PhytoKeys 255: 1–22 (2025)
DOI: 10.3897/phytokeys.255.140014

Research Article

Pollen and morphometric analysis reveal *Solanum tavinuuyuku* (Solanaceae), a new dioecious species from Mesoamerican *Solanum* sect. *Anarrhichomenum*

Jacob Bryant[1], Mariana Vazquez-Alonso[2], Eric J. Tepe[1]

1 *Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221, USA*
2 *Department of Botany, Connecticut College, New London, Connecticut 06320, USA*
Corresponding author: Jacob Bryant (bryan2jo@mail.uc.edu)

Abstract

Solanum tavinuuyuku, of the Sierra Madre del Sur ecoregion of South-Central Mexico, is a viny, node-rooting species of the Potato clade, in the subclade *Solanum* sect. *Anarrhichomenum*. *Solanum tavinuuyuku* is distinguished from its relatives by possessing long and narrow, lanceolate, ovate to falcate, 1–3-foliate leaves with (5–)7–10 secondary veins; large, persistent pseudo-stipules found at nearly every node on herbarium material; and white, violet-tinged corollas particularly pronounced in the floral bud. *Solanum tavinuuyuku* is functionally dioecious, producing functional, tricolporate pollen and non-functional, inaperturate pollen in short and long-styled flowers, respectively, joining its close relative *S. appendiculatum* as one of only two documented dioecious species in the entire Potato clade. Separation of *S. tavinuuyuku* from its closest ally, the simple-leaved *S. ionidium*, is based on morphometric and geographic evidence presented here.

Resumen

Solanum tavinuuyuku, de la ecorregión de la Sierra Madre del Sur en el centro-sur de México, es una especie trepadora con raíces adventicias en los nodos que pertenece al clado de la papa, en el subclado *Solanum* sección *Anarrhichomenum*. *Solanum tavinuuyuku* se distingue de sus parientes por poseer hojas largas y estrechas, lanceoladas, ovadas a falcadas, de 1 a 3 foliolos con (5–)7 a 10 venas secundarias; grandes pseudoestípulas persistentes encontradas en casi todos los nodos en material de herbario; y corolas violetas, particularmente pronunciadas en el capullo floral. *Solanum tavinuuyuku* es funcionalmente dioica, produciendo polen tricolporado funcional y polen inaperturado no funcional en flores de estilo corto y largo, respectivamente, uniéndose a su pariente cercano *S. appendiculatum* como una de las únicas dos especies dioicas documentadas en todo el clado de la papa. La separación de *S. tavinuuyuku* de su aliado más cercano, *S. ionidium* de hojas simples, de las cuales muchas muestras fueron identificadas anteriormente, se basa en evidencia morfométrica y geográfica presentada aquí.

Key words: Dioecy, morphometrics, Mexico, palynology, Potato clade

Academic editor: Leandro Giacomin
Received: 22 October 2024
Accepted: 11 March 2025
Published: 4 April 2025

Citation: Bryant J, Vazquez-Alonso M, Tepe EJ (2025) Pollen and morphometric analysis reveal *Solanum tavinuuyuku* (Solanaceae), a new dioecious species from Mesoamerican *Solanum* sect. *Anarrhichomenum*. PhytoKeys 255: 1–22. https://doi.org/10.3897/phytokeys.255.140014

Introduction

Solanum L. is one of the most species-rich and diverse genera of the angiosperms, encompassing ca.1200 species, and is the largest genus in the Solanaceae (Frodin 2004; Gagnon et al. 2022; Hilgenhof et al. 2023; Messeder et al. 2024; Moonlight et al. 2024). Apart from eminent crop species, potato, tomato and eggplant, the genus also includes many under-studied groups and new species continue to be discovered (e.g. Särkinen et al. (2015); Martine et al. (2016a, 2016b); Särkinen and Knapp (2016); Lacey and Martine (2017); Gouvêa et al. (2018, 2019, 2020); McDonnell et al. (2019); McClelland et al. (2020); Knapp et al. (2022); Tovar and Giacomin (2022); Williams et al. (2022); Tovar et al. (2024)).

Within *Solanum*, 12–13 major lineages have been resolved using modern molecular approaches (Weese and Bohs 2007; Särkinen et al. 2013; Gagnon et al. 2022). A total revision of the entire genus has not been undertaken since Candolle’s (1852) *Prodromus*, but progress is being made to that end by many authors, including by the team behind the Solanaceae Source web resource (www.solanaceaesource.org). Through that initiative, systematic work on the genus has advanced considerably, with workers revising infrageneric groups across the *Solanum* phylogeny (e.g. Levin et al. (2006); Tepe and Bohs (2011); Stern et al. (2013); Wahlert et al. (2014); Clark et al. (2015); Aubriot et al. (2016); Knapp et al. (2019); Spooner et al. (2016); Vorontsova and Knapp (2016)). One of the largest lineages of the ‘non-spiny’ species of *Solanum* is the Potato clade, comprising 12–13 well-supported subclades including the lesser-known and monophyletic *Solanum* sect. *Anarrhichomenum* (Tepe et al. 2016). *Solanum* sect. *Anarrhichomenum* has not been the subject of a comprehensive taxonomic revision and many previous taxonomic works have not recognized it as a single cohesive group. In his monograph of the wild potatoes, Correll (1962) organized some species of *Solanum* sect. *Anarrhichomenum* Bitter under *Solanum* series *Appendiculata* Rydb. (of sect. *Basarthrum* Bitter), but excluded the Mexican species *S. ionidium* Bitter. Nee (1993) was the first to suggest kinship between *S. appendiculatum* Dunal and *S. ionidium* and placed *S. ionidium* with the other four traditionally recognized species in Mesoamerica of what is now recognized as *Solanum* sect. *Anarrhichomenum* (*S. appendiculatum*, *S. skutchii* Correll, *S. tacanense* Lundell, *S. subvelutinum* Rydb. and *S. ionidium*). These Mesoamerican species make up a species complex defined by their morphological similarity to, and common ancestry with, the more well-known *S. appendiculatum* (Correll 1962; Nee 1999; Murillo-Pérez and Rodríguez 2021) and the new species described in this paper joins this group. More recent studies have clarified sectional limits and workers have contributed additional South American species, *S. complectens* M.Nee and G.J.Anderson (Nee et al. 2006) and *S. baretiae* Tepe (Tepe et al. 2012) to the clade. Species of this section are defined by their scandent, viny habit, rooting at the nodes, orange–red globose fruits and a single or, more rarely, paired, but strongly anisophyllous pseudo-stipules at each node along the stem (Tepe et al. 2016).

Solanum appendiculatum, a close relative of the new species proposed here, was the first dioecious species to be discovered within *Solanum* and has been the focal interest of many studies investigating the evolution of dioecy in *Solanum* (Anderson and Gensel 1979; Anderson and Levine 1982; Zavada and Anderson 1997; Wu et al. 2021). Staminate and pistillate plants of *S. appendiculatum* were originally described as three distinct taxa separated by differences in floral morphology (i.e. filament connation and style length) (Correll 1962), but were later determined to be a single dioecious species, based on cross compatibility and biosystematic studies in a series of landmark papers (e.g. Anderson and Gensel (1979); Anderson and Levine (1982)). Taken from the conclusions of these investigations are a series of morphological indicators that can be reliably used to determine the presence of dioecy in *Solanum*, even when cryptic, as in *S. appendiculatum*. Common to most known dioecious *Solanum* is stylar dimorphism and the production of inaperturate, non-functional pollen in the anthers of long-styled flowers and functional, tricolporate pollen in short-styled flowers (Anderson and Levine 1982; Anderson and Symon 1989; Anderson et al. 2015).

The Mesoamerican *Solanum* sect. *Anarrhichomenum* species complex remains poorly understood and is currently under revision by the authors. This study reports some of the results gleaned from ongoing systematic work on the complex and presents *S. tavinuuyuku*, a morphologically unique, dioecious *Solanum* species from Mexico, a center of diversification for Solanaceae (Rodríguez 2004; Villaseñor 2016) and the first new species of Mesoamerican *Solanum* sect. *Anarrhichomenum* to be described in over 70 years. Alongside the morphological and geographical analyses, we report the results from these investigations into the reproductive biology of *S. tavinuuyuku*, following the invaluable work of Anderson and colleagues (Anderson and Levine 1982; Anderson and Symon 1989; Zavada and Anderson 1997; Anderson et al. 2015).

Materials and methods

Sampling and measurement

Observations were based on examination of 38 herbarium specimens on loan and/or specimen images from European and Central, South and North American Herbaria (AAU, ARG, B, BH, BM, BR, C, CORD, F, G, GH, LL, MEXU, MICH, MO, NY, OAX, SERO, UC, UGAC, US, WIS, XAL). Physical specimens were measured with a standard ruler and, for smaller structures, a 1-cm micro-ruler with 0.1 mm marks (Ted Pella, Inc., Redding, CA) under a Leica M80 dissecting microscope. Three specimens of the new species were observed and measured using digitized collections from BM, MEXU, NY (Suppl. material 1). In total, all 20 specimens of the eight known collections of *S. tavinuuyuku* were sampled for this study, along with 18 collections of *S. ionidium*, and 12 collections of *S. appendiculatum* (Suppl. material 1). Specimens of *S. appendiculatum* were limited to collections that were sympatric with *S. tavinuuyuku* or were highly similar in leaf characteristics.

Morphometrics

Factor Analysis of Mixed Data (FAMD) was conducted and visualized in Rstudio (R Core Team 2021) using the packages “FactoMineR” (Le et al. 2008) and “factoextra” (Kassambara and Mundt 2020), respectively, on a total of 15 morphological and two geographic variables (Suppl. material 2). FAMD was selected as the most appropriate tool for exploratory multivariate analysis over the more common PCA because it can incorporate mixed data regimes (Pages 2004). Of the total 15 variables, 12 morphological and one geographic variable were continuous measures and one morphological (corolla color) and one geographic variable (mountain range where specimens were collected) were categorical. Characters such as stamen length and width and style length were excluded from analysis due to their relationship to dioecy (Correll 1962; Anderson and Levine 1982; Anderson et al. 2015). On unifoliate specimens, the absence of lateral leaflets was represented by zeros in the data matrix. Highly co-linear variables such as leaf, leaflet and pseudo-stipule length and width are represented in our study by a ratio.

To prepare for FAMD, the data were first imputed and dimensions were visualized in a scree plot. The ten most influential variables contributing to each dimension were visualized as barplots. FAMD results were plotted in an ordination plot with 95% confidence interval (CI) ellipses based on a multivariate t-distribution grouped by taxonomic classification and a biplot of quantitative variables. Afterwards, multiple-comparison Dunn's tests were conducted on all variables included in morphometric analysis using the base "FSA" package in R (Ogle et al. 2022) and visualized as a series of boxplots.

Mapping

All accessions used in the morphological study were then mapped alongside an additional 46 accessions of *S. appendiculatum* and *S. ionidium* to visualize the distribution of *S. tavinuuyuku* in comparison to those of its relatives *S. appendiculatum* and *S. ionidium* in Mexico (Fig. 1). Thirty-seven of these collections were of *S. appendiculatum*, seven were derived from digital images provided by Mexican herbaria (OAX and UGAC) and the rest were directly examined by the authors (Suppl. material 1). Nine additional accessions of *S. ionidium* were also included, all of which were received as digital images from Mexican herbaria (XAL and OAX) (Suppl. material 1). In total, combining supplementary accessions and those included in morphometric analyses, 82 data points were mapped, encompassing all known collections of *S. tavinuuyuku*, along with selected accessions of *S. appendiculatum* and *S. ionidium*. Maps were constructed in ArcGIS Pro using reference coordinates from herbarium labels or georeferences derived from location descriptions using the online GEOLocate application (Rios and Bart 2010).

Palynology

Pollen grains were retrieved from all available herbarium collections (Suppl. material 1) to investigate the presence of dimorphic pollen in *S. tavinuuyuku*. Three specimens represented staminate, short-styled plants, while the remaining two were long-styled, pistillate plants. Pollen grains were extracted with permission by removing individual anthers from flowers in debris packets on herbarium specimens and then perturbing the anther to release the grains, scraped from a presenting anther or retrieved by inserting an insect pin into the distal end of the anther. Pollen grains were then either mounted directly in a 1:1 glycerine-water solution or treated using a modified acetolysis procedure (Erdtman 1960; Bryant 2025) Both untreated and treated pollen samples were viewed using phase contrast and scanning electron microscopy (SEM) with a Nikon Labophot-2 phase contrast scope and Apreo 2 SEM, respectively.

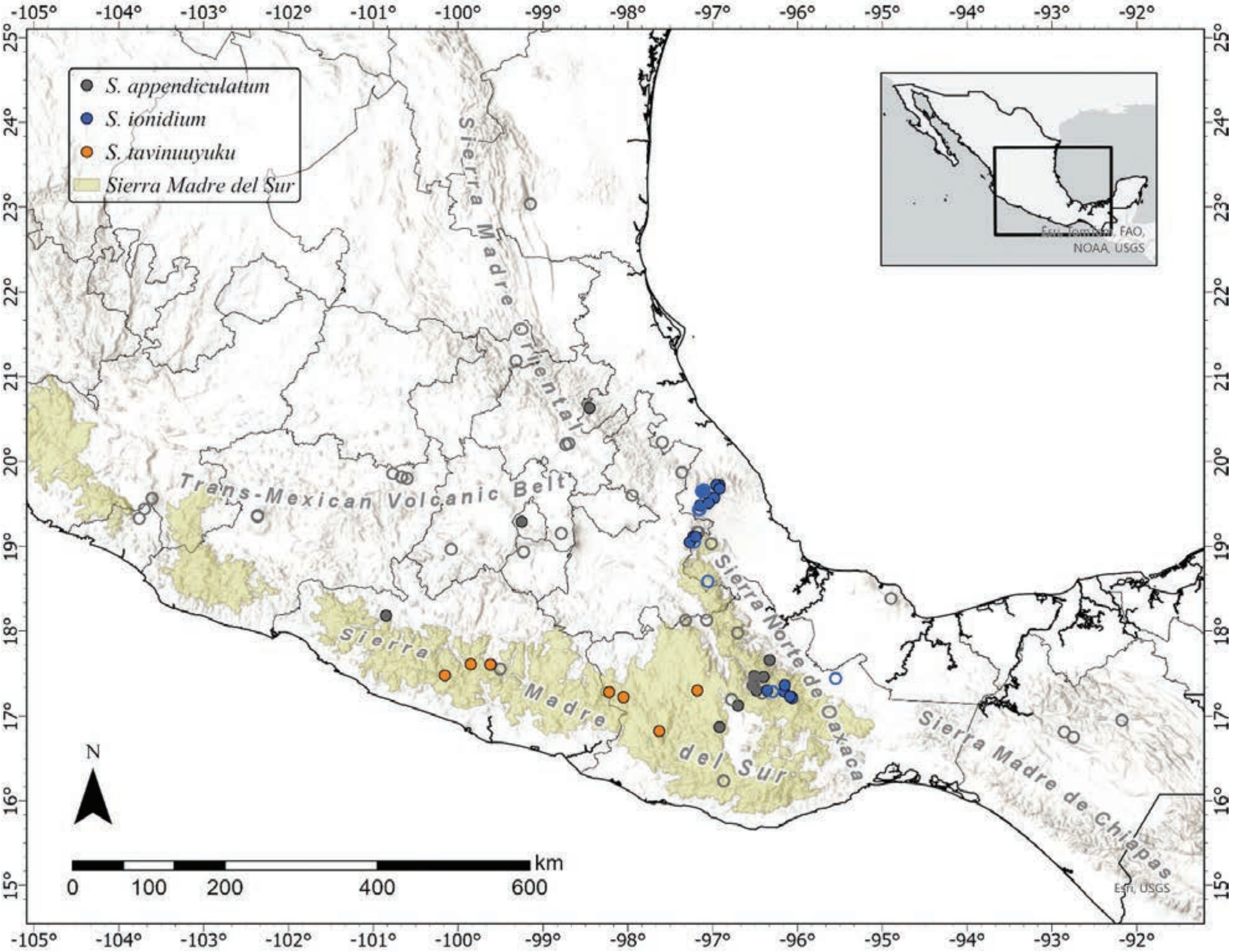

Figure 1. Distribution map of the three species of *Solanum* sect. *Anarrhichomenum* included in this study. The map is composed of collection locations of 82 combined accessions of *Solanum tavinuuyuku* (orange); *Solanum ionidium* (blue) and *Solanum appendiculatum* (grey). Specimens included in the morphometric analyses are indicated by filled circles. Supplementary accessions are indicated by open circles. The Sierra Madre del Sur biogeographic region was mapped using shapefiles of biogeographic provinces provided in Morrone et al. (2017).

Throughout this paper, specimen barcodes and accession numbers are cited in brackets. Barcode numbers are listed after an herbarium acronym, whereas accession numbers include the number only. When digital collections of the new species were used, these collections are indicated by the '[photo]' notation.

Results

Morphometrics

For multivariate species delimitation, the first two dimensions were retained in FAMD analysis accounting for 40.7% of total variance in the data. Results revealed a distinct separation of *S. tavinuuyuku* from its close relatives *S. ionidium* and *S. appendiculatum* in morphospace (Fig. 2). Variables contributing to the separation included number of leaflets, region of collection, pedicel length, lateral leaflet length/width ratio, number of flowers per inflorescence, leaf length/width ratio, corolla color and number of secondary veins (Fig. 3). All accessions of *S. tavinuuyuku* clustered together around positive eigenvalues

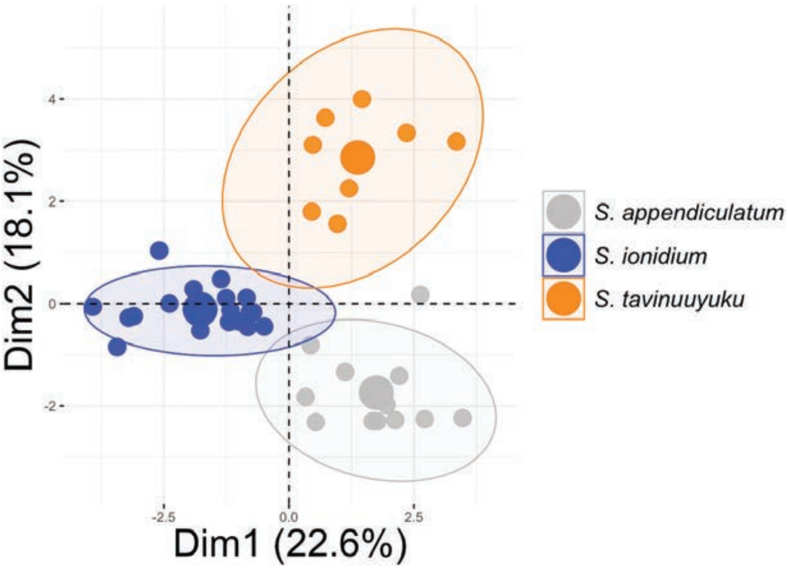

Figure 2. Factor analysis of mixed data (FAMD) ordination plot of *Solanum tavinuuyuku* and sympatric or near sympatric species from *Solanum* sect. *Anarrhichomenum* with 95% CI ellipses.

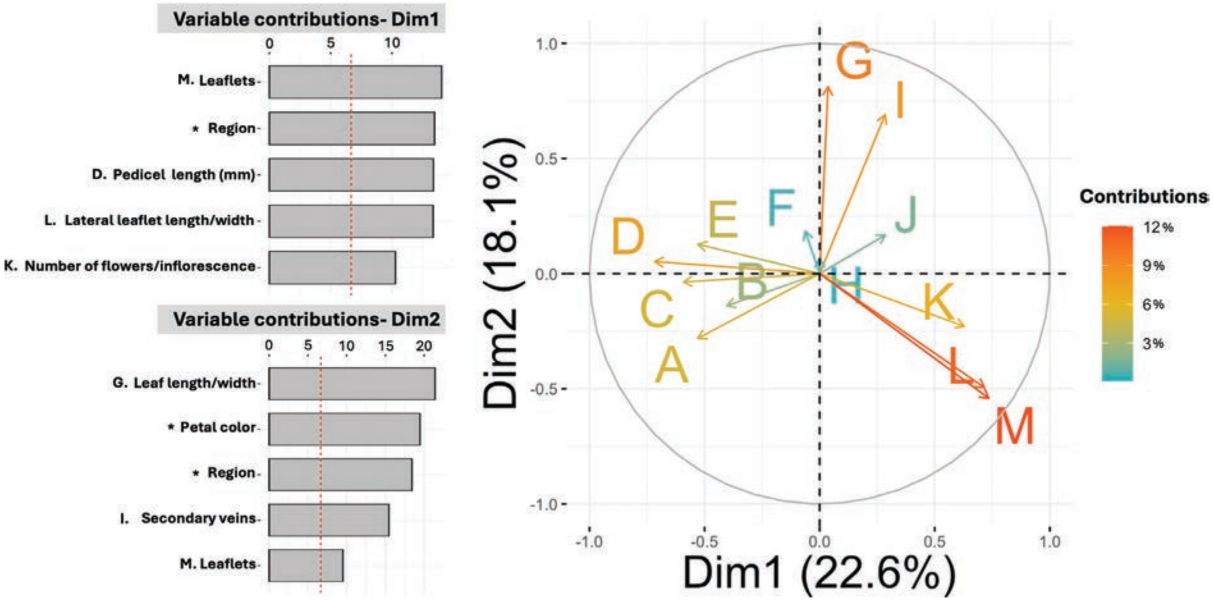

Figure 3. Barplots and a biplot of the contributions of influential variables in FAMD. Barplots represent the variable contributions of the top five most influential categorical and quantitative variables in FAMD analysis. Influential quantitative variables are labelled **A –M** and can be found in the biplot, while variables marked with an asterisk (*) represent influential categorical variables, which are not represented in the biplot. Peduncle length (cm) (**A**); pseudo-stipule length/width ratio (**B**); calyx lobe width (mm) (**C**); pedicel length (cm) (**D**); flower diameter (**E**); calyx length (mm) (**F**); leaf length/width ratio (**G**); petiole length (cm) (**H**); number of secondary veins (**I**); elevation (m) (**J**); flowers per inflorescence (**K**); lateral leaflet length/width ratio (**L**); number of leaflets (**M**).

along Dim 1 and Dim 2 and most collections fell within or near the 95% CI ellipse for that species (Fig. 2). Conversely, *S. ionidium* collections clustered around negative eigenvalues along Dim 1 and demonstrated a near complete separation from *S. tavinuuyuku* (Fig. 2). *Solanum appendiculatum* occupied an intermediate space between *S. tavinuuyuku* and *S. ionidium* along Dim 1, but fell

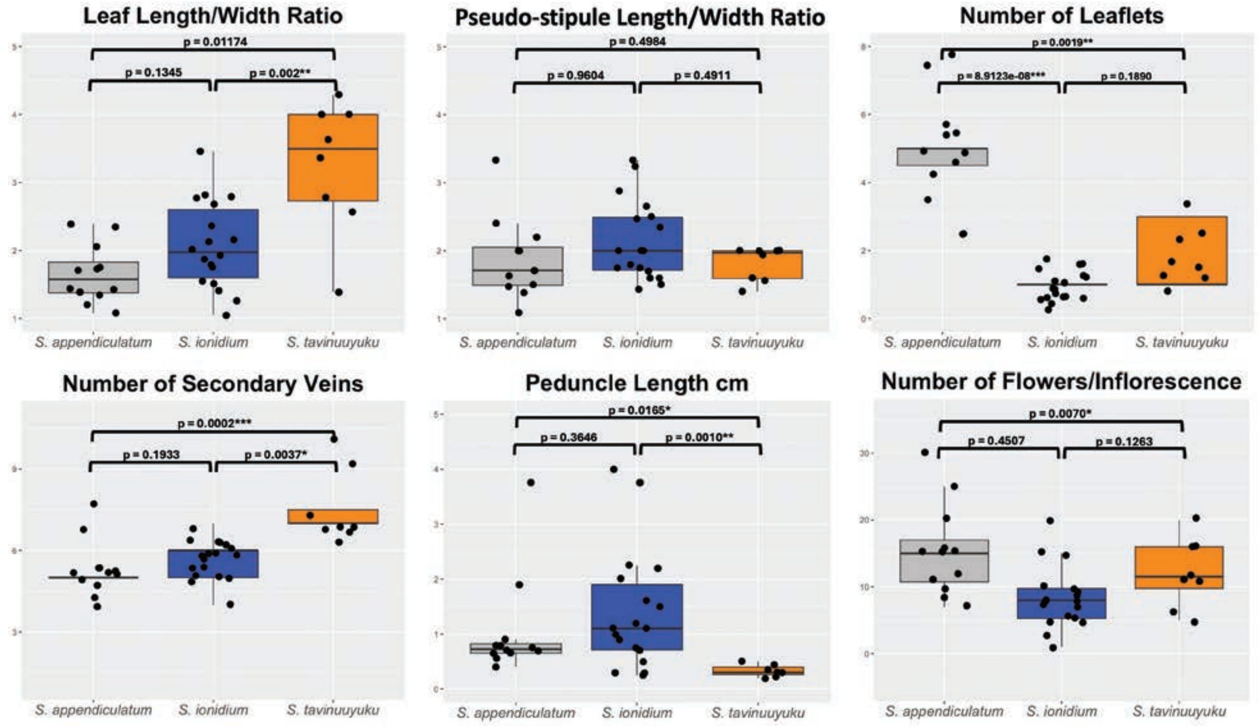

Figure 4. Boxplots of important morphological characters distinguishing *Solanum tavinuuyuku* from *Solanum appendiculatum* and *Solanum ionidium.* The upper and lower bounds of the box represent the upper and lower quartiles, respectively. The middle line within the box represents the median value. Maximum and minimum values are represented by the extent of the upper and lower "whiskers". Bars above each box within each graph constitute pairwise comparisons between species. P-values are derived from the results of multiple comparison Dunn's tests and were adjusted using Bonferroni correction (Suppl. material 2). Asterisks represent a significant result in Dunn's tests. A single asterisk (*) = $p < 0.05$; double asterisks (**) = $p < 0.005$; triple asterisks (***) = $p < 0.0005$.

within negative eigenvalues along Dim 2 (Fig. 2), differing from both its relatives in leaflet dimensions, number of leaflets, leaf length/width ratio and flowers per inflorescence (Fig. 3). Non-parametric Dunn's tests for multiple comparisons supported differences between the new species and one or both relatives in seven of the fifteen variables included in FAMD (Fig. 4; Suppl. material 2). Of these, *S. tavinuuyuku* differed ($p < 0.05$) from *S. ionidium* in four morphological variables and from *S. appendiculatum* in six morphological variables, as well as the region of collection between both species (Suppl. material 2).

Palynology

Inaperturate pollen of rounded shape (20–[23.3]–25 μm × 21.00–[23.17]–27.00 μm), was observed in two of the five samples included in this palynological study (*Croat 45517* and *Calzada 19434*) and convexly triangular, tricolporate pollen (20–[23.33]–25 μm × 21.00– [23.17]–27.00 μm) in the remaining three (*Calzada 19831*, *Rzedowski 159* and *Tenorio 1414*). The inaperturate condition was only observed in the anthers of long-styled flowers, whereas tricolporate pollen was found in the anthers of short-styled plants (Fig. 5). The combination of stylar and pollen dimorphism (inaperturate and aperturate) suggests that *S. tavinuuyuku* is likely to be dioecious.

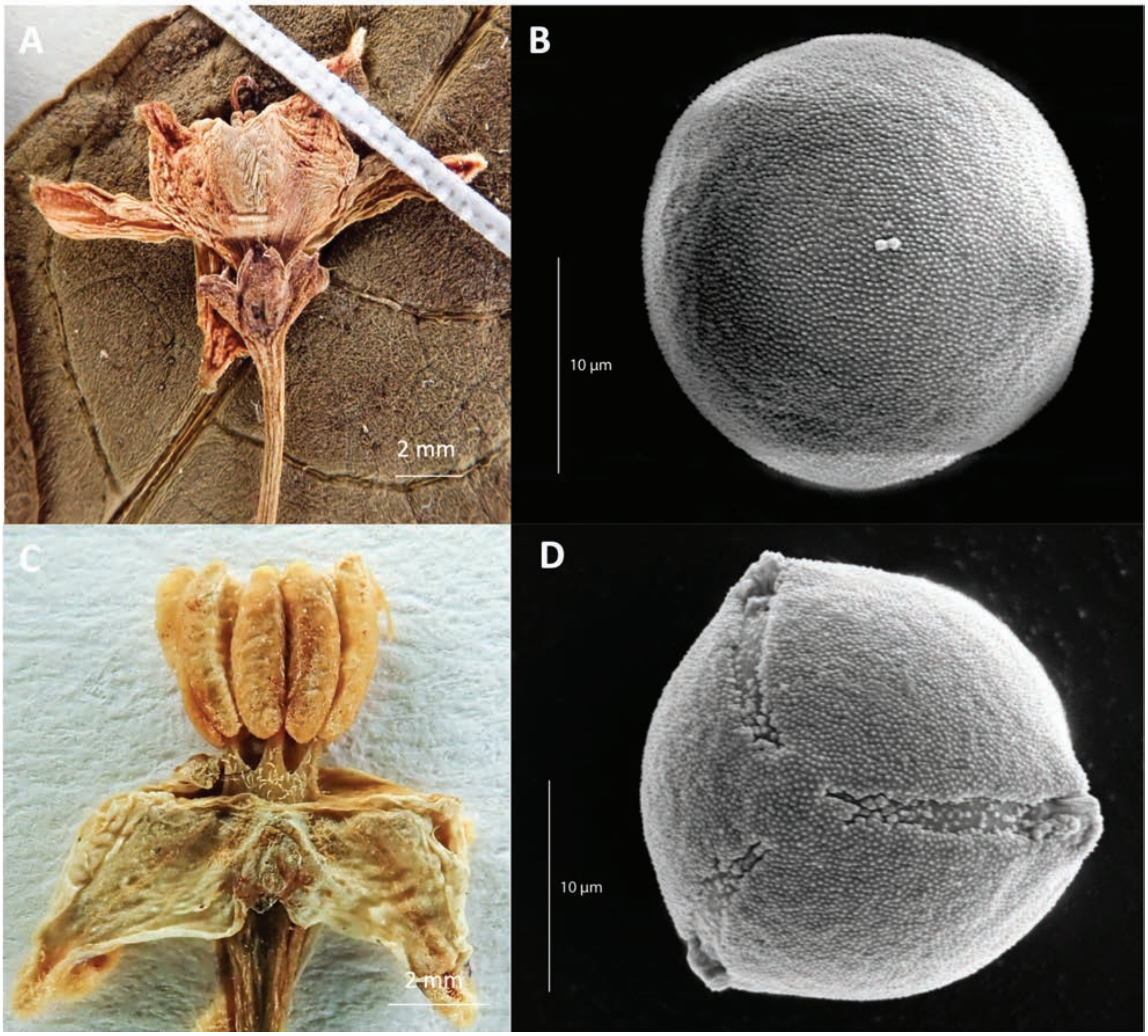

Figure 5. Dimorphic flowers and pollen grains representative of the pistillate and staminate sex of *Solanum tavinuuyuku*. **A** Pistillate flower (Croat 45261a) **B** SEM of inaperturate pollen from a functionally pistillate flower (Calzada 19434) **C** staminate flower (Rzedowski 159) **D** SEM of tricolporate, aperturate pollen from a functionally staminate flower (Calzada 19831) at 3,500× magnification.

Taxonomic treatment

Solanum tavinuuyuku **J.M.Bryant & Tepe, sp. nov.**
urn:lsid:ipni.org:names:77359685-1
Fig. 6

Diagnosis. *Solanum tavinuuyuku* resembles *Solanum ionidium* Bitter, but is distinguished from that species by its long and narrow, lanceolate, ovate to falcate, simple to 3-foliate leaves with 7–10 secondary veins, large and persistent pseudo-stipules, sparse to dense pubescence and white to violet-tinged corolla color that is especially pronounced in the floral bud.

Type. México. Oaxaca • Distr. Santiago Juxtlahuaca. Mpio. Santiago Juxtlahuaca. Loc. Puerta de Luz, hacia la torre microondas, entrada por Santa

Figure 6. *Solanum tavinuuyuku* **A** habit of flowering branch **B** habit of fruiting branch **C** pseudo-stipules with pubescence **D** abaxial leaf surface showing pubescence on the mid- and secondary veins **E** inflorescence **F** staminate flower **G** staminate flower with staminal column opened to reveal pistil **H** pistillate flower and detail of the pistil, with stigma magnified **I** calyx with pubescence **J** infructescence with mature berries **K** seed. [**A, C–G** drawn from Calzada 19831 **B, J** drawn from Calzada 19434 **H** drawn from Croat 45517 **I** drawn from Croat 45261a **K** drawn from Rzedowski 159]. Art by Ericka Belén Cortez Castro.

Rosa, via San Miguel Cuevas a El Manzanal, 17.13°N, 98.03°W, 2405 m elev., 18 Apr 1995 (fl), *J. I. Calzada 19831* (holotype: MEXU! [892615]; isotypes: LL! [00218235], NY! [NY00751674]).

Description. Vine, trailing along ground or climbing on other vegetation to 10 m or more, rooting at the nodes. ***Stems*** woody, sparsely to densely pubescent with long, transparent to pale tawny, unbranched, eglandular, multicellular simple hairs (termed finger-hairs in Seithe and Anderson (1982)), 0.5–0.8 mm long, more densely pubescent at the nodes, the stems often with white lenticels. ***Sympodial units*** plurifoliate, not geminate. ***Leaves*** distichous, simple to 3-foliate pinnately compound, tending towards 3-foliate on juvenile vegetative shoots and simple on mature shoots, the blades 2–14 × 1–5 cm, chartaceous, the margins flat to somewhat revolute, not ciliate, lamina sparsely to densely pubescent to glabrescent abaxially and adaxially, but on average, more densely pubescent adaxially, on veins and on developing leaves, the rachis and petiolules densely pubescent with multicellular finger hairs; simple leaves 3.8–11 cm × 1.5–3.5 cm, ovate, lanceolate to narrowly elliptic in shape, somewhat falcate on some specimens, the bases acute to rounded, symmetrical to asymmetrical, the apices acute to acuminate, the acumen 0.3–1 cm long, venation pinnate, (5–)7–10 pairs of secondary veins, secondary veins curved; compound leaves include an apical leaflet and a single pair of lateral leaflets, the distal lateral leaflet often smaller than its counterpart or completely absent; apical leaflets of compound leaves 5.5–9 cm × 2–3 cm, substantially larger than lateral leaflets, ovate to lanceolate, the bases acute, rounded to nearly truncate, symmetrical to asymmetrical, the apices acuminate, the petiolules 1.5–2.5 mm; lateral leaflets 2–4.5 cm × 0.5–2 cm, elliptic to ovate, the apices acute, the petiolules nearly lacking to 1 mm; interjected leaflets absent; petioles 0.3–5(–8.6) cm, densely pubescent. ***Pseudo-stipules*** present at nearly every node, single, persistent, 3–5 × 6.5–10 mm, reniform or more rarely subovate, the apices sharply acute, pubescence resembling that of the leaves. ***Inflorescences*** terminal corymbose-cymes, 2–3 × branched, 1.5–2.2 cm × 2–2.5 cm in flower, 3.5–6 cm × 2.5–4 cm in fruit, with 6–15 flowers distanced 0.5–3 mm apart, the axes glabrous, glabrescent or less frequently moderately pubescent, the peduncle 1–5(–11) mm, becoming extra-axillary with added sympodia, occasionally presenting a single bract-like leaf opposite the branching point; pedicels 0.2–1.2 cm in flower, 1–1.5 cm in fruit, articulated at the base, 0.5 mm wide across the base, expanded distally in flower and fruit up to 2 mm, moderately pubescent in flower with scattered, long, slender, finger hairs, 0.3–0.8 mm, glabrescent to glabrous in fruit. ***Flowers*** pentamerous, apparently perfect, but dimorphic, morphologically complete, with anthers from pistillate flowers producing inaperturate pollen. ***Calyx*** campanulate, 1.5–2.5 mm × 1.5–3(–6) mm in developing buds, 3–3.5 mm × 3–4 mm on mature flowers, the tube 1–1.5 mm long in flower and fruit, 5-lobed, the lobes triangular with acute to apiculate apices, 1–1.5 mm × 1–1.5 mm in flower, 1.5–2.5 mm × 1.5–2.5 mm in fruit, the margins somewhat scarious, the lobes with short, stocky, clustered pubescence on the apices, 0.02–0.3 mm and often sparsely to densely pubescent with long, slender, appressed, finger hairs, 0.3–0.7 mm, across the entire calyx, glabrescent in fruit. ***Corolla*** 0.8–1.2 cm in diameter, stellate, 5-lobed, reflexed or flat at anthesis, the tube 1.5–2.5 mm, the lobes 3.5–6 mm × 2–2.5 mm, ovate to triangular, acute at apices, puberulent on apices and along the distal half to third of the margins, violet with white along petal margins (as visible on herbarium material), violet color pronounced on developing buds. ***Stamens*** equal, with filaments 0.3–1 mm long, free to partially fused to half the entire filament length, pubescent; anthers 2.8–3.1 mm × 1–1.2 mm, oblong with retuse apices, cordate at the bases, connivent, yellow, with large apical pores, developing into introrsely dehiscent longitudinal slits with age. ***Ovary*** glabrous; the style on short-styled, staminate flowers 1–3 mm or nearly absent, far shorter than stamens, cylindrical, the stigma slightly clubbed or truncate; the style on long-styled, pistillate flowers 5.5–6 mm, exserted above the stamens, curved at the tip, cylindrical, the stigma capitate. ***Fruits*** 0.3–1 cm in diameter, globose, green when immature to bright orange-red at maturity, glabrous. ***Seeds*** 3.5–4 mm × 2.2–3 mm, flattened, rounded to teardrop-shaped, with a 0.2–0.5 mm wide wing around the margins, the thickened part of the seed 2.5–3 mm × 2–2.5 mm, rounded to oval-shaped, lenticular, light to medium brown, the surface smooth, the wing yellowish-tan to translucent near the margins, with radial striations, 15–20 seeds per fruit.

Distribution and ecology. *Solanum tavinuuyuku* appears to be endemic to south-central Mexico and has been collected in extreme western Oaxaca (Mixteca and Sierra Sur Regions) through central Guerrero (La Montaña and Centro Regions). These areas are a part of the Sierra Madre del Sur ecoregion, where *S. tavinuuyuku* grows in the understory of pine-oak forests and montane cloud forests, from 1700 to 2840 m in elevation (Fig. 7).

Phenology. *Solanum tavinuuyuku* appears to flower year-round. Flowering specimens have been collected January, April, August, October and December; fruiting specimens are from January, August and October.

Etymology. The epithet *tavinuuyuku* is of Mixtec origin and is derived from a combination of the Mixtec words *tavi nuu yuku*, which can be roughly translated to mean “found growing in the mountains”. We acknowledge that the very act of naming of natural objects is political. Taxonomists have and are still often guilty of acquiring these natural entities as their own intellectual possession, dictating, in a sense, the language in which we communicate about the world we inhabit. Nonetheless, it is our intent that by choosing this name, we honor the contributions of all indigenous nations and peoples in documenting and conserving local biodiversity, particularly the Mixtec people who inhabit these landscapes. Although we were not able to discover a local name for this plant, we propose an epithet sourced from a language familiar to the people who have undoubtedly interacted with it for millennia.

Conservation statement. IUCN Red List Criteria (IUCN 2022) suggest a status of vulnerable (VU) for *S. tavinuuyuku*, based on criteria B1 a, b (i, iii) with an extent of occurrence (EOO) of ca. 18,582 km^2. The species is known from only eight localities and is either quite rare or particularly under-collected (Fig. 1). The Sierra Madre del Sur ecoregion is composed primarily of rugged terrain, can be quite remote and thankfully is a conservation priority in Mexico (Arriaga Cabrera et al. 2000). However, the forest habitats where *S. tavinuuyuku* grows are under threat from ranching and agricultural activities in the area (Toledo-Aceves et al. 2011) which may impact the demographic and ecological stability of the species in the future if current trends continue.

Specimens examined. Mexico. Guerrero • 2 km al NE del Campamento El Gallo; estribaciones SW del Cerro Teotepec, 2650 m elev., 27 Jan 1965 (fl, fr), *J.*

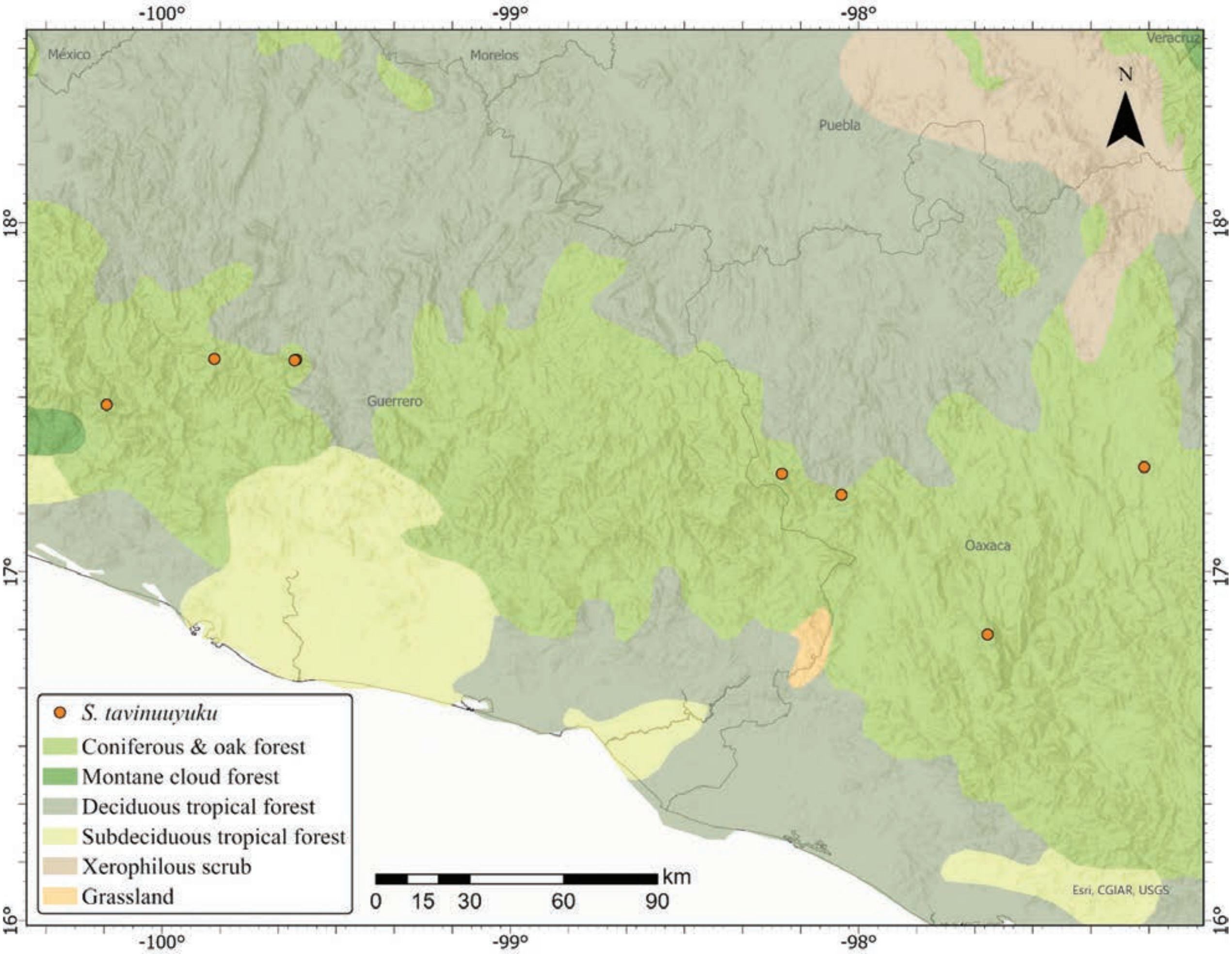

Figure 7. Distribution of *Solanum tavinuuyuku* in Mexico in the States of Oaxaca and Guerrero, within Coniferous and oak forest potential vegetation (Rzedowski 1990).

