

# When the absence of evidence is not the evidence of absence: *Nasa* (Loasaceae) rediscoveries from Peru and Ecuador, and the contribution of community science networks

Tilo Henning<sup>1</sup>, Rafael Acuña-Castillo<sup>2,3</sup>, Xavier Cornejo<sup>4</sup>, Paúl Gonzáles<sup>5</sup>, Edgar Segovia<sup>6</sup>, Akira Armando Wong Sato<sup>7</sup>, Maximilian Weigend<sup>8</sup>

<sup>1</sup> Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374, Müncheberg, Germany

<sup>2</sup> Escuela de Biología, Universidad de Costa Rica, Apdo. Postal 11501-2060, San Pedro de Montes de Oca, San José, Costa Rica

<sup>3</sup> Herbario Luis A. Fournier Origgí, Centro de Investigación en Biodiversidad y Ecología Tropical (CIBET), Universidad de Costa Rica, Apdo. Postal 11501-2060, San Pedro de Montes de Oca, San José, Costa Rica

<sup>4</sup> Herbario GUAY, Departamento de Botánica, Facultad de Ciencias Naturales, Universidad de Guayaquil, P.O. Box 09-01-10634, Guayaquil, Ecuador

<sup>5</sup> Laboratorio de Florística, Departamento de Dicotiledóneas, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Av. Arenales 1256, Jesús María, Peru

<sup>6</sup> Universidad Católica de Cuenca, CIITT, 42VM+PJ4, Ricaurte, Azuay, Ecuador

<sup>7</sup> Plant Ecology Division, CORBIDI, Calle Santa Rita 105 Of. 2, Urb. Huertos de San Antonio Monterrico, Lima, Surco, Peru

<sup>8</sup> Nees-Institut für Biodiversität der Pflanzen, Universität Bonn, Meckenheimer Allee 171, 53115, Bonn, Germany

Corresponding author: Tilo Henning ([HenningTilo@web.de](mailto:HenningTilo@web.de))



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## Abstract

Documentation of plant taxa has long been subject to the temporal and spatial selectivity of professional research expeditions, especially in tropical regions. Therefore, rare and/or narrowly endemic species are sometimes known only from very few and very old herbarium specimens. However, these taxa are very important from a conservation perspective. The lack of observations of living plants and confirmation of the actual occurrence of taxa hinders the planning and implementation of effective conservation measures. Community science networks have recently made tremendous contributions to documenting biodiversity in many regions across the globe. The rediscovery of six species of *Nasa* (Loasaceae) from Peru and Ecuador primarily via the platform iNaturalist, is reported.

**Key words:** Andes, conservation, iNaturalist, specimen, threatened species

## Introduction

In the past, taxonomic work was almost exclusively based on physical herbarium specimens. Herbarium specimens often lay undisturbed for decades or centuries, depending on the off chance of a specialist revising the holdings of a given collection (e.g., Cornejo 2017). Digitisation of specimens has dramatically improved access to herbarium collections, and nowadays specimens deposited in a herbarium may be accessible directly via the internet, rendering the comparison to type specimens dramatically easier than when physical access was still required (Hedrick et al. 2020). But this still means that a scientist with the necessary equipment and the required research and collection permits, has

to encounter the plant in the wild, spend time and resources preparing a specimen and deposit it in a public repository, which then might be digitised at some stage. Much information is lost in the process, of course, and depending on the details recorded on the herbarium label, characters such as plant size or flower colour may be imperfectly captured. Herbarium collections remain an invaluable resource for taxonomic, floristic and systematic studies, but – by their very nature – reflect the actual distribution patterns imperfectly with a tremendous time lag (Bebber et al. 2010). If a species has gone uncollected for decades, especially in areas modified by human activities, specimen-based studies will suggest the species is extinct (Wood 2007; De Lírío et al. 2018; Pitman et al. 2022). This assumption could be reinforced if field searches of localities on herbarium labels fail to locate the species in its former habitat (Weigend 2000; Weigend and Rodríguez 2003).

The mostly tropical Andean genus *Nasa* Weigend is particularly relevant in this context: Due to its urticant nature and soft, quickly degrading leaves, the plants are difficult to collect. Additionally, the species of this genus tend to be rare, narrowly endemic, and highly seasonal – further reducing the likelihood of herbarium documentation. The genus as such is very widely distributed in tropical America from Veracruz (Mexico) to Antofagasta (Chile) on the western side of the Andes and Santa Cruz (Bolivia) in the east (Weigend 2001). However, *Nasa* reaches its highest diversity in the Amotape-Huancabamba Zone (AHZ), a region that encompasses southern Ecuador and northern Peru (Weigend 2002). In this phytogeographical zone, the highest diversity and density of taxa per unit of area is found and also the most range-restricted taxa (Mutke et al. 2014), some of which are endemic to a single known locality such as a single mountain summit or forest fragment (e.g., *Nasa glabra* (Weigend) Weigend, *N. kuelapensis* Weigend, *N. laxa* (J.F.Macbr.) Weigend, *N. pongalamesa* Weigend, *N. sanagoranensis* T.Henning, Weigend & A.Cano, *N. urentivelutina* Weigend). Further north or south of the AHZ, the distribution ranges of the taxa tend to cover larger areas, and thus, these species are less likely to be under threat (Mutke et al. 2014). On the other hand, many of the species that are restricted to the AHZ can be considered under some threat category according to national red list assessments (Rodríguez and Weigend 2006; Cornejo and Suin 2011).

In the course of systematic studies on predominantly Neotropical Loasaceae during the last decades, dozens of taxa previously unrecognized by science have been described. *Nasa* is the largest, most species-rich genus in the family (Weigend et al. 2006), with 55 of the 97 species and 21 subspecies described in the last ca. 25 years (e.g. Henning and Weigend 2011; Henning et al. 2011; Henning et al. 2019). Many of the species described during the 19<sup>th</sup> and the first half of the 20<sup>th</sup> century remained enigmatic – only known from the type collection or from a very limited number of often poorly preserved specimens. Some of these taxa, such as *Nasa aspiazui* (J.F.Macbr.) Weigend, *Nasa modesta* Weigend, *Nasa panamensis* Weigend, *Nasa rugosa* (Killip) Weigend subsp. *rugosa*, and *Nasa rufipila* Weigend have highly distinctive characters, but for decades no new specimens have been included in public collections. Despite targeted field work by the authors and overall growing collection activities in the Neotropics, the species seem to have vanished from the localities where they were originally collected. Now, however, some of those taxa have recently been rediscovered, *Nasa colanii* Dostert & Weigend, *N. hastata* (Killip) Weigend



and *N. solaria* (J.F. Macbr.) Weigend from Peru, *N. ferox* Weigend, the typical subspecies of *Nasa humboldtiana* (Urb. & Gilg) Weigend and *N. ramirezii* (Weigend) Weigend from Ecuador.

Different factors have lately come into play that resulted in these surprising rediscoveries that are summarised in this article. With the ongoing infrastructural development of the Andean countries, many new roads have increased accessibility, even to very remote areas and remaining habitat fragments (Torres Trujillo 2016; Corporación Andina de Fomento 2020). National research activities are steadily increasing in scope and efficiency. In addition, there is a vibrant scene of botanists, naturalists, environmentalists and hobbyists in, e.g., Peru and Ecuador, with a growing interest in nature and biodiversity and nature tourism, both by national and international citizens, and there has been a constant growth trend in the last decade (Santiago Chávez et al. 2017; Daries et al. 2021). Most importantly, it is no longer only professional botanists preparing herbarium specimens contributing to our understanding of biodiversity. Global networking and the increasing use of free data repositories and biodiversity networks have tremendously facilitated the presentation and availability of valuable data such as geo-referenced occurrence records and photos. iNaturalist is a particularly valuable platform for the exchange of (photographic) occurrence records. It is now considered one of the most influential community science projects (Aristeidou et al. 2021) and has already contributed towards the identification, location and description of previously unrecognised species (Winterton 2020; Alvarado Cárdenas et al. 2021). In addition to professional scientific collaborations for *N. colanii* and *N. humboldtiana*, a range of rediscoveries reported here come from fellow users of iNaturalist sharing their field images to discuss them with others.

The combination of recent field studies and a revision of digital data repositories considerably expands our understanding of the distribution patterns and status of several rare and/or putatively extinct taxa in *Nasa*.

## Materials and methods

The data for the five species included in this study were obtained from field trips, the iNaturalist.org platform, various literature references (see next sections) and material deposited at BM, E, F, GH, GOET, GUAY, HA, K, M, MO, MOL, OXF, P, S, US, USM and W. The type material and the protologues of all the species included here were examined. TH, RAC and MW have used iNaturalist to make their own field observations available to the scientific community and began curating other observations in their field of expertise. Four of the six taxa were rediscovered in this way: *Nasa ferox*, *N. hastata*, *N. ramirezii* and *N. solaria*. In the case of the two other species, *Nasa colanii* and *N. humboldtiana*, the fellow scientists AAWS from Lima, Peru and XC from Guayaquil, Ecuador directly approached TH, RAC and MW in order to help/confirm their identifications. Data for *N. colanii* has subsequently been uploaded to iNaturalist.

Field trips in Ecuador and Peru were conducted to:

- The montane forest remnant at El Corazón (2°03'S, 78°54'W), 2500–2800 m (3167 m on google earth), in the province of Chimborazo, western Andes of Ecuador, during the months of July 2021 and August 2022, by XC.

- The NW buffer zone of El Cajas National Park (2°46'51.5"S, 79°15'56.6"W), 3826–3835 m, the province of Azuay, western Andes of Ecuador, during July 2021. *Nasa* specimens grew on a 30° inclined, NW oriented slope, on a rocky outcrop at the foot of a naked rocky peak, in the valley of the Río Cajas. Vegetation among the rocks was dominated by *Polylepis reticulata* Hieron. (Rosaceae), *Gynoxys miniphylla* Cuatrec. (Asteraceae), *Gynoxys cuicochensis* Cuatrec. (Asteraceae), and another indetermined Asteraceae, and a thick layer of bryophytes covered the ground. This habitat was surrounded by grasslands and disturbed habitat (used for llama ranching). The average daytime temperature at this locality was 13 °C, night-time temperatures averaged < 0 °C and monthly precipitations varied from 35 mm in August, to 157 mm in March.
- The buffer zone of the Cordillera de Colán National Sanctuary (5°37'50.96"S, 78°15'20.84"W), 2600 m (1715 m on google earth), near the "Refugio Lechucita" of the Cordillera de Colán, in the department Amazonas, Peru, during September and December 2019 by AAWS. Rainfall is abundant and constant throughout the year with monthly averages of 91.9–226.8 mm, the forest is located on a slope oriented NE to SW. The months with the least rainfall are September, October and November, when the highest temperatures occur.
- The relict forest of Zárate (11°55'46.25"S, 76°29'36.55"W), 1400–3550 m, in the department Lima, Peru, in April 2009 by PG. Zárate has an average annual temperature of 12 °C and annual average precipitation of 360 mm, the forest is located on a slope oriented S and W, inclined between 45 and 90°, dominated by *Oreopanax oroyanus* Harms (Araliaceae), *Myrcianthes quinqueloba* (McVaugh) McVaugh (Myrtaceae), *Escallonia resinosa* Pers. (Escalloniaceae) and *Prunus rigida* Koehne (Rosaceae).
- The Arahua village (11°37'17"S, 76°40'15"W), 2450 m (2495 m on google earth), department Lima, Peru, in April 2011 by PG. Arahua has an average annual temperature of between 5–20 °C and annual average precipitation of 800 mm, and its shrubland is located on a slope oriented W, inclined between 20 and 45°, dominated by *Alonsoa meridionalis* Druce (Scrophulariaceae), *Ambrosia arborescens* Mill. (Asteraceae), *Baccharis sternbergiana* Steud. (Asteraceae), *Calceolaria angustiflora* Ruiz & Pav. (Calceolariaceae), *Mutisia acuminata* Ruiz & Pav. (Asteraceae), *Ophryosporus peruvianus* (J.F.Gmel.) R.M.King & H.Rob. (Asteraceae) and *Vasconcellea candicans* A.DC. (Caricaceae)
- The Santa Rosa de Quives district (11°34'13.05"S, 76°42'12.23"W), 2450 m (2100 m on google earth), department Lima, Peru, in June 2012 by PG. Santa Rosa de Quives has an average annual temperature between 13–20 °C and annual average precipitation of 400 mm. The shrubland is located on a slope oriented S, inclined between 30 and 50°, dominated by *Barnadesia dombeyana* Less. (Asteraceae), *Chionopappus benthamii* S.F.Blake (Asteraceae), *Jungia pauciflora* Rusby (Asteraceae), *Paracalia jungioides* (Hook. & Arn.) Cuatrec. (Asteraceae), *Lomanthus cantensis* (Cabrera) P.Gonzáles (Asteraceae), *Calceolaria angustiflora* and *Escallonia resinosa*.
- The relict forest at Huarimayo (11°30'29.59"S, 76°42'35.98"W), 2800–3000 m, department Lima, Peru, in May 2015 and May 2022, by PG. Huarimayo. It has an average annual temperature of 12 °C and annual

average precipitation of 350 mm; the forest is located on a slope oriented SW, inclined between 45 and 90°, dominated by *Oreopanax oroyanus*, *Myrcianthes quinqueloba*, *Escallonia resinosa*, *Prunus rigida* and *Cervantesia bicolor* Cav. (Santalaceae).

- The archaeological complex of Rupac (11°19'23.00"S, 76°46'52.97"W), 3033–3099 m, department Lima, Peru, in April and May 2018. Rupac has an average annual temperature of 12 °C and annual average precipitation of 500 mm. Its shrubland is located on a slope oriented W, inclined between 20 and 60°, dominated by *Alonsoa meridionalis*, *Calceolaria angustiflora*, *Lomanthus subcandidus* (A.Gray) B.Nord. (Asteraceae), *Mutisia acuminata*, *Siphocampylus tupaiformis* Zahlbr. (Campanulaceae), and *Vasconcellea candicans*.

## Results

***Nasa colanii* Dostert & Weigend, Revista Peru. Biol. 13(1): 73 (2006).**

Fig. 1A, B

**Type.** PERU. Amazonas: Provincia Bagua, Cordillera Colán SE of La Peca, ca. 3000 m, 25 Sep 1978, *P. Barbour* 3573 (holotype: MO! [acc. # 2796329]; isotype: USM [acc. # 000462]).

The *Nasa triphylla*-group also includes two subsistent taxa with reflexed trichomes from montane rainforest, namely *Nasa aequatoriana* (Urb. & Gilg) Weigend and *Nasa colanii*. *Nasa aequatoriana* is well documented from Ecuador (Weigend 2000), while *Nasa colanii* was known only from a single Peruvian collection from 1978 (Dostert and Weigend 1999; Croat et al. 2021). *Nasa colanii* is probably the one species reported from the most inaccessible region of all the species here discussed – the Cordillera de Colán in northern Peru, near to the Ecuadorean Border.

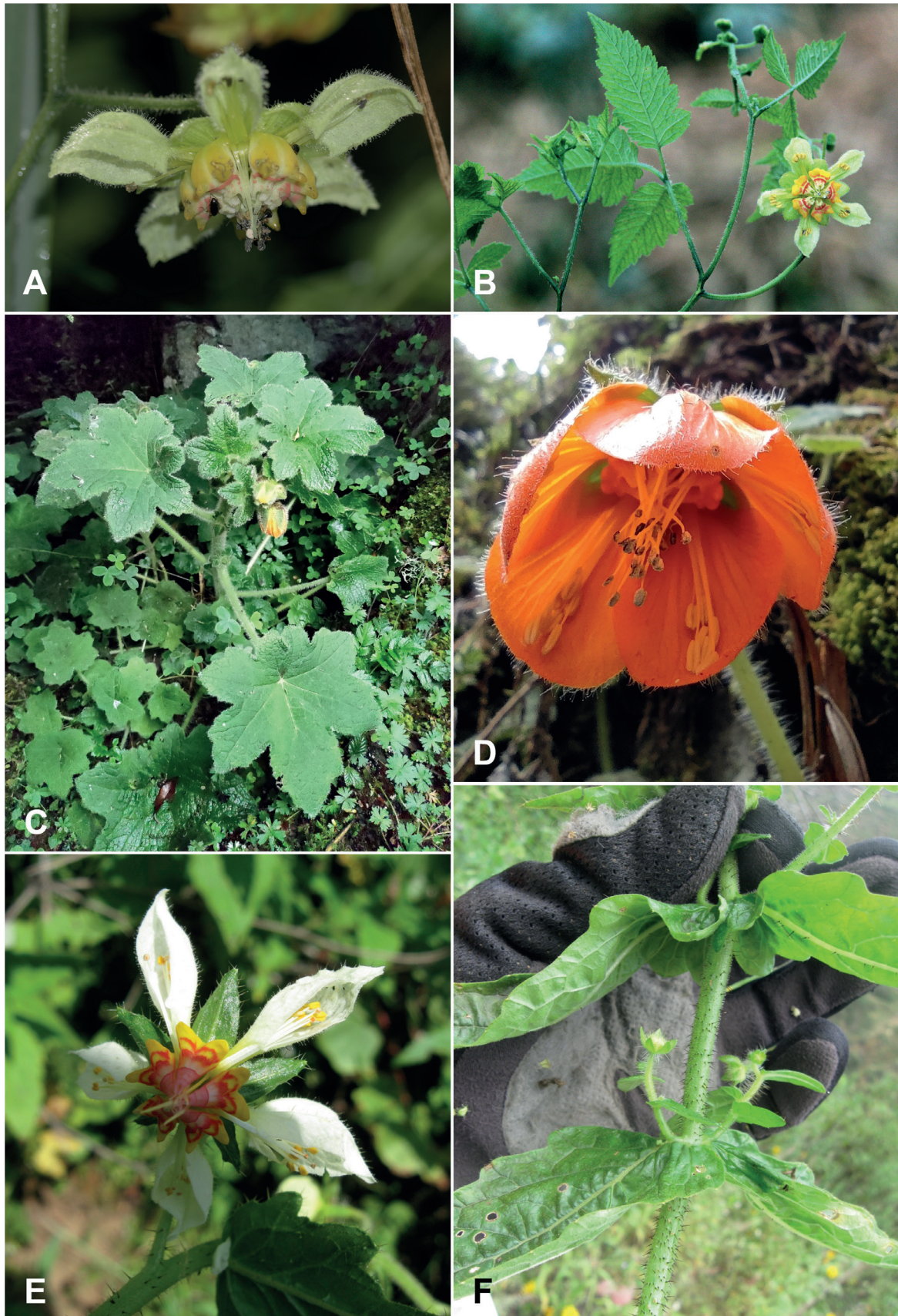
In the field, *Nasa colanii* differs from vegetatively similar *Nasa aequatoriana* by its much shorter, pale greenish-white petals (Fig. 1A, B). Additionally, the nectar scales of *N. colanii* are yellow and white with red transversal stripes, as in other species of the *Nasa triphylla* complex, but much paler with a very narrow red band only (Fig. 1A).

*Nasa colanii* was found on creek banks in rocky soils in a cloud forest ecosystem located in the buffer zone of the Cordillera de Colán National Sanctuary (5°37'50.96"S, 78°15'20.84"W) at an elevation of 2605 m, near the Refugio Lechucita. This taxon had previously only been reported once in 1978 from the same region, possibly from the same locality (Rodríguez and Weigend 2006; Wong Sato et al. 2021). This species has probably not been collected since, due to its apparent narrow endemism and a lack of scientific exploration of this area (Rodríguez and Weigend 2006; C. Olivera, pers. comm., 2021).

**Additional specimens examined.** PERU. Amazonas: Provincia Utcubamba, Distrito Cajaruro, buffer zone of the Cordillera de Colán National Sanctuary, ca. 2605 m, 5°37'50.96"S, 78°15'20.84"W 21 Dec 2019, A.A. Wong Sato 53 (MOL).

**Photographic record.** PERU. Amazonas: Provincia Utcubamba, Distrito Cajaruro. Buffer zone of the Cordillera de Colán National Sanctuary; observation by A. A. Wong Sato, 21 Dec 2019 (Wong Sato 53, MOL): <https://www.inaturalist.org/observations/143281337>.





**Figure 1.** **A, B** *Nasa colanii* **C, D** *Nasa ferox* **E, F** *Nasa hastata* **A** flower of *N. colanii* **B** flowering branch of *N. colanii* **C** habit of *N. ferox* **D** flower of *N. ferox* **E** flower of *N. hastata* **F** node with the characteristic, semiamplexicaulous leaves of *N. hastata*, Photo credits: **A, B** A. A. Wong Sato **C, D** E. Segovia **E, F** P. Gonzáles.

***Nasa ferox* Weigend, Revista Peru. Biol. 13(1): 74 (2006).**

Fig. 1C, D; fig. 8 in Weigend 1996b (as *Loasa peltata*)

**Type.** ECUADOR. Azuay: Cantón Cuenca, Contrayerba, 3600–3800m, s.d., F.C. Lehmann 7943 (holotype: US 00603973!; isotypes: F No. 578096!, K 000372883!).

Described only in 2000 (Weigend et al. 2006) from specimens collected by F. C. Lehmann probably in the 1880s (possibly May 1887, according to Cribb 2010, Lehmann was in Contrayerba at least twice). Specimens of this species were considered as belonging to *Loasa ranunculifolia* Kunth (Urban and Gilg 1900) or *Loasa peltata* Urb. & Gilg (Weigend 1996b). The species was known with certainty only from the area of Contrayerba, in the province of Azuay close to the NW border of what is now Parque Nacional Cajas and had not been reported for ca. 130 years. Given the location of the park close to the city of Cuenca, and the fact that the important road 582 goes through the park makes it particularly surprising that the species has not been reported in such a long time, even more so if we consider the numerous botanical expeditions that have been carried out in the general region. New photographs uploaded by ES to iNaturalist in 2022 clearly show living plants of this species previously known only from dried specimens. Judging from the pictures now available, the species seems closely allied to *Nasa jungiifolia* (Weigend) Weigend from just a little bit further south in Azuay, but differs from it in the smaller stature of the plants (20–70 cm, Fig. 1C) and the shorter, wider and fleshy, deep orange petals (Fig. 1D) (versus taller plants to >1 m in height and narrower, long acuminate, membranous, pale orange petals in *N. jungiifolia*). The habitat of the living plants of *N. ferox* is located in a rock outcrop at the foot of a vertical rocky cliff, with nearby pastures, some used for llama ranching. The substrate is covered by a dense layer of mosses along with succulent *Peperomia* spp. (Piperaceae), *Stellaria* spp. (Caryophyllaceae), *Oxalis* spp. (Oxalidaceae) and ferns. Tall shrubs and small trees such as *Polylepis reticulata*, *Gynoxys* spp. (Asteraceae), and an undetermined Asteraceae were the main woody species of this habitat. The slope in the site is low, about 30 degrees. *Nasa ferox* is not an abundant species; only a very small population of about ten fertile plants growing in four spots near the borders of the rock zone could be found, with the plants growing always in sheltered places, in rock crevices, near big rocks or at the base of dense, taller, shrub aggregations. Some regeneration was observed at the end of the rain season in July, with a few seedlings growing among the moss carpets that covered the rocks. We also saw some infertile plants growing near the fertile ones in two of the spots. Footprints and dung from llamas and bovines on the trails nearby, show that livestock roam the area.

**Additional specimens examined.** ECUADOR. Azuay: Province unknown: „Andes of Ecuador“, R. Pearce 1862 (K); Provincia Azuay, Cantón Cuenca, Reserva de la Biosfera Macizo del Cajas, 3835 m, 12 Jan 2022, E. Segovia 3239-CMP40 (HA); Reserva de la Biosfera Macizo del Cajas, 3823 m, 07 Jul 2022, E. Segovia 4890-CMP40 (HA).

**Photographic records.** ECUADOR. Azuay: Cantón Cuenca, Reserva de la Biosfera Macizo del Cajas, 2.78118S, 79.26592W, 3835 m, E. Segovia, Jan 2022, <https://www.inaturalist.org/observations/105051734> (<https://www.gbif.org/occurrence/3465963568>); Reserva de la Biosfera Macizo del Cajas, 2.778691S, 79.266028W, 3825 m, K. Montesinos, 21 May 2022, <https://www.inaturalist.org/observations/145275636> (<https://www.gbif.org/occurrence/4011672795>);



Reserva de la Biosfera Macizo del Cajas, 2.782492S, 79.267025W, 3823 m, E. Segovia, Jul 2022, <https://www.inaturalist.org/observations/125099917> (<https://www.gbif.org/occurrence/3858810457>); Reserva de la Biosfera Macizo del Cajas, 2.734655S, 79.259702W, G. Normand, Apr 2022, <https://www.inaturalist.org/observations/112870994> (<https://www.gbif.org/occurrence/3764320941>).

***Nasa hastata* (Killip) Weigend, T.Henning & R.H.Acuña, comb. nov.**

urn:lsid:ipni.org:names:77322127-1

Fig. 1E, F; also see Weigend and Rodríguez 2003: fig. 18A–F

*Loasa hastata* Killip, J. Wash. Acad. Sci. 18(4): 92 (1928). Basionym. Type: Peru. Lima. Provincia Huarochirí, Matucana, 2500 m, Apr–May 1922, *J.F. Macbride & W. Featherstone* 416 (holotype: F! [acc. # 516950!]; isotype: US! [00115210, acc. # 1230340]).

**Type.** Based on *Loasa hastata* Killip.

Like *N. solaria* (J.F.Macbr.) Weigend (see below), this species was collected by J. F. Macbride and W. Featherstone in what is now the province of Huarochirí in the department of Lima. No subsequent collections were known of this species, and it was considered extinct in the wild (Weigend and Rodríguez 2003). The affinities of this morphologically distinctive Central Peruvian endemic remain obscure. Its leaf shape is unique in *Nasa* and the nectar scales are also very distinctive (Fig. 1E, F). Weigend and Rodríguez (2003) considered it as part of the *Nasa stuebeliana* (Urb. and Gilg) Weigend species group, a group that is otherwise most likely monophyletic and mostly restricted to the southern half of the AHZ. Recent field studies by PG yielded new observations of this taxon from the districts of Arahua and Santa Rosa de Quives in the province of Canta, showing that it is still present in the department of Lima.

In Arahua, this species is restricted to a narrow altitudinal range between 2450 and 2500 m on shrubland. Only one sterile individual was recorded and photographed in April 2009. Two years later (2011) a small population of five flowering individuals was encountered and photographed by Elizabeth Gonzáles, the sister of PG. Another expedition to the site in April 2015 by PG and Tim Böhnert (Bonn) yielded only two sterile individuals. In Santa Rosa de Quives, PG and his colleague Eduardo Navarro walked a 500 m trail collecting plants across the shrubland for four hours and only recorded a single individual of *Nasa hastata*. The two localities are only 7 km apart and are separated by a mountain ridge that reaches 3,500 m.

**Note.** For obvious reasons iNaturalist limits the designation of taxon names to scientific names from external curated data sources such as IPNI (ipni.org). This helpful functionality revealed a nomenclatural issue with the name *Nasa hastata*, which was not available. Instead only the basionym “*Loasa hastata*” could be found in IPNI, together with the remark that due to an incorrect citation of the basionym reference, *Nasa hastata* was a name not validly published which had been used in previous publications (Weigend in Weigend 1998: 164; Weigend and Rodríguez 2003: 377; Weigend in Weigend et al. 2006: 75). The nomenclatural problem is solved here by our validation of the combination.

This is a nice example of how meaningful linkage of individual databases not only offers quickly accessible information in a convenient form. It provides different dimensions of error avoidance by ensuring the correct spelling of names and authorities, but also revealing profound nomenclatural issues as in the present case.

**Additional specimens examined. PERU. Lima:** Provincia Canta, Distrito Arahua, Arahua y alrededores, matorral, 2450 m, 11°34'13.05"S, 76°42'12.23"W, 28 Apr 2011, *P. Gonzáles et al.* 1469 (USM); Distrito Santa Rosa de Quives, a 3.5 km de Pichu Pichu, matorral dominado por *Jungia amplistipula* (Asteraceae) y *Barnadesia dombeyana*, 2165–2363 m, 11°34'13.05"S, 76°42'12.23"W, 6 Jun 2012, *P. Gonzáles & E. Navarro* 1873 (USM [acc. # 275320]); *Unknown* 625 (USM [acc # 174383]).

**Photographic record. PERU. Lima:** Provincia Canta, Arahua, P.Gonzáles, 30 Apr 2009, <https://www.inaturalist.org/observations/100632141>; Arahua, 28 Apr 2011 (*Gonzáles et al.* 1469, USM), <https://www.inaturalist.org/observations/139056447>; Santa Rosa de Quives, 6 Jun 2012, (*Gonzáles and Navarro* 1873, USM acc. # 275320), <https://www.inaturalist.org/observations/139059041>.

### ***Nasa humboldtiana* (Urb. & Gilg) Weigend subsp. *humboldtiana***

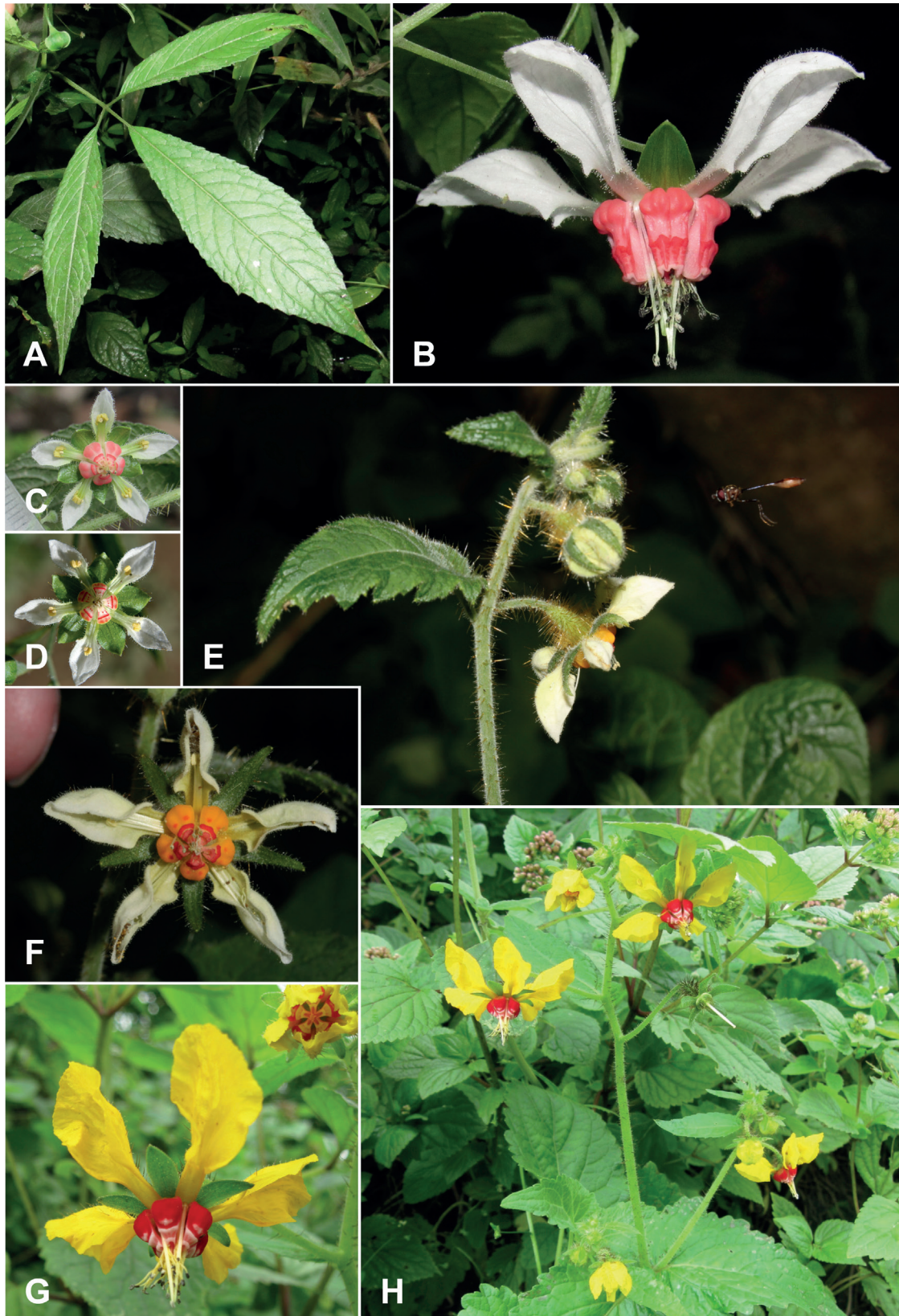
Fig. 2A, B

*Loasa humboldtiana* Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 240, pl. 5, fig. 40 (1900). Type. Ecuador. Chimborazo: Cantón Chunchi?, in andibus ecuadorensibus, Llalla, Aug 1859, *R. Spruce* 6002 (lectotype, designated in Weigend 1996a: 232: P! [00123885]; isoelectotypes: B[destroyed, F Neg No. 10196!], BM! [BM000021453], E! [E00085319], F! [acc. #1540659], GH! [00076022], GOET!, K! [K000372786, K000372787, K000372788], M! [0113254], OXF!, P [P02273159] S! [acc. # S-R-8215], W! [0053328, 1889-0113217]).

**Type.** Based on *Loasa humboldtiana* Urb. & Gilg.

*Nasa humboldtiana* belongs to the *Nasa triphylla* (Juss.) Weigend complex (Dostert and Weigend 1999), a natural group of herbaceous annuals found in the undergrowth of humid forest and disturbed sites in the northern Andes and Central America. This group is characterized by compound leaves (Fig. 2A), a trait that is very rare in *Nasa* (Acuña-Castillo et al. 2021). The highest number of species in this complex is in the AHZ, only two out of 24 taxa (including subspecies) grow exclusively outside this region (Dostert and Weigend 1999).

*Nasa* (*Loasa*) *humboldtiana* has been very poorly understood since its original description, with only two known collections from the 19<sup>th</sup> century (Weigend 1996a). In recent years, extensive field studies yielded several new taxa closely allied to *Nasa humboldtiana*. In a synopsis of the *Nasa triphylla* complex (Dostert and Weigend 1999), *N. humboldtiana* was expanded to include three additional infraspecific taxa namely subsp. *roseoalba* (Weigend) Dostert (originally described as a distinct species, *Loasa roseoalba* Weigend: Weigend 1996a), subsp. *tricolor* Dostert & Weigend and subsp. *obliqua* Dostert & Weigend. Later, targeted field work led to the discovery of two additional subspecies.



**Figure 2.** **A–D** *Nasa humboldtiana* **E, F** *Nasa ramirezii* **G, H** *Nasa solaria* **A** trifoliolate leaf of *N. humboldtiana* subsp. *humboldtiana* **B** flower of *N. humboldtiana* subsp. *humboldtiana* **C** flower of *N. humboldtiana* cf. subsp. *obliqua* from Chimborazo/Ecuador **D** flower of *N. humboldtiana* subsp. *obliqua* from Cajamarca/Peru **E** inflorescence of *N. ramirezii* with putative pollinator **F** flower of *N. ramirezii* **G** flower of *N. solaria* **H** habit of *N. solaria*. Photo credits: **A–C** X. Cornejo **D** T. Henning **E, F** R. Ripley **G, H** P. Gonzáles.



*Nasa humboldtiana* subsp. *subtrifoliata* Weigend & T.Henning and subsp. *glandulifera* Weigend & T.Henning are endemic to small forest remnants in northern Peru and under imminent threat of extinction (Henning and Weigend 2009).

*Nasa* (*Loasa*) *humboldtiana* was described in 1900 (Urban and Gilg 1900), and the most recent herbarium collection by Richard Spruce dated back to Aug. 1859 from what today is the southern part of the province of Chimborazo, Ecuador (Spruce 1908 p. 222 & 249–250). *Nasa humboldtiana* subsp. *humboldtiana* differs most notably from the other subspecies by its remarkably different nectar scales, with a rectangular (instead of tapering) scale neck (Henning and Weigend 2009). In 2021, one of us (XC) collected material clearly referable to the typical subspecies *humboldtiana* in a ravine within a conserved remnant of montane Andean forest in El Corazón, a private property located in the province of Chimborazo (Fig. 2A, B), ca. 30 km straight line from the type locality of this taxon, which signifies the rediscovery of the taxon after 162 years. The same population, represented by fewer than five individuals, has been observed in the field flowering at least twice (XC), most recently in August 2022.

**Additional specimens examined. ECUADOR. Chimborazo:** Cantón Pallatanga, Reserva El Corazón, a montane Andean forest, ca. 2700 m, 2°3'S, 78°54'W, 10 Jul 2021, X. Cornejo & J. Josse 9388 (GUAY).

***Nasa humboldtiana* (Urb. & Gilg) Weigend subsp. *obliqua* Weigend, Revista Peru. Biol. 13(1): 75 (2006).**

Fig. 2C, D

**Type.** PERU. Cajamarca: Provincia Hualgayoc [Prov. Santa Cruz], Monte Seco, 1800 m, J. Soukup 3826 (holotype: US! [00604255, acc. # 1985252]).

Collected in the Reserva El Corazón, but in a semi open forest/grassland ecosystem with scattered native tree remnants adjacent to the forest where *Nasa humboldtiana* subsp. *humboldtiana* occurs, and on the same day, XC also collected and photographed another subspecies of *Nasa humboldtiana*, which is here tentatively assigned to *N. humboldtiana* cf. subsp. *obliqua* (Fig. 2C). This taxon had previously only been reported from a single area in northern Peru, namely the Prov. Santa Cruz in the department of Cajamarca (Fig. 2D). The new record in Ecuador lies ca. 550 km north of the original collection on the western slope of the Peruvian Andes, and additional studies are required to confirm its identity. It may also represent a separate and novel taxon or unusually small-flowered plants of the widespread SW Ecuadorean endemic *Nasa humboldtiana* subsp. *roseoalba*, whose type locality is near Chillanes (Bolívar, Ecuador) ca. 20 km to the northwest. This taxon was even collected previously in El Corazón in sterile condition in Feb. 2017 (R. Acuña & D. Guilcapi 1725, QCA, BONN). The leaflet texture and leaflet base shape of the newly collected plants appear to be somewhat intermediate between more typical *N. humboldtiana* subsp. *roseoalba* and *N. humboldtiana* subsp. *obliqua*.

**Additional specimens examined. PERU. Cajamarca:** Provincia Santa Cruz, Monte Seco, 1500 m, N. Dostert 98/154 (CPUN, F, M, USM); La Florida, above Monteseco, 1200–1500 m, 5 May 2003, M. Weigend et al. 7554 (B, HUT, USM, M); Near Agua Azul, 5 May 2003, Weigend et al. 7569 (B, HUT, M, USM); **ECUADOR. Chimborazo:** Reserva El Corazón, a montane Andean forest, ca. 2700 m, 2°03'S, 78°54'W, 10 Jul 2021, X. Cornejo & J. Josse 9389 (GUAY).

***Nasa ramirezii* (Weigend) Weigend, Revista Peru Biol. 13(1): 80 (2006).**

Fig. 2E, F; fig. 6 in Weigend 1996a (as *Loasa ramirezii*)

*Loasa ramirezii* Weigend, Sendtnera 3: 231 (1996). Type. Colombia. Nariño: Municipio Tangua, 5 km S of Tangua [vertiente al otro lado del valle sur de Tangua], 2600 m, M. Weigend & B.R. Ramírez 3280 (holotype: M! [M-0113266]; isotypes: COL!, PSO! [PSO0000004, PSO0000005]).

**Type.** Based on *Loasa ramirezii* Weigend.

The rediscovery of *Nasa ramirezii* is here reported for Ecuador only. It was described in 1996 based on a collection made in the southern Colombian department Nariño by MW and cultivated plants thereof. From Ecuador, however, only a small number of very old specimens was known which all lack proper locality information. These four collections all go back to the 19<sup>th</sup> century and could only tentatively be assigned to this species, whose occurrence was only secured from a few small patches in southern Colombia so far. Until now, this taxon could only be assumed to occur in northern Ecuador, and given the widespread habitat destruction in this region, it was suspected that it might be extinct there. Two recent observations uploaded to iNaturalist revealed the first photographs of living plants from Ecuador and the first exact locality information. The taxon is apparently restricted to a small area in the province of Imbabura and has been repeatedly observed in the Conrayaro forest.

Two independent observations have been recorded from the same area by Ruth Ripley in March 2018 and Mony León in April 2023, respectively. The former shared the following detailed occurrence data with us: flowering plants of *Nasa ramirezii* were found on the path to the Cascada de Conrayare, in San Alfonso de Iruguincho, in Timbuyacu, to the southwest of Cerro de Añaburo at elevations between 2700–3000 m. This is located in San Miguel de Urcuquí County, Imbabura Province in NW Ecuador. The area is dominated by Andean forest and some common angiosperms include for example *Barnadesia* sp., *Bomarea* sp. (Alstroemeriaceae), Ericaceae, *Geranium* sp. (Geraniaceae), Melastomataceae, *Oxalis* sp., *Peperomia* sp., *Phyllanthus* sp. (Phyllanthaceae), *Salvia* sp. (Lamiaceae) and *Siparuna* sp. (Siparunaceae).

**Additional specimens examined.** **COLOMBIA. Valle de Cauca:** Popayan, Western slopes of the Sotara Volcanoe, 2400 m, *Lehmann* 6205 (K); **Nariño:** Tangua, Tapialquer, 2250–2500 m, *B.R. Ramirez s.n.* (PSO); 5km south of Tangua in a coffee plantation, 2600 m, *M. Weigend* 3280 & *B.R. Ramirez* (M, COL, PSO); Tajumbina, Mpio de la Cruz, 2630 m, *Buenavides s.n.* (PSO); Mpio Consaca, Mpio de Coriaco, 1820 m, *Ramirez s.n.* (PSO); Mpio de Consaca, Seccion de Coriaco, 1820 m, *Guarin* 407 (PSO). **ECUADOR. Province unknown:** “Andes of Quito”, *Jameson* 79 (K); “Andes of Cuenca at 10,000 feet in woods, July 1840” *Jameson* 289 (K); Without locality, *Jameson s.n.* anno 1840 (E); “Loasa sp. Nova de Huayaquil [Guayaquil]”, *Ruiz & Pavón s.n.*, leg. Tafalla (BM, G).

**Photographic records.** **ECUADOR. Imbabura:** San Miguel de Urcuquí, Conrayaro forest, 0.427288S, 78.270863W, 14 Mar 2018, R. Ripley <https://www.inaturalist.org/observations/12800711> (<https://www.gbif.org/occurrence/3466042315>); W 78.278315, 0.436863, 2 Apr 2023, M. León <https://www.inaturalist.org/observations/153269256>.



***Nasa solaria* (J.F.Macbr.) Weigend, Revista Peru. Biol. 13(1): 80 (-81) (2006)**  
Fig. 2G, H

*Loasa solaria* J.F.Macbr, Publ. Field Mus. Nat. Hist., Bot. Ser. 13(4): 163 (1941).  
Type. Peru. Lima: Provincia Huarochirí, San Miguel de Viso, ca. 2750 m, May 1922, J.F. Macbride & W. Featherstone 577 (holotype: F! [acc. # 517105]; isotype: US! [00115216, acc. # 1230343]).

**Type.** Based on *Loasa solaria* J.F.Macbr.

Nine species of *Loasa* originally described in the Flora of Peru (Macbride 1941) are today included in *Nasa*. All of these are still considered distinctive and accepted as good species (Weigend et al. 2006). Although all were described from relatively few specimens (some collected by Macbride himself), seven have been rediscovered and studied in the wild and/or in cultivation in the past decades. Two species, however, still remain unknown (or almost so) in the wild: *Nasa aspiazui* from Junín (collected by A. Weberbauer) and *N. solaria* from Lima (collected by J. F. Macbride and W. Featherstone). *Nasa solaria* is the only species in the genus with the combination of entire, shallowly lobed leaves and flowers with deep yellow petals and bright red nectar scales and cannot be confused with any other species (Fig. 2G, H). *Nasa solaria* is morphologically quite aberrant in the genus, and this has rendered a morphological placement difficult. Plastid DNA (obtained after the rediscovery of the species) seems to indicate it could be allied to the morphologically plesiomorphic *Nasa poissoniana* (Urb. & Gilg) Weigend species group (Acuña-Castillo et al. 2021). The original collection of *Nasa solaria* came from the department of Lima, province Huarochirí – and despite its proximity to the national capital, about 80 km, it has been recovered only once in the last century in this area. This and a second collection from the department of Lima in 1998, as well as two earlier collections from the neighbouring department of Ancash, remained undiscovered in the herbarium in Lima (USM) until a targeted search was conducted after the species was recently rediscovered and the first photos of living plants reached us. The area of the original collection has been subject to massive human intervention and land use change, possibly leading to local extinction. Recently, there have been several new collections of this species from the Province of Huarochirí, where the original material came from and the neighbouring Provinces Canta and Huaral, confirming that the species remains rare, but is still present in the area. This species is restricted to an elevational range of ca. 1000 m between 2800 and 3600 m; currently, two populations are known from the undergrowth of relict forests. The other two known localities, Carhua in Prov. Canta and Rupac in the Prov. Huaral, are in shrubland, where very scattered, small trees of *Myrcianthes quinqueloba* and *Escallonia resinosa* can still be found, indicating that these areas were previously covered with forest, but that despite deforestation, *Nasa solaria* still grows there. In the forests of Zárate, Prov. Huarochirí, only two individuals were found. In the forests of Huarimayo, Canta, four populations separated by ca. 300 m in a linear transect were found between the years 2015 and 2022 with a total of only seven individuals. At Rupac, Huaral, one population was recorded with two individuals in April 2018 and five plants in May 2018.

**Additional specimens examined. PERU. Ancash:** Provincia Bolognesi, Acas, monte bajo, borde de chacra, 3600 m, 16 Jun 1969, *E. Cerrate* 7463 (USM 284507); Subida de la Rinconada a la cumbre, camino de Ocros, monte bajo, 3000 m, 2 May 1977, *E. Cerrate* 6646 (USM 271500); **Lima:** Provincia Canta, Carhua, en la carretera hacia Paríamarca, ladera arcillosa con arbustos perennifolios, 3300 m, 3 May 1998, *G. Segovia* 4756 (USM 277067); Distrito San Buenaventura, San José, justo en el límite con Huamantanga, bosque relicto, 2800–3000 m, 11°30'29.59"S, 76°42'35.98"W, 30–31 May 2015, *P. Gonzáles et al.* 3773 (USM 290273); bosque relicto de Huarimayo, bosque relicto, 2877 m, 11°30'29.59"S, 76°42'35.98"W, 12 May 2022, *P. Gonzáles et al.* 10470 (USM); Arriba de San Bartolomé, Monte Zárate, 2900–3000 m, 29 May 1954, *R. Freyre* 9712 (USM 28005); San Bartolomé, Monte de Zárate, matorral y relicto de bosque dominado por *Oreopanax*, *Myrcianthes* entre otros, 1440–3550 m, 11°55'46.25"S, 76°29'36.55"W, 24–26 [25] Apr 2009, *P. Gonzáles et al.* 492 (USM 256800); Provincia Huaral, Distrito. Atavillos Bajo, Pampas, subida a Rupac, ladera con suelo franco-arcilloso, matorral, 3033–3509 m, 11°19'23.00"S, 76°46'52.97"W, 15 Apr 2018, *A. Cano et al.* 22677 (USM 327563); Pampas, en las cercanías al centro poblado y camino al complejo arqueológico de Rupac, ladera con afloramiento rocoso suelo franco-arcilloso a franco-arenoso, matorral, 3033–3099 m, 11°19'23.00"S, 76°46'52.97"W, 7 [May] Jun 2018, *A. Cano et al.* 22723 (USM 327614).

**Photographic record. PERU. Lima:** Provincia Canta, Bosque de Huarimayo, W. Aparco, 31 May 2015 (*P. Gonzáles et al.* 3773, USM acc. # 290273), <https://www.inaturalist.org/observations/139042423> (<https://www.gbif.org/occurrence/3947631714>); Provincia Huarochirí, Bosque de Zárate, 25 Apr 2009 (*P. Gonzáles et al.* 492, USM acc. # 256800), <https://www.inaturalist.org/observations/118647914> (<https://www.gbif.org/occurrence/3802749525>); *P. Gonzáles*, 27 May 2019, <https://www.inaturalist.org/observations/118647465> (<https://www.gbif.org/occurrence/3802781447>); Provincia Huaral, Rupac, *P. Gonzáles*, 15 Apr 2018 (*Cano et al.* 22677, USM acc. # 327563), <https://www.inaturalist.org/observations/139044179> (<https://www.gbif.org/occurrence/3947206738>); *P. Gonzáles*, 7 May 2018 (*Cano et al.* 22723, USM acc. # 327614), <https://www.inaturalist.org/observations/100467047> (<https://www.gbif.org/occurrence/3416222423>).

## Discussion

Ongoing documentation of biodiversity by trained botanists continues to yield important species records – even in, by tropical South American standards, relatively well sampled countries such as Ecuador and highly accessible regions such as the department of Lima in Peru. The rediscovery of *Nasa humboldtiana* subsp. *humboldtiana* after more than 160 years, the tentative range extension of *Nasa humboldtiana* subsp. *obliqua* and the rediscoveries of *Nasa solaria*, and especially *Nasa hastata*, after nearly 100 years, near Lima, are typical examples of these crucial contributions to our understanding of species ranges and conservation.

However, many non-botanists get out into the field, lacking the requisite permits, training and ambition to prepare specimens and deposit them in a public repository. Photographic documentation of biodiversity, however, is a pastime

for many and coincidentally may lead to highly relevant taxon records, particularly if they end up being uploaded into public databases. The records on iNaturalist can be studied by professional taxonomists, who are able to provide accurate determinations, or even recognize undescribed taxa, provided that the images uploaded show diagnostic traits of a taxon in sufficient detail. These digital records efficiently complement the records obtained from scientific collections, such as herbaria or museums, and we agree that the inclusion of these records into GBIF is appropriate.

Of course, where both data sources, photographic records and physical herbarium specimens, are linked, this creates a tremendous added value (Heberling and Isaac 2018). Purely digital occurrence records should not be understood as a substitute for physical specimens (Daru and Rodriguez 2023), but due to a range of reasons (see above) photographic records are often created under conditions where the preparation of physical specimens would be impossible or illegal. Conversely, specimens collected in the field should ideally be supplemented by, for example, an iNaturalist record, and vice versa, provided that collecting a specimen is permitted and justifiable.

Information about a species' geographic range and possibly even abundance can be gathered from specimens kept in scientific collections, taxonomic revisions, field guides and similar works, but this data is often diffuse due to the long time period aggregated into the characterisation of range and abundance. iNaturalist can provide a sharper picture in time and space, since it documents taxa from a specific place and time and usually soon after the observation, providing a much more current view of occurrences. This has helped us and our colleagues to locate areas where the chances of finding and collecting a species are high, saving time and resources during field research. Due to these advantages, data obtained from iNaturalist are increasingly included in professional systematic, floristic, ecological, and conservation studies (Atha et al. 2020; Soteropoulos et al. 2021; Iwanycki Ahlstrand et al. 2022; Smith et al. 2022). Critically, and as in the case of this study, iNaturalist's data has also led to the rediscovery of populations of taxa considered as extinct for many years as is the case of *Gasteranthus extinctus* L.E.Skog & L.P.Kvist (Gesneriaceae) by Pitman et al. (2022), *Scarabaeus sevoistra* Alluaud, 1902 (Coleoptera: Scarabaeinae) by Deschodt et al. (2021), and *Tipulodes annae* Przybyłowicz, 2003 (Lepidoptera, Erebidae) by Alzate-Cano and Hurtado-Pimienta (2021). Hopefully, as more scientists and members of the public contribute to the database, and more professionals get involved in the curation (Callaghan et al. 2022), more undescribed or "long lost" taxa will be found. Our examples of the rediscovery of *Nasa ferox* after 130 years and *Nasa hastata* after 100 years, both "found" on iNaturalist underscore this point.

Conservation in the long term is only possible with the involvement of the human population at large and most importantly of the local communities. Biological education programs and the easy access to tools that permit the nature enthusiasts to document their encounters with nature can raise awareness and attract more people into investigating their natural environment (Echeverria et al. 2021), increasing their appreciation and understanding of biodiversity and ecology. iNaturalist.org has simultaneously become an important contributor to the knowledge of biodiversity and a successful tool for public engagement (Aristeidou et al. 2021).

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### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

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### Author contributions

TH, RA and MW conceptualised the paper and wrote the first draft. XC, PG, AAWS and ES provided their data and added the respective paragraphs. All authors contributed to the manuscript.

### Author ORCIDs

Tilo Henning  <https://orcid.org/0000-0003-1929-3264>

Rafael Acuña-Castillo  <https://orcid.org/0000-0002-8568-8566>

Xavier Cornejo  <https://orcid.org/0000-0002-4081-4047>

Paúl Gonzáles  <https://orcid.org/0000-0002-6799-376X>

Akira Armando Wong Sato  <https://orcid.org/0000-0002-3617-8121>

Maximilian Weigend  <https://orcid.org/0000-0003-0813-6650>

### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Checklist

## Checklist of Orchidaceae from Caquetá, Colombia

Tatiana Arias<sup>1,2</sup>, Jeisson Chaux-Varela<sup>3\*</sup>, Maria del Pilar Camero<sup>3</sup>, R. Alexis Calderon-Álvarez<sup>3,4</sup>, Anyi Carolina Trujillo<sup>3</sup>, Marco A. Correa-Munera<sup>3,5</sup>, Alejandro Zuluaga<sup>6</sup>, Oscar Perdomo<sup>7</sup>, Oscar A. Pérez-Escobar<sup>8</sup>, Edwin Trujillo-Trujillo<sup>7,9</sup>, Janice Valencia-D.<sup>9</sup>

1 Marie Selby Botanical Gardens, Sarasota FL, USA

2 Sociedad Colombiana de Orquideología, Medellín, Antioquia, Colombia

3 Departamento de Biología, Universidad de la Amazonia, Florencia, Caquetá, Colombia

4 Grupo de Investigación en Ecología y Territorio, Departamento de Ecología y Territorio, Pontificia Universidad Javeriana, Bogotá D.C., Colombia

5 Jardín Botánico Uniamazonia and Herbario Enrique Forero – HUAZ, Florencia, Caquetá, Colombia

6 Departamento de Biología, Universidad del Valle, Cali, Valle del Cauca, Colombia

7 Grupo de Investigación en Agroecosistemas y Conservación en Bosques Amazónicos GAIA, Universidad de la Amazonia, Florencia, Caquetá, Colombia

8 Royal Botanic Gardens, Kew, Richmond, UK

9 Laboratorio de Agrobiodiversidad y Malherbología LAMUA, Universidad de la Amazonia, Florencia, Caquetá, Colombia

Corresponding author: Tatiana Arias ([tarias@selby.org](mailto:tarias@selby.org))

## Abstract

A checklist of Orchidaceae from Caquetá, Colombia is presented here. We recorded 98 genera and 418 species, exceeding a previous inventory by 276 species. The checklist is conservative in the number of genera and species by including only taxa that were fully and reliably identified and that are either linked to a corresponding herbarium voucher, a living collection specimen or a photo taken in the field and published in iNaturalist by one of the authors or a collaborator. The documented species diversity in the region could dramatically increase in the next few years with additional collecting efforts in the eastern slopes of the Andes nested in Caquetá. About 9% (418/4600) of all Orchidaceae species recorded for Colombia are reported for this area, showing the important contribution to orchid diversity of Andean-Amazonian foothills of Caquetá.

**Key words:** Alpha diversity, Amazon, Andes, floristic studies, foothills, orchids



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## Introduction

Orchidaceae are one of the most diverse and widely distributed flowering plant families including 25,000–27,000 species and 880 genera (Chase et al. 2015). Colombia has the largest diversity of orchid species in the American tropics (Pérez-Escobar et al. 2022a), hosting ~ 4,600 species that represent ~18% of the known species diversity in the family. The highest level of species richness arises in the northern Andes region of the country (Pérez-Escobar et al. 2022a), where a large number of endemic species occur, accounting for 36.8% of the total species reported for Colombia. With new orchid novelties published annually (Ortiz Valdivieso et al. 2009; Hágater et al. 2013; Pérez-Escobar et al.

\* These authors contributed equally to this work.



2021, 2022b; Vieira-Uribe and Moreno 2022), Colombia is a hotspot for biodiversity conservation (Betancur et al. 2015).

Caquetá, one of Colombia's 32 Departments, is a largely unexplored region with an extraordinary ecosystem diversity, geographically presenting a variety of landscapes, topographic forms and different types of associated vegetation and water sources, including the Amazon plains, valleys, hills, foothills and mountain ranges (Fig. 1). The Department contains four national natural parks, covers part of the Chiribiquete World Heritage Park and 35 recognised



**Figure 1.** Representative landscapes from Caquetá, Colombia. **A** general view of the Andean Piedmont in the Municipality of Florencia **B** Hills and rivers coming down from the eastern slopes of the Andes and flowing into the Amazonian Forest in the Municipality of Belén de los Andaquíes, the Pescado River **C** Hilly slopes from the Andean Piedmont transitioning to the Amazon Forest in the Municipality of Belén de los Andaquíes **D** General view of Amazonia Forest from a hilly slope of the eastern Andes, in the Municipality of Paujil **E, F** Amazonian waterlogged Forest at La Laguna de Peregrinos, Municipality of Solano.



civil society reserves (RUNAP 2022, webpage checked June 2023). Caquetá is placed in the eastern slopes of the Andean foothills, a confluence zone of mountainous and lowland Amazonian landscapes with different communities' composition. The Andean foothills of Caquetá range between 200 and 1000 m a.s.l.

The Colombian Eastern Andean Mountain range transitions along an environmental gradient from foothills to either the Guyana Shield (Meta and Caquetá), the Amazon Basin (Caquetá, Putumayo and Amazonas) or the Orinoco Basin (Arauca, Casanare and Meta) (Hoorn et al. 2010). These ecotones are hyperdiverse because of the evolutionary, biogeographical and ecological processes that operate in a rich array of landscapes (Ruiz et al. 2007; Instituto Geográfico Agustín Codazzi 2010). It is perhaps in the confluence of lowland and mountainous landscapes where the greatest wealth of plant species diversity and endemism occurs in the country (Ruiz et al. 2007; Pérez-Escobar et al. 2022a), but the limited existing orchid inventories underestimate the region's species diversity.

Although Orchidaceae are diverse within Caquetá, few checklists and taxonomic studies focusing on this group are available. For example, the Catalogue of Plants and Lichens of Colombia (Bernal et al. 2016), reported a total of 104 orchid species, whereas "The National Orchid Conservation Plan" presented a count of 142 species (Betancur et al. 2015). Currently, Caquetá is severely affected by deforestation driven by anthropogenic transformations of the natural ecosystems (Jaramillo-Castelblanco 2016; IDEAM 2020). Biological diversity inventories of the Andean-Amazonian Region are, thus, crucial to provide information for habitat conservation strategies in the region.

In this study, we generated a detailed species list of Orchidaceae for the Department of Caquetá, one of the most unexplored areas in Colombia, due to, amongst other factors, difficulties such as security risks and lack of easy access routes to some of its regions and municipalities. This is a collective work developed by more than twelve Colombian botanists during 2019–2023, under the umbrella project "Orquídeas para la Paz" (Orchids for Peace). This programme aims to explore, reproduce and support orchid species recovery, while developing sustainable strategies for business, based on horticulture for vulnerable communities around Colombia. Data obtained here come from living collections, photographs taken from field and an extensive review of herbarium collections around the world.

## Methods

### Study area

Caquetá is located in south-western Colombia, between latitudes 0.7°S–2.9°N and longitudes 71°–76°W. It comprises 16 municipalities including Florencia, Belén de los Andaquíes, El Paujil, Doncello and La Montañita, amongst others (Table 1). This region contains a variety of landscapes and ecosystems ranging from the Eastern Cordillera of the Andes to the Amazonian plains, with elevations ranging from 0–3200 m a.s.l. The mean annual rainfall is about 2179 mm. The mean annual temperature ranges from 27–29 °C (Instituto Geográfico Agustín Codazzi 2010) (Fig. 1).

**Table 1.** Checklist of the Orchidaceae of Caquetá, Colombia. ALB: Albania, BEL: Belen de los Andaquies, CAR: Cartagena el Chaira, CUR: Curillo, DON: Doncello, FLO: Florencia, MIL: Milan, MOR: Morelia, PAU: Paujil, PRC: Puerto Rico, SJF: San Jose del Fragua, SOL: Solano, STA: Solita, SVC: San Vicente del Caguán, VAL: Valparaiso. \* Endemic to Colombia \*\* New report for Colombia.

Species name	Accessions reviewed	Area
<i>Acianthera casapensis</i> (Lindl.) Pridgeon & M.W. Chase	iNaturalist	BEL
<i>Acianthera ciliata</i> (Knowles & Westc.) F. Barros & L. R. S. Guim.	Pabon M. 320 (COAH), Arias T. 851, 852, 914 (HUAZ)	SOL, VAL
<i>Acianthera discophylla</i> (Luer & Carnevali) Luer	Living collection (El Manantial, Florencia)	SOL
<i>Acianthera erinacea</i> (Rchb. f.) A. Doucette	Arias T. 951, Chaux-Varela J. 61 (HUAZ)	FLO, PAU, SVC
<i>Acianthera sicaria</i> (Lindl.) Pridgeon & M. W. Chase	Arias T. 917 (HUAZ)	FLO
<i>Aganisia cyanea</i> (Lindl.) Rchb.f.	Arevalo R. 406 (COAH), Vasco A. 389, 396, 360, 420 (HUA), Trujillo E. 1043 (HUAZ)	BEL, SOL
<i>Aganisia fimbriata</i> Rchb.f.	Torres M.M. 1084 (COAH), Mesa N. & Trujillo E. 24 (HUAZ)	FLO, SOL
<i>Anathallis acuminata</i> (Kunth) Pridgeon & M. W. Chase	Benavides A. 506 (HUA), Arias T. 969 (HUAZ)	DON, SOL
<i>Anathallis brevipes</i> (H. Focke) Pridgeon & M. W. Chase	Arias T. 906 (HUAZ)	CAR
<i>Anathallis spiculifera</i> (Lindl.) Luer	Sanchez M. 1834, 1835 (COAH)	SOL
<i>Anathallis sclerophylla</i> (Lindl.) Pridgeon & M. W. Chase	iNaturalist	SVC
<i>Aspidogyne clavijera</i> (Rchb. f.) Meneguzzo	Cardenas D. 45472 (COAH)	MOR
<i>Aspidogyne confusa</i> (C. Schweinf.) Garay	Castro F. 9890 (COAH)	SOL
<i>Aspidogyne foliosa</i> (Poepp. & Endl.) Garay	Trujillo E. 7026, 7109 (CUVC)	ALB
<i>Aspidogyne jamesonii</i> (Garay) Meneguzzo	Romero 4050 (COL)	CAR
<i>Batemannia colleyi</i> Lindl.	Arevalo R. 43 (COL), Vasco A. 158 (HUA)	FLO, SOL
<i>Beloglottis costaricensis</i> Schltr.	iNaturalist	FLO
<i>Bifrenaria clavijera</i> Rchb.f.	Trujillo E. 1043 (COAH)	BEL
<i>Bifrenaria longicornis</i> Lindl.	Arbelaez E. 907 (COAH), Arevalo R. 417 (COL), Vasco A. 264 (HUA)	SOL
<i>Braemia vittata</i> (Lindl.) Jenny	Duivenvoorden J. 2362, Torres M. 1128 (COAH), Benavides A. 634 (HUA)	SOL
<i>Brachionidium lehmannii</i> Luer **	Arias T. 952 (HUAZ)	PAU
<i>Brassia caudata</i> (L.) Lindl.	Ortiz-Valdivieso M. 596 (HPUJ), Arias T. 857 (HUAZ)	BEL
<i>Bulbophyllum lehmannianum</i> Kraenzl.	Arevalo R. 363 (COL)	FLO
<i>Campylocentrum kuntzei</i> Cogn. ex Kuntze	Correa M. 7136 (COAH, HUAZ)	FLO
<i>Campylocentrum micranthum</i> (Lindl.) Maury	iNaturalist	SVC
<i>Catasetum discolor</i> (Lindl.) Lindl.	Arbelaez E. 238 (HUA), Idobro J. M. 9001 (COL), Arevalo R. 311 (COL), Sastre R. D. 5173 (P)	SOL
<i>Catasetum ochraceum</i> Lindl.	Gonzalez M. F. 2680, 2710 (COL)	SOL
<i>Catasetum roseo-album</i> (Hook.) Lindl.	Barbosa C. 7716 (FMB)	SOL
<i>Catasetum tabulare</i> Lindl.	Perdomo O. 430 (CUVC)	FLO
<i>Catasetum tuberculatum</i> Dodson C.	Aguilar M. 253 (COAH), Arias T. 858 (HUAZ)	FLO
<i>Catasetum villegasii</i> G. F. Carr	Carr G. F. (COAH, USF)	SVC
<i>Cattleya crispa</i> Lindl. **	Living collection (El Manantial, Florencia)	CAR, SJF
<i>Cattleya violacea</i> (Kunth) Rolfe	Trujillo E. 808, Calderon A. A. 263 (HUAZ)	CAR
<i>Chondrorhyncha rosea</i> Lindl.	Schmidt-Mumm K. s.n. (LA)	FLO

Species name	Accessions reviewed	Area
<i>Cleistes abditia</i> G. A. Romero & Carnevali	Palacios P. 582, Castaño N. 3184 (COAH)	FLO, SOL
<i>Cleistes rosea</i> Lindl.	Jaimes M. S. 1269 (COAH), Sanin D. 6454 (COL), Perdomo O. 256 (CUVC), Cumaco L. S. & Trujillo E. 42, Arias T. 990, Chaux-Varela J. 96 (HUAZ)	FLO, PAU
<i>Cleistes tenuis</i> (Griseb.) Schltr.	Aguilar M. 253 (COAH)	SOL
<i>Coryanthes leucocorys</i> Rolfe	iNaturalist	BEL, FLO
<i>Cranichis polyantha</i> Schltr.	Madero 22 (AMES)	N/A
<i>Cryptarrhena lunata</i> R. Br.	iNaturalist	FLO
<i>Cycnoches egertonianum</i> Bateman	Perdomo O. 387 (CUVC)	FLO
<i>Cycnoches haagii</i> Barb. Rodr.	Dodson C. 3249 (COAH)	DON
<i>Cyrtochilum caquetanum</i> P. Ortiz-Valdivieso M, L. E. Álvarez & A. J. Carrillo	Ortiz-Valdivieso M. 1393 (HPUJ)	N/A
<i>Cyrtochilum cimiciferum</i> (Rchb. f.) Dalström	iNaturalist	SVC
<i>Cyrtochilum divaricatum</i> (Lindl.) Dalström	iNaturalist	SVC
<i>Cyrtochilum flexuosum</i> Kunth	Ramirez J. G. 5282 (JAUM), Gentry A. et al. 9046 (MO)	FLO
<i>Cyrtochilum meirax</i> (Rchb. f.) Dalström	Perdomo O. 416, 404 (CUVC), Arias T. 925, 954, Chaux-Varela J. 58, 60, 92(HUAZ)	FLO, PAU, PRC
<i>Cyrtochilum midas</i> Dalström	Perdomo O. 0195 (CUVC)	FLO
<i>Cyrtochilum orgyale</i> Kraenzl.	iNaturalist	SVC
<i>Cyrtochilum porrigens</i> (Rchb. f.) Kraenzl.	Perdomo O. 394, 400 (CUVC), Calderon A. 250, 251 (HUAZ)	PRC, FLO
<i>Cyrtochilum ramosissimum</i> (Lindl.) Dalström	Trujillo E. 7587 (CUVC)	PRC
<i>Cyrtochilum scabiosum</i> Kraenzl.	Cuatrecasas J. 8466 (COL)	FLO
<i>Cyrtochilum trifurcatum</i> Kraenzl.	Perdomo O. 412 (CUVC)	PRC
<i>Cyrtochilum undulatum</i> Kunth	iNaturalist	FLO
<i>Cyrtochilum ventrilabrum</i> Kraenzl.	Perdomo O. 393 (CUVC)	PRC
<i>Cyrtopodium cristatum</i> Lindl.	Betancur J. 1548 (HUA)	SVC
<i>Cyrtopodium palmifrons</i> Rchb. f. & Warm.	Living collection (El Caraño, Florencia)	FLO
<i>Dichaea ancoraelabia</i> C. Schweinf	Perdomo O. 267 (CUVC), Ortiz-Valdivieso M. 531 (HPUJ), Mesa N. & Trujillo E. 03 Arias T. 877 (HUAZ)	BEL, FLO, SOL
<i>Dichaea caquetana</i> Schltr. *	Fernández-Pérez A. 7240 (COL)	FLO
<i>Dichaea hystricina</i> Rchb.f.	Castaño N. 8705 (COAH)	BEL, FLO
<i>Dichaea panamensis</i> Lindl.	Dueñas H. 3060 (COL), Vasco A. 387 (HUA)	SOL
<i>Dichaea pendula</i> (Aubl.) Cogn.	Castaño N. 8678 (COAH), Betancur J. 1916 (COL, HUA)	BEL, SVC
<i>Dichaea picta</i> Rchb.f.	Jimenez E. 11 (COAH)	FLO
<i>Dichaea rendlei</i> Gleason	Franco-Rosselli P. 3825 (COL), Vasco A. 220, 267, 304 (HUA), Betancur J. 13560 (COAH)	SOL
<i>Dichaea sodiroi</i> Schltr.	Ortiz-Valdivieso M. 553 (HPUJ)	FLO
<i>Dichaea splitgerberi</i> Rchb.f.	Trujillo E. 956 (COAH, FMB), Castaño N. 1761, Cardenas D. 40460, 44494, 48527 (COAH)	BEL, PRC, SVC, VAL
<i>Dichaea trinitensis</i> Gleason **	Arias T. 885 (HUAZ)	SOL
<i>Dichaea trulla</i> Rchb.f.	Cardenas D. 42199 (COAH), Betancur J. 20668, Arevalo R. 98, 306 (COL), Vasco A. 305 (HUA), Perez 663 (FMB)	BEL, FLO, SOL
<i>Dimerandra emarginata</i> (G. Mey.) Hoehne	Arias T. 859 (HUAZ)	FLO
<i>Dracula alcithoe</i> Luer & R. Escobar	iNaturalist	BEL
<i>Duckeella adolphii</i> Porto & Brade	Pabon M. 461, 462, Echeverry R. 3297, Palacios P. 691, 537, 1218, Duivenvoorden J. 263 (COAH), Arbelaez E. 64 (HUA)	SOL
<i>Duckeella caquetana</i> Szlach. & Kolan. *	Arbelaez M. V. 64 (COAH, UGDA)	SOL
<i>Duckeella fernandezii</i> Szlach., Kolan. & Baranow *	Fernandez 20065 (COL, UGDA)	SOL

Species name	Accessions reviewed	Area
<i>Elleanthus amethystinoides</i> Garay	Cardenas D. 20257 (COAH)	BEL
<i>Elleanthus ampliflorus</i> Schltr.	Arias T. 1201 (HUAZ)	FLO
<i>Elleanthus aurantiacus</i> Rchb.f.	Castaño N. 7442, 8608, Cardenas D. 46146 (COAH), Mason H. L. 13954, Gentry A. 9036 (COL), Arias T. 1211 (HUAZ)	BEL, FLO, SOL
<i>Elleanthus blatteus</i> Garay	Arias T. 980, 1193, 1217 (HUAZ)	DON, FLO
<i>Elleanthus columnaris</i> Rchb.f.	Fonnegra R. 5465 (HUA, MO)	FLO
<i>Elleanthus conifer</i> (Rchb.f. & Warsz.) Rchb.f.	Jimenez E. 26 (COAH)	FLO
<i>Elleanthus emberanus</i> (Szlach. & Kolan.) J. M. H. Shaw *	Trujillo W. et al. 968 (COAH)	BEL
<i>Elleanthus fractiflexus</i> Schltr.	Castaño N. 8720, Betancur J. 20388, 20544 (COAH), Arias T. 1188 (HUAZ)	BEL, FLO
<i>Elleanthus graminifolius</i> (Barb.Rodr.) Lojtnant	Cardenas D. 20682, Perdomo O. 318, Betancur J. 20682 (COAH) Perdomo O. 318 (CUVC)	BEL, FLO
<i>Elleanthus kermesinus</i> Rchb. f.	Cuatrecasas J. 8766 (COL)	FLO
<i>Elleanthus lancifolius</i> C. Presl.	Ortiz-Valdivieso M. 459 (HPUJ), Araujo E. & Trujillo E. 28 (HUAZ)	BEL, FLO
<i>Elleanthus oliganthus</i> Rchb.f.	Vargas V. A. 99 (COAH), Cardenas D. 42099 (FMB), Cumaco L. S. & Trujillo E. 34, Santofimio L. M. & E. Trujillo E. 04 (HUAZ)	BEL, FLO
<i>Elleanthus robustus</i> Rchb. f.	Arias T. 1190 (HUAZ)	FLO
<i>Elleanthus tillandsioides</i> Barringer	Trujillo W. 968 (FMB), Cardenas D. 41786, 41817 (COAH)	BEL
<i>Encyclia aspera</i> Schltr.	Arevalo R. 210 (COL), 211 (COAH)	SOL
<i>Encyclia chloroleuca</i> Neumann	Living collection (El Caraño, Florencia)	FLO
<i>Encyclia leucantha</i> Schltr.	Pabon M. 927, Arbelaez E. 787 (COAH)	SOL
<i>Encyclia pilosa</i> (C. Schweinf.) Carnevali & I. Ramírez	Arbelaez E. 389 (COAH)	SOL
<i>Epidendrum acuminatum</i> Ruiz & Pav.	Ortiz-Valdivieso M. 4171 (HPUJ)	FLO
<i>Epidendrum acutilobum</i> Hágsater E. & Uribe Veléz *	Kapuler & Hascall 168 (COL), Arias T. 979 (HUAZ)	FLO, DON
<i>Epidendrum amazonicorifolium</i> Hágsater E.	Cardenas D. 40246 (COAH)	FLO
<i>Epidendrum angulatum</i> Hágsater E. & J. Duarte *	Moreno s.n. (AMO)	FLO
<i>Epidendrum angustatum</i> (T. Hashim.) Dodson C.	Ortiz-Valdivieso M. 461 (HPUJ)	FLO
<i>Epidendrum arachnoglossum</i> Rchb. f. ex André	Arbelaez E. 53 (HUA)	SOL
<i>Epidendrum arevalo</i> (Schltr.) Hágsater E.	Hágsater E. s.n. (AMO), Ortiz-Valdivieso M. 473 (HPUJ), Arias T. 1196 (HUAZ)	FLO
<i>Epidendrum armeniacum</i> Lindl. **	Perdomo O. 302 (CUVC)	FLO
<i>Epidendrum aura-usecheae</i> Hágsater, Rinc. -Useche & O. Pérez	Arias T. 1014 (HUAZ)	SVC
<i>Epidendrum barbeyanum</i> Kraenzl.	Ortiz-Valdivieso M. 462 (HPUJ)	FLO
<i>Epidendrum borealistachyum</i> Hágsater E., E. Santiago & C. Fernandez	Sanin D. 6361 (COAH), 6100 (COL), 6558, 6632 (HUA), Correa M. & Aldana J. 7196, Mesa N. & Trujillo E. 16 Arias T. 1199 (HUAZ)	FLO
<i>Epidendrum brachyrepens</i> Hágsater E. **	Betancur J. 2225 (HUA)	SVC
<i>Epidendrum caesaris</i> Hágsater E. & E. Santiago	Estrada J. 668 (COL)	SOL
<i>Epidendrum calanthum</i> Rchb.f. Warsz.	Barbosa C. 8140 (COL)	SOL
<i>Epidendrum calyptrandium</i> Hágsater E., H. Medina & Huamantupa	Cardenas D. 41772 (COAH, FMB)	BEL
<i>Epidendrum chorandrochilum</i> F. Lehm. & Kraenzl.	Perdomo O. 235 (CUVC)	FLO
<i>Epidendrum cleistocoleum</i> Hágsater E. & E. Santiago	iNaturalist	SVC
<i>Epidendrum cochlidium</i> Lindl.	Jimenez E. 1 (HUA)	FLO
<i>Epidendrum compressibulum</i> D. E. Benn. & Christenson **	Arias T. 926 (HUAZ)	FLO



Species name	Accessions reviewed	Area
<i>Epidendrum compressum</i> Griseb.	Cuatrecasas J. 27127 (COL), Trujillo W. 816 (HUA), Mesa N. & Trujillo E. 18 (HUAZ)	CAR, FLO
<i>Epidendrum</i> × <i>communis</i> Hágsater Ined	Arias T. 997; 998 (HUAZ)	SVC
<i>Epidendrum coronatum</i> Ruiz & Pav	Arias T. 863 (HUAZ)	CAR, SJF
<i>Epidendrum cuneatum</i> Schltr.	Arias T. 960 (HUAZ)	CAR, SJF
<i>Epidendrum cupreum</i> F. Lehm. & Kraenzl.	Ortiz-Valdivieso M. 573 (HPUJ)	FLO
<i>Epidendrum cylindraceum</i> Lindl.	Arias T. 1204 (HUAZ)	FLO
<i>Epidendrum erosum</i> Ames & C. Schweinf.	Hoyos s.n. (AMO)	FLO
<i>Epidendrum excisum</i> Lindl.	iNaturalist	SVC
<i>Epidendrum flexuosum</i> G. Mey.	iNaturalist	SVC
<i>Epidendrum filamentosum</i> Kraenzl.	Perdomo O. 272, 413 (CUVC) Arias T. 988, Chaux-Varela J. 34, 98 (HUAZ)	FLO, DON
<i>Epidendrum fimbriatum</i> Kunth	Arias, T. 1198, Calderon A. 256 (HUAZ)	FLO
<i>Epidendrum geminiflorum</i> Kunth	iNaturalist	SVC
<i>Epidendrum ibaguense</i> Kunth	Cardiel J. M. 59 (COL), Arias T. 993 (HUAZ)	DON, FLO, SOL
<i>Epidendrum lacustre</i> Lindl.	Forero E. 9816, Palacios P. 859, 1192, 1201, 1226, Arbelaez E. 53, 731, Duivenvoorden J. 607, Sanin D. 6362 (COAH), Restrepo D. 344 (HUA)	FLO, SOL
<i>Epidendrum longicolle</i> Lindl.	Cumaco L. S. & E. Trujillo E. 16 (HUAZ)	FLO
<i>Epidendrum macrocarpum</i> Rich.	Marin C. 2896 (COAH, COL), Velayos M. 6455 (COL)	FLO, MOR, SOL
<i>Epidendrum macrum</i> Dressler	Ortiz-Valdivieso M 528 (HPUJ, AMO)	FLO
<i>Epidendrum magnicallosum</i> C. Schweinf.	Arevalo R. 46, 91, 165 (COL)	SOL
<i>Epidendrum mamapachae</i> Hágsater, F.O. Espinosa & E. Santiago *	iNaturalist	SVC
<i>Epidendrum melinanthum</i> Schltr.	Plazas L. L. et al. 42 (HUAZ)	FLO
<i>Epidendrum microcapitellatum</i> Hágsater, Medina Tr. & E. Santiago *	iNaturalist	FLO
<i>Epidendrum micronocturnum</i> Carnevali & G.A. Romero	Arevalo R. 40 (COL)	SOL
<i>Epidendrum microphyllum</i> Lindl.	Arevalo R. 201 (COL)	SOL
<i>Epidendrum mora-retanae</i> Hágsater E.	Living collection (El Caraño, Florencia)	FLO
<i>Epidendrum myrmecophorum</i> Barb. Rodr.	Perdomo O. 275, 284 (CUVC), Arbelaez E. 256, 379 (HUA)	CAR, FLO, MOR, SOL
<i>Epidendrum nocturnum</i> Jacq.	Arbelaez E. 888, Franco-Rosselli P. 3730, Sanchez M. 1943, 1942, 1941 (COAH), Cardenas D. et al. 42156 (FMB), Arias T. 874–876, 959, Trujillo E. 815 (HUAZ)	BEL, CAR, DON, FLO, SJF, SOL
<i>Epidendrum orbiculatum</i> C. Schweinf.	Ortiz-Valdivieso M. 573 (HPUJ)	SVC
<i>Epidendrum orchidiflorum</i> Salzm. ex Lindl.	Arbelaez E. 730, 815, 776, Cardenas D. 45063, 48634, Sanin D. 6642 (COAH), Davidse G. 5612, Hermann F. J. 11257, Manson H. L. 13949 (COL), Arbelaez E. 379 (HUA), Araujo D. & Trujillo E. 17, Arias T. 865, Croat T. et al. 100480 (HUAZ)	CAR, FLO, SOL
<i>Epidendrum porphyreonocurnum</i> Hágsater & R. Jiménez	Perdomo O. 179 (CAUP)	FLO
<i>Epidendrum portokaliu</i> Hágsater E. & Dodson C.	Cuatrecasas J. 9002 (COL)	FLO
<i>Epidendrum putumayoense</i> Hágsater E. & L. Sanchéz	Valencia E. & Hágsater E. 11640 (AMO)	N/A
<i>Epidendrum radicans</i> Pav. ex Lindl.	Polania O. L. & Trujillo E. 5 (HUAZ)	FLO
<i>Epidendrum rhodochilum</i> (Schltr.) Hágsater E. & Dodson C.	Trujillo W. s. n. (AMO)	BEL

