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Research Article

Typhonium morangense (Araceae), a new species from the tropical forest of Eastern Nepal

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Abstract

A new species of the aroid genus *Typhonium* Schott has been discovered in Eastern Nepal. This species, named *Typhonium morangense* R.Ojha & S.Rai, is described based on comparative morphological examinations with closely related species from the Indian subcontinent. The new species is currently known only from Pathari Shanishchare Municipality in the Morang district of Eastern Nepal. It is the second species of *Typhonium* observed in Nepal, and the only one endemic to the country. A detailed description, color plate, geographic distribution, phenology, and morphological comparison of the new species with morphologically close species are provided. Given its restricted range and the low number of individuals observed in the wild, this species requires special attention from conservation biologists working in Eastern Nepal.

Key words: Bulbils, Koshi Province, Morang, *Typhonium inopinatum*

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Introduction

The moderately species-rich aroid genus *Typhonium* Schott is expected to comprise around 100 species (Hetterscheid and Sookchaloem 2012), of which 72 species currently have accepted names, according to Plants of the World Online (POWO 2024). Phylogenetic studies have confirmed the placement of this genus in the tribe Areae, belonging to the subfamily Aroideae (Zhao et al. 2023). The genus is widely distributed across tropical to subtropical Asia, extending from the central Himalayas to New Guinea and Australia. More than half of the currently accepted *Typhonium* species are found in the Indochina region (Pham et al. 2023). Due to the high diversity and endemism of the genus in the Indochina region, it has long been considered the center of origin for the genus *Typhonium*. This is further supported by a recent biogeographic reconstruction study by Low et al. (2020), which suggests a middle to early Miocene origin (approximately 17.24 Ma) of the genus in the Indochina region. Within this region, the highest diversity (33 species) and endemism (23 species) are found in Thailand (Hetterscheid and Sookchaloem 2012; Sookchaloem and Maneeanakekul 2018). This center of biodiversity likely harbors several undiscovered species, as indicated by the recent discovery of *Typhonium hangiae* V.D.Nguyen, D.D.Nguyen & V.C.Nguyen and *Typhonium obtusum* Luu, X.B.

Nguyen-Le & H.C. Nguyen in Vietnam (Pham et al. 2023; Luu et al. 2024). Given the known distribution of the genus, only a few species are found in the Himalayas. For example, only one species, *Typhonium trilobatum* (L.) Schott, has been reported in Nepal (Shrestha et al. 2022). As the northwestern distribution border of the genus, Nepal stands out as an important area for studying the diversity of *Typhonium*.

As part of our ongoing efforts to document the diversity of Araceae in Nepal, field surveys were conducted in the tropical forests of Eastern Nepal (Rajapaksha et al. 2023; Rai et al. 2024). In early 2024, a population of small aroids with 2–5 hastate to trilobate leaves and a pinkish-brown inflorescence was encountered. At first glance, these plants resembled the widespread *T. roxburghii* Schott or the more narrowly distributed *T. inopinatum* Prain, known to occur in India, Myanmar, and Thailand, due to the shape of their leaves, the inner color of the spathe, and the appearance of the spadix. However, detailed morphological examinations revealed these plants to be distinct from these two species and any previously described species. Thus, we introduce these plants as a new species endemic to Eastern Nepal.

Materials and methods

To provide detailed information about the plant, we observed several individuals across different patchy stands. Mature individuals were collected to be deposited as herbarium specimens, while some inflorescences were preserved as pickled samples for further study. The morphometric study included a total of 20 individuals. Measurements of various parts of the living plants were taken in the field and photographs were captured using a Nikon Coolpix P900 camera. Micro-morphological measurements of flower parts of the specimens were examined using a dissection microscope. The images of these structures were taken using a mobile phone camera. Relevant literature was consulted to compare the morphology with closely related species and other species of the genus found in neighboring countries (Murata et al. 2010; Hetterscheid and Sookchaloem 2012; Venu and Rao 2014; Nirola and Das 2014; Manudev and Nampy 2022; Pham et al. 2023). Images of *Typhonium* specimens available in virtual herbaria and databases, including KATH (http://plantdatabase.kath.gov.np), CAL, ARUN, BM, P, PE (http://www.cvh.ac.cn), US, and others accessible through https://www.gbif.org and https://plants.jstor.org, were also examined (herbarium acronyms follow Thiers 2024). In addition to studying images of type specimens and protologs, the morphological characterization of putatively related species was based on descriptions provided by Li and Hetterscheid (2010) for *T. roxburghii*; King and Prain (1898), Venu and Rao (2014), and Luu et al. (2024) for *T. inopinatum*; Manudev and Nampy (2022) for *T. bulbiferum* Dalzell; and Murata et al. (2010) for *T. cordifolium* S.Y.Hu.

Taxonomic treatment

***Typhonium morangense* R.Ojha & S.Rai, sp. nov.**
urn:lsid:ipni.org:names:77355953-1
Fig. 1

Type. NEPAL, Koshi Province, Morang District, Pathari Shanishchare Municipality; 26°39'59"N, 87°33'29"E; ca.150 m; 2024.06.03; Sudeep Rai *MP011* [holotype KATH! isotypes KATH! [*MP012*], TUCH!, TURH!].

Diagnosis. *Typhonium morangense* is morphologically closely related to *T. inopinatum* but it differs significantly from the latter species in having (1) smaller habit, 10–24 cm height (vs. 10–45 cm in *T. inopinatum*); (2) globose bulbils on top of petiole and leaves (vs. bulbils absent); (3) a sessile appendix (vs. subsessile); (4) thick, sickle-shaped staminodes (vs. filiform); (5) staminodes half curved downwards (vs. horizontally spread or, slightly curved); (6) ovary white (vs. yellowish) and (7) stigma pink (vs. yellow).

Description. Seasonally dormant small herb, 10–24 cm tall; Tuber 0.8–2 cm long, 1–2 cm diameter, upright, sub-globose to sub-cylindrical with many filiform roots, without rhizomatous offsets. ***Leaves*** 2–5 together; ***petiole*** 10–23 cm long, light brown, grayish brown at base to light green at apex, globose black bulbil on the top of petiole. ***Leaf blade*** entire, hastate or shallowly or deeply tri-lobed or trifoliolate, glabrous, adaxially green, abaxially lighter green, 5–11 cm long, 2–7 cm wide when hastate, 10–12 cm when deeply trilobed, globose black bulbil on the basal margin of the leaf. ***Inflorescence*** solitary, usually 1–2 together, shorter than petiole; ***peduncle*** subterranean, white, 1.2–3.8 cm long; ***spathe*** 5.5–12 cm long, tube and limb separated by a constriction, grayish pink outside, inside dark reddish purple color; ***spathe tube*** ovoid to cylindric, 1–1.5 cm long, 1–1.7 cm diameter, outside grayish pink, inside reddish purple; ***spathe limb*** narrowly triangular, 4.5–10.5 cm long, 1–3 cm wide, smoothly tapering from below middle, apex acute; ***spadix*** 6.5–9.5 cm long, as long as spathe or slightly longer, sessile; ***female zone*** conical, 2–6 mm long, 4–5 mm diameter at the base, slightly pink, ***ovary*** unilocular, white with one dark yellow basal ovule, ***style*** absent, ***stigma*** sessile, disc shape with dark pink over the periphery; ***staminodes*** arranged in three whorls, free at the base, thick sickle-shaped, half-length curved downwards, yellow, glabrous, cover 1/3th of the female zone; ***male zone*** 5–8 mm long, 3–5 mm diameter, cylindrical, ***stamens*** congested, ***thecae*** two, irregular, coral pink to sandy brown color with apical short slits or pores; ***appendix*** usually sessile, 2.7–7.7 cm long, narrowly elongated conical, dark reddish purple, base obliquely truncate, top acute, inside semisolid. ***Fruits*** ovoid to capsule-shaped, 0.2–0.3 cm diameter, 0.3–0.4 cm long, slightly light green at initial stages while turning white after maturity.

Phenology. Flowering in May to June, fruiting July to August.

Etymology. The specific epithet is based on the locality of its discovery, the Morang district of Eastern Nepal.

Distribution and habitat. The new species is known from Pathari Shanishchare municipality, Morang district, growing under the canopy of dense *Shorea robusta* Gaertn. forest at ca. 150 m asl. This species prefers moist shady floors under the dense canopy of forest.

Uses. No reports for utilization by the local human population are known.

Conservation status. The new species has been recorded only from its type locality, where it forms patchy stands of approximately 150 individuals. However, it is highly likely to be present in similar nearby habitats. Together with the type locality, these undiscovered sites are expected to contain several hundreds of individuals. Until further investigation, the species is provisionally designated as “Data Deficient” (DD) following the IUCN standards (IUCN 2024). Conservation biologists active in Eastern Nepal may want to consider this species as requiring attention, as it is a local endemic known only from this region.

Taxonomic notes. The hastate leaf shape, small inflorescence size, and presence of a few staminodes are shared characteristics between *Typhonium inopinatum*

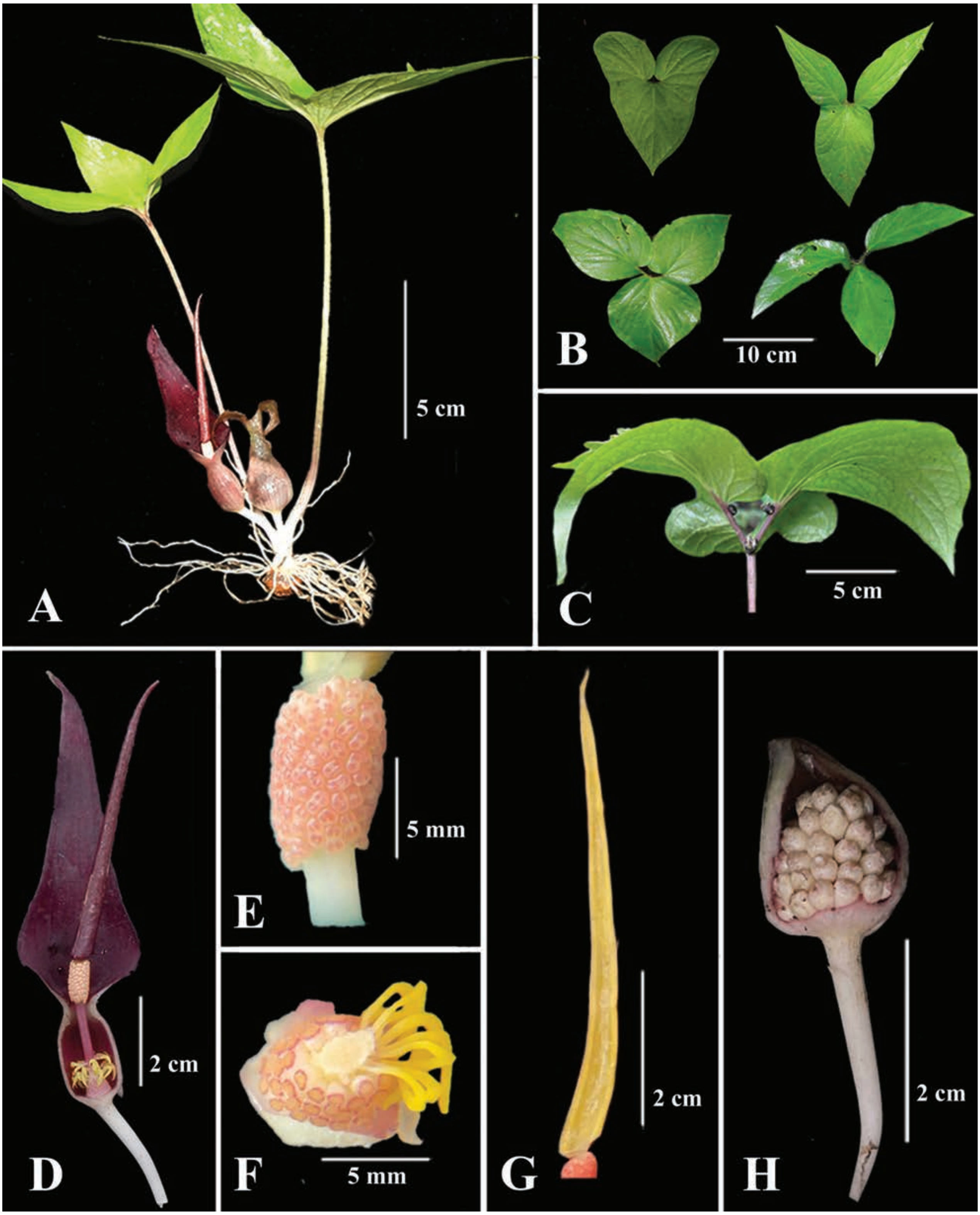

Figure 1. *Typhonium morangense* sp. nov. **A** whole plant **B** different forms of leaf blade **C** bulbils **D** inflorescence **E** male zone **F** female zone and staminodes **G** longitudinal section of appendix **H** fruits.

and *T. morangense*. However, apart from key differences presented in diagnosis, *T. morangense* can be distinguished from *T. inopinatum* by the color of the spathe (grayish pink outside and reddish purple inside vs. basally brownish, apically green), the color of appendix (reddish purple vs. yellow, yellowish-brown), the length of the spadix (as long as or slightly longer than the spathe vs. shorter than the spathe), the female flowers (without style vs. with style). *T. morangense* also shares several similarities with *T. roxburghii*, in the habit and the color of spathe. However, *T. morangense* differs from *T. roxburghii* in several characteristics: the size of the inflorescence (spathe limb 4.5–10.5 × 1–3 cm vs. 13–15 × ~5 cm; appendix 2.7–7.7 cm vs. 12–15 cm), the arrangement of the staminodes (arranged in three whorls vs. more than three whorls; half-length curved downward vs. only the tips pointing downward), the appendix (sessile vs. stipitate), the shape of the ovule (ellipsoid vs. ovoid), and the color of the stigma (pink vs. purple). Although *T. inopinatum* and *T. roxburghii* share several similar characters with *T. morangense*, neither has bulbils.

Bulbils have also been reported in the Indian endemic *T. bulbiferum* and *T. cordifolium* distributed in Myanmar, Thailand, Vietnam, and Cambodia (Nguyen et al. 2022). These two species differ from *T. morangense* by having triangular to sagittate or cordate leaves in *T. bulbiferum* and ovate to elliptic leaves in *T. cordifolium* (vs. hastate, tri-lobed, or trifoliolate leaves in *T. morangense*). Additionally, *T. bulbiferum* has a linear-lanceolate, pale rose spathe with an acuminate apex, and *T. cordifolium* has a narrowly triangular-ovate, dark reddish-purple to purplish spathe with an acuminate and apically curled apex (vs. narrowly triangular, grayish-pink spathe with a dark reddish-purple interior and an acute apex in *T. morangense*).

It is noted that a plant from India with globose bulbils on the lower leaf margin and the petiole apex, which was identified as *T. roxburghii* by Nirola and Das (2014), was recently confirmed to be a misidentification (Manudev and Nampy 2022). This indicates uncertainty in identification due to the presence of bulbils.

A comprehensive morphological comparison of the new species with these related species is presented in Table 1.

Table 1. Morphological differences between *Typhonium morangense* and closely related species.

	T. morangense	*T. bulbiferum*	*T. cordifolium*	*T. inopinatum*	*T. roxburghii*
Height	10–24 cm	15–30 cm	-	10–45 cm	10–35 cm
Leaf blade	hastate or tri-lobed or trifoliolate, entire, 5–11 × 2–12 cm	triangular hastate, sagittate or cordate, mid lobe 5–8 × 3–6 cm	narrowly ovate-elliptic to elliptic, 4–25 × 1.5–4 cm	ovate to triangular or hastate, entire, 8 × 2 cm long	hastate, triangular, shallowly or deeply tri-lobed, 5–17 × 4–14 cm
Bulbils	globose, black on the top of the petiole and leaves base	globose to linear bulbils	present on the apex of the leaf	absent	absent
Spadix	6.5–9.5 cm long, sessile, as long as spathe limb	slender, as long as spathe	as long as spathe	4.3–9 cm long, shorter than spathe limb	subequaling the spathe
Appendix	2.7–7.7 cm long, dark-reddish purple, usually sessile, base obliquely truncate, apex acute	7–14 cm long, pale yellow to cream, stipitate, rounded at the base	3.6–7.7 cm long, brick orange-colored, base not truncate	4–6 cm long, yellow, yellowish-brown, subsessile, base truncate	12–15 cm, narrowly conical, dark purple, truncate, pale red stipe
Spathe tube	ovoid to cylindrical, 1–1.5 cm long, 1–1.7 cm in diameter, outside grayish pink, inside reddish purple	oblong, 1–2 cm long.	1.2 × 1 cm long	ovoid, 2 cm cm long, 1.5 cm wide, greenish inside and outside	-
Spathe limb	narrowly triangular, 4.5–10.5 × 1–3 cm, grayish pink outside, dark reddish purple inside, acute apex	linear-lanceolate, 7–16 × 2–4 cm, hyaline or pale rose, acuminate at apex	narrowly triangular-ovate, 5–9 × 3 cm, dark reddish purple to purplish brown, apex acuminate, upright or recurved, and curled apically	narrowly ovate to lanceolate, 9–10 × 4.5 cm, greenish (light purple outside), green with dark purple streaks and spots inside, apex acute to acuminate	broadly triangular-ovate, 13–15 × ca. 5 cm, purple or purple mixed with a dirty green flush outside, inside deep rich purple, usually twisted apex
Stamens	coral pink to sandy brown	-	creamy white	pale yellow	yellow
Staminod	thick sickle-shaped, arranged in three whorls, half-length curved downwards, acute tip	acinaciformis, curved, uniseriate	cylindrical, spreading, obtuse tip	filiform, horizontally spread and slightly curved, bifurcated pointed or entire tip	filiform, weakly papillose, arranged in 3 whorls, horizontally spread, pointing downward, acute tips

For now, the new species is considered endemic to Nepal, but further research is necessary to confirm its distribution range, particularly the potential occurrence in India. Special attention should be given to critically studying specimens with globose bulbils.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Field investigation, collection, and photography: SR. Specimen examination, description: RO. Original draft writing: RO, SR. Draft improvement and editing: HS.

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Data availability

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

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Research Article

Lilium huanglongense (Liliaceae): a newly-discovered species in north-western Sichuan, China

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Abstract

In this study, we describe *Lilium huanglongense*, a newly-discovered lily species identified following extensive surveys in an undeveloped area of the Huanglong National Nature Reserve in Sichuan, China. This region, located in the Hengduan Mountains of south-western China, is recognised as one of the world's prominent biodiversity hotspots, providing diverse habitats for a wide range of plant species. Morphologically, *L. huanglongense* resembles *Lilium fargesii* Franch., which is distributed in central China, as well as other tepal-recurved members of the section *Lophophora* (Bureau & Franch.) F. T. Wang & Ts. Tang. This section comprises dwarf lilies predominantly found in the alpine scrub of the Hengduan Mountains, extending westwards into the Himalayas. Molecular phylogenetic analyses using both nuclear ITS and chloroplast genomes confirm the independent status of the new species and its placement within the section *Lophophora*. The identification of this new species helps to fill the distribution gap between broad-leaved forest and alpine scrub species within the section, thereby enhancing our understanding of the diversity and distribution history of *Lophophora*.

Key words: Liliaceae, *Lilium huanglongense*, Lophophorum-clade, new species, section *Lophophora*

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Introduction

Lilium, a genus in the tribe Lilieae of the family Liliaceae, comprises herbaceous, bulbous plants with scaled bulbs, dorsifixed anthers and loculicidal capsules (Peruzzi 2016). With approximately 123 recognised species (POWO 2024), the genus is widely distributed across the Northern Hemisphere in Asia, Europe and North America (Liang and Tamura 2000). China, in particular, hosts approximately 55 distinct species according to the latest flora records (Liang 1995; Liang and Tamura 2000), with key distribution areas including northeast, central and south-western China. Amongst these regions, south-western China stands out as a hotspot for the diversity of wild lilies due to its mountainous environment (Gao et al. 2013b, 2015). These mountain ranges and deep valleys constitute an intricate topography, which provides unique habitats for various species.

The Daba Mountains and the Qinling Mountains of central China, as well as the Hengduan Mountains and the Himalayas, form a series of mountain ranges from central to western China, together harbouring the greatest number of lilies in the world (Yundong Gao, unpublished data). Furthermore, the rugged terrain and sparse population in mountainous regions have constrained previous explorations, indicating the potential presence of undiscovered species in these areas. Investigating plant groups within these continuous mountain ranges would enhance our understanding of species diversification and dispersal history amongst the selected plant species, thereby contributing significantly to our overall knowledge of biodiversity.

Our prior investigation elucidated the taxonomic classification of the genus *Lilium* (Gao et al. 2012, 2013b, 2015; Gao and Gao 2016; Yuan and Gao 2024). Specifically, the Lophophorum-clade *sensu* Gao et al. (2013b) was identified, which comprises both the campanulate-flowered *L. oxypetalum* Baker, *L. lophophorum* (Bureau & Franch.) Franch., *L. nanum* Klotzsch, as well as the tepal-recurved *L. fargesii* Franch., *L. stewartianum* Balf. f. & W.W. Sm. and *L. matangense* J.M. Xu that span mountainous regions from central China (broadleaved forests) to the Himalayas (alpine scrub and meadows). This clade is analogous to the subgeneric section *Lophophora* (Bureau & Franch.) F. T. Wang & Ts. Tang, as refined by Watanabe et al. (2021) in their recent work. Most members of this clade, which exhibit recurved perianths, have a limited and sporadic distribution. For example, *L. fargesii* exhibits a notable widespread distribution at mid-elevation areas (second step) in China (mainly in Qinling and Daba Mountains), while *L. matangense* is found further westwards at higher altitudes of over 3000 m, with narrow distribution and very small population sizes and therefore is of conservation value. Additionally, *L. stewartianum* is distributed at an altitude of about 3500 m in the southern Hengduan Mountains (Fig. 1).

With advancements in molecular phylogenetics, the monophyly of the Lophophorum-clade has been confirmed (Gao et al. 2013b; Watanabe et al. 2021); however, our understanding of its composition and evolutionary history remains incomplete. Moreover, the distribution of the entire Lophophorum-clade is disjunctive (Fig. 1), particularly between the northern Hengduan Mountains and the western Qinling Mountains, creating a “gap” where members of this clade have not been previously documented. This relic distribution pattern may suggest the presence of additional undiscovered taxa or those that are already extinct. In recent years, meticulous sampling and analysis of this group have yielded several novel findings. This paper highlights one such discovery: the putative new species *L. huanglongense*, identified by rangers in the Huanglong National Nature Reserve. This discovery can be partially attributed to the increased attention that the Chinese government has directed towards nature reserves and it represents a significant advancement in our understanding of this clade.

Currently, we aim to clarify the status and phylogenetic position of the putative new species by comparing its morphology with that of the most morphologically similar species, in addition to conducting molecular phylogenetic analyses utilising both nuclear markers and chloroplast genomes. Furthermore, the analysis of the morphological and genetic distinctiveness of *L. huanglongense* is expected to offer additional insights into the Lophophorum-clade by addressing the geographic distribution gap observed amongst its members.

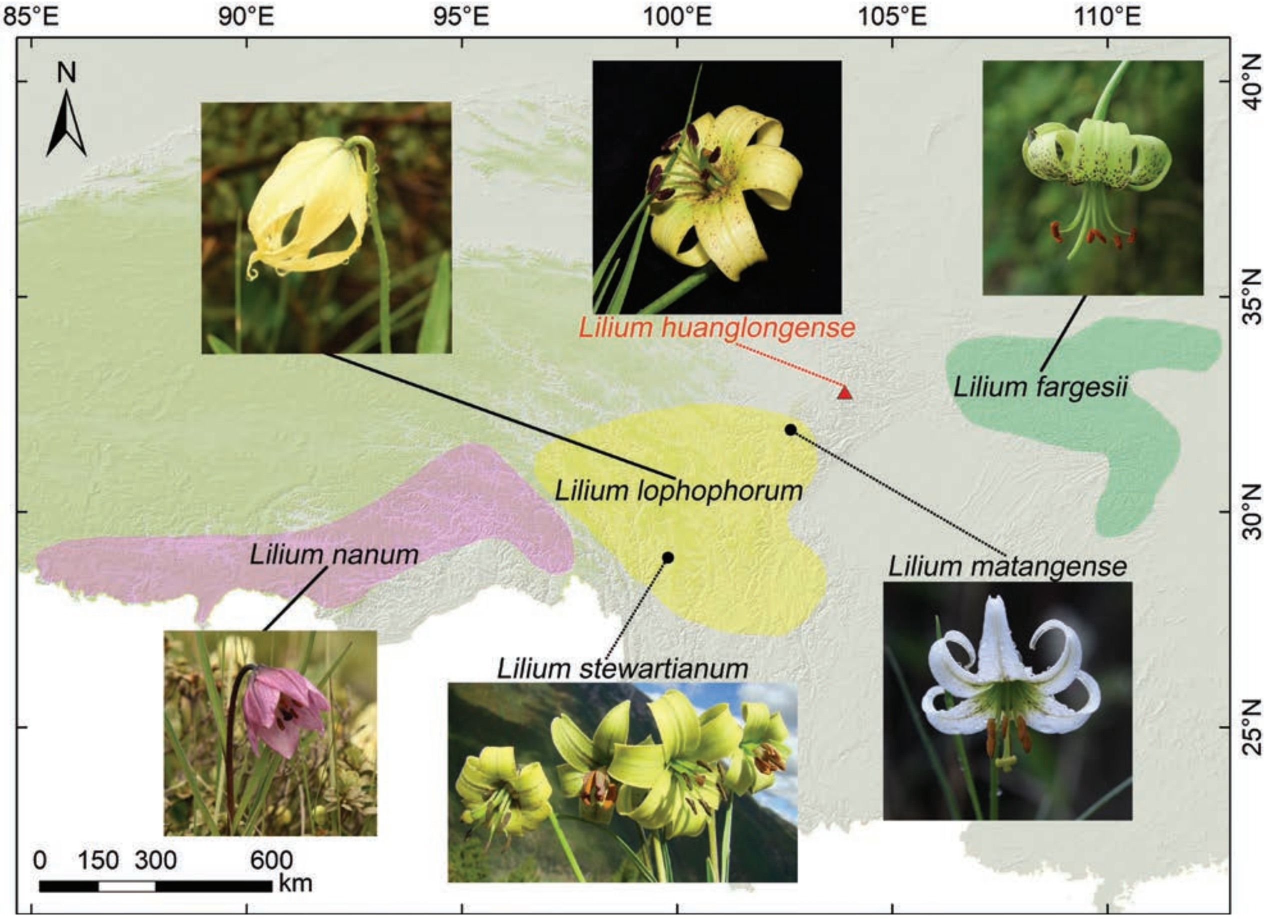

Figure 1. Morphological characteristics and geographic distribution of *Lilium huanglongense* and related species. *Lilium huanglongense* fills the geographic gap west of the Qinling Mountains and at the confluence of the Hengduan Mountains.

Materials and methods

Field sampling

Leaf materials of the new species were collected from the Huanglong National Nature Reserve and temporarily preserved in silica gel for DNA extraction. During fieldwork, we captured many photographs of the individuals and collected three complete specimens for conservation purposes. These images and specimens were used for subsequent measurements and descriptions. The voucher specimens have been deposited in the Herbarium of the Chengdu Institute of Biology (**CDBI**).

Morphological analysis

This study is grounded in an analysis of herbarium specimens, digital specimen images, field observations and relevant literature. We conducted a comprehensive literature review of pertinent taxa using online databases such as Tropicos (https://tropicos.org/) and the Biodiversity Heritage Library (BHL, https://www.biodiversitylibrary.org/), focusing on *Lilium oxypetalum* Baker (Baker 1874), *L. lophophorum* (Bureau & Franch.) Franch. (Franchet 1898), *L. nanum* Klotzsch (Klotzsch and Garcke 1862), *L. fargesii* Franch. (Franchet 1892), *L. stewartianum* Balf. f. & W.W. Sm. (Smith 1923) and *L. matangense* J.M. Xu (Xu 1985).

Specimens were meticulously examined through visits to the CDBI, IBSC, KUN, PE, SZ and WUK Herbaria (acronyms according to Thiers (2024), same below) and by accessing digital images from virtual herbarium platforms, including the China Virtual Herbarium (https://www.cvh.ac.cn/), the Kew Herbarium Catalogue (http://apps.kew.org/herbcat/gotoHomePage.do) and JSTOR Global Plants (https://plants.jstor.org/), as well as online images from herbaria B, E, GH, K and P. This approach aimed to facilitate a comparative analysis of morphological characters based on a substantial number of specimens. Morphological traits were selected, based on taxonomically significant features detailed in the "Flora of China" (Liang and Tamura 2000), including bulbs, stems, leaves and flowers. Specifically, the new species was morphologically compared to the tepal-recurved members of the Lophophorum-clade, namely *L. fargesii*, *L. stewartianum* and *L. matangense*. For comparative analysis, the dimensions of the bulbs, stems, leaves and floral organs were measured from both specimen images and photographs of fresh plants, utilising MATO (Liu et al. 2023) and PS software (Suppl. material 1: table S1). The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were calculated using the GeoCAT software (Bachman et al. 2011).

Molecular phylogeny inference

Genomic DNA was extracted from silica-gel dried leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Allen et al. 2006). Paired-end sequencing libraries were then constructed with insert sizes of approximately 350 bp, followed by sequencing on the DNBSEQ-T7 platform (Beijing Genomics Institute, BGI), with the depth of about 0.1 ~ 0.2 × (10G pair ending reads). About 13 Gb of raw data were filtered by fastp v.0.23.2 (Chen et al. 2018). The Internal Transcribed Spacer (ITS1, 5.8S and ITS2) and chloroplast genome of new species were then assembled using GetOrganelle v.1.7.6.1 (Jin et al. 2020) with default parameters. Chloroplast genomes were annotated and manual corrections were made using Geneious Prime v.2023.1.2 (Biomatters Ltd. Auckland, New Zealand), based on the plastome of *Lilium fargesii* (NC_033908.1).

To deduce the phylogenetic position of the putative new species, we combined newly-generated DNA sequences and published sequences, including thirty-two ITS and twenty-eight cp genome from NCBI (https://www.ncbi.nlm.nih.gov/), to infer phylogenetic relationships, selecting the entire Lophophorum-clade species and 2–3 representative from closely-related clades (Suppl. material 1: table S2) based on previous studies (Gao et al. 2013a; Yuan and Gao 2024). Outgroups included four species of *Fritillaria* and *Cardiocrinum* (Suppl. material 1: table S2).

We utilised the online platform (https://ngphylogeny.fr/, Lemoine et al. (2019)) to construct Maximum Likelihood (ML) phylogenetic trees based on complete plastid sequences. The sequences were analysed through the Advanced Workflow, employing the PhyML + SMS/OneClick method. Detailed workflows for MAFFT, BMGE and PhyML + SMS (Maximum Likelihood-Based Phylogenetic Tree Inference with Intelligent Model Selection) are provided in the Methods section of Lemoine et al. (2019). Bootstrap analysis (FBP + TBE) was conducted with 1000 replicates, while all other parameters were kept at their default settings. ITS sequences were processed using PhyloSuite v.1.2.2 (Zhang et al. 2020). A total of 34 sequences were aligned in batches with MAFFT v.7.313 (Katoh and Standley 2013) using the '--auto' strategy in normal alignment mode. The resultant files were subjected to additional manual corrections using MEGA v.11.0 (Tamura et al. 2021). Subsequent analyses were performed in PhyloSuite, where ambiguous sites and gaps were removed using Gblocks (Talavera and Castresana 2007). The sequences were then concatenated into a single alignment and converted into Nexus format files.

ModelFinder (Kalyaanamoorthy et al. 2017) was used for the selection of the most appropriate evolutionary model. Based on the Akaike Information Criterion, GTR + F + G4 was chosen as the optimal model of nucleotide evolution. Bayesian phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model (2 parallel runs, 10,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in. The construction of the ITS Maximum Likelihood (ML) tree was performed using PhyloSuite, with the sequence file generated through MAFFT and Gblocks. The file was then processed using the A La Carte option on the online tree-building platform to execute the PhyML analysis. Bootstrap analysis was conducted with 1000 replicates and all other parameters were set to their default values. The generated Maximum Likelihood (ML) and Bayesian Inference (BI) (Suppl. material 2) phylogenetic trees were visualised using iTOL v.6 (https://itol.embl.de, Letunic and Bork (2024)).

Results

Morphology comparison (Figs 2–4, Table 1)

Lilium huanglongense shares with *L. lophophorum* a pair of marginal ridges along the central groove on the adaxial surface of tepals (Fig. 2B), which has been recognised as the most important character defining this section (Watanabe et al. 2021). This feature also provides morphological evidence supporting the new species' placement within the Lophophorum-clade. Additionally, the turk's-cap perigone suggests that *L. huanglongense* is more closely related to tepal-recurved species such as *L. fargesii*.

While *L. huanglongense* shares reflexed perianth segments with *L. fargesii*, *L. stewartianum* and *L. matangense*, it differs notably in terms of floral organs.

Table 1. Morphological comparisons of *Lilium huanglongense*, *L. fargesii*, *L. stewartianum*, and *L. matangense*.

Characters		*L. huanglongense*	*L. fargesii*	*L. stewartianum*	*L. matangense*
Bulb	colour	yellow	white	yellow	white
	diam.	1.2–1.5 cm	approximately 1.5 cm	approximately 2.0 cm	1.0–1.5 cm
Stem	length	15–40 cm	20–70 cm	20–50 cm.	23–35 cm
Leaves		5–12 × 0.3–0.7 cm	10–14 × 0.2–0.5 cm	2.5–7 × 0.3–0.4 cm	2–2.5 × 0.5–1 cm
Flower	basal colour	yellow	green, pink	greenish to deep yellow	white
	tube length	shorter	shorter	longer	shorter
	stigma	three-lobed without inflation	three-lobed without inflation	three-lobed with inflation	three-lobed with inflation
	nectar glands	nectaries with cristate projections on both surfaces	nectaries with cristate projections on both surfaces	papillose nectaries that form two ridges along the bases of the inner tepals	inner ones with fimbriate projections on both surfaces of nectaries

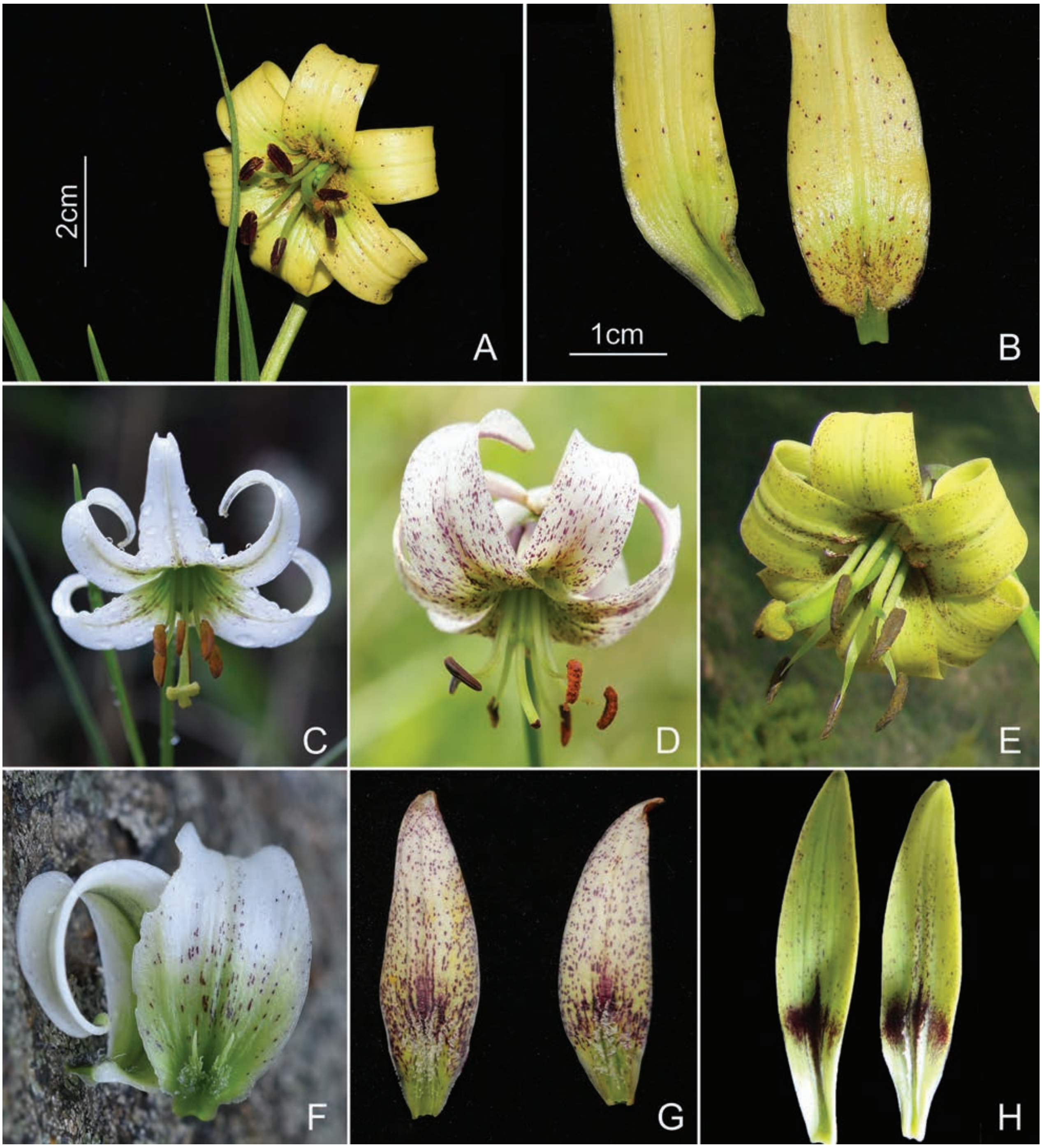

Figure 2. Comparison of floral structures of similar species **A** *Lilium huanglongense* flower **B** tepals of *Lilium huanglongense* with basal nectaries **C** *Lilium matangense* flower **D** *Lilium fargesii* flower **E** *Lilium stewartianum* flower **F** tepals of *Lilium matangense* with basal nectaries **G** tepals of *Lilium fargesii* with basal nectaries **H** tepals of *Lilium stewartianum* with basal nectaries. Photographed by Yundong Gao.

Firstly, the flower of the new species is about 3–4 cm in diameter and, when fully expanded, the perianth is nearly in the same plane as the androgynophore (Fig. 2A), whereas in the other species, the androgynophore is exposed to a greater extent (Fig. 2C–E). Secondly, the stigma of *L. huanglongense* is three-lobed without inflation (Fig. 3F, G), whereas that of *L. matangense* is three-lobed with inflation (Fig. 2C).

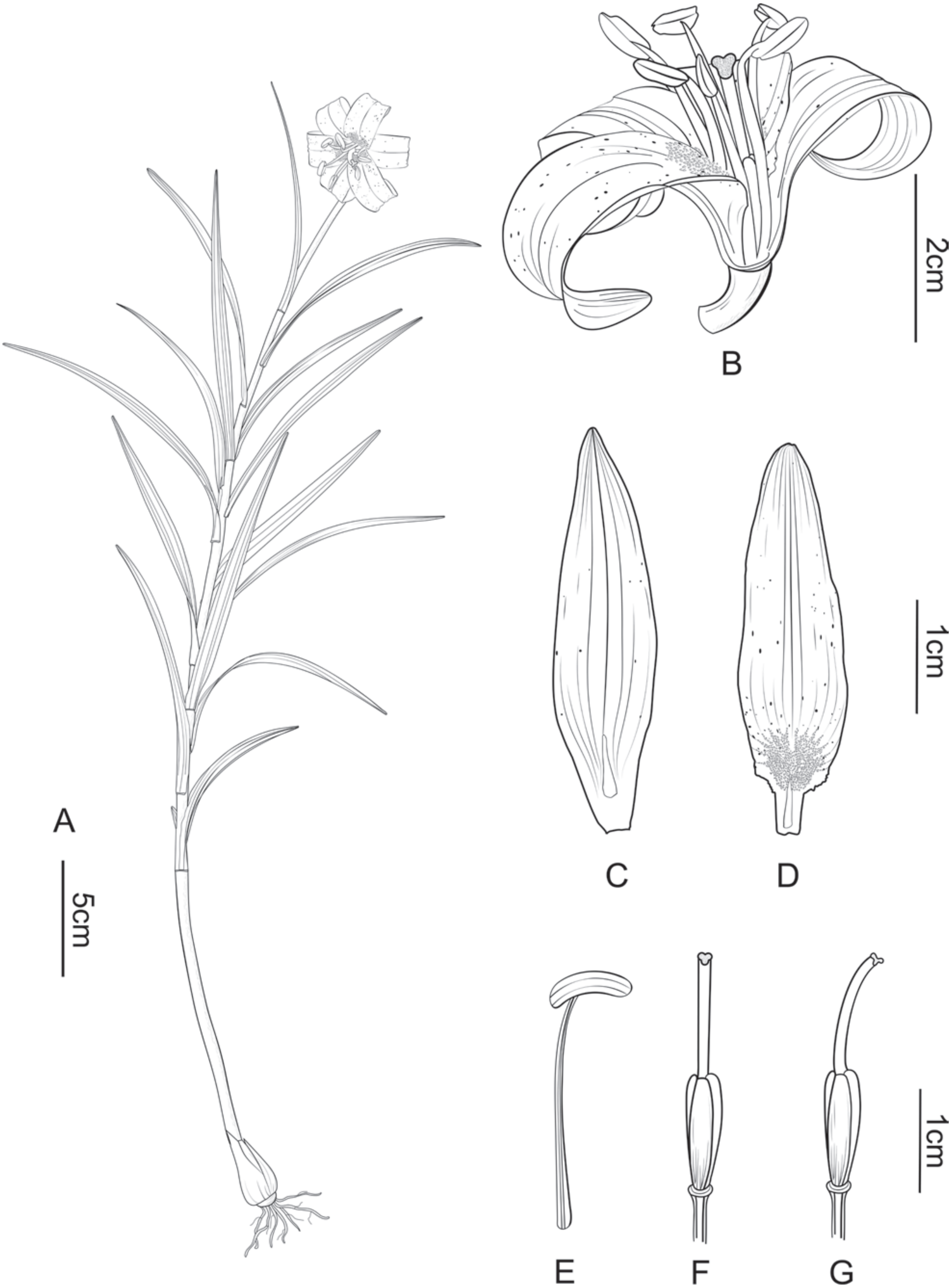

Figure 3. *Lilium huanglongense* T.Wang & Y.D.Gao, sp. nov. **A** habit **B** dissected flower **C** outer perianth segment **D** inner perianth segment **E** stamen **F** pistil (frontal view) **G** pistil (lateral view). Drawn by T. Wang from the holotype.

Figure 4. Habitat of *Lilium huanglongense* and morphological comparison with *Lilium fargesii* **A** habitat destroyed by mudslides **B** flowering plant **C** habit of *Lilium huanglongense* exhibiting a greater abundance of basal leaves, accompanied by wider leaf blades compared to *L. fargesii* **D** habit of *Lilium fargesii*. *Lilium huanglongense*. Photographs were taken by multiple authors of present work.

Furthermore, *L. huanglongense* possesses a greater number of basal leaves compared to *L. fargesii* (Fig. 4C, D). The detailed differences between *L. huanglongense* and the most similar species are listed in Table 1. These morphological differences effectively distinguish the new species from known congeners.

Phylogenetic analyses (Fig. 5)

The analysis was based on molecular data, specifically ITS (ITS1, 5.8S and ITS2) sequences and the complete chloroplast genome. This study utilised two datasets, each including two individuals of the new species. The new species has an ITS sequences with 624 base pairs (bp) in length with a GC content of 61.5%, whereas the chloroplast genome was 152,597 bp long with a GC content of 37.0%. The chloroplast genome comprises double-stranded circular DNA and exhibits a characteristic quadripartite structure, including a large single-copy (LSC) region spanning 81,965 bp, a small single-copy (SSC) region of 17,496 bp and two inverted repeat (IR) regions, each measuring 26,568 bp. We utilised 34 ITS sequences, with lengths ranging from 610 bp to 633 bp prior to alignment and, after alignment correction, the sequence lengths were 641 bp with 223 variable sites and 411 conserved sites. In addition, we analysed 30 complete chloroplast genomes with sequence lengths ranging from 151,655 bp to 153,235 bp before alignment and 157,060 bp after alignment correction, containing 6,417 variable sites and 148,565 conserved sites.

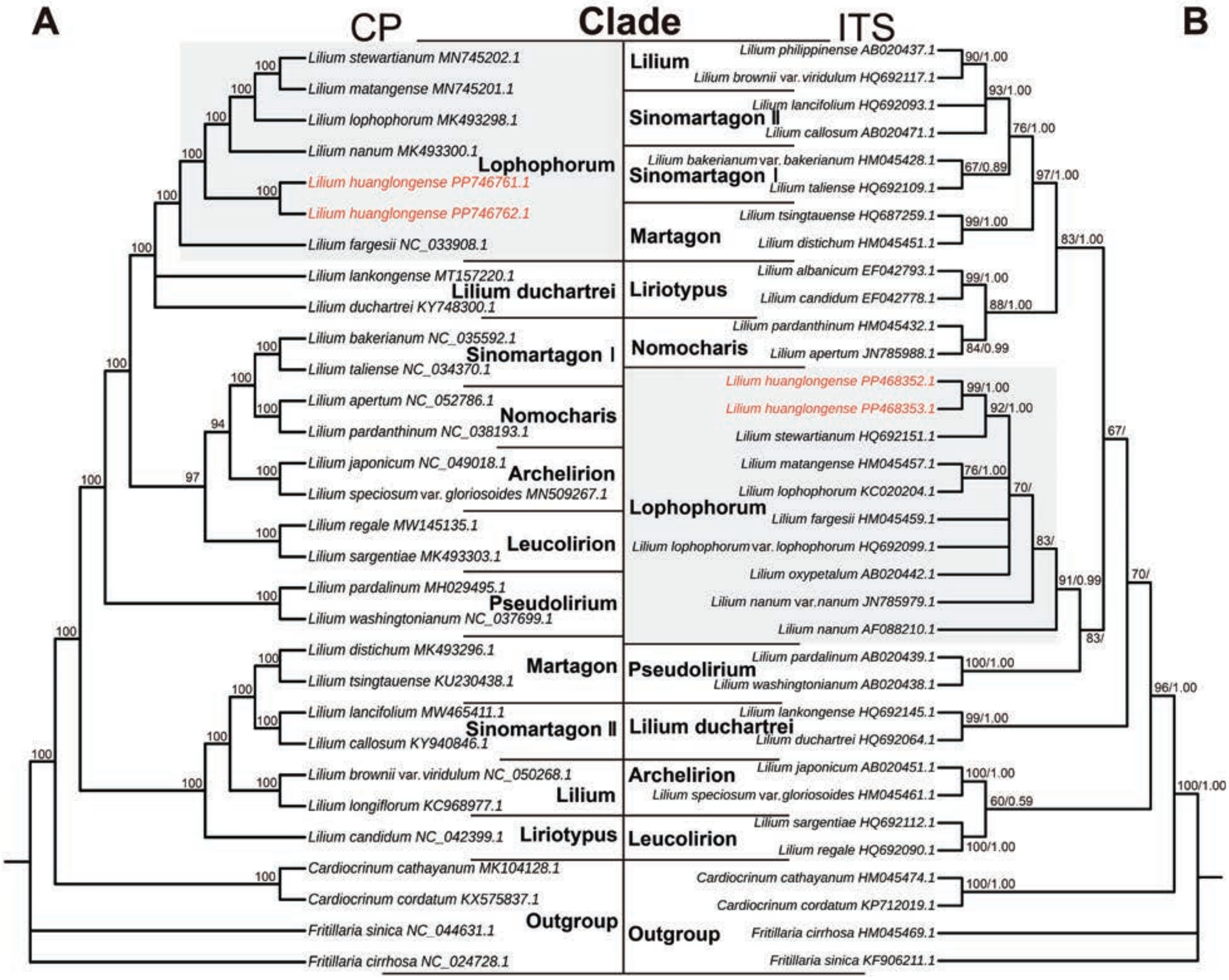

Figure 5. Maximum Likelihood (ML) phylogenetic analysis of selected species of *Lilium* based on **A** complete plastome DNA and **B** nuclear ITS sequence. Numbers at nodes indicate bootstrap percentages (BS) for ML. In **B**, the values to the left of the "/" represent the bootstrap support (BS), while those to the right indicate the Bayesian posterior probability (PP).

The phylogenetic analysis indicates that the Lophophorum-clade is monophyletic, supported by both chloroplast and ITS phylogenies, with support values of 100% (Fig. 5A) and 91%/0.99 (Fig. 5B, Suppl. material 2), respectively. These results are consistent with previous works (Gao et al. 2013a; Yuan and Gao 2024). In the plastid phylogeny, *L. fargesii* is resolved as sister to all other species within the Lophophorum-clade. Within this successively branching clade, both individuals of *L. huanglongense* form a monophyletic group, which is sister to *L. nanum*, *L. lophophorum*, *L. matangense* and *L. stewartianum*. In the ITS phylogeny, *L. huanglongense* is sister to *L. stewartianum*, whereas in the plastid phylogeny, *L. stewartianum* forms a clade with *L. matangense* (Fig. 5).

Taxonomic treatment

***Lilium huanglongense* T.Wang & Y.D.Gao, sp. nov.**
urn:lsid:ipni.org:names:77356314-1
Figs 1–4, 6, Table 1, Suppl. material 1: table S1
Common name: 黄龙百合 huang long bai he

Type. China • Sichuan: Songpan County, Huanglong National Natural Reserve, 30 June 2023, *Y.D. Gao GYD2023001* (holotype: CDBI 0285062) (Fig. 6).

Diagnosis. *Lilium huanglongense* is most similar to *L. fargesii* and *L. stewartianum*, but can be distinguished from *L. fargesii* by its yellow tepals and stamens that are longer than the pistil and, in contrast to *L. stewartianum*, *L. huanglongense* lacks a deep, slender floral tubes (the height of the cone formed by the connivent tepals) and has a trilobed, non-inflated stigma. (Table 1, Suppl. material 1: table S1)

Description. Bulb ca. 1.2–1.5 cm in diam., ovoid; scales 1.5–3 × ca. 8 mm, lanceolate, yellow. Stem 15–40 cm long, smooth, basal part red, red colour gradually fading and becoming green with reddish-brown speckles towards the apex of stem. Leaves 5.0–12.0 × 0.3–0.7 cm, scattered, mostly in middle and distal parts of stem, linear, margin recurved, smooth. Flowers actinomorphic, solitary, ca. 4–5 cm in diameter, nodding, Tepals 3.0–3.5 × 0.7–1.0 cm, lanceolate, margin revolute, yellow, with scattered, purple or purplish-brown spots mainly concentrated in the basal part; inner tepal nectaries with cristate projections on both surfaces, green; outer ones glabrous, with a green glistening nectarial channel at the base. Filaments 2–2.5 cm, glabrous; anthers 7–9 × ca. 2 mm, narrowly oblong, brown. Ovary 0.8–1 × ca. 0.3 cm, cylindrical. Style 0.8–1.2 cm, shorter than filaments, three-lobed without inflation, curved upwards. Capsule ca. 2 × 1.5 cm, oblong.

Phenology. Flowering from June to July.

Habitat and distribution. Occurring in alpine meadows on limestone slopes near streams, at altitudes of 3000–3300 m. This species is only known from three locations (one destroyed) in Huanglong National Nature Reserve, Songpan, NW Sichuan.

Etymology. The epithet adopted here is derived from Huanglong National Natural Reserve, the site of discovery of this species.

Conservation status and IUCN preliminary assessment. We conducted surveys in collaboration with staff from the Huanglong National Nature Reserve in Sichuan Province, China, covering approximately 150 km^2. The species

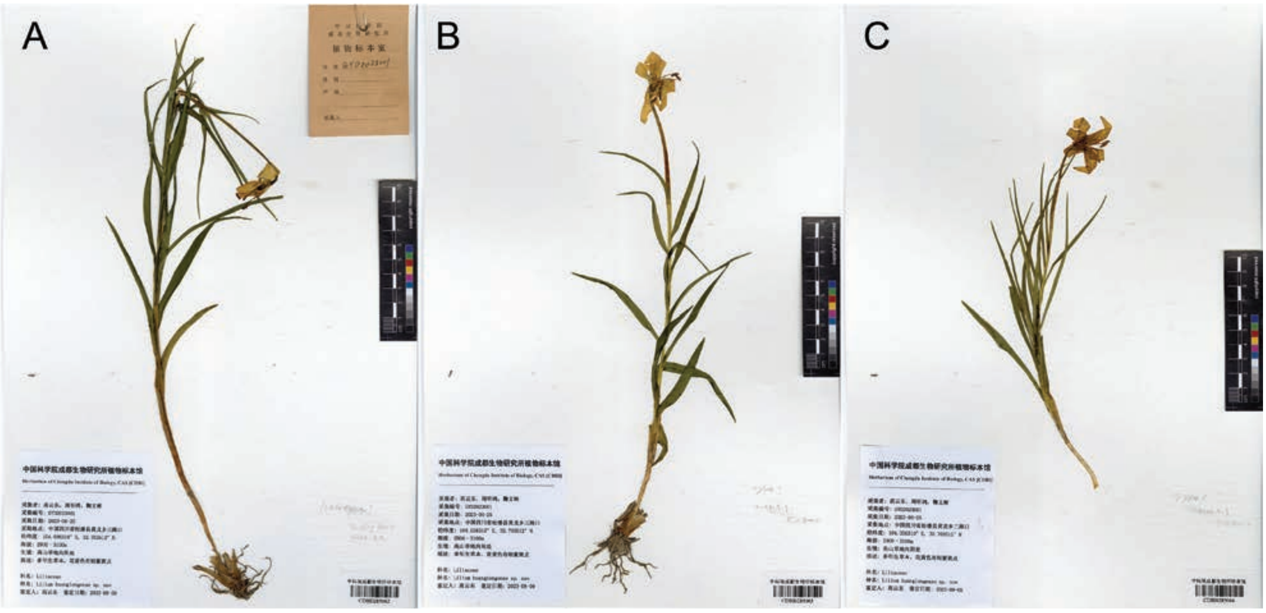

Figure 6. Type Specimens of *Lilium huanglongense* T.Wang & Y.D.Gao, sp. nov. **A** holotype CDBI0285062 **B** type CDBI0285063 **C** type CDBI0285064.

L. huanglongense was found at only three locations. The Extent of Occurrence (EOO) for this species was calculated to be approximately 5.361 km^2, while its Area of Occupancy (AOO) was estimated at around 0.509 km^2. During our field surveys, we observed that the species' habitat is highly unstable due to annual summer floods and rockfalls. In the summer of 2023, one of the previously known sites was completely destroyed by a mudslide, resulting in the loss of all individuals at that location (Fig. 4A). At the remaining two locations, we recorded approximately 30 mature individuals in total each year. Given the limited distribution, small population size and the instability of its habitat, we propose that *L. huanglongense* be classified as Critically Endangered (CR, B1i+ii, C1) according to IUCN Red List Criteria (IUCN 2024).

Discussion

Previously, we documented the presence of two distinct flower morphologies within the Lophophorum-clade, which may reflect parallel evolution as lilies rapidly adapt to diverse environments (Gao et al. 2015; Yuan and Gao 2024). This suggests that the former classification of *Lilium* into subgenera, based solely on floral morphological differences, may not be entirely valid (Watanabe et al. 2021). The Lophophorum-clade further supports the notion that parallel evolution is prevalent within the genus *Lilium* (Gao et al. 2015; Yuan and Gao 2024). Consequently, caution is warranted when assessing subgeneric affinities, based exclusively on morphological characteristics in this genus.

Molecular phylogenetic analysis demonstrated that *Lilium huanglongense* occupies a distinct position within the Lophophorum-clade. The Maximum Likelihood (ML) tree, based on chloroplast data, shows *L. huanglongense* as sister to *L. nanum*, *L. lophophorum*, *L. matangense* and *L. stewartianum* (Fig. 5A). In contrast, both the ML and Bayesian Inference (BI) trees, based on ITS data, place *L. huanglongense* and *L. stewartianum* in a monophyletic group (Fig. 5B), revealing a discordance between nuclear and plastid phylogenies (Fig. 5).

This discrepancy may result from incomplete lineage sorting (ILS) and introgression. However, our previous studies suggest that plastid phylogeny better reflects the geographic relationships amongst species, with introgression being a more plausible explanation (Gao et al. 2013b, 2015).

Within the plastid genome tree, *Lilium fargesii* occupies the earliest diverging position within the *Lophophorum*-clade (Fig. 5A). The emergence of *L. huanglongense* may have occurred during times of environmental fluctuation, such as the Quaternary Ice Age, followed by subsequent environmental isolation from its potential ancestral species during interglacial periods (Davis et al. 2005). This aligns with the observed distribution pattern, indicating that *L. huanglongense* is confined to a unique geographic position within the entire clade (Fig. 1), similar to other tepal-recurved members (e.g. *L. matangense*, *L. stewartianum*, Fig. 1) that are sporadically distributed in the Hengduan Mountains region. The limited population size and few populations of these tepal-recurved members of the Lophophorum clade suggest that a widely-distributed common ancestor may have existed previously, with the current relic pattern resulting from long-term isolation.

Morphologically, *Lilium huanglongense* is characterised by its compact stature, pale yellow perianth (Fig. 2A), flattened and delicate floral structure and dense basal foliage, distinguishing it from morphologically similar species. The pale yellow perianth of *L. huanglongense* sets it apart from *L. matangense* (Fig. 2C) and *L. fargesii* (Fig. 2D), which typically exhibit flowers with perianth colours ranging from white to green. Additionally, its relatively small size and flattened floral structure (short tube length) differentiate it from *L. stewartianum* (Fig. 2E), which features a deep, slender floral funnel (the height of the cone formed by the connivent tepals). Our comparisons also revealed significant differences in the proportions of floral organs amongst these species. *Lilium huanglongense* has flattened floral parts, with the flowers and stamens nearly in the same plane (Fig. 2A). In contrast, the pistils and stamens of similar species, such as *L. fargesii*, *L. stewartianum* and *L. matangense*, clearly protrude from the perianth.

The specialised perigone structure in *L. huanglongense* is likely the result of localised plant-environment interactions, particularly with its pollinators. These pollinators play a crucial role in driving morphological evolution (Van der Niet and Johnson 2012; Van der Niet et al. 2014) and have influenced the delicate floral features of *L. huanglongense*, such as its pale yellow perianth and flattened floral structure, which may have evolved to attract specific pollinators in its habitat. *Lilium huanglongense* exhibits a shorter style than *L. fargesii* and a shorter floral tube (the height of the cone formed by the connivent tepals) than *L. stewartianum*. These differences in floral morphology may have evolved in response to varying pollinators, indicating that pollination syndrome may play a key role in the speciation process of this new species.

Geographically, *Lilium huanglongense* bridges the distribution gap between *L. fargesii*, native to central China and other species inhabiting the south-western alpine mountains (Fig. 1). The entire Lophophorum-clade extends over a broad geographic range from Hunan (*L. fargesii*) in the east to the western Himalayas (*L. nanum*) in the west. This distribution encompasses mesic broadleaf forests in the central Daba Mountain system, alpine scrublands in the south-western Hengduan Mountains and extends to the alpine scrub meadows of the Himalayas (Gao et al. 2013b). The divergence of the Lophophorum clade is likely due to historical geological and climatic changes in the Qinling-Dabashan-Hengduan-Himalayan region (Gao et al. 2013b; Xing and Ree 2017). This region, characterised by a series of mountain ranges and diverse ecological niches, spans central and south-western China and supports substantial biodiversity.

In conclusion, *Lilium huanglongense* is a morphologically and molecularly distinct new species within the Lophophorum-clade. This discovery not only contributes to the diversity of the genus, but also fills a geographical gap west of the Qinling Mountains, at the confluence of the Hengduan Mountains. However, our understanding of the Lophophorum-clade remains incomplete. To enhance our comprehension of its phylogenetic relationships and gain a comprehensive understanding of the biogeographic processes involved, further literature reviews, fieldwork and additional collection of morphological and molecular data are necessary.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

All authors contributed equally to this study and made a significant contribution to the overall result.

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Data availability

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

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Supplementary material 1

Supplementary information

Authors: Ting Wang, Yumei Yuan, Ting-Hong Zhou, Yundong Gao

Data type: xlsx

Explanation note: **table S1.** Comparison of measurements and morphology of specimens of *Lilium huanglongense*, *Lilium stewartianum*, *Lilium fargesii* and *Lilium matangense*. **table S2.** GenBank accession numbers for sequences utilised in phylogenetic analyses.

Link: https://doi.org/10.3897/phytokeys.252.135155.suppl1

Supplementary material 2

Phylogenetic tree constructed from 34 ITS sequences using Bayesian analysis

Authors: Ting Wang, Yumei Yuan, Ting-Hong Zhou, Yundong Gao

Data type: pdf

Link: https://doi.org/10.3897/phytokeys.252.135155.suppl2

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Research Article

Gastrochilus obovatifolius (Orchidaceae, Aeridinae), a new species from the Daba Mountains of Chongqing, China

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Abstract

Gastrochilus obovatifolius, a new species discovered in the Dabashan of northeast Chongqing (China), is described and illustrated. The novelty morphologically resembles *G. affinis*, *G. balangshanensis* and *G. heminii* in having a glabrous and smooth epichile. but can be distinguished by the unique obovate shape of the leaves, smaller epichile, and flower color. The results of molecular phylogenetic analysis, based on nuclear ribosome internal transcribed spacer (nrITS) and four chloroplast DNA fragments (*mat*K, *psb*A–*trn*H, *psb*M–*trn*D, and *trn*L–F) of 55 *Gastrochilus* species, support the inclusion of *G. obovatifolius* in *G.* sect. *Microphylli*, being most closely related to *G. balangshanensis* from Sichuan. The new species is a trunk epiphyte in evergreen broad-leaved forest.

Key words: Epidendroideae, Flora of Chongqing, *Gastrochilus* sect. *Microphylli*, phylogeny, Qinling-Daba Mountains, taxonomy

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Introduction

The orchid genus *Gastrochilus* D.Don (Don 1825: 32) part of the mostly Asian vandoid subtribe Aeridinae, comprises approximately 80 species distributed in tropical and subtropical Asia, from India and Sri Lanka to East Asia and south to Indonesia (Tsi 1996, 1999; Liu et al. 2019; Zhang et al. 2022; Ya et al. 2023; POWO 2024; Zhang et al. 2024a, b). Among these, around 60 species are found in China (including approximately 40 endemic), where new species continue to be regularly described (Chen et al. 2009; Liu 2017; Liu et al. 2019, 2023; Li et al. 2022; Ya et al. 2023; Zhang et al. 2024a, b; Zhou et al. 2024). A recent systematic study has classified *Gastrochilus* into six sections: *Gastrochilus* sect. *Gastrochilus*, *G.* sect. *Pseudodistichi* Jun Y. Zhang & H. He, *G.* sect. *Brachycaules* Q. Liu & J.Y. Gao

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[*] The authors contributed equally to this paper.

ex Jun Y. Zhang & H. He, *G.* sect. *Acinacifolii* Q. Liu & J.Y. Gao ex Jun Y. Zhang & H. He, *G.* sect. *Microphylli* (Benth. & Hook. f.) Seidenf., and *G.* sect. *Caespitosi* Z. H. Tsi (Zhang et al. 2024a). These sections can be delimited by leaf shape and length, type of epichile hairs and margin, and by whether the epichile surface is smooth or not (Zhang et al. 2024a). Recently, the Mountains of Southwest China have emerged as a fertile ground for new species discovery in *G.* sect. *microphylli*, namely the Hengduan Mountains (Liao et al. 2022, Ya et al. 2023, Zhang et al. 2024b), but the Qinling-Daba Mountains, to the east, have received less attention.

The Qinling-Daba Mountains (QDM) constitute a comprehensive geographical and geomorphic entity, serving as a natural demarcation between northern and southern China. These mountains function as an important corridor between the Hengduan Mountains and the eastern plains, separating the warm temperate zone from the subtropical zone (Zhu 1958, Zhang 2019). This region is acknowledged as one of China's foremost biodiversity hotspots, hosting a total of 9,491 species of seed plants, accounting for 39% of the overall seed plant diversity present in China (Zhang et al. 2022a). Moreover, the area is home to 3,585 endemic species, which represent 37% of the vascular plant species found within this region (Zhang et al. 2022b). In March 2024, during a plant survey in Chengkou County, Northeast Chongqing, at the southern foothills of the QDM, we encountered an unknown species of *Gastrochilus* with distichous, alternate, and obovate leaves, and small flowers with dark purple stripes. The smooth and glabrous epichile pointed to its inclusion in *G.* sect. *Microphylli* (Seidenfaden 1988). Some living plants were put into cultivation at Guilin Botanical Garden for further study. Standard herbarium taxonomy, including a comprehensive literature review on the genus (Chen et al. 2009; Liu 2017; Lee et al. 2019; Liu et al. 2019; Rao et al. 2019; Wu et al. 2019; Ormerod and Kumar 2020; Li et al. 2021; Nguyen et al. 2021; Averyanov et al. 2022; Chen et al. 2022; Dey et al. 2022; Liao et al. 2022; Zhang et al. 2022, 2024a, b; Lee et al. 2023; Li et al. 2023; Liu et al. 2023; Ya et al. 2023; Zhou et al. 2024), confirmed that it represents a new species of *Gastrochilus*. In parallel, we investigated the phylogenetic position of *G. obovatifolius* sp. nov., which is here described and illustrated.

Materials and methods

Morphological analyses

Specimens of the novelty were collected during our field expedition to the Chongqing Municipality in 2024. Photographs were taken using a Nikon D7200 digital camera (Japan). Morphological descriptions and measurements were based on living plants and dried herbarium specimens deposited at CGMC and IBK (herbarium acronyms follow Thiers 2024). This material was compared to relevant specimens, including types, presented in Zhang et al. (2024b), housed at CDBI, K, KUN and PE. Descriptive terminology follows Beentje (2016).

Genomic DNA extraction and sequencing

Leaf material for DNA extraction was dried using silica gel (Chase and Hills 1991). Genomic DNA was extracted using a modified CTAB protocol (Chen et al. 2014). The total genomic DNA sample was sent to Majorbio in China (http://www.majorbio.com/) for library construction and next-generation sequencing. Short-insert (350 bp) paired-end read libraries preparation and 2 × 150 bp sequencing were performed on an Illumina (HiSeq4000) genome analyzer platform. Approximately 1 Gb of raw data for the new species was filtered using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/download.html) to obtain high-quality clean data by removing adapters and low-quality reads.

Plastid genome and ribosomal DNA (rDNA) assembly and annotation

Complete chloroplast genome and ribosomal genome data were assembled using GetOrganelle v.1.7.7.0 (Jin et al. 2020). ITS sequence extraction was performed by ITSx v.1.1.3 (Bengtsson-Palme et al. 2013). The plastid genome was preliminarily annotated using CPGAVAS2 (Shi et al. 2019), with *Gastrochilus fuscopunctatus* (Hayata) Hayata (GenBank: NC_035830) as the reference genome. The annotation results were confirmed using Geseq (Tillich et al. 2017). The genome map of the new species was drawn by OGDRAW (Greiner et al. 2019). The genome sequences were deposited in GenBank (accession numbers: PP942372, PP949380).

Phylogenetic analyses

To investigate the phylogenetic position of this species, we extracted five DNA regions (ITS, *mat*K, *psb*A–*trn*H, *psb*M–*trn*D, and *trn*L–F) from assembled rDNA and complete plastid genome sequences of the new species. Additionally, we downloaded sequence data used in Zhang et al. (2024a) and Ya et al. (2023) from GenBank (Suppl. material 1). This resulted in 95 accessions representing 61 taxa in total, with 55 taxa belonging to *Gastrochilus* as the ingroup, and six Aeridinae species in the genera *Luisia* Gaudich., *Saccolabium* Blume, *Holcoglossum* Schltr. and *Pomatocalpa* Breda as the outgroup. All sequences were aligned using MAFFT and the five aligned DNA markers were concatenated in Phylosuite v.1.2.3 (Katoh and Standley 2013; Zhang et al. 2020; Xiang et al. 2023). The substitution model was determined in Phylosuite v.1.2.3 using ModelFinder (Kalyaanamoorthy et al. 2017; Zhang et al. 2020; Xiang et al. 2023) and the evolutionary best fit model (GTR+F+I+G4) was selected using the corrected Akaike Information Criterion (AICc). Bayesian Inference (BI) was conducted using MrBayes in Phylosuite v.1.2.3 (Ronquist et al. 2012; Zhang et al. 2020; Xiang et al. 2023). The Markov chains were run for 1,000,000 generations, with sampling every 1,000 generations and a burn-in of 0.25. Four Markov chains with two runs were executed. The Maximum Likelihood (ML) phylogenetic trees were generated in IQ-TREE with 1000 bootstrap replicates in Phylosuite v.1.2.3 (Guindon et al. 2010; Minh et al. 2013; Nguyen et al. 2015; Zhang et al. 2020; Xiang et al. 2023).

Results

The complete plastid genome sequences of *Gastrochilus obovatifolius* sp. nov. comprise 146,248 bp (Fig. 1). The characteristics and statistics of the plastid genome are summarized in Suppl. materials 2, 3. The aligned nrITS matrix is 683 nucleotides long with 207 variable sites, and the combined four plastid markers

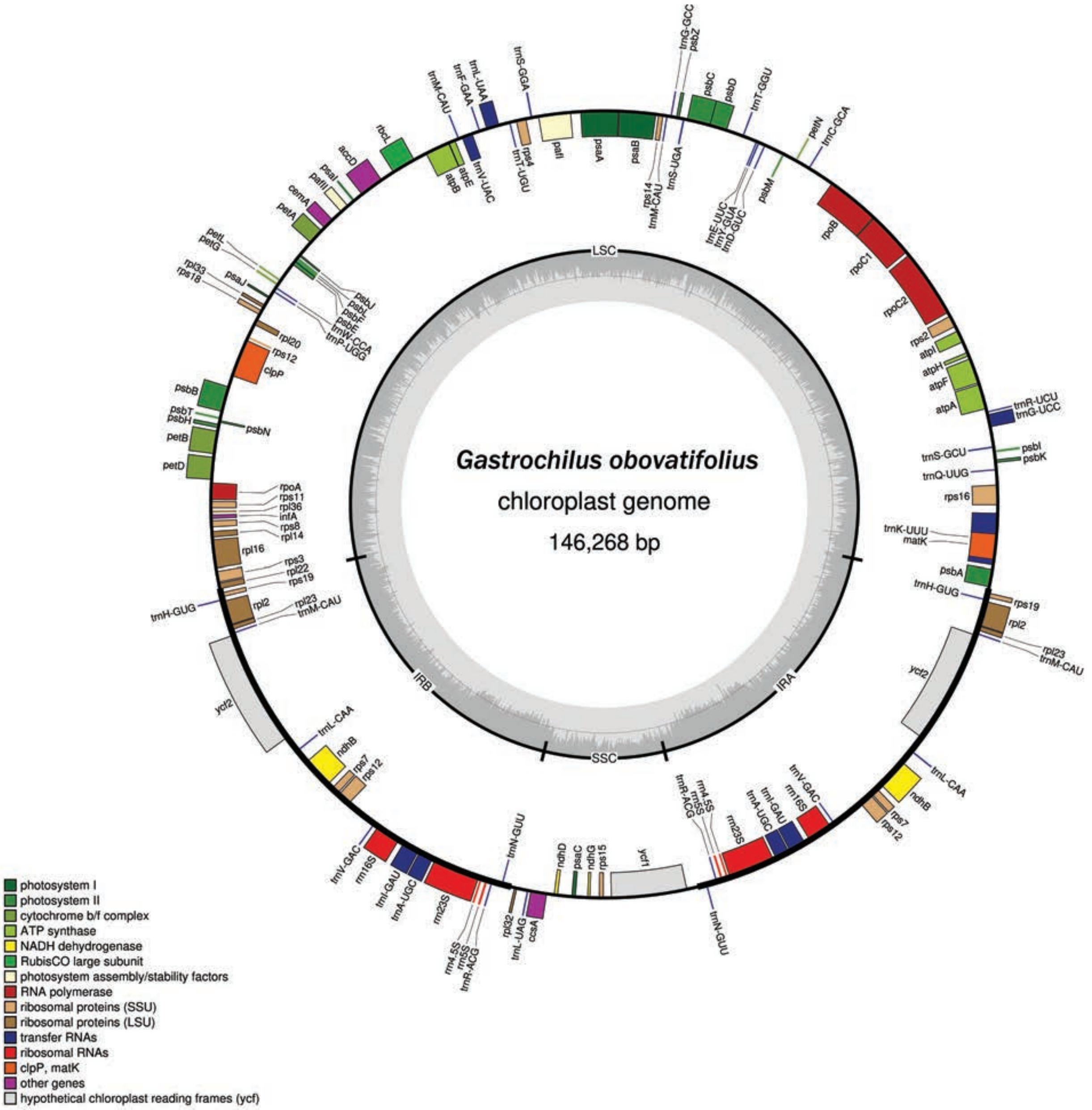

Figure 1. Plastome map of *Gastrochilus obovatifolius*. The thick lines on the outer complete circle represent the inverted repeat regions (IRa and IRb). The gray gradient in the innermost circle represents the GC content. Genes on the outside and inside of the map are transcribed in clockwise and counter directions, respectively.

matrix including 3,431 nucleotides in length with 343 variable sites, consists of 805 bp for *mat*K, 677 bp for *psb*A–*trn*H, 950 bp for *psb*M–*trn*D, and 999 bp for *trn*L–F, respectively. The characteristics and statistics of the five plastid markers are summarized in Suppl. material 4. The resulting tree topology (Fig. 2) aligns with previous phylogenetic analyses of *Gastrochilus* (Zhang et al. 2024a, b). The new species and *G. balangshanensis* Jun Y.Zhang, B.Xu & Yue H.Cheng form a monophyletic pair (PP/BS = 0.94/82) sister to *G. heminii* and *G. bernhardtianus* (PP/BS = 0.56/42), within *G.* sect. *Microphylli* (PP/BS = 0.79/89) (Fig. 2).

A morphological comparison of *G. obovatifolius*, *G. balangshanensis*, *G. heminii*, and *G. affinis* is presented in Table 1, which further substantiates the recognition of *G. obovatifolius* as a new species.

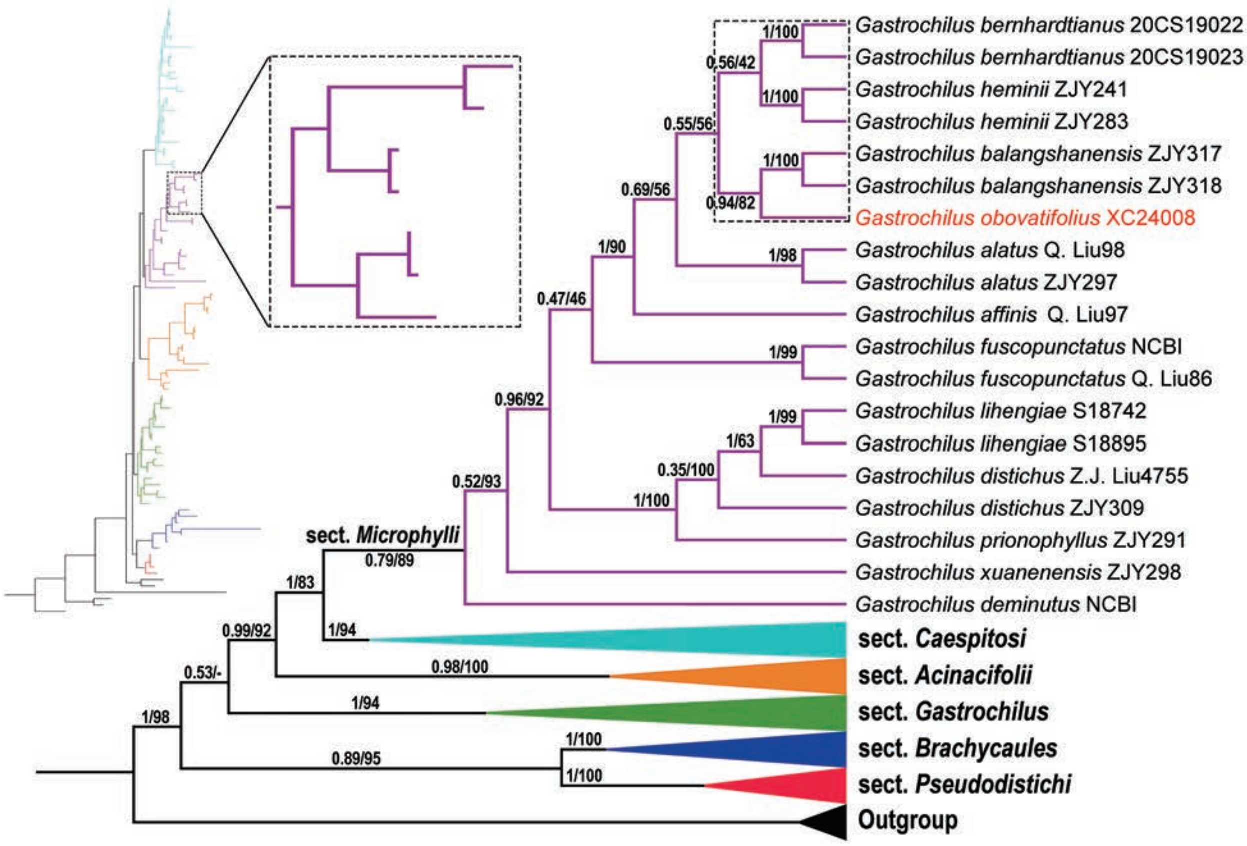

Figure 2. Bayesian tree from analysis of combined nrITS and four-plastid (*mat*K, *psb*A–*trn*H, *psb*M–*trn*D, and *trn*L–F) marker data of 55 species of *Gastrochilus*. The bootstrap values (BS) of ML and posterior probabilities (PP) of BI are listed at each node. The new species is highlighted in red, dotted lines shows its phylogenetic relationship with three related species.

Table 1. Morphological comparison between *Gastrochilus obovatifolius* and three morphologically similar species in *G.* sect. *Microphylli*.

Characters	*G. obovatifolius*	*G. affinis*	*G. balangshanensis*	*G. heminii*
Leaves	obovate, 1.4–1.6 × 0.6–0.8 cm	elliptic to narrowly oblong, 1.2–2.6 × 0.3–0.7 cm	nearly elliptic, 0.9–1.5 × 0.4–0.8 cm	narrowly oblong or oblong-falcate, 0.9–2.3 × 0.3–0.5 cm
Dorsal sepal	elliptic, ca. 5.0 × 4.0 mm, apex obtuse	elliptic-oblong, 3.0–5.0 × 1.0–1.3 mm, apex obtuse	elliptic, 5.6–6.4 × 4.8–5.2 mm, apex obtuse	elliptic-oblong, ca. 2.4 × 1.5 mm, apex obtuse
Lateral sepals	elliptic, slightly oblique, ca. 5.0 × 3.0 mm, apex obtuse	elliptic-ovate, slightly oblique and incurved, 3.5–4 × 0.7–1.3 mm, apex obtuse	elliptic, 5.6–6.4 × 4.8–5.2 mm, apex obtuse	elliptic-oblong, ca. 2.4 × 1.5 mm, apex obtuse
Petals	oblong, ca. 6.0 × 3.0 mm	ovate-elliptic to elliptic, 3.0.–4.0 × 1.0–1.3 mm	oblong, 5.0–5.8 × 4.0–4.4 mm	narrowly oblong, ca. 2.6 × 1.3 mm
Epichile	reniform, 10.0–12.0 × 6.0–8.0 mm, revolute, margin erose, median patch dark purple with 2 low ridges	subtriangular, ca. 8.0 × 4.5 mm, decurved, margin finely erose at base, median patch brown to purplish-brown, with 2 thick ridges	reniform, 10.0–12.0 × 5.5–6.5 mm, revolute, margin erose, median patch purple-red with two inconspicuous ridges	reniform, 4.2–6.5 × 2.0–3.0 mm, revolute, margin erose, median patch purple-red with irregular folds
Hypochile	sub-hemispherical, 4.0–4.5 × 4.0–4.2 mm, dorsally compressed, slightly bent outward, obtuse at the apex	obconical, 3.0–4.0 × 2.0–3.0 mm, dorsally compressed, slightly bent outward, subacute to obtuse and shortly bifid at apex	sub-hemispherical, 6.0–8.0 × 5.8–7.5 mm, dorsally compressed, obtuse-rounded at the apex	subconical, 2.0–2.4 × 1.6–2.0 mm, dorsally compressed, slightly bent outward, splits into two conical sacs at the apex
Distribution	Chengkou, NE. Chongqing	Gaoligongshan, NW. Yunnan, Xizang; India, Nepal	Wenchuan, Central Sichuan	Wenchuan, Central Sichuan

Taxonomic treatment

Gastrochilus obovatifolius C.Xiong, X.Y.Fu & S.R.Yi, sp.nov.

urn:lsid:ipni.org:names:77356371-1
Figs 3, 4

Diagnosis. *Gastrochilus obovatifolius* is most similar to *G. balangshanensis*., but differs by the longer stem (3–5 vs. 1.5–3.5 cm), obovate leaves (vs. nearly elliptic), and smaller sepals (ca. 5.0 × 3.0–4.0 vs. 5.6–6.4 × 4.8–5.2 mm) and hypochile (4.0–4.5 × 4.0–4.2 vs. 6.0–8.0 × 5.8–7.5 mm).

Type. China • Chongqing: Chengkou County (城口县), Dong'an Town (东安镇), Lizishuping (栗子树坪), 31°42'N, 109°11'E, alt. ca. 1650 m, 30 March 2024, *Si-Rong Yi et al. YSR2703* (holotype IBK!, isotype CGMC!).

Description. ***Epiphytic herbs***, monopodial, pendent, with a short stem, 3.0–5.0 cm long, leafy. ***Roots*** vermiform, slender, 4.0–6.0 cm long and ca. 1 mm in diameter. ***Stems*** short, branched, green, glabrous, up to 5 cm long and ca. 2.0 mm in diameter, covered with sheathing leaf bases; sheaths with purplish-red spots; internodes 1.2–1.8 mm long. ***Leaves*** alternate, distichous, obovate, fleshy, 1.4–1.6 × 0.6–0.8 cm, margin entire, apex acute, obscurely serrate; young leaves yellow-green with a few purplish-red spots, mature leaves green with hardly any purplish-red spots. ***Inflorescences*** 1–2 racemes, usually arising from the axil of an upper leaf, 2.0–2.5 cm long, 1–2-flowered; peduncle 0.8–1.2 cm long; bracts 1.0–2.0 mm long. ***Flowers*** 1.2–1.4 × 1.0–1.2 cm, yellow-green, with dark purple stripes on petals and sepals, raised abaxially along the midrib; pedicel and ovary yellowish-green with purple-red spots, 1.5–1.6 cm long. ***Dorsal sepal*** elliptic, concave, ca. 5.0 × 4.0 mm, apex obtuse; ***lateral sepals*** similar to dorsal sepal, slightly oblique, ca. 5.0 × 3.0 mm, apex obtuse; petals oblong, concave, ca. 6.0 × 3.0 mm, apex obtuse. ***Labellum*** epichile reniform, yellow-green with purplish-red spots, 1.0–1.2 × 0.6–0.8 cm, revolute, margin erose, smooth and glabrous above, median patch, thickened, with 2 low ridges, dark purple; hypochile sub-hemispherical, yellow-green, mouth with lateral purplish-red markings, sac with purplish-red spots on the underside, obtuse at the apex, 4.0–4.5 mm tall, 4.0–4.2 mm in diameter, dorsally compressed, slightly bent outward. ***Column*** stout, ca. 2.0 × 1.2 mm; rostellum bilobed; anther cap galeate with curved emarginate beak, ca. 1.0 × 1.2 mm; pollinia 2, ca. 0.8 × 0.6 mm, yellow, nearly spherical, entire, with a depression in the center; stipe elongate, ca. 1.2 mm long, viscidium yellow, elliptic, ca. 0.8 × 0.4 mm. ***Capsule*** not seen.

Phenology. Flowering from March to April.

Etymology. The specific epithet '*obovatifolius*' refers to the highly distinctive obovate leaves. The suggested Chinese common name is "dào luǎn yè pén jù lán (倒卵叶盆距兰)".

Distribution and ecology. The new species has only been recorded in Chengkou County, northeast Chongqing Municipality, bordering Shaanxi Province, China (Fig. 6). It grows as a trunk epiphyte on *Quercus engleriana* Seemen, in evergreen broad-leaved forest at an elevation of 1620–1650 m a.s.l. (Fig. 5). Besides *Q. engleriana*, the most frequent co-occurring angiosperm species include *Fargesia spathacea* Franch., *Hepatica henryi* (Oliv.) Steward, *Pieris japonica* (Thunb.)

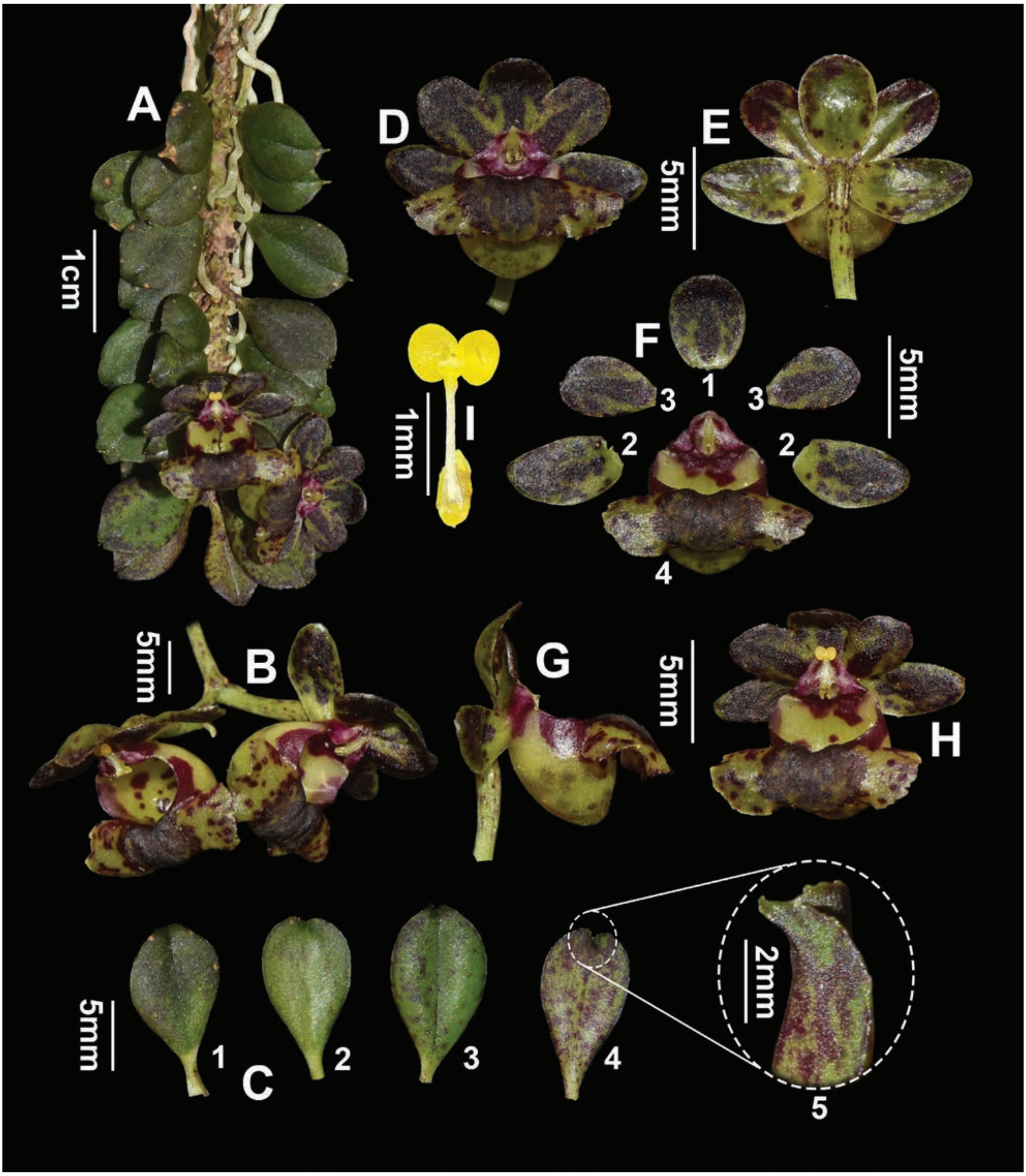

Figure 3. *Gastrochilus obovatifolius* C.Xiong, X.Y.Fu & S.R.Yi, sp. nov. *in vivo*. **A** flowering plant **B** inflorescence **C** leaves (C1–C3: front view, C4: back view, C5: close-up of leaf apex) **D** flower (front view) **E** flower (back view) **F** dissected flower (F1: dorsal sepal; F2: lateral sepals; F3: petals; F4: labellum) **G** flower (side view) **H** flower (top view) **I** pollinarium.

D. Don ex G. Don, *Rhododendron adenopodum* Franch., and *Zanthoxylum dimorphophyllum* Hemsl.

Additional specimens examined (paratypes). China • Chongqing: Chengkou County (城口县), Dong'an Town (东安镇), Dongjiachang (董家厂), 31°42'N, 109°12'E, alt. ca. 1620 m, 8 April 2024, *Si-Rong Yi et al. YSR2733* (IBK!, CGMC!).

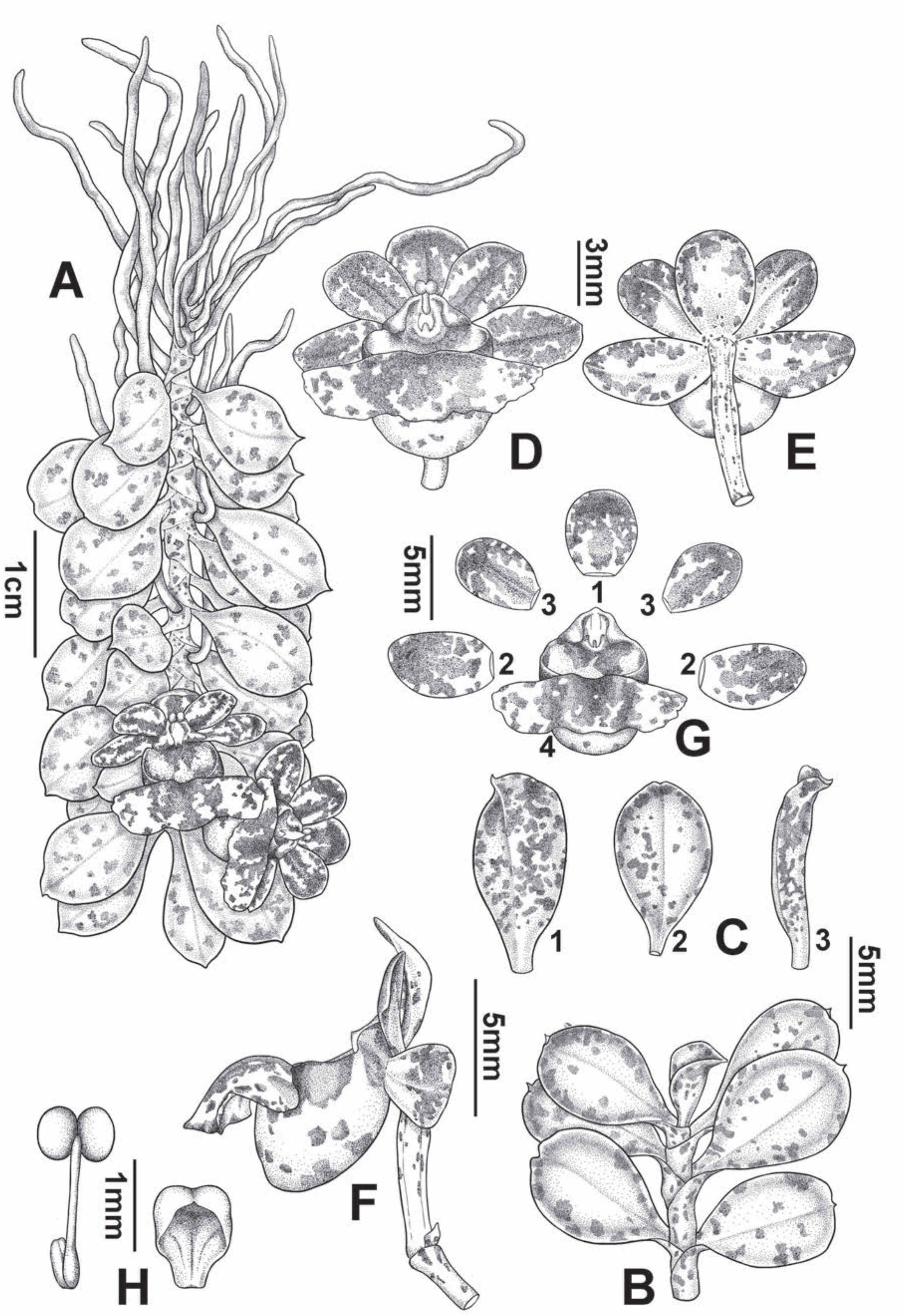

Figure 4. *Gastrochilus obovatifolius* C.Xiong, X.Y.Fu & S.R.Yi, sp. nov., an overview **A** habit **B** stem apex **C** leaves (C1: front view, C2: back view, C3: side view) **D** flower (side view) **E** flower (front view) **F** flower (back view) **G** dissected flower (G1: dorsal sepal; G2: lateral sepals; G3: petals; G4: labellum) **H** pollinarium and anther cap.

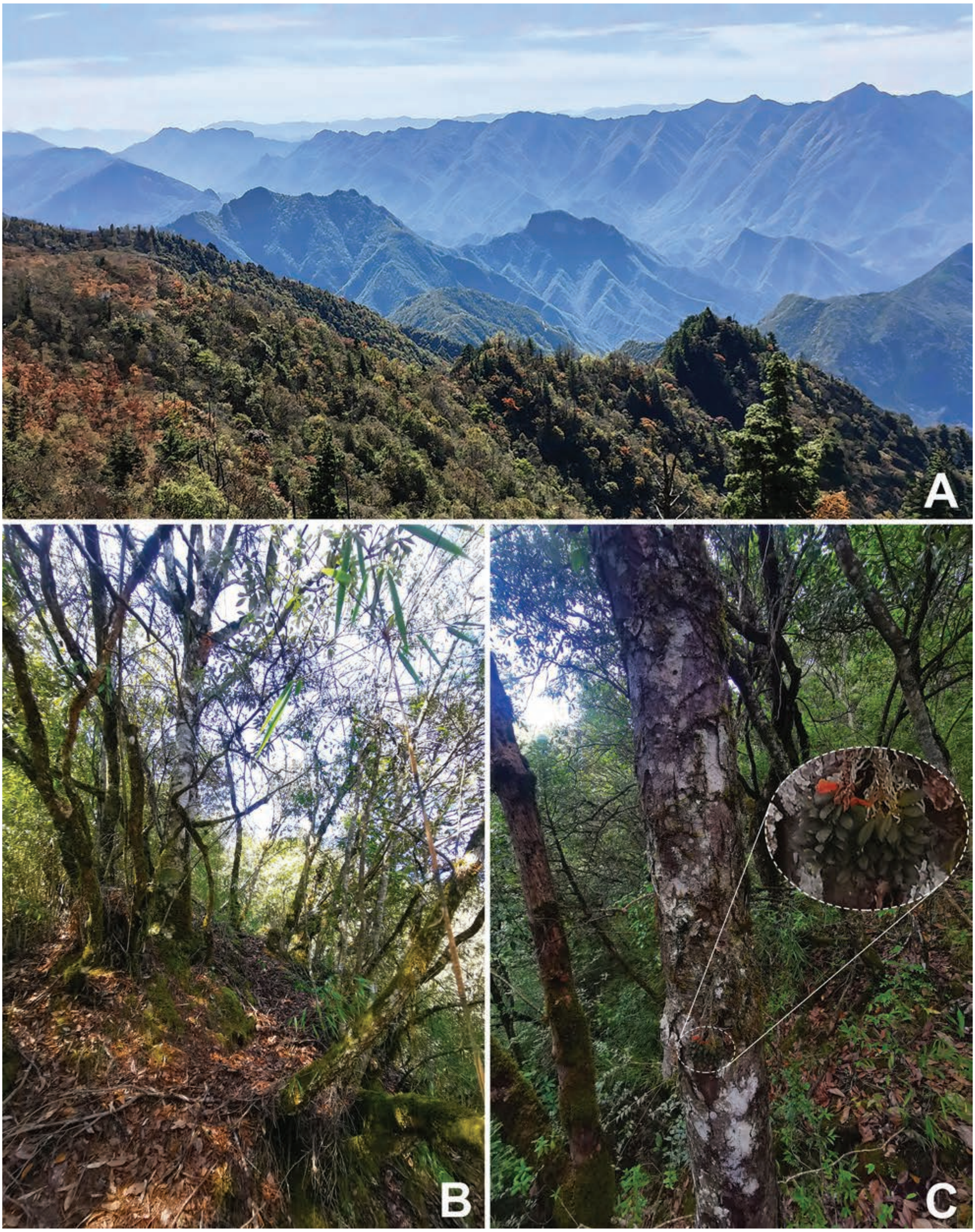

Figure 5. *Gastrochilus obovatifolius*, habitat and plants in situ. **A** broad-leaved temperate forest in the Daba Mountains of Chongqing **B** a woodland trail lined with *Fargesia spathacea*, *Quercus engleriana*, *Pieris japonica*, *Rhododendron adenopodum*, and *Zanthoxylum dimorphophyllum* **C** *G. obovatifolius* growing as a trunk epiphyte on *Q. engleriana*.

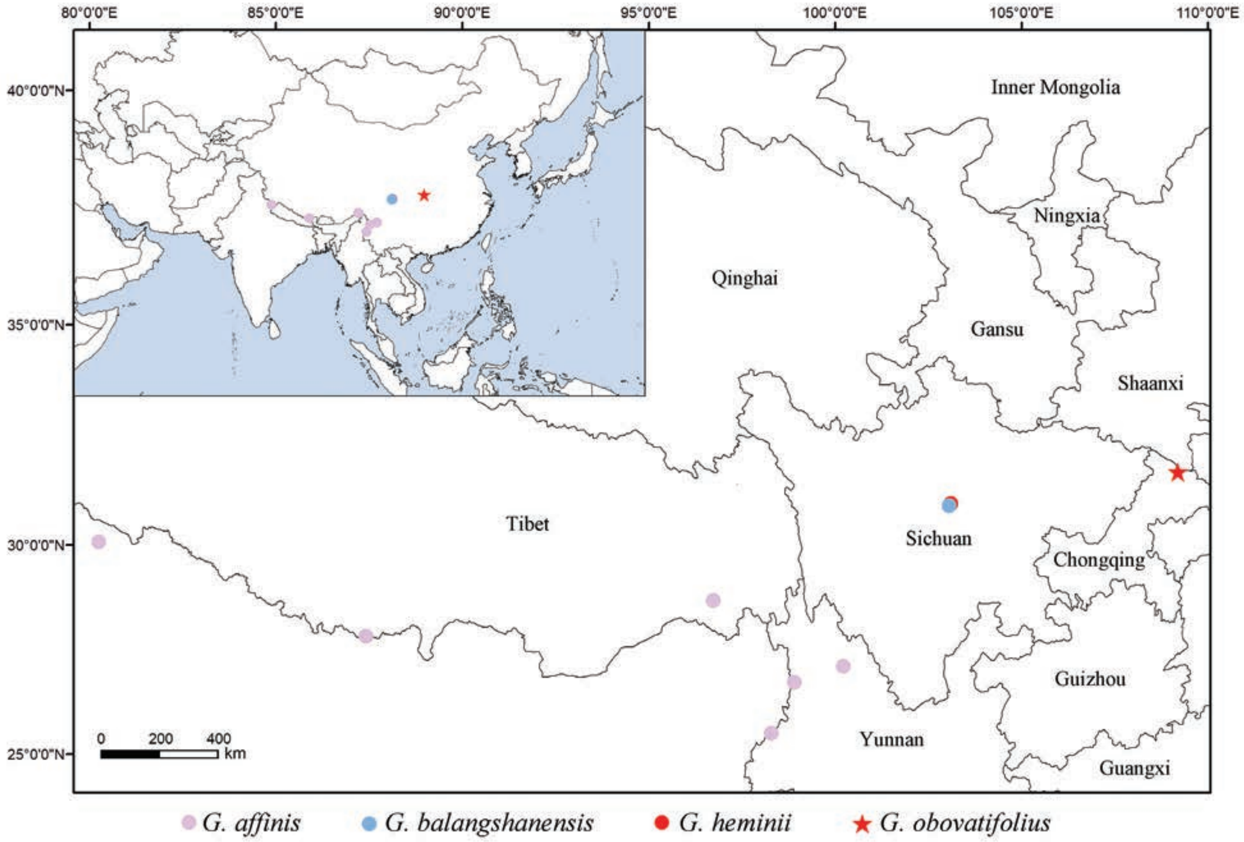

Figure 6. Distribution map of *Gastrochilus obovatifolius* and three related species of *G.* sect. *Microphylli* in southwestern China and adjoining areas.

Discussion

The phylogenetic analysis revealed that the newly discovered *Gastrochilus obovatifolius*, along with 12 other *Gastrochilus* species (comprising 19 accessions), form a clade classified as *Gastrochilus* sect. *Microphylli*. This clade is distinguished by floral characteristics such as diminutive flowers (petals and sepals < 6 mm long), a nearly reniform glabrous epichile with central irregular projections, and in some species, a hypochile split into two conical protrusions (Zhang et al. 2024a). The obovate leaves of *G. obovatifolius* set it apart from any other species in *Gastrochilus* sect. *Microphylli*, which predominantly exhibit lanceolate, oblong, or ovate leaves.

Gastrochilus sect. *Microphylli* now encompasses a total of 16 species, including 13 distributed in southern and southwestern China. Among these 13 species, only *G. xuanenensis* Z. H. Tsi (Tsi 1982) and the newly described *G. obovatifolius* are known to be restricted to the Qinling-Daba Mountains (QDM), while the Hengduan Mountains (HDM) harbor a greater diversity, with 11 out of the 13 identified species (Zhang et al. 2024b). The high endemism rate in the QDM and its adjacent regions (Zhang et al. 2022a, b), contrasts with the relatively low diversity of *G.* sect. *Microphylli* species in this area, which may suggest some potential for the identification of new species. Accordingly, there is a pressing need for further field research and investigations focused on *Gastrochilus* and other epiphytic orchids in this region.

In Chengkou County, where *G. obovatifolius* was discovered, two other species of *Gastrochilus* are present: *G. fargesii* (Kraenzl.) Schltr. and *G. formosanus* (Hayata) Hayata. These species are classified under *G.* sect. *Acinacifolii* and *G.* sect. *Caespitosi*, respectively. They can be easily differentiated from *G. obovatifolius* by their foliar and floral characteristics, including the longer leaves (5–15 cm long) and the differently textured epichile, which is densely covered with papillate hairs.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualisation and Methodology: Chi Xiong. Investigation: Ya Huang, Hong-Jing Zhang, Si-Rong Yi. Data Curation: Xiao-Ying Fu, Yi-Chen Wang, Yu-Bing Yang. Software and Visualisation: Xiao-Ying Fu, Ke Tan. Writing-Original draft: Chi Xiong, Xiao-Ying Fu. Writing-Review and Editing: Ke Tan, Si-Rong Yi. All authors have read and approved the final manuscript.

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Data availability

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

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Supplementary material 1

Detailed information of material, voucher and GenBank accession numbers

Authors: Chi Xiong, Xiao-Ying Fu, Ke Tan, Ya Huang, Hong-Jing Zhang, Yi-Chen Wang, Yu-Bing Yang, Si-Rong Yi

Data type: docx

Link: https://doi.org/10.3897/phytokeys.252.133501.suppl1

Supplementary material 2

Genes encoded in the plastid genome of *Gastrochilus obovatifolius* C.Xiong, X.Y.Fu & S.R.Yi

Authors: Chi Xiong, Xiao-Ying Fu, Ke Tan, Ya Huang, Hong-Jing Zhang, Yi-Chen Wang, Yu-Bing Yang, Si-Rong Yi

Data type: docx

Link: https://doi.org/10.3897/phytokeys.252.133501.supp2

Supplementary material 3

Summary of the whole plastid genome of *Gastrochilus obovatifolius* C.Xiong, X.Y.Fu & S.R.Yi

Authors: Chi Xiong, Xiao-Ying Fu, Ke Tan, Ya Huang, Hong-Jing Zhang, Yi-Chen Wang, Yu-Bing Yang, Si-Rong Yi

Data type: docx

Link: https://doi.org/10.3897/phytokeys.252.133501.suppl3

Supplementary material 4

Statistics for the molecular datasets used in this study

Authors: Chi Xiong, Xiao-Ying Fu, Ke Tan, Ya Huang, Hong-Jing Zhang, Yi-Chen Wang, Yu-Bing Yang, Si-Rong Yi

Data type: docx

Link: https://doi.org/10.3897/phytokeys.252.133501.suppl4

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DOI: 10.3897/phytokeys.252.139783

Research Article

Spiradiclis scorpiura (Rubiaceae), a new species from Guangxi, China

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Abstract

Spiradiclis scorpiura (Rubiaceae), a new calcareous species found in Guangxi, China, is described and illustrated. This new species is similar to *S. coccinea*, *S. scabrida*, and *S. purpureocaerulea* in having pubescent stems and subglobose capsules, but it is easily distinguished by its cincinnous inflorescence and its triangular, 1–2 mm long, pubescent bracteoles. According to the IUCN criteria, it is considered Data Deficient (DD) until more information becomes available. Photographs, an illustration, a distribution map, and a comparative table with the most similar species are provided.

Key words: Daxin County, limestone, new species, sinkhole, taxonomy

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Introduction

Spiradiclis Blume closely resembles *Ophiorrhiza* L., and the two genera are in the tribe Ophiorrhizeae based on morphological characteristics (Verdcourt 1958; Darwin 1976; Lo 1999; Chen and Taylor 2011; Wu et al. 2019) and molecular evidence (Bremer 2009; Rydin et al. 2009; Wikström et al. 2013; Razafimandimbison and Rydin 2019). Razafimandimbison and Rydin (2019) suggested that *Spiradiclis* is a synonym of *Ophiorrhiza*. However, we consider that the delimitation and relationship of the two genera still need further research, and since *Spiradiclis* is morphologically different from *Ophiorrhiza* by its linear-oblong or subglobose capsules with four valves (vs. obcordate and compressed capsules with two valves), we prefer the traditional concept of *Spiradiclis*, thereby considering it separate from *Ophiorrhiza*.

There are a total of 62 *Spiradiclis* species, according to Plants of the World Online (POWO 2024). They are distributed in southeastern Asia, including Bhutan, China, India, Indonesia, Myanmar, and Vietnam. Most of the species are distributed in China and are native to the south and southwest of the country. In the last decade, more than 20 new species of *Spiradiclis* have been discovered in China (e.g., Wang 2016; Zhang et al. 2018; Pan et al. 2019; Tong et al. 2020; Cai et al. 2022; Nong et al. 2024).

