardi – Isernia): forest dominated by turkey oak, beech, locally with silver fir plantations. Scaly limestones, marls, clays, with calcirudites and calcarenites in the upper part (Oligocene).
- Petacciato Marina (Petacciato – Campobasso): coastal sand dunes (Pleistocene).
- S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia): surroundings of the Abbey and agricultural and forestry environments adjacent to the sources of the Volturno river. Travertines and Quaternary deposits.
- Saepinum-Altilia (Saepinum – Campobasso): Archaeological site of a Roman settlement at the foot of the Matese Massif. Fluvial-lacustrine deposits consisting of yellowish marls and incoherent sands (Quaternary).
- Santuario Italico di Pietrabbondante (Isernia): Archaeological site of a monumental Samnite cult complex built with limestone blocks. Micaceous sandstones alternating with silty clays with arenaceous lenses (Miocene).
- Valle Fiorita, Le Mainarde (Pizzone – Isernia): beech forests and pastures on dolomitic or calcareous-dolomitic substrate (Jurassic), micritic limestones (Jurassic – Cretaceous) and Quaternary moraine deposits.
- Venafro (Isernia): urban center.

Floristic inventory

MARCHANTIIDAE

Apopellia endiviaefolia (Dicks.) Nebel & D.Quandt (Pelliaceae) – Collemeluccio (Pescolanciano – Isernia), 41.713289°N, 14.341452°E, m 910, stream, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.596206°N, 14.325515°E, m 660, riverbed, 22 July 2023.

Conocephalum conicum (L.) Lindb. (Conocephalaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595795°N, 14.325796°E, m 670, wet limestone, 22 July 2023.

Frullania dilatata (L.) Dumort. (Frullaniaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023; Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691022°N, 13.970888°E, m 1480, *Acer pseudoplatanus* bark, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595127°N, 14.327225°E, m 660, *Acer campestre* bark, 22 July 2023; S. Donato (Carpinone – Isernia), 41.606914°N, 14.324097°E, m 840, *Ulmus minor* bark, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759301°N, 14.213789°E, m 1040, *Fagus sylvatica* bark, 23 July 2023; Venafro (Isernia), 41.483172°N, 14.038686°E, m 250, *Ligustrum lucidum* bark, 24 July 2023.

**Frullania tamarisci* (L.) Dumort. (Frullaniaceae) – Collemeluccio (Pescolanciano – Isernia), 41.710659°N, 14.347213°E, m 960, *Quercus cerris* bark, 21 July 2023. Species confirmed for the bryoflora of Molise. Epiphytic species, reported in all Italian regions although not recently confirmed everywhere.

Lejeunea cavifolia (Ehrh.) Lindb. (Lejeuneaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, limestone in a gorge, 22 July 2023.

Lophocolea heterophylla (Schrad.) Dumort. (Lophocoleaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708221°N, 14.353014°E, m 920, rotting stump, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.765041°N, 14.215632°E, m 1030, rotting log, 23 July 2023.

**Marchantia quadrata* Scop. (Marchantiaceae) – Montedimezzo (Vastogirardi – Isernia), 41.760218°N, 14.210153°E, m 1010, limestone, 23 July 2023. Species new for the bryoflora of Molise. Common lithophilous species, occurring in almost all Italian regions.

Metzgeria furcata (L.) Dumort. (Metzgeriaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691242°N, 13.970067°E, m 1570, *Fagus sylvatica* bark, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708232°N, 14.352978°E, m 930, *Pyrus pyraster* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595902°N, 14.325103°E, m 640, *Acer campestre* bark, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760078°N, 14.207411°E, m 990, *Abies alba* bark, 23 July 2023.

Plagiochila porelloides (Torrey ex Nees) Lindenb. var. *porelloides* (Plagiochilaceae) – Montedimezzo (Vastogirardi – Isernia), 41.759652°N, 14.215372°E, m 1050, *Fagus sylvatica* forest, 23 July 2023.

**Porella arboris-vitae* (With.) Grolle (Porellaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595899°N, 14.325057°E, m 630, limestone in a gorge, 22 July 2023. Species new for the bryoflora of Molise. Occurring in most Italian regions but less widespread in the southern ones (absent in Apulia and Basilicata, no recent confirmation in Calabria and Sicily).