Rzedowski and McVaugh 159 (CORD, F, MEXU [photo]) • Mun. Eduardo Neri, along highway between Milpillas (on Highway 95) and Atoyac de Alvarez, 3.7mi W of turn-off on to road to Chichihualco, 17.08°N, 99.73°W, 2325 m elev., 14 Jan 1979 (fl, fr) *T.B. Croat 45621-a* (BM [photo], F, MO, NY, US) • Along road between Milpillas at Highway 95 and Atoyac de Alvarez (near Highway 200) 3.5 miles west of junction with road to Chichihualco, 2425 m elev., 12 Jan 1979 (fl), *T.B. Croat 45517* (MO) • 15 km al E de Pto. del Gallo, Carr. Filo de Caballo. Mpio. Chichihualco, 3090 m elev., 18 Aug 1982 (fl), *L. Hernandez S. and C. Romero de T. 1414* (MEXU [photo], NY). **Oaxaca** • Distr. Santiago Juxtlahuaca, Mpio: San Martin Peras, 17.18°N, 98.11°W, 2565 m elev., 10 Oct 1994 (fl, fr), *J.I. Calzada 19434* (LL, MEXU [photo], NY) • Mun. San Martin Peras, Loc. Dt. de Juxtlahuaca. A 3 km de la desviación a San Martin Peras, hacia Coicoyán de las Flores, 17.28°N, 98.22°W, 3 Dec 1992 (fl) *Torres et al. 14098* (MEXU [photo]) • Distr. Putla, Mpio. Santa Cruz Itundujia, A 7.37 km en LR (SE) de Santa Cruz Itundujia, 16.82°N, 97.63°W, 2865 m elev., 1 Aug 2008 (fl, fr) *Gutierrez et al. 3075* (MEXU [photo], SERO [photo]).

Key to species of *Solanum* sect. *Anarrhichomenum* in Mexico

1 Leaves unifoliate **2**
– Leaves (2–)3–7 foliate **3**

2 Leaves broadly ovate with bases cordate to rounded, symmetrical and secondary veins 5– 7. Corollas white. Collected in the Sierra Norte de Oaxaca in the Sierra Madre del Sur ecoregion and in the Sierra Madre Oriental on Mexico's gulf coast .... ***S. ionidium***

– Leaves lanceolate to narrowly elliptic with bases rounded, asymmetrical to symmetrical and secondary veins (5–)7–10. Corollas white, often tinged with violet. Collected in the southern mountains of the Sierra Madre del Sur ecoregion.. ***S. tavinuuyuku***

3 Anthers oblong with retuse apices..**4**

– Anthers oblong with aristate apices .... ***S. skutchii***

4 Apical leaflet with secondary veins (5–)7–10, over double the length of the lateral leaflets.. ***S. tavinuuyuku***

– Apical leaflet with secondary veins 5–7, slightly larger than lateral leaflets or roughly equivalent in size and shape... .... ***S. appendiculatum* (incl. *S. subvelutinum*, *S. tacanense*)**

Discussion

Solanum tavinuuyuku is the first species to be added to Mesoamerican *Solanum* sect. *Anarrhichomenum* in nearly 70 years and joins the *S. appendiculatum* species complex, a morphologically and taxonomically complex group of understudied vines. *Solanum tavinuuyuku* is restricted to the unique Sierra Madre del Sur ecoregion in south-central Mexico (Aragón-Parada et al. 2021) that spans the political State of Guerrero and south-western Oaxaca and is the only 1–3 foliate species of *Solanum* sect. *Anarrhichomenum* in that area. *Solanum tavinuuyuku* is also of considerable interest as it is only the second documented dioecious species in the Potato clade and amongst the greater so-called 'non-spiny' *Solanum* (Weese and Bohs 2007), apart from *S. appendiculatum*.

Comparative morphology and geography of sympatric species

Most collections of *S. tavinuuyuku* were originally identified as *S. ionidium*, which was once believed to be the only unifoliate species amongst Mesoamerican *Solanum* sect. *Anarrhichomenum*. Superficial similarities in the leaves and the lack of sufficient systematic study of this group have made it easy to misidentify unifoliate collections of *S. tavinuuyuku*, especially when working with herbarium material. *Solanum ionidium* has not been the subject of any focused taxonomic studies since Bitter initially described the species (Bitter 1913) and its placement in *Solanum* sect. *Anarrhichomenum* is relatively recent (Nee 1999). Despite the similarities in leaf morphology, however, these two species are quite distinct.

Morphologically, *S. tavinuuyuku* differs from *S. ionidium* in five out of the fifteen morphological characters assessed in this study. Long and narrow leaves, (5–)7–10 secondary veins, shorter peduncles (ca. 0.3 cm in *S. tavinuuyuku* vs. ca. 1.5 cm in *S. ionidium*) and violet-tinged flowers can reliably be used to distinguish the new species from *S. ionidium* and these characters favoured separation in morphometric analyses. *Solanum ionidium* is also differentiated from the new species by possessing exclusively unifoliate leaves, ciliate leaf margins, vegetative parts with glabrous or sparsely pubescent indument and longer pedicels on average (ca. 1.3 cm in *S. ionidium* vs. 0.9 cm in *S. tavinuuyuku*).

Furthermore, *S. tavinuuyuku* is not sympatric with *S. ionidium*, which inhabits only the north-eastern extent of the Sierra Madre del Sur in northern Oaxaca (Sierra Norte de Oaxaca), but has principally been collected throughout the Sierra Madre Oriental mountains in the State of Veracruz (Fig. 1). *Solanum tavinuuyuku* occupies pine-oak habitats in the southern mountains of the Sierra Madre del Sur of south-western Oaxaca and grows throughout the mountains that span the State of Guerrero (Fig. 7), but does not cross the Valley of Oaxaca into Sierra Norte de Oaxaca (Fig. 1).

Solanum tavinuuyuku does grow in sympatry with its close relative, *S. appendiculatum* (Fig. 1), and may be confused with that species on specimens with primarily 3–foliate leaves or that lack lateral leaflets (which happens very rarely). However, it is very rare to find a collection of *S. appendiculatum* that has apical leaflets as long as a unifoliate leaf or apical leaflet of *S. tavinuuyuku*. Moreover, the apical leaflets of *S. appendiculatum* have longer petiolules, 5–6 secondary veins on average (vs. 7–10 in *S. tavinuuyuku*) and are almost always elliptic–ovate with symmetrical bases (vs. ovate–lanceolate leaves with asymmetrical–symmetrical bases as in *S. tavinuuyuku*).

On 3-foliate specimens, *S. tavinuuyuku* is distinguished by very long apical leaflets and smaller lateral leaflets (ca. 0.51 cm × ca. 0.25 cm in *S. tavinuuyuku* vs. ca. 1.94 cm × ca. 0.82 cm in *S. appendiculatum*) that are less than half the length of the apical leaflet on average. Some compound leaves may only have a single lateral leaflet paired with a small indentation or gap on the apical leaflet giving the leaf an asymmetrical appearance, a feature which is not apparent in *S. appendiculatum*. Due to their small size, the lateral leaflets can be overlooked on herbarium material. This is never the case with *S. appendiculatum*, which has lateral leaflets roughly equivalent to, or only slightly smaller than the apical leaflet (the apical leaflet is ca. 2.5× longer than the lateral leaflet in *S. tavinuuyuku* vs. the apical leaflet ca. 1.20× longer than the lateral leaflet length in *S. appendiculatum*) and is clearly a compound-leaved species of 3–7 leaflets (vs. the often unifoliate presentation of *S. tavinuuyuku*). *Solanum appendiculatum* does often produce violet-tinged flowers in populations collected in the Central American Volcanic Arch, but produces mostly white flowers in the northern part of its range, including the Sierra Madre del Sur populations. In sympatric populations, *S. appendiculatum* can be differentiated from *S. tavinuuyuku* by corolla color, both in the floral bud and in flower, longer peduncles (ca. 0.33 cm in *S. tavinuuyuku* vs. ca. 1.05 cm in *S. appendiculatum*) and smaller pseudo-stipules (ca. 6.94 mm × ca. 3.83 mm in *S. tavinuuyuku* vs. ca. 5.32 mm ×. ca. 3.02 mm in *S. appendiculatum*).

Solanum tavinuuyuku also grows in sympatry with *S. morelliforme* Bitter & Muench and some collections of *S. tavinuuyuku* have been mistaken for that species, particularly due to its epiphytic, viny habit and long, lanceolate, simple leaves. However, *S. morelliforme* is a member of *Solanum* sect. *Petota* (Spooner et al. 2004), the tuber-bearing species of the Potato clade, which can easily be separated from *Solanum* sect. *Anarrhichomenum* by their tubers, articulation of the pedicel above the base and lack of adventitious roots at the nodes.

Dioecy in *Solanum*

Dioecy has been identified in four of the thirteen infrageneric major and minor clades of *Solanum*, comprising only ca. 16 species to date (Anderson et al. 2015; Williams et al. 2022; Gagnon et al. 2022; Hilgenhof et al. 2023), with the majority occupying the so-called "spiny" *Solanum* group or subg. *Leptostemonum* Bitter (Knapp et al. 1998; Martine et al. 2006, 2009, 2019; Anderson et al. 2015). All known dioecious species in *Solanum* are morphologically hermaphroditic, though some species are strongly dimorphic and others are cryptic, as is the case with *S. appendiculatum* (Anderson and Levine 1982). In a collection of ground-breaking studies, Anderson and collaborators provided a suite of morphological and reproductive indicators that are empirically strong, reliable lines of evidence that indicate dioecy in *Solanum* (Anderson and Gensel 1979; Anderson and Levine 1982; Levine and Anderson 1986; Anderson and Symon 1989; Mione and Anderson 1992; Zavada and Anderson 1997; Anderson et al. 2015). These studies, along with others (Knapp et al. 1998; Martine et al. 2009; Wu et al. 2021), have characterized functionally female plants as possessing flowers that are capable of bearing fruit with long styles exserted above the androecium, well-developed, capitate stigmas and anthers that produce non-functional inaperturate pollen or no pollen at all. Inaperturate pollen can be recognized by their rounded shape and lack of apertures (Anderson and Gensel 1979) and, in at least some species, are typically less nutritious and possess lower abundances of some amino acids essential for pollen tube development (Mattioli et al. 2012, 2018; Ndem-Galbert et al. 2021). The mechanical inhibition of pollen tube growth, resulting from the improper development of apertures and a reduced amino acid content, plays a key role in producing the feminizing phenotype of inaperturate pollen grains in dioecious *Solanum* species (Zavada and Anderson 1997; Ndem-Galbert et al. 2021). Functionally male plants, on the other hand, have flowers with short styles that are equal to or shorter than the androecium with underdeveloped, more cylindrical stigmas, that normally do not bear fruit with viable seeds. Pollen produced by these individuals is tricolporate and fully functional (Anderson and Levine 1982; Martine et al. 2016b) and has a convexly triangular shape in polar view (Anderson and Gensel 1979; Knapp et al. 1998).

The dioecious sexual system of *S. tavinuuyuku* was first suggested by the pattern of floral dimorphism described above. For example, two reliable collections with both flowers and fruits connected on the same branch have long-styled flowers and well-developed capitate stigmas (*Croat 45621-a* [BM, F, MO, NY, US], *Gutierrez 3075* [MEXU, SERO] and *Calzada 19434* [LL, MEXU, NY]). Pollen grains derived from the anthers of two similar representative pistillate plants (*Croat 45517* [MO] and *Calzada 19434* [LL, MEXU, NY]) completely lacked apertures (Fig. 6), resembling the rounded inaperturate grains from pistillate plants of *S. appendiculatum* (Anderson and Gensel 1979; Knapp et al. 1998). In contrast, short-styled flowering collections do not have fruits present on the same branch, have styles that do not exceed the androecium and have truncated and underdeveloped stigmas (*Calzada 19831* [LL, MEXU, NY], *Tenorio 1414* [MEXU, NY], and *Torres 14098* [MEXU]). Two of these three specimens (*Calzada 19831* [LL, MEXU, NY], and *Tenorio 1414* [MEXU, NY]) were available for direct observation (the other obtained as a digital image) and pollen sampled from these specimens all appeared tricolporate and convexly triangular (Fig. 6), much like the pollen of staminate plants of *S. appendiculatum* (Anderson and Gensel 1979; Knapp et al. 1998).

One sheet of *Rzedowski 159* (F) appears at first glance to violate the expected morphological pattern typical of dioecy in *Solanum*. This exceptional specimen presents both a short-styled flowering shoot and a fruiting shoot. The shoots are mounted on the same herbarium sheet, but are disconnected. When examined, pollen grains sampled from both sheets of this collection rendered convexly triangular, tricolporate pollen, as would be expected from a short-styled, staminate flower. Taken together, it is highly unlikely that the flowering and fruiting shoot are from the same individual. It is not uncommon for collectors to intentionally take from a multitude of individuals representative of a population or unintentionally mount together two individuals that have become intertwined. Due to its anomalous presentation, inconsistency with the morphological pattern evident in the majority of *S. tavinuuyuku* specimens and pollen morphology, this collection is likely a mixture composed of a functionally male flowering and female fruiting individual, representing a population rather than an individual.

Although investigations into pollen viability and tube growth in inaperturate grains, seed set in short-styled flowers and genomic signatures of sex would be ideal for definitively determining dioecy (Anderson and Levine 1982; Mione and Anderson 1992; Anderson et al. 2015; Wu et al. 2021), the combination of floral and pollen dimorphism in a robust sample of known collections of this species is strong enough to confidently describe *S. tavinuuyuku* as dioecious.

Acknowledgements

We would like to thank the curators and workers of the following herbaria for their specimen loans and/or digitized images of specimens needed for this study: AAU, ARG, B, BH, BM, BR, C, CORD, F, G, GH, LL, MEXU, MICH, MO, NY, OAX, SERO, UC, UGAC, US, WIS and XAL. We would also like to thank Ericka Belén Cortez Castro for her beautiful illustration and assistance in photographing and measuring specimens at MEXU. We also would like to thank David Lentz for assistance with SEM and Alicia Ortiz for providing the Mixtec name. Lastly, we would like to give our appreciation to Theresa Culley and Takuya Konishi at the University of Cincinnati, as well as two anonymous reviewers and Leandro Giacomin for providing helpful and constructive comments which greatly improved the final manuscript.

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 partially funded by the US National Science Foundation (NSF DEB-1020509, DEB-1146119 and DEB-1442075).

Author contributions

Conceptualization: JB. Formal analysis: JB. Methodology: JB. Supervision: EJT. Visualization: MVA, JB. Writing – original draft: JB. Writing – review and editing: EJT, MVA, JB.

Author ORCIDs

Jacob Bryant https://orcid.org/0009-0004-7462-8359
Mariana Vazquez-Alonso https://orcid.org/0000-0002-1285-5773
Eric J. Tepe https://orcid.org/0000-0002-8493-0736

Data availability

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

References

Anderson GJ, Gensel PG (1979) Pollen morphology and the systematics of *Solanum* section *Basarthrum*. Pollen et Spores 18: 533–556.

Anderson GJ, Levine DA (1982) Three taxa constitute the sexes of a single dioecious species of *Solanum*. Taxon 31(4): 667–672. https://doi.org/10.2307/1219682

Anderson GJ, Symon DE (1989) Functional dioecy and andromonecy in *Solanum*. Evolution; International Journal of Organic Evolution 43(1): 204–219. https://doi.org/10.2307/2409175

Anderson GJ, Anderson MKL, Patel N (2015) The ecology, evolution and biogeography of dioecy in the genus *Solanum*: With paradigms from the strong dioecy in *Solanum polygamum*, to the unsuspected cryptic dioecy in *Solanum conocarpum.* American Journal of Botany 102(3): 471–486. https://doi.org/10.3732/ajb.1400486

Aragón-Parada J, Rodríguez A, Munguía-Lino G, De-Nova JA, Salinas-Rodríguez MM, Carrillo-Reyes P (2021) Las plantas vasculares endémicas de la Sierra Madre del Sur, México. Botanical Sciences 99(3): 643–660. https://doi.org/10.17129/botsci.2682

Arriaga Cabrera L, Espinoza-Rodriguez JM, Aguilar-Zuñiga C, Martínez-Romero E, Gómez-Mendoza L, Loza EL (2000) Regiones terrestres prioritarias de Mexico. CONABIO, Mexico DF.

Aubriot X, Singh P, Knapp S (2016) Tropical Asian species show the Old World clade of “spiny solanums” (the Leptostemonum clade: Solanaceae) is not monophyletic. Botanical Journal of the Linnean Society 180: 1–27. https://doi.org/10.1111/boj.12412

Bitter G (1913) Repertorium Specierum Novarum Regni Vegetabilis 11: 484–485. https://doi.org/10.1002/fedr.19130113102

Bryant JB (2025) Pollen Acetolysis. https://doi.org/10.17504/protocols.io.n92ld8o4nv5b/v2 [accessed 7 March 2025]

Candolle AP de (1852) Prodromus systematis naturalis regni vegetabilis. Paris: Victoris Masson.

Clark JL, Nee M, Bohs L, Knapp S (2015) A revision of *Solanum* section *Aculeigerum* (the *Solanum wendlandii* group, Solanaceae). Systematic Botany 40: [1–36] 1102–1136. https://doi.org/10.1600/036364415X690148

Correll DS (1962) The Potato and its wild relatives: Section Tuberarium of the genus *Solanum*. Texas Research Foundation. https://doi.org/10.1007/BF02861992

Erdtman G (1960) The acetolysis method- a revised description. Svensk Botanisk Tidskrift 54: 561–564.

Frodin D (2004) History and concepts of big genera. Taxon 53(3): 753–776. https://doi.org/10.2307/4135449

Gagnon E, Hilgenhof R, Orejuela A, McDonnell A, Sablok G, Aubriot X, Giacomin LL, Gouvêa YF, Bragionis T, Stehmann JR, Bohs L, Dodsworth S, Martine C, Poczai P, Knapp S, Särkinen T (2022) Phylogenomic discordance suggests polytomies along the backbone of the large genus *Solanum*. American Journal of Botany 109(4): 580–601. https://doi.org/10.1002/ajb2.1827

Gouvêa YF, Giacomin LL, Stehmann JR (2018) A sticky and heavily armed new species of *Solanum* (*Solanum* subg. *Leptostemonum*, Solanaceae) from eastern Brazil. PhytoKeys 111: 103–118. https://doi.org/10.3897/phytokeys.111.28595

Gouvêa YF, Stehmann JR, Knapp S (2019) *Solanum medusae* (Solanaceae), a new wolf-fruit from Brazil, and a key to the extra-Amazonian Brazilian Androceras/Crinitum clade species. PhytoKeys 118: 15–32. https://doi.org/10.3897/phytokeys.118.31598

Gouvêa YF, Amorim de Paula LF, Stehmann JR, Giacomin LL (2020) *Solanum hydroides* (Solanaceae): A prickly novelty from the land of the sugar loaves, central Brazilian Atlantic Forest. PhytoKeys 139: 63–76. https://doi.org/10.3897/phytokeys.139.46635

Hilgenhof R, Gagnon E, Knapp S, Aubriot X, Tepe EJ, Bohs L, Giacomin LL, Gouvêa YF, Martine CT, Orejuela A, Orozco CI, Peralta IE, Särkinen T (2023) Morphological trait evolution in *Solanum* (Solanaceae): Evolutionary lability of key taxonomic characters. Taxon 72(4): 811–847. https://doi.org/10.1002/tax.12990

IUCN (2022) Guidelines for using the IUCN red list categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf [accessed 25 Jan 2023]

Kassambara A, Mundt F (2020) factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. https://CRAN.R-project.org/package=factoextra

Knapp S, Persson V, Blackmore P (1998) Pollen morphology and functional dioecy in *Solanum* (Solanaceae). Plant Systematics and Evolution 210(1–2): 113–139. https://doi.org/10.1007/BF00984731

Knapp S, Barboza GE, Bohs L, Särkinen T (2019) A revision of the Morelloid Clade of *Solanum* L. (Solanaceae) in North and Central America and the Caribbean. PhytoKeys 123: 1–144. https://doi.org/10.3897/phytokeys.123.31738

Knapp S, Chiarini F, Cantero JL, Barboza GE (2022) The Morelloid clade of *Solanum* L. (Solanaceae) in Argentina: Nomenclatural changes three new species and an updated key all taxa. PhytoKeys 164: 33–66. https://doi.org/10.3897/phytokeys.164.54504

Lacey LM, Martine CT (2017) *Solanum jobsonii*, a novel andromonoecious bush tomato species from a new Australian national park. PhytoKeys 82: 1–13. https://doi.org/10.3897/phytokeys.82.12106

Le S, Josse J, Husson F (2008) FactoMineR: An R package for multivariate analysis. Journal of Statistical Software 25(1): 1–18. https://doi.org/10.18637/jss.v025.i01

Levin RA, Myers NR, Bohs L (2006) Phylogenetic relationships among the “spiny solanums” (*Solanum* subgenus *Leptostemonum*, Solanaceae). American Journal of Botany 93(1): 157–169. https://doi.org/10.3732/ajb.93.1.157

Levine DA, Anderson GJ (1986) Evolution of dioecy in an American *Solanum*. In: D’Arcy WG (Ed.) Solanaceae: biology and systematics. Columbia University Press, New York, NY, 264–273.

Martine CT, Vanderpool D, Anderson GJ, Les DH (2006) Phylogenetics of andromonoecious and dioecious Australian species of *Solanum* subgenus *Leptostemonum* section *Melongena*: Inferences from ITS sequence data. Systematic Botany 31: 410–420. https://doi.org/10.1600/036373644406777585801

Martine CT, Anderson GJ, Les DH (2009) Sex-bending aubergines: Molecular phylogenetics of cryptically dioecious *Solanum* in Australia. Australian Systematic Botany 22(2): 107–120. https://doi.org/10.1071/SB07039

Martine CT, Frawley E, Cantley JT, Jordon-Thaden IE (2016a) *Solanum watneyi*, a new bush tomato species from the Northern Territory, Australia named for Mark Watney of the book and film “The Martian”. PhytoKeys 61: 1–13. https://doi.org/10.3897/phytokeys.61.6995

Martine CT, Cantley J, Frawley E, Bulter A, Jordon-Thaden IE (2016b) New functionally dioecious bush tomato from northwestern Australia, *Solanum ossicruentum*, may utilize “trample burr” dispersal. PhytoKeys 63: 19–29. https://doi.org/10.3897/phytokeys.63.7743

Martine CT, Jordon-Thaden IE, McDonnell AJ, Cantley JT, Hayes DS, Roche MD, Frawley ES, Gilman IS, Tank DC (2019) Phylogeny of the Australian *Solanum dioicum* group using seven nuclear genes, with consideration of Symon’s fruit and seed dispersal hypotheses. PLoS One 14(4): e0207564. https://doi.org/10.1371/journal.pone.0207564

Mattioli R, Biancucci M, Lonoce C, Costantino P, Trovato M (2012) Proline is required for male gametophyte development in *Arabidopsis*. BMC Plant Biology 12(1): 236. https://doi.org/10.1186/1471-2229-12-236

Mattioli R, Biancucci M, El Shall A, Mosca L, Costantino P, Funck D, Trovato M (2018) Proline synthesis in developing microspores is required for pollen development and fertility. BMC Plant Biology 18(1): 356. https://doi.org/10.1186/s12870-018-1571-3

McClelland DHR, Nee M, Knapp S (2020) New names and status for Pacific spiny species of *Solanum* (Solanaceae, subgenus *Leptostemonum* Bitter; the Leptostemonum Clade). PhytoKeys 145: 1–36. https://doi.org/10.3897/phytokeys.145.48531

McDonnell AJ, Wetreich HB, Cantley JT, Jobson P, Martine CT (2019) *Solanum plastisexum*, an enigmatic new bush tomato from the Australian Monsoon Tropics exhibiting breeding system fluidity. PhytoKeys 124: 39–55. https://doi.org/10.3897/phytokeys.124.33526

Messeder JVS, Carlo TA, Guojin Z, Tovar JD, Arana C, Huang J, Huang CH, Ma H (2024) A highly resolved nuclear phylogeny uncovers strong phylogenetic conservatism and correlated evolution of fruit color and size in *Solanum* L. The New Phytologist 243(2): 765–780. https://doi.org/10.1111/nph.19849

Mione T, Anderson GJ (1992) Pollen-ovule ration and breeding system evolution in *Solanum* section *Basarthrum* (Solanaceae). American Journal of Botany 79(3): 279–287. https://doi.org/10.1002/j.1537-2197.1992.tb14549.x

Moonlight PW, Baldaszti L, Cardoso D, Elliott A, Särkinen T, Knapp S (2024) Twenty years of big plant genera. Proceedings of the Royal Society: Biological Sciences. https://doi.org/10.1098/rspb.2024.0702

Morrone JJ, Escalante T, Rodríguez-Tapia G (2017) Mexican biogeographic provinces: Map and shapefiles. Zootaxa 4277(2): 277–279. https://doi.org/10.11646/zootaxa.4277.2.8

Murillo-Pérez G, Rodríguez A (2021) Dichotomous keys to the species of *Solanum* (Solanaceae) in Mexico. Botanical Sciences 99(2): 413–446. https://doi.org/10.17129/botsci.2713

Ndem-Galbert JR, Hall JE, McDonnell AJ, Martine CT (2021) Differential reward in “male” versus “female” pollen of functionally dioecious *Solanum*. American Journal of Botany 108(11): 2282–2293. https://doi.org/10.1002/ajb2.1765

Nee M (1993) Flora de Veracruz: Solanaceae II. Instituto de Ecologia, Xalapa, Ver.

Nee M (1999) Synopsis of *Solanum* in the new world. Solanaceae IV: advances in biology and utilization. Royal Botanic Gardens, Kew, 285–333.

Nee M, Bohs L, Knapp S (2006) New species of *Solanum* and *Capsicum* (Solanaceae) from Bolivia, with clarification of nomenclature in some Bolivian Solanum. Brittonia 58(4): 322356. https://doi.org/10.1663/0007-196X(2006)58[322:NSOSAC]2.0.CO;2

Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis. R package version 0.9.3. https://github.com/fishR-Core-Team/FSA

Pages J (2004) Analyse factorielle de donnees mixtes. Revue de Statistique Appliquee 52: 93–111.

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Rios NE, Bart HL (2010) GEOLocate (Version 3.22) [Computer software]. Tulane University Museum of Natural History, Belle Chasse, LA.

Rodríguez A (2004) Solanáceas. In: García-Mendoza AJ, et al. (Eds) Biodiversidad de Oaxaca. Instituto de Biología, UNAM-Fondo Oaxaqueño para la Conservación de la Naturaleza-World Wildlife Fund, México, 297–303.

Rzedowski J (1990) Vegetación potencial, escala 1:4000000. En: Vegetación potencial. Tomo II, Seccion IV, 8.2 Atlas de Mexico (1990–1992). Instituto de Geografía, UNAM, Mexico.

Särkinen T, Knapp S (2016) Two new non-spiny *Solanum* (Solanaceae) from the Gran Chaco Americano and a key for the herbaceous glandular-pubescent solanums from the region. PhytoKeys 74: 19–33. https://doi.org/10.3897/phytokeys.74.10159

Särkinen T, Bohs L, Olmstead RG, Knapp S (2013) A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a data 1000-tip tree. BMC Evolutionary Biology. https://doi.org/10.1186/1471-2148-13-214

Särkinen T, Gonzáles P, Knapp S (2015) Four new non spiny *Solanum* species (Solanaceae) species from South American. PhytoKeys 44: 39–64. https://doi.org/10.3897/phytokeys.44.8693

Seithe AK, Anderson GJ (1982) Hair morphology and the relationships of species in *Solanum* sect. *Basarthrum*. Plant Systematics and Evolution 139: 229–256. https://doi.org/10.1007/BF00989327

Spooner DE, Van den Berg RG, Rodríguez A, Bamberg J, Hijmans RJ, Lara-Cabrera SI (2004) Wild Potatoes (*Solanum* section *Petota*) of North and Central America. Systematic Botany Monographs 68: 1–209. [+ 9 plates] https://doi.org/10.2307/25027915

Spooner DE, Alvarez N, Peralta IE, Claussen AM (2016) Taxonomy of wild Potatoes and their relatives in southern South America (*Solanum* sects. *Petota* and *Etuberosum*). Systematic Botany Monographs 100: 1–240.

Stern S, Giacomin LL, Stehmann JR, Knapp S (2013) A revision of *Solanum* section *Gonatotrichum* Bitter (Solanaceae). Systematic Botany 38: 471–496. https://doi.org/10.1600/036364413X666624

Tepe EJ, Bohs L (2011) A revision of *Solanum* section *Herpystichum.* Systematic Botany 36(4): 1068–1087. https://doi.org/10.1600/036364411X605074

Tepe EJ, Ridely G, Bohs L (2012) A new species of *Solanum* named for Jeanne Baret, an overlooked contributor to the history of botany. PhytoKeys 8(0): 37–47. https://doi.org/10.3897/phytokeys.8.2101

Tepe EJ, Anderson GJ, Spooner DM, Bohs L (2016) Relationships among wild relatives of the tomato, Potato, and pepino. Taxon 65(2): 262–276. https://doi.org/10.12705/652.4

Toledo-Aceves T, Meave JA, González-Espinosa M, Ramírez-Marcia N (2011) Tropical montane cloud forests: Current threats and opportunities for their conservation and sustainable management in Mexico. Journal of Environmental Management 92(3): 974–981. https://doi.org/10.1016/j.jenvman.2010.11.007

Tovar JD, Giacomin LL (2022) A new species and new records of *Solanum* (Solanaceae) from Colombia. PhytoKeys 195: 63–73. https://doi.org/10.3897/phytokeys.195.78832

Tovar JD, Knapp S, Giacomin LL (2024) A new species and a key to members of the Geminata clade (*Solanum* L.; Solanaceae) in Columbia. Acta Botanica Brasílica 38: e20240092.

Villaseñor JL (2016) Checklist of the native vascular plants of Mexico. Revista Mexicana de Biodiversidad 87(3): 559–902. https://doi.org/10.1016/j.rmb.2016.06.017

Vorontsova MS, Knapp S (2016) A revision of the "spiny solanums", *Solanum* subgenus *Leptostemonum* (Solanaceae) in Africa and Madagascar. Systematic Botany Monographs 99: 1–432.

Wahlert GA, Chiarini F, Bohs L (2014) Phylogeny of the Carolinense clade of *Solanum* (Solanaceae) inferred from nuclear and plastid DNA sequences. Systematic Botany 39(4): 1208–1216. https://doi.org/10.1600/036364414X682599

Weese T, Bohs L (2007) A three-gene phylogeny of the genus *Solanum* (Solanaceae). Systematic Botany 32(2): 445–463. https://doi.org/10.1600/036364407781179671

Williams TM, Hayes J, McDonnell AJ, Cantley JT, Jobson P, Martine CT (2022) *Solanum scalarium* (Solanaceae), a newly-described dioecious bush tomato from Judbarra/Gregory National Park, Northern Territory, Australia. PhytoKeys 216: 103–116. https://doi.org/10.3897/phytokeys.216.85972

Wu M, Haak DC, Anderson GJ, Hahn MW, Moyle LC, Guerrero RF (2021) Inferring the genetic basis of sex determination from the genome of a dioecious nightshade. Molecular Biology and Evolution 38(7): 2946–2957. https://doi.org/10.1093/molbev/msab089

Zavada MS, Anderson GJ (1997) The wall and aperture development of pollen from dioecious *Solanum appendiculatum*: What is inaperturate pollen? Grana 36(3): 129–134. https://doi.org/10.1080/00173139709362600

Supplementary material 1

Specimen accessions used in morphometrics, mapping, and dioecy studies

Authors: Jacob Bryant, Mariana Vazquez-Alonso, Eric J. Tepe

Data type: xlsx

Link: https://doi.org/10.3897/phytokeys.255.140014.suppl1

Supplementary material 2

Post-hoc Dunn's Tests results comparing morphological and geographic characters

Authors: Jacob Bryant, Mariana Vazquez-Alonso, Eric J. Tepe

Data type: xlsx

Link: https://doi.org/10.3897/phytokeys.255.140014.suppl2

Supplementary material 3

Accession information in DarwinCore format

Authors: Jacob Bryant, Mariana Vazquez-Alonso, Eric J. Tepe

Data type: csv

Link: https://doi.org/10.3897/phytokeys.255.140014.suppl3

Supplementary material 4

Character matrix of morphological variables used in multivariate statistical analyses

Authors: Jacob Bryant, Mariana Vazquez-Alonso, Eric J. Tepe

Data type: csv

Link: https://doi.org/10.3897/phytokeys.255.140014.suppl4

PhytoKeys 255: 23–34 (2025)
DOI: 10.3897/phytokeys.255.151041

Research Article

A new cherry species (*Prunus*, Rosaceae) from south-western Ecuador

Álvaro J. Pérez[1], Jorge Andrés Pérez-Zabala[2], Katya Romoleroux[1], David A. Espinel-Ortiz[1,3], Chaquira Romoleroux[1], Natasha Albán-Vallejo[1]

1 *Herbario QCA, Laboratorio de Botánica Sistemática, Facultad de Ciencias Exactas, Naturales y Ambientales, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre 1076 y Vicente Ramón Roca, 170525, Quito, Ecuador*
2 *Herbario Gabriel Gutiérrez Villegas (MEDEL) y Semillero de estudios taxonómicos de plantas de Colombia, Facultad de Ciencias, Universidad Nacional de Colombia, Cra 65, 59A-110, Medellín, Colombia*
3 *Bonn Institute of Organismic Biodiversity, University of Bonn, Meckenheimer Allee 170, 53115 Bonn, Germany*
Corresponding author: Katya Romoleroux (kromoleroux@puce.edu.ec)

Abstract

Prunus luxurians, a new species from Buenaventura Reserve at El Oro province in Ecuador is described and illustrated. Additionally, notes on its geographical distribution, ecology, conservation status, and taxonomic affinities are documented. *Prunus luxurians* has some vegetative and floral similarities with other Andean species, but the unique combination of oblong-lanceolate leaves with prominent secondary and tertiary veins, densiflorous floriferous shoots less than 5 cm long, sepals with two marginal glands and flowers with turbinate hypanthium clearly differentiates it from the rest. This is the first species of *Prunus* described from the western flank at elevation below 1500 m, and particularly from a humid spot surrounded by dry areas (Tumbesian influence). Other taxonomic novelties of *Prunus* on this flank can be expected, so further botanical exploration is needed to better understand the diversity of the genus in the region.

Resumen

Se describe e ilustra *Prunus luxurians*, una nueva especie de la Reserva Buenaventura en la provincia de El Oro en Ecuador. Además, se documentan notas sobre su distribución geográfica, ecología, estado de conservación y afinidades taxonómicas. *Prunus luxurians* comparte algunas similitudes vegetativas y florales con otras especies andinas, pero la combinación única de hojas oblongo-lanceoladas con venas secundarias y terciarias prominentes, brotes floríferos densifloros de menos de 5 cm de largo, sépalos con dos glándulas marginales y flores con hipanto turbinado la diferencian fácilmente del resto. Esta es la primera especie de *Prunus* descrita del flanco occidental a una altitud inferior a 1500 m, y particularmente de un lugar húmedo rodeado de zonas secas (influencia tumbesina). Se espera que existan otras novedades taxonómicas de *Prunus* en este flanco, por lo que es necesaria una mayor exploración botánica para comprender mejor la diversidad del género en la región.

Key words: Buenaventura Reserve, El Oro, Neotropics, plant taxonomy

Academic editor: Hanno Schaefer
Received: 21 February 2025
Accepted: 12 March 2025
Published: 4 April 2025

Citation: Pérez ÁJ, Pérez-Zabala JA, Romoleroux K, Espinel-Ortiz DA, Romoleroux C, Albán-Vallejo N (2025) A new cherry species (*Prunus*, Rosaceae) from south-western Ecuador. PhytoKeys 255: 23–34. https://doi.org/10.3897/phytokeys.255.151041

Introduction

Prunus L. is almost a worldwide distributed genus with around 450 species (Pérez-Zabala 2015) which includes several appreciated fruit crops (e.g. almonds, cherries, plums), ornamentals (e.g. cherry blossom), timber sources (e.g. black cherry) and medicinal ones (e.g. African cherry). It is characterized by a base haploid chromosome number = 8, presence of cyanogenic glycosides, foliar glands, deciduous stipules, monocarpellate solitary pistil and drupaceous fruit (Kalkman 1966; Potter et al. 2007). The species level taxonomy of *Prunus* has been characterized by vague descriptions, minimal or absent taxonomic discussions and typification uncertainty in several old names (McVaugh 1951; Shaw and Small 2004). A major taxonomic revision is particularly complicated considering that around 1230 species' scientific names have been published under *Prunus*, and more than 2000 names when considering those published under other previously segregated genera and putative hybrids (IPNI 2024). The complexity of the species circumscriptions could be related to natural and human-driven hybridization in some groups (Shaw and Small 2004; Dickinson et al. 2007; Rohrer 2015), underscored phenotypic plasticity (Bortiri et al. 2006), but also to a lack of taxonomic assessment evidenced in many traditional descriptions with limited morphological evaluation that understates potential important traits such as, for instance, leaf venation architecture (Pérez-Zabala 2015) and floral micromorphology (Kalkman 1966).

Although *Prunus* has been traditionally considered as a mainly north temperate genus of deciduous species with marginal diversity of evergreen taxa in mountain regions of the subtropics and tropics (Pennington and Dick 2004), recent studies have shown that similar or even a higher number of species can be found in the tropics (mostly in the Americas, followed by Asia and one species in Africa) from lowlands to around 4000 m (Kalkman 1966; Pérez-Zabala 2015, 2022). All the tropical species and many subtropical ones have been traditionally grouped in the subgenus *Laurocerasus* (Duh.) Rehd. based on the evergreen condition and the racemose inflorescence. Particularly in the Neotropics, the most important contribution to the knowledge of the genus has been the revisionary work by (Koehne 1915) who recognized 59 taxa, 39 of them as novelties. However, Koehne himself recognized his work as provisional since the revision was based on just 180 specimens mainly stored in European herbaria (most of the species were known from one or few specimens) and made practical sub-treatments with keys by geographic units (countries or group of them), annotating that a consolidated series classification will require further work. After Koehne`s revision, the taxonomic contributions have been mostly isolated publications of new species with no, or minimal, taxonomic discussion, for instance those for Peru (Macbride 1934), Guatemala (Johnston 1938), Colombia (Cuatrecasas 1950), Mexico (Lundell 1968), Paraguay (Basualdo and Zardini 1992) and Venezuela (Li and Aymard 1997). After those works, the last three published novelties of the genus in the region were proposed by Pérez-Zabala (2007) for Colombia based on a previous full taxonomic revision for the country (Pérez-Zabala 2005).

A renewed effort for understanding the diversity and evolution of *Prunus* in the Neotropics has been done recently based on field work and a comprehensive study of more than 4000 collections in herbaria worldwide most of which have been collected during the last 40 years (Pérez-Zabala 2022) and as a result numerous allegedly new species have been identified. Particularly, for the flora of Ecuador, Romoleroux (1996) identified only five native species of *Prunus*, a number that could be considered an underestimation since neighboring countries like Peru and Colombia have more than 30 species each. They also contain the regions where most of the diversity would be expected, along the gradient of the Amazonian flank of the Andes and in inter Andean valleys (Pérez-Zabala 2022). After field work and the examination of some collections from a premontane cloud forest in El Oro province placed in the western flank of the Andes in southern Ecuador, the authors found substantial differences compared to previously published taxa known to the country and with respect to any other species of the genus. Consequently, a new species is proposed here, a full description is provided, morphological differences with similar species are discussed, some ecological aspects of the taxa are examined, and its conservation status is evaluated.

Materials and methods

Upon botanical exploration of one of the authors (Á.J.Pérez) in the cloud forest remnants at the Sambotambo Birón area, Reserve Buenaventura, owned and managed by the Jocotoco Foundation (https://www.jocotoco.org.ec) in El Oro province, during December 2021, a small population of an unknown species of *Prunus* was found along the forest remnants borders of the region. To asses taxonomic affinities of the new species we consulted taxonomic literature of South American species and particularly the synopsis of the genus for the Neotropics (Pérez-Zabala 2022), examined specimens at Herbaria GUAY, LOJA, QCA and QCNE (acronyms follow Thiers (2024)), consulted photographic records on iNaturalist (https://www.inaturalist.org), and studied high-resolution images of type material for Neotropical taxa (Tropicos database, https://www.tropicos.org/ and the JSTOR global Plants website http://plants.jstor.org).

