

# *Thesium muasyae* (Santalaceae), a new species from the limestone fynbos of the Overberg, South Africa

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## Abstract

*Thesium muasyae*, a new species of the family Santalaceae, is described and illustrated. This species has unique morphological and ecological characters, differentiating it from other closely related species of the genus, such as *T. karoocicum*. These characters include plants forming compact shrubs to about 30 cm tall with glabrous surfaces; leaves recurved, to about 4 cm long, terete to triangular, apiculate; flowers placed in lax spikes or borne solitary; and style up to about 2.5 mm long. Ecologically, *T. muasyae* is endemic to the limestone fynbos in the Overberg, Bredasdorp District, South Africa. Molecular phylogenetic evidence places this species in Subgenus *Frisea* Section *Barbata*, as closest sister to *T. hispidulum* + *T. karoocicum*. A preliminary conservation Red List assessment suggests that *T. muasyae* is Critically Endangered, based on its population size, area of occupancy and extent of occurrence.

## Keywords

Endemic, Greater Cape Floristic Region, systematics, taxonomy, Thesiaceae

## Introduction

*Thesium* L. is the largest genus in the family Santalaceae with > 360 species (the Angiosperm Phylogeny Group IV 2016; POWO 2022). Species in this genus are root hemi-parasites found in Africa, Asia, Australia, Europe, South America and by introduction, North America (Nickrent et al. 2010; García et al. 2018; Zhigila

et al. 2020; POWO 2022). The Cape of South Africa is its origin and centre of diversity (Pilger 1935; Moore et al. 2010). *Thesium* species are found in several biomes and is abundant in the Fynbos (especially in fynbos and renosterveld vegetation types), Albany Thicket, and Succulent Karoo (Hill 1925; Manning and Goldblatt 2012). The species inhabit different substrates but are mainly found on areas with sandstone, quartz, shale, deep coastal sand and limestone (Manning and Goldblatt 2012). Species in the genus exhibit diverse growth habits including erect, suberect to prostrating herbs, rhizomatous shrublets under 10 cm tall and shrubs to small trees to about 2 m tall (Hill 1925). The genus is diagnosed by a combination of complex morphological characters such as leaves without distinct petioles that are adpressed to the stems, flowers usually with external glands between the perianth lobes, ovaries with the placental column twisted or straight, and fruits indehiscent nutlets with prominent veins and persistent perianth segments (Hill 1915; García et al. 2018).

Recent molecular phylogenetic analyses for Santalaceae, with robust taxa and loci sampling of *Thesium*, supported a monophyletic genus (Moore et al. 2010; García et al. 2018; Zhigila et al. 2020). The sampling comprised all of the Greater Cape Floristic Region (GCFR) species and worldwide representatives of *Thesium* using four plastid (*trnL-F*, *matK*, *rpl32-trnL* and *rbcL*) and one nuclear (ITS) DNA regions. Based on this well-developed phylogeny, Zhigila et al. (2020) confirmed the monophyly of *Thesium* and hypothesized five subgenera within the genus namely *Hagnothesium* (A.DC.) Zhigila, Verboom and Muasya, *Thesium* L., *Discothesium* (A.DC.) Zhigila, Verboom and Muasya, *Psilotheresium* (A.DC.) Zhigila, Verboom and Muasya and *Frisea* (Rchb.) Hendrych, with the subgenus *Frisea* having the highest number of species (103 species, most of which are South African) and *Hagnothesium* being endemic to the GCFR. Within the GCFR, molecular data revealed genetic variations for some taxa that may represent different species. These data have spurred our interest for further field surveys in the botanically rich but poorly explored renosterveld and limestone fynbos patches of the Overberg.

Eleven species are currently recorded in the limestone fynbos and renosterveld of the Overberg region, South Africa (Curtis-Scott et al. 2020). Five of them, *T. dmmagiae* Zhigila, Verboom and Muasya, *T. nigroperiatum* Zhigila, Verboom and Muasya, *T. quartzicolum* Zhigila, Verboom and Muasya, *T. rhizomatum* Zhigila, Verboom and Muasya and *T. stirtonii* Zhigila, Verboom and Muasya are endemics (Zhigila et al. 2019a). Except for *T. quartzicolum* and *T. stirtonii* (found on quartz outcrops of the Overberg), these species are confined to the shale scrubs or ecotones of shale and limestone slopes south east of the Vanderstalkraal Private Farm. In this paper, we describe a fifth species endemic to the limestone outcrops of the Overberg in the GCFR. The illustrations, distribution, molecular phylogenetic relationships and preliminary conservation status are presented. This work forms part of the series of the published works on the GCFR *Thesium* species (Zhigila et al. 2019a, 2019b, 2020) as well as the larger project to revise the entire genus (see Mashego and le Roux 2018; Visser et al. 2018; Lombard et al. 2021).

## Materials and methods

### Morphological assessments

The morphological assessments of the new species were carried out on our field collections and on herbarium specimens deposited at BOL, NBG (including SAM and STE vouchers) and PRE (codes as indicated by Thiers 2022), as well as online voucher materials (JSTOR 2022). Micromorphological characters were observed using a hand lens (10×) or under stereomicroscope Leica S9i with Nikon DS-5M Camera attached. The holotype of *T. muasyae* was deposited at BOL and duplicates distributed to NBG, PRE and K. Morphological terms were adopted from the recent *Thesium* taxonomic treatments of García et al. (2018), Zhigila et al. (2020) and Lombard et al. (2021).

### Molecular work and barcoding

Whole genome DNA was extracted from the silica-gel dried leaf materials collected during our fieldworks between 2019 and 2021. The extraction was performed using a modified CTAB protocol (Doyle and Doyle 1987) as amended by Zhigila et al. (2020). The following primers: ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3'), ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') (White et al. 1990), trnL-C (5'-CGA AAT CGG TAG ACG CTA CG-3') and trnL-F (5'-TT TGA ACT GGT GAC ACG AG-3') were used to amplify and sequence the regions (Taberlet et al. 1991). The PCR mix per 30 µl reaction volume included 19.3 µl distilled H<sub>2</sub>O, 3 µl of 10× buffer, 1.25 µl MgCl<sub>2</sub>, 1.2 µl dNTP, 1 µl BSA, 1 µl DMSO, 0.9 ul each of forward and reverse primers, 0.3 µl kappa taq and 1.2 µl DNA template. For amplification, the PCR thermal condition included a 2-min initial denaturing step at 94 °C, then 30 cycles of 1 min denaturation, followed by annealing for 1 min at 50 °C, extension for 2 min at 72 °C, further extension for 7 min at 72 °C, and kept at 4 °C as amended by Zhigila et al. (2020). Sequencing reactions for both reverse and forward reactions were performed at the Stellenbosch University Sequencing Facility using the amplification primers.

Forward and reverse reaction sequences were assembled using Chromaspro version 2.1.5 (Technelysium 2017) and were aligned in MAFFT online service (Kuraku et al. 2013; Katoh et al. 2019). Each DNA locus was assessed and edited manually with the package BioEdit v. 7.2.6 (Hall 1999). The newly generated DNA sequences were deposited at the GenBank public repository with accession numbers OM746331–OM746335 for nrITS, and OM857946 and OM857954 for *trnL-F*. Tree files including sequences from previous studies were submitted to TreeBase (study number TB2:S24838) and are provided as Suppl. material 1.

A model-based Bayesian method (MrBayes) was used for the phylogenetic analyses on XSEDE v.3.2.6 (Ronquist et al. 2017) using the Cyber-Infrastructure for Phylogenetic Research (CIPRES) V.3.3 (Miller et al. 2010) platform. The GTR+G, since it is deemed to be the best-fit nucleotide substitution model with the Akaike and Bayesian Information Criterion, was selected as determined in jModelTest2 (Darriba et al.

2012). In two independent runs for the Markov Chain MCMC permutations, four simultaneous chains were initiated with a random tree run for  $50^7$  generations with the trees sampled at every  $10^3$  generations. Discarding burn-in trees of 25%, summaries of 50% majority-rule consensus trees were held. For Maximum Likelihood (ML) analyses, jModelTest2 selected GTR+G for nrITS, GTR+I+G for *trnL-F* and GTRGAMMA for combined dataset as the best-fitting models. The package RAxML v8 (Stamatakis 2014) was used for all analyses. Setting petitions for each region and the combined dataset with 1000 replicates of bootstrap analysis. In both BI and ML analyses, the posterior probabilities (PP) and percentage bootstrap support (BS) values respectively were used to indicate support for clades. The phylogenetic trees were visualised and edited in FigTree v1.4.4 (Rambaut 2018).

## Conservation assessments

The preliminary conservation Red List status for the species was determined using the IUCN guidelines (IUCN 2017). The extent of occurrence (EOO) and areas of occupancy (AOO) of the new species was assessed using the software Geospatial Conservation Assessment Tool (GeoCAT), with the default cell size of  $2 \times 2$  km matrix (Bachman et al. 2011; GeoCAT 2021).

## Results

### Taxonomic treatment

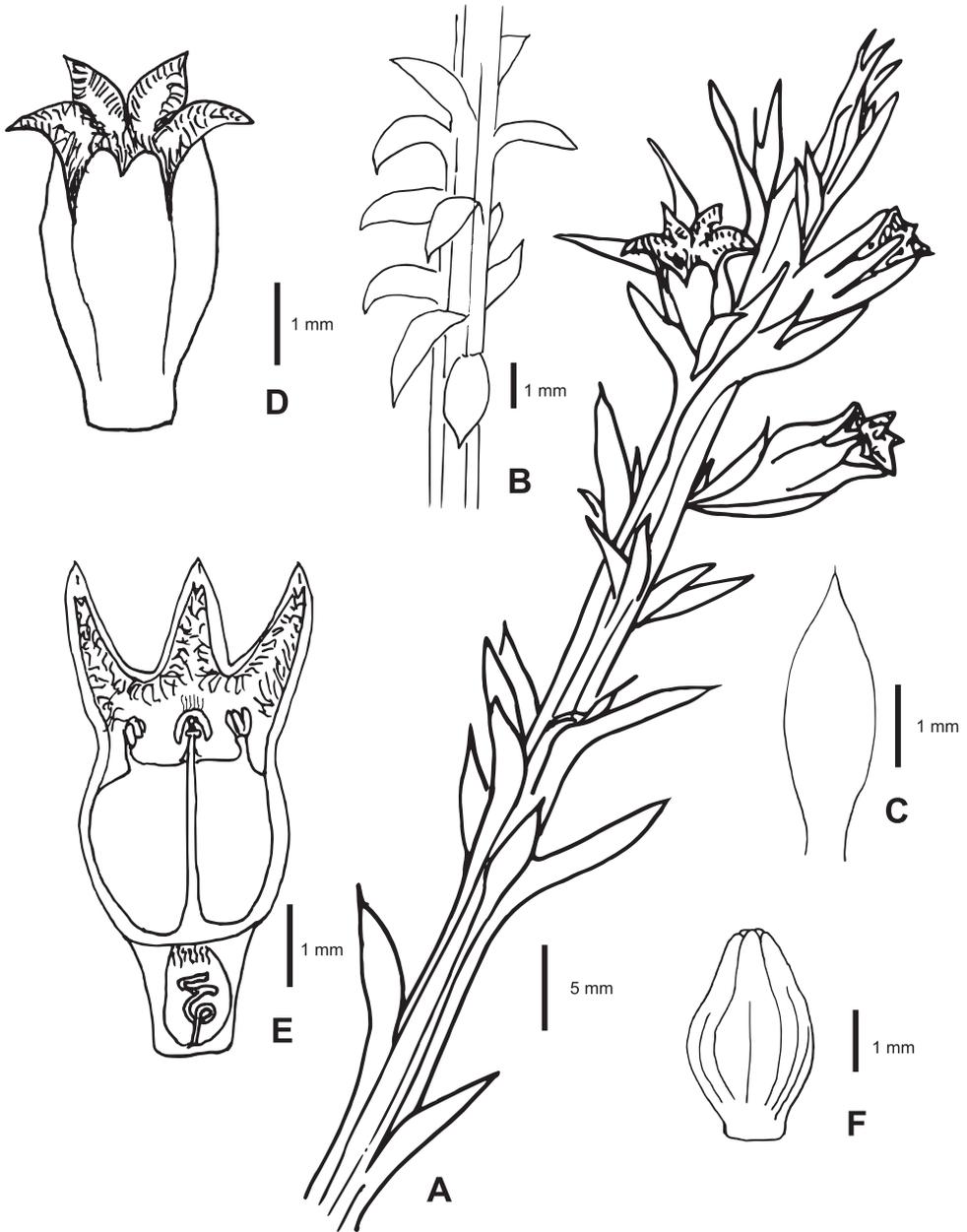
#### *Thesium muasyae* Zhigila, sp. nov.

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Figs 1, 2

**Type.** SOUTH AFRICA. Western Cape Province, Bredasdorp District, on limestone ridges, south east of Vanderstelskraal Farm, Overberg,  $34^{\circ}24'53.2''\text{S}$ ,  $20^{\circ}15'10.5''\text{E}$  [ $34.41478^{\circ}\text{S}$ ,  $20.25292^{\circ}\text{E}$ ]; elev. 60 m; 21 October 2021, *D.A. Zhigila & A.M. Muasya 1308* (holotype, BOL; isotypes: K, NBG, PRE).

**Diagnosis.** *Thesium muasyae* is morphologically similar to *T. karoicum* Compton (1931). Both species have robust woody habits, well-developed terete to triangular and imbricate leaves, elongated styles, conspicuous external glands between the perianth lobes, persistent perianth segments longer than the fruits, and elaiosomes (Table 1). However, *T. muasyae* differs from *T. karoicum* in its branching pattern being intricate to sympodial, stems and leaves glabrous, leaves recurved, flowers in lax elongated terminal spikes or racemes in leaf and bract axils, patelliform flowers with post-staminal trichomes attached to the anthers (versus branching pattern divaricate to virgate, stems and leaves minutely scabrous, leaves erect, flowers in terminal capitate head or clusters, urceolate flowers with post-staminal trichomes free from anthers in *T. karoicum*). Further the two do not overlap in distribution and ecology, *T. muasyae* is restricted



**Figure 1.** *Thesium muasyae* sp. nov. **A** twig showing inflorescence arrangements **B** branchlet showing vegetative arrangements **C** bract **D** flower lateral view **E** flower dissected longitudinally **F** fruit. Line drawing by Pia M. Eibes.

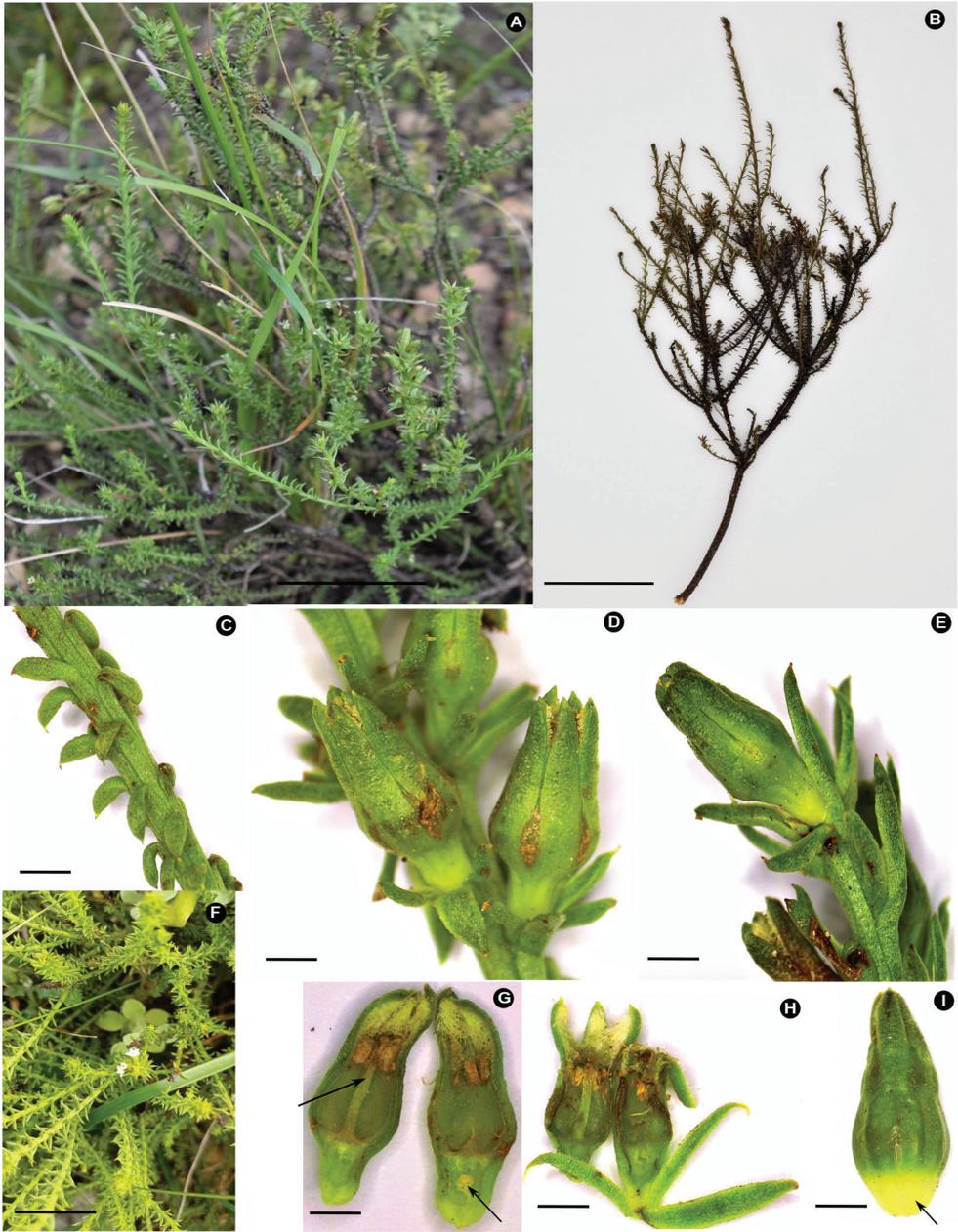
to the Overberg limestone outcrops whereas *T. karoicum* is found on the Sandstone Mountains of the Succulent Karoo. *Thesium muasyae* is also similar to *T. sonderianum*, but differs in the branching pattern being sympodial to intricate, plant surface glabrous, leaf apex apiculate, inflorescences solitary spikes on branchlets, perianth ex-

**Table 1.** Main differentiating morphological features of *Thesium muasyae* from its most-similar congeners.

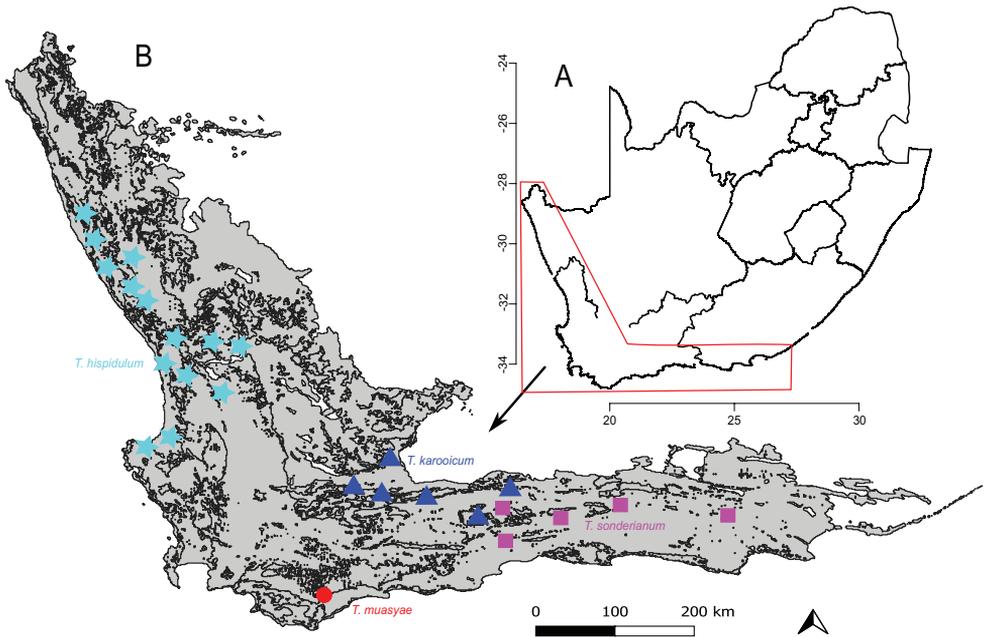
	<i>T. muasyae</i>	<i>T. karoicum</i>	<i>T. sonderianum</i>	<i>T. hispidulum</i>
Plant height	10–30 cm	10–70 cm	50–100 cm	10–50 cm
Branching pattern	sympodial to intricate	divaricate	dichotomous	decumbent
Plant surface	Glabrous	minutely pubescent	minutely pubescent	pubescent
Leaf curvature	recurved or straight	recurved	recurved	recurved or straight
Leaf margin	Terete	scabrous	scabrous	scabrous
Leaf apex	Apiculate	acuminate	acutely mucronate	acuminate
Inflorescent type	elongated lax or solitary spikes	globose spikes	globose spike	globose spikes
External glands	present	present	absent	absent
Style length	1–2.5 mm	0.5–1.5 mm	1–2.5 mm	0.3–0.4 mm
Style	above anther	below anther	below anther	below anther
Anther	exserted	exserted	partly exserted	inserted
Post-staminal trichomes	attached to anthers	free from anthers	attached to anthers	attached to anthers
Fruit length	4–7 mm	4–10 mm	5–10 mm	3–4 mm
Fruit ribs	5-ribbed	10-ribbed	5-ribbed	10-ribbed
Substrate	limestone slopes	sandstone and shale	sandstone	sandstone and shale
Biome	Limestone Fynbos	Succulent Karoo	Grassland	Sandstone Fynbos

ternal glands present, stigma above the anthers, found on limestone slopes (versus dichotomously branched, plant surface minutely pubescent, leaf apex acutely mucronate, inflorescences terminal globose spikes, perianth external glands absent, stigma below the anthers, and restricted to the grasslands in *T. sonderianum*). Comparisons of important morphological characters of *T. muasyae*, *T. hispidulum*, *T. karoicum* and *T. sonderianum* are presented in Table 1.

**Description.** A perennial shrub, arising from woody rootstock, glabrous, to about 30 cm tall. **Stems** woody, erect to suberect, much branched, 3.0–5.0 mm in diameter, deeply grooved longitudinally. **Branches** 10–20 in number, mainly from the base, scarcely grooved, angled from  $> 45^\circ$  to  $< 90^\circ$ , branching pattern intricate to sympodial. **Leaves** terete to triangular, somewhat succulent, adpressed to the branchlets, lanceolate or oblanceolate or somewhat triangular,  $1.5\text{--}3 \times 0.5\text{--}1.5$  cm, basally decurrent, midrib inconspicuous, not keeled but recurved, margins not distinct or entire, apically apiculate. **Inflorescences** a lax terminal spike or flowers solitary in leaf and bract axils. Bracts 2–4, leaf-like, slightly adnate to the base of peduncle, linear to lanceolate,  $1.0\text{--}2.0 \times 0.3\text{--}0.5$  mm, margin entire, apex acute to acuminate, green; bracteoles bract-like, but smaller, adpressed to the pedicel, shorter than flower length. **Flowers** patelliform, on short peduncles, 5-merous,  $2.0\text{--}5.5 \times 1.5\text{--}5.0$  mm, perianth lobe segments lanceolate, external gland conspicuously elongated between perianth lobe segments,  $2.0\text{--}2.5 \times 1.0\text{--}1.2$  mm, lobe apex uncinata, obtuse, incurved, perianth lobe apical trichomes present, lobe margins entire, lobe internal colour white, external colour greenish black; hypanthium clearly marked, to about 0.5 mm long, hypanthium length longer than perianth lobe tube and wider. **Stamens** equal flower merosity, 0.2–0.3 mm long, staminal filaments exserted slightly above stigmas, attached to the perianth lobe walls by a tuft of trichomes, downwardly-directed basal trichomes absent. **Style** together with



**Figure 2.** Morphological features of *Thesium muasyae* **A** whole plant in habitat **B** type material *D.A. Zhigila & A.M. Muasya 1308* **C** branchlet and leaves **D** fruiting branchlet **E** fruit lateral view **F** inflorescences and leaves **G** flower longitudinal section showing long style in relation to anthers and twisted placental column **H** flower subtended by bracts **I** eliosome on fruit. Photographs by Daniel A. Zhigila. Scale bars: 0.5 mm.



**Figure 3.** Map of **A** South Africa with the red outline indicating the Greater Cape Floristic Region (GCFR) **B** the GCFR showing the type locality (red solid circle) of *Thesium muasyae* and of the congener species, *T. hispidulum* (aqua solid stars), *T. karooicum* (blue solid triangles) and *T. sonderianum* (fuchsia solid squares).

stigma 4–6 mm long; placental column twisted. **Fruits** subglobose to oblong, ovary portion oval, 5.0–8.0 × 4.5–5.5 mm, green to creamy green, glabrous with 10 conspicuous longitudinal ribs, reticulate veins prominent, pedicels enlarging into elaiosomes, persistent perianth segments equal to longer than the fruit.

**Distribution and ecology.** *Thesium muasyae* was collected on the limestone ridges, south east of Vanderstelskraal Farm, Overberg, Bredasdorp District, Western Cape Province, South Africa (Fig. 3, triangles) at elevations less than 80 m above sea level. This species occurs on limestone and shale-limestone ecotone scrubs. The limestone soil in the type locality is characterised mainly by calcium carbonate, tiny fossils and other fossilized debris from the coastal limestone of the Bokkerveld Group (Finch et al. 2014; Penn-Clarke et al. 2018). Physically, the limestone soil is grey to whitish brown.

**Phenology.** The collections were made in October with fruits and few flowers. Based on the average of 40 days from flowering to fruiting stage in *Thesium* species (pers. obs.), we can then extrapolate the flowering period to be between August and November.

**Etymology.** The specific epithet ‘*muasyae*’ honors Professor A. Muthama Muasya for his immense contribution to the floristics and taxonomy of the Overberg and Cape plant species.

**Conservation status.** We estimated a total of 10–20 individuals of *T. muasyae* in a single population over an extent of 0.0 km<sup>2</sup> and the area of occupancy of about 5.0 km<sup>2</sup>. Although this species is on a private farm, grazing from livestock is an immediate threat. In addition, the entire Overberg Renosterveld habitat is considered as Endangered due to intense agricultural activities and the areas being fire-prone (von Staden 2015; Topp and Loos 2019). These threats together with the GeoCat geographical range estimations translate to the criterion B2, Critically Endangered category of the IUCN (2017) for *T. muasyae*.

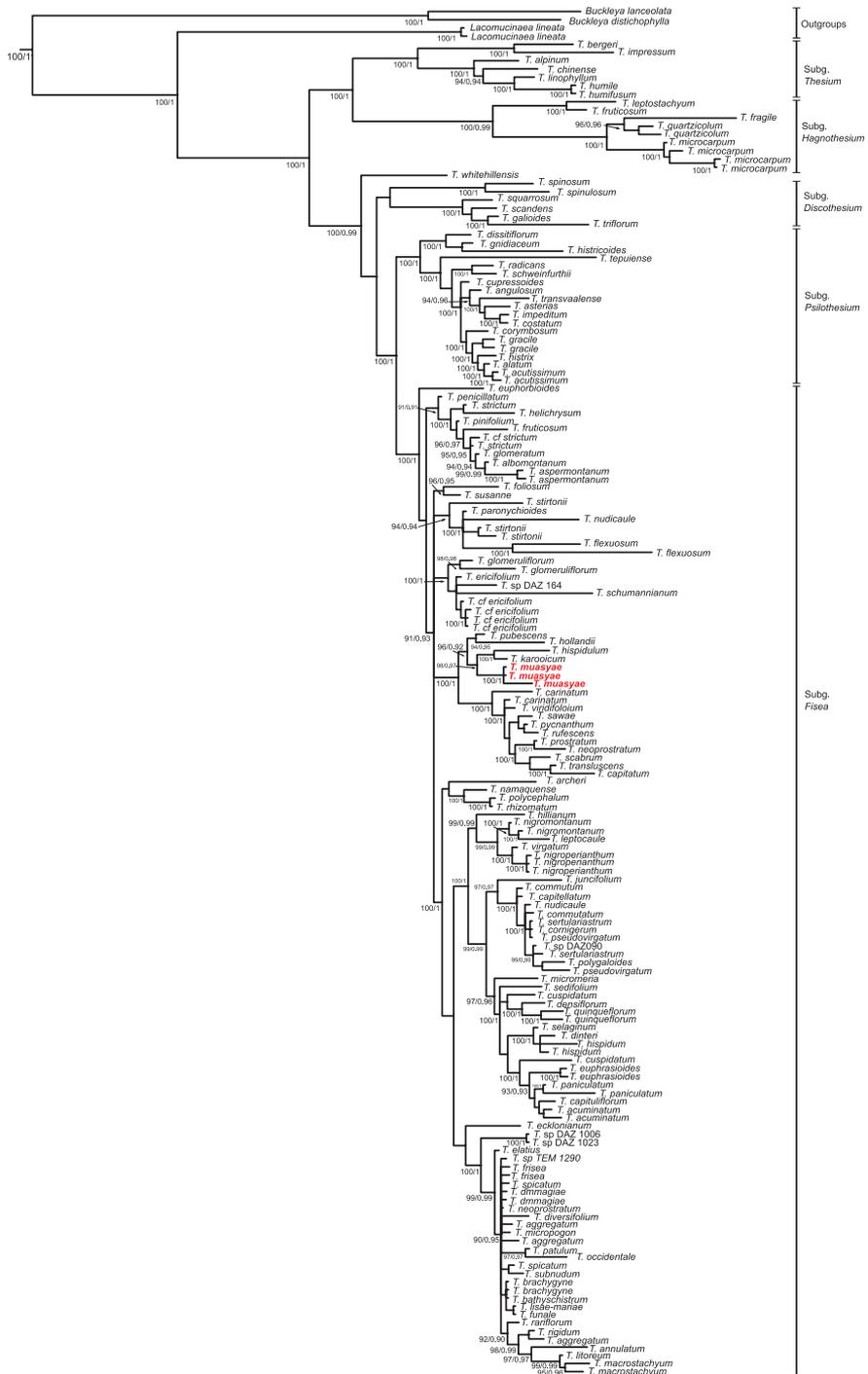
**Additional specimens examined.** SOUTH AFRICA. Western Cape Province, Bredasdorp District, on limestone ridge, south east of the Vanderstelskraal Farm, 34°24'52.1"S, 20°15'8.1"E [34.41447°S, 20.25225°E], elev. 63 m, 21 October 2021, *D.A Zhigila & A.M Muasya* 1312 (BOL!); 34°24'53.2"S, 20°15'10.5"E [34.41478°S, 20.25292°E], elev. 65 m, *A.M Muasya & D.A Zhigila* 8276 (BOL).

### Phylogenetic placement

The Maximum Parsimony and Bayesian analyses placed *T. muasyae* (red bold on Fig. 4) in a clade consisting of *T. karoicum*, *T. hollandii* and *T. hispidulum* with strong bootstrap and posterior probability values (BS = 100% and PP = 0.99 respectively). This clade is in the Subgenus *Frisea*, Section *Barbata*. The molecular placement supports the morphological similarities of *T. muasyae* and the congener species as stated in Table 1 and the diagnosis section above.

### Discussion

The morphological characters suggest that *T. muasyae* fits into section *Barbata* (Hill 1915), in the subgenus *Frisea* (Reichenbach 1828; Zhigila et al. 2020). Species in this leafy clade (sensu Moore et al. (2010)) share morphological characters such as stem transverse sections grooved, leafy stems (not scattered), linear to lanceolate leaves, determinate inflorescences, flowers having tuft of trichomes at perianth lobe apices, and flower shape patelliform, conspicuous external glands between perianth lobes, elongated perianth tubes and anthers attached to the tubes by post-staminal hairs and style 4–6 mm long (Hill 1915). However, *T. muasyae* differs from species in this clade in its growth height being < 30 cm tall (versus the typical 30–120 cm tall in *Barbata* clade), sympodial to intricate branching pattern (versus usually virgate or fastigiata branching patterns), leaves terete to triangular (versus leaves with distinct upper or lower surfaces to sometimes triangular), leaf apices apiculate (versus predominantly acute to acuminate), flower solitary, in leaf axils and terminal heads (versus raceme-like, cymose or globose spikes in most other *Barbata* species). The results of the molecular analyses are congruent with the previous studies (e.g. Moore et al. 2010; García et al. 2015; Zhigila et al. 2020) and support the morphological evidence to recognise *T. muasyae* as novel to science.



**Figure 4.** A 50% majority-rule consensus tree for Santalaceae that include the new species *Thesium muasyae* (red bold) based on a combined nrITS and plastid *trnL-F* regions obtained from Bayesian Inference. Numbers on the nodes indicate clades with bootstrap and posterior probability support values of > 95% and 0.90 respectively.

In the last five years, nine new species and several new records of *Thesium* have been discovered from the Overberg Region (Zhigila et al. 2019a 2019b; Lombard et al. 2021). Most of these new taxa are evolutionary unique and having narrow ranges. For example, narrow-ranged and critically endangered *T. rhizomatium* and *T. nigrop-eriathum* are endemic to the limestone and ecotones of limestone fynbos and shale renosterveld of the type locality of *T. muasyae*. Hence, these new generic records have expanded our understanding of the biogeographic coverage and habitat diversity of species in the genus *Thesium*.

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## Supplementary material I

### Supplementary tree

Authors: Daniel A. Zhigila, A. Muthama Muasya

Data type: (phyl. file)

Explanation note: Tree files including sequences from previous studies.

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# Typification of six names in *Camellia* (Theaceae)

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## Abstract

Six names in *Camellia* or its synonym *Thea*, including *Camellia reticulata* f. *simplex*, *C. symplocifolia*, *Thea forrestii*, *T. lanceolata* var. *stenophylla*, *T. megacarpa* and *T. yunnanensis* are lectotypified here with nomenclatural and taxonomic notes provided.

## Keywords

*Camellia*, lectotypification, *Thea*

## Introduction

Species of *Camellia* L. (Theaceae) are of great economic value through the productions of tea and seed oil, as well as cultivation for their beautiful flowers. The genus has been taxonomically revised at least six times (e.g. global species: Sealy 1958; Chang 1981; Chang and Bartholomew 1984; Ming 2000; species in China: Chang 1998; Ming and Bartholomew 2007), but the types of many taxa remain unclear. Some of them have recently been typified (Zhao et al. 2017a, b, 2018, 2019; Zhao 2021, 2022). Following these works, six more names in the genus with similar problems are identified and they are lectotypified below.

## Materials and methods

Taxonomic literature, especially protologues, and specimens at Herbaria A, BM, E, G, K, KUN, L, MO, NY, P, PE, TCD, U and US (acronyms based on Thiers 2022, continuously updated) were examined. The most informative specimen was selected as the lectotype, based on Art. 9 of the Shenzhen Code (Turland et al. 2018, hereafter ICN).

## Typification of names

### 1. *Camellia reticulata* Lindl. f. *simplex* Sealy, Revis. Gen. *Camellia* 183. (1958)

**Lectotype.** (designated here): China. Yunnan: Northeast of Tengyueh (Tengchong), 20°10'N, 6000 ft alt., March 1913, G. Forrest 9715 (K!; Fig. 1).

**Notes.** Sealy (1958: 183) established the new form to represent the wild plants of *C. reticulata* since the latter was described based on a plant with semi-double flowers (Zhao 2022). A gathering, Forrest 9715, was designated as the type of the form. Sealy (1958: 182) cited “Forrest 9715 (E, K)” on the page before the protologue, so the collection Forrest 9715 at Herbaria E and K are syntypes of *C. reticulata* f. *simplex* (Art. 40 Note 1 of the ICN). A single sheet of Forrest 9715 was found at E with barcode E00117770 (see <http://data.rbge.org.uk/herb/E00117770>). However, the single specimen of the collection at K bears Sealy’s drawing of a flower and his notes on the morphology, so it is selected above as the lectotype of the form (Fig. 1).

Ming (2000: 278) recognised *C. reticulata* f. *simplex* as a heterotypic synonym of *C. reticulata*. Though the original material of the species was derived from the cultivated plant, the current broad circumscription for *C. reticulata* covers wild plants under the ICN since the nomenclatural type does not need to be the most typical or representative of a natural species (Art. 7.2 of the ICN). However, it is better to name cultivars according to the *International Code of Nomenclature for Cultivated Plants* (Art. 28 Note 2; Brickell et al. 2016).

### 2. *Camellia symplocifolia* Griff., Itin. Pl. Khasyah Mts. 40, No. 652. (1848)

**Lectotype.** (designated here): India. Khasya Hills, W. Griffith s.n. (TCD0018254!; Fig. 2).

**Notes.** Griffith (1848) described *C. symplocifolia* under his catalogue number 652 and stated that the original material was collected at Churra, Khasyah. Though “Icon no. 31” was cited in the protologue and this citation directs to a drawing, fig. 2 of plate DCIV (“*C. simplicifolia*”) in *Icones Plantarum Asiaticarum* (Griffith 1854), the single drawing cannot be considered as the holotype because the corresponding specimens that were collected and used by Griffith to prepare the description, although uncited, may still exist (see Art. 9 Ex. 2 of the ICN). Furthermore, fig. 2 of plate DCIV (Griffith



Figure 1. Lectotype of *Camellia reticulata* Lindl. f. *simplex* Sealy (G. Forrest 9715 at K). Photo: D.W. Zhao.

1854) is a poor drawing of leaves and flowers, which can hardly illustrate the key features of the taxon and is, therefore, unsuitable to serve as the lectotype. The specimens of potential original material collected by Griffith in Khasya Hills were found at BM, K and TCD. Unfortunately, none of the specimens bears the name “*C. symplocifolia*” or the catalogue number. Considering the name was published in Griffith’s posthumous papers, it would be unsurprising that the original material of the name had not been clearly labelled. Based on the protologue and the drawing, one of the specimens, *Griffith s.n.* (TCD0018254; Fig. 2), with flower materials (e.g. flower buds, androecia and gynoecia) and barcoded, is selected above as the lectotype of *C. symplocifolia*. Additionally, the specimen (TCD0018254; Fig. 2) can be easily distinguished from *C. caudata* Wall. that was listed on the same page of Griffith (1848) by its subsessile flower buds and glabrous filaments, whereas the latter bears pedicellate flower buds and hairy filaments.

*Camellia symplocifolia* is treated as a heterotypic synonym of *C. kissi* Wall. by Sealy (1958: 197), Chang (1981: 35) and Ming (2000: 303). I agree with this treatment.

### 3. *Thea forrestii* Diels, Notes Roy. Bot. Gard. Edinburgh 5: 284. (1912) ≡ *Camellia forrestii* (Diels) Cohen-Stuart

**Lectotype.** (designated here; first-step designated by Sealy 1958: 54): China. Yunnan: South end of Tsu-hsiong-fu (Chuxiong) Valley, 7000–8000 ft alt., February 1903, *G. Forrest 314* (E00284407!; its image is available at <http://data.rbge.org.uk/herb/E00284407>).

**Notes.** Diels (1912) cited a single gathering, *G. Forrest 314*, as the type of *T. forrestii* in the protologue. Cohen-Stuart (1916) transferred this species into *Camellia* without any comment on the type. However, three duplicates of *G. Forrest 314* were found at E (E00284407), K (K000380522) and P (P04511547), so they are syntypes of *T. forrestii* (Art. 40 Note 1 of the ICN). When Sealy (1958: 54) indicated “*Forrest 314* (E, K; type of *C. forrestii*)”, such citation may act as the first-step lectotypification under Art 9.17 of the ICN. It is further narrowed to a single specimen at E (E00284407) here as the second-step lectotype of *T. forrestii*.

### 4. *Thea lanceolata* (Blume) Pierre var. *stenophylla* Merr., Enum. Philipp. Fl. Pl. 70 (1923).

**Lectotype.** (designated here): Philippines. Luzon: Ilocos Norte Province, Bangui to Claveria, August 1918, *M. Ramos 33005* (US 00113903 [the image is available at <http://n2t.net/ark:/65665/34f38884f-74b3-4f15-8e0b-db0f884e39b1>]!).

**Notes.** Merrill (1923: 70) cited a single collection, “*B.S. 33005 Ramos*” in the protologue without specifying the corresponding herbarium. Two duplicates of this gathering were found at K and US, so they are syntypes (Art. 40 Note 1 of the ICN) of *T. lanceolata* var. *stenophylla*. The specimen at US bearing barcode 00113903 is selected above as the lectotype of this taxon because a fragment of fruit is attached on a branchlet of the sheet.



**Figure 2.** Lectotype of *Camellia symplocifolia* Griff. (Griffith s.n., TCD0018254). The red arrow indicates one position of the androecium and gynoecium. Image scanned by Ms J. Stone (TCD) and adapted by D.W. Zhao.

Due to a lack of materials, Sealy (1958: 145) did not give this variety a formal taxonomic treatment. Keng (1989: 68) identified it as a heterotypic synonym of *C. lanceolata* (Blume) Seem. and claimed that the latter was “understandably unlikely to be a homogeneous taxon” based on its widespread geographic distribution. I generally agree with Keng’s (1989) broad circumscription of *C. lanceolata* until more data are available (see below).

### 5. *Thea megacarpa* Elmer, *Leafl. Philipp. Bot.* 5: 1842. (1913)

**Lectotype.** (designated here): Philippines. Palawan: Puerto Princesa (Mt. Pulgar), March 1911, *Elmer 12822* (E00504323!; its image is available at <http://data.rbge.org.uk/herb/E00504323>).

**Notes.** A single collection, *Elmer 12822*, was cited in the protologue (Elmer 1913: 1843) without indicating where the specimens were conserved. Ten duplicates of *Elmer 12822* housed at various Herbaria A (00025101), BM, E (E00504323), G (G00354856), K, MO (705490), NY (00385756), P (P04511437), U (U 0226169) and US (00113904) were found, so they are the syntypes of *T. megacarpa* (Art. 40 Note 1 of the ICN). The citation of Ming (2000: 228), “Type:... *A.D.E. Elmer 12822* (K, E, BM, P)”, did not validate the lectotypification because the single herbarium in which the type was deposited was not specified (Art. 9.22 of the ICN). The specimen at E (E00504323), which bears immature fruit and seeds, is selected as lectotype.

Cohen-Stuart (1916: 68) transferred *Thea megacarpa* into *Camellia*. Sealy (1958: 142) treated it as a heterotypic synonym of *C. lanceolata*. Chang and Ren (1991: 68) thought that *Elmer 12822* “much differed from” *C. lanceolata* because the former bore “free filaments and [a] thicker pericarp”, whereas the latter bore “totally united filaments” and a “thinner pericarp”. However, flowers, including filaments, were absent in all the specimens of *Elmer 12822* examined above and there was no description of flower parts in the protologue (Elmer 1913: 1842–1843). Later, Ming (2000: 228) recognised the plant as a subspecies of *C. furfuracea* (Merr.) Cohen-Stuart.

However, as a native and the single representative of *Camellia* in the Malay Archipelago, *C. lanceolata* holds a specific phylogenetic position (Zhao et al. 2022). The plants under the broad circumscription of this taxon show a continuous variation in the size and shape of the morphological characters. For instance, the length of the leaf blade can vary from 2 cm (e.g. *Beaman 8977* at K) to 13 cm (e.g. the lectotype of *T. megacarpa*, *Elmer 12822* at E), but the elements of flower and fruit, such as the filament tube, the hairy ovary and the furfuraceous surface of the pericarp, are generally similar amongst them. Since there is no clear correlation between morphological variation and geographic distribution and molecular phylogenetic analysis of the plants is absent, I provisionally agree with the broad definition of *C. lanceolata* and place *T. lanceolata* var. *stenophylla* and *T. megacarpa* in its synonymy.

**6. *Thea yunnanensis* Pit. ex Diels, Notes Roy. Bot. Gard. Edinburgh 5: 284. (1912)  
≡ *Camellia yunnanensis* (Pit. ex Diels) Cohen-Stuart**

**Lectotype.** (designated here; first-step designated by Sealy 1958: 163): China. Yunnan: Ta long tan, 10 October 1889, *Delavay s.n.* (P01903507!; its image is available at <https://science.mnhn.fr/institution/mnhn/collection/p/item/p01903507>).

**Notes.** Two gatherings, *Delavay s.n.* and *G. Forrest 430*, were cited in the protologue (Diels 1912: 284), so they are syntypes of *T. yunnanensis* (Art. 9.6 of the ICN). When Sealy (1958: 163) cited “*Delavay 15 Oct. 1889* (P, type)”, his lectotypification must be accepted, but may be considered as the first-step (Art. 9.17 of the ICN) because three specimens of Delavay’s collection matching the description of *T. yunnanensis* were found at P (P01903507, P01903508 and a single specimen consisting of two sheets: P06614716 [1/2] & P06614717 [2/2]; Art. 8.3 of the ICN, also see Ex. 7). However, the three specimens of *Delavay s.n.* were all collected on 10 October 1889 and two of them, P01903507 and P01903508, bear Pitard’s handwriting “*Thea yunnanensis* sp. n.”, so the date of collection recorded in the protologue, “15 October 1889”, is probably incorrect and the correct date is 10 October 1889 which was recorded on the labels. Specimen with barcode P01903507 bears a drawing of a dissected flower and is designated above as the second-step lectotype of *T. yunnanensis* (Art. 9.17 of the ICN).

