

Research Article

# Decussiphycus sinensis sp. nov. (Bacillariophyceae, Mastogloiales) – a new species described from China, with comments on phylogenetic position of the genus

Andrei Mironov<sup>1,2,3®</sup>, Anton Glushchenko<sup>2®</sup>, Elena Kezlya<sup>2®</sup>, Yevhen Maltsev<sup>2®</sup>, Anton Iurmanov<sup>2®</sup>, Yan Liu<sup>1®</sup>, Maxim Kulikovskiy<sup>2®</sup>

1 College of Life Science and Technology, Harbin Normal University, Harbin, 150080, Heilongjiang Province, China

2 K.A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, Russia

3 Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1, building 12, Moscow, 119234, Russia

Corresponding author: Yan Liu (yanliuhrb@hotmail.com)

#### Abstract

During the study of freshwater diatom communities in Hainan Province, China, we uncovered an unknown diatom species of the genus *Decussiphycus*, which is described as *Decussiphycus sinensis* **sp. nov.** herein. The description is based on LM and SEM investigations; morphologically, the new species is compared to other taxa belonging to the genus. We complemented the description with the results of a molecular analysis based on SSU rDNA and *rbcL* sequencing. Molecular data is acquired for *Decussiphycus* for the first time. Hereby, we discuss the phylogenetic relationships between this genus and its closest allies – *Aneumastus* and *Mastogloia*, demonstrating the affinity of *Decussiphycus* within the order Mastogloiales.

**Key words:** Bacillariophyceae, *Decussiphycus*, Hainan Province, new species, phylogeny, pore occlusions

#### Introduction

Throughout the years, diatomists praised different kinds of evidence while describing new species and genera or analyzing the taxonomy and phylogeny of high-rank groups (i.e. families and orders). While some taxonomists valued chloroplast morphology (Mereschkowsky 1903; Heinzerling 1908; Cox 1987, 2015), others appraised valve features (Cleve 1891; Hustedt 1930; Mann 1984; Kociolek and Stoermer 1988; Krammer 2002). However, diatom taxonomy has nowadays shifted towards the adoption of a "narrow" species concept (Mann 1999). Most of all, this trend influenced the taxonomic revision of the "catch-all" genera, e.g., *Navicula* Bory. Several genera, for example *Placoneis* Mereschkowsky and *Geissleria* Lange-Bertalot & Metzeltin were separated from *Navicula* after re-examinations of chloroplast and valve morphology (Mereschkowsky 1903; Lange-Bertalot and Metzeltin 1996). Another genus introduced in this way is *Decussata* (R.M. Patrick) Lange-Bertalot *nom. inval.*, which has been recently renamed (Guiry and Gandhi 2019) as *Decussiphycus* Guiry & Gandhi in accordance



Academic editor: Kalina Manoylov Received: 25 November 2024 Accepted: 21 January 2025 Published: 18 March 2025

**Citation:** Mironov A, Glushchenko A, Kezlya E, Maltsev Y, Iurmanov A, Liu Y, Kulikovskiy M (2025) *Decussiphycus sinensis* sp. nov. (Bacillariophyceae, Mastogloiales) – a new species described from China, with comments on phylogenetic position of the genus. PhytoKeys 254: 1–19. https://doi. org/10.3897/phytokeys.254.142654

**Copyright:** <sup>©</sup> Andrei Mironov et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). with Art. 20.2 of the ICN (Shenzhen Code; Turland et al. 2018). Similarly, the mentioned nomenclature alteration was applied by Wynne (2019) to replace *Delicata* Krammer *nom. inval.* with *Delicatophycus* M.J. Wynne. Lately, this approach was criticized by da Silva (2024) and thus, the validity of the names *Delicatophycus* and *Decussiphycus* remains unclear until the publication of the Madrid edition of the International Code of Nomenclature for Algae, Fungi, and Plants.

Originally, *Decussiphycus* emerged from *Navicula placenta*-group (Patrick 1959) and was later granted with a status of an independent genus by Lange-Bertalot (2000). Edlund et al. (2006) emended the description of *Decussiphycus* (at the time – *Decussata nom. inval.*), focusing on both chloroplast and valve characters to distinguish it from other genera. According to their diagnosis, *Decussiphycus* includes diatoms with two chloroplasts of complex configuration (H-shaped in girdle view, each with four apically elongated lobes) near each pole. The valves are flat, rectangular in girdle view, equipped with a narrow mantle, circular central area, filiform raphe, crozier-shaped proximal raphe ends and distal ends deflected in opposite directions. The most notable feature of the genus is decussate or quincunx arrangement of striae (Edlund et al. 2006). Several ultrastructural features of *Decussiphycus* should be listed as well: slight sinuous discontinuity of the raphe near the apices, perforated bands of cingulum (each with two rows of poroids), areolae occluded by "circular convex hymene" (sensu Edlund et al. 2006).

After its emergence at the genus-level, Decussiphycus has been considered to represent the order Mastogloiales D.G. Mann, which has been originally made up of Aneumastus D.G. Mann & Stickle and Mastogloia Thwaites ex W. Smith, solely (Round et al. 1990). Subsequently, Cox (2015) elaborated a new concept of the order by emending the descriptions of Achnanthaceae Kützing and Mastogloiaceae Mereschkowsky. Therefore, Mastogloiales was supplemented with a monoraphid genus Craspedostauros Cox. Cox's system implied that the synapomorphies of Craspedostauros and biraphid mastogloioid diatoms are cribrate areolae and presence of two H-shaped chloroplasts. However, the relevance of Cox's proposals is still being contested. Similarly, until today, the accurate phylogenetic position of the genus Decussiphycus has been doubtful due to the complex morphology of the genus (i.e. intricate structure of pore occlusions) and, most importantly, the lack of molecular data. In this paper, we provide the results of a new molecular analysis based on SSU rDNA and rbcL sequencing, demonstrating the phylogenetic position of Decussiphycus for the first time defining its position within the order Mastogloiales.

Hitherto, the genus *Decussiphycus* has been comprised by only three morphologically close taxa – *Decussiphycus placenta* (Ehrenberg) Guiry & Gandhi, *Decussiphycus placenta* var. *obtusus* (F. Meister) Guiry & Gandhi and *Decussiphycus hexagonus* (Torka) Guiry & Gandhi. One unknown *Decussiphycus* species was found during the survey in the area of Wuzhishan Mountain in the Province of Hainan, China. Notably, as multiple studies revealed (Kociolek et al. 2015; Kulikovskiy et al. 2015, 2018; Glushchenko and Kulikovskiy 2017; Glushchenko et al. 2017a, 2019, 2020; Liu et al. 2018; Maltsev et al. 2019; Mironov et al. 2024), diatom communities in the region of South China and surrounding territories are characterized by considerable level of diversity. Several species of the order Mastogloiales, e.g. *Aneumastus laosica* Glushchenko, Kulikovskiy & Kociolek and *Aneumastus genkalii* Glushchenko, Kulikovskiy & Kociolek, were described from this area (Glushchenko et al. 2017b), too. In this study, based on unique combination of valve features and molecular data, we describe one more species of this order – *Decussiphycus sinensis* Glushchenko, Maltsev, Mironov, Liu & Kulikovskiy sp. nov.

# Methods

#### Sample collection and preparation

In the current study, we investigated a single sample of diatom biofilms, collected from an unnamed mountain stream at the slope of Wuzhishan Mountain, Hainan Province, China. The sample was treated with 10% hydrochloric acid to remove carbonates and then washed with deionized water for 12 h. To remove the organic matter, boiling in concentrated hydrogen peroxide (37%) was applied. Furthermore, the sample was washed with deionized water four times with 12 h intervals. It was then decanted and filled with deionized water up to 100 ml; the suspension was pipetted onto coverslips. Afterwards, it was left for drying at room temperature. A permanent sample was mounted in Naphrax® (refractive index = 1.73). Live material was viewed with a Zeiss Axio Scope A1 microscope with mounted Axiocam ERc 5s camera (Zeiss, Germany) and equipped with an oil immersion EC Plan-NEOFLU-AR objective (x100, n.a. 1.3) for epifluorescent microscopy (EFM) and an oil immersion Plan-apochromatic objective (x100, n.a. 1.4; Nomarski differential interference contrast) for LM of cleaned material.

Later, a part of the suspension was spread onto aluminum stubs after air-drying at room temperature for 24 h in order to prepare SEM stubs. The stubs were then sputter-coated with 50 nm of Au by the means of Eiko IB 3 apparatus (Eiko Engineering, Japan). For SEM investigations, we applied the TESCAN Vega III (TESCAN, Brno, Czech Republic) in the Borissiak Paleontological Institute of the Russian Academy of Science. The suspension and slides analyzed herein are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Sciences, Moscow, Russia.

The terminology of the valve follows Lange-Bertalot (2001), Edlund et al. (2006), Stancheva and Temniskova (2006), Kulikovskiy et al. (2016).

## **Culturing and DNA preparation**

The monoclonal strain Ca68 was established by micropipetting a single cell under a Zeiss Axio Vert. A1 inverted microscope (with × 10 objective). The strain was cultivated in WC liquid medium (Guillard and Lorenzen 1972) in Petri dishes at 23 °C with an alternating 12-hour light and dark photoperiod.

Genomic DNA was extracted with Chelex100 Chelating Resin (Bio-Rad Laboratories, Hercules, CA, USA) with primers D512for and D978rev for SSU rDNA (Zimmermann et al. 2011); dp7- (Daugbjerg and Andersen 1997) and *rbc*L404+ (Ruck and Theriot 2011) for *rbc*L. ScreenMix (Evrogen, Moscow, Russia) was utilized for the PCR. Amplified material was visualized by horizontal electrophoresis in agarose gel (1.0%) stained by the SYBR<sup>™</sup> Safe (Life Technologies, Carlsbad, CA, USA). Sequencing procedure was conducted with a Genetic Analyzer 3500 instrument (Applied Biosystems, Waltham, MA, USA).

#### **Molecular analysis**

Molecular analysis, as performed in this study, follows the algorithms described in Mironov et al. (2024) and Tseplik et al. (2024).

The dataset for multigene analysis was comprised of 29 concatenated SSU rDNA and *rbcL* sequences, selected for available lineages of 25 representatives of Mastogloiales sensu Cox (2015) and four diatom species from Thalassiosiro-phycidae Round & R.M. Crawford chosen as the outgroups (taxa names and Accession Numbers are given in Fig. 1). The SSU rDNA and *rbcL* sequences were aligned in separately by the means of the G-INS-I algorithm using the Mafft ver. 7



**Figure 1.** Phylogenetic position of *Aneumastus, Mastogloia* and *Decussiphycus* species based on BI from an alignment of 29 sequences and 1,353 characters (*rbcL* and SSU rRNA genes). Values of PP below 0.9 are hidden. Strain numbers (if available) and GenBank numbers are indicated for all sequences.

software (RIMD, Osaka, Japan) (Katoh and Toh 2010). The dataset used in further analysis included 1,795 and 1,493 nucleotide sites for nuclear SSU rDNA, and plastid *rbcL* regions, respectively. After that, unpaired regions were eliminated, and the resulting aligned SSU rDNA sequences were combined with the *rbcL* sequences into a united matrix for concatenated SSU rDNA and *rbcL*. Alignments used for phylogenetic analyses are presented in supplementary files (Suppl. material 1).

The Bayesian inference (BI) method was conducted with Beast ver. 1.10.1 software (BEAST Developers, Auckland, New Zealand) (Drummond and Rambaut 2007). Most suitable partition-specific substitution models, shape parameter a and a proportion of invariable sites (pinvar) were found out with the help of the Bayesian information criterion (BIC) in jModelTest ver. 2.1.10 software (Vigo, Spain) (Darriba et al. 2012). During the BIC-based model selection procedure, we chose the following models, shape parameter a and a proportion of invariable sites (pinvar): GTR+G+I,  $\alpha$  = 0.4710 and pinvar = 0.5970 for SSU rDNA; TPM1uf+G+I,  $\alpha$  = 0.3960, and pinvar = 0.7310 for the first codon position of the *rbcL* gene; JC+I, pinvar = 0.8690 for the second codon position of the *rbcL* gene; GTR+G+I,  $\alpha$  = 1.1260, and pinvar = 0.2320 for the third codon position of the rbcL gene. Besides, the HKY and F81 models were applied instead of TPM1uf and JC, respectively, as the most similar suitable options for BI. Speciation procedure was performed by a Yule process tree prior. Five MCMC analyses were conducted for 5 million generations (burn-in 1,000 million generations). Tracer ver. 1.7.1 software (MCMC Trace Analysis Tool, Edinburgh, United Kingdom) (Drummond and Rambaut 2007) was utilized for the convergence diagnostics. Furthermore, the initial 15% trees were eliminated, while the rest were retained for final chronogram construction (with 90% Bayesian posterior probabilities - PP). The Bayesian phylogenetic topology for the rbcL and SSU rRNA genes tree is attached as a supplementary file (Suppl. material 2). Phylograms were viewed and edited with FigTree ver. 1.4.4 (University of Edinburgh, Edinburgh, United Kingdom) and Adobe Photoshop CC ver. 19.0 software.

## Results

#### Molecular phylogeny of Decussiphycus

Phylogeny of the Mastogloiales sensu Cox (2015), based on SSU rDNA and rbcL sequencing, is demonstrated in Fig. 1. As illustrated, three genera of the order - Aneumastus, Mastogloia and Decussiphycus comprise an independent monophyletic group (clade AMD, highlighted in green), which is highly statistically supported (posterior probability, PP = 0.98). In our molecular analysis, the genus Decussiphycus was represented by a single newly acquired strain Decussiphycus sinensis sp. nov., which is positioned in a separate node, as a basal taxon within the group. The genus Aneumastus is demonstrated as monophyletic in the phylogram, with maximum statistical support. On the contrary, molecular data reveals the paraphyly of the genus Mastogloia, confirming the assumptions of Kezlya et al. (2024). This revelation, perhaps, indicates the necessity of further re-evaluation of Mastogloia. Another independent clade on the phylogram (highlighted in blue) corresponds to the genus Tetramphora Mereschkowsky, including 3 strains. The relationship between these clades is also strongly supported (PP = 1.0). The clade comprising Achnanthes and Craspedostauros (highlighted in red) is isolated from the AMD clade the most. That clade, in turn, is also supported with maximum rate.

#### **Species description**

# *Decussiphycus sinensis* Glushchenko, Maltsev, Mironov, Liu & Kulikovskiy, sp. nov.

Figs 2-6

**Holotype.** Slide 09153 in herbarium of MHA, Main Botanical Garden, Russian Academy of Science, Moscow, Russia, represented here by Fig. 4C.

**Isotype.** Slide 08909 in herbarium of MHA, Main Botanical Garden, Russian Academy of Science, Moscow, Russia.

**Type.** CHINA. Hainan Province, unnamed stream at the northern slope of Wuzhishan Mountain, biofilms on rocks, 18.9815°N, 109.6854°E, 470 m asl, leg. Y. Liu, 12.07.2014. Slide 09153 from oxidized culture strain no. Ca68, isolated from sample THHN 2014043.

**Representative specimens.** Strain Ca68 (slides 09153); sample THHN 2014043 (slide 08909).

**Sequence data.** GenBank accession numbers PV016799 (strain Ca68, partial SSU rRNA gene sequence, V4 region); PV021297 (strain Ca68, partial rbcL sequence).

**Description.** *Live cells* (Fig. 2A–N). Cells solitary. Nucleus located centrally within a cytoplasmic bridge between the central nodules (Fig. 2A, E, I, white arrows). Each cell contains two chloroplasts of complex configuration, each of which is located at in the apical valve regions (Fig. 2A, E, I, black arrows). In the valve face view, both of the plastids are invaginated along the apical axis to create a central plastid isthmus. In the girdle view, each chloroplast is H-shaped, with four clearly visible arms that extend along the surface of the valve, in its plane (Fig. 2M, white arrows). The arms reach the cingulum region. The four lobes can be discerned in valve view as well (Fig. 2G, white arrows).

LM (Figs 3, 4). The post-initial valve has a linear shape with slightly convex margins and bluntly curved ends; length – 77.5  $\mu$ m, width – 16.9  $\mu$ m. Distal raphe ends recurved in opposite directions, terminating to the valve face (Fig. 3A). Valves linear–elliptical to elliptical with broadly rounded ends. Length 32.1–69.1  $\mu$ m, width 13.9–18.3  $\mu$ m. Axial area narrow, linear. Central area transapically oval to circular. Sometimes, a few randomly located areolae, visible with careful focusing, are positioned at the central area (Fig. 3H, black arrow). Raphe filiform, straight to slightly undulate. Central raphe ends drop-shaped. Distal raphe ends deflected to the valve margin. Striae are decussate, formed by clearly visible areolae forming the right quincunx. Areolae are arranged in multiple rows – a transapical row and two oblique rows which cross each other at angles of 60°–80°. Transapical striae 21–23 in 10  $\mu$ m. Occasionally, residual cingula can be found separated from the valve (Fig. 4L).

**SEM, external view** (Fig. 5A–F). Valve face is flat. Central raphe ends slightly tilted to one side and lie in asymmetrical depressions (Fig. 5C, white arrows). Distal raphe ends oppositely deflected (Fig. 5A, white arrowheads), bordered by small silica folds (Fig. 5D, E, white arrowheads). Areolae small, rounded, their diameters are slightly larger near the axial area (Fig. 5D, E, black arrows) and smaller towards the valve margin (Fig. 5D, E, white arrows). The apex of the valve is equipped with a single isolated row of areolae, situated behind the distal raphe ends (Fig. 5D, E, black arrowheads). Cingulum composed of open 3–4 girdle bands (Fig. 5F).



**Figure 2. A–N** *Decussiphycus sinensis* sp. nov. LM, DIC. Strain Ca68 **A–H** valve face view **A–H** girdle view **A, E, I** live cells. White arrows point to the nucleus, black arrows show the two apically located plastids **B–D, F–H, J–N** chloroplast auto-fluorescence. White arrows show the four lobes of each plastid. Scale bar: 10 µm.

Two rows of areolae are located on each girdle band (Fig. 5B, black arrowheads). Girdle band areolae are smaller than areolae at the valve and arranged alternately or, sometimes, chaotically. Rarely, one of the rows is interrupted. Notably, each areola in the girdle bands is covered with a layer of silica (Fig. 5F, black arrowheads).



**Figure 3.** A–L *Decussiphycus sinensis* sp. nov. LM, DIC. Size diminution series. Slide 08909 (from sample EHHN 2014043). Post-initial valve (A). Black arrow shows the randomly located areolae (H). Scale bar: 10 µm.

**SEM**, *internal view* (Fig. 6A–F). The valve margins is shallow (Fig. 6D, E, black arrows). The striae continue onto the valve margins (Fig. 6B, D, E, white arrows). The interstriae are slightly raised relatively to the striae. Oblique ribs of the quincunx system are located near the central area. They are more elevated in comparison to the transapical interstriae (Fig. 6C, black arrowheads). Areolae vary in size, shape, and type of occlusions. Areolae located closer to the sternum are distinguished by a larger diameter, round shape and presence



**Figure 4. A–L** *Decussiphycus sinensis* sp. nov. LM, DIC. Slide 09153 (from strain Ca68). Holotype (**C**) A residual cingulum separated from the valve (**L**). Scale bar: 10 µm.

of flat, slightly depressed, rounded silica formations (Fig. 6D, E, black arrows). Areolae, located near the valve margin are transapically elongated, covered with oval, raised silica caps ("convex hymene", sensu Edlund et al. 2006) (Fig. 6D, E, white arrows). Transapical areolae density – 18–20 in 10  $\mu$ m. Raphe slits, straight, lying on the raised sternum. The sternum widens towards the valve apex (Figs 6D–F). Central raphe ends straight, not expanded (Fig. 6C, white arrows). Distal raphe ends terminate with well-expressed, horse-shoe-shaped helictoglossae (Fig. 6F, white arrow).

**Etymology.** The specific epithet refers to the name of the country where this species was discovered.

Distribution. So far, the species is known only from the type locality.

**Ecology.** The species was located in a mountain stream with temperature of 26.7 °C, pH = 7.64 and conductivity =  $60 \mu$ S/cm.

**Comments.** Specimens of *D. sinensis* sp. nov. from wild population were  $32.1-69.1 \mu m \log$ ,  $13.9-18.3 \mu m$  wide, with striae width of  $21-23 in 10 \mu m$ . Specimens from culture are characterized by smaller valves:  $41.5-46.1 \mu m \log$ ,  $15.5-17.1 \mu m$  wide; striae  $22-23 in 10 \mu m$ , which corresponds to material from wild population.



**Figure 5. A**–**F** *Decussiphycus sinensis* sp. nov. SEM, external view. Slide 09153 (from strain Ca68) **A** the entire valve; note the oppositely deflected distal raphe fissures **B** details of girdle bands structure; note the two rows of areolae (black arrowheads) **C** details of the central area; proximal raphe fissures expanded, slightly tilted to one side (white arrows) **D**, **E** details of the valve apex; note the distal raphe ends with silica folds (white arrowheads), areolae larger near the axial area (black arrows) and smaller towards the margin (white arrows), an isolated row of areolae (black arrowheads) **F** girdle bands; note the double rows of areolae with silica caps (black arrowheads). Scale bars: 10 μm (**A**, **F**); 0.5 μm (**B**); 2 μm (**C**–**E**).



Figure 6. A–F *Decussiphycus sinensis* sp. nov. SEM, internal view. Slide 09153 (from strain Ca68) A the entire valve B details of striae structure near the valve margin; note the shallow margin (white arrows) C details of the central area; note proximal raphe fissures (white arrows) and ribs of the quincunx system (black arrowheads) D, E details of the valve apex; note the shallow valve margin (black arrows) with striae continuing onto it (white arrows), areolae with round occlusions near the sternum (black arrowheads) and oval occlusions towards the margin (white arrowheads). Apical expansions of the sternum indicated by asterisks F details of the valve apex; note the horseshoe-shaped helictoglossa (white arrow) and apical expansion of the sternum (asterisk). Scale bars: 10 µm (A); 2 µm (B–F).

#### New combination in the genus Decussiphycus

*Decussiphycus obtusus* (F. Meister) Glushchenko, Maltsev, Mironov, Liu & Kulikovskiy, comb. et stat. nov.

**Basionym.** *Navicula placenta* var. *obtusa* F. Meister 1932. Kieselalgen aus Asien, p. 37, pl. 13, fig. 99.

**Synonym.** *Decussiphycus placenta* var. *obtusus* (F. Meister) Guiry & Gandhi, 2019.

#### Discussion

# Morphological comparison *Decussiphycus sinensis* sp. nov. with similar species

*D. sinensis* sp. nov. shares a number of similarities with other representatives of the genus (see Table 1), e.g., shape of the central area, decussate striae arrangement, along with ultrastructural valve features – morphology and location of chloroplasts, structure of girdle bands, areolae occluded by "circular convex hymene" (sensu Edlund et al. 2006). Generally, *D. sinensis* sp. nov. and similar species differ from each other, primarily, by valve outlines and morphology of apices.

*D. sinensis* sp. nov. resembles *D. placenta* by valve width  $(13.9-18.3 \mu m in D. sinensis$  sp. nov. vs. 14-20  $\mu m$  in *D. placenta*) and striae density (21-23 in 10  $\mu m$  in *D. sinensis* sp. nov. vs. 20-25 in 10  $\mu m$  in *D. placenta*). However, *D. sinensis* sp. nov. differs from *D. placenta* by broadly rounded and unprotracted apices, while in *D. placenta* valve apices are distinctly protracted, narrowly rostrate to subcapitate (e.g. Lange-Bertalot 2001: p. 452, Pl. 108, figs 11-13, Pl.109, fig. 4).

Among *D. sinensis* sp. nov. and *D. hexagona*, striae densities are comparable: 21-23 in 10 µm in *D. sinensis* sp. nov. vs. 20-25 in 10 µm in *D. hexagona* (Table 1). Regarding the remaining features of the valve, *D. sinensis* sp. nov. differs from *D. hexagona* most prominently. The valves of *D. sinensis* sp. nov. are linear-elliptic to elliptic, valve outlines convex; the valves of *D. hexagona* are mostly linear or linear-elliptic, with weakly convex to nearly parallel outlines (Table 1). Valve width in *D. sinensis* sp. nov. is 13.9-18.3 µm, which significantly exceeds valve width in *D. hexagona* – 9-13 µm (see Table 1). Central area in *D. sinensis* sp. nov. is transapically oval to round, and transapically elliptic in *D. hexagona* (see Table 1). Finally, the two species differ by the shape of apices: they are broadly rounded in *D. sinensis* sp. nov., but rostrate to bluntly rounded in *D. hexagona* (e.g. Lange-Bertalot 2001: p. 452, Pl. 108, figs 14–17).

Both *D. placenta* var. *obtusus* and the newly described species are characterized by narrow axial and circular central areas, as well as broadly rounded valve apices. At the same time, the species obviously differ in valve width  $(13.9-18.3 \,\mu\text{m} \text{ in } D. \text{ sinensis} \text{ sp. nov. vs. } 21-25 \,\mu\text{m} \text{ in } D. \text{ placenta var. obtusus})$  and striae density (21-23 in 10  $\mu\text{m}$  in *D. sinensis* sp. nov. vs. 20 in 10  $\mu\text{m}$  in *D. placenta* var. obtusus). The other ultrastructural morphological features of *D. placenta* var. obtusus are not studied yet.

	D. sinensis sp. nov.	D. placenta	D. hexagona	D. obtusus
Valve shape	linear-elliptic to elliptic	broadly elliptic	linear to linear-elliptic	elliptic
Valve ends	broadly rounded	abruptly protracted, narrow- ly rostrate to subcapitate	narrowed to a wedge, finally obtusely rounded	broadly rounded
Length, µm	32.1-69.1	35-60	25-44	44-60
Width, µm	13.9–18.3	14-20	9-13	21-25
Transapical striae in 10 µm	21-23	20-25	20-25	20
Axial area	narrow, linear	narrow, linear	narrow, linear	narrow, linear
Central area	transapically oval to circular	rather small, broadly elliptic in outline	transapically elliptic	circular
Shape of areolae near the sternum, internally	distinguished by a larger diam- eter, rounded, covered with flat, slightly recessed rounded silica plates	rounded, transapically elon- gated and are covered with raised rounded silica plates	rounded, covered with raised silica caps	n.d.
Shape of areolae near the valve margin, internally	become transapically elongated, covered with oval, raised silica caps	n.d.	n.d.	n.d.
Ecology	confined to lotic ecosystems	confined to lotic ecosystems	confined to lotic ecosystems, found in acidi- fied freshwaters, aerophile	confined to lotic ecosystems
Distribution	Southeast Asia, China, Hainan (type locality)	widely distributed	widely distributed	Southeast Asia, Nepal
References	This study	Lange-Bertalot 2001; Ku- likovskiy et al. 2016	Lange-Bertalot, 2000, 2001; Stancheva and Temniskova 2006; Kulikovskiy et al. 2016	Meister 1932

Table 1. Comparison of morphological features of D. sinensis sp. nov. and related species.

#### On the taxonomy of Decussiphycus obtusus comb. et stat. nov.

As illustrated by F. Meister (Meister 1932: taf. 13, fig. 99), the morphology of *Navicula placenta* var. *obtusa* is clearly different from *Decussiphycus placenta* according to its current conception. The most prominent difference is expressed in the shape of apices. Therefore, we propose transferring *Navicula placenta* var. *obtusa* to the genus *Decussiphycus* and endowing it with a new status.

#### On the molecular phylogeny of Decussiphycus

As described in the introduction, throughout the history of diatom science, taxonomists gave preferences to various types of evidence for their inquiries: from chloroplast characters, to valve structure, to, as nowadays, molecular data. On this challenging course, several mistakes were made, which, consequently, led to misunderstanding some taxa's systematics and phylogeny. For instance, E. J. Cox (2015) made an attempt to assess the system of diatoms relying on chloroplast morphology. One of her assumptions revolved around *Achnanthes* Bory. In her study, Cox (1999) presumed the homology between *Achnanthes* and *Mastogloia* based on the similarities in plastid arrangement, structure of areolae (presence of cribrate occlusions) and, partially, presence of stauros. However, further molecular analysis (Ashworth et al. 2017), involving the discussed genera, alongside *Craspedostauros* and *Staurotropis* Paddock, did not prove Cox's hypothesis. As authors demonstrated, *Achnanthes* and *Craspedostauros* are closely allied, rather than related to *Mastogloia*. The same evidence has been acquired as

the result of our molecular investigation. In this study, we supplement the monophyly of Mastogloiales, comprising it of three genera – Aneumastus, Decussiphycus and Mastogloia. In fact, as our analysis demonstrate, genera of Mastogloiales sensu Cox (2015) scatter into three groups: Craspedostauros+Achnanthes clade, Tetramphora clade and Aneumastus+Mastogloia+-Decussiphycus (AMD) clade. According to molecular data, the latter group must be treated as the natural order Mastogloiales.

At the same time, interrelationships within the discussed *AMD* group are still fully obvious. Taxonomic composition of *Mastlogloia*, which is the most species-rich genus of the *AMD* clade (Loir and Navarino 2013), is of particular interest. *Mastlogloia* includes several species with unique morphological features, i.e. *Mastogloia fimbriata* (T.Brightwell) Grunow lacking external terminal raphe fissures and an apical septum (Pennesi et al. 2016), or *Mastogloia cyclops* Voigt, possessing a distinctive stigma (Stephens and Gibson 1980a). The latter case has been recently investigated by Kezlya et al. (2024) who utilized a combined analysis of valve ultrastructure and molecular data to propose a new genus – *Stigmagloia* Glushchenko, Kezlya, Kapustin & Kulikovskiy. Undoubtedly, further morphological and molecular investigations of different species-groups within *Mastogloia* (Stephens and Gibson 1980a, 1980b), could bring novel insights into the phylogeny of the genus itself, as well as the order Mastogloiales in general.

# Conclusions

Our research describes a new species, *Decussiphycus sinensis* sp. nov., and proposes a new combination – *Decussiphycus obtusus* comb. et stat. nov. The new species description is based on a thorough investigation of valve morphology by means of LM and SEM, supplemented with the results of a two-gene molecular analysis. Thus, the new species can be distinguished by a combination of valve features (i.e. valve outlines, shape of apices, ultrastructure of areolae) and molecular data. In addition, we have made an attempt to investigate the morphological and molecular boundaries of the order Mastogloiales and discuss its relations with genera *Craspedostauros* and *Achnanthes*, underscoring the need for further research in this field.

## Acknowledgements

The authors express their gratitude to senior researcher R.A. Rakitov (instrument analytics room of the Borissiak Paleontological Institute of the Russian Academy of Science, PIN RAS) for assistance in working with a scanning electron microscope.

Authors also express their gratitude to Wolf-Henning Kusber for his valuable advice in discussing nomenclature problems of genera *Delicatophycus* and *Decussiphycus*.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

Publication is based on research carried out with financial support by the National Natural Science Foundation of China (32470214) for culturing and LM and Russian Science Foundation (24-14-00165, https://rscf.ru/project/24-14-00165/ accessed on 22 November 2024) for molecular analysis and SEM, and by the framework of state assignment of the Ministry of Science and Higher Education of the Russian Federation (theme 122042700045-3) for finishing manuscript.

#### Author contributions

Conceptualization: MK, AM, AG. Data curation: AI, MK, AG. Formal analysis: AM, AG, EK. Funding acquisition: MK, YL. Investigation: AM. Methodology: YM. Project administration: YL. Supervision: YM, MK. Validation: EK, AI. Visualization: YM, AG. Writing - original draft: AM. Writing - review and editing: YM, YL, MK, EK.

## **Author ORCIDs**

Andrei Mironov I https://orcid.org/0000-0001-9936-0652 Anton Glushchenko I https://orcid.org/0000-0002-3876-3455 Elena Kezlya I https://orcid.org/0000-0002-5263-9338 Yevhen Maltsev I https://orcid.org/0000-0003-4710-319X Anton lurmanov I https://orcid.org/0000-0002-0270-8737 Yan Liu I https://orcid.org/0000-0001-8556-5040 Maxim Kulikovskiy I https://orcid.org/0000-0003-0999-9669

## **Data availability**

All relevant data can be found within the article text and its Supplementary files.

# References

- Ashworth MP, Lobban CS, Witkowski A, Theriot EC, Sabir MJ, Baeshen MN, Sabir JS, Jansen RK (2017) Molecular and morphological investigations of the stauros-bearing, raphid pennate diatoms (Bacillariophyceae): *Craspedostauros* EJ Cox, and *Staurotropis* TBB Paddock, and their relationship to the rest of the Mastogloiales. Protist 168(1): 48–70. https://doi.org/10.1016/j.protis.2016.11.001
- Cleve PT (1891) The diatoms of Finland. Acta Societatia pro Fauna et Flora Fennica 8(2): 1–68. https://doi.org/10.5962/bhl.title.64355
- Cox EJ (1987) *Placoneis* Mereschkowsky: The re-evaluation of a diatom genus originally characterized by its chloroplast type. Diatom Research 2(2): 145–157. https://doi.or g/10.1080/0269249X.1987.9704994
- Cox EJ (1999) *Craspedostauros* gen. nov., a new diatom genus for some unusual marine raphid species previously placed in *Stauroneis* Ehrenberg and *Stauronella* Mereschkowsky. European Journal of Phycology 34(2): 131–147. https://doi.org/10.1080/09670269910001736192
- Cox EJ (2015) Coscinodiscophyceae, Mediophyceae, Fragilariophyceae, Bacillariophyceae (Diatoms). In: Frey W (Ed.) Syllabus of plant families. Adolf Engler's Syllabus der Pflanzenfamilien. 13<sup>th</sup> edn. Photoautotrophic eukaryotic algae Glaucocystophyta, Cryptophyta, Dinophyta/Dinozoa, Heterokontophyta/Ochrophyta, Chlorarachniophy-

ta/Cercozoa, Euglenophyta/Euglenozoa, Chlorophyta, Streptophyta p.p. Borntraeger Science Publishers, Stuttgart, 64–103.

- da Silva WJ (2024) Is *Delicata* Krammer a validly published genus name? Trying to put light on Article 20.2. Taxon 00(5): 1–7. https://doi.org/10.1002/tax.13174
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/ nmeth.2109
- Daugbjerg N, Andersen RA (1997) A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbcL* sequence data. Journal of Phycology 33(6): 1031–1041. https://doi.org/10.1111/j.0022-3646.1997.01031.x
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): 214. https://doi.org/10.1186/1471-2148-7-214
- Edlund MB, Brant LA, Levkov Z, Nakov T (2006) An emended description of *Decussata* (Patrick) Lange-Bertalot & Metzeltin that includes protoplast organization and detailed valve and cingulum ultrastructure. Diatom Research 21(2): 269–280. https:// doi.org/10.1080/0269249X.2006.9705669
- Glushchenko AM, Kulikovskiy MS (2017) *Amphipleura vavilovii*: A new diatom species of the family Amphipleuraceae from Laos. Inland Water Biology 10(1): 17–21. https://doi.org/10.1134/S1995082916040064
- Glushchenko A, Kulikovskiy M, Kociolek JP (2017a) New diatom species from *Gomphonema subtile* group in Southeast Asia. Phytotaxa 329(3): 223–232. https://doi.org/10.11646/phytotaxa.329.3.3
- Glushchenko A, Kulikovskiy M, Okhapkin A, Kociolek JP (2017b) *Aneumastus laosica* sp. nov. and *A. genkalii* sp. nov. two new diatom species from Laos (Southeast Asia) with comments on the biogeography of the genus. Cryptogamie. Algologie 38(3): 1–17. https://doi.org/10.7872/crya/v38.iss3.2017.183
- Glushchenko A, Kulikovskiy MS, Kuznetsova IV, Kociolek JP (2019) Karthickia verestigmata gen. et. sp. nov. – an interesting diatom frustular morphology similar to several different cymbelloid genera. Phycologia 58(6): 605–613. https://doi.org/10.1080/00 318884.2019.1626605
- Glushchenko AM, Kociolek JP, Kuznetsova I, Kulikovskiy M (2020) Four new species from the genus *Kobayasiella* Lange-Bertalot from waterbodies of Southeast Asia. Diatom Research 35(4): 363–376. https://doi.org/10.1080/0269249X.2020.1842252
- Guillard RRL, Lorenzen CJ (1972) Yellow-green algae with chlorophyllide c1,2. Journal of Phycology 8(1): 10–14. https://doi.org/10.1111/j.0022-3646.1972.00010.x
- Guiry MD, Gandhi K (2019) *Decussiphycus* gen. nov.: A validation of "*Decussata*" (R.M. Patrick) Lange-Bertalot (Mastogloiaceae, Bacillariophyta). Notulae Algarum 94: 1–2. https://doi.org/10.1080/0269249X.2006.9705669
- Heinzerling O (1908) Der Bau der Diatomeenzelle mit besonderer Beruchsichtigung der ergastischen Gebilde und der Beziehung des Baues zur Systematik. Bibliotheca botanica, Stuttgart, 1–88.
- Hustedt F (1930) Bacillariophyta (Diatomeae) Zweite Auflage. In: Pascher A (Ed.) Die Süsswasser-Flora Mitteleuropas. Heft 10. Verlag von Gustav Fischer, Jena, 1–466.
- Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26(15): 1899–1900. https://doi.org/10.1093/bioinformatics/btq224
- Kezlya E, Glushchenko A, Kapustin D, Maltsev Y, Doan-Nhu H, Kulikovskiy M (2024) Stigmagloia lobbanii gen. et sp. nov. (Bacillariophyceae, Mastogloiales), a new stigma-bearing diatom genus separated from Mastogloia. Phytotaxa 677(1): 49–65. https://doi.org/10.11646/phytotaxa.677.1.2

- Kociolek JP, Stoermer EF (1988) A preliminary investigation of the phylogenetic relationships among the freshwater, apical pore field-bearing cymbelloid and gomphonemoid diatoms (Bacillariophyceae). Journal of Phycology 24(3): 377–385. https:// doi.org/10.1111/j.1529-8817.1988.tb00187.x
- Kociolek JP, Glushchenko A, Kulikovskiy M (2015) Typification, valve ultrastructure, and systematic position of *Gomphonema gomphopleuroides* Amosse ex Kociolek, Glushchenko & Kulikovskiy, an endemic diatom from Southeast Asia. Diatom Research 30(3): 247–255. https://doi.org/10.1080/0269249X.2015.1072583
- Krammer K (2002) *Cymbella*. In: Lange-Bertalot H (Ed.) Diatoms of Europe. Vol. 3. A.R.G. Gantner Verlag K.G., Ruggell, 1–584.
- Kulikovskiy M, Glushchenko A, Kociolek JP (2015) The diatom genus *Oricymba* in Vietnam and Laos with description of one new species, and a consideration of its systematic placement. Phytotaxa 227(2): 120–134. https://doi.org/10.11646/phytotaxa.227.2.2
- Kulikovskiy MS, Glushchenko AM, Genkal SI, Kuznetsova IV (2016) Identification book of diatoms from Russia. Filigran, Yaroslavl, 1–804.
- Kulikovskiy M, Glushchenko A, Kuznetsova I, Kociolek JP (2018) Description of the new freshwater diatom genus *Okhapkinia* gen. nov. from Laos (Southeast Asia), with notes on family Sellaphoraceae Mereschkowsky 1902. Fottea 18(1): 120–129. https://doi.org/10.5507/fot.2017.021
- Lange-Bertalot H (2000) Transfer to the generic rank of *Decussata* Patrick as a subgenus of *Navicula* Bory. Iconographia Diatomologica 9: 670–673.
- Lange-Bertalot H (2001) *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lato, *Frustulia*. Diatoms of Europe 2: 1–526.
- Lange-Bertalot H, Metzeltin D (1996) Indicators of oligotrophy. 800 taxa representative of three ecologically distinct lake types, carbonate buffered-Oligodystrophic-weakly buffered soft water with 2428 figures on 125 plates. Iconographia Diatomologica 2: 1–390.
- Liu Y, Kociolek JP, Glushchenko A, Kulikovskiy M, Fan Y (2018) A new genus of Eunotiales (Bacillariophyta, Bacillariophyceae: Peroniaceae), *Sinoperonia*, from Southeast Asia, exhibiting remarkable phenotypic plasticity with regard to the raphe system. Phycologia 57(2): 147–158. https://doi.org/10.2216/17-21.1
- Loir M, Novarino G (2013) Marine Mastogloia Thwaites ex W. Sm. and Stigmaphora Wallich species from the French Lesser Antilles. Koeltz Scientific Books, Königstein, 1–133.
- Maltsev E, Andreeva S, Podunaj J, Kulikovskiy M (2019) Description of *Aneumastus mongolotusculus* sp. nov. (Bacillariophyceae, Mastogloiales) from Lake Hovsgol on the basis molecular and morphological investigation. Nova Hedwigia. Beiheft 148: 21–33. https://doi.org/10.1127/nova-suppl/2019/035
- Mann DG (1984) An ontogenetic approach to diatom systematics. In: Mann DG (Ed.) Proceedings of the 7<sup>th</sup> Symposium on Recent and Fossil Diatoms. O. Koeltz, Koenigstein, 113–144.
- Mann DG (1999) The species concept in diatoms. Phycologia 38(6): 437–495. https:// doi.org/10.2216/i0031-8884-38-6-437.1

Meister F (1932) Kieselalgen aus Asien. Verlag von Gebrüder Borntraeger, Berlin, 56 pp. Mereschkowsky C (1903) Uber *Placoneis*, ein neues Diatomeen-Genus. Beihefte zum Botanischen Centralblatt 15(1): 1–30.

Mironov A, Glushchenko A, Maltsev Y, Genkal S, Kuznetsova I, Kociolek JP, Liu Y, Kulikovskiy M (2024) Reassessment of pore occlusion in some diatom taxa with re-evaluation of *Placoneis* Mereschkowsky (Bacillariophyceae: Cymbellales) and description of two new genera. PeerJ 12(e17278): 1–62. https://doi.org/10.7717/peerj.17278

- Patrick RM (1959) New species and nomenclatural changes in the genus *Navicula* (Bacillariophyceae). Proceedings of the Academy of Natural Sciences of Philadel-phia 111: 91–108.
- Pennesi C, Poulin M, Totti C (2016) Phylogenetic relationships and biogeography of the diatom genus *Mastogloia* (Bacillariophyceae): Revision of the section ellipticae including the description of new taxa. Protist 167(2): 148–173. https://doi.org/10.1016/j.protis.2016.02.003
- Round FE, Crawford RM, Mann DG (1990) The Diatoms. Biology & Morphology of the Genera. Cambridge University Press, Cambridge, 1–747.
- Ruck EC, Theriot EC (2011) Origin and evolution of the canal raphe system in diatoms. Protist 162(5): 723–737. https://doi.org/10.1016/j.protis.2011.02.003
- Stancheva R, Temniskova D (2006) Observations on *Decussata hexagona* (Torka) Lange-Bertalot (Bacillariophyta) from Holocene sediments in Bulgaria. Nova Hedwigia 82(1–2): 237–246. https://doi.org/10.1127/0029-5035/2006/0082-0237
- Stephens FC, Gibson RA (1980a) Ultrastructural studies of some *Mastogloia* (Bacillariophyceae) species belonging to the groups *Undulatae*, *Apiculatae*, *Lanceolatae* and *Paradoxae*. Phycologia 19(2): 143–152. https://doi.org/10.2216/i0031-8884-19-2-143.1
- Stephens FC, Gibson RA (1980b) Ultrastructural studies on some *Mastogloia* (Bacillariophyceae) species belonging to the group *Inaequales*. Journal of Phycology 16: 354–363. https://doi.org/10.1111/j.1529-8817.1980.tb03045.x
- Tseplik N, Maltsev Y, Glushchenko A, Genkal S, Mironova E, Kulikovskiy M (2024) New cavum-bearing *Planothidium* (Achnanthidiaceae, Bacillariophyceae) specie from the Kamchatka Peninsula, Russia. Phytotaxa 665(3): 271–281. https://doi.org/10.11646/phytotaxa.665.3.9
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Koeltz botanical books, Glashütten, 1–253. https://doi.org/10.12705/Code.2018
- Wynne MJ (2019) *Delicatophycus* gen. nov.: A validation of "*Delicata* Krammer" inval. (Gomphonemataceae, Bacillariophyta). Notulae Algarum 97: 1–3.
- Zimmermann J, Jahn R, Gemeinholzer B (2011) Barcoding diatoms: Evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. organisms Diversity & Evolution 11: 173–192. https://doi.org/10.1007/s13127-011-0050-6

## **Supplementary material 1**

# Alignment of the rbcL and SSU rRNA genes used for phylogenetic analyses in this study

#### Author: Andrei Mironov

Data type: txt

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.142654.suppl1

# **Supplementary material 2**

#### The Bayesian phylogenetic topology for the rbcL and SSU rRNA genes tree

Author: Andrei Mironov

Data type: txt

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.142654.suppl2

# PhytoKeys

**Short Communication** 

# *Ferula groessingii* (Apiaceae), a new synonym of *Ferula licentiana* var. *tunshanica*

Lei Yang<sup>1,2,3</sup>, Wen-Jun Li<sup>1,2,3</sup>

1 State Key Laboratory of Ecological Safety and Sustainable Development in Arid Lands, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

2 Xinjiang Key Lab of Conservation and Utilization of Plant Gene Resources, Urumqi, 830011, China

3 College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100049, China

Corresponding author: Wen-Jun Li (liwenjunao@ms.xjb.ac.cn)

#### Abstract

A comprehensive evaluation of the diagnostic characters employed in distinguishing *Ferula groessingii* from *F. licentiana* var. *tunshanica* has led to the conclusion that the two taxa are indeed conspecific. As a result, *F. groessingii* is hereby recognized as a new synonym of *F. licentiana* var. *tunshanica*. This reclassification is supported by a comprehensive comparison of taxonomic features, morphological evidence, and distribution data. The study confirms that key morphological traits including plant height, a hairy coat of stems and leaves, number of inflorescences, and fruit vittae, are critical for the identification of this species complex.

Key words: Apiaceae, China, Ferula, new synonym, taxonomy

# Introduction

*Ferula* L. is one of the largest genera in the Apiaceae family, encompassing approximately 220 species globally (https://powo.science.kew.org/results?q=ferula). It is mainly distributed in the southern part of Europe, Northern Africa, Central Asia, and the Mediterranean region, with Central Asia being the biodiversity hotspot of this genus (Pimenov and Leonov 1993).

*Ferula groessingii* Riedl & Riedl-Dorn was originally described as a new species endemic to China based on a single fruiting collection made by Licent from Taiqinggong in Qingdao, Shangong Province (Fig. 1). In the protologue, Riedl and Riedl-Dorn (1987) compared *F. groessingii* with *F. bungeana* Kitagawa (1956) and *F. rigidula* Candolle (1830), but they overlooked *F. licentiana* Handel-Mazzetti (1933) and *F. tunshanica* Anon. (Jiangsu Institute of Botany 1982), which are distributed in Shanxi, Shaanxi, Henan, Hebei, Hubei, Shandong, Anhui and Jiangsu provinces of China (Fig. 3). Furthermore, since its publication, the name has not received attention and was not included in the Flora Reipublicae Popularis Sinicae (Shen 1992) or in the Flora of China (She and Watson 2005). The name *F. groessingii* reappeared only in Pimenov's checklist of the Apiaceae



Academic editor: Alexander Sennikov Received: 3 January 2025 Accepted: 23 February 2025 Published: 18 March 2025

**Citation:** Yang L, Li W-J (2025) *Ferula groessingii* (Apiaceae), a new synonym of *Ferula licentiana* var. *tunshanica*. PhytoKeys 254: 21–27. https://doi. org/10.3897/phytokeys.254.145845

**Copyright:** © Lei Yang & Wen-Jun Li. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0)



Figure 1. The holotype specimen of F. groessingii (https://www.jacq.org/detail.php?ID=191435).

in China (Pimenov 2017). However, for the 30 years following its publication, no other literature mentioned this species name.

During our studies and exploration of the specimens of *Ferula* in China, we found that *F. groessingii* is morphologically very similar to *F. licentiana* var. *tunshanica*. The objective of this study is to confirm the relationship between *F. groessingii* and *F. licentiana* var. *tunshanica*.

# **Material and methods**

We collected specimens of Ferula groessingii from the type locality in 2023 to 2024. These specimens were deposited at the Herbarium of Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences (XJBI). For the detailed collection information, including coordinates, please refer to the "Specimens examined" section. We carefully checked and studied the specimens from NAS and PE in person, while materials from KUN and WUK were examined through photographs on the Chinese Virtual Herbarium (CVH) website (https://www.cvh.ac.cn/index.php). We also conducted detailed comparisons of F. licentiana var. tunshanica plant images available on the Plant Photo Bank of China (PPBC) website (https://ppbc.iplant.cn/ sp/24536). Photographs were taken in the field using a Nikon Z7 II camera. Hairs in the stems and leaves were examined using a dissecting microscope Phenix XTL-165 in the laboratory. In order to prepare tissue sections, the mature fruits were softened before being embedded in paraffin. Following staining, the sections were mounted with neutral balsam, observed under a microscope, and photographed for analysis. Additionally, we compared the protologue of F. groessingii with our observed results.

# Results

We found a clear resemblance between *F. groessingii* and *F. licentiana* var. *tunshanica* (Figs 1, 2, Table 1). Field sampling conducted at the type locality of *F. groessingii* and its detailed comparisons with *F. licentiana* var. *tunshanica* revealed that its characters are nearly identical. Key diagnostic morphological features such as the plant being glabrous throughout, with an average height of about 2 m, a solitary and slender stem, as well as the morphology of the inflorescence and fruit vittae were found to be consistent between two taxa (She and Watson 2005). The distribution of *F. groessingii* matches completely with that of *F. licentiana* var. *tunshanica*. The latter is predominantly found on sunny mountain slopes in Shandong, Anhui and Jiangsu provinces (Fig. 3). Consequently, based on these findings, we propose *F. groessingii* be treated as a synonym of *F. licentiana* var. *tunshanica*.

# Discussion

The scientific name *F. licentiana* var. *tunshanica* has an intriguing taxonomic backstory. It was initially published as *F. tunshanica* Su in the Flora of Jiangsu (Vol. 2: 935. 1982), where "Su" was explicitly stated to represent a collective author (Jiangsu Institute of Botany). Subsequent treatments, whether the invalid reduction to a variety by Liu et al. (1987) or the inadvertent valid publication (Shen 1992; Yu et al. 2010), correctly cited the name authorship. However, in the Flora of China (She and Watson 2005), "Su" was mistakenly interpreted as referring to Song-Wang Su (S.W. Su) who confirmed not to be the author of this taxon, a misinterpretation that has persisted to this day. With no traceable natural person attributable to the name and in accordance with ICN 2018 Art. 46.9 Ex. 45, the correct citation for *F. tunshanica* should be *Ferula tunshanica* Anon. (in Fl. Jiangsu 2: 935. 1982).



**Figure 2**. Morphology of *F. groessingii* **A** habitat **B** basal leaves **C** inflorescence **D** individual **E** flowering stage **F** cauline leaves **G** mericarps, dorsal and commissural aspects **H** cross section of mericarp: (a) median rib, (b) lateral rib, (c) marginal rib, (d) endosperm, (e) vallecular vitta, (f) commissure vitta. Scale bar: 1 mm.



**Figure 3.** Distribution of *F. licentiana*, *F. licentiana* var. *tunshanica* and *F. groessingii*. The map is based on a standard map downloaded from the National Natural Resources Ministry's Standard Map Service website. The boundaries of the base map have not been modified.

Characters	F. licentiana var. licentiana	F. licentiana var. tunshanica	F. groessingii
Habit	1.2–1.8 m	Smaller	1.5–1.7 m
Stem	stem solitary, slender, usually flexuose, paniculate-branched, lower branches alternate, upper branches verticillate		stem glabrous, with a diameter of only 10 mm near the base, branched from the upper part
Leaves	both surfaces glabrous, upper leaves reduced, bladeless, sheaths lanceolate, embracing		both surfaces of the leaves are smooth and glabrous
Inflorescences	terminal umbel short-pedunculate, lateral umbels 1–3, simple or opposite, exceeding terminal, rays 7–11, umbellules 7–11flowered.	rays fewer, 3–7	short peduncle, with 5–7 rays; lateral umbels are often opposite, with long peduncles that exceed the central umbel, and have 7–9 rays.
Fruit	oblong or oblong-obovate, 10–15 mm; lateral broadly winged	fruits are also smaller, less than 10 mm	elliptical or oblong, measuring 4.5 × 8 millimeters to 5.5 × 9 millimeters, with a thickness of 0.5–0.7 millimeters. The top of the fruit is slightly notched, with a filiform dorsal rib, and the vittae in each furrow are very broad.
Vittae	3–4 per furrow, 4–8 toward commissure	1–3 per furrow, 4–6 toward commissure	commissural vittae are 4-6

Table 1. Morphological comparisons of F. licentiana, F. licentiana var. tunshanica and F. groessingii.

#### **Taxonomic treatment**

*Ferula licentiana* var. *tunshanica* (Anon.) R.H.Shan & Q.X.Liu ex K.M.Shen, Fl. Reipubl. Popularis Sin. 55(3): 114 (1992).

- = Ferula tunshanica Anon. in Fl. Jiangsu 2: 935 (1982).
- = Ferula groessingii Riedl & Riedl-Dorn, Linzer Biol. Beitr. 19(2): 485 (1987), syn. nov. Type. CHINA. Shandong province: Qingdao city, Taiqinggong, 12 August 1936, Licent E 13413 (holotype W, image!)

**Type.** CHINA. • Jiangsu province: Tongshan, Maocun, 22 June 1974, Wen-Zhe Fang & Ping-Ping Ling et al. 74020 (holotype: NAS00042894!).

Specimens examined. CHINA. · Shandong: city. Qingdao, county Laoshan (36°08.17'N, 120°40.85'E), 18 Jun 2023, Wen-jun Li & Lei Yang LS20230618001 (XJBI,barcodeXJBI00162552!;XJBI00162553!;XJBI00162554!;XJBI00162555!; XJBI00162556!; XJBI00162557!); city. Jinan, Hushan Forest Park, 11 Jun 2015, Xiao-wei Xin Lilan794 (KUN, image! barcode KUN1480754); city. Jinan, Foyu Valley, 8 Jun 1977, Chang-gi Yuan 83 (NAS, barcode NAS00021792!; NAS00021793!; NAS00021795!; NAS00021796!; NAS00021801!); city. Jinan, 12 Jun 1964, s.n. 64036 (NAS, barcode NAS00021719!; NAS00021720!). · Henan: city. Lingbao, Jiaoyuan Valley, 27 Jun 1974, Zhi-xin Hu 17213 (WUK, image! barcode WUK0416132; WUK0416133; WUK0297545). • Hubei: city. Zaoyang, county Shangdang, mountain Yazishan, 13 Jun 2018, Shen-lan Li GanQL1240 (KUN, image! barcode KUN1457994). · Jiangsu: mountain Tongshan, 10 Jul 1976, Ren-hua Shan 7607E (NAS, barcode NAS00021800!). · Shannxi: mountain Huashan, Yuquanyuan, 26 May 1956, Kun-jun Fu & Ben-zhao Guo 10101 (WUK, image! barcode WUK0092684); county Huayin, mountain Huashan, 24 Jun 1974, Kun-jun Fu 17207 (WUK, image! barcode WUK0416139; WUK0416140; WUK0297551); mountain Huashan, Suoluopin, 10 May 1961, Zhiwuxi 104 (WUK, image! barcode WUK0483271); mountain Huashan, Yukou, 1 Jun 1956, Kun-jun Fu & Ben-zhao Guo 10148 (WUK, image! barcode WUK0099592); county Huayin, Xianyu, 23 Jun 1974, Kun-jun Fu 17205 (WUK, image! barcode WUK0416128; WUK0416129; WUK0297553); county

Huayin, Huayingongshe, 28 Jun 1974, Kun-jun Fu 17215 (WUK image!, barcode WUK0416130; WUK0416131). • **Shanxi**: county Huairen, 24 May 1938, W.Y.Hsia 4373 (PE, barcode PE00756177!).

# Acknowledgments

Our sincere thanks to the staff of NAS, W, XJBI, KUN, WUK, PE, CVH and PPBC for their help in specimen examination and Dr. Hafiz Muhammad Wariss for modifying the English in this article.

# **Additional information**

## **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

# Funding

This study was supported by grants from the Western Young Scholars Project of the Chinese Academy of Sciences (2022-XBQNXZ-003), the Tianshan Youth Program of Xinjiang Uygur Autonomous Region (2022TSYCCX0088), the Capacity building project of strategic biological resources program of Chinese Academy of Sciences (KFJ-BRP-017-089).

## Author contributions

Conceptualization: WJL. Data curation: LY, WJL. Writing – original draft: LY. Writing – review and editing: WJL

## **Author ORCIDs**

Lei Yang <sup>(i)</sup> https://orcid.org/0009-0003-8888-5893 Wen-Jun Li <sup>(i)</sup> https://orcid.org/0000-0002-2932-0783

## **Data availability**

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

# References

Candolle AP (1830) Prodromus systematis naturalis regni vegetabilis, sive, Enumeratio contracta ordinum generum specierumque plantarum huc usque cognitarium, juxta methodi naturalis, normas digesta, vol. 4. Treuttel et Würtz, Paris, 683 pp.

 Handel-Mazzetti H (1933) Kleine Beiträge zur Kenntnis der Flora von China. Österreichische. Botanicheskii Zhurnal 82: 245–254. http://www.jstor.org/stable/43335945
Jiangsu Institute of Botany (1982) Flora of Jiangsu, vol. 2. Phoenix Science Press, Nanjing.

- Kitagawa M (1956) Notulae fractae ob foram Asiae orientalis (9). Shokubutsu Kenkyu Zasshi 31: 302–306. https://doi.org/10.51033/jjapbot.31\_10\_4049
- Liu QX, Shan RH, Sheh ML (1987) Study on the species of genus *Ferula* L. in north and east China. In: Nanjing Botanical Garden, Mem. Sun Yat Sen (Ed.) Bulletin of the Nanjing Botanical Garden, Mem. Sun Yat Sen. Phoenix Science Press, Nanjing, 27–38.

- Pimenov MG (2017) Updated checklist of Chinese Umbelliferae: Nomenclature, synonymy, typification, distribution. Turczaninowia 20(2): 106–239. https://doi. org/10.14258/turczaninowia.20.2.9
- Pimenov MG, Leonov MV (1993) The genera of the Umbelliferae: a nomenclator. Royal Botanic Gardens, Kew, UK, 156 pp.
- Riedl H, Riedl-Dorn C (1987) Der Verwandtschaftskreis von *Ferula rigidula* De Candolle in China. Linz Biological Contributions 19: 483–494.
- She ML, Watson MF (2005) *Ferula*. In: Wu, ZY, Raven PH, Hong DY (Eds) Flora of China, vol. 14. Science Press, Beijing, 174–181.
- Shen GM (1992) *Ferula*. In: Shan RH, She ML (Eds) Flora Reipublicae Popularis Sinicae, vol. 55. Science Press, Beijing, 85–120.
- Yu WB, Peng XM, Li JP (2010) Nomenclatural notes on *Ferula tunshanica* (= *F. licentiana* var. *tunshanica*) (Apiaceae). Phytotaxa 13(1): 59–60. https://doi.org/10.11646/phytotaxa.13.1.6



**Research Article** 

# A new species of *Pleurothallis* (Pleurothallidinae, Orchidaceae) from the historic sanctuary of Machupicchu, Perú

Alexander Damián-Parizaca<sup>1,2®</sup>, Marco Federico Monteros<sup>3,4,5,6®</sup>, Daxs Coayla Rimachi<sup>2</sup>, Joseph Walston<sup>1®</sup>, Nicole Mitidieri-Rivera<sup>1®</sup>

- 1 Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, Wisconsin, USA
- 2 Inkaterra Asociacion Victor Larco Herrera, 130 Miraflores, Lima, Peru
- 3 Fundacion EcoMinga, Mariscal Foch 7-21 y Juan León Mera, Quito, Ecuador
- 4 Reserva: The Youth Land Trust, Washington, D.C., USA
- 5 Instituto Nacional de Biodiversidad (INABIO), Rumipamba 341 y Av. De los Shyris, Quito, Ecuador
- 6 Grupo Científico Calaway Dodson: Investigación y Conservación de Orquídeas del Ecuador, Quito, 170510, Pichincha, Ecuador

Corresponding author: Alexander Damián-Parizaca (adamian.pz@gmail.com)

#### Abstract

*Pleurothallis machupicchuensis*, a new species from Cusco, Perú, is described and illustrated. Information regarding its distribution, habitat, and phenology is provided. Morphologically, *P. machupicchuensis* closely resembles *P. scurrula* and *P. sannio* but can be distinguished mainly by the morphology of the lip. The new species features an ovate, obtuse lip with a prominent bilobed, reniform glenion, in contrast to the oblong-ovate, acute lip with a small rounded glenion observed in *P. scurrula*, and the ovate, rounded lip with an oblong glenion in *P. sannio*.

Key words: Andes, biodiversity, Cusco, Inkaterra

#### Introduction

The genus Pleurothallis R. Br. is among the largest groups within the subtribe Pleurothallidinae Lindl. comprising nearly 500 recognized species, with new taxa being described regularly (e.g. Karremans and Vieira-Uribe 2020). Classifying such a megadiverse group has posed significant challenges to numerous botanists, including Lindley (1842, 1859) and Luer (1998, 1999, 2005), who proposed the earliest and most recent morphology-based classification systems of Pleurothallis. As noted by Karremans (2016), both Lindley and Luer acknowledged the limitations of their classifications, recognizing the absence of more satisfactory solutions given the available data. A major breakthrough in the classification of Pleurothallis was achieved with the work of Pridgeon and colleagues (Pridgeon et al. 2001), who introduced the first DNA-based classification system for Pleurothallidinae. This molecular approach demonstrated that a natural classification of Pleurothallis was attainable, although it required extensive modifications to Luer's circumscription. Subsequent revisions, initiated by Pridgeon himself (Pridgeon and Chase 2001) and expanded upon by various authors over the past two decades (e.g. Karremans et al. 2013; Karremans 2019), have successfully



Academic editor: Timothée Le Péchon Received: 19 November 2024 Accepted: 12 February 2025 Published: 20 March 2025

**Citation:** Damián-Parizaca A, Monteros MF, Coayla Rimachi D, Walston J, Mitidieri-Rivera N (2025) A new species of *Pleurothallis* (Pleurothallidinae, Orchidaceae) from the historic sanctuary of Machupicchu, Perú. PhytoKeys 254: 29–40. https://doi.org/10.3897/ phytokeys.254.142116

**Copyright:** <sup>©</sup> Alexander Damián-Parizaca et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). established the monophyly of *Pleurothallis* by refining its circumscription. These efforts have also clarified its phylogenetic placement within a well-supported clade that includes *Pabstiella* and *Stelis*. However, the infrageneric relationships within *Pleurothallis* remain largely unresolved (Pridgeon 2005; Karremans 2016).

One of the most challenging groups to circumscribe within *Pleurothallis* is *Pleurothallis* sect. *Macrophyllae-Fasciculatae* Lindl. Originally established by Lindley (1859) to include species characterized by terete-angulate stems, cordate leaves, and fasciculate flowers, this section was later incorporated into a broader circumscription as a section within *Acronia* C. Presl by Luer (2005). Recent phylogenetic studies utilizing Sanger-based DNA sequencing have demonstrated that species assigned to Luer's *Acronia* are distributed across multiple clades, indicating its non-monophyly (Wilson 2011; 2013; Pridgeon et al. 2011). These findings have also uncovered a highly intricate evolutionary history, shaped by its recent diversification (~5 Ma) and natural hybridization events within the group (Pérez-Escobar et al. 2017; Pupulin et al. 2021). Given the absence of a robust phylogenetic framework for *Pleurothallis*, recent authors have increasingly treated *Pleurothallis* sect. *Macrophyllae-Fasciculatae* as an informal assemblage or a collection of morphological complexes rather than a monophyletic, phylogenetically well-defined group (Pupulin et al. 2021; Wilson et al. 2022).

Although *Macrophyllae-Fasciculatae* continues to be referenced in the literature, either at the subsectional (e.g., Baquero et al. 2024; Sierra-Ariza 2024) or sectional (Belfort-Oconitrillo et al. 2024) level within *Pleurothallis*, we concur with previous authors that it is more appropriate to recognize this group as an assemblage of species complexes with poorly defined boundaries. Accordingly, in this manuscript, we adopt the definition proposed by Pupulin et al. (2021), who described members previously assigned to *Pleurothallis* sect. *Macrophyllae-Fasciculatae* as a group of *Pleurothallis* species distinguished by their tall growth habit and fasciculate inflorescences borne above the leaf from a spathaceous, occasionally erect bract. However, it is crucial to point out that recent analyses have questioned the suitability of the term "fasciculate" in describing the inflorescences of this group, suggesting instead that these structures are more accurately interpreted as single-flowered co-florescences (Rojas-Alvarado and Karremans 2024).

The absence of a well-defined circumscription presents a significant challenge in assessing the true diversity of this group. However, previous estimates suggest it comprises approximately 300 species endemic to the Neotropics, occupying a broad range of biomes and ecosystems, from sea level to elevations exceeding 3,000 meters (Pridgeon 2005; Wilson et al. 2022). Despite its extensive distribution, the highest species diversity is believed to occur in South America, particularly in the Central and Northern Andes. This is reflected in the frequent discovery of new species and records from Andean countries (e.g., Jiménez et al. 2023; Ocupa-Horna et al. 2023a). While some regions, such as Colombia (Karremans et al. 2023), have recently published comprehensive or updated assessments of Pleurothallidinae diversity, Perú still lacks a modern taxonomic assessment for this group. For instance, Luer's monograph on Acronia sect. Macrophyllae-Fasciculatae (Lindl.) Luer recorded approximately 30 species for Perú (Luer 2005). However, as demonstrated in other orchid genera native to the country (e.g., Lepanthes [Ocupa-Horna et al. 2023b]; Acianthera [Damián-Parizaca et al. 2018]), this estimate is likely incomplete. Ongoing field exploration and taxonomic research are anticipated to reveal many additional species.

One of Perú's most orchid-rich sites is the Historic Sanctuary of Machu Picchu (SHM), a protected area encompassing approximately 30,000 hectares and home to around 400 orchid species (Collantes et al. 2007). This diversity continues to be revised, with new species reported regularly (e.g., *Stelis machupicchuensis*, Collantes et al. 2017; *Telipogon machupicchuensis*, Nauray and Christenson 2003). During the 7<sup>th</sup> Scientific Conference on Andean Orchids, held in Machupicchu Pueblo in November 2024, one of the authors (MM) observed an undescribed *Pleurothallis* species growing in the garden of the Inkaterra Machu Picchu Pueblo Hotel. This species is formally described herein, along with a detailed comparison to its closest morphological relatives.

# Materials and methods

To assess whether the plant observed at the Inkaterra Machupicchu Pueblo Hotel represents an undescribed species, we conducted a detailed morphological analysis. Initially, we compared Pleurothallis taxa previously described and recorded in Perú, subsequently expanding our assessment to include other Andean taxa. Given the absence of an updated taxonomic treatment of species formerly assigned to Pleurothallis sect. Macrophyllae-Fasciculatae we relied primarily on Luer's monograph of Acronia, supplementing it with a review of recently described Pleurothallis species published post-Luer. Recognizing that vegetative traits often provide limited diagnostic utility for distinguishing closely related species, we focused our analysis on floral characteristics. Morphological descriptions follow the framework of Wilson et al. (2022) complemented by the updated inflorescence terminology for Pleurothallidinae proposed by Rojas-Alvarado and Karremans (2024). Additionally, we conducted a preliminary herbarium review using the Tropicos.org, Global Biodiversity Information Facility (GBIF), and Atrium databases. Many herbarium specimens lacked flowers or were too small to exhibit diagnostic traits aligning with our concept of the proposed new species. A physical examination of the CUZ herbarium similarly failed to uncover any specimens resembling P. machupicchuensis inedit.

To supplement our analysis, we searched for live photographs of *Pleurothallis* across several databases, including GBIF, iNaturalist, Flickr, and Atrium. When available, the metadata accompanying these photographs was collected and incorporated into our downstream analysis. Distribution maps were generated in R Studio using a modified script from Damián-Parizaca and Mitidieri-Rivera (2023). For specimens lacking coordinates, we approximated collection locations based on label or protologue information. The final maps were edited in Adobe Photoshop® v.22.5.1. Line drawings, including both line work and stippling, were produced from photographs of dissected perianths using a Wacom Intuos Wireless Graphics Tablet in Adobe Photoshop CS6.v13. Additionally, a Lankester Composite Dissection Plate (LCDP) was created from macro photographs taken with a Nikon D810 camera, and the images were subsequently edited and arranged in Adobe Photoshop.

To further investigate labellum morphology, we used Scanning Electron Microscopy (SEM). Two flowers were dehydrated through a stepwise transition from 70% to 100% ethanol, with fifteen-minute increments at 80%, 95%, and 100%, followed by critical-point drying with a Leica EM CPD300. The samples were mounted onto aluminium SEM stubs, sputter-coated with platinum-palladium

using a Leica EM ACE600, and imaged with a FEI Quanta 200 scanning electron microscope at 20 kV accelerating voltage and a 10–12 mm working distance. SEM imaging was conducted at the Newcomb Imaging Center, Department of Botany, University of Wisconsin–Madison.

# Results

#### **Taxonomic treatment**

*Pleurothallis machupicchuensis* Damián-Parizaca, Monteros & Coayla, sp. nov. urn:lsid:ipni.org:names:77358955-1 Figs 1, 2, 3A, 4

**Type.** PERÚ • Cusco, Prov. Urubamba, Aguas Calientes, property of the Machupicchu Pueblo Hotel, 2000 m, 30 November 2023, *Daxs Coayla 001* (*holotype*: CUZ).

**Diagnosis.** *Pleurothallis machupicchuensis* is most similar to *Pleurothallis scurrula* Luer but differs by the obtuse dorsal sepal (vs. acute), the acute synsepal apex (vs. obtuse to rounded), the falcate petals (vs. oblong-ovate), the ovate lip (vs. oblong), and the reniform bilobed glenion (vs. oblong non bilobed).