Porella cordaeana (Huebener) Moore (Porellaceae) – Collemeluccio (Pescolanciano – Isernia), 41.709271°N, 14.348962°E, m 960, *Acer campestre* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.757923°N, 14.222516°E, m 1090, *Fagus sylvatica* bark, 23 July 2023.

Porella platyphylla (L.) Pfeiff. (Porellaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.692884°N, 13.976827°E, m 1460, *Fagus sylvatica* bark, 20 July 2023; Carpinone (Carpinone – Isernia), 41.594300°N, 14.327743°E, m 650, limestone, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759582°N, 14.215802°E, m 1040, *Fagus sylvatica* bark, 23 July 2023.

Radula complanata (L.) Dumort. (Radulaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595902°N, 14.325103°E, m 640, *Acer campestre* bark, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.757968°N, 14.220791°E, m 1090, *Acer campestre* bark, 23 July 2023.

BRYIDAE

Alleniella complanata (Hedw.) S.Olsson, Enroth & D.Quandt (Neckeraceae) – Collemeluccio (Pescolanciano – Isernia), 41.708764°N, 14.355037°E, m 910, *Abies alba* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595902°N, 14.325080°E, m 630, *Acer campestre* bark, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759652°N, 14.216534°E, m 1050, *Fagus sylvatica* bark, 23 July 2023.

Anomodon viticulosus (Hedw.) Hook & Taylor (Anomodontaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.711649°N, 14.344926°E, m 960, sandstone in *Quercus cerris* forest, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.596139°N, 14.325433°E, m 620, limestone in riverbed, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.764931°N, 14.224414°E, m 1080, limestone in *Fagus sylvatica* forest, 23 July 2023.

**Antitrichia curtipendula* (Hedw.) Brid. (Antitrichaceae) – Montedimezzo (Vastogirardi – Isernia), 41.758233°N, 14.219474°E, m 1060, *Fagus sylvatica* bark, 23 July 2023. Species confirmed for the bryoflora of Molise. Occurring in all central-southern regions except Umbria and without confirmation also in Apulia. It is an indicator species of "forest continuity" and therefore typical of old-growth forests even in the Mediterranean environment (Brunialti et al. 2010).

Barbula unguiculata Hedw. (Pottiaceae) – Carpinone (Isernia), 41.595265°N, 14.327999°E, m 650, limestone, 22 July 2023.

Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen (Brachytheciaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691242°N, 13.970067°E, m

1570, rotting stump, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708221°N, 14.353014°E, m 920, rotting stump, 21 July 2023.

Brachythecium glareosum (Bruch ex Spruce) Schimp. (Brachytheciaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, limestone in a gorge, 22 July 2023.

Brachythecium tommasinii (Sendtn. ex Boulay) Ignatov & Huttunen (Brachytheciaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023.

Calliergonella cuspidata (Hedw.) Loeske (Pylaisiaceae) – Montedimezzo (Vastogirardi – Isernia), 41.760474°N, 14.209178°E, m 990, wet meadow, 23 July 2023.

Cirriphyllum crassinervium (Taylor) Loeske & M.Fleisch (Brachytheciaceae) – Collemeluccio (Pescolanciano – Isernia), 41.712074°N, 14.343940°E, m 940, sandstone in *Quercus cerris* forest, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, gorge, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759987°N, 14.207582°E, m 990, sandstone in *Abies alba* forest, 23 July 2023.

Cratoneuron filicinum (Hedw.) Spruce (Amblystegiaceae) – M. Capraro (Capracotta – Isernia), 41.808247°N, 14.258868°E, m 1430, fountain, 21 July 2023; S. Pietro Avellana (Isernia), 41.791451°N, 14.182452°E, m 960, fountain, 23 July 2023.

**Cryphaea heteromalla* (Hedw.) D.Mohr (Cryphaeaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023. Species new for the bryoflora of Molise. Uncommon species in central-southern Italy, absent or not confirmed in Umbria, Marche, Apulia and Basilicata.

Ctenidium molluscum (Hedw.) Mitt. (Myuriaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595899°N, 14.325057°E, m 630, limestone in a gorge, 22 July 2023.

