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Living in extreme environments: distribution of *Lycium humile* (Solanaceae), an endemic halophyte from the Altiplano-Puna region, South America

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Abstract

Very few Solanaceae species are able to grow in saline soils; one of them is *Lycium humile*. This species is endemic to the Altiplano-Puna region (Central Andes, South America) where there are multiple extreme environmental conditions such as hypersaline soils. Here we present an updated description and distribution of *L. humile* including its new record for Bolivia at the edges of "Salar de Uyuni", the largest salt flat in the world; we discuss its ecological role in saline environments by analyzing soil salinity and cover-abundance values of the studied sites. According to IUCN criteria, we recommend a category of Least Concern for *L. humile*, but the growing development of lithium mining in saline environments of the Altiplano-Puna region may potentially threaten exclusive communities.

Keywords

Andes, Argentina, Bolivia, Chile, conservation status, new record, saline soil, salt-tolerant

Introduction

Lycium L. is the only member of tribe Lycieae (Atropina clade, Solanaceae; Hunziker 1977; Levin et al. 2011; Särkinen et al. 2013) and comprises nearly 90 woody species commonly found in arid, sub-arid and even saline environments (Bernardello 2013). It is a cosmopolitan genus with its greatest diversity occurring in extratropical areas, in southern South America, southern Africa, and southwestern North America (Stiefkens et al. 2020). In South America, the shrub endemic to the Altiplano-Puna region *Lycium humile* Phil. preferentially inhabits saline soils with a distribution circumscribed to Argentina and Chile (Bernardello 1986, 2013).

The Altiplano-Puna region of the Central Andes is considered a cold desert, with high elevation (3700 m average elev.), extreme temperatures (which can reach –30 °C) and daily temperature fluctuations, low and irregular precipitation (even < 50 mm per year), high evaporation and UV radiation, and low nutrient availability (Alonso 2013; Malatesta et al. 2016). In addition, this region is characterized by numerous salt flats and shallow saline lakes, called salars, with very high salt concentrations (Alonso and Rojas 2020). The edges of these salars, which have rather wet soils in some periods, support specific plant communities comprising *L. humile* together with other species such as *Distichlis humilis* Phil., *Distichlis spicata* (L.) Greene and *Frankenia triandra* J.Rémy (Cabrera 1957; Luebert and Gajardo 2000; Tálamo et al. 2010; Carilla et al. 2018). Thus, *L. humile* is one of the few vascular plant species growing at these edges since, even though the water table is near to the surface, plants need to cope with high salinity (> 200 mM NaCl) that promote physiologically dry soils. Halophytes, like *L. humile*, are the only plants able to grow in these environments because they have different mechanisms that enhance root water uptake by decreasing their water potential (Palchetti et al. 2020).

As part of a study of salt tolerance in South American Solanaceae species, we carried out extensive explorations in the Altiplano-Puna region to check the presence and abundance of Solanaceae, in extreme saline environments. Therefore, the aims of this study are: 1) to update the distribution and description of *L. humile*, 2) to document a new record for Bolivia, and 3) to discuss the ecological role of this species in saline environments of the Altiplano-Puna region.

Methods

Field trips

Field collections were performed in Argentina, Bolivia and Chile during 2015 to 2019, comprising expeditions carried out in Jujuy, Salta and Catamarca provinces (Argentina), Antofagasta region (Chile) and Potosí department (Bolivia), between 2000 and 4000 m elevation.

Morphology, vernacular names and uses

Species description was based on *Lycium* monograph (Bernardello 1986) and field observations of 16 populations. Phenology was recorded by observation of plants in the field and herbaria specimens; plant-consuming animals were also annotated. Vernacular names and uses are based on the following authors, Aldunate et al. (1981), Bernardello (1986, 2013), Villagrán and Castro (2003), Philippi (2008), Medina (2012) and Gamboa Fuentes (2014).

Distribution

Distribution was plotted using QGIS 2.8 (QGIS Development Team 2018), based on data from *Lycium* monograph (Bernardello 1986), revised, digitized or original herbarium specimens (BA, BAA, CONC, CORD, E, EIF, F, K, L, LIL, LP, LPB, MA, MO, SGO, SI; acronyms following Thiers 2021), GBIF (2020) database, and data from field trips. Non-georeferenced localities were checked by the authors. No *L. humile* voucher was found in the Bolivian herbaria LPB and USZ. Analyzed data are available as supplementary information (see Suppl. material 1: Palchetti et al. SF1).

Habitat and ecology

Local abundance of *L. humile* and different edaphic variables (pH, electric conductivity [EC], Na^+, Cl^-, Ca^{2+} and Mg^{2+}) were evaluated in seven sites of Argentina, where geographic coordinates were recorded; voucher specimens were collected and deposited at CORD herbarium. Local abundance was calculated as estimated *L. humile* cover percentage in thirty 10 m × 10 m plots at each site. Fifteen random soil sub-samples at 0–20 cm depth were taken from each site and pooled as a composite sample for saturation extract analysis (Richards 1954) performed by LabSA, FCA-UNC (Córdoba, Argentina).

Conservation status

The conservation status was assessed applying the IUCN (2019) criteria. The extent of occurrence and area of occupancy were calculated using a convex hull in QGIS 2.8 (QGIS Development Team 2018) and the Geospatial Conservation Assessment Tool, GeoCAT (Bachman et al. 2011), respectively.

Taxonomic treatment

***Lycium humile* Phil., Fl. Atacam. 43. 1860.**
Fig. 1

Type. Chile. [Antofagasta: Province of Antofagasta], Ad aquas [Profetas], December [1853]–January [1854], *R.A. Philippi s.n.*, pro parte (lectotype, inadvertently designated as 'type' by Muñoz Pizarro 1960, pg. 116: SGO! [SGO000004448, acc. # 055683; Fig. 2], isolectotype: W n.v. [cited as isosyntype by Bernardello (1986)].

Description. Dwarf shrubs, prostrate or ascending, up to 20 cm high, often forming dense and extensive mats, over 5 m. Subterranean organs well-developed with tangled and woody roots and rhizomes. Stems grayish-yellow, unarmed, glabrous, much branched, with slightly arched aerial branches and some stoloniferous branches. Leaves alternate or fasciculate, succulent, obovate or spatulate, light green, 2–16 mm long, 1–4 mm wide, glabrous or with occasionally glandular trichomes, sessile. Flowers 5-merous (rarely 4- or 6-merous), solitary, perfect; calyx tubular, zygomorphic, glabrous, bilabiate or irregularly toothed, the tube 3–5 mm long, the lobes sub-triangular, 1–2 mm long, ciliate at the margins; corolla white, sometimes with purple lines within, narrowly infundibuliform to tubular, barely zygomorphic, glabrous outside, the tube 12–15.5 mm long, 3–3.5 mm wide, glabrescent near the insertion of the stamens inside, the lobes 2.5–3.5 mm long, 2.5–4 mm wide, ovate, with sparse cilia on the edge; stamens inserted at 2/3 from the base, at different levels, filaments unequal in length, some exserted, others included or barely exserted, with few simple hairs at their bases; ovary with prominent red-orange nectary at the base, style exserted or scarcely exserted. Berry subglobose, ca. 7–8 mm in diameter, blackish; seeds irregular, polyhedral, pale brown, up to 25 per fruit, the episperm smooth, without marked cells.

Phenology. Flowering late September-March; fruiting late December-April.

Vernacular names and uses. Bálsamo finito, ch'ampita (Villagrán and Castro 2003); jume (Philippi 2008; Medina 2012); sacha uva or sachauva (Villagrán and Castro 2003; Bernardello 2013); tomatillo (Villagrán and Castro 2003; Medina 2012); uvilla (Villagrán and Castro 2003); walcha (Aldunate et al. 1981; Villagrán and Castro 2003); waycha (Villagrán and Castro 2003; Medina 2012), wicha (Villagrán and Castro 2003; Medina 2012; Gamboa Fuentes 2014). This species has been reported as fodder (Aldunate et al. 1981; Villagrán and Castro 2003; Gamboa Fuentes 2014) and probably has medicinal uses associated with rituals to remove evils (Villagrán and Castro 2003; Medina 2012). Fruits have been reported as edible (Philippi 2008) and may have tinctorial properties (Bernardello 1986; Villagrán and Castro 2003; Medina 2012; Gamboa Fuentes 2014).

Distribution. *Lycium humile* is distributed in the Andean region, southern South America (Argentina, Bolivia and Chile), at 2300–4100 m elevation (Fig. 3). In northwestern Argentina, it inhabits sites at 3000–4000 m elev. in the provinces of Catamarca (Antofagasta de la Sierra and Belén departments) and Salta (Los Andes department), and there is also a specimen collected in Jujuy province, Tumbaya department (Ancibor and Ruthsatz 65; BAA). In northeastern Chile, it occurs throughout the species' elevation range, in the regions of Antofagasta (Antofagasta and El Loa provinces) and Atacama (Copiapó and Chañaral provinces). In southwestern Bolivia, it grows in Potosí department, at the edges of Salar de Uyuni at ca. 3600 m elev. Previous publications (Bernardello 1986; Rodriguez et al. 2018; Zuloaga et al. 2019) have cited the species in the Chilean region of Tarapacá, however after studying herbarium material from Chile we consider its presence on this administrative region as doubtful (see discussion).

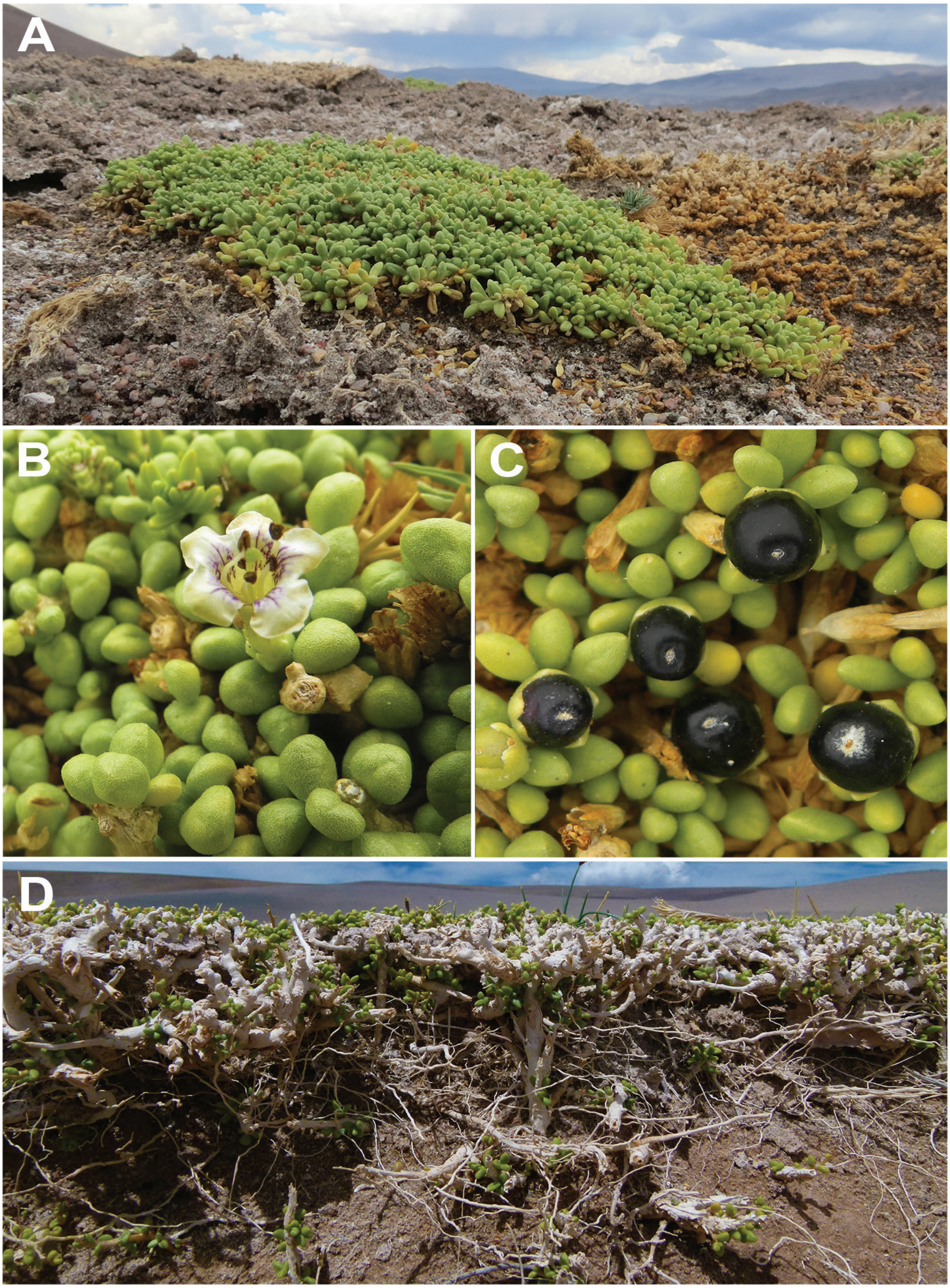

Figure 1. *Lycium humile* **A** overview of a plant in its habitat **B** flower **C** fruits **D** plant architecture.

Habitat and ecology. The species preferentially grows in saline clay soils, and less frequently in sandy soils. It is commonly found in saline mudflats of salars (Fig. 4). In the analyzed sites of the Altiplano-Puna region where *L. humile* grows, soils showed

Figure 2. Lectotype of *Lycium humile* Phil. (SGO 055683). Digital image by courtesy of the Museo Nacional de Historia Natural.

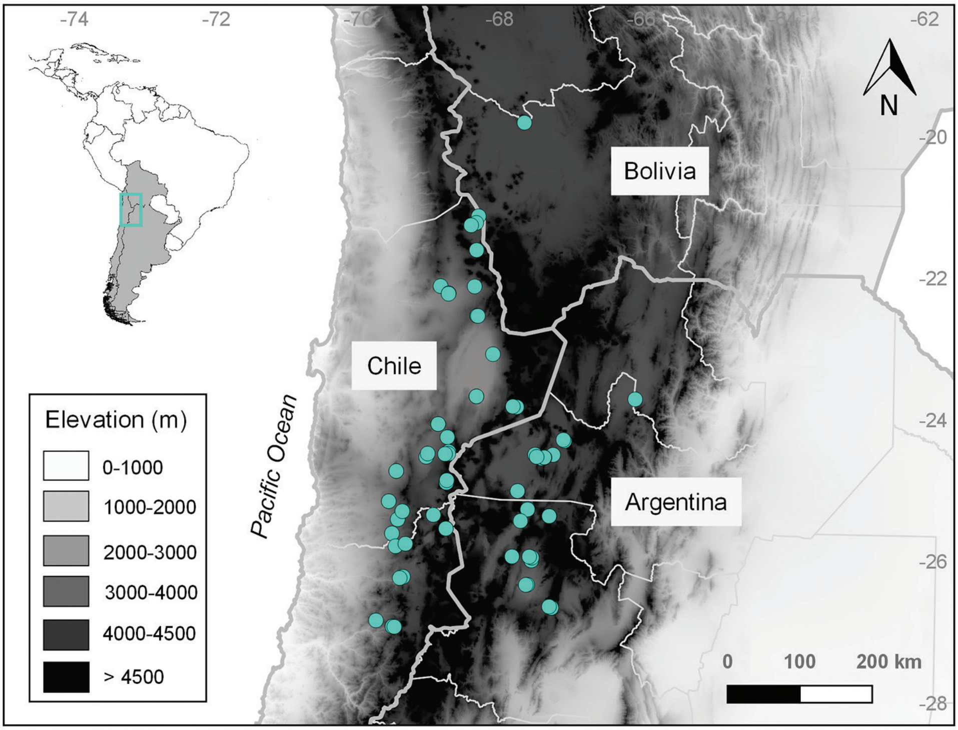

Figure 3. Distribution map of *Lycium humile* (light-blue circles) in the Altiplano-Puna region.

very high salinity reaching high EC (~300 dS/m) and Na⁺ (~30 g/L) values, and low vegetation cover, with an average bare soil of 55 (SD 20.9) %. *Lycium humile* showed an average cover percentage of 22.9 (SD 11) % with a maximum value of ~40% in Salar de Antofalla (Catamarca, Argentina, Table 1). The species occurs in plant communities with low species richness, along with Amaranthaceae, *Nitrophila australis* Chodat & Wilczek and *Salicornia pulvinata* R.E.Fr.; Asteraceae, *Baccharis acaulis* (Wedd. ex R.E.Fr.) Cabrera; Frankeniaceae, *Frankenia triandra*; Juncaginaceae, *Triglochin concinna* Burtt Davy; and Poaceae, *Distichlis humilis* and *D. spicata*.

Conservation status. According to the IUCN criteria (IUCN 2019), a category of Least Concern (LC; B, C and D criteria) is recommended for *Lycium humile*, based on its extent of occurrence of 190,477 km^2, area of occupancy of more than 2,000 km^2, and large population size with more than 10,000 mature individuals observed. Large mining operations in the Andes may produce a continuing decline of area, extent and/or quality of habitat (Schiaffini 2013; Liu et al. 2019) which could adversely affect some subpopulations located at specific sites of the salars. But considering its widespread occurrence and healthy populations found in several locations within protected areas in Argentina (Laguna Blanca Biosphere Reserve, Lagunas Altoandinas y Puneñas de Cata-

Table 1. Edaphic variables of saturated paste extract, bare soil and soil covered by *L. humile* for each study site. Specimen vouchers were deposited at CORD.

Site	**Geographical coordinates**	**Specimen voucher**	**Edaphic variables**						**Bare soil (%)**	**Soil covered by *L. humile* (%)**
			Cl$^-$ (mg/L)	**Ca^{2+} (mg/L)**	**Mg^{2+} (mg/L)**	**Na$^+$ (mg/L)**	**pH**	**EC (dS/m)**		
Laguna Pasto Ventura	26°44.0133'S; 67°9.4433'W	Barboza G.E. et al. 4725	1134.0	48.0	14.0	2144.1	7.4	99.2	15.6	16.9
Carachi Pampa	26°26.0633'S; 67°29.38'W	Barboza G.E. et al. 4304	14512.4	1500.0	1518.8	12000.0	9.8	112.5	70.6	17.7
Salar de Antofalla	25°31.93'S; 67°34.855'W	Barboza G.E. et al. 4313	23181.5	480.0	60.8	14875.0	8.7	160.0	52.8	39.2
Salar del Hombre Muerto	25°27.8017'S; 67°10.37'W	Barboza G.E. et al. 4309b	31311.0	1800.0	741.2	17125.0	8.3	182.5	55.5	34.4
Salar Tolar Grande	24°35.575'S; 67°23.4783'W	Barboza G.E. et al. 4749	3200.0	25.0	4.0	20281.7	7.9	203.3	61.5	12.8
Los Colorados	24°35.4217'S; 67°8.2083'W	Barboza G.E. et al. 4347	45191.5	440.0	2940.3	25375.0	8.5	290.0	82.0	28.1
Salar del Diablo	24°37.8667'S; 67°15.7667'W	Barboza G.E. et al. 4349	46576.0	580.0	437.4	29375.0	8.0	290.0	47.2	11.2
Total average			23586.6	696.1	816.6	17310.8	8.4	191.1	55.0	22.9
Standard deviation (SD)			18516.6	690.5	1082.3	8977.8	0.7	76.8	20.9	11.0

marca Ramsar Site and Los Andes Provincial Reserve) and Chile (Llullaillaco National Park), this activity may not represent a current threat to this species at regional scale.

New country record. Bolivia. Potosí: Dept. Daniel Campos, Uyuni, entrando a Coqueza por el propio salar de Uyuni; 9°54.2333'S; 67°37.3667'W; 3665 m elev.; 13 Dec. 2017; G.E. Barboza 4868 (CORD00086059; LPB).

Taxonomic note. R.A. Philippi (1860) described *L. humile* based on his plant collections from his trip to the Atacama Desert during the summer of 1853–1854. The protologue mentioned three localities in Antofagasta, Chile: "primum prope Cachiyuyal 25°22' lat. m. 4000 p.s.m. legi, deinde ad aquam Profetas dictam 24°45' lat. m., 9000 p.s.m., in valle Chaco 25°15' lat. m., 8500 p.s.m.". Of these three syntypes, Muñoz Pizarro (1960) found a single sheet at SGO (acc. # 055683; Fig. 2) that matches the species and the protologue, being designated by him as "type" of *L. humile*. We consider his citation as an inadvertent lectotypification (see article 7.11 in Turland et al. 2018).

The sheet SGO 055683 bears two labels with different localities, collectors and dates. One label reads "*Lycium humile* Ph., incolis Jume, Ad aquas [Profetas] deserti frequens, Decembri. Januario, Ph.", it agrees with the protologue. The other label reads "*Lycium humile* Ph., Juntas arriba, januar 1885, F. Ph.", which belongs to a collection done by F. Philippi after the description of the species (1860) and is therefore not original material. All material mounted on this single herbarium sheet belongs to *L. humile* and it is impossible to recognize the branches which were gathered by R.A. Philippi. In fact, the sheet also has a label written by Muñoz Pizarro indicating that there is a mix ('mezcla!'), which likely refers to mixed material.

After the work of Muñoz Pizarro, Bernardello (2013) selected as the lectotype of *L. humile* the specimen SGO 055684, whose label reads "Encantada, Chaco". Although

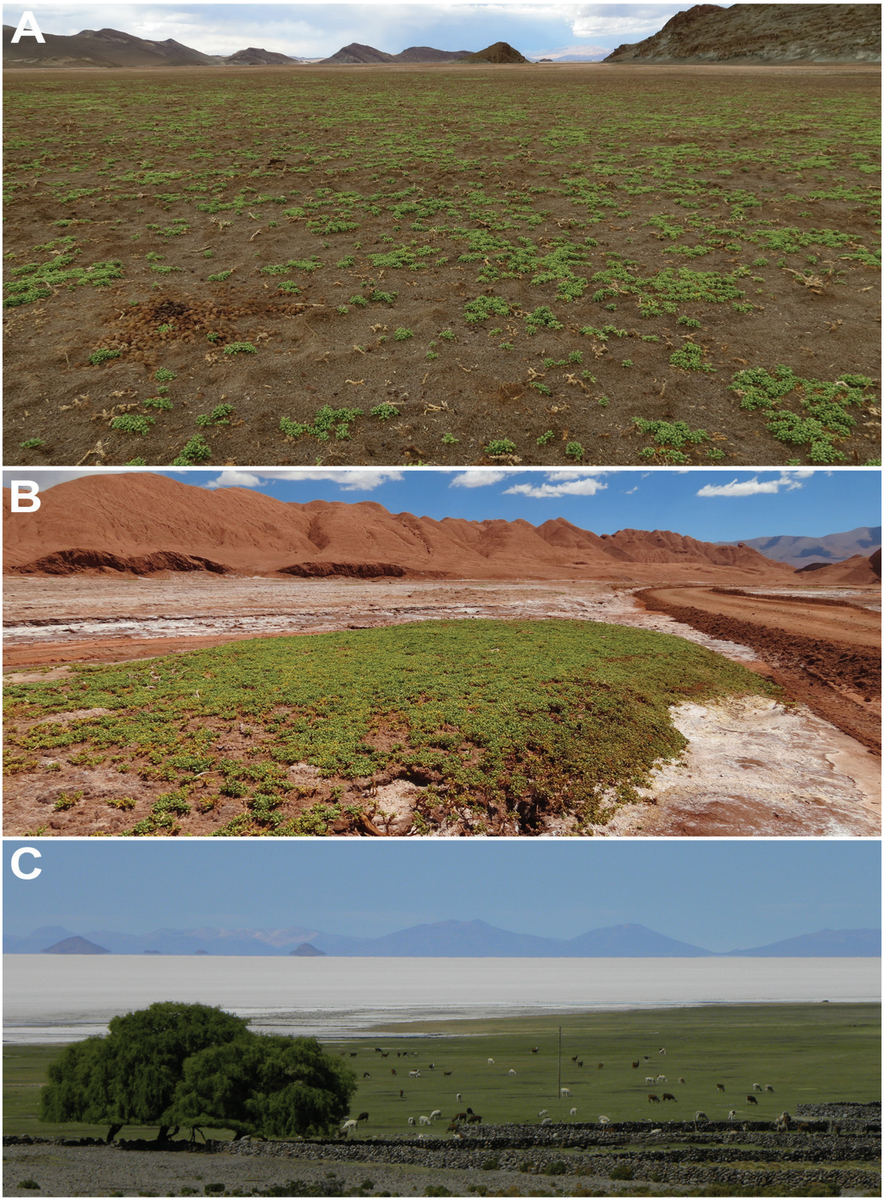

Figure 4. Saline environments of the Altiplano-Puna region (South America) in which *Lycium humile* grows **A** Salar del Hombre Muerto (Catamarca, Argentina) **B** Los Colorados (Salta, Argentina) **C** Salar de Uyuni (Potosí, Bolivia).

the second locality "Chaco" is mentioned in the protologue, this specimen cannot be considered as lectotype because it was collected by K. Reiche (inferred by the handwriting of the collector; Muñoz-Schick et al. 2012) after the publication of the protologue and therefore it is not original material (see article 9.4 in Turland et al. 2018).

Discussion

Lycium humile is easily identified by the prostrate or even mat-forming growth habit and very succulent leaves, and during summer, by the numerous white flowers and blackish berries, which also grow almost on the soil surface. In addition, aerial organs represent a smaller part of the total plant architecture than the very well-developed subterranean organs which may help to reduce water loss (Palchetti et al. 2021).

Lycium is the Solanaceae genus with the highest number of reported taxa growing in saline environments on a global scale (eHALOPH 2021) within the family. In this study, we document that *L. humile* is widely distributed in the Altiplano-Puna region and grows almost exclusively in saline soils. Despite phytosociological studies done in salars of the Bolivian Altiplano (Navarro 1993), *L. humile* was not previously recorded in this area. Our new record not only constitutes the northernmost point of the species distribution range but also offers a valuable contribution to the global knowledge of the halophytic vegetation, since this species grows at the edges of northern Salar de Uyuni. This is the largest salt flat in the world and has amazingly extreme environmental conditions, such as hypersalinity, intense UV irradiance, high lithium concentration and low precipitation, that promote the development of extreme halophiles (Haferburg et al. 2017; Vargas-Cuentas and Roman-Gonzalez 2017).

Further botanical explorations are encouraged to increase the collection in Bolivia and also, to verify if the species grows in the region of Tarapacá, which would represent its northern limit of distribution in Chile. This is because the only specimens related to this area are probably duplicates (K000586026 and CORD00021076) and were labelled without a precise locality. The label of the specimen at K reads "*Lycium humile* Ph. Chili, Com. R.A. Philippi 2/1888. Tarapacá", while the label of the specimen at CORD states "*Lycium humile* Phil. Chile. Prov. Tarapacá: Tarapacá. Leg. R.A. Philippi". These collections were probably collected by F. Philippi and C. Rahmer, during the expedition to the province of Tarapacá in 1885 and distributed (communicated) by R.A. Philippi in 1888. Several specimens from this trip were distributed to foreign herbaria, only with the name of the species and "Tarapacá", despite the trip covering the High Andes from Copiapó to Pica, going through Antofagasta de la Sierra (Argentina). As there are no other specimens from Tarapacá region, the presence of the species in this region is doubtful. A similar situation occurs in Jujuy province (Argentina), where the only specimen was collected 50 years ago and, despite our expeditions, we have not found the species in the surrounding area of the specimen collection site or in other saline environments of this province such as Salinas Grandes and Salar de Olaroz.

In the studied sites, *L. humile* was one of the dominant species, reaching covers higher than 35% in sites with high bare soil (> 50%). This evidences its essential role as primary producer in this extreme ecosystem since *L. humile* contributes greatly to the composition and structure of vegetation in saline environments of the Altiplano-Puna region. In fact, *L. humile* is the characteristic species of an association and alliance described in Chile (Luebert and Gajardo 2000). At the same time, the branches and leaves of *L. humile* provide material for the construction of shelters for rodents of the genus

Ctenomys (Rodentia, Ctenomyidae), and food for lizards of the genus *Liolaemus* (Squamata, Liolaemidae), that feed on the berries, facilitating the dispersal and germination of seeds (Abdala et al. 2021). Other South American animals that feed on *L. humile*, e.g. camelids (such as "vicuña" and "llama") consume aerial organs while birds (like *Muscisaxicola*; Passeriformes, Tyrannidae) eat the berries (pers. obs.); in this sense seed dispersal by birds has been previously reported (Teillier 2000). Thus, beyond our suggested conservation status category of Least Concern for *L. humile*, saline environments of the Altiplano-Puna region should be considered as priority areas for conservation, since the growing development of mining activities may potentially threaten exclusive plant communities (pers. obs.). Especially with the rise of lithium mining during the last eight years due to the increasing demand for lithium-ion batteries (Eftekhari 2017; Kim et al. 2019), which could affect some *L. humile* populations (e.g. in Antofalla, Catamarca, Argentina and in Salar de Atacama, Antofagasta, Chile). In fact, lithium mining has led to socio-environmental conflicts and even it has been proposed as one of the major causes of local environmental degradation (Schiaffini 2013; Liu et al. 2019).

Halophytes represent fewer than 2% of the Angiosperms (Bromham et al. 2020) and within Solanaceae, barely 1.7% of total species are halophytes (Moray et al. 2015). However, there is a growing interest in salt-tolerant plants, not only because saline environments are major contributors to biodiversity (Flowers and Muscolo 2015), but also because these plants represent a powerful tool to understand salt tolerance, a potential source of salt-responsive genes and promoters (Jha et al. 2019), and an alternative source of food, oil raw material, bioenergy and secondary metabolites (Shamsutdinov et al. 2017; Nikalje et al. 2019). Because of the high economic importance of Solanaceae as food and drug sources (e.g. potato, tomato, eggplant, pepper, tobacco), the understanding of their mechanisms of salinity tolerance are fundamental for genetic improvement. Thus, the key role of *L. humile* in saline environments of the Central Andes and its high salt tolerance make it a potential experimental model to study tolerance to salinity and responses to multiple stresses in Solanaceae.

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Supplementary material 1

Occurences of *Lycium humile* in South America

Authors: María Virginia Palchetti, Juan José Cantero, Vanezza Morales-Fierro, Gloria E. Barboza, Andrés Moreira-Muñoz

Data type: occurences

Explanation note: Living in extreme environments: distribution of *Lycium humile* (Solanaceae), an endemic halophyte of the Altiplano-Puna region, South America.

Link: https://doi.org/10.3897/phytokeys.185.71377.suppl1

PhytoKeys 185: 17–26 (2021)
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RESEARCH ARTICLE

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Dryopteris wulingshanensis (Dryopteridaceae), a new species from Hunan, China

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Abstract

Dryopteris wulingshanensis, a new species growing on limestone in the Wulingshan Mountains, Hunan, China, is described and illustrated. This species is most similar to *D. jishouensis* and *D. gymnophylla* on general morphological traits, such as the form of scales, rhizome and sori, but differs by the number of vascular bundles at the base of the petiole, length to width ratio of lamina, stalk length of basal pinnae, division of the lamina, apex form of the pinnule and habitat. Moreover, molecular phylogenetic analysis using the chloroplast *rbc*L gene suggested that *D. wulingshanensis*, as the sister group of *D. jishouensis*, is a monophyletic clade. According to its restricted geographic range, small populations and few individuals, *D. wulingshanensis* should be considered endangered, according to the IUCN Red List criteria.

Keywords

New taxon, *rbc*L, subg. *Dryopteris*, phylogeny, fern

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[*] These authors contributed equally for this study.

Introduction

Dryopteris Adans. (1763: 20, 551) is one of the largest fern genera with about 400 species, widely distributed all over the world (Wu et al. 2013). Based on molecular phylogenetic evidence, several genera are nested within *Dryopteris*, such as *Acrophorus* C. Presl, *Acrorumohra* (H. Itô) H. Itô, *Diacalpe* Blume, *Dryopsis* Holttum & P. J. Edwards, *Nothoperanema* (Tagawa) Ching and *Peranema* D. Don (Zhang and Zhang 2012; Zhang et al. 2012). Most species in *Dryopteris* share a short rhizome and catadromous arrangement of frond segments, compared to its sister genus, *Arachniodes* Blume, which has long-creeping rhizomes and anadromous laminae (Zhang et al. 2012; Wu et al. 2013). The species of this genus usually grow in forests, open vegetation and, occasionally, in the rocky area of temperate and tropical regions (Fraser-Jenkins 1986; Kramer et al. 1990; Wu et al. 2013). In China, the genus is widely distributed, especially in south-western regions, with about 167 species with 60 endemic species in four subgenera (*D.* subg. *Pycnopteris*, *D.* subg. *Nothoperanema*, *D.* subg. *Dryopteris*, and *D.* subg. *Erythrovariae*) (Wu et al. 2013).

During 2016–2021, we surveyed ferns in the Wulingshan Mountains, which occupy the border zone of four Provinces in China (Hubei, Chongqing, Guizhou and Hunan). This region, as one of the biodiversity hotspots, nurtures a large number of endemic plants and preserves many relict plants (Chen et al. 2004; Yan and Zhou 2021). When we arrived at the Pangu Peak of Dehang Scenic Area, Jishou City, Hunan, an epipetric species that grows in the limestone crevices caught our attention. It is most similar to *Dryopteris gymnophylla* (Baker) C. Chr. and *Dryopteris jishouensis* G.X. Chen & D.G. Zhang but differs by the length to width ratio of lamina, stalks of the basal pinnae, apex form of pinnules and habitat. Moreover, we found this unknown species was also distributed in Mt. Tianmenshan and Zhongli Grand Canyon of Zhangjiajie City, Hunan, China. In order to infer the phylogenetic position of this species, the chloroplast *rbc*L sequences of 32 individuals, representing 11 closely related species, were analyzed. Based on morphological and molecular phylogenetic evidence, we describe it as a new species, named *Dryopteris wulingshanensis* J.P. Shu, Y.H. Yan & R.J. Wang and illustrate it here.

Materials and methods

DNA extraction and sequencing

A total of 32 samples, representing 11 species of the genus *Dryopteris*, were analyzed to infer the phylogenetic relationships amongst the unknown species and its closest relatives. *Dryopteris aemula* (Aiton) Kuntze was sampled as an outgroup based on the previous phylogenetic studies of the genus *Dryopteris* (Zhang and Zhang 2012; Zhang et al. 2012). The *rbc*L gene of 18 individuals were newly sequenced and submitted on the GenBank (Table 1), and the others were obtained from GenBank database. Total genomic DNA was extracted from silica gel-dried leaves by using a DNA secure Plant Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocols. The primers and amplification reaction of *rbc*L gene followed the protocols of Shu et al.

Table 1. Information of 18 samples newly sequenced in this study.

Taxon	Voucher specimen	Locality	*rbc*L
Dryopteris jishouensis	JSL3612	Guangxi, China	MZ444597
	JSL3610	Guangxi, China	MZ444596
	JSL3607	Guangxi, China	MZ444595
	ZXL6320	Hunan, China	MZ444598
	ZXL6317	Hunan, China	MZ444593
	YYH7842	Guizhou, China	MZ444594
Dryopteris wulingshanensis	ZXL6320-3	Hunan, China	MZ444607
	ZXL6320-1	Hunan, China	MZ444606
	JSL3935	Hunan, China	MZ444605
	JSL3926	Hunan, China	MZ444604
Dryopteris gymnophylla	ZXL6626	Jiangxi, China	MZ444602
	JSL4354	Zhejiang, China	MZ444599
	JSL3320	Anhui, China	MZ444600
	JSL3393	Anhui, China	MZ444601
	JSL3949	Anhui, China	MZ444603
Dryopteris chinensis	JSL3359	Anhui, China	MZ444610
	JSL3329	Anhui, China	MZ444609
	JSL2983	Anhui, China	MZ444608

(2017). Sequencing reactions were set up to obtain both the forward and reverse sequences, and then sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, USA).

Molecular phylogenetic analysis

The consensus sequences were generated using SeqMan v7.1.0 (DNASTAR, USA) and then 32 sequences used for phylogenetic analysis were aligned with BioEdit v7.2.0 (Hall 1999). The Maximum Likelihood (ML) phylogenetic tree was constructed by IQ-TREE v2.1.3 (Minh et al. 2020), the best-fit model (K2P+I) was chosen according to Bayesian Information Criterion (BIC) with ModelFinder (Kalyaanamoorthy et al. 2017) and the branch support values of ultrafast bootstrap (UFBoot) approximation was performed with 1000 repetitions. Each bootstrap tree was optimized using a hill-climbing Nearest Neighbor Interchange (NNI) search, based directly on the corresponding bootstrap alignment to reduce the risk of overestimating branch supports with UFBoot (Hoang et al. 2017). The Bayesian Inference (BI) species tree was constructed by MrBayes v3.2.7 (Ronquist et al. 2012) with the GTR + I + G model. Four Markov Chain Monte Carlo (MCMC) chains were run simultaneously for two million generations, and sampled every 100 generations. The convergence was assessed with the average standard deviation of split frequencies lower than 0.01.

Results and discussion

A total of 32 samples were used for phylogenetic analysis, based on chloroplast *rbc*L gene and the length of sequence alignments was 1,204 bp after removing the missing or gap sites. The phylogenetic relationships amongst *D. wulingshanensis* and its rela-

Table 2. The morphological comparison of *Dryopteris wulingshanensis*, *D. jishouensis* and *D. gymnophylla*.