Description. Plant epiphytic, caespitose, erect 15 cm tall. Roots slender, flexuose, up to 0.1 cm in diameter. Ramicauls erect, 10-15 cm long, 0.1 cm in diameter, slightly curved at the apex forming an angle of about 100° in the abscission layer, enclosed by two basal papyraceous, sulcate, brownish, tubular sheaths, 4.0-6.5 cm long. Leaf borne at the apex of the ramicaul, suberect to nearly horizontal, pale green, slightly coriaceous, deflexed toward the base, somewhat concave, lanceolate, with a short concavity at the base of the spathe, margins entire, acuminate, 5.0-7.7 × 2.0-3.0 cm, base sessile, cordate, lobes equal. Inflorescence a single-flowered coflorescence borne erect from a depressed, conduplicate, oblong, obtuse, sub-erect spathe at the base of the leaf, 0.8-1.0 cm long, striate, brownish, dry-papyraceous when mature, concealing peduncle, branch system, pseudopeduncle and pedicel; pseudopeduncle terete, up to 0.4 cm long; pedicel yellowish, flexuose, terete, 1.0-1.5 cm long; ovary terete, blackish to brownish, furrowed, 0.5-0.7 cm long. Flowers non-resupinate, spreading, yellowish to citrine colored, column yellowish to whitish, anther cap and stigma yellowish, lip overall yellowish sometimes with reddish margins. Dorsal sepal erect slightly convex, ovate, glabrous, obtuse, 3-veined, 1.0 × 0.5 cm. Lateral sepals connate into a broadly ovate synsepal, centrally concave-channeled, obtuse, glabrous, margins entire, 0.8 × 0.6-0.7 cm, 4-veined. Petals strongly reflexed, falcate, 1-veined, conspicuously papillate on the margins,  $0.6-0.7 \times 0.1$  cm. Labellum overall ovate, obtuse at the apex, slightly ascending, strongly verrucose-bullate towards the margins, papillae grouped into clumps of around 5-6 papillae, clumps join together to form nearly horizontal linear groups that follow down to the base, centrally sulcate, base acutely deflexed upon itself hinged to the column-foot, 0.3 × 0.2 cm; glenion bilobed, hourglass-shaped, ca. 400 µm wide, ca. 150 µm at its shortest, and ca. 250 µm at its longest length, papillae at the glenion boundary smooth and notably larger and taller, width 25 µm and length 35 µm, papillae found outside of the glenion are highly textured, width 25 µm. Column short, stout, complanate, 0.2 cm long, minutely papilose, rostellar flap long, linear, obtuse. Anther apical, incumbent, anther cap



Figure 1. Composite digital plate of *Pleurothallis machupicchuensis* **A** habit **B** flower **C** lip and column in lateral view **D** dissected flower **E** lip adaxial view **F** anther and pollinia **G** petal margin. Prepared from the holotype by Alexander Damián-Parizaca.



Figure 2. A map of known localities of *Pleurothallis machupicchuensis* and *Pleurothallis scurrula* B panoramic view of the Machu Picchu Historical Sanctuary C Inkaterra Machu Picchu Pueblo Hotel Garden. Photographs by (B) Inkaterra, (C) Daxs Coayla.

cucullate, ovate, 2-celled, 0.8 × 0.6 mm. *Stigma* apical, bilobed, reniform. *Pollinia* two, narrowly pyriform, 0.1 cm long attached to an elliptic viscidium. *Fruit* unknown.

**Phenology.** This species has been observed flowering in two seasons: April and from October to December.

**Etymology.** The epithet honors the *llaqta* Machupicchu, an Inka citadel in Cusco, southern Peru, located within the Urubamba Province, where *P. machupicchuensis* is locally distributed.

**Distribution and habitat.** *Pleurothallis machupicchuensis* primarily grows on trees of the genus *Clusia* L. within typical montane forest vegetation. Known populations are located along the Urubamba and Usmubamba Rivers in the provinces of Urubamba and La Convención, respectively, as well as within the Machu Picchu Historic Sanctuary, at elevations ranging from 2000 to 2500 meters (Fig. 2).



**Figure 3.** Line drawings of the species treated in the present study **A** *P. machupicchuensis* prepared by MM based on the holotype **B** *P. phyllocardioides* (=*P. graciliscapa*) **C** *P. sannio* **D** *P. scurrula.* (**B**–**D**) from Luer (2005) courtesy of the Missouri Botanic Gardens Press.



Figure 4. Scanning electron micrographs of *Pleurothallis machupicchuensis* lip **A** general view of the lip **B** detail closeup of the superior part of the lip which overlaps with the column **C**, **D** glenion **E**, **F** lateral sides of the lip. Prepared from holotype *D*. *Coayla 001* (CUZ) by Joseph Walston.

**Conservation status.** Although the known distribution of *Pleurothallis mach-upicchuensis* is within the Machu Picchu Sanctuary, sufficient information is still lacking to assess its preliminary conservation status. Therefore, we recommend classifying this species as Data Deficient (DD) according to the IUCN (2022) criteria. **Taxonomic notes.** *Pleurothallis machupicchuensis* is readily distinguished by the combination of a large dorsal sepal (relative to the synsepal), non-resupinate
flowers, falcate petals, and an ovate lip that is conspicuously sulcate and features a prominent bilobed glenion (Figs 1, 2A). The species was first photographed in 1998 at the Inkaterra Pueblo Hotel and has since been mistakenly identified as Pleurothallis phyllocardioides Schltr. (Collantes et al. 2007; Ochoa 2023). Luer (2005) recognized P. phyllocardioides as part of a species complex widely distributed from Central America throughout the Andes. This complex is characterized by having short and non-reflexed petals measuring 2-3 mm, and an ovate-oblong lip with an unlobed glenion. These features differ significantly from strongly reflexed petals measuring 6-7 mm, and the ovate lip with a bilobed glenion observed in P. machupicchuensis (Fig. 3A, B). Furthermore, Andean populations of P. phyllocardioides reported by Luer (2005) are found in submontane forests below 1200 m altitude, whereas P. machupicchuensis has so far been documented exclusively in montane forests above 2000 m. Additionally, P. phyllocardioides appears to be restricted to Central America (M. Wilson, pers. comm.), and Peruvian populations previously described as Pleurothallis graciliscapa C. Schweinf. (= P. phyllocardioides sensu Luer 2005) show minimal divergence from the typical Central American morphotype, rendering their resemblance to P. machupicchuensis less plausible.

The Peruvian *Pleurothallis scurrula* is the most morphologically similar species to *P. machupicchuensis* but can be readily distinguished, primarily by its petal shape. In *P. scurrula*, the petals are oblong-ovate, whereas in *P. machupicchuensis*, they are conspicuously falcate. The two species are also differentiated by the morphology of the labellum, which is clearly rounded in *P. scurrula* and obtuse in *P. machupicchuensis*. Furthermore, *P. machupicchuensis* possesses a conspicuous bilobed glenion, contrasting with the minute, rounded glenion of *P. scurrula*. Another morphologically similar species is the Colombian *Pleurothallis sannio* Luer & R. Escobar, which can be distinguished by its slightly shorter flowers, with sepals measuring 8 mm in length (versus 10 mm in *P. machupicchuensis*), and its obovate synsepal (versus ovate). Most notably, *P. sannio* lacks a bilobed glenion and a central furrow on the lip, features that are diagnostic of *P. machupicchuensis* (Table 1, Fig. 3C).

**Other records.** PERÚ • Cusco, Prov. Urubamba, Aguas Calientes, Catarata de Mandor, December 20, 2021 [flower] https://www.inaturalist.org/observations/103258849; • ibid. January 5, 2013, [flower] https://www.inaturalist.org/observations/96696628; • Prov. La Convencion, Usmabamba, February 10, 2020

	P. machupicchuensis	P. sannio	P. scurrula
Leaf shape	lanceolate, apex acuminate	narrowly ovate, apex acute	ovate, apex acute
Leaf dimensions (cm)	5-7.7 × 2-3	9−10 × 2.5−3 cm	5-8 × 2-3
Spathe dimensions (cm)	0.8-1.0	1.5	1-1.5
Dorsal sepal shape	ovate, apex obtuse	ovate, apex obtuse	ovate-triangular, apex acute
Dorsal sepal dimensions (mm)	10 × 5	8 × 5.5	7 × 9
Synsepal shape	ovate, apex obtuse	obovate	ovate, apex rounded
Synsepal-dimensions (mm)	8 × 6-7	8 × 4	7 × 9
Petals shape	falcate, apex acuminate	obliquely triangular-ovate, apex acute	oblong-ovate, apex acute
Petals dimensions (mm)	6-7 × 1	6 × 2	7 × 3
Lip shape	ovate, apex obtuse	ovate, apex rounded	oblong-ovate, apex rounded
Lip dimensions (mm)	3×2	3 × 2.5	3 × 2
Glenion	hourglass-shaped, bilobed	oblong	rounded

Table 1. Morphological comparison of Pleurothallis species morphologically similar to P. machupicchuensis.

[flower] https://www.inaturalist.org/observations/68871779; • Prov. Urubamba, Machu Picchu Historic Sanctuary, 1998, reported by the Inkaterra Association Research Team, pl. 114 as *Pleurothallis phyllocardioides* Schlechter (Collantes et al. 2007); • Machupicchu Historical Sanctuary, at 1900–2500 m, March 24, 2012 [flower] https://bit.ly/3WtKfEB; • Cusco, Aguas Calientes, 26 April 2010 [flower] https://bit.ly/4ao1Vak.

## Acknowledgments

AD acknowledges the Department of Botany at the University of Wisconsin-Madison, particularly Kenneth Cameron, for financial support to attend the 7<sup>th</sup> Scientific Conference on Andean Orchids. We extend our gratitude to Mario Sanchez for his assistance in revising *Pleurothallis* specimens at CUZ and to Mark Wilson for corroborating the identity of the newly proposed species and providing valuable feedback on an earlier version of this manuscript. Finally, we sincerely appreciate the insightful comments and suggestions from Gustavo Rojas and an anonymous reviewer, which greatly enhanced the final version of this work.

## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This work was supported by the Department of Botany at the University of Wisconsin-Madison, particularly Kenneth Cameron.

#### **Author contributions**

ADP: writing original manuscript, finance support, data collection, field investigation. MM: field investigation, data collection. DC: field investigation. JW: Resources. NM: Writing, review & editing.

## Author ORCIDs

#### **Data availability**

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

## References

Baquero L, Monteros M, Iturralde G, Jimenez M, Dueñas R (2024) *Pleurothallis markgruinii*, a new Pleurothallidinae (Orchidaceae) from Northwestern Ecuador. Edinburgh Journal of Botany 81: 1–18.

- Belfort-Oconitrillo N, Salguero G, Oses L, Gil-Amaya K, Rojas-Alvarado G, Chinchilla IF, Diaz-Morales M, Pupulin F, Bogarin D, Karremans AP (2024) New species and records of Orchidaceae from Costa Rica IV. Lankesteriana 24(2): 141–192. https://doi. org/10.15517/lank.v24i2.60686
- Collantes B, Soto C, Koechlin J (2007) Orchids INKATERRA at Machupicchu-Pueblo Hotel. Inkaterra Asociacion, Lima, Perú, 338 pp.
- Collantes B, Farfán J, Martel C (2017) *Stelis machupicchuensis* (Orchidaceae), a new species from S Peru. Willdenowia 47(2): 167–174. https://doi.org/10.3372/wi.47.47208
- Damián-Parizaca A, Mitidieri-Rivera N (2023) *Vanilla cameroniana* (Orchidaceae, Vanilloideae), a new species from French Guiana and new records from the Guiana Shield. Phytotaxa 609(3): 222–232. https://doi.org/10.11646/phytotaxa.609.3.5
- Damián-Parizaca A, Mitidieri-Rivera N, Chiron G (2018) A taxonomic synopsis of *Acianthera* (Orchidaceae Pleurothallidinae) in Perú, including two new species. Anales del Jardín Botánico de Madrid. Real Jardín Botánico 75(1): 1–21. https://doi.org/10.3989/ajbm.2449
- IUCN (2022) Guidelines for Using the IUCN Red List Categories and Criteria, version 15.1. http://www.iucnredlist.org/documents/RedListGuidelines.pdf [accessed 10.11.2024]
- Jiménez MM, Velez-Abarca L, Mashendo-Jimbicti V, Garzón-Suárez X, Monteros MF, Wilson M (2023) Five new species of *Pleurothallis* (Orchidaceae: Pleurothallidinae) in subsection *Macrophyllae-Fasciculatae* from Southeastern Ecuador. Phytotaxa 607(3): 161–181. https://doi.org/10.11646/phytotaxa.607.3.1
- Karremans AP (2016) Genera Pleurothallidinarum: An updated phylogenetic overview of Pleurothallidinae. Lankesteriana 16(2): 219–241. https://doi.org/10.15517/lank. v16i2.26008
- Karremans AP (2019) To be, or not to be a *Stelis*. Lankesteriana 19(3): 281–343. https:// doi.org/10.15517/lank.v19i3.40082
- Karremans AP, Vieira-Uribe S (2020) Pleurothallids Neotropical Jewels Vol. I. Imprenta Mariscal, Quito, 320 pp.
- Karremans AP, Bakker FT, Pupulin F, Solano-Gómez R, Smulders MJ (2013) Phylogenetics of *Stelis* and closely related genera (Orchidaceae: Pleurothallidinae). Plant Systematics and Evolution 299(1): 151–176. https://doi.org/10.1007/s00606-012-0712-7
- Karremans A, Moreno JS, Gil-Amaya K, Gutiérrez Morales N, Espinosa F, Mesa S, Restrepo E, Rincón-González M, Serna A, Sierra-Ariza M, Vieira-Uribe S (2023) Colombian Orchidaceae: A catalogue of the Pleruthallidinae. Lankesteriana 23(2): 181–400. https://doi.org/10.15517/lank.v23i2.56158
- Lindley J (1842) Pleurothallis. Edwards's Botanical Register 28: 67-84.
- Lindley J (1859) Folia Orchidacea. An enumeration of the known species of orchids. *Pleurothallis* 9–12. Published for the author by J. Matthews, London.
- Luer CA (1998) Icones Pleurothallidinarum V: Systematics of subgen. *Pleurothallis* sect. *Abortivae*, sect. *Truncatae*, sect. *Pleurothallis*, subsect. *Acroniae*, subsect. *Pleurothallis*, subgen. *Dracontia*, subgen. *Anciferia*. Monographs in Systematic Botany from Missouri Botanical Garden 72: 1–121.
- Luer CA (1999) Icones Pleurothallidinarum XVIII. Systematics of *Pleurothallis*, subgen. *Pleurothallis*, Sect. *Pleurothallis*, Subsect. *Antenniferae*, Subsect. *Longiracemosae*, Subsect. *Macrophyllae-Racemosae*, Subsect. *Perplexae*, Subgen. *Pseu-dostelis*, Subgen. *Acuminatia*. Addenda to *Dracula*, *Lepanthes*, *Masdevallia* and *Pleurothallis*. Miscellaneous new species of *Dryadella*, *Lepanthes* and *Pleurothallis*. Monographs in Systematic Botany from the Missouri Botanical Garden 76: 1–182.

- Luer CA (2005) Icones Pleurothallidinarum XXVII: *Dryadella* and *Acronia* section *Macro-phyllae-Fasciculatae*. In: Monographs in Systematic Botany from Missouri Botanical Garden 103: 1–311.
- Nauray W, Christenson EA (2003) *Telipogon* from Perú. Two New Species from Machupicchu and Environs. Orchids 72: 696–698.
- Ochoa J (2023) Las Orquideas de Machupicchu-Guia de Campo. Grafica IBF EIRL, Cusco, Perú, 258 pp.
- Ocupa-Horna L, Wilson M, Jimenez M (2023a) A new species of *Pleurothallis* (Orchidaceae: Pleurothallidinae) in subsection *Macrophyllae-Fasciculatae* found in Carpish Montane Forest Regional Conservation Area, Perú. Phytotaxa 629(2): 163–175. https://doi.org/10.11646/phytotaxa.629.2.6
- Ocupa-Horna L, Thoerle L, Schuiteman A, Martel C (2023b) A revised checklist of *Lepanthes* (Orchidaceae) from Perú, including new country records. Brittonia 75(2): 159–179. https://doi.org/10.1007/s12228-022-09737-5
- Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke NJ, Antonelli A (2017) Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. The New Phytologist 215(2): 891–905. https://doi.org/10.1111/nph.14629
- Pridgeon AM, Solano R, Chase MW (2001) Phylogenetic relationships in Pleurothallidinae (Orchidaceae): Combined evidence from nuclear and plastid DNA sequences. American Journal of Botany 88(12): 2286–2308. https://doi.org/10.2307/3558390
- Pridgeon AM (2005) Subtribe Pleurothallidinae. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (Eds) Genera Orchidacearum. Vol. 4 Epidendroideae (Part One). Oxford University, Oxford, 319–422. https://doi.org/10.1093/oso/9780198507123.001.0001
- Pridgeon AM, Chase MW (2001) A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). Lindleyana 16(4): 235–271.
- Pupulin F, Aguilar J, Belfort-Oconitrillo N, Díaz-Morales M, Bogarín D (2021) Florae Costaricensis subtribui Pleurothallidinis (Orchidaceae) Prodromus II. Systematics of the *Pleurothallis cardiothallis* and *P. phyllocardia* groups, and other related groups of Pleurothallis with large vegetative habit. Harvard Papers in Botany 26(1): 203–295. https://doi.org/10.3100/hpib.v26iss1.2021.n14
- Rojas-Alvarado G, Karremans AP (2024) A typological and morphological analysis of the Pleurothallidinae (Orchidaceae) inflorescences. Botanical Review 90(3): 221–250. https://doi.org/10.1007/s12229-024-09303-6
- Sierra-Ariza MA (2024) *Pleurothallis cop-biodiversitatis* (Pleurothallidinae), a new species from Colombia. Lankesteriana: International Journal on Orchidology: 259–265. https://doi.org/10.15517/lank.v24i3.61038
- Wilson M (2011) Barcoding the species of *Pleurothallis* subsection *Macrophyllae-Fasciculatae*. Lankesteriana 11: 3. https://doi.org/10.15517/lank.v11i3.18306
- Wilson M, Belle C, Dang A, Hannan P, Kellogg L, Kenyon C, Low H, Mochizuki A, Nguyen A, Sheade N, Shan L, Shum A, Stayton T, Volz C, Vosburgh B, Wellman H, Woolley MA (2013) A preliminary phylogenetic analysis of *Pleurothallis* sensu lato based upon nuclear and plastid sequences. Lankesteriana 13: 139. https://doi.org/10.15517/lank.v0i0.11568
- Wilson M, Larsen B, Moreno JS, Ward R, Riksen JA, Piña L, Sierra-Ariza MA, Jiménez MM, Rincón-Gonzalez M, Galindo-Tarazona R, Suárez HG, Haelterman D (2022) New species of *Pleurothallis* (Orchidaceae: Pleurothallidinae), a new country record, and labellar morphology in the P. cardiostola-P. lilijae complex of subsection *Macrophyllae-Fasciculatae*. Harvard Papers in Botany 27(2): 187–220. https://doi.org/10.3100/hpib.v27iss2.2022.n10



**Research Article** 

# *Phragmotheca centinelensis* (Malvaceae, Malvoideae or Matisioideae), a newly-discovered, endangered canopy tree species from a cloud forest in Pacific Ecuador

Juan Ernesto Guevara-Andino<sup>10</sup>, Dawson M. White<sup>20</sup>, Nigel C. A. Pitman<sup>30</sup>, Juan-Carlos Cerón<sup>40</sup>, Andrea Fernández<sup>50</sup>, Daniel Navas-Muñoz<sup>40</sup>, William S. Alverson<sup>60</sup>

- 1 Grupo de Investigación en Ecología y Evolución en los Trópicos-EETrop- Universidad de las Américas, Quito 170124, Ecuador
- 2 Harvard University Herbaria, 22 Divinity Ave., Cambridge, MA 02476, USA
- 3 Collections, Conservation & Research, Field Museum of Natural History, 1400 S. Lake Shore Dr., Chicago, IL 60605, USA
- 4 Herbario Nacional del Ecuador (QCNE), Instituto Nacional de Biodiversidad, Av. Río Coca E6-115 e Isla Fernandina, Quito, Ecuador
- 5 Herbario QCA, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre 1076 y Roca, Apartado 17-01-2184, Quito, Ecuador
- 6 WIS Herbarium, University of Wisconsin-Madison, 430 Lincoln Dr., Madison WI 53706, USA

Corresponding author: Juan Ernesto Guevara-Andino (juan.guevara@udla.edu.ec)

#### Abstract

During floristic inventories in remnant cloud forests of the Centinela Ridge of the Chocó Region of Ecuador, where less than 7 km<sup>2</sup> of forest patches remain across an area of approximately 500 km<sup>2</sup>, we recently collected a new species in the genus *Phragmotheca* Cuatrec. We describe and illustrate this new species and contrast its morphology with known congeneric species. Due to its small range, threatened habitat and active targeting by loggers, this species is assessed as Endangered under IUCN Criterion B1B2ab(I,ii,iii,v).

#### Resumen

Durante inventarios florísticos en los remanentes de bosques nublados de la cordillera Centinela, en la región del Chocó en Ecuador, donde menos de 7 km<sup>2</sup> de parches de bosque permanecen en un área de aproximadamente 500 km<sup>2</sup>, recientemente recolectamos una nueva especie del género *Phragmotheca* Cuatrec. Describimos e ilustramos esta nueva especie y contrastamos su morfología con las especies congéneres conocidas. Debido a su distribución restringida, hábitat amenazado y la explotación activa por parte de madereros, esta especie se evalúa como En Peligro según el Criterio B1B2ab(i,ii,ii,v) de la UICN.

**Key words:** Biological collections, Centinela, deforestation, endemism, extinction, Malvatheca clade, Matisieae, Matisioideae

Palabras clave: Colecciones biológicas, Centinela, deforestación, endemismo, extinción, clado Malvatheca, Matisieae, Matisioideae

## Introduction

The genus *Phragmotheca* Cuatrec. (Malvaceae, Malvoideae or Matisioideae) comprises 11 known species (Table 1): four in lowland Amazonian forests of Colombia, Ecuador and Peru; two in the Andean highlands of Colombia; and five



Academic editor: Laurence J. Dorr Received: 30 November 2024 Accepted: 12 February 2025 Published: 21 March 2025

**Citation:** Guevara-Andino JE, White DM, Pitman NCA, Cerón J-C, Fernández A, Navas-Muñoz D, Alverson WS (2025) *Phragmotheca centinelensis* (Malvaceae, Malvoideae or Matisioideae), a newly-discovered, endangered canopy tree species from a cloud forest in Pacific Ecuador. PhytoKeys 254: 41–59. https://doi.org/10.3897/ phytokeys.254.143106

#### Copyright: ©

Juan Ernesto Guevara-Andino et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

in low- and mid-elevation Pacific-slope forests of Colombia and eastern Panama (Alverson 1991; Fernández-Alonso 1996; Fernández-Alonso & Jaramillo-Mejía 1999, Fernández-Alonso et al. 2017). Species are generally large canopy trees (to 50 m) with the exception of P. lemniscata Fern. Alonso, which is a treelet in the Chocó Region of Colombia. Phragmotheca is closely related to Matisia Bonpl. and Quararibea Aubl. The three genera consistently form a clade in phylogenetic analyses (/Matisieae in Baum et al. (1998, 2004)) and have been placed in the Matisieae K.Schum. (Schumann 1895; Reveal 2012) within the Malvoideae (sensu Baum et al. (1998, 2004); the /Malvoideae clade of Alverson et al. (1999)). A new revision of ranked names within Malvaceae (Colli-Silva et al. 2025) proposes that the /Matisieae clade be renamed Matisioideae, but one of us [WSA] considers this change to be unjustified at present (see Appendix 1). Phragmotheca differs from the other two genera by its conspicuous internal partitioning of staminal thecae into foveae or septa (cf. fig. 1 in Alverson (1991) and figs 4, 8, 9 and 12 in Fernández-Alonso (1996)), a trait that also is found in other "basal" genera of the /Malvatheca clade sensu Baum et al. (1998), i.e. the clade comprising the /Bombacoideae and /Malvoideae clades). Phragmotheca has long apical staminal lobes and five carpels, as does its presumably closest relative Matisia. (Quararibea has short apical staminal lobes and 2-4 carpels). However, unlike Matisia, Phragmotheca species also have thicker, prominently ribbed endocarps surrounding the five seeds (cf. fig. 1 in Fernández-Alonso (1996) and Fig. 5E, F).

*Phragmotheca* is a conspicuous floristic element of the humid forests of the Chocó Region of Colombia and Ecuador and the western Amazon. It has been hypothesised that lowland humid forests of Panama, Colombia and Ecuador constitute its centre of diversification (Gentry 1986; Fernández-Alonso 1998). Forests of the Chocó Region of Ecuador are subject to one of the highest deforestation rates in the world. Few remnants persist, accounting for only 15% of the original vegetation cover (Finer and Mamani 2019). Since 2021, we have been carrying out floristic inventories in one of the most iconic and imperilled areas within the Chocó Region of Ecuador: the Centinela (or Montañas de IIa) area. Originally this area had ~ 500 km<sup>2</sup> of forest, but less than 7 km<sup>2</sup> remains. In the last 10 years, several new tree species have been described from material collected in Centinela highlighting the relevance of this area for botanical exploration (Torke and Perez 2013; Cornejo 2023; Fernández-Alonso and Cornejo 2024).

During one of our inventories, we collected a specimen of *Phragmotheca* that exhibits remarkable characteristics, clearly different from those of known species in the Chocó (Table 2). This is the first new species of *Phragmotheca* described in the 21<sup>st</sup> century (Table 1). Thus, our results highlight the importance of botanical exploration and conservation not just in poorly-collected sites in the Neotropics, but specifically in Neotropical cloud-forest remnants.

#### Methods

After our initial fieldwork during the floristic inventories, we collected additional, fertile specimens of this unknown species of *Phragmotheca*. We also made an exhaustive search of *Phragmotheca* specimens deposited in Ecuador's three largest herbaria: Herbario de la Universidad Católica del Ecuador (QCA), Herbario Alfredo Paredes de la Universidad Central del Ecuador (QAP) and Herbario Nacional del Ecuador (QCNE; abbreviations according to Thiers (2019)). Table 1. Species of Phragmotheca Cuatrec. (Malvaceae) known prior to the publication of P. centinelensis J.C.Cerón,A.Fernández & J.E.Guevara. Country codes: CO – Colombia, EC – Ecuador, PA – Panama, PE – Peru.

Species	Publication	Distribution	Country	Altitude (m)
P. siderosa Cuatrec.*	1946	Chocoan	CO, EC	< 50 (-620)
P. fuchsii Cuatrec.	1971	Chocoan	CO	< 50
P. leucoflora D.R.Simpson	1982	Amazonian	EC, PE	150-260
P. amazónica (W.S.Alverson) Fern.Alonso**	1991	Amazonian	CO, EC, PE	115-150
P. ecuadorensis W.S.Alverson	1991	Amazonian	EC	250-450
P. mammosa W.S.Alverson	1991	Chocoan	CO, PA	100-800
P. hydra Fern.Alonso	1996	Chocoan	CO	50-100
P. lemniscata Fern.Alonso	1996	Chocoan	CO	180
P. rubriflora Fern.Alonso	1996	Andean	CO	750
P. siderotricha Fern.Alonso	1996	Amazonian	PE	320-700
P. mambitana Fern.Alonso & R.Jaram.	1999	Cordilleran	CO	1700-1800

\* The elevational range of *Phragmotheca siderosa* includes that of subsp. *megacarpa* (Fernández-Alonso 1996). \*\**Phragmotheca amazonica* was originally published as *P. mammosa* subsp. *amazonica* (Alverson 1991).

 

 Table 2. Diagnostic characters of Phragmotheca Cuatrec. in the Chocó Region of Ecuador and Colombia, including P. centinelensis J.C.Cerón, A.Fernández & J.E.Guevara and morphologically similar relatives.

Characters	P. centinelensis	P. hydra	P. lemniscata	P. rubriflora	P. siderosa	
Leaf blade shape	orbicular to oblong- elliptic	cordate-orbicular	broadly elliptic to suborbicular	lanceolate-oblong to narrowly obovate-oblong	ovate to elliptic or broadly elliptic	
Abaxial leaf surface indument	sparsely distributed long- branched fasciculate hairs, lepidote-stellate scales and stellate- fasciculate hairs	densely covered by long-branched fasciculate hairs	densely covered by lepidote scales	stellate-lepidote	densely echinate- stellate	
Primary leaf veins at base (not counting submarginals)	5	5	3	2	5	
Secondary leaf veins (per side)	5	4-5	4-5	6-8	4-5	
Calyx tube shape	narrowly campanulate	cylindrical	tubular- fusiform	tubular-cylindrical	tubular	
Flower length, including pedicel (cm)	3.3-5.2	2.8-2.9	> 8	6.5-7.0	ca. 9.0	
Petal colour	orange or pink	white or yellowish- white	white or yellowish-white	red	white or pale yellowish-brown	
Staminal column length, with lobes (cm)	2.9-3.6	1.7	7.0-7.2	6.5	ca. 8.0	
Staminal column lobe length (cm)	1.3-1.6	0.9-1.0	1.1-1.3	1.2-1.3	1.5	
Thecae per staminal column lobe	6 (relatively uniform in size)	3 (2 large plus 2 basal that are shared with adjacent lobes)	4–6 (quite variable in size)	7 (three pairs per lobe plus one basal that is shared)	_	
Number of fovea (septa) per theca	13-14	15-24	few – many (> 50)	many	_	
Fruit shape, length and width (cm)	ovoid to broadly ovate, $5.9-7.0 \times 4.0-5.3$	broadly ovate, 5.2 × 6.0-6.2	spherical, 7.0 × 4.0-4.5	apparently spherical, ca. 4.0–3.8	broadly ellipsoid to sub-obovoid, 6.5–8.0 × 6.3–6.8	

To gather habit and morphological information, we consulted digitised specimen images of the genus *Phragmotheca* available at the Field Museum virtual herbaria (https://collections-botany.fieldmuseum.org) and 339 images from an archive maintained by one of us [WSA], with additional specimens seen at AMAZ, COL, F, GUAY, HUA, JAUM, MEDEL, MO, MOL, PMA, SCZ, USM and WIS.

To determine the range of the new species for a conservation status assessment using IUCN criteria, we estimated the Extent of Occurrence and Area of Occupancy using the package conR (Dauby et al. 2017). Habitat fragmentation was estimated using the most current deforestation data available for western Ecuador from the online platform Mapa Interactivo (MAATE 2024). We first downloaded ecosystem layers for western Ecuador, as well as deforestation maps for the period 1990–2022. Habitat reduction was then assessed by combining these layers with the layers of Extent of Occurrence (EOO) and Area of Occupancy (AOO) using the clip tool in the ArcGis software (ESRI 2011). The clip tool overlays a range size map of EOO (Extent of Occurrence) and AOO (Area of Occupancy) with boundary layers, which, in this case, include deforestation scenarios from 1990 to 2022 and ecosystem layers. This process refines the potential habitat by limiting it to forest fragments. Habitat loss was then calculated as the area outside the combined boundaries defined by the deforestation and ecosystem layers. Conservation assessment was done following IUCN Standards and Petitions Committee (2022).

#### Results

#### **Taxonomic treatment**

*Phragmotheca centinelensis* J.C.Cerón, A.Fernández & J.E.Guevara, sp. nov. urn:lsid:ipni.org:names:77359001-1 Figs 1–5

**Diagnosis.** The new species is morphologically similar to *Phragmotheca hydra* Fern. Alonso, but differs by its orbicular to oblong-elliptic (vs. orbicular-cordate) leaves with a mixture of long-branched fasciculate hairs and lepidote-stellate scales in the axils of the mid-vein and secondary veins on the abaxial leaf surface (vs. fasciculate hairs only); larger flowers  $(3.5-5.0 \text{ [including the pedicel]} \times 2.9-3.5 \text{ vs. } 2.8-2.9 \times 2.8-3.0 \text{ cm})$  with glabrous, concave-spoon-like (vs. linear spathulate) petals; longer, glabrous staminal column (2.9-3.6 vs. 1.7 cm and densely covered by stellate hairs); staminal lobes each bearing 6 thecae (vs. 3-4 thecae); glabrous (vs. sparsely covered with long-branched, fasciculate hairs) style; subcapitate (vs. subacute) stigma; narrower, patelliform fruiting calyx (3.3-3.9 vs. 4-4.5 cm in diameter); and ovoid (vs. globose) fruits that are proportionately more slender ( $5.0-7.0 \times 4.0-5.3 vs. 5.2 \times 6.0-6.2 cm$  in diameter).

The semi-cordate leaf bases of this new species resemble those of *Phrag-motheca lemniscata* Fern.Alonso, but *P. centinelensis* differs by its larger size (canopy trees to 35 m vs. small treelets); indument of abaxial leaf surfaces (sparse fasciculate hairs, lepidote-stellate scales and stellate-fasciculate hairs vs. dense lepidote scales); floral calyces (narrowly campanulate vs. tubular-fusiform); petals (orange or pink, glabrous, glossy, distally concave, non-reflexed, with slightly convolute margins vs. white-cream, linear-spathulate, distally reflexed, internally covered by a mixture of stellate and lepidote scales); shorter



Figure 1. Phragmotheca centinelensis J.C.Cerón, A.Fernández & J.E.Guevara, sp. nov. Image of the holotype at QCNE (*J.C. Cerón, A. Fernández, D. Navas, & L. Narváez* 4643).

staminal column (2.9–3.6 vs. 7.0–7.2 cm long, including the apical lobes) with apical lobes differing in indument (densely covered by septate simple hairs vs. glabrous); fruiting calyces (patelliform and covered by lenticels vs. discoid sub-cupular); and fruits (broadly ovoid vs. globose).

**Type. ECUADOR – Santo Domingo de los Tsáchilas •** (fl, fr, J.C. Cerón, A. Fernández, D. Navas, & L. Narváez 4643 (holotype: QCNE!, isotypes: F!, MO!, QCA!, WIS!)); Parroquia El Esfuerzo, Recinto Milton Murillo, Finca del Sr. Marlon Sarango, fragmento de bosque intervenido rodeado de pastizales; 0°35'54.53"S, 79°13'27.23"W; alt. 824 m; 01 Nov 2023.

Description. Canopy trees to 25-35 m tall; trunk cylindrical, longitudinally fissured and forming thin plates, buttressed to 7 m height (Fig. 2A), outer bark reddish with dispersed, irregular grey spots (Fig. 2B), internal bark fibrous (outer half reddish-brown, inner half yellow-cream; Fig. 2C); branches: main branches off trunk verticillate, smaller branches glabrous, terete, longitudinally fissured, with granular lenticels. Stipules 2-3 mm long, broadly triangular, caducous. Leaves alternate, clustered at the tips of the branchlets; petioles 3.6-12.2 cm long, 1-3.5 mm in diameter, terete, finely striate, densely pubescent, pulvinate at both ends. Leaf blades (Fig. 3A, B) glaucous when young, pale green abaxially when mature, coriaceous, entire, orbicular to oblong-elliptic,  $(6-)6.7-31.1(32) \times$ (5-)7.6-29.3(-30) cm, semi-cordate to deeply cordate at base with slightly asymmetric lobes 0.5-4.3 cm depth, the apex obtuse to shortly apiculate; the abaxial surface densely covered by a mixture of lepidote-stellate scales, stellate-fasciculate trichome and fasciculate trichomes; venation conspicuous on the abaxial surface; primary veins palmate near leaf base (Fig. 3D) with 5 basal nerves, 3-4 submarginal nerves slightly ascending to the leaf margin and with 5 pairs of secondary veins arising from mid-rib in distal 2/3 of blade, these with barbate tufts of fasciculate trichomes and sessile lepidote-stellate scales in their axils (Fig. 3G); tertiary venation also prominent on the abaxial surface (Fig. 3E), forming a conspicuous reticulum (inconspicuous on the adaxial surface), with golden lepidote-stellate scales on the lamina between nerves (Fig. 3F). Flowers slightly zygomorphic, solitary (Fig. 4A, B), borne opposite to leaves on short branches (brachyblasts), 3.3-5.2 cm long (including the pedicels), the pedicels 1.2-1.4 cm long, 0.3-0.4 cm in diameter, finely striate, covered with sessile stellate-lepidote scales and fasciculate hairs; floral bracts absent below the calyx. Calyx narrowly campanulate (Fig. 4C), 2.3-3.3 × 0.9-1.2 cm, densely covered by a brownish, floccose indument (imparting a granular appearance), broadly acuminate and 4-lobed at summit, internally covered with adpressed sericeous hairs. Petals spathulate, distinctly bilobed at apex, strongly concave distally (Fig. 4B, D), the apex obtuse or rounded, the margins slightly convolute, 3.5-4.1 × 1.5-2.0 cm, orange or pink, glabrous, glossy, 2-4 inner corolla lobes covering the staminal tube and non-reflexed corolla tube lobes. Staminal column 2.9-3.6 cm long, the tube  $(16.5-)16.8-20.6(-21.0) \times 0.4-0.6$  mm long, orange, with 5 linear terminal lobes (Fig. 4G), each lobe 12.9-15.8 × 2.5-2.8 mm, orange or pink and bearing 6 thecae in two parallel lines on the adaxial surface, these surrounded by long, unbranched, septate hairs (Fig. 4G-I), with 14-16 foveae per theca. **Ovary** elongate, 1.5–1.7 mm long, 5-carpellate, glabrous, the style not exceeding staminal filaments in length, longitudinally 6-sulcate, the stigma capitate, spongy, glandular. *Fruiting pedicel* short, terete, 7.3-8.9 × 5.8-6.1 mm. Fruiting calyx patelliform and slightly dentate (Fig. 5A, B), surrounding only the



Figure 2. Phragmotheca centinelensis J.C.Cerón, A.Fernández & J.E.Guevara A trunk B outer bark C inner bark. Photos of the type individual (*Cerón et al. 4643*) by Andrea Fernández and Juan Carlos Cerón.

basal 1/5 of the fruit and beset with cream-coloured lenticels. *Fruit* ovoid to broadly ovoid,  $5.9-7 \times 4-5.3$  cm, the exocarp pale, smooth, finely fissured longitudinally (Fig. 5C) cream-coloured, sparsely granular with short, fasciculate hairs; the mesocarp fibrous-pulpy, white or cream-coloured and exuding yellow-ish mucilage (Fig. 5D, E), the endocarp densely fibrous, forming a woody pyrene with each of the five seeds (Fig. 5F). Mature *seeds* elliptic,  $4-4.8 \times 1.7-2$  cm.

**Phenology.** Flowering from November to December; fruiting between February and March.

**Distribution.** *Phragmotheca centinelensis* is a large tree of non-flooded habitats in the Chocó cloud forests of Ecuador occupying a narrow altitudinal band between 600 and 1000 m elevation. It occupies mostly highly dissected terrain in the ridges of the Centinela area (Figs 6, 7). According to our tree species inventory, *P. centinelensis* appears to be relatively rare in the region and possibly endemic to the Ecuadorian Chocó; the only two known populations are in cloud forest remnants in the western Andean foothills of Centinela. Although we know of no record of *P. centinelensis* from Colombia, it may occur in similar habitats of the adjacent Chocó Department. Despite its rarity, distinctive features make it conspicuous in the field, notably the large buttresses up to 7 m height, its reddish outer bark and the longitudinal fissures on the trunk.



**Figure 3**. *Phragmotheca centinelensis* J.C.Cerón, A.Fernández & J.E.Guevara **A** leaves, adaxial surface **B** leaves, abaxial surface **C** terminal branch showing the terminal bud and lenticels **D** basal leaf veins, abaxial surface (8×) **E** tertiary and quaternary venation, abaxial surface (8×) **F** stellate-lepidote scales, abaxial surface of leaf (100×) **G** barbate axil of midvein and secondary vein, with tufts of long-branched fasciculate hairs and stellate-lepidote scales, abaxial leaf surface. Photos of the type individual (*Cerón et al. 4643*) by Andrea Fernández and Juan Carlos Cerón.



Figure 4. Phragmotheca centinelensis J.C.Cerón, A.Fernández & J.E.Guevara A flowering branch B flower with reflexed, concave-spoon-like petals C campanulate flowering calyx D distal part of petal showing the diagnostic concave form that is characteristic of this new species E flower bud (8×) F close-up of flower bud indument, a mix of stellate-lepidote scales and fasciculate hairs G apical lobes of staminal column (note sterile tips), each with two parallel rows of thecae and the shorter style in centre H close-up of apical lobes with elongate, foveate thecae (15×); I elongate, septate hairs on the thecae (100×).

Habitat and ecology. Some of the most conspicuous floristic elements of the Centinela cloud forests include the families Fabaceae, Lecythidaceae and Malvaceae with dominant tree species including Ocotea insularis (Meisn.) Mez, Ruagea glabra Triana & Planch., Guatteria sp., Aegiphila alba Moldenke, Trichilia surinamensis (Miq.) C.DC., Socratea exorrhiza (Mart.) H.Wendl. and Iriartea deltoidea Ruiz & Pav. The genera Eschweilera Mart. ex DC., Inga Mill., Gustavia L., Matisia, and Quararibea are also both dominant and species-rich groups in this area. It is remarkable that Phragmotheca centinelensis co-exists locally with several other species within the /Matisieae clade, including M. palenquiana (A.Robyns) W.S.Alverson,



**Figure 5.** *Phragmotheca centinelensis* J.C.Cerón, A.Fernández & J.E.Guevara **A** fruiting branch **B** patelliform fruiting calyx **C** apex of the fruit showing the fine longitudinal fissures **D** longitudinal view of the fruit showing yellow exudate from the mesocarp **E** transverse section of the fruit showing the exocarp, fibrous mesocarp and very thick endocarp tissue surrounding the seeds **F** two pyrenes (seeds with some residual fibrous-woody endocarp and mesocarp tissue).

*M. coloradorum* Benoist, *M. giacomettoi* Romero, *M. castano* H.Karst. & Triana and *Q. casasecae* Fern.Alonso & Castrov., *Q. grandifolia* (Little) Cuatrec. perhaps due to similar dispersal mechanisms or pollination syndromes. Natural regeneration of *P. centinelensis* seems to be rare in the forest fragments of the Centinela area. Gravity seems to be the prominent mechanism of primary dispersion. We found a high density of fruits and seeds underneath parent trees with abundant signs of predation by rodents or other small mammals, nearly all fruits with bite marks.



**Figure 6. A** Distribution of *Phragmotheca centinelensis* J.C.Cerón, A.Fernández & J.E.Guevara, its relatives in the Chocó Region of Ecuador and Colombia and two morphologically similar Amazonian species **B** cloud-forest habitat of the new species in Ecuador.

There may be secondary dispersal due to scatter-hoarding by these mammals. The heavily armoured endocarps may be an adaptation to facilitate this, but more study is needed. Our current observations suggest a high mortality of seeds and seedlings due to a negative density dependence process via Janzen-Connell phenomena (Forrister et al. 2019).

**Conservation status.** Phragmotheca centinelensis is known from only three confirmed populations in the western foothills of the Ecuadorian Andes in the few remnants of cloud forests of the Montañas de Ila, more specifically in areas outside of Centinela Ridge (Fig. 7). This small population, within a 150-ha patch of forest, represents the type locality. In this area, only three adult individuals have been observed, all of them with a diameter at breast high > 30 cm. Another subpopulation of four individuals has been reported at the southern end of the Centinela Ridge, ca. 15 km from the confirmed population. Based on field observations by other botanists, we believe there to be a second population occurring in a large fragment of forest (2500 ha) in the private Mashpi Reserve, 85 km north of Centinela (A. Pérez and G. Toasa, pers. comm.). We reviewed a sterile Phragmotheca herbarium specimen from the Mashpi Reserve and confirmed it belongs to the new species described herein. A third population is located in small patch of cloud forest at 1100-1200 m elevation, near the town of Cielo Verde in Imbabura Province, approximately 15 km north of Mashpi Reserve. This forest is part of the buffer zone from Los Cedros Reserve with approximately 4500 ha protecting the last remnants of Chocó cloud forests in western Ecuador.

Our estimates of extent of occurrence (EOO) and area of occupancy (AOO) are 520 km<sup>2</sup> and 16 km<sup>2</sup>, respectively (Bachman et al. 2011) (Fig. 7). Two of the three known confirmed populations of Phragmotheca centinelensis are not formally protected in the Ecuadorian Protected Area National System (SNAP). Our analysis demonstrates that, from 1990 to 2022, deforestation across the range of this species has reduced its EOO and AOO in 21% approximately. Furthermore, based on estimated EOO and AOO (Fig. 7) and historic deforestation over the last 34 years, we found that 34% of its potential habitat has disappeared. Thus, on the basis of current deforestation rates in the Chocó Region (Finer and Mamani 2019) and habitat loss, our assessment of P. centinelensis's global threat status is Endangered [EN] under IUCN Criterion EN B1ab(i,ii,iii)+2ab(i,ii,iii). It is important to note that historical deforestation of the Centinela Ridge predates the 1970s, but several large forest fragments remained interspersed in a matrix of cacao, balsa and Gmelina L. plantations (Dodson and Gentry 1991). However, at present, deforestation was occurring even during our visits: at the time of our floristic inventories in 2021, one of the three adult individuals which we recorded was being removed by loggers.

**Etymology.** We named the new species in honour of the iconic site that the renowned botanists Alwyn Gentry and Calaway Dodson visited more than 40 years ago in the Centinela area, close to Santo Domingo de los Colorados in the western foothills of the Ecuadorian Andes. At the time they visited an area known as Centinela del Pichincha, they observed a severely fragmented land-scape, which led them to conclude that ongoing deforestation had wiped out almost all the remaining forests in the region. After the publication of their seminal paper (Dodson and Gentry 1991), the term "Centinelan extinction" was coined and popularised by Wilson (1992). The term aimed to reflect the global extinction of a high number of endemic plant species, many of them undescribed, following high levels of forest fragmentation.



Figure 7. Map showing the Extent of Occurrence (EOO), Area of Occupancy (AOO) and available habitat of *Phragmotheca centinelensis* J.C.Cerón, A.Fernández & J.E.Guevara in western Ecuador. Inset: Location of Santo Domingo de las Tsáchilas Province within Ecuador.

Additional specimens examined. ECUADOR – Santo Domingo de los Tsáchilas • J.C. Cerón et al. 4684 (QCA, QCNE, F); Parroquia El Esfuerzo, Recinto Milton Murillo, Finca del sr. Marlon Sarango. bosque siempreverde piemontano de la cordillera occidental de los Andes (BsPn01), Fragmento de bosque intervenido rodeado de pastizales; 0°26'15.35"S, 79°13'38.57"W; alt. 840 m; 01 Nov 2023; fl • Santo Domingo de los Tsáchilas: J.C. Cerón et al. 4688 (QCA, QCNE, F); Parroquia Centinela del Pichincha, Comunidad Santa Rosa, bosque siempreverde piemontano de la cordillera occidental de los Andes (BsPn01), bosque maduro intervenido; 0°39'51.15"S, 79°18'48.37"W; alt. 682 m; 01 Nov 2023; fr. • Pichincha: Reserva Masphi • PDRBA 43 (QCA); Colecciones en la parcela 7 de 60 × 60 m del transecto altitudinal para el monitoreo de la dinámica forestal. Bosque Montano Bajo. Árbol, placa 7294; 0°10'6.63"N, 78°52'34.2402"W; alt. 800–900 m; 2–25 Aug 2019. • Imbabura: Cielo Verde • PDRBA 285 (QCA); Colecciones en la parcela 7 de 60 × 60 m del transecto altitudinal para el monitoreo de la dinámica forestal. Bosque Montano Bajo. Árbol, placa 7294; placa 586; 0°15'26.2074"N, 78°55'6.096"W; alt. 1100–1200 m; 10–22 Jul 2021.

#### Discussion

Based on morphology and geographic distribution, *Phragmotheca centinelensis* is a new species distinct from other morphologically similar congeneric species in the Chocó and Amazon Regions.

Phragmotheca centinelensis is one of many new species described from remnant forests in western Ecuador over the last 15 years (Cornejo 2009a, 2009b,; Palacios 2012; Torke and Perez 2013; Guevara-Andino and Fernández-Fernández 2020; Couvreur et al. 2022; Fernández-Alonso and Cornejo 2024; Clark et al. 2024). Its discovery adds valuable new insight into the region's conservation value and floristic richness, as well as providing another piece of the puzzle to understand the historical biogeography and evolution of members of the /Matisieae clade (the > 100 species included in the genera Matisia, Phragmotheca and Quararibea). For example, M. palenquiana (A.Robyns) W.S.Alverson was described (as a Quararibea) from specimens collected in 1974 by Gentry and Dodson at the Río Palenque Biological Station, very close (60 km) to the type locality of P. centinelensis. The diagnosis in the original article (Robyns 1976) notes the similarity of the new species to M. bicolor Ducke, an Amazonian species. A third, very closely-related species, with similarly distinctive flowers and fruits, was recently discovered in Costa Rica notwithstanding many decades of dedicated botanical collecting efforts there: M. tinamastiana A. Estrada & Cascante (Estrada and Cascante (1998), with an updated distribution in Fernández-Alonso and Campos-Pineda (2023)). Triplets of closely-related species distributed east of the Andes in the Amazon, west of the Andes in Colombia and Ecuador and northwest into isthmian Costa Rica and Panama are evident in several genera of the / Bombacoideae and /Malvoideae clades (sensu Baum et al. (1998, 2004)), which invites further investigation once additional molecular phylogenies for these genera are available.

## Conclusion

*Phragmotheca centinelensis* is a distinct and endangered new species of canopy tree found in forest remnants of western Ecuador. Discovery and documentation efforts for rare species such as this are critical to their conservation and to the value they add to our understanding of floristics, historical biogeography and the evolutionary diversification of Neotropical cloud forests.

## Acknowledgements

We thank the staff of the QCA and QCNE herbaria for their support and for facilitating loans of material that made this study possible. The organisations that house those herbaria, Instituto Nacional de la Biodiversidad and Pontificia Universidad Católica del Ecuador, respectively, also provided valuable support for this work. We acknowledge the Ministerio del Ambiente, Agua y Transición Ecológica for granting collection and research permits to Universidad de las Américas under Genetic Resources Access Agreement number MAATE-DBI-CM-2021-0187.

## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This work is part of the Centinela Rebirth: Restoring Threatened Tree Species in Western Ecuador project and has been supported by Universidad de las Américas Research Grant 514.C.XIV.24 to JEGA, the Mohammed Bin Zayed Species Conservation fund to DMW, Fundación Jocotoco, and Franklinia Foundation.

#### Author contributions

J. E. Guevara-Andino: Conceptualisation, Methodology, Validation, Formal, Analysis, Investigation, Resources, Writing – Original draft, Visualisation, Supervision, Project Administration, Funding Acquisition. D. M. White: Conceptualisation, Methodology, Investigation, Resources, Supervision, Project Administration, Funding Acquisition. N. C. A. Pitman: Conceptualisation, Methodology, Investigation, Resources, Supervision, Funding Acquisition. J. C. Cerón: Investigation, Formal Analysis, Data curation. A. Fernández: Investigation, Formal Analysis. D. Navas-Muñoz: Formal Analysis, Visualisation, Data curation. W. S. Alverson: Writing (original text regarding the /Matisieae clade), Review and Editing.

#### Author ORCIDs

Juan Ernesto Guevara-Andino <sup>©</sup> https://orcid.org/0000-0002-5433-6218 Dawson M. White <sup>®</sup> https://orcid.org/0000-0002-0670-9390 Nigel C. A. Pitman <sup>®</sup> https://orcid.org/0000-0002-9211-2880 Juan-Carlos Cerón <sup>®</sup> https://orcid.org/0009-0007-4986-4802 Andrea Fernández <sup>®</sup> https://orcid.org/0009-0002-0445-2867 Daniel Navas-Muñoz <sup>®</sup> https://orcid.org/0009-0001-1761-9147 William S. Alverson <sup>©</sup> https://orcid.org/0000-0001-7793-0386

#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information. Coding for the IUCN analyses and distributional maps will be submitted to Dryad.

## References

- Alverson WS (1991) A synopsis of *Phragmotheca* (Bombacaceae), with two new species and a new subspecies. Brittonia 43(2): 73–87. https://doi.org/10.2307/2807297
- Alverson WS, Whitlock BA, Nyffeler R, Bayer C, Baum DA (1999) Phylogeny of the core Malvales: Evidence from *ndh*F sequence data. American Journal of Botany 86(10): 1474–1486. https://doi.org/10.2307/2656928
- Bachman S, Moat J, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Baum DA, Alverson WS, Nyffler R (1998) A durian by any other name: Taxonomy and nomenclature of the Core Malvales. Harvard Papers in Botany 3(2): 315–330.

- Baum DA, DeWitt Smith S, Yen A, Alverson WS, Nyffeler R, Whitlock BA, Oldham RL (2004) Phylogenetic relationships of Malvatheca (Bombacoideae and Malvoideae; Malvaceae sensu lato) as inferred from plastid DNA sequences. American Journal of Botany 91(11): 1863–1871. https://doi.org/10.3732/ajb.91.11.1863
- Carvalho-Sobrinho JG, Alverson WS, Alcantara S, Queiroz LP, Mota AC, Baum DA (2016) Revisiting the phylogeny of Bombacoideae (Malvaceae): Novel relationships, morphologically cohesive clades, and a new tribal classification base on multilocus phylogenetic analyses. Molecular Phylogenetics and Evolution 101(2016): 56–74. https://doi.org/10.1016/j.ympev.2016.05.006
- Clark JL, Fernández A, Zapata JN, Restrepo-Villarroel C, White DM, Pitman N (2024) *Amalophyllon miraculum* (Gesneriaceae), an exceptionally small lithophilous new species from the western Andean slopes of Ecuador. PhytoKeys 242: 307–316. https://doi.org/10.3897/phytokeys.242.118069
- Colli-Silva M, Pérez-Escobar OA, Ferreira CDM, Costa MTR, Gerace S, Coutinho TS, Yoshikawa VN, Antonio-Domingues H, Hernández-Gutiérrez R, Bovini MG, Duarte MC, Cheek M, Chase MW, Fay MF, Christenhusz MJM, Dorr LJ, Schoeplein C, Cocoran M, Roy S, Cable S, McLay T, Maurin O, Forest F, Baker WJ, Antonelli A (2025) Taxonomy in the light of incongruence: An updated classification of Malvales and Malvaceae based on phylogenomic data. Taxon, Early View [published 23 Jan 2025]. https://doi. org/10.1002/tax.13300
- Cornejo X (2009a) Two new species of *Pentagonia* (Rubiaceae, Hippotideae) from Colombia and Ecuador. Novon 19(1): 25–31. https://doi.org/10.3417/2006060
- Cornejo X (2009b) Amyris centinelensis and Zanthoxylum bonifaziae: Two new species of Rutaceae from western Ecuador. Harvard Papers in Botany 14(2): 161–166. https://doi.org/10.3100/025.014.0207
- Cornejo X (2023) *Eschweilera podoaquilae*: A new species of Lecythidaceae from northwestern Ecuador. Phytotaxa 579(2): 139–142. https://doi.org/10.11646/phyto-taxa.579.2.8
- Couvreur TLP, Cornejo X, Zapata JN, Loor A (2022) Two new magnoliid (Annonaceae, Lauraceae) tree species from Manabí, western Ecuador. Blumea 67(2): 97–108. https://doi.org/10.3767/blumea.2022.67.02.02
- Dauby G, Stévart T, Droissart V, Cosiaux A, Deblauwe V, Simo-Droissart M, Sosef MSM, Lowry PP 2<sup>nd</sup>, Schatz GE, Gereau RE, Couvreur TLP (2017) ConR: An R package to assist large-scale multispecies preliminary conservation assessments using distribution data. Ecology and Evolution 7(24): 11292–11303. https://doi.org/10.1002/ece3.3704
- Dodson CH, Gentry AH (1991) Biological extinction in western Ecuador. Annals of the Missouri Botanical Garden 78(2): 273–295. https://doi.org/10.2307/2399563
- ESRI (2011) ArcGIS Desktop, Release 10. Environmental Systems Research Institute, Redlands, California.
- Estrada A, Cascante A (1998) *Matisia Tinamastiana* (Bombacaceae): Una nueva especie arborescente del Pacífico Central de Costa Rica. Brenesia 49–50: 79–85. https:// biblioteca.museocostarica.go.cr/articulo.aspx?id=2519&art=9154
- Ezedin Z (2024) A conspectus of Angiosperm supertribes. Harvard Papers in Botany 29(1): 63-78. https://doi.org/10.3100/hpib.v29iss1.2024.n8
- Fernández-Alonso JL (1996) Contribuciones al conocimiento del género *Phragmotheca* Cuatr. (Bombacaceae-Quararibeae). Caldasia 18(3): 253–284. https://repositorio. unal.edu.co/handle/unal/31015

- Fernández-Alonso JL (1998) Redescripción del "Piscande", Pachira patinoi (Dugand & Robyns) Fern.-Alonso comb. nov. (Bombacaceae) y notas sobre su hábitat y distribución. Revista de la Academia Colombiana de Ciencias Exactas 22(82): 7–12. https:// doi.org/10.18257/raccefyn.22(82).1998.2874
- Fernández-Alonso JL, Campos-Pineda E (2023) *Matisia gentryi* and *M. tinamastiana* (Malvaceae), two species newly recorded from Panama and an updated key to *Matisia* in this country. Checklist 19(6): 1013–1020. https://doi. org/10.15560/19.6.1013
- Fernández-Alonso JL, Cornejo X (2024) *Quararibea centinelae* (Malvaceae), una nueva especie endémica de Centinela, occidente de Ecuador. Harvard Papers in Botany 29(1): 79–85. https://doi.org/10.3100/hpib.v29iss1.2024.n9
- Fernández-Alonso JL, Jaramillo-Mejía R (1999) Nueva especie de zapote del monte (*Phragmotheca*, Bombacaceae) en bosques premontanos del centro de Colombia. Caldasia 21(2): 125–131. https://repositorio.unal.edu.co/bitstream/handle/unal/31131/17513-55778-1-PB.pdf?sequence=1
- Fernández-Alonso JL, Fernández-Hilario R, Reynel-Rodríguez C (2017) Redescripción del zapotillo rosado del Perú, *Phragmotheca sidereotricha* Fern. Alonso (Malvaceae) y notas sobre su hábitat y distribución. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 41(160): 319–325. https://doi.org/10.18257/ raccefyn.498
- Finer M, Mamani N (2019) Saving the Ecuadorian Chocó. MAAP: 102. https://www. maapprogram.org/choco/
- Forrister DL, Endara MJ, Younkin GC, Coley PD, Kursar TA (2019) Herbivores as drivers of negative density dependence in tropical forest saplings. Science 363(6432): 1213–1216. https://doi.org/10.1126/science.aau9460
- Gentry A (1986) Species richness and floristic composition of Choco region plant communities. Caldasia 15(71–75): 71–91. https://www.jstor.org/stable/43406071
- Guevara Andino JE, Fernández-Fernández D (2020) A new rare and endemic species of *Sloanea* (Elaeocarpaceae) from the Chocó region of Ecuador. PhytoKeys 160: 131–139. https://doi.org/10.3897/phytokeys.160.54993
- IUCN Standards and Petitions Committee (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.1.
- MAATE: Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador (2024) Mapa interactivo. http://ide.ambiente.gob.ec/mapainteractivo/ [accessed 14 Nov 2024]
- Palacios, W (2012) Cuatro especies nuevas de árboles del Ecuador. Caldasia 34(1): 75–85. https://revistas.unal.edu.co/index.php/cal/article/view/36428
- Reveal JL (2012) Matisieae and Matisiinae. Indices Nominum Supragenericorum Plantarum Vascularium. http://www.plantsystematics.org/reveal/pbio/fam/allspgfileM. html [accessed 14 Nov 2024]
- Robyns A (1976) Bombacaceae neotropicae novae VII: A new species of *Quararibea* from Ecuador. Bulletin du Jardin Botanique National de Belgique 46(1/2): 235–236. https://doi.org/10.2307/3667417
- Schumann K (1895) Matisieae. In: Engler A, Prantl K (Eds) Die Natürlichen Pflanzenfamilien III, 6: 58, 63–65. https://www.biodiversitylibrary.org/item/56529
- Stevens PF (2001 onwards) Angiosperm Phylogeny Website, version 17, updated 4 Oct 2024. Missouri Botanical Garden, St. Louis. https://www.mobot.org/MOBOT/research/APweb/
- Thiers B (2019) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden, New York. https://sweetgum.nybg.org/science/ih/

Torke B, Perez A (2013) Notes on the genus *Swartzia* (Leguminosae) in Ecuador, with descriptions of two new species. Phytotaxa 147: 13–25. https://doi.org/10.11646/ phytotaxa.147.1.2

Wilson EO (1992) The Diversity of Life. Belknap Press, Cambridge.

#### Appendix 1

This appendix considers the option of placing the genus *Phragmotheca* Cuatrec. and two closely-related genera in the /Matisieae clade (*Matisia* and *Quararibea*, Baum et al. (2004)), in the Matisieae within the Malvoideae, versus placing it in the newly-published Matisioideae (Colli-Silva et al. 2025). We use forward slashes ("clademarks," as described in Baum et al. (1998)) for the names of clades, to avoid confusion with ranked names, which are, in many cases, otherwise identical.

Recent phylogenetic studies consistently indicate a clade comprising one branch with all members of the traditional family Malvaceae and a second branch with most members of the traditional family Bombacaceae (excluding *Durio* Adans. and relatives). This clade was given the name /Malvatheca (fig. 2, in Baum et al. (2004)). Its two major branches, the /Malvoideae and /Bombacoideae clades, were given stem-based phylogenetic definitions (appendix in Baum et al. (1998)). Ranked names for these two clades, Malvoideae and Bombacoideae, came into general use by researchers in the last two decades and in authoritative references such as Stevens (2001 onwards). Colli-Silva et al. (2025) acknowledge the close phylogenetic relationship of these two major lineages, but they constrain the application of Malvoideae to the distal portion of the /Malvoideae clade, which then necessitates recognition of the /Matisieae clade as a subfamily, Matisioideae. However, this narrowed application of the ranked name Malvoideae has consequences that may not be desirable.

Under their proposal, the /Malvoideae clade would no longer have a ranked name available for it, despite being a lineage with a complex and interesting pattern of floral, fruit and biogeographic evolution and despite its sister lineage retaining its subfamilial rank: Colli-Silva et al. (2025) did not elevate the three currently recognised tribes of Bombacoideae (Adansonieae Horan, Bernoullieae Carv.-Sobr. and Bombaceae Kunth.; Carvalho-Sobrinho et al. (2016)) to subfamilies, notwithstanding molecular and morphological support comparable to their Matisioideae and Malvoideae.

The second set of consequences may obtain when better phylogenetic trees are available for the base of the /Malvatheca clade. For example, the genera *Ochroma* Sw. (1 sp.) and *Patinoa* Cuatrec. (4 spp.) have been recovered in some phylogenetic studies (Alverson et al. 1999; Baum et al. 2004; Carvalho-Sobrinho et al. 2016) as basal branches of the /Malvoideae clade (a quite plausible result, given aspects of their morphology) and phylogenetic placements of the genera *Chiranthodendron* Larreat. (1 sp.), *Fremontodendron* Coville (3 spp.) and *Septotheca* Ulbr. (1 sp.) are still unclear. With the exception of *Fremontodendron*, no representatives of these genera were included in the study by Colli-Silva et al. (2025). If any of these are found to be basal branches of the /Malvoideae clade, then they will require one or more new subfamily names despite comprising only one or a few species. In contrast, the broader concept of Malvoideae that has been in general use over the last two decades would not require the designation of additional, tiny subfamilies. Alternatively, assigning the rank of supertribe (Ezedin 2024) to the segment of the malvoid lineage that has echinate pollen (i.e. the Malvoideae of Colli-Silva et al. (2025)) would also avoid these problems. If, in the future, more comprehensive phylogenetic studies find that the /Matisieae clade is in a polytomy with the /Bombacoideae and /Malvoideae clades or attaches outside of the /Malvatheca clade, it would make sense to elevate the current tribe, Matisieae, to subfamilial status. However, that is not our current understanding of relationships.

Thus, narrowing the definition of Malvoideae and elevating the Matisieae to subfamilial status seems premature or even unwarranted and does not add significant insight into the evolution of these groups. Assignment of ranks is arbitrary, but requires adequate justification, especially since changing them can create confusion through creating multiple meanings of the same name(s). Therefore, until convincing phylogenetic studies are available that include representatives of at least *Ochroma, Patinoa* and *Septotheca*, we prefer to retain the concepts of Malvoideae and Matisieae that have been in general use during the last two decades.

## **Supplementary material 1**

#### Conservation assessment and georeferenced data

- Authors: Juan Ernesto Guevara-Andino, Dawson M. White, Nigel C. A. Pitman, Juan-Carlos Cerón, Andrea Fernández, Daniel Navas-Muñoz, William S. Alverson Data type: xlsx
- Explanation note: This file contains information regarding herbarium specimens and habitat reduction analyses used for the description of the new tree species *Phragmotheca centinelensis*. The different spreadsheets contains georeferenced data, collectors' name, herbaria names and data on habitat reduction, deforestation, extent of occurrence and area of occupancy.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.143106.suppl1



**Research Article** 

# Aristolochia zhuhaiensis, a self-supporting new species of Aristolochiaceae from Guangdong, China and notes on Aristolochia thwaitesii

Yi-Fan Wang<sup>10</sup>, Zi-Rui Guo<sup>20</sup>, Sven Landrein<sup>30</sup>, Joyce G. Onyenedum<sup>10</sup>, Shuai Liao<sup>40</sup>

- 1 Department of Environmental Studies, New York University, New York 10012, USA
- 2 Suzhou Lianhelvyou Ecological Agriculture Development Co., LTD, Suzhou 215026, Jiangsu, China
- 3 Kadoorie Farm and Botanic Garden (KFBG) Corporation, Hong Kong S.A.R., China
- 4 South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, Guangdong, China
- Corresponding authors: Joyce G. Onyenedum (jgo5750@nyu.edu); Shuai Liao (shuai.liao.cn@gmail.com)

#### Abstract

A self-supporting new species of Aristolochiaceae, *Aristolochia zhuhaiensis* Y.Fan Wang & Z.R.Guo, is described and illustrated from Zhuhai City, Guangdong Province, China. Morphologically, this species exhibits vegetative characters, including leaf blade shape and growth form, that are similar to *Aristolochia thwaitesii* Hook., the only other known taxon in *Aristolochia* subg. *Siphisia* in Asia that is non-twining and self-supporting, native to southern Guangdong, particularly the Hong Kong Special Administrative Region. However, *A. zhuhaiensis* can be readily distinguished from *A. thwaitesii* by its unique floral morphology. The new species features a campanulate to funnel-shaped calyx limb densely covered with porcelain-white granular pustules. In contrast, *A. thwaitesii* has an elongated, concave tubular calyx limb with an adaxial surface that transitions from purple to pale yellow or white towards the apex, densely covered with recurved fleshy prickles. *Aristolochia zhuhaiensis* is endemic to Zhuhai City and has not been observed elsewhere. Detailed illustrations, photographic plates, a distribution map, a digitized holotype voucher specimen, a comparative table, and an identification key are provided to facilitate the differentiation of *A. zhuhaiensis* from *A. thwaitesii*.

**Key words:** Aristolochia subg. Siphisia, conservation status, fieldwork, morphology, taxonomy

## Introduction

Aristolochia L., the largest genus in Aristolochiaceae with more than 600 species distributed across tropical, subtropical, and temperate regions (Huber 1993; Neinhuis et al. 2005; Wanke et al. 2006), includes three recognized monophyletic subgenera: *Aristolochia*, *Pararistolochia* (Hutch. & Dalziel) Schmidt, and *Siphisia* (Duch.) Schmidt (González and Stevenson 2002; Wanke et al. 2006; Buchwalder et al. 2014; Ohi-Toma and Murata 2016). Subgenus *Siphisia*, notable for its morphological distinctiveness—strongly curved perianth, three-lobed gynostemium, and paired anthers (González and Stevenson 2000; 2002)—and



Academic editor: Elton John de Lirio Received: 17 October 2024 Accepted: 28 February 2025 Published: 21 March 2025

**Citation:** Wang Y-F, Guo Z-R, Landrein S, Onyenedum JG, Liao S (2025) *Aristolochia zhuhaiensis*, a self-supporting new species of Aristolochiaceae from Guangdong, China and notes on *Aristolochia thwaitesii*. PhytoKeys 254: 61–76. https://doi.org/10.3897/ phytokeys.254.139616

**Copyright:** <sup>©</sup> Yi-Fan Wang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). high species diversity in the Indochina Peninsula and southeastern Himalayas, has been a focal point of taxonomic debate (Pfeifer 1966; González 1999; Huang et al. 2003; Ohi-Toma et al. 2006; Wanke et al. 2006; González et al. 2014). While systematic and molecular studies have consistently supported its monophyly within *Aristolochia sensu lato* (González and Stevenson 2002; Wanke et al. 2006), Zhu et al. (2019) conducted new phylogenetic analyses using more comprehensive genetic datasets and proposed its reinstatement to genus rank as *Isotrema* Raf., based on infra- and inter-genic markers. Since its publication, this treatment has been adopted by some scholars, primarily in new species descriptions (Li et al. 2019; Cai et al. 2020; Huang et al. 2022), contrasting with the prevailing *Aristolochia sensu lato* framework used in broader botanical studies (Phan et al. 2021; Van Do et al. 2021a; Van Do et al. 2021b). Here, while acknowledging the valid arguments for both perspectives, we adhere to the *Aristolochia sensu lato* framework, which aligns with long-standing taxonomic convention and robustly integrates both morphological and molecular evidence.

In Aristolochia s.l., the majority of species exhibit a climbing habit, either as herbaceous climbers or woody lianas (Wagner et al. 2014; Trueba et al. 2015). Wagner et al. (2014) demonstrated that the climbing habit, prevalent across most species in the genus, is a derived trait that evolved from herbaceous or shrub-like ancestors and likely facilitated the rapid diversification of *Aristolochia* s.s. and subg. *Siphisia*. Within subg. *Siphisia*, the evolution of shrub-like traits in certain species has been proposed to originate from climbing *Aristolochia* ancestors. Wagner et al. (2012) developed two hypotheses to explain the emergence of these traits, both based on the premise that climbing is the plesiomorphic state for subg. *Siphisia*. Notably, all non-climbing members of subg. *Siphisia* included in these studies were restricted to North and Central America.