**Dialytrichia mucronata* (Brid.) Broth. (Pottiaceae) – Carpino Waterfall (Carpinone – Isernia), 41.596167°N, 14.325479°E, m 670, riverbed, 22 July 2023. Species new for the bryoflora of Molise. Widespread in most of Italy but, in the South, absent or not confirmed in Apulia, Basilicata and Calabria.

Dicranum scoparium Hedw. (Dicranaceae) – Montedimezzo (Vastogirardi – Isernia), 41.765284°N, 14.214614°E, m 1000, *Fagus sylvatica* forest, 23 July 2023.

Didymodon acutus (Brid.) K.Saito (Pottiaceae) – M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023.

Encalypta streptocarpa Hedw. (Encalyptaceae) – M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023.

Eucladium verticillatum (Brid.) Bruch & Schimp. (Pottiaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595725°N, 14.326035°E, m 630, limestone in a gorge, 22 July 2023.

**Eurhynchium striatum* (Hedw.) Schimp. (Brachytheciaceae) – Montedimezzo (Vastogirardi – Isernia), 41.759432°N, 14.216534°E, m 1060, soil in *Fagus sylvatica* forest, 23 July 2023. Species new for the bryoflora of Molise. Common and widespread species, recorded for all Italian regions except Molise but not recently confirmed in Basilicata.

Exsertotheca crispa (Hedw.) S.Olsson, Enroth & D.Quandt (Neckeraceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023; Collemeluccio (Pescolanciano

– Isernia), 41.708429°N, 14.352935°E, m 900, *Abies alba* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595709°N, 14.324656°E, m 640, limestone in a gorge, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760078°N, 14.207411°E, m 990, *Abies alba* bark, 23 July 2023.

**Fabronia pusilla* Raddi (Fabroniaceae) – S. Donato (Carpinone – Isernia), 41.606914°N, 14.324097°E, m 840, *Ulmus minor* bark, 22 July 2023; Venafro (Isernia), 41.483807°N, 14.038659°E, m 250, *Quercus ilex* bark, 24 July 2023. Species new for the bryoflora of Molise. Epiphytic species, recorded for all southern Italian regions except Molise, and not recently confirmed in Apulia.

Fissidens taxifolius Hedw. (Fissidentaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708421°N, 14.352954°E, m 910, soil in *Abies alba* and *Quercus cerris* forest, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.763878°N, 14.214164°E, m 1020, slope, 23 July 2023.

Flexitrichum flexicaule (Schwägr.) Ignatov & Fedosov (Flexitrichaceae) – M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023.

**Fontinalis antipyretica* Hedw. (Fontinalaceae) – Collemeluccio (Pescolanciano – Isernia), 41.713289°N, 14.341452°E, m 910, stream, 21 July 2023; Venafro (Isernia), 41.484137°N, 14.042546°E, m 220, stream, 24 July 2023. Species confirmed for the bryoflora of Molise. Typically rheophilic species, occurring in all regions, but not confirmed for Campania, and Basilicata in southern Italy.

Grimmia pulvinata (Hedw.) Sm. (Grimmiaceae) – Santuario Italico (Pietrabbondante – Isernia), 41.73965°N, 14.38737°E, m 1010, wall, 21 July 2023; Carpinone (Isernia), 41.593098°N, 14.328484°E, m 680, 22 July 2023; Archaeological site of Saepinum-Altilia (Sepino – Campobasso), 41.432723°N, 14.618568°E, travertine, m 550, 22 July 2023.

**Gymnostomum aeruginosum* Sm. (Pottiaceae) – Montedimezzo (Vastogirardi – Isernia), 41.759640°N, 14.212513°E, m 1040, road enbankment, 23 July 2023. Species new for the bryoflora of Molise. Lithophilous species, quite common in northern Italy but absent or not confirmed for Umbria, Lazio, Campania, and Basilicata in central-southern Italy.

Homalothecium lutescens (Hedw.) H.Rob. (Brachytheciaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023; M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023; Carpinone (Isernia), 41.594312°N, 14.327453°E, m 630, limestone, 22 July 2023.