During field surveys in Daxin County, Guangxi, in July 2024, a *Spiradiclis* population was found in flower and fruit that was morphologically similar to *Spiradiclis coccinea* H.S.Lo. However, this newly collected *Spiradiclis* is distinctly different from *S. coccinea* by its elliptic leaves and cincinnous inflorescence. Therefore, this population was suspected to represent a new species. This was confirmed by more observations, the examination of specimens of closely related *Spiradiclis* species from the herbaria PE, IBK, GXMI, and KUN, and by consulting relevant literature. Hence, we confirm that the unusual plant is a species of *Spiradiclis* new to science, and the newly discovered taxon is here described as a new species.

Materials and methods

Fieldwork was carried out in Daxin County, Guangxi, to document the new species in its natural habitat. In addition, studies of herbarium material of various *Spiradiclis* species were conducted at PE, IBK, GXMI, and KUN, and relevant literature was consulted (Lo et al. 1983; Wang 2002; Wang et al. 2015; Wu et al. 2015, 2016, 2019; Pan et al. 2016; Liu et al. 2017; Zhang et al. 2018; Wen et al. 2019; Li et al. 2021; Song et al. 2022). Additional related *Spiradiclis* species were examined based on online images from the Kew Herbarium Catalogue (http://apps.kew.org/herbcat/gotoHomePage.do) and JSTOR Global Plants (http://plants.jstor.org/). Morphological characteristics of stems, leaves, pedicels, flowers, receptacles, gynoecia, and carpels were used to distinguish *Spiradiclis* species in this study.

The description is based on the type specimens. Measurements were made with a tape measure and callipers. The structure of the indumentum and its distribution were observed and described using a dissecting microscope at magnifications of more than 20×. Additional information on locality, habitat, ecology, plant form, and fruits was collected in the field. The preliminary conservation threat assessment followed IUCN Categories and Criteria (IUCN 2022).

Results and discussion

Taxonomy

***Spiradiclis scorpiura* Y.Nong & L.Wu, sp. nov.**
urn:lsid:ipni.org:names:77356517-1
Figs 1–4

Chinese name. xiē wěi luó xù cǎo (蝎尾螺序草).

Diagnosis. *Spiradiclis scorpiura* is most similar to *S. coccinea* but is different in its densely pubescent young stems that become glabrous when older (vs. glabrous or subglabrous), its cincinnous inflorescences (vs. cymose), its triangular, 1–2 mm long, pubescent bracteoles (vs. subulate, 3–4 mm long, glabrous), its calyx puberulent outside (vs. glabrescent outside), and its capsule 3–4 mm in diam. (vs. 4.5–5.5 mm in diam.).

Type. China • Guangxi, Daxin County, 22°54'06"N, 106°50'02"E, alt. 504 m, at the rim of the top of a sinkhole, 11 July 2024, flowering, *Y. Nong NY2024071101* (GXMI). (***Holotype*:** GXMI! 051187; isotype: IBK!).

Description. Subshrubs, perennial, 20–50 cm tall, rooting near base, stems ascending; stems densely pubescent when young but glabrous when old.

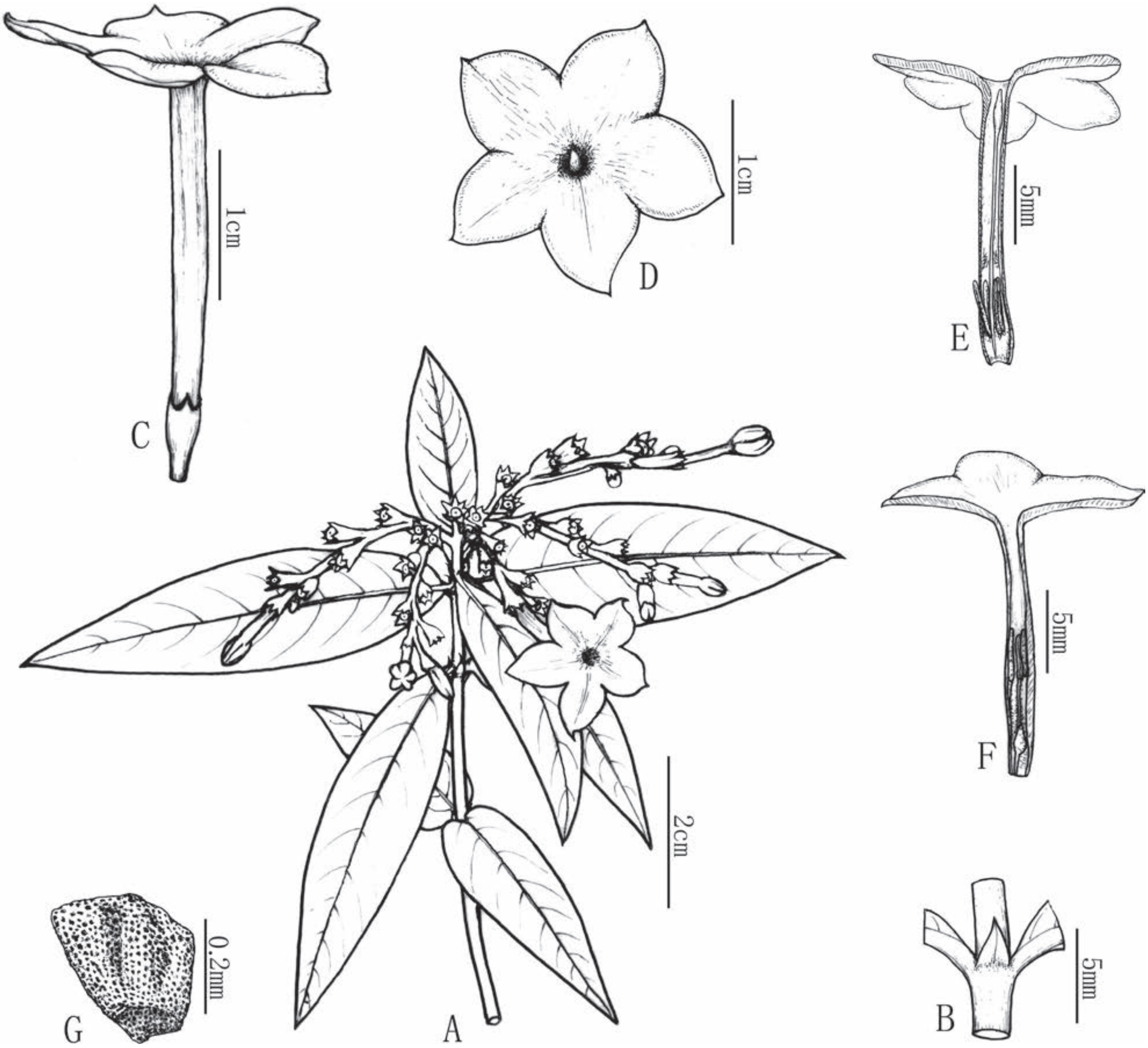

Figure 1. Line drawing of *Spiradiclis scorpiura* Y.Nong & L.Wu **A** flowering plant **B** stipule **C** flower **D** long-styled flower, frontal view, showing corolla lobes and stigma **E** longitudinally opened long-styled flower, showing the position of the stamens and the style and stigma **F** longitudinally opened short-styled flower, showing the position of the stamens and the style and stigma **G** seed (Drawn by Xin-cheng Qu).

Leaves opposite; petiole 1–2 mm long, sparsely pubescent; blade drying papery, adaxially olive green, abaxially yellowish green, elliptic, 3–7 × 0.5–1.5 cm, sparsely pubescent or glabrous on both surfaces, margin entire, base cuneate, apex acuminate; secondary veins 8–12 on each side of the midrib, midrib concave adaxially and prominently convex abaxially; stipules triangular, 1–2 mm long, glabrous outside, apex acute. Inflorescences terminal, cincinnous, 3–6 branched, 3–44-flowered, pubescent; peduncles 0.6–1 cm long, pubescent; pedicels short, c. 1 mm long, pubescent; bracteoles triangular, 1–2 mm long, pubescent outside. Flowers distylous. Calyx pubescent; hypanthium portion obovate, 1–2 mm long, with 5 straight ridges; lobes 5, triangular or ovate-lanceolate, 1–1.5 mm long. Corolla purple, slenderly salverform-funnelform, glabrous or pubescent outside; tube 15–18 mm long, lobes broadly ovate to suborbicular, 6–8 mm long. Stamens 5. Style filiform, stigma clavate,

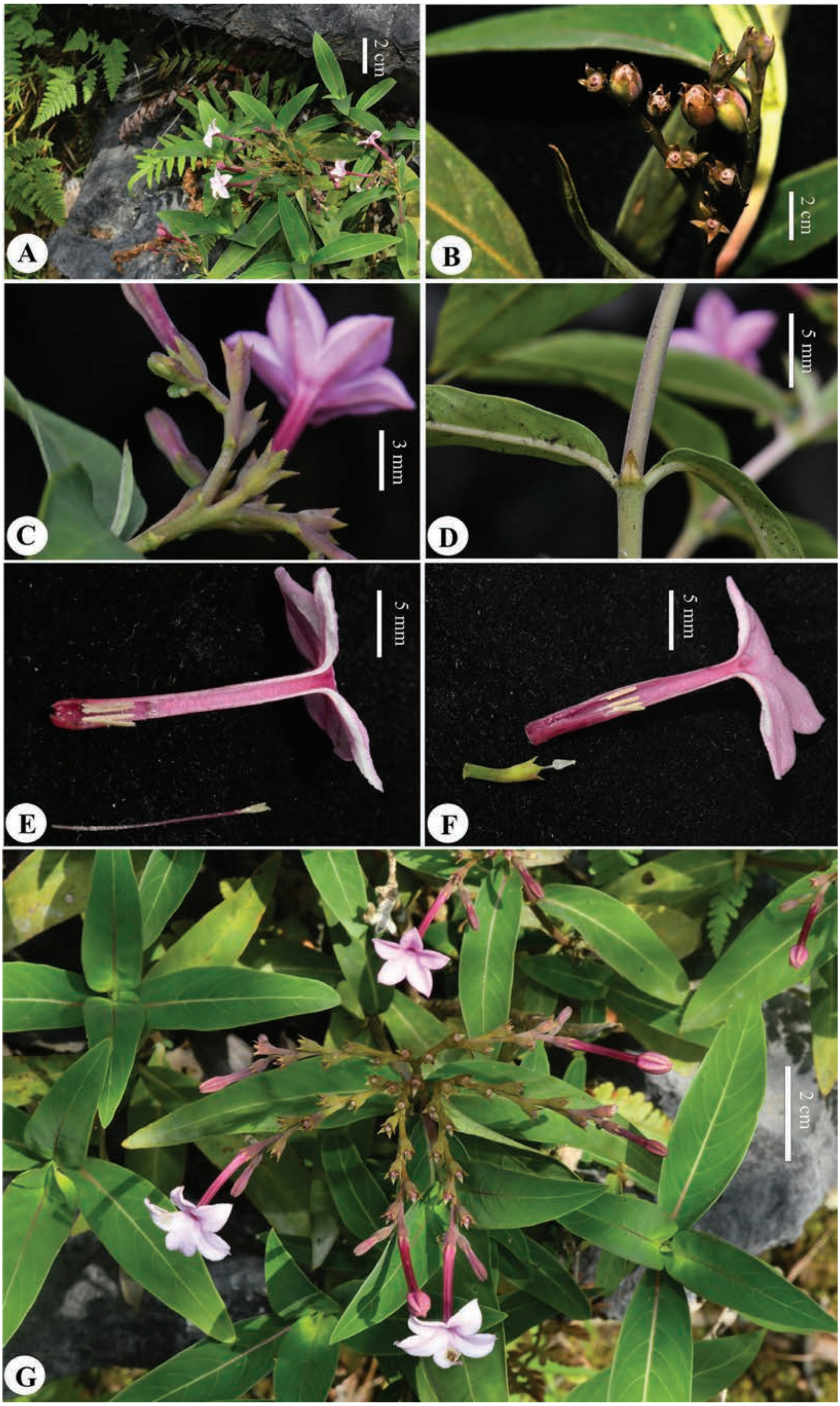

Figure 2. *Spiradiclis scorpiura* Y.Nong & L.Wu **A** habit **B** detail of fruiting plant **C** inflorescence **D** stipule **E** longitudinally opened, long-styled flower **F** longitudinally opened, short-styled flower **G** flowering plant (photographed and edited by You Nong).

标本采集记录签(Record label)
采集号(No.)：NY2024071101
标本份数(Number of specimens)：6 份
采集人(Collector)：农友、韦贵元
采集日期(Date)：2024 年 7 月 11 日
采集地点(Location)：广西大新县硕龙镇巧家屯
环境(Environment)：天坑口
出现多度(Abundance)：一般
生活型(Life-form)：亚灌木
株高(Height)：
枝、叶(Branches and leaves)：
花(Flower)：紫色
果实及种子(Fruits and seeds)：
科名(Family)：茜草科
植物名（Name）：
备注（Remark）：螺序草属

Figure 3. Holotype specimen of *Spiradiclis scorpiura* Y.Nong & L.Wu, *Y. Nong NY2024071101* (GXMI! 051187).

2-lobed, lobes linear, c. 2 mm long. Long-styled flowers: corolla tube with pilose ring above stamens inside; stamens born near the base of the tube, anthers sessile or subsessile, c. 3 mm long; style c. 1.5 cm long or slightly longer. Short-styled flowers: corolla tube pubescent near the base inside;

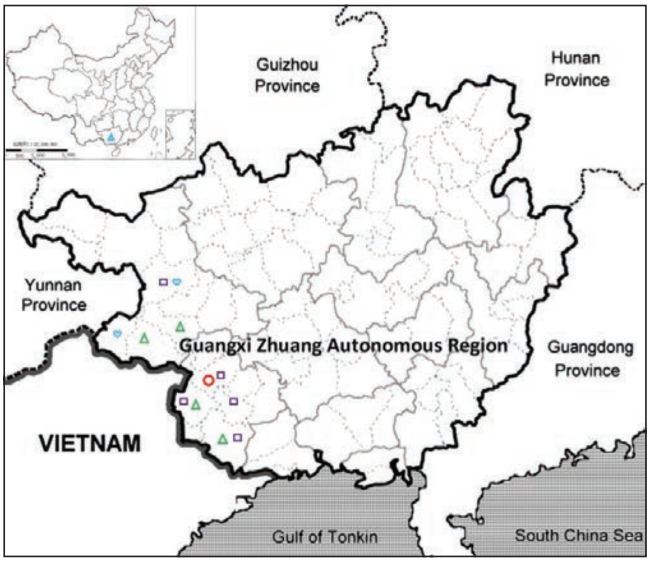

Figure 4. Distribution of *Spiradiclis scorpiura* (red circle), *S. coccinea* (green triangle), *S. scabrida* (blue heart), and *S. purpureocaerulea* (purple square) in Guangxi (blue triangle in insert map), China.

stamens born in the middle of the tube, anthers sessile or subsessile, c. 2 mm long; style c. 4 mm long. Capsules subglobose, 3–4 mm in diam., glabrescent, valves 4. Seeds numerous, angular, c. 0.2 mm in diam.

Phenology. Flowering and fruiting in June–July.

Etymology. The specific epithet “*scorpiura*” refers to the terminal, cincinnous inflorescences of the new species.

Distribution and habit. Known only from southeast Guangxi, China. The species has only been found at the rim of a sinkhole at elevations of 504 m.

Preliminary IUCN red list category. Data available for the new species, only known from the type locality and the type specimens, are insufficient to assess its conservation status. According to the IUCN Criteria (IUCN 2022), it is considered Data Deficient (DD) until more information becomes available. *Spiradiclis scorpiura* is currently known from a single, relatively large population. Further collection and monitoring are necessary to allow more conclusive estimations about the rarity and vulnerability of the species.

Comparison with other *Spiradiclis* species

In addition, *S. scorpiura* also looks similar to *S. scabrida* D.Fang & D.H.Qin, but is different by its shorter petioles (1–2 mm vs. 2–5 mm long), its cincinnous inflorescences (vs. corymbose-cymose), and its triangular, 1–2 mm long, pubescent bracteoles (vs. linear, 2–5 mm long, glabrous). *Spiradiclis scorpiura* is also similar to *S. purpureocaerulea* H.S.Lo, but it differs in its densely pubescent young stems that become glabrous when old (vs. densely pubescent young and old stems), its elliptic leaves, pubescent or glabrous on both surfaces (vs. ovate, adaxially densely strigose-hispidulous, abaxially densely pubescent), its 1–2 mm long petioles (vs. 5–20 mm long), its cincinnous inflorescences (vs. congested-cymose), and its triangular, 1–2 mm long, pubescent bracteoles (vs. narrowly lanceolate, 4–5 mm long, densely pubescent). More detailed morphological differences amongst the similar species are shown in Table 1.

Table 1. Main morphological differences between *Spiradiclis scorpiura*, *S. coccinea, S. scabrida,* and *S. purpureocaerulea*.

Morphological traits	*S. scorpiura*	*S. coccinea*	*S. scabrida*	*S. purpureocaerulea*
Stems	densely pubescent when young but glabrous when old	glabrous or subglabrous	pubescent to glabrescent	densely pubescent
Leaves	elliptic, sparsely pubescent or glabrous on both surfaces	narrowly elliptic-oblong or elliptic-oblong, glabrous on both surfaces	ovate, narrowly ovate, or lanceolate, abaxially glabrous or occasionally sparsely strigillose or scabridulous at least on principal veins	ovate, adaxially densely strigose-hispidulous, abaxially densely pubescent
Length of petioles	1–2 mm	1–2 mm	2–5 mm	5–20 mm
Stipules	triangular, 1–2 mm long, glabrous outside	triangular, rapidly narrowed to subulate, 4–5 mm long, glabrous outside	subtriangular, 0.7–1 mm long, subglabrous outside	subulate, 2–3 mm long, pubescent outside
Inflorescence	cincinnous, 3–6-branched, pubescent	cymose, with more than 10 flowers, pubescent	corymbose-cymose, 3-24-flowered, pubescent, puberulent, or glabrescent	congested-cymose, densely pubescent
Bracteoles	triangular, 1–2 mm long, pubescent outside	subulate, 3–4 mm long, glabrous outside	linear, 2–5 mm long, glabrous outside	narrowly lanceolate, 4–5 mm long, densely pubescent outside
Calyx	puberulent outside; hypanthium portion obovate, 1–2 mm long, with 5 straight ridges; lobes triangular or ovate-lanceolate, 1–1.5 mm long	glabrescent outside; hypanthium portion obconic, 1.2–1.5 mm long; lobes narrowly lanceolate, 1.7–2 mm long	puberulent outside; hypanthium portion obovate, 1–1.5 mm long; lobes ovate-lanceolate, 1–1.5 mm long	pubescent outside; hypanthium portion obconic-globose, c. 2 mm long; lobes narrowly lanceolate, 4–4.5 mm long
Corolla	tube 15–20 mm long, lobes broadly ovate to suborbicular, 6–8 mm long	tube 15–18 mm long, lobes broadly ovate to suborbicular, 4.5–6 mm long	tube 25–26 mm long; lobes ovate, c. 3.5 mm long	tube 19–21 mm long; lobes subovate, c. 6 mm long
Capsule	subglobose, 3–4 mm in diam.	subglobose, 4.5–5.5 mm in diam.	subglobose, 3–4 mm in diam.	subglobose, 4–4.5 mm in diam.

Acknowledgements

The authors are grateful to Xiang-Chun Lan for fieldwork assistance and Xin-Cheng Qu for the line drawing (Guangxi Institute of Traditional Medical and Pharmaceutical Sciences, Nanning).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Data curation: YN; Funding acquisition: YN and CGX; Investigation: YN, CGX, and YGW; Methodology: YN, LQL, and YGW; Project administration: YN and YJL; Supervision: QMH, LW; Visualisation: YN, LQL, QXC; Writing—original draft: YN; Writing—review and editing: YN.

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Data availability

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

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PhytoKeys 252: 51–63 (2025)
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Research Article

Lespedeza jianghuensis (Fabaceae), a new species from riparian meadows of Yangtze River basin, China

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Abstract

Lespedeza jianghuensis (Fabaceae), from Yangtze River basin, China, is described and illustrated as a new species. It is a dwarf shrublet occurring in the riparian meadows along the banks of rivers and lakes. It is morphologically similar to *L. cuneata*, *L. lichiyuniae*, and *L. caraganae*, but differs by its prostrate or decumbent habit, angulate but not furrowed stems, and small stipules (ca. 1 mm). Phylogenetic analyses based on combination of five chloroplasts fragments and ITS sequence confirmed that it belongs to *Lespedeza* sect. *Junceae*. We also evaluate its conservation status as Least Concern (LC).

Key words: Fabaceae, morphological traits, new species, taxonomy, wetland

Introduction

The genus *Lespedeza* Michx. (Fabaceae) is characterized by trifoliolate leaves, small pea-like flowers, and single-seeded pod (Huang et al. 2010). The genus was first described by Michaux (1803). It has a primarily East Asian distribution, with the majority of the ca. 40–50 recognized species found in China, Japan, Korea, and adjacent regions (Ohwi and Kitagawa 1983; Ohashi 2005; Ohashi et al. 2009a, 2009b). A few species occur in North America, where the genus likely originated and then dispersed to Asia in the past (Xu et al. 2012). Taxonomic delimitation within *Lespedeza* has been challenging, with ongoing debate about species boundaries and relationships (Ohashi 2005; Xu et al. 2011). Based on the results of distribution and molecular phylogenetic analyses, Ohashi and Nemoto (2014) re-circumscribed the subgenera, confining *L.* subg. *Lespedeza* to North America and *L.* subg. *Macrolespedeza* to Asia.

China is a major center of diversity for *Lespedeza*, harboring 27 species, including 14 endemic species (Huang et al. 2010). Recent field investigations and taxonomic studies have led to the discovery and description of new species

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from various regions of China, such as *L. jiangxiensis* Bo Xu bis, X. F. Gao & Li Bing Zhang from Jiangxi Province (Xu et al. 2013), *L. pseudomaximowiczii* D. P. Jin, Bo Xu bis & B. H. Choi from Anhui, Henan and Zhejiang Province (Jin et al. 2018) and *L. danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang from Guangdong Province (Zhao et al. 2021). These findings highlight the need for continued exploration and taxonomic research on *Lespedeza* in China.

During a botanical survey in the middle and lower reaches of Yangtze River, from 2021 to 2023, we found an unknown *Lespedeza* plant in riparian meadows. It resembles *L. cuneata* in the white flowers, narrow leaflets with pinnate lateral veins, but differs by its procumbent habit and smaller stipules. After carefully examining specimens and literature, together with a molecular phylogenetic analysis based on combination of five plastid fragments and Internal Transcribed Spacers (ITS), we describe and illustrate it as a new species.

Materials and methods

Morphological study

The morphological characters were examined, based on the living plants and specimens deposited in the herbaria CSFI, HIB, HNNU, IBSC, JXCM, JXAU, NAS, and PE, herbarium acronyms as in Thiers (2021). Online databases such as CVH (https://www.cvh.ac.cn/), PPBC (http://ppbc.iplant.cn/) and JSTOR (https://www.jstor.org/) were also examined to document more records of the new species.

Phylogenetic analyses

Two representative individuals from different populations were selected for molecular analyses, *Jia-Xiang Li et al. 2023101903* from Jiangxi Province, and *Song Huang et al. HD-25* from Hubei Province. A total of 29 accessions, representing 28 species of *Lespedeza*, two species of *Kummerowia* [*K. stipulacea* (Maxim.) Makino and *K. striata* (Thunb.) Schindl.] and three species of *Campylotropis* [*C. hirtella* (Franch.) Schindl., *C. macrocarpa* (Bunge) Rehder., and *C. polyantha* (Franch.) Schindl.], were sampled for outgroup comparison. Most sequences except the new species were downloaded from GenBank. Total genomic DNA was extracted from silica-gel-dried leaf material using the modified CTAB procedure (Doyle and Doyle 1987), with reference to the assembly of the original sequences by Xue et al. (2023). Phylogenetic reconstruction of the new species and related taxa was carried out using nuclear DNA internal transcribed spacer sequences (ITS) linked to five chloroplast loci (*rpl16*, *rpl32-trnL*, *rps16-trnQ*, *trnL-F*, *trnK/matK*), analyses method of combined chloroplast and ITS sequences data with reference to Xu et al. (2012). Taxa sampled and GenBank accession numbers for the six datasets are listed in Appendix 1. The phylogenetic relationships were estimated by generating a Maximum likelihood (ML) trees using RAxML-HPC2 (8.2.12) with the GTRGAMMA model, and the support for individual nodes in the phylogenetic tree was assessed with1,000 bootstrap replicates (Stamatakis 2014). The resulting tree was visualized in TreeGraph 2 (Stöver and Müller 2010).

Results

Molecular phylogenetics

Lespedeza (BS = 86), *Kummerowia* (BS = 100), and *Campylotropis* (BS = 100) were all recovered as monophyletic in the phylogenetic tree of this study (Fig. 1). sect. *Macrolespedeza* taxa were clustered into a single clade (clade C) as sister to the sect. *Junceae* taxa, of which was divided into two clades (i.e., clade A and B) (BS = 32). The putative new species is deeply embedded in clade B and is considered to be a member of subclade B-1 consisting of *L. lichiyuniae* T. Nemoto, H. Ohashi & T. Itoh, *L. cuneata* (Dum. Cours.) G. Don, *L. caraganae* Bunge. (BS = 80, Fig. 1).

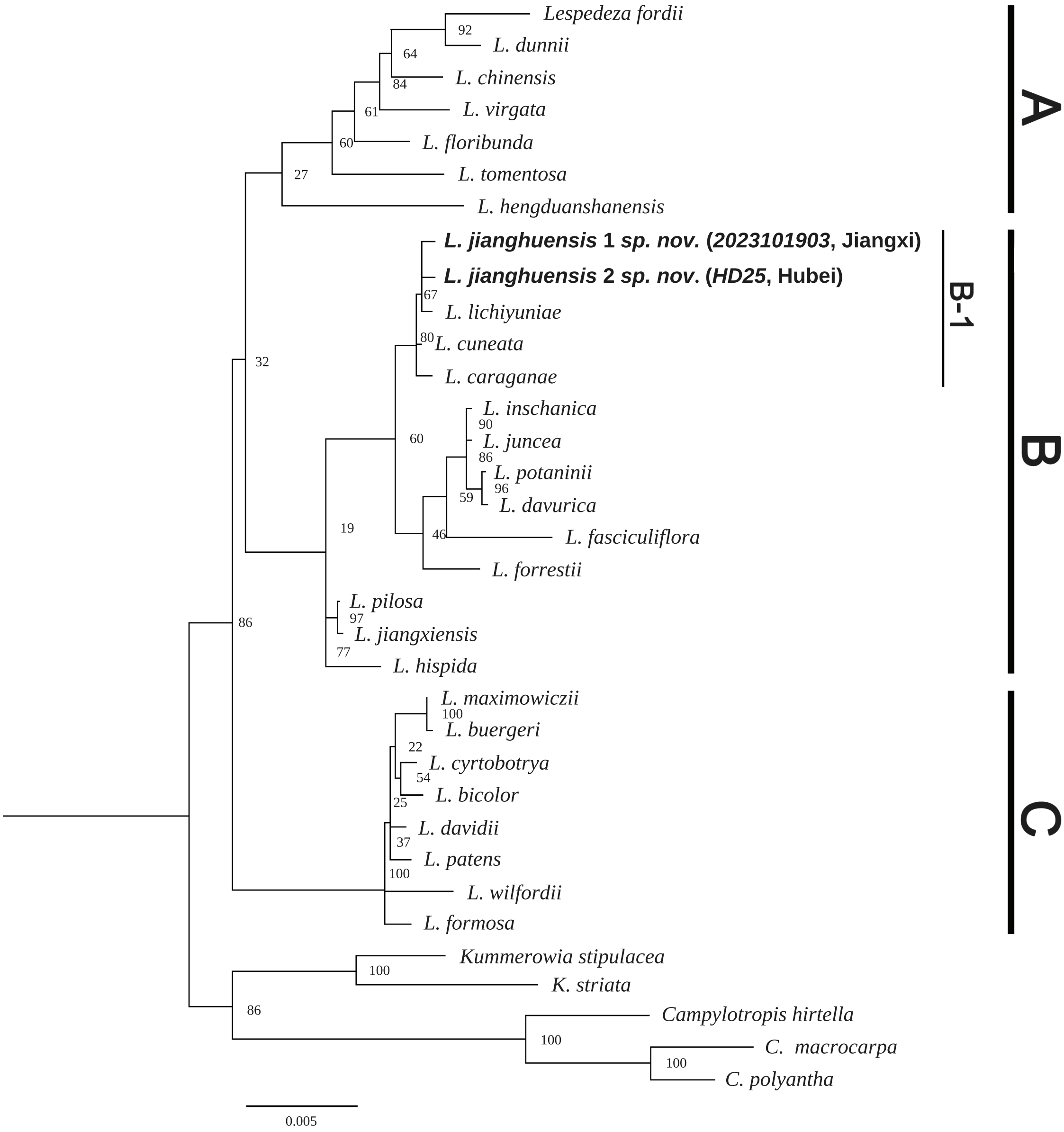


Figure 1. Phylogenetic relationships amongst 33 species of *Lespedeza*, *Kummerowia* and *Campylotropis* based on combined chloroplast and ITS sequences using Maximum Likelihood analysis. The new species in this study is indicated in boldface, with specimen collection numbers and provinces sampled indicated in parentheses. Numbers above branches indicate Maximum likelihood bootstrap values (before slash).

Morphological comparison

Table 1 summarizes a detailed morphological comparison of the new species with three closely related species within subbranch B-1. These species have laterals veins to the margin of leaflets. Morphologically, the new species is most similar to *L. cuneata*, sharing features such as white flowers, and narrow leaflets. However, the new species differs from the latter by the procumbent habit, green or purplish black stems, sparse leaves along the branches, sparse hairs on the stems and lower side of leaflets, and short stipules ca. 1 mm (Table 1).

Taxonomic treatment

***Lespedeza jianghuensis* Song Huang, Jia X. Li & B. Pan bis, sp. nov.**
urn:lsid:ipni.org:names:77356518-1
Figs 2–4

Type. China • Jiangxi, Nanchang, Jinxian County, Qinglan Lake, 15–30 m a.s.l., 19 October 2023, *Jia-Xiang Li et al. 2023101903* (holotype: CSFI080349!, isotype HITBC!).

Diagnosis. *L. jianghuensis* is morphologically most similar to *L. cuneata*, in having narrow leaflets and straight lateral veins to the margin, but differs from the latter by its prostrate or decumbent habit (vs. erect or ascending), stems strongly branched at the base (vs. stems simple), branches with appressed-pubescent (vs. densely ascending-pubescent), leaves sparse (vs. leaves crowded), leaflets narrowly obovate, terminal leaflets 5–16 mm long (vs. cuneate or linear-cuneate, terminal leaflets 7–30 mm long in *L. cuneata*), abaxial surfaces of leaves pubescent with obvious veins (vs. densely whitish pubescent, veins indistinct), stipules triangular, 0.7–1 mm long (vs. lanceolate, 1–4 mm long in *L. cuneata*) and calyx lobes narrowly triangular (vs. lanceolate) (Fig. 5).

Description. Shrub, prostrate or procumbent, much branched at the base, up to 60 cm long (Fig. 3). Stem slightly angular, green when young, appressed white pubescent, purplish black when old, late glabrous. Trifoliolate; stipules persistent, triangular, 0.7–1 × 0.4–0.8 mm; petiole 1–6 mm long, appressed pubescent; pulvini 0.3–0.7 mm, appressed pubescent. Leaflets glabrous above, adpressed hairy beneath, late glabrous, pinnate veins clear on both side,

Table 1. Morphological comparison of *Lespedeza jianghuensis* and its closely related species.

Character	*L. jianghuensis*	*L. cuneata*	*L. lichiyuniae*	*L. caraganae*
Habit	Prostrate or decumbent, up to 60 cm tall	Erect or ascending, up to 100 cm tall	Erect or ascending, up to 120 cm tall	Erect, up to 50 cm tall
Stem	Appressed- pubescent, subglabrous when old, green to purplish black, angular but not furrowed	Grayish green, densely ascending pubescent, multiple furrowed	Green to reddish brown, ascending- or appressed-pubescent, multiple furrowed	Grayish green, adpressed shortly hairy, multiple furrowed
Leaflet	Narrowly obovate, 5–16 × 2–4(–5) mm, glabrous above, adpressed hairy below	Cuneate or linear-cuneate, (7–)10–30 × 2–7 mm, subglabrous above, densely adpressed hairy below	Narrowly obovate, 4.6–28 × 1.6–7.7 mm, glabrous above, rather densely sericeous below	Oblong-linear, 20–40 × 2–4 mm, subglabrous above, adpressed hairy below
Stipule	Triangular, 0.7–1 mm	Lanceolate, 1–4 mm	Linearly triangular, 1–4 mm	Subulate, 2.5 mm
Flower color	White	White	Pale purple	White

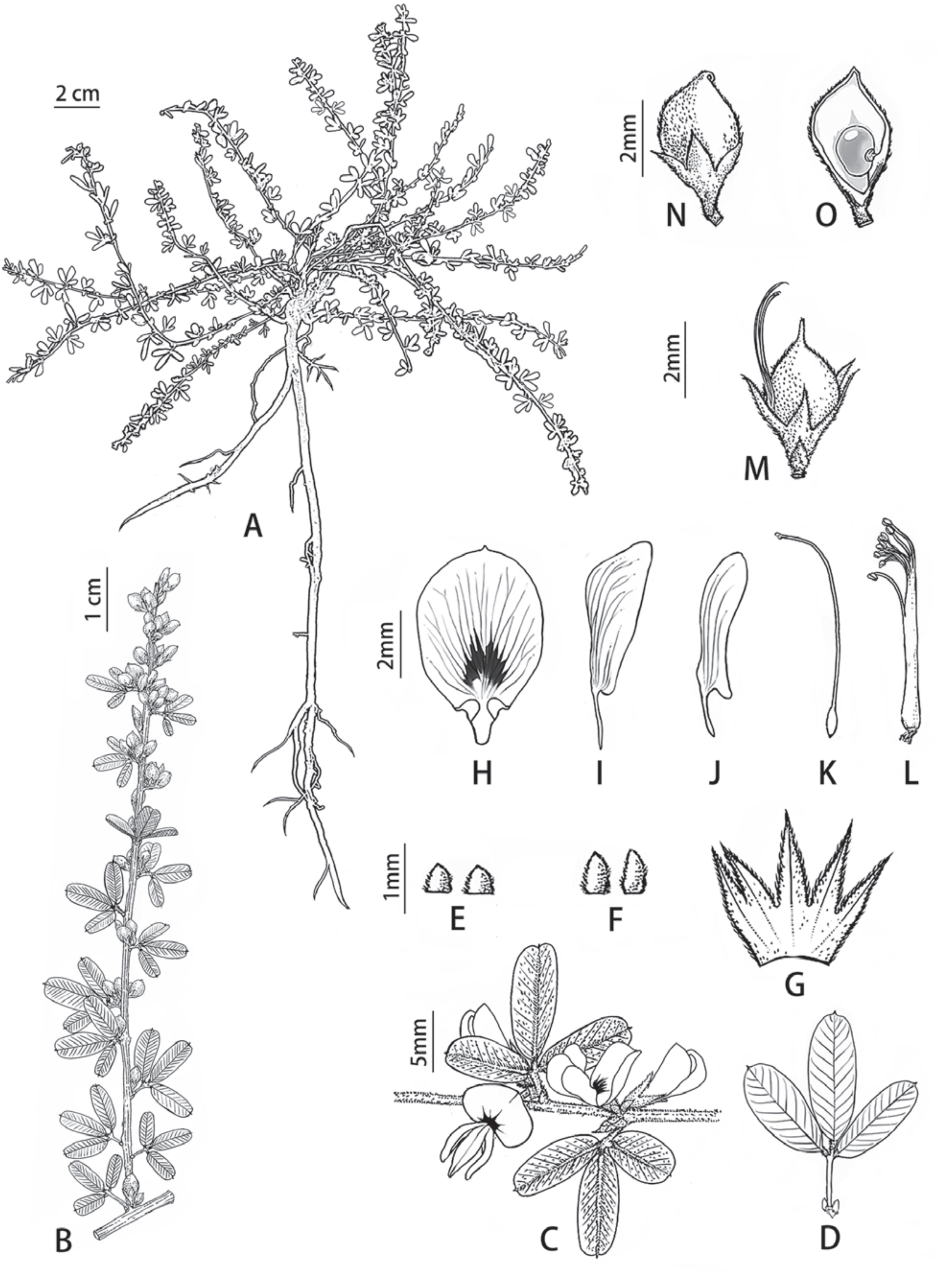


Figure 2. *Lespedeza jianghuensis* Song Huang, Jia X. Li & B. Pan bis, sp. nov. **A** habit **B** fruiting branch **C** flowering branch **D** leaf **E** bracts **F** bracteoles **G** calyx **H** standard **I** keel-petal **J** wing **K** pistil **L** stamens **M** pod of chasmogamous flower **N** pod of cleistogamous flower **O** seed. Illustrated by Mei-Qian Chen.

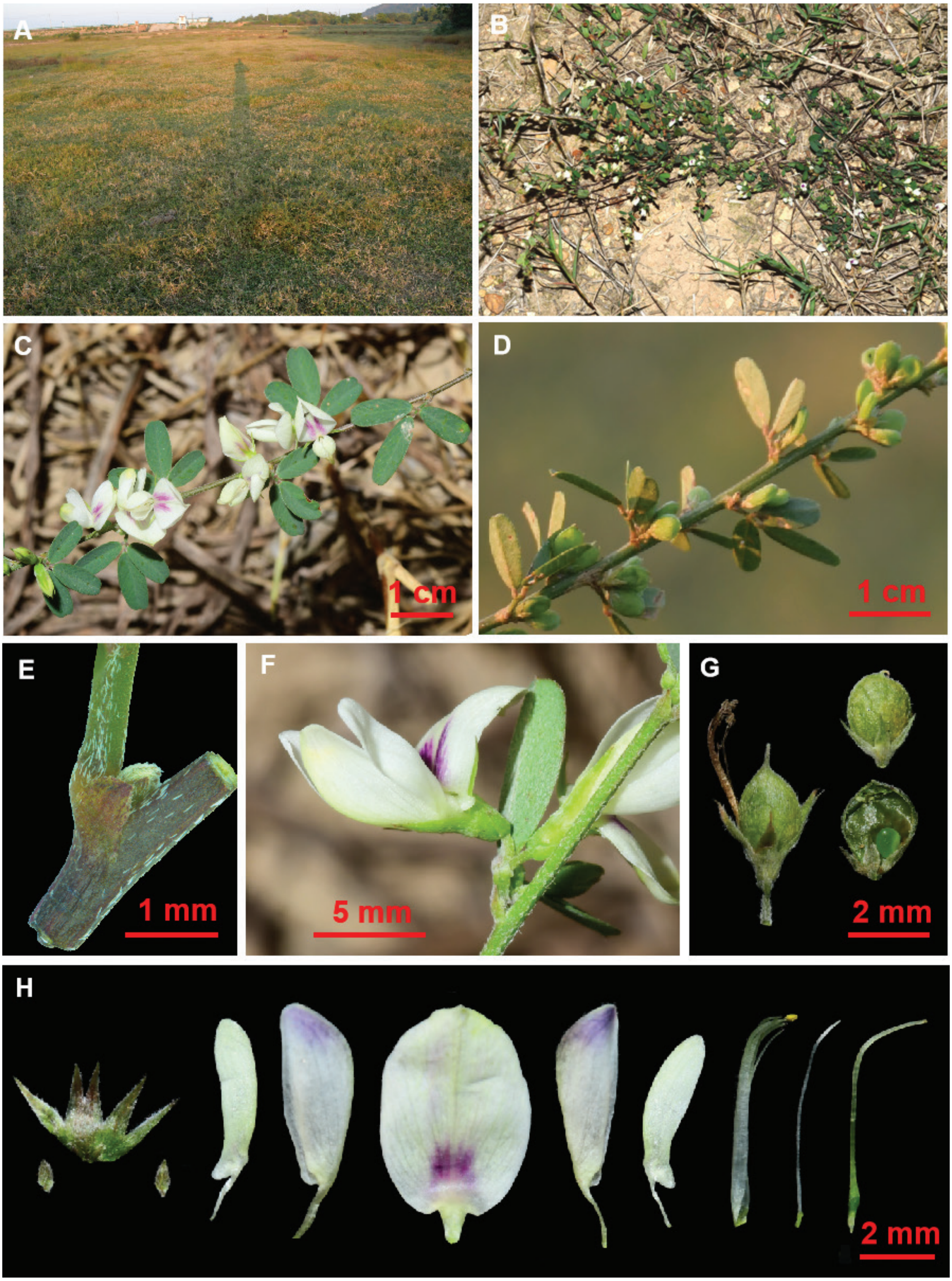
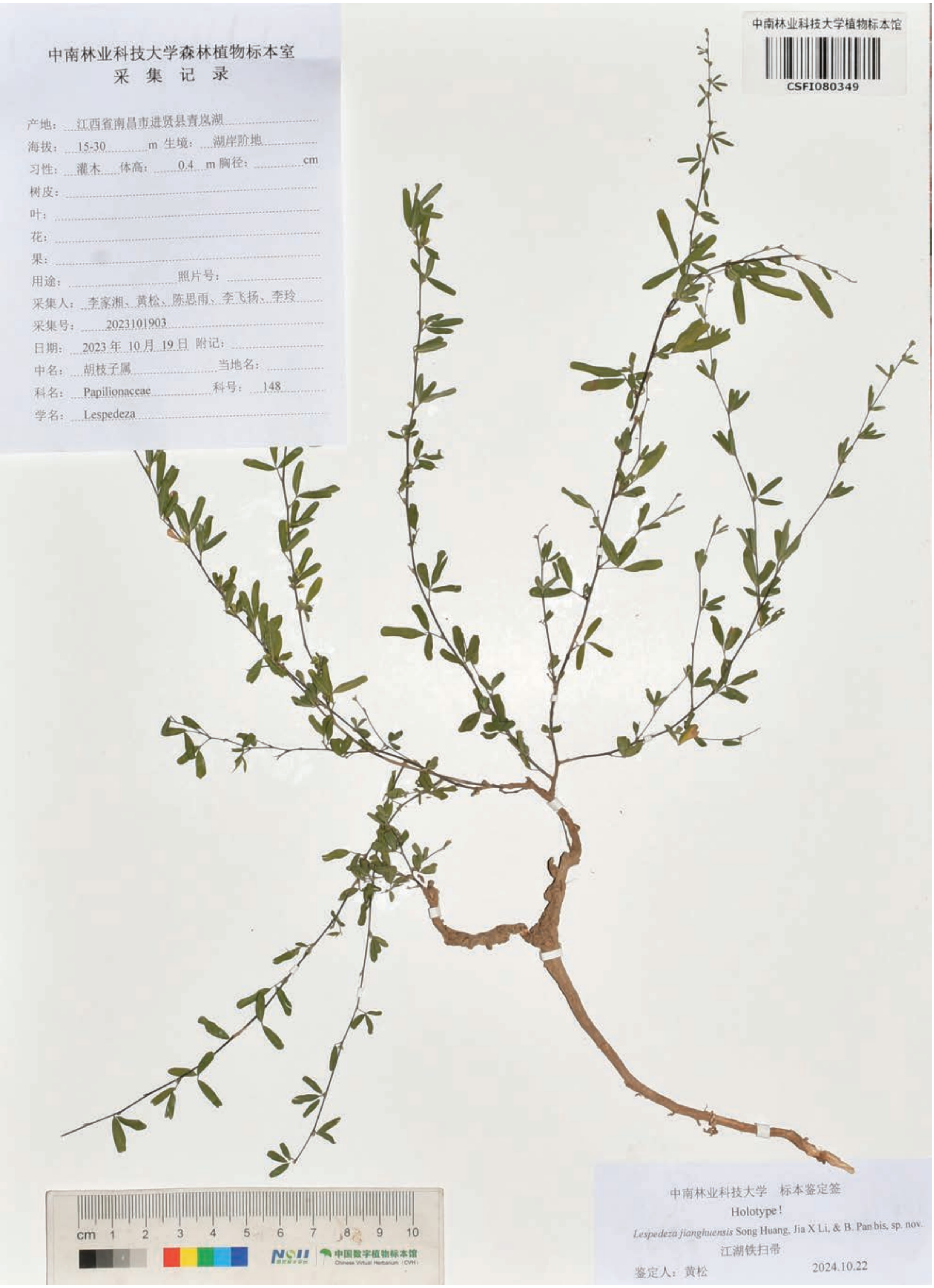


Figure 3. Morphology and habitat of *Lespedeza jianghuensis* Song Huang, Jia X. Li & B. Pan bis, sp. nov. **A** habitat **B** habit **C** flowering branch **D** fruiting branch **E** stipule **F** lateral view of flower **G** fruit and seed, pod of chasmogamous flowers (left), pods of cleistogamous flowers (right) **H** flower dissections, from left to right (bracts and anatomical calyx; wing, keel-petal and standard; stamens and pistil). Photographed by Jia-Xiang Li and Song Huang.



中南林业科技大学森林植物标本室
采集记录

产地：江西省南昌市进贤县青岚湖
海拔：15-30 m 生境：湖岸阶地
习性：灌木 体高：0.4 m 胸径：cm
树皮：
叶：
花：
果：
用途： 照片号：
采集人：李家湘、黄松、陈思雨、李飞扬、李玲
采集号：2023101903
日期：2023 年 10 月 19 日 附记：
中名：胡枝子属 当地名：
科名：Papilionaceae 科号：148
学名：Lespedeza

中南林业科技大学 标本鉴定签
Holotype !
Lespedeza jianghuensis Song Huang, Jia X Li, & B. Pan bis, sp. nov.
江湖铁扫帚
鉴定人：黄松 2024.10.22

Figure 4. Holotype specimen of *Lespedeza jianghuensis* Song Huang, Jia X. Li & B. Pan bis, sp. nov.

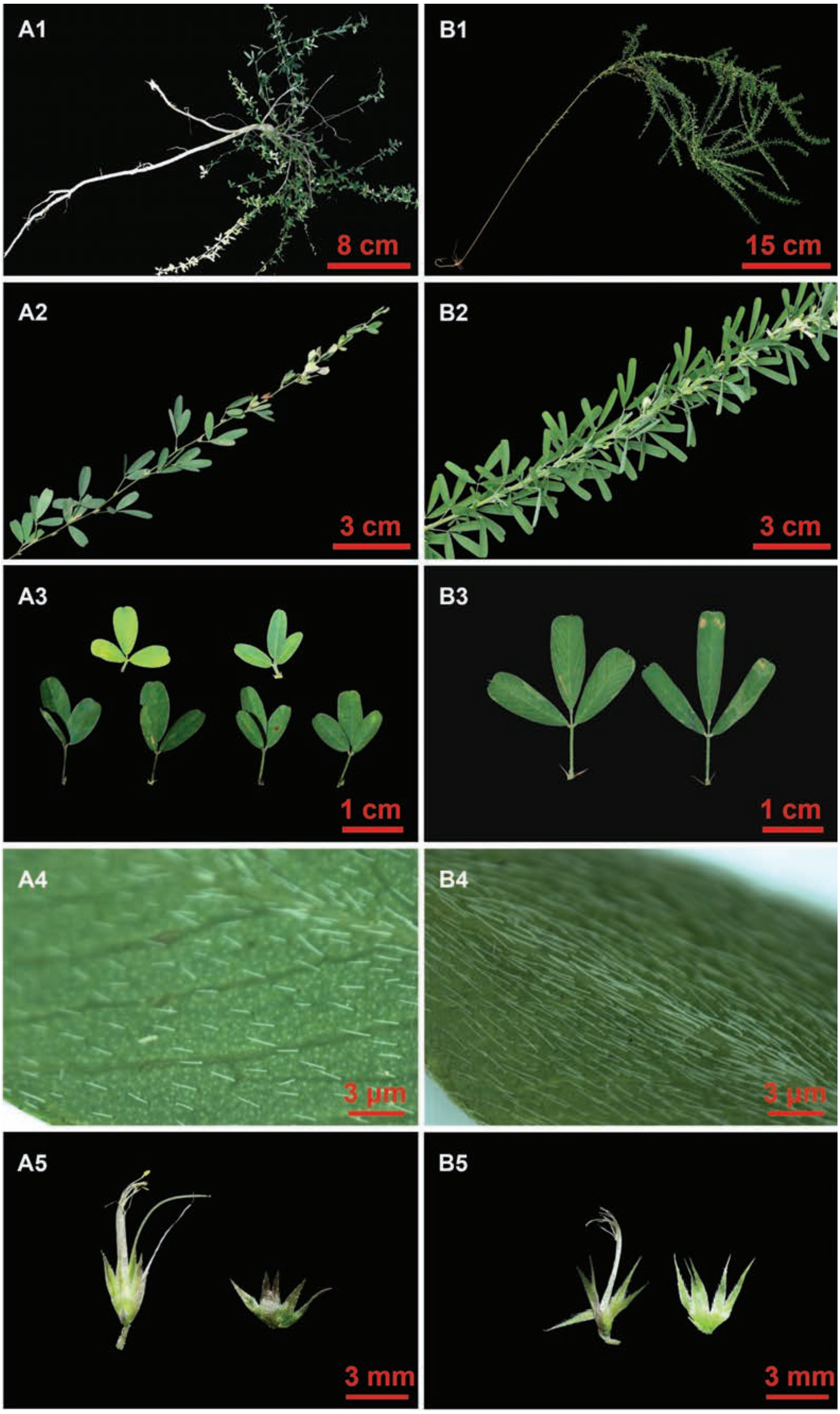


Figure 5. Morphological comparison between *Lespedeza jianghuensis* Song Huang, Jia X. Li & B. Pan bis, sp. nov. (**A1–A5**) and *L. cuneata* (Dum. Cours.) G. Don (**B1–B5**) **A1, B1** plant habit **A2, B2** branches **A3, B3** adaxial view of leaf **A4, B4** abaxial surface of leaflet **A5, B5** calyx. Photographed by Song Huang.

(6–)8–14 pairs, reaching margin of leaflets, narrowly obovate, 5–16 × 2–4(–5) mm, apex obtuse or slightly emarginate, apiculate, terminal leaflets slightly larger than lateral ones; pulvinus 0.3–0.6 mm; rachis short, 0.5–1.0 mm. Racemes axillary, not exceeding the leaves, with 2–6 flowers, pedicels very short (shorter than 1 mm); bracts ovate-triangular, 0.3–0.6 mm; bracteoles 2 at base of calyx, ovate-triangular, 0.5–1 mm, equal to or shorter than calyx tube, appressed hairy. Flowers 5–9 mm; calyx campanulate, 2.5–4.5 mm, 5-lobed, lobes narrowly triangular, 1–2.8 mm, appressed hairy, margins ciliate, lateral and lowest calyx lobes lobed to below middle, upper lobes split about 1/3 of calyx; corolla white; standard oval, base with purple spot, reflexed at anthesis, 5.5–8 mm, with a claw and 2 auricles at base, lamina 5–7 × 4–4.5 mm, obtuse with a point at apex; wings narrowly elliptic, slightly shorter than the keel petals, 5–6.5 mm, lamina 4–6 × 1–2 mm, slightly auriculate at base, with a 1–1.5 mm claw; keel 5.5–8 mm, often tinged purple at apex, lamina 4–6.5 × 1–2.5 mm, narrowly obovate to obovate, obtuse at apex, abruptly narrowed to claw, claw ca. 1.5–2 mm.; diadelphous stamens (9+1), 6–7.5 mm; pistil, 6.5–8 mm, ovary obovate, glabrous; cleistogamous flowers clustered in the lower leaf axils of the stem, sessile or subsessile; Pods indehiscent, 1-seeded, densely adpressed hairy. Chasmogamous pods oval, 3–4.5 × 1.5–2.5 mm, with a straight beak at apex. Cleistogamous pods nearly round, 2.5–3.5 × 1.8–2.5 mm, 2–3 times longer than persistent calyx, with a curved beak at the apex, beak ca. 0.3 mm long,

Geographical distribution and habitat. *Lespedeza jianghuensis* is widely distributed in the middle reaches of Yangtze River basin in China (Fig. 6). such as Hunan, Jiangxi and Hubei Provinces. It grows on river floodplains, lakeshore terraces, mudflats, marshes, headlands, and other grasslands and wastelands close to rivers and lakes at elevation from 7 to 39 meters, and it is subject to seasonal flooding in summer.

Phenology. Flowering September to October; fruiting October to November.

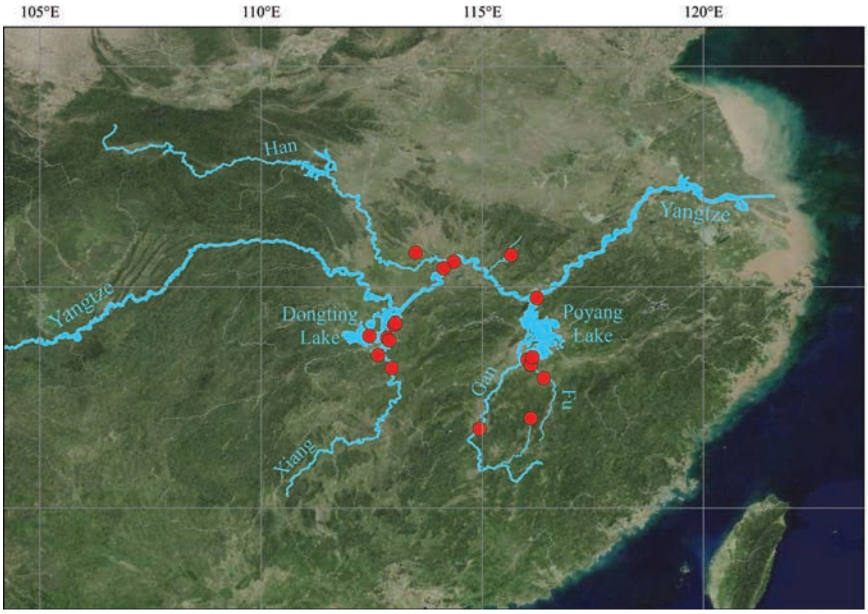


Figure 6. Geographic distribution (red dots) of *Lespedeza jianghuensis* Song Huang, Jia X. Li, & B. Pan bis, sp. nov.

Etymology. The specific epithet "*jianghuensis*" refers to the distribution area, with "jiang" standing for Yangtze River and its tributaries, and "hu" standing for the lakes in Yangtze River Basin.

Chinese name (assigned here). jiāng hú tiě sào zhou (江湖铁扫帚).

Conservation status. The new species is widely distributed along the Yangtze River, and it is likely to be more common and widespread than currently documented. It is therefore not considered to be in imminent conservation danger. The wild population of the new species is presumed to be around 50,000, and there is no known population size or population decline. It is evaluated as Least Concern (LC) according to IUCN Red List criteria (IUCN Standards and Petitions Committee 2022).

Additional specimens examined. China. Hubei: Wuhan City, Wuchang District, Hesheng Bridge, 12 Jul. 1958, *Ying-Han Zhang 842* (HIB0041554). Yingcheng City, Yihe Township, Xier Village, 4 Oct. 2024, *Song Huang et al. HD-25* (HITBC); **Hunan**: Changsha city, Yuelu Mountain, along the Xiang River, 10 Aug. 1972, *Lin-Han Liu 8060* (HNNU00017822); Ningxiang City, Wangcheng District, Tuntou Lake, 5 Oct. 2024, *Song Huang et al. HD-26* (HITBC); Xiangyin County, Qingtan Township, 8 Aug. 2001, *Ke-Ming Liu & Guang-Wan Hu 24013* (HNNU00017820); Xiangyin County, Xianglu Mountain, 24 Oct. 2021, *Ang Liu & Guo-Hui Zhou DTH0298* (CSFI080351); Yueyang County, Lujiao Town, 26 Oct. 2021, *Ang Liu & Guo-Hui Zhou DTH0373* (CSFI080350); Yueyang County, Zhongzhou township, 21 Sep. 2024, *Song Huang et al. HD-05* (HITBC). **Jiangxi**: Fengcheng City, Dalan Town, Wangzhou Village, 1 Oct. 2024, *Song Huang et al. HD-20* (HITBC); Fengcheng City, Yuandu Town, Changyuan Village, 30 Sep. 2019, *Xiao-Lang Du 360981190930092LY* (JXCM0010153); Ganzhou City, Ningdu County, Xiaotian Township, 24 Oct. 1958, *Qi-Ming Hu 5622* (IBSC0180254); Ji'an City, Taihe County, by the Gan River, 15 Sep. 1980, *South Meadow group 297* (PE01791044); Jinxian County, Luoxi Township, Nanyang Village, 2 Oct. 2024, *Song Huang et al.* HD-21 (HITBC); Nanchang County, Wenshen Town, Cangtou Village, 2 Oct. 2024, *Song Huang et al. HD-24* (HITBC); Nanchang County, 28 Aug. 1940, *Migo H*. (NAS00387673, NAS00387675, NAS00387676); Yichun City, Tonggu County, Yingchao Village, 14 Oct. 1996, *Zheng-Ming Tao et al. 960460* (JXAU0007890).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SH. Formal analysis: HYZ. Investigation: PZ. Methodology: BP. Project administration: JXL. Resources: YTZ. Software: FS. Supervision: AL. Validation: LQZ. Visualization: MQC. Writing - original draft: SH. Writing - review and editing: BP, SH, JXL.

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Data availability

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

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Appendix 1

Table A1. GenBank accession numbers for the 28 species of *Lespedeza* and 5 outgroups.

Taxon	ITS	*rpl16*	*rpl32-trnL*	*rps16-trnQ*	*trnL-F*	*trnK/matK*
***Lespedeza* Michx.**						
L. bicolor Turcz.	JN402405	JN402497	JN402589	JN402683	JN402777	JN402872
L. buergeri Miq.	JN402406	JN402500	JN402590	JN402684	JN402778	JN402873
L. caraganae Bunge	JN402410	JN402502	JN402594	JN402688	JN402782	JN402877
L. chinensis G. Don	JN402416	JN402508	JN402600	JN402694	JN402788	JN402883
L. cuneata (Dum. Cours.) G. Don	JN402417	JN402509	JN402601	JN402695	JN402789	JN402884
L. cyrtobotrya Miq.	JN402422	JN402514	JN402606	JN402700	JN402794	JN402889
L. davidii Franch.	JN402428	JN402520	JN402612	JN402706	JN402800	JN402895
L. davurica (Laxm.) Schindl.	JN402425	JN402517	JN402609	JN402703	JN402797	JN402892
L. dunnii Schindl.	JN402431	JN402523	JN402615	JN402709	JN402803	JN402898
L. fasciculiflora Franch.	JN402432	JN402524	JN402616	JN402710	JN402804	JN402899
L. floribunda Bunge	JN402436	JN402528	JN402620	JN402714	JN402808	JN402903
L. fordii Schindl.	JN402440	JN402532	JN402624	JN402718	JN402812	JN402907
L. formosa (Vogel) Koehne	JN402443	JN402535	JN402627	JN402721	JN402815	JN402910
L. forrestii Schindl.	JN402448	JN402540	JN402632	JN402726	JN402820	JN402915
L. hengduanshanensis (C. J. Chen) Bo Xu bis, X. F. Gao & Li Bing Zhang	JN402434	JN402526	JN402618	JN402712	JN402806	JN402901
L. inschanica (Maxim.) Schindl.	JN402452	JN402544	JN402636	JN402730	JN402824	JN402919
L. jianghuensis Song Huang, Jia X. Li, & B. Pan bis (*2023101903*, Jiangxi)	PQ932036	PQ963378	PQ963378	PQ963378	PQ963378	PQ963378
L. jianghuensis Song Huang, Jia X. Li, & B. Pan bis (*HD25*, Hubei)	PQ932035	PQ963377	PQ963377	PQ963377	PQ963377	PQ963377
L. jiangxiensis Bo Xu bis, X. F. Gao & Li Bing Zhang	JN402455	JN402546	JN402639	JN402733	JN402827	JN402922
L. juncea (L. f.) Pers.	JN402457	JN402548	JN402641	JN402735	JN402829	JN402924
L. lichiyuniae T. Nemoto, H. Ohashi & T. Itoh	JN402461	JN402552	JN402645	JN402739	JN402833	JN402928
L. maximowiczii C. K. Schneid.	JN402464	JN402555	JN402648	JN402742	JN402836	JN402931
L. patens Nakai	JN402466	JN402557	JN402650	JN402744	JN402838	JN402933
L. pilosa (Thunb.) Siebold & Zucc.	JN402467	JN402558	JN402651	JN402745	JN402839	JN402934
L. potaninii Vassilcz.	JN402469	JN402560	JN402653	JN402747	JN402841	JN402936
L. tomentosa (Thunb.) Siebold ex Maxim.	JN402476	JN402565	JN402659	JN402753	JN402848	JN402943
L. virgata (Thunb.) DC.	JN402480	JN402569	JN402663	JN402757	JN402852	JN402947
L. wilfordii Ricker	JN402484	JN402573	JN402667	JN402761	JN402856	JN402951
L. hispida (Franch.) T. Nemoto & H. Ohashi	JN402450	JN402542	JN402634	JN402728	JN402822	JN402917
***Kummerowia* Schindl.**						
K. striata (Thunb.) Schindl.	MN192633	JN402583	JN402677	JN402771	JN402866	JN402961
K. stipulacea (Maxim.) Makino	JN402676	JN402582	JN402676	JN402770	JN402865	JN402960
***Campylotropis* Bunge**						
C. macrocarpa (Bunge) Rehder	MN721989	NC044100	NC044100	NC044100	NC044100	NC044100
C. hirtella (Franch.) Schindl.	JN402491	JN402580	JN402674	JN402768	JN402863	JN402958
C. polyantha (Franch.) Schindl.	LC740845	NC064519	NC064519	NC064519	NC064519	NC064519

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Research Article

A new species of genus *Chiloschista* (Aeridinae, Vandeae, Epidendroideae, Orchidaceae) from Sumatra Island, Indonesia

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Abstract

Chiloschista tjiasmantoi, a new species of epiphytic leafless orchid from the northernmost region of Sumatra Island, Indonesia, is described and illustrated. The flower of this new species is morphologically close to *C. javanica*, but differs in having oblong-obovate petals, narrowly oblique oblong side lobes with truncate to obtuse apex, and a different shape of lip sac.

Key words: Aceh, leafless orchid, morphology, Southeast Asia

Introduction

The Indonesian archipelago is known to be one of the most important orchid diversity hotspots in the world, and it continues to generate new species discoveries, which indicates that there are still many potential areas that need to be explored (e.g., Metusala 2017a; Metusala and Supriatna 2017; Metusala 2019a, 2019b; Metusala and O'Byrne 2020; Saputra et al. 2020; Metusala et al. 2021; Metusala 2024). Recent botanical explorations in Indonesia have usually focused on a few popular, orchid taxa, while groups such as mycoheterotropic and leafless orchids have been comparatively neglected, presumably due to their less attractive habitus, often cryptic growth habit and small flower size (Metusala and Supriatna 2017; Metusala 2020a; Suetsugu et al. 2021; Panday et al. 2022).

The leafless orchid genus *Chiloschista* Lindl. (1832: 1522) was established in 1832 with *C. usneoides* (D.Don) Lindl. (1832: 1522) as its type, and there are now 30 accepted species distributed from the Indian subcontinent through to Southeast Asia and Australia (Dalstrom and Kolanowska 2020; POWO 2024). In previous treatments, the genus was classified under various subtribes, such as Sarcochilinae (Schlechter 1926), Aeridinae (Dressler 1993), and Phalaenopsidinae (Szlachetko 2003). However, recent phylogenetic studies showed that *Chiloschista* is a monophyletic genus, clustered with *Phalaenopsis*, within the subtribe Aeridinae of the tribe Vandeae (Zou et al. 2015; Liu et al. 2023).

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Species of this genus are characterized by monopodial growth, typically as epiphytic or lithophytic herbs. Plants are often seen as a cluster of numerous terete or flattened photosynthetic roots that radiate from a very short stem. *Chiloschista* flowers are either ephemeral or last up to several days, 3-lobed, saccate or spurred, and usually have a thickened hairy internal callus. Their columns are subterete and short, but have a rather long foot. The anther caps have 2 filiform setae and 4 pollinia in 2 closely appressed pairs (Cockburn et al. 1985; Chen and Wood 2009; Wood 2014; Dalstrom and Kolanowska 2020).

Prior to this article, Indonesia has 4 accepted species: *C. javanica* Schltr. (1919: 275) is recorded only from Java Island; *C. phyllorhiza* (F.Muell) Schltr. (1921: 492) is distributed across Java, the Lesser Sunda Islands, and Sulawesi; *C. taeniophyllum* (J.J.Sm.) Schltr. (1921: 492) is endemic to Maluku (Ambon Island and Banda Islands); and *C. treubii* (J.J.Sm.) Schltr. (1921: 492) that is also endemic to Maluku (Seram Island and Wokam Island of the Aru Islands Regency) (POWO 2024). There is no prior record of any *Chiloschista* species from Sumatra, Borneo, and New Guinea Islands.

During a botanical inventory conducted by the author in 2019, several living specimens of *Chiloschista* were found growing on semi-open coffee plantation close to the forest in Aceh Province, in the northernmost region of Sumatra Island. *Chiloschista* roots usually turn darker when wet and look like the color of tree bark, which makes them difficult to find, such that their small but bright flowers are often important to their detection. Several plants, including individuals with flowers, were collected as herbarium and living specimens for Purwodadi Botanic Gardens in East Java. Further observations of the flowering specimens found that this taxon represents an undescribed species with flower characteristics morphologically similar to *C. javanica* and *C. sweelimii*. Here I describe it as a new species, as well as the first record of this genus on Sumatra Island. A key to the five species of *Chiloschista* in Indonesia is also included.

Materials and methods

Morphological measurements were conducted with a loupe and a ruler accurate to 0.5 mm. Environmental data were collected using thermohygrometer and lux–meter. The specimens were also compared with closely related species (*C. javanica* and *C. sweelimii*). Observation and morphological studies have been done by examining the relevant literature sources, illustrations, living and herbarium specimens, and photographs. These literature sources included protologues and descriptions of the relevant taxa: *C. javanica* (Smith 1905a), *C. sweelimii* (Teck 2016), *C. taeniophyllum* (Smith 1905b), *C. treubii* (Smith 1912), and *C. phyllorhiza* (Mueller 1866; Smith 1939). Photographs were taken with a Sony DSC-W70. Detailed morphological observations were conducted using stereomicroscope Olympus SZX7 with camera EP50. Terminology for morphological description follows Harris and Harris (2001). Conservation status was assessed using the IUCN Red List Category and Criteria. The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were estimated based on GeoCAT (https://geocat.iucnredlist.org/). Six specimens of *Chiloschista* sp. from Aceh were used for examinations (*RIO 9117*; *9118*; *9119*; *9121*; *9123*; *9124*).

Additional examined specimens

1. *Chiloschista sweelimii*. Malaysia • Malay Peninsula; *Lim*, *S.L. s.n.*; K 000891272 • Malay Peninsula; *Ong FRI 75458* (KEP).
2. *Chiloschista javanica*. Indonesia • Java; *Docters van Leeuwen s.n.*, L 1500077 • Java; *leg. ign. s.n.*; L 0264545 • Java; *Docters van Leeuwen 2402*; BO 0057026 • Java; *Docters van Leeuwen 2402*; BO 0057027 • Java; *leg. ign. s.n.*; BO 0057030 • Java; Docters *van Leeuwen s.n.*; BO 0057031 • Java; *Docters van Leeuwen s.n.*; BO 0057037 • Java; *leg. ign. s.n.*; BO 0057038.

Taxonomic treatment

Chiloschista tjiasmantoi **Metusala, sp. nov.**
urn:lsid:ipni.org:names:77356634-1
Figs 1A, B, 2, 3, 4B, E

Type. Indonesia • Sumatra: Aceh Province, c. 900 m, *RIO 9118* (holotype, BO!) (detailed localities are not shown here for conservation purpose).

Diagnosis. *Chiloschista tjiasmantoi* is morphologically similar to *C. javanica*, but differs in having oblong-obovate petals (vs. broadly elliptic to ovate petals), narrowly oblique oblong side lobes with truncate to obtuse apex (vs. relatively straight triangular side lobes with obtuse apex), a lip sac that has a “V” shape in longitudinal section view with a narrow angle of about 45–50° (vs. a lip sac that has an “L” shape in longitudinal section view with a wide angle of about 90°), and a very narrow cavity between the apex of the hairy callus and the thick curved front lobe of the lip (vs. a rather broad cavity).

Description. Epiphytic herb. Roots numerous, spreading, terete to slightly flattened, 2.0–3.5 mm in diameter, greyish-green when wet and becoming grayish-white when dry, grow radially from a short stem as the central, and mature individuals can grow elongated to reach more than 30.0 cm. Stem reduced, very short, erect, simple 2.0–4.0 mm long, up to 3.0 mm in diameter, densely covered with dry stem bracts. Stem bracts are triangular active, and persistent, and encircle the stem tightly. Leaves one or two, 4–7 mm long, deciduous, unseen in cultivation. Inflorescence axillary, arising among the roots gap, pendulous, up to 31.0 cm long in total, peduncle c. 2.0 mm in diam. near base, terete, densely covered with short white hairs, purplish near base and becoming purplish green toward apex, sometimes branched at the base, up to 30 flowered per rachis, flowers arranged spirally in a slightly zig-zag pattern and open simultaneously, each flower can last up to 5 days; flower bracts triangular, 2.0–3.0 mm long × 1.5–2.0 mm wide, acuminate to caudate, pubescent, greenish and soon becoming brown when old. Pedicel and ovary about 2.0 mm long, terete, brownish-green to purplish-green, covered with whitish hairs. Flower rather thin-textured, 1.0–1.2 cm high × 1.0–1.2 cm wide, open widely, sepals and petals yellowish cream or yellow with orange or reddish spots, labellum yellowish cream with reddish or orange spots on their sac, column yellowish green with orange tinge on its foot. Dorsal sepal oblong-elliptic, 5.0–6.0 mm long × 4.0 mm wide, obtuse to rounded, both surfaces pubescent. Petals oblong-obovate, 5.0–6.0 mm long × 3.0–4.0 mm wide, truncate to rounded, both surfaces pubescent. Lateral sepals

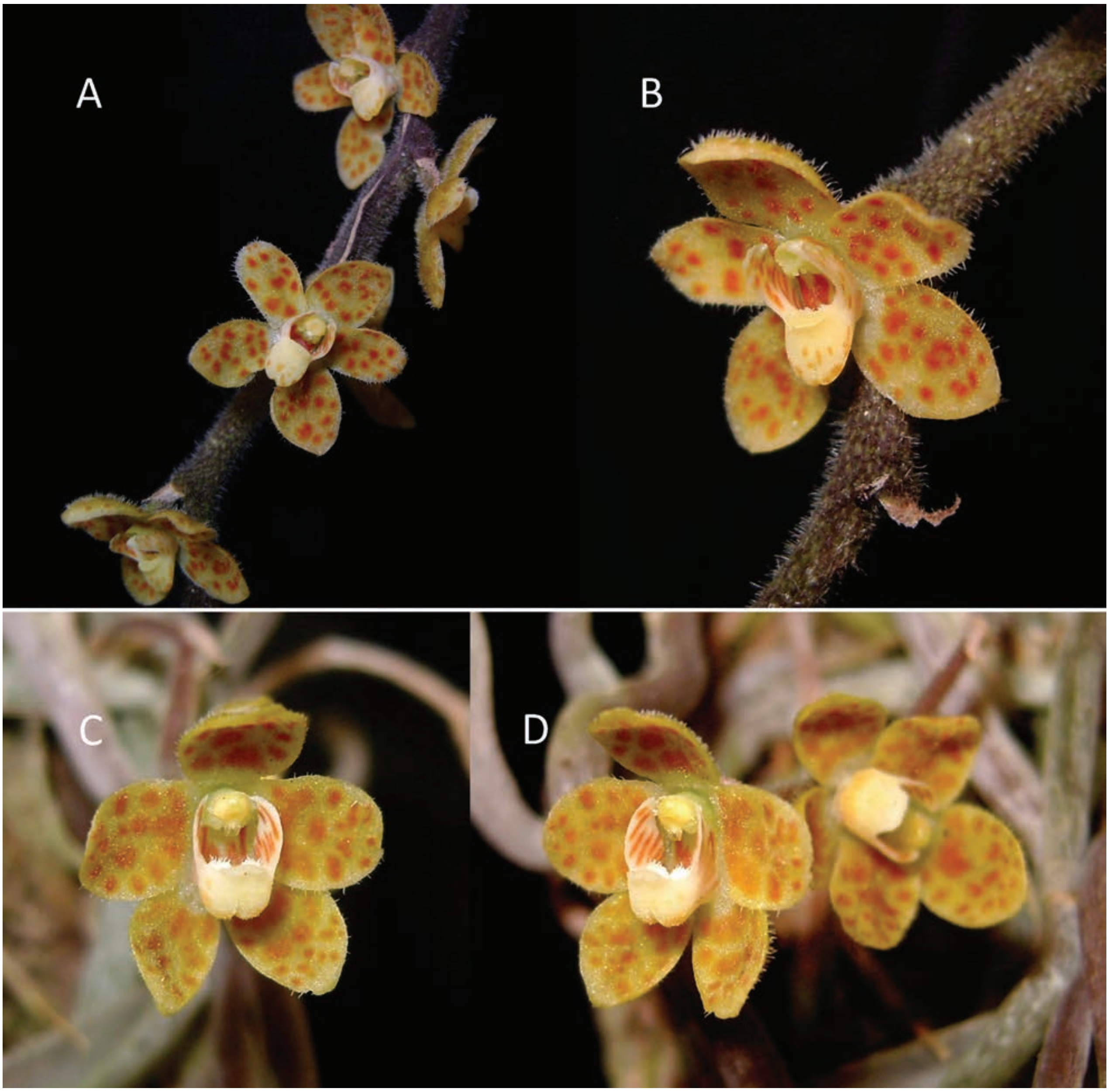

Figure 1. *Chiloschista tjiasmantoi* sp. nov. (**A, B**) and *Chiloschista javanica* (**C, D**) **A** inflorescence **B** flower, oblique view. **C** flower, front view **D** flower, oblique view. Photos by Destario Metusala.

obliquely oblong-elliptic, 6.0 mm long × 4.0–5.0 mm wide, obtuse, both surfaces pubescent. Lip immobile, minutely papillose externally, 3-lobed, deeply saccate, indistinctly canaliculated ventrally, 2.8–3.2 mm long, 4.0–4.5 mm high (from side lobes apex to basal part of sac), 2.0–2.5 mm wide at front; side lobes erect to slightly curved inwards, obliquely oblong to rather falcate, c. 2.0 mm long × c. 1.5 mm wide near base and narrowed gradually to about 1.0 mm near apex, apex truncate to rounded, yellowish cream with red streaks on internal surface; front lobe subtrapezoid, short c. 1.0–1.5 mm × 1.5 mm, apex truncate to slightly emarginate, curved; sac ovate to subrectangular from front view, “V” shape in longitudinal section view, 2.0–2.5 mm long × 2.0–2.3 mm wide, apex truncate or retuse or slightly bilobed, yellowish cream with pale reddish or pale orange spots on external surface around the apex; callus a fleshy thickening

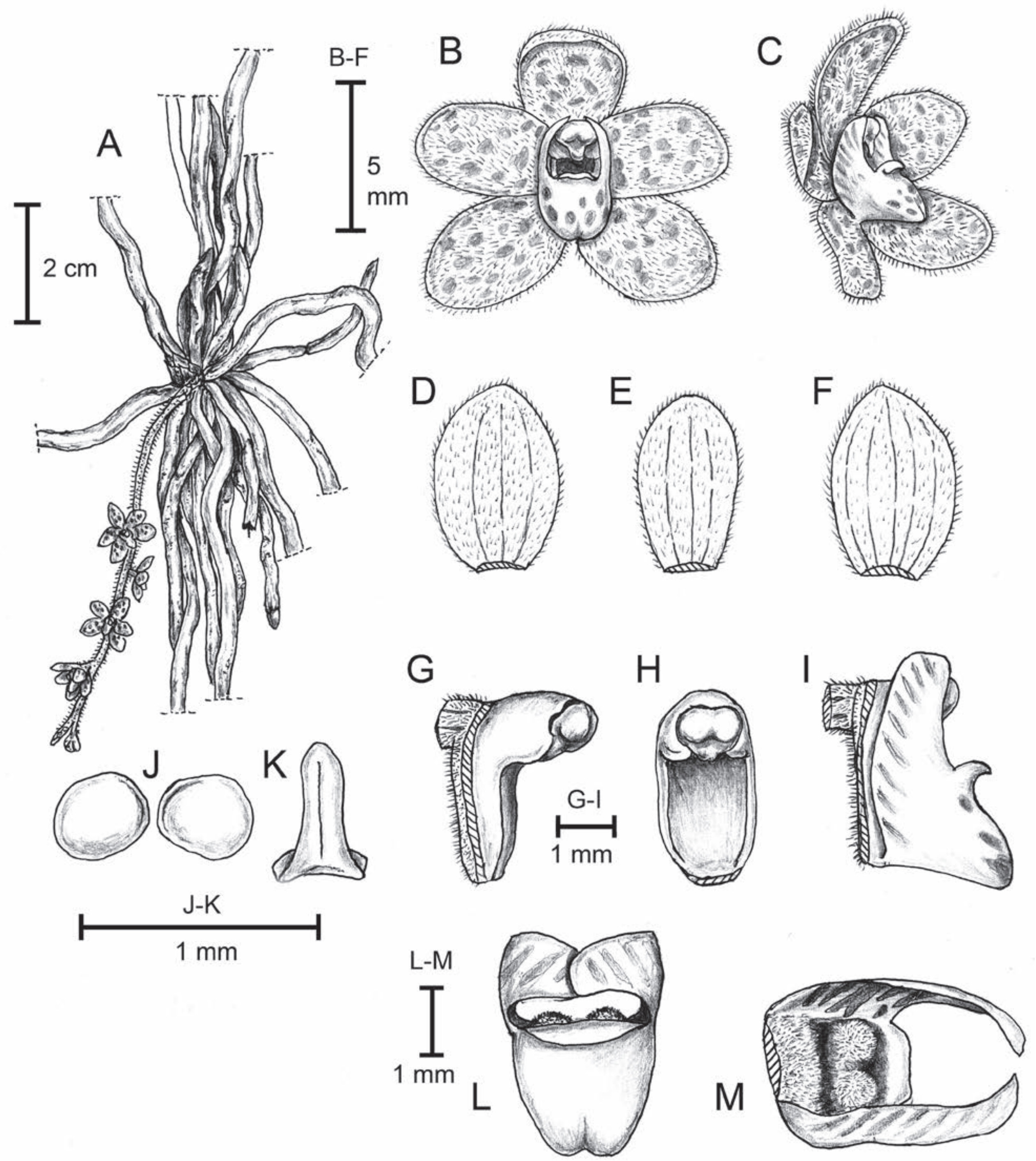

Figure 2. *Chiloschista tjiasmantoi* sp. nov. **A** habitus with inflorescence **B** flower, natural shape, front view **C** flower, natural shape, oblique view **D** dorsal sepal, flat shape **E** petal, flat shape **F** lateral sepal, flat shape **G** column and foot, oblique view **H** column and foot, front view **I** column and lip, side view **J** pollinia **K** stipe and viscidium **L** lip, above view **M** lip interior, back view. Line drawing by Destario Metusala from *RIO 9118*.

arise from basal to middle of the internal front wall, split into two oblong hairy callus that rises up to sac opening, creating a small narrow cavity between apex of the hairy callus and the thick curved front lobe of the lip; column short 1.5–2.0 mm long (excluding the anther cap), foot about 2.2–2.5 mm long; anther cap cucullate, yellowish or cream, c. 1.5 mm × 1.5 mm; pollinarium two unequal globose on a narrowly linear to triangular stipe, yellow. Fruit not seen.

Figure 3. *Chiloschista tjiasmantoi* sp. nov. Flowering plants *in situ*. Photos by Alfajaruddin.

Distribution, habitat and phenology. Based on the existing data, the distribution of *Chiloschista tjiasmantoi* may be restricted to Aceh Province, the most northern part of Sumatra Island. This new species is currently only known from five locations in two different regencies at elevation ranging from 700–1000 m. The populations of this species were mostly found growing epiphytically on old coffee trees (*Coffea* spp.) and shade trees (*Leucaena* spp.) in the local coffee plantations, together with *Vanda pumila* (Orchidaceae), in a windy and semi–opened wet habitat with medium sunlight intensity. Flowering recorded in mid-July, early November to late December.

Etymology. The specific epithet "tjiasmantoi" honors Wewin Tjiasmanto, the chairman of Tjiasmanto Conservation Fund and a philanthropist concerned with the Indonesian plant conservation.

Cultivation. *Chiloschista tjiasmantoi* seems rather difficult to cultivate at lower elevations (300 m. a.s.l). However, it was successfully grown by attaching it to a slab of tree fern with a top dressing of moss to prevent the roots from drying out, under a light intensity of about 30–75% with good air circulation and humidity levels of about 80% or more.

Discussion. This new species is morphologically similar to *C. javanica* (Fig. 1C, D, 4C, F) and *C. sweelimii* (Fig. 4A, D). However, there is a geographical separation among these three species. *C. tjiasmantoi* is thus far only recorded from Aceh Province, the northernmost region of Sumatra Island; *C. sweelimii* occurs in the Malay Peninsula and Vietnam, and *C. javanica* is endemic to Java Island.

Chiloschista tjiasmantoi differs from *C. javanica* in having narrower oblong-obovate petals, narrowly oblique oblong side lobes with truncate to obtuse apex, a lip sac that has a "V" shape in longitudinal section view with a narrow angle of about 45–50°, a narrow cavity between the apex of the hairy callus and the thick curved front lobe of the lip. Meanwhile, *C. javanica* has broadly elliptic to ovate petals, relatively straight triangular side lobes with obtuse apex, a lip

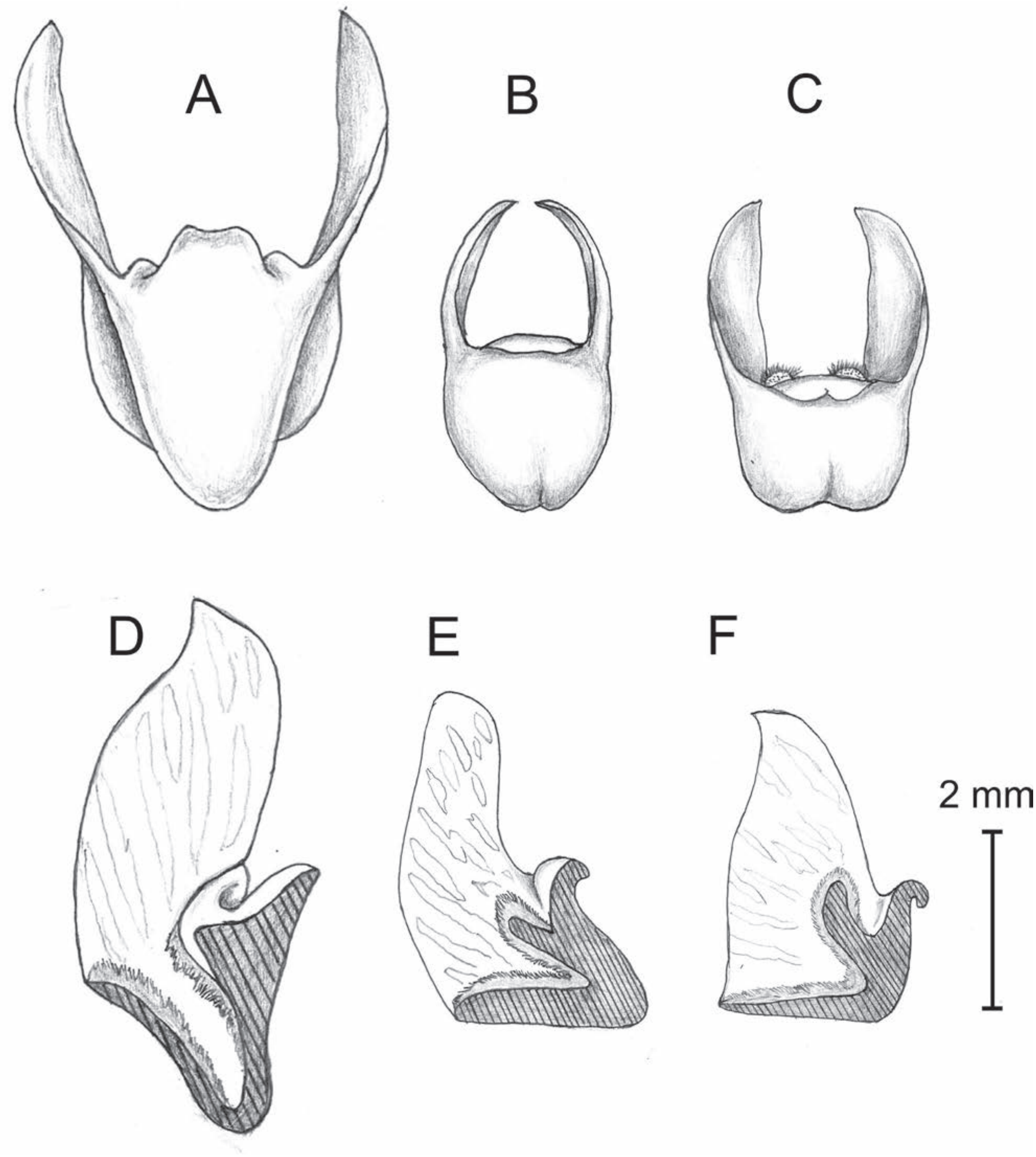

Figure 4. Lip of three *Chiloschista* species **A** *C. sweelimii*, natural shape, front view **B** *C. tjiasmantoi*, natural shape, front view **C** *C. javanica*, natural shape, front view **D** *C. sweelimii*, longitudinal section, flat shape, side view **E** *C. tjiasmantoi*, longitudinal section, flat shape, side view **F** *C. javanica*, longitudinal section, flat shape, side view. **A** and **D** redrawn after Ong Poh Teck in Teck (2016). Drawn by Destario Metusala.

sac that has an "L" shape in longitudinal section view with a wide angle of about 90°, and a rather broad cavity between the apex of the hairy callus and the thick curved front lobe of the lip.

Furthermore, *C. tjiasmantoi* differs from *C. sweelimii* in having sepals and petals with hairs on both sides, oblong-obovate petals, a broadly oblong-elliptic dorsal sepal, basal front lobe without any callus, an ovoid to subrectangular lip's sac from front view with a truncate to slightly bilobed apex, and an oblong fleshy thickening that arises from basal to middle of the internal front wall. This thickening then divides into a 2-lobed hairy callus that rises near the sac opening, creating a cavity between the apex of the hairy callus and the thick curved front lobe. In contrast, *C. sweelimii* has sepals and petals with hairs only on their adaxial surface, ovate to orbicular petals, an ovate dorsal sepal, basal front lobe with large callus on either side (each c. 1.5 × 1.0 mm), a triangular shaped lip's sac from front view with pointed obtuse apex, and the absence of prominent internal callus inside the lip's sac (Teck 2016). A comparison between the new species and its morphological allies is shown in Table 1.

This new species will be the fifth *Chiloschista* species in Indonesia, and the first record of this genus on Sumatra Island. With the exception of *C. phyllorhiza*, the species are endemic to the country and appear to have restricted distributions. Although the flowers of some species appear nearly identical in appearance, the internal structure of the lip can be very different, which suggests this would be a good key character for further identification of this genus (Gyeltshen et al. 2019; Dalstrom and Kolanowska 2020). A deeper investigation into its floral morphological variation needs to be carried out in the future, as this is essential to support species delimitation of Indonesian *Chiloschista* (Metusala 2020b; Metusala et al. 2020).

Conservation. *Chiloschista tjiasmantoi* was recorded from five locations in Aceh Province. Based on currently available data, the Extent of Occurrence (EOO) of this species is 117.01 km^2 with user-defined cell width = 2 km (criterion B1: < 5,000 km^2) and Area of Occupancy (AOO) value of 20.00 km^2 (criterion B2: < 500 km^2). Their natural habitat is threatened by land conversion, especially from large-scale coffee plantations. Existing populations were estimated to be less than 2,500 mature individuals (≤ 250 in most subpopulations) where they mostly found attached to the branches of coffee trees and its shade trees (*Leucaena* spp.). Unfortunately, coffee plantations provide a vulnerable habitat for this new orchid species as they can be pruned or cut down at any time. Moreover, many coffee farmers believe that this orchid is a harmful parasitic plant and eradicate them by clearing the coffee branches from any epiphytic plants. This species has also been found traded on domestic online platforms as an ornamental orchid, although currently in small quantities as its habit and small flowers are considered less attractive to Indonesian hobbyists. In many cases, unsustainable commercial harvesting may have a significant impact on the viability of wild orchid populations (Hinsley et al. 2018). Therefore, I consider this species to likely be in the Endangered category B1 and B2+ab (i,ii,iii,iv), C2+a (i) (IUCN Red List Categories and Criteria).

It has been well-documented that ecosystems in the highland of Aceh Province are severely affected by climate change, particularly through increased temperatures (Schroth et al. 2015). This could also have a major impact on various sensitive plant species, such as many highland epiphytic orchids, including natural populations of *Chiloschista tjiasmantoi*. Therefore, conservation-based researches are needed to determine the vulnerability of these species to climate change-related pressures, especially drought stress (Al Farishy et al. 2017; Arimy et al. 2017; Metusala 2017b; Metusala et al. 2017; Suffan et al. 2021; Ishmah et al. 2021; Trimanto et al. 2023).

Table 1. Morphological comparison among the new species and its allies.

Characters	*C. tjiasmantoi* Metusala	*C. javanica* Schltr	*C. sweelimii* Holttum
Flower colour	Sepals and petals yellowish cream or yellow with orange or reddish spots, lip yellowish cream with reddish or orange spots on their sac and side lobes with pale reddish orange stripes	Sepals and petals yellowish cream or yellow with orange or reddish spots, lip white or cream with pale reddish or orange spots on their sac and side lobes with pale reddish orange stripes	Sepals and petals yellow with orange-brown blotches, lip white on their sac and side lobes with reddish-orange stripes
Dorsal sepal	Oblong-elliptic, 5.0–6.0 mm long × 4.0 mm wide, obtuse to rounded, both surfaces pubescent but abaxial with shorter hairs	Elliptic, 5.0–6.0 × 3.0–3.5 mm, obtuse to rounded, adaxial surface pubescent, abaxial with scattered shorter hairs	Ovate, 5.0–6.0 × 3.5–5.0 mm, obtuse to acute, only adaxial surface pubescent
Lateral sepals	Obliquely oblong-elliptic, 6.0 mm long × 4.0–5.0 mm, obtuse, both surfaces pubescent but abaxial with shorter hairs	Elliptic, 4.0–5.0 mm × 3.0–3.5 mm, obtuse to rounded, adaxial surface pubescent, abaxial with scattered shorter hairs	Broadly elliptic to broadly ovate, 5.0–6.0 × 3.5–5.0 mm, obtuse to acute, only adaxial surface pubescent
Petals	Oblong-obovate, 5.0–6.0 mm long × 3.0–4.0 mm wide, truncate to rounded, both surfaces pubescent but abaxial with shorter hairs	Broadly elliptic to ovate, 4.0–4.5 × 3.0–3.5 mm, obtuse to rounded, adaxial surface pubescent, abaxial with scattered shorter hairs	Ovate-orbicular, 4.0–5.0 × 3.0–4.5, obtuse, adaxial surface sparsely pubescent
Side lobes	Obliquely oblong, slightly falcate, *c.* 1.5 mm wide at base and narrowed gradually to about 1.0 mm near apex, truncate to obtuse	Triangular, relatively straight, *c.* 1.9 mm wide at base and narrowed gradually to about 0.8 mm near apex, acute	Obliquely oblong, slightly falcate, c. 2 mm wide at base and narrowed gradually to about 0.9 mm near apex, acute to obtuse
Front lobe	Broadly subtrapezoid, short, *c.* 1.0–1.5 × 1.5 mm, apex truncate to slightly emarginate, curved, base without any callus	Broadly subtrapezoid, short, *c.* 1.0–1.5 × 1.5 mm, apex truncate to slightly emarginate, curved, base without any callus	Broadly triangular, short, *c.* 2.0 × 1.0 mm, apex retuse, base with large callus on either side (*c.* 1.5 × 1.0 mm)
Lip's sac	Ovate to subrectangular from front view, forming a "V" shape in longitudinal section view with a narrower angle (45–50°), *c.* 2.0–2.5 × 2.0–2.3 mm, apex retuse to slightly bilobed	Subrectangular from front view, forming an "L" shape in longitudinal section view with a wider angle (90°), *c.* 2.0–2.3 × 2.7–3.0 mm, apex retuse	Triangular from front view, forming a "V" shape in longitudinal section view with a narrower angle (45–50°), *c.* 4.0–5.0 × 3.0–3.5 mm, apex obtuse
Internal lip's sac ornament	An oblong fleshy thickening arises from basal to middle of the internal front wall, split into 2 lobed hairy callus that rises to sac opening, creating a small narrow cavity between apex of the hairy callus and the thick curved front lobe	A large and high oblong fleshy thickening arises from basal to middle of the internal front wall, split into 2 lobed long protruding hairy callus that rises to sac opening, creating a cavity between apex of the hairy callus and the thick curved front lobe	Internal callus absence

Key to the species of *Chiloschista* in Indonesia

1 Roots very flattened with a glabrous inflorescence's peduncle **2**
– Roots more or less terete with a hairy inflorescence's peduncle **4**
2 Sepals and petals narrowly elliptic, the lip's sac conical with pointed apex ....***C. taeniophyllum***
– Sepals and petals broadly elliptic, the lip's sac hemispherical with rounded or slightly bilobed apex **3**
3 Lip's sidelobes obliquely quadrangular with sac apex rounded ....***C. treubii***
– Lip's sidelobes oblong with sac apex slightly bilobed ....***C. phyllorhiza***
4 Lip's sac has a "V" shape in longitudinal section view with a narrow angle of about 45–50° ....***C. tjiasmantoi***
– Lip's sac has an "L" shape in longitudinal section view with a wide angle of about 90°..***C. javanica***

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Additional information

Conflict of interest

The author has declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

The author solely contributed to this work.

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Data availability

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

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Research Article

Petrocodon curvitubus, a new species of Gesneriaceae from Guangxi, China

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Abstract

A new species of Gesneriaceae, *Petrocodon curvitubus* J.X.Wei, B.Pan & T.Ding, **sp. nov.** from Guangxi, China, is described and illustrated. The new species is morphologically similar to *P. lui* and *P. tenuitubus*, but can be easily distinguished by its elliptic to oblong-ovate leaves, corollas with two purple longitudinal stripes, and conical ovary.

Key words: China, Gesneriaceae, Guangxi, limestone flora, new species

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Introduction

The genus *Petrocodon* Hance was established in 1883, initially containing only one species, *Petrocodon dealbatus* Hance (Hance, 1883). For over a century, the genus remained monotypic until two new species, *P. ferrugineus* Y.G. Wei and *P. multiflorus* F. Wen & Y.S. Jiang, were discovered in southern China (Wei 2007; Wei et al. 2010). In 2011, the range and morphological diversity of the genus were further expanded based on molecular evidence (Wang et al. 2011; Weber et al. 2011), which led to the incorporation of several monotypic genera, such as *Calcareoboea* C.Y.Wu ex H.W.Li, *Dolicholoma* D. Fang & W.T. Wang, *Paralagarosolen* Y.G. Wei, and *Tengia* Chun, as well as all species of the small genus *Lagarosolen* W.T. Wang, four species of *Didymocarpus* Wall., one species of *Wentsaiboea* D. Fang & D.H. Qin, one species of *Primulina* Hance, and recently all but the type species of *Allocheilos* W.T.Wang (Wang et al. 2011; Weber et al. 2011; Xu et al. 2014; Liu et al. 2024). In recent years additional taxa have been discovered in *Petrocodon* such as *P. villosus* Xin Hong, F.Wen & S.B.Zhou, *P. rubrostriatus* K. Tan, X.Q. Song & M.X. Ren, *P. jiangxiensis* F. Wen, L.F. Fu & L.Y. Su, *P. pulchriflorus* Y.B.Lu & Q.Zhang (Hong et al. 2014; Lu et al. 2017; Su et al. 2019; Tan et al. 2023). According to the data from the Gesneriaceae Resource Centre (GRC 2024), there are currently 55 species and one variety of *Petrocodon* (*Petrocodon dealbatus* var. *denticulatus* (W.T. Wang) W.T. Wang) recognized worldwide. These species are mainly distributed in southwestern China, on the Indochina Peninsula, northern Thailand, northern Laos, and northern

Vietnam. Notably, *P. flavus* D.J. Middleton & Sangvir. is endemic to Thailand; *P. leveilleus* (Fedde) X.X. Bai & F. Wen and *P. hispidus* (W.T.Wang) A. Weber & Mich. Möller are distributed in China and Vietnam; *P. bonii* (Pellegr.) Mich. Möller & A. Weber is distributed in northeastern Thailand and Vietnam; and the remaining *Petrocodon* species are endemic to China (Pellegrin 1926; Li 1982; Vu 2006; Weber et al. 2011; Middleton et al. 2015; Vu 2017; Xin et al. 2021; Xiong et al. 2024).

In January 2024, the authors collected an unidentified species of Gesneriaceae with a purple corolla growing on a stone wall at the entrance of a karst cave in Debao County, Guangxi. This species had a slender corolla, and its curved corolla tube showed a certain similarity to those of *Primulina curvituba* B. Pan, Li H. Yang & M. Kang (Yang et al. 2017). However, further detailed observation showed that the stigma of the newly collected species was bilobed with oval lobes, unlike the chiritoid stigma of *Primulina* Hance. Other morphological characteristics, such as alternate leaves in basal rosettes, bilobed corolla with two fertile stamens, and capsules dehiscing into four valves, indicate that the species belongs to *Petrocodon* (Weber et al. 2011, 2020). We collected two flowering individuals as voucher specimens, carefully observed plants in the wild, and the herbarium specimens in the laboratory. We also compared them with the digital specimens of the Chinese Virtual Herbarium (https://www.cvh.ac.cn/), JSTOR Global Plants (http://plants.jstor.org), and the latest publication on *Petrocodon* taxa. This species can be clearly distinguished from other accepted *Petrocodon* species. The curved shape of the corolla tube is similar to *P. lui* (Yan Liu & W.B. Xu) A. Weber & Mich. Möller and *P. tenuitubus* W.H. Chen, F. Wen & Y.M. Shui (Xu et al. 2010; Chen et al. 2019), but it can be distinguished from *P. lui* by leaf shape, corolla coloration, bract, corolla tube, stamen, and pistil size. It is different from *P. tenuitubus* in leaf shape and color, number of inflorescences and bracts, corolla shape and indumentum, and pistil shape. Therefore, we describe it as a new species of *Petrocodon*, *P. curvitubus*.

Materials and methods

We collected two specimens from the type locality and deposited them at IBK. The morphological measurements and descriptions of the new species are based on plants observed in the field. Additionally, we compared the new species with digital specimens from the Chinese Virtual Herbarium and JSTOR Global Plants and checked the relevant literature (e.g., Xu et al. 2010; Chen et al. 2019). The morphological data for *P. lui* and *P. tenuitubus* used in comparison came from the protologues (Xu et al. 2010; Chen et al. 2019) and their specimens. We also thoroughly examined the type specimen of *P. tenuitubus* (isotype: IBK [IBK00417055]), the type of *P. lui* (holotype: IBK [IBK00406224], isotype: IBK [IBK00406225], paratype: IBK [IBK00406226], [IBK00406227], [IBK00406228], [IBK00406229]). Furthermore, the endangered category of this new species was assessed according to the criteria of the IUCN Red List (IUCN 2024). This new species is described using the terminology used by Wang et al. (1998) and Harris and Harris (2001).

Taxonomic treatment

Petrocodon curvitubus **J.X.Wei, B.Pan & T.Ding, sp. nov.**
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Figs 1–3

Diagnosis. *P. curvitubus* is florally similar to *P. lui* and *P. tenuitubus*. However, it can be distinguished from these by leaf characteristics, with leaves elliptic to oblong-ovate, apex obtuse or slightly acuminate, base cuneate (vs *P. lui*, ovate or broadly ovate; apex subacute or obtuse, base cordate to shallowly cordate, oblique; vs *P. tenuitubus*, ovate to orbicular; apex obtuse, base cordate symmetrical or asymmetrical), corollas with purple stripes (vs absent in *P. lui* and *P. tenuitubus*), corolla lobes obovate-elliptic to oblong, apex acuminate (vs *P. lui*, lobes oblong, obovate to suborbicular, apex obtuse; vs *P. tenuitubus*, lobes narrowly ovate to ovate, apex obtuse), and ovary conical, ca. 2.5 × ca. 0.8 mm (vs *P. lui*, linear, ca. 3 × 1.5 mm; vs *P. tenuitubus*, ovoid, 1–2 × 1–1.5 mm) (Table 1).

Description. Perennial herbs. Rhizome short and straight, subterete, 1–3.8 cm long, 7.5–9 mm in diameter. Leaves 10–18, basal, alternate, and congested at rhizome apex; petiole terete, adaxially pale green, abaxially pale brown, 2.5–6.4 cm long, 2–2.5 mm in diameter, densely white pubescent. Leaf blade herbaceous, elliptic to oblong-ovate, 2.1–13.3 × 1.1–7.5 cm, apex obtuse or slightly acuminate, base cuneate, margin crenulate, adaxially surface pale green, abaxially grayish green, both surfaces densely white pubescent, lateral veins pinnate 5–8 pairs on each side, adaxially concave, abaxially prominent. Inflorescences 3–6, axillary, 1–5-branched, cymose, (4-)10–16(-22)-flowered; peduncles 2.2–6.8 cm long, 2–2.5 mm in diameter, densely white pubescent; bracts 2, linear, opposite, 4–5 × ca. 0.5 mm, outside densely white pubescent, inside glabrous; pedicels 3–8 mm long, 0.3–0.7 mm in diameter, densely white pubescent. Calyx 5-lobed from base, pale green, lobes linear to triangular, 4–6 × ca. 0.5 mm, outside densely white pubescent, inside glabrous; Corolla pale purple, zygomorphic, 1.8–2.2 cm long, both surfaces sparsely puberulent, with two dark purple longitudinal stripes in the corolla mouth; corolla tube slenderly tubular, strongly curved downwards at base (1–3 mm from the base), then bent forwards, 1–1.3 cm long, 1.5–2.5 mm in diameter at the mouth, 1 mm in diameter at the base, adaxial lip 2-lobed to near base, obovate-elliptic, 0.5–0.7 cm long, ca. 0.3 mm in diameter, apex acuminate; abaxial lip 3-lobed to base, oblong, 0.6–1.1 cm long, 2.5–4 mm in diameter, apex acuminate. Stamens 2, adnate about 6 mm from corolla base, glabrous; anthers pale yellow, ca. 1.5 mm long, dorsifixed, cohering apically; filaments ca. 1 mm long, white, glabrous. Staminodes 3, ca. 0.1 long, white, adnate 2.5 mm above corolla base. Disc annular, ca. 0.5 mm high, orange-yellow. Pistil ca. 5.5 mm long, ovary conical, ca. 2.5 mm long, ca. 0.8 mm in diameter, style ca. 3 mm long, ovary and style densely covered with short glandular and eglandular hairs, stigma bilobed, lobes oval, ca. 0.5 mm long. Capsule 0.5–1 cm long, about 2 mm wide, dehiscing 4-valved. Seeds not seen.

Type. China • Guangxi Zhuang Autonomous Region, Baise City, Debao County, Longguang Town, 23°5'N, 106°44'E, 454 m a.s.l., growing on rock wall at the

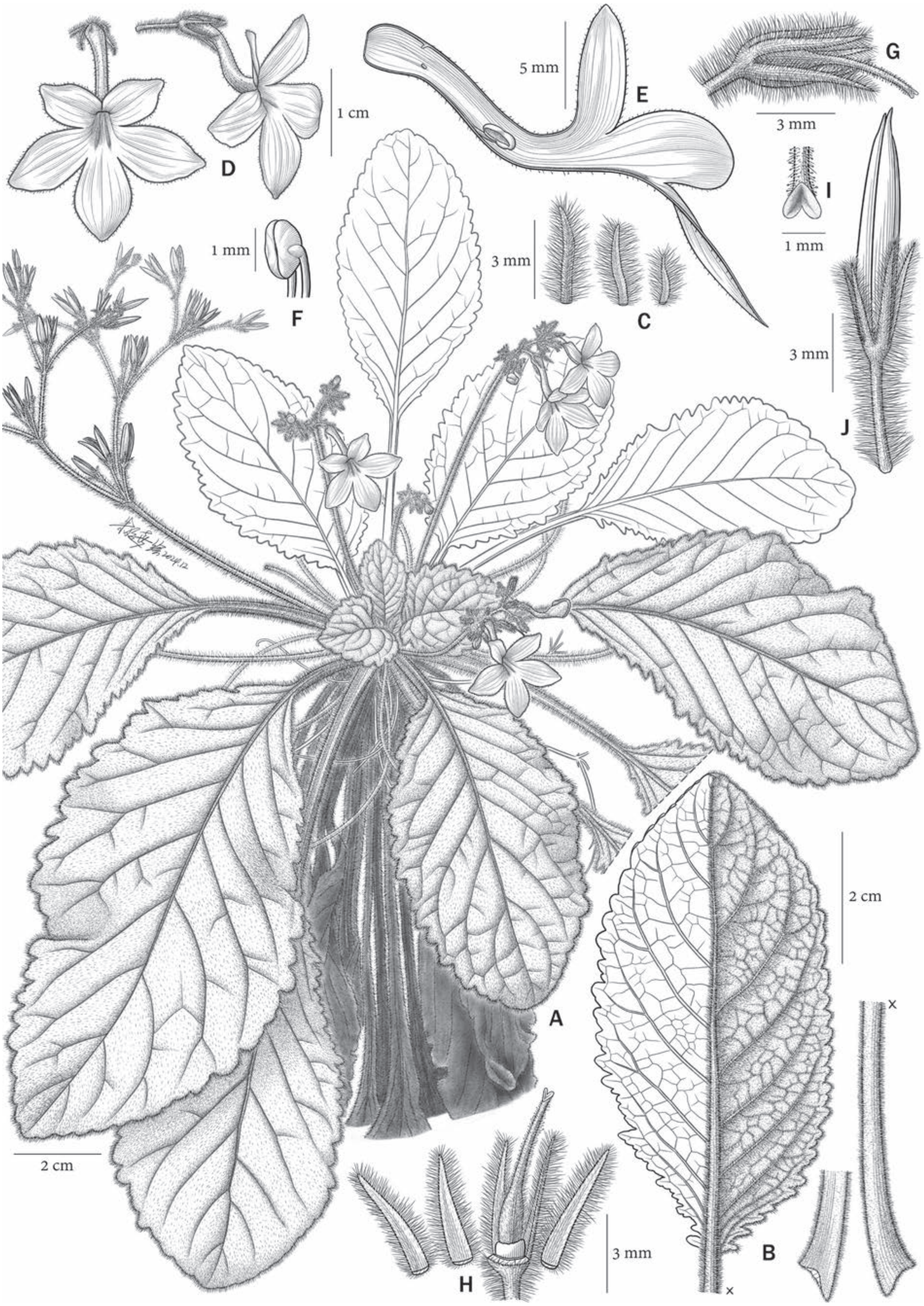

Figure 1. *Petrocodon curvitubus* J.X.Wei, B.Pan & T.Ding, sp. nov. **A** plant in flower **B** abaxial leaf surface and petiole **C** bracts **D** frontal view of corolla and side view of flower showing strongly curved corolla tube **E** longitudinal section of corolla showing the position of stamens **F** stamens with cohering anthers **G** pistil and calyx **H** pistil with sepals dissected **I** stigmas **J** fruit.