In Asia, nearly all *Aristolochia* subg. *Siphisia* species are climbers, with one notable exception: *Aristolochia thwaitesii* Hook. This species, previously the only documented self-supporting subshrub of Asian subg. *Siphisia*, is primarily found in coastal regions of Hong Kong SAR, China (Tutcher 1905; Dunn and Tutcher 1912; Barringer 1993; Xia 2007), and has also been reported in neighboring Guangdong cities such as Jiangmen and Zhuhai (Ma 1989; Huang et al. 2003). However, upon reviewing related literature and examining available voucher specimens of *A. thwaitesii*, we found that nearly all were collected from Hong Kong, with only two vegetative specimens from Guangdong (IBSC0127924 & SYS00159983). Beyond this, no fertile specimens or living collections from mainland China exist.

During a 2023 late spring expedition in Doumen District, Zhuhai City, as part of a traditional Chinese medicine survey, we discovered an *Aristolochia* species that is an erect non-climbing subshrub with leaf morphology very similar to *A. thwaitesii*. The plants have been collected and cultivated by locals for their dried roots, harvested for medicinal purposes. Based on its vegetative characteristics, we tentatively identified it as *A. thwaitesii* and collected samples for ex situ conservation, as this species is listed as Vulnerable under IUCN criteria A2c (China Plant Specialist Group 2019).

In late January 2024, the cultivated plants began to flower, revealing floral morphological features significantly different from *Aristolochia thwaitesii*. These differences, when compared to original descriptions and herbarium specimens, suggested it could potentially be a new species. Subsequent fieldwork from February to April across multiple locations in Zhuhai City uncovered several flowering populations of this *Aristolochia* species, all with consistent floral features matching those of the specimens we collected in 2023 from Doumen District and now under cultivation.

This unknown species is morphologically similar to *A. thwaitesii* in terms of plant habit, vegetative traits, and its connate geniculate perianth, but it is readily distinctive due to its floral morphology. It has a campaniform-infundibuliform calyx limb densely covered with porcelain-white granular pustules, whereas *A. thwaitesii* has an elongated concave tubular calyx limb with an adaxial surface transitioning from purple to pale yellow or white toward the calyx limb apex, fully covered with recurved fleshy prickles. This unknown species has been found exclusively in coastal dwarf forests situated in hilly areas, Zhuhai City.

Based on these findings, this study concludes that the newly discovered *Aristolochia* represents a new species, which is described and illustrated herein.

## Material and methods

The specimens examined in this study were collected from Doumen District, Zhuhai, Guangdong Province, China, and deposited at the Herbarium of the South China Botanical Garden (IBSC), Chinese Academy of Sciences. Morphological comparisons, including flower shape, color, and longitudinal sections of floral organs, were conducted against the original descriptions and voucher specimens of *Aristolochia thwaitesii*.

The conservation status assessment followed the IUCN Red List Categories and Criteria (IUCN 2012) and the latest Guidelines for Using the IUCN Red List Categories and Criteria (IUCN 2024). The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were calculated using GeoCAT (Geospatial Conservation Assessment Tool) (Bachman et al. 2011), incorporating field data, ecological literature, and known distribution records to evaluate the species' range and potential threats.

## **Taxonomy treatment**

Aristolochia zhuhaiensis Y.Fan Wang & Z.R. Guo, sp. nov. urn:lsid:ipni.org:names:77358999-1 Figs 1–3

**Type.** CHINA. • Guangdong Province: Zhuhai City, Doumen District, under forest in coastal hills, elevation 90 m, 16 Feb 2024, Y.Fan Wang & Z.R. Guo *yw00036* (holotype: IBSC1041409!; isotypes: IBSC1041410!, IBSC1041411!).

**Diagnosis.** Aristolochia zhuhaiensis shares morphological similarities with *A. thwaitesii*, both of which are distributed in neighboring regions and represent the only two known self-supporting, non-twining *Aristolochia* subg. *Siphisia* species in Asia. In terms of vegetative characteristics, these two congeners are highly similar; however, they can be readily distinguished by their markedly different floral morphologies. *Aristolochia zhuhaiensis* is characterized by a campaniform-infundibuliform calyx limb densely covered with porcelain-white granular pustules. It also features a conspicuous platform-shaped area beneath the perianth throat, which is white, glabrous, waxy, and slightly elevated



Figure 1. Illustration of Aristolochia zhuhaiensis A habit in situ B inflorescence in early development C inflorescence D lateral view of the flower E longitudinal section of the flower F front view of the bud G, H front view of the flower, showing the adaxial side of the calyx limb covered with porcelain-white granular pustules I–J gynostemium, from female stage (I) to male stage (J) K leaves (adaxial), L. Leaves (abaxial). Images B–E, I were provided by Mr. Yiwen Jiang.

upward. In contrast, *A. thwaitesii* possesses an elongated, concave tubular calyx limb with an adaxial surface that transitions from purple to pale yellow or white towards the apex. This species is entirely covered with recurved fleshy prickles and lacks the distinctive platform structure found in the calyx of *A. zhuhaiensis*. Detailed morphological comparisons between the new species and *A. thwaitesii* are shown in Figs 4, 5 and Table 1.



Figure 2. Line drawing of *Aristolochia zhuhaiensis* by Ms. Yushan Cai **A** habit, showing a subshrubby growth form with basal inflorescences **B** roots fusiform or globose **C**, **D** front and lateral view of the flower **E** bud **F** bud sprouts in early development **G**, **H** gynostemium **I** adaxial side of the leaf **J** abaxial view of the leaf, pinnate venation.

1 2 3 4 5 6 Jandan Jandan	7 8 9 10 GUANGDONG
me M	
	JW 00036
₩ <sup>按院华御</sup> ∰ 938558 册 标本馆	中国科学院华南植物园标本馆(1880) Aristolochiaceae 马兜特科_24 图式标本(2024-124) Aristolochiaceae 建成大规则。 botterma shahainense Yi Fan Wang & Z. R. Guo 建立人 课料 2024-11-28 建筑 课料 2024-11-28 环境地: 中国、广家省、珠海市、中口区、沿海林下 林谷教、3,生活型、洋湖大、各社: holotype & isotypes <b>法</b> 成本 9 元,

Figure 3. Holotype of Aristolochia zhuhaiensis Y.Fan Wang & Z.R. Guo (IBSC 1041409).

**Description.** Erect subshrub. Roots fusiform or globose. Stems terete, densely rusty villous, becoming glabrous when lignified. Leaf blades spathulate, narrowly oblanceolate, or lanceolate, with an apex acute to acuminate,  $12-16 \times 3-3.5$  cm. Almost glabrous to subglabrous adaxially, densely brown-villous abaxially. Venation pinnate, with 10-12 secondary veins on each side, leaves



**Figure 4.** Comparison of the floral morphology of *Aristolochia zhuhaiensis* and *A. thwaitesii* **A**–**D** *A. zhuhaiensis* **A** front view of the flower **B** longitudinal section of the flower **C** porcelain-white granular pustules on adaxial calyx limb **D** gynostemium, stigma apex slightly hooked inward **E**–**H** *A. thwaitesii* **E** front view of the flower **F** longitudinal section of the flower **G** recurved purple fleshy prickles on adaxial calyx limb **H** gynostemium with stigma apex not curved.

papery; petiole approximately 0.6–1.0 cm long, densely rusty villous. Basal inflorescence, fasciculate; flower count varies significantly depending on plant nutrient status, ranging from solitary to up to 20. Pedicels approximately 1–1.2 cm long, initially erect, then pendulous, densely rusty villous. Bracteole 1, lanceolate,  $1-2 \times 0.4-0.5$  mm, densely rusty villous on both sides, sessile.



Figure 5. Comparison of the vegetative morphology of Aristolochia zhuhaiensis and A. thwaitesii A-CA. thwaitesii A adaxial (bottom right) and abaxial (top left) side of the leaf B petiole and stem C habit in situ D-FA. zhuhaiensis D adaxial (top left) and abaxial (bottom right) side of the leaf E petiole, nodes and stem F habit in situ.

Perianth zygomorphic, densely covered with grey indumentum abaxially, campanulate,  $0.9-1.2 \times 0.8-1.0$  cm; dark purplish, arachnoid white villous. Calyx tube geniculately curved, yellowish to brownish abaxially; utricle to geniculation: 2.5-3.5 cm, light purplish with dark veins, subglabrous adaxially; geniculation to throat: 2.5-3.0 cm, dark purple, glabrous, velvety adaxially. Calyx limb

Characters	Aristolochia zhuhaiensis	Aristolochia thwaitesii				
Distribution	Zhuhai, Guangdong, China	Hong Kong SAR, China				
Habit	Erect subshrub	Erect subshrub				
Leaf	·					
shape	Spathulate, narrowly oblanceolate, or lanceolate, with an apex acute to acuminate	Spathulate, narrowly oblanceolate, or ob- long-oblanceolate, with an apex acuminate to obtus				
size	12-16 × 2-2.5 cm	10−15 × 2.5−3 cm				
pubescence (adaxial side)	Almost glabrous to subglabrous adaxially, densely brown-villous abaxially	Subglabrous adaxially, densely brown-villous abaxially				
venation	Pinnate, 10–12 secondary veins each side	Pinnate, 10–12 secondary veins each side				
Inflorescence						
inflorescence	Basal inflorescence; flowers in dense fascicles; fascicles with variable flower count	Basal inflorescence; flowers in dense fascicles; fascicles with variable flower count				
flower count	Solitary to 20 flowers	Solitary to 30-40 flowers				
Perianth	·	·				
calyx utricle (adaxial side)	Campanulate, 0.9–1.2 × 0.8–1.0 cm; dark purplish, arachnoid white villous	Cylindric to spherical, 0.8–1.0 × 0.6–0.8 cm; dark purplish, arachnoid white villous				
calyx tube (adaxial side, U–G–T: utricle to geniculation to throat)	U–G: 2.5–3.5 cm, light purplish with dark veins, subglabrous; G–T: 2.5–3.0 cm, dark purple, glabrous, velvety	U–G: 1.8–2.8 cm, purplish with white patches, subglabrous, slightly ridged with veins; G–T: 2.0–2.5 cm, dark purple to almost black, glabrous, velvety				
calyx limb (adaxial side)	Calyx limb 3.0-4.0 cm long, 4.5-5.5 cm in diameter, flaring to campaniform-infundibuliform; densely covered with porcelain-white granular pustules; beneath the throat forms a glabrous and unarmed raised platform	Calyx limb elongate to concave tubular shape, 3.5– 4.0 cm long, 2.0–2.5 cm in diameter; fully covered with recurved fleshy prickles; Calyx margin white to pale yellow, 3 dentate; dark purple inside tubular calyx limb without any platform-like Structure				
calyx throat	Throat ca. 6–9 mm in diameter; purple, cordate-shaped	Throat ca. 5–8 mm in diameter, purple to black; elliptic-shaped				
Gvnostemium	Lobe apex hooked inward	Lobe apex obtuse				

Table '	1. Mor	phological	comparison	and distr	ibution rang	e of Ari	istolochia.	zhuhaiensis	and A.	thwaitesii.
---------	--------	------------	------------	-----------	--------------	----------	-------------	-------------	--------	-------------

3.0–4.0 cm long, 4.5–5.5 cm in diameter, flaring to campaniform-infundibuliform, densely covered with porcelain-white granular pustules; beneath the throat forms a glabrous and flat area that lies on the soil, waxy; abaxial side of calyx limb brownish to yellowish, villous with ridged veins. Apex of the calyx limb 3-dentate; when the flower is near withering, the calyx apex rolls outward. Throat approximately 6–9 mm in diameter, purple, cordate-shaped. Stamens 6 in one series, fully adnate in 3 pairs to the style column to form a gynostemium, opposite the stigma lobes; anthers oblong, approximately 2.0–3.0 mm long, extrorse. Gynostemium approximately 4.5–5 mm long, 4–5 mm in diameter, fleshy; lobes 3, hooked inward at the apex; margin crenate-rugose. Ovary inferior, cylindric, 6–8 mm long, 3–4 mm in diameter, densely rusty villous abaxially.

**Distribution and habitat.** The new species has been discovered exclusively in Zhuhai City, Guangdong Province, China (Fig. 6). It is typically found near creeks within forests on coastal hill slopes, at elevations ranging from 50 to 100 meters. To date, three populations have been identified, all situated in the southern region of Zhuhai City: two within Doumen District and one in Jinwan District, each in proximity to the coastal areas. This species commonly occurs near water sources such as creeks and mountain streams, usually thriving under dense canopy cover. Associated species typically include *Nepenthes mirabilis* (Lour.) Druce, *Drosera spatulata* Labill., *Strophanthus divaricatus* (Lour.) Hook. & Arn., *Pteris vittata* L., and *Rhodomyrtus tomentosa* (Aiton) Hassk.



Species Distribution Map: Aristolochia zhuhaiensis & A. thwaitesii Occurrences in Guangdong Province and Hong Kong SAR

**Figure 6.** Distribution map of *Aristolochia zhuhaiensis* Y.Fan Wang & Z.R. Guo, sp. nov., highlighting its geographically close yet isolated distribution, with *A. thwaitesii* primarily found in Hong Kong SAR and *A. zhuhaiensis* observed in Zhuhai City.

**Phenology.** Flowering from January to early March, fruit or seed not seen. **Etymology.** The epithet '*zhuhaiensis*' refers to the type locality (Zhuhai City), where the new species has been discovered. Given Zhuhai's status as a highly populated and urbanized city, the discovery of a new species is both a rare and welcome occurrence.

Vernacular name. During our initial encounter with this new species, local cultivators referred to it by the Cantonese vernacular name "shí qín qú" (石蠄蝶), indicating that the root, harvested for medicinal purposes, has a surface texture so coarse it resembles a toad's skin. Interestingly, as our survey extended further, we discovered that the Hakka people refer to the species as "shù zăi lián" (树仔莲), which translates literally to "shrubby herb medicine." These two distinct ethnic groups each have their own vernacular name for the same species. For clarity and consistency in scientific communication with the broader public, we propose the Chinese name "zhū hǎi guān mù tōng" (珠海关木通), reflecting the literal meaning of its epithet and its geographical origin.

**Conservation status.** Since we first discovered and recognized it as a new species, we have conducted more than five extensive field trips covering the coastal mountain hills, including all major coastal cities within the Greater Bay Area, particularly focusing on Zhuhai, and its neighboring cities Jiangmen and Shenzhen. Despite these efforts, we only located three extant populations, all of which are confined to Zhuhai. As previously mentioned, this species has long been recognized by local medicinal practitioners and has been exploited for

generations to treat snake bites, according to local testimony. The traditional belief in its medicinal properties has made this species highly sought after, leading to its scarcity in the wild in recent years.

Our investigation revealed that *A. zhuhaiensis* is morphologically challenging to distinguish from its close congener, *A. thwaitesii*, when not in flower. Local collectors report sometimes to travel to outlying islands near Hong Kong or the Pearl River Estuary to harvest this plant. We hypothesize that in these cases, it remains unclear whether the collected individuals are *A. zhuhaiensis* or *A. thwaitesii*, especially as both species primarily exhibit vegetative growth for much of the year, and the collectors focus mainly on harvesting the tuberous roots based on our field survey.

Aristolochia zhuhaiensis faces several severe threats. While A. thwaitesii is already classified as Vulnerable (VU) according to the China Plant Specialist Group (2019), the conservation status of A. zhuhaiensis appears to be even more precarious. The three populations we identified are situated on very small hills in close proximity to urban areas. These hills have limited area and gentle slopes and are completely encircled by urban developments, including residential communities, real estate projects, tourist attractions, airport, and farmlands, as well as other anthropogenic landscapes. Furthermore, these habitats have been heavily invaded by artificial fast-growing *Eucalyptus* plantations. Our field observations indicate that A. zhuhaiensis exclusively grows under native coastal low shrubland along riparian zones and does not thrive in secondary forests or plantation environments. Habitat loss due to its proximity to human activity is likely one of the primary threats to its survival.

During our multiple fieldwork expeditions in the region, we observed a substantial number of flowers produced by robust individuals, with some bearing up to 20 flowers in a single fascicle at the basal stem. However, we found no evidence of fruiting or seed production. The basal flowering habit might indicate specific pollinator requirements, a topic that warrants further ecological study.

Another aspect of the potential ecological significance of *A. zhuhaiensis* is its possible role as a host plant for the Chinese windmill butterfly *Byasa alcinous* Klug, which is also associated with *A. thwaitesii*. This host relationship with *A. thwaitesii* has been confirmed by the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong SAR (Anon 2010). Considering the morphological similarities between *A. zhuhaiensis* and *A. thwaitesii*, as well as the natural range of *B. alcinous* extending into Zhuhai (Wu and Bai 2001), *A. zhuhaiensis* could also provide a vital food source for the butterfly. Further ecological studies are needed to confirm this potential interaction.

Currently, the three known populations of *A. zhuhaiensis* are located in the coastal region of Zhuhai City, each in close proximity and restricted to an area of less than 10 km<sup>2</sup>, at elevations ranging from 50 to 100 meters. Based on these populations, the estimated extent of occurrence (EOO) is 0.335 km<sup>2</sup> (<100 km<sup>2</sup>), and the area of occupancy (AOO) is 12.000 km<sup>2</sup>. No fruiting or seed production has been observed over the past two consecutive years, and each population comprises fewer than ten individuals. Based on these findings, we recommend classifying *A. zhuhaiensis* as Critically Endangered (CR) under the IUCN Red List criteria B1ab(i,ii,iii,iv,v); D1. This recommendation reflects its extremely limited distribution, ecological significance, close association with urban areas, vulnerability to habitat loss and medicinal exploitation, and the absence of observed sexual reproduction.

Additional specimen of Aristolochia zhuhaiensis examined. CHINA. • Guangdong Province: Zhuhai City, Doumen District, under forest in coastal hills, elevation 68 m, 2 Mar 2024, Y.Fan Wang & Z.R. Guo *yw00077* (paratype: IBSC1041412!).

**Specimens of Aristolochia thwaitesii examined.** CHINA. • Hongkong: Wong Nai Chung Gap, 1903, W.J. Tutcher 979 (IBSC0127925), 4 Jul 2000, Y.W. Lam 1633 (HK38320); Lantau Island, 16 Mar 1909, W.J. Tutcher 8273 (IBSC0127926 & HK23774). UK. Cultivated at Kew, Royal Botanic Gardens, *s.coll. s.n.*, April 1858 (K003691508); • Tze Kong Bridge, Repulse Bay, Southern District, Hong Kong Island, 1 April 2024 Y.Fan Wang *yw00068* (IBSC!); Tze Kong Bridge, Repulse Bay, Southern District, Hong Kong Island, 2 April 2024 Y.Fan Wang *yw00069* (IBSC!).

Notes. Aristolochia thwaitesii has been confirmed, by our fieldwork, to occur in the Hong Kong region, specifically on Hong Kong Island and Lantau Island (Fig. 6). Although there are records indicating its discovery in Jiangmen and Zhuhai (Ma 1989; Huang et al. 2003), no living plant collection, or photograph of the flower has provided conclusive evidence of its presence there. The only two voucher specimens (IBSC0127924 & SYS00159983), collected in 1973 and 1979, almost 50 years ago, are solely vegetative specimens, lacking flowers. Following the confirmation of A. zhuhaiensis, we re-examined these two voucher specimens and embarked on a field expedition in early July 2024 to Dawanshan Island, where voucher IBSC0127924 was originally collected. Despite extensive efforts, we failed to locate any Aristolochia individuals. Administratively, Wanshan Island is under Zhuhai's jurisdiction, but geographically it lies closer to the known distribution of A. thwaitesii in Hong Kong than to the range of A. zhuhaiensis that we have documented. As for voucher SYS00159983, despite multiple extensive field surveys in Jiangmen City, no specimens have been found. Given the current lack of definitive evidence, such as a living specimen or a photograph confirming the floral morphology, we have refrained from assigning either voucher to A. thwaitesii or A. zhuhaiensis until further conclusive data can clarify their identity.

Given the proximity of this species to urban areas and the lack of any form of protection or monitoring in its natural habitats, combined with its long history of medicinal exploitation, we have opted not to disclose the precise locations of these populations in this paper to safeguard their conservation. We strongly urge local authorities to implement policies or legislation to protect this rare species. Researchers requiring material or information for scientific purposes may contact the authors directly for collaboration and information sharing.

#### Key to Aristolochia zhuhaiensis and morphologically close species

- 1 Habit erect subshrub......2
- Habit climbing shrub or woody liana ......3
#### 

- ple, with a bright yellow throat.....**A. championii**

### Acknowledgements

We are extremely grateful for the support of this project, which was funded in part by a grant from the New York University Center for Environmental and Animal Protection (CEAP). We also extend our sincere thanks to Mr. Yiwen Jiang for his initial acute recognition of the species, his invaluable assistance in connecting us with local communities, and for providing essential images of the plant. Our gratitude goes to Ms. Yushan Cai for her accurate and exquisite scientific illustrations of this species. We also appreciate the assistance of Dr. Ji-Hong Li at Kadoorie Farm and Botanic Garden (KFBG, Hong Kong) during our fieldwork, as well as Mr. Huaneng Li for his help in locating one of the wild populations. Lastly, we express our heartfelt thanks to Dr. Israel Lopes da Cunha Neto, Dr. Lena Hunt, Ms. Angelique Acevedo, Mr. Zachary Kozma, and Ms. Hannah Ratcliff from the Onyenedum Lab at New York University for their continuous support and botanical guidance.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

This work was supported by a grant from the New York University Center for Environmental and Animal Protection (CEAP). This research was also funded by a fellowship from the China Postdoctoral Science Foundation (2024M753278) to SL.

### Author contributions

Conceptualization: YFW. Data curation: ZRG, YFW. Formal analysis: YFW. Funding acquisition: SL, YFW, JGO. Methodology: JGO, YFW, SL. Project administration: JGO, SL. Resources: SL. Supervision: JGO, SL, SL. Visualization: ZRG. Writing - original draft: YFW. Writing - review and editing: JGO, SL, SL.

### Author ORCIDs

Yi-Fan Wang <sup>©</sup> https://orcid.org/0009-0003-1339-1856 Zi-Rui Guo <sup>©</sup> https://orcid.org/0009-0004-1817-9827 Sven Landrein <sup>©</sup> https://orcid.org/0000-0003-0028-2450 Joyce G. Onyenedum <sup>©</sup> https://orcid.org/0000-0002-1047-9807 Shuai Liao <sup>©</sup> https://orcid.org/0000-0002-3876-8002

### Data availability

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

### References

- Anon (2010) Eco-Blog: Butterfly. Hong Kong Live Eco-map by Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong Special Administrative Region. http://117.18.78.138/ecolog.asp?AnimalID=2&lang=cht&offset=14 [September 4, 2024]
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Barringer K (1993) On Aristolochia thwaitesii Hooker (Aristolochiaceae). Novon 3(4): 321–323. https://doi.org/10.2307/3391376

- Buchwalder K, Samain M-S, Sankowsky G, Neinhuis C, Wanke S (2014) Nomenclatural updates of *Aristolochia* subgenus *Pararistolochia* (Aristolochiaceae). Australian Systematic Botany 27(1): 48–55. https://doi.org/10.1071/SB13042
- Cai L, Dao ZL, Zhu XX (2020) *Isotrema hei* (Aristolochiaceae), a new Species from Yunnan, China. Annales Botanici Fennici 57(1–3): 125–129. https://doi.org/10.5735/085.057.0117
- China Plant Specialist Group (2019) *Aristolochia thwaitesii* (amended version of 2004 assessment). https://doi.org/10.2305/IUCN.UK.2004.RLTS.T46528A144785442.en [August 18, 2024]
- Dunn ST, Tutcher WJ (1912) Flora of Kwangtung and Hongkong (China). Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) Additional Series 10: 1–370. https://doi.org/10.5962/bhl.title.57063
- González F (1999) A phylogenetic analysis of the Aristolochioideae (Aristolochiaceae). Ph.D. City University of New York. https://www.proquest.com/docview/304498487/ abstract/E04AE34AD52D40CCPQ/1 [June 12, 2024]
- González F, Stevenson DW (2000) Perianth development and systematics of *Aristolochia*. Flora (Jena) 195(4): 370–391. https://doi.org/10.1016/S0367-2530(17)30995-7
- González FA, Stevenson DW (2002) A phylogenetic analysis of the subfamily Aristolochioideae (Aristolochiaceae). Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 26: 25–59. https://doi.org/10.18257/raccefyn.26(98).2002.2752
- González F, Wagner ST, Salomo K, Symmank L, Samain M-S, Isnard S, Rowe NP, Neinhuis C, Wanke S (2014) Present trans-Pacific disjunct distribution of *Aristolochia* subgenus *Isotrema* (Aristolochiaceae) was shaped by dispersal, vicariance and extinction. Journal of Biogeography 41(2): 380–391. https://doi.org/10.1111/jbi.12198
- Huang SM, Kelly LM, Gilbert MG (2003) Aristolochiaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, Vol. 5. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 246–269.

- Huang YS, Qin K, Zou CY, Huang ZP, Liu Y (2022) *Isotrema haimingii*, a new species of Aristolochiaceae from Dayaoshan Mountain of Guangxi, China. Taiwania 67: 380–385. https://doi.org/10.6165/tai.2022.67.380
- Huber H (1993) Aristolochiaceae. In: Kubitzki K, Rohwer JG, Bittrich V (Eds) The families and genera of vascular plants. Springer, Berlin, 129–137. https://doi.org/10.1007/978-3-662-02899-5\_10
- IUCN Species Survival Commission (SSC) (2012) IUCN Red List categories and criteria, version 3.1, 2<sup>nd</sup> edn. https://portals.iucn.org/library/node/10315
- IUCN Standards and Petitions Committee (2024) Guidelines for Using the IUCN Red List Categories and Criteria (Version 16). https://www.iucnredlist.org/documents/ RedListGuidelines.pdf [June 13, 2024]
- Li RT, Wang ZW, Wang J, Zhu XX, Xu H (2019) *Isotrema sanyaense*, a new species of Aristolochiaceae from Hainan, China. PhytoKeys 128: 85–96. https://doi.org/10.3897/ phytokeys.128.35042
- Neinhuis C, Wanke S, Hilu KW, Müller K, Borsch T (2005) Phylogeny of Aristolochiaceae based on parsimony, likelihood, and Bayesian analyses of *trnL-trnF* sequences. Plant Systematics and Evolution 250: 7–26. https://doi.org/10.1007/s00606-004-0217-0
- Ohi-Toma T, Murata J (2016) Nomenclature of *Isotrema*, *Siphisia*, and *Endodeca*, and their related infrageneric taxa of *Aristolochia* (Aristolochiaceae). Taxon 65(1): 152–157. https://doi.org/10.12705/651.11
- Ohi-Toma T, Sugawara T, Murata H, Wanke S, Neinhuis C, Murata J (2006) Molecular Phylogeny of *Aristolochia* sensu lato (Aristolochiaceae) based on Sequences of *rbcL*, *matK*, and *phyA* Genes, with Special Reference to Differentiation of Chromosome Numbers. Systematic Botany 31(3): 481–492. https://doi.org/10.1600/036364406778388656
- Pfeifer HW (1966) Revision of the North and Central American Hexandrous Species of Aristolochia (Aristolochiaceae). Annals of the Missouri Botanical Garden 53(2): 115– 196. https://doi.org/10.2307/2394940
- Phan LK, Wanke S, Neinhuis C, Van Do T (2021) *Aristolochia luudamcui* (Aristolochiaceae), a new species from northern Vietnam. Phytotaxa 527(1): 67–74. https://doi. org/10.11646/phytotaxa.527.1.7
- Trueba S, Rowe NP, Neinhuis C, Wanke S, Wagner ST, Isnard S, Friedman EWE (2015) Stem Anatomy and the Evolution of Woodiness in Piperales. International Journal of Plant Sciences 176(5): 468–485. https://doi.org/10.1086/680595
- Tutcher WJ (1905) Description of some New Species, and Notes on other Chinese Plants. The Journal of the Linnean Society. Botany 37: 66. https://doi. org/10.1111/j.1095-8339.1905.tb00824.x
- Van Do T, Lai HV, Le KD (2021a) Aristolochia quangnamensis (Aristolochiaceae), a new species from central Vietnam. Annales Botanici Fennici 58(4–6): 267–273. https://doi.org/10.5735/085.058.0412
- Van Do T, Nguyen HV, Le KD (2021b) *Aristolochia vuquangensis* (Aristolochiaceae), a new species from Central Vietnam. Phytotaxa 500(1): 37–44. https://doi.org/10.11646/ phytotaxa.500.1.5
- Wagner ST, Isnard S, Rowe NP, Samain M-S, Neinhuis C, Wanke S (2012) Escaping the lianoid habit: Evolution of shrub-like growth forms in *Aristolochia* subgenus *Isotrema* (Aristolochiaceae). American Journal of Botany 99(10): 1609–1629. https://doi. org/10.3732/ajb.1200244
- Wagner ST, Hesse L, Isnard S, Samain M-S, Bolin J, Maass E, Neinhuis C, Rowe NP, Wanke S (2014) Major trends in stem anatomy and growth forms in the perianth-bearing

Piperales, with special focus on *Aristolochia*. Annals of Botany 113(7): 1139–1154. https://doi.org/10.1093/aob/mcu044

- Wanke S, González F, Neinhuis C (2006) Systematics of Pipevines: Combining Morphological and Fast-Evolving Molecular Characters to Investigate the Relationships within Subfamily Aristolochioideae (Aristolochiaceae). International Journal of Plant Sciences 167(6): 1215–1227. https://doi.org/10.1086/508024
- Wu CS, Bai JW (2001) A review of the genus *Byasa* Moore in China (Lepidoptera: Papilionidae). Oriental Insects 35(1): 67–82. https://doi.org/10.1080/00305316 .2001.10417288
- Xia NH (2007) Aristolochiaceae. In: Hu QM, Wu DL (Eds) Flora of Hong Kong, Vol. 1. Agriculture, Fisheries and Conservation Department, Hong Kong, 62–65.
- Zhu XX, Li XQ, Liao S, Du C, Wang Y, Wang ZH, Yan J, Zuo YJ, Ma JS (2019) Reinstatement of *Isotrema*, a new generic delimitation of *Aristolochia* subgen. *Siphisia* (Aristolochiaceae). Phytotaxa 401(1): 1–23. https://doi.org/10.11646/phytotaxa.401.1.1

## PhytoKeys

**Research Article** 

# *Carex yankouensis*, a new species of Cyperaceae from limestone landform in northern Guangdong, China

Ang Liu<sup>10</sup>, Jian-jun Zhou<sup>2</sup>, Lei Wu<sup>1</sup>, Xun-lin Yu<sup>1</sup>

1 Central South University of Forestry & Technology, Changsha 410004, Hunan, China

2 Hunan Agriculture and Forestry Industry Survey and Design Institute Co., Ltd, Changsha 410007, Hunan, China

Corresponding authors: Lei Wu (wuleiibk@163.com); Xun-lin Yu (csfuyuxl@163.com)

#### Abstract

*Carex yankouensis*, a new species of Cyperaceae (*Carex* section Rhomboidales) from the limestone landform in northern Guangdong, China is described and illustrated. The new species is similar to *C. brevicuspis* C. B. Clarke, but differs in having shorter culms (10-15 cm vs 20–55 cm) and spikes (1-1.5 cm vs 3.7-7 cm), leaves wider (15-20-35 mm vs 5-10 mm) and lighter colored (pale green or yellow-green vs dark green), nutlet beak oblique (vs erect or slightly curved), and slightly thickened (vs thickened) style base. Following the IUCN Red List Criteria (IUCN 2024), *Carex yankouensis* is assessed as 'Data Deficient (DD)'.

Key words: Carex, limestone landform, new species, taxonomy

### Introduction

*Carex* L., belonging to Cyperaceae, encompasses approximately 2000 species which are distributed across all continents except Antarctica. Notably, the treatment in Flora of China features 527 of these species, with an impressive 260 being exclusive to China (Dai et al. 2010). Undoubtedly, *Carex* stands as one of the most diverse genera among seed plants worldwide, yet the intricacies of its classification pose significant challenges. Despite these difficulties, recent years have witnessed the publication of numerous novel *Carex* species for China (Li et al. 2022; Lu and Jin 2022; Lu et al. 2023; Li et al. 2024; Qiu et al. 2024) and other parts of the world.

In Flora of China (Dai et al. 2010), species of the genus *Carex* are classified into 42 sections and three subgenera. The treatment includes section Rhomboidales with 43 species. However, the taxonomic revisions of the section have not been entirely resolved yet. Jin and Zheng (2013) revised this section and recognized 40 species, along with six subspecies and four varieties, but due to the widespread distribution of the species of this section, new species may still exist in some special geomorphic areas, such as limestone regions. Indeed, some recently published species of this section, such as *C. duanensis* Z.C.Lu, Y.F.Lu & X.F.Jin (Lu et al. 2024), are distributed in such limestone landform areas.

In July 2021, during our investigation in the limestone area of northern Guangdong, we collected a distinct species of *Carex* which they grew on the walls of



Academic editor: Pedro Jiménez-Mejías Received: 4 November 2024 Accepted: 2 March 2025 Published: 24 March 2025

Citation: Liu A, Zhou J-j, Wu L, Yu X-I (2025) *Carex yankouensis*, a new species of Cyperaceae from limestone landform in northern Guangdong, China. PhytoKeys 254: 77–85. https://doi.org/10.3897/ phytokeys.254.140929

**Copyright:** © Ang Liu et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). a karst cave. Although it neither bloomed nor bore fruit, the specimens were striking by their wide leaves, which reached a width of up to 35 mm, and the obvious small transverse veins between the leaf veins. We took two plants back to Changsha City for further observation and research. Fortunately, in November of that year, these two plants bloomed and provided us with mature nutlets for our research in March of the following year. At the same time, we also went to the corresponding phenological period to collect voucher specimens from the type locality. Through phenological observation and morphological research, we finally confirmed that this was a new species of *C*. sect. Rhomboidales.

### Material and methods

The specimens are mainly stored in the Herbarium of Forest Plants in Central South University of Forestry and Technology (CSFI). The morphological observation of the new species is based on field investigations, cultivated plants from the type locality, and specimen studies. Morphological research includes the length of rhizomes, the length, width and color of leaves, number of spikes, and the shape, size of bracts, glumes, utricles, and nutlets. We also use SEM to observe the nutlets which come from the holotype specimen we collected to ensure that the relevant descriptions were true. The sample preparation process and operating procedures of SEM refer to previous research by Lu et al. (2024). The conservation status of this new species is based on field observations in accordance with IUCN Red List guidelines (IUCN 2024).

### **Taxonomic treatment**

*Carex yankouensis* X.L.Yu, A.Liu & J.J.Zhou, sp. nov. urn:lsid:ipni.org:names:77359144-1

Figs 1, 2, 3

**Diagnosis.** This new species is similar to *C. brevicuspis* C. B. Clarke, but differs from it in having shorter culms (10-15 cm vs 20-55 cm) and spikes (1-1.5 cm vs 3.7-7 cm), leaves much wider (15-20-35 mm vs 5-10 mm) and lighter colored (pale green or yellow-green vs dark green), nutlet beak oblique (vs straight or slightly curved), and style base slightly thickened (vs conspicuously thickened) (Referring to Fig. 5, Table 1).

**Type.** CHINA • Guangdong: Qingyuan City, Yingde County, Jiulong Town, Yankou, in dry limestone, elevation ca. 100–200 m, 4 April 2022, *Ang Liu* LAYD01 (Holotype CSFI!, isotype HIB!, CSH! & ZJFC!) (Referring to Fig. 4).

**Description.** Perennial herbs. *Rhizome* short, stout. *Culms* 10–15 cm tall, blunt trigonous, smooth, base clothed with brown fibrous sheaths. *Leaves* up to 30 cm long and 15–20(-35) mm wide, longer than culms, blades papery, soft, broadly linear, pale green or yellow-green, flat, margin entire, apex acuminate or tailed, distinctly transverse veins between the leaf veins. *Bracts* leaflike, much shorter than inflorescence, sheathing. *Spikes* 4–5, distant, the proximal spike usually nearly basal and far from the distal ones; terminal spike staminate, 1–3(-4) cm, linear, with a peduncle ca. 3 cm; lateral spikes mostly pistillate, sometimes with several male flowers at apex, 1–1.5 × 0.6–0.8 cm, narrowly cylindric, densely flowered, the proximal-most one with a peduncle 3–5 cm,

smooth. **Glumes** ca.  $3 \times 1$  mm, staminate and pistillate ones similar in morphology, linear-lanceolate, pale, edges transparent, green 3-veined costa ending at apex, the tip rounded. **Utricles** ca.  $5 \times 2$  mm (including beak), longer than or nearly equaling glumes, obliquely patent, the body ovoid or obovoid, pale green, the walls herbaceous, the surface sparsely pubescent, many veined, contracted at both ends, the apex abruptly contracted into a ca. 3 mm long beak,



Figure 1. *Carex yankouensis* sp. nov. A habit B plants, transverse veins between the leaf veins extremely distinct in the red circle C rhizome D inflorescences E spikelet in flower F spikelet in fruit G female glumes and stamens H yong utricles, Male glume and stigmas I nutlets. Photographs by Ang Liu. Scale bars: 5 mm.



**Figure 2.** SEM micromorphology of nutlets of *Carex yankouensis* sp. nov. **A** overview **B** angle constricted at middle **C** sexine ornamentation. The nutlets are from the holotype: *Ang Liu* LAYD01, CSFI 076290. Scale bars: 1 mm (**A**); 500 μm (**B**); 50 μm (**C**).



Figure 3. Carex yankouensis sp. nov. A plant B glumes C female glume and stamens D young utricle and stigmas E utricle F nutlet. Drawn by PhD Jing Tian; based on the holotype: Ang Liu LAYD01, CSFI 076290 and cultivated plants from type locality.



Figure 4. Holotype of Carex yankouensis sp.nov. (Ang Liu LAYD01, CSFI 076290).



Figure 5. Morphological comparison of *Carex yankouensis* sp. nov. (A1–A3) and *C. brevicuspis* (B1–B3) A1, B1 plants A2, B2 infructescence A3, B3 nutlets. Photographs by Ang Liu.

orifice 2-lobed with sharp teeth. *Nutlets* ca. 2 × 1.5 mm, black-purple, ovate, trigonous, with 3 angles constricted at middle, faces concave at base, the epidermic cells forming an ornamentation of irregular polygons, base curved stipitate, apex abruptly contracted into an oblique beak, beak ca. 1 mm, slightly annulate at orifice; style base slightly thickened; stigmas 3.

**Phenology.** Flowers observed from November to December, fruits from March to April.

Characters	Carex yankouensis sp. nov.	C. brevicuspis
Culms	10–15 cm high	20–55 cm high
Leaves	15–20(-35) mm wide	5–10 mm wide
	papery, soft	papery, hard
	pale green or yellow-green	dark green
	transverse veins between the leaf veins extremely distinct	transverse veins between the leaf veins diddly distinct
Spikes	terminal spike staminate, 1–3(-4) cm	terminal spike staminate, 2.5–4 cm
	lateral spikes 1−1.5 × 0.6−0.8 cm	lateral spikes 3.7–7 × 0.9–1 cm
Nutlets	beak oblique	beak straight or slightly curved
	style base slightly thickened	style base conspicuously thickened

Table 1. Comparison of morphological characters between Carex yankouensis sp. nov. and C. brevicuspis.

**Etymology.** The epithet of this new species is derived from the type locality. 'Yankou' is the locality name, which means the entrance of a karst cave in Chinese.

**Distribution and habitat.** This new species is currently only found in the limestone landform areas of Jiulong Town, and usually grows on the walls of limestone.

Additional specimens examined (Paratypes). CHINA • Guangdong: Qingyuan City, Yingde County, Jiulong Town, Hui long Park, in dry limestone, elevation ca. 120 m, 9 November 2023, *Ang Liu* LAYD06 (CSFI!).

**Conservation status.** At present, we have only found two populations with a total of about 200 individuals in the limestone areas of Jiulong Town. However, there are vast limestone landforms near the type location, and there may be distribution of this new species in these areas. Of course, we need a broader and deeper investigation to confirm that. According to the IUCN red list criteria (IUCN 2024), the conservation status of the new species should be better categorized as 'Data Deficient (DD)'.

### Acknowledgements

Thanks for PhD Jing Tian's exquisite preparation of the line drawing and constructive opinions from reviewers and editors.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

No funding was reported.

### Author contributions

All authors have contributed equally.

### **Author ORCIDs**

Ang Liu b https://orcid.org/0000-0001-6281-7145

### **Data availability**

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

### References

- Dai LK, Liang SY, Zhang SR, Tang YC, Tetsuo K, Gordon CT (2010) Carex. In: Wu ZY, Raven PH (Eds) Flora of China, Vol. 23. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, Beijing, 285–461. https://www.iplant.cn/info/Carex?t=foc
- IUCN (2024) Guidelines for Using the IUCN Red List Categories and Criteria. version 16. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. http://www.iucnredlist.org/resources/redlistguidelines
- Jin XF, Zheng CZ (2013) Taxonomy of *Carex* section Rhomboidales (Cyperaceae). Science Press, Beijing, 1–237.
- Li YY, Chen YP, Jiang LQ, Liu ED, Luo Y, Peng H (2022) *Carex malipoensis* (Cyperaceae), a new species from southeast Yunnan, China. PhytoKeys 188: 19–29 https://doi. org/10.3897/phytokeys.188.75401
- Li YL, Deng SW, Luo JC, Li MX, Zou LT, Zeng QG, Chen HF (2024) *Carex qingyuanensis* (Cyperaceae), a new species from Guangdong, China, PhytoKeys 241: 155–168. https://doi.org/10.3897/phytokeys.241.117734
- Lu YF, Jin XF (2022) Notes on *Carex* (Cyperaceae) from China (VIII): five new species and a new variety from southern and south-western China. PhytoKeys 188: 31–47. https://doi.org/10.3897/phytokeys.188.77776
- Lu YF, Lu ZC, Duan YH, Zhang K, Jin XF (2023) Notes on *Carex* (Cyperaceae) from China (IX): three new species of section *Mitratae s.l.* PhytoKeys 225: 153–164. https://doi.org/10.3897/phytokeys.225.101410
- Lu ZC, Lu YF, Chang SL, Mo ML, Jin XF (2024) *Carex duanensis* (*Carex* sect. Rhomboidales), a new species of Cyperaceae from limestone areas of Guangxi, China. Phyto-Keys 241: 221–228. https://doi.org/10.3897/phytokeys.241.121098
- Qiu XD, Lu YF, Jin XF (2024) Carex linanensis (sect. Mitratae), a new species of Cyperaceae from Zhejiang, East China. PhytoKeys 241: 121–130 https://doi.org/10.3897/ phytokeys.241.119176

## PhytoKeys

**Research Article** 

# Typification of names of *Weinmannia* (Cunoniaceae) of species described by José Cuatrecasas from Colombian collections

Carmen Ulloa Ulloa<sup>10</sup>, Andrea Chaspuengal-Morales<sup>2</sup>, Francisco Fajardo-Gutiérrez<sup>30</sup>, Nora H. Oleas<sup>20</sup>

1 Missouri Botanical Garden, 4344 Shaw Blvd., St. Louis, Missouri 63110, USA

2 Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb), Facultad de Ciencias de Medio Ambiente, Universidad Tecnológica Indoamérica, Machala y Sabanilla, EC170301 Quito, Ecuador

3 Botanischer Garten Berlin, Freie Universität Berlin, Königin-Luise-Straße 6-8, 14195 Berlin, Germany

Corresponding author: Nora H. Oleas (noraoleas@uti.edu.ec)

#### Abstract

We propose 18 new lectotype designations for names of *Weinmannia*, five of which are second-step typifications involving four inadvertent first-step lectotype designations, and 13 of them are designations of one single duplicate at the herbarium where deposited. José Cuatrecasas described all, but one, of the taxa from collections made in Colombia. We clarify the lectotypification for *Weinmannia parvifoliolata* Cuatrec. Finally, we propose one lectotype designation for *W. cochensis* described by Georg Hieronymus.

Key words: Andes, Colombia, Cuatrecasas, lectotypes, nomenclature, South America

### Introduction

The genus *Weinmannia* L. is the largest in the family Cunoniaceae and consists of an estimated 90 species mostly found in the Americas with two species on the Mascarene Islands (Harling 1999; Pillon et al. 2021). Bernardi (1961, 1963) was the latest author to review *Weinmannia* worldwide. A modern comprehensive taxonomic monograph for *Weinmannia* is lacking. In the last three decades, regional or country level synopses or checklists have been published for Latin America: Bolivia (Harling and Fuentes 2014), Brazil (Santos-Silva et al. 2020), Colombia (Bernal 2016; Fajardo-Gutiérrez et al. 2020), Ecuador (Bradford 1999; Harling 1999), Peru (Zarucchi 1993), Venezuela (Hokche et al. 2008; Bradford and Berry 1998), and the Southern Cone (Hopkins 2008); as well for Mexico and Central America (Morales 2010), and the Caribbean (Acevedo-Rodríguez and Strong 2012); and a summary checklist for the Western Hemisphere by Ulloa Ulloa et al. (2017).

Weinmannia is a genus with extremely variable morphological characters and ongoing phylogenetic studies do not support Bernardi's sections based on leaf morphology. Neither are Bradford's (2002) sections supported when based on inflorescence characteristics (Fajardo-Gutiérrez in preparation; Pillon et al. 2021). Moreover, *Weinmannia* seems to be an assemblage of various species complexes and possible hybrids making species delimitations difficult.



Academic editor: Hanno Schaefer Received: 22 August 2024 Accepted: 28 January 2025 Published: 26 March 2025

**Citation:** Ulloa Ulloa C, Chaspuengal-Morales A, Fajardo-Gutiérrez F, Oleas NH (2025) Typification of names of Weinmannia (Cunoniaceae) of species described by José Cuatrecasas from Colombian collections. PhytoKeys 254: 87–98. https://doi.org/10.3897/ phytokeys.254.135362

**Copyright:** © Carmen Ulloa Ulloa et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

During an ongoing synoptical study of the Ecuadorian species of Weinmannia, as part of a larger project investigating the genus for Latin America, we realized that most species' names described by José Cuatrecasas from Colombia need typification. As part of his studies on Andean plants, Cuatrecasas (1933, 1940, 1941, 1942, 1948[1949]) described 37 species and varieties of Weinmannia from Colombia, most of them based on his collections. He usually cited his gatherings as "Typus" or "Type", without specifying how many duplicates he studied, or in which herbaria he deposited his collections; in other words, his new taxa were based on syntypes (Turland et al. 2018: Art 9.6). Duplicates of Cuatrecasas' collections were distributed to various herbaria in the Americas and Europe. In his review of the American species of Weinmannia, Bernardi (1961, 1963) cited some of those type collections in specific herbaria, but in most cases we found that a second-step lectotypification was required (Turland et al. 2018: Art. 9 Ex. 14). Harling (1999) in his treatment for the Flora of Ecuador series provided typification of names applied to species of Weinmannia occurring in Ecuador. The establishment of nomenclatural types is crucial for any discussion of taxonomic problems such as species delimitation, character evolution reconstruction, hybridization, and to improve field and herbarium identifications. In this contribution, we propose lectotypification of 14 species and four varietal names in order to fix the application of those names in anticipation of the publication of future taxonomic studies; we also clarify one previous lectotypification.

### **Material and methods**

We assessed the names first by reviewing the protologues and studying most of the type material deposited in the following herbaria: B, COL, F, MA, MO, P, (acronyms according to Thiers 2024). We also verified all the type images in JSTOR Global Plants (plants.jstor.org), and websites at G, P, US, and W. The names are arranged in alphabetical order of their basionyms. For each we provide the most recent accepted species name given by Bernal (2016) in the catalogue of plants and lichens of Colombia. José Cuatrecasas Arumí (1903 Camprodon, Spain-1996 Washington D.C.) was an important botanist working on the flora of tropical America. In the 1930s, Cuatrecasas worked at the Real Jardín Botánico in Madrid; from 1939 to 1947, he was associated with academic institutions in Colombia; from 1947 to 1955 with the Field Museum in Chicago. He continued his prolific career at the United States National Herbarium in Washington D.C. (Robinson et al. 1996). For this reason, we decided to designate most lectotypes in COL or F herbaria.

At the end, we include one typification of a species name described by Georg Hieronymus from Colombian collections. References to Article numbers and examples ("Art.") in the following are according to the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) by Turland et al. (2018).

### Nomenclatural treatment

The following specific and varietal names proposed by Cuatrecasas between 1933 and 1948 have not been typified until now. We also clarify the lectotype designation of *Weinmannia parvifoliolata* Cuatrec.

### *Weinmannia balbisiana* Kunth var. *subovata* Cuatrec., Lloydia 11(3): 201. 1948 [1949].

**Type.** COLOMBIA • Comisaría del Caquetá, Cordillera Oriental, Quebrada del rio Hacha, abajo de Gabinete, 2100–2250 m. alt. 23-III-40, *J. Cuatrecasas 8562* (lectotype, designated here: F [barcode F0063208F image!]; isolectotypes, COL [barcode COL000001488!]), F [barcode F0063209F image!], F [barcode F0063210F image!].

**Note.** Cuatrecasas (1948[1949]) cited the single collection *J. Cuatrecasas* 8562 housed at F as the type; however, in the herbarium F there are three duplicates of *J. Cuatrecasas* 8562; these three specimens are syntypes so we chose one of them for the lectotypification (cf., Art. 40 Ex. 3). This variety is a synonym of *W. balbisiana* Kunth according to Bernal (2016: 1126).

### Weinmannia bogotensis Cuatrec., Ciencia (México) 1: 253. 1940.

≡ Weinmannia auriculata var. bogotensis (Cuatrec.) Bernardi, Candollea 18: 325. 1963.

**Type.** COLOMBIA • Dep. Cundinamarca, vertiente oriental de la Sierra de Bogotá en la quebrada de los Santos, 3000 m, 28-I-1940, *J. Cuatrecasas 8005* (lectotype, first-step designated by Bernardi (1963: 325) as type, second-step lectotype designated here F [barcode F0063212F image!]; isolectotypes, BC [barcode BC623896 image!], COL [barcode COL000001489!], COL [barcode COL000001490!], F [barcode F0063213F image!], P [barcode P00697246!], U [barcode U0001478 image!], US [barcode US00097243 image!]).

**Note.** Cuatrecasas (1940) did mention the collection *J. Cuatrecasas 8005* as the type, but he did not cite the herbarium housing the type. Subsequently, Bernardi (I.c.) cited *J. Cuatrecasas 8005* housed at F as the type; however, the F herbarium has two duplicates of the preceding collection. It is construed here that Bernardi inadvertently did the first-step lectotypification process, and we herewith select one duplicate for the second-step lectotypification. This species is a synonym of *W. auriculata* D. Don in Bernal (2016: 1126), and it is likely a hybrid-origin taxon (Fajardo-Gutiérrez et al. 2020; Miranda and Fuentes 2012).

### Weinmannia caquetana Cuatrec., Caldasia 2: 19, fig. 7. 1941.

= *W. subsessiliflora* subsp. *caquetana* (Cuatrec.) Bernardi, Candollea 17: 144. 1961.

**Type.** COLOMBIA • Comisaría del Caquetá, Quebrada del Río Hacha, 2100-2250 m. alt., 23-III-1940, *J. Cuatrecasas 8561* (lectotype, first-step designated by Bernardi (1961: 144) as type, second-step lectotype designated here, F [barcode F0063216F image!]; isolectotypes, COL [barcode COL000001512!], F [barcode F0063215F image!], US [barcode US00097250 image!]).

**Note.** For the type, Cuatrecasas (1941) cited three collections, viz., "23-III-1940, *J. Cuatrecasas* no. 8561", "22-III-40, *J. Cuatrecasas*, 8599-A", and "26-III-1940, *J. Cuatrecasas*, 8714". Bernardi (1961: 144) cited *J. Cuatrecasas* 8561 as the type housed in the F Herbarium. We found two duplicates of this collection in the F herbarium and chose one of them for the second-step lectotypification. Bernal (2016: 1128) treats this species as a synonym of *W. subsessiliflora* Ruiz & Pav.

### Weinmannia crenata C. Presl var. caliana Cuatrec., Ciencia (México). 1: 254. 1940.

W. sorbifolia Kunth var. caliana (Cuatrec) Cuatrec., Lloydia 11(3): 204. 1948 [1949].

**Type.** COLOMBIA • Dep. El Valle, Hoya del río Cali, Bosques "El Recuerdo" 1800 m. [s.d.], *J. M. Duque-Jaramillo 1555* (lectotype, designated here, US [barcode 00097255 image!]; isolectotypes, VALLE [barcode VALLE000201 image!], VALLE [barcode VALLE000200 image!]).

**Note.** In the protologue, Cuatrecasas (1940) did not mention a number or a herbarium for the J.M. Duque J. [José María Duque Jaramillo] collection. However, when Cuatrecasas (1948 [1949]) made the new combination in *W. sorbifolia* Kunth, he did mention '*Duque 1555*' housed in the US herbarium, but without designating it as the type. We chose this duplicate as the lectotype. This variety is a synonym of *W. sorbifolia* Kunth in Bernal (2016: 1128).

### *Weinmannia cundinamarcensis* Cuatrec., Revista Acad. Colomb. Ci. Exact. 5: 32, fig. 18. 1942.

**Type.** COLOMBIA • Cordillera Oriental; Departamento Cundinamarca; extremo sudeste de la Sabana de Bogotá, en San Miguel, bosque a 2800-3000 m. alt., colect 10 sept. 1941, *J. Cuatrecasas & R. Jaramillo 12033* (lectotype, first step designated by Bernardi (1961: 142) as type, second-step lectotype designated here: F (barcode F0063222F image!); isolectotypes, F (barcode F0063221F image!), F (barcode F0063223F image!), COL (barcode COL0000014932!), COL (barcode COL000001493!), COL (barcode COL00001494!), GH (barcode GH00043352 image!), P (barcode P02441813 image!), US (barcode US00097252 image!).

**Note.** In the protologue, Cuatrecasas (1942) cited a single gathering as the type (*"Typus*: ... 10 sept. 1941, *J. Cuatrecasas & R. Jaramillo 12033."*), but he did not mention an institution where the type was deposited. Bernardi (1961: 142) mentioned an F duplicate as type (*".... Cuatrecasas 12033* (typus F,")). We, however, found three duplicates of that collection in the F herbarium, and therefore is construed here that Bernardi inadvertently did the firststep lectotypification process, and we herewith select one duplicate for the second-step lectotypification. *Weinmannia cundinamarcensis* is known only from the type locality and is accepted by Bernal (2016: 1126). However, due to intermediate characters, Fajardo-Gutiérrez et al. (2020) suggested this taxon likely is of hybrid-origin.

### Weinmannia duquei Cuatrec., Caldasia 2: 25, figs. 10E, 14. 1941.

**Type.** COLOMBIA • Departamento del Valle; Hoya del río Cali, "El Recuerdo", 2500 m. alt., *J.M. Duque-Jaramillo s.n.* (lectotype, designated here: COL [barcode COL000001495!]; isolectotype (fragment) G [barcode G00357679 image!]).

**Note.** Cuatrecasas (1941) cited a single collection as the type but did not mention the herbarium housing the type (*"Typus: ...* legit *Duque-Jaramillo* [s.n.; s.d.]."). Therefore, we designate the specimen housed at the COL herbarium as the lectotype. *Weinmannia duquei* was considered a synonym of *W. chryseis* Diels (a species described from Peru) in Bernal (2016: 1126).

### Weinmannia magnifolia Cuatrec., Caldasia 2: 25, figs. 10D, 13. 1941.

**Type.** COLOMBIA • Dep. de Huila, entre Gabinete y Andalucía, 2300-2200 m. alt., 24-III-1940, *J. Cuatrecasas 8590* (lectotype, designated here: COL [barcode COL000001498!]; isolectotypes, COL [barcode COL000001499!], F [barcode F0063243F image!], F [barcode F0063244F image!], P [barcode P00697262!], U [barcode U0082345 image!], US [barcode US00097274 image!], US [barcode US00097275 image!]).

**Note.** Cuatrecasas (1941) cited a single collection as the type but did not mention the herbarium housing the type (*"Typus: ...* 24-III-1940, *J. Cuatrecasas*, *8590."*). Bernal (2016: 1127) listed the "isotype" as ("IT: COL, F"). Since there was no holotype, there was no isotype. Although Bernal's usage of the type term "isotype" is correctable to lectotype (see Art. 9.10) his citation of two herbaria and the lack of usage of the phrase "designated here" (or its equivalent) do not constitute any inadvertent lectotypification (see Art. 7.11). Furthermore, at COL, we found two duplicates of that collection, and we chose one of them, which agrees well with the protologue, for the lectotypification. This species was accepted in Bernal (2016: 1127).

### Weinmannia myrtifolia Cuatrec., Caldasia 1(2): 16, figs. 4, 5B. 1941.

**Type.** COLOMBIA • Cordillera Oriental, vert. oriental, Dep. de Cundinamarca, Bosque en Juiquín, Quebrada Amarilla, bajo el páramo de Guasca, 2840 m. alt., 2-VI-1940, *J. Cuatrecasas 9451* (lectotype, designated here, COL [barcode COL000001501!]; isolectotypes, F [barcode F0063252F image!], F [barcode F0063253F image!], P [barcode P02441812!], U [barcode U0082346 image!], US [barcode US00097280 image!], US [barcode US00997637 image!]).

**Note.** Cuatrecasas (1941) cited a single collection as the type but did not mention the herbarium housing the type (*"Typus: ...* 2-VI-1940, *J. Cuatrecasas*, 9451."). He also cited a paratype collection as *"Ya en prensa este trabajo, el Dr. A. Dugand ha recolectado en el mismo Páramo de Guasca y en lugar próximo al del tipo unos ejemplares (Dugand 2969: Cordillera oriental: Páramo de Guasca; vertiente oriental, 3100 metros alt., Julio 20, 1941)". Bernal (2016: 1126-1127) merely mentioned that the type was housed in COL, F, U, and US herbaria and treated this species as a synonym of <i>W. karsteniana* Szyszył. We selected the duplicate deposited at COL as the lectotype.

### *Weinmannia ovalis* Ruiz & Pav. var. *petiolata* Cuatrec., Lloydia 11(3): 203. 1948 [1949].

**Type.** COLOMBIA • Dep. del Valle, Cordillera Occidental, Los Farallones, Quebrada de Las Nieves, lomas parameras sobre la mina El Diamante 3100–3120 m, 31-VII-1946, *J. Cuatrecasas 21820* (lectotype, designated here: F [barcode V0334900F image!]; isolectotypes, F [barcode V0334901F image!], COL [barcode COL000076221!], P [P05518589 image!]).

**Note.** Cuatrecasas (1948 [1949]) did cite the collection *J. Cuatrecasas 21820* housed at the F Herbarium as the type; however, in the F Herbarium there are two duplicates of the collection, and both specimens lack the holotype annotation, and thus constitute syntypes; we chose one of them for the lectotypification (cf. Art. 40 Ex. 3). This variety is a synonym of *W. elliptica* Kunth according to Bernal (2016: 1126).

### Weinmannia parvifoliolata Cuatrec., Caldasia 2: 21, figs. 9, 10A. 1941.

**Type.** COLOMBIA • Cordillera Oriental de Colombia, en el filo divisorio entre la Comisaría del Caquetá y Departamento del Huila, 2300–2400 m. alt., 22-III-1940, *J. Cuatrecasas 8486-A* (lectotype, designated by Bernardi (1961: 147): F [barcode F0063261F image!], isolectotypes: COL [barcode COL000001502!]; US [barcode US00097286 image!], US [barcode US00997638 image!]).

**Note.** Although Cuatrecasas (1941) cited a single collection as the type, he did not mention the herbarium housing the type (*"Typus: ... 22-III-1940, J. Cuatrecasas, 8496-A."*). Bernardi (1961: 147) assumed that the holotype was at the COL Herbarium and mentioned the duplicate at the F Herbarium as an *"isotype"*. Since there was no holotype, there was no isotype, nonetheless, Bernardi's usage of the type term *"isotype"* is correctable to lectotype (see Art. 9.10). Therefore, Bernardi's citation of *"isotype"* is treated here as the inadvertent lectotype designation; Bernal (2016: 1127) merely mentioned the isotype *("IT: COL, F")* and accepted this species.

### Weinmannia penicillata Cuatrec., Caldasia 2: 13, figs. 1, 2. 1941.

= W. ovalis var. penicillata (Cuatrec.) Cuatrec., Lloydia 11(3): 203. 1948 [1949].

**Type.** COLOMBIA • Cordillera Oriental sobre el filo divisorio entre el Dept. del Huila y la Comisaría del Caquetá, en Gabinete, 2300-2450 m alt., 21-III-1940, *J. Cuatrecasas 8430* (lectotype, designated here, F [barcode F0063264F image!]; isolectotypes, CM [barcode CM0754 image!], COL [barcode COL000001503!], F [barcode F0063263F image!], G [barcode G00357648 (fragment) image!], US [barcode US00097287 image!]).

**Note.** Cuatrecasas (1941) cited a single collection as the type but he did not mention the herbarium housing it (*"Typus: ...* 21-III-1940, *J. Cuatrecasas, 8430."*). Subsequently, he mentioned (Cuatrecasas 1948 [1949]) that the type was housed in the F Herbarium. We, however, found two duplicates of that col-

lection at F. Therefore, it is interpreted that Cuatrecasas (1948 [1949]) inadvertently did the first-step lectotypification process, and we herewith do the second-step lectotypification process. Bernal (2016: 1126) treated this species as a synonym of *W. elliptica* Kunth.

### Weinmannia queremalensis Cuatrec., Lloydia 11(3): 193. 1948 [1949].

**Type.** COLOMBIA • Departamento del Valle, Cordillera Occidental, vertiente occidental, Hoya del río Digua, lado izquierdo del río San Juan en la region de Querermal, 1540–1650 met. alt., Quebradita del Km. 51, 25-II-1947 colect. *J. Cuatrecasas 23723* (lectotype, first-step designated by Bernardi (1963: 310) as type, second-step lectotype designated here, F [barcode F0063272F image!]; isolectotypes, BC [barcode BC623891 image!], COL [barcode COL000001504!], COL [barcode COL000001506!], COL [barcode COL00001499!], F [barcode F0063273F image!], P [barcode P00697257 image!], U [barcode U0001482 image!], US [barcode US00097295 image!], US [barcode US00097296 image!], VALLE (not seen).

**Note.** Within the protologue, Cuatrecasas (1948 [1949]) did cite a single collection as the type (*"Type*: ... 25-II-1947 colect. J. Cuatrecasas 23723."); he however, mentioned three herbaria (F, VALLE, US) as housing the type, and he thus did not indicate the holotype and only cited syntypes. Bernardi (1963: 310) mentioned *Cuatrecasas 23723* ("F; typus *W. queremalensis*"); we, however, found two duplicates of this collection in that herbarium. Bernardi inadvertently did the first-step lectotypification process, and we herewith select one duplicate for the second-step lectotypification. *Weinmannia queremalensis* is considered a synonym of *W. latifolia* C. Presl. in Bernal (2016: 1127), who cited the type as housed in COL, F, US, and VALLE herbaria.

### Weinmannia sclerophylla Cuatrec., Caldasia 2: 17, figs. 5C, 6. 1941.

■ W. sorbifolia Kunth var. sclerophylla (Cuatrec.) Cuatrec., Lloydia 11(3): 204. 1948 [1949].

**Type.** COLOMBIA • Cordillera Oriental de Colombia, vertiente occidental, Dep. del Huila, entre Gabinete y Andalucía, bosque 2300-2200 m. alt., 25-III-1940, *J. Cuatrecasas 8691* (lectotype, designated here, COL [barcode COL000001518!]; isolectotypes, F [barcode F0063277F image!], F [barcode F0063278F image!], F [barcode F0063279F image!], US [barcode US00097302 image!], US [barcode US00997640 image!]).

**Note.** For the type designation, Cuatrecasas (1941) cited three collections (*"Typus: ...* 25-III-1940, *J. Cuatrecasas, 8691.* También en id. id., hondonada del Abra de San Andrés, 2100-1900 m. alt., 24-III-40, *J. Cuatrecasas, 8601 y 8654"*) but he did not mention any herbaria. We chose *J. Cuatrecasas 8691*, conserved at the COL Herbarium, as the lectotype. Bernal (2016: 1128) merely mentioned that the type is at the COL Herbarium and treated this species as a synonym of *W. sorbifolia* Kunth.

### Weinmannia sibundoya Cuatrec., Caldasia 2: 23, figs. 10C, 12. 1941.

**Type.** COLOMBIA • Comisaría del Putumayo, bosque paramero en el filo de la Cordillera en el extremo E. del valle de Sibundoy, La Cabaña, 2800 m. alt., 2-I-1941, *J. Cuatrecasas 11624* (lectotype, designated here, COL [barcode COL000001507!]; isolectotypes, F [barcode F0063280F image!], US [00097305 image!]).

**Note.** Cuatrecasas (1941) cited two collections as the type (*"Typus: ... 2-I-1941, J. Cuatrecasas, 11624. También en id. id., no. 11527."*) and did not mention any herbarium. Neither Bernardi (1961: 165) nor Harling (1999: 22) designated a lectotype. We herewith designate *Cuatrecasas 11624,* conserved at the COL Herbarium, as the lectotype. *Weinmannia sibundoya* is considered a synonym of *W. multijuga* Killip & A.C.Sm. in Harling (ibid.) and Bernal (2016: 1127).

### Weinmannia subvelutina Cuatrec., Caldasia 2: 15, figs. 3, 5A. 1941.

= W. rollottii Killip var. subvelutina (Cuatrec.) Bernardi, Candollea 18: 327. 1963.

**Type.** COLOMBIA • Comisaría del Putumayo, lado sur de la Laguna de la Cocha en La Quebrada de Santa Lucía, 2850 m. alt. 8-1-1941. *J Cuatrecasas 11816* (lectotype, designated here, COL [barcode COL000001513!]; isolectotypes, F [barcode F0063288F!], F [barcode F0063287F!], G [barcode G00357673 image! fragment], US [barcode US00097312 image!]).

**Note.** Cuatrecasas (1941) cited a single collection as the type but did not mention the herbarium housing the type (*"Typus: ...* 8-I-1941, *J. Cuatrecasas, 11816."*). We herewith designate the COL specimen as the lectotype. We add that Bernardi (1963: 327-328) mentioned *"Cuatrecasas 11816* (COL, F), cum capsulis (typi numerus)." Since he cited two herbaria and the plural term "typi", his citation does not constitute a typification. *Weinmannia subvelutina* is considered a synonym of *W. rollottii* Killip in Bernal (2016: 1128).

### Weinmannia tamana Cuatrec., Revista Acad. Colomb. Ci. Exact. 5: 32. 1942.

**Type.** COLOMBIA • Cordillera Oriental, Departamento de Santander, Páramo de Tamá, vertiente de Samaria, 2600-2900 m. alt., colect. 29 oct. 1941, *J. Cuatrecasas, R. E. Schultes & E. Smith 12726* (lectotype, designated here, COL [barcode COL000001508!]; isolectotypes, F [barcode F0063291F image!], F [barcode F0063292F image!], GH [barcode GH00043362 image!], P [barcode P02441810 image!], U [barcode U0007918 image!], US [barcode US00097313 image!].

**Note.** Cuatrecasas (1942) cited a single collection as type, but he did not mention in which herbarium the type was housed ("Typus: ... *J. Cuatrecasas, R. E. Schultes & E. Smith 12726.*"). Bernardi (1961: 176) cites a duplicate at the F Herbarium, but he does not designate it as a type. We chose the duplicate deposited at the COL Herbarium as the lectotype. Bernal (2016: 3027) treats this species as a synonym of *W. fagaroides* Kunth.

### Weinmannia tolimensis Cuatrec., Trab. Mus. Ci. Nat., Ser. Bot. 26: 18, figs 10, 11. 1933.

**Type.** COLOMBIA • Andes, Cordillera Central ad 3.000 m. alt. in monte Tolima, inter El Salto et La Selva, 16-V-1932, *J. Cuatrecasas 2737* (lectotype, designated here, MA [barcode MA235618!]).

**Note.** Cuatrecasas (1933) did cite a single collection ("legi [*J. Cuatrecasas*] *no.* 2737.") but did not use the term type or mention the herbarium housing the collection. We herewith designate the MA Herbarium specimen as the lecto-type. *Weinmannia tolimensis* is an accepted species in Bernal (2016: 1128).

### Weinmannia tolimensis Cuatrec. var. latifoliolata Cuatrec., Lloydia 11(3): 204. 1948 [1949].

**Type.** COLOMBIA • Dep. del Cauca; Cordillera Central vertiente occidental Cabeceras del río Palo, Quebrada de Santo Domingo, bosquecillo subiendo al páramo, 2950-3150 m. alt., 13-XII-1944, *J. Cuatrecasas 19256* (lectotype, designated here: F [barcode F0063295F image!]; isolectotypes, F [barcode F0063294F image!], US [barcode US00097315 image!]).

**Note.** Cuatrecasas (1948 [1949]) cited the single collection *J. Cuatrecasas 19256* housed at the F Herbarium as the type; however, there are two duplicates of this collection at that herbarium, and we chose one of them for the lectotyp-ification (cf. Art. 40 Ex. 3). This variety is a synonym of *W. tolimensis* Cuatrec. according to Bernal (2016: 1128).

### Name described by Georg Hieronymus

The following name, included by Harling (1999) in his treatment of Cunoniaceae for Ecuador, is typified here.

### Weinmannia cochensis Hieron., Bot. Jahrb. Syst. 21(3): 310. 1895.

**Type.** COLOMBIA • "Páramo dicta ad fluvium Rio de Cocha, … in regione suprema silvarum, alt. s. m. 3200 m, mense Augusto fructifera collecta est." A. Stübel 357 (lectotype, designated here, B [barcode B\_10\_9009851!]).