The botanical description follows terminology used by Stearn (2004), the leaf morphology type was based on the terms of Hickey and King (2000) and Wilhelm and Rericha (2020), leaf architecture (including venation) was based on Ellis et al. (2009) and glandular position follows Pérez-Zabala (2005). We retained the use of the concept of specialized shoot named flowering shoot instead of the more general term inflorescence, considering the comparable vegetative vs. flower shoot structure in *Prunus* (including both deciduous and evergreen species) in terms of the presence of basal cataphylls homologous to leaves (including fused stipules) (Kalkman 1966), proleptic growth from resting lateral buds developing asynchronously with respect to the subtending leaves (Costes et al. 2014), a predetermined length and number of flowers, and flowers subtended by bracts homologous to reduced leaves (or sometimes well-developed leaves) (Kalkman 1966). Finally, to determine the conservation status of this new species, we followed the guidelines for the use of categories and criteria of the IUCN Red List version 16 (IUCN Standards and Petitions Committee 2024) based on the indicators of criterion B; Geographical distribution is represented as extent of occurrence (B1) and/or area of occupancy (B2).

Taxonomy

Prunus luxurians Pérez-Zab., Á.J.Pérez, Romol. & N.Albán, sp. nov.

urn:lsid:ipni.org:names:77359711-1
Figs 1–3

Type. Ecuador. El Oro: • Cantón Santa Rosa, Parroquia Torata, Buenaventura Reserve, Jocotoco Foundation, 03°33'54.7"S, 79°46'47.5"W, 1300–1400 m, 22 Dec 2021 (fl), *Á.J. Pérez, P. Mena-Olmedo, A. de la Cruz, J. Zambrano & L. Aguilar 11743* (holotype: QCA; isotypes: LOJA, QCNE).

Diagnosis. *Prunus luxurians* has a unique combination of leaves oblong lanceolate, cernuous in posture, around three times longer than wider, with prominent secondary and tertiary veins, secondary veins curved toward the margin and apparently eucamptodromous but becoming brochidodromous distally, base rounded to subcordate, floriferous shoots erect, densiflorous, less than 5 cm long, sepals with 2 marginal glands and flowers with turbinate hypanthium. *Prunus integrifolia* (Presl.) Walp. share with the new species the leaf shape and transverse posture, number of secondary veins, erect floriferous shoots and anthers of similar size; but, the new species has leaves shorter than 14 cm (vs. more than 15 cm.), chartaceous (vs. coriaceous), with conspicuous venation (vs. relatively obscure), glands submarginal and separated from the midrib (vs. attached to the midrib), turbinate hypanthium (vs. wide turbinate) and erect pedicels (vs. recurved) (Fig. 4).

Description. ***Tree*** up to 11 m tall; main trunk bark brown, grooved, with brown to light brown lenticels. Most recent growth units 2–2.6 mm diam. at base, with 8–9 leaves per unit, glabrous, angled, reddish brown in vivo; lenticels (0.2–) 0.5–0.9 × 0.1–0.4 mm, elliptic to narrowly elliptic, slightly protuberant, density up to 32 per centimeter (at the base of last growth units); cataphylls 2.8–3 × 3.5–4.1 mm, trapezoidal, trilobed at the apex, glabrous. ***Leaves*** alternate, distichous on plagiotropic shoots, transversal posture cernuous when fresh; petioles 3.5–5.9 × 1–1.4 (–1.8) mm long, flexuous, longitudinally grooved, glabrous; stipules paired, 1.3–1.5 mm at the base (scar), ca. 3.5 mm long, oblong falcate, apex acute, apparently deciduous when blades start expanding; leaf-blades (6.1–) 10–13.65 × (2.9–) 3.5–4.65 cm, around three times longer than wide, oblong-lanceolate, equilateral, chartaceous, conduplicate longitudinally, base rounded to subcordate, apex acute; margin entire to slightly repand; upper surface lustrous, impressed to slightly bulliform, glabrous; lower surface opaque, slightly rugose, glabrous; midrib 0.6–0.9 mm wide, slightly depressed above, prominent below, secondary vein framework apparently eucamptodromous but becoming brochidodromous distally, secondary veins 7–14 pairs, departing at 70–80 degrees from the midrib and progressively curving up to 30 degrees after the first quarter and almost parallel to the margin towards the end, slightly depressed above, raised below; intersecondaries 1–3 per intercostal area, following up the proximal trajectory of secondaries up to 20–30% of their length; tertiary intercostal opposite percurrent sinuous, obtuse with respect to the midvein, epimedial tertiary alternate percurrent and perpendicular to midvein, distal course basiflexed, slightly depressed above, impressed below, exterior tertiary veins looped; quaternary and higher order veins inconspicuous; leaf glands 2, elliptic to circular, 0.5–1 × 0.5–1 mm, dark brown, sub-basal below the first pair of secondary veins, distanced (0.3–) 1–1.2 (–3.4) mm from the midrib and 0.2–0.9 mm from the margin. ***Floriferous***

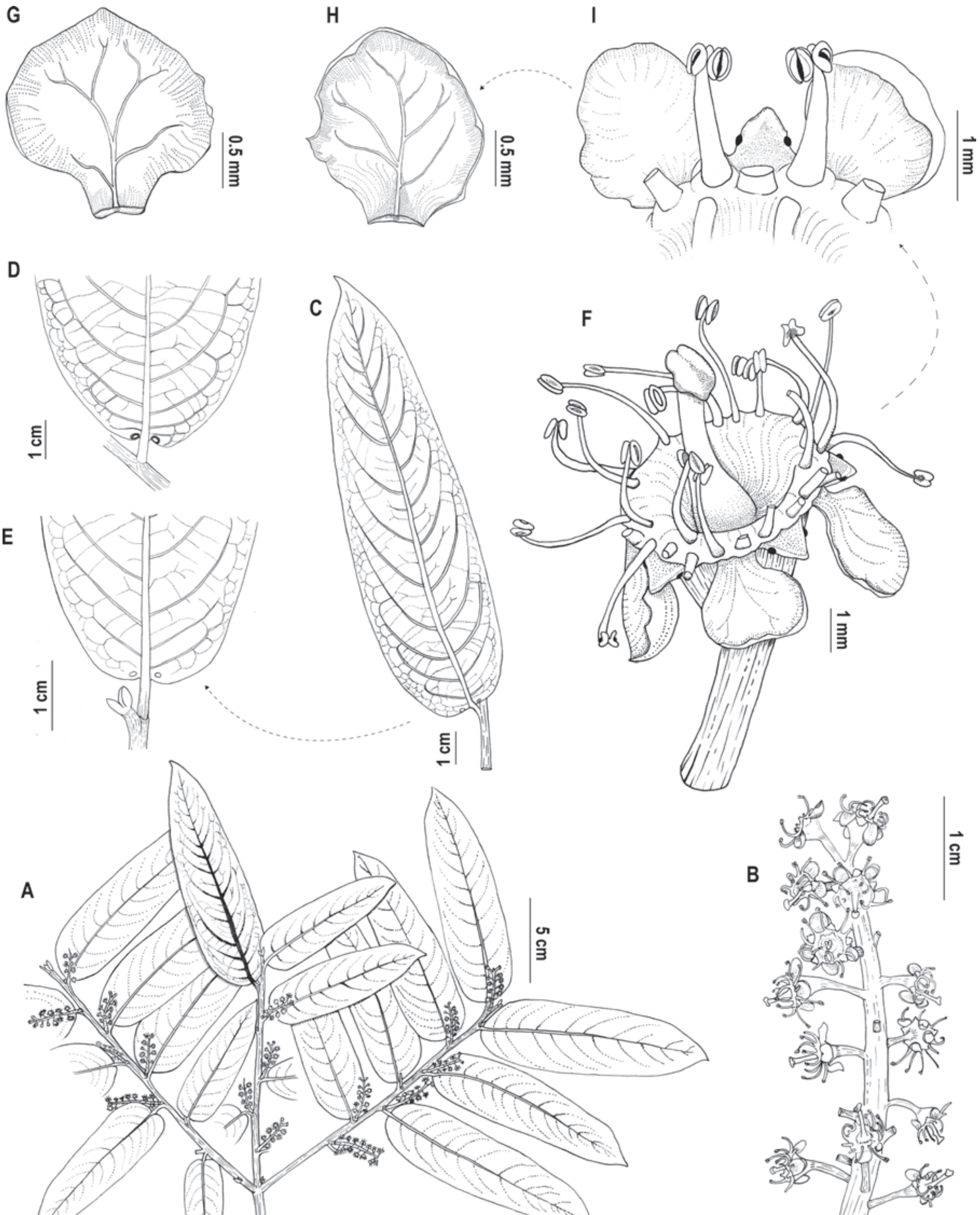

Figure 1. *Prunus luxurians* Pérez-Zab., Á.J.Pérez, Romol. & N.Albán **A** habit **B** floriferous shoot **C** leaf lower surface **D, E** leaf glands **F** flower **G** petal lower surface **H** petal upper surface **I** detail of petals and sepals (notice marginal glands). **A–I** based on *Á.J. Pérez et al. 11743* (QCA). Illustrations by Natasha Albán.

shoots with a single axis, erect to suberect, present on the axils of all leaves and cataphylls of the current growth units, axis (17–) 32–44.5 × 2.4–2.8 mm at the base and keeping a similar width until the middle, light green in vivo, glabrous, 14–27 flowered with groups of 3–4 flowers closer together; first flower at ca

Figure 2. *Prunus luxurians* Pérez-Zab., Á.J.Pérez, Romol. & N.Albán **A** habit **B** branch with leaves and floriferous shoots **C** flowers. Photos by Á.J. Pérez.

8 mm from the base; cataphylls 1.6–3.7 × 1.2–4.1 mm, glabrous, trapezoidal, floral bracts not seen. ***Flowers*** pedicellate, pedicels 1.5–5.4 × 0.3–1.4 mm, straight, thickened at apex, white, glabrous; hypanthium 1.5–2.1 × 2–3.5 mm, turbinate, pale yellow, glabrous outside; sepals 0.7–1.2 × 0.7–1.5 mm, broadly triangular, margin entire, apex acute, glabrous outside and inside, slightly reflexed, with two marginal subapical glands that turn black when dry; petals 1.5–2.4 × 1.1–2 mm, widely obovate, 0.4–0.6 (–0.8) mm wide at the basal claw, white, glabrous, apex rounded, margin entire, and slightly involute, venation with 4–6 lateral branches little conspicuous, eglandular; stamens 19–26, in two series, the outer series (3–3.2 mm long) longer than the inner one (1.5–2 mm long), glabrous, filaments 1.4–3.5 mm long, white, anthers 0.4–0.6 × 0.3–0.4 mm, basifixed with indorse dehiscence, elliptic-oblong, yellow; pistil 2–4.5 mm long, ovary 0.3–1.2 × 0.2–1.4 mm, glabrous, style 0.3–2.7 mm long, as long as or shorter than stamens, glabrous, stigma 0.5–1.0 mm wide, discoid-lobed. ***Fruit*** not seen.

Etymology. We use the epithet *luxurians* to refer to the profuse blooming and outstanding beauty of this species at its flowering time that make it very conspicuous at the lower and mid-strata of the forest where it inhabits.

Distribution, habitat and ecology. Known thus far only from the type locality in El Oro province, south-western Ecuador, a montane forest remnant at the Sambotambo-Birón area along the road to Cerro Pelado, between 1300–1600 m (Fig. 5). According to the Ministerio del Ambiente del Ecuador (2013) the type locality lies in the Catamayo-Alamor evergreen piedmont forest (BsPn02) that harbors high diversity and endemism (Cerón et al. 1999; Myers et al. 2000) as a result of the Andes and Tumbesian region influence. In the Sambotambo-Birón area a total of five individuals of *Prunus luxurians* were observed growing on the borders of the forest remnants co-occurring with the following tree species: *Guatteria microcarpa* Ruiz & Pav. (Annonaceae), *Dictyocaryum lamarckianum* (Mart.) H.Wendl., *Wettinia kalbreyeri* (R.Bernal) R.Bernal (Arecaceae), *Guarea kunthiana*

FLORA DEL ECUADOR
Á.J. Pérez, P. Mena-Olmedo, A. de la Cruz, J. Zambrano & L. Aguilar
11743 ROSACEAE
Prunus sp.
EL Oro
Cantón Santa Rosa, Parroquia Torata, Reserva Buenaventura, Fundación Jocotoco. Colecciones en los remanentes de bosque montano del sector Sambotambo-Birón. Especies representativas: *Dictyocaryum lamarkianum, Roupala montana, Guarea kunthiana*
03°33'54.7"S 79°46'47.5"W Alt: 1300-1400 m 22/12/2021
Arbol 11 m, flores blancas (FOTO + DNA sample)
HERBARIO QCA - Escuela de Ciencias Biológicas - PUCE

Figure 3. *Prunus luxurians* Pérez-Zab., Á.J.Pérez, Romol. & N.Albán holotype collection *Á.J. Pérez et al. 11743* (QCA).

A.Juss. (Meliaceae), *Roupala montana* Aubl. (Proteaceae) and the recently described *Magnolia buenaventurensis* Á.J.Pérez & E.Rea (Magnoliaceae) and *Begonia jocotocoi* Á.J.Pérez & Tebbitt (Begoniaceae) (Pérez et al. 2023, 2024).

Phenology. Flowering from October to January and probably fruiting in July to August (the latter according to testimonies of people living in the area).

Conservation status. Known only from a single herbarium collection of a population of around five adults that we observed growing on the edges of

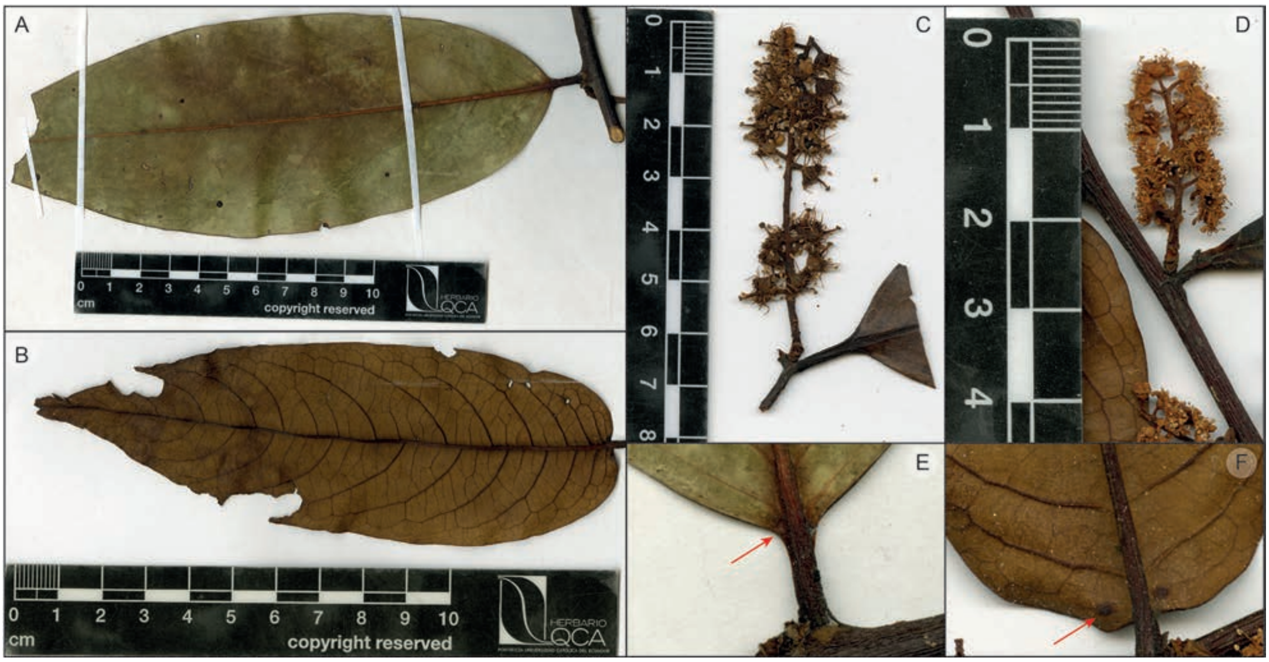

Figure 4. Differences between *Prunus luxurians* Pérez-Zab., Á.J.Pérez, Romol. & N.Albán (**B**, **D**, **F**) and *Prunus integrifolia* (Presl.) Walp. (**A**, **C**, **E**). **A, B** leaf abaxial surface **C, D** floriferous shoots **E, F** leaf gland. **A**, **E** sample *Lægaard 17716* (QCA), **B**, **D**, **F** *Á.J. Pérez et al. 11743* (QCA), and **C** *Salinas 3188* (QCA).

the forest remnants in the Buenaventura Reserve, close to the road to Cerro Pelado in the Sambotambo-Birón area. Based on the available information, and according to the IUCN Red List Criteria and Guidelines (IUCN Standards and Petitions Committee 2024), *Prunus luxurians* is preliminarily assessed as Critically Endangered (CR B2ab(iii)) because of its limited area of occupancy (AOO < 5 km2) and a single known population of around five individuals. Additionally, mining and farming activities currently threaten the forest remnants around the type locality. Priority reproduction studies and germination assays are needed for attempting *ex-situ* conservation of this promising ornamental species.

Discussion

Prunus luxurians has similarities with some Andean species that display oblong lanceolate leaves, transverse posture cernuous, secondary veins exmedially curved and apparently eucamptodromous but distally becoming brochidodromous, two leaf glands placed around the leaf base, floriferous shoots erect or suberect, and anthers around 0.5 mm long like *P. integrifolia*, *P. rugosa* Koehne, *P. pearcei* Rusby and *P. pleiantha* Pilger. However, all these four species have larger leaves (generally starting from 15 cm), glands attached to the midrib, longer flowering shoots (which may be also subflexuous) and recurved floral pedicels. Other apparently eucamptodromous species are *P. schultzeae* Pilger from the Amazon foothills below 500 m in Pastaza and Napo (Ecuador), and *P. sana* Macbr. and *P. williamsii* Macbr. from mid-elevation (700–1200 m) Amazon flanks in northern Peru and Ecuador, and Cuzco in central Peru, respectively. However, they all have elliptic leaves with acuminate to very acuminate apex, impressed secondary veins, and little conspicuous tertiary veins, flexuous floral axis, and smaller flowers with all parts smaller than *P. luxurians*. On the other hand, other

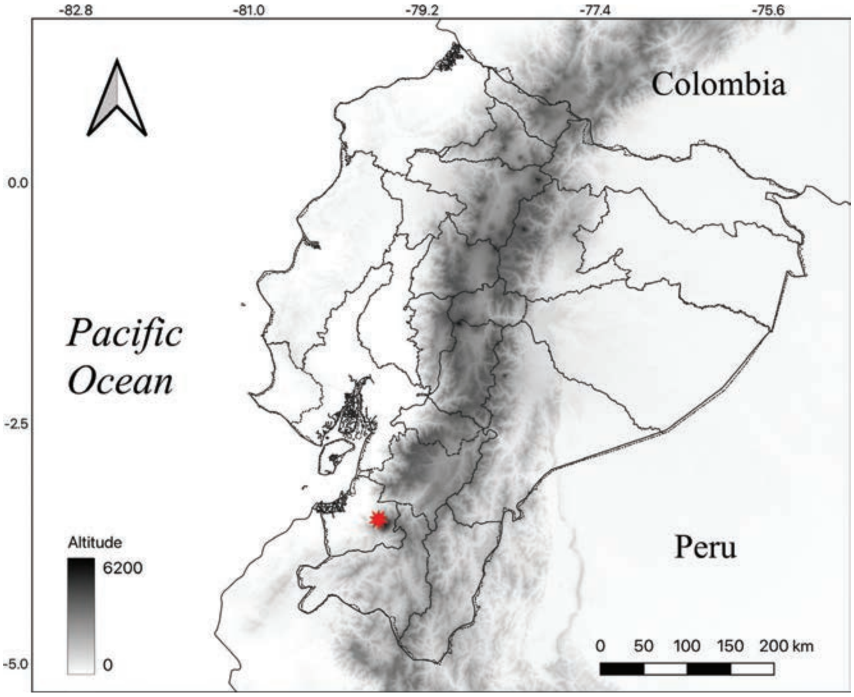

Figure 5. Distribution map of *Prunus luxurians* Pérez-Zab., Á.J.Pérez, Romol. & N.Albán (red star) from south-western Ecuador. Map generated by Á.J. Pérez & E. Rea.

Andean species with oblong to lanceolate leaves (*P. opaca* (Benth.) Walp. and *P. littlei* Pérez-Zab.) generally have smaller leaves (up to 10 cm long), a clearly brochidodromous secondary vein framework (relatively impressed and then obscure when dry), flexuous floral axes and a campanulate hypanthium. Finally, this is the first tropical species in which glands have been observed in the sepals, but certainly, this character could have been unnoticed in previous descriptions and may be present in some other Neotropical species. Marginal sepal glands (sometimes as part of tooth) are present in Eurasian species like *P. armeniaca* L., *P. cerasifera* Ehrh., *P. cerasus* L., *P. domestica* L., *P. glandulosa* Thunb., *P. spinosa* L., *P. subhirtella* Miq., *P. yedoensis* Matsum., and in the North American species *P. americana* Marshall, *P. andersonii* A. Gray, *P. fremontii* S. Watson, *P. hortulana* L.H. Bailey, *P. mexicana* S. Watson, *P. murrayana* E.J. Palmer, *P. nigra* Aiton, *P. rivularis* Scheele, *P. subcordata* Benth., and *P. texana* D. Dietr. (Rohrer 2015).

Most of the species with a wide range of occurrences in the Andes grow above 1800 m (ca *P. integrifolia*, *P. littlei* and *P. opaca*), whereas species described from the eastern foothills of the Andes below 1800 m have a relatively restricted distribution (ca *P. amplifolia* Koehne, *P. sana*, *P. williamsii*). *Prunus luxurians* is the first species of *Prunus* to be described from the western flanks, at an altitude of less than 1500 m, and in particular from a humid spot surrounded by dry areas (the latter known as the Tumbesian influence). Only three species of *Prunus* are known from the west flanks of the Andes: *Prunus megacarpa* Pérez-Zab, *P. rigida* Koehne and *P. subcorymbosa* Koehne. *Prunus megacarpa* grows in very humid forests between 1600 and 1900 m in northwestern Colombia, and is characterized by obovate leaves, multiple leaf glands and very large fruits. *Prunus rigida* is found in humid or sub-humid forest or shrublands above 2800 m from Cajamarca to Lima, and has entire to dentate leaves, impressed venation, erect floral axis and anthers larger than 1 mm long. The last species, *P. subcorymbosa*, is widely distributed from Bolivia to Venezuela from 800 to 2500 m, and it has pubescent buds, young leaves and floral axes, flowering shoots generally branched from the axils of the cataphylls, hypanthium widely campanulate and pubescent, and fruits prolate and longer than 15 mm. The western flanks of the Andes in Colombia and Ecuador have been poorly explored although a high diversity is expected due to the presence of the hyperdiverse extremely humid Choco biogeographic region from eastern Panama to central Ecuador (Pérez-Escobar et al. 2019), and the special local climatic conditions found in the transition from humid to dry forest in southern Ecuador (Cerón et al. 1999). Few collections of *Prunus* have been recorded from the western flanks of the Andes, and some of those seen by the authors correspond to taxonomic novelties, indicating that further botanical exploration is necessary to get a better picture of the diversity of the genus in the region.

Acknowledgements

Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador issued permit MAATE-ARSFC-2024-0434. ÁJP thanks the Jocotoco Foundation for logistical and fieldwork support, and José León, Patricio Mena-Olmedo, Amado de la Cruz, Jorge Zambrano, Diego Cabrera and Leonardo Aguilar, Jocotoco staff. We thank Carolina Parreño and Carla Rodríguez for their help in digitizing the herbarium material, Edison Rea for his assistance of the distribution map, and Xavier Cornejo, Hugo Navarrete and Hanno Schaefer for their suggestions in improving 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 PUCE - Pontificia Universidad Católica del Ecuador.

Author contributions

Concept idea KR and AJP; field work AJP; taxonomic species description KR, JAP, AJP and NA; figures elaboration AJP, NA, DAEO and KR; first draft KR, JAP, AJP, NA, DAEO and CR; editing and final draft DAEO, JAP and KR.

Author ORCIDs

Álvaro J. Pérez https://orcid.org/0000-0002-0644-9111
Jorge Andrés Pérez-Zabala https://orcid.org/0000-0003-1388-7694
Katya Romoleroux https://orcid.org/0000-0002-0679-9218
David A. Espinel-Ortiz https://orcid.org/0000-0003-3405-0375
Chaquira Romoleroux https://orcid.org/0009-0003-0278-3790
Natasha Albán-Vallejo https://orcid.org/0009-0008-1506-8768

Data availability

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

References

Basualdo I, Zardini E (1992) Prunus ravenii Zardini & Basualdo, Prunus douglasii Basualdo & Zardini, Rubus paraguariensis (Chodat & Hassler) Basualdo & Zardini: Novedades en la familia Rosaceae para Paraguay. Candollea 47: 251–255. https://doi.org/10.5169/seals-879573

Bortiri E, Vanden Heuvel B, Potter D (2006) Phylogenetic analysis of morphology in Prunus reveals extensive homoplasy. Plant Systematics and Evolution 259(1): 53–71. https://doi.org/10.1007/s00606-006-0427-8

Cerón CE, Palacios W, Valencia R, Sierra R (1999) Las Formaciones Naturales de la Costa del Ecuador. In: Sierra R (Ed.) Propuesta Preliminar de un Sistema de Clasificación de Vegetación para el Ecuador Continental. Proyecto INEFAN/GEF-BIRF y EcoCiencia, Quito, 55–78.

Costes E, Crespel L, Denoyes B, Morel P, Demene M-N, Lauri P-E, Wenden B (2014) Bud structure, position and fate generate various branching patterns along shoots of closely related Rosaceae species: A review. Frontiers in Plant Science 5. https://doi.org/10.3389/fpls.2014.00666

Cuatrecasas J (1950) Contributions to the flora of South America II. Fieldiana. Botany 27: 56–58.

Dickinson TA, Lo E, Talent N (2007) Polyploidy, reproductive biology, and Rosaceae: Understanding evolution and making classifications. Plant Systematics and Evolution 266(1–2): 59–78. https://doi.org/10.1007/s00606-007-0541-2

Ellis B, Daly D, Hickey L, Johnson K, Mitchell J, Wilf P, Wing S (2009) Manual of Leaf Architecture. Cornell University Press CABI, GB. https://doi.org/10.1079/9781845935849.0000

Hickey M, King C (2000) The Cambridge Illustrated Glossary of Botanical Terms. Cambridge University Press, 1–208.

IPNI (2024) International Plant Names Index. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. http://www.ipni.org [March 19, 2024]

IUCN Standards and Petitions Committee (2024) 16 Guidelines for Using the IUCN Red List Categories and Criteria. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf [December 11, 2024]

Johnston IM (1938) Some undescribed species from Mexico and Guatemala. Journal of the Arnold Arboretum 19(2): 117–128. https://doi.org/10.5962/bhl.part.17089

Kalkman C (1966) The Old World species of Prunus subg. Laurocerasus including those formerly referred to Pygeum. Blumea 13: 1–115. https://repository.naturalis.nl/pub/526237

Koehne E (1915) Zur Kenntnis von Prunus grex Calycopadus und grex Gymnopadus sect. Laurocerasus. Botanische Jahrbücher fur Systematik 52: 279–333.

Li CL, Aymard G (1997) A new species and a new combination in Prunus subgenus Lauracerasus (Rosaceae) from the Venezuelan Guayana. BioLlania, Ed. Espec. 6: 449–454.

Lundell CL (1968) Studies of Tropical American Plants V. Wrightia 4: 79–96.

Macbride JF (1934) New or renamed spermatophytes mostly Peruvian. Candollea 5: 362–367.

McVaugh R (1951) A Revision of the North American Black Cherries (Prunus Serotina Ehrh., and Relatives). Brittonia 7(4): 279. https://doi.org/10.2307/2804698

Ministerio del Ambiente del Ecuador (2013) Sistema de clasificación de los ecosistemas del Ecuador continental. Subsecretaría de Patrimonio Natural, Quito, 1–232.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Pennington RT, Dick CW (2004) The role of immigrants in the assembly of the South American rainforest tree flora. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 359(1450): 1611–1622. https://doi.org/10.1098/rstb.2004.1532

Pérez ÁJ, Rea E, Burgess KS, Mena-Olmedo P, Cabrera L, León J, Vázquez-García JA (2023) A new endemic Magnolia species (M. sect. Talauma, Magnoliaceae) from the south-western montane forest remnants of Ecuador. Phytotaxa 592(2): 119–126. https://doi.org/10.11646/phytotaxa.592.2.5

Pérez ÁJ, Mena-Olmedo P, Cabrera L, León J, Tebbitt MC (2024) A new endemic Begonia species (sect. Knesebeckia, Begoniaceae) from the south-western montane forest remnants of Ecuador. Phytotaxa 666(3): 216–222. https://doi.org/10.11646/phytotaxa.666.3.5

Pérez-Escobar OA, Lucas E, Jaramillo C, Monro A, Morris SK, Bogarín D, Greer D, Dodsworth S, Aguilar-Cano J, Sanchez Meseguer A, Antonelli A (2019) The Origin and Diversification of the Hyperdiverse Flora in the Chocó Biogeographic Region. Frontiers in Plant Science 10: 1328. https://doi.org/10.3389/fpls.2019.01328

Pérez-Zabala JA (2005) Revisión taxonómica del género *Prunus* L. (Rosaceae) para Colombia. Master thesis. Universidad Nacional de Colombia.

Pérez-Zabala JA (2007) Studies on the genus Prunus (Rosaceae) in the Neotropics: taxonomic and nomenclature changes for Colombia. Anales del Jardín Botánico de Madrid 64: 177–190. https://doi.org/10.3989/ajbm.2007.v64.i2.173

Pérez-Zabala JA (2015) Prunus. In: Davidse G, Sousa Sánchez M, Knapp S, Chiang Cabrera F (Eds) Flora Mesoamericana, Saururaceae a Zygophyllaceae. Universidad Nacional Autónoma de México, México, 31–47.

Pérez-Zabala JA (2022) Taxonomic Diversity, Phylogeny, and Diversification of the Environmental Niche of the Genus Prunus L. with emphasis on the New World Tropics. PhD thesis. UC Davis. https://www.proquest.com/openview/978e23cde15cd00c2848ca6fe591f389/1?pq-origsite=gscholar&cbl=18750&diss=y

Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA, Campbell CS (2007) Phylogeny and classification of Rosaceae. Plant Systematics and Evolution 266(1–2): 5–43. https://doi.org/10.1007/s00606-007-0539-9

Rohrer JR (2015) Prunus. In: Flora of North America Editorial Commitee (Ed.) Flora of North America North of Mexico. New York and Oxford, 352–378.

Romoleroux K (1996) 56 Flora of Ecuador Rosaceae. In: Harling G, Andersson L (Eds) University of Gothenburg, Göteborg, Sweden; Riksmuseum, Stockholm, Sweden; Pontificia Universidad Católica del Ecuador, Quito, Ecuador, 1–151.

Shaw J, Small RL (2004) Addressing the “hardest puzzle in American pomology:” Phylogeny of Prunus sect. Prunocerasus (Rosaceae) based on seven noncoding chloroplast DNA regions. American Journal of Botany 91(6): 985–996. https://doi.org/10.3732/ajb.91.6.985

Stearn WT (2004) Botanical Latin: History, Grammar, Syntax, Terminology and Vocabulary. David & Charles.

Thiers BM (2024) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. https://sweetgum.nybg.org/science/ih/ [December 11, 2024]

Wilhelm G, Rericha L (2020) Illustrated Glossary of Botanical Terms. Flora of the Chicago Region, A Floristic and Ecological Synthesis. fouth. Indiana Academy of Sciences and Conservation Research Institute, USA, 1–43. http://conservationresearchinstitute.org/forms/CRI-FLORA-Glossary.pdf [December 11, 2024]

PhytoKeys 255: 35–40 (2025)
DOI: 10.3897/phytokeys.255.138826

Research Article

Lagerstroemia yangchunensis (Lythraceae), a new species from Guangdong, China

Bao-Huan Wu[1,2,3*], Xiu-Ting Liu[4*], Zi-Yu Bao[1,2,3], Xiao-Yan Ma[1,2,3], Ying Chen[1,2,3], Guo-Di Chen[5], Xing Hu[1,2,3], Guo-Feng Liu[1,2,3], Se-Ping Dai[1,2,3]

1 *Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou 510405, China*
2 *Guangzhou Horticultural Plant Germplasm Resource Nursery, Guangzhou 510405, China*
3 *Guangzhou Collaborative Innovation Center on Science-Tech of Ecology and Landscape, Guangzhou 510405, China*
4 *Ting Jie Natural History Studio,Yangjiang 529500, China*
5 *Yangjiang Municipal People's Government Taiwan, Hong Kong and Macao Affairs Bureau, Yangjiang 529500, China*
Corresponding authors: Guo-Di Chen (13380861111@189.cn); Xing Hu (373108011@qq.com)

Abstract

A new species from southern China, *Lagerstroemia yangchunensis* B.H.Wu & G.D.Chen, **sp. nov.**, is described in the present paper. This species is morphologically similar to *L. duperreana* Pierre ex Gagnep., but can be distinguished by its coarse bark with vertical fissures, calyx lobes glabrous inside, and flowers with shorter pseudopedicels and petals with longer claws. Detailed morphological characteristics, habitat information, and comparisons with similar species are provided.

Key words: Crape myrtle, Flora, new species, Yangchun

Academic editor: Peter de Lange
Received: 9 October 2024
Accepted: 15 March 2025
Published: 8 April 2025

Citation: Wu B-H, Liu X-T, Bao Z-Y, Ma X-Y, Chen Y, Chen G-D, Hu X, Liu G-F, Dai S-P (2025) *Lagerstroemia yangchunensis* (Lythraceae), a new species from Guangdong, China. PhytoKeys 255: 35–40. https://doi.org/10.3897/phytokeys.255.138826

Introduction

Lagerstroemia L. (Lythraceae) is a significant genus known for its ornamental value, notably for its vibrant summer blooms. Taxonomic revisions of *Lagerstroemia* have been extensive, commencing with Koehne's monograph (Koehne 1883, 1903) and followed by subsequent revisions by Furtado and Srisuko (1969), which recognized 53 species. Subsequently, regional taxonomic updates (Lee and Lau 1983; Hewson 1990; Qin and Graham 2007; De Wilde and Duyfjes 2013, 2014, 2016; De Wilde et al. 2014) and additional species descriptions (Zhou et al. 2004; Gu et al. 2012, 2015; Deepu and Pandurangan 2017; Pham et al. 2017; De Wilde and Duyfjes 2019; Wu et al. 2023) have refined our understanding of the genus. Currently, the genus comprises ca. 52 species (Wu et al. 2023).

In recent years, we carried out several field surveys to gather germplasm resources of *Lagerstroemia* in China. In 2024, we found an unknown *Lagerstroemia* species with coarse and vertically fissured bark, a character uncommon in *Lagerstroemia* species of Guangdong. After extensive morphological comparisons and taxonomic analyses, we confirmed a new species which we formally describe herein.

---

* These authors contributed equally to this work.

Materials and methods

Morphological characteristics were analyzed based on observations of the living plants in the field and dried specimens in herbaria. 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 South China Botanical Garden (IBSC), South China Agricultural University (CANT), Sun Yat-Sen University (SYS) and China National Botanical Garden (CNBG).

Taxonomic treatment

***Lagerstroemia yangchunensis* B.H.Wu & G.D.Chen, sp. nov.**
urn:lsid:ipni.org:names:77359970-1
Fig. 1

Type. China • Guangdong: Yangchun City, Chunwan Town, Xinglong Country, in forest, on sunny slope. 22.3327°N, 112.0097°E, 205 m a.s.l., 28 Jun 2024 (fl), *B.H. Wu Lg2024142* (holotype: IBSC!; isotypes: CANT!, CNBG!, SYS!).

Diagnosis. *Lagerstroemia yangchunensis* is morphologically similar to *L. duperreana*, but distinguished by its coarse bark with vertical fissures, calyx lobes glabrous inside, and flowers with shorter pseudopedicels and petals with longer claws.

Description. Trees to 15 m tall. Bark greyish brown, thick and coarse, vertically fissured; branchlets glabrous, terete. Leaves alternate, rarely subopposite; petiole 4–8 mm long, glabrous; leaf blade papery, margin entire, elliptic-oblong, oblong, rarely obovate, 10–14 × 3.5–5 cm, base cuneate to acute, apex acute, acuminate, rarely obtuse, adaxial surface green, glabrous, abaxial surface pale green, slightly pubescent along midvein, lateral veins brochidodromous, 10–15 on each side of midvein. Inflorescences paniculate, terminal or axillary; panicles 9–22 cm long, puberulous with whitish brown hairs. Pedicels 2–5 mm long, densely whitish tomentose; flower buds densely whitish tomentose, obovoid (excluding pseudopedicels), 7–9 mm long, pseudopedicel 2–3 mm long; flowering calyx tubes (excluding pseudopedicels) cup-shaped, 6–8 mm long, outside densely whitish tomentose, distinctly 12-ridged, inside glabrous, lobes 6, triangular to slightly acuminate, 2.5–4.3 × 2–3.5 mm, reflexed, epicalyx minute, pseudopedicel 2.5–5 mm long; petals 6, crumpled, ovate to broadly ovate, 15–20 mm long including 9–10 mm long claw; stamens 30–40, dimorphic, with 6 stamens longer (21–24 mm long), thicker and the lower half reddish purple in color, the remaining stamens shorter (10–13 mm long), thinner and white in color, filaments glabrous; ovary glabrous, styles 22–30 mm long, glabrous, stigmas small. Capsules oblong, smooth, 16–19 mm long, ca. 10 mm in diameter, loculicidally dehiscent, 5-valved. Seeds 7–10 mm long including wing.

Phenology. Flowering from June to July, fruiting after July.

Distribution and habitat. *Lagerstroemia yangchunensis* is hitherto known from its type locality, Chunwan Town, Yangchun City of Guangdong; only 4 individuals were found in the population. It grows in forest on sunny slopes at ca. 200 m elevation.

Etymology. The species epithet “yangchunensis” refers to Yangchun County, the locality where this species was discovered.

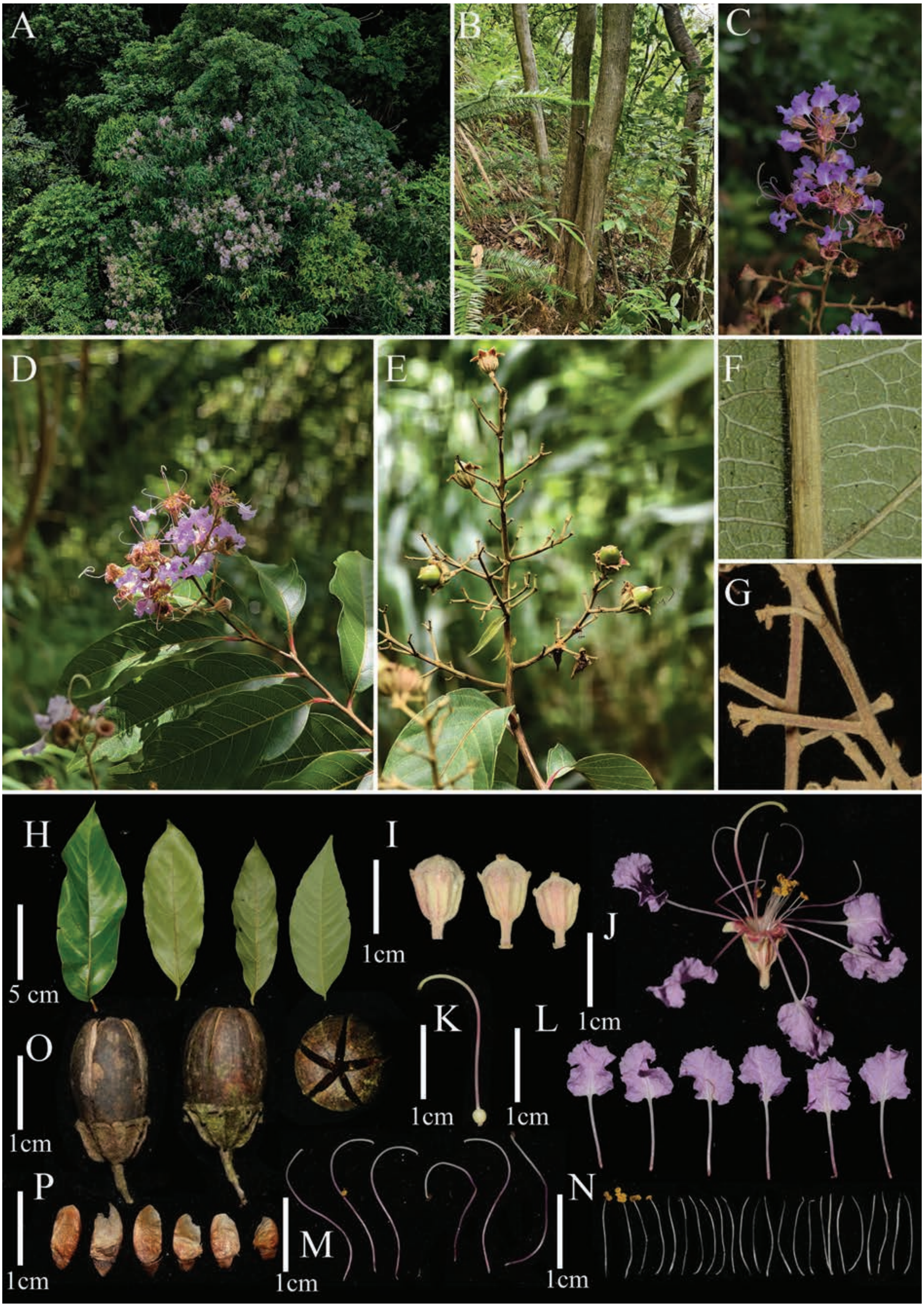

Figure 1. *Lagerstroemia yangchunensis* **A** canopy view during flowering period **B** stems **C** flowering branchlet of the inflorescence **D** flowering branch **E** fruiting branch **F** part of the abaxial leaf surface **G** floral axes **H** leaves **I** flower buds **J** flower **K** gynoecium **L** petals **M** longer stamens **N** shorter stamens **O** capsules **P** seeds. Photographed by Guo-Di Chen and Bao-Huan Wu.

Table 1. Morphological Comparison of *Lagerstroemia yangchunensis* and other similar species.