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# *Lepisorus medioximus* (Polypodiales, Polypodiaceae), a new species from Shan State of Myanmar

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## Abstract

A new species of the species-rich fern genus *Lepisorus* (Polypodiales, Polypodiaceae) has been found to occur in Shan state, Myanmar. *Lepisorus medioximus* is described based on morphological characters and phylogenetic evidence. Phylogenetic analyses showed that the specimens of *L. medioximus* formed a distinct clade nested in the *Pseudovittaria* clade. The morphological comparison demonstrated that the species is distinct from phylogenetically related species, namely *L. elegans*, *L. contortus*, and *L. tosaensis*, in the morphology of the rhizome scales, size, and shape of the lamina, position of sori, and paraphyses.

## Keywords

Epiphyte, integrative taxonomy, micromorphology, overlooked species diversity, phylogeny

## Introduction

The genus *Lepisorus* (J. Sm.) Ching (Polypodiaceae) occurs throughout Eastern and Southern Asia with range extensions towards the Pacific islands including Hawai'i and towards tropical Afromadagascar (Ching 1933; Zink 1993; Wang et al. 2012). Taking into account the various taxonomic studies and the recent proposal to expand the generic circumscription by including all genera of the tribe Lepisoreae such as *Lemmaphyllum* C. Presl,

*Lepidomicrosorium* Ching & K.H. Shing, *Neocheiropteris* Christ, *Neolepisorus* Ching, *Paragramma* (Blume) T. Moore, and *Tricholepidium* Ching (Zhao et al. 2020) *Lepisorus* can be currently recognized as one of the most species-rich genera among genera in Polypodiaceae, comprising ca. 90 species in 18 sections (PPGI 2016; Fujiwara et al. 2020; Zhao et al. 2020). Whereas the core of the genus *Lepisorus* (*Lepisorus* s.s.) is easily recognized by its unique suite of morphological characters including creeping rhizomes covered by clathrate scales, simple leaves, and sori covered with scale-like paraphyses (Ching 1933; Qi et al. 2013; Zhao et al. 2020), some controversy still exists concerning the broader circumscription to avoid the need to recognize the genus *Ellipinema* Li Bing Zhang & Liang Zhang (Zhang et al. 2020). Despite significant progress (Wang et al. 2010a; Wei et al. 2017; Zhang et al. 2020; Zhao et al. 2020), taxonomic uncertainty is arguably not restricted to the generic classification but affects the estimation of the total species diversity that is expected to be still underestimated due to the difficulty in the taxonomic classification of this genus. Several characters utilized as key information on species delimitation show high variation within some species (Wang et al. 2010b). In particular, the identification of species relies on a few diagnostic characters such as the shape of the lamina, position of sori, and structure of the rhizome scales and paraphyses. Unfortunately, these characters are hardly diagnosable in the field. As a consequence, some species have been frequently misidentified or overlooked, as exemplified by the recent reclassification of Japanese *L. thunbergianus* (Kaulf.) Ching and relatives (Fujiwara et al. 2018), and the rediscovery of *Lepisorus cespitosus* Y.X. Lin previously known only as type specimens (Fujiwara et al. 2020).

Yunnan has been increasingly recognized as the diversity hotspot of *Lepisorus* s.s. (Wang et al. 2012; Fujiwara et al. 2020). While sufficient explorations of the *Lepisorus* diversity have been made available for China and India, this cannot be claimed for the regions south and southwest of Yunnan, namely Laos and Myanmar. These two countries as well as Thailand show extremely low species diversity of the genus despite being expected to harbor a notable diversity of this genus (Suppl. material 1: Table S1). This can be attributed to not only the difficulty in the taxonomic classification of this genus but also the underestimation of whole fern species diversity due to fewer flora surveys previously conducted. Thus, overlooked species are expected to occur in these regions.

The Shan state of Myanmar is the focus of this study. The Shan state covers 155,800 km<sup>2</sup> which is almost a quarter of the whole area of Myanmar and is mainly comprised of a hilly plateau bordering Yunnan, China in the north, Laos in the east, and Thailand in the south. Shan state has been in historical times much less surveyed than Yunnan Province of China although the latter is known for its rich diversity of ferns including *Lepisorus*. Thus, we expect to retrieve not only new records but also some new fern species that are putative endemics to Shan State. To make this expectation tested, floristic inventories were carried out across the Shan state in September 2019. Two unusual specimens of *Lepisorus* were collected in Pin Laung Township, Ka Thaung (upper) located in the southern part of the state, which were recognized as a putative new species. This proposal was studied by consulting checklists of Myanmar and adjacent areas (Dickason 1946; Lindsay and Middleton 2012; Qi et al. 2013; Khine et al. 2017; Khine and Schneider 2020; Hori 2021; Vongthavone et al. 2021), and careful comparison of morphological characters with previously described species

by consulting specimens and species protologues (e.g., Ching 1933; Bir and Trikha 1969; Yu and Lin 1996, 1997; Lin 2000; Qi et al. 2013). Besides morphological diagnostics, we employed DNA sequences to identify genotypic evidence supporting these two specimens as distinct species from any other species that are previously described.

## Materials and methods

### Morphology

The morphology of the two specimens of *Lepisorus* sp. (Hori et al. 108225 and 108229) was compared to descriptions and specimens of species sharing similarities in the main diagnostic features, namely rhizome scales, the size and shape of the lamina, the position of sori, and paraphyses. The morphological observation was conducted using a stereomicroscope. Voucher specimens were deposited in MBK, HITBC, and RAF.

### DNA extraction and chloroplast DNA region sequencing

Total DNA for each of the two specimens was extracted from silica dried leaves using the CTAB method according to Doyle and Doyle (1987). Four plastid regions, *rbcL* gene, *rbcL-atpB* intergenic spacer, *rps4-trnS* intergenic spacer, and *trnL-trnF* region including the *trnL* intron and the *trnL-trnF* intergenic spacer were amplified according to the protocol provided (Wang et al. 2010b) using ExTaq (TaKaRa Bio Inc., Shiga, Japan). The PCR products were purified using Illustra ExoStar 1-Step (GE Healthcare, Wisconsin, USA) and used as templates for Sanger sequencing. Reaction mixtures for sequencing were prepared using the SuperDye v3.1 Cycle Sequencing Kit (ADS). The reaction mixtures were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems).

### Phylogenetic analyses

To unveil the phylogenetic position of the new *Lepisorus*, a genus-level phylogeny was reconstructed incorporating a total of 88 species of *Lepisorus* including species representing clades previously treated as distinct genera, namely *Lemmaphyllum*, *Lepidomicrosorium*, *Neolepisorus*, *Neocheiropteris*, *Paragramma*, and *Tricholepidium*, retrieved from the sequence matrices assembled in previous studies (Wang et al. 2010b; Fujiwara et al. 2018, 2020; Zhao et al. 2020) (Suppl. material 2: Table S2). *Leptochilus ellipticus* (Thunb.) Noot., *Microsorium punctatum* (L.) Copel., and *Bosmania membranacea* (D. Don) Tets. were included as outgroup taxa. Sequences of each plastid region were separately aligned using MAFFT (Katoh and Standley 2013) followed by manually editing in Aliview (Larsson 2014), and subsequently merged into a concatenated matrix using SequenceMatrix (Vaidya et al. 2011). GTR +I +G was adopted as the best substitution model as selected based on AIC using jModelTest 2.1.10 (Darriba et al. 2012). Phylogenetic hypothesis reconstruction was performed with three different methods: maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP).

ML analyses were performed using IQ-TREE v.1.6 (Nguyen et al. 2015) with default settings. 1000 ultrafast bootstrap replicates were conducted to infer node robustness (Hoang et al. 2018). For the BI method, MrBayes 3.2.6 (Ronquist et al. 2012) was employed by applying two runs of four MCMC chains for 100,000,000 generations with samples taken every 1000 generations. Tracer 1.6 (Rambaut and Drummond 2013) was used to evaluate the samples trees with a focus on convergence. The first 25% were discarded as burn-in. The MP analysis was performed using a heuristic approach with TBR branch-swapping, as implemented in MEGAX (Kumar et al. 2018). Ten initial trees were generated by the addition of randomly selected sequences. The robustness of each branch was assessed by bootstrap analysis calculating 1000 replicates.

## Results and discussion

The combined dataset of *rbcL*, *rbcL-atpB*, *rps4-trnS*, and *trnL-F* contained 4,617 bp of which 744 sides were parsimoniously informative. The optimal log-likelihood for the reconstructed phylogeny inferred by the ML method was  $\ln = -21430.138$ . The topologies were congruent among the phylogenetic hypothesis obtained using the three distinct phylogenetic inference approaches. The result showed that two specimens of *Lepisorus* from the Shan state of Myanmar formed a clade with a bootstrap value of 100% (ML ultrafast bootstrap value = 100%, MP bootstrap value = 100%) and BI posterior probability of  $p = 1.0$ . This clade was nested in the subclade of the sect. *Pseudovittaria* clade (Fig. 1) (Zhao et al. 2020) that included *L. contortus* (H. Christ) Ching, *L. elegans* Ching & W.M.Chu, *L. lineariformis* Ching & S. K. Wu, and *L. nyalaensis* Ching & S. K. Wu. While the latter two species were highly distinct from the new species in their linear to linear-lanceolate lamina, the accumulated substitution event causing a rather long branch separated the two specimens from *L. elegans*—the morphologically most similar species of this clade (Fig. 1).

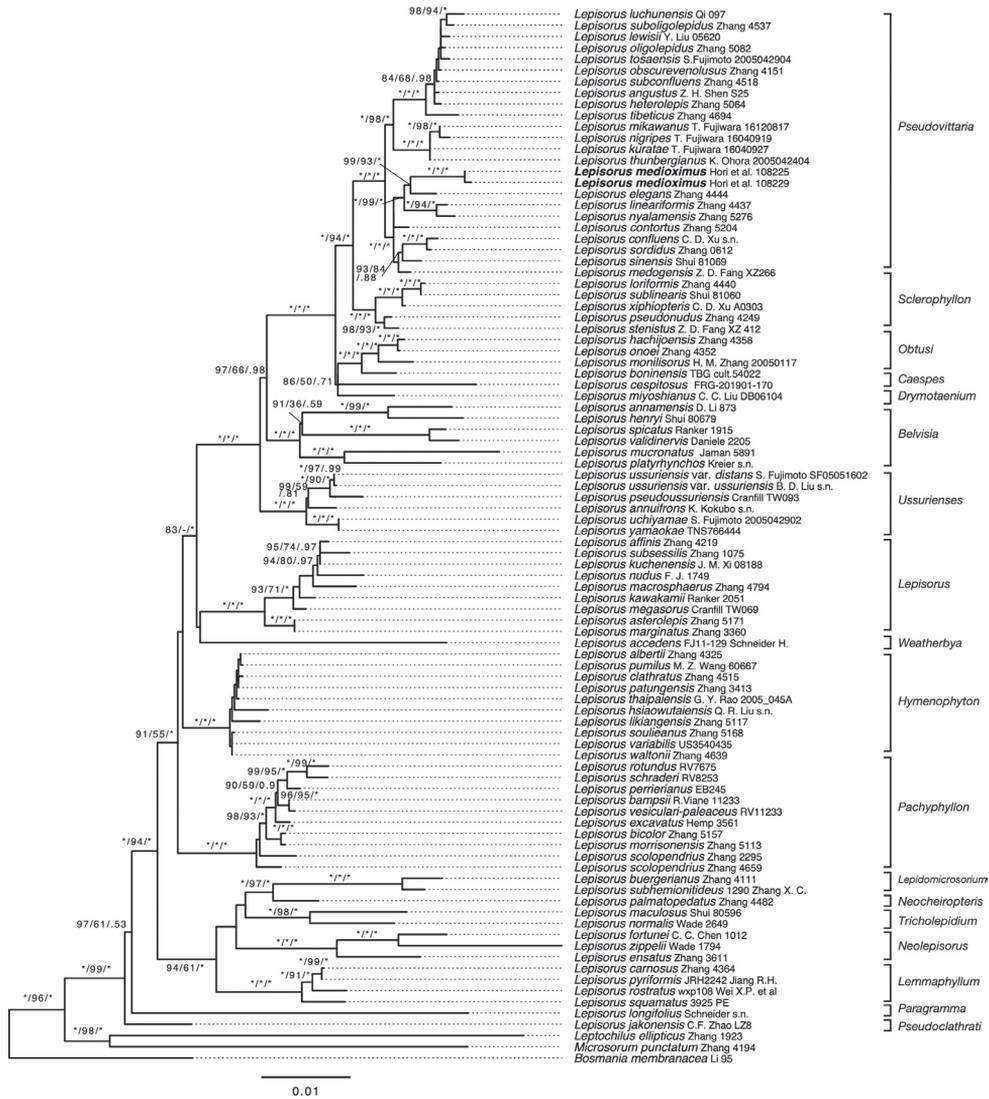
The morphological comparison revealed that the new species was similar to *L. elegans*, *L. contortus*, and *L. tosaensis* (Makino) H. Itô, species from the sect. *Pseudovittaria*, consistent with the result of phylogenetic analyses. However, the species was discernible from the similar species in lanceolate, pale brown rhizome scales, very short stipe, lanceolate lamina widest at the proximal 1/3, sori located closely to the costa and restricted to the 3/4 distal part of the lamina, and ovate-lanceolate paraphyses (Table 1 and Fig. 2).

### *Lepisorus medioximus* T.Fujiw., K.Hori & Khine, sp. nov.

urn:lsid:ipni.org:names:77300053-1

Figs 2, 3

**Diagnosis.** The new species differs from similar species, *Lepisorus elegans* and *L. contortus*, by the combination of the following morphological characteristics: the lanceolate laminae with the widest at proximal 1/3 of the lamina, sori closer to costa, sori on distal



**Figure 1.** Maximum likelihood tree of *Lepisorus* based on the combined dataset of *rbcL*, *rbcL-atpB*, *rps4-trnS* and *trnL-F*. The number on each branch indicates support values as follows: ML bootstrap support/MP bootstrap support/BI posterior probability. The classifications of genus and section for *Lepisorus* follows Zhao et al. (2020) and Fujiwara et al. (2020).

3/4 of the lamina, and ovate-lanceolate, ovate to orbicular clathrate paraphyses with entire margins. The species is discernible from *L. tosaensis* by pale-brown lanceolate rhizome scale with a narrow opaque band, remote fronds, and lanceolate leaf scales.

**Type.** MYANMAR. Shan state: Pin Laung Township, Ka Thauung upper, 19°57'58.5"N, 96°31'09.1"E, alt. ca. 904 m, 26 Sep. 2019, K. Hori, P.K. Khine ["Kine"], T. Fujiwara, M. Nagashima, P.P. Shwe & A.K. Moe 108225 (holotype: MBK0328223!, isotype: HITBC! and RAF!).

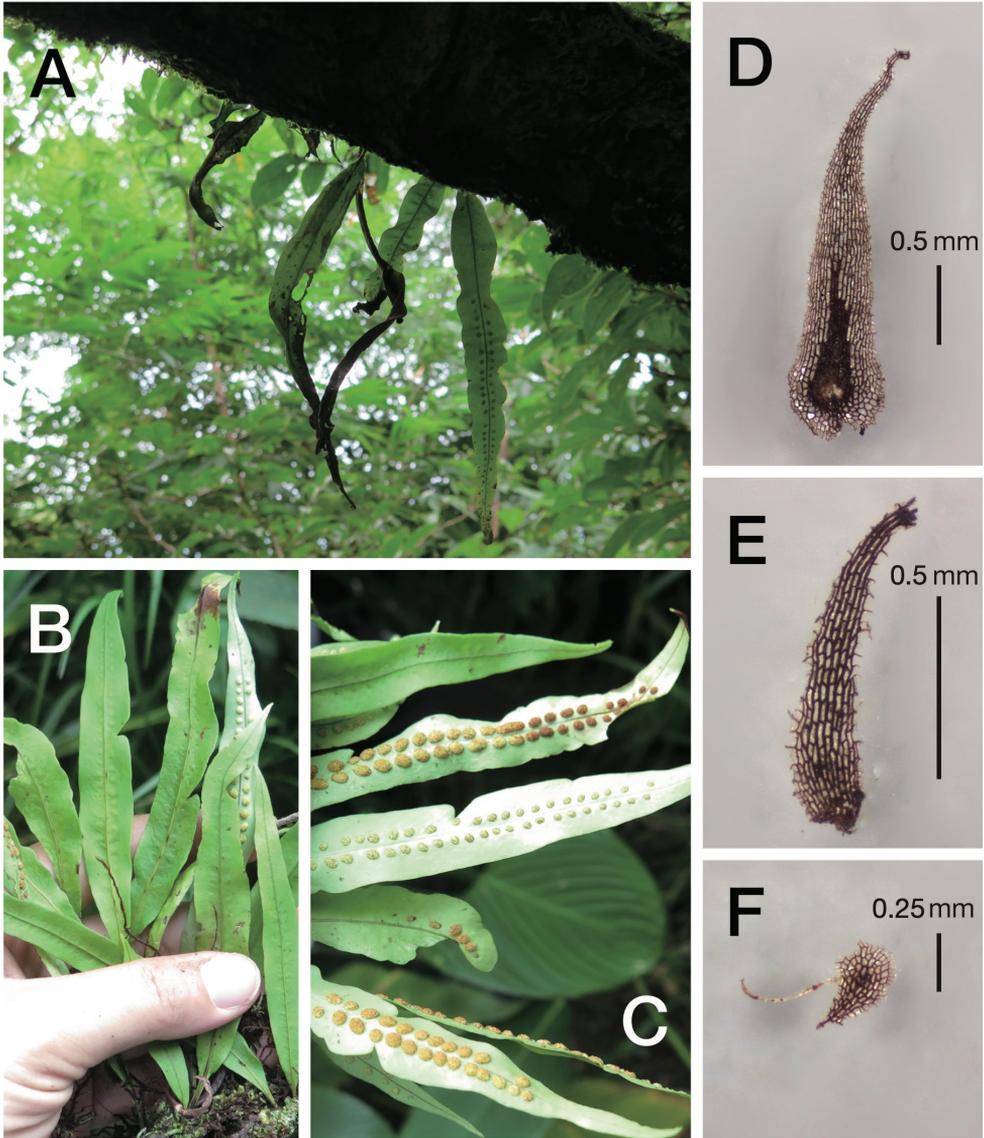
**Table 1.** Comparison of morphological characters between *Lepisorus medioximus* and three related species.

	<i>Lepisorus medioximus</i>	<i>Lepisorus elegans</i>	<i>Lepisorus contortus</i>	<i>Lepisorus tosaensis</i>
<b>Rhizome scale</b>	Lanceolate, pale brown, iridescent, clathrate with short and narrow, dark brown opaque band, margin entire to subentire, lumina large	Lanceolate, yellow-brown, iridescent, almost clathrate, sometimes with narrow, brown opaque band, margin entire to subentire, lumina large	Broadly lanceolate, pale-brown, clathrate with narrow, brown opaque band, margin denticulate, lumina small	Lanceolate or broadly lanceolate, iridescent, opaque dark brown with clathrate margin, lumina small
<b>Fronds</b>	Remote, 0.5–1.5 cm apart	Remote, 0.5–2 cm apart	Remote, 0.5–2 cm apart	Fronds clustered
<b>Stipe</b>	Stipe short, straw-colored to deep brown, 0.4–0.8 cm long	Stipe straw-colored to deep brown, 1–5 cm long	Stipe normally straw-colored, less often brown, 2–5 cm long	Stipe straw-colored, 1–3 cm long
<b>Laminae</b>	Lanceolate, widest at the proximal 1/3 of lamina, base cuneate, slightly decurrent, apex long caudate	Lanceolate, widest at middle, base cuneate, slightly decurrent, apex long caudate	Linear-lanceolate to lanceolate, widest at middle, base cuneate, decurrent, apex shortly acuminate	Lanceolate to broadly lanceolate, widest at middle, base cuneate, decurrent, apex acuminate
<b>Leaf scale</b>	Lanceolate, brown, clathrate	Lanceolate, brown, clathrate	Ovate, pale brown, clathrate	Ovate, brown, clathrate
<b>Sori</b>	On distal 3/4 of lamina, close to costa, orbicular to ovate	Restricted to distal 1/3–1/2 of lamina, midway between costa and margins, orbicular	Restricted to distal half, slightly closer to costa, orbicular, or slightly ovate	Restricted to distal half of lamina, close to costa, orbicular
<b>Paraphyses</b>	Ovate-lanceolate, ovate to orbicular, brown, clathrate, lumina large, margin entire	Orbicular, brown, lumina small, usually opaque, sometimes clathrate, margins with awn-spines	Orbicular, brown, clathrate with center dark brown, thick and opaque, margin denticulate	Orbicular, brownish, clathrate, central lumina small, margin denticulate

**Description.** Plant epiphytic. Rhizomes long creeping, 0.10–0.15 cm in diam., densely scaly, sometimes naked when old; Rhizome scales lanceolate, pale-brown, iridescent, clathrate with short and narrow, dark brown, opaque center band, 2.1–2.8 mm long × 0.4–0.6 mm wide, margin entire to subentire, apex acuminate, lumina large. Fronds remote, up to 1.5 cm apart; stipe short, 0.4–0.8 cm long, 0.6–1.0 mm diam., straw to dark brown colored; Lamina lanceolate, abaxially grayish-green, adaxially light green when fresh, 8–16 cm long × 0.9–1.5 cm wide, widest at proximal 1/3 of lamina, thinly leathery, adaxially glabrous, abaxially sparsely scaly, lamina base attenuate, decurrent, apex long caudate; costa raised on both sides, veinlets obscure; Leaf scales lanceolate, brown, clathrate, 0.8–1.4 mm long × 0.1–0.3 mm wide, margin denticulate, apex acuminate; Sori on distal 3/4 of lamina, very close to costa, orbicular or elliptic, 0.17–0.35 mm long × 0.12–0.23 mm wide, occasionally sunken on abaxial side of lamina; Paraphyses ovate-lanceolate, ovate to orbicular, brown, clathrate, lumina large, margin entire, 0.19–0.28 mm in diam.

**Etymology.** The epithet ‘medioximus’ refers to the sori attached to the middle location on lamina.

**Distribution.** This species is only known from the type locality in Myanmar, Shan state.

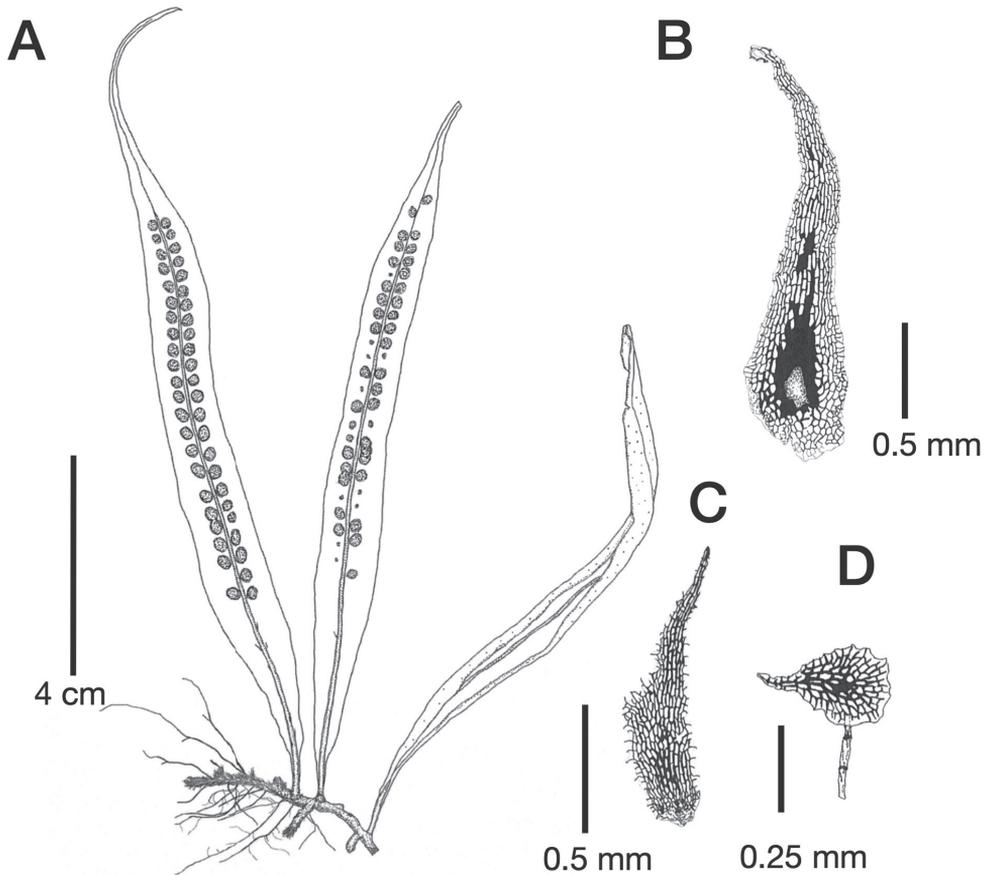


**Figure 2.** Living plant of *Lepisorus medioximus* T.Fujiw., K.Hori & Khine **A** habit **B** adaxial side of lamina **C** abaxial side of lamina with sori **D** rhizome scale **E** leaf scale **F** paraphyses.

**Habitat.** Epiphyte on tree trunks and branches in evergreen to sub-evergreen forest.

**Additional specimens examined (paratypes).** MYANMAR. Shan state: Pin Laung Township, Ka Thaung upper, 19°57'58.5"N, 96°31'09.1"E, alt. ca. 904 m, 26 Sep. 2019, K. Hori, P.K. Khine ["Kine"], T. Fujiwara, M. Nagashima, P.P. Shwe & A.K. Moe 108229 (MBK 0328227!, HITBC! and RAF!).

**Note.** Until now, we have not discovered additional specimens from other localities despite our exhaustive search focusing on herbarium specimens collected in all



**Figure 3.** *Lepisorus medioximus* T.Fujiw., K.Hori & Khine (holotype, Hori et al. 108225 = MBK0328223) **A** habit **B** rhizome scale **C** leaf scale and **D** paraphyses.

parts of Myanmar and the Yunnan province of China. We specifically checked not only the Myanmar *Lepisorus* specimens deposited to the Makino Botanical Garden (MBK), the Institute of Botany, Chinese Academy of Sciences at Beijing (PE) and the Royal Botanic Gardens (K) but also the *Lepisorus* specimens of Dickason collection deposited in the United States National Herbarium (US), the Natural History Museum (BM), and Naturalis Biodiversity Center (L). Given the observation of more than 50 individuals of the species at the type locality, we expected this species to be abundant in this poorly collected area. Further inventories in Shan state and the adjacent areas should be necessary to find new localities of the species and evaluate the conservation status of the species. Reflecting our limited knowledge, the IUCN red list status of this species is given as “Data Deficient” instead of “Critical Endangered”. The latter status would assume a restriction of this species range to the two localities recorded.

## Acknowledgements

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## Supplementary material I

### Table S1

Authors: Tao Fujiwara, Phyo Kay Khine, Kiyotaka Hori, Thant Shin, Noriaki Murakami, Harald Schneider

Data type: Excel datasheet

Explanation note: A checklist for *Lepisorus* s.s. (sensu stricto) species occurrence in Yunnan and the adjacent regions of Indochina.

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Link: <https://doi.org/10.3897/phytokeys.201.84911.suppl1>

## Supplementary material 2

### Table S2

Authors: Tao Fujiwara, Phyo Kay Khine, Kiyotaka Hori, Thant Shin, Noriaki Murakami, Harald Schneider

Data type: Excel datasheet

Explanation note: Summary table for the information of species used in the phylogenetic analyses in this study.

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Link: <https://doi.org/10.3897/phytokeys.201.84911.suppl2>

# Two new freshwater species of *Surirella* (Bacillariophyta) from the Wuling Mountains, China

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## Abstract

Two sympatric *Surirella* species found at the same specific locality in the Wuling Mountains of China are documented with light and scanning electron microscope. Both species are new to science and named *S. wufluminensis* **sp. nov.** and *S. suiningensis* **sp. nov.** *Surirella wufluminensis* has large frustules that are either clockwise or counterclockwise twisted when viewed with the light microscope, and possesses distinctive fibulae, mound-like outgrowths on the valve surface throughout, raised longitudinal ridges on both sides of the raphe, and two helictoglossa-like processes at one apex internally. *Surirella suiningensis* has narrowly ovate valve outline, distinctive fibulae, troughs alternating with crests from pole to pole, and two helictoglossa-like processes at one apex internally. These two species do not produce costae on the valve surface in contrast to many species in *Surirella*. This study provides a further two examples of the wide range of morphological diversity in the genus *Surirella*.

## Keywords

Helictoglossa-like process, new species, *Surirella*, twisted frustule, ultrastructure

## Introduction

The diatom genus *Surirella* Turpin (1828) includes numerous taxa commonly found in benthic habitats (Hustedt 1930; Krammer and Lange-Bertalot 1991; but see also Hustedt 1942 for a consideration of species from large lakes that may be planktonic). Formal morphological (Ruck and Kociolek 2004) and molecular (Ruck et al. 2016) analyses suggested that the former diagnosing features of *Surirella* and closely-related genera were not supported and that some species of *Surirella*, including its generic type *S. striatula* Turpin and the Pinnatae groups of *Surirella*, are more closely related to some species previously included in *Campylodiscus* C.G. Ehrenberg ex Kützing than they are to other species previously included in *Surirella* (the Fastuosoid group) (Ruck and Kociolek 2004; Ruck et al. 2016).

Wang (2018) considered the surirelloid diatoms from inland habitats of China and recognized 47 different taxa (including only two new species) within Surirellaceae: nine taxa in *Cymatopleura* W. Smith, 29 taxa in *Surirella*, four taxa in *Stenipterobia* Brébisson ex Van Heurck, and five taxa in *Campylodiscus* Ehrenberg ex Kützing. Kociolek et al. (2020) listed 33 freshwater *Surirella* taxa described from China before 2000, including those described by Mereschkowsky (1906) and Skvortzov (1927; 1929a, b, c; 1930, 1938, 1976). Post-2000 until 2019 another 3 new taxa were described (Kulikovsky et al. 2012; Liu et al. 2019). Among the above 36 taxa listed by Kociolek et al. (2020), only three species, *Cymatopleura xinjiangiana* Q-M You & J. P. Kociolek, *C. aquastudia* Q-M You & J. P. Kociolek, *Surirella tientsinensis* Skvortzov, were also mentioned in Wang (2018). And most recently Liu et al. (2021) described a new species from China and included the record of another species in the flora of the country.

There are a few taxa in *Surirella* sensu stricto possessing ‘twisted’ frustules, such as *S. aquastudia* (Kociolek & Q. You) Kociolek, *S. xinjiangiana* (Q. You & Kociolek) Kociolek, and *S. dongtingensis* Bing Liu & Ector (You et al. 2017; Liu et al. 2021). They often appear to different visual discrepancies due to the degree of twist or their position relative to the observer. There are also a few taxa in *Surirella* sensu stricto without costa-stria bundles (sensu Liu et al. 2019), such as *S. stalagma* M.H. Hohn & Hellerman and *S. atomus* Hustedt (English 2011). In this study, we describe two new species belonging to *Surirella* sensu stricto characterized by twisted frustules and the valves lacking costae (thickened siliceous ribs).

## Materials and methods

The study site is at the course of Wu River running through Suining County, located in the Wuling Mountains of China under a sub-tropical to warm temperate type climate. At the sampling site, epilithic algae were collected from numerous submerged stones showing yellow-brown surfaces indicating the presence of diatoms. Each stone was placed on a plastic plate and its surface was brushed using a toothbrush, with the brushed-off diatom samples being washed into the plate. The diatom samples were transferred into a 100 mL sampling bottles and fixed with 70% ethanol. Two samples

were collected from each site. Together with the sample collection, temperature, pH, and conductivity were measured *in situ* with a portable multimeter (HQ40D, HACH Company)—details are presented below in the ‘Distribution and ecology’ section of the species description.

Specimens for permanent slides were air-dried onto coverslips then mounted onto microscope slides using Naphrax. The slides were examined and specimens photographed using a Leica DM3000 light microscope (LM) at  $\times 1000$  magnification (objective NA 1.25) and a Leica MC190 HD digital camera. The holotype slides are deposited in the Natural History Museum, London, United Kingdom (BM) and isotypes slides are kept in the Herbarium of Jishou University, Hunan, People’s Republic of China (JIU). For scanning electron microscopy (SEM) observation several drops of the selected cleaned diatom material were air-dried onto glass coverslips that were then attached to aluminium stubs using double-sided conductive carbon strips and sputter-coated with platinum for 20 seconds (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged using a field emission scanning electron microscopy Sigma HD (Carl Zeiss Microscopy) available at Huaihua University, China.

Terminology related to valve morphology follows Ruck and Kociolek (2004) and Van de Vijver et al. (2013), and costa-stria bundle (CSB), over-fibula costa (OFC) follows Liu et al. (2019).

## Results

**Class Bacillariophyceae Haeckel**

**Order Surirellales D.G. Mann**

**Family Surirellaceae Kützing**

**Genus *Surirella* Turpin**

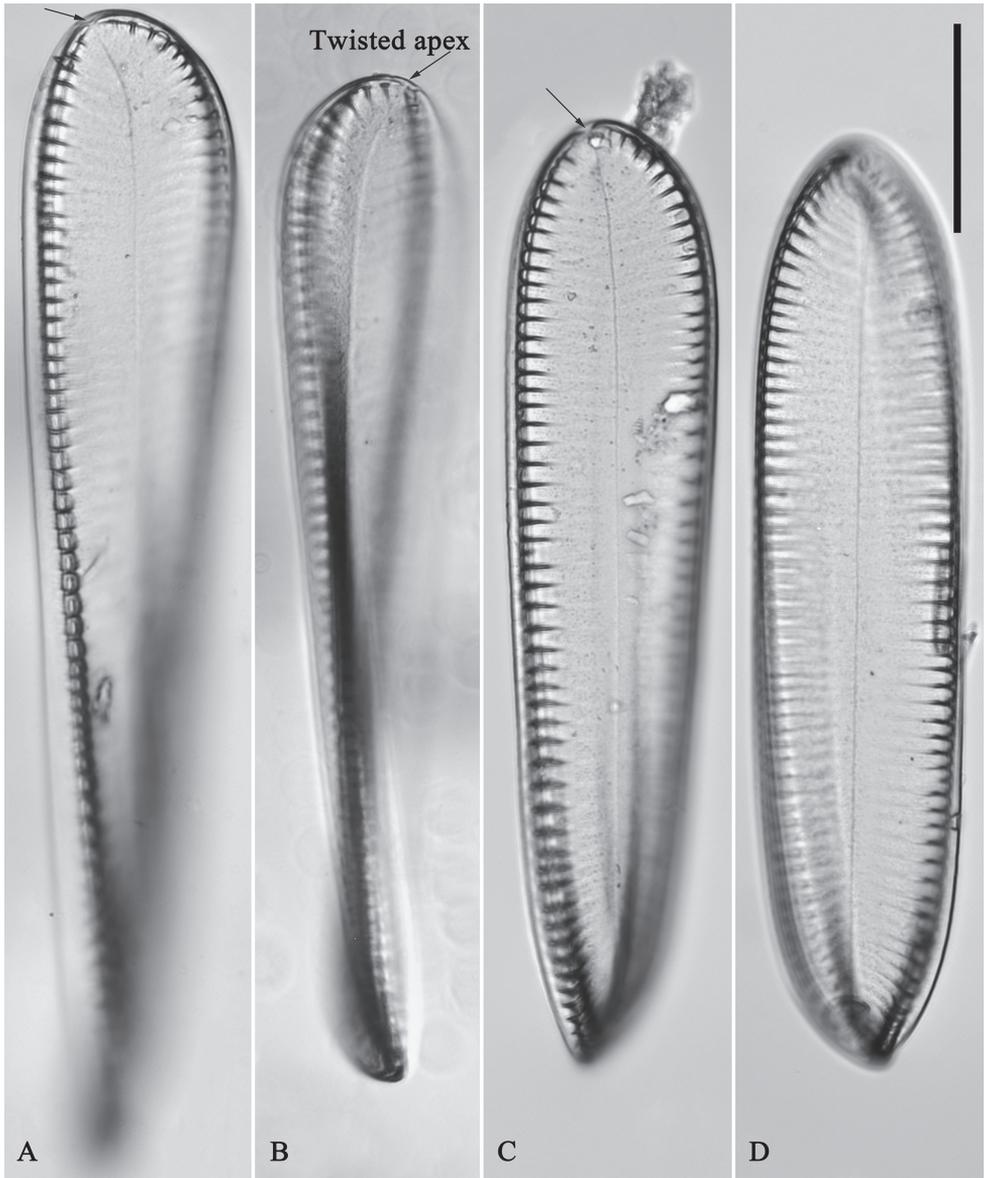
***Surirella wufuminensis* Bing Liu & Kociolek, sp. nov.**

Figs 1–4

**Holotype.** *Holotype* BM! 81892, specimen circled on slide, illustrated as Fig. 1D; isotype JIU! G202106, specimen circled on slide, illustrated as Fig. 1C.

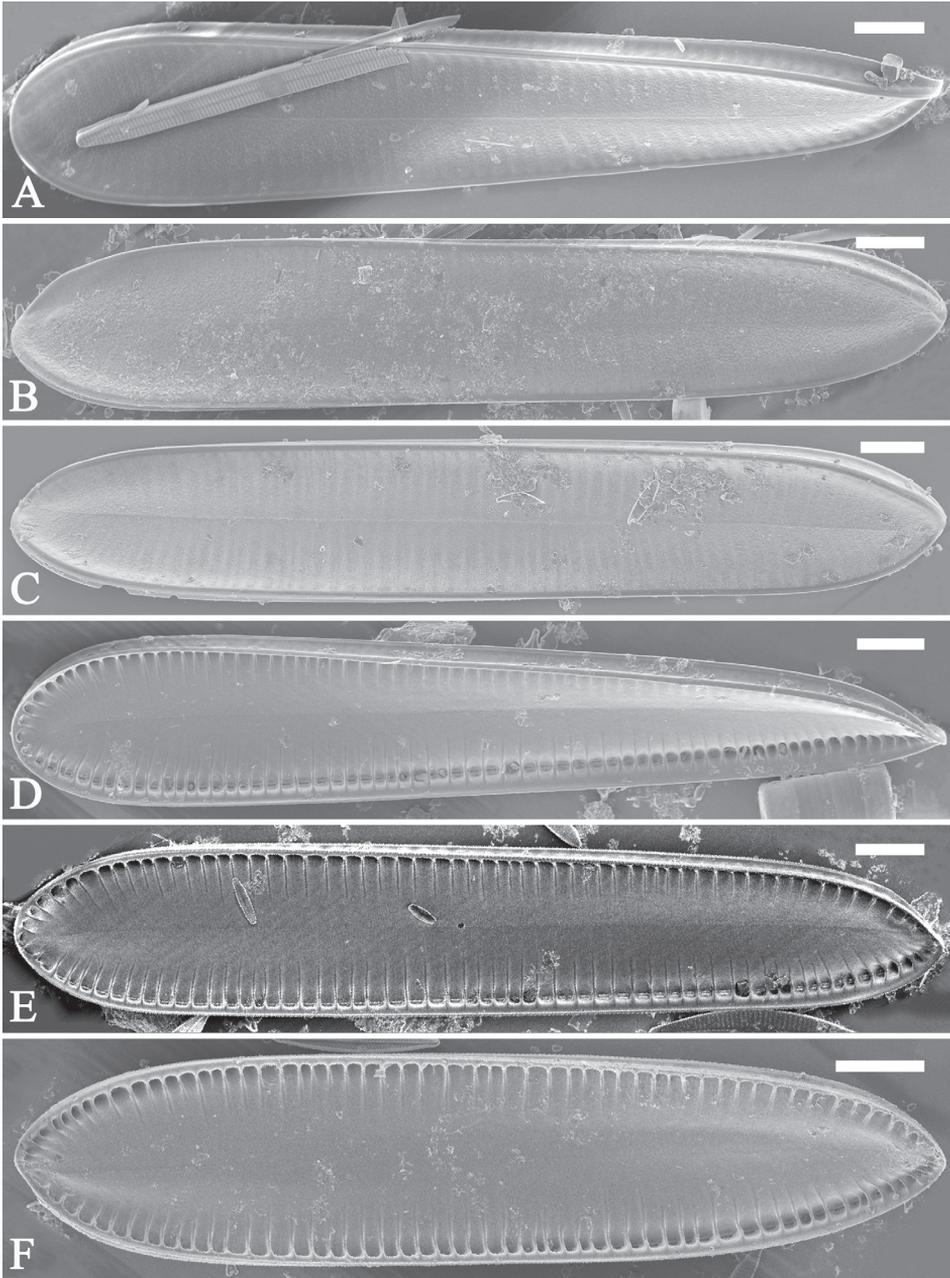
**Type locality.** CHINA. Hunan province: the course of Wu River, a sampling point at Changpu Town, Suining County,  $26^{\circ}34.59'N$ ,  $110^{\circ}09.19'E$ , 300 m a.s.l., collected by Bing Liu, March 22, 2021.

**Description.** *LM* (Fig. 1). Valves twisted, sometimes exhibiting linear-lanceolate valve outline (Fig. 1D), with twisted and deflected apices (Fig. 1A–C). Valve dimensions ( $n = 27$ ): 198–295  $\mu\text{m}$  long, 41–50  $\mu\text{m}$  wide. Valve face smooth without undulations, valve midline sigmoid, fibulae very distinctive, 20–28 in 100  $\mu\text{m}$ . The degree of rotation (twist) of the valves differs (Fig. 1A–D) as each position relative to the observer is different. Two type valves are observed under LM: one is counterclockwise twisted (Fig. 1A, C), the other is clockwise twisted (Fig. 1B, D), also seen in SEM (Fig. 2A–F).



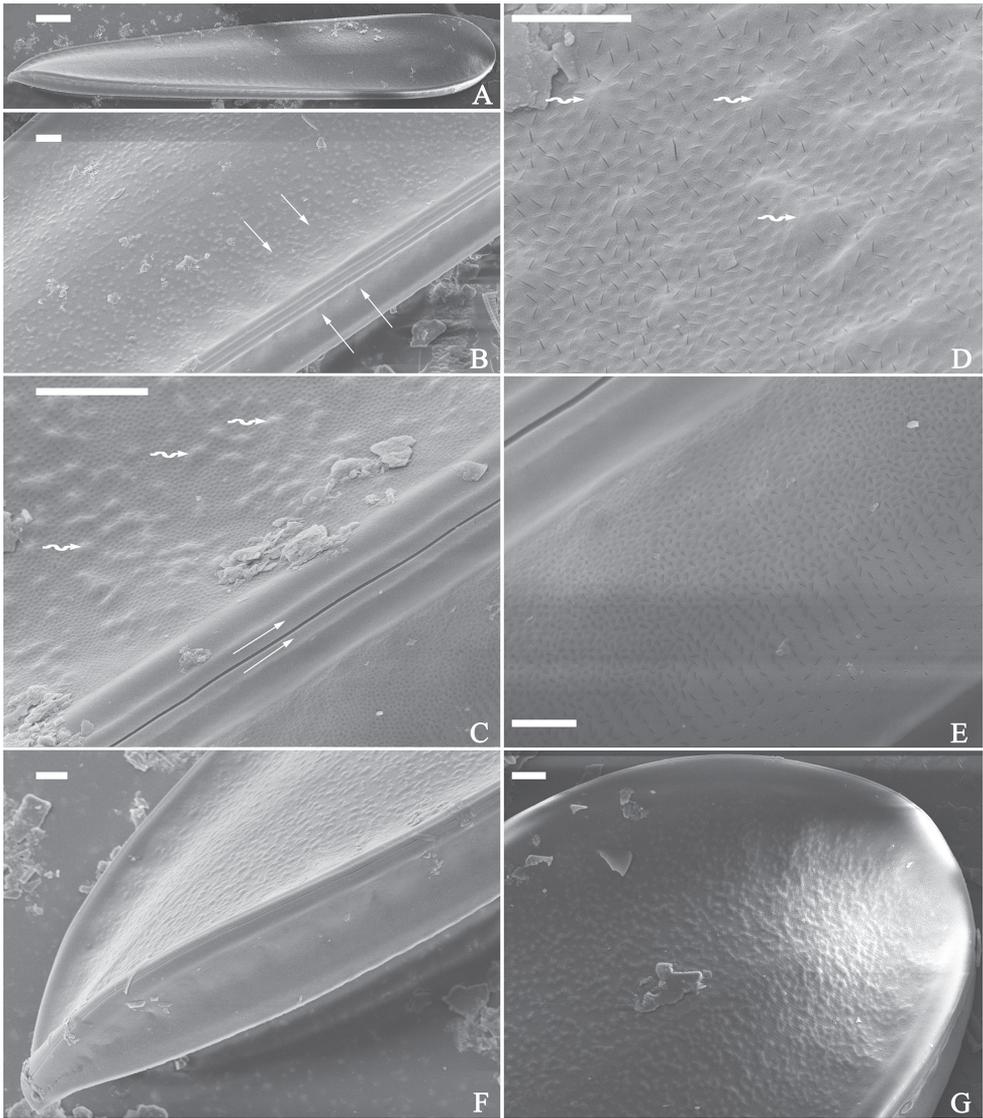
**Figure 1.** *Surirella wufluminensis* sp. nov., LM,  $\times 400$  **A, C** two counter-clockwise twisted valves **B, D** two clockwise twisted valves. Micrograph of the holotype specimen (Fig. A) and isotype specimen (Fig. D). Scale bar: 50  $\mu\text{m}$ .

**SEM** (Figs 2–4). Valve twisted, but visual discrepancies of degree of twist differs (Fig. 2A–F). Valve surface flat without undulations but mound-like siliceous outgrowths scattered throughout (Figs 2A–C, 3A–D). Very shallow depressions close to mantle, corresponding to beneath, internal fibulae (Fig. 3B, four arrows). Raised longitudinal ridges produced on both sides of raphe (Fig. 3C). Externally, costae (thickened silica ribs) absent, external openings of areolae slit-like on both valve face and mantle (Fig. 3D, E).