Species characters	*D. jishouensis*	*D. wulingshanensis*	*D. gymnophylla*
Vascular bundles at the base of petiole	2–3	About 5	7–8
Length/width of the fronds	1.7–2.0 times	1.3–1.6 times	1–1.2 times
Stalk of basal pinnae	Usually shorter than 1.5 cm	With a long stalk, usually up to 3cm or more	With a long stalk, usually up to 3cm or more
Division of the lamina	3× pinnate	4× pinnate- pinnatipartite	3× pinnate-pinnatipartite
Pinnules	Obtuse	Acuminate	Acuminate
Habitat	Epipetric	Epipetric	Terrestrial

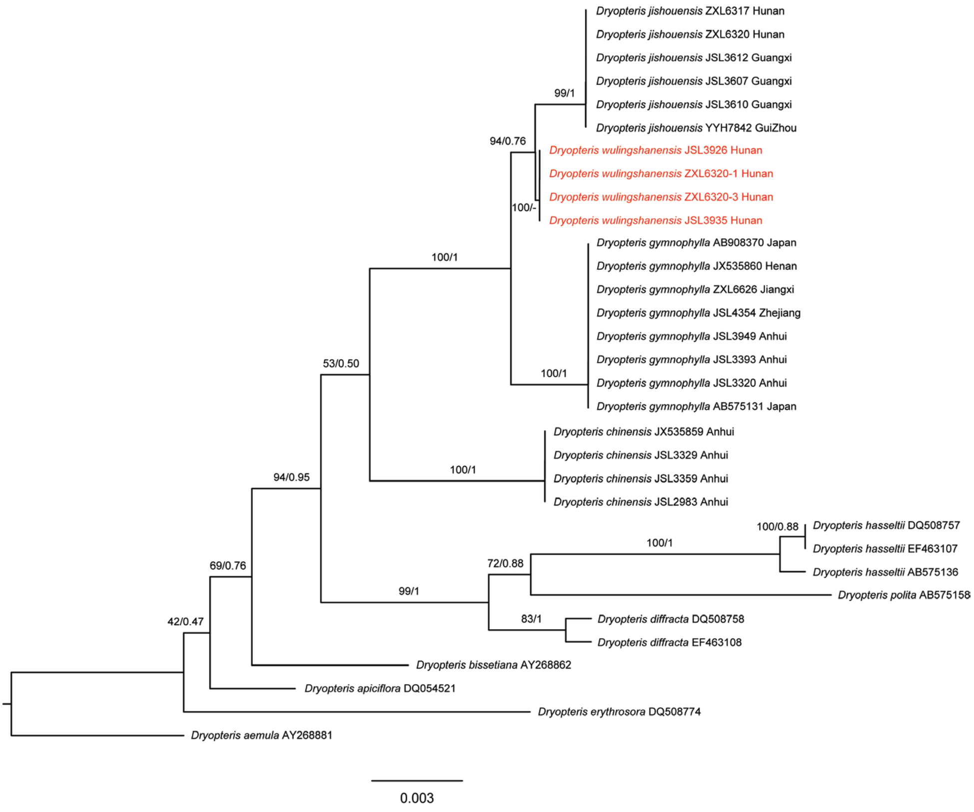

Figure 1. The phylogenetic relationships amongst the new species *Dryopteris wulingshanensis* and its relatives. The topology was the Maximum Likelihood (ML) tree and bootstraps support values (BS) and posterior probability (PP) are showed on the branches.

tives based on ML and BI algorithms, showed a tree topology to be nearly the same as previous studies (Zhang and Zhang 2012; Zhang et al. 2012). Our results indicated the new species *D. wulingshanensis* was a monophyletic clade (BS/PP = 100/-) and was sister to *D. jishouensis* (Fig. 1). Morphologically, the new species was similar to *D. jishouensis* and *D. gymnophylla* on the general morphological characteristics, but differs by the vascular bundles at the base of petiole, length/width ratio of the fronds, stalk length of basal pinnae, division of the lamina, pinnules and habitat (Fig. 2, Table 2).

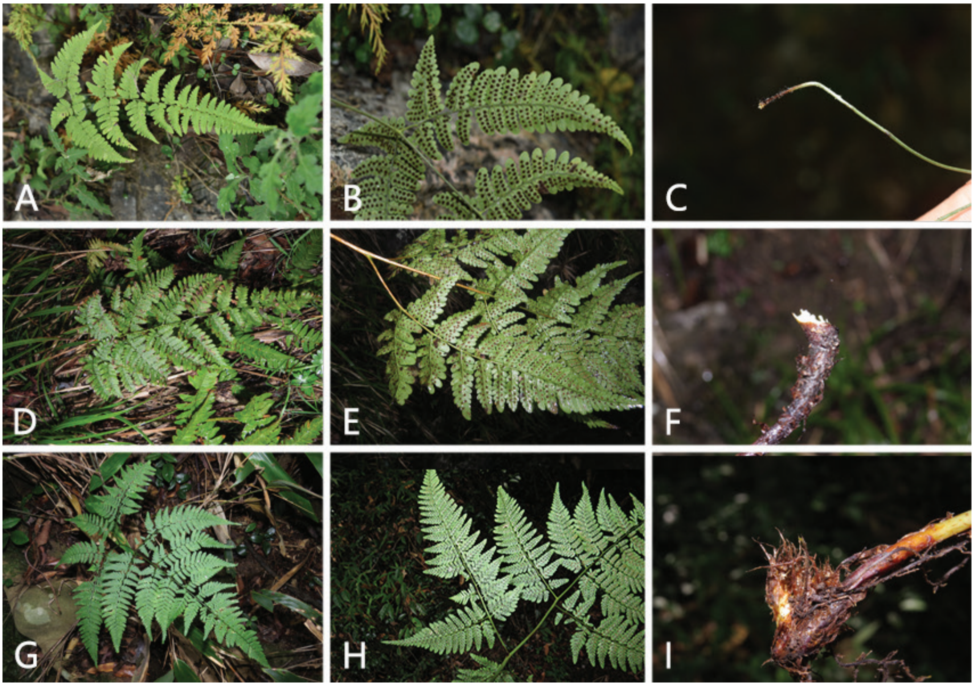

Figure 2. Morphological comparison amongst *Dryopteris wulingshanensis*, *D. jishouensis* and *D. gymnophylla* **A–C** *D. jishouensis* (type locality, Hunan **A** plants and habitats **B** stalks of basal pinnae **C** scales at base of stipe) **D–F** *D. wulingshanensis* (Zhangjiajie, Hunan **D** plants and habitats **E** stalks of basal pinnae **F** scales at base of stipe) **G–I** *D. gymnophylla* (Zhejiang **G** plants and habitats **H** stalks of basal pinnae **I** scales at base of stipe).

Taxonomic treatment

Dryopteris wulingshanensis **J.P.Shu, Y.H.Yan & R.J.Wang, sp. nov.**
urn:lsid:ipni.org:names:77222479-1
Figs 3, 4
武陵山鳞毛蕨

Type. China. Hunan: Wulingshan Mountains Zhongli Grand Canyon, Sangzhi County, Zhangjiajie City. 29°39'10.08"N, 110°37'04.29"E, 900 m alt., 26 June 2021, *Y.-H. Yan & Z.-R. Gu*, *YYH24468* (holotype, IBSC; isotypes, NOCC!, CSH!,PE!).

Diagnosis. The morphology of *D. wulingshanensis* was intermediated between *D. jishouensis* and *D. gymnophylla*, but more similar to the former. *Dryopteris wulingshanensis* and *D. jishouensis* both grow in alkaline soil, but the former's fronds are ovate, length/width 1.3–1.6 times, tripinnate to quadripartite; the latter's fronds are ovate-lanceolate to triangular-lanceolate, length/width 1.7–2.0 times or more, bipinnate to tripinnatisect. *Dryopteris gymnophylla* grows in acidic soil, the fronds are pentagonal, usually length/width 1–1.2 times, tripinnate to quadripinnnatisect (Fig. 2, Table 2).

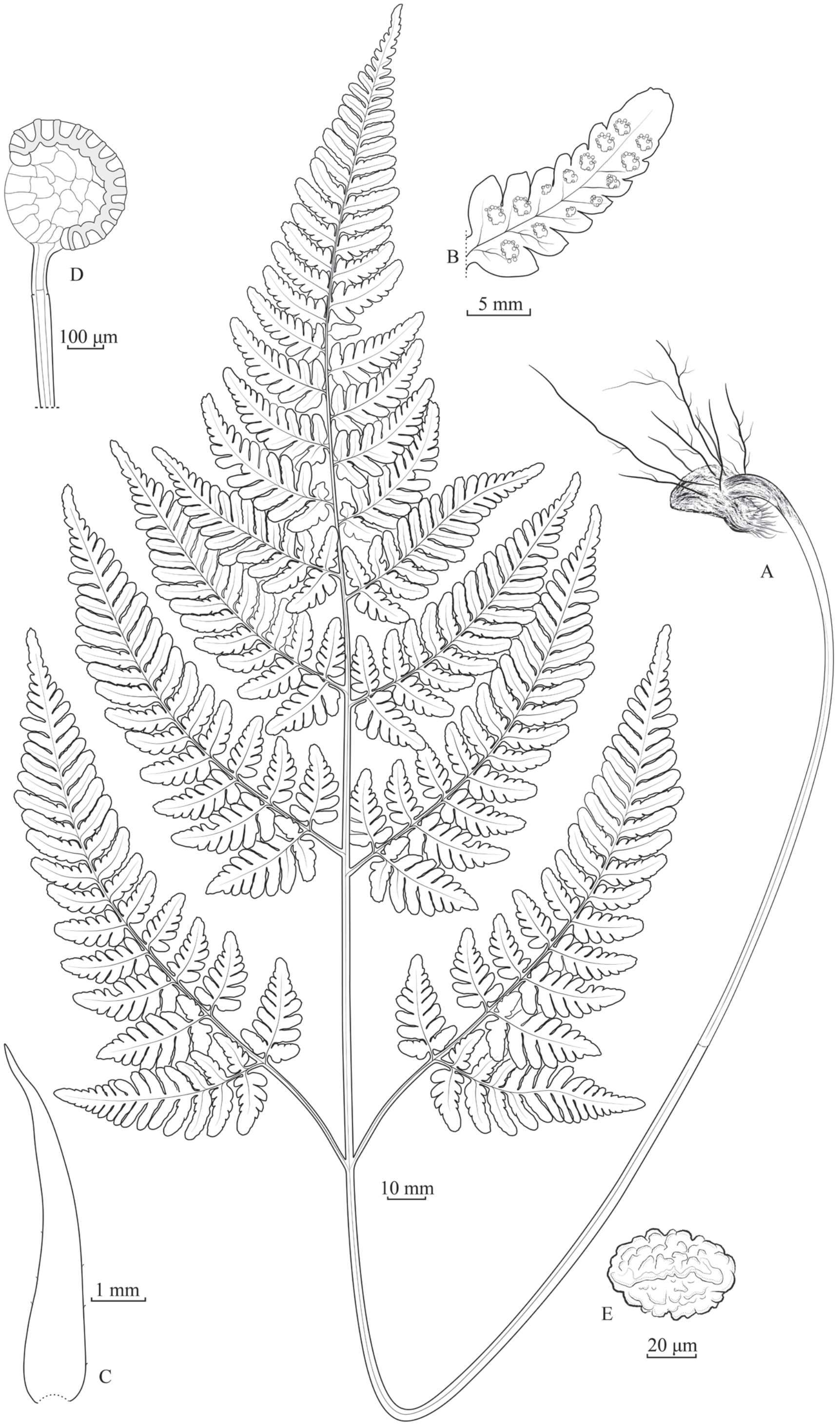

Figure 3. *Dryopteris wulingshanensis* J.P. Shu, Y.H. Yan & R.J. Wang **A** habit **B** pinnule with sori **C** scale at base of stipe **D** sporangium **E** spore (drawn by Li-Jun Chen, based on the type material at IBSC).

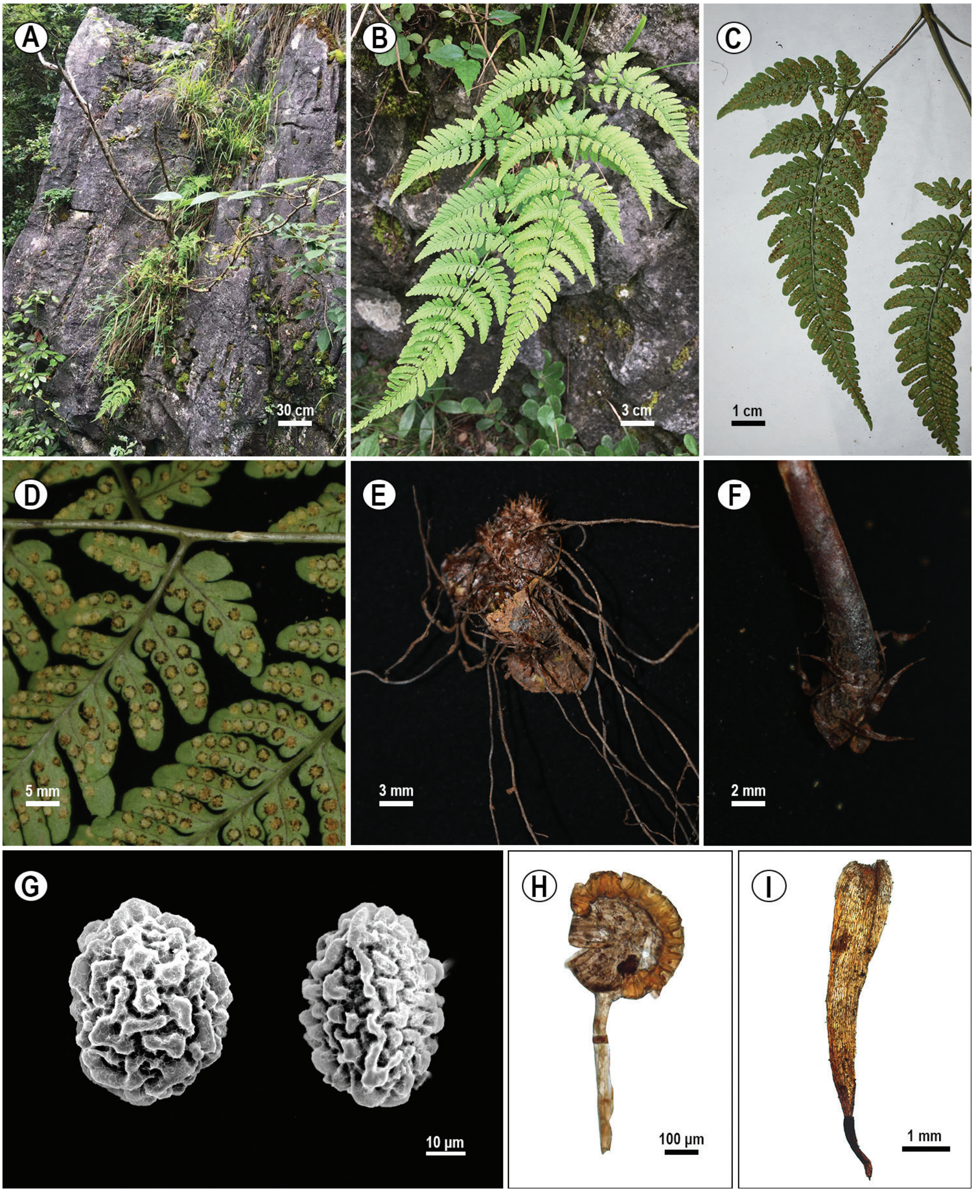

Figure 4. The habitat and morphological characters of *Dryopteris wulingshanensis* J.P. Shu, Y.H. Yan & R.J. Wang **A** habitat **B** lamina **C** basal pinna **D** sori **E** rhizome **F** base of stipe **G** spore (**Left** distal pole, **Right** proximal pole) **H** sporangium **I** scale.

Description. Rhizome short-creeping, apex scaly; **scales** dark brown, lanceolate, margin entire or 1–2 dentate at base. **Frond** approximate, (40–)65–70 cm; stipe (23–)31–36 cm, medial diameter 1.5–2 mm, basal scales similar to rhizome scales, antrorse, glabrous, stramineous to brown-stramineous, ventrally grooved; **lamina** ovate, (19–)32–36 × (12–)19–28 cm, about 1.3–1.6 times as long as wide, base round or cordate, apex acuminate, tripinnate to quadripartite (premature lamina only bipinnate to tripinnatisect); **pinnae** 6–8 pairs, oblique, distant, falcate, stipitate, basal pair longest and largest, opposite to nearly opposite, significantly falcate, stipe (1.2–)3–4.5(–7) cm, deltate-lanceolate, (8–)12–23 × (3.6–)7–11 cm, apex long-acuminate, base broad-cuneate to round, asymmetric, basiscopic pinnules longer than acroscopic pinnules, suprabasal pinnae with pinnules similar; **pinnules** 7–8 pairs, discrete, oblique to explanate, falcate, base broadly cuneate, pedicellate, basiscopic ones largest, (3–)5–10 × (1.5–)2.2–4 cm, stipe (1.5–)4–9 mm, trigonal oblong, apex obtuse, ultimate pinnules (lobe) 7–10 pairs, oblique to explanate, trigonal oblong, basiscopic ones longer than acroscopic ones, exstipitate, round-obtuse, base broad-cuneate, connate, pinnatifid to partite, (9–)14–20 (4)8–10 mm; **lobes** 2–7 pairs, 1.5–2 mm wide, oblong, entire, round, others ultimate pinnules lobate to pinnatifid or crenate, base broad-cuneate to decurrent; other pinnae decrescent, opposite to alternate, trigonal lanceolate to oblong lanceolate; **rachis** and costa glabrous, stramineous, adaxially sulcate, lamina papyraceous, glabrous; **veins** pinnate, single or dichotomous; **sori** round, on apices of veinlets, nearly incision; **indusia** orbicular-reniform, margin premorse, brown, persistent.

Additional specimens examined (paratypes). China. Hunan: Pangu Peak, Dehang Scenic Area, Jishou City, Xiangxi Tujia and Miao Autonomous Prefecture. 28°20'50.046"N, 109°34'34.522"E, 914 m alt., 26 Jun 2016, *X.-L. Zhou* et al. *ZXL6320-1*, *ZXL6320-3* (CSH!); Tianmenshan Scenic Area, Zhangjiajie City. 5 April 2016, *H.-J. Wei* et al. *JSL3926*, *JSL3935* (CSH!).

Distribution and habitat. *Dryopteris wulingshanensis* is endemic to the Wulingshan mountains in Jishou and Zhangjiajie Cities, Hunan, China. It is epipetric in limestone crevices at an elevation of 700–1000 m in evergreen and deciduous broad-leaved mixed forest. The associated ferns include: *Cheilanthes patula* Baker, *Cyrtomium fortunei* J. Sm., *Cyrtomium nephrolepioides* (Christ) Copel., *Lemmaphyllum drymoglossoides* (Baker) Ching, *Polystichum tsus-simense* (Hook.) J. Sm., *Pronephrium penangianum* (Hook.) Holttum, *Pyrrosia petiolosa* (Christ) Ching and *Woodwardia unigemmata* (Makino) Nakai and associated seed plants include: *Celtis sinensis* Pers., *Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W. Hill, *Davidia involucrata* Baill., *Ficus sarmentosa* var. *henryi* (King ex Oliv.) Corner, *Hydrangea strigosa* Rehder, *Loropetalum chinense* (R. Br.) Oliv., *Mallotus philippinensis* (Lam.) Muell. Arg., *Miscanthus sinensis* Andersson, *Platycarya strobilacea* Siebold & Zucc., *Rhus chinensis* Mill. and *Viburnum cylindricum* Buch.–Ham. ex D. Don.

Etymology. The specific epithet "*wulingshanensis*" is derived from the name of type locality Wulingshan Mountains, where the new species is found.

IUCN Conservation Assessment. EN(B1ab(iii)). *Dryopteris wulingshanensis* is only known from three locations of Wulingshan Mountains in Jishou and Zhangjiajie Cities, Hunan, China. Based on its restricted geographic range, small populations and few individuals, *Dryopteris wulingshanensis* should be considered endangered under the IUCN Red List criteria (IUCN 2019).

Acknowledgements

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RESEARCH ARTICLE

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Rubus kaznowskii (Rosaceae), a new bramble species from south-central Poland

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Abstract

Based on field research in south-central Poland, supplemented with a review of herbarium materials, we identified a stable bramble biotype with a range large enough (190 km distance between the outermost stands) to be described as a new regional agamic species, *Rubus kaznowskii* **sp.nov.** It belongs to the series *Subthyrsoidei* (sect. *Corylifolii*). Although *R. kaznowskii* has a unique combination of features, it can be potentially mistaken for *R. gothicus*. It differs from the latter species in many aspects, including: pruinose primocanes, denser indumentum of the abaxial leaf surface, and more curved prickles on the petiole. *R. kaznowskii* has mainly been observed on rusty soils, in habitats of mixed coniferous and mixed broadleaf forests, usually in sunny places, along forest margins and roads, in clearings and roadside thickets.

Keywords

distribution, ecology, genome size, morphology, taxonomy

Introduction

The genus *Rubus* is one of the taxonomically most complex group of angiosperms. It encompasses 400–500 sexual species and more than twice as many agamic species (Weber 1995). In Europe, among more than 750 bramble species (Kurtto et al. 2010), there are only four sexual diploids; the remaining species are apomictic polyploids (Kollmann et al. 2000; Potter et al. 2007; Krahulcová et al. 2013; Sochor et al. 2015, 2019). More or less facultative apomixis in the genus enables the emergence of new clonal lineages. The identification of brambles is hindered by their high morphological plasticity, which depends on local environmental conditions and the phenological or developmental phase. Attempts to apply traditional species concept to countless morphological forms of brambles caused long-term stagnation in their systematics, which was broken by the so-called "Weberian reform". Although every stabilised biotype with a well-defined range deserves a status of agamic species (Haveman and de Ronde 2013), only biotypes with a range exceeding (20–)50 km between the outermost stand are officially described as new taxa (Weber 1995; Holub 1997).

A recent study on *Rubus* flora of Poland is based on this modern concept of agamic species (Zieliński 2004). However, it remains incomplete because not all regions of the country have been thoroughly explored in this respect. Zieliński (2004) mentioned 90 *Rubus* species from Poland. Since this time, 17 other species have been added to this list. Among them, there are 7 newly described bramble species (Zieliński and Trávníček 2004; Zieliński et al. 2004a; Trávníček et al. 2005; Maliński et al. 2014; Wolanin et al. 2016, 2020; Kosiński et al. 2018) and 10 species new for the Polish flora (Zieliński and Trávníček 2004; Zieliński et al. 2004a; 2004b, Trávníček and Zázvorka 2005; Kosiński 2006, 2010; Kosiński and Oklejewicz 2006; Kosiński and Zieliński 2013; Oklejewicz et al. 2013; Kosiński et al. 2014; Maliński et al. 2015). Together with the new taxon described here, the current Polish *Rubus* flora consists of 108 species.

During field studies in the Małopolska Upland (south-central Poland), we came across a remarkable bramble morphotype from the section *Corylifolii* Lindley, which was different from any other species of the section. The subsequent herbarium survey showed that this bramble had been relatively frequently collected in some areas where detailed floristic investigations were carried out. This allows us to suppose that the so far known area of its occurrence may be still somewhat underestimated.

We classified this bramble to ser. *Subthyrsoidei* (Focke) Focke (sect. *Corylifolii*). The series includes species that are supposed to originate by hybridisation of *Rubus caesius* L. and species of the series *Discolores* (P.J. Müller) Focke or *Rhamnifolii* (Bab.) Focke (Weber 1995; Zieliński 2004). Most of the 37 species known in ser. *Subthyrsoidei* have their centre of distribution in north-central Europe, mainly Germany, especially the eastern part, Denmark, southern Sweden, south-western Poland, Czechia and Slovakia (see the map "AFE 4586" in Kurtto et al. 2010). The series is represented in Poland by six species; they occur in the western part of the country, particularly in Lower Silesia. Only one species, *R. kuleszae* Ziel., has been confirmed so far in the area covered by the field research under this study (Zieliński 2004). The representatives of the series are characterised by the low-arching first-year stems with relatively uniform prickles and scattered to numerous stalked glands; the terminal leaflets of the first-year stem are typically elliptic or obovate, the leaves are green to grey-tomentose beneath, and long-stalked glands are present on the inflorescence axis and pedicels (Weber 1995; Zieliński 2004).

Methods

Field studies were conducted mainly during 2014 and 2019. The position of each stand (latitude, longitude and elevation) was determined using a handheld GPS unit. The distribution category was assigned following Weber (1995) and Kurtto et al. (2010). Voucher specimens collected during the studies (including specimens used for morphological investigations) were deposited in the Herbarium of the Institute of Dendrology, Kórnik (KOR). Distribution maps were compiled in the QGIS 3.16 software (QGIS Development Team 2020), using grid squares following the principles presented in the Atlas Florae Europaeae, AFE (Lampinen 2001), and the Atlas of Distribution of Vascular Plants in Poland, ATPOL (Zając 1978; Verey 2017). We also searched for previous records of *R. kaznowskii* from the studied area in the herbarium of the Jagiellonian University (KRA). The morphological description was based on the revision of all herbarium specimens; some characteristics (e.g., features of flowers) were studied on plants growing in the field and in garden collections. We examined well-developed first-year stems (primocanes) and inflorescences. Additional reference material for the comparative study of similar species was obtained from the Herbarium of the Institute of Dendrology in Kórnik (KOR), which has the most comprehensive collection of brambles occurring in Poland and neighbouring areas (accessible at: https://rcin.org.pl/dlibra/collectiondescription/478).

For the nuclear DNA content estimation in *R. kaznowskii*, flow cytometry was used. Leaves of *R. kaznowskii* and the internal standard *Solanum lycopersicum* L. 'Stupicke' (1.96 pg/2C DNA; Doležel et al. 1992) were chopped together with a razor blade in a Petri dish in 500 µl of a staining buffer (CyStain PI OxProtect 05–5027). The nuclei suspension was filtered through a 30-µm mesh (CellTrics, Sysmex) into a clean tube. Subsequently, the samples were stained with 1.5 ml of a staining buffer containing propidium iodide and RNase (CyStain PI OxProtect 05–5027) and 1% β-mercaptoethanol (Sigma) and incubated for 50 min at room temperature in the dark. After incubation, the samples were analysed using a CyFlow Space flow cytometer (Sysmex) equipped with a 532-nm green laser. At least 5,000 nuclei were read for each sample; DNA ploidy was established by comparison of the 2C DNA content in the new species with that of the triploid *R. crispomarginatus* Holub, tetraploid *R. prissanicus* Kosiński, Maliński & Ziel. and hexaploid *R. capitulatus* Utsch (Kosiński et al. 2018).

Taxonomic treatment

Rubus kaznowskii **Kosiński & Ziel., sp. nov.**
urn:lsid:ipni.org:names:77222480-1
Figs 1–5; Suppl. material 1: Figs S2–S5

Type. Poland. Łódź Province: Łask District, 250 m SE of Gucin village, alongside the road in the pine forest, 186 m alt., 51°32'46"N, 19°14'54"E, 14 Jul 2014, P. Kosiński & J. Zieliński s.n.(holotype: KOR 51366; isotype: KOR 55451).

Diagnosis. *Rubus kaznowskii* can be mistaken for the similar *R. gothicus*. However, it differs from the latter species in several aspects. Primocanes of *R. kaznowskii* are pruinose and covered with usually smaller and slightly curved (not straight) prickles, more numerous hairs and stalked glands. Prickles on the petiole are strongly curved and smaller. Similarly, the inflorescence axis in *R. kaznowskii* is covered by smaller prickles and usually more numerous stalked glands than that in *R. gothicus*. The serration of leaf blade margins is more regular and finer; the apex of the terminal leaflet is more gradually narrowed and shorter. The abaxial surface of the lamina (yellowish when dry) is covered by a denser indumentum and has distinct whitish, protruding veins. Although indumentum in both species consists predominantly of fasciculate hairs, their proportion to simple hairs in *R. kaznowskii* is higher than that in *R. gothicus*. The lack of long hairs in the indumentum, both fasciculate and simple, allows distinguishing *R. kaznowskii* not only from *R. gothicus* but also from other Polish representatives of *Subthyrsoidei*. For detailed differences between *R. kaznowskii* and *R. gothicus*, see Table 1.

Some similarities may link *R. kaznowskii* with two other species from the series *Subthyrsoidei*, *R. holandrei* P.J.Müller (= *R. grossus* H.E.Weber) and *R. storhii* H.E.Weber & M.Ranft., which has not been found in Poland until now. Both species differ from *R. kaznowskii* by the absence of stalked glands on the vegetative stems (*R. kaznowskii* – usually numerous), less hairy leaf undersides, straight or slighty curved prickles on the petioles (*R. kaznowskii* – hooked), and flower colours: the former has white, and the latter pink petals (*R. kaznowskii* – white or pinkish-white and pinkish in the bud).

Description. Shrub, usually up to 70 cm tall. First-year stems low arching, 4–6(–8) mm in diameter, angulate, with flat sides (sometimes slightly furrowed or bluntly angled), flushed violet-red or purple on the side exposed to the sun, more or less pruinose, without or with scattered 0.4–0.6-mm long fasciculate hairs (up to 15 per 1 cm length of stem side); stalked glands and acicles up to 0.4 mm long, rather numerous: usually 5–15(–25) per 1 cm length of stem side; prickles somewhat uneven and shorter than stem diameter, (2.5–)3–5(–6) mm long, quite numerous, 10–15(–19) per 5 cm of stem length, usually little curved, rarely straight and declining, abruptly tapering from the 2.5–3.5-mm broad base; intermixed with sparse to quite numerous stout small prickles. Leaves on the first-year stem moderately large (14–)18–20(–25)-cm long, 5-foliolate, indistinctly pedate, with flat or somewhat convex (when alive) leaflets arrangement; leaflets partly imbricate with flat or slightly convex/concave laminas;

Table 1. Main morphological differences between *Rubus kaznowskii* and *Rubus gothicus*.

Features	*R. kaznowskii*	*R. gothicus*
Primocane		
Wax layer	More or less pruinose	Unpruinose
Hairs (number per 5 cm of the stem length)	(0–)10–50 fasciculate hairs	0–10 simple hairs
Stalked glands (number per 5 cm of the stem length)	25–75	1–10
Prickles	Usually slightly curved and shorter than stem diameter	Usually straight, the longest as long as the stem diameter or longer
Leaf		
Apex of the terminal leaflet	Gradually narrowed, 0.8–1.5 cm	Abruptly narrowed, 1.5–2.0 cm
Blade margin of the terminal leaflet	Usually slightly periodical	Usually distinctly periodical
Prickles on petiole	Strongly curved, not longer than petiole diameter	Slightly curved or straight and declining, longer than petiole diameter
Indumentum cover (abaxial side of the lamina)	Dense (above 50%)	Sparse (below 50%)
Long simple (>0.5 mm), large fasciculate (>0.35 mm), and glandular trichomes (abaxial side of the lamina)	Absent	Numerous
Short (<0.25 mm) and medium (0.25–0.5 mm) simple hairs (abaxial side of the lamina)	Numerous	Rare
Inflorescence axis		
Prickles length/rachis diameter ratio	1–2	>2
Prickles form	slightly curved	slightly curved or straight and declining
Stalked glands (number per 5 cm of the axis length)	20–40(–50)	10–25(–50)

matt green and subglabrous above (with 0–10 adpressed simple hairs per 1 cm^2, especially towards leaf margin and on main veins, and with scattered subsessile glands); light or whitish- or yellowish-green and soft to the touch beneath because of dense indumentum (50–90% of the intercostal area) of fasciculate hairs and less numerous simple hairs, with clearly visible bright and protruding veins. Terminal leaflet lamina (7–)8–10(–11)-cm long, usually ovate and widest below the middle (37–50% of its length); shallowly cordate at the base, gradually narrowed, with an apex 8–15 mm long; petiolule moderately, (17–)20–35(–39) mm, long (25–35% of its lamina length). Basal leaflets mostly sessile or sometimes with short petiolules up to 1–2 mm long; their lamina narrowly ovate to obovate, usually shorter than petioles (about 90% of their length on average). Leaf margin periodically serrate, with incisions 2–3(–5) mm deep, sometimes with 1–2 quite distinct lobes near the middle of the lamina; teeth usually triangular, broader than long, with a thin narrow apex. Petioles sparsely hairy, with scattered stalked glands and usually with 9–13 strongly curved, 1–2-mm long prickles. Stipules filiform, with scattered hairs and stalked glands. Inflorescence up to 20–30 cm long, broadly paniculate, rounded near apex, with erectopatent lateral branches up to 7–10 cm long; usually leafless above, with 3(–5)-foliolate leaves below. Inflorescence axis hairy with adpressed small fasciculate (stellate) hairs and scattered longer both fasciculate and simple hairs; stalked glands 50–80 per 1 cm of the axis length, 0.2–0.5 mm long; prickles usually 5–10 per 5 cm of axis length, declining, slender, subulate, slightly curved, 2–3 mm long (1–2 × as long as the axis thickness). Flower pedicels (5–)10–20(–25) mm long, densely hairy and with 40–80 stalked glands; prickles 4–8(–14), slender, slightly curved and declining, 1–2 mm long. Sepals pat-


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Rubus kaznowskii Kosiński & Ziel

POLAND, Łódź Province, Łask District: 250 m SE of Gucin village,
alongside the road in the pine forest.
Elevation 186 m, 51° 32' 46"N, 19° 14' 54"E, AFE 34UCC4, ATPOL DD94

14-07-2014 (2014/1)

P. Kosiński, J. Zieliński

HOLOTYPUS

Imaged with number:
KOR023001

Figure 1. Holotype of *Rubus kaznowskii* (KOR 51366).

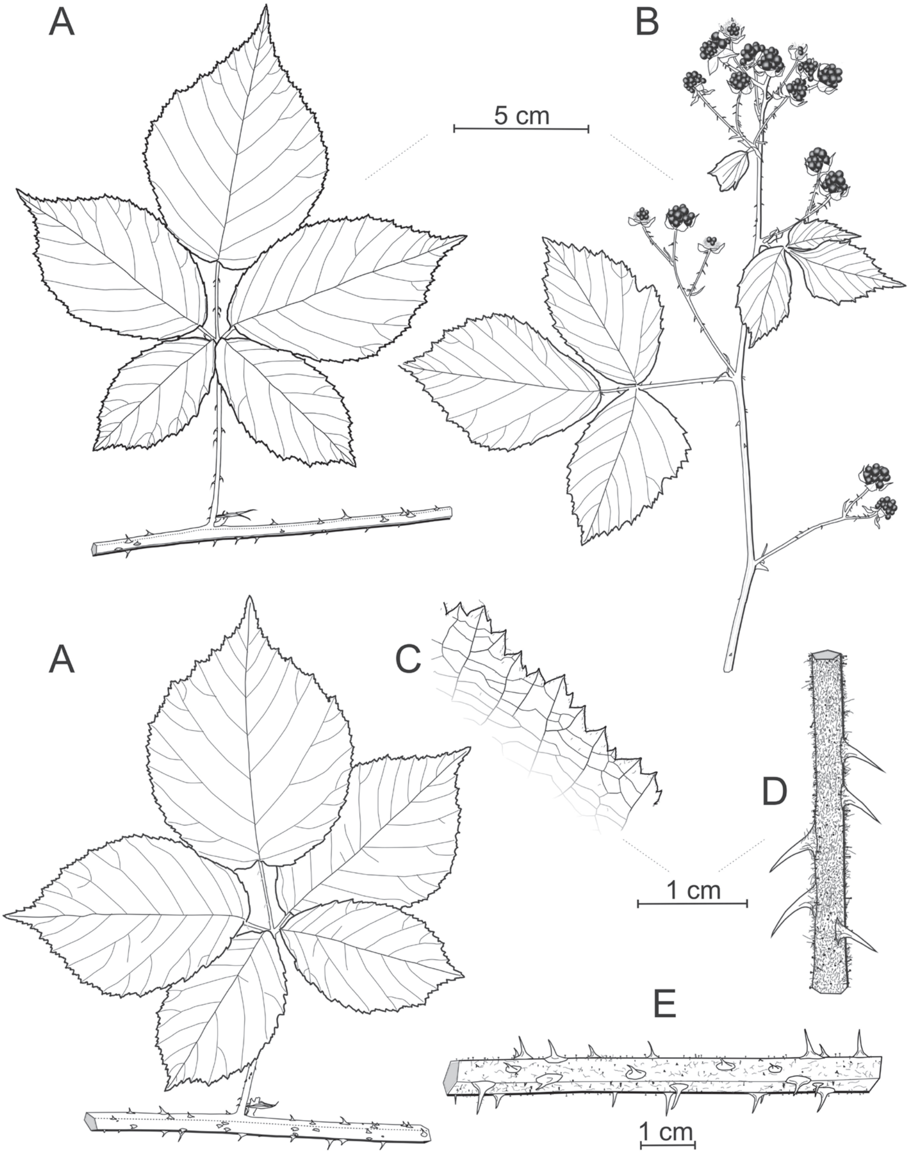

Figure 2. *Rubus kaznowskii* **A** segments of the primocane **B** infructescence **C** detail of the terminal leaflet margin **D** detail of the inflorescence axis **E** detail of the first-year stem. Illustration by Piotr Kosiński.

ent after anthesis, 6–9 mm long, grey-green-felted with numerous stalked glands and several pricklets on the outer surface. Petals white or pinkish-white, wrinkled, broadly ovate or obovate, 12–13 mm broad and 14–16 mm long; often present an additional, incomplete whorl composed of smaller petals. Stamens longer or as long as the yellow-

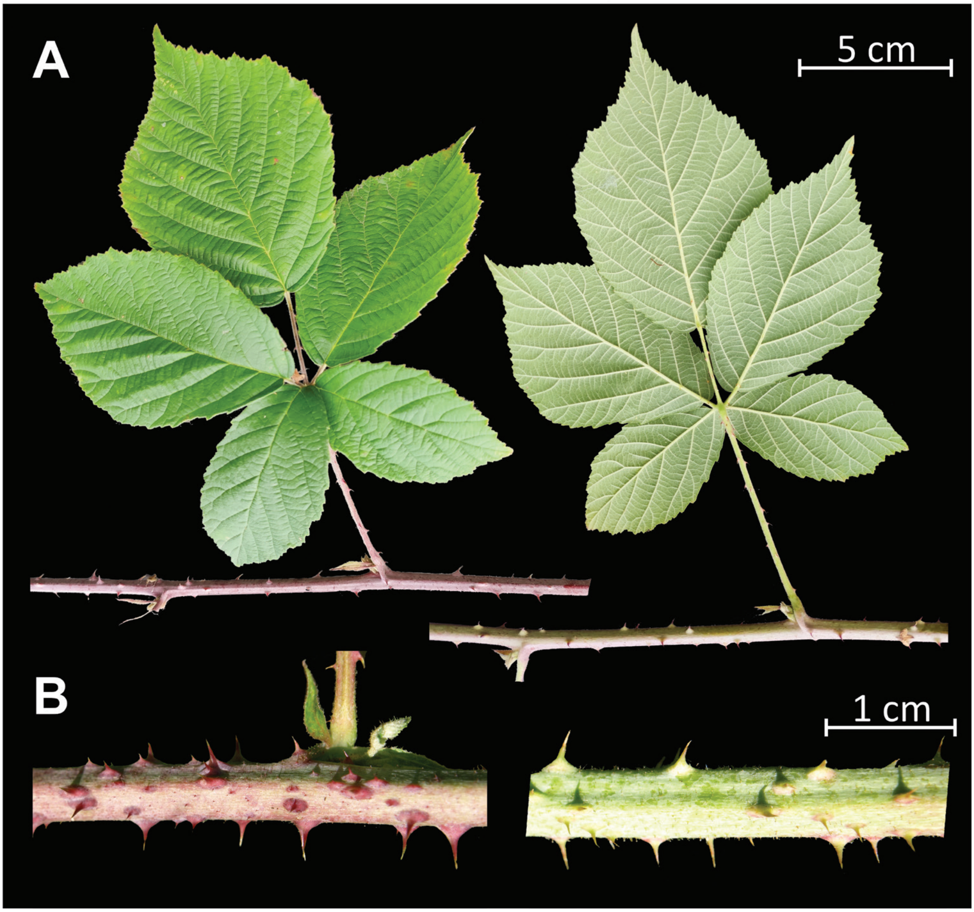

Figure 3. Fragments of primocanes **A** and primocane stems **B** of a living specimen of *Rubus kaznowskii*. Photos by Piotr Kosiński.

ish-green styles; anthers glabrous, yellowish-white; filaments whitish. Carpels glabrous or sparsely hairy, receptacle usually with long hairs protruding among carpels. Flowering June-July.

Genome size. The nuclear DNA content of *R. kaznowskii* is 2C = 1.43 ± 0.01 pg. Comparison of its PI fluorescence with triploid, tetraploid, and hexaploid *Rubus* species, of which ploidy was previously established by chromosome counting, revealed that *R. kaznowskii* is DNA tetraploid. In the European *Rubus* flora, tetraploids predominate (2n = 28) (Kollmann et al. 2000; Potter et al. 2007; Krahulcová et al. 2013; Sochor et al. 2015, 2019). Tetraploidy also prevails in the ser. *Subthyrsoidei*. In Poland, there are only two pentaploid species of the series, *R. kuleszae* Ziel. and *R. wahlbergii* Arrh., which are distinguished from their remaining representatives by larger leaves (Zieliński 2004).