Characters	*L. yangchunensis*	*L. duperreana*	*L. speciosa*
Bark	greyish brown, thick, coarse, vertically fissured	light brown grey, thin, mottled and dimpled	light brown, black when old, coarsely vertically cracked and slightly flaking
Leaf shape	elliptic-oblong, oblong, rarely obovate	oblong, elliptic-oblong, sometimes obovate	elliptic, elliptic-oblong, or elliptic-ovate
Leaf size	10–14 cm long, 3.5–5 cm wide	8–12(–15) cm long, 3–5 (–10) cm wide	10–25 cm long, 6–12 cm wide
Leaf surface	adaxial surface glabrous, abaxial surface slightly pubescent along midvein	glabrous	glabrous
Number of lateral veins	10–15 on each side	8–10 on each side	(6–)8–14 on each side
Petiole length	4–8 mm	3–5 mm	6–15 mm
Panicle length	densely whitish tomentose	glabrous	glabrous
Flower buds	densely whitish tomentose, obovoid, 7–9 mm long, pseudopedicel 2–3 mm long	short densely whitish hairy, obovoid, ca. 6 mm long, pseudopedicel 6–12 mm long	short-farinose, subglobose, ca. 0.5 mm long, pseudopedicel 3–8 mm long
Calyx tube	6–8 mm long, distinctly 12-ridged	5–6 mm long, (indistinctly) 12-ridged	calyx tube 6–8 mm long, distinctly 12-ridged
Calyx lobe	2.5–4.3 mm long, glabrous within	ca. 3 mm long, densely whitish hairy in the upper part within	up to 8 mm long, glabrous within
Petal length	15–20 mm long, including 9–10 mm long claw	10–15 mm long, including ca. 5 mm long claw	ca. 20 mm long, including ca. 5 mm long claw

Vernacular name. The proposed Chinese name for *Lagerstroemia yangchunensis* is 阳春紫薇 (yáng chūn zǐ wēi).

Discussion. *Lagerstroemia yangchunensis* is morphologically similar to *L. duperreana*; however, it can be easily distinguished from the latter species by several characteristics, such as bark coarse and vertically fissured, calyx lobes glabrous inside and flowers with shorter pseudopedicels and petals with longer claws. A detailed comparison is summarized in Table 1.

Having large inflorescences with flowers boasting vibrant purple colors, *Lagerstroemia yangchunensis* is a potential ornamental species for urban landscaping or *Lagerstroemia* breeding. However, its restricted distribution to the edge of a managed woodland in Chunwan Town poses significant threats to its survival. The small and isolated population, coupled with interference from human activities in its habitat, makes this species highly vulnerable to extinction. To ensure the species' long-term viability and promote its sustainable development, urgent conservation measures, such as habitat protection, seed banking, ex situ cultivation, and public awareness campaigns, are necessary.

Additional specimen examined. China • Guangdong Province: Yangchun City, Chunwan Town, 22.3327°N, 112.0097°E, 205 m a.s.l., 28 Jun 2024 (fl), *B.H. Wu Lg2024141*; *ibid.*, 28 June 2023, *B.H. Wu Lg2024143*, *Lg2024144*.

Acknowledgements

We thank Dr. You-Pai Zeng for introducing the authors to each other, which initiated this work. We are grateful to the curators of herbaria IBSC, P, PE and XBGH 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 Science and Technology Program from Forestry Administration of Guangdong Province (Grant no. 2023KJCX002), and Social Development Project of Guangzhou Municipal Science and Technology Bureau (Grant no. 202206010058).

Author contributions

Bao-Huan Wu, Xiu-Ting Liu and Xing Hu are the main authors of the article, and everyone else participated in the writing. Xing Hu, Xiu-Ting Liu and Guo-Di Chen participated in the taxonomic confirmation of the new species and field investigation, and Se-Ping Dai is the leader of the research project, responsible for promoting the project.

Author ORCIDs

Bao-Huan Wu https://orcid.org/0000-0002-0498-0603
Xiu-Ting Liu https://orcid.org/0009-0000-3383-5336
Zi-Yu Bao https://orcid.org/0009-0002-9768-2839
Xiao-Yan Ma https://orcid.org/0009-0007-7855-0984
Xing Hu https://orcid.org/0009-0001-8286-101X

Data availability

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

References

De Wilde WJJO, Duyfjes BEE (2013) Miscellaneous information on *Lagerstroemia* L. (Lythraceae). Thai Forest Bulletin (Botany) (41): 90–101.

De Wilde WJJO, Duyfjes BEE (2014) *Lagerstroemia* (Lythraceae) in Malesia. Blumea 59(2): 113–122. https://doi.org/10.3767/000651914X685357

De Wilde WJJO, Duyfjes BEE (2016) Survey of *Lagerstroemia* L. (Lythraceae) in Indochina (excl. Thailand) with the description of *Lagerstroemia densiflora*, sp. nov., a new species from Vietnam. Adansonia 38: 241–255. https://doi.org/10.5252/a2016n2a8

De Wilde WJJO, Duyfjes BEE (2019) Three new species of Lythraceae and one new species of Stemonaceae described from Indochina. Blumea 64(2): 177–182. https://doi.org/10.3767/blumea.2019.64.02.08

De Wilde WJJO, Duyfjes BEE, Phonsena P (2014) Lythraceae. In: Santisuk T, Newman M (Eds) Flora of Thailand, vol. 11. The Forest Herbarium Department of National Parks, Wildlife and Plant Conservation, Bangkok, 547–597.

Deepu S, Pandurangan AG (2017) Lectotypification and note on the status of *Lagerstroemia intermedia* (Lythraceae). Phytotaxa 296: 297–300. https://doi.org/10.11646/phytotaxa.296.3.11

Furtado CX, Srisuko M (1969) A revision of *Lagerstroemia* L. (Lythraceae). Gardens' Bulletin (Singapore) 24: 185–334.

Gu C-H, Ji M-C, Ma D-D (2012) *Lagerstroemia menglaensis* sp. nov. (Lythraceae) from Xishuangbanna, Yunnan Province, China. Nordic Journal of Botany 30: 299–301. https://doi.org/10.1111/j.1756-1051.2011.01276.x

Gu C-H, Ma D-D, Zhu X-D, Tembrock LR, Wang X (2015) *Lagerstroemia densa* (Lythraceae), a new species from Daqing Mountain, Guangxi Zhuang Autonomic Region, China. Phytotaxa 204(3): 230–234. https://doi.org/10.11646/phytotaxa.204.3.6

Hewson HJ (1990) *Lagerstroemia*. In: George AS (Ed.) Flora of Australia, Vol. 18. AGPS Press, Canberra, 110–113.

Koehne BAE (1883) Lythraceae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 4(1): 12–37.

Koehne BAE (1903) Lythraceae. Das Pflanzenreich 17: 1–326.

Lee S-K, Lau L-F (1983) Lythraceae. In: Fang W-P, Chang C-Y (Eds) Flora Reipublicae Popularis Sinicae, Vol. 52. Science Press, Beijing, 67–111.

Pham TT, Tagane S, Chhang P, Yahara T, Souradeth P, Nguyen TT (2017) *Lagerstroemia ruffordii* (Lythraceae), a new species from Vietnam and Cambodia. Acta Phytotaxonomica et Geobotanica 68: 175–180. https://doi.org/10.18942/apg.201705

Qin H-N, Graham SA (2007) *Lagerstroemia*. In: W C-Y, Raven PH (Eds) Flora of China, 13. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis, 277–281.

Wu B-H, Hu X, Tu W-H, Wang W, Dai S-P (2023) *Lagerstroemia stenophylla* (Lythraceae), a new species from China. PhytoKeys 234: 127–133. https://doi.org/10.3897/phytokeys.234.111861

Zhou S-B, Guo X-H, Qin W-H (2004) A new species of *Lagerstroemia* (Lythraceae) from Anhui Province. Bulletin of Botanical Research 24: 392–393.

PhytoKeys 255: 41–50 (2025)
DOI: 10.3897/phytokeys.255.148471

Research Article

Sinosasa damingshanensis (Poaceae, Bambusoideae), a new combination supported by morphological and molecular evidence

Yi-Ting Zhang[1], Shu-Yan Lin[1], Zheng-Yang Niu[2], Wei-Xin Jiang[3], Nian-He Xia[2], Yu-Long Ding[1]

1 *College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China*
2 *Key Laboratory of Plant Resources Conservation and Sustainable Utilization, Guangdong Provincial Key Laboratory of Digital Botanical Garden, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China*
3 *Guangxi Key Laboratory of Forest Ecology and Conservation, Key Laboratory of National Forestry and Grassland Administration on Cultivation of Fast-Growing Timber in Central South China, College of Forestry, Guangxi University, Nanning 530004, China*
Corresponding authors: Nian-He Xia (nhxia@scbg.ac.cn); Yu-Long Ding (ylding@vip.163.com)

Abstract

Morphological characteristics and phylogenetic analyses clearly revealed that *Chimonobambusa damingshanensis* should be a member of the genus *Sinosasa*, rather than *Chimonobambusa*, and is a distinct species close to *Sinosasa huapingensis* and *S. mingyueshanensis*. Morphologically, it differs from all the other known *Sinosasa* species by internodes initially with white pubescent, culm leaf auricles absent and triangularly subulate sheath blades, characteristics that are unusual in this genus. And this morphological distinction warrants recognition of *Ch. damingshanensis* as a new combination of *Sinosasa*.

Key words: Arundinarieae, new combination, phylogeny, taxonomy

OPEN ACCESS

Academic editor: Wei Lim Goh
Received: 1 February 2025
Accepted: 10 March 2025
Published: 8 April 2025

Citation: Zhang Y-T, Lin S-Y, Niu Z-Y, Jiang W-X, Xia N-H, Ding Y-L (2025) *Sinosasa damingshanensis* (Poaceae, Bambusoideae), a new combination supported by morphological and molecular evidence. PhytoKeys 255: 41–50. https://doi.org/10.3897/phytokeys.255.148471

Introduction

Sinosasa L.C.Chia ex N.H.Xia, Q.M.Qin & Y.H.Tong (Poaceae, Bambusoideae), was first recognized as a genus of the temperate woody bamboo (i.e. Arundinarieae) by Q. M. Qin et al. (2021), and segregated from the genus *Sasa* Makino and Shibata (1901). It is characterized by racemose synflorescences, 3 stamens, 2 stigmas per floret, branches solitary and supranodal ridge strongly raised (Qin et al. 2021). So far, 8 species endemic to subtropical areas of China (Qin et al. 2021; Li et al. 2023), were included in the genus.

Chimonobambusa damingshanensis Hsueh & W. P. Zhang (1988) was described based on the only collection *C. J. Hsueh 8605* from Daming Mountain, Nanning, Guangxi Province, China. It is widely accepted in the flora accounts and monographs (Ohrnb 1990; Zhu et al. 1994; Hsueh and Wang 1996; Li and Stapleton 2006; Yi et al. 2008; Yi et al. 2009; Maria et al. 2018). In the protologue, it is described as “Rhizoma ampnipodiale; culmi graciles, erecti, caespitosi…; nodis culmorum plane protuberantibus, nodis vaginarum…basi 1-2 nodis radicibus aeriis…; Rami 1-3… Rami florum foliati; Ramuli florum basi 4-5-bracteati…”. All the important characters provided by the authors fit well with the circumscription of *Chimonobambusa*. After careful examination of the types

(SWFC), we realized that the racemose synflorescence with real spikelets were misinterpreted as pseudospikelets and then the species *Ch. damingshanensis* is definitely not a member of the genus *Chimonobambusa*. In order to clarify the identity, we launched a special expedition to the type locality of the species, i.e. Daming Mountain, Nanning City, Guangxi Province of China, where only one bamboo with leptomorph rhizome, culm nodes prominent, culm sheath blades extremely small and foliage leaves with very long ligule, was found and it matches the protologue of *Ch. damingshanensis* very well. It is easily recognized as a member of *Sinosasa* rather than *Chimonobambusa* from branch compliment 1 per node, very prominent culm nodes and long ligule of foliage leaves. So, after comprehensive study, including morphological investigation, phenological observation, and phylogenetic reconstruction, *Chimonobambusa damingshanensis* was herein formally treated as a member of the genus *Sinosasa*, rather than *Chimonobambusa*.

Materials and methods

The specimens of *Chimonobambusa damingshanensis* were obtained from field trips in 25 December of 2024. Its type specimen, *C. J. Hsueh 8605*, deposited in the Herbarium of Southwest Forestry University (SWFC), was examined. Some detailed characters, e.g. sheath ligule, were observed with hand lens and stereomicroscope (Leica S6D).

A total of 24 species representing 10 genera of the tribe Arundinarieae (Bambusoideae) were utilized to reconstruct a phylogeny based on complete chloroplast (cp) genome, among which *Bambusa sinospinosa* McClure and *B. emeiensis* L. C. Chia & H. L. Fung were set as the outgroup taxa. All these sequences were previously published in GenBank. Accession numbers and voucher information are listed in Table 1.

By using the Plant Genomic DNA Kit (TSINGKE), total genomic DNA of *Ch. damingshanensis* was extracted from young and healthy leaves, and then sent to Novogene for DNA sequencing under the Illumina NovaSeq 6000 platform. A total of 40 G genome skimming data was used to assemble the complete chloroplast genome by GetOrganelle v 1.7.4 (Jin et al. 2018) using *Amborella trichopoda* Baill. (accession number: NC_005086) and *Chimonobambusa luzhiensis* (J. R. Xue & T. P. Yi) T. H. Wen & Ohrnb. (accession number: NC_062708) set as the reference, with k-mer values of 21, 45, 65, 85, 105, 125 bp. The Bandage software (Wick et al. 2015) was employed to graphically visualize the assembled chloroplast. The complete chloroplast (cp) genome was annotated, and manually corrected in Geneious v9.1.4 (Kearse et al. 2012).

The matrix of all the whole chloroplast genomes was aligned in MAFFT v. 7.490 (Katoh and Standley 2013). Phylogenetic trees were constructed by using Maximum Likelihood (ML) and Bayesian Inference (BI). ML analysis was generated by IQ-TREE v.1.6.8 (Nguyen et al. 2015). BI analysis was generated by using MrBayes v 3.2.6 under the Akaike information criterion (AIC) (Ronquist et al. 2012). The best substitution model of TPM1uf was defined by jModeltest2 2.1.6 (Darriba et al. 2012).

Posterior Probability (PP) was obtained from Metropolis-coupled Markov Chain Monte Carlo (mcmc nruns = 2; ngen = 10,000,000; printfreq = 1,000; samplefreq = 1,000; nchains = 4; 25% burn-in).

Table 1. List of 24 bamboo taxa sampled in the present study with related voucher and GenBank accession information.

Taxon	Voucher information	Accession number
Ingroup		
Acidosasa purpurea (Hsueh & T.P. Yi) Keng f.	Zhang08023 (KUN)	HQ337793
Ampelocalamus actinotrichus (Merr. & Chun) S.L. Chen, T.H. Wen & G.Y. Sheng	MPF10003 (KUN)	MF066245
Chimonobambusa angustifolia C.D. Chu & C.S. Chao	Wu20210053 (YAFG)	OK040768
Chimonobambusa damingshanensis Hsueh & W. P. Zhang	WM241225 (NF)	PV021571
Chimonobambusa hejiangensis C.D. Chu & C.S. Chao	GACP (NMGU)	MT884004
Chimonobambusa purpurea Hsueh & T.P. Yi	LW20200602-01 (CAAF)	MW030500
Chimonobambusa quadrangularis (Fenzi) Makino	CIMPC-RFM-20210302 (CMPC)	MW928533
Chimonobambusa sangzhiensis (B.M. Yang) N.H. Xia & Z.Y. Niu	NZY109 (IBSC)	OM867788
Chimonobambusa tumidissinoda Ohrnb.	MPF10083 (KUN)	MF066244
Chimonobambusa utilis (Keng) Keng f.	Not provided by the author	OK040769
Hsuehochloa calcareus (C.D. Chu & C.S. Chao) D.Z. Li & Y.X. Zhang	MPF10050 (KUN)	KJ496369
Indocalamus sinicus (Hance) Nakai	ZMY037 (KUN)	MF066250
Indosasa crassiflora McClure	BH58 (IBSC)	OK558536
Indosasa shibataeoides McClure	MPF10028 (KUN)	MF066251
Oligostachyum shiuyingianum (L.C. Chia & But) G.H. Ye & Z.P. Wang	DZL09122 (KUN)	JX513423
Pleioblastus amarus (Keng) Keng f.	Zhang Yu-QuC373 (SANU)	MH988736
Pleioblastus maculatus (McClure) C.D.Chu & C.S.Chao	MPF10161 (KUN)	JX513424
Sasa veitchii Rehder	LC1325 (ISC)	KU569975
Sinosasa fanjingshanensis N.H. Xia, Q.M.Qin & J.B. Ni	BH124 (IBSC)	OP850348
Sinosasa gracilis B.M.Yang	LX153 (IBSC)	OP973764
Sinosasa guangxiensis (C.D.Chu & C.S.Chao) N.H. Xia, Q.M. Qin & X.R. Zheng	CZY173 (IBSC)	OP850352
Sinosasa longiligulata (McClure) N.H. Xia, Q.M. Qin & J.B. Ni	CZY163 (IBSC)	OP850351
Outgroup		
Bambusa emeiensis L.C. Chia & H.L. Fung	Zhang08019 (KUN)	HQ337797
Bambusa sinospinosa McClure	Li043 (KUN)	MK679807

Results

The chloroplast genome size of *Chimonobambusa damingshanensis* is 139,964 bp and those of all the samples ranged from 139,394 bp (*Bambusa multiplex* L. C. Chia & H. L. Fung) to 140,013 bp (*Sinosasa gracilis*) with an alignment of 159,910 bp. The phylogenetic tree topology generated by ML and BI analyses was somewhat congruent, differing only in the support values of the nodes, so only the ML tree was shown with nodal support values from both methods labelled (Fig. 1). As shown in the majority-rule consensus tree, *Chimonobambusa damingshanensis* is distantly related to other *Chimonobambusa* species, but forms a monophyletic clade with four *Sinosasa* species with nodal support (BS = 77% and PP = 1.00).

Morphologically, *Chimonobambusa damingshanensis* resembles *Sinosasa huapingensis* N.H.Xia, Q.M. Qin & Y.H. Tong and *Sinosasa mingyueshanensis* N.H.Xia, Q.M.Qin & X.R. Zheng in having branches solitary, culm leaf auricles absent, foliage leaf auricles and oral setae present (Figs 2, 3). However, it can be easily distinguished from the latter two species by the morphological characters (Table 2).

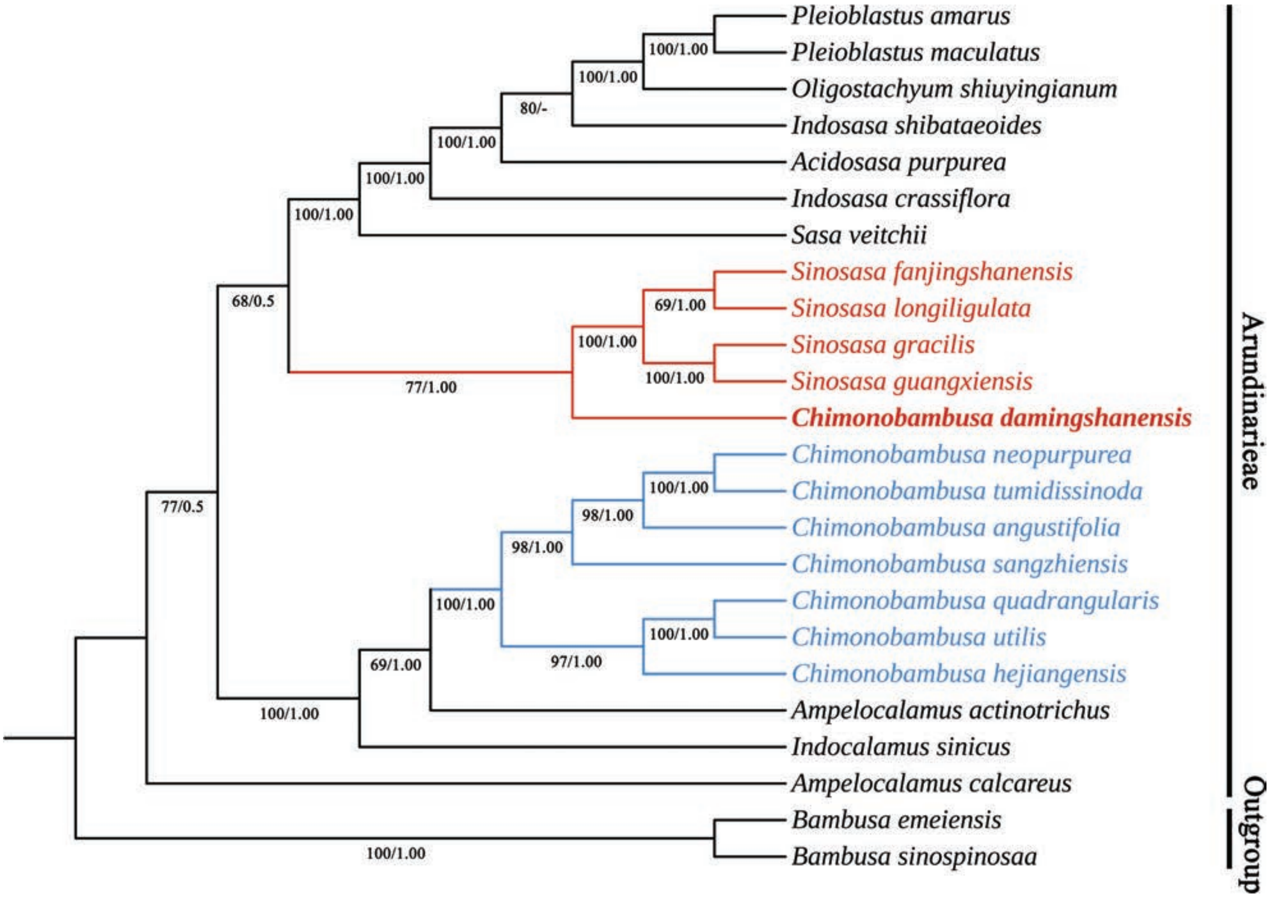

Figure 1. Phylogenetic relationships among *Chimonobambusa damingshanensis* and other 21species of Arundinarieae, based on plastid genome dataset with Maximum Likelihood and Bayesian analysis. Numbers above branches indicate maximum likelihood bootstrap support (BS) and posterior probabilities (PP), respectively.

Table 2. Morphological comparison of *Chimonobambusa damingshanensis* and two related species.

Morphology	*Ch. damingshanensis*	*S. huapingensis*	*S. mingyueshanensis*
Young culm internodes	white pubescent gradually glabrous	sparsely brown hirsute gradually deciduous	upper part initially densely brown strigose, gradually glabrescent
Sheath blades	Triangularly subulate	Lanceolate	Lanceolate
Foliage leaf sheaths	Glabrous	Puberulent	Glabrous
Oral setae	4–8, 2.5–6 mm	2–4, 2–6 mm	4–6, 10–20 mm
Number of leaves on ultimate branch	5–10	8–17	6–7
Number of secondary veins of blades	3–4 (6) pairs	5–7 pairs	6–7 pairs
blades both surfaces	glabrous	adaxially glabrous, abaxially puberulent	glabrous

Discussion

Our phylogenetic analysis demonstrated that *Sinosasa* is monophyletic (Fig. 1), consistent with the previous studies (Zeng et al. 2010; Guo et al. 2021). *Chimonobambusa damingshanensis* are described in the protologue as having 2 stigmas, and are observed in the type specimens as having true spikelets and 3 stamens. Molecular evidence from the plastid and morphological evidence further confirmed that *Chimonobambusa damingshanensis* should be a member of *Sinosasa* rather than *Chimonobambusa*, and thus a new combination in *Sinosasa* is proposed.

Taxonomic treatment

***Sinosasa damingshanensis* (Hsueh & W.P.Zhang) N.H.Xia & Y.L.Ding, comb. nov.**
urn:lsid:ipni.org:names:77359971-1
Figs 2–4

Basionym. *Chimonobambusa damingshanensis* Hsueh & W. P. Zhang, Bamb. Res. 7(3): 5. (1988).

Lectotype (here designated). China • Guangxi, Nanning, Wuming, Daming Mountain, 12 Nov. 1986, *C. J. Hsueh 8605 fl.* (Lectotype: SWFC!).

Revised description. Shrubby bamboos. Rhizomes leptomorph, rhizome internodes cylindrical, 2.45–4.75 cm long, nearly solid; nodes prominent, 2–4 roots at each node; rhizome bud ovate, ca. 4 mm high. Culms 1.5–2 m tall, 5–8 mm in diam; internodes terete, 5.4–13 cm long, initially with white pubescent gradually glabrous; supranodal ridge conspicuous, intranodes glabrous, 3–4 mm tall, infranodal region with a creamy-yellow and sericeous ring; branches solitary at each branching node. Culm bud solitary, triangular-ovate, sunken into supranodal ridge. Culm leaf sheaths persistent or tardily deciduous, papery, shorter than or as long as internode, abaxially glabrous or sparsely strigose, 6.8–13.3 cm high; sheath scar flat or slightly prominent; auricles and oral setae absent; blades erect, triangularly subulate, 1.6–4.5 mm high, glabrous. Foliage leaves 5–10 per ultimate branch; foliage leaf sheaths glabrous, purple or purple-green; auricles linear, 1–2 mm wide; oral setae erect or curled, 4–8, pale yellow, 2.5–6 mm long, easily deciduous when old; ligules developed, (3.3) 6–10 (–16) mm high, papery. Blades lanceolate, papery, 12.5–31.7 × 1.0–4.9 cm, both surfaces glabrous, margins serrulate along both sides, secondary veins 3–4 (6) pairs, significantly elevated on the lower leaf surface. The unit of the inflorescence raceme-like; lemma papery, ovate-lanceolate, ca. 10 mm long, glabrous; palea shorter than lemma, 6–7 mm long, 2-keeled; 2 stigmas, 3 stamens; ovary ellipsoid; style short. Fruit unknown.

Notes. *Chimonobambusa damingshanensis* Hsueh & W. P. Zhang was originally described based on *C.J. Hsueh 8605* which contains multiple specimens. According to the ICN (Turland et al. 2018) Art. 8.1, “The type (holotype, lectotype, or neotype) of a name of a species or infraspecific taxon is either a single specimen conserved in one herbarium or other collection or institution, or a published or unpublished illustration”, *C.J. Hsueh 8605 fl.* (SWFC, floriferous specimen) is designated here as the lectotype of *Ch. damingshanensis*. It has flower specimens and is more complete.

Phenology. New shoots produced during April to May.

Chinese name. 大明山华赤竹(Chinese pronunciation: dà míng shān huá chì zhú).

Distribution and habitat. Broad-leaved forests; It is endemic to Daming Mountain in Wuming District, Guangxi, China.

Additional specimen examined. *Sinosasa damingshanensis*: China • Guangxi: Nanning, Wuming, Daming Mountain, 23°29'43.9"N, 108°26'14.0"E, alt. 1224–1445 m a.s.l., 25 December 2024, *Y. L. Ding & Y. T. Zhang WM241225* (NF!).

Figure 2. *Chimonobambusa damingshanensis* Hsueh & W. P. Zhang. **A**–**D** *C.J. Hsueh 8605* (SWFC!). Photo by Yi-Ting Zhang.

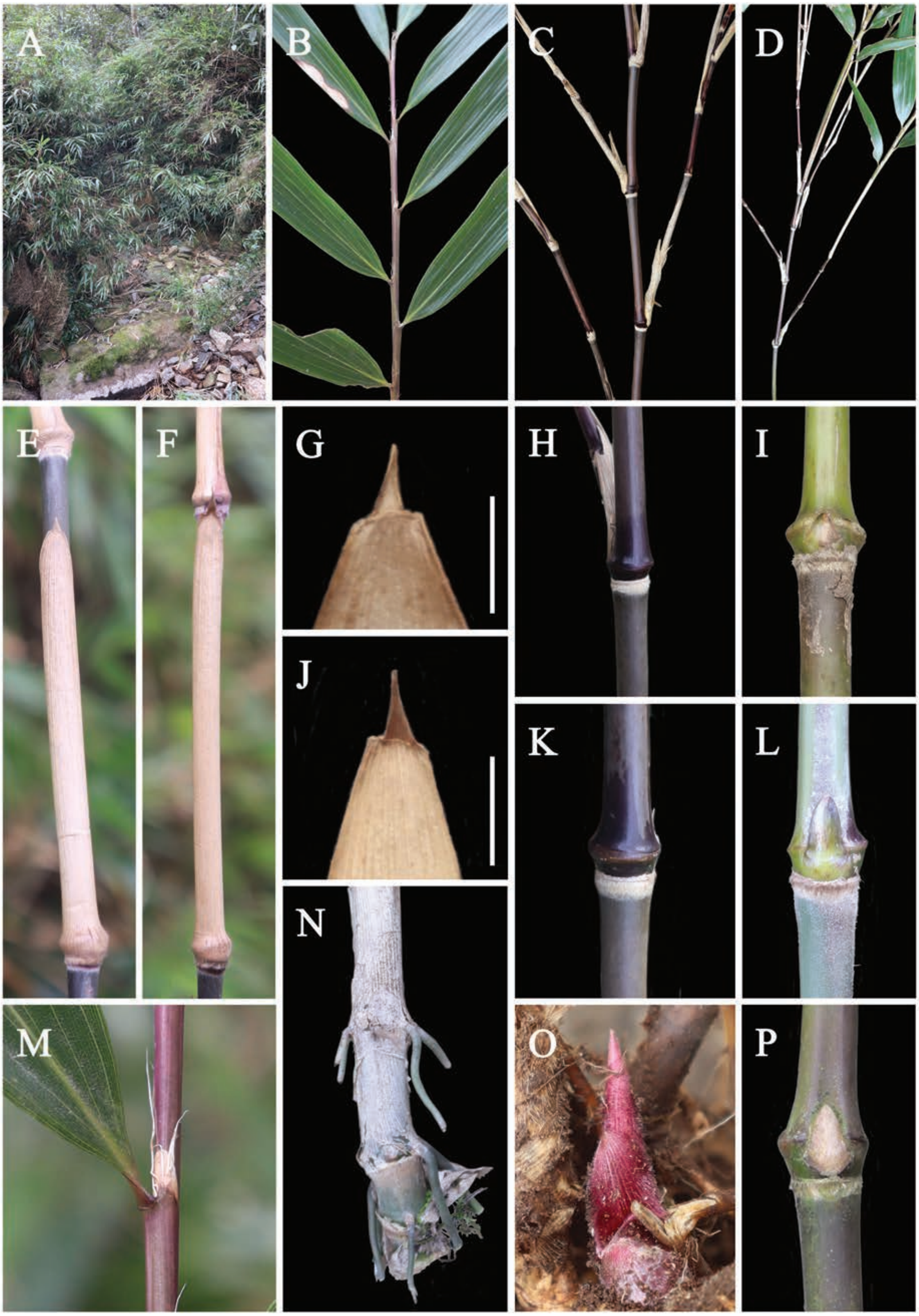

Figure 3. *Sinosasa damingshanensis* **A** habitat and plants **B** foliage leaf branches **C** solitary branch **D** leafy branchlet and culm **E, F** culm and culm sheath **G** adaxial view of sheath blade **H** branch complements with a solitary primary axis **I, L, P** culm bud **J** abaxial view of sheath blade **K** culm node **M** leaf sheath, ligule and oral setae **N** basal culm nodes with root thorns (uncommon) **O** development of bamboo shoot under the ground. Scale bars: 1 cm (**G, J**).

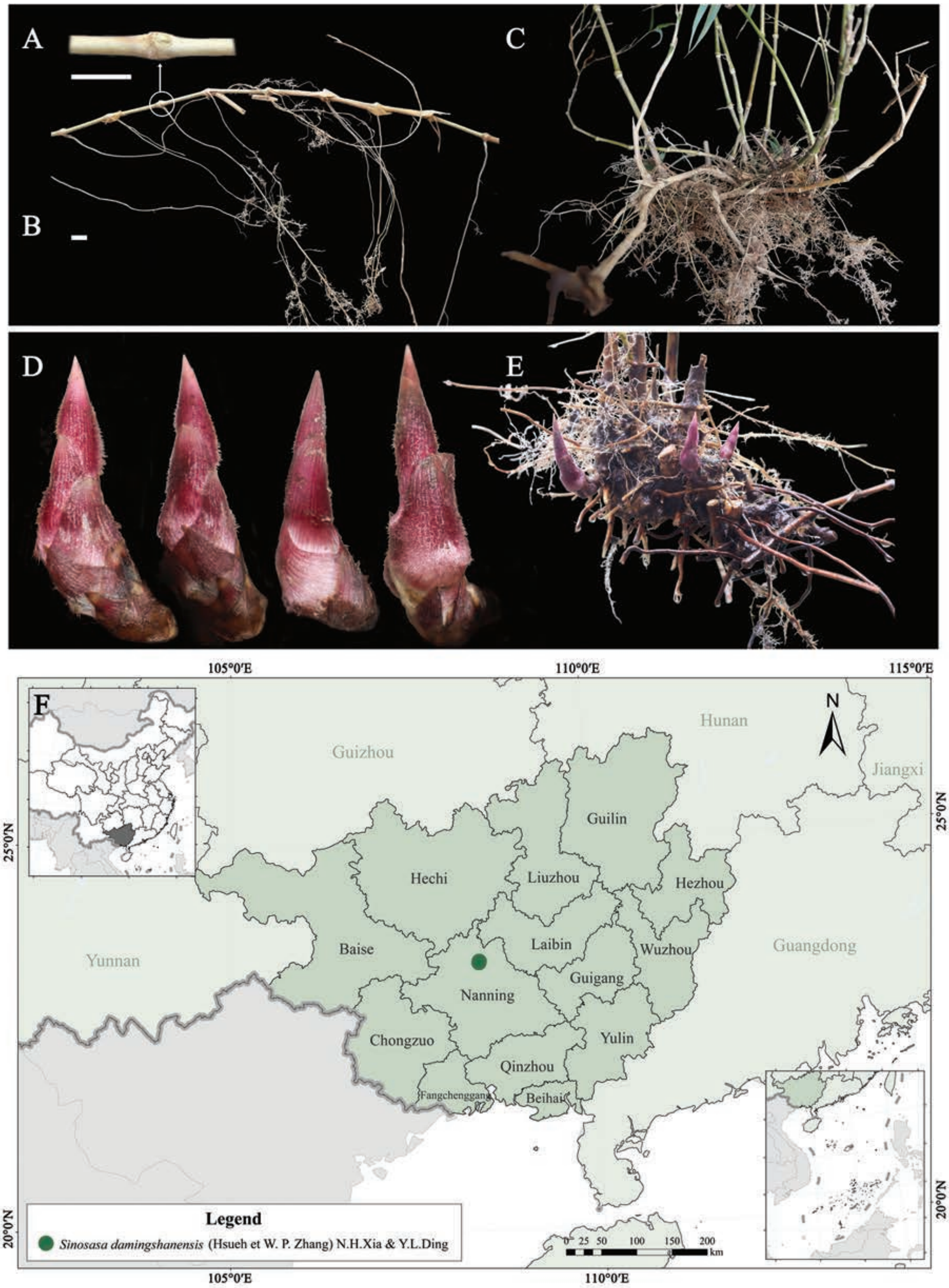

Figure 4. *Sinosasa damingshanensis* **A** rhizome bud **B, C, E** rhizome **D** development of bamboo shoot under the ground **F** distribution map of *Sinosasa damingshanensis* in Nanning, Guangxi, China. Scale bars: 1 cm (**A, B**).

Acknowledgments

We are very grateful to Min Zhang from National Nature Reserve of Daming Mountain for assisting with sample collection during fieldwork.

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 Key research projects of Yibin, Research and Integrated Demonstration of Key Technologies for Smart Bamboo Industry (YBZD2024-1), the Strategic Research and Consulting Project of China Academy of Engineering (2024-XZ-49).

Author contributions

Yu-Long Ding, Nian-He Xia, Shu-Yan Lin, Wei-Xin Jiang, and Yi-Ting Zhang conducted fieldwork and specimen identification. Yi-Ting Zhang and Zheng-Yang Niu performed the molecular analyses and phylogenetic reconstruction. Both authors contributed equally to writing the manuscript.

Author ORCIDs

Yi-Ting Zhang https://orcid.org/0000-0002-7068-9830
Shu-Yan Lin https://orcid.org/0000-0001-6326-2516
Zheng-Yang Niu https://orcid.org/0000-0003-0281-1504
Wei-Xin Jiang https://orcid.org/0000-0001-6447-8087
Nian-He Xia https://orcid.org/0000-0001-9852-7393

Data availability

All of the data that support the findings of this study are available in the main text. The newly generated sequences were deposited in the GenBank database (accession numbers PV021571).

References

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

Guo C, Ma PF, Yang GQ, Ye XY, Guo Y, Liu JX, Liu YL, Eaton DAR, Guo ZH, Li DZ (2021) Parallel ddRAD and genome skimming analyses reveal a radiative and reticulate evolutionary history of the temperate bamboos. Systematic Biology 70(4): 756–773. https://doi.org/10.1093/sysbio/syaa076

Hsueh CJ, Wang ZP (1996) *Chimonobambusa*. In: Keng PC, Wang ZP (Eds) Flora Reipublicae Popularis Sinicae (Vol. 9). Science Press, Beijing, 324–344.

Hsueh CJ, Zhang WP (1988) A Taxonomical Study of *Chimonobambusa* Makino, China. Bamboo Research 7(3): 5–7.

Jin JJ, Yu WB, Yang JB, Song Y, Yi TS, Li DZ (2018) GetOrganelle: a simple and fast pipeline for de novo assembly of a complete circular chloroplast genome using genome skimming data. bioRxiv 256479. https://doi.org/10.1101/256479

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

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford, England) 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Li DZ, Stapleton CMA (2006) *Chimonobambusa* Makino. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, vol. 22. Science Press, Beijing, 152–161.

Li X, Ni JB, Cai ZY, Tong YH, Xia NH (2023) *Sinosasa gracilis* (Poaceae, Bambusoideae), a new combination supported by morphological and phylogenetic evidence. PhytoKeys 226: 53–63. https://doi.org/10.3897/phytokeys.226.101164

Makino T, Shibata K (1901) On Sasa, a new genus of Bambuseae, and its affinities. Botanical Magazine Tokyo 15(168): 18–31. https://doi.org/10.15281/jplantres1887.15.168_18

Maria SV, Lynn GC, John D, Rafaël G, William JB (2018) World Checklist of Bamboos and Rattans. Science Press, Beijing, 41.

Nguyen LT, Schmidt HA, Haeseler V, 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

Ohrnb D (1990) Gen. Chimonobambusa 42. The bamboo of the word: a preliminary study of the names and woody bamboos (Bambusoideae Nees v. Esenb.) documented in lists and maps. Langweid am Lech, 179 pp.

Qin QM, Tong YH, Zheng XR, Ni JB, Xia NH (2021) *Sinosasa* (Poaceae: Bambusoideae), a new genus from China. Taxon 70(1): 27–47. https://doi.org/10.1002/tax.12422

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna 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

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 [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

Wick RR, Schultz MB, Justin Z, Holt KE (2015) Bandage: Interactive visualization of de novo genome assemblies. Bioinformatics (Oxford, England) 31(20): 3350–3352. https://doi.org/10.1093/bioinformatics/btv383

Yi TP, Shi JY, Ma LS, Wang HT, Yang L (2008) Illustrated Flora of Bambusoideae in China. Science Press, Beijing, 268.

Yi TP, Ma LS, Shi JY, Wang HT, Yang L, Liu L (2009) Bamboo Subfamily Tribe and Genus Identification Key. Science Press, Beijing, 76.

Zeng CX, Zhang YX, Triplettd JK, Yang JB, Li DZ (2010) Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of molecular divergence. Molecular Phylogenetics and Evolution 56(2): 821–839. https://doi.org/10.1016/j.ympev.2010.03.041

Zhu SL, Ma NX, Fu MY (1994) Iconographia bambusoidearum sinicarum. China Forestry Publishing House, Beijing, 158 pp.

PhytoKeys 255: 51–73 (2025)
DOI: 10.3897/phytokeys.255.140805

Research Article

Morphological and phylogenetic analysis approach to three new species and a new section of *Astragalus* (Fabaceae) from Mongolia

Dariganga Munkhtulga[1], Shukherdorj Baasanmunkh[2], Nudkhuu Nyamgerel[2], Jong Ho Park[2], Zagarjav Tsegmed[2], Komiljon Sh. Tojibaev[3], Hyeok Jae Choi[2]

1 *Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar 14201, Mongolia*
2 *Department of Biology and Chemistry, Changwon National University, Changwon 51140, Korea*
3 *Institute of Botany, Academy of Sciences of Uzbekistan, Tashkent 100125, Uzbekistan*
Corresponding authors: Shukherdorj Baasanmunkh (baasanmunkh.sh@gmail.com); Hyeok Jae Choi (hjchoi1975@changwon.ac.kr)

Abstract

Astragalus L. is the largest genus worldwide, comprising more than 3,100 species belonging to 250 sections. In Mongolia, approximately 130 species, including 15 endemic and 25 subendemic species have been previously recognized from 42 sections and 6 subgenera. In this study, we investigated several species within section *Laguropsis* in Mongolia based on extensive morphological analyses and molecular evidence. Based on these results, we describe three new species and a new section. Two of the newly described species, *A. oyunicus* and *A. teshigicus*, belong to the section *Laguropsis*, whereas the remaining species, *A. uvsicus*, is the type species of the new section *Uvsicus*. Furthermore, our findings revealed that (i) *A. tamiricus*, previously considered endemic to Mongolia, is an additional synonym of *A. laguroides*, and (ii) *A. gobi-altaicus*, previously a synonym of *A. laguroides*, is an independent species. Finally, we provide taxonomic nomenclature, morphological observations, distribution maps and wild photo illustrations of each species.