**Note.** In the protologue, Hieronymus (1895) cited five Alfons Stübel collections from Colombia, considered as syntypes: "crescit ad Cuchilla de Santo Domingo inter urbem Popayan et altiplanitiem Páramo de Huila (coll. columb. n. 290" [B barcode B\_10\_9009849!, F barcode F0063217F image! fragment]; "in altiplanitie Páramo dicta ad fluvium Rio de Cocha, ..." (coll. columb. n. 354 [B barcode B\_10\_9009852!]), "et haud procul a loco ultimo indicato in regione suprema silvarum, alt. s. m. 3200 m, ..." (coll. columb. n. 357 et 360 d [B barcode B\_10\_9009850!]); "prope lacum Laguna de Pasto alt. s. m. 2800 m" (coll. columb. n. 365 [B!]." Bernardi (1961: 179) cited two of those collections at Herbarium B (Stübel 290, 357), but did not designate a type, and Harling (1999: 39) cited all five aforementioned collections as syntypes, but he was unable to study any of

them. The specimen here chosen as lectotype, *A. Stübel 357*, deposited in the Berlin Herbarium, matches the original description well and has inflorescences; furthermore, the area where it was collected can be easily discerned today based on the label information: *"Excursión de Pasto a la Laguna Grande de Cocha y al Cerro Patascoy (falso): región superior de bosques, 3200 m, viii, 1869."* The specific epithet refers to *'Laguna de la Cocha' in Nariño, Colombia. The other syntypes have rather imprecise label localities, lack inflorescences, or are fragments only.* This species is accepted in Bernal (2016: 1126) and Harling (1999: 22).

### Acknowledgments

Chaspuengal thanks the Center for Conservation and Sustainable Development of the Missouri Botanical Garden for a Shirley Graham Fellowship that allowed her to study *Weinmannia* in St. Louis during the spring of 2024. We thank the collection's managers of the mentioned herbaria for facilitating visits or loans. We thank F. Barrie and G. Davidse for consultation on the *Code*. We thank K. Gandhi, F. Almeda, and an anonymous reviewer for their detailed review comments.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

Oleas thanks Universidad Tecnológica Indoamérica for funding support Grant number INV-0019-003-006 (2021-2026).

### **Author contributions**

Conceptualization: CUU. Data curation: ACM, CUU, FFG. Formal analysis: FFG, CUU. Funding acquisition: NHO, ACM. Investigation: ACM, CUU, FFG. Methodology: CUU. Project administration: NHO. Resources: NHO. Validation: CUU, FFG. Writing – original draft: NHO, CUU, FFG. Writing – review and editing: FFG, NHO, ACM, CUU.

### **Author ORCIDs**

Carmen Ulloa Ulloa () https://orcid.org/0000-0003-2453-8131 Francisco Fajardo-Gutiérrez () https://orcid.org/0000-0002-2235-3910 Nora H. Oleas () https://orcid.org/0000-0002-1948-4119

### Data availability

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

### References

Acevedo-Rodríguez P, Strong MT (2012) Catalogue of seed plants of the West Indies. Smithsonian Contributions to Botany 98: i–xxvi, 1–1192. https://doi.org/10.5479/ si.0081024X.98.1

- Bernal R (2016) Cunoniaceae. In: Bernal R, Gradstein SR, Celis M (Eds) Catálogo de Plantas y Líquenes de Colombia I. Universidad Nacional de Colombia (Sede Bogotá), Instituto de Ciencias Naturales, Bogotá, 1126–1129.
- Bernardi L (1961) Revisio generis Weinmanniae. Pars I: Sectio Weinmanniae. Candollea 17: 123–189.
- Bernardi L (1963) Revisio generis Weinmanniae. Pars II: Sectio Simplicifoliae. Candollea 18: 285–334.
- Bradford JC (1999) Cunoniaceae. In: Jørgensen PM, León-Yánez S (Eds) Catalogue of the Vascular Plants of Ecuador. Missouri Botanical Garden Press, St. Louis, 419–420.
- Bradford JC (2002) Molecular phylogenetics and morphological evolution in Cunonieae (Cunoniaceae). Annals of the Missouri Botanical Garden 89(4): 491–503. https://doi. org/10.2307/3298592
- Bradford JC, Berry PE (1998) Cunoniaceae. In: Berry PE, Hoist BK, Yatskievych K (Eds) Flora of the Venezuelan Guayana, Vol. 4. Missouri Botanical Garden Press, St. Louis. 462–469.
- Cuatrecasas J (1933) Plantae novae Colombianae. Trabajos del Museo Nacional de Ciencias Naturales (y Jardin Botanico). Serie Botanica 26: 1–30.
- Cuatrecasas J (1940) Nuevos encenillos de Colombia. Ciencia (Mexico) 1: 253-254.
- Cuatrecasas J (1941) Estudios sobre plantas andinas, II. Caldasia 2: 13-27.
- Cuatrecasas J (1942) Notas a la Flora de Colombia, V. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 5(17): 16–39.
- Cuatrecasas J (1948 [1949]) Studies in South American Plants I. Lloydia 11: 185-225.
- Fajardo-Gutiérrez F (in preparation) Expelled by the Antarctic ice: biogeographic history of Cunonieae tribe during the Cenozoic. [unpublished manuscript]
- Fajardo-Gutiérrez F, Olaya Ramírez TL, Calbi M (2020) Flora de Bogotá: Cunoniaceae. Pérez-Arbelaezia 21(1): 177–215.
- Harling GW (1999) 78. Cunoniaceae. In: Harling GW, Andersson L (Eds) Fl. Ecuador. University of Göteborg, Göteborg, 61: 1–72.
- Harling GW, Fuentes AF (2014) Cunoniaceae. In: Jørgensen PM, Nee MH, Beck SG (Eds) Catálogo de las Plantas Vasculares de Bolivia, Monographs in Systematic Botany from the Missouri Botanical Garden. Missouri Botanical Garden Press, St. Louis, 127(1): 540–542.
- Hieronymus G (1895) Plantae Stubelianae novae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 21(3): 306 –369. https://www.biodiversitylibrary.org/page/205877
- Hokche O, Berry PE, Huber O [Eds] (2008) Nuevo Catálogo de la Flora Vascular de Venezuela. Fundación Instituto Botánico de Venezuela, Caracas, 1–859.
- Hopkins HCF (2008) Cunoniaceae. In: Zuloaga FO, Morrone O, Belgrano MJ (Eds) Catálogo de las Plantas Vasculares del Cono Sur, Monographs in Systematic Botany from the Missouri Botanical Garden. Missouri Botanical Garden Press, St. Louis, 107(2): 1982–1983.
- Miranda T, Fuentes AF (2012) Avances en el estudio de hibridación en *Weinmannia* L.(-Cunoniaceae) para Bolivia. In Memorias II Congreso Boliviano de Botánica.
- Morales JF (2010) Sinopsis del género *Weinmannia* (Cunoniaceae) en México y Centroamérica. Anales del Jardin Botanico de Madrid 67(2): 137–155. https://doi. org/10.3989/ajbm.2247
- Pillon Y, Hopkins HCF, Maurin O, Epitawalage N, Bradford J, Rogers ZS, Baker WJ, Forest F (2021) Phylogenomics and biogeography of Cunoniaceae (Oxalidales) with complete generic sampling and taxonomic realignments. American Journal of Botany 108(7): 1181–1200. https://doi.org/10.1002/ajb2.1688

- Robinson HE, Funk VA, Pruski JF, King RM (1996) José Cuatrecasas Arumí. Compositae Newsletter 29: 1–30.
- Santos-Silva F, Cardoso PH, Cabral A (2020) Cunoniaceae in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. http://floradobrasil2020.jbrj.gov.br/reflora/floradobrasil/FB7125 [Accessed 03.04.2024]
- Thiers B (2024 [continuously updated]) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http:// sweetgum.nybg.org/ih [Accessed 03.04.2024]
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF [Eds] (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. https://doi.org/10.12705/Code.2018
- Ulloa Ulloa C, Acevedo-Rodríguez P, Beck SG, Belgrano MJ, Bernal R, Berry PE, Brako L, Celis M, Davidse G, Gradstein SR, Hokche O, León B, León-Yánez S, Magill RE, Neill DA, Nee MH, Raven PH, Stimmel H, Strong MT, Villaseñor Ríos JL, Zarucchi JL, Zuloaga FO, Jørgensen PM (2017) An integrated assessment of vascular plants species of the Americas. Science 358 (6370): 1614–1617. [Online Suppl. Materials: 1–23 + 1–2497], f. 1–4 [f. S1–5] https://doi.org/10.1126/science.aao0398
- Zarucchi JL (1993) Cunoniaceae. In: Brako L, Zarucchi JL (Eds) Catalogue of the Flowering Plants and Gymnosperms of Peru, Monographs in Systematic Botany from the Missouri Botanical Garden. Missouri Botanical Garden Press, St. Louis, 45: 384–387.



Research Article

# *Primulina nanlingensis* (Gesneriaceae), a new species from the Limestone Karst of Guangdong, China

Jin-Chu Luo<sup>1,2\*®</sup>, Yuan-Qiu Li<sup>3\*®</sup>, Ya-Li Li<sup>1,2®</sup>, Ming-Zhao She<sup>1,2®</sup>, Yang-Jin Zeng<sup>3</sup>, Fa-Guo Wang<sup>1®</sup>, Hong-Feng Chen<sup>1®</sup>

- 1 Guangdong Provincial Key Laboratory of Applied Botany, State Key Laboratory of Plant Diversity and Specialty Crops, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
- 2 University of Chinese Academy of Sciences, Beijing 100049, China
- 3 Guangdong Shimentai National Nature Reserve, Yingde 511500, China

Corresponding author: Hong-Feng Chen (h.f.chen@scbg.ac.cn)

### Abstract

*Primulina nanlingensis*, a new species of Gesneriaceae from the Karst of Guangdong, China, is described and illustrated. This species is morphologically similar to *P. versicolor*, but can be distinguished by its larger crenate-margined leaves, fewer flowers per cyme and overall cyme number, ovate-lanceolate bracts with shallow serrations, calyx lobes with 1–3 teeth per side, stamens densely glandular at base and tip and pistil densely glandular-puberulent. It also resembles *P. pengii*, but has significant differences in its longer corolla, pale yellow corolla colour and ovate-lanceolate bracts. Phylogenetic analyses with ITS and *trn*L-F sequences revealed that *P. nanlingensis* is sister to *P. versicolor* and *P. pengii*, but isolated from its morphological relatives. The phylogenetic and morphological relationships with similar species are discussed, including detailed descriptions, photographs and distribution information. According to the IUCN Red List Criteria, the new species is assessed as Near Threatened [NT].

Key words: Gesneriaceae, limestone flora, morphology, phylogeny, taxonomy

### Introduction

The genus *Primulina* Hance (1883) was initially monotypic, with its type specimen collected from the Lianjiang River Basin in northern Guangdong, China. The second species, *P. guangxiensis* Yan Liu & W.B.Xu, was described in 2011, based solely on morphological characteristics (Liu et al. 2011). Drawing upon molecular and morphological evidence, *Primulina* underwent subsequent revision and expansion, resulting in the identification of 123 species and eight varieties (Wang et al. 2011; Weber et al. 2011). The revision included numerous species from *Chirita* sect. *Gibbosaccus*, two species from *Wentsaiboea* D.Fang & D.H.Qin and *Chiritopsis* W.T.Wang which were consolidated into *Primulina* (Zhou et al. 2016; Xu et al. 2023). Species within the genus *Primulina* are perennial herbs distinguished by their rhizomatous stems, fleshy opposite leaves, corolla with an infundibuliform tube and 2-lipped lobe, two stamens with coherent anthers and



Academic editor: Alan Paton Received: 23 December 2024 Accepted: 3 March 2025 Published: 26 March 2025

Citation: Luo J-C, Li Y-Q, Li Y-L, She M-Z, Zeng Y-J, Wang F-G, Chen H-F (2025) *Primulina nanlingensis* (Gesneriaceae), a new species from the Limestone Karst of Guangdong, China. PhytoKeys 254: 99–111. https://doi.org/10.3897/ phytokeys.254.145138

**Copyright:** © Jin-Chu Luo et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). a characteristic chiritoid stigma (Wang et al. 2011; Yang et al. 2023). As of February 2025, the genus *Primulina* has 244 accepted species, thereby constituting the largest genus in the Chinese Gesneriaceae (Chen et al. 2024; GRC 2025).

Typically residing in the sheltered and moist conditions of karst formations, such as caves and similar microhabitats (Xu et al. 2021), these species are predominantly restricted to limestone niches in southern and south-western China, as well as northern Vietnam (Kang et al. 2014; Li et al. 2019). Globally, the genus includes over 170 species that are endemic to karst regions (Xu et al. 2021; Pan et al. 2022). Guangdong Province is home to 114 species of Gesneriaceae, with a significant proportion of 48 species belonging to *Primulina* (Song et al. 2023). Since 2017, four new species of *Primulina* have been described in Guangdong Province, these being *P. effusa* F.Wen & B.Pan, *P. anisocymosa* F.Wen, Xin Hong & Z.J.Qiu, *P. huangjiniana* W.B.Liao, Q.Fan & C.Y.Huang and *P. liangwaniae* B.M.Wang & Y.H.Tong (Pan et al. 2017; Hong et al. 2019; Huang et al. 2020; Tong et al. 2023).

During a botanical survey conducted in 2024 within the Shimentai Nature Reserve, an unidentified species of *Primulina* was discovered on two limestone hills. The species was subsequently introduced and cultivated in the greenhouses of the South China Botanical Garden, Chinese Academy of Sciences. From April to June of 2024, the plant exhibited continuous flowering with beautiful pale yellow flowers. After rigorous comparison of this material with herbarium specimens and consultation of relevant references and monographs (Guo et al. 2015; Pan et al. 2016), we confirmed that it represents a new species of *Primulina*, which we describe in this study. Phylogenetic analysis, utilising ITS and *trnL*-F sequences, confirmed its position within the genus.

### Material and methods

### Morphological observation

The material of this new species was collected during a botanical survey conducted at Shimentai Nature Reserve, Yingde City, Guangdong Province. The species was cultivated for further morphological study at the South China Botanical Garden, Chinese Academy of Sciences. Morphological assessments of the new species were carried out using herbarium specimens, with measurements taken from fresh samples. Comparative morphology was conducted with morphologically similar species, utilising both living plants and specimens from institutions such as IBSC, KUN, PE and IBK, as well as digital images from JSTOR Global Plants (http://plants.jstor.org/). Indumentum characteristics were examined by an Olympus-SZ61 stereomicroscope and Olympus-BX43 optical microscope, with photographic documentation accomplished using a Nikon D810 camera.

### **Molecular sampling**

We collected one individual from each of the two natural populations within the protected area and dried the fresh leaf samples using silica gel. Genomic DNA was extracted from the dried leaves using a modified CTAB protocol (Doyle and Doyle 1987). Based on recent phylogenetic studies (Pan et al. 2022; Yang et al. 2023; Chen et al. 2024), we retrieved ITS and *trn*L-F sequences for 124 *Primulina* species from GenBank to determine the phylogenetic position of the new species. *Petrocodon ainsliifolius* W.H.Chen & Y.M.Shui and *Petrocodon hancei* (Hemsl.) A.Weber & Mich.Möller were selected as outgroup taxa for the analysis (Chen et al. 2024). Corresponding GenBank accession numbers are presented in Table 1.

### **Phylogenetic analyses**

We constructed Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic trees for *Primulina nanlingensis* and 124 *Primulina* species using ITS and *trn*L-F sequences with PhyloSuite v.1.2.2 (Zhang et al. 2020). Sequences were aligned using MAFFT v.7.471 (Katoh et al. 2019) in PhyloSuite v.1.2.3 and refined with Gblocks 0.91b (Talavera and Castresana 2007), prior to their combination. The optimal nucleotide substitution model was determined using ModelFinder (Kalyaanamoorthy et al. 2017) in PhyloSuite v.1.2.3. The ML tree was built using IQ-tree v.1.6.12 (Nguyen et al. 2015) with the TN+F+R3 model and 5000 bootstrap replicates. The BI tree was constructed using MrBayes v.3.2.6 (Ronquist et al. 2012) with the HKY+F+I+G4 model, running two chains for 3,000,000 generations and sampling every 1000 generations. Both models were selected, based on the Bayesian Information Criterion (BIC). Finally, tree visualisation was performed using ITOL v.7 (https://itol.embl.de/).

Species	ITS	trnL-F	Species	ITS	trnL-F
Petrocodon ainsliifolius	KF202291	KF202298	Primulina lunglinensis var. amblyosepala	MK747105	MK746281
Petrocodon hancei	KY796057	KY796059	Primulina lungzhouensis	KY394931	KY393525
Primulina alutacea	KY394847	KY393441	Primulina lutea	JX506921	JX506813
Primulina argentea	KY394848	KY393442	Primulina lutvittata	MK369978	MK369993
Primulina baishouensis	KY394849	KY393443	Primulina mabaensis	KY394937	KY393531
Primulina balansae	MK747141	MK746274	Primulina macrodonta	JX506923	JX506815
Primulina beiliuensis	KY394850	KY393444	Primulina maculata	KU220604	KU220609
Primulina beiliuensis var. fimbribracteata	KY394851	KY393445	Primulina malipoensis	MK747123	MK746240
Primulina bicolor	KY394852	KY393446	Primulina medica	KY394940	KY393534
Primulina bipinnatifida	KY394853	KY393447	Primulina melanofilamenta	MK747158	MK746277
Primulina brachytricha var. magnibracteata	MK369979	MK369994	Primulina minor	MK747160	MK746290
Primulina carinata	KY394858	KY393452	Primulina minutimaculata	KY394941	KY393535
Primulina cataractarum	MW900263	MW960358	Primulina moi	KF498115	KY393536
Primulina chizhouensis	KY394860	KY393454	Primulina mollifolia	KY394943	KY393537
Primulina confertiflora	MK747101	MK746253	Primulina nandanensis	KY394947.1	KY393541
Primulina cordata	KC190200	KC190207	Primulina nanlingensis_YD1	PQ740297	PQ759014
Primulina cordistigma	MK747118	MK746251	Primulina nanlingensis_YD2	PQ740298	PQ759015
Primulina crassirhizoma	KY394864	KY393458	Primulina ningmingensis	KY394949	KY393543
Primulina crassituba	MK747147	MK746230	Primulina obtusidentata	KF498096	KY393544

Table 1. Species names and GenBank accession numbers of ITS and trnL-F DNA sequences used for analysis.

Species	ITS	trnL-F	Species	ITS	trnL-F
Primulina curvituba	MK747137	MK746242	Primulina ophiopogoides	KF498062	KY393545
Primulina danxiaensis	JX506886	JX506778	Primulina orthandra	MK747128	MK746286
Primulina depressa	KY394869	KY393463	Primulina pengii	KU220603	KU220610
Primulina diffusa	KY394871	KY393465	Primulina petrocosmeoides	KY394953	KY393547
Primulina dongguanica	KY394872	KY393466	Primulina pinnatifida	KY394954	KY393548
Primulina dryas	KY394875	KY393469	Primulina polycephala	KY394955	KY393549
Primulina eburnea	JX506891	JX506783	Primulina porphyrea	KU173793	KU173799
Primulina efusa	MK369976	MK369991	Primulina pseudoeburnea	KY394958	KY393552
Primulina fengkaiensis	MK369975	MK369990	Primulina pseudoglandulosa	KF498138	KY393482
Primulina fengshanensis	MK369970	MK369985	Primulina pseudoheterotricha	JX506933	JX506824
Primulina fimbrisepala	JX506894	JX506786	Primulina pseudolinearifolia	MK747140	MK746280
Primulina fimbrisepala var. mollis	JX506895	JX506787	Primulina pseudoroseoalba	KY394959	KY393553
Primulina fordii	MG727881	MG727878	Primulina pungentisepala	KY394962	KY393556
Primulina fordii var. dolichotricha	MK747125	MK746247	Primulina purpurea	KY394964	KY393558
Primulina glandaceistriata	MK747114	MK746256	Primulina qingyuanensis	KY394965	KY393559.1
Primulina glandulosa	KY394887	KY393481	Primulina renifolia	KY394966	KY393560
Primulina gongchengensis	KY394889	KY393483	Primulina roseoalba	KY394972	KY393566
Primulina grandibracteata	MK747121	MK746266	Primulina rosulata	KU528874	KU528884
Primulina guihaiensis	KY394893	KY393487	Primulina rubribracteata	KU173791	KU173797
Primulina halongensis	KY394895	KY393489	Primulina secundiflora	MK747119	MK746279
Primulina hedyotidea	JX506905	JX506797	Primulina shouchengensis	KY394980	KY393574
Primulina heterochroa	KY394898	KY393492	Primulina sichuanensis	MK747162	MK746264
Primulina hochiensis	JX506903	JX506795	Primulina sinovietnamica	MK369973	MK369988
Primulina huaijiensis	KF498127	KY393495	Primulina spinulosa	KF498063	KY393576
Primulina hunanensis	KU220602	KU220608	Primulina subulata	KY395020	KY393579
Primulina jiangyongensis	KY394902	KY393496	Primulina subulata var. guilinensis	KY394967	KY393561
Primulina jingxiensis	KY394903	KY393497	Primulina subulatisepala	MK747122	MK746246
Primulina jiulianshanensis	OP243287	OP243283	Primulina suichuanensis	KY395021	KY393580
Primulina jiuwanshanica	MK747116	MK746260	Primulina tabacum	KY395023	KY393582
Primulina juliae	MG727889	MG727873	Primulina tenuituba	KY395025	KY393584
Primulina langshanica	KY394907	KY393501	Primulina tsoongii	KY395029	KY393588
Primulina latinervis	KY394908	KY393502	Primulina verecunda	KY395031	KY393590
Primulina lechangensis	KY394910	KY393504	Primulina versicolor	MK747155	MK746252
Primulina leeii	KY394911	KY393505	Primulina vestita	MK747156	MK746282
Primulina lepingensis	KY394913	KY393507.1	Primulina villosissima	KY395032	KY393591
Primulina lianpingensis	MH343910	MH344542	Primulina wenii	MK747148	MK746284
Primulina lijiangensis	KY394919	KY393513	Primulina wentsaii	KY395033	KY393592
Primulina linearicalyx	MH032854	MH032841	Primulina wuae	MK747159	MK746265
Primulina linearifolia	KY394921	KY393515	Primulina xiziae	KY395038	KY393597
Primulina longgangensis	JX506916	JX506808	Primulina yangchunensis	KY395039	KY393598
Primulina longicalyx	KY394927	KY393521	Primulina yangshanensis	KY395040	KY393599
Primulina longii	JX506917	JX506809	Primulina yangshuoensis	KY395042	KY393601
Primulina longnanensis	OP243286	0P243282	Primulina yingdeensis	KU528876	KU528886
Primulina longzhouensis	JX506918	JX506810	Primulina yungfuensis	JX506957	JX506848
Primulina lunglinensis	KY394930	KY393524	Primulina zhoui	MK747104	MK746222

### Result

### Molecular phylogenetic studies

The ITS matrix, consisting of 128 sequences with an aligned length of 952 base pairs (bp), contained 52.5% variable sites and 34.5% informative sites. Similarly, the *trn*L-F matrix had an aligned length of 1027 bp, with 20.8% variable sites and 9.2% informative sites. Additionally, the combined ITS and *trn*L-F matrix had a total aligned length of 1689 bp and featured 38.8% variable sites and 24% informative sites. The two populations of the new species from Shimentai National Nature Reserve form a monophyletic group (BS = 100%, PP = 1.00), where BS stands for bootstrap support and PP stands for posterior probability. They also form a sister clade with *P. versicolor* and *P. pengii* (BS = 100%, PP = 1.00). All three form a strongly-supported clade with *P. alutacea*, *P. suichuanensis* and *P. polycephala* (Fig. 1).

### **Taxonomic treatment**

### Primulina nanlingensis J.C.Luo & H.F.Chen, sp. nov.

urn:lsid:ipni.org:names:77359322-1 Figs 2, 3

**Type.** CHINA • Guangdong Province, Yingde City, Shimentai National Nature Reserve, 23°28'N, 113°05'E, 620 m elev., growing on top of a cliff on a limestone hill, 7 May 2024 (fl.), *J.C. Luo & H.F. Chen LJC00501* (holotype: IBSC; isotypes: IBSC).

Diagnosis. The new species is similar to Primulina versicolor F.Wen, B.Pan & B.M.Wang in terms of flower shape and corolla colour, but easily distinguished from the larger leaf blades  $(10-21 \times 7-19 \text{ cm vs. } 8-18 \times 6.5-16.5 \text{ cm})$  with a crenate margin (vs. entire); notably lower number of flowers (3-4 cymes, 4-8 flowered vs. 4–8 cymes, 4–24 flowered or more); bracts ovate-lanceolate (vs. broadly oval or suborbicular), with shallowly serrate margins above the middle (vs. entire margins); calyx lobes densely glandular on both surfaces (vs. outside glandular-pubescent inside nearly glabrous) and with 1-3 inconspicuous teeth each side (vs. 3-5-serrate); longer pistil (3.2-3.5 cm vs. 2.5-2.8 cm) and glandular-puberulent (vs. puberulent); filaments white (vs. pale yellow) with densely glandular at base and tip, sparser mid-section (vs. only upper half sparsely glandular-puberulent). Additionally, while the leaf morphology of this new species resembles that of P. pengii W.B.Xu & K.F.Chung, it differs in having a longer corolla length (4.2-5.2 cm vs. 2.8-3.6 cm), pale yellow corollas (vs. white) and ovate-lanceolate bracts with slightly serrate edges above the middle (vs. cordate bracts with entire margins).

**Description.** Herbs, perennial rhizome subterete,  $5-8 \text{ cm} \times 1.5-2.5 \text{ cm}$ , internodes indistinct. Leaves 6-8, opposite at top of rhizome; blade green, succulent to thickly chartaceous, ovate or broadly ovate to elliptic,  $10-21 \times 7-19 \text{ cm}$ , apex obtuse or subacute, margin crenate, base slightly oblique or symmetrical, leaf surface and petiole densely pubescent, abaxial surface densely villous and the veins on the abaxial surface densely pubescent; lateral veins 4-6-nerved on each side; petiole cross section sub-semicircular or compressed,  $2.5-9.5 \text{ cm} \times 0.8-1.5 \text{ cm}$ . Cymes axillary, 3-4, 4-8 flowers per cyme; bracts 2 opposite, ovate-lanceolate,  $4.1-4.4 \text{ cm} \times 2.2-2.4 \text{ cm}$ , outer side is shallowly serrate, apex



**Figure 1.** Phylogenetic tree of *Primulina* generated using Maximum Likelihood (ML) analysis and Bayesian Inference (BI) of the combined ITS and *trn*L-F sequences. Numbers on branches indicate bootstrap support ( $\geq$  50%) from ML and posterior probabilities ( $\geq$  0.50, rounded to two decimal places) from Bayesian Inference (BI) analyses, while values (< 50% / 0.50) below this threshold are represented by a dash (–). \* indicates the new species.



Figure 2. *Primulina nanlingensis* J.C.Luo & H.F.Chen A plants in natural habitat B habit in flowering C cyme and frontal view of corolla D the adaxial and abaxial surface of leaf blades E outside surface of bracts F frontal view of corolla G side view of corolla H opened corolla showing stamens, staminodes and colour I stamens J stigma K outside and inside surface of calyx lobes L pistil with calyx lobes and pistil without calyx lobes M infructescence.

acuminate, outside pubescent, inside sparsely pubescent; peduncle 5–12.5 cm long, 4–6 mm across, densely pubescent; pedicel 1.2–2.8 cm long, glandular-puberulent. Calyx 5-lobed nearly to the base, 8–15 mm × 1.6–2.5 mm, lanceolate, light green, both surfaces densely glandular-puberulent, with 1–3 inconspicuous



Figure 3. *Primulina nanlingensis* J.C.Luo & H.F.Chen A habit B side view of corolla C frontal view of corolla D opened corolla E stamens F pistil with calyx lobes G outside surface of calyx lobes H outside surface of bracts I capsule. Drawn by Mrs. Yunxiao Liu based on J.C. Luo & H.F. Chen LJC00501.

teeth on each side. Corolla 4.2-5.2 cm long, pale yellow, throat dark yellow with 2 pale purple stripes, with 3 patches at the sinuses of the 2 upper lip lobes, patches light brown outside and dark purple inside, sometimes the dark purple patches absent and these patches are glandular, outside densely glandular-pubescent; tube infundibuliform, 3.2-3.8 cm long, orifice ca. 1.3 cm in diameter; limb distinctly 2-lipped, adaxial lip 2-lobed bifid to over the middle, lobes oblong, 5-7 mm × ca. 6 mm; abaxial lip 3-lobed to one-third from the top, lobes oblong, 4-6 mm × ca. 4 mm. Stamens 2, adnate to 1.3-1.5 cm above the base of the corolla base; anthers elliptic, 2.5-3 mm long, densely glandular; filaments ca. 15 mm long, white, with the base and upper part sparsely covered with glandular-puberulent; staminodes 3, two lateral ones adnate to ca. 1.3 cm above the corolla base, ca. 7 mm long, densely glandular-pubescent, apex bent, the central one adnate to ca. 3 mm above the base of corolla base, ca. 2 mm long, glabrous; disc annular, ca. 1 mm high, yellow, glabrous. Pistil 3.2-3.5 cm long; ovary linear, ca. 1.8-2.2 cm long, densely glandular-puberulent; style 0.8-1.2 cm long, sparse glandular-puberulent. Stigmas 2-lobed, 2.8-3.6 mm long, shallowly lobed, lobes ca. 1 mm long. Capsule green, mature dark brown, 3.2-4.5 cm × 1.5-2.2 mm, with persistent calyx lobes at base, densely white-villous and pubescent.

Phenology. Flowering from late April to early June, fruiting from June to August. Distribution and ecology. Primulina nanlingensis is known only from two separate limestone hills in the Shimentai National Nature Reserve, Yingde City, Guangdong Province, China. Companion species were calcareous herbs such as Selaginella effusa Alston, S. delicatula (Desv.) Alston., Pilea peltata Hance and Ficus sarmentosa var. henryi (King ex Oliv.) Corner etc.

**Etymology.** The species epithet refers to the type locality, the Nanling Mountains.

Vernacular name. 南岭报春苣苔 (Chinese name); Nán Lǐng Bào Chūn Jù Tái (Chinese pronunciation).

**Provisional conservation status.** At present, only two populations of *Primulina nanlingensis* have been discovered on limestone hills in the Shimentai National Nature Reserve, where a substantial area of 35 km<sup>2</sup> has been identified as suitable habitat for the species. The two naturally distributed populations are no more than 10 km apart and each population consists of no more than 100 mature individuals. Currently, the two populations are stable, as the habitat is under protection by the administrators of the scenic area. However, considering the overall low number of individuals across the species populations and the conservation measures in place, it could be provisionally classified as Near Threatened [NT] according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2024).

Additional specimens examined. CHINA • Guangdong Province, Yingde City, Shimentai National Nature Reserve, 23°28'N, 113°05'E, 620 m elev., growing on top of a cliff on a limestone hill, 25 May 2024 (fl.), *J.C. Luo & H.F. Chen LJC00502* (IBSC).

### Discussion

The karst regions of southern and south-western China, as well as northern Vietnam, are hotspots for diversity of *Primulina* species, predominantly consisting of endemic species with limited populations confined to isolated sites (Kang et al. 2014; Tong et al. 2020; Wei et al. 2022). The Nanling Mountain

Part	P. nanlingensis	P. versicolor	P. pengii			
Leaf blade	ovate or broadly ovate to elliptic, 10– 21 cm × 7–19 cm, margin crenate	broadly oval or nearly cordate, 8–18 cm × 6.5–16.5 cm, margin entire	ovate to broadly ovate, 14–25 cm × 9.5–15 cm, the margin shallowly repand to crenate			
Cyme	3-4, 4-8-flowered	4–8, 4–24-flowered or more	3-4, 4-12-flowered			
Bracts	ovate-lanceolate, 4.1–4.4 cm × 2.2–2.4 cm, shallowly serrate above the middle, apex acuminate, outside pubescent, inside sparsely pubescent	broadly oval or suborbicular, 5–5.5 cm × 4.4–5 cm, apex acute, outside densely appressed pubescent, inside nearly glabrous, margin entire	cordate, 2.6–3.2 cm × 2.5–3 cm, the margin entire to shallowly repand, the apex acute, outside pubescent, inside sparsely pubescent			
Corolla	pale yellow, 4.2–5.2 cm, outside densely glandular-pubescent, inside nearly glabrous; throat dark yellow with 2 pale purple stripes	canary yellow, 3.5–4.2 cm, outside densely glandular-pubescent, inside nearly glabrous; throat dark yellow with 2 brownish-purple stripes	white, 2.8–3.6 cm long, outside glandular pubescent, inside sparsely puberulent, with 2 pale purple stripes			
Calyx lobes	8−15 mm × 1.6−2.5 mm, both surfaces densely glandular, with 1−3 inconspicuous teeth each side	8.5 mm × 2 mm, outside densely glandular-pubescent, inside nearly glabrous, margin 3–5-serrate	8–10 mm × ca. 2 mm, outside glandular pubescent, inside sparsely pubescent, margin serrulate			
Stamens	filaments ca. 15 mm long, white, densely glandular at base and tip, sparser mid-section, anthers elliptic, 2.5–3 mm long, densely glandular	filaments ca. 12.5 mm long, pale yellow, glabrous, but the upper half of filament sparsely glandular puberulent, anthers semicircular, 5–6 mm long, glabrous	filaments ca. 14 mm long, white, sparsely puberulent, anthers reniform, ca.4 mm long, puberulent			
Pistul	3.2–3.5 cm long, densely glandular- puberulent	2.5–2.8 cm long, densely puberulent	2.4–3.1 cm long, densely puberulent			

Table 2. Comparisons of Primulina nanlingensis to P. versicolor and P. pengii, respectively.



Figure 4. Morphological comparison between *Primulina nanlingensis* (A1–A4), *P. versicolor* (B1–B4, photos by Fang Wen.) and *P. pengii* (C1–C4, photos by Weibin Xu). Legends: Leaf blade (A1, B1, C1); Cyme (A2, B2, C2); Corolla and Calyx lobes (A3, B3, C3); Bracts (A4, B4, C4).

Range serves as a habitat for *Primulina*, where the complex topography and soil heterogeneity foster a high level of species diversity and endemism (Wang et al. 2017). The type localities for both *P. nanlingensis* and *P. versicolor* are situated within Yingde City, with a distance of over 50 km between them. Furthermore, *P. pengii* is found in Yangshan County, Guangdong Province, which is more than 120 km away from *P. nanlingensis*. Notably, *P. nanlingensis* closely resembles
*P. versicolor* in both flower shape and colour, but differs in several internal floral structures, such as the pistil with glandular-pubescent and stamens densely glandular at the base and tip, sparser in the middle, fewer flowers per cyme and overall cyme number. Phylogenetic analysis shows that they are closely related, yet their morphological differences suggest that *P. nanlingensis* represents a new species. Detailed comparisons of the three species are provided in Table 2 and Fig. 4. Finally, given the small population size and restricted distribution to just two locations, conservation efforts for *P. nanlingensis* are of utmost importance. In all, *P. nanlingensis*, as a newly-discovered species in *Primulina*, not only enhances the plant diversity in Naning Mountain, but also provides valuable insights for further study on the local adaptation in karst regions.

### Acknowledgements

We would like to express our gratitude to Yunxiao Liu (IBSC) for preparing the line drawing. We are grateful to Dr. Weibin Xu (Guangxi Institute of Botany, Chinese Academy of Sciences) for providing photos of *Primulina pengii* and to Dr. Fang Wen (same institute) for photos of *P. versicolor* and his guidance on plant identification.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

This study was financially supported by the Science and Technology Projects in Guangzhou (E33309) and Guangdong Flagship Project of Basic and Applied Basic Research (2023B0303050001).

### **Author contributions**

Hong-Feng Chen, Yuan-Qiu Li, Jin-Chu Luo: Conceptualisation, Methodology; Hong-Feng Chen, Yuan-Qiu Li, Yang-Jin Zeng, Jin-Chu Luo, Ming-Zhao She: Field investigation, Materials collection; Jin-Chu Luo: Data analyses and visualisation; Jin-Chu Luo: Manuscript writing; Hong-feng Chen, Yuan-Qiu Li, Ya-Li Li, Fa-Guo Wang: Manuscript revision. All authors have read and approved the manuscript.

### Author ORCIDs

Jin-Chu Luo <sup>©</sup> https://orcid.org/0009-0003-0526-8962 Yuan-Qiu Li <sup>©</sup> https://orcid.org/0009-0005-8135-500X Ya-Li Li <sup>©</sup> https://orcid.org/0000-0003-4667-5241 Ming-Zhao She <sup>©</sup> https://orcid.org/0009-0003-1223-6037 Fa-Guo Wang <sup>©</sup> https://orcid.org/0000-0002-9326-8000 Hong-Feng Chen <sup>©</sup> https://orcid.org/0000-0002-8415-3260

### **Data availability**

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

### References

- Chen DY, Zeng XH, Cai QL, Wu XG, Gao YY, Xu GL (2024) *Primulina adenopoda* (Gesneriaceae), a new species from the limestone karst area of Jiangxi Province, China. Taiwania 69(1): 74. https://doi.org/10.22541/au.170663663.32946717/v1
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Fang D, Qin DH (2004) *Wentsaiboea* D. Fang & DH Qin, a new genus of the Gesneriaceae from Guangxi, China. Journal of Systematics and Evolution 42(6): 533–536.

GRC (2025) Gesneriaceae Resource Centre. Royal Botanic Garden Edinburgh. https:// padme.rbge.org.uk/grc/data/checklists.org/ [Accessed 25 February 2025]

Guo J, Pan B, Liu J, Xu WB, Chung KF (2015) Three new species of *Primulina* (Gesneriaceae) from limestone karsts of China based on morphological and molecular evidence. Botanical Studies 56(1): 1–13. https://doi.org/10.1186/s40529-015-0115-5

- Hance HF (1883) New Chinese Cyrtandreae. Le Journal de Botanique 21: 165–170.
- Hong X, Keene J, Qiu ZJ, Wen F (2019) *Primulina anisocymosa* (Gesneriaceae), a new species with a unique inflorescence structure from Guangdong, China. PeerJ 7: e6157. https://doi.org/10.7717/peerj.6157
- Huang CY, Meng KK, Guo JQ, Chen F, Liao WB, Fan Q (2020) *Primulina huangjiniana* (Gesneriaceae), a new species from Guangdong, China. Guangxi Zhi Wu 40(10): 1429–1437. https://doi.org/10.11931/guihaia.gxzw202003032
- IUCN Standards and Petitions Committee (2024) Guidelines for using the IUCN Red List categories and criteria. Version 16: Prepared by the Standards and Petitions Committee in March 2024.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler AV, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Kang M, Tao J, Wang J, Ren C, Qi Q, Xiang QY, Huang H (2014) Adaptive and nonadaptive genome size evolution in Karst endemic flora of China. The New Phytologist 202(4): 1371–1381. https://doi.org/10.1111/nph.12726
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Li S, Xin ZB, Chou WC, Huang Y, Pan B, Maciejewski S, Wen F (2019) Five new species of the genus *Primulina* (Gesneriaceae) from limestone areas of Guangxi Zhuangzu Autonomous Region, China. PhytoKeys 127: 77–91. https://doi.org/10.3897/phytokeys.127.35445
- Liu Y, Xu WB, Huang YS (2011) *Primulina guangxiensis* sp. nov. (Gesneriaceae) from a karst cave in Guangxi, China. Nordic Journal of Botany 29(6): 682–686. https://doi. org/10.1111/j.1756-1051.2011.01089.x
- Nguyen LT, Schmidt HA, Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Pan B, Wang BM, He JY, Wen F (2016) Primulina versicolor and P. alutacea spp. nov. (Gesneriaceae), two new species with yellow flowers from northern Guangdong, China. Edinburgh Journal of Botany 73(1): 25–37. https://doi.org/10.1017/ S0960428615000268
- Pan B, Zou LL, Zhang RL, Kang M, Wen F (2017) Primulina effusa F. Wen & B. Pan a new species of Gesneriaceae from limestone areas of South China. Guangxi Zhi Wu 37(10): 1250–1256. https://doi.org/10.11931/guihaia.gxzw201706006

- Pan B, Wang BM, Yang LH, Lai BD, Li PW (2022) *Primulina fangdingii* (Gesneriaceae), a new species from Guangxi, China. Nordic Journal of Botany 2022(10): e03684. https://doi.org/10.1111/njb.03684
- Ronquist FM, Teslenko P, van der Mark DL, Ayres A, Darling S, Höhna B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic infe-rence and model selection across a large model space. Systematic Biology 61(3): 539– 542. https://doi.org/10.1093/sysbio/sys029
- Song ZQ, Ye W, Dong SY, Jin ZC, Zhong XJ, Wang Z, Zhang BY, Xu YC, Chen WL, Li SJ, Yao G, Xu ZF, Liao S, Tong YH, Zeng YP, Zeng YB, Chen YS (2023) A dataset on inventory and geographical distributions of higher plants in Guangdong, China. Shengwu Duoy-angxing 31(9): 23177. https://doi.org/10.17520/biods.2023177
- Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164
- Tong YH, Wang BM, Vu TC, Xia NH (2020) *Primulina elegans* (Gesneriaceae), a new species from North Vietnam. Nordic Journal of Botany 38(11): e02942. https://doi.org/10.1111/njb.02942
- Tong YH, Ni JB, Wang BM (2023) *Primulina liangwaniae* (Gesneriaceae), a new species from Guangdong, China. Phytotaxa 630(2): 154–158. https://doi.org/10.11646/phytotaxa.630.2.7
- Wang YZ, Mao RB, Liu Y, Li JM, Dong Y, Li ZY, Smith JF (2011) Phylogenetic reconstruction of Chirita and allies (Gesneriaceae) with taxonomic treatments. Journal of Systematics and Evolution 49(1): 50–64. https://doi.org/10.1111/j.1759-6831.2010.00113.x
- Wang J, Ai B, Kong H, Kang M (2017) Speciation history of a species complex of *Primulina eburnea* (Gesneriaceae) from limestone karsts of southern China, a biodiversity hot spot. Evolutionary Applications 10(9): 919–934. https://doi.org/10.1111/eva.12495
- Weber A, Middleton DJ, Forrest A, Kiew R, Lim CL, Rafidah AR, Sontag S, Triboun P, Wei YG, Yao TL, Möller M (2011) Molecular systematics and remodeling of *Chirita* and associated genera (Gesneriaceae). Taxon 60(3): 767–790. https://doi.org/10.1002/tax.603012
- Wei YG, Do VT, Wen F (2022) A Checklist to the Plants of Northern Vietnam. China Forestry Publishing House, Beijing, 383 pp.
- Xu MZ, Yang LH, Kong HH, Wen F, Kang M (2021) Congruent spatial patterns of species richness and phylogenetic diversity in karst flora: Case study of *Primulina* (Gesneriaceae). Journal of Systematics and Evolution 59(2): 251–261. https://doi. org/10.1111/jse.12558
- Xu GL, Chen TR, Wu XG, Cai WL, Zhuo XH, Zeng NG, Ding L (2023) Primulina arcuata, a new species of Primulina (Gesneriaceae) from the Danxia Landform in Jiangxi, China. Taiwania 68(4): 391–397. https://doi.org/10.6165/tai.2023.68.391
- Yang ZM, Chou WC, Stephen M, Wei YG (2023) *Primulina nymphaeoides* (Gesneriaceae), a new species from Guangxi, China. Taiwania 68(1): 44–50. https://doi.org/10.6165/ tai.2023.68.44
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang G (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhou DS, Zhou JJ, Li M, Yu XL (2016) *Primulina suichuanensis* sp. nov. (Gesneriaceae) from Danxia landform in Jiangxi, China. Nordic Journal of Botany 34(2): 148–151. https://doi.org/10.1111/njb.00956



Research Article

# A realigned taxonomy for the *Schiedea kauaiensis* – *S. perlmanii* species pair (Caryophyllaceae) based on recent collections and new analyses that require nomenclatural changes for both species

Warren L. Wagner<sup>10</sup>, Stephen G. Weller<sup>2</sup>

1 Department of Botany, MRC-166, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA

2 Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA

Corresponding author: Warren L. Wagner (wagnerw@si.edu)

#### Abstract

The Kaua'i species Schiedea kauaiensis H. St. John was previously characterized by a geographical range including a number of Napali Coast valleys and the Limahuli, Wainiha, and Manoa valleys in the northern part of the island whereas the closely related S. perlmanii W. L. Wagner & Weller occurs in the Anahola area and on Ha'upu on the windward (eastern) side of Kaua'i. The primary characteristic distinguishing them is a subshrub habit for S. kauaiensis vs a vining habit in S. perlmanii. In several localities from northern Kaua'i including Limahuli, Wainiha, and Manoa valleys, populations were known only from herbarium specimens but were included within S. kauaiensis in part because these localities were closest to the Napali Coast valleys, which encompasses the remainder of the range of the species. Recent field work resulting in discovery of new populations and cultivation of plants from Limahuli and Manoa has shown that plants from these three northern localities do not represent S. kauaiensis but rather fit with S. perlmanii. Two of the collections from this northern area are the types of S. wichmanii H. St. John and S. kauaiensis. Since these names were published earlier, we must adopt here the earliest name, S. kauaiensis, for the plants formerly known as S. perlmanii leaving the species from the Napali Coast valleys without a name and described here as a new species, S. napaliensis W. L. Wagner & Weller.

Key words: Caryophyllaceae, Conservation, Hawaiian Islands, Kaua'i, Schiedea

### 

Academic editor: Gian Pietro Giusso del Galdo Received: 31 January 2025 Accepted: 10 March 2025 Published: 26 March 2025

**Citation:** Wagner WL, Weller SG (2025) A realigned taxonomy for the *Schiedea kauaiensis* – *S. perlmanii* species pair (Caryophyllaceae) based on recent collections and new analyses that require nomenclatural changes for both species. PhytoKeys 254: 113–123. https://doi.org/10.3897/ phytokeys.254.148438

**Copyright:** This is an open access article distributed under the terms of the CC0 Public Domain Dedication.

### Introduction and discussion

The Kaua'i Island pair of closely related species of *Schiedea* Cham. & Schltdl. in sect. *Mononeura* W.L. Wagner & Weller, *S. kauaiensis* and *S. perlmanii* were distinguished by growth habit and geography in the most recent revision of the genus (Wagner et al. 2005), where *S. perlmanii* was first published. In that publication, *Schiedea kauaiensis*, a subshrub, was noted as occurring on the northwestern to northern part of the island and *S. perlmanii* had a vining habit and occurred from the southeastern to northeastern part of the island. In general, *S. perlmanii* occurred in lower elevation mesic vegetation and was known from only a few collections from the Ha'upu area of southeastern Kaua'i and from a single collection in 1952

disjunct from Ha'upu in the Anahola area above Papa'a in northeastern Kaua'i. Schiedea kauaiensis was known from scattered areas of Kaua'i, including Wainiha-Manoa ridge (type locality), Limahuli, Kalalau, Nualolo, and Mahanaloa valleys on the northern to western side, and Olokele Valley and the Wahiawa Mountains in the central region, in open areas of diverse mesic forest at a wider range of elevations. However, there were only three herbarium collections available for study from the northern areas of Limahuli, Wainiha, and Manoa valleys and we had no direct field observations or cultivated plants in the greenhouse for study. Since these localities were close to the eastern end of the range of S. kauaiensis and the primary distinguishing character (habit) was not clear on herbarium specimens, they were included in the delimitation of S. kauaiensis. After publication of the Schiedea monograph in 2005, new collecting activity on the eastern and northern areas of Kaua'i, especially from 2014, and 2021-2023, led to the rediscovery of populations of S. perlmanii on Kawalumakua (near Papa'a) and of S. kauaiensis in Manoa and Limahuli valleys. Collectors (Heintzman and Wood) noted that the collections from Manoa were morphologically atypical and very close to S. perlmanii.

Recent comprehensive sampling across the genus used a variety of greenhouse, field and laboratory studies to gain additional insights into the evolution of breeding systems in Schiedea. The phylogenetic focus of the project employed a suite of modern DNA sequencing tools to generate trees of hypothesized evolutionary relationships for all species in the genus. This was coupled with additional field and greenhouse studies to explore breeding system evolution, ranging from chemical analyses of floral scent to evening field observations of Schiedea flowers in the wild to better understand whether organisms such as moths might act as pollinators. The project also used progeny from crosses among the few remaining individuals of S. kauaiensis from several Napali Coast valleys for outplanting in natural areas. This focus allowed us to examine more closely the morphology of many species, including S. kauaiensis and S. perlmanii. A preliminary analysis of genomic data from ca. 25 samples of all known localities of these two species strongly supports the inclusion of populations from Limahuli and Manoa with S. perlmanii rather than with populations of S. kauaiensis from the Napali Coast (McDonnell et al., pers. comm.). With support from both morphological and genomic data we here move these three populations for inclusion in a recircumscribed species consisting of largely windward populations and resulting in a more narrowly circumscribed species on the NW leeward Napali valleys.

### **Realigned taxonomy**

We here utilize the information published previously in the *Schiedea* monograph (Wagner et al. 2005) with various updates to support the realigned classification with the removal of populations from Limahuli, Wainiha, and Manoa valleys on the northern part of Kaua'i from the circumscription of what is described here as a new species, *S. napaliensis*. Because the type of *S. kauaiensis* was from Wainiha Valley, populations from Moloa'a and Ha'upu, which were formerly placed in *S. perlmanii*, will in the revised circumscription bear the oldest name *S. kauaiensis*, with *S. perlmanii* becoming a synonym of *S. kauaiensis*.

Following the new alignment of populations, the next step was to reexamine the morphological characters of all collections from throughout the ranges of both species. In addition to inflorescence size and habit, variation in flower

Character	S. kauaiensis	S. napaliensis           Erect to ascending subshrubs 3–10 dm tall, few branched, glabrous throughout, except glandular-t puberulent throughout inflorescence						
Habit	Vine, stems 6–12 dm long (in cultivation eventually to 15+ dm long), sprawling when young to reclining when longer, at least sparingly branched, glabrous throughout or sparsely short-puberulent in inflorescence							
Leaf shape	Narrowly ovate or lanceolate to elliptic-lanceolate	Oblong-elliptic						
Leaf length/width	Blades 4–11.5 cm long, 2–2.8 cm wide	Blades 7.5–15 cm long, 1.8–4.1 cm wide						
Inflorescence	Inflorescence terminal, with 40–60 flowers, 20–35 cm long and nearly as wide, laterally-directed or pendent, the tertiary and higher level internodes or pedicels weakly spreading	Inflorescence terminal, with 27–70 flowers, 20–48 cm long, branches spreading						
Bracts	Bracts subulate, the lowermost of central axis narrowly elliptic, falcate, green and purple-tinged or purple, the lowermost ones 2–17 mm long, those of branches and flowers 1.5–4.5 mm long, purple, the adaxial surface puberulent	Bracts subulate, the lowermost of the central axis elliptic-lanceolate, recurved and often twisted, as green as the leaves, the lower ones 30–45 mm long, those of the branches and flowers 5–18 mm long, glandular-puberulent						
Pedicels	Pedicels (5–)13–15 mm long	Pedicels (7–) 10–23 mm long						
Sepals	Sepals 2.2–3 mm long, ovate, green, sometimes purple- tinged toward apex or irregularly purple throughout, opaque, strongly reflexed, apex attenuate	Sepals 4.3–4.8 mm long, lanceolate, green, opaque, strongly reflexed, apex long-attenuate						
Capsules	Capsules 2.5–2.8 mm long, ovoid	Capsules 3.1–3.8 mm long, narrowly ovoid						
Seeds	Seeds ca. 1.2 mm long	Seeds ca. 1.3 mm long						

**Table 1.** Comparison of morphological and geographical/ecological characters of the subclade of species of *Schiedea kauaiensis* and *S. napaliensis* (sect. *Mononeura*).

size and pubescence in particular differentiated *S. kauaiensis* and *S. napaliensis*. A summary of most useful characters for distinguishing the two species is presented in Table 1.

750-950 m Diverse mesic forest

### Schiedea kauaiensis H. St. John, Phytologia 64: 177. 1988.

Figs 1-3

400-640 m Mesic shrubland to mesic forest

Elevation/ habitat

- Schiedea nuttallii var. pauciflora O. Deg. & Sherff in Sherff, Bot. Leafl. 7: 6. 1952. Type. Hawaiian Islands, Kaua'i: Forest Reserve, ridge behind Papa'a, 16 Jan 1952, O. Degener & A. B. Greenwell s .n. (holotype: F-1451309, F-1451310, originally mounted on a single sheet [photo: F!], but now on 2 sheets [photos: F!]; isotypes: B! BISH-2 sheets!, F!, K, NY-2 sheets!, PH-2 sheets!, US- 2 sheets!).
- Schiedea nuttallii var. lihuensis Sherff, Bot. Leafl. 9: 3. 1954. Type. Hawaiian Islands, Kaua'i: [southeastern Kaua'i] "mauka of Gap?, near Lihue," 1911, J. M. Lydgate s. n. (holotype: BISH-501710!, photo: F!, isotypes: BISH-2 sheets!)
  Schiedea wichmanii H. St. John, Phytologia 64: 178. 1988. Type. Hawaiian Is-
- Schiedea wichmanii H. St. John, Phytologia 64: 178. 1988. Type. Hawaiian Islands, Kaua'i: Limahuli Valley, E wall, 60° slope, locality on dark soil and loose rock with remnant 'Ohi'a, Eugenia, Uluhe, Santalum [pyrularium A. Gray], Diospyros, Psychotria, and Hibiscus, 1300 ft [395 m], 13 Sep 1978, S. Perlman & C. Wichman, Jr. 219 (holotype: BISH-522858!; isotype: BISH!, PTBG!). [Sterile specimens, flowers liquid preserved.]
- Schiedea perlmanii W. L. Wagner & Weller, Syst. Bot Monogr. 72: 71. 2005. Type. Hawaiian Islands, Kaua'i: Mt. Ha'upu, near Queen Victoria's profile, W of head of Victoria, *Diospyros-Metrosideros* lowland mesic forest, 1700–1950 ft [515–590] m, 27 Feb 1992, *S. Perlman* 12614 (holotype: US-3252201!; isotypes: AD, BISH!, PTBG!).

Type. Hawaiian Islands, Kaua'i • Wainiha-Manoa ridge, wet forest near edge of pali, 2000 ft [610 m], 30 Jul 1977, C. Christensen 290 (holotype: BISH-522854!).

Description. Vine; stems 6-12 dm long (in cultivation eventually to 15 dm or more long), sprawling when young to reclining when longer, at least sparingly branched, internodes deep purple or purplish green, glabrous throughout, except bracts, sepals, and sometimes the pedicel sparsely short-puberulent. Leaves opposite; blades 4-11.5 cm long, 2-2.8 cm wide, narrowly ovate or lanceolate to elliptic-lanceolate, weakly glossy, green or yellowish green, sometimes purple-tinged, especially on lower surface, weakly coriaceous and rubbery, chartaceous when dry, with only the midvein evident, the midvein ± slightly excentric, usually reddish purple, margin entire, slightly thickened and becoming revolute toward the base, apex acute to acuminate; petioles 0.8-0.9 cm long, purple, weakly ± grooved. Inflorescence terminal, with 40-60 flowers, 20-35 cm long and nearly as wide, diffuse, laterally-directed or pendent, the tertiary and higher level internodes or pedicels weakly spreading; bracts subulate, the lowermost of central axis narrowly elliptic, falcate, green and purple-tinged or purple, the lowermost ones 2-17 mm long, those of branches and flowers 1.5-4.5 mm long, purple, the adaxial surface puberulent; pedicels 13-15 mm long at anthesis, weakly flattened, very weakly angled just below the flower and often sparsely short-puberulent. Flowers hermaphroditic, usually pendent. Sepals 2.2-3 mm long, elongating to 4 mm long in fruit, ovate, green, sometimes purple-tinged toward apex or irregularly purple throughout, opaque, strongly reflexed and convex in the proximal 1/4, producing a conspicuous transverse bulge, the distal part broadly navicular, oriented at 5° to 30° angle to the pedicel, abaxial side sparsely puberulent toward the base, the adaxial side puberulent, primarily near the midrib, margins conspicuously scarious, ciliate, apex attenuate, inconspicuously slightly twisted. Nectary base 0.7-0.9 mm long, yellow, the nectary shaft 4.5 mm long, gently recurved, at 90° angle to the axis, apex deeply bifid to ca. 1/2 their length. Stamens 10; filaments dimorphic, the antisepalous whorl 6.2–6.3 mm long, the alternate whorl 5 mm long; anthers 0.75-0.8 mm long, subequal, pale yellow. Styles 3. Capsules 2.5-2.8 mm long, ovoid. Seeds ca. 1.2 mm long, orbicular-reniform, compressed, the surface rugose. Chromosome number unknown.

**Distribution.** (Fig. 3). Kaua'i, known from mesic shrubland in four disjunct areas on windward Kaua'i: 1) near the summit of the Hoary Head Mountains (Ha'upu), 2) from a collection from above Papa'a made over 50 years ago and more recently Moloa'a Forest Reserve, Anahola, 3) as well as Wainiha, Limahuli, and Manoa valleys, and 4) from an old collection of a population in Wahiawa Mountains (Hi'i Mts) in the central region; ca. 400–640 m.

**Specimens examined. Hawaiian Islands. Kaua'i:** Koloa District: Hi'i Mts., *s.d., Lydgate s.n.* (BISH). Lihue District: Ha'upu summit, slopes of N facing side near top, [21°55'33.6"N, 159°24'1.9"W], *Perlman et. al. 12917* (PTBG), *Wood et al. 18289* (PTBG), *Wood et al. 18449* (PTBG); Mt. Ha'upu, N facing cliffs above Kipu, between Queen Victoria's Profile and Mt. Ha'upu summit, *Perlman 17563* (BISH, NY, PTBG, US), *Wood et al. 11435* (PTBG); Mt. Ha'upu, slopes above Kipu ranch, to W of Queen Victoria's Profile, *Perlman 17439* (PTBG), *17449* (PTBG); windward Ha'upu, just E of Ha'upu Peak, Kipu, below "Queen Elizabeth's [Queen Victoria's] Profile, *Warshauer 1184* (BISH). Kawaihau District: Moloa'a



**Figure 1.** Schiedea kauaiensis **A** habit, stem with leaves and inflorescence **B** branch of inflorescence **C** flower in early anthesis, male stage **D** flower in later anthesis, female stage. Reproduced from fig. 25 of Wagner et al. 2005; drawn from cultivated plant from *Perlman 12917* grown in the greenhouse of the University of California at Irvine. Illustration by Alice Tangerini.



**Figure 2**. Comparison of inflorescence pubescence **A** *Schiedea kauaiensis*, showing sparse short hairs **B** *S. napaliensis*, glandular-puberulent throughout. Although seeds of *S. kauaiensis* and *S. napaliensis* are similar in size, mature capsules of *S. napaliensis* are larger and contain more seeds than *S. kauaiensis*. Drawn from herbarium specimen of *Perlman* 17563 (US) and *Perlman* 472 (**A**), and *Perlman* 12074 (US) (**B**). Illustration by Alice Tangerini.

Warren L. Wagner & Stephen G. Weller: Schiedea kauaiensis realigned taxonomy



Figure 3. Distribution map (Kaua'i, Hawaiian Islands) of *Schiedea kauaiensis* and *S. napaliensis* showing extant and extirpated locations as of 2024 for populations represented by collections. Reproduced from Fig. 26 of Wagner et al. (2005).

Forest Reserve, near Kawalumakua Peak [22°09'14.4"N, 159°20'08.4"W], *Wood et al.* 14705 (PTBG), [22°09'19.9"N, 159°20'31.1"W], *Wood et al.* 14718 (BISH, PTBG, US), *Wood et al.* 14719 (PTBG), *Tangalin* 3361 (PTBG). Hanalei District: Manoa valley, above falls in valley to East of Limahuli, 530 m, *Perlman et al.* 23977 (PTBG); Wainiha valley, on ridge 1300 ft. S of Kulanaililia, top of ridge, [22°12'20.5"N, 159°34'5.1"W], *Christensen* 317 (BISH); Limahuli Valley, E wall, 60° slope, locality on dark soil and loose rock with 'Ohi'a, *Eugenia*, Uluhe, *Santalum* [*pyrularium* A. Gray], *Diospyros, Psychotria*, and *Hibiscus*, 1300 ft [395 m], 16 August 1978, *S. Perlman* & *C. Wichman*, *Jr.* 218 (BISH); Lower Limahuli valley, up subgulch on W side of valley [22°13'02.9"N, 159°34'59.6"W], *Perlman* & *Bender* 17370 (PTBG).

**Cultivated specimens. Kaua'i:** Ha'upu summit, slopes of N facing side near top, *Perlman et.al.* 12917 [cult. *Wagner & Shannon* 6795] (BISH, GH, NY, PTBG, US); SE portion of Moloa'a Forest Reserve, Anahola upper gulch [22°'15"N, 159°33"W], *Heintzman KP06012199* (US); from a cutting from Upper Manoa Valley, *Heintzman KP05052302* (PTBG), *Wood & DeMotta* 18282 (PTBG).

#### Schiedea napaliensis W.L.Wagner & Weller, sp. nov.

urn:lsid:ipni.org:names:77359323-1 Figs 2-4

**Type. Hawaiian Islands, Kaua'i** • Mahanaloa Valley, up valley from old horse trail, S side of valley, [22°12'35.6"N, 159°34'27.2"W], 10 July 1991, *S. Perlman & J. Obata 12074* (holotype: PTBG-1000046146!; isotypes: BISH, MO, US!).

Description. Erect to ascending subshrubs 3-10 dm tall; stems fewbranched, glabrous, becoming sparsely then moderately glandular-puberulent in the inflorescence, the internodes purple. Leaves opposite; blades 7.5-15 cm long, 1.8-4.1 cm wide, oblong-elliptic, light green or yellowish green, adaxial surface slightly glossy, the abaxial surface glossy, slightly thickened and rubbery, chartaceous when dry, usually slightly undulate, with only the midvein evident or sometimes with an additional pair of inconspicuous, smaller, looping veins, the midvein ± slightly excentric, margin entire, slightly thickened and weakly revolute, especially toward the base, apex acute to weakly acuminate, base gradually attenuate; petioles 0.5-1.1 cm long, pale green, purple toward the base, weakly ± grooved. Inflorescence terminal, with 27-70 flowers, 20-48 cm long, diffuse, flowers widely spaced, branches spreading, progressively more densely puberulent to apex, the hairs straight, erect, 0.1-0.35 mm long; bracts subulate, the lowermost of the central axis elliptic-lanceolate, as green as the leaves, recurved and often twisted, the lower ones 30-45 mm long, those of the branches and flowers 5-18 mm long; pedicels (7-) 10-23 mm long, elongating slightly in fruit, slightly asymmetrically flattened. Flowers hermaphroditic. Sepals 4.3-4.8 mm long, lanceolate, green, opaque, strongly reflexed and convex in the proximal 1/4, producing a small transverse bulge, the distal part shallowly concave, oriented ca. 40° to 80° angle to the pedicel, glandular-puberulent, a few of the hairs sometimes non-glandular, margins scarious, ciliate, apex long-attenuate. Nectary base 0.6-0.9 mm long, dark yellow, the nectary shaft 3-4.5 mm long, gently recurved, at 90° to the axis, apex deeply bifid, sometimes divided nearly to the base. Stamens 10; filaments dimorphic, the antisepalous whorl 7.5 mm long, the alternate whorl 5.3-5.8 mm long; anthers 0.75-0.85 mm long, subequal, pale yellow. Styles 3. Capsules 3.1-3.8 mm long, narrowly ovoid. Seeds ca. 1.3 mm long, orbicular-reniform, compressed, the surface rugose. Chromosome number unknown.

**Etymology.** Specific epithet refers to the geographic region of the Napali Coast valleys where this species occurs.

**Specimens examined. Hawaiian Islands. Kaua'i**: Waimea District: Olokele Valley, *Lydgate 12* (BM-BM013854574); Kopiwai, Ku'ia Valley, [22°08'9.6"N, 159°41'32.7"W], *Hobdy 200* (BISH, US); Ku'ia Natural Area Reserve, in Mahanaloa Valley, N facing slope of valley N of Milolii Ridge, above confluence with Pa'aiki Valley, [22°08'1.4"N, 159°41'48.5"W], *Lorence & Wood 7620* (BISH [2], MO, PTBG); Mahanaloa Valley, above confluence of Kuia & Mahanaloa stream, 756 m, *Wood 7430* (PTBG, US); Mahanaloa Valley, below confluence of Kuia & Mahanaloa stream, 700 m, *Tangalin & Demotta 1981* (PTBG); Mahanaloa Valley, East from Weller #2, 701 m, *Tangalin & Aguraiuja 1953* (PTBG); Ku'ia Valley, a tributary of Mahanaloa Valley, 200 ft inside Ku'ia, right side slope, [22°8'17.2"N, 159°42'3"W], *Perlman 472* (BISH, US); Makaha Valley, 823 m, *Wood et al. 15662* (PTBG), 790 m, *Wood & Perlman 17429* (PTBG);



**Figure 4.** Schiedea napaliensis **A** habit, stem with leaves **B** inflorescence **C** portion of inflorescence **D** flower in early anthesis, male stage **E** flower in later anthesis, male stage **F** flower in female stage; Reproduced from fig. 27 of Wagner et al. 2005; drawn from cultivated plant from *Perlman 12074* grown in the greenhouse of the University of California at Irvine. Illustration by Alice Tangerini.

Makaha valley, near bottom of gulch, North facing slope, 772 m, *Perlman et al. 25234* (PTBG, US); Nuololo, north facing slopes above drainage, 954 m, *Wood & Query 14517* (PTBG), *Wood et al. 15266* (BISH, PTBG, US), *Wood et al. 15568* (BISH, PTBG, US), *Wood et al. 15670.01* (PTBG). Hanalei District, Kala-lau Valley, in back of valley, native cliffs and ridges, along ridge, [22°09'7.2"N, 159°37'42.8"W], *Wood et. al. 1973* (PTBG, US).

**Cultivated specimens.** Kaua'i. Mahanaloa Valley, up valley from old horse trail, S side of valley, *Perlman & Obata 12074* [cult. *Wagner & Shannon 6805*] (BISH, PTBG, US), *Perlman & Obata 12074* [cult. 1991, *Weller & Sakai s.n.*] (US).

**Distribution.** (Fig. 3). *Schiedea napaliensis* occurs in Waimea and Hanalei districts in the Napali Coast valleys of Makaha, Nualolo, Mahanaloa, Ku'ia, Pa'aiki, and Kalalau, and formerly in the Olokele Valley in the Waimea District, in open areas of diverse mesic forest; 750–950 m.

### **Conservation status**

Only a single naturally established plant of S. *napaliensis* occurs in the wild at present. The causes for the decline of this species include browsing by introduced ungulates, erosion resulting from ungulate activity, and consumption of seedlings by introduced mollusks. Using seed collections or plants propagated in tissue culture at the Lyon Arboretum, plants representing three localities (Nualolo, Mahanaloa, and Makaha) were used in a greenhouse crossing program to produce outcrossed seeds for restoration efforts. Numerous plants have been introduced into protected areas on Kaua'i by the Plant Extinction Prevention Program, Division of Forestry and Wildlife, State of Hawai'i, and appear to be growing well. Whether these plants will produce seeds capable of establishing new generations of plants remains to be seen.

### Acknowledgements

We are grateful to Alice Tangerini for her excellent illustrations. We thank Tim Flynn (PTBG), Barb Kennedy (BISH), Kevin Faccenda (BISH), and Sandy Knapp (BM) for examining historic or type specimens to verify identification and for providing information on current holdings of these two species. We thank Matthew J. Keir (State Botanist, State of Hawaii, Division of Forestry and Wildlife) and Ken Wood (National Tropical Botanical Garden) for supporting this work, and Scott Heintzman (Plant Extinction Prevention Program) for information about populations of *S. kauaiensis* and suggesting the potential need for tax-onomic realignment of the species discussed in this study. We appreciate the review comments from Bruce Baldwin, Rich Rabeler, and John Powers that helped clarify the text and suggested corrections.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

This work was supported by National Science Foundation grants DEB-1753659, DEB-1753664, DEB-1750373 and DEB-1752785: Collaborative Research: Unlocking the evolutionary history of Schiedea (carnation family, Caryophyllaceae): Rapid radiation of an endemic plant genus in the Hawaiian Islands (PIs: A. Sakai, S. Weller, N. Wickett, M. Moore, W. Wagner).

### Author contributions

Warren L. Wagner / lead author; Stephen G. Weller / co-author and coordination of information from greenhouse cultivation and conservation status.

### **Author ORCIDs**

Warren L. Wagner D https://orcid.org/0000-0001-5012-8422

### **Data availability**

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

### References

Wagner WL, Weller SG, Sakai AK (2005) Monograph of *Schiedea* (Caryophyllaceae–Alsinoideae). Systematic Botany Monographs 72: 1–169.



**Research Article** 

### Taxonomic treatment on *Garcinia sopsopia* (Section *Brindonia*, Clusiaceae) in Thailand, with a new synonym and three lectotypifications of its synonyms

Chatchai Ngernsaengsaruay<sup>1,2®</sup>, Pichet Chanton<sup>3®</sup>, Weereesa Boonthasak<sup>4</sup>, Nittaya Mianmit<sup>5</sup>, Tharnrat Kaewgrajang<sup>6®</sup>

- 1 Department of Botany, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
- 2 Biodiversity Center, Kasetsart University (BDCKU), Chatuchak, Bangkok 10900, Thailand
- 3 Suan Luang Rama IX Foundation, Nong Bon Subdistrict, Prawet District, Bangkok, 10250, Thailand
- 4 Royal Park Rajapruek, Highland Research and Development Institute (Public Organization), Chiang Mai 50100, Thailand
- 5 Department of Forest Management, Faculty of Forestry, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand
- 6 Department of Forest Biology, Faculty of Forestry, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand

Corresponding author: Chatchai Ngernsaengsaruay (fsciccn@ku.ac.th)

### Abstract

*Garcinia sopsopia* belongs to the section *Brindonia* in the family Clusiaceae. The fruits, young shoots and leaves are edible and have a sour taste. Morphological description and illustrations are provided, along with notes on distribution, habitats and ecology, phenology, a preliminary conservation assessment, etymology, vernacular names, uses and specimens examined. *Garcinia mckeaniana* is a newly-synonymised name under *G. sopsopia*. Three synonyms of *G. sopsopia* are here lectotypified, including *G. paniculata*, *G. rhumicowa* and *G. mckeaniana*.