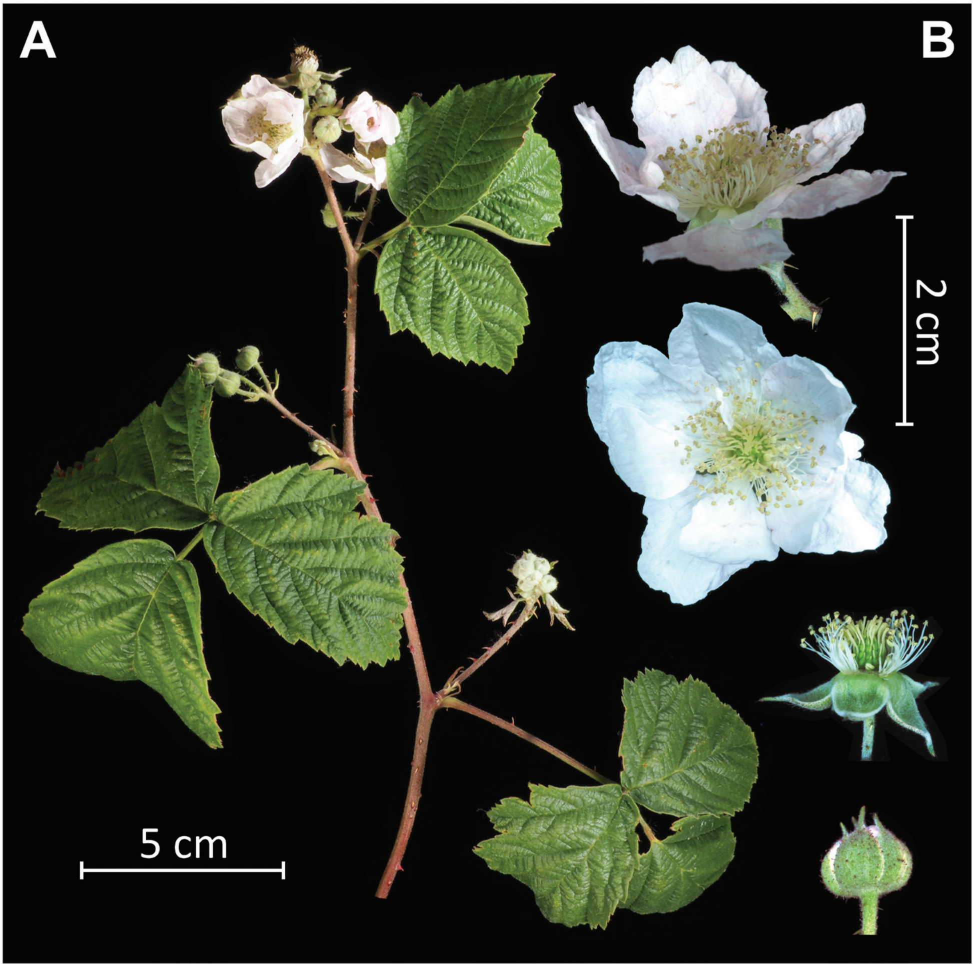

Figure 4. Inflorescence **A** and flowers **B** of a living specimen of *Rubus kaznowskii*. Photos by Piotr Kosiński.

Eponymy. The epithet "kaznowskii" refers to Kazimierz Kaznowski (1876–1943), teacher, naturalist and batologist from the Świętokrzyskie (Holy Cross Mts) region (Poland); the oldest known herbarium specimen of *R. kaznowskii* was collected by him.

Distribution and ecology. *Rubus kaznowskii* is a regionally distributed bramble species. The distance between outermost stands reaches more than 190 km. More than two-thirds of all its stands are located in the Kielce Upland, and one-fifth of them in the Central Masovian Upland, between the Warta and Vistula rivers. It was confirmed in nine AFE units: 34UDB4, 34UEB2, 34UDB1, 34UCC4, 34UDB3, 34UEB1, 34UDB2, 34UEB3, 34UEC2 (Fig. 6). The elevation of *R. kaznowskii* stands ranges from 135 to 325 m. A more detailed map of the distribution of the species in the ATPOL grid is presented in Suppl. material 1: Fig. S1.

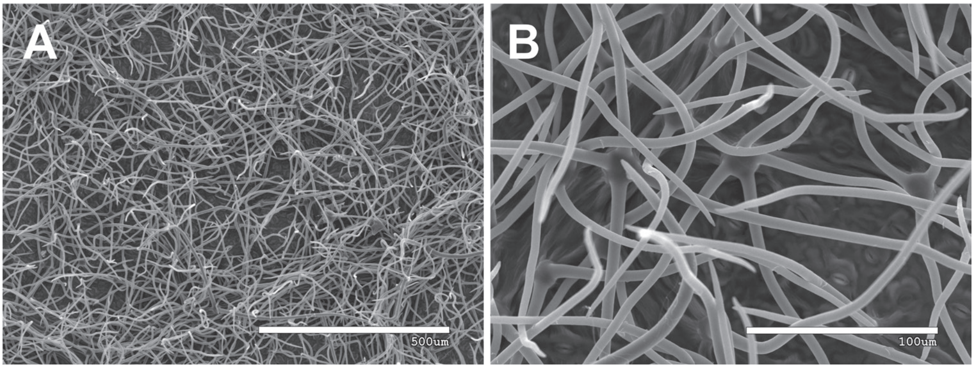

Figure 5. Abaxial side of a leaflet of *Rubus kaznowskii* in SEM. General **A** and more detailed **B** views of indumentum cover in the intercostal area assessed as dense, with the predominance of fasciculate hairs over simple hairs. Photos by Dominik Tomaszewski.

The species occurs mainly on rusty soils (brunic arenosols), in semi-dry to mesic habitats of mixed coniferous and mixed broadleaf forests, preferring open places with favourable light conditions: along forest margins and in clearings and in roadside thickets, among others (Suppl. material 1: Figs S6, S7).

Preliminary conservation status. *Rubus kaznowskii* is a moderately hemerophilic species. No pressures or threats are evidenced. According to IUCN Criteria (IUCN 2021), we propose to include it in the category of Least Concern (LC).

Specimens examined. Poland. Łódź Province, Łask District: 2 km N of Kamostek, pine-oak forest, roadside thickets, 181 m a.s.l., 51°31'46"N, 19°04'12"E, 10 Sep 2019, *P. Kosiński*, *T. Maliński* & *J. Zieliński* (KOR 55452); Opoczno District: Wierzchowisko, roadside thickets, 228 m a.s.l., 51°13'04.26"N, 20°13'04.45"E, 23 Aug 2010, *A. Trojecka-Brzezińska* (KRA); Piotrków District: 0.5 km NW of Poniatów, broadleaved forest margin, 203 m a.s.l., 51°23'55.33"N, 19°45'02.22"E, 14 Jul 2014, *P. Kosiński* & *J. Zieliński* (KOR 51359); NW outskirts of Poniatów, edge of the brdoadleaved forest, 203 m a.s.l., 51°23'55"N, 19°45'02"E, 14 Jul 2014, *P. Kosiński* & *J. Zieliński* (KOR 51357); SEE of Piotrków Tybunalski, edge of the pine plantation on mixed deciduous forest habitat, 203 m a.s.l., 51°23'46"N, 19°45'29"E, 24 Aug 1999, *J. Zieliński* (KOR 41323); **Mazovia Province**, Białobrzegi District: Ksawerów Stary, roadside thickets, 135 m a.s.l., 51°38'58"N, 21°07'42"E, 13 Sep 2019, *P. Kosiński*, *T. Maliński* & *J. Zieliński* (KOR 55454); Ostrowiec District: Łysowody near Ćmielów, roadside thickets, 203 m a.s.l., 50°54'22"N, 21°33'55"E, 8 Aug 2002, *R. Piwowarczyk* (KRA 0354524); Radom District: W of Młodocin Mniejszy, pine forest, 194 m a.s.l., 51°20'25"N, 21°01'10"E, 3 Aug 2005, *M. Nobis* (KRA); E outskirts of Waliny, alongside the railway and road with the ditch, 189 m a.s.l., 51°21'07.8"N, 21°00'37"E, 4 Aug 2005, *M. Nobis* (KRA); 2.8 km SSE of Maliszów, mixed coniferous forest, 194 m a.s.l., 51°16'08"N, 21°07'53"E, 30 Aug 2002, *M. Nobis* (KRA); Kolonia Dąbrówka Zabłotnia, pine forest margin, 182 m a.s.l., 51°19'06.52"N, 21°03'17.71"E, 24 Jun 2003, *M. Nobis* (KRA 0318820); Kresy near Wierzbica, railway gorge, 203 m a.s.l., 51°16'22.55"N, 21°01'21.32"E, 23 Jun 2003, *M. Nobis* (KRA 0318832); 2 km W of Pakosław, pine-oak forest, 220 m a.s.l.,

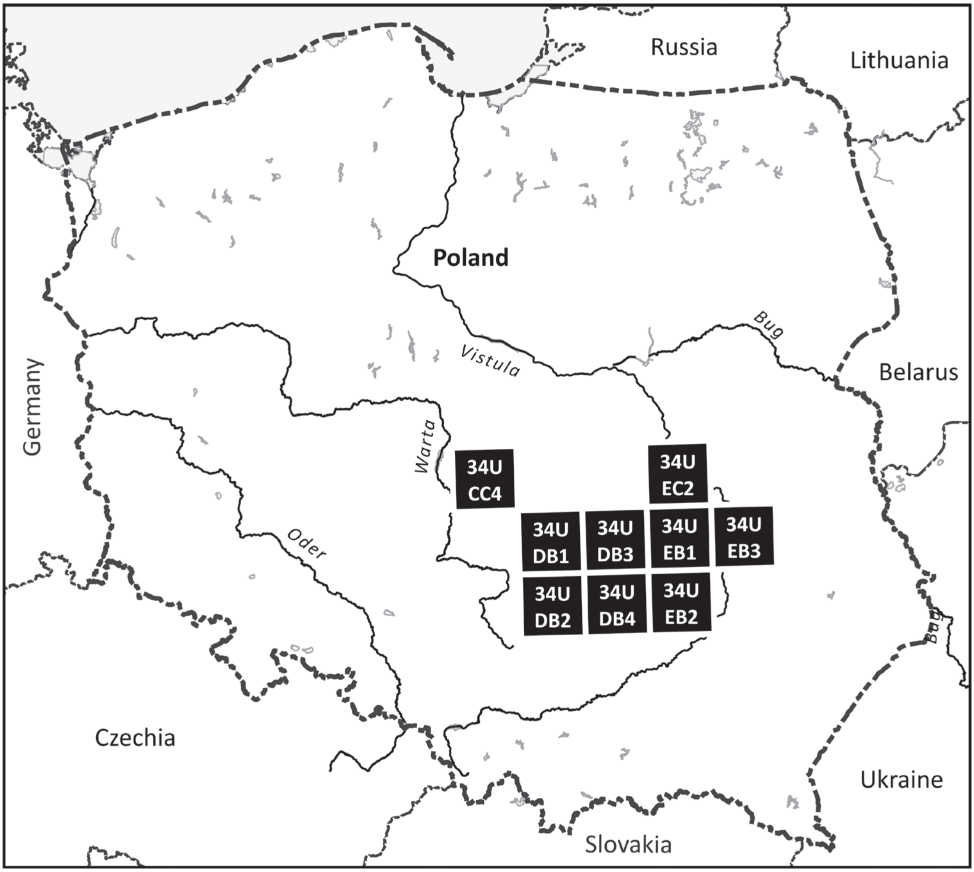

Figure 6. Distribution of *Rubus kaznowskii* based on Atlas Florae Europaeae (AFE) grid system.

51°12'22.23"N, 21°07'58.29"E, 2 Jul 2004, *M. Nobis* (KRA 0317627); S of Malczew, sandpit, 179 m a.s.l., 51°21'23.68"N, 21°09'55.12"E, 27 Aug 2005, *M. Nobis* (KRA 0365787); Skarżysko District: N of Kierz Niedźwiedzi, forest margin, 228 m a.s.l., 51°10'53"N, 20°57'06.6"E, 10 Aug 2003, *M. Nobis* (KRA 0318815); Szydłowiec District: Szydłowiec, roadside thickets near the artificial lake, 219 m a.s.l., 51°13'07.16"N, 20°51'20.65"E, 22 Jul 2003, *M. Nobis* (KRA 0320963); 1 km N of Zaława, roadside thickets, 205 m a.s.l., 51°16'04.64"N, 20°45'59.97"E, 22 Jun 2004, *M. Nobis* (KRA 0317573 & 0317574); between Aleksandrów and Budki I, roadside thickets/forest margin, 242 m a.s.l., 51°12'50.98"N, 20°46'18.60"E, 25 Aug 2003, *M. Nobis* (KRA 0318819); Wymysłów, roadside thickets, 218 m a.s.l., 51°14'09.39"N, 20°49'08.00"E, 1 Aug 2003, *M. Nobis* (KRA 0318822); Wola Zagrodnia, roadside thickets, 226 m a.s.l., 51°14'43.59"N, 20°44'49.88"E, 11 Jun 2003, *M. Nobis* (KRA 0321855); Lipienice, alongside railway and road, 205 m a.s.l., 51°14'45.8"N, 20°58'41.6"E, 18 Aug 2002, *M. Nobis* (KRA); W part of Gąsawy Rządowe Niwy, forest margin, 240 m a.s.l., 51°11'57.64"N, 20°55'29.66"E, 31 Jul 2003, *M. Nobis* (KRA 0320129); Gąsawy Rządowe, forest margin, 237 m a.s.l., 51°12'16.47"N, 20°56'46.8"E, 8 Aug 2003, *M. Nobis* (KRA 0318823); Zwoleń District: 0.5 km NWW of Górki, pine plantation on the mixed deciduous forest habitat, 161 m a.s.l., 51°19'59"N, 21°35'39"E, 13 Sep 2019, *P. Kosiński*, *T. Maliński* & *J. Zieliński* (KOR 55453); Przyłęk, alongside the forest road with ditch, 143 m a.s.l., 51°18'30"N, 21°44'50"E, 1 Aug 2012, ? (KOR 55461); **Świętokrzyskie Province**, Jędrzejów District: 3 km NW of Zagórze, roadside thickets, 259 m a.s.l., 50°36'41.4"N, 20°10'33.7"E, 8 Aug 2011, *B. Piwowarski* (KRA); 2 km E of Małogoszcz, 1 km N of Bocheniec, pine-oak forest, 249 m a.s.l., 50°48'37"N, 20°18'27"E, 11 Jun 2011, *G. Łazarski* (KRA 0472546); Kielce District: 5.8 km NNW of Mniów, 300 m of Gliniany Las, silver fir forest/hornbeam-oak forest, 325 m a.s.l., 51°01'50"N, 20°24'28"E, 23 Aug 2012, *M. Podgórska* (KOR 55457); 0.5 km NNE of Milechowy, pine-oak forest, 262 m a.s.l., 50°49'38"N, 20°20'10"E, 4 Sep 2014, *G. Łazarski* (KRA 0472934); between Szewce and Łaziska, forest margin, 306 m a.s.l., 50°51'00.23"N, 20°27'41.69"E, 15 Jul 2014, *P. Kosiński* & *J. Zieliński* (KOR 51383); 2 km SW of Łaziska, Piekoszów, thickets at the edge of the wet, mixed forest, 313 m a.s.l., 50°51'29"N, 20°20'10"E, 2 Aug 2012, *G. Łazarski* (KRA 0472550); W of Niestachów, slopes of Mt Otrocz, pine plantation on the mixed deciduous forest habitat, 321 m a.s.l., 50°50'24"N, 20°42'60"E, 28 Jun 1934, *K. Kaznowski* (KOR 10598); between Raków and Sadków, edge of the pine plantation on the mixed deciduous forest habitat, 276 m a.s.l., 50°42'40.07"N, 21°03'33.83"E, 17 Jul 2014, *P. Kosiński* & *J. Zieliński* (KOR 51365); Masłów, pine plantation on the oak forest habitat, 328 m a.s.l., 50°54'30"N, 20°42'30"E, 12 Aug 1987, *J. Zieliński* (KOR 31452); Stara Słupia, roadside thickets, 319 m a.s.l., 50°51'37.7"N, 21°04'35.5"E, 16 Sep 1986, *R. Kapuściński* (SKPN); Słowik, roadside thickets, 244 m a.s.l., 50°50'08"N, 20°32'24"E, 5 Jul 1932, *K. Kaznowski* (KOR 10609); Końskie District: 2 km NE of Gaworczów, wilderness “Kopaczka”, thermophilous oak forest, 264 m a.s.l., 51°17'34"N, 20°27'34"E, 20 Jul 2012, *M. Podgórska* (KOR 55458); 1 km W of Cisownik, clearing of pine and hornbeam-oak forests, 290 m a.s.l., 51°05'17"N, 20°25'07"E, 18 Jul 2010, *M. Podgórska* (KRA); Opatów District: 2.2 km S of Julianów, edge of the pine-oak forest on the mixed deciduous forest habitat, 194 m a.s.l., 50°52'50"N, 21°36'50"E, 13 Sep 2019, *P. Kosiński*, *T. Maliński* & *J. Zieliński* (KOR 55457); Ostrowiec District: Łysowody near Ćmielów, roadside thickets, 215 m a.s.l., 50°54'07"N, 20°26'32"E, 8 Aug 2002, *R. Piwowarczyk* (KRA 0354532); Łysowody near Ćmielów, roadside thickets, 215 m a.s.l., 50°54'20"N, 21°33'20"E, 8 Aug 2002, *R. Piwowarczyk* (KRA 0354531); Skarżysko District: 1.5 km S of Grzybowa Góra Mała, roadside thickets/pine forest margin, 237 m a.s.l., 51°7'30.42"N, 20°56'29.80"E, 30 Jun 2003, *M. Nobis* (KRA 0318817); Skarżysko-Kamienna, Piękna street, roadside thickets, 228 m a.s.l., 51°07'12.37"N, 20°54'24.81"E, 21 Aug 2003, *M. Nobis* (KRA 0318828 & 0318829); Starachowice District: Jagodne Małe, forest margin, 227 m a.s.l., 51°08'44.49"N, 20°59'41.41"E, 11 Jul 2003, *M. Nobis* (KRA 0320966 & 0320965); Marcinków, railway embankment, 228 m a.s.l., 51°05'34.88"N, 20°58'54.03"E, 9 Aug 2003, *M. Nobis* (KRA 0320960); Wąchock, thickets by ruins of the manor house, 214 m a.s.l., 51°4'41.9"N, 21°00'55"E, 29 Jul 2002, *M. Nobis* (KRA); W of road between Lubienia and Marcule, along the road in the mesic deciduous forest, 242 m a.s.l., 51°04'23.88"N, 21°11'41.77"E, 7 Aug 2002, *M. Nobis* (KRA 0322795 & 0322796); Szydłowiec District: S of Barak Niwy, roadside thickets/forest margin, 252 m a.s.l., 51°10'49.45"N, 20°51'31.49"E, 26 Jun 2003, *M. Nobis* (KRA 0318818); Gąsawy Rządowe Niwy, roadside thickets/forest margin, 237 m a.s.l., 51°11'25.89"N, 20°56'41.12"E, 11 Jul 2003, *M. Nobis* (KRA 0318824).

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Supplementary material 1

Map and photos

Authors: Piotr Kosiński, Tomasz Maliński, Marcin Nobis, Magdalena Rojek-Jelonek, Dominik Tomaszewski, Monika Dering, Jerzy Zieliński

Data type: species data

Link: https://doi.org/10.3897/phytokeys.185.71193.suppl1

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RESEARCH ARTICLE

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Lespedeza danxiaensis (Fabaceae), a new species from Guangdong, China, based on molecular and morphological data

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Abstract

Lespedeza danxiaensis (Fabaceae), a new species from Danxiashan National Nature Reserve in Guangdong Province, is described and illustrated. The new species is morphologically similar to *Lespedeza pilosa*, but it can be easily distinguished by its thin leathery leaflets and long peduncles. Phylogenetic analysis based on ITS confirmed that the new species belongs to *Lespedeza* subg. *Macrolespedeza*. The new species is the first known species of *Lespedeza* endemic to Danxia landform and is currently only known from Mount Danxia, Guangdong.

Keywords

Danxia landform, Guangdong, Leguminosae, new species, taxonomy

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[*] The authors contributed equally to this work.

Introduction

Lespedeza Michx. (Fabaceae) is a member of the subtribe Lespedezinae (Hutch.) Schub. of the tribe Desmodieae (Benth.) Hutch. The genus is characterised by shrubs, subshrubs or perennial herbs with tri-foliolate leaves (Huang et al. 2010; Ohashi and Nemoto 2014). *Lespedeza* has a disjunct distribution being present in both East Asia and North America, and consists of 46 species including the recently described *L. pseudomaximowiczii* D. P. Jin, Bo Xu bis & B. H. Choi and *L. hengduanshanensis* (C.J. Chen) Bo Xu bis, X.F. Gao & Li Bing Zhang (Ohashi and Nemoto 2014; Xu et al. 2014; Jin et al. 2018). The genus is traditionally divided into two subgenera, viz. *Lespedeza* subg. *Lespedeza* and *L.* subg. *Macrolespedeza* (Maxim.) H. Ohashi, based on the presence or absence of cleistogamous flowers (Ohashi 1982; Huang et al. 2010; Xu et al. 2012). Molecular phylogenetic studies, using nrITS and five chloroplast fragments (*rpl*16, *rpl*32-*trn*L, *rps*16-*trn*Q, *trn*L-F and *trn*K/*mat*K), showed that subg. *Lespedeza* is paraphyletic since the North America taxa (belonging to *L.* subg. *Lespedeza*) are sister to East Asia taxa that included members of both subgenera (Xu et al. 2012). Based on these results, Ohashi and Nemoto (2014) re-circumscribed both subgenera and confined *L.* subg. *Lespedeza* to North America, while *L.* subg. *Macrolespedeza* was confined to Asia.

During a botanical expedition to Danxiashan National Nature Reserve, Renhua County, Guangdong Province from May to October, 2020, we discovered an unknown species of *Lespedeza*. It is similar to *L. pilosa* (Thunb.) Siebold & Zucc. in indumentum (densely villous throughout), procumbent stems and ovate to obovate leaflets, but differs from the latter by its leathery leaflets, pinkish corolla and longer peduncles of chasmogamous flowers. After carefully checking specimens and literature, together with a molecular phylogenetic analysis based on Internal Transcribed Spacers (ITS), we demonstrated it is indeed a new species; thus here, we describe and illustrate it.

Materials and methods

Morphological study

The morphological characters were examined, based on the living plants and specimens kept in the herbaria **IBSC**, **NPH**, **SWFC** and **SYS**, herbarium acronyms as in Thiers (2021).

Taxon sampling and molecular analyses

Three individuals of *L. danxiaensis* were collected from Danxiashan National Park, Guangdong province, China from July to September in 2020 (Fig. 1). Voucher specimens were deposited in the Herbarium of Sun Yat-sen University (SYS). The nuclear DNA Internal Transcribed Spacers (ITS) was used for reconstructing the phylogeny of the new species and its related taxa (Xu et al. 2012). A total of 45 ac-

Figure 1. Satellite image for the location of *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang.

cessions, representing 33 species of *Lespedeza* [including two nominal species viz. *L. nipponica* Nakai and *L. japonica* L. H. Bailey, which had been synonymised with *L. formosa* (Vogel) Koehne (Hatusima 1967) or *L. thunbergii* (DC.) Nakai (Ohashi et al. 2009)] and one species of a related genus, *Campylotropis macrocarpa* (Bunge) Rehder was sampled for outgroup comparison. The GenBank accession numbers are listed in Appendix I. Most sequences were downloaded from GenBank, except for the new species, which was newly sequenced in the present study. Three samples of the new species were sequenced and were identical, of which only one sequence (MZ468553) was selected for the phylogenetic analysis. Genomic DNA was extracted from silica-gel-dried leaves using the modified 2 × CTAB procedure of Doyle and Doyle (1987). The ITS sequences were amplified with primer pairs ITS4/ITSA, with PCR amplification and sequencing following Xu et al. (2012). The phylogenetic relationships were assessed using the Maximum Likelihood (ML) method, which was constructed using the programme IQ-TREE (Nguyen et al. 2015).

Results

Molecular phylogenetics

The aligned sequences of ITS for phylogenetic analyses are 702 bp in length. *Lespedeza* was recovered as monophyletic in the resulting phylogenetic tree in this study (LP: 100, Fig. 2). The North American *Lespedeza* taxa were clustered into a clade

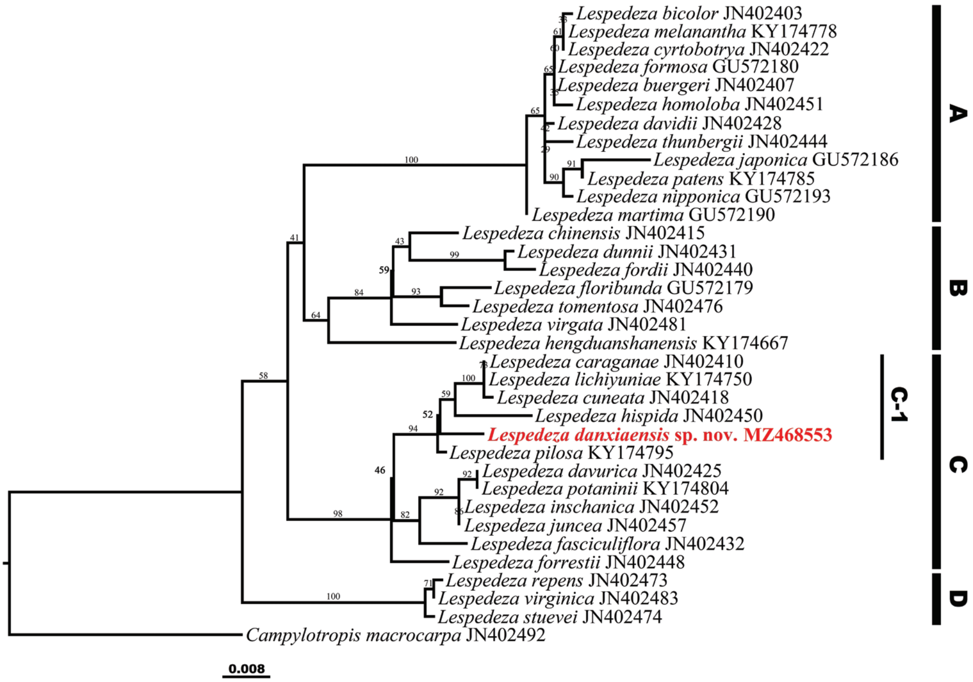

Figure 2. Phylogenetic relationships amongst 33 species of *Lespedeza* and *Campylotropis macrocarpa* based on ITS sequences using Maximum Likelihood analysis, bootstrap value of the Maximum Likelihood (LP) are shown along the branches. The new species described in this study is shown in bold and red type.

(clade D) as sister to the Asian taxa, of which were divided into three clades (viz. clade A, B and C) (LP: 100, Fig. 2). The putative new species is deeply nested within the clade C and was strongly supported as a member of subclade C-1 consisting of *L. caraganae* Bunge, *L. cuneata* G. Don, *L. hispida* (Franch.) T. Nemoto & H. Ohashi, *L. lichiyuniae* T. Nemoto, H. Ohashi & T. Itoh and *L. pilosa* (Thunb.) Siebold & Zucc. (LP = 94, Fig. 2).

Morphological comparison

A detailed morphological comparisons of the new species with the five closely related species within subclade C-1 are summarized in Table 1. In morphology, the putative new species is most similar to *L. pilosa*, sharing such features as procumbent stem, ovate to obovate leaf blades, and plant covered densely villous indumentum. However, the new species differs from the latter by leathery leaflets, longer peduncles of chasmogamous flowers, and pink to pale purple corolla (Table 1, Fig. 3). The other four species included in subclade C-1 could be easily distinguishable from the new species by their habits (stem erect vs. stem procumbent), narrow leaf shape (oblong-linear to narrowly obovate leaf vs. ovate, obovate to subrounded), and shorter peduncles (0.5–1.0 mm vs. 11–28 mm) (Table 1).

Table 1. Morphological comparison of *Lespedeza danxiaensis* with its closest relatives.

Characters	*L. danxiaensis*	*L. pilosa*	*L. caraganae*	*L. cuneata*	*L. hispida*	*L. lichiyuniae*
Habit	Procumbent	Procumbent	Erect	Erect or ascending	Erect or ascending	Erect or ascending
Leaf texture	Leathery or thin leathery	Papery	Papery	Papery	Papery	Papery
Leaf shape	Ovate, obovate to subrounded	Broadly obovate or obovate	Oblong-linear	Cuneate or linear-cuneate	Narrowly obtriangular or narrowly obovate	Narrowly obovate
Adaxial surface of leaflet	Pubescent with ± adpressing hairs, more dense along the margin	White ascending-pilose	Subglabrous	Subglabrous	Glabrous	Glabrous
Abaxial surface of leaflet	Densely pubescent with ± adpressing hairs and more dense along the veins	White ascending-pilose	Adpressed hairy	Densely adpressed hairy	Densely adpressed or ascending pubescent	Densely appressed hairy
Peduncles of chasmogamous flowers (mm)	(2–) 11–28	0.5–1	0.5–1	Short	Ca. 1	Short
Flower color	Pink to pale purple	Yellowish white or white	White or yellow	Yellowish or white	White	Pink or pale purple

Taxonomic treatment

Lespedeza danxiaensis Q. Fan, W.Y. Zhao & K.W. Jiang, sp. nov.

urn:lsid:ipni.org:names:77222602-1
丹霞铁马鞭

Type. China. Guangdong: Renhua County, Danxiashan National Nature Reserve, 24°56'N, 113°45'E, 290 m a.s.l., 30 Sept 2020, *Q. Fan 18409* (holotype, SYS!; isotypes IBSC!, NPH!, SWFC!, SYS!). (Figs 3, 4)

Diagnosis. *L. danxiaensis* is most similar to *L. pilosa* morphologically both being densely villous throughout, and having procumbent stems with ovate to obovate leaflets, but differs from the latter by its leathery leaflets with obviously concave veins (vs. leaflets papery, veins slightly concave), pink to pale purple corolla (vs. corolla yellowish-white to white, with purple spots at base of the standard) and longer peduncles of chasmogamous flowers (1.1–2.8 cm vs. peduncles of chasmogamous flowers rather short, 0.5–1.0 mm in *L. pilosa*).

Description. Perennial herbs, evergreen, with densely erect or ascending villous hairs throughout, turn sparse when old. **Stems** procumbent or ascending, woody at base, 50 cm tall. **Leaves** alternate, 3-foliolate; stipules persistent, ovate-triangular to triangular-lanceolate, apex acute, 3.5–4.5 mm, with 3–5 veins, sparsely pubescent; petioles 1.4–3.8 cm, densely pubescent; rachis 0.5–1.3 cm, densely pubescent, leaflets leathery, adaxially green, pubescent with ± adpressed hairs, more dense along the margin, abaxially greyish-green, more densely pubescent with ± adpressing hairs and more dense along the veins, lateral veins 8–12 pairs, obviously concave adaxially and prominent abaxially; terminal leaflets slightly larger than lateral ones, ovate to obovate, 2.2–3.8 × 1.5–2.5 cm, obtuse at apex, apiculate, rounded at base; lateral leaflets ovate to sub-

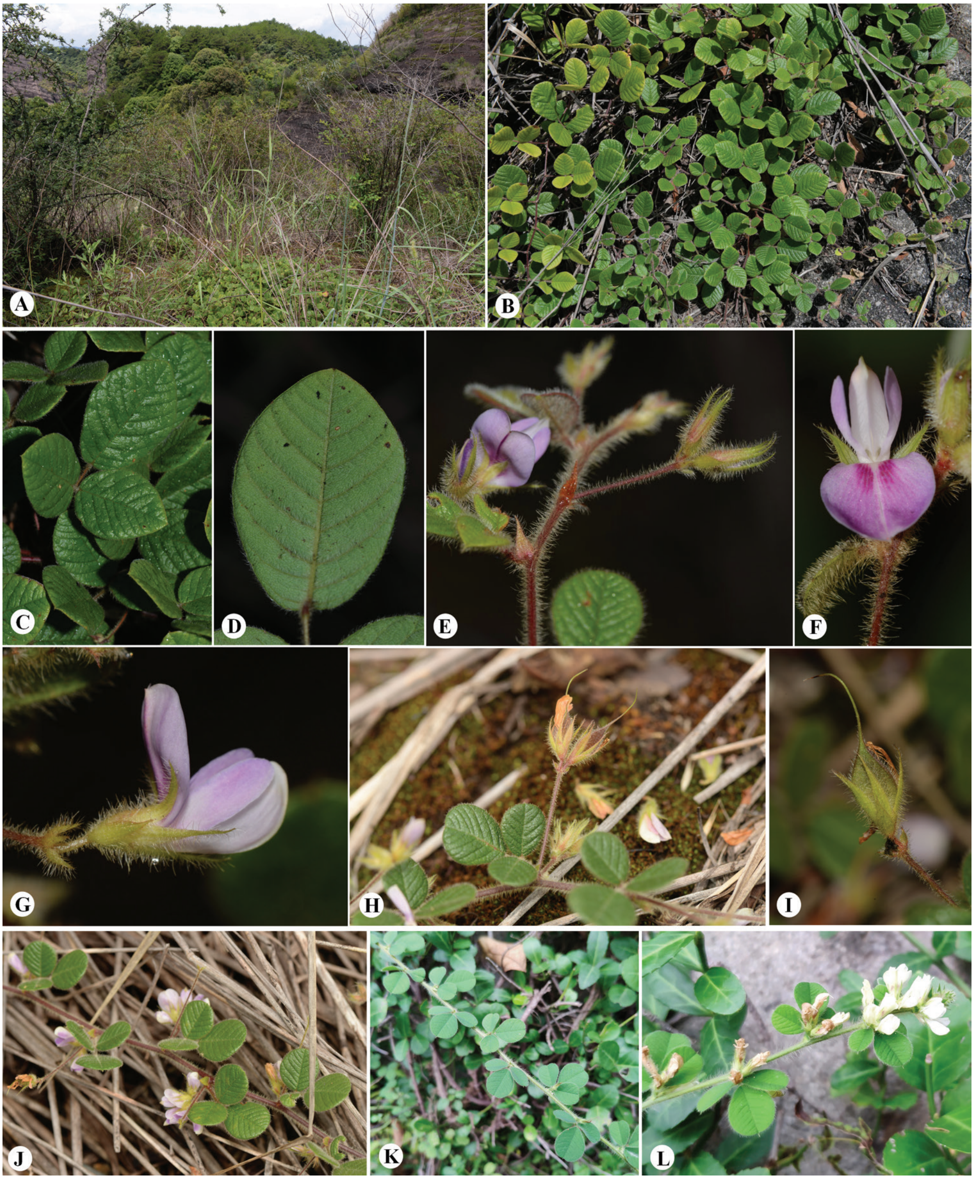

Figure 3. *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang and *L. pilosa* (Thunb.) Siebold & Zucc. *L. danxiaensis* (**A–J**) **A** habit, bushwood on the mountaintop of *Danxia* landform **B** plant, stems procumbent **C** adaxial view of leaf, surface green, leather **D** abaxial view of one leaflet, surface greyish-green with densely pubescent **E** flowering branchlet with flower bud, stipule triangular-lanceolate, apex acute **F** front view of flower **G** lateral view of flower, bracteoles long ovate, sepals narrowly lanceolate **H** fruiting branchlet, show the long peduncles **I** fruit, densely pubescent, stamens persistent **J** flowering branchlet, peduncles usually longer than 1 cm, flower pink to pale purple, young branch reddish brown *L. pilosa*(**K–L**) **K** branchlet with unripe fruit, leaf papery **L** flowering branchlet, peduncles short, flower white, young branch green. (Photographs: **A-J** by Qiang Fan **K–L** by Kai-Wen Jiang).

rounded, 1.7–3.0 × 1.4–2.3 cm; petiolule ca. 1 mm; the leaves on flowering branches obviously smaller (with rachis 2–5 mm long; terminal leaflets obovate, 1.2–1.8 × 0.8–1.7 cm, apex obtuse or emarginate, broadly cuneate at base, lateral ones rounded to obovate, 0.9–1.5 × 0.7–1.2 cm). **Inflorescence** a pseudoraceme, 1–2 axillary, with 2–4 flowers per inflorescence, 2-flowered per node; peduncles of chasmogamous flowers slender and pubescent, (0.2–)1.1–2.8 cm, those of cleistogamous flowers reduced to 1–4 mm, on upper part of stems sometimes reduced; bracts 2 per node, narrowly ovate-triangular to broadly triangular, acute at apex, 1.5–3.3 mm, sparsely pubescent adaxially, glabrous abaxially, 3–5-veined. **Pedicel** 0.5–2.0 mm, pubescent; bracteoles 2, adnate to the base of the calyx, shorter than the calyx tube, oblong-ovate to ovate-lanceolate, 3.5–5.5 mm, sparsely pubescent, 5(–7)-veined. **Calyx** deeply 5-lobed almost to the base, densely pubescent adaxially, glabrous abaxially; tube ca. 1 mm; lobes lanceolate, sub-equal, 7–8 × ca. 1 mm, acute at apex. **Corolla** exserted (absent in cleistogamous flowers), pink to pale purple; standard pale purple, with dark purple spots at base, longer than wings and keels, inflexed-auriculate at base, lamina 7.5–8.0 × 6.5–7.0 mm, broadly elliptic to sub-orbicular, apex obtuse or emarginate, attenuate to a claw ca. 1 mm long at base; wings pale purplish-white, slightly shorter than keels, 7.5–8.3 mm with lamina 5.5–6.0 × 2.3–2.6 mm, narrowly ovate, obtuse at apex, slightly auriculate at base, with a basal claw ca. 2.5 mm; keel petals white to pale purplish-white, 7.5–8.5 mm with lamina 5.5–6.0 × 2.8–3.0 mm, obovate to elliptic, obtuse at apex, attenuate to a claw ca. 2.5 mm at base. **Stamens** glabrous, (9+1) diadelphous, ca. 9 mm, curved upwards in distal part; staminal tubes ca. 5 mm; anthers uniform, ovate, ca. 0.5 mm. **Pistils** ca. 10 mm, longer than stamens (shorter than stamens in cleistogamous flowers); ovary narrowly elliptic, shortly stipitate, style filamentous, curved upwards in distal part, ascending-pubescent; stigma terminal, capitate. **Pods** brownish, 1-seeded, elliptic, style persistent at apex, rostrate, 7–9 × ca. 3 mm, densely ascending-pubescent; those of cleistogamous flowers not seen. **Seeds** ovate, ca. 3.0 × ca. 1.4 mm.

Phenology. Flowering from June to October, fruiting from September to December.

Etymology. The specific epithet refers to Mount Danxia, the locality of the type collection. The Chinese name of the new species is here given as 丹霞铁马鞭 (Dān xiá tiĕ mǎ biān), in which "丹霞" is the Chinese name for Mount Danxia, as well as "铁马鞭" being the common name for *Lespedeza pilosa* and its allies.