Key words: *Astragalus*, DNA barcoding, flora of Mongolia, new section, new species

Academic editor: Stephen Boatwright
Received: 8 November 2024
Accepted: 20 March 2025
Published: 8 April 2025

Citation: Munkhtulga D, Baasanmunkh S, Nyamgerel N, Park JH, Tsegmed Z, Tojibaev KSh, Choi HJ (2025) Morphological and phylogenetic analysis approach to three new species and a new section of *Astragalus* (Fabaceae) from Mongolia. PhytoKeys 255: 51–73. https://doi.org/10.3897/phytokeys.255.140805

Introduction

Astragalus L., which belongs to the Fabaceae family, is one of the largest genera of angiosperms, with more than 3,100 species belonging to 250 sections (Podlech and Zarre 2013; POWO 2024). This genus is widely distributed in Asian countries (Maassoumi and Ashouri 2022), particularly in Iran with 850 species followed by 400 in China (Xu and Podlech 2010), 320 in Kazakhstan (Abdulina 1998; Perezhogin et al. 2023), 273 in Uzbekistan (Tojibaev et al. 2015; Sennikov et al. 2016), 270–280 in Kyrgyzstan (Sytin and Lazkov 2018), 150 in Pakistan (Khan et al. 2023), and 90 in Siberia (Vydrina 2006). A recently published checklist of the Tian Shan flora contains at least 222 *Astragalus* species, 82 of which are endemic (Sennikov and Tojibaev 2021). A distinctive feature of the flora of the Mediterranean, Iran, and Central Asia is the rich diversity of species of the genus *Astragalus*. This genus occupies first place in the spectrum of leading genera in the Iran-Turanian flora (Tojibaev et al. 2015). Central Asia is home to at least 650 *Astragalus* species. Morphologically relatively homogeneous and diverging no earlier than the Late Miocene, it comprises some of the highest diversification rates documented thus far in angiosperms (Folk et al. 2024). Taxonomic identification of *Astragalus* is difficult and complicated worldwide. However, a number of new species have been discovered based on morphology (Sytin and Lazkov 2018; Yang et al. 2024) and in combination with morphology and molecular pieces of evidence (Gao et al. 2009; Bagheri et al. 2016, 2022; Erkul et al. 2022). In addition, researchers have studied the seed testa morphology of selected *Astragalus* species to explore their taxonomic significance (Shemetova et al. 2018; Kashyap et al. 2021).

Recently, numerous studies have conducted molecular analyses, such as DNA barcoding, including that on the internal transcribed spacer (ITS), two chloroplast regions (*matK* and *rbcL*) (Gao et al. 2009; Zhang and Jiang 2020; Bagheri et al. 2022, 2023; Erkul et al. 2022; Baasanmunkh et al. 2024a) and comparative complete plastomes (Tian et al. 2021; Moghaddam et al. 2023). According to their research, ITS markers have been successfully identified in most *Astragalus* species, outperforming chloroplast regions (*matK* and *rbcL*) in terms of recognition (Bagheri et al. 2016; Zhang and Jiang 2020; Baasanmunkh et al. 2024a).

In Mongolia, 127 *Astragalus* species belonging to 42 sections and 6 subgenera were recognized (Ulziykhutag 1989, 2003, 2004; Baasanmunkh et al. 2022). Additionally, three new records of *Astragalus* were recently found in the Mongolian flora based on morphological and molecular evidence (Baasanmunkh et al. 2024a, b). In general, *Astragalus* exhibits relatively high endemism in Mongolia compared to other major genera (Baasanmunkh et al. 2021). In particular, 15 and 23 species are currently endemic and subendemic to the country (Baasanmunkh et al. 2021, 2022), respectively.

The section *Laguropsis* Bunge comprises approximately 44 species distributed in Central Asian countries and Russia (Siberia) (Podlech and Zarre 2013; Tojibaev et al. 2015). In the past, several new species of the section *Laguropsis* were discovered in Mongolia, including *A. gobi-altaicus* N.Ulziykh (Ulziykhutag 1990) and *A. tamiricus* N.Ulziykh. (in Biazrov et al. 1989). Recently, two new species, *A. admirabilus* Pyak & E.Pyak and *A. liuaiminii* Z.Z.Yang & Q.R.Liu, were described from the Altai Mountains (Russia and Mongolia) (Pyak and Pyak 2019) and Xinjiang (China) (Yang et al. 2024), respectively. In Mongolia, 17 species from the section *Laguropsis* were reported, including two endemic species (Baasanmunkh et al. 2021; 2022). Among these, *A. gobi-altaicus*, endemic to Mongolia, has been treated as a synonym for *A. laguroides* Pall. by Xu and Podlech (2010).

In the present study, we focused on the *A. laguroides* complex, the most closely related species within the section *Laguropsis*. Based on our results, we describe three new species and one new section of *Astragalus* from Mongolia based on extensive morphological analysis and nrDNA barcoding.

Materials and methods

Taxon sampling

We have been conducting field surveys since 2017 to collect fresh samples of *Astragalus*, including detailed wild photographs and leaves across Mongolia. We collected more than 500 herbarium specimens of *Astragalus* that were deposited in the herbaria of the National University of Mongolia (UBU). In addition, we examined herbarium specimens from the following herbaria: ALTB, LE, MW, NS, GFW, TASH, UBA, UBU (Thiers 2023) and FloraGREIF (https://floragreif.uni-greifswald.de). A point distribution map was produced based on the herbarium specimens using ArcGIS (Esri 2012).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel-dried leaves using the CTAB method (Doyle and Doyle 1987). A total of 25 samples were extracted from eight species. The nuclear ITS region (White et al. 1990) was used for amplification and sequencing. PCR was performed as previously described (Baasanmunkh et al. 2024a). PCR products were sequenced in both directions by Macrogen (Seoul, Korea). DNA sequences were visually checked and manually trimmed in Geneious Prime 2024.0.7 (www.geneious.com). Automatic alignment of the trimmed sequences using the ClustalW (Thompson et al. 2002) algorithm and construction of a consensus dataset were performed using BioEdit Sequence Alignment Editor v.7.2.5 (Hall et al. 2011). The DNA sequences generated in this study have been deposited in GenBank (www.ncbi.nlm.nih.gov).

Phylogenetic analysis

The constructed ITS dataset included 20 *Astragalus* species, including 8 newly sequenced *Astragalus* species from Mongolia, with previously sequenced species from Zhang and Jiang (2020) and Baasanmunkh et al. (2024a). *Phyllolobium balfourianum* (N.D.Simpson) M.L.Zhang & Podlech was selected as an outgroup according to Zhang and Jiang (2020). Detailed information on the sample taxa, GenBank accession numbers, and references for each sample are provided in Table 1. Phylogenetic analyses were conducted using maximum parsimony (MP) methods in RAxML v.8.2.11 (Stamatakis 2014) as implemented in Geneious, with the best-scoring maximum likelihood (ML) tree algorithm and 1000 bootstrap replicates. The reconstructed trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results and discussion

We partially revised the section *Laguropsis* belonging to the subgenus *Cercidothrix* in Mongolia based on morphological and molecular analyses in the present study. Here, we described three new species, *A. oyunicus*, *A. teshigicus*, and *A. uvsicus*, from eastern, northern, and western Mongolia. In addition, we described a new section, *Uvsicus*, based on *A. uvsicus*. Furthermore, the taxonomic status of some species, such as *A. tamiricus*, was previously endemic to Mongolia and an additional synonym of *A. laguroides*. In contrast, *A. gobi-altaicus* was synonymous with *A. laguroides*, an independent species from *A. laguroides*.

DNA barcoding

The aligned ITS region was 598 bp long with 174 variable characteristics, 21 of which were parsimony informative. The ITS1 sub-region included an 11 bp parsimony-informative site, which was slightly variable compared to the others. The phylogenetic tree showed that the ITS sequence data supported

Table 1. Detailed information on species, section, subgenus name, GenBank accession numbers, and references of the samples used in this study.

No	Species name	Specimen code	Section	Country	GenBank accession number	Reference
subgenus *Cercidothrix*						
1	*Astragalus dilutus*	UBU0004768	*Laguropsis*	Mongolia	PQ492291	this study
2	*A. dilutus*	UBU0003441	*Laguropsis*	Mongolia	PQ492292	this study
3	*A. dilutus*	UBU0032545	*Laguropsis*	Mongolia	PQ492293	this study
4	*A. dilutus*	UBU0042005	*Laguropsis*	Mongolia	PQ492294	this study
5	*A. dilutus*	UBU0042001	*Laguropsis*	Mongolia	PQ492295	this study
6	*A. dilutus*	UBU0036425	*Laguropsis*	Mongolia	PQ492296	this study
7	*A. dilutus*	UBU0042002	*Laguropsis*	Mongolia	PQ492297	this study
8	*A. teshigicus*	UBU0002138	*Laguropsis*	Mongolia	PQ492298	this study
9	*A. tamiricus*	UBU0039103	*Laguropsis*	Mongolia	PQ492299	this study
10	*A. tamiricus*	UBU0010820	*Laguropsis*	Mongolia	PQ492300	this study
11	*A. tamiricus*	UBU0040555	*Laguropsis*	Mongolia	PQ492301	this study
12	*A. laguroides*	UBU0026340	*Laguropsis*	Mongolia	PQ492302	this study
13	*A. laguroides*	UBU0026328	*Laguropsis*	Mongolia	PQ492303	this study
14	*A. laguroides*	UBU0006948	*Laguropsis*	Mongolia	PQ492304	this study
15	*A. laguroides*	UBU0039105	*Laguropsis*	Mongolia	PQ492305	this study
16	*A. gobi-altaicus*	UBU0012226	*Laguropsis*	Mongolia	PQ492314	this study
17	*A. gobi-altaicus*	UBU0030804	*Laguropsis*	Mongolia	PQ492315	this study
18	*A. ochrias*	UBU0006593	*Laguropsis*	Mongolia	PQ492306	this study
19	*A. lupulinus*	UBU0026235	*Laguropsis*	Mongolia	PQ492310	this study
20	*A. oyunicus*	UBU0034202	*Laguropsis*	Mongolia	PQ492311	this study
21	*A. oyunicus*	UBU0039102	*Laguropsis*	Mongolia	PQ492312	this study
22	*A. oyunicus*	UBU0014416	*Laguropsis*	Mongolia	PQ492313	this study
23	*A. uvsicus*	UBU0039100	*Uvsicus*	Mongolia	PQ492307	this study
24	*A. uvsicus*	UBU0039101	*Uvsicus*	Mongolia	PQ492308	this study
25	*A. uvsicus*	UBU0027685	*Uvsicus*	Mongolia	PQ492309	this study
26	*A. ammodytes*		*Ammodytes*	Mongolia	OR527932	Baasanmunkh et al. (2024a)
27	*A. gubanovii*		*Macrotrichoides*	Mongolia	OR527933	Baasanmunkh et al. (2024a)
28	*A. testiculatus*		*Mixiotricha*	Mongolia	OR527928	Baasanmunkh et al. (2024a)
29	*A. hypogaeus*		*Trachycercis*	Mongolia	OR527930	Baasanmunkh et al. (2024a)
30	*A. junatovii*		*Trachycercis*	Mongolia	OR527929	Baasanmunkh et al. (2024a)
31	*A. teskhemicus*		*Trachycercis*	Mongolia	OR527931	Baasanmunkh et al. (2024a)
subgenus *Hypoglottis*						
32	*A. danicus*		*Hypoglottis*	Russia	OQ106945	Unpublished
33	*A. laxmannii*		*Hypoglottis*	China	MT923540	Unpublished
34	*A. tibetanus*		*Hypoglottis*	Kazakhstan	OQ106946	Unpublished
subgenus *Astragalus*						
35	*A. bhotanensis*		*Brachycephali*	China	MF044289	Zhang and Jiang (2020)
36	*A. skythropos*		*Skythropos*	China	MF044266	Zhang and Jiang (2020)
37	*Phyllolobium balfourianum*			China	MF044295	Zhang and Jiang (2020)

the monophyletic status of the genus *Astragalus* with strong bootstrap support (Fig. 1). A newly sequenced *Astragalus* species from Mongolia nested within a species from the subgenus *Cercidothrix*. Molecular phylogenetic studies show that most *Astragalus* is monophyletic (Kang et al. 2003). Our phylogenetic results showed the same topology as in a previous study.

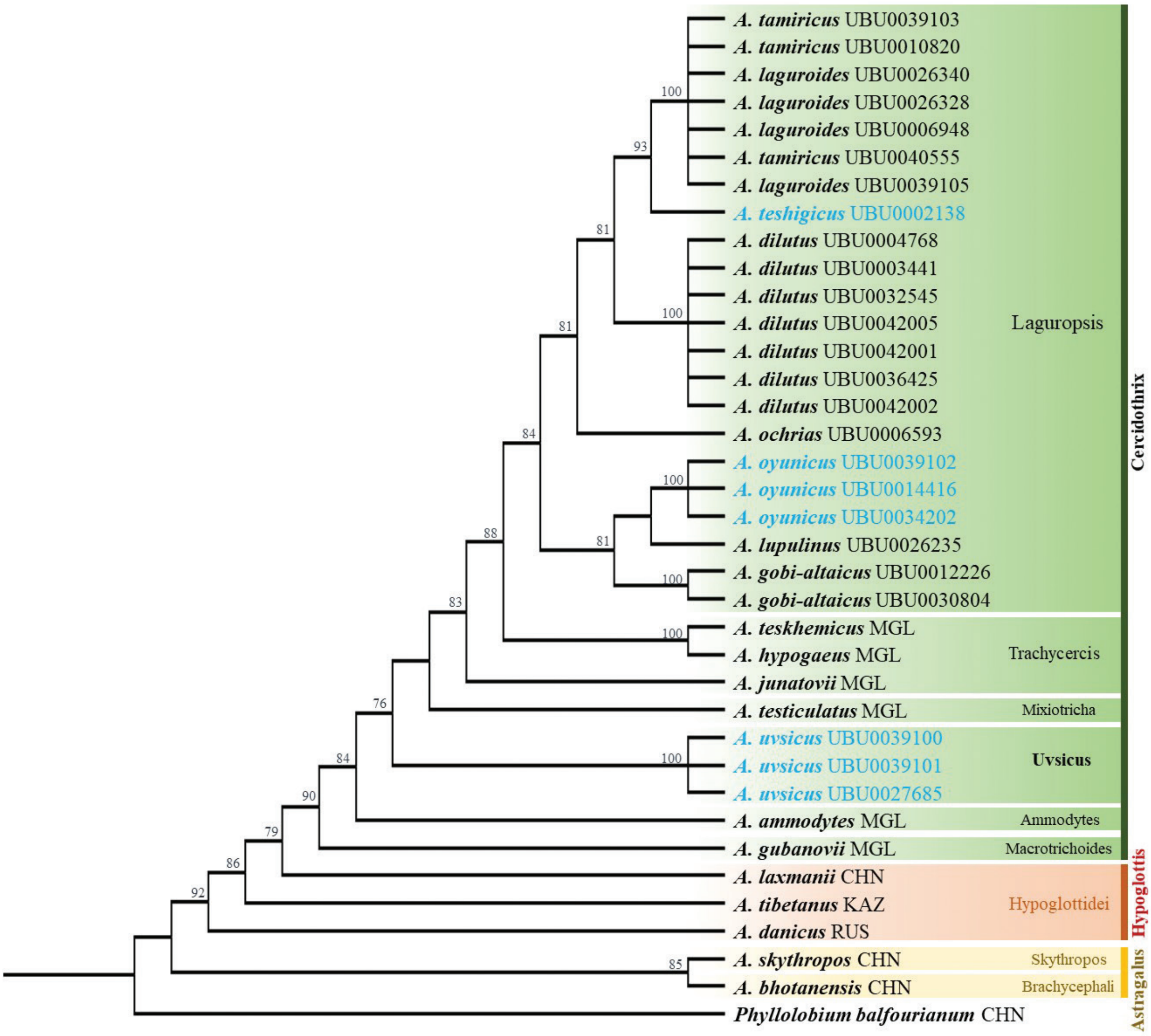

Figure 1. Phylogenetic tree of *Astragalus* species based on ITS sequences. Newly sequenced samples are indicated with UBU specimen code. The bootstrap support values above 70% are shown at branch level. CHN, MGL, KAZ, and RUS samples are from China, Mongolia, Kazakhstan, and Russia, respectively.

Taxonomic treatment

***Astragalus* subgenus *Cercidothrix* Bunge, section *Laguropsis* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 137 (1868). (Fig. 2)**

***Astragalus laguroides* Pall., Reise Russ. Reich. 3(2): App. 750 (1776).**
Fig. 2

≡ *Astragalus lagurus* Pall., Sp. Astragal.: 18 (1800).
≡ *Saccocalyx laguroides* (Pall.) Steven, Bull. Soc. Imp. Naturalistes Moscou 4: 269 (1832).
≡ *Tragacantha laguroides* (Pall.) Kuntze, Revis. Gen. Pl. 2: 945 (1891).
= *Astragalus tamiricus* N.Ulziykh. in L.G.Byazrov & al., Fl. Khangaya: 124 (1989). syn. nov. • Type. Mongolia. Khangai region: • Arkhangai province, Ikhtamir soum, Khoid Tamir river, monasterium Tzetza-Van, shrublands, 19 August 1926, *N. Pavlov* (Holotype LE01017820!, Paratype LE01017821!, Paratype LE01017822!, Isotype MW0593230!).

Type. Mongolia. circa Selenga river, *P.S. Pallas* [BM! (Fig. 6D)].

Diagnosis. *Astragalus laguroides* is a type species in the section *Laguropsis* (Podlech and Zarre 2013). This species is widely distributed in central and western Mongolia. According to the description of *A. tamiricus*, some morphological characteristics, such as racemes, leaflets, stipules, and flowers, including calyx, standard, keel, and wings, are slightly smaller than *A. laguroides* (Biazrov et al. 1989). However, our studied samples of *A. tamiricus*, collected from the type location in Arkhangai Province, clustered with *A. laguroides* based on our phylogenetic tree (Fig. 1). Therefore, we treated *A. tamiricus* as an additional synonym of *A. laguroides* in this study.

Description. Plants perennial 8–15(–20) cm tall, acaulescent or nearly so, in vegetative parts covered with medifixed, appressed white hairs. Stems, if present, up to 1 cm, angular-sulcate, densely hairy. Stipules 6–8(–10) mm, narrowly triangular-acuminate, adnate to the petiole for c. 3–4 mm, otherwise free from each other, covered with spreading or ascending hairs. Leaves 3–14(–18) cm; petiole 1.5–5(–8) cm, like the rachis rather densely to densely, more rarely loosely hairy. Leaflets in 3–5(–8) pairs, in the basal leaves often only in 1–2 pairs, narrowly elliptic to more rarely elliptic, 8–25(–40) × 3–6 mm, acute, on both sides rather densely appressed hairy. Peduncles 2–12 cm, rather densely covered with symmetrically to asymmetrically bifurcate, appressed to subappressed white hairs, toward the raceme sometimes also with some black hairs mixed in. Racemes ovoid, 2–4(–6) cm long, densely many-flowered. Bracts linear-acute, 4–8 mm, covered with asymmetrically bifurcate, at the margins with basifixed white hairs. Calyx at anthesis tubular, soon becoming ovoid-inflated, 11–14 mm, loosely to rather densely covered with asymmetrically bifurcate to basifixed, tangled, spreading white hairs 1–2 mm, at outer side of the teeth and sometimes at the nerves of the tube also with black hairs; teeth subulate, 3–4 mm. Petals violet. Standard 17–20 mm; blade 6–7 mm wide, narrowly obovate, apex emarginate. Wings slightly shorter than standard, ca. 14–18 mm long; blades narrowly oblong, rounded, 5.5–7 × 1.5–2 mm; auricle 1 mm, claw 9–10 mm. Keel 13–15 mm; blades 4–4.5 × 1.8–2.2 mm; claw 9–10 mm. Ovary sessile, narrowly ellipsoid. Pods enclosed in the calyx, sessile, oblong, (6) 7–8 × 2–3 mm long, keeled ventrally, slightly and widely grooved dorsally, with a hooked beak c. 1 mm, unilocular; covered with subappressed to spreading white hairs 1–1.5 mm, smaller black hairs amount to greater than the apex.

Distribution. China (Inner Mongolia, Xinjiang), Russia and Mongolia (Podlech and Zarre 2013) (Fig. 9).

Habitat. This species grows in debris, stony slopes, trails, pebble beds, rocky and stony screes.

Additional specimens examined. Mongolia. Khuvsgul region: • Selenge province, 13 km west from Tsagaannuur soum, 50°6'0"N, 105°21'0"E, 757 m, 12 June 2012, *D. N. Shaulo* (NS0010107) • Khangai region: Khangai, at the spring between Oit-beise and Lugan-kure, 47°48'13.17"N, 101°58'23.88"E, 1471 m, 11 July 1926, *N. Ikkonnikov-Galitzky* (LE01017821) • Arkhangai province, Tuvshruulekh soum, 47°26'43.6"N, 101°55'9.5"E, 1572 m, 20 June 1977, *N. P. Guricheva* (MW0183266, MW0183267) • Arkhangai province, Tsenkher soum, Urd Tamir river, 48°23'59.9"N, 97°9'4.9"E, 2118 m, 11 July 1978, *I. A. Gubanov* (MW0183264) • Bulgan province, Khishig-Undur soum, Teg river, 48°48'17.9"N, 103°31'49.4"E, 1209 m, 05 July 1974, *O. V. Jurba* (MW0183270) • Bulgan

Figure 2. *Astragalus laguroides* in Mongolia **A** general habit **B** raceme **C** flower **D** bracts **E** standard **F** wings **G** keel **H** pistil **I** stamens **J** pod **K** leaves **L** leaflet **M** omission of medifixed hairs on the upper side of the leaf **N** stipules. (Photo credits: S.Baasanmunkh and D.Munkhtulga).

province, Mogod soum, Nomgon mountain, 48°16'1.17"N, 102°55'44.67"E, 1706 m, 02 July 1980, *Sh. Dariimaa* (UBA) • Bulgan province, 9 km south east from Unit soum, 49°15'15"N, 102°56'41.6"E, 1238 m, 17 June 1964, *Khishgee* (UBA) • Bulgan province, Khangal soum, Burgastain davaa, 49°10'22.99"N, 104°28'38.27"E, 1433 m, 04 August 1978, *B. Mandakh & Sh. Dariimaa* (UBA). Arkhangai province, Khangai soum, 47°50'0.132"N, 99°21'6.876"E, 2135 m, 05 August 2020, *Sh. Baasanmunkh* (UBU0010817, UBU0032212) • Arkhangai province, Battsengel soum, 47°45'53.208"N, 101°56'38.904"E, 1420 m, 20 August 2020, *Sh. Baasanmunkh* (UBU0010820) • Arkhangai province, Ikh Tamir soum, Tamir river, 47°36'27.9"N, 101°8'17.3"E, 1420 m, 30 June 2023, *D. Munkhtulga* (UBU0039991–UBU0039998, UBU0040555, UBU0040556) • Arkhangai province, Ikh Tamir soum, Tamir river, 1666 m, 28 May 2024, *B. Oyuntsetseg, G. Bayarmaa & D. Munkhtulga* (UBU0039103, UBU0039104) • Dauria region: • Tuv province, 3 km northwest from Bornuur soum, 48°32'7.3"N, 106°8'1.5"E, 1208 m, 07 June 1973, *K. Kloss* (GFW45636) • Tuv province, Unjuul soum, Argal mountain, 47°59'8.22"N, 105°55'2.1"E, 1445 m, 19 July 1973, *Ch. Sanchir* (UBA) • Selenge province, Dulaankhan soum, Yruu river, Dulaankhan mountain, 49°56'3.1"N, 106°12'21.5"E, 750 m, 09 August 1982, *E. Ganbold* (UBA) • Mongolian Altai region: • Khovd province, Darvi soum, Sutai mountain, 46°23'59.1"N, 94°0'38.3"E, 1900 m, 18 August 1984, *I. A. Gubanov* (MW0183284) • Gobi-Altai province, Tsetseg soum, 46°19'3.817"N, 93°2'35.239"E, 1430 m, 12 July 2019, *B. Oyuntsetseg* (UBU0006948) • Gobi-Altai province, Tseel soum, Baga Tayan, 45°24'54"N, 95°52'4.8"E, 1893 m, 07 July 2019, *B.Oyuntsetseg*, *Sh.Baasanmunkh* (UBU0005920, UBU0005922) • Middle Khalkh region: • Avzaga mountain, 48°1'53.05"N, 103°14'40.64"E, 1917 m, 12 June 1971, *V. I. Grubov, N. Ulziikhutag & Sh. Dariimaa* (UBA) • Tuv province, 7 km south from Unjuul soum, 46°49'12"N, 105°30'36"E, 1552 m, 30 July 1973, *T. I. Kazantseva* (NS0025349) • same locality, 7 June 1974, *T. K. Gordeeva*, (NS0025347, NS0025348) • Tuv province, Buren soum, Ikher-Uhaa, 46°59'13.9"N, 105°3'28.1"E, 1377 m, 22 July 1976, *Tumurtogoo* (UBU0039987) • Depression of Great Lakes region: • Khovd province, Myangad soum, 48°15'7.898"N, 91°54'14"E, 1185 m, 26 August 1984, *I. A. Gubanov* (MW0183279) • Khovd province, Chandmani soum, Jargalant khairkhan mountain, 47°49'23.2"N, 92°24'15.1"E, 1836 m, 19 August 2003, *M. Schnittler* (GFW45638) • Khovd province, 5 km south west from Khar-Us Lake, 47°42'59.8"N, 91°58'59.902"E, 1459 m, *S. A. Dyachenko & P. A. Kosachev* (ALTB1100016497) • Valley of Lakes region: Bayankhongor province, 30 km west from Arvaikheer city, 46°3'50.4"N, 102°30'18"E, 2130 m, 1974, *Ts. Jamsran*, (UBU0026328) • Uvurkhangai province, Guchin-Us soum, 45°24'8.399"N, 102°14'55.298"E, 1575 m, 23 July 1978, *G. N. Ogureeva* (MW0183268) • Bayankhongor province, Bogd soum, 45°31'30"N, 101°6'28.8"E, 1569 m, 12 July 2019, *Batsaikhan* (UBU0006948) • Bayankhongor province, Bumbugur soum, 46°13'8.4"N, 99°6'43.2"E, 1994 m, 05 July 2019, *B. Oyuntsetseg, Sh. Baasanmunkh* (UBU0006680) • Bayankhongor province, Buutsagaan soum, 46°11'36.5"N, 99°10'1.5"E, 1880 m, 29 May 2024, *B. Oyuntsetseg, G. Bayarmaa & D. Munkhtulga* (UBU0039105–UBU0039112) • Gobi Altai region: • Bayankhongor province, Buutsagaan soum, south slope of Bogd mountain, 44°57'42.38"N, 100°12'10.31"E, 2725 m, 02 July 1977, *E. Ganbold* (UBA) • Bayankhongor province, Bogd soum, Orog lake, 44°59'19.1"N, 100°52'50.999"E, 1100 m, 16 July 2013, *R. Tungalag & Ts. Tsendeekhuu* (UBU0021615) • Trans-Altai Gobi region: • Bayankhongor province, Shinejinst soum, Djinst mountain, Altai tsagaan khaalga, 44°25'37.582"N, 99°19'6.24"E, 2190 m, 07 September 1979, *Ch. Sanchir* (UBA) • Govi-Altai province Tseel soum, Aj Bogd mountain, Ar zuslan river, 44°41'12.221"N, 95°14'9.481"E, 2400 m, 09 August 1984, *Kh. Buyan-Orshikh & Yu. G. Evtipeev* (UBA) • Bayankhongor province, Shinejinst soum, Djinst mountain, 44°26'28.4"N; 99°13'36"E, 2000 m, 14 July 1979, *I. A. Gubanov* (MW0593124, MW0593125).

Astragalus gobi-altaicus **N.Ulzyikh., Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol., n.s., 95(2): 83 (1990).**
Fig. 3

Diagnosis. *Astragalus gobi-altaicus* was first described in East Gobi, Mongolia (Ulziykhutag 1990). Later, this species was treated as a synonym of *A. laguroides* by Xu and Podlech (2010). However, based on our extensive morphological studies, *A. gobi-altaicus* can be distinguished from *A. laguroides* by its inflorescence being oblong-cylindrical (vs. globose or ovate), leaflets that are broadly elliptical or oblong-obovate, more rarely sub-oval (vs. leaflets oblong, narrowly elliptical, or lanceolate), calyx teeth 4–6 mm long (vs. calyx teeth 3–4) (Table 2). In addition, the phylogenetic tree supported that *A. gobi-altaicus* was different from *A. laguroides*, which was more similar to *A. oyunicus* and *A. lupulinus* (Fig. 1).

Type. Mongolia. Umnugovi province, Noyon soum, Noyon-Bogd mountain, 08 September 1979, *V. I. Grubov, A. Muldaschev & Sh. Darijmaa 1964* [Holotype LE01016096! (Fig. 6F)] • Umnugovi province, Nomgon soum, Khurkh mountain, 42°43'16.6"N, 105°06'17.4"E, 1425 m, 28 June 1980, *I. A. Gubanov 5941* [Paratype MW0593122!] • Umnugovi province, Khurmen soum, Bayan-Undur, 42°33'18.1"N, 103°54'38.1"E, 1413 m, 06 August 1981, *I. A. Gubanov 3376* [Paratype MW0593121!, MW0593120!].

Description. Plants perennial 8–12 cm tall, acaulescent, with merely white, in vegetative parts distinctly warty hairs. Stipules whitish, 3–4 mm, triangular, nearly free from the petiole, not connate behind the stem, appressed hairy, at the margins with basifixed hairs. Leaves (–3)8–14 cm; petiole 1–4 cm, like the rachis slender, loosely to rather densely covered with medifixed, appressed hairs 0.5–1 mm. Leaflets in 2–4 pairs, in the basal leaves often in 1–2 pairs only, oblanceolate or narrowly elliptic to elliptic, 7–18 × 5–10 mm, mucronate, on both sides densely covered with medifixed, appressed hairs 1.5–2(–3) mm. Peduncles 3–6 cm, slightly angular-sulcate, loosely to rather densely covered with medifixed, appressed hairs, partly glabrescent with age. Raceme oblong to oblong-cylindrical 3–8 cm long, densely many-flowered. Bracts whitish, 3–5 mm, narrowly linear, covered with mostly basifixed hairs. Calyx 11–14 mm, tubular at beginning of anthesis, soon ovoid-inflated, rather densely covered with subbasifixed to basifixed, rigid, spreading, straight white hairs 2–3 mm; teeth filiform, 4–5 mm. Standard (–9)14–18(–20) mm; blade c. 5 mm wide, elliptic, narrowly triangular toward the slightly emarginate tip, slightly constricted below the middle, at the base obtusely angularly passing into the claw. Wings 12–15 mm; blades narrowly oblong, obtuse, c. 5.5 × 1.5 mm; auricle 1 mm, claw 8 mm. Keel 10–12 mm; blades obliquely obovate 3.5 × 2 mm; claw c. 8 mm. Ovary sessile. Pods enclosed in the calyx, sessile, oblong, 7–8 × 3–4 mm long, with a hooked beak c. 2–2.5 mm, unilocular; covered with tangled, spreading only white hairs.

Distribution. Endemic to Mongolia. This species is currently known from the South Gobi in Mongolia (Fig. 9).

Habitat. This species grows in debris desert steppe, mountain slopes, sandy desert steppes, sayr slopes and granite slopes of hillocky areas.

Additional specimens examined. Mongolia, Gobi Altai region: • Umnugovi province, Tsogt-Tsetsii soum, Tsetsii mountain, 43°30'48.38"N, 105°41'18.85"E,

Figure 3. *Astragalus gobi-altaicus* in Mongolia **A** general habit **B** raceme **C** flower **D** calyx **E** bracts **F** standard **G** wings **H** keel **I** pistil **J** stamens **K** pod **L** pod valve **M** leave, abaxial view **N** leave, adaxial view **P** leaflet, abaxial view **O** leaflet, adaxial view **Q** omission of medifixed hairs on the upper side of the leaf **R** stipules, outside view **S** stipules, inside view. (Photo credits: D.Munkhulga and B.Oyuntsetseg).

1727 m, 28 May 1962 (UBA) • Umnugovi province, Nomgon soum, Baruun tsohiotiin Khudag, 42°41'14.1"N, 105°13'8.5"E, 1463 m, 17 May 1962 (UBA) • Umnugovi province, Bulgan soum, Dundsaikhan mountain, Khaalgiin sair, 43°44'16.53"N, 103°34'10.88"E, 2229 m, 25 June 1962 (UBA) • Umnugovi province, 20 km southeast from Khurmen soum, 43°5'55.7"N, 104°11'39.4"E, 1631 m, 06 July 1973, *Ts. Jamsran* (UBU0026387) • Umnugovi province, Bayandalai soum, northern part of

Zuramtai mountain, 1715 m, 05 September 1976, *Ch. Sanchir* (UBA) • Bayankhongor province, Shinejinst soum, Jinst mountain, 44°26'28.4"N, 99°13'36"E, 2000 m, 14 July 1979, *I. A. Gubanov* (MW0593124, MW0593125) • Umnugovi province, Noyon soum, Noyon Bogd mountain, 43°12'17.35"N, 101°50'39.68"E, 2200 m, 08 September 1979, *V. I. Grubov & Sh. Dariimaa* (UBA) • Umnugovi province, Sewrei soum, Zuulun mountain, 43°38'49.19"N, 102°20'57.46"E, 1900 m, 26 August 1982, *I. A. Gubanov* (MW0593123) • Umnugovi province, Dalanzadgad soum, Khachig mountain, 42°59'35.53"N, 105°40'35.27"E, 1800 m, 01 September 1982, *I. A. Gubanov* (MW0183265) • Umnugovi province, Khankhongor soum, Chanaijn Kharaa mountain, 44°22'48"N, 104°16'48"E, 1242 m, 22 June 1988, *H. D. Knapp* (GFW45639) • Umnugovi province, Bayandalai soum, Ikh Argalant mountain, 43°10'32.24"N, 103°35'24.93"E, 1910 m, 16 June 2007, *Ts. Jamsran* (UBU0030804) • Umnugovi province, Bayan-Ovoo soum, 42°56'57.5"N, 106°9'13.3"E, 1170 m, 08 June 2013, *Ch. Sanchir* (UBU0032442) • Alashan Gobi region: • Umnugovi province, Alashaa Gobi, Khalzan mountain, 42°10'18.32"N, 105°15'43.97"E, 1363 m, 31 July 1989, *I. A. Gubanov* (MW0183261) • Umnugovi province, Nomgon soum, Borzon govi, 42°33'35.14"N, 105°9'58.8"E, 1208 m, 2003, *Ariuntuya* (UBU0012226).

Astragalus oyunicus **D.Munkhtulga & S.Baasanmunkh, sp. nov.**

urn:lsid:ipni.org:names:77359973-1

Fig. 4

Diagnosis. The new species is close to *A. gobi-altaicus*, but differs by its leaves having leaflet narrowly elliptic, 15–18 × 4–7 mm, apex acute (vs. oblanceolate or narrowly elliptic to elliptic, 7–18 × 5–10 mm, apex mucronate) and raceme oblong to oblong-cylindrical, 3–8 cm long (vs. ovoid to oblong, 3–4 cm long). It is also similar to *A. laguroides* in leaf shape and general habit, but differs in its stipules broadly triangular-acuminate, 4–6 mm long (vs. narrowly triangular-acuminate, 5–8(–10) mm long) and pod densely covered with spreading white and fewer black hairs 2 mm (vs. covered with subappressed to spreading white hairs 1–1.5 mm, smaller black hairs amount greater than the apex) (Table 2).

Type. MONGOLIA. Khentii province, Bayankhutag soum, 46°54'10.2"N, 110°12'51.9"E, 1166 m, 05 August 2023, *B. Oyuntsetseg & D. Munkhtulga Khen-2023* [Holotype UBU0039102! (Fig. 6C)] • Ulaanbaatar city, Bayanzurkh district, Gachuurt village, 47°55'52.3"N, 107°07'51.2"E, 1467 m, *Yondon* [Isotype UBU0034202!].

Description. Plants perennial, 5–15 cm tall, acaulescens, in vegetative parts covered with ± medifixed, appressed white hairs c. 1 mm. Rootstock divided with short to elongated blackish brown branches. Stipules broadly triangular-acuminate, 4–6 mm, shortly adnate to the petiole, otherwise free from each other, densely covered with hairs up to 1.5 mm, at the margins also with basifixed hairs. Leaves 5–12(–15) cm; petiole 2–4 cm, like the rachis covered with medifixed, appressed hairs. Leaflets in 3–5 pairs, narrowly elliptic, 15–18 × 4–7 mm, on both sides densely hairy, apex acute. Peduncles 3–7 cm, rather densely to densely covered with symmetrically to asymmetrically bifurcate, flexuose, subappressed white hairs. Racemes ovoid to oblong 3–4 cm long. Bracts whitish, 5–6 mm, narrowly triangular, with strongly asymmetrically bifurcate, ± spreading white hairs. Calyx at beginning of anthesis tubular, later

Figure 4. *Astragalus oyunicus* in Mongolia **A** general habit **B** raceme **C** flower **D** calyx **E** standard **F** wings **G** keel **H** stamens **I** pistil **J** pod **K** pod valve **L** laeve **M** leaflet **N** stipules. (Photo credits: D.Munkhtulga).

on ovoid-inflated, 10–12 mm long, covered with basifixed, spreading, straight white and fewer black hairs 1–2 mm; teeth subulate, 2–3 mm. Petals violet. Standard 18–20 mm; blade 5–6 mm wide, obovate, slightly constricted in the middle, emarginate, at the base gradually narrowed. Wings 14–16 mm; blades narrowly oblong, obtuse, 5.5–6.5 × 2–2.5 mm; auricle c. 1.5 mm; claw c. 9 mm. Keel 12–14 mm; blades 4–4.5 × 3 mm; claw 8–8.5 mm. Ovary sessile. Legumes enclosed in the calyx, 6–7 × 3–4 mm, with a hooked beak c. 1 mm, unilocular; densely covered with spreading white and fewer black hairs c. 2 mm.

Table 2. Morphological comparisons of five *Astragalus* species in Mongolia.

Characters	*A. laguroides*	*A. gobi-altaicus*	*A. oyunicus*	*A. teshigicus*	*A. uvsicus*
Leaves	3–14(–18) cm long	8–14 cm long	5–12(–15) cm long	8–18 cm long	3–8(–12) cm long
Leaflets	3–5(–8) pairs, narrowly elliptic, 8–25(–40) × 3–6 mm, both surfaces rather densely hairy, apex acute	2–4 pairs, broadly-elliptical or oblong-obovate, 7–12 × 3–5 mm, on both sides densely hairy, apex mucronate	3–5 pairs, narrowly elliptic, 15–18 × 4–7 mm, on both sides densely hairy, apex acute	4–6 pairs, elliptical, 14–20 × 5–8 mm, on both sides densely covered with medifixed, subappressed hairs, acute to rarely obtuse	2–5 pairs, narrowly elliptic, 8–11(–14) × 3–5 mm, rather densely covered with medifixed, appressed hairs, apex acute
Stipules	5–8(–10) mm long, narrowly triangular-acuminate, covered with spreading or ascending hairs	3–4 mm long, narrowly triangular-acuminate, rather densely appressed hairy, at the margins with basifixed hairs	4–6 mm long, broadly triangular-acuminate, loosely covered with spreading or ascending hairs	4–5 mm long, broadly triangular-acuminate, densely covered with spreading or ascending hairs	6–8 mm long, narrowly triangular-acuminate, covered with spreading or ascending hairs
Raceme	ovoid to oblong, 2–4(–6) cm long	oblong-cylindrical, 3–8 cm long	ovoid to oblong, 3–4 cm long	oblong or oblong-cylindrical, 4–7(–8) cm long	globose or ovate, 3–4 cm long
Calyx	11–14 mm long, densely covered with asymmetrically bifurcate to basifixed, tangled, spreading white hairs 1–2 mm, at outer side of the teeth and sometimes at the nerves of the tube also with black hairs; teeth 3–4 mm	12–16 mm long, densely covered with subbasifixed to basifixed, spreading, straight white hairs 2–3 mm; teeth 4–6 mm	10–12 mm long, covered with basifixed, spreading, straight white and fewer black hairs 1–2 mm; teeth 2–3 mm	12–15 mm long, rather densely covered with spreading, straight white hairs 2 mm; teeth 2–3 mm	11–14 mm long, covered with basifixed, spreading white and black hairs 1–2 mm; teeth 2–3 mm
Standard	17–20 mm long, emarginate	14–18(–20) mm long, slightly emarginate	18–20 mm long, emarginate	20–24 mm long, emarginate	15–18 mm long, emarginate
Wings	14–18 mm long	12–16 mm long	14–18 mm long	17–22 mm long	13–15 mm long, obtuse
Keels	13–15 mm long	10–14 mm long	12–16 mm long	14–18 mm long	12–14 mm long
Pods	sessile, oblong, (–6)7–8 × 2–3 mm, with a hooked beak c. 1 mm, unilocular; covered with subappressed to spreading white hairs 1–1.5 mm, smaller black hairs amount greater than the apex	sessile, oblong, 7–8 × 3–4 mm, with a hooked beak 2–2.5 mm, unilocular; covered with tangled, spreading white hairs	sessile, oblong, 6–7 × 3–4 mm, with a hooked beak c. 1 mm, unilocular; densely covered with spreading white and fewer black hairs c. 2 mm	sessile, oblong, 5–6 × 2–3 mm, with a hooked beak c. 1 mm, unilocular; densely covered with spreading white hairs	sessile, linear, 6.5–7 × 2–3 mm, with beak c. 1 mm, bilocular, loosely covered with spreading white hairs c. 1 mm, smaller black hairs amount greater than the apex

Distribution. Endemic to Mongolia (Fig. 9).

Habitat. This species grows in stony mountain slopes and low isolated rounded hills of the steppe region.

Etymology. The species is named after Prof. Batlai Oyuntsetseg who is a botanist in Mongolia.

Additional specimens examined. Mongolia. Mongolian Dauria region: • Ulaanbaatar city, Chingeltei district, Zuun salaa, 23 August 1963 (UBU0032447) • Ulaanbaatar city, Narangiin enger, 25 July 1967, *L. Purevsuren* (UBU0026331, UBU0026338) • Ulaanbaatar city, Songinokhairkhan district, Tolgoit, Narangiin enger, 10 August 1967, *Tsogoo* (UBU0026224, UBU0032448, UBU0032449, UBU0039988-UBU0039990) • Khentii province, Jargalant Khan soum, Kherlen river, 07 July 1979, *V.I. Grubov & A. Mundaashev 321* (UBA) • Ulaanbaatar city, Chingeltei district, Shadivlan, 48°01'00"N, 106°54'01"E, 1520 m, 06 June 2021, *B. Oyuntsetseg & A. Anudari* (UBU0014416) • Middle Khalkh region: Tuv province, Buren soum, Ikher-Ukhaa, 22 July 1976, *Tumurtogoo* (UBU0039987) • Govisumber province, Sumber soum, 23 July 1992, *B. Oyuntsetseg & Oyunchimeg* (UBU0031855) • Khentii province, Tsenkhermandal soum, Batkhairkhan mountain, 47°42'30"N, 109°05'34.8"E, 1459 m, 28 May 2020, *D. Munkhtulga* (UBU0031854).