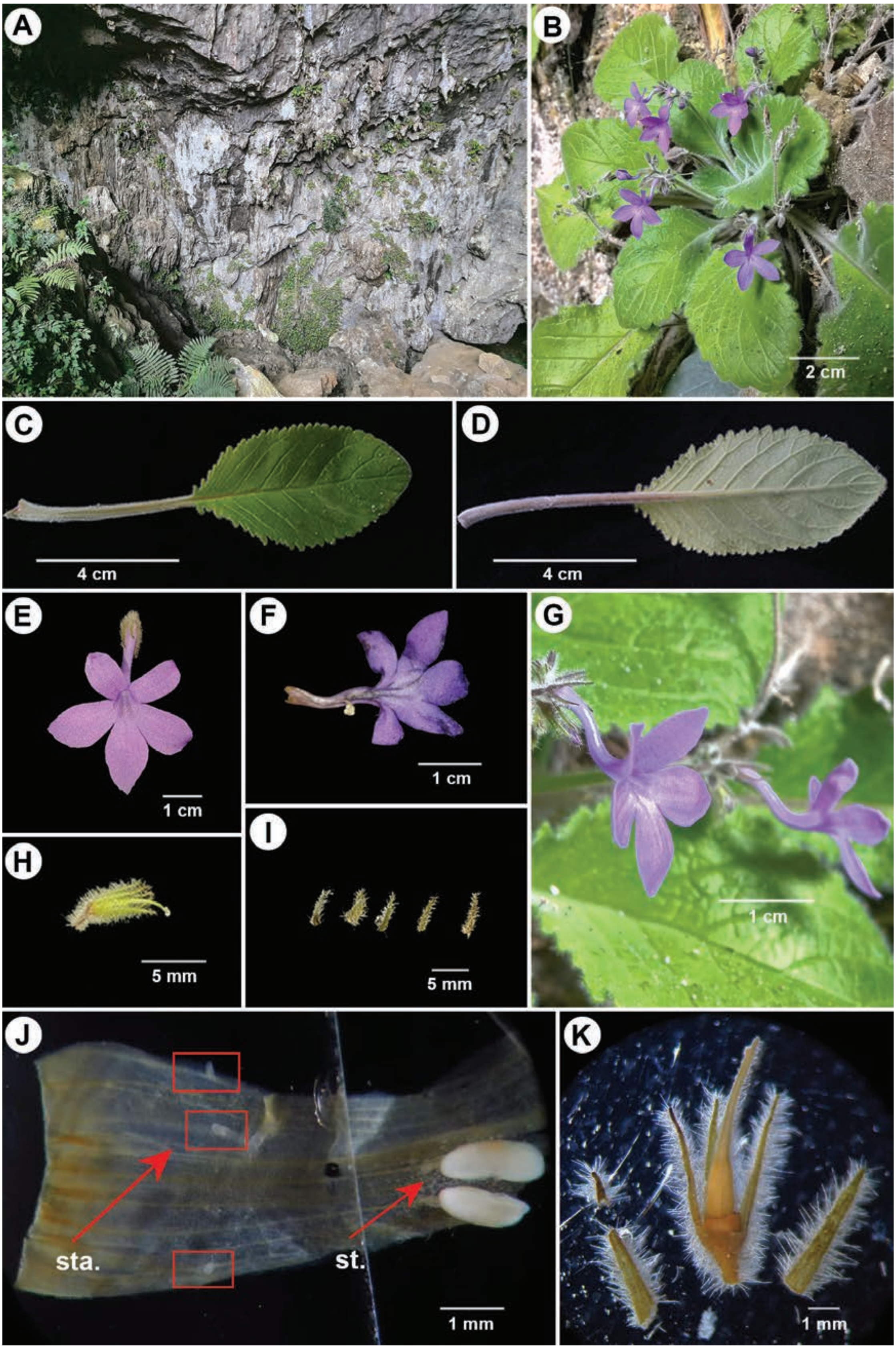

Figure 2. *Petrocodon curvitubus* J.X.Wei, B.Pan & T.Ding, sp. nov. **A** habitat **B** plant in flower **C** adaxial leaf surface **D** abaxial leaf surface **E** frontal view of flower **F** opened corolla **G** side view of flower showing strongly curved corolla tube **H** pistil and calyx **I** sepals **J** stamens (st.) and staminodes (sta.) from a pickled specimen **K** pistil and calyx with partly dissected sepals from a pickled specimen.

Figure 3. Herbarium type specimens of *P. curvitubus* J.X.Wei, B.Pan & T.Ding, sp. nov. **A** WJX001 **B** WJX002.

Table 1. Comparison of characters among *P. curvitubus*, *P. lui*, and *P. tenuitubus*.

Character	*P. curvitubus* sp. nov.	*P. lui*	*P. tenuitubus*
Leaf	elliptic to oblong-ovate; apex obtuse or slightly acuminate, base cuneate	ovate or broadly ovate; apex subacute or obtuse, base cordate to shallowly cordate, oblique	ovate to orbicular; apex obtuse, base cordate symmetrically or asymmetrically
Leaf adaxially	densely white pubescent	glabrous or sparsely puberulent	densely strigose
Leaf abaxially	densely white pubescent	puberulent	tomentose
Peduncles	densely white pubescent	puberulent	glandular hairs
Bracts	2, adaxially densely white pubescent	2, adaxially puberulent	3, adaxially tomentose
Pedicels	densely white pubescent	puberulent	densely glandular hairs
Corolla tube	slenderly tubular, strongly curved near base, 1–1.3 cm long, 1.5–2.5 mm in diameter at the mouth, with two dark purple longitudinal stripes, stripes glabrous	slender, curved, 1.0–1.5 cm long, ca. 5–6 mm in diameter at the mouth, with two yellow stripes, stripes densely covered with glandular hairs	slender, curved, 0.7–1.6 cm long, 6–8 mm in diameter at mouth, with two yellow stripes, stripes glabrous
Corolla	lobes obovate-elliptic to oblong, apex acuminate	lobes oblong, obovate to suborbicular, apex obtuse	lobes narrowly ovate to ovate, apex obtuse
Filaments	glabrous	sparsely glandular	glabrous
Ovary	conical, ca. 2.5 × ca. 0.8 mm	linear, ca. 3 × 1.5 mm	ovoid, 1–2 × 1–1.5 mm

entrance of karst cave. 26 January 2024, flowering, *WJX001* (holotype: IBK! IBK00470322), *WJX002* (paratype: IBK! IBK00470323).

Phenology. Flowering from January to March.

Etymology. The epithet originates from the strongly curved corolla tube.

Vernacular name. Wān Guǎn Shí Shān Jù Tái (弯管石山苣苔), the first two words, "Wān Guǎn," mean the corolla tube is strongly curved, and the following four words, "Shí Shān Jù Tái," mean *Petrocodon* in Chinese.

Distribution and habitat. *Petrocodon curvitubus* can only be found at its type locality, Longguang Township, Debao, Baise, Guangxi. It grows on a limestone wall at the entrance of a karst cave at an altitude of 454 m. The average annual temperature in Debao County is 19.5 °C, and the average annual precipitation is 1456.2 mm.

Preliminary conservation assessment. Currently, only one population that consists of about 200 mature individuals of *P. curvitubus* has been discovered. This population is concentrated on the stone wall at the entrance of a karst cave that is located in Longguang Township, Debao, Baise, Guangxi. The area occupied (AOO) is about 100 m^2, which is significantly smaller than the smallest AOO unit of the IUCN (2024) (10 km^2 for Critically Endangered under B2). Additionally, we also observed that the habitat of *P. curvitubus* is located near a village and is vulnerable to human activities. Frequent human impacts may lead to a reduction in mature individuals of this species and the decline of the population. According to the IUCN Red List Categories and Criteria (IUCN 2024), the endangered level of this new species is preliminarily assessed as “Critically Endangered” [CR, B2a,b (iii,v)].

Discussion

A curved corolla tube can be found in Chinese Gesneriaceae not only in *Petrocodon*, but also in *Primulina* and *Oreocharis* Benth, as exemplified by *P. curvituba*, *Oreocharis curvituba* J.J. Wei & W.B. Xu (Wei et al. 2016) and *Oreocharis pumila* (W.T. Wang) Mich. Möller & A. Weber (Möller et al. 2011). It has been hypothesized that a curved corolla tube may represent a pollinator shift and an adaptation to a long-tongued pollinator of these plants (Guo and Wang 2014; Yang et al. 2017). Research on genera such as *Aquilegia* L. and *Oreocharis* has shown that pollinator shifts often drive the evolution of floral traits (Huang 2007; Whittall and Hodges 2007; Jin et al. 2021). However, whether the curved corolla tube of *P. curvitubus* is specifically adapted to certain pollinators requires further investigation. Future studies could include the construction of phylogenetic relationships and floral trait analyses among Gesneriaceae species with curved corolla tubes to better understand their adaptation and evolutionary trajectories.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Investigation: Bo Pan. Writing—original draft: Jin-Xin Wei. Writing—review and editing: Tao Ding.

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Data availability

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

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Research Article

Plastome-based subgenus-level phylogenetic backbone of hawthorns: insights into the maternal position and taxonomic synopsis of *Crataegus shandongensis* (Rosaceae, Maleae)

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Abstract

The recent recognition of the five-subgenera classification within *Crataegus* has prompted discussion about the maternal phylogenetic relationships among these subgenera, with inconsistencies in taxon sampling, marker selection, and inference methods contributing to differing interpretations. In this study, we performed deep genome skimming sequencing and assembled 63 whole plastomes, including 58 from *Crataegus* and five from related genera as the outgroups. We employed multiple phylogenetic inference methods (Maximum Likelihood and Bayesian Inference) to reconstruct an accurate phylogeny. The whole plastome-based, maternally inherited trees consistently supported two major clades within *Crataegus*: one comprising *C.* subg. *Crataegus* and *C.* subg. *Brevispinae*, the other encompassing the remaining three subgenera. Within the latter clade, *C.* subg. *Sanguineae* and *C.* subg. *Americanae* formed a sister group, which together were sister to *C.* subg. *Mespilus*. Our analysis also revealed a close maternal relationship between *C. shandongensis* and *C. pinnatifida* var. *major*, suggesting the shared maternal ancestry. Furthermore, we updated the description of *C. shandongensis* based on extensive specimen examination and designated the lectotype for this species. This comprehensive taxonomic synopsis, supported by both phylogenomic and morphological analyses, provides a robust foundation for future taxonomic and evolutionary studies of the Shandong hawthorn.

Key words: Chloroplast genome, *Crataegus*, deep genome skimming, lectotype, taxonomy

Introduction

The hawthorn genus *Crataegus* L. represents a diverse group of deciduous shrubs and small trees within the family Rosaceae, playing a significant ecological role as a source of food and habitat for various pollinators, birds, and mammals.

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* These authors have contributed equally to this work.

In comparison to the morphologically similar genus *Hesperomeles* Lindl., which is endemic to South America, *Crataegus* is native to temperate regions of North America, Europe, and Asia. These two genera can be easily distinguished by their distinct distributions, although they share several morphological traits, including the presence of thorns, polypyrenous drupes, shoot dimorphism, clusters of small, fragrant white or pink flowers, and bright red or orange fruits (Robertson et al. 1991; Gu and Spongberg 2003; Kalkman 2004; Phipps 2014). The genus *Crataegus* is taxonomically challenging, with the reported number of species varying from 140 to 230 (Phipps et al. 1990; Gu and Spongberg 2003; Phipps 2014). This variability is attributed to several factors, primarily widespread hybridization and polyploidization, especially among North American species (Dickinson 2018; Liston et al. 2021). These processes have resulted in a dynamic genetic landscape, complicating the classification and study of *Crataegus* and making it a compelling subject for botanical and taxonomic research.

Recent molecular phylogenetic and morphological studies have consistently supported the merging of *Crataegus* and *Mespilus* L. (Lo et al. 2007) and proposed a comprehensive subgeneric classification of *Crataegus*, including five subgenera: *C.* subg. *Crataegus*, *C.* subg. *Americanae* El Gazzar, *C.* subg. *Brevispinae* (Beadle) Ufimov & T.A.Dickinson, *C.* subg. *Mespilus* (L.) Ufimov & T.A.Dickinson, and *C.* subg. *Sanguineae* Ufimov (Ufimov and Dickinson 2020). All the Chinese *Crataegus* species belong to two subgenera: *C.* subg. *Crataegus* and *C.* subg. *Sanguineae* (Yu and Gu 1974; Gu and Spongberg 2003). Despite these advances, plastid-based phylogenies have yielded conflicting topologies for these five subgenera due to differences in sampling strategies, sequencing regions, and analytical methods (Lo et al. 2007; Zarrei et al. 2014; Liston et al. 2021).

Lo et al. (2007) sequenced four plastid regions—*trn*G-*trn*S, *psb*A-*trn*H, *trn*H-*rpl*2, and *rpl*20-*rps*12—and analyzed the phylogeny of *Crataegus* using Maximum Parsimony (MP) and Maximum Likelihood (ML) methods. These approaches resulted in differing topologies (Fig. 1A vs. 1B). The MP analysis identified two major clades: one comprising subg. *Americanae* and subg. *Sanguineae*, and the other containing subg. *Crataegus*, subg. *Brevispinae*, and subg. *Mespilus*. Conversely, the ML analysis, while confirming the sister relationship between subg. *Americanae* and subg. *Sanguineae*, left other inter-subgeneric relationships unresolved. Zarrei et al. (2014) used four plastid sequences (*trn*L-F, *trn*G-*trn*S, *rpl*2-*trn*H, and *rpl*20-*rps*12) with the MP method, producing yet another topology in which subg. *Mespilus* was basal, followed by subg. *Brevispinae*, subg. *Crataegus*, subg. *Sanguineae*, and subg. *Americanae* (Fig. 1C), and this topology was confirmed by the inclusion of more data (Zarrei et al. 2015). More recently, Liston et al. (2021) analyzed 24 plastomes (14 diploids and ten allotetraploids) and found strong support for subg. *Crataegus* as the basal clade, followed by a clade containing subg. *Mespilus*, subg. *Americanae*, and subg. *Sanguineae* (Fig. 1D). These discrepancies underscore the complexities involved in resolving *Crataegus* phylogeny, which may be attributed to factors such as sampling limitations, methodological differences, and the impact of hybridization and rapid early radiation (Ufimov and Dickinson 2020).

Based on the morphological similarity, Gu and Spongberg (2003) placed *Crataegus shandongensis* F.Z.Li & W.D.Peng in *C.* subg. *Crataegus*, and Li and Peng (1986) noted its close morphological relationship with *C. cuneata* Siebold & Zucc. in the protologue. However, the accurate phylogenetic placement of *C. shandongensis* has not been thoroughly estimated since its publication (Li and Peng 1986).

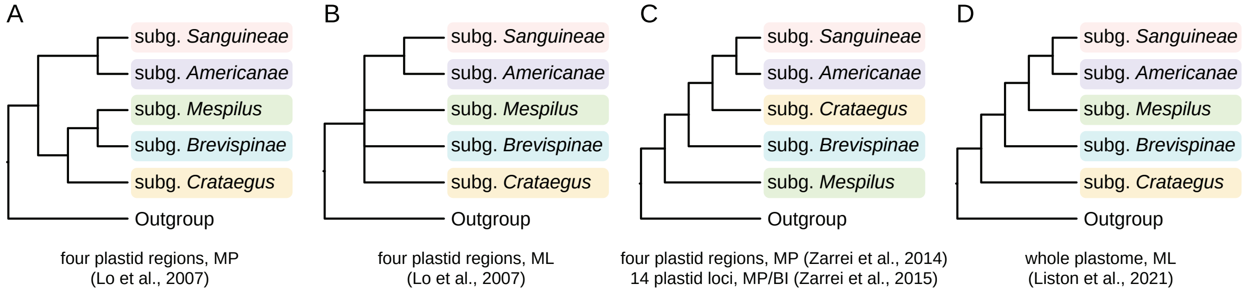


Figure 1. Phylogenetic hypotheses for the five subgenera of *Crataegus* adapted from prior studies: *C.* subg. *Americanae*, *C.* subg. *Brevispinae*, *C.* subg. *Crataegus*, *C.* subg. *Mespilus*, and *C.* subg. *Sanguineae*. **A, B** Maximum Parsimony (MP) and Maximum Likelihood (ML) trees inferred from four plastid regions (*trn*G-*trn*S, *psb*A-*trn*H, *trn*H-*rpl*2, and *rpl*20-*rps*12), as presented by Lo et al. (2007). **C** MP tree estimated from four plastid regions (*trn*L-F, *trn*G-*trn*S, *rpl*2-*trn*H, and *rpl*20-*rps*12; Zarrei et al. 2014). Additionally, MP tree and Bayesian Inference (BI) tree inferred from 14 plastid loci (*trn*G-*trn*S, *rpl*2-*trn*H, *rpl*20-*rps*12, *trn*L-*trn*F, *atp*B-*rbc*L, *rps*16 intron, *rpl*16 intron, *trn*C-*ycf*6, *acc*D, *rpo*C1, *atp*F-*atp*H, *mat*K, *rbcL*a, and *psb*A-*trn*H; Zarrei et al. 2015). **D** ML tree inferred from whole plastome sequences, as described by Liston et al. (2021).

Maternally inherited plastomes have been widely used for phylogenetic inference in angiosperms, particularly within the Rosaceae family. Nikiforova et al. (2013) pioneered the use of whole plastomes to address evolutionary questions in Rosaceae. Advances in sequencing technologies have significantly enhanced the application of plastome data. Zhang et al. (2017) assembled 132 plastomes from across the Rosaceae family, establishing a robust phylogenetic framework that has informed subsequent studies. The PhyloAI team further contributed by assembling plastome and nuclear ribosomal DNA (nrDNA) sequences from deep genome skimming (DGS) data (Liu et al. 2021). Their work has addressed numerous taxonomic and evolutionary questions within Rosaceae, notably in the tribe Maleae (Liu et al. 2019, 2020a, 2020b, 2022, 2023a, 2023b; Jin et al. 2023, 2024a, 2024b; Wang et al. 2024) and *Prunus*-related lineages (Hodel et al. 2021, 2022; Su et al. 2021). This successful application of plastome-based phylogenetic studies enhances our confidence in exploring the maternally subgeneric relationships and phylogenetic placement of *Crataegus shandongensis*.

A comprehensive taxonomic synopsis is crucial for understanding species diversity, supporting conservation efforts, and addressing ecological challenges. It provides a solid foundation for scientific communication and informed decision-making. A refined synopsis of a species should include accurate typification, an updated description and diagnosis, an evaluated conservation status, continuous field observations, and specimen examinations across major herbaria worldwide. In this study, we aim to provide an updated taxonomic synopsis of *Crataegus shandongensis*. In its protologue, Li and Peng (1986) designated the gathering *W.D. Peng 84001*, collected on 15 May 1984, as the type for *C. shandongensis*, specifying that the type was deposited in the herbarium of the Forestry School of Shandong Province, with an “isotype” sent to the China National Herbarium (PE). However, specimens from the Forestry School of Shandong Province herbarium have since been transferred to the herbarium of Shandong Agricultural University (SDAU). A comprehensive examination of the specimens at SDAU by one of our authors, Wei-Dong Peng, found multiple duplicates of *W.D. Peng 84001*, including an inflorescence branch collected on 15 May 1984 and an infructescence branch collected on 2 July 1984. In accordance with Article 40.2 of the International Code of Nomenclature for Algae, Fungi, and Plants (ICN, Turland et al. 2018), when the type is indicated by reference to an entire gathering, or a part thereof, that consists of more than one specimen, those specimens are syntypes. Accordingly, we conclude that the four duplicates of the gathering *W.D. Peng 84001*, collected on 15 May 1984, qualify as syntypes, thereby necessitating the lectotypification of this name.

In this study, we aim to 1) clarify the maternally inherited phylogenetic relationships among *Crataegus* subgenera using multiple inference methods; 2) resolve the phylogenetic placement of *Crataegus shandongensis* through plastome-based phylogenomic analyses in the framework of the hawthorns and 3) provide an updated taxonomic synopsis, including revised typification and morphological description, to facilitate accurate identification and conservation efforts.

Materials and methods

Taxon sampling

To clarify the maternal relationship among the five subgenera of *Crataegus* and determine the phylogenetic position of *C. shandongensis*, we conducted a comprehensive taxon sampling strategy across the genus. This included all currently recognized subgenera: *C.* subg. *Crataegus*, *C.* subg. *Americanae*, *C.* subg. *Brevispinae*, *C.* subg. *Mespilus*, and *C.* subg. *Sanguineae* (Ufimov and Dickinson 2020). Specifically, we sampled 16 individuals representing nine species from *C.* subg. *Crataegus*. We also sampled 11 individuals from *C.* subg. *Americanae*, two from *C.* subg. *Brevispinae*, two from *C.* subg. *Mespilus*, and 27 from *C.* subg. *Sanguineae*. In total, our sampling encompassed 58 individuals from 34 species across all subgenera. To provide a broader phylogenetic context, we included five representative species as the outgroup: *Amelanchier cusickii* Fernald, *Hesperomeles goudotiana* Killip, *Peraphyllum ramosissimum* Nutt., *Malacomeles denticulata* (Kunth) Decne., and *Malus domestica* (Suckow) Borkh., resulting in a total of 63 samples (Liu et al. 2020b; Liston et al. 2021). Such extensive sampling significantly enhances the robustness and reliability of our phylogenetic analyses, providing a strong foundation for understanding the evolutionary relationships within *Crataegus*.

For novel data generation, we performed DGS on two newly collected samples, *Crataegus brachyacantha* Sarg. & Engelm. and *C. shandongensis*. Additionally, we incorporated 36 whole plastome datasets from GenBank at the National Center for Biotechnology Information (NCBI) and 25 plastome datasets from the study of Liston et al. (2021), detailed in Table 1. This integrated dataset provided a robust framework for analyzing maternal genetic relationships within *Crataegus* and for evaluating the phylogenetic placement of *C. shandongensis*.

DNA extraction, library preparation, and DGS sequencing

We extracted whole genomic DNA from herbarium specimens of *Crataegus brachyacantha* and *C. shandongensis* using a modified CTAB (mCTAB) protocol, based on the methods described by Doyle and Doyle (1987) and adapted by Li et al. (2013). This extraction was performed at the Plant DNA and Molecular Identification Platform (PDMIP) at the Institute of Botany, Chinese Academy of Sciences (IBCAS). Skilled technicians ensured the successful extraction of high-quality DNA from the often degraded material of herbarium specimens.

Table 1. The *Crataegus* accessions used in this study are detailed below. Bold rows denote samples sequenced by our PhyloAI team. Accession numbers marked with an asterisk (*) indicate plastomes sequenced specifically for this study. Notably, all 25 plastomes assembled by Liston et al. (2021) had not been submitted to GenBank, and their corresponding accession numbers were absent in this table.

Subgenera	Species	Accession number	Location	Voucher	Publication
subg. *Americanae*	*C. calpodendron* (Ehrh.) Medik.	–	Massachusetts, USA	*T.A. Dickinson 2002-07A*	Liston et al. 2021
subg. *Americanae*	*C. chrysocarpa* Ashe 1	–	Idaho, USA	*E.Y.Y. Lo EL-122*	Liston et al. 2021
subg. *Americanae*	*C. chrysocarpa* 2	–	Washington, USA	*J. Coughlan JC174*	Liston et al. 2021
subg. *Americanae*	*C. crus-galli* L.	–	Georgia, USA	*N. Talent NT489*	Liston et al. 2021
subg. *Americanae*	*C. macracantha* Koehne 1	–	Washington, USA	*J. Coughlan JC168*	Liston et al. 2021
subg. *Americanae*	*C. macracantha* 2	–	Colorado, USA	*N. Talent NT347*	Liston et al. 2021
subg. _Americanae_	**_C. marshallii_ Eggl. 1**	**MK920294**	**Minnesota, USA**	**_J. Wen 14051_ (US)**	**Liu et al. 2019**
subg. *Americanae*	*C. marshallii* 2	MK920293	USA	*J.B. Nelson 26961* (US)	Liu et al. 2019
subg. *Americanae*	*C. opaca* Hook. & Arn.	–	Louisiana, USA	*T.A. Dickinson 2003-33*	Liston et al. 2021
subg. *Americanae*	*C. punctata* Jacq.	–	Ontario, Canada	*M.A. Purich 81*	Liston et al. 2021
subg. *Americanae*	*C. triflora* Chapm.	–	Alabama, USA	*T.A. Dickinson 2003-23*	Liston et al. 2021
subg. *Brevispinae*	*C. brachyacantha* Sarg. & Engelm. 1	–	Louisiana, USA	*C. Reid 5203*	Liston et al. 2021
subg. _Brevispinae_	**_C. brachyacantha_ 2**	**PQ283266***	**Texas, USA**	**_J.W. Hardin & R.M. Brown 521_ (US03513565)**	**This study**
subg. _Crataegus_	**_C. cuneata_ Siebold & Zucc. 1**	**OR915925**	**Hubei, China**	**_C.M. Zhao et al. EX2548_ (PE01857312)**	**Wang et al. 2024**
subg. *Crataegus*	*C. cuneata* 2	MZ504723	–	–	Unpublished
subg. *Crataegus*	*C. hupehensis* Sarg. 1	MW201730	Beijing, China	BJLGY-2020-SZ001	Hu et al. 2021a
subg. _Crataegus_	**_C. hupehensis_ 2**	**OR915900**	**Hubei, China**	**_B.B. Liu 2322_ (PE02070251)**	**Wang et al. 2024**
subg. *Crataegus*	*C. laevigata* (Poir.) DC.	OM232780	United Kingdom	–	Liu et al. 2022
subg. *Crataegus*	*C. monogyna* Jacq. 1	ON641281	Portugal	LISE:96340	Unpublished
subg. *Crataegus*	*C. monogyna* 2	–	Ontario, Canada	*T.A. Dickinson 2003-79*	Liston et al. 2021
subg. *Crataegus*	*C. pinnatifida* var. *major* N.E.Br. 1	KY419945	–	Zhang sd148	Zhang et al. 2017
subg. *Crataegus*	*C. pinnatifida* var. *major* 2	MZ494513	Liaoning, China	JD1H	Hu et al. 2021b
subg. *Crataegus*	*C. rhipidophylla* Gand.	OM232778	Turkey	–	Liu et al. 2022
subg. *Crataegus*	*C. scabrifolia* (Franch.) Rehder 1	OP021659	China	SWFU20210783MFY	None
subg. _Crataegus_	**_C. scabrifolia_ 2**	**OR915927**	**Yunnan, China**	**_G.P. Yang 333_ (PE01438424)**	**Wang et al. 2024**
subg. _Crataegus_	**_C. scabrifolia_ 3**	**OR915928**	**Yunnan, China**	**_Y.L. Shui et al. 64833_ (PE01438422)**	**Wang et al. 2024**
subg. _Crataegus_	**_C. shandongensis_ F.Z.Li & W.D.Peng**	**PQ283267***	**Shandong, China**	**_s.coll. s.n._ (PE)**	**This study**
subg. _Crataegus_	**_C. songarica_ K.Koch 1**	**OR915930**	**Xinjiang, China**	**_Z.M. Zhang 222_ (PE01153664)**	**Wang et al. 2024**
subg. _Crataegus_	**_C. songarica_ 2**	**OR915929**	**Xinjiang, China**	**_Y.R. Lin 74898_ (PE01153668)**	**Wang et al. 2024**
subg. *Mespilus*	*C. germanica* (L.) Kuntze 1	MK920295	–	*M.D. Tidestrom 14120* (US)	Liu et al. 2019
subg. *Mespilus*	*C. germanica* 2	–	California, USA	*T.A. Dickinson s.n.*	Liston et al. 2021
subg. _Sanguineae_	**_C. altaica_ (Loudon) Lange 1**	**OR915923**	**Xinjiang, China**	**_Z.M. Zhang 263_ (PE01153350)**	**Wang et al. 2024**
subg. _Sanguineae_	**_C. altaica_ 2**	**OR915924**	**Xinjiang, China**	**_D.Y. Hong et al. 0136_ (PE01153341)**	**Wang et al. 2024**
subg. _Sanguineae_	**_C. aurantia_ Pojark.**	**OR897856**	**Gansu, China**	**_X.G. Sun et al. 2708_ (PE01841632)**	**Wang et al. 2024**
subg. *Sanguineae*	*C. bretschneideri* C.K.Schneid.	MW963339	Beijing, China	BJLGY-2020SZ002	Zheng et al. 2021
subg. *Sanguineae*	*C. chungtienensis* W.W.Sm. 1	ON032469	Yunnan, China	YUNCM2021051701	Wu et al. 2022
subg. *Sanguineae*	*C. chungtienensis* 2	KY419947	–	Zhang sd147	Zhang et al. 2017
subg. *Sanguineae*	*C. douglasii* Lindl. 1	–	Ontario, Canada	*E. Lo EL-11*	Liston et al. 2021
subg. *Sanguineae*	*C. douglasii* 2	–	Idaho, USA	*E. Lo EL-170*	Liston et al. 2021
subg. *Sanguineae*	*C. douglasii* 3	–	Oregon, USA	*J. Coughlan JC224*	Liston et al. 2021
subg. _Sanguineae_	**_C. kansuensis_ E.H.Wilson 1**	**OR915910**	**Shanxi, China**	**_D.M. Kong k0229_ (PE02070251)**	**Wang et al. 2024**
subg. *Sanguineae*	*C. kansuensis* 2	MF784433	–	PGP00008	Zhang et al. 2020
subg. *Sanguineae*	*C. maximowiczii* C.K.Schneid. 1	MZ494512	Liaoning, China	MSZ1H	Hu et al. 2021b

Subgenera	Species	Accession number	Location	Voucher	Publication
subg. *Sanguineae*	***C. maximowiczii* 2**	**OR915918**	**Jilin, China**	***B.B. Liu et al. 4499* (PE)**	**Wang et al. 2024**
subg. *Sanguineae*	*C. nigra* Waldst. & Kit.	–	Québec, Canada	*T.A. Dickinson 2318-50*	Liston et al. 2021
subg. *Sanguineae*	*C. oresbia* W.W.Sm.	ON032470	Yunnan, China	YUNCM2021051702	Wu et al. 2022
subg. *Sanguineae*	*C. rivularis* Nutt. 1	–	Idaho, USA	*E. Lo EL-199*	Liston et al. 2021
subg. *Sanguineae*	*C. rivularis* 2	–	Nevada, USA	*T.A. Dickinson 2007-02*	Liston et al. 2021
subg. *Sanguineae*	*C. rivularis* 3	–	New Mexico, USA	*N. Talent NT357*	Liston et al. 2021
subg. *Sanguineae*	*C. saligna* Greene 1	–	Utah, USA	*T.A. Dickinson 2004-05*	Liston et al. 2021
subg. *Sanguineae*	*C. saligna* 2	–	Colorado, USA	*T.A. Dickinson 2001-07*	Liston et al. 2021
subg. *Sanguineae*	***C. sanguinea* Schrad.**	**OR915926**	**Inner Mongolia, China**	***Chifeng Collection Team 2-Z29* (PE02044283)**	**Wang et al. 2024**
subg. *Sanguineae*	*C.* sp.	OM232779	Canada	–	None
subg. *Sanguineae*	*C. suksdorfii* (Sarg.) Kruschke 1	–	California, USA	*J. Coughlan JC033*	Liston et al. 2021
subg. *Sanguineae*	*C. suksdorfii* 2	–	Washington, USA	*P.F. Zika 18485*	Liston et al. 2021
subg. *Sanguineae*	***C. wilsonii* Sarg. 1**	**OR915931**	**Sichuan, China**	***Bashan Collection Team 4974* (PE01872493)**	**Wang et al. 2024**
subg. *Sanguineae*	*C. wilsonii* 2	–	Massachusetts, USA	*T.A. Dickinson s.n.*	Liston et al. 2021
subg. *Sanguineae*	***C. wilsonii* 3**	**OR915932**	**Sichuan, China**	***Bashan Collection Team 5683* (PE01872495)**	**Wang et al. 2024**
outgroup	***Amelanchier cusickii* Fernald**	**MN068257**	**Washington, USA**	***J.W. Thompson 14588* (US1889072)**	**Wang et al. 2024**
outgroup	*Hesperomeles goudotiana* Killip	MN068271	Colombia	*J. Cuatrecasas 5035*	Unpublished
outgroup	***Peraphyllum ramosissimum* Nutt.**	**KY420011**	**Nevada, USA**	***B. Maguire & A.H. Holmbren 25288* (US03513221)**	**Wang et al. 2024**
outgroup	***Malacomeles denticulata* (Kunth) Decne.**	**MN068267**	**Mexico**	***A. Ventura 3359* (US03513083)**	**Wang et al. 2024**
outgroup	*Malus domestica* (Suckow) Borkh.	–	–	–	Liston et al. 2021

DNA libraries were then prepared using the NEBNext® Ultra™ II DNA Library Prep Kit, involving careful quality control to ensure the libraries were suitable for high-throughput sequencing. This preparation included fragmentation, end-repair, and adapter ligation of the DNA to create libraries compatible with the sequencing platform. The DGS sequencing was conducted on the BGISEQ-500 platform at Frasergen in Wuhan, China. Each sample generated approximately 20 gigabases (Gb) of raw data with 150 base pair (bp) paired-end reads, providing a comprehensive whole genomic DNA dataset for in-depth genomic analysis.

The raw sequencing data have been archived in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1155316. The corresponding voucher specimens are preserved in the PE and the United States National Herbarium (US), ensuring that both the genomic data and physical specimens are accessible for future research and validation.

Raw data cleaning and quality control

The raw sequencing data were initially processed to enhance quality by trimming low-quality reads and removing adapter sequences using Trimmomatic v. 0.39 (Bolger et al. 2014). This tool applied various filtering steps, including removing sequences below a specified quality threshold (LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, MINLEN:36) and eliminating adapter contaminants that could interfere with subsequent analyses. This trimming process retained only high-quality, reliable reads, forming a robust dataset for further genomic work.

Following trimming, we assessed the quality of the clean reads using FastQC v. 0.12.1 (Andrews 2010, available at https://www.bioinformatics.babraham.

ac.uk/projects/fastqc/). FastQC provided detailed reports on various quality metrics, including read length distribution, GC content, sequence duplication levels, and potential overrepresented sequences. This quality assessment was crucial for validating the effectiveness of the trimming process and ensuring that the data met the necessary standards for assembly.

We proceeded with chloroplast genome assembly only after confirming the integrity and quality of the clean reads. The careful preprocessing and quality control ensured that our assembly was based on the most accurate and reliable sequence data, thereby enhancing the robustness and reliability of the subsequent genomic analyses.

Plastome assembly and annotation

Given the variability in sequencing coverage across samples, we employed the Successive Approach combining Reference-based and *de novo* assembly (SARD approach, Liu et al. 2023b), a method that performs well even for the low-coverage data. This assembly approach has been successfully applied to a range of angiosperm families, including Amaryllidaceae (Lou et al. 2022a), Magnoliaceae (Liu et al. 2020e; Wang et al. 2020), Rosaceae (Liu et al. 2019, 2020b, 2022, 2023a, 2023b; Jin et al. 2023, 2024b), and Vitaceae (Liu et al. 2021).

We utilized two distinct programs for automatic plastome assembly, each employing different algorithms: mapping-and-*de novo* assembly with GetOrganelle v. 1.7.7.0 (Jin et al. 2020) and seed-and-extend assembly with NOVOPlasty v. 3.6 (Dierckxsens et al. 2017). Initially, GetOrganelle v. 1.7.7.0 was used with default parameters to assemble plastomes, successfully generating a high-quality circular plastome for *Crataegus shandongensis*. For the non-circular *C. brachyacantha* sample, NOVOPlasty was applied with parameters set to “Genome Range = 120000-200000”, “K-mer = 31”, “Read Length = 150”, and “Insert size = 300” using the *rbc*L sequence of *C. brachyacantha* (accession number: KC251218) as the seed and the plastome of *C. pinnatifida* Bunge (accession number: MN102356) as the reference. This method did not produce a circular plastome either. Consequently, we employed the SARD approach for assembly. We aligned plastome-related reads from the raw reads of *C. brachyacantha* to the reference plastome of *C. maximowiczii* C.K.Schneid. (accession number: NC065485) using Geneious Prime v. 2023.2 (Kearse et al. 2012) to generate a consensus sequence. Concurrently, we performed *de novo* assembly with SPAdes v. 3.13.1, applying error correction and K-mer lengths of 21, 33, 55, and 77 (Bankevich et al. 2012). Finally, we mapped all contigs assembled from SPAdes, GetOrganelle, and NOVOPlasty to the consensus sequence, resulting in a high-quality, complete circular plastome.

The plastomes were then annotated using GeSeq (Tillich et al. 2017). All assembled plastomes from this study have been submitted to GenBank, with accession numbers provided in Table 1.

Data matrix generation and phylogenetic inference

In this study, we utilized the whole plastome dataset for phylogenetic inference. We applied concatenation-based method for estimation. We retained only one of the inverted repeats (IRs) from each plastome of *Crataegus* and outgroups, including all 63 plastomes. The alignment of these plastomes was performed with MAFFT v. 7.520 (Nakamura et al. 2018) using the "--auto" parameter and then refined with trimAl v. 1.4.1 (Capella-Gutiérrez et al. 2009) to exclude poorly aligned regions, applying parameters "-gt 0.8 -st 0.001".

For concatenation-based phylogenetic inference, we utilized various programs and algorithms for accurate estimation, including ML and Bayesian Inference (BI). Optimal evolutionary models were determined using PartitionFinder2 (Lanfear et al. 2014, 2017; Stamatakis 2014) with parameters for "linked branch lengths", "Akaike Information Criterion model (AICc)", and "greedy search" approach (Lanfear et al. 2012). We inferred ML trees using IQ-TREE2 v. 2.2.2.7 (Minh et al. 2020), with 1,000 ultrafast bootstrap support assessments (Minh et al. 2013) and the SH-aLRT test (Anisimova and Gascuel 2006) for evaluating tree topology reliability. Additionally, we used RAxML v. 8.2.13 (Stamatakis 2014) for ML tree inference, with parameters "-f a -p 12345 -x 12345" and 200 rapid bootstrap replicates. For BI analysis, MrBayes 3.2.7a (Ronquist et al. 2012) was employed, with Markov Chain Monte Carlo (MCMC) analysis set to run for 10 million generations, sampling trees every 1,000 generations and discarding the initial 25% of samples.

Morphological analysis

To update the taxonomic synopsis of *Crataegus shandongensis*, we examined specimens housed at several related herbaria, including Lushan Botanical Garden (LBG), PE, Qufu Normal University (QFNU), and SDAU. Notably, we focused on duplicates of the type collection (*W.D. Peng 84001*) at the herbarium SDAU. Key diagnostic traits, including leaf morphology, inflorescence structure, and fruit characteristics, were documented and measured using a stereo microscope.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.z612jm6nc.

Results and discussion

DGS sequencing and data generation

Our PhyloAI team generated 17 DGS raw datasets for the genus *Crataegus*, including newly sequenced data for *C. brachyacantha* and *C. shandongensis* (Table 1). The DGS sequencing produced 19.89 Gb of data for *C. brachyacantha* and 16.46 Gb for *C. shandongensis*, with coverage depths of 24.16× and 19.99×, respectively, based on the estimated genome size of 823.41 Mb for *C. pinnatifida* var. *major* N.E.Br. (Zhang et al. 2022). This high coverage ensures reliable plastome assembly and accurate data for phylogenetic analyses.

We assembled complete circular plastomes for two species (Table 1), marking the first plastome assemblies for *Crataegus brachyacantha* in the monotypic subgenus *C.* subg. *Brevispinae* and for the China-endemic *C. shandongensis* in *C.* subg. *Crataegus*. Each plastome in this study featured two IRs that were complementary, separated by a small single copy (SSC) and a large single copy (LSC) region (Fig. 2). Overall, the plastomes encoded 133 genes:

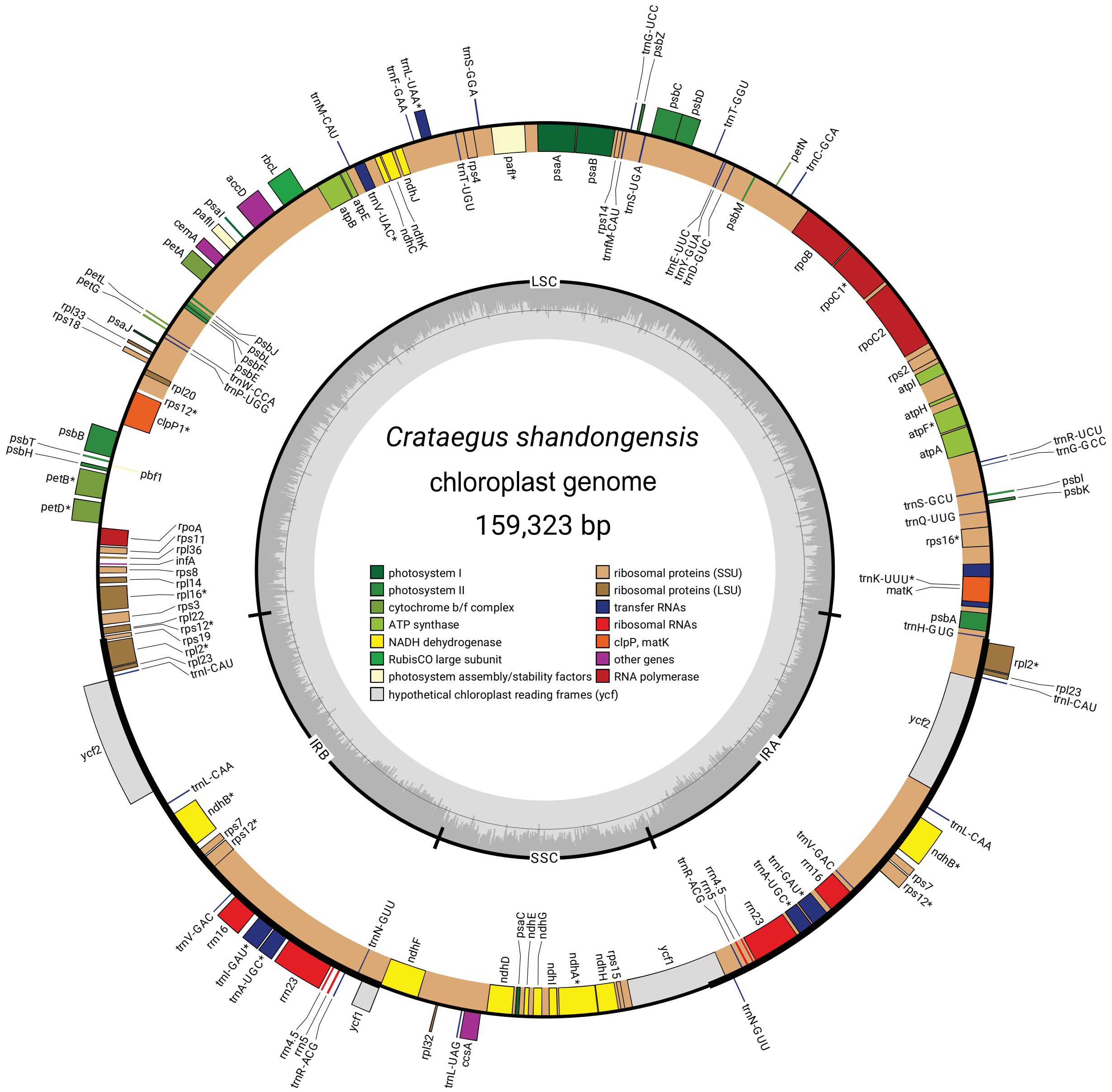


Figure 2. Gene map of the *Crataegus shandongensis* chloroplast genome, with genes inside the circle transcribed clockwise and those outside transcribed counterclockwise. The dark gray inner circle indicates GC content, while the light gray outer circle denotes AT content. Different gene types are represented in various colors. LSC, large single copy; SSC, small single copy; IRA, inverted repeat region A; IRB, inverted repeat region B.

88 protein-coding genes, 37 tRNA genes, and eight rRNA genes (Fig. 2). No significant rearrangements or gene losses were found in all the representative species of all five subgenera. These plastomes provide crucial insights into species relationships within *Crataegus* and are essential for accurately resolving the phylogenetic relationships among the five currently recognized subgenera. To enhance the precision of our phylogenetic analyses, we performed extensive data cleaning, resulting in whole plastome dataset, with alignment lengths of 130,986 bp. These high-quality plastome datasets are invaluable resources for future research in the phylogenetics, taxonomy, and evolutionary biology of *Crataegus*, underscoring the utility of DGS in studying non-model organisms and advancing our understanding of their biodiversity.

Well-resolved maternal phylogenetic relationship among five subgenera in *Crataegus*

In this study, we collected whole plastome data covering all five currently recognized subgenera of *Crataegus* to accurately estimate their maternal relationships. We constructed three trees based on the whole plastome dataset using two phylogenetic inference methods (ML and BI, Fig. 3, Suppl. materials 1–3). All whole plastome-based trees revealed consistent topologies, dividing the five *Crataegus* subgenera into two well-supported clades, designated as Clade I and Clade II (Fig. 3, Suppl. materials 1–3). Clade I comprises three subgenera, and *C.* subg. *Mespilus* is sister to a combined clade including *C.* subg. *Americanae* and *C.* subg. *Sanguineae* (bootstrap support (BS) = 96, SH-aLRT support/ultrafast bootstrap support (SH-aLRT/UFBoot) = 94.6/99, posterior probabilities (PP) = 1; Suppl. materials 1–3). *Crataegus* subg. *Americanae* and *C.* subg. *Sanguineae* were consistently resolved as sister groups in all three trees with robust support (BS = 100, SH-aLRT/UFBoot = 100/100, PP = 1; Fig. 3, Suppl. materials 1–3). Clade II includes two subgenera, *C.* subg. *Crataegus* and *C.* subg. *Brevispinae* (BS = 93, SH-aLRT/UFBoot = 94.1/99, PP = 1; Fig. 3; Suppl. materials 1–3).

In *Crataegus* subg. *Sanguineae*, the plastome with GenBank accession number OM232779, initially identified as *C. mollis* (Torr. & A.Gray) Scheele, was assembled from whole genomic data (SRA accession no. SRR3130998) provided by the Harvard University Arnold Arboretum. Unexpectedly, this sample clusters with *C.* subg. *Sanguineae* rather than with *C.* subg. *Americanae*, which contradicts previous morphological evidence, such as differences in mature fruit color (Phipps 2014). This discrepancy raises the possibility of misidentification or DNA contamination, possibly due to issues during field collection or sample labeling. The GenBank record only lists "USA" as the locality and lacks voucher specimen information, which limits our ability to verify its identity. We recommend that future submissions to GenBank include detailed locality and voucher information to improve verification and reproducibility. Liu et al. (2022) utilized this plastome as an outgroup in an apple study but did not detect its anomalous placement due to limited taxon sampling. The broader sampling in our current study, which included increased taxonomic representation and better resolution, has brought this inconsistency to light. To confirm the identity and phylogenetic placement of OM232779, further investigation is required. Until such data are available, we recommend interpreting the phylogenetic position of this sample with caution, particularly for any downstream analyses or conclusions that may rely on this placement.

Phylogenetic position and taxonomic synopsis of *Crataegus shandongensis*

All three phylogenetic trees consistently supported a close maternal relationship among *Crataegus shandongensis*, *C. pinnatifida* var. *major*, and *C. scabrifolia* (Franch.) Rehder (Fig. 3; Suppl. materials 1–3). Notably, *C. scabrifolia* appeared in two separate clades (Fig. 3; Suppl. materials 1–3), suggesting multiple maternal sources for this species. *Crataegus shandongensis* is endemic to Shandong, *C. scabrifolia* is found in South and Southwest China (including Chongqing,

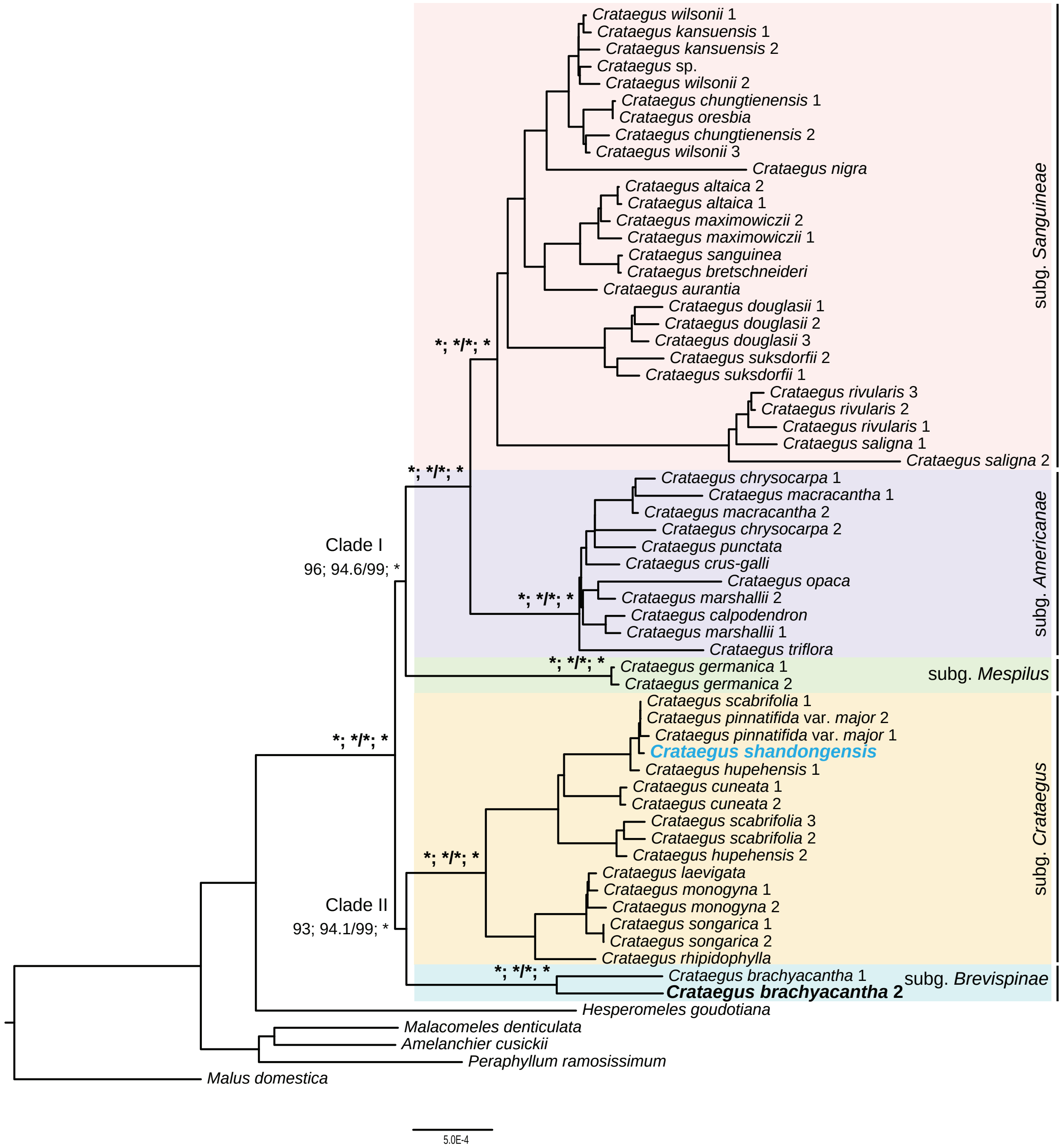


Figure 3. Phylogenetic tree of *Crataegus* estimated using the Maximum Likelihood (ML) algorithm with RAxML based on the whole plastome dataset. Support values for key nodes are shown next to the branches: Bootstrap (BS) from RAxML, SH-aLRT support and ultrafast bootstrap from IQ-TREE2, and posterior probability (PP) from Bayesian analysis based on whole plastome dataset. Asterisks (*) indicate full support (100; 100/100; 1). Detailed support values refer to Suppl. materials 1–3.

Guangxi, Guizhou, Sichuan, and Yunnan), and *C. pinnatifida* var. *major* is distributed across South, Southeast, East, and North China, as well as the Korean Peninsula and the Far East of Russia. Given their distributions, it is plausible to infer that *C. shandongensis* is more closely related to *C. pinnatifida* var. *major* maternally.

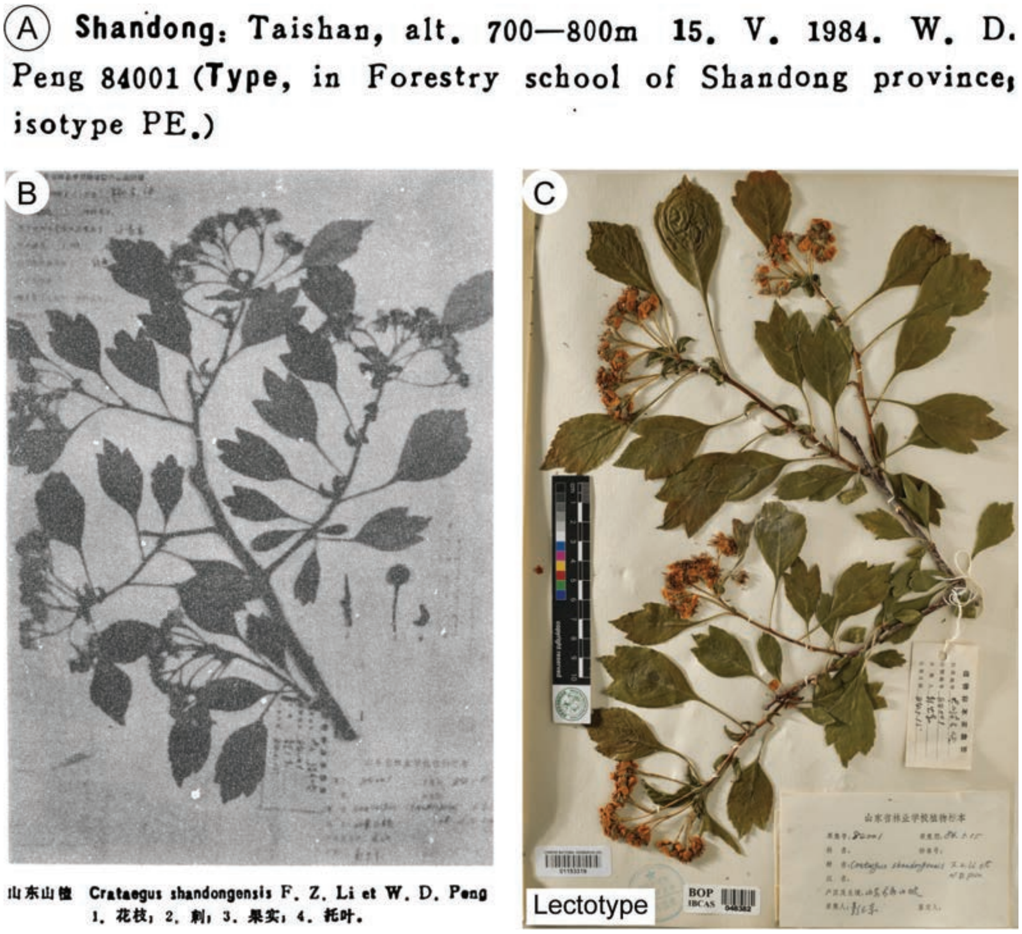
Li and Peng (1986) labeled two gatherings, the flowering branch collected on 15 May 1984 and the fruiting branch collected on 2 July 1984, to be *W.D. Peng 84001*. This practice of collecting both flowering and fruiting specimens from the same individual was common in the 20th century, especially in collections by E.H. Wilson in China (Liu et al. 2020c, 2020d; Lou et al. 2022b; Wang et al. 2024). Early plant collectors often deemed it essential to obtain specimens from the same individual in different stages of development. Many collectors attached permanent tags to the plants to track these collections and sometimes retained the original collector's number for subsequent collections, noting the different collection dates and/or collectors. This practice has significantly impacted the accurate typification of related species. For instance, in the case of *Photinia schneideriana* Rehder & E.H.Wilson, Wilson combined branches collected in May and October 1907 onto one sheet and designated it as the type for *P. schneideriana*. This action contradicted Article 8.2 of ICN (Turland et al. 2018), a case that has been thoroughly discussed (Liu and Hong 2017; Lou et al. 2022b). Fortunately, Li and Peng (1986) clearly indicated the type information (Fig. 4A), specifying the gathering collected on 15 May 1984 as type, with type deposited in the Forestry School of Shandong Province herbarium and isotype sent to PE. It should be noted that the sense of isotype in the protologue of Li and Peng (1986) was syntype (Art. 40.2 in Turland et al. 2018). Thus, the presence of specimens with the same collector number but different collection dates does not affect the valid publication of *C. shandongensis*.

The authors provided an image of a specimen that includes both an inflorescence branch and fruit (Fig. 4B). However, we were unable to locate this particular duplicate mentioned by the original authors. We suspect that this specimen may have been lost or destroyed during the transfer from the herbarium of the Forestry School of Shandong Province to the SDAU herbarium. Fortunately, we identified three additional duplicates in the herbarium SDAU and one in PE, and all these four duplicates are syntypes (Figs 4C, 5A–C). According to Article 9.12 of ICN (Turland et al. 2018), syntypes and isosyntypes share equal priority for lectotypification. Consequently, we selected a well-preserved duplicate in PE (barcode 01153319) as the lectotype (Fig. 4C), as the three duplicates in SDAU have been severely damaged by insects, particularly impacting the petals of the flowers (Fig. 5A–C).

Crataegus shandongensis **F.Z.Li & W.D.Peng, Bull. Bot. Res., Harbin 6: 149. 1986.**
Chinese name: Shandong hawthorn; 山东山楂

Type. China. Shandong, Taian, Taishan, alt. 700–800 m, 15 May 1984, *W.D. Peng 84001* (***lectotype***, **designated here**: PE [barcode 01153319!, Fig. 4C]; isolectotypes: SDAU!, Fig. 5A–C).

Description. Shrubs 1–2 m tall, thorns 1–1.2 cm long or absent; branchlets initially pubescent and then glabrous, older branches gray-brown, sparsely lenticellate, glabrous. Stipules herbaceous, falcate, margin glandular-serrate. Leaves simple, deciduous; petioles 1.5–4 cm long, narrowly winged, glabrous; blades 4–8 cm long, 2–4 cm wide, obovate or long-elliptic; base cuneate, apex acuminate; usually 3-lobed, rarely 5-lobed or unlobed; margins irregularly double-serrate above the middle part; venation craspedodromous; glabrous adaxially, except for the loosely hairy midrib, pinnate veins slightly impressed above, prominent below, veins entering the tip of the serrations. Inflorescences corymb,



Shandong: Taishan, alt. 700—800m 15. V. 1984. W. D. Peng 84001 (Type, in Forestry school of Shandong province; isotype PE.)

山东山楂 **Crataegus shandongensis** F. Z. Li et W. D. Peng
1. 花枝; 2. 刺; 3. 果实; 4. 托叶.

Figure 4. Type specimens of *Crataegus shandongensis*. **A** typification information of *Crataegus shandongensis* in the protologue (Li and Peng 1986) **B** duplicate of *W.D. Peng 84001* shown in the protologue (Li and Peng 1986) **C** lectotype. Collection number: *W.D. Peng 84001*; Collection date: 15 May 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong, China.

7–18 flowered, 4 cm long, 8 cm wide; pedicels and peduncles white-pubescent; bracts linear-lanceolate 2–3 mm long, margin glandular-serrate, caducous; hypanthium externally white-pubescent; small flower 2 cm in diameter; petals suborbicular, white, about 5 mm long, very shortly clawed; sepals triangular, apex caudate-acuminate, 4–6 mm long, nearly as long as the calyx tube, white-pubescent adaxially; stamens 20; styles 5, base white-villous. Fruits globose, 10–15 mm in diameter, red, glabrous; sepals persistent, reflexed; nutlets 5, flat on both sides, grooved on the back.

Distribution. China (Shandong: Mount Tai).

Conservation status. *Crataegus shandongensis* is currently known from a single extant population situated along the slope of Mount Tai. Despite thorough field observations over the past 38 years by one of the authors (Wei-Dong Peng), no additional populations have been found. Although Mount Tai has been under continuous protection for over 5,000 years due to its profound cultural significance, which has helped to preserve the natural habitat to some extent, the species remains restricted to this limited area. According to the IUCN Red List Criteria (IUCN 2022), specifically criteria B1ab(iii)+2ab(iii), *C. Shandongensis* meets the thresholds for Critically Endangered (CR) status. These criteria pertain to the restricted geographic range (criterion B1) and the limited area

Figure 5. Type specimens of *Crataegus shandongensis*. **A** isolectotype. Collection number: *W.D. Peng 84001*; Specimen accession number: 2365; Collection date: 15 May 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong **B** isolectotype. Collection number: *W.D. Peng 84001*; Specimen accession number: 2364; Collection date: 15 May 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong **C** isolectotype. Collection number: *W.D. Peng 84001*; Specimen accession number: 2368; Collection date: 15 May 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong **D** paratype (PE [barcode 01153320]). Collection number: *W.D. Peng 84001*; Collection date: 2 July 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong **E** paratype (LBG [barcode 00021556]). Collection number: *W.D. Peng 84001*; Collection date: 2 July 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong. **F** Paratype (SDAU!). Collection number: *W.D. Peng 84001*; Collection date: 2 July 1984; Collector: W.D. Peng; Collection location: Taishan, Shandong.

of occupancy (criterion B2), coupled with an ongoing decline in habitat quality (subcriterion ab(iii)). The restricted distribution and the absence of additional populations justify the classification of *C. shandongensis* as CR.

Additional specimens examined (paratypes). China. Shandong, Mount Tai (Taishan), altitude 700–800 m, 2 July 1984, *W.D. Peng 84001* (PE [barcode 01153320!, Fig. 5D], LBG [barcode 00021556!, Fig. 5E], SDAU! [Fig. 5F]).

Conclusions

In summary, our plastome-based phylogenomic analyses of all five recognized subgenera have provided a detailed clarification of their phylogenetic relationships. We successfully resolved the maternal lineage of *Crataegus shandongensis*, confirming its distinct position within the genus and deepening our understanding of its phylogenetic context. Furthermore, all Shandong hawthorn-related specimen examinations throughout the Chinese herbaria enabled us to develop a comprehensive taxonomic synopsis for *C. shandongensis*, including its typification and an updated species description.

While the maternally inherited plastomes are valuable for elucidating maternal lineages, they have limitations in addressing complex evolutionary processes such as hybridization and polyploidization (Guo et al. 2022). As we transition into the phylogenomic era—marked by the utilization of hundreds or thousands of single-copy nuclear genes (SCNs)—substantial progress is anticipated in plant systematics (Liu et al. 2021). Research integrating both maternally inherited plastid DNA and biparentally inherited SCNs has already highlighted the importance of detecting conflicts between these compartments, offering new insights into hybridization and polyploidy (Lo et al. 2007; Liston et al. 2021; Jin et al. 2023). Additionally, Doyle (2022) provided a critical review for accurately inferring species trees using coalescent methods, emphasizing the integration of diverse genomic resources, including SCN genes, plastid protein-coding genes, and mitochondrial genes. This study provides a crucial foundation for the phylogenomic era, offering a robust framework to facilitate and advance future research endeavors.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

B.B.L conceptualized and led the project. The data assembly and phylogenomic analysis were conducted by X.H.L and S.Y.X. C.X was responsible for the experimental work. The initial draft of the manuscript was prepared by B.B.L, S.Y.X, X.H.L, G.N.L, D.K.M, and L.Z. F.R.Z and W.D.P checked the specimens in the herbarium SDAU. B.L provided valuable insights and feedback on nomenclatural issues. All authors approved the final manuscript.

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Data availability

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

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Supplementary material 1

Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Maximum Likelihood analysis using RAxML

Authors: Xiao-Hua Lin, Si-Yu Xie, Guang-Ning Liu, Dai-Kun Ma, Fei-Ran Zou, Wei-Dong Peng, Chao Xu, Bing Liu, Liang Zhao, Bin-Bin Liu

Data type: pdf

Explanation note: Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Maximum Likelihood analysis using RAxML. The numbers displayed above each branch represent the bootstrap support value.

Link: https://doi.org/10.3897/phytokeys.252.136506.suppl1

Supplementary material 2

Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Maximum Likelihood analysis using IQ-TREE2

Authors: Xiao-Hua Lin, Si-Yu Xie, Guang-Ning Liu, Dai-Kun Ma, Fei-Ran Zou, Wei-Dong Peng, Chao Xu, Bing Liu, Liang Zhao, Bin-Bin Liu

Data type: pdf

Explanation note: Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Maximum Likelihood analysis using IQ-TREE2. The numbers displayed above each branch represent the SH-aLRT support and ultrafast bootstrap support values, respectively.

Link: https://doi.org/10.3897/phytokeys.252.136506.suppl2

Supplementary material 3

Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Bayesian Inference analysis using MrBayes

Authors: Xiao-Hua Lin, Si-Yu Xie, Guang-Ning Liu, Dai-Kun Ma, Fei-Ran Zou, Wei-Dong Peng, Chao Xu, Bing Liu, Liang Zhao, Bin-Bin Liu

Data type: pdf

Explanation note: Whole plastome-based phylogenetic backbone of *Crataegus* estimated through Bayesian Inference analysis using MrBayes. The numbers displayed above each branch represent the posterior probability.

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Research Article

Primula yanbianensis (Primulaceae), a new species in *Primula* sect. *Cortusoides* from Sichuan, China

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Abstract

Primula yanbianensis T.Shuai, Lei Cai & Z.K.Wu, a new species of Primulaceae from Sichuan, China, is described and illustrated. Morphological evidence supports *P. yanbianensis* as a member of *P.* sect. *Cortusoides*, which is characterised by lack of farina, generally well furnished with multicellular hairs, subrounded leaf blades are more or less lobed, with distinct petiole and a deeply cordate base and campanulate or narrowly campanulate calyx not accrescent after anthesis. The new species is characterised by having umbel in whorls of 1–2, scape usually lower than leaf clusters, bracts broadly ovate and rose to pink petals distinctly veined. The distribution, morphological comparison with closely-related species and conservation status of the new species are also provided.

Key words: New species, *Primula* sect. *Cortusoides*, Sichuan, yan bian bao chun

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Introduction

Primula L. is one of the largest genera in Primulaceae, encompassing ca. 545 species worldwide (POWO 2024). Most *Primula* species are native to temperate and alpine regions of the Northern Hemisphere, with only a few species occurring in the Southern Hemisphere (Hu 1990; Hu 1994; Richards 2002). The south-western region of China (especially the Himalayan-Hengduan Mountains) is the centre of diversity of the genus, with more than 300 species recorded, which are distributed mainly in western Sichuan, eastern Xizang and north-western Yunnan (Hu 1990; Hu and Kelso 1996; Richards 2002).

Primula Sect. *Cortusoides* Balf. f. (39: 140, 1913) (Balfour 1913), comprising about 25 species worldwide and 19 species being recorded in China (of which *P. pelargoniifolia* G.Hao, C.M.Hu & Z.Y.Liu and *P. longipilosa* Ze H.Wang & H.Peng are two newly-described species in recent years (Xu et al. 2014a; Wang et al. 2022), is widely distributed in central and eastern Asia, but its distributional centre is in SW China (Hu 1990; Xu et al. 2014a; Wang et al. 2022). Morphologically, the section is characterised by the plants being efarinose, but generally well furnished with multicellular hairs, subrounded leaf blades are more or less lobed, with distinct petiole and a deeply cordate base and campanulate or narrowly campanulate calyx not accrescent after anthesis (Smith and Fletcher 1944).

As one of the hotspots of biodiversity in China, Sichuan has over ca. 170 species of *Primula* distributed all over its range (Wu 1999). With the increased exploration of the region, many new additions of *Primula* species have constantly been reported in Sichuan over the past two decades (Hu and Ceng 2003; Hu and Hao 2006; Wu et al. 2013; Xu et al. 2014b, 2015, 2016a, 2016b, 2016c, 2017a, 2017b, 2019; Ju et al. 2018, 2021, 2023; Yuan et al. 2018; Li et al. 2023; Luo et al. 2023).

During a botanical expedition to Yanbian, Sichuan, south-western China in June 2017, we discovered a peculiar *Primula* population with long white multicellular hairs all over the plant, subrounded leaf blades being more or less lobed, with distinct petiole and a deeply cordate base and narrowly campanulate calyx. These features indicate that it should be a member of the *P.* Sect. *Cortusoides*. However, its scape is shorter than the leaves, umbel is in whorls of 1–2, bracts are broadly ovate and rose to pink petals are distinctly veined, all of which indicate it to be a distinct species. We surveyed the site and adjacent area again and collected specimens in July 2020 and June 2024. After a full observation of the morphological characteristics and comparing the relevant literature and specimens for related species, we found that this species did not match the known species of *Primula* and convinced us that this plant is novel and previously undescribed. Therefore, we describe and illustrate the plant as a new species here.

Materials and methods

The morphological observation, measurements and description of the new species were based on both dried specimens and living plants from Yanbian County, Sichuan Province. Morphological comparisons with closely-related species were made by reviewing specimens from major herbaria, such as PE, P, E, KUN, SM and NY and relevant literature reports (Hu 1990; Hu and Kelso 1996; Xu et al. 2014a; Wang et al. 2022). The information regarding the type specimens involved in this study is as follows: *P. longipilosa*, Gengma TCM Resources Survey Exped. 5309260482, KUN1536784, KUN!; *P. neurocalyx* Franch., P.G. Farges 1369, P00649666, P!; *Primula sinomollis* Balf.f. & Forrest, Forrest, G. 5523, E00024096, E!. All morphological characters of *P. yanbianensis* and its morphologically similar species in the *P.* sect. *Cortusoides*, including *P. neurocalyx*, *P. longipilosa* and *P. sinomollis*, were measured using a Vernier caliper by living plants from their type localities . The conservation status of the new species was assessed following the guidelines of the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2024).

Taxonomic treatment

***Primula yanbianensis* T.Shuai, Lei Cai & Z.K.Wu, sp. nov.**
urn:lsid:ipni.org:names: 77356677-1

Diagnosis. The new species is most similar to *P. neurocalyx*, *P. longipilosa* and *P. sinomollis*, sharing multicellular hairs covering the plant, subrounded leaf blade are more or less lobed, with distinct petiole and a deeply cordate base and campanulate or narrowly campanulate calyx. However, the new species is distinguished from the latter three mainly by its scape usually being lower than leaf clusters, umbel is in whorls of 1–2, broadly ovate bracts and rose to pink petals are distinctly veined (Figs 1–3). The main morphological distinctions between *P. yanbianensis*, *P. neurocalyx*, *P. longipilosa* and *P. sinomollis* are summarised in Table 1.

Type. CHINA • Sichuan: Panzhihua City, Yanbian County, Yongxing Town. 27°4'32.95"N, 101°24'17.75"E, 1530 m alt., 28 June 2017 (fl.), *Zhikun WU & Lei Cai*, L.Cai2017070; 15 July 2020 (fl.), *Zhikun WU*, ZKWu2020053 (***holotype*****:** KUN!; ***Paratype*****:** KUN!).

Description. A perennial herb with a short and usually inconspicuous rhizome and numerous robust fibrous roots, densely covered throughout with multicellular hairs. ***Leaves*** all rising from the root, forming a rosette, leaves including the petiole 10–25 cm long, petiole 5–18 cm, clothed with long spreading soft multicellular hairs, slightly sheathed at the base; leaf blade suborbicular to reniform, 4–12 cm long, 3–10 cm wide, apex obtuse, base cordate to deeply cordate, the leaf margin is undulate and lobed, the upper surface of the leaf sparsely septate hairs, while the lower surface covered with multicellular hairs along all the veins, mid-rib and 4, 5 pairs of pinnate lateral veins slightly impressed above, prominent below, reticulation open and rather feebly

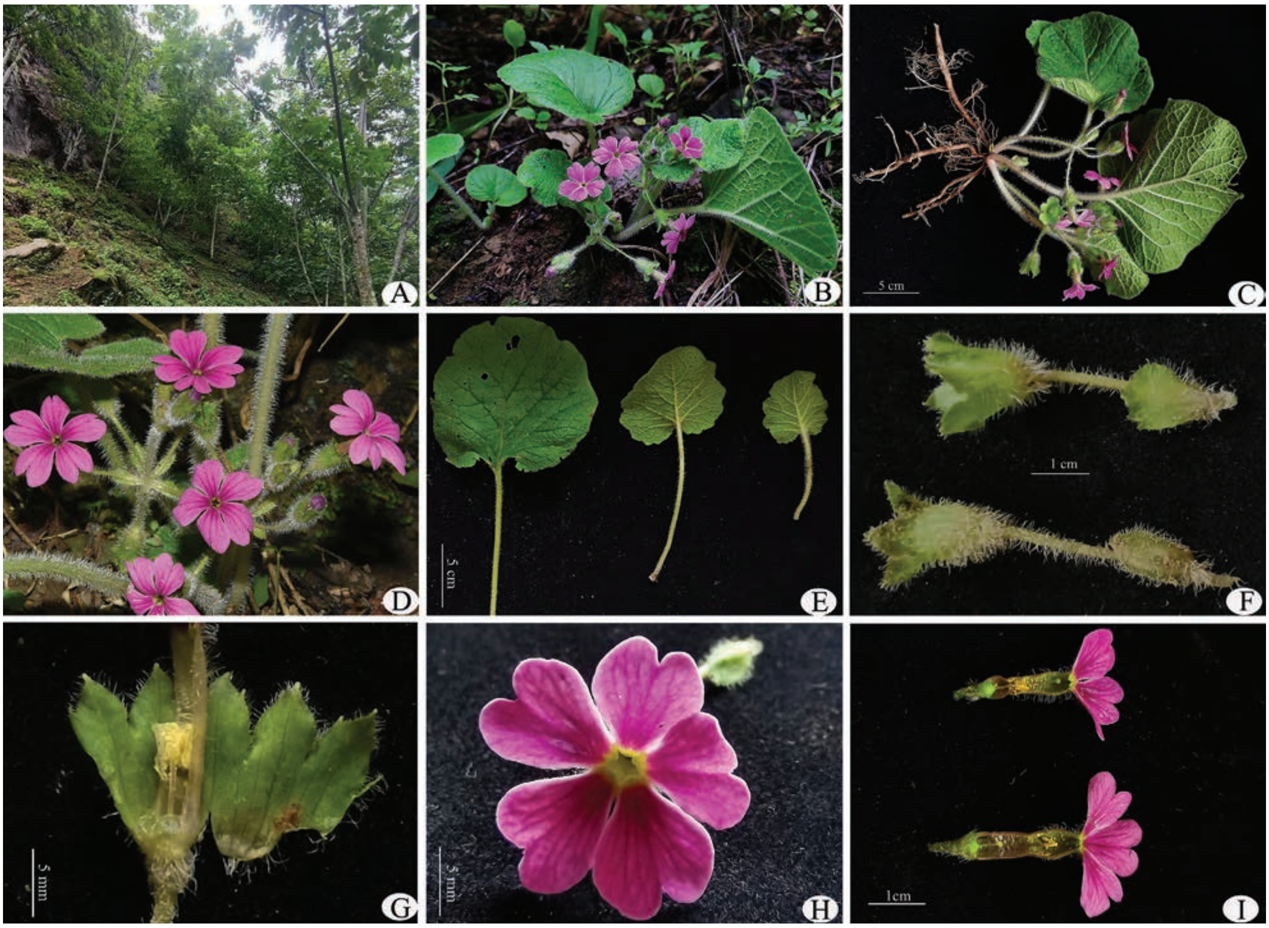


Figure 1. *Primula yanbianensis* sp. nov. **A** habitat **B** habit in flowering **C** fresh plant with roots **D** inflorescence **E** leaves, left: upper surface, right: lower surface **F** bracts and calyx **G** calyx and stamens **H** flower, front view **I** pin flower and thrum flower. Photographed by Z.K.Wu.

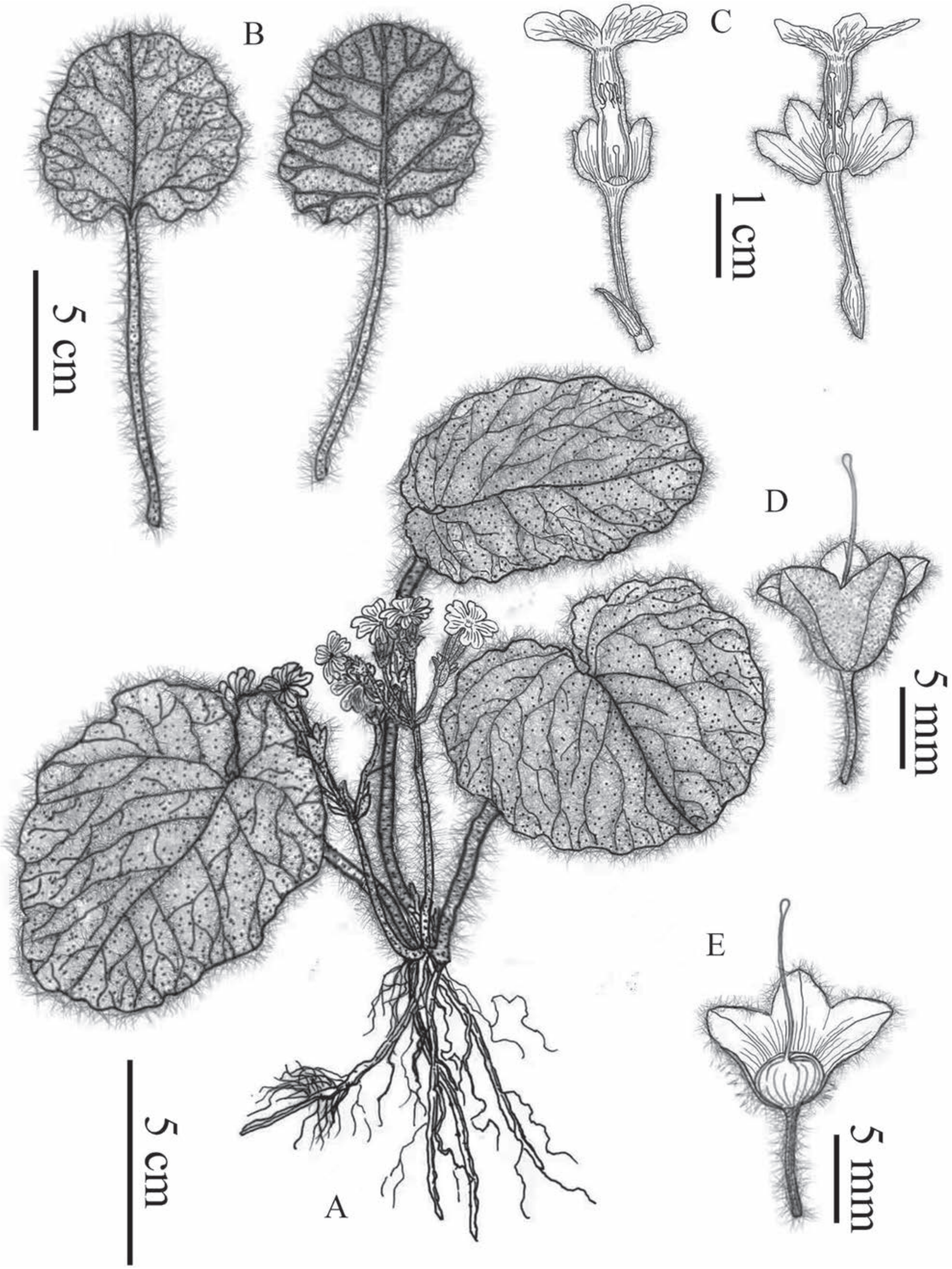


Figure 2. *Primula yanbianensis* sp. nov. **A** habit **B** leaves, left: upper surface, right: lower surface **C** flower, left: thrum flower, right: pin flower **D** calyx and stigma **E** calyx and ovary. Drawn by Ms. Xiang-Li Wu.

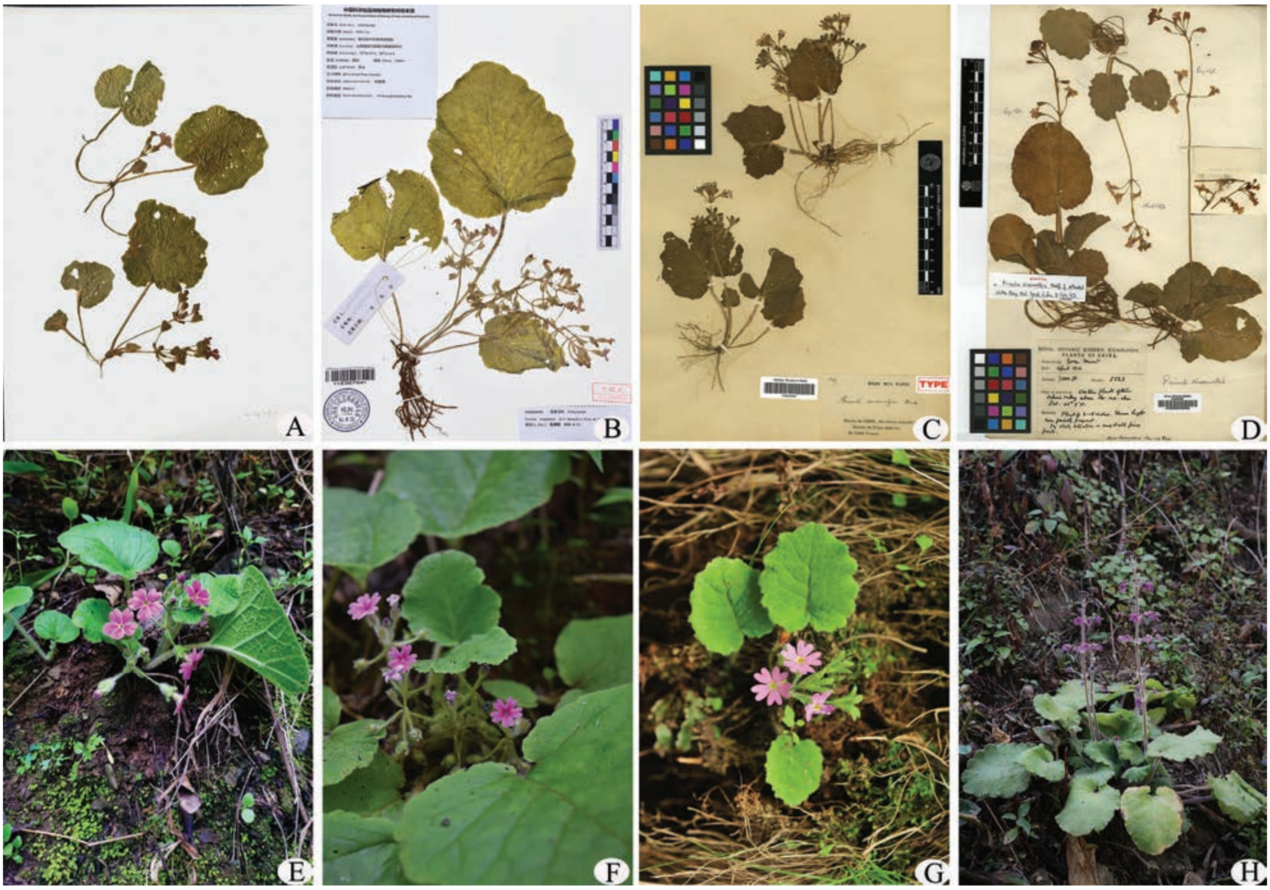


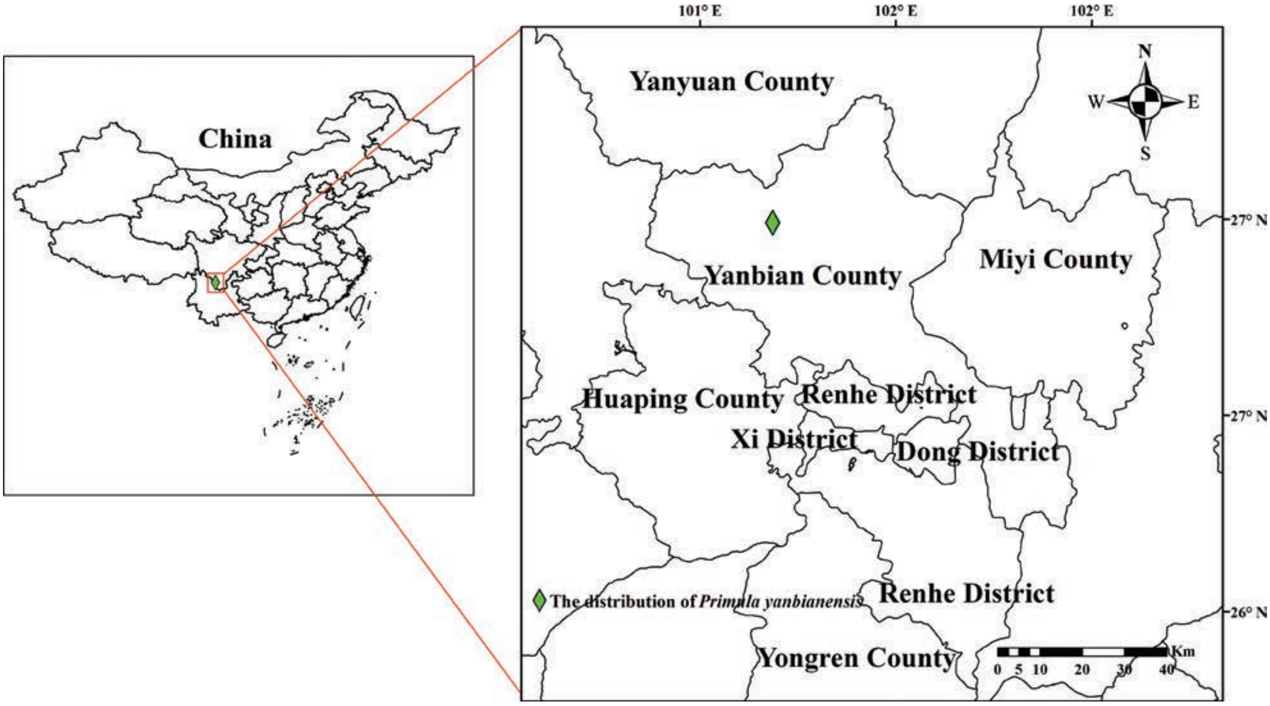
Figure 3. *Primula yanbianensis* and three of its allies **A** holotype of *P. yanbianensis* (L.Cai 2017070, KUN!) **B** holotype of *P. longipilosa* (Gengma TCM Resources Survey Exped. 5309260482, KUN!) **C** type of *P. neurocalyx* (P.G. Farges 1369, P!) **D** syntype of *P. sinomollis* (Forrest, G.5523, E!) **E** *P. yanbianensis* **F** *P. longipilosa* **G** *P. neurocalyx* **H** *P. sinomollis*. **E, G, H** photographed by Z.K. Wu from their type locality, **F** photographed by Li Chen from its type locality.

developed. ***Scapes*** 6–13 cm long, 1, 2 rising from the middle of the rosette leaves, shorter than leaves, densely covered with long soft multicellular hairs, carrying 1, 2 superposed umbels each with 3–5 flowers. ***Bracts*** broadly ovoid, 3–6 mm long, usually shorter than half of the pedicel, with long soft multicellular hairs. ***Pedicel*** 1, 2 cm, with a dense covering of long multicellular hairs. ***Flower*** heterostylous. ***Calyx*** narrowly campanulate to campanulate, 6–8 mm long, covered with long multicellular hairs abaxially, inner surface glabrous, cut to middle, lobes broadly ovate, with 3–5 prominent veins, apex with short cusp. ***Corolla*** rose to pink, tube 1.2–1.8 cm long, with long soft multicellular hairs outside, limb 1.5–2 cm in diameter, lobes obcordate, 6–8 mm in diameter, with prominent veins from yellow mouth, apex deeply emarginate. ***Pin flowers*****:** tube 1.2–1.5 cm long, style 10–12 mm long, stamens at ca. 3 mm above the base of corolla tube; ***thrum flowers*****:** tube 1.2–1.8 cm long, style 3–5 mm long, stamens at 2/3 length of corolla tube, ca. 10–12 mm above the base of corolla tube. Ovary globose (Figs 1, 2). ***Capsule*** unknown.

Distribution and habitat. This new species is currently only known from the type locality near Qingyi Road, Yongxing Town, Yanbian County, Panzhihua City, Sichuan Province, China and is mostly found on grassy slopes along the valley forest margin, at altitudes of 1500–1650 m (Fig. 1; Map 1).

Table 1. Comparison of main morphological characters amongst *P. yanbianensis*, *P. neurocalyx*, *P. longipilosa* and *P. sinomollis*.

Characters	*P. yanbianensis*	*P. longipilosa*	*P. neurocalyx*	*P. sinomollis*
Leaf blade	suborbicular to reniform, 3–10 cm in diameter, adaxially with sparsely multicellular hairs, abaxially with multicellular hairs along veins	cordate to broad cordate, 4–16 cm in diameter, covered with white soft multicellular hairs on both sides	orbicular or broadly ovoid, 3–7 cm in diameter, adaxially with sparsely multicellular hairs, abaxially with long hairs along veins	broadly elliptic to suborbicular, 3–8 cm in diameter, adaxially with appressed multicellular hairs, abaxially with long hairs along veins
Bract	broadly ovoid, 3–6 mm long	narrowly lanceolate, 5–15 mm long	linear-lanceolate, 6 mm long	linear to linear-lanceolate, 6–8 mm long
Scape	usually shorter than leaf clusters	shorter than or almost equal to leaf clusters	slightly longer than or equal to leaf cluster	usually much longer than leaf clusters
Inflorescence	umbels, 1–2 whorled, with each round containing 3–5 flowers	racemes mostly, sometimes umbels, 2, 3 rising from the rosette leaves, each scape has 7–25 flowers arranged in a racemose inforescence	umbels, 1, 2 whorled, with each round containing 3–7 flowers	umbels, 3–10 whorled, with each round containing 3–9 flowers
Calyx	6–8 mm long, lobes broadly ovate	6–10 mm long, lobes triangular to ovate-triangular	7–9 mm long, lobes rectangular to rectangular-lanceolate, herbaceous	5, 6.5 mm long, distinctly 5-ribbed, lobes narrowly lanceolate
Flowers	heterostylous, corolla rose to pink with distinctive veins on all part of lobes, yellow eye	heterostylous, corolla pink to pink rose, with veins prominent at base of lobes, inconspicuous at apex, purple eye	homostylous, corolla red-purple, lack veins on lobes, yellow eye	heterostylous, corolla light red to magenta or purple-red, lack veins on lobes, purple eye
Flowering time	June to July	July	May to July	April



Map 1. Location of the distribution of *Primula yanbianensis* in Yanbian County, Sichuan.

Phenology. Flowering occurs from June to July.

Etymology. The specific epithet of the new species is taken from the Chinese Pinyin “yanbian”, the name of the county in south-western Sichuan, China, where the type specimen was collected (Map 1).

Vernacular name. Chinese mandarin: yan bian bao chun (盐边报春).

Conservation status. This new species is a rare species with a restricted distribution. Currently, only one population with less than 150 individuals has been found in the type locality. We have observed a steady decline in the territory area of the habitat due to road construction and grazing, based on the latter two field expeditions conducted in 2020 and 2024. Therefore, in combination with the information currently available and in accordance with the guidelines for the use of the IUCN Red List Categories and Criteria (IUCN 2024), the conservation status of this new species has been assessed as ‘Critically Endangered’ (CR B1ab(i, iii)).

Additional specimens examined (*paratypes*). • The same locality as holotype, 18 June 2024, *Tian Shuai*, TSh 2024045 (KUN!).

Discussion

In the subgenus *Auganthus* of *Primula*, which encompasses sections such as *Auganthus*, *Monocarpicae*, *Cortusoides*, *Malvacea*, *Pycnoloba*, *Obconicolisteri*, *Reinii*, *Ranunculoides* and *Bullatae*, many species exhibit similar leaf characteristics. These include long petioles and multicellular hairs with leaf blades that are suborbicular to reniform in shape and deeply cordate at the base. Consequently, when encountering a plant within this subgenus, it is essential to determine the appropriate section for classification. In these closely-related sections, variations in calyx structure can be instrumental in distinguishing them from one another. Upon discovering *P. yanbianensis* in Yanbian, we considered whether it might belong to sect. *Malvacea*; however, its calyx traits are distinctly different from those found within the *Malvacea* series of *Primula*. The calyx characteristics of members belonging to sect. *Malvacea* serve as key diagnostic features that differentiate them from other sections: they possess foliaceous calyxes with more or less prominent well-developed reticulate venation and an obconical tube that expands into broad spreading lobes. During fruiting stages, the calyx enlarges to form a platter-like expansion at its center where a globose capsule rests; notably thickened throughout its entirety. Although *P. malvacea* is also present in southwestern Sichuan and adjacent northwestern Yunnan, which exhibiting significant morphological variation in inflorescences and flowers, and we observed both verticillate inflorescence types and racemose inflorescence types within this region as well. Nevertheless, the foliaceous calyx traits of *P. malvacea* are markedly distinct from the campanulate calyx characteristic of *P. yanbianensis* (Fig. 1D). This distinction is why we did not classify this new species under sect. *Malvacea*; instead based on its campanulate calyx morphology we have classified *P. yanbianensis* within sect. *Cortusoides*.

Morphologically, *P.* sect. *Cortusoides* is characterised by a lack of farina, the plants are generally covered with multicellular hairs, the petiole is strongly conspicuous, the leaf blades are suborbicular to reniform, the margins are lobed, the bases are cordate to deeply cordate and the calyxes are campanulate or narrowly campanulate (Smith and Fletcher 1944). These traits make it easy to determine at the sectional level. However, some traits are overlapping in the closely-related species in this section, making it difficult to identify these species in field, for example, *Primula mollis* group (*P. mollis* Nutt.ex Hook., *P. sinomollis*, *P. neurocalyx* and *P. longipilosa*), *Primula vaginata* group (*P. vaginata* Watt, *Primula septemloba* Franch., *Primula palmata* Hand.-Mazz. and *Primula loeseneri* Kitag.), as well as *Primula cinerascens* group (*Primula cinerascens* Franch., *Primula polyneura* Franch., *Primula scopulicola* G.Hao, C.M.Hu & Y.Xu and *Primula sieboldii* E.Morren). The new species *P. yanbianensis* is similar to *P. longipilosa*, *P. neurocalyx* and *P. sinomollis*, but *P. yanbianensis* can be easily separated from these three species by its umbel being in whorls of 1–2, scapes usually lower than the leaf clusters, bracts broadly ovate and rose to pink petals with conspicuous veins. Overall, the new species is most closely related to *P. longipilosa* morphologically, but *P. longipilosa* is mainly distributed in Yunnan near the border to Myanmar (Wang et al. 2022), whereas the new species is distributed in Sichuan and no similar populations have been found in the intermediate area between these two. Therefore, *P. yanbianensis* might have diverged from their ancestors after geographical isolation, but its phylogenetic relationships need further investigation.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

All authors participated in the expedition, T. Shuai and Y-F Chen did the measurement, T. Shuai and Z.K.Wu wrote the manuscript.

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Data availability

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

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Short Communication

Caelospermum versus *Coelospermum* in Rubiaceae (Gentianales): their etymologies explained

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Abstract

Caelospermum is the original spelling of the generic name, as it appeared in the protologue, but in literature and online databases, the variant spelling *Coelospermum* is often used. However, the confusion about the spelling is caused by a misinterpretation of the etymology. Here, we demonstrate that the original spelling by Blume should be retained.

Key words: Carl Ludwig Blume, etymology, Greek, Latin, nomenclature, orthography, Rubiaceae

Caelospermum versus *Coelospermum*

Caelospermum Blume is a genus within the Rubiaceae family that is found in Southeast Asia and Oceania and currently consists of 12 species (POWO 2025). The genus is included in the tribe Morindeae, subfamily Rubioideae (Razafimandimbison and Rydin 2024). The circumscription of the genus has been controversial but a broadly delimited *Caelospermum* can be characterised by the mostly lianescent habit, corolla tubes with narrow longitudinal slits alternating with the lobes, ovaries simple or 2–20(–30)-connate, primarily bilocular with biovular locules, later becoming 4-locular by the development of secondary septa, drupaceous fruits, concavo-convex to planoconvex pyrenes with a narrow slit in the lower part, and flattened seeds with a narrow basal wing (Johansson 1988). Razafimandimbison et al. (2009) confirmed Johansson's concept of *Caelospermum* with molecular phylogenetic methods, although enlarging it by treating the Australian genus *Pogonolobus* F.Muell. as a synonym.

The generic name was originally spelled as *Caelospermum* in the protologue (Blume 1826: 994; Fig. 1). The genus was mentioned to be closely related to *Morinda* L. and *Gynochtodes* Blume and a single species (i.e. the type species), *Caelospermum scandens* Blume, was described from Nusa Kambangan island in Indonesia. There was no explanation of the etymology of the generic name, but it seems evident that it is derived from Latin "caelum" (= heaven, sky) and "sperma" (= seed). Although Blume mentioned some characters of the pyrenes (Fig. 1), this etymology did not seem to make sense.

Shortly after, the spelling of the generic name was changed by other taxonomists. Both de Candolle (1830: 468) and Richard (1834: 209) corrected the

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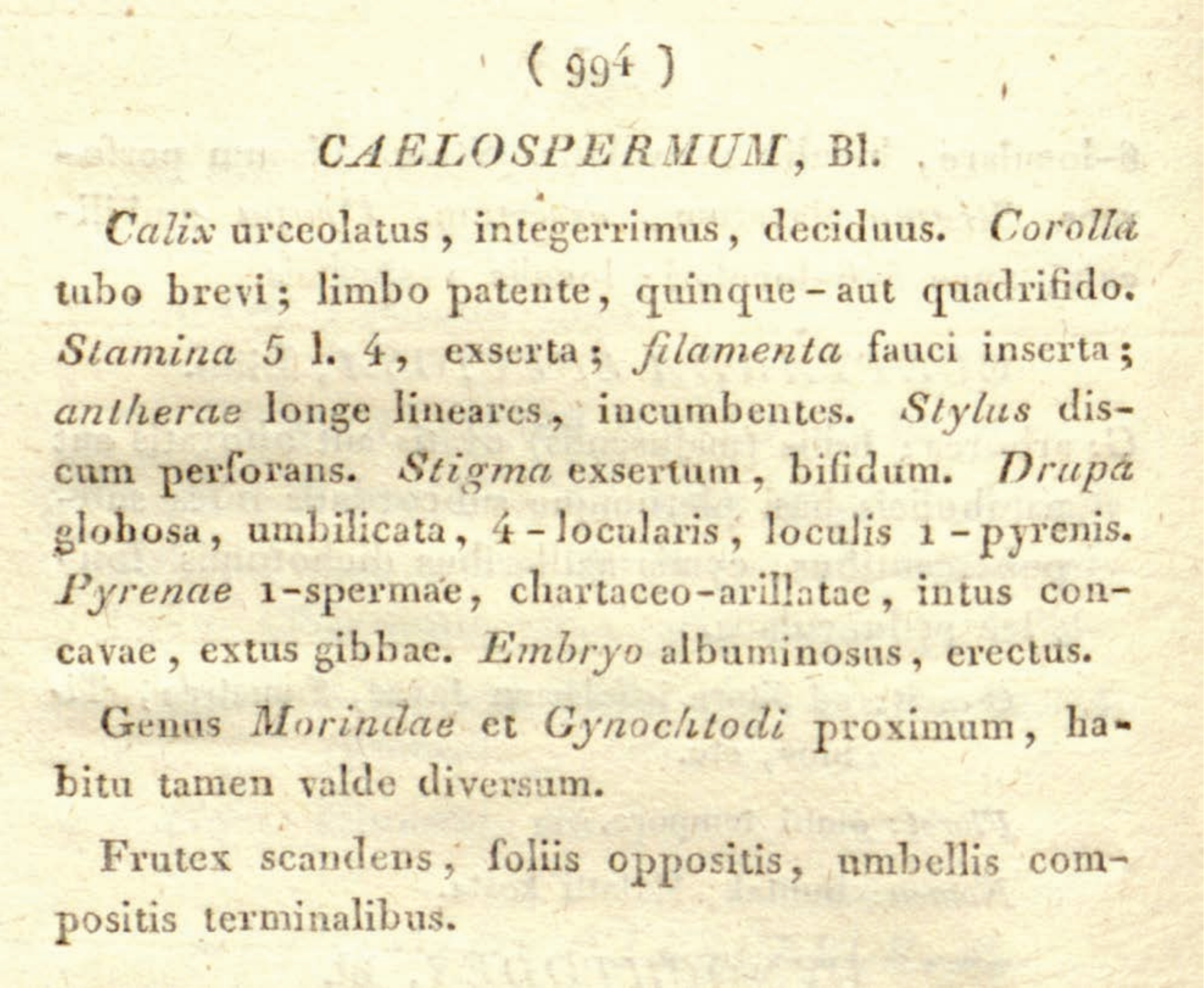

(994)

CAELOSPERMUM, Bl.

Calix urceolatus, integerrimus, deciduus. *Corolla* tubo brevi; limbo patente, quinque-aut quadrifido. *Stamina* 5 l. 4, exserta; *filamenta* fauci inserta; *antherae* longe lineares, incumbentes. *Stylus* discum perforans. *Stigma* exsertum, bifidum. *Drupa* globosa, umbilicata, 4-locularis, loculis 1-pyrenis. *Pyrenae* 1-spermae, chartaceo-arillatae, intus concavae, extus gibbae. *Embryo* albuminosus, erectus.

Genus *Morindae* et *Gynochtodi* proximum, habitu tamen valde diversum.

Frutex scandens, foliis oppositis, umbellis compositis terminalibus.

Figure 1. The original publication of the generic name *Caelospermum* Blume (from Blume 1826).

name to *Coelospermum*, however, without commenting on the reason. Hooker (1873: 119) also used *Coelospermum* but he mentioned *Caelospermum* between brackets. Throughout literature, both spelling variants have been used, although it seems that *Coelospermum* is more prevalent, especially in recent molecular phylogenetic studies (e.g. Razafimandimbison et al. 2008, 2009). The monographer of the genus first used *Coelospermum* (Johansson 1987). In his revision of the genus, however, he used the original spelling *Caelospermum* and listed the name *Coelospermum* in synonymy (Johansson 1988), without explaining why he made this change. Other authors also used both variants in different publications (e.g. Halford and Ford 2004 vs Halford and Ford 2009). Mouly and Fleurot (2021) used the spelling *Coelospermum* but explicitly mentioned *Caelospermum* as an orthographical variant. The origin of the change in spelling is found in the interpretation of the etymology. Subsequent authors interpreted the name as derived from Greek “koilos” (= concave, hollow), not Latin “caelum” (= heaven, sky). Since Blume (1826)’s description included the phrase “Pyrenae ... intus concavae ...”, it was interpreted that Blume meant “hollow-seeded” (IPNI 2025).

Besides in literature, the names are also entered in online databases about nomenclature and taxonomy. The International Plant Names Index (IPNI 2025) lists both *Caelospermum* and *Coelospermum* but both names have two entries and there is confusion about the correct spelling. In the nomenclatural notes of *Caelospermum* (https://www.ipni.org/n/34272-1), it says to use *Coelospermum*. However, the entry *Caelospermum* (https://www.ipni.org/n/116925-3) remarks that there is an orthographical variant *Coelospermum*. The entry *Coelospermum* (https://www.ipni.org/n/327850-2) has a remark in which IPNI chooses to correct *Caelospermum* to *Coelospermum*. The entry *Coelospermum* (https://www.ipni.org/n/85291-3) says the opposite because the remark says that *Coelospermum* is the orthographical variant. Tropicos (Tropicos.org 2025) is clearer about the spelling: *Coelospermum* (https://www.tropicos.org/name/40037911) is considered to be the orthographical variant of *Caelospermum* (https://www.tropicos.org/name/40034119). The World Flora Online (WFO 2025) lists *Caelospermum* (https://www.worldfloraonline.org/taxon/wfo-4000008840) and several subordinate taxa, but all those names are annotated as unchecked names. The status of *Coelospermum* (https://www.worldfloraonline.org/taxon/wfo-4000005905) and its species are indicated as accepted names. The Plants of the World Online (POWO 2025 only lists *Coelospermum* (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:327850-2).

The interchangeability of cael- (from "caelum") and coel- (from "koilos") is also noted for other generic names in angiosperms. Hasskarl (1855: 139) published the name *Koilodepas* Hassk. (Euphorbiaceae), which is derived from Greek "koilos" (= concave) and "depas" (= beaker) and refers to the cupulate calyx. He later Latinised the name to *Coelodepas* Hassk. (Hasskarl, 1857). However, Bentham and Hooker (1880: 313) published the name as *Caelodepas* Benth. & Hook.f., with reference to *Coelodepas* Hassk. and *Koilodepas* Hassk. Other examples are *Caelebogyne* J.Sm. / *Coelebogyne* J.Sm., *Caelestina* Cass. / *Coelestina* Cass., *Caelia* G.Don. / *Coelia* Lindl., *Caelocline* Auct. ex Steud. / *Coelocline* A.DC., *Caeloglossum* Steud. / *Coeloglossum* Lindl., and *Caelogyne* Wall. ex Steud. / *Coelogyne* Lindl. (Steudel 1840: 247; IPNI 2025). In almost all cases, the variant coel- is preferred (POWO 2025; WFO 2025). *Caelestium* Yurtseva & Mavrodiev (Polygonaceae) has no variant because the etymology is explicitly stated in the protologue: "… from "caelestis" (= heavenly, divinus)" (Yurtseva and Mavrodiev 2019: 73).

Regarding *Caelospermum*, the correction of Blume's spelling of the name is the result of a misinterpretation of the etymology. *Caelospermum* can indeed be derived from Latin "caelum" but it should be translated as "vault of heaven" (Lewis and Short 1891). In ancient cosmology, the celestial vault is portrayed as a vast solid dome arching above the Earth. The name would then refer to arched pyrenes (Fig. 2), which corresponds well with the characters in the protologue (i.e. "Pyrenae … intus concavae, extus gibbae") and in Johansson (1988)'s revision (i.e. "endocarp … concavo-convex to planoconvex"). The interpretation that the name would be derived from Greek "koilos" is conceivable but not necessarily correct.