Distribution, ecology and habitat. *Lespedeza danxiaensis* is currently known only from a few populations on Mount Danxia in Renhua County, Guangdong Province of China. It was observed to occur in bushwood on the mountaintop of Danxia landform at elevations between 270 and 310 m; plants in association included *Osteomeles subrotunda* K. Koch, *Abelia chinensis* R. Br., *Lagerstroemia indica* L., *Selaginella tamariscina* (P. Beauv.) Spring etc.

Conservation status. The known localities of *Lespedeza danxiaensis* are in Danxiashan National Nature Reserve where they are well protected. However, its population size is quite small. There are fewer than 100 individuals surviving in an area of about 200 m^2 in the currently known localities. We carried out several field surveys in 2020

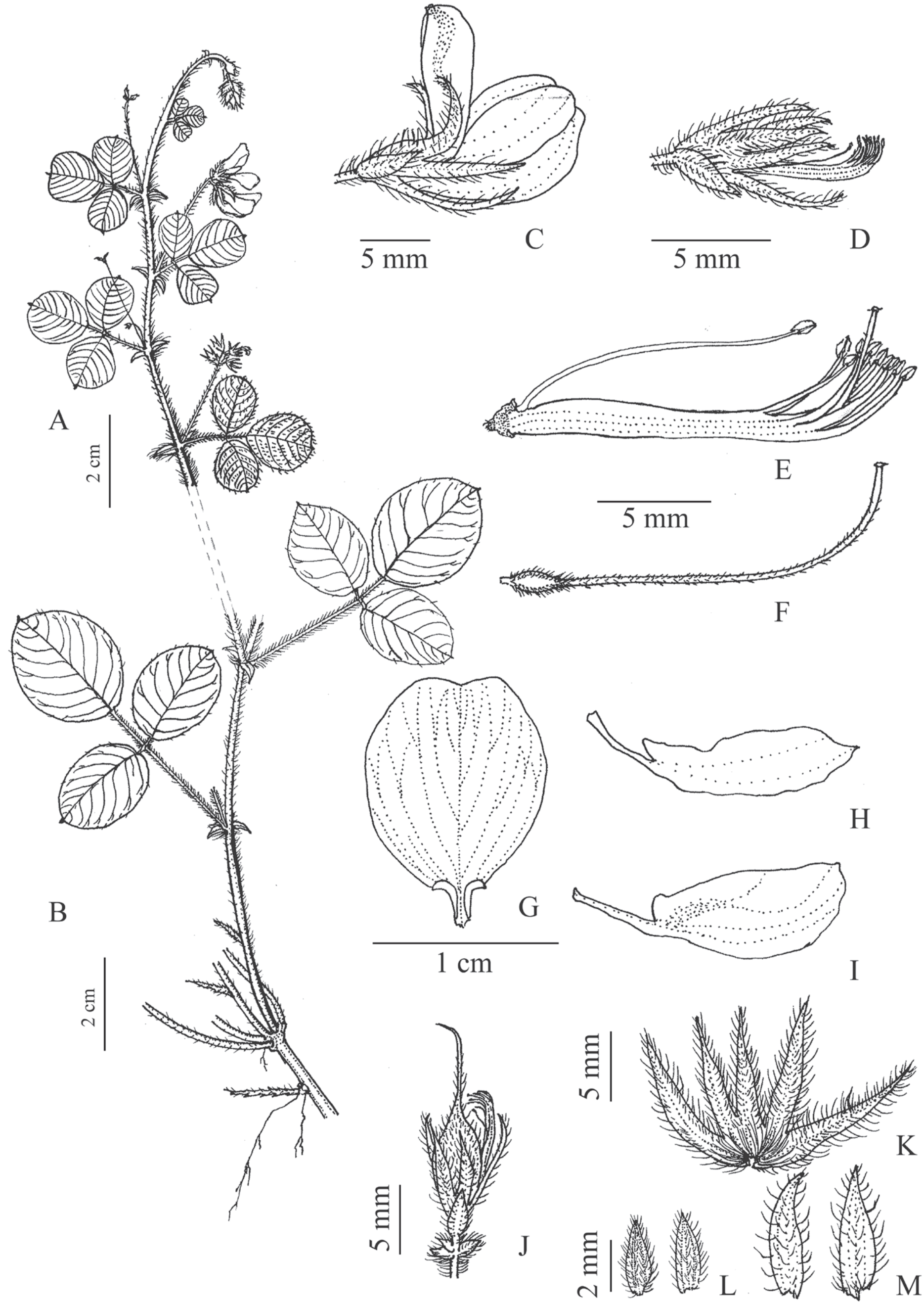

Figure 4. *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang **A** upper portion of plant **B** lower portion of plant **C** chasmogamous flower **D** cleistogamous flower **E** staminal tube **F** pistil **G** standard **H** wing-petal **I** keel-petal **J** chasmogamous fruit **K** abaxial view of calyx, flattened **L** bracts **M** bracteoles. (Drawn by Rong-En Wu).

from May to October, but no other populations were found. Due to its limited distributional range and small population size, *Lespedeza danxiaensis* is here recommended as **Critically Endangered (CR, B2a)** according to IUCN Categories (IUCN Standards and Petitions Subcommittee 2019).

Additional specimens examined (paratypes). China. Guangdong: Renhua County, Danxiashan National Nature Reserve, 24°56'N, 113°45'E, 290 m a.s.l., 3 July 2020, *Q. Fan 18027* (IBSC, NPH, SWFC, SYS); *ibid*., 14 August 2020, *Q. Fan & Y. S. Huang 18130* (IBSC, NPH, SYS).

Discussion

It is obvious that the new species belongs to *Lespedeza* due to its persistent bracts with two flowers inside, non-articulate pedicels, and 1-seeded pods (Fig. 3). Our molecular phylogenetic results further support the inclusion of the new species within *Lespedeza* subg. *Macrolespedeza* re-circumscribed by Ohashi and Nemoto (2014) (Fig. 2). The most conspicuous character of *L. danxiaensis* is its procumbent stems. There are only three procumbent *Lespedeza* species formerly recorded in China, i.e., *L. fasciculiflora*, *L. hengduanshanensis*, and *L. pilosa*. However, the former two species, occurring in western China (northwestern Yunnan, western Sichuan and Tibet) (Huang et al. 2010; Xu et al. 2014), are distantly related to the new species in the phylogenetic tree (Fig. 2). The third species *L. pilosa* is close to the new species, but they differ in the leaf texture, flower color, and the peduncle length of the chasmogamous flowers as described above. In addition, the ITS sequences of the three individuals of the new species are identical and no heterozygous sites were detected in these sequences, indicating that *L. danxiaensis* is not of hybrid origin, but a distinct species.

Lespedeza danxiaensis is current only known from the type locality, i.e. Mount Danxia, and only one population with fewer than 100 individuals was found by the authors. They grow in the special habitat of the Danxia landform, confined to the sub-top area of a peak. The special habitat may lead the phenomenon in which the number of this species is extremely small, thus the conservation of the species, including *ex situ* and *in situ* conservation, is urgently needed. *Lespedeza danxiaensis* has a procumbent habit, usually growing in patches on the ground, and is drought-tolerant. Our observations found that the above-ground part of the species survives drought by dropping many leaves during the dry season. Thus, this species may be suitable as a slope protection or soil-and-water conservation plant, which has potential development and application value.

Acknowledgements

We are deeply grateful to Mrs Rong-En Wu for her excellent illustrations in the manuscript. We thank the curators and staff of herbaria IBSC, NPH, SWFC and SYS for accepting and storing the type specimens. This study was supported by Guangdong Provincial Construction Project of Agricultural Science and Technology Innovation and Extension System (2020KJ264), Guangdong Special Fund for Natural Resources Management and Ecological Forestry Construction (2021GJGY001), the Foundation of Administrative Committee of Danxiashan National Nature Park (2016-0293) and the project of the Fourth Survey of Chinese Traditional Medicine Resources (2019-302-001 and 2019-303-001).

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Appendix I

Table A1. List of the GenBank accession numbers of the ITS sequences of sampled species in this study.

Species	GenBank Accession Number
Campylotropis macrocarpa (Bunge) Rehder	JN402492
Lespedeza bicolor Turcz.	JN402403
Lespedeza buergeri Miq.	JN402407
Lespedeza caraganae Bunge	JN402410
Lespedeza chinensis G. Don	JN402415
Lespedeza cuneata G. Don	JN402418
Lespedeza cyrtobotrya Miq.	JN402422
Lespedeza danxiaensis Q. Fan, W. Y. Zhao & K. W. Jiang	MZ468553 MZ468554 MZ468555
Lespedeza davidii Franch.	JN402428
Lespedeza davurica (Laxm.) Schindl.	JN402425
Lespedeza dunnii Schindl.	JN402431
Lespedeza fasciculiflora Franch.	JN402452
Lespedeza floribunda Bunge	GU572179
Lespedeza fordii Schindl.	JN402440
Lespedeza formosa (Vogel) Koehne	GU572180
Lespedeza forrestii Schindl.	JN402448
Lespedeza frutescens (L.) Hornem.	JN402454
Lespedeza hengduanshanensis (C.J. Chen) Bo Xu bis, X.F. Gao & Li Bing Zhang	KY174667
Lespedeza hirta (L.) Hornem.	JN402449
Lespedeza hispida (Franch.) T. Nemoto & H. Ohashi	JN402450
Lespedeza homoloba Nakai	JN402451
Lespedeza inschanica Schindl.	JN402452
Lespedeza japonica L.H. Bailey	GU572186
Lespedeza juncea (L. f.) Pers.	JN402457
Lespedeza lichiyuniae T. Nemoto, H. Ohashi & T. Itoh	KY174750
Lespedeza maritima Nakai	GU572190
Lespedeza melanantha Nakai	KY174778
Lespedeza nipponica Nakai	GU572193
Lespedeza patens Nakai	KY174785
Lespedeza pilosa Siebold & Zucc.	KY174795
Lespedeza potaninii V.N. Vassil.	KY174804
Lespedeza repens W.P.C. Barton	JN402473
Lespedeza stuevei Nutt.	JN402474
Lespedeza thunbergii (DC.) Nakai	GU572186
Lespedeza tomentosa Siebold ex Maxim.	JN402476
Lespedeza virgata DC.	JN402481
Lespedeza virginica (L.) Britton	JN402483

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RESEARCH ARTICLE

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Causonis sessilifolia (Vitaceae), a new species from Thailand

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Abstract

A new species, *Causonis sessilifolia*, from Thailand is described, based on morphological and phylogenetical methods. A full description, conservation assessment, a key, images and phylogenetic tree are provided. Diagnostic characters for this species are sessile leaves that are sometimes opposite and inflorescence insertion interfoliar.

Keywords

Causonis, Thailand, new taxon, taxonomy

Introduction

The genus *Cayratia* Juss. in the broad sense (s.l.) has consistently been found to be paraphyletic (Wen et al. 2007; Trias-Blasi et al. 2012; Lu et al. 2013; Parmar et al. 2021). To maintain the monophyly within Vitaceae, the species in *Cayratia* sect. *Discypharia* Suess. (Suessenguth, 1953; Latiff, 1981) which was later treated as *Cayratia* subg. *Discypharia* (Suess.) C.L.Li (Li 1996, 1998; Chen et al. 2007), were placed in the newly-re-instated genus *Causonis* Raf. (Wen et al. 2013; Parmar et al. 2021). This genus can be separated from the *Cayratia* s.l. as it lacks a distinct membrane enclosing the ventral infolds in seeds (Wen et al. 2013; Parmar et al. 2021). *Causonis* can be distinguished from other Vitaceae genera by plants being hermaphroditic, flowers 4-merous, inflorescences mostly axillary, but sometimes pseudo-axillary.

Causonis comprises about 16 species and four varieties and is found in tropical, subtropical and temperate Asia to Australia including the Pacific Islands (Parmar et al. 2021). Trias-Blasi and Parnell (2020) reported two *Causonis* species in Thailand, but the specimen from Nakhon Sawan in the northern floristic region of Thailand, was found to be morphologically distinct from other species reported from the region.

In autumn 2019, an expedition was conducted in a 5-hectare area adjacent to Kriangkrai River in the Nakhon Sawan Province (Fig. 1), because of concerns relating to high levels of deforestation in the lowland floodplain forests in Thailand. Additionally, very few specimens had been collected from this habitat. Generally, these areas are flooded every year during September-November when the water increases by 1–3 metres. This species was found in an open area along the riverbank.

Methods

This study is based on the material collected in October 2019 in Nakhon Sawan. Morphological characters were studied using a hand lens (30–60× magnification) and stereomicroscope and documented by photography. Collected specimens were thoroughly compared with protologues and types of all *Causonis* species occurring in Thailand and neighbouring regions. Additionally, herbarium material of *Causonis*, deposited in K, was studied (herbarium codes according to Thiers 2021). Herbarium vouchers for this study are deposited in BKF. The description follows the style and level of details outlined in Trias-Blasi and Parnell (2020), while the general terminology is based on Beentje (2016). The conservation assessment is based on the most recent version of the guidelines of IUCN Standards and Petitions Subcommittee (IUCN 2012).

Genomic DNA for the new taxon was extracted from silica-gel-dried leaf material using DNeasy Plant Mini Kit Qiagen (Qiagen, Hilden, Germany) following manufacturers' protocols. All other sequences for different taxa were downloaded from GenBank. The DNAs were amplified for four chloroplast loci (*atpB-rbcL*, *trnC-petN*, *trnH-psbA* and *trnL-F*) following the protocols in Lu et al. (2018). The PCR products were purified and examined on a 1% agarose gel before being sent to Majorbio Company in Beijing, China, for sequencing on a Roche 454 sequencer with the same PCR amplification primers using a standard GS FLX Titanium sequencing kit XL+ (454 Life Sciences, Branford, CT, USA). The voucher specimens and the sequences' GenBank accession numbers are provided in Table 1.

Geneious 8.1.9 was used to assemble forward and reverse sequences (Kearse et al. 2012). Geneious was also used to edit contiguous sequences and check chromatograms for base validation. Following that, sequences were aligned using MAFFT 1.3.1 (Katoh et al. 2002) and then manually adjusted in Geneious. On the CIPRES Science Gateway (Miller et al. 2010), phylogenetic analyses were first performed for individ-

Figure 1. Map of the specimen collected (black circle).

ual DNA loci using the Maximum Likelihood (ML) approach in RAxML-HPC2 on XSEDE (8.2.12) using the GTR + G model with 1000 bootstrap replicates (Stamatakis 2014). Single tree analyses did not detect well-supported topological conflicts amongst individual DNA loci (i.e. ML BS < 70%; Hillis and Bull 1993). As a result, for further phylogenetic analyses, the four chloroplast DNA loci were concatenated. For the four chloroplast (4cp) dataset, partitioned ML and Bayesian Inference (BI) analyses were performed and the best fitting models for individual data partitions were selected using PhyML 3.0 (Guindon et al. 2010) with the Akaike Information Criterion (AIC). The nucleotide substitution model GTR + G was found to be the most suitable for *trnC-petN* and *trnL-F* and HKY85 + G for *atpB-rbcL* and *trnH-psbA*. MrBayes 3.2.6 was used to conduct Bayesian analysis on the CIPRES Science Gateway (Ronquist et al. 2012). For a total of 10,000,000 generations, four Markov Chain Monte Carlo analyses were conducted, with one tree sampled every 1,000 generations. The standard deviation between the split frequencies was found to be less than 0.01, indicating that enough generations had been completed. Following the burn-in of the first 25% of trees, the remaining trees were used to determine a 50% majority-rule consensus tree and posterior probabilities (PP). The trees obtained from ML and BI were analysed for topological conflicts through FigTree v.1.4.4 (Rambaut 2018).

Taxonomic treatment

Causonis sessilifolia Trias-Blasi & G.Parmar, sp. nov.

urn:lsid:ipni.org:names:77222603-1

Diagnosis. Morphologically, *Causonis sessilifolia* and *Causonis japonica* (Thunb. ex Murray) Raf. share similarity in possessing 5-foliolate leaves, but the former taxon has sessile leaves and 2–5-furcate tendrils (vs. petiolate leaves and 2–3-furcate tendrils in *C. japonica*).

Table 1. Voucher information and GenBank accession numbers for the sequences used in this study.

Taxon	Voucher No.	Locality	*atpB-rbcL*	*trnC-petN*	*trnH-psbA*	*trnL-F*
Causonis australasica L.M.Lu & Jackes	*AU015* (PE)	Australia, Queensland	MW408585	MW408375	MW408696	MW408491
Causonis clematidea (F.Muell.) Jackes	*Wen 12184* (US)	Australia, New South Wales, Sydney (cult.)	KC166297	KC166475	KC166552	KC166625
Causonis corniculata (Benth.) J.Wen & L.M.Lu	*YSL4758* (PE)	China, Taiwan	MW408551	MW408342	MW408665	MW408460
Causonis daliensis (C.L.Li) G.Parmar & L.M.Lu	*VN2014116* (PE)	Vietnam, Lam Dong	MW408540	MW408333	MW408654	MW408450
Causonis fugongensis (C.L.Li) G.Parmar & L.M.Lu	*CPG36648* (PE)	Myanmar, Kachin	MW408564	MW408355	MW408676	MW408473
Causonis japonica (Thunb.) Raf. var. *japonica*	*Wen 8537* (US)	Japan, Chiba	KC166313	KC166488	KC166564	KC166637
Causonis japonica var. *pseudotrifolia* (W.T.Wang) G.Parmar & J.Wen	*Wen 8085* (US)	China, Chongqing	AB234920	KC166498	KC166573	AB235006
Causonis maritima (Jackes) Jackes	*AU020* (PE)	Australia, Queensland	MW408567	MW408358	MW408679	MW408476
	Wen 9403 (US)	China, Taiwan	KC166314	JF437193	JF437079	JF437299
Causonis mollis (Wall. ex M.A.Lawson) G.Parmar & J.Wen	*CPG23617* (PE)	Vietnam, Vinh Phuc	MW408535	MW408328	MW408650	MW408445
Causonis sessilifolia Trias-Blasi & G.Parmar	Poopath & Duangjai 2511	Thailand, Nakhon Sawan	OK338627	OK338628	OK338629	OK338630
Causonis timoriensis var. *mekongensis* (C.Y.Wu ex W.T.Wang) G.Parmar & L.M.Lu	*CPG18926* (PE)	China, Yunnan	MW408580	MW408370	MW408692	MW408486
Causonis trifolia (L.) Mabb. & J.Wen	*CPG27533* (PE)	China, Yunnan	MW408575	MW408365	MW408687	–
	CPG38701 (PE)	India, Kerala	MW408576	MW408366	MW408688	–
	LA17 (PE)	Laos, Luang Namtha	MW408577	MW408367	MW408689	MW408484
	Wen 7488 (US)	Thailand, Chiang Mai	KC166323	KC166500	KC166574	AB235007
	CPG19178 (PE)	Indonesia, Bali	KC428757	KC428783	KC428800	KC428819
Pseudocayratia pengiana Hsu & J.Wen	*YSL4764* (PE)	China, Taiwan	MW408587	MW408377	MW408698	MW408493
Pseudocayratia speciosa J.Wen & L.M.Lu	*Wen 12026* (US)	China, Fujian	KC166377	–	KC166616	KC166682

"–" represents missing sequences.

Type material. THAILAND. Northern floristic region: Nakhon Sawan, Muang, Kriangkrai subdistrict, abandoned area at the bridge of Kriangkrai Canal, 15°44'40"N, 100°11'9"E, 23 October 2019, M. Poopath & S. Duangjai 2511 (holotype, BKF! (SN229663 (Fig. 5)), isotype BKF! (SN229662)).

Description. Slender herbaceous climber. *Stem* cylindrical, 2–5 mm diameter, branched, glabrous, young stems purplish-green, hairy with some bent hairs to glabrous; tendril 2–5-furcate, slender, wiry, leaf-opposed, cylindrical, with a straight section, then bifurcating and coiling, 5–10 cm long, glabrous. *Leaves* compound, pedately 5-foliolate, alternate or opposite; petiole absent, central petiolule 0.5–1.5(–3) cm long, middle petiolules sessile, lateral petiolules 0.5–2 mm long, mostly glabrous, sometimes with bent hairs; central leaflet blade lanceolate to slightly rhombic, 2–5 by 1–2.5 cm, base cuneate; middle leaflet blade lanceolate, 1–3 by 0.5–1.5 cm; lateral leaflet blade lanceolate often with a single asymmetric acute lobe, 0.5–1.75 by 0.2–1.25 cm, base cuneate; margin broadly denticulate, apex acuminate to mucronate; adaxial and abaxi-

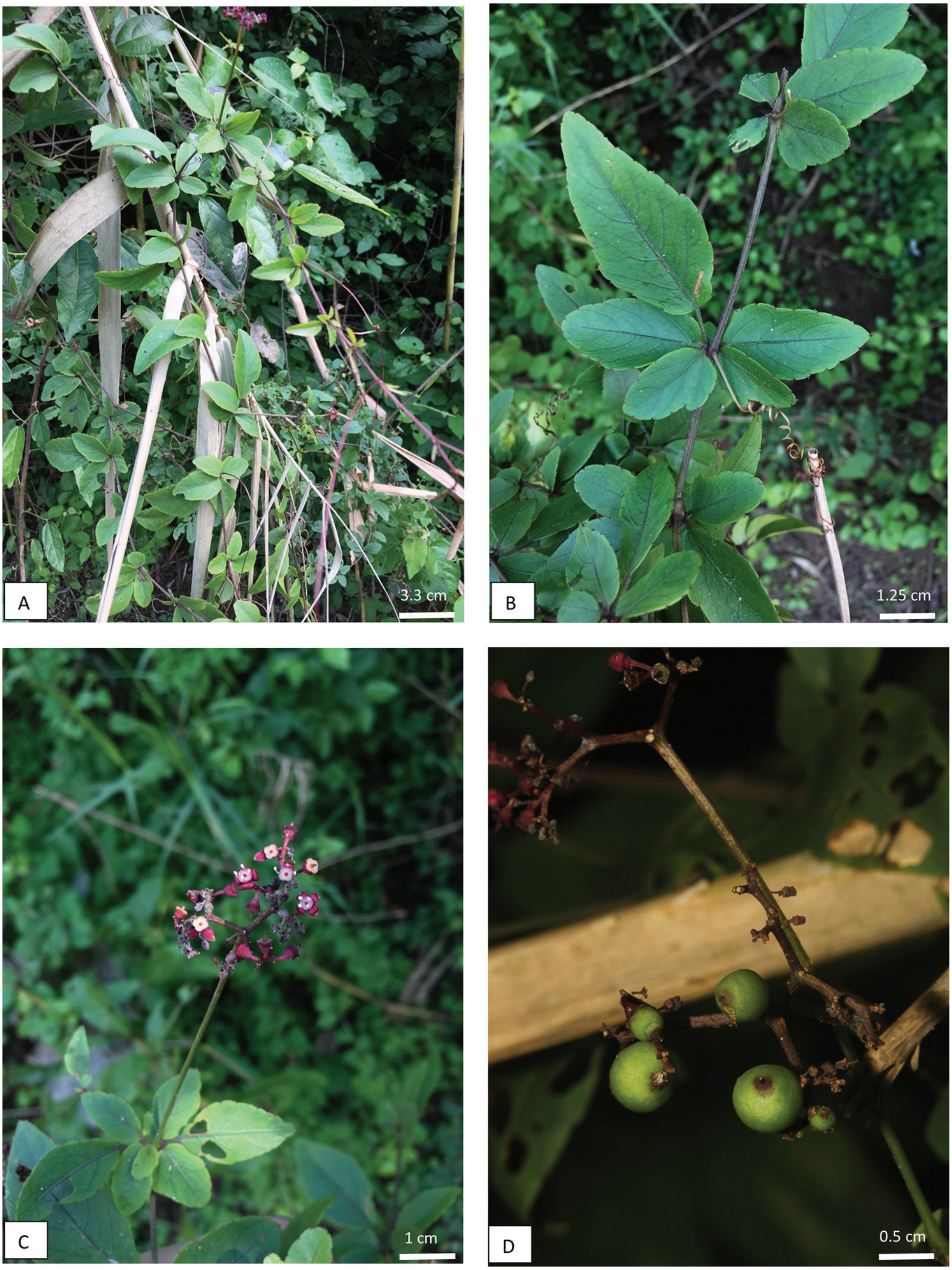

Figure 2. *Causonis sessilifolia* **A** habit **B** pedately 5-foliolate leaves **C** flowers and opposite leaves **D** fruits. Images: Sutee Duangjai and Manop Poopath.

al sides glabrous, mid-rib raised on upper surface, each leaflet with 2–5 pairs of lateral veins, if leaflet lobed then lower lateral vein may be more distinct. *Inflorescence* ramified, interfoliar or pseudo-terminal, mostly dividing dichotomously, with numerous ramifications, 0.7–2 by 1–2.5 cm, lax, erect; peduncle 2–8 cm long, glabrous, pedicel

Figure 3. *Causonis sessilifolia*. Habitat. Images: Manop Poopath.

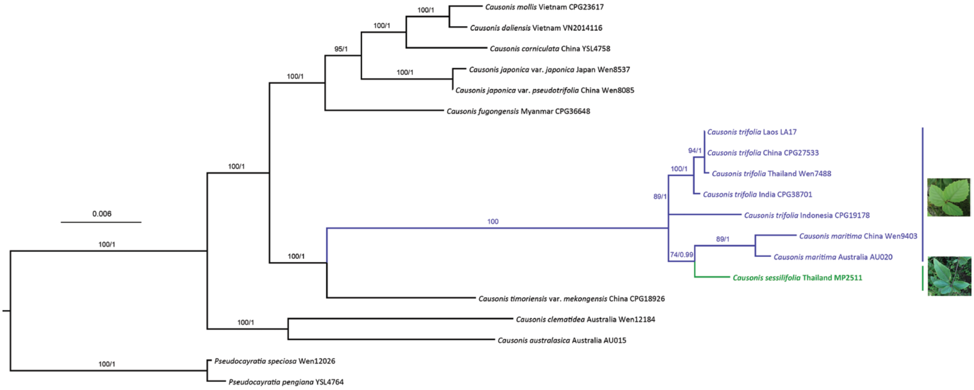

Figure 4. Phylogeny of selected species of *Causonis* including *C. sessilifolia*, based on the combined chloroplast dataset (*atpB-rbcL, trnC-petN, trnH-psbA* and *trnL-F*). Maximum Likelihood bootstrap values and Bayesian posterior probability values are indicated above branches respectively.

0.75–2 mm long, glabrous. Buds ovoid, 1–2 by 1–2 mm. *Calyx* cupuliform, entire, margin sinuate, 0.5–1 by 1–1.25 mm, glabrous, dark red. *Corolla* petals 4, ovate, 1.5–2 by 1.25–2 mm, apex cucullate, glabrous, dark red. *Stamens* 4; filaments flattened, broadening at the base, 1 mm long, cream; anthers elliptic, medifixed, 0.5–1 mm long, cream. *Ovary* adnate to the disc; disc with 4 distinct lobes, cupular, 0.5 by 1.5 mm,

Figure 5. Holotype of *Causonis sessilifolia*.

glabrous, dark red outside and whitish inside. *Style* conical, 0.5 mm long; stigma inconspicuous, dark red. *Fruit* berry, globose, 4–7 mm in diameter, glabrous, smooth, green. *Seeds* 2, 4–5 by 3 mm, adaxial side with two faces, abaxial side convex and ovate with a linear chalazal knot (Fig. 2).

Phenology. Flowering and fruiting in October.

Etymology. The specific epithet "*sessilifolia*" refers to the sessile leaves of the taxon.

Distribution and habitat. Thailand (Northern floristic region, Nakhon Sawan). Lowland floodplain forest, along the bank of canal in open areas; 30 m alt. (Figs 1, 3).

Conservation status. This species is only known from the type locality and, therefore, has an Area of Occupancy (AOO) and Extent of Occupancy (EOO) of 4 km^2. This suggests that it might be Critically Endangered as the AOO is less than 10 km^2 and is only found in one location. The species has been found to grow outside any protected areas and in an abandoned area next to a canal. This could mean the species is more vulnerable than others as it is unprotected. Additionally, all the surrounding areas are used for agriculture and, therefore, it is likely this forested area might also be transformed for this use. Due to this threat, the restricted AOO and number of locations, we think that this taxon could be driven to being Critically Endangered or Extinct in a very short time; therefore, we assess the taxon as VUD2 (IUCN 2012).

Taxonomic remarks. Phylogenetically, this pedately 5-foliolate leaved species lies in a clade previously known with species of exclusively trifoliolate leaves such as *Causonis trifolia* (L.) Mabb. & J.Wen and *Causonis maritima* (Jackes) Jackes (Parmar et al. 2021), but *C. sessilifolia* lacks the petiolate leaves found in *C. trifolia* and *C. maritima*. In particular, this species is phylogenetically most closely related to *C. maritima* (ML BS = 74%; Bayesian PP = 0.99; Fig. 4), but morphologically differs from it in having pedately 5-foliolate leaves (vs. trifoliolate), leaves sometimes opposite (vs. leaves always alternate), leaves sessile (vs. leaves petiolate), inflorescence insertion interfoliar (vs. axillary), calyx and corolla glabrous (vs. hairy).

Key to *Causonis* in Thailand (including *Causonis maritima*)

1 Leaves 3-foliolate **2**
– Leaves 5-foliolate **3**
2 Tendrils 2–3-furcate, tips without adhesive disc; hooked hairs confined to mid-vein on adaxial surface .... **1. *C. maritima***
– Tendrils 3–5-furcate, tips with adhesive disc; hairs all over abaxial and adaxial surfaces .... **2. *C. trifolia***
3 Petiole present; inflorescence leaf-opposed, pseudoaxillary or axillary .... **3. *C. japonica***
– Petiole absent; inflorescence interfoliar .... **4. *C. sessilifolia***

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Leaf epidermal micromorphology in *Aspidistra* (Asparagaceae): diversity and taxonomic significance

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Abstract

Micromorphological characters of leaf epidermis were investigated in 69 species of *Aspidistra* using scanning electron microscopy. Sculpture of epidermis varies from smooth to verrucose and rugose in the genus. The abaxial epidermis of some species bears papillae, whereas the adaxial surface uniformly lacks the papillae. Sculpture type of epidermis and density of papillae are generally found to be stable characters at a species level. The infraspecific variation of epidermis sculpture, where present, ranges from smooth to verrucose or from verrucose to rugose. Micromorphological characters of leaf epidermis are shown to have potential taxonomic significance in *Aspidistra*; in combination with the type of shoot structure, they allow to subdivide the species into 13 groups. The groups are largely incongruent with floral morphological traits. An identification key to the studied species of *Aspidistra* based on vegetative characters (gross leaf and shoot morphology and characters of leaf epidermis) is presented.

Keywords

Aspidistra, epidermis, identification key, papillae, SEM

Introduction

Aspidistra Ker Gawl., belonging to the family Asparagaceae, is a large genus of herbaceous plants which inhabits tropical and subtropical forests of Asia. In our estimate, the genus comprises about 200 species. *Aspidistra* is remarkable for its extremely diverse flower morphology (Tillich 2005, 2008, 2014; Averyanov and Tillich 2012; Tillich et al. 2017; Tillich and Averyanov 2018). Floral characters are most important in taxonomy of *Aspidistra*, whereas vegetative characters are rarely used for species identification: most of the species have similar habit and are hardly distinguishable without flowers. The majority of representatives of *Aspidistra* are characterized by creeping rhizome without aerial shoots. Only a few species have erect stem (e.g. *A. erecta* Yan Liu & C.I Peng and *A. globosa* Vislobokov & Nuraliev), representing a group easily recognizable by vegetative morphology (Vislobokov et al. 2016). Another non-floral character that was shown to have taxonomic significance in *Aspidistra* is the distribution of foliage leaves along the shoot (De Wilde and Vogel 2006; Averyanov and Tillich 2014). Shoots of *Aspidistra* consist of repeatedly developing elementary shoots, each elementary shoot bearing several cataphylls followed by one to several foliage leaves (Vislobokov et al. 2014, 2017). The species of *Aspidistra* can be divided into two groups: the first group is characterized by solitary leaves (i.e., one foliage leaf per elementary shoot), and in the second group the leaves are arranged in tufts (i.e., 3–5 foliage leaves per elementary shoot). Additionally, these groups of species differ in the gross morphology of leaf. In most species of the first group, the leaf is divided into petiole and blade (e.g. *A. arnautovii* Tillich, *A. formosa* (Tillich) Aver. & Tillich, *A. subrotata* Y.Wan & C.C.Huang), with blade of various shape. By contrast, all species of the second group have narrowly elliptic or linear leaves lacking a petiole but gradually tapering towards base (e.g. *A. carnosa* Tillich, *A. hainanensis* W.Y.Chun & F.C.How, *A. viridiflora* Vislobokov & Nuraliev). The second group is much smaller with respect to species number than the first one.

Despite the usefulness of the vegetative characters of *Aspidistra* outlined above, they are far from being enough for identification to the species level, because numerous species often share the same combination of these characters. Thus, the precise identification of *Aspidistra* in non-flowering condition in most cases is impossible at the current state of knowledge. At the same time, identification of sterile plants of *Aspidistra* appears to be highly demandable due to several features of reproductive biology of this genus. The flowers in *Aspidistra* are usually developed at the ground level, often hidden by leaf litter, and their search requires special efforts (Tillich 2005). The field recognition of species of *Aspidistra* is also complicated by their common sympatric occurrence: three or four (and up to six) species are often recorded in a given forest, where they sometimes grow side by side forming mixed populations (Nuraliev et al. 2017). Moreover, the flowering takes place only in a particular season, which differs among the species, so that only a part of individuals of the genus (if any) are usually observed to produce flowers in a given forest. A widely used technique of specimen identification in *Aspidistra* is collecting sterile living material and obtaining the flowers under cultivation (Averyanov and Tillich 2014, 2015; Vislobokov et al. 2019b). However, this method requires a preliminary estimation of the number of the species inhabiting a given area.

Micromorphological characters, including those of vegetative organs, sometimes appear sufficiently diverse to serve as a useful instrument for taxonomy and species identification. This approach has already been successfully applied for *Dracaena* Vand. ex L., another genus of Asparagaceae (Klimko et al. 2018), as well as for certain genera of Caryophyllaceae, Lamiaceae and Myrtaceae (Haron and Moore 1996; Mostafavi et al. 2013; Krawczyk and Głowacka 2015). To date, micromorphology has never been investigated in *Aspidistra*. In the present study, we investigated micromorphological characters of leaf epidermis in this genus. Our goals were (1) to evaluate diversity of adaxial and abaxial leaf epidermis in *Aspidistra* including cell sculpture and density of papillae; (2) to determine infraspecific variation of these characters; (3) to analyze taxonomic significance of characters of leaf epidermis by delineating species groups on the basis of these characters; (4) to compile an identification key to the studied species of *Aspidistra* based on vegetative features, including leaf and shoot morphology and the characters of leaf epidermis.

Materials and methods

Specimens

Fully developed foliage leaves were collected from living plants of *Aspidistra* found in nature (during fieldwork in China and Vietnam) as well as cultivated in the Botanical Garden Munich-Nymphenburg (BGMN), the Botanical Institute of the Russian Academy of Sciences (BIN), the Main Botanical Garden of the Russian Academy of Sciences (MBG) and the Singapore Botanic Gardens (SBG) (Appendix 1). The leaves were fixed and stored in 70% ethanol. All the studied specimens possessed floral material, and identification of the plants used in this study was verified by investigation of floral structure. In total, 113 specimens representing 69 species of *Aspidistra* were involved in the study. Of them, 45 specimens represent type material (including types and paratypes). Each species was represented by 1 to 8 specimens, and a total of 22 species (ca. 32%) were represented by two or more specimens.

Scanning electron microscopy (SEM)

For SEM, a single fragment ca. 5×5 mm was cut out from the leaf blade by a razor blade for each specimen. The fragment was taken from the central part of the leaf blade (equidistant from petiole/leaf base and leaf apex), equidistantly from midvein and leaf margin, between secondary veins. The dissected material was transferred from 70% ethanol to 100% acetone via 80% and 96% ethanol followed by an 1: 1 mixture of ethanol (96%) and acetone (100%). The material was critical-point dried using an HCP-2 critical point dryer (Hitachi, Japan). Dried samples were divided in two equal parts by a razor blade, which then were mounted onto stubs with different sides exposed, using double-sided sticky tape. The mounted specimens were coated with gold using an Eiko IB-3 ion-coater (Eiko Engineering, Japan) and observed using a CamScan 4DV (CamScan, UK) scanning electron microscope at Moscow State University.

Morphological traits

The following traits of leaf epidermis were investigated: (1) size and shape of epidermal cells; (2) fine relief of the outer periclinal cell wall: micro-sculpture of epidermis surface; and (3) curvature of outer periclinal wall: presence and density of papillae on epidermis. Size of epidermal cells and density of stomata were measured once for each specimen. Density of papillae was measured by counting number of papillae within a frame 100×100 μm in at least two repeats in each specimen. Standard terminology of surface sculpturing patterns mainly follows Barthlott (1981).

The identification key was compiled on the basis of original data on leaf micromorphology and data on gross vegetative morphology available from Liang and Tamura (2000), Li (2004) and the species protologues.

Results

Diversity of micromorphological traits

Leaf epidermis of examined species of *Aspidistra* consists of elongated tetragonal cells 60–160 μm long and 10–40 μm wide with straight boundaries. The leaves are amphistomatic. Stomata are of anomocytic type. Density of stomata is 50–170 per 1 mm^2 on abaxial surface, and very low on adaxial side (less than 10 per 1 mm^2). Guard cells are 25–40 μm long and 5–10 μm wide.

Epidermis surface is smooth or sculptured to various degrees (Figs 1–4). Within the observed variation, we recognize two types of epidermis sculpture: *verrucose* and *rugose*. Verrucose surface bears rough irregularities, short wide ridges or projections (e.g. Figs 2e, j, k, o, 3q, r). Rugose surface has numerous fine narrow tortuous folds (e.g. Figs 3e-g, 4m).

Epidermis of about a half of the studied specimens is smooth on both surfaces. In the other specimens, the adaxial and abaxial epidermis is either uniformly or differently micro-ornamented (or one of the surfaces is smooth). Usually the sculpture is pronounced to a greater extent on the abaxial surface than on the adaxial one. Verrucose sculpture, if present, is usually found on both sides, or only abaxially with smooth adaxial epidermis. Rugose epidermis is usually expressed on both leaf sides or rarely only on the abaxial side, with the adaxial side being verrucose. Thus, only the abaxial epidermis is illustrated (Figs 1–4).

Adaxial epidermis of foliage leaves of all studied specimens is uniformly epapillate. Abaxial epidermis of about a half of the studied specimens bears papillae (e.g. Figs 3o, 4n, s). Papillae are hemispherical, 10–30 µm in diameter, with one to several of them per epidermal cell. Density of papillae varies from 1 to 45.5 papillae per 0.01 mm^2. We recognize three categories of papillae density: low, 1–3.5 papillae per 0.01 mm^2; medium, 4–7.5 papillae per 0.01 mm^2; high, 8–45.5 papillae per 0.01 mm^2.