Species name	Accessions reviewed	Area
<i>Epidendrum rhombochilum</i> L.O. Williams	Betancur J. et al. 20224 (COAH)	BEL
<i>Epidendrum rigidum</i> Jacq.	Ortiz-Valdivieso M. 471 (HPUJ), Polania O. L. & Trujillo E. 1, Cumaco L. S. & Trujillo E. 15 (HUAZ), Groenendijk J. s.n. (MA)	FLO, SOL
<i>Epidendrum rugulosum</i> Schltr.	Sanin D. 6640 (HUA)	FLO
<i>Epidendrum sanctae-rosae</i> Hágsater E., Sauleda, Uribe Vélez & E. Santiago	Perdomo O. 322, 424 (CUVC)	FLO
<i>Epidendrum saxatile</i> Lindl.	Trujillo E. et al. 1038 (COAH), Arias T. 967 (HUAZ)	BEL, DON
<i>Epidendrum sculptum</i> Rchb.f.	Cardenas D. 41772 (COAH, FMB)	BEL
<i>Epidendrum secundum</i> Jacq.	Betancur J. 20457 (COAH), Correa M. et al. 4605 (HUAZ), Barbosa C. et al. s.n. (MA)	BEL, FLO, PRC, SOL
<i>Epidendrum spilotum</i> Garay & Dunst.	Escobar R. 5270 (AMO)	N/A
<i>Epidendrum stenobractistachyum</i> Hágsater E. & E. Santiago	Cuatrecasas J. 8426 (COL)	FLO
<i>Epidendrum strobiliferum</i> Rchb.f.	Cardenas D. 46422 (COAH)	SOL
<i>Epidendrum teuscherianum</i> A. D. Hawkes **	Chaux-Varela J. 102, 103, 108 (HUAZ)	DON
<i>Epidendrum tridens</i> Poepp. & Endl.	Franco-Rosselli P. et al. 3730 (COL)	SOL
<i>Epidendrum tumuc-humaciense</i> (Veyret) Carnevali & G. A. Romero	Arbelaez E. & Castro F. 888, Barbosa C. et al. 8154, Castro Viejo F. et al. 335, Franco-Rosselli P. et al. 3636 (COL)	SOL
<i>Epidendrum uleananodes</i> Hágsater E. **	Groenendijk 33 (COAH)	SOL
<i>Epidendrum vinosum</i> Schltr. **	iNaturalist	PAU
<i>Epidendrum whittenii</i> Hágsater E. & Dodson C.	Coca et al. 9207b (FAUC)	SJF
<i>Epistephium hernandii</i> Garay	Arbelaez E. 276 (COAH)	SOL
<i>Epistephium parviflorum</i> Lindl.	Castro F. 10974, Palacios P. 864 (COAH)	SOL
<i>Epistephium subrepens</i> Hoehne	Duivenvoorden J. 215, Gentry A. 65171, Ospina H. 1141, Palacios P. 581, 760, 2437, Restrepo D. 9 (COAH)	SOL
<i>Eriopsis biloba</i> Lindl.	Arbelaez E. 172 (COAH), Palacios P. 2415 (COL), Barbosa C. 7631 (FMB)	SOL
<i>Eriopsis sceptrum</i> Rchb.f. & Warsz.	Cardenas D. 46424 (COAH)	SOL
<i>Erycina glossomystax</i> (Rchb.f.) N. H. Williams & M. W. Chase	Arias T. 961 (HUAZ)	DON
<i>Erycina pumilio</i> (Rchb. f.) N. H. Williams & M. W. Chase	Atwood J. T. & Mora D. s.n.	N/A
<i>Erycina pusilla</i> (L.) N. H. Williams & M. W. Chase	von Sneidern K. 1075, Castroviejo S. 322, Perez-Arvelaez E. 370 (COL), Betancur J. 2349 (HUA)	BEL, FLO, SVC
<i>Eulophia alta</i> (L.) Fawc. & Rendle	Perdomo O. 342 (CUVC), Betancur J. 2214 (HUA)	BEL, SVC
<i>Galeandra macroplectra</i> G. A. Romero & Warford	Galeano G. 2249 (COL)	SOL
<i>Galeandra stangeana</i> Rchb. f.	Franco-Rosselli P. 3860 (COL)	SOL
<i>Galeottia negrensis</i> Schltr.	Mendoza H. 10283, Cardenas D. 48408 (COAH)	SOL, SVC
<i>Gongora atropurpurea</i> Hook.	iNaturalist	SVC
<i>Gongora portentosa</i> Linden & Rchb. f.	Living collection (El Caraño, Florencia)	FLO
<i>Habenaria mesodactyla</i> Griseb.	Franco-Rosselli P. 2410 (COL)	SOL
<i>Habenaria monorhiza</i> Rchb. f.	Orozco C. I. 2768 (COL), Goudot J. s.n. (P), Arias T. 756, 992, 1011, Chaux-Varela J. 107 (HUAZ)	FLO, DON, PAU, PRC, SOL, SVC
<i>Habenaria pratensis</i> Rchb. f.	iNaturalist	CAR
<i>Houlletia lowiana</i> Rchb. f.	Cardenas D. 46013 (COAH)	BEL
<i>Houlletia sanderi</i> Rolfe	Perdomo O. 307 (CUVC)	FLO
<i>Hylaeorchis petiolaris</i> (Schltr.) Carnevali & G. A. Romero	iNaturalist	BEL

Species name	Accessions reviewed	Area
<i>Ionopsis satyrioides</i> (Sw.) Rchb.f.	Perdomo O. 345 (CUVC)	ALB, BEL
<i>Ionopsis utricularioides</i> (Sw.) Lindl.	Trujillo E. 846 (COAH), Idobro J. M. 8590 (COL)	CAR
<i>Jacquiniella globosa</i> (Jacq.) Schltr.	Living collection (El Caraño, Florencia)	FLO
<i>Jacquiniella teretifolia</i> (Sw.) Britton & P. Wilson	Betancur J. 20543, 20658 (COAH)	BEL, FLO
<i>Koellensteinia graminea</i> Rchb.f.	iNaturalist	SVC
<i>Laelia rosea</i> (Lindl.) C.Schweinf.	Calderon A. 260, 261, Chaux-Varela J. 43 (HUAZ)	CAR, FLO
<i>Lepanthes agglutinata</i> Luer	Cardenas D. 41869 (COAH, FMB, MO, NYCB), Trujillo E. 7691, 7705, 7949 (CUVC)	BEL, FLO, PRC
<i>Lepanthes auriculata</i> Luer	Trujillo E. 7693 (CUVC)	PRC
<i>Lepanthes delhierroi</i> Luer & Hirtz **	Arias T. 1186 (HUAZ)	FLO
<i>Lepanthes florenciana</i> J. S. Moreno & Hoyos *	Hoyos D., López O. & Fonseca A. 945 (COAH, HUAZ)	FLO
<i>Lepanthes forceps</i> Luer & R. Escobar	Living collection (El Caraño, Florencia)	FLO
<i>Lepanthes hirtzii</i> Luer	Trujillo E. 7687 (CUVC)	PRC
<i>Lepanthes mucronata</i> Lindl.	Arias T. 1234 (HUAZ)	FLO
<i>Lepanthes nontecta</i> Luer **	iNaturalist	SVC
<i>Lepanthes tachirensis</i> Foldats	Arias T. 1232 (HUAZ)	FLO
<i>Lepanthes wageneri</i> Rchb. f.	Trujillo E. 7689 (CUVC)	PRC
<i>Lockhartia acuta</i> Rchb.f.	iNaturalist	CAR
<i>Lockhartia micrantha</i> Rchb. f.	Betancur J. 2226 (COL, HUA), Arias, T. 989 (HUAZ)	DON
<i>Lycaste fuscina</i> Oakeley **	iNaturalist	SVC
<i>Lycaste macrobulbon</i> Lindl.	iNaturalist	SVC
<i>Lycaste macrophylla</i> Lindl.	iNaturalist	SVC
<i>Lycomormium fiskei</i> H.R. Sweet	Ortiz-Valdivieso M. 1365 (HPUJ)	N/A
<i>Lycomormium schmidtii</i> Á. Fernández	Fernandez 7248 (COAH)	FLO
<i>Macradenia purpureoestrata</i> G. Gerlach	Romero 4082 (COL)	PRC
<i>Macroclinium manabinum</i> (Dodson C.) Dodson C. **	iNaturalist	FLO
<i>Malaxis histionantha</i> (Link, Klotzsch & Otto) Garay & Dunst.	iNaturalist	SVC
<i>Masdevallia amanda</i> Rchb. f. & Warsz.	Perdomo O. 395 (CUVC), Ortiz-Valdivieso M. 4185 (HPUJ), Arias T. 1187 (HUAZ)	FLO, PRC
<i>Masdevallia constricta</i> Poepp. & Endl. **	iNaturalist	FLO
<i>Masdevallia ensata</i> Rchb. f.	Trujillo E. 7698 (CUVC)	FLO
<i>Masdevallia picturata</i> Rchb.f.	Arias T. 1230 (HUAZ)	FLO
<i>Masdevallia tubulosa</i> Lindl.	Polania & Trujillo E. 10 (HUAZ), Trujillo E. 7696 (CUVC)	FLO, PRC
<i>Masdevallia virgo-cuencae</i> Luer & Andreetta	Perdomo O. 423 (CUVC)	FLO, SVC
<i>Maxillaria acuminata</i> Lindl.	Perdomo O. 199 (CUVC)	FLO
<i>Maxillaria aequiloba</i> Schltr.	Trujillo E. 7561, Perdomo O. 399 (CUVC)	FLO, PRC
<i>Maxillaria alba</i> Lindl.	Ortiz-Valdivieso M. 467 (HPUJ)	BEL
<i>Maxillaria anceschiana</i> Molinari	Correa M & Trujillo E. 5344 (HUAZ)	FLO
<i>Maxillaria aureoglobula</i> Christenson	Perdomo O. 292, 349 (CUVC), Chaux-Varela J. 36 (HUAZ)	BEL, FLO
<i>Maxillaria aurea</i> (Poepp. & Endl.) L. O. Williams	Cardenas D. 48633 (COAH), Sanin D. 6569 (COL), Perdomo O. 411 (CUVC), Diaz et al. 38, Santofimio L. M. & Trujillo E. 12, Molina A. 31, Arias T. 1202 (HUAZ)	FLO, PRC
<i>Maxillaria auyantepuiensis</i> Foldats	Trujillo W. 1041 (COAH), Ortiz-Valdivieso M. 556 (HPUJ)	BEL, FLO
<i>Maxillaria bicallosa</i> (Rchb. f.) Garay	Betancur J. 20637 (COAH), Perdomo O. 370, 384 (CUVC), Chaux-Varela J. 35 (HUAZ)	BEL, FLO
<i>Maxillaria bolivarensis</i> C. Schweinf.	Trujillo E. 957 (COAH), Perdomo O. 346, 382 (CUVC)	BEL, FLO

Species name	Accessions reviewed	Area
<i>Maxillaria brachybulbon</i> Schltr.	Perdomo O. 285 (CUVC)	FLO
<i>Maxillaria buchtienii</i> Schltr.	Perdomo O. 316 (CUVC)	FLO
<i>Maxillaria camaridii</i> Rchb. f.	Cardenas D. 41896, 44563 (COAH), Trujillo E. 812 (HUAZ)	BEL, CAR, FLO, SVC
<i>Maxillaria carinulata</i> Rchb. f.	Santofimio L. M. & Trujillo E. 6 (HUAZ)	FLO
<i>Maxillaria cassapensis</i> Rchb. f.	Perdomo O. 401, Trujillo E. 7700 (CUVC)	PRC
<i>Maxillaria crassifolia</i> (Lindl.) Rchb.f.	Arevalo R. 68, 157, Franco-Rosselli P. 4153 (COL), Perdomo O. 370 (CUVC)	BEL, SOL, PRC
<i>Maxillaria cruentata</i> (Arévalo & Bergq.) Molinari & Mayta *	Arevalo R. 1080 (COL, WIS)	SOL, SVC
<i>Maxillaria cryptobulbon</i> Carnevali & J.T. Atwood	Londoño 871 (UDBC)	PRC
<i>Maxillaria cuzcoensis</i> C. Schweinf. **	Dodson C. 3255 (SEL)	FLO
<i>Maxillaria discolor</i> (G. Lodd. ex Lindl.) Rchb. f.	Arevalo R.218, Betancur J. 20650, Pabon M. 544(COAH), Vasco A. 342 (HUA), Arias T. 848, 849, 853, 867, Chaux-Varela J. 38, Trujillo E. 806 (HUAZ)	BEL, SOL, CAR, PRC, SVC
<i>Maxillaria dunstervillei</i> Carnevali & I. Ramirez J. G. **	Castaño N. 8572 (COAH)	BEL, PRC
<i>Maxillaria ecuadorensis</i> Schltr.	Perdomo O. 274 (CUVC), Santofimio L. M. & Trujillo E. 10 (HUAZ)	FLO
<i>Maxillaria egertoniana</i> (Bateman) Molinari	Living collection (El Manantial, Florencia)	SJF
<i>Maxillaria embreei</i> Dodson C.	Castaño N. 8843 (COAH), Perdomo O. 321 (CUVC), Araujo D. & Trujillo E. 3, Arias T 1219 (HUAZ)	BEL, FLO, SOL
<i>Maxillaria equitans</i> (Schltr.) Garay	iNaturalist	CAR
<i>Maxillaria erikae</i> Molinari **	Perdomo O. 0167 (CUVC)	FLO
<i>Maxillaria exaltata</i> (Kraenzl.) C. Schweinf.	Ortiz-Valdivieso M. 4172 (HPUJ), Araujo D. & Trujillo E. 32 (HUAZ)	FLO
<i>Maxillaria fractiflexa</i> Rchb. f.	Perdomo O. 391 (CUVC)	PRC
<i>Maxillaria imbricata</i> Barb. Rodr.	Arias, T. 957, 958, 970, 1016 (HUAZ)	DON, FLO, PAU, SVC
<i>Maxillaria inaequisepala</i> (C. Schweinf.) Molinari	Prado et al. 614 (FMB)	SOL
<i>Maxillaria kegelii</i> Rchb.f.	Arevalo R. 276 (COAH), Correa M. 9932 (HUAZ)	SOL
<i>Maxillaria lepidota</i> Lindl.	iNaturalist	FLO
<i>Maxillaria longipetala</i> Ruiz & Pav.	iNaturalist	SVC
<i>Maxillaria longipetiolata</i> Ames & C. Schweinf.	Perdomo O. 262 (CUVC), Trujillo E. 1041 (HUAZ)	BEL, FLO
<i>Maxillaria longissima</i> Lindl.	Perdomo O. 233 (CUVC), Araujo D. & Trujillo E. 4, Arias T. 1191 (HUAZ)	FLO
<i>Maxillaria mapiriensis</i> (Kraenzl.) L. O. Williams	Perdomo O. 323 (CUVC), Arias T. 965 (HUAZ)	FLO, DON
<i>Maxillaria meridensis</i> Lindl.	Cuatrecasas J. 9121 (COL), Araujo D. & Trujillo E. 4, Arias T.12078, Chaux-Varela J. 90, Cumaco L. S. & Trujillo E. 32, Pinilla J. et al. 32, Plazas L. L. et al. 37 (HUAZ)	FLO
<i>Maxillaria nasuta</i> Rchb. f.	Living collection (El Manantial, Florencia)	SOL
<i>Maxillaria notylioglossa</i> Rchb. f.	Perdomo O. 179 (CUVC)	FLO
<i>Maxillaria novoeae</i> Molinari	Perdomo O. 0173 (CUVC)	FLO
<i>Maxillaria nubigena</i> (Rchb. f.) C. Schweinf.	Correa M & Trujillo E. 4903, Santofimio L. M. & Trujillo E. 16 (HUAZ)	FLO
<i>Maxillaria obtusa</i> (Lindl.) Molinari	Barbosa C. 7543 (COAH), Franco-Rosselli P. et al. 3814 (COL, MO)	SOL
<i>Maxillaria parkeri</i> Hook.	Gentry A. 65290 (COAH, MO) Arevalo R. 154, 267, 325 (COL), Prado L. F. 526, 542 (COAH, MO, COL)	SOL
<i>Maxillaria parviflora</i> (Poepp. & Endl.) Garay	Gentry A. 65290 (COAH, MO) Arevalo R. 154, 267, 325 (COL) Arias T. 1001, Chaux-Varela J. 104 (HUAZ)	BEL, DON, FLO, SVC



Species name	Accessions reviewed	Area
<i>Maxillaria pendens</i> Pabst **	Living collection (El Caraño, Florencia)	FLO
<i>Maxillaria pergracilis</i> (Schltr.) Schuit. & M.W. Chase	<i>Perdomo O. 372</i> (CUVC)	BEL, FLO
<i>Maxillaria plicata</i> Schltr.	<i>Arias, T. 1205</i> (HUAZ)	FLO
<i>Maxillaria porrecta</i> Lindl.	<i>Perdomo O. 273, 340</i> (CUVC), <i>Arias T. 928, 963, 1204, Polania D. &amp; Trujillo E. 6</i> (HUAZ)	BEL, DON, FLO
<i>Maxillaria proboscidea</i> Rchb. f. **	<i>Arias T. 850, 868</i> (HUAZ)	SOL
<i>Maxillaria pterocarpa</i> Barb. Rodr.	<i>Dodson C. 3247</i> (SEL)	FLO
<i>Maxillaria ringens</i> Rchb. f.	<i>Ortiz-Valdivieso M 529</i> (HPUJ)	FLO
<i>Maxillaria sanantonioensis</i> Christenson *	Living collection (El Caraño, Florencia)	FLO
<i>Maxillaria setigera</i> Lindl.	<i>Cardenas D. 48631</i> (COAH)	FLO
<i>Maxillaria soulangeana</i> Molinari	Living collection (El Caraño, Florencia)	BEL
<i>Maxillaria splendens</i> Poepp. & Endl.	<i>Ortiz-Valdivieso M. 554, 4241</i> (HPUJ)	FLO
<i>Maxillaria striata</i> Rolfe	<i>Trujillo E. s.n.</i> (CUVC)	FLO
<i>Maxillaria subrepens</i> (Rolfe) Schuit. & M. W. Chase	<i>Arevalo R. 87</i> (COL)	SOL
<i>Maxillaria tenuis</i> C. Schweinf.	<i>Arevalo R. 85, 362</i> (COAH, COL)	BEL, SOL
<i>Maxillaria uncata</i> Lindl.	<i>Arevalo R. 213</i> (COAH), <i>Arias, T. 915</i> (HUAZ)	SOL
<i>Maxillaria villosa</i> (Barb. Rodr.) Cogn.	<i>Prado 508, Restrepo 866</i> (COAH)	SOL
<i>Maxillaria violaceopunctata</i> Rchb. f.	<i>Sastre R. D. 5061</i> (P)	SOL
<i>Miltoniopsis phalaenopsis</i> (Linden & Rchb. f.) Garay & Dunst.	<i>Cabezas 1752</i> (JBB)	SVC
<i>Muscarella cryptophyta</i> (Barb.Rodr.) Bogarín & Karremans **	<i>Arias T. 918</i> (HUAZ)	FLO
<i>Muscarella samacensis</i> (Ames) Luer	<i>Ortiz-Valdivieso M. 474</i> (HPUJ), <i>Chaux-Varela J. 53</i> (HUAZ)	FLO
<i>Myoxanthus affinis</i> (Lindl.) Luer	Living collection (El Manantial, Florencia)	SJF
<i>Myoxanthus cimex</i> (Luer & R. Escobar) Luer	<i>Perdomo O. 266, 415</i> (CUVC)	FLO
<i>Myoxanthus merae</i> (Luer) Luer **	<i>Arias T. 1020</i> (HUAZ)	SVC
<i>Myoxanthus monophyllus</i> Poepp. & Endl.	<i>iNaturalist</i>	SVC
<i>Myoxanthus reymondii</i> (H. Karst.) Luer	<i>Arias T. 974, Chaux-Varela J. 86</i> (HUAZ)	DON
<i>Myoxanthus xiphion</i> Luer	<i>Perdomo O. 180, 414</i> (CUVC)	FLO
<i>Notylia barkeri</i> Lindl. **	<i>Arias T. 846, 847</i> (HUAZ)	SOL
<i>Notylia platyglossa</i> Schltr.	<i>Perdomo O. 271</i> (CUVC)	VAL
<i>Notylia sagittifera</i> (Kunth) Link, Klotzsch & Otto	<i>iNaturalist</i>	MIL
<i>Octomeria colombiana</i> Schltr.	<i>Trujillo E. 1039</i> (COAH, HUAZ), <i>Arias T.973, Chaux-Varela J. 87</i> (HUAZ)	DON, BEL
<i>Octomeria erosilabia</i> C. Schweinf.	<i>Arevalo R 84</i> (COL), <i>Arevalo R. 242, van der Wal 231 M.</i> (COAH), <i>Vasco A. 242, 255</i> (HUA)	SOL
<i>Octomeria exigua</i> C. Schweinf.	<i>Arevalo R. 356</i> (COL), <i>Gonzalez M. F. 2693</i> (COAH, COL)	FLO, SOL
<i>Octomeria grandiflora</i> Lindl.	<i>Arevalo R. 367</i> (COL), <i>Mesa N. &amp; Trujillo E. 07</i> (HUAZ)	FLO, SOL
<i>Octomeria minor</i> C. Schweinf.	<i>Vasco A. 188, 203</i> (COL)	SOL
<i>Octomeria scirpoidea</i> (Poepp. & Endl.) Rchb.f.	<i>Cardenas D. 6854</i> (COAH), <i>Arevalo R. 273</i> (COL), <i>Vasco A. 202</i> (HUA)	SOL
<i>Octomeria surinamensis</i> H. Focke	<i>Arevalo R. 90, 152, 246, 266, 348</i> (COL)	SOL
<i>Octomeria taracuana</i> Schltr.	<i>Velayos 6421</i> (MA), <i>Franco-Rosselli P. 4148</i> (COL)	SOL
<i>Octomeria tridentata</i> Lindl.	<i>Dodson C. 3245</i> (SEL)	FLO
<i>Odontoglossum paniculatum</i> Dalström & Deburghgr.	<i>iNaturalist</i>	SVC
<i>Oliveriana brevilabia</i> (C. Schweinf.) Dressler & N.H. Williams	<i>iNaturalist</i>	SVC

Species name	Accessions reviewed	Area
<i>Oncidium abortivum</i> Rchb. f.	Betancur J. 2197 (HUA)	SVC
<i>Oncidium alexandrae</i> (Bateman) M. W. Chase & N.H. Williams	Luteyn J. L. et al. 4958, Sanin D. 6395 (COL), Calderon A. 248 (HUAZ), Gentry A. et al. 9183 (MO)	FLO, PRC
<i>Oncidium baueri</i> Lindl.	Trujillo E. 549 (COAH, HUAZ), Calderon A. 249 Arias T. 995, 1214 (HUAZ)	DON, FLO, SOL
<i>Oncidium citrinum</i> Lindl.	Ortiz-Valdivieso M 550 (HPUJ)	FLO
<i>Oncidium eliae</i> (Rolfe) M. W. Chase & N. H. Williams	Perdomo O. 409 (CUVC)	FLO, PRC
<i>Oncidium ensatum</i> Lindl. **	iNaturalist	SVC
<i>Oncidium fuscatum</i> Rchb. f.	Correa M. et al. 5113 (HUAZ)	FLO
<i>Oncidium gramineum</i> (Poepp. & Endl.) M. W. Chase & N. H. Williams	Perdomo O. 362, 3623 (CUVC), Arias T. 920, Chaux-Varela J. 52 (HUAZ)	BEL, FLO
<i>Oncidium orthotis</i> Rchb. f.	Perdomo O. 348, 405 (CUVC)	BEL, PRC
<i>Oncidium poikilostalex</i> (Kraenzl.) M. W. Chase & N. H. Williams	iNaturalist	SVC
<i>Oncidium putumayense</i> (P. Ortiz) M. W. Chase & N. H. Williams	iNaturalist	SVC
<i>Oncidium sphacelatum</i> Lindl.	Betancur J. 1666 (HUA)	SVC
<i>Ornithocephalus bryostachys</i> Schltr. **	Hoyos F. s.n. (HUAZ)	FLO
<i>Otoglossum globuliferum</i> (Kunth) N. H. Williams & M. W. Chase	Cardenas D. 48646 (COAH)	FLO
<i>Otoglossum serpens</i> (Lindl.) N. H. Williams & M. W. Chase	Ramirez J. G. 5204 (COAH), Perdomo O. 249, Trujillo E. 7623 (CUVC)	FLO
<i>Palmorchis guianensis</i> (Schltr.) C. Schweinf. & Correll	Duivenvoorden J. 949 (MO, COAH)	SOL
<i>Palmorchis puber</i> (Cogn.) Garay	Cardenas D. 48406, Castro F. 11280 (COAH)	SOL, SVC
<i>Paphinia cristata</i> (Lindl.) Lindl.	Trujillo E. 3858 (HUAZ)	SOL
<i>Paphinia lindeniana</i> Rchb. f.	Bernal R. 533 (COL)	SJF
<i>Peristeria guttata</i> Knowles & Westc.	Sanchez M. 28 (HUAZ)	CAR
<i>Platystele alucitae</i> Luer	Sanin D. 6490 (COAH)	FLO
<i>Pleurothallis bivalvis</i> Lindl.	Arias, T. 972 (HUAZ)	DON, FLO
<i>Pleurothallis bicornis</i> Lindl.	Arias T. 1229 (HUAZ)	FLO
<i>Pleurothallis chloroleuca</i> Lindl.	iNaturalist	SVC
<i>Pleurothallis cordata</i> (Ruiz & Pav.) Lindl.	Perdomo O. 422, 398 (CUVC), Arias T. 1221 (HUAZ)	FLO, PRC
<i>Pleurothallis discoidea</i> Lindl.	Arias, T. 953 (HUAZ)	PAU
<i>Pleurothallis languida</i> Luer & R. Escobar	iNaturalist	SVC
<i>Pleurothallis manicosa</i> Luer & R. Escobar	iNaturalist	BEL
<i>Pleurothallis matudana</i> C. Schweinf.	Living collection (El Manantial, Florencia)	SJF
<i>Pleurothallis microcardia</i> Rchb.f.	Betancur J. 20415 (COAH)	BEL, SVC
<i>Pleurothallis octavioi</i> Luer & R. Escobar	Arias T. 1192 (HUAZ)	FLO, SVC
<i>Pleurothallis phalangifera</i> (C. Presl) Rchb. f.	Trujillo E. 7946 (CUVC)	FLO
<i>Pleurothallis pruinosa</i> Lindl.	iNaturalist	BEL
<i>Pleurothallis ruberrima</i> Lindl.	Jimenez E. 33 (COAH)	FLO
<i>Pleurothallis ruscifolia</i> (Jacq.) R. Br. in W. T. Aiton	Castaño N. 9230 (COAH)	BEL, FLO
<i>Pleurothallis sandemanii</i> Luer	Living collection (El Caraño, Florencia)	FLO
<i>Pleurothallis sphaerantha</i> Luer **	Living collection (El Manantial, Florencia)	FLO
<i>Polycynis barbata</i> (Lindl.) Rchb. f.	Perdomo O. 354, 378 (CUVC)	BEL, FLO
<i>Polyotidium huebneri</i> (Mansf.) Garay	Benavides A. 1292 (HUA)	SOL
<i>Polystachya foliosa</i> (Hook.) Rchb.f. in Walp.	Cardenas D. 24841, Rodriguez W. D. 6973, Sanin D. 6465 (COAH), Arias T. 855, 877, 1002, Trujillo E. & Marin 183, Cumaco L. S. & Trujillo E. 23, Trujillo E. et al. 1512 (HUAZ)	CAR, FLO, SOL, SVC

Species name	Accessions reviewed	Area
<i>Polystachya stenophylla</i> Schltr.	Trujillo E. et al. 824, Mesa N. & Trujillo E. 8 (HUAZ)	CAR, MON
<i>Ponthieva fertilis</i> (F. Lehm. & Kraenzl.) Salazar	iNaturalist	SVC
<i>Prescottia cordifolia</i> Rchb.f.	Diaz J. 369 (COAH)	PRC
<i>Prescottia stachyodes</i> (Sw.) Lindl.	Cuatrecasas J. 9070 (COL)	FLO
<i>Prosthechea aemula</i> (Lindl.) W. E. Higgins	Romero R. 4136 (COL, MO)	CAR
<i>Prosthechea chimborazoensis</i> (Schltr.) W. E. Higgins	Mendoza H. 497 (FMB)	SOL
<i>Prosthechea cochleata</i> (L.) W. E. Higgins	iNaturalist	SVC
<i>Prosthechea crassilabia</i> (Poepp. & Endl.) Carnevali & I. Ramírez	Stevenson P. 961 (COAH), Arias T. 994 (HUAZ)	FL, PAU, SVC
<i>Prosthechea grammatoglossa</i> (Rchb.f.) W. E. Higgins	Arias, T. 1018 (HUAZ)	SVC
<i>Prosthechea pygmaea</i> (Hook.) W. E. Higgins	iNaturalist	SVC
<i>Prosthechea tigrina</i> (Linden ex Lindl.) W. E. Higgins	Living collection (El Caraño, Florencia)	FLO, SVC
<i>Prosthechea venezuelana</i> (Schltr.) W. E. Higgins	Living collection (El Manantial, Florencia)	N/A
<i>Prosthechea vespa</i> (Vell.) W. E. Higgins	Arevalo R. 198, Castaño N. 8544, Cardenas D. 48640 (COAH), Perdomo O. 253, 367 (CUVC), Santofimio L. M. & Trujillo E. 8, 15 (HUAZ)	BEL, FLO, SOL
<i>Psilochilus macrophyllus</i> (Lindl.) Ames	Betancur J. 20322, Castaño N. 7500, 8785 (COAH)	BEL
<i>Psychopsis sanderæ</i> (Rolfe) Lückel & Braem	Living collection (El Manantial, Florencia)	NA
<i>Pterostemma escobarii</i> (Dodson C.) M. W. Chase & N. H. Williams	iNaturalist	SVC
<i>Rodriguezia bracteata</i> (Vell.) Hoehne	Living collection (El Manantial, Florencia)	ALB, BEL, FLO, SJF
<i>Rodriguezia claudiae</i> Chiron **	iNaturalist	SJF
<i>Rodriguezia chasei</i> Dodson & D. E. Benn.	iNaturalist	SVC
<i>Rodriguezia lanceolata</i> Ruiz & Pav.	Diaz et al. 101 (UDBC)	FLO, SOL
<i>Rudolfiella floribunda</i> (Schltr.) Hoehne	Ortiz-Valdivieso M. 999 (HPUJ)	PRC
<i>Rudolfiella picta</i> (Schltr.) Hoehne	Perdomo O. 280, 343 (CUVC)	BEL, FLO
<i>Sacoila lanceolata</i> (Aubl.) Garay	Castro F. 67608 (COAH)	PRC
<i>Sarcoglottis neillii</i> Salazar & Tobar **	Calderon A. 264 (HUAZ)	FLO
<i>Scaphosepalum cimex</i> Luer & Hirtz **	Living collection (El Caraño, Florencia)	FLO
<i>Scaphyglottis aurea</i> (Rchb.f.) Foldats	Velayos 6870 (COL)	SOL
<i>Scaphyglottis bidentata</i> (Lindl.) Dressler	Giraldo 3311 (COAH), Estrada 666, Velayos 6514 (COL)	BEL, SJF
<i>Scaphyglottis boliviensis</i> (Rolfe) B. R. Adams	Perdomo O. 428 (CUVC), Arias T. 871, 903 (HUAZ)	BEL, FLO, CAR
<i>Scaphyglottis caquetana</i> Szlach. & Kolan. *	Cardenas D. et al. 6899 (COAH)	SJF
<i>Scaphyglottis graminifolia</i> (Ruiz & Pav.) Poepp. & Endl.	Perdomo O. 418 (CUVC), Arias T. 905, Chaux-Varela J. 37 (HUAZ)	FLO, SJF, SOL
<i>Scaphyglottis imbricata</i> (Lindl.) Dressler	Living collection (El Manantial, Florencia)	SJF
<i>Scaphyglottis longicaulis</i> S. Watson	Cumaco L. S. & Trujillo E. 7, Mesa N. & Trujillo E. 2, Santofimio & Trujillo E. 11 (HUAZ)	BEL, SJF
<i>Scaphyglottis obtusisepala</i> Szlach. & Kolan. *	Trujillo E. 1042 (COAH)	BEL
<i>Scaphyglottis prolifera</i> (Sw.) Cogn.	Aguilar M. 251 (COAH)	FLO
<i>Scaphyglottis punctulata</i> (Rchb. f.) C. Schweinf.	Trujillo E. 7664, 7863 (CUVC), Arias T. 966, 1200, 1209 (HUAZ)	DON, FLO, PRC
<i>Scaphyglottis stellata</i> Lodd. ex Lindl.	Cardenas D. 48550, 6932, Gonzalez M. F. 2617, Rodriguez M. 3641, Stevenson P. 1399, Velayos M. 6412 (COAH), Arevalo R. 88 (COL), Trujillo E. 885 (HUA), Arias T. 869, 905, 962, Chaux-Varela J. 42 (HUAZ), Cardenas D. 6932 (MO)	CAR, DON, FLO, SJF, SOL, SVC
<i>Sertifera purpurea</i> Lindl. & Rchb.f.	Sanin D. 6098 (COAH)	FLO
<i>Sobralia biflora</i> Ruiz & Pav.	Trujillo E. 887 (HUAZ)	SJF

Species name	Accessions reviewed	Area
<i>Sobralia crocea</i> (Poepp. & Endl.) Rchb. f.	Cardenas D. 42426 (FBM, COAH), Betancur J. 20496 (COAH), Trujillo E. 7985 (CUVC)	BEL, FLO
<i>Sobralia decora</i> Bateman	Arevalo R. 346 (COAH)	SOL
<i>Sobralia fimbriata</i> Poepp. & Endl.	Perdomo O. 337 (CUVC)	BEL
<i>Sobralia fragrans</i> Lindl.	Palacios P. 2578 (COL), Castaño N. 7582, Cardenas D. 46381, Betancur J. 20594 (FMB)	BEL, SOL
<i>Sobralia granitica</i> G. A. Romero-Gonzalez MF & Carnevali	Castro F. 10841 (COAH), Arias T. 1015 (HUAZ)	SOL, SVC
<i>Sobralia klotzschiana</i> Rchb. f.	Betancur J. 20458 (COAH), Romero R. 4053 (COL), Arias T. 991, 1015 (HUAZ)	BEL, PAU
<i>Sobralia leucoxantha</i> Rchb. f.	Polania D. & Trujillo E. 4 (HUAZ)	FLO
<i>Sobralia liliastrum</i> Lindl.	Arbelaez E. 326, Cardenas D. 4135, Palacios P. 1164 (COAH), Franco-Rosselli P. 3237, 2417, 3718 (COL), Cumaco L. S. & Trujillo E. 33, Pabon M. 971 (HUAZ), Cardiel J. M. 1010 (MA)	FLO, SOL
<i>Sobralia macrophylla</i> Rchb. f.	Cuatrecasas J. 9119, Fernandez A. 20079 (COL), Vasco A. 306 (HUA), Mesa N. & Trujillo E. 06, 22 (HUAZ)	FLO, SOL
<i>Sobralia roezlii</i> Rchb. f.	iNaturalist	FLO
<i>Sobralia sessilis</i> Lindl.	Living collection (El Manantial, Florencia)	BEL
<i>Sobralia violacea</i> Linden ex Lindl.	Barbosa C. 8109, Gonzalez M. F. 2270, Palacios P. 2932, 2880 (COL)	SOL
<i>Sobralia virginalis</i> Peeters & Cogn. In Cogn. & Goos.	iNaturalist	FLO, SVC
<i>Specklinia grobyi</i> (Lindl.) F. Barros	Escobar R. 5051 (MO)	SVC
<i>Specklinia picta</i> (Lindl.) Pridgeon & M. W. Chase	Cardenas D. et al. 48247 (COAH), Arevalo R. 217 (COL)	SOL, SVC
<i>Stanhopea candida</i> Barb. Rodr.	Arias T. 854 (HUAZ)	SOL
<i>Stelis aviceps</i> Lindl.	Cardenas D. et al. 41680 (COAH), 41773 (FMB)	BEL
<i>Stelis kefersteiniana</i> (Rchb.f.) Pridgeon & M. W. Chase	iNaturalist	SOL
<i>Stelis lindenii</i> Lindl.	Ortiz-Valdivieso M. 536 (HPUJ)	BEL
<i>Stelis oblonga</i> (Ruiz & Pav.) Willd.	Sanin D. 6492 (COAH, HUA)	FLO
<i>Stelis purpurea</i> (Ruiz & Pav.) Willd.	Perdomo O. 268, 315 (CUVC)	FLO, SVC
<i>Stelis superbiens</i> Lindl.	Perdomo O. 368 (CUVC)	BEL
<i>Stenia pallida</i> Lindl.	Arias, T. 913, 987 (HUAZ)	SJF, DON
<i>Telipogon pogonostalis</i> Rchb. f.	Arias, T. 981 (HUAZ)	DON
<i>Telipogon polymerus</i> Rchb. f. *	Trujillo E. 7636, Perdomo O. 403 (CUVC)	FLO, PRC
<i>Telipogon selbyanus</i> N. H. Williams & Dressler **	Perdomo O. 418 (CUVC)	FLO
<i>Trichocentrum cebolleta</i> (Jacq.) M. W. Chase & N. H. Williams	Arias T. 909, 1000, 1010, Chaux-Varela J. 40 (HUAZ)	DON, FLO, SVC
<i>Trichocentrum helicanthum</i> (Kraenzl.) J. M. H. Shaw	Living collection (El Manantial, Florencia)	N/A
<i>Trichocentrum nanum</i> (Lindl.) M.W. Chase & N. H. William	Living collection (El Manantial, Florencia)	SVC
<i>Trichocentrum nudum</i> (Bateman ex Lindl.) M. W. Chase & N. H. Williams	Living collection (El Manantial, Florencia)	FLO
<i>Trichocentrum pulchrum</i> Poepp. & Endl.	Perdomo O. 276 (CUVC)	FLO
<i>Trichosalpinx orbicularis</i> (Lindl.) Luer	Franco-Rosselli P. 4179, Palacios P. 2450, Arevalo R. 341 (COL), Cardenas D. 42195 (FMB)	BEL, PRC, SOL
<i>Trizeuxis falcata</i> Lindl.	iNaturalist	BEL
<i>Tubella multicuspidata</i> (Rchb.f.) Archila	Cardenas D. et al. 41704 (COAH)	BEL
<i>Tubella pusilla</i> (Kunth) Archila	Arias T. 870 (HUAZ), Perdomo O. 397 (CUVC)	CAR, PRC
<i>Vanilla bicolor</i> Lindl.	Idobro J. M. 11423 (COAH)	SOL



Species name	Accessions reviewed	Area
<i>Vanilla guianensis</i> Splitg.	Barona A. 4863 5192 (COAH)	CAR, SOL
<i>Vanilla odorata</i> C. Presl.	Barona A. 4864 (COAH)	SOL
<i>Vanilla palmarum</i> (Salzm. ex Lindl.) Lindl.	Barona 4620, Cardenas D. et al. 48604 (COAH)	BEL, SVC
<i>Vanilla penicillata</i> Garay & Dunst. in Dunst. & Garay	Franco-Rosselli P. 4258 (COL)	SOL
<i>Vanilla pompona</i> Schiede	Cardiel J. M. 1089 (COL)	SOL
<i>Vanilla sprucei</i> Rolfe	Barona A. 3124, Duivenvoorden J. 719, Restrepo D. 441 (COAH)	SOL
<i>Vanilla trigonocarpa</i> Hoehne	Barona A. 3125, 4611, 4612, 4613, 4615, 4616, 4617, 4618, 4619 (COAH)	BEL
<i>Warczewiczella amazonica</i> Rchb. f. & Warsz.	Alzate F. 980 (FAUC)	MIL
<i>Wulfschlaegelia calcarata</i> Benth.	Cardenas D. et al. 48513 (COAH), Blanco M. et al. 233 (COAH, HUAZ)	FLO
<i>Xerorchis amazonica</i> Schltr.	Barona A. 1483 (COAH)	SOL
<i>Xerorchis trichorhiza</i> (Kraenzl.) Garay	Franco-Rosselli P. 4240 (COL)	SOL
<i>Xylobium foveatum</i> (Lindl.) G. Nicholson	Perdomo O. 324 (CUVC)	FLO
<i>Xylobium leontoglossum</i> (Rchb. f.) Rolfe	Trujillo E. et al. 7867 (CUVC)	FLO

## Field expeditions

To expand the checklist of Orchidaceae that occur in Caquetá, we carried out a total of 12 field expeditions between 2019 and 2023 for the project “Orquídeas para la Paz.” Two expeditions explored part of the Alto Fragua Indi Wasi National Natural Park in collaboration with the Park and ten expeditions explored montane, premontane forests and lowland Amazonian Forest of the Department. Fertile specimens were collected and prepared for herbaria according to techniques used for orchid collections, that includes the preservation of flowers in spirit collections, photographs and tissue collections for ongoing DNA analyses. The specimens were deposited at either the Universidad de la Amazonía (HUAZ) or the Universidad del Valle (CUCV) Herbaria (acronyms according to Thiers 2020). Duplicate collections were made for other herbaria when possible. Living specimens, collected when flowers were not found, were taken to local nurseries at El Caraño, Florencia, located at 950 m a.s.l. for cold weather orchids, or El Manantial, Florencia, located at 300 m a.s.l. for warm weather orchids. Once they flowered, they were photographed, identified and herbarium specimens were made. All collections were deposited under the collection permit of the Universidad de la Amazonia (permit number 01691 October 2020; Indi Wasi National Park memorandum No. 20182200004943) by Alexis Calderón, Marco Correa and Edwin Trujillo.

## Resources used

Databases and herbaria were used to find herbarium specimens that were examined to back-up observations without vouchers. Available literature, as well as local, regional and national catalogues were used to find herbarium specimens collected in the region. Only records with a herbarium specimen were considered for this checklist; living specimens and iNaturalist records by one of the authors or collaborators and with a specialist identification were considered in the list, but only as “tentative” until the herbarium collection is available.

To carry out online consultations in herbaria, search criteria were considered using the keywords: “Caquetá”, “Orchidaceae”, “tropical humid forest”, “botanical expeditions”, “Amazon region” and “Caquetá River.” The “advanced search” option was used for most of the herbaria consulted, since it allows for a more direct search for information. International herbaria consulted, either in person or online, included: Harvard University Oak Ames Herbarium (AMES), Herbario del Instituto Chinoín (AMO), Berlin (B), Royal Botanic Gardens Kew Herbarium (KEW), University of California, Los Angeles Herbarium (LA), the Real Jardín Botánico de Madrid (MA), Naturalis Biodiversity Center (NL), Herbarium Utrecht (U), New York Botanical Garden (NY), the Muséum national d’Histoire naturelle in Paris (P), Marie Selby Botanical Gardens (SEL), Tropicos (2002) of the Missouri Botanical Garden (MO), Gdansk University (UGDA), University of Florida Herbarium (USF), W-Reichenbach (Vienna) and University of Wisconsin Herbarium (WIS).

Colombian herbaria included: Instituto Amazónico de Investigaciones Científicas – SINCHI (COAH), Herbario Nacional Colombiano (COL), Herbario de la Universidad del Cauca (CAUP), Herbario de la Universidad del Valle (CUCV), Herbario de la Universidad de Caldas (FAUC), Herbario Federico Medem-Bogotá (FMB), Herbario de la Pontificia Universidad Javeriana (HPUJ), Herbario de la Universidad de Antioquia (HUA), Herbario Enrique Forero (HUAZ) de la Universidad de la Amazonia, Jardín Botánico José Celestino Mutis de Bogotá (JBB), Universidad de los Llanos (LLANOS), Universidad de Nariño (PSO), Universidad Surcolombiana (SURCO), Herbario Forestal de la Universidad Distrital Francisco José de Caldas (UDBC) and Universidad Pedagógica y Tecnológica de Colombia (UPTC). Other checked databases included GBIF (Global Biodiversity Information Facility, 2022), IDigBio (Integrated Digitized Biocollections) and BRAHMS at Marie Selby Botanical Gardens (Software for Natural History management).

### Name validation and data curation

Correct scientific names of species were assigned, based on the World Flora Online (WFO 2022), except for the genus *Tubella* (Luer) Archila, which was accepted as valid following Bogarín et al. (2018). All names were supported by herbarium specimens, photographs uploaded on iNaturalist ([www.inaturalist.com](http://www.inaturalist.com)) or living collections in one of the local nurseries that supported their presence in Caquetá with reliable taxonomic determination.

All records obtained from herbaria, databases and literature were carefully curated regarding their scientific name, locality, collector and collection number. For localities, names of municipalities were verified and updated. To assign a name to duplicates with different identification, the provenance of each assigned name was investigated, paying particular attention to plants identified by experts, curators and recognised taxonomists, besides using the date of determination and its citation in a publication. Lastly, using photographs and a list of taxonomic groups within orchids, we reached out to as many specialists as possible for their species expertise (see Acknowledgements). This final dataset was used for analyses.

### Data analysis

Collection records were georeferenced as precisely as the information allowed, since in many records, the location is not clearly specified, a frequent

situation in orchids, old collections and those made by amateurs. Due to the lack of standardised geographical coordinates, only the number of species in municipalities is reported for this study. Georeferenced records and species distribution maps were constructed for the 16 municipalities and are available upon request.

## Results

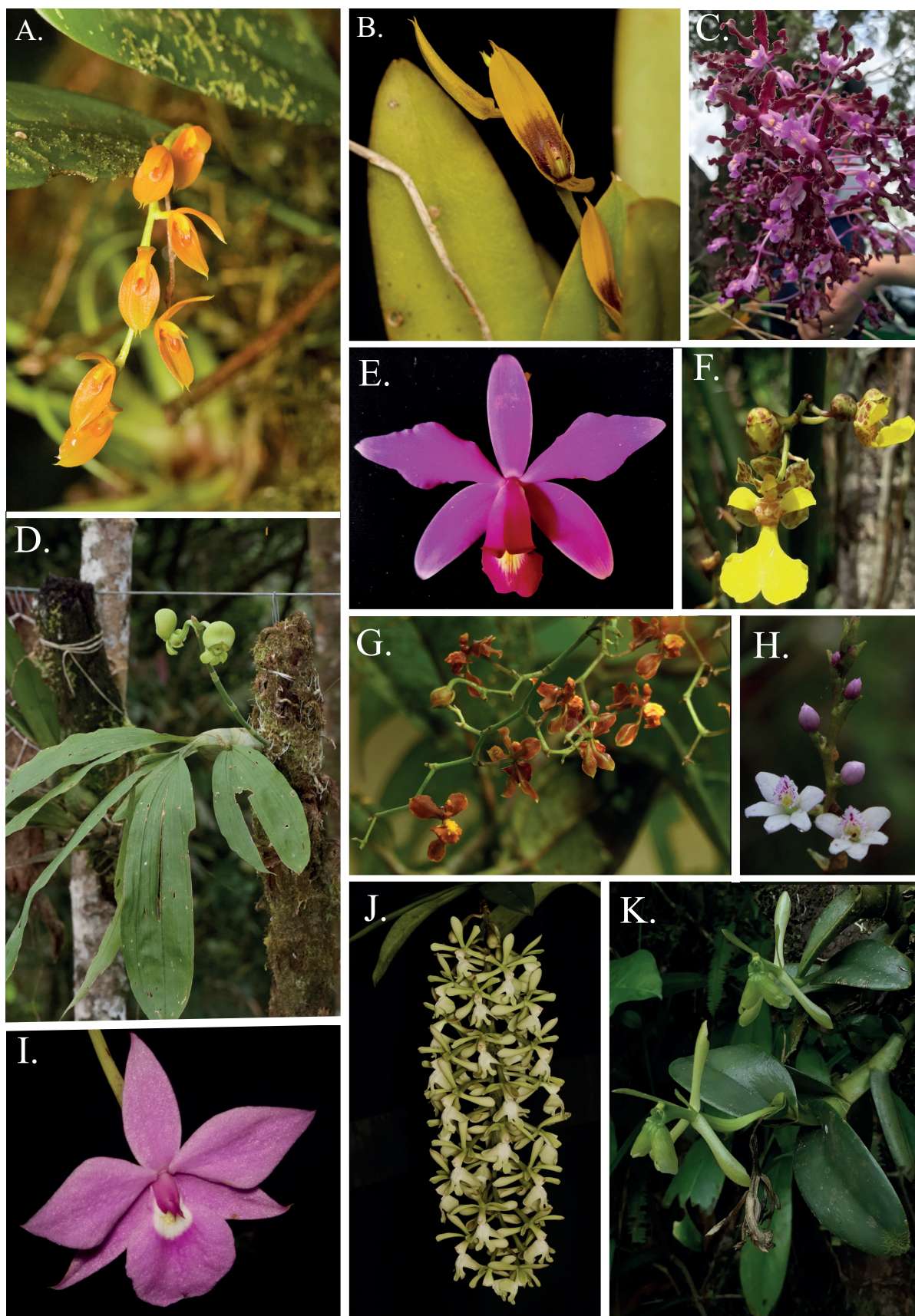
A total number of 228 fertile specimens were collected in the field (collections made by Arias T., Chaux-Varela J., Perdomo O., Trujillo E. and Correa M.), 692 herbarium specimens were reviewed in different herbaria, 100 photographs and two living collections accessed for specimen identifications. The most abundant species in the field were *Epidendrum nocturnum* Jacq., *E. lacustre* Lindl., *Maxillaria discolor* (G. Lodd. ex Lindl.) Rchb. f. and *Scaphyglottis stellata* Lodd. ex Lindl. Some of the rarest ones only observed in the field once were *Cyrtochilum caquetanum* P. Ortiz, L. E. Álvarez & A. J. Carrillo, *Masdevallia ensata* Rchb. f. and *Paphinia cristata* (Lindl.) Lindl. In “Orquídeas para la Paz” expeditions, 98 species (collections made by Arias T. and Chaux-Varela J.) representing 29 genera were collected. Living individuals that were not flowering in the field were brought to El Manantial (300 m a.s.l.) and El Caraño (950 m a.s.l.) both located in the Municipality of Florencia. Herbaria collections are actively being made once orchids start flowering. A total of 55 species are available at El Manantial and 60 at El Caraño.

We report 418 species belonging to 98 genera represented in 744 herbarium collections including duplicates. Eighty-two species are new reports for Caquetá since they have not been vouchered until this study (Table 1, collections exclusively made by one of the authors of this paper). The most species-rich genera were *Epidendrum* L. (68 spp.), *Maxillaria* Ruiz & Pav. (59 spp.), *Pleurothallis* R.Br. (16 spp.), *Elleanthus* C. Presl (14 spp.), *Sobralia* Ruiz & Pav. (14 spp.) (Table 1, Figs 2–5). Most genera found in Caquetá (75) have one to three species (Fig. 5). We found two introduced species around urban areas of Florencia, *Arundina graminifolia* (D. Don) Hochr. and *Dendrobium nobile* Lindl.; these were not included in the species list.

Eighty-one species are included as “tentative” because they have been accurately identified, but lack a herbarium voucher. Fifty-three out of these 81 were included as potentially distributed in the Caquetá because records produced by the authors and collaborators in the field through photographs were not documented with herbarium vouchers. Such photographic records were submitted to iNaturalist (see <https://www.inaturalist.org/projects/orquideas-del-caqueta>). Twenty-seven species including 15 in El Manantial and 12 in El Caraño, are part of our living collections. They were collected fertile in the field and identified, but have not been photographed or documented with herbarium vouchers to date.

For the Municipality of Florencia, 192 species were recorded, followed by Solano with 108 and Belén de los Andaquíes with 77 (Fig. 4). Five municipalities including Albania, Morelia, Valparaíso, Milán and La Montañita have three or less records and there are no orchid botanical collections for Curillo and Solita (Table 1). Fourteen species are endemic to Colombia and from those, 11 have new herbarium vouchers made in this project. Twenty nine are new reports for Colombia and 15 of those have new herbarium vouchers as a result of this





**Figure 2.** Representative Orchidaceae species from Caquetá, Colombia. **A** *Acianthera casapensis* **B** *Acianthera ciliata* **C** *Laelia rosea* **D** *Catasetum tuberculatum* **E** *Cattleya violacea* **F** *Trichocentrum nudum* **G** *Cyrtorchilum porrigens* **H** *Epidendrum fimbriatum* **I** *Dimerandra emarginata* **J** *Epidendrum coronatum* **K** *Epidendrum difforme*.

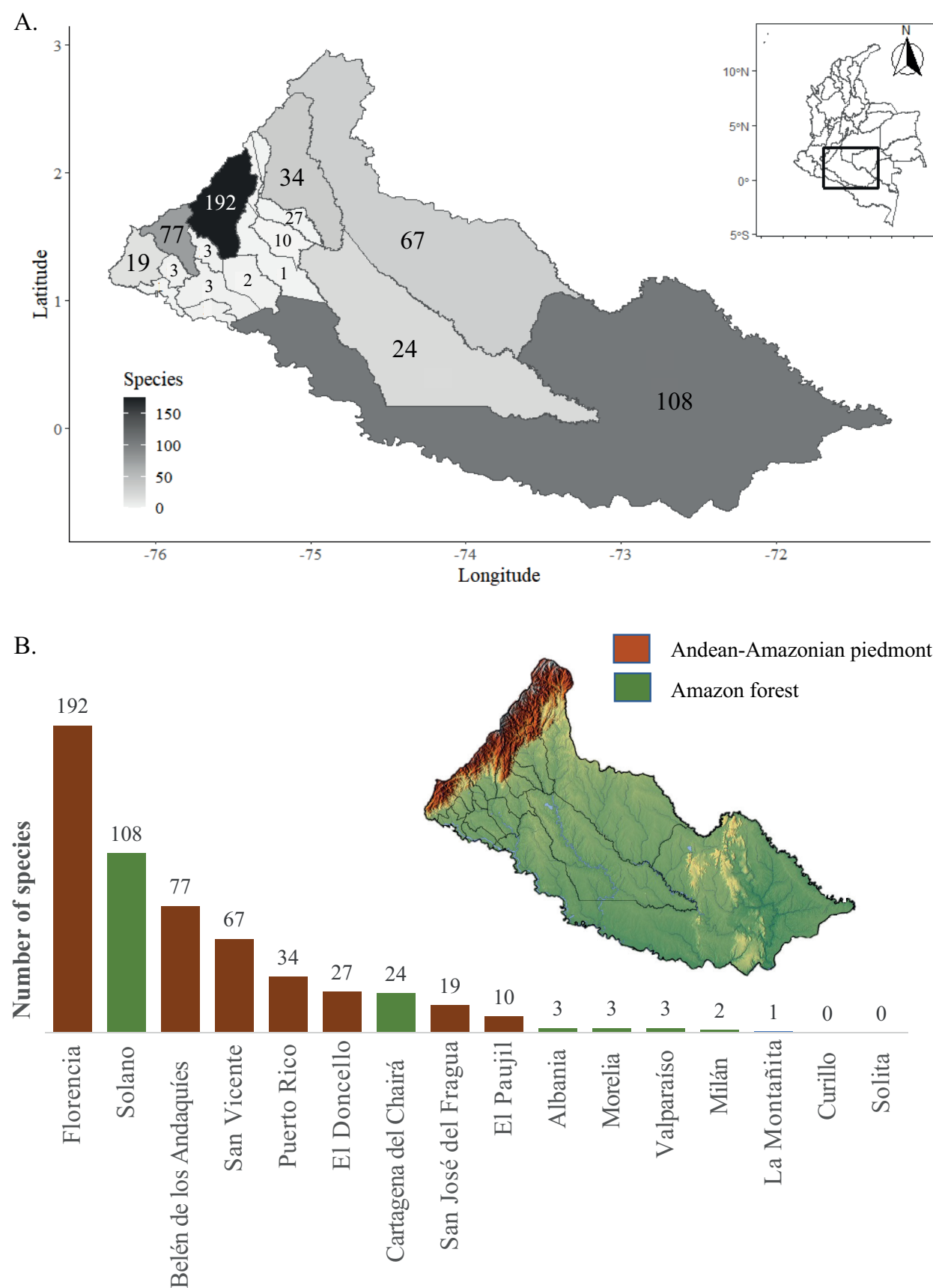




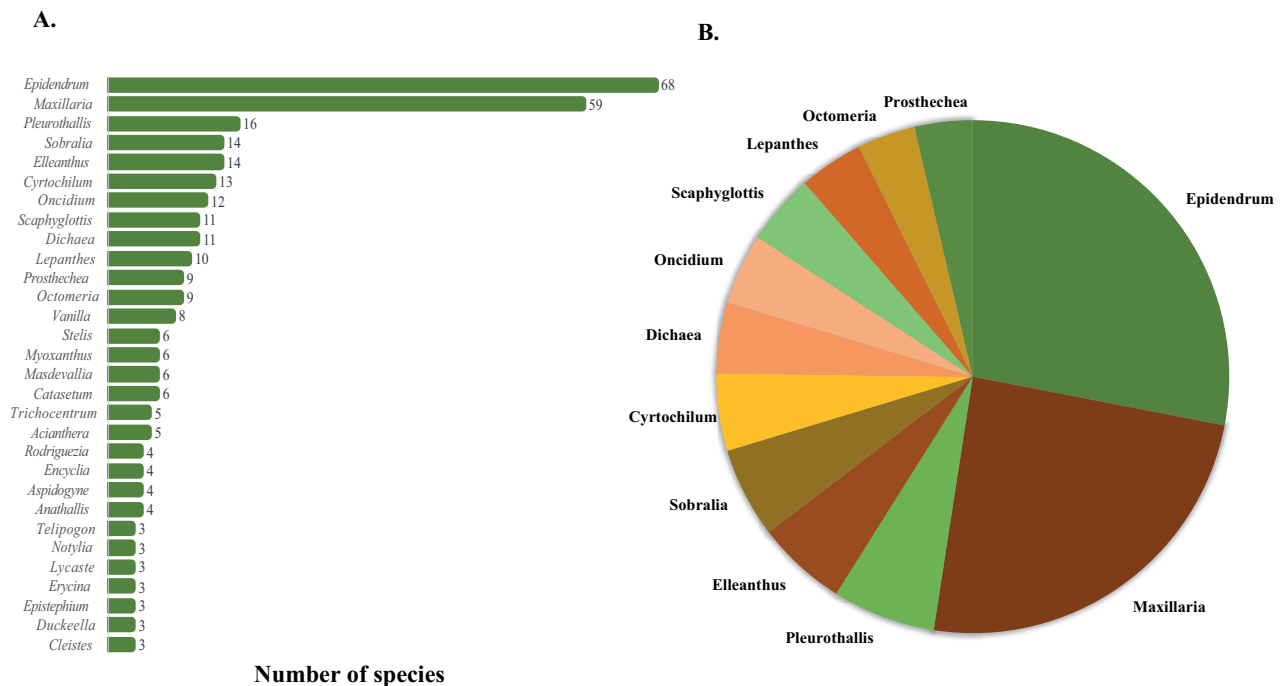
**Figure 3.** Representative Orchidaceae species from Caquetá, Colombia. **A** *Galeandra macroplecta* **B** *Maxillaria aureoglobula* **C** *Maxillaria egertoniana* **D** *Maxillaria equitans* **E** *Maxillaria parkeri* **F** *Maxillaria parviflora* **G** *Notylia barkeri* **H** *Oncidium alexandrae* **I** *Octomeria grandiflora* **J** *Prosthechea chimborazoensis* **K** *Stanhopea candida* **L** *Sobralia macrophylla*.

project (Table 1). *Masdevallia virgo-cuencae* Luer & Andreetta (VU), *Miltoniopsis phalaenopsis* (Linden & Rchb. f.) Garay & Dunst. (VU) and *Oncidium alexandrae* (Bateman) M. W. Chase & N. H. Williams (EN) were included in the Red List of Colombian orchid species (Calderón-Sáenz 2007).

Most collections made in Caquetá have been deposited at HUAZ, which currently holds 210 orchid specimens, while COAH holds 207 orchid specimens and COL 109 orchid specimens. Seven international herbaria had between 1–12 collections from Caquetá (Table 1).



**Figure 4.** Distribution of orchid species number in Caquetá municipalities. **A** Orchid species distribution by municipality and a heatmap of species richness found in each of the municipalities **B** Number of orchid species found in each municipality and their geographic position (Andean Piedmont or Amazonian Basin).



**Figure 5.** Number of orchid species in most abundant genera of Caquetá, Colombia **A** number of species in the most species rich genera of Caquetá **B** pie chart showing the proportion of species in genera with 9 to 70 species.

## Discussion

A total of 276 new species records of Orchidaceae were added to the previous orchid report of Betancur et al. (2015), who cited 142 species. The great diversity of Orchidaceae species in Caquetá might be explained by spatial heterogeneity and phytophysiognomies in this region (Etter et al. 2006). The significant diversity of *Epidendrum* (68/1000) and *Maxillaria* (59/570) was expected because these are some of largest Neotropical Orchidaceae genera with regards to species number (Fig. 5, Suppl. material 1: table S1). The four most species-rich genera account for 40% of the total species, but they represent 4.12% of the total genera. Forty-three genera included only one species for the region, which corresponds to 19% of the total species and 42% of the total genera. Species in genera, such *Encyclia* Hook. and *Stelis* Sw., were challenging to identify and additional taxonomic work is required. One widely distributed and unpublished hybrid *Epidendrum* × *communis* Hágsater Ined. was added to the list after specialist advice (Hágsater, pers. comm.)

During the construction of this list, we left out collections made by Werner Hopp (Schlechter 1924) since they were collected in Putumayo in 1921 and 1922 when Putumayo was part of the Caquetá intendancy. In 1991, Putumayo was politically recognised as a Department and, as such, it is no longer part of Caquetá. Additionally, some species collected by Hopp were deposited in the Berlin Herbarium (B) and destroyed during Second World War (Suppl. material 1. table S2).

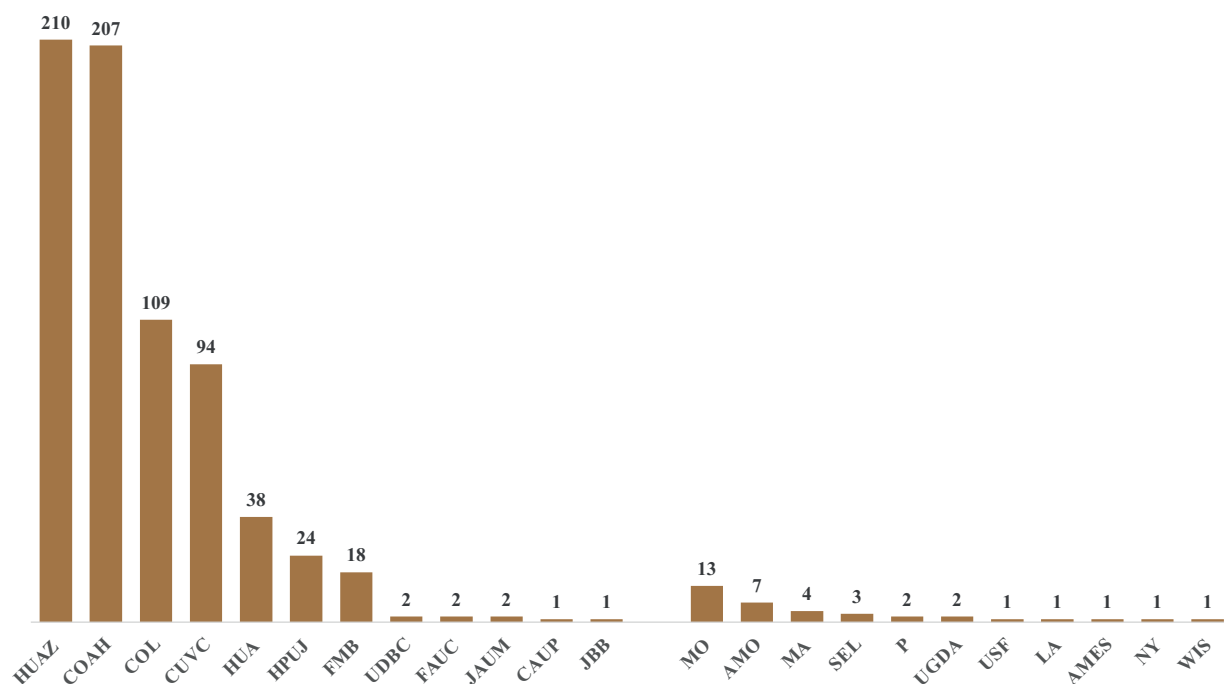
Most orchid species documented in Caquetá are found in the Florencia Municipality (192 spp.). This could be explained by the convenience of collecting around cities and the wide altitudinal gradient in this municipality. We present collections numbers by municipality because conservation strategies might differ between political boundaries in Colombia. Regional Autonomous Corporations (CAR) are the main environmental authority. They are responsible for im-

plementing policies and plans from the Ministry of Environment and are granted administrative and financial autonomy. Entities responsible for the formulation of such conservation strategies might benefit more from having such information presented following Departmental divisions. By depositing 140 herbarium specimens at HUAZ (duplicates will be sent to other herbaria) in the framework of this study, we substantially increased its orchid collection to 210 species, positioning HUAZ as the first local herbarium with more herbarium collections from Caquetá than any other herbaria (Fig. 6, Suppl. material 1: table S1).

Five of the municipalities of Caquetá, representing ~ 20% of the total geographical area of the Department, had zero to three herbarium collections or species reported (Fig. 4). The areas in the north-eastern part of the eastern Andean Mountain range still need extensive exploration. These areas include the National Natural Park Cordillera de Los Picachos, where landmines were

### Colombia national herbaria

### International herbaria



**Figure 6.** Number of orchid species in the Colombian national herbaria and international herbaria. Instituto Amazónico de Investigaciones Científicas – SINCHI (COAH), Herbario Nacional Colombiano (COL), Herbario de la Universidad del Cauca (CAUP), Herbario de la Universidad del Valle (CUCV), Herbario de la Universidad de Caldas (FAUC), Herbario Federico Medem-Bogotá (FMB), Herbario de la Pontificia Universidad Javeriana (HPUJ), Herbario de la Universidad de Antioquia (HUA), Herbario Enrique Forero (HUAZ) de la Universidad de la Amazonia, Jardín Botánico José Celestino Mutis de Bogotá (JBB), Universidad de los Llanos (LLANOS), Universidad de Nariño (PSO), Universidad Surcolombiana (SURCO), Herbario Forestal de la Universidad Distrital Francisco José de Caldas (UDBC), and Universidad Pedagógica y Tecnológica de Colombia (UPTC). Harvard University Oak Ames Herbarium (AMES), Herbario del Instituto Chinoín (AMO), Berlin (B), Royal Botanic Gardens Kew Herbarium (KEW), University of California, Los Angeles Herbarium (LA), the Real Jardín Botánico de Madrid (MA), Naturalis Biodiversity Center (NL) - Botany (Herbarium Utrecht), New York Botanical Garden (NY), the Herbarium Paris of the Museum National D'Histoire Naturelle (P), Marie Selby Botanical Gardens (SEL), TROPICOS database (access 2022) of the Missouri Botanical Garden (MO), Gdansk University (UGDA), University of Florida Herbarium (USF), W-Reichenbach (Vienna) and University of Wisconsin Herbarium (WIS).



planted by rebel groups during the long armed conflict that lasted for decades and these have not been removed to date. Orchid diversity could significantly increase with the development of intensive exploration in these mountainous ecosystems and a thorough exploration of the Amazonian Forest canopy. For instance, Departments like Antioquia and Huila have been catalogued as having the largest orchid diversity (Betancur et al. 2015); however, these areas of Colombia have not been extensively explored for decades.

During our expeditions, two species *Cattleya violacea* (Kunth) Lindl. and *Trichocentrum lanceanum* (Lindl.) M. W. Chase & N. H. Williams have been found only in La Laguna del Chaira in the Cartagena del Chaira Municipality. We doubt these species have a natural distribution there. Rather, we suspect they were introduced during the massive effort to bring orchids to La Laguna del Chaira during the 1980s, during which “uninformed” reintroductions of non-native species could have taken place.

This checklist places Caquetá as the eighth Department in Colombia in terms of genera diversity (98 genera) from its original position in the National Plan of Orchid Conservation (15<sup>th</sup> place, 62 genera). As for the ranking in species number for Colombia, Caquetá goes from position 17<sup>th</sup> (142 spp.) to position 9<sup>th</sup> (418 spp.) (Betancur et al. 2015). Caquetá has many orchid genera (98/258 in Colombia) with few species each, 76% of genera having around 1–3 species. Each of these genera include a unique clade distributed in a relatively small area of Colombia. This could be of particular interest in conservation, prioritising evolutionary history over species diversity (Arponen 2012). Caquetá would be one of the regions of Colombia where there are more different genera represented in clades than in other Colombian regions. This work supplies valuable evidence to promote conservation efforts and politics for habitat preservation of the Colombia Andean Piedmont.

Caquetá has lost approximately 30% of its original area due to human impacts, such as cattle ranching. National parks in Caquetá make up 65% of the protected remnants. In the last 50 years, expansion of the agricultural frontier for the establishment of grazing lands, wood extraction and illegal coca crops have destroyed many ecosystems, greatly impacting all national parks. Florencia, for example, is currently undergoing consistent expansion of farming lands ultimately leading to the decimation of natural ecosystems (IDEAM 2020).

## Conclusion

Our floristic study is a needed contribution towards a better understanding of the diversity of Colombian orchids. The checklist provides a set of freely-available data on orchid diversity in Caquetá. Furthermore, our study is a baseline panorama of orchid species diversity in the Department, identifying groups of interest for further taxonomic work, especially those which have not been monographed. Lastly, the information provided could enhance local conservation strategies for endangered floristic elements in the Department by adding to a more complete overview of the high orchid diversity in the region.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions


Conceptualization: TA. Data curation: TA, JVD, ACT, OP, ETT. Formal analysis: TA, ETT, JVD. Funding acquisition: TA. Investigation: AZT, ETT, MACM, ACT, JCV, TA, MPC, JVD, OP. Methodology: TA, OP, ETT, RACÁ, JCV, MPC, ACT. Project administration: MPC, RACÁ, TA. Supervision: JVD, TA. Validation: TA, OAPE, ACT, AZT, ETT, JVD. Visualization: TA. Writing - original draft: TA. Writing - review and editing: OP, TA, ETT, OAPE, JVD, AZT.

### Author ORCIDs

Tatiana Arias  <https://orcid.org/0000-0001-8261-0635>


R. Alexis Calderón-Álvarez  <https://orcid.org/0000-0001-5277-3070>

Marco A. Correa-Munera  <https://orcid.org/0000-0003-2683-7733>

Alejandro Zuluaga  <https://orcid.org/0000-0002-5874-6353>

Oscar Perdomo  <https://orcid.org/0000-0003-1260-1965>

Oscar A. Pérez-Escobar  <https://orcid.org/0000-0001-9166-2410>

Edwin Trujillo-Trujillo  <https://orcid.org/0000-0002-5601-540X>

Janice Valencia-D.  <https://orcid.org/0000-0003-2508-9825>

### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Supplementary data

Authors: Tatiana Arias, Jeisson Chaux-Varela, Maria del Pilar Camero, R. Alexis Calderón-Álvarez, Anyi Carolina Trujillo, Marco A. Correa-Munera, Alejandro Zuluaga, Oscar Perdomo, Oscar A. Pérez-Escobar, Edwin Trujillo Trujillo, Janice Valencia-D.

Data type: tables (excel document)

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Link: <https://doi.org/10.3897/phytokeys.229.102737.suppl1>



# *Malus* includes *Docynia* (Maleae, Rosaceae): evidence from phylogenomics and morphology

Guang-Ning Liu<sup>1,2\*</sup>, Dai-Kun Ma<sup>3\*</sup>, Chao Xu<sup>3</sup>, Jian Huang<sup>4</sup>,  
Bin-Jie Ge<sup>5</sup>, Qiang Luo<sup>6</sup>, Yu Wei<sup>1,2</sup>, Bin-Bin Liu<sup>3</sup>

1 Beijing Botanical Garden, Xiangshan-Wofosi Road, Beijing 100093, China

2 Beijing Floriculture Engineering Technology Research Centre, Beijing, China

3 State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, No. 20. Nanxincun, Xiangshan Road, Beijing 100093, China

4 CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Xishuangbanna 666303, China

5 Eastern China Conservation Center for Wild Endangered Plant Resources, Shanghai Chenshan Botanical Garden, No.3888 Chenhua Road, Songjiang District, Shanghai 201602, China

6 Institute of Agricultural Sciences, Xichang College, No. 1. Xuefu Road, Anning Town, Xichang City, 615013, China

Corresponding authors: Bin-Bin Liu ([liubinbin@ibcas.ac.cn](mailto:liubinbin@ibcas.ac.cn)); Yu Wei ([weiyu@chnbg.cn](mailto:weiyu@chnbg.cn))

## Abstract

*Docynia* has been treated as a separate genus or merged into *Cydonia* or *Docyniopsis*. Our phylogenomic evidence from 797 single-copy nuclear genes and plastomes confirmed the sister relationship between *Docynia* and *Docyniopsis*. By integrating the phylogenomic and morphological evidence, we propose to accept a broad generic concept of *Malus* and merge *Docynia* into *Malus*. Three new combinations are also made here: *Malus delavayi* (Franch.) B.B.Liu, *M. indica* (Wall.) B.B.Liu and *M. longiunguis* (Q.Luo & J.L.Liu) B.B.Liu.

**Key words:** *Docynia*, *Malus*, nomenclatural transfer, phylogenomics, taxonomy



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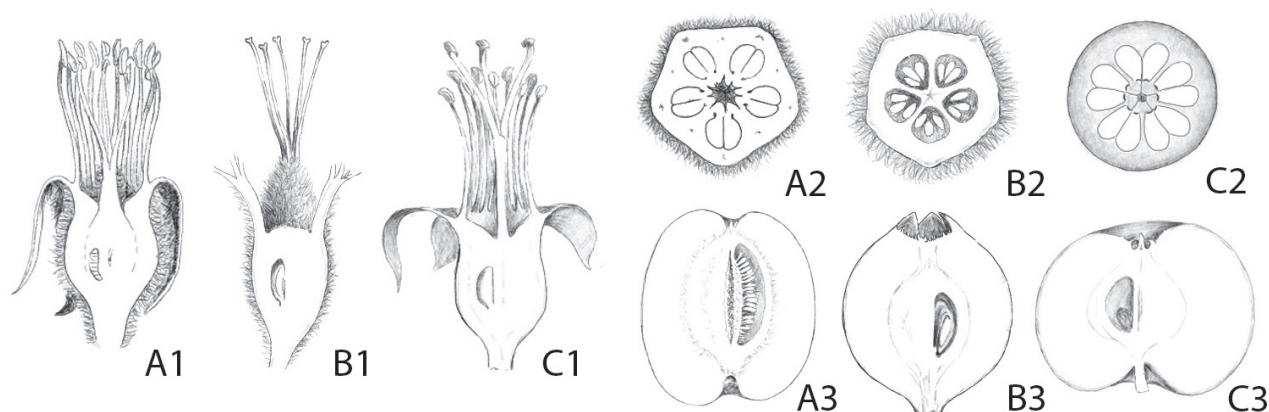
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## Introduction

*Docynia* Decne. is a genus belonging to the apple subtribe Malinae and this genus is endemic to East and Southeast Asia (Yu and Ku 1974; Phipps et al. 1990; Gu and Spongberg 2003). Due to the easily distinguished multiple ovules per locule, 3–10 in *Docynia* (Fig. 1B2, B3) versus two in *Malus* Mill. (Fig. 1C2, C3), *Docynia* has been recognised as a separate genus in a series of taxonomic treatments (i.e. Decaisne (1874); Focke (1888); Koehne (1893); Rehder (1940, 1949); Yu and Ku (1974); Robertson et al. (1991); Kalkman (2004)). However, due to the multiple ovules per locule shared with *Cydonia* Mill. (Fig. 1A2, A3), Spach (1834) and Wenzig (1883) proposed an alternative taxonomic treatment, merging *Docynia* into *Cydonia*.

Recent phylogenetic and phylogenomic studies presented strong topological discordance amongst nuclear/plastid genes and showed cytonuclear conflicts (referring to fig. 1 in Liu et al. (2022)). *Docynia* is closely related to *Docyniopsis* (C.K.Schneid.) Koidz. (= *Malus* sect. *Docyniopsis* C.K.Schneid.), based on the plastomes and the nuclear sequences (Lo and Donoghue 2012; Liu et

\* These authors have contributed equally to this work.



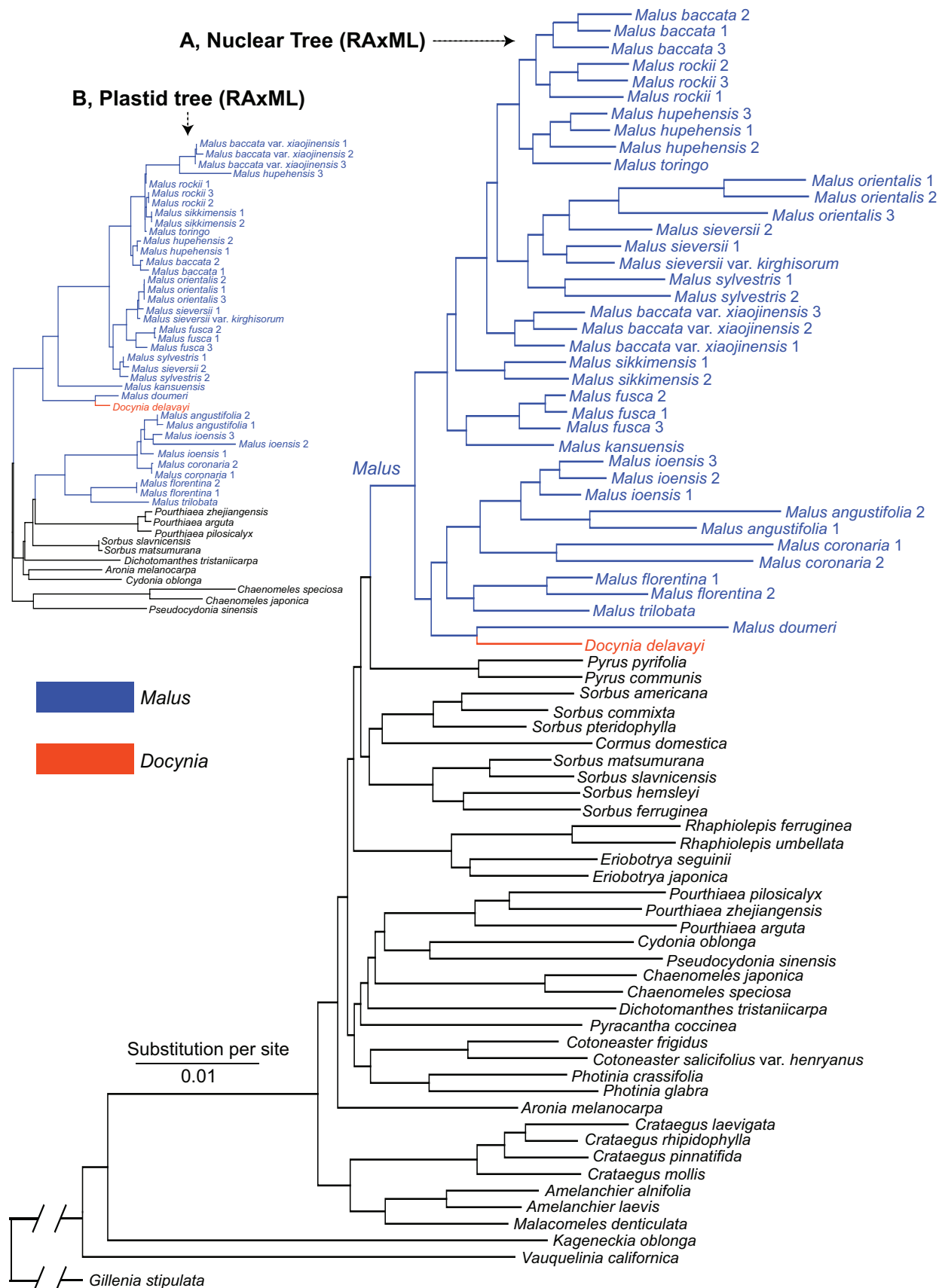
**Figure 1.** Morphological comparison amongst *Cydonia* (A1-A3), *Docynia* (B1-B3) and *Malus* (C1-C3) **A1, B1, C1** longitudinal section of carpel **A2, B2, C2** cross-section of fruit **A3, B3, C3** longitudinal section of fruit.

al. 2019, 2020a, 2020b, 2022; Jin et al. 2023). Several shared morphological characteristics have also supported their close relationship, i.e. cone-shaped non-adnate part of the ovaries (Fig. 1B1, C1), fully connate carpels (Fig. 1B1, C1), incurved and persistent calyx, numerous scattered sclereids throughout the flesh, juvenile leaves deeply lobed and similar flavonoid chemistry (Williams 1982; Robertson et al. 1991; Kalkman 2004). However, Jin (2014) proposed an alternative phylogenetic inference, based on the whole plastome, the sister relationship between *Docynia* and *Cydonia*. Additionally, Xiang et al. (2017) inferred a close relationship between *Docynia* and *Eriolobus* M.Roem., based on the transcriptomic data and this result provided another line of evidence for Schneider's (1906) taxonomic transfer. However, Xiang et al. (2017) sampled only four apple-related species, *Malus baccata* (L.) Borkh., *M. domestica* (Sukow) Borkh., *Docynia delavayi* (Franch.) C.K.Schneid. and *Eriolobus trilobatus* M.Roem., the inferred phylogenomic topology based on this limited taxon sampling; thus, an accurate species relationship was not presented. Therefore, the argument that *Docynia* should be transferred to the genus *Eriolobus*, based on a strongly-supported sister relationship between the two taxa is untenable, as this evidence with limited taxon sampling is insufficient to justify a taxonomic reclassification proposed by Schneider (1906). Liu et al. (2022) sampled 39 individuals representing 18 wild species and provided a robust backbone of the apple and its allies in the framework of the tribe Maleae integrating 797 single-copy nuclear genes (SCN genes) and whole plastome data (Fig. 2). This phylogenomic analyses resolved the phylogenetic position of *Docynia*, placing it within *Malus* sensu lato (Liu et al. 2022).

In this study, we aim to transfer three currently-recognised species of *Docynia* to *Malus*.

## Materials and methods

We sampled 77 individuals in the framework of Maleae, of which 39 were apple-related species and the other 38 were outgroup species. All these 77 samples were performed for deep genome skimming (DGS) sequencing with 5-10G data for each sample. We assembled the whole plastome using NOVOPlasty v. 4.3.1 (Dierckxsens et al. 2016) and a successive assembly approach (Liu et al. 2021).



**Figure 2.** Maximum Likelihood (ML) tree of *Malus* within Maleae inferred from RAxML analysis using the concatenated 797 single-copy nuclear genes (SCNs) supermatrix (A), the upper left inset is a portion of the RAxML tree of *Malus*, based on the 78 concatenated plastid coding sequences (CDSs) supermatrix. (Adapted from figs 2 & 5 in Liu et al. (2022)).

Given the rich genomic resources in various lineages of Rosaceae, we screened 797 nuclear SCN genes from six genomes, *Malus baccata*, *M. domestica*, *Pyrus betulifolia* Bunge, *P. bretschneideri* Rehder, *P. ussuriensis* Maxim. × *P. communis* L. and *P. pyrifolia* (Burm.f.) Nakai. We assembled these 797 nuclear SCN genes for these 77 samples using HybPiper pipeline v. 1.3.1 (Johnson et al. 2016). The assembled sequences were then cleaned with a series of procedures, such as trimAL v. 1.2 (Capella-Gutiérrez et al. 2009), AMAS v. 1.0 (Borowiec 2016), TreeShrink v. 1.3.9 (Mai and Mirarab 2018) and Spruceup (Borowiec 2019). We combined the concatenated and coalescent-based methods for accurate phylogenetic inference. As for the concatenated-based method, we performed Maximum Likelihood (ML) tree inference using RAXML 8.2.12 (Stamatakis 2014) and IQ-TREE2 v. 2.1.3 (Minh et al. 2020). The shrunken trees from TreeShrink (Mai and Mirarab 2018) were used as input to estimate a coalescent-based species tree with ASTRAL-III (Zhang et al. 2018). The detailed parameters refer to the materials and methods in Liu et al. (2022).