In Rubiaceae, the origins of the generic names by Blume are not always obvious (e.g. *Metabolos* Blume; Bremekamp 1952: 30) and Blume never explained their etymologies in the protologues, except for eponyms. We will therefore never be able to definitively ascertain what Blume actually intended. Ultimately, both cael- and coel- can refer to a concave feature, and can therefore be used in that sense. However, their etymologies are different, so they should not be considered as orthographical variants. In that case, the original spelling of the name should be retained (ICN Art. 60.1; Turland 2019). In conclusion, the original spelling *Caelospermum* Blume is correct and should be used.

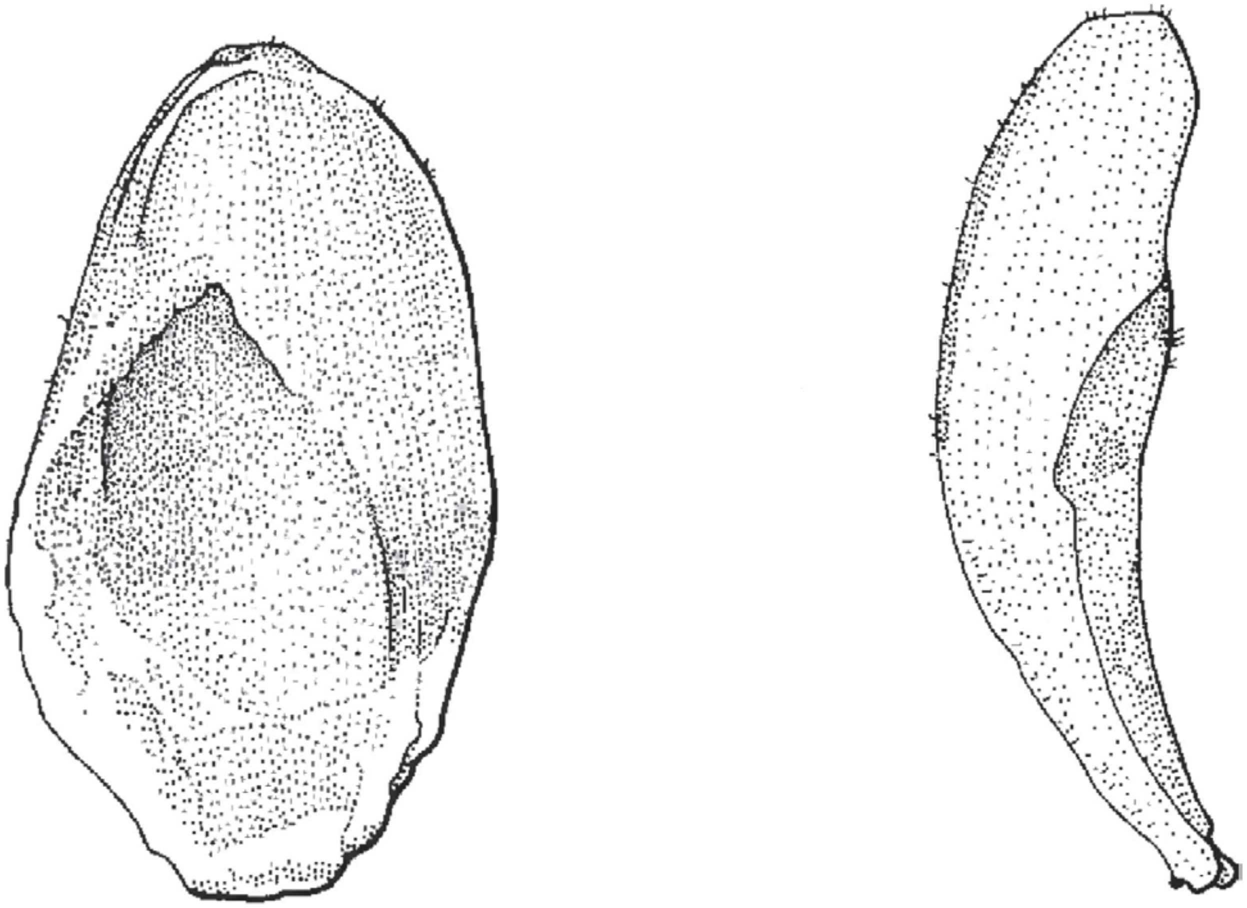

Figure 2. The concave pyrene with a basal marginal groove of *Caelospermum purpureum* Halford & A.J.Ford in adaxial (left) and lateral view (right) (reproduced from Halford and Ford 2009: fig. 1J, K with permission from the editor).

Additional information

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Author contributions

Conceptualization: BV, ER. Investigation: BV. Writing - original draft: BV. Writing - review and editing: BV, ER.

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Data availability

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

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Research Article

Two new species of *Ocotea* (Lauraceae) with clustered leaves from the Uxpanapa region, Mexico

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Abstract

Two new species of *Ocotea* (Lauraceae) from Southern Mexico are described and illustrated. Based on their floral morphology, vegetative indument, and micromorphological attributes, both species are circumscribed within the *Ocotea helicterifolia* group. Within this clade, the two species are morphologically similar to those with clustered leaves. Both species are endemic to the species-rich yet fragmented forests of the Uxpanapa region in Mexico, and therefore, their populations are threatened. Here, the morphological similarities of both species and their conservation status are discussed.

Key words: Endemic, floristic refuge, Tehuantepec Isthmus, tropical rainforests, Veracruz

Introduction

Lauraceae, with around 55 genera and ca. 3000 species, is one of the most ecologically important families of woody plants in tropical and subtropical forests (Rohwer 1993; Chanderbali et al. 2001). Members of the Lauraceae family pose taxonomic challenges for botanists due to the small size and complex internal structure of their flowers, the absence of local taxonomic treatments and the difficulty of following the available dichotomous keys (van der Werff 1991; van der Werff and Richter 1996; Rohwer et al. 2009; Liu et al. 2017; Rohde et al. 2017). Consequently, many herbarium specimens are undetermined, incorrectly determined, or without updated nomenclature. Furthermore, the current estimates of Lauraceae diversity in many tropical areas do not consider the latest botanical collections and exploration efforts, leading to an underestimation of its species richness (van der Werff 2020).

The genus *Ocotea* Aubl. is the most species-rich neotropical Lauraceae genera. It has a very broad concept and has been considered a catch-all genus for taxa that cannot be confidently placed in more clear-cut Lauraceae genera (Rohwer 1991; Lorea-Hernández 2002; van der Werff 2002). Moreover, phylogenetic analyses consistently show that *Ocotea* is a polyphyletic genus (Trofimov et al. 2019; Penagos-Zuluaga et al. 2021). Thus, within *Ocotea* several groups of species can be recognized (van der Werff 2002). In recent years, some of them have been elevated to the rank of genus based on phylogenetic and morphological evidence (Trofimov et al. 2019; Trofimov and Rohwer 2020).

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One of the best-defined groups in *Ocotea* is the so-called *Ocotea helicterifolia* group, which consists of around 40 Mexican and Central American tree species (Rohwer 1991, van der Werff 2002). It is characterized by the combination of mostly pubescent leaves, hermaphroditic flowers with spreading tepals, short-filamented and papillose stamens, anthers with locelli arranged in two superposed pairs, and fruit cupules with a single margin (van der Werff 2002). Additionally, a recent study revealed micromorphological characteristics supporting this delimitation; *Ocotea* helicterifolia group species share a rhombic-shaped stomatal complex and a narrowly lip-shaped aperture field of stomata (Trofimov and Rohwer 2018). Ecologically, a significant number of species are found in cloud forests at high altitudes, whilst only some species inhabit lowland tropical forests (Rohwer 1991; Lorea-Hernández and van der Werff 2002; van der Werff 2002; Lorea-Hernández 2005). Moreover, phylogenetic analyses suggest that the *Ocotea helicterifolia* group is a sister clade to the genus *Damburneya* and is not related to other species of *Ocotea* (Trofimov et al. 2016; Penagos-Zuluaga et al. 2021).

During a field study in Southern Mexico, two new species of *Ocotea* were collected in the tropical rainforest of the Uxpanapa region. In the context of the genus, the two new species belong to the *O. helicterifolia* group and present a unique combination of morphological features, which allows them to be distinguished from any other species. These interesting species are herein described and illustrated, and their morphological relationships with other species of *Ocotea* are discussed.

Materials and methods

We visited and reviewed the specimens of *Ocotea* deposited at the MEXU herbarium, Universidad Nacional Autónoma de México (UNAM). Also, we consulted the digitized type specimens available at JSTOR Global Plants (http://plants.jstor.org/). Thus, the new species were recognized using the unique combination of features criterion (Donoghue 1985) through comparisons with morphologically similar species and literature review (e.g. Rohwer 1991, van der Werff 2002). Finally, species descriptions were elaborated following the terminology presented in Hickey (1973).

We analyzed the stomata features of both species using a scanning electron microscope (SEM) at the Biodiversity Photography and Microscopy Laboratory (LANABIO) at UNAM. Leaf material for SEM was rehydrated with hot water for an hour and immersed in distilled water with a drop of biological soap to be subjected to an ultrasonic bath for two rounds of 90 seconds to remove debris. Rehydrated and clean samples were then rinsed in two soaks of distilled water. Then, samples were dehydrated gradually with alcohol (ethanol) solutions of 10, 30, 50, 70, 80, 90 and 100% for 30 minutes each. Finally, samples were critical-point dried using extra-dry CO_2, mounted on metal sample holders, and covered with gold. Sample observation was conducted with a Hitachi SU1510® (Hitachi City, Japan) scanning electron microscope.

Based on all known localities for the new species, we assessed their conservation status by calculating their extent of occurrence (EOO) and their area of occupancy (AOO) using the GeoCAT tool (Bachman et al. 2011) and applying the IUCN Red List categories and criteria and the guideline (IUCN 2012; 2024).

Results

Two new species of *Ocotea* belonging to the *Ocotea helicterifolia* group

The morphological characteristics of both species suggest that they belong to the *Ocotea helicterifolia* group. Specifically, both species share pubescent leaves, hermaphroditic flowers with spreading tepals, short-filamented and papillose stamens and anthers with locelli arranged in two superposed pairs. This circumscription was further confirmed by their rhombic-shaped stomatal complexes (Fig. 1).

Within the *Ocotea helicterifolia* group, both species are morphologically similar to *Ocotea bourgeauviana* (Mez) van der Werff, *Ocotea congregata* van der Werff, *Ocotea tonii* (Lundell) van der Werff, and *Ocotea verticillata* Rohwer, with which they share the clustered leaves. However, both species independently exhibit a unique combination of morphological characteristics, allowing us to conclude that they are new species for science (see Table 1). Thus, both species are described and illustrated below.

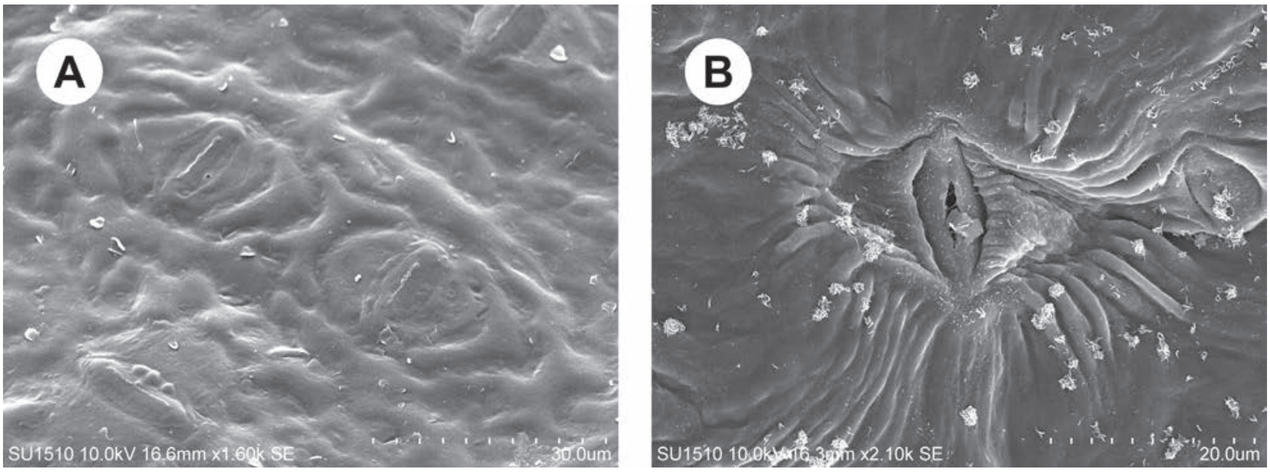

Figure 1. SEM photos of the stomatal complex **A** *Ocotea bella* **B** *Ocotea caelestis*.

Taxonomic treatment

Ocotea bella Reveles & Ortiz-Rodr., sp. nov.

urn:lsid:ipni.org:names:77356682-1
Figs 2, 3

Type. Mexico • Veracruz, Uxpanapa, 700 m southwest from Progreso Chapultepec village, 17°14'12.1"N, 94°18'23.4"W, 73 m, 14 Apr 2024, *Hurtado-Reveles L.* 1994 (holotype: MEXU; isotypes: NY, MO).

Diagnosis. Similar to *Ocotea congregata* in having clustered leaves with slightly bullate upper surface and condensed inflorescences, but differs from it in its narrowly elliptic to obovate leaves with acute to cuneate basis and shorter petioles, and by its smaller flowers with white tepals and reddish stamens (Table 1).

Description. ***Trees***, evergreen, 10–20 m tall, 15–20 cm DBH, bark smooth to slightly striate; twigs terete, densely covered with erect and adpressed, brown to light–brown trichomes, terminal buds 5–10 × 5–7 mm, densely covered with erect and adpressed golden sericeous trichomes. ***Leaves*** alternate-verticillate, clustered near the apex of branches (pseudo-verticillate), 3–8 × 13–30 cm, chartaceous,

Table 1. Morphological differences among *Ocotea helicterifolia* group species with clustered leaves.

Traits	*Ocotea bella*	*Ocotea caelestis*	*Ocotea bourgeauviana*	*Ocotea congregata*	*Ocotea tonii*	*Ocotea verticillata*
Leaf size	13–30 × 3–8 cm	7–17 × 2–5 cm	10–22 × 3–7.5 cm	9–17 × 4–7 cm	15–25 × 5–6 cm	14–26 × 4.5–14.5 cm
Leaf apex shape	acuminate to caudate	acuminate to caudate	acuminate to caudate	obtuse to acute	acuminate	acuminate
Leaf base shape	acute to cuneate	acute to cuneate	cuneate	rounded or obtuse	rounded	obtuse to slightly cordate
Upper leaf surface texture	bullate	smooth	smooth	bullate	smooth	smooth
Petiole length	7–14 mm	5–8 mm	5–10 mm	10–25 mm	Up to 6 mm	3–8 mm
Flower diameter	5.5–6.5 mm	4–5 mm	5–6 mm	7–9 mm	5.5–6.5 mm	7–10 mm
Tepal outer surface	pubescent	glabrous	glabrous	pubescent	pubescent	pubescent towards the base
Tepal inner surface	pubescent towards the base	pubescent towards the base	pubescent	pubescent towards the base	pubescent	pubescent
Stamen color	reddish	white	White	white	white	white
Anthers	Without a sterile tip	With a sterile tip	Without a sterile tip	Without a sterile tip	With a sterile tip	With a sterile tip
Staminode shape	stipitiform	capitate	clavate	stipitiform	clavate	stipitiform

narrowly elliptic to obovate, apex acuminate to caudate, base acute to cuneate, often asymmetrical, upper surface slightly bullate, sparsely covered with erect light–brown trichomes, lower surface densely covered with erect light–brown trichomes, midrib slightly raised on the upper surface, prominently raised on the lower surface; secondary veins 9–12 on each side, slightly impressed above, prominently raised below; petiole swollen, 7–14 mm long, densely covered with erect and adpressed, brown to light–brown trichomes. ***Inflorescences*** condensed panicles, borne in the axils of distal leaves on recent growth, ca. 10 cm long, peduncles ca. 3 mm long, pedicels minute, the main axis, peduncles, pedicels and bracts densely covered with erect light–brown trichomes. ***Flowers*** bisexual, ca. 6 mm in diam.; tepals 6, subequal, white in anthesis, oblong, 2–2.5 × 1–1.5 mm, outer surface sparsely covered with adpressed, light–brown trichomes, inner surface glabrous or with trichomes only at the base; stamens 9, in three whorls, reddish in anthesis, ca. 1.5 mm long, filaments a third to half the length of the anther, densely covered with long hairs, as long as the filaments or as long as the stamens, anthers 4–locular, pollen sacs arranged in two pairs, one above the other, those in the outer two whorls introrse, those of the third whorl latrorse-extrorse, stamens of the third whorl with two globose glands at the base, staminodes minute, stipitiform, sometimes absent, pistil glabrous, ovary ovoid, 1 mm long, 0.6 mm in diam, style glabrous, ca. 0.1 mm long, stigma barely trifid. ***Fruits*** (immature), ellipsoid, 5–7 × 6–8 mm, cupule trumpet-shaped, 15–20 × 6–9 mm, some tepals persistent.

Phenology. This species has been found with flowers in April and with fruits in June and July.

Etymology. The specific epithet refers to the remarkable beauty (from Latin *bella* = beautiful or pleasant) of the flowers and leaves of this species.

Distribution. *Ocotea bella* is known only from the Uxpanapa region in Veracruz, in Southern Mexico (Fig. 4).

Ecology and habitat. *Ocotea bella* inhabits tropical rainforests on reddish clay-rich soils, with a mean annual temperature of 25 °C and a mean annual precipitation of 3450 mm. It has been collected at the base of north-facing hill slopes near a small stream.

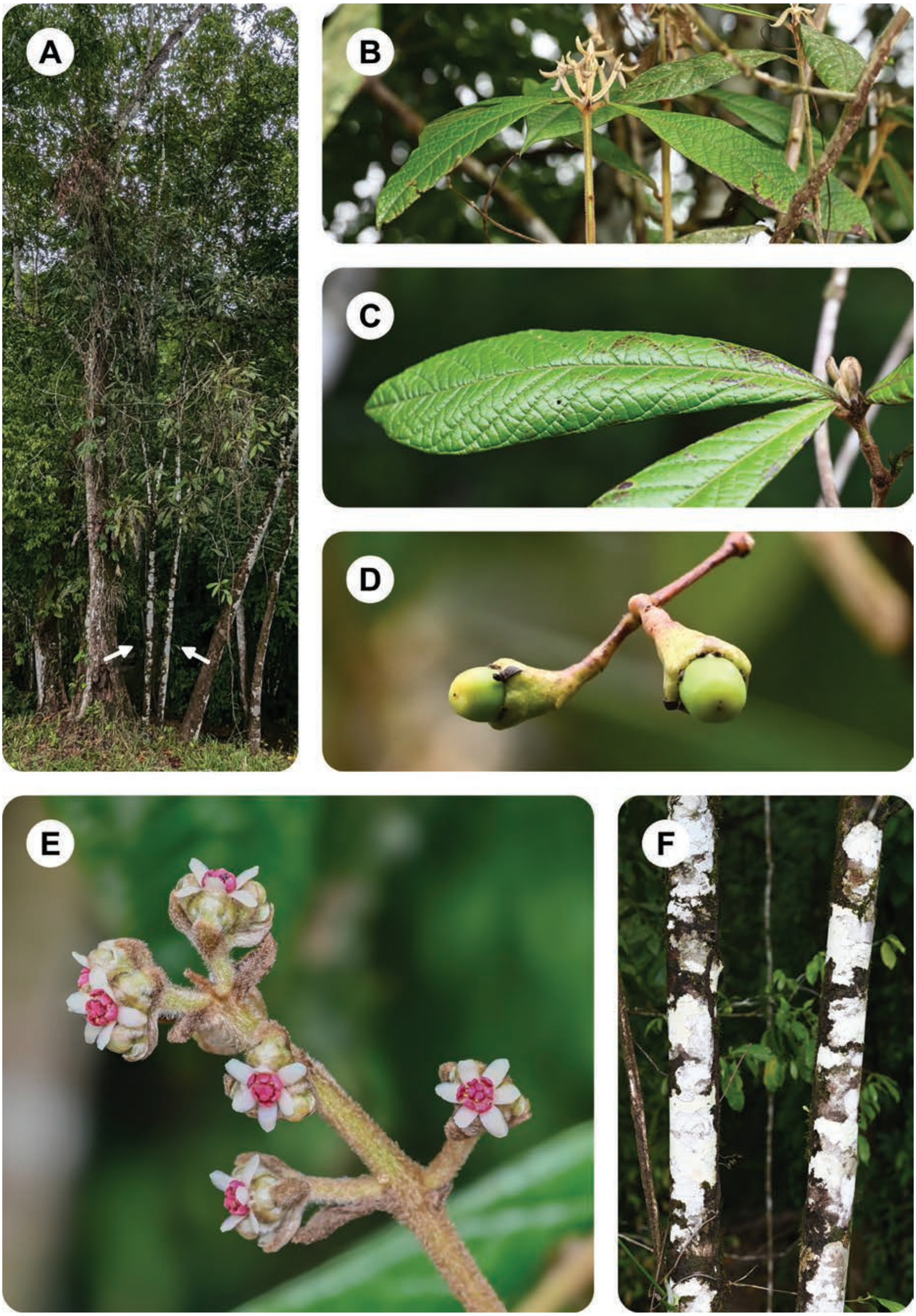

Figure 2. *Ocotea bella* Reveles & Ortiz-Rodr **A** general view of the tree (arrows indicate the specimen, which is a single individual with a bifurcated trunk at the base) **B** distal part of twig, with leaves and young growth **C** upper leaf surface **D** young fruits **E** inflorescence **F** trunks.

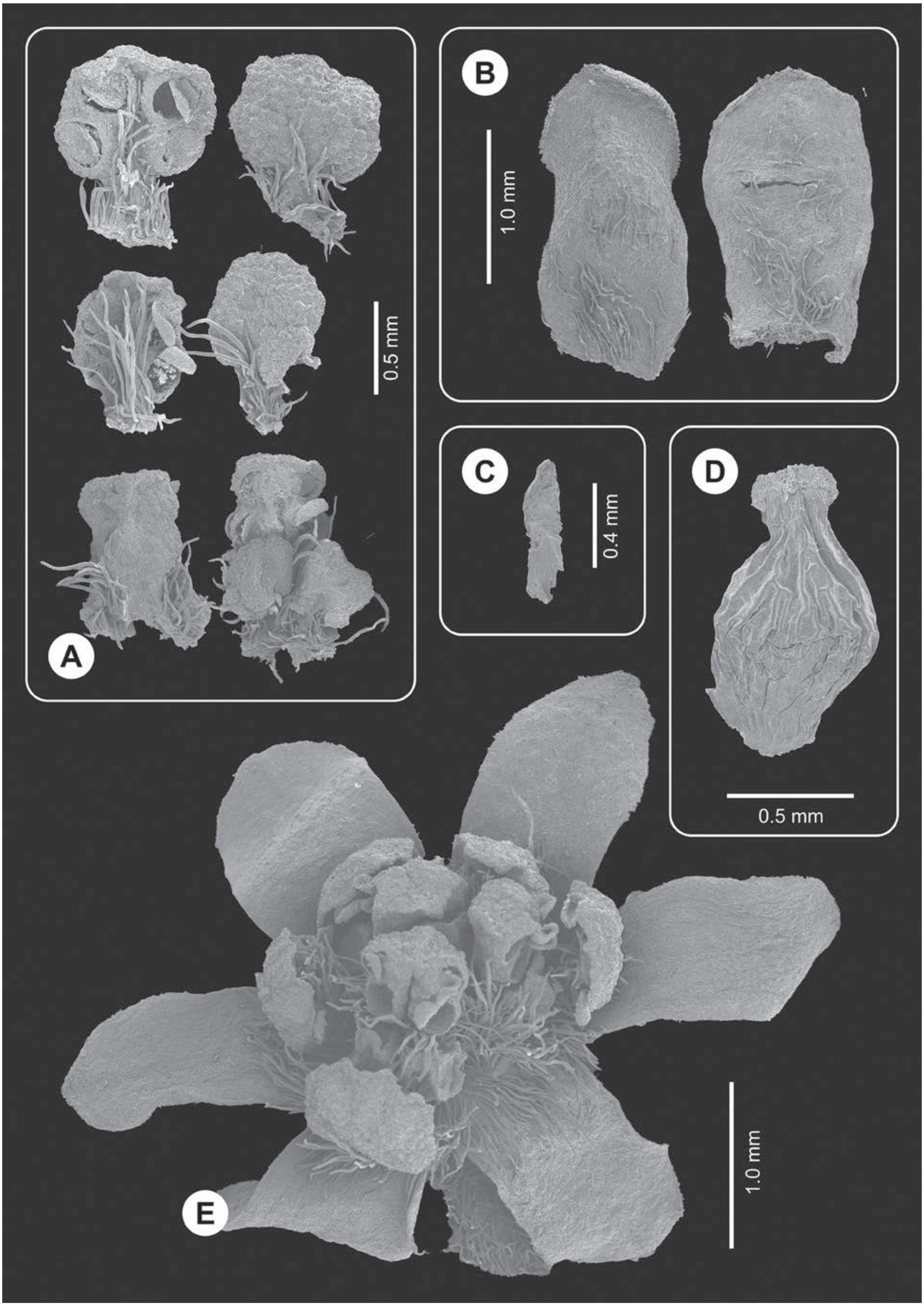

Figure 3. Flowers and floral parts of *Ocotea bella* (SEM photos) **A** stamens, adaxial and abaxial view, first to third whorl, in pairs from top to bottom **B** external view of tepals **C** staminode **D** pistil **E** general flower view (one whorl II stamen missing).

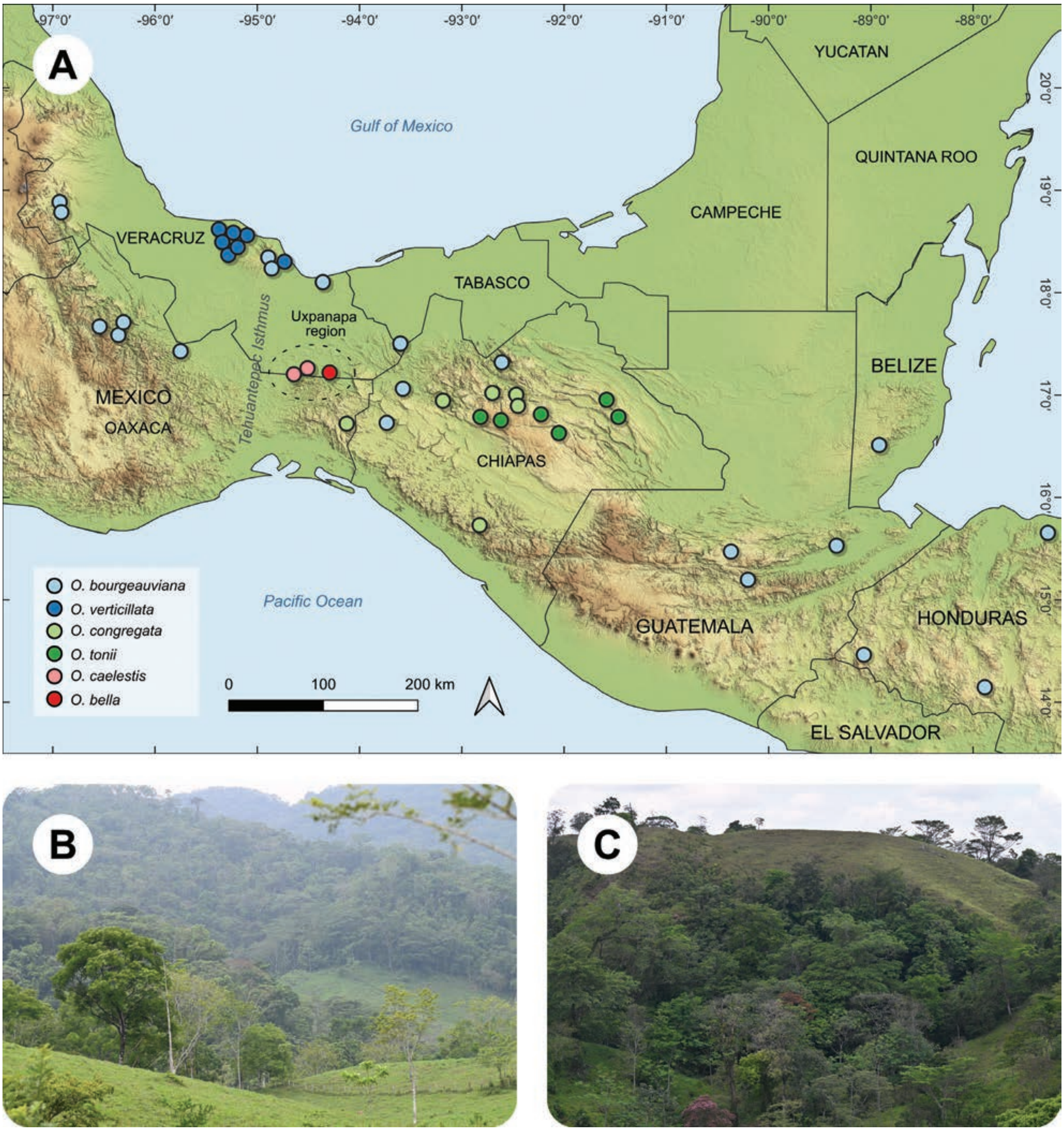

Figure 4. A distribution of the *Ocotea helicterifolia* group species with clustered leaves **B** habitat of *Ocotea bella* at the type locality **C** habitat of *Ocotea caelestis* at the type locality. Distribution localities are based on specimen records at the MEXU Herbarium.

Conservation status. We currently lack the necessary information to assess definitively the conservation status of *Ocotea bella*. However, based on application of the criteria established by the IUCN (IUCN 2012, 2024), we conclude tentatively that the species is Critically Endangered [CR B2ab(ii,iii)]. Its area of occupancy (AOO) is 4.0 km, within the limits of Critically Endangered status under criterion B. The species is known from a single location (*sensu* IUCN 2024), also within the limit of the Critically Endangered status. In addition, the species appears to be rare. We found only one mature individual within an area of approximately 2 km^2. Moreover, this species is not found within any protected natural areas, and it faces threats from deforestation and unsustainable practices such as logging, fires, and illegal settlements. Thus, we project that the ongoing loss of its habitat will induce a strong continuous decline of its AOO.

Notes. Among the species of *Ocotea helicterifolia* group, the reddish stamens of *Ocotea bella* are unique and distinguish it very well from any other species. It is an unusual character among neotropical Lauraceae. Additionally, the clustered leaves in this group seem restricted to northern Mesoamerica, ranging from Mexico to Honduras. Among the species with clustered leaves, the species most similar to *Ocotea bella* is *Ocotea congregata* van der Werff, endemic to Chiapas, Mexico (Fig. 4). Vegetatively, *Ocotea congregata* is distinguished from *Ocotea bella* by its smaller, broadly elliptical leaves, with rounded basis, obtuse to acute apex, and long petioles (Table 1). Additionally, *Ocotea congregata* prefers montane forests (around 1500 m altitude) whilst *Ocotea bella* is restricted to lowland forests (200–300 m altitude).

Ocotea caelestis **Ortiz-Rodr. & Reveles, sp. nov.**

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Figs 5, 6

Type. Mexico • Veracruz, Uxpanapa, 5 km south from Poblado 2 village, 17°11'28.4"N, 94°38'34.4"W, elev. 195 m, 1 May 2023, *Hurtado-Reveles L.* 1995 (holotype: MEXU; isotypes: NY, MO).

Diagnosis. Similar to *Ocotea bourgeauviana* in having clustered leaves and a glabrous outer surface of the tepals, but differs from it in its smaller leaves and flowers, adaxial surface only pubescent at the base, tongue-shaped stamens with a sterile tip and with pubescent filaments (Table 1).

Description. ***Trees***, evergreen, 4–8 m tall, 8–12 cm DBH; bark smooth to slightly striate; twigs terete, densely covered with erect and adpressed, golden trichomes, glabrous with age, terminal buds 5 × 5 mm, densely covered with erect and adpressed golden trichomes. ***Leaves*** aggregated to alternate-verticillate, clustered near the apex of branches, 2–5 × 7–18 cm, coriaceous, elliptic to lanceolate, base acute to attenuate, apex acute to caudate, upper surface smooth, glabrous, lower surface densely covered with erect golden trichomes, midrib slightly raised on the upper surface, prominently raised on the lower surface; secondary veins 5–8 on each side, slightly impressed above, prominently raised below; petiole swollen, 5–8 mm long, densely covered with erect and adpressed, golden trichomes. ***Inflorescences***, paniculate–cymose, axillary, 4–8 cm long, pedicels 1–3 mm long, the main axis, peduncles, pedicels and bracts densely covered with erect light–brown trichomes. ***Flowers*** bisexual, 4–5 mm in diam., tepals 6, subequal, ovate to elliptic, 2–3 × 1–2 mm, outer surface glabrous, inner surface with sparse, brown sericeous trichomes only near the base, stamens 9, in three whorls, white, ca. 1.5 mm long, filaments very short to anthers almost sessile, densely covered with small trichomes, anthers tongue-shaped, with a sterile tip which extends for ca. a third of the length of the total length of the anther, 4–locular, pollen sacs in two pairs above each other, inner stamens with two globose glands at the base, staminodes capitate, ca. 0.8 mm long, filament short and pubescent, pistil glabrous, ovary ovoid, 10 × 8 mm, style glabrous, ca. 0.5 mm long. ***Fruit*** unknown.

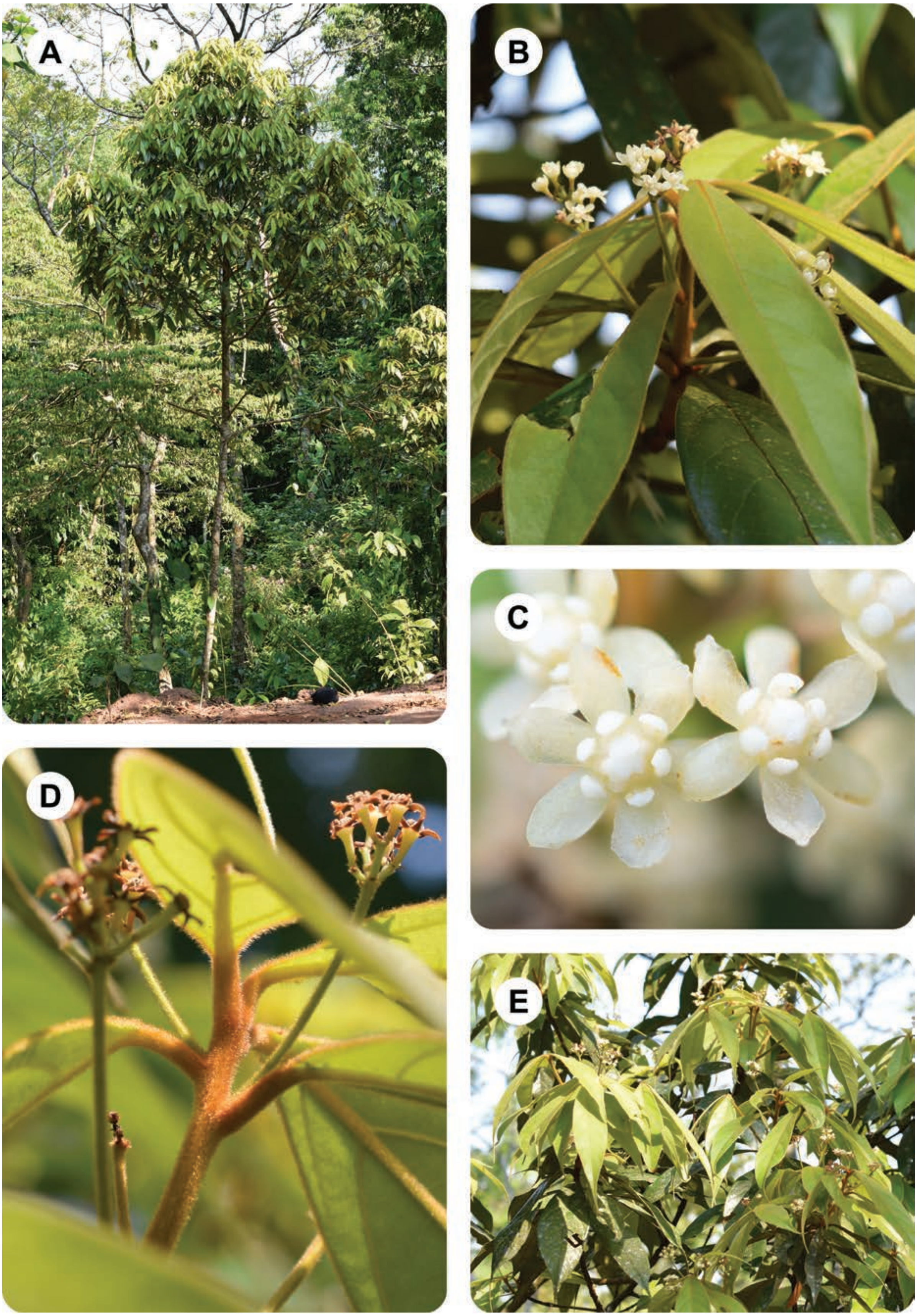

Figure 5. *Ocotea caelestis* Ortiz-Rodr. & Reveles **A** general view of the tree **B** tip of flowering branch, showing leaf and inflorescence arrangement **C** flowers **D** apical part of recent growth showing the indument **E** branches with young and mature leaves.

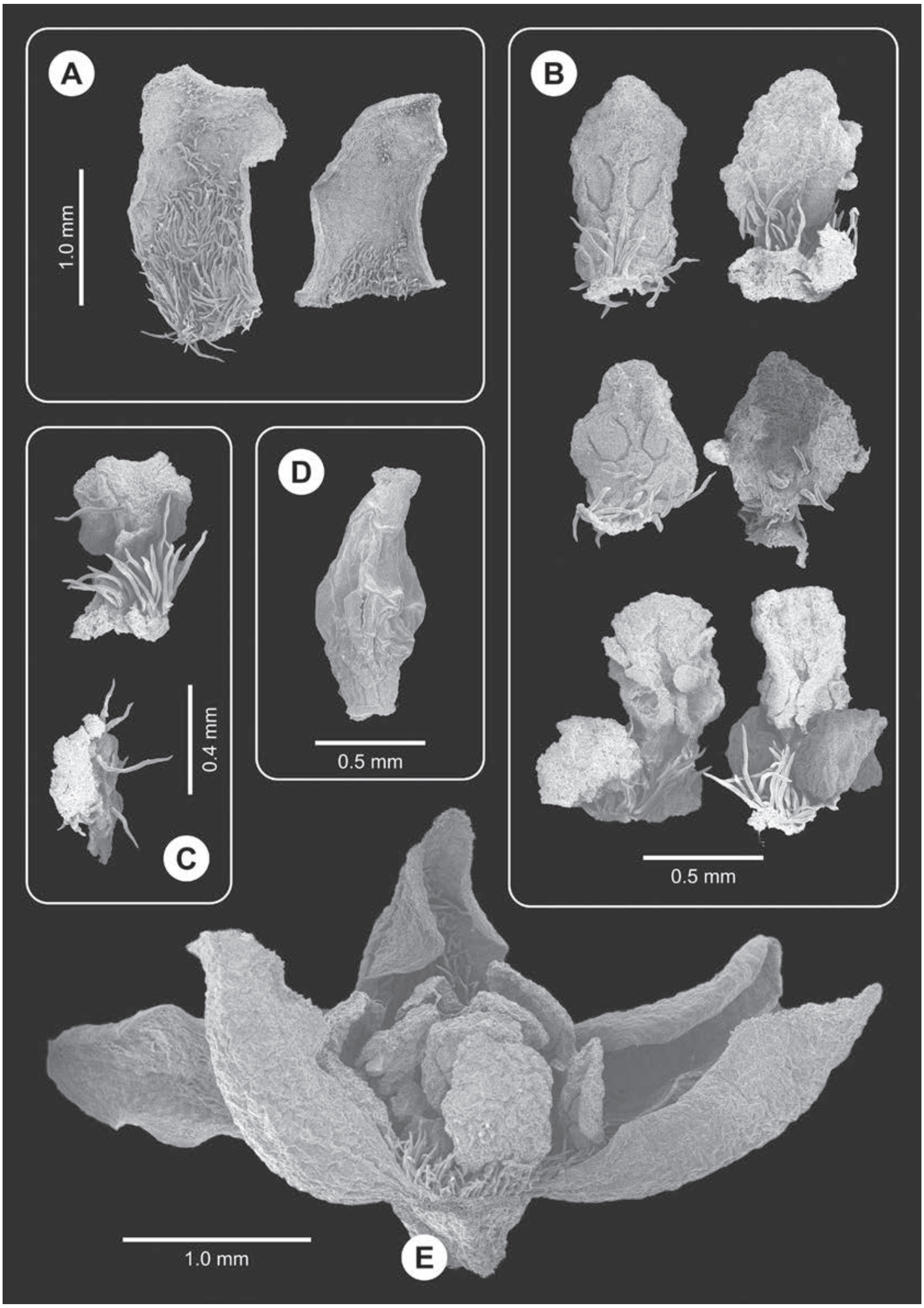

Figure 6. Flowers and floral parts of *Ocotea caelestis* (SEM photos) **A** adaxial view of tepals **B** stamens, adaxial and abaxial view, first to third whorl, in pairs from top to bottom **C** staminode abaxial and side view **D** pistil **E** flower, lateral view, one tepal of first whorl (in front) removed.

Phenology. This species has been collected with flowers from April to May.

Etymology. The specific epithet refers to the ethereal appearance of this species (from the latin *caelestis*= heavenly appearance). The fine and dense golden indument in young leaves and twigs gives it a particular brightness during the first hours of the day. Moreover, inflorescences in living specimens notably rise above the flaccid leaves, which combined with their soft whitish color and almost glassy look gives them an ethereal appearance.

Distribution. *Ocotea caelestis* is known only from the Uxpanapa region in Veracruz, Southern Mexico (Fig. 4).

Ecology and habitat. *Ocotea caelestis* inhabits the tropical rainforests established on deep soils, with mean annual temperatures of 25 °C and mean annual precipitation of around 3250 mm. It occupies areas close to the hill ridges.

Conservation status. Based on the criteria established by the IUCN (IUCN 2012, 2024), we conclude tentatively that the species is Critically Endangered [CR B2ab(ii,iii,v)]. Its area of occupancy (AOO) is 8.0 km^2, within the limits of the Critically Endangered status under criterion B. The species is known from one location (*sensu* IUCN 2024), also within the limit of Critically Endangered status. Furthermore, only two adult individuals within an approximate area of 1 km^2 have been found. The oldest collection near the type locality is more than 30 years old, suggesting that the species may persist in forest patches. Since the species is not found within any protected area, the increasing land use for livestock farming in the region is likely its main threat.

Additional specimens examined. Mexico • Veracruz, Jesús Carranza, south from Poblado 2 village, 17°12'00"N, 94°38'30.0"W, elev. 200 m, 12 Apr 1987, Tom Wendt & H. Hernández G. 5622 (MEXU).

Notes. Within the *Ocotea helicterifolia* group, tongue-shaped stamens with a sterile tip have been reported only in *Ocotea botrantha* Rohwer, *Ocotea sinuata* (Mez) Rohwer, *Ocotea tonii* (Lundell) van der Werff, *Ocotea verticillata* Rohwer and now in *Ocotea caelestis*. The clustered leaves relate *Ocotea caelestis* to *Ocotea verticillata*, from which it differs in its smaller leaves, with acute to cuneate basis, smaller flowers, outer surface of tepals glabrous, inner surface pubescent only at the base, and capitate staminodes shape (see Table 1).

Vegetatively, *Ocotea caelestis* is very similar to *Ocotea bourgeauviana* (Mez) van der Werff and could hardly be separated in the absence of flowers. Flowering specimens of the two species can be easily distinguished. Flowers in *O. bourgeauviana* have trichomes distributed throughout the inner surface of the tepals (only at the base in *Ocotea caelestis*), and its stamens are not tongue-shaped and do not have a sterile tip. Moreover, both species are allopatrically distributed (Fig. 4).

Discussion

Taxonomic novelties within a hyper-diverse region: Uxpanapa, Mexico

The isolated mountains of the Tehuantepec Isthmus in the Mexican states of Chiapas, Oaxaca, and Veracruz have long fascinated taxonomists due to their high level of endemism and species richness (Wendt 1987; Salazar 1999; Molina-Paniagua et al. 2023). In particular, the region of Uxpanapa, located between the states of Veracruz and Oaxaca, is considered a center of speciation and harbors many endemic species (Wendt 1987). Speciation processes in the Uxpanapa region may have been influenced by its role as a refuge for wet-tropical species during Pleistocene climatic fluctuations, as well as by its topographical complexity (Wendt 1987; Molina-Paniagua et al. 2023).

Only in the last two decades, several new plant species have been described from this region [e.g. Ceratozamia *dominguezii* Pérez-Farr. & Gut.-Ortega (Zamiaceae), *Desmopsis terriflora* G.E. Schatz, T. Wendt, Ortiz-Rodr. & Mart.-Vel. (Annonaceae), *Ixchelia uxpanapana* (T. Wendt) Wahlert & H.E. Ballard (Violaceae), *Lobelia lithophila* Senterre & Cast.-Campos (Campanulaceae), *Magnolia uxpanapana* A. Vázquez, Padilla-Lepe & Gallardo-Yobal (Magnoliaceae), *Mortoniodendron uxpanapense* Dorr & T. Wendt (Malvaceae), *Peperomia nopalana* G. Mathieu, *P. trichobracteata* G. Mathieu & T. Krömer & *P. xalana* G. Mathieu (Piperaceae), *Styrax uxpanapensis* P.W. Fritsch (Styraceae) (Dorr and Wendt 2004; Fritsch 2005; Senterre and Castillo-Campos 2008; Wahlert et al. 2015; Jimeno-Sevilla et al. 2018; Pérez-Farrera et al. 2021; Martínez-Velarde et al. 2023; Vázquez-García et al. 2024)]. An interesting pattern is the high level of divergence between species endemic to the Uxpanapa region and their closest relatives distributed outside this region (e.g. Senterre and Castillo-Campos 2008; Martínez-Velarde et al. 2023). Of course, the species described here fit this pattern well. *Ocotea bella* has flowers with red stamens and white petals, features absent in other species of the *Ocotea helicterifolia* group. Also, only *Ocotea caelestis* has the combination of clustered leaves with acute to cuneate basis and tongue-shaped stamens with a sterile tip.

Despite all of this, the Uxpanapa region is considered poorly surveyed, with intermittent exploration periods, old and unrepresentative collections and few specimens per species (Carvajal-Hernández 2018). Consequently, it is not surprising that new botanical explorations are leading to the discovery of new taxa. Considering the biological context, the Uxpanapa region's future is alarmingly concerning. It is an area highly impacted by humans and vulnerable to climate change (Aguilar-López et al. 2016). Ongoing efforts to study and catalogue its biodiversity need to be strengthened, and effective conservation programs must be implemented (Carvajal-Hernández 2018).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

LHR & AEOR conceived the idea, conducted the fieldwork, analysed the data and wrote the final version of the manuscript.

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Data availability

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

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Research Article

Ovicula biradiata, a new genus of Compositae from Big Bend National Park in Trans-Pecos Texas

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Abstract

Here, we describe and illustrate a new monospecific genus of Compositae, *Ovicula biradiata* **gen. et sp. nov.**, from the Chihuahuan Desert in Big Bend National Park, Texas. *Ovicula biradiata* is a very locally abundant, yet range-limited, spring annual herb found in coarse calcareous alluvium. Based on its pistillate ray florets, pappus of hyaline, aristate scales, tomentose foliage and slightly saucer-shaped to flat, epaleate receptacle, we determine that the new species has affinities with the Helenioid subtribe Tetraneurinae in the Heliantheae alliance. Molecular phylogenetic analysis of nrDNA (ITS) sequence data supports the phylogenetic position of *Ovicula biradiata* within the subtribe Tetranuerinae, where it is resolved as the sister lineage to the genus *Psilostrophe*. We also present detailed habitat information, high-resolution images captured using a dissecting microscope and scanning electron micrographs of vegetative and reproductive characters of *Ovicula biradiata* and related taxa, as well as provide an updated key to the genera of Tetraneurinae. Finally, we discuss the significance of this remarkable discovery for community science, biodiversity exploration and plant conservation in the Chihuahuan Desert.

Resumen

Se ilustra y describe un nuevo género monoespecífico de Compositae, *Ovicula biradiata* **gen. et sp. nov.**, del Desierto Chihuahuense en el Parque Nacional Big Bend, Texas. *Ovicula biradiata* es una hierba annual muy localmente abundante, pero con un rango limitado, florece en la primavera y se encuentra en aluviones calcáreos gruesos. Con base en sus flores pistiladas con corola radiada, vilano de escamas aristadas y hialinas, follaje tomentoso y receptáculo ligeramente en forma de platillo a plano y epaleado, determinamos que la nueva especie tiene afinidades con Helenieae subtribu Tetraneurinae, en la alianza Heliantheae. Análisis cladísticos moleculares de secuencias del ADN ribosomal nuclear (ITS) apoyan la posición filogenética de *O. biradiata* dentro de la subtribu Tetraneurinae, donde resulta el lineage hermano del género *Psilostrophe*. También presentamos información detallada sobre el hábitat, imágenes de alta resolución usando un microscopio de disección y micrografías electrónicas de barrido de caracteres vegetativos y reproductivos de *O. biradiata* y taxa relacionados, así como una clave actualizada para los géneros de Tetranuerinae. Finalmente, discutimos la significancia del descubrimiento en relación a la ciencia ciudadana, exploración de biodiversidad, y la conservación de plantas en el Desierto Chihuahuense.

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Key words: Asteraceae, biodiversity, calciphile, Chihuahuan Desert, Helenieae, taxonomy, Tetraneurinae

Palabras clave: Asteraceae, biodiversidad, calcifilo, Desierto Chihuahuense, Helenieae, taxonomía, Tetraneurinae

Introduction

The Chihuahuan Desert is the largest and most biologically diverse warm desert in North America (Bell et al. 2014). Big Bend National Park is in southern Brewster County, Texas, bordered to the south by the Rio Grande. Its 801,165 acres (324,220 hectares) include some of the best representation of Chihuahuan Desert microhabitat diversity in the United States, including within the Chisos Mountain range (highest elevation ca. 7825 ft. (2385 m)) and numerous smaller peaks separated by low desert bajadas. Eighty-nine plant species of conservation concern are found in the park (Louie 1996; Poole et al. 2007, Texas Parks and Wildlife Department 2024). Many of these species of concern have limited distributions that extend into adjacent areas in Mexico or into Chihuahuan Desert habitats to the immediate north and east of the park boundary (Powell and Worthington 2018).

Previous floristic and wildflower studies centred in and near Big Bend National Park, include McDougall and Sperry (1951), Warnock (1970, 1974, 1977), Fenstermacher et al. (2008), Morey (2008, 2024), Hardy (2009), Weckesser and Terry (2014) and Powell and Worthington (2018). Wauer (1973, 1997) explored many off-the-trail areas searching for plants and animals of natural history interest. Potentially relevant floristic treatises of wider range include Correll and Johnston (1970), Henrickson et al. (1997), Turner et al. (2003), Turner (2013), Eason (2018) and Allred et al. (2020). Even though the park has been rather thoroughly botanised in the vicinity of most accessible areas, additional new plant discoveries are possible because of its extensive habitat diversity in stretches of remote terrain.

On 2 March 2024, while traversing cross country in search of rare plant populations, the first author photographed an anomalous composite and posted images on iNaturalist. These diminutive plants, observed during the peak of their growing season, were inconspicuous annuals, from less than one centimetre to 3–7 centimetres across, prostrate and densely white-woolly, matching the whitish colour of their calcareous gravel substrate (Figs 1–9). Following a review, Park authorities granted us permission to collect a few individuals for further study. From the limited material on hand and the photos we had taken, we were able to discern characters suggestive of relationships to the tribe Helenieae Lindl. (sensu Baldwin et al. (2002)), especially *Tetraneuris* Greene. These characters included obconic fruits with five paleaceous, aristate scales and pistillate ray florets with maroon linear markings (Bierner and Turner 2006; Funk et al. 2009; Spellenberg and Zucker 2019). To test the hypothesised relationship to Helenieae, we carried out a more detailed study of inflorescence and fruit characters using scanning electron microscopy and sequenced nrDNA sequence data for one DNA gene region, the Internal Transcribed Spacer (ITS). Here, we present morphological, micro-anatomical and molecular phylogenetic evidence that supports description of this plant as a new genus and species of subtribe Tetraneurinae Rydb.

Methods

Field and herbarium collections

After the National Park Service granted a research permit for this study (BIBE-2024-SCI-0015), plants were collected from two field locations, briefly dried and deposited in the A. Michael Powell (SRSC) and California Academy of Sciences (CAS) Herbaria for mounting and further study. To our knowledge, besides these collections, this previously unknown species has not been deposited in herbaria before. Dried vegetative and reproductive material of putative close relatives was obtained for detailed morphological study and DNA sequencing from herbarium specimens at SRSU and CAS. Sampling included representatives of genera in subtribe Tetraneurinae, i.e. *Amblyolepis* DC., *Baileya* Harv. & A. Gray ex Torr., *Hymenoxys* Cass., *Psilostrophe* DC. and *Tetraneuris* Greene. A complete list of specimens sampled and GenBank accession numbers is presented in Table 1.

Morphological study

We examined morphological characters from field collections of the new species and exsiccate of putative close relatives using dissecting microscopy. Images of microscopic features were captured using a Leica M60 stereomicroscope (Leica Camera, Wetztlar, Germany) outfitted with a digital camera.

Table 1. Specimen voucher data and GenBank accession numbers for herbarium material used in molecular phylogenetic analyses and scanning electron microscopy.

Taxon	Purpose	Accession number	Collector	Collector number	Date	GenBank accession number
Psilostrophe bakeri Greene	DNA/SEM	CAS 818281	A. Cronsquist	11645	13 June 1980	PQ144335
Baileya pauciradiata Harv. & A. Gray	SEM	CAS 288097	H.S. Gentry		25 Feb 1933	
Tetraneuris acaulis (Greene) K.F. Parker var. *arizonica*	SEM	CAS 731062	J. Henrickson	10576	4 June 1973	
Psilostrophe sparsiflora (A.Gray) Nelson	DNA	CAS 5935742	M. Butterwick	7526	19 May 1981	PQ144336
Tetraneuris scaposa (DC.) Greene	DNA/SEM	CAS 700109	B. Turner	15128	5 June 1983	PQ144339
Baileya multiradiata harv. & A. Gray	SEM	CAS 713832	P. Munz	13688	3 May 1935	
Amblyolepis setigera DC.	SEM	CAS 765096	B. Ertter	5598	13 March 1985	
Psilostrophe villosa Rydb. ex Britton	DNA	CAS 507562	P. Raven	19297	7 June 1964	PQ144334
Hymenoxys cooperi Cockerell var. *cooperi*	SEM	CAS 1005608	J. Henrickson	10521	4 June 1973	
Psilostrophe mexicana R.C. Br.	DNA	CAS 720959	J.L. Villaseñor	1591	23 September 1982	PQ144337
Psilostrophe gnaphalodes DC.	DNA	CAS 701425	S. Sunderberg	1214	15 August 1981	PQ144338
Ovicula biradiata Manley	DNA/SEM	SRSC 00058752	D. Manley	2	17 April 2024	PQ144333

Table 2. Comparison of morphology amongst genera of subtribe Tetraneurinae.

Character/taxon	*Hymenoxys*	*Tetraneuris*	*Amblyolepis*	*Psilostrophe*	*Ovicula*	*Baileya*
Life span	Annual, biennial or perennial	Annual or perennial	Annual	Biennial, perennial or shrubby	Ephemeral annual	Annual, biennial or perennial
Stems	5–120+ long; erect, often branched; glabrous or pilose	5–50 cm long; erect, or plants, acaulescent; sparsely to densely pilose	Usually 12–50 cm long; erect to decumbent; sparsely to moderately pilose	8–50+ cm long; spreading to erect; often densely woolly	1–4 cm long; prostrate; densely woolly	Usually 15–75 cm long; mostly erect; woolly
Leaves/blades	Basal and cauline; simple or 1–2-pinnately lobed; glabrous or pilose	Basal or basal and cauline; linear to lanceolate; glabrous or pilose	Cauline; linear to spatulate; pilose	Basal and cauline; linear to spatulate; sparsely to densely woolly	Basal ovate, involute to nearly folded; densely woolly	Basal and cauline; linear to ovate, often pinnately lobed; often densely woolly
Heads/ peduncles	Single or several; peduncles 0.4–20+ cm long	Single or several; peduncles 0.5–40+ cm long	Usually single; peduncles to 20 cm long	Single or in clusters; peduncles 0.5–60+ cm long	Single; sessile or peduncles 1 mm long	Single or several; peduncles 2–12 cm long
Involucres	2.5–30 mm wide	6–20 mm wide	12–20 mm wide	2–7 mm wide	To 4–6 mm wide	5–25 mm wide
Phyllaries	2–3-seriate; sparsely to moderately pilose	3-seriate; sparsely to densely pilose	2-seriate; sparsely to moderately pilose	1–2-seriate; densely woolly	3-seriate; densely woolly	2-seriate; moderately to densely woolly
Ray florets	(3–)8–13+; corollas yellow to orange, corollas yellow to orange, nerves colourless or greenish; ray floret corollas 0.7–12 mm wide	None or 7–27; corollas yellow, nerves colourless, greenish, sometimes reddish-brown to maroon; ray floret corollas 2.5–6 mm wide	Usually 8–13; corollas yellow, nerves colourless or greenish, sometimes darker than background of laminae; ray floret corollas 4.5–10 mm wide	1–8; corollas yellow, nerves greenish, sometimes darker than background; ray floret corollas 3–20 mm wide	2 (-3); corollas whitish, nerves maroon; ray floret corollas 0.6–1 mm wide	5–7 or 20–55; corollas yellow, nerves colourless to greenish; ray floret corollas 4–7 mm wide
Disc florets	Usually 25–50+; corollas yellow to brownish-yellow, 1.5–7.4 mm long; pubescent distally, trichomes to 0.2 mm long	20–250+; corollas yellow, purplish distally, 1.6–3 mm long; pubescent mainly distally, trichomes to 0.1–0.2 mm long	20–-50; corollas yellow, 5–7 mm long; essentially glabrous distally	5–25+; corollas yellow to orange, 3.5–5.5 mm long; pubescent distally, trichomes 0.1–-0.2 mm long	10–12; corollas pale yellow, ca. 2–-3 mm long; tomentose distally, trichomes 0.3–0.5 mm long	10–20, or 40–100+; corollas yellow, 2.5–4 mm long; densely pubescent distally, trichomes to ca. 0.2 mm long
Cypselae	Obconic or obpyramidal, 1.4–4.7 mm long; glabrous or pilose	Obconic or obpyramidal, 1.5–4 mm long; moderately to densely tomentose	Obconic, 3–4.5 mm long, prominently 10-ribbed; ribs densely tan-tomentose	Cylindrical to clavate or obpyramidal, striate-ribbed, 2.5–4 mm long; glabrous, gland-dotted, or villous	Obconic-obpyramidal, faintly ribbed, 1.5–2 mm long; densely tomentose	Narrowly obpyramidal, 3–4 mm long, weakly ribbed or striate; glandular
Pappus	None or of 2–11(–15) usually aristate, obovate to lanceolate, scales, 0.8–4.3 mm long	Usually 4–8 aristate lanceolate, obovate, to oblanceolate, scales, 1–4.5 mm long	5–6 ovate scales 2–3.5 mm long	4–8 oblong, elliptic or lanceolate, scales, 1.5–3.2 mm long	5 aristate, oval scales, to 2 mm long	Usually absent, rarely scales
Base chromosome number	$x = 15$	$x = 15$	$x = 19$	$x = 16$	$x = ?$	$x = 16$

Morphology was compared with representatives of all recognised genera of tribe Helenieae (Table 2). In addition, surface morphology of floral and vegetative structures was analysed and imaged using a Hitachi SU3500 Scanning Electron Microscope (SEM; Hitachi, Tokyo, Japan) at the California Academy of Sciences. Initially, inflorescence and fruit structures were disassembled under a dissecting microscope and loaded on to an 18 mm pin-mounted SEM stub using double sticky tape. To enhance the electron conductivity of samples, we then used a Cressington Sputter Coater 108 (Cressington, Watford, UK) at a vacuum pressure of 0.8 Pa to apply a 5 nm layer of gold-palladium to the sample for 50 seconds. We observed traits of potential phylogenetic informativeness following Robinson (1981) and King and Robinson (1970) at 15 kV and a working distance of 7 mm, under automated controls for focus, contrast and stigmation.

DNA extraction, amplification and sequencing

Following removal of the woolly indumentum under a dissecting microscope, fresh, field-collected leaves were dried for one week using silica gel and pulverised in a Qiagen tissue lyser (Qiagen, inc., Valencia, California) with a mixture of zircon beads and autoclaved sand. Genomic DNA was extracted using the DNEasy plant mini-kit (Qiagen, inc., Valencia, California) in the Center for Comparative Genomics at the California Academy of Sciences. We followed the provided protocol with a modified incubation in a cell-lysis buffer extended to 16 hours. A Polymerase Chain Reaction (PCR) master mix containing 9.1 ul H_20, 0.3 ul DnTPs, 0.15 ul Taq polymerase, 0.75 ul $MgCl_2$, 1.5 ul 10× PCR buffer and 0.6 ul Bovine serum Albumin (BVA) was combined with two primers for amplifying the Internal Transcribed Spacer region (ITS), ITS4 and LEU (White et al. 1990). Two ul of undiluted genomic DNA was combined with the PCR master mix and transferred to a thermal cycler programmed to the following conditions: 97 degrees for 1 min; 40 cycles of 97 degrees for 10 sec, 48 degrees for 30 sec, 72 degrees C for 20 seconds; and 72 degrees C for 7 minutes. Post-PCR products were checked for successful amplification using gel electrophoresis and unpurified PCR-product was forward and reverse Sanger sequenced by Genewiz (Azenta US Inc., Burlington, MA).

Following an initial search of the NCBI BLAST database to confirm a close match between our ITS sequence and putative closely-related taxa, we visually aligned the ITS sequence for the new species with the Baldwin et al. (2002) published data matrix for epaleate tribes of the Heliantheae alliance. Once we recovered strong evidence for the sister relationship of the new species with *Psilostrophe* in tribe Helenieae, we generated additional sequence data for all recognised minimum-rank taxa of *Psilostrophe* and *Tetraneuris scaposa* (DC.) Greene using leaf tissues sampled from herbarium specimens. For these additional samples, we followed an extraction and amplification procedure identical to that described above. Selection of a model of molecular substitution and Maximum Likelihood (ML) inference of a phylogenetic tree, based on aligned data matrices of ITS, was inferred using IQTREE2 (Minh et al. 2020) and bootstrap support for nodes was calculated, based on 1000 iterations using fast-bootstrapping.

Results

Phylogenetic relationships

Preliminary searches of the NCBI nucleotide BLAST database showed a significant match between ITS sequences of the new species and core members of subtribe Tetraneurinae, including *Psilostrophe cooperi* (A. Gray) Greene (88.69%), *Baileya multiradiata* Harv. & A. Gray (88.89%), *Tetraneuris acaulis* Greene (88.48%) and *Hymenoxys lemmonii* Cockerell (88.68%). The ML phylogenetic tree, based on the ITS alignment from Baldwin et al. (2002), resolved the new species with very high (98 bs) support as nested in tribe Helenieae, where it was more closely related to *Psilostrophe* than other members of subtribe Tetranuerinae (Fig. 10). The new species + *Psilostrophe*, in turn, form the sister lineage to the clade containing *Amblyolepis setigera* DC., *Tetraneuris acaulis*, *Tetraneuris scaposa* (DC.) Greene, *Hymenoxys ambigens* var. *floribunda* (A.Gray) W.L. Wagner, *Hymenoxys hoopesii* (A. Gray) Bierne, and *Hymenoxys lemmonii* Cockerell. Addition of DNA sequence data (ITS) for five previously unsampled taxa of *Psilostrophe* resolves all currently recognised minimum-rank taxa in this genus as a monophyletic group separate from and sister to the new species. Amongst taxa of *Psilostrophe*, the narrowly endemic *P. bakeri* is resolved as sister to the rest of the genus, with *P. sparsiflora* next to diverge, followed by *P. cooperi*. Relationships amongst the highly-nested taxa *P. mexicana*, *P. gnaphalodes*, *P. tagetina* and *P. villosa* were not well supported with ITS data alone.

Micro-anatomy

Micro-morphological features targeted using SEM for their value in evaluating phylogenetic relationships in the Heliantheae alliance included the surface texture of cypselae, pappus elements, trichomes, style trichomes, stigmatic surface, pollen shape and glands of vegetative and reproductive structures. A comparative table (Table 2) of these characteristics for genera of Tetraneurinae is given along with plates of SEM images (Figs 11, 12). Micro-anatomical features of the new species revealed by SEM include the dentate margins and pleated structure of hyaline pappus scales, style branch apices with terminal papillae and short stipitate glands that are present on the abaxial surface of ray and disc corolla lobes. Two types of trichomes were observed. Cypselae trichomes appear stiff, linear and end in a bifurcate tip. These conform with the cypselar trichomes observed in many other Compositae, also called twin hairs (“Zwillingshaare”) by Hess (1938). Trichomes on leaf tissues have a dilatated base (foot) that is notably wider than the rest of the structure, which has an elongated, flagellate body, an apex that ends in a simple, unbranched tip and a flexible, convoluted, helical structure, presumably giving the plant its characteristic woolly appearance. These trichomes conform to the oblique septate flagellate trichome type identified by Ramayya (1962), which occur in many groups of Compositae and often render the plant surface tomentose. Pollen grains of the new species measure approximately 20 micrometres in diameter and are oblate spheroidal in shape, with evenly spaced, symmetrical echinate projections.

SEM images of representatives of related genera revealed similarities in the paleaceous and finely pleated structure of the pappus (Figs 11H, 12A, E, I), pollen (Figs 11D, 12D), short-stalked stipitate glands (Figs 11C, 12C), stiff cypselae trichomes with forked tips (Figs 11A, 12D), presence of the flexible helical trichomes that give the new species its woolly appearance (Fig. 11B) and style branch apices with sweeping papillae (Figs 11**I**, 12H). Some consistent differences that were noted between the new species and its sister genus *Psilostrophe* included the vestiture of disc and ray floret corolla lobes, which consist of papillae in *Psilostrophe*, whereas the new species possesses helical trichomes along ray throats and on abaxial surfaces of disc lobes (Figs 11F, 12F). Finer variability was evident in the size and shape of the apex in paleaceous pappus elements at shallower taxonomic scales (Figs 11H, 12A, E, **I**).

Taxonomic treatment

Ovicula biradiata **Manley, gen. et. sp. nov.**
urn:lsid:ipni.org:names:77356807-1
Figs 1–8

Description. ***Annuals***, small, flowering plants usually 1–2(–3) cm tall, from less than 1 cm wide to 3–7 cm across, whole plants densely white-tomentose. ***Root*** single, thread-like, 0.5–1 mm wide at the plant base. ***Stems*** unbranched, erect or branches, if evident, lateral, prostrate, spreading 1–4 cm in one or more directions, internodes ca. 1 cm long. ***Leaves*** basal, mostly in tight clusters or at nodes on short stems, proximal leaves spreading, distal ascending, petioles 1–4 mm long, blades ovate, 4–7 × 2.5–5 mm, entire, planar, gently involute or nearly conduplicate. ***Heads*** heterogamous, borne singly, essentially sessile (peduncles to ca. 1 mm long), obscured by woolly leaves. ***Involucres*** 5–7 × 4–6 mm, broadly funnelform to campanulate or subglobose. ***Phyllaries*** in 3 series, ca. 1–2 in outer series, ca. 1–2 in second series, outer slightly spreading, those in outer 2 series 3–4 × 2–3 mm, ovate, inner series ca. 7, linear, ca. 2 mm wide, with scarious margins ca. 0.5 mm wide, densely white-tomentose. ***Receptacles*** ca. 1 mm across, slightly saucer-shaped to flat, sometimes with a very small conic enation from near centre, otherwise basically smooth or with faint floret scars, epaleate. ***Ray florets*** 2(–3) per head, 3–6 × 0.6–1 mm long, positioned on opposing sides, pistillate and fertile, strap-like; corolla tube 2–3 mm long, densely pilose distally, with wavy trichomes 0.3–1 mm long, laminae 3–6 × 0.6–1 mm, 3-lobed, whitish, markedly 4-nerved proximally, 6-nerved distally, nerves maroon, proximal portion of the abaxial ray laminae densely covered with sessile or short stipitate glandular trichomes. ***Disc florets*** 10–12 per head, perfect and fertile; corolla pale yellow, ca. 2–3 mm long, tube 0.6–0.9 mm long, throat 1.6–1.8 mm long, lobes 5, 0.1–0.3 mm long, distalmost throat and lobes densely pilose with wavy trichomes 0.3–0.5 mm long; anthers yellow, distal anther appendage narrowly obovate to subsagittate; style tip appendage truncate, apex papillate. ***Cypselae*** of ray and disc florets similar, 1.5–2 mm long, obconic-obpyramidal, slightly compressed or obscurely 4–5-angled (prismatic), ribs 4–5, densely pubescent with straight, ascending-appressed, silvery trichomes 0.5–0.9 mm long, minutely forked at tip, partially obscuring the bases of pappus scales. ***Pappus*** of ray and disc florets similar, scales 5, ca. 1–3 × 0.8–1 mm, ovate, hyaline, with an apical arista ca. 1 mm long; the scales spreading when dry (Figs 1–7). Chromosome number unknown.

Similar to members of tribe Helenieae (sensu Baldwin et al. (2002)), especially *Tetraneuris*, with its annual habit, radiate heads, phyllaries in 3 series,

Figure 1. First photograph of *Ovicula biradiata* taken by Deb Manley on 2 March 2024.

convex, epaleate receptacles, ray florets pistillate and fertile, strap-like 3-lobed ray floret laminae with prominent, coloured veins, cypselae obconical, faintly ribbed and pappus of hyaline aristate scales, disc florets perfect and fertile, corollas yellow, 5-lobed; differs from other Helenieae genera by its smaller size, shorter stems, tightly clustered small leaves, greater tomentum density and smaller, sessile heads with only 2(–3) ray florets.

Type. USA • Texas: Brewster Co.; Big Bend National Park, low gravelly limestone exposure, eroded alluvial flats, NE of Dagger Mt.; elev. 800 m, 20 Apr 2024, *Debra Manley 2*, with *C. Whiting*, *C. Hoyt*, *P. Manning*, and *S. Menzies*; holotype: SRSC 00058752 (BIBE 61799); isotype: CAS 1352777 (BIBE 61820).

Paratypes. USA • Texas; Brewster Co.: Big Bend National Park, low gravelly limestone exposure, eroded alluvial flats, NE of Dagger Mt.; elev. 792.5 m, 20 Apr 2024, *Debra Manley 3*, with *C. Whiting, C. Hoyt, P. Manning,* and *S. Menzies*; BIBE 61800 (SRSC 00058751).

Figure 2. Researchers examining individuals in habitat. **A** Patty Manning scanning the ground in appropriate habitat for individuals of *Ovicula biradiata* **B** NPS botanist Carolyn Whiting photographing *O. biradiata* (circled in red). Photos by Cathy Hoyt on 20 April 2024.

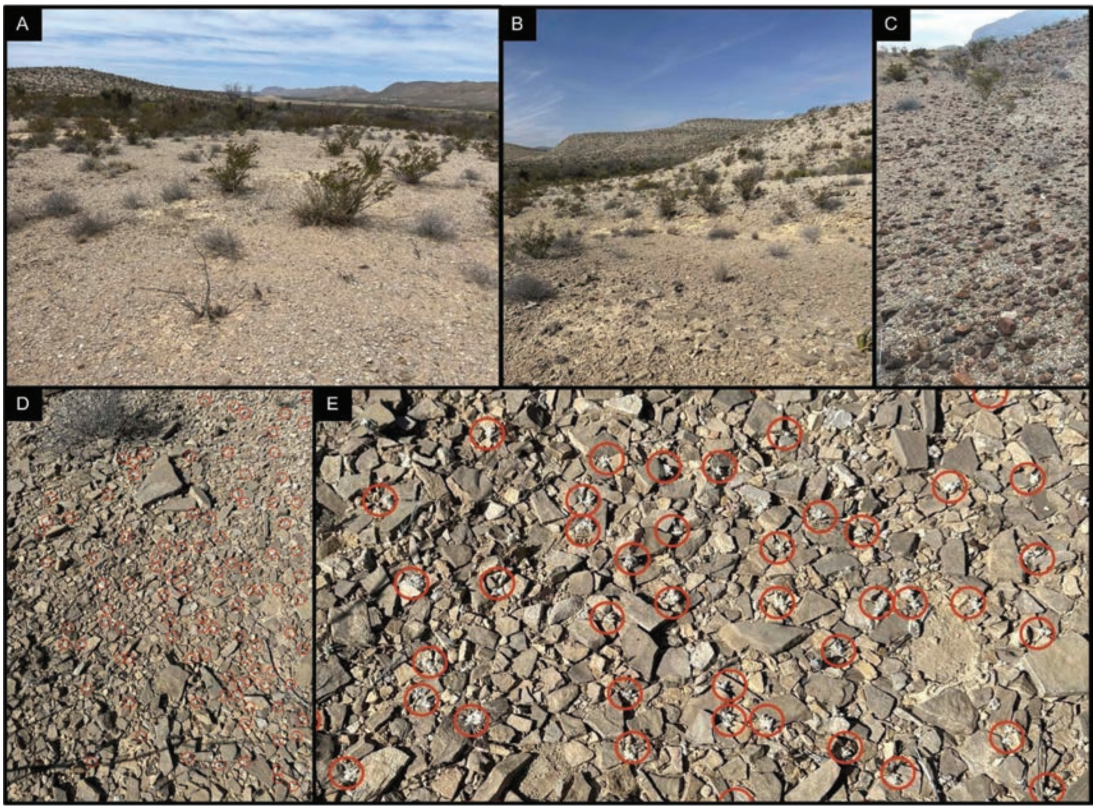

Figure 3. Known habitat of *Ovicula biradiata*. **A, B** Habitat with evident pediment slopes **C** slight habitat variation with iron-bearing rocks present in calcareous cobbles **D** overview of population locality with individual plants circled in red **E** close-up of individual plants in habitat illustrating cryptic appearance amongst calcareous surficial deposits. Photos by James Bailey (**A**) in April 2024 and Deb Manley on 20 April 2024 (**B–E**).

Etymology. The generic name from Latin *Ovis* "sheep" and -*cula* (diminutive ending) references the dense woolly indumentum of this new plant. The name honours the desert bighorn sheep (*Ovis canadensis nelsonii*), an iconic, but threatened desert animal that is currently rebounding in this part of the Chihuahuan Desert, providing hope for other rare species like *O. biradiata*. The specific epithet *biradiata* references the typically two conspicuous ray florets, occasionally three per head, positioned on opposing margins of the capitulum (Figs 1, 4). A recommended common name for *O. biradiata* is "woolly devil", in reference to the woolly indumentum, the proximity of populations to the locality known as Devil's Den and the tendency for the ray florets to resemble horns.

Distribution and phenology. *Ovicula biradiata* is known from limestone pediments of eastern Big Bend National Park where only three small populations have been found. Within these subpopulations, individual plants were abundant, but short-lived, indicating an ephemeral life history. The species was discovered on 2 March 2024 when plants were in full flower (Fig. 1). It is not presently known how early the plants may produce flowers, but, in the same general area, there are other species in several families that may bloom in early February or even earlier. By late May, after a period of warm and dry weather, the delicate annual plants had ceased vegetative growth and only desiccated inflorescences could be found (Fig. 4).

Habitat and associated taxa. The general area of the three known locations for the new taxon, as so far observed, consists of a broad floodplain composed

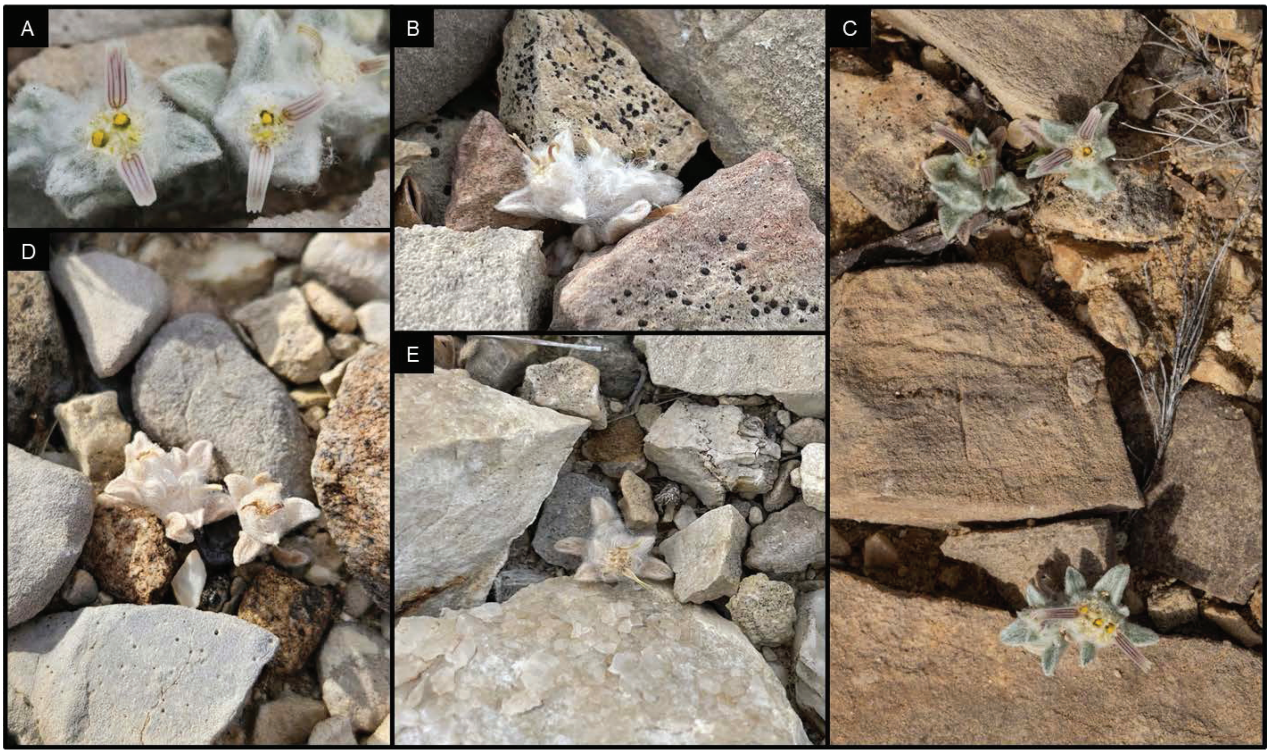

Figure 4. Images of *Ovicula biradiata* individuals representing the "small" growth habit that occurs most frequently in all three known locations. Photographs by James Bailey in April 2024 (**A**), Kelsey Wogan on 27 April 2024 (**B, E**), Cathy Hoyt on 2 March 2024 (**C**), Dana Sloan on 27 April 2024 (**D**).

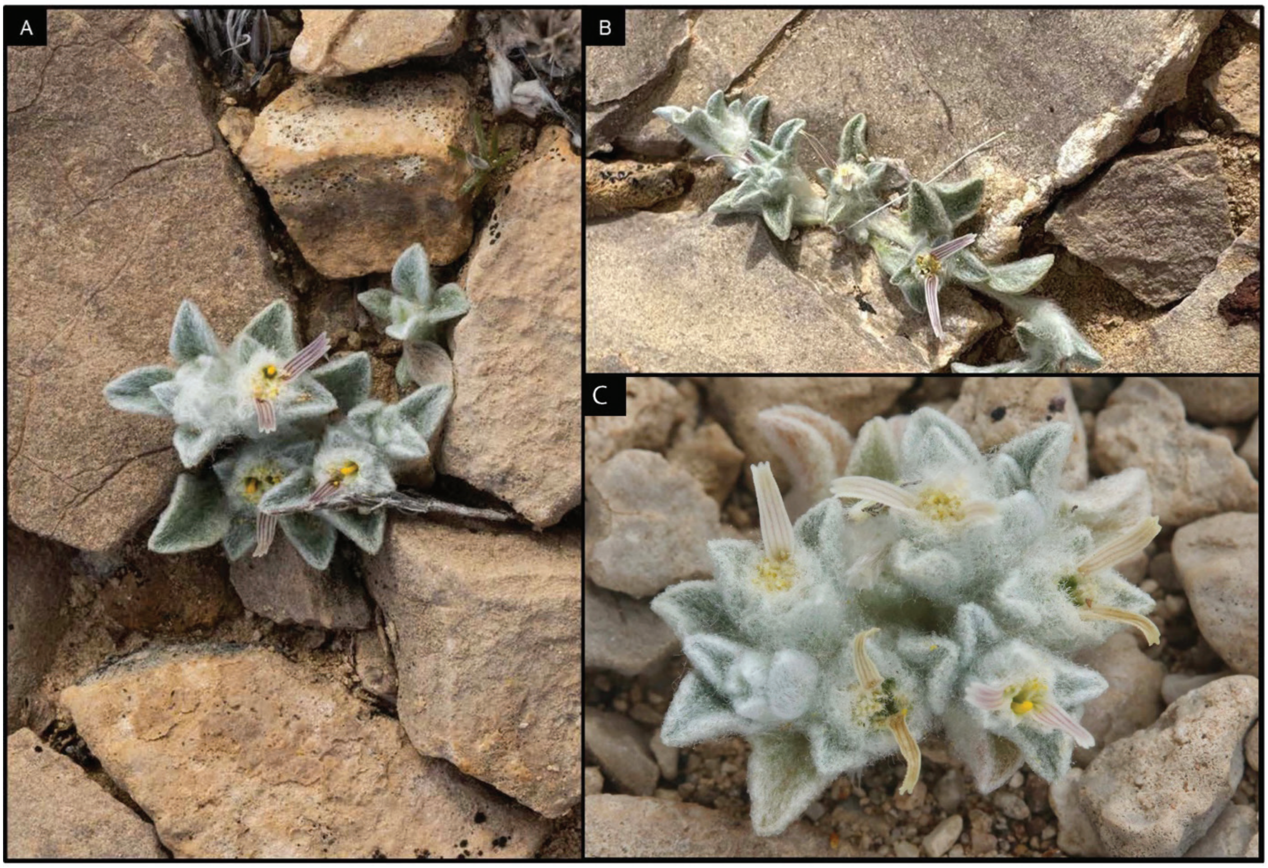

Figure 5. Examples of moderately sized individuals found occasionally throughout the known locations. Photographs by Cathy Hoyt (**A)** & Deb Manley (**B**) on 2 March 2024 and James Bailey in April 2024 (**C**).

Figure 6. Largest individuals of *Ovicula biradiata* encountered by researchers in known localities thus far. Photographs by Deb Manley (**A, B**) on 20 April 2024 & A. Michael Powell (**C**) on 27 April 2024.

Sul Ross State Univ. Herbarium (SRSC)

Ovicula biradiata D. L. Manley
Holotype
A.M. Powell Nov 2024

PLANTS OF TRANS-PECOS TEXAS
Brewster County

Gen. et sp. nov. Asteraceae

Big Bend Natl. Park, low gravelly to rocky limestone exposure, eroded alluvial flats, Chihuahuan Desert scrub, NE of Dagger Mt.; 2610 ft. (795.53 m); associated plants, *Agave lechuguilla, Larrea, Krameria, Plantago, Oenothera, Nerisyrenia camporum, Bouteloua, Grusonia aggeria, Aristida, Dasyochloa pulchella, Ariocarpus fissuratus.*

Plants annual, locally abundant on the limestone exposure; woolly, tiny, mostly inconspicuous, larger plants to ca. 5 cm across, with 1-few short, prostrate stems, spreading; disc corollas pale yellow, ray floret ligules whitish with maroon nerves.

Deb Manley 2 20 Apr 2024
with C. Whiting, C. Hoyt, P. Manning, and S. Menzies

Figure 7. Scan of the Holotype of *Ovicula biradiata*.

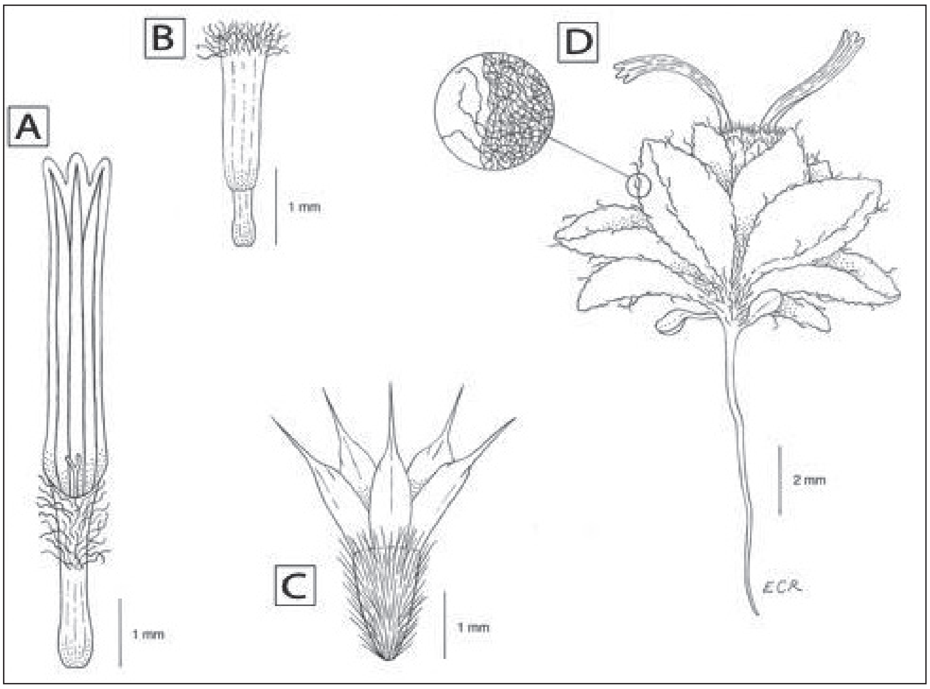

Figure 8. Line drawing of *Ovicula biradiata* gen. et. sp. nov. **A** Ray floret without cypsela **B** disc floret without cypsela **C** cypsela **D** habit with close up of leaf surface illustrating nature of indumentum. Illustration by Ellen Ruggia, based on material from the paratype (Manley 3).

of fine sand and clay sediments and braided with drainage. This alluvial basin terrain is fringed with low, gravel-capped pediments which then extend into foothills and steeper slopes of a flanking limestone mountain range. The locations are within 625 m of each other and occur where a shallow layer of mixed alluvial gravel and stones overlie bedrock of the Boquillas Formation. This composite substrate occurs on both the Ernst and San Vicente members of the formation and the observed habitat exposures consist of thinly-bedded limestone, carbonate shale and siltstone overlain by Quaternary gravel, which is a heterogeneous mix of surrounding geologic substrates. One site includes a significant presence of iron-bearing rocks. The known locations receive full sun throughout the day with very little shade provided by the sparse vegetation or the flat topography (Figs 3, 4).

Widely-distributed species noted in the habitat include *Vachellia vernicosa* (Britton & Rose) Seigler & Ebinger, *Larrea tridentata* (DC.) Coville, *Tiquilia greggii* (Torr. & A. Gray) A.T. Richardson, *T. hispidissima* (Torr. & A. Gray) A.T. Richardson, *Agave lechuguilla* Torr., *Thymophylla acerosa* (DC.) Strother, *Plantago* sp. L., *Oenothera* sp. L., *Physaria* sp. (Nutt.) A. Gray, *Nerisyrenia camporum* Greene, *Krameria* sp. Loefl., *Bouteloua* sp. Lag., *Aristida* sp. L., *Dasyochloa pulchella* (Kunth) Willd. ex Rydb., *Ariocarpus fissuratus* K. Schum., *Echinocactus horizonthalonius* Lem., *Opuntia* sp. (L.) Mill. and *Grusonia aggeria* (Ralston & Hilsenb.) E.F. Anderson. Cryptobiotic soil is present in the habitat as well. See Figs 2, 3 for habitat photos and Fig. 9 for a distribution map.

Conservation. *Ovicula biradiata* is, so far, known only from within a small area in a seldom accessed part of Big Bend National Park. Nevertheless, the extremely narrow range and ephemerality of the species suggests that it is highly sensitive to variable weather patterns. Recently, this part of the Chihuahuan Desert has

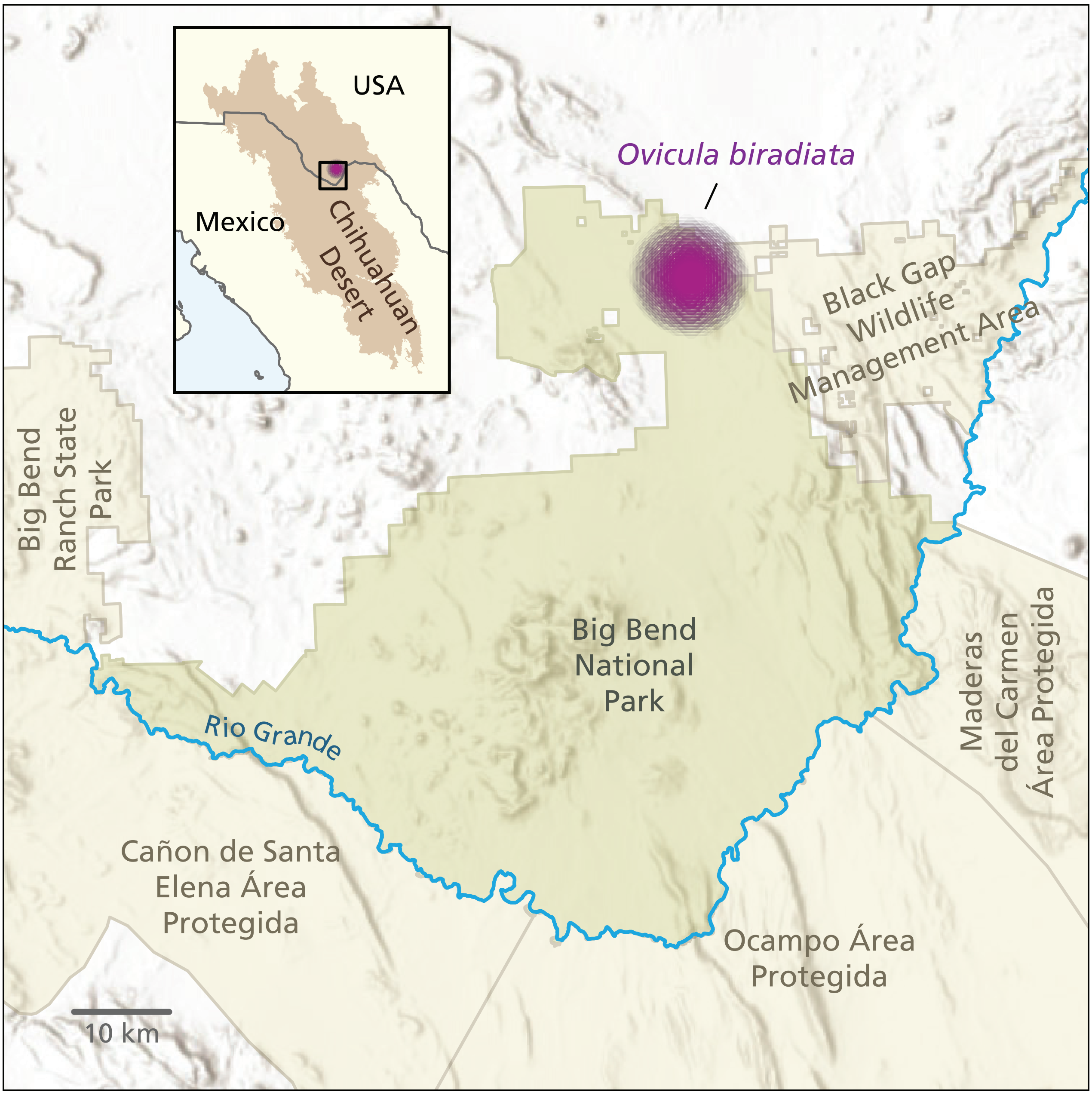

Figure 9. Approximate range map of *Ovicula biradiata*. Geographical location of the known range of *O. biradiata* in Big Bend National Park in Brewster County, Texas. The boundaries of the park are illustrated in dark green. The purple dot marks the approximate area where three sub populations, each with abundant individuals of *O. biradiata*, were located. An inset illustrates the location of the study site within the Chihuahuan Desert.

been under severe drought conditions and aridity is predicted to increase in this region due to climate change (Climate Change Response Program 2024). Under current IUCN guidelines for assessment of conservation status (IUCN Standards and Petition Committee 2022), *O. biradiata* would, therefore, preliminarily qualify as being vulnerable (VU) and under a high threat of extinction. More study is needed on the reproductive biology and population structure of *O. biradiata*, as well as potential threats to its habitat, to determine if the species should be listed by the U.S. Fish and Wildlife Service under the Federal Endangered Species Act. Due to the extreme sensitivity of the known collection sites the geocoordinates of the locality have been withheld and the locality is obscured on the map (Fig. 9).

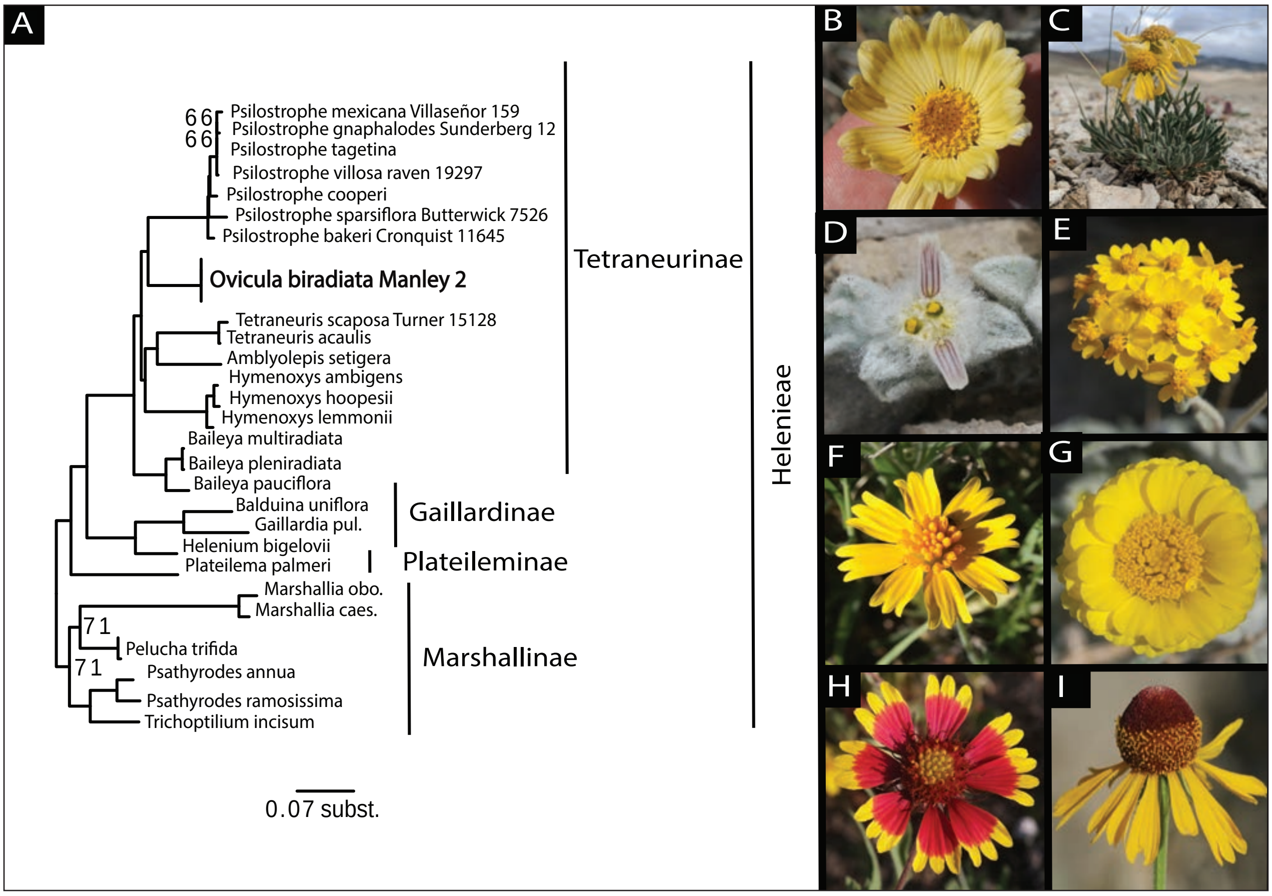

Figure 10. Phylogenetic relationships of *Ovicula biradiata* and representative photographs of genera of Helenieae. **A** Maximum Likelihood (ML) phylogenetic tree of Helenieae, based on an aligned matrix of nrDNA sequence data from the Internal Transcribed Spacer region. ITS sequences generated as part of this study have collector numbers indicated to the right. Subtribes are indicated with text. *Ovicula biradiata* is in bold **B** *Tetraneuris scaposa* **C** *Hymenoxys cooperi* **D** *Ovicula biradiata* **E** *Psilostrophe tagetina* **F** *Amblyolepis setigera* **G** *Baileya pleniradiata* **H** *Gaillardia pulchella* **I** *Helenium amphibolum*. Photographs by Peri Lee Pipkin (**C**) and James Bailey (**B, D–I**).

Discussion

The discovery of *Ovicula biradiata* underscores that the task of documenting and describing plant diversity is far from finished in the Chihuahuan Desert. Furthermore, that discoveries are not limited to unexplored or unpopulated regions and that interest and purposeful attention may still reveal novelties in places such as National Parks that might be considered "well-trodden" or fully understood. Encounters with novel plant species sufficiently different from their relatives to warrant description at generic rank are very uncommon in North America, but when they do occur, these often tend to be rare species associated with arid or edaphic micro-habitats where selection for unique growth forms is most pronounced (Stebbins 1952). Past examples of unique monospecific genera discovered in deserts or unique soils include *Apacheria chiricahuensis* C.T. Mason, *Dedeckera eurekensis* Reveal & J.T. Howell, *Megacorax gracielanus* S. Gonzalez & W. Wagner and *Yermo xanthocephalus* Dorn. Conservation management of *O. biradiata* will depend on gathering more detailed observations of its habitat specialisation, population size, reproductive biology, geographic range and life cycle and these are data that should be gathered with urgency.

As drought conditions continue to increase in frequency and severity, opportunities to observe annual plants, including *O. biradiata*, may occur less frequently.

Evolutionary implications

With the addition of *Ovicula biradiata*, subtribe Tetranuerinae contains six genera and 46 minimum rank taxa, making it the most diverse subtribe of Helenieae (Baldwin 2009). Extant diversity in this group is concentrated in western North America where they are distributed across a broad range of habitats from high mountains to low deserts (Baldwin and Wessa 2000). An ephemeral, annual life history has evidently evolved multiple times in this group apart from *O. biradiata*, as in *Baileya pauciradiata* Harvey & A. Gray, *Tetraneuris linearifolia* Greene and *Amblyolepis setigera* DC., *O. biradiata* stands out amongst other members of Tetraneurinae, however, for its minute stature, sessile heads and densely woolly foliage that effectively camouflages the plant into a background of coarse calcareous gravel. A salient, visually conspicuous characteristic of *O. biradiata* is its ephemeral ray florets, which usually appear in pairs (Figs 1–6).

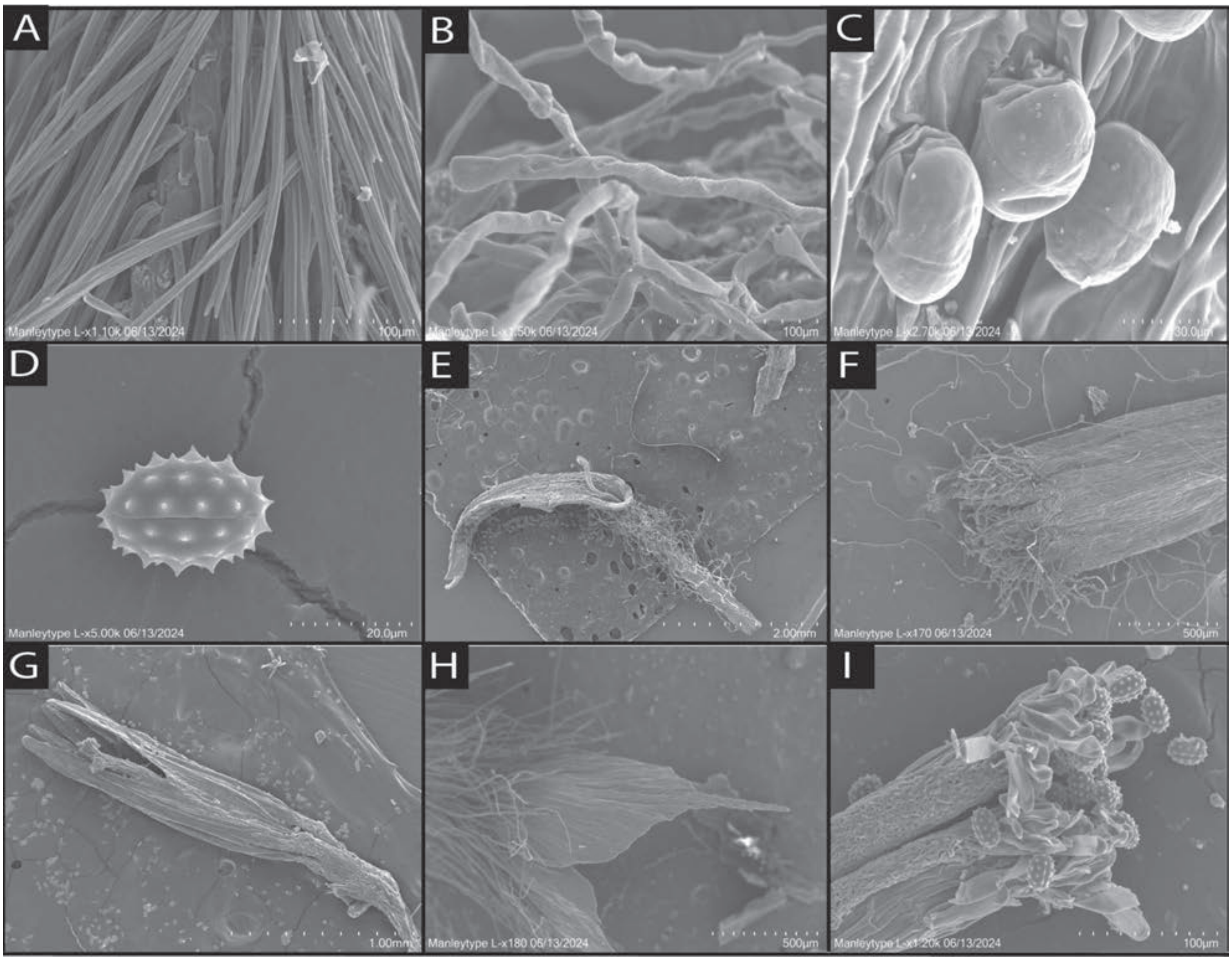

Figure 11. Scanning electron micrographs (SEM) of *Ovicula biradiata*. **A** Cypsela trichomes appear stiff, linear and end in a bifurcate (forked) tip **B** trichomes on leaf surface with a flexible, helical structure **C** short-stalked capitate glands on abaxial surface of ray corolla **D** pollen **E** ray floret without cypsela **F** disc corolla apex **G** anther column and exserted stigma **H** pappus palea tip with fine pleated serrations **I** style branch apex, with papillate trichomes sweeping pollen grains.

Phylogenetic relationships

Morphological features of *Ovicula biradiata* initially appeared to suggest a close link between the new genus and *Tetraneuris*, including maroon linear markings on the ray floret corollas (typically only visible on the abaxial face of the ray lamina in *Tetraneuris*), cypselae with a dense indument of fork-tipped trichomes and pappus of 4–6 hyaline, aristate scales. Some combination of these traits is present in other genera of subtribe Tetraneurinae, however, suggesting they may be shared ancestral characteristics. Molecular phylogenetic (ITS) data support a more distant relationship between *O. biradiata* and *Tetraneuris* than was expected from morphology and resolves the new genus as the sister lineage to the paper flowers (*Psilostrophe*). *Ovicula biradiata* bears resemblance to *Psilostrophe* in terms of its dense tomentose

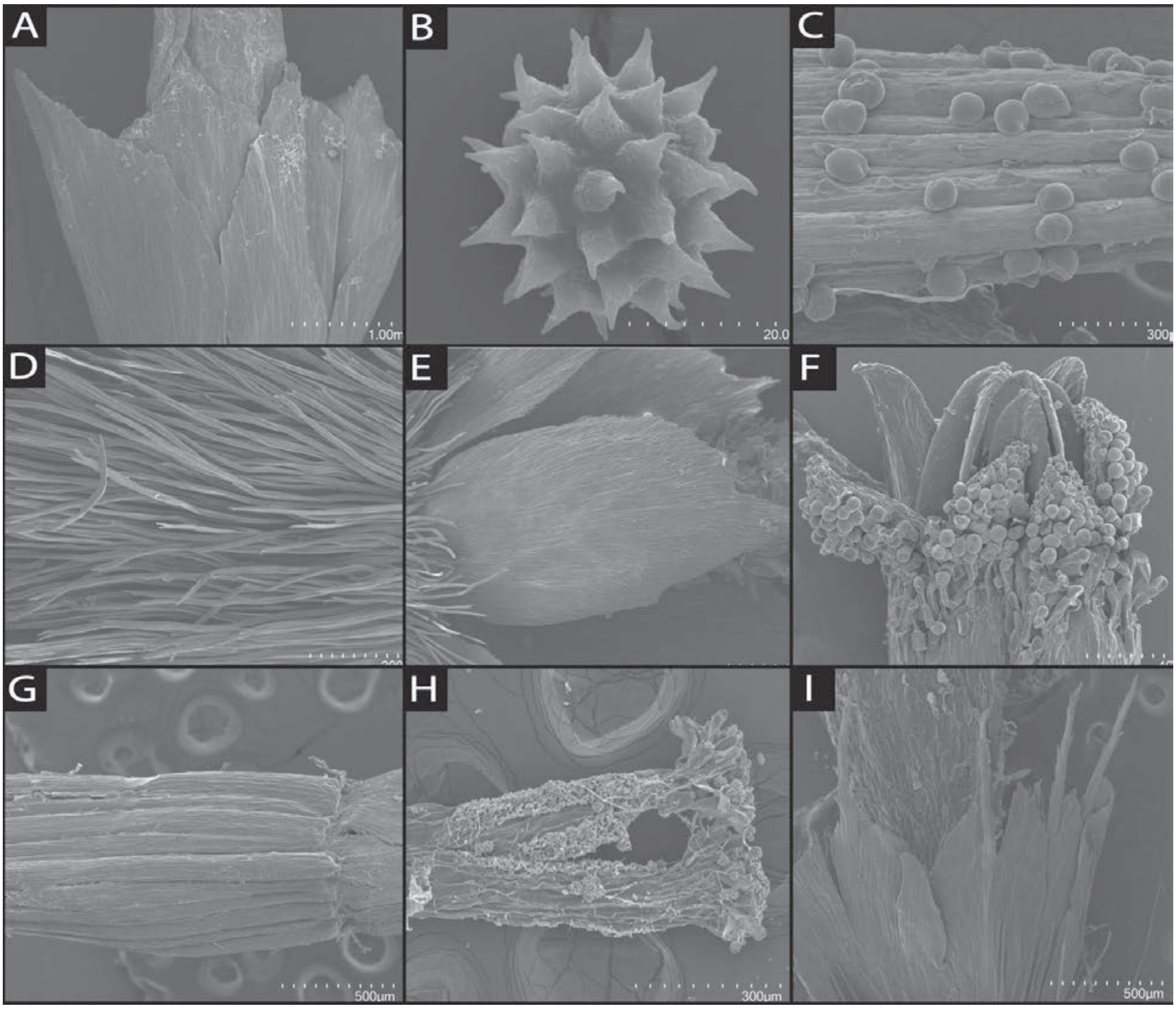

Figure 12. Scanning electron micrographs (SEM) of various genera of Tetraneurinae. **A** Pappus of *Amblyolepis setigera* **B** pollen of *A. setigera* **C** short-stalked capitate glands on disc corolla of *Baileya pauciradiata* **D** stiff, twin hairs on cypsela of *Hymenoxys cooperi* **E** hyaline, aristate palea-like pappus of *Hymenoxys cooperi* **F** vesicular trichomes on abaxial surface of disc corolla lobes in *Psilostrophe bakeri* **G** ridges on the surface of a cypsela in *P. bakeri* **H** sweeping papillate trichomes on style branch apices in *Tetraneuris scaposa* **I** paleaceous pappus of *T. scaposa* with terminal, antrorsely setose bristle.

trichomes, leaves that are both basal and cauline and typically non-scapiform heads. ITS is a relatively easy-to-sequence DNA region that has been used for decades to resolve relationships at a variety of scales in Compositae, yet it represents only one line of genetic evidence. The possibility that conflicting relationships amongst genera of Tetraneurinae may be supported by alternative DNA regions or potentially reveal a role for other processes such as hybridisation in producing enigmatic evolutionary lineages like *O. biradiata*, are hypotheses that are worth exploring in future studies.