Additionally, we have found that shape and density of papillae on the secondary veins (not employed in the main part of our study) is different in some specimens from those of the epidermis located between the secondary veins.

The main results of investigation of each species (epidermis sculpture and the presence and density of papillae) are presented in Appendix 1.

Infraspecific variation

Size and shape of epidermal cells of investigated species does not reveal any species-specific pattern: their infraspecific variation is nearly as broad as interspecific variation.

The micro-sculpture of abaxial and adaxial epidermis is, in contrast, generally constant (fixed) at a species level. These traits show stability in at least 16 out of 22 species of *Aspidistra* represented by two or more specimens in the present study. We found that only in several species the sculpture of at least one leaf side is variable; it varies either between smooth and verrucose, or between verrucose and rugose. We have not observed any species with variation between smooth and rugose sculpture. For example, in two investigated specimens of *A. arnautovii* adaxial epidermis is verrucose, whereas in the other three specimens (Fig. 4g) the epidermis is smooth (Appendix 1, group X). Epidermis sculpture of *A. formosa* also varies from verrucose to rugose at adaxial and abaxial surface (Fig. 4r). Finally, two studied specimens of *A. erosa* Aver., Tillich, T.A.Le & K.S.Nguyen have verrucose adaxial surface but differ in having rugose vs. verrucose surface of abaxial epidermis (Figs 2j, 3a).

Presence and density of papillae is also stable in 16 out of 22 species of *Aspidistra* represented by two or more specimens. Density of papillae on abaxial epidermis varies only slightly within some species. Appreciable variation was found in *A. arnautovii* (4–7 papillae per 0.01 mm^2; Fig. 4g), *A. bogneri* (38–45.5 papillae per 0.01 mm^2; Fig. 4n), *A. lutea* Tillich (4–6 papillae per 0.01 mm^2; Fig. 4b) and *A. opaca* Tillich (4–5.5 papillae per 0.01 mm^2; Figs 3q, 4k); nevertheless, each of these species fits a single category of papillae density (low, medium or high) proposed above. The most significant variation of this feature was found in *A. connata* Tillich (0–5 papillae per 0.01 mm^2), *A. hainanensis* (0–1.5 papillae per 0.01 mm^2; Figs 1b, j), *A. oviflora* Aver. & Tillich (0–2.5 papillae per 0.01 mm^2; Fig. 1d, l) and *A. subrotata* (0–6 papillae per 0.01 mm^2; Fig. 2q, 3r, 4e); they are categorized as showing variation from absence of papillae to low or medium density of papillae.

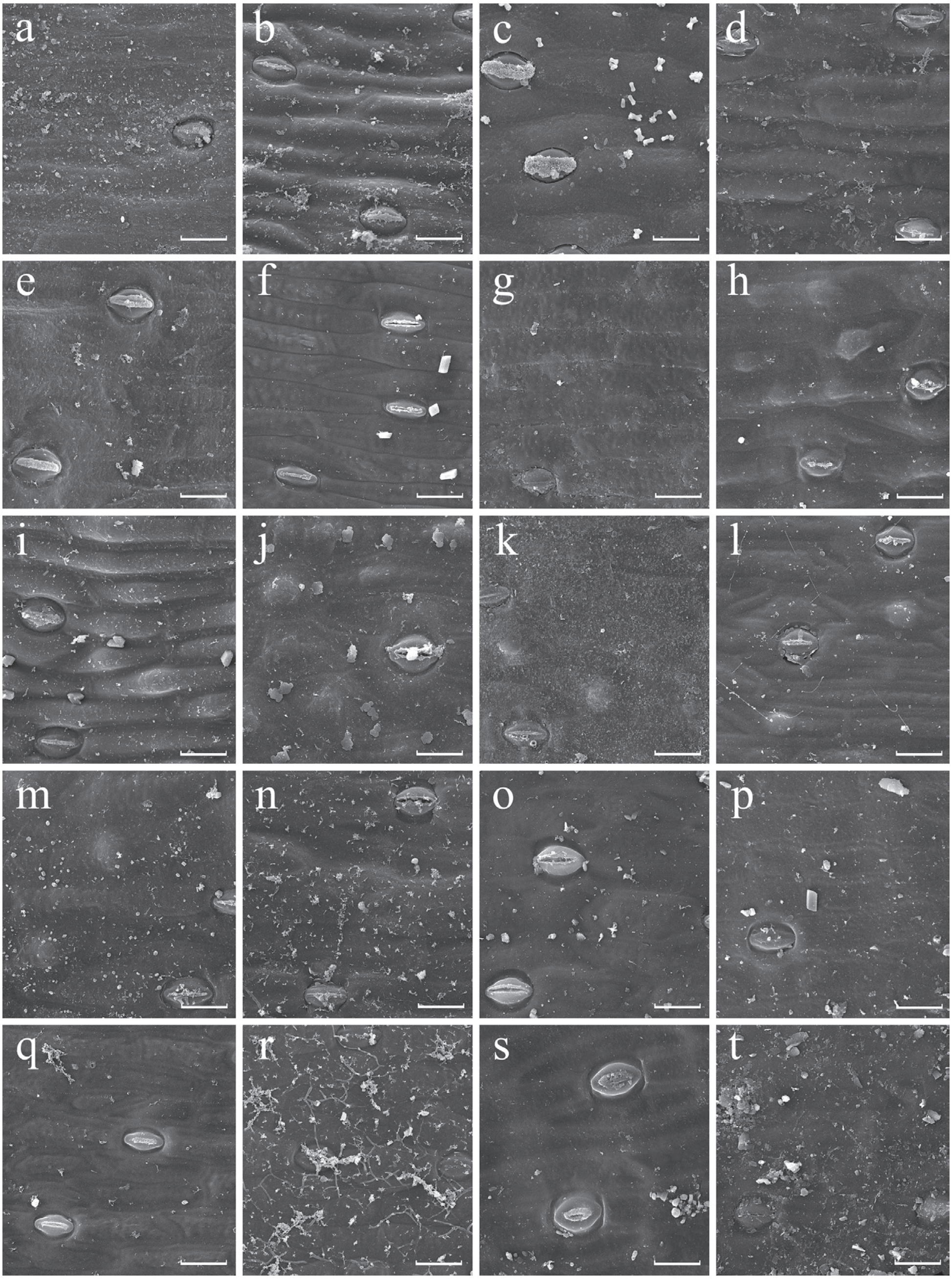

Figure 1. SEM images of abaxial leaf epidermis of *Aspidistra*; morphological groups I (a–g), II (h–m) and III (n–t) (partly). **a** *A. graminifolia* **b** *A. hainanensis* (s.n.) **c** *A. linearifolia* **d** *A. oviflora* (13394) **e** *A. triradiata* **f** *A. viridiflora* **g** *A. yingjiangensis* **h** *A. carnosa* **i** *A. cylindrica* **j** *A. hainanensis* (11/1394) **k** *A. longifolia* **l** *A. oviflora* (2018.14340.01) **m** *A. larutensis* **n** *A. atroviolacea* **o** *A. clausa* **p** *A. claviformis* **q** *A. dolichanthera* (2016.12354.01) **r** *A. erecta* **s** *A. jingxiensis* **t** *A. lurida*. Scale bars: 30 µm.

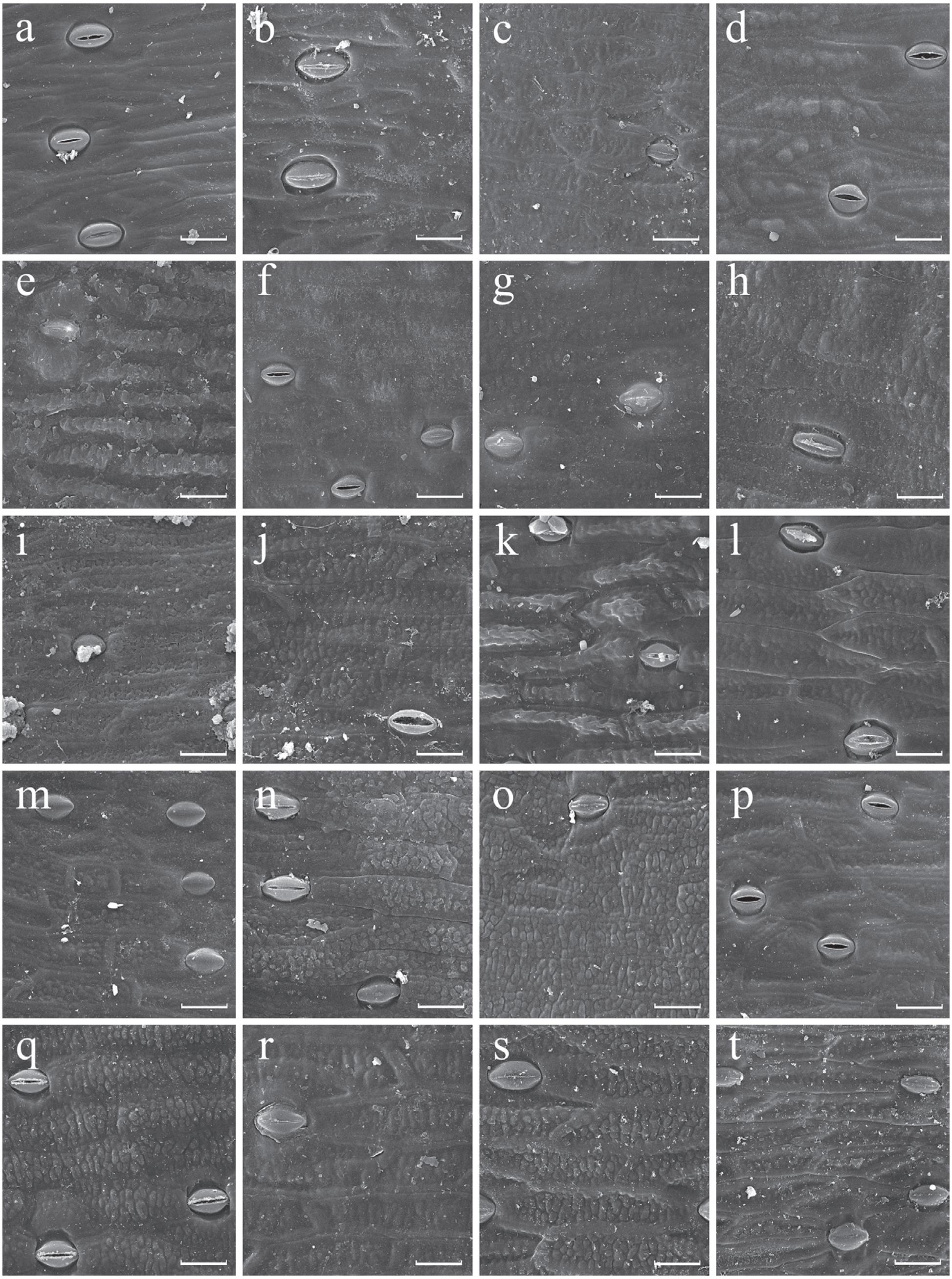

Figure 2. SEM images of abaxial leaf epidermis of *Aspidistra*; morphological groups III (a–c) (partly) and IV (d–t). **a** *A. petiolata* **b** *A. renatae* **c** *A. sessiliflora* **d** *A. basalis* **e** *A. lateralis* **f** *A. medusa* **g** *A. typica* **h** *A. connata* (V/0490) **i** *A. bella* **j** *A. erosa* (JLS2972) **k** *A. globosa* **l** *A. gracilis* **m** *A. laotica* (20122018) **n** *A. mirostigma* **o** *A. phanluongii* (2015.11347.01) **p** *A. sarcantha* (JLS2962) **q** *A. subrotata* (2015.11350.01) **r** *A. sutepensis* **s** *A. truongii* (2013/2461) **t** *A. vietnamensis*. Scale bars: 30 μm.

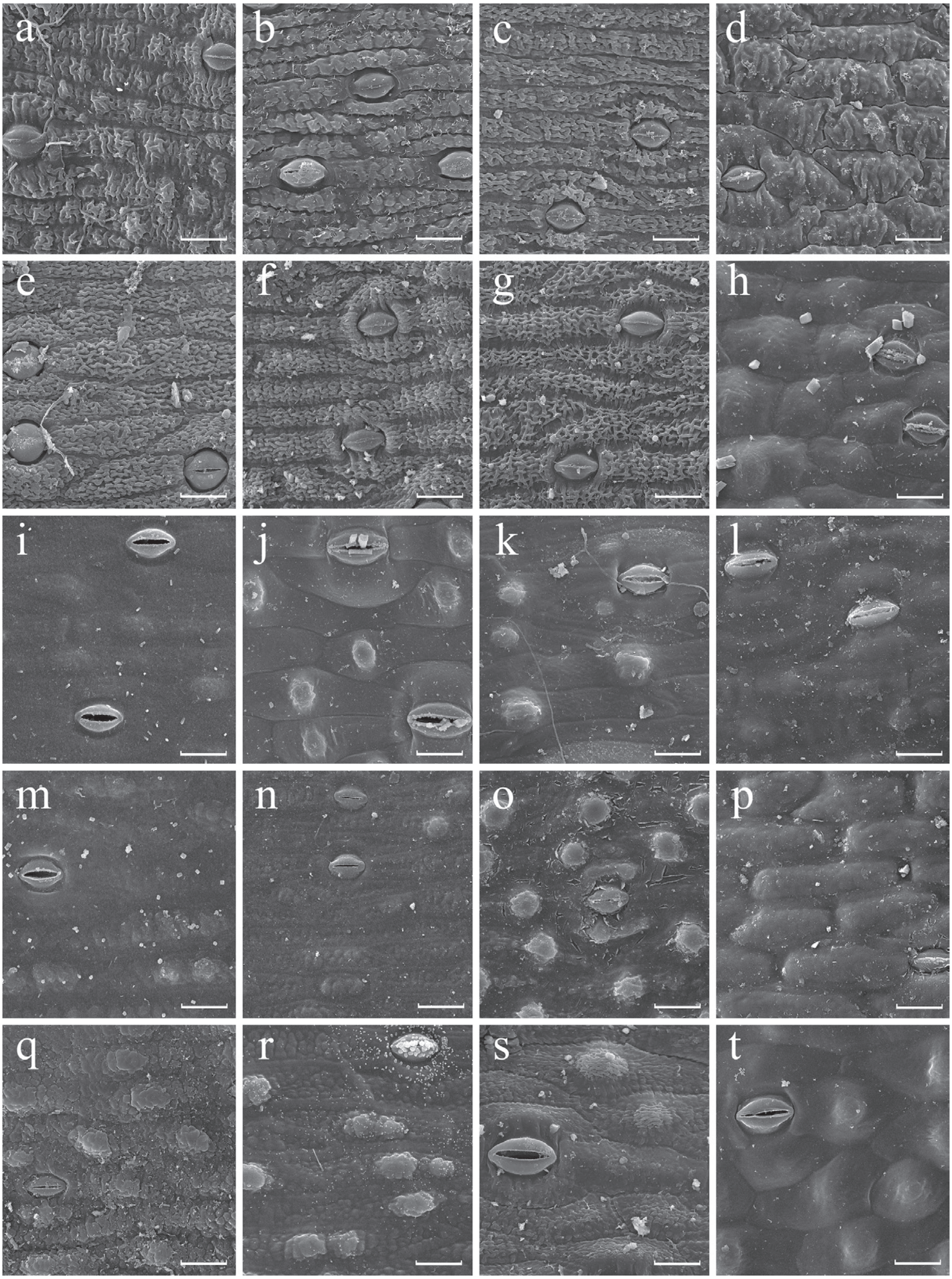

Figure 3. SEM images of abaxial leaf epidermis of *Aspidistra*; morphological groups V (a–g), VI (h–l, o), VII (m, n, p–r), VIII (s) and IX (t) (partly). **a** *A. erosa* (JLS2906) **b** *A. locii* (86-169) **c** *A. lubae* **d** *A. xuansonensis* **e** *A. corniculata* **f** *A. foliosa* (97/2360) **g** *A. multiflora* **h** *A. fungilliformis* (2016.12350.02) **i** *A. geastrum* **j** *A. longipetala* **k** *A. papillata* **l** *A. tillichiana* **m** *A. connata* (97/2358) **n** *A. semiaperta* **o** *A. bicolor* **p** *A. clausa* **q** *A. opaca* (18035) **r** *A. subrotata* (A.s.5) **s** *A. minor* **t** *A. hekouensis*. Scale bars: 30 µm.

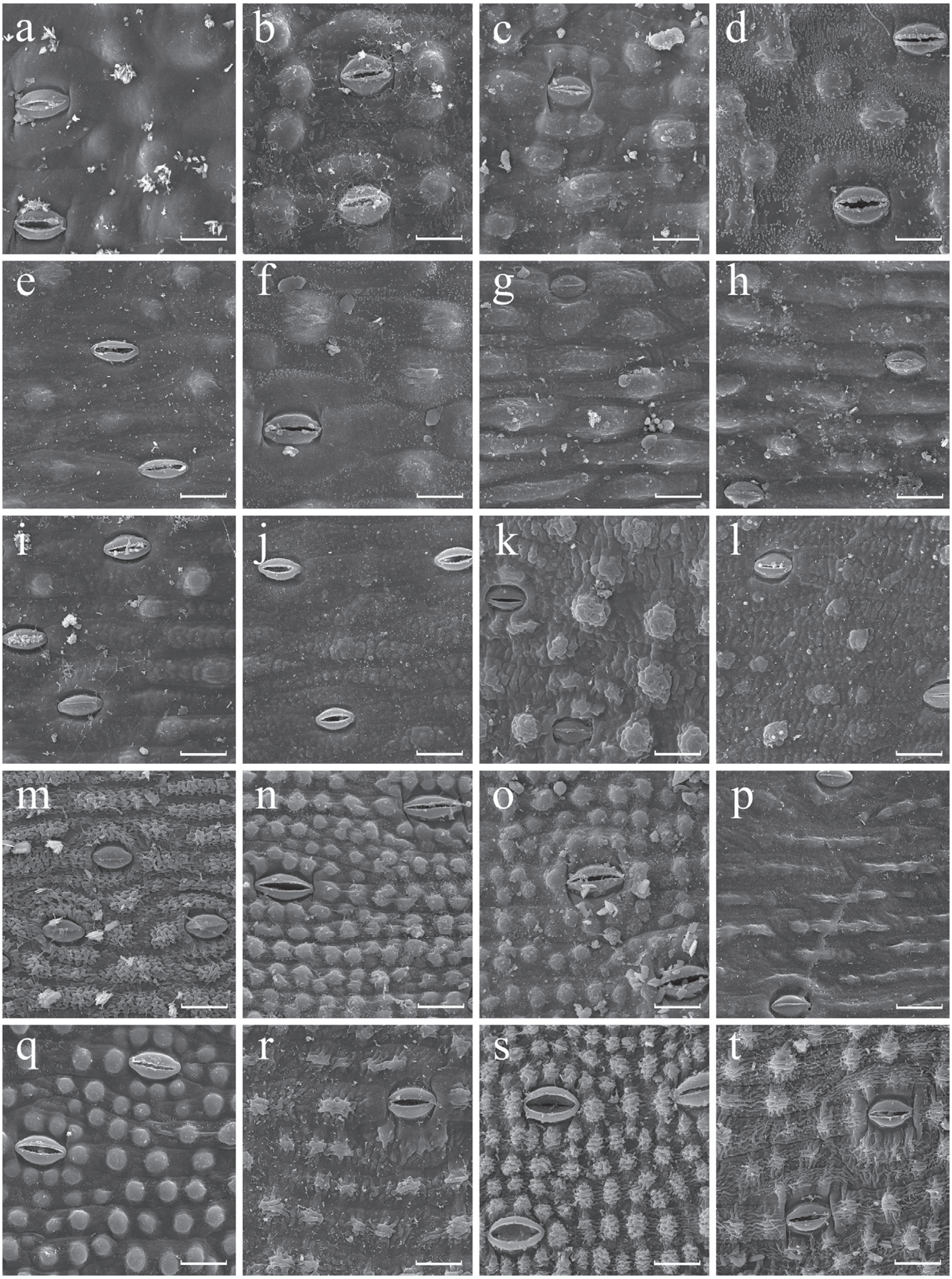

Figure 4. SEM images of abaxial leaf epidermis of *Aspidistra* morphological groups IX (a–f) (partly), X (g–l), XI (m), XII (n–q) and XIII (r–t). **a** *A. longanensis* **b** *A. lutea* (96/3126) **c** *A. sinensis* **d** *A. stricta* **e** *A. subrotata* (20121895) **f** *A. superba* **g** *A. arnautovii* (18566) **h** *A. connata* (96/3119) **i** *A. nutans* **j** *A. subrotata* (JLS2989) **k** *A. opaca* (2013/2460W) **l** *A. subrotata* (04/1769) **m** *A. zinaidae* **n** *A. bogneri* (9311) **o** *A. grandiflora* **p** *A. letreae* **q** *A.magnifica* **r** *A. formosa* (2015.11376.01) **s** *A. jiewhoei* (JLS1218) **t** *A. marasmioides* (2015.11354.01). Scale bars: 30 µm.

Discussion

Combinations of vegetative characters found in *Aspidistra*

We built a space of logical possibilities for all the studied specimens with regard to the following characters of vegetative morphology: type of shoot, sculpture of adaxial and abaxial epidermis, and density (and presence) of papillae. The specimens showed 23 combinations of these traits. Considering infraspecific variation of some traits, we combined the studied specimens of *Aspidistra* into 13 groups (Appendix 1) in order to make specimens of the same species belong to one group. This method allowed categorizing specimens of 62 species, whereas 7 species (*A. clausa* Vislobokov, *A. connata*, *A. erosa*, *A. hainanensis*, *A. opaca*, *A. oviflora*, *A. subrotata*) showed too high variation of traits so that specimens of each species got into several groups. The brief description of these groups is presented in Table 1.

Some species possess a unique combination of traits, e.g. *Aspidistra minor* Vislobokov, Nuraliev & M.S.Romanov (Fig. 3s, group VIII) is readily distinguishable from all other studied species by leaf epidermis finely rugose on both sides bearing abaxially papillae of low density. *Aspidistra zinaidae* Aver. & Tillich (Fig. 4m, group XI) likewise possesses a unique set of traits, which is similar to that of *A. minor* and differs in medium density of papillae.

We recognized no correlation between the morphological groups of species outlined above and geographical distribution of the species.

The availability of recognition of the morphological groups indicates that the characters under study show an infraspecific variation that is narrow enough, and the interspecific diversity that is broad enough to be applied to taxonomy for most of the studied species of *Aspidistra*. In other words, the characters possess a taxonomic signal. The identification key provided below is a reflection of this conclusion.

Table 1. Groups of the examined specimens of *Aspidistra* recognized here, and traits on which the groups are based.

Group	Type of shoot (leaves solitary/tufted)	Sculpture of adaxial epidermis	Sculpture of abaxial epidermis	Density of papillae on abaxial epidermis
I	tufted	smooth/verrucose	smooth/verrucose	no papillae
II	tufted	smooth	smooth/verrucose	low
III	solitary	smooth/verrucose	smooth	no papillae
IV	solitary	smooth/verrucose	verrucose	no papillae
V	solitary	verrucose/rugose	rugose	no papillae
VI	solitary	smooth/verrucose	smooth	low
VII	solitary	smooth/verrucose	verrucose	low
VIII	solitary	rugose	rugose	low
IX	solitary	smooth	smooth	medium
X	solitary	smooth/verrucose	verrucose	medium
XI	solitary	rugose	rugose	medium
XII	solitary	smooth	smooth	high
XIII	solitary	verrucose/rugose	verrucose/rugose	high

Correlations between leaf micromorphology and floral structure

Most of the groups of species outlined here on the basis of vegetative characters do not show any correlation with floral traits. We were able to recognize only several cases of such correlation, which are addressed below.

Aspidistra mirostigma Tillich & Škorničk., *A. phanluongii* Vislobokov and *A. sarcantha* Aver., Tillich, T.A.Le & K.S.Nguyen are similar in floral groundplan and shape: they share trimerous flowers with urceolate perigone, short style and wide stigma with its margin adjoined to the wall of perigone tube (Vislobokov et al. 2013; Leong-Škorničková et al. 2014; Averyanov et al. 2019). These species are demonstrated here to share epapillate leaf epidermis with verrucose sculpture at adaxial and abaxial surface, all belonging to group IV (Fig. 2n, o, p). The micromorphological features are thus in concordance with floral features in this group of species. At the same time, group IV comprises 13 more species, and the floral diversity of the entire group is remarkable high.

Aspidistra atroviolacea Tillich and *A. renatae* Bräuchler (treated as *A. atroviolacea* var. *renatae* (Bräuchler) Tillich & Aver. by Tillich and Averyanov 2018) are similar in having dark violet campanulate perigone and mushroom-shaped pistil (i.e. with slender style and hemispherical stigma) (Bräuchler and Ngoc 2005; Tillich 2005). Both species have epapillate smooth leaf epidermis (Figs 1n, 2b; belonging to group III), again in concordance with the floral features.

A group of five species, *A. corniculata* Vislobokov, *A. erosa*, *A. foliosa* Tillich, *A. lubae* Aver. & Tillich and *A. multiflora* Aver. & Tillich, shares trimerous flowers with mostly reddish-purple campanulate perigone and mostly white mushroom-shaped pistil (Tillich 2005; Averyanov and Tillich 2014, 2015; Averyanov et al. 2019; Vislobokov et al. 2019a). This group is uniformly characterized by finely rugose surface of abaxial leaf epidermis and absence of papillae (Figs 3a, c, e, f, g; group V). On the other hand, *A. locii* Arnautov & Bogner (Fig. 3b, group V) and *A. xuansonensis* Vislobokov (Fig. 3d; group V) have the same micromorphological traits, but possess distinctly different flowers.

Taxonomically uncertain groups of species in *Aspidistra* in the light of micromorphological data

The general stability of the micromorphological characters at the species level demonstrated here in *Aspidistra* allows to discuss taxonomy of complicated groups of species with employment of the newly obtained data.

Representatives of *Aspidistra* with tufted leaves (i.e., with several foliage leaves per elementary shoot) were considered to form a group of closely related species (De Wilde and Vogel 2006; Tillich and Averyanov 2012). Various authors proposed different taxonomic decisions to accommodate the diversity of plants with this morphology. The entire group was regarded as a single variable species *A. longifolia* Hook.f. s.l. by Phonsena and De Wilde (2010). Tillich and Averyanov (2012), in contrast, outlined the SE Asian part of this group as *A. hainanensis* species complex comprising several species (and excluded *A. longifolia* s.str. decribed from Assam, India from this complex). Here we follow the latter viewpoint, as it describes better the floral variation of these plants. Within our study, the specimens of *Aspidistra* with tufted leaves form two morphological groups (group I and II) which differ from each other in the presence of papillae on leaf epidermis (absence vs. presence with low density). The leaf epidermis of all these species is usually smooth but slightly tuberous in some cases. Although both characters vary within *A. hainanensis* species complex, we consider the variation not to be very significant for species delimitation, because it does not exceed the range of infraspecific variation found in some other species (e.g. *A. arnautovii* and *A. subrotata*). Thus, our data do not contradict the idea of phylogenetic closeness of these species.

Several other taxa with uncertain boundaries form a group here referred to as *A. subrotata* species complex. It includes *A. subrotata* with several described infraspecific taxa and *A. connata* with two proposed varieties. *Aspidistra connata* was recently suggested to be treated as a synonym of *A. subrotata*, as the absence of any considerable differences in their floral structure was shown during investigation of extensive material (Averyanov et al. 2018). *Aspidistra subrotata* is one of the most widely distributed species of the genus: it was originally described from China (Wan and Huang 1987), and subsequently reported from numerous localities in Vietnam (Tillich 2005, 2014), Thailand (Phonsena and De Wilde 2010) and Laos (Averyanov and Tillich 2017). *Aspidistra connata* is also known from China (Xu et al. 2010) and Vietnam (Tillich 2005; Leong-Škorničková et al. 2014). Both species inhabit diverse habitats and show extremely high diversity in size of flowers and leaves, shape of the leaf blade, and shape and coloration of the stigma (Averyanov and Tillich 2017). In the present study, *A. subrotata* and *A. connata* are expediently treated as distinct species in order to compare their micromorphological characters. Eight specimens of *A. subrotata* and four specimens of *A. connata* were investigated. We demonstrate that both species show very high diversity of leaf micromorphology. In both species, sculpture of epidermis varies between smooth and verrucose (but never rugose) adaxially as well as abaxially, and the abaxial side varies from being completely epapillate to having medium density of papillae. Accordingly, specimens of each species fall into several morphological groups (*A. subrotata* – IV, VI, VIII, IX; *A. connata* – IV, VI, IX). Thus, features of leaf micromorphology do not provide any clues for delimitation of *A. subrotata* complex; on the other hand, they do not contradict the idea of distinctness of *A. subrotata* and *A. connata*, because the variation found in each species is higher than the variation found in most other species of *Aspidistra*.

Key for identification of the studied species of *Aspidistra* based on epidermis micromorphology and gross vegetative morphology

1 Leaves grouped on shoot by 3–5 (3–5 foliage leaves per elementary shoot), sessile; blade narrowly elliptic to linear, 1–5 cm wide, 15–50 times as long as wide..**2**

– Leaves solitary (one leaf per elementary shoot), petiolate or sessile; blade of various shape **6**

2 Adaxial epidermis epapillate; abaxial epidermis with sparse papillae (papillae density low: 1–3.5 papillae per 0.01 mm^2) **3**

– Adaxial and abaxial epidermis epapillate **4**

3 Epidermis smooth adaxially, verrucose abaxially .... ***A. larutensis***

– Epidermis smooth on both sides .... ***A. carnosa*, *A. hainanensis*, *A. longifolia*, *A. oviflora***

4(2) Adaxial and abaxial epidermis smooth .... ***A. graminifolia*, *A. hainanensis*, *A. linearifolia*, *A. oviflora*, *A. triradiata***

– Epidermis verrucose at least on one side **5**

5 Epidermis smooth adaxially, verrucose abaxially .... ***A. viridiflora***

– Epidermis verrucose on both sides .... ***A. yingjiangensis***

6(1) Adaxial and abaxial epidermis smooth and epapillate; leaf petiolate **7**

– Epidermis sculptured (verrucose or rugose) or papillate at least on one side; leaf petiolate or sessile **9**

7 Plant with aerial erect to ascending stem ca. 50 cm high .... ***A. erecta***

– Plant without aerial stem **8**

8 Blade narrowly lanceolate to narrowly elliptic, 1.5–4.5 cm wide .... ***A. atroviolacea*, *A. clausa*, *A. renatae***

– Blade ovate to elliptic, 5–15 cm wide... ***A. claviformis*, *A. dolichanthera*, *A. jingxiensis*, *A. lurida*, *A. petiolata*, *A. sessiliflora***

9(6) Adaxial and abaxial epidermis epapillate **10**

– Adaxial epidermis epapillate, abaxial epidermis papillate **23**

10 Epidermis verrucose at least on one side (and never rugose) **11**

– Epidermis finely rugose at least on one side **18**

11 Plant with erect stem **12**

– Plant without erect stem **14**

12 Aerial stem up to 50 cm high .... ***A. globosa***

– Aerial stem 3–20 cm high **13**

13 Aerial stem 3–5 cm high, blade 8–16 cm long .... ***A. laotica***

– Aerial stem ca. 20 cm high, blade 20–25 cm long .... ***A. lateralis***

14(11) Blade narrowly elliptic to narrowly lanceolate, 8–20 times as long as wide **15**

– Blade elliptic to ovate, 2–7 times as long as wide **16**

15 Petiole 3–5 cm long .... ***A. basalis***

– Petiole 15–32 cm long .... ***A. erosa*, *A. gracilis*, *A. subrotata* var. *angustifolia***

16(14) Epidermis verrucose on both sides.... ***A. bella*, *A. mirostigma*, *A. phanluongii*, *A. sarcantha*, *A. subrotata*, *A. sutepensis*, *A. truongii*, *A. vietnamensis***

– Epidermis verrucose on one side, smooth on the other side **17**

17 Petiole longer than blade .... ***A. medusa***

– Petiole equal to or shorter than blade .... ***A. connata*, *A. typica***

18(10) Epidermis finely rugose on both sides **19**

– Epidermis verrucose adaxially, finely rugose abaxially **20**

19 Blade 1.5–3.7 cm wide .... ***A. corniculata*, *A. foliosa***

– Blade 4–10 cm wide .... ***A. multiflora***

20(18) Blade elliptic, 5–11 cm wide .... ***A. locii***, ***A. xuansonensis***

– Blade lanceolate, 1.5–5 cm wide **21**

21 Blade equal to or insignificantly longer than petiole .... ***A. erosa***

– Blade 2–10 times as long as petiole **22**

22 Blade 12–20 cm long, 4–6 times as long as wide .... ***A. lubae* var. *lubae***

– Blade 20–35 cm long, 8–13 times as long as wide .... ***A. lubae* var. *lancifolia***

23(9) Density of papillae on abaxial epidermis low (1–3.5 papillae per 0.01 $\text{mm}^{2)}$ **24**

– Density of papillae on abaxial epidermis medium to high (4–45.5 papillae per 0.01 mm^2) **26**

24 Epidermis finely rugose on both sides .... ***A. minor***

– Epidermis smooth or verrucose on both sides **25**

25 Epidermis smooth on both sides .... ***A. fungilliformis***, ***A. geastrum***, ***A. longipetala***, ***A. papillata***, ***A. tillichiana* var. *latifolia***

– Epidermis verrucose at least on one side .... ***A. bicolor***, ***A. clausa***, ***A. connata***, ***A. opaca***, ***A. semiaperta***, ***A. subrotata***

26(23) Density of papillae on abaxial epidermis medium (4–7.5 papillae per 0.01 mm^2) **27**

– Density of papillae on abaxial epidermis high (8–45.5 papillae per 0.01 mm^2) **30**

27 Epidermis finely rugose on both sides .... ***A. zinaidae***

– Epidermis smooth or verrucose on both sides **28**

28 Epidermis verrucose at least on one side .... ***A. arnautovii***, ***A. connata***, ***A. nutans***, ***A. opaca***, ***A. subrotata***

– Epidermis smooth on both sides **29**

29 Petiole 2–5 cm long, blade 3–7 times as long as petiole .... ***A. lutea***, ***A. sinensis***

– Petiole 9–28 cm long, blade equal to or insignificantly longer than petiole ... . ***A. hekouensis***, ***A. longanensis***, ***A. stricta***, ***A. subrotata***, ***A. superba***

30(26) Epidermis finely rugose (rarely verrucose) on both sides **31**

– Epidermis smooth on both sides **33**

31 Rhizome with very short internodes (foliage leaves crowded); blade 20–30 × 8.5–13 cm .... ***A. jiewhoei***

– Rhizome with long internodes (foliage leaves spaced 8–25 mm apart); blade 10–17 × 5–8 cm **32**

32 Petiole 10–15 cm long, blade 10–12 × 5 cm; epidermis finely rugose on both sides .... ***A. marasmioides***

– Petiole 15–20 cm long, blade 14–17 × 7–8 cm; epidermis finely rugose or verrucose at least on one side .... ***A. formosa***

33(30) Blade narrowly lanceolate, 1.5–3 cm wide, 23–30 times as long as wide .... ***A. letreae***

– Blade lanceolate, elliptic or ovate, 5–14 cm wide, 6–9 times as long as wide **34**

34 Petiole absent or inconspicuous .... ***A. bogneri***

– Petiole distinctly present, usually 30 cm long or longer .... ***A. grandiflora, A. magnifica***

Conclusions

Micromorphological characters of leaf epidermis show sufficiently high diversity in the genus *Aspidistra*, and relatively low infraspecific variation in most of its species. The following variable characters are recognized: sculpture of adaxial and abaxial epidermis (smooth, verrucose and rugose) and the presence and density of papillae at abaxial side of leaf (absent, with low, medium and high density). Combined with characters of gross vegetative morphology, they allow recognition of 13 basic types of vegetative morphology in *Aspidistra*. We constructed an identification key for species of *Aspidistra* in sterile condition on the basis of the newly obtained micromorphological data and earlier known macromorphological traits. The key allows to identify a species to a group containing one to eight species. The results demonstrate considerable taxonomic significance of micromorphological features in *Aspidistra*.

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Appendix 1

Table A1. Species and specimens of *Aspidistra* examined, and traits of vegetative morphology. The species are arranged according to their belonging to the morphological groups, and in the alphabetical order within the groups. Note that some species are represented in more than one group.