Astragalus teshigicus **D.Munkhtulga & S.Baasanmunkh, sp. nov.**

urn:lsid:ipni.org:names:77359974-1
Fig. 5

Type. Mongolia. Bulgan province, Teshig soum, Baga Baysgalan, 49.9679, 102.5867, 1128 m, 05 June 2018, *B. Oyuntsetseg & Sh. Baasanmunkh NW11* [Holotype UBU0002138! (Fig. 6B), Isotype UBU0002139!].

Diagnosis. The morphological features of *A. teshigicus* are similar to that of *A. laguroides* but differ by its raceme oblong or oblong-cylindrical (vs. ovoid), calyx rather densely covered with spreading, straight only white hairs 2 mm (vs. densely covered with asymmetrically bifurcate to basifixed, tangled, spreading white hairs 1–2 mm, at outer side of the teeth and sometimes at the nerves of the tube also with black hairs), leaves 8–18 cm (vs. 3–14 cm long), leaflets in 4–6 pairs, elliptical, 14–20 × 5–8 mm (vs. 3–5 pairs, narrowly elliptic, 8–25 × 3–6 mm) (Table 2).

Description. Plants perennial, 8–20 cm tall, acaulescent. Stems up to 1 cm, densely appressed white hairy. Stipules 4–5 mm long, broadly triangular-acuminate, densely covered with spreading or ascending hairs. Leaves 8–18 cm; petiole 3–7 cm, like the rachis covered with appressed white hairs. Leaflets in 4–6 pairs, elliptical, 14–20 × 5–8 mm, on both sides densely covered with medifixed, subappressed hairs, acute to rarely obtuse. Peduncles 6–12 cm, with medifixed, appressed hairs. Racemes oblong or oblong-cylindrical (3.5) 4–7 (8) cm long, rather densely many-flowered. Bracts scarious, 5.5–6 mm, narrowly triangular, sparsely covered with ascending only white hairs, at the margins covered with spreading white hairs up to 1.5–2 mm. Calyx 12–15 mm, at the beginning of anthesis tubular, soon ovoid-inflated, densely covered with basifixed, ± spreading, rigid white hairs 1.5-2 mm; teeth subulate, 2–3 mm. Petals purplish. Standard 20–24 mm; blade 4.5–5.5 mm wide, obovate, slightly constricted in the middle, slightly emarginate, at the base gradually narrowed. Wings 17–22 mm; blades oblong, rounded, 5–5.5 × 2–3 mm; auricle c. 1 mm; claw 8–9 mm. Keel 14–18 mm; blades 4–4.5 × 2–3 mm; claw c. 8 mm. Ovary sessile. Legumes enclosed in the calyx, oblong, 4–5.5 × 2–2.5 mm, with a hooked beak c. 1 mm, unilocular; densely covered with spreading white hairs.

Distribution. Endemic to Mongolia. This species is found only in a single location in the northern part of the country (Fig. 9).

Habitat. This species grows in steppe areas including mountain slopes, rocky and stony mountains.

Etymology. The species epithet refers to the location where the species was collected.

Astragalus **subgenus** ***Cercidothrix*** **Bunge, section** ***Uvsicus*** **D.Munkhtulga, S.Baasanmunkh & H.J.Choi, sect. nov.**

urn:lsid:ipni.org:names:77359975-1

Type. *A. uvsicus* D.Munkhtulga, S.Baasanmunkh & H.J.Choi, sp. nov.

Figure 5. *Astragalus teshigicus* in Mongolia **A** general habit **B** raceme **C** bract **D** calyx **E** standard **F** keel **G** wings **H** pistil **I** stamens **J** leave **K** omission of medifixed hairs on the upper side of the leaf **L** stipules, inside view **M** stipules, outside view **N** pod. (Photo credits: D.Munkhtulga and B.Oyuntsetseg).

Description. Perennials, herbaceous, acaulescent, with bifurcate hairs. Stipules adnate, like rachis densely to rather densely hairy. Leaves with appressed hairs. Inflorescens with a well developped peduncle, densely mostly many-flowered. Bracteoles absent. Calyx tubular at beginning of anthesis, mostly soon elongating and becoming ovoid to globose-inflated. Legumes enclosed in the calyx, sessile, bilocular.

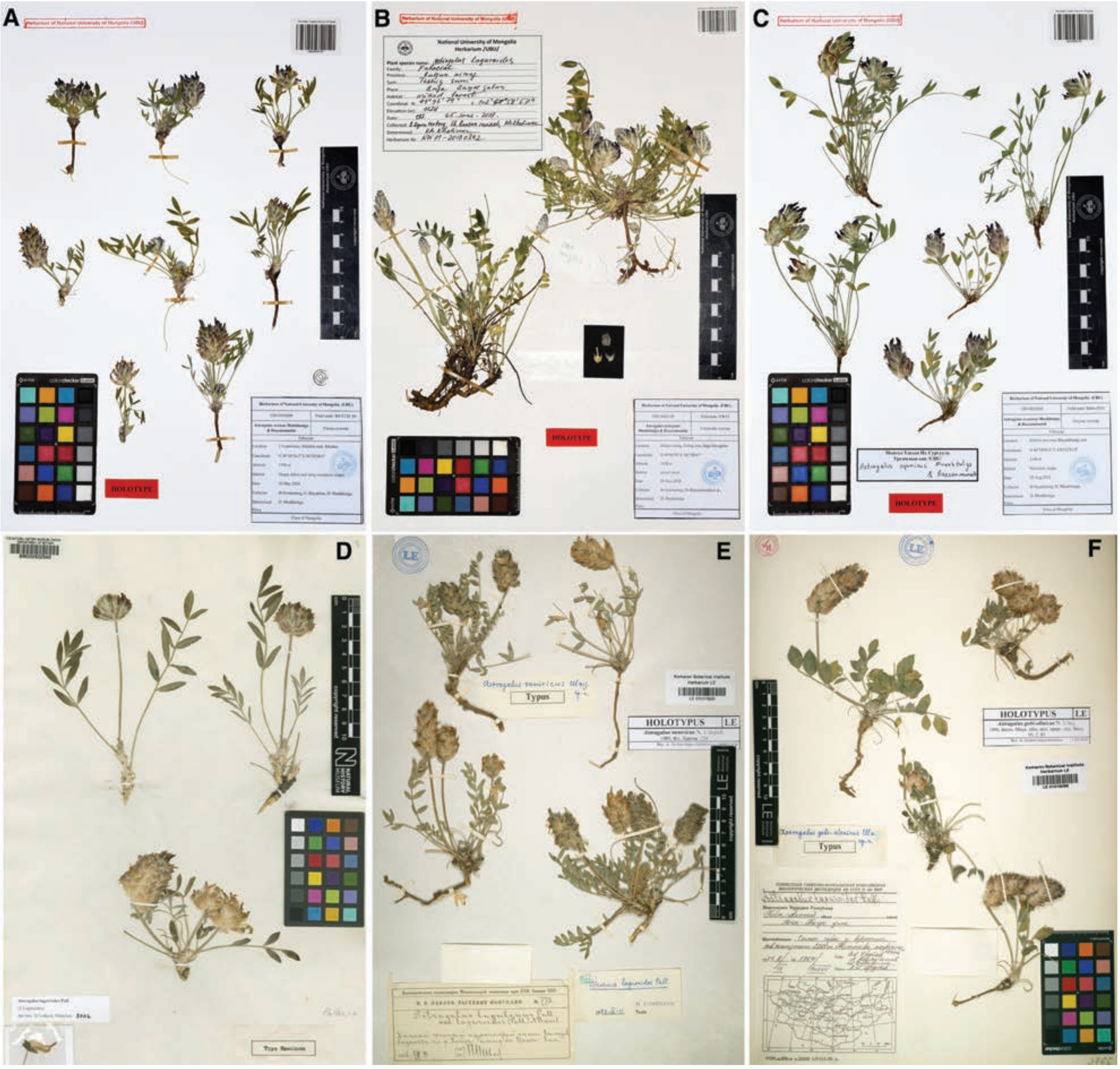

Figure 6. Type specimens of *Astragalus* species **A** *A. uvsicus* (UBU0039100) **B** *A. teshigicus* (UBU0002138) **C** *A. oyunicus* (UBU0039102) **D** *A. laguroides* (BM000632540) **E** *A. tamiricus* (LE01017820) **F** *A. gobi-altaicus* (LE01016096).

Astragalus uvsicus **D.Munkhtulga, S.Baasanmunkh & H.J.Choi, sp. nov.**
urn:lsid:ipni.org:names:77359976-1
Fig. 7

Diagnosis. *Astragalus uvsicus* is morphologically similar to *A. beitashanensis* W. Chai & P. Yan (Zhai and Yan 2010) and *A. laguroides*, but can be distinguished by its leaflet 2–5 pairs, narrowly elliptic, acute 8–11(–14) × 3–5 mm (*A. beitashanensis*, vs. 3–5 pairs, elliptic to suborbicular, obtuse, 4–9 × 3–6 mm; vs. *A. laguroides*, 3–5 pairs, narrowly elliptic, acute 8–25 × 3–6 mm) Legumes linear, 5–6 × 2–3 mm, with a beak c. 1 mm, bilocular, loosely covered with spreading, straight hairs (*A. beitashanensis*, legumes oblong, c. 8 mm, unilocular, very densely covered with spreading, basifixed hairs; *A. laguroides*, legumes oblong, (6) 7–8 × 2–3 mm long, with a hooked

Figure 7. *Astragalus uvsicus* in Mongolia **A** general habits **B** raceme **C** standard **D** wings **E** keel **F** pistil **G** stamens **H** pod **I** pod valve **J** leave **K** leaflet **L** stipules. (Photo credits: D.Munkhtulga).

beak c. 1 mm, unilocular; covered with subappressed to spreading hairs). At the section level, the *Uvsicus* section is differentiated by its plants covered with medifixed hairs (vs. covered with semi-appressed pilose in *Mixiotricha*), peduncle not longer than leaves, raceme many-flowered (vs. peduncle very short, raceme few-flowered in *Trachycercis* and *Mixiotricha*), legumes enclosed in the calyx (vs. calyx ruptured by legumes in *Macrotrichoides*), and bilocular (vs. unilocular in *Laguropsis*) (Table 3). The general habits of selected sections within the subgenus *Cercidothrix* are shown in Fig. 8.

Figure 8. General habits of some selected *Astragalus* species in Mongolia **A** *A. laguroides* **B** *A. gobi-altaicus* **C** *A. oyunicus* **D** *A. teshigicus* **E** *A. ochrias* **F**. *A. dilutus* **G** *A. lupulinus* (**A–G** sect. *Laguropsis*) **H** *A. junatovii* (sect. *Trachycercis*) **I** *A. teskhemicus* (sect. *Trachycercis*) **J** *A. testiculatus* (sect. *Mixiotricha*) **K** *A. gubanovii* (sect. *Macrotrichoides*) **L** *A. uvsicus* (sect. *Uvsicus*) (Photo credit: A, C, F, G, I, J, K, L by D. Munkhtuglga; A, B, D, E, H by B. Oyuntsetseg).

Type. MONGOLIA. Depression of Great Lakes: • Uvs province, Malchin soum, Khuiten valley, 49°29'36.2"N, 93°10'18.5"E, 1550 m, 30 May 2024, *B. Oyuntsetseg, G. Bayarmaa, & D. Munkhtulga* [Holotype UBU0039100! (Fig. 6A)] • Uvs province, Malchin soum, Surtiin zuslan, 49°37'0.6"N, 93°3'46.6"E, 1611 m, 10 June 2021, *D. Munkhtulga* [Isotype UBU0027685!].

Description. Plants perennial, 5–12 cm tall, acaulescens or nearly so, covered with medifixed hairs. Rootstock with a pluricipital root-crown. Stipules narrowly triangular-acuminate, 5–7 mm, shortly adnate to the petiole, otherwise free from each other, covered with strongly asymmetrically bifurcate,

Table 3. Morpholgical comparisions of five *Astragalus* sections in Mongolia.

Characters	*Uvsicus*	*Laguropsis*	*Trachycercis*	*Mixiotricha*	*Macrotrichoides*
Plants	appressed pilose	appressed pilose	appressed pilose	semiappressed pilose	appressed pilose
Raceme	many flowered	many flowered	few flowered	few flowered	few flowered
Peduncle	long	long	very short	very short	long
Calyx	vesicularly inflated	vesicularly inflated	not inflated	not inflated	slightly inflated
Pods	bilocular	bilocular and unilocular (in *A. laguroides*, *A. oyunicus*, *A. teshigicus*, *A. gobi-altaicus*)	Bilocular	bilocular	bilocular

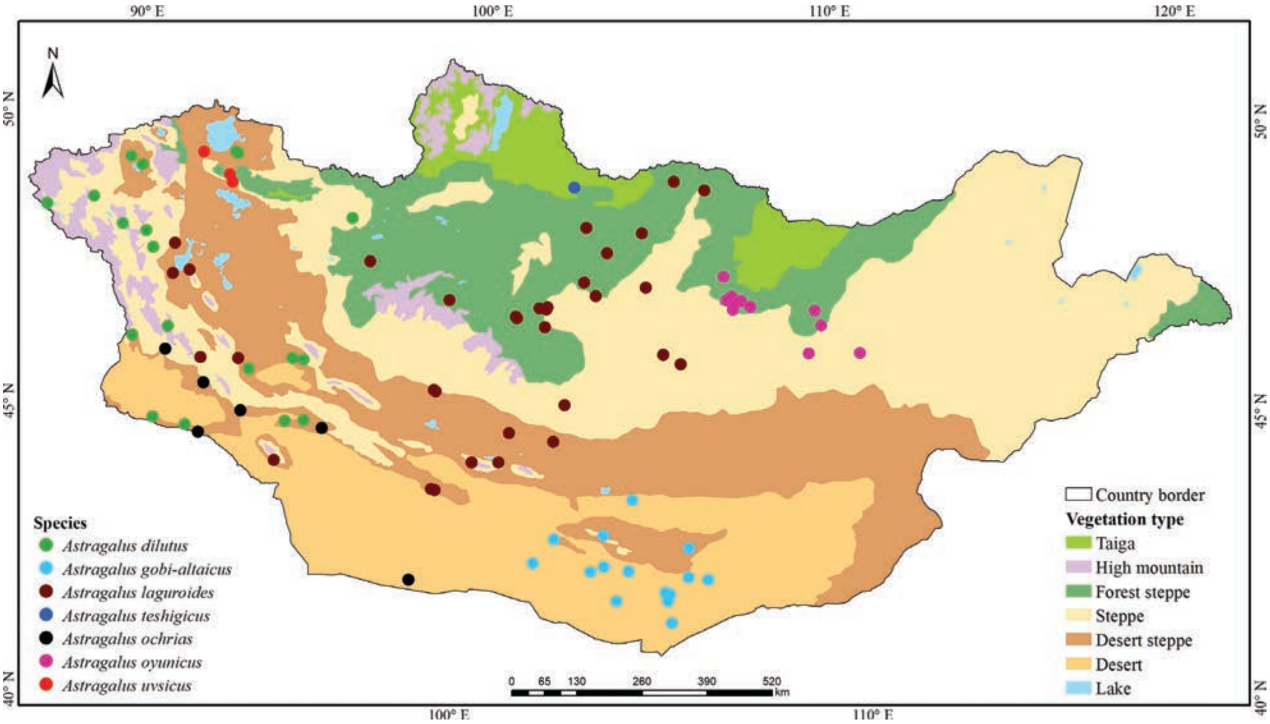

Figure 9. Distribution map of the studied *Astragalus* species from the sections *Laguropsis* and *Uvsicus* in Mongolia.

subappressed white hairs up to 2 mm. Leaves 3–8 (12) cm; petiole 2–3 cm, like the rachis covered with medifixed, appressed hairs. Leaflets in 2–5 pairs, narrowly elliptic, 8–12 × 3–5 mm, apex acute, rather densely covered with medifixed, appressed hairs c. 1 mm. Peduncles 2–6 cm, with medifixed, appressed hairs. Racemes globose or ovate 3–4 cm long. Bracts scarious, 2–3 mm, narrowly triangular, white and few black hairy. Calyx at beginning of anthesis tubular, later on ovoid-inflated, 11–14 mm, with distinct, elevated longitudinal nerves, loosely covered with subbasifixed, spreading white and black hairs 1–2 mm; teeth subulate, 2–3 mm. Petals violet. Standard 15–18 mm; blade 3–4 mm wide, obovate, slightly constricted in the middle, emarginate, at the base gradually narrowed into the rather long claw. Wings 13–15 mm; blades narrowly oblong, obtuse, 5.5–6.5 × 1.1–2 mm; auricle c. 1 mm; claw 7.5–8.5 mm. Keel 12–14 mm; blades 4–5 × 3 mm. Ovary sessile. Legumes enclosed in the calyx, linear, c. 5–6 × 2–3 mm, with a beak c. 1 mm, bilocular, loosely covered with spreading, straight white hairs c. 1 mm, smaller black hairs amount greater than the apex.

Distribution. Endemic to Mongolia. This species is found in the Khyargas and Uvs lakes in the depression of great lakes region in Mongolia (Fig. 9).

Habitat. This species grows in stony mountain slopes and near rocks, sayr slopes.

Etymology. The species epithet refers to the location where the species was collected.

Additional specimens examined. MONGOLIA. Depression of Great Lakes: • Uvs province, Tarialan soum, Turgen river, 49°56'10.9"N, 92°15'22.7"E, 900 m, 31 August 1984, *I. A. Gubanov 9338* (MW0183285).

Conclusion

Based on morphological characteristics and molecular analysis, we described three new species, *A. oyunicus*, *A. teshigicus*, and *A. uvsicus*, from Mongolia in this study. In addition, a new section of *Uvsicus* is described based on *A. uvsicus*, supported by morphological and molecular evidence. We conclude that morphological characteristics and size are important for distinguishing between closely related *Astragalus* species. In addition, we confirmed the finding of several previous studies (Bagheri et al. 2016; Zhang and Jiang 2020; Baasanmunkh et al. 2024a) that the nrITS primer is quite well distinguished between species at the section and subgenus levels. Finally, we partially studied the species of the section *Laguropsis*; however, we will continue to study the remaining species in Central Asian countries.

Acknowledgements

We extend thanks to Profs. Batlai Oyuntsetseg and Gun-Aajav Bayarmaa (National University of Mongolia) who greatly helped our field expeditions in the last several years. The authors would also like to thank the three anonymous reviewers for their insights, which helped improve 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 study was supported by research grants from the Korea Basic Science Institute (National Facilities and Equipment Center) grant funded by the Ministry of Education (Grant No. 2023R1A6C101B022), the Foundational and Protective Field of Studies Support Project at Changwon National University in 2024, and taxonomic revision of polymorphic plant families of the flora of Uzbekistan (FZ-20200929321).

Author contributions

SB designed this study. DM, SB, and NN wrote the original draft of the manuscript. DM, SB, ZT, and HCJ were collected the samples. DM, SB, NN, JHP, and ZT analyzed the data. SB, KST, and HJC revised the manuscript. All the authors have revised and agreed to the published version of the manuscript.

Author ORCIDs

Dariganga Munkhtulga https://orcid.org/0009-0009-0175-203X
Shukherdorj Baasanmunkh https://orcid.org/0000-0003-4224-9376
Nudkhuu Nyamgerel https://orcid.org/0000-0002-9110-423X
Jong Ho Park https://orcid.org/0009-0003-9053-8591
Zagarjav Tsegmed https://orcid.org/0000-0002-6024-4918
Komiljon Sh. Tojibaev https://orcid.org/0000-0003-2846-5777
Hyeok Jae Choi https://orcid.org/0000-0001-6315-0071

Data availability

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

References

Abdulina SA (1998) Spisok sosudistykh rasteniy Kazakhstana [checklist of vascular plants of Kazakhstan]. In: Kamelin RV (Ed.). Almaty: Alma-Ata, 18–21.

Baasanmunkh S, Urgamal M, Oyunstsetseg B, Grabovkaya-Borodina A, Oyundelger K, Gundegmaa V, Tsegmed Z, Kechaykin AA, Pyak AI, Zhao LQ, Choi HJ (2021) An updated checklist of endemic vascular plants of Mongolia. Diversity 13: 619. https://doi.org/10.3390/d13120619

Baasanmunkh S, Urgamal M, Oyuntsetseg B, Sukhorukov AP, Tsegmed Z, Son DC, Erst A, Oyundelger K, Kechaykin AA, Norris J, Kosachev P, Ma J-S, Chang KS, Choi HJ (2022) Flora of Mongolia: Annotated checklist of native vascular plants. PhytoKeys 192: 63–169. https://doi.org/10.3897/phytokeys.192.79702

Baasanmunkh S, Munkhtulga D, Nyamgerel N, Oyuntsetseg B, Undruul A, Tsegmed Z, Knyazev MS, Choi HJ (2024a) Taxonomic studies on the genus *Astragalus* (Fabaceae) from Mongolia (I): Two new records of *A. testiculatus* and *A. teskhemicus* based on morphological and molecular evidence. Biology Bulletin 51: 969–979. https://doi.org/10.1134/S1062359023605748

Baasanmunkh S, Munkhtulga D, Oyuntsetseg B, Undruul A, Tsegmed Z, Kechaykin AA, Choi HJ (2024b) *Astragalus arbuscula* (Fabaceae) new to Mongolian flora. Korean Journal of Plant Taxonomy 54: 212–216. https://doi.org/10.11110/kjpt.2024.54.3.212

Bagheri A, Maassoumi AA, Rahiminejad MR, Blattner FR (2016) Molecular phylogeny and morphological analysis support a new species and new synonymy in Iranian *Astragalus* (Leguminosae). PLoS ONE 11(3): e0149726. https://doi.org/10.1371/journal.pone.0149726

Bagheri A, Maassoumi AA, Noroozi J, Blattner FR (2022) *Astragalus* sect. *Elvendia* (Fabaceae), a new tragacanthic section recorded from Mt. Alvand, a center of endemism in W Iran. Plant Biosystems 156(5): 1260–1268. https://doi.org/10.1080/11263504.2022.2036846

Bagheri A, Maassoumi AA, Brassac J, Blattner FR (2023) Dated phylogeny of *Astragalus* Section *Stereothrix* (Fabaceae) and allied taxa in the *Hypoglottis* Clade. Biology (Basel) 12: 138. https://doi.org/10.3390/biology12010138

Biazrov LG, Ganbold E, Gubanov IA, Ulziikhutag N (1989) Flora of Khangay. Nauka, Leningrad, 191 pp. [in Russian]

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Erkul SK, Duman H, Ateş MA (2022) *Astragalus oksutdagensis* (Fabaceae), a new species from Turkey. Nordic Journal of Botany 2022: e03237. https://doi.org/10.1111/njb.03237

Esri (2012) Advancing the power of geography. For business, government, and society. https://www.esri.com/enus/about/about-esri/overview

Folk RA, Charboneau J, Belitz M, Singh T, Kates HR, Soltis DE, Soltis PS, Guralnick RP, Siniscalchi CM (2024) Anatomy of a mega-radiation: Biogeography niche evolution in *Astragalus*. American Journal of Botany 111(3): e16299. https://doi.org/10.1002/ajb2.16299

Gao T, Pang XH, Chen SL (2009) Authentication of plants in *Astragalus* by DNA Barcoding technique. Planta Medica 75: PB21. https://doi.org/10.1055/s-0029-1234433

Hall T, Biosciences I, Carlsbad C (2011) BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60–61.

Kang Y, Zhang ML, Chen ZD (2003) A preliminary phylogenetic study of the subgenus *Pogonophace* (*Astragalus*) in China based on ITS sequence data. Journal of Integrative Plant Biology 45(2): 140–145.

Kashyap S, Sahu CK, Verma RK, Chaudhary LB (2021) Taxonomic application of macro and micro morphological characters of seeds in *Astragalus* L. (Galegeae, Fabaceae) in India. Phytotaxa 502(2): 191–207. https://doi.org/10.11646/phytotaxa.502.2.8

Khan A, Sultan A, Zarre S, Ishaq K (2023) *Astragalus pseudoanisacanthus*, a new species of section Poterion from northern Balochistan (Pakistan). Phytotaxa 622: 239–251. https://doi.org/10.11646/phytotaxa.622.4.1

Maassoumi AA, Ashouri P (2022) The hotspots and conservation gaps of the mega genus *Astragalus* (Fabaceae) in the Old-World. Biodiversity and Conservation 31(8–9): 2119–2139. https://doi.org/10.1007/s10531-022-02429-2

Moghaddam M, Wojciechowski MF, Kazempour-Osaloo S (2023) Characterization and comparative analysis ofthe complete plastid genomes of four *Astragalus* species. PLoS ONE 18: e0286083. https://doi.org/10.1371/journal.pone.0286083

Perezhogin YV, Borodulina OV, Erokhin NG, Kubentaev SA (2023) New locations of species of the genus *Astragalus* L. in Kazakhstan. Bulletin of the Karaganda University 110: 107–112. https://doi.org/10.31489/2023bmg2/107-112

Podlech D, Zarre S (2013) A taxonomic revision of the genus *Astragalus* L. (Leguminosae) in the Old World (3-Volume Set). Vienna Natural History Museum / Naturhistorisches Museum Wien Publishing, 1–2439.

POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ [accessed 01 October 2024]

Pyak AI, Pyak EA (2019) A new species of *Astragalus* section *Laguropsis* (Fabaceae: Galegeae) from a crossborder highland region of Altai Mountains in Russia and Mongolia. Phytotaxa 414(4): 194–198. https://doi.org/10.11646/phytotaxa.414.4.6

Rambaut A (2012) FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology. Edinburgh.

Sennikov A, Tojibaev K (2021) Checklist of Vascular Plants of the Tian-Shan Mountain System. Korea National Arboretum, Pocheon, 607 pp.

Sennikov A, Tojibaev K, Khassanov F (2016) The Flora of Uzbekistan project. Phytotaxa 282(2): 107–118. https://doi.org/10.11646/phytotaxa.282.2.2

Shemetova T, Erst A, Wang W, Xiang K, Vural G, Aytac Z (2018) Seed morphology of the genus *Astragalus* L. from North Asia. Turkish Journal of Botany 42(6): 710–721. https://doi.org/10.3906/bot-1802-25

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sytin A, Lazkov G (2018) *Astragalus issykkulensis* (Fabaceae), a new species from Kyrgyzstan. Turczaninowia 21(2): 257–261. https://doi.org/10.14258/turczaninowia.21.2.21

Thiers B (2023) Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff, New York. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [Accessed October 15, 2024]

Thompson JD, Gibson TJ, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. Current Protocols in Bioinformatics. In: Baxevanis AD, Pearson WR, Stein LD, Stormo GD, Yates III JR (Eds) John Wiley & Sons, New York, 2.3.1–2.3.22. https://doi.org/10.1002/0471250953.bi0203s00

Tian C, Li X, Wu Z, Li Z, Hou Xand Li FY (2021) Characterization and comparative analysis of complete chloroplast genomes of three species from the genus *Astragalus* (Leguminosae). Frontiers in Genetics 12: 7054842. https://doi.org/10.3389/fgene.2021.705482

Tojibaev KS, Beshko NY, Azimova DE, Turginov OT (2015) Distribution patterns of species of the genus *Astragalus* L. (sect. *Macrocystis*, *Laguropsis* and *Chaetodon*) in the territory of Mountain Middle Asian province. Turczaninowia 18: 17–38. https://doi.org/10.14258/turczaninowia.18.2.3

Ulziykhutag N (1989) Bobovye Mongolskoi Narodnoi Respubliki. PhD Thesis, Vol. 2.

Ulziykhutag N (1990) The new species and sections belonging to *Astragalus* genus from Mongolia. 2. subspecies *Cercidithrix* Bunge. Bulletin of Moscow Society of Naturalists, Biological Series 95: 75–86.

Ulziykhutag N (2003) Legumes of Mongolia (taxonomy, ecology, geography, phylogeny and economic value). Mongolia, Ulaanbaatar, Bembi San, 588 pp.

Ulziykhutag N (2004) Plants of Central Asia: plant collection from China and Mongolia, in *Astragalus*. CRC Press, Boca Raton, Florida 8c: 204–232.

Vydrina SN (2006) *Astragalus* L. In: Polozhij AV, Malyschev LI (Eds) Flora of Siberia. Vol. 9. Fabaceae (Leguminosae). Science Publishers, Enfield, New Hampshire, 57–69.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Xu LR, Podlech D (2010) *Astragalus* L. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, Vol. 10. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 328–453.

Yang ZZ, Liu QR, Liu Z, Xiang J-shan, Li X-lan (2024) *Astragalus liuaiminii*, a new species of *Astragalus* (Fabaceae) from Xinjiang, China. PhytoKeys 243: 209–214. https://doi.org/10.3897/phytokeys.243.119707

Zhai W, Yan P (2010) *Astragalus beitashanensis*, a new species of Fabaceae from Xinjiang, China. Novon 20: 21–22. https://doi.org/10.3417/2008017

Zhang D, Jiang B (2020) Species identification in complex groups of medicinal plants based on DNA barcoding: A case study on *Astragalus* spp. (Fabaceae) from southwest China. Conservation Genetics Resources 12(3): 469–478. https://doi.org/10.1007/s12686-019-01119-6

PhytoKeys

PhytoKeys 255: 75–83 (2025)
DOI: 10.3897/phytokeys.255.143043

Research Article

Syzygium triflorum (Myrtaceae), a new species from Vietnam

Dang Hung Cuong[1], Nguyen Thi Kim Thanh[2], Nguyen Huu Hiep[1], Dang Ngoc Huyen[1], Thanh Truong Hoang[3], Shuichiro Tagane[4]

1 *Institute of Tropical Ecology, Joint Vietnam-Russia Tropical Center and Technology Research Center, Hanoi, Vietnam*
2 *Faculty of Biology, University of Science, Vietnam National University, Hanoi, Vietnam*
3 *Forest Science Institute of Central Highlands and South of Central Vietnam (FSIH), Lam Dong, Vietnam*
4 *Kagoshima University Museum, Kagoshima University, Korimoto, Kagoshima, Japan*
Corresponding author: Truong Hoang Thanh (truonghoangthanhld@gmail.com)

Abstract

Syzygium triflorum T.T.Hoang, Kim Thanh, S.Tagane & D.H.Cuong, **sp. nov.**, from Kon Chu Rang Nature Reserve, Vietnam, is described and illustrated. The new species is distinguished from related species, *Syzygium skiophilum*, by its having 4-angled and reddish-grey twigs, oblong leaves with an attenuate apex, less secondary veins of 26–30 pairs, exclusively axillary inflorescences, lanceolate-ovate bracts, sessile flowers, obconical hypanthium (slightly pyriform in flower buds) and more stamens of 18–28. The flowers and fruits were observed in the rainy season, July 2024. This new species grows under a mixed broad-leaved and coniferous forest dominated by *Dacrydium elatum* and *Dacrycarpus imbricatus*.

Key words: Flora, Indochina, Kon Chu Rang Nature Reserve, Myrtales

Introduction

Syzygium Gaertn. is the largest genus in the family Myrtaceae, with over 1,200 species primarily found in tropical and subtropical zones. The genus is generally characterised by trees or shrubs, branches sympodial, sometimes 2–4-ridged; leaves simple, opposite, gland-dotted and with intramarginal veins; inflorescences usually paniculate; hypanthium obconic, funnel-shaped or sometimes clavate; calyx lobes and petals 4 or 5; stamens many; ovary inferior; fruits berry (mostly drupaceous) and 1–2-seeded (Hyland 1983; Chantaranothai and Parnell 1994; Pham 2000; Parnell and Chantaranothai 2002; Biffin et al. 2006; Chen and Craven 2007; Govaerts et al. 2008; Craven and Biffin 2010; Ashton 2011; Soh and Parnell 2015).

The genus *Syzygium* in the Indochinese Region was first revised by François Gagnepain under the genus *Eugenia* P. Micheli ex L., identifying 55 species for the Flore générale de l'Indochine (Gagnepain 1914, 1917, 1920). Merrill and Perry (1938) later updated Indochinese *Eugenia* with synopsis, new species and new records and, lastly, transferred *Eugenia* to *Syzygium*. Soh and Parnell (2015) published a revision of Indochinese *Syzygium* (Cambodia, Laos and Vietnam) and enumerated 56 species, based on collections

Academic editor: James W. Byng
Received: 29 November 2024
Accepted: 12 March 2025
Published: 10 April 2025

Citation: Cuong DH, Thanh NTK, Hiep NH, Huyen DN, Hoang TT, Tagane S (2025) *Syzygium triflorum* (Myrtaceae), a new species from Vietnam. PhytoKeys 255: 75–83. https://doi.org/10.3897/phytokeys.255.143043

from the 1940s to 2010. Recent research has added 17 new species from Indochina since 2011 (Soh and Parnell 2011; Tagane et al. 2015, 2018; Chantaranothai 2024; Soh et al. 2024; Le et al. 2024), indicating that the diversity of *Syzygium* in the region is still unclarified and there could be more species waiting to be discovered.

In Vietnam, amongst 56 species treated in the most recent taxonomic revision by Soh and Parnell (2015), 49 species were recorded from the country, representing the highest species diversity in Indochina. Since then, eight species have been newly described from Vietnam: *S. honbaense* Tagane, V.S. Dang & Yahara, *S. phamhoangii* Tagane, V.S. Dang & Yahara, *S. yersinii* Tagane, V.S. Dang & Yahara, *S. chantaranothaianum* W. K. Soh & J. Parn., *S. cucphuongense* W. K. Soh & J. Parn., *S. quoctrianum* W. K. Soh, H.V. Sam & J. Parn., *S. samianum* W. K. Soh & J. Parn. and *S. ngheanense* N.S. Ly, N.D. Do & T.H. Le) and three species were recorded for the first time: *S. foxworthianum* (Ridl.) Merr. & L.M. Perry, *S. angkae* (Craib) Chantar. & J. Parn. and *S. thorelii* (Gagnep.) Merr. & L.M. Perry (Soh and Parnell 2011; Tagane et al. 2018; Chantaranothai 2024; Soh et al. 2024; Le et al. 2024). These taxa comprise approximately 60 species that have been inhabiting Vietnam until now.

During a botanical survey and ecological fieldwork conducted in Kon Chu Rang Nature Reserve, Gia Lai Province, Central Highland Vietnam in 2024, the authors collected an unknown *Syzygium* species. After thoroughly comparing this collected sample with available herbarium specimens and reviewing relevant literature, it did not match any previously described species worldwide and we here describe it as a new species, *S. triflorum*.

Materials and methods

The specimens of new species were compared with similar species through a literature review, examined from dried specimens from the herbaria, for example, DLU, FOF, HNU, KAG and VNM and online images of the type specimens (downloaded from herbaria, for example, K, P and websites such as JSTOR Global Plants (https://plants.jstor.org/), Tropicos (https://www.tropicos.org/), GBIF (https://www.gbif.org/; https://powo.science.kew.org/), Asian Plant (https://asianplant.net/), Singapore Biodiversity Online Database (https://m.singapore.biodiversity.online/) and Chinese Virtual Herbarium (https://www.cvh.ac.cn/).

The measurement of the description is based on the fresh and dry materials we collected from Kon Chu Rang Nature Reserve. The scientific name and terms follow Hyland (1983), Beentje (2010) and Turland et al. (2018) (Shenzhen code) and the authors mentioned above. The colour photographs are taken under a Canon 600D camera and a Meiji Techno EM-32 stereomicroscope. The plant specimens were deposited at DLU (Da Lat University Herbarium), HNU (VNU University of Science) and the Herbarium of The Joint Vietnam-Russia Tropical Science and Technology Research Center in Ha Noi.

Conservation Assessments are based on the conservation assessments following the recommendations of the IUCN Standards and Petitions Subcommittee (IUCN 2024) and IUCN Red List Categories and Criteria (IUCN 2012).

Taxonomic treatment

***Syzygium triflorum* T.T.Hoang, Kim Thanh, S.Tagane & D.H.Cuong, sp. nov.**
urn:lsid:ipni.org:names:77360036-1

Type. Vietnam. Gia Lai Province, K'Bang District, Son Lang Commune, Kon Chu Rang Nature Reserve, in coniferous forest, 200 m from Bai Chay station along the path, 14°28'44.5"N, 108°34'19.2"E, 1015 m a.s.l., 4 July 2024, *T.T.Hoang, N. T. K. Thanh & D.H. Cuong DLU1005* (holotype DLU! isotypes HNU [HNU025528!, HNU025529!], the Herbarium of The Joint Vietnam-Russia Tropical Science And Technology Research Center) (Figs 1, 2).

Diagnosis. *Syzygium triflorum* could be confused with *S. skiophilum* (Duthie) Airy Shaw distributed in Thailand, Malay Peninsula and Borneo (Airy Shaw 1949; Parnell and Chantaranothai 2002; Ashton 2011). Generally, *S. triflorum* is similar to *S. skiophilum* because they both show small tree habit and short cymose inflorescences with tiny flowers. However, *S. triflorum* differs from *S. skiophilum* in having 4-angled and reddish-grey twigs (vs. terete and blackish-brown in *S. skiophilum*), oblong leaf blades (vs. elliptic-lanceolate, obovate or oblanceolate), fewer secondary veins (26–30 pairs vs. ca. 45 or 14–18 pairs), exclusively axillary inflorescences (vs. temirnal and axillary), lanceolate-ovate bracts (vs. linear), sessile or subsessile flower (vs. with short pseudostalk ca. 1 mm long), obconical hypanthium (slightly pyriform in flower buds) (vs. funnel-shaped) and more stamens (18–28 vs. 8 stamens) (Table 1, Fig. 3).

Description. Trees, 1.5–3 m tall, all part glabrous. Young twigs 4-angled, reddish-grey. Leaves opposite; petioles 1–2 mm long, concave adaxially; blades oblong, 7.5–8.5 cm × 2.0–2.2 cm, dark green and shiny adaxially, pale green, with numerous dark green gland dots abaxially, coriaceous, base cuneate, margin entire, apex attenuate with a rounded acute tip 1.5–2 mm long, mid-rib impressed adaxially, prominent abaxially, secondary veins faint, 26–30 pairs, tertiary veins faintly visible, intramarginal vein 1, 0.6–0.8 mm from the margin. Inflorescences short cymose, axillary or on old branchlets behind leaves, 3–4 mm long, clusters of 3 (rarely reducing to 1) flowers; peduncle 0.8–1 mm long, terete; bracts lanceolate-ovate, 0.5–0.7 mm long, pale green to dark red on the upper half, apex rounded. Flower buds pyriform. Flowers 2.8–3.2 mm × 1.8–2.0 mm, light green, sessile or with pseudostalks to 0.5 mm long; bracteoles triangular, 0.2–0.3 mm long, dark red on the upper half, caducous. Hypanthium obconical, 2.0–2.2 mm × 1.8–2.0 mm, light green. Sepals 4, very broadly triangular, 0.2–0.3 mm long, light green, persistent, apex acute. Petals 4, free, suborbicular, 0.8 mm × 1.0 mm, light green, membranous, concave adaxially. Stamens 18–28 in a single whorl, 0.8–1 mm long, filaments white, anthers ellipsoid, ca. 0.1 mm long, cream-coloured. Ovary 2-locular, many ovules per locule, placentas axile; style and stigma 0.8–1.0 mm long, positioned lower than the stamens. Fruits (immature) globose, 4 × 4 mm, calyx disc convex. Seeds not seen.

Flowering and fruiting. The flowers and fruits were observed during the rainy season in July 2024. At this time, the fruits were not fully matured.

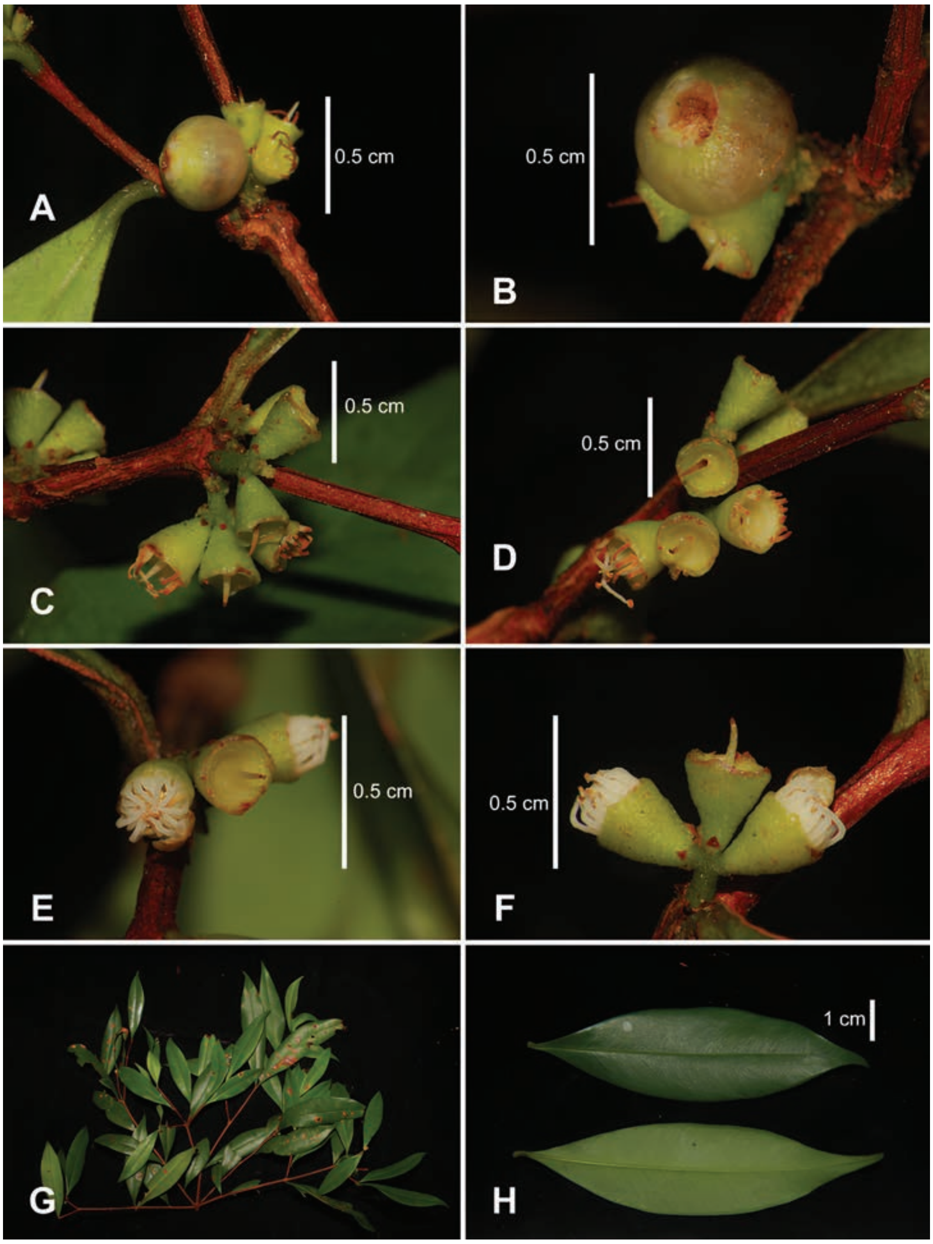

Figure 1. *Syzygium triflorum* T.T.Hoang, Kim Thanh, S.Tagane & D.H.Cuong **A** immature fruit in lateral view **B** immature fruits in top view **C** hypanthia in lateral view (after petals and most stamens had fallen off) **D** hypanthia with calyx disc and styles in top view (after petals and most stamens had fallen off) **E** inflorescence in top view, showing flowers before anthesis (left and right) **F** inflorescence in lateral view (3 flowers in cluster) **G** leafy branches **H** leaves, adaxial (upper) and abaxial (bottom) surface. Photos: H. T. Truong and H. C. Dang.