Micro-anatomy

Micro-anatomical observations enabled by SEM revealed several characteristic features noted as diagnostic for the tribe (e.g. as Gaillardiinae in Robinson (1981)). These include a style apex with sweeping papillae, stigma with two receptive lines and oblate spheroidal pollen with regular echinate spines. Some characters revealed by SEM images for *O. biradiata*, include the pleated, serrate structural anatomy of the aristate pappus scales and foliar trichomes with a flagellate, helical body, which make up the plants woolly-tomentose indumentum. Short-stipitate glands present on the ray and disc corollas of *O. biradiata* resemble those found in many Compositae, which are often associated with sesquiterpene lactone synthesis (Robinson 2009). Phytochemical studies of Compositae, including members of tribe Helenieae, have yielded unique chemical compounds (e.g. Helenolins) with potential for anti-inflammatory and anti-cancer activity. The presence of short-stipitate glands in *O. biradiata* suggests this new species might contain secondary metabolites worthy of study for their potential medicinal value.

Finally, to encourage further study of this fascinating group, we present an updated key to the genera of Tetraneurinae, including *Ovicula*, based on information compiled from floras and observations of herbarium specimens in SRSC and CAS:

Key to the genera of Tetraneurinae

1 Phyllaries in 2 series, outer herbaceous, inner very short, hyaline, scale-like; cypselae 10-ribbed, the ribs densely tan-pubescent; herbage notably pleasant-scented; annuals; proximal leaves usually oblanceolate to broadly spatulate, semi-clasping, blades with long brownish trichomes, mostly on the margins.. ***Amblyolepis***

– Phyllaries in 1–3 series, all herbaceous, inner not short, sometimes with scarious margins; cypselae 2–5-ribbed or angled, often weakly so, faintly striate in some taxa, the ribs or angles gland-dotted, naked or pubescent with long or short trichomes, these whitish or silvery; herbage lacking notable scent; annuals, biennials or perennials; proximal leaves of various shapes, not clasping, trichomes if present not long and brownish, instead white or colourless, often densely tomentose **2**

2 Foliage glabrous or tomentellous (sparsely hairy), at the base often woolly, densely silky in *H. subintegra*; outer phyllaries usually partially connate; ray corollas ultimately withering and falling.............................. ***Hymenoxys***

– Foliage densely woolly to tomentellous, sometimes glabrous; outer phyllaries distinct; ray corollas usually persistent in fruit, except readily dislodged in *Ovicula* **3**

3 Ray florets 2(–3), corollas white with maroon nerves, readily dislodged from developing cypselae, laminae 0.6–1 mm wide; plants minute annuals, usually 1–2(–3) cm tall, 1–7 cm wide, branches if present lateral, prostrate, whole plants densely woolly; leaves crowded basally, entire, 4–7 mm long, 2.5–5 mm wide .... ***Ovicula***

– Ray florets 1–55, rarely 0, corollas yellow or orange, with yellow or maroon nerves, usually persistent in fruit, laminae 0.7–20 mm wide; plants annuals, biennials or perennials, 5–100 cm tall (except 2–40 cm in several species of *Tetraneuris*), stems ascending to erect, scapiform in most *Tetraneuris*, glabrous or tomentellous to densely wololy; leaves basal or cauline, densely woolly or not, entire to pinnately lobed, 20–120 mm long, 5–30 mm wide...**4**

4 Pappus absent; leaf blades woolly, mostly 3-lobed or pinnate ....***Baileya***

– Pappus of 4–8 hyaline scales; leaf blades tomentellous to woolly, margins mostly entire, sometimes toothed or lobed..**5**

5 Ray florets 7–27, except none in *T. verdiensis*; disc florets 20–200+; plants tomentellous to somewhat woolly, scapiform, except stems erect in the annual *T. linearifolia*; heads mostly single ....***Tetraneuris***

– Ray florets usually 1–6; disc florets usually 5–17; plants woolly, not scapiform; heads single (in *P. cooperi*) or in clusters with peduncles 0.5–2.5 cm or more long...***Psilostrophe***

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

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Author contributions

Conceptualization: AMP, KAW, DLM, IHLM. Data curation: KAW, AMP, DLM, ACC, KP, IHLM. Formal analysis: IHLM, KP. Funding acquisition: IHLM. Investigation: AMP, KAW, DLM, CVW. Methodology: IHLM. Project administration: CVW, AMP, DLM, KAW. Supervision: AMP. Visualization: KAW, KP. Writing – original draft: IHLM, AMP. Writing – review and editing: DLM, IHLM, AMP, ACC, KP, KAW, CVW.

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Data availability

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

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Research Article

No end to endemism – contributions to the difficult *Nasa* Weigend Series *Alatae* (Loasaceae). A new species from Peru and the rehabilitation of "*Loasa*" *calycina* Benth.

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Abstract

A new species of *Nasa* ser. *Alatae* (Urban & Gilg) Weigend from Northern Peru is described and illustrated. *Nasa katjae* **sp. nov.** was at first encountered by an observation on iNaturalist and subsequently collected in the humid Andean forests near Colasay in the province of Jaén (Cajamarca, Peru). Whilst comparing the new species with closely related *Nasa loxensis* (Kunth) Weigend, a taxon widespread in Southern Ecuador (and tentatively adjacent Peru), a reevaluation of the status of earlier synonymized *Loasa calycina* Benth. became necessary. Consequently, *Nasa calycina* **comb. nov.** is rehabilitated at species level and *Nasa loxensis* is redefined.

Resumen

Describimos e ilustramos una nueva especie de *Nasa* ser. *Alatae* (Urban & Gilg) Weigend del norte de Perú. *Nasa katjae* **sp. nov.** fue inicialmente encontrada gracias a la plataforma de ciencia ciudadana iNaturalist y posteriormente recolectada en los bosques húmedos andinos cerca de Colasay en la provincia de Jaén (Cajamarca, Perú). Al comparar la nueva especie con *Nasa loxensis* (Kunth) Weigend, un taxón estrechamente relacionado y ampliamente distribuido en el sur de Ecuador (y posiblemente en Perú adyacente), fue evidente que una reevaluación del estado de *Loasa calycina* Benth., anteriormente sinonimizada, era necesaria. En consecuencia, proponemos la rehabilitación de *Nasa calycina* **comb. nov.** a nivel de especie y redefinimos a *Nasa loxensis* basados en la evidencia acumulada.

Key words: Amotape-Huancabamba Zone, Andes, Cajamarca, cloud forest, Cornales, iNaturalist, Loasoideae, narrow-endemic

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Introduction

Human expansion and technological advances have brought even some of the most remote natural habitats within immediate reach, while also exposing them to the imminent threat of destruction. As a result, new taxa are being discovered that often occur only in hard-to-reach, small-scale habitats, and most of them are threatened with extinction before they are even described (Brown et al. 2023). On a positive note, digitisation and the advent of online repositories have immensely facilitated and accelerated the initial report of taxa, increasing the chances that a taxon is discovered, scientifically described, and potentially protected before it disappears due to human caused habitat alteration and destruction (Henning et al. 2023). All of this came to fruition in the context of the discovery of a new species of *Nasa* Weigend (Weigend et al. 2006, Loasaceae subfam. Loasoideae), described here. It has a very narrow range and has very specific habitat requirements, making it vulnerable to human disturbance. The forest to which it is putatively endemic is unprotected and under pressure from agricultural expansion and climate change. A local Peruvian naturalist, Carlos Pérez Peña first reported the species photographically on the citizen science platform iNaturalist (http://www.inaturalist.org), it was then assessed by specialists, and subsequently collected and documented. The important efforts by interested citizens that make their discoveries available in public repositories, complement traditional herbarium, laboratory and field research carried out by specialists and have opened a new venue for scientists of all academic levels to document and improve the understanding of the enormous biodiversity in the tropics.

Nasa ser. *Alatae* (Urban & Gilg) Weigend

Although revised only in 2000 (Weigend 2000a, 2000b, 2001), *Nasa* ser. *Alatae* has repeatedly been expanded by new taxa since (Weigend 2004b; Rodríguez and Weigend 2004) due to continuous collection efforts, mostly in northern Peru. The series is restricted to four countries: Panama (1 sp.), Colombia (7 spp.), Ecuador (8 spp.) and Peru (19 spp.) and the species number has more than tripled from only 11 during the first half of the 20th century (sensu Urban and Gilg 1900; Urban 1911; Macbride 1941, three additional names accepted at the time are currently synonymised under other names) to 35 within less than a decade (1996–2004). This sudden increase is due in part to Maximilian Weigend and his colleagues focusing on this group of plants and conducting targeted field campaigns, and in part to the fact that reachability and access to more remote forest areas is now much easier than in the past as mentioned before. Overall, Peru has 16 endemic species and, including the species also found in southern Ecuador, 17 species occur in the so-called Amotape-Huancabamba Zone (AHZ, Fig. 1A, Table 1), an area spanning from northern Peru into southern Ecuador (Fig. 1A), that is a known hotspot of biodiversity and is characterised by an extremely high rate of narrow endemism (Weigend 2002, 2004a) and is thus representing the most important area of distribution for this group.

Nasa ser. *Alatae*, as originally defined, is morphologically comparably homogeneous. Species of this series were grouped in this taxon by Urban and Gilg (as *Loasa* Adans. sect. *Loasa* (≡ *Euloasa*) ser. *Alatae*) by virtue of their simple, pinnately veined leaves in combination with corollas with erect, often red or or-

Figure 1. A Distribution of *Nasa katjae* and similar species. Left: Location of the forest system and the habitat of *N. katjae* (red star) in Peru and the Amotape-Huancabamba Zone, orange area: approximate distribution of *N. loxensis*, cyan area: *N. calycina*. Right: collections sites of *N. katjae* in the forest north of the town of Colasay **B–D** habitat of *N. katjae* **B** small stream at "Agua fria" **C** path crossing a small stream at the type locality **D** flowering *N. katjae* among dense vegetation (left). Map data 2024 (C) Google. All photos by J. P. Allen.

Table 1. Currently accepted species considered part of *Nasa* ser. *Alatae*. ** – the type species of the series; * – species of uncertain affiliation to the series. Species present in the Amotape-Huancabamba Zone (AHZ) are highlighted in bold.

Species	Distribution
***N. amaluzensis* (Weigend) Weigend**	Ecuador, Peru
***N. anderssonii* Weigend**	Ecuador, Peru
N. auca (Weigend) Weigend	Ecuador
N. campaniflora (Triana & Planchon ex. Urb. & Gilg) Weigend	Colombia
***N. carnea* (Urb. & Gilg) Weigend**	Peru
***N. dillonii* Weigend**	Peru
N. dolichostemon (Urb. & Gilg) Weigend	Colombia
***N. driessleae* Weigend**	Peru
***N. glabra* (Weigend) Weigend**	Ecuador
***N. lambayequensis* Weigend**	Peru
N. lehmanniana (Urb. & Gilg) Weigend	Colombia
N. lenta (J.F. Macbr.) Weigend	Peru
*N. limata** (J.F. Macbr.) Weigend	Peru
***N. longivalvis* E. Rodr. & Weigend**	Peru
N. loxensis **(Kunth) Weigend (incl. Loasa calycina Benth.)**	Ecuador
***N. nubicolorum* Weigend**	Peru
***N. olmosiana** (Gilg ex J.F. Macbr.) Weigend**	Ecuador, Peru
N. panamensis Weigend	Panama
*N. pascoensis** Weigend	Peru
***N. pongalamesa* Weigend**	Peru
N. profundiserrata Weigend	Colombia
N. puma-chini (Weigend) Weigend	Colombia, Ecuador
N. rubrastra (Weigend) Weigend	Colombia, Ecuador
***N. sagasteguii* Weigend**	Peru
***N. solata* (J.F. Macbr.) Weigend**	Peru
***N. stolonifera* Weigend**	Peru
N. tingomariensis (J.F. Macbr.) Weigend	Peru
N. trianae (Urb. & Gilg) Weigend	Colombia
***N. urentivelutina* Weigend**	Peru
***N. victorii* Weigend**	Peru

ange petals and very distinctive nectar scale wings bent 45°–90° relative to the nectar scale back (Gilg 1894; Urban and Gilg 1900; Fig. 2F). Due to these traits, recognizing a member of *Nasa* ser. *Alatae* would be relatively straightforward, and some species could even be grouped in morphologically distinctive informal groups such as “carnea”, “lehmanniana”, “campaniflora” (Weigend 2000b; Weigend and Gottschling 2006). However, taxa within the same group are sometimes difficult to tell apart as their diagnostic characters are often relatively inconspicuous in herbaria, even if consistent. The comparatively few specimens of some taxa available in collections (something that is typical for *Nasa*: Henning et al. 2023) also make it difficult to assess the morphological variability of the species. *Nasa* ser. *Alatae* includes many species adapted to wet premontane or montane forests (Weigend 2000a). An ecosystem rarely exploited by Loasaceae outside the tropical mountains, but also one that remains quite understudied due to difficulties in access and the complex topography of many mountain ranges, particularly the Andes. These are probably the reasons why assigning some specimens to accepted taxa often remains doubtful or impossible, despite recent and thorough revisionary works (Weigend 2000a, 2000b, 2001, 2004b).

The phylogenetic relationships of *Nasa* ser. *Alatae* are not fully resolved. According to available molecular data, in particular plastid markers, most of the species currently considered as part of this section appear to belong in one of the four major clades of *Nasa* (Clade II in Acuña-Castillo et al. 2021). However, a few species traditionally included in this series (*Nasa olmosiana* (Gilg ex J.F.Macbr.) Weigend, *N. pascoensis* Weigend and, sometimes, *N. limata* (J.F. Macbr.) Weigend) seem to be more closely related to "non-*Alatae*" species, at least according to plastid data. To complicate matters, a group of morphologically very different taxa, traditionally placed in the large, plesiomorphic series *Saccatae* (Urban & Gilg) Weigend (e.g. *Nasa sanchezii* T.Henning & Weigend) and the smaller series *Carunculatae* (Urb. & Gilg) Weigend (e.g. *Nasa macrothyrsa* (Urb. & Gilg) Weigend) have repeatedly been retrieved as nested within the same clade as the majority of *Nasa* ser. *Alatae* spp. (Weigend and Gottschling 2006; Acuña-Castillo et al. 2021). Whether this is a result of past hybridisation events or convergent evolution is still unclear.

The complex diversity of the group

Nasa is a relatively ancient genus (its crown age dating back to ca. 28 Ma), which probably originated in the proto–Central Andes, in mid-elevation, seasonally dry scrub habitats (Acuña-Castillo et al. 2019, 2021). Its history has been shaped by frequent dispersals and habitat shifts, especially in the AHZ which has acted mostly as a cradle for this genus (Acuña-Castillo et al. 2021). *Nasa* ser. *Alatae*, which is primarily adapted to forest habitats, is often affected by habitat changes and (de)fragmentation in the dynamic landscape of the Andes, and this is especially noticeable in the very complex mosaic of habitats in the AHZ. As a result, the members of this group have a patchy and often very narrow distribution and tend to show morphological variability between different disjunct local populations. That is the case of our current concepts of *Nasa loxensis* and *N. olmosiana* and of *N. puma-chini* (Weigend) Weigend and *N. rubrastra* (Weigend) Weigend to a lesser extent. To add another layer of complexity, in the Central Cordillera of Colombia, the very different *N. trianae* (Urb. & Gilg) Weigend and *N. profundiserrata* Weigend could hybridize (Weigend 2001), so it is possible that other taxa may do the same where their ranges meet. The delimitation of species is therefore difficult, especially considering the incomplete sampling of suitable habitats in some of the areas with the highest diversity and the resulting limited herbarium evidence.

Besides a few clearly recognisable species such as e.g. *Nasa dillonii* Weigend, *N. driessleae* Weigend and *N. urentivelutina* Weigend that are well characterised by single or a combination of stable striking morphological characters, many taxa seem to show a relatively broad intraspecific variability. Moreover, for many members of the group, the morphological spectrum is limited, and taxon delimitation often is based on evolutionary unstable character complexes that are prone to a certain degree of plasticity (e.g. petal shape and orientation, indumentum). Earlier taxonomic studies were based on the generally scarce herbarium material (cf. Urban and Gilg 1900), sometimes complemented by observations from a limited number of field trips (Macbride 1941). Thanks to the advent of freely accessible online repositories (e.g. iNaturalist), an ever-increasing number of photographic reports are now available. These low-threshold ways of making observations available across all levels of professionalism have become an invaluable and rapid source of information that complements the more sluggish traditional sources of evidence (Henning et al. 2023). We are conscious that repositories like iNaturalist also have their shortcomings (particularly in groups that are poorly curated, i.e. not critically assessed by specialists, or for observations where the diagnostic traits of an organism are not evident), and that they cannot substitute traditional herbarium collections and fieldwork. But, from our experience, observations that show diagnostic traits and that have been curated and evaluated by regional or international specialists of a certain group, have proven to be an extremely useful complement to more traditional, and more individualistic, field- and herbarium studies. To gather a similar volume of evidence as iNaturalist, would have taken weeks of fieldwork following months or years of planning. Consequently, to the already known diversity of *Nasa* ser. *Alatae*, information is constantly added by new occurrence reports and geo-referenced photographic evidence. On the one hand, this leads to a consolidation of certain taxa whose morphological and geographical characterisation is confirmed by the growing evidence. On the other hand, for some taxa the morphological spectrum and, often in tandem, the known distribution is constantly expanding, often to a point where the original description becomes inadequate, and a new taxonomic assessment becomes unavoidable. It must be said that until this day, the overall picture is thus getting more and more complex and has not yet reached a point where species boundaries and distribution patterns become necessarily clearer for all taxa of the group. However, what is apparent is that the alpha-taxonomy of the group is not yet fully resolved and that the diversity and level of endemism as already indicated in earlier works (e.g. Weigend 2000a) appears endless. With every forest fragment that becomes accessible, new evidence is presented that constantly expands the morphological spectrum. To ultimately resolve the systematics of ser. *Alatae*, thorough molecular studies down to the population level will be needed. Unfortunately – and this is, in our opinion, the biggest shortcoming of online-only repositories – usually there is no physical sample created in the context of these photographic occurrence records, which would be an essential prerequisite for such an endeavor.

The need for names

Peru has recently been determined as one of the global “darkspots” of plant diversity (Ondo et al. 2024) meaning that it is not only a hotspot of known phytodiversity, but also a region that harbors a large amount of expected unknown diversity that should be prioritised in order to determine the diversity and distribution of plant taxa. The assignment of this status is largely due to the large geographical share that Peru has in the Amotape-Huancabamba Zone with its exceptional rate of endemism and its diverse and small-scale habitats (see Henning et al. 2021 for references). The AHZ is one of the main regions that accounts for the Peruvian “areas housing most undescribed and poorly geolocated species” (definition “darkspots” of plant diversity – Ondo et al. 2024) that define the whole country as a darkspot of plant diversity.

Due to the urgent need for conservation in general and that of the remaining forest fragments of the Andes in the Amotape-Huancabamba Zone in particular, it is imperative to assess the respective local biodiversity. To put the logic of current protection mechanisms in simple terms: widespread taxa are less in need of protection than narrow endemics and, logically, we can only protect taxa that are validly described, preferably at the species level. And because time is running out, we cannot wait until the systematics of certain groups are solved by means of elaborate and lengthy molecular studies with their taxonomic consequences only subsequently resolved (if at all), which continues to take up valuable time. It would, however, also not make sense to hastily delimit and describe numerous aberrant local forms based on a single observation (given a type specimen is available) and thereby further complicate matters. But it is most desirable and urgent to describe taxa and thus make their names available for biodiversity assessments and potential conservation measures if their taxonomic status is apparent based on the ("good") characteristics observed. In our opinion this is the case here. The new species *Nasa katjae* sp. nov. that we formalise below, shows a unique combination of characters that clearly and unambiguously separates it from the known taxa of the group. It is most likely a narrowly endemic taxon and thus makes it a valuable component of the flora of the unprotected forest patch it has been found in, which is increasingly threatened (see below). Consequently, *Nasa katjae* sp. nov. is described here as new to science and illustrated, its affinities with related taxa are discussed, all known aspects of its ecology are presented, and the known distribution and its potential threat status are detailed.

Methods

Plant collection

The type collection near Colasay in September 2023 was carried out based on the collection permit RD-000130-2023-DGGSPFFS-DGSPF.

Revised material

We carried out extensive studies of herbarium specimens, or high-resolution images of herbarium specimens from the following herbaria (Thiers 2024): AAU, CHEP, E, F, GB, HA, HUT, K, LOJA, NY, P, QCA, US and USM. Additional studies were carried out on observations from iNaturalist.org.

Conservation assessment

The tentative assessment of the conservation status of the *Nasa* species was made using the International Union for Conservation of Nature criteria (IUCN Standards and Petitions Committee 2024). For *N. loxensis* and *N. calycina* comb. nov., the area of occupancy (AOO) and the extent of occurrence (EOO) was calculated using GeoCat (Bachman et al. 2011). A cell width of 5 km was used to calculate the AOO. Since the suitable habitat of narrowly endemic *N. katjae* sp. nov. merely has the size of one grid, both values have been estimated according to the size of the forest fragment.

Results and discussion

Formal taxonomy

Nasa katjae **T.Henning, J.P.Allen & R.H.Acuña, sp. nov.**
urn:lsid:ipni.org:names:77356918-1
Figs 2, 3C

Type. Peru • Departamento Cajamarca, Provincia Jaén, Distrito Colasay, Above Colasay near Agua Fria. In dense forest, along the trail to Agua Fria in streams, climbing in the vegetation. Just below the peak at ca. 2500 m, -5.93990, -79.05400, 01. Sep. 2023, D. B. Montesinos 10003, T. Henning, J. P. Allen (Holotype: HUT No. HUT-64640!, Isotype USM).

Diagnosis. The new species is morphologically most similar to *Nasa calycina* comb. nov. (see below) and differs from it in its very elongated stems, subscandent habit, proportionately broader leaf blades with a conspicuously deeply cordate base, sepals and petals almost twice as long (to 4 cm and 4.5 cm respectively), sepals and petals of equal length and nectar scales with 3 conspicuous apical dorsal threads up to 5 mm long.

Description. Plants to 1.5–3 (–4) meters tall, covered with scabrid, and stinging trichomes, glochidiate trichomes restricted to the abaxial surface of the leaves along the veins. Stinging hairs (setae) scattered all over the plant but most densely on the stem, ovary, sepals (= fruit) and along the veins of the leaves. Apical parts of the petals set with few glandular hairs. Stems upright when young or growing in open areas, climbing through and leaning on adjacent vegetation when growing in dense undergrowth, base slightly woody. Leaves opposite, petiolate, petiole up to 13 cm long, leaf blades pinnately veined, 7–11 × 5–10 cm, widely ovoid to triangular, with 3–7 obtuse triangular lobes on each side, the lower ones up to 3 cm wide and 1.5 cm long, gradually decreasing in size towards the apex, the upper ones inconspicuous, margins serrate, each tooth with a hydatode, base conspicuously cordate (sinus to 1 cm deep). Inflorescence a monochasial or dichasial cyme, bracts alternate to 3 × 1.5 cm, one per flower, smaller than vegetative leaves, base shallowly cordate to truncate. Sepals 5, persistent, long acuminate, green, up to and 3.5–5 × 0.6–0.8 cm when fruiting, with 3 main veins, temporarily spreading in early anthesis, closely fitting on the petals later and further contracting in fruit. Petals 5, scarlet red, shallowly cymbiform, oblanceolate, base narrower than the limb but claw poorly differentiated, 3.5–4.5 × 1–1.5 cm, with 3 evident main veins, gaps between petals let the nectar sacs and scale bases visible when calyx lobes removed. Nectar scales 5, orange, 19–21 mm long and 5–6 mm wide at base, with 2 distinct, broadly ovoid, seemingly smooth (when fresh) nectar sacs at the base, each one as wide as nectar scale back (3 mm in diameter), nectar scale back rectangular, narrow 15 × 3 mm, straight papillose, margins with even longer papillae, ending in 3 conspicuous, distinct dorsal threads, inserted apically, up to 5 mm long and with two horizontal wings, seemingly smooth, 7 × 4 mm and diverging 90–120° from the back. Staminodes 2 per scale, c. 22 mm long, slightly sigmoid, base papillose, apex filiform. Stamens in 5 antepetalous fascicles with 10–20 each, anthers whitish when shedding pollen. Ovary broadly conical, with a rounded base, 5 × 5 mm, with 3 parietal placentae. Stigma lobes 3, decurrent on the style surface. Fruit a broadly clavate capsule with a

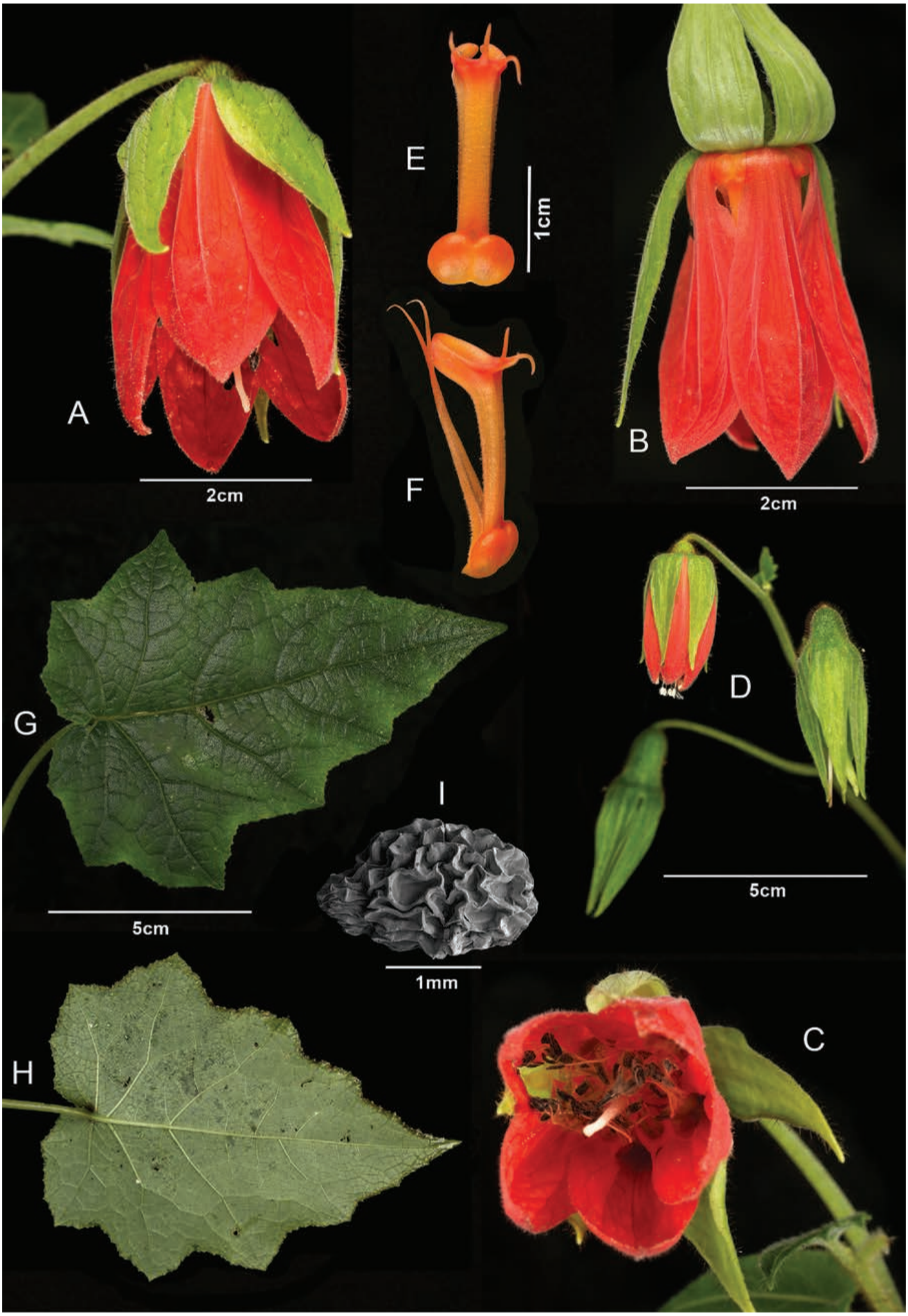

Figure 2. Lankester Composite Dissection Plate (LCDP) of *Nasa katjae* **A** Flower, fronto-lateral view **B** flower, lateral view, sepals lifted **C** frontal view in late anthesis **D** inflorescence, not the elongated sepals on the young fruits **E** nectar scale, dorsal view **F** nectar scale lateral view with free inner staminodes **G** leaf adaxial surface **H** leaf abaxial surface **I** seed. Credit: **A–H** J. P. Allen **I** Y. Malkowsky.

Figure 3. Comparison of flowers of *Nasa katjae* with its closest relatives in their actual size ratio **A** *Nasa loxensis* **B** *Nasa calycina* **C** *Nasa katjae*. Photo credits: **A** M. Ackermann **B** R. Ripley **C** J. P. Allen.

globose base, 20–25 mm long (without sepals) and 8–10mm wide at sepal insertion, opening with three apical valves. Seeds numerous, ovoid, 2.5 mm long and 1.5 mm wide, testa black and reticulate.

Paratypes. PERU, CAJAMARCA, Prov. Jaén, Dist. Colasay • Sector Aguas frias, bosque montano húmedo, 5°56'14.69"S, 79°03'19.33"W, 2542 m, 28 March 2024, *José Luis Marcelo-Peña, Marisela Rojas, Robert Zurita 11923* (ISV) • Ditto, 5°56'23.07"S, 79°03'14.28"W, 2550 m, 30 March 2024, *José Luis Marcelo-Peña, Marisela Rojas, Robert Zurita 12127* (ISV) • Ditto, 5°56'23"S, 79°03'14"W, 2550 m, 30 March 2024, *José Luis Marcelo-Peña, Marisela Rojas, Robert Zurita 12128* (ISV).

Photographic evidence (iNaturalist): **PERU**, CAJAMARCA, Prov. Jaén, Dist. Colasay • -5.92834, -79.05414, April 2022, *biomonstrando*, http://www.inaturalist.org/observations/143704890 (type locality) • -5.93739, -79.0351, December 2023, *biped_cub*, http://www.inaturalist.org/observations/194322389 • -5.93739, -79.04139, December 2023, *biped_cub*, http://www.inaturalist.org/observations/194324117.

Affinities. The species appears to be morphologically closest to *Nasa calycina* comb. nov., a species endemic to a small area of southern Ecuador, in SE Loja and adjacent Zamora-Chinchipe. This taxon has been considered a synonym of the more northerly *Nasa loxensis* (Weigend 1996; Jørgensen and León-Yánez, 1999; Weigend 2000b), but detailed examination of their floral morphology showed that both taxa are best treated as closely related, but separate species (see below). The species can be found in forest edges and relatively open understories of wet montane “cloud” forest. Habit, leaf shape, perianth parts size and proportions, as well as size and insertion of the nectar scale dorsal threads separate the new species from *Nasa calycina* comb. nov. (Table 2), rehabilitated to species level in this article (see below).

Our first encounter with this species took place on iNaturalist (http://www.inaturalist.org/observations/143704890), where, at first, we suspected it was an unusual *Nasa loxensis* (a species whose concept has been quite broadly applied

Table 2. Comparison of the species most similar to *Nasa katjae* sp. nov.

	Nasa loxensis	***Nasa calycina***	***Nasa katjae***
max. plant height m	1.5	1	1.5–4
sepal : petal ratio	0.25–0.5 : 1	0.6–0.8 : 1	0.9–1.1 : 1
corolla shape	closed, tapered towards the apex	half open, cylindrical	half open to closed, campanulate to barrel-shaped
max. petal length × width mm	38 × 10	20 × 10	45 × 15
nectar scales length × width mm	11–16 × 3	10 × 3	19–21 × 5–6
dorsal threads on nectar scales	short (>1 mm) or absent	short (>1 mm) or absent	conspicuous to 5 mm long

in the most recent revisions of Loasaceae of Ecuador: Weigend 1996; Jørgensen and León-Yánez 1999; Weigend 2000b). After a more thorough analysis, it is evident to us that both species could be quite closely related but have important and consistent differences that support the distinctiveness of this new taxon. However, the most striking difference that was impossible to assess in the iNaturalist observations, but obvious at the natural habitat, is the sheer size of the plants. Whilst a few plants along the path resemble the typical upright and medium-sized habit of most species of Ser. *Alatae* (e.g. *N. loxensis*), some specimens found off the path in dense vegetation, were leaning on and climbing through the dense undergrowth (e.g. bamboo). These plants can grow several meters long until they reach open areas to present their flowers freely, which are then presumably visited and pollinated by hummingbirds. Another striking character unique to the new species are the conspicuous elongated sepals. Although sepal length is subject to a large variation in many species of *Nasa* and usually of moderate systematic value, the characteristic sepals found in *Nasa katjae* are exceptional. They are as long as the petals and, in some cases, even longer and the persistent calyx closes after flowering and shows a further elongation (Fig. 2D). Whilst the function of the long calyx in fruiting remains unknown (possibly moisture regulation), the calyx lobes are visually striking during anthesis and greatly influence the floral display. They are initially nestled up to the petals and start spreading and bending out their apices with beginning anthesis, giving the flower an elegant, slightly bell-shaped appearance. The sepals (half-) spread in peak anthesis and bend back after the flower sheds and fruit development starts.

Etymology. The new species is named after Katja Lohse, beloved partner of the first author, mother of their children and steady supporter of his scientific endeavors.

Distribution and ecology. *Nasa katjae* has so far only been collected a few times. The type collection was made about 5 km north of Colasay (Cajamarca, Peru, Fig. 1A). It grows only in, and at the edges of small streams in the highest part of this isolated forest fragment (Fig. 1 B–D). It is associated with the typical floristic elements in these “bosques montanos húmedos” such as *Chusquea* sp., *Fuchsia* sp., *Vernonanthura* sp., *Saurauia* sp., *Miconia* spp. and *Viburnum* sp. The forest in the uppermost regions is largely intact and only disturbed by small paths and some recent, small-scale clearings. However, the increasing negative impact of livestock farming is evident from the cattle tracks visible everywhere along the paths and already within the forest fragments.

The plants are adapted to the very dense vegetation by a flexible growth habit. In the few more open areas where the path crosses the streams, the plants grow upright and begin to flower at a height of 1 to 1.50 m. In the wettest parts, where the dense primary vegetation consists mainly of impenetrable bamboo thickets (*Chusquea* sp.), the plants continue to grow in length until they reach an open area to display their flowers. Although difficult to measure, some individuals easily reach a size of more than 4 m. The flowers are typical "hummingbird-pollinated" flowers thus having open space around them is vital for successful pollination. All *Nasa* species are protandrous and exhibit a thigmonastic stamen movement and staggered stamen maturation (Weigend et al. 2010; Henning and Weigend 2012). In the taxa pollinated by hummingbirds, this movement is reduced to a minimum both geometrically (i.e. the angle the stamens bend) and temporally (the number of moved stamens = pollen packets in time). *N. katja* sp. nov. has erect petals (in contrast to spreading petals in insect-pollinated taxa), therefore the stamen hardly move towards the center, and the pollen dispersal is limited to a successive maturation and dehiscence of the anthers. This is a secondary adaptation to the unreliable visitation probability and the random and long distances hummingbirds transport the pollen (Henning et al. 2018). However, we could not observe any visits to the plants. Ripe fruits with full seed set were found.

The new species must be considered narrowly endemic to the overall area. It has been found twice at a spring area called "Agua Fria" which is the type locality. Only very recently, two new observations have come to our knowledge that, according to the locality-data on iNaturalist, have taken place some approximately 2.5 km further east. However, it is very likely that the species follows the streams downhill through the forest and inhabits suitable areas within the primary forest in all directions.

Phenology. The known populations have been visited four times now by different people between February 2023 and March 2024. All visitors report flowering and fruiting plants, and it can be assumed that the plants flower throughout the year without a substantial break reflecting the constant climate and minor seasonal variation in temperature and precipitation in this wet montane forest.

Preliminary conservation status. The forest near Colasay represents the southeasternmost part of a larger forest system that is often referred to as the "Bosques Montanos de Jaén". A former protected area called "Área de Conservación Municipal de Bosque de Huamantanga" was recently expanded to become the "Área de Conservación Regional Páramos y Bosques Montanos de Jaén y Tabaconas" (D.S. N°005-2021-MINAM see https://www.gob.pe/institucion/sernanp/normas-legales/1896920-005-2021-minam, Fig. 1A) and covers almost 32000 hectares of the north-western parts of this forest system in the districts of Sallique, Chontalí and San José del Alto. Unfortunately, the southern district of Colasay and the surrounding forests are not yet part of this protected area. Although large areas are still intact, recent deforestation and the expansion of agricultural land are threatening the forests at higher altitudes, which not only harbor great biodiversity, but also represent an important watershed whose streams are the main tributaries for a number of important river systems. There is no evidence that *N. katjae* is present in other parts of the larger forest system. Given the peculiar habitat preference and the lack of other collections or reports of the species, even in a wider area, *N. katjae* appears to be very narrowly endemic. It presumably only occurs along a small number of streams in a few remote spots in the south-eastern part of the forest system and seems isolated from the other parts by the steep topography and its adaptation to flowing water. The forest patch north of Colasay has an extension of approximately 25–30 km^2. It is only connected to the northwards running chain of forests in the west at lower altitudes that might represent a barrier for high-altitude taxa. The Extent of Occurrence (EOO) can thus be estimated as this single forest patch and the Area of Occupancy (AOO) is even smaller, and it appears possible that only a small number of populations exists at and near the type locality. Due to the visible expansion of agricultural activities from the lower towards the higher altitudes, the forest is being pushed back from all sides and is getting even more isolated from the other forests further north. Given these observations, the new species must be considered as Critically Endangered (CR) based on the criteria A3 (conditions *c* and *d*) B1 and B2 (conditions *a* and *b*) according to the IUCN guidelines (2024). Unlike in *N. loxensis* and *N. calycina* comb. nov., we did not use Geocat to calculate the EOO and AOO for *N. katjae*. Since the whole forest only has the size of one grid cell (25 km^2) a comparison would be pointless and misleading. In fact, the de facto suitable habitat is much smaller, since only the uppermost parts of the forest appear moist enough.

About the status and a new combination for *Loasa calycina* Benth.

***Nasa calycina* (Benth.) R.H.Acuña & T.Henning, comb. nov.**
urn:lsid:ipni.org:names:77356919-1
Fig. 3B

Basionym. *Loasa calycina* Benth., Pl. Hartw. [Bentham]: 132. 1844.

Holotype. Ecuador • [Prov. Loja] Mountains near Loxa, July [1841], C.T. Hartweg s.n. (Holotype: K barcode K000372874!)

Description. Plants to ca. 1 meter tall, covered with scabrid, glochidiate and stinging trichomes, glandular trichomes inconspicuous or absent. Stems erect, cylindrical to ca. 1 cm diam. Leaves opposite, petiolate, petiole 2–4 cm, leaf blades pinnately veined, 6–15 × (2–)3–7 cm, narrowly ovoid to ovoid or triangular, with 4–8 lobes per side, in some leaves shallow and poorly defined, triangular, from wider than long to longer than wide, apices acute, the largest of a leaf 0.5–2.5 × 0.5–2 cm, the second or third usually the largest, becoming progressively smaller apically, margins serrate, each tooth with a hydatode, base cuneate, rounded or truncate, apex acuminate. Inflorescence a monochasial or dichasial cyme, 10–50 cm long, bracts alternate, one per flower, usually shorter and proportionally much narrower than the vegetative leaves, elliptical, 1.5–4 × 0.3–0.7 cm, diminishing in length, and particularly width, towards the inflorescence apex. with 4–7, broadly triangular, shallow, often indistinct lobes, base cuneate to rounded. Pedicels 1.5–2.5 cm long in anthesis, straight, often horizontal but ranging between 45° above or below the horizontal, the apex deflexed. Flowers deflexed, 3–6 per inflorescence branch. Sepals 5, narrowly triangular or ovate, apex acuminate, yellowish-green, 1.2–2 × 0.5–0.7 cm, with 3 main veins, as long or slightly shorter than the petals. Petals 5, orange red to carmine, shallowly cymbiform, oblanceolate, base narrower than the limb but claw short and poorly differentiated (to 4 mm wide), 1.5–2 × 0.6–1 cm, with 3 evident main veins, tip rounded or obtuse, basal gaps between petals leave the scale bases visible (gaps often not visible because they are either very small or hidden by the sepals), corolla more or less cylindrical, as wide basally as distally. Nectar scales 5, orange or red, 10 mm long and 3 mm wide at base, with 2 distinct, broadly ovoid, seemingly smooth (when fresh) nectar sacs at the base, each one as wide as the nectar scale back (ca. 2.5 mm in diameter), nectar scale back rectangular, narrow, 5.5 × 2 mm, straight, surface papillose, with 0, 2 or 3 short filiform threads, inserted subapically, < 1 mm long and with two horizontal wings, 3–4 × 1.5 mm. Staminodes 2 per scale, to 14 mm long, slightly sigmoid, narrowing apically, base papillose, apex filiform. Stamens in 5 antepetalous fascicles with 8–10 each, filaments to 12 mm long, anthers 1 × 0.5 mm, elliptical, cream before dehiscence, whitish when shedding pollen, black after pollen is shed. Ovary broadly conical to hemispherical, with a rounded base, ca. 5–6 × 5–6 mm, with 3 parietal placentae. Stigma lobes 3, shortly decurrent on the style surface, style to ca. 15 mm long. Fruit a broadly clavate to conical capsule on an erect pedicel, 3.5–5 cm long, with persistent sepals and a shortly tapering base, mature capsule 20–30 × 10–12 mm (width at sepal insertion), including an elongated conical apical projection, opening by the three apically dehiscing valves. Seeds not seen.

Notes. *Loasa calycina* has been considered a synonym of *Nasa loxensis* since at least 1996 (Weigend 1996; Jørgensen and León-Yánez 1999). The most important differences between both species are evident in the proportions of the calyx and corolla elements with *N. calycina* having proportionally longer sepals, ca. ≥75% as long as the petals, while *N. loxensis* has sepals that are less than half the length of the petals (Table 2, Fig. 3). Also, the corolla in living anthetic flowers has a different shape in both species, as in *N. calycina* petals are arranged in a cylindrical shape with a proportionally wide corolla opening, while in *N. loxensis* the corolla tapers gradually, so distally the corolla is narrower than basally, with the petals leaving a very narrow opening (Fig. 3A, B).

In the latest and most authoritative treatment of Loasaceae of Ecuador, Weigend (2000b) discussed briefly the significant morphological variability of his broad concept of *Nasa loxensis*. But he recommended maintaining it until either more specimens from areas became available or it became clear that few or no additional specimens were known.

Weigend (2000b) acknowledged the differences in floral proportions of some specimens from Loja (that we consider fit well under the concept of *Loasa calycina* of Bentham) from those of Azuay (e.g. near Cuenca and Cajas National Park) that conform better to Kunth's concept of *Loasa loxensis*. However, it is important to note that the type material of both *Nasa loxensis* and *Nasa calycina* come from near the city of Loja, where still today plants that adjust very closely to these specimens grow. The wider availability of more specimens in the mosaic of habitats (from pretty much pristine to significantly anthropogenically modified) around the city of Loja, and further north, along with photographs of living plants in platforms like iNaturalist have allowed us to confirm the relatively modest but seemingly consistent morphological differences between *Nasa loxensis* and *N. calycina*. The lack of florally intermediate specimens in possible areas of contact near Loja suggests that hybridisation is so infrequent, that truly intermediate specimens have not been recorded so far. From the specimens and photographs available we know that both species approach each other but appear to be sympatric in only one locality, between La Argelia and La Palma, SW of the city of Loja, and no evident intermediates in floral characters have been observed. Living plants that fit closely Hartweg's type material of *Loasa calycina* have been collected most frequently from what is now Podocarpus National Park

GenBank accessions AY285722.1, MK333044.1, MK333010.1, MK332976.1, MK332944.1 and AY769214.1 identified as belonging to *Nasa loxensis* and used in the studies of Weigend et al. (2004), Weigend and Gottschling (2006) and Acuña-Castillo et al. (2019, 2021) belong to *Nasa calycina* (voucher: *J.R. Grant & L. Struwe 01-4063*).

Specimens examined. Ecuador. Loja • Carretera Loja-Yangana, desvío al Parque Nacional Podocarpus, E del Nudo de Cajanuma, 2880–3000 m, 14 March 1989, *A. Freire-Fierro 1323* (GB, QCA) • Parque Nacional Podocarpus, E of Nudo de Cajanuma, hectare plot near "Centro de información", c. 2900 m, 15 April 1989, *B. Eriksen 91176* (QCA) • Parque Nacional Podocarpus sector Cajanuma, 2900 m, 30 June 1996, *P. Lozano 449* (Loja) • Cajanuma, 21 February 1985, *F. Vivar 2310* (Loja) • Parque Nacional Podocarpus, above Nudo de Cajanuma, 2800–3000 m, 14–15 May 1988, *B. Øllgaard et al. 74090* (AAU, Loja) • Parque Nacional Podocarpus, S of Loja, wet montane forest at the "Centro de Información", E of Nudo de Cajanuma, 2850–1950 m, 21–22 February 1985, *B. Øllgaard et al. 57847* (AAU, Loja) • Cajanuma, 04°06'24"S, 79°11'22"W, 2600 m, 31 January 2002, *P. Lozano et al. E-600* (Loja) • Entrada a Cajanuma, sendero a las Lagunas, 3100 m, 11 June 2002, *P. Lozano et al. E-1650* (Loja) • Uritushinga, 2800 m, 14 February 1978, *F. Vivar 898* (Loja) • Sitio Ventanas, Cordillera Oriental, Or. Loja, ca. 2400 m, 27 December 1947, *R. Espinosa & A. Espinosa 2279* (Loja) • Road La Argelia (southern Loja)-La Palma, along crest of mountain range just SW of Loja, 2700–2950 m, 4 March 1989, *B. Øllgaard et al. 90792* (AAU, Loja) • Zamora-Chinchipe: Parque Nacional Podocarpus (San Francisco entrance); trail leading west from San Francisco, 03°59'24"S, 079°05'48"W, 2100 m, 16 February 2001; *J.R. Grant & L. Struwe 01-4063* with *C. Rosales* (Loja, QCA) • Área del Parque Nacional Podocarpus, Cajanuma, rotundamente " El Mirador", 3000 m, s.d., *Rbu & SL 107* (QCA).

Photographic evidence (iNaturalist): (Note: due to *Nasa loxensis* s.l. being considered threatened by the IUCN, it was not possible to obtain the precise locality of some observations): **Ecuador**. Loja • -4.113259,-79.174943, April 2024, *Daniel Arias-Cruzatty*, http://www.inaturalist.org/observations/2057414 • April 2022, *Lilia Cueva Cueva*, http://www.inaturalist.org/observations/114005635 • December 2020, *prengelv*, http://www.inaturalist.org/observations/67754076 • Parque Nacional Podocarpus, Cajanuma, sendero a las Lagunas del Compadre, cerca del páramo, December 2020, *Angel Hualpa Erazo* http://www.inaturalist.org/observations/67015645 • Parque Nacional Podocarpus, Cajanuma, December 2020, *Amarú Ramóm Salcedo*, http://www.inaturalist.org/observations/66984658 • 04°07'3.34"S, 079°09'53.21"W, 3088 m, February 2017, *dennisronsse*, http://www.inaturalist.org/observations/37683383 • Near Cajanuma Refuge, September 2007, *Ruth Ripley*, http://www.inaturalist.org/observations/35868409 • ditto, http://www.inaturalist.org/observations/35868095 • ditto, http://www.inaturalist.org/observations/34983697 • ditto, http://www.inaturalist.org/observations/34981561 • Cerca del Refugio Cajanuma, -4.115681 -79.171616, February 2019, *Bodo Nuñez Oberg*, http://www.inaturalist.org/observations/20486169 • Cajanuma Field Station, and trail to Mirador, 04°06'45"S, 079°10'37"W, December 2007, *Jason Grant*, http://www.inaturalist.org/observations/20486169.

Distribution. *Nasa calycina* is endemic to Loja and Zamora-Chinchipe (Fig. 1), mostly known from areas near or within Podocarpus National Park (mostly around the Cajanuma sector), where *Nasa loxensis* is missing. There is a single record between La Argelia and La Palma in an unprotected area where it seems to meet the southernmost range of *Nasa loxensis*. Further south in Loja (and reaching Piura, Peru), both taxa seem to be replaced by *Nasa amaluzensis*, a species that is florally quite close to *Nasa calycina* but differs significantly in leaf morphology.

Phenology. Flowering has been recorded during September and mostly from December to July.

Tentative conservation assessment. The known range of *Nasa calycina* is diminutive, but most of the populations of the species appear to be protected within the Podocarpus National Park, where it is frequently encountered in the Cajanuma sector. We still recommend considering this species as EN B2abiii, due to a reduced AOO (125 km^2), the reduced number of known locations where the species can be found (only four) and the inferred decline of habitat quality where the only known population not found within or in the immediate vicinity of Podocarpus National Park grows.

***Nasa loxensis* (Kunth) Weigend in Weigend et al. Revista Peru. Biol. 13(1): 77. 2006.**

Fig. 3A

Basionym. *Loasa loxensis* Kunth, Nov. Gen. Sp. 6: 116.1823.

Holotype. Ecuador • [Prov. Loja] Loxa, [Aug?. 1802], A.v. Humboldt & A. Bonpland 3349 (Holotype: P barcode P00679495!)

Description. Plants to ca. 1.5 m tall, covered with scabrid, glochidiate and stinging trichomes, glandular trichomes inconspicuous or absent. Stems erect, cylindrical, to 1 cm diam., base woody. Leaves opposite, petiolate, petioles 1–6 cm long, leaf blades pinnately veined, 3–14 × 2–8.5 (–11.5) cm, triangular to ovate, with 1–6 lobes per side, widely triangular to ovate, apices acute or rounded, the largest of a leaf, 0.5–2(–4.5) × 0.4–2(–3) cm, the first or second often the largest (the third less commonly so), progressively smaller apically, margins serrate to denticulate, each tooth with a hydatode, base rounded, truncate or shallowly cordate (sinus to 5 mm deep), apex acuminate. Inflorescence a monochasial or dichasial cyme, 7–30 cm long, bracts alternate, one per flower, usually shorter and proportionally much narrower than the vegetative leaves, elliptical, 1–8 × 0.2–3.2 cm, diminishing in length, and particularly width, towards the inflorescence apex with 4–7, broadly triangular, shallow to very shallow lobes, base rounded. Pedicels 2.5–3.6 cm long in anthesis, often horizontal but ranging to 45° above or below the horizontal, the apex deflexed. Flowers deflexed, up to 7 per inflorescence branch. Sepals 5, ovate triangular, apex acute to acuminate, green, 0.7–1.3 × 0.4–0.7 cm, with three main veins, evidently much shorter than the petals. Petals 5, orange to red, shallowly cymbiform, base narrower than the limb but claw short and poorly differentiated (to 4 mm wide), 2.5–3.8 × 0.8–1 cm, with 3 evident main veins, tip acuminate, basal gaps between petals leave the scale bases visible (gaps often visible, sepals too short to hide them), corolla wider at base, tapering distally. Nectar scales 5, yellow to orange red, going from lighter yellow on the back to darker orange on sacs and red orange on neck, 11–16 mm long and ca. 3 mm wide at the base, basally on back with two depressed globose sacs 2 mm in diam., nectar scale back rectangular narrow, papillose, dorsal threads 0, 2 or 3, short, < 1 mm long (very rarely longer), inserted subapically, scale neck thickened, slightly recurved, laterally protracted into two horizontal wings 5 mm long and 1 mm wide. Staminodia 2 per scale, 17 mm, narrowing apically, apex filiform, more or less sigmoid, slightly papillose, white. Stamens, usually extending beyond the petals during the male phase, in 5 antepetalous fascicles of 13–16 each, filaments to 30+ mm long, anthers 1.5 mm long and 1 mm wide, elliptical, cream before dehiscence, whitish when shedding pollen, black after pollen is shed. Ovary broadly conical or hemispherical, with a rounded base, to 7 × 5–6 mm, with 3 parietal placentae. Stigma lobes 3, shortly decurrent on the style surface, style to ca. 30 mm long. Fruit a clavate or cylindrical capsule with persistent sepals, pedicel erect, 25–55 mm long, mature capsule 21–27(–30) x 11–13(–14) mm (width at sepal insertion) including a short or elongated conical apical projection, opening by the three apically dehiscing valves, base rounded, less commonly tapering. Seeds numerous, dark brown, ovoidal, testa reticulate.

Notes. For detailed information on identification, ecology etc. see notes under *Nasa calycina*. Across its distributional range, our redefined *Nasa loxensis* seems to show high uniformity in floral characters, although the observed leaf morphology can be significantly more diverse. The most unusual (and variable) leaf morphologies have been reported from the southernmost populations directly to the west and south of the city of Loja (Weigend 2000b). Some plants in this area have leaf blades with very prominent, long lobes and deep sinuses, and are occasionally as wide as long. The reasons for the high foliar variability in this part of the range are unknown. Living plants that closely resemble the type material of *Nasa loxensis* (*Humboldt & Bonpland 3349*) have been collected from localities to the west and north of the city of Loja.

Specimens examined. Ecuador. Cañar • Parroquia Rivera, sector Santo Tomás, vía a Monay, propiedad de Luis Méndez, 2754 m, 12 October 2000, *A. Verdugo et al. 230* (HA) • Azuay Sevilla de Oro, 10–12 km N of village, 2750–2850 m, 11 September 1976, *B. Øllgaard & H. Balslev 9331* (AAU) • The Eastern Cordillera, 1–8 km north of the village of Sevilla de Oro, 2400–2700 m, 27 July – 12 August 1956, *W. Camp E-4339* (P) • Gualaceo a Macas, a 12 km de Gualaceo, 6 August 1986, *A. Freire-Fierro 251* (GB) • West of Patul 3 km between Huahualcay and Río Patul below Pasas de Pinglión, 2670–3275 m, 19 May 1943, *J. Steyermark 52611* (F) • Mountains near Cuenca, August 1864, *W. Jameson s.n.* (E, US) • Rio Machangara, NW Cuenca, quebrada vegetation, 3000–3100 m, 18 September 1967, *B. Sparre 18609* (US) • Sayausid, ca. 3000 m, 1 April 1968, *G. Harling et al. 7942* (GB) • Mountains above Sayausid, 3000–3200 m, 18 March1974, *G. Harling & L. Andersson 12604* (GB) • Above Sayausí, at first bridge over Río Tomebamba. Secondary scrub, 3200 m, 3 March 1985, *G. Harling & L. Andersson 22702* (GB, QCA) • Above Sayausí, trail to Cajas, 3300 m, 20 July 1939, *C. Penland & R. Summers 1074* (F, US) • Río Mihuir (Miguir?) 1 km below Miguir on road Cuenca – Molleturo. Secondary scrub, 3400 m, 8 March 1985, *G. Harling & L. Andersson* 22915 (GB, QCA) • Cuenca, Cajas, Laguna Llaviuco (=Laguna Surocucho). Directly on the roadside, 3000–3150 m, September – October 1995, *M. Weigend & S. Horn 3830* (F, QCA) • Area Nacional Recreacional Cajas, Sect. Llaviuco, 3300 m, 8 January 1991, *S. León et al. 2509* (QCA) • Surucucho, 2800 m, March 1967, *F. Vivar et al. 514* (Loja) • Cuenca, Sayausí, sector Dudahuayco, captación de agua. En sitios abiertos y pastizales, 3060 m, 20 July 2006, *A. Verdugo & D. Minga 1678* (HA) • Cuenca, Sayausi. Road to Llaviucu lagoon, close to the intersection with the Cuenca-Molleturo road. Common in degraded vegetation on the roadside, 3024 m, 18 February 2017, *R. Acuña & H. Garzón 1732* (QCA) • Las Cajas: near Laguna Llaviuco Montane forest and disturbed areas along the road, 3100–3200 m, 12 September 1983, *B. Boysen Larsen & B. Eriksen 45098* (AAU) • Parque Nacional Cajas, Laguna de Llaviuco, 3170 m, 9 June 2011, *C. Ulloa et al. 2137* (HA, QCA) • Fierroloma, Zorrocucho, en bosque primario, 3200 m, 15 January 1997, *D. Minga 86* (HA) • Dudahuaycu, Mazán, 3500 m, 6 March 1991, *G. Chacón 93* (HA) • 14 July 1994, *G. Chacón 94* (HA) • A 1 km del control de la vía a Loja, partidero del lado derecho hacia Yanasacha, bosque secundario y pajonal, zona lluviosa, 3000–3200m, 26 June 1978, *J. Jaramillo & J. Boeke 412* (QCA) • Río Matadero valley near entrance to Parque Nacional de Cajas, 2900 m, 28 December 1979, *L. Holm-Nielsen 20928* (AAU) • Sunsun-Yanasacha. Vía a mina de Caolín, cerca el río. En el sotobosque, 3100 m, 16 June 1999, *F. Serrano et al. 718* (HA) • Vía partidero a Quinoas-Surocucho, 3000 m, 5 February 1978, *F. Ortiz & J. Jaramillo 76* (QCA) • Portete del Tarqui and environs, remnants of montane forest, 2600–2700 m, 24 February 1993, *G. Harling & B. Ståhl 26666* (QCA) • Tarqui, near the monument, 2600 m, 5 February 1982, *G. Harling et al. 20247* (GB) • Victoria del Portete, sector Aguarongo y/o Caspishitana. En el sotobosque, 3584 m, 26 April 2006, *A. Verdugo & D. Minga 904* (HA) • 15 km SW of Cuenca on road to Giron, 8 km SE on dirt road from Hacienda Tarquí turnoff, km 22, to Patococha, 2950 m, 30 May 1990, *P. Peterson & E. Judziewicz 9359* (QCA) • Patacocha, 7–8 km by trail S of Hacienda Tarqui at Inquis, 3050–3100 m, 29 January 1988, *U. Molau et al. 2750* (GB) • Victoria del Portete, Río Portete, sector captación de agua, 2778 m, 25 April 2006, *A. Verdugo & D. Minga 887* (HA) • Sigsig to Gualaquiza, Rio Altarurcu (20 km E of Sigsig), 2800 m, 13 April 1968, *G. Harling et al. 8309* (GB) • Cumbe, 2900 m, 22–24 April 1968, *G. Harling et al. 8692* (GB) • 13 km S of Cumbe, 3300 m, 9 Jun 1979, *B. Løjtnant et al.14417* (AAU, GB) • Along Pan-American Highway, 40 km south of Cuenca, 3300 m, 20 September 1944, *I. Wiggins 10761* (US) • LOJA: Saraguro, camino Panamericana-Huashapamba, montaña húmeda, 2800–3000 m, 31 September 2004, *A. Macas s.n.* (CHEP) • Vicinity of Las Juntas, 28 September 1918, *J. Rose et al. 23189* (US) • Between La Toma and Loja, 1800–2600m, 4 September 1923, *A. Hitchcock 21370* (US) • Road Catamayo (La Toma)-Loja, km 9 past junction with old road. Bosque húmedo remnants and secondary scrub, 2550 m, 28 April 1997, *G. Lewis 3216* (E, LOJA) • West of Loja, just over pass to Catamayo (Toma); 1 km south of Loma de Trigal, scrub forest, 5 May 1986, *M. Baker 6959* (NY, US) • Cerro Villonaco, 2600–2750 m, 12 April 1974, *G. Harling & L. Andersson 13461* (GB) • W Slope, 10 February 1985, *G. Harling & L. Andersson 21862* (GB, QCA) • 1 March 1947, *R. & A. Espinosa 1316* (LOJA) • Cerro Uritusinga. Loja-La Palma, km 18–20. Montane forest, primary and secondary forest under heavy pressure, exploited to produce charcoal, 2910–3000 m, 30 November 1994, *P. Jørgensen et al. 1040* (LOJA) • Loja, 8 November 1876, *E. André K-546* (F) • Parte alta de Hacienda Montecristi, unos 40 km NE de Loja, curso del Río Zamora hacia el oriente, 8 June 1947, *R. Espinosa 1471* (LOJA) • Cantón Catacocha, El Almendral, Hacienda La Hamaca, 1800–2200 m, 16 April 1944, *M. Acosta Solís 7902* (F) • Chepel, 2200 m, s.d., *R. Espinosa 1978* (LOJA) • PROV. UNKNOWN: Herb. de Pérou, s.d. *J. de Jussieu s.n.* (P) • Équateur et Pérou, s.d., *M. Grisar s.n.* (P) • *Warszewicz s.n.* (F neg, No. 10202).

Photographic evidence (iNaturalist): (Note: due to *Nasa loxensis* s.l. being considered threatened by the IUCN, it was not possible to obtain the precise locality of some observations): ECUADOR • AZUAY: -2.98455, -79.07776, April 2023, *Kabir Montesinos*, http://www.inaturalist.org/observations/154122854 • Laguna Sorocuchu, -2.84466, -79.14866, April 2023, *Kabir Montesinos*, http://www.inaturalist.org/observations/153135367 • W del P.N. Cajas, -2.89167, -79.36357, May 2021, *Kabir Montesinos*, http://www.inaturalist.org/observations/80194774 • Cerca del Portete de Tarqui, -3.08651, -79.13816, March 2024, *Kabir Montesinos*, http://www.inaturalist.org/observations/204670994 • Cerca del Portete de Tarqui, -3.08931, -79.13622, November 2022, *Kabir Montesinos*, http://www.inaturalist.org/observations/141022114 • Carachula, -3.14969, -79.35685, June 2023, *Kabir Montesinos*, http://www.inaturalist.org/observations/167995363 • Loja: Washapamba, -3.671097, -79.252418, May 2023, *LostInCR*, http://www.inaturalist.org/observations/178891174 • camino de Washapamba a Cerro de Torre-3.682952, -79.244846, April 2022, *Rudy Gelis*, http://www.inaturalist.org/observations/118712124 • Province Undetermined: Aug.2019, *manuelganzhi*, http://www.inaturalist.org/observations/31622038 • July 2020, *Jonathan Aguirre Pesantez*, http://www.inaturalist.org/observations/67707816 • June 2022, *Jonathan Aguirre Pesantez*, http://www.inaturalist.org/observations/121346890 • September 2022, *bb_593*, http://www.inaturalist.org/observations/134805560 • October 2022, *bb_593*, http://www.inaturalist.org/observations/137584820 • February 2022, *Edgar Segovia*, http://www.inaturalist.org/observations/106356391.

There are two observations from southern Cajamarca in Peru that are tentatively placed here. Since no herbarium material was available so far, we refrain from reporting a Peruvian distribution of this species for the time being. **Peru** • Cajamarca, -6.47573, -79.01225, August 2022, *manuelroncal*, http://www.inaturalist.org/observations/131296479 • -6.23583, -79.07411, August 2007, *barbetboy*, http://www.inaturalist.org/observations/15334620.

Distribution. *Nasa loxensis* has a wide distribution in southern Ecuador, found in the provinces of Cañar, Azuay, Loja and apparently Morona-Santiago (Fig. 1). It is one of the most frequently collected species of *Nasa* in Ecuador as it can be found growing near human settlements, frequently visited national parks, and in both pristine and degraded habitats. At its southernmost distribution limits, it is suddenly replaced by *Nasa calycina,* farther to the SW, *Nasa amaluzensis* replaces both taxa. Further north in Tungurahua and Cotopaxi, similar *Nasa auca* can be found. As indicated above, a disjunct distribution at the southern end of the Amotape-Huancabamba Zone in Peru (Cajamarca, Prov. Chota) appears possible but needs further examination and material.

Phenology. Flowering has been recorded every month of the year.

Tentative conservation assessment. We recommend considering this species as NT. Although both its AOO (900 km^2) and EOO (12.193 km^2) could suggest a Vulnerable (VU) status like Cornejo and Suin (2011) recommended previously, these seem to be the only criteria that could justify such assessment as no other criterion appears to support a high-risk category. *Nasa loxensis* can be seen frequently in the W and S of the city of Cuenca and seems to be able to withstand some habitat degradation. Seemingly healthy populations are protected within the current limits of Cajas National Park.

Key to the species of *Nasa* ser. *Alatae* present in the Amotape-Huancabamba Zone (modified and extended from Weigend 2004b)

1 Calyx tube and fruit without stinging hairs or, if with them, few, unevenly distributed and mostly restricted to base of the ovary/fruit....................... **2**

– Calyx tube and fruit usually densely covered with stinging hairs, these evenly distributed across the outer surface of the ovary/fruit.................. **4**

2 Plants with evident stinging hairs; stems always lacking prominent ridges; mature capsules clavate, base acute.................................... ***N. anderssonii***

– Plants mostly without evident stinging hairs; stems, at least when young, with prominent ridges; mature capsules subglobose to broadly cylindrical, base rounded.. **3**

3 Petals ovate, narrowing gradually distally; leaf lobes usually obvious .... ***N. glabra***

– Petals obovate, broadly rounded distally; leaf lobes often not obvious .... ***N. victorii***

4 Leaves pentagonous with acute leaf lobes; stem densely covered with uniseriate glandular hairs, especially in distal portion; stinging hairs to 5 mm long .... ***N. amaluzensis***

– Leaves ovate to widely ovate, if widely ovate then leaf lobes always rounded to acuminate; stem without or with relatively few uniseriate glandular hairs; stinging hairs typically under 4 mm long.. **5**

5 Corolla yellow; leaves with white lines along primary and secondary veins adaxially, lamina dark green .... ***N. driessleae***

– Corolla pink, orange, or red, never yellow; leaves without white lines along veins or, if with white veins, then lamina bright green **6**

6 Inflorescence with large, sessile, semi-amplexicaul bracts ... ***N. olmosiana***

– Inflorescence with petiolate or sessile bracts, never semi-amplexicaul ... **7**

7 Lamina very densely pubescent, velvety to the touch with numerous stinging hairs between the trichomes; vegetative shoots with numerous, mostly alternate, spirally inserted leaves (rarely opposite); stiffly erect, sparsely branched shrub.. ***N. urentivelutina***

– Lamina hairy and sometimes densely so, but never velvety to the touch; leaves never alternate, always opposite; plant often branched from base... **8**

8 Dark green calli below insertion of petiole present; petals either very narrow (5–6 × as long as wide) or widely ovate, carnose and completely lacking stinging hairs on back; capsule clavate; pedicel elongating post-anthetically.. **9**

– Dark green calli below insertion of petiole absent, petals not narrow (< 4 × as long as wide) nor carnose; capsule variable in shape; pedicel not elongating post-anthetically **11**

9 Petals ovate (< 4 × as long as wide), carnose .... ***N. sagasteguii***

– Petals linear (5 > 6 × as long as wide), membranaceous **10**

10 Leaves ovate, up to 90 × 50 mm; Peru: Piura................................. ***N. solata***

– Leaves widely ovate to subcircular, 90–150 mm long and wide; Peru: Cajamarca .... ***N. dillonii***

11 Petals half-spreading and corolla star-shaped; floral scales white or pale yellow **12**

– Petals erect and corolla tubular to campanulate; floral scales white, pale yellow, orange, red, or deep pink.. **14**

12 Sparsely branched, stiffly erect herb; petals pink; Peru: Cajamarca .... ***N. carnea***

– Much-branched stoloniferous herb or sub-shrub; petals orange or reddish-pink; Peru: La Libertad (Bolívar) south to Áncash............................ **13**

13 Stem and leaf veins with numerous yellow to red stinging hairs and scabrid hairs; petals orange, narrowly oblong, slightly cymbiform; capsule apical valves much shorter than the capsule below sepal insertion.... ***N. stolonifera***

– Stem and leaf veins mostly without stinging hairs but with scabrid and glochidiate hairs; petals reddish-pink, narrowly ovate, deeply cymbiform; capsule apical valves about as long as the capsule below sepal insertion .... ***N. longivalvis***

14 Petals pale pink .... ***N. pongalamesa***

– Petals orange or red, never pale pink **15**

15 Petals narrowing strongly basally (claw well differentiated), leaving most of the nectar scales (both base and back) exposed; growing in Jalca .... ***N. lambayequensis***

– Petals narrowing weakly basally (claw poorly differentiated), usually hiding most of the nectar scale (only the bases exposed), growing in montane forest and subpáramo **16**

16 Petals widely ovate, half-spreading; corolla campanulate; Peru: Amazonas .... ***N. nubicolorum***

– Petals oblong, oblanceolate to narrowly ovate, erect; corolla conical, tubular to narrowly campanulate; Southern Ecuador and Peru: Cajamarca ... **17**

17 Corolla scarlet red, tubular (beginning anthesis) to slightly campanulate (carpellate phase); sepals as long as petals; nectar scales with long dorsal filiform appendages to ca. 5 mm long; plants erect to subcandent up to 4 m long .... ***N. katjae* sp. nov.**

– Corolla orange or bright red; sepals shorter than petals; nectar scales without dorsal filiform appendages, or these short, to ca. 1 mm long; plants less than 2 m tall **18**

18 Corolla conical, petals tapering towards the apex, leaving only a small opening for the pollinator; sepals half as long as petals; flowers up to 4 cm long .... ***N. loxensis***

– Corolla, cylindrical, petals straight, leaving an obvious opening for the pollinator; sepals ¾ as long as petals; flowers to ca. 2 cm long .... ***N. calycina* comb. nov.**

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

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Data availability

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

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Research Article

Sinosenecio tongziensis (Asteraceae), a new species from north Guizhou, China

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Abstract

Sinosenecio tongziensis R.B.Zhang, Tan Deng & Ying Liu (Asteraceae), a new species from Tongzi County in northern Guizhou, China, is described and illustrated. It closely resembles *S. changii* in the subscapigerous habit, ovate-oblong and pinnately veined leaf lamina, and simple to compound terminal corymbs, yet differs markedly by the texture of leaf lamina (membranous vs. papery), the number of lateral veins (8–10 vs. 10–18), and the indumentum on the stems, leaves, and inflorescences (pubescent with 2–5 mm long, uniseriate, spreading hairs vs. sparsely white arachnoid to densely white tomentose). Phylogenetic analysis indicates that *S. tongziensis* is related to *S. bodinieri*, *S. nanchuanicus*, *S. confervifer*, and *S. globiger* var. *adenophyllus*.

Key words: Flora of Guizhou, ITS, new taxon, taxonomy

Introduction

Sinosenecio B. Nordenstam (Asteraceae, Senecioneae) is a genus comprising 48 species (Chen et al. 2011; Liu and Yang 2012; Liu et al. 2019; Zou et al. 2020; Chen et al. 2022; Peng et al. 2022; Su et al. 2023a; Su et al. 2023b), characterized by subscapiform or leafy stems, palmately or rarely pinnately veined leaf lamina, solitary to numerous capitula, and often ecalyculate involucres (Peng et al. 2022). Multiple lines of evidence indicate that *Sinosenecio* as currently defined is polyphyletic (Wang et al. 2009; Liu 2010; Liu and Yang 2011a, b; Ren et al. 2017). Species with a base chromosome number of $x = 30$ and strictly polarized endothecial cell wall thickenings are phylogenetically close to the tussilaginoid genera, while the remaining species with $x = 24$ (rarely 13) and both polarized and radial thickenings are closely related to *Nemosenecio* (Kitamura) B. Nordenstam and *Tephroseris* (Reichenbach) Reichenbach.

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During a field expedition in 2020, a previously undocumented *Sinosenecio* species was discovered in Tongzi County, north Guizhou, China. At first glance, it closely resembles *Sinosenecio changii* (B. Nordenstam) B. Nordenstam, a species with $x = 24$, in the subscapigerous habit, pinnately veined and ovate-oblong leaf lamina, and simple to compound terminal corymbs. Based on these characteristics, we determined that this plant represents a new species, described here as *S. tongziensis* with a report on its floral micromorphological characters. Additionally, we performed phylogenetic analysis using nuclear ribosomal internal transcribed spacer (nrITS) sequence data to explore its phylogenetic affiliation in the genus.

Material and methods

Morphological comparison

For the description of the new species, living plants and dried specimens were examined and measured. The terminology followed Chen (1999), Chen et al. (2011), and Hickey and King (2021). Type specimens of the new species were deposited in ZY, PE, and SCFI. Morphological data of related species were gathered through field work and by examining high-resolution photographs of herbarium specimens from BNU, CAS, CDBI, CSFI, GZTM, HGAS, HWA, IBK, JIU, JJF, KUN, PE, SCFI, WH, ZY. Additionally, observations of living plants were supplemented using resources from online databases such as National Specimen Information Infrastructure platform (Ministry of Science and Technology of China 2013) and the Plant Photo Bank of China website (Institute of Botany of Chinese Academy of Sciences 2008).

Phylogenetic analysis

To test the phylogenetic affiliation of *S. tongziensis*, we assembled an ITS dataset containing 55 accessions representing *S. tongziensis*, 39 species of *Sinosenecio*, four of *Nemosenecio*, six of *Tephroseris*, and an outgroup *Petasites tricholobus* Franch. The nrITS sequences of *S. tongziensis* was newly generated for this study, while the remaining sequences were downloaded from GenBank. GenBank accession numbers are provided in Suppl. material 1.

Leaf material of the new species was collected and dried with silica-gel for DNA extraction. Total DNA was extracted using a modified CTAB procedure (Doyle and Doyle 1987). The nrITS region of *S. tongziensis* was amplified and sequenced using primers ITS1 and ITS4 (Doyle and Doyle 1987) following the procedure described in Peng et al. (2022). Sequences were aligned in MEGA7 (Kumar et al. 2016). The final matrix contained 630 characters. The best-fitting model GTR+G was selected based on Akaike information criterion (AIC) in MrMTgui (Nuin 2004). Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 (Ronquist et al. 2012), with four simultaneous Markov chain Monte Carlo (MCMC) chains run for 2,000,000 generations, sampling one tree every 100 generations. We verified that the average deviation of split frequencies had reached a value below 0.01. The first 25% trees were discarded as burn-in and the remaining trees were used to construct a majority-rule consensus tree with Bayesian posterior probabilities (PP).

Taxonomic treatment

Sinosenecio tongziensis R.B.Zhang, Tan Deng & Ying Liu, sp. nov.

urn:lsid:ipni.org:names:77356936-1
Figs 1, 2

Type. China • Guizhou Province, Tongzi County, Guancang Town, Xianrenshan Mountain, elev. 1,200–1,300 m, growing on slopes beneath forests in karst areas, 27 April 2020, Chong-Bo Ma ZRB1607 (fl.) (***holotype*****:** ZY!; ***isotype*****:** PE!), • 5 May 2024, Ren-Bo Zhang ZRB2661 (fl.) (***paratype*****:** ZY!, SCFI!), • 25 May 2024, Ren-Bo Zhang ZRB2676 (fr.) (***paratype*****:** ZY!).

Diagnosis. Resembles *S. changii* in the subscapigerous habit, ovate-oblong and pinnately veined leaf lamina, and simple to compound terminal corymbs, but differs by membranous leaf lamina (vs. papery), 8–10 lateral veins (vs. 10–18), and stems and leaves pubescent with 2–5 mm long, uniseriate, spreading hairs (vs. sparsely white arachnoid to densely white tomentose) (Table 1).

Description. ***Herbs***, scapigerous. Fresh ***rhizomes*** 6–16 mm in diam., clad in persistent brown petiole bases. ***Stems*** 1 to 3, erect, scapiform, 15–40 cm tall, ribbed, villous with 2–5 mm long, uniseriate, spreading hairs. ***Leaves*** several, radical, rosulate, densely villous as the stems; petiole 3–9 cm long; blade (broadly) ovate, obovate, or ovate-oblong, 4–14 × 3–10 cm, membranous, villous, densely so along veins, pinnately veined, lateral veins 4–5 pairs, base cordate, margin repand with mucronulate teeth, long ciliate. ***Capitula*** 4–29, arranged in terminal simple to compound corymbs; peduncles 1–5 cm long, slender, (sparsely) pubescent, with 3–20 mm long, linear or linear-spatulate bracts. ***Involucres*** campanulate, 4–7 × 4–7 mm, not calyculate; phyllaries ca. 13, lanceolate, oblanceolate or subelliptic, 4–6 × 1–1.6 mm, herbaceous with membranous margins, abaxially pubescent with uniseriate, spreading hairs, apically acuminate, ciliate at the apex and on upper margin. ***Ray florets*** 10–13; corolla tube 2.5–3.5 mm long, glabrous; ray yellow, oblong, 7–10 × 1.2–2.2 mm, 4-veined, apically 3-denticulate. ***Disk florets*** many; corolla yellow, 4–6 mm long, with 2–3 mm tube and campanulate limb; lobes ovate-lanceolate, ca. 0.8 mm long, apically acute. ***Anthers*** oblong, ca. 1.1 mm long, base obtuse to rounded, appendages lanceolate. ***Styles*** ca. 2.5 mm long in ray florets and 4–4.5 mm long in disk florets, branches recurved, ca. 0.8 mm long. ***Achenes*** cylindric, 1.6–2.0 mm long, inconspicuously ridged, glabrous. ***Pappus*** capillary-form, uniform, white, persistent, 2.4–4.0 mm long.

Floral micromorphological characters. The filament collar of *S. tongziensis* consisted of uniformly sized cells (Fig. 3E), and the anther endothecial cell wall thickenings were polarized and radial (Fig. 3F), lending strong support for the phylogenetic affiliation of *S. tongziensis* in subtribe Tephroseridinae (see below).

Phenology. Flowering from April to May, fruiting from May to June.

Etymology. The specific epithet is derived from the type locality, Tongzi County, Guizhou Province, China.

Vernacular name. The proposed Chinese name is “桐梓蒲儿根”, pronounced as “tóng zǐ pú ér gēn”.

Distribution and ecology. The new species is endemic to Guizhou Province and is currently known only from the type locality, Xianrenshan Mountain, Tongzi County. It grows on slopes beneath the forests in karst areas at elevations of 1,200–1,300 m.

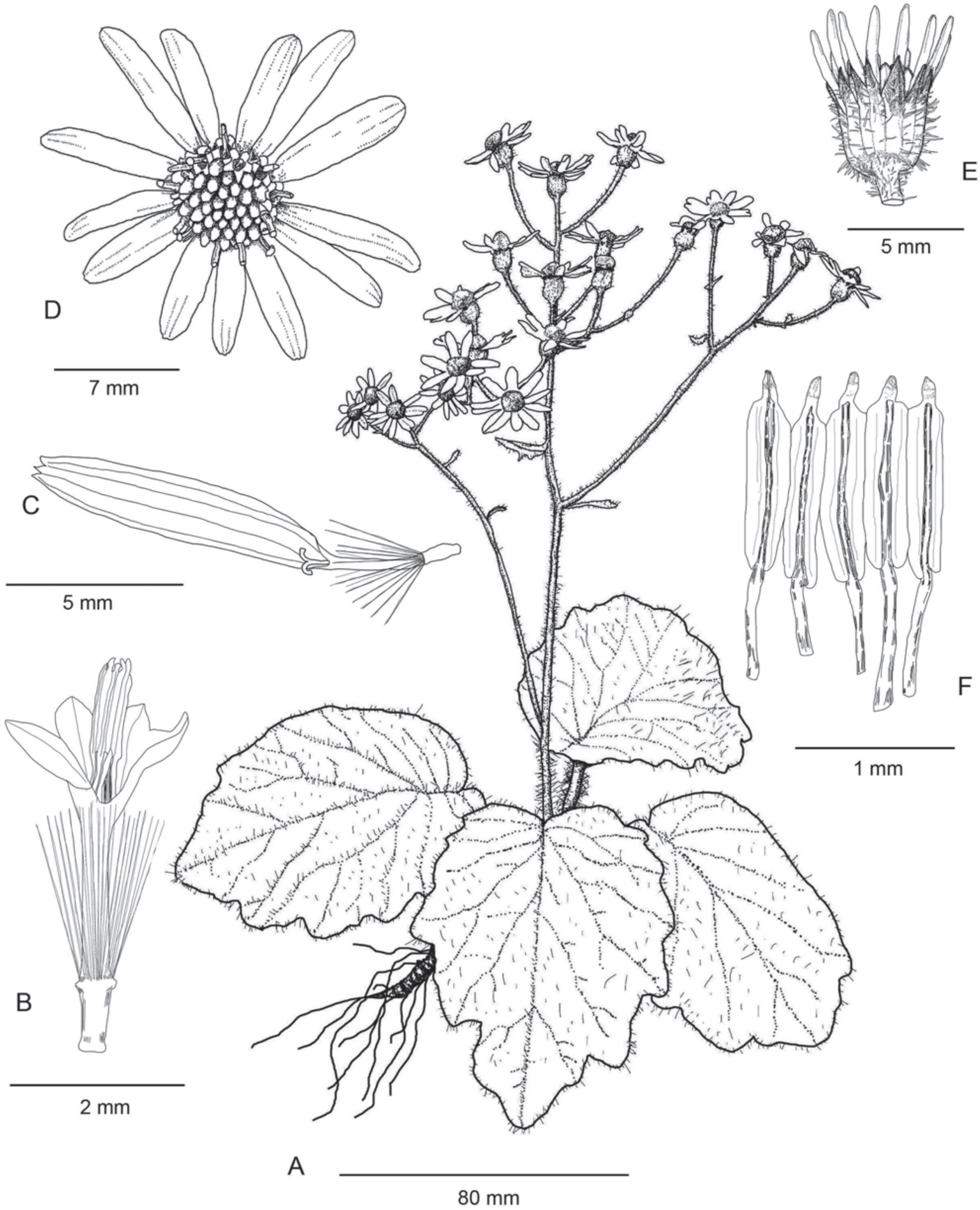

Figure 1. Line drawing of *Sinosenecio tongziensis* **A** habit **B** a disk floret **C** a ray floret **D** top view of a capitulum **E** involucre **F** stamens. All from *Chong-Bo Ma ZRB1607* (PE, ZY). Drawn by Tan Deng.

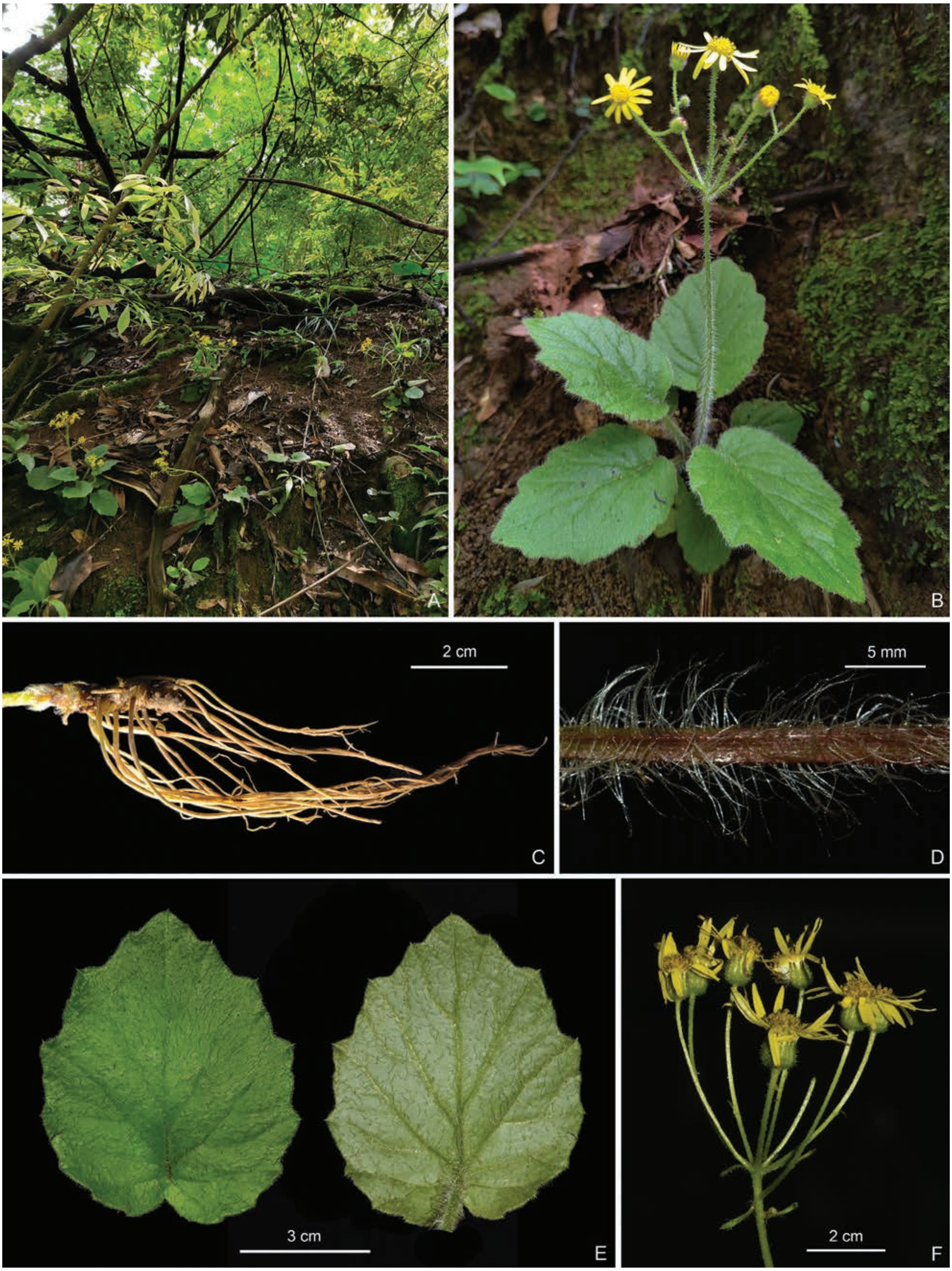

Figure 2. *Sinosenecio tongziensis* **A** habitat **B** habit **C** rhizome and fibrous roots **D** close-up of a petiole **E** adaxial (left) and abaxial (right) leaf surface **F** inflorescence (Photographed by Ying Liu and R.B. Zhang).

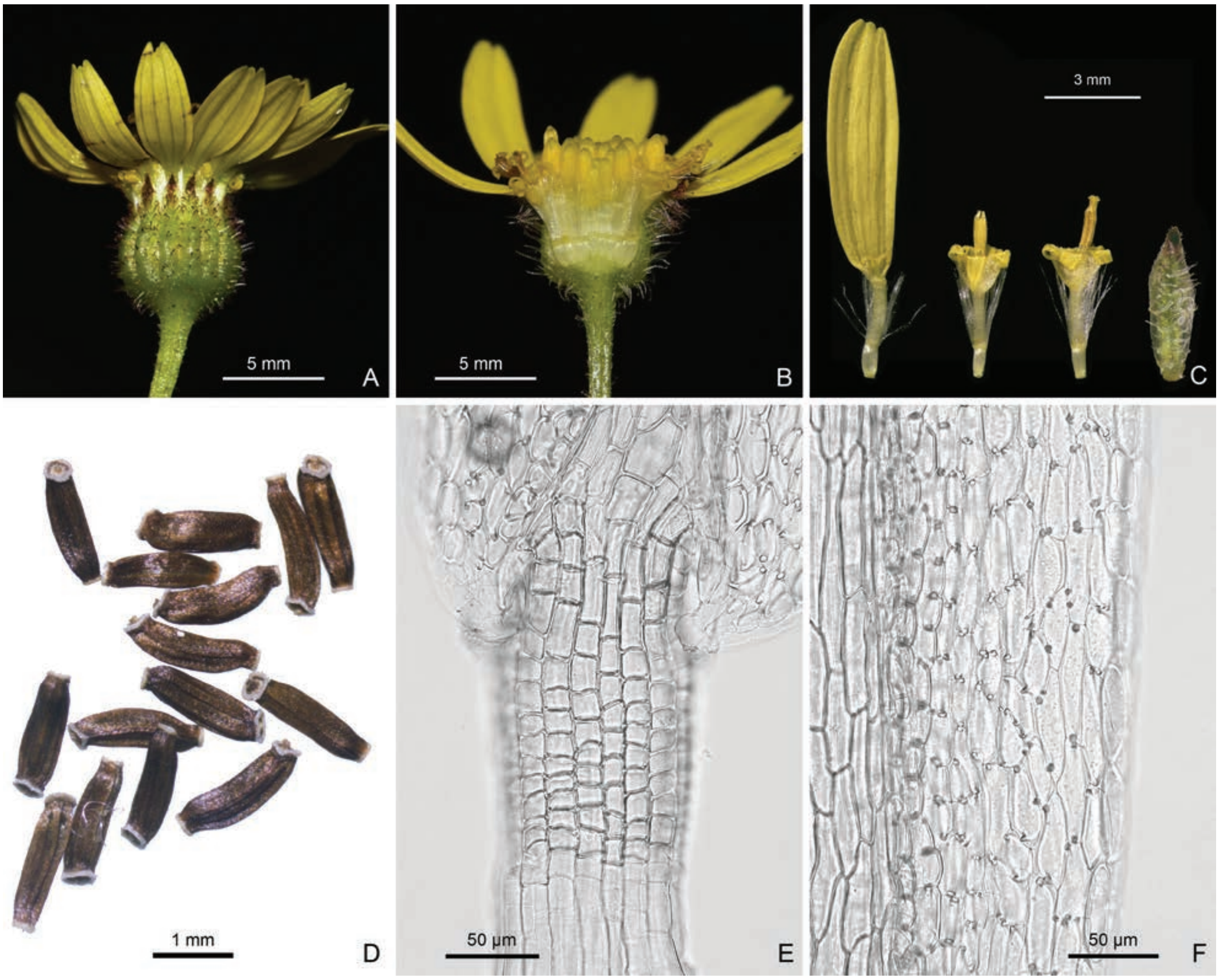

Figure 3. Capitulum, florets, achenes, and floral micromorphological characters of *Sinosenecio tongziensis* **A** side view of a capitulum **B** longitudinal section of a capitulum **C** from left to right, a ray floret, two disc florets, and abaxial surface of a phyllary **D** achenes **E** uniformly-sized cells of filament collar **F** polarized and radial anther endothecial cell wall thickenings (Photographed by Ying Liu and R.B. Zhang).

Table 1. Comparison of *Sinosenecio tongziensis* and *S. changii*.

Characters	*S. tongziensis*	*S. changii*
Number of cauline leaf	0	usually 1
Indumentum on stems and leaves	Villous with 2–5 mm long, uniseriate, spreading hairs	Sparsely white arachnoid to densely white tomentose
Length of petiole	3–9 cm	2.5–4 cm
Shape of lamina	Ovate, obovate, or ovate-oblong	Ovate-oblong
Size of lamina	4–14 × 3–10 cm	2.5–9 × 2–6 cm
Texture of lamina	Membranous	Papery
Morphology of lamina margin	Long ciliate	Not ciliate
Length of peduncle	1–5 cm	1–4.5 cm
Shape of involucre	Campanulate	Broadly campanulate to hemispheric
Number of phyllaries	13	13
Number of ray florets	10–13	12–18
Achene length	1.6–2.0 mm	ca. 1.5 mm
Flowering	Apr–May	May–Jul
Fruiting	May–Jun	Jun–Aug
Distribution	N Guizhou	S Chongqing, N Guizhou, SE Sichuan

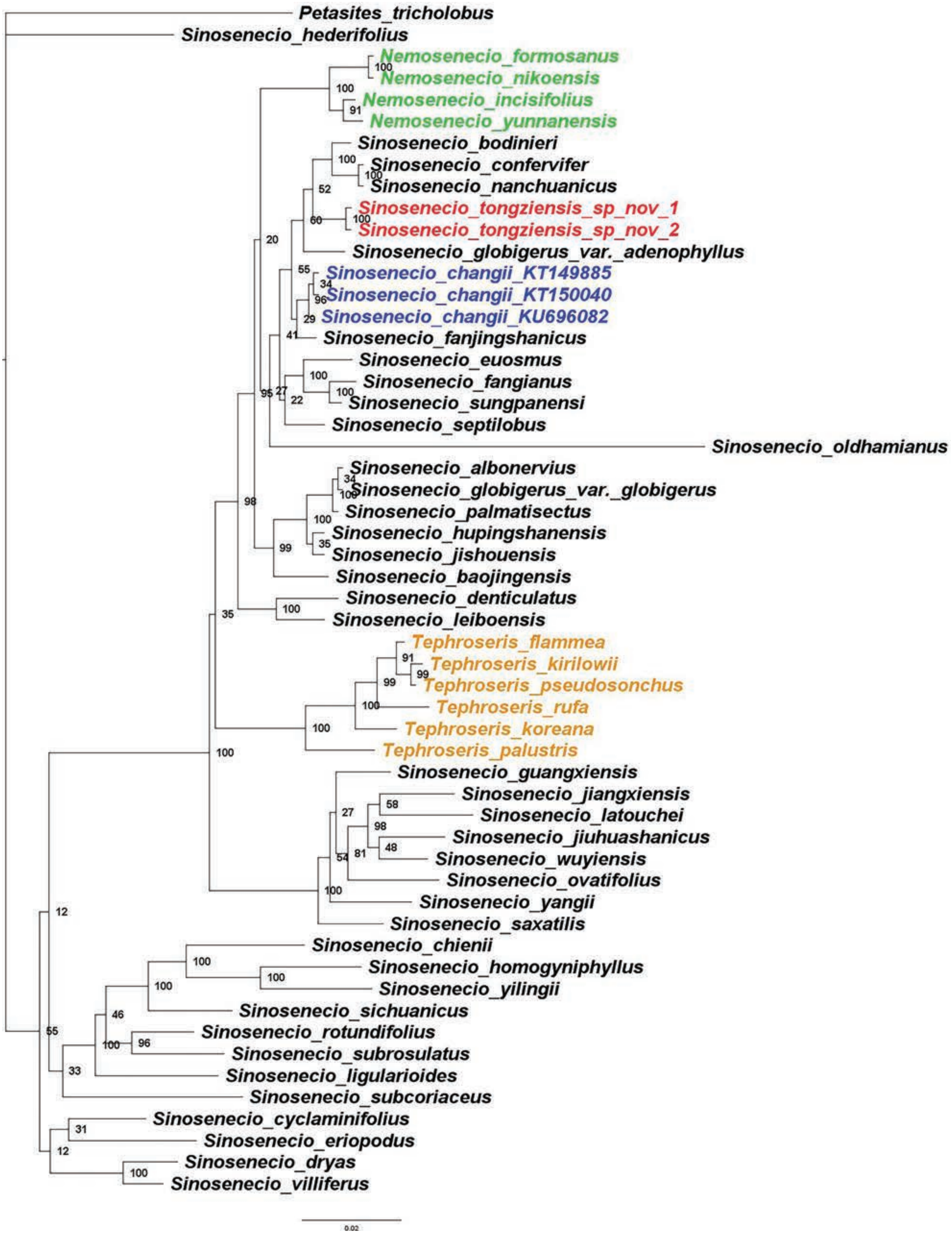

Figure 4. Bayesian phylogenetic tree based on ITS sequence data, showing the phylogenetic position of *Sinosenecio tongziensis*. Numbers at the nodes are Bayesian posterior probabilities. *Nemosenecio* and *Tephroseris* are highlighted in green and yellow, while *S. tongziensis* and *S. changii* are noted in red and blue respectively. GenBank accession numbers were provided for three accessions of *S. changii*.

Conservation status. *Sinosenecio tongziensis* is currently recorded only at the type locality. Thousands of individuals are scattered across the mid-slope and at the base of the mountain, with approximately 600 to 700 mature individuals. Given its narrow distribution and relatively low number of mature plants, *S. tongziensis* may be more appropriately categorized as vulnerable (VU) according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

Phylogenetic affiliation. In the phylogenetic tree, *Nemosenecio* and *Tephroseris* were resolved as monophyletic (Fig. 4). Together with some species of *Sinosenecio*, these genera constituted a well-resolved clade representing subtribe Tephroseridinae (Fig. 4), conforming to previous studies (Wang et al. 2009; Ren et al. 2017; Zou et al. 2020). Despite its close resemblance to *S. changii*, *S. tongziensis* instead formed a weakly supported lineage (PP = 0.6) with *S. bodinieri* (Vaniot) B. Nordenstam, *S. confervifer* (H. Léveillé) Y. Liu & Q. E. Yang, *S. nanchuanicus* Z. Y. Liu, Y. Liu & Q. E. Yang, and *S. globiger* var. *adenophyllus* C.Jeffrey & Y.L.Chen (Fig. 4). It can be easily distinguished from these related species by the pinnately veined leaves (vs. palmately).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: RBZ. Data curation: QLD. Formal analysis: YL, RXW. Funding acquisition: LH. Investigation: RBZ. Methodology: ZMQ, QLD, DJX, YL. Project administration: LH. Resources: ZMQ. Software: RXW. Supervision: RBZ. Visualization: TD. Writing - original draft: RBZ. Writing - review and editing: YL.

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Data availability

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

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Supplementary material 1

Supplementary information

Authors: Ren-Bo Zhang, Tan Deng, Ying Liu, Da-Jun Xie, Ruo-Xun Wei, Lin He, Quan-Li Dou, Zheng-Min Qian

Data type: xlsx

Link: https://doi.org/10.3897/phytokeys.252.141719.suppl1

PhytoKeys 252: 197–206 (2025)
DOI: 10.3897/phytokeys.252.138994

Research Article

On the identity and typification of *Viola tenuis* Bentham (Violaceae)

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Abstract

Viola tenuis Benth. was first described by George Bentham in 1842, without any type specimen designated, but with a collection record by Richard Brinsley Hinds in 1841. Currently, *Viola tenuis* is considered a synonym of another species according to Plants of the World Online (POWO). However, based on a combination of morphological and molecular evidence, we propose reinstating *Viola tenuis* as a distinct species. Our analysis places it within *Viola* sect. *Plagiostigma* subsect. *Diffusae*, where it shows morphological similarities to five other species endemic to Guangdong Province, China. We designated the type of *V. tenuis* here and assigned additional KFBG specimens to the species.

Key words: Conservation, nomenclature, typification, *Viola* sect. *Plagiostigma* subsect. *Diffusae*

Introduction

Viola sect. *Plagiostigma* subsect. *Diffusae*, which is mainly distributed in Southeast Asia, has undergone a long and controversial history in classification. Wilhelm Becker first published this subsection as an unranked group *Diffusae* (Becker 1923) and it was then reclassified as *V.* sect. *Nominium* ser. *Diffusae* (Van Steenis 1934). Thereafter, Wang (1991) elevated the rank of this taxon as *V.* sect. *Diffusae* (W.Becker) C.J.Wang in *Flora Reipublicae Popularis Sinicae*. Recently, Marcussen et al. (2022) placed it in *V.* sect. *Plagiostigma* as subsect. *Diffusae* (W.Becker) C.C.Chang. The diversity of *Diffusae* species in China has long been underestimated, as 19 species have been discovered recently, 10 of which are new to science (Huang 2023). Amongst them, there is an unresolved name, *V. tenuis* Benth., published by George Bentham in 1842, whose identity we resolved here.

Viola tenuis, a species having typical characters of *V.* subsect. *Diffusae*, with long peduncles, ovate-oblong leaf blades, thin rhizomes and flowers with short spurs and marginated style, was first collected in Hong Kong by Mr. Hinds and described by George Bentham in 1842 (Bentham 1842). Wilhelm Becker (1907) described a later homonym, *V. tenuis* W.Becker, from Brazil and reclassified the Hong Kong species as *V. diffusa* subsp. *tenuis* (Benth.) W.Becker (Becker 1921). The name *V. tenuis* Benth. was also accepted as a valid species in *Taiwania* (Wang and Huang 1990), but treated as a synonym of *V. diffusa* Ging.

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in "Flora Reipublicae Popularis Sinicae" (Wang 1991), "Flora of China" (Chen et al. 2007) and Plants of the World Online (Govaerts 2023).

Based on field surveys, molecular and morphological analyses, we provide evidence supporting the recognition of *V. tenuis* Benth. as a distinct species and its name should not be treated as a synonym. In this paper, we present updated descriptions and illustrations for the species.

Materials and methods

We conducted field investigations and observations in Guangdong and Hong Kong during the flowering and fruiting periods of *Viola tenuis*. Leaf material of the species and its relatives were collected and stored in zip-lock plastic bags with silica gel for comparisons and taxonomical treatment. We used a micrometer and a stereomicroscope to observe and measure morphological features to compile detailed descriptions, based on both fresh and dry specimens. Voucher specimens were deposited in the Herbarium of Sun Yat-sen University (SYS) and the Herbarium of Kadoorie Farm and Botanic Garden (KFBG).

Total DNA was extracted by using the modified CTAB method (Doyle and Doyle 1987). Previously reported primers ITS1 and ITS4 (White et al. 1990) were used to amplify the regions of partial internal transcribed spacer 1, 5.8S ribosomal RNA gene and partial internal transcribed spacer 2. PCR amplifications were performed following Fan et al. (2015). We downloaded the sequences of the species and related ones from NCBI and aligned them using MEGA 6.0 (Tamura et al. 2013) with ClusterW, then manually adjusted the alignment. Phylogenetic inferences were carried out with Maximum Likelihood (ML) using IQ-TREE 2.0.3 (Minh 2020). SYM+I+I+R2 was selected as the best–fit model and 2, 000 bootstraps were conducted to evaluate the confidence.

Results

The aligned length of our ITS sequences was 724 bps in total. *Viola tenuis* belongs to the monophyletic group *V.* subsect. *Diffusae* (BS = 100%, Fig. 1) and specimens of *V. tenuis* from Shenzhen and Hong Kong form a sister group with *V. heyuanensis* Yan S.Huang, Q.L.Ye & Q.Fan (BS = 90%).

Taxonomic treatment

***Viola tenuis* Benth. in London J. Bot. 1: 482 (1842)**
Fig. 2

≡ *Viola diffusa* subsp. *tenuis* W.Becker in Philipp. J. Sci. 19(6): 714 (1921).

Type. CHINA • Hong Kong, 1841, *Hinds s.n.* (holotype: K000370141 [photo!]).

Additional specimens examined. CHINA • Hong Kong, Sai Kung, 06 May 2016, *Danial Hang HKY0012* (KFBG, Fig. 3c); • Hong Kong, Sai Kung, 13 Mar 2018, *Jingang Liu JG0615* (KFBG, Fig. 3b); • Hong Kong, Sai Kung, 21 Jul 2023, *Jinlong Zhang JL1488* (KFBG); • Zhuhai, Dawanshan Island, 07 Nov 1973, *Yue73 73-3160* (IBSC); • Zhuhai, Dawanshan Island, 04 Nov 1973, *Yue73 73-3055* (IBSC); • Zhuhai, Dangan Island, 12 Jul 2017, *Ming Tan Tu TY3390* (CDBI);

Figure 1. Maximum Likelihood (ML) tree of the new species and related species. Bootstrap values of the Maximum Likelihood are shown along the branches. Outgroups: *Melicytus obovatus*. *Viola tenuis* are marked in grey.

• Shenzhen, Qiniangshan Mountain, 18 Mar 2005, *Shouzhou Zhang & Liangqian Li 0259* (SZG); • Shenzhen, Qiniangshan Mountain, 17 May 2005, *Shouzhou Zhang & Liangqian Li 1528* (SZG); • Shenzhen, Qiniangshan Mountain, 16 Apr 2005, *Shouzhou Zhang & Liangqian Li 0196* (SZG); • Shenzhen, Qiniangshan Mountain, 16 Apr 2019, *Q. Fan 18910* (SYS);

Etymology. It is hypothesised that the specific epithet refers to the plant’s thin rhizomes.

Vernacular name. We propose a Chinese name, Xì Jīng Jǐn Cài (细茎堇菜), to reflect the thin rhizomes of this species.