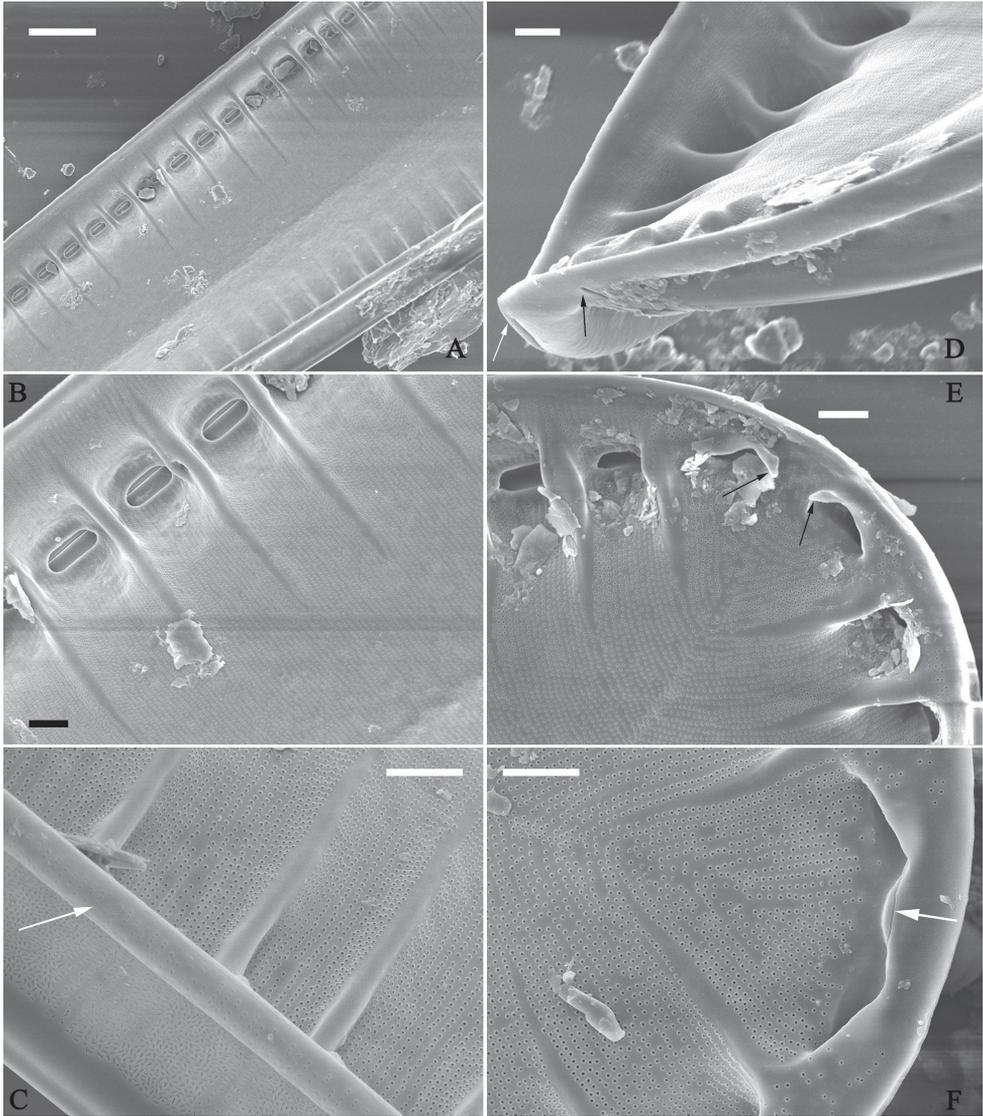
**Figure 2.** *Surirella wufluminensis* sp. nov., SEM **A–C** three valves in external view; note the visual discrepancies of twist degrees between them as the position of each valve relative to the observer differs **D–F** three valves in internal view; note the visual discrepancies of twist degrees between them. Scale bars: 20  $\mu\text{m}$ .

External distal raphe endings interrupted at both apices (Figs 3F, G, 4D). Internally, distal raphe endings interrupted only at one apex where two helictoglossa-like processes present (Fig. 4E), raphe continuous at another apex (Fig. 4F). Mantle with same striation patterns



**Figure 3.** *Surirella wufluminensis* sp. nov., external view, SEM **A** a whole valve **B** middle detail from **A** showing the shallow depressions on the valve face correspond to the ones on the mantle (four arrows) **C–E** details from Fig. B showing mound-like siliceous outgrowths on the valve surface (**C, D**, three wavy arrows respectively), raised longitudinal ridges on both sides of the raphe (**C**, two arrows), slit-like external openings of areolae on both valve face and mantle (**D–F**) **F, G** apical details from **A**, note interrupted terminal raphe endings (see also Fig. 4D). Scale bars: 20  $\mu\text{m}$  (**A**); 3  $\mu\text{m}$  (**B, C, F, G**); 1  $\mu\text{m}$  (**D, E**).

as valve face and its base margin thickened (Figs 3E, F, 4C). Fibulae distinctive, extending from mantle, spanning 1/3 to 1/2 of half valve width except near two apices where fibulae meeting at midline (Figs 2D–F, 4A). Uniseriate striae 40–42 in 10  $\mu\text{m}$  (measured in SEM images,  $n = 3$ ). Internal openings of areolae rounded, rimmed (Fig. 4C, F). One portula and ca. 15–23 uniseriate striae located between two adjacent fibulae (Fig. 4A, B).



**Figure 4.** *Surirella wufluminensis* sp. nov., internal view, SEM **A–C** middle details from Fig. 2D, note that fibulae extend 1/3 to 1/2 of half valve width (**A**), one portula between two adjacent fibulae (**A, B**), uniseriate striae, and valve mantle with thickened base margin (**C**, white arrow) **D, E** apical details from Fig. 2D, note two interrupted external terminal raphe endings (**D**, two arrows), two helictoglossa-like processes (**E**, two arrows) **F** another apex showing continuous raphe at one apex (white arrow). Scale bars: 5  $\mu\text{m}$  (**A**); 2  $\mu\text{m}$  (**B–F**).

**Etymology.** Named after Wu River, where the species was found.

**Ecology and distribution.** Epilithic in a mountain river with oligotrophic waters. The following environmental parameters were measured in the field. Conductivity was  $99.7 \pm 0.3 \mu\text{S}\cdot\text{cm}^{-1}$ , pH was  $7.9 \pm 0.1$  and water temperature was  $13.2 \pm 0.2 \text{ }^\circ\text{C}$ .

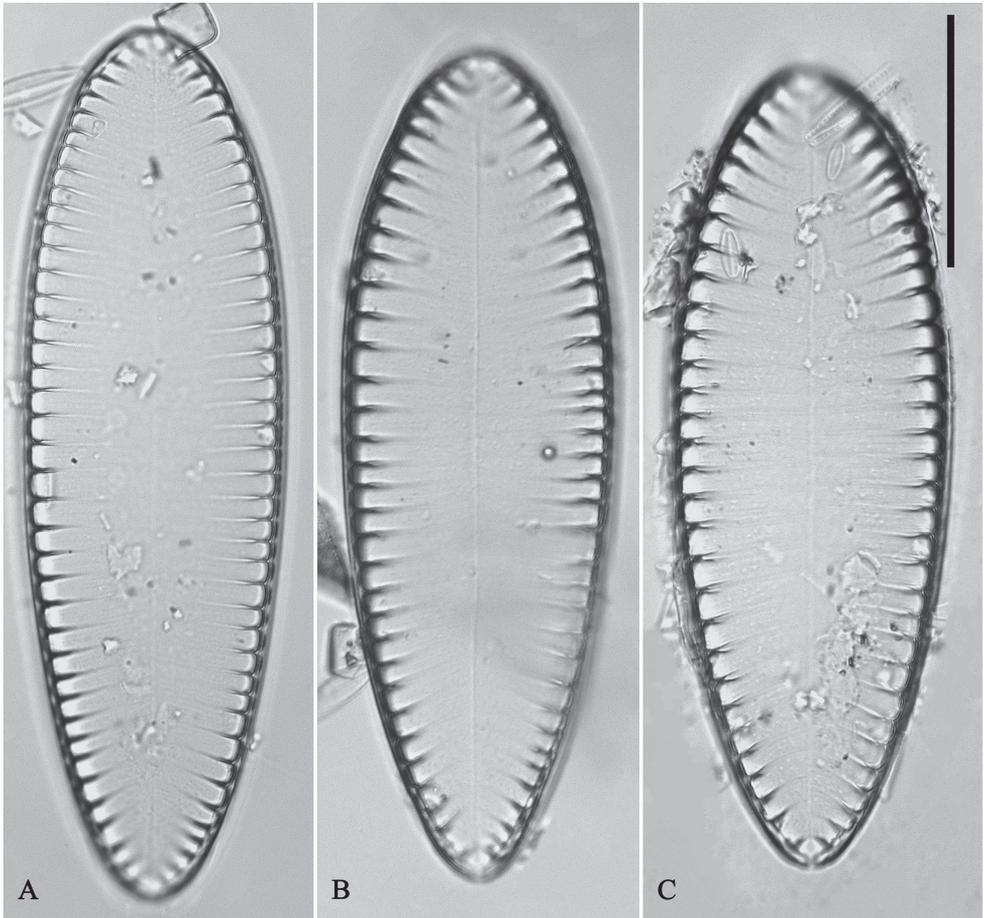
***Surirella suiningensis* Bing Liu & D.M. Williams, sp. nov.**

Figs 5–8

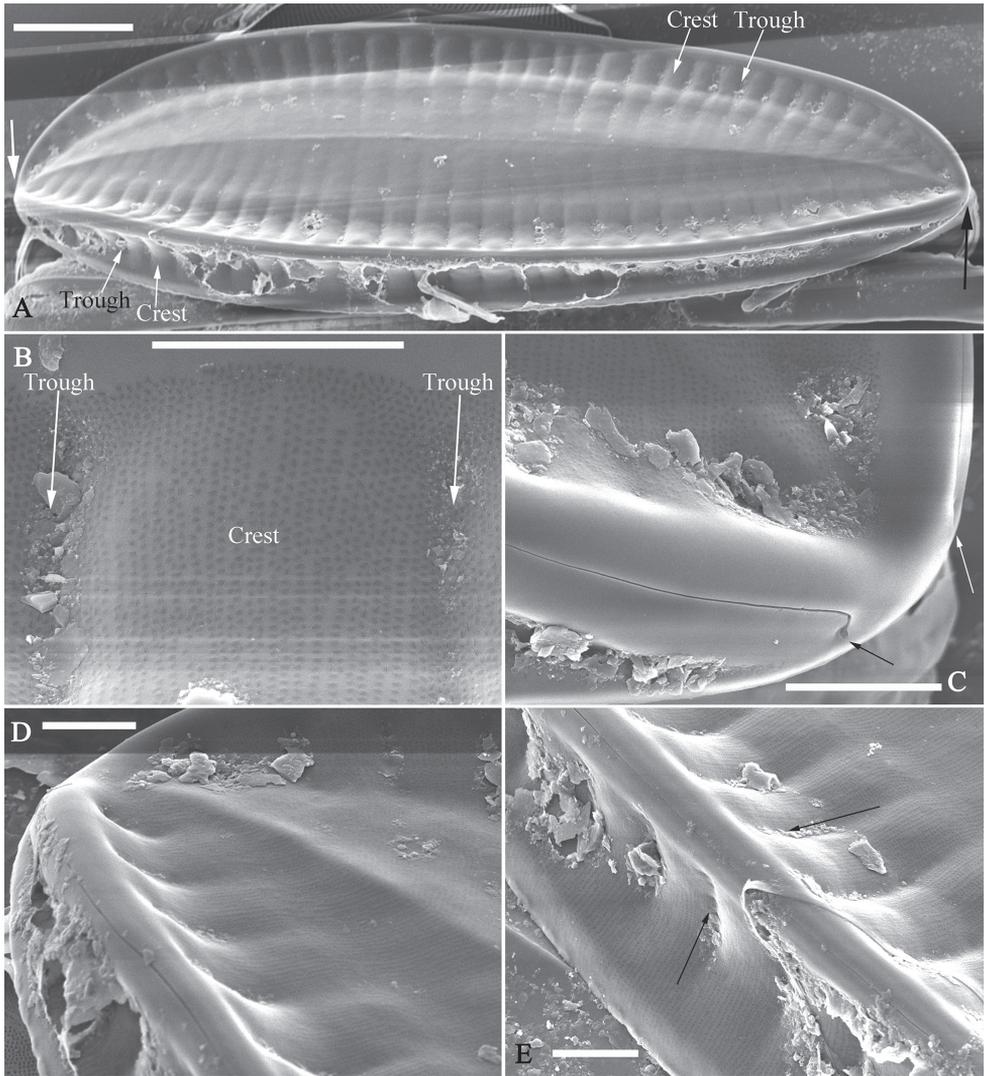
**Holotype.** *Holotype* BM! 81893, specimen circled on slide, illustrated as Fig. 5B; isotype JIU! G202107, specimen circled on slide, illustrated as Fig. 5A.

**Type locality.** CHINA. Hunan Province: the course of Wu River, a sampling point at Changpu Town, Suining County, 26°34.59'N, 110°09.19'E, 300 m a.s.l., collected by Bing Liu, March 22, 2021.

**Description.** *LM* (Fig. 5). Valves narrowly ovate, heteropolar, with downward deflecting head and foot poles; headpole broadly rounded whereas footpole narrowly rounded. Valve dimensions (n = 8): 148–173 µm long, 49–55 µm wide (measured at

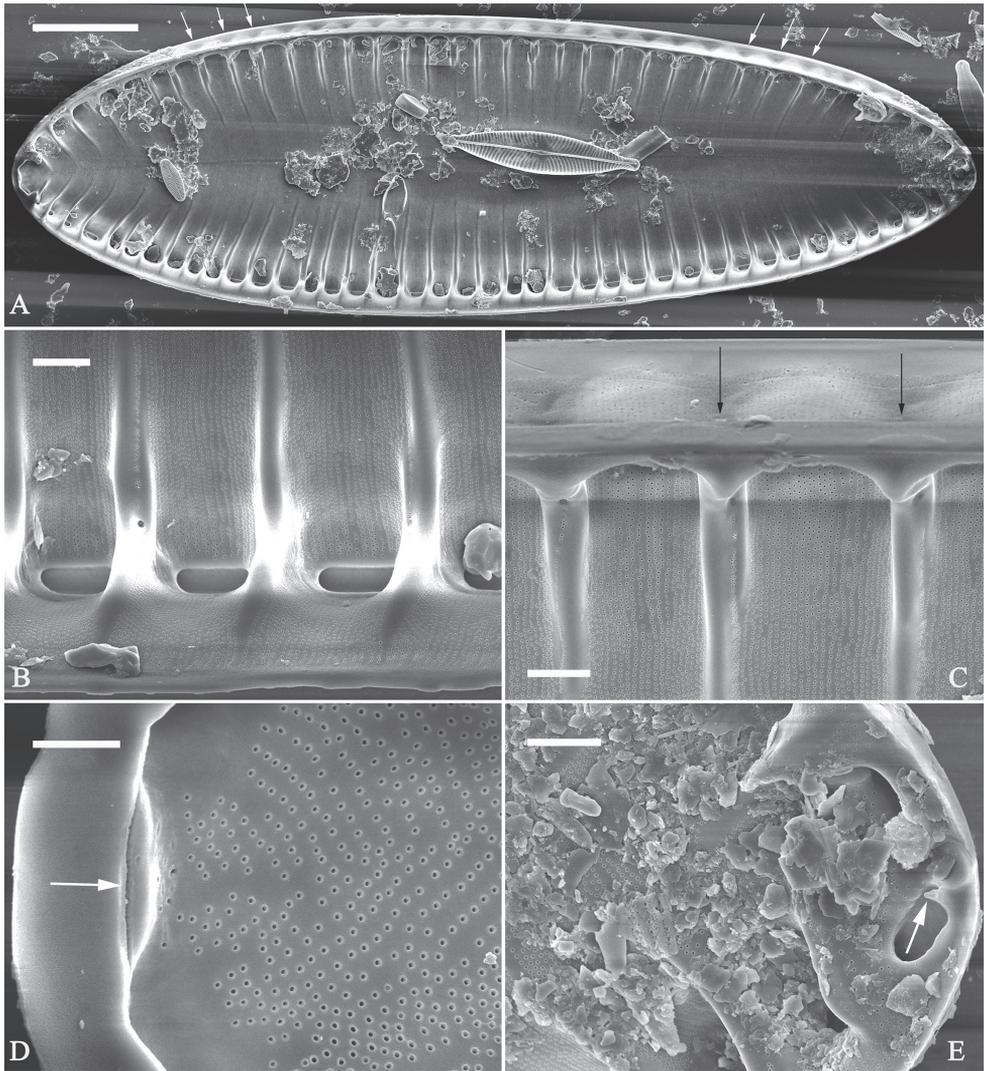


**Figure 5.** *Surirella suiningensis*, sp. nov., LM. ×400 **A–C** three valves showing narrow-ovate outline, slightly heteropolar valves, straight valve median line, downward deflecting head and foot poles, distinctive fibulae spanning 70–80% of half valve width except near each apex where a few fibulae meet at median line. Micrograph of holotype (**B**). Scale bar: 50 µm.



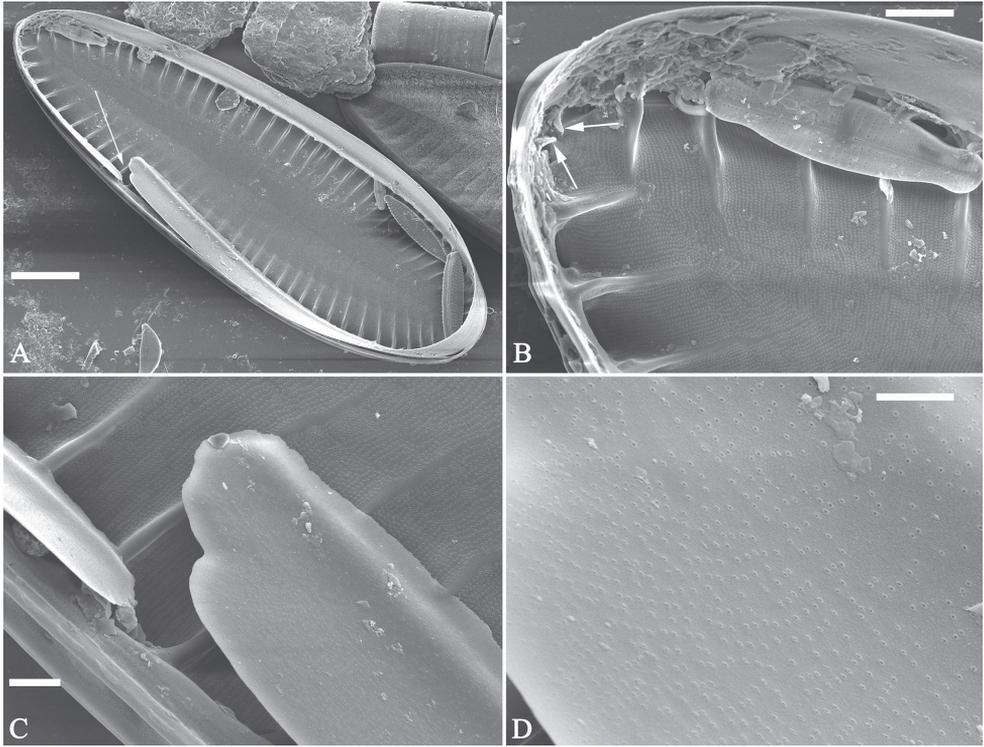
**Figure 6.** *Surirella suiningensis* sp. nov., external view, SEM **A** one valve showing distinct continuous siliceous median ridge, downward deflecting head and foot poles (two arrows), and pattern of troughs alternating with crests **B** detail from **A** showing two troughs and one crest **C, D** apical details from **A**, note two curved terminal raphe fissures (**C**, two arrows) **E** marginal detail of **A**, note the recessed valve mantle with thickened base margin, no fenestrae existed, troughs on valve surface corresponding to those on the mantle (two black arrows). Scale bars: 20  $\mu\text{m}$  (**A**); 2  $\mu\text{m}$  (**B–E**).

widest valve part). Valve face appearing smooth, without undulations and costae. Valve midline straight, downward deflecting at each apex. Fibulae very distinctive, parallel in valve middle part, radiate at two apices, 20–24 in 100  $\mu\text{m}$ . Fibulae extending from mantle towards midline, spanning 70–80% of half valve width except near each apex where few fibulae nearly meet at median line.



**Figure 7.** *Surirella suiningensis*, sp. nov., internal view, SEM **A** one complete valve showing fibulae extending close to median line and fibulae corresponding to the sinking of mantle (i.e. troughs, six arrows) **B** detail from **A** showing only one portula between two adjacent fibulae and uniseriate striae **C** sinking of mantle against the fibulae (two arrows) **D, E** apical details from **B**, note the raphe continuous at one apex (**D**, arrow) whereas interrupted at the other apex (**E**, one arrow pointing at a helictoglossa-like process). Scale bars: 20  $\mu\text{m}$  (**A**); 2  $\mu\text{m}$  (**B, C, E**); 1  $\mu\text{m}$  (**D**).

**SEM** (Figs 6–8). Valve smooth, canal raphe system located around valve margins, slightly raised (Fig. 6A). Median line slightly raised, continuous from pole to pole, downward deflecting at both apices (Fig. 6A). Valve surface producing shallow troughs and higher crests (Fig. 6A, B), and troughs alternating with crests from pole to pole.



**Figure 8.** **A–D** *Surirella suiningensis*, sp. nov., internal view, SEM **A** one valve with valvocopula, note the open nature of valvocopula (arrow) **B** apical detail from **A**, note two helictoglossa-like processes (two arrows) **C** detail of open part of valvocopula **D** detail of valvocopula showing the rimmed areolae internally. Scale bars: 20  $\mu\text{m}$  (**A**); 4  $\mu\text{m}$  (**B**); 1  $\mu\text{m}$  (**C, D**).

Costae (thickened siliceous ribs) absent (Fig. 6B). Each trough corresponding to its internal fibula, each crest composed of c. 16–27 uniseriate striae (Fig. 6B, E). Distal raphe endings curved (Fig. 6C, D). Mantle with same pattern as valve surface, troughs align with each other (Fig. 6E). Internally, fibulae evident, extending from mantle to median line, spanning 70–80% of half valve width. Striae uniseriate, 47–51 in 10  $\mu\text{m}$ . External openings of areolae slit-like (Fig. 6B) while internal openings rounded and rimmed (Fig. 7D). One portula and 16–27 uniseriate striae located between two adjacent fibulae (Fig. 7B). Sinking of mantle against fibulae present (Fig. 7C). Raphe continuous at one apex whereas interrupted at other apex (Figs 7E, 8B). Valvocopula open (Fig. 8A, C) and rimmed areolae internally (Fig. 8D).

**Etymology.** Named after Suining County of Hunan Province, where the species was found.

**Ecology and distribution.** Epilithic in a mountain river with oligotrophic waters. *Surirella suiningensis* was found in the same sampling site with *S. wufluminensis*, for the environmental parameters, see above.

## Discussion

The structure of the valves in the two new species argues for their placement in the genus *Surirella*, in the sense of Ruck et al. (2016). Both have direct communication between the raphe opening and the valve interior via simple portulae, a feature recognized by Ruck et al. (2016) as a synapomorphy for the genus. The structure of the raphe, which is discontinuous in both taxa, is akin to species in the Pinnatae group (= sensu stricto group) of *Surirella* (Ruck and Kociolek 2004; Ruck et al. 2016), suggesting they might be more closely related to species in that group than species in *Cymatoleura* W. Smith. The distinct presence of an internal helictoglossa-like process at the raphe terminations in the two species can be considered as a differentiating character from other “typical” *Surirella* species, which has, so far, been reported only in a few species such as *Surirella robusta* Ehrenberg, *S. splendida* (Ehrenberg) Kützing, *S. rumrichorum* Metzeltin & Lange-Bertalot (1998, figs 216: 2, 3; 219: 7), and *S. hinziae* Cvetkoska, Levkov & P.B. Hamilton (in Cvetkoska et al. 2015, p. 187, fig. 32).

*Surirella aquastudia*, *S. xinjiangiana*, and *S. dongtingensis* all have twisted frustules and all produce undulations on the valve surface from pole to pole. Previously, based on the presence of valve undulations, they would have been placed in the genus *Cymatopleura*. *Surirella wufluminensis* on the other hand, lacks undulations on its valve surface. Other similar species demonstrating valves with torsion include the former *Surirella spiralis* Kützing (Wang 2018) – this species is now recognized as a member of *Iconella*, as *I. spiralis* (Kützing) Ruck & T. Nakov. The only taxa similar to *S. wufluminensis*, in the sense of having twisted valves and lacking central valve undulations, are *S. subcontorta* Hustedt (in Schmidt 1942, plate 356, figs 1, 2), described from the African Rift Lake of Tanganyika, and *S. uninodes* Skvortzov (1937, p. 360) from Lake Baikal. *Surirella subcontorta*, as illustrated and described by (Hustedt 1942) is much wider at the ‘headpole’ than *S. wufluminensis*. *Surirella uninodes* is twisted near the middle of the valve, quite unlike *S. wufluminensis* (Hustedt 1942).

With regards to *S. suiningensis*, the ovate valve outline is reminiscent of *Surirella davidsonii* A.W.F. Schmidt, *S. elegans* Ehrenberg, *S. slesvicensis* Grunow (in Schmidt 1875, plate 21, fig. 19) as well as *Iconella guatimalensis* (Ehrenberg) Ruck & Nakov. *Surirella suiningensis* has narrow-ovate valve outline (i.e., its headpole is only slightly wider than its footpole) while *S. davidsonii* has an ovate valve outline (i.e. its headpole is wider than its footpole). SEM images of *S. davidsonii* have been published by Metzeltin and Lange-Bertalot (1998). *Surirella elegans* produces a lanceolate central region which *S. suiningensis* lacks. *Surirella slesvicensis*, described from a swamp in Europe, is similar to *S. suiningensis* in shape and overall proportions. De Toni (1892) suggested *S. slesvicensis* may be conspecific with *S. elegans* and *S. subalpina* Donkin (1869, p. 292) described from the U.K. There are no records of *S. slesvicensis* or *S. subalpina* being studied with electron microscopy (Gaul et al. 1993; Henderson and Reimer 2003).

*Surirella wufluminensis* and *S. suiningensis* occur together in the same freshwater habitat. Their associated species comprise *Pinnularia hustedtii* Meister (see Williams et al. 2022, p. 294), *Diatoma vulgaris* Bory de Saint-Vincent (1824, p. 461), *Tabularia*

*fonticola* (Hustedt) C.E. Wetzel & D.M. Williams (in Vigneshwaran et al. 2020, p. 179), and some species of *Fragilaria* Lyngbye (1819, p. 182), *Gyrosigma* Hassall (1845, p. 435), *Navicula* Bory de Saint-Vincent (1822, p. 128), *Nitzschia* Hassall (1845, p. 435), *Sellaphora* Mereschkowsky (1902, p. 186) and among others.

## Acknowledgements

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# Micromorphological leaf epidermal traits as potential taxonomic markers for infrageneric classification of *Oxytropis* (Fabaceae)

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## Abstract

The characteristics of the leaf epidermis have proven to be useful criteria to support taxonomic studies within Fabaceae. However, there are few systematic studies on the taxonomic significance of leaf epidermis of *Oxytropis* DC. Here, we used light and scanning electron microscopy to investigate leaf epidermal characteristics of 18 species of genus *Oxytropis* from the Northeastern Margin of Qinghai-Tibet Plateau. Our examination showed two main types of leaf epidermal cells: polygonal and irregular, and four different patterns of anticlinal walls: straight-arched, sinuate, undulate, and sinuate. All species studied possess anomocytic stomata. Two trichome shapes were identified: strip-like trichomes, that were present only in *O. ciliata*, and cylindrical trichomes, present in all other species. Epidermal cell shape and anticlinal wall pattern were constant within species and are useful for species delimitation within genus *Oxytropis*, when combined with other macroscopic traits. The shape of trichomes can be useful for distinguishing *O. ciliata* from the other investigated species. Stomatal type was the same within the genus and may be used to elaborate the phylogenetic relationships between genera in combination with data on stomata from other genera. Cluster analysis results were largely consistent with the classification of species and sections based on macro morphological data, indicating that foliar epidermis characteristics of *Oxytropis* can be used as markers for taxonomic identification at the infrageneric classification level. Lastly, our results support the delineation of the section *Leucopodia* as an independent section but do not support the merging of section *Gobicola* into section *Baicalia*.

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\* These authors contributed to the work equally and should be regarded as co-first authors.

**Keywords**

China, cluster analysis, leaf epidermis, LM, *Oxytropis*, SEM, taxonomy

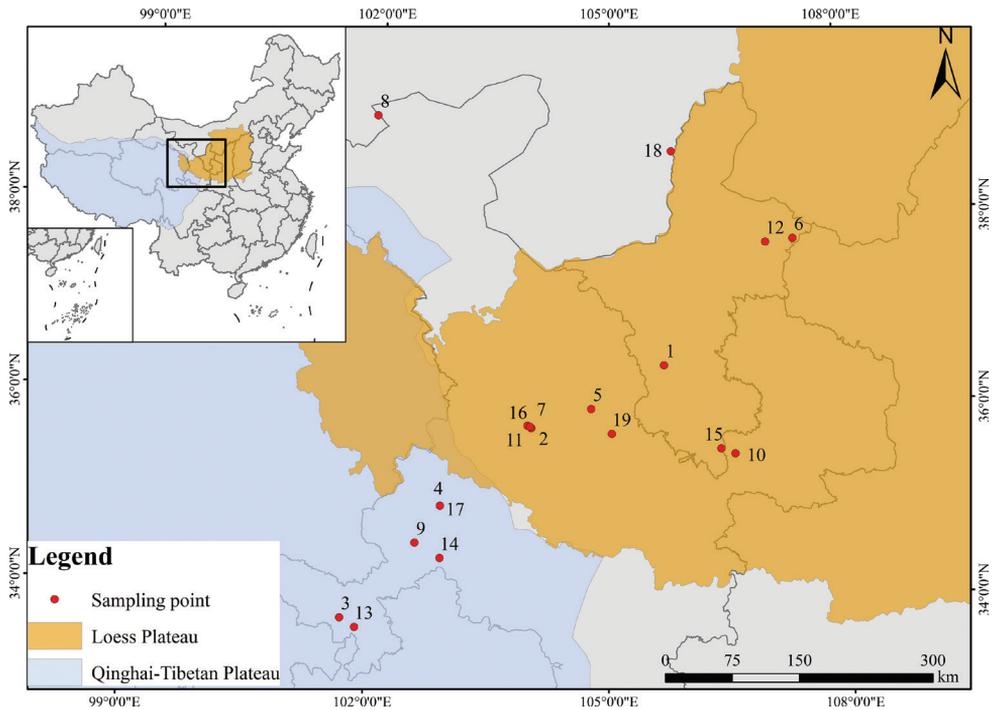
**Introduction**

Genus *Oxytropis* DC. is one of the largest groups within Fabaceae, with approximately 330 species occurring in the cold mountainous regions of Europe, Asia, and North America, and also concentrated in Central Asia (Polhill 1981; Zhu et al. 2010). The genus was established by De Candolle (1802), who distinguished it from *Astragalus* based on differences in keel-petals and legumes. In China, *Oxytropis* species are mainly distributed in the north and northwest regions (Zhang 1998; Zhu and Ohashi 2000). However, there is some debate regarding the delimitation and identification of species within this genus. In China, the genus was first reported by Peter-Stibal (1937), who recorded two subgenera, 11 sections, and 27 species. Wang and Tang (1955) recorded 27 species of *Oxytropis* and 1 variety in China. In contrast, in Flora Reipublica Popularis Sinicae (FRPS), Zhang (1998) divided *Oxytropis* into six subgenera, 22 sections, and 146 species. While Zhu and Ohashi (2000) recognized 125 species and 4 varieties, a recent study (Zhu et al. 2010) on the Flora of China (FOC) reported that the genus *Oxytropis* consisted of three subgenera and 20 sections containing 133 species. Therefore, different species delimitations in China have been proposed by taxonomists, whereby the infrageneric delimitation of *Oxytropis* remains controversial.

Leaf epidermal anatomical features, such as epidermal cell shape, epicuticular waxes (Barthlott et al. 1998; Wissemann 2000; Tomaszewski and Zieliński 2014; Tomaszewski et al. 2019), stomatal complexes (Carpenter 2005; Alvarez et al. 2009; Yang et al. 2012; Nisa et al. 2019), and trichomes (Webster et al. 1996; Hu et al. 2012; Eiji and Salmaki 2016; Mannethody and Purayidathkandy 2018; Ashfaq et al. 2019), are all useful diagnostic and taxonomic characteristics. The taxonomic relevance of the foliar epidermal characteristics of Fabaceae is well documented (Zou et al. 2008; Alege and Shaibu 2015; Silva et al. 2018; Shaheen et al. 2020). Zou et al. (2008) found that epidermal characteristics can be used to distinguish genus *Bauhinia* from *Cercis*. Similarly, Chukwuma et al. (2014) described the presence of glandular trichomes and found that they could be used as a distinguishing feature between genera *Centrosema* and *Clitoria*. Consistently, in genus *Lotus*, epidermal micromorphological features are useful and informative for distinguishing between sections *Simpetaria* and *Microlotus* (Stenglein et al. 2003).

Leaf epidermal features are also valuable for classification at the species level in Fabaceae. For example, Silva et al. (2018) found that leaflet anatomy is taxonomically useful at both genus and species levels in the Dipterygeae clade. Similarly, Rashid et al. (2019) concluded that the combination of leaf epidermal characteristics and other traits has potential for taxonomic resolution at the species level in the tribe *Trifolieae*. Additionally, in *Crotalaria*, leaf characteristics, such as texture, venation pattern, and epidermis, have shown potential for aiding the circumscription of some species (Devecchi et al. 2014).

Previous studies have investigated different aspects of *Oxytropis*, including cytology (Ledingham 1957, 1960; Ledingham and Rever 1963; Ranjbar et al. 2010; Liu et al. 2011; Martin et al. 2015), molecular phylogeny (Jorgensen et al. 2003; Archambault and Strömviik 2012; Dizkirici et al. 2016), pollen (Zhu and Ohashi 2000; Wang 2005; Ceter et al. 2013), and seed micromorphology (Solum and Lockerman 1991; Bojňanský and Fargašová 2007; Meyers et al. 2013; Erkul et al. 2015). On the other hand, relatively few studies on leaf epidermal anatomy have been reported in *Oxytropis* species (Karaman et al. 2009; Lu 2011), which described leaf epidermal traits by light or scanning electron microscopy but lacked systematic analysis. In addition, the Northeastern Margin of the Qinghai-Tibet Plateau is located in the transition zone between the Qinghai-Tibet Plateau and the Loess Plateau, the two main distribution areas of *Oxytropis* in China (Fig. 1). This region includes the northeastern part of the Qinghai-Tibet Plateau and the western part of the Loess Plateau (Tian et al. 2021) (Fig. 1). It is one of the regions with rich diversity of *Oxytropis* (Zhang 1998; Zhu et al. 2010). However, little research has been conducted on the epidermal traits of *Oxytropis* in this region. Thus, we provide the first systematic comparison and microscopic investigation of 18 species of *Oxytropis* from this region using light and scanning electron microscopy to elucidate the taxonomic significance of leaf micromorphology and test the recent taxonomic treatment (Zhu et al. 2010).



**Figure 1.** Map of study area. Numbers represent sample codes, as shown in Table 1.

## Materials and methods

All leaf samples were obtained from specimens deposited at the herbarium of the Northwest Normal University. The materials investigated are listed in Table 1, and the infrageneric classification by Zhu et al. (2010) was adopted (Fig. 1). Those used for analysis by light microscopy were soaked in water at 37 °C for 12–18 h; removed and placed under a dissecting microscope to separate the epidermal tissue from the leaf body, followed by maceration in 1% safranin solution. To check the consistency of the epidermal structure under a light microscope (DM6 B Leica, Leica Microsystems S.L.U., Barcelona, Spain), at least 20 slides were prepared from different parts of a single leaf, and from different leaves of each species. The number and size of the stomata on each slide were counted. Materials for observation by scanning electron microscopy (SEM) were mounted directly on the stubs without treatment. After gold sputtering, the specimens were examined and imaged using a field emission (FE-SEM) Zeiss Ultra Plus instrument (Zeiss, Germany). Quantitative and qualitative traits were selected when performing cluster analysis.

Leaf epidermal terminology was based on the classification proposed by Dilcher (1974) and Wilkinson (1980). Stomatal index (SI) was calculated using the following equation:

$$SI = S/E + S \quad (1)$$

where, SI is the stomatal index, S is the number of stomata per unit area, and E is the number of epidermal cells per unit area. Stomatal density (SD) was expressed as the number of stomata per unit leaf area.

## Data analysis

Statistical data was processed by the Origin 2021 software (OriginLab Corporation 2021). The raw data matrix includes quantitative and qualitative characters, and qualitative traits were coded using a presence/absence (0/1) matrix (Table 2). The Euclidean distance is one of the most commonly used distance measurement methods in hierarchical clustering, which can reflect the absolute difference of individual numerical characteristics and is suitable for the analysis that needs to reflect the difference from the numerical size of the dimension (Raymond and Sylvia 1993; Farhana and Safwana 2018). The Ward error sum of squares method applies the idea of ANOVA to classification, and the obtained clustering information is more abundant and rarely affected by abnormal data (Ward 1963; Szekely and Rizzo 2005). Therefore, the Ward's method was used for cluster analysis using the squared Euclidean distance to interpret the morpho-anatomical similarity among species in this study.

**Table 1.** Source of materials.

Section	Code	Species	Locality	Coordinates	Habitat	Voucher
Section <i>Xerobia</i>	1	<i>O. ciliata</i>	Yueliang Mountain	36°25'41.85"N, 105°42'23.71"E	Valley	X. Zhao 1947
Section <i>Polyadena</i>	2	<i>O. muricata</i>	Maxian Mountain	35°47'46.48"N, 103°58'12.64"E	Sunny hillside	X. Zhao 1903
Section <i>Falcicarpae</i>	3	<i>O. falcata</i>	Awangcang wetland park	33°45'32.85"N, 101°41'6.58"E	Riverside	X. Zhao 1842
Section <i>Baicalia</i>	4	<i>O. ochrantha</i>	Dangzhou grassland	34°56'54.09"N, 102°53'8.74"E	Alpine meadow	X. Zhao 1813
	5	<i>O. bicolor</i>	Tiemu Mountain	35°58'32.21"N, 104°46'31.40"E	Sunny hillside	X. Zhao 1927
	6	<i>O. racemosa</i>	Yanchi	37°43'52.02"N, 107°23'55.77"E	Desert sandy land	X. Zhao 1946
	7	<i>O. myriophylla</i>	Maxian Mountain	35°47'46.48"N, 103°58'12.64"E	Valley	X. Zhao 1833
Section <i>Lycotriche</i>	8	<i>O. aciphylla</i>	Jijiquan nature reserve	38°59'43"N, 101°55'39"E	Desert sandy land	X. Zhao 1924
Section <i>Eumorpha</i>	9	<i>O. imbricata</i>	Taohe river	34°33'28.66"N, 102°34'53.99"E	Riverside	X. Zhao 1940
	10	<i>O. coerulea</i>	Taitong Mountain	35°30'8.94"N, 106°35'54.90"E	Border of Forest	X. Zhao 1832
Section <i>Mesogaea</i>	11	<i>O. xinglongshanica</i>	Xinglong Mountain	35°46'20.53"N, 104°1'2.49"E	Valley	X. Zhao 1913
	12	<i>O. glabra</i>	Rabah Lake National Nature Reserve	37°42'3.19"N, 107°2'33.46"E	Desert sandy land	X. Zhao 1950
	13	<i>O. kansuensis</i>	Azi Test Station of LZU	33°39'57.96"N, 101°52'22.44"E	Alpine meadow	X. Zhao 1819
	14	<i>O. melanocalyx</i>	Guanggai Mountain	34°24'23.35"N, 102°53'58.80"E	Alpine meadow	X. Zhao 1956
	15	<i>O. taohensis</i>	Liupan Mountain	35°33'21.81"N, 106°25'21.54"E	Border of Forest	X. Zhao 1838
	16	<i>O. ochrocephala</i>	Xinglong Mountain	35°47'5.17"N, 104°0'0.67"E	Beside farmland	X. Zhao 1828
	17	<i>O. ochrocephala</i>	Dangzhou grassland	34°56'54.11"N, 102°53'8.81"E	Alpine meadow	X. Zhao 1812
Section <i>Oxytropis</i>	18	<i>O. latibracteata</i>	Helan Mountain	38°39'46.59"E 105°49'20.25"N	Border of Forest	X. Zhao 1951
Section <i>Leucopodia</i>	19	<i>O. squammulosa</i>	Shaochagou	35°42'57.20"N, 105°2'21.20"E	Arid steppe	X. Zhao 1928

## Results

### Epidermal cell characters

Epidermal cell traits varied within a wide range. The shape varied from polygonal to irregular with straight arched, sinuolate, undulate, and sinuate wall patterns (Table 3; Figs 2–4). Polygonal cells with straight-arched walls were common in most taxa and were predominant in *O. racemosa*, *O. glabra*, *O. kansuensis*, *O. melanocalyx*, *O. taohensis*, *O. ochrocephala* (XLS), *O. ochrocephala* (HZ), *O. myriophylla*, *O. aciphylla*, *O. imbricata*, *O. xinglongshanica*, and *O. ciliata* (Figs 2–4). In turn, irregular sinuolate walls were predominant in *O. ciliata*, *O. muricata*, *O. ochrantha*,

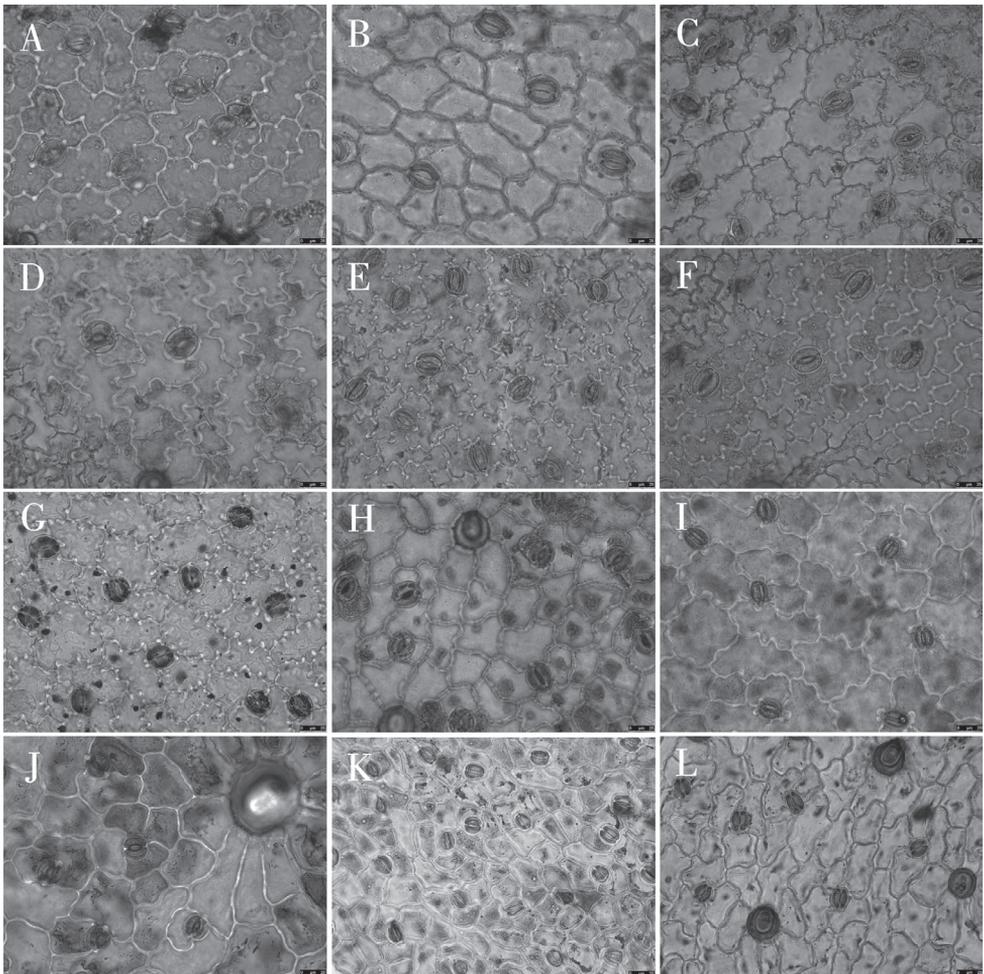
**Table 2.** Matrix of qualitative leaf epidermal characters of *Oxytropis* species.