## Results and discussion

The phylogenetic relationship between *Docynia* and *Malus* has been controversial for two centuries. Our results revealed that all these nine nuclear and plastid trees in our study (Liu et al. 2022) demonstrated the paraphyly of *Malus* s.s., with *Docynia* nested within it (Fig. 2) and this was also confirmed in several recent molecular studies (Lo and Donoghue 2012; Xiang et al. 2017; Liu et al. 2020a). The sister relationship between *Docynia* and *Malus doumeri* A.Chev. (= *Docyniopsis*) was confirmed either in the nuclear or plastid trees, except for the conflicting phylogenetic placement of the *Docynia*-*Docyniopsis* clade. As indicated in our previous study (Liu et al. 2022), the close relationship between *Docynia* and *Docyniopsis* (Fig. 3) was also supported by the morphological evidence, such as cone-shaped non-adnate part of the ovaries (Figs 1B1, C1, 3I), fully connate carpels (Figs 1B1, C1, 3K), incurved and persistent calyx (Fig. 3A, F, I, J), numerous scattered sclereids throughout the flesh, juvenile leaves deeply lobed and similar flavonoid chemistry (Williams 1982; Robertson et al. 1991; Kalkman 2004).

Despite the monophyly of narrowly-circumscribed small genera in *Malus* s.l., including *Chloromeles* (Decne.) Decne., *Docynia*, *Docyniopsis*, *Eriolobus* M.Roem. and *Malus* sensu stricto, we believe that such narrow generic concepts may be impractical for use by botanists, ecologists, conservation biologists and horticulturalists. Given the prevalence of reticulations in angiosperms, we recommend integrating multiple lines of evidence for accurate taxonomic treatments, including morphology, phylogenomics, cytology, biogeography and ecology, as proposed by integrative systematics (Wen et al. 2017). Traditionally, taxonomic circumscription was often focused solely on the taxonomic community, with little consideration given to its broader implications. However, today there is a growing recognition that taxonomic circumscription can have far-reaching effects on many aspects of biology, including conservation, ecology and evolution. By considering the needs of the broader biological community, taxonomic circumscription can help to ensure that taxonomic classifications are more valuable and relevant to a wider range of researchers and practitioners. An excessive inclination towards separating genera can hinder





**Figure 3.** Structural comparison of the represented species in *Malus* sect. *Docyniopsis*, *M. doumeri* **A** inflorescence branch with young fruits **B** undeveloped leaves **C** flower **D** sepals (five) **E** petals **F** longitudinal section of flower **G** filament **H, K** cross-section of fruit in different stages **I** infructescence branch **J** the projected pome at apex and the persistent sepals. All photos credit to Bin-Jie Ge.

the advancement of research programmes for understanding evolution across all descendants stemming from a common ancestor. Additionally, by educating the general public about the importance of taxonomic circumscription, we can help foster a greater appreciation for biodiversity and its role in understanding and conserving it. In summary, taxonomic circumscription today should be viewed as a tool for serving the needs of both the taxonomic community and the broader biological community, as well as educating the general public about the importance of biodiversity and taxonomy (Wen et al. 2015, 2017; Funk 2018).

With all these considerations, we propose using the broad generic concept of *Malus*, which includes all members of *Malus* sensu Gu and Spongberg (2003) and the species in *Docynia*. We here formally transferred the three currently-recognised species of *Docynia* to *Malus* in the following text.

## Taxonomic treatment

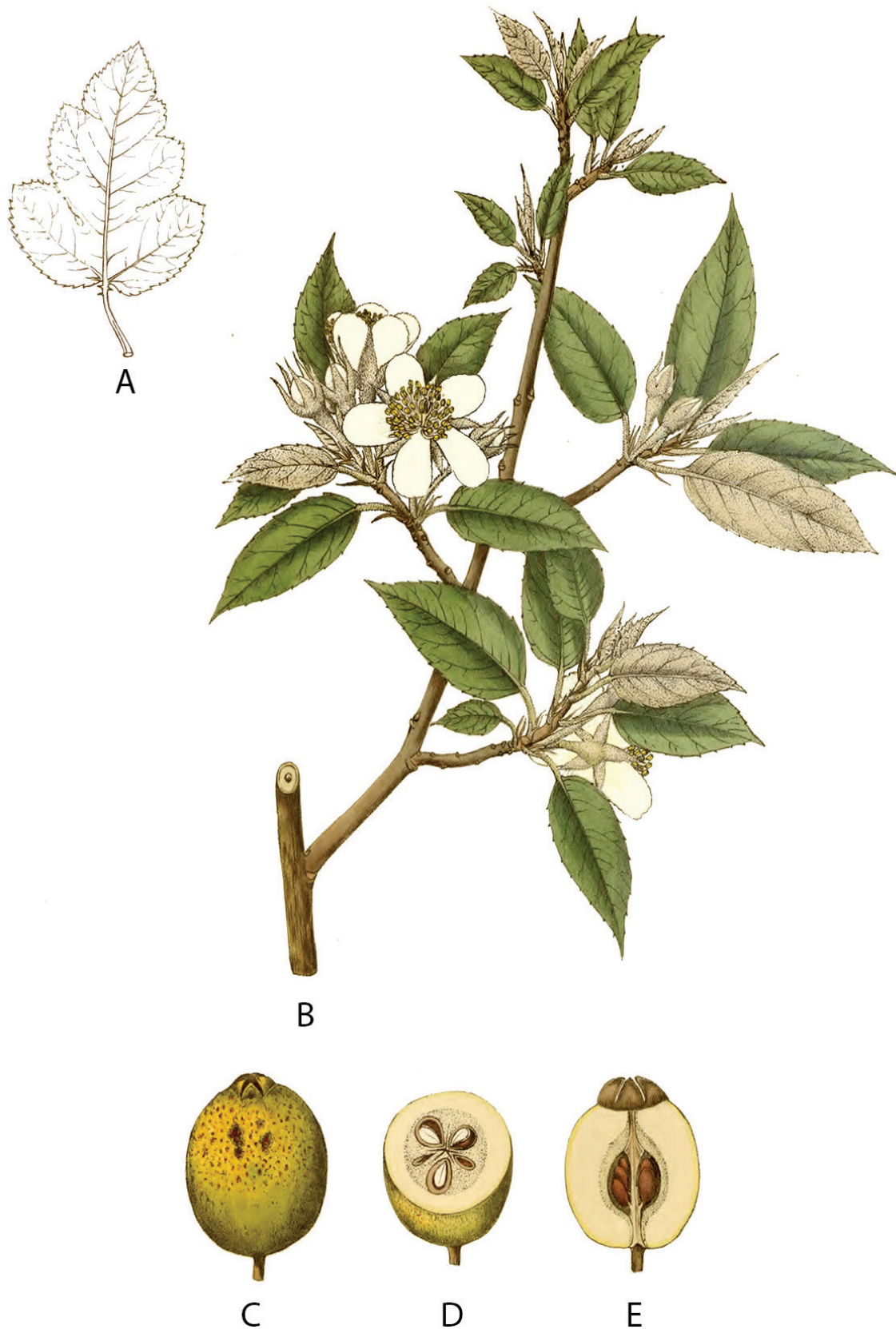
### ***Malus indica* (Wall.) B.B.Liu, comb. nov.**

urn:lsid:ipni.org:names:77322788-1

Figs 4, 5

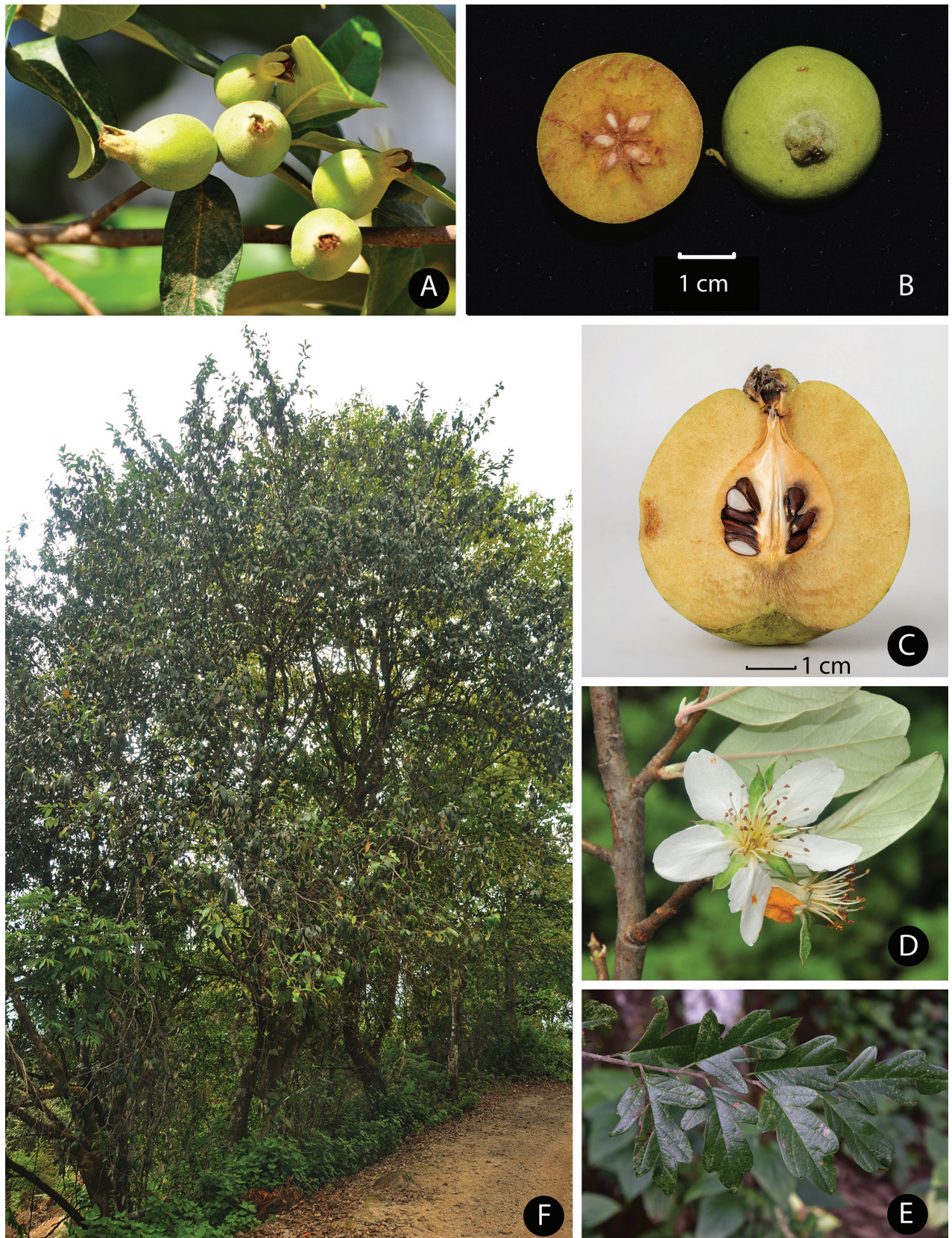
Chinese name: 多依; pinyin (spelled as it sounds): duo yi

- ≡ *Pyrus indica* Wall., Pl. Asiat. Rar. (Wallich) 2(8): 56 (1831). Type: Tab. 173 (holotype, Fig. 4). INDIA. "Khasia reg. temp. alt. 6000 pds", J.D. Hooker & T. Thomson 510 (**epitype, designated here**: M [barcode M0213698]!). Note 1. Image of the epitype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0213698>.
- ≡ *Cydonia indica* (Wall.) Spach, Hist. Nat. Vég. (Spach) 2: 158 (1834). Type: Based on *Pyrus indica* Wall.
- ≡ *Docynia indica* (Wall.) Decne., Nouv. Arch. Mus. Hist. Nat. 10: 131 (1874). Type: Based on *Pyrus indica* Wall.
- ≡ *Eriolobus indica* (Wall.) C.K.Schneid., Ill. Handb. Laubholzk. 1: 728 (1906). Type: Based on *Pyrus indica* Wall.
- = *Docynia griffithiana* Decne., Nouv. Arch. Mus. Par. 10: 131 (1874). Type: INDIA. "Himalaya oriental.", Griffith 2082 (holotype: P [barcode P01819345]!; isotypes: E [barcode E00010836]!, K, CAL [accession no. 153563]). Image of the holotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.p01819345>.
- = *Docynia indica* var. *griffithiana* (Decne.) Ghora, Bull. Bot. Surv. India 47(1–4): 150 (2005). Type: Based on *Docynia griffithiana* Decne.
- = *Docynia hookeriana* Decne., Nouv. Arch. Mus. Par. 10: 131 (1874). Type: INDIA. "Khasia, regio temp. alt. 5000 pds.", J.D. Hooker & T. Thomson 511 (holotype: P [barcode P01819346]!). Image of the holotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.p01819346>.
- = *Pyrus rufifolia* H.Lév., Bull. Géogr. Bot. 25: 46 (1915), [*Pirus*]. Type: CHINA. Yunnan: "flâne des coteaux arides à Lou-Pou, 3050 m, Juin 1912", E.E. Maire s.n. (holotype: E [barcode E00010835]!). Image of the holotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00010835>.
- = *Docynia rufifolia* (H.Lév.) Rehder, J. Arnold Arbor. 13: 310 (1932). Type: Based on *Pyrus rufifolia* H.Lév.



**Figure 4.** Holotype of *Malus indica* (redrawn from the illustration of Pl. Asiat. Rar. (Wallich 1831). 2: t. 173, 1831) **A** lobed-leaf **B** inflorescence branch **C** fruit **D** cross-section of fruit **E** longitudinal section of fruit.







- = *Malus docynioides* C.K.Schneid., Bot. Gaz. 63: 400 (1917). Type: CHINA. Sichuan: “Szechuan australis: inter Kua-pie et Ta-tiao-ko, alt. ca. 2700 m, 23 Maji 1914”, C.K. Schneider 1349 (holotype: K [barcode K000758093]!; isotype: A [barcode 00026465]!). Image of the holotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000758093>.
- = *Docynia docynioides* (C.K.Schneid.) Rehder, J. Arnold Arbor. 2(1): 58 (1920). Type: Based on *Malus docynioides* C.K.Schneid.

**Distribution.** Bhutan, China (Sichuan and Yunnan), India, Myanmar, Nepal, Pakistan, Sikkim, Thailand and Vietnam.

**Note 1.** In the protologue of *Pyrus indica*, Wallich (1831) did not designate a specimen as the holotype, but only provided an illustration, which is considered to be the holotype (Fig. 4). However, the accurate identification of this species will be significantly impeded due to the limited morphological details in the illustration compared to the specimens (Turland et al. 2018). Consequently, it becomes necessary to select a single specimen as the epitype in order to distinguish it from its closest relatives, such as *Malus delavayi* and *M. longiunguis*. Decaisne (1874) cited two specimens (*J.D. Hooker & T. Thomson 509* and *J.D. Hooker & T. Thomson 510*) while transferring this species to *Docynia* as *Docynia indica*. Therefore, herein, we select a well-preserved specimen in the herbarium M (*J.D. Hooker & T. Thomson 510*: M0213698) as the epitype.

***Malus delavayi* (Franch.) B.B.Liu, comb. nov.**

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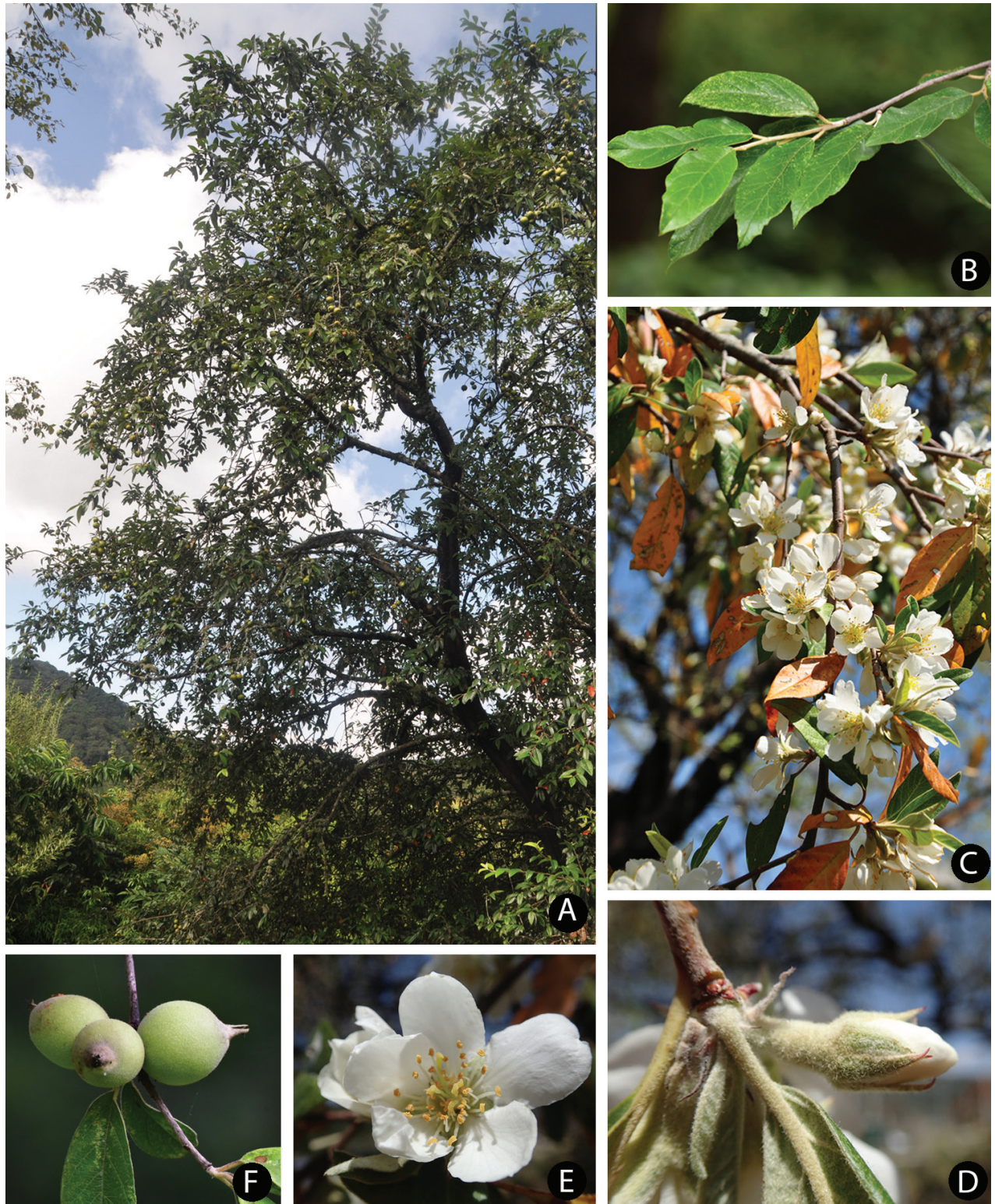
Fig. 6

Chinese name: 云南多依; pinyin (spelled as it sounds): yun nan duo yi

- ≡ *Pyrus delavayi* Franch., Pl. Delavay.: 227, t. 47 (1890), [*Pirus*]. Type: CHINA. Yunnan: “in montibus calcareis ad Mao-kou-tchang, supra Tapin-tze, prope Tali, alt. 2200 m.”, 14 April 1884, P.J.M. Delavay 466 (**lectotype, designated here**: P [barcode P01819347]!; isoelectotype: L [barcode L0019412]!); ibidem, P.J.M. Delavay 890 (syntype: K [barcode K000758091]!); in silvis ad orientem versus montis Hee-chan-tong, alt. 2500 m, 5 April 1887 (syntypes: K [barcode K000758090]!, K [barcode K000758092]!). Image of the lectotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.p01819347>.
- ≡ *Eriolobus delavayi* (Franch.) C.K.Schneid., Ill. Handb. Laubholz. 1: 727 (1906). Type: Based on *Pyrus delavayi* Franch.
- ≡ *Docynia delavayi* (Franch.) C.K.Schneid., Repert. Spec. Nov. Regni Veg. 3: 180 (1906). Type: Based on *Pyrus delavayi* Franch.
- ≡ *Cydonia delavayi* (Franch.) Cardot, Bull. Mus. Natl. Hist. Nat. 24: 63 (1918). Type: Based on *Pyrus delavayi* Franch.
- = *Cotoneaster bodinieri* H.Lév., Bull. Géogr. Bot. 25: 44 (1915). Type: CHINA. Yunnan: “montagnes près de la frontière du Kouy-Tchéou; à Kiang-Ty”, 9 April 1897, G. Bodinier s.n. (holotype: E [barcode E00010834]!; isotype: A [barcode 00026464]!). Image of the holotype available from <https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00010834>.

**Distribution.** China (Guizhou, Sichuan, and Yunnan).





**Figure 6.** *Malus delavayi* (Franch.) B.B.Liu **A** overview of tree **B** leaf branch **C** inflorescence branch **D** flower buds **E** flower **F** young fruits. Photo credits to Jian Huang.





**Figure 7.** *Malus longiunguis* (Q.Luo & J.L.Liu) B.B.Liu **A** fruit branch **B** flower **C** young fruit. Photos credit to Qiang Luo.

***Malus longiunguis* (Q.Luo & J.L.Liu) B.B.Liu, comb. nov.**

urn:lsid:ipni.org:names:77322790-1

Fig. 7

Chinese name: 长爪多依; pinyin (spelled as it sounds): chang zhua duo yi

≡ *Docynia longiunguis* Q.Luo & J.L.Liu, Bull. Bot. Res., Harbin 31(4): 389 (2011).

Type: CHINA. Sichuan: Xichang, Lushan, alt. 1860 m, 18 March 2010, Q. Luo 010304 (holotype: PE [barcode 02362758]!). Note 2.

**Distribution.** China (Sichuan).

**Note 2.** In the protologue, the holotype is indicated as being deposited in the herbarium of Xichang College (HXCH, Luo et al. 2011); however, this holotype specimen was then sent to the China National Herbarium (PE).

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**Additional information**

**Conflict of interest**

No conflict of interest was declared.

**Ethical statement**

No ethical statement was reported.

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## Author contributions

G.N.L. designed and led the project. B.B.L. and Y.W. supervised the study. G.N.L., D.K.M., and C.X. drafted the manuscript. J.H., B.J.G., and Q.L. provided the photos in this paper. All the authors approved the final manuscript.

## Author ORCIDs

Guang-Ning Liu  <https://orcid.org/0009-0009-0765-0392>

Dai-Kun Ma  <https://orcid.org/0009-0005-5523-508X>

Chao Xu  <https://orcid.org/0000-0002-9678-4772>

Jian Huang  <https://orcid.org/0000-0002-2253-4586>

Bin-Jie Ge  <https://orcid.org/0000-0002-4232-3567>

Qiang Luo  <https://orcid.org/0000-0001-5043-2539>

Yu Wei  <https://orcid.org/0000-0002-2013-4829>

Bin-Bin Liu  <https://orcid.org/0000-0002-0297-7531>

## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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# *Rosa funingensis* (Rosaceae), a new species from Yunnan, China

Ling-Na Zheng<sup>1,2,3\*</sup>, Le Luo<sup>1,2,3\*</sup>, Yu-Wei Tang<sup>1,2,3</sup>, Chao Yu<sup>1,2,3</sup>, Pei-Feng Lyu<sup>1,2,3</sup>, Xue-Sen Liu<sup>1,2,3</sup>, Qi-Xiang Zhang<sup>1,2,3</sup>, Yu-Yong Yang<sup>2,3,4,5</sup>

1 Beijing Key Laboratory of Ornamental Plants Germplasm Innovation & Molecular Breeding, Beijing Laboratory of Urban and Rural Ecological Environment, Engineering Research Center of Landscape Environment of Ministry of Education, Beijing 100083, China

2 National Engineering Research Center for Floriculture, Beijing 100083, China

3 Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants of Ministry of Education, Beijing 100083, China

4 School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China

5 Kunming Yang Chinese Rose Gardening Co., Ltd, Kunming 6500871, China

Corresponding author: Le Luo (luolebjfu@163.com)

## Abstract

A new species *Rosa funingensis* and its variant *R. funingensis* f. *rosea*, both collected from Yunnan Province, China, are, for the first time, documented and illustrated in this study. Morphological analysis in comparison with two related species in the wild, *R. gigantea* and *R. rubus*, presents distinguishable features through leaf surfaces, inflorescences and the shape of styles. *R. funingensis* leaf surfaces are abaxially villous, purple-red, pale green when mature, adaxially glabrous, dark green; inflorescences solitary or 2–5(7) in corymbose cyme; and styles connate into a column or not, exserted.

**Key words:** molecular evidence, morphology, new species, *Rosa*, wild germplasm



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## Introduction

There are about 150–200 species of roses around the world, widely distributed throughout the Northern Hemisphere, with Central and Southwest Asia being the centres of distribution of the genus (Rehder 1951; Ku and Robertson 2003; Quest-Ritson and Quest-Ritson 2003). China has 95 species of the genus *Rosa*, of which 65 species are endemic (Ku and Robertson 2003); there are also 34 varieties, totalling 129 taxa of roses (Liu and Lian 2014). The number of wild rose species in China are found to decrease gradually from the southeast to the southwest and northwest of the country (Yu and Lu 1985). Yunnan is one of the main distribution centres and differentiation centres of *Rosa* (Xu 2001), with 41 species and 17 varieties of wild *Rosa* (Chen and Li 2006).

On 5 April 2018, a unique species of *Rosa* was discovered during an investigation of wild rose resources in Funing County, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, China. It shared certain morphological characteristics with *R. gigantea* and *R. rubus*, while being distinguished in terms of leaf, inflorescence and shape of styles. Subsequently, this species was introduced to the Kunming South Tropical Garden (Kunming Nanguo Shanhua) Horticulture Technology Co. Ltd., Yunnan Province for fur-

\* These authors contributed equally to this work and should be considered co-first authors.

ther observation and study. After a thorough examination over a period of three years, it was determined that the specific morphological characteristics of this species and its variant were stable, indicating that they were, indeed, new to the *Rosa* genus. In 2021, phylogenetic relationships were analysed after collecting the specimens to confirm their status as a new species within the genus *Rosa*. As a result of this research, the new species was described and named as *Rosa funingensis* L. Luo & Y. Y. Yang. Additionally, a form of this new species was identified and documented as *Rosa funingensis* L. Luo & Y. Y. Yang f. *rosea* L. Luo & Y. Y. Yang, characterised by its light salmon-pink flowers that fade to white.

## Materials and methods

### Field observations, comparative morphology

We studied living plants of the new species in their natural habitats and documented their known distribution ranges. Morphological descriptions and illustrations were based on mature foliage, fresh flowering material and mature fruit of living plants and dried specimens of *R. funingensis* and *R. funingensis* f. *rosea*.

### Phylogenetic analysis

Sixteen taxa of the genus *Rosa*, including *Rosa funingensis* and two outgroups (*Fragaria vesca* and *Potentilla tanacetifolia*) were used to reconstruct a phylogenetic tree. Sequences of *R. Chinensis* 'Old Blush' (sequence number: SRR6175515), *Fragaria vesca* (sequence number: SRR12536045) and *Potentilla tanacetifolia* (sequence number: SRR8208352) were downloaded from GenBank. The other 14 taxa were selected from six sections. Their complete genomic DNA was extracted from silica-gel-dried leaves using the CTAB method (Porebski et al. 1997) and sequenced using Illumina NovaSeq.

The sequenced data were quality-controlled to obtain clean data. Genome alignment was performed using MINIMAP2 v.2.21. PCR de-duplication and SNP detection were performed using gatk v.4.2.0.0. SNP annotation was performed using ANNOVAR v.2020-06-07. The processed SNP data were analysed in VCFTOOLS v.0.1.17 for *Fst* analysis (*fst-window-size* 100000 *fst-window-step* 10000), *theta $\pi$*  analysis (*window-pi* 100000 *window-pi-step* 10000) and Tajima's *D* analysis (*TajimaD* 100000). The resulting data were analysed in MEGA11 (Tamura et al. 2021). The evolutionary history was inferred by using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-9601.46) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. There were a total of 3560 positions in the final dataset.



## Results

### Phylogenetic analysis

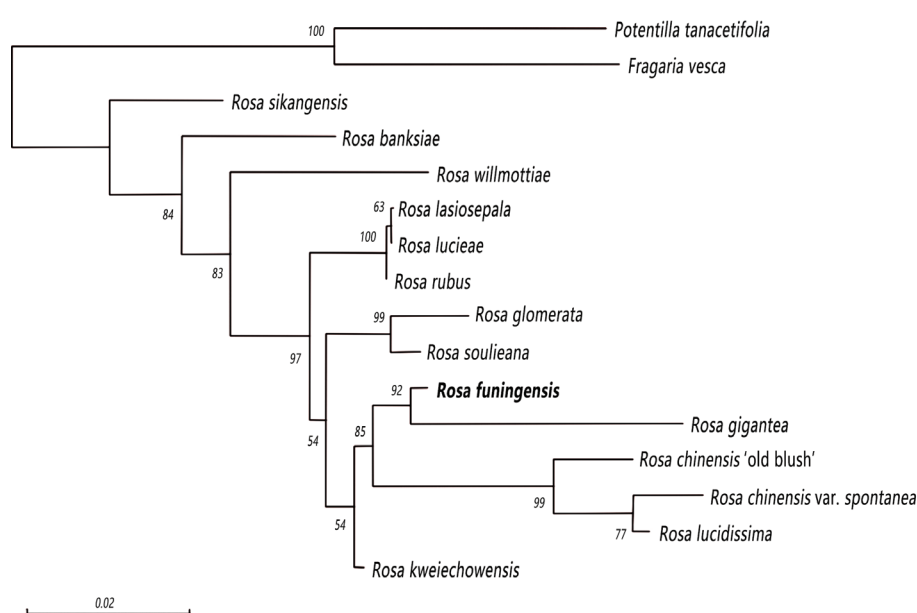
The ML phylogenetic tree (Fig. 1) showed that *Rosa glomerata*, *R. soulieana* (sect. *synstylae*) and *R. kweichowensis* (sect. *microphyllae*) and all the species of sect. *chinenses* formed a well-supported clade (purple), with the sister group of *R. lasiosepala*, *R. luciae* and *R. rubus* of sect. *synstylae* (orange). The putative new species, *R. funingensis*, is placed into a well-supported clade with *R. gigantea* (sect. *chinenses*) and formed a larger clade with sect. *chinenses*.

### Discussion

*Rosa funingensis* is often in association with *R. rubus* and *R. gigantea* in the wild where there are no other members of the genus *Rosa* present. The overlap of the flowering period of *R. rubus* (late March to late April) and *R. gigantea* (March) generates the possibility of natural hybridisation to produce offspring.

Morphologically, *Rosa funingensis* shares similarities with both *R. rubus* and *R. gigantea*, but it is not exactly the same as either one, which provides further evidence that *R. funingensis* may be a natural hybrid. The molecular evolutionary tree also supports this inference.

The discovery of this new species enriches the resources of *Rosa* and provides new materials for interspecific hybridisation. Hybridisation between sections has been a great challenge in rose breeding, making wild *Rosa* resources not sufficiently exploited (Zhao et al. 2015). The hybridised experiment during 2020–2022 between *R. funingensis* and the wild species of sect. *chinenses* shows that it is, indeed, a good breeding material. The inclusion of *R. funingensis* as a new germplasm resource for breeding between sect. *chinenses* and



**Figure 1.** The Maximum Likelihood tree, based on SNPs data. Numbers above branches are ML bootstraps. Grey represents the outgroup, light blue represents Sect. *Pimpinellifoliae*, green represents Sect. *Banksianae* and yellow represents Sect. *Cinnamomeae*. Orange represents Sect. *Synstylae*, purple represents Sect. *Chinenses* and dark blue represents Sect. *Microphyllae*. The new species is shown in bold.

sect. *synstylae* has the potential to enhance the genetic diversity and improve the breeding outcomes of the genus *Rosa*.

Additionally, during our field research, we also found plants that are similar to *R. funingensis*, but with smaller leaflets (7–9); stipule margin covered with sparsely glandular hairs; flowers showing light salmon-pink at the beginning and turning white at the later stage; hip obovoid. We speculate that these plants may be a form of *R. funingensis*, with an increased number of leaflets and this is currently under observation.

### Taxonomic treatment

***Rosa funingensis* L. Luo & Y.Y. Yang, sp. nov.**

urn:lsid:ipni.org:names:77322791-1

Figs 2–4

**Type.** CHINA, MUYANG TOWN, FUNING COUNTY, WENSHAN ZHUANG AND MIAO AUTONOMOUS PREFECTURE, YUNNAN PROVINCE, 23°25'27"N, 105°21'15"E, 1396 m a.s.l., 31 March 2021, Y. Y. Yang (Holotype BJFC00107680!).

**Diagnosis.** *Rosa funingensis* is mostly similar to *R. gigantea*. However, *Rosa funingensis* differs significantly from *R. gigantea* by having leaves abaxially villous, purple-red, pale green when mature, adaxially glabrous, dark green (vs. both surfaces glabrous), rachis and petiole shortly prickly, glandular hairs and villous (vs. sparsely shortly prickly and glandular pubescent), inflorescences solitary or 3–5(7) in corymbose cyme (vs. solitary or 2 or 3 and fasciculate) and styles connate into a column or not (vs. free) (Table 1).

**Description.** *Rosa funingensis*: Shrubs climbing, new branches 5–6 m long. **Branchlets** green, young stems purple-red on sunny side, glabrate; prickles scattered, slightly curved, robust, flat, gradually tapering to broad base. Leaflets including petiole 12–14 cm; stipules mostly adnate to petiole, free parts lanceolate, villous or with short dentate glands at margin, apex acuminate, dry and shrinking when old; rachis and petiole shortly prickly, glandular hairs and villous hairs. **Leaves** usually 5–7, often 3 near inflorescence, leaflets obovate or oblong, 3–4 × 2–2.5 cm, apex acuminate, leaves leathery, adaxially glabrous, dark green, abaxially villous, purple-red, pale green when mature, margin with sharp single serrations. **Inflorescences** solitary or several in cyme; peduncle with pedicels 1–2 cm, densely glandular hairs; bracts linear, apex acuminate, 1 × 0.3 cm, margin glandular hairy, with prominent mid-vein. **Flowers** 7–9 cm in diam.; sepals 5, ovate-lanceolate, abaxially glandular-pubescent, apically caudate, adaxially villous, margin glandular hairs, occasionally linearly divided, reflexed; petals 5, single, white, nearly cordate, apex emarginate, with strong sweet fragrance. Styles connate into a column or not, exserted, light red and the stigma is light yellow. **Hips** yellow, subglobose, 1.2–1.5 cm in diam., glabrous.  $2n = 14$ .

**Phenology.** Flowering in early April, fruiting from July to October.

**Etymology.** The species epithet refers to Funing County, where the new species was first discovered. The variant with pink flower colour is proposed to be named "*Rosa funingensis* f. *rosea*".

**Distribution and habitat.** New species are currently known from Funing County, Wenshan Zhuang and Miao Autonomous Prefecture in eastern Yunnan, at elevations between 400 m and 1400 m. They grow on hillsides, roadsides and riversides.

**Table 1.** Morphological comparisons of *Rosa funingensis*, *R. gigantea* and *R. rubus*.

	<i>R. funingensis</i>	<i>R. gigantea</i>	<i>R. rubus</i>
Leaflet number	5–7	5–9	3–5
Branch	glabrous	glabrous	pubescent when young, glabrous when old
Leaf surface	abaxially pubescent, adaxially glabrous	both surfaces glabrous	abaxially pubescent or glandular, adaxially usually glabrous, rarely pubescent
Rachis and petiole	shortly prickly, glandular hairy and pubescent	sparsely shortly prickly and glandular	pubescent with sparse small curved prickles
Stipule margin	pubescent and glandular	glabrous, or glandular only at free parts	pubescent and glandular
Inflorescence	solitary or 2–5(7) in cyme	solitary	10–25 in cyme
Pedice	glandular	glabrous or glandular	pubescent and glandular
Flower size (diameter)	7–9 cm	8–9 cm	4–5 cm
Styles	connate into a column or not	free	connate into a column
Hip colour and size (diameter)	yellow, 1.2–1.5 cm	yellow, 2.5–2.8 cm	red, 1.0–1.5 cm



**Figure 2.** Specimens of *Rosa funingensis* and *R. funingensis* f. *rosea* **A** *R. funingensis* **B** *R. funingensis* var. *rosea*.

**Conservation status.** Based on currently available data, the newly-described *Rosa funingensis* species and its variants should be assigned to the ‘Data Deficient’ (DD) category of IUCN (2022). The precise conservation status of the population(s) has not been determined. Further explorations are needed to assess its distribution and conservation status. The known distribution of this species is limited. The type locality of this new species is an unprotected mountainous area. Increasing human activities and habitat destruction may cause a threat to the existence of this rare species.





**Figure 3.** *Rosa funingensis* and *R. funingensis* f. *rosea* **A** plant **B** leaves **C** stipule **D** branches and prickles **E**, **F** two different Inflorescences **G**, **H** two different styles **I–K** flowering process of *R. funingensis* **L–N** flowering process of *R. funingensis* f. *rosea* **O**, **P** hips.

***Rosa funingensis* L. Luo & Y.Y. Yang f. *rosea* L. Luo & Y.Y. Yang, f. nov.**

urn:lsid:ipni.org:names:77322792-1

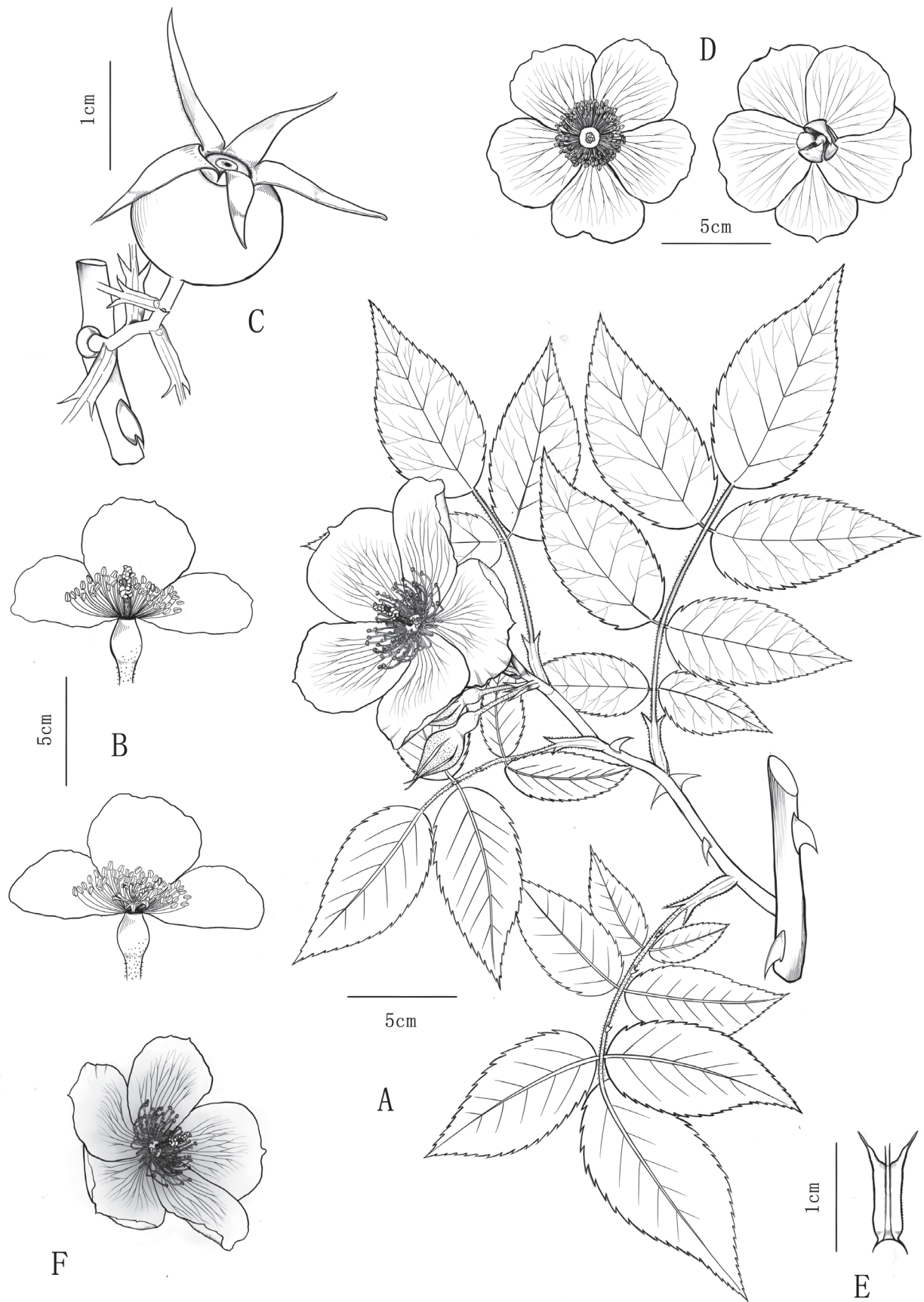
Figs 2–4

**Type.** CHINA, Muyang Town, Funing County, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, 1396 m a.s.l., 23°25'27"N, 105°21'15"E, 31 March 2021, Y. Y. Yang (Holotype BJFC00107675!)

**Description.** *Rosa funingensis* f. *rosea*: Flowers light salmon-pink and fading to white.

**Etymology.** The variant with pink flower colour is proposed to be named "*Rosa funingensis* f. *rosea*".





**Figure 4.** Illustration of *Rosa funingensis* and *Rosa funingensis* f. *rosea* **A** whole plant **B** floral anatomy **C** hip **D** flowers of *R. funingensis* **E** stipule **F** flowers of *R. funingensis* f. *rosea*. Drawn by Y. W. Tang.

### Identification key to taxa in *R. sect. Chinenses* and *Rosa rubus*

- 1 Styles connate into a column; sepals pinnately lobed; mature hips red..... *R. rubus*
- Styles free or connate into a column; sepals often entire, occasionally pinnately lobed; mature hips yellow .....2
- 2 Young stems glabrous, leaflets 5–7–(9); flowers always open wide and flat and are floppy in full bloom; hips globose or depressed-globose .....3
- Young stems pubescent or glabrous; leaflets 3 – 5 – (7); flowers hardly completely open, often high-centred (bowl-shaped) in full bloom; hips ovoid, obovoid or globose.....4
- 3 Leaves glabrous; stipule margin glabrous, or only glandular at free parts; flowers solitary; styles free ..... *R. gigantea*
- Leaves abaxially pubescent; stipule margin pubescent and glandular; flowers solitary or 2–5(7) in cyme; styles connate into a column or not .... *R. funingensis*
- 4 Young stems pubescent; leaflets 3–(5) ..... *R. lucidissima*
- Young stems glabrous; leaflets (3)–5–(7) ..... *R. chinensis* var. *spontanea*

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### Additional information

#### Conflict of interest

The authors have declared that no competing interests exist.

#### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: LL. Data curation: LNZ. Funding acquisition: LL, QXZ. Investigation: XSL, YYY, PFL. Methodology: LNZ. Project administration: LL. Resources: LL. Supervision: CY. Visualization: YWT, LNZ. Writing - original draft: LNZ. Writing - review and editing: PFL.

### Author ORCIDs

Ling-Na Zheng  <https://orcid.org/0000-0002-3850-5413>

Le Luo  <https://orcid.org/0000-0002-8143-1931>

Yu-Wei Tang  <https://orcid.org/0000-0001-9722-2299>

Chao Yu  <https://orcid.org/0000-0002-8096-5333>

Pei-Feng Lyu  <https://orcid.org/0000-0001-9969-5356>

Xue-Sen Liu  <https://orcid.org/0000-0002-0240-2551>

Qi-Xiang Zhang  <https://orcid.org/0000-0001-5690-0065>

Yu-Yong Yang  <https://orcid.org/0000-0002-4134-6847>

## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Comparisons of *R. funingensis*, *R. gigantea*, and *R. rubus*

Authors: Ling-Na Zheng

Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.229.101052.suppl1>



## *Rosa davurica* var. *rubro-stipulata* (Rosaceae), the correct name for *R. davurica* var. *alpestris*

Young-Soo Kim<sup>1</sup>, Dong Chan Son<sup>1</sup>

<sup>1</sup> Division of Forest Biodiversity and Herbarium, Korea National Arboretum, Pocheon 11186, Republic of Korea

Corresponding author: Dong Chan Son ([sdclym@korea.kr](mailto:sdclym@korea.kr))

### Abstract

The name *Rosa davurica* var. *alpestris* (Nakai) Kitag. was published in 1979 as a new combination based on *R. rubro-stipullata* var. *alpestris* Nakai. It is generally accepted as a deciduous shrub occurring in Russia, Manchuria, Japan, and the northern part of the Korean Peninsula and is distinguished by the presence of eglandular leaves. *Rosa rubro-stipullata* var. *alpestris* was originally described as a new variety with a leaf size relatively smaller than that of *R. rubro-stipullata* var. *rubro-stipullata*. However, the observation of various specimens showed the leaf size of var. *alpestris* to be of minor importance, and it was included in var. *rubro-stipullata* as a synonym. Due to the priority of autonyms, a new combination is required to replace *R. davurica* var. *alpestris*. Additionally, it should be noted that the epithet “*rubro-stipullata*” is derived from the Latin word “*stipula*” rather than “*stipulla*.” Therefore, for this variety, we propose a new combination, *R. davurica* var. *rubro-stipulata* (Nakai) D. C. Son & Y. S. Kim, **comb. nov. & stat. nov.**

**Key words:** autonym, nomenclature, priority, Shenzhen Code



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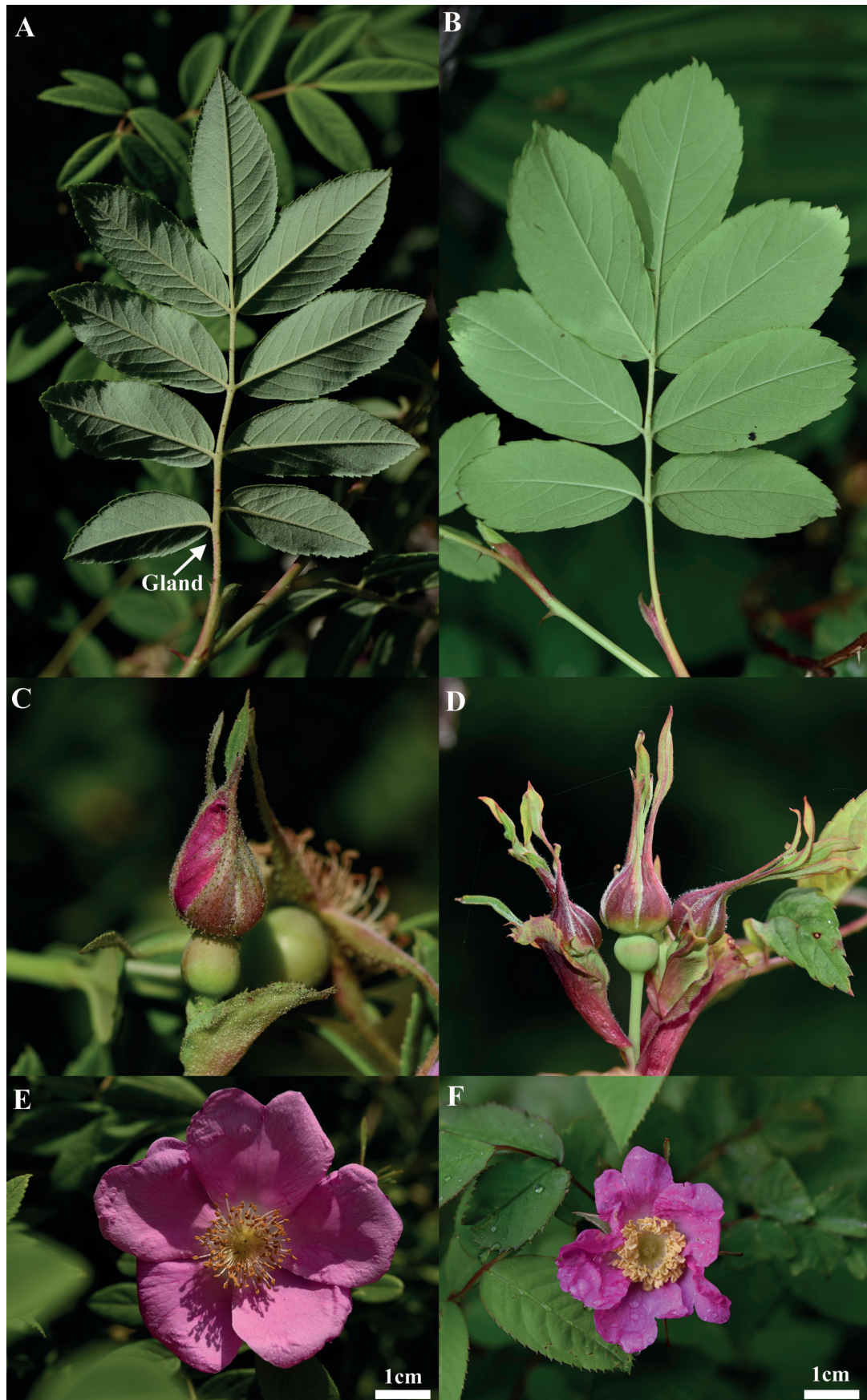
Citation: Kim Y-S, Son DC (2023)

*Rosa davurica* var. *rubro-stipulata* (Rosaceae), the correct name for *R. davurica* var. *alpestris*. PhytoKeys 229: 71–76. <https://doi.org/10.3897/phytokeys.229.105786>

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*Rosa davurica* var. *alpestris* (Nakai) Kitag. is a deciduous shrub distributed through Russia, Manchuria, Japan, and the northern part of the Korean Peninsula. *Rosa davurica* Pall. is remarkable because of the variable shape of its leaflets, from narrowly to broadly elliptic, and the absence or presence of glands on their lower surface. The varietal name is commonly applied to plants of *R. davurica* with eglandular leaflets (Kitagawa 1979; Lee 2003; Ohba 2001). Further morphological observations showed that var. *alpestris* is readily distinguished from var. *davurica* by the presence of eglandular rachis and petiole, abaxial surface of calyx lobe sparsely glandular or eglandular, and flower 2~3 cm in diam. (Fig. 1; Table 1).

*Rosa davurica* var. *alpestris* (Nakai) Kitag. was published in 1979, as a new combination based on *R. rubro-stipullata* var. *alpestris* Nakai. *Rosa rubro-stipullata* var. *alpestris* Nakai was originally described as a new variety with a leaf size relatively smaller than that of *R. rubro-stipullata* var. *rubro-stipullata* (Nakai 1916). However, based on several specimens, including type specimens of var. *alpestris* and var. *rubro-stipullata*, we observed that although the leaf size of var. *alpestris* was smaller than that of var. *rubro-stipullata*, this character does not correlate with any other morphological trait or geographical feature, and it



**Figure 1.** Morphological characters distinguishing *R. davurica* (A, C, E) and *R. davurica* var. *alpestris* (B, D, F) A, B leaves C, D calyx lobe E, F flower. Photo Credits: Dong-Hyuk Lee.

**Table 1.** Morphological differences among *Rosa davurica* and *R. davurica* var. *alpestris*.

	Characters	<i>R. davurica</i>	<i>R. davurica</i> var. <i>alpestris</i>
Leaflet	Presence of gland on abaxial surface	Glandular	Eglandulose
Rachis	Presence of gland on surface	Glandular	Eglandulose
Petiole	Presence of gland on surface	Glandular	Eglandulose
Calyx lobe	Density of hair on abaxial surface	Densely glandular	Sparsely glandular or eglandulose
Flower	Diameter (cm)	4~5	2~3

is not taxonomically worthy of being recognized as a variety. Therefore, it is reasonable to regard *R. rubro-stipullata* var. *alpestris* as a synonym of *R. rubro-stipullata* var. *rubro-stipullata*. In practice, *R. rubro-stipullata* var. *rubro-stipullata* has been treated as a synonym of *R. davurica* var. *alpestris* in the literature (Kitagawa 1979; Ohba 2001; Chang et al. 2014; Korea National Arboretum 2020; POWO 2023; WFO 2023).

According to the rules of the ICN (Turland et al. 2018), “An autonym is treated as having priority over the name(s) of the same date and rank that upon their valid publication established the autonym,” and a new combination is required to replace *R. davurica* var. *alpestris* because of the priority of the autonym (see ICN Article 11.6 Ex. 28). Meanwhile, the epithet “*rubro-stipullata*” is derived from the Latin word “*stipula*” rather than “*stipulla*”, hence it should be corrected to “*rubro-stipulata*” (see ICN Article 60.1). Therefore, for this variety, we propose a new combination, *Rosa davurica* var. *rubro-stipulata* (Nakai) D. C. Son & Y. S. Kim.

## Taxonomic treatment

***Rosa davurica* var. *rubro-stipulata* (Nakai) D. C. Son & Y. S. Kim, comb. nov. & stat. nov.**

urn:lsid:ipni.org:names:77322794-1

*Rosa rubro-stipulata* Nakai, Bot. Mag. (Tokyo) 30: 242 (1916). Basionym.

**Type.** KOREA. Chagang-do: 牙得嶺 (江界側) [Adeuk-ryeong (Ganggye)], July 5, 1914, T. Nakai 1824 (lectotype, designated by Momiyama and Ohba (1988: 10): TI00022345, photo!); KOREA Hamgyongnam-do: 牙得嶺 (長津側) [Adeuk-ryeong (Chang-jyu)], July 6, 1914, T. Nakai 1820 (syntype: TI00022346, photo!). Fig. 2.

= *Rosa rubro-stipulata* var. *alpestris* Nakai, Bot. Mag. (Tokyo) 30: 242 (1916); *Rosa marretii* var. *alpestris* (Nakai) Uyeki, Woody Pl. Distr. Chosen: 51 (1940); *Rosa davurica* var. *alpestris* (Nakai) Kitag., Neolin. Fl. Manshur. 382 (1979). **Type.** KOREA. Hamgyeongbuk-do: 長白山 (Baekdusan), August 1913, T. Mori 77 (lectotype, designated by Momiyama and Ohba (1988: 11): TI00022341, photo!); KOREA. Hamgyeongbuk-do: 長白山 (Baekdusan), August 1913, T. Mori 114 (syntype: TI00022342, photo!); KOREA. Ryanggang-do: 崔哥嶺 (Choiga-ryeong), August 1913, T. Mori 206 (syntype: TI00022343, photo!); KOREA. Ryanggang-do: 神武城 – 無頭峯 (Shinmusung – Mudubong), August 8, 1914, T. Nakai 1816 (syntype: TI00022344, photo!). Fig. 3.





Figure 2. Type specimens of *Rosa rubro-stipulata* var. *rubro-stipulata* A lectotype (T100022345) B syntype (T100022346).





Figure 3. Type specimens of *Rosa rubro-stipulata* var. *alpestris* A lectotype (TI00022341) B–D syntype (TI00022342, TI00022343, TI00022344).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

All authors contributed to this work.

### Author ORCIDs

Young-Soo Kim  <https://orcid.org/0000-0002-8524-2441>

Dong Chan Son  <https://orcid.org/0000-0002-6773-0580>







### Data availability

All of the data that support the findings of this study are available in the main text.

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# Taxonomic and ecological remarks on *Solenopsis bivonae* species complex (Campanulaceae)

Salvatore Brullo<sup>1</sup>, Cristian Brullo<sup>1</sup>, Salvatore Cambria<sup>1</sup>, Valeria Tomaselli<sup>2</sup>, Alessandro Crisafulli<sup>3</sup>, Giuseppe Siracusa<sup>1</sup>, Pietro Minissale<sup>1</sup>, Gianpietro Giusso del Galdo<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Catania, via A. Longo 19, Catania, Italy

<sup>2</sup> Dipartimento di Biologia, Università di Bari Aldo Moro, via Orabona 4, 70125 Bari, Italy

<sup>3</sup> Dipartimento ChiBioFarAm, Università degli Studi di Messina, Via Stagno d'Alcontres, 98100 Messina, Italy

Corresponding author: Salvatore Brullo ([salvo.brullo@gmail.com](mailto:salvo.brullo@gmail.com))

## Abstract

The populations usually attributed to *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan are investigated from a taxonomical and morphological viewpoint. Within this species complex, four new subspecies occurring in Sicily and Calabria are recognized, such as subsp. *bivonae*, subsp. *madoniarum*, subsp. *peloritana* and subsp. *brutia*. In addition, a new species from Cyprus described as *S. meikleana* and *S. bacchettiae* from Sardinia must be included in this group. The synonymy, typification, description, seed testa morphology, chorology, ecology, illustrations, conservation status, and examined specimens for each taxon are provided. Besides, the analytical keys, distribution maps, and phytosociological arrangement regarding these taxa are given too.

**Key words:** ecology, Lobelioideae, Mediterranean flora, *Solenopsis*, taxonomy



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## Introduction

*Solenopsis* C.Presl is a very peculiar genus of Campanulaceae, belonging to subfam. Lobelioideae, distributed in the Mediterranean and Macaronesian territories. Within this genus, two well-distinct groups can be recognized, which differ in habit and in flower structure (Crespo et al. 1998; Brullo et al. 2023a, b). The first one is characterized by a caulescent or subcaulescent habit with leaves all inserted on the scape and flowers with corolla provided by lobes slightly divaricated at the top. Conversely, the second one shows a stemless habit with leaves arranged in basal rosette and flowers with corolla provided by lobes markedly patent at the top. The only exception is represented by a species showing intermediate characters between the two groups since it has an erect scapose habit and flower corolla with clearly divaricated lobes at the top. The first group includes only annual species, such as *Solenopsis laurentia* C.Presl, widespread in the Mediterranean area and the Canary Islands, represented by several subspecies examined by Brullo et al. (2023a), to which *S. mothiana* C.Brullo, Brullo & Giusso, showing a punctiform distribution in Sicily (Isola Grande dello Stagnone), must be added. According to Brullo et al. (2023b), the second group includes many more species, such as

*S. bivonae* (Tineo) M.B. Crespo et al. from Sicily and South Italy, and recorded also from Cyprus, *S. bacchettae* Brullo et al. from Sardinia, *S. minuta* C. Presl from Crete, *S. balearica* (E. Wimm.) Aldasoro et al. from Majorca, *S. corsica* (Meikle) M.B. Crespo et al. from Corse and N. Sardinia and *S. antiphonitis* Hadjik. & Hand from N. Cyprus. Recently, *S. minuta* has been observed in Cyprus by Christodoulou et al. (2020). Besides, *S. bicolor* (Batt.) Greuter & Burdet must be mentioned, occurring in Tunisia and Algeria, which is characterized by intermediate features between the two groups (Crespo et al. 1998). In the frame of taxonomic research on this genus, the populations currently attributed to *S. bivonae* (Tineo) M.B. Crespo, Serra & Juan are here investigated. In particular, this study of living material from several Mediterranean localities (Sicily, South Italy, Sardinia and Cyprus), and cultivated plants in the Botanical Garden of Catania emphasized the close morphological relationships among them. These investigations show that *S. bivonae* must be considered a species-complex, within which several morphologically well-distinct taxa can be identified. In order to verify the realistic distribution of these taxa, several herbarium materials were examined from all localities where the populations of this species were previously recorded. In particular, according to literature data (Pignatti 1982; Crespo et al. 1998; Brullo and Guarino 2018; Cambria et al. 2019), *S. bivonae* s.l. occurs in a scattered way in various Mediterranean territories, such as Sicily, South Italy, Sardinia, and Cyprus. As concerns the Sardinian populations, previously attributed to *S. bivonae* (Crespo et al. 1998), they have been described by Brullo et al. (2023b) as *S. bacchettae*, species well differentiated from the populations growing in Sicily, where occur *S. bivonae* s.s., described by Tineo (1827) as *Laurentia bivonae* on material collected along the Oreto River near Palermo, which represents its *locus classicus*. In particular, *S. bacchettae* differs from *S. bivonae* s.s. apart from some relevant features (hairy leaves, larger flowers, different coloured corolla, ultrastructure of pollen grains, and testa seed), also from the ecological point of view. In fact, *S. bacchettae* occurs prevalently along the small streams with flowing waters, while *S. bivonae* s.s. is localized on dripping walls or peat bogs. As regards the other populations of *S. bivonae*, significant morphological differences were observed in the individuals occurring in Sicily, South Italy and Cyprus, which allow for them to be treated as distinct taxa. Based on literature and herbarium data, *S. bivonae* in Sicily was recorded in many more stands than where it occurs today. Effectively, the populations of this species are linked to wet and very specialized natural habitats (dripping walls), many of which have now completely disappeared due to anthropic pressure. Currently, as proved by personal surveys, this species is still present in the *locus classicus*, where it has now become extremely rare due to pollution factors, as well as in other Sicilian stands. Other small populations of this species occur also along the Sosio river (Chiusa Sclafani), Ficuzza and in various localities of the Madonie massif, confirming previous literature data (Bivona-Bernardi 1806; Tineo 1827; Gussone 1843; Strobl 1883; Lojacono Pojero 1903; Marcenò et al. 1985; Gianguzzi et al. 2004; Giardina et al. 2007). Recently, a new population was observed by Cambria et al. (2020) near Vallone Pirtuso (Peloritani Mountains), as well as at Monte Canalotto near Piazza Armerina (unpublished record). As concerns the Sicilian populations, three taxa treated as distinct subspecies can be distinguished. Significant morphological features allow to differentiate these taxa, which show a well circumscribed dis-



tribution and peculiar ecological requirements. In particular, the populations of type subspecies (subsp. *bivonae*) occur at low altitudes, from sea level up to ca. 250 m a.s.l. (Oreto and Sosio rivers), while a second new subspecies (subsp. *madoniarum*) is widespread in the Madonie massif and in a small isolated stand near Piazza Armerina, where it grows at an altitude of 700–1600 m a.s.l. Finally, the third new subspecies (subsp. *peloritana*) is localized in a punctiform mountain locality of the Peloritani range at an elevation of 600–700 m a.s.l. The only continental populations of *S. bivonae* s.l. occur in North Calabria (S Italy), where Longo (1893, 1902) collected it along the banks of the Lao River near Laino Castello and Laino Borgo, while later Peruzzi and Gargano (2003) recorded it always along the Lao River, but below the village of Papasidero. From the taxonomical point of view, the Calabrian plants are clearly distinct from the other subspecies occurring in Sicily. Therefore, they are treated as a new subspecies of *S. bivonae* (subsp. *brutia*). Regarding the Cyprus populations, they were attributed by several authors (Poech 1842; Kotschy 1862; Unger and Kotschy 1865; Boissier 1875; Holmboe 1914) to *Laurentia tenella* DC., while Lindberg (1946) and Osorio-Tafall and Seraphim (1973) referred them to *Laurentia minuta* (L.) DC. Finally, these populations were identified by Crespo et al. (1998) and Christodoulou et al. (2020) as *Solenopsis bivonae*. Wimmer (1948), in his revision of Lebelioideae, recognized three sections within the genus *Laurentia* Adans., and in particular he included the taxa previously attributed to the genus *Solenopsis* in the sect. *Solenopsis* (C.Presl) Hendl. Besides, this author referred the Cyprus populations to *Laurentia minuta* (L.) DC. f. *nobilis*, quoting this taxon also from Palermo in Sicily, without the indication of the nomenclatural type. Afterward, Meikle (1979) used the Wimmer's name, transferring this taxon to the genus *Solenopsis* and treating it as a subspecies, proposing the new combination *S. minuta* subsp. *nobilis* (F.E.Wimmer) Meikle, designating as lectotype a specimen collected by Kotschy in Cyprus (W). Moreover, Meikle (1979, 1985) mentioned this taxon apart from Cyprus, also in Sicily, emphasizing, however, that the Sicilian populations are quite variable, while its presence in Sardinia remains doubtful. Based on our morphological investigations, the populations of Cyprus are clearly differentiated from those present in Sicily and Calabria and can be attributed to a distinct species, described as *S. meikleana*, which is usually linked to dripping wet walls or river banks.

## Material and methods

The morphological investigations were conducted on wild plants collected in several Mediterranean territories (Sicily, South Italy, Sardinia, and Cyprus) and cultivated in the Botanical Garden of Catania (Italy). The morphological features were analyzed based on at least twenty individuals for each examined population, with well-developed vegetative and reproductive structures. The living material was observed under a Zeiss Stemi SV 11 Apo stereomicroscope at 6–66× magnification, provided with a drawing device. Electron micrographs (SEM) were obtained under a Zeiss EVOL LS10 scanning electron microscope at an accelerating voltage of 10 kV; ten seeds were directly mounted onto aluminum stubs with double adhesive tape and coated with gold prior to observation. The seed surface sculpturing terminology mainly followed Barthlott (1981, 1984) and Gontcharova et al. (2009). The vouchers are deposited in the Herbarium of the

University of Catania (CAT). The herbarium codes are according to Thiers (2020). Phytosociological investigations were carried out using the method of the Sigmatis school (Braun-Blanquet 1928), while for the syntaxonomical arrangement, Mucina et al. (2016) was followed. The conservation status of the species was calculated with GeoCAT (Geospatial Conservation Assessment Tool) software (Bachman et al. 2011) and according to IUCN guidelines (IUCN 2022).

## Taxonomic treatment

### 1. *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan, Pl. Syst. Evol. 210(3–4): 219. 1998.

- ≡ *Lobelia bivonae* Tineo, Cat. Pl. Hort. Reg. Panorm.: 279, 1827.
- ≡ *Laurentia bivonae* (Tineo) Pignatti, Giorn. Bot. Ital. 111:54, 1977.
- ≡ *Lobelia tenella* Biv., Sic. Pl. Cent. I: 53. 1806, non L., Mantissa Alt.: 120, 1771.
- ≡ *Laurentia tenella* A. DC., Prodr. 7(2): 410, 1839, p.p.
- ≡ *Solenopsis laurentia* subsp. *tenella* (A. DC.) O. Bolòs et al., Fl. Manual Paisos Catalans: 1215. 1990, p.p.
- ≡ *Laurentia gasparrinii* (Tineo) Strobl subsp. *tenella* (A. DC.) O. Bolòs & Vigo, Collect. Bot. (Barcelona) 14:102, 1983, p.p.
- ≡ *Solenopsis bivonaeana* C.Presl, Prodr. Mon. Lobel.: 32. 1836, p.p.

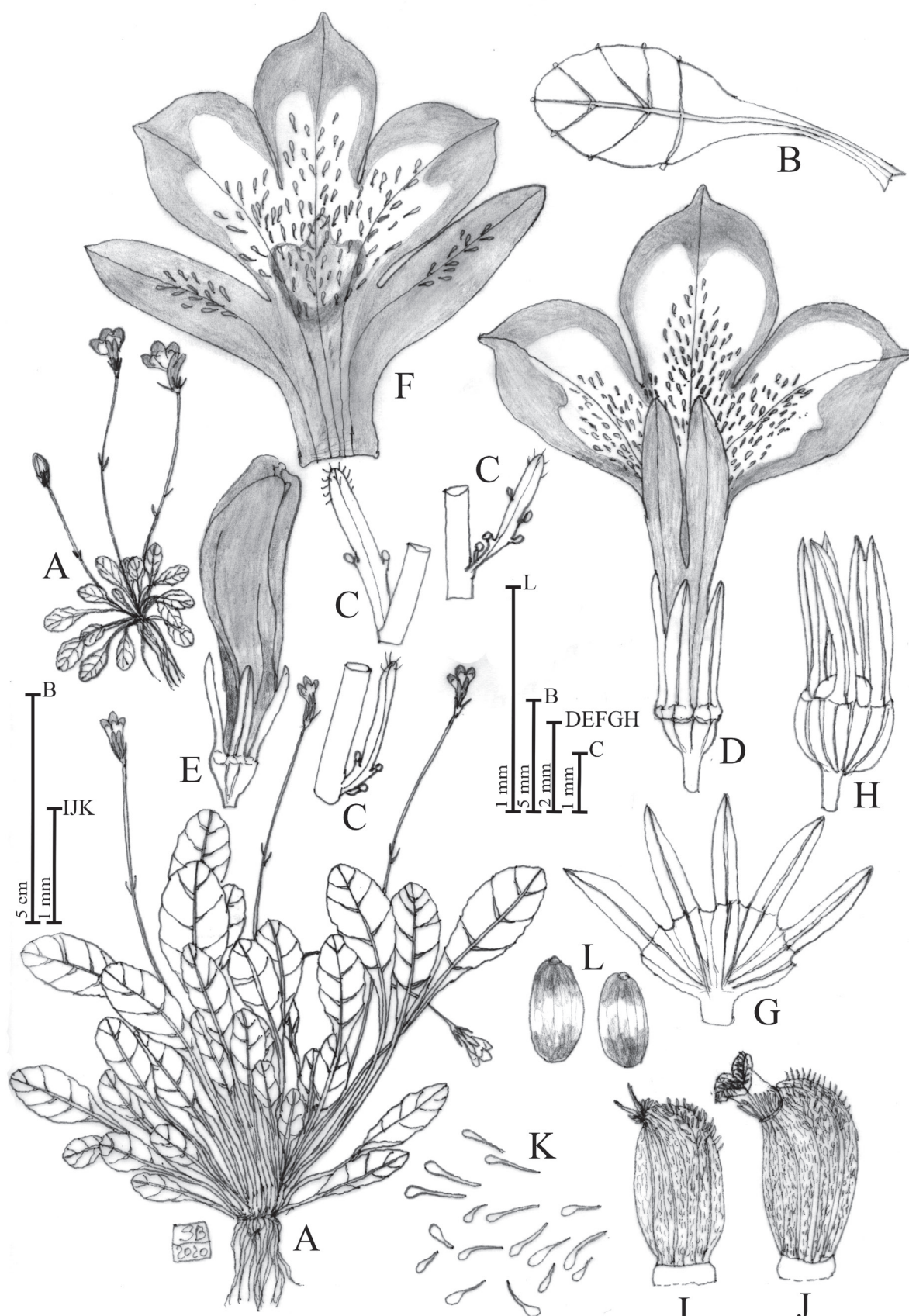
**Type.** *Lobelia* 33\* *tenella* Bivona, Cent. 1. p. 53. n. 58. Ad margines fluminis Oreti, Bivona Bernardi (lectotype: BM, designated by Crespo et al. 1998).

**Description.** Perennial herb, acaulescent, rosulate, with 2–12.5 cm in diameter, provided with fibrose slender roots. Leaves 10–100 mm long, oblanceolate to spatulate, with blade entire or weakly crenate, glabrous, 4–40 × 2–15 mm, with petiole 3–60 mm long. Floral pedicels 2–11 cm, 2–3 times longer than leaves, with 1–2 bracteoles, 1.8–5.5 mm long, 0.1–0.7 mm wide, with glands at the margin. Calyx 3–5 mm long, with linear–lanceolate lobes, 2–4 mm long. Corolla 8.5–14.5 mm long, bilabiate, with tube 3.5–5 mm long, 0.9–1.5 mm in diameter; upper lip with two lobes linear–lanceolate, 3–6 mm long, 1.2–2.4 mm wide, bluish–lilac to dark lilac; lower lip trilobed, 5–9 mm long, widely edged in bluish–lilac and irregularly white in the central part until the base, covered by papillae in the ventral face. Stamen filaments free, 4–5.5 mm long, anthers violet, connate into a tube 1.4–1.9 mm long, wholly encapsulating the stigma; the two lower anthers are smaller, each appendiculate at the top with a tuft of hairs, closing a narrow fissure; the three upper anthers are curved. Ovary fused with the calyx tube; style whitish, 4–7 mm long; stigma pale lilac, bifid, papillate, with a ring of hairs just under the base. Capsule 1.6–3 mm long. Seeds more or less ellipsoid, shining, 0.40–0.50 × 0.2–0.3 mm.

#### 1.1. *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan, Pl. Syst. Evol. 210(3–4): 219. 1998. subsp. *bivonae*

Figs 1, 6A, 7A, 8C, 9A

**Description.** Basal rosette 2–12.5 cm in diameter, with leaves 12–100 mm long, spatulate, with blade 6–40 × 4–15 mm and petiole 5–60 mm long; floral



**Figure 1.** *Solenopsis bivonae* subsp. *bivonae* **A** habit **B** leaf **C** bracts **D** flower in dorsal view **E** bud **F** open corolla **G** open calyx **H** calyx and capsule **I** anther in lateral view **J** anther in lateral view with exerted stigma **K** unicellular papillae occurring in the ventral face of the corolla **L** seeds. Drawn by Salvatore Brullo.

pedicels 5–11 cm, with (1) 2 bracteoles, very close near the middle, 2–2.4 mm long, 0.3–0.5 mm wide, hairy at the apex, with 1–4 stipulated glands at the margin per side; calyx 3–4 mm long, with lobes 2–3 mm long; corolla 10–12 mm long, with tube lilac, 4–5 mm long, ca. 1 mm in diameter; upper lip with lobes 3.5–4.5 mm long, 1.3–1.7 mm wide, bluish–lilac, acute at apex, provided in the ventral face with papillae in the central part, 0.25–0.6 mm long; lower lip 5–7 mm long, with a small greenish–yellow macula at the base, slightly bordered of brown at base, lobes ovate and mucronate at the apex, 3.5–4.5 × 3–4 mm, covered by not very dense papillae for more than the lower half; stamen filaments 4–4.5 mm long, anther connate into a tube 1.5–1.8 mm long; the two lower anthers are without papillae at basis; the three upper anthers with hairs in the upper part of the back; style 4–4.5 mm long; capsule smooth, 1.6–2 mm long; seeds ellipsoid–fusiform, brownish, 0.40–0.45 × 0.2–0.25 mm.

**Iconography.** Bivona-Bernardi (1806) tav. 2, sub *Lobelia tenella*; Boccone (1697) tav. 27, fig. top right, sub *Rapunculus aquaticus, minimus, repens, alpinus, bellidis folio, flore caeruleo inaperto*; Brullo et al. (2023b) Figs 2C, D, 4.

**Etymology.** It is dedicated to Antonino Bivona Bernardi, Sicilian botanist (1770–1837), who first described this species.

**Phenology.** Flowering late April to September, fruiting May to September.

**Distribution and ecology.** According to herbarium investigations and our field survey, this taxon occurs in North–West Sicily (Fig. 10), in the surroundings of Palermo, especially along the banks of the Oreto River, where it is today very rare, while it is very widespread along the Sosio river near Chiusa Sclafani. Previously, it was reported from Alcamo, where unfortunately it is extinct. As concerns its ecological requirements, it is localized at an elevation between 10 and 250 m, growing on calcareous vertical wet rocky places affected by permanent dripping (Fig. 8A). The plant community characterized by this hygrophyte can be referred to the phytosociological class *Adiantatea capilli–veneris* Br.-Bl. in Br.-Bl., Roussine and Nègre 1952 (cfr. Cambria 2020). This habitat is floristically differentiated by a moss carpet where grow also *Adiantum capillus–veneris* L. and *Samolus valerandi* L.

**Conservation status.** Currently, this taxon's result is circumscribed in Sicily to two wet stands (Oreto and Sosio rivers), where it is very rare in the first locality and quite spread in the second one. Overall, this plant results in it being seriously threatened since it is linked to wetlands potentially subject to anthropic pressure, which tends to alter the water regime, prejudicing its survival. Therefore, in agreement with Conti et al. (1997), who quoted it as *S. minuta* subsp. *nobilis*, it can be treated as Endangered (EN), following IUCN criteria (IUCN 2022).

**Additional specimens examined.** ITALY, SICILY. Palermo in herbosis uliginosis, August 1888, *H. Ross* s.n. (PAL–GREUTER 8699, AMD43927); Sicile, 1831, *M. Tineo* s.n. (P00260397); Mondello, In locis hyeme inundatis, 1847, *M. Alb. de Franqueville* s.n. (P00260381); Orethus fluvius, locus rivulos, 1846, *M. Alb. de Franqueville* s.n. (P00260380); Palermo, s.d., *Tineo* s.n. (FI); Fiume Oreto, s.d., *Tineo* s.n. (FI); Fiume Oreto presso Palermo, s.d., *Parlatore* s.n. (FI); Palermo ad ripas F. Oreto, 25 April 1888, *N. Guzzino* 3068 (AMD43928); Palermo: fiume Oreto, 13 May 1888, *D. Lanza* s.n. (AMD43930); Palermo ad fluviorum margines, May, *A. Todaro* s.n. (L2993294, FI, RO); Palermo, ad aquae dulcis in herbosis uliginosis, June 1895, *H. Ross* 42 (L2993297, O-V2262582, FI); ex Sicilia, s. d., *G. Gussone* s.n. (L2993298); Palermo in herbosis uliginosis, June 1888, *H. Ross*



s.n. (L2993299, RO); Palermo, ad rivulorum margines, August, A. *Todaro* 463 (U1178908, P03406807, FI, RO); Fiume Oreto in humidis marginis, s.d., A. *Todaro* s.n. (U1178909); Palermo al fiume Oreto (Sicilia), in maritimis ad muros humidus, July 1881, M. *Lojacono* s.n. (P04608258, P00260396, MPU255098, FI); Ad muros madidos, Palermo, July, M. *Lojacono* s.n. (PAL39476); Palermo, June 1889, A. *Todaro* s.n. (P00260403); In humidis ad muros prope Panormum, 20 May 1855, E. & A. *Huet du Pavillon* (O-V2263343, FI); Lungo l'Oreto a Palermo, 22 August 1902, A. *Mazza* s.n. (FI); Panormi, ad rivulos alla Guadagna, September 1869, F. *Parlatore* s.n. (FI); Palermo a S. Maria di Gesù, in luoghi umidi, 1 May 1895, *Biondi* s.n. (FI); Fiume Oreto presso la Guadagna e S. Erasmo, June 1834, F. *Parlatore* s.n. (FI); Palermo alla Guadagna, 29 September 1868, F. *Parlatore* s.n. (FI); In humidis Palermo, s.d., A. *Todaro* s.n. (RO); Fiume Oreto, 1817, *Tineo* s.n. (RO); Fiume Oreto, Palermo, 38°5'18.17"N, 13°20'35.45"E, 46 m, 29 July 2018, S. *Cambria* s.n. (CAT); Alcamo in humentibus arenosis, May, *Citarida* 241 (RO); Fiume Sosio, S. Carlo, Chiusa Sclafani, 19 August 1995, G. *Certa* s.n. (PAL89386); Contrada Tagliarini près du fleuve Sosio, commune de Prizzi, province de Palerme, Sicile, Altitude: m. 640 environ. Le long bords humides. 20 August 1996, G. *Certa* s.n. (PAL39475); Fiume Sosio, 28 August 1986, G. *Spampinato* s.n. (CAT037289); Fiume Sosio, località S. Carlo (Chiusa Sclafani), 37°38'22.13"N, 13°15'59.66"E, 223 m, 10 July 2018, S. *Cambria* & G. *Di Gregorio* s.n. (CAT).

**1.2. *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan subsp. *madoniarum* Brullo, C. Brullo, Cambria, Tomaselli, Minissale & Giusso del Galdo, subsp. nov.**

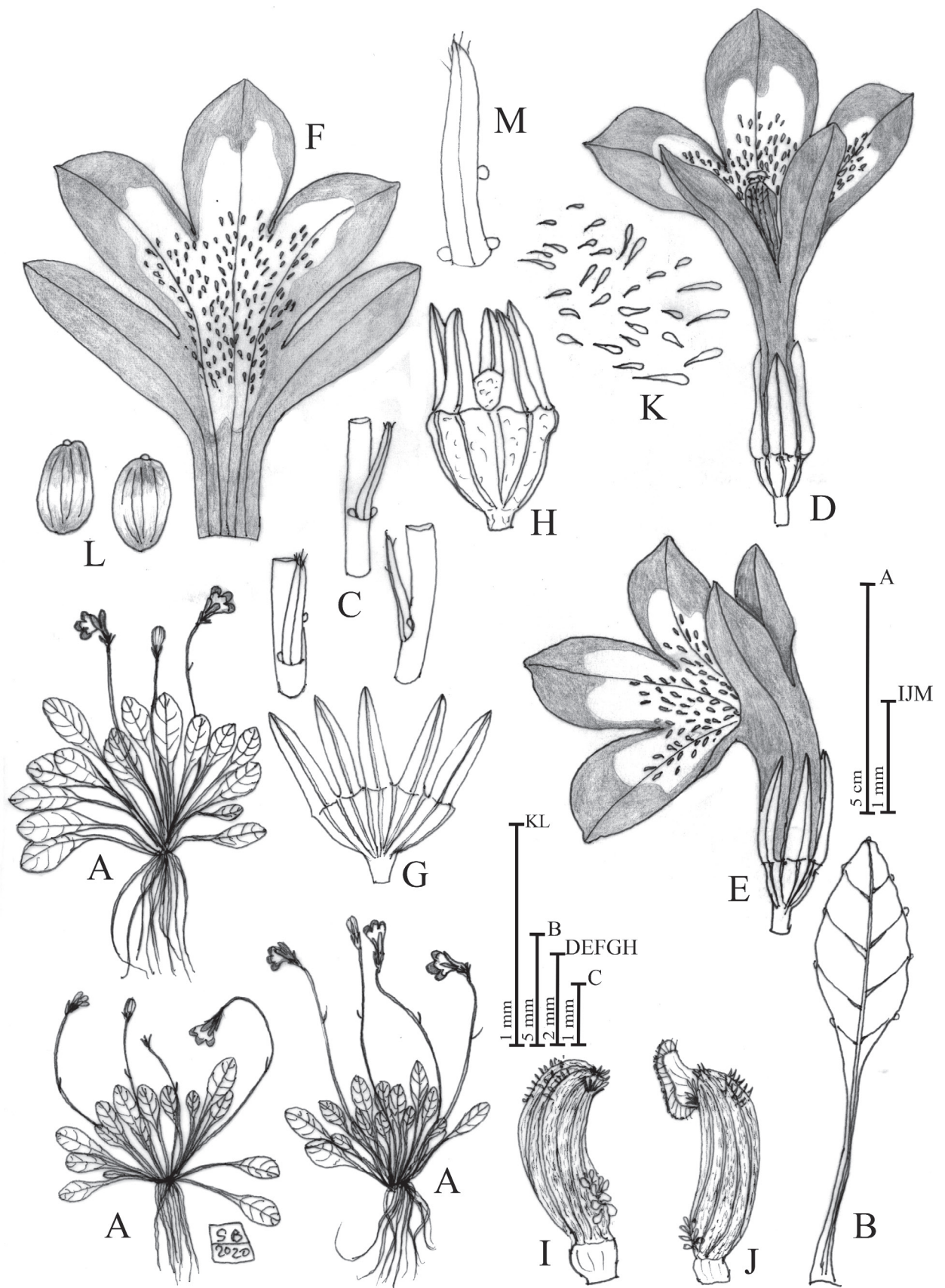
urn:lsid:ipni.org:names:77323164-1

Figs 2, 6D, 7B, 8D, 9B

**Type.** ITALY. SICILY. Madonie, laghetto sopra Piano Zucchi, 37°52'43.40"N, 14°0'12.87"E, 1259 m a.s.l., 15 July 2018, S. *Cambria* s.n. (holotype CAT).

**Diagnosis.** It differs from the type in having leaves arranged in a smaller rosette with shorter blade, shorter floral pedicel, provided with a single bracteole glabrous or with few apical hairs and 1–2 basal sessile glands, corolla smaller with upper lip lobes without glands in the ventral face and lower lip lobes shorter, obtuse, provided with dense and shorter papillae, with anther tube papillose at the basis and longer capsule. Conversely, the type is characterized by leaves arranged in a larger rosette with longer blade, longer floral pedicel, provided with (1)2 bracteoles with several hairs at the apex and 1–4 lateral stipulated glands, corolla larger with upper lip lobes with glands in the ventral face and lower lip lobes longer, acute, provided with lax and longer papillae, anther tube without papillae at the basis and shorter capsule.