Description. Perennial, stoloniferous herb with basal leaves rosulate, 5–8.5 cm tall. Rhizome erect or obliquely erect, rather slender, dark purple, with dense remains of stipules and petioles. Lateral stolons purple or light green, spreading and elongating, usually producing adventitious roots, with an apical rosette of leaves. Stipules ca. 8 mm, adnate to petioles for about 1/3 of their length at base, linear-lanceolate, acute at apex, with margins pinnatifid, glabrous or rarely puberulous. Petioles 2–4 cm, with wings narrow, glabrous

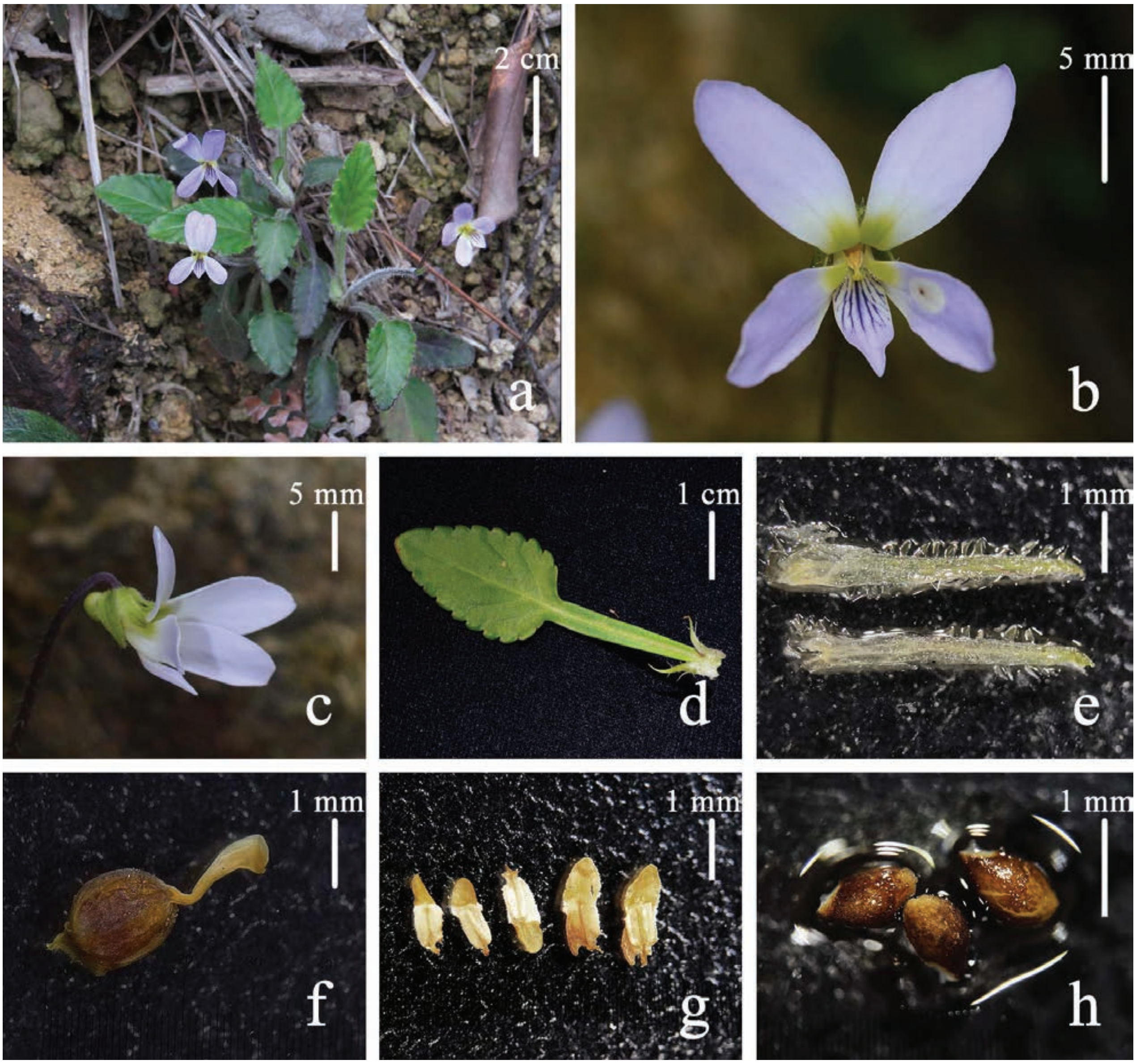

Figure 2. *Viola tenuis* Benth. **a** habitat and habit (photograph in Hong Kong, J. Zhang) **b** flower, front view **c** flower, side view **d** leaf and stipules **e** bracteoles **f** ovary and stigma **g** stamens **h** seeds.

or sparsely puberulous along the margin. Leaf blades ovate or ovate-oblong, 1.0–2.4 × 1.5–3.3 cm, glabrous or densely puberoulous; margin finely crenate; base wedge-shaped to truncated; apex obtuse, rarely acuminate. Chasmogamous flowers ca. 1.5 cm in diam.; peduncles slender, 6–7 cm long, glabrous, much higher above the basal leaves, with two opposite bracteoles above middle; bracteoles linear-lanceolate, ciliate, 5.5–6 mm long, apex acuminate or obtuse. Sepals green, ciliate, lanceolate, 1.5–1.7 × 4–5.5 mm, with entire margin, acuminate apex and truncate, with extremely short semicircular appendages. Petals purple, anterior one with apparent violet lines, posterior and lateral ones with a yellow to green patch at base; posterior petals, narrowly ovate to oblong, 4.5 × 12 mm, glabrous, with entire margin and obtuse apex; lateral petals with straight to slightly clavate hairs at the base, oblong, ca. 1.3 cm long, with entire margin, apex obtuse; anterior petal spatulate, 8 mm long, with a short, round spur, interior side of the spur puberulent. Stamens 5, unequal, puberulent;

Figure 3. a holotype of *Viola tenuis* Benth. (K000370141) **b, c** specimen of *V. tenuis* in KFBG (JG0615, HKY0012).

anther thecae ca. 0.9 mm long, with terminal appendages ca. 0.6–0.85 mm long; posterior appendages (nectar spurs) of the two anterior stamens ca. 0.95 mm long, triangular. Ovary ovoid, ca. 1.25 × 1.49 mm, glabrous; style clavate, ca. 1.35 mm long, conspicuously geniculate at base; stigma glabrous, with thickened lateral margins, shortly beaked at apex. Cleistogamous flowers ca. 3.2 mm long; peduncles ca. 6.5 mm long; bracteoles linear-lanceolate, puberulous, ca. 2.8 mm long, apex acute. Sepals green, glabrous or puberulous, lanceolate, ca. 0.8 × 3.2 mm, with entire margin, acuminate obtuse. Capsules glabrous, light green to yellow, ovoid to oblong, ca. 9 mm long. Seeds brown, oblongoid, ca. 1.4–1.55 mm long, with tubercles and inconspicuous elaiosomes.

Phenology. Chasmogamous flowers from April to May, rarely October, cleistogamous flowers from May to September and fruits from April to October.

Distribution and habitat. *Viola tenuis* is currently known from Hong Kong, Shenzhen and Zhuhai in China (Fig. 4). The species grows on rocks or in rocky crevices at altitudes of 100–700 m a.s.l.

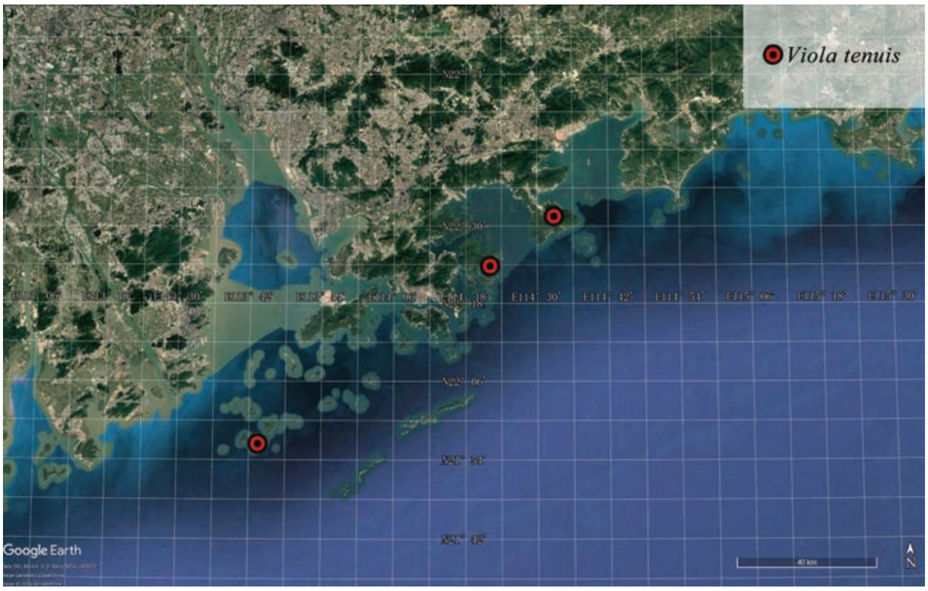

Figure 4. Distribution of *Viola tenuis* Benth.

Conservation status. As its area of occupancy is estimated less than 20 km^2, the conservation status of *Viola tenuis* should be considered as Vulnerable (VU) according to IUCN Red List criteria (D2; IUCN 2024).

Discussion

Becker (1921), when merging *Viola tenuis* into *V. diffusa* as a subspecies, described its leaves as roundish or ovate with a distinctly cordate base and long petioles. While accepting the subspecies, he suggested it might simply be a small-leaved variety of *V. diffusa*. However, Bentham (1842), when publishing this species, described it as having ovate-oblong leaves with a truncate base, along with rhizomes and stolons; he specifically mentioned that the leaves are similar to those of *V. patrinii* Ging. Although the original description did not include a type designation, there is only one specimen collected by Richard Brinsley Hinds in Hong Kong in 1841 (Fig. 3a). As the individuals on it also have narrow and truncate leaves, the distinct morphological characteristics clearly distinguish it from *V. diffusa* and other species of the *Diffusae* group. Based on our field surveys, *V. tenuis* Benth. is endemic to Guangdong and Hong Kong (Figs. 3 and 4) and forms a paraphyletic group with *V. diffusa* (Fig. 1), so it is more appropriate to be treated as a separate species. Both the name *Viola tenuis* proposed by Becker in 1907 and *Viola* × *tenuis* proposed by Karl Richter in 1891 are illegitimate later homonyms, based on Shenzhen Code Art. 11.4 and 53.1 (Turland et al. 2017).

In our phylogenetic tree, *V. tenuis*, *V. huizhouensis* Yan S.Huang & Q.Fan, *V. chaozhouensis* Yan S.Huang, J.H.Ding & Q.Fan, *V. guangzhouensis* A.Q.Dong, J.S.Zhou & F.W.Xing, *V. longissima* Yan S.Huang & Q.Fan and *V. heyuanensis* form a monophyletic group (Fig. 1). The latter three species have aerial stems, while the former three do not. The colour and hairiness of the leaf surface, the thickness of the rhizome and the size of the flowers can distinguish the first three species, but the relationship between morphological characters and the phylogeny still needs more investigation and evidence. These species are dis-

Table 1. Morphological comparisons between *Viola tenuis*, *V. diffusa* and *V. heyuanensis*.

Species name	*Viola tenuis*	*Viola diffusa*	*Viola heyuanensis*
Leaf blade	Ovate to ovate-oblong, 1.0–2.4 × 1.5–3.3 cm, glabrous or puberulous; finely crenate margin; wedge-shaped to truncated base; obtuse apex, rarely acuminate	Ovate to oblong, 1.7–2.5 × 3.0–4.9 cm, nearly glabrous; crenate margin; wedge-shaped base, gradually tapering into petiole	Narrowly ovate to triangular, 1.8–3.2 × 1.3–1.8 cm, densely pubescent; coarsely serrate margin; cuneate to truncate base, rarely cordate; obtuse or acuminate apex
Petioles	2–4 × ca. 0.1–0.2 cm, glabrous or sparsely puberulent, narrow wings	1.6–2.0 × 0.35–0.47 cm, sparsely pubescent, broad wings	2–4.5 cm, densely pubescent, narrow wings
Aerial stem	Absent	Absent	Aerial stems solitary or several and fasciculate, erect, slender
Rhizome	Erect or obliquely erect, rather slender, dark purple, with dense remains of stipules and petioles	Absent, with fibrous root	Erect or obliquely erect, rather stout, reddish-brown rarely branched
Flower	Chasmogamous flowers ca. 1.5 cm in diam.	Chasmogamous flowers ca. 0.8 cm in diam.	Chasmogamous flowers ca 1.2 cm diam.
Seeds	Brown, oblongoid, with tubercles, 1.4–1.55 mm long	Yellow, obovoid, with smooth surface, 1–1.2 mm long	Brown, obovoid, with sparse tubercles, 1–1.5 mm long

tributed in the mountainous area of Guangdong Province and appear to have undergone adaptive radiation (Huang et al. 2023). We hypothesise that sea level change might have influenced their diversification (Holbourn et al. 2018).

Based on our field surveys, molecular and morphological analyses (Table 1), we provide strong evidence supporting the recognition of *V. tenuis* Benth. as a distinct species and, thus, that this name should not be treated as a synonym.

There is no type specimen of *V. tenuis* Benth. designated in the protologue (Bentham 1842), which was entitled as ‘Enumeration of plants collected from Hong Kong, by RB Hinds, determined and described by George Bentham, ESQ’. According to the publication, we determined K000370142 (Fig. 2a) in the Royal Botanic Garden Kew as the holotype of *V. tenuis* (Shenzhen Code, Art. 7.9, Art. 8.1), as it is the only specimen collected by Hinds and identified as *Viola tenuis* by G. Bentham.

Key to *Viola* subsect. *Diffusae*

1 Leaf base conspicuously decurrent to the petiole **2**
– Leaf base inconspicuously or not decurrent **3**
2 Seeds with a conspicuous elaisome .... ***Viola pendulipes***
– Elaisome inconspicuous or absent **4**
3 Leaf blades oblong to oblong–lanceolate, distinctly longer than broad .... ***V. yunnanensis***
– Leaf blades ovate or orbicular, about as long as broad **5**
4 Flowers 20–35 mm across **6**
– Flowers 4–20 mm across **7**
5 Flowers with a yellow to green patch at the base .... ***V. qingruii***
– Flowers without a yellow to green patch at the base **8**
6 Plants with distinct aerial stems **9**
– Plants with short or no aerial stem **10**
7 Leaf blades abaxially purple .... ***V. jinggangshanensis***
– Leaf blades light green **11**
8 Stipules triangular; flowers white .... ***V. lucens***
– Stipules linear to linear-lanceolate; flowers light pink .... ***V. changii***

9 Leaf blades abaxially purple .... ***V. guangzhouensis***
– Leaf blades light green..**12**
10 Leaves papery when dried .... ***V. nanlingensis***
– Leaves thinly leathery when dried **13**
11 Seeds yellow to light brown, with smooth surface..................................**14**
– Seeds dark brown, with tubercles **15**
12 Posterior petals shorter than lateral ones; aerial stems 5–7 cm tall......... ....***V. heyuanensis***
– Posterior petals longer than lateral ones; aerial stems up to 40 cm tall ....***V. longissima***
13 Leaves abaxially green; rhizomes slender ....***V. tenuis***
– Leaves abaxially dark purple **16**
14 Lateral petals bearded .... ***V. diffusa***
– Lateral petals glabrous .... ***V. amamiana***
15 Lateral petals bearded .... ***V. pricei***
– Lateral petals glabrous .... ***V. nagasawae***
16 Leaf apex acute; leaf blade glabrous or pubescent only along veins ....***V. chaozhouensis***
– Leaf apex obtuse; leaf blade densely covered with white pubescence....... ....***V. huizhouensis***

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

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

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Supplementary material 1

GenBank accessions for phylogenetic analysis

Authors: Yan-Shuang Huang, Stephan W. Gale, Jinlong Zhang, Qiang Fan

Data type: docx

Link: https://doi.org/10.3897/phytokeys.252.138994.suppl1

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Research Article

Taxonomic revision and phylogenetic relationships of *Thinouia* (Sapindaceae), a neotropical genus

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Abstract

A taxonomic revision of *Thinouia* (Sapindaceae) is presented, including typification and descriptions for all accepted species. Phylogenetic analyses based on molecular data confirm that the genus is monophyletic and suggest two main clades with *T. cazumbensis* sister to the other species of the genus. Thirteen species of *Thinouia* are accepted, including a new species from Amazonia. The genus is distributed across continental tropical America, with its highest diversity found in South America. Its species can be distinguished by morphological details of leaves, indumentum, inflorescences, flowers, and fruits. We present a comprehensive review of the morphology and geographical distribution of the genus, along with an identification key, distribution maps, conservation risk assessments, illustrations, and comments on the ecology and taxonomy for all species, gathered from the literature and fieldwork.

Key words: Lianas, Neotropical flora, Paullinieae, Sapindales, Sapindoideae

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Introduction

Thinouia Triana & Planch. is a small genus of Neotropical lianas, comprising 13 species distributed from southern Mexico to southern Brazil and Argentina, across a wide variety of habitats including savannas, evergreen forests and rainforests in the Neotropical region (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2011; Medeiros et al. 2020). The genus is monophyletic and currently is placed in tribe Paullinieae, alongside *Cardiospermum* L., *Lophostigma* Radlk., *Paullinia* L., *Serjania* Plum. ex Miller, and *Urvillea* Kunth (Acevedo-Rodríguez et al. 2017; Medeiros et al. 2020). *Thinouia* is characterized by the presence of umbelliform and racemiform thyrses, actinomorphic flowers with marginal or bifid petal appendages, isopolar tricolporate pollen grains, and schizocarpic fruits that split into three mericarps, each with a distal wing (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2017; Medeiros et al. 2020).

After extensive work in the field, herbarium, and molecular laboratory (Acevedo-Rodríguez et al. 2017; Medeiros et al. 2020), we present an updated taxonomic revision of *Thinouia* based on novel molecular phylogenetic data. Since the last taxonomic revision of the genus (Radlkofer 1931–1934), almost 100 years ago, new species have been described; more information regarding distribution, morphology and ecology have been gathered; and the phylogenetic relationships among the species have been analyzed. In this revision, we provide morphological descriptions for all taxa, along with an identification key, illustrations, distribution maps, conservation risk assessments, and comments on their ecology, nomenclature and taxonomy.

Material and methods

Taxonomy

Morphological descriptions and phenology of species are based on fieldwork observations gathered by the senior author, and on the study of herbarium specimens deposited at the following herbaria: ALCB, BHCB, CEPEC, COL CVRD, ESA, F, FUEL, GH, HCF, HSTM, HUEFS, IAN, ICN, INPA, F, JPB, K, NY, MBM, MBML, MEDEL, MEXU, MG, MICH, MO, P, QCNE, R, RB, RBR, RON, SP, SPF, U, UB, UFACPZ, UPCB, USM, US, and VIES (according to Thiers 2022). Our analyses included the samples deposited in the Laboratory of Botany and Plant Ecology of the Federal University of Acre (LABEV). The indumentum terminology follows Beentje (2010), leaf and fruit terminology follow Radford et al. (1974), leaf venation terminology follows Ellis et al. (2009), and inflorescence terminology follows Weberling (1989). The species concept follows the evolutionary species concept of de Queiroz (2007) and we considered isolated evolutionary lineages diagnosed by morphological characters to be separate species.

Maps were elaborated using ArcGIS 10.5 software (ESRI 2016), geographical coordinates were obtained from herbaria specimens, and shapefiles were obtained from The Americas Base Map (Bletter et al. 2004) and WWF (2022). The distribution of *Thinouia* across biomes in the Neotropics is based on Olson et al. (2001). For each species, one representative specimen per municipality is cited. An Index to Numbered Collections Studied is included in Appendix 1.

The conservation status for each species was evaluated according to criteria adopted by the International Union for Conservation of Nature (IUCN 2022). For *Thinouia tomocarpa* and *T. silveirae* the extent of occurrence (EOO) and area of occupancy (AOO) were calculated using Kew's GeoCAT tool (available at: <https://geocat.iucnredlist.org/>) (Bachman et al. 2011). For the other species, the EOO and AOO were calculated in collaboration with the CNCFlora (available at: <http://www.cncflora.jbrj.gov.br/portal>), adopting EOO for the weedy species and AOO, based on 2 km cell width, for the remaining species. Collections that could not be properly georeferenced were excluded from EOO and AOO calculations.

Phylogenetic reconstruction

The phylogenetic analysis included the same markers as in Acevedo-Rodríguez et al. (2017) and Medeiros et al. (2020): the plastid marker trnL intron and nuclear ribosomal internal transcribed spacer (ITS). The genomic DNA was extracted using DNA NucleoSpin Plant II kit (Machery-Nagel, GmbH & Co. KG, Dueren, Germany) following the manufacturer's protocol. Approximately 60 mg of leaf tissue were pulverized with Tissuelyzer (Qiagen, Dusseldorf, Germany) for 3 min at 60 hz. PCR amplification used the primers and the protocols described in Acevedo-Rodríguez et al. (2017). PCR products were purified and sequenced by Macrogen (Seoul, South Korea). All sequences, vouchers and GenBank accession numbers are summarized in Appendix 2.

The alignments were performed using Muscle (Edgar 2004) using the default parameters implemented in Geneious software (Kearse et al. 2012). Poorly aligned regions were manually adjusted and removed in case they could not unambiguously be aligned. We used jModelTest 2.0 (Guindon et al. 2010; Darriba et al. 2012) and the Akaike information criterion (AIC) to select the best-fit model of nucleotide substitution for each dataset. GTR+I+G was selected as the best model for the ITS dataset, whereas HKY+G was selected as the best model for the trnL dataset. Bayesian Inference (BI) analyses were conducted using MrBayes 3.2.2 (Ronquist et al. 2012) in the online CIPRES Science Gateway interface (Miller et al. 2015) with four Markov chain Monte Carlo (MCMC) runs using a random starting tree and 10 million generations, with a sampling frequency of one every 1000th generations. We used Tracer 1.7 (Rambaut et al. 2018) to check for convergence of the MCMC and to check for stationarity. We discarded 25% of the trees as burn-in.

Since the morphology of petal appendages was an important character in Radlkofer's infrageneric classification of the genus (1878), we performed an ancestral character state reconstruction analysis of this structure with Mesquite v. 3.61 (Maddison and Maddison 2019), to demonstrate if Radkofer's classification is good. We analyzed the size attributes of the petal appendages (longer than the petals vs. shorter or equal to the petals) using two complementary approaches, parsimony of ancestral states, and likelihood of ancestral states. For the likelihood of ancestral states method, the current probability model was selected.

Results

Taxonomic history

Thinouia was described by Triana and Planchon (1862) based upon *Thouinia scandens* Cambess., a species that they transferred into a new genus, *Thinouia*. Although no explanation is given by Triana and Planchon for the origin of the name *Thinouia*, it seems to be an anagram of *Thouinia*, a genus that honors André Thouin [1747–1824] a distinguished French botanist. The genus wasn't immediately recognized by contemporaries of Triana & Planchon, e.g., Bentham and Hooker (1862) or Baillon (1874), until Radlkofer [1829–1927], the acclaimed specialist of Sapindaceae, published a new species and a synopsis of the genus in 1878, when he recognized seven species. In his treatment of Sapindaceae for Martius' "Flora Brasiliensis" (Radlkofer 1892–1900), Radlkofer monographed *Thinouia*, recognizing 11 species. In his posthumous publication of Sapindaceae for Engler's "Das Pflanzenreich" (1931–1934), his treatment of *Thinouia* was published verbatim from his treatment for "Flora Brasiliensis".

Radlkofer (1878) organized the genus in two sections. *Thinouia* sect. *Lepidodine* (= sect. *Thinouia*), presented a single species, *T. myriantha*, and was characterized by the presence of a petaloid appendage that is longer than the petals; *Thinouia* sect. *Petalodine*, with five species, was characterized by a petaloid appendage that is shorter than the petals. *Thinouia obliqua* Radlk. wasn't classified in this scheme because its flowers were unknown at the time. In 1890, recognizing the affinity of *Thinouia* with other genera of climbing Sapindaceae, Radlkofer placed *Thinouia* in a monotypic subtribe (Thinouieae) within tribe Paullinieae, which in turn was placed in his series Eusapindaceae (= subfam. Sapindoideae). In his 1931–1934 monograph, Radlkofer presented a brief taxonomic history of the genus and recognized the following 11 species: *Thinouia compressa* Radlk., *T. coriacea* Radlk., *T. mucronata* Radlk., *T. myriantha* Triana & Planch., *T. obliqua* Radlk., *T. paraguayensis* (Britton) Radlk., *T. repanda* Radlk., *T. sepium* S. Moore, *T. scandens* (Cambess.) Triana & Planch., *T. ternata* Radlk., and *T. ventricosa* Radlk.

Following Radlkofer's work, few changes have taken place in the taxonomy of the genus. Based on the striking morphology of the fruits, Standley and Record (1936) described *Thinouia tomocarpa* from British Honduras (now = Belize), a discovery that expanded the known occurrence of the genus outside South America. Croat (1976) recorded *T. myriantha* from Panama while placing *T. tomocarpa* as a synonym of this species. In your work de la Cruz and Dirzo (1987) recorded *T. tomocarpa* for Veracruz, Mexico. Ferrucci (1991), in her treatment of Sapindaceae for the "Flora de Paraguay", recorded the distribution of *T. compressa* and *T. mucronata* for Paraguay for the first time and synonymized *T. repanda* under *T. mucronata* and *T. sepium* under *T. paraguayensis*.

While revising *Serjania* sect. *Platycoccus*, and based on a morphological-based cladistic analysis, Acevedo-Rodríguez (1993) proposed *Thinouia* as a member of tribe Cupanieae, in the vicinity of *Allosanthus* Radlk. This placement was later reversed, and *Allosanthus* was placed in synonymy with *Thinouia* (Acevedo-Rodríguez et al. 2011), a decision that is supported by recent molecular studies (Acevedo-Rodríguez et al. 2017, Medeiros et al. 2020). Ferrucci and Somner (2008) described *Thinouia restingae* Ferrucci & Somner, a species endemic to SE Brazil and placed in section *Petalodine*. They also presented a key to the species of *Thinouia* occurring in Brazil. In 2020, *T. cazumbensis* H. Medeiros was described from the state of Acre, Brazil (Medeiros et al. 2020).

Distribution and ecology

Thinouia is a relatively small genus of Neotropical lianas comprising 13 species distributed from southern Mexico to southern Brazil and Argentina, across a wide variety of habitats, including savannas, evergreen forests and rainforests in the Amazon, Central America, and Brazilian Atlantic Forest (Fig. 1). The occurrence of *Thinouia* in Central America and on the Guiana Shield is almost entirely due to two species (*T. trifoliolata* and *T. myriantha*) that are widely distributed, and in southern Mexico it is represented by only one species (*T. tomocarpa*). The Amazon region and the Brazilian Atlantic Forest are major centers of diversity for the genus with four and eight species, respectively. Furthermore, some species also occur in cerrado vegetation, in gallery or seasonally dry forests.

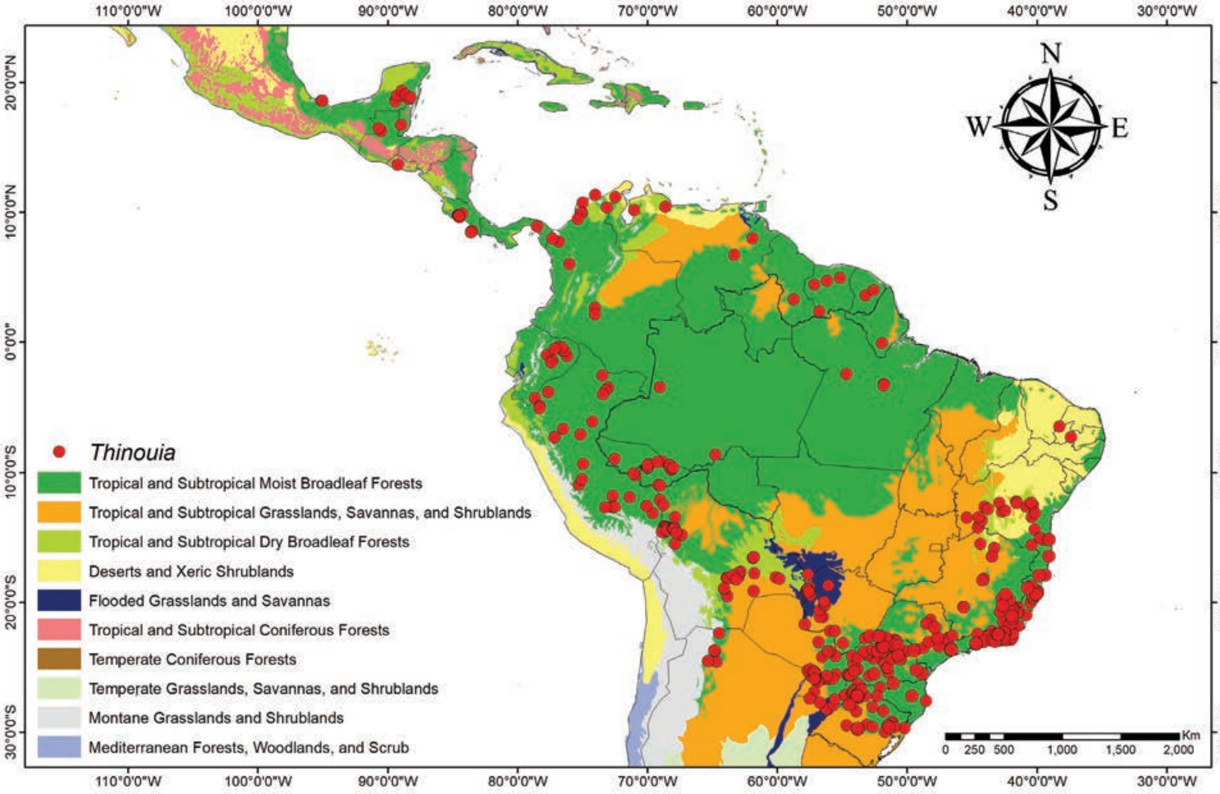

Figure 1. Distribution of *Thinouia* across biomes in the Neotropics. Shapefile WWF 2022 (Olson et al. 2001).

Morphology

Life form, stem morphology and macroanatomy

All species of *Thinouia* are tendrilled lianas that often reach the canopy of the forest. However, in the absence of phorophytes, some species may grow as arching shrubs.

Stems are woody, cylindrical (Fig. 2A) or ribbed (Fig. 2B), reaching up to ca. 13 cm in diam. and 15 m in length (e.g., *Thinouia scandens*). During initial stages and early secondary growth, stems in all species have a regular anatomy (here termed simple, Fig. 2A) that is produced by the activity of a single, continuous cambium. This regular anatomy continues through the plant's entire lifespan in some species (e.g., *Thinouia cazumbensis*, *T. compressa*, *T. myriantha*), but in a number of species (e.g., *T. obliqua*, *T. restingae*, *T. scandens*), secondary vascular cylinders develop from new cambia within the pericycle during late secondary growth. These secondary vascular cylinders (= peripheral cylinders), which aren't connected to the initial vascular cylinder (= central cylinder), grow in thickness in a manner similar to the one described for the central cylinder (Tamaio and Somner 2010). The combination of these vascular cylinders form a cable-like structure, where some of the peripheral cylinders project as ribs along the external surface of the stem (Fig. 2B). The peripheral cylinders (5–7 larger ones and numerous smaller ones), are produced continuously, and in a cross-section of the stem are shown as having diameters that relate to their developmental stages (Fig. 2B). This anatomical variant is referred to by Tamaio and Somner (2010) as the corded vascular system and by Angyalossy et al. (2015) as neoformed secondary vascular cylinders (neo formations).

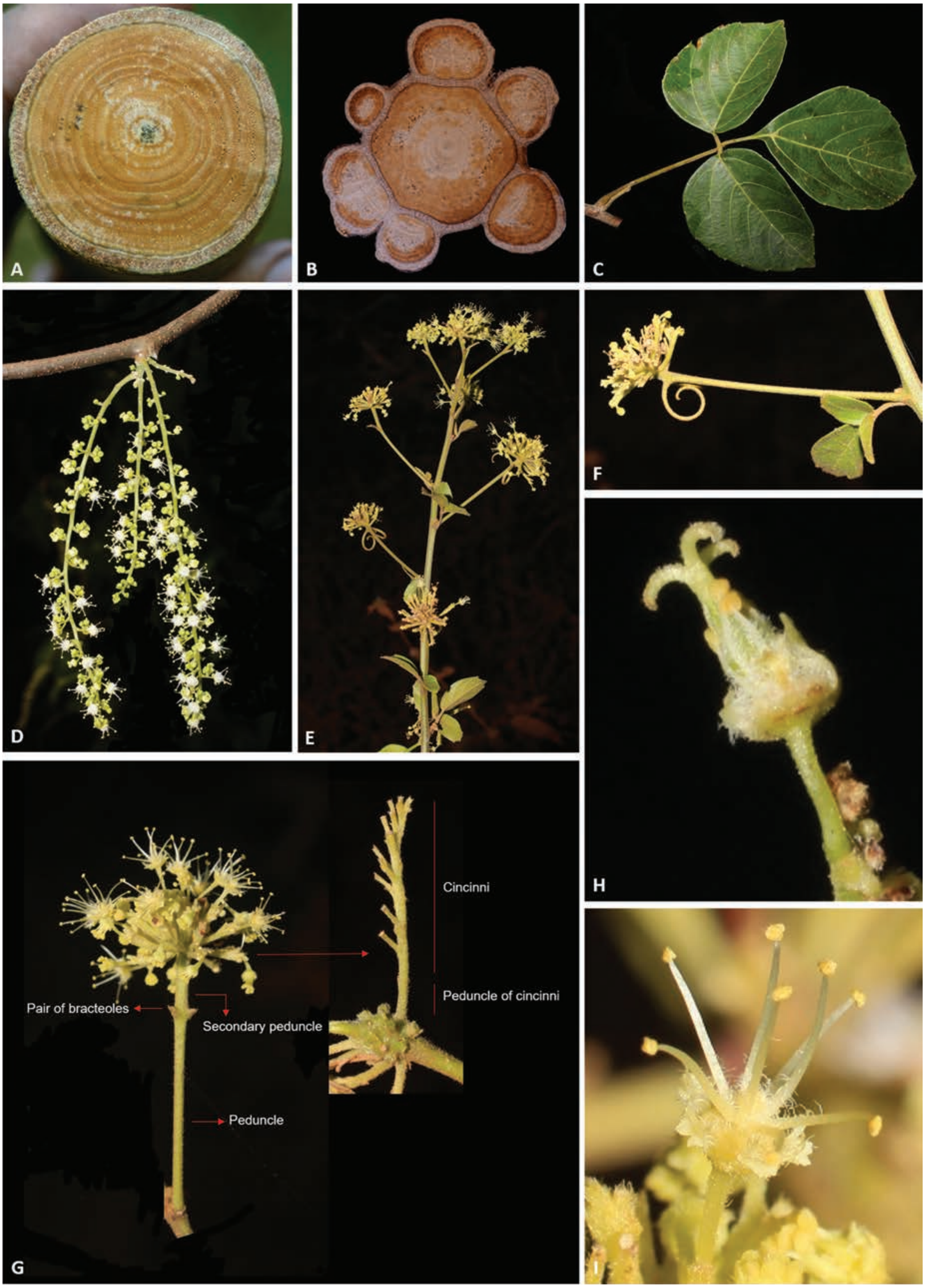

Figure 2. A Cylindrical simple stem in *Thinouia paraguayensis* **B** lobed stem with a central vascular cylinder and six neoformed peripheral ones in *T. scandens* **C** trifoliolate leaf of *T. obliqua* **D** cauliflorous inflorescence in *T. trifoliolata* **E** terminal frondobracteate synflorescence in *T. silveirae* **F** umbelliform, long-peduncled thyrse with tendrils in *T. silveirae* **G** diagram of an umbelliform inflorescence with terminology **H** pistillate flower **I** staminate flower [Acevedo-Rodríguez 16750 (**A**) 3701 (**B**); Medeiros 3832 (**C**) 3331 (**D**) 4496 (**E–G**, **I**) 4464 (**H**); photos: **A, B** by P. Acevedo-Rodríguez **C–I** by H. Medeiros].

Thinouia may be confused with woody species from the closely related genera *Paullinia* or *Serjania* but may be distinguished from these by the presence of neoformed secondary vascular cylinders. These are characterized by the presence of sclereids in the center, instead of a pith as in the peripheral cylinders in species of *Paullinia* and *Serjania* (Tamaio and Somner 2010, Cunha Neto et al. 2017). The latter two genera may have neoformed secondary vascular cylinders, but these are formed symmetrically around the central cylinder instead of randomly, as in *Thinouia* (Acevedo-Rodríguez et al. in press).

Stipules, leaves, trichomes and indumentum

The shape of stipules seems to be a conserved character as all species show minute, triangular or deltate stipules, axillary .

Leaves in all species are trifoliolate (Fig. 2C), with the terminal leaflet consistently different from the lateral ones. Leaflets are elliptic to obovate, acute, decurrent, subcuneate, truncate, obtuse, or rounded at the base, and acute, acuminate, rounded, emarginate or obtuse and frequently mucronate at the apex. Most species have subtriplinerved venation.

The indumentum is variable regarding both type (simple, arachnoid or capitate) and density (glabrous to tomentose) of trichomes. Indumentum variation in the cavity of the seed locule is of great utility in distinguishing different species.

Inflorescences and flowers

Inflorescences in *Thinouia* are umbelliform (Fig. 2E) or racemiform thyrses (Fig. 2D) often bearing a pair of circinate tendrils at the apex of the peduncle (Fig. 2F), which always bears a diminute bract at base of the peduncle and two bracteoles at the apex or at the base of the secondary peduncle (Fig. 2G). Thyrses are axillary and simple, or terminal and forming a synflorescence. Additionally, *T. trifoliolata* may be cauliflorous (Fig. 2D). Flowers are grouped in cincinni (Fig. 2G).

Thinouia flowers are actinomorphic (Fig. 2I) and do not show much variation across species, with the exception of *T. obliqua* where the number of stamens is 6–7 (instead of 8, like in the other species). Flower size typically ranges between 2 to 5 mm long. Sepals are variable in terms of pubescence and shape, but normally they are abaxially pubescent and adaxially glabrous.

The corolla consists of five free petals, mostly spatulate or less frequently lanceolate, deltoid or obdeltate. Petals are accompanied by petaloid appendages that are either adnate to their adaxial basal surface or are a prolongation of the petal's margin. The appendages are appressed against the filaments in a way that seems to restrict access to the nectary disc. They are bilaterally symmetrical, sometimes branched distally, and vary in size, being either shorter or slightly, to significantly, longer than the petals.

The androecium consists of 8 stamens (6–7 in *Thinouia obliqua*) with basally connate filaments of equal length, which are usually white or cream. Stamens are 1.5 to 4.8 mm long (from the base of the united filaments to the anther apex). The anthers are yellow, ca. 0.5 mm long, ellipsoid, dorsifixed, and introrse, opening by longitudinal slits; they are glabrous, glandular or villous (Fig. 2I). Pollen grains are isopolar, obtusely triangular in polar view and subspherical in equatorial view, tricolporate, with elongate colpi nearly reaching the poles, and striate (Ferrucci and Anzotegui 1993; Acevedo-Rodríguez et al. 2017). The gynoecium is superior, syncarpic and tricarpellate; the ovary in most species is pubescent and the style is usually longer than the three papillose and terete stigmata (Fig. 2H); the ovules are solitary with axial placentation.

Fruits, seed and embryo

The fruit in *Thinouia* is a stipitate schizocarp that splits into three mericarps, each with a distal wing and a proximal locule where the seed is located (Fig. 3). Young and not fully mature fruits are often greenish or reddish rose, turning straw-colored when mature (Fig. 3F). Mericarp size varies from 3 to 7.5 cm in length, but there is little variation in shape between species (Fig. 3). The locule is consistently subglobose or lenticular although horizontally flattened in *T. compressa* (Fig. 3B), and in *T. myriantha* (Fig. 3D) the wing is distal but follows the locule along the dorsal margin; however, in some species the wing is not formed on the dorsal margin of the locule, forming an indentation on that region (Fig. 3C, E, H). The pericarp is glabrous or less often puberulous to pubescent. The cavity of the seed locule is glabrous or pubescent. Seeds are trigonous-ellipsoid or lenticular-ellipsoid, exarillate, with a small hilum (Acevedo-Rodríguez et al. 2017). The embryo is consistently ovoid, showing folded cotyledons. 2n = 28 (Urdampilleta et al. 2008).

Phylogenetic relationships

The ITS dataset included 31 terminals and the alignment had a length of 781 bp; the trnL dataset included 31 terminals and was 616 bp long; the combined dataset included 31 terminals and had a length of 1398 bp. Only the topology from the combined analysis is described here (Fig. 4), as our separate analyses of each locus did not reveal any strongly supported incongruences.

Based on Acevedo-Rodríguez et al. (2017) and Medeiros et al. (2020), the monophyly of *Thinouia* is strongly supported and the group is recovered as sister to the other genera in tribe Paullinieae. In the current phylogenetic reconstruction of *Thinouia* (Fig. 4), we analyzed all 13 species of the genus, nine of which are represented by more than one accession. In all instances, the species form a single monophyletic lineage. *Thinouia cazumbensis* was recovered as sister to a clade containing the remaining species of the genus and with a high posterior probability (PP = 0.8). Within this group there were two clades, here called Clade II and Clade III. Clade II is strongly supported (PP = 1) and includes *T. tomocarpa* as sister to *T. myriantha* and the new species *T. silveirae*. Clade III has strong support (PP = 1), and contains two subclades; the first subclade included *T. obliqua*, successively followed by *T. ternata*, and *T. paraguayensis* + *T. mucronata*. The second subclade included *T. scandens* as sister to a group formed by *T. trifoliolata*, *T. restingae*, *T. compressa*, and *T. ventricosa* (PP = 1).

The results of the petal appendage type reconstruction based on MP and ML were largely consistent, with the MP analysis displayed in Fig. 5. They revealed that petal appendages shorter or equal to the petals represent the ancestral state of *Thinouia*. The size of appendages relative to the petals, the trait that differentiates *T.* sect. *Lepidodine* from sect. *Petalodine* according to Radlkofer (1878), appears to have evolved just one time.

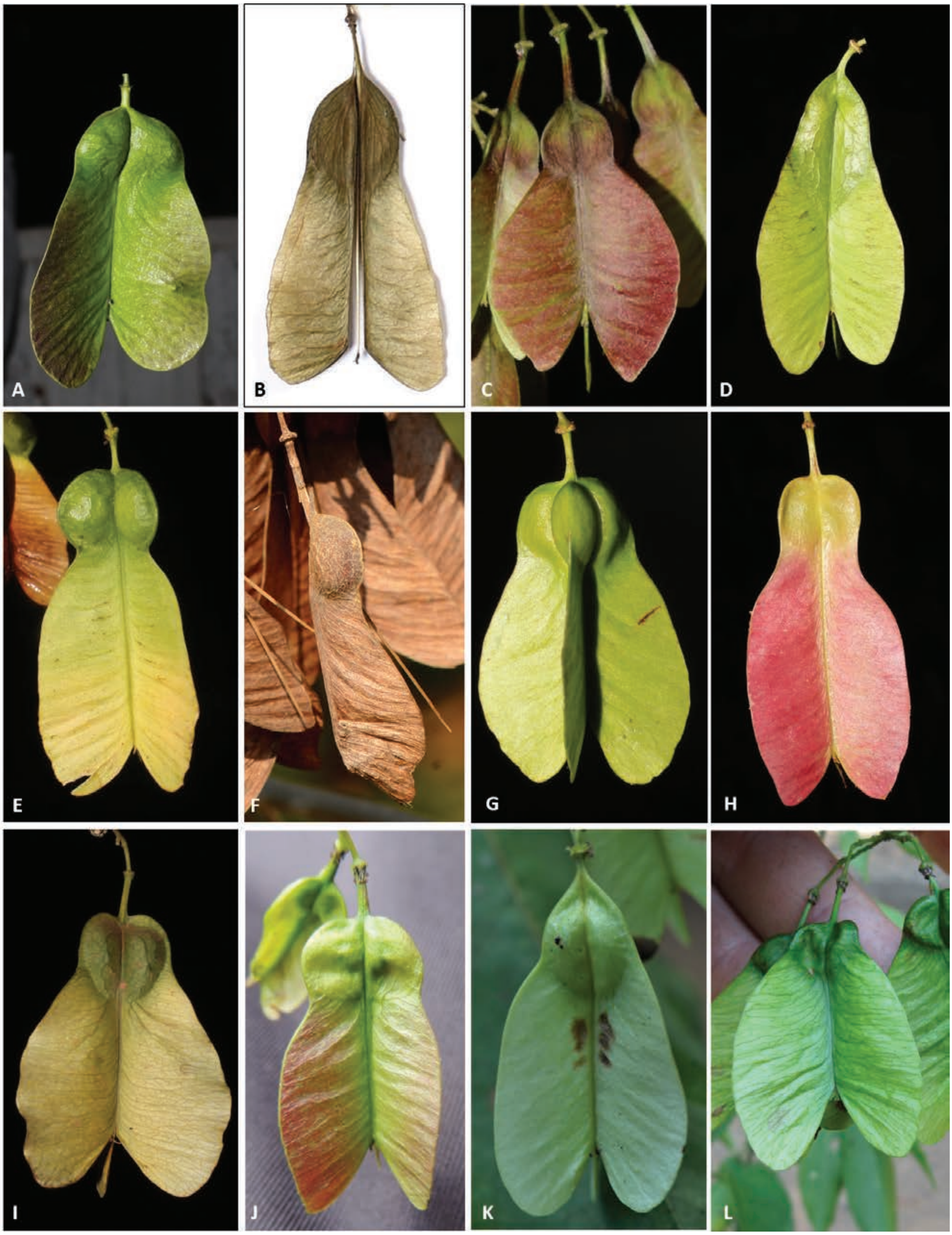

Figure 3. Color and shapes of schizocarps in *Thinouia*. **A** *T. cazumbensis* **B** *T. compressa* **C** *T. mucronata* **D** *T. myriantha* **E** *T. obliqua* **F** *T. paraguayensis* **G** *T. restingae* **H** *T. scandens* **I** *T. silveirae* **J** *T. ternata* **K** *T. trifoliolata* **L** *T. ventricosa* [Acevedo-Rodríguez 16750 (**F**) 17159 (**K**); Cálio 70 (**B**); Daneu 746 (**J**); Ferrucci s.n. (**L**); Figueira 927 (**C**); Medeiros 3401 (**A**) 3330 (**D**) 3832 (**E**) 4453 (**G**) 4473 (**H**) 2191 (**I**); photos: **F, K** by P. Acevedo-Rodríguez; J by L. Daneu **L** by M. S. Ferrucci **C** by M. Figueira **A, B, D, E, G, H, I** by H. Medeiros].

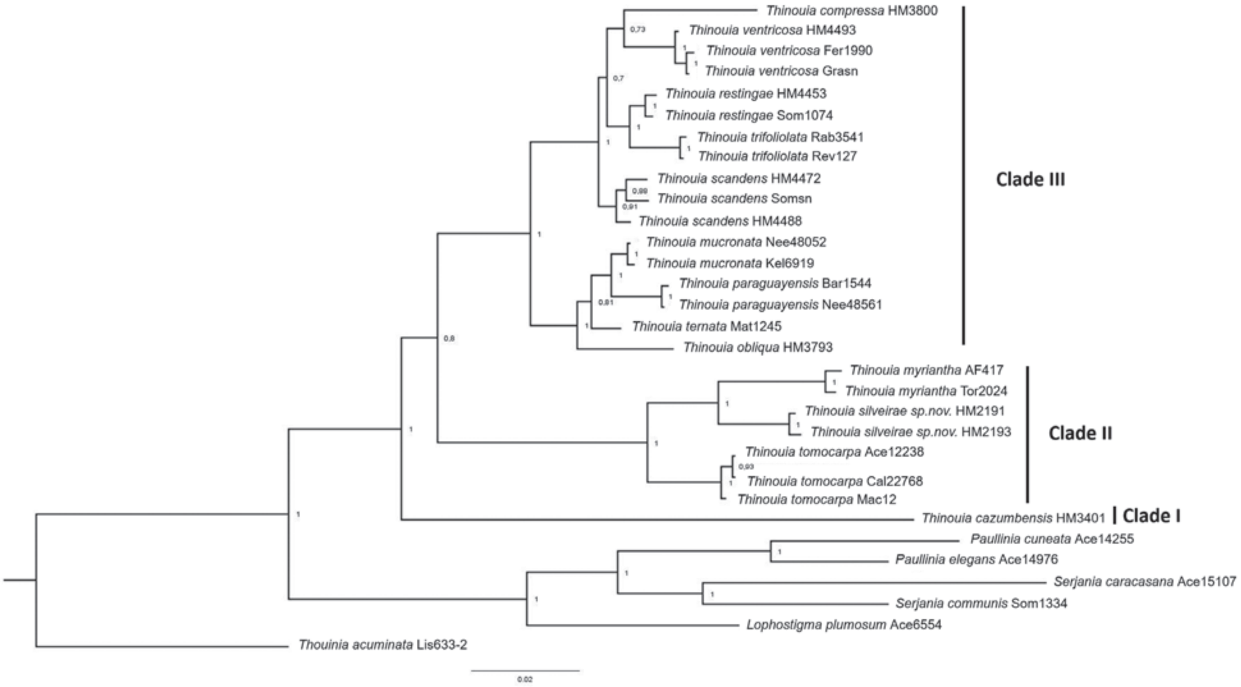

Figure 4. Maximum clade credibility tree from a Bayesian analysis of the combined two-marker dataset for *Thinouia* and outgroups. Bayesian posterior probability values are indicated above the branches.

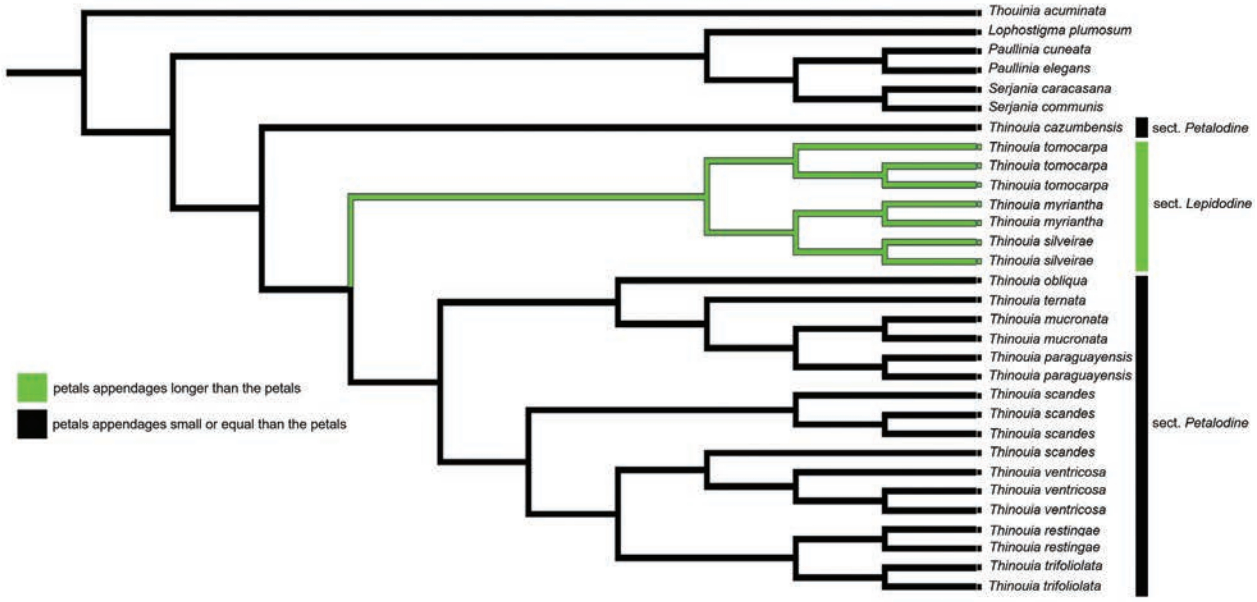

Figure 5. Maximum parsimony ancestral state reconstruction using Mesquite v.3.70. Ancestral states are shown as the colors of the branches (see legend).

Taxonomic treatment

Thinouia Triana & Planchon, Ann. Sci. Nat. Bot. Ser. 4, 18: 368. 1862

Carpidiopterix H. Karsten, Fl. Columb. 2: 45. 1863. Type: *Carpidiopterix macroptera* (Cassar.) H. Karst. = *Thinouia scandens* (Cambess.) Trian. & Planch.
Allosanthus Radlk. in A. Engler., Pflanzenr. IV. 165. (Heft 98): 1157. 1933. Type: *Allosanthus trifoliolatus* Radlk. = *Thinouia trifoliolata* (Radlk.) Acev.-Rodr. & Ferrucci

Type. *Thinouia myriantha* Triana & Planchon.

Description. Lianas or seldom shrubs with arched branches; climbing with the aid of a pair of circinate tendrils that are proximal to the floriferous part of the inflorescence and homologous to a cincinnus. Stems terete or ribbed, lenticellate; cross-sections of branches and young stems simple, i.e., with single vascular cylinders, some species developing a compound stem where 1–5(7–8) vascular cylinders are formed within the cortex, these becoming wider as the stem grows wider. Stipules minute, axillary, deltate to lanceolate, early deciduous. Leaves alternate, trifoliolate; petioles and petiolules unwinged. Inflorescences of umbelliform or racemiform thyrses, often bearing a pair of tendrils at the base of the rachis, axillary or forming a frondobracteate synflorescence on distal portion of branches, rarely cauliflorous, peduncle and secondary peduncle, sometimes sessiles; flowers produced in cincinni; pedicels articulated. Flowers actinomorphic; calyx cup-shaped, partly connate, sepals 5, valvate, of equal size; petals 5, obovate to spatulate, with a pair of short appendages, smaller or equal than the petals or longer than the petals, sometime clawed; disc extrastaminal, annular, rarely 5-lobed; stamens 6–8, filaments equal or in two unequal series, anthers dorsifixed; pollen isopolar, obtusely triangular in polar view, subspherical in equatorial view, tricolporate, with elongated colpi nearly reaching the poles, striate; ovary 3-carpellate, the ovules solitary with axile placentation; style elongated with 3 papillose stigmatic branches. Fruit schizocarpic, stipitate, splitting into 3 mericarps, each with a distal wing, the seed locus subglobose, lenticular or rarely flattened; seed trigonous-ellipsoid, or lenticular-ellipsoid, exarillate, with a small hilum.

Key to the species of *Thinouia*

1a Inflorescence racemiform **2**

2a Epicarp glabrous; seed locule cavity sparsely ferruginous pubescent, with simple, capitate and arachnoid trichomes; nectary disc annular to lobed; petal appendages 0.8–1 mm long .... ***T. trifoliolata* (12)**

2b Epicarp strigose; seed locule cavity glabrous; nectary disc 5-lobed; petal appendages rudimentary, < 0.5 mm long .... ***T. cazumbensis* (1)**

1b Inflorescence umbelliform **3**

3a Petal appendages longer than the petals **4**

4a Seed locule subglobose, flattened at the base, epicarp pubescent at the locule, the wing puberulous or pilose; cavity of seed locule densely villous .... ***T. silveirae* (9)**

4b Seed locule slightly flattened, epicarp sparsely pubescent at the locule, the wing sparsely pubescent; cavity of seed locule glabrous or rarely sparsely pilose, with capitate trichomes **5**

5a Stamens with filament villous throughout; petal appendages marginal. Belize, southern Mexico, El Salvador, and Honduras .... ***T. tomocarpa* (11)**

5b Stamens with filament villous on the lower half; appendages basal. Costa Rica to Amazon basin .... ***T. myriantha* (4)**

3b Petal appendages shorter than or equal to the petals **7**

7a Fruit locule flattened .... ***T. compressa* (2)**

7b Fruit locule subglobose **8**

8a Cavity of seed locule with arachnoid trichomes **9**

9a Leaflets with entire margins, or rarely with basally one-toothed margins; venation brochidodromous, without domatia on the abaxial side of secondary vein axils..***T. restingae* (7)**

9b Leaflets with entire or serrate margins, venation semicraspedodromous, with domatia on the abaxial side of secondary vein axils............***T. ventricosa* (13)**

8b Cavity of seed locule with capitate trichomes **10**

10a Petal appendages distally bifurcated; stamens 6–7 ....***T. obliqua* (5)**

10b Petal appendages distally entire; stamens 8...**11**

11a Fruits > 4.5 cm long.. ***T. scandens* (8)**

11b Fruits < 4.5 cm long..**12**

12a Leaflet secondary veins semi-craspedodromous; lateral leaflet decurrent at the base .... ***T. mucronata* (3)**

12b Leaflet secondary veins craspedodromous; lateral leaflet truncate or rounded at the base **13**

13a Mericarps 2.5 cm wide; epicarp glabrous; terminal leaflet widely elliptic, obtrullate or ovate. SE and NE Brazil..................................... ***T. ternata* (10)**

13b Mericarps 1.3–2.3 cm wide; epicarp glabrous or with sparse, simple trichomes on the locule and ventral margin of the wing; terminal leaflet broadly ovate. Western Brazil, Bolivia and Paraguay ....***T. paraguayensis* (6)**

1. *Thinouia cazumbensis* H. Medeiros, PhytoKeys 165: 118. 2020.

Fig 6, 13A

Type. Brazil. Acre. • Mun. Sena Madureira, Reserva Extrativista do Cazumbá-Iracema, Núcleo Cazumbá, castanhal coletivo, 20 July 2018, *H. Medeiros et al. 3401* (holotype: RB!, isotypes: INPA!, SPF!, UFACPZ!, US!).

Description. Tendrilled liana, 6–8 m long; stem puberulent, with yellowish to whitish indumentum, lenticellate; cross-section simple, cylindrical. Leaves trifoliolate; stipules ca. 2 mm long, linear, triangular to lanceolate, hirsute-tomentose; petiole 2–8.5 cm long, canaliculate; terminal petiolule 1.2–1.7 cm long, tomentose or tomentulose, lateral petiolules 0.2–0.8 cm long; leaflets glabrous on both sides, the secondary venation eucamptodromous but distally craspedodromous; secondary veins 7–8 pairs, subalternate or alternate, spacing irregular, sometimes with domatia on abaxial secondary vein axils; intersecondaries present; tertiary veins reticulate; margins entire to dentate-serrate, with 2–4 teeth reduced to inconspicuous glands, ciliate; terminal leaflet 12–13 × 6.5–7.5 cm, oblong, the apex acute, mucronate, the base truncate or rounded to obtuse; lateral leaflet 9.5–11.5 × 4.8–5.7 cm, oblong or ovate-rhomboidal, the apex acute, mucronate, the base truncate or rounded. Thyrses axillary, racemiform, 8.5–16 cm long; peduncle 1.1–2.8 cm long; rachis 7.5–16 cm long; cincinni numerous, sessile. Flowers ca. 2 mm long, pedicel ca. 0.5 mm long; sepals ca. 1 mm long, connate at the base, lobes ovate, acute, glabrous and with prominent veins on the adaxial surface, abaxial surface villous; petals ca. 1.5 mm long, obovate, obtuse, not clawed, glabrous on the central part and villous on the margins; appendages rudimentary, ca. 0.3 mm long, bifid, shorter than the petals, adnate to central portion of petal, villous; nectary disc glabrous, 5-lobed, the lobes ca. 1 mm long. Staminate flower with stamens 8, ca. 1.5 mm

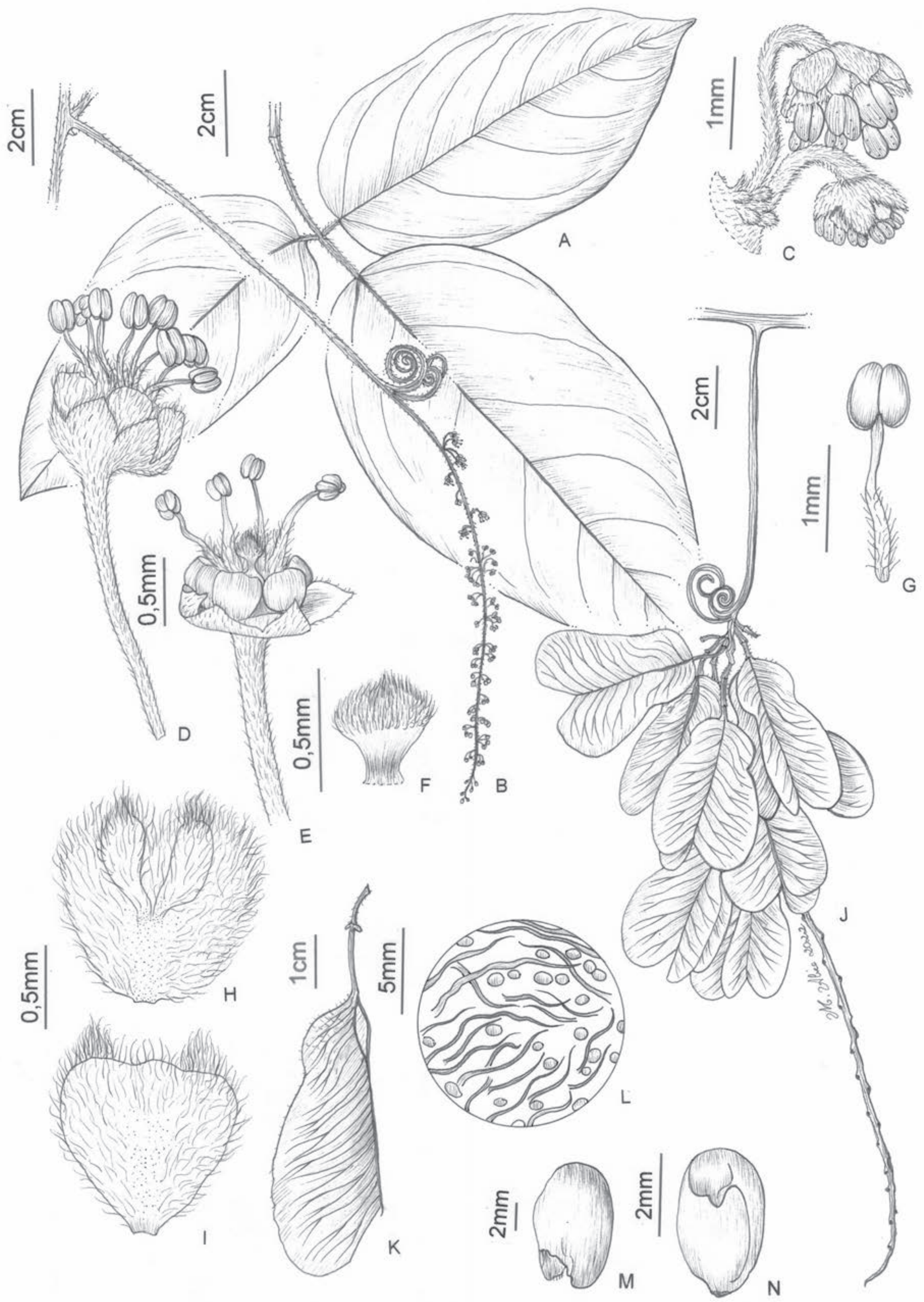

Figure 6. *Thinouia cazumbensis* H. Medeiros **A** leaf **B** inflorescence racemiform with tendrils **C** portion of cincinni **D** staminate flower showing sepals, petals and stamens **E** staminate flower with petals removed showing a 5-lobed nectary disc and pistillode **F** pistillode **G** stamen of staminate flower **H** petal with minute appendages, frontal [adaxial] view **I** petal in dorsal [abaxial] view **J** infructescence **K** mericarp **L** epicarp densely strigose and with glandular trichomes **M** seed **N** embryo (**A**–**N** from *Medeiros 3401*). Illustration by Maria Alice de Rezende.

long, the filaments villous for more than half of their length, the anthers glabrous; pistillode ca. 1.5 mm long. Pistillate flower with staminodes ca. 1 mm long; pistil ca. 1.5 mm long, the style villous, the ovary puberulent. Fruits ovate, chartaceous, 5–5.5 × 2–2.3 cm; stipe 2–3 mm long; seed locule slightly subglobose, 1.2–1.4 × 1.1–1.4 cm; epicarp densely strigose, with simple and capitate trichomes on cocci, strigose on wings; cavity of seed locule glabrous. Seeds trigonous-ovoid, ca. 6 × 4 mm, basally attached, glabrous.

Distribution, habitat and phenology. *Thinouia cazumbensis* is known from the type and from a collection from the state of Pará, Brazil, in non-flooded tropical and subtropical moist broadleaf forests. It occurs in the Reserva Extrativista do Cazumbá-Iracema where it is an infrequent liana that reaches the canopy of the open ombrophilous forest with abundant bamboo (*Guadua* spp.) (Fig. 13A). Collected in flower during July and September, and in fruit in July.

Notes. *Thinouia cazumbensis* is differentiated from most species of *Thinouia* by the racemiform thyrses (Fig. 6B) and the 5-lobed nectary disc, a character recorded for the first time in the genus (Fig. 6E).

Conservation status. The species is still only known from a single locality each in Acre and Pará and it is categorized as Data Deficient (DD) according to IUCN (2022). Further field studies are needed to evaluate its conservation status more accurately.

Additional specimen examined. Brazil. Pará • Rio Jarí, Monte Dourado, terra firme forest, 17 Sep 1968, N. T. Silva 1022 (IAN, US).

2. *Thinouia compressa* Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München 8: 282. 1878

Figs 7, 13A

Thinouia coriacea Britton, Bull. Torrey Bot., Club 16: 191. 1889. Type: Bolivia. Guanai, May 1886, *H. H. Rusby 550* (lectotype, designated here: NY! [NY00387407], isolectotypes: GH [GH10593] [image!], MICH [MICH1115486] [image!], NY! [NY00387406]).

Type. Brazil. [**Rio de Janeiro?**] • Cantagallo, Jul 1832, *L. Riedel 513* (lectotype, designated here: US! [US00169545], isolectotypes: AAH!, LE? [not seen], NY! [NY5169]).

Description. Tendrilled liana; stem cylindrical, striate, puberulous, with ferruginous, rounded or elliptic lenticels; cross-section simple. Leaves trifoliolate; stipules ca. 0.8 mm long, triangular, tomentose; petiole 2–3 cm long, terete, pubescent; terminal petiolule 0.2–0.5 cm long, canaliculate, lateral petiolules 0.2–0.3 cm long; leaflets with adaxial surface glabrous or puberulous, abaxial surface puberulous or pubescent, the leaflet secondary venation semicraspedodromous or craspedodromous; secondary veins 4–5 pairs, subalternate or opposite, spacing irregular, with domatia on the abaxial side of secondary vein axils; intersecondaries present or absent; tertiary veins irregular reticulate; margins serrate or dentate-serrate, subrevolute, with (3)6–8 teeth reduced to inconspicuous glands; terminal leaflet 3.7–5.5 × 2.3–3.7 cm, ovate-rhomboid, symmetrical or asymmetrical, the apex obtuse or acuminate, mucronate, the base decurrent; lateral leaflets 3–4.8 × 2.2–3 cm, ovate, asymmetrical, the apex

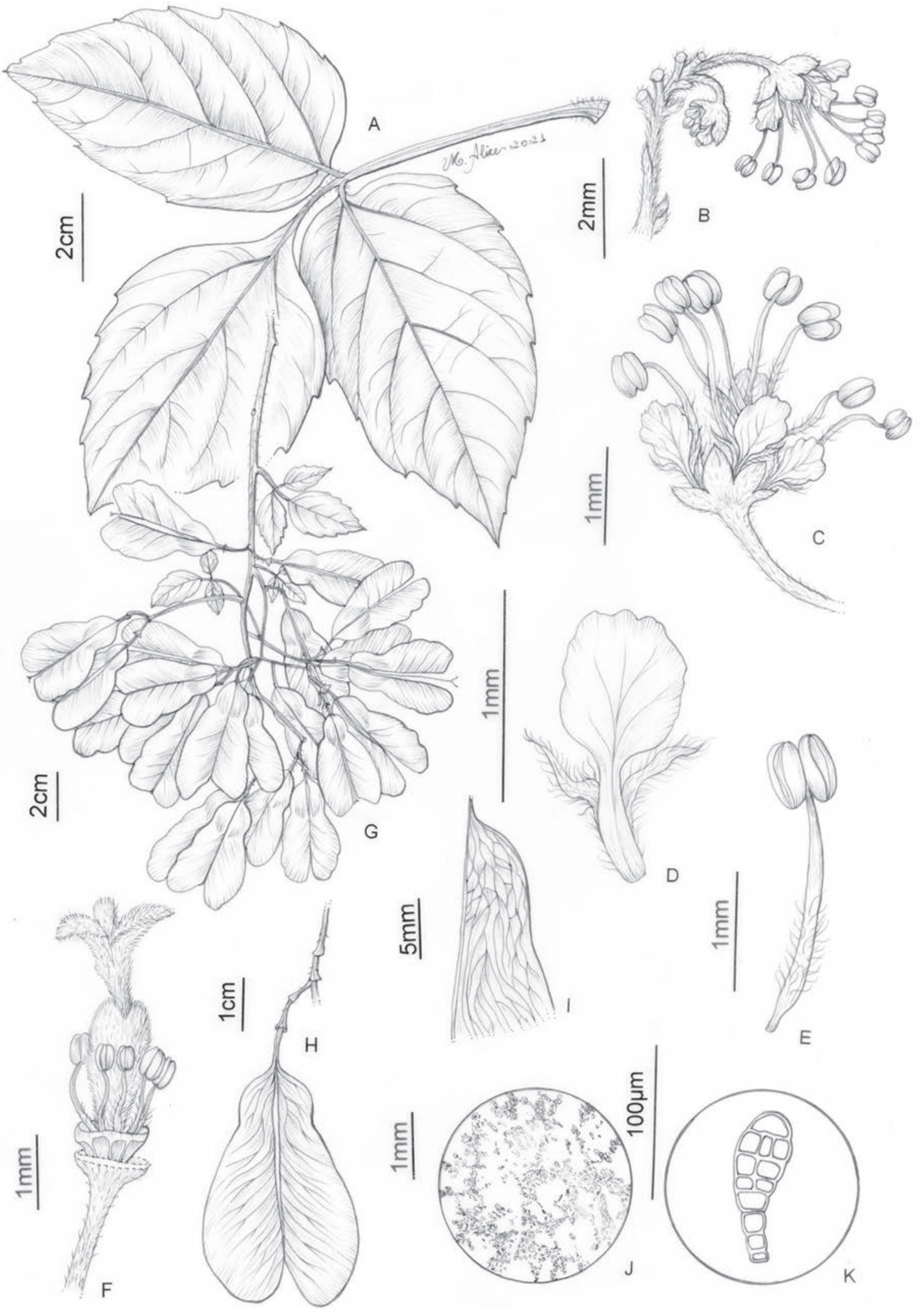

Figure 7. *Thinouia compressa* Radlk. **A** leaf **B** cincinnus **C** staminate flower **D** petal with bifid appendage, dorsal [abaxial] view **E** stamen **F** pistillate flower with perianth removed showing nectary disc, staminodes and gynoecium **G** infructescence **H** fruit **I** detail of mericarp showing locule **J** detail of indumentum in seed locule cavity **K** Capitate trichome with uniseriate stalk and multicellular terminal cells (**A, G–K** from *França 4971* **B–F** from *Hatschbach 67777*). Illustration by Maria Alice de Rezende.

obtuse or acuminate, mucronate, the base subcuneate to obtuse. Thyrses axillary or terminal, umbelliform, 1.7–4 cm long; peduncle 0.7–2.4 cm long; secondary peduncle subsessile or 0.2–0.5 cm long; cincinni numerous, peduncle of cincinni 2–3.5 mm long, tomentose. Flower 2.5–4.5 mm long, pedicel 1–2 mm long, pilose or tomentose; sepals 1–1.25 mm long, connate at base, deltate, abaxially pilose, adaxially glabrous; petals 1–2.5 mm long, spatulate, distally orbiculate, clawed, the margin erose, adaxially glabrous with glands; petal appendages rudimentary, bifid, shorter than the petals, ca. 0.5 mm long, villous; nectary disc glabrous, annular. Staminate flower with stamens 8, ca. 2.5 mm long, the filaments villous on lower half, the anthers pilose to glabrous; pistillode ca. 0.6 mm long, villous at the apex. Pistillate flower with staminodes ca. 1.7 mm long, villous more than half of their length; pistil 3 mm long, villous. Fruits 3–5 × 2.2–2.3(2.7) cm; accrescent pedicel 3–5 cm long; stipe 5–8 mm long; seed locule flattened; epicarp glabrous or with sparse simple trichomes; cavity of seed locule with capitate, ferruginous trichomes with uniseriate stalk and multicellular terminal cells. Seed ellipsoid, 6–7.5 × 3.8–4.4 mm, basally attached, glabrous.

Distribution, habitat and phenology. *Thinouia compressa* is known from tropical and subtropical moist broadleaf forests, dry broadleaf forests, grasslands, savannas, shrublands, and xerophytic shrublands in Bolivia, Brazil, and Paraguay, along streams, roadside thickets, semi-deciduous forests, and caatinga vegetation in Brazil (Bahia, Ceará and Pernambuco), (Fig. 13A). Flowering from February to August, and fruiting from March to August.

Notes. *Thinouia compressa* is easily distinguished from other species of *Thinouia* by its fruits with flattened seed locules (vs. subglobose or lenticular). *Thinouia compressa* is similar to *T. paraguayensis*. They both have leaflets with mucronate apex, margins dentate-serrate, flowers 3–5 mm long, and fruits 2.5–5 cm long, with seed ellipsoid. However, *Thinouia compressa* differs from the *T. paraguayensis* by the adaxially glabrous or puberulous leaflets and the spatulate-orbiculate petals (vs. puberulous, pubescent-tomentose only along the veins, and spatulate-obovate petals).

Conservation status. *Thinouia compressa* occurs from Northeastern Brazil to Paraguay and Bolivia within an EOO of 3,207,299.55 km^2 and an AOO of 140.00 km^2. This species occurs in dry tropical forests, a habitat that is under threat as it is being converted into large-scale agricultural plantations and urban development. Despite this, the EOO values and the number of threat situations extrapolate the thresholds for inclusion of the species in a threat category. Moreover, there are no data on population declines for the application of other criteria. Thus, it should be regarded as Least Concern (LC).

Selected specimens examined. Bolivia. La Paz • Prov. Franz Tamayo, 2 km W de la Hacienda Ubito, 850 m, 13 Jul 1993, Kessler et al. 3983 (LPB, US). **Brazil. Bahia** • Mun. Brejolândia, 5 km to the North of Tabocas, 1 Mar 1980, Harley et al. 21997 (CEPEC, SPF) • Mun. Correntina. Distrito de São Manoel do Norte, 479 m, 8 Apr 2005, Miranda et al. 706 (HUEFS) • Mun. Coribe, 545 m, 10 Apr 2007, Queiroz et al. 12715 (RB) • Mun. Espigão Mestre, 3 km S of Cocos, 14 Mar 1972, Anderson et al. 36945 (F, MO, U, UB) • Mun. Ibiquera, Gruta da Lapinha, 610–710 m, 1 Jan 2004, França et al. 4971 (RB) • Mun. Iraquara, Gruta da Lapa Doce, 19 Mar 2019, Medeiros and Sousa 3800 (RB, SPF, UFACPZ) • Mun. Oliveira dos Brejinhos, Canabrava, 16 Mar 1998, Hatschbach et al. 67777 (BHCB, HCF, MBM, RB, UB, UFACPZ) • Mun. Macaúbas, Estrada para Canatiba, 600–800 m, 20 Apr 1996, Hatschbach et al. 65076 (CEPEC, MBM) • Mun. Machado Portello, 19–23 Jun 1915, Rose et al. 19992 (US). **Ceará** • Crato, 1910, Löfgren 625 (R); Serra de Saturité, 24 Aug 1908, Ducke s.n (INPA 12613, UB). **Espírito Santo** • Mun. Alegre, São João do Norte, 25 Jun 2008, Kollmann 11067 (CEPEC). **Mato Grosso do Sul** • Mun. Bonito, Projeto Guaicurus, 14 Mar 2003, Hatschabach et al. 74746 (HUEFS, MBM, MEXU, SPF). **Minas Gerais** • Mun. Caratinga, Fazenda Macedônia/Cenibra-Ipaba, 21 Aug 1991, Stehmann and Soares s.n. (MBM 227949) • Mun. Januária, 13 km by road W of Januária, 19 Apr 1973, Anderson et al. 9187 (F, UB) • Mun. Leopoldina, Domingos Pisoni, 30 Mai 1936, Barreto 7558 (BHCB, F, R) • Mun. Marliéria, Parque Estadual do Rio Doce, 28 May 2001, Stehmann 2959 (ESA) • Mun. Santo Hipólito, 5 km de Santo Hipólito em direção à Monjolos, 14 Aug 1998, Rapini 628 (SPF) • Mun. São João del-Rei, Serra do Lenheiro, 17 Jun 1996, Alves 4981 (R) • Mun. Tombos, Fazenda Antilhas, 30 May 1941, Oliveira 387 (UB). **Paraíba** • Mun. Maturéia, Pico do Jabre, 1225 m, 11–13 Jul 2007, Agra et al. 6985 (JPB). **Pernambuco** • Entre Salgueiro, Cedro e Jardim, 17 May 1971, Heringer 725 (R, RB, UB). **Rio de Janeiro** • Mun. Barra, Fazenda Boa Esperança, 650 m, 8 Mai 2013, Bovini et al. 3824 (CTES, RB, UFAPZ, US) • Mun. Santa Maria Madalena, 16 km antes do portal de Santa Maria Madalena, 17 Jun 2004, Calió et al. 70 (CTES, K, NY, MBM, RB, SPF). **Paraguay. Guaira** • Cordillera de Ybytyruzú, 28 May 1989, Zardini and Velásquez 12165 (MO, US) • Road to Polilla, 800 m, 23 Jul 1989, Zardini and Velásquez 13669 (MO, US). **Paraguari** • Acahay Massif, Easternmost Peak, 13 Jan 1992, Zardini and Aquino 29700 (MO, US). **Primavera** • High woodland, 27 Jul 1956, Woolston 840 (US). **San Pedro** • Línea Caraguatay, 2 Oct 1987, Zardini and Benítez 3333 (MO, US) • Yaguarete Forest, 152 m, Zardini and Guerrero 48472 (MO, P, US) • Colonia Primavera, 17 Jul 1956, Woolston 664 (P).

3. ***Thinouia mucronata*** **Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München 8: 282. 1878**

Figs 8, 13B

Thinouia repanda Radlk. in Engler & Prantl, Nat. Pflanzenfam. 3 (5): 308. 1895. Type: Paraguay. Yaguaron, February 1877, *B. Balansa 2488* (lectotype, designated by Ferrucci 2021, pg. 401: P [P04795732] [image!], isolectotypes: K [K000634087] [image!], P [P04795732] [image!]), G [G00008258] [image!].

Type. Brazil. São Paulo • Campinas, 1875, *H. Mosén 3953* (lectotype, designated by Massing and Miotto 2020, p. 160: S [S17-1112] [image!], isolectotypes: P [P06695515] [image!], S [S17-1101] [image!], S [S17-1104] [image!].

Description. Tendrilled liana; stem puberulous or glabrescent, lenticels ferruginous, rounded or elliptic; cross-section simple or with neo formations when mature. Leaves trifoliolate; stipules minute, ca. 0.5 mm long, pubescent, triangular; petiole 1–5.5 cm long, canaliculate or sometimes semiterete, glabrous or puberulous; terminal petiolules 0–0.3 cm long, lateral petiolules 0–0.2 cm long; leaflets glabrous, puberulous along veins or sparsely pubescent on both surfaces, the leaflet secondary venation semi-craspedodromous; secondary veins 3–5 pairs, with irregular spacing and domatia on the abaxial side of secondary vein axils; intersecondaries presents; tertiary veins irregular reticulate

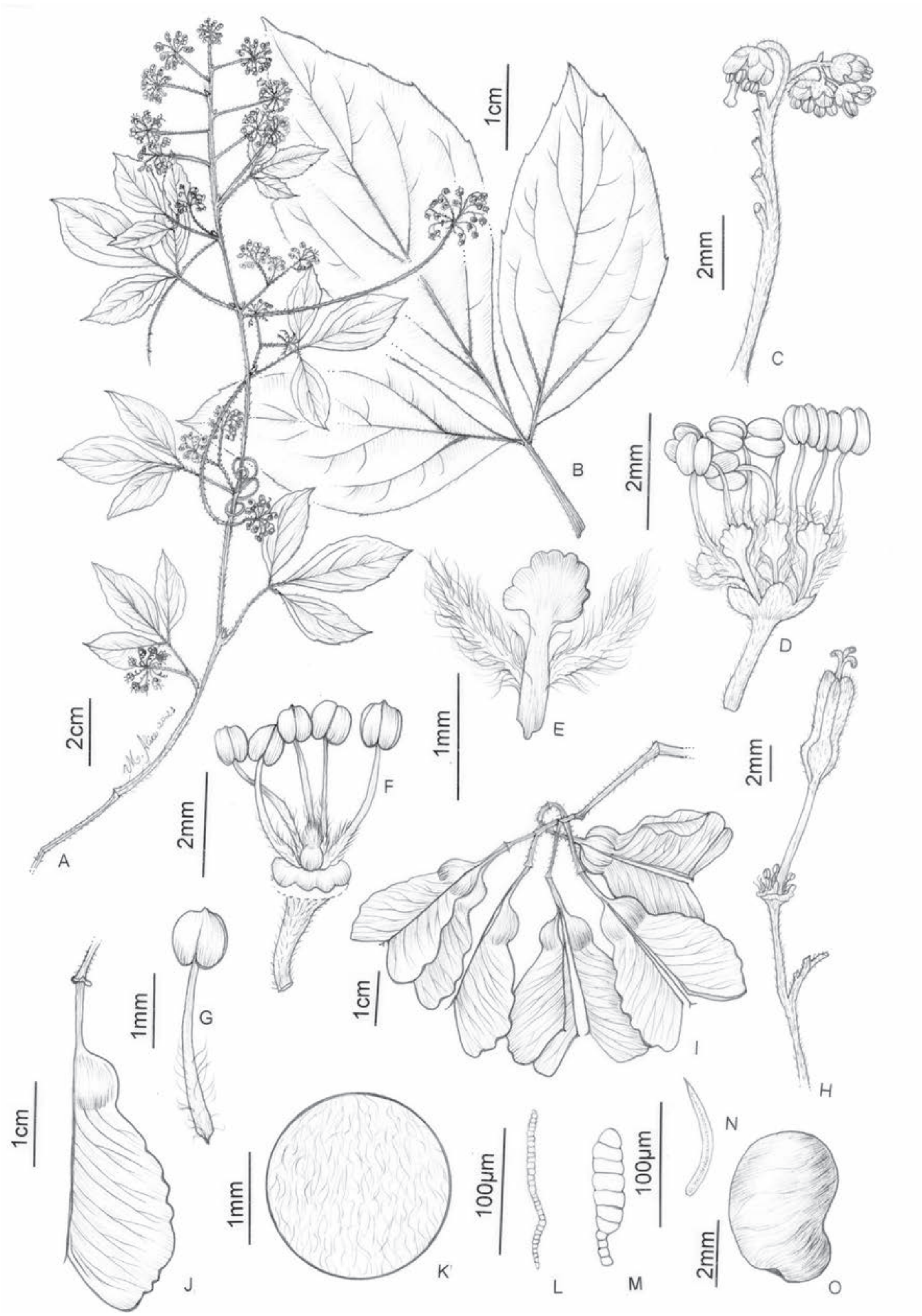

Figure 8. *Thinouia mucronata* Radlk. **A** distal portion of flowering branch **B** leaf showing leaflet venation **C** cincinnus **D** staminate flower **E** petal with bifid appendage, dorsal [abaxial] view **F** staminate flower with perianth removed showing nectary disc, stamens and pistillode **G** stamen **H** pistillate flower with long-stipitate, developed gynoecium **I** infructescence **J** mericarp **K** indumentum detail of seed locule cavity **L**, **M** capitate trichomes with uniseriate stalk and unicellular terminal cells **N** simple trichome from locule cavity **O** seed (**A, C–G** from *Bianek 229* **B** from *Caxambu 5215* **H** from *Kozera 3718* **I–O** from *Caxambu 6486*). Illustration by Maria Alice de Rezende.

or alternate-percurrent; margins subentire to repand-serrate, glabrous or ciliate, revolute, the teeth 2–5(8–12), sometimes reduced to inconspicuous glands; terminal leaflet 3–9.7 × 1.5–5 cm, ovate or oblong, the apex acute to obtuse, mucronate, the base decurrent; lateral leaflets 3–7.7 × 1–4.3 cm, ovate or oblong, asymmetrical, the apex acute or obtuse, mucronate, the base decurrent. Thyrses axillary or terminal, umbelliform, 2.5–7.8 long; peduncle 1.5–6.5 cm long; secondary peduncle 0–0.5 cm long; cincinni numerous; peduncle of cincinnus 2.5–4 mm long. Flower ca. 3–5 mm long; pedicel 2–2.5 mm long, glabrous or puberulous; sepals 0.5–0.8 mm long, connate at the base, deltoid, abaxially puberulous and adaxially villous; petals 1.5–1.7 mm long, spatulate, erose, clawed, abaxially glabrous and adaxially villous; appendages ca. 1 mm long, shorter than the petals, bifid, villous; nectary disc glabrous, annular. Staminate flower with stamens 8, 2.5–3.5 mm long, the filaments villous on lower half, the anthers 0.4–0.5 mm long, glabrous; pistillode <0.5 mm long, villous. Pistillate flower with staminodes ca. 1.5 mm long, with same indumentum as the stamens; pistil 0.5–1 mm long, villous. Fruits chartaceous, 2.2–3.8 × 1.5–2.6 cm; accrescent pedicel 5–6(7) mm long; stipe (4)5–8 mm long; seed locule subglobose; epicarp glabrous, sometimes puberulous on the locule, rarely tomentose on stipe and base of fruits; cavity of seed locule villous with simple and capitate trichomes with uniseriate stalk and unicellular or multicellular terminal cells. Seed 5–6 × 3–4 mm, ellipsoid.

Distribution and habitat. *Thinouia mucronata* is known from tropical and subtropical moist broadleaf forests; tropical and subtropical dry broadleaf forest; tropical and subtropical grassland, savannas and shrublands; and desert and xeric shrublands in northern Argentina, southern Bolivia, SE Brazil, and Paraguay (Fig. 13B), in cerrado, chaco, gallery, ombrophilous dense, semi-deciduous and deciduous forests. Flowering from December to March and fruiting from December to August.

Notes. This species is strongly supported as sister to *T. paraguayensis* (Fig. 4). They both have simple stems or with neo formations when mature, leaflets with mucronate apex, flowers 3–5 mm long, and chartaceous fruits, with seed locule subglobose. However, *T. mucronata* is distinguished by the semi-craspedodromous secondary venation and 3–5 pairs of secondary veins, where basal secondary veins form an acute angle with the midvein, (vs. craspedodromous and 4–5(6) pairs of secondaries, basal secondary veins form an obtuse angle with the midvein).

Conservation status. *Thinouia mucronata* possesses a broad EOO of 3,328,158.18 km^2 and an AOO of 320.00 km^2, with more than 10 known localities. The EOO values and the number of threat situations extrapolate the thresholds for the inclusion of the species in a threat category. Moreover, there are no data on population declines for the application of other criteria. Thus, it should be regarded as Least Concern (LC).

Selected specimens examined. Argentina. Jujuy • Dep. El Carmen, Abra de Santa Laura, 14 Feb 1972, Cabrera et al. 22085 (F) • Dep. General San Martín, Parque Nacional Calilegua, 18 Apr 1998, Vanni et al. 4179 (CTES, F, US) • Dep. Ledesma, Parque Nacional Calilegua, 1107 m, 30 Aug 2012, Coulleri et al. 384 (CTES, NY, RB, UFACPZ, US). **Misiones** • Dep. Apóstoles, Ayo. Chimiray y ruta 40, 9 Feb 1993, Tressens et al. 4409 (CTES, US) • Dep. Cainquás, Ruta 14, km 252, 26 Jan 1950, Schwindt 3087 (US) • Dep. Candelaria, Loreto, 2 Feb 1948, Montes

100B (US) • Dep. Guaraní, Predio Guaraní, 27 Apr 1999, Tressens et al. 6164 (CTES, MBM) • Dep. Iguazú, Acesso al Parque Nacional Iguazú, 23 Feb 2001, Vanni et al. 4518 (CTES, ESA, F, MEXU, US) • Dep. Montecarlo, Colonia Guatambú, 25 Feb 2001, Keller 622 (CTES, MEXU) • Santa Ana, 11 Jan 1913, Rodríguez 715 (F). **Salta** • Dep. La Caldera, El Ucumar, ruta 9, 12 Mar 1982, Schinini 22301 (CTES, F, ICN). **Bolivia. Chuquisaca** • Prov. Sud Cinti, Trayecto Las Abras-Cañón Verde, 903–1031 m, 6 Feb 2006, Lozano and Peñaranda 2128 (HSB, MO, US) • Prov. Tomina, Las Casas, 1280 m, 18 Apr 2005, Gutiérrez et al. 1200 (HSB, MO). **Santa Cruz** • Prov. Andrés Ibáñez, 10 km E of Cotoca, 350 m, 27 Jul 1994, Nee 45374 (NY, US) • Prov. Cordillera, 2.5 km W of railroad and 3.5 km w of the Santa Cruz-Abapó, 570 m, 24 May 2005, Nee 53120 (NY, US) • Prov. Florida, 14.2 km NE of Achira Camping, 1800 m, 17 Jan 1998, Nee 48052 (MEXU, NY, US). **Tarija** • Prov. Arce, 29.2 km S of Emboroza-Sidras road on road to Bermejo, 21 Apr 1983, Solomon 9966 (US) • Prov. O’Connor, Abra de la Cuesta de San Simón, 30 Apr 1983, Krapovickas et al. 39043 (F). **Brazil. Bahia** • Mun. Boa Nova, Fazenda Cotermaia, 8 Mar 2003, Fiaschi et al. 1395 (CEPEC, NY, SPF) • Mun. Maracás, 13–22 km ao S de Maracás, 27 Apr 1978, Mori et al. 10061 (CEPEC, MO, NY, RB) • Mun. Planaltino, ca. 6 km W de Nova Itarana, 790 m, 14 May 2001, França et al. 3507 (CEPEC, HUEFS). **Mato Grosso do Sul** • Mun. Amambai, Rio Iguatemi, 13 Feb 1983, Hatschbach 46203 (INPA, MBM, MG, MO, NY) • Mun. Antônio João, Fazenda Cervo, 11 Jun 2006, Barbosa and Silva 1448 (HUEFS, MBM, MG, RB) • Mun. Ponta Porã, Fazenda Itamarati, 9 Mar 2004, Hatschbach et al. 76943 (MBM, RB). **Minas Gerais** • Without locality, s.d., Claussen 521 (P). **Paraná** • Mun. Arapongas, Campinho, 29 Jan 1997, Kinupp 217 (FUEL, MBM) • Mun. Bom Sucesso, 460 m, Proença 82 (ICN) • Mun. Campo Mourão, RPPN Ana Tramujas, 667 m, 9 Jun 2009, Siqueira et al. 204 (HCF, MBM) • Mun. Céu Azul, Parque Nacional do Iguaçu, 674 m, 18 Jun 2015, Caxambu et al. 6559 (HCF) • Mun. Cianorte, Estrada Cambuci, 12 Feb 2013, Rosado 371 (HCF, HUEM) • Mun. Diamante do Norte, Estação Ecológica do Caiuá, 24 Feb 2006, Zeiden 34 (HUEM, RB) • Mun. Doutor Camargo, Rio Ivai, 16 May 1969, Hatschbach et al. 21527 (MBM) • Mun. Entre Rios do Oeste, Linha Divisa, 229 m, 29 Jan 2015, Siqueira et al. 1385 (MBM) • Mun. Fênix, RPPN Vila Rica, 16 May 2014, Caxambu et al. 5215 (MBM) • Mun. Figueira, Fazenda São Pedro, 26 Apr 2001, Pavão s.n. (FUEL, RB422905) • Mun. Foz do Iguaçu, Parque Nacional do Iguaçu, 203 m, 4 Jun 2015, Caxambu et al. 6486 (HCF) • Mun. Goioerê, RPPN Moreira Sales, 27 Mar 2017, Siqueira et al 2162 (HCF) • Mun. Guaira, Parque Nacional de Sete Quedas, 21 Mar 1982, Kirizawa et al. 756 (SP) • Mun. Guarapuava, Estrada para Campo Mourão km 7, 6 Feb 1969, Hatschbach 21007 (MBM, NY) • Mun. Ibiporã, Fazenda Doralice, 24 Apr 2003, Urdampilleta 148 (FUEL, HCF) • Mun. Jaboti, Água Branca, 18 Mar 1994, Hatschbach et al. 60563 (MBM, MEXU, MO) • Mun. Londrina, Fazenda Ramses-Distrito de São Luiz, 12 Mar 2003, Urdampilleta et al. 118 (FUEL, HCF) • Mun. Mamboré, Fazenda São Domingos, 16 May 1967, Lindeman and Haas 5325 (MBM, RB) • Mun. Maringá, 517 m, 16 Jan 2013, Proença 89 (ICN) • Mun. Medianeira, Estrada para Santa Helena km 10, 8 Feb 1969, Hatschbach 21077 (MBM, SP) • Mun. Moreira Sales, Fazenda Moreira, 24 Mar 2007, Marques s.n. (HCF 5351, MBM) • Mun. Palotina, Parque Estadual de São Camilo, 20 Jan 2011, Kozera and Cardozo 3770 (MBM) • Mun. Pitanga, 19 Feb 2005, Bianek 229 (HCF, MBM) • Mun. Planalto, Estrada de terra próximo ao Rio Capanema, s.d., Rodriguea et al. 133 (ESA) • Mun. Porto Rico, Mata ciliar do Rio Paraná, 15 Jan

1987, Soares-Silva et al. 26 (FUEL, RBR) • Mun. Primeiro de Maio, Mata Santa Rosa-Distrito de Ibiaci, 11 May 1998, Francisco s.n. (FUEL, MBM 338060, RBR) • Mun. Rolândia, Córrego dos Carangueijos, 24 Jan 1997, Kinnup s.n. (FUEL27790, R) • Mun. São Jorge do Oeste, Rio Iguaçu-Salto Osório, 10 Jun 1968, Hatschbach and Guimarães 19360 (MBM, NY) • Mun. Sertaneja, Fazenda Tangará, 21 May 1999, Francisco s.n. (FUEL, MBM338061) • Mun. Telêmaco Borba, Dec 2011, Bonaldi 506 (MBM) • Mun. Tuneiras do Oeste, Fazenda Água do Índio, 22 Jan 2004, Bianek 172 (HCF) • Mun. Turvo, Propriedade da família Rickli, 1104 m, 27 Feb 2009, Caxambu et al. 2524 (HCF, MBM) • Mun. Ubiratã, Sítio Invicta, 409 m, 9 Jan 2009, Sekine et al. 97 (HCF, MBM) • Mun. Umuarama, Serra Dourada, 19 Jan 1967, Hatschbach 15753 (MBM). **Rio de Janeiro** • Mun. Cabo Frio, Estação Radiogoniométrica de Campos Novos, 12 Jun 2009, Somner et al. 1354 (RBR) • Mun. Miguel Pereira, Sítio Xapuri, 12 Jan 2006, Menescal 103 (RB, UFAPZ) • Mun. Paraty, Estrada do Cabral, 19 Apr 1994, Marquete 1692 (RB, UFACPZ) • Mun. São Pedro da Aldeia, Ilha dos Macacos, 14 Apr 2019, Bastos and Neves F5 (RB). **Rio Grande do Sul** • Mun. Arroio do Tigre, Barragem de Itaúba, 19 Apr 1978, Lise 5860 (F) • Mun. Caxias do Sul, Caravágio, 27 Jan 1999, Kegler et al. 153 (US) • Mun. Giruá, Granja Sodol, 20 Dec 1966, Hagelund 4947 (ICN) • Mun. Ibarama, 11 Feb 2013, Proença 123 (ICN) • Mun. Iraí, Ladera con selva frente al Balneario Oswaldo Cruz, 29 Jan 1992, Krapovickas and Cristóbal 44016 (CTES, MBM) • Mun. Jaboticaba, IFN-Conglomerado 752-2-10-37, 24 May 2013, Maihack s.n. (RB613850) • Mun. Jaguari, Rio Jaguari, 5 Jan 2011, Durigon and Ferreira 456 (ICN) • Mun. Linha Nova, Roseiral, 1 Feb 2018, Massing 125 (ICN) • Mun. Maquiné, 6 Jun 2013, Proença 115 (ICN) • Mun. Pontão, Projeto de Assentamento Encruzilhada Natalino III e IV, 30 Aug 2008, Grings 346 (ICN) • Mun. Nova Petrópolis, 3 Apr 2018, Massing 224 (ICN) • Mun. Santa Maria, BR-158, 20 Feb 2019, Figueira et al. 927 (RB, SMDB) • Mun. Santa Rosa, 8 Jul 1965, Hagelund 3678 (ICN) • Mun. São Jeronimo, Faz. Do Conde, 17 May 1982, Abruzzi 655 (F) • Mun. Tenente Portela, Parque Estadual do Turvo, Mar 1982, Bueno et al. s.n. (ICN 2808) • Mun. Três de Maio, Feb 1967, Hagelund 5243 (ICN) • Mun. Tupanciretã, Projeto de Assentamento Tarumã, 7 Feb 2008, Grings 1272 (ICN) • Mun. Vale do Sol, Linha XV de Novembro, 27 Feb 1993, Jarenkow 2343 (MBM, PEL). **Santa Catarina** • Mun. Aberlado Luz, Comunidade da Grama, 110 m, 14 Apr 2009, Stival-Santos 588 (HCF) • Mun. Belmonte, 17 Mar 2011, Durigon 607 (ICN) • Mun. Florianópolis, Cachoeira do Bom Jesus, 6 May 1970, Klein 8690 (MBM, PEL) • Mun. Itapiranga, Rio Piperiguaçú, 26 Dec 1973, Karhs and Schenkel s.n. (ICN2810) • Mun. Seara, Nova Teutônia, 11 Jan 1944, Glaumaum 289 (RB). **São Paulo** • Mun. Campinas, 30 Jan 1995, Novaes 3202 (SP) • Mun. Coronel Macedo, Bairro dos Costas, 24 Jan 1996, Souza et al. 10426 (BHCB, ESA) • Mun. Guariba, Acesso da estrada SP 255 para Guariba, 11 Mar 1991, Cordeiro et al. 834 (SP) • Mun. Piracicaba, Mata da Pedreira ESALQ/USP, 20 Apr 1985, Catharino 288 (ESA, MBM, SP) • Mun. Monte Alegre, Amparo, 7 Apr 1943, Kuhlmann 610 (S) • Mun. Socorro, Entre Saltinho - Monte Sião, 9 May 1995, Tamashiro et al. 1015 (ESA, SP, SPF) • Mun. Teodoro Sampaio, Parque Estadual do Morro do Diabo, 23 Jun 1994, Pastore et al. 514 (SP, SPF). **PARAGUAY. Canendiyú** • Mbaracayu Natural Reserve, 10 Jun 1998, Zardini and Chaparro 48588 (MEXU, MO). **Concepción** • Estancia Primavera, 28 Jun 2002, Zardini and Guerrero 59086 (FACEN, MO, US). **Guairá** • Colonia Independencia. Camino a San Gervacio, 25 Mar 1993, Schinini et al. 27933 (CTES, MBM, US). **Itapúa** • Ruta 1, 65 km de Encarnación, 17 Aug 1980, Ferrucci 161 (CTES, F, NY, US). **Misiones** • San Ignacio. Rutal, 5 km de San Ignacio, 30 Mar 1981, Ferrucci and Schinini 179 (CTES, F, MBM). **Paraguari** • Parque Nacional Ybycui, 13 Jan 1983, Hahn et al. 1072 (MO, NY); Acahay Massif. Easternmost Peak, Zardini and Tilleria 29772 (MO, PY, US). **San Pedro** • Yaguareté forest, 20 Jun 1995, Zardini et al. 42893 (MO, PY, US) • San Estanislao. Estancia La Manina, 13 Feb 1975, Pedersen 11042 (MBM).

4. *Thinouia myriantha* Triana & Planch., Ann. Sci. Bot. Ser. 4, 18: 369. 1862

Figs 9, 10, 13C

Carpidiopterix macroptera sensu H. Karst., Fl. Columb. 2: 45. 1863, not *Thouinia macroptera* Casar.

Type. COLOMBIA. Prov. de Bogotá • Tocaima, Limba, alt. 450 m, May 1857, *J. Triana s.n.* (lectotype, designated here: MPU [2 sheets] [MPU010893, MPU010892] [image!]).

Description. Tendrilled liana 10–15 m; stem cylindrical, 10–12 cm diam., puberulous and ferruginous, lenticels rounded or elliptic and ferruginous; cross-section simple. Leaves trifoliolate; stipules minute, ca. 0.4 mm long, tomentose; petiole 1.8–6 cm long, terete, striate, pubescent, puberulous to glabrescent; terminal petiolule 1–4 cm long, lateral petiolules 0.3–1.5 cm long; leaflets glabrous on both sides, sometimes with sparse trichomes along veins; the leaflet secondary venation eucamptodromous or mixed semicraspedodromous at the apex; secondary veins (4)5–6 pairs, subalternate or alternate, spacing irregular, with domatia on abaxial side of axils; intersecondaries present; tertiary veins irregular reticulate to mixed alternate-opposite percurrent; margins entire to repand-serrate (rarely serrate), with (2)4–8 teeth per side, reduced to inconspicuous glands; terminal leaflet 6.5–17.5(22) × 3.5–10.5(11.3) cm, elliptic-ovate, apex acute or obtuse, long-apiculate, base truncate, obtuse or rounded; lateral leaflets 5.5–15.5(17) × 2–8.9(9.5) cm, ovate, asymmetrical, apex obtuse or acute and acuminate without drip tip, base truncate or rounded, sometimes cordate. Thyrses axillary or terminal, umbelliform, 1.5–10 cm long, peduncle 1–8.6 cm long, secondary peduncle 0.1–1.8 cm long; cincinni numerous, peduncle of cincinni 0.4–0.5 cm long, tomentose. Flower 1–2.5 mm long, pedicel 0.8–3 mm long, pubescent or tomentose; sepals 0.5–1 mm long, oblong-ovate, abaxially villous or slightly villous and adaxially glabrous; petals 0.4–0.8 mm long, lanceolate to obdeltoid, not clawed, villous; petal appendages 0.7–1.5 mm long, longer than the petals, bifid, villous; nectary disc annular, glabrous. Staminate flowers with stamens 8, 2–3 mm long, filaments villous on lower half, anthers glabrous to sparsely villous; pistillode ca. 0.5 mm long, villous. Pistillate flower with staminodes 8, ca. 1.5 mm long, with same indumentum as stamens; pistil ca. 4.5 mm long, ovary tomentose, stigma and styles tomentose. Fruits chartaceous, 2–5.4 × 1–3.2 cm; accrescent pedicel 1.6–3.5 mm long; stipe 2–5.1 mm long; seed locule slightly flattened; epicarp sparsely pubescent; cavity of seed locule with simple unicellular, multicellular and ferruginous trichomes. Seed 6–7.2 × 3.2 mm, ellipsoid, flat.

Distribution and habitat. *Thinouia myriantha* is a species widely distributed from Costa Rica to South America, skirting the Amazon basin (i.e., peri-Amazonian distribution sensu de Granville 1992), known from tropical and subtropical

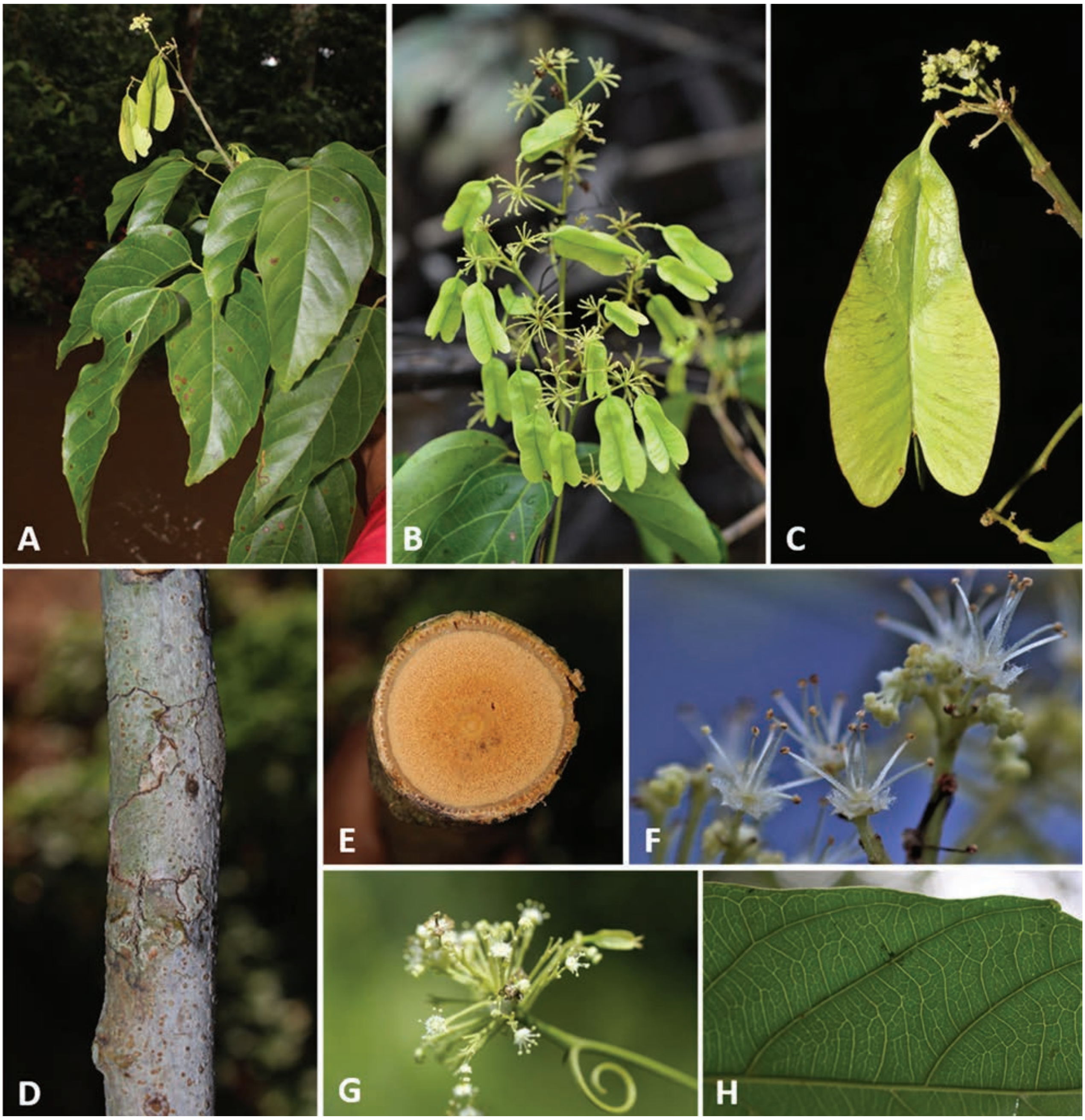

Figure 9. *Thinouia myriantha* Triana & Planch. **A** fertile branch **B** fruiting branch **C** immature fruits **D** cylindrical stem **E** cross-section of the simple stem **F** staminate flower **G** detail of inflorescence **H** detail of the margin and secondary veins [Acevedo-Rodríguez 17136 (**B**) 17128 (**F**) 14262 (**G**); Medeiros 3330 (**A, C–E** and **H**); photos: **A, C, D, E, H** by H. Medeiros **B, F–G** by P. Acevedo-Rodríguez].

moist broadleaf forests in Costa Rica, Panama, Colombia, Venezuela, the Guianas, Ecuador, Peru, Bolivia, and Brazil (Fig. 13C). Flowering from November to February, and fruiting from December to August.

Notes. According to our phylogenetic analyses, *Thinouia myriantha* is sister to *T. silveirae* (see comments in *T. silveirae*), with both species forming a clade that is sister to *T. tomocarpa*. These species together form a clade characterized by the (1) petal appendages that are longer than the petal; (2) fruit cocci that are slightly flattened; (3) cross-section of stem simple; and (4) size and shape of leaflets. The species in this clade, however, are differentiated by the distribution of hairs on the stamens. *Thinouia myriantha* is distinguished by the filaments that are villous only on lower half, white in *T. tomocarpa*, and the stamens that are villous from the base to the connective (see comments in *T. tomocarpa*). *Thinouia myriantha*, as currently recognized, has an ample distribution with substantial morphological variation, and possibly includes more than one species. The resolution of this issue requires the analysis of additional data, including more molecular, morphometry and anatomical data to fully understand if there is some congruence between phylogenetic results and morphological variation.