Homalothecium sericeum (Hedw.) Schimp. (Brachytheciaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023; Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691090°N, 13.973024°E, m 1500, limestone in *Fagus sylvatica* forest, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708115°N, 14.353047°E, m 940, *Fagus sylvatica* bark, 21 July 2023; Carpinone (Isernia), 41.593418°N, 14.326554°E, m 650, limestone, 22 July 2023; Archaeological site of Saepinum-Altilia (Sepino – Campobasso), 41.432934°N, 14.618218°E, *Quercus ilex* bark, m 550, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759825°N, 14.210826°E, m 1020, *Quercus cerris* bark, 23 July 2023.

Hypnum andoi A.J.E.Sm. (Hypnaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.596139°N, 14.325433°E, m 620, riverbed, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760078°N, 14.207411°E, m 990, *Abies alba* bark, 23 July 2023.

Hypnum cupressiforme Hedw. var. *cupressiforme* (Hypnaceae) – Archaeological site of Saepinum-Altilia (Sepino – Campobasso), 41.432880°N, 14.618264°E, m 540, wall, 22 July 2023.

Isothecium alopecuroides (Dubois) Isov. (Lembophyllaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708764°N, 14.355037°E, m 910, *Abies alba* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759301°N, 14.213789°E, m 1040, *Fagus sylvatica* bark, 23 July 2023.

**Isothecium myosuroides* Brid. (Lembophyllaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023. Species new for the bryoflora of Molise. Uncommon and localized species, absent in Abruzzo and Basilicata, concerning southern Italy.

Kindbergia praelonga (Hedw.) Ochyra (Brachytheciaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708421°N, 14.352954°E, m 910, soil in *Abies alba* and *Quercus cerris* forest, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595795°N, 14.325796°E, m 670, limestone (riverbed), 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759266°N, 14.213635°E, m 1040, *Fagus sylvatica* forest, 23 July 2023.

**Leptodon smithii* (Hedw.) F.Weber & D.Mohr (Neckeraceae) – Collemeluccio (Pescolanciano – Isernia), 41.710659°N, 14.347213°E, m 960, *Quercus cerris* bark, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595746°N, 14.326148°E, m 640, limestone in a gorge, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.763857°N, 14.214217°E, m 1010, *Acer campestre* bark, 23 July 2023; Venafro (Isernia), 41.483985°N, 14.038472°E, m 240, *Quercus ilex* bark, 24 July 2023. Species new for the bryoflora of Molise. Epiphytic and lithophilous species, common and sometimes abundant, especially on bark, more common and occurring in all the regions of southern Italy.

Lescuraea incurvata (Hedw.) E.Lawton (Leskeaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023.

Leucodon sciuroides (Hedw.) Schwägr. (Leucodontaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023; Carpino Valley, Schioppo Waterfall (Carpinone – Isernia), 41.592008°N, 14.329460°E, m 680, *Acer campestre* bark, 22 July 2023; Archaeological site of Saepinum-Altilia (Sepino – Campobasso), 41.432880°N, 14.618264°E, *Quercus ilex* bark, m 550, 22 July 2023.

Lewinskya affinis (Schrad. ex Brid.) F.Lara, Garilleti & Goffinet (Orthotrichaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023.

**Lewinskya speciosa* (Nees) F.Lara, Garilleti & Goffinet (Orthotrichaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708232°N, 14.352978°E, m 930, *Pyrus pyraster* bark, 21 July 2023. Species new for the bryoflora of Molise. Epiphytic species, widespread, albeit scattered, throughout Italy. It is absent in Umbria, Apulia, and Sardinia, and is not confirmed for Lazio.

Lewinskya striata (Hedw.) F.Lara, Garilleti & Goffinet (Orthotrichaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023; Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691690°N, 13.976543°E, m 1450, *Fagus sylvatica* bark, 20 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.757283°N, 14.227680°E, m 1110, *Hedera helix* bark, 23 July 2023.

Mnium stellare Hedw. (Mniaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691090°N, 13.973024°E, m 1500, limestone in *Fagus sylvatica* forest, 20 July 2023.

**Neckera pumila* Hedw. (Neckeraceae) – Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.758233°N, 14.219474°E, m 1060, *Fagus sylvatica* bark, 23 July 2023. Species new for the bryoflora of Molise. Rare species, associated with well-preserved forest environments. In southern Italy it occurs only in Abruzzo, Campania, Apulia, Calabria, and the major islands.