**Description.** Basal rosette 3.5–8 cm in diameter, with leaves 15–45 mm long, oblanceolate to oblanceolate–spatulate, with blade 4–20 × 2–8 mm and petiole 8–25 mm long; floral pedicels 2–5(9) cm, with one bracteole near the middle, 1.8–2.2 mm long, 0.1–0.3 mm wide, with few hairs at the apex, with 1 or 2 sessile glands at the base and rarely one sessile gland at the margin; calyx 3–4 mm long, with lobes 2–3.5 mm long; corolla 8.5–10 mm long, with tube lilac, 3.7–4.5 mm long, 0.9–1.3 mm in diameter; upper lip with lobes 3–4 mm



**Figure 2.** *Solenopsis bivonae* subsp. *madoniarum* **A** habit **B** leaf **C** bracts **D** flower in dorsal view **E** flower in lateral view **F** open corolla **G** open calyx **H** calyx and capsule **I** anther in lateral view **J** anther in lateral view with exerted stigma **K** unicellular papillae occurring in the ventral face of the corolla **L** seeds **M** bract detail. Drawn by Salvatore Brullo.

long, 1.2–1.7 mm wide, bluish–lilac, obtuse or slightly mucronate at apex, provided in the ventral face with dense papillae in the lower half, 0.1–0.24 mm long; lower lip 5–6 mm long, with a large yellowish macula at the base, bordered at the base by a brown band, lobes ovate and obtuse or slightly mucronate at the apex,  $2.5\text{--}3.5 \times 1.6\text{--}2.5$  mm, covered by dense papillae in the lower half; anther connate into a tube 1.4–1.6 mm long; the two lower anthers are papillose at the base; style 4.5–5.5 mm long; capsule 2.7–3 mm long; seeds obovoid-ellipsoid, pale brown,  $0.40\text{--}0.46 \times 0.24\text{--}0.26$  mm.

**Etymology.** The epithet derives from Madonie, a massif of North Sicily, where this taxon is rather spread.

**Phenology.** Flowering late May to October, fruiting June to October.

**Distribution and ecology.** Based on herbarium data and field investigations, this taxon is distributed in the Madonie massif, where it is localized in many places at 700–1600 m of altitude (Fig. 10), represented mainly by peat bogs, dominated by *Sphagnum* sp. pl., *Aulacomnium palustre* (Hedw.) Schwägr. and *Polytrichum commune* Hedw. Here, it characterized an orophilous plant community belonging to *Scheuchzerio palustris*–*Caricetea fuscae* R. Tx. 1937, as emphasized by Raimondo et al. (1980, 2021). Sometimes, as near Petralia Soprana or Piazza Armerina, it occurs also on calcareous vertical wet rocky places affected by permanent dripping, where it is a member of vegetation of the class *Adiantetea capilli-veneris*, dominated by *Adiantum capillus-veneris* and several bryophytes.

**Conservation status.** This taxon shows a scattered distribution, occurring mainly in some localities within the Madonie Regional Park. Besides, it is a species closely linked to small wet stands fed by water springs, whose collecting leads to the destruction of the habitat and the disappearance of the vegetation that characterizes it. It shows an EOO of 410 km<sup>2</sup> and an AOO of 20 Km<sup>2</sup>. Therefore, according to B criterion, we propose to consider this taxon as Endangered [EN – B1ab(iii)+2ab(iii)] (IUCN 2022)].

**Additional specimens examined (paratypes).** ITALY, SICILY. Madonië, van Portella Mandarinini naar Geraci Siculi, op bult in moeras, c. 1400 m., 9 June 1983, J. Mennema 2962 (L2993484); Ad rivulos et fontes Montium Nebrodensium (alla fontana di S. Nicolò sul M. Pietrafucile, 24 June 1840, *De Heldreich* s.n. (P00260388; WAG1507801, FI); Italie, Sicile, Prov. Palermo, entre Portella Mandarinini (1206 m) et Geraci (1070 m) en passant pour la base de Punta Argentiera (1450 m), 9 June 1983, A. Charpin, M. Dittrich & D. Jeanmonod 96449 (PAL); Ad aquas scaturientes Madoniarum 3500', 6 August 1874, G. Strobl s.n. (FI); Ad scaturigines frigidas Nebrodes acque delle Favare di Petralia, July 1888, M. Lojacono 319 (FI); Madonie presso il passo della Botte, July 1904, F. Cava s.n. (FI); Madonie a Vulpignano, alla Favara, a Polizzi presso alla Pietà, June 1840, F. Parlato s.n. (FI); A montibus nebrodensibus, s.d., Schouw s.n. (G-DC00239486); Contrada Scorzone (Geraci Siculo), 22 June 2004, R. Galeasi s.n. (CAT000194); Piano Pomo (Madonie-PA), 31 July 1990, Bartolo, Brullo, Pulvirenti, Scelsi, Spampinato s.n. (CAT037288); Madonie, Portella Mandarinini, sfagnete, 37°51'55"N, 14°07'04"E, 1247 m, 15 July 2018, S. Cambria s.n. (CAT); Madonie, Petralia Soprana, sorgente Cataratta, parete umida, 37°49'37.26"N, 14°4'11.39"E, 1166 m, 15 July 2017, S. Cambria s.n. (CAT); Piazza Armerina, Monte Canalotto, presso l'abbeveratoio, 37°28'6.55"N, 14°22'41.04"E, 771 m, 16 October 2021, S. Cambria & D. Azzaro s.n. (CAT).

**1.3. *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan subsp. *peloritana* Brullo, C.Brullo, Cambria, Tomaselli, Crisafulli, Minissale & Giusso del Galdo, subsp. nov.**

urn:lsid:ipni.org:names:77323165-1

Figs 3, 6B, 7C, 8E, 9C

**Type.** ITALY. SICILY. Monti Peloritani, Vallone Passo Pirtuso, S. Lucia del Mela, 38°4'59"N, 15°18'28"E, 559 m, 19 July 2020, S. Cambria, A. Crisafulli & F. Anania s.n. (holotype CAT).

**Diagnosis.** It differs from the type in having longer bracteoles, glabrous, provided with apical gland, longer calyx with longer lobes, larger corolla with denser and spread glands in the ventral face, larger upper lip lobes and lower lip lobes, within the lower lip a yellow macula at the base, slightly bordered of red-brown, longer style and larger capsule. Conversely, the type is characterized by shorter bracteoles, hairy apex without gland, shorter calyx with shorter lobes, smaller corolla with more scattered glands in the ventral face, smaller upper lip lobes and lower lip lobes, within the lower lip a greenish-yellow macula at the base, slightly bordered of brown, shorter style and smaller capsule.

**Description.** Basal rosette 4–10 cm in diameter, with leaves 15–55 mm long, with blade 7–23 × 4–10 mm and petiole 5–30 mm long; floral pedicels 5.5–11 cm, with 2 bracteoles, 3–5.5 mm long, 0.4–0.7 mm wide, glabrous, with one terminal gland and 1–2 stipulated glands at the margin per side; calyx 4–5 mm long, with lobes 3.2–4 mm long; corolla 12–14.5 mm long, with tube green with lilac ribs, 3.5–4 mm long, ca. 1.5 mm in diameter; upper lip with lobes 5–6 mm long, 2–2.4 mm wide, dark lilac, provided in the ventral face with papillae in the central part, 0.1–0.4 mm long; lower lip 8–9 mm long, with a small yellow macula at the base, slightly bordered of red-brown at base in the upper part or sometimes with central red-brown spot, lobes obovate, the central one 5.5–6.5 × 4–4.5 mm, the lateral ones 4.5–5.5 × 4–4.2 covered by very dense papillae almost until the apex; stamen filaments 4.5–4.7 mm long, anther connate into a tube 1.7–1.9 mm long; the three upper anthers with scattered hairs in the upper part of the back; style 6.5–7 mm long; capsule smooth, 2.5–3 mm long; seeds ellipsoid, 0.45–0.50 × 0.24–0.26 mm.

**Etymology.** The epithet derives from Peloritani, a chain of North-eastern Sicily, where this taxon is localized.

**Phenology.** Flowering June to August, fruiting July to August.

**Distribution and ecology.** It grows on metamorphic vertical wet rocky stands affected by permanent dripping. It is a member of a plant community of the class *Adiantetea capilli-veneris*, dominated by *Adiantum capillus-veneris*, associated with *Samolus valerandi* L., *Lysimachia nemorum* L., *Hypericum hircinum* L. subsp. *majus* (Aiton) N. Robson and several bryophytes. In this stand, it is localized exclusively along a short watercourse of the Mela valley (Peloritani chain) at an elevation of 600–700 m (Fig. 10), where several individuals of this taxon were surveyed.

**Conservation status.** This taxon is known for one stand of the Peloritani chain, along a short wet wall, where about one hundred well-developed individuals were observed. This population is very isolated and inaccessible and it does not seem subject to immediate threats. It shows an EOO of 4 km<sup>2</sup> and an AOO of 4 Km<sup>2</sup>. Therefore, according to the B criterion (IUCN 2022), we propose to consider this taxon as Critically Endangered category [(CR – B1ab(iii)+2ab(iii))].





**Figure 3.** *Solenopsis bivonae* subsp. *peloritana* **A** habit **B** leaves **C** bracts **D** open corolla **E** flower in lateral view **F** flower in dorsal view **G** bud. **H** open calyx **I** calyx and capsule **J** seeds **K** unicellular papillae occurring in the ventral face of the corolla **L** anther in lateral view **M** anther in ventral view. Drawn by Salvatore Brullo.

**1.4 *Solenopsis bivonae* (Tineo) M.B.Crespo, Serra & A.Juan subsp. *brutia* Brullo, C.Brullo, Cambria, Tomaselli, Minissale & Giusso, subsp. nov.**

urn:lsid:ipni.org:names:77323166-1

Figs 4, 6C, 7D, 8F, 9D

**Type.** ITALY. CALABRIA. Rive del fiume Lao, presso Papasidero (Cosenza), 39°52'10.96"N, 15°54'7.93"E, 130 m, 06 August 2018, S. Brullo, D. Puntillo & D. Uzunov s.n. (holotype CAT).

**Diagnosis.** It differs from the type in having leaves arranged in a smaller rosette, shorter leaves, with oblanceolate to oblanceolate-spathulate blade, shorter petiole, shorter floral pedicel, glabrous bracteoles, located in the upper half, provided with sessile apical gland, two basal glands and 0–2 lateral glands, corolla in the lower lip with a green macula at the base and provided with three dark blue spots above, lobes papillose up to the apex, longer staminal filaments, glabrous anther tube, longer style, slightly tuberculate capsule, reddish–brown and larger seeds. Conversely, the type is characterized by leaves arranged in a larger rosette, longer leaves, with spathulate blade, longer petiole, longer floral pedicel, bracteoles hairy at the apex, located in the middle, provided with 1–4 stipulated lateral glands, corolla in the lower lip with a greenish-yellow macula at the base, without spots, lobes papillose for more than the lower half, shorter staminal filaments, anther tube hairy at the apex, shorter style, smooth capsule, brownish and smaller seeds.

**Description.** Basal rosette 2.5–7 cm in diameter, with leaves 10–58 mm long, oblanceolate to oblanceolate-spathulate, with blade 5–22 × 2–10 mm and petiole 3–36 mm long; floral pedicels 3–6 cm, with 2 bracteoles, spaced in the upper half, 2–3 mm long, 0.25–0.35 mm wide, glabrous with a sessile gland at the apex, with 2 basal sessile glands and 0–2 sessile glands at the margin; calyx 3.5–5 mm long; corolla 11–12 mm long, with tube white-lilac, 4.5–5 mm long, 1–1.2 mm in diameter; upper lip with lobes 4–4.5 mm long, 1.4–1.8 mm wide, sub-obtuse at apex, provided in the ventral face with papillae in the central part, 0.16–0.6 mm long; lower lip 6.5–7 mm long, greenish at the throat, surmounted by three distinct dark blue spots, slightly bordered of brown at base, lobes 3.5–5 × 2.5–3.5 mm, covered by not very dense papillae often almost to the apex; stamen filaments 5–5.5 mm long, anther connate into a tube 1.5–1.6 mm long; the three upper anthers glabrous in the upper part of the back; style 6–6.5 mm long; capsule slightly tuberculate, 2.3–3 mm long; seeds ellipsoid, reddish–brownish, 0.46–0.50 × 0.26–0.3 mm.

**Phenology.** Flowering June to September, fruiting June to September.

**Etymology.** The specific epithet refers to “Brutia,” the Latin name of Calabria, territory where this taxon grows.

**Distribution and ecology.** This taxon was surveyed in the lower reaches of Lao river (North Calabria), at elevations of 130–350 m, where it grows on rocky metamorphic outcrops (Fig. 10). It likes humid and shady stands covered by a dense moss carpet, associated mainly to *Adiantum capillus-veneris*. As for the other subspecies previously examined, it is linked to hygrophilous communities of the *Adiantetea capilli-veneris* too. From a phytogeographical point of view, it should be noted that this taxon is the only *Solenopsis* with a perennial habit, localized in a continental territory since all the others occur exclusively in big Mediterranean islands (Crespo et al. 1998).





**Figure 4.** *Solenopsis bivonae* subsp. *brutia* **A** habit **B** leaves **C** bracts **D** flower in dorsal view **E** open corolla **F** open calyces **G** calyx and capsule **H** anther in lateral view **I** anther in lateral view with stigma. **J** seeds **K** unicellular papillae occurring in the ventral face of the corolla. Drawn by Salvatore Brullo.

**Conservation status.** The populations of this subspecies are rare and all circumscribed to the banks of Lao river in North–West Calabria. Based on recent field surveys, its presence in the three hitherto known locations has been confirmed in only one of them (near Papasidero), while in the other two, it seems to have disappeared (Laino–Castello and Laino–Borgo). It shows an EOO of 9.51 km<sup>2</sup> and an AOO of 12 Km<sup>2</sup>. Therefore, in addition to its rarity and the considerable reduction of its current range, according to B criterion (IUCN 2022), we propose to consider this taxon as Endangered [EN – B1ab(iii)+2ab(iii)].

**Additional specimens examined (paratypes).** ITALY, CALABRIA. Valle del Lao (sopra le rocce e in altri luoghi umidi lungo il f. Lao ai piedi lo Borgo-Laino-Castello), 18 August 1892, *B. Longo s.n.* (RO); Sulle rocce umide lungo il fiume Lao alla Maradosa (Laino Castello), 27 September 1900, *B. Longo s.n.* (RO); Sopra una roccia umida lungo il fiume Lao (Laino Castello-Cosenza), 16 August 1902, *B. Longo s.n.* (RO).

## 2. *Solenopsis meikleana* Brullo, C.Brullo, Cambria, Tomaselli, Minissale & Giusso, sp. nov.

urn:lsid:ipni.org:names:77323167-1

Figs 5, 6E, 7F, 8G, 9E

*Laurentia tenella* Auct. Fl. Cypr., non A. DC. Prodr. 7(2): 410, 1839.

*Laurentia minuta* Auct. Fl. Cypr., non A. DC. Prodr. 7(2): 410, 1839.

*Laurentia minuta* (L.) DC. f. *nobilis* Wimmer, Ann. Naturhist. Mus. Wien 56:333, 1948, p.p.

*Solenopsis minuta* (L.) C. Presl subsp. *nobilis* (Wimmer) Meikle, Kew Bull. 34(2): 374, 1979, p.p.

*Solenopsis bivonae* (Tineo) M. B. Crespo, Serra & A. Juan, Pl. Syst. Evol. 210(3–4): 219. 1998, p.p.

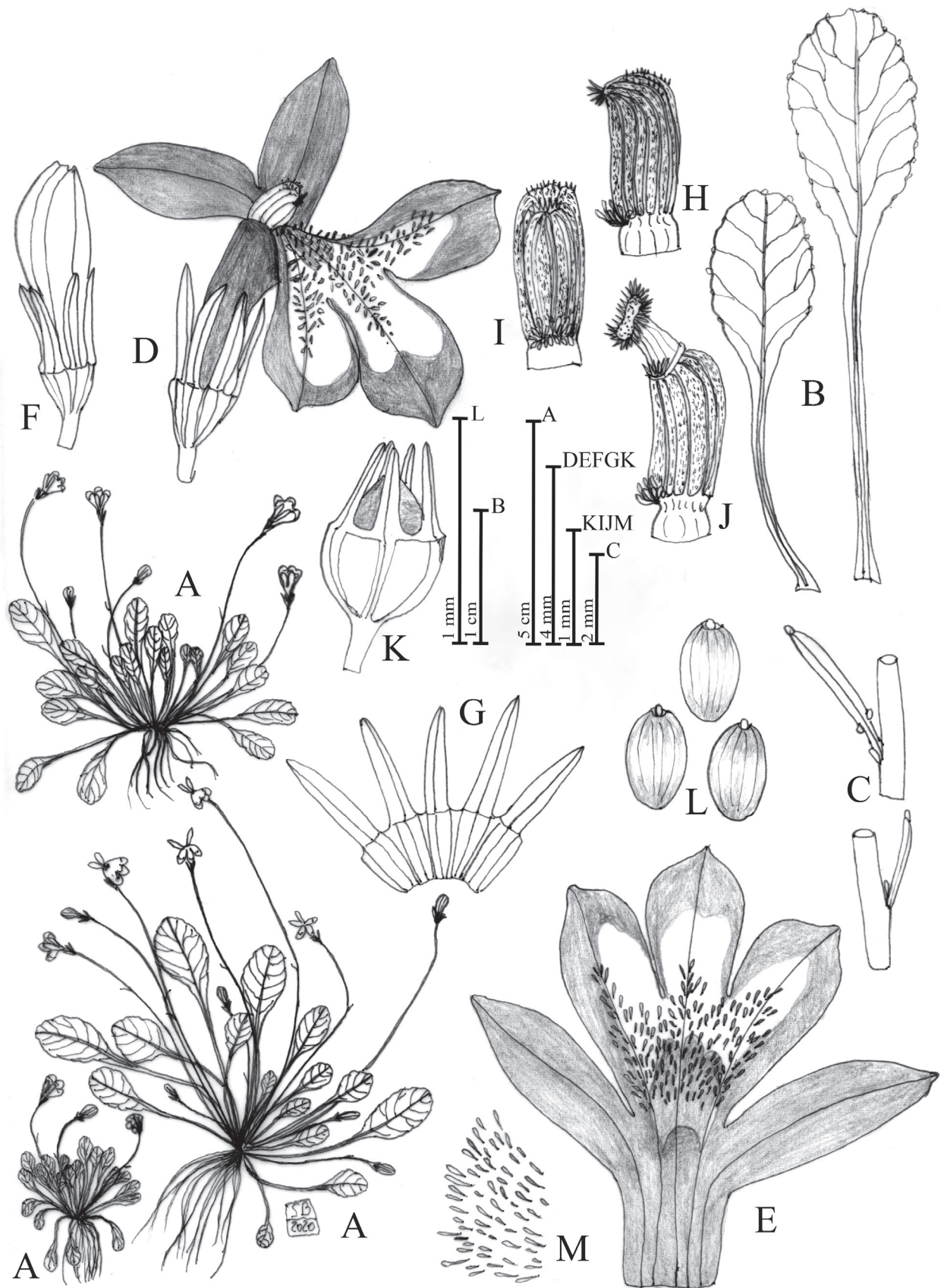
*Solenopsis bivonae* Christodoulou et al., Cypricola 17: 1, 2020, p.p.

**Type.** CYPRUS. Mesa Potamos Falls, 34°53'31.88"N, 32°54'32.37"E, 960 m, 6 June 2019, *S. Cambria s.n.* (holotype CAT).

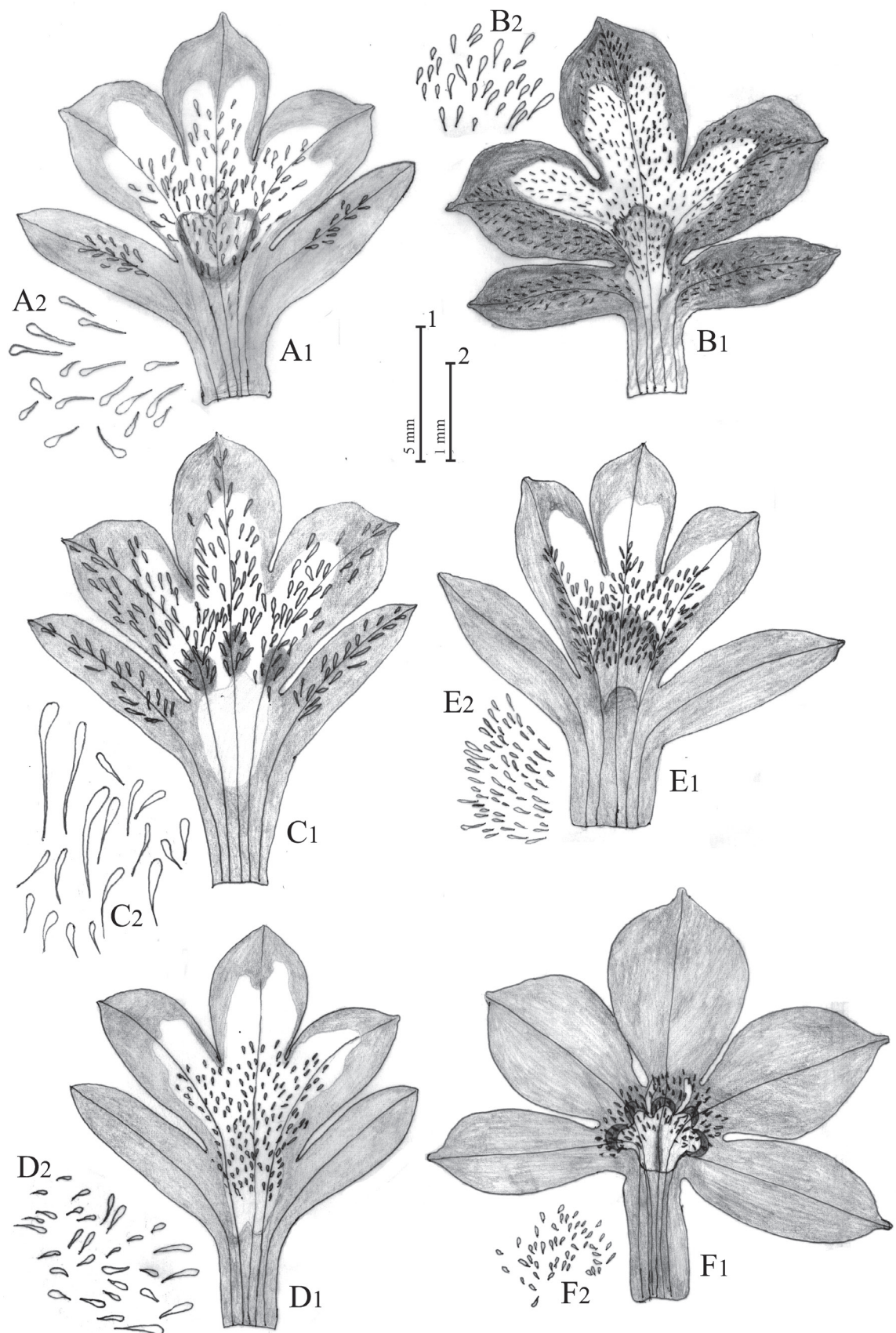
**Diagnosis.** It differs from *Solenopsis bivonae* in having glabrous and longer bracteoles, provided with apical sessile glands and 1–2 glands per side, pale blue or pale violet corolla, with upper lip lobes without papillae, lower lip lobes oblong, smaller, provided with shorter glands, anther tube shorter and papillose at the base, shorter style and longer capsule. Conversely, *S. bivonae* is characterized by shorter bracteoles, hairy at the apex and with 1–4 glands per side, bluish-lilac corolla, with upper lip lobes with papillae in the ventral face, lower lip lobes linear-lanceolate, larger, provided with longer glands, anther tube longer, without basal papillae, longer style and shorter capsule.

**Description.** Basal rosette 2.5–11 cm in diameter, with leaves 10–75 mm long, oblanceolate–spathulate, with blade glabrous or covered by scattered hyaline hairs, 6–30 × 4–15 mm and petiole 5–50 mm long; floral pedicels 2–12 cm, subequal to 3 times longer than leaves, with 1–2 bracteoles, 2–8 mm long, 0.2–0.6 mm wide, glabrous, with 1–2 stipulated glands at the margin per side; calyx 3–5 mm long, with lobes 1.5–3 mm long; corolla 10–12 mm long, with tube green-violet, 3–5 mm long, 1.1–1.3 mm in diameter; upper lip



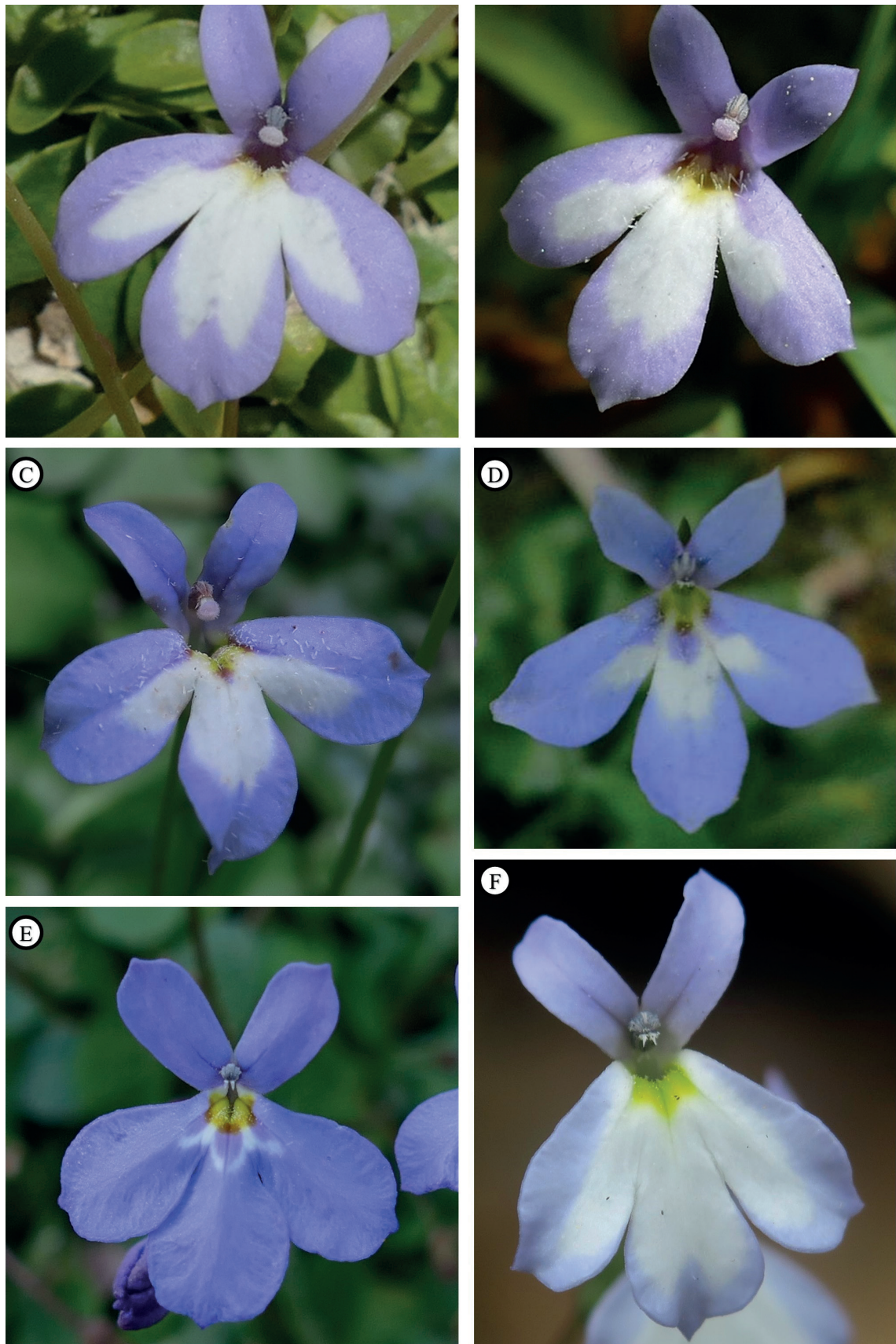


**Figure 5.** *Solenopsis meikleana* sp. nov. **A** habit **B** leaves **C** bracts **D** flower in lateral view **E** open corolla **F** bud **G** open calyx **H** anther in lateral view **I** anther in ventral view **J** anther in lateral view with stigma **K** calyx and capsule **L** seeds **M** unicellular papillae occurring in the ventral face of the corolla. Drawn by Salvatore Brullo.



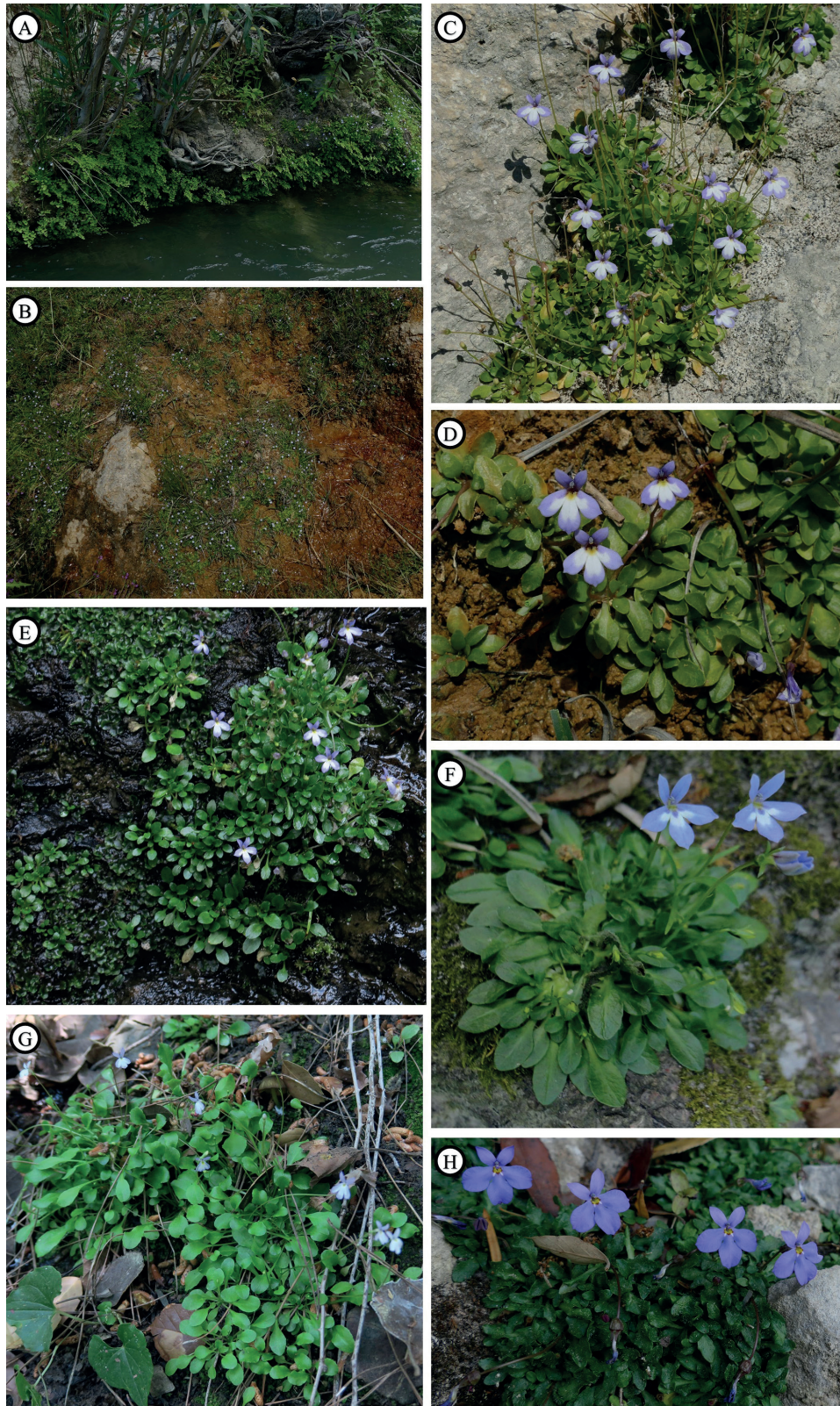
**Figure 6.** Open corolla (1) and detail of corolla papillae (2) of *Solenopsis bivonae* subsp. *bivonae* (A), *S. bivonae* subsp. *peloritana* (B), *S. bivonae* subsp. *brutia* (C), *S. bivonae* subsp. *madoniarum* (D), *S. meikleana* (E) and *S. bacchettiae* (F). Drawn by Salvatore Brullo.





**Figure 7.** Flowers in frontal view in natural habitat of *Solenopsis bivonae* subsp. *bivonae* (A), *S. bivonae* subsp. *madoniarum* (B), *S. bivonae* subsp. *peloritana* (C), *S. bivonae* subsp. *brutia* (D), *S. bacchettiae* (E) and *S. meikleana* (F). Photographed by Salvatore Cambria (A–C, E, F) and Lorenzo Peruzzi (D).





**Figure 8.** Natural habitat along the Sosio river (Sicily) colonized by *Solenopsis bivonae* subsp. *bivonae* (A). Natural habitat in Madonie massif (Sicily) colonized by *S. bivonae* subsp. *madoniarum* (B). Habit of *S. bivonae* subsp. *bivonae* from Sosio River (C). Habit of *S. bivonae* subsp. *madoniarum* from Madonie massif (D). Habit of *S. bivonae* subsp. *peloritana* from Mela River, Sicily (E). Habit of *S. bivonae* subsp. *brutia* from Lao River, Calabria (F). Habit of *S. meikleana* from Cedar Valley, Cyprus (G). Habit of *S. bacchettae* from Seui, Sardinia (H). Photographed by Salvatore Cambria (A–E, G, H) and Lorenzo Peruzzi (F).





**Figure 9.** Habit of living plants of *Solenopsis bivonae* subsp. *bivonae* from Sosio river (A), *S. bivonae* subsp. *madoniarum* from Madonie massif (B). *S. bivonae* subsp. *peloritana* from Mela River (C). Habit of *S. bivonae* subsp. *brutia* from Lao River (D). *S. meikleana* from Cedar Valley, Cyprus (E). *S. bacchettiae* from Seui, Sardinia (F).

with lobes 1.5–1.7 mm wide, pale blue to pale-violet, without papillae; lower lip 5–5.5 mm long, lobes oblong, obtuse at the apex, 2.5–3.5 × 1.4–2.2 mm, covered by papillae 0.05–0.3 mm long; stamen filaments 3–5 mm long, an-

ther connate into a tube 1–1.5 mm long; the two lower anthers are papillose at basis; the three upper anthers with scattered hairs in the upper part of the back; style 3.5–4 mm long; capsule 3–3.2 mm long; seeds broadly ellipsoid, 0.40–0.46 × 0.24–0.29 mm.

**Iconography.** Meikle (1985), plate 65.

**Phenology.** Flowering March to October, fruiting April to October.

**Etymology.** It is dedicated to Robert Desmond Meikle (1923–2021), author of the “Flora of Cyprus,” who dealt with the taxonomy of the genus *Solenopsis*.

**Distribution and ecology.** This species occurs in western Cyprus, where it is localized in very moist environments such as river banks, springs, waterfalls, and dripping walls (Fig. 10). Usually, it grows from hills to mountain belts up to an elevation of 1600 m, on ophiolitic rocky outcrops covered by bryophyte carpets and ferns, particularly *Adiantum capillus-veneris*. This vegetation can be referred to the *Adiantetea capilli-veneris* class for its floristic and ecological peculiarity.

**Conservation status.** This species, endemic to Cyprus, shows a scattered distribution in the western part of the island. It is a perennial hygrophyte, usually occurring in the wet rocky stands, which are always subject to dripping waters. Regarding conservation, the habitat characterized by this species is subject to synanthropic threats, represented mainly by the uptake of springs or the waters of streams, which allow its survival. The species shows an EOO of 1298 km<sup>2</sup> and an AOO of 288 Km<sup>2</sup>. Therefore, according to B criterion (IUCN 2022), we propose to consider this taxon as Endangered [EN – B1ab(iii)+2ab(iii)].

**Additional specimens examined (paratypes).** **CYPRUS.** Iter Cyprium, Mont Troodos 5000–6400', 10 June 1912, *M. Haradjian s.n.* (L2993291); frequens ad fontes in pago Moni inter Larnaca et Limassol, 28 April 1862, *T. Kotschy* 576 (L2993300, G-BOIS00781682); Troodos, valley Caledonian falls. On rocks next to the falls. 34°54'N, 32°52'E, Alt. 1350, 22 July 1995, *J.J. Wieringa* 3330 and *M.I.D. Janzen* (WAG 1335512); Iter Cyprium, pr. Galata, 16 June 1880, *Sintenis et Rigo* 742 (P00260376); Ganze voicin de la Gratiola et de la Bonnaga in insula Cypri in humidis maritimis, 1837, *M. Aucher-Eloy s.n.* (P00260370); in Cypro, s.d., *M. Aucher-Eloy* 3854 (P00260371, G-BOIS00781706); In humidis in insulae Cypri, 1836, *M. Aucher (Eloy) s.n.* (G-DC00329488); Ins. Cypro, in valle fluminis prope Galata, 16 June 1880, *Sintenis et Rigo* 742 (P00260379; FI); Cyprus, near Phini. On dripping tufa by roadside, 5 June 1962, *R.D. Meikle* 2874 (P00242688); Zypern: Trooditissa Monastery, Division 2 (sensu Meikle 1979, 1985), at the waterfall in hairpin bend E of Monastery, wet rocks, 1315 m (L: 32°50'33"E/ B: 34°54'45"N), 24 Sep. 2010, *Hand* 5739 (B100342825); Insule Cypri, Nikosia, pr. le gauche a Kordukis, 28 March 1905, *J. Holmboe* 292 (O-V2262581); Insulae Cypri, Troodos: Pasha Livadia, 12 July 1905, *J. Holmboe* 1075 (O-V2262581); Cedar Valley, Cedar hiking path, 34°59'28.58"N, 32°41'19.65"E, 1126 m, 7 June 2019, *S. Cambria s.n.* (CAT).

### 3. *Solenopsis bacchettiae* Brullo, C.Brullo, Tavilla, Siracusa & Cambria, Nord. J. Bot. 40 (12): 2, e03773.

Figs 6F, 7E, 8H, 9F

*Laurentia tenella* Moris, Fl. Sardo: 542, 1840–1843, non A. DC. Prodr. 7(2): 410, 1839.



**Figure 10.** Geographical distribution of *Solenopsis bivonae* subsp. *bivonae* (black dots); *S. bivonae* subsp. *madoniarum* (red dots); *S. bivonae* subsp. *peloritana* (pale blue dot); *S. bivonae* subsp. *brutia* (dark blue dot); *S. meikleana* (purple dots); *S. bacchettiae* (green dots).

*Solenopsis bivonae* auct. Flora Sarda, non M. B. Crespo, Serra & A. Juan, Pl. Syst. Evol. 210: 219, 1998.

*Solenopsis minuta* (L.) C.Presl subsp. *minuta* sensu Arrigoni, Fl. Is. Sard. 4: 532, 2013, non C. Presl (C. Presl 1836, p. 32).

**Type.** ITALY. SARDINIA. Montarbu di Seui, lungo la strada sterrata ad est di Bruncu Arrascialei, su pareti umide, 986 m, 39°24'09"N, 9°53'32"E, 23 July 2018, S. Cambria s.n. (holotype: CAT, isotypes: CAT, CAG).

**Description.** It differs from *S. bivonae* in having a basal rosette 3–10 cm in diameter, with leaves 12–60 mm long, hairy mainly on the blade, which is 5–25 × 2–12 mm and petiole 7–35 mm long; floral pedicels 2.5–7.5 cm, with bracteoles, in the lower half, 3–5.5 mm long, 0.4–0.5 mm wide, with 1–4 sessile glands at the margin per side; calyx (3.5)4–6.5 mm long, with lobes 2–3.5 mm long; corolla 13–16 mm long, uniformly dark blue–lilac, with tube blue–lilac, 5–6 mm long, 1–1.5 mm in diameter; upper lip with ovate-lanceolate lobes 5–7 mm long, 2.4–4 mm wide, obtuse and mucronate at apex, without papillae; lower lip 7–10 mm long, with a large yellowish 5-lobed macula at the base, bordered in the lobes by a triangular brown macula, with two thin white strips in the central part of the throat, rarely replaced by a white halo, lobes 5–8 × 3–5 mm, only at throat covered by dense papillae 0.1–0.2 mm long; stamen filaments 5–7 mm long, anther connate into a tube 1.4–1.7 mm long; style 6–8 mm long; capsule tuberculate, 3–4 mm long; seeds pale brown, 0.50–0.52 × 0.3–0.32 mm.

**Iconography.** Brullo et al. (2023b), Fig. 1.

**Phenology.** Flowering May to August, fruiting June to September.

**Etymology.** This species is dedicated to Gianluigi Bacchetta, an active botanist from Cagliari University and an expert on the Sardinian flora.



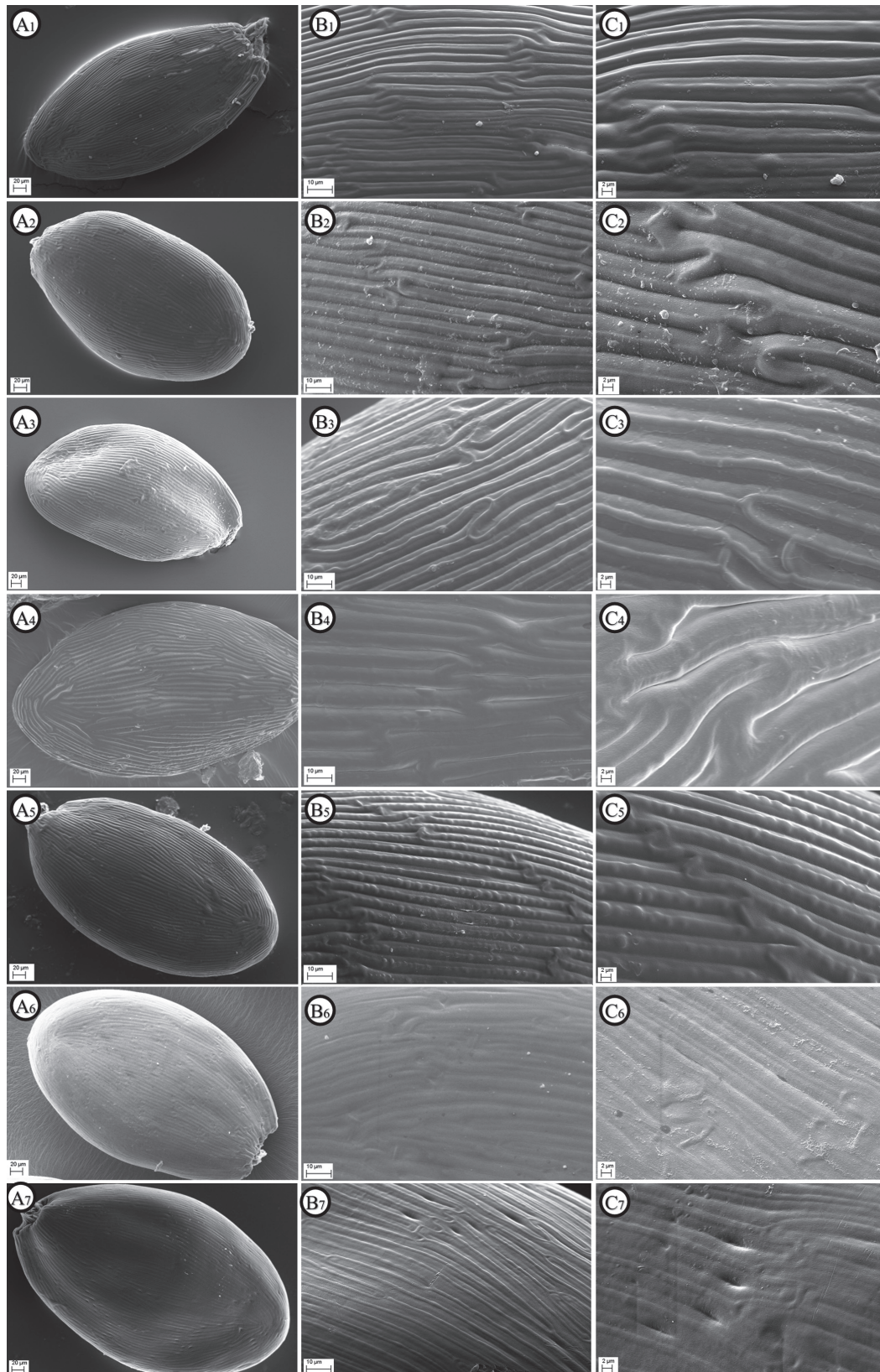
**Distribution and ecology.** According to Brullo et al. (2023b), *Solenopsis bacchettae* is distributed in central–east Sardinia, where it is localized on carbonatic substrates (Fig. 10). It grows exclusively on damp soils along or near small streams with fresh water at 700–1000 m a.s.l., where it is a member of a plant community rich in endemic hygrophilous species.

**Conservation status.** This species shows a scattered distribution, currently represented by few locations, where an estimated population of around 1000 individuals occurs. Based on IUCN (2022) criteria, Brullo et al. (2023b) proposed to treat it as an endangered species (EN).

**Additional specimens examined.** See Brullo et al. (2023b).

### Seed micromorphology

According to literature (Murata 1992, 1995; Haridasan and Mukherjee 1993; Serra and Crespo 1997; Crespo et al. 1998; Brullo et al. 2013, 2023b), the ornamentations of the seed coat in the Lobelioideae, subfamily of Campanulaceae, show a relevant taxonomical value and phylogenetic importance. Overall, the testa structure of mature seeds within this subfamily shows well-defined and constant ornamentations in every taxon. The seed coat sculptures are characterized by long, narrow cells (50–150  $\mu\text{m}$  long) separated by longitudinal furrows. From the SEM observations, the seeds of *Solenopsis bivonae* subsp. *bivonae* (Fig. 11A1) have an ellipsoid–fusiform shape, narrowing towards the basal and apical ends, having a size of  $0.40\text{--}0.45 \times 0.20\text{--}0.25$  mm. As concerns its seed testa, the cells have periclinal walls distinctly convex, 4–5  $\mu\text{m}$  wide, crossed by a marked convex central ridge 1.4–1.8  $\mu\text{m}$  wide, with anticlinal walls linear and deeply grooved (Fig. 11B1–C1). The seeds of *S. bivonae* subsp. *madoniarum* (Fig. 11A2, A3) show an obovoid–ellipsoid shape, rounded at the apical end, with a size of  $0.40\text{--}0.46 \times 0.24\text{--}0.26$  mm. As concerns its seed testa, the cells have periclinal walls distinctly convex, 5.5–8.0  $\mu\text{m}$  wide, crossed by a marked convex central ridge 0.8–1.6  $\mu\text{m}$  wide, with anticlinal walls linear and deeply grooved (Fig. 11B2–C2, B3–C3). The seeds of *S. bivonae* subsp. *peloritana* (Fig. 11A4) have an ellipsoid shape, rounded at the apical end, with a size of  $0.45\text{--}0.50 \times 0.24\text{--}0.26$  mm. As concerns its seed testa, the cells have periclinal walls distinctly convex and smooth, 6.4–10.0  $\mu\text{m}$  wide, without a central ridge, with anticlinal walls linear and deeply grooved (Fig. 11B4–C4). The seeds of *S. bivonae* subsp. *brutia* (Fig. 11A5) have an ellipsoid shape, rounded at the apical end, with a size of  $0.46\text{--}0.50 \times 0.26\text{--}0.30$  mm. As concerns its seed testa, the cells have periclinal walls distinctly convex, 4.4–6.0  $\mu\text{m}$  wide, crossed by a marked convex central ridge 1.4–2.0  $\mu\text{m}$  wide with a row of distinct tubercles and with anticlinal walls linear and deeply grooved (Fig. 11B5–C5). The seeds of *S. meikleana* (Fig. 11A6) have a broadly ellipsoid shape, rounded at the apical end, with a size of  $0.40\text{--}0.46 \times 0.24\text{--}0.29$  mm. Regarding its seed testa, the cells have periclinal walls slightly convex, 5.0–8.3  $\mu\text{m}$  wide, crossed by an evanescent convex central ridge 0.8–1.2  $\mu\text{m}$  wide, and with anticlinal walls linear and slightly grooved (Fig. 11B6–C6). The seeds of *S. bacchettae* (Fig. 11A7) have an ellipsoid shape, rounded at the apical end, with a size of  $0.50\text{--}0.52 \times 0.30\text{--}0.32$  mm. As concerns its seed testa, the cells have periclinal walls usually quite flat, 4.0–4.5  $\mu\text{m}$  wide, crossed by a slightly convex central ridge 1.0–1.6  $\mu\text{m}$  wide and with anticlinal walls linear and slightly grooved (Fig. 11B7–C7).



**Figure 11.** SEM images of seed shape (**A**  $\times 250$ ) and detail of seed testa (**B**  $\times 1000$  and **C**  $\times 2000$ ) regarding: **1.** *Solenopsis bivonae* subsp. *bivonae* from Sosio river, Sicily. **2.** *S. bivonae* subsp. *madoniarum* from Madonie massif, Sicily. **3.** *S. bivonae* subsp. *madoniarum* from Piazza Armerina, Sicily. **4.** *S. bivonae* subsp. *peloritana* from Mela river, Sicily. **5.** *S. bivonae* subsp. *brutia* from Lao river, S. Italy. **6.** *S. meikleana* from Cedar Valley, Cyprus. **7.** *S. bacchettiae* from Seui, Sardinia. Images made by Giuseppe Siracusa.

## Phytosociological remarks

Based on our field observations during the surveys on the populations belonging to the *Solenopsis bivonae* group, it was possible to verify that they were always localized in very specialized humid habitats, limited to very circumscribed surfaces. As previously highlighted, three main habitats can be recognized, where usually the examined populations of *Solenopsis* occur. In particular, they are represented by dripping rocky walls, peat bogs, and edges of streams or springs. As concerns the wet rocky environments, the surface is usually covered by a bryophytic layer, where individuals of *Adiantum capillus-veneris* more or less densely grow. According to Deil (1995, 1996, 1998), these habitats represent conservative environments that remain very stable in time and space, unaffected by climate change in neither geological nor current climatic variation in the Mediterranean area. Besides, these wet stands host several vicarious taxa having a Tertiary origin (Deil 1995, 1996, 1998) belonging, in particular, to *Primula*, *Hypericum* sect. *Adenosepalum*, *Pinguicula*, and relictual tropical ferns, such as *Woodwardia radicans* (L.) Sm., *Pteris vittata* L., *P. cretica* L. and *Osmunda regalis* L. Indeed, the current floristic composition of these peculiar hygrophilous communities results from evolutionary processes within the single taxa rather than recent changes in the environmental and ecological conditions. Therefore, the plant communities within which these species now grow must be considered the impoverished remains of those dating back to the Tertiary. Due to the climatic changes during the Quaternary and the recent Holocene, these phytocoenoses generally occupy much smaller areas than in the past, remaining almost constant in their floristic composition. At the same time, the taxa that characterize them have undergone significant speciation processes, always remaining linked to the same ecological context and maintaining their phytosociological role. These communities, due to their floristic set, structure, and ecological requirements, must be referred to the phytosociological class *Adiantetea capillis-veneris* Br.-Bl. in Br.-Bl., Roussine and Nègre 1952, syntaxon distributed mainly in the Mediterranean area and Western Asia (Braun-Blanquet et al. 1952; Brullo et al. 1989; Deil 1989, 1998; de Foucault 2015). Floristically, this syntaxon is differentiated mainly by the occurrence of *Adiantum capillus-veneris* L., *Samolus valerandi* L., which grow together with several bryophytes, among them *Eucladium verticillatum* (With.) Bruch & Schimp., *Conocepalum conicum* (L.) Dumort., *Pellia endiviifolia* (Dicks.) Dumort., *P. epiphylla* (L.) Corda, *Scorpiurum circinatum* Fleischer & Loeske, *Rhynchostegiella tenella* (Dicks.) Limpr. and *Eurhynchium praelongum* (Hedw.) Schimp. As concerns the *Solenopsis* species treated by us in this paper, most of them are closely related to these environments belonging to the *Adiantetea capillis-veneris*, which is here represented by the order *Adiantetalia capillis-veneris* Br.-Bl. ex Horvatic 1934 and the alliance *Adiantion capillis veneris* Br.-Bl. ex Horvatic 1934. From the syntaxonomical point of view, the *Solenopsis* species occurring in these wet environments can be considered local characteristics of five different new associations; they are: (A) *Adianto capilli-veneris-Solenopsietum bivonae*, (B) *Adianto capilli-veneris-Solenopsietum madoniari*, (C) *Adianto capilli-veneris-Solenopsietum peloritanae*, (D) *Adianto capilli-veneris-Solenopsietum brutiae*, (E) *Adianto capilli-veneris-Solenopsietum meikleanae*. Their floristic composition, structure, ecology, and chorology are examined for each of them, and their nomenclatural type is provided.



A– *Adiantum capilli-veneris*–*Solenopsietum bivonae* ass. nov. hoc loco (Table 1, association A)

Holotypus: rel. 7, hoc loco.

Characteristic species: *Solenopsis bivonae* subsp. *bivonae*.

Structure and ecology: This association occurs at an elevation of 10–250 m a.s.l. in the calcareous rocky walls subject to dripping by groundwater, partially covered by a bryophytic carpet mainly represented by *Eucladium verticillatum*, *Pellia endiviifolia*, *Rhynchostegiella tenella*, and *Scorpiurum circinatum*. It is differentiated physiognomically by the dominance of *Solenopsis bivonae* subsp. *bivonae*, which with its leaf rosettes covers most of these small surfaces, usually mixing with *Adiantum capillus-veneris* and *Samolus valerandi*. The stands colonized by this vegetation are localized especially along water-courses in the cooler and shadier places.

Distribution: The association was surveyed along the Sosio river near Chiusa Sclafani, where it is quite frequent, and the Oreto River near Palermo, where, however, it is currently very rare.

B– *Adiantum capilli-veneris*–*Solenopsietum madoniari* ass. nov. hoc loco (Table 1, association B)

Holotypus: rel. 10, hoc loco.

Characteristic species: *Solenopsis bivonae* subsp. *madoniarum*.

Structure and ecology: This association is localized in a habitat very similar to those colonized by the previous one but linked to stands with higher elevation (700–1200 m a.s.l.). This vegetation shows a lower coverage of *Adiantum capillus-veneris* and a more developed bryophytic layer, characterized by *Eucladium verticillatum* and *Pellia endiviifolia*. This habitat is represented by vertical rocky walls with dripping waters coming from small springs.

Distribution: This association is quite rare, and was observed in a few mountain localities, like near Piazza Armeria and Petralia Soprana.

C– *Adiantum capilli-veneris*–*Solenopsietum peloritanae* ass. nov. hoc loco (Table 1, association C)

Holotypus: rel. 14, hoc loco.

Characteristic species: *Solenopsis bivonae* subsp. *peloritana*.

Structure and ecology: It is a sub-mountain association closely linked to metamorphic vertical rocky walls with dripping groundwaters at an elevation of 600–700 m a.s.l. The bryophytic layer is represented by *Pellia epiphylla* and *Conocephalum conicum*, where *Adiantum capillus-veneris*, *Solenopsis bivonae* subsp. *peloritana* and *Samolus valerandi* grow, often with high values of coverage.

Distribution: This association is exclusive of a small stand in the Tyrrhenian slope of the Peloritani range.

D– *Adiantum capilli-veneris*–*Solenopsietum brutiae* ass. nov. hoc loco (Table 1, association D)

Holotypus: rel. 17, hoc loco.

**Table 1.** (A) *Adiantum capilli-veneris*-*Solenopsis bivonae*; (B) *Adiantum capilli-veneris*-*Solenopsis madoniarum*; (C) *Adiantum capilli-veneris*-*Solenopsis peloritanae*; (D) *Adiantum capilli-veneris*-*Solenopsis brutiae*; (E) *Adiantum capilli-veneris*-*Solenopsis meikleanae*.

Relevé number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	*	23	24	25	26	27
Exposure	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	O	O	S	S	S	S	S	S	S	S	S
Elevation (m)	230	230	230	230	230	230	230	230	40	771	771	771	1166	600	670	700	130	130	130	1200	1200	1200	1200	1200	1200	1200	1200	1200
Surface (mq)	50	50	50	50	50	50	50	50	10	20	20	20	10	20	20	20	5	5	5	0.6	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.8
Coverage (%)	60	80	90	100	100	80	80	80	70	60	60	60	60	70	60	70	100	100	100	80	90	90	90	90	100	100	100	90
Association	A	A	A	A	A	A	A	A	A	B	B	B	B	C	C	C	D	D	D	E	E	E	E	E	E	E	E	E
Char. Association																												
<i>Solenopsis bivonae</i> subsp. <i>bivonae</i>	3	4	4	4	3	1	3	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Solenopsis bivonae</i> subsp. <i>madoniarum</i>	.	.	.	.	.	.	.	.	.	3	3	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Solenopsis bivonae</i> subsp. <i>peloritana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	3	3	2	.	.	.	.	.	.	.	.	.	.	.	.
<i>Solenopsis bivonae</i> subsp. <i>brutia</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	3	3	.	.	.	.	.	.	.	.	.
<i>Solenopsis meikleana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	3	3	2	3	2	2	2
<i>Carex troodi</i> Turill	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	+	1	1	1	1	1	.
Char. All. ( <i>Adiantum capilli-veneris</i> ) and Cl. ( <i>Adiantum capilli-veneris</i> )																												
<i>Adiantum capillus-veneris</i> L.	2	1	3	4	4	4	3	2	3	1	.	+	2	2	1	2	3	2	3	1	1	1	1	+	+	+	1	1
<i>Eucladium verticillatum</i> (With.) Bruch & Schimp.	.	1	.	.	.	+	+	1	2	2	2	1	1	.	.	+	2	2	2	3	2	2	2	3	3	3	1	2
<i>Samolus valerandi</i> L.	+	.	1	.	+	1	+	.	+	1	1	1	+	2	1	2	.	.	.	+	.	.	+	+	.	+	.	+
<i>Pellia epiphylla</i> (L.) Corda	.	.	.	.	.	.	.	.	.	.	.	.	.	2	+	1	2	2	1	2	4	2	1	2	3	4	3	3
<i>Pellia endiviifolia</i> (Dicks.) Dumort.	1	1	+	+	1	+	1	.	.	.	+	1	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Conocephalum conicum</i> (L.) Dumort.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	+	1	1	+	.	.	.	.	.	.	.	.	.	.
Other species																												
<i>Eurhynchium praelongum</i> (Hedw.) Schimp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	2	1	1	2	2	2	3	2	2	1	3
<i>Scorpiurum circinatum</i> Fleischer & Loeske	+	.	.	.	+	.	+	+	.	.	.	.	.	.	.	.	.	.	.	+	1	+	+	+	.	+	.	.
<i>Rhynchostegiella tenella</i> (Dicks.) Limpr.	+	+	.	.	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	+	1	.	.	.	1	.	.
<i>Hypericum hircinum</i> L.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.
<i>Eupatorium cannabinum</i> L.	.	.	.	+	.	+	+	.	+	.	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.
<i>Lotus tenuis</i> Waldst. & Kit. ex Willd.	.	+	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Equisetum arvense</i> L.	+	+	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Bryum</i> sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	.	.	.	.	.
<i>Mentha pulegium</i> L.	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Relevé number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Exposure	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	O	O	S	S	S	S	S	S	S	S
Elevation (m)	230	230	230	230	230	230	230	230	40	771	771	771	1166	600	670	700	130	130	130	1200	1200	1200	1200	1200	1200	1200	1200
Surface (mq)	50	50	50	50	50	50	50	50	10	20	20	20	10	20	20	20	5	5	5	0.6	0.5	0.5	0.4	0.4	0.5	0.5	0.8
Coverage (%)	60	80	90	100	100	80	80	80	70	60	60	60	60	70	60	70	100	100	100	80	90	90	90	100	100	100	90
Association	A	A	A	A	A	A	A	A	A	B	B	B	B	C	C	C	D	D	D	E	E	E	E	E	E	E	E
<i>Crepis leontodontoides</i> All.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Pulicaria dysenterica</i> (L.) Bernh.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Lysimachia nemorum</i> L.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.	.	.	.
<i>Agrostis stolonifera</i> L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
<i>Mycelis muralis</i> (L.) Dumort.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
<i>Brachypodium sylvaticum</i> (Huds.) P.Beauv.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
<i>Fissidens taxifolius</i> Hedw.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1	.	.	.	.	.	.	.	.	.	.	.
<i>Carex</i> sp.	.	.	1	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Potentilla reptans</i> L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	1	.	.	.	.	.	.	.	.
<i>Centaurium pulchellum</i> (Sw.) Druce	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Dittrichia viscosa</i> (L.) Greuter subsp. <i>viscosa</i>	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Chenopodium album</i> L.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Carex pendula</i> Huds.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
<i>Angelica sylvestris</i> L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.
<i>Helosciadium nodiflorum</i> (L.) W.D.J.Koch	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
<i>Viola alba</i> Besser subsp. <i>dehnhardtii</i> (Ten.) W.Becker	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
<i>Hypericum tetrapterum</i> Fr.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Rel. 1–8: Sosio River (Chiusa Sciafani), 10/07/2018  
 Rel. 9: Oreto River (Palermo), 29/07/2018  
 Rel. 10–12: Mt. Canalotto (Piazza Armerina), 16/10/2021  
 Rel. 13: Cataratta Spring (Petràlia Soprana), 15/07/2017  
 Rel. 14–16: Passo Pirtuso Valley, S. Lucia del Mela, 19/7/2020  
 Rel. 17–19: Lao River, Papasidero (Calabria), 6/08/2018  
 Rel. 20–27: Caledonia Waterfalls, Cyprus 06/10/1988



Characteristic species: *Solenopsis bivonae* subsp. *brutia*.

Structure and ecology: This association was surveyed on metamorphic wet rocky outcrops along the banks of perennial water-courses at an elevation of 130–350 m a.s.l. Physiognomically, this vegetation is dominated by *Adiantum capillus-veneris* and *Solenopsis bivonae* subsp. *brutia*, which grow on a well-developed bryophytic layer, characterized by *Pellia epiphylla*, *Eucladium verticillatum*, *Conocephalum conicum*, and *Eurhynchium praelongum*.

Distribution: This association was observed in North Calabria, along the banks of the lower reaches of the Lao river.

#### E– *Adiantum capilli-veneris*–*Solenopsietum meikleanae* ass. nov. hoc loco (Table 1, association E)

Holotypus: rel 22, hoc loco.

Characteristic species: *Solenopsis meikleana* and *Carex troodi* Turril.

Structure and ecology: This association usually is linked to wetlands represented mainly by waterfalls and dripping walls, often near the spring, where it grows on ophiolitic substrata at an elevation of 1000–1600 m a.s.l. The vegetation is localized prevalently in the stands not directly affected by the water flow, liking less damp surfaces. In the bryophytic layer, the more frequent species are *Eucladium verticillatum*, *Pellia epiphylla*, *Eurhynchium praelongum*, and *Scorpiurum circinatum*, while among the vascular plants, the endemic *Solenopsis meikleana* and *Carex troodi* are dominant, growing together with *Adiantum capillus-veneris*.

Distribution: This association is endemic to the western part of the island of Cyprus, which is localized in very specialized damp habitats.

As concerns *Solenopsis bivonae* subsp. *madoniarum*, in Sicily it is more widespread in the peatlands, an uncommon and peculiar habitat, currently exclusive of the mountain belt of Madonie massif at an elevation of 1200–1600 m a.s.l. In this area, the bog mosses dominated by *Sphagnum* sp. pl. are circumscribed to small surfaces with groundwater emerging or fed by springs, limitedly to highly acidic substrates with siliceous origin. These stands, locally known as tremulous lands, host a very specialized vegetation dominated by a thick and deep layer of *Sphagnum* species, which is here represented mainly by *S. auriculatum* Schimp. and *S. inundatum* Russow [= *S. obesum* (Wilson) Warnst], which are associated with *Aulacomnium palustre* (Hedw.) Swaegr., *Polytrichum commune* Hedw., *Bryum pseudotriquetrum* (Hedw.) P. Gaertn. et al., *Philonotis fontana* (Hedw.) Brid., *Callirgoniella cuspidata* (Hedw.) Loeske, etc. (Raimondo and Dia 1978; Raimondo et al. 2021). The phytosociological relevés carried out by some of the authors, always on the Madonie massif (Table 2), agree quite well with those previously published by Petronici et al. (1978) and Raimondo et al. (1980, 2021). This vegetation, where *Solenopsis bivonae* subsp. *madoniarum* grows together with the bryophytes mentioned above, was attributed by Raimondo et al. (2021) to a new association proposed as *Sphagno auriculati*–*Caricetum echinatae* and arranged in the *Caricion fuscae* Koch, 1926, an alliance of the *Scheuchzerio palustris*–*Caricetea fuscae* Tüxen, 1937. This class is distributed in the Euro–Siberian territory, reaching the Mediterranean region, limited to restricted mountain stands, which assume a relict meaning.

**Table 2.** *Sphagno auriculati-Caricetum echinatae*.

Relevé number	1	2	3	4	5	6	7	8	9	10
Elevation (dam)	138	138	138	138	138	138	140	140	140	140
Surface (mq)	5	5	2	4	4	4	5	2	2	4
Coverage (%)	100	100	100	100	100	100	100	100	100	100
Char. Association										
<i>Solenopsis bivonae</i> subsp. <i>madoniarum</i>	1	2	3	3	2	1	2	1	3	1
<i>Carex paniculata</i> L.	.	.	+	.	1	1	+	+	+	.
Char. All. ( <i>Caricion nigrae</i> ) and Ord. ( <i>Caricetalia nigrae</i> )										
<i>Aulacomnium palustre</i> (Hedw.) Swaegr.	1	2	+	+	2	1	1	3	2	2
<i>Carex punctata</i> Gaudin	+	+	.	1	1	1	1	.	.	+
Char. Cl. ( <i>Scheuchzeria-Caricetea nigrae</i> )										
<i>Sphagnum inundatum</i> Russow	3	3	2	2	3	4	3	3	3	4
<i>Carex echinata</i> Murray	4	2	3	3	1	3	1	2	2	2
<i>Carex demissa</i> Hornem.	+	.	1	+	+	.	+	.	+	.
<i>Polytrichum commune</i> Hedw.	2	1	.	.	2	.	.	1	1	.
<i>Deschampsia caespitosa</i> (L.) P.Beauv.	.	.	.	1	1	.	.	.	.	.
Other species										
<i>Juncus fontanesii</i> J. Gay	3	5	3	2	4	3	4	4	4	3
<i>Poa trivialis</i> L.	1	1	+	1	+	1	1	1	+	1
<i>Mentha aquatica</i> L.	+	1	+	2	1	1	2	3	2	1
<i>Holcus lanatus</i> L.	2	2	1	2	1	2	2	2	1	1
<i>Juncus conglomeratus</i> L.	1	2	3	2	2	1	3	2	2	1
<i>Festuca circumediterranea</i> Patzke	2	2	2	1	1	2	+	.	+	1
<i>Juncus striatus</i> Schousb. ex E.Mey.	.	1	1	+	+	2	1	1	2	1
<i>Bryum pseudotriquetrum</i> (Hedw.) P. Gaertn. et al.	1	.	1	1	1	1	1	1	1	+
<i>Hypericum tetrapterum</i> Fr.	.	.	.	2	1	+	1	2	2	1
<i>Dactylorhiza maculata</i> (L.) Soó subsp. <i>saccifera</i> (Brongn.) Diklić	1	2	2	2	2	2	.	.	.	.
<i>Carex remota</i> L.	.	+	.	+	.	1	.	+	.	+
<i>Carex ovalis</i> Gooden	.	.	.	.	.	1	1	+	+	+
<i>Bellis hybrida</i> Ten.	.	1	1	1	1	+	.	.	.	.
<i>Dactylis glomerata</i> L.	.	.	.	.	.	.	2	2	2	1
<i>Isolepis setacea</i> (L.) R.Br.	.	.	.	.	.	.	1	2	1	1
<i>Trifolium repens</i> L.	.	.	.	.	.	.	+	1	+	+
<i>Philonotis fontana</i> (Hedw.) Brid.	.	.	.	.	.	.	2	2	2	1
<i>Ranunculus fontanus</i> C.Presl.	.	.	.	.	.	.	+	+	1	.
<i>Cirsium creticum</i> (Lam.) d'Urv. subsp. <i>triumfettii</i> (Lacaita) K.Werner	.	+	.	+	+	.	.	.	.	.
<i>Pulicaria dysenterica</i> (L.) Bernh.	.	.	.	2	1	1	.	.	.	.
<i>Jungermannia gracillima</i> Sm.	1	.	+	.	.	1	.	.	.	.
<i>Lycopus europaeus</i> L.	.	.	.	.	.	.	+	2	1	.
<i>Utricularia australis</i> R.Br.	1	.	+	.	.	1	.	.	.	.
<i>Cynosurus cristatus</i> L.	.	.	.	.	.	.	1	1	1	.
<i>Galium palustre</i> L. subsp. <i>elongatum</i> (C.Presl) Arcang.	.	.	.	.	.	.	+	.	+	.
<i>Bechnum spicant</i> (L.) Sm.	+	.	.	.	.	+	.	.	.	.
<i>Lysimachia nemorum</i> L.	.	.	.	.	2	1	.	.	.	.

Rel. 1–10, Portella Mandarinini, Madonie, 31.7.1990

**Table 3.** Diagnostic characters of taxa belonging to *Solenopsis bivonae* group.

Taxa	<i>S. bivonae</i> subsp. <i>bivonae</i>	<i>S. bivonae</i> subsp. <i>madoniarum</i>	<i>S. bivonae</i> subsp. <i>peloritana</i>	<i>S. bivonae</i> subsp. <i>brutia</i>	<i>S. meikleana</i>	<i>S. bacchettiae</i>
Characters						
Leaf rosula diameter (cm)	2–12.5	3.5–8	4–10	2.5–7	2.5–11	3–10
Occurrence of stolons	no	no	no	no	yes	no
Leaf indumentum	glabrous	glabrous	glabrous	glabrous	glabrous to hairy	hairy
Leaf shape	spathulate	oblanceolate– spathulate	spathulate	oblanceolate– spathulate	oblanceolate– spathulate	oblanceolate– spathulate
Leaf length (mm)	12–100	15–45	12–55	10–58	10–75	12–60
Leaf petiole length (mm)	5–60	8–25	5–30	3–36	5–50	7–35
Leaf blade size (mm)	6–40 × 4–15	4–20 × 2–8	7–23 × 4–10	5–22 × 2–10	6–30 × 4–15	5–25 × 2–12
Floral pedicel length (mm)	5–11	2–5(9)	5.5–11	3–6	2–12	2.5–7.5
Bracteole number	1(2)	1	2	2	1–2	1–2
Bracteole size (mm)	2–2.4 × 0.3–0.5	1.8–2.2 × 0.1–0.3	3–5.5 × 0.4–0.7	2–3 × 0.25–0.45	2–8 × 0.2–0.6	3–5.5 × 0.4–0.5
Bracteole apex	hairy	few hairs	glabrous with one gland	glabrous with one gland	glabrous with one gland	hairy
Bracteole lateral glands	1–4	1–2	1–2	1–3	1–2	1–4
Calyx length (mm)	3–4	3–4	4–5	3.5–5	3.5	(3.5)4–6.5
Calyx lobes length (mm)	2–3	2–3.5	3.2–4	2–3	1.5–3	2–3.5
Corolla length (mm)	10–12	8.5–10	12–14.5	11–12	10–12	13–16
Corolla tube length (mm)	4–5	3.7–4.5	3.5–4	4.5–5	3–5	5–6
Corolla tube diameter (mm)	ca. 1	0.9–1.3	ca. 1.5	1–1.2	1.1–1.3	1–1.5
Corolla tube colour	lilac	lilac	green	white–lilac	green–violet	blue–lilac
Corolla upper lip shape	linear–lanceolate	linear–lanceolate	linear–lanceolate	linear–lanceolate	linear–lanceolate	ovate–lanceolate
Corolla upper lip size (mm)	3.5–4.5 × 1.3–1.7	3–4 × 1.2–1.7	5–6 × 2–2.4	4–4.5 × 1.4–1.8	3.5–4.5 × 1.5–1.7	5–7 × 2.4–4
Corolla upper lip papillae	yes	no	yes	yes	no	no
Corolla upper lip colour	bluish–lilac	bluish–lilac	dark lilac	bluish–lilac	pale blue to pale violet	dark blue–lilac
Corolla upper lip apex	acute	obtuse	acute	subobtuse	acute	obtuse
Corolla lower lip length (mm)	5–7	5–6	8–9	6.5–7	5–5.5	7–10
Corolla lower lip colour	bluish–lilac, white in central part	bluish–lilac, white in central part	bluish–lilac, white in central part	bluish–lilac, white in central part	pale blue to pale violet, white in central part	uniformly dark blue–lilac, rarely with a basal white alone
Corolla lower lip macula	greenish–yellow bordered of brown at base	yellowish, bordered of brown at base	yellow, bordered of red–brown above, with a central red– brown spot	greenish, with three distinct dark blue spots, bordered of brown	greenish–yellow	yellowish, 5 lobed, bordered of brown
Lobes of lower lip shape	ovate and mucronate	ovate and obtuse	obovate and mucronate	ovate and mucronate	oblong–obtuse, mucronate	widely ovate, mucronate
Lobes of lower lip size (mm)	3.5–4.5 × 3–4	2.5–3.5 × 1.6–2.5	4.5–6.5 × 4–4.5	3.5–5 × 2.5–3.5	2.5–3.5 × 1.4–2.2	5–8 × 3–5
Papillae of lower lip lobes	covering more than lower half	covering the lower half	covering almost until the apex	covering almost until the apex	covering more than lower half	covering only the throat
Papillae density	not very dense	very dense	very dense	not very dense	not very dense	very dense
Papillae length	0.25–0.6	0.1–0.24	0.1–0.4	0.16–0.6	0.05–0.3	0.1–0.2
Staminal filament length (mm)	4–4.5	4–4.5	4.5–4.7	5–5.5	3–5	5–7
Anther tube length (mm)	1.5–1.8	1.4–1.6	1.7–1.9	1.5–1.6	1–1.5	1.4–1.7
Anther tube basal papillae	no	yes	no	no	yes	no
Anther tube dorsal hairiness	yes	yes	yes	yes	yes	yes
Style length (mm)	4–4.5	4.5–5.5	6.5–7	6–6.5	3.5–4	6–8
Capsule length (mm)	1.6–2	2.7–3	2.5–3	2–3.3	3–3.2	3–4
Capsule surface	smooth	smooth	tuberculate	tuberculate	smooth	tuberculate
Seeds size (mm)	0.40–0.45 × 0.20–0.25	0.40–0.46 × 0.24–0.26	0.44–0.50 × 0.24–0.26	0.46–0.50 × 0.26–0.30	0.40–0.46 × 0.24–0.29	0.5–0.52 × 0.3–0.32



### Key to the taxa belonging to the *Solenopsis bivonae* group

Basing on the morphological diacritical characters listed in Table 3, the following analytical key has been performed.

- 1 Leaves always hairy; corolla uniformly dark blue–lilac, with upper lip ovate–lanceolate, 2.4–4 mm wide; papillae localized only in the throat..... ***S. bacchettae***
- Leaves glabrous, rarely subglabrous; corolla pale blue–violet to bluish–lilac with lower lip white in the basal part, with upper lip linear–lanceolate, 1.2–2.4 wide; papillae spread along the lips ..... **2**
- 2 Bracteoles 2–8 mm long; corolla tube green–violet; corolla lips pale blue to pale violet; corolla throat uniformly greenish–yellow; corolla lower lip with lobes oblong, anther tube 1–1.5 mm long; style 3.5–4 mm long ..... ***S. meikleana* sp. nov.**
- Bracteoles 1.8–2.5 mm long; corolla tube white–lilac to lilac; corolla lips bluish–lilac; corolla throat yellowish to greenish bordered of brown; corolla lower lip with lobes ovate; anther tube 1.4–1.9 mm long.; style 4–7 mm long ..... **3**
- 3 Bracteoles 3–5.5 mm long; corolla 12–14.5 mm long, with upper lip 5–6 mm long and lower lip 8–9 mm long; style 6.5–7 mm long..... ***S. bivonae* subsp. *peloritana***
- Bracteoles 1.8–3 mm long; corolla 8.5–12 mm long, with upper lip 3–4.5 mm long and lower lip 5–7 mm long; style 4–6.5 mm long..... **4**
- 4 Floral pedicel with one bracteole; corolla with lobes of the upper lip without papillae and lobes of lower lip 2.5–3.5 mm long and 1.6–2.5 mm wide; papillae up to 0.24 mm long; anther tube provided by basal papillae..... ***S. bivonae* subsp. *madoniarum***
- Floral pedicel with two bracteoles or rarely with one bracteole; corolla with lobes of upper lip partially covered by papillae and lobes of lower lip 3.5–5 mm long and 2.5–4 mm wide; papillae up to 0.6 mm long; anther tube without basal papillae ..... **5**
- 5 Floral pedicel 3–6 mm long; bracteoles glabrous at the apex; lower lip of corolla with macula bordered with three distinct dark blue spots; staminal filament 5–5.5 mm long; style 6–6.5; capsule 2–3.3 mm long ..... ***S. bivonae* subsp. *brutia***
- Floral pedicel 5–11 mm long; bracteoles hairy at the apex; lower lip of corolla with macula without dark spots; staminal filament 4–4.5 mm long; style 4–4.5, capsule 1.6–2 mm long..... ***S. bivonae* subsp. *bivonae***

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## Additional information

### Conflict of interest

No conflict of interest was declared.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: GPGG, CB, VT, PM, SB. Data curation: SC, SB. Formal analysis: CB, SB. Funding acquisition: PM. Investigation: CB, SB, GPGG, SC, AC, VT. Methodology: GS, VT, SC, SB. Project administration: SB. Resources: SB. Software: CB, GS. Supervision: GPGG, SB, CB, PM. Writing - original draft: SB. Writing - review and editing: CB, SC, PM, SB, VT, GPGG.

### Author ORCIDs

Salvatore Brullo  <https://orcid.org/0000-0003-2568-7278>

Salvatore Cambria  <https://orcid.org/0000-0002-3828-1552>

Valeria Tomaselli  <https://orcid.org/0000-0001-9121-9558>

Alessandro Crisafulli  <https://orcid.org/0000-0003-4398-345X>

Pietro Minissale  <https://orcid.org/0000-0002-4047-4169>

Gianpietro Giusso del Galdo  <https://orcid.org/0000-0003-4719-3711>

### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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# *Anthemis* sect. *Hiorthia* (Asteraceae) on Kriti Island, Greece: high ploidy levels and a new species

Katerina Goula<sup>1</sup>, Theophanis Constantinidis<sup>1</sup>

<sup>1</sup> Section of Ecology & Systematics, Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, 15784 Athens, Greece  
Corresponding author: Katerina Goula ([agoula@biol.uoa.gr](mailto:agoula@biol.uoa.gr))

## Abstract

A morphological and karyological investigation of the *Anthemis* sect. *Hiorthia* representatives of Kriti (Greece) revealed that three different species are found on the island, all endemic, and each characterised by a different ploidy level based on the haploid series of  $x = 9$ . *Anthemis abrotanifolia*, the species with the widest distribution, is tetraploid with  $2n = 4x = 36$ . *A. samariensis*, a local endemic of the Lefka Ori, was found being decaploid, with  $2n = 10x = 90$ , the highest number ever recorded in *Anthemis*. The recently discovered population on Mt. Kedros (south-central Kriti) is morphologically distinct from all the *Anthemis* entities growing on Kriti; it also differs from the variable and widespread *A. cretica* group. It is here described as a new species, *A. pasiphaes* Goula & Constantinidis. It is a hexaploid, with  $2n = 6x = 54$ . All chromosome numbers are reported for the first time. Polyploidy might have acted as a reproductive barrier among these perennial species, complementing isolation by spatial distance and evolutionary divergence. Further, it might have contributed adaptation advantages to these three predominately mountain species.

**Key words:** Anthemideae, chromosomes, Greek endemic, karyology, Mediterranean area, taxonomy



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## Introduction

Among the biodiversity hotspots in the Mediterranean Basin, Kriti or Crete (Greece) has a prominent position (Médail 2017). No fewer than 2240 plant taxa have been recorded on this large continental island (Kougioumoutzis et al. 2020), where the percentage of endemism is the highest known in Greece (Dimopoulos et al. 2013). The island's geographical isolation, permanent since the early Pliocene (Greuter 1972; Sakellariou and Galanidou 2016), combined with mountain isolation due to Kriti's uplifting and rugged topography, have played a significant role in plant endemism (Legakis and Kypriotakis 1994). The mountains of Kriti, in particular, have served both as refugia for old montane species and as cradles for plant diversification (Greuter 1972; Trigas et al. 2013).

One of the richest families in endemics on the island is Asteraceae, with at least 29 taxa endemic to Kriti, 13 of them restricted to mountainous areas (see Strid 2016b). With respect to the genus *Anthemis*, there are 14 taxa on the island (Strid 2016b; Goula and Constantinidis 2021); five of them, i.e., *A. abrotanifolia* (Willd.) Guss., *A. filicaulis* (Boiss. & Heldr.) Greuter, *A. glaberrima* (Rech.f.)

Greuter, *A. samariensis* Turland, and *A. tomentella* Greuter, being regional endemics confined to Kriti and the nearby islets (Strid 2016b). *Anthemis* sect. *Hiorthia* (DC.) R.Fernandes, formed mainly by perennial species of high altitude (Oberprieler 1998), was represented up to now by two endemic species in Kriti: *A. abrotanifolia* and *A. samariensis*. The latter is the *Anthemis* species that was most recently described on the island (Turland 2008). This chasmophyte was initially found in only two adjacent but distinct localities in the Lefka Ori, the richest area of Kriti in numbers of local endemic taxa (Montmollin and Iatrou 1995). The two subpopulations seem to face no threats by human activities or grazing. However, the species was assessed as “Vulnerable” according to the IUCN Red List criteria, due to its very restricted distribution area (Turland 2009). Five years after the description of *A. samariensis*, another population of a perennial *Anthemis* was discovered by Vangelis Papiomytoglou on Mt. Kedros, approximately 58 km as the crow flies ESE of the type locality of *A. samariensis* in the Lefka Ori. It was reported by Strid and Tan (2017) as conspecific with *A. samariensis*. Mt. Kedros is located in the central-southern part of Kriti, reaching 1776 m a.s.l., and is closer to Mt. Psiloritis (2456 m, the highest mountain of Kriti) than to the Lefka Ori.

In 2018, during field work focused on the taxonomic diversity of Greek *Anthemis*, the first author visited Mt. Kedros to collect material from this particular population. When this material was compared to *A. samariensis* from the *locus classicus*, the extent of the noticed morphological divergence led us to a more thorough examination of the samples. In addition, a karyological survey of the Mt. Kedros population, as well as those of *A. abrotanifolia* and *A. samariensis*, was carried out in order to explore and understand chromosome diversity and ploidy levels of all *A. sect. Hiorthia* members found in Kriti. The results are presented in this study.