In the current study, it was found that only a single collection has been made in the type locality region and that only the specimen housed in MPU was located, so that the MPU specimen is designated here as the lectotype.

Conservation status. *Thinouia myriantha* possesses a broad EOO of 5,040,364.60 km^2 and AOO of 168.00 km^2, with more than 10 threat situations and records in conservation units. The EOO values and the number of threat situations extrapolate the threshold for the inclusion of the species in a threat category. Furthermore, several conservation units protect the species. Thus, *T. myriantha* was considered as Least Concern (LC).

Selected specimens examined. BOLIVIA. La Paz • Prov. Abel Iturralde, Parque Nacional Madidi, 535 m, 19 May 2001, Macía et al. 4542 (US) • Prov. Sud Yungas, Sapecho ca. 5m E del Río Beni, 28 Mar 1986, Beck 13309 (US). **BRAZIL. Acre** • Mun. Bujari, Rio Antimary, 12 Dec 2016, Frazão et al. 417 (SPF, UFACPZ) • Mun. Marechal Thaumaturgo, Rio Bagé, 5 Dec 2000, Daly et al. 10444 (NY, UFACPZ, US) • Mun. Rio Branco, Área de Proteção Ambiental Lago do Amapá, 20 Nov 2017, Medeiros et al. 3788 (RB, SPF, UFACPZ) • Mun. Sena Madureira, Reserva Extrativista do Cazumbá-Iracema, 4 Dec 2019, Medeiros et al. 4300 (NY, RB, SPF, UFACPZ, US) • Mun. Mâncio Lima, Parque Nacional da Serra do Divisor, 8 Dec 2022, Medeiros et al. 4898 (NY, RB, SPF, UFACPZ, UPCB). **Amazonas** • Mun. São Paulo de Olivença, Camatian, 23 Jan 1949, Fróes 23958 (IAN, UB). **Pará** • Mun. Belterra, Flona do Tapajós, 7 Feb 2017, Torke et al. 2024 (HSTM, RB, UFAPZ) • Mun. Parauapebas, Serra dos Carajás, 10 Jan 1995, Rodrigues 1632 (IAN) • Mun. Santarém, Floresta Nacional do Tapajós, 29 Apr 2010, Nascimento 33 (IAN) • Mun. Vitória do Xingu, 3°18'S, 51°47'W, 7 Jan 2015, Gonçalves PSACF_EX04702 (RB, UFACPZ). **Rondônia** • Mun. Porto Velho, Parque Nacional do Mapinguari, 13 Dec 2013, Silveira et al. 500 (INPA, RB, RON). **COLOMBIA. Atlántico** • Cerca a Usiacurí, 2 Jan 1949, Molina et al. 19At075 (COL, US) • Barranquilla and vicinity, Dec 1934, Elias 1266 (F-2, MEDEL, US). **Bolivar** • Loma de los Colorados near San Juan de Nepomuceno, 31 Dec 1992, Gentry et al. 78457 (U, US) • Cartagena. Cerro de la Popa, 4 Feb 1962, Saraiva and Johnson 42 (COL). **Magdalena** • Delta of Magdalena river, 28 May 1935, Dugand 872 (F) • Roadside 10 km north of Codazzi, 23 Nov 1943, Haught 3867 (COL, RB, US) • Along stream near La Paz, 12 Jan 1944, Haught 3962 (COL, MO, US) • Barro Blanco, 29 Nov 1945, Haught 4746 (COL, F, MEDEL, NY, US) • Santa Marta. Agua Dulce Road, 2 Jan 1898, Smith 882 (F, MO, NY, U, US). **Meta** • Sierra de La Macarena, Río Guapaya, 21 Jan 1950, Philipson et al. 2197 (COL, MEDEL, US). **COSTA RICA. Puntarenas** • Parque Nacional Corcovado Sirena Trail, 6 Jan 1989, Kernan 867 (F, MEXU, MO, US). **San José** • Carara National Park, 3 Apr 1993, Gentry et al. 79370 (MO). **ECUADOR. Napo** • Tiputini Biodiversity Station, 18 Mar 1998, Burnham et al. 1671 (F, QCNE, US) • La Joya

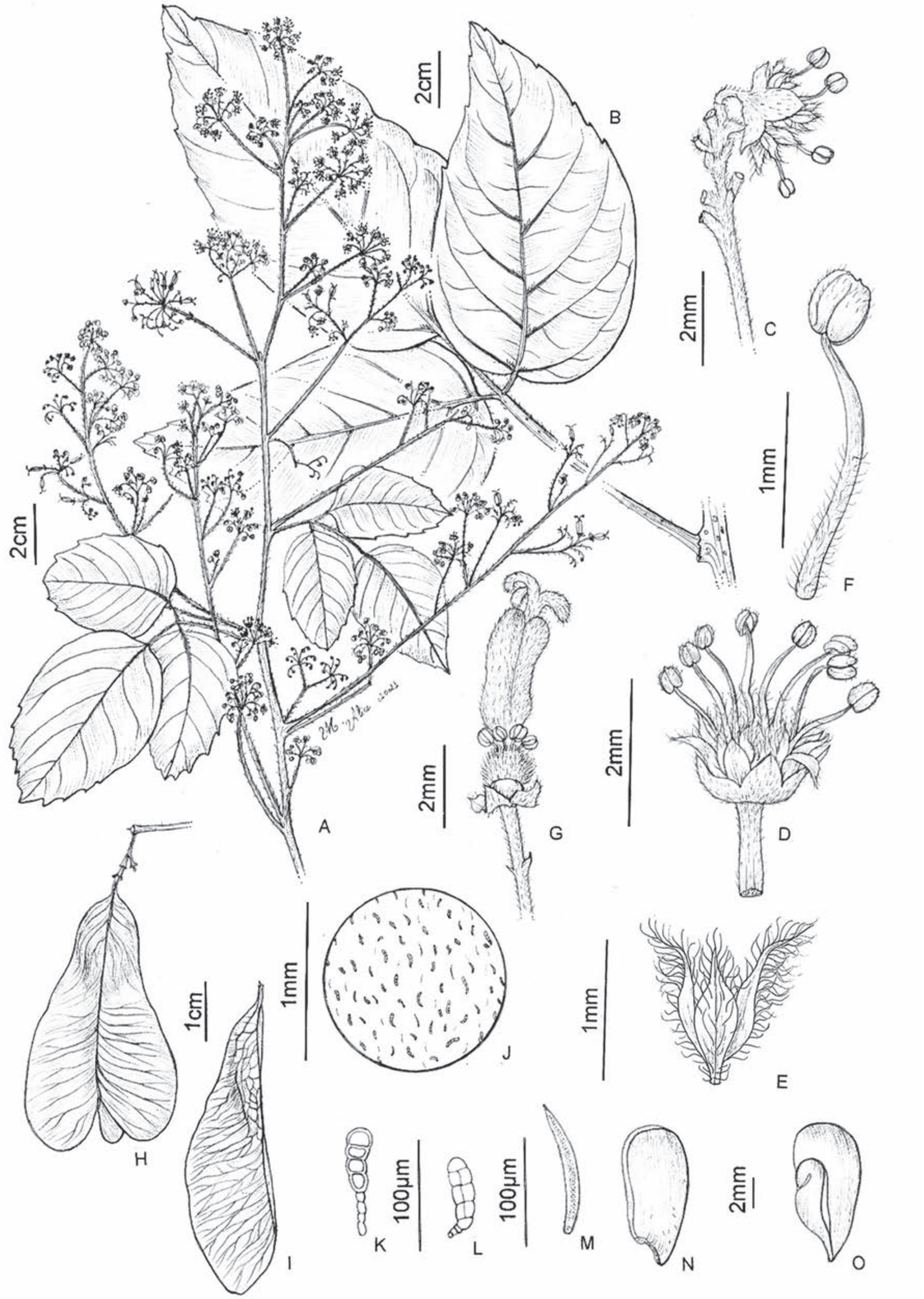

Figure 10. *Thinouia myriantha* Triana & Planch. **A** flowering branch **B** leaf **C** portion of inflorescence, displaying a cincinnus and staminate flower **D** staminate flower **E** petal with bifid appendage, dorsal [abaxial] view **F** stamen of staminate flower **G** young fruit showing nectary disc **H** fruit **I** Mericarp **J** indumentum detail of locule cavity **K** capitate trichome with uniseriate stalk and unicellular terminal cells from locule cavity **L** capitate trichome with uniseriate stalk and multicellular terminal cells from locule cavity **M** simple trichome from locule cavity **N** seed **O** embryo (**A–F** from *Costa 359* **H–O** from *Medeiros 3330*). Illustration by Maria Alice de Rezende.

de los Sachas. Comunidad Indillama, 250 m, 14–28 Jan 1994, Grijalva et al. 450 (QCNE, MO, US) • Orellana. Via a los Pozos Gacela, 250 m, 8 Aug 1993, Palacios 11034 (QCNE, US). **French Guiana** • Haut Camopi Bauin Oyapock, 1 Feb 1949, De La Rue s.n. P0669512 (P) • Aratai River, 1–2 km downstream, 22 Feb 2003, Acevedo-Rodríguez et al. 12359 (NY, US). **Guatemala** • Izabal bet Virginia and Lago Izabal, 50–100 m, 4 Apr 1940, Steyermark 38774 (F). **Guyana. [Without Region]** • Kanuku Mts., Rapununi R., Crabwood Cr., 100 m, 3 Jul 1995, Jansen-Jacobs et al. 4314 (F, MO, P, U, US). **U. Takutu-U. Essequibo** • Trewa River 0–5 km N of confluence of Rewa and Kwitaro Rivers, 90 m, 26 Feb 1997, Clarke 3964 (U, US). **Panama. Panamá** • Barro Colorado Is., Zetek, 26 Feb 1971, Foster 2204 (F) • Serrania de Majé, 300 m, 28 Jan 1984, Churchill and Nevers 4441 (MO). **Peru. Cuzco** • Prov. Cuzco, Campamento San Martín-C, 467 m, 15 Jan 1997, Acevedo-Rodríguez et al. 8788 (USM) • Prov. La Convención, Distrito Echarate, Chahuares, 805 m, Huamantupa and Carrión 9075 (MO). **Loreto** • Prov. Manu, Puerto Maldonado, Los Amigos Biological Station, 24 Nov 2003, Maceda et al. 1097 (US) • Prov. Maynas, Iquitos, Estación Experimental del Instituto de Investigaciones de la Amazonía Peruana, 24 Aug 1990, Vásquez et al. 14290 (MO, US) • Prov. Requena, Jenaro Herrera, 26 Feb 2010, Zárate 14005 (USM). **Madre de Dios** • Cocha Cashu Station, 350 m, 26 Nov 1980, Foster 5905 (F, NY) • Tambopata Tourist Camp, 260 m, Gentry and Ortiz 78338 (MO) • Trail from CICRA to Cocha Lobos, 9 Aug 2003, Acevedo-Rodríguez et al. 14262 (NY, US). **San Martín** • Fundo Pampahermosa (Huicte), 10 Jun 1964, Schunke-Vigo 6533 (F, USM). **Ucayali** • Upper Ucayali, Mashea, s.d., Tessmann 3314 (NY). **Suriname. Brokopondo** • Brownsberg, Mazaronitop, 23 Nov 2003, van Andel et al. 4485 (U, US). **Sipaliwini** • Sipaliwini Region, Voltzberg Nature Reserve, 100 m, Hoffman et al. 5308 (US) • Wilhelmina-Gebergte, 5 Jun 1926, B. W. 6988 (U) • In montibus Bakhuis inter flum. Kabalebo et Coppename Sinistruim, 30 Dec 1964, Florschütz and Maas 2577 (U). **Venezuela. Delta Amacuro** • Río Grande 37 km, este Noreste de El Palmar, 320 m, 10 Feb 1964, Steyermark 93135 (F, NY, P, U, US, VEN) • El Palmar-Raudal trail 2–6 km SW of Río Ganame, 22 Nov 1955, Wurdack and Monachino 38715 (US). **Zulia** • Alrededores de la Represa Burro Negro, 12 Feb 1980, Bunting 8744 (NY). **Yaracuy** • Los Cañizos, plains of the Yaracuy river, 50 m, Jan 1920, Pittier 8758 (NY, US).

5. *Thinouia obliqua* Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München. 8(3): 282. 1878

Figs 11, 12, 13D

Type. **Peru** • 1875, *Ruiz and Pavón 916* (lectotype, designated here: MA [MA813132] [image!], isolectotypes: B†? [F-Negative239294] [image!], F [V0438017F] [image!], F [V031742F] [image!], FI [FI004651] [image!], G [G00008259] [image!], MA [MA813131] [image!], MA [MA813133] [image!], MA [MA817669] [image!], MA [MA817670] [image!]).

Description. Tendrilled liana, up to 40 m long (fide *Mexia 6675*); stem cylindrical-striate or 4–5 sulcate, 10–12 cm diam., pubescent, densely lenticellate, rounded and ferruginous; cross-section simple when young or with neo formations when mature. Leaves trifoliolate; stipules minute, ca. 1 mm long, tomentose; petioles 1–6.5 cm long, terete, striate, pubescent; terminal petiolule

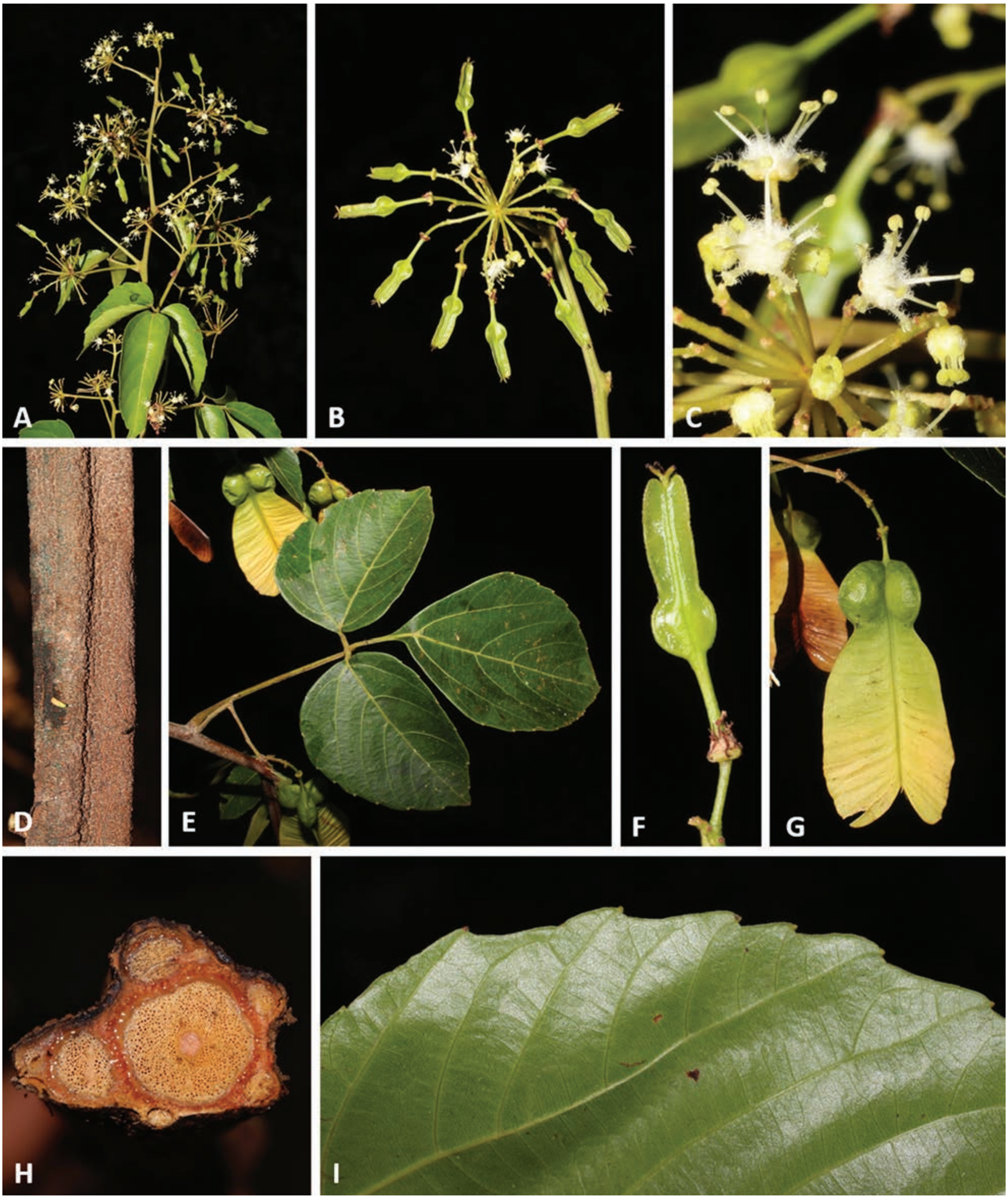

Figure 11. *Thinouia obliqua* Radlk. **A** fertile branch **B** inflorescence branch with immature fruits **C** staminate flower **D** sulcate stem **E** leaf **F** immature fruit **G** mature fruit **H** stem with neo formations, central vascular cylinder surrounded by 7 neoformed, peripheral vascular cylinders of various sizes **I** leaflet margin [Medeiros 3332 (**A–D, I**) 3832 (**E–H**); photos: by H. Medeiros].

0.1–1.1 cm long, lateral petiolules 0.1–1.1 cm long; leaflets with the adaxial side glabrescent or puberulous, puberulous or pubescent in the veins, the abaxial side glabrescent, puberulous or pubescent, puberulous or pubescent on the veins; the leaflet secondary venation craspedodromous; secondary veins (4)5–6 pairs, subalternate or alternate, spacing irregular, domatia present in abaxial surface of secondary vein axils; intersecondaries present; tertiary veins alternate percurrent; margins dentate-serrate, with (6)7–12 teeth on one side, reduced to inconspicuous glands; terminal leaflet 4.9–13.6 × 2.9–5.8 cm, elliptic-ovate or ovate-rhomboidal, symmetrical or asymmetrical, the apex acute to acuminate, mucronate, the base rounded to obtuse, sometimes slightly decurrent; lateral leaflets 3.6–11.1 × 1.8–7 cm, ovate, asymmetrical, the apex acute to acuminate, mucronate, the base truncate to rounded. Thyrses axillary or terminal, umbelliform, 2–5 cm long; peduncle 1–3 cm long; secondary peduncle subsessile or 0.1–0.6 cm long; cincinni numerous, peduncle of cincinnus 0.4–0.8 cm long, pubescent. Flower 4–5.5 mm long, pedicel 1.5–2.5 mm long, pilose or pubescent; sepals 0.5–1 mm long, deltoid, villous on both surfaces; petals 1.7–2.2 mm long, spatulate, clawed, glabrous or villous; petal appendages 0.7–1 mm long, shorter than the petals, bifid, distally bifurcated, villous; nectary disc annular, glabrous. Staminate flowers with stamens 6–7, 2–3.5 mm long, the filaments villous on lower half, the anthers papillose, glabrous or pilose; pistillode 0.5–1 mm long, villous. Pistillate flower with staminodes 6–7, 1.5–2 mm long; pistil 3.5–4.5 mm long, the ovary glabrous, the stigma and styles villous. Fruits chartaceous, 3.5–5.5 × 2.3–2.7 cm; accrescent pedicel 3.4–5 mm long; stipe ca. 2 mm long; seed locule subglobose; epicarp glabrous or with sparse trichomes; cavity of seed locule with simple or capitate, ferruginous trichomes; capitate trichomes with uniseriate stalk and multicellular terminal cells. Seed 6–6.7 × 5.5 mm, ellipsoid or subglobose, glabrous.

Distribution and habitat. *Thinouia obliqua* is known from Tropical and Subtropical Moist Broadleaf Forests, between 140–1000 m in the Amazon basin in western Brazil, Bolivia, Colombia, Ecuador and Peru (Fig. 13D). Flowering from October to May, and fruiting from January to August.

Notes. Although our phylogenetic reconstruction suggests that *T. obliqua* and *T. myriantha* belong to different clades, these two species are vegetatively similar and are partly sympatric. Nevertheless, *T. obliqua* is differentiated by the long-spatulate petals, 1.7–2.2 mm long; petal appendages that are shorter than the petals, 0.7–1 mm long, bifid and distally branched; and stamens 6–7 (vs. small obdeltoid petals, ca. 0.7 cm long; petal appendages longer than the petals, 1–1.5 mm long, bifid and not branched distally; stamens 8). In addition, stems in *T. obliqua* present neo formations, simple in *T. myriantha*.

In the protologue for *T. obliqua*, Radlkofer (1878) mentioned that the material used in the description was collected by *Ruiz and Pavón 916* in Peru. Of the ten duplicates of this collection known to us, we are selecting a duplicate at MA [MA813132] as lectotype because it is more representative.

Popular name. Macote, Macote Negro (Peru), Pacaguasca (Ecuador).

Conservation status. *Thinouia obliqua* occurs in the Western Amazon and possesses a broad EOO of 1,000,444.55 km^2 and an AOO of 104.00 km^2. It is protected by several conservations units, such as the Reserva Extrativista do Cazumbá-Iracema in the state of Acre (Brazil), Parque Nacional Natural Tinigua in the Department of Meta (Colombia), and Los Amigos Biological Station in the Department of Madre de Dios (Peru). Thus, *T. obliqua* should be regarded as Least Concern (LC).

Selected specimens examined. Bolivia. Beni • Prov. Ballivián, Río Beni above confluence with Río Quiquibey, 320 m, 22 May 1990, Daly et al. 6570 (US). **La Paz** • Prov. Franz Tamayo, Serranía de Chepite, 700 m, Killeen et al. 3825 (LPB,

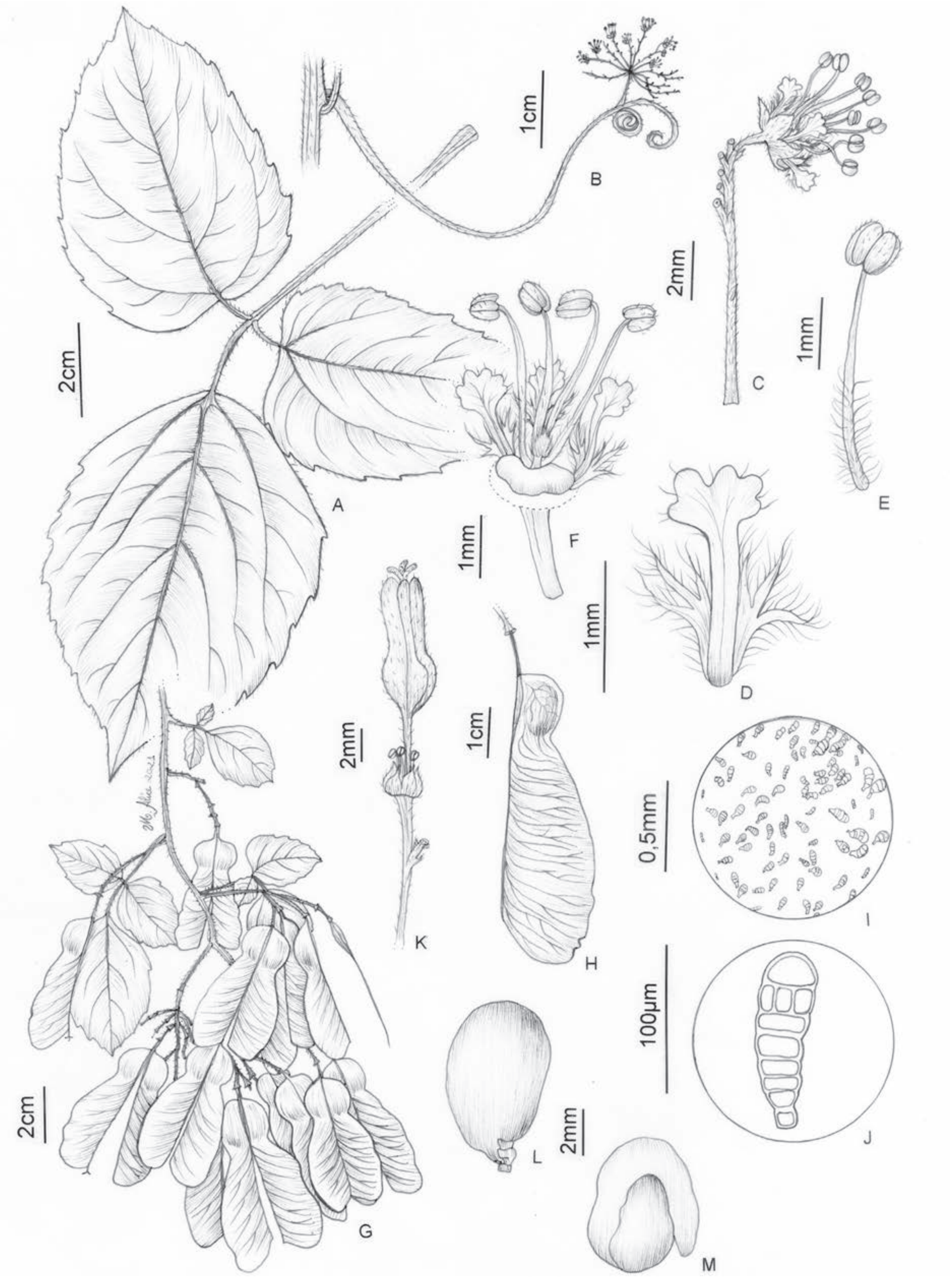

Figure 12. *Thinouia obliqua* Radlk. **A** leaf **B** inflorescence with coiled tendrils **C** cincinnus with a staminate flower **D** petal with bifid and distally branched appendage, dorsal [abaxial] view **E** stamen of staminate flower **F** staminate flower with portion of perianth removed showing nectary disc, pistillode, petals with appendages and stamens **G** infructescence **H** mericarp **I** indumentum detail of locule cavity **J** capitate trichome with uniseriate stalk and multicellular terminal cells in locule cavity **K** pistillate flower with young fruit **L** seed **M** embryo (**A, G–M** from *Medeiros 3832* **B–F** from *Medeiros 3332*). Illustration by Maria Alice de Rezende.

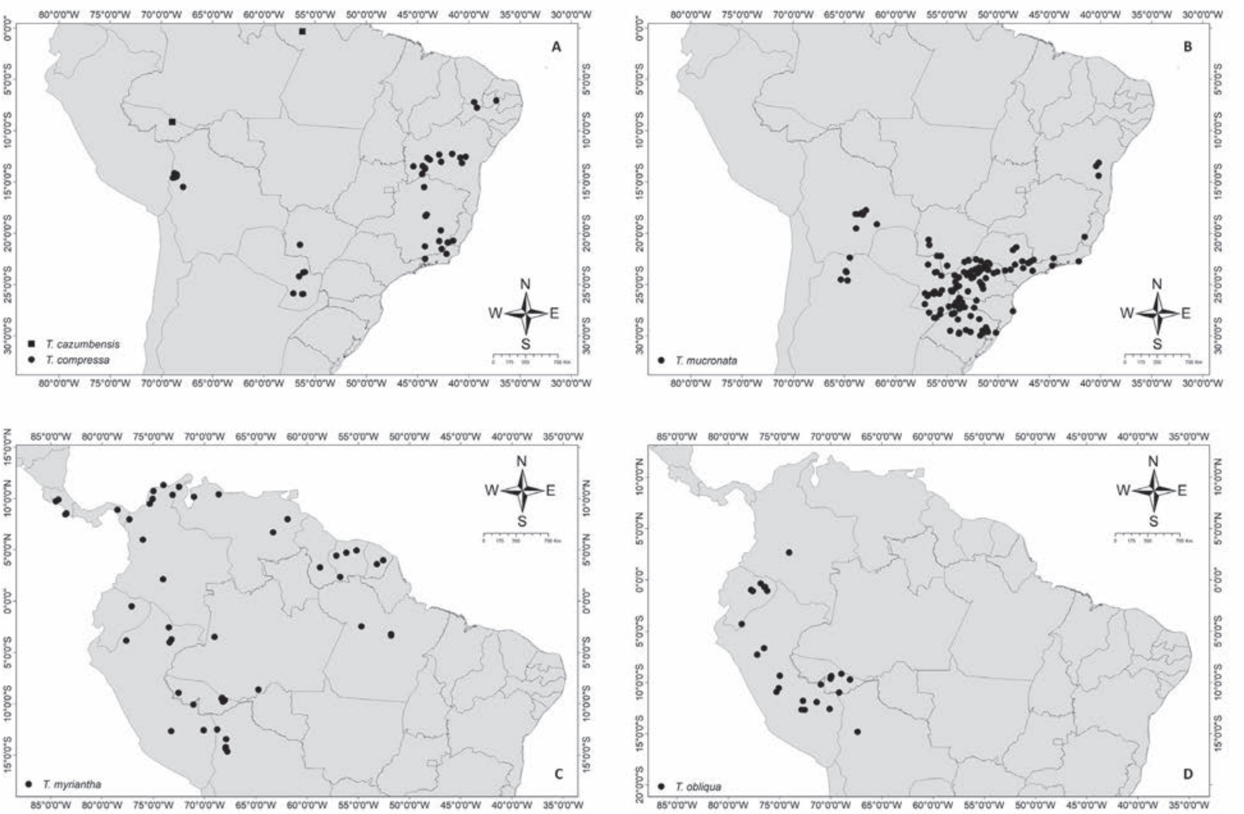

Figure 13. Distribution maps of species from *Thinouia* **A** *Thinouia cazumbensis* and *T. compressa* **B** *T. mucronata* **C** *T. myriantha* **D** *T. obliqua*.

F, MO, US). **Brazil. Acre** • Mun. Brasiléia, Rio Acre, 22 Mar 1998, Daly et al 9727 (MO, NY, UFACPZ) • Mun. Bujari, Riozinho do Andirá, 21 Jan 2018, Medeiros et al. 3328 (RB, SPF, UFACPZ) • Mun. Mâncio Lima, Parque Nacional da Serra do Divisor, 6 Dec 2022, Medeiros et al. 4884 (INPA, NY, RB, SPF, UFACPZ, UPCB) • Mun. Manoel Urbano, Parque Estadual Chandless, 180 m, 3 Apr 2019, Medeiros et al. 3810 (NY, RB, SPF, UFACPZ, US) • Mun. Sena Madureira, RESEX Cazumbá-Iracema, 3 Jan 2019, Medeiros et al. 3792 (RB, SPF, UFACPZ, US). **Colombia. Meta** • Centro de Investigaciones Ecológicas La Macarena, Apr 2000, Stevenson 2144 (NY). **Ecuador. Los Rios** • Bet Quevedo and Naranjal, 90 m, 7 Nov 1934, Mexia 6675 (F). **Napo** • 5 km of Las Sachas, 300 m, Baker et al. 5993 (QAME, QCNE, MO, NY) • Yasuní Forest Reserve, 240–310 m, 29 Jun 1995, Acevedo-Rodríguez and Cedeño 7577 (F, MO, US) • Estación Científica Yasuní, 200–300 m, 8 Nov 1997, Romoleroux and Baus 3200 (QCA, US) • Parque Nacional Yasuní, 200–300 m, 21 Jan 1998, R. J. Burnham and A. Krings 1569 (QCNE, F, MICH, US) • Estación Biológica Jatun Sacha, 450 m, 17 Feb 1988, Cerón 3657 (QCNE, MO, US) • Aguarico. Reserva Etnica Huaorani, 240 m, Aulestia 3367 (QCNE, MO, US). **Zamora-Chinchipe** • Nangaritza Cantón, Parroquia Zurmi, 1000 m, 13 Dec 2001, Clark et al. 6466 (QCNE, US). **Peru. Ayacucho** • Río Apurimac Valley, near Kimpitiriki, 400 m, 10 May 1929, Killip and Smith 22969 (F, NY). **Cuzco** • Prov. Convención, Echarate, 900m, 3 Feb 1939, Sork et al. 10496 (F). **Huánuco** • Vicinity of Tingo María, 10 Aug 1959, Mathias and Taylor 3991 (F, USM) • Ca. 30 km, SW of Pucallpa-Tingo María road, 300 m, Gentry and Diaz 58605 (F, MO, NY, USM). **Junín** • Between Azupizu and Santa Rosa, 650 m, 28 Jun 8 Jul 1929, Killip and Smith 26139 (NY) • Puente Perene, 600 m, 9 May 1961, Schunke-Vigo 4104

(MO). **Pasco** • Prov. Oxapampa, Carretera Oxapamapa-Paucartambo, 730 m, 11 Jun 2003, Rojas et al. 1147 (HOXA, MO, USM). **San Martín** • Prov. Chazuta, Río Huallaga, 260m, Apr 1935, Klug 4058 (F, MO, NY) • Prov. Juan Jui, Alto Río Huallaga, 400–800 m, Apr 1936, Klug 4293 (F, NY, USM) • Prov. Pachiza, Río Huayabamba, 1 Aug 1959, Mathias and Taylor 3980 (F, USM) • Prov. San Martín, Quebrada Mamonaquinha to junction with Río Mayo, 250 m, 25 May 1986, Knapp et al. 7405 (MO, USM) • Saposoa, Monte Real, 400 m, 7 Jul 1958, Woytkowski 5065 (MO). **Ucayali** • Middle Ucayali, Boca de Yarina, s.d., Tessmann 3500 (NY) • Purús, Río la Novia, 22 Feb 2002, Schunke-Vigo and Graham 14868 (MO, USM).

6. *Thinouia paraguayensis* (Britton) Radlk. in Engler & Prantl, Nat. Pflanzenfam. 3(5): 308. 1895

Figs 14, 19A

Thouinia paraguayensis Britton, Ann. New York Acad. Sci. 7: 75. 1893

Thinouia sepium S. Moore, Trans. Linn. Soc. London, Bot. 4: 341. 1895. Type: Brazil. Mato Grosso. 1891–1892, *S. Moore 1076* (lectotype, designated here: BM [BM000838100] [image!]; syntype: Brazil. Mato Grosso. 1891–1892, *S. Moore 943,* B†).

Type. Paraguay • Central Paraguay. Road to Lambare, 05 May 1889, *T. Morong 625a* (lectotype, here designated: NY! [NY02684301], isolectotypes: F [V0361740F] [image!], MO [MO101264008]).

Description. Tendrilled liana; stem cylindrical, striate, puberulous, pubescent or tomentose, with yellowish to whitish indumentum, lenticels sparse, rounded or elliptic; cross-section simple or with neo formations when mature. Leaves trifoliolate; stipules minute, ca. 0.5 mm long, tomentose, deltoid; petiole 2.6–4.2 cm long, canaliculate, pubescent or tomentose; petiolules with keel in the middle, terminal petiolule 0.7 cm long, lateral petiolules 0.1–0.5 cm long; leaflets with the adaxial side puberulous, pubescent-tomentose only on the veins, the abaxial side pubescent or tomentose, discolorous; the leaflet secondary venation craspedodromous; secondary veins 4–5(6) pairs, opposite or alternate, spacing irregular, domatia sometimes present in abaxial surface of secondary vein axils; tertiary veins alternate percurrent; margins dentate-serrate, with 7–14 teeth per side, reduced to inconspicuous glands; terminal leaflet 4.4–6.5(10) × 2.5–5(8.5) cm, broadly ovate, apex obtuse or acuminate, mucronate, base decurrent or cuneate; lateral leaflets 3.5–4.5 × 1.8–4.4 cm, ovate, apex obtuse or acuminate, rarely retuse, mucronate, base truncate or rounded. Thyrses axillary or terminal, umbelliform, 1–6(7.5) cm long; peduncle 0.7–4(6) cm long; secondary peduncle (0)0.1–0.7 cm long; cincinni numerous, peduncle of cincinnus 1–3 mm long. Flower 3–5 mm long, pedicel ca. 2.3 mm long, villous; sepals ca. 0.5 mm long, connate at the base, deltate, abaxially villous, adaxially glabrous; petals ca. 1.6 mm long, spatulate, clawed, erose, adaxially glabrous or villous on the central part; appendages ca. 0.6 mm, shorter than the petals, bifid, sometimes bifurcate distally, villous; nectary disc annular, glabrous. Staminate flower with stamens 8, ca. 2.5 mm long, the filaments villous on lower half, the anthers papillose, glabrous, sometimes puberulous; pistillode ca. 0.6 mm long, villous at the apex. Pistillate flower with staminodes ca. 1 mm long, villous; pistil 2 mm long, puberulous or villous. Fruits chartaceous, 2.6–4.2 × 1.3–2.3 cm; accrescent pedicel 4.2–6.9 mm long; stipe 5.2–6.4 mm long; seed locule subglobose; epicarp glabrous or with sparse simple trichomes in the locule and ventral side of the wing; cavity of seed locule with sparse capitate trichomes; capitate trichomes with uniseriate stalk and unicellular terminal cells. Seed ellipsoid, 5.4–6.4 × 3.7–4.5 mm, basally attached, glabrous.

Distribution, habitat and phenology. *Thinouia paraguayensis* is known from tropical and subtropical dry broadleaf forests; tropical and subtropical grasslands, savannas, shrublands, and flooded grasslands and savannas in Bolivia, Brazil, and Paraguay (Fig. 19A), along roadside thickets, cerrado, chaco, gallery forests, and semi-deciduous forests. Flowering from December to April, and fruiting from January to August.

Notes. *Thinouia paraguayensis* is morphologically similar to *T. mucronata* and *T. ternata*. To distinguish it from *T. mucronata*, refer to the comments under that species. From *T. ternata* it is distinguished by the terminal leaflets that are <5.2 cm long (vs. >5.2 cm long), the lateral petiolules 0.1–0.5 cm long (vs. 2.1–4.4 cm long), the fruit 1.3–2.3 (vs. 2.5) cm wide), epicarp glabrous or subglabrous with sparse simple trichomes in the locule and ventral side of the wing (vs. glabrous).

In the protologue of the species (Morong and Britton 1893), Britton indicated the locality and number of specimens, but without specifying herbaria. The specimen NY02684301 is chosen here as the lectotype of *Thinouia paraguayensis* (Britton) Radlk. The author of the basionym, Dr. Nathaniel Lord Britton, worked as the director-in-chief of the New York Botanical Garden and probably examined this specimen to describe the species.

For *Thinouia sepium*, a synonym of *T. paraguayensis*, the specimen *S. Moore 1076* (BM000838100) is chosen here as the lectotype. The description of this species was based on two collections (*S. Moore 943* and *1076*), both collections from the same place, however the collection *S. Moore 1076* has flowers and fruits and was the only collection we were able to locate.

Conservation status. *Thinouia paraguayensis* has an EOO of 403,484.82 km^2 and an AOO of 148.00 km^2. In central-eastern Brazil and Paraguay, where the species is found, the major threat affecting this species is to convert forest areas to agricultural land (large-scale plantations), and urban expansion. Despite this, the EOO values and the number of threat situations approach the thresholds for the inclusion of the species in a threat category. There are no data on population decline for the application of other criteria, thus, it should be regarded as Least Concern (LC).

Selected specimens examined. Bolivia. Santa Cruz • Prov. Andrés Ibáñez, 7 km NW of Puerto Pailas, 300 m, 10 Feb 1994, Nee et al. 44866 (NY, US) • Prov. Chiquitos, Parque Histórico Santa Cruz La Vieja, 5 Apr 2006, Ferrucci et al. 2535 (CTES, UEC) • Prov. Cordillera, Cabezas, 20 Jan 1945, Peredo 22 (MO, NY) • Prov. Ñuflo de Chaves, Estancia San Miguelito, 260 m, 21 Dec 1995, Fuentes 1484 (MEXU) • Velasco, 58 km W de Roboré, 110 m, 19 Jul 2013, Ferrucci et al. 3128 (CTES, US). **Brazil. Mato Grosso** • Vicinity of Estancia Miranda, 22 Jun 1979, Prance et al. 26298 (CEN, NY). **Mato Grosso do Sul** • Mun. Corumbá, Baia de Tamengo, 8 Aug 1979, Claudio 454 (RB, UFACPZ, US) • Mun. Ladário, Estrada da Manga, 150 m, 3 Jun 1998, Damasceno Júnior et al. 1518 (COR) • Mun. Porto Murtinho, Estrada para o Rio Apa, 14 Jun 2006, Barbosa 1544 (HCF, HUEFS,

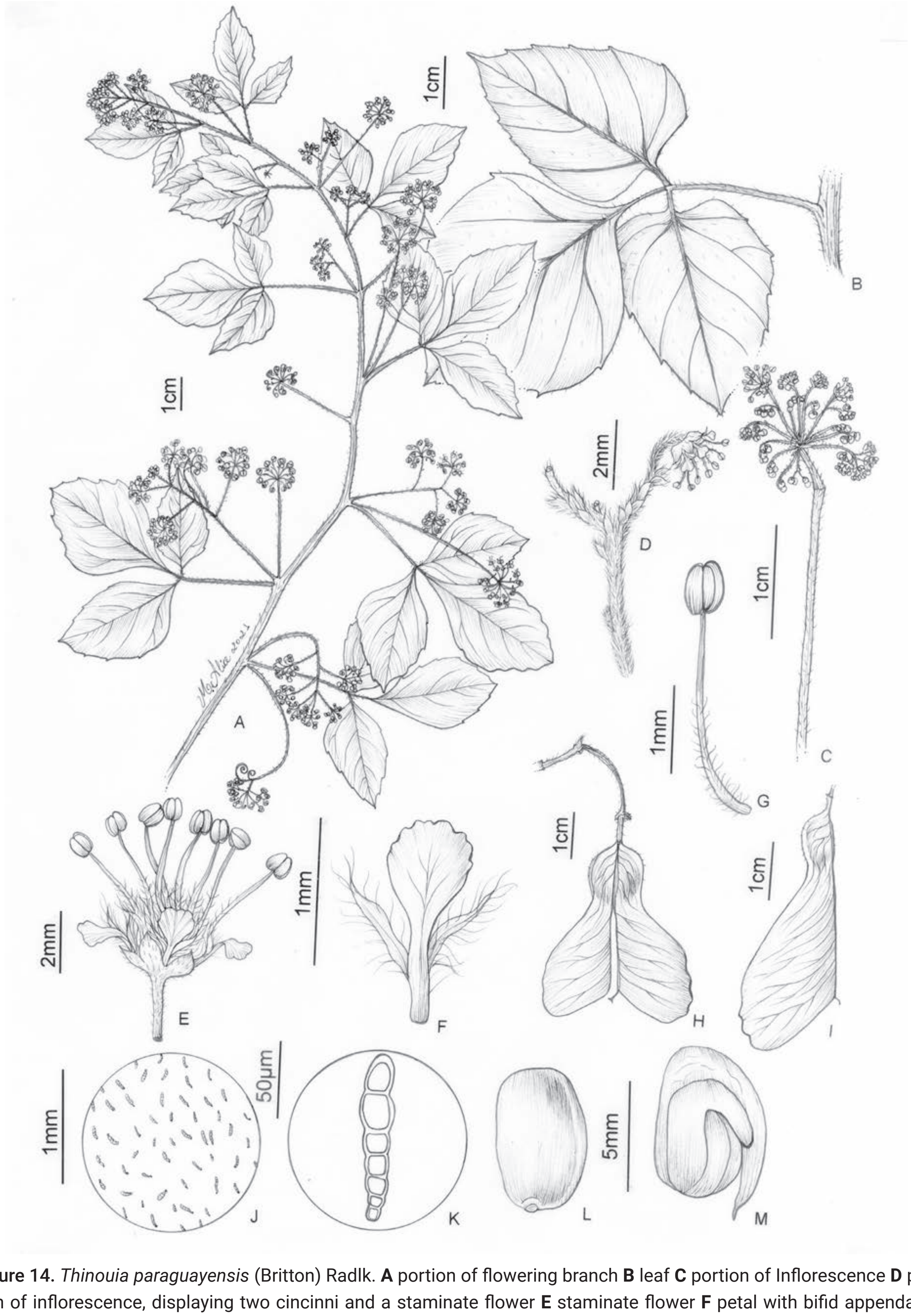

Figure 14. *Thinouia paraguayensis* (Britton) Radlk. **A** portion of flowering branch **B** leaf **C** portion of Inflorescence **D** portion of inflorescence, displaying two cincinni and a staminate flower **E** staminate flower **F** petal with bifid appendage, dorsal [abaxial] view **G** stamen of staminate flower **H** fruit **I** mericarp **J** indumentum detail of locule cavity **K** capitate trichome with uniseriate stalk and unicellular terminal cells from locule cavity **L** seed **M** embryo (**A–G** from *Ferrucci 178* **H–M** from *Hatschbach 49244*). Illustration by Maria Alice de Rezende.

MBM, MEXU). **Paraguay. [without Department]** • Cordillera de Altos, 1898–1899, Hassler 3745 (NY, P). **Central** • Estero del Ypoá, 10 Feb 1990, Zardini and Velázquez 18791 (US) • Jardín Botánico y Zoológico, 20 Jun 1990, Pérez 68 (US). **Cordillera** • Río Salado basin, 21 Jul 1990, Zardini and Velázquez 22320 (AS, MO, US) • Altos. 21 Mar 1990, Mereles 3842 (FCQ, MO). **Paraguarí** • Ruta 1.5 km N de Quindy, 29 Mar 1981, Ferrucci et al. 178 (F, MBM) • Cerro Mbatoví, 2 Jul 1988, Zardini 5433 (MO) • Cerro Palacios, 9 Jul 1988, Zardini 5651 (MO, US) • Acahay. Macizo Acahay, 5 Jan 1989, Zardini et al. 9207 (MO, PY, US). **San Pedro** • Alto Paraguay, 21 Jan 1961, Woolston et al. 1248 (HUEFS, SP).

7. *Thinouia restingae* Ferrucci & Somner, Brittonia 60(4): 371. 2008

Figs 15, 19A

Type. Brazil. Rio de Janeiro • Município Saquarema, Reserva Ecológica Estadual de Jacarepiá, próximo à lixeira, 11 May 1994, *D. Araújo 10025* (holotype: RB! [724823], isotype: CTES [CTES0013570] [image!]).

Description. Tendrilled liana; stem cylindrical-striate or 4–5 lobed, glabrous or puberulous, lenticels rounded and ferruginous; cross-section simple when young or with neo formations when mature. Leaves trifoliolate; stipules minute, ca. 0.5 m long, puberulous, triangular; petiole 1.5–4 cm long, canaliculate, glabrous, rarely puberulous; leaflets glabrous on both sides; the leaflet secondary venation brochidodromous; secondary veins 8–10 pairs, alternate, spacing irregular; intersecondaries present; tertiary veins irregular reticulate; margins entire, sometimes undulate and rarely with 1 tooth on the base; terminal petiolule 0.1–0.5 cm long, lateral petiolules 0.1–0.2 cm long; terminal leaflet 5–8 × 2.7–3.3 cm, oblong, symmetrical or asymmetrical, apex obtuse, rounded or emarginate, mucronate, base acute to decurrent; lateral leaflet 4.7 × 2.4 cm, oblong or oblong-ovate, asymmetrical, apex obtuse, rounded or emarginate, mucronate, base decurrent or rounded. Thyrses axillary or terminal, umbelliform, 0.8–2.8 cm long, peduncle 1–4.2 cm long, secondary peduncle subsessile 0.1–0.7 cm long, cincinni numerous, peduncle of cincinnus 0.2–0.8 cm long, tomentose to glabrescent. Flower 3–5 mm long, pedicel 1.4–4 mm long, glabrous; sepals ca. 1.3 mm long, deltoid, glabrous or sometimes glabrescent, ciliate; petals ca. 2 mm long, spatulate, erose at apex, clawed, adaxially glandular and with sparse simple trichomes; petal appendages 1–1.5 mm long, shorter than the petals, bifid, sometimes distally branched, villous; nectary disc annular, glabrous. Staminate flowers with stamens 8, ca. 4.8 mm long, filaments villous on lower half, anthers glabrous; pistillode ca. 0.3 mm long, villous. Pistillate flower with staminodes 8, ca. 2 mm long; pistil ca. 3 mm long, ovary adpressed-pubescent along dorsal edges, stigma and style puberulous. Fruits chartaceous, 3.6–4.2(5.4) × 2.6–3 cm; accrescent pedicel 3–4 mm long; stipe 3–6 mm long; seed locule lenticular; epicarp glabrous; cavity of seed locule densely villous with arachnoid, biseriate, simple or branched trichomes. Seed 6.8–7.3 × 5.2–5.6 mm, obovoid to ellipsoid, glabrous.

Distribution, habitat and phenology. *Thinouia restingae* is endemic to tropical and subtropical moist broadleaf forests in SE of Brazil, in restinga vegetation and ombrophilous and semi-deciduous forests in the states of Bahia, Espírito Santo, and Rio de Janeiro (Fig. 19A). Flowering from October to December, and fruiting from January to August.

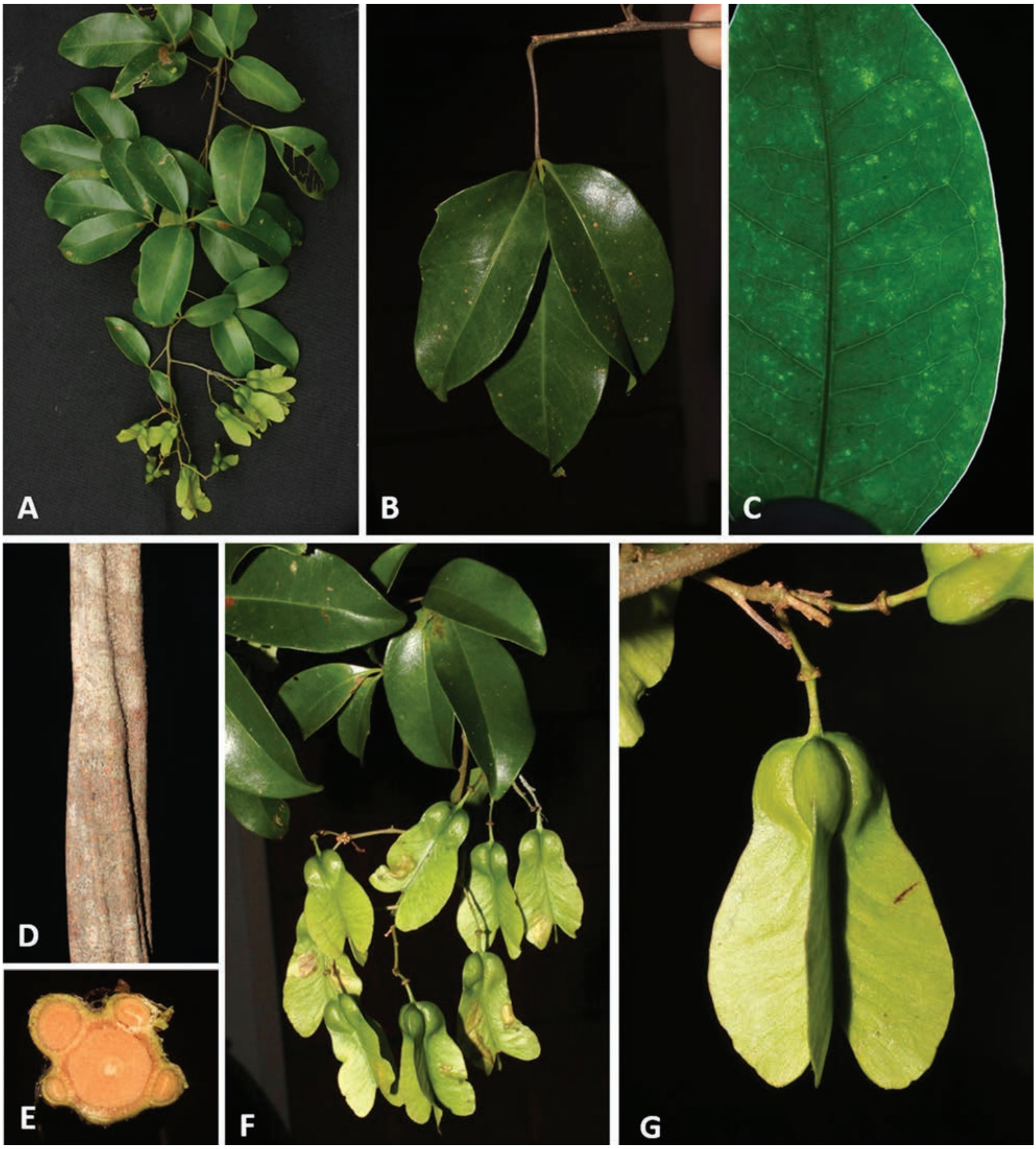

Figure 15. *Thinouia restingae* Ferrucci & Somner **A** fertile branch **B** leaf **C** detail of the secondary veins and leaflet margin **D** lobed stem **E** cross-section of stem with neo formations **F** fruiting branch **G** mature schizocarp [Medeiros 4453 (**A–G**); photos: **A** by C. Toledo **B–G** by H. Medeiros].

Notes. *Thinouia restingae* and *T. ventricosa* have similar fruit morphology and are the only species with densely arachnoid trichomes on the locule cavity. *Thinouia restingae* however, is distinguished from *T. ventricosa* by the entire or rarely basally 1-toothed margins (vs. dentate-serrate with 2–3(4) teeth), 8–10 pairs of secondary veins (vs. 3–5 pairs), secondary veins framework brochidodromous (vs. semicraspedodromous), and the absent of domatia (vs. domatia on abaxial side of secondary vein axil).

Conservation status. *Thinouia restingae* is represented by records distributed by the Atlantic Coast within an EOO of 95,642.60 km^2 and AOO of 58.00 km^2 in antropically modified restinga vegetation in the states of Rio de Janeiro and Espírito Santo, and less frequent in the semi-deciduous and ombrophilous forests in the states of Bahia, Espírito Santo and Rio de Janeiro. Thus, it should be regarded as Vulnerable [VU, B2ab(ii,iii,iv)], due to its range of distribution being less than 100 km^2 and the number of locations being ≤10. Additionally, these species have a continually declining habitat quality, principally in the restinga vegetation where it faces intense pressure from human occupation.

Selecte specimens examined. BRAZIL. Bahia • Mun. Jussarí, Rod. Jussarí/Palmira, 11 Feb 2003, Paixão et al. 211 (CEPEC). **Espírito Santo** • Mun. Aracruz, Retiro Serra Peladinha, 14 Dec 2007, Mansano et al. 480 (RB) • Mun. Nova Venécia, Área de Proteção Ambiental Pedra do Elefante, 18 Feb 2008, Forzza et al. 5089 (CEPEC, CTES, MBML, RB, UPCB) • Mun. Piúma, Estrada entre Marataízes e Piúma, 29 Nov 2006, Souza et al. 32487 (RB) • Mun. Sooretama, Reserva Biológica de Sooretama, 12 Feb 2021, Medeiros and Toledo 4475 (RB). **Rio de Janeiro** • Mun. Armação de Búzios, Praia Gorda, 16 Dec 1998, Fernandes et al. 195 (RB) • Mun. Cabo Frio, Parque Ecológico Municipal do Mico Leão Dourado, 29 May 2003, Fernandes et al. 855 (RB, RBR) • Mun. Campos dos Goytacazes, Morro do Itaoca, 361 m, 15 Feb 2021, Medeiros and Toledo 4453 (RB) • Mun. Maricá, Reserva Ecológica de Jacarepiá, 27 Feb 2021, Medeiros and Toledo 4491 (RB) • Mun. Rio das Flores, Estrada para Rio das Flores, 2 Aug 2006, Marquete et al. 3775 (RB, UFAPZ) • Mun. Rio das Ostras, Praia Virgem, 5 May 2016, Somner et al. 1826 (RB, RBR) • Mun. Saquarema, Reserva Ecológica Estadual de Jacarepiá, 28 Sep 1990, Somner et al. 616 (RB).

8. *Thinouia scandens* (Cambess.) Triana & Planch., Ann. Sci. Bot. Ser. 4, 18: 369. 1862

Figs 16, 19B

Thouinia scandens Cambess. in A. de Saint-Hilaire, Flora Brasiliae Meridionalis 1: 384. 1828. *Thinouia scandens* forma *genuina* nom. invalid.

Paullinia caudata Vell., Fl. Fluminensis 159. 1829 ("1825"); *Fl. Fluminensis Icones 4: tab. 31*. 1831 ("1827"). *Thinouia scandens* forma *caudata* (Vell.) Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München. 8(3): 282. 1878. (lectotype, designated here: Brasil. [Rio de Janeiro or São Paulo] no locality or habitat given; [illustration] Original parchment plate *Flora Fluminensis* in the Manuscript Section of the Biblioteca Nacional do Rio de Janeiro [cat. no.: mss1198653_029] and later published in Vellozo, *Fl. Fluminensis Icones 4: tab. 31*. 1831. [image!]).

Thinouia scandens forma *racemosa* Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München. 8(3): 282. 1878. *Thouinia macroptera* Casar., Nov. Stirp. Bras. 5: 45. 1843. *Carpidiopterix macroptera* (Casar.) H. Karst., Fl. Columb. 2: 45. 1863. Type: Brazil. "Habitat circa Rio de Janeiro, s.d. [before 1840], *Riedel s.n* [*Casaretto Herb. no. 2479*] (lectotype, designated here: TO [image!]).

Thinouia scandens forma *areolata* Radlk. nom. nud.

Type. BRASIL. Rio de Janeiro • "Nascitur in sylvis primaevis provinciae Rio de Janeiro, "Florebat Februario", *Saint-Hilaire s.n.* (lectotype, here designated: MPU [MPU010891] [image!]).

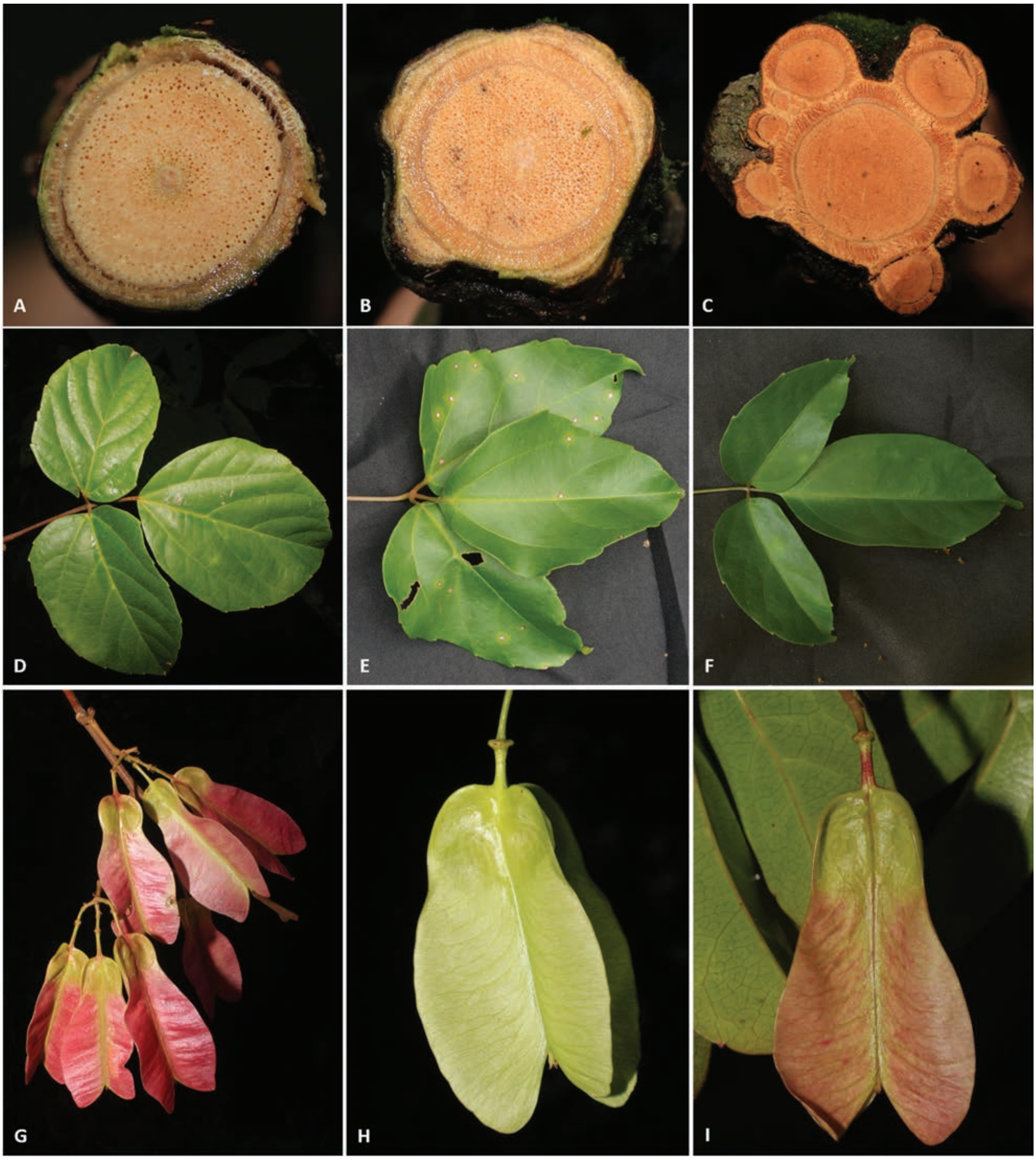

Figure 16. Morphological variation in *Thinouia scandens* (Cambess.) Triana & Planch. **A** stem cross-section cylindrical, simple **B** stem cross-section obtusely pentagonal, showing early neo formation **C** stem cross-section ribbed (lobed), showing neo formations at different stages of development **D–F** leaf morphological variation **G–I** fruit morphological variation [Medeiros 4450 (**E, H**) 4451 (**B**) 4473 (**A, D, G**) 4481 (**C**) 4486 (**F**) 4488 (**I**); photos: by H. Medeiros].

Description. Tendrilled liana; stem cylindrical or 4–5 lobed, glabrous, pubescent or tomentose, lenticels rounded, elliptic or oblong; cross-section simple or with neo formations when mature. Leaves trifoliolate; stipules ca. 0.5 mm long, tomentose, triangular; petiole 5.6–9.1 cm long, semiterete to canaliculate with keel in the middle, striate, glabrous to pubescent; terminal petiolule 0.8–3.7 cm long, canaliculate with keel in the middle, striate; lateral petiolules with keel in the middle 0.4–1.4 cm long; leaflets with the adaxial side glabrous and the abaxial side glabrous, glabrescent, puberulous or tomentose; the leaflet secondary venation craspedodromous to semicraspedodromous, secondary veins 5–7 pairs, alternate or subalternate, spacing irregular, domatia on abaxial surface of secondary vein axils commonly on lowermost pair of secondary veins; intersecondaries present; tertiary veins alternate percurrent or mixed opposite-alternate percurrent; margins entire, dentate or serrate with 3–9 teeth, reduced to inconspicuous glands; terminal leaflet 5.2–11.7 × 3.1–7 cm, obovate, oblong, oblong-ovate or ovate to very widely ovate, symmetrical or asymmetrical, apex rounded, obtuse, acute or acuminate, mucronate or sometimes emarginate, base rounded to obtuse; lateral leaflets 4.3–12.7 × 2.7–8.1 cm, ovate, oblong, elliptic or lanceolate, asymmetrical, the apex rounded, obtuse, acute or acuminate, mucronate, base truncate or obtuse to rounded. Thyrses axillary or terminal, umbelliform, 2–4 cm long, peduncle 1.5–2 cm long, secondary peduncle 0.2.–0.5 cm long; cincinni numerous, peduncle of cincinnus 0.6–0.7 cm long, tomentose. Flower 4.5–6 mm long; pedicel 2–3 mm long, pilose or sparsely pubescent; sepals ca. 0.5 mm long, oblong-ovate, abaxially pilose or sparsely pubescent, adaxially glabrous or sometimes villous, ciliate; petals 1.6–2.5 mm long, oblong-spatulate, clawed, erose, adaxially glandular, abaxially glabrous; appendages 0.7–1.5 mm long, shorter than the petals, villous; nectary disc annular, glabrous. Staminate flowers with stamens 8, 2.8–3 mm long, the filaments villous on lower half, the anthers 0.5–0.6 mm long, glabrous, papillose; pistillode ca. 0.5 mm villous. Pistillate flower with staminodes 8, 1.5–1.7 mm long, villous throughout; pistil ca. 4.5 mm long, the ovary adpressed-pubescent along dorsal edges, the stigma and style tomentose. Fruits chartaceous, 4.3–7.5 × 2.5–4.1 cm; accrescent pedicel 2.3–6.1 cm long; stipe 4.3–9.7 cm long; seed locule subglobose to lenticular; epicarp glabrous; cavity of seed locule pubescent with capitate trichomes; capitate trichomes with uniseriate stalk and multicellular terminal cells. Seed ellipsoid, 6–7.4 × 4.3–5.6 cm, glabrous.

Distribution and habitat. *Thinouia scandens* is endemic to tropical and subtropical moist broadleaf forests in SE Brazil, between 40–1200 m, in gallery forests, semi-deciduous forests, *tabuleiro* forests and ombrophilous forests in Bahia, Espírito Santo, Minas Gerais, São Paulo and Rio de Janeiro states (Fig. 19B). Flowering from December to April and fruiting from January to October.

Notes. *Thinouia scandens* is easily recognized by the leaflets with entire, dentate or serrate margins, with 3–9 pairs teeth per side or with teeth reduced to inconspicuous glands; venation craspedodromous to semicraspedodromous; fruits 4.3–7.5 cm long; and locule cavity densely pubescent with capitate trichomes. Radlkofer (1878, 1931–1934) recognized several taxonomic forms within *T. scandens* based on variations of leaf size, form and pubescence. However, our study found that these characters overlap. Additionally, in our molecular phylogenetic studies the samples representing *T. scandens* were recovered with very strong support in a clade. Therefore, we considered the forms proposed by Radlkofer as synonyms of *T. scandens*.

In the protologue of the *Thinouia scandens* (Cambess.) Triana & Planch. only the place of collection was indicated without mentioning a particular specimen. We have found three specimens that correspond to the collector and identified by the basionym species author: MPU010891, P00754921, and P00754922. Of these, only in the former does the locality correspond with that of the protologue and therefore has been chosen as the lectotype. For the name *Paullinia caudata* Vell., we have selected as the lectotype the illustration of Vellozo (1831, t. 31), which is the only original material available for this name, *Paullinia caudata*. This illustration clearly fits in the *Thinouia scandens* due its elliptic to ovate leaves with margins entire to dentate-serrate and the long fruits. *Thinouia scandens* f. *racemosa* was based upon two elements, i.e., the illegitimate *Paullinia racemosa* of Vellozo (1831, t. 29) and a collection of Riedel s.n (the type of *Thouinia macroptera* Casar.). We have selected the latter as the lectotype, as it is preferable to an illustration.

Conservation status. *Thinouia scandens* possesses a broad EOO of 180,117.85 km^2 and an AOO of 136.00 km^2, with many known locations. However, the ombrophilous forest vegetation in SE Brazil, where the species is found, is subject to continuing decline in area and quality of habitat due to anthropic pressure. Despite this, there are no data on population decline to apply other criteria and several conservation units in Brazil protect the species. Thus, it should be regarded as Least Concern (LC).

Selected specimens examined. Brazil. Bahia • Mun. Almadina, Serra do Corcovado, 20 Mar 2006, Paixão et al. 909 (CEPEC, RBR) • Mun. Nova Viçosa, Estrada para Nova Viçosa, 22 Feb 2021, Medeiros and Toledo 4486 (RB, SPF, UFACPZ) • Mun. Porto Seguro, Estação Veracel, 12 Oct 2006, Colman et al. 21 (ALCB) • Mun. Una, Reserva Biológica do Mico-Leão, 10 Mar 1993, San't Ana et al. 291 (CEPEC, SP). **Espírito Santo** • Mun. Alegre, São João do Norte, 17 Mar 2009, Couto et al. 1131 (MBML, VIES) • Mun. Linhares, Estrada paralela ao Rio Doce, 18 Feb 2021, Medeiros and Toledo 4472 (RB, SPF, UFAPCPZ) • Mun. Nova Venécia, Área de Proteção Ambiental Pedra do Elefante, 329 m, Fraga et al. 2083 (CEPEC, CTES, MBML, RB, UPCB) • Mun. Regência, Reserva Biológica de Comboios, 24 Jan 1990, Folli 1076 (US) • Mun. São Mateus, Próximo de Boa Esperança, s.d, Maguanini and Mattos s.n. (RB 87950). **Minas Gerais** • Mun. Alto Caparaó, Parque Nacional do Caparaó, 23 Apr 1998, Leoni 3950 (RB) • Mun. Carangola, PCH-Carangola, Feb 2007, Leoni et al. 63 (RB, UFACPZ) • Mun. Faria Lemos, Fazenda Santa Rita, 14 May 2006, Silva 122 (RB) • Mun. Francisco Sá, ca. 5 km NE of Francisco de São, 950 m, Irwin et al. 23129 (MG) • Mun. Ipaba, s.d., Pujals s.n. (MBM 420905). **Rio de Janeiro** • Mun. Guapimirim, Parque Nacional da Serra do Órgãos, 734 m, 14 Feb 2021, Medeiros and Toledo 4452 (NY, RB, SPF, UFACPZ, US) • Mun. Itaperuna, Estrada para São Lourenço, 7 Jun 2004, Marquete et al. 3501 (IBGE, HRB) • Mun. Mangaratiba. Trilha para o Mirante, 17 Aug 2017, Somner and Acevedo-Rodríguez 1851 (RBR) • Mun. Natividade, Mata de São Vicente, Morro da Torre, 11 Jun 2006, Gonçalves et al. s.n. (RB 00594985) • Mun. Niterói, Morro do Cavalhão, 28 Aug 1888, Glaziou et al. 17499 (P) • Mun. Nova Friburgo, Macaé de Cima, 9 Oct 1993, Vieira and Gurken 429 (ESA, MBM, NY, RB) • Mun. Paraty, Estrada para o morro do Corisquinho, 10 Mar 1994, Campos 14 (CTES, RB, RBR) • Mun. Petrópolis, Estrada velha Rio Petrópolis, 1 Sep 1990, Somner et al. 585 (RBR) • Mun. Piraí, Distrito de Cacaria, 27 May 2009, Somner et al. 1339 (RBR) • Mun. Rio Bonito, Distrito de Brasília, Fazenda Cachoeira, 13 Aug 1986, Acevedo-Rodríguez et al. 1436 (MO, NY, RB) • Mun. Rio de Janeiro, Horto Florestal, 28 Apr 1924, Florestal s.n. (RB 148992, S) • Mun. Teresópolis, Parque Nacional da Serra dos Órgãos, 14 Feb 2021, Medeiros and Toledo 4450 (NY, RB, SPF, UFACPZ, US). **São Paulo** • Equipe Morro das Pedras, 1917, Brade 7923 (R) • Mun. São Paulo. Nativa do Jardim Botânico, 25 Feb 1942, Hoehne s.n. (SP 46367).

9. *Thinouia silveirae* H. Medeiros, sp. nov.

urn:lsid:ipni.org:names:77356941-1
Figs 17, 18, 19B

Diagnosis. *Thinouia silveirae* is most closely related to *T. myriantha* but differs from the latter by its mericarps with lenticular locule that are flat at the base, the pubescent epicarp and the densely villous locule cavity with capitate trichomes, and the petal appendage adnate to the marginal portion (vs. mericarps with slightly flattened locule; epicarp glabrous, locule cavity sparsely pilose or glabrous with scattered capitate trichomes, and petal appendage adnate to the basal portion).

Type. Brazil. Acre • Mun. Xapuri. Ramal que dá acesso a pousada do Seringal Cachoeira, 9 Jul 2021, *H. Medeiros, C. G. Silva and M. H. Oliveira 4496* (holotype: RB!, isotypes: INPA!, NY!, SPF!, RON!, UFACPZ!, US!).

Description. Tendrilled liana, 10–15 m long; stem 3–5 cm diam., cylindrical, striate, tomentose, glabrescent when mature, lenticels rounded or elliptic; cross-section simple. Leaves trifoliolate; stipules ca. 1 mm long, tomentose, triangular; petiole 5.8–12.4 cm long, terete or angular, striate, tomentose or pubescent; terminal petiolule 2–4.5 cm long, semiterete or terete, striate; lateral petiolules 0.7–1.9 cm long; leaflets with adaxial side glabrous or glabrescent, sometimes pubescent along veins, the abaxial side glabrescent, puberulous or pubescent; the leaflet secondary venation eucamptodromous but semicraspedodromous toward the apex; secondary veins 5–6(7) pairs, alternate or subalternate, spacing irregular, with domatia on the abaxial side of secondary vein axils; intersecondaries present; tertiary veins alternate percurrent; margins entire or sparsely dentate, with 3–6 vestigial teeth reduced to inconspicuous glands; terminal leaflet 8.6–16.6 × 6–11.6 cm, elliptic-ovate or ovate, symmetrical or asymmetrical, the apex acuminate, sometimes emarginate, the base rounded; lateral leaflet 7.8–15.6 × 5.7–10 cm, elliptic or ovate, asymmetrical, the apex acuminate, sometimes rounded to retuse, the base truncate or slightly cordate. Thyrse umbelliform, axillary or terminal; solitary when axillary, 3.5–5.5 cm long, with a pair of circinate tendrils on distal portion of peduncle; peduncles 1.5–3.5 cm long; secondary peduncles 0.1–0.7 cm long; cincinni numerous, peduncle of cincinnus 1.7–4.7 mm long. Flowers 2.5–7.3 mm long, pedicel 1.2–3.5 mm long, pilose to villous; sepals ca. 0.7 mm long, connate at the base, deltate, abaxially villous, adaxially glabrous; petals ca. 0.5 mm long, obdeltoid to widely obtrullate, not clawed, villous; appendages ca. 0.9 mm long, longer than the petals, marginal and bifid, villous; nectary disc glabrous, annular. Staminate flower with stamens 8, ca. 2.7 mm long, the filaments villous at the base, the anthers ca. 0.5 mm long, papillose, glabrous or puberulous; pistillode ca. 0.5 mm long, villous. Pistillate flower with staminodes 0.8–1.2 mm long, villous; pistil ca. 1.5 mm long, villous. Fruits chartaceous, 4–6.1 × 2.5–3.9 cm; accrescent pedicel 2.6–5.3 cm long; stipe 4.2–9.4 cm long; seed locule lenticular but flattened at the base; epicarp pubescent at the locule, puberulous or pilose at the wing; cavity of seed locule densely villous, with capitate trichomes with uniseriate stalk and multicellular terminal cells. Seed ellipsoid 7.5–9.5 × 4.1–6.2 mm, glabrous.

Distribution and habitat. *Thinouia silveirae* is known only from the southwestern Amazonian region, in the states of Acre and Rondônia, Brazil (Fig.19B); in tropical and subtropical moist broadleaf forests. Flowering from June to July, and fruiting from July to September.

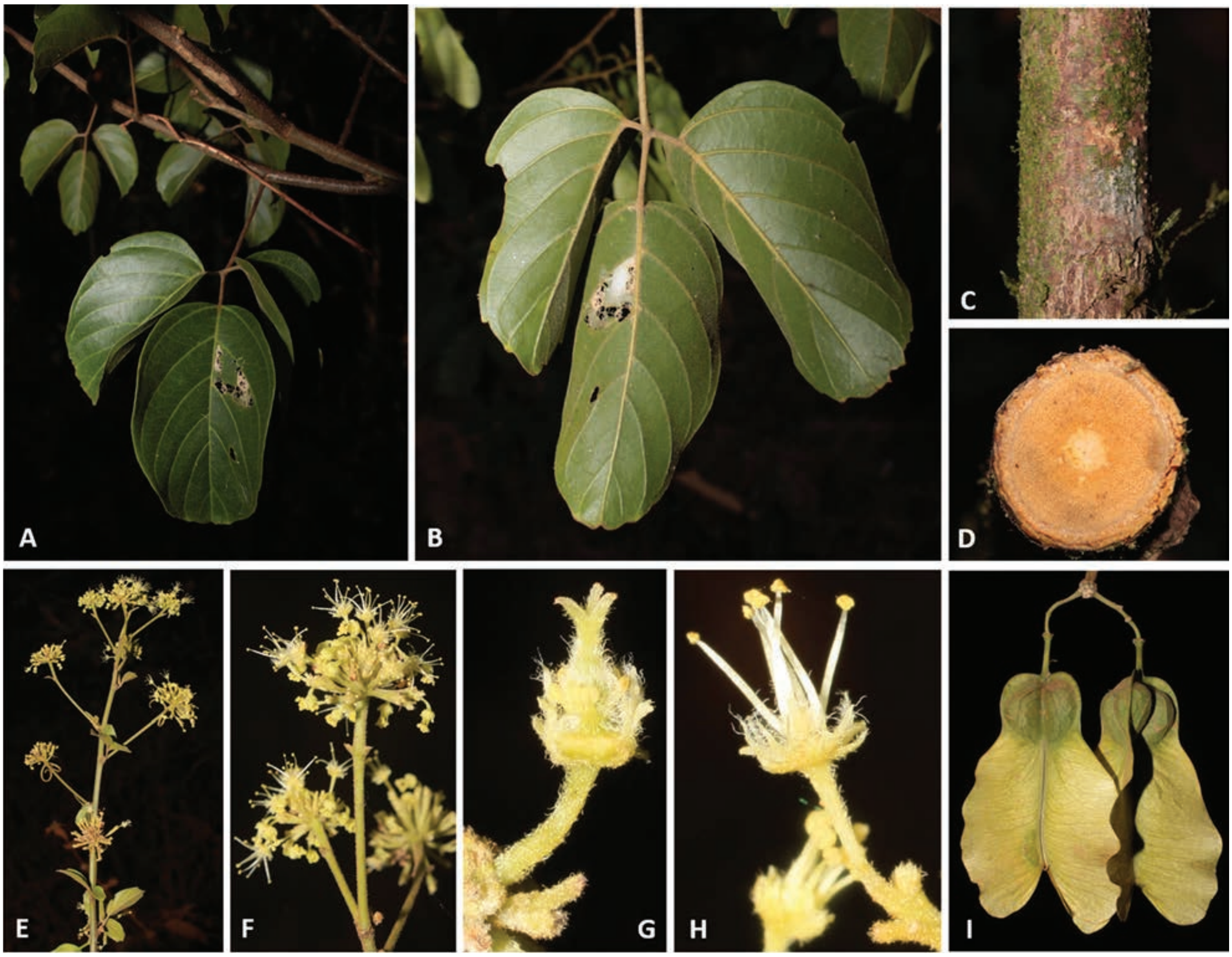

Figure 17. *Thinouia silveirae* H. Medeiros **A** vegetative branch **B** leaf showing abaxial surface **C** stem with lenticellate bark **D** stem cross-section **E** distal synflorescence **F** distal portion of synflorescence **G** mature fruits **H** staminate flower **I** pistillate flower [Medeiros 2191 (**A–D, G**) 4496 (**E–F, H–I**); photos by H. Medeiros].

Etymology. The specific epithet honors Dr. Marcos Silveira, professor and ecologist, at the Universidade Federal do Acre, who has made significant contributions to the floristic studies of Acre and SW Amazonia.

Notes. *Thinouia silveirae* is strongly supported as sister to *T. myriantha* (Fig. 4) by our phylogenetic analyses of molecular data. In addition, both species share similar morphology, including elliptic to ovate leaflets, the presence of domatia, leaflet margins that are dentate to serrate, an annular and glabrous disc, and petal appendages that are longer than the petals (Fig. 5). *Thinouia silveirae* is differentiated from this close relative by its mericarps which have: (1) inflated, pubescent cocci that are flattened at the base; (2) wings that are puberulous or pilose; and (3) a densely villous seed locule cavity with simple and capitate trichomes.

Conservation status. *Thinouia silveirae* possesses a broad EOO of 59,955.343 km^2 and an AOO of 12.00 km^2. Although this species is known only from a few collections, its conservation status is here treated as Least Concern (LC) due to its occurrence within two conservation units, the Reserva Extrativista do Cazumbá-Iracema in the state of Acre, and the Floresta Nacional do Jamari in the state of Rondônia.

Selected specimens examined. Brazil. Acre • Mun. Sena Madureira, RESEX Cazumbá-Iracema, 31 Jul 2017, Medeiros et al. 2189 (RB, SPF, UFACPZ, US)

Figure 18. *Thinouia silveirae* H. Medeiros **A** portion of flowering branch **B** leaf **C** indumentum on leaflets **D** staminate flower with portion of perianth removed showing nectary disc **E** pistillate flower **F** petal with bifid and marginal appendage, dorsal [abaxial] view **G** petal with bifid appendage, frontal [adaxial] view **H** stamen of staminate flower **I** fruit **J** mericarp **K** indumentum of epicarp **L** indumentum detail of locule cavity **M** capitate trichomes with uniseriate stalk and multicellular terminal cells from locule cavity **N** simples trichomes on locule cavity **O** seed **P** embryo (**A–H** from *Medeiros 4496* **I–P** from *Medeiros 2191*). Illustration by Maria Alice de Rezende.

• Mun. Xapuri, Estrada para o Seringal Cachoeira, 8 Sep 2017, Medeiros et al. 2193 (INPA, RB, SPF, UFACPZ). **Rondônia** • Mun. Itapoã do Oeste, Floresta Nacional do Jamari, 28 Aug 2017, Medeiros et al. 2191 (RB, RON, SPF, UFACPZ, US).

10. *Thinouia ternata* Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München 8: 282. 1878

Figs 19D, 20, 21

Banisteria ternata Vell., Fl. Fluminensis: 159. 1829 (“1825”) (nom. illeg.).

Type. Brazil. Minas Gerais • Lagoa Santa, s.d. *Warming s.n.* (lectotype, designated here: P [P06695484] [image!] – epitype, designated here: Brazil. Bahia. Rui Barbosa. ARIE Serra do Orobó, Fazenda Bom Jardim [12°19'43"S, 40°28'34"W] 591 m, 21 Apr 2006, *D. Cardoso and K. S. Matos 1245* RB! [RB01464762]; isoepitype: NY!, HUEFS [HUEFS108414] [image!]).

Description. Tendrilled liana; stem pubescent or tomentose and glabrescent when mature, lenticels round or elliptic; cross-section simple. Leaves trifoliolate; stipules ca. 0.5 mm long, tomentose, triangular; petiole 3.5–7.7 cm long, terete or semiterete and keeled along the middle, striate, pubescent to tomentose; terminal petiolule 0.3–0.7 cm long, semiterete; lateral petiolules 0.2–0.5 cm long; leaflets with adaxial side tomentose to glabrescent, sometimes strigose, the abaxial side tomentose; leaflet secondary venation craspedodromous; secondary veins 4–6 pairs, alternate or subalternate, spacing irregular, with domatia on the abaxial side of secondary veins axils; intersecondaries present; tertiary veins mixed opposite-alternate percurrent or alternate percurrent; margins dentate or serrate, with (7)8–12 vestigial teeth on each side, reduced to inconspicuous glands; terminal leaflet 5.3–10.6 × 4.4–5.6 cm, broadly elliptic, obtrullate or ovate, the apex acuminate and mucronate, the base decurrent; lateral leaflet 4.2–8.9 × 2.3–3.8 cm, ovate, asymmetrical, the apex acute to acuminate, mucronate, the base truncate to rounded. Thyrses axillary or terminal, umbelliform, 1.5–5.3 cm long; peduncle 0.5–4.2 cm long; secondary peduncle 0.1–0.5 cm long; cincinni numerous, peduncle of cincinnus 2–3 mm long, tomentose. Flower 5–5.2 mm long, pedicel 2–3 mm long, pilose or sparsely pilose; sepals ca. 0.5 mm long, triangular, abaxially pilose or sparsely pubescent, adaxially glabrous or sometimes villous, ciliate; petals 1–1.7 mm long, oblanceolate, spatulate, clawed, erose, adaxially villous and abaxially glabrous; petal appendages <0.5 mm long, shorter than the petal, bifid, villous; nectary disc annular, glabrous. Staminate flowers with stamens 8, ca. 2.5 mm long, the filaments villous on lower half, the anthers ca. 0.5 mm long, glabrous, papillose; pistillode ca. 0.4 mm long, villous. Pistillate flower with staminodes 8, ca. 1 mm long, the filaments villous ca. on lower half, the anthers ca. 0.3 mm, glabrous, papillose; pistil ca. 4 mm long, glabrous at base, villous from middle to apex. Fruits chartaceous, 3–4 × 2.5 cm; accrescent pedicel 4.7–5.2 cm long; stipe 6.2–7 mm long; seed locule lenticular; epicarp glabrous; cavity of seed locule glabrous or with sparse simple or capitate trichomes with uniseriate stalk and unicellular terminal cells. Seed ellipsoid 5.7–7 × 3.6–4.7 cm, glabrous.

Distribution and habitat. This species is endemic to tropical and subtropical Brazil, found in moist broadleaf forests in ombrophilous, semi-deciduous and

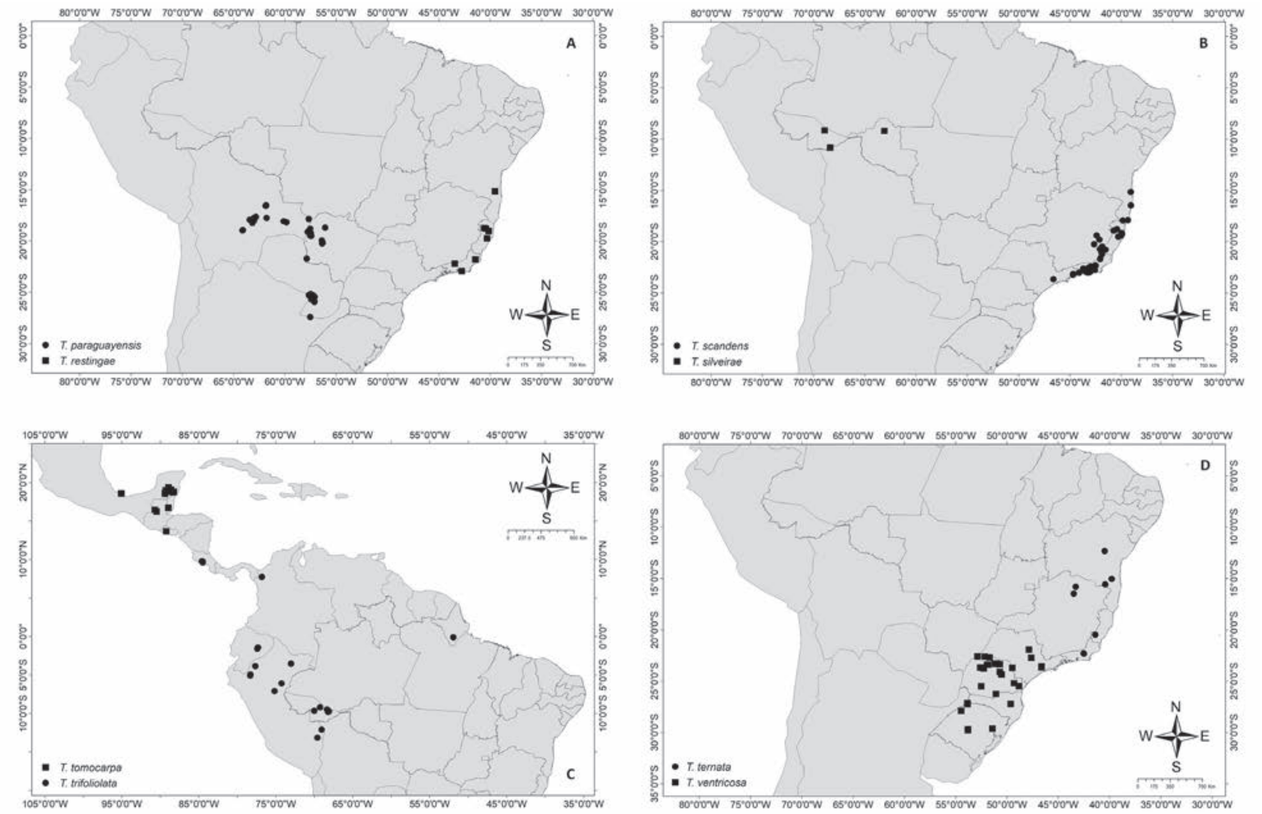

Figure 19. Distribution maps of *Thinouia* species **A** *Thinouia paraguayensis* and *T. restingae* **B** *T. scandens* and *T. silveirae* **C** *T. tomocarpa* and *T. trifoliolata* **D** *T. ternata* and *T. ventricosa*.

deciduous forests, and along streams, on limestone outcrops, and forest margins, in Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro states (Fig. 18D), at 215–950 m elevation. Flowering from February to April, and fruiting from May to October.

Notes. *Thinouia ternata* is sister to *T. mucronata* and *T. paraguayensis* (see comments under *T. paraguayensis*) (Fig. 4). They are morphologically similar, with simple stems, leaflets with mucronate apex, flowers 3–5 mm long, and fruits chartaceous. *Thinouia ternata* can be distinguished from *T. mucronata* by the lateral leaflet that are truncate to rounded at the base (vs. decurrent) and by the craspedodromous (vs. semi-craspedodromous) venation.

Banisteria ternata Vell. is the older name for this species. However, as it is a later homonym of *Banisteria ternata* DC., therefore the name is illegitimate. Consequently, the name *Thinouia ternata* should be credited to Radlkofer (1878), not a combination based on Vellozo's name. In the protologue of *T. ternata* Radlk., Radlkofer cited a collection of Warming from Minas Gerais in addition to the name of Vellozo. We designate the collection of Warming deposited at P as the lectotype, as a specimen is preferable over an illustration. Additionally, we designated an epitype because the lectotype does not have the necessary features in order to fix this name with certainty to a given species.

Conservation status. *Thinouia ternata* is represented by a few records from four states of Brazil's Atlantic Forest, with an EOO of 264,322.39 km^2 and an AOO of 28.00 km^2 in the anthropically modified semi-deciduous forests. Thus, it should be regarded as Vulnerable [VU, B2ab(ii, iii, iv)], due to its range of distribution being less than 100 km^2 and the number of locations being ≤10. Additionally,

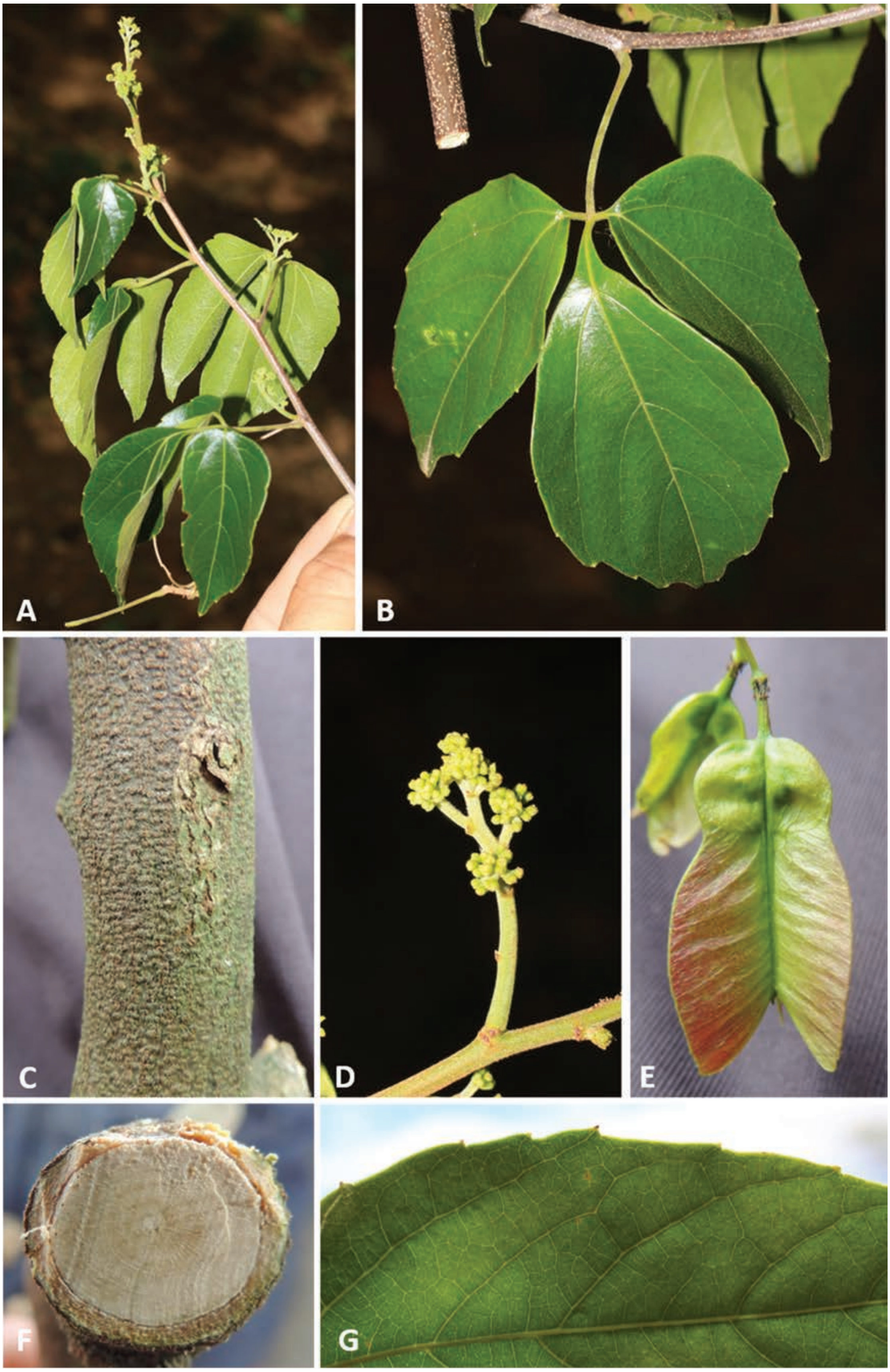

Figure 20. *Thinouia ternata* Radlk. **A** fertile branch **B** leaf **C** stem with lenticellate bark **D** portion of young synflorescences **E** mature fruit **F** stem cross-section, simple **G** partial view of leaflets showing secondary veins and serrulate margins [Medeiros 4489 (**A, B, D, G**); Daneu 746 (**C, E–F**); photos: **A, B, D, G** by H. Medeiros **C, E, F** by L. Daneu].

Figure 21. *Thinouia ternata* Radlk. **A** flowering branch **B** leaf **C** staminate flower **D** petal with bifid appendage, dorsal [abaxial] view **E** stamen of staminate flower **F** infructescence **G** indumentum detail of locule cavity **H** capitate trichomes with uniseriate stalk and unicellular terminal cells from locule cavity **I** pistillate flower with portion of perianth removed showing the gynoecium and nectary disc **J** seed **K** embryo (**A–E** from *Amorim 3580* **H–M** from *Cardoso 1245*). Illustration by Maria Alice de Rezende.

these species have continuously declining habitat quality, principally in the restinga vegetation where it faces intense pressure from human occupation.

Selected specimens examined. Brazil. Bahia • Mun. Itabuna, Margem do Rio Pardo, 23 May 1968, Belém 3597 (CEPEC, F, IAN, NY) • Mun. Itajú do Colônia, ca. 81km de Santa Cruz da Vitória, 25 Feb 2021, Medeiros and Toledo 4489 (CEPEC, RB, SPF, UFACPZ, US). **Espírito Santo** • Mun. Muniz Freire, Vieira Machado, 17 Feb 1993, Souza 439 (CVRD). **Minas Gerais** • Serra do Espinhaço, ca. 5 km NE of Francisco de Sá, 13 Feb 1969, Irwin et al. 23219 (NY, UB) • Mun. Janaúba, Ribeirão Poções, 13 Feb 1991, Hatschbach 55100 (MBM) • Mun. Pains, 613 m, 12 Jul 2006, Borges et al. 224 (RB, RBR) • Fazenda Amargoso, 750–810 m, 25 May 2003, Melo et al. 625 (BHCB, RB). **Rio de Janeiro** • Mun. Nova Friburgo, caminho para a Pedra do Cão Sentado, 27 Oct 1986, Somner et al. 544 (RBR).

11. *Thinouia tomocarpa* Standley, Field Mus. Nat. Hist., Bot. Ser. 12: 411. 1936

Figs 19C, 22

Type. Belize • Temash River, 6 Feb 1935, *W. A. Schipp 1336* (holotype: F [0361405F] [image!], isotypes: G [G00008257] [image!], G [G0008262] [image!], G [G00008260] [image!], G [G00008261] [image!], MICH [MICH1115487] [image!], K [K000634088] [image!], NY! [NY00387405], S [S-R-11015] [image!]).

Description. Tendrilled liana; stems cylindrical, striate, tomentose to glabrescent, lenticels round or elliptical; cross-section simple. Leaves trifoliolate; stipules minute, < 0.5 mm long; petiole ca. 4.8 cm long, terete, puberulous; terminal petiolules 2–2.2 cm long, canaliculate; lateral petiolules 0.8–1 cm long; leaflets glabrous on both surfaces; leaflet secondary venation semicraspedodromous to eucamptodromous, secondary veins 4–6 pairs, alternate, spacing irregular, domatia wanting; intersecondaries present; margins repand-serrate, sometimes only at the apex, with (2) 4–6 teeth on each side, reduced to inconspicuous glands; terminal leaflet 6–8 × 5 cm, elliptic or oblong-ovate, sometimes asymmetrical, the apex long-apiculate or obtuse, the base rounded to obtuse; lateral leaflets 6.5 × 3.9–4.2 cm, elliptic or ovate, sometimes asymmetrical, the apex long-apiculate or obtuse, with a gland at the apex, the base truncate to rounded. Thyrses axillary or terminal, umbelliform, 3.5–6 cm long; peduncle 1.8–3.6 cm long; secondary peduncle sessile or 0.1–0.7 cm long; cincinni numerous, peduncle of cincinnus 2–5 mm long, tomentose. Flower 3–4 mm long, pedicel 1.9–2.4 mm long, tomentose; sepals ca. 0.7 mm long, deltate, abaxially villous and adaxially glabrous; petals 0.6–1.3 mm long, obdeltate, not clawed, villous; appendages 1.4–1.6 mm long, longer than the petals, bifid, marginal, villous; nectary disc annular to slightly lobed, glabrous. Staminate flowers with stamens 8, ca. 4 mm long, the filaments villous throughout, the anthers sparsely villous; pistillode ca. 1 mm long, tomentose. Pistillate flowers with staminodes 8, ca. 2 mm long, villous throughout, the anthers sparsely villous; pistil 1.2–1.4 mm long, villous, the ovary villous, the stigma and style villous. Fruits chartaceous, 3–8 × 1.7–4 cm; accrescent pedicel 2.4–3.8 mm long; stipe ca. 4.3 mm long; seed locule subglobose, but flattened at the base, sometimes slightly flattened, puberulous; seed locule cavity densely covered with capitate trichomes; these with uniseriate stalk and unicellular terminal cells. Seed ellipsoid, 2.5–3.5 × 1.5–2 mm, basally attached.

Figure 22. *Thinouia tomocarpa* Standley **A** flowering branch **B** leaf **C** cincinnus with pistillate flowers **D** staminate flower **E–F** petal with bifid appendage, dorsal [abaxial] and frontal [adaxial] views **G** stamen **H** flower with petals removed showing nectary disc **I** young fruit **J** mature fruit **K** mericarp **L** indumentum detail of locule cavity **M** seed (**A–I** from *Manríquez 495* **J–M** from *Breedlove 50858*). Illustration by Maria Alice de Rezende.

Distribution and habitat. This species is known from southern Mexico (Chiapas & Veracruz), the Yucatan Peninsula in Mexico (Campeche, Quintana Roo) and Belize and in El Salvador, in tropical and subtropical moist broadleaf forests at 120–830 m elevation (Fig. 19C). Flowering from February to April, and fruiting from April to May.

Notes. *Thinouia tomocarpa* was described by Standley and Record (1936) based on the morphology of the fruit, which appeared as if its apex has been cut off by shears, hence the name *tomocarpa* [from the Greek word *tomus*, meaning 'cutting']. This species is otherwise very similar to *T. myriantha*. Although Croat (1976) lumped *T. tomocarpa* in the synonymy of *T. myriantha*, we are resurrecting it because (1) morphologically, *T. tomocarpa* and *T. myriantha* are differentiated by the stamens which are villous throughout in the former but villous only on the lower half in the latter (2) petal appendage is marginal in *T. tomocarpa* (vs. basal); and (3) in our molecular phylogenetic studies show that samples representing *T. tomocarpa* form a monophyletic group with very strong support which is sister to a clade containing *T. myriantha* and *T. silveirae* (Fig. 4).

Conservation status. *Thinouia tomocarpa* possesses an EOO of 227,986.147 km^2 and an AOO of 56.00 km^2, with more than 10 known locations. The EOO values and the number of threatening situations approach the thresholds for classifying the species in a threat category. Beyond this, there are no data on population declines for the application of other criteria, thus it should be regarded as Least Concern (LC).

Selected specimens examined. Belize. Cayo • Chiquibul Forest 4 km on the road from Las Cuevas, 12 Apr 2003, MacMaster et al. 12 (MEXU). **El Salvador. La Libertad** • Laderas de La Laguna, 6 May 1987, R. Cruz 44 (MEXU). **Mexico. Campeche** • Mun. Calakmul, 7.5 km al W de Flores Magón, 175 m, 12 Mar 2002, Soto et al. 22768 and 22770 (MEXU) • Mun. Hopelchén, a 3 km al N de Zoh-Laguna camino a Dzibalchén, 200 m, 1 Apr 1996, Alvaro and Martínez 268 (MEXU, MO). **Chiapas** • Mun. Ocosingo, Ribera del río Chajulillo al sur de la Estación, 22 Feb 199, Colín 2369 (MEXU) • Mun. Palenque, near side road to Agua Azul 0 km South of Palenque, 13 Apr 1981, Breedlove 50858 (MEXU). **Quintana Roo** • 18 km sobre camino a Tomas Garrido, 8 May 1980, Téllez and Cabrera 2134 (MEXU) • 3 km al sur de La Pantera, por la vía corta a Mérida, 21 Mar 1981, Cabrera 1672 (MEXU) • 12 Km al N de San Felipe Bacalar, 22 Mar 1983, Cabrera 4530 (MEXU, NY) • Mun. Jóse María Morelos, a 11.9 km al SE de La aguada La Presumida, 150 m, 12 Mar 2004, Álvarez et al. 8113 (MEXU) • Mun. Othón P. Blanco, La Pantera, 10 Apr 1998, Granados and Chí 641 (MEXU). **Veracruz** • Mun. San Andrés Tuxtla, Estación de Biología Tropical Los Tuxtlas, 27 Mar 1983, Manriquez 495 (MEXU, NY).

12. *Thinouia trifoliolata* [sic, as *trifoliata*] (Radlk.) Acev.-Rodr. & Ferrucci., Syst. Bot. 42(1): 111. 2017

Figs 19C, 23, 24

Allosanthus trifoliolatus Radlk. in A. Engler, Pflanzenr. [Heft 98f] 4, Fam. 165: 1157. 1933.

Type. Peru • Stromgebiet des Maranon, Santiago-Mundung am Pongo de Manseriche, ca. 77°30'W, 1924, *G. Tessmann 4462* (lectotype, here designated: B

[2 sheets] [B100673676, B100673675] [image!], syntype: Peru. Stromgebiet des Maranon, Santiago-Mundung am Pongo de Manseriche, ca. 77°30'W, 1924, *G. Tessmann 4444*, B [B10067362] [image!], NY! [NY4206164]).

Description. Tendrilled liana; stem cylindrical, striate, glabrous or puberulous, lenticels round or elliptical, sometimes with whitish or mucilaginous exudate; cross-section simple. Leaves trifoliolate; stipules < 0.5 mm long; petiole 5–7 cm long, terete, glabrous or subglabrous, sometimes pulvinate at base; terminal petiolules 1.6–2.8 cm long, canaliculate, sometimes pulvinate; lateral petiolules 0.7–1.9 cm long, canaliculate, sometimes pulvinulate; leaflets glabrous on the both sides; leaflet secondary venation eucamptodromous, secondary veins 5–8 pairs, alternate or subalternate, spacing irregular, without domatia; intersecondaries present; tertiary veins mixed opposite-alternate percurrent or alternate percurrent; margin entire, sometimes serrate at the apex; terminal leaflet 7–14.5 × 3.4–7.2 cm, elliptic or oblong, symmetrical or asymmetrical, the apex acuminate, rarely rounded to retuse, with an apical gland, the base obtuse; lateral leaflets 6.8–12.5 × 3.3–6.8 cm, elliptic or oblong, the apex acuminate, with an apical gland, the base acute or obtuse. Thyrses cauliflorous, axillary or terminal, racemiform, 4–5.2 cm long; bracts ca. 2.8 mm, linear-lanceolate, pubescent, glabrescent; peduncle 0–0.2 cm long, tomentose; cincinni numerous, peduncle of cincinnus ca. 0.1 cm long, tomentose. Flower ca. 3.5 mm long; pedicel 1.5–2 mm long, glabrous to strigose; sepals ca. 1 mm long, deltate to obdeltate, abaxially strigose or glabrous, adaxially glabrous; petals ca. 1 mm long, obtrullate, not clawed, erose, ciliate along margins; appendages 0.8–1 mm long, shorter than the petal, villous; nectary disc annular, lobed, glabrous. Staminate flowers with stamens 8, ca. 3 mm long, the filaments villous on lower half, the anthers ca. 0.5 long, glabrous, papillose; pistillode ca. 0.5 mm long, villous on the apex. Pistillate flowers with staminodes 8, ca. 1 mm long, villous on lower half; pistil ca. 1.5 mm long, the ovary villous at the apex, the style and stigma villous. Fruits chartaceous, 3–5.1 × 2–2.8 cm; accrescent pedicel ca. 2 cm long; stipe 2–2.5 mm long; seed locule lenticular, sometimes the base flattened; epicarp glabrous; seed locule cavity sparsely ferruginous-pubescent, with simple, capitate and arachnoid trichomes. Seed 7.5 × 4–4.5 mm, obovoid, glabrous.

Distribution and habitat. *Thinouia trifoliolata* is known from Costa Rica, Colombia, Ecuador, Peru, and Brazil (Fig. 19C), in tropical and subtropical moist broadleaf forests at 100–320 m elevation, in dense and open ombrophilous forests. Flowering from November to February, and fruiting from December to March.

Notes. Unlike most members of the clade in which *T. trifoliolata* emerges (Fig. 4) the inflorescence of *T. trifoliolata* is racemiform. This character is also found in *T. cazumbensis*, a species sympatric with *T. trifoliolata*, so in the absence of molecular data both species could be interpreted as closely related. However, our molecular phylogenetic analyses strongly supports that this character is homoplaseous, i.e., it evolved independently within the clades where these two species belong. Despite sharing a similar inflorescence, *T. trifoliolata* differs from *T. cazumbensis* by its lobed annular disc and sparsely ferruginous pubescent locule cavity (vs. 5-lobbed disc and glabrous locule cavity).