Ecology. This species grows in a mixed broad-leaved and coniferous forest so far known only from Kon Chu Rang Nature Reserve. The forests are dominated by *Dacrydium elatum* (Roxb.) Wall. ex Hook. and *Dacrycarpus imbricatus* (Blume) de Laub. (Podocarpaceae), along with other angiosperms, such as *Exbucklandia populnea*, *Rhodoleia championii* (Hamamelidaceae), *Magnolia mediocris* (Magnoliaceae), *Castanopsis* spp. (Fagaceae) and *Schima superba* (Theaceae). Many small trees grow in the understorey with *Syzygium triflorum* sp. nov., including *Ixora* sp., *Lasianthus* spp. (Rubiaceae), *Eurya* sp. (Pentaphylacaceae) and *Eriobotrya bengalensis* (Rosaceae). The habitat receives approximately 60–70% of the total light from the understorey at an elevation around 1,000 m a.s.l.

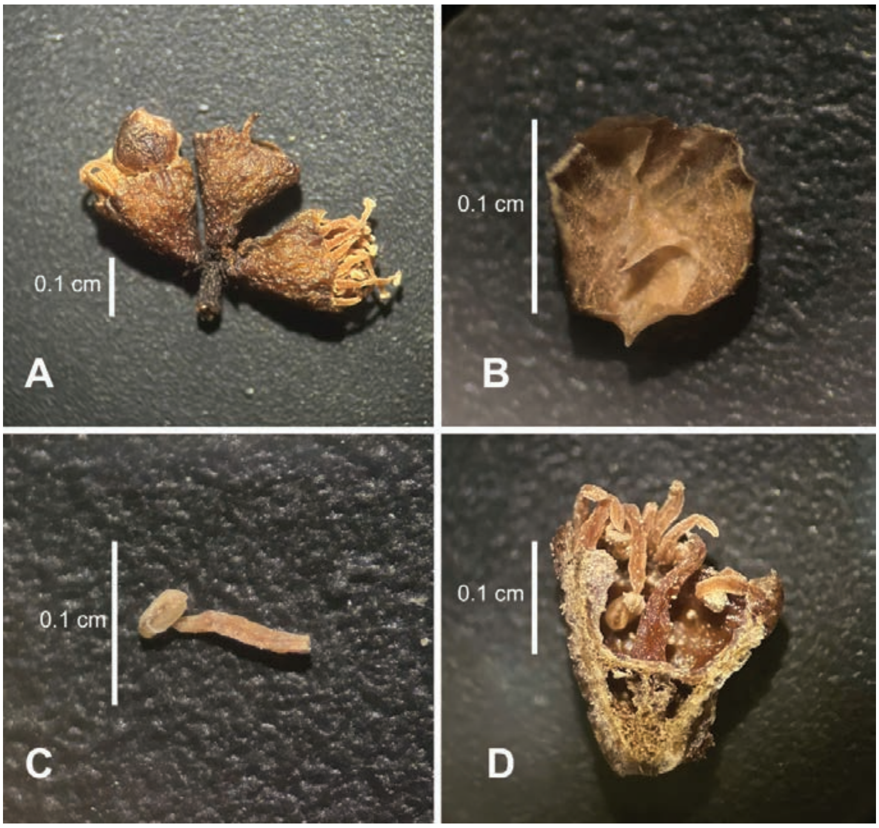

Figure 2. The dried flowers of *Syzygium triflorum* T.T.Hoang, Kim Thanh, S.Tagane & D.H.Cuong **A** 3-flowered inflorescence, in lateral view (with an orbicular petal in the left flower) **B** petal, adaxial surface **C** stamen (shorter one) **D** longitudinally dissected flower (with placenta axile). Photos taken by H. T. Truong and H. C. Dang.

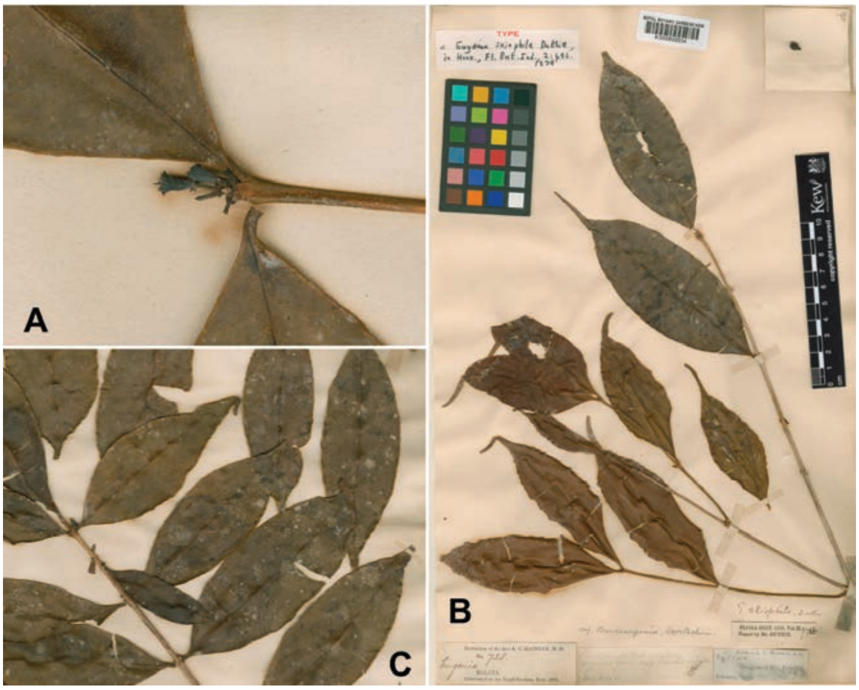

Figure 3. Syntypes of *Syzygium skiophilum* (Duthie) Airy Shaw **A** hypanthium of *A.C. Maingay 738/2* (K000800033) **B** leaves of *A.C. Maingay 738* (K000800034) and **C** *A.C. Maingay 738/2* (K000800033). Image reproduced with permission of © The Board of Trustees of the RBG, Kew. http://specimens.kew.org/herbarium/K000800033; http://specimens.kew.org/herbarium/K000800034.

Table 1. Morphological comparison between *Syzygium triflorum* and *S. skiophilum*. The charactersistics of *S. skiophilum* was from Parnell and Chantaranothai (2002), Ashton (2011), as well as specimens shown in the Appendix 1.

Parts	***Syzygium triflorum* sp. nov.**	***Syzygium skiophilum* (Duthie) Airy Shaw**
Twigs	4-angled, reddish-grey	round, dark brown, terete
Leaves	Oblong 7.5–8.5 cm × 2.0–2.2 cm	elliptic-lanceolate, obovate or oblanceolate 7–9 cm × 2.5–3 cm
– Secondary veins	26–30 pairs	ca. 45 pairs or 14–18 pairs
– Apex	attenuate with rounded acute tip 1.5–2 mm long	prominently caudate to caudate-acuminate with slender acumen, ca. 15 mm long.
– Petiole	1–2 mm long	3–6 mm long
Flowers	2.8–3.2 mm × 1.8–2.0 mm	–
– pseudostalks	0–0.5 mm	1 mm
– Colour	yellowish-green	yellowish
– Bracts	lanceolate-ovate, 0.5–0.7 mm long	linear, ca. 3 mm long
– Hypanthium	obconical, 2.0–2.2 mm × 1.8–2.0 mm	ovate-triangular, subacute, 2.8–3.3 mm
– Sepals	triangular, 0.2–0.3 mm long	0.5–0.8 mm long
– Number of stamens	18–28 in 1 whorl	8
Fruits	globose, 4 × 4 mm	ellipsoid, 6 × 4 mm

Vernacular. Trâm ba hoa (three-flowered syzygium), Trâm hoa nhỏ (tiny-flowered syzygium)

Etymology. The species epithet refers to its number of flowers in a inflorescence, which is usually in a cluster of three.

Preliminary conservation status

This species is currently known with only a few populations being found, each consisting of about 30–40 individuals, within a 10-hectare (0.01 km^2) stand of the mixed broad-leaved and coniferous forest dominated by *Dacrydium elatum* and *Dacrycarpus imbricatus* at Kon Chu Rang Nature Reserve. The total number of the mature individuals are approximately 200, living in understorey and strictly protected near the Bai Chay ranger station.

The primary natural threat in this area might be random fires, which could cause extreme fluctuations in the number of mature individuals. However, this risk is less prevalent in broad-leaved forests and the mixed broad-leaved and coniferous forests of Kon Chu Rang. Fires have not been recorded in these forests in the Nature Reserve for at least 30 years. Therefore, this species is temporarily regarded as safe in this forest stand.

All five IUCN criteria have been considered, but there is no fully direct or indirect information about the species' current status or possible threats. Considering the known population is very small, restricted to a 10-hectare stand and the number of mature individuals approximately 200 trees, Endangered (EN) is categorised for the preliminary conservation status of this species according the criteria D (IUCN 2012).

Discussion

Although many new taxa have been described over the past two decades and phylogenetic molecular classifications were studied, the classification of *Syzygium* remains complex due to the genus' megadiverse nature, confusing morphology, misidentified specimens and the lack of morphological data

(Biffin et al. 2006; Craven and Biffin 2010; Ahmad et al. 2016; Brambach et al. 2017; Tagane et al. 2018; Widodo and Veldkamp 2021). The morphological characters of fruit structures, seed structures, placentation position and the number of ovules may provide novel insights into evolutionary relationships and the higher systematic classification of *Syzygium* (Briggs and Johnson 1979; Hyland 1983; Biffin et al. 2006), but these characteristics are incomplete in *S. skiophilum*. Therefore, the additional data of the distinctive morphology of *S. skiophilum* and *S. triflorum* sp. nov. may provide evidence of an evolutionary trend to shorter petioles, smaller flowers and one stamen whorl of the genus *Syzygium*.

The short cymes consisiting of three small flowers and single whorl stamens with a tendency to reduce the number of stamens are observed in both *S. triflorum* and *S. skiophilum* in Southeast Asia (Figs 1, 2, 3). The reduction to a single whorl of stamens with tiny stamens occurs in many *Syzygium* species (e.g. *S. chemunjianum*, *S. dhaneshiana*, *S. phamhoangii* and *S. phoukhaokhouayense*) (Shareef et al. 2012; Tagane et al. 2018), but the extreme reduction to a single whorl of stamens along with extremely short cymose is particularly happening in *S. triflorum* and *S. skiophilum*. We suggest that the number of stamen whorls is essential for distinguishing different species and might imply an evolutionary trend within *Syzygium*.

Acknowledgements

The authors thank the manager and staff of the Kon Chu Rang Nature Reserve for their special permission and assistance with our botanical and ecological surveys in the protected areas.

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 Joint Vietnam-Russia Tropical Center and Technology Research Center (project code ST.Đ1.05/24)..

Author contributions

All authors have contributed equally.

Author ORCIDs

Dang Hung Cuong https://orcid.org/0000-0003-1208-737X
Nguyen Thi Kim Thanh https://orcid.org/0000-0003-2948-6385
Nguyen Huu Hiep https://orcid.org/0009-0002-4377-3161
Dang Ngoc Huyen https://orcid.org/0000-0001-9742-4675
Thanh Truong Hoang https://orcid.org/0000-0002-8799-5818
Shuichiro Tagane https://orcid.org/0000-0002-1974-7329

Data availability

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

References

Ahmad B, Baider C, Bernardini B, Biffin E, Brambach F, Burslem D, Byng JW, Christenhusz M, Florens FBV, Lucas E, Ray A, Ray R, Smets E, Snow N, Strijk JS, Wilson PG (2016) *Syzygium* (Myrtaceae): Monographing a taxonomic giant via 22 coordinated regional revisions. PeerJ Preprints 4: 1–13. https://doi.org/10.7287/peerj.preprints.1930v1

Airy Shaw HK (1949) Additions to the flora of Borneo and other Malay Islands. XX. The Myrtaceae of the Oxford University Expedition to Sarawak, 1932. Kew Bulletin 4: 117–125. https://doi.org/10.2307/4119049

Ashton PS (2011) Myrtaceae. In: Soepadmo E, Saw LG, Chung RCK, Kiew R (Eds) Tree Flora of Sabah and Sarawak, Volume 7. Sabah Forestry Department, Forest Research Institute Malaysia (FRIM), Sarawak Forestry Department, Malaysia, 87–330. https://doi.org/10.26525/TFSS7002

Beentje H (2010) The Kew Plant Glossary: An Illustrated Dictionary of Plant Identification Terms. Bibliovault OAI Repository, the University of Chicago Press, 1–160.

Biffin E, Craven LA, Crisp MD, Gadek PA (2006) Molecular systematics of *Syzygium* and allied genera (Myrtaceae): Evidence from the chloroplast genome. Taxon 55(1): 79–94. https://doi.org/10.2307/25065530

Brambach F, Byng JW, Culmsee H (2017) Five new species of *Syzygium* (Myrtaceae) from Sulawesi, Indonesia. PhytoKeys 81: 47–78. https://doi.org/10.3897/phytokeys.81.13488

Briggs BG, Johnson LAS (1979) Evolution in Myrtaceae – evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102: 157–256. https://biostor.org/reference/67846

Chantaranothai P (2024) Notes on the genus *Syzygium* (Myrtaceae) from Cambodia, Thailand, Vietnam, China and Taiwan. PhytoKeys 244: 151–161. https://doi.org/10.3897/phytokeys.244.118657

Chantaranothai P, Parnell J (1994) A revision of *Acmena*, *Cleistocalyx*, *Eugenia s.s.* and *Syzygium* (Myrtaceae) in Thailand. Thai Forest Bulletin. Botany 21: 1–123. https://li01.tci-thaijo.org/index.php/ThaiForestBulletin/article/view/29683

Chen J, Craven LA (2007) Myrtaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China Vol. 13. Science Press, Beijing and Missouri Botanical Garden Press, St Louis, Missouri, 321–359.

Craven LA, Biffin E (2010) An infrageneric classification of *Syzygium* (Myrtaceae). Blumea 55(1): 94–99. https://doi.org/10.3767/000651910X499303

Gagnepain F (1914) *Eugenia* nouveaux d’Indo-Chine. Notulae Systematicae 3: 316–336. https://www.biodiversitylibrary.org/page/4519008#page/9/mode/1up

Gagnepain F (1917) Classification des *Eugenia*. Bulletin de la Société Botanique de France 23: 94–103. https://doi.org/10.1080/00378941.1917.10836016

Gagnepain F (1920) Myrtaceae. In: Lecomte H (Eds) Flore générale de l’Indochine 2. Masson, Paris, 788–864. https://www.biodiversitylibrary.org/page/31329242

Govaerts R, Sobral N, Ashton P, Barrie F, Holst BK, Landrum LL, Matsumoto K, Fernanda Mazine F, Nic Lughadha E, Proença C, et al. (2008) World Checklist of Myrtaceae. Kew Publishing, Royal Botanic Gardens, Kew, 1–455.

Hyland BP (1983) A revision of *Syzygium* and allied genera (Myrtaceae) in Australia. Australian Journal of Botany Supplementary Series 13(9): 1–164. https://doi.org/10.1071/BT8309001

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK. https://portals.iucn.org/library/node/7977

IUCN (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

Le TH, Nguyen TC, Tran TTN, Do ND, Ly NS (2024) *Syzygium ngheanense* (Myrtaceae: Myrtoideae), a new species of *Syzygium* from Central Vietnam. Phytotaxa 641: 228–234. https://doi.org/10.11646/phytotaxa.641.3.5

Merrill ED, Perry LM (1938) On the Indo-Chinese species of *Syzygium* Gaertner. Journal of the Arnold Arboretum 19: 99–116. https://doi.org/10.5962/p.185381

Parnell J, Chantaranothai P (2002) Myrtaceae. In: Santisuk T, Larsen K (Eds) Flora of Thailand Volume 7. The Forest Herbarium, Royal Forest Department, Bangkok, 778–914. https://www.researchgate.net/publication/255980114_Myrtaceae

Pham HH (2000) Cây cỏ Việt Nam-An Illustrated Flora of Vietnam, Vol. 2. Youth Publication, Ho Chi Minh City.

Shareef SM, Santhosh KES, Shaju T (2012) A new species of *Syzygium* (Myrtaceae) from the Southern Western Ghats of Kerala, India. Phytotaxa 71: 28–33. https://doi.org/10.11646/phytotaxa.71.1.5

Soh WK, Parnell J (2011) Three new species of *Syzygium* (Myrtaceae) from Indochina. Kew Bulletin 66: 557–564. https://doi.org/10.1007/s12225-011-9305-9

Soh WK, Parnell J (2015) A revision of *Syzygium* Gaertn. (Myrtaceae) in Indochina (Cambodia, Laos and Vietnam). Adansonia Sér. 3 37(2): 179–275. https://doi.org/10.5252/a2015n2a1

Soh WK, Sam HV, Parnell JAN (2024) Three new species of *Syzygium* Gaertn. (Myrtaceae) from Cambodia and Vietnam. Adansonia 46(4): 29–36. https://doi.org/10.5252/adansonia2024v46a4

Tagane S, Toyama H, Chhang P, Nagamasu H, Yahara T (2015) Flora of Bokor National Park, Cambodia I: Thirteen new species and one change in status. Acta Phytotaxonomica et Geobotanica 66: 95–135. https://doi.org/10.18942/apg.KJ00010001424

Tagane S, Dang S, Souladeth P, Nagamasu H, Toyama H, Naiki A, Fuse K, Tran H, Yang CJ, Prajaksood A, Yahara T (2018) Five new species of *Syzygium* (Myrtaceae) from Indochina and Thailand. Phytotaxa 375: 247–260. https://doi.org/10.11646/phytotaxa.375.4.1

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 [Eds] (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code). Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. https://doi.org/10.12705/Code.2018

Widodo P, Veldkamp J (2021) The confusing taxonomy and nomenclature of *Syzygium confusum* complex (Myrtaceae). Reinwardtia 20: 43–49. https://doi.org/10.14203/reinwardtia.v20i2.4160

Appendix 1

Specimens examined for comparison with the new species. Malaysia. Penang, Government Hill, 1871, *Maingay, A.C. 738* (TYPE) (K [K000800034]); 1871, *Maingay, A.C. 738/2* (TYPE) (K [K000800033]); Perak, Nov 1884, *Scortechini 491* (barcode-00071230); Perak, Taiping, *s.n.* (BM [BM000944235]), Benedetto Scortechini; Perak, Larut, *s.coll. s.n.* (K [K000800032]). **Indonesia**. Java, Jan 1910, *s. coll. 52* (K [K000800189]).

PhytoKeys

PhytoKeys 255: 85–102 (2025)
DOI: 10.3897/phytokeys.255.146942

Research Article

Festuca silana (Poaceae), a new species from the Sila plateau in Italy

Mattia Pallanza[1,2], Simone Orsenigo[1,2], Nicola Maria Giuseppe Ardenghi[3], Liliana Bernardo[4], Petr Šmarda[5], Petra Šarhanová[5], Graziano Rossi[1], Bruno Foggi[6]

1 *Department of Earth and Environmental Sciences, University of Pavia, Via Sant'Epifanio 14, 27100 Pavia, Italy*
2 *National Biodiversity Future Centre, 90133 Palermo, Italy*
3 *Botanic Garden, University Museum System, University of Pavia, Via Sant'Epifanio 14, 27100 Pavia, Italy*
4 *Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Pietro Bucci, 87030 Arcavacata di Rende (Cosenza), Italy*
5 *Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic*
6 *Department of Biology, University of Florence, Via G. La Pira 4, 50121 Firenze, Italy*
Corresponding author: Mattia Pallanza (mattia.pallanza02@universitadipavia.it)

Abstract

A new hexaploid species of fine-leaved fescue from *Festuca* sect. *Festuca* (*Festuca silana* Ardenghi, Pallanza & Foggi, **sp. nov.**) endemic to the Sila plateau is described. The new taxon shows morphological affinities with species of the *F. marginata* group from which it can be distinguished by higher ploidy, ecology, and leaf cross section anatomy. ddRADseq data suggests *F. silana* could be a local hexaploid descendant/derivate of the widespread diploid *F. marginata*. Its distribution is restricted to several localities in the Sila highlands of the Southern Apennines, Italy.

Key words: Calabria, endemic, fescue, flow cytometry, Italy, morphometry, new species, ploidy, RAD sequencing

Academic editor: Marcin Nobis
Received: 16 January 2025
Accepted: 5 February 2025
Published: 10 April 2025

Citation: Pallanza M, Orsenigo S, Ardenghi NMG, Bernardo L, Šmarda P, Šarhanová P, Rossi G, Foggi B (2025) *Festuca silana* (Poaceae), a new species from the Sila plateau in Italy. PhytoKeys 255: 85–102. https://doi.org/10.3897/phytokeys.255.146942

Introduction

Festuca L. (Poaceae, Loliinae) is one of the most species-rich genera of grasses (Poaceae), containing worldwide around 680 accepted species (Govaerts 2024). Within the *Festuca* genus, “fine-leaved fescues” represent the most important group, with approximately 450 species (Foggi and Tison 2014; Kellogg 2015). The taxonomy and systematics of *Festuca* has historically been regarded by botanists as difficult, due to the morphological convergence of unrelated taxa, phenotypic plasticity, complex nomenclature and a lack of clear universally accepted diagnostic features between taxa. The first solid foundation to modern taxonomic *Festuca* studies was posed by Eduard Hackel in his “*Monographia Festucarum Europaearum*” (Hackel 1882). Hackel introduced novelties in the approach to identification and classification of fescues, including the study of the leaf cross-section, one of the main investigation tools still in use in modern festucology. Since then, many scholars have dedicated their efforts toward the taxonomy and systematics of *Festuca*, integrating the initial morphometrical approach with more modern techniques stemming from technologicals and scientific advances. These include analysis of the relative genome size (Šmarda et al. 2008; Martínez-Sagarra et al. 2021), chromosome number (Šmarda and Kočí 2003; Ardenghi et al. 2016), DNA sequencing (Boeuf et al. 2022, Kriuchkova et al. 2023, Mucko et al. 2024) and electron microscopy (Ortúñez and Cano-Ruiz 2013). Although the implementations of these new methods emanate from improved floristic records and checklist (Roleček et al. 2019; Gudkova et al. 2023; Bednarska et al. 2024), to the conservation and applications of the taxa studied (Ardenghi et al. 2017), most recent works have focused on groups of species, using an integrated approach combining multivariate morphometry, ploidy level and genetics (Šmarda et al. 2007; Foggi et al. 2012; Ardenghi et al. 2024; Bednarska et al. 2024).

During an extensive sampling conducted across the European Mediterranean basin in 2014 by N.M.G. Ardenghi, an unusual taxon belonging to the fine-leaved fescues was collected in different localities of the Sila plateau (Italy). The entity was already known and reported by Sarfatti in the “Prodoromo della Flora della Sila” under *Festuca ovina* L. subsp. *laevis* Hack. var. *gallica* (Hack.) St.-Yves subvar. *costei* St.-Yves (≡ *Festuca costei* (St.-Yves) Markgr.-Dann.) based on the original identification by Markgraf-Dannenberg (Sarfatti 1959). However, this taxon has been overlooked until now. A preliminary study of morphology and leaf cross section anatomy of both fresh samples and herbaria specimens including the holotype for *F. costei*, led us to question the identification proposed by Markgraf-Dannenberg. In fact, these fescues differ from *F. costei* in a series of readily identifiable features, especially in the leaf cross-section (e.g. number of vascular bundles, development and organization of the sclerenchyma, etc.) and rather appear to be morphologically in between the *F. stricta* and the *F. marginata* groups. In particular, the Sila entity appears to have the typical cross sections of the former and the overall morphology of the latter.

Due to the unusual combination of leaf cross-section anatomy and general morphology, we initially thought it could be a taxon of the *F. stricta* group (i.e.: *F. stricta* Host, *F. rupicola* Heuff., *F. trachyphylla* (Hack.) R.P.Murray) which present similar patterns in the organization of the tiller leaves sclerenchyma. This is especially true for *F. trachyphylla*, which is also the only species from the stricta group to normally present 7 vascular bundles in the cross section, a shared characteristic with the Sila specimens. Nevertheless, the samples still share numerous morphological similarities (e.g. smooth leaf blades, glabrous spikelets, etc.) with *Festuca marginata* (Hack.) K. Richt., which occurs in similar types of habitats across Italy.

To better understand the relationships among these different taxa, we chose to apply an integrated approach combining classical morphometry, ploidy level analysis using flow cytometry and ddRADseq sequencing. The results from these combined analyses support that the taxon from the Sila plateau is a new endemic species.

Materials and methods

Materials

Overall, 177 individuals representing 25 populations of five different taxa (*F. marginata*, *F. rupicola*, *F. stricta*, *F. trachyphylla* and the proposed new species) ranging from the Alps to the Apennines and from fresh and herbarium material (BRNU, FI, G, MSNM, PAV; Herbaria codes follow Thiers 2024, updated continuously) were morphologically studied (Suppl. material 1: tables S4, S5). Specimens were carefully selected to ensure a balanced representation of the different taxa. Fifty of the freshly collected individuals from the same populations were also analyzed using flow cytometry.

Morphometric analysis

We selected 35 morphological characters for the analysis (Table 1), choosing those considered as diagnostic for fine-leaved fescues.

Measurements were performed according to the standards described in Foggi 1999 (which complies with Hackel 1882; Saint-Yves 1913; Ellis 1976; Wilkinson and Stace 1991) with minor modifications. To account for intraindividual variation, we chose to either keep the highest recorded value for the sample or to use the mean value calculated from three different measurements depending on the morphological character. Characters concerning leaf cross sections follow a design similar to Bednarska et al. (2024). Spikelet, floret and other microscopic characters were observed under a Eurotek NB-50T stereomicroscope at magnifications of 8x–10x. Leaf cross sections were studied with a Carl Zeiss Axiostar Plus microscope at magnifications of 40x–100x. Both microscopes were coupled with a camera (ToupTek USB3.0 Eyepiece Camera S3CMOS05000KPA) and all observed characters were measured using Toupview ver. 4.11.19728.20211022 software.

Each quantitative character was tested for normality of distribution within taxa using the Shapiro-Wilk test. Some characters had non-normal distribution for some taxa, however, since the chosen analyses have been shown to be robust to violation of the normality of distribution assumption (Klecka 1980), we decided to continue without transforming the data. To further support this decision, it has to be clear that non-fitted variables would have to be transformed as a whole, independently from the taxon, leading to a weakened perception of the actual morphological variability among taxa. Characters were also tested for significant correlations (>0.95) via Spearman's non-parametric coefficient. High correlation was found only between characters related to the tiller leaves' scabridity (Sk_Deg, Sk_Ext, Ep_Ind). Among them, we decided to keep only the density of the abaxial epidermis indumentum as observed in the cross section (Ep_Ind) in the analyses as it was the least subjective to measure. We also decided not to include the culm length and tiller leaf blade length (Clm_L and LfB_L respectively) due to their high dependency on environmental factors such as grazing, trampling and wildfires. However, these characters were systematically measured in all samples and used in the morphological description of the new species. After preliminary data manipulation, 31 of the original characters were used to perform the analyses.

A PCoA utilizing Gower's distances (Gower 1971) was used to visualize the pattern of morphological variation among the studied taxa. Following the PCoA, a jackknifed canonical discriminant analysis (CDA) was performed (Krzanowski 1990), taking into account the morphological groups individuated with the PCoA and the ploidy levels inferred with flow cytometry. All characters invariant within the groups were excluded from the CDA dataset (bringing down the characters used to 25) as it is one of the fundamental assumptions to avoid any type of distortion in Discriminant Analyses.

Table 1. Morphological characters used in multivariatue analyses and their coding. QD = quantitative discrete, QC = quantitative continuous, BI = binary, CO = ordinal.

Code	Character	Type
Clm_L	Culm length	QC
LfB_L	Tiller leaf blade length	QC
Pan_L	Panicle length	QC
Pan_Sk	Panicle scabridity	CO
Pan_Pb	Pubescence at the base of the panicle	CO
Clf_L	Culm leaf blade length	QC
Sh_Nd	Sheath to node distance	QC
InfBr_L	Inferior branch of the panicle length	QC
TilSh_Pb	Tiller sheaths pubescence	CO
Nd_Pr	Node pruinosity	BI
Sh_Pr	Tiller sheaths pruinosity	BI
Sh_Col	Tiller sheaths color	CO
Sk_Deg	Tiller leaves scabridity degree	CO
Sk_Ext	Percentage of tiller leaves scabrid surface	QC
Sc_Org	Sclerenchyma organization	CO
Sc_CT	Central sclerenchyma strand thickness	CO
Sc_MT	Marginal sclerenchyma strands thickness	CO
KlMR	Keel to middle rib distance	QC
Klmg	Keel to margins distance	QC
VB_N	Number of vascular bundles	QD
R_N	Number of accessory ribs	QD
Shp	Leaf cross-section outline shape	CO
Ep_Und	Presence of epidermal undulations	BI
Ep_Ind	Richness of epidermal indumentum	CO
LH	Longest hair in the abaxial surface of the cross-section	QC
Sp_L	Spikelet length	QC
LGl_L	Lower glume length	QC
UGl_L	Upper glume length	QC
UGl_hW	Upper glume half-width	QC
Lm_L	Lemma length	QC
Lm_hW	Lemma half width	QC
A_L	Awn length	QC
Gl_Pb	Glumes pubescence	CO
Lm_Pb	Lemma pubescence	CO
Sp_Sh	Spikelet shininess	CO

All statistical analyses were computed with R (R Core Team 2024) in RStudio 2024.4.2.764 (Posit team 2024) using the MorphoTools2 package (Šlenker et al. 2022).

Ploidy estimation

The ploidy level was measured in 50 samples (1–4 per population) using flow cytometry with DAPI dye. The youngest and most well-preserved leaves were selected from representative individuals of both fresh plants and herbarium vouchers (no older than one year). Samples were then co-chopped with the standard (*Lycopersicum esculentum* "Stupické polní tyčkové rané") in a Petri dish containing 0.5 mL Otto I buffer (0.1M citric acid, 0.5% Tween 20; Otto

1990) using a razor blade. The nuclei suspension was then filtered through a 50 µm nylon mesh before 1 mL of Otto II buffer (0.4M Na2HPO4 · 12H2O) supplemented with 2 µg/mL DAPI was added. Samples were analyzed with a CyFlow ML flow cytometer (Partec GmbH, Germany) equipped with a UV light-emitting diode (365 nm, Sysmex Partec GmbH) at the Department of Botany and Zoology, Masaryk University, Brno, Czech Republic. To confirm the ploidy level, choromosome counts were performed using the Fuelgen protocol. In brief, Squash preparations were made on root tips obtained from germinating seeds. The root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

DNA extraction and ddRAD sequencing

Genomic DNA was extracted from silica gel-dried leaves or herbarium specimens from 14 samples of the same species included in the morphometric analyses (Suppl. material 1: table S7). DNA quality was assessed by 1.5% agarose gel electrophoresis, and concentration was measured using a Qubit 2 Fluorometer with the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific).

The double digest restriction site-associated DNA (ddRAD) library preparation protocol was adapted from Sochor et al. (2024) with modifications. Briefly, 100 ng of genomic DNA was digested with SbfI-HF and MseI restriction enzymes in rCutSmart buffer (New England Biolabs) at 37 °C for 3 hours, followed by enzyme inactivation at 80 °C for 20 minutes. Immediately afterwards, P1 and P2 adapters, corresponding to the restriction sites of the respective enzymes, were ligated using T4 DNA ligase (New England Biolabs) at 16 °C overnight, with subsequent heat inactivation at 65 °C for 10 minutes. The P1 adapter for each sample contained a unique barcode for individual identification. The DNA concentration of each restricted sample was measured using the Qubit fluorometer, and equimolar amounts of digested DNA from all samples were pooled. The pooled samples were purified with 1.2 × SPRI magnetic beads and used as a template for PCR enrichment. PCR amplification was performed in four 20 µl reactions (18 cycles each) using Phusion HF PCR Mastermix (New England Biolabs) and standard Illumina P1-i5 (5’-AATGATACGGCGACCACCGA-3’) and P2-i7 (5’-CAAGCAGAAGACGGCATACGA-3’) primers. The protocol for each step is available in the Suppl. material 1: table S6. The final amplified products were size-selected using SPRI magnetic beads, with left and right side selections at 0.5 × and 0.9 × ratios, following the manufacturer’s protocol. The final ddRAD library was sequenced at the CEITEC facility (Brno, Czech Republic) on an Illumina NextSeq platform using a mid-output configuration with 300 cycles. Sequencing utilized a portion of the platform’s capacity, generating approximately 30,000,000 paired-end reads.

Phylogenomic analysis

Paired-end reads generated by Illumina sequencing of the ddRAD library were demultiplexed and analyzed using iPyRAD v.0.9.97 (Eaton and Overcast 2020). Quality control parameters were configured as follows: trimming was performed for bases with a quality score below Q20, allowing up to five low-quality bases per read. The Phred Q score offset was set to 33. The minimum read depth for base calling and majority-rule consensus was set to 6, with a maximum read depth per sample capped at 10,000. The sequence similarity threshold was specified as 0.90, permitting a maximum of one base mismatch in barcodes. Adapter sequences were strictly filtered out, and a minimum read length of 35 bp was required. Consensus sequence assembly was conducted with the following parameters: a maximum of six alleles per consensus sequence to accommodate diploid and polyploid species in the dataset; a maximum of 5% uncalled bases and 5% heterozygous sites per consensus sequence; and a minimum of seven samples sharing data at a given locus (allowing up to 46% missing data). Locus filtering parameters were set to allow a maximum of 20% SNPs per locus, up to eight indels per locus, and a maximum of 20% heterozygous sites per locus.

Phylogenomic relationships among individuals based on ddRAD data were inferred using RAxML v.8.2.12 (Stamatakis 2014). The GTRCAT substitution model was employed, and bootstrap analysis with 1,000 replicates was performed to construct a maximum likelihood tree. The resulting phylogeny was visualized using FigTree v.1.4.4 (Rambaut 2015).

Results

Morphometric analysis

The first two axes of the exploratory PCoA account for 45.23% of the variability. Three main clusters can be observed in the biplot (Fig. 1): species of *F. stricta* group, the Sila hexaploid and the diploid *F. marginata*. The hexaploid taxon from the Sila plateau appears morphologically as an in-between entity but still clearly distinct from the other two groups. In particular, the taxon seems to share with the species of the *F. stricta* group the tendency to develop decurrent to confluent sclerenchyma strands (Sc_Org), as well as presenting undulations between epidermal cells (Ep_Pap) and longer awns (A_L). However, these are the only similarities between the two groups, as the Sila specimens resemble more, on a superficial level, species of the *F. marginata* group. They lack numerous other features typical

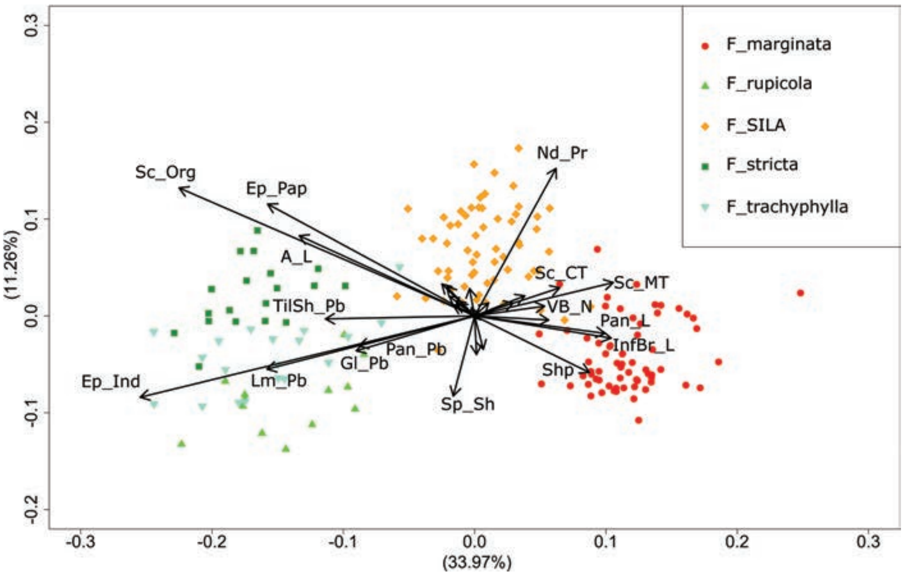

Figure 1. Biplot for the PCoA performed with Gower's distance.

of the *F. stricta* taxa, such as scabrid leaf blades due to the presence of barbs on the adaxial indumentum (Ep_Ind) and an overall pubescence observed in the panicle (Lm_Pb, Gl_PB, Pan_Pb) and tiller sheats (TilSh_Pb). Compared to the new species, *F. marginata* tends to develop discrete thickened sclerenchyma strands (Sc_Org, Sc_MT, Sc_CT), a larger number of vascular bundles (VB_N), bigger panicles(Pan_L, InfBr_L) and leaves in a more elongated conduplicate "V" shape (Shp). Finally, the Sila taxon seems to be overall a more pruinous plant (Nd_Pr, Sp_Sh) compared to all the other species analyzed. Based on the clustering of the taxa in the biplot, we decided to merge the species from the *Festuca stricta* aggregate into a single group to avoid distortions in the following discriminant analysis.

The jackknifed CDA (Fig. 2) fully supports the grouping hypothesis indicated by the scatterplot of the PCoA with 97.74% classification success in 177 samples (Table 2). Again, the new species appears as a distinct morphological in-between entity of the other considered species.

Table 2. Confusion matrix for the DA performed on the three morphological groups individuated by the PCoA.

Taxon	N	*F. marginata*	*F. sila*	*F. stricta* group	correct	%
F. marginata	65	65	0	0	65	100.00
F. silana	61	3	58	0	58	95.08
F. stricta group	51	0	1	50	50	98.04
Total	177	68	59	50	173	97.74

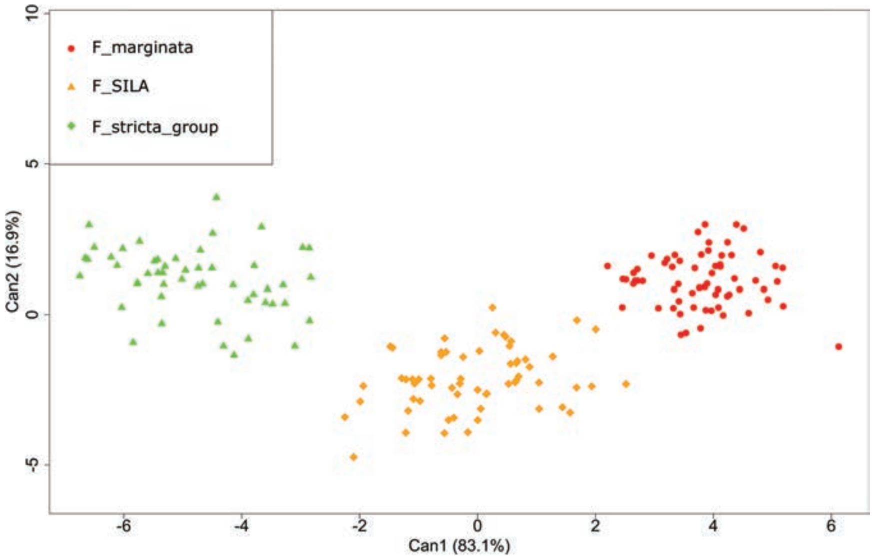

Figure 2. Scatterplot for the DA performed on the three morphological groups individuated by the PCoA.

Ploidy estimation

Overall, 50 of the freshly collected individuals were studied with flow cytometry. All 20 individuals of *F. stricta* s.l. (*F. stricta*, *F. trachyphylla* and *F. rupicola*) were hexaploid and all *F. marginata* samples were diploid (17 individuals) in accordance with previous works (Portal 1999; Arndt 2008, Šmarda et al. 2008; Ardenghi et al. 2016, 2024). All 13 individuals assumed as *F. silana* were hexaploid.