## Materials and methods

Plant material, which included flowering and fruiting samples, was collected during two field trips on Mt. Kedros, in spring and summer of 2018. Dried specimens prepared from these samples were deposited in ATHU (the acronym follows Thiers 2022, continuously updated) and were examined thoroughly, in comparison with specimens of *Anthemis samariensis* from its *locus classicus* preserved at the herbarium of the Mediterranean Agronomic Institute of Chania (MAIC). The morphological diversity within the *A. sect. Hiorthia* members was also studied based on specimens collected between 2017 and 2021 (Goula, unpublished material), as well as specimens and digital specimen images provided by the herbaria ATH, ATHU, B, BM, BR, E, GOET, JE, K, MNHN, MO, NHMC, P, TAU, TAUF, UPA, W, WAG, and WU. The concept of the *A. cretica* entities and the protologues of subspecific taxa attributed to this name, together with descriptions and nomenclatural comments, were studied in historic and recent literature (Linnaeus 1737, 1753, 1763; Bieberstein 1808; Boissier 1875; Eig 1938; Fernandes 1975; Grierson and Yavin 1975; Fernandes 1976; Franzén 1986; Franzén 1991; Greuter et al. 2003; Greuter 2006+; Turland 2008; Strid 2016a, b).

In order to investigate the chromosome numbers of *Anthemis* sect. *Hiorthia* of Kriti, mature achenes originating from populations of *A. abrotanifolia* on Mt. Psiloritis, *A. samariensis* on the Lefka Ori and plants of Mt. Kedros were cultivated in pots at the facilities of the Department of Biology, National and Kapodistrian University of Athens. Root tips from the seedlings were treated with a combined

cycloheximide 0.0009% and 8-hydroxyquinoline 0.0006% solution for three hours, fixed in Carnoy's solution for at least 24 hours and stored in ethanol 70% at -20 °C. To obtain photographs of metaphase plates, root tips were hydrolyzed in HCl 1N at 60 °C for 12 minutes, placed in Feulgen stain for up to two-and-a-half hours and squashed over microscope slides with a few drops of acetic acid 45%. Idiograms were constructed from photographs of at least three different metaphase plates (Goula and Constantinidis 2021). Construction of the idiograms was conducted using the KaryoType software, ver. 2.0 (Altınordu et al. 2016).

## Results

### Morphology

The morphological characters of the population on Mt. Kedros made it stand out as different from all known taxa of *Anthemis* sect. *Hiorthia* from Kriti. Its closest relative, regarding morphology, seems to be *A. samariensis*, although it also appears to share common features with taxa of the variable *A. cretica* group from the Greek mainland and Anatolia. The morphological differences among members of *A.* sect. *Hiorthia* of Kriti are summarised in Table 1. *Anthemis cretica* subsp. *cretica*, the member of the *A. cretica* group morphologically and geographically nearest to the Mt. Kedros population, has also been added for comparison reasons.

**Table 1.** Morphological differences between the *Anthemis* sect. *Hiorthia* members of Kriti Island and *A. cretica* subsp. *cretica* from Peloponnisos (Greece). All measurements in mm.

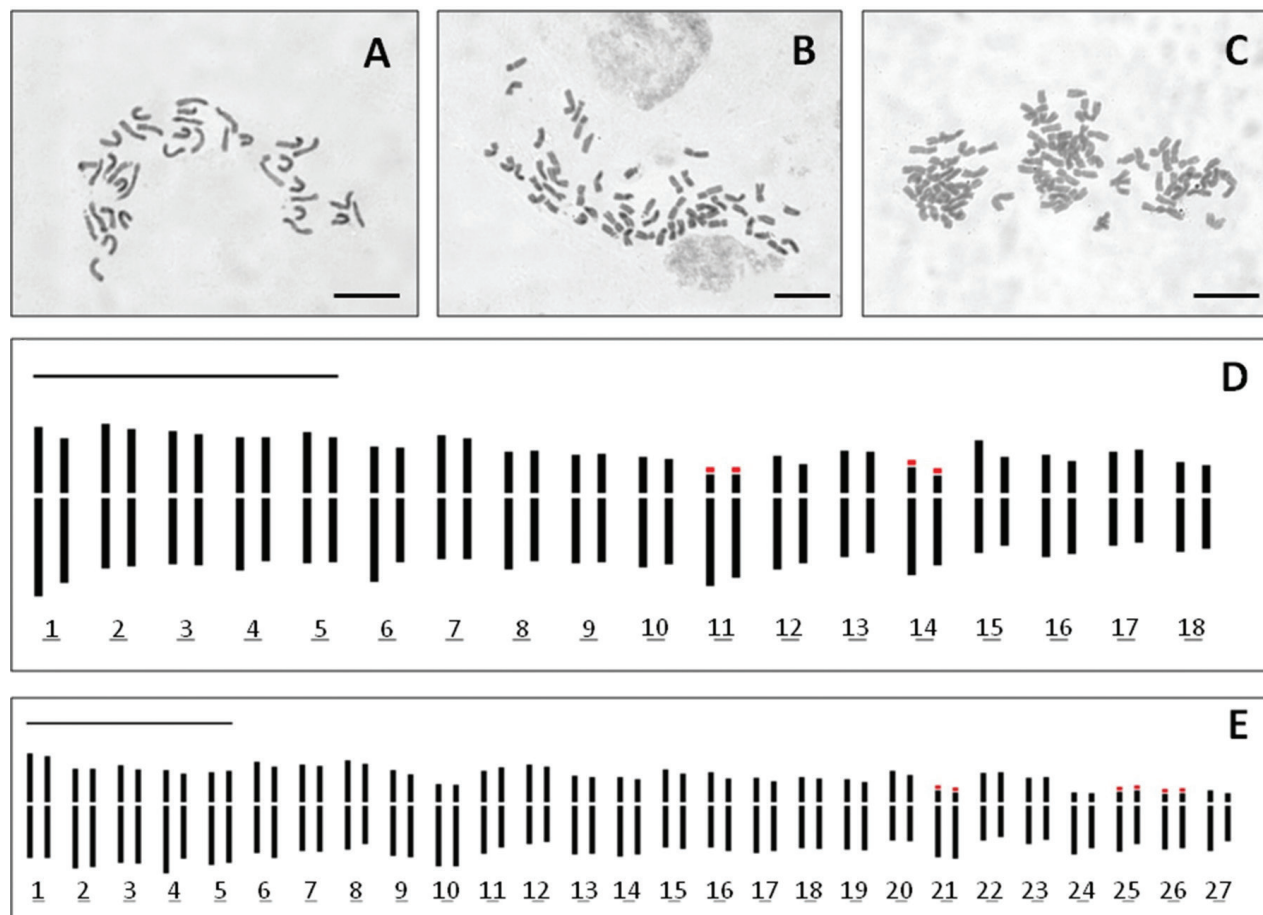
		<i>Anthemis abrotanifolia</i>	<i>Anthemis samariensis</i>	Mt. Kedros population	<i>Anthemis cretica</i> subsp. <i>cretica</i>
Stem indumentum		sericeous	usually glabrous to subglabrous	woolly	sericeous
Leaf indumentum		sericeous	villous	woolly	sericeous
Lobed leaves along stem		present	usually absent	present	present
Leaf dimensions	Outline	15–40 × 10–20	20–45 × 15–20	20–30 × 14–16	15–40 × 8–15
	Petiole width	0.3–0.6	1.5–2	0.5–1	0.3–0.6
	Ultimate lobes width	0.3–0.8	1–1.8	0.7–1.5	0.3–0.6
Number of primary leaf segments		5–7	usually 7	usually 7	(7–)9–15
Involucral bracts dimensions	Length	3–5	4–6	3.5–5	2–5
	Width	0.8–1.4	2–2.5	1.2–1.5	1.3–2
	Margin width	ca. 0.1	0.3–0.5	0.1–0.3	ca. 0.1
Receptacular scales	Length	3–3.5	6.5–7	4–6	3.5–4
	Width	0.9–1.3	ca. 1	0.7–1	ca. 1
	Apex	truncate to cuneate	emarginate	usually cuneate	cuneate
	Arista	0.1–0.2	ca. 1	1.5–2	0.5
Number of ligulate florets		0–8	8–14	14–20	14–16(–20)
Ligulate florets dimensions	Tube length	1.6–2	1.5–2	2–2.5	1.8–2
	Tube width	0.5–0.7	0.5–0.7	ca. 1	0.5–0.7
	Ligule width	1–2.5	5–6.5	3–5	3–4
Achene dimensions	Length	1.3–2	(2.1–)2.3–2.5(–2.8)	1.8–2.6	1.4–2
	Width	0.5–0.8	0.8–1	0.5–0.8	0.8–1
Pappus dimensions	Corona	0.1	0.2	0.2–0.4	0.3–0.6
	Auricle	absent	1.5	0.5–1	0.3–0.6
Pappus on achenes of ligulate florets		acute, dentate rim with 2–3 larger teeth	3-lobed auricle	denticulate auricle	denticulate to lobed oblique corona

In addition to the morphological differences of Table 1, the Mt. Kedros *Anthemis* and the variable *A. cretica* group also exhibit two noteworthy qualitative dissimilarities, as follows: a) the stem leaves of the Mt. Kedros population are clearly petiolate, whereas the stem leaves of *A. cretica* have a pair of lobes at or near their rachis base, thus appearing as almost sessile, and b) the receptacular scales of the Mt. Kedros population are aristate, in contrast to the acute to acuminate scales of the *A. cretica* group. A comparison of the Mt. Kedros population with the morphologically most relevant *A. cretica* subspecies, i.e., *A. cretica* subsp. *cretica*, and also *A. cretica* subsp. *carpatica* (Willd.) Grierson and *A. cretica* subsp. *cassia* (Boiss.) Grierson, reveals additional qualitative and quantitative differences. *Anthemis cretica* subsp. *cretica*, particularly those populations from the mountain areas of Peloponnisos that are geographically closest to Kriti, differ further in that they bear a large number of primary leaf segments (up to 15 vs. usually 7 in the Mt. Kedros *Anthemis*) with much narrower ultimate lobes (0.3–0.6 mm vs. 0.7–1.5 mm in the Mt. Kedros *Anthemis*), narrower involucre (7–11 mm vs. 10–12 mm in the Mt. Kedros *Anthemis*), a conical, acute receptacle, smaller outer and inner achenes (1.4–2 mm with a pappus up to 0.6 mm vs. 1.8–2.6 mm with a pappus up to 1 mm in the Mt. Kedros *Anthemis*), and in the shape of the corona on the achenes of the ligulate florets (see Fig. 4, F1 & F3). *Anthemis cretica* subsp. *carpatica* differs from the Kedros population in its indumentum, varying from totally glabrous to slightly sericeous, and the completely different pappus shape, consisting of an acute rim or a very small, ca. 0.2 mm corona, with no auricle. *Anthemis cretica* subsp. *cassia* from E and S Anatolia, Syria and Lebanon (Grierson and Yavin 1975; Greuter 2006+) has wider ultimate leaf lobes (usually 2–3.5 mm vs. 0.7–1.5 mm in the Mt. Kedros *Anthemis*), shorter pappus on the achenes (0.5 mm vs. up to 1 mm in the Mt. Kedros *Anthemis*), whereas its involucre bracts margin may be pale, in contrast to the Mt. Kedros population with its always dark bract margin.

## Karyology

The examination of metaphase plates of the three *Anthemis* sect. *Hiorthia* members of Kriti revealed three distinct ploidy levels. *Anthemis abrotanifolia* from Mt. Psiloritis was found to be tetraploid with  $2n = 4x = 36$  (Fig. 1a). The Mt. Kedros population was found to be hexaploid with  $2n = 6x = 54$  (Fig. 1b). All the cultivated plants of *A. samariensis* from the Lefka Ori were found to be decaploid with the surprising number of  $2n = 10x = 90$  (Fig. 1c). The chromosome numbers of all taxa are reported here for the first time. The decaploid chromosome level was unknown up to now in *Anthemis* and is therefore reported here as new. The large chromosome number of *A. samariensis* complicated its idiogram construction and the detailed study of chromosome morphology. The idiograms of *A. abrotanifolia* and the Mt. Kedros population are shown in Fig. 1 (1d and 1e, respectively). The karyotype of *A. abrotanifolia* consists of 22 metacentric, ten submetacentric and four subtelocentric chromosomes that bear satellites (karyotype formula:  $2n = 4x = 22m + 10sm + 4st^{4sat}$ ). The karyotype of the Mt. Kedros population consists of 24 metacentric, 18 submetacentric and 12 subtelocentric chromosomes. Six of the latter bear satellites (karyotype formula:  $2n = 6x = 24m + 18sm + 12st^{6sat}$ ). In





**Figure 1.** Metaphase plates and idiograms of *Anthemis* sect. *Hiorthia* members in Kriti **A** *A. abrotanifolia* from Mt. Psiloritis **B** *Anthemis* population from Mt. Kedros **C** *A. samariensis* from Lefka Ori **D** idiogram of *A. abrotanifolia* **E** idiogram of Mt. Kedros population. Scale bars: 10 µm.

Greek representatives of *A. sect. Hiorthia*, tetraploids are more common. The karyotypes of the Greek tetraploid ( $2n = 4x = 36$ ) *A. cretica* populations (various subspecies) also consist of 24 metacentric chromosomes, but usually with four submetacentric and eight subtelocentric chromosomes that bear four to six satellites (Goula, unpublished data). The only hexaploid *A. cretica* subspecies in Greece (*A. cretica* subsp. *carpatica*) has a different karyotype structure from that of the Mt. Kedros population, formulated as  $2n = 6x = 28m + 14sm + 10st^{2sat} + 2t^{2sat}$ . *Anthemis cretica* subsp. *cretica* from Peloponnisos is tetraploid with a karyotype formula of  $2n = 4x = 24m + 6sm + 6st^{6sat}$  (Goula, unpublished data).

## Discussion

The morphological distinction of the Mt. Kedros *Anthemis* population from the other two members of *A. sect. Hiorthia* of Kriti and the related *A. cretica* group, coupled with the different ploidy levels revealed in our study, allow the recognition of a new species described here as *Anthemis pasiphaes* Goula & Constantinidis (see below). According to current knowledge, this new species is endemic to Mt. Kedros (Fig. 2) and adds a new narrow endemic to the flora of Kriti. As a rule, local and regional Greek endemics are more common in the southern

parts of the country, particularly Kriti and Peloponnisos, following an increase of the endemism rate observed in a north to south direction (e.g., Georghiou and Delipetrou 2010). The center of *Anthemis* diversity is in SW Asia (Lo Presti et al. 2010) and that of the heterogenous *A. cretica* group, to which both *A. samariensis* and *A. pasiphaes* presumably link, is apparently in Anatolia (Franzén 1991).

In order to decide on the taxonomic position of *Anthemis pasiphaes* we considered the discussion provided by Turland (2008) in the case of *A. samariensis* and the arguments presented below. We concluded that *A. pasiphaes* would better be kept as a separate species and not fall under the variability of the *A. cretica* group for the reasons explained below.

- a. Geological evidence indicates that Kriti has been permanently isolated from continental Greece and SW Asia since the early Pliocene, about 4 mya. By that time, it appears that the *Anthemis cretica* clade had already been separated from the rest of *Anthemis*, although diversification within its clade began about 2 mya (Lo Presti and Oberprieler 2009). It is plausible that the speciation of the *A. sect. Hiorthia* members on Kriti (*A. abrotanifolia*, *A. pasiphaes*, *A. samariensis*) was the result of geographical vicariance events of a local clade. Allopatric speciation due to isolation in mountain ranges drove diversification in other groups of Asteraceae rich in endemic species as well (e.g. *Centaurea*, López-Vinallonga et al. 2015).
- b. The *Anthemis cretica* assemblage is a diverse group of taxa with a complicated phylogenetic, taxonomic, and nomenclatural history. Concepts related to the group have changed over the decades. Most of the nowadays accepted subspecific entities classified under *A. cretica* appeared as new combinations in Grierson (1975). Grierson (1974) delved into the labyrinth of the historic literature on *A. cretica* and elucidated several species names, which, as he characteristically mentioned “had suffered a history of misapplication”. However, the origin and taxonomic category of the *A. cretica* lectotype specimen (Herb. Clifford: 415, *Anthemis* 2, BM000647187!) remains unresolved. Linnaeus (1763), cited “Habitat in Italia Helvetia” as the origin area of the species (under *A. montana* L., an illegitimate replacement name for *A. cretica*), whereas Franzén (1986), after examining material from the entire *A. cretica* distribution area, considered the East Aegean Islands as a more probable origin area. Furthermore, it is not yet clarified whether the Linnaean lectotype specimen was collected from a wild population or belongs to a cultivated specimen, i.e., it possesses possible distorted characters. According to the points presented above, the concept of the *A. cretica* needs further elucidation and is rather built on a shaky foundation. As of today, the number and rank of taxa within the *A. cretica* collective species are not yet fully resolved and large databases are not in full accordance. Euro+Med Plantbase (Greuter 2006+), for example, accepts 25 subspecies within *A. cretica*, compared to the World Flora Online (WFO 2022), which accepts 23 subspecies. Morphological differences between infraspecific *A. cretica* entities and certain corresponding species of *A. sect. Hiorthia* may be quite vague. For example, our field experience with some *Anthemis* populations of N Greece, particularly those of high mountains, makes distinguishing



**Figure 2.** Distribution of *Anthemis* sect. *Hiorthia* on Kriti Island. Pink dot: *A. samariensis*, red dot: *A. pasiphaes*, yellow dots: *A. abrotanifolia*. Based on Strid (2016b) and additional specimens in ATHU, MAIC and NHMC. Background map data: Google, SIO, NOAA, U.S. Navy, NGA, GEBCO.

between *A. cretica* subsp. *carpatica* and *A. pindicola* Halácsy problematic. Grierson (1975) himself characterised his *A. cretica* group classification as “possibly oversimplified” and “tentative” and underlined the necessity of a biosystematic study within the group. The inclusion of new taxa within an even broader *A. cretica* complex would add intricacy to the whole structure. Cutting-edge molecular tools, when used, would presumably help in elucidating phylogeny and would offer a classification scheme in compliance with evolutionary patterns.

- c. Polyploidy is one of the reproductive barriers responsible for isolating plant populations, and at the same time a driving force of speciation (Rieseberg and Willis 2007). Within the Mediterranean Basin in particular, polyploidy has played a major role in the diversification of several plant genera (e.g., Tomasello and Oberprieler 2022). In *Anthemis*, polyploidy has been recorded almost exclusively in *A. sect. Hiorthia*, where it is evolutionarily important (Kuzmanov et al. 1981). Hexaploids ( $2n = 6x = 54$ ) and octoploids ( $2n = 8x = 72$ ) have been recorded in only one representative of this section: *A. cretica* subsp. *carpatica* (Küpfer 1974; Baltisberger 1993). In Greece, tetraploid cytotypes ( $2n = 4x = 36$ ), along with the typical diploid number ( $2n = 2x = 18$ ), are more common, but hexaploid cytotypes also occur within the *A. cretica* complex (Goula and Constantinidis 2019). In our case, the three different, high ploidy levels of the *Anthemis* on Kriti (*A. abrotanifolia*, *A. pasiphaes*, *A. samariensis*) corroborate their morphological differentiation forming reproductive barriers and thus supporting their specific rank.

Incidence of polyploidy in plants depends on various factors, among them the climate and the life form (Rice et al. 2019). High chromosome numbers are more common within certain families, e.g., Asteraceae (Semple and Watanabe 2009). Within tribe Anthemideae, in particular, several genera have been reported to form extensive polyploid complexes as, e.g., *Leucanthemum* with ploidy levels varying from 2x to 22x (see Oberprieler et al. 2009). The higher genetic diversity provided through polyploidy improves environmental adaptation and tolerance, resulting in the ability of plants to colonise and be successful in harsh environmental contexts (Meudt et al. 2021). Polyploidy in the three *Anthemis* sect. *Hiorthia* representatives of Kriti, restricted to calcareous stony slopes (*A. abrotanifolia*) or cliffs in mountain regions (*A. pasiphaes* and *A. samariensis*), seems to offer an advantage in adapting to and surviving in hostile habitats.

### Taxonomic treatment

#### ***Anthemis pasiphaes* Goula & Constantinidis, sp. nov.**

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**Diagnosis.** Member of *Anthemis* sect. *Hiorthia* related to *A. samariensis*, but differing in its woolly indumentum, presence of lobed leaves on flowering stems, longer aristae (1.5–2 mm) on receptacular scales, and presence of denticulate auricle on achenes of ligulate florets.

**Type.** GREECE. Kriti: Nomos Rethimnou, Eparchia Amariou. Mt. Kedros, ca. 2 km linear distance S of Gerakari village, vertical limestone rocks facing N, on the northern slopes of the mountain, 1265 m a.s.l., 35°11'N, 24°36'E, 29 April 2018, Goula, Kofinas, Papanikolaou & Papiomytoglou 2379 (holotype, ATHU). Figs 3–5.

**Description.** Perennial herb with stock covered in last year's leaf sheaths. Indumentum woolly,  $\pm$  appressed, hairs medifixed. Glands present in most parts of plant. Stems simple or branched; leafy non-flowering shoots present at anthesis. Flowering stems decumbent to erect, simple, 10–25 cm tall, angled, woolly, greyish-green, with successively smaller and less dissected leaves up to middle, and entire, scale-like leaves up to almost below capitulum. Leaves somewhat aromatic with golden stalked glands on leaf surface, greyish-green, up to 6 cm long, with both surfaces woolly; petiole up to 3 cm long and 0.5–1 mm wide; leaf blade 2-pinnatisect, ovate in outline, 2–3 cm  $\times$  ca. 1.5 cm; primary segments usually 7, each one divided into 2–5 ultimate lobes; ultimate lobes narrowly oblanceolate to obovate, 0.7–1.5 mm wide, apex subacute with minute cartilaginous cusp, usually hidden below the dense trichomes. Capitulum solitary, radiate. Involucre hemispherical, 10–12 mm wide. Involucral bracts imbricate, greyish-green, lanceolate, 3.5–5  $\times$  1.2–1.5 mm, outer surface villous with dark green or dark brown midvein; margin dark brown, 0.1–0.3 mm wide, membranous, densely and minutely lacerate, apex dark brown to black, acute to acuminate. Receptacle hemispherical becoming hemispherical-conical, apex obtuse. Receptacular scales narrowly oblanceolate, navicular, 4–6  $\times$  0.7–1 mm, scarious, apex usually cuneate or emarginate, midvein straw coloured, prominent, leading to arista (1–)1.5–2 mm long. Ligulate florets 14–20; tube green, cylindrical,



2–2.5 mm × ca. 1 mm; ligules patent at anthesis, later reflexed, white, oblong to oblong-obovate, 10–15 × 3–5 mm, spotted with sessile glands. Disk florets yellow, spotted with sessile glands; tube cylindric, 3–3.5 mm long (including the lobes), 0.5–0.8 mm wide; lobes 5, triangular, 0.5–0.7 mm long; lower part of disk florets swollen and spongy at maturity. Achenes straw-coloured, narrowly obconic-oblong. Achenes of disk florets weakly 4-angled, slightly curved, 1.8–2.5 mm long, excluding pappus, 0.5–0.8 mm wide, more or less longitudinally ribbed; pappus oblique, forming short lacerate corona 0.2–0.4 mm wide and lacerate auricle adaxially; auricle scarious, 0.5–0.8 mm long, densely and finely longitudinally veined. Achenes of ligulate florets more curved and more prominently ribbed, 2.3–2.6 mm long excluding pappus, surface characters as in achenes of disk florets, but additionally sessile glands present; pappus as in achenes of disk florets, but auricle entire, 0.8–1 mm long, with lacerate apex.  $2n = 6x = 54$ .

**Distribution and habitat.** *Anthemis pasiphaes* is apparently endemic to Mt. Kedros, restricted to its northern part (Fig. 2). It grows on steep, calcareous cliffs, mostly inaccessible even to the numerous goats that graze the area. Currently known only from the type locality, at 1265 m a.s.l., but presumably also occurring higher up, on the same slope. It grows together with other endemics of Kriti, like *Crepis auriculifolia* Spreng., *Dianthus juniperinus* subsp. *pulviniformis* (Greuter) Turland, *Erysimum raulinii* Boiss., *Lomelosia albocincta* (Greuter) Greuter & Burdet, *Sesleria doerfleri* Hayek, and *Stachelina petiolata* (L.) Hilliard & B.L.Burt.

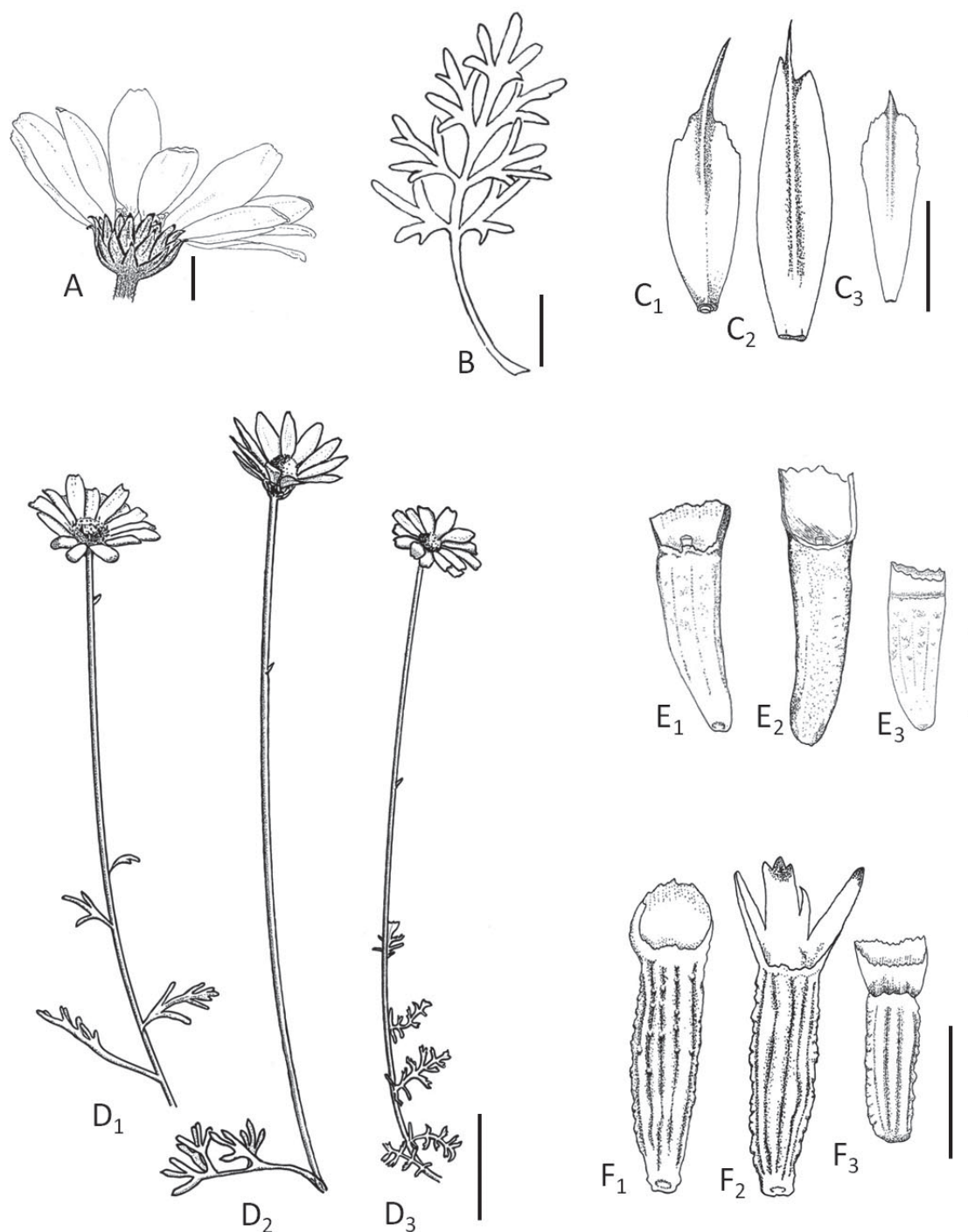
**Etymology.** The specific epithet derives from the female name Pasiphaë and consists of the Greek words *πάσι* (= all) and *φάος/φῶς* (= light), i.e., “she who illuminates everyone”. Pasiphaë was daughter of Helios (god of the Sun), wife of King Minos, Queen of Kriti and immortal, according to Greek mythology.

**Phenology.** Flowering from late April to early June; fruiting from June to July.

**Conservation status.** *Anthemis pasiphaes* is currently known from the type locality only. This single population is considerably small, with no more than 50 individuals counted, and restricted to practically inaccessible cliffs. The species has not been recorded elsewhere, although there are several similar habitats around in Kriti, which is botanically one of the best explored regions of Greece (Strid and Tan 2017). Potentially suitable localities on Mt. Kedros have not revealed any additional populations so far. The presence of grazing animals in the area is very apparent, limiting the small population to very steep cliffs. Neither mature individuals nor leaf rosettes were observed in localities accessible to goats. A rock-climbing area on the southern slopes of Mt. Kedros is not a threat to *A. pasiphaes* at present; however, the northern part of the mountain is also suitable for rock-climbing activities that would potentially destroy the only known population. Although we counted a very small number of mature plants, it is possible that more plants are present, very locally, on cliffs surrounding the *locus classicus*, given that they are not accessible to goats and retain enough moisture and some shade to permit uninterrupted growth of *A. pasiphaes*. If distribution and frequency of mature plants follow the same patterns we observed during field work, we may then estimate with some certainty that the total population of the species is fewer than 250 mature individuals. Therefore, the species meets the Criterion D



Figure 3. Holotype of *Anthemis pasiphaes* Goula & Constantinidis (ATHU).



**Figure 4.** *Anthemis pasiphaes* Goula & Constantinidis and comparison with *A. samariensis* and *A. cretica* subsp. *cretica* **A** capitulum **B** leaf **C** receptacular scales **D** flowering stems **E** achenes of disk florets **F** achenes of ligulate florets **C**<sub>1</sub>–**F**<sub>1</sub> *A. pasiphaes* **C**<sub>2</sub>–**F**<sub>2</sub> *A. samariensis* **C**<sub>3</sub>–**F**<sub>3</sub> *A. cretica* subsp. *cretica*. Scale bars: 5 mm (**A**); 1 cm (**B**); 2 mm (**C**, **F**); 3 cm (**D**); 1 mm (**E**). Drawn by N.A. Katsaros. *A. pasiphaes* was drawn from the holotype (Goula et al. 2379) and Goula & Katsaros (2644), both in ATHU, *A. samariensis* from material collected from the type locality (Ap. Kal. 9685, MAIC) and *A. cretica* subsp. *cretica* from material collected on Mt. Parnonas (Goula & Katsaros 2610, ATHU).

(number of mature individuals <250) following the IUCN Guidelines for the assessment of taxa known only from the type locality (IUCN Standards and Petitions Committee 2022). The IUCN Red List category of Endangered seems suitable (EN D).





**Figure 5.** *Anthemis pasiphaes* at its locus classicus **A** plant growing on a vertical rock **B** capitulum. Photo taken on 29.04.2018 by K. Goula. Scale bars: 5 cm (**A**); 5 mm (**B**).

### A revised key to genus *Anthemis* and the related genus *Cota* from Kriti (including surrounding islets)

- 1 Achenes compressed, rhombic in transverse section; leaf segments pectinate.....**2**
- Achenes not compressed, orbicular or sub-quadrangular in transverse section; leaf segments usually not pectinate.....**3**
- 2 Receptacular scales straw colour at maturity; plant usually erect .....**C. altissima**
- Receptacular scales purplish-brown to almost black at maturity; plant usually procumbent.....**C. melanolepis**
- 3 Plant annual, non-flowering shoots absent at anthesis.....**4**
- Plant perennial, non-flowering shoots present at anthesis .....**13**
- 4 Receptacle without scales.....**5**
- Receptacle with scales present at least on upper part.....**6**
- 5 Leaves somewhat fleshy, lobes obtuse; ligules absent; achenes caducous, with a ca. 0.5 mm long corona ..... **A. ammanthus** subsp. **ammanthus**
- Leaves not fleshy, lobes acute; ligules occasionally present; achenes with a ca. 1 mm long corona, outer achenes persistent, inner caducous..... **A. filicaulis**
- 6 Receptacle without scales in lower part; achenes cylindrical to turbinate, tuberculate, pappus absent ..... **A. cotula**
- Receptacle with scales all over, at least when young; achenes with a different combination of characters ..... **7**



- 7 Receptacular scales hairy..... ***A. ammanthus* subsp. *paleacea***
- Receptacular scales glabrous ..... **8**
- 8 Peduncles not or slightly clavate in fruit; at least inner achenes not firmly attached to receptacle ..... **9**
- Peduncles clavate in fruit; achenes firmly attached to receptacle or involucre indurate at maturity..... **11**
- 9 Plants slender; ligules not more than 5 mm or absent; margin of involucre bracts pale; achenes not or obscurely ribbed..... **10**
- Ligules always present, longer than 7 mm; involucre bracts usually with dark margin; achenes with 7–10 distinct ribs ..... ***A. chia***
- 10 Ligules always present, pinkish at least beneath; receptacular scales linear-lanceolate; achenes with a fimbriate corona ..... ***A. glaberrima***
- Ligules absent or, if present, white; receptacular scales linear-setaceous; achenes with an entire to lacerate corona..... ***A. tomentella***
- 11 Stems erect; receptacle conical, elongated; achenes with a thickened marginal rim, pappus absent..... ***A. arvensis* subsp. *incrassata***
- Stems prostrate to ascending; receptacle shortly conical; achenes with acute rim and a corona at least 0.3 mm long..... **12**
- 12 Ligules absent; capitula discoid ..... ***A. rigida* subsp. *rigida***
- Ligule present, white; capitula radiate..... ***A. rigida* subsp. *ammanthiformis***
- 13 Involucre 4–7 mm long, ligules usually absent; disk florets yellow or pink; achenes without pappus or with a very short acute rim..... ***A. abrotanifolia***
- Involucre 8–12 mm long, ligules present, large; disk florets yellow; achenes with a 0.5–1.5 mm long auricle..... **14**
- 14 Flowering stems glabrous at least at middle part, leafless except for small, scale-like leaves; receptacular scales with an arista ca. 1 mm long; achenes of ligulate florets with a 3-lobed auricle ..... ***A. samariensis***
- Flowering stems woolly, bearing dissected leaves usually up to middle; receptacular scales with an arista 1.5–2 mm; achenes of ligulate florets with an entire auricle ..... ***A. pasiphaes***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Conceptualization: KG. Data curation: KG. Formal analysis: KG. Methodology: TC. Supervision: TC. Writing - original draft: KG. Writing - review and editing: TC.

## Author ORCIDs

Katerina Goula  <https://orcid.org/0000-0001-9207-3570>

Theophanis Constantinidis  <https://orcid.org/0000-0001-9704-3864>

## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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# A new species of *Amorphophallus* (Araceae) from northeastern Thailand

Wilawan Promprom<sup>1,2</sup>, Wannachai Chatan<sup>1,2</sup>, Pattana Pasorn<sup>2,3</sup>,  
Narueset Prasertsri<sup>4</sup>, Thinnakon Angkahad<sup>4</sup>

<sup>1</sup> Department of Biology, Faculty of Science, Maharakham University, Kantharawichai District, Maharakham Province, 44150, Thailand

<sup>2</sup> Plant and Innovation Research Unit, Maharakham University, Maharakham, 44150, Thailand

<sup>3</sup> Walai Rukhavej Botanical Research Institute, and Center of Excellence in Biodiversity Research, Maharakham University, Maha Sarakham 44150, Thailand

<sup>4</sup> Department of Geo-Informatics Technology, Faculty of Informatics, Maharakham University, Maharakham 44150, Thailand

Corresponding author: Wannachai Chatan ([wannachaichatan@gmail.com](mailto:wannachaichatan@gmail.com))

## Abstract

*Amorphophallus sakonnakhonensis* Chatan & Promprom, a new species from northeastern Thailand, is described and illustrated. The new species is most similar to *A. harmandii* Engl. & Gehrm. and *A. linearis* Gagnep., but it is distinguished by the combination of characters as follows: clear differences with *A. harmandii* are shorter style; disc-like, slightly smooth surface, concave centre, ca. 0.2 × 0.1 mm stigma; slightly cylindrical, slightly narrower upper part of staminate flower zone; slightly cylindrical to elongate-fusiform, erect or slightly erect, creamy white appendix. The clearly distinct morphology with *A. linearis* are disc-like, slightly smooth surface, concave centre, ca. 0.2 × 0.1 mm stigma; elliptic or obovate leaflet; 1–3 cm long, creamy white appendix. The preliminary conservation status was assessed, and the distinct characteristics of similar species were discussed.

**Key words:** *Amorphophallus sakonnakhonensis*, Araceae, aroid, plant diversity, plant taxonomy



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## Introduction

*Amorphophallus* Blume ex Decne. (Decaisne 1834) is a genus in the family Araceae that contains about 200–250 species (Hettterscheid 2012; Claudel et al. 2017; POWO 2023) and which is distributed from tropical west Africa, subtropical eastern Himalayas, throughout subtropical and tropical Asia into tropical western Pacific and northeastern Australia (Hettterscheid and Ittenbach 1996; Sedayu et al. 2010; Hettterscheid 2012), and 62 species are currently recorded for Thailand (POWO 2023). *Amorphophallus* species usually grow in tropical humid forests, seasonal forests, grass savannahs and secondary forests. Commonly, the members of *Amorphophallus* do not produce their leaves and inflorescences simultaneously (i.e. the inflorescence is produced first and lasts for a short period, and then the leaf is produced, and leaves are only produced for one year for some species.)

During field surveys of plant diversity and medicinal plants in northeastern Thailand in 2019–2022, and our investigations in Sakon Nakhon Province, we collected *Amorphophallus* specimens which were not readily identifiable. After the herbarium specimens and living plants were carefully investigated, we concluded that these were not representatives of any previously-named plant species. Consequently, a new species is described here.

## Materials and methods

Plant material was collected during field surveys in Khok Si Suphan District, Sakon Nakhon Province in 2019–2022. Morphological observations of the new species were carried out on living plants from the field, as well as on herbarium specimens in BKF and BK. This study consulted the relevant taxonomic literature (such as Gagnepain 1942; Hetterscheid and Ittenbach 1996; Li et al. 2010; Hetterscheid 2012, etc.). The preliminary conservation status of the new species was assessed by applying the criteria (IUCN 2022) given.

## Taxonomic treatment

### *Amorphophallus sakonnakhonensis* Chatan & Promprom, sp. nov.

urn:lsid:ipni.org:names:77323169-1

Figs 1–4

**Type.** THAILAND. Sakon Nakhon Province: Khok Sri Suphan District, northeastern Thailand, 300–320 m elev., 16°59'45.5"N, 104°15'46.9"E, 24 April 2021, *W. Chatan* 2897 (holotype: BKF!; isotype: BK!); Khok Sri Suphan District, northeastern Thailand, *W. Chatan* 3073 (paratype: BKF!), 16°59'45.5"N, 104°15'49"E, 29 June 2022.

**Diagnosis.** *Amorphophallus sakonnakhonensis* is most similar to *A. harmandii* Engl. & Gehrm. and *A. linearis* Gagnep. The essential differences with *A. harmandii* are shorter style (ca. 1 mm vs. 2–3 mm long); disc-like, slightly smooth surface, concave centre, ca. 0.2 × 0.1 mm stigma vs. depressed, shallowly bilobed, ca. 0.6 × 1.5 mm; slightly cylindrical, narrower upper part staminate flower zone vs. fusiform-conical or lageniform with distinctly dilated basal haft; slightly cylindrical to elongate-fusiform, erect or slightly erect, creamy white appendix vs. very narrowly conical, near myosuroid or fusiform conical or slightly sigmoidally curved forward in the lower half, white or greenish yellow.

The essential differences with *A. linearis* are disc-like, slightly smooth surface, concave centre, ca. 0.2 × 0.1 mm stigma vs. capitate, globose, 1.0–1.3 × 0.9–1.5 mm; elliptic or obovate leaflet vs. linear less often lanceolate; 1–3 cm long, creamy white appendix vs. 18–50 cm long, creamy white or green.

**Description.** Perennial herb, narrowly elongated, not branching, 5–10 cm long and 0.4–1.0 cm diam., skin light yellowish to yellowish brown with few or many fibrous lateral roots. Both flowering and leafing plants of small stature, up to 12 cm high. Leaves solitary. Petiole 2.5–2.8 × 0.2–0.3 cm, smooth, white near base and greenish-purple or dark dull red to dark red or rust-coloured at upper part; leaf blade up to 10 cm diam., rachis narrowly winged throughout; leaflet elliptic or obovate, ca. 0.5–3.0 × 0.3–0.5 cm, adaxial side green, abaxial side pale green, apex acuminate. Inflorescence solitary, developed before leaf; peduncle 3.0–5.5 cm long, 2.0–2.5 mm diam., white near base and greenish





**Figure 1.** *Amorphophallus sakonnakhonensis* Chatan & Promprom. **A** habitat and habit **B** habit.

to greenish-purple at upper part; spathe broadly ovate, both sides dull greenish yellow or creamy white excepting for the deep purple red or bluish purple at base adaxially, 2.0–2.2 × 1.5–1.8– cm, erect, embracing and close to spadix and upper part moving slightly away from spadix during anthesis, smooth on both surfaces, except for lower part verrucate adaxially; spadix subequal or longer than spathe, 2–3 cm long; pistillate and staminate flower zones contiguous, but flowers are slightly distant from each other at connection zone. Pistillate flower zone cylindrical, ca. 1.0 × 0.5 cm, axis green, most flowers slightly distant from each other; ovary depressed, shallow bilobed, ca. 1 × 1 mm, light green; style short, thick, ca. 1 mm long; stigma ca. 0.2 × 0.1 mm, creamy or yellowish, disc-like, surface slightly smooth, centre concave. Staminate flower zone slightly cylindrical, 1.5–2.0 × 0.6–0.8 cm, upper part narrower; staminate flowers fused to a synandrium consisting of 3–5 stamens, creamy or yellowish; filaments united into a short column, ca. 1.0 × 0.8–1.2 mm, whitish or creamy.





**Figure 2.** *Amorphophallus sakonnakhonensis* Chatan & Promprom. **A** whole plant **B, C** tuber and inflorescence from the same plant **D** spadix **E** infructescence and fruits **F** seeds.

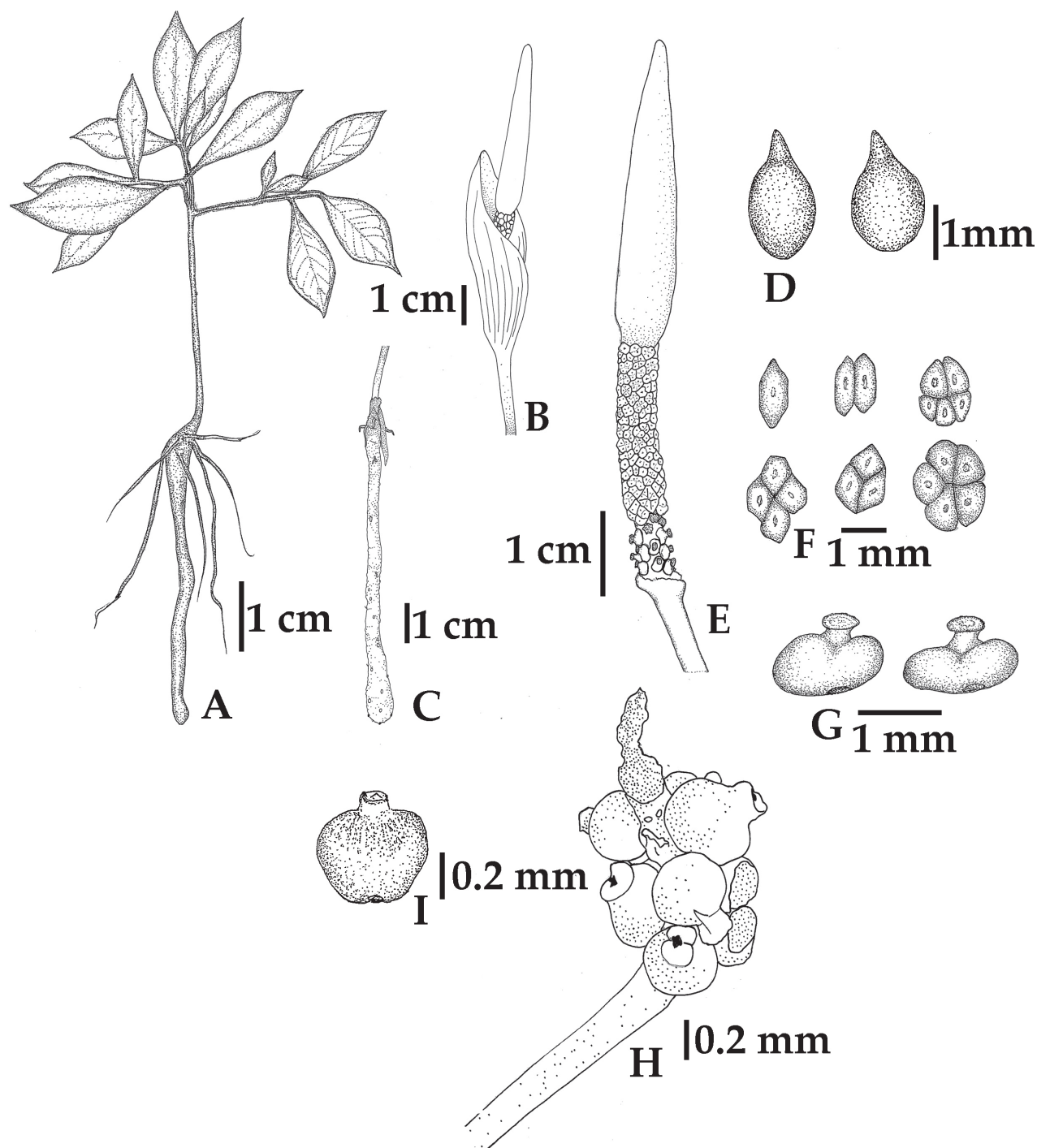
Appendix slightly cylindrical to elongate-fusiform, 1–3 × 0.8–1.0 cm, erect or slightly erect, creamy white; apex obtuse to slightly acute; surface smooth. Berry green when young and mature, conical to broadly pyriform, shallowly bilobed, ca. 0.5 × 0.5 cm, upper part elongated, odourless, surface verrucate. Seed ellipsoid or ovoid, 2.5–3.5 × 1.0–1.3 mm, apex conical.

**Flowering and fruiting.** Flowering in May–June and fruiting in June–August.

**Distribution.** The new species is endemic to Thailand and is known from only the type locality, Khok Sri Suphan District, Sakon Nakhon Province, northeastern Thailand (Fig. 4).

**Ethymology.** Specific epithet of *A. sakonnakhonensis* refers to the type locality, Sakon Nakhon Province, northeastern Thailand.

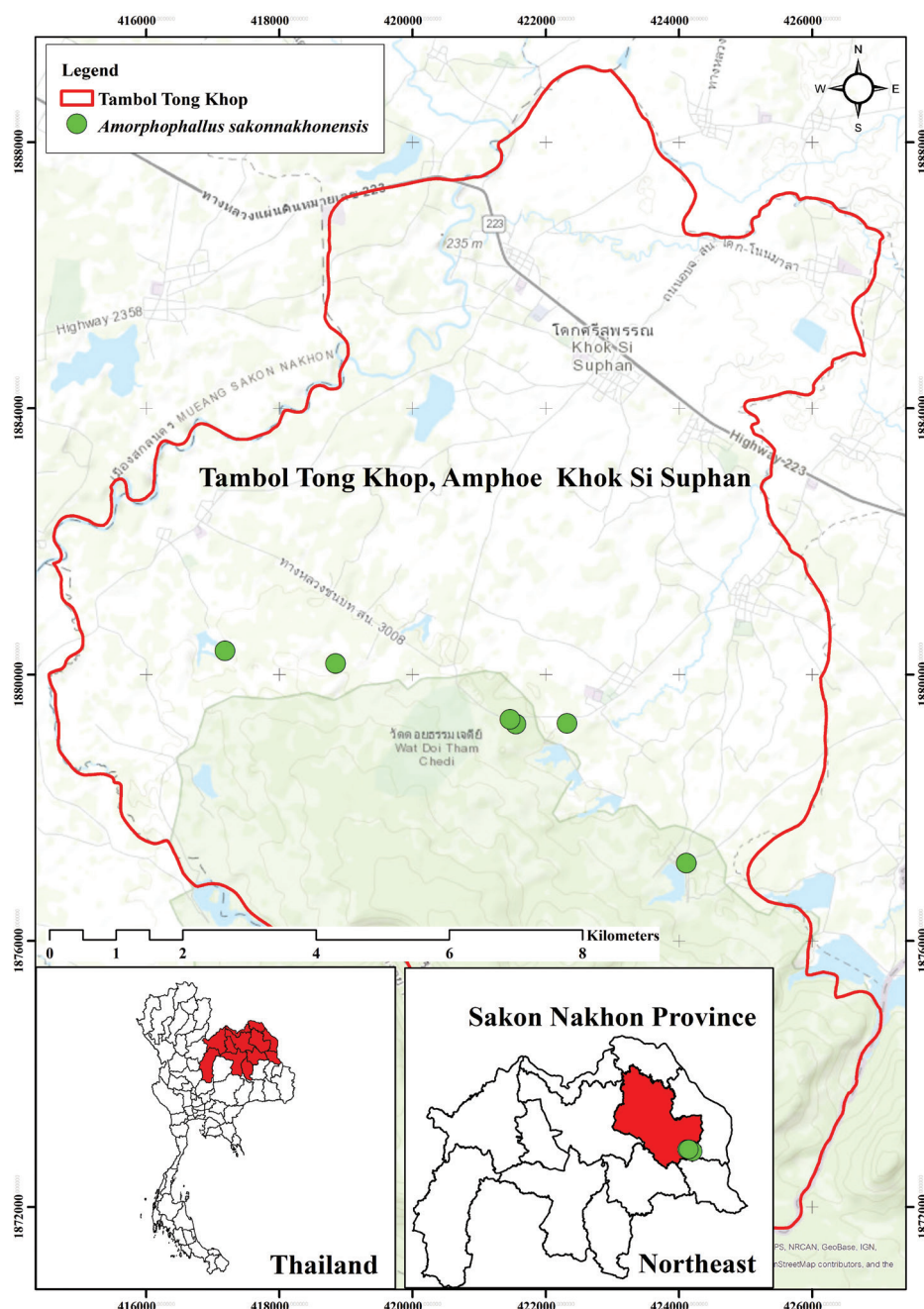
**Vernacular name.** The Vernacular name of the new species is Buk Noi.



**Figure 3.** *Amorphophallus sakonnakhonensis* Chatan & Promprom. **A** whole plant **B, C** tuber and inflorescence from the same plant **D** seeds **E** spadix **F** staminate flowers **G** pistillate flowers **H** infructescence and fruits **I** fruit.

**Preliminary conservation status.** According to the fieldwork, one population of *Amorphophallus sakonnakhonensis* was found at the type locality in Khok Sri Suphan District, Sakon Nakhon Province, northeastern Thailand. However, further explorations are needed for a proper conservation assessment because more information on its distribution and population status is required. Therefore, the species has been preliminarily assigned to Data Deficient (DD) category according to The Guidelines for Using The IUCN Red List Categories and Criteria (IUCN, 2022).





**Figure 4.** Distribution of *Amorphophallus sakonnakhonensis* Chatan & Promprom (green circle).

**Discussion.** *Amorphophallus sakonnakhonensis* Chatan & Promprom is similar to *A. harmandii* Engl. & Gehrm. (Engler 1911) and *A. linearis* Gagnep. (Gagnepain 1941), the plants from Thailand and Indochina (Hettterscheid 2012). The new species would be placed in subgenus *Metandrium* (Claudel et al. 2017), based on the hypothesis on the phylogenetic affinity of the species, which clearly lies with the *A. harmandii* alliance, close to that species, particularly seen by the near-unique fruits (green, warty). Similarities to *A. harmandii* and *A. linearis* consist of the narrowly elongated tuber, spadix longer than spathe, and contiguous nature of the staminate and pistillate flower zones. The different morphological characters are described below. The main differences



of the new species from *A. harmandii* are that it has shorter style; disc-like, slightly smooth surface, concave centre stigma; slightly cylindrical staminate flower zone; slightly cylindrical to elongate-fusiform, erect or slightly erect, creamy white appendix. The new species is also different from *A. linearis* by its disc-like, slightly smooth surface, concave centre stigma; elliptic or obovate leaflet; 1–3 cm long, creamy white appendix. In addition, the size of these organs of the new species are smaller or slightly smaller, including tuber, petiole, leaf blade, peduncle, spathe, spadix, staminate flower zone and appendix. More details of morphological differences amongst *A. sakonnakhonensis*, *A. harmandii* Engl. & Gehrm. and *A. linearis* Gagnep. are presented in Table 1.

**Table 1.** Morphological differences amongst *Amorphophallus sakonnakhonensis*, *A. harmandii* Engl. & Gehrm. and *A. linearis* Gagnep.

Characters	<i>Amorphophallus sakonnakhonensis</i> Chatan & Promprom	<i>A. harmandii</i> Engl. & Gehrm.	<i>A. linearis</i> Gagnep.
Tuber	5–10 cm long and 0.4–1.0 cm diam., not branching	ca 40 cm long and 2 cm diam., not or occasionally branching	10–45 cm long and 1–2 cm diam., not or occasionally branching
Petiole	2.5–2.8 × 0.2–0.3 cm	20–80 cm by 4–10 mm	25–85 by 0.5–1.5 cm
Leaf blade	up to 10 cm diam.	36–74 cm diam.	30–80 cm diam.
Leaflet	elliptic or obovate, ca 0.5–3.0 × 0.3–0.5 cm	elliptic, elliptic-lanceolate or lanceolate, 2–20 × 1–7 cm	linear, less often lanceolate, 3–17 × 0.2–1.0 cm
Peduncle	3.0–5.5 cm long, 2.0–2.5 mm diam.	peduncle 6–18 cm long, 3–4 mm diam.	19–55 cm long, 0.3–1 cm diam.
Spathe	broadly ovate, 2.0–2.2 × 1.5–1.8 cm, smooth on both surfaces, except for the base verrucate adaxially	ovate to orbicular, 5–13 × 5–10.5 cm, base densely verrucate, verrucae small adaxially	elongate-triangular or lanceolate, 7–16 × 3.5–6 cm, base within densely clothed with irregular, laterally compressed, ridge-like, sometimes flaky, often confluent, warts, often with short or long hair-like branches
Spadix	2–3 cm long	8–15 cm long	12–57 cm long
Female flowers	slightly distant from each other; style ca. 1 mm long; disc-like, slightly smooth surface, concave centre, ca. 0.2 × 0.1 mm stigma	closed to each other; style 2–3 mm long; depressed, shallowly bilobed, ca 0.6 × 1.5 mm stigma	closed to each other; style 1–2 mm long; capitate globose, 1.0–1.3 × 0.9–1.5 mm stigma
Staminate flower zone size	1.5–2.0 × 0.6–0.8 cm; slightly cylindrical, narrower upper part	3–6 × 1–1.7 cm; fusiform-conical or lageniform with distinctly dilated basal haft	2.5–8 × 0.4–2 cm; conical or slightly lageniform
Appendix	slightly cylindrical to elongate-fusiform; 1–3 × 0.8–1.0 cm, erect or slightly erect; creamy white, never green	very narrowly conical, near myosuroid or fusiform conical or slightly sigmoidally curved forward in the lower half; 5–18 cm × 5–16 mm; creamy white or greenish yellow	myosuroid, base slightly narrowed, 18–50 × 0.5–1.6 cm, at first erect, nodding after anthesis; creamy white or green;

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Conceptualization: WC. Data curation: PP, NP, WP, WC. Formal analysis: TA, WP. Funding acquisition: WC.

## Author ORCIDs

Wilawan Promprom  <https://orcid.org/0000-0001-8821-2267>

Wannachai Chatan  <https://orcid.org/0000-0002-3941-7626>

Pattana Pasorn  <https://orcid.org/0009-0007-3808-7331>

Narueset Prasertsri  <https://orcid.org/0009-0009-1798-1291>

Thinnakon Angkahad  <https://orcid.org/0009-0002-1439-8518>

## Data availability

All of the data that support the findings of this study are available in the main text.

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# Seven *Epithemia* taxa (Bacillariophyta) from Lake Akan (Japan) and their salinity tolerances

Takashi Chiba<sup>1</sup>, Yoshifumi Horie<sup>2</sup>, Akihiro Tuji<sup>3</sup>

<sup>1</sup> College of Agriculture, Food and Environment Sciences, Department of Environmental and Symbiotic Science, Rakuno Gakuen University, Bunkyo-dai 582, Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

<sup>2</sup> Research Center for Inland Seas (KURCIS), Kobe University, Fukaeminami-machi, Higashinada-ku, Kobe, Hyogo 658-0022, Japan

<sup>3</sup> Department of Botany, National Museum of Nature and Sciences, Tsukuba, Ibaraki 305-0005, Japan

Corresponding author: Takashi Chiba (t-chiba@rakuno.ac.jp)

## Abstract

The ecologies (salinity tolerance) of many diatoms are largely unknown, despite their potential to contribute to more detailed paleoenvironmental reconstructions. This study therefore aimed to investigate the relationship between diatom species and salinity. We cultured seven cosmopolitan benthic diatom species obtained from Lake Akan, a freshwater inland lake in Japan: *Epithemia adnata*, *E. frickei*, *E. gibba*, *E. operculata*, *E. sorex*, *E. sp.* and *E. turgida*. Each species was cultured at eleven salinities between 0‰ and 50‰. *Epithemia adnata*, *E. frickei* and *E. sorex* had the highest growth rate at a salinity of 3‰, with no further increase observed above 25‰. However, *E. gibba* had the highest growth rate at a salinity of 5‰, with no increase at salinities  $\geq 30$ ‰. These results suggest that *E. adnata*, *E. frickei*, *E. gibba*, and *E. sorex* grow in freshwater to brackish-water environments. *Epithemia operculata* and *E. sp.* proliferated at all salinities, indicating that they can adapt to hypersaline environments. However, *E. turgida* did not survive in salinities  $>10$ ‰, making it the species with the narrowest salinity tolerance range. These results provide new knowledge that improves the understanding of the ecology of these species in modern environments and offer insights into paleoenvironmental reconstructions through diatom analysis.

**Key words:** Diatom, *Epithemia* genus, Lake Akan, salinity



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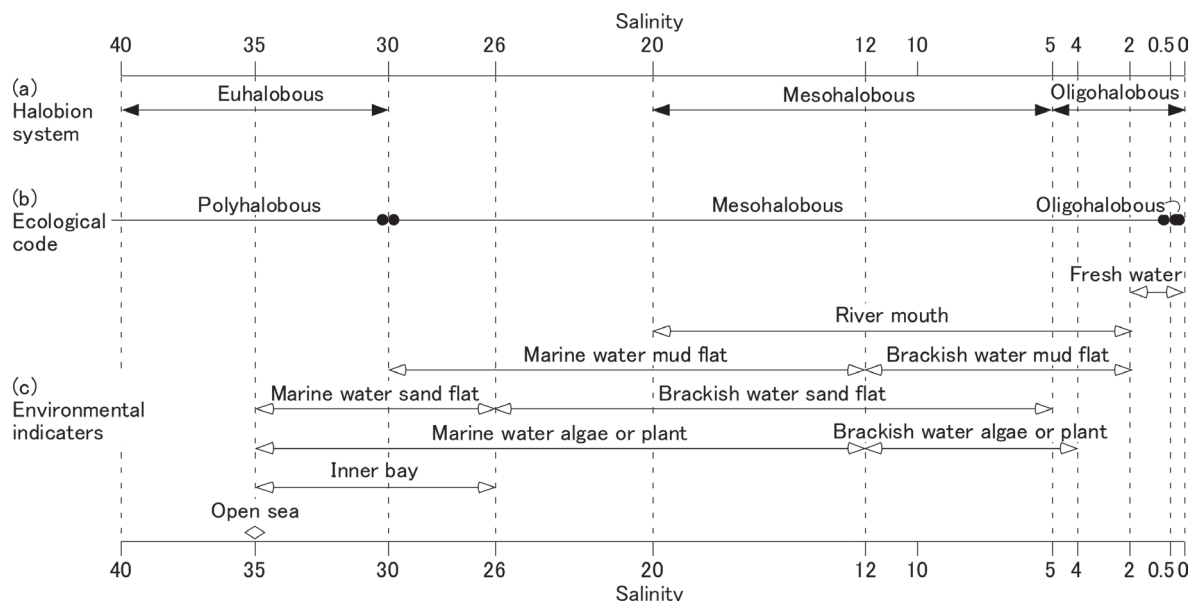
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## Introduction

Diatoms are unicellular algae that are used as environmental indicators because of their adaptive radiation through the aquatic environment (Vos and De Wolf 1993; Van Dam et al. 1994). They are used as indicators in modern and paleoenvironmental reconstructions of sedimentary environments because their siliceous ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ; amorphous silica) frustules are well preserved in sediments, and they appear as fossil diatoms (Kolbe 1927; Vos and De Wolf 1988; Chiba and Sawai 2014) (Fig. 1). Diatoms are particularly useful indicators for reconstructions of coastal environmental changes, such as changes in paleo-sea level (Smol and Stoermer 2010). However, many diatom species still have unknown ecologies (Round et al. 1990); despite their abundance in Holocene sediments, it is unclear whether they are freshwater or brackish-water



**Figure 1.** The correspondence of salinity ranges between among halobion system, ecological code and environmental indicators. **a** shows salinity ranges of halobion system (Kolbe 1927). Polyhalobous, mesohalobous and oligohalobous correspond to marine, brackish-water and freshwater respectively **b** shows salinity ranges of ecological code (Vos and De Wolf 1988) **c** shows salinity ranges of ten diatom assemblages in environmental indicators (Kosugi 1988) (modified from Chiba and Sawai 2014).

species. Although it is important to determine this characteristic in brackish-water environments, it can be challenging to determine through fieldwork alone. However, culture experiments are an effective method for clarifying these uncertainties. (Smayda 1969; Yamamoto et al. 2017).

Owing to the difficulty of taxonomy, the genus *Epithemia*, a taxon within raphid diatoms (Bacillariophyta), has been continuously described as a new species because of its high diversity and numerous framework recombinations (You et al. 2009; Stancheva et al. 2013; Vishnyakov et al. 2014; Ruck et al. 2016; Rybak et al. 2020). Moreover, the information accumulated for this genus is underutilised in paleoenvironmental reconstructions. On the other hand, the genus *Epithemia* in current taxonomy is widely adapted and distributed from freshwater to marine environments.

For example, *Epithemia gibba* (Ehrenberg) Kützing, formerly known as *Rhopalodia gibba* (Ehrenberg) O. Müller, is a cosmopolitan benthic species (epipelon species on sand grains or aquatic plants) commonly found in lakes, rivers, and coastal regions worldwide; however, it prefers waters with relatively high electrolyte concentrations (Patrick and Reimer 1975) and alkalinity (Van Dam et al. 1994). Ruck et al. (2016) proposed taxonomic changes in Rhopalodiales and Surirellales based on molecular phylogenetic analysis. Rhopalodiaceae was integrated with Surirellaceae and the paraphyletic genus *Rhopalodia* was integrated with *Epithemia* (Kamakura and Sato 2018; Kamakura et al. 2021). However, in reconstructions of paleocoastal environments from Holocene sediments, *E. gibba* is sometimes classified as a freshwater species (Zalat and Vildary 2007; Tsoy et al. 2015), although it often appears in sediments and is sometimes classified as a brackish-water species (Chiba et al. 2016; Nazarova et al. 2022). Understanding the ecology (salinity range) of this species would make it important in paleoenvironmental reconstructions; however, detailed knowledge of its salinity tolerance, as well as those of *E. adnata* (Kützing) Brébisson and *E. sorex* Kützing (El Hamouti



and Gibert 2012; Roy and Keshri 2015; Chiba et al. 2016) is lacking, despite their potential to provide more detailed paleoenvironmental reconstructions.

In this study, therefore, we conducted culture experiments using seven *Epithemia* species isolated from the water of Lake Akan, an inland lake in eastern Hokkaido, Japan, and investigated in detail the relationships between their growth rates and salinity.

Materials and methods

Samples

Samples were obtained in July 2022 from the shore of Lake Akan (Fig. 2; 43°26'25.80"N, 144°5'6.70"E), eastern Hokkaido, Japan, using 10-mL syringes. Lake Akan is an inland volcanic lake that serves as a habitat for rare algae such as *Aegagropila linnaei* Kützing (e.g., Wakana et al. 2021). It has a salinity, pH, and temperature of 0‰, 8.0, and 21 °C, respectively (Table 1), with an electrical conductivity of 366 (μS/cm) and sand as the bottom sediment. Kawashima and Mayama (2002) previously reported several species of the genus *Epithemia* among six taxa from Lake Akan.

Table 1. Water quality in the sampling locality.

	GPS coordinates	Temperature (°C)	Salinity (‰)	Electric conductivity (μS/cm)	pH
Sampling locality	43°26'25.80"N, 144°5'6.70"E	21	0	366	8.0

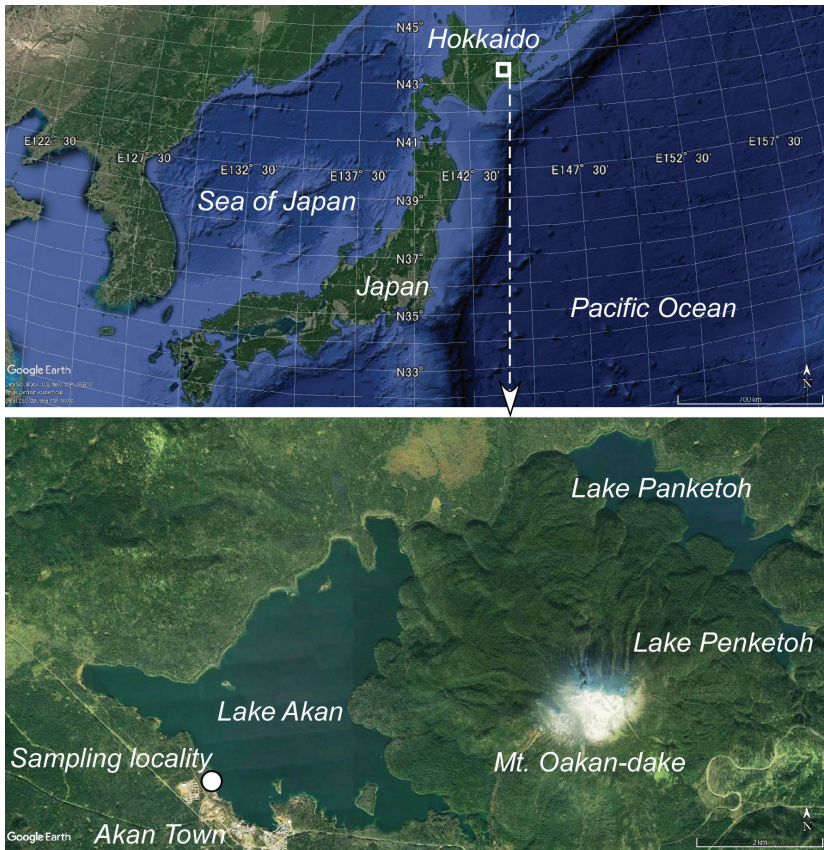


Figure 2. Sampling locality.

## Isolation and culture

Vegetative cells of *E. adnata*, *E. frickei*, *E. gibba*, *E. operculata*, *E. sorex*, *E. sp.* and *E. turgida* were isolated from samples using Pasteur pipettes and an inverted microscope (CK-2, Olympus, Tokyo, Japan). The cells of these seven species were attached to aquatic plants and sand-size grains in Lake Akan, so they are considered epiphytic and benthic species, respectively. Cells of each species were transferred into 11 wells of a 12-well culture plate (VTC-P12, AS ONE CORPORATION, Osaka, Japan) to establish strains. The salinities in the wells were set to 0‰, 1‰, 3‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰, 35‰, and 50‰, according to Yamamoto et al. (2017). Many wells were set to salinities  $\geq 10‰$  because, although these species were reported from freshwater, we wanted to test the upper limits of their salinity tolerances. The pH of all wells was set to 8.3 (weakly alkaline). The culture medium was based on Bold's Basal Medium (BBM) (Stein 1973), and its concentration was adjusted by adding artificial seawater and silica. The cultures were maintained in an incubator at 15 °C under 12-h light:12-h dark conditions.

## Experimental protocol

The experimental protocol followed that of Tuji (2000). Each well of the 12-well cultures plates that we used had an area of 3.8 cm<sup>2</sup> and depth of 2 cm. Medium (1 mL) was pipetted into each well of a 12-well plate prepared in triplicate for each salinity, and pre-incubated strains exhibiting logarithmic growth were inoculated into each well at a starting density of one cell per well (Yamamoto et al. 2017). The long-term culture experiments were initiated on 15 July 2022 without a salinity acclimation period, and the number of diatoms was counted at least once every seven days after inoculation until growth stopped and stationary phase was confirmed. The experimental period varied for each strain but did not exceed 60 d, with all experiments completed by 15 September 2022.

The specific growth rates during the exponential growth period were calculated using the following equation (Shimizu 2006):

$$\mu[d^{-1}] = \text{Log}_e(N/N_0)/(t - t_0)$$

where  $\mu$  is the growth rate,  $t_0$  and  $t$  are the initial and final days of the exponential growth period, respectively, and  $N_0$  and  $N$  are the cell numbers at  $t_0$  and  $t$ , respectively. After completing all experiments, the strains were boiled in H<sub>2</sub>O<sub>2</sub> to remove organic material, washed, and mounted on permanent slides. The frustules were examined using an optical microscope (BH-2, Olympus), and a scanning electron microscope (JSM-6390LV, JEOL Ltd., Tokyo, Japan) was used to identify the species. Morphological analysis of *Epithemia* species was conducted according to Krammer and Lange-Bertalot (1988), Kawashima and Mayama (2002), and You et al. (2009). Some *Epithemia adnata* (Kützinger) Brébisson varieties are indistinguishable from *E. frickei* Krammer. Therefore, we selected those varieties that could be morphologically distinguished by observation with an inverted microscope and used them for the experiment.

## Results

In this section, we first show the morphological characteristics of isolated and cultured species (Figs 3–9) and describe their salinity tolerance (Fig. 10).

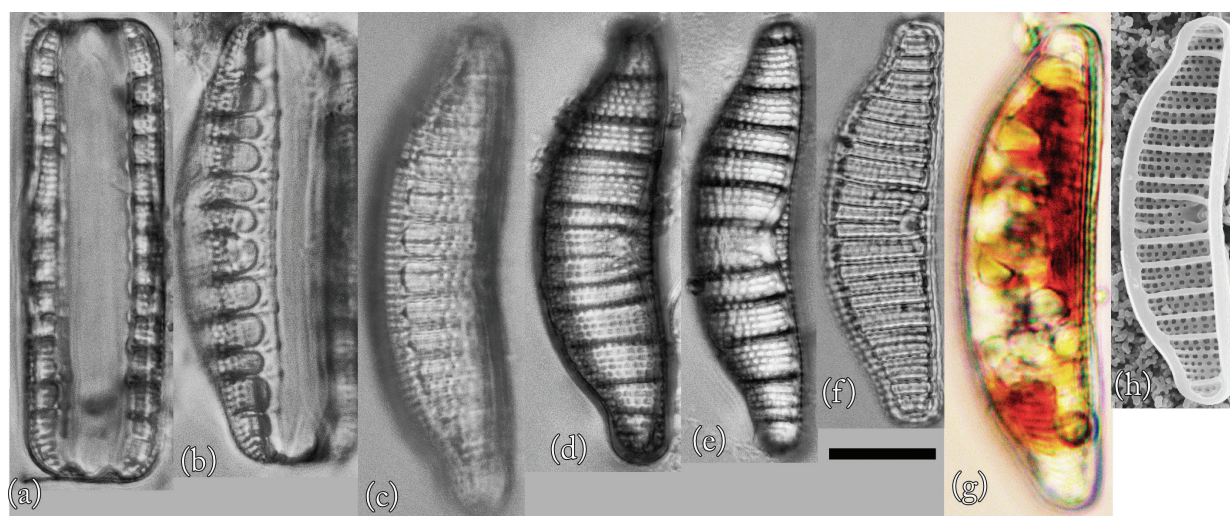
### *Epithemia adnata* (Kützing) Brébisson

This species is reported by Kawashima and Mayama (2002). Taxonomic examination confirmed that this species is *Epithemia adnata* (Fig. 3). Individuals observed in culture had universal morphological characteristics recognized worldwide (Krammer and Lange-Bertalot 1988). However, many individuals were close to the smallest size reported thus far.

**Light microscopy (LM) morphology.** Valves were approximately centred on the dorsum, the dorsal margin was slightly convex, the ventral margin was slightly concave or straight, and the apex was broad and rounded (Krammer and Lange-Bertalot 1988). Individuals were 24–52 µm in length and 7–10 µm in width ( $n = 50$ ). The raphe was biarcuate; that is, bent inwards from the pole towards the dorsal side, but never reaching its edge. There were 12–17 areola in 10 µm, 13–17 striae in 10 µm, and 4–6 costae in 10 µm, with 3–6 striae between adjacent costae. The costae were nearly parallel or slightly radial.

**Scanning electron microscopy (SEM) morphology.** The external raphe was surrounded on both sides by thin silica strips, forming a V-shaped structure in the middle of the ventral margin (Krammer and Lange-Bertalot 1988). In the external valve view, a regular and uniform arrangement of dome-shaped caps was connected at the apical and transapical ends. These dome-shaped caps were usually four to eight in number and formed a single areola. The very complex structure of these areolas made their identities and boundaries more complex (Krammer and Lange-Bertalot 1988). The raphe fissure was located approximately the same distance from both edges of the apical region. A hyaline band was present on the dorsal side of the fissure and ran along the length of the valve (Krammer and Lange-Bertalot 1988).

**Proliferative salinity.** This species grew at all salinities from 0‰ to 50‰. The growth rate was highest at a salinity of 3‰, and there was almost no growth at salinities of 25‰ or higher (Fig. 10).



**Figure 3.** LM (a–g) and SEM (h) images of *Epithemia adnata*. Scale bar: 10 µm.



### *Epithemia frickei* Krammer

This is the first record of this species in Lake Akan. Morphological examination confirmed that this species is *Epithemia frickei* (Fig. 4). Individuals observed in culture had universal morphological characteristics recognized worldwide (Krammer and Lange-Bertalot 1988). However, many individuals were close to the relatively smaller size reported to date.

**LM morphology.** Morphological features included many characteristics similar to those of *E. adnata*, including dorsoventral flaps, a slightly convex dorsal margin, and a slightly concave ventral margin. However, apices were slightly rounded and very slightly protruding. Individuals were 15–28  $\mu\text{m}$  in length and 7–10  $\mu\text{m}$  in width ( $n = 50$ ). The raphe was essentially double arched; that is, bent inwards from the bar towards the dorsal side, but not reaching its end. There were 11–16 areola in 10  $\mu\text{m}$ , 13–17 striae in 10  $\mu\text{m}$ , and 4–6 costae in 10  $\mu\text{m}$ , with 3–6 striae between adjacent costae. The costae were almost parallel or slightly radial.

This species resembles *E. adnata*, but it is smaller. In addition, the areola density is coarse. Furthermore, the overhang of the valve end is not as large as that of *E. adnata*, and it converges. Individuals with frustules similar in length to those of *E. adnata* tended to have wider frustules. These features can be identified by LM.

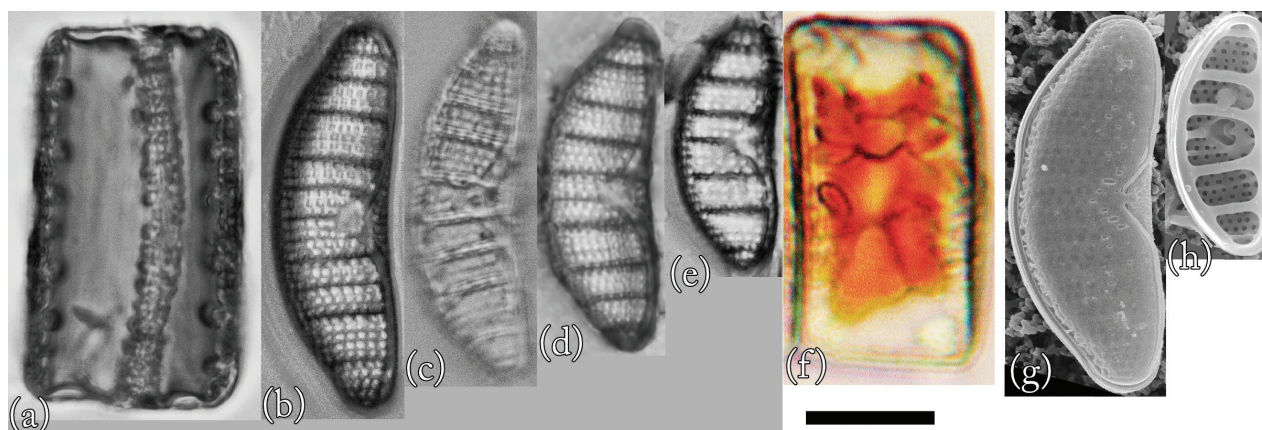


Figure 4. LM (a–f) and SEM (g and h) images of *Epithemia frickei*. Scale bar: 10  $\mu\text{m}$ .

**SEM morphology.** The overhang on the frustule end of this species was much smaller than that of *E. adnata* and converged. In addition, the shapes of areolae were more random than those of *E. adnata*. The external raphe was bounded on both sides by thin bands of silica, and the raphe formed a V-shaped structure in the centre of the ventral margin (Krammer and Lange-Bertalot 1988; Kawashima and Mayama 2002). The raphe fissure was located approximately the same distance from both edges of the apical region. (Krammer and Lange-Bertalot 1988; Kawashima and Mayama 2002). These characteristics are similar to those of *E. adnata*. However, the sequence interval of areolae within one of the striae lines of this species tended to be slightly wider than those of *E. adnata*.

**Proliferative salinity.** This species grew at all salinities from 0‰ to 50‰. The growth rate was highest at a salinity of 3‰, and there was almost no growth of this species at salinities of 25‰ or higher. This characteristic is similar to that of *E. adnata*.



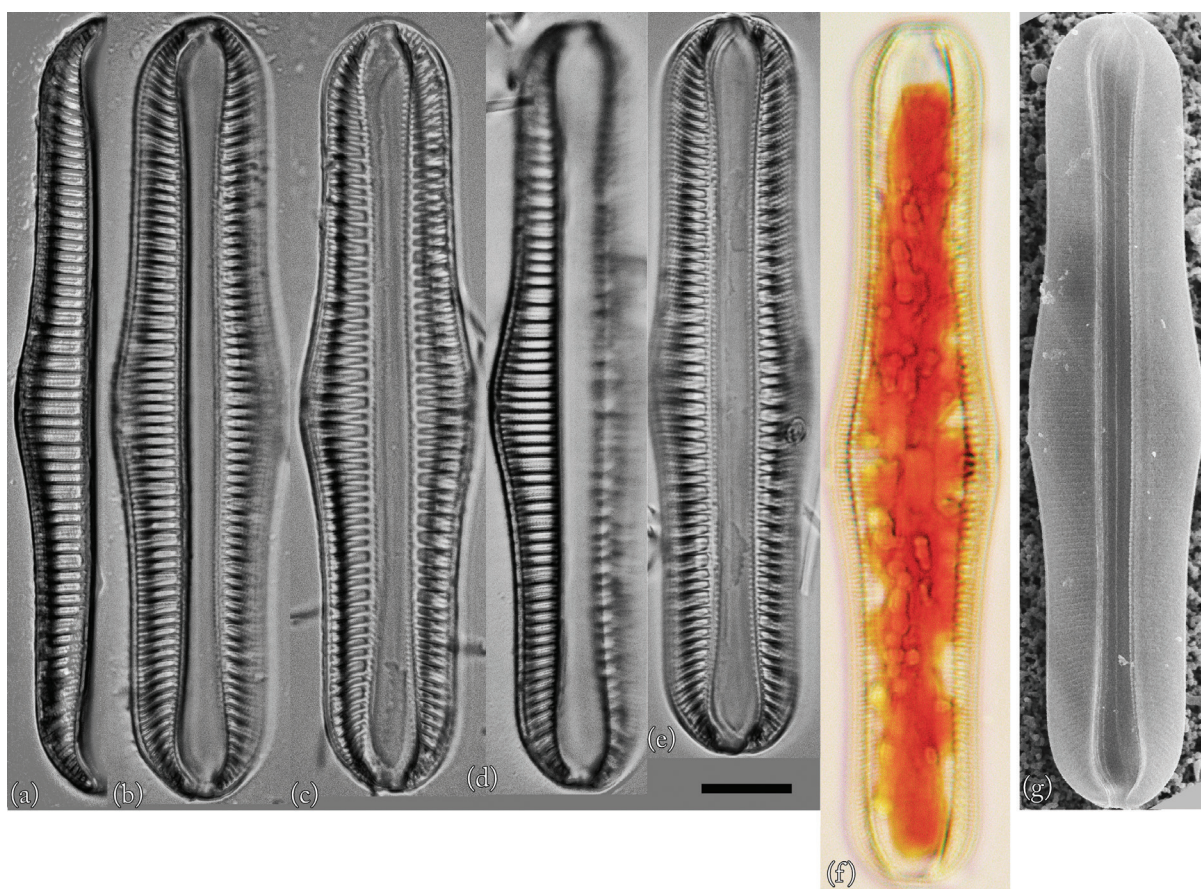
***Epithemia gibba* (Ehrenberg) Kützing**

This species is reported as *Rhopalodia gibba* by Kawashima and Mayama (2002). Morphological examination confirmed that this species is *Epithemia gibba* (Fig. 5). Individuals observed in culture had universal morphological characteristics recognized worldwide (Krammer and Lange-Bertalot 1988). However, many individuals were also close to the smallest size reported to date (Krammer and Lange-Bertalot 1988; Kawashima and Mayama 2002).

**LM morphology.** Valves were linear or bracket-shaped, and apices were bent towards the ventral margin (Krammer and Lange-Bertalot 1988; You et al. 2009). The central valve was inflated, and it was slightly indented towards the ventral margin at the central nodule. Individuals were 74–96  $\mu\text{m}$  in length and 7–9  $\mu\text{m}$  in width ( $n = 50$ ). The raphe was biarcuate; that is, branches curved from the poles inwards towards the dorsal side, but they never reached the margin. There were 14–17 areolae in 10  $\mu\text{m}$ , 13–17 striae in 10  $\mu\text{m}$ , and 7–9 costae in 10  $\mu\text{m}$ , with 2–3 striae between adjacent costae. Costae were near-parallel or radiated slightly.

**SEM morphology.** Some individuals had spherical silica deposits on the surface of the valves, whereas others had none. Areolae were usually indistinct (Kawashima and Mayama 2002), but some had bands arranged parallel to the valves, and some had bands that were depressed inwards.