Species	Living specimens studied		Herbarium voucher	Country of origin	Type of shoot (leaves solitary / tufted)	Sculpture of adaxial epidermis	Sculpture of abaxial epidermis	Density of papillae distribution on abaxial epidermis**	Group
	Greenhouse hosting specimen*	Garden accession number; field collector's number (if differs from that of herbarium voucher)							
A. graminifolia	BIN	1285	*Averyanov AL 84* (isotype, MW: MW0751740)	Vietnam	tufted	smooth	smooth	no papillae	I
A. hainanensis	BIN	s.n.	-	unknown	tufted	smooth	smooth	no papillae	
A. linearifolia	BGMN	2011/0980; *Joschko s.n.*	-	unknown	tufted	smooth	smooth	no papillae	
A. oviflora	MBG	2013.12433.01	*Vislobokov 13062* (MW: MW0735044)	Vietnam	tufted	smooth	smooth	no papillae	
A. oviflora	BIN	13394; *Maisak TM1052*	*Averyanov et al. CPC5425* (isotype, LE: LE01050400)	Vietnam	tufted	smooth	smooth	no papillae	
A. triradiata	MBG	2016.12342.01	*Vislobokov et al. G30* (MW: MW0753785)	China	tufted	smooth	smooth	no papillae	
A. viridiflora	MBG	2015.11381.01	*Nuraliev et al. 1280* (holotype, MW: MW0754789)	Vietnam	tufted	smooth	verrucose	no papillae	
A. yingjiangensis	BIN	251964	-	unknown	tufted	verrucose	verrucose	no papillae	
A. carnosa	BGMN	96/3122W; *Arnautov 96-102*	*Tillich 4476* (holotype, M: M0213536)	Vietnam	tufted	smooth	smooth	low	II
A. cylindrica	MBG	2015.11382.01	*Kuznetsov et al. 1357* (holotype, MW: MW0595646)	Vietnam	tufted	smooth	smooth	low	
A. hainanensis	BGMN	11/1394	*Tillich 5717* (M: MSB159621)	unknown	tufted	smooth	smooth	low	
A. longifolia	BGMN	15/1593	-	unknown	tufted	smooth	smooth	low	
A. oviflora	MBG	2018.14340.01	-	unknown	tufted	smooth	smooth	low	
	BIN	14386	*Averyanov et al. CPC7491* (LE: LE01050072)	Vietnam	tufted	smooth	smooth	low	
A. larutensis	BGMN	s.n.	-	unknown	tufted	smooth	verrucose	low	
A. atroviolacea	BGMN	97/2363	*Bogner 2309* (paratype, M: M0213530)	Vietnam	solitary	smooth	smooth	no papillae	III
A. clausa	MBG	2014.12431.01	*Vislobokov 14097* (holotype, MW: MW0595637)	Vietnam	solitary	smooth	smooth	no papillae	
A. claviformis	MBG	2016.12351.01	*Vislobokov et al. G71* (MW: MW0753784)	China	solitary	smooth	smooth	no papillae	
A. connata	BGMN	V/0490	-	unknown	solitary	verrucose	smooth	no papillae	
A. dolichanthera	MBG	2016.12354.01	*Vislobokov et al. G74* (MW: MW0753743)	China	solitary	smooth	smooth	no papillae	

Species	Living specimens studied		Herbarium voucher	Country of origin	Type of shoot (leaves solitary / tufted)	Sculpture of adaxial epidermis	Sculpture of abaxial epidermis	Density of papillae distribution on abaxial epidermis**	Group
	Greenhouse hosting specimen*	Garden accession number; field collector's number (if differs from that of herbarium voucher)							
A. dolichanthera	BIN	s.n.	*Averyanov CBL1675* (LE: LE01054609)	Vietnam	solitary	smooth	smooth	no papillae	III
A. erecta	MBG	2016.12349.01	*Vislobokov et al. G61* (MW: MW0753774)	China	solitary	smooth	smooth	no papillae	
A. jingxiensis	MBG	2016.12348.01	*Vislobokov et al. G51* (MW: MW0753783)	China	solitary	smooth	smooth	no papillae	
A. lurida	BIN	2225	*s.coll., s.n.* (LE: LE01050063)	unknown	solitary	smooth	smooth	no papillae	
A. petiolata	SBG	20121925; *Leong-Škorničková JLS1622*	-	Vietnam	solitary	smooth	smooth	no papillae	
A. renatae	BGMN	04/1772	*Brauchler et al. 3000* (holotype, M: M0243611)	Vietnam	solitary	smooth	smooth	no papillae	
A. sessiliflora	BIN	14448	*Averyanov AL271* (holotype, LE: LE01050440)	China	solitary	smooth	smooth	no papillae	
A. basalis	BGMN	2011/1397	*Tillich 5720* (holotype, M: MSB159619)	China	solitary	smooth	verrucose	no papillae	IV
A. bella	MBG	2019.14670	*Averyanov et al. CPC7484* (paratype, LE: LE01042157)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. erosa	SBG	*Leong-Škorničková JLS2972*	-	unknown	solitary	verrucose	verrucose	no papillae	
A. globosa	from nature	-	*Kuznetsov et al. 1444* (paratype, MW: MW0595640)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. gracilis	BGMN	2011/1395	*Tillich 5718* (holotype, M: MSB159618)	China	solitary	verrucose	verrucose	no papillae	
A. laotica	SBG	20122018	*Leong-Škorničková et al. JLS1802* (SING: 0192256)	Laos	solitary	verrucose	verrucose	no papillae	
	BIN	1311(1313)	*Averyanov LA-VN554* (holotype, LE: LE01032172)	Laos	solitary	verrucose	verrucose	no papillae	
A. lateralis	BGMN	97/2382; *Bogner 2492*	*Tillich 4361* (holotype, M: MSB004977)	Vietnam	solitary	smooth	verrucose	no papillae	
A. medusa	SBG	*Leong-Škorničková JLS3096*	-	Laos	solitary	smooth	verrucose	no papillae	
A. mirostigma	SBG	*Leong-Škorničková JLS1571*	*Leong-Škorničková et al. JLS-1571* (holotype SING)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. phanluongii	MBG	2015.11347.01	*Nuraliev 874* (MW: MW0735057)	Vietnam	solitary	verrucose	verrucose	no papillae	
	from nature	-	*Vislobokov M0211* (paratype, MW: MW0591735)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. sarcantha	SBG	*Leong-Škorničková JLS2914*	-	Vietnam	solitary	verrucose	verrucose	no papillae	
	SBG	JLS2962	*Leong-Škorničková et al. JLS2962* (SING: 0240186)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. subrotata var. *subrotata*	BGMN	97/2376 II	*Tillich 4461* (M: M0213561)	Vietnam	solitary	verrucose	verrucose	no papillae	
	MBG	2015.11350.01	*Vislobokov 13067* (MW: MW0735071)	Vietnam	solitary	verrucose	verrucose	no papillae	IV

Species	**Living specimens studied**		**Herbarium voucher**	**Country of origin**	**Type of shoot (leaves solitary / tufted)**	**Sculpture of adaxial epidermis**	**Sculpture of abaxial epidermis**	**Density of papillae distribution on abaxial epidermis****	**Group**
	Greenhouse hosting specimen*	**Garden accession number; field collector's number (if differs from that of herbarium voucher)**							
A. subrotata var. *angustifolia*	MBG	2015.11365.01	*Vislobokov 13097* (MW: MW0735079)	Vietnam	solitary	verrucose	verrucose	no papillae	IV
A. sutepensis	BGMN	05/2338	*Tillich 5081* (M: MSB126218)	Thailand	solitary	verrucose	verrucose	no papillae	
A. truongii	BGMN	2013/2461	*Leong-Škorničková et al. HB17* (paratype, SING: 0188968)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. truongii	BGMN	2013/2461W	*Rybkova et al. HB17 6484* (M: M0210863)	Vietnam	solitary	verrucose	verrucose	no papillae	
	SBG	*Leong-Škorničková JLS1047*	-	Vietnam	solitary	verrucose	verrucose	no papillae	
A. typica	MBG	2017.12835.06	*Tillich 5996* (M)	Vietnam	solitary	smooth	verrucose	no papillae	
A. vietnamensis	BIN	786	*Averyanov et al. HAL12116a* (holotype, LE: LE01050347)	Vietnam	solitary	verrucose	verrucose	no papillae	
A. corniculata	from nature		*Vislobokov 18089* (isotype, MW: MW0595715)	Vietnam	solitary	rugose	rugose	no papillae	V
A. erosa	SBG	*Leong-Škorničková JLS2906*	-	Vietnam	solitary	verrucose	rugose	no papillae	
A. foliosa	BGMN	97/2360; *Bogner 2502*	*Tillich 4471* (holotype, M: M0213541)	Vietnam	solitary	rugose	rugose	no papillae	
	BGMN	97/2353	*Bogner 2803* (M)	Vietnam	solitary	rugose	rugose	no papillae	
A. locii	BGMN	96/3121; *Arnautov 86-112*	*Tillich 4359* (M: MSB004976)	Vietnam	solitary	verrucose	rugose	no papillae	
	BIN	86-169	*Arnautov 86-112* (LE: LE01055472)	Vietnam	solitary	verrucose	rugose	no papillae	
A. lubae var. *lancifolia*	BIN	CPC1566	*Averyanov et al. CPC1566* (holotype, LE: LE01050389)	Vietnam	solitary	verrucose	rugose	no papillae	
A. lubae var. *lubae*	BIN	13266	*Averyanov et al. CPC6962* (LE: LE01049991)	Vietnam	solitary	verrucose	rugose	no papillae	
A. multiflora	MBG	2015.11375.01	*Vislobokov 14045* (MW: MW0750152)	Vietnam	solitary	rugose	rugose	no papillae	
A. xuansonensis	MBG	2015.11366.01	*Vislobokov 13102* (paratype, MW: MW0591740)	Vietnam	solitary	verrucose	rugose	no papillae	
A. bicolor	BGMN	97/2352; Bogner 2503	*Tillich 4462* (holotype, M: M0213531)	unknown	solitary	verrucose	smooth	low	VI
A. fungilliformis	MBG	2016.12350.02	*Vislobokov et al. G63* (MW: MW0753782)	China	solitary	smooth	smooth	low	
	MBG	2016.12355.01	*Vislobokov et al. G82* (MW: MW0753780)	China	solitary	smooth	smooth	low	
A. geastrum	BGMN	97/2375G	*Tillich 4598* (holotype, M: M0213544)	Vietnam	solitary	smooth	smooth	low	
A. longipetala	MBG	2017.13459.01	*Vislobokov et al. G111* (MW: MW0754777)	China	solitary	smooth	smooth	low	
A. papillata	from nature		*Vislobokov 18087* (MW: MW0756241)	Vietnam	solitary	smooth	smooth	low	
A. tillichiana var. *latifolia*	BIN	13270	*Averyanov et al. CPC6841* (holotype, LE: LE01050358)	Vietnam	solitary	smooth	smooth	low	VI

Species	Living specimens studied		Herbarium voucher	Country of origin	Type of shoot (leaves solitary / tufted)	Sculpture of adaxial epidermis	Sculpture of abaxial epidermis	Density of papillae distribution on abaxial epidermis**	Group
	Greenhouse hosting specimen*	Garden accession number; field collector's number (if differs from that of herbarium voucher)							
A. clausa	from nature		*Vislobokov 18090* (MW: MW0756227)	Vietnam	solitary	verrucose	verrucose	low	VII
A. connata	BGMN	97/2358	*Bogner 2495* (M)	Vietnam	solitary	smooth	verrucose	low	
A. opaca	from nature		*Vislobokov 18035* (MW: MW0756235)	Vietnam	solitary	verrucose	verrucose	low	
A. semiaperta	BIN	11614	*Averyanov et al. CPC1566b* (LE: isotype, LE01050394)	Vietnam	solitary	smooth	verrucose	low	
A. subrotata var. *subrotata*	from nature		*Vislobokov et al. A.s.5* (MW: MW0735059)	Thailand	solitary	verrucose	verrucose	low	
A. subrotata var. *angustifolia*	MBG	2015.11355.01	*Vislobokov et al. A.s.1* (MW: MW0735069)	Thailand	solitary	verrucose	verrucose	low	
A. minor	MBG	2018.14282.01	*Nuraliev et al. 1966A* (holotype, MW: MW0595716)	Vietnam	solitary	rugose	rugose	low	VIII
A. hekouensis	BGMN	95/2832; *Bogner 2216*	*Tillich 5005* (M: M0213547)	China	solitary	smooth	smooth	medium	IX
A. longanensis	BIN	260301	-	Vietnam	solitary	smooth	smooth	medium	
A. lutea	BGMN	96/3126; *Arnautov 76-140*	*Tillich 4481* (holotype, M: M0213551)	Vietnam	solitary	smooth	smooth	medium	
	BIN	17304	*Tillich 5003 Arnautov 76-140* (paratype, LE: LE01049990)	Vietnam	solitary	smooth	smooth	medium	
A. sinensis	BIN	13659	*Arnautova s.n.* (holotype, LE: LE01050480)	China	solitary	smooth	smooth	medium	
A. stricta	BGMN	96/3124; *Arnautov 88-110*	*Tillich 4367* (holotype, M: M0213560)	Vietnam	solitary	smooth	smooth	medium	
A. subrotata var. *subrotata*	SBG	20121895; *Leong-Skornickova JLS1558*	-	Vietnam	solitary	smooth	smooth	medium	
A. superba	BGMN	97/2369T; *Bogner 2346*	*Tillich 4480* (paratype, M: M0213562)	Vietnam	solitary	smooth	smooth	medium	
A. arnautovii var. *arnautovii*	BIN	18566	*Averyanov AL466ED4* (LE: LE01048630)	Vietnam	solitary	smooth	verrucose	medium	X
	BGMN	96/3123; *Arnautov 88-115*	*Tillich 4474* (holotype, M: M0213528)	Vietnam	solitary	smooth	verrucose	medium	
	MBG	2015.11353.01	*Vislobokov 13042* (MW: MW0735025)	Vietnam	solitary	smooth	verrucose	medium	
A. arnautovii var. *catbaensis*	BGMN	96/3125; *Arnautov 88-144*	*Tillich 4460* (M: M0213526)	Vietnam	solitary	verrucose	verrucose	medium	
	MBG	2015.11352.01	*Vislobokov 13040* (MW: MW0735030)	Vietnam	solitary	verrucose	verrucose	medium	
A. connata	BGMN	96/3119; *Arnautov 85-722*	*Tillich 4470* (holotype, M: M0213538)	Vietnam	solitary	smooth	verrucose	medium	
	BIN	*Arnautov 85-722*	*Tillich 5486* (paratype, LE: LE01050022)	Vietnam	solitary	smooth	verrucose	medium	
A. nutans	BIN	13281	*Averyanov et al. CPC7158b* (holotype, LE: LE01032173)	Vietnam	solitary	smooth	verrucose	medium	
A. opaca var. *opaca*	BGMN	2013/2460W; *Rybkova et al. 180*	-	Vietnam	solitary	verrucose	verrucose	medium	
	BGMN	97/2359; *Bogner 2491*	*Tillich 4468* (holotype, M: M0213554)	Vietnam	solitary	verrucose	verrucose	medium	

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	Greenhouse hosting specimen*	Garden accession number; field collector's number (if differs from that of herbarium voucher)							
A. opaca var. *opaca*	SBG	20111766	JLS1121	Vietnam	solitary	verrucose	verrucose	medium	X
A. opaca var. *rugosa*	BGMN	13/2460; *Rybkova et al. 180*	-	Vietnam	solitary	verrucose	verrucose	medium	
A. subrotata var. *subrotata*	SBG	*Leong-Škorničková JLS2989*	-	Vietnam	solitary	smooth	verrucose	medium	
A. subrotata var. *subrotata*	BGMN	04/1769	-	Vietnam	solitary	verrucose	verrucose	medium	
A. zinaidae	BIN	7242	*Averyanov et al. HAL11111b* (holotype, LE: LE01050435)	Vietnam	solitary	rugose	rugose	medium	XI
A. bogneri	BIN	9311	-	Vietnam	solitary	smooth	smooth	high	XII
	BGMN	97/2374W	*Bogner 2500* (paratype, M: M0213534)	Vietnam	solitary	smooth	smooth	high	
	BGMN	97/2404	*Bogner 2805* (paratype, M: M0213535)	Vietnam	solitary	smooth	smooth	high	
	SBG	20120086; *Leong-Škorničková JLS1436*	-	Vietnam	solitary	smooth	smooth	high	
A. grandiflora	BIN	11590	*Harder et al. DKH 8123* (holotype, LE: LE01054814)	Vietnam	solitary	smooth	smooth	high	
A. letreae	SBG	*Leong-Škorničková JLS2977*	-	Vietnam	solitary	smooth	smooth	high	
A. magnifica	MBG	2016.12423.01; *Nuraliev 1672*	*Romanov et al. s.n.* (MW)	Vietnam	solitary	smooth	smooth	high	
A. formosa	SBG	20120060; *Leong-Škorničková JLS1384*	-	Vietnam	solitary	verrucose	verrucose	high	XIII
	BGMN	1997/2367	*Tillich 5280* (holotype, M: M0213543)	unknown	solitary	verrucose	verrucose	high	
	SBG	*Leong-Škorničková JLS3178*	-	Vietnam	solitary	verrucose	verrucose	high	
	MBG	2015.11376.01	*Vislobokov 14062* (MW: MW0750154)	Vietnam	solitary	verrucose	rugose	high	
	BIN	s.n.	*Averyanov et al. CPC6412* (LE: LE01050398)	unknown	solitary	rugose	rugose	high	
A. jiewhoei	SBG	*Leong-Škorničková JLS1218*		Vietnam	solitary	rugose	rugose	high	
	SBG	20122069	*Leong-Skornickova JLS1871* (holotype, SING, isotype, M: M0225616)	Vietnam	solitary	rugose	rugose	high	
A. marasmioides	BGMN	96/3118; *Arnautov 88-117*	*Tillich 4458* (holotype, M: M0213552)	Vietnam	solitary	rugose	rugose	high	
	MBG	2015.11354.01	*Vislobokov 13046* (MW: MW0735052)	Vietnam	solitary	rugose	rugose	high	

* For description of the abbreviations, see Materials and methods.

** Estimated as number of papillae per 0.01 mm^2: low = 1–3.5, medium = 4–7.5, high = 8–45.5.

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Studies in Austral Bryaceae (Bryopsida). III. A Preliminary Account with Keys to *Rosulabryum* J.R. Spence in Chile

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Abstract

A preliminary study of the genus *Rosulabryum* J.R. Spence in Chile is presented, with brief species descriptions, notes on ecology and distributions, and a taxonomic key. The following 12 species are confirmed with vouchered specimens; *Rosulabryum andicola* (Hook.) Ochyra, *Rosulabryum billarderii* (Schwägr.) J.R. Spence, *Rosulabryum campylothecium* (Taylor) J.R. Spence, *Rosulabryum capillare* (Hedw.) J.R. Spence, *Rosulabryum coloratum* (Müll. Hal.) J.R. Spence, *Rosulabryum densifolium* (Brid.) Ochyra, *Rosulabryum longidens* (Thér.) J.R. Spence, *Rosulabryum macrophyllum* (Cardot & Broth.) Ochyra, *Rosulabryum perlimbatum* (Cardot) Ochyra, *Rosulabryum puconense* (Herzog & Thér.) J.R. Spence, *Rosulabryum rubens* (Mitt.) J.R. Spence, and *Rosulabryum torquescens* (Bruch *ex* De Not.) J.R. Spence. *Rosulabryum canariense* (Brid.) Ochyra is tentatively excluded as the Chilean material can be referred to *R. coloratum*. Similarly, *Rosulabryum viridescens* (Welw. & Duby) Ochyra is tentatively excluded since the Chilean plants do not match the African type, but instead appear to be atypical plants of *R. campylothecium*.

Keywords

Bryaceae, Chile, mosses, *Rosulabryum*

Introduction

With a known moss flora of more than 900 species, Chile has a rich but incompletely documented bryoflora (Müller 2009). Recent studies involving herbarium collections and field work (Larraín 2016; Ireland et al. 2017; Drapela and Larraín 2020; Larraín et al. 2020) suggest that many more species will be discovered. The country has a great diversity of climates, geological substrates, and vegetation communities, ranging from tropical arid deserts in the north, montane and alpine climates along the Andean Mountain chain, Mediterranean-climate regions in the center of the country, to temperate and subantarctic forests and moorlands in the south.

The Bryaceae is a large world-wide family that occurs in a wide variety of habitats and climates and can represent as much as 10% of the species richness in local and regional moss floras. Chile has an extremely rich and incompletely known Bryaceae flora, with an estimated 85 species, based on revisions and recent collecting (Ochi 1980, 1982; Müller 2009; Ireland et al. 2017; Spence 2020a). These field studies have also revealed species that appear to be new to science, mostly in the genera G*emmabryum* J.R. Spence & H.P. Ramsay, *Ochiobryum* J.R. Spence & H.P. Ramsay, and *Plagiobryoides* J.R. Spence.

One of the most easily recognized and common genera is *Rosulabryum* J.R. Spence, which is well represented in the southern hemisphere. The genus currently consists of ca. 75 described species (J. Spence unpublished data), with 12 well documented species in Chile. However, much of the country remains under collected, especially in the north and in the Andes, thus it is likely that additional species will be found with more intensive field work. Of particular note is the presence of a diverse montane *Rosulabryum* flora in the northern and central Andes which may extend southward into the Chilean Andes.

Rosulabryum occurs in a clade with *Brachymenium* Schwägr. sect. *Brachymenium*, sister to a clade consisting of *Plagiobryum* Lindb., *Plagiobryoides* J.R. Spence and *Ptychostomum* Hornsch. (Pedersen et al. 2006). The molecular studies that include more than one species in the genus include Cox and Hedderson (2003), Pedersen et al. (2003) and Pedersen et al. (2006). These studies show a well-supported *Rosulabryum* clade using several different species. However, the relationships and phylogenetic position of the *Rosulabryum* and the related *Ptychostomum* clades remain unresolved at this time.

Method and materials

Collections were obtained from various herbaria, primarily CONC, MO and NY. Additional field work in the Tierra del Fuego region of Chile (Magellanes Province) has been completed and collections are currently being studied in the author's personal herbaria. Much of the recent floristic work has been done in the southern regions of Bío Bío, Aisén and Magallanes (e.g., Larraín 2016; Ireland et al. 2017; Drapela and Larraín 2020; Spence 2020a; unpublished data). Nomenclature for the species documented in this study follows Spence (2020b). This study is a preliminary treatment of the genus for Chile to help guide identification of collections and field work.

Results

Of the 12 species documented in this study, their distributions include four main elements, 1) widespread temperate, 2) southern temperate, 3) subantarctic/*Nothofagus*, and 4) Neotropical montane. The widespread element includes *R. capillare*, *R. rubens* and *R. torquescens*. Southern temperate species include *R. billarderii*, *R. campylothecium*, and *R. macrophyllum*, with the first two extending to Australasia. The subantarctic element is represented by *R. perlimbatum*, which extends to southern New Zealand. The Neotropical montane element includes *R. andicola* and *R. densifolium*. An endemic element may also exist, as currently *R. longidens* and *R. puconense* are endemic to Chile, with potentially one record of the former reported from Argentina which needs to be verified. *Rosulabryum coloratum* has an anomalous distribution, as it occurs in montane regions of Bolivia as well as lower elevations of south-central Chile. In general, these distributions conform at least in part to well documented and similar distributions among the vascular plant flora of Chile (Moreira-Muñoz 2011). Overall, the diversity of *Rosulabryum* in Chile is similar to that in other well studied regions, including Australasia (14 spp.) and North America (16 spp.), but appears to be more diverse than the western Palearctic (8 spp.).

Rosulabryum is characterized by a combination of predominantly gametophyte characters, including mostly rosulate habit to sometimes evenly foliate stems, ovate to obovate or spathulate leaves, a well-developed limbidium, serrulate, serrate or denticulate distal leaf margins, costa with well-developed stereid band, nodding capsule, well-developed peristome with appendiculate cilia, small spores, rhizoidal tubers, and filiform gemmae in the leaf axils (Mohamed 1979; Spence 1996). In terms of gametophyte morphology, *Rosulabryum* is most similar to *Rhodobryum*. *Rhodobryum* differs in producing distinctive stolons, a weakly developed to nearly absent stereid band in the costa, and a lack of rhizoidal tubers and leaf axil gemmae. Both genera often have polysetose perichaetia, but this feature is much more common in *Rhodobryum*. In addition, the chromosomes of *Rhodobryum* are significantly different in structure not only from *Rosulabryum* but the rest of the family (cf. Ramsay and Spence 1996).

Key to *Rosulabryum* of Chile

1 Filiform gemmae present, usually in axils of sterile shoot leaves **2**
1’ Gemmae absent **3**
2 Plants large, at least some leaves > 3 mm, distal margins sharply serrate; costa of innovations short-excurrent; predominantly terricolous .... ***Rosulabryum andicola* (in part)**
2’ Plants small, leaves < 2 (2.5) mm, distal margins serrulate, costa of innovation leaves long excurrent; terricolous or lignicolous-epiphytic, often on tree trunks and large branches .... ***Rosulabryum longidens***

3 Plants small, leaves mostly < 2 (2.5) mm, spirally twisted around stem when dry, costa excurrent in long ± straight awn .... ***Rosulabryum capillare***

3’ Plants small to robust, if spirally twisted around stem then leaves > 3 mm or costa short-excurrent... **4**

4 Plants small, leaves < 2 mm, distal leaf margins smooth to serrulate, stems rosulate to somewhat evenly foliate, leaves often with reddish tints; rhizoidal tubers red to orange or red-brown, cell walls protuberant............................ **5**

4’ Plants medium to large, leaves mostly > 2.5mm, distal leaf margins finely serrulate to sharply serrate or denticulate, stems variable but often rosulate, leaves mostly green to yellow-green, rarely somewhat reddish-tinged, rhizoidal tuber color various, cells smooth, lacking protuberant walls **6**

5 Sexual condition dioicous; costa excurrent in medium-length awn, limbidium distinct, leaf margins serrulate, rhizoidal tubers red, cell walls strongly protuberant, diameter to ca. 260 (280) µm; capsules 2–3 mm long .... ***Rosulabryum rubens***

5’ Sexual condition synoicous or polyoicous with single sex shoots, costa percurrent to short excurrent in short awn, limbidium weak, sometimes absent distally, margins smooth to finely serrulate; rhizoidal tubers red-brown, red to orange, cell walls weakly protuberant, diameter to 600 µm; capsules 4–6 mm long ....***Rosulabryum puconense***

6 Limbidium weak distally, of 1 row or sometimes nearly absent; leaves concave; distal laminal cells firm-walled to often incrassate............................... **7**

6’ Limbidium distinct distally, usually of 2 or more rows; leaves flat or weakly concave; distal laminal cells thin to firm-walled but usually not incrassate **10**

7 Plants in tight rosulate tufts when dry, leaves imbricate, golden-yellow or yellow-green; costa excurrent in long ± straight awn; rhizoidal tubers lacking .... ***Rosulabryum campylothecium***

7’ Plants in loose rosulate to comal tufts or stems evenly foliate, leaves somewhat imbricate to shrunken or twisted when dry, colors various but not golden-yellow; costa excurrent in short often recurved awn; tubers present............. **8**

8’ Plants ± evenly foliate; leaves spirally twisted around stem when dry; leaf apex acute, costa excurrent in short recurved awn; tubers brown to red-brown ....***Rosulabryum coloratum***

8 Plants in one or more interrupted rosulate tufts; leaves weakly imbricate to irregularly contorted when dry; leaf apex broadly acute to rounded or obtuse; costa excurrent in short recurved or straight awn; tubers red, scarlet, brown or yellow-brown.. **9**

9 Sexual condition autoicous or rarely synoicous; leaf broadly acute at tip, costa tapering towards leaf tip, leaves not strongly keeled; rhizoidal tubers bright red to scarlet .... ***Rosulabryum canariense***

9’ Sexual condition dioicous or rhizo-autoicous; leaf rounded-obtuse at tip, costa very thick to apex, leaves strongly keeled; rhizoidal tubers brown to yellow-brown ....***Rosulabryum viridescens***

10 Sexual condition synoicous or rarely autoicous; plants medium-sized, leaves 2–3 mm, obovate, costa excurrent in medium-length awn; rhizoidal tubers scarlet to red .... ***Rosulabryum torquescens***

10’ Sexual condition dioicous or rhizo-autoicous; plants medium to robust, leaves mostly > 3 mm, ovate, obovate to spathulate, costa percurrent to excurrent in short awn; rhizoidal tubers dark red, brown to red-brown......................... **11**

11 Limbidium very wide, (3) 6–8 rows, often imparting whitish-hyaline border to leaf **12**

11’ Limbidium narrower, (2) 3–4 rows, colored to clear, not imparting whitish-hyaline border to leaf **13**

12 Leaves spirally twisted around stem when dry, distal margins sharply serrate; limbidium 3–4 rows; filiform leaf axil gemmae usually present .... ***Rosulabryum andicola* (in part)**

12 Leaves irregularly contorted to somewhat imbricate when dry, distal margins serrulate; limbidium 4–8 rows; filiform leaf gemmae absent .... ***Rosulabryum perlimbatum***

13 Plants elongate evenly foliate, to 8–10 cm, leaves narrowly ovate, distal margins sharply serrate to denticulate, teeth often double .... ***Rosulabryum densifolium***

13’ Plants rosulate, not evenly foliate, mostly < 2 cm long, leaves obovate to broadly ovate, distal margins serrulate to singly serrate............................. **14**

14 Leaves ovate to oblong, ±imbricate when dry; distal margins serrulate.......... .... ***Rosulabryum macrophyllum***

14’ Leaves obovate to spathulate, irregularly contorted when dry; distal margins sharply serrate .... ***Rosulabryum billarderii***

Species accounts

1. *Rosulabryum andicola* (Hook.) Ochyra

Remarks. A widespread African-Neotropical species found from Chile north to the Southwestern US. This species replaces *R. billarderii* in warmer climates in Chile and is rare south of the Maule Region. It is found principally on damp to dry soil or soil over rock, rarely on fallen logs and tree stumps. The species is characterized by dioicous sexual condition, strongly rosulate stems, large leaves that are spirally twisted around the stem when dry, a costa excurrent in a short awn, a very well-developed wide limbidium, sharply serrate distal leaf margins, and brown filiform gemmae in the leaf axils of sterile shoots. The rhizoidal tubers are red to red-brown and can be > 1 mm in diameter. (Illustrations: Mohamed 1979: 422, as *Bryum andicola*; Magill 1987: 382, as *B. andicola*).

Representative specimen examined: Region VIII, Bío Bío Province, Ecological Reserve Coligual, 37°23'S 71°40'W, ca. 630 m, on forest floor, clay banks along road with adjacent native forest, *R.R. Ireland & G. Bellolio 35234*, 21 Nov. 2002 (MO).

2. ***Rosulabryum billarderii*** **(Schwägr.) J.R. Spence**

Remarks. The common widespread species of large *Rosulabryum* from central Chile south to Aisén. The species is also found in Argentina, the Falkland Islands, Australia, New Zealand and Macquarie Island (Spence and Ramsay 2019). In the Magellanes Region it is largely replaced by *R. perlimbatum*. The species occurs in a variety of forested and semi-open habitats, often in shade on mesic to damp soil banks, fallen logs, tree stumps and soil-covered ledges. It is characterized by dioicous sexual condition, strongly rosulate stems, large leaves that are irregularly contorted when dry, a costa excurrent into a short awn, a strong limbidium, serrate leaf margins, and brown to red-brown rhizoidal tubers (Illustrations: Mohamed 1979: 407, as *Bryum billarderii*; Spence and Ramsay 2006: 335, as *R. billarderii*; Fife 2015: 84, as *R. billarderii*).

Representative specimens examined. Aisén Region, Provincia Capitán Prat, cruzando pasarela Río Ñadis, en bosque de *Nothofagus dombeyi*, 47°29'49"S, 72°56'51"W, alt. ca. 70 m, sobre tronco caído en claro del bosque, *J. Larraín & R. Vargas 26799*, 19 Ene 2007 (CONC): Region VIII, Bío Bío Province, road from Tomeco to Florida, 2 km N from Hwy 0–50, 36°57'S 72°40'W, alt. ca. 190 m, on soil bank, R.R. Ireland & G. Bellolio 32401 (MO).

3. ***Rosulabryum campylothecium*** **(Taylor) J.R. Spence**

Remarks. A common temperate species in the central and south regions of Chile from Coquimbo Region to at least the Los Lagos Region but absent from colder subantarctic climates. It also occurs in Australia and New Zealand (Spence and Ramsay 2019). The species is found on exposed to partially shaded damp to drying soil, sand, and rock ledges, often in open woodlands or on cliffs and outcrops. The species is characterized by dioicous sexual condition, medium-sized imbricate golden to yellow-green strongly concave leaves, a costa excurrent in a long often denticulate awn, a weak or nearly absent limbidium distally, serrate distal leaf margins, and the absence of rhizoidal tubers (Illustrations: Spence and Ramsay 2006: 335, as *R. campylothecium*; Fife 2015: 85, as *R. campylothecium*).

Representative specimens examined. Region VIII, Prov. Ñuble, Cobquecura, rock cliffs near ocean, 36°05'S 72°48'W, alt. ca. 0 m, on cliff, *R.R. Ireland & G. Bellolio 32374*, 9 Oct 2001 (MO): Region VIII, Prov. Concepción, Puda Beach, 36°29'S 72°54'W, alt. ca. 0 m, *R.R. Ireland & G. Bellolio 32267*, 7 Oct. 2001 (MO).

4. ***Rosulabryum capillare*** **(Hedw.) J.R. Spence**

Remarks. A worldwide temperate species found in a wide variety of habitats, but most often on damp shaded soil or fallen logs and tree stumps. Its distribution in Chile remains poorly understood but it is common in the more temperate southern regions such as Bío Bío (Ireland et al. 2017). It is characterized by dioicous sexual condition, rosulate stems, small obovate leaves that are spirally twisted around the stem when dry, a costa excurrent into a medium to long awn, moderately well-developed limbidium, serrate distal leaf margins, and small brown rhizoidal tubers (Illustrations: Syed 1973: 270 as *Bryum capillare*; Spence and Ramsay 2006: 336, as *R. capillare*; Hallingbäck et al. 2008: 349, as *B. capillare*; Fife 2015: 86, as *R. capillare*; Lüth 2019: 923 as *Ptychostomum capillare*; Holyoak 2021: 72, as *P. capillare*).

Representative specimens examined. Region VIII, Prov. Concepción, Bellavista Creek, San José Farm, 36°41'S 72°56'W, alt. ca. 90 m, on rotten log, stream with adjacent native forest with predominantly *Nothofagus*, *Aetoxicon punctatum* & *Peumus boldus*, *R.R. Ireland & G. Bellolio 33903*, 23 Nov. 2001 (MO): Region VIII, Bío Bío Province, Saltillo del Itata, small falls on Itata River, 37°04'S 72°09'W, ca. 210 m, on brick wall beside poplar trees by river, *R.R. Ireland & G. Bellolio 34950*, 28 Oct. 2002 (MO).

5. ***Rosulabryum coloratum*** **(Müll. Hal.) J.R. Spence**

Remarks. A relatively uncommon species found principally in semi-arid regions in the central and northern regions of Chile and adjacent areas of Bolivia, found on sandy soil or soil over rock, often near streams. The species is characterized by its dioicous sexual condition, evenly foliate stems, large narrowly ovate leaves that are spirally twisted around the stem when dry, a weak limbidium distally, and brown to red-brown rhizoidal tubers. Ochi (1980) had synonymized it with *R. canariense* but it is treated here as a good species (see below under *R. canariense*). (Illustration: Ochi 1980: 148, as *Bryum canariense*).

Representative specimens examined. Aisén Region, Provincia Capitán Prat, río Baker, pasando balsa Baker y luego camino río abajo, en bosque en cerro detrás de la casa de don Delmiro, junto a riachuelo, 47°15'46"S, 72°42'54"W, alt. ca. 100 m, en el suelo junto al arroyo, *J. Larraín & R. Vargas 26697*, 18 Ene 2007 (CONC); Region VIII, Prov. Ñuble, road from Cobquecura to Quiríhue, 24 km SE of Cobquecura, Mengel Creek, 36°13'S 72°36'W, alt. ca. 360 m, on sandy soil beside creek, *R.R. Ireland & G. Bellolio 32323*, 8 Oct 2001 (MO).

6. ***Rosulabryum densifolium*** **(Brid.) Ochyra**

Remarks. A robust species distributed throughout the Neotropics, primarily along the mountain chains from Mexico south to Chile, and throughout the Andes (Ochi 1980). It is common at higher elevations, often above 3000 m, but can also occur in the lowlands, and is found along streams and in wetland areas on wet soil or rock. The species is characterized by its dioicous sexual condition, long evenly foliate stems with large narrowly ovate leaves, a costa excurrent into a medium to long awn, strong limbidium, sharply serrate to denticulate distal leaf margins, with the teeth often double, and brown to red-brown rhizoidal tubers (Ochi 1967: 30, as *Bryum densifolium*).

Representative specimen examined. Bío Bío Region, Prov. Ñuble, Renegado River at Aserradero bridge, 36°54'S 71°28'W, alt. ca. 1000 m, on muddy soil bank beside river, *R.R. Ireland & G. Bellolio 30831*, 9 Dec 2002 (MO).

7. ***Rosulabryum longidens*** **(Thér.) J.R. Spence**

Remarks. A common species in the central regions of Chile, from the Bío Bío north to at least Coquimbo, usually on tree trunks and larger branches, stumps and fallen trees in forests, occasionally on soil. The species is characterized by dioicous sexual condition, small leaves, rosulate fertile stems, with numerous rosulate to evenly foliate innovations with leaves spirally twisted around the stem when dry, strong excurrent costa in medium to long awn which is sometimes red, brown to red-brown filiform gemmae in the leaf axils of sterile shoots, and small red to red-brown rhizoidal tubers. Ochi (1982) synonymized this with the Neotropical *R. pseudocapillare* (Besch.) Ochyra, but *R. longidens* is quite distinct from that species. The single report from Argentina (Ochi 1982; as *R. pseudocapillare*) needs to be re-examined as it may be a different species, thus *R. longidens* may be a Chilean endemic. It is the only epiphytic species in the genus in Chile. There are no known illustrations of this species.

Representative specimen examined. Region VIII, Bío Bío Province, Lago Falls as south side of Laja River, 37°12'S 72°22'W, alt. ca. 170 m, on tree stump, *R.R. Ireland & G. Bellolio 34815*, 23 Oct. 2002 (MO); Region VIII, Prov. Arauco, Huillinco Falls, 37°45'S 73°22'W, alt. ca. 130 m, on base of large *Nothofagus* sp., falls and rock cliff in native forests, *R.R. Ireland & G. Bellolio 33662*, 12 Nov. 2001 (MO).

8. ***Rosulabryum macrophyllum*** **(Cardot & Broth.) Ochyra**

Remarks. An uncommon species of moist to wet soil in the subantarctic moorlands and *Nothofagus* forests in southern Chile, reaching north to the Aisén Region (Ochi 1982; Larraín 2016)). The species is characterized by dioicous sexual condition, rosulate stems, large ovate leaves that are ±imbricate when dry, costa excurrent into a short awn, a fairly well developed limbidium, finely serrulate distal leaf margins, and brown to red-brown rhizoidal tubers. The species is also known from the Falkland Islands and Argentina (Illustrations: Ochi 1967: 33, as *Bryum macrophyllum*).

Representative specimen examined. Región de Aisén, Provincia de Aisén, Comuna de Cisnes Parque Nacional Queulat, sector Angostura Risopatrón, sendero Los Colonos, bosque de *Amomyrtus luma-Nothofagus nitida-Laureliopsis philippiana*, sobre tronco podrido en la orilla del lago, 44°14'04"S, 72°30'24"W, elev. 130 m, *J. Larraín 43684*, *with R. Vargas*, *E. Muñoz & J.F. Croxatto*, 14 dic 2019 (CONC).

9. ***Rosulabryum perlimbatum*** **(Cardot) Ochyra**

Remarks. A common and widespread species of the subantarctic forests and moorlands of the Magallanes and Aisén Regions on damp to wet soil, fallen logs, and tree stumps (Ochi 1982). Reports from further north are likely misidentifications and are either *R. andicola* or *R. billarderii*. This is a robust species with dioicous sexual condition, rosulate stems, sometimes with 2+ interrupted clumps along stems, large leaves that are contorted to somewhat imbricate when dry, serrulate distal leaf margins, an extremely wide limbidium of 4+ rows distally, and brown to red-brown rhizoidal tubers. The wide limbidium often gives the leaves a white-margined appearance. The species is also found in Argentina, the Falkland Islands, and extreme southern New Zealand (Illustrations: Mohamed 1979: 422, as *Bryum perlimbatum*; Fife 2015: 87, as *R. perlimbatum*).

Representative specimen examined. Prov. Antártica Chilena, Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, Isla Hoste, Península Dumas, Bahía Ibáñez, Caleta Yekadahby, 55°03'47"S, 68°25'19"W, on soil in shade in moist *Nothofagus betuloides-N. pumilio-Maytenus magellanica* forest on E-facing slope with extensive rock outcrops, *J.R. Spence 6059*, 15 January 2013(CAS).

10. *Rosulabryum puconense* (Herzog & Thér.) J.R. Spence

Remarks. This species was synonymized under *R. capillare* by Ochi (1980). However, it differs in significant ways. It is characterized by small rosulate to evenly foliate stems, small leaves that are somewhat imbricate to irregularly contorted when dry, costa excurrent in a short awn, a weak to sometimes absent limbidium, and smooth to finely serrulate distal leaf margins. Perhaps most striking are its sexual condition and rhizoidal tubers. Most collections are synoicous, but some are single-sex and thus would be considered dioicous. The species likely has the same sexual systems as *R. torquescens* and can be described as polyoicous. The rhizoidal tubers are similar to those produced by *R. rubens*, with often protuberant cell walls, but they are much larger. Some tubers are up to 600 µm across, with colors varying from red-brown to red or orange. *R. puconense* seems to have a preference for mesic to dry soil under shrubs and is especially common along the coasts from central Chile south to the Tierra del Fuego region. Although currently a Chilean endemic, it may ultimately be found in southern Argentina. There are no known illustrations of this species.