**Key words:** Dioecy, exudate containing canals, glandular wavy lines, Guttiferae, lectotypifications, Malpighiales, synonymisation, taxonomy

### Introduction

*Garcinia* L. is a group of evergreen trees, occasionally shrubs, which are usually dioecious, but sometimes polygamo-dioecious. It also has obligately and facultatively agamospermous species (Ngernsaengsaruay et al. 2023a). The genus comprises approximately 400 species (Gaudeul et al. 2024; POWO 2025) and is the largest genus in the Clusiaceae Lindl. (Guttiferae Juss.). It is a pantropically distributed genus and has centres of diversity located in Africa (Madagascar), Australasia and Southeast Asia (Sweeney and Rogers 2008; Gaudeul et al. 2024). In Asia, *Garcinia* is most diverse in the Malesian Region, but also spreads north into southern China, west to India and east to the Micronesian islands (Nazre et al. 2018).

In Thailand, the genus *Garcinia* was enumerated by Craib (1925), with 20 species. Gardner recorded six species in northern Thailand (Gardner et



Academic editor: Manuel Luján Received: 26 January 2025 Accepted: 10 March 2025 Published: 26 March 2025

**Citation:** Ngernsaengsaruay C, Chanton P, Boonthasak W, Mianmit N, Kaewgrajang T (2025) Taxonomic treatment on *Garcinia sopsopia* (Section *Brindonia*, Clusiaceae) in Thailand, with a new synonym and three lectotypifications of its synonyms. PhytoKeys 254: 125–141. https://doi.org/10.3897/ phytokeys.254.147960

**Copyright:** © Chatchai Ngernsaengsaruay et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

al. 2000) and 23 species (including five unidentified species) in Peninsular Thailand (Gardner et al. 2015). A taxonomic revision of Garcinia in Thailand has recently been undertaken by the first author as part of the Flora of Thailand project. Ngernsaengsaruay and Suddee (2016, 2022) described new species, G. nuntasaenii Ngerns. & Suddee from north-eastern and G. santisukiana Ngerns. & Suddee from eastern Thailand, respectively. Ngernsaengsaruay (2022) recognised three species in G. section Brindonia (Thouars) Choisy in Thailand, i.e. G. atroviridis Griff. ex T. Anderson, G. lanceifolia Roxb. and G. pedunculata Roxb. ex Buch.-Ham. Ngernsaengsaruay et al. (2022a, 2023a) published additional new species records from Peninsular Thailand, G. dumosa King and G. exigua Nazre, respectively. Ngernsaengsaruay et al. (2022b) described G. siripatanadilokii Ngerns., Meeprom, Boonthasak, Chamch. & Sinbumr. as a new species from Peninsular Thailand. Garcinia section Xanthochymus (Roxb.) Pierre was revised for Thailand, with four native species: G. dulcis (Roxb.) Kurz, G. nervosa (Miq.) Miq., G. prainiana King and G. xanthochymus Hook. f. ex T. Anderson (Ngernsaengsaruay et al. 2023b). Garcinia section Garcinia L. was treated for Thailand, with three species and one variety, i.e. two native species: G. celebica L. and G. exigua and one cultivated species: G. mangostana L. var. mangostana, including excluded and unplaced species, G. anomala Planch. & Triana (Ngernsaengsaruay et al. 2024a). Ngernsaengsaruay et al. (2024b) published an additional new species record from Peninsular Thailand, G. minutiflora Ridl. Finally, Garcinia sections Dicrananthera Pierre and Macrostigma Pierre were revised for Thailand. Three species were enumerated, i.e. one species, G. thorelii Pierre, belongs to the section Dicrananthera and two species, G. nuntasaenii and G. prainiana, are in the section Macrostigma (Ngernsaengsaruay and Chanton 2024). The sectional level taxonomy in Garcinia was recently updated by Gaudeul et al. (2024) and Sweeney and Gaudeul (2024).

Oxycarpus sopsopia Buch.-Ham. was described in 1826 (Buchanan-Hamilton 1826) and transferred to the genus Garcinia by Mabberley (1977). Mabberley (1977) selected the syntype F. Buchanan-Hamilton 1120 housed at E [E00438015] collected at Goalpara, India, "habitat in sylvis Camrupae orientalis" as the lectotype. The same author synonymised Garcinia paniculata (G. Don) Roxb. under G. sopsopia. Garcinia sopsopia belongs to the section Brindonia (Jones 1980; Gaudeul et al. 2024). Garcinia mckeaniana was described by William Grant Craib based on the syntypes, A. F. G. Kerr 3470 and A. F. G. Kerr 3504 collected in Doi Suthep, Thailand, at elevations of 1,200–1,550 m a.m.s.l. (Craib 1924).

We examined the protologues, types and general specimens of *Garcinia sopsopia* and *G. mckeaniana* and found that *G. mckeaniana* shares vegetative and reproductive characters with *G. sopsopia*. *Garcinia sopsopia* (basionym: *Oxycarpus sopsopia*) is the earliest name for the species. Therefore, *G. mckeaniana* is treated here as a new synonym of *G. sopsopia*.

In this paper, we provide a taxonomic treatment on *Garcinia sopsopia* in Thailand that includes synonymisation, lectotypifications, a detailed morphological description and illustrations, along with notes on distribution, habitats and ecology, phenology, a preliminary conservation assessment, etymology, vernacular names, uses and specimens examined.

### Materials and methods

The collected specimens were examined by consulting taxonomic literature (e.g. Anderson (1874); Kurz (1877); Pierre (1883); Vesque (1889, 1893); Engler (1893); Craib (1924); Kanjilal et al. (1934); Gagnepain (1943); Maheshwari (1964); Mabberley (1977); Jones (1980); Long (1984); Singh (1993) and by comparing with herbarium specimens housed in the following herbaria: AAU, BKF, BM, C, CMUB, K, P, QBG and those included in the virtual herbarium databases of A, GH, AAU, BM, BR, CAL, E, G, K, L, P, The Wallich Catalogue Online and MICH (from GBIF, https://www.gbif.org/). All herbarium codes follow Thiers (2024, continuously updated). All specimens cited have been seen by the authors unless stated otherwise. The taxonomic history of the species was compiled using the literature and online databases (IPNI 2025; POWO 2025). The morphological characters, distribution, habitats and ecology, phenology and uses were described from historic and newly-collected herbarium specimens and the author's observations during fieldwork. The vernacular names were compiled from the specimens examined and literature (e.g. Pooma and Suddee (2014)). Thailand floristic regions follow Flora of Thailand Vol. 4(3.3) (The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation 2023). The preliminary assessment of conservation status was performed following the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2024) combined with GeoCAT analysis (Bachman et al. 2011) and field information.

### **Results and discussion**

### **Taxonomic treatment**

**Garcinia sopsopia (Buch.-Ham.) Mabb., Taxon 26(5–6): 529. 1977.** Figs 1–3

- = Oxycarpus sopsopia Buch.-Ham., Mem. Wern. Nat. Hist. Soc. 5(2): 345. 1826.
   Type. lectotype (designated by Mabberley (1977)), India, Assam, Goalpara,
   "habitat in sylvis Camrupae orientalis", 1 Jun 1808, F. Buchanan-Hamilton 1120, E image! [E00438015]. Fig. 4A.
- = Stalagmitis paniculata G. Don, Gen. Hist. 1: 621. 1831.
- = Garcinia paniculata (G. Don) Roxb., Fl. Ind. 2: 626. 1832.
- = Garcinia bobee-cowa Choisy, Descr. Guttif. Inde: 35. 1849.
- Stalagmitis boobicowa G. Don, Gen. Hist. 1: 621. 1831, nom. nud. Type. lecto-type (designated here), India, cultivated in Calcutta Botanical Garden (H.B.C.) (originally from Sylhet, Bangladesh), ♂ fl., s.d., Wallich Cat. 4857B, G image! [G00726286]; isolectotypes: CAL image! [CAL0000065167]; isolectotype: K! [K001104077]. Fig. 4B.
- Garcinia rhumicowa Choisy, Descr. Guttif. Inde: 35. 1849. Type. lectotype (designated here), Bangladesh, Sylhet, ♂ fl., s.d., F. De Silva, Wallich Cat. 4858B, G image! [G00726295]; isolectotypes: BR image! [BR0000036486748], CAL image! [CAL0000065165], K! [K000677604, K001104080], P images! [P04701880, P04701886] (cited as "Garcinia bhumicowa Roxb." on the label, as a nom. nud.). Fig. 5A.



Figure 1. Garcinia sopsopia **A** branchlets, leaves and staminate inflorescences with flower buds and open flowers **B** staminate inflorescences with flower buds and open flowers **C**, **D** fully open staminate flowers **E** fruits **F** seed. Drawn by Wanwisa Bhuchaisri.

Garcinia mckeaniana Craib, Bull. Misc. Inform. Kew 1924(3): 84. 1924. Type. lectotype (designated here), Thailand, Chiang Mai, Doi Suthep, ♂ fl., A. F. G. Kerr 3470, K! [K000677701]; isolectotypes: BM image! [BM000611632], P! [P05061534]), syn. nov. Fig. 5B.

**Description.** *Habit* evergreen trees, dioecious, 8–20 m tall, 50–120(–150) cm gbh; exudate pale yellow, sticky; branches decussate, horizontal or nearly horizontal; young branchlets green, 4-angular to slightly 4-angular, glabrous. *Bark* brown or reddish-brown, smooth or slightly rough; inner bark red or reddish-pink. *Terminal bud* concealed between the bases of the uppermost pair



**Figure 2.** *Garcinia sopsopia* **A** habit and habitat **B** slashed bark with yellow exudate **C** branchlets and leaves **D** branchlets and leaves: upper leaf surfaces (left) and lower leaf surfaces (right). Photos: Chatchai Ngernsaengsaruay.

of petioles. *Leaves* decussate; lamina elliptic, elliptic-oblong, narrowly elliptic or oblanceolate-obovate,  $9.5-23 \times 4-10.5$  cm, apex acuminate or acute, base cuneate, sometimes obtuse, margin entire or repand, subcoriaceous, slightly bullate, dark green above, paler below, glabrous and shiny on both surfaces, midrib slightly raised (proximal part) and flattened (distal part) above, raised



Figure 3. *Garcinia sopsopia* **A** branchlets, leaves and staminate inflorescences with flower buds and open flowers **B** staminate inflorescences with flower buds and open flowers **C**, **D** fully open staminate flowers **E** branchlets, leaves and ripe fruits **F** ripe fruits **G** seeds. Photos: Chatchai Ngernsaengsaruay.

below, secondary veins 8-12 each side, 0.7-2 cm apart from each other, curving towards the margin and connected in distinct loops and united into an intramarginal vein, flattened above, raised below, intersecondary veins usually absent, tertiary veins scalariform, veinlets reticulate, visible on both surfaces, with scattered brown gland dots on both surfaces, interrupted long wavy lines (glandular wavy lines, also called exudate containing canals) present, of differing lengths, running across the secondary veins to the apex, visible on both surfaces especially on the lower surface of dry leaves; petiole green, 0.9-1.8 cm long, 1.2-4 mm diam., not grooved, glabrous, with a basal appendage clasping the branchlet; in fresh leaves, brittle when crushed; in young leaves, brownish-red, turning pale green, glossy. Inflorescences terminal, a thyrse with many to numerous flowers, 4-12 cm long, glabrous; bracts early caducous, triangular,  $1-1.8 \times 1-1.7$  mm; peduncle 1.2-2.8 cm long, 1-3 mm diam., 4-angular; rachis 3.6-8 cm long, 1-2.7 mm diam., 4-angular. *Flowers* unisexual, 4-merous; bracteoles early caducous; sepals and petals decussate, concave, glabrous. Flower buds green, subglobose to globose, 2.8-5 mm diam. Staminate flowers in a much-branched thyrse (3.5–11.5 cm wide), with decussate branches, fully open flowers 0.9-1.6 cm diam.; pedicel green, 1.7-3.8 mm long, 0.5-2 mm diam., 4-angular; sepals 4, green, thinly coriaceous; outer sepals broadly ovate or ovate, 1.8-3 × 1-2 mm, apex rounded; inner sepals broadly elliptic, elliptic or suborbicular, 2-3.2 × 1.3-2.3 mm, apex rounded; the outer pair slightly smaller than the inner pair; petals 4, pale yellow to yellow, slightly thick and fleshy, oblong, 3-5.8 × 2.6-4.7 mm, subequal (thicker and longer than sepals), apex rounded, gradually reflexed after anthesis; stamens numerous, united into a single central 4-sided or weakly 4-lobed bundle surrounding a pistillode, bundle 3-4 × 2.6-3.8 mm; filaments very short; anthers 4-thecous, small, longitudinally dehiscent; pistillode creamish-white, mushroom-shaped, 1.3-2.7 mm long; rudimentary ovary small; sterile stigma, sessile, slightly convex, radiate, shallowly 5-7-lobed, 0.5-1 mm diam., papillate. Pistillate flowers in a shortbranched thyrse, fully open flowers same as or slightly larger than staminate flowers; pedicel green, short and thick (slightly shorter and thicker than in staminate flowers), 4-angular; sepals and petals same as or slightly larger than in staminate flowers; staminodes absent; pistil mushroom-shaped, ovary globose or subglobose, 2-3 mm diam., glabrous, 5-7-locular; stigma sessile, convex, radiate, shallowly 5-7-lobed, papillate. Fruits berries, green, turning bright yellow, when ripe, glabrous and glaucous, cut fruits with a sticky yellow exudate, globose or subglobose, 4.5-7 × 4.3-6.3 cm, sometimes oblique, asymmetrical, without or with a short, thick beak and concave at the apex, with 6-8 longitudinal sutures, pericarp fleshy, 0.7-1.2 cm thick; persistent stigma dark brown or blackish-brown, 2.5-4 mm diam., indistinctly lobed, papillate; persistent sepals 2-4.5 × 3-5.7 mm, larger than in flowering material; fruiting stalk short and thick, 3–4.5 mm long, 5–7 mm diam. Seeds 3–7, sometimes aborted (1–2), dark brown mottled with paler irregular lines, semi-ellipsoid, 1.8-3 × 0.8-1.2 cm, rounded at both ends, with a yellow fleshy pulp.

**Distribution.** India (Assam, Meghalaya), Nepal, Bhutan, Bangladesh, Myanmar, Vietnam, Laos, Thailand. The distribution record of *Garcinia sopsopia* was published without coordinates, but it includes a textual description of its location (Fig. 6).

**Distribution in Thailand. Northern:** Chiang Mai, Nan, Phrae, Uttaradit, Tak, Kamphaeng Phet; **North-eastern:** Loei. Fig. 6.



**Figure 4.** Lectotype of *Garcinia sopsopia* and isolectotype of *Garcinia paniculata* **A** *Garcinia sopsopia*, *F. Buchanan-Hamil-ton 1120* (E00438015) from Goalpara, "habitat in sylvis Camrupae orientalis", Assam, India, lectotype selected by Mabberley (1977) **B** *Garcinia paniculata*, a synonym of *Garcinia sopsopia*, *Wallich Cat. 4857B* (K001104077) cultivated in Calcutta Botanical Garden (H.B.C.), India (originally from Sylhet, Bangladesh), isolectotype selected here. Photos: © 2018 Royal Botanic Garden Edinburgh, https://data.rbge.org.uk/herb/E00438015 (**A**), © The Board of Trustees of the RBG, Kew (**B**).

**Habitat and ecology.** This species is found in lower montane rain forests, lower montane pine-oak forests and dry evergreen forests, sometimes along streams, at elevations of 500–1,550 m a.m.s.l.

Phenology. Flowering in September to January; fruiting in March to June (August).

**Conservation status.** *Garcinia sopsopia* is widely distributed from India, Nepal to Myanmar and Vietnam. It is known from many localities and has a large EOO of 1,779,647.52 km<sup>2</sup> and an AOO of 128 km<sup>2</sup>. In Thailand, this species is known to be naturally distributed in the northern and the north-eastern regions and has an EOO 79,178.24 km<sup>2</sup> and an AOO of 56 km<sup>2</sup>. Therefore, we consider the conservation assessment here as Least Concern (LC).

**Etymology.** The specific epithet of *Garcinia sopsopia* is derived from "Sopsopiya Bengalensium" (Buchanan-Hamilton 1826; Mabberley 1977). The specific epithet of *G. paniculata* is a Latin word meaning with a branched-racemose or cymose inflorescence (Gledhill 2002). However, from our examination of specimens, the staminate inflorescence of this species is a terminal, many-branched thyrses and the pistillate inflorescence is a terminal, short-branched thyrses. The specific epithet of *G. mckeaniana* honors James W. McKean, MD (1860–1949). He was an American doctor and missionary who pioneered leprosy work in Thailand, including the construction of the Chiang Mai Leprosarium in 1908.



**Figure 5.** Isolectotype of *Garcinia rhumicowa* and lectotype of *Garcinia mckeaniana* **A** *Garcinia rhumicowa*, a synonym of *Garcinia sopsopia*, *F. De Silva*, *Wallich Cat. 4858B* (K001104080) from Sylhet, Bangladesh (cited as "Garcinia bhumicowa Roxb." on the label, as a nom. nud.), isolectotype selected here **B** *Garcinia mckeaniana*, a new synonym of *Garcinia sopsopia*, *A. F. G. Kerr 3470* (K000677701) from Doi Suthep, Chiang Mai Province, Thailand, lectotype selected here. Photos: © The Board of Trustees of the RBG, Kew.

He began his work in Chiang Mai in 1889 and remained there for his entire missionary life, carrying out general medical and evangelical work (https://leprosyhistory.org/database/person99).

**Vernacular names. Ma da** (ມະດະ) (Uttaradit, from the specimen *P. Kanchanapan 30*); Boobee-Kowa, Bubi Kowa (India); Sochopa-tenga, Sosopatenga (Assam); Sopsopia garcinia, Sopsop garcinia (English).

**Uses.** The fruits (pericarp and fleshy pulp surrounding the seeds), young shoots and leaves are edible and have a sour taste. In India, it is often cultivated for its edible fruits and the leaves are also said to be edible (Jones 1980; Singh 1993). In Assam, the ripe fruits are eaten raw (Baruah et al. 2021), the fleshy pulp being used for making refreshing drinks (Brahma et al. 2022). The leaves are used to treat roundworm (Baruah et al. 2021). Moderately hard wood is used for house construction and firewood (Baruah et al. 2021). In Vietnam, five xanthones were isolated and identified from *Garcinia sopsopia* for the first time. Garcinone E and bannanxanthone E displayed a significant inhibitory effect against the growth of bacterium *Staphylococcus aureus* (Nguyen et al. 2020).



**Figure 6.** Distribution of *Garcinia sopsopia*. It is known from India (Assam, Meghalaya), Nepal to Myanmar and Vietnam. In Thailand, this species is known to be naturally distributed in the northern and the north-eastern regions. Photo: Pichet Chanton and Chatchai Ngernsaengsaruay.

**Lectotypifications.** *Garcinia paniculata* was named by William Roxburgh, found in a few gardens about Calcutta, was originally from Sylhet ("Silhet" or "Sillet"), where the species is indigenous and known to the natives by the name Boobee-Kowa (Roxburgh 1832). He did not choose a holotype nor did he mention the collector number and the name of the herbaria where the specimen was housed. He also did not provide a description or diagnosis; later, the name *G. paniculata* was validly described by Choisy (1849). The name *G. paniculata* has been lectotypified in a first-step by Maheshwari (1964), who cited "Type: ex Sylhet, E. Pakistan, cult. in Indian Botanic Garden, Calcutta", without citing a specimen or herbarium and in a second-step by Mohanan et al. (2023) using the specimen *Wallich 4857*, noted locality as "HBC (Calcutta Herbarium)" at CAL [CAL0000065167], with an isolectotype at K [K001104077]. However, we located the specimen *Wallich Cat*.

4857 representing three different materials, which are distinguished by 4857A, 4857B and 4857C (in The Wallich Catalogue). The specimen *Wallich Cat.* 4857A (K [K001104076]) is from Calcutta Botanical Garden (H.B.C.), Buchanan-Hamilton's Herbarium; *Wallich Cat.* 4857B (CAL [CAL0000065167], G [G00726286], K [K001104077]) is from H.B.C.; and *Wallich Cat.* 4857C (BR [BR0000036486724], G [G00726273] and K [K001104078]) is from Sylhet ("Silhet" or "Sillet") and, following Art. 9.6 of the ICN (Turland et al. 2018), these are syntypes. We think Maheshwari's and Mohanan et al.'s typifications are mistaken. Since the name *G. paniculata* was validly described by Choisy (1849), a Swiss botanist working in Geneve, it is more plausible that he analysed material from G. Therefore, the specimen *Wallich Cat.* 4857B at G [G00726286], is selected here as the lectotype, with isolectotypes at CAL [CAL0000065167] and K [K001104077], following Art. 9.3 and 9.12 of the ICN (Turland et al. 2018).

*Garcinia rhumicowa* was named by Jacques Denys Choisy, based on the specimen *Wallich Cat. 4858* collected from Calcutta Botanical Garden (H.B.C.) and Sylhet ("Sillet") (Choisy 1849). We located the specimen *Wallich Cat. 4858* which represents two different materials collected from two different localities and which are distinguished by *4858A* and *4858B* (in The Wallich Catalogue). The specimen *Wallich Cat. 4858A* (CAL [CAL0000065164, CAL0000065168], K [K001104079]) is from Calcutta Botanical Garden and *Wallich Cat. 4858B* (BR [BR0000036486748], CAL [CAL0000065165], G [G00726295], K [K000677604, K001104080], P [P04701880, P04701886]) is from Sylhet and, following Art. 9.6 of the ICN (Turland et al. 2018), these are syntypes. It is more plausible that Jacques Denys Choisy (1799–1859), a Swiss botanist working in Geneve, analysed material from G. The specimen *Wallich Cat. 4858B* at G [G00726295] should be considered as a lectotype, with isolectotypes at BR [BR0000036486748], CAL [CAL00000677604, K001104080] and P [P04701880, P04701886], following Art. 9.3 and 9.12 of the ICN (Turland et al. 2018).

*Garcinia mckeaniana* was described by William Grant Craib, who cited two gatherings, *A. F. G. Kerr* 3470 and *A. F. G. Kerr* 3504 collected from Doi Suthep, at elevations of 1,200–1,550 m a.m.s.l. (Craib 1924). He did not mention the name of the herbaria where the materials were housed and, following Art. 9.6 of the ICN (Turland et al. 2018), these are syntypes. We located the materials *A. F. G. Kerr* 3470 (1,200 m a.m.s.l.) at BM [BM000611632], K [K000677701] and P [P05061534] and *A. F. G. Kerr* 3504 (1,550 m a.m.s.l.) at BM [BM000611633] and K [K000677702]. The material *A. F. G. Kerr* 3470 at K [K000677701] is better preserved and more complete than the others and is designated here as the lectotype, with isolectotypes at BM [BM000611632] and P [P05061534], following Arts. 9.3 and 9.12 of the ICN (Turland et al. 2018).

**Notes.** According to previous studies (e.g. Anderson (1874); Kurz (1877); Craib (1924); Gagnepain (1943); Maheshwari (1964); Mabberley (1977); Jones (1980); Long (1984); Singh (1993)) and based on the specimens that we examined, *Garcinia mckeaniana* and *G. sopsopia* are similar and the vegetative and reproductive characters are overlapping between the two taxa. Therefore, *G. mckeaniana* is not morphologically distinguishable from *G. sopsopia* and is treated here as a new synonym.

*Garcinia sopsopia* is recognised by its staminate flowers in terminal, much-branched thyrses with many to numerous flowers; pistillate flowers in terminal, short-branched thyrses (raceme-like), fewer in number of flowers than staminate; tetramerous flowers; numerous stamens (in staminate flowers) united into a single central 4-sided or weakly 4-lobed bundle surrounding a pistillode; the leaves with scalariform tertiary veins; and characters of fruits.

A comparison of morphological characters of *Garcinia sopsopia* in Thailand with previous studies is summarised in Table 1.

Additional specimens examined. THAILAND. Northern. · Chiang Mai [Doi Suthep, ♂ fl., 2 Jan 1915, A. F. G. Kerr 3504 (BM [BM000611633], K [K000677702]); • ibid., fl., 6 Oct 1958 (as Garcinia sp.), T. Sorensen et al. 5492 (C); • ibid., ♂ fl., 26 Dec 1987 (as G. mckeaniana), J. F. Maxwell 87-1648 (L [L2416545]); • Doi Suthep-Pui National Park, fr., 19 Apr 1991 (as G. mckeaniana), J. F. Maxwell 91-361 (AAU, A [GH00429134], P [P05061535]); • Huai Khok Ma, Doi Suthep-Pui National Park, fr., 8 Jun 1995 (as G. mckeaniana), S. Kopachon S128 (CMUB); • ibid., 18 Jun 2003 (as G. mckeaniana), J. F. Maxwell et al. 4 (CMUB); • Doi Suthep-Pui National Park, between Doi Suthep Temple and Chang Khian Valley, ♂ fl., 9 Oct 1997 (as G. aff. propinqua), P. Sidisunthorn & S. Gardner 2371 (CMUB); • near Wat Phra That Doi Suthep, Doi Suthep-Pui National Park, ∂ fl., 29 Sep 2013 (as G. mckeaniana), Tong Lau 1 (CMUB); Khun Chang Khian, Mueang District, 👌 fl., 29 Oct 1994 (as Garcinia sp.), BGO. Staff 2456 (QBG); • ibid., 3 fl., 29 Oct 1994 (as Garcinia sp.), W. Nanakorn et al. 2479 (BGO. Staff 2479) (AAU, QBG); Doi Angka, Mae Ka Pak drainage, 🖒 fl., 18 Nov 1930 (as G. mckeaniana), H. B. G. Garrett 607 (BKF, C, K, L [L2416546]); • Doi Inthanon, fr., 21 Mar 1996 (as Garcinia sp.), BGO. Staff 6204 (QBG); • Huai Sai Lueang Waterfall, Doi Inthanon, fr., 22 Mar 2002 (as Garcinia sp.), T. Wongprasert et al. 023-37 (BKF); • Doi Inthanon, Mae Chaem Distritct, along stream, near Huai Sai Lueang Waterfall, at an elevation of 1,060 m a.m.s.l., fr., 24 May 2023, C. Ngernsaengsaruay & T. Kaewgrajang G57-24022023 (BKF)]; • Nan [Hue Wao, fr., 10 March 1921 (as G. mckeaniana), A. F. G. Kerr 5065 (BKF, BM, K); • Doi Phu Kha National Park, 👌 fl., 13 Jan 2000, P. Srisanga 1275 [AAU & QBG (as Garcinia sp.), BKF & CMUB (as G. pedunculata)]; • ibid., fr., 27 May 2000 (as G. pedunculata), P. Srisanga 1481 (QBG); •Tham Sakoen National Park, Yot Subdistrict, Song Khwae District, fl., 16 Dec 2010 (as G. pedunculata), W. La-ongsri & N. Romkham 1282 (QBG)]; • Phrae [Mae Kray, 3 fl., 10 Jan 1972 (as G. mckeaniana), C. F. van Beusekom et al. 4788 (BKF, C, K, P [P05062052])]; • Uttaradit [Khao Phlueng, fl., 20 Dec 1943 (as G. mckeaniana), P. Kanchanapan 30 (BKF)]; • Tak [Ler Tor Royal Project Area, Mae Ramat District, at an elevation of 1,250 m a.m.s.l., A fl., 14 Dec 2024, C. Ngernsaengsaruay et al. G58-14122024 (BKF)]; • Kamphaeng Phet [Khlong Lan, Mae Wong National Park, 3 fl., 10 Oct 1999 (as G. plena), M. van de Bult 380 (CMUB)]; North-eastern. · Loei [Lone Tae, Phu Luang Wildlife Sanctuary, fr., 17 May 1998 (as Garcinia sp.), T. Wongprasert s.n. (BKF124471); • ibid., fr., Aug 1998 (as Garcinia sp.), T. Wongprasert s.n. (BKF126762)].

INDIA. • Cultivated in Calcutta Botanical Garden (H.B.C.), ♂ fl., 31 Dec 1814 (as *G. paniculata*), *Wall. Cat.* 4857A (Buchanan-Hamilton's Herbarium) (K-W [K001104076]); • ibid., ♂ fl., s.d. (as *G. bhumicowa*), *Wallich Cat.* 4858A (CAL [CAL0000065164, CAL0000065168], K-W [K001104079]); • ibid., fl., s.d. (as *G. bhumicowa*), *Unknown s.n.* (E [E00839542]); ibid., fl., Dec 1814 (as *G. paniculata*), *F. Buchanan-Hamilton* 1022 (E [E00839543]); • ibid., ♂ fl., s.d. (as *G. paniculata*), *Unknown s.n.* (K [K000677603], L [L2417597], P [P04701882]); • Assam, ♂ fl., 1863 (as *G. paniculata*), *C. Jenkins* (*Herb. L. Pierre* 4578) (P [P04701879, P04701887]); • ibid., s.d. (as *G. paniculata*), *C. Jenkins s.n.* (P [P04701884, P04701885]); • ibid., ♂ fl., 1865 (as *G. paniculata*), *C. Jenkins s.n.* (G [G00726260]); • Meghalaya, (East)

Characters	In this study	Axillary (Wight 1838)					
Position of staminate inflorescences	Terminal in agreement with Craib (1924), Kanjilal et al. (1934), Gagnepain (1943), Maheshwari (1964), Jones (1980) and Long (1984)						
Pistillate flowers In a short-branched thyrse and the number pistillate flowers in each inflorescence is for than staminate flowers in agreement with J (1980)		In a spike (Roxburgh 1832; Brandis 1906), in a short few-flowered, spike-like raceme (spicate raceme), rarely branched (Kurz 1877; Kanjilal et al. 1934; Maheshwari 1964; Singh 1993) or in a raceme (Long 1984)					
Colour of flowers Yellow in staminate and pistillate flowers		Pure or dull white (Anderson 1874; Brandis 1906; Maheshwari 1964; Singh 1993) or white in staminate flowers and yellow in pistillate flowers (Jones 1980)					
Pistillode	Present in agreement with Gagnepain (1943)	Absent (Jones 1980; Singh 1993)					
Number of seeds per fruit	3-7	3–5 (Maheshwari 1964; Singh 1993) or 4 (Anderson 1874; Kurz 1877; Kanjilal et al. 1934)					

Table 1. A con	nparison of mo	phological c	haracters of	Garcinia sopsopia ir	n Thailand with	previous studies.
----------------	----------------	--------------	--------------	----------------------	-----------------	-------------------

Khasi Hills, Cherrapunjee,  $\bigcirc$  fl., 24 Jul 1952 (as *G. paniculata*), *W. N. Koelz* 30814 (L [L2417594], MICH [1507203]); • Khasia, Regio trop., young fr., 4 Dec 1850 (as *G. paniculata*), *J. D. Hooker & T. Thomson s.n.* (K [K003668822]); • ibid., young fr., s.d. (as *G. paniculata*), *J. D. Hooker & T. Thomson s.n.* (G [G00726242], L [L2417595, L2417596], P [P04701883]); • Khasia, Regio trop, Churra, young fr., 16 Jun 1850 (as *G. paniculata*), *J. D. Hooker & T. Thomson 943* (K [K003668814]); East India,  $\bigcirc$  fl., s.d. (as *G. paniculata*), *W. Roxburgh s.n.* (BM [BM000611602], K [K000677602]); • Garo Hills, Tura Mountain, fl., s.d. (as *G. paniculata*), *N. E. Parry 881* (K [K003668815]).

**NEPAL.** • Locality unspecified, fl., s.d. (as *G. paniculata*), *N. Wallich s.n.* (CAL [CAL0000065163]).

**BHUTAN.** • Sarbhang District, Burborte Khola near Phipsoo, young fr., 18 Mar 1982, A. J. C. Grierson & D. G. Long 3845 (E [E00170196], K [K001331949]); • Gaylegphug District, Lodrai Khola near Gaylegphug, 21 Mar 1982, A. J. C. Grierson & D. G. Long 3887 (E [E00170197], K [K003668996]).

BANGLADESH. • Sylhet, ♂ fl., s.d. (as G. paniculata), Wall. Cat. 4857C (BR [BR0000036486724], K-W [K001104078]); Chittagong, fl., 1874 (as G. paniculata), W. Schleich s.n. (K [K003668821]); • Chittagong Hill Tracts, fr., Mar 1880 (as G. paniculata), J. S. Gamble 7800 (K [K003668823]); • Ponasari, Kelatuli, 2 Sep 1944 (as G. paniculata), J. Sinclair 3717 (E [E00839545]); • Cultivated in East Bengal, fl., s.d. (as G. paniculata), Herbarium of the late East India Company 852 (K [K003668818], P [P04701881]).

MYANMAR. • Mon State, Amherst [Kyaikkhami] District, Dawna Range, Ta-Ok Plateau, fr., 23 Mar 1909 (as *G. cowa*), *J. H. Lace* 4754 (E [E00839544]); • Kachin State, Myitkyina District, Nammina to Namma, fr., 7 Mar 1910 (as *G. paniculata*), *J. H. Lace* 5172 (E [E00839546]); • Sandoway District, Arakan Yoma, fl., 17 Jan 1931 (as *G. cowa*), *Bals* 11938 (K [K003668816]); • Locality unspecified, Feb 1872 (as *G. cowa*), Presented by the Council of King's College s.n. (K [K003668817]).

**VIETNAM.** • Tonkin, O. de Chapa, Quan Hóa District, Xinh mun, fl., 14 Aug 1926 (as G. *mckeaniana*), *M. E. Poilane 12929* (K, P [P05061533]).

LAOS. • Xieng Khuang, fl., 18 Nov 1920 (as *G. mckeaniana*), *M. Poilane 2330* (K, P [P04899369]); • Khammouan, Nam Theun, Kaeng Luang, fl., 3 Nov 2005 (as *Garcinia* sp.), *M. F. Newman et al. LAO836* (BKF, L [L2409472, L2409473], P [P04897552]); • Khammouan, fl., 4 Nov 2005 (as *Garcinia* sp.), *M. F. Newman et al. LAO855* (BKF165806, BKF165964, BKF168376, L [L2409466, P04897550].

### Acknowledgements

We would like to thank the curators and staff of the following herbaria AAU, BKF, BM, C, CMUB, K, P and QBG for their assistance during visits and allowing access to the herbarium specimens and those included in the virtual herbarium databases of Harvard University Herbaria (A, GH), AAU, BM, BR, CAL, E, G, K, L, P, The Wallich Catalogue Online and MICH (from GBIF). We are grateful to the plant collectors of *Garcinia sopsopia*. We also would like to thank Wanwisa Bhuchaisri for the line drawings, Nopadol Somsri (Head), Audom Jadsarikit, Banthat Nawasaichon and Somphol Fasangklangdong, staff of Forest Development Project, Royal Forest Department under Lerto Royal Project Development Center for their facilitation and kind help in the field-work.

### **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

This research was funded by the Princess Sirindhorn Center for Sustainable Development, Kasetsart University and the International SciKU Branding (ISB), Faculty of Science, Kasetsart University.

### **Author contributions**

Conceptualisation: CN. Data curation: CN, PC. Formal analysis: CN. Funding acquisition: CN, NM. Investigation: CN, NM, TK. Methodology: CN, PC, WB. Project administration: CN. Resources: CN, PC. Writing – original draft: CN, PC. Writing – review and editing: CN, PC, WB, NM, TK.

### **Author ORCIDs**

Chatchai Ngernsaengsaruay <sup>©</sup> https://orcid.org/0000-0002-7131-976X Pichet Chanton <sup>©</sup> https://orcid.org/0009-0001-7325-6109 Tharnrat Kaewgrajang <sup>©</sup> https://orcid.org/0000-0001-7736-3596

### Data availability

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

### References

- Anderson T (1874) Guttiferae. In: Hooker JD (Ed.) The Flora of British India Vol 1 (2). L. Reeve & Co. Ltd., London, 258–278.
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity Science. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

- Baruah S, Barman P, Basumatary S, Bhuyan B (2021) Diversity and ethnobotany of genus *Garcinia* L. (Clusiaceae) in Assam, Eastern Himalaya. Ethnobotany Research and Applications 21(33): 1–14. https://doi.org/10.32859/era.21.33.1-14
- Brahma J, Islary A, Ray S (2022) Traditional importance and unique properties of *Garcinia* spp. found in Assam, India. The Pharma Innovation Journal SP-11(3): 10–17.
- Brandis D (1906) Guttiferae. Indian Trees: An Account of Trees, Shrubs, Woody Climbers, Bamboos and Palms Indigenous or Commonly Cultivated in the British Indian Empire, 1<sup>st</sup> edn. Archibald Constable & Co. Ltd., London, 48–56. https://doi.org/10.5962/bhl. title.50463
- Buchanan-Hamilton F (1826) Commentary on the Herbarium Amboinense. Memoirs of the Wernerian Natural History Society 5(2): 307–383.
- Choisy JD (1849) Description des Guttiferes de l'Inde, recueillies par le Dr. Wallich et de quelques Guttiferes peu connus d'Amerique precedee d'observations generales sur cette famille. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 12(2): 381–440.
- Craib WG (1924) Contributions to the Flora of Siam. Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew 1924(3): 81–98. https://doi.org/10.2307/4111779
- Craib WG (1925) Florae Siamensis Enumeratio: A List of the Plants Known from Siam with Records of Their Occurrence Vol 1(1). Polypetalae. Bangkok Times Press, Bangkok, 198 pp.
- Engler A (1893) Guttiferae. In: Engler HGA, Prantl KAE (Eds) Die Natürlichen Pflanzenfamilien. Verlag von Wilhelm Engelmann, Leipzig 3(6): 194–242
- Gagnepain F (1943) Guttiféres. In: Humbert H, Gagnepain F (Eds) Supplément a la Flore Générale de L'Indo-Chine Vol 1(3). Muséum National D'Histoire Naturelle, Phanérogamie, Paris, 254–277.
- Gardner S, Sidisunthorn P, Anusarnsunthorn V (2000) A Field Guide to Forest Trees of Northern Thailand. Kobfai Publishing Project, Bangkok, 545 pp.
- Gardner S, Sidisunthorn P, Chayamarit K (2015) Forest Trees of Southern Thailand Vol 1 (A–Es). Kobfai Publishing Project, Bangkok, 748 pp.
- Gaudeul M, Sweeney P, Munzinger J (2024) An updated infrageneric classification of the pantropical species-rich genus *Garcinia* L. (Clusiaceae) and some insights into the systematics of New Caledonian species, based on molecular and morphological evidence. PhytoKeys 239: 73–105. https://doi.org/10.3897/phytokeys.239.112563
- Gledhill D (2002) The Names of Plants, 3<sup>rd</sup> edn. Cambridge University Press, UK, 326 pp. https://doi.org/10.1017/CB09780511754951
- Ha NTT, Cuong PV, Anh LTT, Tra NT, Cham BT, Son NT (2020) Antimicrobacterial xanthones from *Garcinia mackeaniana* leaves. Vietnam Journal of Chemistry 58(3): 343–348. https://doi.org/10.1002/vjch.2019000183
- IPNI (2025) International plant names index. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. http://www.ipni.org
- IUCN Standards and Petitions Committee (2024) Guidelines for Using the IUCN Red List Categories and Criteria Version 6. Prepared by Standards and Petitions Committee. https://www.iucnredlist.org/resources/redlistguidelines
- Jones SW (1980) Morphology and Major Taxonomy of *Garcinia* (Guttiferae). Ph.D. Thesis, University of Leicester and British Museum (Natural History), London, 474 pp.
- Kanjilal UN, Kanjilal PC, Das A (1934) Guttiferae. Flora of Assam Vol 1. Prabasi Press, Calcutta, 103–115.

- Kurz S (1877) Guttiferae. Forest Flora of British Burma Vol 1. The Superintendent of Government Printing, Calcutta, 86–96. https://doi.org/10.5962/bhl.title.52413
- Long DG (1984) Guttiferae. In: Grierson AJC, Long DG (Eds) Flora of Bhutan Vol 1(2). Royal Botanic Garden, Edinburgh, 366–371.
- Mabberley DJ (1977) Francis Hamilton's commentaries with particular reference to Meliaceae. Taxon 26(5–6): 523–540. https://doi.org/10.1002/j.1996-8175.1977. tb03833.x
- Maheshwari JK (1964) Taxonomic Studies on Indian Guttiferae III. The Genus *Garcinia* L. s.l. Bulletin of the Botanical Survey of India 6: 107–135. https://doi.org/10.20324/ nelumbo/v6/1964/76375
- Mohanan N, Shameer PS, Sabu T (2023) Taxonomic revision of *Garcinia* (Clusiaceae) in India. Rheedea 33(3): 101–158. https://doi.org/10.22244/rheedea.2023.33.03.01
- Nazre M, Newman MF, Pennington RT, Middleton DJ (2018) Taxonomic revision of *Garcin-ia* section *Garcinia* (Clusiaceae). Phytotaxa 373(1): 1–52. https://doi.org/10.11646/phytotaxa.373.1.1
- Ngernsaengsaruay C (2022) Lectotypifications of three names in *Garcinia*, synonymy of *Garcinia pedunculata* and detailed descriptions of three species in *Garcinia* section *Brindonia* (Clusiaceae). Diversity 14(7): 1–29. https://doi.org/10.3390/d14070556
- Ngernsaengsaruay C, Chanton P (2024) A taxonomic revision of *Garcinia* sections *Dicrananthera* and *Macrostigma* (Clusiaceae) in Thailand. PhytoKeys 248: 339–360. https://doi.org/10.3897/phytokeys.248.130311
- Ngernsaengsaruay C, Suddee S (2016) *Garcinia nuntasaenii* (Clusiaceae), a new species from Thailand. Thai Forest Bulletin (Botany) 44(2): 134–139. https://doi.org/10.20531/TFB.2016.44.2.09
- Ngernsaengsaruay C, Suddee S (2022) *Garcinia santisukiana* (Clusiaceae), a new species from Thailand. Kew Bulletin 77(2): 121–125. https://doi.org/10.1007/s12225-021-09979-8
- Ngernsaengsaruay C, Duangnamon D, Boonthasak W (2022a) *Garcinia dumosa* (Clusiaceae), a new record for Thailand, with associated lectotypifications. Thai Forest Bulletin (Botany) 50(1): 66–74. https://doi.org/10.20531/tfb.2022.50.1.07
- Ngernsaengsaruay C, Meeprom N, Boonthasak W, Chamchumroon V, Sinbumroong A, Wessapak P, Duangjai S (2022b) *Garcinia siripatanadilokii* (Clusiaceae), a new species from Peninsular Thailand. Kew Bulletin 77(4): 905–913. https://doi.org/10.1007/ s12225-022-10059-8
- Ngernsaengsaruay C, Boonthasak W, Meeprom N, Tetsana N, Suddee S (2023a) *Garcinia exigua* (Clusiaceae), a new record for Thailand. Thai Forest Bulletin (Botany) 51(1): 36–44. https://doi.org/10.20531/tfb.2023.51.1.05
- Ngernsaengsaruay C, Chanton P, Chaiprasongsuk M, Leksungnoen N (2023b) A taxonomic revision of *Garcinia* section *Xanthochymus* (Clusiaceae) in Thailand. PeerJ 11(e16572): 1–54. https://doi.org/10.7717/peerj.16572
- Ngernsaengsaruay C, Chanton P, Leksungnoen N, Chaiprasongsuk M, Thunthawanich R (2024a) A taxonomic revision of *Garcinia* section *Garcinia* (Clusiaceae) in Thailand. PhytoKeys 244: 175–211. https://doi.org/10.3897/phytokeys.244.126207
- Ngernsaengsaruay C, Tetsana N, Suddee S, Boonthasak W, Meeprom N (2024b) *Garcinia minutiflora* (Clusiaceae), a new record from Peninsular Thailand, with lecto-typification. Thai Forest Bulletin (Botany) 52(2): 72–79. https://doi.org/10.20531/tfb.2024.52.2.10
- Pierre JBL (1883) Flore Forestiére de la Cochinchine Vol 1(5, 6). Octave Doin, Éditeur, Paris, t. 65–83, 84–96.

- Pooma R, Suddee S (Eds) (2014) Tem Smitinand's Thai Plant Names, Revised Edition 2014. Office of the Forest Herbarium, Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Bangkok, 806 pp.
- POWO (2025) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/
- Roxburgh W (1832) Polyandria Monogynia. In: Carey W (Ed.) Flora Indica or Descriptions of Indian Plants Vol 2. W. Thacker & Co., Calcuttta, India; Parbury, Singapore; Allen & Co., London, 521–644. https://doi.org/10.5962/bhl.title.6633
- Singh NP (1993) Clusiaceae (Guttiferae nom. alt.). In: Sharma BD, Sanjappa S (Eds) Flora of India Vol 3. Deep Printers, Ramesh Nagar, New Delhi, 86–151.
- Sweeney PW, Gaudeul M (2024) Revisiting the infrageneric classification of *Garcinia* L. (Clusiaceae): an updated sectional name and new, legitimate names in *Garcinia* for *Allanblackia gabonensis* (Pellegr.) Bamps and *A. parviflora* A. Chev. PhytoKeys 243: 63–66. https://doi.org/10.3897/phytokeys.243.124751
- Sweeney PW, Rogers ZS (2008) Nomenclatural notes on *Garcinia* (Clusiaceae) from Madagascar and the Comoros. Novon, A Journal for Botanical Nomenclature 18(4): 524–537. https://doi.org/10.3417/2006146
- The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation (2023) Floristic Regions and Provinces of Thailand. Flora of Thailand Vol 4(3.3). Prachachon Co. Ltd., Bangkok, 715 pp.
- Thiers B (2024, continuously updated) Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code) Adopted by the Nineteenth International Botanical Congress Shenzhen, China, 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. https://doi.org/10.12705/Code.2018
- Vesque J (1889) Epharmosis sive materiae ad instruendam anatomiam systematis naturalis 2 (Gentalia foliaque Garciniearum et Calophyllearum). Vincennes, Delapierre, 1–29. [pl. 1–162]. https://doi.org/10.5962/bhl.title.828
- Vesque J (1893) Guttiferae. In: de Candolle ALPP, de Candolle ACP (Eds) Monographiae Phanerogamarum, Prodromi Nunc Continuatio, Nunc Revisio Vol 8. Sumptibus G. Masson, Paris, 1–669.
- Wight R (1838) Illustrations of Indian Botany; or Figures Illustrative of Each of the Natural Orders of Indian Plants Vol 1(1–8). Published by J.B. Pharoah, Madras, 1–130.

## PhytoKeys

**Research Article** 

# *Camellia shuangbaiensis* (Theaceae), a new species from Yunnan, China

Zi-Yuan Li<sup>1\*</sup>, Bao-Huan Wu<sup>2,3,4\*</sup>, Shang Qu<sup>5</sup>, Le-Shan Du<sup>1</sup>, Hai-Ou Liu<sup>1</sup>, He-Xiang Duan<sup>6</sup>, Fei-Fei Li<sup>7,8</sup>, Wen-Hui Liu<sup>1</sup>

- 1 Institute of Ecology, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
- 2 Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou 510405, China
- 3 Guangzhou Horticultural Plant Germplasm Resource Nursery, Guangzhou 510405, China
- 4 Guangzhou Collaborative Innovation Center on Science-tech of Ecology and Landscape, Guangzhou 510405, China
- 5 Beijing Adonis Environmental Protection Technology Co., LTD, Beijing 100035, China
- 6 Yunnan Research Academy of Eco-environmental Sciences, Kunming 650034, China
- 7 Beijing Botanical Garden, Beijing, 100093, China
- 8 Key Laboratory of National Forestry and Grassland Administration on Plant Ex situ Conservation, Beijing, 100093, China

Corresponding authors: Fei-Fei Li (lifeifei30761@126.com); Wen-Hui Liu (wenhui211@126.com)

#### Abstract

A new species of the genus *Camellia* (Theaceae), *Camellia shuangbaiensis* G.P.Yang & B.H.Wu, **sp. nov.**, from the central region of Yunnan Province of China is described. *Camellia shuangbaiensis* is morphologically similar to *C. mileensis* and *C. hongkongensis*, but it can be distinguished by its smaller leaves with an ovate, abaxially tomentose lamina, and 14–16 bracteoles and sepals.

Key words: Camellia, Flora, new taxon, Shuangbai County, taxonomy

### Introduction

*Camellia* Linnaeus (1753: 698) (Theaceae) is a genus of evergreen trees or shrubs predominantly distributed across the tropical and subtropical regions of Asia (Chang and Ren 1998; Ming and Bartholomew 2007). Renowned for their ornamental value and economic importance, particularly in tea production, *Camellia* species have been cultivated for a long history in China, and China harbors the richest diversity of *Camellia*, with over 80% of the recorded species within its region (Chang and Ren 1998; Ming and Bartholomew 2007). From 2020 to 2025, there were 8 new *Camellia* species described from China (Liu et al. 2020a, 2020b; Xu et al. 2020; Yu et al. 2021; Ye et al. 2022; Zhang et al. 2022; Chen et al. 2023; Lin et al. 2024). Moreover, a study using floral pigments and multivariate analyses suggested that the Xinan District in China, encompassing Yunnan province, is presumed to be the origin site of red-flowered *Camellia* species (Li et al. 2013).

During our field investigations in Yunnan Province in 2024, we found an unknown *Camellia* species with red flowers with 3 distinct styles and brown fruits

Academic editor: Bo Li Received: 19 December 2024 Accepted: 27 February 2025 Published: 26 March 2025

Citation: Li Z-Y, Wu B-H, Qu S, Du L-S, Liu H-O, Duan H-X, Li F-F, Liu W-H (2025) *Camellia shuangbaiensis* (Theaceae), a new species from Yunnan, China. PhytoKeys 254: 143–149. https://doi.org/10.3897/ phytokeys.254.144891

**Copyright:** © Zi-Yuan Li et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

\* These authors contributed equally to this work.

with furfuraceous surfaces. Through extensive morphological comparisons and taxonomic analyses, we have confirmed that these specimens represent a new species, which we formally describe in this document.

### Materials and methods

Morphological comparisons of the putative new species with related species were conducted using living plants, relevant literature, and herbarium specimens. Measurements were conducted manually with rulers or using Digimizer version 4.6.0 (MedCalc Software, Mariakerke, Belgium). The voucher specimens were deposited in the herbarium of China National Botanical Garden (**CNBG**), the herbarium of South China Botanical Garden (**IBSC**) and the herbarium of Sun Yat-sen University (**SYS**).

### Results

### **Taxonomic treatment**

Camellia shuangbaiensis G.P.Yang & B.H.Wu, sp. nov. urn:lsid:ipni.org:names:77359329-1 Fig. 1 Chinese name: 双柏山茶 (Shuang Bai Shan Cha)

**Type. CHINA · Yunnan:** Shuangbai County, Damaidi Township, in ravine. 24°22.44'N, 101°50.84'E, 1740.262051 m a.s.l., 6 June 2024 (fl.), *S. Qu and G.P. Yang Lg2024132* (holotype: CNGB!; isotypes: IBSC!).

**Diagnosis.** *Camellia shuangbaiensis* morphologically resembles *C. mileensis* T.L.Ming and *C. hongkongensis* Seem., but it can be distinguished from the latter two species by its leaves with ovate shape, tomentose abaxial surface and rounded leaf base and bracteoles/sepals 14–16.

**Description.** Small evergreen tree, 2–4 m tall; bark greyish brown, rough; current-year branchlets densely covered with whitish pubescence. Leaf blades ovate to elliptic, leaf apex acuminate to acute, leaf base round, 3.5-6.5 × 2.3-4 cm, leaf blade leathery, adaxially dark green, pubescent along the midrib, abaxially light green, tomentose, more or less punctate; midrib prominent on both surfaces, secondary veins 5-6 pairs, elevated on adaxial surface and impressed on abaxial surface; petiole 2-5 mm long, pubescent. Flowers solitary or sometimes 2 or 3-clustered, terminal, subterminal or axillary, 2-4 cm in diameter, sessile. Bracteoles and sepals 14-16, semipersistent, outside brownish silky pubescent, inside glabrous; outer bracteoles and sepals broadly semiorbicular, rarely apex bifid; inner bracteoles and sepals suborbicular to oblong-elliptic. Petals 6-7, red, basally slightly connate, oblong-elliptic to obovate-elliptic, apex round, 7-11 × 4-5 mm, outside white silky pubescent along ridge. Stamens 33-40, 1.5-2.8 cm long, glabrous; outer filament whorl basally connate for 1-1.5 cm. Ovary 3-loculed, about 2.5-3.3 mm in diameter, tomentose. Styles 3, distinct, glabrous, 1.4-1.75 cm long. Capsule ovoid or subglobose, surface furfuraceous, 1.7-1.9 cm in diameter; pericarp ca. 2 mm thick.

Phenology. Flowering in February, fruiting in June.

**Etymology.** The specific epithet "shuangbaiensis" refers to Shuangbai County of Yunnan Province, the type locality of the new species.


Figure 1. Camellia shuangbaiensis A flowering individual and habitat B stem C flowering branch D fruiting branches E tomentose and punctate leaf abaxial surface F pubescent midvein on the leaf abaxial surface G leaves H branchlet I flower buds J fruits K bracteoles and sepals L pistil M androecium N petals. Photographed by Shang Qu and Bao-Huan Wu. Scale bars: 1 cm.

**Distribution and habitat.** Presently, *Camellia shuangbaiensis* is only known from its type locality, Shuangbai County of central Yunnan. It is distributed in mountain slope mixed forest habitat at altitudes of 1500–2000 m a.s.l.

Additional specimens examined. CHINA • Yunnan: Shuangbai County, Damaidi Town, Damaidi Country, 24°22.44'N, 101°50.84'E, 1740 m a.s.l., 5 February. 2024 (fl.), *S. Qu and G.P. Yang Lg2024133* (CNGB; SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024135* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024136* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024136* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024137* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024138* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024138* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024139* (SYS); • ibid., 11 June 2024 (young fr.), *S. Qu and F.F. Li Lg2024140* (SYS).

# Discussion

Morphologically, *Camellia shuangbaiensis* closely resembles *C. mileensis* and *C. hongkongensis*. However, it can be distinguished by its ovate leaves with tomentose adaxial surface, rounded leaf bases, and 14–16 bracteoles/sepals. Detailed morphological comparisons among *C. shuangbaiensis* and its relatives are presented in Table 1.

Section *Furfuracea* was initially circumscribed based on *C. furfuracea* (Merr.) Cohen-Stuart (Chang 1981), and primarily characterized by its furfuraceous (scaly) fruit surface. Ming (1999, 2000) merged this section into Sect. *Heterogenea*, however, molecular phylogenetic analyses (Xiao and Parks 2003; Vijayan et al. 2009; Zhao et al. 2023) have consistently rejected this taxonomic treatment, and a broader sect. *Furfuracea* including *C. hong-kongensis* was supported.

Characters	Camellia shuangbaiensis	C. mileensis	C. hongkongensis
Habit	small trees, 2–4 m tall	shrubs to 2 m tall	trees to 10 m tall
Leaf blade	ovate to elliptic, abaxially tomentose, more or less punctate, pubescent along midvein, adaxially pubescent along midvein	elliptic, oblong, or lanceolate, abaxially sparsely villous along midvein, punctate, adaxially glabrous	oblong, oblong–elliptic, or oblong– lanceolate, both surfaces glabrous
Leaf length	3.5–6.5 cm	6–6.5 cm	6–12.5 cm
Leaf width	2.5–4 cm	2.5-3 cm	2-4 cm
Petioles	3–5 mm,pubescent	3–5 mm,hirtellous	7–13 mm, glabrous
Leaf margin	crenate-serrulate	serrulate	entire or obscurely undulate- denticulate
Leaf apex	acuminate to acute	bluntly and shortly caudate	acuminate to shortly acuminate
Leaf base	Rounded	cuneate to broadly cuneate	cuneate to obtuse
Bracteoles and sepals	14-16,semipersistent	9-10,semipersistent	11-12,semipersistent
Petals	6–7, red, obovate to broadly obovate, 1.2–1.9 × 1.4–3.5 cm	7–8, white or pale pink, obovate, 2–2.5 × 1.4–1.8 cm	6−7, red, broadly obovate, 3−3.5 × 1.5−2.3 cm
Stamens	filament whorl 1.5–2.8 cm,outer filament whorl basally connate for 1–1.5 cm	filament whorl 1.8 cm, outer filament whorl basally connate for 0.9–1.4 cm	filament whorl 2.5–3 cm,outer filament whorl basally connate for 1.3–2 cm
Styles	3, distinct, 1.3-1.7 cm	3,distinct, ca. 1.7 cm	3,distinct,2.8-3.3 cm
Ovaries	2.5–3 mm in diam., densely white tomentose	1.5 mm in diam., white tomentose,	ca. 2 mm in diam., densely tomentose
Fruit	1.7–2 cm in diam., furfuraceous, pericarp ca. 2 mm thick	1.2–1.5 cm in diam., furfuraceous, pericarp ca. 1.5 mm thick	2–3 cm in diam., furfuraceous, pericarp 3–4 mm thick

Table 1. Morphological comparison of Camellia shuangbaiensis and similar species.

Both *C. hongkongensis* and *C. shuangbaiensis* align with Sect. *Furfuracea* species in terms of their furfuraceous fruit surfaces and 3 free styles. However, their red petals clearly distinguish them from the species of Sect. *Furfuracea*.

Although *C. hongkongensis* was previously classified under Sect. *Camellia* (Chang 1981; Chang and Ren 1998; Ming et al. 2000; Ming and Bartholomew 2007), recent molecular studies (Xiao and Parks 2003; Vijayan et al. 2009; Zhao et al. 2023) have supported to place it within Sect. *Furfuracea*. Considering the morphological similarities between the two species, *C. shuangbaiensis* is likely also a member of Sect. *Furfuracea*.

*Camellia shuangbaiensis* is currently found only in the Damaidi Township, where it grows along the edges of dry-hot river valleys. With a population of just over 100 individuals, the species is restricted to areas adjacent to roadsides, which are heavily impacted by human activities. Additionally, some individuals have been observed to be parasitized by *Scurrula* species. Considering its restricted distribution and vulnerability to parasitic plants and invasive species, *C. shuangbaiensis* faces severe survival threats, which are further exacerbated by its proximity to vehicular pathways on mountain slopes.

These factors necessitate urgent and comprehensive conservation measures. Despite these challenges, the species' striking floral characteristics render it a promising candidate for ornamental camellia breeding programs, highlighting the importance of implementing targeted ecological management strategies to ensure its long-term survival in situ.

# Acknowledgements

We are deeply grateful to Mr. Guo-Ping Yang for his efforts in discovering this new species and for conducting field investigations. We are grateful to the curators of herbaria IBSC, KUN, PE, XBGH and SYS for access to the specimens and the digital images of specimens.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

#### Funding

This study was supported by Global Environment Fund (GEF) Building Sustainability into PA Reforms to Conserve Globally Significant Biodiversity in China Project (No. GEF 9461), Biodiversity survey and assessment project of the Ministry of Ecological Environment, Yunnan Provincial Environmental Protection Special Funds Project, Science and Technology Program from Forestry Administration of Guangdong Province (Grant no. 2023KJCX002), Social Development Project of Guangzhou Municipal Science and Technology Bureau (Grant no. 202206010058).

#### **Author contributions**

Zi-Yuan Li and Bao-Huan Wu are the main authors of the article, and everyone else participated in the writing. Shang Qu, Le-Shan Du, Hai-Ou Liu, He-Xiang Duan, Fei-Fei Li and Wen-Hui Liu participated in the taxonomic confirmation of the new species and field investigation, and Fei-Fei Li and Wen-Hui Liu are the leaders of the research projects, responsible for promoting the project.

#### Author ORCIDs

Zi-Yuan Li <sup>®</sup> https://orcid.org/0009-0002-4941-0505 Bao-Huan Wu <sup>®</sup> https://orcid.org/0000-0002-0498-0603 Shang Qu <sup>®</sup> https://orcid.org/0009-0008-5531-5487 Le-Shan Du <sup>®</sup> https://orcid.org/0000-0002-4418-4011 Hai-Ou Liu <sup>®</sup> https://orcid.org/0000-0003-1148-1615 He-Xiang Duan <sup>®</sup> https://orcid.org/0000-0003-1453-1403 Fei-Fei Li <sup>®</sup> https://orcid.org/0000-0003-4733-6268 Wen-Hui Liu <sup>®</sup> https://orcid.org/0009-0006-2043-6129

#### **Data availability**

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

# References

- Chang HT (1981) A Taxonomy of the Genus *Camellia*. Sunyatsen University Natural Science, Guangzhou 1: 1–43.
- Chang HT, Ren SX (1998) Theaceae. In: Chang HT (Ed.) Flora Reipublicae Popularis Sinicae, Vol. 49(3). Science Press, Beijing, 3–34.
- Chen HL, Wei SJ, Tang SQ, Liu Y (2023) *Camellia wumingensis* (Theaceae), a neglected species from Guangxi, China. Taiwania 68(4): 472–479. https://doi.org/10.6165/ tai.2023.68.472
- Li JB, Hashimoto F, Shimizu K, Sakata Y (2013) Chemical taxonomy of red-flowered wild Camellia species based on floral anthocyanins. Phytochemistry 85: 99–106. https:// doi.org/10.1016/j.phytochem.2012.09.004
- Lin M, Ye QL, Zhang ZJ, Liao WB, Fan Q (2024) *Camellia zijinica* (Theaceae), a new species endemic to Danxia landscape from Guangdong Province, China. PhytoKeys 237: 245–255. https://doi.org/10.3897/phytokeys.237.114768
- Linnaeus C (1753) Species Plantarum 2. Holmiae: Impensis Laurentii Salvii, Stockholm, 561–1200.
- Liu ZW, Ye PM, Li ZH, Wei XJ, Liu ED, Zhang ZR, Ma JL, Yang SX (2020a) *Camellia zhaiana* (sect. *Longipedicellata*), a new species of Theaceae from Guangxi, China. Phytotaxa 460(3): 225–229. https://doi.org/10.11646/phytotaxa.460.3.5
- Liu ZW, Chai SF, Wu FY, Ye PM, Jiang CJ, Zhang ZR, Yu XQ, MA JL, Yang SX (2020b) *Camellia rostrata*, a new species of yellow camellias from Southwest China. Phytotaxa 459(1): 61–68. https://doi.org/10.11646/phytotaxa.459.1.6
- Ming TL (1999) A systematic synopsis of the genus *Camellia*. Acta Botanica Yunnanica 21: 149–159.
- Ming TL, Gu ZJ, Zhang WJ, Xie LS (2000) Monograph of the Genus *Camellia*. Yunnan Science and Technology Press, Yunnan.
- Ming TL, Bartholomew B (2007) Theaceae. In: Wu ZY, Raven PH (Eds) Flora of China (Vol. 12). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 367–412.

- Vijayan K, Zhang WJ, Tsou CH (2009) Molecular taxonomy of Camellia (Theaceae) inferred from nrITS sequences. American Journal of Botany 96(7): 1348–1360. https:// doi.org/10.3732/ajb.0800205
- Xiao TJ, Parks CR (2003) Molecular analysis of the genus *Camellia*. International Camellia Journal 26: 57–65.
- Xu ZG, Yuan DY, Tang YC, Wu L, Zhao YL (2020) Camellia hainanica (Theaceae) a new species from Hainan, supported from morphological characters and phylogenetic analysis. Pakistan Journal of Botany 52(3): 1025–1032. https://doi.org/10.30848/ PJB2020-3(31)
- Ye PM, Huang YS, Huan JF, Liu ED, Ma JL, Yang SX (2022) *Camellia hainingii*, a new species of Theaceae in China. Phytotaxa 558(3): 291–296. https://doi.org/10.11646/ phytotaxa.558.3.5
- Yu XQ, Liu ED, Liu ZW, Xiao B, Ma JL, Yang SX (2021) *Camellia luteocalpandria* (Theaceae), a new species and the first discovery of sect. *Calpandria* in China. Phytotaxa 489(2): 223–228. https://doi.org/10.11646/phytotaxa.489.2.11
- Zhang Q, Wu FY, Huang YH, Ma JL, Yang SX (2022) *Camellia piloflora* (Theaceae), a new yellow *camellia* from Guangxi, South China. Phytotaxa 574(2): 179–184. https://doi.org/10.11646/phytotaxa.574.2.7
- Zhao DW, Hodkinson TR, Parnell JAN (2023) Phylogenetics of global *Camellia* (Theaceae) based on three nuclear regions and its implications for systematics and evolutionary history. Journal of Systematics and Evolution 61(2): 356–368. https://doi. org/10.1111/jse.12837



**Research Article** 

# Resolving a nearly 95-year-old enigma: Transfer of the littleknown Japanese moss *Arctoa schistioides* to *Kiaeria falcata* (Rhabdoweisiaceae, Bryophyta)

Wen-Zhuan Huang<sup>10</sup>, Jia-Yi Zheng<sup>10</sup>, Xin-Rui Xia<sup>10</sup>, Xin-Yin Ma<sup>10</sup>, Tian-Xiong Zheng<sup>20</sup>, Yu-Huan Wu<sup>10</sup>

1 College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

2 Hattori Botanical Laboratory, Obi 6-1-26, Nichinan, Miyazaki 889-2535, Japan

Corresponding authors: Tian-Xiong Zheng (txzheng@hattorilab.org); Yu-Huan Wu (yuhuanwu@hznu.edu.cn)

#### Abstract

Taxonomic uncertainties regarding rare species often impede effective biodiversity conservation. One such taxonomic uncertainty is the 95-year-old mystery surrounding *Arctoa schistioides* (Broth. ex Ihsiba) Ihsiba. Since its initial publication in 1929, this species has not been subjected to any further discoveries and is, thus, classified as "doubtful taxa" or "insufficiently known taxa" to date. Assessing the taxonomic status of this species is essential for determining whether a conservation strategy should be implemented. In this study, we examined the holotype of *A. schistioides* and treated this species as a new synonym of *Kiaeria falcata* (Hedw.) I.Hagen, a widely distributed species in the Northern Hemisphere, by providing detailed description, illustration and taxonomic notes. Our findings not only resolve this long-standing mystery, but also enhance our understanding of Japanese mosses and the global distribution of bryophytes.

Key words: Bryophyta, Dicranum schistioides, doubtful species, new synonym, taxonomy

# Introduction

Japan is renowned for its extensive diversity of mosses and is regarded as one of the global centres of moss diversity (Geffert et al. 2013). In the most recent checklist, Suzuki (2016) documented 1,270 species across 342 genera within the Japanese moss flora; however, 149 species were classified as "doubtful taxa" in this checklist. Resolving the uncertainties surrounding these questionable species is crucial for enhancing our understanding of Japanese moss diversity and the global distribution of bryophytes.

Arctoa schistioides (Broth ex Ihsiba) Ihsiba is one such enigmatic species, possessing a noteworthy taxonomic history. In 1907, S. Okamura collected an interesting specimen from Mt. Iwaki, Japan (Fig. 1), which was initially identified by V.F. Brotherus as a new species and later compiled and published by Ihsiba (1929) as *"Dicranum schistioides* Broth. ex Ihsiba". Subsequently, Ihsiba (1932) transferred this name to *Aratoa schistioides*. Sakurai (1954) provided a comprehensive catalogue on Japanese mosses, but did not recognise the species. Iwatsuki and Noguchi (1973) listed all genera and species of mosses in Japan and considered



Academic editor: Matt von Konrat Received: 11 November 2024 Accepted: 6 March 2025 Published: 28 March 2025

**Citation:** Huang W-Z, Zheng J-Y, Xia X-R, Ma X-Y, Zheng T-X, Wu Y-H (2025) Resolving a nearly 95-year-old enigma: Transfer of the little-known Japanese moss *Arctoa schistioides* to *Kiaeria falcata* (Rhabdoweisiaceae, Bryophyta). PhytoKeys 254: 151–159. https://doi.org/10.3897/ phytokeys.254.141498

**Copyright:** © Wen-Zhuan Huang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Wen-Zhuan Huang et al.: Transfer Arctoa schistioides to Kiaeria falcate

MOSSES OF JAPAN HERBARIUM S. OKAMURA
Dicramum schistioides Broth
HAB. <u>Mt. Iwaki', Mutsu</u> , NO. DATE <u>Aug 2, 07</u> , DET. Broth 1273 COLL.SHUTAI OKAMURA JAPAN

Figure 1. Specimen label of the holotype of Arctoa schistioides (Broth. ex Ihsiba) Ihsiba (S. Okamura s.n. [NICH 37785]).

*A. schistioides* a well-established taxon, which was followed by Sekine (1982). Since then, the taxonomic status of *A. schistioides* started to be questioned. Crosby et al. (1999) classified this name as "insufficiently known" in "A Checklist of the Mosses". In subsequent versions of the Japanese moss checklist (e.g. Iwatsuki (2004, 2011); Suzuki (2016)), the distribution and record of *A. schistioides* in Japan was considered doubtful. In fact, this species has not been subjected to any additional discoveries or descriptions in the 95 years since its publication (Ihsiba 1929), resulting in very limited knowledge about it. To date, the status of *A. schistioides* remains an enigmatic issue requiring further resolution.

Accurate species identification is crucial for biodiversity conservation, particularly amongst rare taxa that are taxonomically uncertain due to insufficient study (Ding et al. 2018; Li et al. 2023). Erroneous classification and misidentification may overlook endangered species that warrant protection (Gibson et al. 2019). Conversely, management actions stemming from incorrect species identification can waste resources and funding (Solow et al. 2011). Therefore, further assessment is necessary to clarify the taxonomic status of *Arctoa schistioides*.

# Material and methods

Specimen of Arctoa schistioides (= Dicranum schistioides), probably the holotype (S. Okamura s.n.; NICH 37785), was borrowed from NICH and morphologically examined. Notes on the nomenclatural status and collection site of this specimen were provided below. The specimen was examined by using a stereomicroscope (Leica EZ4; Leica, Wetzlar, Germany) and a compound microscope (Leica DM6 B; Leica, Wetzlar, Germany). Microscopic pictures were captured using a digital camera (Leica DFC450 C; Leica, Wetzlar, Germany) attached to the compound microscope. The plant pictures were taken using a stereomicroscope (Keyence VHX-6000; Keyence, Osaka, Japan).