**Nogopterium gracile* (Hedw.) Crosby & W.R.Buck (Leucodontaceae) – Collemeluccio (Pescolanciano – Isernia), 41.711649°N, 14.344926°E, m 960, sandstone in *Quercus cerris* forest, 21 July 2023. Species new for the bryoflora of Molise. Species occurring in all central-southern Italian regions, although not confirmed in Abruzzo.

Orthotrichum anomalum Hedw. (Orthotrichaceae) – Santuario Italico (Pietrabbondante – Isernia), 41.73965°N, 14.38737°E, m 1010, limestone wall, 21 July 2023.

Orthotrichum cupulatum Hoffm. ex Brid. var. *cupulatum* (Orthotrichaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023; Santuario Italico (Pietrabbondante – Isernia), 41.73965°N, 14.38737°E, m 1010, limestone wall, 21 July 2023.

Orthotrichum diaphanum Schrad. ex Brid. (Orthotrichaceae) – S. Donato (Carpinone – Isernia), 41.596139°N, 14.324110°E, m 820, *Acer campestre* bark, 22 July 2023.

Orthotrichum stramineum Hornsch. ex Brid. (Orthotrichaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691022°N, 13.970888°E, m 1480, *Acer pseudoplatanus* bark, 20 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759301°N, 14.213789°E, m 1040, *Fagus sylvatica* bark, 23 July 2023.

**Oxyrrhinchium hians* (Hedw.) Loeske (Brachytheciaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, gorge, 22 July 2023. Species new for the bryoflora of Molise. A common species throughout Italy and occurring in all regions.

Palustriella commutata (Hedw.) Ochyra (Amblystegiaceae) – Carpino Valley, Schioppo Waterfall (Carpinone – Isernia), 41.592292°N, 14.331917°E, m 690, travertine, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760378°N, 14.210020°E, m 1040, fountain, 23 July 2023; S. Pietro Avellana (Isernia), 41.791451°N, 14.182452°E, m 960, fountain, 23 July 2023.

Plagiomnium affine (Blandow ex Funck) T.J.Kop. (Mniaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595795°N, 14.325796°E, m 670, limestone, 22 July 2023.

**Plagiomnium rostratum* (Schrad.) T.J.Kop. (Mniaceae) – Carpino Waterfall (Carpinone – Isernia), 41.596167°N, 14.325479°E, m 670, riverbed, 22 July 2023. Species new for the bryoflora of Molise. A very common species throughout Italy and occurring in all regions.

Plagiomnium undulatum (Hedw.) T.J.Kop. (Mniaceae) – Montedimezzo (Vastogirardi – Isernia), 41.760218°N, 14.210153°E, m 1020, wet slope, 23 July 2023.

**Plagiothecium nemorale* (Mitt.) A.Jaeger (Plagiotheciaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691090°N, 13.973024°E, m 1500, limestone in *Fagus sylvatica* forest, 20 July 2023. Species new for the bryoflora of Molise. Very common in northern Italy, it is however not so widespread in the South, where it occurs only in Campania, Calabria, Sardinia, and Sicily.

Plasteurhynchium meridionale (Schimp.) M.Fleisch (Brachytheciaceae) – Carpino Valley, Schioppo Waterfall (Carpinone – Isernia), 41.591650°N, 14.328361°E, m 680, limestone in *Quercus cerris* forest, 22 July 2023.

**Plasteurhynchium striatulum* (Spruce) M.Fleisch (Brachytheciaceae) – Collemeluccio (Pescolanciano – Isernia), 41.711649°N, 14.344926°E, m 960, sandstone in *Quercus cerris* forest, 21 July 2023. Species new for the bryoflora of Molise. A very common lithophilous species throughout Italy and occurring in all regions.

Pseudoscleropodium purum (Hedw.) M.Fleisch (Brachytheciaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708421°N, 14.352954°E, m 910, *Abies alba* and *Quercus cerris* forest, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, gorge (soil), 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760474°N, 14.209178°E, m 990, meadow, 23 July 2023.

Pterigynandrum filiforme Hedw. (Pterigynandraceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691690°N, 13.976543°E, m 1450, *Fagus sylvatica* bark, 20 July 2023; Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759652°N, 14.216534°E, m 1050, *Fagus sylvatica* bark, 23 July 2023.

Ptychostomum capillare (Hedw.) Holyoak & N.Pedersen (Bryaceae) – Petacciato Marina (Petacciato – Campobasso), 42.03455°N, 14.856025°E, coastal sand dune, 24 July 2023.