Representative specimen examined. Prov. Antártica Chilena, Comuna Cabo de Hornos, Isla Grande de la Tierra del Fuego, Bahía Yendegaia, NNE shore opposite Caleta Ferrari, 54°50'28"S, 68°47'52"W, on dry exposed soil over rock outcrops near ocean, *J.R. Spence 6042*, 13 January 2013 (CAS): Region VIII, Bío Bío Province, Saltillo del Itata, small falls on Itata River, 37°04'S 72°09'W, ca. 210 m, on soil bank among poplar trees by river, *R.R. Ireland & G. Bellolio 34940*, 28 Oct. 2002 (MO).

11. *Rosulabryum rubens* (Mitt.) J.R. Spence

Remarks. A rare species generally found in disturbed habitats, especially on disturbed soil and concrete, and possibly introduced from the northern hemisphere. There is only one record from Chile in disturbed habitats in the capitol Santiago (Ochi and Mahu

1988, at HO, not seen!). It is characterized by its dioicous sexual condition, rosulate to somewhat evenly foliate stems, small leaves that are irregularly contorted when dry, costa excurrent into a medium-length awn, a narrow but usually distinct limbidium, and serrulate distal leaf margins. The plants are often reddish-tinged. The rhizoidal tubers are diagnostic; predominantly red, with strongly protuberant cell walls, from 100–280 µm in diameter, often arising from lower leaf axils or at the base of the stem on short rhizoids. The species has a scattered world-wide temperate distribution (Illustrations: Crundwell and Nyholm 1964: 630, as *Bryum rubens*; Lüth 2019: 938 as *Ptychostomum rubens*; Holyoak 2021: 232, as *P. rubens*).

12. *Rosulabryum torquescens* (Bruch *ex* De Not.) J.R. Spence

Remarks. A widespread warm temperate to subtropical species in the northern hemisphere, Africa, Australasia and South America (Spence and Ramsay 2019). Although there are few collections from Chile, the species is likely to be common in the Mediterranean-climate regions of the country as far south as Los Lagos. It is found on soil, soil over rock and occasionally wood, including burnt wood, generally in exposed areas, often along road cuts. It is characterized by synoicous or rarely autoicous sexual condition, rosulate stems, medium-sized leaves that are irregularly contorted when dry, a costa excurrent in a medium to long awn, strong limbidium, serrate distal leaf margins, and scarlet, bright red to orange rhizoidal tubers (Illustrations: Syed 1973, 308 as *Bryum torquescens*; Spence and Ramsay 2006: 347, as *R. torquescens*; Hallingbäck et al. 2008: 350, as *B. torquescens*; Lüth 2019: 939 as *Ptychostomum torquescens*; Holyoak 2021: 257, as *P. torquescens*).

Representative specimens examined. Los Lagos Region, Chiloé, communa de Ancud, Estación Biológica Senda Darwin, Al fondo de al “ciudad de los Muertos”, Sobre tronco caido ed bosque Quemado, 41°52'S 73°39'W, 300 m, *J. Larraín 23424*, 2 Feb. 2003 (CONC): Region VIII, Prov. Concepcion, Park “Jorge Alessandri” (Compania Manufacturera de Papeles y Cartones), on soil in clearing, 36°56'S 73°09'W, ca. 200–490 m, *R.R. Ireland & G. Bellolio 32819*, 19 Oct. 2001 (MO).

Excluded species

Rosulabryum canariense (Brid.) Ochyra. Ochi (1980) placed *R. coloratum* into synonymy under this species. Here they are treated as specifically distinct (see above under *R. coloratum*). True *R. canariense* is autoicous or synoicous, has interrupted rosulate tufts along the stem, irregularly contorted obovate leaves, and bright red, scarlet or orange rhizoidal tubers. I have not seen any material from Chile in collections examined in this study with these characters, thus it is tentatively excluded from South America, but is retained in the key pending additional field work. It is a northern hemisphere Mediterranean-climate species, also reported from South Africa, although the plants there are somewhat distinct from northern hemisphere plants.

Rosulabryum viridescens (Welw. & Duby) Ochyra. Ochi (1977) reported this species based on the type of *Bryum hamatum* Dusén, an illegitimate name as there was no formal description. More recently, I have identified several collections from the Bío Bío Region as this species. However, having recently obtained material of *R. viridescens* from South Africa, it is clear that the South American material is not that species (cf. Magill 1987). Most of the recently named collections are atypical specimens of *R. campylothecium*, while the *B. hamatum* type may be an aberrant form of *R. macrophyllum* or *R. perlimbatum* characterized by imbricate ovate leaves. Thus *R. viridescens* in tentatively excluded from Chile and South America, although it is retained in the key.

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RESEARCH ARTICLE

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Calceolaria flavida (Calceolariaceae) a new endemic species to central Chile

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Abstract

A new species of *Calceolaria* sect. *Cheiloncos* endemic to central Chile is described. A comparison with the morphologically similar species *Calceolaria asperula* and *Calceolaria petioalaris* is made, and a key as well as detailed images to differentiate them is provided. The species is only known from the Natural Sanctuary Cerro El Roble, which is part of the coastal mountain range of central Chile and can be considered as Critically Endangered (CR) under the IUCN categories and criteria.

Keywords

Chilean Mediterranean hotspot, Cerro el Roble, endemism, montane flora, South America, taxonomy

Introduction

Calceolaria Linnaeus is the largest genus within Calceolariaceae with approx. 250 species distributed from Mexico to Southern Chile and Argentina (Molau 1988; Cosacov et al. 2009). The centre of diversity of the genus is found in Peru (Molau 1988). The genus includes herbs and shrubs characterised by opposite leaves and bilabiate corollas with a saccate lower lip with an infolded lobe normally bearing the elaiophore, a highly specialised, oil-producing structure involved in pollination (Vogel 1974).

The latest and most comprehensive revision of *Calceolaria* for Chile since Reiche (1911), published by Ehrhart (2000), was followed by the publication of new species segregated from the *C. integrifolia* complex (Ehrhart 2005), the revision of *Calceolaria* section *Calceolaria* (Puppo and Novoa 2012) and by *Calceolaria philippii* Eyzaguirre (2014). In Chile, there are 61 currently recognized species of *Calceolaria*, ten of which are further separated in a total of 30 subspecies (Rodriguez et al. 2018). Out of a total of 81 taxa, 60 (74%) are endemic to Chile. *Calceolaria* in Chile presents a wide distribution, from the latitudes of Arica y Parinacota region (18°35'S) to Magallanes region (54°50'S), and from the coast to the high elevations of the Andes (0–4300 m). Unsurprisingly, the area of most diversity is central Chile, from the Coquimbo region to the Araucania region (Ehrhart 2000). High levels of endemism in plants are common in the biogeographic area of central Chile which is recognized as a biodiversity hotspot (Myers et al. 2000; Arroyo et al. 2004).

Infrageneric classification within *Calceolaria* has been a subject of several works (Bentham 1846; Wettstein 1891; Kränzlin 1907; Pennell 1951). Molau (1988), working on the monograph of the tropical species of *Calceolaria*, restructured previous classifications and divided *Calceolaria* into three subgenera. *Calceolaria* subgenus *Calceolaria* comprises mainly species found in tropical regions, while *Calceolaria* subgenus *Cheiloncos* (Wettstein) Pennell and *Calceolaria* subgenus *Rosula* (Descole & Borsini) Molau comprise mostly temperate species. Subsequently, Ehrhart (2000), organized the species native to Chile in four sections: *Calceolaria* sect. *Calceolaria* (one species, *Calceolaria pinnata* L. subsp. *pinnata*), *Calceolaria* sect. *Kremastocheilos* Witasek (one species, *Calceolaria uniflora* Lam.), *Calceolaria* sect. *Tenella* C. Ehrhart (one species, *Calceolaria tenella* Poepp. & Endl.), and *Calceolaria* sect. *Cheiloncos* Wettstein (47 species). The latter section, comprising most of the species present in Chile, was further divided into 14 informal Greges, based on vegetative and reproductive characters. More recently, molecular (Andersson 2006) and combined molecular and morphological studies (Cosacov et al. 2009), confirmed the subgeneric classification and some of the sections proposed by Molau (1988) as monophyletic, while most of the sections were found to be polyphyletic, and sections *Tenella* and *Kremastocheilos* sensu Ehrhart (2000) had little support. Due to the lack of resolution and the low sampling of Chilean species in Andersson (2006) and Cosacov et al. (2009), the classification proposed by Ehrhart (2000), particularly for *Calceolaria* sect. *Cheiloncos*, is yet to be supported by studies with more extensive sampling.

The aim of this work is to describe a new species of *Calceolaria*, endemic to central Chile, assess its conservation status and provide a key for correct identification.

Methods

Between the austral Spring of 2018–2020, several botanical explorations were carried out in the coastal mountain range of central Chile, between the limits of Valparaiso region and the Metropolitan region, in the Natural Sanctuary "Cerro El Roble", 75 km northeast of Santiago's urban area (Fig. 1). Specimens of *Calceolaria* that could

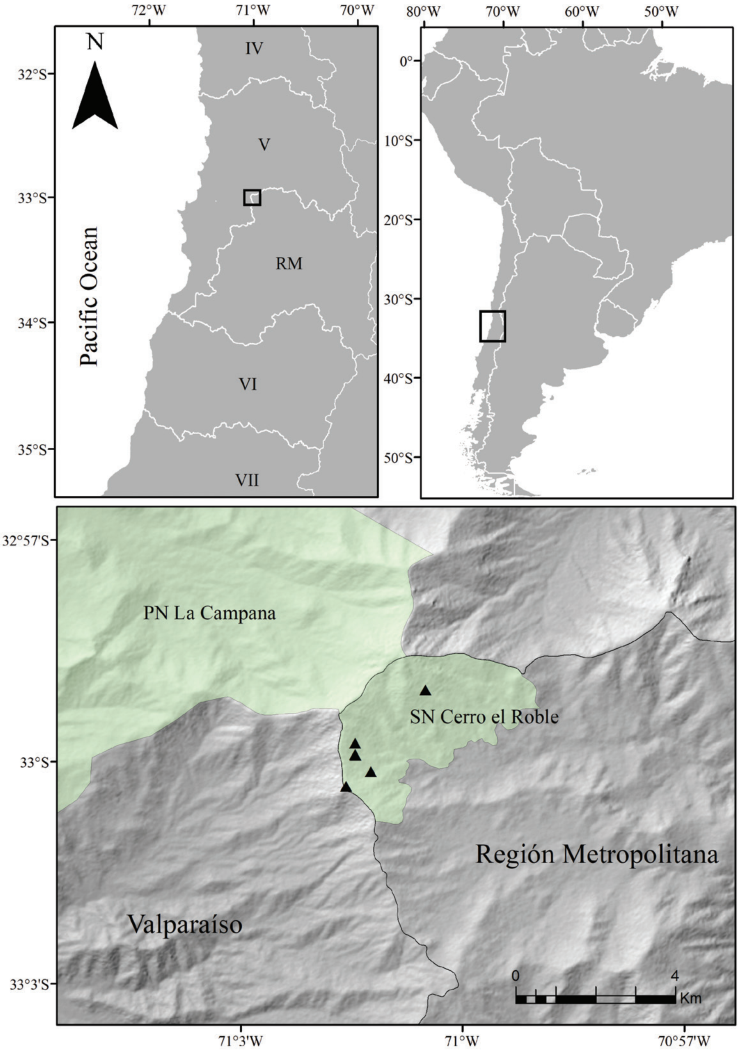

Figure 1. Distribution map of *Calceolaria flavida* (triangles) in Chile. Roman numbers represent administrative regions (IV Coquimbo, V Valparaiso, RM Metropolitan Region, VI O'Higgins, VII Maule). Green polygons represent protected areas (La Campana National Park and Natural Sanctuary Cerro El Roble).

not be assigned to any of the described species of the genus were found flowering in two sites close to the summit (1722–1729 m and 1766 m). The climate of the study site is classified as Mediterranean type with a rainfall regime characterized by an annual mean precipitation of 656 mm, a water deficit of 897 mm, and a 7-month dry season (Donoso et al. 2010). The soil is mainly composed of weathered granitic rocks (Brüggen 1950). The vegetation of this area is characterized by a relict deciduous forest dominated by *Nothofagus macrocarpa* (Nothofagaceae), and surrounded by sclerophyllous forest and scrub of *Quillaja saponaria* (Quillajaceae) and *Lithraea caustica* (Anacardiaceae) (Latorre-Beltrán 2012). At lower elevations, on the bottom of creeks with permanent flooding by groundwater, dense swamp forests of *Drimys winteri* (Winteraceae) and *Luma chequen* (Myrtaceae) can be found. At the summit, a relict andean scrub dominated by *Chuquiraga oppositifolia* (Asteraceae) and *Azorella prolifera* (Apiaceae) is found (Ministerio del Medio Ambiente 2018).

Specialised literature on systematics and taxonomy of *Calceolaria* was consulted (Witasek 1905; Valenzuela 1969; Ehrhart 2000; Ehrhart 2005). Herbarium specimens were collected and deposited at SGO (Lavandero 372; Lavandero & Santilli 201027). A systematic examination of selected specimens of *Calceolaria* found at SGO, EIF, CONC, as well as online digital images of specimens available on E, PH and US (acronyms following Thiers 2021) was carried out to search for more collections that could be morphologically coincident with the species. Herbarium specimens with similar morphology were found at SGO identified as *Calceolaria asperula* and *Calceolaria* aff. *asperula*. A thorough examination and dissection of the type specimen of *Calceolaria asperula* Phil. (SGO 055831) was performed, due to discrepancies with the schematic representation of the flower and the description of the species by Ehrhart (2000) and to confirm the identity of the new species.

The description and key were prepared after examining all available specimens. Description was made based on terminology following Ehrhart (2000) and Ehrhart (2005).

The assessment of the conservation status of the species was made using the International Union for Conservation of Nature (IUCN 2017) criteria. The extent of occurrence (EOO) and area of occupancy (AOO) were calculated using GeoCat (Bachman et al. 2011).

Results

Following the morphological comparison of the plant collected with the specimens found in the consulted herbaria, we reached the conclusion that the individuals found in Cerro El Roble represent a new species. The new species is vegetatively similar to *Calceolaria asperula* Philippi and to *Calceolaria petioalaris* Cavanilles, both species endemic to Central Chile, belonging to *Calceolaria* sect. *Cheiloncos*, group B, Grex X (C. dentatae) and Grex XI (C. petioalaris) sensu Ehrhart (2000), respectively. The three species have in common the growth form and other vegetative characters. They are perennial herbs with a lignified base, without any woody shoots aboveground that last from one growing season to the next one, with non-branching shoots aboveground, new shoots early in the season with very short internodes, giving them the appearance of a rosette-like structure (these internodes elongate later in the season and the rosette disappears), and ovate leaves and serrate margins, covered in glandular hairs.

Nevertheless, both leaf texture and indumentum and flower morphology differ considerably among the three species (Figs 2–4). The secondary and tertiary venation of the new species is visibly impressed on the adaxial side and prominent on the abaxial side of the lamina (Fig. 2C–D). The leaf indumentum is formed by long and densely arranged glandular and eglandular trichomes, which gives a glutinous and sticky texture. Freshly collected material can hardly be separated from the paper in which it is dried. The leaf texture and indumentum is similar to *Calceolaria asperula* (Fig. 2A–B), but the latter has a deeply impressed venation on the upper surface, forming deep cavities, giving the most rugose aspect of the three species. *Calceolaria petioalaris*, has a venation slightly impressed on the adaxial side and slightly prominent on the abaxial side, with leaf indumentum composed of short glandular and eglandular trichomes, which give a less glutinous and sticky texture; freshly collected material can easily be separated from the paper in which it is dried (Fig. 2E–F). The flower lips of the new species are rounded in shape, saccate, and the upper lip is narrower and longer than half the length of the lower lip (Fig. 3C–D), while the flower lips of *C. petioalaris* are squared, flat and almost equal in width, and the upper lip is shorter than the lower lip (Fig. 3E–F). The length of the stamens of the new plant and *C. petioalaris* is similar, while *C. asperula* presents much shorter filaments (Fig. 4A, C, E). The new species shows an elaiophore similar to *C. petioalaris* and different from the one of *C. asperula* which has an elaiophore made of dispersed oil producing trichomes (Fig. 4B, D, F), a character that is unique among *Calceolaria* found in Chile (Ehrhart 2000).

The dissection of the type specimen of *Calceolaria asperula* (SGO 055831) showed that the lips differ in size, being the upper lip less than half the size of the lower lip (Suppl. material 1). This contrasts with the schematic representation of the flower of *Calceolaria asperula* found in Ehrhart (2000). The dissection also confirms that the elaiophore of *Calceolaria asperula*, is formed by dispersed oil producing trichomes (Suppl. material 1: Fig. S1D–E).

Taxonomic treatment

Calceolaria flavida Lavandero & Santilli, sp. nov.

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Figures 2C–D, 3C–D, 4C–D, 5, 6B-C

Diagnosis. *C. flavida* is most similar to *C. asperula* and *C. petioalaris* in growth habit and in having leaves of similar shape covered in glandular hairs. *C. flavida* can easily be distinguished from *C. asperula* in having pale yellow corolla (vs. bright yellow), the upper lip longer than half the length of the lower lip (vs. upper lip shorter than half the length of the lower lip), anthers much shorter than filaments and opening towards the distal part of the upper lip (vs. anthers as long as filaments and opening toward the style) and an elaiophore with densely arranged oil-producing trichomes (vs. dispersed oil producing trichomes). It can be distinguished from *C. petioalaris* by its reddish stems (vs. green), secondary and tertiary veins of the adaxial side of leaf lamina visibly impressed (vs. secondary and tertiary veins of the adaxial side of leaf lamina slightly impressed), pale yellow corolla (vs. bright yellow), upper lip narrower than lower lip seen from above (vs. upper lip as wide as lower lip), lips rounded in shape (vs. squared), saccate upper lip (vs. flat), and style inserted in corolla (vs. exserted).

Type. CHILE. Región Metropolitana, Cerro El Roble, 1674 m, 32°59'54" S - 71°01'27" W, 17-12-2006, *N. García & M. Muñoz 3836* (holotype SGO 157641!)

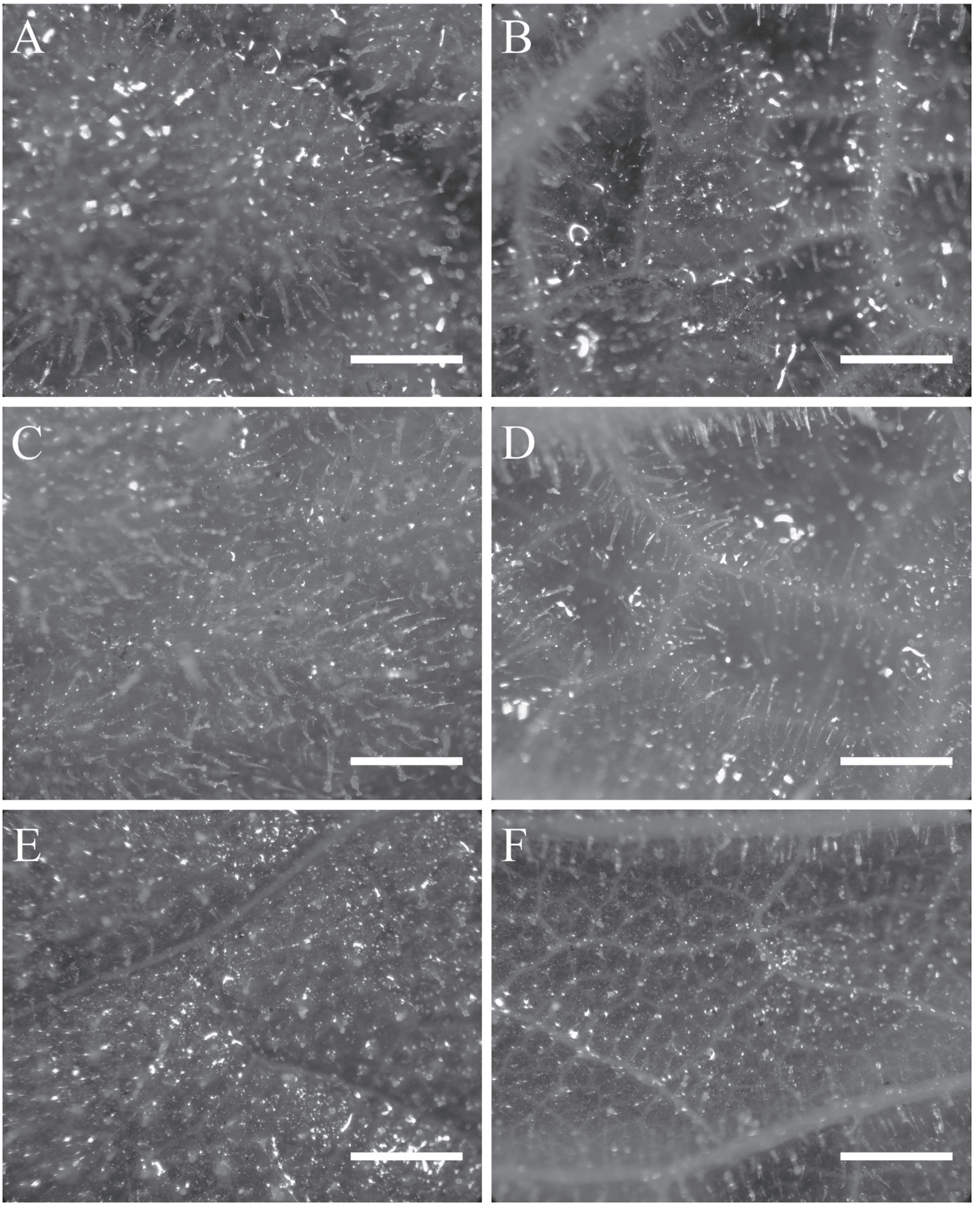

Figure 2. Indumentum type in leaves of *Calceolaria* **A, C, E** upper leaf surface **B, D, F** lower leaf surface **A, B** *Calceolaria asperula* (Lavandero 409 (SGO)) **C, D** *Calceolaria flavida* (Lavandero & Santilli 201027 (SGO)) **E, F** *Calceolaria petioalaris* (B. Rosende s/n). Scale bar: 1 mm.

Description. ***Perennial*** herb up to 60 cm; base lignified, growth form type 6 sensu Ehrhart (2000). ***Stems*** reddish, erect, lower vegetative part not branched, densely covered with glandular hairs accompanied by much longer regular hairs; internodes very short at the beginning of the growing season, giving the aspect a rosette, these

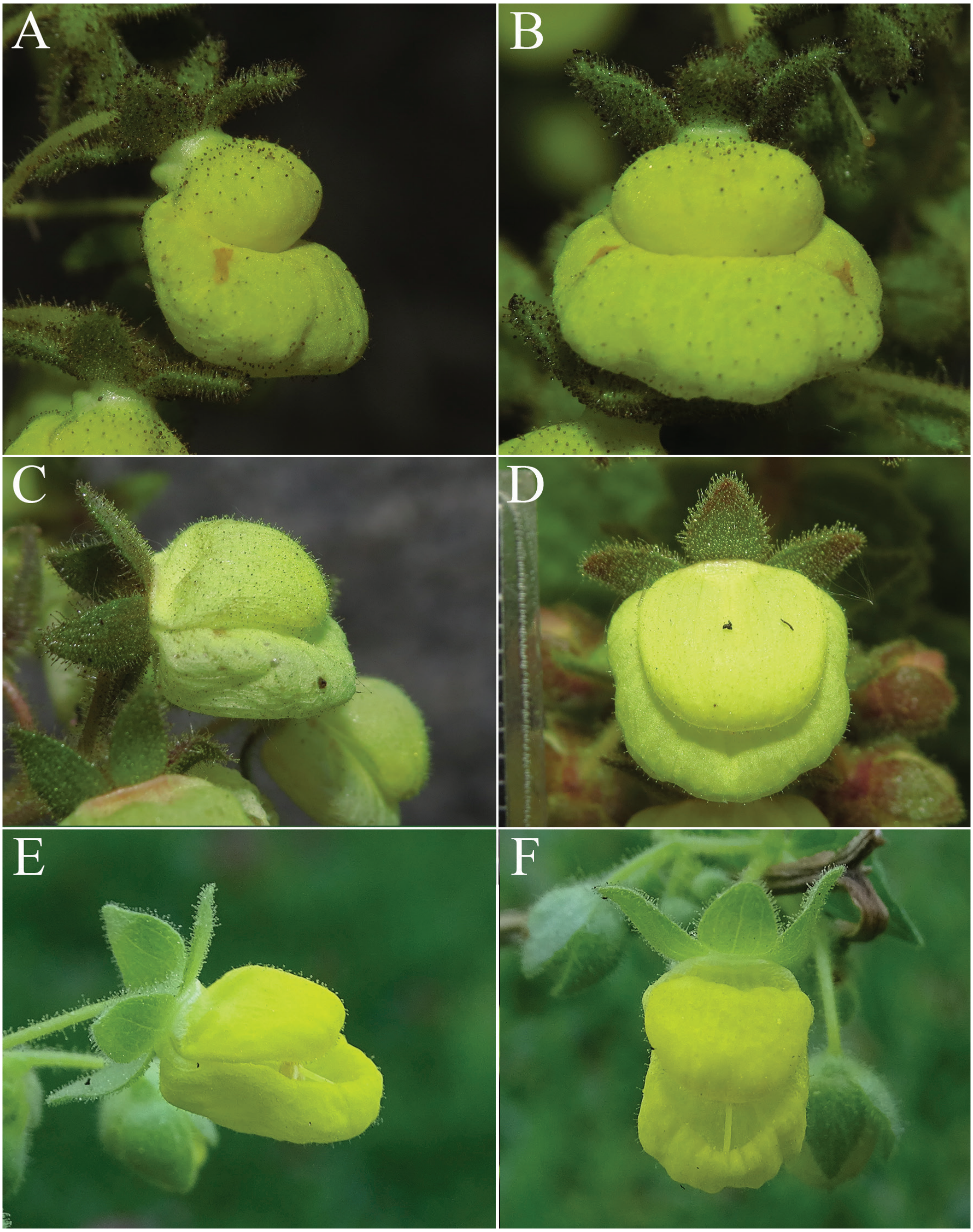

Figure 3. Lateral and frontal view of flowers (from left to right) of *Calceolaria* **A, B** *Calceolaria asperula* (Lavandero 409 (SGO)) **C, D** *Calceolaria flavida* (Lavandero & Santilli 201027 (SGO)) **E, F** *Calceolaria petioalaris* (B. Rosende s/n).

internodes extend throughout the growing season, being progressively longer towards the apex; stems renewing from the lignified base every season. ***Leaves*** opposite, green; lower leaves lanceolate, petiolate, base cuneate, apex acute; upper leaves ovate, sessile

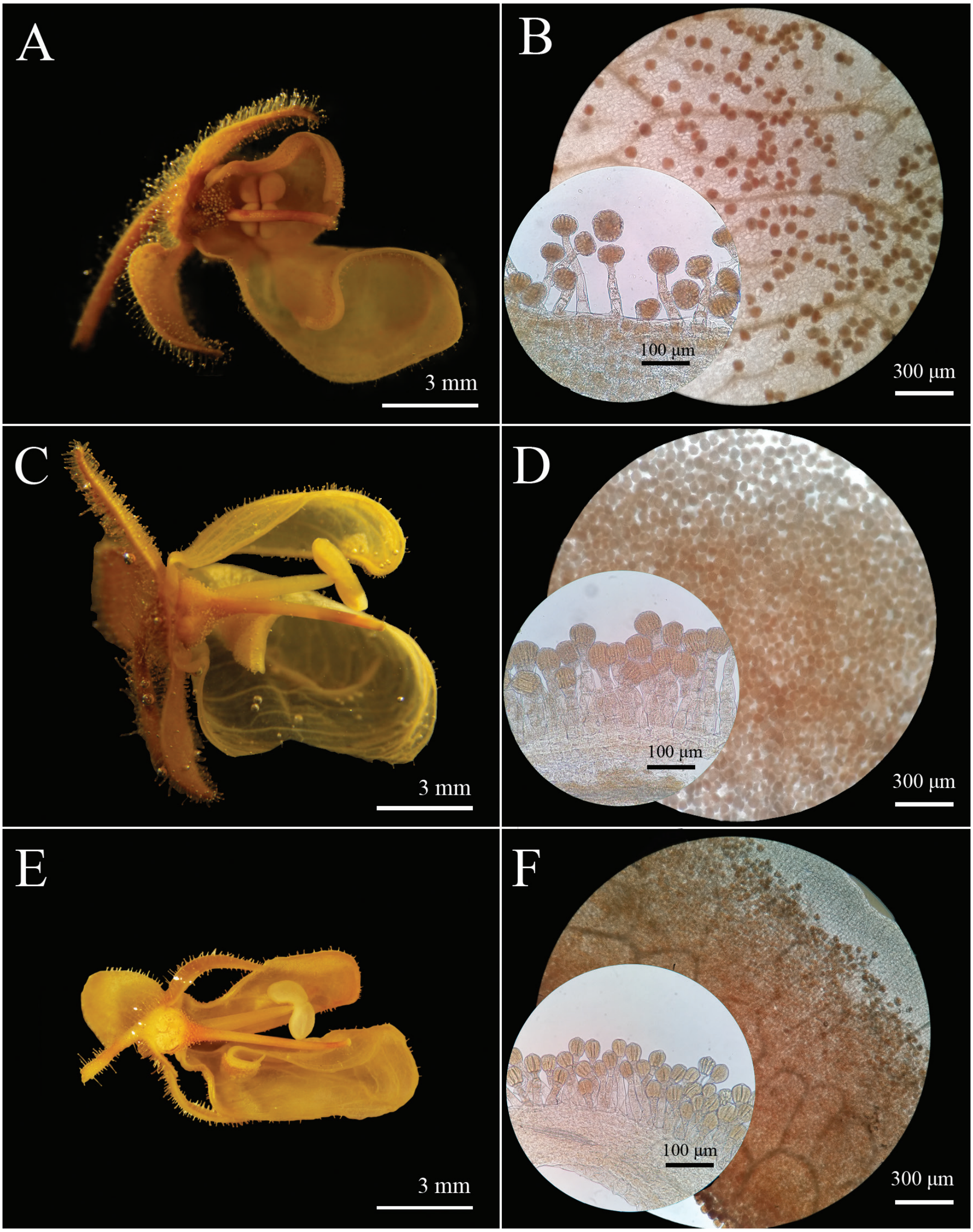

Figure 4. Lateral cross-section view of flowers of *Calceolaria* and detail of elaiophores **A, B** *Calceolaria asperula* (Lavandero 409 (SGO)) **C, D** *Calceolaria flavida* (Lavandero & Santilli 201027 (SGO)) **E, F** *Calceolaria petioalaris* (B. Rosende s/n).

to partially amplexicaul, base subcordate, apex acute; (1.7–)2–7(–8.5) × (1.2–)2.5–3.5(–4) cm, margins serrate or slightly biserrate, lamina hirsute, trichomes glandular; venation impressed in the upper surface and prominent in the lower surface, secondary and tertiary veins of the adaxial side of leaf lamina visibly impressed. ***Synflorescence***

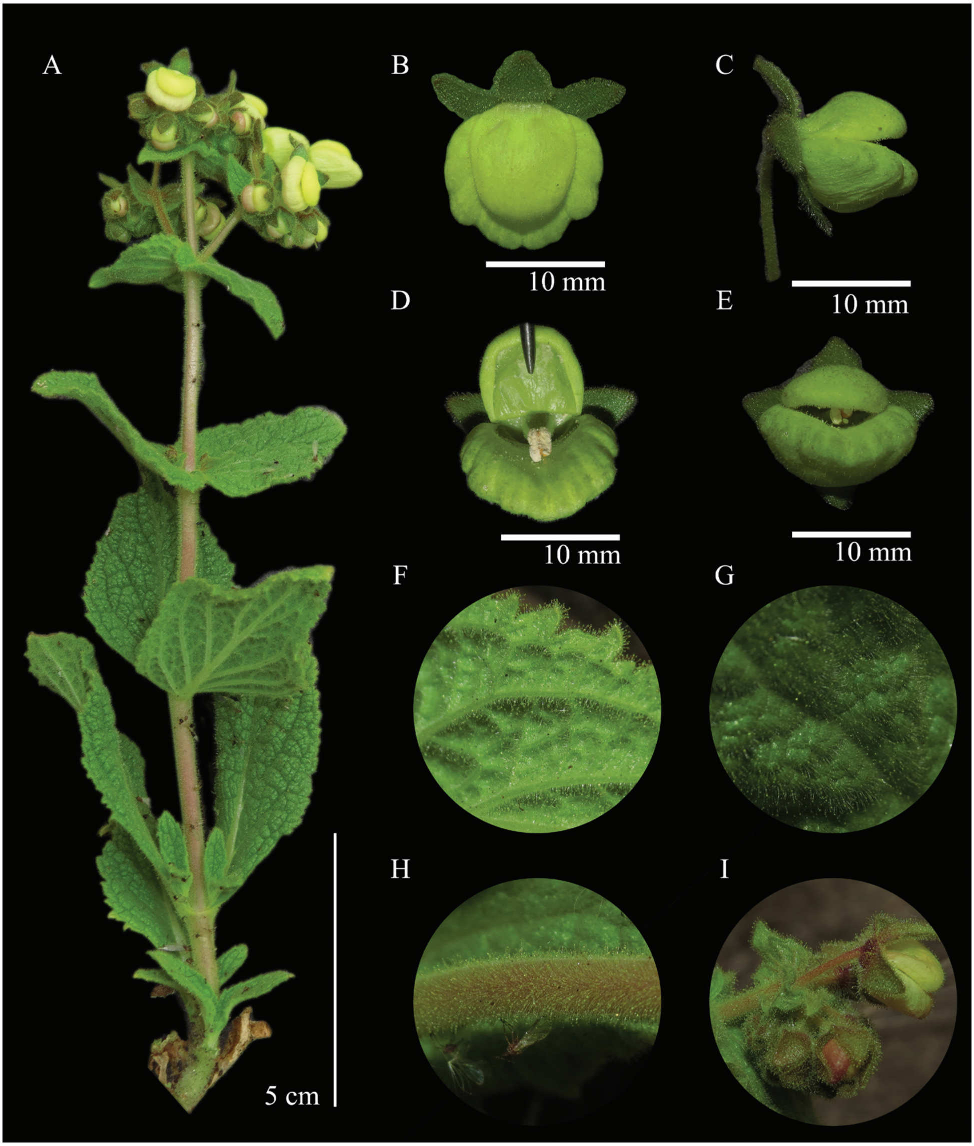

Figure 5. *Calceolaria flavida* **A** habit **B** upper side view of flower **C** lateral view of flower **D** frontal view of flower with upper lip open **E** frontal view of flower **F** detail of abaxial side of leaf **G** detail of adaxial side of leaf **H** detail of stems **I** detail of early-flowering inflorescence.

not conspicuously elevated from the vegetative part, up to 32 cm tall including the basal internode of the main florescence; basal internode 46–85 mm and as long as the internodes between the leaves at most; main inflorescence composed of 1–3 pairs of 15–19-flowered cymes; hypopodia 3.4–6.4 cm; pedicels 6.5–10.2(–20) mm; cyme bracts sessile, 14–30 × 8–25 mm, subordinate bracts sessile, 5–9 × 3–6 mm. ***Sepals*** green, ovate, 6.5–7.3 × 3.7–4.2 mm, densely covered in glandular hairs on both sides. ***Corolla*** pale yellow, evenly covered in glandular hairs, longitudinal axes of the lips par-

Figure 6. Habitat of *Calceolaria flavida* **A** NW-facing slopes dominated by *Puya coerulea var. coerulea*, *Lithraea caustica* and *Gochnatia foliolosa* (Natural Sanctuary Cerro El Roble, Región Metropolitana, Chile) **B, C** habit of *Calceolaria flavida*.

allel to each other, the upper lip longer than half the length of the lower lip and close to one another; lower lip saccate, rounded and lobed, 9.0–10.5 (length) × 9.2 (width) × 6.0–6.2 (height) mm; aperture narrow and oval, facing the upper lip, depression of the upper side almost absent; upper lip saccate, rounded to truncate seen from above, narrower than lower lip seen from above, 6.8–9.3 × 8.0–9.2 × 4.0–4.1 mm; aperture wide and almost reaching the sides of the lip. ***Elaiophore*** type 1 (sensu Ehrhart 2000), same length as the opening of the lower lip, 7.6 × 2.4 mm, folded inwards into the lower lip and covering the end of the lobe; oil-producing trichomes 190–245(-270) µm long, stalk generally (3–)4–6(–7)-celled and glandular head 38–44-celled, densely arranged, forming a well-defined and compact cushion. ***Stamens*** 2, included in the upper lip, stamens and style almost parallel, forming an acute angle; filaments 5.1–5.4 mm; anthers shorter than filaments, dithecal, basifixed, with line of dehiscence opening towards the distal part of the upper lip, 2.7–3.3 × 1.2–1.4 mm; ***Gynoecium*** (ovary + style) 6.0 mm; ovary densely covered by glandular hairs; style inserted in upper lip, 4.3 mm; stigma inconspicuous. ***Capsule*** conic, acuminate, 5.3–5.6 × 3.4–3.7 mm, with sparse glandular hairs. ***Seeds*** globose, 520–600 × 280–340 µm, seed surface type 3 (sensu Ehrhart 2000).

Habitat and distribution. *C. flavida* seems to be endemic to the Natural Sanctuary Cerro El Roble (33°00'S 71°01' W), which is part of the coastal mountain range of central Chile (Fig. 1). It can be found on slopes with N-NW orientation at elevations of 1450–2200 m. *Calceolaria flavida* grows on soils of granitic origin, between rocks in open areas within sclerophyllous scrub dominated by *Puya coerulea* Lindl. *var. coerulea* (Bromeliaceae), *Lithraea caustica* (Molina) Hook. & Arn. and *Gochnatia foliolosa* (D. Don) D. Don ex Hook. & Arn (Asteraceae) (Fig. 6A).

Phenology. The species was found flowering between October and January.

Etymology. TThe specific epithet flavida is a singular, feminine, nominative Latin adjective alluding to pale yellow colour of corolla.

Conservation status. *C. flavida* can be considered as Critically Endangered (CR) under the IUCN categories and criteria B1ab(iii). The criterion B1 was selected because its extent of occurrence is < 100 km^2 (0.995 km^2). The criterion "a" was selected because it is known to exist at only one location (=1). The criterion "b(iii)" was selected because there is a projected decline in the area, extent and quality of habitat. Climate change and the persistent drought that has been affecting Central Chile represent a threat to plants that grow in the region. Starting in 2010, the Chilean territory between the Coquimbo and Araucanía Regions has experienced a rise in temperature and a precipitation deficit of approximately 30% causing visible deterioration of non-irrigated vegetation as well as increasing the likeability of forest fires (Garreaud, 2015). The species grows within the Natural Sanctuary Cerro El Roble.