# Results

Based on morphological observations, *Arctoa schistioides* exhibits no morphological differences from *Kiaeria falcata* (Hedw.) I.Hagen. Therefore, we treat *A. schistioides* as a new synonym of *K. falcata*.

#### **Taxonomic treatment**

# *Kiaeria falcata* (Hedw.) I.Hagen, Kongel. Norske Vidensk. Selsk. Skr. (Trondheim) 1914(1): 112. 1915.

- Arctoa schistioides (Broth. ex Ihsiba) Ihsiba, Classif. Mosses Japan: 130.
  1932. syn. nov.
- *≡ Dicranum schistioides* Broth. ex Ihsiba, Cat. Mosses Japan: 43. 1929.

**Type.** JAPAN • Aomori Prefecture, Hiromae City, Mt. Iwaki, 2 Aug 1907, *S. Okamura s.n.* (*holotype*: NICH 37785!), (Figs 2, 3).

**Description.** Plants small, in loose tufts. Stems 5–8 mm, simple, cross-section of stem rounded to oval, diameter 0.11–0.15 mm, central strand present. Leaves homomallous, falcate-secund when dry, erect-spreading when moist. Leaves lanceolate at base, gradually tapering into a channelled acumen; costa excurrent as an awn, mamillose dorsally, in transverse section with differentiated guide cells, with dorsal and ventral epidermis and few substereids on dorsal side of guide cells or poorly differentiated; margins plane or slightly incurved distally, entire or crenulate in distal part of acumen; lamina unistratose, occasionally partially 2-stratose in distal portion, margins 1-stratose; distal and median laminal cells short rectangular to subquadrate, with moderately thickened walls,  $(6-)8-15(-19) \times 4-8 \mu m$ ; basal juxtacostal cells elongate-rectangular, moderately thick-walled, non-porose,  $30-50 \times 5-9 \mu m$ ; alar cells gradually enlarged, not sharply differentiated, unistratose, non-porose,  $24-45 \times 14-20 \mu m$ .

Autoecious. Perigonia terminal closely located below the perichaetia. Perigonial leaves small, ovate-lanceolate to triangular,  $0.65-0.75 \times 0.45-0.55$  mm, costa present or absent; Perichaetial leaves with sheathing base, abruptly into a channelled acumen. ca. 3.2 mm long. Sporophyte single in perichaetium. Seta straight, 5.5–7.5 mm long, yellowish-brown. Capsules obovate, curved and strumose, smooth when dry; Exothecial cells irregular, short rectangle, thick walled; Calyptra not seen; Operculum not seen; Annulus persistent, one row of small cells; Peristome teeth to 0.35 mm long, orange-brownish below, whitish in distal portion, divided into two prongs to the middle, vertically pitted-striolate below, papillose above. Spores 14–17  $\mu$ m.



Figure 2. Arctoa schistioides (Broth. ex Ihsiba) Ihsiba A plants B capsules, arrows shows strumose C spores D, E, F peristome teeth G perigonial leaves H perichaetial leaves I annulus J exothecial cells. All from the holotype (S. Okamura s.n. [NICH 37785]).

**Notes.** The nomenclatural status of the cited specimen (*S. Okamura s.n.*; NICH 37785) should be stated first. According to the protologue of *Dicranum schistioides* (Ihsiba 1929), the type specimen of this species was collected from "津軽富士" (Tsugarufuji; in English), which is another name for Mt. Iwaki (岩木山; in Japanese) in Aomori Prefecture, Japan (Tokuhisa 1978). During this study, we extensively searched the bryological collection of NICH and located only one specimen of *D. schistioides*, namely "*S. Okamura s.n.*" (NICH 37785), which was detected by V. F. Brotherus and collected by S. Okamura from



Figure 3. Arctoa schistioides (Broth. ex Ihsiba) Ihsiba A leaves B apex C cross section of stem D cross sections of leaf E upper laminal cells of leaf F middle laminal cells of leaf G basal juxtacostal cells H alar cells. All from the holotype (S. Okamura s.n. [NICH 37785]).

"Mt. Iwaki, Mutsu" (Fig. 1). Since Aomori Prefecture was administratively part of "Mutsu" (Sanseidohenshuusho 1975), we thus deemed that this specimen shares the same collection site as recorded in the protologue of *D. schistioides* (Ihsiba 1929). Furthermore, as the original collection of S. Okamura was supposedly deposited in NICH (Vitt et al. 1985), it can be thus inferred that the present specimen is the holotype of *D. schistioides* (Art. 9.1; Turland et al. 2018). As Mt. Iwaki (Fig. 1) is now included in Hiromae City, Aomori Prefecture, we provided a corrected type citation above.

In addition, nomenclature of *Dicranum schistioides* also needs a brief discussion here. This species is nomenclaturally valid although it was only described in Japanese at the time of publication (Art. 39.1; Turland et al. 2018). Later, it was transferred to the genus *Arctoa* without providing a basionym or replaced synonym (Ihsiba 1932). However, the taxonomic authority of its basionym "(Broth.)" and Japanese name "tz t t t" were clearly given, which should be regarded as an indirect reference (Arts. 38. 14 & 41.3; Turland et al. 2018), giving *A. schistioides* a valid taxonomic status.

Arctoa schistioides is distinguished by the following characteristics: (1) a strumose capsule (Fig. 2A, B), (2) a smooth capsule when dry (Fig. 2B); (3) the

presence of a central strand (Fig. 3C), (4) gradually enlarged alar cells (Fig. 3A, H), (5) a nearly homogeneous costa structure without stereids (Fig. 3D), (6) mamillose leaf subula (Fig. 3B, D), (7) distal laminal cells that are subquadrate to short rectangular (Fig. 3E), (8) elongate-rectangular basal juxtacostal cells (Fig. 3G), (9) a persistent annulus comprised of small cells (Fig. 2I), (10) perigonia located just below the perichaetia (Fig. 2A) and (11) irregular, short rectangle, thick-walled exothecial cells (Fig. 2J). These characteristics imply that *A. schistioides* actually belongs to *Kiaeria falcata* due to the lack of distinct morphological differences between the two species (Newmaster 2007a; Brugués and Ruiz 2012; Lüth 2019).

Morphologically, Arctoa schistioides may be confused with A. fulvella (Dicks.) Bruch & Schimp. due to their similar plant morphology and leaf shape (Noguchi 1987; Newmaster 2007a, 2007b; Lüth 2019). However, the alar cells of A. schistioides are gradually enlarged and not sharply differentiated (Fig. 3A, H), whereas those of A. fulvella are clearly delimited and well differentiated (Noguchi 1987; Gao et al. 1999; Ochyra and Buck 2003; Newmaster 2007b; Lüth 2019). Additionally, these two species can be distinguished by their capsule morphology: A. schistioides exhibits smooth capsules when dry, characterised by a distinct strumose (Fig. 2A, B). In contrast, A. fulvella possesses distinctly ribbed capsules when dry and lacks a strumose structure (Noguchi 1987; Ochyra and Buck 2003; Newmaster 2007b; Lüth 2019). Notably, both species share peristome teeth that are divided into two prongs at the mid-point (Fig. 2D; Noguchi (1987); Newmaster (2007b)); however, this division is not always conspicuous in A. fulvella, as the teeth occasionally appear undivided and perforated near the middle (Gao et al. 1999; Ochyra and Buck 2003; Lüth 2019).

Arctoa schistioides is easily confused with A. blyttii (Bruch & Schimp.) Loeske. However, the leaves of A. schistioides are homomallous and falcate-secund when dry (Fig. 2A), while those of A. blyttii are erect-spreading and flexuose (Newmaster 2007a; Brugués and Ruiz 2012). Additionally, the perigonia of A. schistioides are situated just below the perichaetia (Fig. 2A), whereas those of A. blyttii are terminal on a separate branch or positioned far below the perichaetia (Newmaster 2007a; Brugués and Ruiz 2012). Another distinguishing feature is that the exothecial cells of A. schistioides are irregular, short rectangular and thick-walled (Fig. 2J), while those of A. blyttii are rectangular and thin-walled (Brugués and Ruiz 2012). Furthermore, the annulus of A. schistioides is persistent and consists of a single row of small cells (Fig. 2I), whereas the annulus of A. blyttii is deciduous and composed of three rows of large cells (Brugués and Ruiz 2012, 2015).

Arctoa schistioides is also morphologically similar to A. starkei (F. Weber & D. Mohr) Loeske and A. glacialis (Berggr.) Fedosov, Jan Kučera & M. Stech. However, the upper laminal cells of the latter two species are long and rectangular and their capsules are ribbed or grooved when dry (Newmaster 2007a). In contrast, the upper-middle cells of A. schistioides are short rectangular to subquadrate (Fig. 3E) and its capsules are smooth when dry (Fig. 2A, B). Additionally, *Kiaeria falcata* var. serratifolia Sakurai, a taxon endemic to Japan, can only be distinguished from A. schistioides by its serrate leaf margins (Sakurai 1952), whereas the latter species are smooth or crenulate leaf margins in the distal part of the acumen (Fig. 3A, B).

In conclusion, we propose Arctoa schistioides as a new synonym of Kiaeria falcata.

# Acknowledgements

We are grateful to the staff of NICH for searching necessary specimens.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This research was funded by National Natural Science Foundation of China, grant number 32270215 and Grant-in-Aid for Scientific Research (Specially Designated Research Promotion) of the Japan Society for the Promotion of Science (JSPS).

#### Author contributions

Wen-Zhuan Huang conceived the study. Wen-Zhuan Huang and Tian-Xiong Zheng wrote the original draft. Jia-Yi Zheng, Xin-Rui Xia and Xin-Yin Ma took the photomicrographs and conducted the measurements of morphological characters. Wen-Zhuan Huang, Tian-Xiong Zheng and Yu-Huan Wu reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

#### Author ORCIDs

Wen-Zhuan Huang <sup>®</sup> https://orcid.org/0000-0001-5871-5699 Jia-Yi Zheng <sup>®</sup> https://orcid.org/0009-0000-6222-8818 Xin-Rui Xia <sup>®</sup> https://orcid.org/0009-0006-7708-7716 Xin-Yin Ma <sup>®</sup> https://orcid.org/0000-0003-0876-4497 Tian-Xiong Zheng <sup>®</sup> https://orcid.org/0000-0002-1963-2302 Yu-Huan Wu <sup>®</sup> https://orcid.org/0000-0002-8139-2194

#### Data availability

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

# References

- Brugués M, Ruiz E (2012) The genus *Kiaeria* in Spain, including *K. blyttii* (Bruch et Schimp.) Broth. new to Spain. Cryptogamie. Bryologie 33(3): 263–266. https://doi.org/10.7872/cryb.v33.iss3.2012.263
- Brugués M, Ruiz E (2015) *Kiaeria* I. Hagen. In: Brugués M, Guerra J (Eds) Flora Briofítica Ibérica Volumen II. Universidad e Murcia, Murcia, 69–72.
- Crosby MR, Magill RE, Allen B, He S (1999) A Checklist of Mosses. Missouri Botanical Garden, St. Louis, 1–315.
- Ding X, Xiao JH, Li L, Conran JG, Li J (2018) Congruent species delimitation of two controversial gold-thread nanmu tree species based on morphological and restriction site-associated DNA sequencing data. Journal of Systematics and Evolution 57(3): 234–246. https://doi.org/10.1111/jse.12433
- Gao C, Vitt DH, He S (1999) Dicranaceae. In: Gao C, Crosby MR, He S (Eds) Moss Flora of China Vol. 1. Sphagnaceae–Leucobryaceae. Science Press, Beijing & Missouri Botanical Garden, St. Louis, 91–241.
- Geffert JL, Frahm JP, Barthlott W, Mutke J (2013) Global moss diversity: Spatial and taxonomic patterns of species richness. Journal of Bryology 35(1): 1–11. https://doi. org/10.1179/1743282012Y.000000038
- Gibson KJ, Streich MK, Topping TS, Stunz GW (2019) Utility of citizen science data: A case study in land-based shark fishing. PLoS ONE 14(12): e0226782. https://doi. org/10.1371/journal.pone.0226782
- Huang WZ, Xu H, Ma XY, Zhu RL (2023) *Dicranum hengduanensis* (Dicranaceae, Bryophyta), a new species with fragile leaves from the Hengduan Mountains in China. The Bryologist 126(2): 226–235. https://doi.org/10.1639/0007-2745-126.2.226
- Huang WZ, Shen C, Xu H, Shu L, Sulayman M, Wu YH, Zhu RL (2024) A synopsis of *Dicranum* Hedw. (Dicranaceae, Bryophyta) in China, with special references to four species newly reported and re-evaluation of *Dicranum psathyrum* Klazenga. Plants 13(13): 1957. https://doi.org/10.3390/plants13131759

Ignatova EA, Fedosov VE (2008) Species of *Dicranum* (Dicranaceae, Bryophyta) with fragile leaves in Russia. Arctoa 17(1): 63–83. https://doi.org/10.15298/arctoa.17.05

Ihsiba E (1929) Catalog of mosses of Japan. Nishigahara Kankokai, Tokyo, 1–295.

 Ihsiba E (1932) Classification of mosses of Japan. Nishigahara Kankokai, Tokyo, 1–176.
 Iwatsuki Z (2004) New catalog of the mosses of Japan. The Journal of the Hattori Botanical Laboratory 96: 1–182. https://doi.org/10.18968/jhbl.96.0\_1

- Iwatsuki Z (2011) A list of Japanese names of mosses. Part II. Hattoria 2: 47–115. https://doi.org/10.18968/hattoria.2.0\_47
- Iwatsuki Z, Noguchi A (1973) Index Muscorum Japonicarum. The Journal of the Hattori Botanical Laboratory 37: 299–418. https://doi.org/10.18968/jhbl.37.0\_299
- Li DQ, Lu J, Liang H, Zhu DH, Fan DM, Kou YX, Yang Y, Zhang ZY (2023) The rare *Fagus chienii* is conspecific with *F. hayatae* based on molecular and morphological evidence. Plant Diversity 45(5): 544–551. https://doi.org/10.1016/j.pld.2023.01.003
- Lüth M (2019) Mosses of Europe A Photographic Flora. Michael Lüth, Freiburg, 1–1306. Newmaster SG (2007a) *Kiaeria* I. Hagen. In: Flora of North America Editorial Committee (Eds) Flora of North America. Volume 27. Bryophyta, part 1. Oxford University Press, Oxford, 420–423.
- Newmaster SG (2007b) Arctoa Bruch & Schimper. In: Flora of North America Editorial Committee (Eds) Flora of North America. Vol. 27. Bryophyta, part 1. Oxford University Press, Oxford, 362–363.

- Noguchi A (1987) Illustrated Moss Flora of Japan. The Hattoria Botanical Laboratory, Hiroshima, 1–242.
- Ochyra R, Buck WR (2003) Arctoa fulvella, new to Tierra del Fuego, with notes on trans-American bipolar bryogeography. The Bryologist 106(4): 532–538. https://doi. org/10.1639/0007-2745(2003)106[532:AFNTTD]2.0.CO;2
- Sakurai K (1952) Observations of the genus *Kiaeria, Paraleucobryum, Orthodicranum,* and *Dicranoloma* in Japan. Botanical Magazine Tokyo 65(771–772): 251–256. https://doi.org/10.15281/jplantres1887.65.251

Sakurai K (1954) Muscologia Japonica. Iwanami Shoten, Tokyo.

- Sanseidohenshuusho (1975) Konsaisu Chimei Jiten. Sanseido Press, Tokyo.
- Sekine Y (1982) Nihon san Senrui no Kensaku. Houjou Shokan, Tokyo.
- Solow A, Smith W, Burgman M, Bout T, Wintle B, Roberts D (2011) Uncertain sightings and the extinction of the ivory-Billed woodpecker. Conservation Biology 26(1): 180– 184. https://doi.org/10.1111/j.1523-1739.2011.01743.x
- Suzuki T (2016) A revised new catalog of the mosses of Japan. Hattoria 7: 9–223. https://doi.org/10.18968/hattoria.7.0\_9
- Tokuhisa T (1978) Konsaisu Nippon Sanmei Jiten. Sanseido Press, Tokyo.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. https://doi. org/10.12705/Code.2018
- Vitt DH, Gradstein SR, Iwatsuki Z (1985) Compendium of Bryology. A World Listing of Herbaria, Collectors, Bryologists, and Current Research. Verlag von J. Cramer, Braunschweig.



#### **Research Article**

# *Pyrus zhaoxuanii* (Rosaceae), A new pear species from Danxiashan Mountain, Guangdong, China

Xiao-Wei Yi<sup>1,2</sup>, Ying-Yu Wu<sup>1,2</sup>, Qiang Fan<sup>1,2</sup>, Fang Chen<sup>3</sup>, Zai-Xiong Chen<sup>3</sup>, Bin-Bin Liu<sup>4,5</sup>, Cui-Ying Huang<sup>1,2</sup>

- 1 State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Stress Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China
- 2 National Park and Nature Education Research Institute, Sun Yat-sen University, Guangzhou 510275, China
- 3 Guangdong Danxiashan National Nature Reserve Administration, Shaoguan 512300, China
- 4 State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
- 5 China National Botanical Garden, Beijing 100093, China

Corresponding authors: Cui-Ying Huang (hcying7@mail.sysu.edu.cn); Bin-Bin Liu (liubinbin@ibcas.ac.cn)

#### Abstract

*Pyrus zhaoxuanii* is described as a new species from Guangdong Province, China, within the genus *Pyrus*, specifically under *P.* subg. *Pashia*. Although it shares morphological similarities with *P. calleryana*, *P. zhaoxuanii* can be distinguished by its uniquely small, obovate, leathery leaves, which have an obtuse apex and short petioles. A phylogenetic analysis based on single nucleotide polymorphisms (SNPs) indicated that *P. zhaoxuanii* forms an independent branch within *Pyrus* and is categorized in the Oriental clade, *P.* subg. *Pashia*. Currently, this species has only been recorded in the Danxiashan National Nature Reserve. Considering its potential distribution and population size, we recommend classifying this species as Least Concern (LC) according to the IUCN Red List classifications and criteria.

Key words: Danxia landscape, new species, phylogeny, Pyrus



Academic editor: Hugo de Boer Received: 30 September 2024 Accepted: 14 March 2025 Published: 28 March 2025

Citation: Yi X-W, Wu Y-Y, Fan Q, Chen F, Chen Z-X, Liu B-B, Huang C-Y (2025) *Pyrus zhaoxuanii* (Rosaceae), A new pear species from Danxiashan Mountain, Guangdong, China. PhytoKeys 254: 161–173. https://doi. org/10.3897/phytokeys.254.138039

**Copyright:** <sup>©</sup> Xiao-Wei Yi et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

# Introduction

The genus *Pyrus* L. belongs to the apple tribe Maleae in the Rosaceae family (Zhang et al. 2017; Liu et al. 2020a, 2022; Wang et al. 2024). Currently, at least 25 confirmed species of *Pyrus* have been identified worldwide, with 14 species recorded in China (Gu and Spongberg 2003; Jin et al. 2024a). As one of the most important fruit trees, pears have a cultivation history spanning possibly 3,000 years (Pu and Wang 1963; Teng 2011). *Pyrus* species exhibit self-incompatibility, necessitating cross-pollination from different flowers, which increases genetic heterozygosity among individual plants. Interspecific hybridization is common within *Pyrus*, leading to a blend of genes and extensive genetic variation among species (Jin et al. 2024b). This frequent introgression is prevalent in the apple tribe Maleae, including apples, pears, and their relatives (Phipps et al. 1991; Lo and Donoghue 2012; Liu et al. 2019, 2020b, 2023; Jin et al. 2023). This genetic complexity complicates determining relationships between

different *Pyrus* species (Westwood and Bjornstad 1971), making their classification particularly challenging. Furthermore, many geographical subspecies have historically been regarded as "species" (Westwood and Challice 1978).

*Pyrus calleryana* Decne., commonly known as Callery pear, is native to eastern and southern China, Korea, and Japan (Bell and Zimmerman 1990; Gu and Spongberg 2003) and is frequently used as a rootstock for pear trees. The Flora of China recognizes four varieties of *P. calleryana*: *P. calleryana* var. *calleryana*, *P. calleryana* var. *integrifolia* T.T.Yu, *P. calleryana* var. *koehnei* (C.K.Schneid.) T.T.Yu, and *P. calleryana* var. *lanceolata* Rehder (Gu and Spongberg 2003).

To clarify the classification of the genus *Pyrus*, a previous study examined the diversity of *Pyrus* and the independent domestication of Asian and European pears (Wu et al. 2018). This study analyzed 113 individuals and constructed a phylogenetic tree of major *Pyrus* species, categorizing them into Asian and European pears, resulting in six distinct groups. Among these, *P. calleryana*, *P. pashia* Buch.-Ham. ex D.Don, and *P. betulifolia* Bunge were classified as Asian pear Group II, indicating their close phylogenetic relationships. These three species exhibit relatively limited domestication and share characteristics of small, undomesticated fruits. Building on this research, an updated infrageneric classification divided pears into two subgenera: *P. subg. Pashia* (Asian pears) and *P. subg. Pyrus* (European pears) (Jin et al. 2024a). All members of Group II fall under *P. subg. Pashia*.

During our investigation in Danxiashan Mountain, we found two types of Callery pear (*P. calleryana*) with different morphological characteristics. Individuals on the gentle slopes at the foot of the mountain are consistent with the normal *P. calleryana* phenotype morphologically, while those on the steep slopes and cliffs exhibit distinct traits, including small, obovate, thick, leathery leaves with obtuse tips and short petioles. Additionally, some branchlets have evolved into thorns, and the plant's stature has become shrubbier.

Initially, we hypothesized that these plants were ecotypes of *P. calleryana*. However, as our research progressed, it became clear that this plant should be classified under *P.* subg. *Pashia* as a new branch in the phylogenetic tree. Based on morphological characteristics and phylogenetic analyses, we propose that it represents a new species, which we describe and illustrate here.

# Methods and materials

#### Samples collection, DNA extraction, and sequencing

Four individuals of the putative new species and six individuals of *Pyrus calleryana* were collected from four locations in Mount Danxiashan, Renhua County, Shaoguan City, Guangdong Province (Table 1, Fig. 1). Fresh leaf material from each individual was dried and stored in silica gel. Total genomic DNAs were extracted using CTAB method (Doyle and Doyle. 1987). The extracted genomic DNAs were assessed for integrity, purity, and concentration using agarose gels and Qubit 4.0 with Qubit<sup>®</sup> DNA Assay Kit (Life Technologies). The qualified DNA samples (≥ 50 ng) were then sent to Jierui Biotech (Guangzhou, China) for paired-end library preparation, followed by genome skimming sequencing on Illumina Xplus (Illumina Inc.; San Diego, California, USA), adhering to the standard Illumina sequencing protocol. Voucher specimens for each individual were deposited in the herbarium of Sun Yat-sen University (SYS).

Pop.ID	Collection number	Location	Geographical ordination	individual
L1	101	Shaoshishan	24°58'15"N, 113°44'50"E	2
S1	102	Shaoshishan	24°58'29"N, 113°44'10"E	2
L2	201	Bazhai	25°00'27"N, 113°39'54"E	2
S2	202	Bazhai	25°00'24"N, 113°40'07"E	1
L3	301	Heshangzhai	25°02'09"N, 113°45'45"E	1
L4	401	Yanyan	25°00'54"N, 113°37'42"E	1
S4	402	Yanyan	25°00'54"N, 113°37'42"E	1

Table 1. Sample collection information.

\*The Pop.ID "L" means "large leaves" representing *P. calleryana*; "S" means "small leaves" representing *P. zhaoxuanii*.



Figure 1. Distribution of Pyrus zhaoxuanii and Pyrus calleryana in Danxiashan Mountain.

# Morphological study

The morphological study documented images of the new species during its flowering and fruiting stages. The morphological characteristics of the putative new species were compared with those of *P. calleryana*, as well as with specimens from other species within the *Pyrus* genus. The plant specimens used in this study were obtained from the herbaria P, PE, SYS, NAS that herbarium acronym as per BIEN 4.2 (https://bien.nceas.ucsb.edu/ bien/data-contributors/herbaria/). Voucher specimens are preserved in the herbarium of Sun Yat-sen University (SYS).

#### **Phylogenetic analyses**

#### Other nuclear genome data acquisition

We downloaded re-sequencing data of the nuclear genome of 22 individuals, including 20 representative species of *Pyrus*, such as *P. pashia* and *P. betulifo-lia*, as well as the closely related genus, *Malus pumila*, from the NCBI nucleo-tide database (Suppl. material 1). The fasterq-dump program in SRA Toolkit v. 3.1.0 (https://github.com/ncbi/sra-tools/wiki/Home) was used to convert the SRA-formatted files to paired-end FASTQ format files.

Constructing a phylogenetic tree based on nuclear genome data

The raw data from the ten individuals sequenced in our study, together with the genomic data for 22 other species downloaded from NCBI, were used to reconstruct a phylogenetic tree. The genome of Pyrus bretschneideri Rehder (GeneBank sequence number: GCF\_019419815.1) served as the reference. The FASTQ formatted paired-end sequencing data (1 and 2) were mapped to the reference genome using BWA v. 0.1.17 (Li 2013). The resulting mapping files were converted into BAM format using SAMtools v. 1.6 (Danecek et al. 2021), and the mapping results were sorted while removing PCR duplicate sequences. BCFtools v. 1.9 (Danecek et al. 2021) was employed to generate a set of candidate variant positions (SNPs and indels), followed by variant detection. The VCF file was normalized and filtered with the parameters "-s LOWQUAL -e 'QUAL<20 || INFO/DP <5' " to remove variants with quality values (QUAL) less than 20 or depth (DP) less than 5. All single nucleotide polymorphisms (SNPs) in the VCF file were extracted, and the SNP data of the 32 individuals were combined into one file and filtered twice. The parameters were set to "-i 'DP>=5 & DP<=100 & QUAL>=30' -s LOWQUAL" and "-i 'MAF>=0.05"" to retain sites with a DP between 5 to 100, QUAL of at least 30, and a minor allele frequency of at least 5%. A custom Perl script was utilized to select sites present in at least 75% of the individuals. The VCF file was converted to PHYLIP format using vcf2phylip.py (Ortiz 2019), and the phylogenetic tree was reconstructed using IQ-TREE v. 2.1.4 (Minh et al. 2020) based on the maximum likelihood method. The parameters were set to "-m MFP+ASN -bb 2000" (Kalyaanamoorthy et al. 2017), and the best fitting model for DNA replacement was determined to be TVM+I+R4, calculated using the Bayesian information criterion.

# **Results and discussion**

A total of 1,164,026 SNPs were generated for the 32 samples. The phylogenetic tree inferred from these SNPs closely resembled findings from previous SNP-based research (Wu et al. 2018), defining five subclades with high support values (Fig. 2). Furthermore, our results also correspond with the ortholog-based phylogenomic studies (Jin et al. 2024a, 2024b). Those *P. calleryana* individuals we collected, along with its varieties, formed a sister group to the putative new species. Ultimately, the putative new species, *P. calleryana*, *P. pashia*, *P. betulifolia*, and *P. pseudopashia*, collectively formed Group 1, which showed high support values and belonged to the *P.* subg. *Pashia* (Jin et al. 2024a). This phylogenetic tree indicates that pronounced genetic differentiation exists between the new species and other species within Group 1.



**Figure 2**. Phylogenetic tree built based on SNPs, *Malus pumila* as the outgroup. Among them, those marked red are *P. zhaoxuanii*, and those marked blue are *P. calleryana* that we collected in Danxia Mountain. The numbers displayed on the diagram represent the bootstrap confidence level of the phylogenetic tree.

The putative new species is most similar to the *Pyrus calleryana*, both morphologically and molecularly. Both species possess small fruits, five white petals, and corymb inflorescences. However, compared to *P. calleryana*, the putative new species has smaller leaves  $(19-31 \times 10-15 \text{ mm vs. } 40-80 \times 35-60 \text{ mm})$ , obovate leaves (vs. broadly ovate or ovate, rarely narrowly elliptic), apex obtuse (vs. apex acuminate, rarely acute), shorter petioles (2–12 mm vs. 20–40 mm), distinct stem thorns (vs. nearly no stem thorns), and a shrubby habit (vs. tree habit) (Table 2).

Morphologically, a common characteristic of Group 1 in our study is small fruit size, which distinguishes them from other groups. Within Group 1, *Pyrus zhaoxuanii* differentiates from other species due to its small, obovate leaves. When we first encountered it at Mount Danxia, we suspected it was an ecotype of *P. calleryana*. However, *P. zhaoxuanii* has established a new branch on the phylogenetic tree based on SNP data. Molecular phylogenetics supports its classification as an independent species rather than an ecotype of *P. calleryana*.

Feature	P. zhaoxuanii	P. calleryana
Leaf Size	19−31 × 10−15 mm	40-80 × 35-60 mm
Leaf Texture	Leathery, thick	Papery, thin
Leaf Shape	obovate, rarely elliptical	Elliptical or ovate
Leaf Apex	blunt, rarely acuminate	Acuminate, rarely acute
Leaf Base	Cuneate	Round to wide cuneate
Inflorescence Number	2-7(8)	6-12
Pedicel	Tomentose	Glabrous
Petiole	Short	Long
Branch Thorn	0-11	0-4
Mean Branch Thorn	5	0.2

Table 2. Morphological comparison of P. zhaoxuanii, and P. calleryana.

\*The Pop.ID "L" means "large leaves" representing *P. calleryana*; "S" means "small leaves" representing *P. zhaoxuanii*.

#### **Taxonomic treatment**

# Pyrus zhaoxuanii X.W.Yi, B.B.Liu & Q.Fan, sp. nov.

urn:lsid:ipni.org:names:77359363-1 Figs 3-6 Chinese name. 昭璇梨

**Type.** CHINA. • Guangdong Province, Shaoguan City, Danxiashan National Nature Reserve, 25°0'28.26"N, 113°39'42.80"E, alt. 380 m, 24 February 2024, *Y.Y. Wu et al. DNPC4016* (holotype: SYS!; isotypes: SYS!, PE!).

**Diagnose.** *Pyrus zhaoxuanii* is similar to *P. calleryana*, but can be differentiated by its small, obovate leaves, short petioles, pronounced stem thorns, and shrubby habit.



**Figure 3.** Flower and pome of *Pyrus zhaoxuanii* **A** frontal dissection of the flower **B** stamens **C** longitudinal section of the flower **D** front view of the entire flower **E** cross and longitudinal sections of the fruit **F** cross-section of the ovary. Scale bars: 1 cm (**A**, **E**); 5 mm (**B**, **C**, **D**, **F**).



**Figure 4**. *Pyrus zhaoxuanii* **A** plant, stem with many branches and thorn **B** habitat, growing on the edge of cliffs or steep slopes **C** branches with many subdivisions and thorns **D** lenticels on young branches **E** surface of the stem **F**, **G** leaves during the fruiting period **H**, **I** leaf buds and bud scales with hair **J**, **K** flowers **L**, **M** fruits **I** comparison of *P*. *zhaoxuanii* and *P*. *calleryana* (left: *P*. *zhaoxuanii*, right: *P*. *calleryana*).

**Description.** Deciduous shrubs or small trees, 2–5 m high, with lateral branches; bark dark gray to brownish with vertical splits; much-branched; Twigs smooth, spiny, covered with linear lenticels. Leaf buds long ellipsoid, with 5–7 hairy scales outside. Leaves fascicled on short branches; petiole 2–12 mm



**Figure 5.** Line drawing of *Pyrus zhaoxuanii* **A** flowering plant **B** fruiting plant **C** flower **D** appearance of fruit **E** leaf **F** pistil **G** longitudinal section of flower **H** longitudinal and cross section of fruit **I** bud scales **J** stamens. Scale bars: 5 cm (**A**, **B**); 1 cm (**C**, **D**, **E**, **F**, **G**); 2 cm (**H**); 2 mm (**I**); 5 mm (**J**). Illustrated by Si-Rui Pan.

long; Leaf blade obovate, rarely elliptic, (14) 19-31 (45) × (7) 10-15 (23) mm, margin obtusely serrate, base cuneate, apex blunt or round, rarely acuminate, leathery in quality. Corymb 2–8 flowered, receptacle cup-shaped, covered with short hairs; sepals are triangular, ca. 4 mm long, woolly, with rust-colored velutinous on the margins, bending downwards at the apex; flower 12–17.5 (29.5) mm in diameter; Petals 5,  $5-8 \times 5-6$  mm, pure white, glabrous, obovate, margin slightly sinuate, apex rounded; filament 3–7 mm long; anthers purple to pink; style 2, 4–6.5 mm long, glabrous; flower stalk (19) 21–35 mm long. Pome small, spheroid, reddish-brown to brownish-black, ca. 1 cm long; fruiting pedicel 18–36 mm long. Seeds ovate, blackish, ca. 2 × 5 mm.

**Phenology.** Flowering was observed from February to March, while fruiting occurred from September to October.



Figure 6. Holotype of Pyrus zhaoxuanii, Y.Y. Wu et al. DNPC4016 (SYS).

**Etymology.** *Pyrus zhaoxuanii* is named in honor of Prof. Zhao-Xuan Zeng (1921–2007), a famous geographer of South China Normal University, who made significant contribution to the study of danxia landscape.

**Distribution and habitat.** The new species is currently known only from its type locality, Mount Danxiashan, Renhua County, Guangdong Province, China. It typically grows on steep slopes at altitudes of 200–600 m above sea level.

**Conservation status.** The new species is a common shrub found on the steep slopes of Mount Danxia. Most individuals are located within the Danxia Nature Reserve, which is well protected, and we observed no active threats or ongoing declines in population size. According to the Guidelines for Using the IUCN Red List Categories and Criteria, v. 16 (IUCN Standards and Petitions Committee 2024), we suggest classifying *P. zhaoxuanii* as Least Concern(LC).

Additional specimens examined (Paratypes): CHINA. • Guangdong Province, Shaoguan City, Danxiashan National Nature Reserve, 113°39'50.56"N, 25°0'27.81"E, alt. 328 m, 24 February 2024, Y.Y. *Wu et al. DNPC4014* (SYS!); CHINA. • Guangdong Province, Shaoguan City, Danxiashan National Nature Reserve, 24°58'28.73"N, 113°44'10.29"E, alt. 285 m, 26 September 2023, Y.Y. *Wu* & Q. Fan 102 (SYS!).

# Acknowledgements

We are grateful to the Guangdong Danxiashan National Nature Reserve for their support in collecting all materials. Our appreciation extends to Li-Juan Liao and Chu-Hui Zhang for their assistance in fieldwork. We would like to express our heartfelt thanks to the Biological Museum of Sun Yat-sen University for their help with specimen preparation and binding. Additionally, we greatly appreciate the assistance provided by Si-Rui Pan in the illustration.

# Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

# Funding

This study was supported by the Guangdong Provincial Special Research Grant for the Creation of National Parks (2021GJGY034), the National Natural Science Foundation of China (32370225, 32370216, 32270216, and 32000163), the Innovation and Entrepreneurship Training Program for College Students (33000-11220004), and the Youth Innovation Promotion Association Chinese Academy Sciences (2023086).

# **Author contributions**

Investigation: ZXC, FC. Validation: QF. Visualization: YYW. Writing - original draft: XWY. Writing - review and editing: BBL, CYH.

#### Author ORCIDs

Xiao-Wei Yi <sup>©</sup> https://orcid.org/0009-0004-8867-7418 Qiang Fan <sup>®</sup> https://orcid.org/0000-0003-4254-6936 Bin-Bin Liu <sup>®</sup> https://orcid.org/0000-0002-0297-7531 Cui-Ying Huang <sup>®</sup> https://orcid.org/0009-0009-6134-2032

#### **Data availability**

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

# Reference

- Bell RL, Zimmerman RH (1990) Combining Ability Analysis of Juvenile Period in Pear. HortScience 25: 1425–1427. https://doi.org/10.21273/HORTSCI.25.11.1425
- Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021) Twelve years of SAMtools and BCFtools. Giga-Science 10: giab008. https://doi.org/10.1093/gigascience/giab008
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small amounts of fresh leaf tissue. https://www.semanticscholar.org/paper/A-rapid-DNA-isolation-procedurefor-small-amounts-Doyle-Doyle/5c9d80b422d61d0a3e454392568297e67717c861 [October 5, 2024]
- Gu C-Z, Spongberg SA (2003) Pyrus Linnaeus. In: Wu Z-Y, Raven PH (Eds) Flora of China, Vol. 9. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 173–179. http://www.iplant.cn/info/Pyrus?t=foc
- IUCN Standards and Petitions Committee (2024) Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf [Accessed 25.03.2024]
- Jin Z-T, Hodel RGJ, Ma D-K, Wang H, Liu G-N, Ren C, Ge B-J, Fan Q, Jin S-H, Xu C, Wu J, Liu B-B (2023) Nightmare or delight: Taxonomic circumscription meets reticulate evolution in the phylogenomic era. Molecular Phylogenetics and Evolution 107914. https://doi.org/10.1016/j.ympev.2023.107914
- Jin, Z-T, Ma D-K, Liu G-N, Hodel RGJ, Jiang Y, Ge B-J, Liao S, Duan L, Ren C, Xu C, Wu J, Liu B-B (2024a) Advancing Pyrus phylogeny: Deep genome skimming-based inference coupled with paralogy analysis yields a robust phylogenetic backbone and an updated infrageneric classification of the pear genus (Maleae, Rosaceae). Taxon, tax.13163. https://doi.org/10.1002/tax.13163
- Jin Z-T, Lin X-H, Ma D-K, Hodel RGJ, Zhao L, Ren C, Duan L, Xu C, Wu J, Liu B-B (2024b) Unraveling the Web of Life: Incomplete lineage sorting and hybridization as primary mechanisms over polyploidization in the evolutionary dynamics of pear species. https://doi.org/10.1101/2024.07.29.605463
- Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285
- Li H (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. https://doi.org/10.48550/arXiv.1303.3997
- Liu B-B, Hong D-Y, Zhou S-L, Xu C, Dong W-P, Johnson G, Wen J (2019) Phylogenomic analyses of the Photinia complex support the recognition of a new genus Phippsiomeles and the resurrection of a redefined Stranvaesia in Maleae (Rosaceae). Journal of Systematics and Evolution 57: 678–694. https://doi.org/10.1111/jse.12542
- Liu B-B, Liu G-N, Hong D-Y, Wen J (2020b) Eriobotrya Belongs to Rhaphiolepis (Maleae, Rosaceae): Evidence From Chloroplast Genome and Nuclear Ribosomal DNA Data. Frontiers in Plant Science 10: 1731. https://doi.org/10.3389/fpls.2019.01731
- Liu B-B, Campbell CS, Hong D-Y, Wen J (2020a) Phylogenetic relationships and chloroplast capture in the Amelanchier-Malacomeles-Peraphyllum clade (Maleae,

Rosaceae): Evidence from chloroplast genome and nuclear ribosomal DNA data using genome skimming. Molecular Phylogenetics and Evolution 147: 106784. https://doi.org/10.1016/j.ympev.2020.106784

- Liu B-B, Ren C, Kwak M, Hodel RGJ, Xu C, He J, Zhou W-B, Huang C-H, Ma H, Qian G-Z, Hong D-Y, Wen J (2022) Phylogenomic conflict analyses in the apple genus Malus s.l. reveal widespread hybridization and allopolyploidy driving diversification, with insights into the complex biogeographic history in the Northern Hemisphere. Journal of Integrative Plant Biology 64: 1020–1043. https://doi.org/10.1111/jipb.13246
- Liu G-N, Ma D-K, Zhang Y, Hodel RGJ, Xie S-Y, Wang H, Jin Z-T, Li F-X, Jin S-H, Zhao L, Xu C, Wei Y, Liu B-B (2023) Phylogenomic analyses support a new infrageneric classification of Pourthiaea (Maleae, Rosaceae) using multiple inference methods and extensive taxon sampling. Taxon 72: 1285–1302. https://doi.org/10.1002/tax.13083
- Lo EYY, Donoghue MJ (2012) Expanded phylogenetic and dating analyses of the apples and their relatives (Pyreae, Rosaceae). Molecular Phylogenetics and Evolution 63: 230–243. https://doi.org/10.1016/j.ympev.2011.10.005
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015
- Ortiz EM (2019) vcf2phylip v2.0: convert a VCF matrix into several matrix formats for phylogenetic analysis. scite.ai. https://doi.org/10.5281/zenodo.2540861
- Phipps JB, Robertson KR, Rohrer JR, Smith PG (1991) Origins and Evolution of Subfam. Maloideae (Rosaceae). Systematic Botany 16: 303–332. https://doi. org/10.2307/2419283
- Pu F, Wang Y (1963) Pomology of China. Vol. 3. Pears. Shanghai Sci. and Technol. Press, Shanghai, China. [in Chinese]
- Teng Y (2011) The Pear industry and research in China. Acta Horticulturae (909): 161–170. https://doi.org/10.17660/ActaHortic.2011.909.16
- Wang H, Li X-Y, Jiang Y, Jin Z-T, Ma D-K, Liu B, Xu C, Ge B-J, Wang T, Fan Q, Jin S-H, Liu G-N, Liu B-B (2024) Refining the phylogeny and taxonomy of the apple tribe Maleae (Rosaceae): Insights from phylogenomic analyses of 563 plastomes and a taxonomic synopsis of Photinia and its allies in the Old World. PhytoKeys 242: 161–227. https://doi.org/10.3897/phytokeys.242.117481
- Westwood MN, Bjornstad HO (1971) Some Fruit Characteristics of Interspecific Hybrids and Extent of Self- Sterility in Pyrus. Bulletin of the Torrey Botanical Club 98: 22. https://doi.org/10.2307/2483493
- Westwood MN, Challice JS (1978) Morphology and Surface Topography of Pollen and Anthers of Pyrus Species1. Journal of the American Society for Horticultural Science 103: 28–37. https://doi.org/10.21273/JASHS.103.1.28
- Wu J, Wang Y, Xu J, Korban SS, Fei Z, Tao S, Ming R, Tai S, Khan AM, Postman JD, Gu C, Yin H, Zheng D, Qi K, Li Y, Wang R, Deng CH, Kumar S, Chagné D, Li X, Wu J, Huang X, Zhang H, Xie Z, Li X, Zhang M, Li Y, Yue Z, Fang X, Li J, Li L, Jin C, Qin M, Zhang J, Wu X, Ke Y, Wang J, Yang H, Zhang S (2018) Diversification and independent domestication of Asian and European pears. Genome Biology 19: 77. https://doi.org/10.1186/ s13059-018-1452-y
- Zhang S-D, Jin J-J, Chen S-Y, Chase MW, Soltis DE, Li H-T, Yang J-B, Li D-Z, Yi T-S (2017) Diversification of Rosaceae since the Late Cretaceous based on plastid phylogenomics. The New Phytologist 214: 1355–1367. https://doi.org/10.1111/nph.14461

# **Supplementary material 1**

#### GeneBank accession numbers of the sampled species used in this study

Authors: Xiao-Wei Yi, Ying-Yu Wu, Qiang Fan, Fang Chen, Zai-Xiong Chen, Bin-Bin Liu, Cui-Ying Huang

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.138039.suppl1

# PhytoKeys

**Research Article** 

# *Thismia aliasii* (Thismiaceae), a new species from Terengganu, Peninsular Malaysia

Mat Yunoh Siti-Munirah<sup>10</sup>, Shakri Mohamad Alias<sup>2</sup>

1 Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia

2 Jabatan Perhutanan Negeri Terengganu, 21500 Kuala Terengganu, Terengganu, Malaysia

Corresponding author: Mat Yunoh Siti-Munirah (sitimunirah@frim.gov.my)

#### Abstract

A new mycoheterotrophic species, *Thismia aliasii*, is described and illustrated. This species inhabits a hill dipterocarp forest in mountains of eastern Peninsular Malaysia. *Thismia aliasii* differs from other *Thismia* species by the following features: tepals equal in size and shape with different length of their appendages, appendages of the outer tepals shorter than those of the inner tepals (3.5 mm vs. ca. 26–32 mm long), stamen supraconnective at apex with three long filiform appendages and two acute appendages, and the margins of individual connectives abaxially raised into a conspicuous rib. With respect to floral morphology, *T. aliasii* should be placed to *Thismia* subsect. *Odoardoa*. According to the categories and criteria of the IUCN Red List, *T. aliasii* is provisionally classified as Critically Endangered (CR).

**Key words:** Achlorophyllous plants, endemism, Gunung Chemerong, monocots, Terengganu, *Thismia* subsection *Odoardoa* 

# Introduction

The fully mycoheterotrophic monocot family Thismiaceae is mainly confined to tropical and subtropical regions. Species of the family are small herbs that usually inhabit shady, humid environments, often hidden under the leaf litter (Merckx et al. 2024). The genus *Thismia* Griff., often referred to as "fairy lanterns" (Wapstra et al. 2005), is remarkable due to its unusual flower appearance. The unique flower design of the genus is related to the specialized pollination mechanisms that involve small insects such as fungus gnats (belonging to the genus *Corynoptera*) (Guo et al. 2019). *Thismia* flowers were also visited and possibly pollinated by a wide range of other invertebrates, such as scuttle flies (Phoridae, Diptera) (Yudina et al. 2021).

The genus *Thismia* comprises about 116 species (Besi et al. 2024; Chung et al. 2024; Chunyang et al. 2024; Nuraliev and Sennikov 2024; POWO 2024; Siti-Munirah et al. 2024; Ya et al. 2024). It is the most widespread and species-rich genus within the family and has a high degree of endemism. Its range extends from tropical and subtropical Asia to northern and eastern Australia and New Zealand, and from Costa Rica to tropical South America with an isolated occurrence in North America (POWO 2024). Many of its species are considered extremely rare, with scattered



Academic editor: Michal Hroneš Received: 1 September 2024 Accepted: 20 February 2025 Published: 31 March 2025

**Citation:** Siti-Munirah MY, Mohamad Alias S (2025) *Thismia aliasii* (Thismiaceae), a new species from Terengganu, Peninsular Malaysia. PhytoKeys 254: 175–188. https://doi. org/10.3897/phytokeys.254.136085

#### Copyright:

© Mat Yunoh Siti-Munirah & Shakri Mohamad Alias. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). distributions, and some may already be extinct, but there are also species with quite wide ranges and multiple known localities (Dančák et al. 2020).

The state of Terengganu is currently known to be the richest of the Peninsular Malaysian states in the species diversity of *Thismia* (Table 1, Fig. 1). All the 13 species known from Terengganu are present in the northern part of the state. The southern Terengganu, in contrast, is known to be inhabited by only two species of *Thismia*, both found in the vicinity of hill and mountain range known as Gunung (Gn.) Chemerong, which is part of the Hutan Lipur Gn. Chemerong (HLGC) or Chemerong Forest Eco Park and also well known as Chemerong-Berembun-Langsir (CBL) mountain range. This area is, in turn, a part of the Pasir Raja Forest Reserve (FR). One of these two species is *T. aseroe* (Fig. 2D), whereas the other one is *T. aliasii* described here as new to science.

# Materials and methods

The assessment is based on the material collected in Gn. Chemerong, Hulu Terengganu, Terengganu (Fig. 3). *Thismia aliasii* was first discovered during a field trip of the second author to CBL in 2019. The specimens were preserved in 70% ethanol and stored in the Kepong Herbarium (KEP). Three flowering individuals and one fruiting plant were collected and studied in total. Morphological characteristics were examined and measurements performed using an Olympus SZ61 stereomicroscope and high-resolution macrophotography. The measurements were made on fresh and spirit material.

# **Taxonomic account**

Thismia aliasii Siti-Munirah, sp. nov.

urn:lsid:ipni.org:names:77359488-1 Figs 4–6

**Diagnosis.** *Thismia aliasii* is very similar to the species of the *T*. subsection *Odoardoa*, as the tepals are the same in shape and size. However, the tepal appendages of the new species are of unequal length, the inner ones are longer than the outer ones, while the tepal appendages of the other species are of

No.	Species	Locality in Terengganu	Status of Endemism
1.	T. alba Holttum ex Jonker	Taman Negeri Kenyir, Tembat FR	No
2.	T. aliasii Siti-Munirah	Gn. Chemerong (Pasir Raja FR)	Endemic
3.	T. arachnites Ridl.	Taman Negeri Kenyir	No
4.	T. aseroe Becc.	Gn. Chemerong (Pasir Raja FR)	No
5.	T. brunneomitroides Suetsugu & Tsukaya	Taman Negeri Kenyir	No
б.	T. clavigeroides Chantanaorr. & Seelanan	Taman Negeri Kenyir	No
7.	T. domei Siti-Munirah	Hulu Telemong FR	Endemic
8.	T. javanica J.J.Sm.	Hulu Telemong FR, Tembat FR	No
9.	T. kenyirensis Siti-Munirah & Dome	Taman Negeri Kenyir	Endemic
10.	T. latiffiana Siti-Munirah & Dome	Gn. Sarut (Hulu Nerus FR)	Endemic
11.	T. ornata Dančák, Hroneš & Sochor	Gn. Padang	No
12.	T. sitimeriamiae Siti-Munirah, Dome & Thorogood	Gn. Sarut (Hulu Nerus FR)	Endemic
13.	T. terengganuensis Siti-Munirah	Hulu Telemong FR	Endemic

Table 1. Thismia of Terengganu state (Siti-Munirah and Dome 2019, 2021, 2022, 2023a, 2023b; Siti-Munirah et al. 2021).



Figure 1. Thismia species occurring in the state of Terengganu A Thismia alba B T. arachnites C T. aseroe D T. domei E T. javanica F T. kenyirensis G T. latiffiana H T. sitimeriamiae I T. terengganuensis. Photos by Siti-Munirah (A–G, I) and Dome (H).



**Figure 2.** Landscapes of Gn. Chemerong and the species of *Thismia* found there **A** the Chemerong waterfall with a height of 370 m **B** the Chemerong River not far from the habitat of *T. aliasii* **C** *Thismia aliasii*, an individual discovered by Mohamad Alias in 2019 (FRI 91119) **D** *Thismia aseroe* (FRI 79116) found on the trail at Hutan Lipur Chemerong. Photos by Siti-Munirah (**A**, **B**, **D**) and Mohamad Alias (**C**).

equal length. In addition, in the new species the margins of the individual connectives are raised abaxially into the conspicuous rib, whereas connectives are almost flat abaxially in the rest of the species.

**Type.** MALAYSIA. • Peninsular Malaysia: Terengganu, Hulu Terengganu District, Hutan Simpan Pasir Raja, Chemerong Forest Eco Park, Gunung Chemerong, 4°39'33.2"N, 102°58'58.6"E, elev. ca 640 m, 26 July 2023, *Siti-Munirah, FRI* 79119 (holotype KEP!, spirit collection, barcode no. SC13201).



Figure 3. Gunung Chemerong (black circle) in Terengganu, the only known locality of *Thismia aliasii*.

Description. Achlorophyllous herb, up to ca. 11 cm tall, mostly glabrous (where not stated otherwise). Roots vermiform, unbranched, ca. 1.5 mm in diameter, light brown. Stem erect, up to 56 mm long, 1.8-2 mm in diameter, white-cream to light brown, bearing 1-2 flowers. Leaves up to 5, spirally arranged (arranged denser at stem base and looser at apex), triangular to narrowly triangular (shorter at stem base and longer and narrower at stem apex), scale-like, apex pointed, margin almost entire to slightly irregularly serrate, up to 7 mm long, ca. 2 mm wide at base, colored similar to stem. Flowers terminal and solitary, or in 2-flowered terminal inflorescence, actinomorphic, ca. 58 mm long (including ovary, floral tube, tepals and tepal appendages when erected). Involucral bracts 3, similar to upper leaves, triangular to narrowly triangular, scale-like, acute, with entire margin, 8 mm long, ca. 2.5 mm wide at base, white-brownish/light brown. Pedicel to 1.5 mm long at anthesis, to ca. 4 mm long after anthesis, white-brownish. Floral tube obovoid funnel-shaped, 20 mm long, ca. 4 mm wide at base, ca. 8 mm wide at middle, ca. 10 mm wide distally; outer surface with minute glands, orange to sepia-brown, with 12 darker longitudinal ribs; inner surface smooth or rough, almost similar color to the outer



**Figure 4**. *Thismia aliasii* **A** plant with flower and roots **B** top view of flower showing tepals and annulus **C** bract (abaxial view) **D** leaf (abaxial view) **E** style and stigma **F** stamens (outer view) **G** stamens (inner view) **H** fruiting plant. All drawn by Mohamad Aidil Noordin from spirit material, FRI 91119.

surface, with transverse bars. **Tepals** 6, free, spreading, triangular, apex acute, 7 mm long, ca. 1–1.5 mm wide (ca. 1.5 mm at base), smooth, color similar to flower tube (dark brown at apex, yellow-orange at back), each apically bearing a tentacle-like appendage 0.5 mm wide and narrowing towards the apex; **appendage of outer tepal** up to 3.5 mm long, dark brown; **appendage of inner tepal** ca. 26–32 mm long, brownish. **Annulus** moderately raised, hexagonal in outline,


Figure 5. *Thismia aliasii* **A** flowering plant (over-brightened by a camera flash) **B** anthetic flower, top view **B1** annulus, top view **B2** tip of appendage of outer tepal **B3** tip of appendage of inner tepal **C** distal portion of flower, lateral view **C1** flower, top view **D** floral tube (with its apex removed), side view **D1** outer surface of floral tube (showing tiny glands) **E** inner surface of flower tube, pistil and ovary **E1** longitudinal section of ovary and pistil with stigma **F** fruiting plant **F1** seeds in capsule **F2** seeds. Photos by Siti-Munirah (**A, C1**: FRI 79119; **A1, B–E**: FRI 79167; **E1, F1, F2**: FRI 91119, spirit material) and Mohamad Alias (**F**: FRI 91119).

ca. 6 mm in diameter, with ring width ca 1.5 mm, brown-orange, aperture ca. 3 mm in diameter. Stamens 6, pendulous from annulus; filaments laterally pale orange, otherwise translucent white; connectives broad and flattened, fused laterally into a tube, shortly papillose, translucent white, with hairs around thecae, with margins abaxially raised to form conspicuous ribs along the sutures between the connectives, ribs distally slightly protruding beyond the apex of supraconnective; interstaminal glands conspicuous, placed between bases of lateral appendages; supraconnective with 3 filiform apical appendages (central appendage ca. 2 mm long and paired appendages ca. 2.4 mm long) and with 2 acute hook-like subapical appendages ca. 0.6 mm long positioned adaxially between the apical appendages and the lateral appendage; lateral appendage skirt-like, projecting towards the floral tube and not reaching the supraconnective apex, translucent white and brown on sides, lateral margin wavy. Ovary red, pale cream proximally and blackish distally; unilocular; placentas 3, free, column-like, arising at ovary base; ovules numerous. Style dark blackish-orange, ca. 1.2 mm long; stigma ca. 1.4 mm long, papillose, 3-lobed, erect, lobes ± rectangular, bifurcate at the apex, black-greenish. Fruit dehiscent, cup-shaped, ca. 6 mm high, ca. 7 mm in diameter, pale brownish, darker in the upper part. Seeds long fusiform ca. 0.75 mm long, ca. 0.25 mm wide.

Additional specimens examined. MALAYSIA. • Peninsular Malaysia: Terengganu, Hulu Terengganu District, Pasir Raja FR, Chemerong Forest Eco Park, Gunung Chemerong, elev. ca 642 m, 3 October 2019, Mohamad Alias, FRI 91119 (KEP spirit collection, barcode no. SC13202); elev. ca 640 m, 26 July 2023, Siti-Munirah, FRI 79167 (KEP spirit collection, barcode no. SC13203).

**Distribution.** Endemic to Terengganu, Peninsular Malaysia. Currently only known from the type locality, Gunung Chemerong (Figs 2, 3, 7).

**Ecology.** The species inhabits moist shady areas of upper hill dipterocarp forest on moist soil at elevation of 640 m a.s.l. Flowering and fruiting recorded in July and October. The population was found in moist, shaded areas next to the main hiking trail to the summit of Gunung Chemerong Berembun Langsir at altitudes of 640 m above sea level. The species was found a few hundred meters away from the main river.

**Etymology.** The species is named after Mr. Alias (the second author), a current ranger of the Terengganu Forestry Department (JPNT), who is also a freelance photographer (known as John Sp) and who was the first to discover the species.

**Conservation status.** Since 2019, several surveys have been conducted at an area of 4 hectares, but the species was observed only twice, with a total of 5 individuals recorded. The main threat to the population is the degradation of habitat quality due to hiking activities, as the population is found near the main hiking trail which is heavily used by hikers ascending to the summits of Gunung Chemerong Berembun Langsir. Therefore, according to IUCN Red List (IUCN 2024) this species is assessed as Critically Endangered, CR B2ab(iii), D. More surveys are needed to determine the population size of the species.

**Notes.** *Thismia aliasii* is easily recognized within the genus by the combination of the following characters: vermiform roots, almost uniform flower coloration (light to dark orange to sepia-brownish red), inner tepals free from each other, unequal tepal appendages (appendages of the outer tepals being shorter than those of the inner tepals), stamens each with 3 long filiform apical appendages, 2 acute subapical appendages and a lateral appendage, and connectives



**Figure 6**. *Thismia aliasii* **A** longitudinally dissected stamen tube showing the margins of individual connectives abaxially raised into conspicuous ribs (spirit material) **A1** stamens, view from inside **B**, **C** stamens, view from outside **D**, **E** stamen tube, oblique view from below and view from below (showing apical appendages and longitudinal ribs). Yellow arrows: filiform apical appendages; red arrows: acute hook-like subapical appendage; orange arrows: anthers. All photos by Siti-Munirah (**A**: FRI 91119; **A1**, **B–E**: FRI 79167).



Figure 7. Habitat and habit of *Thismia aliasii* **A** plants in situ **B** plant with flower bud **C** plant with anthetic flower. All photos by Siti-Munirah.

laterally thickened into conspicuous abaxial interstaminal ribs. Within the infrageneric classification of Kumar et al. (2017), *T. aliasii* is assigned here to *Thismia* subgenus *Thismia* section *Thismia* subsection *Odoardoa* Schltr., as long as it has free tepals equal in shape and size. The new species probably belongs to clade 5 defined by Shepeleva et al. (2020), which is characterized by free inner tepals and the presence of appendages of the outer and inner tepals. In addition, the fact that the appendages of the inner tepals are much longer than those of the outer tepals make *T. aliasii* similar to *T. neptunis* Becc., (Sochor at al. 2018), which belongs to *T.* subgen. *Thismia* sect. *Thismia* subsect. *Brunonithismia* Jonker according to Kumar et al. (2017), but was recovered as a member of clade 5 by Shepeleva et al. (2020).

The connectives of most *Thismia* species are usually flat and without any processes abaxially, making *T. aliasii* unique not only among *T.* subsect. *Odoardoa* but within the entire genus. The only other exceptions are species from *T.* sect. *Geomitra* (Becc.) Kumar & S.W. Gale which have ribs in the center of each stamen but differ by coralliform roots and flower mitre.

We summarize *Thismia* subsect. *Odoardoa* to comprise 23 species based on the above-mentioned characteristics and following the classifications of Kumar et al. (2017) and Shepeleva et al. (2020) with additions from Chantanaorrapint et al. (2016), Hroneš et al. (2018), Nishioka et al. (2018), Dančák et al. (2020), Siti-Munirah and Dome (2019), Siti-Munirah et al. (2024), Ya et al. (2024):

# A checklist of *Thismia* subg. *Thismia* sect. *Thismia* subsect. *Odoardoa* Schltr., 1921.

Type species: -Thismia aseroe Becc., 1878.

Species included:

- 1) T. alba Holttum ex Jonker, 1948.
- 2) T. aliasii Siti-Munirah, 2025. (this study)
- 3) T. annamensis K.Larsen & Aver., 2007.
- 4) T. aseroe Becc., 1878.
- 5) T. bifida M. Hotta, 1967.
- 6) T. bryndonii Tsukaya, Suetsugu & Suleiman, 2017.
- 7) T. chrysops Ridl., 1895.
- 8) T. claviformis Chantanaorr. & J.Wai, 2016.
- 9) T. cornuta Hroneš, Sochor & Dančák, 2018.
- 10) T. domei Siti-Munirah, 2019.
- 11) T. filiformis Chantanaorr., 2012.

12) T. fumida Ridl., 1890.

- 13) T. grandiflora Ridl., 1895.
- 14) T. hexagona Dančák, Hroneš, Kobrlová & Sochor, 2013.

T. hexagona var. grandiflora Tsukaya, Suleiman & H.Okada, 2014.

- 15) T. inconspicua Sochor & Dančák, 2017.
- 16) T. kinabaluensis T.Nishioka & Suetsugu, 2018.
- 17) T. lauriana Jarvie, 1996.

18) T. malayana Siti-Munirah, Hardy-Adrian, Mohamad-Shafiq & Irwan-Syah, 2024.

19) *T. mullerensis* Tsukaya & H.Okada, 2005.

20) T. ophiuris Becc., 1878.

- 21) T. ornata Dančák, Hroneš & Sochor, 2020.
- 22) T. pallida Hroneš, Dančák & Rejzek, 2018.
- 23) T. racemosa Ridl., 1915.

Ten species of the subsection occur in Peninsular Malaysia (*T. alba, T. aseroe, T. chrysops, T. domei, T. fumida, T. grandiflora, T. malayana T. ornata, T. racemosa* and *T. aliasii*), with five of them (*T. alba, T. aliasii*, *T. aseroe, T. domei* and *T. ornata*) occurring in the state of Terengganu. Whereas most of these species are characterized by tepal appendages equal in size and shape, in *T. aliasii* the appendages of the inner tepals are longer than those of the outer tepals. In addition, the stamen connectives laterally raised to form a conspicuous interstaminal rib are a unique feature of *T. aliasii* that is not found in the other species of *T. subsect. Odoardoa*.

# Acknowledgements

This research was conducted under the Flora of Peninsular Malaysia Project (FPM) at the Forest Research Institute Malaysia (FRIM), Kepong, funded by the Ministry of Natural Resources and Environmental Sustainability (NRES) under the 12<sup>th</sup> Malaysian Plan (SPPII No. P23085100018003) and the Nagao Research Grant to partially fund Thismia research. We thank the Jabatan Perhutanan Negeri Terengganu (JPNT) and Pejabat Hutan Daerah Dungun, MARDI team, as well as Dome Nikong of WildDome for their continuous support of Thismia research in Terengganu. Special thanks to Zubir Ahmad MI and Angan A for their help in searching for Thismia in the field and to the flora team (KEP (Imin K and Syahida-Emiza S) and KBG (Suhaida M, Khairil Azuar AK and Ahmad Norsidar AH)) and Zainul Ariffin AS and Amirul I for their support in the field work of Gn. Chemerong. We thank Michal Hroneš, Martin Dančák, Maxim Nuraliev and Sahut Chantanaorrapint for constructive comments on our manuscript, Mohamad Aidil Noordin for botanical drawings, Wendy SY Yong for assistance in conservation status assessment, and Mohd Faizal MT for encouragement and great help in many ways to the first author.

# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

# Funding

This research was funded by the Ministry of Natural Resources and Environmental Sustainability (NRES) under the 12<sup>th</sup> Malaysian Plan (SPPII No. P23085100018003) and the Nagao Research Grant to partially fund *Thismia* research.

# **Author contributions**

All authors have contributed equally.

# **Author ORCIDs**

Mat Yunoh Siti-Munirah D https://orcid.org/0000-0002-5062-9988

# Data availability

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

#### References

- Besi EE, Qistina Anis MJ, Syamil AR, Mohd Zulkifly S, Ag Shaffie AA, Go R (2024) *Thismia perlisensis* (Thismiaceae), a new red-annulus *Thismia* species from Peninsular Malaysia. Phytotaxa 675(1): 1–10. https://doi.org/10.11646/phytotaxa.675.1.1
- Chantanaorrapint S, Wai JS, Poopath M, Tetsana N (2016) *Thismia claviformis* (Thismiaceae), a new species from the Thai-Malay Peninsula. Phytotaxa 280(3): 292–296. https://doi.org/10.11646/phytotaxa.280.3.8
- Chung SW, Hsu TC, Lin CW, Yang TYA, Fanerii M, Pitisopa F, Li CW (2024) Three new species and new records of *Thismia* (Thismiaceae) in The Solomon Islands. Taiwania 69(3): 336–347. https://doi.org/10.6165/tai.2024.69.336
- Chunyang L, Haijun Y, Wenhao Q, Han X (2024) *Thismia jinzun* (Thismiaceae), A New Species of *Thismia* Genus from Hainan Island, China. Phytotaxa 664(4): 273–280. https://doi.org/10.11646/phytotaxa.664.4.4
- Dančák M, Hroneš M, Sochor M (2020) *Thismia ornata* and *T. coronata* (Thismiaceae), two new species from Sarawak, Borneo. Willdenowia 50(1): 65–76. https://doi. org/10.3372/wi.50.50106
- Guo X, Zhao Z, Shing Mar S, Zhang D, Saunders RMK (2019) A symbiotic balancing act: Arbuscular mycorrhizal specificity and specialist fungus gnat pollination in the mycoheterotrophic genus *Thismia* (Thismiaceae). Annals of Botany 124(2): 331–342. https://doi.org/10.1093/aob/mcz087
- Hroneš M, Rejžek M, Sochor M, Svátek M, Kvasnica J, Egertová Z, Pereira JT, Nilus R, Dančák M (2018) Two new species of *Thismia* subsect. *Odoardoa* (Thismiaceae) from Borneo. Plant Ecology and Evolution 151(1): 110–118. https://doi. org/10.5091/plecevo.2018.1387
- IUCN Standards and Petitions Committee (2024) Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf
- Kumar P, Gale SW, Li J, Bouamanivong S, Fischer GA (2017) *Thismia nigricoronata*, a new species of Burmanniaceae (Thismieae, Dioscoreales) from Vang Vieng, Vientiane Province, Laos and a key to subgeneric classification. Phytotaxa 319(3): 225–240. https://doi.org/10.11646/phytotaxa.319.3.2
- Merckx VSFT, Gomes SIF, Wang D, Verbeek C, Jacquemyn H, Zahn FE, Gebauer G, Bidartondo MI (2024) Mycoheterotrophy in the wood-wide web. Nature Plants 10(5): 710–718. https://doi.org/10.1038/s41477-024-01677-0
- Nishioka T, Suetsugu K, Repin R, Kitayama K (2018) *Thismia kinabaluensis* (Thismiaceae), a new species from Mt. Kinabalu, Sabah, Borneo. Phytotaxa 360(2): 174–178. https://doi.org/10.11646/phytotaxa.360.2.10
- Nuraliev MS, Sennikov AN (2024) A checklist of *Thismia* section *Labiothismia* (Thismiaceae), reinstated to include Asian species earlier placed in the neotropical *T.* sect. *Glaziocharis*. Phytotaxa 662(1): 93–101. https://doi.org/10.11646/phytotaxa.662.1.7
- POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. https://powo.science.kew.org/ [Retrieved 1 September 2024]
- Shepeleva EA, Schelkunov MI, Hroneš M, Sochor M, Dančák M, Merckx VSFT, Kikuchi IABS, Chantanaorrapint S, Suetsugu K, Tsukaya H, Mar SS, Luu HT, Li H-Q, Logacheva MD, Nuraliev MS (2020) Phylogenetics of the mycoheterotrophic genus *Thismia* (Thismiaceae: Dioscoreales) with a focus on the Old World taxa: Delineation of novel natural groups and insights into the evolution of morphological traits. Botanical Journal of the Linnean Society 193(3): 287–315. https://doi.org/10.1093/botlinnean/boaa017

- Siti-Munirah MY, Dome N (2019) *Thismia domei* and *T. terengganuensis* (Thismiaceae), two new species, and *T. javanica*, a new record from Terengganu, Peninsular Malaysia. PhytoKeys 124: 123–137. https://doi.org/10.3897/phytokeys.124.34728
- Siti-Munirah MY, Dome N (2021) *Thismia* in Nerus Forest Reserve. Conservation Malaysia Bulletin 33: 8–9. https://www.mybis.gov.my/pb/4663
- Siti-Munirah MY, Dome N (2022) *Thismia latiffiana* (Thismiaceae), an unusual new species from Terengganu, Peninsular Malaysia. PhytoKeys 188: 105–114. https://doi.org/10.3897/phytokeys.188.77061
- Siti-Munirah MY, Dome N (2023a) *Thismia kenyirensis* (Thismiaceae), a new species from Taman Negeri Kenyir, Terengganu, Peninsular Malaysia. PhytoKeys 221: 61–72. https://doi.org/10.3897/phytokeys.221.98571
- Siti-Munirah MY, Dome N (2023b) *Thismia* (Thismiaceae) in Kenyir State Park, Terengganu. In: Ahmad et al. (Eds) Proceeding of Kenyir State Park Scientific Expedition. Universiti Malaysia Terengganu Press, Kuala Nerus, Terengganu, 49–56.
- Siti-Munirah MY, Dome N, Thorogood CJ (2021) *Thismia sitimeriamiae* (Thismiaceae), an extraordinary new species from Terengganu, Peninsular Malaysia. PhytoKeys 179: 75–89. https://doi.org/10.3897/phytokeys.179.68300
- Siti-Munirah MY, Hardy-Adrian C, Mohamad-Shafiq S, Irwan-Syah Z, Hamidi AH (2024) *Thismia malayana* (Thismiaceae), a new mycoheterotrophic species from Peninsular Malaysia. PhytoKeys 242: 229–239. https://doi.org/10.3897/phytokeys.242.120967
- Sochor M, Egertová Z, Hroneš M, Dančák M (2018) Rediscovery of *Thismia neptunis* (Thismiaceae) after 151 years. Phytotaxa 340(1): 71–78. https://doi.org/10.11646/ phytotaxa.340.1.5
- Wapstra M, French B, Davies N, O'Reilly-Wapstra J, Peters D (2005) A bright light of the dark forest floor: observations of the fairy lanterns *Thismia rodwayi* F. Muell. (Burmanniaceae) in Tasmanian forests. University of Tasmania. Tasmanian Field Naturalist Club Inc 127: 2–18. https://hdl.handle.net/102.100.100/590442
- Ya J-D, Chen H-Y, Zhang W, Zhu R-B, Cai J, Yu W-B (2024) Phylogenetic and biogeographical analyses of *Thismia* (Thismiaceae) support *T. malipoensis* as the eighth species in China. –. Willdenowia 54(1): 47–63. https://doi.org/10.3372/wi.54.54102
- Yudina SV, Vislobokov NA, Nuraliev MS (2021) Evidences of a mixed pollination strategy in Vietnamese species of *Thismia* (Thismiaceae: Dioscoreales). Wulfenia 28: 109–128.