Species	Adaxial epidermis						Abaxial epidermis						
	Shape of trichomes	Ornamentation of trichomes	Inner margin of stomatal rim	Waxy layer of epidermal cells	Shape of anticlinal walls	Pattern of trichomes	Shape of stomatal rim	Inner margin of stomatal rim	Waxy layer of epidermal cells	Ornamentation of stomatal rim	Inner margin of stomatal rim	Waxy layer of epidermal cells	Shape of anticlinal walls
<i>O. ciliata</i>	0	0	0	1	0	0	2	2	1	1	1	1	2
<i>O. muricata</i>	1	1	1	1	0	0	1	1	0	0	0	0	1
<i>O. falcata</i>	1	1	0	2	0	1	1	1	0	0	0	0	1
<i>O. ochrantha</i>	1	0	0	1	0	0	1	0	1	1	1	0	3
<i>O. bicolor</i>	1	1	1	1	0	0	1	1	1	1	1	0	3
<i>O. nemosa</i>	1	1	0	2	1	2	1	1	0	0	0	0	3
<i>O. myriophylla</i>	1	0	0	1	1	2	1	0	0	0	2	1	2
<i>O. aciphylla</i>	1	1	0	2	1	2	1	1	0	0	0	1	2
<i>O. tmbriata</i>	1	1	0	0	1	2	1	1	0	1	1	1	2
<i>O. coerulea</i>	1	1	0	3	0	0	2	0	0	0	0	0	1
<i>O. xinglongshanica</i>	1	1	0	2	1	2	1	1	0	2	2	1	2
<i>O. glabra</i>	1	1	1	0	1	2	1	1	0	0	0	0	3
<i>O. kansuensis</i>	1	1	0	0	1	2	1	1	0	0	0	0	3
<i>O. melanocalyx</i>	1	1	0	2	1	2	1	1	0	1	1	0	3
<i>O. naochensis</i>	1	1	0	2	1	2	1	1	0	0	0	0	3
<i>O. ochrocephala</i> (XLS)	1	1	0	2	1	2	1	1	0	1	1	0	1
<i>O. ochrocephala</i> (HZ)	1	1	0	2	1	2	1	1	0	1	1	0	1
<i>O. latibracteata</i>	1	1	1	1	0	3	1	1	0	1	1	0	1
<i>O. squamulosa</i>	2	2	0	0	0	3	2	2	0	0	0	0	1

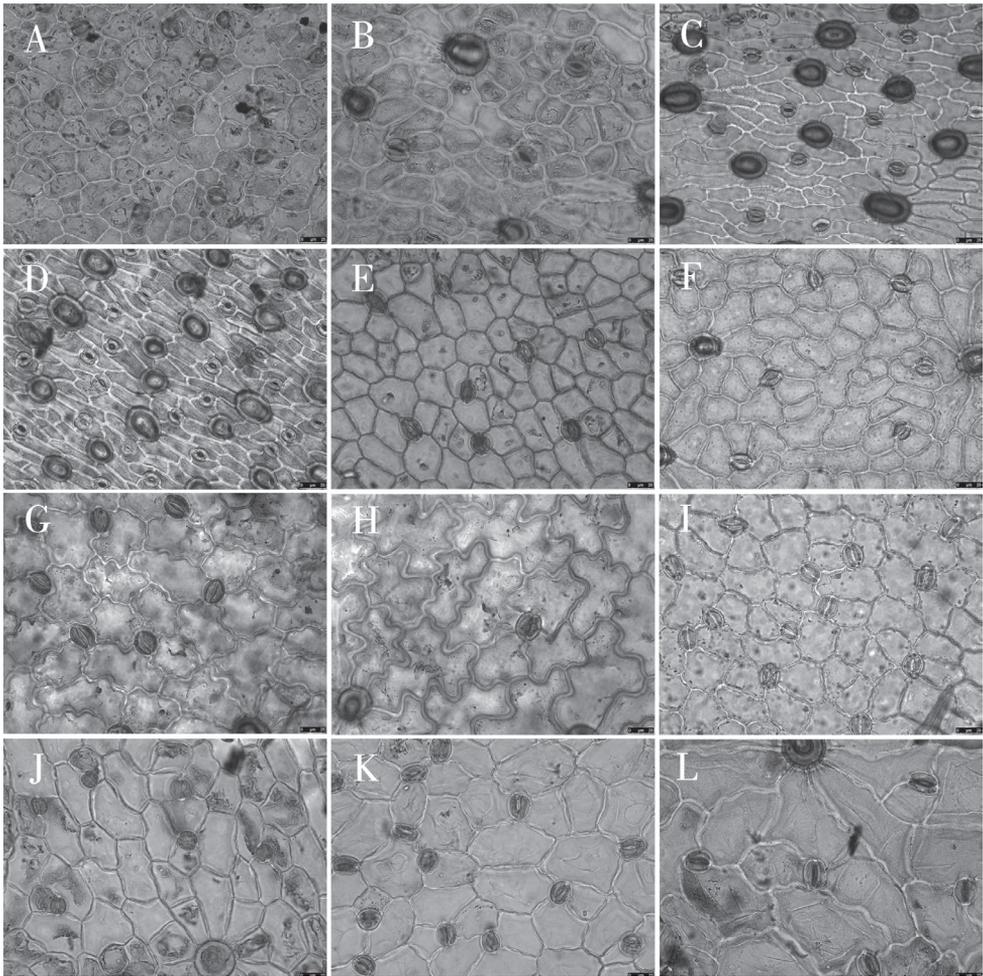
Note: Shape of trichomes: strip-like 0, cylindrical 1, absent 2; Ornamentation of trichomes: striation 0, striation and granular 1, absent 2; Inner margin of outer stomatal rim: undulate 0, smooth 1; Ornamentation of outer stomatal rim: granular 0, smooth 1, scale-like 2, banded sediment 3; Waxy layer of epidermal cells: granular 0, smooth 1, scale-like 2; Shape of cells: irregular 0, polygonal 1; Pattern of anticlinal walls: sinuate 0, sinuate 1, straight arched 2, Undulate 3.

*O. bicolor*, and *O. coerulea* (Figs 2, 3), and irregular undulate walls were predominant in *O. latibracteata*, *O. squammulosa*, *O. ochrantha*, *O. bicolor*, *O. racemosa*, *O. glabra*, *O. kansuensis*, *O. melanocalyx*, and *O. taochensis* (Figs 2–4). Lastly, irregular sinuate walls were predominant in *O. falcata*, *O. muricata*, *O. coerulea*, *O. ochrocephala* (XLS), *O. ochrocephala* (HZ), *O. latibracteata*, and *O. squammulosa* (Figs 2–4).

In addition, SEM analysis showed that, based on the shape, the waxy layer on epidermal cells could be separated into three groups (Table 4): a smooth waxy layer was found in epidermal cells of *O. ciliata*, *O. muricata*, *O. bicolor*, *O. myriophylla*, *O. glabra*, *O. ochrantha*, *O. ochrocephala* (XLS), *O. ochrocephala* (HZ), *O. imbricata*, *O. melanocalyx*, and *O. latibracteata* (Figs 5–9); a granular waxy layer was observed in



**Figure 2.** Light microscope photographs of epidermal cells in *Oxytropis* DC. **A, B** adaxial and abaxial epidermal cells of *O. ciliata* **C, D** adaxial and abaxial epidermal cells of *O. muricata* **E, F** adaxial and abaxial epidermal cells of *O. falcata* **G, H** adaxial and abaxial epidermal cells of *O. ochrantha* **I, J** adaxial and abaxial epidermal cells of *O. bicolor* **K, L** adaxial and abaxial epidermal cells of *O. racemosa*.

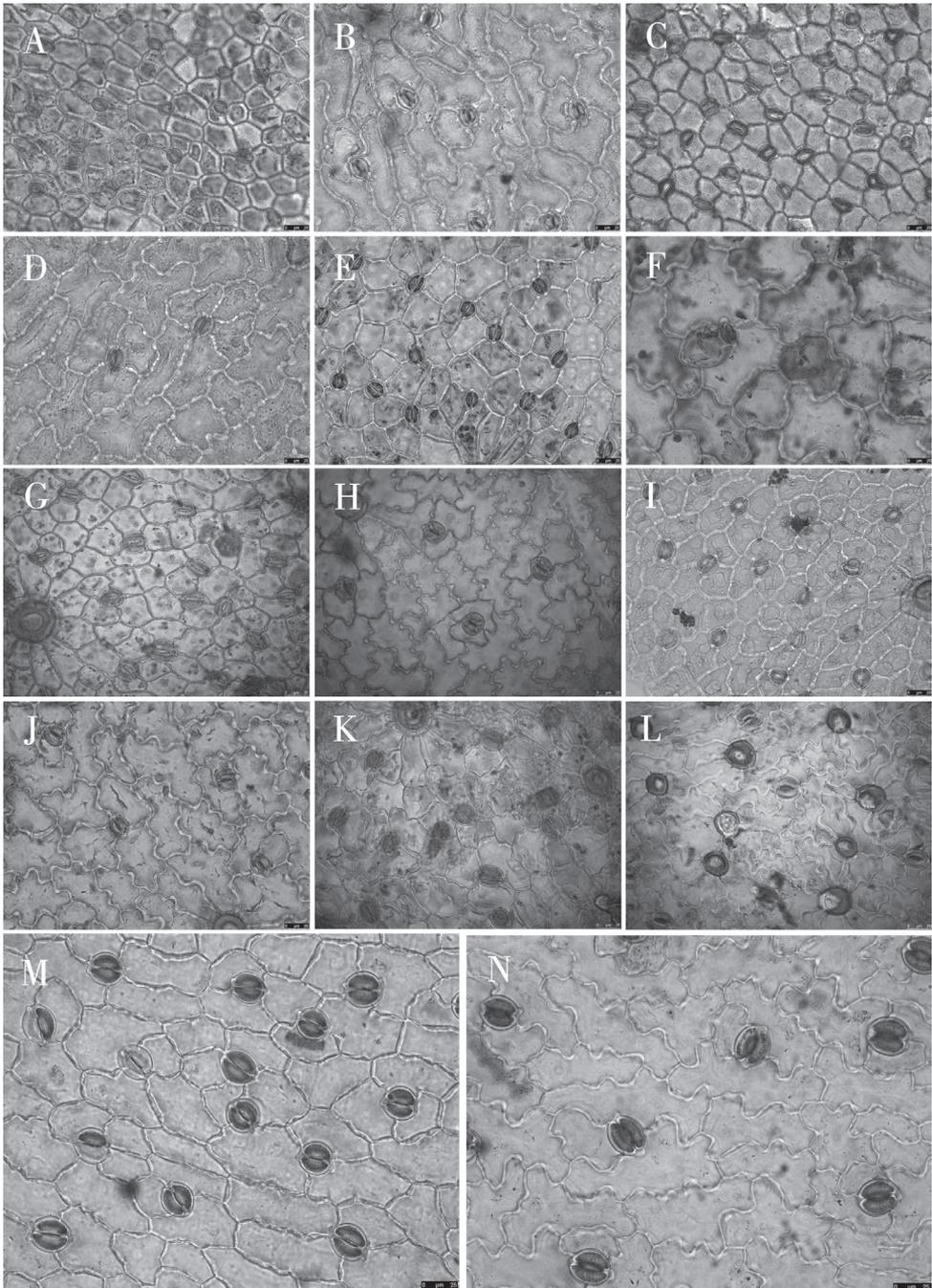


**Figure 3.** Light microscope photographs of epidermal cells in *Oxytropis* DC. **A, B** adaxial and abaxial epidermal cells of *O. myriophylla* **C, D** adaxial and abaxial epidermal cells of *O. aciphylla* **E, F** adaxial and abaxial epidermal cells of *O. imbricata* **G, H** adaxial and abaxial epidermal cells of *O. coerulea* **I, J** adaxial and abaxial epidermal cells of *O. xinglongshanica* **K, L** adaxial and abaxial epidermal cells of *O. glabra*.

those of *O. ochrantha*, *O. imbricata*, *O. kansuensis*, *O. racemosa*, *O. muricata*, *O. falcata*, *O. aciphylla*, *O. glabra*, *O. kansuensis*, *O. ochrocephala* (XLS), *O. ochrocephala* (HZ), and *O. squammulosa* (Figs 5–9), and finally, a scale-like waxy layer was observed in epidermal cells of *O. falcata*, *O. racemosa*, *O. aciphylla*, *O. xinglongshanica*, *O. melanocalyx*, *O. taohensis*, *O. ochrocephala* (XLS), and *O. ochrocephala* (HZ) (Figs 5–9).

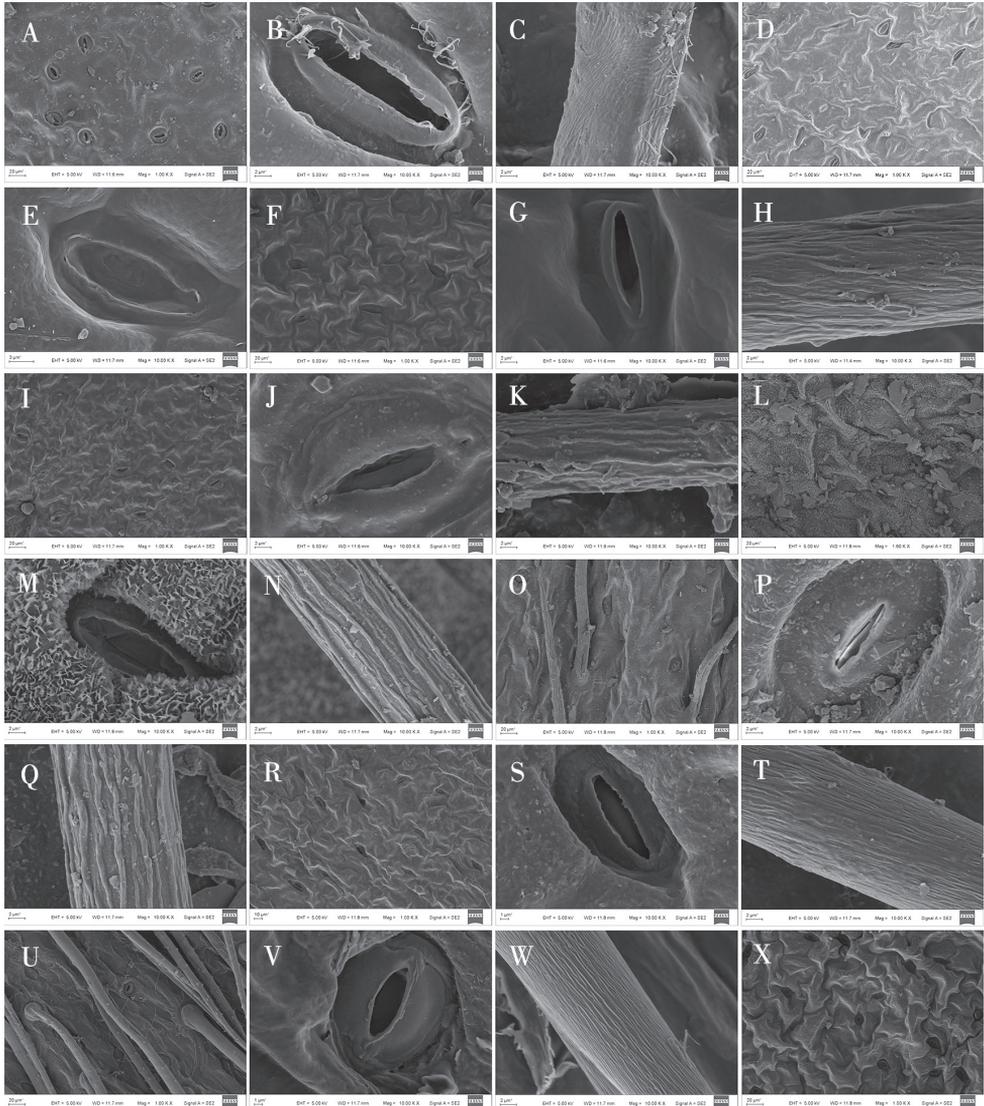
### Stomatal characters on the epidermis

With respect to stomata, all species of *Oxytropis* studied here were anomocytic, and stomatal index (SI) and stomatal density (SD) of the adaxial epidermis were greater than those of the abaxial epidermis (Table 3). In most of the examined species,



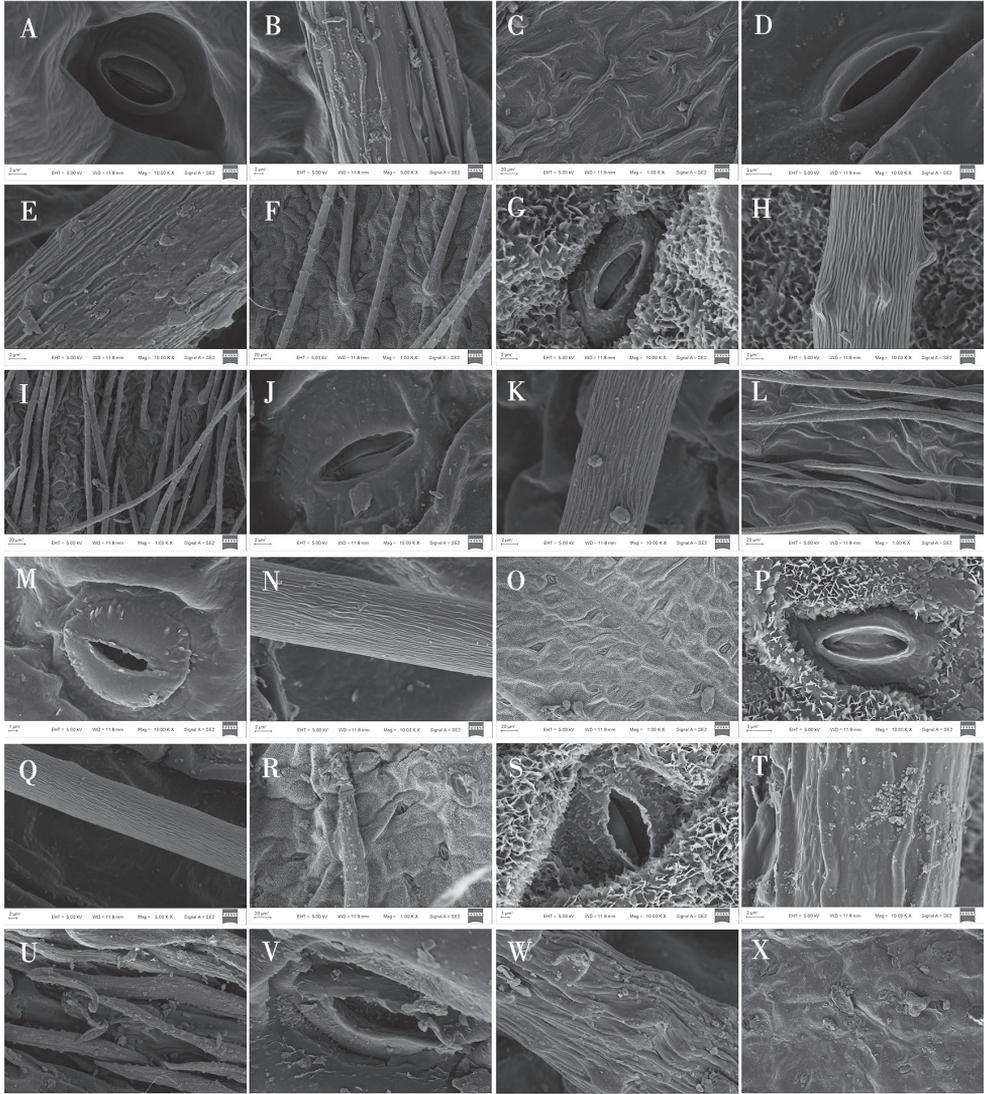
**Figure 4.** Light microscope photographs of epidermal cells in *Oxytropis* DC. **A, B** adaxial and abaxial epidermal cells of *O. kansuensis* **C, D** adaxial and abaxial epidermal cells of *O. melanocalyx* **E, F** adaxial and abaxial epidermal cells of *O. taobensis* **G, H** adaxial and abaxial epidermal cells of *O. ochrocephala* (XLS) **I, J** adaxial and abaxial epidermal cells of *O. ochrocephala* (HZ) **K, L** adaxial and abaxial epidermal cells of *O. latibracteata* **M, N** adaxial and abaxial epidermal cells of *O. squammulosa*.

remarkable variation was observed in stomatal size and number. Specifically, SD was lowest in *O. coerulea* (Table 3; Fig. 3) and largest in *O. melanocalyx* (Table 3; Fig. 4). Meanwhile, SI was highest (0.26) in *O. melanocalyx*, and lowest (0.003) on the abaxial surface of *O. coerulea* (Table 3; Figs 3, 4). Stomatal size was largest in *O. muricata* and smallest in *O. aciphylla* (Table 3; Figs 2, 3). As per SEM



**Figure 5.** Scanning electron microscope photographs of epidermal cells in *Oxytropis* DC. **A–C** adaxial epidermal cells of *O. ciliata* **D, E** abaxial epidermal cells of *O. ciliata* **F–H** adaxial epidermal cells of *O. muricata* **I–K** abaxial epidermal cells of *O. muricata* **L–N** adaxial epidermal cells of *O. falcata* **O–Q** abaxial epidermal cells of *O. falcata* **R–T** adaxial epidermal cells of *O. ochrantha*. **U–W** abaxial epidermal cells of *O. ochrantha* **X** adaxial epidermal cells of *O. bicolor*.

observation, the inner margin of the outer stomatal rim was either undulate or smooth (Table 4). Five species, including *O. ciliata*, *O. muricata*, *O. ochrantha*, *O. glabra*, and *O. latibracteata*, showed smooth and undulating inner margins of the outer stomatal ledge (Figs 5, 7, 9), while *O. bicolor* showed only a smooth inner margin of the outer stomatal ledge (Figs 5, 6). In contrast, the remaining species



**Figure 6.** Scanning electron microscope photographs of epidermal cells in *Oxytropis* DC. **A, B** adaxial epidermal cells of *O. bicolor* **C-E** abaxial epidermal cells of *O. bicolor* **F-H** adaxial epidermal cells of *O. racemosa* **I-K** abaxial epidermal cells of *O. racemosa* **L-N** adaxial epidermal cells of *O. myriophylla* **O-Q** abaxial epidermal cells of *O. myriophylla* **R-T** adaxial epidermal cells of *O. aciphylla* **U-W** abaxial epidermal cells of *O. aciphylla* **X** adaxial epidermal cells of *O. imbricata*.

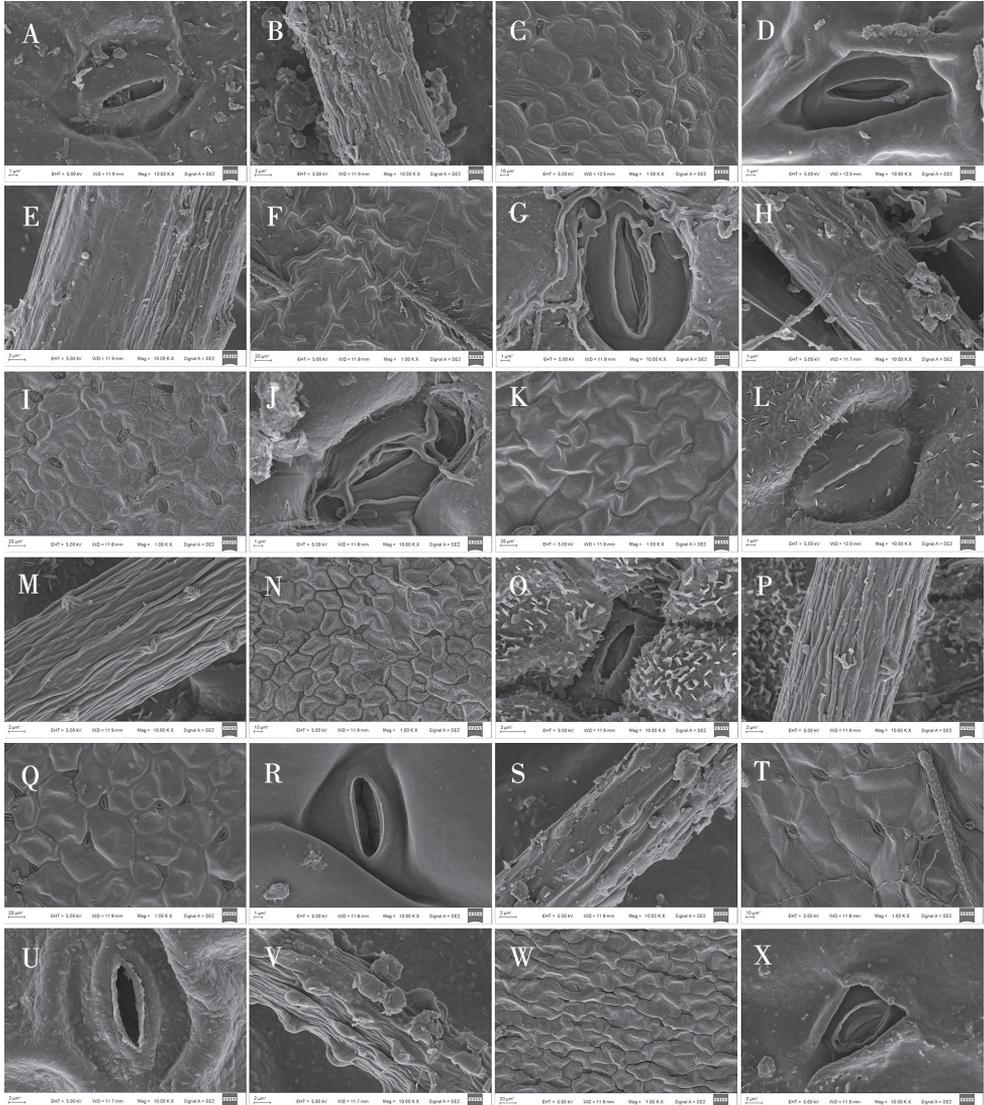
Table 3. Characteristics of the leaf epidermis of *Oxytropis* under light microscopy (surface view).

Species	Adaxial epidermis						Abaxial epidermis							
	Shape of cells	Pattern of anticlinal walls	Type of stomata	Mean stomatal density / (mm <sup>2</sup> )	Mean stomatal index / %	Mean stomatal size /mm <sup>2</sup>	Shape of cells	Pattern of anticlinal walls	Type of stomata	Mean stomatal density / (mm <sup>2</sup> )	Mean stomatal index / %	Mean stomatal size /mm <sup>2</sup>	Adaxial and abaxial SD ratio	Adaxial and abaxial SI ratio
<i>O. ciliata</i>	Irregular	Sinuolate	Anomocytic	131.77	0.21	639.84 (27.88×22.95)	Polygonal	Straight arched	Anomocytic	63.54	0.11	748.35 (30.2×24.78)	2.07	1.90
<i>O. muricata</i>	Irregular	Sinuolate	Anomocytic	129.92	0.17	647.79 (29.01×22.33)	Irregular	Sinuolate	Anomocytic	66.14	0.11	810.79 (31.61×25.65)	1.96	1.54
<i>O. filicata</i>	Irregular	Sinuolate	Anomocytic	170.87	0.17	571.99 (26.42×21.65)	Irregular	Sinuolate	Anomocytic	81.99	0.1	669.96 (28.94×23.15)	2.08	1.7
<i>O. ochrantha</i>	Irregular	Sinuolate	Anomocytic	156.4	0.19	511.08 (24.69×20.7)	Irregular	Undulate	Anomocytic	95.08	0.1	541.54 (25.69×21.08)	1.64	1.9
<i>O. bicolor</i>	Irregular	Sinuolate	Anomocytic	110.24	0.23	448.58 (24.58×18.25)	Irregular	Undulate	Anomocytic	77.17	0.16	497.51 (23.59×21.09)	1.42	1.43
<i>O. racemosa</i>	Polygonal	Straight arched	Anomocytic	292.82	0.18	312.63 (19.18×16.3)	Irregular	Undulate	Anomocytic	97.15	0.09	357.39 (21.7×16.47)	3.01	2
<i>O. myriophylla</i>	Polygonal	Straight arched	Anomocytic	250.79	0.15	410.40 (21.83×18.8)	Polygonal	Straight arched	Anomocytic	33.07	0.03	423.75 (23.82×17.79)	7.58	5
<i>O. aciphylla</i>	Polygonal	Straight arched	Anomocytic	369.29	0.16	253.77 (16.84×15.07)	Polygonal	Straight arched	Anomocytic	234.94	0.11	257.21 (17.45×14.74)	1.57	1.45
<i>O. imbricata</i>	Polygonal	Straight arched	Anomocytic	139.17	0.14	409.05 (22.7×18.02)	Polygonal	Straight arched	Anomocytic	81.3	0.11	372.01 (21.96×16.94)	1.71	1.27
<i>O. coerulesca</i>	Irregular	Sinuolate	Anomocytic	152.95	0.21	526.83 (25.28×20.84)	Irregular	Sinuolate	Anomocytic	0.69	0.0031	514.8 (26.4×19.5)	221.66	67.74
<i>O. xinglongshanica</i>	Polygonal	Straight arched	Anomocytic	209.45	0.19	403.65 (21.89×18.44)	Polygonal	Straight arched	Anomocytic	67.18	0.09	389.68 (21.03×18.53)	3.11	2.11
<i>O. glabra</i>	Polygonal	Straight arched	Anomocytic	173.62	0.25	442.83 (24.08×18.39)	Irregular	Undulate	Anomocytic	92.32	0.21	517.17 (26.95×19.19)	1.88	1.19
<i>O. kansuensis</i>	Polygonal	Straight arched	Anomocytic	412.7	0.22	251.78 (17.87×14.09)	Irregular	Undulate	Anomocytic	63.39	0.13	389.15 (22.25×17.49)	6.51	1.69
<i>O. melanocalyx</i>	Polygonal	Straight arched	Anomocytic	383.53	0.26	376.79 (21.73×17.34)	Irregular	Undulate	Anomocytic	39.96	0.09	368.32 (22.68×16.24)	9.59	2.88
<i>O. taochensis</i>	Polygonal	Straight arched	Anomocytic	202.56	0.21	418.08 (23.37×17.89)	Irregular	Undulate	Anomocytic	36.99	0.12	373.49 (21.88×17.07)	5.47	1.75

Species	Adaxial epidermis						Abaxial epidermis							
	Shape of cells	Pattern of anticlinal walls	Type of stomata	Mean stomatal density / (mm <sup>2</sup> )	Mean stomatal index /%	Mean stomatal size /mm <sup>2</sup>	Shape of cells	Pattern of anticlinal walls	Type of stomata	Mean stomatal density / (mm <sup>2</sup> )	Mean stomatal index /%	Mean stomatal size /mm <sup>2</sup>	Adaxial and abaxial SD ratio	Adaxial and abaxial SI ratio
<i>O. ochrocephala</i> (XLS)	Polygonal	Straight arched	Anomocytic	265.95	0.2	388.29 (21.56×18.01)	Irregular	Sinuate	Anomocytic	58.25	0.11	418.50 (22.72×18.42)	4.56	1.81
<i>O. ochrocephala</i> (HZ)	Polygonal	Straight arched	Anomocytic	289.37	0.21	419.94 (22.91×18.33)	Irregular	Sinuate	Anomocytic	57.87	0.11	448.21 (23.64×18.96)	5.0003	1.90
<i>O. latibaccata</i>	Irregular	Undulate	Anomocytic	147.64	0.16	485.93 (24.53×19.81)	Irregular	Sinuate	Anomocytic	93.21	0.1	544.02 (26.03×20.9)	1.58	1.6
<i>O. squamulosa</i>	Irregular	Undulate	Anomocytic	226.67	0.22	465.37 (22.58×20.61)	Irregular	Sinuate	Anomocytic	99.9	0.15	542.38 (25.84×20.99)	2.26	1.46

Note: XLS (Xinglongshan population); HZ (Hezuo population)

had an undulate inner margin of the outer stomatal ledge. Ornamentation of the outer stomatal ledge was smooth or granular in most species under this study. *O. coerulea* was a notable exception with a banded sediment ornamentation of the outer stomatal ledge (Fig. 7).

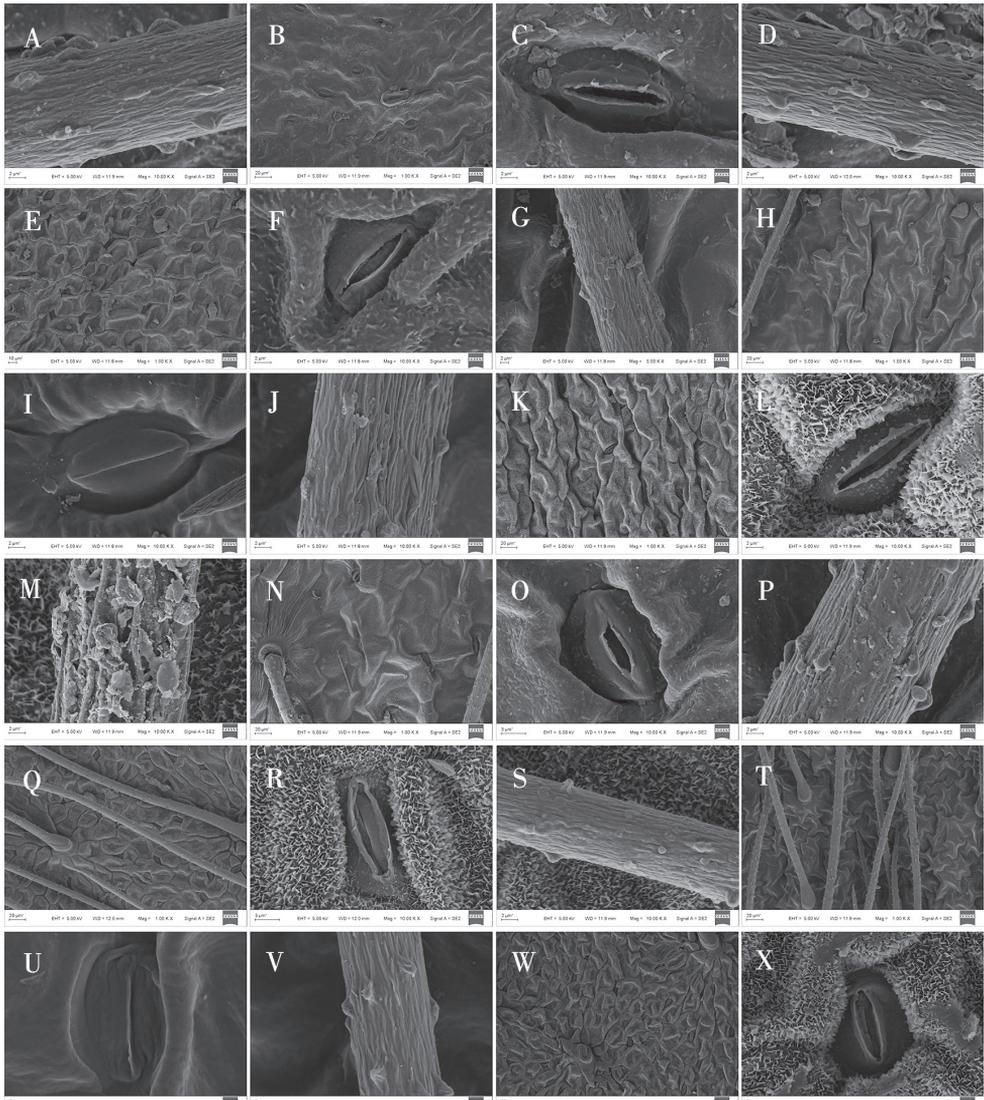


**Figure 7.** Scanning electron microscope photographs of epidermal cells in *Oxytropis* DC. **A, B** adaxial epidermal cells of *O. imbricata* **C–E** abaxial epidermal cells of *O. imbricata* **F–H** adaxial epidermal cells of *O. coerulea* **I, J** abaxial epidermal cells of *O. coerulea* **K–M** adaxial epidermal cells of *O. xinglongshanica* **N–P** abaxial epidermal cells of *O. xinglongshanica* **Q–S** adaxial epidermal cells of *O. glabra* **T–V** abaxial epidermal cells of *O. glabra* **W–X** adaxial epidermal cells of *O. kansuensis*.

**Table 4.** Characteristics of the leaf epidermis of *Oxytropis* under scanning electron microscopy.

Species	Adaxial epidermis				Abaxial epidermis					
	Shape of trichomes	Ornamentation of trichomes	Inner margin of outer stomatal ledge	Ornamentation of outer stomatal ledge	Waxy layer of epidermal cells	Shape of trichomes	Ornamentation of trichomes	Inner margin of outer stomatal ledge	Ornamentation of outer stomatal ledge	Waxy layer of epidermal cells
<i>O. elitata</i>	strip-like	striation	undulate	smooth	smooth	absent	absent	smooth	smooth	smooth
<i>O. muricata</i>	cylindrical	striation with granular	smooth	smooth	smooth	cylindrical	striation with granular	undulate	granular	granular
<i>O. falcata</i>	cylindrical	striation with granular	undulate	granular	granular	cylindrical	striation with granular	undulate	granular	granular
<i>O. ochrantha</i>	cylindrical	striation	undulate	smooth	smooth	cylindrical	striation	smooth	smooth	smooth
<i>O. bicolor</i>	cylindrical	striation with granular	smooth	smooth	smooth	cylindrical	striation with granular	smooth	smooth	smooth
<i>O. nucemosa</i>	cylindrical	striation with granular	undulate	granular	granular	cylindrical	striation with granular	undulate	granular	granular
<i>O. myriophylla</i>	cylindrical	striation	undulate	granular	granular	cylindrical	striation	undulate	granular	scale-like
<i>O. aciphylla</i>	cylindrical	striation with granular	undulate	scale-like	granular	cylindrical	striation with granular	undulate	granular	granular
<i>O. tibricata</i>	cylindrical	striation with granular	undulate	granular	granular	cylindrical	striation with granular	undulate	granular	granular
<i>O. everulea</i>	cylindrical	striation with granular	undulate	banded sediment	banded sediment	absent	absent	undulate	banded sediment	smooth
<i>O. xinglongshanica</i>	cylindrical	striation with granular	undulate	scale-like	scale-like	cylindrical	striation with granular	undulate	scale-like	granular
<i>O. glabra</i>	cylindrical	striation with granular	smooth	smooth	smooth	cylindrical	striation with granular	undulate	granular	granular
<i>O. kansuensis</i>	cylindrical	striation with granular	undulate	granular	granular	cylindrical	striation with granular	undulate	granular	granular
<i>O. melanoclype</i>	cylindrical	striation with granular	undulate	scale-like	scale-like	cylindrical	striation with granular	undulate	granular	smooth
<i>O. taoshensis</i>	cylindrical	striation with granular	undulate	scale-like	scale-like	cylindrical	striation with granular	undulate	granular	granular
<i>O. ochrocephala</i> (XLS)	cylindrical	striation with granular	undulate	scale-like	scale-like	cylindrical	striation with granular	undulate	smooth	smooth
<i>O. ochrocephala</i> (HZ)	cylindrical	striation with granular	undulate	scale-like	scale-like	cylindrical	striation with granular	undulate	smooth	smooth
<i>O. latibracteata</i>	cylindrical	striation with granular	smooth	smooth	smooth	cylindrical	striation with granular	undulate	smooth	smooth
<i>O. squamulosa</i>	absent	absent	undulate	granular	granular	absent	absent	undulate	granular	granular

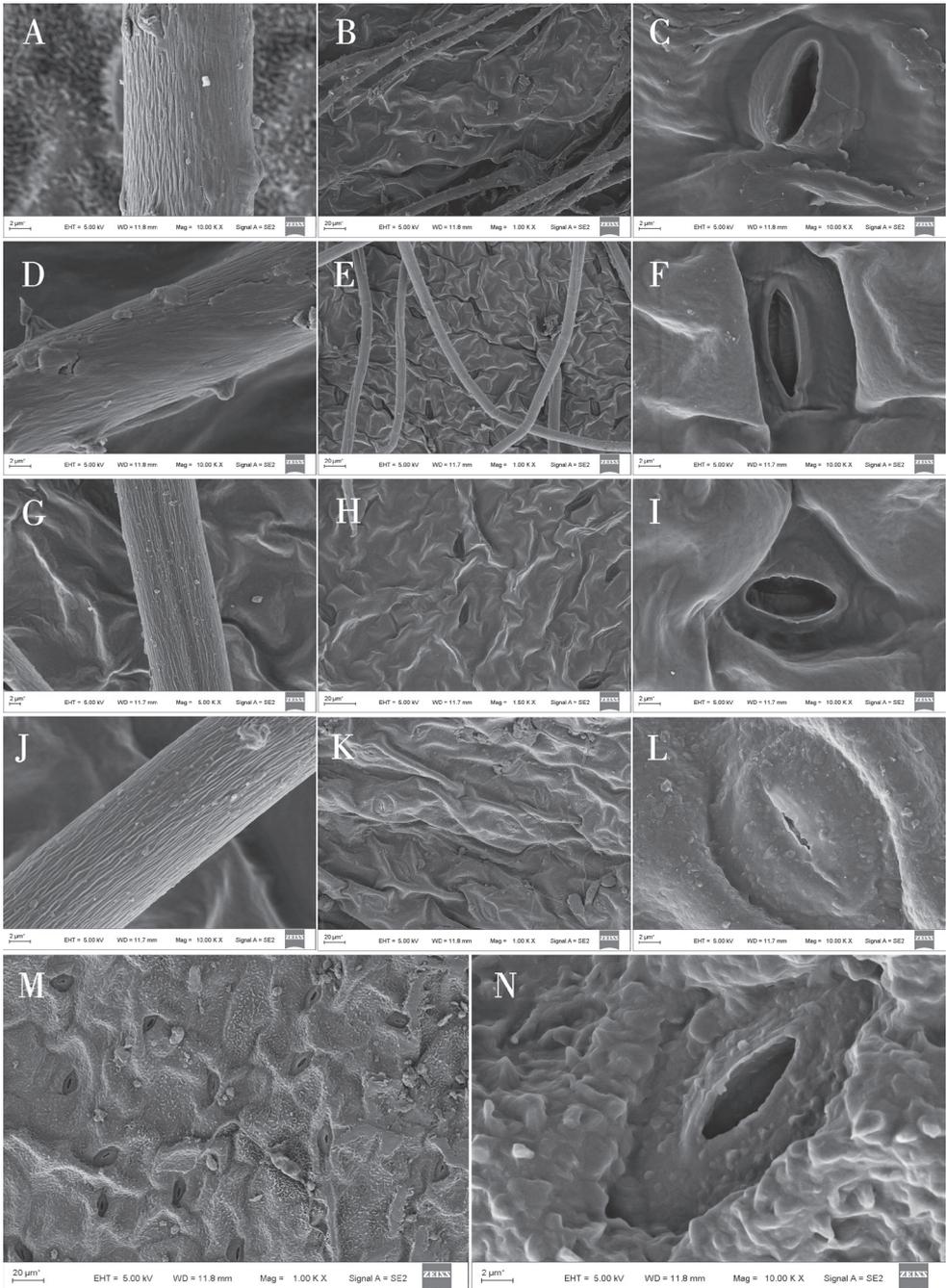
Note: XLS (Xinglongshan population); HZ (Hezuo population).



**Figure 8.** Scanning electron microscope photographs of epidermal cells in *Oxytropis* DC. **A** adaxial epidermal cells of *O. kansuensis* **B–D** abaxial epidermal cells of *O. kansuensis* **E–G** adaxial epidermal cells of *O. melanocalyx* **H–J** abaxial epidermal cells of *O. melanocalyx* **K–M** adaxial epidermal cells of *O. taochensis* **N–P** abaxial epidermal cells of *O. taochensis* **Q–S** adaxial epidermal cells of *O. ochrocephala* (HZ) **T–V** abaxial epidermal cells of *O. ochrocephala* (HZ) **W–X** adaxial epidermal cells of *O. ochrocephala* (XLS).

### Trichome characters on the epidermis

Most of the species observed showed trichomes, except for *O. squammulosa* (Table 4; Fig. 9). Two trichome shapes were identified in this genus. Strip-like trichomes, that were present only in *O. ciliata* (Table 4; Fig. 5), and cylindrical trichomes, that



**Figure 9.** Scanning electron microscope photographs of epidermal cells in *Oxytropis* DC. **A** adaxial epidermal cells of *O. ochrocephala* (XLS) **B–D** abaxial epidermal cells of *O. ochrocephala* (XLS) **E–G** adaxial epidermal cells of *O. latibracteata* **H–J** abaxial epidermal cells of *O. latibracteata* **K–L** adaxial epidermal cells of *O. squammulosa* **M–N** abaxial epidermal cells of *O. squammulosa*.

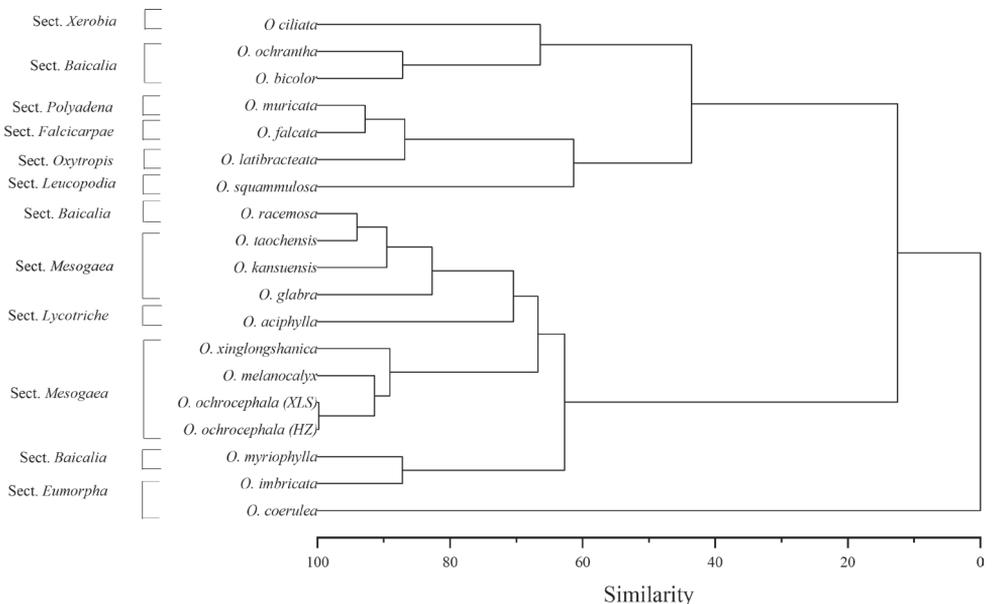
were present in all other species (Table 4). Trichrome ornamentation of *O. ochrantha*, *O. ciliata*, and *O. myriophylla* was striate (Table 4; Figs 5, 6), while the remaining species were striate and granular (Table 4).