Ptychostomum imbricatulum (Müll.Hal.) Holyoak & N.Pedersen (Bryaceae) – M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023; Petacciato Marina (Petacciato – Campobasso), 42.03455°N, 14.856025°E, coastal sand dune, 24 July 2023.

Pulvigera lyellii (Hook. et Taylor) Plášek, Sawicki & Ochyra (Orthotrichaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.759301°N, 14.213789°E, m 1040, *Fagus sylvatica* bark, 23 July 2023.

Rhynchostegiella tenella (Dicks.) Limpr. (Brachytheciaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595746°N, 14.326148°E, m 640, limestone in a gorge, 22 July 2023; Venafro (Isernia), 41.484437°N, 14.040032°E, m 240, wall, 24 July 2023.

Rhynchostegium murale (Hedw.) Schimp. (Brachytheciaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023.

Rhynchostegium riparioides (Hedw.) Cardot (Brachytheciaceae) – M. Capraro (Capracotta – Isernia), 41.808247°N, 14.258868°E, m 1430, fountain, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.596167°N, 14.325479°E, m 670, riverbed, 22 July 2023.

Schistidium apocarpum (Hedw.) Bruch & Schimp. (Grimmiaceae) – M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023.

**Schistidium crassipilum* H.H.Blom (Grimmiaceae) – S. Donato (Carpinone – Isernia), 41.597420°N, 14.327143°E, m 710, limestone, 22 July 2023. Species new for the bryoflora of Molise. The Italian distribution of this species is not yet fully known, although it occurs in all southern regions.

Sciuro-hypnum reflexum (Starke) Ignatov & Huttunen (Brachytheciaceae) – Montedimezzo (Vastogirardi – Isernia), 41.765041°N, 14.215632°E, m 1030, rotting log, 23 July 2023.

**Scleropodium touretii* (Brid.) L.F.Koch (Brachytheciaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595117°N, 14.327231°E, m 650, gorge, 22 July 2023. Species new for the bryoflora of Molise. Occurring in all southern Italian regions.

**Scorpiurium circinatum* (Bruch.) M.Fleisch. & Loeske (Brachytheciaceae) – Carpino Waterfall (Carpinone – Isernia), 41.595797°N, 14.325848°E, m 670, travertine, 22 July 2023; Carpino Valley, Schioppo Waterfall (Carpinone – Isernia), 41.592322°N, 14.331873°E, m 700, travertine, 22 July 2023; Venafro (Isernia), 41.484437°N, 14.040032°E, m 240, wall, 24 July 2023. Species new for the bryoflora of Molise. Species widespread throughout Italy and occurring in all southern regions.

**Syntrichia papillosa* (Wilson) Jur. (Pottiaceae) – S. Vincenzo al Volturno (Rocchetta a Volturno – Isernia), 41.648760°N, 14.084814°E, m 540, *Populus nigra* bark, 20 July 2023. Species new for the bryoflora of Molise. This is a species that becomes progressively rarer in southern Italy, where it occurs only in Abruzzo, Basilicata, Calabria, and Sicily.

Syntrichia ruralis (Hedw.) F.Weber & D.Mohr var. *ruralis* (Pottiaceae) – Santuario Italico (Pietrabbondante – Isernia), 41.73965°N, 14.38737°E, m 1010, limestone wall, 21 July 2023; M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023; Carpinone (Isernia), 41.593875°N, 14.326723°E, m 650, 22 July 2023; Archaeological site of Saepinum-Altilia (Sepino – Campobasso), 41.432878°N, 14.6182798°E, soil, m 550, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.764896°N, 14.225773°E, m 1090, *Fagus sylvatica* bark, 23 July 2023; Venafro (Isernia), 41.483905°N, 14.039010°E, m 240, wall, 24 July 2023.

**Syntrichia virescens* (De Not.) Ochyra (Pottiaceae) – S. Donato (Carpinone – Isernia), 41.606914°N, 14.324097°E, m 840, *Ulmus minor* bark, 22 July 2023. Species new for the bryoflora of Molise. Epiphytic species with very irregular distribution in Italy. It is recorded only for Abruzzo, Apulia, Sardinia, and Sicily in southern Italy.