**Research Article** 

# *Impatiens maolanensis* (Balsaminaceae), a new species of *Impatiens* in a tiankeng from Guizhou, China

Bai-Zhu Li<sup>1\*®</sup>, Qin-Ying Wen<sup>2,3\*®</sup>, Jin-Dong Wang<sup>2,3\*®</sup>, Xiao-Xiang Huang<sup>4</sup>, Zhi-Bin Xiong<sup>4</sup>, Zhi-Juan Deng<sup>2,3</sup>, Yin Yi<sup>2,3®</sup>, Xiao-Xin Tang<sup>2,3®</sup>

- 1 School of Life Sciences, Central China Normal University, Wuhan 430079, China
- 2 Key Laboratory of National Forestry and Grassland Administration on Biodiversity Conservation in Karst Mountainous Areas of Southwestern China, School of Life Science, Guizhou Normal University, Guiyang 550025, China
- 3 Key Laboratory of Plant Physiology and Developmental Regulation, School of Life Science, Guizhou Normal University, Guiyang 550025, China
- 4 Maolan National Nature Reserve Administration Bureau, Qiannan Buyei and Miao Autonomous Prefecture 558400, China

Corresponding author: Xiao-Xin Tang (tangxiaoxin@gznu.edu.cn)



Academic editor: Eberhard Fischer Received: 28 November 2024 Accepted: 23 February 2025 Published: 1 April 2025

**Citation:** Li B-Z, Wen Q-Y, Wang J-D, Huang X-X, Xiong Z-B, Deng Z-J, Yi Y, Tang X-X (2025) *Impatiens maolanensis* (Balsaminaceae), a new species of *Impatiens* in a tiankeng from Guizhou, China. PhytoKeys 254: 189–200. https://doi.org/10.3897/ phytokeys.254.142981

**Copyright:** <sup>©</sup> Bai-Zhu Li et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

#### Abstract

Impatiens maolanensis Z.B.Xiong & Q.Y.Wen (Balsaminaceae), a new species of Impatiens subg. Clavicarpa from Maolan National Nature Reserve, Guizhou, China, is described. The new species grows in a tiankeng (a large, naturally formed pit) connected to a dried-up underground river. I. maolanensis is similar to I. auriculata Chang Y. Xia & S. X. Yu, I. liboensis K. M. Liu & R. P. Kuang and I. tianlinensis S. X. Yu & L. J. Zhang, but differs from the latter three species in terms of orange-red flowers, roots, stems, bracts, dorsal petals, lateral sepals, lower sepals, pollen grains and seeds micromorphology. The micromorphological characteristics and surface patterning of pollen grains and seeds of the new species were examined using scanning electron microscopy (SEM). Pollen grains of I. maolanensis are triangular-round in polar view and elliptical in equatorial view. The pollen exine has an irregular and relatively smooth reticulate ornamentation, and under high magnification, granular protrusions can be observed. Seeds of I. maolanensis are black and narrowly ellipsoid. The seed coat has reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes. Morphological and micromorphology evidence support the establishment of the new species. Our study provides detailed information on the new species, including morphological characteristics, phenology, photographs, palynology, seed micromorphology, etymology, habitat and distribution, and conservation assessment.

Key words: Balsaminaceae, morphology, new species, SEM, taxonomy

#### Introduction

The genus, *Impatiens* L., belonging to the family Balsaminaceae, is a large genus of angiosperms with approximately 1,000 species around the world (Grey-Wilson 1980; Janssens et al. 2006; Yu et al. 2016). It comprises two subgenera, subg. *Clavicarpa* S. X. Yu ex S. X. Yu & Wei Wang and subg. *Impatiens* 

<sup>\*</sup> These authors contributed equally to this work.

(Yu 2012; Yu et al. 2016; Zeng et al. 2016). *Impatiens* is widely distributed in the Northern Hemisphere, primarily in tropical and subtropical regions (Yu 2012). Most species within this genus are herbaceous plants, characterized by distinct floral structures consisting of one or two pairs of lateral sepals, a dorsal petal, two lateral united petals, and a lower sepal with a nectar spur (Yu et al. 2016). The morphological variation within *Impatiens* is exceedingly intricate, presenting numerous challenges to plant taxonomists (Chen 2001; Ruchisansakun et al. 2015; Li et al. 2022).

In China, more than 270 species of Impatiens are discovered, distributed across the country, with a concentration primarily in the southwest. Among them, more than 240 species are endemic to China, and the majority are narrow endemics, particularly in karst areas such as Yunnan, Guizhou, Sichuan, and Guangxi, where the endemism phenomenon is particularly pronounced (Yu 2012). Over recent years, a plethora of new species has been successively discovered in the areas (Yuan et al. 2011; Kuang et al. 2014; Cai et al. 2015; Luo et al. 2015; Tan et al. 2015; Ding et al. 2016; Cho et al. 2017; Xia et al. 2019; Qin et al. 2020; Gu et al. 2021; Liao et al. 2021, Song et al. 2021a, b; Yuan et al. 2022; Wang et al. 2022a, 2022b; Yuan et al. 2022; Zhang et al. 2023a; Hu et al. 2024; Song et al. 2024). Thus, the plants of Impatiens can be characterized by the phrase "one species per mountain, one species per cave, one species per karst basin" (where "karst basin" denotes a small basin encircled by multiple limestone hills within a karst landscape) (Yu 2012). Traditionally, morphological characteristics, pollen grains and seed micromorphology have been considered essential factors for distinguishing species and classifying them within Impatiens (Lu and Chen 1991; Yu 2012; Zeng et al. 2016).

In October 2024, during a field survey conducted at the Maolan National Nature Reserve, Libo County, Qiannan Buyei and Miao Autonomous Prefecture, Guizhou, China, we collected a species of Impatiens in a tiankeng connected to a dried-up underground river. According to Yu Shengxiang's classification, this species belongs to the subgenus Clavicarpa S. X. Yu ex S. X. Yu & Wei Wang of Impatiens in family Balsaminaceae (Yu 2012; Yu et al. 2016). Plants belonging to this subgenus are generally perennial herbs, distinguished by racemes with more than 5 flowers, 4 lateral sepals, 4-loculed carpels with a single seed per locule, clavated fruits, elliptical seeds, and 3-colpated pollen grains as key characteristics (Yu 2012). Impatiens maolanensis is similar to I. auriculata Chang Y. Xia & S. X. Yu, I. liboensis K. M. Liu & R. P. Kuang and I. tianlinensis S. X. Yu & L. J. Zhang in terms of morphology, floral structure, and capsule shape (Yu 2012; Kuang et al. 2014; Zeng et al. 2015; Zeng et al. 2016). However, it exhibits significant differences from the latter three species in terms of orange-red flowers, roots, stems, bracts, dorsal petals, lateral sepals, lower sepals, pollen grains, and seeds micromorphology.

Therefore, in early November 2024, we conducted a survey in Guizhou to collect flowering materials of the species. After careful observation of morphological characteristics, pollen grains and seed morphology, and following a comprehensive comparison with known *Impatiens* species (*I. auriculata*, *I. liboensis* and *I. tianlinensis*) (Chen 2001; Yu 2012; Kuang et al. 2014; Zhang et al. 2014; Zeng et al. 2015; Zeng et al. 2016; Zhang et al. 2023b) we confirmed this species as a new species and provide its description below.

# Material and methods

#### Morphological and micromorphological analysis

This study integrates data from herbarium specimens, digitized specimen images, field observations, and taxonomic literature. Specimens were meticulously examined through visits to the Institute of Botany, Chinese Academy of Sciences (**PE**), Guangxi Institute of Botany (**IBK**), Guangxi Medicinal Botanical Garden (**GXMG**), and Hunan Normal University (**HNNU**) (herbarium acronyms follow *Index Herbariorum*; Thiers, 2025). Digital images were additionally sourced from the Chinese Virtual Herbarium (**CVH**; https://www.cvh.ac.cn/). The taxonomic description followed the terminology used by Chen (2001) and Yu (2012). The holotype voucher specimens were stored at the Herbarium of Guizhou Normal University (**GZNU**). The conservation status of the new species was assessed following the guidelines of the IUCN Red List Categories and Criteria (IUCN 2024).

At the same time, we collected plant specimens, mature pollen grains, and seeds of I. maolanensis, I. auriculata and I. liboensis from Maolan National Nature Reserve, Libo County, Qiannan Buyei and Miao Autonomous Prefecture, Guizhou, China. We measured the plant height with a tape measure and used a vernier caliper to measure the flower characteristics of fresh plants. The collected mature pollen grains and seeds were enveloped in absorbent paper and subsequently placed into paper bags with silica gel for drying. Dried pollen grains and seeds were carefully attached to stubs with the aid of double-sided adhesive tape and then thinly coated with gold, approximately 2 nm in thickness, using a MSP-1S sputter coater for a duration of 90 seconds. The pollen grains and seeds coated with gold were subsequently observed and photographed using a HITACHI-SU8600 scanning electron microscope. The polar axis and equatorial axis diameters of 30 pollen grains, as well as the length and width of the seeds, were measured respectively. Micromorphological characteristics of the pollen grains and seeds were described following the methods outlined by Walker and Doyle (1975), Wang and Wang (1983), and Lu and Chen (1991). The morphological and pollen grains' micromorphological comparison was conducted between I. maolanensis and I. tianlinensis; the latter species was described in detail by Zeng et al (2015, 2016).

#### Results

#### **Taxonomic treatment**

#### Impatiens maolanensis Zhibin Xiong & Qinying Wen, sp. nov.

urn:lsid:ipni.org:names:77359625-1 Figs 1, 3A-D, 4A-C

**Diagnosis.** Impatiens maolanensis is similar to I. auriculata (Figs 2A–G, 3E–H, 4D–F), I. liboensis (Figs 2H–N, 3I–L, 4G–I) and I. tianlinensis (Zeng et al. 2015, 2016) but its characteristics are significantly different from the latter three species in terms of orange-red flowers, thick fibrous root, stem with leaf scars and nodes, petioles, sessile or nearly sessile, bracts, outer lateral sepals, dorsal petal, lower sepal, and fruit color (Table 1).

Characters	I. maolanensis	I. auriculata	I. liboensis	I. tianlinensis
Habitat	perennial	annual	perennial	perennial
Plant height	25–35 cm	50–160 cm	22-50 cm	50-80 cm
Root	thick fibrous root	fibrous root	globose or irregular underground tuber	-
Stem	erect, branched, with leaf scars and nodes	erect, branched	erect, unbranched	fleshy, erect, simple, robust; inferior nodes swollen
Leaves	over-oblong or ovate-lanceolate	oblong-ovate	ovate-oblong or nearly oblong	obovate to oblanceolate
petiole	sessile or nearly sessile	sessile or nearly sessile	1.5-5 cm long	(0.5-) 1–2 cm long (or upper leaves subsessile) with several short clavate glands
Flower color	orange-red and vertical stripes	yellow-red spots and markings	white or pink white	yellowish or cream
Inflorescence	4-flowered	3-11 flowered	3–7 flowered	3-5 (-7) flowered
Bracts	ovate with long-cuspidate apex, red	ovate or oblong-ovate, green	ovate or oblong-ovate, green	ovate, acute, deciduous
Outer lateral sepals	obliquely ovate, dark red	obliquely ovate, green	obliquely ovate, white-pink	ovate, symmetric, yellowish- green
Inner lateral sepals	linear-lanceolate, orange adaxial surface, orange with a red stripe on abaxial surface,	linear-lanceolate, yellow-green	linear-lanceolate, white-pink	sickle-shaped, inaequilateral, apex acuminate or caudate
Dorsal petals	oblong, orange, cuneate at base, obtuse and emarginate at apex, with abaxial midvein thickened and a vertical red stripe on both adaxial and abaxial surface	obovate or nearly round, yellow- green, obtuse at apex, emarginate, with abaxial midvien thickened	obovate, white-pink, round at base, obtuse at apex, emarginate, with abaxial midvien thickened, obviously but narrowly carinate	ovate, apex obtuse, base broadly cuneate, midrib obvious, with a slight dorsal crest
Lateral united petals	basal lobes, orange, nearly oblong, obtuse at apex; disal lobes, orange- red spots, obovate-oblong, obtuse at apex and near middle retuse, with abaxial auricle inflexed, suborbicular	basal lobes, yellow-red spots, nearly oblong, obtuse at apex; disal lobes, yellow-red spots, obovate- oblong, obtuse at apex, with with abaxial auricle inflexed, semi-ovate	basal lobes, white-yellow and red spots, nearly oblong, acute at apex; disal lobes, white-yellow and red spots, obovate-oblong or obliquely obvate, obtuse at apex and near middle retuse,with abaxial auricle inflexed, suborbicular	basal lobes, yellow-red spots, oblong; disal lobes, yellow-red spots, elliptic, yellow-apex emarginate, middle of inner margin without appendage
Lower sepal and spur	saccate, orange-red, with an incurved or spiraled spur, 3–4 cm long, with a vertical red stripe on the top and base of surface	funnel, yellow-red spots and markings, with a long inwardly curved spur	saccate, white-pink spots, with an incurved spur, 1.2–1.5 cm long	saccate, abruptly constricted into an involute spur, 1–1.5 cm long
Capsule	obovate-clavate, drak green-dark red, with a red beak at apex	obovate-clavate, green, with a beak at apex	obovate-clavate, dark green, swollen in the upper part rostellate at apex	hammer-shaped
flowering season	September to November	October to December	August to November	September to November
fruiting season	October to December	November to January	September to November	September to November
Pollen grains	triangular-round in polar view, three-colpate, exine has an irregular reticulate ornamentation	nearly triangular in polar view, three-colpate, exine has an irregular reticulate ornamentation	nearly triangular in polar view, three-colpate, exine has an irregular reticulate ornamentation	triangular in polar view, exine has an irregular reticulate ornamentation, and there are hardly any granular protrusions within the mesh under high magnification
Seeds	narrowly ellipsoid, 4.5 mm long, 2.42 mm wide, black, reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes	ellipsoid, 4.22 mm long, 2.71 mm wide, brown, reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes	ellipsoid, 3.48 mm long, 1.76 mm wide, brown, reticulate ornamentation with sunken meshes, folded base, and no granular protrusions within the meshes	ellipsoid

#### Table 1. Detailed comparison of I. maolanensis, I. auriculata, I. liboensis and I. tianlinensis.

Type. CHINA • Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture (黔南布依族苗族自治州), Libo County (荔波县), Maolan National Nature Reserve (茂兰国家级自然保护区), Karst terrain, 25°19'59"N, 108°2'58"E, alt. 541 m, 28 October 2024, *Zhibin Xiong and Qinying Wen* (holotype: GZNU2024102801!, isotypes: GZNU2024102802!, GZNU2024102803!).

**Etymology.** The specific epithet 'maolanensis' refers to the locality where this new species was discovered, located in Maolan National Nature Reserve, Libo County, Guizhou Province, China. The new species is named '茂兰凤仙花' in Chinese.



**Figure 1.** Habitat and morphology of *Impatiens maolanensis* Zhi-Bin Xiong & Q.Y.Wen, sp. nov. **A–C** habitat **D** plant **E** front view of flower **F–H** different views of flower **I** anatomy of flower **J** root **K** capsule **L** seed (Photographed by Zhi-Bin Xiong and Qin-Ying Wen).



Figure 2. Impatiens auriculata A front view of flower B, C different views of flower D habitat E root F capsule G anatomy of flower. Impatiens liboensis H front view of flower I, J different views of flower K habitat L root M capsule N anatomy of flower (Photographed by Qin-Ying Wen and Bai-Zhu Li).



**Figure 3.** Scanning electron micrographs of pollen grains. *Impatiens maolanensis* Zhi-Bin Xiong & Q.Y.Wen, sp. nov. **A** group view, ×1000 **B** polar view, ×3000 **C** equatorial view, ×3000 **D** exine ornamentation, ×7000. *Impatiens auriculata* **E** group view, ×1000 **F** polar view, ×3000 **G** equatorial view, ×3000 **H** exine ornamentation, ×7000. *Impatiens liboensis* **I** group view, ×1000 **J** polar view, ×3000 **K** equatorial view, ×3000 **L** exine ornamentation, ×7000 (Photographed by Bai-Zhu Li).

Description. Plants perennial, 25-35 cm tall. Roots fibrous, 0.5 cm thick or thicker, up to 9 cm long. Stem robust, erect, branched, 0.4 cm thick or thicker, with leaf scars and nodes. Leaves alternate, densely arranged at the top of stem, glabrous, deep green, membranous, 9-12.5 cm long, 2.5-4 cm wide; petiole, sessile or nearly sessile, with two glands at base; lamina 10.4–13.6 cm long, 3.1–3.7 cm wide, over-oblong or ovate-lanceolate, acuminate to cuspidate at apex, cuneate at base, with crenate margin, setose between marginal teeth; lateral veins in 6–9 pairs. Inflorescences in upper leaf axils, racemose, 2–4 flowered, peduncle 5-7 cm long, dark green to dark red; pedicels 1-2 cm long, dark red; bract 1, at base or middle of pedicel, persistent, bracts ovate with long-cuspidate apex, 3-5 mm long, dark red. Flowers orange-red, 4-5 cm long. Lateral sepals 4; outer lateral sepals 2, obliquely ovate, 1.5 cm long, 0.7 cm wide, dark red, acute at apex, with abaxial midvein slightly thickened; inner lateral sepals 2, linear-lanceolate, 1.5 cm long, 0.28 cm wide, orange adaxial surface, orange with a red stripe on abaxial surface, recurved at apex. Lower sepal saccate, gradually elongates into an incurved or spiraled spur, 3-4 cm long, with a vertical red stripe on the top and base of surface, 4 cm long (excluding spur); mouth obliguely upwards, 1.2-1.5 cm wide, acute at apex. Dorsal petal oblong, 1.2 cm in diameter, orange, cuneate at base, obtuse and emarginate at apex, with abaxial midvein thickened and a vertical red stripe on both adaxial and abaxial surface. Lateral united petals sessile, orange with red spots, 2-lobed, 2.3-2.6 cm long; basal lobes, orange, nearly oblong, 1.1 cm long, 0.5 cm wide, obtuse at apex; disal lobes, orange-red spots, obovate-oblong, 2.1 cm long, 0.9 cm wide, obtuse at apex and near middle retuse, with abaxial auricle inflexed, suborbicular. Stamens 5, ca. 5 mm long; filaments linear; anther small, ovate, obtuse at apex. Ovary superior, 4-carpellate, erect with axile placentation, fusiform, red, ca. 5 mm long; stigma four-lobed, red. Capsule obovate-clavate, with a red beak at apex, deep green to dark red, 1.5-2 cm long, 4-valved, fleshy. Seeds narrowly elliptical, 4.5 mm long, 2.42 mm wide, black.



**Figure 4.** Scanning electron micrographs of seeds. *Impatiens maolanensis* Zhi-Bin Xiong & Q.Y.Wen, sp. nov. **A** whole view, ×30 **B** partial view, ×1000 **C** partial view, ×5000. *Impatiens auriculata* **D** whole view, ×30 **E** partial view, ×1000 **F** partial view, ×5000. *Impatiens liboensis* **G** whole view, ×30 **H** partial view, ×1000 **I** partial view, ×5000 (Photographed by Bai-Zhu Li).

**Phenology.** Flowering season: September to November. Fruiting season: October to December.

Palynology. Pollen grains of Impatiens maolanensis, I. auriculata, I. liboensis and I. tianlinensis are three-colpate, with an exine with irregular reticulate ornamentation. Pollen grains of I. maolanensis are triangular-round in polar view and elliptical in equatorial view, with a polar: equatorial ratio of 28.4-32.5: 28.2-32.1 µm. The pollen exine has an irregular and relatively smooth reticulate ornamentation, and under high magnification, granular protrusions can be observed (Fig. 3A-D). Pollen grains of *I. auriculata* are nearly triangular in polar view and elliptical in equatorial view, with a polar: equatorial ratio of 27.5-32: 26.3-31.2 µm. The pollen exine has an irregular reticulate ornamentation and holes (Fig. 3E-H). Pollen grains of I. liboensis, are subellipsoid in polar view and elliptical in equatorial view, with a polar: equatorial ratio of 29.6-32.8: 28.7-32.6 µm. The pollen exine has an irregular reticulate ornamentation and holes (Fig. 31-L). Pollen grains of I. tianlinensis are triangular in polar, with a polar: equatorial ratio of 29.62-30.47: 12.68-13.54 µm. The pollen exine has an irregular reticulate ornamentation and holes, and there are hardly any granular protrusions within the mesh under high magnification (Zeng et al. 2015, 2016).

**Seed micromorphology.** Seeds of *Impatiens maolanensis*, narrowly ellipsoid, 4.5 mm long, 2.42 mm wide, black. The seed coat has reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes (Fig. 4A–C). Seeds of *I. auriculata*, ellipsoid, 4.22 mm long, 2.71 mm

wide, brown. The seed coat has reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes (Fig. 4D–F). Seeds of *I. liboensis*, ellipsoid, 3.48 mm long, 1.76 mm wide, brown. The seed coat has reticulate ornamentation with sunken meshes, folded base, and no granular protrusions within the meshes (Fig. 4G–I).

**Habitat and distribution.** Impatiens maolanensis has only been observed within the Maolan National Nature Reserve, Libo County, Qiannan Buyei and Miao Autonomous Prefecture, Guizhou Province, China (Figs 1A–C, 5). Its uniqueness lies in its growth within a tiankeng (a large, naturally formed pit or depression in the earth's surface) at an altitude of 541 m, within the typical karst area. To reach this location, one must first pass through a karst cave, descend approximately 100 m to a dried-up underground river, and then continue for about 200 m to reach the tiankeng where the species grows. In terms of ecological coexistence, *I. maolanensis* grows alongside various plants such as *Sphagnum* sp., ferns, bamboo, *Pilea* sp., and *I. liboensis*.

**Conservation status.** Our study documented a single population of 18 mature individuals within Guizhou Province, but no expanded surveys have been conducted in adjacent regions or potential habitats (e.g., karst landscapes in Guangxi or Yunnan). The conservation status of Impatiens maolanensis is currently assessed as Data Deficient (DD) under IUCN Red List Categories and Criteria. While the observed population size suggests potential vulnerability, the lack of comprehensive geographic sampling prevents robust assessment against extinction risk criteria (e.g., CR D).

Similar species. The new species, I. maolanensis is similar to I. auriculata, I. liboensis and I. tianlinensis in floral morphology. All species have alternate leaves, inflorescences, 4 lateral sepals, superior ovaries, 4 carpels, fusiform or obovate-clavate fruits, and three-colpate pollen grains. Nevertheless, the new species is easily distinguishable. Unlike the latter three species, I. maolanensis has a thick fibrous root; stem robust, with leaf scars and nodes; petioles, sessile or nearly sessile; flowers orange-red; bracts and outer lateral sepals, dark red; inner lateral sepals with a red stripe on abaxial surface; dorsal petal oblong, cuneate at base, obtuse and slightly emarginate at apex, with a red stripe on both the adaxial and abaxial surfaces; disal lobes, obovate-oblong, obtuse at apex and near middle retuse, orange with red spots; lower sepal saccate, gradually elongates into an incurved or spiraled spur, mouth obliguely upwards, and a red stripe on the top and base of surface; stigma red; capsule obovate-clavate, with a red beak at apex. The plant height of I. maolanensis is similar to I. liboensis, but significantly lower than I. auriculata and I. tianlinensis. The pollen grains' micromorphology of I. maolanensis differ from the latter three species in that the pollen grains are triangular-round in polar view. The pollen exine has an irregular and relatively smooth reticulate ornamentation, and the meshes are almost without holes. The seeds' micromorphology of I. maolanensis differs from I. liboensis and I. auriculata in that the seeds are black and narrowly ellipsoid. The seed coat has reticulate ornamentation with slightly sunken meshes, folded base, and granular protrusions within the meshes. I. maolanensis, I. liboensis and I. auriculata can be found within the Maolan National Nature Reserve in Libo County (Fig. 5). However, I. maolanensis is only discovered growing in a semi-shaded tiankeng, while I. auriculata grows near water, and I. liboensis grows under forests in shady and damp places or beside ditches. More detailed comparison of the four species is presented in Table 1.



Figure 5. Map shows the sampling location of the three Impatiens species (Drawn by Jin-Dong Wang).

#### Discussion

Based on morphological characteristics and molecular evidence, Yu et al (2016) divided the genus *Impatiens* into subgen. *Clavicarpa* and subgen. *Impatiens*. Our new species, *I. maolanensis* belongs in sect. *Clavicarpa*, subgen. *Clavicarpa* because of its 4-flowered, racemose inflorescence, 4 lateral sepals, lower sepal saccate or funnel, 4-carpellate ovary, obovate-clavate capsule, three-colpate pollen grains. The endemic species of *Impatiens* in limestone areas such as Yunnan, Guangxi, and Guizhou are the most abundant, and they are strongly differentiated by edge effects and small habitat changes. Some endemic species of limestone areas have grown into perennial fruticeous-herbaceous plants over a long evolutionary process, which is particularly special for herbaceous *Impatiens*. *I. maolanensis* is exclusively found in the tiankeng of karst areas, and it exhibits very distinct differences from similar species. It is known to exist as a single population comprising only 18 mature individuals. Therefore, we propose the establishment of a specialized conservation area to ensure the survival and promote the propagation of *I. maolanensis*.

#### Acknowledgements

The authors thank Ding Wang, Hai-Lan Wu and Jin-Wei Luo for their assistance in the field.

#### Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This research was funded by various sources, including the National Natural Science Foundation of China (32360330, 32360262), China Scholarship Council (202308520101), Guizhou Provincial Program on Commercialization of Scientific and Technological Achievements (QianKeHeChengGuo [2022]010), Water-Fertilizer Coupling and Biodiversity Restoration in Karst Rocky Desertification (QianJiaoJi[2023]004), Key Laboratory of Environment Friendly Management on Alpine Rhododendron Diseases and Pests of Institutions of Higher Learning in Guizhou Province ([2022]044), The Joint Fund of the National Natural Science Foundation of China and the Karst Science Research Center of Guizhou Province (U1812401).

#### Author contributions

Bai-Zhu Li: Conceptualization (lead); Investigation (equal); Software (lead); Methodology (lead); Data curation (lead); Formal analysis (lead); Visualization (lead); Writing-original draft (lead); Writing-review and editing (lead); Project administration (lead). Qin-Ying Wen: Investigation (equal); Software (supporting); Data curation (supporting); Formal analysis (supporting); Project administration (supporting). Jin-Dong Wang: Investigation (supporting); Software (supporting). Zhi-Bin Xiong: Investigation (lead). Xiao-Xiang Huang: Investigation (supporting). Zhi-Bin Xiong: Investigation (equal); Data curation (supporting). Zhi-Juan Deng: Data curation (supporting); Formal analysis (supporting). Yin-Yi: Supervision (lead); Funding acquisition (lead). Xiao-Xin Tang: Conceptualization (supporting); Methodology (supporting); Formal analysis (supporting); Writing-original draft (lead); Writing-review and editing (lead); Supervision (lead); Funding acquisition (lead). Xiao-Xin Tang: Conceptualization (supporting); Methodology (supporting); Formal analysis (supporting); Writing-original draft (lead); Writing-review and editing (lead); Supervision (lead); Funding acquisition (lead).

#### Author ORCIDs

Bai-Zhu Li <sup>©</sup> https://orcid.org/0000-0002-7227-097X Qin-Ying Wen <sup>©</sup> https://orcid.org/0009-0007-0438-1073 Jin-Dong Wang <sup>©</sup> https://orcid.org/0009-0005-8020-3266 Yin Yi <sup>©</sup> https://orcid.org/0000-0003-3079-334X Xiao-Xin Tang <sup>©</sup> https://orcid.org/0000-0003-0569-0453

#### Data availability

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

#### References

- Cai XZ, Hu GW, Cong YY (2015) *Impatiens xanthinoides* (Balsaminaceae), a new species from Yunnan, China. Phytotaxa 227(3): 261–267. https://doi.org/10.11646/phyto-taxa.227.3.5
- Chen YL (2001) Balsaminaceae. Flora Reipublicae Popularis Sinicae Vol. 47(2). Science Press, Beijing, 1–243.
- Cho SH, Kim BY, Park HS, Phourin C, Kim YD (2017) *Impatiens bokorensis* (Balsaminaceae), a new species from Cambodia. PhytoKeys 77(3): 33–39. https://doi.org/10.3897/phytokeys.77.11345
- Ding B, Gadagkar SR, Wang JC, Zhang M, Guo H, Yu SX (2016) *Impatiens wawuensis* (Balsaminaceae): A new species from Sichuan, China. Phytotaxa 273(4): 293–298. https://doi.org/10.11646/phytotaxa.273.4.5

Grey-Wilson C (1980) Impatiens of Africa. A. A. Balkema, Rotterdam, 57 pp.

- Gu JZ, Yang N, Peng Y, Song Y, Guo SW, Cong YY (2021) *Impatiens shangjiangensis* (Balsaminaceae), a new species from Yunnan, China. Annales Botanici Fennici 58(4–6): 197–201. https://doi.org/10.5735/085.058.0401
- Hu HF, Xu J, An MT, Guo Y, Yang JW (2024) *Impatiens beipanjiangensis* (Balsaminaceae), a new species from Guizhou, China. PhytoKeys 241: 201–213. https://doi. org/10.3897/phytokeys.241.113700
- IUCN (2024) Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. http://www.iucnredlist.org/ documents/RedListGuidelines.pdf
- Janssens S, Geuten K, Yuan YM, Song Y, Küpfer P, Smets E (2006) Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. Systematic Botany 31(1): 171–180. https://doi.org/10.1600/036364406775971796
- Kuang RP, Duan DL, Gu JZ, Cai XZ, Cong YY, Liu KM (2014) *Impatiens liboensis* sp. nov. (Balsaminaceae) from Guizhou, China. Nordic Journal of Botany 32(4): 463–467. https://doi.org/10.1111/j.1756-1051.2013.00194.x
- Li BZ, Li Y, Li ZF, Xiang MD, Dong X, Tang XX (2022) The complete chloroplast genome of *Impatiens mengtszeana* (Balsaminaceae), an endemic species in China. Mitochondrial DNA. Part B, Resources 7(2): 367–369. https://doi.org/10.1080/23802359.202 1.1994894
- Liao RL, Cai L, Yu ZY, Wang YH, Sun WB (2021) *Impatiens wutaishanensis* (Balsaminaceae), a new species from southeast Yunnan, China. PhytoKeys 176(4): 43–53. https:// doi.org/10.3897/phytokeys.176.58825
- Lu YQ, Chen YL (1991) Seed morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic significance. Zhiwu Fenlei Xuebao 29(3): 252–257. https://doi. org/10.3767/000651905X622699
- Luo Q, Wang TJ, Zhao LH (2015) *Impatiens shimianensis* sp. nov. (Balsaminaceae) from Sichuan, China. Nordic Journal of Botany 33(2): 204–208. https://doi.org/10.1111/ njb.00573
- Peng S, Rono PC, Yang JX, Wang JJ, Hu GW, Wang QF (2021a) Description of a new species and lectotypification of two names in *Impatiens* sect. *Racemosae* (Balsaminaceae) from China. Plants 10(9): 1812. https://doi.org/10.3390/plants10091812
- Peng S, Cong YY, Tian J, Zhang CF, Wang QF (2021b) *Impatiens bullatisepala* (Balsaminaceae), a new species from Guizhou, China. Phytotaxa 500(3): 217–224. https://doi. org/10.11646/phytotaxa.500.3.5
- Qin Y, Xia CY, Yang XD, Triboun P, Son HT, Wu XX, Pei ZX, Yu SX (2020) *Impatiens macrantha* (Balsaminaceae) sp. nov., a new species from limestone areas in Guangxi, China. Nordic Journal of Botany 38(10): e2784. https://doi.org/10.1111/njb.02784
- Ruchisansakun S, van der Niet T, Janssens SB, Triboun P, Techaprasan J, Jenjittikul T, Suksathan P (2015) Phylogenetic Analyses of Molecular Data and Reconstruction of Morphological Character Evolution in Asian *Impatiens* Section Se*meiocardium* (Balsaminaceae). Systematic Botany 40(4): 1063–1074. https://doi. org/10.1600/036364415X690102
- Song YX, Peng S, Cong YY, Zheng YM (2021a) *Impatiens rapiformis*, a new species of Impatiens with root tuber from Yunnan, China. Nordic Journal of Botany 39(5): e03151. https://doi.org/10.1111/njb.03151
- Song YX, Cong YY, Kuang RP, Peng YC, Zheng YM (2021b) *Impatiens sterilis*, a rare new species of *Impatiens* with reduced sterile inflorescences from Yunnan, China. Taiwania 66(1): 48–52. https://doi.org/10.6165/tai.2021.66.48

- Song XQ, Song BN, Fu MX, Wang JC, Liu JY, Qin WR, Jiang YZ, Fan L, Yang B (2024) *Impatiens yingjingensis* (Balsaminaceae), a new species from Sichuan, China. PhytoKeys 242: 293–306. https://doi.org/10.3897/phytokeys.242.119702
- Tan YH, Liu YN, Jiang H, Zhu XX, Zhang W, Yu SX (2015) *Impatiens pandurata* (Balsaminaceae), a new species from Yunnan, China. Botanical Studies 56(29): 29. https://doi. org/10.1186/s40529-015-0108-4
- Thiers B (2025 continuously updated) Index Herbariorum. https://sweetgum.nybg.org/ science/ih/ [accessed 11 November 2024]
- Walker JW, Doyle JA (1975) The bases of angiosperm phylogeny: Palynology. Annals of the Missouri Botanical Garden 62(3): 664–723. https://doi.org/10.2307/2395271
- Wang KF, Wang XZ (1983) An introduction to palynology. Peking University Press, Beijing, 205 pp.
- Wang Z, Wu PP, Liu CC, Guo K, Yu SX (2022a) *Impatiens nushanensis* (Balsaminaceae), a new species from Yunnan, China. Phytotaxa 545(2): 186–196. https://doi. org/10.11646/phytotaxa.545.2.7
- Wang Q, Xu RH, Zhang Y, Li XC (2022b) Impatiens chenmoui (Balsaminaceae), a new species from southern Yunnan, China. PhytoKeys 214: 83–95. https://doi.org/10.3897/ phytokeys.214.94898
- Xia CY, Sudhindra RG, Zhao XL, Van Do T, Zhu XY, Qin Y, Deng HP, Yu SX (2019) *Impatiens maculifera* sp. nov. (Balsaminaceae) Yunnan, China. Nordic Journal of Botany 37(8): e2422. https://doi.org/10.1111/njb.02422
- Yu SX (2012) Balsaminaceae of China. Peking University Press, Beijing.
- Yu SX, Janssens SB, Zhu XY, Lidén M, Gao TG, Wang W (2016) Phylogeny of *Impatiens* (Balsaminaceae): Integrating molecular and morphological evidence into a new classification. Cladistics 32(2): 179–197. https://doi.org/10.1111/cla.12119
- Yuan Y, Song Y, Ge X (2011) *Impatiens qingchengshanica* (Balsaminaceae), a unique new species from China and its phylogenetic position. Botanical Studies 52(2): 225–230. https://doi.org/10.1080/00380768.2011.567398
- Yuan Y, Chen H, Luo Q (2022) Impatient sikaiensis sp. nov. (Balsaminaceae) from Sichuan, China. Nordic Journal of Botany 2023(2): e03724. https://doi.org/10.1111/ njb.03724
- Zeng L, Liu YN, Gogoi R, Zhang LJ, Yu SX (2015) *Impatiens tianlinensis* (Balsaminaceae), a new species from Guangxi, China. Phytotaxa 227(3): 253–260. https://doi. org/10.11646/phytotaxa.227.3.4
- Zeng L, Yan RY, Zhang M (2016) Taxonomic significance of the pollen morphology of subg. *Clavicarpa (Impatiens*, Balsaminaceae). Guihaia 36(10): 1245–1252. https:// doi.org/10.11931/guihaia.gxzw201507037
- Zhang S, Gao SP, Zhang X, He HF (2014) Pollen morphology and its relationship to taxonomy of 13 species in the *Impatiens* (Balsaminaceae) from Ya'an of Sichuan, China. Xibei Zhiwu Xuebao 34(3): 502–508. [In Chinese]
- Zhang LJ, Yu SX, Liu YN, Gogoi R, Zeng L (2023a) *Impatiens tianlinensis* (Balsaminaceae), a new species from Guangxi, China. Phytotaxa 227(3): 253–260. https://doi. org/10.11646/phytotaxa.227.3.4
- Zhang Q, Zhao QY, Gu ZJ, Huang HQ, Yan B, Huang MJ (2023b) Studies on pollen micromorphology of *Impatiens* plants in Southwest Sichuan. Yuan Yi Xue Bao 50(8): 1664–1678. https://doi.org/10.16420/j.issn.0513-353x.2022-0716

# PhytoKeys

**Research Article** 

# *Lilium brunneum* (Liliaceae): a newly discovered species in northwestern Yunnan, China

Ting Wang<sup>1,20</sup>, Xiuying Shen<sup>30</sup>, De Wang<sup>30</sup>, Ying Zhao<sup>30</sup>, Xiaomei Qu<sup>30</sup>, Yundong Gao<sup>1,20</sup>

- 1 CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610213, China
- 2 University of Chinese Academy of Sciences, Beijing 100049, China

3 Fugong Administrative Sub-Bureau of Gaoligongshan National Nature Reserve, Nujiang Prefecture, Nujiang 673499, China

Corresponding author: Yundong Gao (gaoyd@cib.ac.cn)

#### Abstract

*Lilium brunneum* represents a newly discovered and described lily species with a limited distribution in isolated alpine regions of north-western Yunnan, China. The recognition of this new species as a distinct entity is supported by both morphological and molecular data. Furthermore, the investigation of this region, identified as a 'hotspot' due to its high diversity and endemicity within the genus *Lilium*, is anticipated to provide greater insight into the processes of speciation and the maintenance of species boundaries in this genus. Further fieldwork aimed at exploring these regions is expected to discover additional new species and therefore warrants special attention and resources.

Key words: Hengduan Mountains, Liliaceae, Lilium brunneum, Lilium souliei complex, new species

#### Introduction

The southwestern region of China, which encompasses the Hengduan Mountains and the Tibetan Plateau, is renowned for its numerous mountain ranges. Amongst these, the Hengduan Mountains and Eastern Himalaya Mountain ranges are recognised as two of the world's biodiversity hotspots (Marchese 2015; Yu et al. 2020). Over an extended period, beginning in the late Miocene, the Hengduan Mountains experienced complex orogeny, notably characterised by rapid uplift, resulting in a highly rugged terrain and significant environmental heterogeneity (Wang et al. 2012). This diverse ecological landscape has led to the region being described as a cradle of evolution due to its remarkable biodiversity (Lu et al. 2018). While these mountainous regions provide favorable conditions for the survival of wildlife, their remote and rugged nature poses challenges to exploration efforts. The extensive expanse of unexplored regions presents opportunities for discovering previously unidentified or unrecognised species, largely due to a lack of investigation and sampling.

The genus *Lilium* L., a prominent member of the monocot family Liliaceae, comprises approximately 123 species (POWO 2024; WFO 2025). This genus consists of non-climbing, bulbous herbaceous plants (Peruzzi 2016) and



Academic editor: Lorenzo Peruzzi Received: 24 January 2025 Accepted: 15 March 2025 Published: 3 April 2025

Citation: Wang T, Shen X, Wang D, Zhao Y, Qu X, Gao Y (2025) *Lilium brunneum* (Liliaceae): a newly discovered species in north-western Yunnan, China. PhytoKeys 254: 201–219. https://doi.org/10.3897/ phytokeys.254.147769

**Copyright:** © Ting Wang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). is distributed across mid to high latitudes of the Northern Hemisphere (Liang and Tamura 2000; Skinner 2002). Extant species are primarily concentrated in temperate regions of the Northern Hemisphere, while East Asia hosts a notably greater diversity of *Lilium* species compared to Central Asia, Europe and North America (Gong et al. 2017; Dhiman et al. 2020). The Hengduan Mountains and the Himalayas are considered to be the central distribution area of *Lilium* (De Jong 1974; Du et al. 2014, 2017). However, the taxonomic status of several species in the Hengduan Mountain region remains unclear and requires clarification.

In our previous studies focusing on this region – particularly upon members of the former genus *Nomocharis* Franchet – we discovered that campanulate-flowered species, such as *Lilium souliei* (Franch.) Sealy, and its close relatives, are nested within the Nomocharis-clade (Gao et al. 2013b, 2015; Yuan and Gao 2024). This finding reveals unexpected relationships given the significant differences in phenotypic characteristics. These alpine lilies, characterised by their dwarf habit and bell-shaped, nodding flowers, include *L. souliei*, *L. saccatum* S.Yun Liang, *L. medogense* S.Yun Liang, *L. paradoxum* Stearn and *L. georgei* (W.E. Evans) Sealy. Several of these species were only described in the last century, with few records existing other than the type specimens, resulting in potential taxonomic confusion within this group. Consequently, we will refer to this group as the *L. souliei* complex for brevity.

According to field records accumulated and verified by the authors, the *L. souliei* complex is distributed from north-western Yunnan Province to south-eastern Tibet, traversing the Hengduan and extending into the Eastern Himalaya Mountain ranges (Dhiman et al. 2020). Amongst these, *L. souliei* has the broadest distribution, thriving in alpine grass and shrub habitats at elevations of 2800–4000 m above sea level (Wu et al. 2012), primarily in the central part of the Hengduan Mountains. *L. saccatum* is predominantly found in the shrub and grassland slopes of the Eastern Himalaya at approximately 3900 m (Liang 1987). In contrast, *L. paradoxum* and *L. medogense* exhibit much narrower distributions. *L. paradoxum* is endemic to southeastern Tibet, with limited populations at around 3500 m (Stearn 1956), while *L. medogense* is confined to alpine wetland habitats around 3600 m (Liang 1985) in Motuo (Medog) County, Xizang, China (Fig. 1).

With further investigations in the Hengduan Mountains and surrounding areas, coupled with advancements in molecular phylogenetics, we recognise that this is an opportune time to clarify the status of several monophyletic yet taxonomically ambiguous lily groups in the region. In this context, the *L. souliei* complex serves as an exemplary focus for detailed investigation and taxonomic revision. Through a comprehensive review of the literature and examination of herbarium specimens, the author, Gao, proposes that *L. georgei* (W.E. Evans) Sealy, first discovered by George Forrest in 1924 along the Myanmar-China border (Sealy 1950), also belongs to this complex.

In the summers of 2023 and 2024, the authors undertook two consecutive field trips to the remote areas of the Gaoligong Mountains in Yunnan (mid to southern part of the Hengduan Mountains), aiming to rediscover this lily and collect additional information and materials since no records exist beyond those documented by Forrest. Unfortunately, *L. georgei* was not located, as access to the type locality is currently restricted; however, a new species belonging to this group was discovered and is described in this paper.





In the present study, we utilise both morphological and molecular data to elucidate the status and phylogenetic position of the putative new species by comparing its morphology with that of closely related taxa. Simultaneously, this study aims to provide a more informative overview of the *L. souliei* complex, highlighting distinguishing characteristics amongst species and clarifying geographic distribution patterns. We hope this research will illuminate the diversity of lilies in the Hengduan Mountains and adjacent areas, contributing to and enriching the catalogue of the genus *Lilium*.

# Materials and methods

#### **Field sampling**

The new species was observed and photographed exclusively in two locations: Fugong County, Yunnan, China, in the Gaoligong Mountains (on the border with Myanmar) and near Zhiziluo in the Biluoxueshan Range; and material was collected for herbarium specimens and molecular study. At these locations, mature individuals were counted, and the extent of their distribution was estimated to support a conservation assessment using GeoCAT software (Bachman et al. 2011) and IUCN criteria. The collected images and specimens were subsequently used for measurements and descriptions. Voucher specimens have been deposited in the herbarium of the Chengdu Institute of Biology (**CDBI**, Fig. 5A). Fresh leaves were collected and rapidly dried using silica gel.

#### Morphological analysis

A comprehensive review of the relevant taxa was conducted through online databases, including Tropicos (https://tropicos.org/) and the Biodiversity Heritage Library (BHL, https://www.biodiversitylibrary.org/). Specimens were accessed through both physical and online herbarium collections, including the CDBI, E, K, KUN, IBSC, P, PE and SZ herbaria (acronyms according to Thiers 2024); the Chinese 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/). This methodology was designed to facilitate a morphological comparison analysis based on a large and diverse set of specimens. The morphological traits selected for analysis were informed by key taxonomic features outlined in the Flora of China (Liang and Tamura 2000), including characteristics of the bulb, stem, leaf and flower. Eighteen morphological traits of the new species and its closely related taxa were measured using MATO (Liu et al. 2023). A list of morphological characters and their acronyms used in analyses is shown in (Suppl. material 1: table S1).

Principal component analysis (PCA) was performed on the standardised data for 18 quantitative traits (Suppl. material 1) using the built-in prcomp() function in R version 4.3.1 to achieve dimensionality reduction and feature extraction. The factoextra (Kassambara and Mundt 2017) and ggplot2 (Wickham et al. 2016) packages were installed and the fviz\_pca\_biplot() function was used to generate a biplot combining principal components and variables.

#### 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 a depth of about  $0.1 \sim 0.2 \times (10G \text{ pair ending reads})$ . Approximately 10-12 Gb of raw data were filtered and evaluated using fastp v0.23.2 (Chen et al. 2018) and FastQC (https://www.bioinformatics.babraham.ac.uk/projects/ fastqc/) with default parameters. Chloroplast and Internal Transcribed Spacer (ITS) sequences were assembled from the clean data using GetOrganelle v1.7.6.1 (Jin et al. 2020). Chloroplast sequences were selected with the correct orientation through multiple sequence alignment using Mafft v7 (https://mafft.cbrc.jp/ alignment/software/, (Katoh et al. 2019)) and the chloroplast genome was annotated using Geneious Prime v2023.1.2 (Biomatters Ltd, Auckland, New Zealand).

To infer the phylogenetic position of the newly described species, newly generated DNA sequences were combined with publicly available sequences, including thirty-four ITS and thirty-three chloroplast genomes from NCBI (https:// www.ncbi.nlm.nih.gov/). Based on previous research (Yuan and Gao 2024), we selected the majority of species from the Nomocharis-clade (In particular, as many individuals as possible of *Lilium souliei*, *L. saccatum*, *L. medogense* and *L. paradoxum*), as well as representative species from 2–3 closely related clades. The outgroup included three species from *Fritillaria*, *Cardiocrinum* and *Notholirion* (Suppl. material 2: table S2). Whenever possible, the ITS and chloroplast sequences used for phylogenetic analysis were derived from the same individual. Maximum Likelihood (ML) analyses of both chloroplast and ITS data were conducted using an online platform (https://ngphylogeny.fr/; Lemoine et al. 2019). Sequences were analysed with an advanced workflow utilising the PhyML + SMS/OneClick method. The MAFFT, BMGE and PhyML + SMS workflow (for Maximum Likelihood phylogenetic tree inference based on smart model selection) was employed (Lemoine et al. 2019). Bootstrap analysis (FBP + TBE) was performed with 1000 replicates, with all other parameters set to default.

Bayesian Inference (BI) phylogenetic trees based on chloroplast and ITS sequences were constructed using PhyloSuite v1.2.2 (Zhang et al. 2020). Sequences were aligned using the "auto" strategy under normal alignment mode with MAFFT v7.313 (Katoh and Standley 2013) and the resulting files were further adjusted manually using MEGA v11.0 (Tamura et al. 2021). Gblocks (Talavera and Castresana 2007) was applied within PhyloSuite to remove ambiguous sites and gaps. ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the most appropriate evolutionary model. According to the Bayesian Information Criterion (BIC), GTR+F+I+G4 was selected as the optimal model for chloroplast data. Bayesian phylogenetic analysis was performed using Mr-Bayes 3.2.6 (Ronquist et al. 2012) with a partitioned model (two parallel runs, 2,000,000 generations), discarding the first 25% of sampled data as burn-in. For ITS data, SYM+G4 was identified as the best nucleotide evolution model based on BIC. Bayesian phylogenetic inference was conducted using MrBayes 3.2.6 (Ronquist et al. 2012) with a partitioned model (two parallel runs, 10,000,000 generations) and the first 25% of sampled data were discarded as burn-in.

The resulting Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic trees were visualised using FigTree v1.4.0.

#### Results

#### Morphological comparison

Figs 2-5, Table 1

*Lilium brunneum* (Fig. 2) is distinctly different from *L. medogense* (Fig. 3A) and *L. paradoxum* (Fig. 3B), as the latter species are characterised by their taller plants with larger flowers of different coloration, and the possession of whorled foliage (Table 1). In particular, the whorled leaves of the latter two species are distinctive features not found in other members of the *L. souliei* complex. Principal component analysis (PCA) was used to assess the four species of *Lilium* – *L. brunneum* (Fig. 3C), *L. souliei* (Fig. 3D), *L. saccatum* (Fig. 3E) and *L. georgei* (Fig. 5B) – which are most similar to one another.

The resulting PCA plot visually illustrates the distribution of the species within the reduced-dimensional space defined by the principal components. The first two components collectively account for 81.4% of the total variance in the dataset (PC1: 70.1%; PC2: 11.3%), demonstrating that this visualisation provides a robust representation of the dataset's variability (Fig. 4). PC1 is primarily driven by pistil length (PL), stamen length (STL) and filament length (F), while PC2 is predominantly influenced by pedicel length (P) and corolla width (CW). The PCA clearly separates *L. souliei, L. saccatum, L. georgei* and *L. brunneum* based on the analysed variables, supporting their morphological distinctiveness (Fig. 4).



**Figure 2.** Habitat and morphology of *Lilium brunneum* T.Wang & Y.D.Gao, sp. nov. **A** habitat **B** habit **C** pedicel **D** leaf **E** bulb **F** transverse section of the flower (a) adaxial surface of the petal (b) abaxial surface of the petal (c) **G** anatomy of the petal (d) pistil and stamen (e) **H** bulb with scale.



Figure 3. Comparison of several closely related species. A Lilium medogense B L. paradoxum C L. brunneum D L. souliei E L. saccatum F anatomical diagram of the flower of L. brunneum G anatomical diagram of the flower of L. souliei H anatomical diagram of the flower of L. saccatum.



**Figure 4.** Principal components analysis (PCA) biplot displaying different traits and 96 individuals on PC1 and PC2. For abbreviations and codes of different traits (see Suppl. material 1).

Characters		L. brunneum	L. medogense	L. paradoxum	L. souliei	L. georgei	L. saccatum		
Bulb	length (cm)	3.4 (3.3-3.6)	2.9 (2.6-3.4)	2.5 (1.6-3.4)	2.8 (1.9-3.6)	5.0 (3.8-6.3)	2.9 (2.5-3.2)		
	width (cm)	1.5 (1.4–1.6)	2.6 (2.4-2.9)	2.2 (0.9-3.5)	1.5 (1.0-2.4)	3.1 (2.4-3.9)	1.8 (1.4–2.1)		
Pedicel	length (cm)	1.6 (0.7-2.6)	4.9 (3.8-6.0)	4.6 (2.3-6.2)	5.1 (2.8-10.5)	3.8 (3.1-4.9)	2.2 (1.8-2.6)		
Stem	length (cm)	22.3 (16.6-27.9)	45.6 (37.4-52.1)	40.5 (19.7-66.9)	16.8 (9.5-25.2)	28.1 (18.7–36.5)	24.5 (13.2-40.7)		
Leaf	middle leaf length (cm)	3.4 (2.6-4.4)	5.2 (4.7-6.1)	3.8 (2.1-7.0)	4.0 (2.3-6.6)	4.7 (3.0-6.4)	3.0 (1.9–3.8)		
	middle leaf width (cm)	0.7 (0.5–1.0)	1.8 (1.5–2.3)	1.2 (0.7–2.1)	0.8 (0.5–1.2)	1.6 (1.2–2.1)	1.0 (0.6–1.2)		
	arrangement	scattered	verticillate	verticillate	scattered	scattered	scattered		
Flower	corolla width (cm)	2.0 (1.3-3.0)	5.6 (4.3-7.4)	5.5 (3.9-6.8)	4.0 (2.5-5.1)	4.2 (3.8-4.9)	2.5 (1.8–3.1)		
	basal colour	brown to light brown	yellow	purple	purple-red	purplish-blue	purple-red		
	filaments and ovary	closely appressed	closely appressed	closely appressed	spreading	closely appressed	closely appressed		

**Table 1.** Morphological comparisons of the *Lilium* species studied (The table presents the mean values of the measurements, with the corresponding minimum and maximum ranges provided in parentheses).



Figure 5. Comparison of specimens of *Lilium brunneum* and *L. georgei* **A** holotype of *L. brunneum* (CDBI0299797) **B** holotype of *L. georgei*. (https://data.rbge.org.uk/herb/E00381818).

#### **Phylogenetic analyses**

Figs 6, 7

The analysis was based on molecular data, specifically ITS (ITS1, 5.8S and ITS2) sequences and the complete chloroplast genome. We utilised forty-one ITS sequences, with lengths ranging from 610 bp to 633 bp prior to alignment. After alignment correction, the sequence lengths were 647 bp with 245 variable sites and 395 conserved sites. In addition, we analysed thirty-eight complete chloroplast genomes with sequence lengths ranging from 151,083 bp to 152,915 bp before alignment and 158,552 bp after alignment correction, containing 7,643 variable sites and 147,778 conserved sites.

The Bayesian and Maximum Likelihood (ML) trees derived from both chloroplast and ITS data are largely consistent. In the ITS phylogenetic tree, individuals of *L. brunneum* from the two populations form a distinct clade, which is allied with a clade comprising *L. souliei*, *L. yapingense* and another clade containing *L. paradoxum*, *L. medogense* and *L. saccatum* (Fig. 6). The grouping of the latter three species is strongly supported (PP = 0.95, BS = 80%). All the aforementioned species form a well-supported clade (PP = 0.97, BS = 92%, Fig. 6). In the chloroplast consensus tree, the two *L. brunneum* populations cluster together, forming a sister clade with *L. paradoxum*, with moderate support. This support



**Figure 6.** Phylogenetic trees for selected *Lilium* species were constructed based on nuclear ITS sequences using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. The values at the nodes represent Bayesian posterior probabilities (PP) to the left of the slash and bootstrap support values (BS) to the right. The target species is highlighted in red.

significantly increases after the inclusion of *L. paradoxum* (PP = 0.99, BS = 80%, Fig. 7). Additionally, all members of the *L. souliei* complex were found to cluster within a single clade, which is highly supported (PP = 0.99, BS = 100%).

#### **Taxonomic treatment**

Lilium brunneum T.Wang & Y.D.Gao, sp. nov. urn:lsid:ipni.org:names:77359676-1 Figs 2-5 and Table 1 Chinese name: 焦瓣百合 (jiao ban bai he)

**Type.** CHINA · Yunnan: Fugong county, Gaoligongshan Range [高黎贡山] (on the border with Myanmar); near Zhiziluo [知子罗], Biluoxueshan Range [碧罗雪山], 3500–3800 m. 10 July 2023, *Y.D. Gao GYD1524* (holotype CDBI0299797) (Fig. 5A), 11 July 2023, *GYD1530* (paratype CDBI0299796).



**Figure 7**. Phylogenetic trees for selected *Lilium* species were constructed based on the complete chloroplast genomes using both Bayesian Inference (BI) and Maximum Likelihood (ML) methods. The numbers at the nodes indicate Bayesian posterior probabilities (PP) to the left of the slash and bootstrap support values (BS) to the right. The target species is highlighted in red.

**Diagnosis.** *Lilium brunneum* shares morphological similarities with *L. georgei*, *L. souliei*, and *L. saccatum* but exhibits distinct differences that set it apart. Compared to *L. georgei*, *L. brunneum* is characterised by its shorter stature and smaller flowers. In contrast to *L. souliei*, it possesses shorter pedicels, and its filaments are closely appressed to the ovary. When compared to *L. saccatum*, *L. brunneum* is distinguished by its unique petal coloration, which ranges from brown to light brown with a greenish-yellow base. (Table 1, Fig. 2).

**Description.** Perennial herbs with narrowly ovoid bulbs, 1.4-1.7 cm in diam.; scales white, outermost partially purplish-red, lanceolate. 1.5-3 cm × 6-10 mm. Stem erect, 16-28 cm. Leaves 6-12, scattered, narrowly elliptic, lanceolate, margin sometimes sparsely papillose. Flower solitary, nodding, campanulate. Tepals brown to light brown, usually paler towards base, unspotted, basally gibbous, greenish yellow; outer elliptic,  $1.8-2.6 \times 0.5-1.1$  cm, apex shortly pointed; inner 0.6-1.2 cm wide; nectaries greenish yellow, not papillose. Stamens converging, adnate to the ovary; filaments to 1.0 cm, glabrous, green; anthers dorsifixed at approximately the middle, purple-brown, 4-7 mm. Ovary cylindrical,

7–11 mm long, 2–3 mm wide, green; style shorter than ovary, 5–7 mm; stigma swollen. Capsule subglobose, 1.5–2 cm in diam.

**Phenology.** Flowering from June to July; fruiting from August to October.

Habitat and distribution. On open stony alpine meadows and edges of bushes. 3500-3800 m. NW Yunnan (Fugong[福贡]) and bordering Myanmar (Kachin).

**Etymology.** The epithet and Chinese name adopted here both denote the perianth colour of light brown resembling that of caramel.

**Conservation status and IUCN preliminary assessment.** Through extensive field surveys, we identified two populations of *L. brunneum* located in the Gaoligongshan Range (on the border with Myanmar) and near Zhiziluo in the Biluoxueshan Range. The area of occupancy (AOO) was estimated to be approximately 32 km<sup>2</sup>. Although each population contains more than 300 individuals, the number of mature individuals is fewer than 200. *L. brunneum* grows above the snowline at altitudes exceeding 3500 m. However, ongoing global climate warming may result in a reduction of its habitat area. Based on the criteria of the International Union for Conservation of Nature (IUCN 2024), we recommend classifying *L. brunneum* as an endangered species (EN, B2ab(ii+iii), C2a(i)).

# Discussion

The overall size of *Lilium brunneum* is significantly smaller when compared to *L. medogense* and *L. paradoxum*. Based on specimen measurements, the average plant height and corolla width for *L. medogense* are 45.6 cm and 5.6 cm, respectively, while those of *L. paradoxum* are 40.5 cm and 5.5 cm. In contrast, *L. brunneum* displays a distinctly shorter average plant height of 22.3 cm and a corolla width of only 2.0 cm (Table 1, rows 5 and 9). Notably, the former two species can be easily distinguished within the *L. souliei* complex owing to their significantly larger sizes compared to the other members. Furthermore, both species exhibit whorled leaves (typically 3–5 whorls), a characteristic absent in the remaining members of the complex. Specifically, *L. medogense* is distinguished by its bright yellow flowers, making it particularly prominent amongst the complex, as the other members typically present more or less purplish hues. Consequently, these two species were not included in further fine-scale comparisons below, as they cannot be confused with the other members of the complex.

Regarding the remaining members, *L. brunneum* exhibits several distinguishing characteristics when compared to *L. souliei*, *L. saccatum* and *L. georgei*. Principal Component Analysis (PCA) based on morphological measurements from multiple individuals demonstrates clear boundaries between these species (Fig. 4). As a predominant member within the complex, *L. souliei* is positioned in the upper-left quadrant and displays wide morphological variation (Fig. 4), likely associated with its extensive geographic distribution (Fig. 1) facilitating a broad range of variations in response to niche diversity. In comparison to *L. brunneum*, *L. souliei* is characterised by longer pedicels (measured from the flower base to the bract, Fig. 3D), a greater distance between the filaments and ovary (Fig. 3G) and purple-coloured tepals. The PCA plot indicates a complete lack of overlap between *L. brunneum* and *L. georgei* (Fig. 4). Although the collection sites for *L. brunneum* are geographically closest to those of *L. georgei* (Fig. 1), the latter possesses a larger habit, particularly in flower size (Fig. 5B, Table 1), and exhibits more striking colouration, as is noted in the description on the collection label of *L. georgei*, where Forrest wrote: 'flowers that are soft blue-purple throughout, deepest on the exterior' (G. Forrest 24772, https://data. rbge.org.uk/herb/E00381818). These differences collectively support the clear delineation of these two species.

While *L. brunneum* is situated closest to *L. saccatum* in the PCA plot, they remain distinctly isolated without any overlap (Fig. 4). The principal components contributing to this separation are primarily flower organ size (e.g. dimensions O). A detailed comparison between *L. saccatum* and *L. brunneum* can also be found in Table 1 and Suppl. material 1. In addition to these differences, *L. brunneum* exhibits lighter-coloured outer tepal bases (Figs 2C, 3C) and features a pale green style (Fig. 3F), whereas the style of *L. saccatum* is dark purplish-black (Fig. 3H). Notably, their geographic distributions are not connected with, but rather widely separated from, that of *L. souliei* (Fig. 1).

The molecular phylogenetic analysis also supports the distinct status of the new species. While the nuclear ITS region has certain limitations due to its relatively short length (approximately 630 bp), it remains sufficient for species delimitation, although it lacks the resolution needed to clarify detailed relationships amongst selected taxa (Yao et al. 2010; Zhang et al. 2022). In the ITS phylogenetic tree, L. medogense, L. saccatum, L. paradoxum, L. souliei, L. brunneum and L. yapingense Y.D. Gao & X.J. He constitute a robustly supported clade (PP = 0.97, BS = 92%, Fig. 6). As previously mentioned, this clade contains several parallel sub-clades due to insufficient informative sites; however, the separation of each recognised species remains clear. Amongst these species, individuals from the two populations of L. brunneum form a distinct clade that is allied with L. souliei, L. yapingense and a clade consisting of L. paradoxum, L. medogense and L. saccatum (Fig. 6). The convergence of the latter three species is supported by considerable evidence (PP = 0.95, BS = 80%), suggesting a closer relationship compared to the other members. The clustering of L. yapingense within this clade indicates that this dwarf lily, characterised by its whitish to pinkish campanulate flowers, as described by Gao et al. (2013a), may share a common ancestry with the members of the L. souliei complex.

In the chloroplast phylogenetic inferences, the tree topologies generated by Bayesian inference (BI) and maximum likelihood (ML) are congruent. The chloroplast consensus tree demonstrates that the two populations of *L. brunneum* cluster together, forming a sister clade to *L. paradoxum*, with moderate support; this confidence significantly increases upon the inclusion of *L. saccatum* (PP = 0.99, BS = 80%, Fig. 7). Moreover, all members of the *L. souliei* complex are found clustered within the same clade with robust support (PP = 0.99, BS = 100%); however, two species from the former genus *Nomocharis* are also included and positioned as sisters to *L. souliei*. Consequently, under chloroplast phylogenetic analysis, the complex is not monophyletic, revealing incongruence between nuclear ITS and plastid genome data.

According to prior research, such incongruences are common within the genus *Lilium* and the most plausible explanation may involve genetic introgression, with the chloroplast tree providing a more accurate reflection of geographic relationships (Gao et al. 2013b, 2015). For instance, *L. souliei*, which encompasses the broadest distribution range within the complex, overlaps geographically with *L. apertum* Franch. and *L. saluenense* (Balf. f.) S.Y. Liang, which are sister species on the chloroplast tree, receiving strong support (PP = 1.00, BS = 100%). Similarly, although *L. taliense* Franch. is classified within the Sinomartagon-clade in the nuclear ITS phylogeny, it clusters with species from the Nomocharis-clade in the chloroplast tree due to sympatric distributions, likely resulting from introgression via interspecific hybridisation. In fact, *Lilium* species distributed throughout the Hengduan Mountains region have been consistently found clustered within a single clade in both previous and current plastid-based phylogenetic trees (Gao et al. 2015; Duan et al. 2022; Yuan and Gao 2024). This pattern suggests that substantial gene flow has occurred or is ongoing amongst these species within this biodiversity hotspot.

While gene flow can be detected amongst species that are partially sympatric and distributed in the Hengduan Mountains, it is unexpected that several sympatric Lilium species exhibit no evidence of gene exchange. For example, the new species coexists with L. yapingense, which, according to the nuclear phylogeny, is guite closely related to the entire L. souliei complex. However, there is no evidence of interaction between them at the chloroplast genome level, which is unexpected, as introgression would typically be anticipated in such cases. Comparative studies on three gradient-distributed lilies in the same region have shown asymmetric gene flow amongst species, with shared chloroplast genome types (Gao et al. 2020). Although L. brunneum is found near to L. yapingense, they appear to lack gene exchange, as evidenced by the intact nature of their chloroplast genomes in the plastid phylogenetic tree, a surprising observation considering that they share the same locality and blooming period. The latter aspect has been identified as a critical factor influencing the isolation of gene exchanges amongst lilies (Gao et al. 2020; Feng et al. 2025). This scenario indicates the presence of unknown mechanisms that prevent hybridisation amongst these species, which are likely associated with the high levels of endemism observed in this region.

Further examples of this phenomenon can also be observed within the complex itself. While the distribution ranges of *L. saccatum* and *L. medogense* are geographically proximate (Fig. 1), their phylogenetic relationship is relatively distant. *L. saccatum* thrives in shrubby grasslands on mountain slopes at elevations exceeding 3600 m, whereas *L. medogense* is found in fissured clearings at the edges of alpine wetlands interspersed with fir forests at lower elevations. This ecological disparity may suggest the presence of isolating mechanisms beyond niche diversification and habitat isolation; for instance, differences in floral structures potentially driven by their respective pollinator assemblages (Levin 1978; Baack et al. 2015; Liu et al. 2019) may contribute to the maintenance of species boundaries, thereby restricting gene flow amongst these sympatric lilies and further enhancing biodiversity and endemism within the Hengduan Mountains region.

In conclusion, both morphological and molecular evidence confirm that *L*. *brunneum* is a distinct new species. This finding enhances our understanding of *L*. *souliei* and its closely related species, further enriching the catalog of lilies in the Hengduan Mountains region. Additionally, this research contributes to our understanding of species boundaries and the mechanisms contributing to the high levels of endemism observed in this area. Future efforts, including field studies, systematic research with additional data (such as population-level research utilising next-generation sequencing) and analyses of niche diversity, are needed to elucidate the possible undetected species, their origins and the mechanisms maintaining species boundaries.

# Acknowledgements

The authors express their gratitude to Professor Xin-Fen Gao, Dr. Wen-Bin Ju, Mr. Heng-Ning Deng, Jun-Yi Zhang and Ms. Yu-Mei Yuan of the Chengdu Institute of Biology, Chinese Academy of Sciences (CAS), Associate Professor Pan Li from Zhejiang University for their invaluable assistance with sample collection. Special thanks are given to Mr. Melvyn Herbert for his contributions to material collection and linguistic refinement of the manuscript.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

# Funding

This work was supported by the National Natural Science Foundation of China (NSFC Grant No. 32171605) to Yundong Gao, as well as the Natural Science Foundation of Sichuan Province (Grant No. 2023NSFSC0141).

# **Author contributions**

Yundong Gao: Conceptualization, Data curation, Funding acquisition, Investigation, Validation, Writing – original draft and Writing – review and editing. Ting Wang: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft and Writing – review and editing. Xiuying Shen, De Wang, Ying Zhao, Xiaomei Qu: Resources and Investigation.