**Proliferative salinity.** This species grew at all salinities from 0‰ to 50‰. The growth rate was highest at 5‰, and there was almost no growth of this species at salinities of 30‰ or higher (Fig. 10).



**Figure 5.** LM (a–f) and SEM (g) images of *Epithemia gibba*. Scale bar: 10  $\mu\text{m}$ .

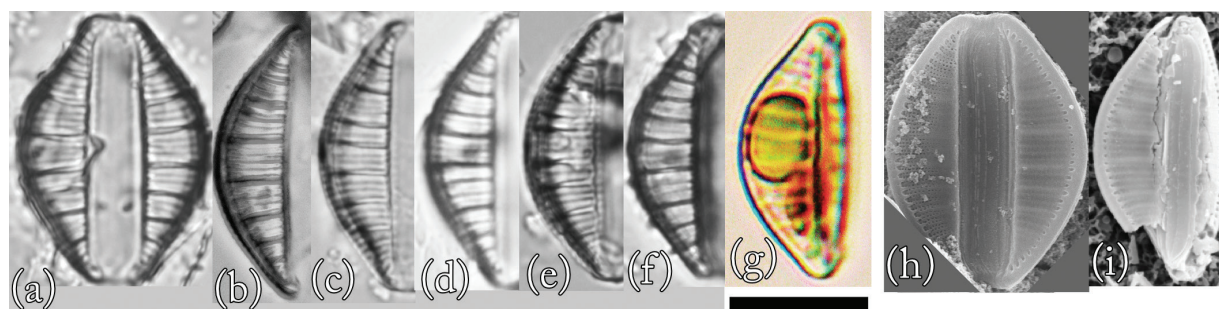


Figure 6. LM (a–g) and SEM (h and i) images of *Epithemia operculata*. Scale bar: 10 µm.

### *Epithemia operculata* (C. Agardh) Ruck & Nakov

This is the first record of this species in Lake Akan. Taxonomic examination confirmed that this species is *Epithemia operculata* (Fig. 6).

**LM morphology.** Frustules were elliptical and close to triangular. The dorsal edge of the valves was arched, but the central part was slightly depressed ventrally. The ventral margin was arcuate at the apices and straight in the axial direction at the centre. The apices of the valves extended and protruded. Individuals were 12–18 µm in length and 6–8 µm in width ( $n = 50$ ). The raphe was biarcuate. There were 13–16 striae in 10 µm and 6–8 costae in 10 µm, with 2 striae between adjacent costae. Costae were nearly parallel or radiated slightly. There were 5–7 costae in 10 µm. This species is similar to *Epithemia rupestris* (W. Smith) Krammer and *Epithemia constricta* W. Smith, as shown by Lange-Bertalot and Krammer (1987) and Krammer and Lange-Bertalot (1988). Although it was difficult to distinguish between small individuals of this species and *E. operculata* by optical microscopic observation, it was possible to distinguish them based on their different curvatures.

**SEM morphology.** There were 36–44 areolae in 10 µm, and double puncta formed the striae (Watanabe et al. 2005; You et al. 2009). The keel was raised above the valve plane and slightly indented towards the ventral margin at the central nodule (You et al. 2009).

**Proliferative salinity.** This species grew at all salinities tested in this study. The growth rate was the fastest at a salinity of 50‰, but no clear peak was observed (Fig. 10).

### *Epithemia sorex* Kützing

This species is reported by Kawashima and Mayama (2002). Taxonomic examination confirmed that this species is *Epithemia sorex* (Fig. 7). Individuals observed in culture had universal morphological characteristics recognized worldwide (Krammer and Lange-Bertalot 1988). Many individuals, however, were close to the smallest size reported to date (Krammer and Lange-Bertalot 1988; Kawashima and Mayama 2002).

**LM morphology.** Frustules were approximately dorsoventral, the dorsal margin was slightly convex, and the ventral margin was slightly concave. The apices of the frustules were thin and rounded. Individuals were 24–32 µm in length and 7–9 µm in width ( $n = 50$ ). The raphe was biarcuate; this is, bent inwards from the pole towards the dorsal side, but not reaching its edge. There were 14–17 areolae in 10 µm, 13–16 striae in 10 µm, and 6–9 costae in 10 µm. Frustules had 2–3 striae between adjacent costae, and costae were almost parallel or slightly radial.



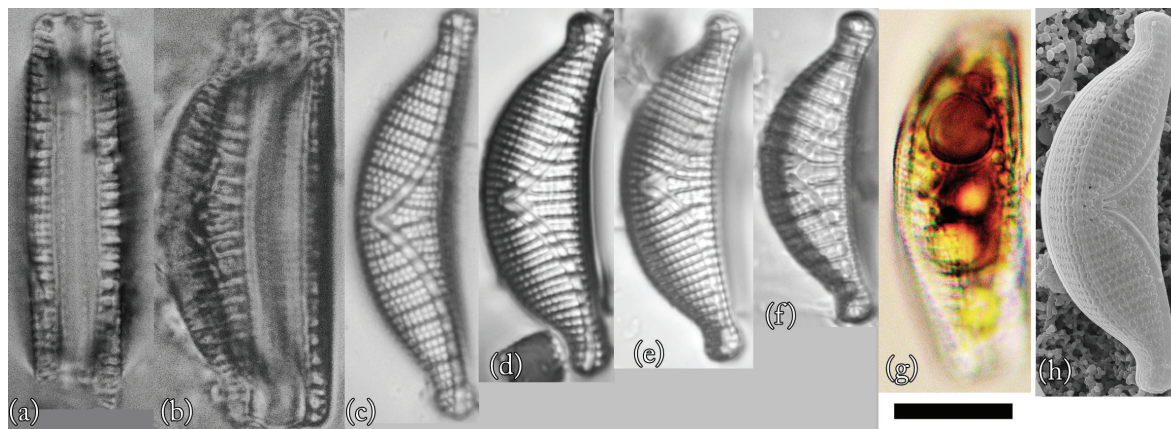


Figure 7. LM (a–g) and SEM (h) images of *Epithemia sorex*. Scale bar: 10 µm.

**SEM morphology.** A wide hyaline band was located dorsal to the raphe fissure. The acroraphe fissure was relatively simple and located in the middle of the pole (You et al. 2009). The external view of the valves showed an arrangement of solid domed caps positioned above the areolae (You et al. 2009).

**Proliferative salinity.** The growth rate was highest at a salinity of 5‰, and there was almost no growth of this species at salinities of 25‰ or higher (Fig. 10).

### *Epithemia* sp.

This is the first description of this species in Lake Akan. Taxonomic examination confirmed that this species is *Epithemia* sp. (Fig. 8).

**LM morphology.** The frustules were broadly elliptical or arcuate and crescent-shaped. The dorsal edge of the valves was arched, but the central part was depressed ventrally. However, the ventral margin was arcuate at the apices and straight in the axial direction at the centre. The apices of the valves extended in an arc and protruded. Individuals were 28–35 µm in length and 6–8 µm in width ( $n = 50$ ). The raphe was biarcuate. There were 4–7 costae in 10 µm. Costae were nearly parallel or radiated slightly. This species is similar to *Epithemia rupestris* (W. Smith) Krammer, as shown by Krammer and Lange-Bertalot (1988). This species is also similar to *Epithemia rumrichiae* (Krammer) Krammer (as *Rhopalodia rumrichiae*; Ohtsuka and Tuji 1999), but the vesicle density was clearly finer. Although it was difficult to distinguish small individuals of this species from those of *E. operculata* by optical microscopic observation, it was possible to distinguish them by their different curvatures.

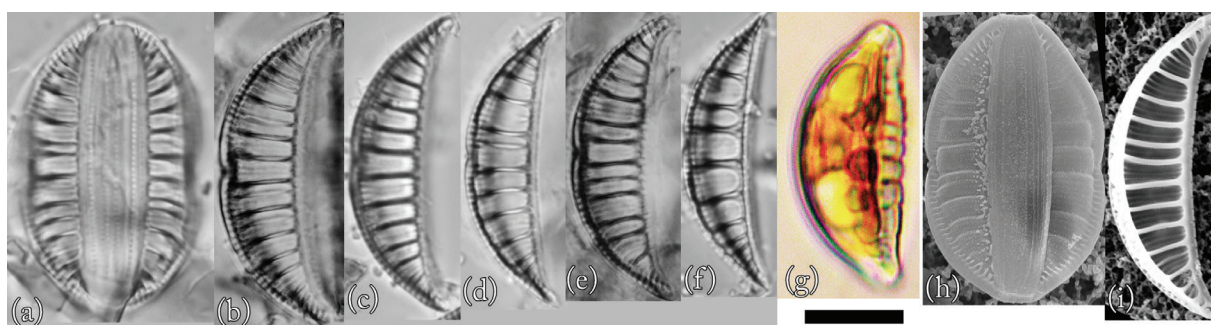


Figure 8. LM (a–g) and SEM (h and i) images of *Epithemia* sp. Scale bar: 10 µm.

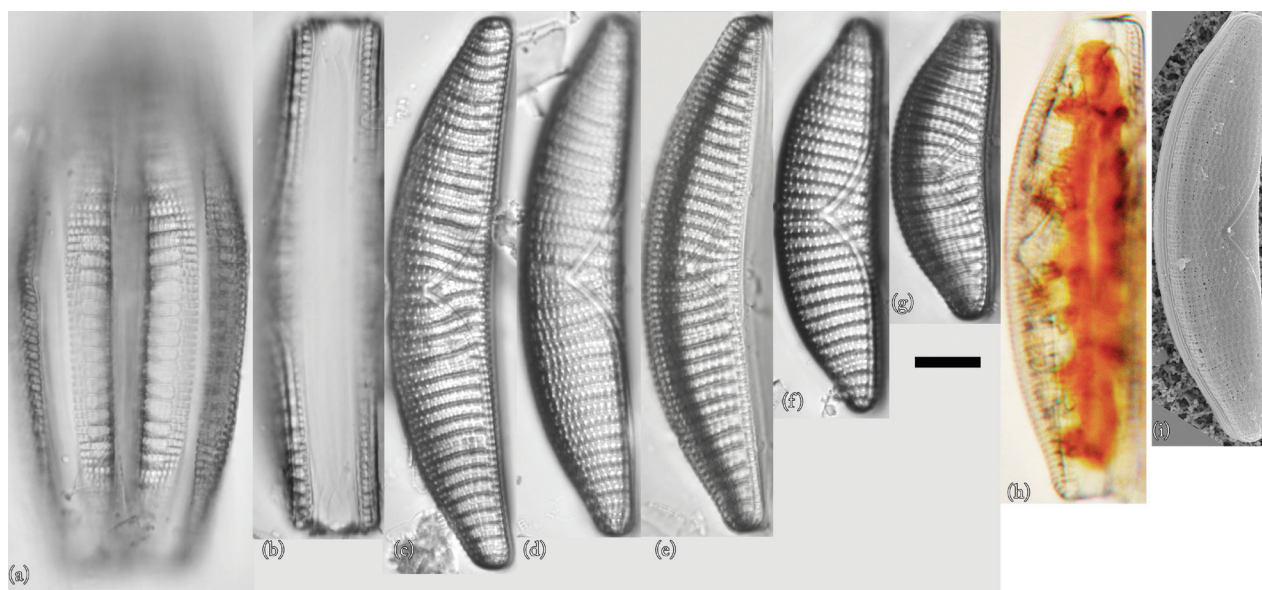
**SEM morphology.** External observation using SEM showed the central part of the valve exhibiting areola and constriction of the central portion. The keel was raised above the valve plane and clearly indented towards the ventral margin at the central nodule. Striae formed two areola rows. There were 38–46 areolae in 10  $\mu\text{m}$ . The areolae were arranged in transapical rows, with double puncta forming the striae. The valves bent inwards on the external margin side of the costae and rose gently into a convex shape between the costae. The surface appeared wavy. The characteristics of this species are similar to those shown in electron micrographs of *Epithemia constricta* W. Smith (You et al. 2009). However, the individuals observed in Lake Akan differed from those in electron micrographs of *E. constricta*, which had radial symmetry, as shown by Lange-Bertalot and Krammer (1987). Therefore, this species was considered to be *E. sp.*

**Proliferative salinity.** This species grew at all salinities tested in this experiment (0–50‰), but it showed the highest growth rate at 20‰ salinity (Fig. 10).

### *Epithemia turgida* Kützing

This species is reported by Kawashima and Mayama (2002). Taxonomic examination confirmed that this species is *Epithemia turgida* (Fig. 9). Individuals observed in culture had universal morphological characteristics recognized worldwide (Krammer and Lange-Bertalot 1988). Many individuals, however, were close to the smallest size reported to date. The central tubercle was located more dorsally than centrally.

**LM morphology.** The valves of this species are similar to those of *E. adnata* and are approximately dorsiventral; the dorsal margin is somewhat convex, the ventral margin is slightly concave, and no protrusion is observed at the apices. Individuals are 48–106  $\mu\text{m}$  in length and 16–18  $\mu\text{m}$  in width ( $n = 50$ ). The biarcuate raphe was located along the ventral border. The central nodule of this species was located more dorsally than centrally (Kawashima and Mayama 2002). There were 8–10 areolae in 10  $\mu\text{m}$ , 9–10 striae in 10  $\mu\text{m}$ , and 4–5 costae in 10  $\mu\text{m}$ , with 2–5 striae between adjacent costae. Costae were near-parallel or radiated slightly.

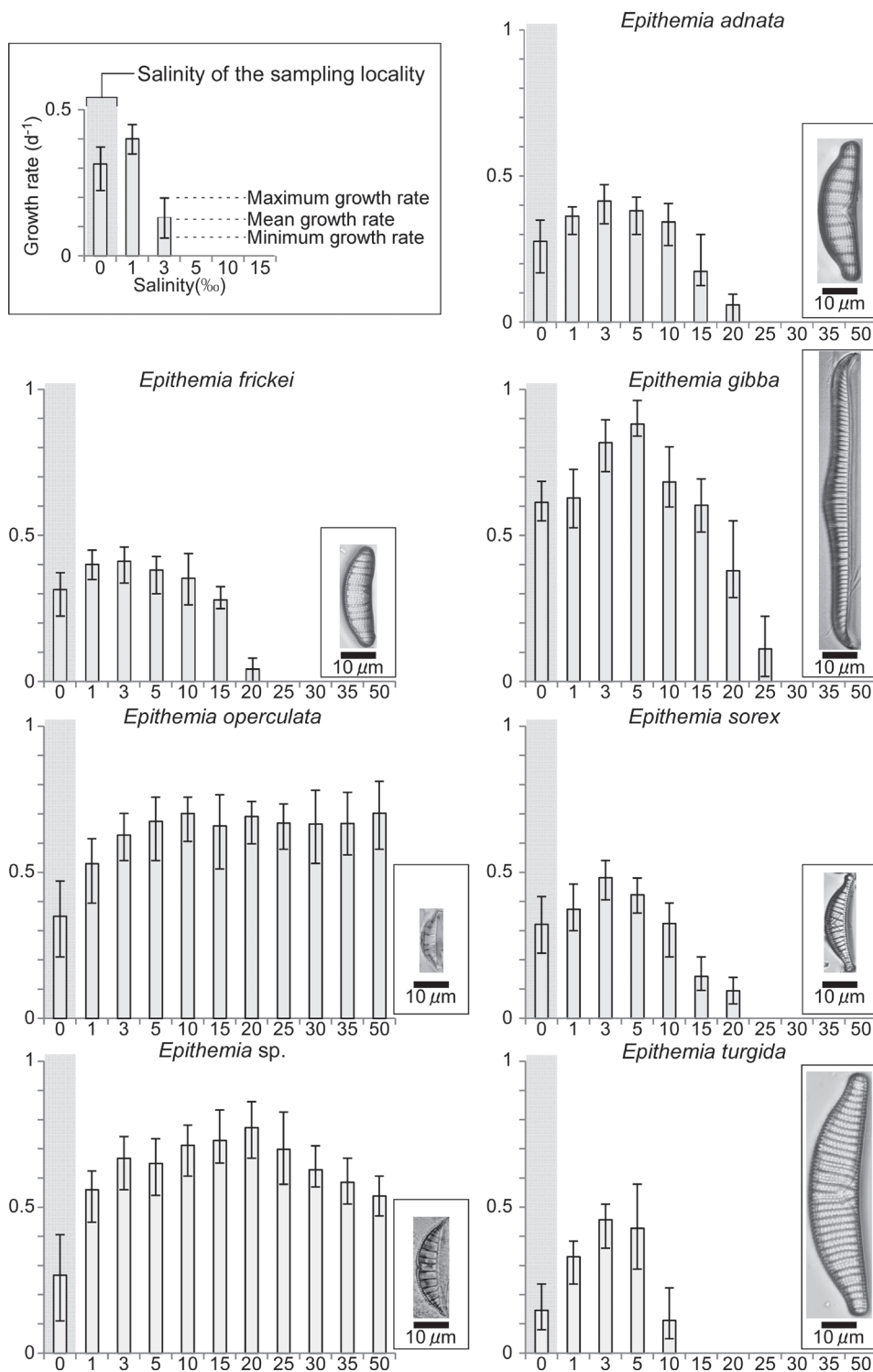


**Figure 9.** LM (a–h) and SEM (i) images of *Epithemia turgida*. Scale bar: 10  $\mu\text{m}$ .



**SEM morphology.** The appearance of the areola structure was similar to those of *E. adnata* and *E. sorex* (Kawashima and Mayama 2002). Valves showed a very regular arrangement of dome-shaped caps connected at the apical and transapical ends. Two small circular portal veins opened into the cell between costae fibula (You et al. 2009).

**Proliferative salinity.** This species had the highest growth rate at a salinity of 3‰. There was almost no growth of this species at salinities of 15‰ or higher (Fig. 10).



## Discussion

The experiments conducted on seven species of *Epithemia* (*E. adnata*, *E. frickei*, *E. gibba*, *E. operculata*, *E. sorex*, *E. sp.* and *E. turgida*) revealed no individual close to the maximum sizes previously described (Krammer and Lange-Bertalot 1988); instead, relatively small ones proliferated. This suggests that these species did not reproduce sexually and did not produce auxospores during the growth period, which may have increased during cell division. However, this observation may have been affected by the limits of the experimental environment; and sexual reproduction may require variations in water temperature and photon flux. The blooming of coastal benthic diatoms may be caused by factors such as water temperature (Admiraal 1977), turbidity (De Seve 1993), and nutrient conditions (Kilroy and Bothwell 2011), which should be further investigated.

*Epithemia adnata* and *E. frickei* grew between salinities of 0‰ and 15‰ and grew slightly after isolation at salinities  $\geq 20$ ‰ (Fig. 10). The growth rates of these species were the highest in the low-salinity brackish water (3‰), which was different from that of the collection site.

*Epithemia gibba* grew slightly after isolation at a salinity of 30‰ but died quickly (Fig. 10). Furthermore, it did not grow at salinities  $\geq 30$ ‰. In contrast, there was growth at a salinity of 0‰, consistent with the classification of Van Dam et al. (1994) and Vos and De Wolf (1993). *Epithemia gibba* is not solely a freshwater species, but it barely grew at salinities  $\geq 25$ ‰, demonstrating its upper limit for salinity tolerance. Although this species is found in freshwater lakes (high electrolytes), our discovery that its optimum salinity was 5‰ classified it as a freshwater to brackish-water rather than a freshwater species.

*Epithemia sorex* grew slightly after isolation at a salinity of 25‰ but died quickly (Fig. 10). Furthermore, it did not grow at salinities  $\geq 25$ ‰. In contrast, growth was observed at a salinity of 0‰, consistent with the findings of Van Dam et al. (1994) and Vos and De Wolf (1993). *Epithemia sorex* is not solely a freshwater species; we found that this species barely grew at salinities  $\geq 25$ ‰, demonstrating its upper limit for salinity tolerance. Although this species is found in freshwater lakes (high electrolytes), our discovery that its optimum salinity was 5‰ classified it as a freshwater to brackish-water rather than a freshwater species.

*Epithemia turgida* grew slightly after isolation at a salinity of 15‰ but died quickly (Fig. 10). Furthermore, it did not grow at salinities  $\geq 15$ ‰. In contrast, there was growth at a salinity of 0‰, consistent with the findings of Van Dam et al. (1994) and Vos and De Wolf (1993). *Epithemia turgida* is not solely a freshwater species; our discovery that this species barely grew at salinities  $\geq 10$ ‰ demonstrated its upper limit for salinity tolerance. Although this species is found in freshwater lakes (high electrolytes), our discovery that its optimum salinity was 3‰ or 5‰ classified it as a freshwater to brackish-water rather than a freshwater species.

*Epithemia operculata* and *E. sp.* were reported for the first time from Lake Akan in this study. They grew at all salinities tested, even at 50‰; however, *E. sp.* grew faster at higher salinities. These two species were not previously recognised by Kawashima and Mayama (2002), and they appear to be very rare. Although it is not clear why these species were found in Lake Akan, a

freshwater lake, they might have been attached to fish transplanted from Lake Abashiri, a northern, brackish lake, to Lake Akan in the 20<sup>th</sup> century.

The differences between water quality at the collection site (growth environment) and in the culture environment have been demonstrated in a previous study (Yamamoto et al. 2017), which focused on diatom species living on a tidal flat. These species proliferated even in freshwater environments but preferred waters with high concentrations of electrolytes. In a study of diatoms in the Fujimae tidal flat, Yamamoto et al. (2017) reported the ecology of seven diatom species that had a range of salinity tolerance wider than that in the growing environment. The high salinity tolerance of these species contributed to their tolerance to possible drought or rapid temperature increases on the surface of tidal flats caused by tidal changes. In this study, the salinity range tested was not as wide as that experienced by diatoms growing on tidal flats, suggesting that the seven species used in this study may not have been able to grow in moist and rapidly changing environments and are not suitable for tidal flat environments.

There are documented discussions for the reasons for discrepancies between the culture environment and natural habitats (Brand 1984; De Jong and Admiraal 1984; Clavero et al. 2000). Such variations in growth may be due to interspecific competition (Lewin and Mackas 1972; Admiraal 1984; De Jong and Admiraal 1984) or the influence of nutrient levels on community composition (Underwood et al. 1998; Skinner et al. 2006). Alternatively, natural distributions may not accurately reflect salinity tolerance because of the presence of physiologically differentiated races or cryptic species within a species, each with its narrower tolerance and optimum salinity. Recent molecular biological approaches have revealed the existence of cryptic and pseudocryptic species contained in *Pseudo-nitzschia delicatissima* (Cleve) Heiden (Orsini et al. 2004; Ruggiero et al. 2015) and *Navicula phyllepta* Kützinger (Créach et al. 2006; Vanelislander et al. 2009;). Therefore, extrapolation of the behavior of individual clones to natural conditions must be undertaken cautiously. Nevertheless, identifying the intrinsic responses of specific clones to salinity gradients is crucial in providing important information that can help clarify the impact of environmental factors on the ecology of benthic diatoms.

Another important ecological aspect of diatoms is that individual growth rates and species composition of brackish-water diatoms differ depending on their habitats (Yamamoto et al. 2017). Some species prefer high salinity, even though they may be found in brackish water, whereas others prefer low salinity, highlighting the importance of classification and culture experiments of such low-salinity, brackish-water species to provide accurate numerical values for the reconstruction of paleoenvironments in lakes and marine coastal areas. However, relying solely on the abundance trends of these species for determining whether environments were freshwater or brackish-water may lead to incorrect identification of the paleoenvironment. Therefore, to ensure a reasonable interpretation of the paleorecord, it is necessary to understand the production trends of other species and add relevant information. Furthermore, these findings are also important in understanding taphonomic processes (Chiba 2014), such as the effect of the concentration of individual sizes on fossil diatom assemblages.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: TC. Data curation: TC. Formal analysis: TC. Funding acquisition: TC. Investigation: TC. Methodology: AT. Project administration: TC. Resources: AT. Supervision: AT, YH. Validation: YH. Visualization: TC. Writing - original draft: TC. Writing - review and editing: AT, YH.

### Author ORCIDs

Takashi Chiba  <https://orcid.org/0000-0001-6431-3645>

Yoshifumi Horie  <https://orcid.org/0000-0002-4558-8624>

Akihiro Tuji  <https://orcid.org/0000-0003-2527-5986>

### Data availability

All of the data that support the findings of this study are available in the main text.

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# *Primulina pingnanensis*, a new species of Gesneriaceae from Guangxi, China

Zheng-Long Li<sup>1,2,3</sup> , Yan-Yun Kuang<sup>1,4</sup> , Qing-Qing Xu<sup>1</sup> , Wei-Chuen Chou<sup>2</sup> , Xin Hong<sup>1,3,5</sup> , Li Ding<sup>5,6</sup> 

<sup>1</sup> Anhui Provincial Engineering Laboratory of Wetland Ecosystem Protection and Restoration, School of Resources and Environmental Engineering, Anhui University, CN-230601, Hefei City, Anhui Province, China

<sup>2</sup> Gesneriad Conservation Center of China (GCCC), Guilin Botanical Garden, CAS, Guilin 541006, China

<sup>3</sup> Yunnan Key Laboratory for Integrative Conservation of Plant Species with Extremely Small Populations, Kunming Institute of Botany, Chinese Academy of Sciences, CN-650201 Kunming, Yunnan, China

<sup>4</sup> State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, CN-210008, Nanjing, China

<sup>5</sup> Gesneriad Committee of China Wild Plant Conservation Association, National Gesneriaceae Germplasm Resources Bank of GXIB, CN-541006 Guilin, Guangxi, China

<sup>6</sup> Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, CN-541006, Guilin City, Guangxi Zhuang Autonomous Region, China

Corresponding authors: Xin Hong ([hongxin1989@vip.qq.com](mailto:hongxin1989@vip.qq.com)); Li Ding ([dingli@gxib.cn](mailto:dingli@gxib.cn))

## Abstract

A new species of *Primulina*, *P. pingnanensis*, from the Guangxi Zhuangzu Autonomous Region, China, is described and illustrated here. It is morphologically similar to *P. orthandra* but has significant differences in the bracts, corolla tube and lobes shape, as well as in the indumentum of the outer surface of the corolla, the filaments, the staminodes and the anthers. Colorful photographs and essential information of this new taxon are also provided, including detailed taxonomic description, distribution, habitat, the comparison table, and the IUCN conservation status. We also discuss a validation of new combination *P. crassifolia* and *Chirita crassifolia*.

**Key words:** Flora of Guangxi, Gesneriaceae, limestone flora, new taxon, *Primulina orthandra*, taxonomy



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## Introduction

As of December 2022, about 250 taxa (including varieties) of *Primulina* Hance had been published worldwide, with 215 species and 18 varieties distributed in China and 18 species in Vietnam (GRC 2022; Wei et al. 2022). Based on the quantity and distribution of the *Primulina* species, it is apparent that the Karst limestone areas in Guangxi, China, are the center of *Primulina* diversity and distribution (Hao et al. 2014; Kong et al. 2017; Wei 2018; Li et al. 2019; Xu et al. 2019). This further indicates that more undescribed *Primulina* species may still be found in the Karst ecosystem of South and Southwest China (Möller 2019), especially in karst caves (Xin et al. 2018; Fu et al. 2022), cliffs and gorges.

Averyanov et al. (2020) described a new species of *Chirita* in North Vietnam, which they named *Chirita crassifolia* Aver. & K.S.Nguyen. In 2022, after verification, Möller and collaborators transferred *C. crassifolia* to *Primulina* as *P. crassifolia* (Anh et al. 2022). *Primulina crassifolia* (Aver. & K.S.Nguyen) T.T.P.Anh, F. Wen &

Mich.Möller, comb. nov. Basionym: *Chirita crassifolia* Aver. & K.S.Nguyen, Pl. Diversity Fl. Veg. Bat Dai Son 254 (2021). We emphatically revise the Latin name from '*Chirita crassifolia*' to '*Primulina crassifolia*' here and provide appropriate notes.

In March 2018, when we were on an expedition in Pingnan County, Guangxi, China, we found an unknown *Primulina*-like plant growing at the crevices of rocks on the top of a limestone cliff. According to its leaf arrangement (rosette), fleshy leaves, tubular corolla, and cracked fruits (capsule with parietal placentation), it should be included in *Primulina*. However, it did not match any known species of this genus. In addition, this species distinguishes from the genus *Petrocodon* by its lingulate stigma, curved filaments, and fleshy leaves. We collected specimens in the field and introduced some living plants into cultivation at the nursery of the National Gesneriaceae Germplasm Resources Bank of GXIB and the Gesneriad Conservation Center of China. The morphology of the cultivated plants has not changed from that of the wild plants during the past three years. We compared the collected specimens and living plants with other known type specimens and living plants of *Primulina*, and we found that it is distinctly different from other species of *Primulina*, especially those distributed in Guangxi and recently published species of *Primulina* (e.g., Du et al. 2021; 2022; Xiong et al. 2022; Pan et al. 2022), which are also different from this new taxon. We then confirmed that it was a new species.

## Material and method

The description is based on specimens collected in the wild. The measurements and morphological characteristics of the new species were taken from the type specimens processed by the authors. The type specimens of the new species were deposited in IBK. We examined *Primulina* specimens in IBSC, K, IBK, KUN, US, PE, and VNMN. We also searched two online herbaria, Chinese Virtual Herbarium (CVH, <http://www.cvh.ac.cn>) and Chinese Field Herbarium (CFH, <http://www.cfh.ac.cn>), to find species with similar morphology. We determined it to be an undescribed taxon. All morphological characters were studied under a dissecting microscope and were described using the terminology used by Wang et al. (1990, 1998).

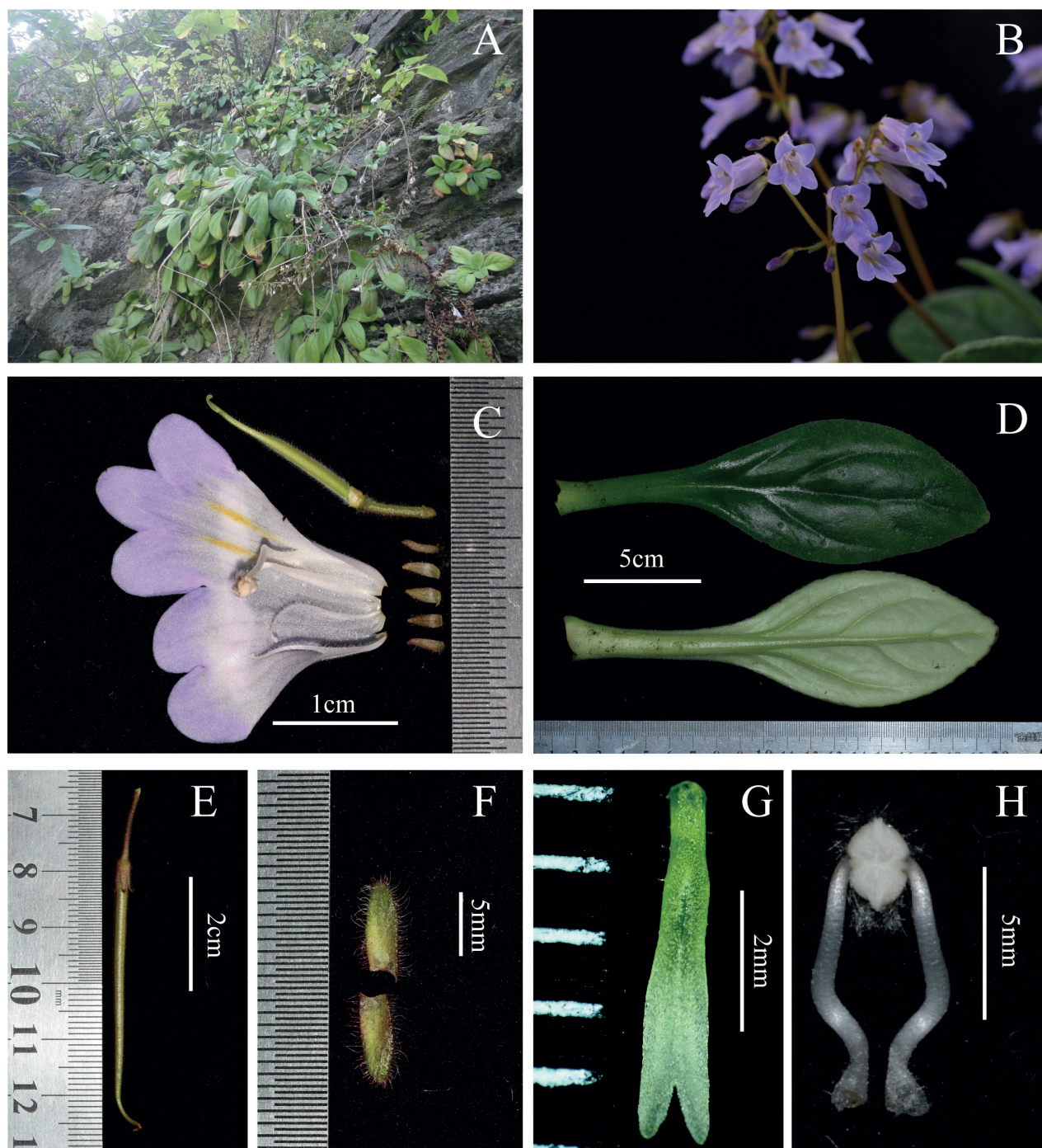
## Taxonomic treatment

***Primulina pingnanensis* Xin Hong, Z.L.Li & W.C.Chou., sp.nov.**

urn:lsid:ipni.org:names:77324168-1

Fig. 1

**Diagnosis.** *Primulina pingnanensis* morphologically resembles *P. orthandra* but is distinguished from the latter by bracts lanceolate (vs. ovate), corolla tube funnel-form and no constriction in the middle (vs. tube near tubular with constriction in the middle), outer corolla surface sparsely white puberulent (vs. glabrous), corolla lobes oblong (vs. broadly ovate), filaments strongly curved at the middle (vs. straight), anthers fused from the entire adaxial surface and sparsely barbate (vs. confluent at apex, glabrous), staminodes obvious, 1–1.3 cm long, sparsely pubescent (vs. ca. 1.5 mm long, glabrous). Detailed morphological comparisons with *P. orthandra* are provided in Table 1 and Fig. 2.



**Figure 1.** *Primulina pingnanensis* Xin Hong, Z.L.Li & W.C.Chou **A** plants in natural habitat **B** cymes **C** opened corolla and dissected calyx lobes with pistil **D** leaves (up: adaxial surface, down: abaxial surface) **E** young fruit **F** bracts **G** stigmas **H** stamens.

**Type.** CHINA. Guangxi Zhuangzu Autonomous Region: Guigang City, Pingnan County, Shuangma Village, 23°37'N, 110°19'E, growing atop a cliff on a limestone hill, 28 March 2018, *Chou Wei-Chuen*, CWC180328-01 (holotype: IBK!; isotypes: AHU!, IBK!).

**Description.** Perennial herb. Rhizomatous stem subterete. **Leaves** basal, opposite, and congested at rhizome apex after years of growth; petiole 5–8 cm long, densely pubescent. Leaf blade dark green, thick herbaceous, elliptic to ovate-elliptic, 15–20 × 6–8 cm, apex acute to obtuse, base broadly cuneate, slightly oblique,



**Table 1.** Diagnostic character differences between *Primulina pingnanensis* and *P. orthandra*.

Character	<i>Primulina pingnanensis</i>	<i>P. orthandra</i>
Bracts	lanceolate	ovate
Tube	funnel-form, no constriction in the middle	tube near tubular, constriction in the middle
Outer corolla surface	sparsely white puberulent	glabrous
Corolla lobes	lobes oblong	lobes broadly ovate
Filaments	strongly bent at the middle	straight
Anthers	fused by the entire adaxial surface and sparsely barbate	confluent at apex, glabrous
Staminodes	obvious, 1–1.3 cm long, sparsely pubescent	ca. 1.5 mm long, glabrous

margin sinuate, densely pubescent on both surfaces; 3–4 pairs of lateral veins on each side of the midrib; adaxially midrib main vein conspicuously sunken and lateral veins inconspicuously sunken, abaxially midrib main vein and lateral vein conspicuously raised. **Cymes** 1–7, axillary, 1–3-branched, 1–8(–16)-flowered; peduncles 18–25 cm long, densely brown puberulent and pubescent; bracts 2, opposite, lanceolate, ca.  $7 \times 2.5$  mm, densely brown pubescent on both surfaces. Pedicels 2.5–4 cm long, densely brown puberulent, and pubescent. Calyx 5-lobed from the base, lobes narrowly lanceolate to triangle, apex acute, ca.  $4 \times 0.9$  mm, outside pubescent, inside glabrous. **Corolla** zygomorphic, lilac, –4.5 cm long, outside sparsely white puberulent, inside glabrous; tube funnel-form, 2.0–3.0 cm long, 1.0–1.3 cm in diameter at the mouth, ca. 5 mm in diameter at the base; limb distinctly 2-lipped; dorsal lip 2-lobed to the middle, lobes ca. 1.0 cm long, apex suborbicular; ventral lip 3-lobed to the middle, lobes oblong, 1.0–1.5 cm long. **Stamens** 2, glabrous, adnate to the corolla tube for ca. 10 mm above the base; filaments 8–10 mm long, extending outwards at the base of the corolla, strongly curved at 90° degrees at the middle, sparsely white pubescent at base; anthers white, ca. 2 mm long, fused by the entire adaxial surface, sparsely barbate; Staminode 3, lateral ones obvious, linear, sparsely pubescent, 1–1.3 cm long, adnate to the corolla for 8 mm above the base; the middle one capitate, ca. 1 mm long, adnate at the corolla base. Disc annular, slightly oblique, ca. 1.5 mm in height, margin repand. **Pistil** 2.8–3.2 cm long; ovary ca. 1.8 cm long, densely white pubescent; style ca. 1 cm long, densely puberulent; stigma trapeziform, ca. 2 mm long, apex slightly 2-lobed, lobes obtuse triangle, ca. 0.4 mm long. **Capsule** 4.5–6 cm long, sparsely brown puberulent, narrowly oblong-ovoid, dehiscing loculicidally into two valves.

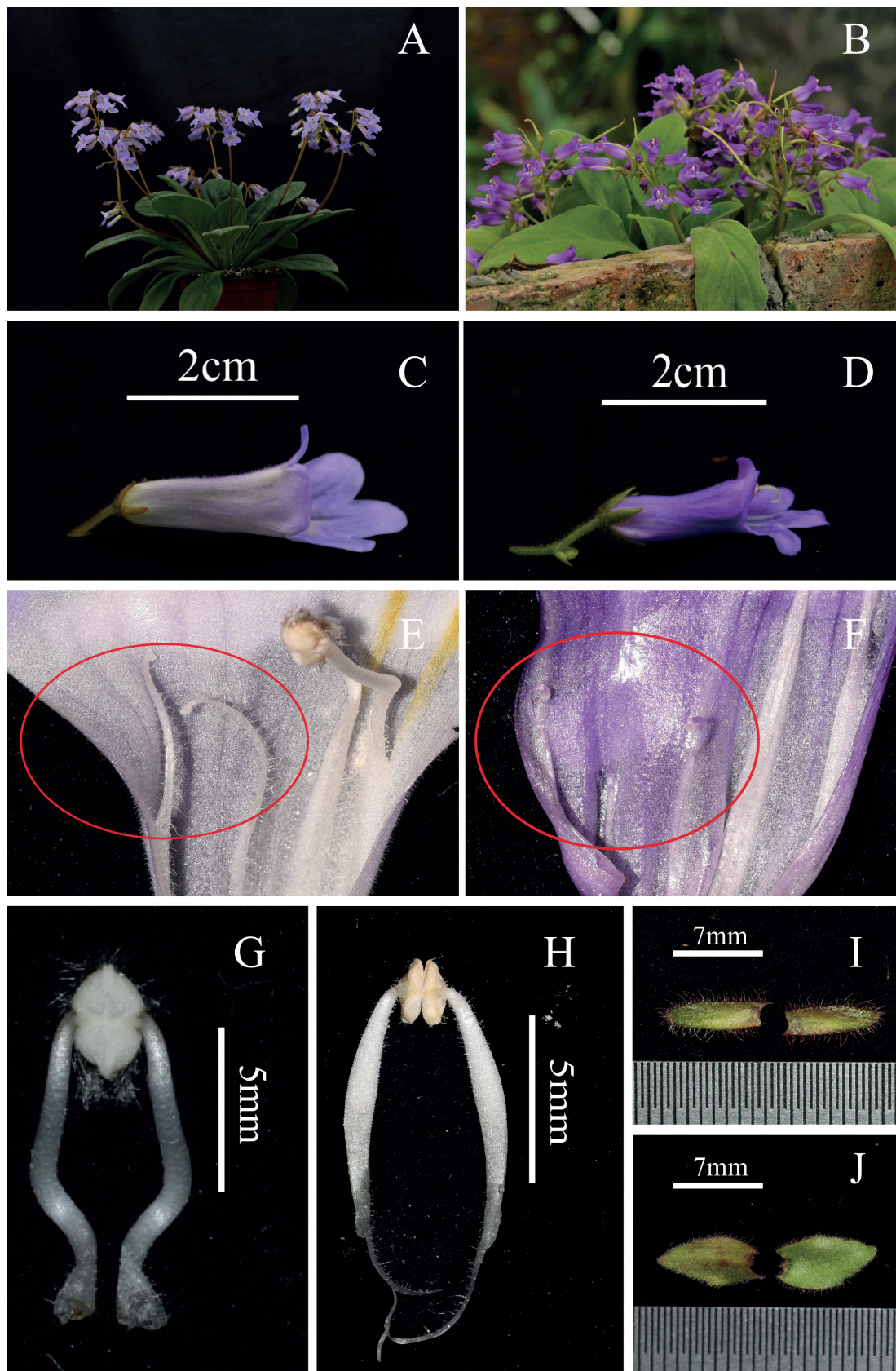
**Phenology.** Flowering from the second half of March to the first half of April, fruiting from June to July.

**Etymology.** The specific epithet is derived from the type locality, Pingnan County. This county is the birthplace of Mr. Chou's mother. Thus, Mr. Chou strongly suggested using "*pingnanensis*" as the scientific name.

**Vernacular name.** Píng Nán Bào Chūn Jù Tái (Chinese pronunciation); 平南报春苣苔 (Chinese name).

**Distribution and habitat.** *Primulina pingnanensis* is only known from the type locality, Shuangma Village, Pingnan County, Guigang City, Guangxi Zhuangzu Autonomous Region, growing on limestone cliffs at an elevation of ca. 50 m. The average temperature in the distribution area is 22.6 °C, while the average annual precipitation is 1050–2100 mm. The habitat is very close to the village with human activities.





**Figure 2.** Morphological comparisons between *Primulina pingnanensis* and *P. orthandra* **A** habit of *P. pingnanensis* **B** habit of *P. orthandra* **C** lateral view of mature flower of *P. pingnanensis* **D** lateral view of mature flower of *P. orthandra* **E** stamens of *P. pingnanensis* **F** stamens of *P. orthandra* **G** filaments of *P. pingnanensis* **H** filaments of *P. orthandra* **I** bracts of *P. pingnanensis* **J** bracts of *P. orthandra*.

**Preliminary conservation assessment.** The terrain in the North and South of Pingnan County is mountainous, while the central area is flat. Very few limestone hills are in the plains, but some mountains are near Shuangma Village. After finding the new species, we conducted several detailed explorations of the area. The current survey results showed that this species has a small population at the top of a limestone mountain near Shuangma Village, with fewer than 200 individuals. According to the results of our field investigations in the type locality and adjacent regions, the EOO and AOO of *Primulina pingnanensis* are about 2 km<sup>2</sup> and 0.1 km<sup>2</sup>, respectively. The severe drought in the second half of 2022 has seriously affected the population. According to the field survey, it is preliminarily estimated that the number of individuals in this population has decreased by 40% or more. More in-depth habitat surveys are warranted to determine if there are more populations nearby. For this current study, we temporarily assess the status of this species as Critically Endangered [CR B1+B2ab (iii, v)], according to the IUCN Red List Categories and Criteria (IUCN 2022).

## Notes

### Validation of new combination in *Primulina*

When the new combination for *Primulina crassifolia* was made by Anh et al. (2022), it was later noticed that the combination was invalid. Therefore, we validate it here.

***Primulina crassifolia* (Aver. & K.S.Nguyen) T.T.P.Anh, F. Wen & Mich.Möller, comb. nov.**

urn:lsid:ipni.org:names:77324169-1

*Chirita crassifolia* Aver. & K.S.Nguyen, Pl. Diversity Fl. Veg. Bat Dai Son 254 (2021). Basionym.

**Type.** Vietnam, Ha Giang Province: Quan Ba district, Tung Vai commune, steep rocky slopes to large and deep cave composed of highly eroded marble-like white limestone, elevation 900–1000 m a.s.l., 17 October 2018, L. Averyanov, Nguyen Sinh Khang, T. Maisak, Truong Duc Thieu, VR 938 (holotype: LE!; isotypes: HN!, LE!).

## Discussion

The Karst and Danxia landforms from south & southwest China to the north Indo-China Peninsula are well-known for their high species diversity and endemism levels. Some interesting genera of Gesneriaceae for example, *Primulina*, *Petrocodon* Hance, and *Hemiboea* Clarke have been attracting the attention of botanists and taxonomists in the past decades. *Primulina* speciation is positively associated with changes in past temperatures and East Asian monsoons. Climatic change around the mid-Miocene triggered an early burst (Kong et al. 2017; Xu et al. 2019; Hsieh et al. 2022). There is abundant diversity in the morphology of the vegetative and reproductive organs of *Primulina*. Still, it can be distinguished by its rosette leaves, tubular corolla, parietal placentation, etc. We concluded

that *P. pingnanensis* is an undescribed species of *Primulina* based on extensive investigation in south and southwest China. A comparison of known live plants and specimens of the genus *Primulina*. *P. orthandra* can be regarded as similar to *P. pingnanensis* because they both have similar leaf morphology, calyx, and purple corolla. However, the differing characteristics of the peduncle, bract, stamen, and staminodes can quickly distinguish the two species from each other.

The high species diversity and restricted distributions on limestone habitats have made the calciphilous *Primulina* an ideal study subject for understanding plant radiation on Sino-Vietnamese limestone karsts (Wei et al. 2022). The habitat of *P. pingnanensis* is located in the mountains in the middle of Pingnan County. The rest of the area is a flat plain. The spatial heterogeneity has created a unique species of *Primulina* in Pingnan. This suggests that the karst areas in southern China have acted as both “museums” and “cradles” of plant evolution (Xu et al. 2019). The discovery and publication of this new species supplements the diversity of *Primulina* distributed in Guangxi. It further indicates that there are more undiscovered new species of *Primulina* in the vast karst areas of south China (Hong et al. 2020; Fu et al. 2022).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

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### Author contributions

WC, ZL and LD collected this species. ZL, YK, and QX performed the data analysis. ZL and XH wrote the manuscript. LD and XH revised the manuscript. All authors have read and approved the final manuscript.

### Author ORCIDs

Zheng-Long Li  <https://orcid.org/0009-0009-8988-5725>

Yan-Yun Kuang  <https://orcid.org/0009-0001-4964-3969>

Qing-Qing Xu  <https://orcid.org/0009-0007-8725-9291>

Wei-Chuen Chou  <https://orcid.org/0000-0001-6470-9295>

Xin Hong  <https://orcid.org/0009-0003-9477-2414>

Li Ding  <https://orcid.org/0009-0003-1523-1784>

## Data availability

All of the data that support the findings of this study are available in the main text.

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# A new species of *Atriplex* (Amaranthaceae) from the Indian subcontinent

Alexander P. Sukhorukov<sup>1,2</sup>, Nidhan Singh<sup>3</sup>, Maria Kushunina<sup>2,4</sup>, Maxim A. Zaika<sup>1,2</sup>,  
Alexander N. Sennikov<sup>5</sup>

<sup>1</sup> Department of Higher Plants, Biological Faculty, Moscow State University, Leninskie Gory 1/12, Moscow, 119234, Russia

<sup>2</sup> Laboratory Herbarium (TK), Tomsk State University, Lenin Ave. 36, Tomsk, 634050, Russia

<sup>3</sup> Department of Botany, I.B. College, Panipat, Haryana-136021, India

<sup>4</sup> Department of Plant Physiology, Biological Faculty, Lomonosov Moscow State University, Moscow, 119234, Russia

<sup>5</sup> Botanical Museum, Finnish Museum of Natural History, University of Helsinki, 00014, Helsinki, Finland

Corresponding author: Alexander P. Sukhorukov ([suchor@mail.ru](mailto:suchor@mail.ru))

## Abstract

A new subshrubby  $C_4$ -species from the lowlands and foothills of India, Pakistan and SE Afghanistan, *Atriplex pseudotatarica*, is described and illustrated. Previously, it was incorrectly identified as *A. crassifolia* auct. non C.A.Mey. belonging to a distant  $C_3$ -group of the genus. A phylogenetic analysis based on nrITS and nrETS revealed its position as sister to *A. schugnanica* (sect. *Obionopsis*). Both species share aphyllous inflorescence and smooth bract-like cover, but differ in life form, leaves, seed colour, and geographical distribution. We revised native Indian *Atriplex* species and excluded some of them from the flora of the country. An improved checklist of the native *Atriplex* species in India with their corrected synonymy and nomenclature is given, and a new diagnostic key is provided.

**Key words:** *Atriplex*, Chenopodiaceae-Amaranthaceae, Indian subcontinent, new species



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## Introduction

*Atriplex* L. is the largest genus in the Amaranthaceae clade encompassing ca. 260 species distributed mostly in arid regions of the world (Žerdoner Čalasan et al. 2022). There is a relatively limited number of *Atriplex* species in the Indian subcontinent. The latest treatments for the flora of Pakistan (Ali and Qaiser 2001) and India (Paul 2012) counted 12 and seven species, respectively. Sukhorukov et al. (2019) revised *Atriplex* in the Himalayas and Tibet, and provided many taxonomic changes for the genus in the Indian Himalaya compared to the previous checklists and floras. All *Atriplex* species native to the Himalayas are represented by the annual  $C_4$  species, but they have different origins. Two of them, *A. pamirica* Iljin and *A. centralasiatica* Iljin, are typical Central Asian elements, whereas *A. schugnanica* Iljin originated in the eastern Irano-Turanian region (Žerdoner Čalasan et al. 2022). Subsequently, the first two species are classified within *A. sect. Obione* (Gaertn.) Reichenb., and *A. schugnanica* is a member of *A. sect. Obionopsis* (Lange) Dumort. (Sukhorukov et al. 2022). In comparison to the species distributed in the Himalayas, the species growing in

lowlands and foothills of the Indian subcontinent are still undercollected and poorly known, because the classical authors preferred to stay in the mountains rather than in the plains during the summer time due to harsh climatic conditions in the latter region, and had a preference for species-rich plant diversity in the mountains.

Unusual *Atriplex* plants were noted in the year 2021 in Haryana State (India) by one of the authors (NS) of the present paper. Further *in situ* studies have confirmed an assumption that the specimens cannot be assigned to any known species or their synonyms, and should be described as a new species.

## Materials and methods

### Material investigated

Field studies were carried out in the Haryana State (India). Taxonomic revision of the herbarium material was undertaken in the herbaria BM, CAL (examined as digital images), DD, K, LE, MHA, and MW. Distribution map is based on the specimens cited in the text and was prepared using SimpleMappr online tool (<http://www.simplemappr.net>).

### Sampling of the study, DNA extractions, amplification, and sequencing

Thirty-seven accession numbers were included in the phylogenetic analyses representing *Atriplex* species, and two accession numbers were taken as outgroups from Amaranthaceae. The samples are listed in Table 1. We have reconstructed a part of the global phylogenetic tree originally published by Žerdoner Čalasan et al. (2022) and indicated the position of the new species among its close relatives.

Among 16 species analyzed in *A. sect. Obionopsis* and close relatives (*A. flabellum* Bunge, *A. moneta* Bunge), 15 accessions were represented by ITS and ETS loci (see below) (Table 1). We included only ETS sequences for one species (*A. kalafricanica*). Following Kadereit et al. (2010), we selected *Halimione pedunculata* (L.) Aellen as an outgroup for ITS- and ETS-based molecular phylogenetic analyses. In short, we analyzed 37 ITS and ETS sequences of 19 taxa (Table 1). We obtained two of these sequences (one of ITS and one of the ETS regions of rDNA) as a part of this study (Table 1) and took the remaining ones from the study of Žerdoner Čalasan et al. (2022).

The DNA from a sample of *A. pseudotatarica* collected in the state of Haryana, India (see also the Results section) was extracted from 5–10 mg of dried leaves employing the DNeasy Plant Mini Kit (Qiagen, the city of Valencia, CA, USA), as described in the manual.

PCRs were carried out in Thermal Cycler T100 (Bio-Rad, USA) using the primers and cycling protocols summarized in Table 2.

The PCR cocktail (20 µL) contained 1.5–2 ng of the total DNA, 5 pmol of each primer, 4 µL of Ready-to-Use PCR Master mix 5× MasDDTaqMIX-2025 containing a “hot-start” SmarTaq DNA polymerase (Dialat Ltd., Moscow, Russia).

PCR products were purified with the Cleanup Mini BC023S Kit (Evrogen, Russia) following the manufactured instructions. Sanger sequencing was performed at Evrogen JSC (Moscow, Russia) employing PCR primers (Table 2).



**Table 1.** GenBank accession numbers for the species of *Atriplex* and an outgroup included in the phylogenetic analysis.

Species	ITS	ETS
<i>A. dimorphostegia</i> 377	OM180193	OM179544
<i>A. flabellum</i> 4591	OM180202	OM179553
<i>A. fominii</i> 4216	OM180203	OM179554
<i>A. kalafganica</i> 4223	–	OM179575
<i>A. laciniata</i> 4357	OM180227	OM179577
<i>A. lasiantha</i> 4221	OM180231	OM179580
<i>A. moneta</i> 4592	OM180253	OM179599
<i>A. olivieri</i> 4229	OM180268	OM179612
<i>A. ornata</i> 4508	OM180270	OM179614
<i>A. paradoxa</i> 3917	OM180276	OM179620
<b><i>A. pseudotatarica</i> 9</b>	OQ843457	OQ829353
<i>A. pratovii</i> 4236	OM180288	OM179631
<i>A. pungens</i> 4365	OM180292	OM179635
<i>A. recurva</i> EM391	OM180298	OM179641
<i>A. schugnanica</i> 4367	OM180307	OM179648
<i>A. tatarica</i> 4570	OM180325	OM179665
<i>A. tatarica</i> var. <i>pseudoornata</i> 4373	OM180326	OM179666
<i>A. tornabenei</i> 4375	OM180327	OM179667
Outgroup		
<i>Halimione pedunculata</i> s.n.	OM180349	OM179688

The number next to the taxon indicates the voucher (see Žerdoner Čalasan et al. 2022). We highlighted in bold the binomial of the new species.

## Alignment and phylogenetic analyses

The L-INS-i alignment strategy with default settings of MAFFT version 7.0 (Katoh et al. 2017) was used to align sequences from both datasets (ITS and ETS). Two obtained alignments were manually edited and concatenated in program PhyDe version 0.9971 (Müller et al. 2010). The combined dataset (ITS and ETS) comprises 1032 bp (593 in ITS and 439 in ETS alignment) and 19 taxa.

We reconstructed the ITS plus ETS phylogeny of *Atriplex* sect. *Obionopsis* and two close relatives (*A. flabellum*, *A. moneta*) using the Maximum Likelihood approach (ML; Felsenstein 1973, 1983) and Bayesian Inference (BI; Rannala and Yang 1996). Gaps were treated as missing data. A variant of the General Time Reversal nucleotide substitution model (Tavaré 1986) (GTR + G + I) was automatically selected by jModelTest v.2.0 (Darriba et al. 2012) for each partition (ITS and ETS) following the Akaike Information Criterion (AIC; Akaike 1974). For the ML analyses of concatenated alignment, we employed RAXML v.8 (Stamatakis 2014). ML Bootstrap analysis was conducted with 2500 replicates by the same program.

BI was performed in BEAST v.2.6.7 (Drummond et al. 2002; Bouckaert et al. 2014). Two runs with four chains each were run for 20 million generations for the combined dataset; both chains were sampled every 20,000 generations with a default parameter. Output log files were analysed using TRACER v.1.6

**Table 2.** Primers and cycling protocols.

Marker	Primer	The source of primer	Cycling protocols (modified from Zacharias and Baldwin (2010))
ITS	Forward (ITS-5): 5'-GGA AGT AAA AGT CGT AAC AAG G-3'	White et al. (1990)	96 °C for 1 min; 40 cycles of (96 °C for 10 sec, 48 °C for 30 sec, and 72 °C for 20 sec + 4 sec/cycle); 72 °C for 5 min.
	Reverse (ITS-4): 5'-TCC TCC GCT TAT TGA TAT GC-3'		
ETS	Forward: (ETS-Atr): 5'-CAC GTG TGA GTG GTG ATT GGT T-3'	Zacharias and Baldwin (2010)	96 °C for 1 min; 40 cycles of (96 °C for 10 sec, 60 °C for 30 sec, and 72 °C for 20 sec + 4 sec/cycle); 72 °C for 5 min
	Reverse (18S-E): 5'-GCA GGA TCA ACC AGG TAG CA-3'	Baldwin and Markos (1998)	

(Rambaut et al. 2014) to assess all parameters' convergence and effective sample size (ESS). Ten percent of the samples were removed as burn-in. A maximum clade credibility tree was generated using TREE ANNOTATOR v.2.4.5 (Drummond and Rambaut 2007).

## Results

### *Atriplex pseudotatarica* Sukhor. & Nidhan Singh, sp. nov.

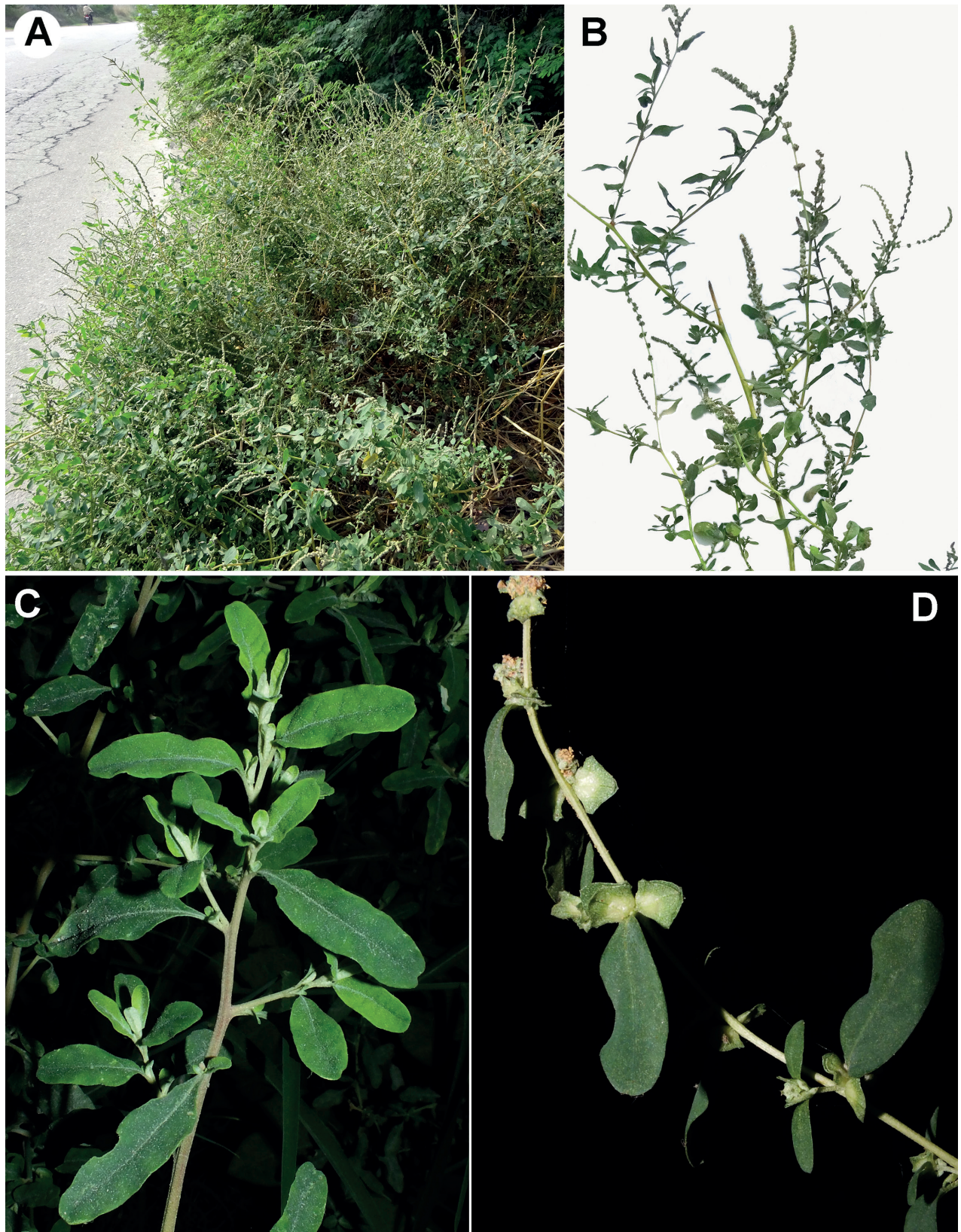
urn:lsid:ipni.org:names:77324170-1

Fig. 1

- *Atriplex crassifolia* auct. non C.A.Mey.
- *Atriplex leucoclada* auct. non Boiss.
- *Atriplex* spp. div. in herb. DD and K.

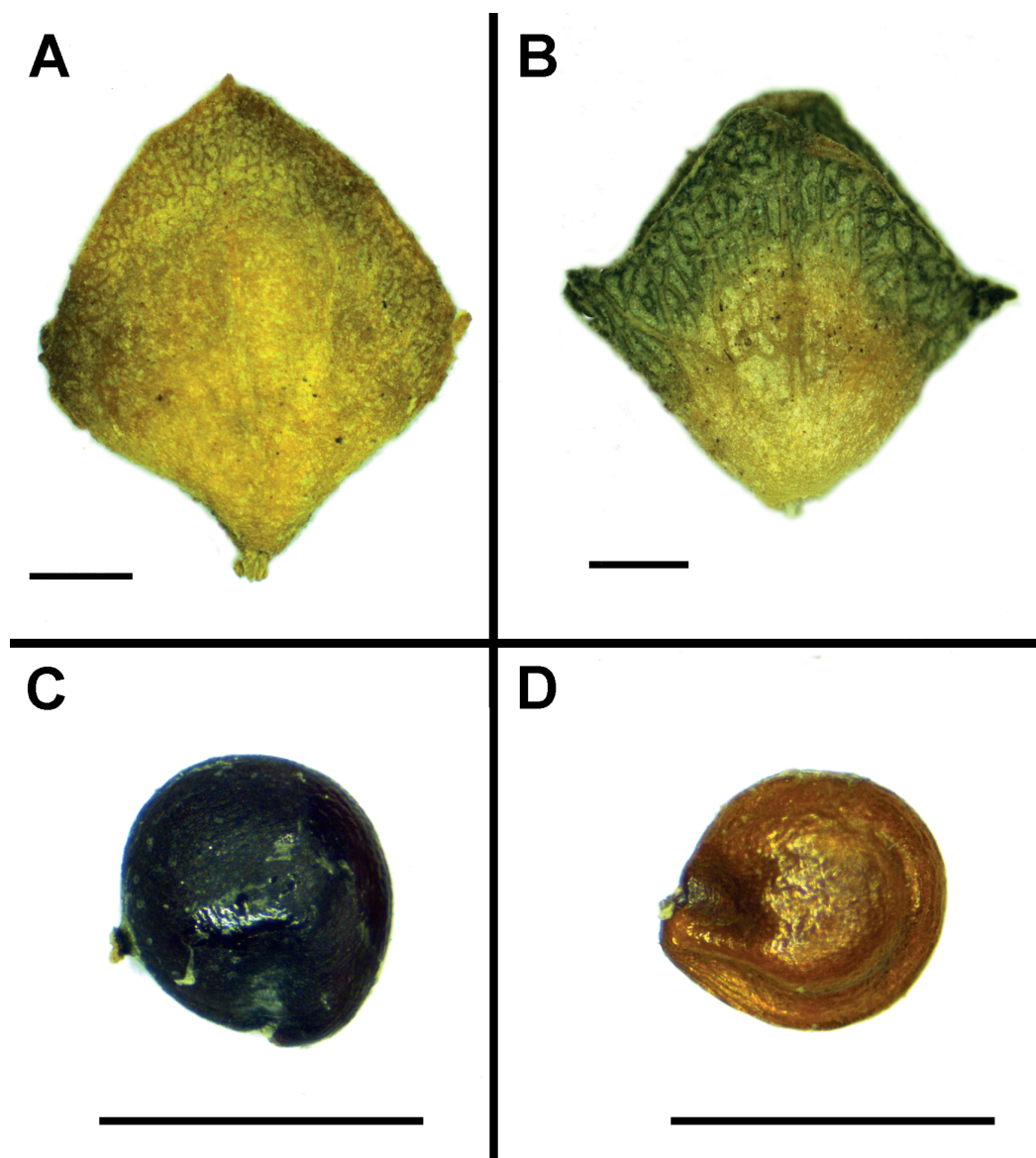
**Type.** INDIA. Haryana, surroundings of Panipat town, near Asan Kalan village, 29°15.1286'N, 76°31.4816'E, 15 Nov 2022 [in flowering and early fruiting stages], *N. Singh & A. Sukhorukov* 9 (Holotype: CAL, isotype: BSD).

**Description.** Monoecious subshrub up to 1.5 m high, branched in upper half; leaves alternate, shortly petiolate; petioles up to 1.0 cm long; blades greyish-silvery on both sides, 1.0–4.0 × 0.5–1.0 cm (much smaller towards inflorescence), oblong or narrowly oblong, entire or shallowly sinuate, with Kranz-anatomy; inflorescences branched, up to 15 cm long, with pseudopposite bracts or with a few small leaves forming pseudowhorls (after fruiting turning into small alternate leaves in younger shoots), aphyllous in other parts; glomerules condensed or slightly interrupted, of both male and female flowers, the latter are also located at the axils of uppermost leaves below the main inflorescence; male flowers stipitate at base, with 5 free perianth segments, anthers 0.25 mm long; bract-like cover of female flowers (Fig. 2A, B) rhombic, entire or scarcely dentate, with or without lateral angles, smooth at the back or rarely with 1–2 very short outgrowths, valves connate to the halfway, sometimes to one third of their length, with indistinct veins, heteromorphic in some other characters: (1) bract-like cover of female flowers located in leaf axils ± indurated in lower half



**Figure 1.** *Atriplex pseudotatarica*. **A** an overview of the plant **B** a twig with the inflorescence **C** a vegetative shoot **D** a shoot at fruiting. Origin of the material **A** Haryana, near Asan Khurd village, Nov 2022 **B** Haryana, near Asan Kalan village, Nov 2022 **C** Haryana, near Asan Kalan village, Aug 2022 **D** Haryana, near Panipat town, Oct 2014. Photographer: **A, B** A. Sukhorukov, **C, D** N. Singh.





**Figure 2.** Bract-like cover (**A, B**) and seeds (**C, D**) of *A. pseudotatarica* **A** cover of a female flower located below the main inflorescence **B** cover of a female flower located in the main inflorescence **C** black seed **D** yellowish-brown seed. Scale bars: 1 mm.

and inflated at fruiting, 4.5–5.5 mm long, rhombic, and (2) bract-like cover of female flowers located in the main inflorescence slightly indurated and not inflated at fruiting, 2.0–4.0 mm long, trilobate and rhombic; seeds heterospermic (Fig. 2C, D): seeds developing in fruits located below the main inflorescence black, slightly elongated (1.1–1.3 × 1.4–1.6 mm), ripening earlier (in November; obs. in Haryana State, India) compared with those of the fruits located in the main inflorescence; seeds in fruits located in the main inflorescence black (similar to those developing below the main inflorescence) or yellowish-brown, 0.8–1.0 mm in diameter, ripening in late November–December.

**Phenology.** Flowering: July–November; fruiting: November–December.

**Habitat.** Saline soils, sands, wasteland, roadsides, 0–2200 m a.s.l. In the natural landscapes in Haryana, *Atriplex pseudotatarica* was observed together with *Bassia indica* (Wight) A.J.Scott, *Suaeda fruticosa* Forssk. (all – Amaranthaceae), and some grasses.



**Etymology.** The specific epithet is chosen due to the resemblance of the new species to *A. tatarica* L., which also has long aphyllous inflorescences.

**Conservation status.** Although there is currently a limited number of collected specimens of *Atriplex pseudotatarica*, this species is clearly more overlooked than rare. Given that it is often found in disturbed habitats, produces a large number of seeds and is naturally adapted to saline substrates, we propose that the species should be assigned to the IUCN Red List category “Least Concern” (IUCN Standards and Petitions Committee 2022).

**Distribution (Fig. 3).** India, Pakistan and SE Afghanistan.

**Specimens examined.** AFGHANISTAN. [Laghman province] Alingar valley, 6000 ft, 1 Sep 1956, W. Thesiger 1693 (BM).

INDIA. Delhi, 23 Oct 1874, *anonymous* 23395 (K); [Himachal Pradesh] Kimawar [Kinnaur], 1884, J.R. Drummond 535 (DD-29978); [Haryana], Karnal, 1885, J.R. Drummond 26479 (K); Haryana, surroundings of Panipat town, nr Asan Khurd village, 29°18.1584'N, 76°31.8779'E, 15 Nov 2022, N. Singh & A. Sukhorukov 7 (CAL, BSD).

PAKISTAN. Lahore, 1846, T.T. Thomson s.n. (K); [Punjab] Rawalpindi, 1872, J.E.T. Aitchinson 224 (K); [Gilgit-Baltistan prov.] Skardu, 7000–8000 ft, 15 Jul [18]92 [early flowering], *without collector's name* 12060 (DD!); [Khyber Pakhtunkhwa prov., Swat Distr.] Shohdara, 11 Nov 1935 [in fruiting stage], R.R. Stewart 15362 (DD-77925); Lahore, common in weedy places, 17 Oct 1938 [in flowering stage], Parker s3436 (DD-81928, DD-81929, K); [Punjab province], nr Attock, 15 Nov 1956, R.R. Stewart 27830 (K).

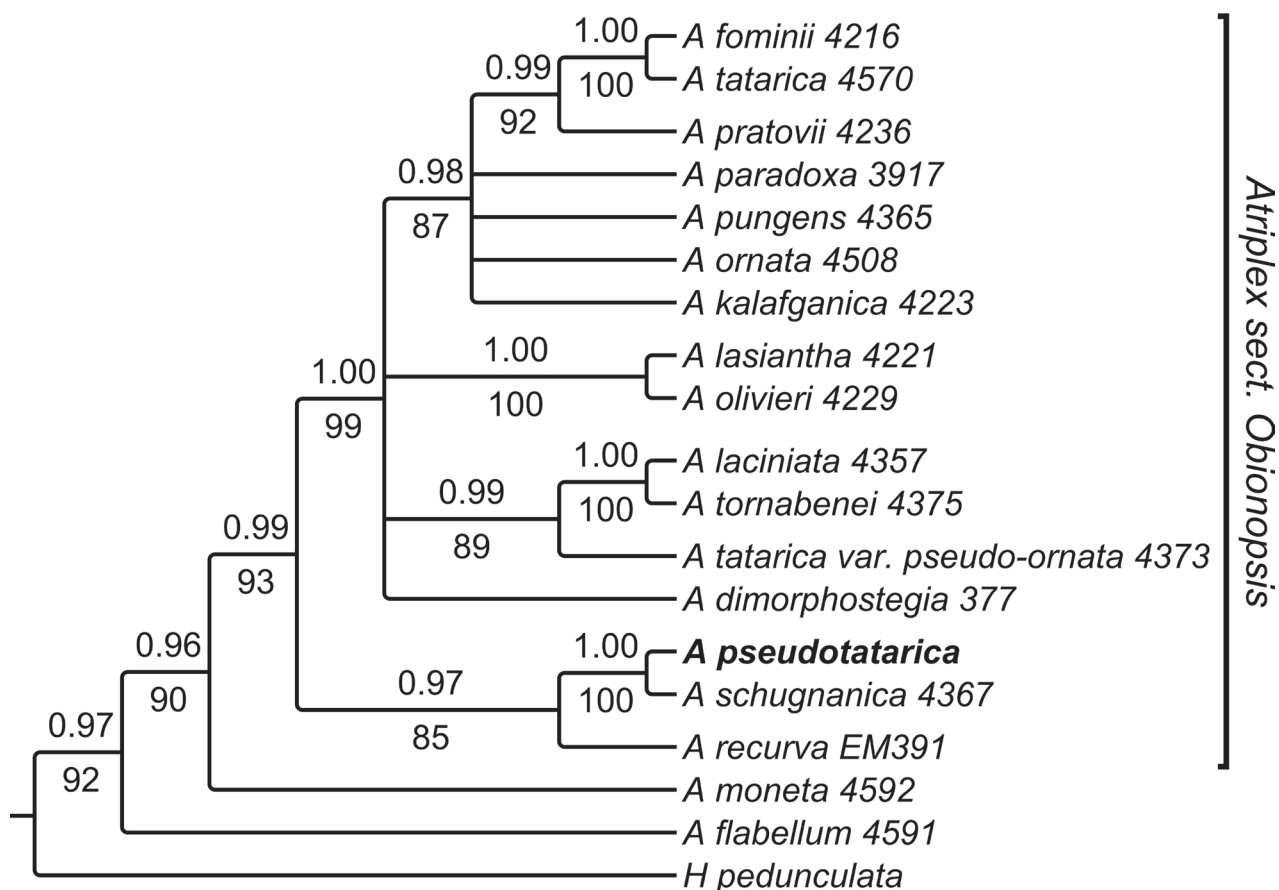
**Notes.** All examined herbarium specimens of *A. pseudotatarica* are represented by upper twigs of the plants. They were mostly incorrectly identified as *A. crassifolia*, or rarely left without identification, as *Atriplex* sp. To date, the name *A. crassifolia* may be found misapplied to some other species attributable to different groups of the genus. *Atriplex crassifolia* is an annual C<sub>3</sub>-species belonging to *A. sect. Teutliopsis* Dumort. (Moser 1934; Iljin 1936; Sukhorukov 2006; Žerdoner Čalasan et al. 2022) with a restricted distribution range in semi-deserts of Kazakhstan and South-West Siberia, Russia (Iljin 1936; Sukhorukov 2006), penetrating into western China (Sukhorukov in Nobis et al. 2016). All other records of *A. crassifolia* reported from Europe are erroneous (Sukhorukov 2006; Sukhorukov et al. 2019). Aellen (1939), Ivanov (1989) and Medvedeva (1996) erroneously applied this name to *A. patens* (Litv.) Iljin, another species from *A. sect. Teutliopsis* (Sukhorukov 2006). The specimens from the Mediterranean area (GBIF Sekretariat 2022; re-identifications in BM!, K!, LE!) belong to *A. tornabenei* Tineo (C<sub>4</sub>-clade, *A. sect. Obionopsis* (Lange) Dumort.: Sukhorukov et al. 2022). The name *A. crassifolia* has also been widely used for the plants growing in lowlands of India and Pakistan (e.g., Hooker (1890), Bamber (1916)), and it is still erroneously applied in recent floras, checklists and ecological studies (Puri et al. 1964; Shetty and Singh 1991; Hussain and Mirza 1993; Jain et al. 2000; Kumar 2001; Paul 2012; Kumar and Singh 2013; Ibrahim 2019). Hooker (1890) stated that *A. crassifolia* is present in both lowlands (“westwards of Jumna [Yamuna] River”) and high mountains. Nevertheless, all records of *A. crassifolia* from the Himalayas refer to C<sub>4</sub>-species from *A. sect. Obione* (Gaertn.) Reichenb.: *A. pamirica* Iljin and *A. schugnanica* Iljin [= *A. pallida* (Moq.) Sukhor. ≡ *Chenopodium pallidum* Moq., nom. rejic. prop.], and those from the lowlands and foothills belong to *A. pseudotatarica*.



Figure 3. Records of *A. pseudotatarica*.

Some of the plants growing in the lowlands of Pakistan also belong to *A. pseudotatarica*, of which some specimens were misidentified as *A. leucoclada* Boiss. Hedge (1997) noted that this species is highly polymorphic in the area covered in “Flora Iranica”, with the extreme forms having smooth, apically trilobate bract-like cover. Unfortunately, he did not indicate where such specimens were collected, but such characters are typical of *A. pseudotatarica*.

**Phylogenetic relationships (Fig. 4).** Based on the combined nrITS and nrETS analyses, *A. pseudotatarica* was found sister to *A. schugnanica*, and both form a subclade within the Eurasian clade, *A. sect. Obionopsis* (Lange) Dumort., which comprises ~ 15  $C_4$ -species predominantly distributed in Irano-Turanian floristic region (Sukhorukov et al. 2022; Žerdoner Čalasan et al. 2022). *Atriplex pseudotatarica* and *A. schugnanica* share some characters typical of the members of *A. sect. Obionopsis* (aphyllous inflorescences, sclerified bract-like cover with the valves connate up to the half of their length, presence of the female flowers in leaf axils and both female and male flowers in the inflorescence), but have several conspicuous morphological differences (Table 3). Additionally, *A. pseudotatarica* is distributed in the lowlands and pre-Himalayan foot-



**Figure 4.** Maximum clade credibility tree from the BEAST analysis of the ITS+ETS *Atriplex* dataset. Bayesian posterior probabilities are given above the branches, bootstrap percentages of the maximum likelihood analyses are given below the branches.

hills, whereas *A. schugnanica* is a typical montane plant growing in the West Himalayas, Karakoram and Pamir at the altitudes of (2000) 2600–4800 m a.s.l. (Iljin 1936; Sukhorukov et al. 2019). In Table 3, we also included other similarly looking  $C_4$  *Atriplex* species; three of them (except *A. tatarica*) are present in the lowlands of Pakistan, and only one (*A. pseudotatarica*) is reaching India. *Atriplex tatarica*, widely distributed in many parts of the “Flora Iranica” area, as well as *A. kalafganica* Aellen (Aellen in Podlech 1975) are also added here because of their morphological resemblance with *A. pseudotatarica*.

The most conspicuous characters of *A. pseudotatarica* are subshrubby life form, pseudopposite leaves or leaves in pseudowhorls below the inflorescence seen at fruiting, and presence of black seeds.

## Discussion

### Genus *Atriplex* in India

A recent revision of the genus in the Himalayan area (Jammu and Kashmir, Himachal Pradesh, Uttarakhand States) has been provided by Sukhorukov et al. (2019, with references therein), and some species (*A. crassifolia*, *A. laciniata* L., *A. rosea* L., *A. sagittata* Borkh. [previously known as *A. nitens* Schkuhr: Paul

**Table 3.** The diagnostic features of *A. pseudotatarica* and similarly looking C<sub>4</sub> *Atriplex* species.

Species / Character	Life form	Leaves	Bract-like cover	Seeds
<i>A. kalafganica</i>	Annual	shortly petiolate, rhombic or ovate, entire or dentate; upper leaves do not form pseudowhorls	with small or prominent outgrowths or smooth	red and brown
<i>A. lasiantha</i>	Annual	petiolate, rhombic or ovate, entire or dentate; upper leaves do not form pseudowhorls	with outgrowths or smooth	red and brown
<i>A. leucoclada</i>	Subshrub	sessile (except lowermost leaves), triangular-deltoid, sinuate-dentate; upper leaves do not form pseudowhorls	with outgrowths	dark brown
<i>A. pseudotatarica</i>	Subshrub	shortly petiolate, oblong, (sub)entire; upper leaves pseudopposite or forming pseudowhorls at fruiting	smooth or with 1–2 small outgrowths	black and brown
<i>A. schugnanica</i>	Annual	petiolate, triangular or rhombic; upper leaves do not form pseudowhorls	smooth or with 1–2 small outgrowths	red and brown
<i>A. tatarica</i>	Annual	petiolate, rhombic, triangular, rarely lanceolate, entire to erose-dentate or lobate; upper leaves do not form pseudowhorls	with small or prominent outgrowths or smooth	red and brown

(2012)] were excluded from this area. The Chenopodiaceae of lowland India are still poorly studied and have not been included in any detailed morphological and chorological studies. Additionally, the plant material from India is old and quite scarce in the European herbaria. All these factors influenced a poor knowledge of some genera like *Atriplex*. Below we provide an improved taxonomic survey of *Atriplex* in the tropical part of India, with some notes on alien species of the genus.

Several alien species of Australian and North American origin were reported from India: *A. amnicola* Paul G. Wilson, *A. nummularia* Lindl., *A. lentiformis* (Torr.) S. Watson (Rani et al. 2013; Kumar et al. 2021). As stated by Singh (2005), many areas in India, especially influenced by a monsoon, are unfavourable for (semi)desert plants such as *Atriplex*. The Rajasthan State and some adjacent areas are of particular interest because they lie in the desert zone that is suitable for *Atriplex* species. We did not find any *Atriplex* specimens in Rajasthan, but several species were reported from this region including subshrubby American *A. lentiformis* (Gupta and Arya 1995), European *A. hortensis* (Bole and Pathak 1988) and two native species, subshrubby *A. stocksii* (Wight) Boiss. (Puri et al. 1964; Bole and Pathak 1988; Arya and Lohara 2016) and annual *A. "crassifolia"* (Puri et al. 1964; Shetty and Singh 1991). *Atriplex crassifolia* sensu Puri et al. (1964) was reported from the vicinity of Jodhpur and described in the diagnostic key as "an annual, male flower clusters axillary or in short leafy spikes", but elsewhere these authors provided a different diagnosis ("male flower clusters in slender leafless interrupted spikes"). We were unable to trace which species should be recognised instead of *A. crassifolia* because these contradictory diagnoses cannot be applied to any species. Shetty and Singh (1991) described it as an annual species with interrupted inflorescences and inflated bract-like covers. These two reproductive characters are also found in *A. pseudotatarica*, but the life form is different in the latter species. Nevertheless, we presume that *A. pseudotatarica* may be present in both Rajasthan and Gujarat due to the records in Haryana State.



## Key to *Atriplex* species native to India

All native species have the  $C_4$  leaf anatomy. No  $C_3$  *Atriplex* species were recorded in India. The alien species are not included in the key because their taxonomy and alien status have not been fully evaluated.

- 1 Stems procumbent, rooting at nodes; leaves (sub)opposite, at least in upper part of the branches, entire (species growing in southern India) ..... ***A. repens***
- Stems erect, not rooting at nodes; leaves alternate, usually undulate, dentate or lobate (species from western, central and northern parts of India) ..... **2**
- 2 Valves of bract-like cover almost free, orbicular; coastal subshrubby plants from western India ..... ***A. stocksii***
- Valves rhombic, connate to the half of their length ..... **3**
- 3 Inflorescence aphyllous or bracteate ..... **4**
- Inflorescence leafy (almost) to the top ..... **5**
- 4 Annual growing at high altitudes (2600–4800 m a.s.l.); leaves triangular or oblong; pseudopposite leaves below inflorescence absent ..... ***A. schugnanica***
- Subshrub growing in lowlands and foothills; leaves oblong; pseudopposite leaves below inflorescence present in flowering and early fruiting ..... ***A. pseudotatarica* sp. nov.**
- 5 Plant with tumble-weed habit; stem erect with spreading branches; leaves rhombic or ovate; bract-like cover of female flowers either smooth or with thorn-like outgrowths (on the same plant) ..... ***A. centralasiatica***
- Plant not forming tumble-weed habit; leaves oblong; bract-like cover smooth or with 1–2 small outgrowths ..... ***A. pamirica***

## List of native *Atriplex* species in India

**1. *Atriplex centralasiatica* Iljin, Act. Inst. Bot. Ac. Sci. USSR, ser. 1, 2: 124 (1936).**

**Holotype.** [KAZAKHSTAN] Lac. Balchasch, prope Aczie [Balkhash Lake, near Aqshi], 19 Sep 1930, E. Czerniakowska 819 (LE!).