Thamnobryum alopecurum (Hedw.) Gangulee (Neckeraceae) – Collemeluccio (Pescolanciano – Isernia), 41.712074°N, 14.343940°E, m 940, sandstone in *Quercus cerris* forest, 21 July 2023; Carpino Waterfall (Carpinone – Isernia), 41.595959°N, 14.325360°E, m 610, limestone in a gorge, 22 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.758205°N, 14.219485°E, m 1060, limestone, 23 July 2023.

**Thuidium tamariscinum* (Hedw.) Schimp. (Thuidiaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708421°N, 14.352954°E, m 910, *Abies alba* and *Quercus cerris* forest , 21 July 2023. Species new for the bryoflora of Molise. Common species in northern Italy, it is becoming increasingly rare southwards, where it is confirmed only for Abruzzo. It is not confirmed in Campania, Apulia, and Calabria.

**Tortella flavovirens* (Bruch.) Broth. var. *flavovirens* (Pottiaceae) – Petacciato Marina (Petacciato – Campobasso), 42.03455°N, 14.856025°E, coastal sand dune, 24 July 2023. Species new for the bryoflora of Molise. Psammophilous species, widespread on coastal dunes and recorded for all coastal regions.

Tortella tortuosa (Hedw.) Limpr. var. *tortuosa* (Pottiaceae) – Le Mainarde, Valle Fiorita (Pizzone – Isernia), 41.691701°N, 13.977099°E, m 1450, limestone in *Fagus sylvatica* forest, 20 July 2023; M. Capraro (Capracotta – Isernia), 41.808316°N, 14.259019°E, m 1420, limestone, 21 July 2023.

Trichostomum brachydontium Bruch (Pottiaceae) – Carpino Valley, Schioppo Waterfall (Carpinone – Isernia), 41.592337°N, 14.331937°E, m 690, limestone in a gorge, 22 July 2023; Venafro (Isernia), 41.484437°N, 14.040032°E, m 240, wall, 24 July 2023.

Zygodon rupestris Schimp. ex Lorentz (Orthotrichaceae) – Collemeluccio (Pescolanciano – Isernia), 41.708628°N, 14.352580°E, m 920, *Abies alba* bark, 21 July 2023; Montedimezzo (Vastogirardi – Isernia), 41.760047°N, 14.207716°E, m 1010, *Juglans regia* bark, 23 July 2023; Venafro (Isernia), 41.483807°N, 14.038659°E, m 230, *Quercus ilex* bark, 24 July 2023.

Discussion

In total, 88 species of bryophytes (13 liverworts and 75 mosses) were found. Of these, 23 are new to the flora of Molise (Aleffi et al. 2023): *Marchantia quadrata*, *Porella arboris-vitae*, *Cryphaea heteromalla*, *Dialytrichia mucronata*, *Eurhynchium striatum*, *Fabronia pusilla*, *Gymnostomum aeruginosum*, *Isothecium myosuroides*, *Leptodon smithii*, *Lewinskya speciosa*, *Neckera pumila*, *Nogopterium gracile*, *Oxyrrhinchium hians*, *Plagiomnium rostratum*, *Plagiothecium nemorale*, *Plasteurhynchium striatulum*, *Schistidium crassipilum*, *Scleropodium touretii*, *Scorpiurium circinatum*, *Syntrichia papillosa*, *Syntrichia virescens*, *Thuidium tamariscinum*, *Tortella flavovirens* var. *flavovirens*. Three species are confirmed for the region (Aleffi et al. 2023): *Frullania tamarisci*, *Antitrichia curtipendula*, *Fontinalis antipyretica*.

All observed species are classified as LC (Least Concern) in the European Red List (Hodgetts et al. 2019) and in the Italian Red-Lists of liverworts (Puglisi et al. 2023) and mosses (Puglisi et al. 2024). In most cases, these species are widely distributed in other regions of central-southern Italy. Less common, and missing from many southern regions, are instead *Porella arboris-vitae*, *Isothecium myosuroides*, and *Syntrichia virescens*. Noteworthy is the presence of *Antitrichia curtipendula* and *Neckera pumila*, two species linked to mature or even old-growth forests, that are locally abundant in the two Natural Reserves of Collemeluccio and Montedimezzo.

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