The description of this species was based on two collections (*Tessmann 4444* and *4462*) from the same place and deposited in the same herbarium (B), how-

Figure 23. *Thinouia trifoliolata* (Radlk.) Acev.-Rodr. & Ferrucci **A** stem with lenticellate bark **B** stem cross-section simple **C** leaf **D** fertile branch with axillary inflorescence **E** cauliflorous inflorescence **F** mature fruits [Medeiros 3331 (**A, D, E**); Acevedo-Rodríguez 17159 (**B, C, F**); photos: **B, C, F** by Acevedo-Rodríguez **A, E, F** by H. Medeiros].

ever the collection *Tessmann 4462* is more representative as it has flowers and complete leaflets. Therefore, we are selecting *Tessmann 4462* as the lectotype.

Conservation status. *Thinouia trifoliolata* possesses a broad EOO of 3,450,116.37 km^2 and AOO of 96.00 km^2, with more than 10 threat situations and records in conservation units. The EOO values and the number of threat situations approach the threshold for the inclusion of the species in a threat category. Thus, *T. trifoliolata* is considered as Least Concern (LC).

Selected specimens examined. Brazil. Acre • Mun. Bujari, Riozinho do Andirá, 28 Nov 2013, Costa et al. 373 (LABEV, NY, RB) • Mun. Manoel Urbano, Parque Estadual Chandless, 20 Jan 2014, Costa et al. 468 (LABEV, NY, RB) • Mun. Sena Madureira, Reserva Extrativista do Cazumbá-Iracema, 13 Dec 2019, Medeiros et al. 4380 (NY, RB, SPF, UFACPZ). **Amapá** • Mun. Mazagão, Reserva Genética de Felipe, 10 Oct 1987, Rabelo et al. 3541 (INPA, HAMAB, MO). **Pará** • Mun. Parauapebas. Floresta Nacional de Carajás, 27 Sep 2022, Obermuller et al. 1920 (MG, NY, RB, UPCB). **Colombia. Antioquia** • Rain forest near Río León, 100 m, 20 Mar 1962, Feddema 1983 (MICH, NY, US). **Costa Rica. Puntarenas**

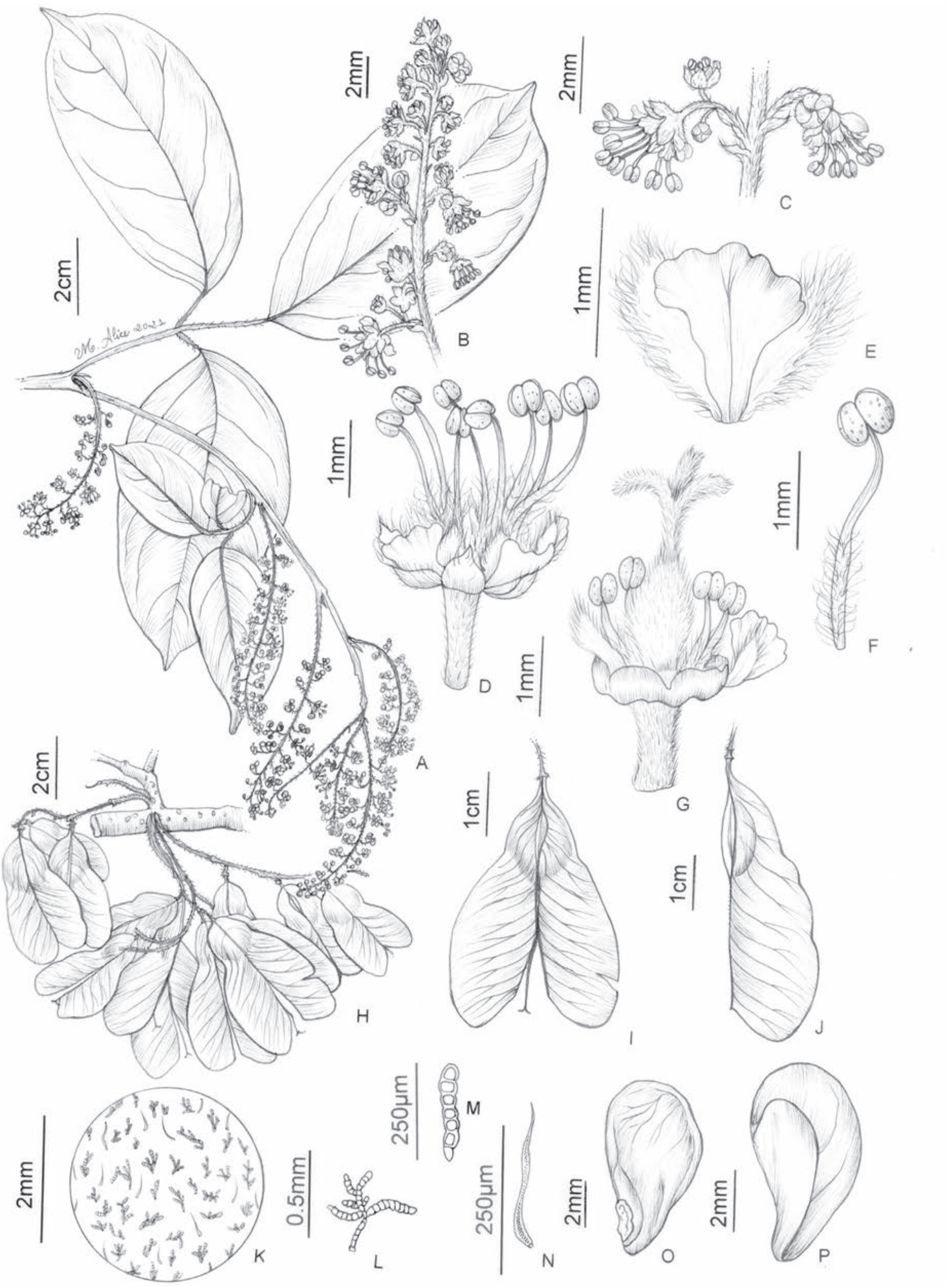

Figure 24. *Thinouia trifoliolata* (Radlk.) Acev.-Rodr. & Ferrucci **A** flowering branch **B** distal portion of inflorescence **C** portion of inflorescence, showing two cincinni with staminate flowers **D** staminate flower **E** petal with bifid appendage, dorsal [abaxial] view **F** stamen **G** pistillate flower with portion of perianth removed showing nectary disc **H** infructescence **I** fruit **J** mericarp **K** indumentum detail of locule cavity **L** arachnoid trichome of locule cavity **M** capitate trichome with uniseriate stalk and unicellular terminal cells of locule cavity **N** simple trichome of locule cavity **O** seed **P** embryo (**A–G** from *Medeiros 3331* **H–P** from *Costa 468*). Illustration by Maria Alice de Rezende.

• Carara National Park, 30–40 m, Grayum et al. 4720 (MO, NY). **San José** • Carara National Park, 5 Apr 1993, Gentry et al. 79454 (MO). **Ecuador. Pastaza** • Pastaza Cantón, Pozo petrolero Villano 2 de ARCO, 400 m, 1 Dec 1991, Hurtado et al. 2885 (US). **Sucumbios** • Pastaza Cantó, Pozo Reserva Faunística Cuyabero, sendero detrás de estación, tierra firme, 265 m, Apr-Oct 1988, Paz y Miño 81023 (MO). **Peru. Amazonas** • Prov. Bagua, Yamayakat Bosque de Rivera, 320 m, 9 Feb 1996, Jaramillo et al. 1143 (MO, US). **Loreto** • Prov. Requena, Cocha Iricahua, margen izquierda del Río Ucayali, 17 Feb 1982, Encarnación 1298 (NY) • Prov. Ucayali, Canchahuayo, 200 m, 30 Nov 1985, Vásquez and Jaramillo 7058 (MO, NY) • Prov. Maynas, Nauta, quebrada Saragosa, 150 m, 11 Dec 1986, Vásquez and Jaramillo 8577 (F, MO, NY) • Iquitos, Caserio Picuruyacu, 11 Feb 1976, Revilla et al. 127 (MO, USM). **Madre de Dios** • Prov. Tambopata, Puerto Maldonado, 26 Nov 2002, Valenzuela and Huamantupa 1063 (US) • San Martín, Juan Jui, Alto Huallaga, 400–800 m, s.d., Klug 4176 (F).

13. *Thinouia ventricosa* Radlk., Sitzungsber. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. München 8: 282. 1878

Figs 19D, 25

Type. Brazil. São Paulo • without locality, *C. F. P. von Martius 1303* (lectotype, designated by Massing and Miotto 2020, pg. 160: M [M0212718] [image!]; isolectotype: P [P02297040] [image!].

Description. Tendrilled liana; stem cylindrical, striate, puberulous, with round or elliptical lenticels; cross-section simple or with neo formations when mature. Leaves trifoliolate; stipules ca. 0.3 mm long, triangular, glabrous or puberulous; petiole 1.5–5.3 cm long, terete or subterete, puberulous or pubescent; petiolules canaliculate, terminal petiolules ca. 0.6 cm long, lateral petiolules 0.2–0.4 cm long, rarely subsessile; leaflets glabrous on both sides or sometimes abaxially pubescent; leaflet secondary venation semicraspedodromous, secondaries 4–5 pairs, alternate, spacing irregular, with domatia on abaxial surface of secondary vein axils, frequently only on the lowermost pair of secondary veins, rarely on other secondaries; intersecondaries presents; tertiary veins irregular reticulate; margins entire to serrate, with 2–3(4) teeth reduced to inconspicuous glands; terminal leaflet 4.7–7.6 × 1.8–2.8 cm, lanceolate, symmetrical or asymmetrical, the apex acute to acuminate, mucronate, the base acute to decurrent, rarely obtuse; lateral leaflets 3.9–7 × 1.5–2.8 cm, lanceolate, asymmetrical, the apex acute to acuminate, mucronate, the base decurrent or rounded. Thyrses axillary or terminal, umbelliform, 2.4–4.5 cm long, peduncle 1–5 cm long, secondary peduncle (0)0.1–0.2 cm long; cincinni numerous, peduncle of cincinnus 0.7–2 mm long. Flowers 2.6–4 mm long, pedicel 2–2.5 mm long, glabrous; sepals ca. 0.5 mm long, connate at the base, deltate, abaxially puberulent, adaxially glabrous, villous at margins; petals ca. 1.4 mm long, short spatulate, abaxially villous on the central part, the rest glabrous; petal appendages rudimentary, bifid, smaller than the petals, villous; nectary disc annular, glabrous, Staminate flower with stamens 8, ca. 3 mm long, filaments villous for little more than half of their length, anthers papillose, glabrous or sparsely pubescent; pistillode ca. 0.9 mm long, pilose on distal half. Pistillate flower

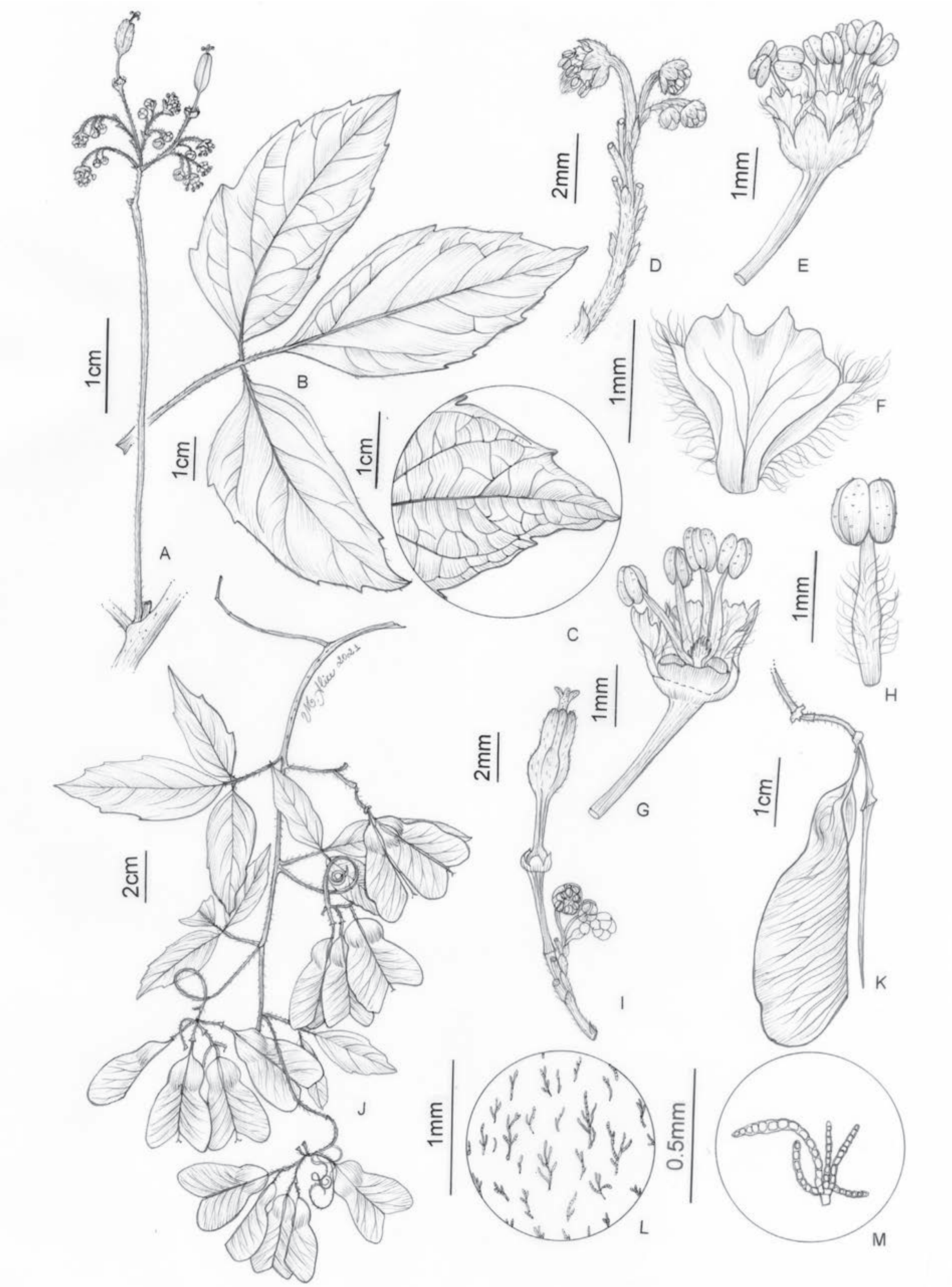

Figure 25. *Thinouia ventricosa* Radlk. **A** axillary inflorescence **B** leaf **C** detail of apex of distal leaflet **D** cincinnus **E** lateral view of staminate flower **F** petal with bifid appendage, dorsal [abaxial] view **G** staminate flower with portion of perianth removed showing nectary disc and pistillode **H** stamen **I** cincinnus with young fruit **J** branch with fruits **K** mericarp **L** indumentum detail of locule cavity **M** arachnoid trichomes of locule cavity (**A, D–I** from *Rosado 222* **B, C** from *Hatschbach 19194* and **J–M** from *Caxambu 5920*). Illustration by Maria Alice de Rezende.

with staminodes ca. 1.2 mm long, villous; pistil ca. 1.3 mm long, pilose on distal half, ovary ca. 1 mm long, style ca. 0.2 mm long. Fruits chartaceous, 3.4–4.7 × 1.8–2.5 cm; accrescent pedicel 5–6.1 mm long; stipe 6.3–8.1 mm long; seed locule lenticular; epicarp glabrous to subglabrous; cavity of seed locule villous, with arachnoid, biseriate, or simple trichomes. Seed ellipsoid, ca. 5.8 × 4.4 mm, basally attached, glabrous.

Distribution, habitat and phenology. *Thinouia ventricosa* is known from tropical and subtropical moist broadleaf forests in the states of Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo, Brazil, and from Argentina (Fig. 18D), in semi-deciduous and ombrophilous forests. Flowering from December to February, and fruiting January to August.

Notes. *Thinouia ventricosa* can be confused with *T. restingae* (see comments under *T. restingae*). Additionally, *T. ventricosa* is similar to *T. scandens* due to the great morphological variability of leaves and fruits. However, *T. ventricosa* is easily distinguished from the latter species by its smaller flowers (2.6–4 mm long vs. 4.5–6 mm long).

Conservation status. *Thinouia ventricosa* possesses a broad EOO of 306,232.35 km^2 and an AOO of 92.00 km^2, with few known locations. However, the semi-deciduous and ombrophilous forests in Southeastern Brazil and Northeastern Argentina, where the species is found, are subject to continuing decline in area and quality of habitat due to anthropic pressure. Despite this, there are no data on population declines to apply other criteria, and several conservation units in Brazil and Argentina protect the species. Thus, it should be regarded as Least Concern (LC).

Selected specimens examined. Argentina. Misiones • El Dorado, Km 31 ruta nac. 12, 10 Jul 1972, Schinini 4904 (MBM). **Brazil. Paraná** • Mun. Cianorte, Estrada Cambuci, 5 Apr 2012, Rosado 59 (HUEM) • Mun. Engenheiro Beltrão, Reserva Florestal de Figueira D'Oeste, 19 Mar 2007, Grande s.n. (MBM 342283) • Mun. Fênix, RPPN Vila Rica, 4 Mar 2015, Caxambu et al. 5920 (HCF) • Mun. Guarapuava, Parque Municipal das Araucárias, 8 Jan 2005, Cordeiro 176 (MBM, ESA) • Mun. Maringá, Parque das Palmeiras, 16 Aug 2019, Rosado 969 (HUEM) • Mun. Morretes, Fartura, 29 Jan 1980, Hatschbach 42750 (F, INPA, MBM, MO, MU, RB, UEC, US, WAG) • Mun. Nova América da Colina, Rio Água Três Barras, 18 May 1998, Francisco et al. s.n. (MBM 338062) • Mun. Rio Bonito do Iguaçu, Fazenda Giacmet-Marodin, 23 Jun 1995, Poliquesi 347 (MBM, MEXU) • Mun. Rio Branco do Sul, Ribeirinha, 7 May 1968, Hatschbach 19194 (F, MBM, NY, P, UPCB, US) • Mun. Sarandi, Condomínio Estância Zaúna, 31 Jul 2019, Rosado 947 (HUEM). **Rio Grande do Sul** • Mun. Derrubada, 15 Aug 2018, Massing 323 (ICN) • Mun. Santa Rosa, 15 Jul 1967, Hagelund 5408 (ICN) • Mun. Tenente Portela, Parque Estadual do Turvo, s.d., Brack et al. 1758 (ICN). **Santa Catarina** • Mun. Porto União, São Miguel, 12 Jul 1962, Reitz et al. 13110 (HBR) • Mun. Rio do Sul, Perto da Cidade, 8 Jul 1964, Reitz et al. 17062 (HBR). **São Paulo** • Without locality, s.d., Helmreichen 58 (F, HUH, NY, WAG) • Mun. Campinas, Prope Campinas, s.d., Mello s.n. (P06695482) • Mun. Itaporanga, Rio Verde, 17 Jun 1990, Hatschbach et al. 54328 (MBM, US) • Mun. Mombuca, Mata do Pinheirinho, 26 Jun 2021, Medeiros and Toledo 4493 (ESA, RB, SPF) • Mun. São Carlos, São Carlos do Pinhal, Jul 1888, Loefgren 705 (SP) • Mun. São Paulo, Nativa do Jardim Botânico, 6 Jan 1940, O. Handro s.n. (SP, US01319028).

Discussion

Phylogenetic relationships

The phylogeny presented here meets the main objective of this study, which was to enhance the understanding of the phylogenetic relationships among *Thinouia* species. Furthermore, it was possible to confirm previous phylogenetic studies that supported the monophyly of *Thinouia*, with *Thinouia* being the sister group to the rest of Paullinieae (Acevedo-Rodríguez et al. 2017; Chery et al. 2019; Medeiros et al. 2020; Cunha Neto et al. 2023). This study provides a baseline for understanding the genus *Thinouia* and the evolution of the tribe Paullinieae. We then discuss the main clades recovered by the phylogenetic analyses and compare them with the infrageneric classification proposed by Radlkofer (1878).

The results of this study support *Thinouia* as monophyletic and indicate *T. cazumbensis* as the sister to all other species of the genus, which splits into two clades (Clade II and III). However, the divergence into the two clades is poorly supported. Clade II is widely distributed in southern Mexico, Central America, and northern South America. In all three of its species (*T. myriantha*, *T. silveirae* and *T. tomocarpa*) the petal appendages are longer than the petals, a character used by Radlkofer (1878) to define section *Lepidodine*. However, the shape of petal appendages of these species is different. *Thinouia myriantha* has petal appendages adnate to the basal portion, while in *T. tomocarpa* and *T. silveirae* the petal appendage is adnate to the marginal portion.

Clade III includes *Thinouia compressa*, *T. mucronata*, *T. obliqua*, *T. paraguayensis*, *T. restingae*, *T. scandens*, *T. ternata*, *T. trifoliolata*, and *T. ventricosa* (PP = 1.0). This clade included species that are almost exclusively distributed in South America with the exception of *T. trifoliolata*, which occurs in lowland rainforests of the Amazon region and Central America. All species in this clade have petal appendages that are smaller than or equal in size to the petals. This character was used by Radlkofer (1878), to define section *Petalodine*, but as this character is also found in *T. cazumbensis*, it cannot be used as a morphological descriptor for sect. *Petalodine* since it seems to be a symplesiomorphic character.

Within tribe Paullinieae, small petal appendages are found in all other genera, but *Thinouia*, which is sister to the rest of the tribe, is the only genus whose petal appendage character was used in an infrageneric classification. Despite the great morphological variation found in petal appendages in Sapindaceae, especially in the tribe Paullinieae (Radlkofer 1895), few studies compare or elucidate the evolutionary changes of petals and petal appendages in Sapindaceae (Leinfellner 1958; Endress and Matthews 2005).

In conclusion, considering that only one of the *Thinouia* sections defined by Radlkofer (1878) is monophyletic, and that there is not enough data at the moment to propose an infrageneric classification, in *Thinouia* we propose to refrain from using one.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: RCF, PAR, HM. Data curation: HM. Formal analysis: HM, JCL. Funding acquisition: RCF. Investigation: RCF, HM, PAR. Methodology: HM, JCL. Project administration: RCF. Software: JCL. Supervision: PAR, RCF. Writing – original draft: HM. Writing – review and editing: JCL, RCF, PAR.

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Data availability

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

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Appendix 1

Index to numbered collection studied

The species epithet comes inside parentheses. The collection numbers for vouchers without collector´s numbers are provided within square brackets with acronym herbarium.

Abbott, J.R. 16326 (paraguayensis).
Abruzzi, M.L. 655 (mucronata).
Acevedo-Rodríguez, P. et al. 1436, 3697 (scandens); 4539, 4578 (mucronata); 7577 (obliqua); 8788, 12359, 14262, 14596, 15037, 17080 (myriantha), 16750 (paraguayensis).
Agra, M.F. 6985 (compressa).
Álvarez, D. 8113 (tomocarpa).
Alvaro, M.P. and Martínez, E. 268 (tomocarpa).
Alves, R.J.V. 4981 (compressa).
Amorim, A.M. 3580 (ternata).
André, Ed. 1841 (myriantha).

Alves, L.M.T. 7 (mucronata).
Anderson, R. 9187 (compressa).
Anderson, W.R. 36988 (compressa).
Araújo, A. 423 (compressa).
Araújo, D. 10025 (restingae).
Armond, N. [R19595] (compressa).
Aulestia, M. 3367 (obliqua).
Baker, M.A. 5993 (obliqua).
Balansa, B. 2486 (paraguayensis); 2488 (mucronata); 36945 (compressa).
Barbosa, E. 1448 (mucronata); 1544 (paraguayensis).
Barreto, K.D. 2010 (mucronata).
Barreto, M. 7558 (compressa).
Bastos, F.M. and Neves, L.F.F. F5 (mucronata).
Baylão, Jr., H.F. 367 (scandens).
Beck, St. G. 13309 (myriantha).
Belém, R.P. 3597 (ternata).
Belshaw, C.M. 3176 (obliqua).
Bianek, A.E. 172, 229 (mucronata).
Bonaldi, R. 506, [MBM375789], [MBM375790] (mucronata);
Borges, R.A.X. 224 (ternata).
Bovini, M.G. 170, 1925 (restingae); 3824 (compressa).
Bowie, J. and Cunningham 254 (scandens).
Brack, P. et al. 1197 (mucronata); 1758 (ventricosa);
Brade, A.C. 7923 (scandens).
Braga, H.N. 701 (restingae).
Braun, B.K. PSACF_EX05036 (myriantha);
Breedlove, D.E. 50858 (tomocarpa).
Bueno, R. [ICN2805], [ICN2808] (mucronata).
Burnham, R.J. 1569 (obliqua); 1671 (myriantha).
Bunting, G.S. 8744 (myriantha).
Cabrera, A.L. 22085, 31483 (mucronata).
Cabrera, E. 1672, 4530 (tomocarpa).
Calió M.F.A. 70 (compressa).
Campos, M.D. 14 (scandens).
Campos, V.A. 5540 (tomocarpa).
Campos Novaes, J. 3202 (mucronata).
Cardoso, D. 1245 (ternata).
Carneiro, J.S. 118 (mucronata).
Carvalho, A.M. 2489 (scandens).
Carvalho, W.B. 265 (scandens).
Catharino, E.L.M. 288 (mucronata).
Caxambu, M.G. 176, 5920 (ventricosa); 304, 306, 2524, 5215, 6486, 6559 (mucronata); Cerón, C.E. 3657, 5453 (obliqua).
Ceteno, P. and Janovec, J.P. 155 (myriantha).
Chagas, F. and Silva 1501 (mucronata).
Churchill, H.W. and Nevers, G. 4441 (myriantha).
Clarke, D. 3964 (myriantha).
Clark, J.L. 6466 (obliqua).
Claudio, 454 (paraguayensis).

Claussen, M. 521 (mucronata).
Colín, S.S. 2369 (tomocarpa).
Colleta, G.D. and Flores, T.B. 520 (scandens).
Colman, L.P. 21 (scandens).
Cordeiro, I. 176 (ventricosa); 834 (mucronata).
Correa de Mello 7 (ventricosa).
Costa, D.S. 359, 756 (myriantha); 373, 468 (trifoliolata); 486 (obliqua).
Coulleri, J.P. 384 (mucronata).
Couto, D.R. 1131 (scandens).
Cruz, R. 44 (tomocarpa).
Daly, D.C. 6570, 9727 (obliqua); 10444 (myriantha).
Daneu, L. 746 (ternata).
Daniel [RBR 32855] (mucronata).
Damasceno Júnior, A.G.1518, 2186 (paraguayensis).
De La Rue, E.A. [P0669512] (myriantha).
Duarte, A.P. 4966 (scandens).
Ducke, A. [INPA12163] (compressa).
Dugand, A. 872 (myriantha).
Durigon, J. 456, 930 (mucronata); 607 (compressa); 977 (trifoliolata).
Ekman, L. 1514 (mucronata).
Elias, Bro. 1266, 1412 (myriantha).
Encarnación, F. 1298 (trifoliolata).
Farney, C. et al. 2173 (restingae).
Feddema, C. 1983 (trifoliolata).
Fernandes, D.S. 195, 361, 855 (restingae).
Ferrucci, M.S.178, 2535, 3128 (paraguayensis); 2726 (mucronata).
Ferrucci, S. 161, 179 (mucronata).
Florschütz, P.A. and Maas, P.J. 255 (myriantha).
Fiaschi, P. 755 (scandens); 1395 (mucronata).
Fiebrig, K. 506, 911 (paraguayensis); 5859, 5978 (mucronata).
Figueira, M. 927 (mucronata);
Folli, D.A. 1021, 1076, 3910, 4162, 4740, 7529 (scandens).
Forzza, R.C. et al. 5089, 5147, 7815 (restingae).
Foster, R. 2204, 5905 (myriantha).
Fraga, C.N. 2083 (scandens).
Francisco, E.M. [MBM338060], [MBM338061] (mucronata); [MBM338062] (ventricosa).
França, F. 3507 (mucronata); 4971 (compressa).
Frazão, A. 417, 418 (myriantha).
Fróes, R.L. 23958, 24586 (myrianhta).
Fuentes, A.C. 1484 (paraguayensis).
Gentry, A.H. 58605 (obliqua); 78338, 78457, 79370 (myriantha); 79454 (trifoliolata).
Giordano, L.C. 1032 (scandens).
Glaziou, A. 857, 3897, 3898, 3899, 3902, 5773, 6864, 8599, 15449, 17499, 18170 (scandens).
Gonçalves, A.C. PSACF_EX04702, PSACF_EX04844 (myriantha).
Gonçalves, M.I.T.M. [RB00594985] (scandens).
Granados, J. and Chí, F. 641 (tomocarpa).

Grande, F. [MBM342283] (ventricosa).
Grayum, M. 4720 (trifoliolata).
Grijalva, A. 450 (myriantha).
Grings, M. 315, 346, 1272 (mucronata).
Gutiérrez J. 1200 (mucronata).
Hagelund, K. 2418, 3678, 3707, 4947, 5243, 8273 (mucronata); 5408 (ventricosa).
Hahn, W. 1072, 1506 (mucronata).
Harley, R.M. 21701, 21997 (compressa).
Hassler, E. 1846, 3745, 8297, 11514 (paraguayensis); 4460 (mucronata).
Hatschbach, G. 15753, 19360, 21007, 21527, 46203, 50380, 60563, 76943 (mucronata); 19194, 42750, 54328 (ventricosa); 49244 (paraguayensis); 55100 (ternata); 65076, 67777, 74746 (compressa).
Haught, O. 3867, 4746 (myriantha).
Heringer, E.P. 725 (compressa).
Hoehne, F.C. 3969, 3971 (paraguayensis); [SP46367] (scandens).
Hoffman, B. 5308, 6091 (myriantha).
Holton, I.F. [NY04219069] (myriantha).
Huamantupa, I. and Carrión, L. 9075 (myriantha).
Hurtado, F. 2885, 3013 (trifoliolata).
Irwin, H.S. 23129 (scandens); 23219 (ternata).
Jacques, E.L. 91 (scandens).
Jansen-Jacobs, M.J. 4314 (myriantha).
Jaramillo, N. 1143 (ventricosa).
Jarenkow, J.A. 2343 (mucronata).
Kaprovickas, A. 39403, 44016 (mucronata).
Karhs and Schenkel [ICN2810] (mucronata).
Karsten, H. [NY04219067] (myriantha).
Kegler, A. 153 (mucronata).
Keller, H.A. 144, 622, 6919 (mucronata).
Kernan, C. 867 (myriantha).
Kessler, M. 3983 (compressa).
Killeen, T. 3825 (obliqua).
Killip, E.P. and Smith, A.C. 22969, 26139 (obliqua).
Kinnup, V.F. 217, [FUEL27790] (mucronata).
Kirizawa, M. 756 (mucronata).
Klein, O.M. [MBM 075952] (mucronata).
Klein, R.M. 8690 (mucronata).
Klug, G. 4058, 4102, 4293 (obliqua); 4176 (trifoliolata).
Kollmann L. 11067 (compressa).
Kozeira, C. and Ribeiro, A. 3716, 3718 (mucronata).
Kozera, C. and Cardozo, A.L. 3770 (mucronata).
Knapp, S. et al. 7405 (obliqua).
Kuhlmann [RB 78342] (mucronata).
Kuhlmann, M. 610 (mucronata).
Kuntze, O. [NY04219055] (mucronata); [NY04219094] (paraguayensis).
Laclette, P. 166, 751 (scandens).
Leoni, L.S. 63, 2134, 3950, 7575 (scandens).
Lima, H.C. 3501 (scandens).
Lindman, C.A.M. 1063 (mucronata).

Lindman, J.C. and Haas, J.H. 4254, 5325 (mucronata).
Linneo, I. and Carreño 1405a (obliqua).
Lise, A. 5860 (mucronata).
Löfgren, A. 625 (compressa), 705 (ventricosa).
Lopes, M.M.M. 1338 (compressa).
Lovato, M.C. [RBR 36323] (mucronata).
Lozano, R. 2056, 2128 (mucronata).
Maceda, A. 1097 (myriantha).
Macía, M.J. 4542 (myriantha).
MacMaster, G. 12 (tomocarpa).
Maguanini A. [RB87950] (scandens).
Maihack, R. [RB613850] (mucronata).
Malme, G.O. 2748 (paraguayensis).
Manriquez, G.I. 495, 3365 (tomocarpa).
Manriquez, G.I. and Sinaca, S.C. 2369 (tomocarpa).
Mansano, V.F. 480 (restingae).
Manzine, F.F.415 (mucronata).
Marques, J.P. [HCF5351] (mucronata).
Marquete, R. 351, 3501 (scandens); 1692 (mucronata); 1826 (restingae).
Martínez, E.S. 18295, 11916 (tomocarpa).
Massing, A.A. 125, 224 (mucronata); 323 (ventricosa).
Mathias, M.E. and Taylor, D. 3479, 3980, 3991, 5395 (obliqua).
Mauad, L.P. 119 (restingae).
Medeiros, H. 2189, 2191, 2193, 4496, 4498 (silveirae); 3328, 3332, 3792, 3810, 3832, 4884 (obliqua); 3330, 3788, 4237, 4300, 4898 (myriantha); 3331, 4380 (trifoliolata); 3401 (cazumbensis); 3800 (compressa); 4450, 4451, 4452, 4472, 4473, 4481, 4486, 4488 (scandens); 4475, 4453, 4491 (restingae), 4489 (ternata); 4493 (ventricosa).
Mello, de C. [P06695482] (ventricosa).
Mello-Silva, R. 1292 (compressa).
Melo, P.H.A. 625 (ternata).
Menescal, A.L. 103 (mucronata).
Mereles, F. 3842 (paraguayensis).
Mexia, Y. 4996 (compressa); 6675 (obliqua).
Mikan 5566 (scandens).
Miranda, E.B. 706 (compressa).
Molina, J.A. 19Bo022, 19At075 (myriantha).
Montes, J.E. 100B, 1871, 2430, 2551 (mucronata).
Moore, S. 1076 (paraguayensis).
Mori, S.A. 10061 (mucronata); 14419, 14426 (compressa).
Morong, T. 625a (paraguayensis).
Mosén, H. 3953 (mucronata).
Mostacedo, B. 3637 (paraguayensis).
Nascimento, E.A.P. 33 (myriantha).
Nee, M. et al. 37644, 44866, 48561 (paraguayensis); 45374, 48052, 53120, 55032 (mucronata).
Nee, M. and Bohs, L. 49447 (mucronata).
Obermuller, F.A. 1920 (trifoliolata).
Ochioni, P. [RB2882] (trifoliolata).

Oliveira, J.E. 387 (compressa).
Ortíz, M. 711 (paraguayensis).
Paixão, J.L. 211 (restingae); 909 (scandens).
Palacios, W. 11034 (myriantha).
Pastore, J.A. 514 (mucronata).
Paula-Souza, J. and Souza V.C. 5694 (scandens).
Pauleiro and Vitorio [RB148994] (scandens).
Pavão, O.C. [RB422905] (mucronata);
Paz, G. and Miño 81023, 81043 (trifoliolata).
Pedersen, T.M. 11042 (mucronata).
Peixoto, A. 4124 (scandens).
Peredo, I. 22 [NY04219096] (paraguayensis).
Pérez B. 68 (paraguayensis).
Philipson, W.R. 2197 (myriantha).
Pirani, J.R. 482, 488 (mucronata); 3758 (compressa).
Pittier, H. 8758 (myriantha).
Pohl 3763, 5566 (scandens).
Poliquesi, C.B. 347 (ventricosa).
Prance, G.T. 26298 (paraguayensis).
Proença, M. 82, 89, 115, 123 (mucronata).
Pujals, A. [MBM420905], [MBM420916] (scandens).
Queiroz, L.P. 2472 (scandens); 9439 (ternata); 12715 (compressa).
Rabelo, B.V. 3541 (trifoliolata).
Rapini, A. 628 (compressa).
Reitz 13110, 17062 (ventricosa);
Revilla, J. 127 (trifoliolata).
Riedel, L. 513 (compressa); [US01318766] (scandens).
Rodriguea C.D.N. 133 (mucronata).
Rodrigues, I.A. 1632 (myriantha).
Rodríguez 715 (mucronata).
Rojas, R. 1147 (5).
Romagnolo, M.B. 3313 (trifoliolata).
Rombous, H.E. 669 (myriantha).
Romoleroux, K. and Baus, E. 3200 (obliqua).
Rosado 59, 222, 223, 224, 947, 969 (ventricosa); 371 (mucronata).
Rose, J.N. 19992 (compressa); 20073 (mucronata).
Rusby, H.H. 550 (compressa).
San´t Ana, S.C. 291 (scandens).
Saint-Hilaire [MPU010891] (scandens).
Saldanha, J. 5513 (scandens).
Salino, A. 3951 (scandens).
Salmazi, L.B. [RBR 32856] (mucronata).
Santos, M.C.F. 150 (scandens).
Saraiva, C. and Johnson, D. 42 (myriantha).
Saucedo, M. 836 (paraguayensis).
Schinni, A. 4904 (ventricosa); 22301, 27933 (mucronata).
Schipp, W.A. 1336 (tomocarpa).
Schott, H.W. 4421 (scandens).
Schunke, J.M. 183 (myriantha).

Schunke-Vigo, J. 4104, 12520, 14868 (obliqua); 6533 (myriantha).
Schwacke 7085 (scandens).
Schwarz, G.J. 7399 (mucronata).
Schwindt, E. 3087 (mucronata).
Sekine, E.S. 97 (mucronata).
Sellow, F. [NY 04219107] (scandens).
Silva, M.F.B. 122 (scandens).
Silva, M.F.F. 169 (myriantha).
Silva, N.T. 1022 (cazumbensis).
Silveira, A.L.P. 500 (myriantha).
Siqueira, E.L. 204, 1385, 2162 (mucronata).
Smith, H.H. 882 (myriantha).
Soares-Silva, L.H. 26 (mucronata).
Solomon, J.C. 9966 (mucronata).
Somner, G.V. 544 (ternata); 585, 1541, 1851, 1339 (scandens); 616, 752, 785, 788, 801, 1074, 1185, 1354, 1495, 1496 (restingae).
Sonia, N. 2083 (paraguayensis).
Sork, H.E. et al. 10496 (obliqua).
Soto, J.C. 22768, 22770 (tomocarpa).
Souza, de V. 439 (ternata).
Souza, V.C. 10426 (mucronata); 32487 (restingae).
Stehmann, J.R. [MBM227949], 2959 (compressa).
Stevenson, P. 2144 (obliqua).
Steyermark, J.A. 38774, 93135 (myriantha).
Stival-Santos, A. 588 (mucronata).
Stutz, L.C. 2331 (mucronata).
Urdampilleta, J.D. 118, 148 (mucronata).
Tamashiro, J.Y. 1015 (mucronata).
Téllez, O. and Cabrera, E. 2134 (11).
Tessmann, G. 3314 (myriantha); 3500 (obliqua); 4444, 4462 (trifoliolata).
Torke, B.M. 2024 (myriantha).
Tressens, S.G. 4409 (mucronata); 6164 (myriantha).
Triana, J. [MPU010893, MPU010892], [P04857748], [P06695510], [BM000838111] (myriantha).
Trigos, R.C. and Sinaca, S.C. 2682 (tomocarpa).
Uittien, H. 6988 (myriantha).
Valenzuela, L. and Humantupa, I. 1063 (trifoliolata).
van Andel, T.T 4485 (myriantha).
Vanni, R. 3621, 3939, 4179, 4518 (mucronata).
Vásquez, R. 14290 (myriantha).
Vásquez, R. and Jaramillo, N. 7058, 8577 (trifoliolata).
Victorio [RB003386344] (scandens).
Vieira, C.M. and Gurken, L.C. 429 (scandens).
von Martius, C.F.P. 1303 (ventricosa).
Zárate, R. 14005 (myriantha).
Zardini, E.M. 5433, 5651, 9207 (paraguayensis); 13513, 42893 (mucronata).
Zardini, E. and Aquino, P. 29700 (compressa); 33097 (paraguayensis).
Zardini, E.M. and Benítez, C. 3333 (compressa).
Zardini, E.M. and Chaparro, I. 48588, 48847 (mucronata).

Zardini, E.M. and Garcete, F. 43321 (compressa).
Zardini, E.M. and Guerrero, L. 48472 (compressa); 59086 (mucronata).
Zardini, E.M. and Soria, N. 4448 (paraguayensis).
Zardini E. and Tilleria 29772 (mucronata).
Zardini, E.M. and Velásquez, C. 12165, 13669 (compressa); 12324 (mucronata); 13053, 18791, 22320 (paraguayensis);
Zardini, E.M. and Vera, M. 45362 (compressa).
Zeiden, D.N.M. 34 (mucronata).
Warming [P06695484] (ternata).
Woolston, A.L. 664, 840, 850 (compressa); 784 (mucronata); 1248 (paraguayensis).
Woytkowski, F. 5065, 7153 (obliqua).
Wurdack, J.J. and Monachino, J.V. 38715 (myriantha).

Appendix 2

Voucher and GenBank information for the taxa included in the phylogenetic analyses

Listed as: taxon, collection, herbarium, place of origin and GenBank accession numbers (ITS, trnL intron). Herbarium acronyms follow Index Herbariorum (Thiers 2022).

Lophostigma plumosum Radlk., Acevedo-Rodríguez 6554 (US), Bolivia, KX584929, KX585020. *Paullinia cuneata* Radlk., Acevedo-Rodríguez 14255 (US), Peru, KX584932, KX585023. *Paullinia elegans* Cambess., Acevedo-Rodríguez 14976 (US), Brazil, KX584933, KX585024. *Serjania caracasana* (Jacq.) Willd., Acevedo-Rodríguez 15107 (US), Mexico, KX584947, KX585038. *Serjania communis* Cambess., Somner 1334 (US), Brazil, KX584950, KX585041. *Thinouia cazumbensis*, Medeiros 3401 (RB) Brazil, MT853074, MT847016. *Thinouia compressa* Radlk., Medeiros 3800 (RB) Brazil, MT853076, MT847018. *Thinouia mucronata* Radlk., Keller 6919 (US), Argentina, KX584971, KX585058. *Thinouia mucronata* Radlk., Nee 48052 (US), Bolivia. *Thinouia myriantha* Radlk., Torke 2024 (HSTM), Brazil, MT853071, MT847013. *Thinouia obliqua* Radlk., Medeiros 3793 (RB) Brazil, MT853075, MT847017. *Thinouia paraguayensis* (Britton) Radlk., Barbosa 1544 (HUEFS), Brazil. *Thinouia paraguayensis* Nee 48561 (US), Bolivia. *Thinouia restingae* Ferrucci & Somner, Somner 1074 (RBR), Brazil, KX584972, KX585060. *Thinouia restingae* Ferrucci & Somner, Medeiros 4453 (RB), Brazil. *Thinouia scandens* (Cambess.) Triana & Planch., Medeiros 4472 (RB), Brazil. *Thinouia scandens* (Cambess.) Triana & Planch., Medeiros 4488 (RB), Brazil. *Thinouia scandens* (Cambess.) Triana & Planch., Somner s.n. (RBR), Brazil. *Thinouia silveirae*, Medeiros 2193 (RB), Brazil, MT853072, MT847014. Brazil. *Thinouia ternata* Radlk., Cardoso 1245 (RB), Brazil. *Thinouia tomocarpa* Standley, Acevedo-Rodríguez 12238 (US), Mexico. *Thinouia tomocarpa* Standley, Soto 22768 (MEXU), Mexico. *Thinouia tomocarpa* Standley, MacMaster 12 (MEXU), Belize. *Thinouia trifoliolata* Acev.-Rodr. & Ferrucci, Rabelo 3541 (MO), Brazil. *Thinouia trifoliolata* Acev.-Rodr. & Ferrucci, Revilla 127 (MO), Peru. *Thinouia ventricosa* Radlk., Ferrucci 1990. *Thinouia ventricosa* Radlk., Grande s.n. (MBM 342283). *Thouinia acuminata* S. Watson, Liston 633-2, ––, EU720478, EU721249.

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Research Article

Orobanche andryalae (Orobanchaceae): a new species from the Canary Islands

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Abstract

A new species of *Orobanche* is described from the Canary Islands. The plant belongs to the complicated Subsection *Minores* and has a distinct combination of morphological features, ecology, and host specificity. The markedly cernuous corolla, high filament insertion and colouration distinguishes *Orobanche andryalae* from closely related species with which it has been previously confused, and which do not occur in the Canary Islands, including *O. amethystea* subsp. *castellana* and *O. calendulae*; these features remain stable in cultivation. *Orobanche andryalae* appears to be parasitic almost exclusively on *Andryala* spp., and occurs on thermophilus volcanic substrates in northern Lanzarote, northwest Tenerife and Jandía in Fuerteventura. An updated key to the *Orobanche* of the Canary Islands is presented, including the new taxon we describe here.

Key words: *Andryala*, broomrape, endemism, Macaronesia, parasitic plant

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Introduction

Broomrapes (genus *Orobanche* L., family Orobanchaceae Vent.) are taxonomically challenging. They are holoparasitic so devoid of functional leaves and other diagnostic characteristics, and many of the characters useful for identification such as stigma and corolla colour, are lost when dry. Herbarium specimens are often determined incorrectly and have inadequate field notes. Particularly challenging are closely related species in the Subsection *Minores* Teryokhin: a group of similar, small-flowered species (corollas typically < 20 mm) (see e.g. El Mokni et al. 2015). Beck-Mannagetta (1930) in his comprehensive monograph of the genus, listed over 345 binomials belonging to this group (to which he referred as the Grex *Minores*) and recognised 20 species, 20 varieties, and a total of 69 forms. Existing phylogenies have not successfully resolved taxa in the Subsection *Minores*. Moreover, genetically distinct host races can be obscured by cryptic morphology (Thorogood et al. 2009). In the absence of a comprehensively sampled and well-resolved phylogeny, a combined approach that considers ecology, host range, and stable morphological features (such as filament insertion and corolla dorsal line) is needed to tease apart taxa in this complicated group.

There are at least seven species of *Orobanche*, and a further seven species of the related genus *Phelipanche* Pomel, recorded to occur in the Canary Islands (BIOTA 2024), but a detailed taxonomic assessment for the area is absent and necessary. Within the Subsection *Minores*, five species have been recorded to occur in the region: *O. minor* Sm., *O. calendulae* Pomel, *O. artemisiae-campestris* Vaucher ex Gaudin (as cf. *O. loricata* – doubtfully, probably in error for the species under consideration) and *O. amethystea* Thuill., together with *O hederae* Duby (also considered, based on molecular evidence, to belong to the similar Subsection *Hederae* Teryokhin). The most widespread of these is *O. minor* which occurs across Europe and the Mediterranean Basin, and is widely naturalised in temperate regions across the globe. This species tends to occur in urban and ruderal sites on all the Canary Islands and may be introduced to the region. *Orobanche minor* is lax, small-flowered (corolla 10–18 mm), and purplish in colour; it grows on a wide variety of hosts from 16 orders (Thorogood et al. 2009). *Orobanche hederae* occurs in damp woods on *Hedera* – but once also reported to grow on *Geranium reuteri* Aedo & Muñoz Garm. (Gilli 1980) - on the western islands (Tenerife, La Gomera, La Palma) and is easily distinguished by its yellow stigma (in other species the stigma is variable but typically pinkish, reddish, purplish), corollas with a distinct constriction behind the corolla mouth, and acute and lacerate corolla lower lip lobes. *Orobanche amethystea* is widespread across Europe and is usually parasitic on *Eryngium campestre* L., but has been recorded on other hosts including various other Apiaceae Lindl., and even *Digitalis* Tourn. ex L. (Plantaginaceae) (Chater and Webb 1972). It is superficially similar to *O. minor* but more robust, with a larger corolla (15–25 mm) that is rather sharply inflected near the base, rather flat along the dorsal line, and geniculate near the tip (rather than with a smoothly-curved dorsal line), with a somewhat 2-lobed upper lip, and high filament insertion. *Orobanche amethystea* has long been recorded to occur on Lanzarote and Tenerife (Reyes-Betancort et al. 2000; Padrón-Mederos et al. 2009); the subspecies reported there is subsp. *castellana* (Reut.) Rouy which differs from the type subspecies in lacking filiform calyx teeth, pinkish-brown (rather than violet) flowers, and the upper corolla lip being less conspicuously divided. However, these plants identified as *O. amethystea* in the Canary Islands lack nearly all the features associated with that species. They also differ markedly from *O. minor* with which it co-occurs, but in a different niche (ruderal sites). The plant in question has only been recorded to parasitize *Andryala perezii* M.Z.Ferreira, R.Jardim, Alv.Fern. & M.Seq. (Ferreira et al. 2014), endemic to Lanzarote and Fuerteventura, and to a lesser extent, on *Andryala pinnatifida* Aiton on Tenerife (a species that occurs on all the Canary Islands) - an unusual but not unquestionable host range for *O. amethystea*; the genus *Andryala* is also the host range of *O. almeriensis* A.Pujadas (González and Pujadas Salvà 1995), which also belongs to the Subsection *Minores*; however this too, is morphologically dissimilar to the material in question (Table 1). The latter author (JARB) has also observed the taxonomic entity in question apparently growing on *Asteriscus sericeus* (L.f.) DC. once, on Pico de la Zarza, the highest peak of Fuerteventura. This population needs revisiting.

Table 1. *Orobanche andryalae* and closely related taxa.

	O. andryalae	***O. calendulae***	***O. almeriensis***	***O. amethystea***	***O. minor***
Host	*Andryala perezii*	*Calendula suffruticosa*	*Andryala ragusina*	*Eryngium campestre*; various Apiaceae; (rarely other hosts)	Numerous hosts, especially Fabaceae, Asteraceae, Apiaceae
Stem	Slender, pale reddish-orange; flowers ± lax, over the upper part of the stem	Slender to robust, brownish red or violet; flowers ± lax, over most of stem	Slender, purple; flowers ± lax, over the upper part of the stem	Slender to robust, purple or pinkish-brown; flowers ± dense, over the upper part of the stem	Slender, purplish to reddish; flowers lax, over most of stem
Floral bracts	10–12 mm, markedly shorter than the corolla	10–15 mm, slightly shorter than the corolla	13–16 mm, ± Equalling the corolla	14–17 mm, markedly exceeding the corolla	6–22 mm ± equalling to exceeding the corolla
Calyx	5–7 mm, segments fused, unequal (rarely entire)	10–15 mm, segments free	9–12 mm, segments fused, unequal	10–13 mm, segments free, unequal	6–14 mm, entire or segments fused, unequal to subequal
Corolla	10–15 mm, pale yellow with faint reddish veins	15–18 mm, cream with pink to violet veins	17–20 mm, cream with purple veins	16–25 mm, cream with purple veins	10–18(20) mm, cream with purple veins
Corolla dorsal line	Strongly cernuous to geniculate	Smoothly-curved	± erect, straight	± erect, straight	Smoothly-curved
Corolla indumentum	Sparsely glandular hairy throughout or basally glabrescent	Glandular hairy, basally glabrescent	Densely glandular hairy, basally glabrescent	Glandular hairy, basally glabrescent or glabrous	Glandular hairy, basally glabrescent
Filaments	Sparsely hairy below, glabrous above; inserted 5 mm above the corolla base	Hairy below, glabrescent above; inserted 2–4 mm above the corolla base	Hairy below, glabrescent above; inserted 2–3 mm above the corolla base	Hairy below, glabrous above; inserted 3–4 mm above the corolla base	Glabrescent or sparsely hairy below; inserted 2–3 mm above the corolla base
Stigma (typically)	Red-orange	Reddish-purple	Violet	Pink to violet	Pink to violet

The closest morphological match to the plants thus far identified as *O. amethystea* subsp. *castellana* in the Canary Islands is in our view, *O. calendulae*: a rather confused taxon that tends to grow on coastal cliffs, primarily parasitizing *Calendula suffruticosa* Vahl aggregate, and related species (and possibly other Asteraceae). It is generally described as having a curved, pale-yellow corolla (although see *taxonomic remarks*, below). Indeed the plant under consideration in the Canary Islands has also been identified as *O. calendulae* (Marrero et al. 1995), notwithstanding the atypical host (*Andryala*). However the plant in Lanzarote has a markedly cernuous (more rarely geniculate) corolla not seen in any other known species in the Subsection *Minores*, as well as distinct colouration and a high filament insertion. On account of this unusual combination of morphological features, and marked distribution and ecology, we examined material from the Canary Islands to resolve this long-standing taxonomic confusion.

Materials and methods

Plant material

Plants were observed in the field in Lanzarote between 2020 and 2023, and then cultivated in 2023–2024 to ensure morphological characteristics were stable (i.e. not a product of environment). Seeds were collected by the second author (MHG) from a population near Haría (29°08'07.4"N, 13°30'18.2"W) and sent to the first author (CJT) who cultivated the plant at the University of Oxford Botanic Garden on 10 potted specimens of *Andryala perezii* grown under glass (ambient light, 15–20 °C). Seventeen spikes of *Orobanche andryalae* emerged in April, 4 months after planting, and each was measured and examined to inform the type description. Specimens were then compared with *O. minor* (growing spontaneously at the University of Oxford Botanic Garden) because this is the most widespread and common species in the Subsection *Minores* and co-occurs (at least on Lanzarote) with *O. andryalae*.

Results

Orobanche andryalae **C.J.Thorogood, M. Hernández González, Rumsey & Reyes-Bet., sp. nov.**

urn:lsid:ipni.org:names:77356942-1

Description. Stems 6–16(25) cm, glandular-hairy, pale orange to light reddish-brown. Stem scarcely swollen below; subterranean bracts broadly triangular, yellow; those above (reduced leaves) rather sparse, brown, 8–15 mm. Flowers 5–15(20), arranged on the upper quarter or third of the stem, lax. Bracts 10–12 mm, rather shorter than the corolla, broadly triangular, brown, glandular-hairy. Calyx 5–7 mm with segments fused, strongly unequal (rarely entire), not exceeding the corolla tube. Corolla 10–15 mm, pale yellow with faint reddish veins and scattered glandular hairs, strongly cernuous when mature, remaining so in fruit, sometimes abruptly geniculate; upper lip bilobed; lower lip 3-lobed, the lateral lobes slightly exceeding the central; all lobes minutely-toothed. Filaments sparsely hairy below, glabrous above; inserted conspicuously (c.5 mm) above the corolla base; anthers ± glabrous. Stigma lobes touching, mid to dark red-orange.

Type. LANZAROTE, Canary Islands, Haría (29°08'07.4"N, 13°30'18.2"W); material grown from seed at the University of Oxford Botanic Garden, Oxford, United Kingdom; April 22, 2024. (holotype ORT 48576!), (isotype OXF! Barcode 002277150).

Distribution, ecology and IUCN Red List status. We examined *O. andryalae* in two locations on the island of Lanzarote, with five specimens at a population near Yé (29°11'46.1"N, 13°29'34.1"W), and about 40 in the other near Haría (29°08'07.4"N, 13°30'18.2"W) in 2020, 2021 and 2022. The plant was also observed in the Valle de Guerra and Teno regions of north Tenerife. Because *O. andryalae* is an annual, and based on observations of related species, we anticipate that numbers may fluctuate markedly from year to year (Rumsey and Thorogood 2023). *Orobanche* seed banks can, however, remain viable for decades (Rumsey and Thorogood 2023). Based on our current observations the species is likely to qualify for a threat status because of its restricted distribution, few locations and very low observed numbers and given that it co-occurs with its narrowly endemic host, *Andryala perezii* on Lanzarote, and on *A. pinnatifida* on Tenerife (records on other endemic Asteraceae including *Asteriscus intermedius* (DC.) Pit. & Proust, *A. sericeus*, and *Crepis canariensis* (Sch.Bip.) Babc. ex Jenkins require further investigation). *Andryala perezii* – the predominant host, is locally common within its restricted range (Ferreira et al. 2014) and further parasite populations are to be expected on Lanzarote (the apparent stronghold for the parasite). *Andryala perezii* also occurs on Fuerteventura, where we have observed *O. andryalae* (but here it was recorded on *Asteriscus sericeus*; this too, requires further examination). *Andryala pinnatifida*, a recorded host in Tenerife, occurs across the western Canary Islands, again indicating *O. andryalae* may be under-recorded. We suggest that in the absence of long-term surveys, *O. andryalae* should, for now, be treated as DD (Data Deficient) (IUCN 2001).

Etymology. *Orobanche andryalae* is named in accordance with its main host species, *Andryala perezii*.

Taxonomic remarks. *Orobanche andryalae* can readily distinguished by its strongly cernuous corolla (Figs 1C, 2C, 3A, 4A), coloration, filament insertion (Figs 1K, 2D), and distinct host and ecology. Importantly, these characteristics remain stable under cultivation (Fig. 3A). The plant is distinct from *O. amethystea* subsp. *castellana*, which it is now clear does not occur in the Canary Islands, and we do not discuss further here. Rather, the closest taxon to *O. andryalae* appears to be *O. calendulae*, which is a somewhat confused taxon, originally documented from Algeria (Pomel 1874), and since recorded from Madeira, Morocco, Portugal and Spain (Chater and Webb 1972), and Tunisia (El Mokni et al. 2023). *Orobanche calendulae* has marked host specificity and ecology, growing on relatives of the *Calendula suffruticosa* aggregate on sea cliffs. Beck-Mannagetta (1930) also recognised a similar entity, *Orobanche mauretanica* Beck, mainly on the basis of distinct calyx characteristics: connate in *O. mauretanica* and entire, or bifid and free in *O. calendulae*. He described a variety of *O. mauretanica* that he named var. *calendulae*, from the Algarve region of Portugal, which is parasitic on *Calendula suffruticosa.* Greuter et al. (1989), however, synonymised *O. mauretanica* under *Orobanche calendulae*, a decision that has been followed rather inconsistently; since that time, floras have also differed somewhat in their descriptions of *Orobanche calendulae*. For example, both Flora Europaea (Chater and Webb 1972) and Flora Iberica (Foley 2001), describe equal calyx segments that are fused at the base; we note that the calyx segments in the holotype from Algeria are bifid but somewhat unequal; in the type description, they are reported to be entire or bifid (Pomel 1874).

The first author (CJT) has observed *Orobanche* populations on sea cliffs in the Algarve growing on *C. suffruticosa* (Fig. 3C, 4C) that may pertain to Beck's *O. mauretanica* var. *calendulae* (= *O. calendulae*). These plants possess pale orange stems, whitish corolla with violet pigmentation, reddish stigmas, apically filiform calyx lobes, and flowers arranged over most of the stem. These features appear to be consistent with the holotype of *O. calendulae* (Pomel 1874). But to complicate matters further, this plant (*O. calendulae* s.l.) co-occurs in the Algarve with *O. minor* (on various hosts), *O. litorea* Guss. (on *Plantago*), *O. balsensis* (J.A.Guim.) Carlón, M.Laínz, Moreno Mor. & Ó.Sánchez (on *Carlina*), and interestingly, *O. amethystea* (on *Eryngium campestre*) (Fig. 3B, 4B); Beck-Mannagetta also recognised a form of *O. mauritanica* on *Eryngium campestre* in the Algarve ('forma *dioristha*'). Presumably this form in fact pertains to *O. amethystea*, but in the absence of material to examine, this remains a mystery. Again, this demonstrates the nontrivial historical confusion surrounding the taxa in this area, and the importance of examining a range of characters, as well as ecology and host identity when describing *Orobanche* Subsection *Minores*.

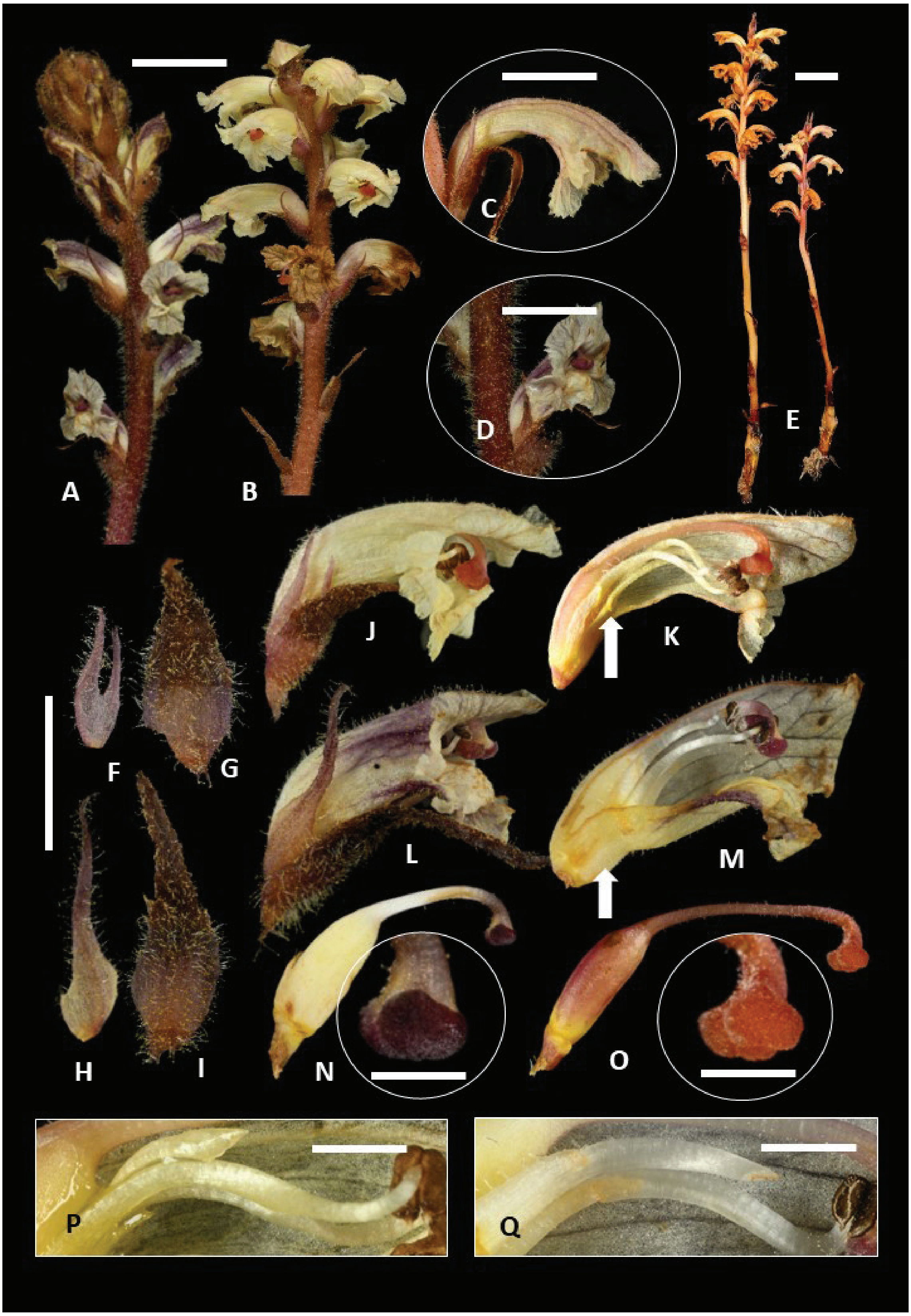

Figure 1. A *Orobanche minor* habit **B** *O. andryalae* habit **C** *O. andryalae* corolla in profile **D** *O. minor* corolla in profile **E** *O. andryalae* whole plant **F, G** calyx and bract of *O. andryalae* **H, I** calyx and bract of *O. minor* **J, K** *O. andryalae* corolla in profile with cross section; arrow indicates filament insertion point **L, M** *O. minor* corolla in profile with cross section; arrow indicates filament insertion point **N** carpel and stigma of *O. minor* **O** carpel and stigma of *O. andryalae* **P** stamen of *O. andryalae* **Q** Stamen of *O. minor.* Scale bars: 15 mm (**A, B**); 7.5 mm (**C, D**); 15 mm (**E**); 15 mm (**F, M**); 5 mm (**N–O**); 4 mm (**P, Q**).

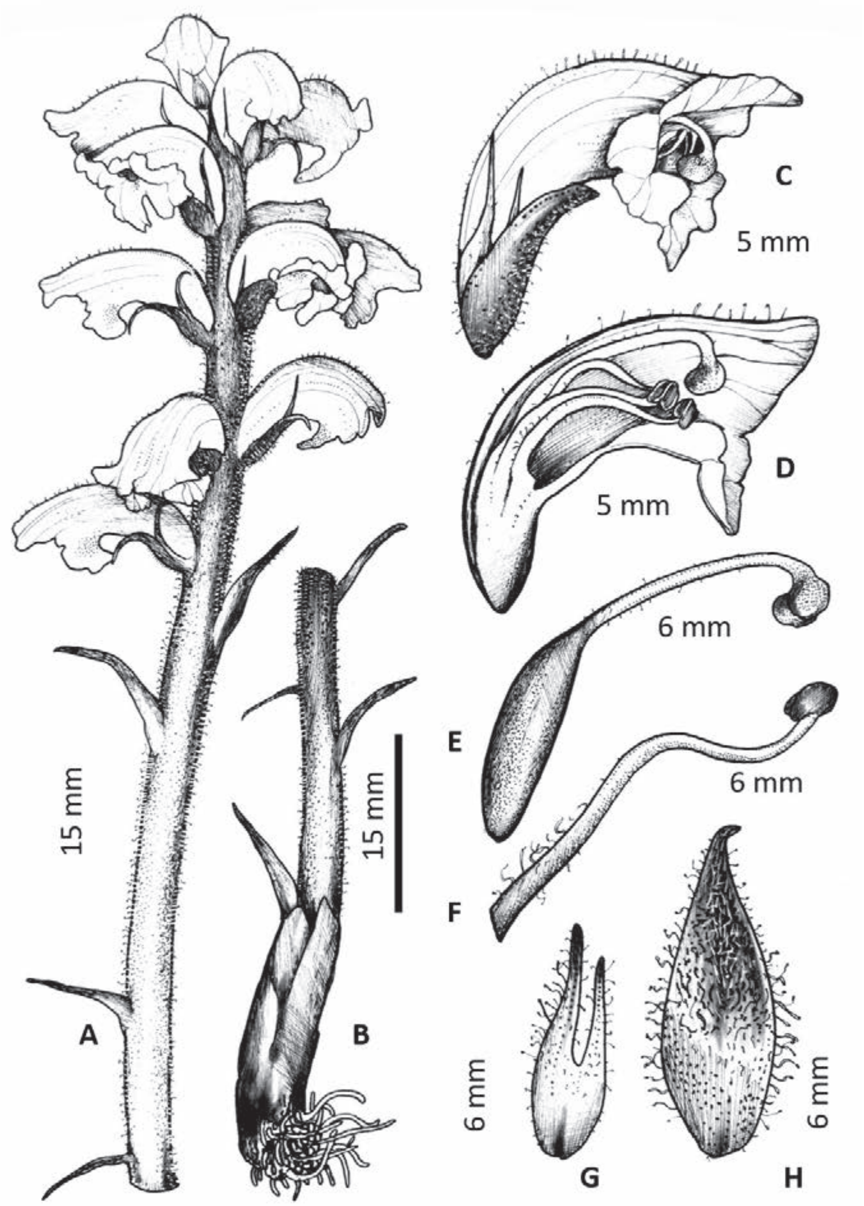

Figure 2. *Orobanche andryalae* **A** habit **B** lower stem **C** corolla **D** corolla cross section **E** carpel **F** stamen **G** calyx lobe **H** bract.

Furthermore, we should note that populations of an unexamined entity in the Subsection *Minores* in Madeira also grow on *Andryala*, in this case *A. glandulosa* Lam. which is endemic to Madeira, Porto Santo and the Desertas. These plants have puberulent, yellowish stems, rather flat-backed corollas, long, apically filiform calyx lobes, and pinkish stigma lobes. Despite their Macaronesian distribution and host, they are clearly distinct from the Canary Island plant under consideration. They do, however, show a superficial similarity to *O. litorea*, and deserve further attention.

Notwithstanding the confusion surrounding the Subsection *Minores*, none of the taxonomic entities considered hitherto in this complex possess the stable combination of features we observe in *Orobanche andryalae* in the Canary Islands: a markedly cernuous corolla, high filament insertion and yellowish-orange colouration with a reddish stigma, and specificity for

Figure 3. A *Orobanche andryalae* in cultivation at the University of Oxford Botanic Garden **B** *O. amethystea* in the Algarve, Portugal **C** *O. calendulae* in the Algarve, Portugal **D** the habitat of *O. andryalae* in Lanzarote: volcanic cliffs (Famara area, northern Lanzarote) **E** *O. andryalae* in Lanzarote, with its host plant *Andryala perezii* **F** *O. andryalae* collected from Fuerteventura (putatively parasitising *Asteriscus sericeus*) **G, H** *O. andryalae* in northwest Tenerife growing on *Andryala pinnatifida* (the corolla dorsal line of the specimen in H is atypical in being less conspicuously cernuous).

Andryala spp. on thermophilous volcanic substrates (Fig. 3D). In advance of a well-resolved phylogeny, it is important that taxonomic entities in the Subsection *Minores* are characterised morphologically and ecologically, to enable robust sampling and nomenclature.

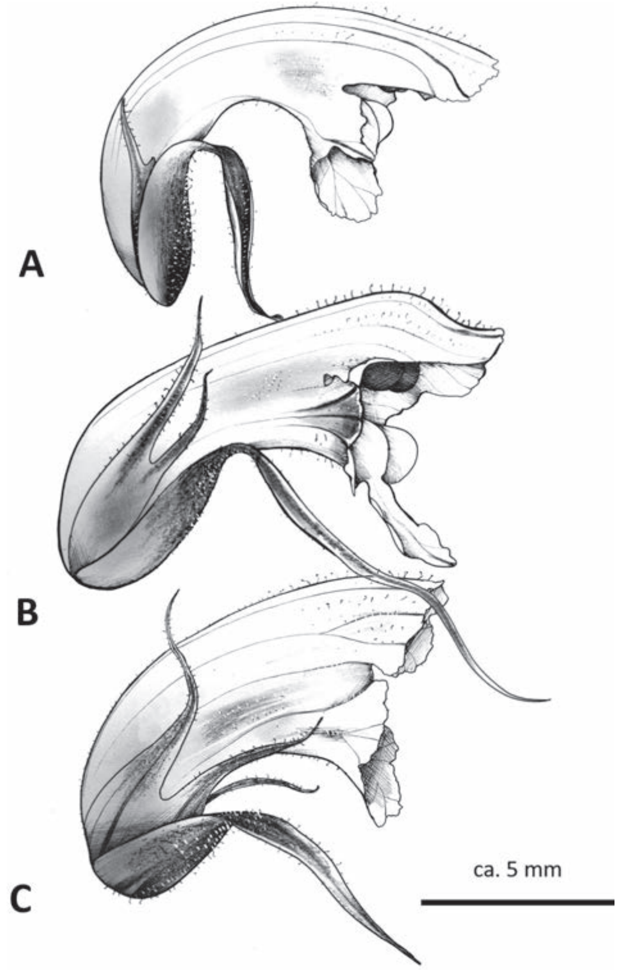

Figure 4. Corollas in profile **A** *O. andryalae* **B** *O. amethystea* **C** *O. calendulae*. Note the scale is indicative only, as corolla length is variable.

Key to the *Orobanche* species known to occur in the Canary Islands

Note that *O. amethystea* is not included as we believe all records of the plant in the Canary Islands pertain to *O. andryalae*.

1 Corolla whitish, distally blue-purple................................ ***Orobanche cernua***
– Corolla whitish to cream with reddish or violet veins (not distally blue-purple)..**2**
2 Flowers fragrant, corolla whitish, tubular-campanulate, lobes with crenate margins .... ***Orobanche crenata***
– Flowers not fragrant, corolla cream to pale yellow, narrowly-tubular, lobes not crenate..**3**
3 Stigma typically yellow, parasitic mainly on ivy in woods............................ .... ***Orobanche hederae***
– Stigma pink or red-orange, not in woods **4**
4 Corolla with an evenly-curved to almost straight dorsal line, filaments inserted 2–3 mm; various hosts, disturbed habitats .... ***Orobanche minor***
– Corolla markedly cernuous (to geniculate), filaments inserted c. 5 mm; on *Andryala* spp. on volcanic substrates .... ***Orobanche andryalae***

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

CT concieved the research and prepared the figures; all authors contibuted to the writing of the manuscript; MHG collected the plant material.

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Data availability

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

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Research Article

A new species of *Sedum* (Crassulaceae, Saxifragineae) from Guangxi, China

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Abstract

A new species, *Sedum guangxiense* **sp. nov.**, discovered in Guangxi Province, China, is described and illustrated. Molecular phylogenetic analyses and morphological differences indicate that this species is well separated from its close relatives in *Sedum*, justifying its recognition as a distinct new species. Morphologically, it closely resembles *Sedum tosaense* and *S. emarginatum* in its leaf shape, inflorescence type and life form, but it can be easily distinguished in its erect stems when young, much larger leaves, narrowly triangular petals, ovate nectar scales and oblong anthers.

Key words: Phylogeny, sect. *Sedum*, *Sedum guangxiense*, taxonomy

Introduction

The genus *Sedum* Linnaeus (1753: 430) (stonecrops) comprises ca. 470 species mainly distributed in the Northern Hemisphere, but some extend to the Southern Hemisphere in Africa and South America (Fu and Ohba 2001). It typically thrives in open, climatically (semi-)arid environments and comprises herbs to (sub-)shrubs with succulent leaves (Stephenson 1994). The molecular study by Messerschmid et al. (2020) showed that *Sedum* is highly polyphyletic and, in order to render *Sedum* monophyletic, about 14 genera derived from within *Sedum* would have to be merged with it. This broader concept of *Sedum* would encompass approximately 755 species, mainly distributed in the Mediterranean, Central America, the Himalayas and East Asia (Stephenson 1994; Thiede and Eggli 2007).

In China, there are approximately 120 species of *Sedum* including 91 endemics which are particularly abundant in the south-western region. *Sedum* is classified into sect. *Filipes* (Fröd.) S.H. Fu (1965: 115), sect. *Oreades* (Fröd.) K.T. Fu (1974: 52) and sect. *Sedum* (Fu and Ohba 2001) which is by far the largest. China stands out as one of the primary centres of biodiversity for *Sedum*, with several studies undertaken (Fu 1965, 1974; Rao 1996; Fu and Ohba 2001). However, ongoing exploration reveals new species within the *Sedum* genus in

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China, indicating that the full extent of its diversity is yet to be uncovered (Zou et al. 2020; Ying 2022a, 2022b; Huang et al. 2023; Meng et al. 2023).

Guangxi Province, located in southern China, boasts a significant biodiversity. The challenge of accurately identifying *Sedum* species within this region is difficult due to the presence of over 17 species. Notably, during the taxonomic revision of *Sedum* in Guangxi, anomalous specimens were collected in Yongfu County, Guangxi Province, China, in 2013. Subsequently, a field survey was conducted in May 2020 to investigate these unidentified specimens. Field observations suggested similarities to *S. tosaense* Makino (1892: 52). However, a thorough examination of both living and herbarium specimens revealed distinctions from *S. tosaense*, particularly in the erect stems during early growth stages, petal morphology, nectar scale shape and style length.

During a field survey of plant resources in Fangcheng City, Guangxi, China, in April 2021, populations of an unknown *Sedum* species were discovered in forests. Through field observations and detailed comparisons, we concluded that this plant is conspecific with the one discovered in Yongfu County, establishing it as a putatively undescribed species. This study intends to determine the phylogenetic position of these plants and to provide a comparative morphological analyses with its close relatives within the genus *Sedum*. To achieve this, phylogenetic analyses were conducted using molecular sequence data from nuclear markers (nrITS) within Eastern Asian *Sedum* sect. *Sedum*. In this study, we clarify the status of the putatively new species by determining its phylogenetic position and comparing its morphology with that of its close relatives.

Material and methods

To examine its phylogenetic relationships and confirm the morphological distinctiveness of this new species from other taxa, voucher specimens and living individuals of *S. guangxiense* were collected from Yongfu County and Fangcheng City. We consulted the relevant literature (Fu 1965, 1974; Rao 1996; Fu and Ohba 2001; Wu et al. 2012; Yang et al. 2012; Xie et al. 2014; Ito et al. 2014a, 2014b, 2017a, 2017b, 2020b; Lu et al. 2019; Zou et al. 2020; Ying 2022a, 2022b; Huang et al. 2023; Meng et al. 2023) and studied herbarium specimens at the Herbaria IBK, IBSC, KUN and PE, therewith comparing our new species with all described Eastern Asian species of *Sedum* sect. *Sedum*.

To determine the phylogenetic position of the new species, nuclear internal transcribed spacer (ITS) sequences of 62 accessions were utilised representing 61 species (Mort et al. 2002; Mayuzumi and Ohba 2004; Ito et al. 2014a, 2017a, 2017b, 2018, 2020a; Xie et al. 2014; Zou et al. 2020; Kim et al. 2023; Huang et al. 2023), including the putatively new species. Fifty-six species belong to *Sedum* sect. *Sedum*, while two species belong to *S.* sect. *Oreades*. These were obtained from GenBank and included in the ITS analysis (Table 1). Based on a phylogenetic study of Crassulaceae (Mayuzumi and Ohba 2004), *Aeonium castello-paivae* Bolle (1859: 240), *A. gomerense* Praeger (1929: 473), *A. lancerottense* Praeger (1932: 190), *A. viscatum* Bolle (1859: 241), *Greenovia aizoon* Bolle (1859: 242) and *Phedimus takesimensis* (Nakai) ‘t Hart (1995: 165) were selected as outgroup members (Table 1).

Table 1. Taxon, locality, accession number and reference for internal transcribed spacer (ITS) sequences of *Sedum*, *Aeonium*, *Greenovia* and *Phedimus* species registered in the GenBank database. The sequences were used for molecular analyses.

Taxon	Origin	Accession number	References
Ingroup			
Sedum section *Oreades*			
S. trullipetalum	Nepal	AB088630	Mayuzumi and Ohba (2004)
S. oreades	Nepal	AB088632	Mayuzumi and Ohba (2004)
Sedum section *Sedum*			
S. actinocarpum	China: Taiwan	LC229265	Ito et al. (2017a)
S. alfredii	China: Guangdong	AB930259	Ito et al. (2014a)
S. arisanense	Japan	LC229273	Ito et al. (2017b)
S. baileyi	China	FJ919935	Unpublished
S. brachyrinchum	China: Taiwan	LC229276	Ito et al. (2017b)
S. boninense	Japan	LC530821	Ito et al. (2020a)
S. bulbiferum	Japan	KM111165	Xie et al. (2014)
S. danjoense	Japan	LC260127	Ito et al. (2017a)
S. emarginatum	China: Anhui	EU592006	Unpublished
S. erythrospermum	China: Taiwan	AB906473	Ito et al. (2014a)
S. formosanum	China: Taiwan	AB930271	Ito et al. (2014a)
S. guangxiense-FC	China: Guangxi	OL693034	This study
S. guangxiense-YF	China: Guangxi	OL693035	This study
S. hakonense	Japan	AB930278	Ito et al. (2014a)
S. hangzhouense	China: Zhejian	LC260130	Ito et al. (2017b)
S. japonicum	Japan	LC229237	Ito et al. (2017a)
S. jinglanii	China: Guangdong	OQ162326	Huang et al. (2023)
S. jiulungshanense	China: Zhejiang	LC229243	Ito et al. (2017a)
S. kiangnanense	China: Zhejiang	LC229244	Ito et al. (2017a)
S. kwanwuense	China: Taiwan	LC229293	Ito et al. (2017a)
S. lineare	Japan	AB088623	Mayuzumi and Ohba (2004)
S. lipingense	China: Guizhou	MN150061	Zhang et al. (2019)
S. lungtsuanense	China: Zhejiang	LC260131	Ito et al. (2017b)
S. mexicanum	Japan	LC229247	Ito et al. (2017b)
S. makinoi	Japan	AB906476	Ito et al. (2014a)
S. microsepalum	China: Taiwan	LC229281	Ito et al. (2017a)
S. morrisonense	China: Taiwan	AB906477	Ito et al. (2014a)
S. mukojimense	Japan	LC530823	Ito et al. (2020a)
S. multicaule	Nepal	AB088631	Mayuzumi and Ohba (2004)
S. nagasakianum	Japan	LC229249	Ito et al. (2017a)
S. nanlingense	China: Guangxi	MN105949	Zou et al. (2020)
S. nokoense	China: Taiwan	AB906478	Ito et al. (2014a)
S. oligospermum	China	LC229250	Ito et al. (2017a)
S. onychopetalum	China: Nanjin	KM111148	Xie et al. (2014)
S. oryzifolium	South Korea	KF954525	Unpublished
S. polytrichoides	China: Anhui	KM111143	Xie et al. (2014)
S. rupifragum	Japan	LC229254	Ito et al. (2017b)
S. sarmentosum	Japan	LC229255	Ito et al. (2017a)

Taxon	Origin	Accession number	References
S. satumense	Japan	LC229256	Ito et al. (2017a)
S. sekiteiense	China: Taiwan	LC229295	Ito et al. (2017a)
S. subtile	Japan	LC229257	Ito et al. (2017a)
S. taiwanalpinum	China: Taiwan	LC229278	Ito et al. (2017a)
S. taiwanianum	China: Taiwan	LC229296	Ito et al. (2017a)
S. tarokoense	China: Taiwan	LC229298	Ito et al. (2017b)
S. tetractinum	China: Zhejiang	LC260135	Ito et al. (2017b)
S. tianmushanense	China: Zhejiang	LC229261	Ito et al. (2017a)
S. tosaense	Japan	AB088620	Mayuzumi and Ohba (2004)
S. triactina	Japan	AB088629	Mayuzumi and Ohba (2004)
S. tricarpum	Japan	LC229259	Ito et al. (2017a)
S. truncatistigmum	China: Taiwan	LC229304	Ito et al. (2017a)
S. uniflorum	Japan	LC530832	Ito et al. (2018)
S. yabeanum	Japan	AB906490	Ito et al. (2014a)
S. zentaro-tashiroi	Japan	AB088619	Mayuzumi and Ohba (2004)
S. wenchuanense	China	ON707681	Unpublished
Outgroups			
Aeonium castello-paivae	Spain: Canary Islands	AY082127	Mort et al. (2002)
Aeonium gomerense	Spain: Canary Islands	AY082133	Mort et al. (2002)
Aeonium viscatum	Spain: Canary Islands	AY082154	Mort et al. (2002)
Aeonium lancerottense	Spain: Canary Islands	AY082143	Mort et al. (2002)
Greenovia aizoon	Spain: Canary Islands	AY082112	Mort et al. (2002)
Phedimus takesimensis	Korea	OP346962	Kim et al. (2023)

The total genomic DNA was extracted from silica gel-dried leaf materials using the CTAB protocol (Doyle and Doyle 1987). DNA sequences of nuclear ribosomal ITS were selected as a marker for molecular phylogenetic studies. The nrITS region was amplified using polymerase chain reactions (PCR) with universal primers, following the protocol of Huang et al. (2017). The complete DNA sequences were submitted to GenBank (Table 1). The ITS sequences obtained by PCR were aligned using MUSCLE version 3.8.31 (Edgar 2004) and were adjusted manually in Bioedit 5.0.9 (Hall 1999). A phylogeny was constructed using Maximum Likelihood (ML) and Bayesian Inference (BI). ML analyses were performed using RAxML v.7.0.4 (Stamatakis et al. 2008) and the substitution model was estimated with ModelTest (Posada and Crandall 1998). BI was conducted in MrBayes 3.2.6 (Ronquist et al. 2012) with the optimal substitution model selected by ModelTest (Posada and Crandall 1998) according to the Akaike Information Criterion. All BI analyses were run for 1,000,000 generations, with four chains in two parallel runs and one tree sampled every 5,000 generations. The convergence of two parallel runs was guaranteed by a splitting frequency of less than 0.005. All other parameters were set to default. The first 25% of the sampled trees corresponding to the burn-in period were discarded and the remaining trees were used to construct a majority rule consensus tree.

Results

Morphological comparison

In morphological terms, two species closely resemble the undescribed species: *S. tosaense* and *S. emarginatum* Migo (1937: 224). It shares similarities with *S. tosaense* in leaf shape, inflorescence type with three branches (each 2-forked) and the basal leaves forming a rosette. However, the newly-discovered species is distinguished by its erect stems when young, narrowly triangular petals, ovate nectar scales, oblong anthers and longer styles. In contrast, the leaves of sterile shoots of *S. tosaense* are rounder, the leaf base is long-attenuate, but shorter, the nectar scales are subquadrangular at the base and the anthers are oblong-ovoid. It is similar to *S. emarginatum* in having spatulate to obovate leaves with a notch or shallow indentation, but differs in having erect stems when young and alternate leaves.

Molecular analysis

ML and BI phylogenetic analyses were performed, based on the ITS sequences of 54 *Sedum* species. This set included two ITS sequences from our undescribed species and 53 ITS sequences from GenBank. A 50% majority rule consensus tree of all post-burn-in trees using the optimal substitution model GTR and Bayesian posterior probabilities was generated (PP, Fig. 1). ML analyses were performed with the substitution model GTRGAMMA and 1,000 rapid bootstrap searches. The topology of the ML tree was highly similar to that of the Bayesian tree and the bootstrap support values for this tree were depicted on Fig. 1 (BS). BI and ML analyses confirmed that the putative new species constituted a well resolved clade, sister to a clade with *S. oligospermum*, *S. sekiteiense* and *S. actinocarpum* and 13 further *Sedum* species (PP = 0.99, BS = 99).

Discussion

The molecular phylogenetic analysis carried out in this study indicates that *S. guangxiense* constitutes a well-supported distinct clade, which is, with high support, sister to a well-supported clade (named clade A in Fig. 1) which includes *S. oligospermum*, *S. sekiteiense*, *S. actinocarpum* and 13 further species (Fig. 1). However, morphologically, *S. guangxiense* can be readily distinguished by its biennial life form (Table 2), erect stems in youth, larger leaves with a retuse apex, usually 5-merous, but sometimes 4- or 6-merous flowers and longer styles. Although it bears resemblance to *S. tosaense* and *S. emarginatum*, these species are distantly related to *S. guangxiense* in the molecular tree (Fig. 1). Specifically, *S. tosaense* and *S. emarginatum* differ in having a perennial life form, prostrate stems when young, smaller leaves with a rounded and emarginate apex, smaller anthers and shorter styles (Table 2). In addition, *S. guangxiense* (Table 2) differs from *S. tosaense* in its ovate (vs. subquadrangular) nectar scales and from *S. emarginatum* in its alternate leaves (vs. opposite and loosely arranged leaves), strongly unequal (vs. subequal) sepals and ovate (vs. oblong) nectar scales.

In recent years, more than 25 new species of *Sedum* have been reported in China, especially in southwest China. This high level of species richness indicates that this area should be further explored to fully unravel the rich biodiversity there.

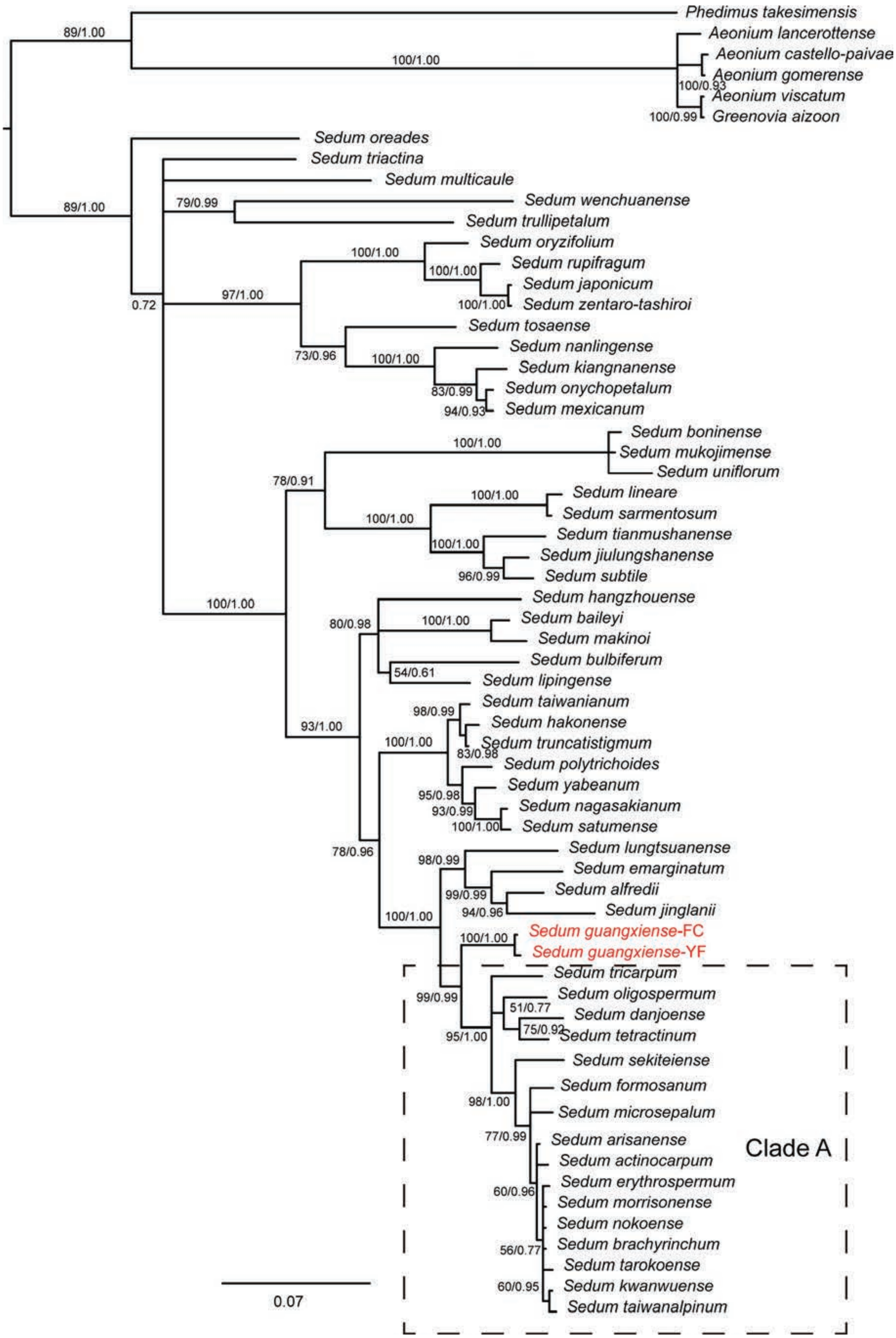


Figure 1. Bayesian phylogenetic tree, based on ITS sequences of Eastern Asian *Sedum*. Bootstrap percentages from the ML analysis (left) and Bayesian posterior probabilities (right) are shown at the nodes.

Table 2. Morphological comparisons between *Sedum guangxiense*, *S. tosaense* and *S. emarginatum,* based on our own measurements of herbarium and living specimens of all three species as well as literature data (Fu and Ohba 2001).

Characters	*S. guangxiense*	*S. tosaense*	*S. emarginatum*
Life form	Biennial	Perennial	Perennial
Flowering stems	Erect when young	Prostrate when young, later erect	Prostrate when young, later erect
Leaf arrangement	Basal leaves forming a rosette	Basal leaves forming a rosette	Basal leaves opposite, loosely arranged
Leaves	Alternate, spatulate to obovate, 1.5–3.5 × 0.5–1 cm, apex usually retuse, rarely round	Alternate, linear-spatulate, 1.2–2 × 0.5–1 cm, apex rounded and emarginate	Opposite, spatulate-obovate to broadly obovate, 1–2 × 0.5–1 cm, apex rounded and emarginate
Inflorescence type	Cyme often 3-branched, each 2-forked	Cyme often 3 (sometimes 4)-branched, each 2-forked	Cymes usually 3-branched, each 2-forked
Flowers	Usually pentamerous, sometimes 4- or 6-merous	Pentamerous	Pentamerous
Carpels	Usually 5, sometimes 4 or 6	5	5
Sepals (colour, shape and size)	Green, spatulate to obovate, strongly unequal, 3–8 × 1–3 mm	Green, linear-spatulate, strongly unequal, 4.5–10 × 2–4.5 mm	Green, spatulate-obovate to broadly obovate, 4–5 × 1–1.5 mm
Petals (colour, shape and size)	Yellow, narrowly triangular, 5–6 × 1.1–1.5 mm	Yellow, narrowly elliptic-lanceolate, 5–6 × 1.5–2.5 mm	Yellow, linear-lanceolate to lanceolate, 6–8 × 1.5–2 mm
Nectar scales (colour, shape and size)	Yellow, ovate, 0.3–0.4 mm	Yellowish, subquadrangular, 0.2–0.4 mm	Yellow, oblong, ca. 0.3–0.6 mm
Anthers (colour, shape and size)	Reddish-brown, oblong, ca. 0.8 mm	Reddish-brown, oblong-ovoid, 0.5–0.6	Reddish-brown, oblong-ovoid, 0.4–0.5
Style length	1.6–2 mm	1–1.2 mm	1.1–1.3 mm
Primary flowering season	April to May	April to May	May to June

Taxonomic treatment

Sedum guangxiense **Yan Liu & C.Y.Zou, sp. nov.**
urn:lsid:ipni.org:names:77356943-1
Figs 2, 3

Type. CHINA • Guangxi: Fangcheng District, Na-suo Town, top of Nanshan. Elevation, 830 m; 21°44'49"N, 108°6'37"E, 24 April 2021. *H. L. Chen ZCY1978* (holotype: IBK!, isotype: PE!).

Diagnosis. *Sedum guangxiense* is similar to *S. tosaense* and *S. emarginatum* in its leaf shape and inflorescence type, but can be distinguished from the latter two by its erect stems in youth (vs. prostrate when young), much larger leaves (1.5–3.5 cm long vs. 1–2 cm), narrowly triangular petals (vs. narrowly elliptic-lanceolate and linear-lanceolate to lanceolate), ovate nectar scales (vs. subquadrangular and oblong) and larger, oblong anthers (rather than oblong-ovoid).

Description. Biennial herb, fleshy, glabrous. Roots fibrous, with several adventitious roots in the leaf axils of the basal nodes. Stems stout, erect when young, with irregular branching, to 10–25 cm tall. Leaves alternate, base spurred, sessile, thick, spatulate to obovate, 1.5–3.5 cm long, 0.5–1 cm wide, apex usually retuse, rarely round, base long-attenuate. Inflorescences terminal, many flowered cymes, usually with three branches, each 2-forked; bracts leaf-like, 5–10 mm long, 2–5 mm wide; flowers usually sessile, usually 5-, sometimes 4- or 6-merous; Sepals usually 5, sometimes 4 or 6, free, green, fleshy, strongly unequal in size, spatulate to obovate, 3–8 mm long, 1–3 mm wide, apex usually retuse, rarely round. Petals usually 5, sometimes 4 or 6, yellow, narrowly

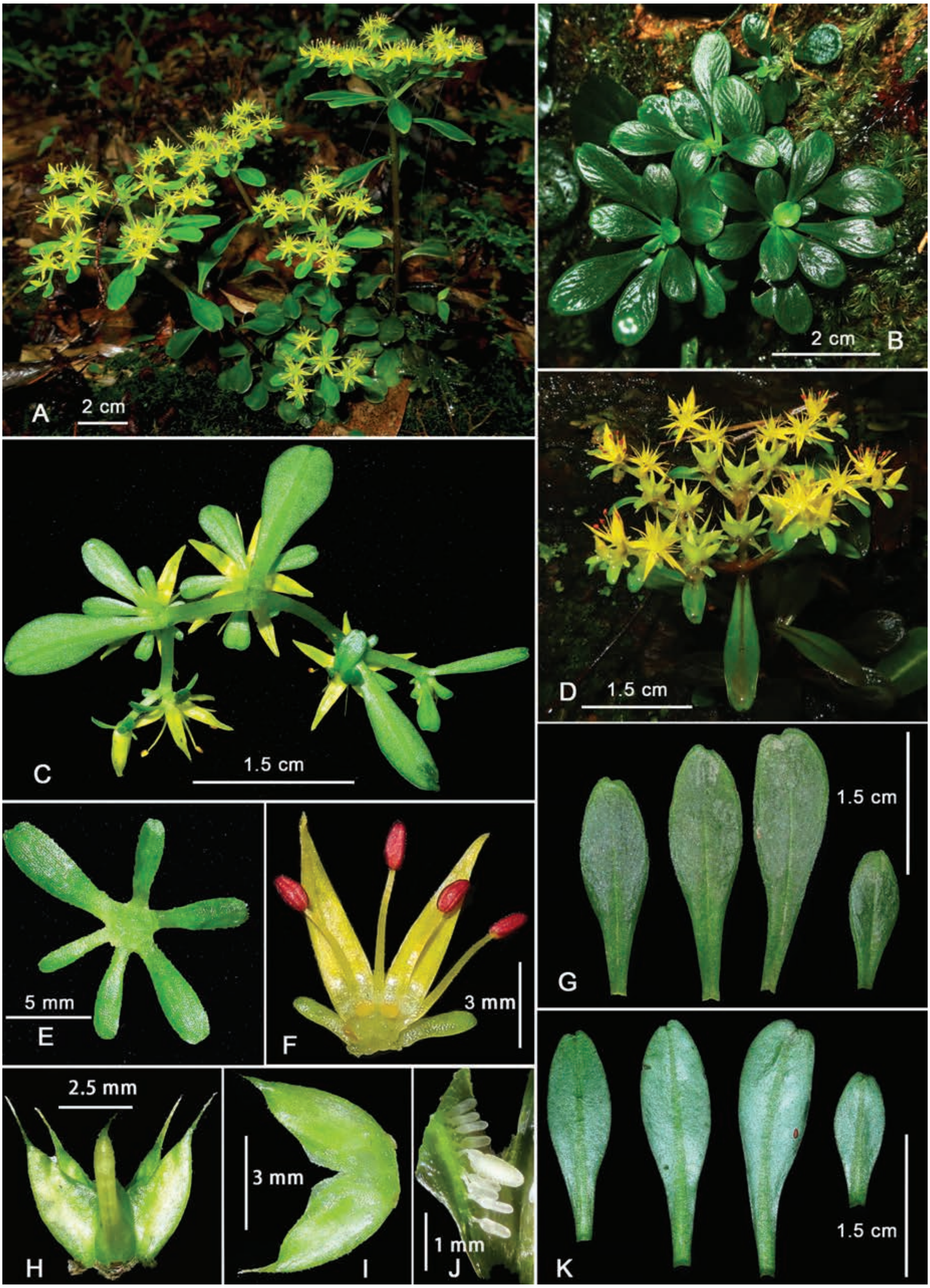
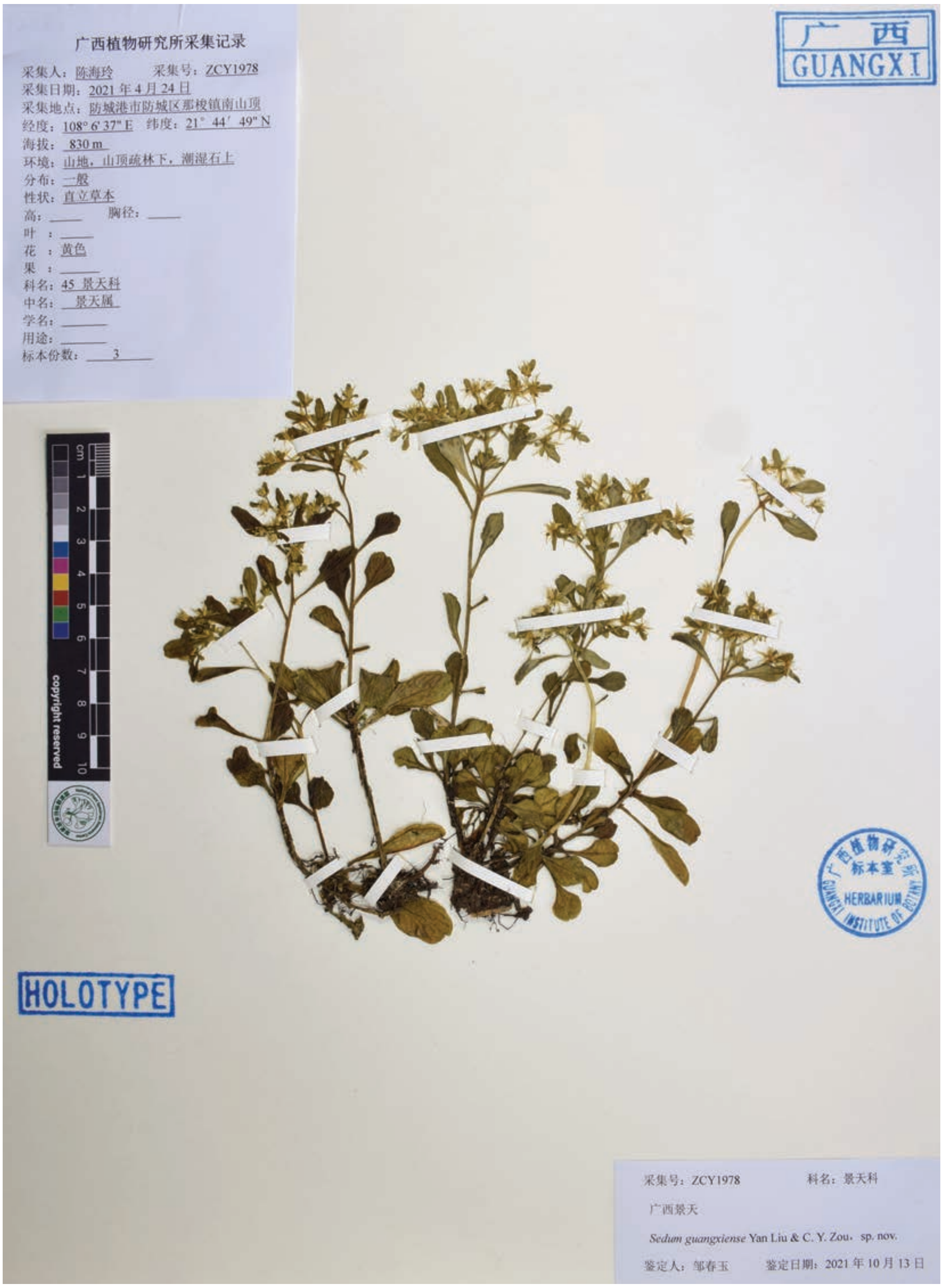


Figure 2. *Sedum guangxiense* **A** plant in flower **B** sterile plant **C** inflorescence from below **D** flowering branch **E** strongly unequal sepals **F** side view of a flower with carpels removed, showing the sepals, petals, stamens and nectar scales **G** adaxial leaf surface **H** unripe follicles **I** carpels connate at the base in longitudinal section **J** ovules **K** abaxial leaf surface. A, photographed by Hai-ling Chen in Fangcheng District (corresponding to the holotype *H. L. Chen ZCY1978*); B, C, photographed by Chun-Yu Zou in Yongfu County (corresponding to the paratype *C. Y. Zou & J. Q. Huang, ZCY1977*).



广西植物研究所采集记录
采集人：陈海玲　　采集号：ZCY1978
采集日期：2021年4月24日
采集地点：防城港市防城区那梭镇南山顶
经度：108° 6′ 37″ E　纬度：21° 44′ 49″ N
海拔：830 m
环境：山地，山顶疏林下，潮湿石上
分布：一般
性状：直立草本
高：＿＿　胸径：＿＿
叶　：＿＿
花　：黄色
果　：＿＿
科名：45 景天科
中名：景天属
学名：＿＿
用途：＿＿
标本份数：3

采集号：ZCY1978　　科名：景天科
广西景天
Sedum guangxiense Yan Liu & C. Y. Zou，sp. nov.
鉴定人：邹春玉　　鉴定日期：2021年10月13日

Figure 3. Holotype of *Sedum guangxiense*, *H. L. Chen ZCY1978* (IBK).

triangular, 5–6 mm long, 1.1–1.5 mm wide, apex acuminate, slightly connate at base. Stamens usually 10, sometimes 8 or 12, shorter than petals, erect at anthesis, arranged in two whorls; antesepalous ones 3.5–4 mm, antepetalous ones 3–3.5 mm; anthers oblong, ca. 0.8 mm long, reddish-brown before dehiscence. Nectar scales ovate, 0.3–0.4 mm. Carpels usually 5, sometimes 4 or 6, connate at the base for ca. 0.3–0.5 mm, gibbous ventrally, 5–6 mm long; styles 1.6–2 mm long. Fruits star-shaped, many seeded follicles, spreading. Flowering April to May, fruiting May to July.

Distribution and habitat. The species is known from Bai-shou Town in Yongfu County and Na-suo Town in Fangcheng District, Guangxi, China. It grows on mossy rocks in secondary broadleaf forests at elevations of 200–550 m (Fig. 4).

Etymology. The specific epithet refers to the distribution in Guangxi Province, China.

Additional specimen examined (paratypes). CHINA • Guangxi: Yongfu County, Bai-shou Town, on mossy rocks along streams, elev. 263 m, 25°11'43.29"N, 110°49'42.89"E, 10 May 2021, *C. Y. Zou & J. Q. Huang, ZCY1977* (IBK!); • Fangcheng District, Na-suo Town, the top of Nanshan, 21°44'49"N, 108°6'37"E, 19 April 2021, *Y. G. Liu, Q. G. Yang & H. L. Cheng 1208* (IBK!).

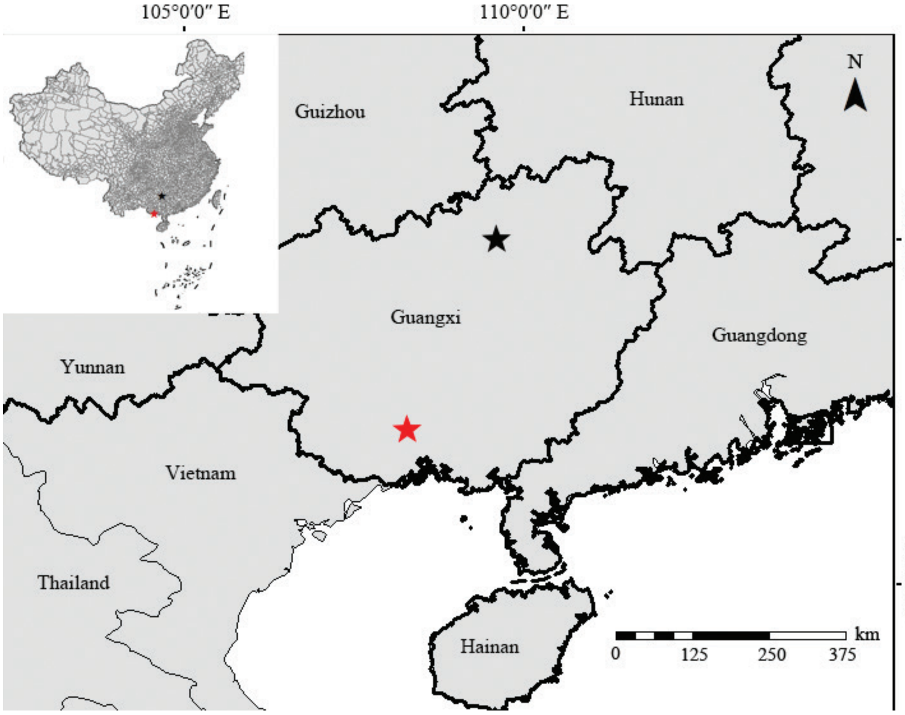


Figure 4. Distribution of *Sedum guangxiense* Yan Liu & C. Y. Zou (pentagram stars) in Guangxi, China. The red pentagram star indicates the locality of the holotype (type locality).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

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

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