This species was reported from India for the first time by Sukhorukov et al. (2019) and is distributed in Jammu and Kashmir State.

**2. *Atriplex pamirica* Iljin, Acta Inst. Bot. Ac. Sc. USSR, ser. 1, 2: 124 (1936).**

≡ *Atriplex tatarica* var. *pamirica* (Iljin) G.L.Chu in Kung & Tsien, Fl. Reipubl. Pop. Sin. 25(2): 46 (1979), nom. inval. (Art. 41.5).

**Holotype.** [TAJIKISTAN] Khargosh, in ripa lac. Kara-kul [bank of Kara-kul Lake], 30 Jul 1878, Yu. Ashurbaev s.n. (LE!).

This species is also restricted to Jammu and Kashmir State (Sukhorukov et al. 2019). A varietal rank of this taxon cannot be accepted, because *A. tatarica* and *A. pamirica* occupy distant positions on the molecular tree (Žer-

doner Čalasan et al. 2022) and belong to different sections, *A. sect. Obionopsis* (Lange) Dumort. and *A. sect. Obione* (Gaertn.) Reichenb., respectively (Sukhorukov et al. 2022).

### 3. *Atriplex pseudotatarica* Sukhor. & Nidhan Singh (this paper).

### 4. *Atriplex repens* Roth, Nov. Pl. Sp.: 377 (1821).

≡ *Obione repens* (Roth) G.L. Chu, Gen. New Evol. Syst. World Chenopod.: 165 (2017). Neotype (designated by Turner (2021: 373)): INDIA. *J.P. Rottler s.n.* (K barcode K001129778!, excluding material marked with a pencil cross; iso-neotype G-DC barcode G00687837).

= *Obione nummularia* Moq., Chenop. Monogr. Enum.: 72 (1840).

≡ *Obione koenigii* Moq. in DC., Prodr. 13(2): 109 (1849), nom. illeg. superfl. Holotype: INDIA. "Ex India orientali", *J.P. Rottler s.n.* (G-DC barcode G00687837, isotype K barcode K001129778!).

– *Atriplex koenigii* Wall., Numer. List: no. 6951 (1832), nom. nud.

– *Atriplex repens* B.Heyne in herb.

**Note.** The name *Obione nummularia* Moq. was validly published on the basis of the only specimen (holotype) originating from India, *J.P. Rottler s.n.* collected in the late 18<sup>th</sup> century and received by A. de Candolle under the name "*Atriplex cristata* Koenig" from M.N. Puerari (now at G-DC).

Zhu et al. (2003) and Zhu and Sanderson (2017) reported the presence of *A. repens* on Hainan Island (southern China); however, the latter species is distributed in the coastal areas in southern India (Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh) and Sri Lanka. The correct name for the plants growing in Hainan and other parts of tropical China as well as in southern Japan is *A. maximowicziana* Makino.

### 5. *Atriplex schugnanica* Iljin, Acta Inst. Bot. Acad. Sc. URSS, ser. 1, 2: 123 (1936).

= *Chenopodium pallidum* Moq., Chenop. Monogr. Enum.: 30 (1840), nom. rejic. prop.

≡ *Atriplex pallida* (Moq.) Sukhor., Phytotaxa 226(3): 288 (2015). Lectotype (designated by Sukhorukov and Kushunina (2014: 14)): [Probably NE INDIA] Voyage de V. Jacquemont aux Indes Orient., *Jacquemont 1377* (P barcode P04993339!, isoelectotypes P barcodes P00606416! P04993338! P05047853!). Image of the lectotype available at: <https://science.mnhn.fr/institution/mnhn/collection/p/item/p04993339>

**Lectotype.** (designated by Sukhorukov and Tscherneva in Sukhorukov (2006: 384)): [TAJIKISTAN] Roschan [Roshan], Usoj [Usoy], in ripa flum. Bartanga [bank of Bartanga River], in decliviis lapidosis [rocky slopes], 20 Aug 1897, S. Korshinsky 4692 (LE!, isoelectotype LE!).

**Note.** The name *Chenopodium pallidum* Moq. was proposed for rejection by Mosyakin and Mandak (2021) due to nomenclatural collisions with its typification (Sennikov 2022). Present in North Himalaya to Pamir Mountains: North

India (Jammu & Kashmir, Himachal Pradesh, Uttarakhand), North Pakistan, Afghanistan, Tajikistan (Sukhorukov et al. 2019).

**6. *Atriplex stocksii* Boiss., Diagn. Pl. Orient., ser. 2, 4: 73 (1859).**

≡ *Atriplex griffithii* var. *stocksii* (Boiss.) Boiss., Fl. Orient. 4: 916 (1879).

≡ *Atriplex griffithii* subsp. *stocksii* (Boiss.) Boulos, Nordic J. Bot. 11(3): 310 (1991).

Lectotype (designated by Hedge (1997: 84)): [PAKISTAN] Scinde [Sindh prov.], *J.E. Stocks* 452 (G-Boiss, isolectotypes K barcode K000898566!, K000898567!).

= *Obione stocksii* Wight, Icon. Pl. Ind. Orient. 5(2): 5, tab. 1789 (1852). Lectotype (designated here): [PAKISTAN] Scinde [Sindh prov.], *J.E. Stocks* 452 (K barcode K000898566!, isolectotypes K barcode K000898567!, G-Boiss).

**Notes.** The species was originally described as *Obione stocksii* Wight based on a single specimen, *J.E. Stocks* 452, collected in present-day Sindh Province of Pakistan. Boissier (1859) re-described the species as *Atriplex stocksii* based on two collections by Stocks from Pakistan and one from southern Iran, *Aucher-Eloy* 5268. In the protologue of *A. stocksii*, Boissier cited the type collection used by Wight but employed the same species epithet. For this reason, the protologue of his species name included the type of Wight's species but Boissier's species name cannot be treated as illegitimate. Boissier explicitly described his species as new and validly published its name in its own right, without any presumed basionym; for this reason, this species name has no basionym even though the potential basionym exists. As Boissier's species name is not superfluous, it cannot be automatically typified by the type of Wight's species name.

Wight (1852) used a single specimen to describe his new species, now stored at K, which is, however, not the holotype due to the availability of another element, i.e. an illustration published in the protologue. Boissier (1859) used three specimens, hence lectotypification is also needed. Hedge (1997: 84) indicated that the type of *A. stocksii* Boiss. is the specimen kept at G-DC, which belongs to the same gathering as the type of *O. stocksii* Wight. The later type designation with the specimen at K, which was made by Omer (2001), has no standing.

The species is reported from Gujarat and Tamil Nadu States (Rao 1986; Paul 2012), but in the latter state its presence is dubious. Also present in Rajasthan State.

**List of *Atriplex* species previously reported from India and hereby excluded from this country**

1. *Atriplex crassifolia* C.A.Mey. Occurs only in Kazakhstan, Russia (South-West Siberia), and western China (Xinjiang). Reported by many old and recent authors (see above).
2. *Atriplex laciniata* L. European coastal plant. Previously reported by Aitchison (1869).
3. *Atriplex sagittata* Borkh. (= *A. nitens* Schkuhr). Species native to temperate regions of Eurasia. Previously reported by Paul (2012, as *A. nitens*).
4. *Atriplex rosea* L. Reported by Hooker (1890) and Paul (2012). Native to the Mediterranean, and Asia Minor, with recent scattered records in the Black Sea region and other parts of Europe (Sukhorukov 2006; Sukhorukov et al. 2022).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions


Conceptualization: APS, ANS. Data curation: MK. Formal analysis: APS, MAZ. Funding acquisition: APS. Investigation: APS, ANS, MK, MAZ, NS. Resources: NS. Visualization: APS, MK, ANS. Writing - original draft: APS, MAZ, ANS. Writing - review and editing: APS, ANS, MK.

### Author ORCIDs

Alexander P. Sukhorukov  <https://orcid.org/0000-0003-2220-826X>

Nidhan Singh  <https://orcid.org/0000-0002-1155-1807>

Maria Kushunina  <https://orcid.org/0000-0003-2541-6494>

Maxim A. Zaika  <https://orcid.org/0000-0003-2633-4730>

Alexander N. Sennikov  <https://orcid.org/0000-0001-6664-7657>

### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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# Review of Orchidaceae of the northern part of Kazakhstan

Serik A. Kubentayev<sup>1</sup>, Petr G. Efimov<sup>2</sup>, Daniyar T. Alibekov<sup>1</sup>, Andrey N. Kupriyanov<sup>3</sup>,  
Klara S. Izbastina<sup>1,4</sup>, Aizhan E. Khalymbetova<sup>5</sup>, Yuri V. Perezhogin<sup>6</sup>

<sup>1</sup> Astana Botanical Garden, 16 Orynbor Str., 010016, Astana, Kazakhstan

<sup>2</sup> Komarov Botanical Institute of the Russian Academy of Sciences, 2 Professor Popov Str., 197022, Saint-Petersburg, Russia

<sup>3</sup> Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences, 18 Sovetsky Ave., 650000, Kemerovo, Russia

<sup>4</sup> S. Seifullin Kazakh Agrotechnical Research University, 62 Zhengis Ave., 010000, Astana, Kazakhstan

<sup>5</sup> L.N. Gumilyov Eurasian National University, 2 Satpayev Str., 010000, Astana, Kazakhstan

<sup>6</sup> A. Baitursynov Kostanay Regional University, 47 Baytursynov Str., 110000, Kostanay, Kazakhstan

Corresponding author: Aizhan E. Khalymbetova (aiscor87@mail.ru)

## Abstract

We present a review of Orchidaceae Juss. of the northern part of Kazakhstan, within the steppe, forest-steppe and semi-desert habitats of the country (Pavlodar, northern Kazakhstan, Kostanay, Akmola, Aktobe, West Kazakhstan, partially Karaganda and East Kazakhstan regions). The investigation is based on herbarium materials, literature data and field observations. We examined material from the following herbarium collections: LE, MW, TK, MHA, SVER, KUZ, ALTB, AA, NUR, KG, KSPI, NS, NSK, MOSP, ORIS, PPIU, totalling 288 herbarium specimens. The paper presents data in the form of revision, focusing on orchids of the northern part of Kazakhstan. It is accompanied by maps indicating localities, notes on habitat preferences, phenology and conservation status. A total of 25 species of 16 genera were recorded, of which eight are included in the Red Book of Kazakhstan (2014). According to our data, we propose to enlarge the number of protected orchids by adding the following nine species: *Corallorhiza trifida*, *Epipactis atrorubens*, *Gymnadenia conopsea*, *Hammarbya paludosa*, *Herminium monorchis*, *Liparis loeselii*, *Malaxis monophyllos*, *Neottia camtschatea* and *Spiranthes australis*. The most widespread species in the studied region are *Dactylorhiza incarnata*, *D. umbrosa* and *Epipactis palustris*. The rarest species (single locality only) are *Epipactis atrorubens*, *E. helleborine*, *Epipogium aphyllum*, *Hammarbya paludosa* and *Herminium monorchis*.

**Key words:** Biodiversity, conservation status, distribution, flora of Kazakhstan, orchid hotspot, rare plants



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## Introduction

Orchids are one of the largest families in the world, numbering, according to various data, from 28,000 to 30,500 species (Chase 2005; Chase et al. 2015; Christenhusz and Byng 2016; Hassler 2023). Due to human encroachment and climate change, as well as other factors, many orchid species are at risk of extinction (Fay 2018; Zizka et al. 2021). Eight species are listed in the Red Book of Kazakhstan (2014).

The diversity of Orchidaceae Juss. in Kazakhstan is low due to the prevalence of an arid climate with a rather harsh temperature regime in the cold period. According to the last vascular plant list of Kazakhstan by Abdulina (1999), there are 31 species of orchids from 18 genera in Kazakhstan. However, several recent additions prove that the diversity of orchids in Kazakhstan is insufficiently studied. The following taxa were discovered in Kazakhstan for the first time since 1999: *Cypripedium* × *ventricosum* Sw. (Kotuhov et al. 2009, 2018), *Epipactis atrorubens* (Hoffm.) Besser (Perezhogin et al. 2015), *Hammarbya paludosa* (L.) Kuntze, *Neottia cordata* (L.) Rich. (Kubentayev et al. 2021).

In recent years, the study of orchids of Kazakh Altai, which accounts for 22 species, has received particular attention (Danilova et al. 2020; Sumbembayev et al. 2020a, b, 2021, 2022, 2023), but orchids are still poorly studied in the northern part of Kazakhstan.

Orchid diversity in the neighbouring countries of Kazakhstan is variable. According to the latest data, there are 1,449 species in China (Zhang et al. 2015), 135 species in Russia (Efimov 2020), 26 species in Mongolia (Baasanmunkh et al. 2021), 10 species in Kyrgyzstan (Lazkov and Sultanova 2014) and nine species in Uzbekistan (Schreder 1941). Khapugin (2020), based on the synthesis of published data on the global distribution of orchids within designated conservation areas, noted the insufficient study of orchids in central and northern Asia as a whole.

Taxonomical and geographical data about orchids presented by Abdulina (1999) and earlier sources are largely outdated. Therefore, we undertook the task of preparing a new, detailed revision of this family for the flora of Kazakhstan. Taking into account that orchid family is notable for numerous rare and protected species, we have provided a detailed revision that includes lists of localities. These lists can subsequently be directly used in documents aimed at establishing the protection of the Kazakhstan flora.

The purpose of this study was to clarify the species diversity and distribution of orchids in the vast territory of the northern part of Kazakhstan, based on the revision of herbarium materials, data from literature and field observations.

## Material and method

Kazakhstan is located in the centre of Eurasia and the current ranking by area is ninth in the world with 2,724,900 km<sup>2</sup>. The territory of Kazakhstan is ecologically diverse, there are important zonal boundaries, including one separating the cold-temperate and temperate territories of northern Eurasia from the warm-temperate and hot-temperate territories of the Ancient Mediterranean (Abdulina 1999).

The presented contribution covers the major part of the country with the exception of the mountainous areas and desert areas, which are very different from the rest of the country and it is necessary to review them separately. In the article, the distribution of separate taxa is given according to both floristic and administrative principles. The studied area includes eight of 14 administrative regions (Fig. 1): Pavlodar, North Kazakhstan, Kostanay, Akmola, Aktobe, West Kazakhstan and partially Karaganda and East Kazakhstan regions). The administrative division of Kazakhstan that succeeded in 2021 is being pursued. Floristic subdivision of the territory follows Pavlov (1956). According to

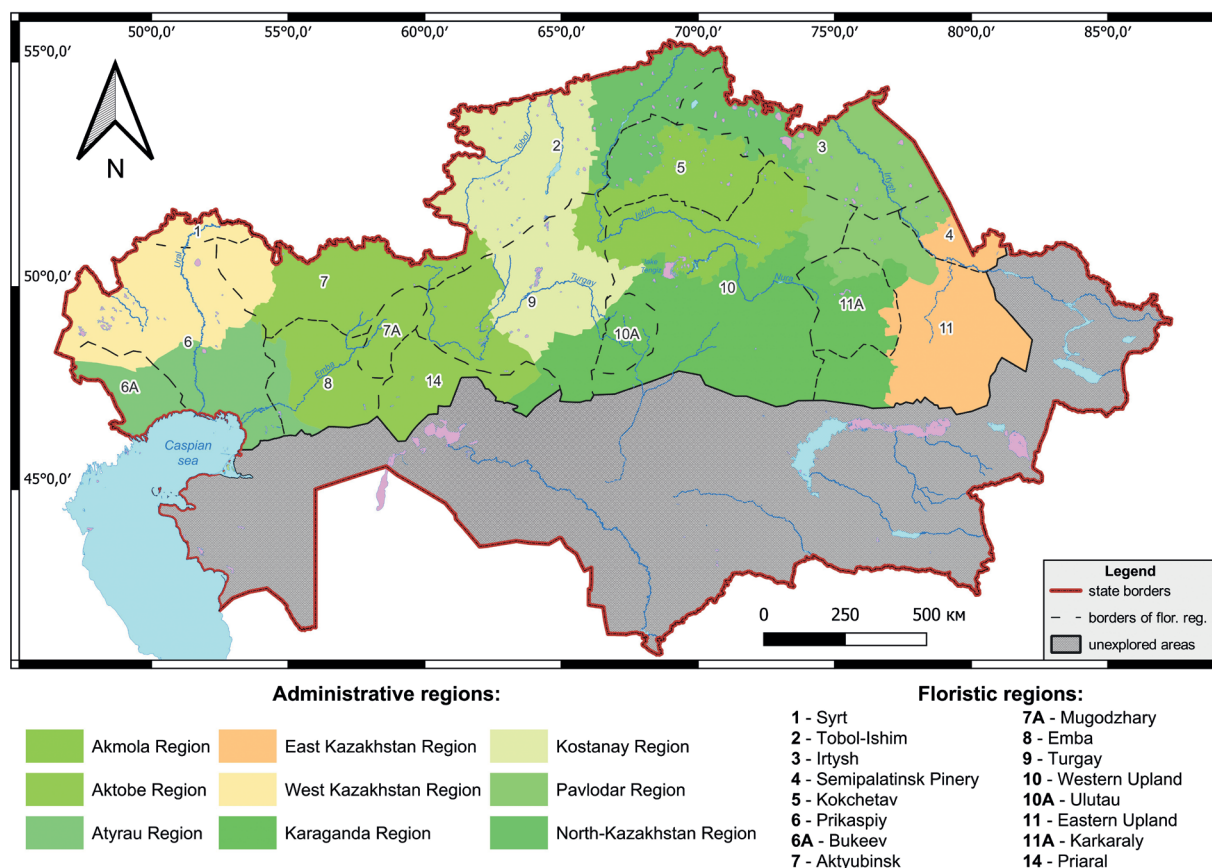


Figure 1. Map of floristic and administrative regions of Kazakhstan.

the latter classification, the studied area includes the following floristic regions (further abbreviated "FR"): Aktyubinsk, Bukeev, Emba, Eastern Upland, Irtysh, Karkaraly, Kokchetav, Mugodzhary, Priaral, Prikaspiy, Semipalatinsk Pinery, Syrt, Tobol-Ishim, Turgay, Ulutau and Western Upland (Fig. 1).

We have studied the following herbarium collections: LE, MW, TK, MHA, SVER, KUZ, ALTB, AA, NUR, KG, KSPI, NS, NSK, MOSP, ORIS and PPIU (acronyms according to Thiers 2022). In addition, we have studied two herbarium collections lacking acronyms: the herbarium of M. Kozybayev North Kazakhstan University, Petropavlovsk, (termed "NKU") and the herbarium of Zhezkazgan Botanic Garden, Zhezkazgan, (termed "ZhBG"). All herbarium collections were studied either personally or after photographs.

The nomenclature of each taxon mostly follows "Plants of the World Online" (POWO 2022).

The conservation status of each species follows the Red Book of Kazakhstan (2014), which assumes three categories of rarity: I – a very rare, critically endangered species; II – a very rare species; III – a rare species with a shrinking range.

Distribution maps of individual species were prepared using ArcMap. Dubious localities (with "question-mark" in the text) are included on the maps as well.

## Results and discussion

According to our data, 25 species of orchids from 16 genera are recorded in the northern part of Kazakhstan. Eight species are listed in the Red Data Book of

Kazakhstan, of which four species are classified under the II category and four species under the III category.

The distribution of the studied species within the floristic regions is as follows: Kokchetav – 14 species, Tobol-Ishim – 13 species, Irtysh – 9 species, Mugodzhary – 8 species, Eastern Upland – 8 species, Karkaraly – 8 species, Semipalatinsk Pinery – 7 species, Aktyubinsk – 2 species, Syrt – 3 species, Western Upland – 3 species, Ulutau – 1 species, Emba – 1 species and Prikaspiy – 1 species (Fig. 2).

The larger number of species in Kokchetav, Tobol-Ishim and Irtysh floristic regions is due to the presence of the more variable spectrum of habitats for orchids, including pine, deciduous and mixed forests, river valleys, sphagnum swamps, flood meadows etc. The Mugodzhary FR, which is also relatively rich in orchids (8 species), is located in the semi-desert zone of Kazakhstan; however, the Urkach and Ber-Chugur places (“place” stands here for the Russian word “urochishche”, which is used for various vernacular toponyms and also for the names of the former settlements) are located here, which include extensive lowlands with birch-aspen forests and sphagnum swamps, a very rare type of habitat in Kazakhstan. The Urkach place is considered to be a unique remnant of fragments of northern vegetation that retreated to the north during dry interglacial times and are evidence of the former vegetation of the Mugodzhary Mountains (Rusanov 1948).

Emba FR and Prikaspiy FR, where only one species of orchids (*Orchis militaris* L.) was found, as well as Turgay FR and Priaral FR, where orchids were not found at all, represent desert and semi-desert zones of Kazakhstan, with high soil salinity. The small number of orchids in Ulutau FR (also one species, *Dactylorhiza incarnata* (L.) Soó (Figs 3A, 5B)), in our opinion, is due to the poor knowledge of this region.

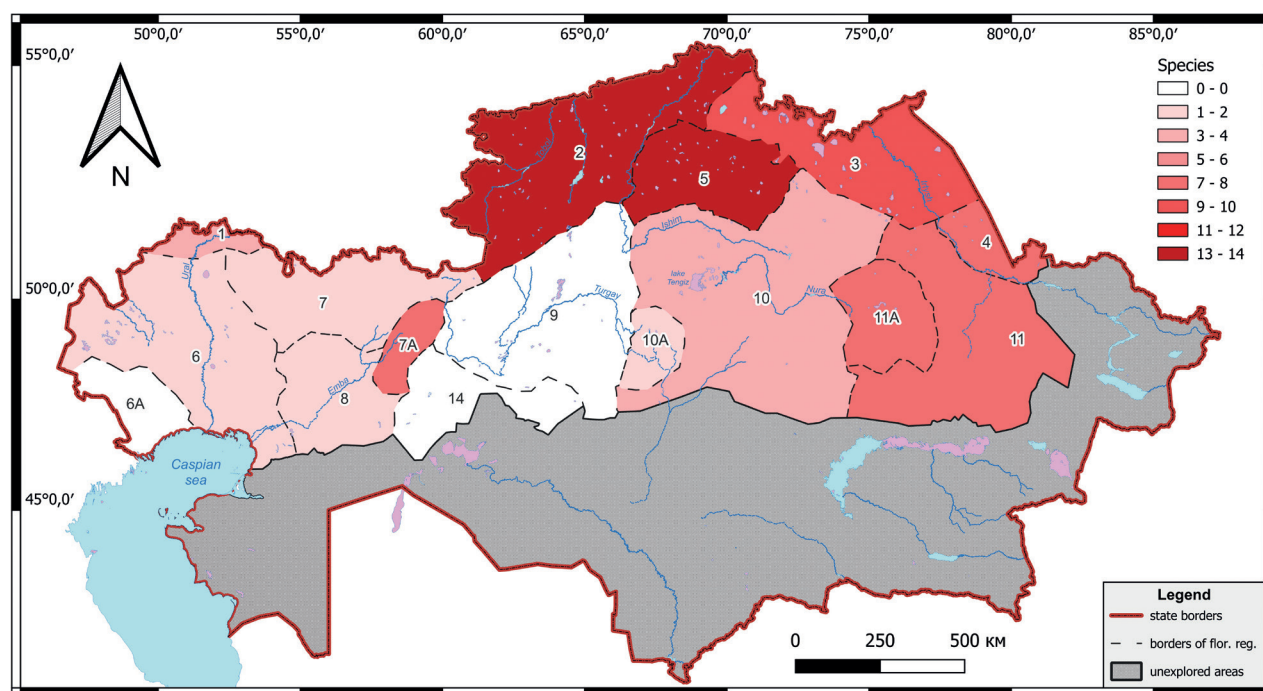


Figure 2. Species abundance of orchids in the floristic regions of the northern part of Kazakhstan.

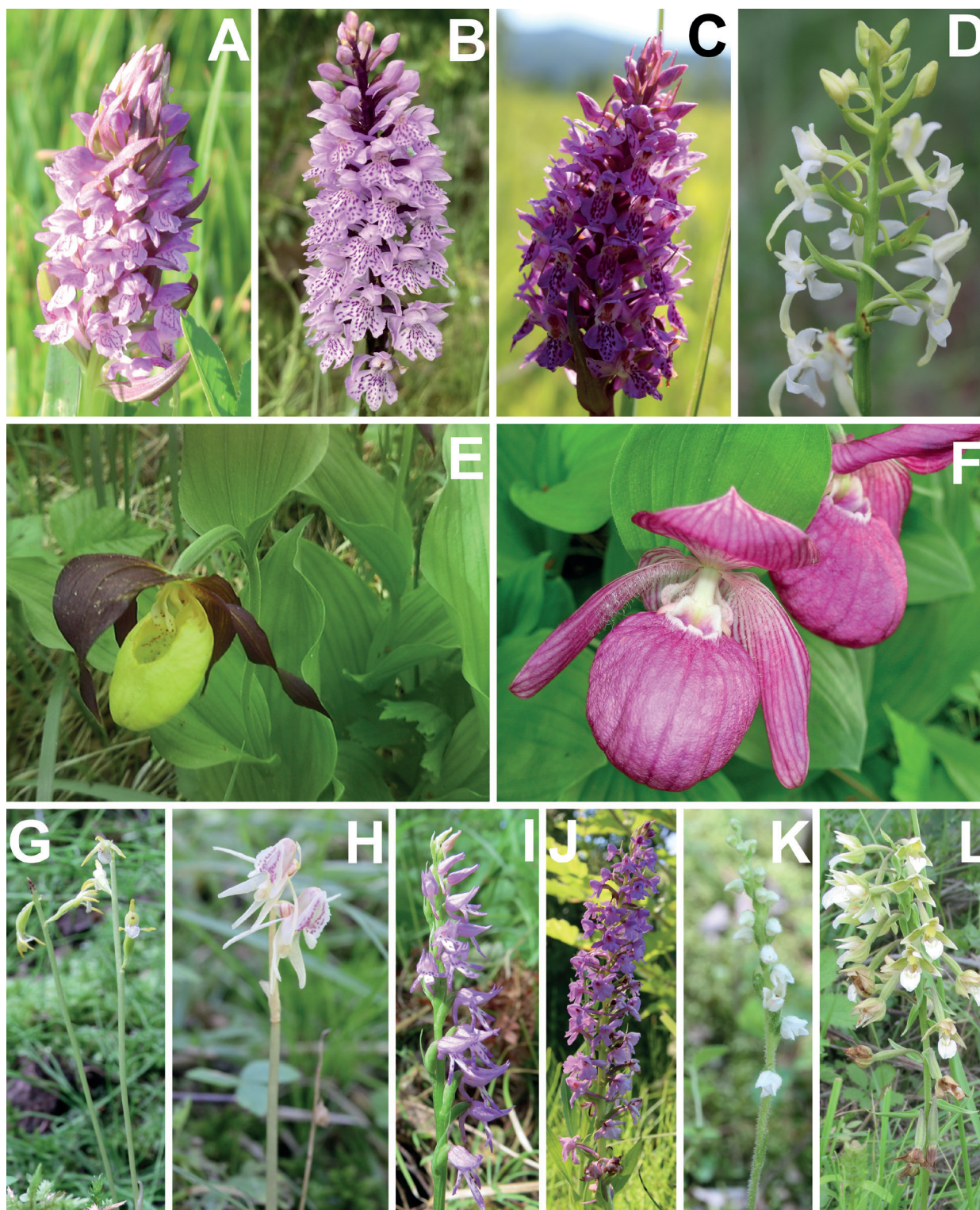


The distribution of the studied species by administrative regions is the following: Akmola – 12 species, Pavlodar – 13 species, Karaganda – 10 species, Kostanay – 10 species, East Kazakhstan – 8 species, North Kazakhstan – 7 species, West Kazakhstan – 3 species and Aktobe – 8 species (Table 1).

Currently, eight species of orchids growing in the northern part of Kazakhstan are included in the Red Book of Kazakhstan (2014): *Cypripedium calceolus* L.

**Table 1.** Summary of orchids distribution in the northern part of Kazakhstan.

Nº	Species	Number of localities	Floristic regions	Administrative regions	Category according to the Red Book of Kazakhstan
1	<i>Corallorhiza trifida</i> Châtel.	9	Kokchetav, Karkaraly	Akmola, Karaganda	–
2	<i>Cypripedium calceolus</i> L.	21	Kokchetav, Tobol-Ishim, Irtysh, Semipalatinsk Pinery	Akmola, North Kazakhstan, Pavlodar, East Kazakhstan	III
3	<i>Cypripedium guttatum</i> Sw.	7	Tobol-Ishim, Irtysh	North Kazakhstan, Pavlodar	II
4	<i>Cypripedium macranthos</i> Sw.	5	Tobol-Ishim, Irtysh, Semipalatinsk Pinery, Kokchetav	North Kazakhstan, Pavlodar, East Kazakhstan	II
5	<i>Dactylorhiza fuchsii</i> (Druce) Soó	39	Kokchetav, Tobol-Ishim, Karkaraly, Irtysh	Akmola, North Kazakhstan, Kostanay, Karaganda, Pavlodar	II
6	<i>Dactylorhiza incarnata</i> (L.) Soó	63	Tobol-Ishim, Eastern Upland, Kokchetav, Syrt, Mugodzhary, Aktyubinsk, Ulutau, Karkaraly, Western Upland, Irtysh	Kostanay, Pavlodar, North Kazakhstan, Akmola, West Kazakhstan, Aktobe, Karaganda, East Kazakhstan	–
7	<i>Dactylorhiza maculata</i> (L.) Soó	7	Kokchetav, Karkaraly, Mugodzhary	Akmola, Karaganda, Aktobe	–
8	<i>Dactylorhiza salina</i> (Turcz. ex Lindl.) Soó	6	Eastern Upland, Western Upland, Tobol-Ishim	Karaganda, Kostanay	–
9	<i>Dactylorhiza sibirica</i> Efimov	2	Eastern Upland	Pavlodar, East Kazakhstan	–
10	<i>Dactylorhiza umbrosa</i> (Kar. & Kir.) Nevski	7	Kokchetav, Tobol-Ishim, Mugodzhary, Western Upland, Eastern Upland	Akmola, Kostanay, Aktobe, Karaganda, East Kazakhstan	–
11	<i>Epipactis atrorubens</i> (Hoffm.) Besser	2	Tobol-Ishim	Kostanay	–
12	<i>Epipactis helleborine</i> (L.) Crantz	1	Mugodzhary	Aktobe	–
13	<i>Epipactis palustris</i> (L.) Crantz	17	Aktyubinskiy, Mugodzhary, Syrt, Tobol-Ishim, Kokchetav, Semipalatinsk Pinery, Karkaraly, Irtysh	Aktobe, West Kazakhstan, Kostanay, Akmola, Pavlodar, East Kazakhstan, Karaganda	III
14	<i>Epipogium aphyllum</i> Sw.	1	Karkaraly	Karaganda	II
15	<i>Goodyera repens</i> (L.) R.Br.	12	Kokchetav	Akmola	–
16	<i>Gymnadenia conopsea</i> (L.) R.Br.	24	Kokchetav, Tobol-Ishim, Irtysh, Semipalatinsk Pinery	Akmola, Kostanay, North Kazakhstan, Pavlodar, East Kazakhstan	–
17	<i>Hammarbya paludosa</i> (L.) Kuntze	1	Mugodzhary	Aktobe	–
18	<i>Hemipilia cucullata</i> (L.) Y.Tang, H.Peng & T.Yukawa	4	Kokchetav, Eastern Upland	Akmola, Pavlodar	–
19	<i>Herminium monorchis</i> (L.) R.Br.	1	Semipalatinsk Pinery, Irtysh	East Kazakhstan, Pavlodar	–
20	<i>Liparis loeselii</i> (L.) Rich.	3	Kokchetav, Mugodzhary, Semipalatinsk Pinery	Akmola, Aktobe, East Kazakhstan	–
21	<i>Malaxis monophyllos</i> (L.) Sw.	8	Tobol-Ishim, Eastern Upland, Karkaraly	Kostanay, Pavlodar, Karaganda	–
22	<i>Neottia camtschatea</i> (L.) Rchb.f.	5	Karkaraly, Eastern Upland	Karaganda, Pavlodar	–
23	<i>Orchis militaris</i> L.	11	Prikaspiy, Mugodzhary, Syrt, Semipalatinsk Pinery, Eastern Upland, Emba	West Kazakhstan, Aktobe, East Kazakhstan	III
24	<i>Platanthera bifolia</i> (L.) Rich.	20	Tobol-Ishim, Kokchetav	Kostanay, North Kazakhstan	III
25	<i>Spiranthes australis</i> (R.Br.) Lindl	4	Tobol-Ishim, Kokchetav, Irtysh	Kostanay, Akmola, Pavlodar	–

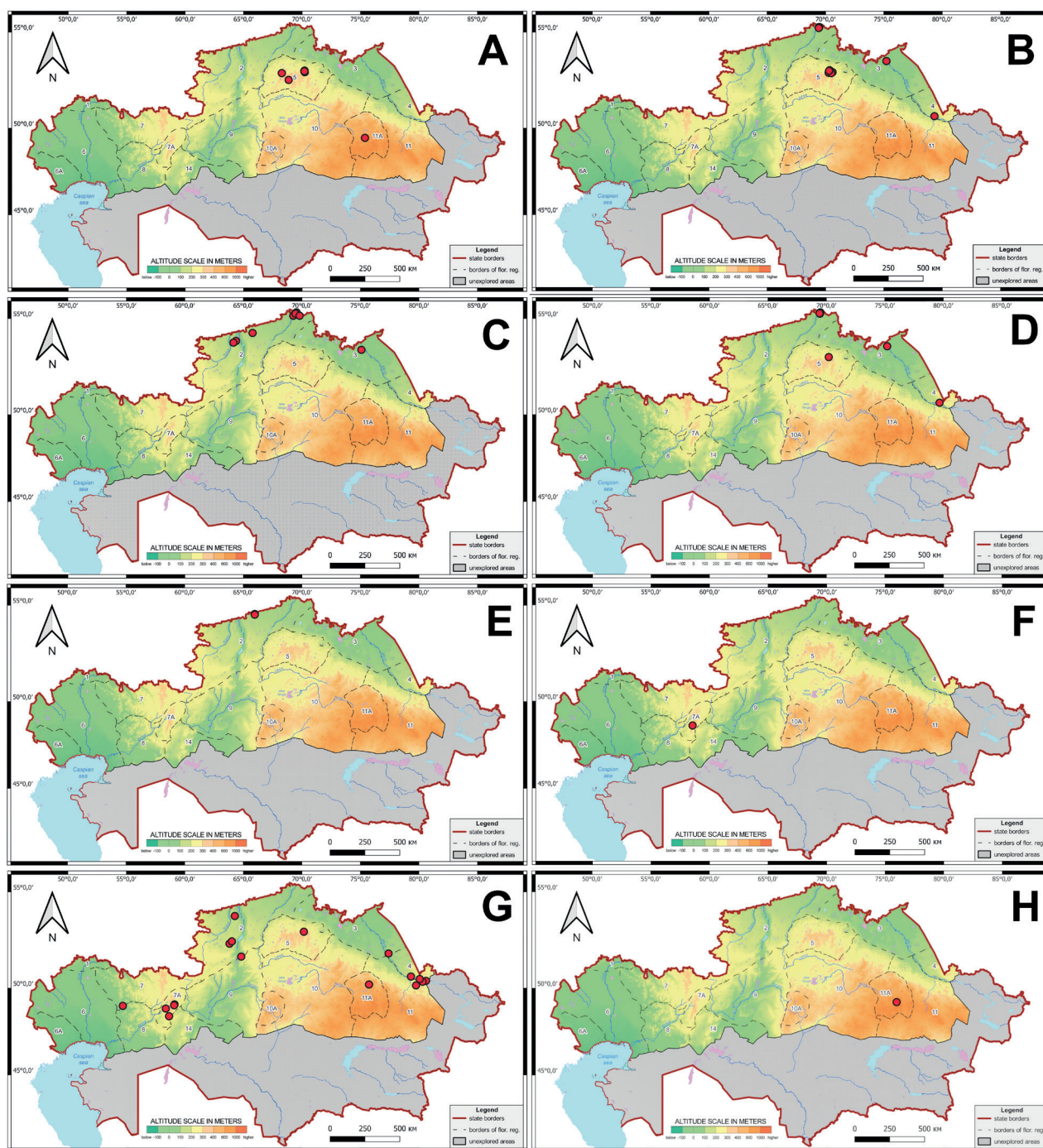


**Figure 3.** Representative taxa of Orchidaceae in the northern part of Kazakhstan **A** *Dactylorhiza incarnata* **B** *D. fuchsii* **C** *D. sibirica* **D** *Platanthera bifolia* **E** *Cypripedium calceolus* **F** *C. macranthos* **G** *Corallorhiza trifida* **H** *Epipogium aphyllum* **I** *Hemipilia cucullata* **J** *Gymnadenia conopsea* **K** *Goodyera repens* **L** *Epipactis palustris*. (Photos: **A–D, G, H, J, K** by S. Kubentayev; **E, F, I, L** by A. Kupriyanov).

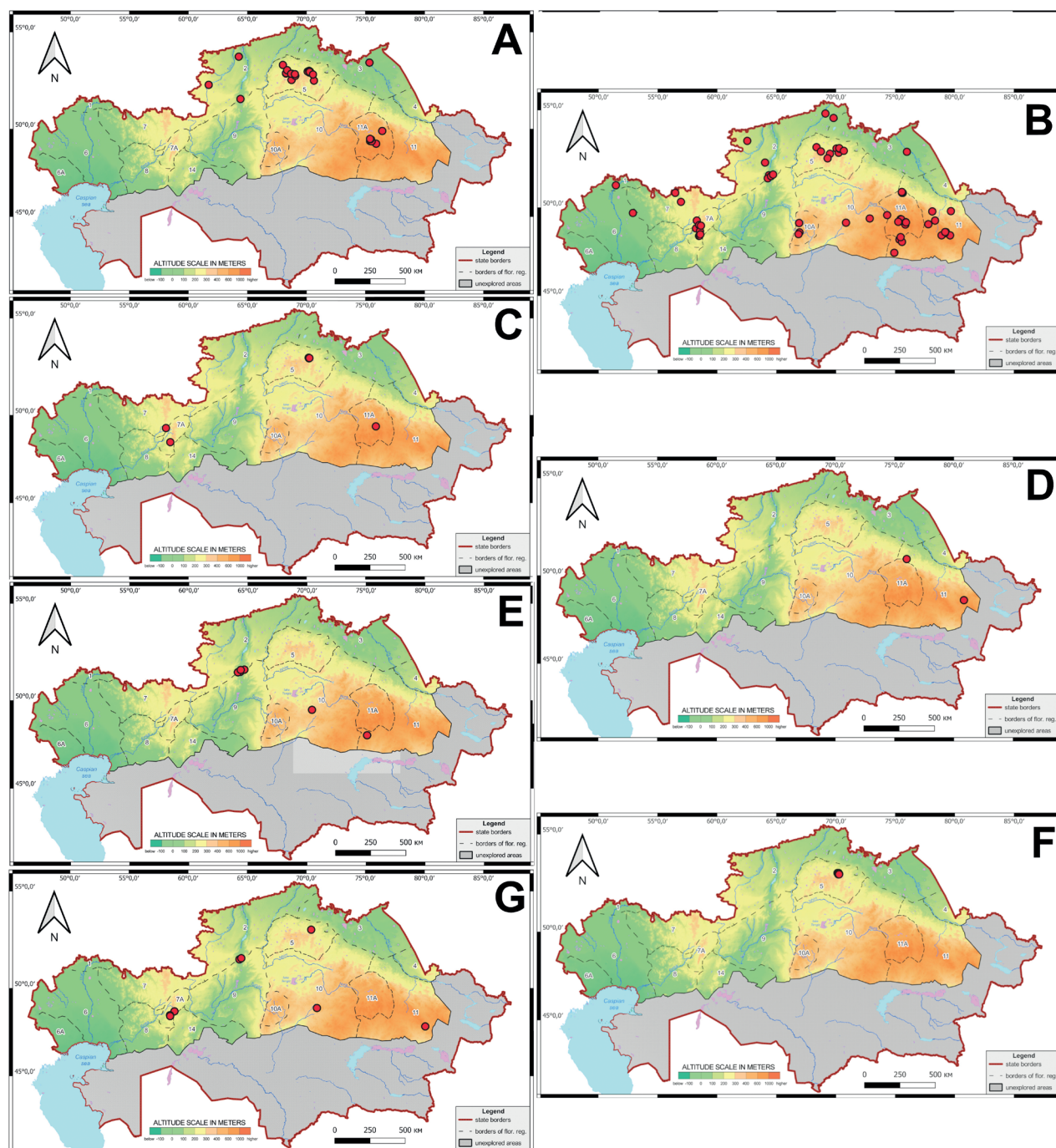
(Figs 3E, 4B), *C. guttatum* Sw., *C. macranthos* Sw. (Figs 3F, 4D), *Dactylorhiza fuchsii* (Druce) Soó (Figs 3B, 5A), *Epipactis palustris* (L.) Crantz (Figs 3L, 4G), *Epipogium aphyllum* Sw. (Figs 3H, 4H), *Orchis militaris* and *Platanthera bifolia*



(L.) Rich (Figs 3D, 6I). We recommend to additionally include in the next edition of the Red Book of Kazakhstan nine species, viz. *Corallorhiza trifida* Chatel. (Figs 3G, 4A), *Epipactis atrorubens*, *Gymnadenia conopsea* (L.) R.Br. (Figs 3J, 6A), *Hammarbya paludosa*, *Herminium monorchis* (L.) R.Br., *Liparis loeselii* (L.) Rich., *Malaxis monophyllos* (L.) Sw., *Neottia camtschatea* (L.) Rchb. f. and *Spiranthes australis* (R.Br.) Lindl. Thus, 17 species of orchids growing in the studied region should be included in the next edition of the Red Book of Kazakhstan.



**Figure 4.** Schematic map of the localities of orchids in the northern part of Kazakhstan **A** *Corallorhiza trifida* **B** *Cypripedium calceolus* **C** *C. guttatum* **D** *C. macranthos* **E** *Epipactis atrorubens* **F** *E. helleborine* **G** *E. palustris* **H** *Epipogium aphyllum*.

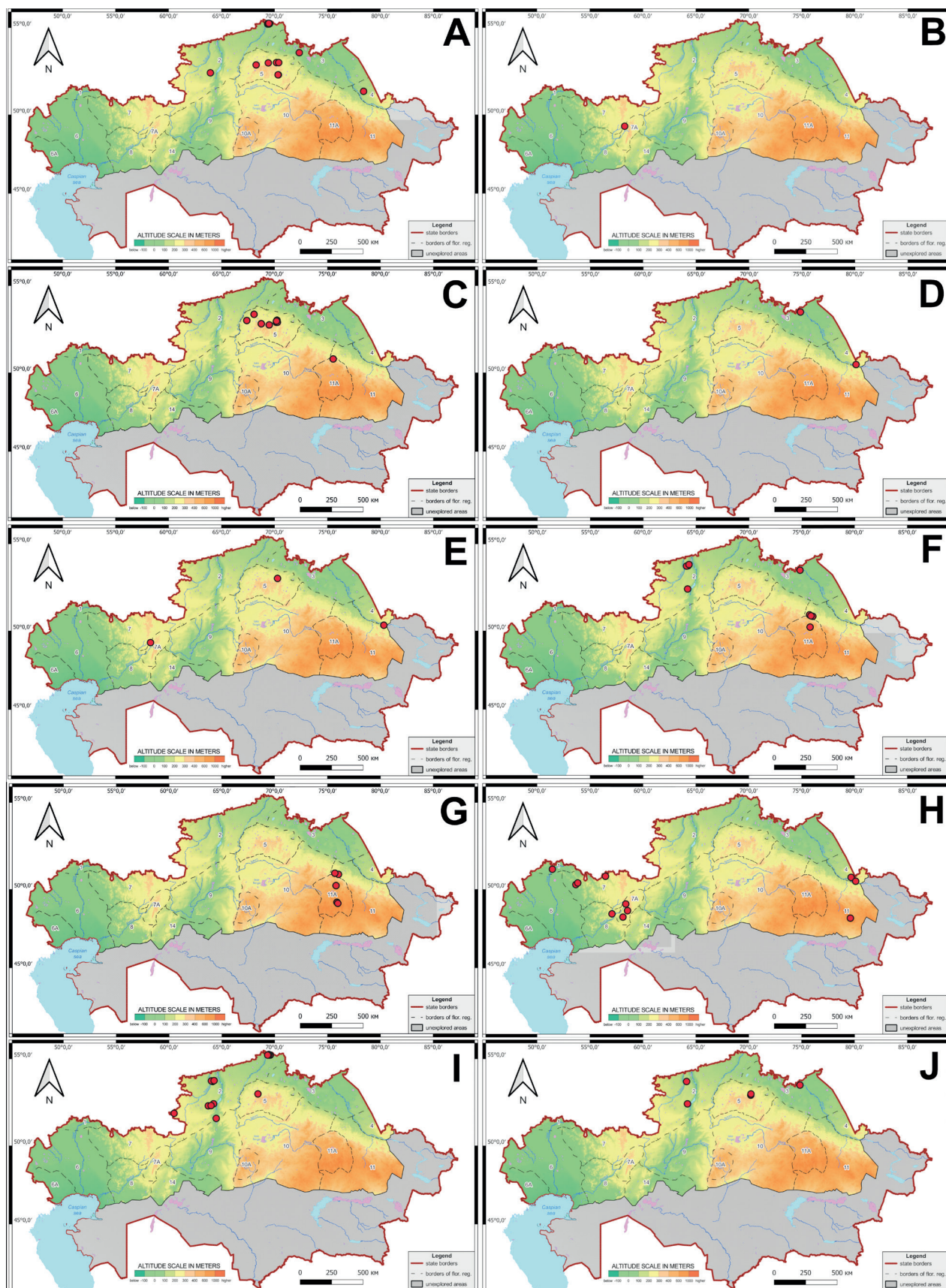


**Figure 5.** Schematic map of the localities of orchids in the northern part of Kazakhstan **A** *Dactylorhiza fuchsii* **B** *D. incarnata* **C** *D. maculata* **D** *D. sibirica* **E** *D. salina* **F** *G. repens* **G** *D. umbrosa*.

Based on our research, we discovered a single herbarium specimen from the Mugodzhary FR. In our assessment, it appears to be *Dactylorhiza ochroleuca* (Wüstnei ex Boll) Holub. However, this finding requires confirmation, as there is a possibility of confusion with hypochromic variants of *Dactylorhiza incarnata*. *Dactylorhiza sibirica* Efimov (Figs 3C, 5D) is reported for the first time for the northern part of Kazakhstan. Many taxa are reported for the first time for particular floristic and administrative regions of the country.

The most widespread species in the studied region are *Dactylorhiza incarnata* (63 localities in 10 FR), *Epipactis palustris* (17 localities in 8 FR),





**Figure 6.** Schematic map of the localities of orchids in the northern part of Kazakhstan **A** *Gymnadenia conopsea* **B** *Hammarbya paludosa* **C** *Hemipilia cucullata* **D** *Herminium monorchis* **E** *Liparis loeselii* **F** *Malaxis monophyllos* **G** *Neottia camtschatea* **H** *Orchis militaris* **I** *Platanthera bifolia* **J** *Spiranthes australis*.

*Dactylorhiza umbrosa* (Kar. & Kir.) Nevski (7 localities in 5 FR). The rarest species (one location only) are *Epipactis atrorubens*, *Epipactis helleborine* (L.) Crantz, *Epipogium aphyllum*, *Hammarbya paludosa* and *Herminium monorchis* (Table 1).

*Dactylorhiza majalis* (Rchb.) P.F. Hunt & Summerh and *D. russowii* (Klinge) Holub, reported earlier for the studied region (Pavlov 1956; Aipeisova 2012, 2013; Kupriyanov 2020), are excluded from the flora of Kazakhstan as erroneous determinations. More recently, Sumbembayev et al. (2023) reported *Dactylorhiza* × *kernerii* (Soó) Soó (= *D. fuchsii* × *D. incarnata*) for the flora of Kazakhstan, based on herbarium collections stored in LE. We believe that those specimens can be rather safely determined as *Dactylorhiza sibirica*, a hybridogenous species originating according to the same hybrid formula.

### ***Corallorhiza trifida* Châtel.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai, Western Tien Shan).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Sandyktau District: Maraldy, near the village of Sandyktau, 5 Jul 1913, *Semenov s.n.* (TK!); Burabay District: “Burabay” State National Nature Park: east shore of Shchuchye Lake, 17 Jun 1972, *Gorchakovskiy s.n.* (SVER 695750!); upper reaches of Imanaevskiy Spring, 2 Aug 1895, *Gordiagin 594* (LE!); near Burabay, 12 Jun 1913, *Drobov 430* (LE!); near Karas’e Lake, 27 Jun 1901, *Gordiagin 514* (LE!); same loc., 20 Jun 2012, *Khrustaleva and Artemova s.n.* (KUZ 02684!); near of Svetloe Lake, 15 Jul 1960, *Denisova 1577* (MW 0816955!); Aiyrtau District: Kokshetau State National Nature Park, Imantau Mountains, “Buyan-Schel” place, 31 May 1973, *Gorchakovskiy s.n.* (SVER 695749!). KARKARALY: **Karaganda Region:** Karkaraly District: Karkaraly Mountains, Alexandrov Klyuch cordon, 31 May 2007, *Kupriyanov et al. s.n.* (KUZ 11464!).

**Habitat and ecology.** Forest swamps, stream valleys, lakesides, sphagnum swamps and wet birch forests.

**Phenology.** Flowering in May–Jun; fruiting in Jul–Aug.

**Conservation status.** Not protected. We recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** *Corallorhiza trifida* is reported for the Kokshetau State National Nature Park for the first time.

### ***Cypripedium calceolus* L.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: “Burabay” State National Nature Park: near Shchuchinsk, Bar-mashinskoe forestry, 12 Jul 2019, *Kubentaev s.n.* (NUR!); same loc., 15 Jun 1965, *Oleneeva and Antoshenko s.n.* (SVER 695751!); same loc., 18 Jun 2012, *Artemova s.n.* (KUZ 02637!); same loc., 8 Jun 2011, *Kupriyanov and Hrustaleva s.n.* (KUZ 01096!); same loc., 2.5 km northeast of Shchuchinsk, 12 Jun 2011, *Hrustaleva s.n.* (KUZ 00884!); Zolotoborskoe forestry, 23 Jun 2016, *Hrustaleva*

and Artemova s.n. (KUZ 02798!); same loc., 3 km south of the Barmashino, 13 Jun 1972, Gorchakovskiy s.n. (SVER 695752!); near Barmashino Lake, 26 Jun 1890, Gordyagin 503 (LE!); the northern shore of Kotyrkol Lake, 19 Jun 1902, Ignatov and Petrovskiy 209 (LE!); same loc., 31 May 1902, Ignatov and Petrovskiy 209 (LE!); located 3–3.5 km east of Katarkol (Kupriyanov 2020); located 2.5 km northeast of Burabay, on the shore of Borovoe Lake (Kupriyanov 2020). TOBOL-ISHIM: **North Kazakhstan Region:** Kyzylzhar District: on the right shore of Ishim River, 75 km north of Petropavlovsk, 5 km north of Krasnoyarka, 16 Jun 1968, Kolodchenko s.n. (AA!, LE!); on the right shore of Ishim River, near Krasnoyarka, Jun 1968, Syzganov et al. s.n. (NKU!); same loc., Jun 1968, Terekhina et al. s.n. (NKU!); same loc., Jun 1968, Freze s.n. (NKU!). IRTYSH: **Pavlodar Region:** Zhelezinskiy District: on the right shore of Irtysh River (Kusnetsov and Pavlov 1958; Kazakh SSR Red Data Book 1981). SEMIPALATINSK PINERY: **East Kazakhstan Region:** Beskaragay District: on the right shore of Irtysh River (Kusnetsov and Pavlov 1958; Kazakh SSR Red Data Book 1981; Red Book of Kazakhstan 2014).

**Habitat and ecology.** Birch and birch-pine forests, forest stream valleys, forest swamps and forest lake shores.

**Phenology.** Flowering in Jun; fruiting in Jul–Aug.

**Conservation status.** It is included in the Red Book of Kazakhstan (category III). It is a rare and endangered species protected in the “Burabay” State National Nature Park, “Sogrov” State Nature Reserve and “Floodplain of the Irtysh River” State Nature Reserve.

**Notes.** Some populations of *Cypripedium calceolus* are located near Shchuchinsk and the village of Burabay, in areas with high recreational activity. These populations require special attention and protection due to the low number of plants in the populations, which can be attributed to the significant anthropogenic impact in these areas (Sultangazina et al. 2014; Kupriyanov 2020).

### *Cypripedium guttatum* Sw.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** TOBOL-ISHIM: **North Kazakhstan Region:** Kyzylzhar District: on the right shore of Ishim River, approximately 75 km north of Petropavlovsk and 5 km north of Krasnoyarka, 16 Jun 1968, Syzganov and Sadvokasova s.n. (LE); the left shore of Ishim River, near Krasnoyarka, 17 Jun 1968, Sidarkina and Galieva s.n. (NKU!); near Vagulino, 12 Jun 1982, Rain and Martyasheva s.n. (NKU!); near Tashkentka, 25 Jun 1982, Vafina et al. s.n. (NKU!). **? Kostanay Region:** ?Uzynkol District (Pugachev 1994), ?Mendykara District (Pugachev 1994). IRTYSH: **Pavlodar Region** [without detailed locality] (Kusnetsov and Pavlov 1958).

**Habitat and ecology.** Wet birch forests.

**Phenology.** Flowering in Jun; fruiting in Jul–Aug.

**Conservation status.** This rare species is included in the Red Book of Kazakhstan (category II) and is protected within the territory of two State Nature Reserves: “Sogrov” and “Floodplain of the Irtysh River”.



**Notes.** The report of *Cypripedium guttatum* for the Kostanay Region is doubtful since we have not found herbarium collections from these areas, including the herbarium of Kostanay Pedagogical University (KSPI), where the Pugachev collections are stored.

### ***Cypripedium macranthos* Sw.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** TOBOL-ISHIM: **North Kazakhstan Region:** Kyzylzhar District: on the right shore of Ishim River, 5 km north of Krasnoyarka, 16 Jun 1968, *Kolodchenko s.n.* (AA!, LE!); on right shore of Ishim River, near Krasnoyarka, Jun 1968, *Shakarova et al. s.n.* (NKU!); same loc., 27 Jun 1987, *Samoylova et al. s.n.* (NKU!). ?KOKCHETAV: **Akmola Region** [without detailed locality] (Semenov 1928; Kusnetsov and Pavlov 1958; Gorchakovskiy 1987). IRTYSH: **Pavlodar Region** [without detailed locality] (Kusnetsov and Pavlov 1958). SEMIPALATINSK PINERY: **East Kazakhstan Region:** Beskaragay District: near Kara-Murza, 16 Jun 1956, *Olovitikova s.n.* (LE!).

**Habitat and ecology.** Birch forests and valleys of forest streams.

**Phenology.** Flowering in Jun; fruiting in Jul–Aug.

**Conservation status.** This very rare species is included in the Red Book of Kazakhstan (category II). It is protected within the “Sogrov” and “Floodplain of the Irtysh River” State Nature Reserves.

**Notes.** According to recent reports (Sultangazina et al. 2014; Kupriyanov 2020) and our field studies, there is currently no confirmation of the presence of *Cypripedium macranthos* within the territory of Kokchetav FR.

One specimen of *C. ×ventricosum* (*C. calceolus* × *C. macranthos*) hybrid was found: “Tobol-Ishim: North Kazakhstan Region: Kyzylzhar District: on the right shore of the Ishim River, near Krasnoyarka, 17 Jun 1968, *Tsykareva s.n.* (AA!)”. This species occurs in areas where parent species co-occur, forming transitional populations with intermediate morphology (Averyanov 1999; Knyazev et al. 2000; Filippov and Andronova 2011; Andronova et al. 2017). This hybrid is reported for the first time in the studied region; Previously it was only reported in the Katon-Karagai District of the East Kazakhstan region in Kazakhstan (Kotuhov et al. 2009, 2018).

### ***Dactylorhiza fuchsii* (Druce) Soó**

(=*Dactylorhiza hebridensis* (Wilmott) Aver., ≡*Dactylorhiza fuchsii* subsp. *hebridensis* (Wilmott) Soó).

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: Near Karas’e Lake, 4 Jul 1937, *Shishkina s.n.* (AA!); same loc., Zolotoborskiy forestry, 21 Jun 1972, *Gorchakovskiy s.n.* (SVER 695767!); Borovskoy forest area, upper reaches of Imanayevsky Creek, 27 Jun 1974, *Gorcha-*



*kovskiy s.n.* (SVER 695769!); same loc., 16 Jun 1972 *Gorchakovskiy s.n.* (SVER 695769!); Barmashinskiy experimental forestry, 1 Aug 1965, *Tyulebergeneva s.n.* (SVER 695763!); Zolotoborsky forestry, eastern shore of Shchuchye Lake, 3 km north of the Medvezhiy cordon, 17 Jun 1972, *Gorchakovskiy s.n.* (SVER 695756!); Burabay forest area, near Akylbay cordon, 10 Jun 1913, *Drobova 308* (LE!); Burabay forest area, near Dorofeyevka, 10 Jun 1913, *Drobova 309* (LE!); “Burabay” State National Nature Park: Borovskoe forestry, 16 Jun 2019, *Kubentayev and Alibekov s.n.* (NUR!); Barmashinskoe forestry, 7 Aug 2020, *Kubentayev et al. s.n.* (NUR!); Temnoborskoe forestry, near Zhukey Lake, north-west coast, 11 Jun 2019, *Kubentayev et al. s.n.* (NUR!); near Burabay, the headwaters of Imanayevsky Creek, 7 Jun 2011, *Kupriyanov s.n.* (KUZ 00816!); near Makinka, 11 Jun 2011, *Kupriyanov s.n.* (KUZ 01111!); Zerendi District: “Kokshetau” State National Nature Park: Zerendi forestry, near Zerenda, 28 May 2020, *Kubentayev and Alibekov s.n.* (NUR!); Oramndybulakskoe forestry, near Karsak, 27 Jun 2020, *Kubentayev and Alibekov s.n.* (NUR!); Zerendi forestry, near Krasniy cordon, 26 Jun 2019, *Kubentayev et al. s.n.* (NUR!); Sandyktauskiy District: Sandyktau forestry, near “Chernichniy log”, 16 Jun 1957, *Gribanov s.n.* (AA!). **North Kazakhstan Region:** Aiyrtau District: Imantau Mount, 7 Aug 1965, *Oleneva and Antoshenko s.n.* (695765 SEVR!); same loc., the valley of the stream flowing down from Imantau Mount, 30 May 1973, *Gorchakovskiy s.n.* (SEVR 695760!); Imantau Mount, Bayan Gorge, 28 Aug 1981, *Gorchakovskiy s.n.* (SEVR 627680!); “Kokshetau” State National Nature Park, near Syrymbet, 10 Aug 2020, *Kubentayev et al. s.n.* (NUR!); near Lobanovo, 27 Jul 2019, *Kubentayev et al. s.n.* (NUR!). **TOBOL-ISHIM: Kostanay Region:** Mendykara District: botanical nature monument “Planting of birch and pine forests near Borovskoye Lake”, Jun 2009, *Perezhogin* (personal observation); Denisovskiy District: Ordzhonikidzevskoye forestry, near Denisovka (Pugachev 1994); Naurzum District: in the “Naurzum” State Nature Reserve, s.d., *KSPI students* (SVER 507474!). **KARKARALY: Karaganda Region:** Karkaraly Mountains, 27 Jun 1843, *Schrenk s.n.* (AA!); same loc., 21 Jun 1890, *Korzhiński s.n.* (LE!); same loc., 1927, *Melville s.n.* (AA!); near Karkaraly, 3 Jul 1937, *Dmitrieva s.n.* (AA!); same loc., same loc., 17 Jul 1987, *Kupriyanov s.n.* (KG!); same loc., 18 Jun 2001, *Ishmuratova s.n.* (KG!); the shore of Pashennoe Lake, 20 Jun 1914, s. *collector 2071* (LE); 70 km southeast of Karkaraly, Kent Mountains, 19 Jul 1968, *Rachkova 784* (LE!); Karkaraly Mountains, Alexandrov Klyuch, 8 Jul 1993, *Mikhailov s.n.* (KG!); Karkaraly Mountains, Karkaralinka River valley, 5 Aug 1989, *Denisova 692* (MW 0816794!). **IRTYSH: Pavlodar Region** [without detailed locality] (Kusnetsov and Pavlov 1958).

**Habitat and ecology.** Moist pine and birch forests, along the shores of forest streams and lakes, forest swamps.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** It is included in the Red Book of Kazakhstan (category II). It is protected on the territories of the “Naurzum” State Nature Reserve; “Kokshetau” State National Nature Park, “Burabay” State National Nature Park, Karkaraly State National Nature Park; Botanical nature monument “Planting of birch and pine forests near Borovskoye Lake”.

**Notes.** During the revision of KUZ herbarium materials, we identified two specimens of *D. fuchsii*, that were previously incorrectly determined as *Dactylorhiza russowii*. Based on these incorrectly misidentified herbarium specimens, *D. russowii* was previously reported for the Kokchetav FR (near Burabay,

the headwaters of Imanayevsky Creek; near Makinka) and in the overall flora of Kazakhstan (Kupriyanov 2020). As a result, there is currently no reliable information regarding the presence of *D. russowii* in Kazakhstan.

### ***Dactylorhiza incarnata* (L.) Soó**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai, Western Tien Shan, Betpak Dala, Balkhash-Alakol Basin, Turanskaya lowlands).

**Specimens examined and literature records.** TOBOL-ISHIM: **Kostanay Region:** Auliekol District: Aman-Karagay pine forest, 1930, *Dmitrieva s.n.* (AA!); Naurzum District: Naurzum-Karagai Mountains, 20 May 1909, *Savich and Kucherovskaya 647* (TK!); Naurzum Reserve, the shore of Small Aksuat Lake, 2 Jul 1949, *Ivleva and Kleshchina s.n.* (TK!); 20 km east of Aksuat, in the upper reaches of Akbulak Stream, 12 Jun 1936, *Voronov 157* (MW 0816813!); Moss swamp amongst springs on the slope of the Ulkendamydy River Valley, 23 Jun 1909, *Kucherovskaya 1125* (LE!); Fedorov District: Between the Traktov and Nazaryev, on the edge of the lake basin (Uballa Lake), 7 Jul 1913, *Korotkiy and Lebedeva s.n.* (LE!). EASTERN UPLAND: **Pavlodar Region:** Bayanaul District: “Bayanaul” State National Nature Park, Bayanaul Mountains, 27 May 2007, *Kupriyanov et al. s.n.* (KUZ 11467!). **North Kazakhstan Region:** Gabit Musrepov District: near the “Ardager” recreation centre, 27 Jun 2019, *Kubentayev s.n.* (NUR!); the City of Petropavlovsk, near Pestroye Lake, 10 Jun 1962, *Troinikova 1336* (MW 0816799!); Akkain District: west of the Borki Village, 29 Jun 1979, *Kolodchenko s.n.* (NKU!). **Karaganda Region:** Aktogay District: the southern tip of the Kyzyl-Rai mountain system, the floodplain of the drying Karatal River, 2 Jul 1969, *Mishchenkova 360* (LE!); same loc., 14 Jul 1974, *Denisova 57* (LE!); same loc., in the Zhenishke River Valley, 27 Jul 1959, *Denisova 224* (LE!); Bektau ata Mountains, *Mikhailov and Alibekov s.n.* (KG!). **East Kazakhstan Region:** Abay District: the Sherubai Valley, Nura River on the shore of the Topar Reservoir, Jun 2006, *Kupriyanov s.n.* (KG!); Degelen Mountains, along the shore of the Uzynbulak Stream, 29 May 1910, *Kucherovskaya 309* (LE!); near the Ak-Jaman Mountains (Zhamantas), 4 Jun 1914, *Shipchinsky 572* (LE!); same loc., 21 Jun 1984, *Grubov et al., 603* (LE!); Chingiz Mountains, Kopa River Valley, 30 May 1914, *Kosinskiy 558* (LE!); the valley of the Chagan River located 1.5 km above the mouth of the Saryzhal River, 14 Jul 1984, *Korobkov 603* (LE!); Chingiztau Mountains upper Bakanas, the sources of the Kyzyluzen on the Barshatas-Abai road, 11 Jun 1984, *Grubov 316* (LE!); Chingiztau Mountains, Bakanas Valley, near Ramadan Village, 9 Jun 1984, *Korobkov 221* (LE!); valley of Namaz River, 28 May 1914, *Kossinsky 485* (LE!); Chingiz Mountains, Munar River Valley, 27 May 1914, *Shipchinsky 345* (LE!); Abraly District: valley between the Zhaksyabrally and Zhamanabrally Mountains, 4 Jun 1910, *Kucherovskaya 668a* (LE!); Semipalatinsk District: the western part of the mountain (Kokon) Kokentau, 15 Jun 1928, *Blumenthal and Zapryagaev 475* (LE!). KOKCHETAV: **Akmola Region:** Zerendi District: near Kostomarovka, 4 Jun 1986, *Mikhailov s.n.* (KG!); Archaly is 5 km from Lesogorskoe Village, 24 Jun 1929, *Grigoriev s.n.* (AA!); “Kokshetau” State National Nature Park, Ormandy Bulak forestry, near Ermakovka, 28 May 2020, *Kubentayev s.n.* (NUR!); Burabay District: near Dorofeevka (Akylbay), 3 Jun 1918, *Drobov 305* (LE!); to the SE from Shchuchya station, 28 Jun 1997, *Gordyagin*

20 (LE!); Burabay, Rashit - cordon, along the shore of Arykpay River, 9 Jun 2011, *Khrustaleva s.n.* (KUZ 00969!); "Burabay" State National Nature Park, 101 block of Borovskoy forestry, 22 Jun 2012, *Khrustaleva and Artemova 02776* (KUZ!); Zolotoborsky forestry, near Tas-Shalkar Lake, 23 Jun 2012, *Khrustaleva and Artemova s.n.* (KUZ 02810!). SYRT: **West Kazakhstan Region:** Terekty District: near Podstepny to the southwest of Uralsk, 1895, *Kulyasov 51* (MW 296063!); near Temirbek, 13 Jun 1908, *Borodin et al. s.n.* (LE!). MUGODZHARY: **Aktobe Region:** Mugalzhar District: the southern tip of the Mugodzhary Mountains, west of Alga-bas railway station, 21-22 Jun 1987, *Skvortsov s.n.* (MHA!); horse farm Emba, 1934, *Buyanov s.n.* (MHA!); Near the north-west of the Mugodzhary Mountains, in a meadow near the sands of Urkach, *Dubinskaya 84* (LE!); Bol'shoy Boktybay Mountains, Berchogur place, 8 Jul 1927, *Rusanov 372* (LE!); The upper reaches of the Chili River (Or) near of the place Dzhaksy-Urkach, 11 Jul 1927, *Krashennnikov 847* (LE!); To the NW from Berchogur Station on the way to Mugojar, 25 Jun 1925, *Krashennnikov 576* (LE!); at the foot of Dau-Tau Mountain, 14 Jun 1936, *Khomutova and Daeva s.n.* (MW 0816798!); near the Ayryuk Mountain, 3 Jul 1927, *Krashennnikov 757* (LE!). AKTYUBINSK: **Aktobe Region:** Martuk District: 7 km from Martuk, 17 Jun 1993, *Panina s.n.* (PPIU); Khobdinsky District: near Bestau Mount, 23 Jul 1934, *Semsel 159* (MW 0816811!). ULUTAU: **Karaganda Region:** Ulytau District: near Ulytau Mountains, 1842, *Schrenk s.n.* (LE!); same loc., 2 Jun 2016, *Nashenova and Ivanov s.n.* (ZhBG!); Arganaty Mountains, 27 Jun 2017, *Kupriyanov and Khrustaleva s.n.* (KUZ 08448!); same loc., floodplain of the River Bazoy, 27 Jun 2017, *Kupriyanov and Khrustaleva s.n.* (KUZ 08449!). KARKARALY: **Karaganda Region:** Karkaraly District: on the salty meadows near the Big Lake, 23 Jul 1890, *Korzhinskiy s.n.* (LE!); Karkaraly Mountains 14 km south of Karkaralinsk, 14 Jun 1959, *Denisova s.n.* (LE!); Karkaraly Mountains on the road between Karkaraly and Zharly, 22 Jun 1991, *Pimenov and Klyukov 29* (MW 0816812!); near Karkaralinsk, near Zhyrym River, 12 May 1914, *Kuchеровskaya 1697* (LE!); near Kent Village, Kent Mountains, 2 Jun 2007, *Kupriyanov et al. s.n.* (KUZ 11468!); same loc., 16 Jun 2001, *Ishmuratova s.n.* (KG!). WESTERN UPLAND: **Karaganda Region:** Abay District: the shore of Talda River, near Amanzholov Farm, 20 Jun 1993, *Mikhailov s.n.* (KG!); Akbastau River Valley, 3 May 2015, *Alibekov s.n.* (KG!); the valley of the Sherubai, Nura River at the shore of the Topar Reservoir, Jun 2006, *Kupriyanov s.n.* (KG!); Shetskiy District: Shopa River Valley, foothills Kusmurn, 25 Jun 1994, *Mikhailov s.n.* (KG!).

**Habitat and ecology.** Wet meadows, flooded saline meadows, valleys of rivers, streams, lakeshores.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected.

**Notes.** *Dactylorhiza incarnata* is the most common orchid species in the studied region, found in almost all areas. However, for Turgay FR, it was recently reported by mistake. The corresponding localities (Sumbembayev et al. 2023) actually refer to the Tobol-Ishim FR. There are currently no known orchid species that occur in Turgay FR. Existing reports of *D. traunsteineri* (Saut. ex Rchb.) Soó for Kazakhstan are most likely based on misidentified *D. incarnata*. Therefore, the report of *D. traunsteineri* for Urkach Plateau in Mugodzhary (Kusnetsov and Pavlov 1958) is possibly based on a herbarium specimen in LE with incomplete label (Alexandri Lehmann Reliquiae botanicae, *Orchis angustifolia*, [det.] Al. Bunge), which was probably collected near the end of May 1842 (Bunge 1847).

During the revision of MHA herbarium materials, we found a herbarium specimen from the Mugodzhary FR (Aktobe Region: Mugalzhar District: the southern tip of the Mugodzhary Mountains, west of Algabaz railway station, 21–22 Jun 1987, Skvortsov s.n. (MHA!)). In our opinion, this specimen corresponds to *Dactylorhiza ochroleuca*. The identification is based on information provided on the herbarium label (“pale, pink-fawn flowers”). However, it is possible that these plants belong to hypochromic variants of *D. incarnata*. Exact determination is possible only through allozyme analysis (Filippov et al. 2017) or DNA analysis.

### ***Dactylorhiza maculata* (L.) Soó**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (?Altai).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: “Burabay” State National Nature Park: the shore of Svetloye Lake, 8 Jun 1960, *Denisova* 1326 (MW 0816814!); Barmashinsky forestry, 7 Jun 2019, *Kubentayev* s.n. (NUR!); same loc., the planning quarter 134, 18 Jun 2012, *Artemova* (KUZ 02650!); the swampy shore of Karas’e Lake, *Khrustaleva and Artemova* s.n. (KUZ 02696!). KARKARALY: **Karaganda Region:** Karkaraly District: Karkaraly Mountains, 12 Aug 2006, *Kupriyanov and Manakov* s.n. (KUZ 11465!). MUGODZHARY: **Aktobe Region:** Mugalzhar District: Mugodzhary Mts, “Urkach” place (*Aipeisova* 2013); Shalkar District: near Ber-Chugur railway station (*Aipeisova* 2013).

**Habitat and ecology.** Sphagnum swamps.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected.

**Notes.** *Dactylorhiza maculata* is often hardly distinguishable from *D. fuchsii*. When they co-occur, they form populations that include plants with intermediate morphology, indicating possible hybridisation. We consider that *D. maculata* is generally a European species, with only isolated occurrences in Asia, particularly in the western part of Siberia and in Kazakhstan. Determining the exact eastern distribution limit of this species is challenging due to its similarity with *D. fuchsii* in this region, where their ranges overlap.

### ***Dactylorhiza salina* (Turcz. ex Lindl.) Soó**

**Distribution in adjacent reg.** Russia (Siberia), Kazakhstan (Altai, Western Tien Shan, Turanskaya lowlands).

**Specimens examined and literature records.** EASTERN UPLAND: **Karaganda Region:** Aktogay District: near Aktogay, 24 Jun 1917, *Harin* s.n. (AA!). WESTERN UPLAND: **Karaganda Region** (without detailed locality) (*Kupriyanov* 2020). TOBOL-ISHIM: **Kostanay Region:** Naurzum District: “Naurzum” State Nature Reserve, near Biragach, 8 Jun 1984, *Zaugol’nova* s.n. (MOSP!); near Naurzum-Karagai, 20 May 1909, *Savich and Kucherovskaya* s.n. (LE!); near Karamenda, the shore of Sarymoyin Lake, 1 Jul 1911, *Borodin* s.n. (LE!); Egin-Bulak spring, north of Naurzum Forest, 22 Jun 1934, *Pavlov* 1396 (MW 0816830!); 5 km south of Aksuat Village, 20 Jun 1945, *Voronov* s.n. (MW 0816882!).



**Habitat and ecology.** Lowlands amongst birch trees, damp saline meadows and floodplains of rivers.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected.

**Notes.** *Dactylorhiza salina* is reported here for the first time for the Tobol-Ishim FR and Kostanay Region. *D. salina* was recently erroneously reported for the Turgay FR (Sumbembayev et al. 2023); in fact, the corresponding localities refer to the Tobol-Ishim FR. There are currently no orchid species known to occur in the Turgay FR. *D. salina* is hardly distinguishable from *D. umbrosa*.

### ***Dactylorhiza sibirica* Efimov**

**Distribution in adjacent reg.** Russia (Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** EASTERN UPLAND: **Pavlodar Region:** Bayanaul District: Bayanaul Mountain Forest, on the slope of the watershed between Jasybai Lake and Sabyndykul, 24 Jun 1979, *Lalayan s.n.* (SVER 627698!). **East Kazakhstan Region:** the village of Kriushi, meadow along a stream, 21 Jul 1928, *Ilyin and Heinrichson s.n.* (LE!).

**Habitat and ecology.** Stream valleys, swampy meadows.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected.

**Notes.** *Dactylorhiza sibirica* is reported for the studied region for the first time. This allopolyploid species was described relatively recently, in 2016, with diploid Siberian *D. fuchsii* and *D. incarnata* as its presumable parental taxa (Efimov et al. 2016). In eastern Kazakhstan, the species was earlier incorrectly determined as *Dactylorhiza baltica* (Klinge) Nevski or *Dactylorhiza* × *kernerii* (Danilova et al. 2020; Sumbembayev et al. 2023).

### ***Dactylorhiza umbrosa* (Kar. & Kir.) Nevski**

**Distribution in adjacent reg.** Russia (Siberia), Kazakhstan (Altai, Western Tien Shan, Balkhash-Alakol Basin, Turanskaya lowlands).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: near the Mirnaya Dolina cordon, the shore of Karabulak Stream, 25 Jun 1937, *Sobolev s.n.* (AA!). TOBOL-ISHIM: **Kostanay Region:** Naurzum District: Nauryzym-Karagay Mountains, 20 May 1909, *Savich and Kuchеровskaya 649* (LE!); “Naurzum” State Nature Reserve, 4 Jun 1938, *Siu s.n.* (MW 0816881!). MUGODZHARY: **Aktobe Region:** Shalkar District: near Ber-Chogur, 10 Jun 1927, *Rusanov s.n.* (AA!); northwest of Ber-Chogur, on the road to Mugodzarsk, 25 Jun 1927, *Krashenninnikov s.n.* (AA!). WESTERN UPLAND: **Karaganda Region:** Zhanaarka District: Sarysu River Valley, “Kara-Agach” place, 13 Jun 1949, *Goloskokov s.n.* (AA!). EASTERN UPLAND: **East Kazakhstan Region:** Ayagoz District: Chingizstau, upper reaches of Kalguta River, 17 Jun 1958, *Gamayunov s.n.* (AA!).

**Habitat and ecology.** Valleys of rivers and streams, along the damp edges of birch and aspen forests, through swamps, salt marshes, wet meadows, in the lowlands amongst birch thickets.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected.

**Notes.** Aipeisova (2012, 2013) reported *D. majalis*. for Mugodzhary (near Ber-Chogur and Mount Boktybai). Although corresponding herbarium specimens were not located, we believe that this report is an obvious mistake. It is more likely that the plants were *D. umbrosa*, as we found herbarium materials collected from the same place (near Ber-Chogur, 10 Jun 1927, *Rusanov s.n* (AA!)). We consider *D. umbrosa* and *D. salina* to be closely-related species and determining plants with certainty can sometimes be challenging.

### ***Epipactis atrorubens* (Hoffm.) Besser**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia).

**Specimens examined and literature records.** TOBOL-ISHIM: **Kostanay Region:** Uzynkol'skiy District: near Krasnye Borki, 12 Jul 1990, *KSPI students 2189* (LE!, KSPI!).

**Habitat and ecology.** Pine forests

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected. The species is very rare and we recommend to include it in the next edition of the Red Book of Kazakhstan.

**Notes.** For the flora of Kazakhstan, the species was reported relatively recently by Perezhugin et al. (2015), based on the herbarium gathering mentioned above. Field studies are necessary to check whether the plant is still extant at that locality or not.

### ***Epipactis helleborine* (L.) Crantz**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai, Western Tien Shan).

**Specimens examined.** ?MUGODZHARY: ?**Aktobe Region:** ?Mugalzhar District: Mugodzhar Mts [without detailed locality] (Kusnetsov and Pavlov 1958; Aipeisova 2013).

**Habitat and ecology.** In mixed and deciduous shady forests, at the forest edges.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected. It requires protection at the regional level.

**Notes.** We did not find any herbarium collections of *E. helleborine* from the studied region and the existing literature reports require confirmation. However, this species is known to be common in mountainous areas of southern and eastern Kazakhstan (Kuznetsov and Pavlov 1958), which are not included in the current revision.

### ***Epipactis palustris* (L.) Crantz**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai, Western Tien Shan).

**Specimens examined and literature records.** AKTYUBINSK: **Aktobe Region:** Uilskiy District: near Uil, 21 Aug 1936, *Nikitin & Deulina s.n.* (LE!). MUGODZHARY: **Aktobe Region:** Mugalzhar District: Along the shore of the Shuldak River, 22 Jun 1927, *Rusanov s.n.* (AA!, LE!); Akzerendy River Valley, 4 Jul 1927, *Rusanov s.n.* (AA!, LE!); Mugodzhary Mts, the Kunduzdy River Valley (left tributary of the Emba), 20 Jul 1957, *Borszczov* 299 (LE!); Shalkar District: Shuldak River Valley (Shet-Irgiz), 29 Jun 1927, *Krashenninikov* 638 (LE!). SYRT: **West Kazakhstan Region:** Chingir- lauskiy District: “Kara-Agach” place, 13 Jun 1950, *Nikishin s.n.* (LE!). TOBOL-ISHIM: **Kostanay Region:** Auliekol District: Aman-Karagay pine forest, 10 Sep 1921, *Pavlov s.n.* (LE!); same loc., near Novonezhinka, 3 Jun 1925, *Rusanov s.n.* (LE!); Mendykara District: Borovskaya water protection forest dacha, near Borovskoye, 08 Jul 1925, *Rusanov s.n.* (LE!); Nauruzymsky District: Ak-Kuchuk River Valley, 1 Aug 1929, *Vernander s.n.* (LE!); KOKCHETAV: **Akmola Region:** Burabay District: Northern swampy shore of Shchuchy Lake, 3 Sep 1981, *Gorchakovskiy s.n.* (SVER 715630!). IRTYSH: **Pavlodar Region:** Akkuli District: the shore of Yamyshevskoye Lake, 26 Jun 1913, *Kucherevskaya* 1048 (LE!). SEMIPALATINSK PINERY: **East Kazakhstan Region:** Semipalatinsk District: 25 km east of Semipalatinsk, Kashtak place, 10 Jul 1933, *Sumnevich s.n.* (TK!); same loc., 20 Aug 1933, *Sumnevich s.n.* (TK!) near the Semeytau Mountains, Northern Spring, 25 Jun 1914, *Mordvinova s.n.* (MOSP!); Borodulikha District: Semeytau Mountains, near the farm, 6 Aug 1928, *Zapryagaev* 1973 (LE!); Beskaragaysky District: near the mouth of the Shagan River, 23 Sep 1928, *Zapryagaev* 2452 (LE!). KARKARALY: **Karaganda Region:** Karkaraly District: Zheltau Mountains (Kupriyanov 2020).

**Habitat and ecology.** Marshy meadows, river valleys, in wet forests.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** This species is included in the Red Book of Kazakhstan (category III). It is protected in the following territories: “Naurzum” State Nature Reserve, “Semey Ormany” State Nature Reserve, “Burabay” State National Nature Park, “Karkaraly” State National Nature Park, as well as the State Nature Reserves of “Floodplain of the Irtysh River”, “Orkash”, “Kokzhide-Kumzhargan” and “Budarinskiy”. It is also protected in the natural monument “Birch and pine plantations forests near Borovskoye Lake”. *Epipactis palustris* is one of the most widely distributed orchids in Kazakhstan. Currently, there is a need to reconsider the necessity of state protection for this species.

### ***Epipogium aphyllum* Sw.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** KARKARALY: **Karaganda Region:** Karkaraly District: Kent Mountains, “Karaagash” place, 4 Aug 1986, *Kupriyanov and Mikhailov s.n.* (KG!).

**Habitat and ecology.** Swampy pine forests, swamps.

**Phenology.** Flowering in Jul–Aug; fruiting in Sep–Oct.

**Conservation status.** This species is included in the Red Book of Kazakhstan (category II) as a rare species found in small numbers within a limited area. In the studied region, it is preserved in the “Karkaraly Biological Reserve”.

**Notes.** The species in the studied region is known from a single locality in central Kazakhstan, as confirmed by the above herbarium sample. This finding was published in 1987 (Kupriyanov and Mikhailov 1987). Currently, further study is necessary to determine whether this locality is still extant.