### Cluster analysis

Cluster analysis reflects the similarity among species based on anatomical characteristics and delimitation of these groups. The phenograms of the quantitative and qualitative data provided four principal clusters (Fig. 10). In the first cluster, *O. ciliata*, *O. ochrantha*, and *O. bicolor* were closely related; in turn, the second cluster included four taxa, *O. falcata*, *O. muricata*, *O. latibracteata*, and *O. squamulosa*. The third cluster comprised *O. racemosa*, *O. glabra*, *O. kansuensis*, *O. aciphylla*, *O. melanocalyx*, *O. taochensis*, *O. ochrocephala* (XLS), *O. ochrocephala* (HZ), *O. xinglongshanica*, *O. myriophylla*, and *O. imbricata*. Lastly, the fourth cluster contained only *O. coerulea*, which was characterized by banded sediments in the outer stomatal ledge.

### Discussion

Leaf characteristics, such as epidermal micro- and macro-hairs, and stomata, are important for the classification of many genera (Dickison 2000; Yang and Lin 2005; Kadiri and Muellner-Riehl 2021). Previous studies have shown that the anatomical features of the leaf epidermis, such as the shape and anticlinal walls of epidermal



**Figure 10.** The dendrogram of *Oxytropis* DC. based on the leaf epidermal characteristics.

cells, are taxonomically significant and can therefore be used for the classification of taxa at the genus or even at the species level (Barthlott et al. 1998; Wissemann 2000; Tomaszewski and Zieliński 2014; Tomaszewski et al. 2019). In this study, there were two main types of leaf epidermal cells: polygonal and irregular; and four different types of pattern of anticlinal walls: straight-arched, sinuolate, undulate, and sinuate. It has been proposed that the pattern of the anticlinal wall may be influenced by habitat; specifically, species in dry environments tend to have a straight arched anticlinal wall, whereas those in humid areas tend to have undulating to sinuous anticlinal walls (Stace 1965; Gifford and Foster 1989). However, in this study, *O. muricata*, *O. falcata*, *O. ochrantha*, *O. bicolor*, and *O. squammulosa* specimens growing in an arid environment exhibited undulate to sinuous anticlinal walls, whereas *O. taochensis* and *O. ochrocephala* specimens found in humid environments exhibited straight arched anticlinal walls. Therefore, our results do not support the aforementioned hypothesis. A similar phenomenon was observed in the study of leaf epidermal traits in Piperales (Song et al. 2020). Furthermore, the shape and anticlinal walls of epidermal cells in *O. ochrocephala* were highly consistent in different populations, indicating that the shape of epidermal cells and the pattern of anticlinal walls were constant within species. Notably, *O. ochrocephala* and *O. kansuensis* are two species easily confused within *Oxytropis*, as they are morphologically difficult to distinguish and they are both abundant in the Qinghai-Tibetan Plateau region (Zhu et al. 2010). However, according to our observations, these two species can be distinguished based on their wall pattern: *O. ochrocephala* has a sinuate anticlinal wall pattern, whereas *O. kansuensis* has an undulating wall pattern. Thus, anticlinal wall pattern might be considered as a useful taxonomic marker for some *Oxytropis* species. However, similar epidermal cell shapes and anticlinal wall patterns exist in other species of the genus *Oxytropis* and other groups of Fabaceae (Zou et al. 2008; Ren et al. 2007). Therefore, epidermal cell shape and anticlinal wall patterns need to be considered in combination with other macro-morphological features classifying the species within the genus *Oxytropis*.

Studies on stomata can have great taxonomic significance for the delimitation of different levels of taxa (Kothari and Shah 1975). Carpenter and Smith (1975) showed that variability in stomatal frequency is taxonomically important at the genus level, whereas Carlquist (1961) emphasized the contribution of stomatal size variation to delimiting species within a genus. In *Oxytropis*, a wide range of variability was observed for stomatal quantitative parameters, such as stomatal density, size, and index. Our results indicated that the quantitative stomatal traits have limited taxonomic value, as they are strongly influenced by environmental factors, such as CO<sub>2</sub> levels and light intensity (Metcalf and Chalk 1950; Royer 2001; Rossatto and Kolb 2010). However, stomatal distribution and types are considered an important taxonomic criterion for taxonomic value, especially at higher taxa (Metcalf and Chalk 1950; Patil and Patil 1987). Thus, for example, we found that the anomocytic stomata type is a common feature in *Oxytropis* that may be used to elaborate the phylogenetic relationships among genera, in combination with stomatal data from other genera. These findings support the concept that genus *Oxytropis* is a monophyletic group (Zhu and Ohashi 2000).

Further, trichomes and their characteristics provide important information for plant identification. The type of indumentum and its presence or absence may serve as diagnostic features for species or genus recognition, as has been recognized in some groups such as Asteraceae (Adedeji and Jewoola 2008; Krak and Mráz 2008), Brassicaceae (Beilstein et al. 2006), Fabaceae (Chukwuma et al. 2014), and Lamiaceae (Eiji and Salmaki 2016). In addition, large plant taxa often share a common pattern of trichome structure. For example, chandelier-shaped trichomes with branches of whorls are characteristic of Platanaceae (Carpenter et al. 2005); peltate or scale-like hairs are typical of Eleagnaceae (Mishra 2009), and three-celled uniseriate hairs are common in Proteaceae (Johnson and Briggs 1975). In the genus *Oxytropis*, the trichome type of the investigated species was simple hair. This is consistent with the results of previous studies on *Oxytropis* (Karaman et al. 2009; Lu 2011). Furthermore, we found that *O. ciliata*, belonging to Section *Xerobia* (Zhu et al. 2010), has strip-like trichomes (margin ciliates) that distinguish this species from other species in this study. The trichome ornamentation of most *Oxytropis* species was consistent, indicating that trichome ornamentation appears to be of a low taxonomic value for distinguishing sections and species. However, owing to sample size limitations, the systematic significance of *Oxytropis* trichomes needs to be based on a more comprehensive sampling.

In this study, six species, including *O. glabra*, *O. kansuensis*, *O. melanocalyx*, *O. taochensis*, *O. ochrocephala*, and *O. xinglongshanica*, all belonging to section *Mesogaea*, clustered together. Our results of cluster analysis are largely consistent with that of the classification of species and sections based on macro morphological data (Zhu et al. 2010), indicating that leaf epidermal micro characteristics might be valuable in understanding systematics of genera at the section level. Bunge (1874) established the section *Gobicola* in 1874, which contained only *O. racemosa*. This treatment was recognized by the FRPS, but section *Gobicola* was merged into section *Baicalia* in Flora IntraMongolica and FOC (Fu 1989; Zhang 1998; Zhu et al. 2010). However, our results do not support the interpretation of Flora IntraMongolica and FOC. In this study, *O. racemosa* and some species of the section *Mesogaea*, such as *O. glabra* and *O. kansuensis*, clustered together into one clade, indicating that the systematic position of *O. racemosa* needs to be reconsidered (Fig. 10). In addition, based on the results of quantitative taxonomy, Wang (2005) advocated that section *Leucopodia*, which only contains *O. squammulosa*, should be merged with section *Xerobia*. Our results clearly do not support this treatment, because *O. squammulosa* did not cluster together with *O. ciliata* in section *Xerobia* (Fig. 10). Moreover, different populations of *O. ochrocephala* clustered together into one group, which demonstrates that leaf epidermal traits are useful for the identification of taxa at the species level. Therefore, foliar epidermis traits of *Oxytropis* can be used as taxonomic markers for identification at the infrageneric classification level.

There is no comprehensive phylogenetic study on the genus *Oxytropis*. Furthermore, although several studies have applied DNA barcodes such as ITS, trnL-F, and psbA-trnH to explore the molecular phylogeny of *Oxytropis* in Northwestern China, the low genetic divergence of the above barcodes among the species makes it difficult to distinguish species within the genus as well as to resolve phylogenetic relationships

between sections (Li et al. 2011; Gao et al. 2013; Lu et al. 2014). Therefore, the reliability of epidermis characters in terms of phylogeny cannot be affirmed. More detailed molecular phylogenetic studies with a broader taxon sampling are required to find correlations between epidermis characteristics and classification of the genus.

## Conclusions

Our results suggest that leaf epidermis can be used as potential taxonomic markers for infrageneric classification of *Oxytropis*. The shape of epidermal cells and the pattern of the anticlinal wall were constant within species, and are useful for species delimitation in the genus *Oxytropis* when combined with other macroscopic traits. Trichome shapes can be useful characteristics to distinguish *O. ciliata* from other investigated species. Although quantitative stomatal characteristics were not effective diagnostic characteristics because of the considerable variation within the same taxa, it nevertheless plays an important role in cluster analysis. Results of cluster analysis are largely consistent with the classification of species and sections based on macro morphological data, indicating that foliar epidermis characteristics of *Oxytropis* can be used as taxonomic identification markers infrageneric classification level. Lastly, our results support the delineation of the sect. *Leucopodia* as an independent section, while not supporting the treatment of merging the sect. *Gobicola* into the sect. *Baicalia*.

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# First record of functional underground traps in a pitcher plant: *Nepenthes pudica* (Nepenthaceae), a new species from North Kalimantan, Borneo

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## Abstract

*Nepenthes pudica*, a new species from North Kalimantan, Indonesia, is described and illustrated. The species belongs to the *N. hirsuta* group (sensu Cheek and Jebb 1999) but exhibits some characters that are unique within the group or even within the genus. Above all, it produces underground, achlorophyllous shoots with well-developed, ventricose lower pitchers that form in soil cavities or directly in the soil. No lower pitchers are formed above ground. The main part of its prey are ants, besides other litter- and soil-inhabiting species of invertebrates. A number of infaunal species were found in both aerial and underground pitchers, mainly Diptera and nematodes. *Nepenthes pudica* is known only from a few neighbouring localities in the Mentarang Hulu district of North Kalimantan, where it grows on ridgetops at an elevation of 1100–1300 m. Its discovery underlines the natural richness of Borneo's rainforest and the necessity to preserve this important ecosystem with its enormous and still undiscovered biodiversity.

## Keywords

Borneo, carnivorous plant, Caryophyllales, Mentarang Hulu, prey composition, taxonomy, underground trap

## Introduction

*Nepenthes* L. is a genus of more than 160 species (Golos et al. 2020) primarily distributed in tropical and subtropical Southeast Asia, with centres of diversity in Borneo, Sumatra, and the Philippines. A small number of species occur in outlying areas, including Madagascar, Seychelles, Sri Lanka, northeastern India, southern China, northeastern Australia, and various islands of the western Pacific Ocean (McPherson et al. 2009). The *Nepenthes* flora of Borneo, with around 40 recognised species, is one of the most species-rich of all. Although the island is still partially covered with extensive primary forest, its area has been rapidly decreasing in recent decades (Miettinen et al. 2011). Commercial logging and subsequent land conversion (mostly for oil palm plantations) drastically reduced the area of pristine old-growth forest from 55.8 Mha in 1973 to 20.6 Mha in 2015 (Gaveau et al. 2016), making the Borneo rainforest one of the most rapidly vanishing ecosystems in the world. The island is botanically relatively well explored in the northern part, i.e. Malaysian Borneo (Sarawak and Sabah) and Brunei, where only remnants of untouched rainforest exist, usually protected as national parks and reserves. In contrast, Indonesian Borneo (Kalimantan) is one of the world's least explored and most threatened biodiversity hotspots, still with vast areas of relatively intact forest (Raes et al. 2009). However, besides the expansion of oil palm plantations, the announced establishment of the new capital of Indonesia, Nusantara, in East Kalimantan might have a serious impact on the vulnerable biota of Borneo (e.g. Teo et al. 2020). The *Nepenthes* flora of Kalimantan is poorly known compared to that of Malaysian and Bruneian Borneo, with relatively few modern records. Thus, the new discoveries that have emerged recently after expeditions to certain remote areas of Kalimantan (Robinson et al. 2019; Golos et al. 2020) are not surprising.

Here we describe a new species of *Nepenthes* from lower montane rainforest in North Kalimantan, Indonesia, which produces well-developed, fully functional and effective underground traps – a strategy as yet unknown in any species of carnivorous plant with pitfall traps. While the majority of carnivorous plants produce their traps above ground or in water, underground traps have up till now been recorded only in the genera *Genlisea* Benth. & Hook.f., *Philcoxia* P.Taylor & V.C.Souza and *Utricularia* L. These genera use three different trapping mechanisms. While *Utricularia* employs actively working sucking utricles (i.e. Poppinga et al. 2016), *Genlisea* employs passive ‘lobster-pot’ type traps (Taylor 1991; Płachno et al. 2008). The adhesive leaves of *Philcoxia* are shallowly buried in sand to receive just enough light to maintain their photosynthetic ability (Pereira et al. 2012). On the other hand, pitfall traps (i.e. traps that rely on gravity) produced from wholly subterranean shoots that have evolved specifically to function underground have not been recorded in carnivorous plants so far (see, e.g. Darnowski et al. 2018).

## Materials and methods

This study is based on plants found in February 2012 in the Mentarang Hulu district of North Kalimantan province, Indonesia. A total of 17 plants were examined across five different sites. Plants were photographed, sampled and subsequently thoroughly

compared with original drawings and descriptions given in protologues of morphologically allied *Nepenthes* species. Specimens of the *Nepenthes hirsuta* group were examined in the herbaria BO, K and L (see Suppl. material 1) and the type material was deposited in BO (herbarium codes according to Thiers 2022).

For scanning electron microscopy (SEM), the representative trap parts were fixed in ethanol and later dehydrated and subjected to critical-point drying using liquid CO<sub>2</sub>. They were then sputter-coated with gold and examined at an accelerating voltage of 20 kV using a Hitachi S-4700 SEM (Hitachi, Tokyo, Japan), which is housed in the Institute of Geological Sciences, Jagiellonian University in Kraków.

Material for prey investigation was sampled from both underground (tree-root cavities) and aboveground pitchers. The entire contents of five lower pitchers and one aerial rosette pitcher was poured out through a 25 µm sieve, immediately fixed in 4% formaldehyde at circa 80 °C, and stored for 14–21 days, before insects and acarids including also larvae were separated and fixed again. The fine content including nematodes, annelids and organic detritus was transferred into glycerine according to De Grisse (1969) and finally mounted onto wax-glycerine slides and examined. Fixed specimens were identified under a light microscope and documented. All individuals that showed signs of digestion were considered prey. Individuals without signs of digestion were identified and assessed as either prey or infauna based on their biology and present life stages (e.g. larvae were mostly considered infauna). All insect and mite preparations are deposited at the Department of Entomology of Moravian Museum Brno. All the nematodes are deposited in the Department of Forest Protection and Wildlife Management in Brno. Permanent slides of *Pristina armata* (Naididae) are deposited at the National Museum in Prague, Czech Republic (Schenková and Čermák 2013).

## Taxonomic treatment

### *Nepenthes pudica* Dančák & Majeský, sp. nov.

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Figs 1–5

**Diagnosis.** *Nepenthes pudica* differs from *N. hispida* Beck in producing short basal underground (vs. aboveground) shoots; ± glabrous (vs. hairy) stems; petiolate (vs. sessile) climbing shoot leaves with auriculate, shortly decurrent (vs. decurrent-amplexicaul) bases; rare (vs. common) upper pitchers; red (vs. green or red blotched) lower pitchers; ± glabrous (vs. hairy) mature pitchers; ventricose (vs. ovoid-ellipsoid) lower pitchers; infundibular (vs. subcylindrical, tapering) upper half of the lower pitcher; 3–5.5 cm (vs. 1.5–3 cm) wide lower pitchers; male flowers in pairs (vs. single or rarely in pairs) and androphore c. 4 mm (vs. 1.5–2 mm) long.

**Type.** INDONESIA. North Kalimantan: Malinau Regency, c. 1110 m a.s.l., 2 February 2012, W. Tjiasmanto, M. Paris & M. Dančák s.n. (BO, holotype BO1985840, isotype BO1985839).



**Figure 1.** *Nepenthes pudica* **A** juvenile rosette pitcher **B** upper pitchers (4 on the right; each from a different plant), intermediate pitcher (1 on the left) **C** habitat with mature plant **D** habitat with lower pitchers excavated from the soil. Photographs by M. Dančák.

**Description.** Terrestrial climber producing climbing shoot and underground basal shoots. *Climbing shoots* up to c. 20 m long, stem glabrous, c. 4–6 mm thick, internodes c. 4 cm long. *Underground basal shoots* short, with reduced, partially or completely achlorophyllous leaves (nanophylls) bearing well-developed lower pitchers,



**Figure 2.** *Nepenthes pudica* **A** detail of lower pitchers excavated from the soil **B** lower pitchers in a cavity under tree roots—note greening of phyllodia formed in presence of low light **C** lower pitchers revealed under a moss mat **D** lower pitchers extracted from a cavity—note achlorophyllous shoot and reduced phyllodia formed in total darkness. Photographs by M. Dančák.

not observed to branch or develop roots. *Rosette leaves* chartaceous, sessile to shortly petiolate, oblanceolate, up to 16 cm long, up to 4 cm wide, apex subobtusate or acute to acuminate, base auriculate, shortly decurrent, glabrous on both sides

but densely hairy with short brown hairs on the margins, tendril up to 16 cm long, uncoiled. **Leaves of climbing shoots** coriaceous, shortly petiolate, oblanceolate, up to 20 cm long, up to 4.5 cm wide, with 2–4 inconspicuous longitudinal veins on each side of the midrib, apex acute, base auriculate, shortly decurrent, glabrous on both sides, margins glabrous, tendril coiling. **Rosette pitchers** produced only briefly on aboveground rosettes, up to 9 cm high, up to 3 cm wide, thin-chartaceous, subcylindrical to ovoid in the lower part. **Lower pitchers** produced exclusively on underground basal shoots, 7–11 cm high, 3–5.5 cm wide, thin-coriaceous, becoming thicker-walled and markedly sturdier when produced at depth, arising abruptly from the uncoiled tendril, ventricose, broadly ovoid to globose in the lower half, infundibular above, clearly widening towards the mouth; eglandular zone of the inner surfaces extending from the mouth almost to the middle of the pitcher; inner surface near the mouth white, conspicuously red blotched, outer surface red-purple, faintly blotched, occasionally entirely off-white when produced at depth; two fringed wings running from the bottom of the pitcher to the mouth at the front; mouth round, rising at the rear into a short neck; peristome cylindrical in section, up to 2 mm wide, inner surface with distinct teeth up to 0.8 mm long, ribs up to 0.5 mm apart, up to 0.2 mm wide; lid broadly ovate, c. 20–30 mm long, c. 20 mm wide, with short spur; large, craterlike nectar glands  $\pm$  elliptic in outline, up to 0.35 mm long, scattered densely in the middle of the lower surface. **Upper pitchers** rarely produced, up to 9 cm high, up to 2 cm wide, thin-coriaceous, arising gradually from the tendril, narrowly infundibular at the base, subcylindrical above; eglandular zone of the inner surfaces covering upper 1/3 of the pitcher; outer surface green, inner surface near the mouth yellowish; two fringed wings running from the middle of the pitcher to the mouth at the front; mouth round, with or without very short neck; peristome cylindrical, up to 1.5 mm wide, inner surface with very short teeth, ribs up to 0.25 mm apart, c. 0.1 mm wide; lid broadly ovate, 11–16 mm long, 9–13 mm wide, with curved spur; craterlike nectar glands as in lower pitchers, up to 0.3 mm long. **Male inflorescence** a raceme, peduncle c. 14 cm, rachis c. 13 cm, partial peduncles 2-flowered, bracts absent, pedicels 4–7 mm long, tepals elliptic, up to 6 by 3 mm; androphore c. 4 mm long, anther head 2.5 by 1.5 mm. **Female inflorescence** unknown. **Infructescence** racemose. **Fruit** a fusiform capsule, reddish brown at maturity, conspicuously glossy, valves of fruits c. 45 by 4 mm. **Seeds** 20–25 mm long.

**Habitat and ecology.** The species occurs on ridgetops over sandstone rocks in lower montane rainforest. The known elevational range is 1100–1300 m a.s.l. The plants frequently grow near trees whose branched roots form cavities covered with a moss layer. Lower pitchers are then copiously produced inside these cavities. If no cavities are available, the pitchers are produced directly in soil, deep litter or under moss cushions. At some sites, *Nepenthes tentaculata* Hook.f. and *N. stenophylla* Mast. grew sympatrically with *N. pudica*, while a species from the *N. fusca* species complex was spotted growing epiphytically in at least one locality.

The subterranean growth habit of *Nepenthes pudica* was consistently observed across the five studied sites but was not shared by the sympatric *Nepenthes* species,



**Figure 3.** *Nepenthes pudica* **A** male flowers **B** male plant with inflorescence **C** infructescence **D** female plant with infructescence. Photographs by M. Dančák.

demonstrating that it was not simply the result of unusual local conditions. The underground shoots of *N. pudica* had no obstacles preventing them from growing upwards, suggesting that they are not negatively gravitropic as is typical of stems. Neither did they show signs of growing towards light, even when concealed only under a soft moss cushion or already slightly chlorophyllous (Fig. 2B). Based on this and their generally lateral character, it might be supposed that they are negatively phototropic rather than positively gravitropic.

**Distribution.** The species is known only from a few adjoining localities in the western part of the Mentarang Hulu district of North Kalimantan, Indonesia. The exact locations have been withheld in order to prevent poaching by unscrupulous commercial collectors.

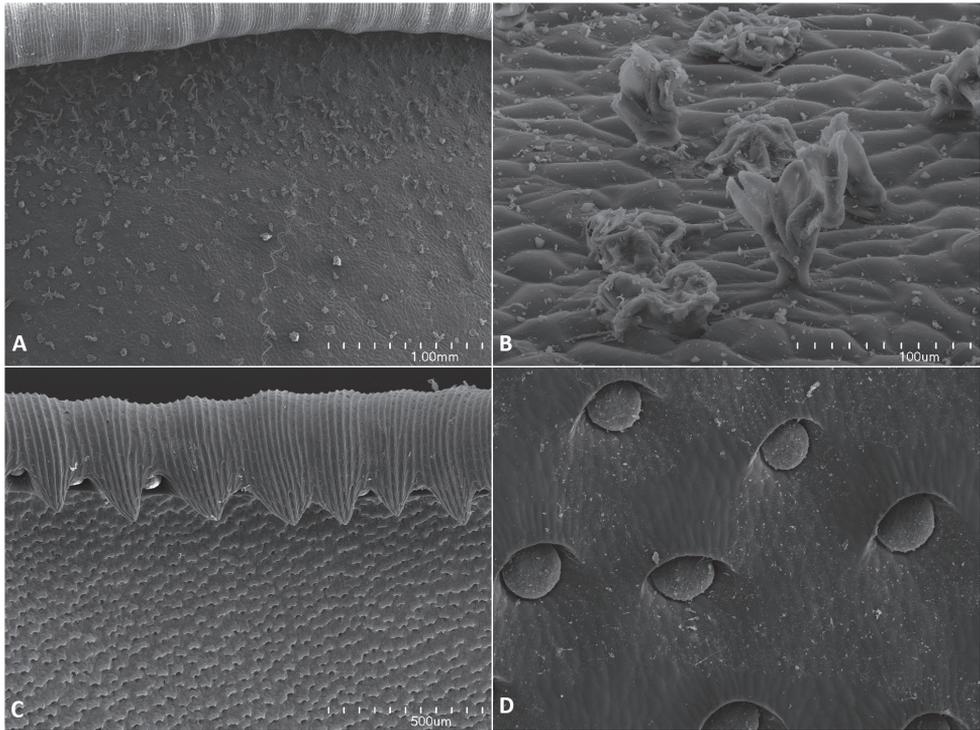
**Etymology.** The specific epithet *pudica* (bashful, shy), is a feminine adjective and alludes to the fact that lower pitchers remain concealed from direct view.

**Table 1.** Main morphological differences between *Nepenthes pudica* and related species, including *N. leptochila*, which is not recognised by most researchers. The characters that best differentiate *N. pudica* from the other species are in bold.

Characteristic	<i>N. hirsuta</i>	<i>N. hispida</i>	<i>N. pudica</i>	<i>N. leptochila</i>
short basal shoots	<b>aboveground</b>	<b>aboveground</b>	<b>underground</b>	<b>aboveground</b>
stem indumentum	<b>hairy</b>	<b>hairy</b>	±glabrous	±glabrous
stem colour	brown	purplish grey	brownish green to purplish	reddish
climbing shoot leaf shape	obovate	oblanceolate to oblong	oblanceolate	obovate-lanceolate
climbing shoot leaf width	3–6 cm	1.8–3.3	up to 4.5 cm	2.5–5.5 cm
climbing shoot leaf	petiolate	<b>sessile</b>	<b>petiolate</b>	shortly petiolate
climbing shoot leaf base	<b>semi-amplexicaul</b>	<b>decurrent-amplexicaul</b>	<b>auriculate, shortly decurrent</b>	auriculate, hardly decurrent
climbing shoot leaf texture	thin-coriaceous	thin-coriaceous	coriaceous	<b>chartaceous</b>
climbing shoot leaf apex	acute or rounded	acuminate to obtuse	acute	acute, obtuse or rounded
longitudinal veins	3–4	3	2–4 not prominent	5
tendrill indumentum	hairy	hairy	hairy or glabrous	glabrous?
upper pitchers	<b>few</b>	<b>common</b>	<b>rare</b>	<b>present</b>
lower pitcher colour	<b>green</b>	<b>green or red blotched</b>	<b>red</b>	?
adult pitcher indumentum	<b>hairy</b>	<b>hairy</b>	±glabrous	glabrous?
lower pitcher shape	<b>ovoid</b>	<b>ovoid-ellipsoid</b>	<b>ventricose</b>	<b>ovoid-ellipsoid</b>
lower half of lower pitcher	ovoid	ovoid-ellipsoid	ovoid to globose	ovoid to globose
upper half of lower pitcher	<b>conical</b>	<b>subcylindrical, tapering</b>	<b>infundibular</b>	<b>~cylindrical, tapering</b>
lower pitcher length	up to 15 cm	5–8.5 cm	7–11 cm	up to 8 cm
lower pitcher width	up to 7 cm	<b>1.5–3 cm</b>	<b>3–5 cm</b>	<b>up to 3 cm</b>
eglandular zone	<b>almost absent</b>	nearly 1/2 of the surface	<b>nearly 1/2 of the surface</b>	<b>1/3 of the surface</b>
peristome width	up to 6 mm	0.5–1.2 mm	up to 2 mm	up to 1.5 mm
peristome in section	cylindrical or flattened	cylindrical	cylindrical	cylindrical or flattened
male flowers	in pairs	<b>single or rarely in pairs</b>	<b>in pairs</b>	?
androphore length	3.5–6 mm	<b>1.5–2 mm</b>	<b>~4 mm</b>	?
ecology	ridgetops	heath forest	ridgetops	?
elevational distribution	<b>0–1000 m</b>	<b>100–800 m</b>	<b>1100–1300 m</b>	<b>~300 m</b>

**Conservation status.** *Nepenthes pudica* is endemic to Borneo. It is known from five closely situated sites, which represent a single location (IUCN 2022). Both the extent of occurrence (EOO) and minimal area of occupancy (AOO) of *N. pudica* are estimated to be less than 4 km<sup>2</sup>. There is uncertainty as to whether the species occurs within Kayan Mentarang National Park, as its borders were not marked in the field at the time of discovery. However, the available maps suggest all the sites are actually located outside the national park, thus legally unprotected. Due to its restricted distribution, small population size and possible habitat loss, the species qualifies to be assigned preliminary conservation status as critically endangered (CR), based on criteria B1 ab(iii) and D of the IUCN Red List categories and criteria (IUCN 2012).

**Prey composition and infauna.** We found 1785 invertebrate individuals belonging to 40 different taxa (Tables 2, 3) in suspensions sampled from five underground pitchers (found in a tree-root cavity) and one aerial rosette pitcher (growing 2 metres above the soil surface and arising from an offshoot of a fallen climbing stem). Necromass of the prey consisted of sclerites of highly digested invertebrates. It contained mainly litter- and soil-inhabiting species as well as a large amount of plant detritus. Among soil- and litter-inhabiting species we observed mites (mostly



**Figure 4.** *Nepenthes pudica*, SEM images of lower pitcher **A** outer wall with outer margin of peristome **B** detail of trichome on the outer wall **C** inner wall and inner margin of peristome showing eglandular zone covered with lunate cells and peristomal glands with peristomal glands **D** inner wall showing glandular zone with digestive glands. SEMs by B.J. Plachno.

from the family Oribatidae), leaf litter-inhabiting beetles (families Scydmaenidae, Pselaphidae, Lioididae, Carabidae) and a single ant of the genus *Anochetus* (subfamily Ponerinae). These taxa are mostly mycophagous, detritophagous, or predators. However, the main and the essential prey component was a species of ant from the subfamily Myrmicinae, probably a species of the genus *Crematogaster*, which is closely associated with *Nepenthes* (Bonhomme et al. 2011). A number of individuals of an ant from the genus *Polyrhachis* were found in the aboveground rosette pitcher in contrast with their rare occurrence in underground traps.

Surprisingly, we found relatively numerous infauna, especially larvae of mosquitoes, nematodes and annelids in both aboveground and underground pitchers (Table 3). We identified three species of mosquitoes from two genera, *Uranotaenia* and *Culex*. Identified nematodes belong to seven families: Aphelenchoididae, Cephalobidae, Diplogastridae, Panagrolaimidae, Plectidae, Rhabditidae (dauer larvae) and Wilsonematidae. The most abundant were members of families Rhabditidae and Diplogastridae detected in the aboveground trap, which were previously recorded from the pitcher fluid of *Nepenthes mirabilis* (Lour.) Druce (Bert et al. 2011). In underground traps, nematodes were rare

**Table 2.** Prey composition of *Nepenthes pudica* based on analysis of five underground pitchers and one aerial pitcher.

Prey composition in traps	traps from root cavity					abovegr.	total
	trap 1	trap 2	trap 3	trap 4	trap 5	trap 6	
Acarina, Oribatidae spp.	1	3	14	c. 100	20		c. 138
Acarina div.	1	1	25				27
Araneae, cf. Lycosidae	1						1
Araneae, cf. Dysderidae	1						1
Araneae					1		1
Arachnoidea, g. sp.		1	1				2
Coleoptera, Aphodiidae g. sp.	1						1
Coleoptera, Carabidae g. sp.				2			2
Coleoptera, cf. Leiodidae				6			6
Coleoptera, Pselaphidae g. sp.		1					1
Coleoptera, Scydmaenidae g. sp.	2	7	4	2			15
Coleoptera, g. sp. 1					3		3
Coleoptera, g. sp. 2			2				2
Diptera, Phoridae g. sp.			1				1
Diptera, Nematocera g. sp.		2		3			5
Diptera, g. sp.					1		1
Hemiptera, Derbidae g. sp.				1			1
Hymenoptera, Chalcidoidea g. sp.			1				1
Hymenoptera, Formicinae: <i>Camponotus</i> cf. <i>gigas</i>						4	4
Hymenoptera, Formicinae: <i>Polyrhachis</i> sp.		3	1		1	17	22
Hymenoptera, Formicinae g. sp.					3	1	4
Hymenoptera, Myrmicinae g. sp. 1	c. 500	11	c. 100	c. 50	c. 700		c. 1361
Hymenoptera, Myrmicinae g. sp. 2		1	1		25		27
Hymenoptera, Ponerinae: <i>Anochetus</i> sp.			1				1
Hymenoptera, Sphecidae g. sp.	2	1					3
<b>Sum of individuals</b>	<b>c. 509</b>	<b>31</b>	<b>c. 151</b>	<b>c. 164</b>	<b>c. 754</b>	<b>22</b>	<b>c. 1631</b>
<b>Sum of taxa</b>	<b>8</b>	<b>10</b>	<b>11</b>	<b>7</b>	<b>8</b>	<b>3</b>	<b>25</b>

**Table 3.** Infauna composition of *Nepenthes pudica* based on analysis of five underground pitchers and one aerial pitcher. (abovegr. = aerial pitcher; L1, L2, L3, L4 – larval stages).

Infauna composition in traps	traps from root cavity					abovegr.	total
	trap 1	trap 2	trap 3	trap 4	trap 5	trap 6	
Diptera, Stratiomyidae (larvae)	1				6		7
Diptera, Culicidae: <i>Uranotaenia</i> sp. 1	2 L1,1 L3,4 L4				4 L3,11 L4		22
Diptera, Culicidae: <i>Uranotaenia</i> sp. 2		9 L3	5 L2,1	1 L1,3			28
			L3,2 L4	L2,7 L4			
Diptera, Culicidae: <i>Culex</i> sp.	4 L4	4 L2,1 L4	3 L4	1 L4			13
Diptera, Acalypttrata			2 L2		1 L1, 4 L2	8 L1	15
Annelida, Naididae: <i>Pristina armata</i>				6			6
Nematoda, Cephalobidae: <i>Heterocephalobus</i> sp.	8						8
Nematoda, Aphelenchida: <i>Aphelenchoides</i> sp. 1				1			1
Nematoda, Aphelenchida: <i>Aphelenchoides</i> sp. 2				1			1
Nematoda, Panagrolaimidae: <i>Propanagrolaimus</i> sp.				8			8
Nematoda, Wilsonematinae: <i>Ereptonema</i> sp.				1			1
Nematoda, Plectidae: <i>Plectus</i> sp.				1			1
Nematoda, Diplogasteridae: <i>Pristionchus</i> sp.						27	27
Nematoda, Rhabditidae (dauer larvae)						16	16
<b>Sum of individuals</b>	<b>20</b>	<b>14</b>	<b>13</b>	<b>30</b>	<b>26</b>	<b>51</b>	<b>154</b>
<b>Sum of taxa</b>	<b>4</b>	<b>2</b>	<b>3</b>	<b>8</b>	<b>3</b>	<b>3</b>	<b>14</b>

and in different compositions compared to the aboveground trap. The most abundant were members of the families Cephalobidae (*Heterocephalobus*) and Panagrolaimidae (*Panagrolaimus*). One of the most interesting inquilines found in the underground pitchers was a new species of annelid worm, *Pristina armata* (family Naididae), described previously by Schenková and Čermák (2013).

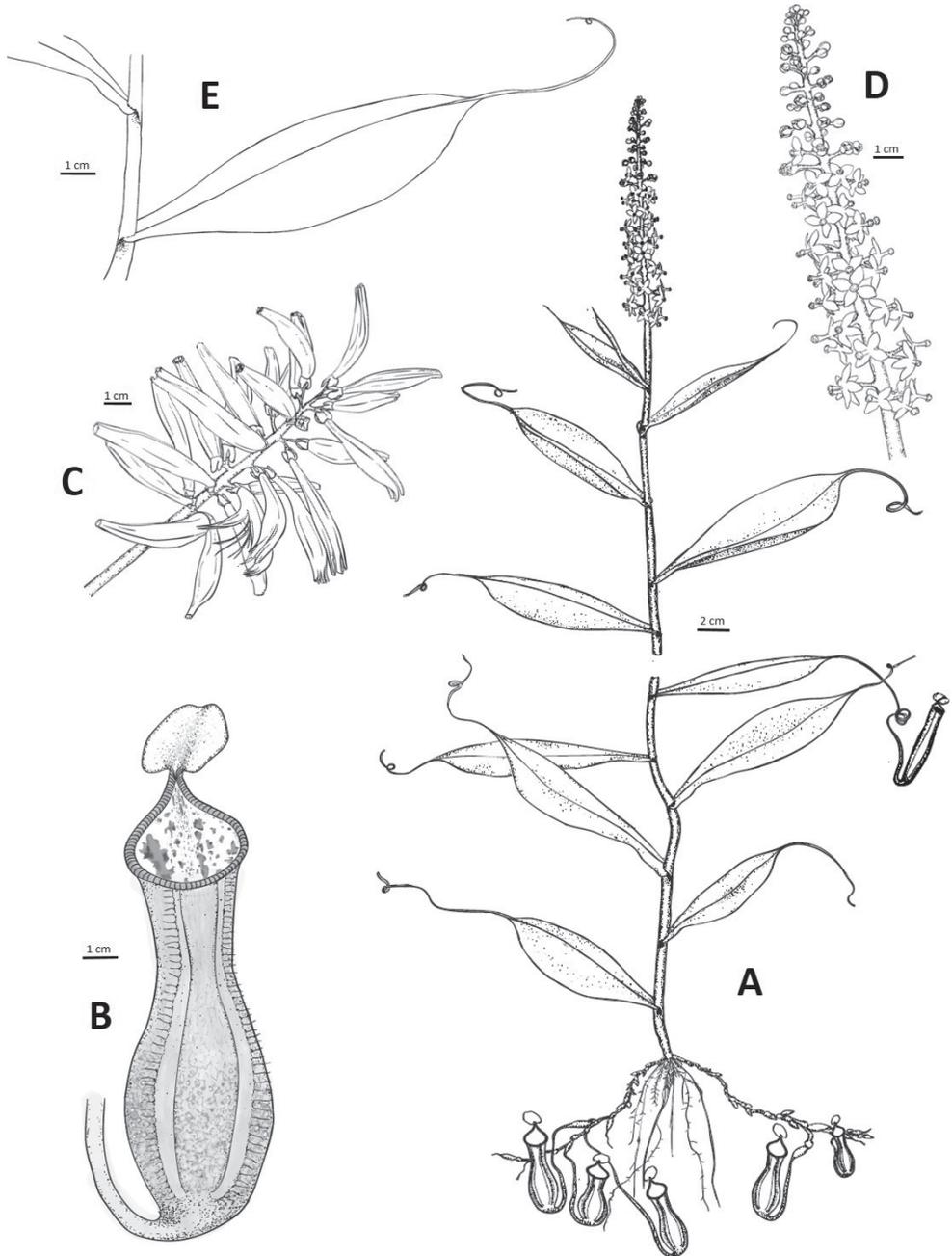
**Selected specimens examined.** See Suppl. material 1.

## Discussion

*Nepenthes pudica* is the first carnivorous species confirmed to use pitfall traps specifically in the subterranean environment. It produces almost exclusively underground pitchers that are well developed and fully functional. Although in some species of *Nepenthes* pitchers are occasionally reported to develop in plant litter or directly in the soil (Salmon 1993; Nerz et al. 1998; Clarke 2001; Ghazalli et al. 2020), no species that specifically targets this environment to this extent has been documented to date. This is not surprising, as pitchers are generally much larger than other types of traps and are rather fragile due to their hollow character. Therefore, they are generally unsuitable for the soil environment, where considerable pressure is needed to form a cavity. As the pitchers of *N. pudica* are of a typical size for the genus, they are by far the biggest underground traps among all known carnivorous plants. While the other genera of carnivorous plants that produce underground traps are, due to the small size of their traps, capable of catching only microscopic or very small prey (Seine et al. 2002; Pereira et al. 2012), the pitchers of *N. pudica* catch prey of the same size as other pitcher plants.

The traps of carnivorous plants are complex and metabolically costly organs that must be produced at the expense of tissues optimised for photosynthesis (Givnish et al. 1984; Pavlovič and Saganová 2015). In pitcher plants, this trade-off often manifests in the separation of primary prey- and light-harvesting structures spatially—e.g. on an intra-leaf level as in most *Nepenthes*—and also temporally, as in the seasonal production of solely photosynthetic leaves by *Cephalotus* Labill. and some *Sarracenia* L., both examples of separation on an inter-leaf but intra-shoot level (McPherson and Schnell 2011; Cross et al. 2019). In *N. pudica*, this ‘division of labour’ is unusually displayed at the level of the shoots. This strategy is analogous to that of certain strongly shoot-dimorphic aquatic *Utricularia*, such as *U. intermedia* Hayne, whose specialised carnivorous shoots penetrate a loose organic sediment while the green stems seek sunlight in clear water near the surface (Adamec 2007).

Each leaf of a typical *Nepenthes* comprises an entirely photosynthetic lamina-like phyllodium and a predominantly carnivorous and only marginally photosynthetic pitcher (Pavlovič et al. 2007, 2009; Karagatzides and Ellison 2009). The unusual architecture of *N. pudica* (Fig. 5A) appears to have largely freed it from the phylogenetic constraint of having functional phyllodia and pitchers in close physical proximity, and thereby allowed it to exploit a novel source of prey in the form of the subterranean environment, limiting competition with sympatric congeners. However, this body plan is likely to come with certain costs. Subterranean pitchers, by virtue of having to



**Figure 5.** *Nepenthes pudica* **A** habit **B** lower pitcher **C** infructescence **D** male inflorescence **E** detail of climbing stem with a leaf. Drawn by Kateřina Janošíková.

displace surrounding substrate as they grow, might be expected to have significantly thicker walls and a higher concentration of structural compounds (e.g. lignin) than those produced above ground. Preliminary observations indicate that underground

pitchers are indeed markedly thicker-walled and sturdier (M. Dančák & M. Golos, pers. observ.). All else being equal, this would increase their construction costs, partly offsetting benefits from carnivory, and likely dictate longer pitcher lifespans, reflecting a greater 'payback time' for recovery of these costs (see Osunkoya et al. 2008). And this does not even consider the additional stem biomass needed for dimorphic shoots. Moreover, the greater separation of the two types of assimilatory organs in *N. pudica* must presumably necessitate two-way exchange of nutrients and photosynthates over much greater mean distances than in species with typical pitcher–phyllodium pairs (see Osunkoya et al. 2007). All told, the benefit from subterranean carnivory must be significant to make up for these additional costs and this is perhaps the reason this strategy is not seen more widely across the genus.

Among *Nepenthes*, the species that come closest to this degree of shoot specialisation are perhaps those in which pitchers produced in low-light conditions near ground level are borne on crowded, greatly reduced phyllodia (the latter sometimes termed 'nanophylls'; Cheek 2015). The best known of these, *N. ampullaria* Jack, additionally produces largely or entirely pitcherless climbing stems (Tan and Wong 1996), mirroring the situation in *N. pudica*, though the latter's production of solely carnivorous shoots appears to be unique among *Nepenthes* and indeed among all pitcher plants. Also of note is the comparatively little-known *N. rhombicaulis* Sh.Kurata of Sumatra, which rarely if ever produces upper pitchers and has been speculated to target underground prey, though until now its lower pitchers have only been documented to develop within dense moss and detritus rather than being truly subterranean (Salmon 1993; Schmid-Hollinger 1994; Clarke 1997a, 2001). This species, which appears to occupy a similar ecological niche to members of the *N. hirsuta* group in Borneo, would be a prime candidate for further investigation in this regard.

Since the discovery of *Nepenthes pudica*, field observations in the Berau region of East Kalimantan (M. Golos, pers. observ. June 2019) have revealed a similar taxon that likewise produces achlorophyllous subterranean shoots bearing nanophylls with reddish pitchers (Fig. 6). This taxon also produces few aerial traps, though it notably differs from the type population of *N. pudica* in growing at considerably lower elevations. Its precise taxonomic affinities have yet to be determined.

As was demonstrated above, the prey of *Nepenthes pudica* consists of various species of soil- and litter-inhabiting fauna. With 25 different taxa, the diversity of identified prey was rather high, which is typical for species growing at higher elevations (Adam 1997). However, ants were the main prey component found in both aerial (subfamily Formicinae) and lower pitchers (subfamily Myrmicinae). At this point, we can assume that *N. pudica* is predominantly an ant specialist, as are the majority of *Nepenthes* species.

Consistently with other *Nepenthes* species, *N. pudica* harbours relatively numerous and diverse infauna in both types of pitchers (154 individuals and 14 identified taxa). Besides mosquitoes, which are commonly associated with pitcher plants (Vong et al. 2021), larvae of aquatic Diptera (family Stratiomyidae and subsection Acalypttrata) were detected as well. The insect-trapping structures of pitcher plants (especially Nepenthaceae and Sarraceniaceae) frequently harbour dipteran larvae, which utilize the food niche in pitchers (Adlassnig et al. 2011). Members of the family Stratiomyidae

are true aquatic organisms inhabiting many kinds of phytotelmata such as tree holes, leaf axils and modified leaves (Greeney 2000); however, their presence in pitchers is not as common in comparison with members of other dipteran families such as Syrphidae, Ceratopogonidae or Chironomidae (Kitching 2000). Rather surprising is the fact that lower pitchers of *N. pudica* also contained abundant dipteran infauna, including mosquitoes. This indicates that the tree-root cavities from which samples were taken were accessible to the outside-living invertebrates. Therefore, even the hidden lower pitchers can serve as a stable and permanent water habitat (phytotelma) similar to other *Nepenthes* species or other plants, e.g. unrelated Bromeliaceae (Thorpe and Rogers 2015), and play an essential role in the development of these symbionts, especially during dry periods. However, underground pitchers produced in compacted substrate (Fig. 2A) would presumably not be similarly accessible to ovipositing insects.

Nematodes formed the other large group of infauna. Identified individuals belonged to families Aphelenchoididae, Cephalobidae, Diplogastridae, Panagrolaimidae, Plectidae, Rhabditidae (dauer larvae) and Wilsonematidae. The most abundant were members of the genus *Pristionchus* (Diplogastridae), detected only in the aboveground trap and obviously associated with the main prey, an ant species of the genus *Polyrhachis*. Species of the genus *Pristionchus* feed selectively on bacteria and fungi decomposing insect carcasses (Rae et al. 2008), including various genera of ants, e.g. *Formica*, *Lasius* and *Myrmica* (Wahab 1962; Ishaq et al. 2021). The nematodes detected in lower pitchers were members of genera generally living in soil and water environments and feeding on bacteria and fungi decomposing organic material (Bongers 1990). The only exception was the genus *Halicephalobus*, the species of which are aquatic but occur in extreme environments (Borgonie et al. 2011; Geraert et al. 1988), various phytotelmata (Andrassy 1952; Körner 1954) or as parasites (Stefanski 1954).