# **Author ORCIDs**

Ting Wang <sup>®</sup> https://orcid.org/0009-0005-2338-7011 Xiuying Shen <sup>®</sup> https://orcid.org/0009-0002-1560-7270 De Wang <sup>®</sup> https://orcid.org/0009-0007-4767-6904 Ying Zhao <sup>®</sup> https://orcid.org/0009-0008-3800-6949 Xiaomei Qu <sup>®</sup> https://orcid.org/0009-0002-8836-6906 Yundong Gao <sup>®</sup> https://orcid.org/0000-0002-0534-2128

# Data availability

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

# References

Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF (2006) A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nature Protocols 1(5): 2320–2325. https://doi.org/10.1038/nprot.2006.384
Baack E, Melo MC, Rieseberg LH, Ortiz-Barrientos D (2015) The origins of reproductive isolation in plants. The New Phytologist 207: 968–984. https://doi.org/10.1111/nph.13424

- Bachman SP, Moat J, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109
- Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics (Oxford, England) 34(17): i884–i890. https://doi.org/10.1093/bioinformatics/bty560
- De Jong PC (1974) Some notes on the evolution of lilies. North Amer. Lily Yearbook 27: 23–28.
- Dhiman MR, Sharma P, Bhargava B (2020) *Lilium*: conservation, characterization, and evaluation. In: Datta SK, Gupta YC (Eds) Floriculture and Ornamental Plants. Springer, Singapore, 1–36. https://doi.org/10.1007/978-981-15-1554-5\_6-1
- Du Y, Wei C, Wang Z, Li S, He H, Jia G (2014) *Lilium* spp. pollen in China (Liliaceae): Taxonomic and phylogenetic implications and pollen evolution related to environmental conditions. PLoS One 9: e87841. https://doi.org/10.1371/journal.pone.0087841
- Du Y, Bi Y, Yang F, Zhang M, Chen X, Xue J, Zhang X (2017) Complete chloroplast genome sequences of *Lilium*: Insights into evolutionary dynamics and phylogenetic analyses. Scientific Reports 7: 5751. https://doi.org/10.1038/s41598-017-06210-2
- Duan Q, Liu F, Gui D, Fan W, Cui G, Jia W, Zhu A, Wang J (2022) Phylogenetic analysis of wild species and the maternal origin of cultivars in the genus *Lilium* using 114 plastid genomes. Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.865606
- Feng Y, Yan C, Tu WQ, Yuan YM, Wang JB, Chen XJ, Liu CQ, Gao YD (2025) Multi-disciplinary evidence illuminates the speciation history of a monophyletic yet dimorphic lily group. Plant Diversity 47(2): 189–200. https://doi.org/10.1016/j.pld.2024.12.005
- Gao YD, Zhou SD, He XJ (2013a) *Lilium yapingense* (Liliaceae), a new species from Yunnan, China, and its systematic significance relative to *Nomocharis*. Annales Botanici Fennici 50(3): 187–194. https://doi.org/10.5735/085.050.0311
- Gao YD, Harris AJ, Zhou SD, He XJ (2013b) Evolutionary events in *Lilium* (including *No-mocharis*, Liliaceae) are temporally correlated with orogenies of the Q–T plateau and the Hengduan Mountains. Molecular Phylogenetics and Evolution 68(3): 443–460. https://doi.org/10.1016/j.ympev.2013.04.026
- Gao YD, Harris AJ, He XJ (2015) Morphological and ecological divergence of *Lilium* and *Nomocharis* within the Hengduan Mountains and Qinghai-Tibetan Plateau may result from habitat specialization and hybridization. BMC Evolutionary Biology 15(1): 147. https://doi.org/10.1186/s12862-015-0405-2
- Gao YD, Harris AJ, Li H, Gao XF (2020) Hybrid speciation and introgression both underlie the genetic structures and evolutionary relationships of three morphologically distinct species of *Lilium* (Liliaceae) forming a hybrid zone along an elevational gradient. Frontiers in Plant Science 11: 576407. https://doi.org/10.3389/ fpls.2020.576407
- Gong X, Hung K-H, Ting Y-W, Hsu T-W, Malikova L, Tran HT, Huang C-L, Liu S-H, Chiang T-Y (2017) Frequent gene flow blurred taxonomic boundaries of sections in *Lilium* L. (Liliaceae). PLoS One 12: e0183209. https://doi.org/10.1371/journal.pone.0183209
- IUCN (2024) Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. http://www.iucnredlist.org/ documents/RedListGuidelines.pdf [Accessed 7 January 2025]
- Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ (2020) GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biology 21(1): 241. https://doi.org/10.1186/s13059-020-02154-5
- Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Kassambara A, Mundt F (2017) factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. https://doi.org/10.32614/CRAN.package.factoextra
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, Gascuel O (2019) NGPhylogeny. fr: New generation phylogenetic services for non-specialists. Nucleic Acids Research 47(W1): W260–W265. https://doi.org/10.1093/nar/gkz303
- Levin DA (1978) The origin of isolating mechanisms in flowering plants. In: Hecht MK, Steere WC, Wallace B (Eds) Evolutionary Biology. Springer US, Boston, MA, 185–317. https://doi.org/10.1007/978-1-4615-6956-5\_4
- Liang SY (1985) A new species of *Lilitum* from Xizang. Journal of Systematics and Evolution 23: 392. https://www.jse.ac.cn/EN/Y1985/V23/I5/392

Liang SY (1987) Lilium L. In: Wu ZY (Ed.) Flora Xizangica. Science Press, Beijing 5: 532–545.

- Liang SY, Tamura M (2000) *Lilium* L. In: Wu ZY, Raven PH (Eds) Flora of China vol. 24. Science Press, Beijing; & Missouri Botanical Garden Press, St. Louis, 135–159.
- Liu CQ, Gao YD, Niu Y, Xiong YZ, Sun H (2019) Floral adaptations of two lilies: Implications for the evolution and pollination ecology of huge trumpet-shaped flowers. American Journal of Botany 106: 622–632. https://doi.org/10.1002/ajb2.1275
- Liu L, Wang Q, Zhang Z, He X, Yu Y (2023) MATO: An updated tool for capturing and analyzing cytotaxonomic and morphological data. The Innovation Life 1(1): 100010. https://doi.org/10.59717/j.xinn-life.2023.100010
- Lu LM, Mao LF, Yang T, Ye JF, Liu B, Li HL, Sun M, Miller JT, Mathews S, Hu H-H (2018) Evolutionary history of the angiosperm flora of China. Nature 554: 234–238. https:// doi.org/10.1038/nature25485

Marchese C (2015) Biodiversity hotspots: A shortcut for a more complicated concept. Global Ecology and Conservation 3: 297–309. https://doi.org/10.1016/j.gecco.2014.12.008

- Peruzzi L (2016) A new infrafamilial taxonomic setting for Liliaceae, with a key to genera and tribes. Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology 150: 1341–1347. https://doi.org/10.1080/11263504.2015.1115435
- POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ [Accessed 28 December 2024]
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Sealy JR (1950) Nomocharis and Lilium. Kew Bulletin 5(2): 273–297. https://doi. org/10.2307/4117245
- Skinner MW (2002) Lilium. In: Flora of North America Editorial Committee (Eds) Flora of North America: Volume 26: Magnoliophyta: Liliidae: Liliales and Orchidales. [Online]. New York and Oxford. http://floranorthamerica.org/Lilium [13 March 2025]

- Stearn WT (1956) Novitates Himalaicae-I. Bull. Brit. Mus. (Nat. Hist.). Bot. London, The Museum 2(3): 65–82.
- Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. https://doi. org/10.1093/molbev/msab120
- Thiers B (2024) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [Accessed 29 December 2024]
- Wang E, Kirby E, Furlong KP, Van Soest M, Xu G, Shi X, Kamp PJ, Hodges KV (2012) Two-phase growth of high topography in eastern Tibet during the Cenozoic. Nature Geoscience 5: 640–645. https://doi.org/10.1038/ngeo1538
- WFO (2025) World Flora Online. http://www.worldfloraonline.org/ [Accessed 12 March 2025]
- Wickham H, Chang W, Wickham MH (2016) Package 'ggplot2.' Create elegant data visualisations using the grammar of graphics. Version 2(1): 1–189. https://github.com/ hadley/ggplot2
- Wu XW, Wang LH, Wu LF, Qu SP, Suh JK, Wang JH (2012) Native species of the genus *Lilium* and the closely related *Nomocharis* in Yunnan, China. Floriculture & Ornamental Biotechnology 6: 28–38.
- Yao H, Song J, Liu C, Luo K, Han J, Li Y, Pang X, Xu H, Zhu Y, Xiao P, Chen S (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One 5: e13102. https://doi.org/10.1371/journal.pone.0013102
- Yu H, Miao S, Xie G, Guo X, Chen Z, Favre A (2020) Contrasting floristic diversity of the Hengduan Mountains, the Himalayas and the Qinghai-Tibet Plateau Sensu Stricto in China. Frontiers in Ecology and Evolution 8. https://doi.org/10.3389/fevo.2020.00136
- Yuan Y, Gao Y (2024) Lilium liangiae, a new species in the genus Lilium (Liliaceae) that reveals parallel evolution within morphology. Frontiers in Plant Science 15. https:// doi.org/10.3389/fpls.2024.1371237
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhang J, Chi X, Zhong J, Fernie A, Alseekh S, Huang L, Qian D (2022) Extensive nrD-NA ITS polymorphism in *Lycium*: Non-concerted evolution and the identification of pseudogenes. Frontiers in Plant Science 13: 984579. https://doi.org/10.3389/ fpls.2022.984579

### **Supplementary material 1**

# Measurement data of Lilium brunneum, Lilium medogense, Lilium paradoxum, Lilium souliei, Lilium georgei, and Lilium saccatum

Authors: Ting Wang, Xiuying Shen, De Wang, Ying Zhao, Xiaomei Qu, Yundong Gao Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.147769.suppl1

# **Supplementary material 2**

#### GenBank accession numbers for sequences utilised in phylogenetic analyses

Authors: Ting Wang, Xiuying Shen, De Wang, Ying Zhao, Xiaomei Qu, Yundong Gao Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.254.147769.suppl2



#### **Research Article**

# Somnuekia flaviflora (Malvaceae, Brownlowioideae), a new genus and species from Thailand

Porntawat Chalermwong<sup>1</sup><sup>(0)</sup>, Sutee Duangjai<sup>2</sup><sup>(0)</sup>, Aroon Sinbumroong<sup>3</sup><sup>(0)</sup>, Theerawat Thananthaisong<sup>4</sup><sup>(0)</sup>, Kunanon Daonurai<sup>4</sup><sup>(0)</sup>, Anusara Kaewmuan<sup>4</sup><sup>(0)</sup>, Manop Poopath<sup>4</sup><sup>(0)</sup>, Wanwisa Bhuchaisri<sup>2</sup>, Kusol Tangjaipitak<sup>2+</sup>, Bhanumas Chantarasuwan<sup>5</sup><sup>(0)</sup>, Chatchai Ngernsaengsaruay<sup>6</sup><sup>(0)</sup>, Sukid Rueangruea<sup>4</sup><sup>(0)</sup>, Somran Suddee<sup>4</sup><sup>(0)</sup>

- 1 Protected Area Regional Office 4 (Surat Thani), Department of National Parks, Wildlife and Plant Conservation, Surat Thani, 84000, Thailand
- 2 Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, 10900, Thailand
- 3 Surat Thani National Park and Protected Area Innovation Center, National Park Innovation Institute, Department of National Parks, Wildlife and Plant Conservation, Surat Thani, 84000, Thailand
- 4 Forest Herbarium (BKF), Department of National Parks, Wildlife and Plant Conservation, Bangkok, 10900, Thailand
- 5 National Science Museum, Thailand, Khlong 5, Klong Luang, Pathumthani, 12120, Thailand
- 6 Department of Botany, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand

Corresponding author: Sutee Duangjai (fforsud@ku.ac.th)

#### Abstract

Academic editor: S. K. Ganesan Received: 7 November 2024 Accepted: 20 March 2025 Published: 3 April 2025

**Citation:** Chalermwong P, Duangjai S, Sinbumroong A, Thananthaisong T, Daonurai K, Kaewmuan A, Poopath M, Bhuchaisri W, Tangjaipitak K, Chantarasuwan B, Ngernsaengsaruay C, Rueangruea S, Suddee S (2025) *Somnuekia flaviflora* (Malvaceae, Brownlowioideae), a new genus and species from Thailand. PhytoKeys 254: 221–243. https://doi. org/10.3897/phytokeys.254.141219

**Copyright:** <sup>©</sup> Porntawat Chalermwong et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Somnuekia is described as a new genus of Malvaceae, currently known only from a few locations in the upper part of Tenasserim in northern and peninsular Thailand. Morphological and molecular phylogenetic analyses, based on plastid DNA sequence regions, support the recognition of this new genus within the Brownlowioideae (Malvaceae). Its distinct phylogenetic position, along with a distinct set of morphological and palynological characteristics, strongly support the recognition of *Somnuekia* as a new genus. A formal description of *Somnuekia flaviflora* is provided along with illustrations, photographs, a distribution map and conservation notes. Furthermore, this new genus is compared to other Asian genera within the subfamily.

Key words: Endemic, monotypic, phylogenetic, taxonomy

### Introduction

In July 2018, Porntawat Chalermwong and his colleague discovered an unknown Malvaceae species along the trail to Dad Fa Waterfall in Tai Rom Yen National Park, Surat Thani Province, peninsular Thailand. In January 2019, they conducted a follow-up survey of the area to collect flowering and immature fruiting specimens. Several characteristics indicated that this species belonged to the expanded family Malvaceae Juss. (Bayer and Kubitzki 2003), including its tree habit, simple leaves, stellate indumentum, 5-locule capsule opening loculicid-ally and subglobose seeds. The leaves of this species resemble those of *Pityranthe trichosperma* (Merr.) Kubitzki, a member of the Brownlowioideae Burrett from China, but its fruit is distinct. This species has a loculicidal capsule, 4.9–6.0 cm long, with five locules, each containing 6–8 seeds (vs. 2–2.5 cm long,

<sup>\*</sup> These authors contributed equally to this work.

<sup>†</sup> Deceased.

with each locule containing 1–2 seeds). Consequently, the unknown species was initially thought to belong to the Brownlowioideae. Leaf samples were then sent to Sutee Duangjai for clarification of the subfamily placement using *ndhF* DNA sequence data. Preliminary results confirmed its placement within the Brownlowioideae. Subsequently, flowering and immature fruiting specimens of the unknown Malvaceae were sent to the Faculty of Forestry at Kasetsart University for classical taxonomic investigation. The samples could not be identified using the key to genera of Brownlowioideae (Bayer and Kubitzki 2003), nor did they match any known genus of the subfamily (Bayer and Kubitzki 2003; Cheek 2007). Therefore, it was considered to represent a new taxon.

The Brownlowioideae, one of ten subfamilies of Malvaceae, consists of nine genera (Colli-Silva et al. 2025) and approximately 97 species (POWO 2025), primarily found in the Old World (Bayer and Kubitzki 2003; Nyffeler et al. 2005). Members of Brownlowioideae are distinguished by stamens with thecae that are divergent at the base, but convergent at the top of the connective (Burret 1926; Bayer et al. 1999; Nyffeler et al. 2005) and sepals fused to form a persistent, campanulate or urceolate calyx (Nyffeler et al. 2005; Cvetković et al. 2021). Colli-Silva et al. (2025) recognised nine genera in the subfamily Brownlowioideae: *Berrya* Roxb., *Brownlowia* Roxb., *Carpodiptera* Griseb., *Christiana* DC., *Diplodiscus* Turcz., *Indagator* Halford, *Jarandersonia* Kosterm., *Pentace* Hassk. and *Pityranthe* Thwaites. The largest genus is *Pentace* (ca. 30 spp.), followed by *Brownlowia* (ca. 29 spp.) and *Diplodiscus* (11 spp.). The remaining genera each contain fewer than 10 species: *Jarandersonia* (eight spp.), *Berrya* (six spp.), *Christiana* (six spp.), *Carpodiptera* (four spp.), *Pityranthe* (two spp.) and *Indagator* (one sp.) (POWO 2025).

Six genera of the subfamily Brownlowioideae are distributed across Asia, New Guinea and the Pacific Islands (Bayer and Kubitzki 2003): Berrya, Brownlowia, Diplodiscus, Jarandersonia, Pentace and Pityranthe. Indagator, a monotypic genus endemic to Australia, was placed close to Diplodiscus, Jarandersonia and Pityranthe by Cheek (2007), based on fruit characteristics. The other two genera, Carpodiptera and Christiana, have a disjunct distribution in Africa and the Americas (Bayer and Kubitzki 2003; Renner 2004) and are genetically close to Berrya (Hernández-Gutiérrez and Magallón 2019; Barbosa-Silva et al. 2021). Only three genera of the Brownlowioideae have been reported in Thailand (Phengklai 1993, as "Tiliaceae"): Berrya, Brownlowia and Pentace, with a total of approximately eight species. In China, one species each of Berrya and Pityranthe (as Hainania trichosperma Merr. or Diplodiscus trichospermus (Merr.) Y. Tang, M.G. Gilbert & Dorr) has been reported (Hung-Ta and Ru-Huai 1989; Bayer and Kubitzki 2003; Tang et al. 2007). In Burma (now Myanmar), only three genera, Berrya, Brownlowia and Pentace, have been recorded (Kurz 1877), with approximately five species. These same genera have also been reported in Indo-China (Gagnepain 1910, 1945). In Malaysia, the subfamily consists of five genera – Berrya, Brownlowia, Diplodiscus, Jarandersonia and Pentace – and includes about 55 species (Tan et al. 2011).

The development of molecular phylogenetic approaches has expanded our understanding of the phylogeny and systematics of Malvaceae *sensu lato* (Alverson et al. 1999; Bayer et al. 1999; Nyffeler and Baum 2000, 2001; Whitlock et al. 2001; Nyffeler et al. 2005; Wilkie et al. 2006; Le Péchon et al. 2010, 2015; Brunken and Muellner 2012; Richardson et al. 2015; Areces-Berazain and Ackerman 2016; Carvalho-Sobrinho et al. 2016;

Hernández-Gutiérrez and Magallón 2019; Dorr and Wurdack 2021; Wu et al. 2023; Hanes et al. 2024; Zhong et al. 2024; Colli-Silva et al. 2025). However, amongst the subfamilies within Malvaceae, only Brownlowioideae remains poorly characterised phylogenetically. The most recent and comprehensive phylogenetic study of Brownlowioideae, conducted by Hernández-Gutiérrez and Magallón (2019), included seven of the 95 species in the subfamily, each representing a different genus. Their results divided the subfamily into two clades. The first clade comprises Brownlowia elata Roxb., Diplodiscus paniculatus Turcz., Jarandersonia clemensiae (Burret) Kosterm. and Pentace polyantha Hassk. The second clade consists of Berrya javanica (Turcz.) Burret, Carpodiptera cubensis Griseb. (Ca. ameliae Lundell) and Christiana africana DC. Due to the lack of phylogenetic studies, generic circumscription within Brownlowioideae has not yet been confirmed using phylogenetic data. Currently, only three complete chloroplast genomes of Pi. trichospermus and 51 plastid DNA sequence accessions for members of Brownlowioideae are available in GenBank (https://www.ncbi.nlm.nih.gov/; accessed April 2024). All prior phylogenetic analyses of the subfamily Brownlowioideae have been based on plastid DNA sequences, such as atpB, ndhF, matK and rbcL (Alverson et al. 1999; Bayer et al. 1999; Nyffeler et al. 2005; Hernández-Gutiérrez and Magallón 2019). Amongst these regions, ndhF has been commonly used as a marker in studies on the phylogenetic relationships within Malvaceae s.l. (Alverson et al. 1999; Whitlock et al. 2001; Pfeil et al. 2002; Nyffeler et al. 2005; Wilkie et al. 2006; Koopman and Baum 2008; Richardson et al. 2015; Hernández-Gutiérrez and Magallón 2019). In this study, we reconstructed a phylogenetic tree of Malvaceae s.l. to determine the subfamily placement of an unknown Thai Malvaceae species. We also evaluated phylogenetic relationships with other genera within the subfamily using DNA sequences from three plastid regions: ndhF, rbcL and the trnL intron and the trnL-trnF spacer (hereafter the trnLF region).

All genera in the Brownlowioideae are delimited, based on fruit characters considered taxonomically essential (Kurz 1877; Kostermans 1960, 1961a, 1961b, 1964, 1970; Kochummen 1973; Hung-Ta and Ru-Huai 1989; Phengklai 1993; Bayer and Kubitzki 2003; Cheek 2007; Tang et al. 2007; Tan et al. 2011; Chung and Soepadmo 2017; Ganesan et al. 2021; Coutinho et al. 2025), whereas floral characters, except for staminode presence, are not useful at the generic level (Kostermans 1960, 1961a, 1961b, 1964, 1970; Cheek 2007). The genus Brownlowia can be distinguished from other related genera - Berrya, Carpodiptera, Christiana, Diplodiscus, Indagator, Jarandersonia, Pentace and Pityranthe – by its apocarpous and loosely connected carpels and indehiscent fruits (Fig. 1). In contrast, the genera Berrya, Carpodiptera, Christiana, Diplodiscus, Indagator, Jarandersonia, Pentace and Pityranthe possess united carpels. The genera Berrya and Pentace have winged capsules (Fig. 1), but the latter also has staminodes. Diplodiscus has exalate capsules, whereas Pityranthe has thin woody capsules. Jarandersonia has spinulose fruits similar to those of Indagator, which has a woody capsule with a shorter spine and both lack wings. Most of the Asian genera, except Berrya, possess five staminodes. The genera Carpodiptera and Christiana have only fertile stamens; their fruits are winged and woody capsules, respectively.

The palynological features of most genera within Brownlowioideae were described by Perveen et al. (2004), who conducted light microscopy (LM) and



Figure 1. Fruit morphology of some Asian genera of the Brownlowioideae **A** Berrya (D. Praphat 124, BKF) **B, C** Brownlowia (**B** S. Pinnin 1548, BKF **C** living plant) **D** Pentace (C. Niyomdham & P. Puudjaa 7500, BKF).

scanning electron microscopy (SEM) for eight genera (*Berrya, Brownlowia, Carpodiptera, Christiana, Diplodiscus, Jarandersonia, Pentace, and Pityranthe* [including *Hainania* Merr.]). The pollen grains of these eight genera are shed as monads with tricolporate apertures (short colpi), suboblate or oblate in shape and typically with a reticulate tectum (Perveen et al. 2004).

To evaluate the taxonomic status of the unknown Thai Malvaceae species, molecular phylogenetic studies were performed to determine its genus and subfamily placement. Additionally, the morphological characteristics of the new taxon were assessed. Based on the results of phylogenetic and morphological analyses, we describe this plant as a new species, *Somnuekia flaviflora*, in a monotypic new genus, *Somnuekia*, belonging to the subfamily Brownlowioideae.

## Materials and methods

# New taxon sampling, morphological investigation, description and geographical distribution

The first set of specimens of the new species was collected by Aroon Sinbumroong and Porntawat Chalermwong in Surat Thani Province, Peninsular Thailand, between January 2019 and January 2023. The second and third sets were collected in Tak Province, northern Thailand, during February and November 2022 by Somran Suddee and his team from BKF. The fourth set, collected by Manop Poopath in Kamphaeng Phet Province, northern Thailand, in 2015, was located in the BKF. Most specimens were preserved in alcohol and pressed and dried at the office in Bangkok. Vouchers were deposited at the Bangkok Herbarium (BK), BKF and the Royal Botanic Gardens, Kew (K) (abbreviations follow Thiers 2020). The collected material was photographed in the field and at the Department of Forest Biology, Faculty of Forestry, Kasetsart University. Morphological studies of the new species were based on observations of living plants, field notes, photographs and dried herbarium specimens. Trichomes on branches, petioles, leaf blades, fruits and seeds were examined from fresh, alcohol-preserved and dried samples using a Zeiss Stemi 508 apochromatic compact stereomicroscope (Carl Zeiss, Germany) and a Zeiss Axioskop 40 microscope (Carl Zeiss, Germany) at the Department of Forest Biology, Faculty of Forestry, Kasetsart University. Images were captured using a Canon EOS700D digital camera and processed with AxioVision SE64 software (Carl Zeiss). The stamen characters and sequential maturation were observed on both fresh and preserved flowers. Pollen samples were taken from a herbarium specimen collected from Surat Thani Province (Chalermwong & Sinbumroong 20230103-01). Pollen morphological characters were observed using LM and SEM at the Department of Botany and the Scientific Equipment Center, Faculty of Science, Kasetsart University. For LM, pollen grains (20 tetrads, 60 grains) were examined with a Zeiss Axioskop 40 microscope (Carl Zeiss, Germany) and images were captured using a Canon EOS700D digital camera. Pollen grains were mounted on stubs with double-sided sellotape, sputter-coated with gold and examined using an FEI Quanta 450 SEM (Hillsboro, OR, USA) at 15.00 kV. Pollen morphology was described following Erdtman (1952). The characters of the unknown Malvaceae taxon were compared with those of six Asian genera within the subfamily Brownlowioideae to clarify morphological similarities and differences.

Digital images of type specimens of other genera in the subfamily Brownlowioideae, available from JSTOR Global Plants (http://plants.jstor.org/), the Herbarium Catalogue, Royal Botanic Gardens, Kew (http://www.kew.org/herbcat), the BioPortal of Naturalis Biodiversity Center (http://bioportal.naturalis.nl/), the Museum National d'Histoire Naturelle-Paris Herbarium, P (https://science.mnhn.fr/institution/ mnhn/collection/p/) and the PE Herbarium (https://petype.myspecies.info), as well as collections in BK and BKF, were examined and compared with the new species. Relevant taxonomic studies (e.g. Kurz (1877); Gagnepain (1910, 1945); Kostermans (1960, 1961a, 1961b, 1964, 1970); Kochummen (1973); Hung-Ta and Ru-Huai (1989); Phengklai (1993); Bayer and Kubitzki (2003); Cheek (2007); Tang et al. (2007); LaFrankie (2010); Tan et al. (2011)) were consulted.

A distribution map based on specimens and field observations was created using ArcMap version 10.3.1 (ESRI). The conservation status of the species was assessed by calculating its extent of occurrence (EOO) and area of occupancy (AOO) using GeoCAT, then evaluated according to the IUCN Red List categories and criteria (IUCN 2022).

#### Molecular phylogenetic analysis

# Sampling, DNA markers, DNA extraction, PCR amplification and sequencing

Since the preliminary results, based on the *ndhF* gene, indicate that the unknown taxon is a member of the Brownlowioideae, then sixteen species (21 samples) of Brownlowioideae, representing nine genera, were analysed. Only the Australian monotypic genus *Indagator* was not included. Thirty-five new sequences were generated for this study, sequences of other Brownlowioideae members were obtained from previous studies. Additional sequences from the complete chloroplast genomes of 21 taxa representing the other eight Malvaceae subfamilies (except only the member of the Matisioideae) were downloaded from GenBank. *Muntingia calabura* L. was used as an outgroup. We used DNA sequences from three plastid regions (*ndhF*, *rbcL* and the *trnLF* region) to investigate the subfamily placement of the unknown Malvaceae taxon from Thailand and its relationships with other genera within the subfamily. Taxon names and GenBank accession numbers are available in Appendix 1.

Leaves from the unknown species and nine other samples were dried and preserved in silica gel (Chase and Hills 1991). Total DNA was extracted from silica-dried leaf samples using either a modified 2 × cetyltrimethyl ammonium bromide (CTAB) procedure (Doyle and Doyle 1987) or the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). DNA quality and quantity were assessed by agarose gel electrophoresis. Double-stranded DNA from three chloroplast genome regions was amplified by polymerase chain reaction (PCR) using six primers for ndhF, four for rbcL and two for the trnL intron and trnL-F spacer region (Table 1). PCR was performed on a C1000 Thermal Cycler (Bio-Rad, Singapore) in a volume of 50 µl, containing 25 µl of 2 × DreamTag Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 21 µl of nuclease-free water, 1 µl of bovine serum albumin (BSA) (New England Biolabs), 1 µl of each primer (20 mmol/l) and 1 µl of template DNA. The thermocycler protocol consisted of an initial 3-min pre-melt at 94 °C, followed by 35 cycles (denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C and extension for 1 min at 65 °C), with a final extension for 10 min at 65 °C.

Amplified products were cleaned using FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Fisher Scientific). The cleaned PCR products were sequenced using the same primers as in the initial amplifications. Sanger sequencing was performed at the Macrogen Sequencing Facility (Macrogen, Inc., Seoul, South Korea).

Region and Primer name	Sequence (5' $\rightarrow$ 3')	Forward or reverse primer	Source				
ndhF gene							
40F	ATATTCATGGATCATACCTTTTGTG	forward	This study				
1080R	TAAAAGGAATGCTGTAAATATTCCG	reverse	This study				
972F	GTCCCAACTGGGTTATATGATG	forward	Alverson et al. (1999)				
1860R	TAAAAGGAATGCTGTAAATATTCCG	reverse	This study				
1300F	GTGACAGTTGGTTGTATTCACCGA	forward	This study				
2140R	TCTTATACCTTTTGTTAAGGATAT	reverse	This study				
rbcL gene							
rbcL1F	ATGTCACCACAAACAGAAAC	forward	Savolainen et al. (2000)				
rbcL724R	TCGCATGTACCTGCAGTAGC	reverse	Fay et al. (1997)				
rbcL636F	TGCGTTGGAGAGACCGTTTC	forward	Kaufmann and Wink (1994)				
rbcL1R	TCCTTTTAGTAAAAGATTGGGCCGAG	reverse	Savolainen et al. (2000)				
trnL intron and trnL-F spacer			·				
С	CGAAATCGGTAGACGCTACG	forward	Taberlet et al. (1991)				
f	ATTTGAACTGGTGACACGAG	reverse	Taberlet et al. (1991)				

Table 4 Duine and	I f DNIA				and a statistic sector as a
Ianie I Primers	A VIU 10T DA2U S	amplification and	1 seguencing	1 OT THE THREE	niastin regions
		unipilitoution une	a ocquerioni	y or the three	piùotiù regiono.

Sequence editing, alignment and phylogenetic analysis

Raw sequences were edited and assembled using AutoAssembler version 1.4.0 (Applied Biosystems). Multiple-sequence alignments were performed with ClustalX (Larkin et al. 2007) and manually adjusted in MacClade 4.07 (Maddison and Maddison 2005). Phylogenetic analyses were conducted using maximum parsimony (MP) and Bayesian Inference (BI; Rannala and Yang (1996); Yang and Rannala (1997)). MP analyses were performed using equally weighted, unordered nucleotide substitutions (Fitch 1971) in PAUP\* version 4.0b10 (Swofford 2002). The most parsimonious trees were identified through heuristic searches with 1,000 replicates of random sequence addition, using tree bisection and reconnection (TBR) swapping and the setting MulTrees = on. TBR swapping was applied to a maximum of 200 trees (nchuck = 200) per replicate. Node support was assessed using bootstrap with 1,000 replicates, the heuristic search with simple addition sequences, TBR swapping (nchuck = 200) and setting MulTrees = off. BI was conducted using MrBayes version 3.2 (Ronquist et al. 2012). Nucleotide substitution models were selected, based on Akaike's Information Criterion (AIC), implemented in MrModelTest version 2.3 (Nylander 2004). Two independent Markov Chain Monte Carlo analyses were performed, each with four simultaneous chains over 10,000,000 generations, sampling one tree every 1,000 generations. The first 25% of trees were discarded as burn-in and the remaining trees were used to construct a majority-rule consensus tree with Bayesian posterior probabilities (PPs).

#### **Results and discussion**

#### Phylogenetic placement of the unknown taxon within Brownlowioideae and its relationships with other genera based on three plastid regions

Phylogenetic placement of the unknown taxon and its relationships with other genera within the subfamily Brownlowioideae were evaluated by analyzing *ndhF*, *rbcL* and the *trnLF* region. The alignment of this dataset consisted of

4,931 characters, with 1,238 variable sites and 495 parsimony informative sites. The MP heuristic search retrieved the 324 most parsimonious trees, with 1,788 steps (consistency index = 0.80; retention index = 0.76). The best-fitting model of nucleotide evolution was GTR + I + G for all regions. Tree topologies generated from the combined data using BI were approximately congruent with those from MP; only the BI majority tree is shown in Fig. 2. In Malvaceae s.l., all nine subfamilies were monophyletic, consistent with previous reports (Alverson et al. 1999; Nyffeler et al. 2005; Hernández-Gutiérrez and Magallón 2019). There were two main clades within Malvaceae s.l.: Byttneriina (PP = 1.0, BS = 98) and Malvadendrina (PP = 1.0, BS = 87). The monophyly of the subfamily Brownlowioideae was strongly supported (PP = 1.0, BS = 100) based on the combined dataset; however, our results do not provide additional insight into the interrelationships beyond those previously published (Alverson et al. 1999; Nyffeler et al. 2005). The sister lineage of Brownlowioideae was not resolved and the subfamily formed a polytomy with the clades Dombeyoideae and Tilioideae (PP = 1.0, BS = 53) and the Bombacoideae/Malvoideae/Sterculioideae clade (PP = 1.0, BS < 50). Phylogenetic analysis of the sequence data of ndhF, rbcL and the trnLF region revealed that the unknown Malvaceae species is a member of Brownlowioideae and confirmed the placement of seven other species: Berrya cordifolia (Willd.) Burret, Brownlowia argentata Kurz, Brownlowia emarginata Pierre, Brownlowia helferiana Pierre, Brownlowia peltata Benth., Brownlowia tersa (L.) Kosterm. and Pentace curtisii King within this subfamily (Fig. 2).

Brownlowioideae contained two clades, Clade I (PP = 1.0, BS = 99) and Clade II (PP = 1.0, BS = 95) (Fig. 2), consistent with the results based on *ndhF* (unpublished data). The first clade included *Br. argentata, Br. elata, Br. emarginata, Br. helferiana, Br. peltata, Br. tersa, D. paniculatus, J. clemensiae, Pe. curtisii, Pe. polyantha* and the unknown Malvaceae. The second clade consisted of *Be. cordifolia, Be. javanica, Ca. cubensis, Ch. africana* and *Pi. trichosperma*. The two accessions of the unknown Malvaceae (*Somnuekia flaviflora*) were monophyletic with strong support (PP = 1.0, BS = 100).

In Brownlowioideae Clade I, J. clemensiae and the unknown taxon (Somnuekia flaviflora) were isolated, while the other samples of the genera Brownlowia, Diplodiscus and Pentace formed a subclade, with D. paniculatus as the sister taxon. Therefore, the monophyly of the genus Brownlowia was not supported. Br. argentata, Br. emarginata and Br. tersa grouped with high support (PP = 1.0, BS = 97). In contrast, Br. elata, Br. helferiana and Br. peltata formed a clade with two species of Pentace (PP = 0.98, BS = 52). The second clade of Brownlowioideae consisted of two species of Berrya, Ca. cubensis, Ch. africana and Pi. trichosperma (PP = 1.0, BS = 94). This clade contained three subclades: the first comprised the three samples of Be. cordifolia, the type species of the genus (PP = 1.0, BS = 100); the second contained Be. javanica, Ca. cubensis and Ch. africana (PP = 1.0, BS = 94); and the third included all samples of Pi. trichosperma (PP = 1.0, BS = 100). The monophyly of the genus Berrya was not supported because Be. javanica grouped with Ca. cubensis and Ch. africana, with high support, rather than with the type species Be. cordifolia. The results (Fig. 2) indicated that the new species is not closely related to Pi. Trichosperma, but belongs to the same clade as Br. argentata, Br. elata, Br. emarginata, Br. helferiana, Br. peltata, Br. tersa, D. paniculatus, Pe. curtisii, Pe. polyantha and J. clemensiae.



Figure 2. Phylogenetic trees of the subfamily Brownlowioideae resulting from analysis of the concatenated dataset (ndhF + rbcL + trnLF region) of 43 samples **A** fifty percent Bayesian majority-rule consensus tree. Posterior probability values are shown above branches and bootstrap percentages for supported clades are shown below branches. Individuals of *Somnuekia flaviflora* are in bold and indicated by arrows. Subfamilies *sensu* Colli-Silva et al. (2025) are labelled where applicable **B** phylogram obtained in Bayesian analysis

#### Morphological study and taxonomic treatment

After confirming that the collected species belonged to the Brownlowioideae (Malvaceae), we examined all collections of this subfamily from Thailand in BK and BKF. In addition, we reviewed virtual collections of nine genera – *Berrya, Brownlowia, Carpodiptera, Christiana, Diplodiscus, Indagator, Jarandersonia, Pentace* and *Pityranthe* – in JSTOR Global Plants, as well as in the K, L, P and PE Herbaria. The collections of the unknown Malvaceae could not be satisfactorily placed in any of the currently described genera within Brown-lowioideae. Furthermore, based on the key to genera of Brownlowioideae and descriptions of recognised genera (Bayer and Kubitzki 2003), the unknown Malvaceae is not referable to any recognised genus in the subfamily and is described here as both a new genus and species. The characteristics of the ovaries, fruits and seeds of this new taxon are not consistent with any genera bearing capsular fruits in the subfamily. Morphological characteristics and habitat data of the new taxon, along with those of closely-related Asian genera, are summarised in Table 2.

The unknown Malvaceae has hermaphroditic flowers with stamens whose thecae are divergent at the base, but convergent at the top of the connective, a characteristic that clearly places it within the subfamily Brownlowioideae. It also has five fusiform staminodes, which are connate at the base with the fertile stamens (Figs 3–5) and differ from those of *Berrya*. The pollen grains of the unknown Malvaceae are grouped in tetrahedral tetrads; however, in SEM-prepared pollen samples, they appeared more or less collapsed (Fig. 6). Only monads with tricolporate (short colpi), suboblate or oblate shapes and typically a reticulate tectum have been reported in the other eight genera (*Berrya*, *Brownlowia*, *Carpodiptera*, *Christiana*, *Diplodiscus*, *Jarandersonia*, *Pentace* and *Pityranthe* 

Character	Somnuekia	Pityranthe	Berrya	Brownlowia	Diplodiscus	Pentace	Jarandersonia
Number of species	1	2	6	29	11	30	8
Distribution	Thailand	Sri Lanka and China (Guangxi to Hainan)	Andaman Is., Bangladesh, Borneo, Cambodia, Christmas I., Fiji, India, Java, Laos, Lesser Sunda Is., Malaya, Myanmar, New Guinea, Northern Territory, Philippines, Queensland, Sri Lanka, Sulawesi, Taiwan, Thailand, Vietnam	Andaman Is., Bangladesh, Borneo, Cambodia, India, Laos, Malaya, Maluku, Myanmar, New Guinea, the Philippines, Solomon Is., Sulawesi, Sumatra, Thailand, Vietnam	Borneo, Malaya, the Philippines	Bangladesh, Borneo, Cambodia, Java, Laos, Lesser Sunda Is., Malaya, Myanmar, the Philippines, Sumatra, Thailand	Borneo
Foliaceous staminodes	present, 5	present, 5	absent	present, 5	present, 5	present, 5	present, 5
Pollen grain	tetrahedral tetrads	monads	monads	monads	monads	monads	monads
Carpels	5-loculed, 6–8 ovules per locule, axile placentation	3–5-loculed, 1(2) ovules per locule, axile placentation	3–5-loculed, 2–6 ovules per locule, axile placentation	5-loculed, 2 ovules per locule, axile placentation	5-loculed, 2 ovules per locule, axile placentation	3–5(10)-loculed, 2 ovules per locule, axile placentation	5-loculed, 2 ovules per locule, axile placentation
Fruits	syncarpous fruit has 5-radiating carpels, exalate capsules	syncarpous fruit lacking wing, thinly woody capsules	syncarpous fruit has winged capsules	apocarpous fruit lacking wing	syncarpous fruit lacking wing, exalate capsules	syncarpous fruit has winged capsules	syncarpous fruit covered with spines bearing setose hairs, capsules

Table 2. Characteristics of the genus Somnuekia and closely-related Asian taxa in the subfamily Brownlowioideae.

[Perveen et al. 2004]). The ovary of the new taxon is ellipsoid in shape and has five carpels, each containing eight ovules with axile placentation.

The unknown Malvaceae has loculicidal dehiscent, cylindrical fruits with five radiating carpels (Figs 3–5), differing from those of *Berrya* (winged capsules vs. wingless capsules), *Brownlowia* (apocarpous vs. syncarpous and indehiscent fruits vs. dehiscent fruits), *Diplodiscus* (two ovules per locule vs. eight ovules per locule), *Jarandersonia* (spinulose fruits vs. non-spinulose fruits), *Pentace* (winged capsules vs. wingless capsules) and *Pityranthe* (five radiating carpels and exalate capsules vs. non-radiating carpels).

#### **Taxonomic treatment**

Based on the morphological and palynological differences between the undescribed taxon and recognised genera in the subfamily Brownlowioideae, as well as its well-supported, isolated phylogenetic placement (Fig. 2), a new genus is proposed here.

#### Subfam. Brownlowioideae Burret

*Somnuekia* Duangjai, Chalermw., Sinbumr. & Suddee, gen. nov. urn:lsid:ipni.org:names:77359677-1

**Type and only known species.** Somnuekia flaviflora Duangjai, Chalermw., Sinbumr. & Suddee

**Diagnosis.** Somnuekia Duangjai, Chalermw., Sinbumr. & Suddee resembles *Pity-ranthe* Thwaites morphologically, but differs from the latter by its unique pollen, having more numerous ovules in each loculus and fruit characters. The detailed distinguishing characters of this new genus and other genera are listed in Table 2.

**Description.** Tree, monoecious. Leaves simple, ovate, cordate or slightly five-angled, with 5–7 basal veins, entire with glandular teeth; with long petioles; stipules filiform, caducous. Flowers bisexual, arranged in terminal panicles; bracts slender, caducous; sepals connected into a campanulate tube, calyx teeth 5; petals 5, spathulate; staminodes 5, fusiform; stamens 25–50, filaments slightly connate at base, with elongated filaments, small anthers, uncommissural antherium; ovary superior, 5 cells, 6–8 ovules per cell; slender style; stigma conical. Capsule cylindrical, loculicidal with 5-radiating carpels, (3)6–8 seeds in each loculus, seeds covered with short stellate scales.

**Etymology.** The genus is named in honour of the Thai dendrologist, Associate Professor Somnuek Pongumphai.

Distribution and habitat. Endemic to Thailand at 200-580 m altitude.

#### *Somnuekia flaviflora* Duangjai, Chalermw., Sinbumr. & Suddee, sp. nov. urn:lsid:ipni.org:names:77359678-1

**Type.** THAILAND • Surat Thani Province, Tai Rom Yen National Park, Dad Fa Waterfall, 8°51'49.6"N, 99°28'41.7"E, ca. 240 m alt., *P. Chalermwong, A. Sinbumroong & A. Issarapakdee 20220317-01* (holotype: BKF! [SN 268094]; isotypes BK!, BKF! [SN268095], K!, SING!). Figs 3–5.



Figure 3. Somnuekia flaviflora A habit B trunk and bark C lower-branch shoot and leaves C1 apical shoot of the lower-branch shoot, showing filiform stipules and trichome covering D terminal bud of upper branch shoot showing scale covering E leaf-like bracts in infructescence F mature flower buds and blooming flower G infructescence with immature and mature capsules H immature capsules with prominent ridges I lateral view of carpel, split longitudinally to show seed arrangement and mature seeds.



Figure 4. Somnuekia flaviflora **A** flowers and flower bud **B**–**D** flowers at different stages of anthesis **B** early blooming flower with indehiscent anther **C** blooming flower with dehiscent anther **D** late blooming flower with dehiscent anther **E** immature capsules with immature seeds, showing reddish-pink stellate scale covering **F** mature capsule with mature seeds, showing dark brown stellate scale covering. Flowers shown in **A**–**C** were taken from branches placed in a plastic bag overnight.



**Figure 5.** Somnuekia flaviflora **A** habit, fruiting branch and leaf-like bracts **B**, **C** leaves **B** leaf showing adaxial surface **C** leaf showing abaxial surface and venation **D** mature flower bud **E**–**K** opened flower and its components **E** opened flower **F** sepals showing pilose adaxial surface **G** petals **H** staminode **I** stamens: anterior view (left), posterior view (middle) and lateral view (right) **J** dissected flower with sepals and petals removed, stamens: anterior view (left), posterior view (middle) and lateral view (right) **K** pistil **L** mature capsules **M** mature capsules at splitting stage **N** carpel in lateral view, split longitudinally to show seed arrangement **O** mature seeds. **A**–**C**, **L**–**O** from *Chalermwong et al. 20220317-01* (type) **D**–**K** from *Sinburnoong & Chalermwong 20190128-01*. Drawings by W. Bhuchaisri. Scale bars: 5 cm (**A**–**C**); 1 cm (**D**, **E**); 5 mm (**F**); 1 cm (**G**); 5 mm (**H**); 1 mm (**I**); 5 mm (**X**); 2 cm (**L**–**N**); 2 mm (**O**).



**Figure 6.** Light and scanning electron micrographs of pollen of *Somnuekia flaviflora* **A** overall view of tetrahedral tetrad (LM, tetrahedral position) **B**–**C** overall view of tetrahedral tetrad and details of exine sculpturing and colpus (SEM, collapsed pollen) **D** detail of exine sculpturing (SEM) showing rugulate-reticulate to reticulate pattern.

Description. Tree to 35 m tall, to 80 cm in diameter at breast height. Bark outer bark greyish-white or brown, smooth and sparsely lenticellate; inner bark light brown, with multiple layers, up to 4.0 cm thick. Twigs lower branches with petiolar scars, densely covered with reddish-brown pilose hairs, later glabrescent, sparsely lenticellate; upper branches densely covered with short greyish-white stellate hairs, later grabrescent and dark brown. Stipules base obliquely ovate, concave, ca. 4 × 3 mm, apex cuspidate, ca. 4-5 mm long, caducous, abaxial hirsute, adaxial glabrous. Leaves spiral, blades ovate, cordate or slightly five-angled, pale green above, greyish-white below, densely covered with stellate scales with scattered reddish-brown pilose hairs along mid-rib and veins below, glabrous or nearly so above, (8.2)10.5-24 × (6.8)9.1-21.5 cm, slightly heart-shaped or truncate at the base, acute or acuminate at the apex, entire or with small teeth and ciliate, with 5-7 basal veins, prominent below, lateral veins 4-6 on each side, tertiary veins scalariform; petiole (3.1)6.8-22.5 cm long, 2.0-5.0 mm in diameter, swollen at either ends, densely covered in reddish-brown pilose hairs for petioles on lower branches or greyish-white stellate scales for petioles of

upper branches. Inflorescences terminal panicles, up to 40 cm long with many lax flowers, peduncles 0.3-0.5 cm in diameter, pedicels, peduncles and rachis covered with greyish-white short stellate scales; bracts and bracteoles small, caducous, with 4-5 leaf-like bracts attached to the rachis. Flowers bisexual, pedicellate; buds ellipsoid or ovoid, 10-14 mm × 4-6 mm, densely covered in greyish-white stellate scales; pedicels 8-13.5 mm × 2-3 mm, densely covered in stellate scales. Calyx lobes 5, lanceolate, greyish-white, 10-14 mm × 3 mm, valvate, apex acute, densely stellate greyish-white abaxially, densely long silver adaxially. Petals 5, yellow, spathulate, ca. 12 × 3 mm, base gradually tapering, apex emarginate, glabrous on both sides. Androgynophore short, cylindrical, ca. 1 mm long, glabrous. Staminodes 5, yellow, fusiform, ca. 8 mm × 0.5 mm, shorter than filaments of fertile stamens, alternipetalous, glabrous. Fertile stamens 25-50; yellow, antepetalous phalanges, filaments slightly connate at base into 5 fascicles, 10-15 mm long, glabrous; anthers dorsifixed, dithecous, longitudinally dehiscent, 1-1.5 mm long. Ovary ellipsoid, ca. 4 mm × 2.5 mm, densely stellately hairy, carpels 5, united, each carpel with 6-8 ovules, with axile placentation; style 1, ca. 7 mm long, glabrous; stigma conical. Infructescences up to 40 cm long, densely covered with greyish-white short stellate scales, leaf-like bracts ovate, 6.8-13.1 cm × 5.4-10 cm, petiole 2.5-7.5 cm long, with 8-22 capsules. Capsules loculicidal, cylindrical, with 5-radiating carpels, 4.9-6.0 cm × 1.5-2.1 cm, with dense stellate scales; stalk 1.4-1.6 cm long, ca. 0.5 mm thick, covered with stellate scales; each carpel flattened, 4.9-6.0 cm × 0.85-1.0 cm, 2-3 mm thick, bulging at seed, each side of pericarp of carpel partially adnate, margins free and covered with stellate scales. Seed (3)6-8 seeds per lip, subglobose, 2.5 × 3.5 × 2.0 mm, covered with short stellate scales, dark brown.

Specimens examined. THAILAND • Surat Thani [Ban Na San District, Tai Rom Yen National Park, nature trail of Dad Fa Waterfall, 8°51'49.6"N, 99°28'41.7"E, 240 m alt., 28 January 2019, fl. and fr., *Sinbumroong & Chalermwong 20190128-01* (BKF), • ibid., 17 March 2022, fr., *Chalermwong et al.* 20220317-01 (BKF! BK! K! SING!), • ibid., 28 April 2022, *Chalermwong & Sinbumroong* 20220428-01 (BKF), • ibid., 3 January 2023, fl. and fr., *Chalermwong & Sinbumroong* 20230103-01 (BKF)]; Tak [Phop Pra District, Namtok Pha Charoen National Park, road to Pa Wai Waterfall, 16°33'55"N, 98°48'47"E, 870 m alt., 19 February 2022, fr., *Thananthaisong et al.* 690 (BKF), • ibid., 9 April 2022, fr., *Kaewmuan et al.* 115 (BKF)]; Tak [Umphang District, Mae Klong Khi Village, 16°33'54"N, 98°55'6"E, 580 m alt., 6 November 2022, fl. and fr., *Thananthaisong et al.* 955 (BKF)]; Kamphaeng Phet [Khlong Lan District, Khlong Lan National Park, Khlong Lan Waterfall, 16°07'49.5"N, 99°16'34.4"E, 200 m alt., 8 April 2015, fr., *Poopath et al.* 1025 (BKF)].

**Distribution.** Endemic. Known from northern and peninsular Thailand. Fig. 7. **Palynology.** The pollen grains of this species are grouped in tetrahedral tetrads. The tetrad is 70.78–95.82 (81.46 ± 5.19) µm in diam. Each pollen grain of the tetrad is isopolar and radially symmetrical, circular in polar view. The pollen aperture is tricolporate, the colpus length is 11.78–27.91 (20.13 ± 3.64) µm and the colpus width is 3.15–8.84 (5.69 ± 1.26) µm. The top pollen is 43.69–62.95 (53.17 ± 5.25) µm in diam. The polar axis length of pollen is 40.38–52.25 (44.80 ± 2.65) µm and the equatorial axis width is 48.36–64.46 (55.93 ± 3.19) µm which are large-sized. The shape of pollen is oblate spheroidal or oblate [P/E ratio = 0.71–0.93 (0.80 ± 0.06)]. The exine thickness is 1.92–5.60 (3.88 ± 0.88) µm and the sculpturing is regulate-reticulate or reticulate (Fig. 6).



**Figure 7.** Known distribution of *Somnuekia flaviflora*. Solid triangles indicate localities of specimen collection; open triangles indicate localities confirmed by field observations by Porntawat Chalermwong and Aroon Sinbumroong. The map was created using ArcMap version 10.3.1 (ESRI).

**Etymology.** The epithet "flaviflora" refers to the yellow corolla and stamens of the new species.

Vernacular name. The Thai name is "Po Sri Somnuek" (ปอศรีสมนึก).

**Ecology.** In limestone foothills or the marginal open places of the tropical rain forests and dry evergreen forests; between 200 and 800 m altitude.

**Conservation status.** According to IUCN (2022) criteria and based on its Area of Occurrence (AOO) of 16 km<sup>2</sup> and Extent of Occurrence (EOO) of 30,507 km<sup>2</sup>, *Somnuekia flaviflora* is assessed a preliminary status as Near Threatened B2ab (ii,iii,v). It occurs in seven locations.

Phenology. Flowering November–February, fruiting November–April.

#### Acknowledgements

We thank the following Herbaria, BM, E, K, L, P and PE for providing online specimen images. As usual, various online resources have been a big help,

including POWO (continuously updated), IPNI (continuously updated), JSTOR-Plants (continuously updated) and the Biodiversity Heritage Library. For providing plant materials that made this study possible, the authors thank the Royal Botanical Gardens at Kew and Geeawit Sichaikhan. This research was partially supported by Kasetsart University Research and Development Institute, KURDI 2014 (Project No. KSD 21.57) and KURDI 2015 (Project No. KSD 69.58) for the second author. The molecular laboratory work was conducted by the second author at the Department of Forest Biology, Faculty of Forestry, Kasetsart University. We are grateful to the Head of the Department for providing facilities and molecular equipment. Additionally, we would like to thank Mr. Michael Cota for editing the English of the initial version of the paper. Finally, we extend our thanks to the editor and the reviewers for their comments on the manuscript.

# Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

No funding was reported.

#### **Author contributions**

PC, SD, AS and SS were involved in study conception and design; PC, SD, AS, TT, KD, AK, MP, KS, BC, SR and SS collected and provided herbarium and field samples for analysis; SD generated and assembled all the molecular data, which he was also responsible for analysing and interpreting; CN prepared pollen samples and palynological analysis; SD and PC drafted the manuscript and critical revisions were provided by SS, SR, MP and CN; SD, AS and SS also wrote generic and species descriptions; WB analysed the conservation status; PC generated the distribution map; SD revised the manuscript and submitted it; All authors except KT have read and approved the final manuscript.

## Author ORCIDs

Porntawat Chalermwong https://orcid.org/0000-0002-6310-4291 Sutee Duangjai https://orcid.org/0000-0002-1490-759X Aroon Sinbumroong https://orcid.org/0000-0001-8504-4106 Theerawat Thananthaisong https://orcid.org/0000-0003-0665-2141 Kunanon Daonurai https://orcid.org/0000-0003-4070-032X Anusara Kaewmuan https://orcid.org/0000-0002-5578-6130 Manop Poopath https://orcid.org/0000-0002-9871-5295 Bhanumas Chantarasuwan https://orcid.org/0000-0002-6106-9875 Chatchai Ngernsaengsaruay https://orcid.org/0000-0002-7131-976X Sukid Rueangruea https://orcid.org/0000-0003-4031-9721

#### **Data availability**

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

#### References

- Alverson WS, Whitlock BA, Nyffeler R, Bayer C, Baum DA (1999) Phylogeny of the core Malvales: Evidence from *ndhF* sequence data. American Journal of Botany 86(10): 1474–1486. https://doi.org/10.2307/2656928
- Areces-Berazain F, Ackerman JD (2016) Phylogenetics, delimitation and historical biogeography of the pantropical tree genus *Thespesia* (Malvaceae, Gossypieae). Botanical Journal of the Linnean Society 181(2): 171–198. https://doi.org/10.1111/boj.12414
- Barbosa-Silva RG, Coutinho TS, Vasconcelos S, da Silva DF, Oliveira G, Zappi DC (2021) Preliminary placement and new records of an overlooked Amazonian tree, *Christiana mennegae* (Malvaceae). PeerJ 9: e12244. https://doi.org/10.7717/peerj.12244
- Bayer C, Kubitzki K (2003) Malvaceae. In: Kubitzki K, Bayer C (Eds) The Families and Genera of Vascular Plants Vol. V, Flowering Plants Dicotyledons Capparales, Malvales and Non-betalain Caryophyllales. Springer, Berlin, 225–311. https://doi. org/10.1007/978-3-662-07255-4\_28
- Bayer C, Fay MF, De Bruijn AY, Savolainen V, Morton CM, Kubitzki K, Alverson WS, Chase MW (1999) Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: A combined analysis of plastid *atpB* and *rbcL* DNA sequences. Botanical Journal of the Linnean Society 129(4): 267–303. https://doi. org/10.1111/j.1095-8339.1999.tb00505.x
- Brunken U, Muellner AN (2012) A new tribal classification of Grewioideae (Malvaceae) based on morphological and molecular phylogenetic evidence. Systematic Botany 37(3): 699–711. https://doi.org/10.1600/036364412X648670
- Burret M (1926) Beiträge zur Kenntnis der Tiliaceen. Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem 9(88): 592–880. https://doi.org/10.2307/3994327
- Carvalho-Sobrinho JG, Alverson WS, Alcantara S, Queiroz LP, Mota AC, Baum DA (2016) Revisiting the phylogeny of Bombacoideae (Malvaceae): Novel relationships, morphologically cohesive clades, and a new tribal classification based on multilocus phylogenetic analyses. Molecular Phylogenetics and Evolution 101: 56–74. https://doi. org/10.1016/j.ympev.2016.05.006
- Chase MW, Hills H (1991) Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon 40(2): 215–220. https://doi.org/10.2307/1222975
- Cheek M (2007) The identity and conservation status of *Indagator fordii* (Brownlowiaceae/Malvaceae-Brownlowioideae, formerly Tiliaceae), a monotypic tree genus from Queensland, Australia. Kew Bulletin 62: 641–645.
- Chung RCK, Soepadmo E (2017) *Brownlowia latifiana* (Malvaceae-Brownlowioideae), a new species from Terengganu, Peninsular Malaysia. Phytotaxa 298(2): 134–146. https://doi.org/10.11646/phytotaxa.298.2.3
- Colli-Silva M, Pérez-Escobar OA, Ferreira CDM, Costa MTR, Gerace S, Coutinho TS, Yoshikawa VN, Antonio-Domingues H, Hernández-Gutiérrez R, Bovini MG, Duarte MC, Cheek M, Chase MW, Fay MF, Christenhusz MJM, Dorr LJ, Schoeplein C, Corcoran M, Roy S, Cable S, McLay T, Maurin O, Forest F, Baker WJ, Antonelli A (2025) Taxonomy in the light of incongruence: An updated classification of Malvales and Malvaceae based on phylogenomic data. Taxon 00(00): 1–25. https://doi.org/10.1002/tax.13300
- Coutinho TS, Barbosa-Silva RG, Dorr LJ (2025) A synopsis of *Christiana* DC. (Malvaceae, Brownlowioideae), with a new species from the Brazilian Atlantic Forest. PhytoKeys 253: 33–56. https://doi.org/10.3897/phytokeys.253.145350
- Cvetković T, Areces-Berazain F, Hinsinger DD, Thomas DC, Wieringa JJ, Ganesan SK, Strijk JS (2021) Phylogenomics resolves deep subfamilial relationships in Malvaceae s.l.

G3: Genes, Genomes. G3 (Bethesda, Md.) 11(7): jkab136. https://doi.org/10.1093/ g3journal/jkab136

- Dorr LJ, Wurdack KJ (2021) Indo-Asian Eriolaena expanded to include two Malagasy genera, and other generic realignments based on molecular phylogenetics of Dombeyoideae (Malvaceae). Taxon 70(1): 99–126. https://doi.org/10.1002/tax.12370
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Erdtman G (1952) Pollen Morphology and Plant Taxonomy Angiosperms. Almqvist and Wiksell, Stockholm, 650 pp. https://doi.org/10.1080/11035895209453507

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). Kew Bulletin 52(1): 111–120. https://doi.org/10.2307/4117844

- Fitch WM (1971) Toward defining the course of evolution: Minimum change for a specific tree topology. Systematic Zoology 20(4): 406–416. https://doi.org/10.2307/2412116
- Gagnepain F (1910) Tiliacées. In: Humbert H (Ed.) Flore Générale de L'Indo-Chine, Museum National d'Histoire Naturelle, Phanérogamie 1(5–6): 523–582.
- Gagnepain F (1945) Tiliacées. In: Humbert H (Ed.) Supplément a la Flore Générale de l'Indo-Chine (Vol. 1). Muséum National d'Histoire Naturelle, Paris, 440–501.
- Ganesan SK, Tsen STL, Chung RCK (2021) *Jarandersonia pereirae* (Malvaceae, Brownlowioideae), a new species from Sabah, Borneo. Annales Botanici Fennici 58(1–3): 189–193. https://doi.org/10.5735/085.058.0121
- Hanes MM, Blanchard Jr OJ, Valencia-D J, McLay T, Abbott JR, McDaniel SF, Barrett RL, Mathews S, Neubig KM (2024) Phylogenetic relationships within tribe Hibisceae (Malvaceae) reveal complex patterns of polyphyly in *Hibiscus* and *Pavonia*. Systematic Botany 49(1): 77–116. https://doi.org/10.1600/036364424X17114831879189
- Hernández-Gutiérrez R, Magallón S (2019) The timing of Malvales evolution: Incorporating its extensive fossil record to inform about lineage diversification. Molecular Phylogenetics and Evolution 140: 106606. https://doi.org/10.1016/j.ympev.2019.106606
- Hung-Ta C, Ru-Huai M (1989) Tiliaceae. In: Hung-ta C (Ed.) Flora Reipublicae Popularis Sinicae. Science Press, Beijing 49(1): 47–123. [in Chinese]
- IUCN (2022) Guidelines for using the IUCN red list categories and criteria. Version 15. Prepared by the Standards and Petitions Subcommittee. https://www.iucnredlist. org/resources/redlistguidelines

Kaufmann M, Wink M (1994) Molecular systematics of the Nepetoideae (Family Labiatae): Phylogenetic implications from *rbcL* gene sequences. Zeitschrift für Naturforschung. C, A Journal of Biosciences 49(9–10): 635–645. https://doi.org/10.1515/znc-1994-9-1015

- Kochummen KM (1973) Tiliaceae. In: Whitmore TC (Ed.) Tree Flora of Malaya, Vol. 2. Malayan Forest Records No. 26. Longman Malaysia Sdn Bhd, Kuala Lumpur, 392–412.
- Koopman M, Baum DA (2008) Phylogeny and biogeography of tribe Hibisceae (Malvaceae) on Madagascar. Systematic Botany 33(2): 364–374. https://doi. org/10.1600/036364408784571653
- Kostermans AJGH (1960) A monograph of the genus *Diplodiscus* Turcz. (Tiliaceae). Reinwardtia 5(3): 255–265.
- Kostermans AJGH (1961a) A monograph of the genus *Brownlowia* Roxb. (Tiliaceae). Communication Forest Research Institute Bogor 73: 1–63.
- Kostermans AJGH (1961b) The genus *Pityranthe* Thwaites (Tiliaceae). Reinwardtia 5(4): 371–373.
- Kostermans AJGH (1964) A monograph of the genus *Pentace* Hasskarl (Tiliaceae). Communication Forest Research Institute Bogor 87: 1–78.

Kostermans AJGH (1970) The genus *Jarandersonia* Kosterm. Reinwardtia 8(1): 17–20. Kurz S (1877) Tiliaceae. Forest Flora of British Burma 1: 152–170. [Office of the Super-

intendent of Government Printing, Calcutta.] https://doi.org/10.5962/bhl.title.52413

- LaFrankie JV (2010) Trees of Tropical Asia: An Illustrated Guide to Diversity. Black Tree Publications, Inc., Philippines, 748.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics (Oxford, England) 23(21): 2947–2948. https://doi.org/10.1093/bioinformatics/btm404
- Le Péchon T, Dubuisson J-Y, Haevermans T, Cruaud C, Couloux A, Gigord LDB (2010) Multiple colonizations from Madagascar and converged acquisition of dioecy in the Mascarene Dombeyoideae (Malvaceae) as inferred from chloroplast and nuclear DNA sequence analyses. Annals of Botany 106(2): 343–357. https://doi.org/10.1093/ aob/mcq116
- Le Péchon T, Dai Q, Zhang L-B, Gao X-F, Sauquet H (2015) Diversification of Dombeyoideae (Malvaceae) in the Mascarenes: Old taxa on young islands? International Journal of Plant Sciences 176(3): 211–221. https://doi.org/10.1086/679350
- Maddison DR, Maddison WP (2005) MacClade 4: Analysis of phylogeny and character evolution. Version 4.07. Sinauer Associates, Sunderland, Massachusetts.
- Nyffeler R, Baum DA (2000) Phylogenetic relationships of the durians (Bombacaceae-Durioneae or /Malvaceae/Helicteroideae/Durioneae) based on chloroplast and nuclear ribosomal DNA sequences. Plant Systematics and Evolution 224(1–2): 55– 82. https://doi.org/10.1007/BF00985266
- Nyffeler R, Baum DA (2001) Systematics and character evolution in *Durio* s. lat. (Malvaceae/Helicteroideae/Durioneae or Bombacaceae-Durioneae). Organisms, Diversity & Evolution 1(3): 165–178. https://doi.org/10.1078/1439-6092-00015
- Nyffeler R, Bayer C, Alverson WS, Yen A, Whitlock BA, Chase MW, Baum DA (2005) Phylogenetic analysis of the Malvadendrina clade (Malvaceae *s.l.*) based on plastid DNA sequences. Organisms, Diversity & Evolution 5(2): 109–123. https://doi. org/10.1016/j.ode.2004.08.001
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Perveen A, Grafström E, El-Ghazaly G (2004) World pollen and spore flora 23. Malvaceae Adams. P.p. Subfamilies: Grewioideae, Tilioideae, Brownlowioideae. Grana 43(3): 129–155. https://doi.org/10.1080/00173130410000730
- Pfeil BE, Brubaker CL, Craven LA, Crisp MD (2002) Phylogeny of *Hibiscus* and the tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of *ndhF* and the *rpl16* intron. Systematic Botany 27: 333–350. https://www.jstor.org/stable/3093875
- Phengklai C (1993) Tiliaceae. In: Smitinand T, Larsen K (Eds) Flora of Thailand 6(1). Royal Forest Department, Bangkok, 10–80.
- POWO (2025–onward) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Available from: http://www.plantsoftheworldonline.org [accessed 20 Febuary 2025]
- Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43(3): 304–311. https://doi.org/10.1007/BF02338839
- Renner S (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. International Journal of Plant Sciences 165(S4): 23–33. https://doi.org/10.1086/383334

- Richardson JE, Whitlock BA, Meerow AW, Madriñán S (2015) The age of chocolate: A diversification history of *Theobroma* and Malvaceae. Frontiers in Ecology and Evolution 3: a120. https://doi.org/10.3389/fevo.2015.00120
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, De Bruijn AY, Sullivan S, Qiu Y-L (2000) Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. Systematic Biology 49(2): 306–362. https://doi.org/10.1093/sysbio/49.2.306
- Swofford DL (2002) PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17(5): 1105– 1109. https://doi.org/10.1007/BF00037152
- Tan HS, Chung RCK, Soepadmo E (2011) A synopsis of *Jarandersonia* (Malvaceae-Brownlowioideae). Gardens' Bulletin (Singapore) 63(1-2): 137-144.
- Tang Y, Gilbert MG, Dorr LJ (2007) Tiliaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol. 12). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 240–263.
- Thiers B (2020) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 25 December 2020]
- Whitlock B, Bayer C, Baum DA (2001) Phylogenetic relationships and floral evolution of the Byttnerioideae ('Sterculiaceae' or Malvaceae *s.l.*) based on sequences of the chloroplast gene *ndhF*. Systematic Botany 26: 420–437.
- Wilkie P, Clark A, Pennington RT, Cheek M, Bayer C, Wilcock CC (2006) Phylogenetic relationships within the subfamily Sterculioideae (Malvaceae /Sterculiaceae- Sterculieae) using the Chloroplast gene *ndhF*. Systematic Botany 31(1): 160–170. https:// doi.org/10.1600/036364406775971714
- Wu M, He L, Ma G, Zhang K, Yang H, Yang X (2023) The complete chloroplast genome of Diplodiscus trichospermus and phylogenetic position of Brownlowioideae within Malvaceae. BMC Genomics 24(1): 571. https://doi.org/10.1186/s12864-023-09680-z
- Yang Z, Rannala B (1997) Bayesian phylogenetic inference using DNA sequences: A Markov Chain Monte Carlo method. Molecular Biology and Evolution 14(7): 717–724. https://doi.org/10.1093/oxfordjournals.molbev.a025811
- Zhong Y, Bai B, Sun Y, Wen K, Qiao Y, Guo L, Deng H, Ye Y, Feng L, Feng X (2024) Comparative genomics and phylogenetic analysis of six Malvaceae species based on chloroplast genomes. BMC Plant Biology 24(1): 1245. https://doi.org/10.1186/ s12870-024-05974-w

## Appendix 1

Species names and GenBank accession numbers of the *ndhF*, *rbcL* and *trnLF* sequences used in this study. Newly-generated sequences are in bold and \* denotes GenBank accession numbers of the complete chloroplast genomes that were used for analysis.

#### Malvaceae:

- Bombacoideae: *Bombax ceiba* L., MG569974\*, MG569974\*, MG569974\*; *Ceiba speciosa* (A.St.-Hil., A.Juss. & Cambess.) Ravenna, MK820674\*, MK820674\*, MK820674\*.
- Brownlowioideae: Berrya javanica (Turcz.) Burret, AF111755, AJ233146, ; Berrya cordifolia (Willd.) L.Laurent, PP750894, PP750871, PP750882; Berrya cordifolia (Willd.) L.Laurent, PP750895, PP750872, PP750883; Berrya cordifolia (Willd.) L.Laurent, PP750896, PP750873, PP750884; Brownlowia argentata Kurz, PP750900, PP750875, PP750887; Brownlowia elata Roxb., AF111756, AJ233147, PP750886; Brownlowia emarginata Pierre, PP750897, PP750878, PP750890; Brownlowia helferiana Pierre, PP750898, PP750879, PP750891; Brownlowia peltata Benth., PP750901, PP750876, PP750888; Brownlowia tersa (L.) Kosterm., PP750902, PP750877, PP750889; Carpodiptera cubensis Griseb., AF111757, -, -; Christiana africana DC., PP750903, AJ233149, - ; Diplodiscus paniculatus Turcz., AF230252, -, - ; Jarandersonia clemensiae (Burret) Kosterm., AF230253, -, -; Pentace curtisii King PP750899, PP750874, PP750885; Pentace polyantha Hassk., AF111758, AJ233156, -; Pityranthe trichosperma (Merr.) Kubitzki, -, AY328195, AY328160; Pityranthe trichosperma (Merr.) Kubitzki, ON813239\*, ON813239\*, ON813239\*; Pityranthe trichosperma (Merr.) Kubitzki, OP572286\*, OP572286\*, OP572286\*; Somnuekia flaviflora sp. nov., PP750892, PP750869, PP750880; Somnuekia flaviflora sp. nov., PP750893, PP750870, PP750881.
- Byttnerioideae: Theobroma grandiflorum (Willd. ex Spreng.) K.Schum., JQ228388\*, JQ228388\*, JQ228388\*.
- **Dombeyoideae:** *Pterospermum kingtungense* C.Y.Wu ex H.H.Hsue, MH606238\*, MH606238\*, MH606238\*; *Pterospermum truncatolobatum* Gagnep., MN533971\*, MN533971\*, MN533971\*.
- Durioneae Becc.: Durio zibethinus L., MG138151\*, MG138151\*, MG138151\*.
- Grewioideae: Corchorus capsularis L., MK251464\*, MK251464\*, MK251464\*; *Microcos chungii* (Merr.) Chun, MN533967\*.
- Helicteroideae s.s.: Reevesia thyrsoidea Lindl., MH939148\*, MH939148\*, MH939148\*.
- Malvoideae: Gossypium stocksii Mast., JF317355\*, JF317355\*, JF317355\*; Hibiscus rosa-sinensis L., MK382984\*, MK382984\*, MK382984\*; Talipariti tiliaceum (L.) Fryxell, MN826059\*, MN826059\*, MN826059\*; Malva parviflora L., MK860036\*, MK860036\*, MK860036\*.
- Sterculioideae: Firmiana major (W.W.Sm.) Hand.-Mazz., MG229069\*, MG229069\*, MG229069\*; Firmiana simplex (L.) W.Wight, MH671308\*, MH671308\*, MH671308\*; Heritiera fomes Banks, MK033519\*, MK033519\*, MK033519\*; Heritiera littoralis Aiton, MK033518\*, MK033518\*, MK033518\*.
- Tilioideae: Craigia yunnanensis W.W.Sm. & W.E.Evans, MN088379\*, MN088379\*, MN088379\*; Tilia amurensis Rupr., KT894772\*, KT894772\*, KT894772\*; Tilia amurensis Rupr., MH169579\*, MH169579\*, MH169579\*; Tilia oliveri Szyszyl., KT894774\*, KT894774\*, KT894774\*.
- Muntingiaceae: Muntingia calabura L., MW038825\*, MW038825\*, MW038825\*.