***Goodyera repens* (L.) R.Br.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai, Western Tien Shan).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: near Karas'e Lake, 19 Jul 1913, *Semenov s.n.* (TK!); same loc., 10 Aug 1973, *Gorchakovskiy s.n.* (SVER 695775!); same loc., 12 Jun 2011, *Kupriyanov s.n.* (KUZ 00893!); same loc., small southern swamp, 26 May 1973, *Gorchakovskiy s.n.* (SVER 695772!); same loc., big swamp, 29 Jun 1974, *Gorchakovskiy s.n.* (SVER 695770!); Sinyukha Mountain, 17 Jun 1912, *Semenov s.n.* (TK!); same loc., north slope, 24 Jun 1929, *Ilyin s.n.* (LE!); Zolotoborskiy forestry, sq. 24, 18 Jun 1972, *Gorchakovskiy s.n.* (SVER 695773!); Borovsky forest area, near Svetloe Lake, 9 Aug 1973, *Gorchakovskiy s.n.* (SVER 695774!); same loc., near Shortankul'skiy peat bog, 5 Aug 1978, *Gorchakovskiy s.n.* (SVER 695776!); Kokchetav Upland, upper reaches of the Imanayevsky Spring, 2 Jul [presumably 1895-1910], *Gordiyagin 593* (LE!); near the top of Ush-Tas Mount, 2 Jul 1896, *Gordiyagin 1105* (LE!); near Burabay, "Burabay" State National Nature Park, Barmashinskoe forestry, 16 Jul 2019, *Kubentayev s.n.* (NUR!).

**Habitat and ecology.** Moist pine, birch and mixed forests.

**Phenology.** Flowering in Jul–Aug; fruiting in Sep–Oct.

**Conservation status.** Not protected. It is necessary to strengthen security measures in the territory of the Shchuchinsko-Borovskaya resort area in the Kokchetav Upland as the habitats of *G. repens* in this area are exposed to strong recreational influences.

**Notes.** *Goodyera repens* (Figs. 3K, 5F) is found only in a limited area within the Kokchetav FR in the studied region.

***Gymnadenia conopsea* (L.) R.Br.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: Kokchetav Upland, near Burabay, "Burabay" State National Nature Park, Barmashinskiy forestry, 12 Jul 2019, *Kubentayev s.n.* (NUR!); Kokchetav Upland, at the top of Mount Sinyukha, 22 Jul 1960, *Denisova 1709* (MW 0816897!); Bulandynskiy District: Otradnenskiy forestry, forest quarter no. 121, 4 Aug 1960, *Denisova 2069* (MW 0816896!); same loc., forest quarter no. 11, 4 Aug 1960, *Denisova 1270* (MW 0816895!); Aryk-Balyk'skiy District: Kokchetav Upland, hills to the south of Imantau Lake, 2 Aug 1960, *Denisova 1817* (MW 0816898!); Zerendi District: 30 km south of Kokshetau, 7 Jul 1957, *Borisova & Rachkovskaya 345* (LE!). TOBOL-ISHIM: **Kostanay Region:** Auliekol District (Auliekol'skiy District): near Aman-Karagay, 27 Apr 1929, *Vernander et al. 331* (LE!).



**North Kazakhstan Region:** Kyzylzhar District: right shore of Ishim River, near Krasnoyarka, 29 Jun 1971, *Sologub and Zelinskaya s.n.* (NKU!); same loc., 29 Jun 1971, *Stepanova and Shahvatova s.n.* (NKU!); same loc., 3 Jul 1971, *Troskina and Shandybina s.n.* (NKU!); same loc., Aug 1973, *Wenzler et al. s.n.* (NKU!); same loc., 6 Jul 1974, *Shushakova and Konovalov s.n.* (NKU!); same loc., 06 Jul 1974, *Gorbunova and Mikheeva s.n.* (NKU!); same loc., 9 Jul 1974, *Sinichkina et al. s.n.* (NKU!); same loc., 13 Jul 1974, *Fomenko s.n.* (NKU!); same loc., 14 Jul 1974, *Sokolovskaya s.n.* (NKU!); same loc., 14 Jul 1974, *Spirenkova and Temirbaeva s.n.* (NKU!). IRTYSH: **Pavlodar Region:** Shcharbaky District: near Aleksandrovka, 1885, *Golde s.n.* (LE!). SEMIPALATINSK PINERY: **East Kazakhstan Region** [without detailed locality] (Kusnetsov and Pavlov 1958).

**Habitat and ecology.** Meadows, birch spikes and grassy pine forests.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sept.

**Conservation status.** Not protected. We recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** The species was seriously under-recorded in earlier treatments, mainly due to incorrect determinations of the existing herbarium specimens. Thus, no reports are available for Kokchetav and Tobol-Ishim FRs, Kostanay, North Kazakhstan and Akmola administrative regions in the floristic accounts of Kazakhstan (Kusnetsov and Pavlov 1958), Kazakh Upland (Karamysheva and Rachkovskaya 1973; Kupriyanov 2020) and Turgay Depression (Pugachev 1994) for this species. However, there exist older literature reports (Semenov 1928 without detailed localities for Petropavlovskiy Uezd (belongs to Tobolsko-Ishimskiy FR) and Kokchetavskiy Uezd (belongs to Kokchetav FR).

### ***Hammarbya paludosa* (L.) Kuntze**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia).

**Specimens examined and literature records.** MUGODZHARY: **Aktobe Region:** Mugalzhar District: Mugodzhary Mts, “Urkach” place, 1 Sep 1934, *Samseev 514* (MW 0816996!).

**Habitat and ecology.** Sphagnum swamps.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected. We recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** *Hammarbya paludosa* was only recently reported for Kazakhstan for the first time (Kubentayev et al. 2021). It was absent in earlier treatments, since the specimen was stored under the name *Microstylis monophyllos* (L.) Lindl. This location is the southernmost part of the area of *H. paludosa*. Fieldwork is necessary to check whether *H. paludosa* is still extant in that locality.

### ***Hemipilia cucullata* (L.) Y.Tang, H.Peng & T.Yukawa**

≡ *Neottianthe cucullata* (L.) Schltr. ≡ *Ponerorchis cucullata* (L.) X.H.Jin, Schuit. & W.T.Jin.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: 3 km north of Shuchinsk, 1978, *Grudzinskaya s.n.* (AA!, NUR!); Borovskoy forest area, near Shortankul'skiy peat bog, 5 Aug 1978, *Gorchakovskiy s.n.* (SVER!); near Balkashino, in the northern part of the forest dacha of B. Tyukty, 9 Aug 1929, *Grigoriev 367* (LE!); the northern slope of Tuyak-Tau Mountain, 13 Jul 1901, *Gordyagin 529* (LE!); near the Ush-Tas Mount top, 2 Jul 1896, *Gordyagin 1105* (LE!); the eastern shore of Svetloe Lake, 16 Jul 2019, *Kubentayev and Alibekov s.n.* (NUR!); near Burabay, "Burabay" State National Nature Park, Akylbayskoye forestry, 16 Jul 2019, *Kubentayev s.n.* (NUR!); same loc., Borovskoe forestry, 16 Jul 2019, *Kubentayev s.n.* (NUR!); Zerendi District: "Kokshetau" State National Nature Park: Jilandinskoe forestry, near the "Gorodok" cordon, 11 Aug 1960, *Denisova 1973* (MW 0816899!); Ayrtau District: near Shokkaragai, 10 Aug 2020, *Kubentayev et al. s.n.* (NUR!). **EASTERN UPLAND: Pavlodar Region:** Bayanaul District: Bayanaul Mountains (*Gorchakovskiy 1987*); same loc., Dzhasybayevsky forestry, 28 Jul 1979, *Lalayan s.n.* (SVER 627695!).

**Habitat and ecology.** Pine and birch forests.

**Phenology.** Flowering in Jul–Aug; fruiting in Sep–Oct.

**Conservation status.** Not protected, but protection is needed since in the studied region the majority of localities fall into the resort area of the Kokchetav Upland. Taking into account that habitats of *H. cucullata* (Figs. 3I, 6C) are exposed to serious recreational loads, we consider it necessary to include the species in the next edition of the Red Book of Kazakhstan.

### *Herminium monorchis* (L.) R.Br.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** SEMIPALATINSK PINERY: **East Kazakhstan Region:** Semipalatinsk District: near Semipalatinsk, 8 Jun 1914, *Mordvinova s.n.* (MOSP!). **IRTYSH: Pavlodar Region** [without detailed locality] (*Kuznetsov and Pavlov 1958*).

**Habitat and ecology.** Forest edges, damp meadows.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sept.

**Conservation status.** Not protected. We recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** *Herminium monorchis* is reported here for the first time for the Semipalatinsk Pinery FR. Previously, in North Kazakhstan, it was reported only for Irtysh FR (*Kuznetsov and Pavlov 1958*). In Kazakhstan, *H. monorchis* is more common in the mountainous regions of eastern and southern Kazakhstan, not included in the current revision.

### *Liparis loeselii* (L.) Rich.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia). Kazakhstan (Balkhash-Alakol Basin).

**Specimens examined and literature records.** KOKCHETAV: **Akmola Region:** Burabay District: near Borovoye, the shore of Chebach'ye Lake, 12 Jun 1913,

*Drobov 431* (LE!). SEMIPALATINSK PINERY: **East Kazakhstan Region**: Semipalatinsk District: near Semipalatinsk, 8 Jun 1914, *Mordvinova s.n.* (MOSP!). MUGODZHARY: **Aktobe Region**: Mugalzhar District: Mugodzhary Mts, “Urkach” place, 21 Aug 1927, *Krashennikov 1230* (LE!).

**Habitat and ecology.** Sedge and sphagnum swamps.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sept.

**Conservation status.** Not protected. The species is extremely rare and we consider it mandatory to include it in the next edition of the Red Book of Kazakhstan.

**Notes.** *Liparis loeselii* is reported here for the first time for Semipalatinsk Pinery and Mugodzhary FRs. Previously, in the studied region, the species was observed only in Kokchetav FR (Karamysheva and Rachkovskaya 1973; Sultangazina et al. 2014; Kupriyanov 2020). The reported localities of *L. loeselii* belong to the type subspecies, whereas the locality in East Kazakhstan represents the newly-described *L. loeselii* subsp. *Orientalis*, which differs from the typical subspecies by having broader leaf blade, more visible petioles and broader fruits (Efimov 2010).

### ***Malaxis monophyllos* (L.) Sw.**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia).

**Specimens examined and literature records.** TOBOL-ISHIM: **Kostanay Region**: Mendykara District: Borovskaya water protection forest dacha, 17 Jul 1925, *Rusanov 1553* (LE!); near Borovskoye (Pugachev 1994); near Kamenskural'skoe (Pugachev and Masyukova 1969); Auliekol District: near Kalininskoye (Pugachev 1994). EASTERN UPLAND: **Pavlodar Region**: Bayanaul District: Bayanaul Mountains, 30 Jun 1913, *Kuchеровskaya s.n.* (LE!); same loc., 10 km west of Bayan-Aul, 19 Jul 1959, *Denisova 436* (LE!); same loc., 19 Jul 1959, *Denisova 427* (MW 0816997!). KARKARALY: **Karaganda Region**: Karkaraly District: Zhisil'tau Mountains, near Egindibulak, 19 Jul 1992, *Kupriyanov s.n.* (KG!).

**Habitat and ecology.** Grassy birch forests, near lakes, along forest streams.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sept.

**Conservation status.** Not protected. We recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** Forms both with one and with two well-developed leaves occur in Kazakhstan, the latter being recognised as a variety of *M. monophyllos* var. *diphyllos* (Cham.) Luer (e.g. by Pavlov 1928). Bayan-Aul and Karkaraly Mts represent the southernmost locality of the species in Central Asia.

### ***Neottia camtschatea* (L.) Rchb.f.**

**Distribution in adjacent reg.** Russia (Siberia), Kazakhstan (Altai, Western Tien Shan).

**Specimens examined and literature records.** KARKARALY: **Karaganda Region**: Karkaraly District: Kent Mountains, near Kent, 1 Jun 2007, *Kupriyanov et al.* (KUZ 11470!); same loc., “Auletas” place, 25 Jul 1992, *Kupriyanov s.n.* (KG!); Zhisil'tau Mountains, near Egindibulak, 16 Jul 1992, *Mikhailov s.n.* (KG!). EASTERN UPLAND: **Pavlodar Region**: Bayanaul District: Bayanaul Mountains, 10 km

west of Bayan-Aul, 19 Jul 1959, *Denisova 444* (MW 0816977!); same loc., 23 Jul 1963, *Denisova 1495* (MW 0816976!).

**Habitat and ecology.** Damp birch-aspen forests along the shores of streams and lakes.

**Phenology.** Flowering in Jun–Jul; fruiting in Aug–Sep.

**Conservation status.** Not protected. The species is extremely rare and we consider it mandatory to include it in the next edition of the Red Book of Kazakhstan.

**Notes.** *Neottia camtschatea* is reported here for the first time for Eastern Upland FR and for Pavlodar Region. Earlier occurrences of the species were confirmed in eastern Kazakhstan (Altai, Tarbagatai), southern Kazakhstan (Dzungarian Alatau, Zailiyskiy Alatau) (Kusnetsov and Pavlov 1958) and central Kazakhstan (Karkaraly) (Kupriyanov 2020).

### *Orchis militaris* L.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia).

**Specimens examined and literature records.** PRIKASPIY: **West Kazakhstan Region:** Chingirlauskiy District: “Kara-Agach”, 23 Jun 1950, *Nikitin s.n.* (LE!); same loc., place valley of the Ural River, headwaters Buldurta River, 21 Jun 2003, *Darbaeva s.n.* (LE!). EMBA: **Aktobe Region:** Mugalzhar District: near Emba, 30 May 1840, *Bunge 1334* (LE!). MUGODZHARY: **Aktobe Region:** Mugodzhary Mts “Urkach” place, near Kumyskul Lake, 10 Jul 1927, *Rusanov 773* (LE!), *s.n.* (AA!); Mugodzhary Mts, near Ayrik (Aipeisova 2012). SYRT: **West Kazakhstan Region:** Bajterekskiy District: south-east of Uralsk, Archiereyskiy site, s.d. *Gremyachenskiy s.n.* (MW 296812!); SEMIPALATINSK PINERY: **East Kazakhstan Region:** Semipalatinsk District: near Semipalatinsk, 8 Jun 1914, *Mordvinova s.n.* (MOSP!); Beskaragajskiy District: near Kanonerka, 12 Jun 1996, *Kupriyanov et al. s.n.* (ALTB!). EASTERN UPLAND: **East Kazakhstan Region:** Abaj District: Akshatau Ridge (Karipbaeva et al. 2015).

**Habitat and ecology.** Sparse birch forests on sandy soils, moist meadows, valleys of rivers and streams, near lakes and forest edges.

**Phenology.** Northern Kazakhstan: Flowering in Jun–Jul; fruiting in Jul–Aug. Western Kazakhstan: Flowering in May–Jun; fruiting in Jun–Jul.

**Conservation status.** It is included in the Red Book of Kazakhstan (category III). It is protected in the territory of the following State Nature Reserves: “Semey Ormany”, “Orkash”, “Kokzhide-Kumzhargan”, “Budarinsky”, “Kirsanovsky” and “Ak-Kuma”. In Kazakhstan, *Orchis militaris* is very rare, the number of individuals in the populations is low. It is necessary to monitor the state of populations.

### *Platanthera bifolia* (L.) Rich.

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** TOBOL-ISHIM: **Kostanay Region:** Mendykara District: Borovskaya water protection forest dacha, 28 Jul 1923, *Rusanov s.n.* (LE!); near Borovskoye, 10 Jul 1977, *Pugachev s.n.* (LE!);



botanical nature monument “Plantations of birch and pine forests near Borovskoye Lake”, 24 Jun 2009, *students* 195 (KSPI!); Zhitikara District: botanical nature monument “Relict larch-birch grove with Sukachev larch”, 10 Jun 2012, *Perezhogin* (personal observation); Auliekol District: near Kazanbasskoye (Pugachev 1994); near Kalininskoye (Pugachev 1994); near Auliekol (Pugachev and Masyukova 1969). **North Kazakhstan Region:** Kyzylzhar District: right shore of Ishim River, near Krasnoyarka, 30 Jun 1971, *Afonina and Litvinenko s.n.* (NKU!); same loc., 29 Jun 1971, *Stepanova and Shakhvatova s.n.* (NKU!); same loc., 29 Jun 1971, *Sologub and Zelinskaya s.n.* (NKU!); same loc., 28 Jun 1971, *Fesan and Kosmagambetova s.n.* (NKU!); same loc., 29 Jun 1971, *Trushcheleva and Shirokikh s.n.* (NKU!); same loc., 26 Jun 1972, *Kudinova and Schneider s.n.* (NKU!); same loc., 24 Jun 1972, *Makayun s.n.* (NKU!); same loc., 27 Jun 1972, *Rosinskaya and Khrushchev s.n.* (NKU!); same loc., 29 Jun 1972, *Shegebaev and Zhampeisov s.n.* (NKU!). **KOKCHETAV: North Kazakhstan Region:** Aiyrtau District: near Lobanovo, Kozhevnyya swamp, 28 May 2020, *Kubentaev et al. s.n.* (NUR!).

**Habitat and ecology.** Forest edges and glades, dry meadows, scrub thickets, thinned forests and the outskirts of bogs.

**Phenology.** Flowering in May–Jul; fruiting in Jul–Aug.

**Conservation status.** The species is included in the Red Book of Kazakhstan (category III) as an endangered species. It is protected on the territory of the nature monument “Stands of birch and pine forests near Borovskoye Lake”; the botanical nature monument “Relict larch-birch grove with Sukachev larch”; Kokshetau State National Nature Park; and the “Sogrov” State Nature Reserve.

**Notes.** There is an old report of *P. bifolia* for Kokchetav FR (without detailed locality) by Semenov (1928), which was omitted from the later floristic accounts of Kazakhstan (Kusnetsov and Pavlov 1958) and Kazakh Upland (Karamysheva and Rachkovskaya 1973; Sultangazina et al. 2014; Kupriyanov 2020). Here, we confirm the old data for this floristic region (and simultaneously, for Kazakh Upland) through our recent gathering from the vicinity of Lobanovo, North Kazakhstan Region.

### ***Spiranthes australis* (R.Br.) Lindl**

**Distribution in adjacent reg.** Russia (European Russia, Ural, Siberia), Kazakhstan (Altai).

**Specimens examined and literature records.** TOBOL-ISHIM: **Kostanay Region:** Mendykara District: 4 km south of Borovskoye, 20 Jun 1925, *Rusanov s.n.* (LE!); same loc., 19 Jun 1925, *Rusanov 1490* (MW 0816970!); Auliekol District: near Kalininskoye (Pugachev 1994). **KOKCHETAV: Akmola Region:** Burabay District: 3 km north of the cordon “Medvezhy”, near Shchuchye Lake, 11 Aug 1973, *Gorchakovskiy s.n.* (SVER 695777!). **IRTYSH: Pavlodar Region** [without detailed locality] (Kusnetsov and Pavlov 1958).

**Habitat and ecology.** On peat bogs.

**Phenology.** Flowering in Jul–Aug; fruiting in Aug–Sept.

**Conservation status.** Not protected. Taking into account the limited expansion and the small number of individuals in the population, we recommend to include this species in the next edition of the Red Book of Kazakhstan.

**Notes.** Currently, field studies are needed to verify the presence of *Spiranthes australis* in the study region, since the species was not observed here for almost 30 years.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

SAK and PGE designed the study and conceived and wrote this article; ANK and YVP analysed the data, revised and finalised the manuscript; SAK, DTA, KSI and AEK analysed the data, conducted the fieldwork and all authors contributed to the interpretation/discussion and revision of the manuscript.

## Author ORCIDs

Serik A. Kubentayev  <https://orcid.org/0000-0002-0369-0591>

Petr G. Efimov  <https://orcid.org/0000-0003-2926-255X>

Daniyar T. Alibekov  <https://orcid.org/0000-0003-1555-1430>

Andrey N. Kupriyanov  <https://orcid.org/0000-0001-5602-2012>

Klara S. Izbastina  <https://orcid.org/0000-0002-6418-1950>

Aizhan E. Khalymbetova  <https://orcid.org/0000-0002-2584-4766>

Yuri V. Perezhogin  <https://orcid.org/0000-0001-6997-8347>

## Data availability

All of the data that support the findings of this study are available in the main text.

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# *Neottia bifidus* (Orchidaceae, Epidendroideae, Neottieae), a new mycoheterotrophic species from Guizhou, China

Mei-Na Wang<sup>1</sup>, Xin-Yi Wu<sup>1</sup>, Cheng-Jiang Tan<sup>2</sup>, Ping Yu<sup>2</sup>, Wen-Hui Rao<sup>1</sup>, Jie-Shan Chen<sup>1</sup>, Jian Li<sup>1</sup>, Jian-Bing Chen<sup>1</sup>

<sup>1</sup> The Orchid Conservation & Research Center of Shenzhen and the National Orchid Conservation Center of China, Shenzhen Key Laboratory for Orchid Conservation and Utilization, Key Laboratory of National Forestry and Grassland Administration for Orchid Conservation and Utilization, Shenzhen, 518114, Guangdong, China

<sup>2</sup> Management Department of Maolan National Nature Reserve, Libo 558400, Guizhou, China

Corresponding authors: Jian Li (lij@cnocc.cn); Jian-Bing Chen (conservation@cnocc.cn)

## Abstract

*Neottia bifidus*, a new mycoheterotrophic orchid, found in Maolan National Nature Reserve in Guizhou Province, China, is described and illustrated here. The new species is close to *N. nidus-avis*, *N. kiusiana* and *N. papilligera* but differs in having a finely pubescent rachis with fewer flowers, a finely pubescent pedicel, and a fishtail-shaped lip that is deeply bilobed to the middle of the lip, with the lobes diverging at an acute angle (45°) to each other and mesochile with many papillae. Additionally, *N. bifidus* is well supported as a new species by molecular phylogenetic results based on ITS and chloroplast genome. The chloroplast genome of the novelty, which contains an LSC region of 33,819 bp, SSC region of 5,312 bp and IRs of 46,762 bp was assembled and annotated. A key to mycoheterotrophic *Neottia* species in China is also provided.

**Key words:** *Neottia bifidus*, new species, Orchidaceae, saprophytic orchid



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## Introduction

The genus *Neottia* Guett. comprises 81 accepted species, including 63 autotrophic species and 18 mycoheterotrophic species (<https://powo.science.kew.org>, Mu et al. 2017; Chen and Jin 2021), distributed widely in north temperate areas with a few species extending into alpine regions in the mountains of tropical Asia (Govaerts et al. 2019; Chen and Jin 2021). East Asia is one of the diversity centers for this genus with more than 70% of *Neottia* species occurring in this region (So and Lee 2020). Formerly, *Neottia* was divided into *Listera* and *Neottia* (Bentham 1881; Pfitzer 1887; Schlechter 1926; Brieger et al. 1974; Dressler 1981; Rasmussen 1982) by the distinct morphological differences possessed by autotrophic plants (*Listera*) with two opposite leaves (sometimes three or more) in the middle of the stem, while mycoheterotrophic plants are achlorophyllous and possess densely fleshy bird's nest like roots. In 2003, Govaerts cited another genus in Tribe Neottieae Lindl., *Holopogon*, as a synonym of *Neottia* (Govaerts 2003).

There are 52 species and one variation of *Neottia* in China, amongst which 14 species are mycoheterotrophic (<https://powo.science.kew.org>, Mu et al. 2017; Chen and Jin 2021). During our fieldwork in the Maolan National Nature

Reserve, Libo County, Guizhou Province, China in 2021, an unknown species of mycoheterotrophic *Neottia* was found in the evergreen broad-leaved forest. Based on morphological characters and molecular evidence, it was considered as a new species of *Neottia* and is described below.

## Materials and methods

Morphological characteristics of the new species were observed, measured and photographed, based on living plants in Maolan National Nature Reserve, Guizhou. The studied specimens are deposited at The National Orchid Conservation Center of China and the Orchid Conservation & Research Center of Shenzhen. The general morphology was derived from fresh specimens and photographs were taken with a DSLR camera. To investigate the systematic position of the new species, the plastid genome and the nuclear ribosomal internal transcribed spacers (nrITS) marker were used in molecular phylogenetic analysis. Total genomic DNA was extracted from fresh flowers and stems (voucher specimens J.B.Chen 00599) using a plant genomic DNA kit and then sent to Novogene (Beijing, China) for the library (350 bp) preparation for genome skimming sequencing. Paired-end (150 bp) sequencing was conducted on the Illumina Hiseq 6000 platform (San Diego, CA), producing approximately 8 Gb reads. The plastid genome was assembled using GetOrganelle (Jin et al. 2020) with the chloroplast genome of *Neottia camtschatea* (L.) Rchb. F.(NC\_030707) and *Neottia listeroides* Lindl. (NC\_030713) as the reference sequences. After assembly, the obtained scaffolds and contigs were annotated by Geneious Prime (Biomatters Ltd., Auckland, New Zealand) (Kearse et al. 2012) and Plastid Genome Annotator (Qu et al. 2019). The annotated complete chloroplast genome was deposited in GenBank with accession number OP279442. nrITS were also sequenced for the new species in this study. The PCR reactions and Sanger Sequencing were performed by Sangon Biotech (Shanghai, China). The primers used in this study are presented in Table 1. In total, 70 species (incl. 29 species of *Neottia*) from seven genera were used for molecular phylogenetic analyses (Table 2). The nrITS dataset consists of six genera and 66 species and the plastid genome dataset consists of five genera and 27 species, respectively. Five species of *Cionisaccus*, *Ophrys* and *Serapias* were selected as outgroup taxa based on Li et al. (2016). All plastid genomes were aligned by MAFFT 7.3 (fft-NS-i  $\times$  1000 strategy) after removing one inverted repeat (IR) region of each sample (Kato and Standley 2013). Poorly-aligned regions were removed by trimAl 1.2 with default settings before phylogenetic analyses (Capella-Gutiérrez et al. 2009). Maximum Likelihood (ML) analyses were conducted in IQTREE 1.6 using the SH-aLRT test and ultrafast bootstrap (UFBoot) feature ( $-alrt$  1000  $-bb$  1000  $-nt$  AUTO) (Nguyen et al. 2015; Hoang et al. 2018).

**Table 1.** Primers used in this study.

Primer	Sequence (5'to3')	Origin
ITS-17SE	ACGAATTCATGGTCCGGTGAAGTGTTTCG	Sun et al. 1994
ITS-26SE	TAGAATTCCCCGGTTCGCTCGCCGTTAC	Sun et al. 1994



**Table 2.** GenBank accession numbers for sequence data, a dash (-) indicates missing data and an asterisk (\*) denotes sequences obtained in this study.

Species	nrITS	cp
<i>Aphyllorchis caudata</i>	FJ454866	-
<i>Aphyllorchis gollanii</i>	MZ463253	-
<i>Aphyllorchis montana</i>	FJ454867	-
<i>Aphyllorchis pallida</i>	MZ463252	-
<i>Cephalanthera bijiangensis</i>	MZ463242	-
<i>Cephalanthera damasonium</i>	AY146446	NC_041179
<i>Cephalanthera epipactoides</i>	KY512499	-
<i>Cephalanthera erecta</i>	MZ463245	-
<i>Cephalanthera exigua</i>	FJ454868	-
<i>Cephalanthera falcata</i>	AB856493	-
<i>Cephalanthera falcata</i> var. <i>flava</i>	MZ463241	-
<i>Cephalanthera humilis</i>	MZ463240	NC_030706
<i>Cephalanthera longibracteata</i>	MK306540	NC_041180
<i>Cephalanthera longifolia</i>	AY146447	NC_030704
<i>Cephalanthera nanchuanica</i>	JN706696	-
<i>Cephalanthera nanlingensis</i>	KT338669	-
<i>Cephalanthera rubra</i>	AY146445	NC_041181
<i>Epipactis albensis</i>	AY154384	NC_041182
<i>Epipactis atrorubens</i>	JN847403	-
<i>Epipactis duriensis</i>	AY351377	-
<i>Epipactis fageticola</i>	AY351382	-
<i>Epipactis flava</i>	FJ454869	-
<i>Epipactis helleborine</i>	MZ463247	MK608776
<i>Epipactis leptochila</i>	FJ454870	-
<i>Epipactis lusitanica</i>	AY351381	-
<i>Epipactis mairei</i>	MZ463250	NC_030705
<i>Epipactis microphylla</i>	FR750399	MH590352
<i>Epipactis muelleri</i>	FJ454871	-
<i>Epipactis palustris</i>	AY146448	NC_041187
<i>Epipactis papillosa</i>	MZ463248	-
<i>Epipactis purpurata</i>	JN847416	MH590354
<i>Epipactis royleana</i>	MZ463249	-
<i>Epipactis thunbergii</i>	MK306477	NC_046817
<i>Epipactis veratrifolia</i>	KF727435	NC_030708
<i>Epipactis voethii</i>	FR750400	-
<i>Neottia acuminata</i>	KT338755	-
<i>Neottia alternifolia</i>	MZ463268	-
<i>Neottia bicallosa</i>	MZ463271	-
<i>Neottia bifidus</i>	OP265395*	OP279442*
<i>Neottia bifolia</i>	MG216639	-
<i>Neottia borealis</i>	MG216431	-
<i>Neottia brevicaulis</i>	MZ463258	-
<i>Neottia camtschatea</i>	KJ023677	NC_030707

Species	nrITS	cp
<i>Neottia cordata</i>	KJ023678	NC_041189
<i>Neottia suzukii</i>	MH321188	NC_041447
<i>Neottia divaricata</i>	MZ463257	-
<i>Neottia fugongensis</i>	MZ463256	NC_030711
<i>Neottia hybrid</i> sp.	MZ463255	-
<i>Neottia japonica</i>	KT338756	NC_041446
<i>Neottia karoana</i>	MZ463270	-
<i>Neottia kiusiana</i>	KT338757	MN537563
<i>Neottia listeroides</i>	MZ463262	NC_030713
<i>Neottia meifongensis</i>	MZ463267	-
<i>Neottia mucronata</i>	MZ463261	-
<i>Neottia nidus-avis</i>	AY351383	JF325876
<i>Neottia nujiangensis</i>	MZ463254	-
<i>Neottia ovata</i>	-	NC_030712
<i>Neottia papilligera</i>	KT338758	-
<i>Neottia pinetorum</i>	KT338759	KU551269
<i>Neottia puberula</i>	MH808061	-
<i>Neottia smallii</i>	AF521058	-
<i>Neottia smithiana</i>	MZ463263	-
<i>Neottia wardii</i>	MZ463260	-
<i>Neottia wuyishanensis</i>	MZ409849	-
<i>Cionisaccus procera</i>	-	MW589517
<i>Ophrys apifera</i>	AY699976	-
<i>Ophrys fusca</i> subsp.	-	AP018716
<i>Ophrys insectifera</i>	AY699950	-
<i>Ophrys sphegodes</i>	-	AP018717
<i>Serapias cordigera</i>	AY364884	-

## Results

The whole chloroplast genome of *N. bifidus* showed a typical quadripartite structure containing a pair of inverted repeats (IRs) separated by a large single-copy (LSC) region and a small single-copy (SSC) region (Fig. 1). The complete plastid genome sequence of *N. bifidus* was 85,893 bp in length containing an LSC region of 33,819 bp, SSC region of 5,312 bp and IRs of 46,762 bp. The chloroplast genome contained 72 genes, including 36 protein-coding genes, 28 tRNA genes and eight rRNA genes (Table 3). The overall GC content is 35%.

The phylogenetic analyses indicated that this unknown species is far from other autotrophic species, but has a better clustering relationship with leafless holomycotrophic species in *Neottia*. The phylogenetic tree, based on the plastid genome, indicated that it is close to *N. kiusiana* T.Hashim. & S.Hatus. (KT338757) with high support (SH-aLRT 100%, UfBoot 100%) and then sister to *N. nidus-avis* (L.) Rich. (JF325876) also with strong support (SH-aLRT 100%, UfBoot 100%) (Fig. 2). The phylogenetic tree, based on nrITS, showed that the new species is sister to *N. kiusiana* and *N. papilligera* Schltr. with high support (SH-aLRT 100%, UfBoot 100%) (Fig. 3).

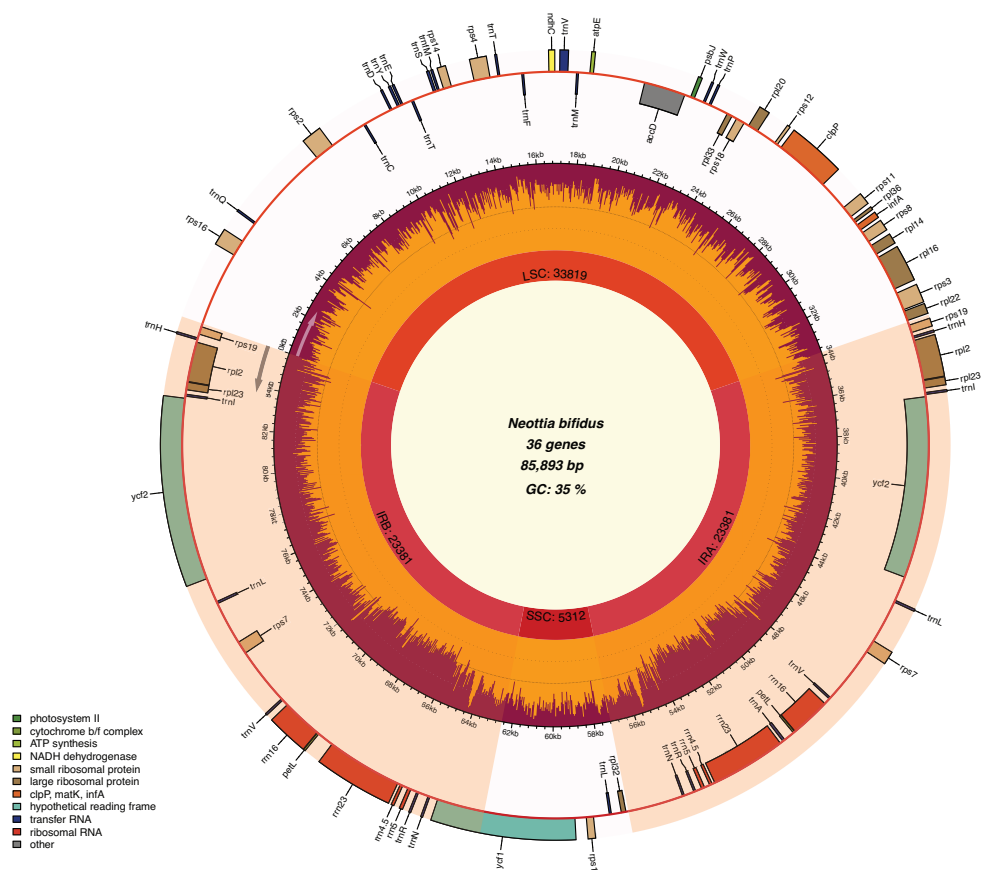
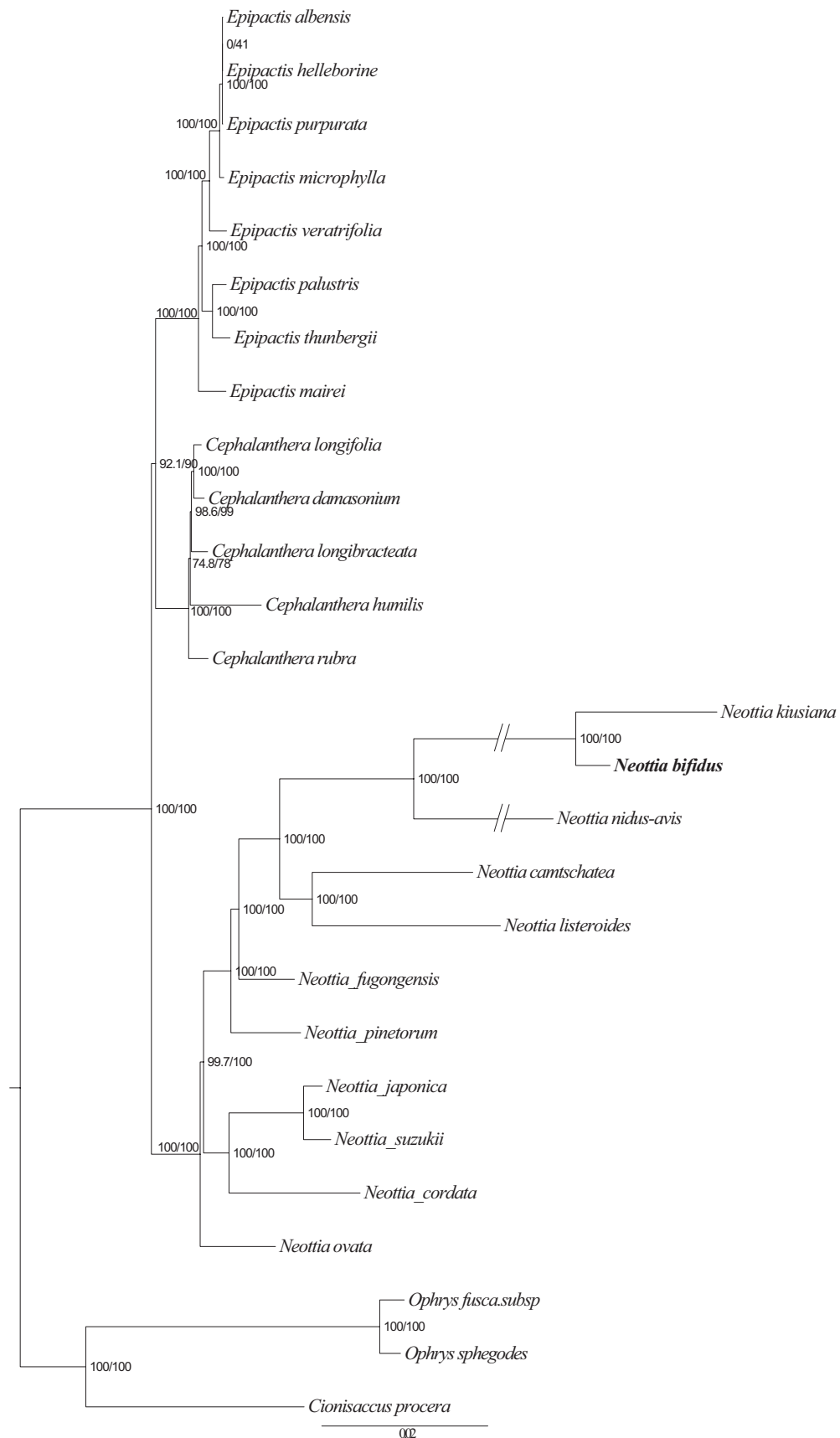


Figure 1. Chloroplast genome map of *N. bifidus*.

Table 3. Genes present in the chloroplast genome of *Neottia bifidus*.

Group of genes	Gene
Photosystem I	-
Photosystem II	<i>psbJ</i>
Cytochrome b/f complex	<i>petL*</i>
ATP synthase	<i>atpE</i>
NADH dehydrogenase	<i>ndhC</i>
Rubis CO large subunit gene	-
RNA polymerase	-
Small ribosomal proteins	<i>rps2, rps3, rps4, rps7*, rps8, rps11, rps12, rps14, rps15, rps16, rps18, rps19*</i>
Large ribosomal proteins	<i>rpl2*, rpl14, rpl16, rpl20, rpl22, rpl23*, rpl32, rpl33, rpl36</i>
tRNA	<i>trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-CAU, trnH-GUG*, trnI-CAU*, trnL-CAA*, trnL-UAG, trnM-CAU, trnN-GUU*, trnP-UGG, trnQ-UUG, trnR-ACG*, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC*, trnV-UAC, trnW-CCA, trnY-GUA</i>
rRNA	<i>rrn4.5*, rrn5*, rrn16*, rrn23*</i>
Translational initiation factor	<i>infA</i>
Subunits of Acetyl-CoA-carboxylase	<i>accD</i>
Protease	<i>clpP</i>
Conserved open reading frames	<i>ycf1, ycf2*</i>

Note: \* means duplicated gene in IRs.



**Figure 2.** Phylogram of *Neottieae*, based on the plastid genome. The numbers near the nodes are the values of SH-aLRT test (left) and the ultrafast bootstrap (right).





**Figure 3.** Phylogram of Neottieae, based on nrITS. The numbers near the nodes are the values of SH-aLRT test (left) and the ultrafast bootstraps (right).

## Taxonomy

### *Neottia bifidus* M.N.Wang, sp. nov.

urn:lsid:ipni.org:names:77324361-1

Figs 4, 5

Chinese name: 鱼尾鸟巢兰

**Type.** China. Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo County, the Maolan National Nature Reserve, 825 m elev., 23 April 2021, J.B.Chen 00599 (holotype: NOCC).

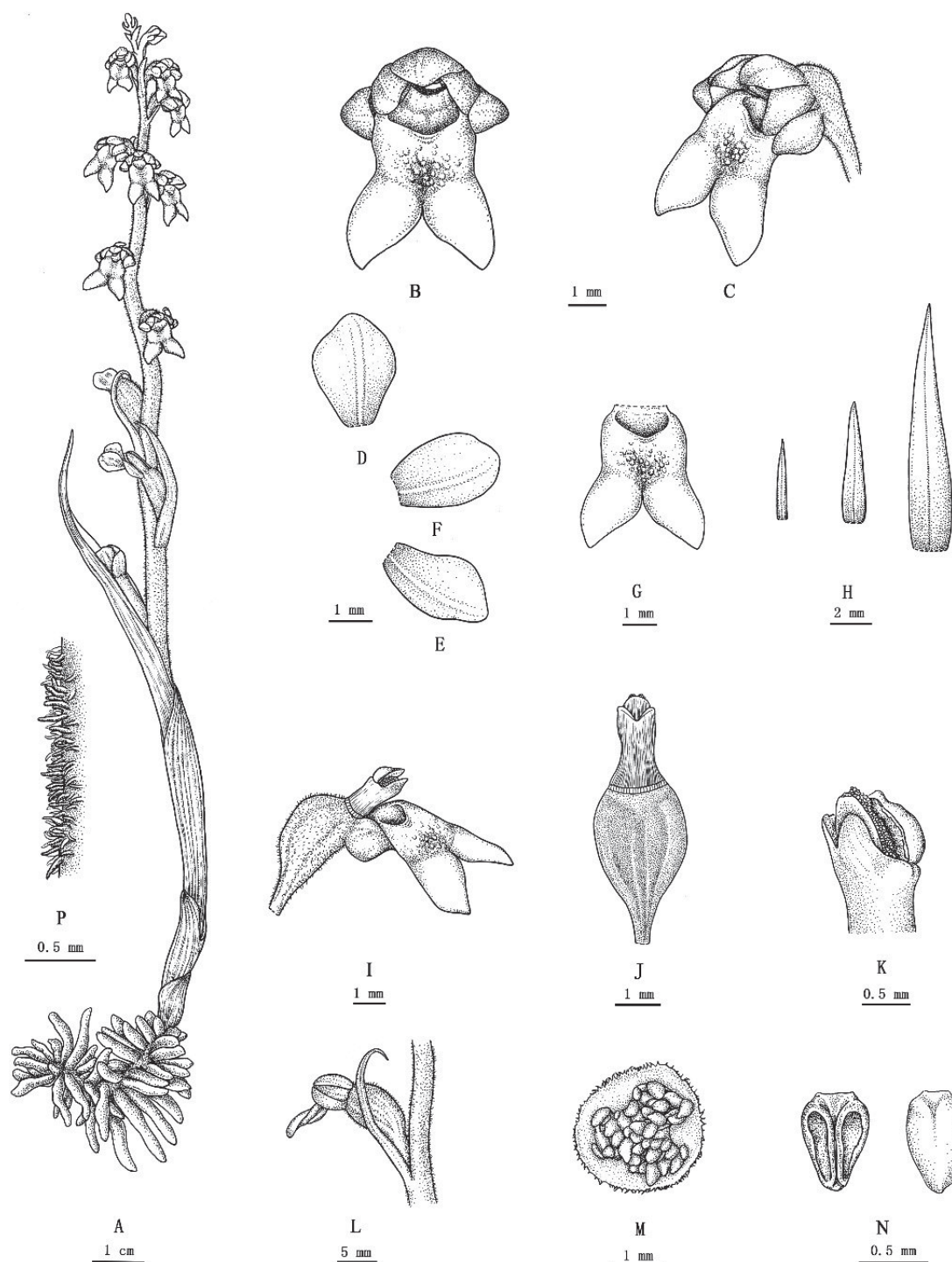
**Diagnosis.** *Neottia bifidus* is morphologically similar to *N. nidus-avis*, *N. kisiana* and *N. papilligera* but differs in having a finely pubescent rachis, with fewer flowers; finely pubescent pedicel; and fish-tail-shaped lip, deeply 2-lobed to the centre of mid-lip, lobes diverging at an acute angle (45°) to one another, mesochile with many papilloses (Table 4).

Terrestrial herbs, leafless, holomycotrophic, 10–19 cm tall. Rhizome short, with many stout, fleshy fascicled roots. Stem erect, terete, leafless, pubescent, with 2–3 sheaths at base; sheaths tubular, 2–3 cm, membranous, glabrous, with 4–7 dark brown veins, upper ones much longer than lower ones; rachis 7–13 cm, pubescent, laxly and irregularly 9–15-flowered; floral bracts membranous, glabrous, narrowly lanceolate, ovate-lanceolate, obtuse to subacute, 0.7–2.1 cm long, lowermost ones much longer than flowers, 1.1–1.3 × 2.6–3 cm, gradually diminishing in upper ones which are shorter than ovaries. Flowers resupinate, pale brown; pedicel and ovary 0.6–1.5 cm long, pubescent. Sepals membranous, ovate to obovate, pale brown, nearly equal in size; dorsal sepal cucullate, 2.3–2.4 × 1.6–1.8 mm, apex obtuse, glabrous; lateral sepals cucullate, strongly cupped, 2.4–2.5 × 1.4–1.5 mm, apex obtuse, glabrous. Petals membranous, ovate to obovate, pale brown, nearly equal in size to dorsal sepal. Lip spreading downwards, subrectangular, 3.8–5 mm long, small and semi-transparent at early anthesis, becoming larger and yellowish-brown at late anthesis, apex deeply 2-lobed to the center of mid-lip; hypochile rectangular, concave at base; mesochile with many papilloses; epichile 2-lobed, lobes extending outwards, triangular, fish-tail-shaped, 2.3–2.5 × 1.5–1.6 mm, diverging at an acute angle (45°) to one another, apex obtuse, margins of apices and inner sides repand or erose. Column cylindrical, 2.8–3 mm long; anther inclined towards rostellum, elliptic, ca. 0.7 mm; stigma ca. 0.9 mm, lamellate, 2-lobed; rostellum shorter than anther. Capsule elliptic, with persistent sepals and petals, 1–1.5 cm long.

**Etymology.** The species epithet refers to the fish-tail-shaped lip of the new species.

**Distribution and habitat.** *Neottia bifidus* is currently known only from the type locality in Libo, Guizhou, China. It grows in humus-rich soil under broad-leaved forests at elevations of 700–900 m and is found growing with *Milusa sinensis* Finet & Gagnep. (Annonaceae), *Platycarya strobilacea* Siebold & Zucc (Juglandaceae), *Michelia martini* (H. Lév.) Finet & Gagnep. ex H. Lév. (Magnoliaceae), *Mallotus philippensis* (Lamarck) Müll. Arg. (Euphorbiaceae), *Symplocos adenophylla* Wall. (Symplocaceae), *Chimonobambusa angustifolia* C. D. Chu & C. S. Chao (Poaceae), *Murraya exotica* L. (Rutaceae), *Gomphandra tetrandra* (Wall.) Sleumer (Stemonuraceae), *Diospyros mollis* Griff. (Ebenaceae), *Strobilanthes hongii* Y. F. Deng & F. L. Chen (Acanthaceae), etc.

**Phenology.** Flowering and fruiting from Apr–May.



**Figure 4.** *Neottia bifidus* M.N.Wang, sp. nov. **A** whole plant **B** flower (front view) **C** flower (side view) **D** dorsal sepal **E** lateral sepal **F** petal **G** lip (front view) **H** bracts **I** ovary, column and lip (side view) **J** ovary and column (ventral view) **K** column **L** fruit with bract **M** fruit (cross section) **N** anther cap **P** hairy on rachis.

**Conservation status.** During our fieldwork, only one population with less than 10 individuals was discovered in Maolan National Nature Reserves (213 km<sup>2</sup>). Most individuals were found growing along the roadside and are easily disturbed by human activities. According to the guidelines for using the IUCN Red List Categories and Criteria (IUCN 2022), the new species should be temporarily assigned as ‘Critically Endangered’ by its limited populations, localities and vulnerable habitats.



**Figure 5.** *Neottia bifidus* M.N.Wang, sp. nov. Photographed by M. N. Wang & W. H. Rao. **A** habit **B** whole plant and hairy on rachis **C** inflorescence **D** flower (front view) **E** ovary and flower (side view) and hairy on ovary **F** dorsal sepal **G**, **H** lateral sepals **I**, **J** petals **K** lip (front view, back view and side view) **L** bract **M** ovary and column **N** column **O** fruit with bract **P** fruit (cross section) **Q** Anther cap.



**Table 4.** Morphological comparison of *Neottia bifidus* and similar species.

Morphological characters	<i>N. bifidus</i>	<i>N. kiusiana</i> (Yukawa et al. 2009)	<i>N. papilligera</i> (Chen et al. 2009)	<i>N. nidus-avis</i> (Jersáková et al. 2022)
Plant height	15–19 cm	6–21 cm	27–30 cm	15–60 cm
Rachis	Rachis densely pubescent, laxly and irregularly 9–15-flowered.	Rachis sparsely glandular hairy, with 10–28 flowers.	Rachis glabrous or pubescent, with much more than 20 flowers.	Rachis glabrous, with much more than 20 flowers.
Pedicel	Pubescent	Glabrous	Glabrous	Glabrous
Lip	Lip 2-lobed to the centre of mid-lip; hypochile without purple dots; mesochile with many papilloses; epichile 2-lobed, lobes triangular, fish-tail-shaped, diverging at an acute angle (45°) to one another.	Lip 2-lobed (not up to the centre of mid-lip); hypochile purple-dotted adaxially; epichile 2-lobed, lobes transversely oblique-rectangular, rectangular or oblong, diverging at an acute angle (45°) to one another.	Lip apex deeply 2-lobed; lobes narrowly oblong, usually twisted, diverging at an obtuse angle (120°–170°) to one another.	Lip apex deeply 2-lobed, diverging at an obtuse angle (120°–170°) to one another.

**Note.** *Neottia bifidus* is morphologically - related to three species, namely, *N. nidus-avis*, *N. kiusiana* and *N. papilligera*, but it is readily distinguished from them, based on morphological characters given in Table 4.

### Key to mycoheterotrophic species of *Neottia* in China

- 1 Stigma terminal; rostellum absent ..... **2**
- Stigma lateral or rarely subterminal; rostellum present, usually above concave stigma ..... **4**
- 2 Flowers purplish-red ..... ***Neottia gaudissartii* (*Holopogon gaudissartii*)**
- Flowers green ..... **3**
- 3 Flowers actinomorphic, lip very similar to the petals ..... ***N. pekinensis* (*Holopogon pekinensis*)**
- Flowers zygomorphic, lip bilobed at the apex, utterly different from the petals ..... ***N. smithiana* (*Holopogon smithianus*)**
- 4 Lip entire; column (excluding anther and rostellum) less than 0.5 mm ..... **5**
- Lip bilobed at apex; column (excluding anther and rostellum) 1.5–4 mm ..... **6**
- 5 Floral rachis glabrous; flowers resupinate ..... ***N. acuminata***
- Floral rachis villous; flowers not resupinate ..... ***N. taibaishanensis***
- 6 Lip distinctly concave at base ..... **7**
- Lip not concave at base ..... **9**
- 7 Apical lobes of lip parallel or diverging at an acute angle to one another... ***N. bifidus***
- Apical lobes of lip diverging at an obtuse angle to one another ..... **8**
- 8 Apical lobes of lip 2.5–3 mm; sinus of lip without a short tooth between lobes ..... ***N. papilligera***
- Apical lobes of lip less than 1 mm; sinus of lip with a short tooth between lobes ..... ***N. brevilabris***
- 9 Lip with a pair of triangular auricles at base ..... ***N. tenii***
- Lip without a pair of auricles at base ..... **10**
- 10 Lip obovate, 6–10 mm wide ..... ***N. megalochila***
- Lip narrowly obovate-oblong or cuneate, 1.5–4 mm wide ..... **11**
- 11 Lip narrowly obovate-oblong, 6–9 × 3–4 mm ..... ***N. listeroides***
- Lip cuneate, 10–12 × 1.5–2 mm ..... ***N. camtschatea***

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Data curation: MNW, XYW. Funding acquisition: MNW. Investigation: MNW, WHR, JL, CJT, PY. Methodology: MNW, XYW. Project administration: JL. Software: XYW. Supervision: JL, JBC. Visualization: JSC. Writing-original draft: MNW. Writing-review and editing: MNW

### Author ORCIDs

Mei-Na Wang  <https://orcid.org/0000-0001-5998-6028>

Xin-Yi Wu  <https://orcid.org/0000-0002-8623-1867>

Cheng-Jiang Tan  <https://orcid.org/0009-0008-4558-4907>

Ping Yu  <https://orcid.org/0009-0003-3319-7145>

Wen-Hui Rao  <https://orcid.org/0000-0002-2177-6700>

Jie-Shan Chen  <https://orcid.org/0009-0000-8859-6053>

Jian Li  <https://orcid.org/0000-0002-0096-6257>

Jian-Bing Chen  <https://orcid.org/0000-0001-9085-0663>

### Data availability

All of the data that support the findings of this study are available in the main text.

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# *Artemisia qingheensis* (Asteraceae, Anthemideae), a new species from Xinjiang, China

Guang-Zhao Jin<sup>1,2,3</sup>, Mariya Sheludyakova<sup>4</sup>, Wen-Jun Li<sup>1,2</sup>, Feng Song<sup>5</sup>, Zhi-Bin Wen<sup>1,2</sup>, Ying Feng<sup>1,2</sup>

<sup>1</sup> State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

<sup>2</sup> The Herbarium of Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

<sup>3</sup> University of Chinese Academy of Sciences, Beijing 100094, China

<sup>4</sup> Komarov Botanical Institute of Russian Academy of Sciences, Prof. Popova 2, Sankt Peterburg 197376, Russia

<sup>5</sup> Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Corresponding authors: Zhi-Bin Wen ([zhibinwen@ms.xjb.ac.cn](mailto:zhibinwen@ms.xjb.ac.cn)); Ying Feng ([luckfy@ms.xjb.ac.cn](mailto:luckfy@ms.xjb.ac.cn))

## Abstract

*Artemisia qingheensis* (Asteraceae, Anthemideae), a new species from Qinghe County, Xinjiang, China, is described and illustrated. We investigated its phylogenetic position and relationships with 35 other species of *Artemisia* using whole chloroplast DNA sequence data. The molecular phylogenetic results and morphological evidence (multi-layered involucre bracts and homogamous capitula with bisexual flowers) showed that the new species belongs to *Artemisia* subgenus *Seriphidium*. A diagnostic table and discussion of morphological characters are provided to distinguish the new species from *A. amoena*, *A. gracilescens*, *A. lessingiana* and *A. terrae-albae*.

**Key words:** *Artemisia* subg. *Seriphidium*, Compositae, new taxon, taxonomy, Xinjiang



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## Introduction

*Artemisia* L. (Asteraceae, Anthemideae), comprising ca. 500 herb and shrub species, is one of the largest genera in the tribe Anthemideae of the family Asteraceae (Bremer and Humphries 1993; Martin et al. 2003; Oberprieler et al. 2009; Vallès et al. 2011). Most *Artemisia* species have important medicinal, ecological and economic values (Duffy and Mutabingwa 2006; Vallès et al. 2011). Recent molecular phylogenetic studies of *Artemisia* have divided it into six subgenera, which are generally accepted: subg. *Artemisia*, subg. *Absinthium* (Miller) Less., subg. *Dracunculus* (Besser) Rydb., subg. *Tridentatae* (Rydb.) McArthur., subg. *Seriphidium* Besser ex Less and subg. *Pacifica* Hobbs & Baldwin (Malik et al. 2017, and references therein).

Subgenus *Seriphidium*, comprising ca. 130 species, is one of the most diverse subgenera and is mainly distinguished from the others by its multi-layered involucre bracts and homogamous capitula with bisexual flowers (Ling 1991). Subgenus *Seriphidium* grows mainly in arid and semi-arid regions in Central Asia and Northwest China (Malik et al. 2017). Thirty-one species and six varieties have been recorded in China (Ling et al. 2011).

During a field expedition in the north-eastern region of the Junggar Basin, located in Xinjiang, China, in 2020, a new population of *Artemisia* from Qinghe County was discovered. After consulting “Flora of China” (Ling et al. 2011) and other relevant literature (Poljakov 1961; Filatova 1966, 1986, 1993, 2007; Ling 1991; Liu 1992; Wei 1999), and after comparing the plants of this population with those of morphologically similar species (Besser 1841; Krascheninnikov 1930; Krascheninnikov and Iljin 1949; Poljakov 1954), we revisited this site at different times in 2021 and 2022 to carry out further observations and sampling with the aim of determining the taxonomic identity of the new population. Following additional morphological and molecular phylogenetic analyses, we concluded that it is different from all other known species of *Artemisia*. Hence, it is here described and illustrated as a new species: *A. qingheensis*.

## Material and method

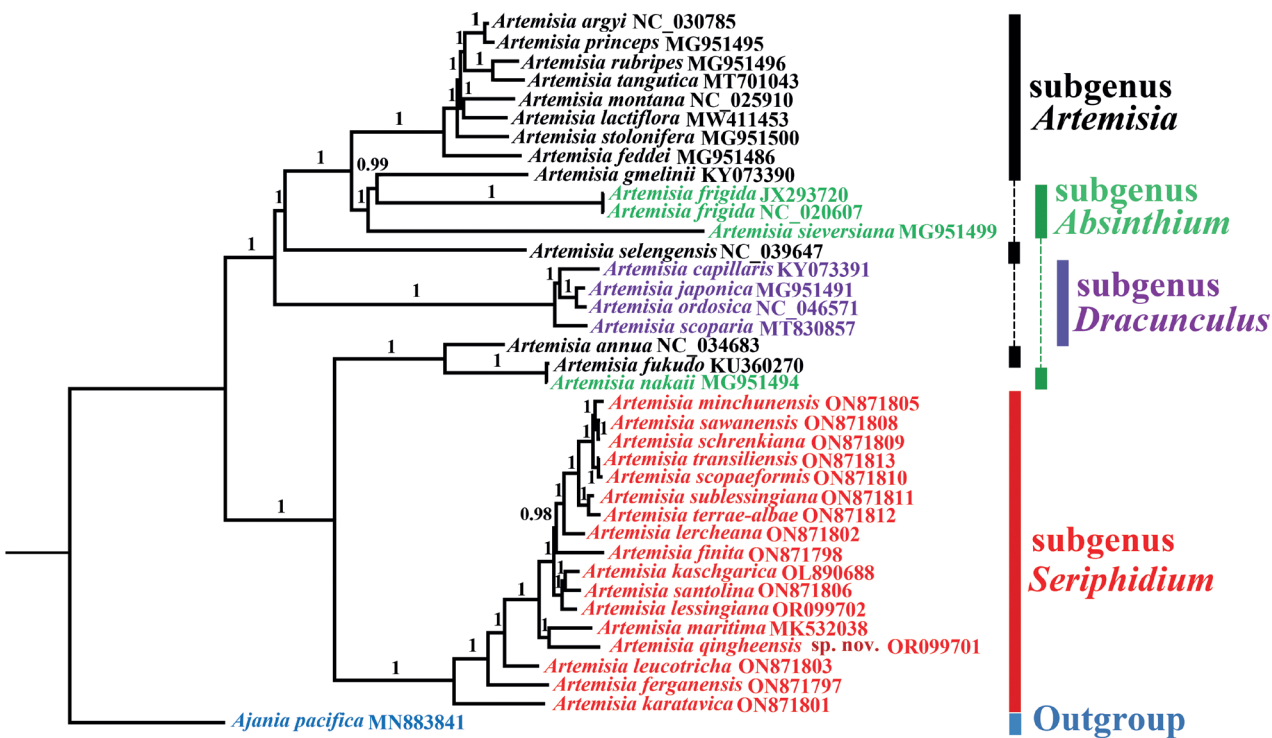
After examining the worldwide list of subg. *Seriphidium* species and their type specimens (Jin 2023), we critically examined specimens (including type material) of *A. gracilescens* Krasch. & Iljin, *A. lessingiana* Besser, and *A. terrae-albae* Krasch. in IBSC, LE, LECB, MW, PE, TK, TASH and XJBI. These species are morphologically most similar to the new taxon.

Chloroplast genomes of 36 *Artemisia* species from four subgenera, including 17 subg. *Seriphidium* species, were used for phylogenetic analysis (Fig. 1). The closely related species *Ajania pacifica* (Table 1) was used as the outgroup (Watson et al. 2002). We included 38 samples in our phylogenetic analyses, 36 of them were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>) and two were newly sequenced for this study: *A. lessingiana* and *A. qingheensis* (Table 1). For both, we extracted total genomic DNA from approximately 100 mg of silica gel-dried leaf material using a modified CTAB method (Doyle and Doyle 1987). Voucher specimens (*A. qingheensis*: No. jgz-099-4; *A. lessingiana*: No. jgz-20220529) were deposited in the Herbarium of the Xinjiang Institute of Ecology and Geography Chinese Academy of Sciences (XJBI). DNA extracts were fragmented for short-insert library construction (300 bp) and sequenced (2 × 150 bp paired-end reads) on DNBSEQ technology platforms at the Beijing Genomics Institute (Shenzhen, China). The raw reads were assessed and edited using FastQC 0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and Trimmomatic 0.35 (Bolger et al. 2014) was used to remove adapters and low quality bases. Finally, a ca. 3 G bp paired-end clean read was obtained for each sample. The clean data was assembled with GetOrganelle v. 1.7.1 (Jin et al. 2020). The complete circular assembly graph was checked using Bandage v. 0.8.1 (Wick et al. 2015). The finished plastid genomes were annotated with Geneious v. 9.1.7 (Kearse et al. 2012). The annotated plastid genomes were submitted to GenBank using Bankit (Table 1).

Genomes were aligned in MAFFT v. 7 (Katoh and Standley 2013). According to the Akaike Information Criterion (AIC), the most appropriate substitution model for the complete chloroplast genome sequence matrix, estimated using jModelTest2 (Darriba et al. 2012), was GTR + I + G. Bayesian Inference (BI) analysis was carried out using MrBayes v.3.2 (Ronquist et al. 2012), with the Markov Chain Monte Carlo simulations algorithm (MCMC) for 20,000,000 generations. The final trees were edited and visualised with FigTree v. 1.4.2 (Rambaut 2012).

**Table 1.** Samples information. Highlighted species newly were sequenced in this study.

Species	GenBank No.	Species	GenBank No.
<i>Ajania pacifica</i>	MN883841	<i>Artemisia minchunensis</i>	ON871805
<i>Artemisia annua</i>	NC_034683	<i>Artemisia montana</i>	NC_025910
<i>Artemisia argyi</i>	NC_030785	<i>Artemisia nakaii</i>	MG951494
<i>Artemisia capillaris</i>	KY073391	<i>Artemisia ordosica</i>	NC_046571
<i>Artemisia feddei</i>	MG951486	<i>Artemisia princeps</i>	MG951495
<i>Artemisia ferganensis</i>	ON871797	<b><i>Artemisia qingheensis</i> sp. nov.</b>	<b>OR099701</b>
<i>Artemisia finita</i>	ON871798	<i>Artemisia rubripes</i>	MG951496
<i>Artemisia frigida</i>	JX293720	<i>Artemisia santolina</i>	ON871806
<i>Artemisia frigida</i>	NC_020607	<i>Artemisia sawanensis</i>	ON871808
<i>Artemisia fukudo</i>	KU360270	<i>Artemisia schrenkiana</i>	ON871809
<i>Artemisia gmelinii</i>	KY073390	<i>Artemisia scopaeformis</i>	ON871810
<i>Artemisia japonica</i>	MG951491	<i>Artemisia scoparia</i>	MT830857
<i>Artemisia karatavica</i>	ON871801	<i>Artemisia selengensis</i>	NC_039647
<i>Artemisia kaschgarica</i>	OL890688	<i>Artemisia sieversiana</i>	MG951499
<i>Artemisia lactiflora</i>	MW411453	<i>Artemisia stolonifera</i>	MG951500
<i>Artemisia lercheana</i>	ON871802	<i>Artemisia sublessingiana</i>	ON871811
<b><i>Artemisia lessingiana</i></b>	<b>OR099702</b>	<i>Artemisia tangutica</i>	MT701043
<i>Artemisia leucotricha</i>	ON871803	<i>Artemisia terrae-albae</i>	ON871812
<i>Artemisia maritima</i>	MK532038	<i>Artemisia transiliensis</i>	ON871813



**Figure 1.** Phylogenetic tree inferred with Bayesian Inference (BI) analyses, using complete chloroplast genome sequences of 37 *Artemisia* species and *Ajania pacifica* as the outgroup. The numbers above the branches are Bayesian posterior probabilities. Coloured vertical lines indicate the subgenus classification of *Artemisia*.

## Results

The new species has multi-layered involucre bracts and homogamous capitula with bisexual flowers and therefore belongs to subg. *Seriphidium*. Its hardened needle-like leaves at maturity distinguish it from morphologically similar species: *A. gracilescens*, *A. lessingiana*, and *A. terrae-albae*. The results of the phylogenetic analyses showed that the new species is nested in a clade formed by subg. *Seriphidium* species (posterior probability (PP) = 1) and that it is the sister group (PP = 1) of *A. maritima* L. (Fig. 1). The new species is more distantly related to *A. lessingiana* and *A. terrae-albae*. In conclusion, the morphological characters and molecular data support the new species as distinct.

## Taxonomic treatment

### *Artemisia qingheensis* G.Z.Jin, sp. nov.

urn:lsid:ipni.org:names:77324802-1

Figs 2A–M, 3

**Type.** CHINA. Xinjiang: Qinghe County, Qinglong Lake, 46°40'N, 90°23'E, barren slopes, 1168.63 m alt., 7 October 2021, *Guangzhao Jin & Lei Yang jgz-17* (holotype: XJBI jgz-17-2, Fig. 3; isotypes: XJBI jgz-17-1, jgz-17-3 and jgz-17-4).

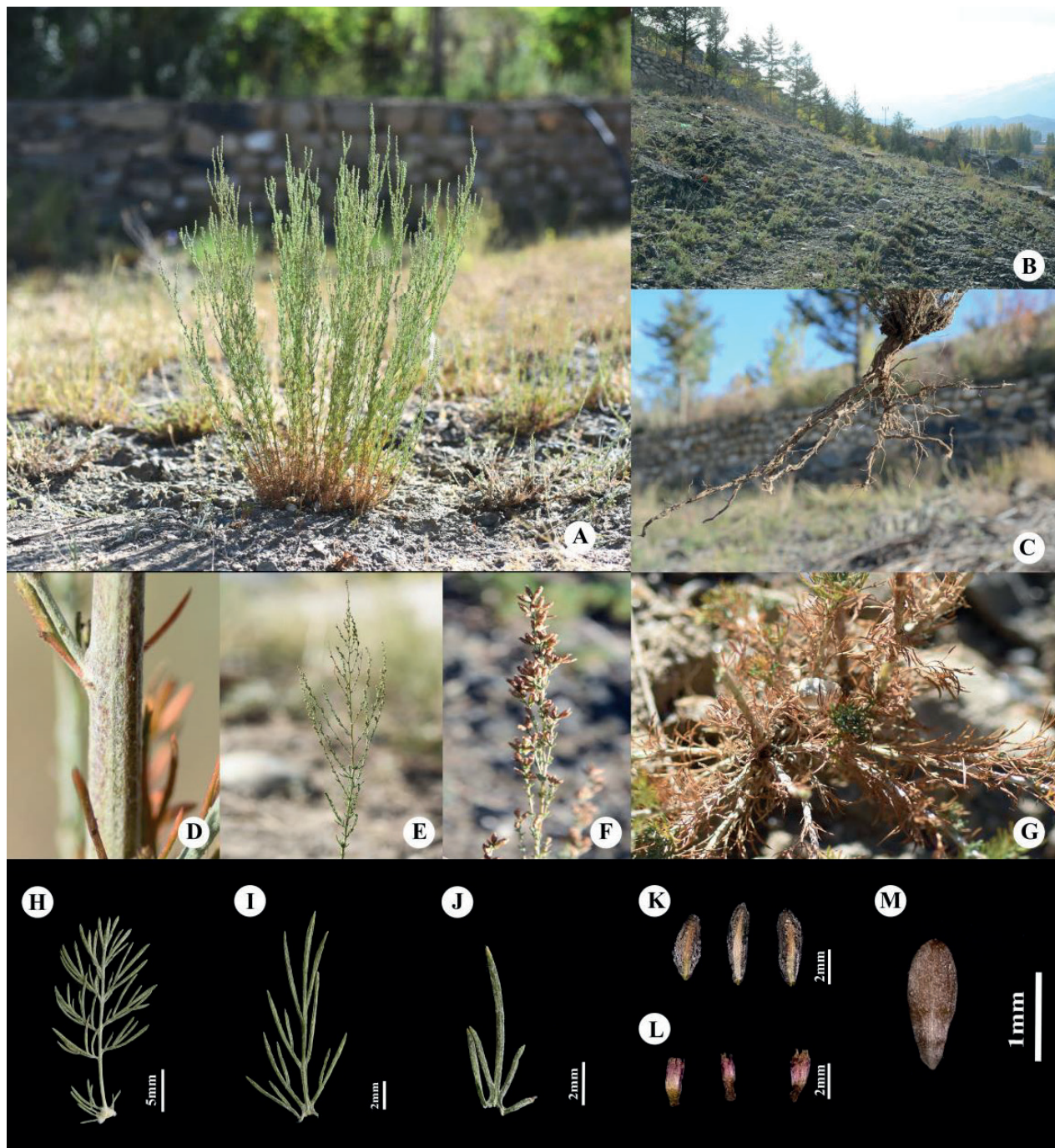
**Description.** Herbs perennial, 10–40 cm tall, with a thick rootstock, grey-white arachnoid pubescent, later glabrescent. Stems numerous, erect and often forming dense clumps, slightly woody proximally, herbaceous distally and with branches distally; branches 3–15 cm long, growing adnate to the stem, occasionally shorter branches. Lower stem leaves: petiole 0.3–1 cm; leaf blade elliptic, 0.5–1.5 cm long, 0.3–1 cm wide, 2-pinnatisect; primary segments 2–4 pairs; ultimate segments narrowly linear, 0.3–0.8 cm long and 0.2–0.5 mm wide, apex acute; petiole base with three-lobed or undivided pseudostipules with linear ultimate segments. Middle stem leaves: leaf blade narrowly ovate, 1 (or 2)-pinnatisect; ultimate segments narrowly linear, 0.5–1.5 cm long and 0.2–0.5 mm wide, apex acute; sessile, base with linear undivided pseudostipules. Upper leaves and leaf-like bracts: three-lobed or undivided, ultimate segments narrowly linear, 0.3–0.8 cm. All leaves greyish-white arachnoid pilose during the vegetative period, nearly glabrous at maturity; developing a needle-like texture at maturity. Inflorescence narrowly spicate or spicate-paniculate. Capitula sessile, numerous, ovoid, 2.5–4 mm long and 1.5–2.5 mm in diam., flowers opening centrifugally. Involucre bracts in 3–4 series, oblong or elliptic, 2–4 mm long and 1.5–2.5 mm wide, subglabrous, margin scarious; outer bracts ovate, inner larger, oblong-elliptic, all bracts with only sparse hairs at apex. Flowers bisexual, 3–6, 2–3.5 mm long and 1–2 mm wide, corolla tubular, purple-red or yellow; anthers linear, apical appendages of anthers subulate. Achenes with inconspicuous fine longitudinal lines, ovoid or obovoid, 1–1.5 mm long and 0.3–0.8 mm wide.

**Distribution and habitat.** *Artemisia qingheensis* is currently only known from Qinghe County, Xinjiang Province, China. It grows on barren slopes at altitudes of 1000 ~ 1500 m.

**Etymology.** *Artemisia qingheensis* is named after its type locality, Qinghe County, Xinjiang Province, China.

**Phenology.** Flowering and fruiting from early September to late October.





**Figure 2.** *Artemisia qingheensis* G. Z. Jin (photographs of the type collection) **A** habit **B** habitat **C** roots **D** stem indumentum **E** compound inflorescence **F** capitula **G** all leaves hardening when mature **H** lower stem leaf **I** middle stem leaf **J** upper leaf **K** involucral bracts **L** florets **M** achene.

**Vernacular name.** 青河绢蒿 (Chinese pinyin: qīng hé juàn hāo). This name is derived from the Chinese name of the type locality.

**Conservation status.** Although field surveys have been conducted in the north-eastern region of the Junggar Basin over a period of three years, we have only discovered three populations of *Artemisia qingheensis* in Qinghe County. Unfortunately, as these populations are next to roads and agricultural land, habitat quality is continuously declining due to man-made interference (e.g.



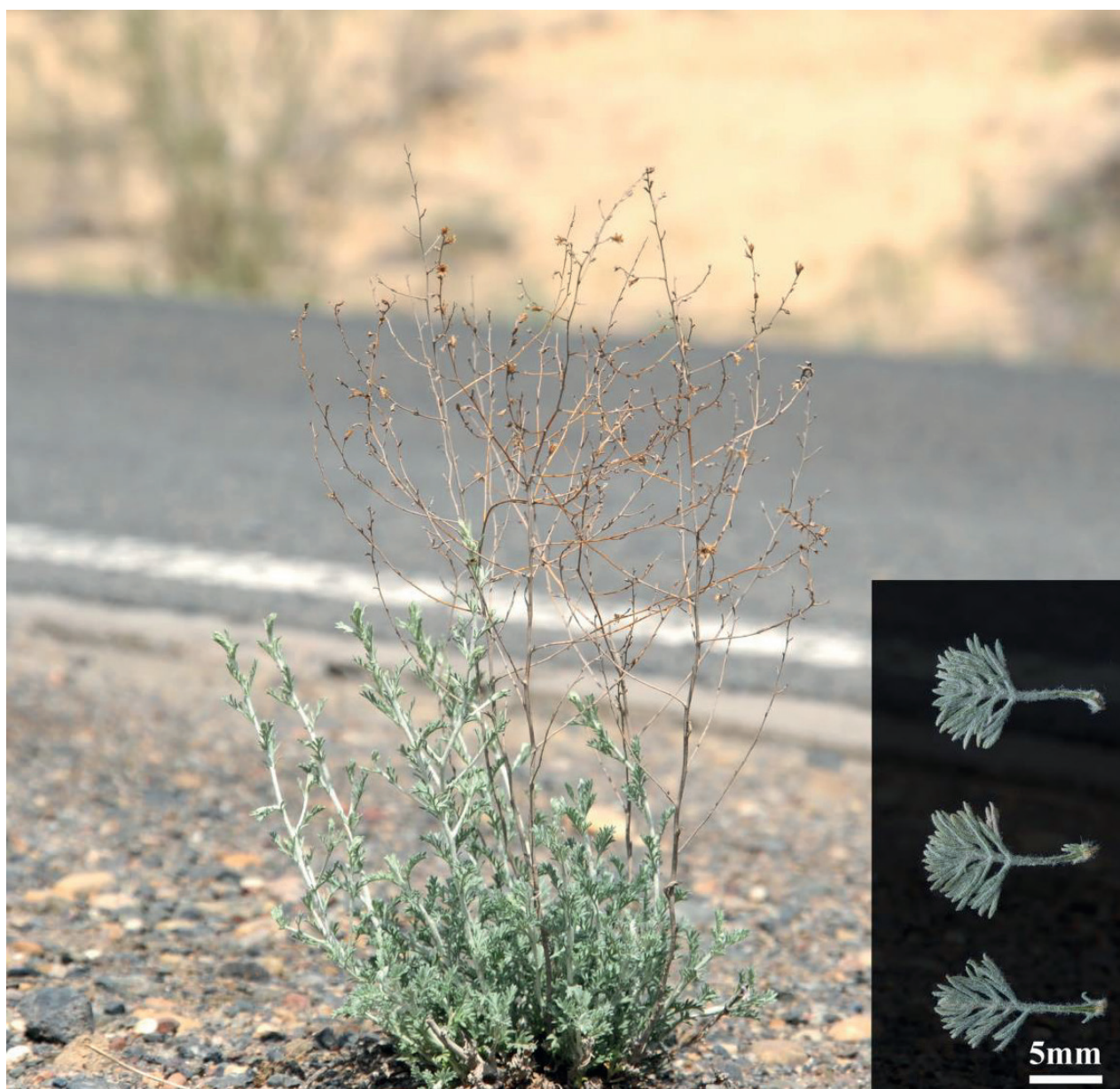


Figure 3. Holotype sheet of *Artemisia qingheensis* sp. nov.



grazing, cultivation and landscape engineering). The possible deterioration of its habitat and the restricted distribution of this species threaten its survival. According to the Guidelines for using the IUCN Red List Categories and Criteria (IUCN 2022), the conservation status of *A. qingheensis* should be assessed as Critically Endangered (CR, B1ab).

**Phylogenetic position and similar species.** *Artemisia qingheensis* belongs to *Artemisia* subg. *Seriphidium* because its involucre is multi-layered, its capitula are homogamous and contain 3–6 bisexual flowers, and these open centrifugally. In addition, our phylogenetic analysis confirmed the inclusion of this new species in subg. *Seriphidium*. *Artemisia qingheensis* is similar to *A. terrae-albae* in its habit, leaf shape, petiole length, capitula shape and corolla colour. However, it can be clearly distinguished from *A. terrae-albae* (Fig. 4)



**Figure 4.** *Artemisia terrae-albae* (voucher specimen: China. Xinjiang: Mongolian Autonomous County of Hoboksar, 379.32 m alt., 8 May 2022, Guangzhao Jin 20220508, XJBI). Inset: Lower stem leaves.

**Table 2.** Morphological comparisons between *Artemisia qingheensis* sp. nov. and morphologically similar species.

Character	<i>A. qingheensis</i>	<i>A. terrae-albae</i>	<i>A. lessingiana</i>	<i>A. gracilescens</i>	<i>A. amoena</i>
Stem	10–40 cm	15–30 cm	18–40 cm	15–30 cm	10–28 cm
Branch	3–15 cm; growing adnate to the stem	3–5 cm; obliquely upward or spreading	3–10 cm; growing adnate to the stem	3–10 cm; growing adnate to the stem	2–3 cm; growing adnate to the stem
Leaf texture	leaves hardening when mature	leaves slightly soft when mature	leaves slightly hardening when mature	leaves slightly soft when mature	leaves slightly soft when mature
Lower leaf	petiole: 0.3–1 cm; leaf blade elliptic, 2-pinnatisect; lobes 2–4 pairs;	petiole: 0.3–1 cm; leaf blade ovate; 1- or 2-pinnatisect; lobes 3–4 pairs	petiole: 2–5 cm; leaf blade oblong-ovate, 1- or 2-pinnatisect; lobes 3–5 pairs	petiole: 0.3–0.5 cm; leaf blade triangular-ovate, 2- or 3-pinnatisect; lobes 2–3 pairs	petiole: 4–8 cm; leaf blade ovate, 1- or 2-pinnatisect; lobes 3–5 pairs
Middle stem leaf	1-pinnatisect	1-pinnatisect	1- or 2-pinnatisect	1- or 2-pinnatisect	1-pinnatisect
Uppermost leaf	three-lobed or undivided	1-pinnatisect	undivided	1- or 2-pinnatisect	undivided
Capitula	ovoid	ovoid	ellipsoidal-ovoid	ellipsoidal	ovoid
Florets	3–6	4–5	5–6	2–5	4–5
Corolla colour	purple-red or yellow	purple-red or yellow	purple-red or yellow	yellow	purple-red or yellow

because its branches grow adnate to the stem (vs. obliquely upward or spreading) and its leaves harden when maturing (vs. leaves slightly soft when mature). This new species is also relatively easy to distinguish from *A. lessingiana* by its shorter petioles 0.3–1 cm (vs. 2–5 cm) and ovate (vs. oblong-ovate) leaf blade.

The new species is similar to *A. gracilescens* in its habit and narrowly spicate or spicate-paniculate inflorescences. However, it is mainly distinguished from *A. gracilescens* by its 2-pinnatisect lowermost leaves and ovate leaf blade (vs. 2- or 3-pinnatisect and leaf blade triangular-ovate), middle stem leaves 1-pinnatisect (vs. usually 1- or 2-pinnatisect), uppermost leaves three-lobed or undivided (vs. 1- or 2-pinnatisect), all leaves hardening when maturing (vs. leaves slightly soft when mature) and ovoid capitula (vs. ellipsoid). Furthermore, this species is also somewhat similar to *A. amoena* Poljakov in its habit and capitula, which are borne in spikes or narrow panicles, but is distinguished by its shorter petioles 0.3–1 cm (vs. 4–8 cm), longer stem branches: 3–15 cm vs. 2–3 cm, and the hardening of the leaves when these mature (vs. leaves slightly soft when mature).

The morphological differences among *A. qingheensis*, *A. terrae-albae*, *A. lessingiana*, *A. gracilescens* and *A. amoena* are summarised in Table 2.

**Additional specimens examined (paratypes).** CHINA. Xinjiang: Qinghe County, Wolf Garden, 1184.85 m alt., 15 October 2020, *Guangzhao Jin & Sheng Zhang jgz-099* (XJBI); Southern suburb of Qinghe County, 1116.96 m alt., 9 October 2021, *Guangzhao Jin & Lei Yang jgz-25* (XJBI).

### Key to *Artemisia qingheensis* and similar species

- 1 Petiole of the lower leaves 0.3–1 cm long ..... **2**
- Petiole of the lower leaves 2–8 cm long ..... **3**
- 2 Branches obliquely upward or spreading, lower leaf 1- or 2-pinnatisect ..... **A. terrae-albae**
- Branches growing adnate to the stem, lower leaf 2- or 3-pinnatisect ..... **4**



- 3 Lower leaf blade oblong-ovate and capitula ellipsoidal-ovoid, branch length 3–10 cm ..... ***A. lessingiana***
- Lower leaf blade ovate and capitula ovoid, branch length 2–3 cm .....  
..... ***A. amoena***
- 4 Leaves harden when maturing, lower leaf blade elliptic ..... ***A. qingheensis***
- Leaves slightly soft when mature, lower leaf blade triangular-ovate .....  
..... ***A. gracilescens***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Formal analysis: SM, LWJ, SF. Writing – original draft: JGZ. Writing – review and editing: FY, WZB.

## Author ORCIDs

Guang-Zhao Jin  <https://orcid.org/0000-0002-2457-2711>

Mariya Sheludyakova  <https://orcid.org/0000-0001-9504-6033>

Wen-Jun Li  <https://orcid.org/0000-0002-2932-0783>

Feng Song  <https://orcid.org/0000-0002-1332-312X>

Zhi-Bin Wen  <https://orcid.org/0000-0003-1727-2996>

Ying Feng  <https://orcid.org/0000-0002-0817-6751>

## Data availability

All of the data that support the findings of this study are available in the main text.

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