Probably the most interesting species living in the pitchers of *Nepenthes pudica* was the annelid worm *Pristina armata* (Naididae), which was described from and found so far only in its lower pitchers. For the description and discussion on its relation to *N. pudica*, see Schenková and Čermák (2013).

The living strategy of *Nepenthes pudica* can be viewed as an advantageous evolutionary adaptation. As carnivorous plants are highly dependent on prey for organic nutrients essential for reproductive success (Zamora et al. 1997), strong selective pressures may have acted on traits related to prey capture (Ellison et al. 2001). Hence, the potentially strong competition for prey and possible environmental limitations in the forest understorey (e.g. dryness affecting ridgetops) might be avoided by moving the traps underground.

*Nepenthes pudica* belongs to the *N. hirsuta* group, which is endemic to Borneo and includes at least two putative close relatives: *N. hirsuta* Hook.f. and *N. hispida*. Another two species are sometimes considered members of this group, namely the Bornean *N. macrovulgaris* J.R.Turnbull & A.T.Middleton and *N. philippinensis* Macfarl. from the island of Palawan (Cheek and Jebb 1999). However, the recent phylogeny of the genus (Murphy et al. 2020), while proving the close relationships of *N. hirsuta* and *N. hispida*, does not support the close affinities of *N. macrovulgaris* and *N. philippinensis*, either mutually or to *N. hirsuta* and *N. hispida*. *Nepenthes hirsuta* and *N. hispida* share a combination of traits that distinguishes this group from the rest of the genus. These are especially the growth form



**Figure 6.** *Nepenthes* sp. with excavated underground traps (bottom left) from a locality in the Berau region of East Kalimantan. Photograph by M.R. Golos.

(well-developed rosetted, non-climbing phase), hairy stem, more or less ovoid shape of the lower pitchers, oblique pitcher mouth,  $\pm$  cylindrical peristome, lid without appendages and flowers usually in pairs (Cheek and Jebb 1999). *Nepenthes pudica*, while possessing most

of these characteristics, shows several unique traits. These are namely a) underground basal shoots (the other species form aboveground basal shoots); b) upper pitchers are only rarely produced in lower parts of the climbing stem; c) lower pitchers are produced exclusively underground; d) the shape of the lower pitchers is ventricose with the lower half ovoid to globose and the upper half infundibular. Another possible member of the *N. hirsuta* group, *Nepenthes leptochila* Danser, was described from northern North Kalimantan (Mt. Djempanga; Danser 1928), but this name is usually considered a heterotypic synonym of *N. hirsuta* (Clarke 1997b; Jebb and Cheek 1997; Cheek and Jebb 2001; Phillipps et al. 2008; McPherson et al. 2009). Nevertheless, the original description mentions several significant differences compared to *N. hirsuta* (e.g. a well-developed eglandular zone inside the pitchers, glabrous stems and pitchers, and much smaller pitchers) so its identity is at least questionable. *Nepenthes leptochila* also bears considerable resemblance to *N. pudica*, especially in being rather glabrous. However, the two taxa differ in all the four previously mentioned characters typical for *N. pudica* and therefore we do not consider them conspecific. For a comparison of critical diagnostic characters of *N. hirsuta* (excluding *N. leptochila*), *N. hispida*, *N. leptochila* and *N. pudica*, see Table 1.

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## Supplementary material I

### List of examined specimens

Authors: Martin Dančák, Luboš Majeský, Václav Čermák, Michal R. Golos, Bartosz J. Płachno, Wewin Tjiasmanto

Data type: docx file

Explanation note: *Nepenthes hirsuta* (including *N. leptochila*) and *Nepenthes hispida*.

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# Resurrection of *Passiflora acuminata* DC. and synonymization of *P. tolimana* Harms, *P. gleasonii* Killip, *P. metae* M. Bonilla, C. Aguirre & Caetano (Passifloraceae) following a study of their morphology and ecogeography

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## Abstract

Within the very uniform series *Laurifoliae*, *Passiflora acuminata* (treated as a synonym of *P. laurifolia* in the *Flora of China*), *P. tolimana*, *P. gleasonii* and *P. metae* appear particularly similar. A review of their descriptions and the associated specimens confirms their lack of morphological differentiation and leads us to formally resurrect *P. acuminata* and place the three other taxa under its synonymy. This taxonomic move is also supported by a revision of 72 additional geolocated specimens (for a grand total of 78) and an analysis of their distribution and habitats. In fact, the bioclimatic space corresponding to the specimens previously assigned to *P. acuminata* encompasses that of all specimens previously assigned to the three other taxa under study. The species range covers a wide region, comprising the lower Amazon and the north of its basin, mostly below 200 m, and, to the west, in the upper Amazon, the Orinoco basin, and along the Andean foothills and valleys, from Venezuela to Peru, at elevations between 100 and 2200 m. In the lowlands, the species appears associated with white sand savannas and water courses. A more complete description is proposed for the species, including its unusual fusiform and slightly ribbed fruit. Another rare trait in the series *Laurifoliae* is that the outer corona filaments tend to be longer than the corolla.

**Keywords**

*Laurifoliae*, subgenus *Passiflora*, taxonomy

**Introduction**

*Passiflora acuminata* DC., *P. tolimana* Harms, *P. gleasonii* Killip, and *P. metae* M. Bonilla, C. Aguirre & C.M. Caetano are very similar taxa that belong to series *Laurifoliae* Killip ex Cervi, 1997 (subgenus *Passiflora*, supersection *Laurifoliae* (Killip ex Cervi) Feuillet & J.M. MacDougal, 2003), which counted 29 species in the classification of MacDougal and Feuillet (2004). The whole series shows a remarkable morphological and ecological uniformity, rendering its taxonomy extremely difficult (Killip 1938). Revising the criteria that delimit it, Rome and Coppens d'Eeckenbrugge (2017) excluded five species from the series *Laurifoliae*. The remaining 24 species are glabrous to pubescent, have stems terete to angular, wingless, sometimes corky on old parts with stipules linear to setaceous, early deciduous. Their leaves are unlobed, oblong-lanceolate, entire to glandular-serrulate, not peltate, and have petioles with 0–2 discoid to oblong sessile glands. Flowers are pendent with two outer series of long campanulate filaments, an involucre of three glandular bracts, free at the base and measuring more than 1 cm long. Since this revision, one more species corresponding to these criteria has been described in Colombia as *P. gustaviana* Ocampo & Molinari, 2017.

*Laurifoliae* species also share habitat preferences, in humid neotropical forests, at low to medium elevations in Central and South America. Furthermore, several species, like *P. nitida* Kunth, *P. riparia* Mart. ex Mast., *P. ambigua* Hemsl., show very extensive distributions (e.g. across the Amazonian basin and/or along the tropical Andes), which favors geographical differentiation and may lead to redundant descriptions in different countries. Rome and Coppens d'Eeckenbrugge (2019) demonstrated such a situation for *P. crenata* Feuillet & Cremers, *P. emiliae* Sacco, *P. fernandezii* L.K. Escobar and *P. pergrandis* Holm-Niels. & Lawesson, and included them in the synonymy of *P. riparia*. Here, we present a similar analysis on *P. acuminata*, *P. tolimana*, *P. gleasonii*, and *P. metae*.

Among these four taxa, the most ancient one is *P. acuminata*, described from Brazil by de Candolle (1828). His very short Latin diagnosis translates as follows: “leaves glabrous, ovate-lanceolate, acuminate, entire; petiole with two glands at the apex; bracts oblong, obtuse, and entire.” De Candolle mentioned a specimen of this species conserved at the National Museum of Natural History of Paris. The latter, without number, had been collected by an anonymous botanist. In *Flora Brasiliensis*, Masters (1872) placed *P. acuminata* as a synonym of *P. laurifolia* L. It was not until 1938 that the species was revived by Killip in *The American species of Passifloraceae*, with a detailed description and citations of several herbarium specimens from Brazil, on which the floral structure can be easily examined. As did Killip, Tillett (2003) distinguished *P. acuminata* from *P. laurifolia* by its radii in two equal series (vs. two unequal series). However, in 2007, this species was again treated as a synonym of *P. laurifolia* in the *Flora of China* (Flora of China Editorial Committee 2007). This rearrangement was

not followed, and many authors (e.g. Vanderplank and Zappi 2011, Silva et al. 2013, 2017, Bonilla Morales et al. 2016) still mention it as a distinct species.

In January 1886, in the municipality of Dolores (Tolima, Colombia), at elevations of 1400–1800 m, Lehmann collected a passionflower with greenish white flowers and a corona of purple filaments. He was careful to note that its glossy dark green leaves had a tough texture and, often, brown nervation. The fruit seemed to remain dark green at maturity with a pleasant and sweet taste. Lehmann drew it on the label as a relatively fusiform and ribbed fruit (Plate 1F), not resembling the globular yellow fruits generally observed in series *Laurifoliae*. Finally, he mentioned that the species is quite common but seems to produce fruit only rarely. A few years later, Harms (1894) described meticulously Lehmann's specimen as *Passiflora tolimana*. For the corona, he mentioned that the outermost filaments are slightly longer than the petals, while the innermost filaments are sometimes fused, forming a membrane.

In 1924, Killip described *Passiflora gleasonii* from a herbarium specimen collected by J.S. De La Cruz in 1923, on the banks of the Pomeroon River in British Guyana. In the original description, he noted that the species had only three series of filaments, with two outer subequal series, capillary, 4–5 cm long, and an inner series, narrowly linear, 1 mm long. Finally, he mentioned that this species is close to *Passiflora nitida* Kunth, but differs from the latter by the outer filaments being narrower and bracts narrowed at both ends, resembling those of *Passiflora vitifolia* Kunth.

In 2016, Bonilla Morales et al. described *P. metae* from two very close sites (within 1 km) at Villavicencio in the Meta department of Colombia. Their description and diagnostic key to other Colombian species underline the glabrous ovary (vs. pubescent in *P. tolimana* and *P. gleasonii*); however, most characters remain the same.

In the present article, we compare the descriptions of *P. acuminata* with those of *P. tolimana*, *P. metae* and *P. gleasonii* and study the type specimens and other herbarium specimens cited in their descriptions. Field observations of flowers and fruits corresponding to *P. gleasonii* from Venezuela, *P. acuminata* from Pará (Brazil), and *P. tolimana* from Ecuador, are presented. We thus show that they cannot be distinguished on their morphology. Based on the examination of 78 geolocalized field observations and herbarium specimens, we analyze their distribution and habitat, using Ecological Niche Modeling tools and available label information. Finally, we resurrect formally *P. acuminata*, provide a complete and precise description of it, and place the names of *P. tolimana*, *P. metae* and *P. gleasonii* in its synonymy.

## Materials and methods

### Morphological analyses

We first compare the descriptions of *P. acuminata* by Killip (1938) and Tillett (2003), *P. tolimana* by Harms (1894) and Killip (1938), the original description of *P. metae* by Bonilla Morales et al. (2016) and those of *P. gleasonii* by Killip (1924, 1938) and Tillett (2003). This comparison is focused on traits relative to stems, stipules, petiole, leaves,

bracts, calyx, corolla, corona (outer and innermost series of filaments), operculum, lamen, ovary, and fruit.

A second comparison bears on 15 of the 17 vouchers associated with the above-mentioned descriptions, focusing on the following traits: petiolar gland position, plant pubescence, bract size, and perianth parts, as observable or mentioned on the voucher label. The specimen Ducke 10528, cited in Killip's description of *P. acuminata*, could not be obtained for examination. The specimen Bonilla 197 (paratype of *P. metae*) had not been deposited at CUVC. No voucher was associated to Tillett's description of *P. gleasonii*.

We further include our own observations on living material from Ecuador (Sucumbíos), Colombia (Meta, Santander), Venezuela (Mérida, Táchira, Zulia), and Pará (Brazil), other vouchers (newly determined), as well as good and precisely geolocalized photographs from several botanists and/or passionflowers enthusiasts.

### Examined herbarium specimens

The following list consists of specimens that were previously assigned either to one of the four taxa under study (*P. acuminata*, *P. tolimana*, *P. gleasonii*, and *P. metae*), or to other *Passiflora* species, and now re-assigned to or kept as *P. acuminata* by the authors.

Previously assigned to *Passiflora acuminata*

**Brazil: Amazonas:** unknown collector s.n. (holotype, P). Lago de Tefé, Manua, 13 Jul 1973, Lleras 16644 (NY). Manaus, along road to Caracaraí, 16 Aug 1986, Croat 62222 (MO). **Pará:** Pará, Ilha de Marajó, Salvaterra, 2 Mar 2012, Costa 534 (MG). 27 Oct–7 Nov 1929, Killip 30272 (US). Obidos, 19–20 Jul 1934, Swallen 5095 (US). Ilha do Mosqueiro, 3–9 Nov 1929, Killip 30572 (US). Santarém, Alter do Chão, between 1998 and 2005, Knowles 1555 (MG). Belém, near Instituto Agronômico do Norte, 28 Oct 1959, Kuhlmann 386 (SPF). Rodovia Belém-Brasília, Km 93, 14 Sept 1959, Kuhlmann 361 (SPF). Santarém, 16 Sept 1999, Cordeiro 4089 (MG). Belém, Parque Estadual do Utinga, 19 Sept 2010, Silva 10 (MG). Belém, Reserva do Mocambo, 23 Nov 2009, Kerpel 8 (MG). Município Almeirim, Mount Dourado, 23 Dec 1986, Pires 1560 (UPCB). South of Instituto Agronômico do Norte, Belém, 25 Nov 1942, Archer 7864 (K). Road from Belém to Vigiúí, 6 Apr 1961, Aubréville 198 (P). Município de Oriximiná, Rio Trombetas, 22 Apr 2007, Salomão 902 (MG). Belém, Parque Estadual do Utinga, 19 Nov 210, Silva 06 (MG). Belém, on lands of IAN, 20 Jun 1944, Silva 243 (IAN). Santarém, shore of Amazon, 15 Mar 1857, Spruce 760 (K). Belém, Fazenda Murutucú, 22–23 May 1924, Zerny s.n. (W). Porto Trombetas, 26 Apr 1987, Knowles s.n. (INPA). South of Belém near mouth of Rio Guama, 14 Aug 1986, Croat 62142 (MO). Cachoeira Porteira, 17 Jan 1991, Knowles 1696 (INPA). Oriximiná, 23 Jan 2013, Koch 528 (RB). **Roraima:** Amazonas (now Roraima), Caracaraí. estrada Manaus, 10 Mar 1978, Silva 4557 (NY). **Colombia: Antioquia:** Municipio San Carlos, Corregimiento Norte del Samaná, 12 Nov 1989, Escobar 8835 (HUA). **Meta:** Municipio de Restrepo, Sector Mirador, 23 Jan 2018,

Ocampo 48 (CUVC). **French Guiana:** Monts Tumuc Humac, 30 Aug 1972, Degranville 1430 (CAY). **Guyana: Demarara-Mahaica region:** Along Linden-Soesdyke Highway, 7 Dec 1986, Pipoly 9142 (US). Berbice savanna near Takama Army Base, 14 Aug 1993, Henkel 2520 (NY). **Cuyuni-Mazaruni;** Kako River, 12 May 2009, Wurdack 4954 (NY). **Upper Demerara-Berbice region:** Linden-Soesdyke Highway, between Dora and Maibia Creek, 21 Jan 1987, Pipoly 9707 (US).

Previously assigned to *Passiflora tolimana*

**Colombia: El Valle:** La Cumbre, 2000 meters, 14–19 May 1922, Pennell 5754 (N), Killip 5594 (N), 1 Oct 1922, Killip 11679 (N). **Guaviare:** 1844, Goudot 10 (P). **Santander:** Municipio de Zapatoca, vereda La Cacica, Reserva La Montaña Mágica “El Poleo”, parte alta, 28 Jul 2014, Díaz 829 (MEDEL). **Tolima:** Dolores, 23 Jan 1886, Lehmann 6060 (K). **Ecuador: Sucumbíos:** Shushufundi, 4 Dec 2014, Rome 538 (LYJB).

Previously assigned to *Passiflora gleasonii*

**Brazil: Acre:** Municipality of Rio Branco, road to Xapurí, 24 Oct 1980, Cid 3035 (NY). **Colombia: Guaviare:** Municipality of San José del Guaviare, Inspección de Puerto Arturo, 25 Aug 1995, Cárdenas 6533 (COL). **Guainía:** Maimachi, Serranía del Naquén, 11 Apr 1993, Madriñán 1014 (MO). **Guyana:** Pomeroron district, Pomeroron River, 14 Jan 1923, De La Cruz 2963 (GH). Essequibo River: Moraballi Creek, 14 Sept 1929, Sandwith 254 (K). **Peru: Pasco:** Oxapampa, 7 Jul 2003, Werff 18100 (MO). **Venezuela: Amazonas:** Department of Río Negro, 9 Mar 1984, Liesner 16501 (MO). **Táchira:** East of Ayarí, 7 Nov 1979, Steyermark 119472 (MO), Cerro de Cuite, along Quebrada La Colorada, 8 Nov 1979, Steyermark 119657 (MO), Cerro of Cuchilla, La Pabellana, 6 Nov 1979, Steyermark 119423 (MO). **Zulia:** 6 km east north-east of Río de Oro, 28 Mar 1982, Liesner 13280 (MO).

Previously assigned to *Passiflora metae*

**Colombia: Meta:** Municipality of Villavicencio: road Caño Pendejo, 541 m, 23 Dec 2013, (fl), M. Bonilla, J. Mosquera, K. Pulido & A. Cajar 167 (Holotype CUVC!).

Previously assigned to *Passiflora ambigua*

**Colombia: Santander:** entre Duitama y Virolín, 6 Nov 1979, Escobar 3056 (HUA).

Previously assigned to *Passiflora laurifolia*

**Brazil: Amazonas:** Manaus; reserva campina - BR174 km 45, Benson 8282 (NY). Igarapé Ipiranga, 4 Jul 1993, Ribeiro 997 (INPA). Estrada para Igarapé do Tinga, 7 Aug

1996, Sothers 883 (INPA). **Pará:** Cachoeira Porteira, 16 Jan 1991, Knowles 1687 (INPA). Mineração Rio do Norte, Porto Trombetas, Mina Saracá, 6 Jun 1999, Miranda 397 (NY). **Colombia: Caquetá:** Orillas del Río Caguán, 12 Apr 1953, Romero-Castañeda 3963 (COL). **Cundinamarca:** Ubala B. Inspección de Policía San Pedro de Jagua, Vereda Soya, 5 Jul 1998, Fernández 16513 (RB). **Meta:** Cordillera de la Macarena, 30 Dec 1950, Idrobo 868 (COL).

Previously assigned to *Passiflora nitida*

**Brazil: Pará:** Município de Oriximiná, Rio Trombetas, 11 Jul 1980, Cid 1461 (INPA). **Colombia: Antioquia:** San Carlos, corregimiento El Jordán, 29 Sept 1989, Velásquez 241 (HUA). San Carlos, Vereda Patio Bonito, Alto El Cerrón, 17 Feb 1998, Correa 85 (HUA). **Caquetá:** Municipio de Doncello, vereda de Buena Vista, 21 May 2003, Castaño 1649 (COL). **Santander:** Barbosa, bosque en BellaVista, 24 Nov 1940, Pérez Arbeláez 8124 (COL).

Previously assigned to *Passiflora riparia*

**Ecuador: Morona Santiago:** ca. 32.5 km S of Gualaquiza on road to Zamora, 4 Feb 1984, Knapp 6242 (QCNE). **Napo:** Archidona, 27 Jun 1968, Holm-Nielsen 1040 (AAU).

Previously assigned to *Passiflora* aff. *guazumifolia*

**Colombia: Boyacá:** Municipio Pajarito, corregimiento de Corinto, 16 Oct 1967, Lozano 935 (COL).

Previously assigned to *Passiflora* sp.

**Peru: Madre de Dios:** Manu, Aguas Calientes, 13 May 1984, Knapp 6441 (K).

## Geolocalized data collected by botanists and/or on iNaturalist

### *Passiflora acuminata*

**Brazil: Acre:** Porto Acre (D. S. Menezes, <https://www.inaturalist.org/observations/41238406>). **Amazonas:** BR174, 1.5 km N of Presidente Figueiredo (A. Adair). AM-240, 1 km S of Vila de Balbina (A. Adair). 5 km N of Oriximina (A. Adair). 1 km NE of Obidos along road (A. Adair). Rodovia Eng. Fernando Guilhaon, Santarém (A. Adair). **Pará:** Santa Bárbara do Pará (B. Ferreira, <https://www.inaturalist.org/observations/34108563> / L. F. Matos (<https://www.inaturalist.org/observations/80518762>)). **Colombia: Antioquia:** Municipio de Remedios, corregimiento La Fragua (J. Restrepo, <https://www.flickr.com/photos/22012266@N02/9906490006/in/photostre>).

am/). **Meta:** Villavicencio, Reserva Forestal Vanguardia (J. Ocampo P.). **Putumayo:** Puerto Leguizamo (M. Molinari & M. Wettges). **Ecuador: Zamora-Chinchi:** Yantzatza (A. M. Hualpa Erazo, <https://www.inaturalist.org/observations/62721105>). **Venezuela: Bolívar:** La Escalera (M. L. Watson, <https://www.inaturalist.org/observations/41457638>). **Mérida:** La Blanca (M. Molinari). Guayabones (M. Molinari). **Táchira:** Santo Domingo airport (M. Molinari). Colón-San Felix road (M. Molinari).

### *Passiflora tolimana*

**Colombia: Cundinamarca:** (N. B. Uribe, <https://www.inaturalist.org/observations/65638815>).

### *Passiflora metae*

**Colombia: Cundinamarca:** El Colegio (geolocalization from the photographer, H. Svoboda) <https://uk.inaturalist.org/photos/>). **Meta:** Buenavista, E of Villavicencio (G. S. Castro, <https://www.inaturalist.org/observations/7356149>).

## Analyses of distribution and habitat

After the analyses of descriptions and reference materials showed that the four species under study could not be distinguished on a morphological basis, a geographical database was constituted, including the localizations of 78 herbarium specimens, photographs and observations listed above. These sites were mapped and a global distribution model was developed, using the MAXENT 3.4.1 software and 19 bioclimatic variable layers from the Worldclim 2.1 database at a 2'30" grid resolution (corresponding roughly to 4.4 × 4.6 km at Equator; <https://www.worldclim.org/data/worldclim21.html>; Fick and Hijmans 2017). MAXENT identifies potential distribution areas based on their similarity in climatic conditions compared to those at the sites where the species has already been observed, hence modeling where conditions are suitable for their development. It infers the probability distribution of maximum entropy (i.e., closest to uniform) subject to the constraint that the expected value of each environmental variable (or its transform and/or interactions) under this estimated distribution matches its empirical average (Phillips et al. 2006). A logistic threshold value equivalent to the 10<sup>th</sup> percentile training presence was retained to separate climatically favorable areas from marginally fit areas. Thresholds of 33 and 67% training presence were used to discriminate “very good” and “excellent” climates. Furthermore, those bioclimatic covariates that most contributed to the model were extracted for the collection sites, and submitted to a Principal Component Analysis to characterize the bioclimatic spaces corresponding respectively to the previous assignments of specimens.

Three environmental parameters were extracted from specimen labels and/or observed from Google Earth (when highly precise geographical coordinates, from direct GPS measures, were available): proximity of water courses, white sands, and degree of perturbation.

## Results and discussion

### Comparative analysis of descriptions

The comparison between the descriptions of *P. acuminata* and the other three taxa is presented in Table 1. The color and pubescence of bracts, and the pubescence of sepals are not given in the table because the descriptions are incomplete regarding these criteria. These traits will be treated in the analysis of herbarium specimens. The series of filaments appear banded with white and purple in all descriptions.

As *P. tolimana* and *P. gleasonii* descriptions were based on herbarium materials, they lack information on perianth color. In the case of *P. tolimana*, the description underestimates the perianth size and lacks the size of the bracts and the operculum.

As shown in Table 1, the comparison of the different descriptions allows no clear distinctions that could justify the consideration of different species. Stems are rounded to angulate, which is the commonest situation in passion flowers. Stipules appear to be short, as compared to the common size in *Laurifoliae*, which is likely related to their observation on dry materials, and slightly longer for *P. metae*, but the information on stipule shape and width or with the scale given in the drawing is very inconsistent for this taxon, hampering any particular conclusion. Petioles are short too, under 2 cm, and their glands are placed close to apex, with the relative exception of Killip's description of *P. tolimana*. For leaves and bracts, their variation in size, shape and distribution of marginal glands is negligible, falling well within the range of variation observed in other widespread species of series *Laurifoliae* (e.g. *P. riparia*; Rome and Coppens d'Eeckenbrugge 2019).

Similarly, taking into account the effect of desiccation on herbarium descriptions, no taxa show a distinct range of variation for sepals, petals, and corona filaments. Killip's description of *P. gleasonii* is unique in mentioning no awn on sepals, which would be exceptional for the whole subgenus *Passiflora*; and it is contradicted by Tillet's description. In fact, minute awns can be seen on sepals of the isotypes conserved at the Gray herbarium and the Missouri Botanical Garden (897955). The respective lengths given for the corona elements clearly indicate that the two outer series of filaments are longer than sepals and petals, an observation that was reported only in Harm's description of *P. tolimana*. The description of the innermost series of filaments, intermediate in size, is not explicit in the descriptions of Harms and Killip, however these authors mention their possible fusion at base, which implies that they are not as short as, and denser than, those intermediate series with few filaments 1–2 mm long. Indeed, the analysis of the *P. gleasonii* holotype shows that the operculum described by Killip is a fourth series of striped filaments, more or less fused. Apart from this confusion in Killip's description, no significant variation has been reported for the operculum and limen. The mention of a 6 mm-long limen by Bonilla Morales et al. (2016) is not supported by the longitudinal flower section drawn and photographed in their paper. In fact, the limen is reduced to a ring widening the trochlea, as in the description of Tillet (2003). Finally, the only potentially significant difference is the report of a glabrous ovary for *P. metae* in the last row of Table 1.

**Table I.** Comparison of published descriptions of *P. acuminata*, *P. gleasonii*, *P. metae*, and *P. tolimana*.

		<i>P. acuminata</i> Killip's description	<i>P. acuminata</i> Tillet's description	<i>P. tolimana</i> Harms' original description	<i>P. tolimana</i> Killip's description	<i>P. gleasonii</i> Killip's original description	<i>P. gleasonii</i> Tillet's description	<i>P. metae</i> original description of Bonilla et al.
stems		terete or subangular	-	rounded to subangular	stem purplish, 5-angled	-	-	terete, striate, glabrous
stipules	length (mm)	about 4	4-8	4-6	4-6	8	8	13
	shape	linear-falcate	narrow-linear, falcate	linear, upper part slightly broader and irregularly glandular-serrate	linear, glandular-serrate toward apex	setaceous	-	linear, falcate, glandular
petiole	length (cm)	about 1	1-1.5	1.1-1.3	1-1.5	up to 2	2	1.7-2
	stipe of glands	-	-	-	-	sessile	sessile or slightly stipitate	sessile
	gland position	at apex	at apex	at apex	in upper half	below apex	2-5 mm below apex	at apex
leaves	size (cm)	7-14 x 2-5	7-16 x 2-8	10-12 x 3-4	10-20 x 3-10	up to 16 x 9	9-16 x 4.4-9	6-19 x 2.2-5.5
	apex	tapering gradually to an acute apex	-	acute or abruptly acuminate, mucronate	abruptly acuminate, mucronate	abruptly acuminate	obtuse, abruptly acuminate	cuspidate
	base	rounded or acutish	acute, rounded, or subcordate	-	narrowed at base	truncate at base	truncate to shallowly cordate	cuneate
	margin	-	-	entire or very finely denticulate-serrate	entire or remotely and obscurely denticulate	remotely and shallowly glandular-serrulate, or subentire	subentire to shallowly glandular-serrulate-dentate	glandular
	shape	lanceolate or oblong-lanceolate	lanceolate-ovate or oblong-lanceolate	oblong-lanceolate	oblong-lanceolate	oblong	oblong-lanceolate to lanceolate-ovate	elliptic
peduncle	length (cm)	3-4	1-5	2.5-3	up to 3	up to 5	-	3-3.5
bracts	size (mm)	25-40 x 10-15	15-40 x 5-15	-	25-35 x 20	about 20 x 4-5	20-35 x 4-14	15-20 x 4.5-5
	shape	obtusate at apex	narrowly elliptic to oblong	spatulate-obovate to oblong with an acute apex	ovate	oblong-elliptic, cuspidate, acuminate	oblong-elliptic, base narrowed, apex rounded, abruptly cuspidate-acuminate	ensiform
	margin	-	entire or glandular	crenate-serrate with 2-3 glands	glandular serrate	glandular-serrate at apex	basal half with large marginal glands, finely serrate	glandular
sepals	size (mm)	20-25 x 6-7	20-30 x 6-10	about 20	15-20 x 7	30-35 x 10	30-35 x 10	30-32 x 9-10
	shape	lanceolate	narrow lanceolate	oblong	oblong	lanceolate, obtuse	lanceolate, obtuse	lanceolate, round at apex
	color	-	whitish green with purple spots	-	greenish white without, light violet within	-	-	adaxially green, abaxially white
	sub-apical awn	yes	yes	yes	yes	no	yes	yes
petals	size (mm)	about 15 mm long	about 15 mm long	similar to sepals or narrower	similar to sepals, slightly narrower	20 x 5	20 x 5	30-32 x 7-8
	color	-	white or lavender	-	light violet	-	-	white

		<i>P. acuminata</i> Killip's description	<i>P. acuminata</i> Tillet's description	<i>P. tolimana</i> Harms' original description	<i>P. tolimana</i> Killip's description	<i>P. gleasonii</i> Killip's original description	<i>P. gleasonii</i> Tillet's description	<i>P. metae</i> original description of Bonilla et al.
series of filaments	number of series	5	5 or 6	-	4 or 5	3	3	5
	outer series length (mm)	30-40	25-40	slightly longer than petals	subequal; slightly exceeding petals	40-50	40-51	38-40
	inner series	2 series with few filaments, setaceous, barely 1 mm long, innermost filaments subulate, 5-6 mm	3 or 4 series, the 2 or 3 outer few, setaceous, less than 1-2 mm, innermost subulate, 5-7 mm	shorter than outer series, the innermost sometimes fused	inner filaments much shorter, united at base	one series linear, 1 mm	linear, 1-2 mm	3d series 2-3 mm, 4th series 0.8-1 mm, innermost series 7.2-7.5 mm, inclined towards androgynophore
oper- culum	length (mm)	4-5	4-5	-	-	7-8	7-8	3
	shape	membranous, slightly incurved, minutely fimbriate	incurved, fimbriate	membranous, inflexed at base, margin erect, short-toothed	membranous, inflexed at base, margin erect, short- toothed	membranous, cleft to the base into linear segments nearly 3 mm wide	cleft to base in segments to 3 mm wide	membranous, strongly incurved, margin fimbriate, white at base, red at apex
limen		tubular, closely surrounding base of gynophore	-	thick base with two rings, the first above base of receptacle, the second 1.5 mm from the first	annular, closely surrounding the base of the gynophore, about 12 mm long, bearing a thickened, annular process about 1.5 mm above its base	borne close to base of gynophore, barely 2 mm high, denticulate	-	6 mm long
ovary		ovoid, minutely puberulent	ovoid, minutely puberulent	tomentose, elliptic, about 5 mm long	ellipsoidal, short- tomentose	ovoid, finely ferruginous- tomentulose	ovoid, finely ferruginous- velutinous	ovary 5-6 x 3 mm, ellipsoid, glabrous, yellowish green
fruit		-	-	-	-	-	globose to ovoid, 7 x 3.5 cm	unknown

## Analysis of specimens associated to the descriptions

Table 2 presents the 15 reference specimens cited in the descriptions of *P. acuminata*, *P. gleasonii*, *P. tolimana* and *P. metae* that could be examined. Six of them were sterile or in buds.

The type of *P. acuminata* shows a very degraded flower. Thus, while we perceive the presence of two equal outer series of filiform filaments, as well as pubescent bracts with glands, it is impossible to observe the pubescence of the ovary and the presence of the inner series of filaments. From the seven specimens associated to *P. acuminata* in Killip's description, only Swallen 5095, Spruce 760, and Burchell 9504 exhibit a set of traits sufficient to allow full confidence in their determination, given the common presence of *P. laurifolia*, also having two glands at the apex of the petiole, in the Pará state of Brazil.

**Table 2.** Comparison of herbarium specimens cited in the descriptions of *P. acuminata*, *P. tolimana*, *P. gleasonii* and *P. metae*.

Specimen, Institution	Origin	Petiole gland position	Bracts	Pubescence	Outer series of filaments	4 <sup>th</sup> series almost perpendicular to the androgynophore, closing the nectary chamber	Hypanthium	Limen	Observations
De Candolle 1828, description of <i>P. acuminata</i>									
<b>Unknown collector s.n., P</b>	Brazil	apex	2.8 × 1 cm, oblong-margin glandular	bracts	two equal filiform, longer than perianth	–	–	–	Flower too damaged to examine the ovary and the inner series
Killip 1938, description of <i>P. acuminata</i>									
<b>Swallen 5095, US</b>	Pará (Brazil)	below apex	3 × 2 cm, ovate, margin glandular	ovary	two equal filiform, longer than perianth	yes	reduced to the nectary chamber	reduced to a ring on the trochlea	–
<b>Spruce 760, K</b>	Pará (Brazil)	apex	4.2 × 3.9 cm, ovate, margin glandular	ovary	two equal filiform, longer than perianth	yes, filaments more or less fused	reduced to the nectary chamber	reduced to a ring on the trochlea	–
<b>Burchell 19504, K</b>	Pará (Brazil)	apex	3 × 1.4 cm, ovate, margin glandular	–	two equal filiform, longer than perianth	yes	reduced to the nectary chamber	reduced to a ring on the trochlea	–
<b>Burchell 9988, K</b>	Pará (Brazil)	apex	absent	–	–	–	–	–	sterile specimen
<b>Killip 30272, US</b>	Pará (Brazil)	apex	–	not examined	–	–	–	–	specimen without flowers
<b>Killip 30572, US</b>	Pará (Brazil)	apex	2.5 × 0.8 cm, lanceolate, margin glandular	not examined	–	–	–	–	specimen without flowers
<b>Hoffmannsegg, BR</b>	Pará (Brazil)	apex	more than 3 × 1 cm, lanceolate	–	–	–	–	–	wrinkled bracts, no flowers
Harms 1894, description of <i>P. tolimana</i>									
<b>Lehmann 6060 (Type), K, B, F</b>	Colombia	apex	11 × 3 mm, ensiform to elliptic, glandular	bracts, ovary	two equal filiform, longer than perianth	yes	reduced to the nectary chamber	reduced to a ring on the trochlea	–
Killip 1938, description of <i>P. tolimana</i>									
<b>Pennell &amp; Killip 5754, US</b>	Colombia	just above middle	–	–	–	–	–	–	<i>P. cf. ambigua</i> , sterile specimen
<b>Killip 5594, US</b>	Colombia	just above middle	–	–	–	–	–	–	<i>P. cf. ambigua</i> , sterile specimen
<b>Killip 11679, US</b>	Colombia	at middle	4.5 × 2.5 cm, oblong	not observable	not observable	not observable	–	not observable	<i>P. ambigua</i>
Killip 1924 & 1938, descriptions of <i>P. gleasonii</i>									
<b>De La Cruz 2963 (Type), US, CM, GH, MO</b>	Guyana	below apex	2 × 0.7 cm, elliptic–lanceolate, margin glandular	bracts – ovary	two equal filiform, longer than perianth	yes, filaments more or less fused	reduced to the nectary chamber	reduced to a ring on the trochlea	–
<b>Sandwith 254, K</b>	Guyana	below apex	elliptic, margin glandular	bracts – ovary	two equal filiform, longer than perianth	yes	reduced to the nectary chamber	reduced to a ring on the trochlea	–
Bonilla & al., 2016, description of <i>P. metae</i>									
<b>Bonilla 167, CUYC</b>	Colombia	apex	–	–	two equal, filiform	yes	–	reduced to a ring on the trochlea	damaged specimen

Among the four specimens associated with Killip's descriptions of *P. tolimana*, only the type Lehmann 6060 is representative. The three other specimens, Pennel 5754, Killip 5594, and Killip 11679, present no positive criteria to confirm their identification (and the flower buds on the latter could not be dissected). On the contrary, they present glands on the middle of the petiole, which indicates that they belong to *P. ambigua*, now known from the Cumbre region where these samples were collected. This confusion explains why Killip's description diverges from the original one on the position of the petiolar glands.

The description of *P. metae* cites the holotype and isotypes Bonilla et al. 187 (deposited at CUVC and at FAUC) and the paratype Bonilla et al. 197 (deposited at CUVC). However, the only specimen available is present at CUVC, where it is given as the holotype and referenced as Bonilla et al. 167. Its flower is in very poor condition and the androgynophore has been broken, which prevents from verification of the glabrousness of the ovary, i.e., the only potentially distinctive trait of this taxon.

Except for the three specimens associated to Killip's description of *P. tolimana*, which are likely representatives of *P. ambigua*, all the examined specimens present leaves with two glands at the petiole apex (or just below), bracts with glandular margins, variable pubescence on the ovary and sometimes on other floral parts as well (bracts, peduncle and calyx), a hypanthium reduced to the length of the nectary chamber, two equal outer series of filaments, longer than the perianth, a fourth series of filaments almost perpendicular to the androgynophore closing the nectary chamber, and a limen reduced to a ring at the base of the trochlea. On the specimens De La Cruz 2963 and Spruce 760, the fourth, innermost, series shows filaments more or less fused together.

## Observations on additional specimens

In total, we have analyzed 55 herbarium specimens and 23 photographed specimens of *P. acuminata*, including herbarium specimens that had been previously determined as representatives of *P. laurifolia* (eight cases), *P. nitida* (five cases), *P. riparia* (two cases), *P. ambigua* (one case), *P. aff. guazumifolia* Jussieu (one case), and two undetermined specimens. These redeterminations were mostly based on hypanthium length (reduced to the nectary chamber in *P. acuminata*), the inner series of filaments, about 1 cm, absent in *P. laurifolia*, *P. riparia* and *P. ambigua*, or parallel to the androgynophore in *P. nitida* (vs. perpendicular in *P. acuminata*), and the position of petiolar nectaries (in apical position vs. median position in *P. ambigua* and *P. riparia*).

Plate 1 presents the high similarity observed for flower and fruit traits, even among materials collected at considerable distances. Photographs A, B, C, D, respectively from Pará (Brazil), Sucumbíos (Ecuador), Mérida (Venezuela) and Guyana, show the same floral structure, which can also be clearly observed on the photograph of the holotype of *P. metae* presented by Bonilla Morales et al. (2016). There are two equal outer series of filaments, longer than the perianth. The inner series are aborted while the innermost series, about 1 cm long, close the entrance of the nectary chamber. The hypanthium is short and reduced to the nectary chamber. The androgynophore is twice wider at its base than above. It can be more or less punctuated with purplish red spots, as can the stamen filaments.



**Plate I.** Flowers and fruits of the four taxa under study **A–C** longitudinal sections of flowers of *P. acuminata*, respectively from Santarém, Pará, Brazil (photograph Luis Otávio Adão Teixeira), from Shushufindi, Sucumbíos (initially determined as *P. tolimana*), Ecuador (photograph David Scherberich); and from Guayabones, Mérida, Venezuela (initially determined as *P. gleasonii*, photograph Miguel Molinari **D** dried flower showing the innermost series of filaments, more or less fused, isotype of *P. gleasonii*, Gray herbarium, Harvard **E, F** dried flower with ensiform bracts and drawing of fruit, holotype of *P. tolimana*, Kew Garden herbarium (photograph Maxime Rome) **G** fruits of a specimen from Venezuela (initially determined as *P. gleasonii*, photograph Miguel Molinari) **H** fruit of *P. acuminata* from Santa Bárbara, Pará, Brazil (photograph Luis Otavio Adão Teixeira). Scale bar: 1 cm.

As mentioned by Lehmann for *P. tolimana* (Killip 1938), fruits are rare, however we could gather observations from Colombia, Brazil and Venezuela (Díaz 829; photographs F, G, H), showing strikingly similar fruits, elliptic to fusiform, slightly ribbed, yellowish green, with a soft epicarp and very thin mesocarp, subhexagonal in cross sectional view. This convergence on an exceptional trait provides further support to the synonymization of the four taxa.

More variation can be observed in bracts and perianth color. Thus, we find various forms, with limited geographic consistency: long and broad bracts north of the Amazon (from the Brazilian states of Pará and Amazonas to the south of Venezuela), small and elliptical bracts in the Brazilian Amazonas, Guyana, Colombia and Ecuador, ensiform to short and wide bracts in Colombia and Venezuela. This diversity, coupled with the under-sampling and poor knowledge of this species at the time of the original descriptions, partly explains the multiplication of taxa. In their study of *P. riparia* and its synonyms, Rome and Coppens d'Eeckenbrugge (2019) also noted the relatively wide intraspecific variability of bract size and shape.

The color of the perianth is predominantly white. However, we found two specimens from Venezuela and Colombia with purplish petals and sepals and exhibiting all of the other characteristics of *P. acuminata* (Liesner 16501 and J. Restrepo's photograph). There is also variation in the darkest color of the outer series of filaments, which can range from pink to dark purple, a variation that is also observed within *P. nitida*.

As observed in the specimens associated to the descriptions, there is some variation in the pubescence of the corolla elements and the ovary. While we could not verify the presence of a glabrous ovary on the type of *P. metae*, we could observe a weak pubescence on the ovary of specimens observed very close to the *locus classicus* in the Colombian department of Meta.

In contrast with several other species in the series *Laurifoliae* (e.g. *P. riparia* and *P. ambigua*), we have not observed flowers gathered in pseudoracemes in the analyzed materials.

## Distribution and ecology

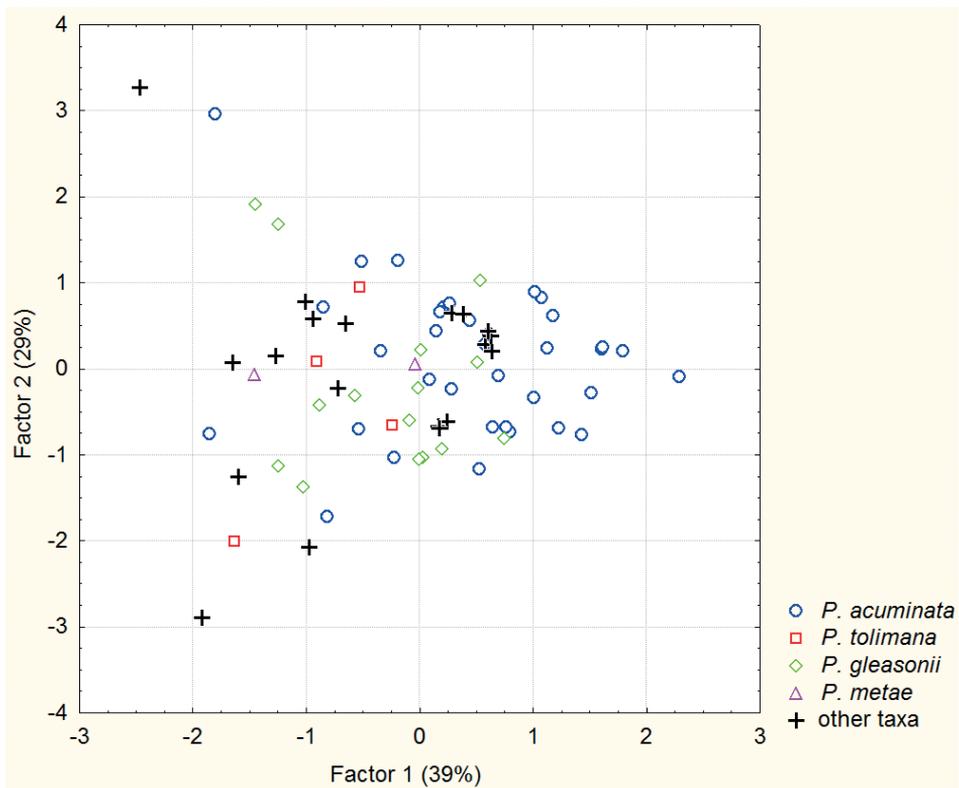
As shown by the global list of examined specimens, *P. acuminata* has so far been documented essentially in Brazil, the country where it was first described. It appears particularly common around Belém, in northeastern Pará and northern Maranhão, but there may be a collecting bias related to the proximity of the Goeldi Museum located at Belém. It had not been collected south of the lower Amazon River (which is consistent with field observation by A. Adair, pers. com.). Elsewhere, in Colombia, Venezuela, and the Brazilian state of Acre, specimens identified as *P. acuminata* correspond to determinations of recent photographs in the framework of the present study, except for Liesner 13280, collected in the Venezuelan state of Zulia, close to Colombia.

A similar situation is observed for *P. tolimana*, described in the eastern Andes of Colombia, with observations and collections relatively concentrated around this same Tolima region and, to the south, in the Andes of Ecuador (including Holm-Nielsen 1040 and Knapp 6242, as redetermined by Rome and Coppens d'Eeckenbrugge 2019), and Peru.

The name of *P. gleasonii* dispersed much further. Indeed, Killip described it from Guyanese specimens, however, all later collections are from Andean foothills, in Colombia and Peru, from Amazonian lowlands, in the southern Orinoco region of Venezuela and Colombia, and western Brazil (upper Amazon and Acre).

The case of *P. metae* is particular as the only geolocalized specimen authenticated by Bonilla Morales is the one photographed by Steven Castro very close to the locus classicus, and very close to specimens of *P. acuminata* with a pubescent ovary.