Additional specimens examined. Chile. Región Metropolitana: Provincia de Chacabuco: Caleu, Cerro El Roble, antes de los potreros, 12 January 2002, *N. García 3863* (EIF); Cerro El Roble, km 5 camino a la cumbre, 1 January 2003, *A. Moreira 863* (SGO); Subida a Cerro El Roble, poco más abajo Portezuelo Rauco, 27 October 2005, *M. Muñoz 4741* (SGO); Caleu, camino a El Roble, 1 km más abajo del corral, 17 December 2006, *N. García & M. Muñoz 3839* (SGO); Cerro El Roble, arriba del refugio a 3.5 km desde la entrada, 29 November 2019, *N. Lavandero 372* (SGO); Cerro El Roble, 27 October 2020, *Lavandero & Santilli 201027* (SGO).

Key of herbaceous *Calceolaria*

Key of herbaceous *Calceolaria* with a woody base and glandular indumentum, taller than 20 cm, with leaves along the stem, not arranged in a rosette, entire with tendency to be sessile, less than five times longer than wide, longer than 25 mm (replace couplet 17 of Ehrhart's key to *Calceolaria* of Chile (Ehrhart 2000)).

1 Leaves with secondary and tertiary veins deeply impressed on the adaxial side; glandular hairs dark, scattered along the corolla; upper lip less than half the length of the lower lip, anthers as long as filaments, and opening toward the style, elaiophore with scattered oil-producing hairs .... ***C. asperula***

– Leaves with secondary and tertiary visibly or slightly impressed on the adaxial side; glandular hairs clear, densely arranged along the corolla; upper lip more than half the length of the lower lip, anthers much shorter than filaments and opening towards the distal part of the upper lip, elaiophore with densely arranged oil-producing hairs..**2**

2 Stems green; leaf margin dentate with sharp teeth, indumentum of short glandular hairs; leaves with secondary and tertiary veins slightly impressed on the adaxial side; corolla bright yellow, upper lip as wide as lower lip seen from above, lips squared in shape, bright yellow, flat; style exserted from corolla..... ***C. petioalaris***

– Stems reddish; leaf margin dentate, with smooth teeth, indumentum of long glandular hairs; leaves with secondary and tertiary veins visibly impressed on the adaxial side; corolla pale yellow, upper lip narrower than lower lip seen from above, lips rounded in shape, pale yellow, saccate; style inserted in corolla....... ***C. flavida***

Discussion

Initial confusion existed regarding the identity of *Calceolaria asperula*. In the protologue of *C. asperula*, Philippi (1895) only gives the diameter of the inferior lip in a short description without mentioning the upper lip. Ehrhart (2000) only illustrates the taxon with a schematic representation of the flower, showing lips of almost equal size, being the upper lip slightly smaller than the lower, and only gives the size of the upper lip seen from above, being 5.5 mm in diameter approximately. Ehrhart (2000) describes it as a species with a unique combination of characters such as the anthers opening towards the style and an elaiophore made of dispersed oil-producing hairs. The dissection of a flower from the type material of *C. asperula* (SGO055831) (Suppl. material 1) shows Ehrhart's (2000) description to be mostly accurate regarding vegetative morphology and elaiophore structure, but the upper and lower lip description is incomplete and imprecise, making the schematic representation of the flower doubtful. This imprecision in Ehrhart's schematic representation of the flower might explain why specimens of *Calceolaria flavida* found in SGO were formerly identified as *C. asperula* or, in some cases, as *Calceolaria* aff. *asperula*.

The classification proposed by Ehrhart (2000) for the species of *Calceolaria* present in Chile, although not yet confirmed by molecular evidence, is however very useful for grouping species based on both vegetative and reproductive characters, the latter having higher weight for the classification at lower levels. Within *Calceolaria* sect. *Cheiloncos*, *Calceolaria flavida* falls into group B, by having an upper lip at least 1/3 as long as the lower lip and anthers shorter than the filaments. At the Grex level, it could be classified within two Greges, Grex X (comprising *C. densifolia* Phil., *C. dentata* Ruiz & Pav., *C. flavovirens* C.Ehrhart, *C. lepida* Phil., *C. morisii* Walp., *C. nitida* Colla, *C. polifolia* Hook, *C. asperula* Phil. and *C. purpurea* Graham) and Grex XI (comprising *C. petioalaris* Cav. and *C. latifolia* Benth.). Among these species, *Calceolaria flavida* has clear affinities with two species, *C. asperula* and *C. petioalaris*, based on leaf shape and growth habit, leaving out all the other species within these two Greges presenting a shrubby habit. The most useful characters to differentiate the new species from the morphologically most similar species *C. asperula* and *C. petioalaris,* proved to be flower related, showing the importance of these stable characters for the taxonomy of *Calceolaria*. From the ecological and geographical perspective, these three species can be clearly distinguished. *Calceolaria petioalaris* is the only one among the three species that associates with meso-hydrophytic conditions, growing most of the time near water courses such as small streams or ravines from the coast up to mid-elevations of the Andean cordillera (50–1800 m), between the Coquimbo and Maule Regions (Ehrhart 2000). *Calceolaria asperula* can be found in more xeric conditions at elevations between 800–2000 m, in both Coastal and Andean Cordilleras, in open and rocky areas among the sclerophyllous montane vegetation, between the Metropolitan and O'Higgins regions. *Calceolaria flavida* is more similar to *C. asperula* in terms of its ecology. It can also be found in xeric conditions associated with sclerophyllous vegetation at mid-elevations (1450–2200 m), but only grows on soils of granitic origin with N-NW orientation of Cerro El Roble, one of the tallest peaks of the Coastal Cordillera of Central Chile.

Out of 81 taxa recognized for Chile, 61 are endemic (Rodriguez et al. 2018; RBG Edinburgh 2021). Most of the endemism is located around Coquimbo and Maule Regions (29°02'S–36°32'S), being Valparaiso and the Metropolitan Region (32°01'S–34°17'S) the most species-rich regions (Rodríguez et al. 2018; RBG Edinburgh 2021). The Coastal cordillera of central Chile is already known to host several species of *Calceolaria* endemic to Chile (Ehrhart 2000; Ehrhart 2005; García 2010; Flores-Toro and Amigo 2013). Moreover, Cerro El Roble hosts two narrow-endemic (Sensu Molau 1988) species of the genus: *Calceolaria caleuana* Muñoz-Schick & Moreira, found on the summit of Cerro El Roble and another locality in the limits of Valparaiso and Coquimbo Regions (Muñoz-Schick and Moreira-Muñoz 2008; Muñoz-Schick and Moreira-Muñoz 2009), and *Calceolaria ascendens* Lindl. subsp. *exigua* (Witasek) Nic. García, a rupicolous taxon found only on rocky outcrops of the coastal Cordillera between 32°42'S – 33°12'S at elevations of 1600–2100 m (García 2010). La Campana National Park, adjacent to Natural Sanctuary Cerro El Roble, is also home to the narrow endemic *Calceolaria campanae* Phillipi, which grows between the rock crevices near the summit of Cerro La Campana. This pattern of diversity found in *Calceolaria* is not unusual for the Chilean flora. Several genera share the same pattern of high diversity and endemism in Central Chile, such as *Senecio* L., *Chaetanthera* Ruiz & Pav., *Haplopappus* Cassini, *Leucheria* Lagascae, *Oxalis* L. and *Adesmia* D.C. (Arroyo et al. 1995; Fuentes et al. 1995). A combination of high climatic heterogeneity due to latitudinal and altitudinal gradients (Armesto et al. 2007), plus the climatic history of the Quaternary, particularly glaciations and the presence of coastal refugia, are the probable drivers for the higher diversity and endemism in this region (Arroyo et al. 1995; Villagrán 1995; Hinojosa and Villagrán 1997; Villagrán and Hinojosa 1997).

The origin and present distribution of *C. flavida* could be related to the series of expansions/contractions and isolation of the vegetation belts in the Coastal Cordillera due to the glacial/interglacial cycles. Since there is no updated phylogeny of *Calceolaria*, no relationships could be inferred for *C. flavida*. Based on its morphology and following the preliminary phylogenetic studies (Cosacov et al. 2009), it could be hypothesized that it belongs to the subgenus *Cheiloncos* sect. *Rugosae* along with the most morphologically similar species *Calceolaria asperula* and *Calceolaria petioalaris*, all endemic to central Chile. A well-resolved phylogeny of the genus could help clarify the relationships among these species and establish a better understanding of the complex evolutionary history of *Calceolaria* in central Chile.

Since *Calceolaria flavida* appears to be a narrow-endemic and our preliminary assessment classifies it as Critically Endangered (CR), further surveys in the Coastal Cordillera of central Chile are needed in order to fully understand its distribution and population size.

Additional specimens examined

Calceolaria asperula. **Chile. Región Metropolitana:** Provincia de Melipilla: Reserva Nacional Roblería del Cobre de Loncha, 18 November 2004, *P. Baxter*, *F. Bustos*, *M.F. Gardner*, *P. Hechenleitner V. & P.I. Thomas 1439* (SGO!, E[photo]!); Reserva Natural Altos de Cantillana, sendero desde refugio el alto a Horcón de Piedra, 28 December 2019, *N. Lavandero 671* (SGO!); Reserva Natural Altos de Cantillana, sendero desde refugio el alto a Horcón de Piedra, 20 November 2020, *N. Lavandero 201120* (SGO!); Provincia Cordillera: Pirque, Reserva Nacional Río Clarillo, Sendero Quebrada Jorquera, 29 January 2013, *T. Christian*, *M.F. Gardner & V. Morales 343* (E [photo]!); Reserva Nacional Río Clarillo, Cajón de los Cipreses, 13 December 2019, *N. Lavandero 409* (SGO!); **Región de O'Higgins**: Cajón de los Cipreses, March 1875, *R.A. Philippi s.n.* (SGO 055831!, PH[photo]!), Provincia del Cachapoal: Rancagua, road from Coya to Mina La Juanita, ca. 8.3 km above retén de Carabineros, 18 January 1995, *L.R. Landrum & J. Martínez 8486* (SGO!); Machalí, Road from Coya to Machalí, 3 January 2009, *M.F. Gardner & S.G. Knees 8453* (E [photo]!); Copada, open rocky slope, 25 January 1925, *F.W. Pennell 12272* (US [photo]!)

Calceolaria petioalaris. **Chile. Región de Coquimbo:** Provincia de Limarí: Camino a Mina Lapislazuli, 14 January 2009, *Fundación Philippi 349* (SGO); Provincia Choapa: Camino Tilama - cuesta Las Palmas, 18 November 2002, *A. Moreira 784*

(SGO): Provincia de Elqui: Illapel, Cuzcuz, 5 November 1985, *M. A. Trivelli s.n* (SGO). **Región de Valparaíso**: Provincia de Petorca: Catemu al N entre Las Majadas y Campamento Cerro Negro, 28 December 2001, *A. Moreira 643* (SGO); Provincia Los Andes: Camino a Portillo, 11 December 2001, *M. Chamy & M. Piovano 15* (SGO); camino a Portillo, 11 December 2001, *M. Chamy & M. Piovano 16* (SGO); Ruta 60, Camino hacia Portillo 21 December 2019, *N. Lavandero 421* (SGO); Provincia de Quillota: Olmué, Parque Nacional La Campana, sector Granizo, 1 February 1998, *Baxter* et al. *s.n* (SGO); Parque Nacional La Campana, sector Granizo, Mina Pronosticada, 6 January 2001, *A. Moreira 510* (SGO); Parque Nacional La Campana, sector Granizo, Mina Pronosticada, March 2001, *A. Moreira 533* (SGO). **Región Metropolitana:** Provincia de Santiago: Hacienda Rinconada de Cerda, Maipú, Quebrada de la Plata, 1 October 1936, *C. Muñoz s.n* (SGO); Cajón del Estero La Leonera, ladera al NO, 27 February 2000, *Arroyo* et al. *201380* (SGO); Provincia Cordillera: Lo Valdés, 28 December 2000, *A. Moreira 498* (SGO); camino al Embalse del Yeso, 21 January 1995, *M. Muñoz & A. Moreira 3704* (SGO); Provincia de Melipilla: Alhué, Reserva Nacional Roblería del Cobre de Loncha, 18 November 2004, *Baxter* et al. *s.n* (SGO); Cuesta Zapata, 7 October 1988, *von Bohlen 512* (SGO), Cuesta Zapata, 4 July 1989, *von Bohlen 581* (SGO); Cuesta Zapata, 18 December 2001, *M. Chamy & M. Piovano 2* (SGO), Cuesta Zapata, 18 December 2001, *M. Chamy & M. Piovano 8* (SGO); Provincia de Chacabuco: Cuesta La Dormida, entre Cruce Caleu y Portezuelo, 4 May 2003, *A. Moreira 958* (SGO). **Región Libertador Bernardo O'Higgins:** Provincia de Colchagua, San Fernando, Sector La Rufina, Zona de Intersección Río Tinguiririca con Río Clarillo, 16 November 2004, *Baxter* et al *s.n* (SGO). **Región del Maule:** Provincia de Curicó: quebrada de los Pejerreyes en Los Queñes, 10 January 1968, *P. Aravena 354* (SGO)

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Supplementary material 1

Figure S1. Type specimen of *Calceolaria asperula* Phil. (SGO 055831)

Authors: Nicolás Lavandero, Ludovica Santilli

Data type: Figure.

Explanation note: **A** complete specimen **B** detail of dried flower **C** detail of rehydrated flower **D** detail of the elaiophore **E** detail of the scattered oil-producing glands of the elaiophore.

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Lectotypification of *Chamaecyparis hodginsii* of the Cupressaceae

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Abstract

Recent phylogenetic studies have suggested that the monotypic *Fokienia* A.Henry & H.H.Thomas is nested within *Chamaecyparis* Spach, which is in agreement with separate morphological studies. Here the authors confirm a previous taxonomic treatment that incorporated *Fokienia hodginsii* (Dunn) A.Henry & H.H.Thomas into *Chamaecyparis* based on the monophyly requirement of taxonomy, i.e. *Chamaecyparis hodginsii* (Dunn) Rushforth. In addition, the type collection of the basionym *Cupressus hodginsii* Dunn was found to contain three sheets of specimens, one in K including a vegetative branch (K000088294) and a separate ovulate cone (K001090486), a second one in A (A00022477), and a third one in IBSC (IBSC0016081). All three specimens are marked with Hongkong Herbarium No. 3505, but only the two specimens in K and IBSC possess similar handwriting of "*Cupressus hodginsii* Dunn". The two specimens should be considered as syntypes according to the Shenzhen Code. The specimen in K is better preserved but it is a mixture according to the collection label: cones from Foochow (Fuzhou) and foliage from Yenping (Nanping). We lectotypified the name *Cupressus hodginsii* with K000088294 because the specimen is well preserved and has enough characters for identification. Moreover, an ovulate cone (K001090486) is on the same sheet.

Keywords

Chamaecyparis hodginsii, lectotypification, nomenclature, taxonomy

Introduction

Dunn (1908) described a new conifer species from Fujian of China, *Cupressus hodginsii* Dunn. Henry and Thomas (1911) believed that this species is different from *Cupressus* L. in the cone scale having only two seeds with two very unequal lateral wings, and should be classified into a separate genus, establishing *Fokienia* A.Henry & H.H.Thomas (generic name is after the provincial name Fujian where the type was collected) and transferred the species into this monotypic genus, i.e. *F. hodginsii* (Dunn) A.Henry & H.H.Thomas. Subsequently this monotypic genus was widely accepted and well known in botanical literature. Both *Flora Reipublicae Popularis Sinicae* (vol. 7, Wang et al. 1978) and *Flora of China* (vol. 4, Fu et al. 1999) treated it as a monotypic genus.

Since 2000, molecular systematic studies have consistently suggested that *Fokienia* is close to *Chamaecyparis* (Gadek et al. 2000; Yang et al. 2012; Qu et al. 2017; Stull et al. 2021). Based on a morphological study, Rushforth (2007) thought that the morphological variation of the genus *Fokienia* overlaps or falls within the range of *Chamaecyparis* including ovule number per cone scale, seed wing symmetry, and time duration for cone ripening, and thus reduced the genus to synonymy with *Chamaecyparis*, and made the combination *Chamaecyparis hodginsii* (Dunn) Rushforth. This morphological taxonomy was supported by a few subsequent phylogenetic studies (Mao et al. 2010, 2012; Jagel and Dörken 2015). Mao et al. (2012) sampled all extant five species of *Chamaecyparis* and the only species of *Fokienia*, assembled a sequence data including chloroplast, mitochondrial, and nuclear ribosomal DNA, and reconstructed a robust phylogeny of Cupressaceae; they demonstrated that *Fokienia* is nested within *Chamaecyparis*, with *Fokienia hodginsii* as sister to a subclade of *Chamaecyparis* including *C. obtusa* and *C. lawsoniana*, which received high bootstrap supports. The taxonomic treatment of Rushforth is acceptable if the monophyly principle is applied, and *Chamaecyparis hodginsii* should be the correct scientific name of the species.

Chamaecyparis hodginsii is a flag species of conservation. Fu (1992) recorded *Fokienia hodginsii* (≡*Chamaecyparis hodginsii*) in his China Plant Red Data Book: Rare and Endangered Plants; Qin et al. (2017) listed this species as threatened (VU). *Chamaecyparis hodginsii* is widely but sporadically distributed in southern China, Laos, and Vietnam. It has become a vulnerable species for a number of reasons, e.g. habitat loss, over exploitation, many old trees died of diseases, and difficult population regeneration (Chen et al. 2018). The recently released List of National Key Protected Wild Plants of China (ver. 2021) follows the treatment of the List of National Key Protected Wild Plants of China (Batch 1, ver. 1999), and lists the protection status of *Fokienia hodginsii* (≡*Chamaecyparis hodginsii*) as Grade II of protection.

In the protologue of *Cupressus hodginsii* Dunn, Dunn (1908) indicated a single collection, i.e. Hongkong Herbarium No. 3505, which should be considered as the type though he did not designate the herbarium where he deposited the type

Figure 1. Lectotype of *Chamaecyparis hodginsii*: Hongkong Herbarium No. 3505 (K000088294).

specimen. We found three duplicates of the collection: one is deposited in the Kew Herbarium (Royal Botanical Garden, Kew) (Fig. 1) and includes a vegetative branch (K000088294) and a separate ovulate cone (K001090486); the label says "cones from large trees at Foochow, foliage from Yenping, Province of Fokien, China"; a second is in the Herbarium of South China Botanical Garden, Chinese Academy of Sciences (IBSC0016081), and a third is in Harvard University Herbaria (A00022477). Farjon (2010) considered the specimen in K is the holotype, which is obviously a mistake. The two duplicates in K and IBSC should be considered as syntypes under Art. 9.6 and 40.2 Note 1 of the *Shenzhen Code* (Turland et al. 2018), both were studied by Dunn because the identification label is marked with his handwriting while the sheet in A should be considered as an isosyntype because it lacks any handwriting label. A lectotype should be designated under Art. 9.11 and 9.12. The specimen in Kew is better preserved, and should be considered as the lectotype. However, that specimen is a mixture according to the collection note that the vegetative branch was collected from "Yenping (Nanping)" and the ovulate cone was from "large trees at Foochow (Fuzhou)". Here we designate the vegetative branch (K000088294) as the lectotype. This lectotype specimen clearly displays a few morphological characters of the species, i.e. dimorphic leaves 4–10 mm long, decussate, facial pairs closely appressed, lateral pairs boat-shaped and having two white depressed stomatal bands abaxially.

Typification

***Chamaecyparis hodginsii* (Dunn) Rushforth, J. Biol. (Vietnam) 29(3): 38 (2007)**
Fig. 1

≡*Cupressus hodginsii* Dunn, J. Linn. Soc., Bot. 38: 367 (1908); *Fokienia hodginsii* (Dunn) A.Henry & H.H.Thomas, Gard. Chron. ser. 3, 49: 67 (1911).

Type. China. Fujian (福建, as "Fokien"): Nanping (南平, as "Yenping" in the protologue), April to June 1905, A.E.N. Hodgins, Hong Kong Herb. 3505 (lectotype: K000088294, designated here; isolectotypes: IBSC0016081, A00022477).

Distribution. China: Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang; Laos; Vietnam.

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Images of the type collection of *Chamaecyparis hodginsii* were obtained from the Chinese Virtual Herbarium (CVH, http://www.cvh.ac.cn/), and Kew Herbarium (http://apps.kew.org/herbcat/goto-HomePage.do). This work was supported by the National Natural Science Foundation of China [No. 31970205 & 31770211], and a Metasequoia funding from the Nanjing Forestry University to Y.Yang.

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Primulina silaniae sp. nov. (Gesneriaceae) from the limestone area of Guizhou Province, China

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Abstract

Primulina silaniae X.X.Bai & F.Wen, a new species of *Primulina* Hance (Gesneriaceae) from the limestone area of Wangmo County, Guizhou Province, is described and illustrated. The new species is similar to *P. spiradiclioides* Z.B.Xin & F.Wen, but can be easily distinguished from the latter by a combination of characteristics, especially in the lateral veins of its leaf and floral shape and tube. At present, three populations in one locality of this new taxon were found, totaling about 600 mature individuals. According to the IUCN Red List Categories and Criteria (Version 3.1), the species is provisionally assessed as Vulnerable [VU D1].

Keywords

flora of Guizhou, karst, new taxon, taxonomy, subtribe, Didymocarpinae

Introduction

Primulina Hance (Gesneriaceae, Didymocarpoideae, Trib. Didymocarpoideae, subtrib. Didymocarpinae) was a monotypic genus until 2011 (Wang et al. 2011; Weber et al. 2011). It was first described and published in 1883 by Henry Fletcher

Hance, a British botanist and the Vice Consul for Foreign Affairs of Huangpu Port in Guangdong (Hance 1883). The type species of this genus is *P. tabacum* Hance, which once was considered a rare cave plant in limestone montane areas and collected in Lianzhou City, Guangdong Province, China (Wang et al. 2013). After 2011, plant taxonomists revised the classification system of *Chirita* and its related genera based on molecular and morphological evidence. Now, as a result of this reorganization and newly described species, the newly defined *Primulina* has become the most diverse genus of Gesneriaceae in China (Li and Wang 2007; Möller et al. 2009, 2011; Wang et al. 2011; Weber et al. 2011). As of October 2021, there were 219 species (excluding infraspecific taxa) (GRC 2021), and most of the species in this genus were discovered from Guangxi, China (Wei 2018).

Although *Primulina* has only been found in southern and southwestern China and northern Vietnam, mainly in limestone areas (Möller et al. 2016), there is still great potential to find undescribed species diversity (Möller 2019). As the main locality of differentiation and diversity of Gesneriaceae in China, many new taxa have been discovered or reported in Guizhou (Xu et al. 2017). From 2019 to October 2021, 21 new species (including infraspecific taxa) of the genus *Primulina* have been described (Wen et al. 2021), including two species from Guizhou Province *P. serrulata* R.B.Zhang & F.Wen from Rongjiang County (Hong et al. 2019) and *P. flexusa* F.Wen, Tao Peng & B.Pan (Peng et al. 2020) from Duyun City (Wen et al. 2021).

In July 2020, when we investigated plants in Wangmo County, we found a plant on moist, shady limestone cliffs that appeared to represent an undescribed species of *Primulina* based on its old fruits and leaves. After regular observation, we photographed the flowers and collected the specimens in November 2020. Comparing these specimens and living plant materials with the type specimens and protologues of all 219 species of *Primulina*, we found that these specimens and plants neither fit the existing protologues nor conformed to the type specimens of these species. Nevertheless, the shape and texture of leaves are most similar to *P. spiradiclioides* Z.B.Xin & F.Wen (Xin et al. 2020); it can be distinguished by a combination of several morphological characters of the leaf margin, lateral veins, leaf indumentum, and floral features. Thus, we confirmed that it represented a new species of *Primulina* and describe and illustrate it here.

Materials and methods

The plant material for description was collected in the field at its type locality in 2020. Morphological observations and dissections of plant material of this new species were made under a stereoscopic microscope (Olympus SZ61, Tokyo, Japan) and measured and described using the terminology used by Wang et al. (1998). Electronic specimens stored in herbaria in China, Vietnam, the United States, and the United Kingdom (e. g. E, GH, HN, IBK, K, IBSC, HITBC, KUN, MO, PE, PH, US, and VNMN) were examined.

Taxonomic treatment

Primulina silaniae **X.X.Bai & F.Wen, sp. nov.**
urn:lsid:ipni.org:names:77233712-1
Figures 1, 2

Diagnosis. The new species is similar to *Primulina spiradiclioides*, but can be easily distinguished from the latter by leaf margin entire with occasionally a few long hairs, glabrous on adaxial and abaxial surfaces (*vs.* margin serrate and not villous, lamina adaxial and abaxial surfaces densely whitish villous), no lateral veins on adaxial and abaxial surfaces (*vs.* inconspicuous on adaxial surfaces, distinctly raised on abaxial surface), pedicels 8–31 mm long (*vs.* ca. 5 mm), calyx inside glabrous (*vs.* sparsely pubescent), bluish-purple corolla lobes, with two short brownish-yellow stripes in the white throat (*vs.* corolla mouth white, forming a conspicuous pentagon), corolla tube slightly curved (*vs.* straight), ovary ca. 6mm long (*vs.* 2–2.5 mm), style 19–22 mm long (*vs.* ca. 2.5 mm), capsule, 8–9 mm long (*vs.* 10–15 mm).

Type. CHINA, Guizhou Province: Wangmo County, Sanglang Town, 25°13'N, 106°26'E, altitude ca. 564 m, November 28, 2020, *Xin-Xiang Bai* et al., *BXX20201128-01*(Holotype: GZAC!; Isotype: GZAC!; IBK!)

Description. Herb perennial, lithophytic. Rhizomatous stem cylindrical, 0.9–1.2 cm long, ca. 0.5 cm in diameter. Leaves basal, fleshy and brittle, 8–18, crowded at the apex of stem; petiole occasionally villous, slightly concave on adaxial surface, 1–4 × 0.2–0.4 cm. Leaf blade oblong or oblanceolate, adaxially dark green, abaxially light green, 4–7 × 0.8–1.0 cm, glabrous on adaxial and abaxial surfaces, margin entire, occasionally a few long hairs, base tapering to petiole, apex acuminate to obtuse; midrib inconspicuous on adaxial surface, conspicuous on abaxial surface, no lateral veins. Cymes axillary, 1–4, 1–4–flowered; peduncle 3.2–12.5 cm long, ca. 1 mm in diameter, densely white pubescent; bracts 2, opposite, oblong, ca. 2.5 × 1 mm, adaxially pubescent, margin entire, apex acute; bracteoles ca. 2 × 0.5 mm, same color and indumentum as bracts; pedicels 8–31 mm long, ca. 0.8 mm in diameter, densely white pubescent; calyx 5-lobed, free from base, lobes equal, pale green, lanceolate to narrowly linear, ca. 9 × 1.5 mm, outside densely white pubescent, inside glabrous, margin entire, apex acute. Corolla infundibuliform, bluish-purple lobed, 3.3–3.6 cm long, two short brownish-yellow stripes with pubescent in white throat of the corolla; tube white, slender tubular, slightly curved, 2.8–3.1 cm long, base ca. 0.2 cm in diameter, mouth ca. 0.4 cm in diameter, tube outside sparsely pubescent in upper parts, lower parts and inside glabrous; limb distinctly 2-lipped, adaxial lip 2-lobed, lobes rounded, margin entire, ca. 6 × 5 mm; abaxial lip 3-lobed, lobes oblong, ca. 9 × 6 mm. Stamens 2, adnate ca. 16 mm above the base of the corolla tube; filaments ca. 6 mm long, terete; anthers coherent by entire adaxial surfaces, elliptic, ca. 2 × 1.5 mm, pale yellow, glabrous; staminodes 3, white, lateral ones ca. 2.1 mm long, adnate ca. 8 mm above the base of the corolla tube, terete, the middle one ca. 1.2 mm long, adnate to ca. 8 mm above the base of the corolla tube. Disk annular, yellow-green, margin entire, ca. 1 mm high. Pistils, ca. 2.6 cm long; style white, linear, 19–22 mm long, densely glandular-pubescent; ovary yellowish-green, ca. 6 mm long, glandular-pubescent. Stigma apex slightly 2-lobed; ca. 2 mm long, ca. 1 mm wide, Capsule narrowly cylindrical, longitudinal dehiscence, 8–9 mm long, ca. 3.5 mm in diameter; calyx and style persistent, white pubescent outside.

Etymology. Tthe epithet 'silaniae' is coined to honour Prof. Si-Lan Dai, the famous horticulturist at the Beijing Forestry University. She is also the former supervisor of one of the authors, Prof. Xin-Xiang Bai. Meanwhile, one of the given names of Prof.

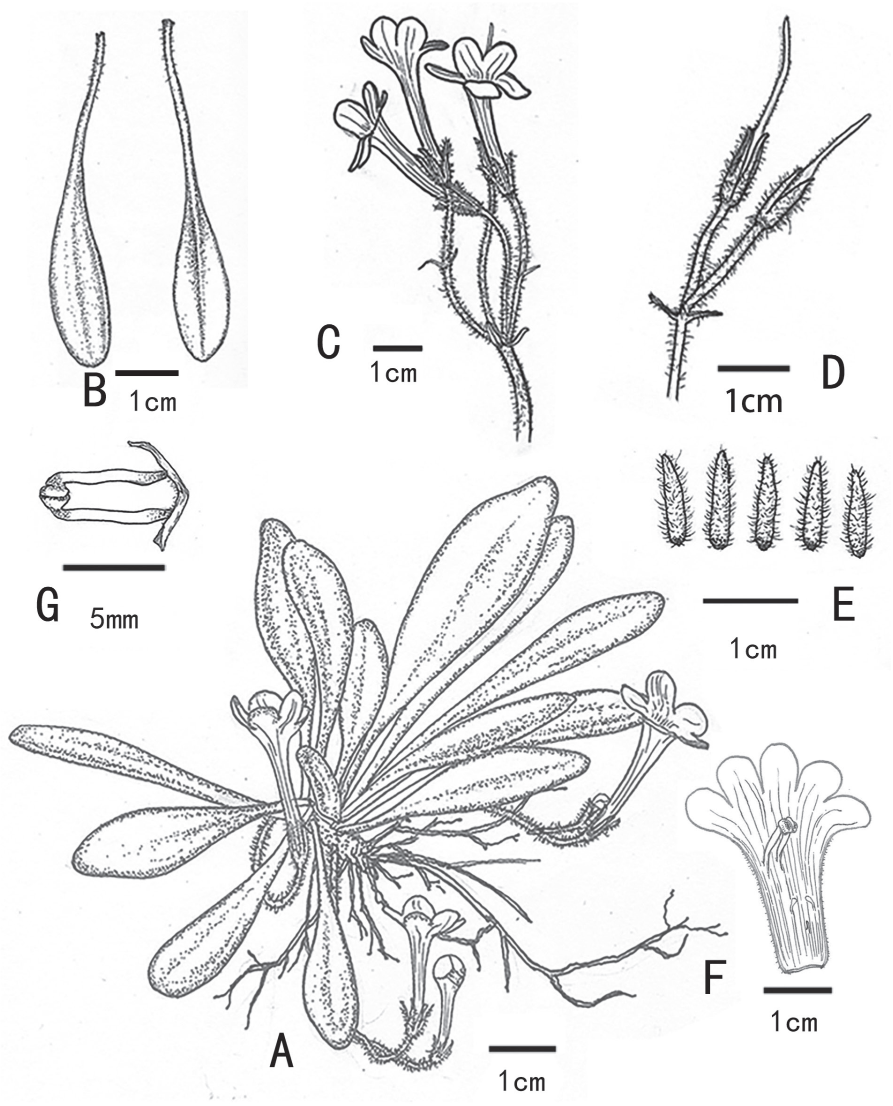

Figure 1. *Primulina silaniae* X.X.Bai & F.Wen, sp. nov. **A** habit **B** adaxial (left) and abaxial (right) surfaces of leaves **C** flowering cyme **D** young capsules **E** dissected calyx lobes **F** half opened corolla cover, showing stamens **G** stamens with cohering anthers. Drawn by Yi Chen from the holotype.

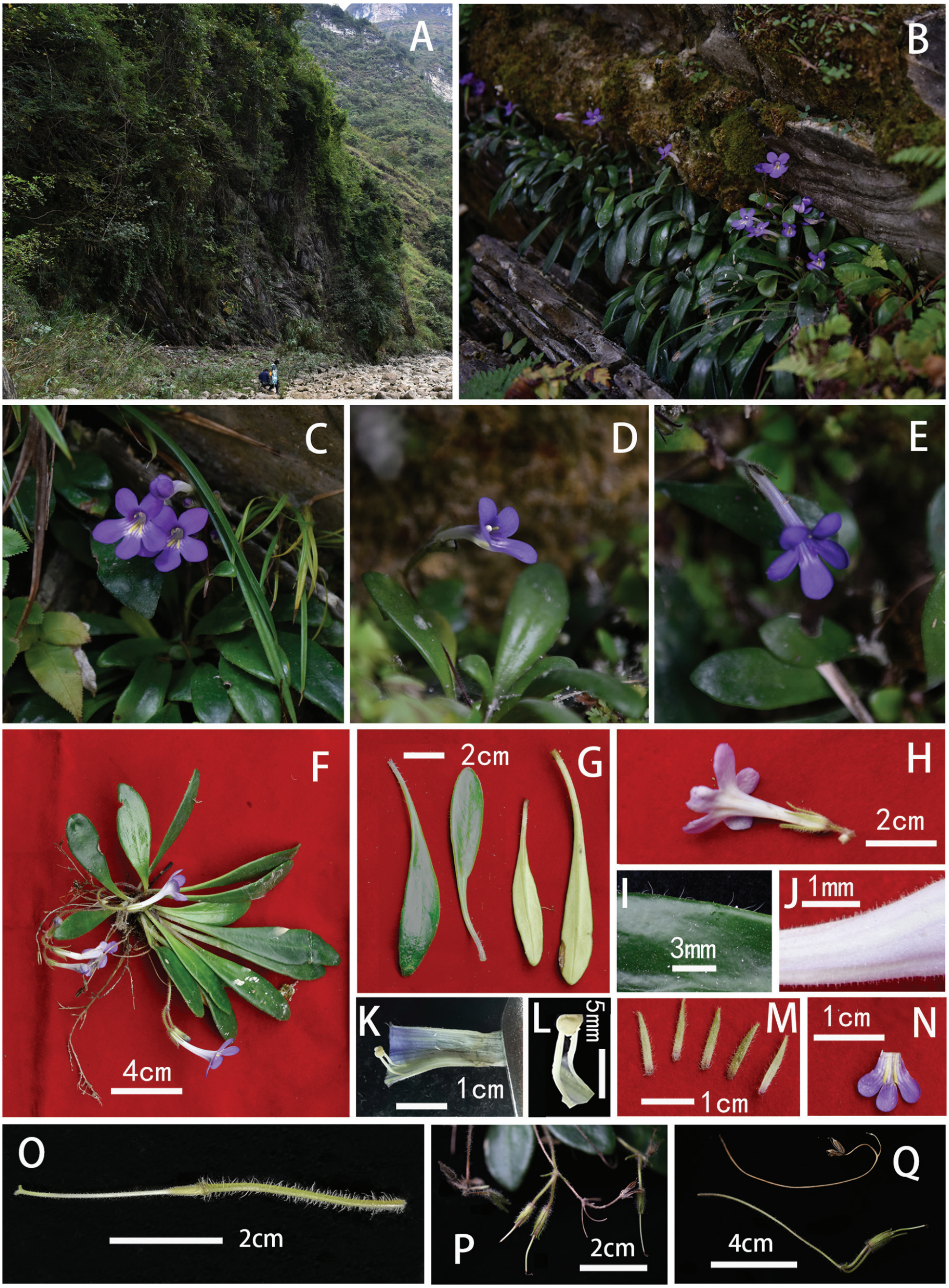

Figure 2. *Primulina silaniae* X.X.Bai & F.Wen, sp. nov. **A, B** habitat **C** front view of flower **D, E** oblique side view of flowers **F** plant **G** adaxial (left two) and abaxial (right two) surfaces of leaves **H** corolla **I** partial, enlarged leaf **J** partial, enlarged corolla tube **K** stamen **L** coherent anthers **M** dissected calyx lobes **N** lower distal part of corolla **O** pistil, pedicel and calyx with sepals removed **P, Q** dehisced and immature fruits. Photographs by Xin-Xiang Bai.

Si-Lan Dai, namely ‘Lan’, shares the same pronunciation in Chinese with the color blue. Thus, the scientific name also hints at the bluish to purplish-blue corolla of this new taxon.

Vernacular name. Sī Lán Bào Chūn Jù Tái (Chinese pronunciation); 思兰报春苣苔 (Chinese name).

Phenology. Flowering from November to February of the following year, fruiting from March to May.

Distribution and habitat. The species has only been found in Wangmo County, the type locality. It grows on moist, shady limestone cliffs at altitudes of 550 to 570 meters.

Provisional IUCN conservation assessment. At present, *Primulina silaniae* is only found in the type locality. There are three populations with ca. 600 matures individuals, all of which grow on moist and shady limestone cliffs. One of the populations has a small number of mature individuals and is located by the roadside, easily disturbed by human activities. It is therefore assessed as Vulnerable [VU D1] according to the IUCN Red List Categories and Criteria (Version 3.1) (IUCN 2012, 2019).

Additional specimens examined. Paratypes. CHINA Guizhou Province: Wangmo County, Sanglang Town, 25°13'N, 106°26'E, 416 m, a.s.l., 22 December 2020, *Xin-Xiang Bai et al.*, *BXX20201122-01* (GZAC!); The same locality, 11 April 2021, *Xin-Xiang Bai et al.*, *BXX20210411-01* (GZAC!); The same locality, 1 November 2020, *Xin-Xiang Bai et al., BXX20201101-01* (GZAC!).

Notes. The leaf of *Primulina silaniae* is fleshy and brittle, glabrous on both sides, with an entire margint that occasionally has a few long hairs, base tapering to petiole, apex acuminate to obtuse; midrib inconspicuous on adaxial surface, conspicuous on abaxial surface, no lateral veins. These characteristics differ from those of other *Primulina* species and can be clearly distinguished from *Primulina spiradiclioides* in morphological characteristics (Table 1).

Table 1. Detailed comparisons of *Primulina silaniae* and *P. spiradiclioides.*

Characters \ species	***P. silaniae***	***P. spiradiclioides***
Leaf-blades	margin entire, occasionally a few long hairs	margin serrate, densely whitish villous
Lateral veins	inconspicuous on adaxial and abaxial surfaces	inconspicuous on adaxial surfaces, distinctly raised on abaxial surface
Leaf indumentum	glabrous on both sides	adaxial and abaxial surfaces densely whitish villous
Pedicels length	8–31 mm	ca. 5 mm
Calyx indumentum	inside glabrous	inside sparsely pubescent
Corolla	bluish-purple lobes, two short brownish-yellow stripes in white throat of corolla	corolla mouth white, forming a conspicuous pentagon
Corolla tube	slightly curved	straight
Ovary length	ca. 6 mm	2–2.5 mm
Style length	19–22 mm	ca. 2.5 mm
Capsule length	8–9 mm	10–15 mm
Stamen insertion	adnate ca. 16 mm above corolla tube base	adnate ca. 1.7 mm above corolla tube base
Calyx indumentum	inside glabrous	inside sparsely pubescent

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