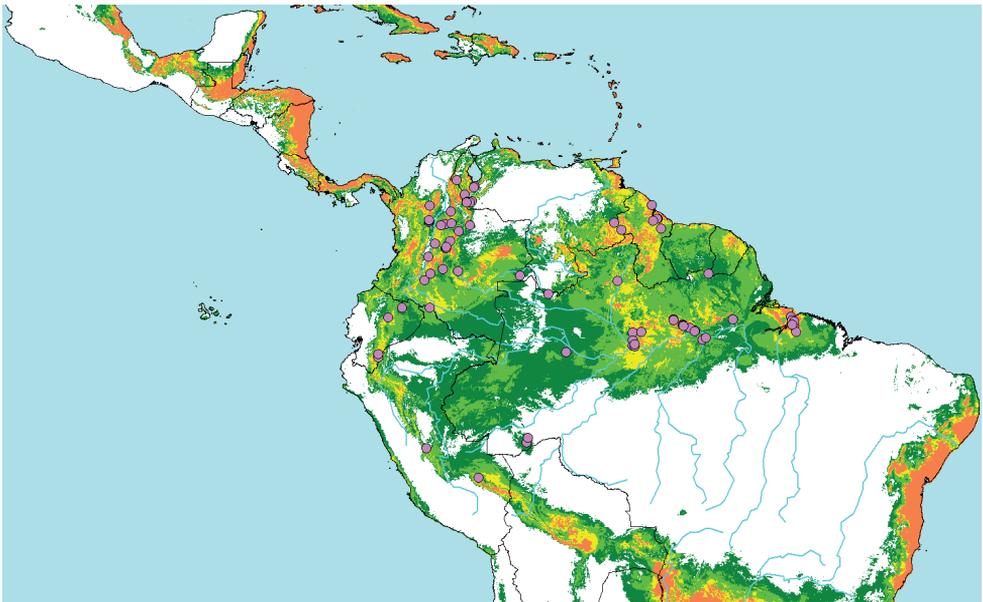
The analysis of the MAXENT model derived from our global dataset (all geolocalized specimens irrespective of their previous assignment) shows that the most determinant bioclimatic variables are the temperature diurnal and annual ranges, the maximum temperature of the warmest month, the mean temperature of the warmest quarter, the precipitation seasonality, with the precipitations of the driest month and those of the driest and coldest quarters. These variables were thence selected for the Principal Component Analysis. The principal plane, accounting for two thirds of the total variance, allowed a fairly good representation of the bioclimatic space of the geolocalized specimens (Fig. 1). The first and main observation is the absence of any



**Figure 1.** Principal plane of the Principal Component Analysis on the eight bioclimatic variables that most contributed to the *P. acuminata* distribution model built from 78 geolocalized specimens. Factors 1 and 2 account for 39 and 29% of total variance respectively. The symbols indicate the initial assignment of specimens.

appreciable differentiation in climatic adaptation between the four taxa under revision. Indeed, the climatic envelope occupied by specimens that had been assigned to *P. acuminata* encompasses that of all the other specimens. Noticeably, the small group of ten specimens showing a score above 1 on the first axis (extreme right of the plane) were all collected close to Belém (seven cases) and Santarém (three cases).

Fig. 2 presents the geographic space corresponding to this climatic envelope, i.e., the potential distribution of *P. acuminata* across South America and Central America. This wide distribution appears to be split between two major regions: to the east, from the Guianas to the lower Amazon River, up to the Rio Negro and southeastern Venezuela, favorable climates are mostly found at low elevations, well under 200 m (except for de Granville 1340, Wurdack 4954, and Watson's observation, from French Guiana, Guyana and Venezuela, at elevations around 500 m); to the west, in the upper Orinoco region and along the Andean foothills and valleys, from western Venezuela to Peru, *P. acuminata* has mostly been reported at elevations comprised between 200 and 2200 m. However, it is also present below 200 m, in Andean valleys and low Orinoquian and Amazonian regions of Venezuela and Colombia. These major, eastern and western, regions are linked by extensive lowland areas where climates are mostly classified as marginal to the species and its presence appears sporadic. However, some of these areas are of difficult access and have been poorly explored (e.g. basins of the Javari River in western Brazil and the upper Amazon River in Peru, except for the vicinity of Iquitos).



**Figure 2.** Potential distribution model for *P. acuminata*; derived from the 78 geolocalized specimens of the present study (pink circles) and MAXENT bioclimatic modeling. Background colors indicate climate suitability: marginal (dark green), favorable (light green; above 10% training omission threshold), very favorable (yellow; above 33% omission threshold); excellent (orange; above 67% omission threshold).

On the other hand, several areas presenting favorable to excellent climates according to the MAXENT model, are not supported by effective observations of the species. In the eastern region, the absence of the species south of the lower Amazon, as observed by A. Adair (pers. com.) is not precisely explained by the model, as the climate appears favorable there. More strikingly, while several highly favorable areas are predicted along the shores of Suriname and French Guiana, the presence of *P. acuminata* has never been confirmed in northern French Guiana despite extensive collecting activity (Rome and Coppens d'Eeckenbrugge 2016, 2020). In the western region, the distribution of specimens mostly correlates with the MAXENT model results for areas along the northern Andes (Colombia and Ecuador). The relative rarity of observations in the climatically favorable upper Orinoco can be easily explained by the lack of botanical exploration in this part of Colombia (Ocampo Pérez et al. 2010). But such an explanation does not hold for the absence of observations in the Colombian Western Cordillera as well as in the Colombian, Ecuadorian and Panamanian Chocó, and further north into Central America, all contiguous areas whose climates appear highly favorable for *P. acuminata*. Indeed, *P. nitida* and *P. ambigua* have been repeatedly collected there, but no *P. acuminata*.

The MAXENT bioclimatic model also points to very favorable areas along the Central Andes, southward to Bolivia and southern Brazil, but they are not met by any observations beyond Peru.

The lack of observations of *P. acuminata* in several extensive areas that combine highly favorable climates and a relatively good level of botanical prospection suggests that the species distribution is significantly constrained by non-climatic factors, justifying further ecological analyses. Before such studies are carried out, our MAXENT distribution model can be considered as a guide for collecting more data.

The ecological information associated with herbarium specimens and the precise geolocalization associated with iNaturalist photograph databases allow inquiring on habitat parameters at a smaller scale, either from label information or from available geographic information (e.g. aerial views).

As for *P. riparia* (Rome and Coppens d'Eeckenbrugge 2019) and *P. nitida*, *P. acuminata* appears associated to riparian habitats. Out of our 78 records, 69 provide information on humidity, with 48 positive cases (close proximity to streams, lakes, ponds, or temporarily flooded areas). Another frequent element of habitat descriptions is the mention of sandy soils, mostly white sand savanna or “campina” habitat (31 cases), particularly in Amazonian and Orinoquian lowlands. Although not signaled in the specimen label, these white sands are very common in the Guyanese region of the *locus classicus* of *P. gleasonii*. In contrast, only one case of clay soil and one of rocky soil are signaled. A rocky soil is also likely for Idrobo 868, collected on the Cordillera Macarena slopes in Colombia. Finally, 51 records point to disturbed vegetations, in contrast with only eleven simply mentioning a forest habitat. The latter is not surprising for a liana, benefitting from clearings in the forest, and easier to observe and collect along roadsides and in open habitats.

This first ecogeographical approach has provided no evidence of differential climatic adaptation among specimens previously assigned to *P. acuminata*, *P. tolimana*,

*P. gleasonii* or *P. metae*. The available herbarium information points to a common association with riparian habitats and/or white sand soils, particularly in lowland areas. Further studies, involving soil information, and more collections, are needed to identify non-climatic factors limiting the presence of *P. acuminata* in a few climatically favorable areas.

### Taxonomic treatment

All previous observations confirm that it is not possible to differentiate several species among the four taxa under study on morphological and/or ecological bases. Thus, it is necessary not only to resurrect *P. acuminata* but also to place the other species as synonyms of the latter. Furthermore, as the type of this species is not sufficiently explanatory, we propose an epitype, Swallen 5095 (Plate 2), presenting all the elements necessary for the morphological understanding of the species, following the suggestion of the international code of nomenclature (Turland et al. 2018).

#### ***Passiflora acuminata* DC., Prodr. [A. P. de Candolle] 3: 328. 1828.**

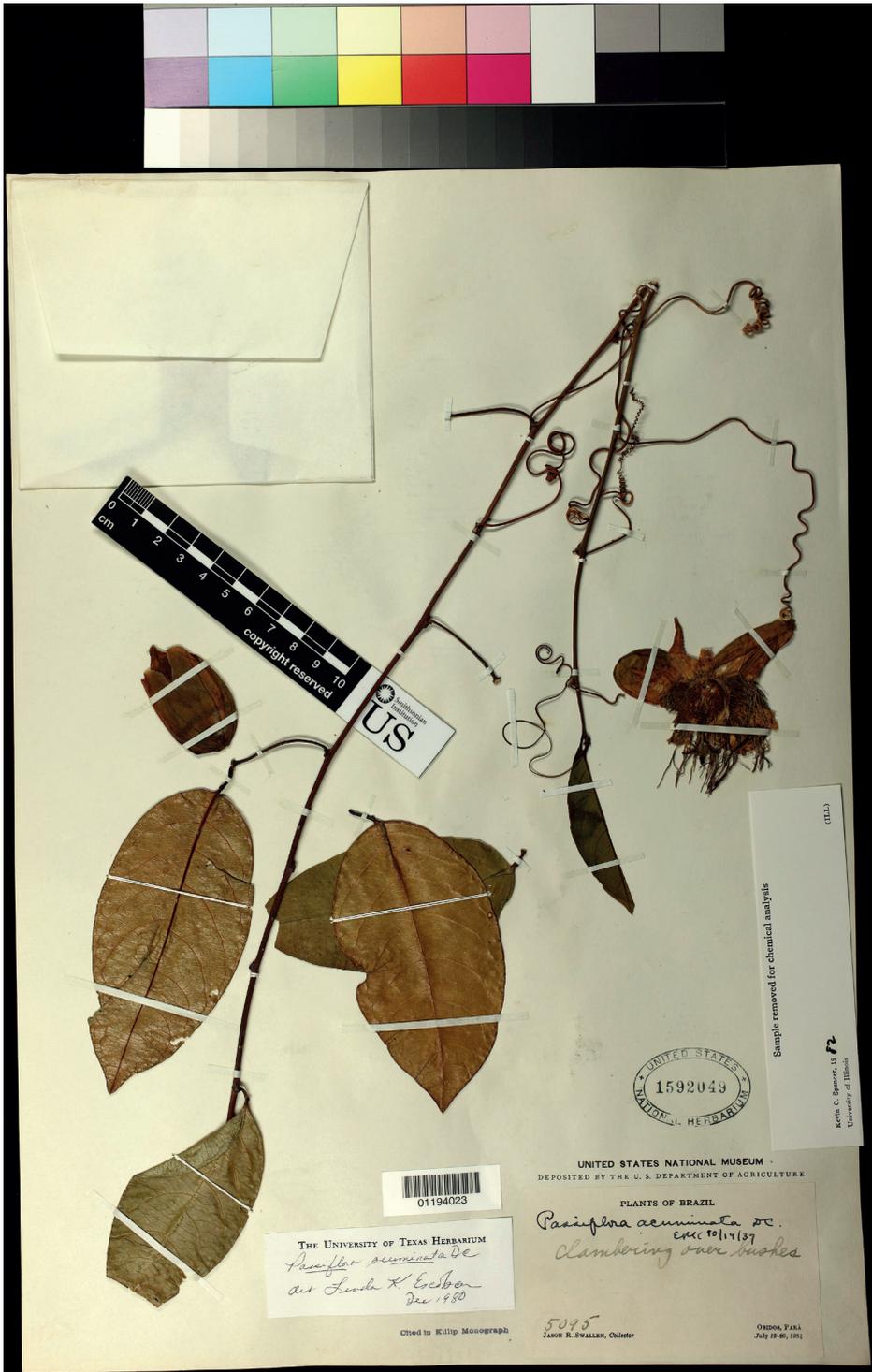
*Passiflora tolimana* Harms, Bot. Jahrb. Syst. 18 (Beibl. 46): 9. 1894, syn. nov. Type: Colombia, Tolima: Dolores, 23 January 1886, *F.C. Lehmann 6060* (holotype: K! [K000323465]; isotypes: B! [B16561], F! [V0066827F])

*Passiflora gleasonii* Killip, J. Wash. Acad. Sci. 14: 112–113. 1924, syn. nov. Type: Guyana: Distr. Pomeroon. Along the Pomeroon River, 14–20 Jan 1923, *J.S. de la Cruz 2963* (holotype: US! [US1123194]; isotypes: CM! [CM164669], GH! [GH3043413], MO! [MO3043413]).

*Passiflora metae* M. Bonilla, C. Aguirre & C. Caetano, Phytotaxa 267(2): 130, f. 1–5. 2016, syn. nov. Type: Colombia, Meta: Villavicencio Municipality: road Caño Pendejo, 541 m, 23 December 2013, *M. Bonilla, J. Mosquera, K. Pulido & A. Cañar 167* (holotype: CUVC! [CUVC067410]).

**Type.** Brazil. Anonymous, s.n. (holotype, P! [P00605761]). Brazil, Pará, Obidos, 19–20 July 1934, *Swallen 5095* (epitype: US! [US1592049]) designated here.

**Description.** Woody liana. Stems rounded to angular, glabrous and green. Tendrils conical, glabrous. Stipules linear, upper part slightly broader and irregularly glandular-serrate, glabrous, 4–18 mm long, about 1 mm wide, deciduous. Petiole 1–2 cm, green to reddish green, slightly canaliculate on the upper part, glabrous, with two sessile and ovate glands (young glands pyramidal) below the apex or at the apex of petiole. Leaves simple, 6–19 × 2.2–9 cm, glabrous throughout, green to dark green, upper surface lustrous, ovate lanceolate to elliptic, cuneate to cordate at base, mucronate and acuminate; margin entire to glandular-serrulate; nerves often reddish. Peduncle terete, green to reddish green, glabrous to slightly pubescent, about 1–2 mm in diameter, 25–50 mm long; pedicel 4–8 mm long. Bracts persistent (until complete ripeness of fruit), ovate to narrow elliptic, apex rounded to acumi-



**Plate 2.** Specimen Swallen 5095 [US01194023], designated as epitype of *Passiflora acuminata* (copyright US National Herbarium).

nate, base cuneate to rounded, glabrous to slightly pubescent, green to dark red, concave, 15–40 mm long, 4–15 mm wide, with a margin glandular to glandular-serrulate. Flowers, axillary, pendulous, about 3 cm long (from the nectary chamber to the ovary apex), solitary. Hypanthium (including the nectary chamber) slightly pubescent, green with red dots outside and white inside, about 5 mm long, with a diameter of about 10 mm at the sepal base. Sepals glabrous to slightly pubescent, oblong, 20–32 mm long, 6–10 mm wide, white (rarely lavender), slightly keel-shaped in distal half with a short to medium awn (1–5 mm long). Petals glabrous, oblong, 15–32 mm long, 5–8 mm wide, white (rarely lavender). Corona of filaments in five series, banded with white and red to purple or dark violet; two major outer series equal 38–51 mm long, the third series 2–3 mm long, the fourth series 0.8–1 mm long, the innermost series, about 10 mm long, with filaments sometimes fused at base, almost perpendicular to the other series, closing the hypanthium entrance. Staminal filaments 9–10 mm long, greenish white with red dots. Ovary pubescent, 5–6 × 3 mm, ellipsoid, yellowish green; three styles (their base can be pubescent), white with red dots, 14–15 mm long, stigmas light yellow. Androgynophore glabrous, greenish white, slightly to densely dotted with red, 20–23 mm long with an enlarged base, with two bulges about 10 mm in diameter. Limen annular, less than 1 mm long. Operculum membranous, translucent-whitish, 3–8 mm long, inflexed at base, the margin erect, short-toothed. Fruit ellipsoid with conical apex to fusiform, pubescent, 6–9 cm long, 3.6–6.5 cm in diameter, triangular to hexagonal in transversal section, slightly ribbed, pericarp 6–10 mm thick; unripe fruit green with minute white dots; ripe fruit yellowish green, minutely dotted, with a sweet translucent pulp. Seeds obovoid, flat, heart-shaped, about 10 mm long.

## Conclusion

Following our morphological and ecogeographic analyses, the name of *P. acuminata* is resurrected and *P. metae*, *P. gleasonii*, and *P. tolimana* are placed as synonyms of this taxon, which reduces the current number of species belonging to series *Laurifoliae* to 18. Thus, this species is unique by its combination of characters: two nectar glands at the apex of petiole, two equal outer series of filaments, longer than petals and sepals, a hypanthium reduced to the nectar chamber, an innermost series of filaments closing the nectar chamber, a slightly pubescent ovary, and an elliptical to fusiform fruit, triangular to hexagonal in transverse section, with a thin pericarp. In the series *Laurifoliae*, its floral structure is similar to that of *P. kapiensis* Rome & Coppens; however the latter has wider leaves, rounded fruits and two glands at the middle of the petiole.

Like several other species in the series, such as *P. laurifolia*, *P. nitida* or *P. riparia*, *P. acuminata* is widely distributed in the basins of the Amazon and the Orinoco and in Andean hillsides, and these four species probably show an appreciable level of sympatry. In the Andes, it might also share habitat with *P. ambigua* (Rome and Coppens d'Eeckenbrugge 2019). Further revision in the series may confirm that *Laurifoliae* species

with a narrow distribution are less frequent than previously thought; this renews our interest in the mechanisms that subtend the evolution of species that are so similar in their morphology and ecology, and thence sympatric in wide areas within the Neotropics.

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# *Oreocharis guangwushanensis*, a new species of Gesneriaceae from Sichuan Province, China

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## Abstract

A new species of *Oreocharis*, *O. guangwushanensis* from the Sichuan Province of south-western China, is described and illustrated here. This new species has a pink corolla that is different from other species of *Oreocharis* in southwest China and, although it is morphologically similar to *O. ronganensis* and *O. reticuliflora*, it has significant differences in the colour and shape of the corolla, the apex of the corolla limb, shape and indumentum of the filaments and a shorter pistil. A detailed description, colour photographs, distribution and habitat, as well as the IUCN conservation status, are also provided.

\* These authors contributed equally to this work.

**Keywords**

Didymocarpoideae, Lithophilous, new taxon, pink flowers, Sichuan flora

**Introduction**

After *Oreocharis* Bentham was redefined by Möller et al. (2011), it has become a large and morphologically diverse genus in Gesneriaceae Rich. & Juss. (Möller et al. 2011, 2014; Chen et al. 2014). There are 136 species and 15 varieties of *Oreocharis* in China, mainly in southern and south-western China (Wen et al. 2021; Yang and Shi 2021; GRC. 2022). Other species are distributed in north-eastern India, northern Vietnam, Japan, Myanmar, Bhutan and Thailand (Wang et al. 1990, 1998; Li and Wang 2004; Möller et al. 2017, 2018; Xu et al. 2017; Chen et al. 2018; Wei 2018).

In July 2020, three authors (ZLL, HJM, ZRY), found this plant in Guangwushan Provincial Nature Reserve during their plant diversity survey. They found this species growing on a large boulder by the roadside, but with only a small number of individuals. On the same day, they found plants of this species in flower at the bottom of a cliff. This population had more individuals than the population on the boulder, with many individuals in flower and fruit, from which they collected specimens. After further investigation, they found that there were only two populations in the Guangwushan Reserve. The author (HX) has seen this plant before, but mistakenly thought it was *Oreocharis farreri* (Craib) Mich.Möller & A.Weber, based on its vegetative habit because it was not in flower.

After consulting some Gesneriaceae monographs (Li and Wang 2004; Wei et al. 2010) and comparing the species with other described congeners (Wang et al. 1990, 1998; Chen et al. 2016; Wei et al. 2016; Do et al. 2017; Li et al. 2017; Yang et al. 2017; Guo et al. 2018; Möller et al. 2018; Pan et al. 2019; Yang et al. 2019; Cai and Dao 2020; Chen et al. 2020; Qin et al. 2020) and specimens of Gesneriaceae deposited at IBSC, IBK, KUN, PE, US and VMN, we referenced the older specimen information and articles from other species. Then we confirmed that it is a new species.

**Materials and methods**

The measurements and morphological characteristics of the new species were taken from the type specimens processed by the authors. We examined *Oreocharis* specimens in IBSC, IBK, KUN, PE, US and VMN, to find species that are morphologically similar. We confirmed it to be an undiscovered species. The type specimens of the new species were deposited in IBK and AHU and living individuals were cultivated at the Gesneriad Conservation Center of China. All morphological characters were studied under a dissecting microscope and were described using the terminology used by Wang et al. (1998).

## Taxonomic treatment

### *Oreocharis guangwushanensis* Z.L.Li & Xin Hong, sp.nov.

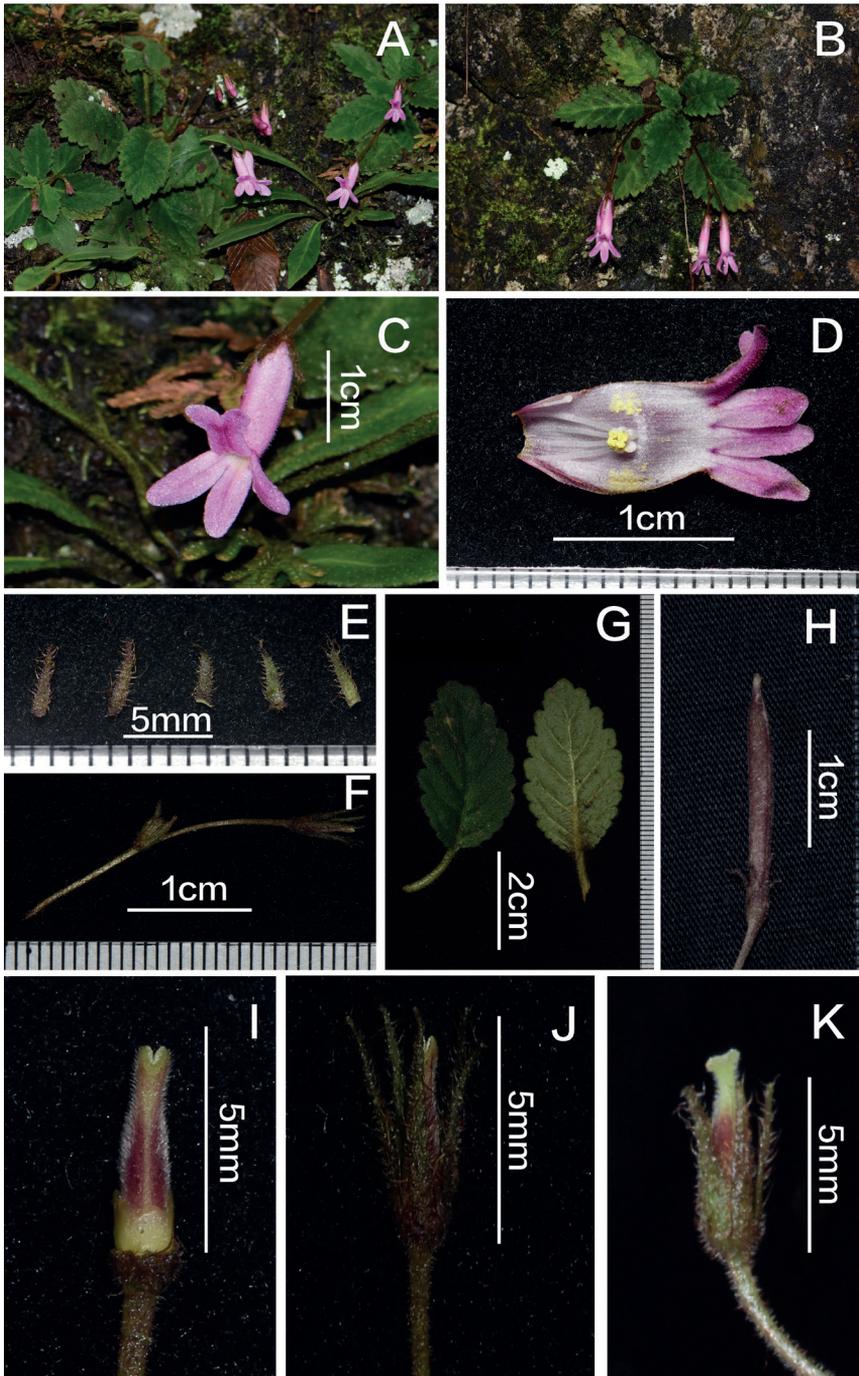
urn:lsid:ipni.org:names:77300515-1

Fig. 1, Table 1

**Diagnosis.** *Oreocharis guangwushanensis* morphologically resembles *O. ronganensis* and *O. reticuliflora*. The new species is vegetatively similar to *O. ronganensis*, but it differs from the latter in that the adaxial lip is 2-lobed to or above the middle (*vs.* lobed to near base), ovary with white pubescence (*vs.* glabrous), anthers in pairs (*vs.* anthers free), shorter tube (9–14 mm *vs.* 20 mm), shorter pistil (5 mm *vs.* 12–15 mm) and filaments strongly twisted and bent at the top (*vs.* linear, straight). The new species resembles *O. reticuliflora* in habit, flower tube and the shape of the calyx, but differs by its pink limbs (*vs.* limbs with a network of violet stripes), filaments strongly twisted and bent at the top (*vs.* linear, straight), anthers in pairs (*vs.* anthers free), ovary with white pubescence (*vs.* glandular-pubescence) and shorter pistil (5 mm *vs.* 10–12 mm).

**Type.** CHINA. Sichuan province: Guangwushan Provincial Nature Reserve, 32°39'N, 106°45'E, 988 m a.s.l., growing on limestone cliffs near a river, 31 July 2020, flowering and fruiting, Hai Jun Ma, *MHJ 21073102* (holotype: IBK; isotype: AHU).

**Description.** Perennial herb. **Leaves** basal, spirally arranged; petiole 2–10 cm long, densely brown pilose and pubescent; lamina thick herbaceous, oblong to lanceolate, 4–8 × 1.6–5 cm, base slightly asymmetrical, wedge-shaped to round, margin serrate, apex obtuse to acute, concave adaxially, dark green, densely brown pubescent; prominent abaxially, light green, densely pubescent, sparsely rust-brown long pilose; ca. 5–6 pairs of lateral veins on each side of the mid rib. **Cymes** 1–3, axillary, pair-flowered, 1–3 branches, 1–3 (–8) flowers; peduncles 4–10 cm long, densely rusty glandular-puberulent and sparsely strigillose; bracts 2, opposite, lanceolate, ca. 4 × 0.9 mm, densely brown pilose. Pedicel 1.5–5 cm long, densely rust-brown pilose and pubescent. Calyx 5-lobed to base, narrowly lanceolate, apex acute, ca. 5 × 0.9 mm, outside brown with brown strigose indumentum, inside green, brown pilose, most densely on the margins. **Corolla**, pink, 1.8–2.6 cm long, densely glandular pubescent on both sides; tube cylindrical, constricted at the throat, slightly upwards curved, 9–14 mm long, 3 mm in diameter at the mouth; limb distinctly 2-lipped; adaxial lip 2-lobed to or above the middle, lobes 2 mm long, apex suborbicular, curving left and right, red stripe in the middle of the two fused upper lobes; abaxial lip 3-lobed to the base, lobes 6 mm long, oblong, with a red line in the middle of the lobes. **Stamens** 4, in pairs, glabrous, adnate to ca. 1–2 mm above the corolla base; filaments 5–7 mm long, strongly twisted and bent at the top, bent close to 270°, hooked, with white glands at the base, filaments of anterior pair 5–6 mm long, of posterior pair 6–7 mm long. Anthers yellow, ca. 0.8 mm long, fused by their entire adaxial surfaces. Staminode 1, linear, ca. 3 mm long, top slightly enlarged, adnate to ca. 2 mm above the corolla base; disc ring-like, about 1.5 mm high, with repand margin. **Pistil** mature ca. 6–7 mm long, at flower opening immature ca. 4.5 mm long, ovary linear-oblong, 2 mm long, reddish-brown, densely white pubescent. Style at pistil maturity ca. 1 mm long, densely pilose. Mature stigma



**Figure 1.** *Oreocharis guangwushanensis* Z.L.Li & Xin Hong **A** flowering plants in natural habitat **B** plant with pair-flowered cymes **C** corolla front view **D** opened corolla **E** dissected calyx lobes, outside brown with rusty strigose indumentum (3 left), inside green with brown pilose indumentum on margins (2 right) **F** peduncle, pedicel and young lateral branch **G** leaves (left: adaxial surface, right: abaxial surface) **H** capsule **I** immature pistil **J** immature pistil with calyx **K** mature pistil with calyx.

**Table 1.** Diagnostic character differences between *Oreocharis guangwushanensis* and its morphologically close relatives *O. ronganensis* and *O. reticuliflora*.

Character	<i>O. guangwushanensis</i> Z.L.Li & Xin Hong	<i>O. ronganensis</i> (K.Y.Pan) Mich.Möller & A.Weber	<i>O. reticuliflora</i> Li H. Yang & X.Z.
<b>Cymes</b>	1–3 (–6)-flowered	4–10-flowered	4–14 (–22)-flowered
<b>Tube</b>	9–14 mm long	ca. 20 mm long	12–15 cm long;
<b>Corolla</b>	corolla pink, with a single stripe in the middle of lower lobes	corolla pink, without stripes	corolla blue-purple, with a network of violet stripes on each lobe
<b>Adaxial lip 2-lobed</b>	adaxial lip 2-lobed to or above the middle	adaxial lip lobed to near base	adaxial lip lobed to near base
<b>Filament attachment</b>	2 mm from corolla base	8–10 mm from corolla base	6–8 mm from corolla base
<b>Stamens and anthers</b>	filaments strongly twisted and bent at the top, anthers in pairs	filaments linear, anthers free	filaments linear, anthers free
<b>Ovary</b>	white pubescent	glabrous	glandular-pubescent
<b>Pistil at maturity</b>	6–7 mm long	12–15 mm long	10–12 mm long

with 2 lobes ca. 0.6 mm long, light green; **Capsule** brownish-red, ca. 2–2.5 cm × 0.2–0.3 cm, sparsely pubescent, narrowly oblong.

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

**Etymology.** The specific epithet is derived from the type locality, Guangwushan Provincial Nature Reserve, Sichuan province, China.

**Vernacular name.** Guāng Wù Shān Mǎ Líng Jù Tái (Chinese pronunciation); 光雾山 马铃苣苔 (Chinese name).

**Distribution and habitat.** The new species has so far been found only in the type locality, Guangwushan Provincial Nature Reserve, Sichuan province, China. In the nature reserve, the average temperature is 16.2 °C, while the average annual precipitation has been calculated as ca. 1200 mm. The forest where *O. guangwushanensis* occurs is a monsoon evergreen broad-leaved forest.

**Preliminary conservation assessment.** Currently, the new species has been observed only from the type locality. After two years of careful investigation, only two small populations of *O. guangwushanensis* have been found. They are less than 200 m apart. In total, ca. 200 mature individuals were present within 4 km<sup>2</sup> (AOO). One population of no more than 40 individuals was growing at a higher altitude of 1124 m on a boulder near a road; the other population on a limestone wall at lower altitude 988 m by a river, close to the scenic area of the Guangwushan Provincial Nature Reserve which attracts many visitors. The locations of both populations are easily accessible and frequently passed by tourists, and thus, the number of individuals is likely to be detrimentally affected. The natural habitat could be disturbed or changed by human activities such as road expansion and other building construction. Following the IUCN Red List Categories and Criteria (IUCN 2019), the new species is provisionally assessed as Critically Endangered [CR B2ab(iii)].

## Acknowledgements

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# Taxonomic notes on *Sorbus megalocarpa* (Rosaceae) and related taxa

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## Abstract

Four *Sorbus* taxa endemic to China, *S. arguta*, *S. guanxianensis*, *S. megalocarpa* var. *megalocarpa* and *S. megalocarpa* var. *cuneata*, are morphologically similar to one another in having large brown fruits with persistent calyx and dense lenticels. In literature, either all of the four taxa were accepted, or two of them, *S. arguta* and *S. megalocarpa* var. *cuneata*, were treated as synonyms of *S. megalocarpa* var. *megalocarpa*, or *S. guanxianensis* alone was dubious. In this study, based on morphological comparison, *S. arguta* is reinstated for its relatively small inflorescence, small fruit and timing of flowering after leaves are unfolded. *S. megalocarpa* var. *cuneata* is confirmed as a synonym and *S. guanxianensis* is proposed as a new heterotypic synonym of *S. megalocarpa*.

## Keywords

resurrection, *Sorbus*, synonymization, taxonomy

## Introduction

*Sorbus* L. (1753: 477; Rosaceae) in the broad sense (*sensu lato*, *s.l.*) comprises about 100 to more than 250 species mainly distributed in northern temperate regions with the center of diversity in China (Phipps et al. 1990; Lu and Spongberg 2003). Although previous molecular studies indicated that *Sorbus s.l.* is highly polyphyletic with species falling into six genera: *Aria* (Pers.) Host, *Chamaemespilus* Medik. (1879: 138), *Cormus* Spach, *Micromeles* Decne. (1874: 168), *Sorbus* and *Torminalis* Medik. (1874: 134; Campbell et al. 2007; Li et al. 2012; Lo and Donoghue 2012; Sun et al. 2018; Ulaszewski et al. 2021), the taxonomic diversity of *Sorbus* in China is being included within a single genus

(Yü and Lu 1974; Lu and Spongberg 2003). The number of species native to China recognized varies tremendously according to different taxonomists (Yü and Lu 1974; Phipps et al. 1990; Lu and Spongberg 2003; Aldasoro et al. 2004; McAllister 2005). For example, in the latest revision of *Sorbus* subg. *Aria* Persoon and *Torminaria* (DC.) Reichenbach, Aldasoro et al. (2004) accepted only 21 species out of the total 31 species and 6 varieties recognized by Lu and Spongberg (2003). The striking inconsistencies in taxonomic treatments have given rise to confusion in species identification and utilization. The controversial delimitation of *S. megalocarpa* Rehder (1915: 266) and its allies, *S. megalocarpa* var. *cuneata* Rehder (1915: 267), *S. arguta* T. T. Yü (Yü and Kuan 1963: 223) and *S. guanxianensis* Ku (1990: 22), is an example here. The four taxa were all accepted by Yü and Lu (1974), Lu and Spongberg (2003). *S. arguta* and *S. megalocarpa* var. *cuneata* were treated as synonyms of *S. megalocarpa* and *S. guanxianensis* was regarded as a doubtful species by Aldasoro et al. (2004). The purpose of this paper is to clarify the taxonomic confusion and to enhance stability of these names based on protologues, related literature studies, original materials examinations and field investigations.

## Materials and methods

Type collections and voucher specimens of *Sorbus arguta*, *S. guanxianensis*, *S. megalocarpa* var. *megalocarpa* and *S. megalocarpa* var. *cuneata* were examined from the following herbaria: A, CDBI, E, GH, IBSC, KUN, NF, PE, WCSBG and US (acronyms follow Thiers continuously updated); virtual images were examined mainly through the website PPBC (<http://ppbc.iplant.cn/>). Morphological comparison presented here is based on analysis of specimens, as well as fresh materials collected by ourselves.

## Taxonomic treatments

### 1. *Sorbus megalocarpa* Rehder, Pl. Wilson. 2(2): 266. 1915.

- ≡ *Aria megalocarpa* (Rehder) H. Ohashi et Iketani, J. Jap. Bot. 68(6): 359. 1993.
- ≡ *Micromeles megalocarpa* (Rehder) Mezhenkyj, NULESU Coll. Fruit Ornament. Pl.: 34. 2018.
- ≡ *Wilsonaria megalocarpa* (Rehder) Rushforth, Phytologia 100(4): 241. 2018.
- = *Sorbus megalocarpa* var. *cuneata* Rehder, Pl. Wilson. 2(2): 267. 1915. Type: CHINA. Sichuan: Western Szechuan, Mupin, 2400–2700 m, 10 October, 1910–11, E.H. Wilson 4215 (lectotype, designated by Aldasoro et al. 2004, pg. 43: K[K000758157]; isolectotype: A[A00112653])
- = *Aria megalocarpa* var. *cuneata* (Rehder) H. Ohashi et Iketani, J. Jap. Bot. 68(6): 359. 1993.
- = *Sorbus guanxianensis* T.C. Ku, Bull. Bot. Res., Harbin 10(3): 22, f. 2. 1990. syn. nov. Type: CHINA. Sichuan: Guanxian (Dujiangyan), 2000 m, 25 August 1987, T.Z. Fu et al. 872102 (holotype: PE[PE00020830]), syn. nov.

- = *Micromeles guanxianensis* (T.C. Ku) Mezhenkyj, NULESU Coll. Fruit Ornament. Pl.: 34. 2018. syn. nov.  
= *Wilsonaria guanxianensis* (T.C. Ku) Rushforth, Phytologia 100(4): 241. 2018. syn. nov.

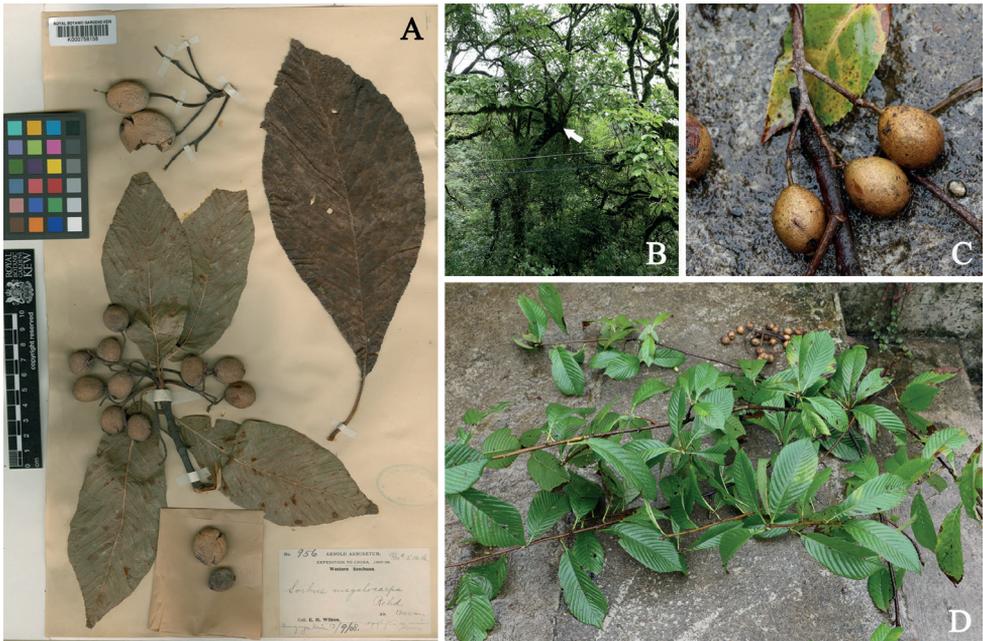
**Type.** CHINA. Sichuan: Western Szechuan, Hung-yah Hsien (Hongyaxian), 1200 m, 12 September 1908, *E.H. Wilson 956* (lectotype, designated by Gabrielian 1978, pg. 220: K[K000758158]; isolectotypes: A[A00112650], E[E00147452], GH[GH00112651], US[US00097467]).

**Notes.** *Sorbus megalocarpa*: *Sorbus megalocarpa* was first published by Rehder (1915: 266). It was transferred to genera *Aria*, *Micromeles* and *Wilsonaria* by Ohashi and Iketani (1993: 359), Mezhenkyj (Mezhenska et al. 2018: 34) and Rushforth (2018: 241) respectively.

Three gatherings collected by Wilson under number “956” were cited in the protologue. The first one was collected at alt. 2200–2600 m., Mupin, in October 1910; the second one was collected at alt. 1200 m., Hung-ya Hsien, on September 12, 1908; and the third one was collected at alt. 2000 m., Mon-kong Ting, on June 19, 1908. Since the author did not indicate holotype for the name, the three gatherings are syntypes according to the Article 9.6 of the *International Code of Nomenclature for algae, fungi and plants (Shenzhen Code)* (Turland et al. 2018). Gabrielian (1978) designated the specimen at K (K000758158; as shown in fig. 1A, plate 62 of Gabrielian 1978), collected from Hung-Ya Hsien, as the lectotype. Four duplicates (A00112650, E00147452, GH00112651, US00097467) out of the eight specimens of “*E. H. Wilson 956*” traced at A, E, GH, K and US, are the isolectotypes here.

*Sorbus megalocarpa* var. *cuneata*: Rehder (1915: 267) differentiated *Sorbus megalocarpa* var. *cuneata* from *S. megalocarpa* var. *megalocarpa* by its “smaller softer fruit” (“fructibus minoribus ovooides circiter 1.5 cm. longis et 1 cm. diam.” and “the more cuneate short-stalked leaves” (“petiolum vix 1 cm”). This variety was recognized by Yü and Lu (1974) and Lu and Spongberg (2003), was transferred to *Aria* by Ohashi and Iketani (1993: 359) and was treated as a synonym of *S. megalocarpa* by Aldasoro et al. (2004). The great variability of leaves and fruits of *S. megalocarpa* was well documented by Aldasoro et al. (2004) and confirmed in our field investigations (Fig. 1B–D). *Sorbus megalocarpa* has elliptic, elliptic-obovate, obovate-oblong leaves with crenate-serrate margins and petiole of 0.7–2 cm long, and large ovoid, ovoid-globose, or sub-globose fruits (1–2.7 cm long, 0.7–2.2 cm in diameter) covered with dense lenticels. The length of petiole and size of fruits of *S. megalocarpa* var. *cuneata* are within the variation range of *S. megalocarpa*. Therefore, we agree with Aldasoro et al. (2004) in reducing *S. megalocarpa* var. *cuneata* to a synonym of *S. megalocarpa*.

*Sorbus guanxianensis*: Ku (1990: 22) published *Sorbus guanxianensis* based on two gatherings, “*T. Z. Fu et al 2102*” (Fig. 2A) and “*Z. L. Zhao 0970*”. In the protologue, Ku (1990) included the diagnostic words “calycis lobi mox decidui”, compared it with *S. alnifolia* (Siebold and Zuccarini) K. Koch in *Sorbus* sect. *Micromeles* and differed it by its larger fruits (about 1.5 cm long), though she assigned it to *Sorbus* sect. *Aria* (Ku 1990). This contradictory taxonomic description led later authors to treat *S. guanxianensis* in different circumscriptions. Phipps et al. (1990) and Lu and

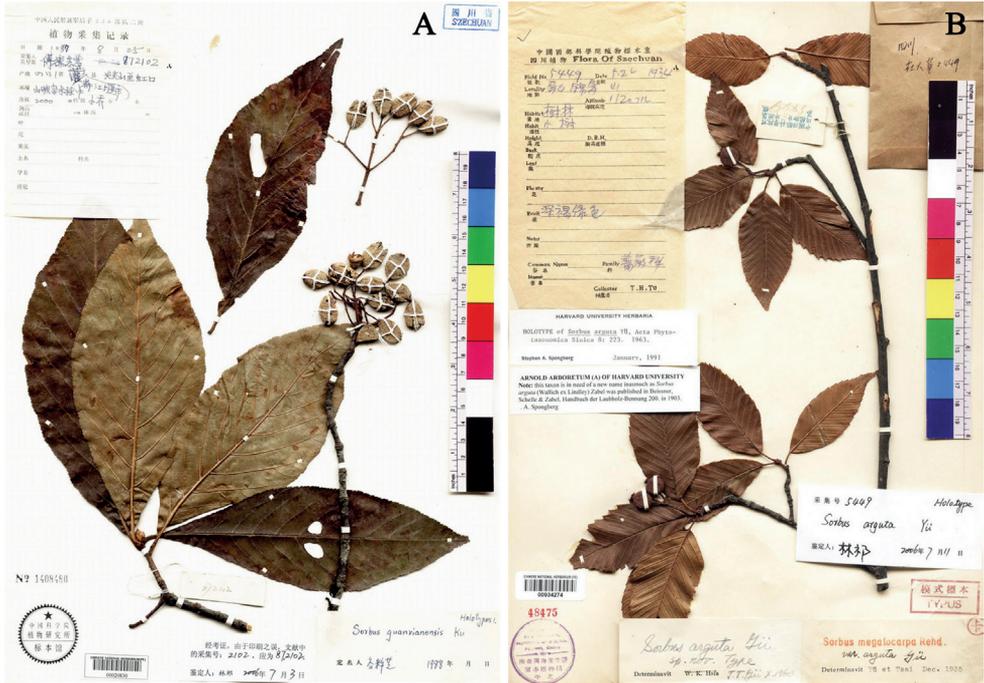


**Figure 1.** *Sorbus megalocarpa* Rehder **A** lectotype (K000758158) **B** an epiphytic plant in the wild (Ya'an, Sichuan, China) **C** fruit (showing the color of fruit and lenticels on it) **D** leaves (showing the variation of leaf shape and petiole length in one plant).

Spongberg (2003) accepted it. Mezhenka et al. (2018: 34) and Rushforth (2018: 241) also recognized it and transferred it to *Micromeles* and *Wilsonaria* respectively. Aldasoro et al. (2004) considered it “a doubtful species” and stated that pomes of *S. guanxianensis* “without lenticels” and “may be a synonym of *S. zahlbruckneri*”.

No specimens of *Sorbus guanxianensis* other than the two gatherings cited in the protologue are available in herbarium. Our examination of the type specimens indicated that characters such as persistent calyx and dense lenticels on pomes were in serious conflict with the description in the protologue and were neglected by Aldasoro et al. (2004). *Sorbus guanxianensis* could easily be distinguished from *S. zahlbruckneri* C. K. Schneider by the leaves which have margins “singly dentate (not double-dentate)” as stated by Aldasoro et al. (2004) themselves. The unusual characters possessed by the type specimens were noted by Rushforth (2018) who stated that *S. guanxianensis* “seems to match Rehder’s *S. megalocarpa* var. *cuneata*” and transferred it to *Wilsonaria* together with *S. megalocarpa*. Morphological similarities (Styles 3 or 4, leaves glabrous or sparsely hair when young, not tomentose, fruit brown, 12–20 mm in diameter, covered in massed contiguous lenticels) stated by Rushforth (2018), and a detailed critical read of the protologues and evaluation of the specimens confirmed that *S. guanxianensis* is conspecific with *S. megalocarpa*. Accordingly, we proposed to reduce *S. guanxianensis* as a heterotypic synonymy of *S. megalocarpa* here.

**Representative specimens examined.** CHINA. Sichuan: Baoxin county, 17 July 1925, *K.L. Chu* 3149 (IBSC); Dayi county, Xiling town, Chadiping, Xiling Snow



**Figure 2.** **A** holotype of *Sorbus guanzianensis* Ku (PE00020830) **B** holotype of *Sorbus arguta* T. T. Yu (PE00934274).

Mountain, 30°38'24.84"N, 103°09'52.33"E, 1471 m, 1 June 2015, *J.D. Ya and X. J. Hu 15CS11089* (KUN); Dujiangyan, Hongkou town, Dashuigou conservation station, 1250 m, *D.H. Zhu, C. Zhang, X.J. Li 4872* (WCSBG); Hongya county, forest farm, July 1992, *Z.W. Wang A00088* (CDBI); Hongya county, Lewu town, Shuanghekou, 2100–2230 m, 2 August 1959, *Z.T. Guan 9120* (PE); Hongya County, Lewu town, Shuanghekou, 2100–2230 m, 3 August 1959, *Z.T. Guan 6814* (PE); Leibo county, Mahu town, 1300 m, 25 May 1959, *238 collection team 0338* (PE); Leibo county, Shahezhou forest farm, 2400 m, 12 August 1972, *238 collection team 0697* (PE); Ya'an city, Yingjing county, Longchigou National Forest Park, Daxiangling, 29°36'21.23"N, 102°50'28.48"E, 1364 m, 19 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1891* (NF); Ya'an city, Yingjing county, Longcanggou National Forest Park, Diecuixi, 29°36'55.01"N, 102°53'42.57"E, 1509 m, 19 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1906* (NF); Ya'an city, Yingjing county, Longcanggou National Forest Park, Diecuixi, 29°36'57.42"N, 102°53'38.74"E, 1512 m, 19 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1907* (NF); Ya'an city, Yingjing county, Longcanggou National Forest Park, Fazhan village, 29°37'04.96"N, 102°53'25.17"E, 1466 m, 20 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1908* (NF); Ya'an city, Yingjing county, Longcanggou town, Fazhan village, 29°38'15.34"N, 102°53'00.64"E, 1359 m, 20 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1914* (NF); Ya'an city, Yingjing county, Longcanggou town, Fazhan village, 29°37'48.37"N, 102°53'13.75"E, 1358 m, 20 September 2020, *X. Chen, X.Y. Wang, C.H. Wang 1915* (NF).

**2. *Sorbus arguta* T.T. Yu, Acta Phytotax. Sin. 8(3): 223. 1963.**

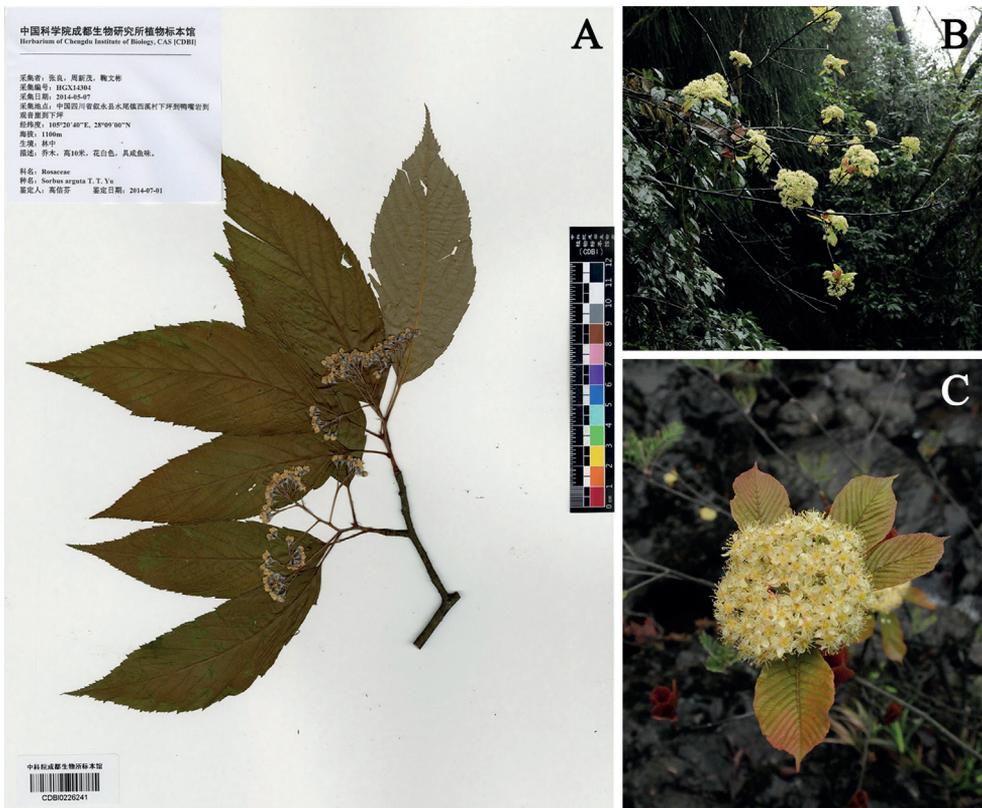
≡ *Micromeles arguta* (T. T. Yu) Mezhenksyj, NULESU Coll. Fruit Ornament. Pl.: 33. 2018.

≡ *Wilsonaria arguta* (T. T. Yu) Rushforth, Phytologia 100(4): 241. 2018.

= *Aria yuarguta* H. Ohashi et Iketani, J. Jap. Bot. 68(6): 361. 1993.

**Type.** CHINA. Sichuan: Pingshan, Chingping Shan, 1120 m, 26 May 1934, *T.H. Tu 5449* (holotype: PE[PE00934274]; isotype: PE[PE00934275])

**Notes.** When describing *Sorbus arguta*, Yü designated “*T. H. Tu 5449*” at PE (Fig. 2B) as the holotype (Yü and Kuan 1963). It was accepted by Yü and Lu (1974), Gabrielian (1978), Phipps et al. (1990) and Lu and Spongberg (2003). Ohashi and Iketani (1993) transferred it to genus *Aria* and proposed a new name *A. yuarguta* H. Ohashi et Iketan (Ohashi and Iketan 1993: 361) for *A. arguta* had been already used by Roemer in 1847 for a different species. Mezhenksyj transferred it to *Micromeles* (Mezhenkska et al. 2018: 33) and Rushforth (2018: 241) transferred it to *Wilsonaria*.



**Figure 3.** Corymbs of *Sorbus arguta* and *S. megalocarpa* **A** flowering specimen of *Sorbus arguta* (CDBI0226241) collected by Liang Zhang, Xinmao Zhou and Wenbin Ju, 7 May 2014 **B** *Sorbus megalocarpa* at blossoming stage, 21 March 2021 **C** detail of the inflorescence of *S. megalocarpa* (**B, C** were taken by Tailun Hu).

However, Aldasoro et al. (2004) argued that: “*S. arguta* is a minor variant of *S. megalocarpa* and does not deserve taxonomic recognition”, and reduced it to a synonym of the later. A detailed study of the original material showed that *Sorbus arguta* is obviously different from *S. megalocarpa*. *Sorbus arguta* has oblong-ovate or ovate-lanceolate leaves with double serrate margins, relatively small corymbs (2–4 cm in diameter) with few flowers, and small sub-globose fruits (1–1.2 cm in diameter) with sparse lenticels, while *S. megalocarpa* has leaves with crenate-serrate margins, large corymbs (10–15 cm in diameter), many flowered (124–258 flowers per inflorescence), and much larger fruits with dense lenticels. Furthermore, *S. arguta* flowers after leaves are unfolded in early May (Fig. 3A, CBDI0226241), whereas *S. megalocarpa* flowers simultaneously with or before the leaves are unfolded in March (Fig. 3B, C). Therefore, *S. arguta* is treated as a distinct species here following Yü and Lu (1974), Gabrielian (1978), Phipps et al. (1990) and Lu and Spongberg (2003).

Representative specimens examined. CHINA. Sichuan: Xinwen county, Xianfeng town, Monkey Bay, 1290 m, 12 May 1959, *Yibin wild economic plants collection team 0368* (CDBI); Xuyong county, Heishuihe Nature Reserve, 1500 m, 6 June 2007, *D.H. Zhu, Z.B. Feng, C. Zhang, F. Wang 20070776* (WCSBG); Xuyong county, Shuiwei town, Xixi village, 28°09'00"N, 105°20'40"E, 1100 m, 7 May 2014, *L. Zhang, X.M. Zhou, W.B. Ju HGX14304* (CDBI); Xuyong county, Shuiwei town, Xixi village, 28°08'01"N, 105°22'20"E, 1230 m, 29 July 2014, *W.B. Ju HGX14833* (CDBI).

## Acknowledgements

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# *Euonymus aquifolium* (Celastraceae): Rediscovered in flowering with respect to its taxonomy, nomenclature, and rarity

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## Abstract

A small population of *Euonymus aquifolium* (Celastraceae) with flowering plants was discovered more than 100 kilometers away from its type locality. The incomplete original description of this species is supplemented here with floral materials since it is known from only two gatherings of fruiting specimens. Its 5-merous flowers and two ovules per locule support its position in *Euonymus*, and this inference is further reinforced by phylogenetic analysis based on the nuclear internal transcribed spacer (ITS) of rDNA. The specific epithet has long been treated as “*aquifolius*” to agree with the generic gender of *Euonymus*. But after examination of the protologues of this and other related species described simultaneously by the same authors, as well as their handwritten annotations attached on the type specimens, we conclude that the epithet *aquifolium* was used as a noun and it should be retained unchanged. Despite this newly discovered population some 100 kilometers away from its type locality, this species is still assessed as Critical Endangered (CR) according to the IUCN Red List Categories and Criteria.

## Keywords

endemic species, *Euonymus aquifolium*, flora of China, floral character, *Glyptopetalum*, nomenclature, taxonomy

\* These authors contributed equally to this work as co-first authors.

## Introduction

*Euonymus aquifolium* Loes. & Rehder in Sargent (1913) was described based on a single gathering<sup>1</sup> collected by E. H. Wilson in 1908 from West Sichuan. It was incompletely known up until now since the three duplicates of the type gathering (*E. H. Wilson 1366*) were branches in fruit, and the other documented gathering (*P. N. Qin et al. 104* collected in 1929) was reported as bearing neither flowers nor leaves (Cheng et al. 1999). The account of this species in the Chinese flora (Cheng et al. 1999; Ma et al. 2008) and Sichuan flora (Chang 1988) was based only on these two gatherings. Being endemic to Sichuan, and without any information on its living population in the wild, it was assessed as a critically endangered species (CR) in the China Biodiversity Red List (Anonymous 2013). However, with its distinctive leaf shape and texture, it would be unusual to be neglected by more recent botanists during their fieldwork in and around the type locality.

Based on uncertain observations on the fruiting materials, the type specimens (from the description in the protologue) and *P. N. Qin et al. 104*, Cheng & Ma in Cheng et al. (1999) transferred this species to the genus *Glyptopetalum* Thwaites (1856). The name *G. aquifolium* (Loes. & Rehder) C.Y.Cheng & Q.S.Ma in Cheng et al. (1999) was followed by Ma et al. (2008) and Anonymous (2013). *Glyptopetalum* is morphologically very close to *Euonymus* Linnaeus (1753), from which it was defined to differ in the exclusively 4-merous flower, single pendulous ovule per locule of ovary, and seed with branched raphe (Thwaites 1856; Ding Hou 1963; Simmons 2004; Ma et al. 2008). Since its publication, some authors either did not mention *Glyptopetalum* (e.g., Kurz 1877; Loesener 1901–1902), or at most treated it as a subdivision of *Euonymus* (e.g., Kurz 1875; Baillon 1877), while others adopted it as a distinct genus and added newly described species or transferring previously described species, mostly from *Euonymus* (e.g., Prain 1891; Ding Hou 1963; Meng et al. 2011). *Glyptopetalum* is a less speciose genus with ca. 20 species than the ~ 130 species of *Euonymus* (Simmons 2004; Ma et al. 2008; Simmons et al. 2012). Subsequent phylogenetic studies revealed unequivocally that the 2–3 sampled *Glyptopetalum* taxa were a clade nested within *Euonymus* (Simmons et al. 2012; Li et al. 2014), such that *Glyptopetalum* should be included within a broadly defined *Euonymus* (Li et al. 2014).

In August 2021, during a field trip of the Second Tibetan Plateau Scientific Expedition in Jiulong County, Sichuan, Southwest China, an area located along the southeastern slope of Mt. Gongga (Minya Konka, the main peak of Hengduan Mountain), a small-sized population of ca. 15 individuals of *Euonymus aquifolium* was unexpectedly encountered by Jun Hu (the first author of this article) and his team members. Some plants were in flower, and the 5-merous flowers instantly reject its identity as a member of *Glyptopetalum*. To better understand this species, its morphological description was thereafter expanded with the observation of living plants and dissection of floral parts. To test whether its generic position inferred using floral characters correspond to the molecular data, a phylogenetic analysis was

conducted by incorporating data from published studies with the addition of samples of this species and its morphologically closest species, *Glyptopetalum ilicifolium* (Franchet 1886) C.Y.Cheng & Q.S.Ma in Cheng et al. (1999), which was collected in 2021 by Chong-Bo Ma & Dong-Liang Lin (*YDYC137* at CDBI) from Xichang, Southwest Sichuan.

## Materials and methods

### Morphological description

*Euonymus aquifolium* was observed as living individuals in the field, and dried herbarium specimens were observed in laboratory, where morphological characters were measured using ImageJ v1.53 k (Schneider et al. 2012). Our description follows the terminology used by Harris and Harris (2001). Voucher specimens were deposited at CDBI (acronym of herbarium follows Thiers 2022).

### DNA extraction, amplification and sequencing

Apart from the newly generated data of *Euonymus aquifolium* and *Glyptopetalum ilicifolium* in this study, all of the other sequences of the 62 samples representing 51 species in molecular phylogenetic analysis were retrieved from GenBank. The accessions are listed in Appendix I. Total DNA was extracted exclusively from silica-gel dried leaves using a Plant DNA Isolation Kit (Cat.No.DE-06111). The same primers and outgroups were used as the phylogenetic analysis of *Euonymus* by Li et al. (2014). The nuclear internal transcribed spacer (ITS) was amplified by polymerase chain reaction (PCR). All DNA samples were sent to TSINGKE Biotech Co. Ltd (Chengdu, China) for sequencing and then deposited to GenBank under the accession number OK172405 for *Euonymus aquifolium* and OM985812 for *Glyptopetalum ilicifolius* (Appendix I).

### Phylogenetic analyses

All sequences were processed with Sequencher v4.1.4 (Gene Codes, Ann Arbor, Michigan, USA), and aligned by using MAFFT v7.475 (Katoh and Standley 2013) with default parameters. Maximum likelihood (ML) and Bayesian inference (BI) methods were applied to infer the gene tree. jModeltest 2.1.6 (Posada 2008) identified GTR+I+G as the best model which selected using the corrected Akaike Information Criterion (AICc). BI analysis was conducted using MrBayes 3.2.7a (Ronquist and Huelsenbeck 2003) with two parallel runs (10 million generations). The first 25% percent of trees from all runs were discarded as burn-in. ML analysis was performed using IQ-TREE v.1.4.241 (Nguyen et al. 2014) with branch support estimated using 2,000 replicates of ultrafast bootstrapping algorithm (UFboot) (Minh et al. 2013).

## Results and discussion

From the field observation, the general morphology of *Euonymus aquifolium* (Figs 1, 2) agreed well with the description in Sargent's (1913) protologue. The fruits were mostly yellowish green, glabrous rather than “squarrose maculate” as documented in the Chinese floras (Cheng et al. 1999; Ma et al. 2008), which might be an error caused by confusing the fruiting characters of *Glyptopetalum ilicifolium* (from our observation of the gathering YDYC137 and referring to Franchet 1886; Cheng et al. 1999; Ma et al. 2008). The flowers were clearly 5-merous, with five sepals, petals, and stamens, and 5-locules in well-developed ovaries. There were 2 ovules at the center of the axis of placenta, and usually only one ovule developing into seed, which might explain the description in the protologue as “1–2 seeds” per locule. We observed that one or more locules generally aborted in fruits, which makes some of the fruits in the pressed specimens appear 4-loculed, or even 2-loculed. For the immature fruits in this newly discovered population, no branched raphe on the seeds was observed. Detailed morphology with special attention to the supplemented floral characters is provided in the following description.

With the addition of newly generated *Euonymus aquifolium* and *Glyptopetalum ilicifolium* sequences, the molecular phylogenetic tree revealed overall similar resolution (BI / ML = 1 / 100; Fig. 3) of *Euonymus* sensu lato as the previous study by Li et al. (2014). Together with the three samples identified as *Glyptopetalum*, i.e., *G. continentale* (Chun and How 1958) C.Y.Cheng & Q.S.Ma in Cheng et al. (1999), *G. rhytidophyllum* (Chun and How 1958) C.Y.Cheng in Cheng et al. (1999), and *G. pallidifolium* (Hayata 1913) Q.R.Liu & S.Y.Meng in Meng et al. (2011), the two newly sampled taxa were resolved in a well-supported clade (1/99) sister to *Euonymus tingens* Wall. in Roxburgh (1824). All four sampled *Glyptopetalum* species are nested within *Euonymus*, which supports synonymization of *Glyptopetalum* with *Euonymus* pending further phylogenetic studies including its generic type *Glyptopetalum zeylanicum* Thwaites (1856). Moreover, our inferred tree reinforces the inference from previous studies that delimitations of sections within *Euonymus* remain problematic (Simmons et al. 2012; Li et al. 2014), except for *E.* sect. *Melanocarya* (Turczaninow 1858) Nakai (1941).

In summary, the results of both floral morphological observation and molecular analysis support its retention in *Euonymus*.

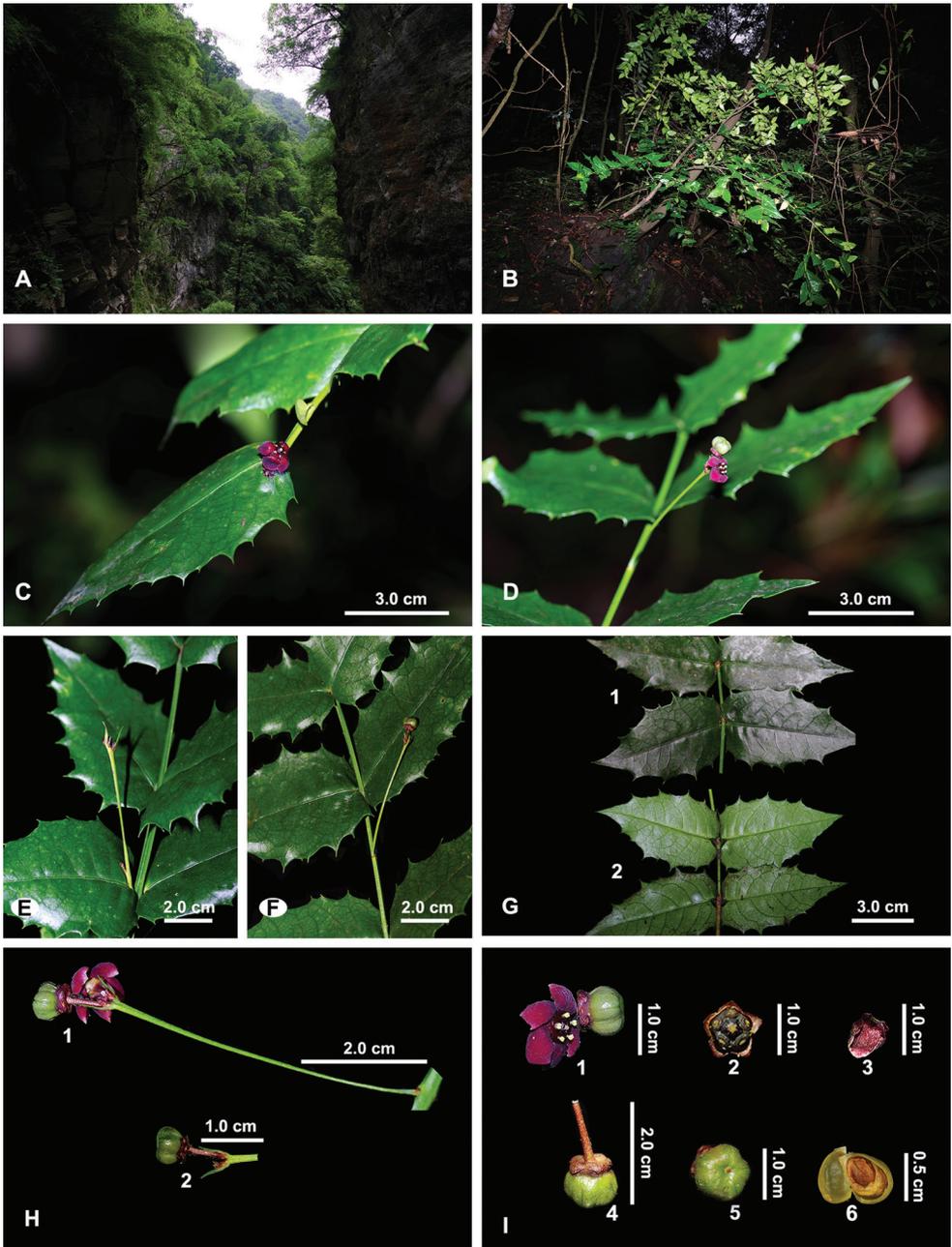
## Taxonomic treatment

### *Euonymus aquifolium* Loes. & Rehder in Sargent 1913: 484

Figs 1, 2

≡ *Glyptopetalum aquifolium* (Loes. & Rehder) C.Y.Cheng & Q.S.Ma in Cheng et al. 1999: 93

**Type.** CHINA. Sichuan [Szechuan]: Wa-shan, on cliffs, elev. ca. 2200 m, in fruiting, November 1908, *E. H. Wilson* 1366 (holotype A00049691 (Fig. 4A); isotypes K000669647 & US00096036).



**Figure 1.** *Euonymus aquifolium* Loes. & Rehder **A** habitat **B** habit on cliff **C** branch with a flower **D** a flower and an immature fruit **E** axillary inflorescence **F** extra-axillary inflorescence **G** leaves in adaxial (G1) and abaxial views (G2) **H** an inflorescence showing peduncle (H1) and pedicel (H2) **I** close-up of a flower and an immature fruit (I1), showing disk and calyx (I2), a detached petal (I3), an immature fruit in side view (I4) and front view (I5), and two immature seeds in a fertile locule with the lower right one covered by aril.

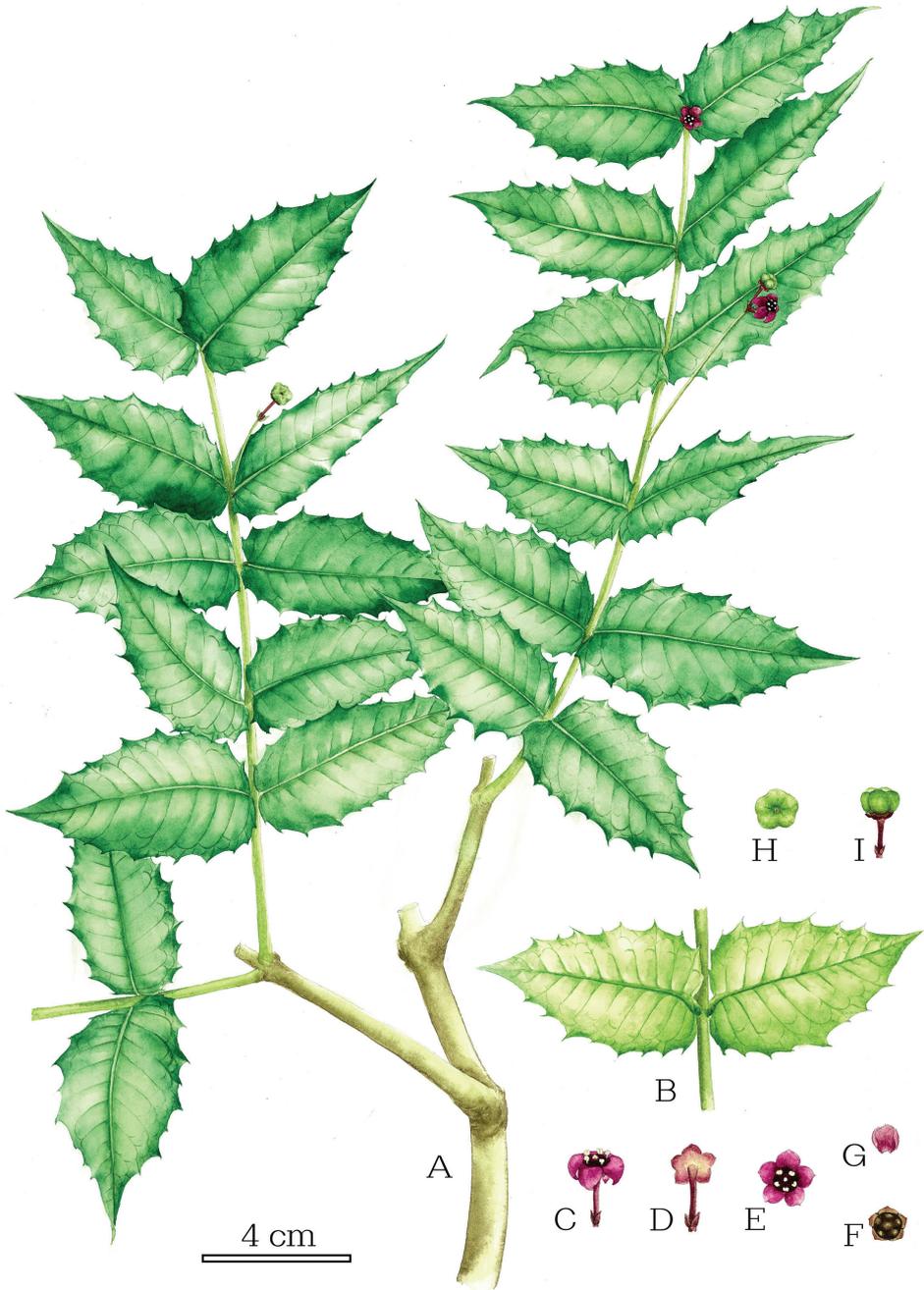
**Description.** Evergreen shrubs, 1–3 m tall, glabrous throughout; young branches firstly 4-angular and green, later turning almost terete and grey-brown. Leaves opposite, leaf blade leathery, oblongly ovate, ovate to orbicularly ovate, 4–7 × 2.5–4.5 cm, uneven on both surfaces, adaxially dark green, abaxially slightly pale green, glossy, margin with clear and irregular large spines, apex acute or short acuminate, base slightly cordate and often marginally clasping the branch, more or less oblique; lateral veins 6–10 pairs, curved and distally ca. 1/4 its length near margin connected with tertiary veins, midrib and lateral veins visibly elevated on both surfaces, transverse veins obscure; subsessile or petiole to 2 mm long. Cymes in leaf axils or extra-axillary, nearly on the adaxial side of branchlet when extra-axillary, with 1 to several (mostly 5–7) flowers; peduncle 1.5–5 cm long; pedicel 0.6–1.2 cm long, usually with two opposite bracteoles at base; bracteoles subulate, 0.4–0.8 cm long, ca. 2 mm wide, persistent. Flowers red-brown, 1–1.5 cm in diameter, 5-merous; calyx 5-lobed to middle, lobes triangular, red-brown, margin with fleshy projections, persistent; petals 5, fleshy, 0.5–0.8 cm long, 0.3–0.6 cm wide, broad-ovate, slightly revolute; disc pentagonal, deep red, fused to ovary; stamens 5 on disk, filaments very short, anthers small and yellow; ovary partly exposed outside disk, reddish brown, style absent, stigma rounded, white with yellowish tinge; ovary 5-locular; ovules 2 per locule. Capsule, subglobose, mostly yellowish green when immature, glabrous, 8–10 mm high, 1.2–1.5 cm in diameter, 5-loculed, sometimes only 4(–2)-loculed owing to infertile of one or more locules, with 2 seeds per locule, or only one seed with another ovule aborted. Seeds brown, oblong, 0.4–0.8 cm long, with orange-yellow aril, more than 1/2 covered by aril.

**Phenology.** Flowering was observed in August, and it could start earlier; fruiting from August to November.

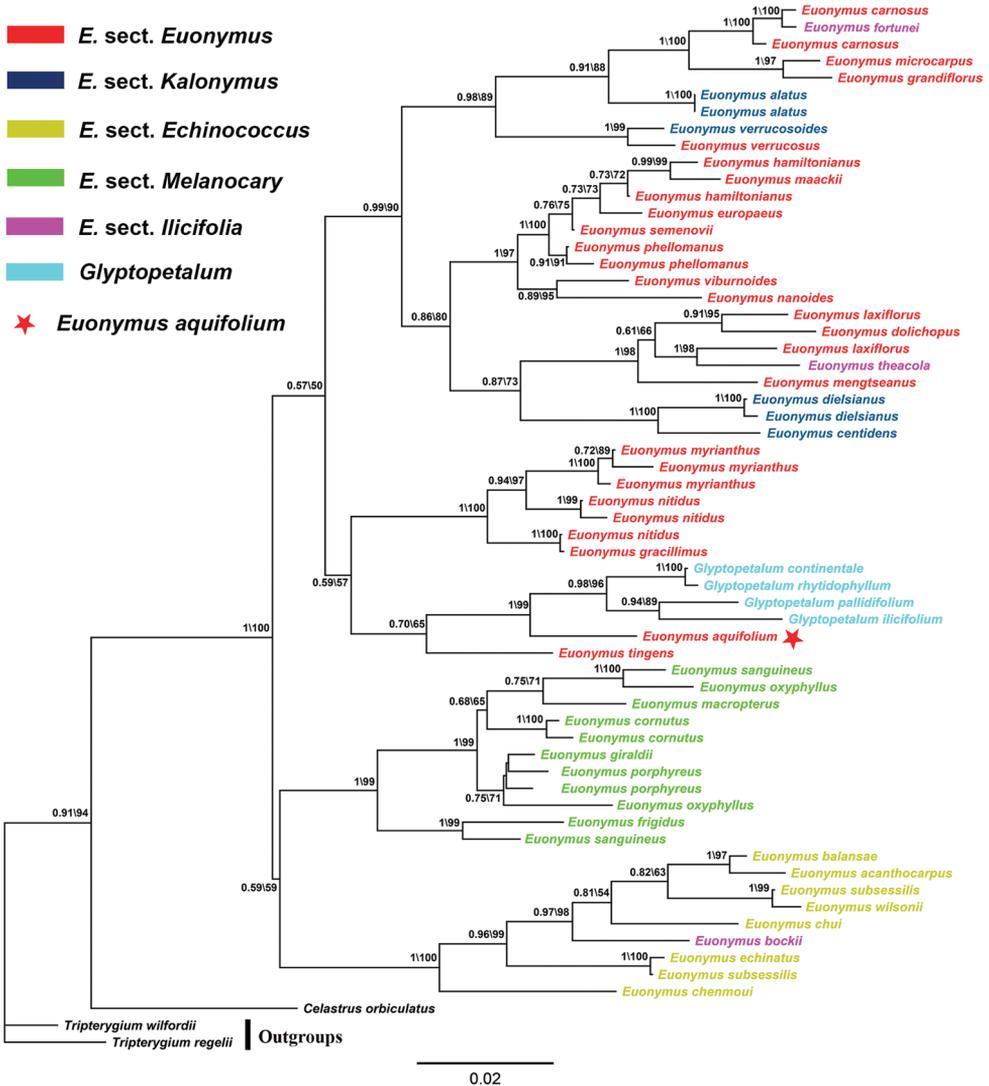
**Habitat.** The newly discovered site (elevation ca. 1850 m) is located under the cliff of a small ditch in the Dadu River Basin. The place is located in the so-called ‘Rain Zone of Western China’, where it is commonly rainy most of the year. The habitat is further shady and humid due to the gorge landform (Fig. 1A, B). Based on the records of type specimens, *Euonymus aquifolium* can grow on the cliffs within the evergreen broadleaf forest or evergreen and mixed deciduous broadleaf forest at an elevational range of 1800–2200 m in Dadu River Basin.

**Additional specimens examined.** CHINA. Sichuan: Jiulong County, Wanba, elev. ca. 1850 m, in flowering, 10 August 2021, *J. Hu et al. hujun20210810B01* (CDBI! NAS! PE!) (Fig. 4B).

**Nomenclatural note.** The species epithet of *Euonymus aquifolium* had been changed to “*aquifolius*” to agree with the generic gender based on the assumption that this epithet was used as adjectival in form, and this was generally followed (e.g., Chang 1988; Cheng et al. 1999; Ma et al. 2008; IPNI 2022). However, when it was originally proposed, the initial letter of the epithet was capitalized as “*Aquifolium*” (Sargent 1913), and that was customary then to indicate the epithet was applied after a proper noun, such as a person or a genus (Clifford and Bostock 2007). *Aquifolium* Miller (1754) is an illegitimate superfluous generic name of *Ilex* Linnaeus (1753), though it could also be used as an adjective (*aquifolius*). Loesener & Rehder also capitalized

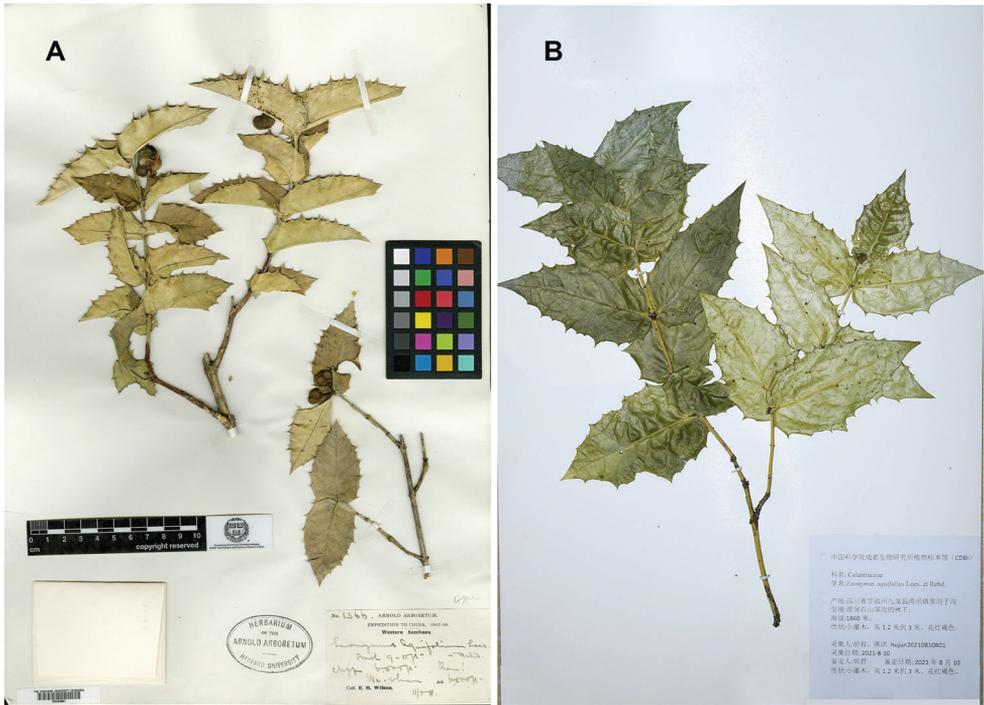


**Figure 2.** *Euonymus aquifolium* Loes. & Rehder **A** flowering and fruiting branches **B** leaves in abaxial view **C** a flower in side view **D** a flower in rear view showing abaxial calyx, attached pedicel and a pair of bracteoles at its base **E** a flower in front view **F** a flower with petals removed showing disk **G** a detached petal **H** an immature fruit in front view **I** an immature fruit in side view [Drawn by Cong-Ying Li from live specimens *J. Hu et al. hujun20210810B01*].



**Figure 3.** Bayesian and ML-based phylogenetic tree inferred from nuclear internal transcribed spacer (ITS) of the 51 sampled taxa identified as species of *Euonymus* and/or *Glyptopetalum*. Values above branches are Bayesian posterior probabilities (> 0.5) / maximum likelihood bootstrap percentages (> 50). Colors of terminal nodes correspond to the five sections of *Euonymus* defined in *Flora of China* (Ma et al. 2008) and the genus *Glyptopetalum*.

the epithet when describing other species in the same publication (Sargent 1913). For example: *Euonymus sargentianus* Loes. & Rehder in Sargent (1913) by naming the epithet as “*Sargentiana*” (named after a person), and *E. oblongifolius* Loes. & Rehder in Sargent (1913) as “*oblongifolia*”. This conclusion is further supported based on a review of the handwriting annotations by those authors on the type material of these

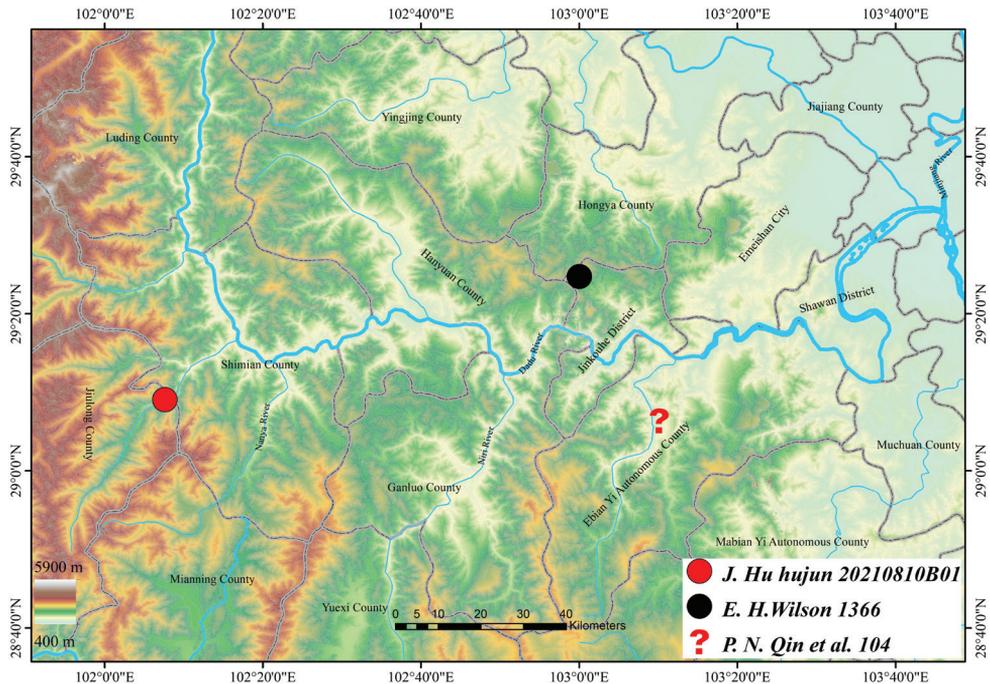


**Figure 4.** Selected specimens of *Euonymus aquifolium* Loes & Rehder **A** holotype of this species (*E. H. Wilson 1366*, A00049691) **B** a duplicate of *J. Hu et al. hujun20210810B01*(CDBI).

taxa, where the species epithets of *E. aquifolium* (*E. H. Wilson 1366*, A) and *E. sargentianus* (*E. H. Wilson 1187*, A) were written in uppercase, while that of *E. oblongifolius* (*E. H. Wilson 3125*, A) was in lowercase. Therefore, the species epithet of *Euonymus aquifolium* should retain its own gender and termination according to Art. 23.5 of ICN (Turland et al. 2018).

**Rarity and conservation status.** The type material of *Euonymus aquifolium* was collected by E. H. Wilson in November 1908 in Washan, Sichuan. Further geographical information concerning the type locality could not be traced by the related references (such as Wilson 1913, 1929; Yin et al. 2010). Owing to the historical vicissitudes, the picture named as “Wa Shan” in Wilson (1913, 1929) was traced by Yin et al. (2010; Fig. 5) to Jinkouhe District, Leshan City, Sichuan. A few botanists and amateurs (K. P. Yin, pers. comm.) had made attempts to find the living plants of this species around this area without result.

Another gathering (*P. N. Qin et al. 104*) identified as this species is recorded in Cheng et al. (1999) without mention of the herbarium where the specimen(s) were deposited. The collectors were a team of younger volunteers assigned by Tsofu Lu (1893–1952), the director of the then newly established Science Institute of West China, to survey in western Sichuan along the water courses in 1929 (Hou 2012), and most of their collections include duplicates bequeathed to the present Chongqing Natural History



**Figure 5.** Distribution map of *Euonymus aquifolium* Loes & Rehder based on three collections.

Museum (CQNM). An extensive search at CQNM did not identify any material from this gathering (Feng Chen, pers. comm.). It is unimaginable that it lacked leaves as commented in Cheng et al. (1999) since this is an evergreen thick leathery leaved plant. Searches by enthusiastic amateurs previously and Jun Hu's team recently for living individuals near the locality of this gathering (Fig. 5) were also unsuccessful.

We conclude that *Euonymus aquifolium* is a rare and vegetatively distinctive species, and this rediscovery uncovered the only presently confirmed living individuals more than 110 years after its description, which are distributed more than 100 km westward from its type locality (Fig. 5). Although it is located in difficult-to-access valleys on, and living on a cliff that is unlikely to be destroyed intentionally, natural hazards might still totally damage its habitat. With just ~ 15 individuals, it should still be assessed as Critically Endangered (CR) according to the IUCN (2022) Red List Categories and Criteria.

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## Appendix I

**Table A1.** The GenBank accessions for DNA sequences used in this paper.

Taxon, GenBank accession No. for ITS.
<i>Euonymus acanthocarpa</i> Franch., KF282154; <i>E. aquifolius</i> Loes. & Rehder, OK172405; <i>E. alatus</i> (Thunb.) Sieb., KF282155, KF282156; <i>E. balansae</i> Sprague, KF282157; <i>E. bochii</i> Loes. ex Diels, KF282158; <i>E. carnosus</i> Hemsl., KF282159, KF282160; <i>E. centidens</i> H. Lev., KF282161; <i>E. chenmoui</i> W. C. Cheng, KF282162; <i>E. chuii</i> Handel-Mazzetti, KF282163; <i>E. cornutus</i> Hemsl., KF282164, KF282165; <i>E. dielsiana</i> Loes. ex Diels, KF282166, KF282167; <i>E. dolichopa</i> Merr. ex J. S. Ma, KF282168; <i>E. echinatus</i> Wall., KF282169; <i>E. europaeus</i> L., KF282170; <i>E. frigida</i> Wall., KF282171; <i>E. giraldui</i> Loes. ex Diels, KF282172; <i>E. gracillimus</i> Hemsl., KF282173; <i>E. grandiflora</i> Wall., KF282174; <i>E. hamiltonianus</i> Wall. ex Roxb., KF282175, KF282176; <i>E. heaeacea</i> Champ. ex Benth., KF282177; <i>E. laxiflora</i> Champ. ex Benth., KF282178, KF282179; <i>E. maackii</i> Rupr., KF282180; <i>E. macroptera</i> Rupr., KF282181; <i>E. mengtseanus</i> (Loes.) Sprague, KF282182; <i>E. microcarpa</i> (Oliv. ex Loes) Sprague, KF282183; <i>E. myrianthus</i> Hemsl., KF282184, KF282185, KF282186; <i>E. nanoides</i> Loes. ex Rehder, KF282187; <i>E. nitidus</i> Benth., KF282188; <i>E. oblongifolius</i> Loes. ex Rehder, KF282189, KF282190; <i>E. oxyphyllus</i> Miq., KF282191, KF282192; <i>E. phellomana</i> Loes. ex Diels, KF282193, KF282194; <i>E. porphyreus</i> Loes., KF282195, KF282196; <i>E. sanguinea</i> Loes. ex Diels, KF282197, KF282198; <i>E. semenovii</i> Regel & Herder, KF282199; <i>E. sp.</i> , KF282200; <i>E. subsessilis</i> Sprague, KF282201, KF282202; <i>E. theacola</i> C. Y. Cheng ex T. L. Xu & Q. H. Chen, KF282203; <i>E. tingens</i> Wall., KF282204; <i>E. verrucosa</i> Scop., KF282205; <i>E. verrucosoides</i> Loes., KF282206; <i>E. viburnoides</i> Prain, KF282207; <i>E. wilsonii</i> Sprague, KF282208; <i>Celastrus orbiculatus</i> Thunb., KF282209; <i>Glyptopetalum continentale</i> (Chun & How) C. Y. Cheng & J. S. Ma, KF282210; <i>G. pallidifolium</i> (Hayata) Q. R. Liu & S. Y. Meng, KF282192; <i>G. rhytidophyllum</i> (Chun & How) C. Y. Cheng, KF282211; <i>Triperygium regelii</i> Sprague & Takeda, KF282212; <i>T. wilfordii</i> Hook. f., KF282213.

<sup>1</sup> The terms gathering, duplicate, and specimen follow the definitions of ICN (Turland et al. 2018).