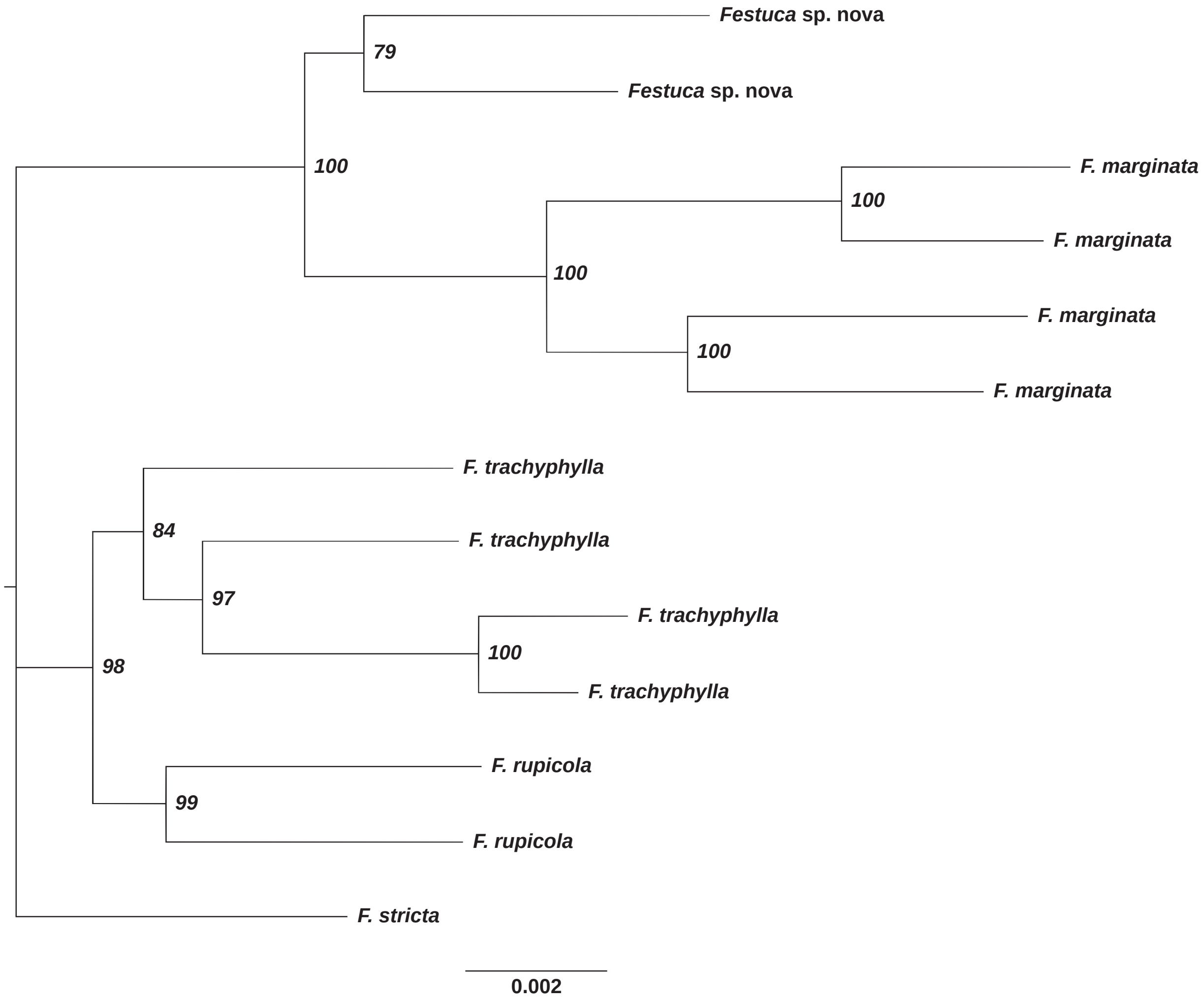

Figure 3. Phylogenetic tree of thirteen *Festuca* individuals, constructed using RAxML based on 2,713 loci and maximum likelihood estimation. The numbers in the nodes represent bootstrap values (%) from 1,000 replicates.

Phylogenomic analysis

A total of 67,353 loci were initially identified, which were reduced to 2,713 after filtering. The primary cause of this reduction was the requirement for a minimum of seven out of 13 samples to contain data for a given locus. Following the first analysis, one sample (F14) with low sequencing coverage was excluded based on quality assessment, and the analyses were repeated without this sample. The number of retained loci per sample ranged from 1,526 to 2,096. The resulting best tree effectively resolved each species, with high bootstrap support for most nodes (Fig. 3).

Taxonomic treatment

Festuca silana **Ardenghi, Pallanza & Foggi, sp. nov**
urn:lsid:ipni.org:names:77360044-1

Typus. ITALY • Sila piccola, Zagarise (Catanzaro), Latteria, Lato W del sentiero, Pendici SW del M. Gariglione (WGS84: 39°07'29.4"N, 16°37'32.8"E), 1587 m, prateria meso-xerofila con roccia granitica affiorante, assieme a *Patzkea paniculata*, 06.07.2014, *N. Ardenghi & L. Bernardo*. (***holotype*****:** PAV-150000!; ***isotypes*****:** FI!, CLU!, W!, G!).

Italy • Sila grande, Spezzano della Sila (CS), Vaccarizzo, Sponda S del Lago Cecita (WGS84: 39°22'13.5"N, 16°30'40.1"E), 1150 m, prato arido con *Astragalus parnassi* subsp. *clabricus*, su sabbie granitiche con affioramenti rocciosi, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*****:** PAV-150002!, PAV-150003!, FI!, CLU!, W!, G!).

Italy • Sila grande, Casali del Manco (CS), Lago Ariamacina, Sponda N del lago (WGS84: 39°20'00.8"N, 16°32'39.0"E), 1327 m, prato arido con *Astragalus parnassi* subsp. *clabricus*, su sabbie granitiche, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*****:** FI!, CLU!, W!, G!).

Italy • Sila grande, San Giovanni in Fiore (CS), Carlomagno, A W della SP211 (WGS84: 39°16'58.1"N, 16°32'36.1"E), 1535 m, prato mesofilo a *Patzkea paniculata*, su substrato granitico con spesso strato di suolo, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*****:** PAV-150001!, FI!, CLU!, W!, G!).

Description. Perennial herb, caespitose to densely caespitose, occasionally pruinose. Tiller shoots intravaginal. Culms (32.0–) 44.7–57.2 (–69.5) cm long and (0.52–) 0.77–1.04 (–1.29) mm in diameter, erect cylindrical, canaliculated, smooth to the touch, with 1 (–2) visible nodes, dark brown to black in color, located in its lower third. Cauline leaves 1 (–2), (1.6–) 2.6–4.0 (–5.3) cm long, sheathing the culm for (5.8–) 8.0–10.3 (–12.8) cm. Basal leaf sheath glabrous, on extremely rare occasions with few sparse hairs, open down to the base, yellowish in color, more rarely veined in red. Ligula 0.5–0.8 mm long, membranaceous, truncate, fringed, with two auricles at the sides. Basal leaf blade (7–) 11.8–21.6 (–31.7) cm long and (0.75–) 0.94–1.06 (–1.36) mm in diameter, smooth, somewhat rigid, conduplicate, bright to dark green. Cross section outline in an open U-V shape. Subepidermal sclerenchyma organized in 3 main strands located at the margins and keel of the leaf blade, thickened and often decurrent or accompanied by secondary strands opposing vascular bundles, forming an interrupted or irregular complete ring. Vascular bundles 7(–9). Ribs 2 (–4). Abaxial surface of the leaf blade covered with a dense indumentum of (0.03–) 0.04–0.07 (–0.13) mm long hairs. Adaxial surface smooth, with small undulations between epidermal cells. Panicle (4–) 6.4–8.6 (–12.5) cm long, cylindrical to pyramidal during anthesis, dense, with 7–27 spikelets; branches 1–5, simple, antrorsely scabrid; nodes 7–12. Spikelets (6.24–) 7.33–8.13 (–8.87) long, laterally flattened, elliptic, green, with (3–) 4–6 (–8) fertile florets. Glumes 2, unequal, lanceolate, glabrous or rarely with few sparse hairs on the margins and apex. Lower glume (2.21–) 2.55–3.12 (–3.90) mm long, with a single nerve. Upper glume (2.46–) 3.68–4.31 (–5.12) mm long, 3-nerved. Lemma (3.82–) 4.81–5.26 (–5.83) mm long, lanceolate, glabrous or more rarely with few sparse hairs near the apex, terminating in an apical awn (1.23–) 1.83–2.52 (–3.30) mm long. Palea 4.53–5.71 mm long, lanceolate, bifid, with 2 finely dentated keels. Anthers 3, (1.72–) 2–2.44 (–2.93) mm long, yellow to orange in color. Ovary glabrous. Stygmas 2, with a feather-like shape. Lodicules 2, bilobed, 0.83–1.06 mm long. Caryopsis 2.42–3.85 mm long, brown to dark orange at maturity, adherent to the palea (Fig. 4). Somatic chromosomes 42 (2n = 6x).

Eponym. The new species is named after the Sila plateau where it typically occurs.

Diagnosis. Differt a *Festuca costei* (St.-Yves ex Litard.) Markgr.-Dann. crassiore sclerenchyma in laminarum margine et carina, plerumque 7 (non (7–) 9–11) fasciculis vascularibus, densioribus et longioribus trichomatibus in abaxiale superficie, minutis undulationibus inter epidermidis cellulas, 42 (non 28) chromosomatibus.

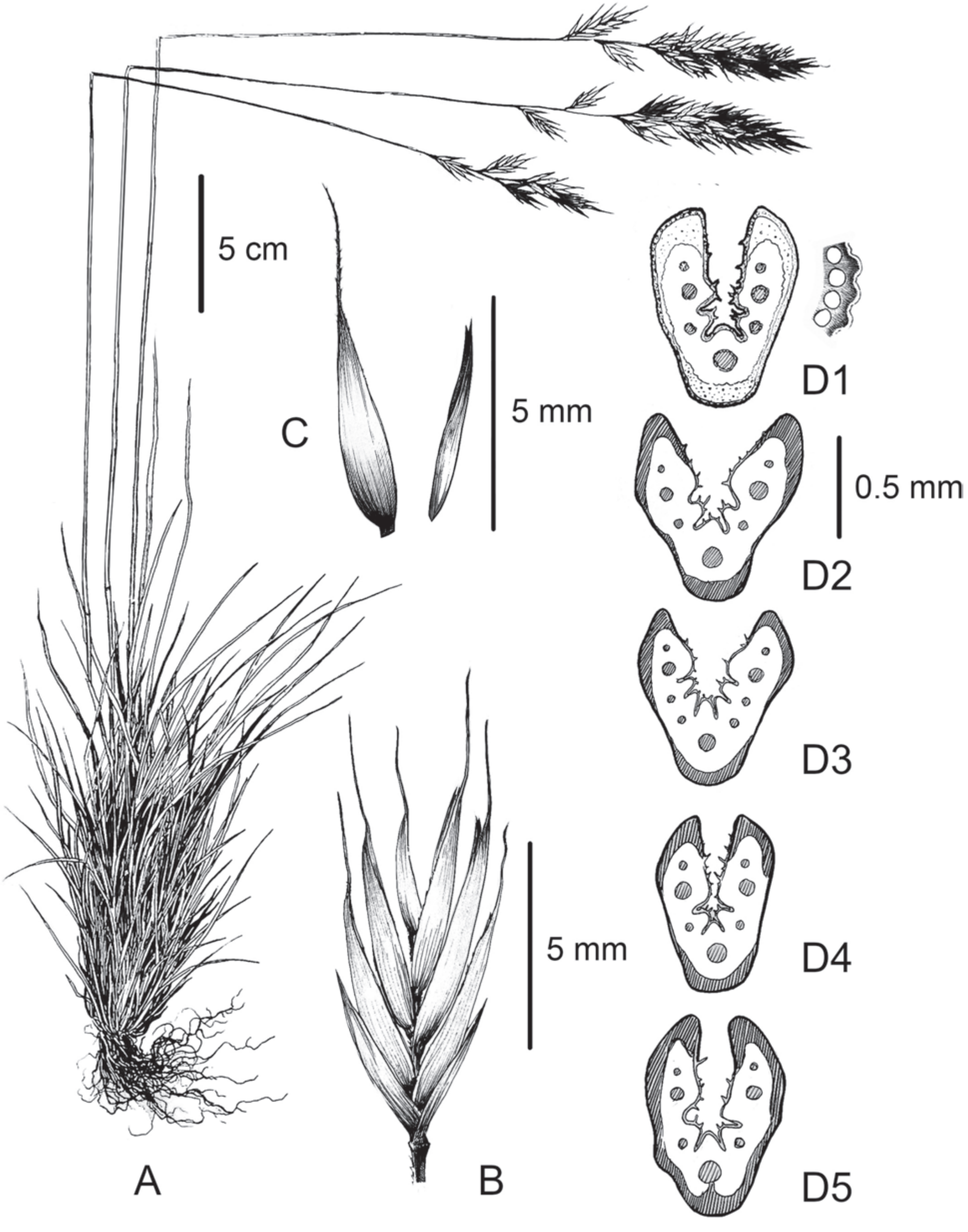

Figure 4. Illustration of *Festuca silana* Pallanza, Ardenghi & Foggi based on the specimens from the locus classicus **A** overall habitus and morphology of the species **B** detailed appearance of the spikelet **C** detailed view of lemma and palea **D1** leaf cross-section of the holotype **D2–D5** different leaves cross-sections showcasing intraspecific variability.

It differs from *Festuca costei* (St.-Yves ex Litard.) Markgr.-Dann. for the thicker sclerenchyma at margins and keel of the leaves, vascular bundles are rarely more than 7 (compared to the (7–) 9–11 in *F. costei*). Abaxial indumentum is more dense and with longer trichomes compared to *F. costei*. It also presents small undulations between epidermal cells. Somatic chromosomes 2n = 6x = 42 instead of 2n = 4x = 28 in *F. costei*.

Differt a *Festuca marginata* (Hack.) K.Richt. sclerenchyma plerumque decurrente vel completum anulum fingente potius quam in tribus discretis filis ordinato, fasciculis vascularibus raro plus quam 7 (non (7-) 9–11 ut solet in F. marginata), longioribus trichomatibus in densiore abaxiale superficie, longioribus aristis, undulationibus inter epidermidis cellulas, 42 chromosomatibus, non 14.

It differs from *Festuca marginata* (Hack.) K.Richt. in the sclerenchyma, usually decurrent or up to forming a complete ring instead of being organized in three discrete strands. Vascular bundles are rarely more than 7 (compared to the (7–) 9–11 in *F. marginata*). Abaxial indumentum is richer and with longer trichomes compared to *F. marginata*. Awns longer compared to *F. marginata*. Undulations are present in between epidermal cells. Somatic chromosomes 2n = 6x = 42 instead of 2n = 2x = 14 in *F. marginata*.

Differt a *Festuca trachyphylla* (Hack.) R.P.Murray sclerenchyma prope margines et carinam crassiore; foliis numquam scabris et colore viridi clariore (non glauco ut solet in *F. trachyphylla*); spiculis numquam pubescentibus; foliarum vaginis quam saepissime glabris (non pubescentibus ut solet in *F. trachyphylla*).

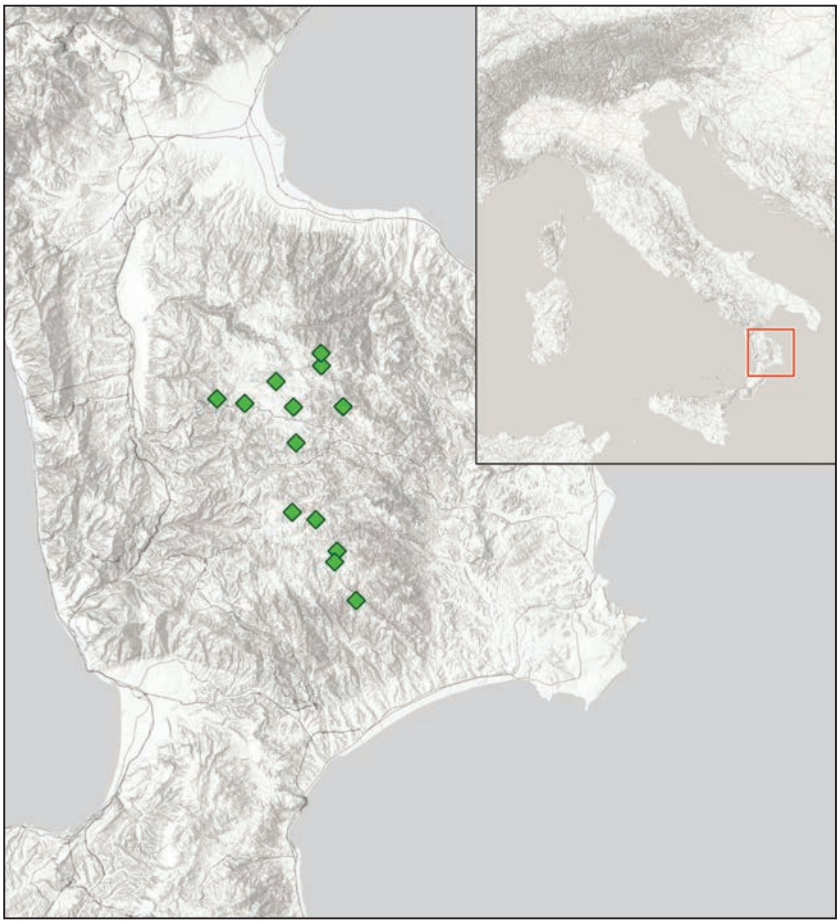

Figure 5. Map of the Sila and known stations of *F. silana* occurring within the plateau.

It differs from *Festuca trachyphylla* (Hack.) R.P.Murray in the sclerenchyma, thicker in the correspondence of margins and keel, the leaf blades never scabrid and of a brighter green color (opposed to the glaucus of *F. trachyphylla*). Spikelets are never pubescent. Leaf sheaths are glabrous except for extremely rare exceptions (opposed to the commonly pubescent in *F. trachyphylla*).

Distribution and ecology. The species is only known from the Sila highland, currently from 13 populations (Fig. 5). It mainly grows in grassland and pasture communities (*Anthemidetalia calabricae* Brullo, Scelsi & Spampinato, 2001) on granitic sands in xerophilic to mesophilic conditions at altitudes between 1100 m and 1600 m. *F. silana* typically occurs in grasslands of xerophile to mesophile conditions, along with other *Festuca* taxa such as *F. marginata* subsp. *marginata*, *F. trachyphylla*, *F. rubra* subsp. *commutata* and *F. cyrnea*.

Conservation status. Although the plant has a distribution limited to the Sila plateau, it is one of the dominant species in the grasslands that it inhabits and lacks any particular threat that could cause a decline in the population. Therefore, it had to be considered as Least Concern (LC) according to IUCN (2012).

Key to the studied species

1 Sclerenchyma strands decurrent sometimes with accessory strands or confluent in an irregular complete ring. Spikelets 7–8.5 mm. Awns usually > 2 mm long. Hexaploid plants **2**

– Sclerenchyma in three discrete strands, never decurrent. Spikelets 6–7 mm. Awns short, generally < 2 mm. Diploid plants. ....***F. marginata* subsp. *marginata***

2 Leaf scabrid, at least in the apicat/upper part. Spikelets and leaf sheaths generally pubescent. Plants glaucous to dark green. **3**

– Leaves completely glatt/smooth, never scabrid. Spikelets glabrous. Leaf sheaths usually glabrous, only very rarely with sparse hair. Plant bright/fresh green. .... ***F. silana***

3 Tiller leaves with 5(rarely 7) vascular bundles. Sclerenchyma of regular thickness throughout its length. Plants of natural areas. **4**

– Tiller leaves with 7–9 (rarely 5) vacular bundles. Sclerenchyma irregularly thickened throughout its length. Plant typical of synantropic or disturbed habitats. ....***F. trachyphylla***

4 Sclerenchyma generally forming a thick continuous ring, more rarely partly interrupted. Leaves strongly scabrid. ....***F. stricta***

– Sclerenchyma in three decurrent strands at the margins and keel, more rarely with accessory strands opposed to the vascular bundles. Leaves moderately to weakly scabrid. ....***F. rupicola***

Discussion

Despite the superficial similarities with other taxa of the *Festuca marginata* group and *F. trachyphylla*, our findings fully support *F. silana* as a standalone species. Attempts to identify the samples of *F. silana* with current keys of the Italian flora led to *F. costei* due to the high importance of the sclerenchyma ring and the general appearance of plants resembling the *F. marginata* group. However, many morphological characters neglected by the keys (such as the number of vascular bundles, the presence of undulations in-between epidermal cells, shorter arms of the leaf cross-section, etc.) easily emerge when looking at the specimens of the two taxa side by side. *F. costei* (= *F. arvernensis* Auquier, Kerguélen & Markgr.-Dann. subsp. *costei* (St.-Yves) Auquier & Kerguélen) has been described for the Massif Central in France and never reported for the Apennines. The separation between the two taxa was also confirmed by the ploidy inference via flow cytometry. *F*. *silana* is always hexaploid, thereby excluding any possibility of it being a particular morphotype of *F. costei*, which has been consistently reported as tetraploid (Auquier and Kerguélen 1977; Portal 1999; Šmarda et al. 2008). Additionally, although *F*. *costei* appears in the most recent checklists of the Italian vascular flora (Bartolucci et al. 2018, 2024), it has been reported for a single locality in the Maritime Alps (Piedmont, Italy; Foggi et al. 2017), but we could not trace any herbarium specimens supporting this data. Moreover, we visited the locality reported by Foggi et al. (2017) and could not find any individuals of this taxon here. Finally, biogeographical considerations were quite weak in supporting this determination and a disjunction between Massif Central – Southern Apennines is not very plausible. In light of these consideration, *F*. *costei* is not a component of the flora of Italy.

Despite the shared hexaploidy and similar leaf cross-sections' anatomy, fescues of the *F. stricta* group differ from *F. silana* in their commonly pubescent tiller leaves' sheaths and spikelets. Also, species of the *F. stricta* group commonly display some level of scabridity in the tiller leaf blades, which never occurs in *F. silana*. Finally, *F. silana* differs from *F. marginata* from the strong morphological distinction in leaf cross section anatomy, especially in sclerenchyma development and structure, as well as for the ploidy level (diploid vs hexaploid). The morphological differences can be attributed to the different ploidy levels, as it has been shown that an increase in ploidy level influences different morphological characters in fine-leaved fescues (Rewicz et al. 2018).

It is reasonable to think that *F. silana* may have originated from autopoliploidy of *F*. *marginata*, which is also present in the Sila plateau and sometimes even within the same localities. This hypothesis is supported by the ddRADseq sequencing results. It should be noted that the *F. marginata* group has recently been revised in Central-Southern Europe (Ardenghi et al. 2024) and all the previously described taxa have been reduced to two single diploid subspecies reflecting major differences in morphology and genome size between the populations in Greece (considered as *Festuca marginata* subsp. *heldreichii* (Hack.) Ardenghi & Foggi) and the remaining European populations (reported under *Festuca marginata* (Hack.) K. Richt. subsp. *marginata*). This newly discovered taxa from the Southern Apennines highlights the need for continued taxonomic research to broaden the understanding of plant diversity of critical groups such as *Festuca* in the Apennines.

Acknowledgments

The authors would like to thank Dr. Jakub Šmerda for helping with DNA isolations, Lucie Horová for the help with the flow cytometry measurements and Noemi Frulio for illustrating the new species. We also would like to thank Dr. Fiona J. White for checking the English of this manuscript.

The Core Facility Genomics of CEITEC Masaryk University is gratefully acknowledged for obtaining the sequencing data presented in this paper.

Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The authors MP and SO were funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union Next Generation EU, Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP F13C22000720007, Project title : "National Biodiversity Future Center - NBFC".

Author contributions

Conceptualization: SO, MP, NMGA, PŠ, BF. Data curation: PŠ, LB, MP. Formal analysis: MP, PŠ, PŠ. Funding acquisition: GR, PŠ, SO. Investigation: PŠ, NMGA, LB, MP, BF. Methodology: LB, BF, NMGA, MP, PŠ, PŠ. Resources: BF, LB, PŠ. Supervision: GR, SO, BF. Writing - original draft: PŠ, SO, LB, BF, NMGA, GR, MP, PŠ. Writing - review and editing: PŠ, MP, GR, PŠ, NMGA, BF, LB, SO.

Author ORCIDs

Mattia Pallanza https://orcid.org/0009-0006-7734-6191
Simone Orsenigo https://orcid.org/0000-0003-0348-9115
Nicola Maria Giuseppe Ardenghi https://orcid.org/0000-0002-4366-5729
Liliana Bernardo https://orcid.org/0000-0002-6703-6409
Petr Šmarda https://orcid.org/0000-0003-1048-9199
Petra Šarhanová https://orcid.org/0000-0002-1006-4003
Graziano Rossi https://orcid.org/0000-0002-5102-5019
Bruno Foggi https://orcid.org/0000-0001-6451-4025

Data availability

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

References

Ardenghi NMG, Šmarda P, Calbi M, Coppi A, Lastrucci L, Lazzaro L, Mugnai M, Quercioli C, Rossi G, Foggi B (2024) Revision of the Festuca marginata "group" (Festuca sect. Festuca, Poaceae) in Southern Europe, with special reference to France, Italy and

Greece. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 158(5): 1192–1214. https://doi.org/10.1080/11263504.2024.2395870

Ardenghi NMG, Fiorini G, Rossi G, Foggi B (2016) Chromosome numbers and karyomorphology of Festuca circummediterranea Patzke group (Poaceae, Loliinae). Phytotaxa 263(3): 255–269. https://doi.org/10.11646/phytotaxa.263.3.6

Ardenghi NMG, Foggi B, Orsenigo S, Maggioni L, Cauzzi P, Rossi G (2017) Festuca and allied genera (Poaceae) as Crop Wild Relatives: Checklists and Red Lists are urgently required. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 151(1): 6–8. https://doi.org/10.1080/11263504.2016.1165753

Arndt S (2008) Novelties in the Festuca valesiaca group (Poaceae) from the central Alps. Plant Systematics and Evolution 271(3–4): 129–142. https://doi.org/10.1007/s00606-007-0588-0

Auquier P, Kerguélen M (1977) Un groupe embrouillé de Festuca (Poaceae): Les taxons désignés par l'épithète "glauca" en Europe occidentale et dans les régions voisines. Lejeunia 89: 1–82.

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Ardenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, Barberis G, Bernardo L, Bouvet D, Bovio M, Cecchi L, Di Pietro R, Domina G, Fascetti S, Fenu G, Festi F, Foggi B, Gallo L, Gottschlich G, Gubellini L, Iamonico D, Iberite M, Jiménez-Mejías P, Lattanzi E, Marchetti D, Martinetto E, Masin RR, Medagli P, Passalacqua NG, Peccenini S, Pennesi R, Pierini B, Poldini L, Prosser F, Raimondo FM, Roma-Marzio F, Rosati L, Santangelo A, Scoppola A, Scortegagna S, Selvaggi A, Selvi F, Soldano A, Stinca A, Wagensommer RP, Wilhalm T, Conti F (2018) An updated checklist of the vascular flora native to Italy. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 152(2): 179–303. https://doi.org/10.1080/11263504.2017.1419996

Bartolucci F, Peruzzi L, Galasso G, Alessandrini A, Ardenghi NMG, Bacchetta G, Banfi E, Barberis G, Bernardo L, Bouvet D, Bovio M, Calvia G, Castello M, Cecchi L, Del Guacchio E, Domina G, Fascetti S, Gallo L, Gottschlich G, Guarino R, Gubellini L, Hofmann N, Iberite M, Jiménez-Mejías P, Longo D, Marchetti D, Martini F, Masin RR, Medagli P, Peccenini S, Prosser F, Roma-Marzio F, Rosati L, Santangelo A, Scoppola A, Selvaggi A, Selvi F, Soldano A, Stinca A, Wagensomme RP, Wilhalm T, Conti F (2024) A second update to the checklist of the vascular flora native to Italy. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology 158(2): 219–296. https://doi.org/10.1080/11263504.2024.2320126

Bednarska I, Cwener A, Šmarda P (2024) Festuca makutrensis (Poaceae) in the flora of the Eastern Europe. Plant Systematics and Evolution 310(5): 33. https://doi.org/10.1007/s00606-024-01913-2

Boeuf R, Hardion L, Šmarda P, Lazare JJ, Thébaud G, Gregor T, Portal R, Höcker R, Mazas SR, Besnard G, Holveck P (2022) Des Carpates aux Pyrénées, centrées sur l'Alsace et les Vosges, analyses génétiques de quelques fétuques à feuilles fines (Festuca sect. Festuca) estimées d'intérêt régional à européen: Considérations taxinomiques et syntaxinomiques. Botanique 8: 37–133.

Brullo S, Scelsi F, Spampinato G (2001) La vegetazione dell'Aspromonte. Studio fitosociologico. Laruffa editore, Reggio Calabria.

Eaton DA, Overcast I (2020) ipyrad: Interactive assembly and analysis of RADseq datasets. Bioinformatics 36(8): 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Ellis RP (1976) A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf blade as viewed in transverse section. Bothalia 12(1): 65–109. https://doi.org/10.4102/abc.v12i1.1382

Foggi B, Tison JM (2014) Festuca L. In: Tison JM, de Foucault B (Eds) Flora Gallica. Flore de France. Biotope Éditions, Mèze, 236–248.

Foggi B, Rossi G, Signorini MA (1999) The Festuca violacea aggregate in the Alps and Apennines (central southern Europe). Canadian Journal of Botany 77(7): 989–1013. https://doi.org/10.1139/b99-054

Foggi B, Parolo G, Šmarda P, Coppi A, Lastrucci L, Lakušic D, Eastwood R, Rossi G (2012) Revision of the Festuca alpina group (Festuca section Festuca, Poaceae) in Europe. Botanical Journal of the Linnean Society 170(4): 618–639. https://doi.org/10.1111/j.1095-8339.2012.01313.x

Foggi B, Rossi G, Ardenghi NMG, Parolo G (2017) Gen. Festuca. In: Pignatti S (Ed.) Flora d'Italia 1. Edagricole, Milano, 550–582.

Govaerts R [Ed.] (2024) WCVP: World Checklist of Vascular Plants. Facilitated by the Royal Botanic Gardens, Kew. https://doi.org/10.48580/dglq4-4nz

Gower JC (1971) A general coefficient of similarity and some of its properties. Biometrics 27(4): 623–637. https://doi.org/10.2307/2528823

Gudkova PD, Kriuchkova EA, Shmakov AI, Nobis M (2023) Preliminary checklist of the genus *Festuca* L. (Loliinae, Pooideae, Poaceae) in the Altai Mountains with outlines for further studies. PhytoKeys 234: 229–274. https://doi.org/10.3897/phytokeys.234.105385

Hackel E (1882) Monographia Festucarum Europaearum. Theodor Fischer, Kassel. https://doi.org/10.5962/bhl.title.15610

IUCN (2012) IUCN Red List categories and criteria: version 3.1, 2nd edn. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

Kellogg EA (2015) Flowering Plants. Monocots. Poaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants 13. Springer. https://doi.org/10.1007/978-3-319-15332-2

Klecka WR 1980 Discriminant analysis (No. 19) Sage University Paper Series on Quantitative Applications in the Social Sciences, 07–019.

Kriuchkova EA, Baiakhmetov E, Nobis M, Gudkova PD (2023) First insight into the phylogeny of fine-leaved Festuca in the Altai Mountain Country based on genome-wide genotyping. Ecology and Evolution 13(4): e9943. https://doi.org/10.1002/ece3.9943

Krzanowski WJ (1990) Principles of multivariate analysis. Clarendon Press, Oxford.

Martínez-Sagarra G, Castro S, Mota L, Loureiro J, Devesa JA (2021) Genome size, chromosome number and morphological data reveal unexpected infraspecific variability in Festuca (Poaceae). Genes 12(6): 906. https://doi.org/10.3390/genes12060906

Mucko M, Lakušić D, Frajman B, Bogdanović S, Doboš M, Ljubičić I, Kuzmanović N, Rešetnik I (2024) Diversification of fescues (Festuca sect. Eskia, Poaceae), a key-component of southern European mountainous grasslands. Taxon 73(6): 1370–1390. https://doi.org/10.1002/tax.13278

Ortúñez E, Cano-Ruiz J (2013) Epidermal micromorphology of the genus Festuca L. subgenus Festuca (Poaceae). Plant Systematics and Evolution 299(8): 1471–1483. https://doi.org/10.1007/s00606-013-0809-7

Otto F (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: Crissman HA, Darzynkiewicz Z (Eds) Methods in Cell Biology 33: 105–110. https://doi.org/10.1016/S0091-679X(08)60516-6

Portal R (1999) Festuca de France. Vals-près-Le-Puy.

Posit team (2024) RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. http://www.posit.co/

R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rambaut A (2015) FigTree: Tree figure drawing tool. http://tree.bio.ed.ac.uk/software/figtree/

Rewicz A, Tomczyk PP, Kiedrzyński M, Zielińska KM, Jędrzejczyk I, Rewers M, Kiedrzyńska E, Rewicz T (2018) Morphometric traits in the fine-leaved fescues depend on ploidy level: The case of Festuca amethystina L. PeerJ 6(2): 5576. https://doi.org/10.7717/peerj.5576

Roleček J, Dřevojan P, Šmarda P (2019) First record of Festuca amethystina L. from the Transylvanian Basin (Romania). Contributii Botanice 54: 91–97. https://doi.org/10.24193/Contrib.Bot.54.6

Saint-Yves A (1913) Les Festuca de la section Eu-Festuca et leurs variations dans les Alpes-Maritimes. Annuaire Conservatoiure et Jardin Botanique de la Ville de Geneve 17: 1–218. https://doi.org/10.5962/bhl.title.15432

Sarfatti G (1959) Prodromo della Flora della Sila (Calabria). Webbia 15(1): 169–248. https://doi.org/10.1080/00837792.1959.10669697

Šlenker M, Koutecký P, Marhold K (2022) MorphoTools2: An R package for multivariate morphometric analysis. Bioinformatics 38(10): 2954–2955. https://doi.org/10.1093/bioinformatics/btac173

Šmarda P, Kočí K (2003) Chromosome number variability in Central European members of the Festuca ovina and F. pallens groups (sect. Festuca). Folia Geobotanica 38(1): 65–95. https://doi.org/10.1007/BF02803128

Šmarda P, Šmerda J, Knoll A, Bureš P, Danihelka A (2007) Revision of central European taxa of Festuca ser. Psammophilae Pawlus: Morphometrical, karyological and AFLP analysis. Plant Systematics and Evolution 266(3–4): 197–232. https://doi.org/10.1007/s00606-007-0532-3

Šmarda P, Bureš P, Horová L, Foggi B, Rossi G (2008) Genome Size and GC Content Evolution of Festuca: Ancestral Expansion and Subsequent Reduction. Annals of Botany 101(3): 421–433. https://doi.org/10.1093/aob/mcm307

Sochor M, Šarhanová P, Duchoslav M, Konečná M, Hroneš M, Trávníček B (2024) Plant kleptomaniacs: Geographical genetic patterns in the amphi-apomictic Rubus ser. Glandulosi (Rosaceae) reveal complex reticulate evolution of Eurasian brambles. Annals of Botany 134(1): 163–178. https://doi.org/10.1093/aob/mcae050

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Thiers BM (2024) [updated continuously] Index Herbariorum. https://sweetgum.nybg.org/science/ih/

Wilkinson MJ, Stace CA (1991) A new taxonomic treatment of the Festuca ovina L. aggregate (Poaceae) in the British Isles. Botanical Journal of the Linnean Society 106(4): 347–397. https://doi.org/10.1111/j.1095-8339.1991.tb02298.x

Supplementary material 1

Occurrences, studied specimens, protocols

Authors: Mattia Pallanza, Orsenigo Simone, Petr Šmarda, Petra Šarhanová

Data type: xlsx

Explanation note: Excel file with a guide sheet describing the content of the different other sheets. A List of herbaria specimens studied as an exploratory part of the research, datasets for the PCoA and DA, Protocols and information on the samples used in the DNA extraction and ddRAD sequencing are available.

Link: https://doi.org/10.3897/phytokeys.255.146942.suppl1

PhytoKeys 255: 103–112 (2025)
DOI: 10.3897/phytokeys.255.142079

Research Article

Sinocrassula obliquifolia (Crassulaceae), a new species from China

Rong-Juan Li[1*], Jing Zhao[1*], Shao-Li Fang[2], Chuan-Jie Huang[1], Miao Luo[1], Zhao-Rong He[1], Xin-Mao Zhou[1], Jia-Guan Wang[1]

1 *School of Ecology and Environmental Science & School of Life Science, Yunnan University, Kunming, 650504, Yunnan, China*
2 *Kunming Coal Design and Research Institute, Baita Road, Panlong District, Kunming, 650011, Yunnan, China*
Corresponding authors: Zhao-Rong He (zhrhe@ynu.edu.cn); Xin-Mao Zhou (xinmao.zhou@ynu.edu.cn); Jia-Guan Wang (wangjiaguan@ynu.edu.cn)

Abstract

Based on a comprehensive morphological and molecular data analysis, we have confirmed and described a new species within the genus *Sinocrassula*, which is distributed in Sichuan Province, China. Morphologically, the new species resembles *Sinocrassula diversifolia* and *S. indica*, but it is distinctly different from them in its asymmetrical leaves, leaf apex with glands, triangular petals that are white at the base and adorned with dense purple-red stripes and spots on the surfaces upward, and rectangular nectar scales. A phylogenetic analysis utilizing four plastid markers and one nuclear marker supports the conclusion that the new species is sister to *S. ganluoensis*.

Key words: Hengduan mountains, phylogeny, rosette, species diversity, taxonomy

OPEN ACCESS

Academic editor: Takuro Ito
Received: 18 November 2024
Accepted: 17 March 2025
Published: 14 April 2025

Citation: Li R-J, Zhao J, Fang S-L, Huang C-J, Luo M, He Z-R, Zhou X-M, Wang J-G (2025) *Sinocrassula obliquifolia* (Crassulaceae), a new species from China. PhytoKeys 255: 103–112. https://doi.org/10.3897/phytokeys.255.142079

Introduction

Crassulaceae are a morphologically diverse and systematically complex group of angiosperms, comprising 35 genera and approximately 1,400 species (Berger 1930; Eggli 2003; Thiede and Eggli 2007; Messerschmid et al. 2020). Known for their heat resistance and rapid growth, members of the Crassulaceae family are highly adaptable and easy to cultivate. This adaptability makes them excellent for soil improvement and essential components in vertical gardens and green roofs (Zhu 2015). *Sinocrassula* A. Berger is a small genus within the family Crassulaceae, comprising approximately ten species distributed across various regions, including Bhutan, China, India, Nepal, Pakistan, Sikkim, and Vietnam (Wang et al. 2012, 2022; Fu and Ohba 2013; Averyanov et al. 2014). Despite the distinct characteristics of *Sinocrassula*, defining monophyletic groups within the genus has proven to be extremely challenging due to the frequent intergradation of morphological traits among taxa, as well as the extensive diversity in morphology and growth habits (Fu and Ohba 2013). Some species of *Sinocrassula* (e.g., *S. indica*) have been used as traditional medicine, or enjoyed as a vegetable and infused in herbal teas (Zhao et al. 2004; Xie and Yoshikawa 2012). In recent years, an increasing number of species of *Sinocrassula* have been discovered in Asia (e.g., *S. jiaozishanensis* Chao Chen, J. Guan Wang & Z.R. He, *S. vietnamensis* Aver. & V.V. Byalt) (Averyanov et al.

---

[*] These authors contributed equally to this work.

2014; Wang et al. 2022). The discovery of new species may provide more medicinal resources and increase the species diversity of *Sinocrassula*.

In July 2023, we conducted two field surveys in Sichuan Province, where we identified two populations of *Sinocrassula* in Shimian and Hanyuan counties. After carefully culturing the plants and performing a morphological study, we discovered that these populations are distinctly different from all other species within the genus. Additionally, we conducted a phylogenetic analysis, which indicated that these populations form a well-supported clade that is sister to *S. ganluoensis* (Li et al. 2024). We propose those populations as a new species of *Sinocrassula*.

Materials and methods

Morphological studies

The living plants of the new species were cultivated in the greenhouse at Yunnan University. Plant morphologies were photographed using a Nikon SMZ1270 stereo microscope (Nikon). Morphological comparisons among the new species and its related species were from field observations, herbarium investigations, and the literature (e.g., Fu and Ohba 2013; Averyanov et al. 2014; Wang et al. 2022). Voucher specimens of the new species have been deposited in the Herbarium of Yunnan University (YUKU; herbarium acronyms follow Index Herbarium by Thiers 2024).

Taxonomic sampling

In order to clarify the phylogenetic position of the new species, two samples of the new species and its putative closely related taxa of *Sinocrassula* from China were included in the phylogenetic analysis. In total, 22 accessions representing 12 species of *Sinocrassula* were used for the phylogenetic analysis. Based on previous phylogenetic studies (Messerschmid et al. 2020; Wang et al. 2022), *Kungia aliciae* (Raym.–Hamet) K. T. Fu was selected as the outgroup.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica-dried material using the TIANGEN plant genomic DNA extraction kit (TIANGEN Biotech., Beijing, China) following the manufacturers' protocols. Four chloroplast DNA markers (*psb*A-*trn*H, *trn*L-F, *rbc*L, *mat*K)] and one nuclear marker (ITS) were amplified and sequenced using previous primers and protocols (Wang et al. 2022).

Phylogenetic analyses

Sequences from GenBank and the newly generated data (Table 1) were aligned using MAFFT v7.450 (Katoh and Standley 2013), followed by manual refinement in BioEdit (Hall 1999). Single alignments were concatenated to a matrix using the 'Concatenate Sequence' plugin in PhyloSuite v1.2.2 (Zhang et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used

Table 1. Species information and corresponding GenBank accession numbers of *Sinocrassula* and close relative genus used in this study.

Species	Voucher	Location	*trn*L-*trn*F	*psb*A-*trn*H	*rbc*L	ITS	*mat*K	Reference
Kungia aliciae	Mayuzumi CH00061 (TI)	Sichuan, China	AB480632	–	–	AB480591	–	Mayuzumi and Ohba 2009
Sinocrassula ambigua	Chen et al. YUS12973 (YUKU)	Deqin, Yunnan, China	PQ629032	PQ629054	PQ629039	PQ611189	PQ629047	Xu et al. 2025
S. ambigua	Chen et al. YUS12672 (YUKU)	Deqin, Yunnan, China	PQ629030	PQ629055	PQ629038	PQ611188	PQ629046	Xu et al. 2025
S. ambigua	Chen et al. YUS6698 (YUKU)	Deqin, Yunnan, China	PQ629035	PQ629059	PQ629040	PQ611190	PQ629048	Xu et al. 2025
S. densirosulata	Chang XC19075 (SZ)	China	MW206800	MW206800	MW206800	–	MW206800	Unknown
S. diversifolia	Chen et al. YUS9407 (YUKU)	Gongshan, Yunnan, China	**PQ629070**	**PQ629074**	**PQ629066**	**PQ623396**	**PQ629062**	This study
S. diversifolia	Chen et al. YUS9477 (YUKU)	Gongshan, Yunnan, China	**PQ629071**	**PQ629075**	**PQ629067**	**PQ623397**	**PQ629063**	This study
S. ganluoensis	Zhao et al. YUS6699 (YUKU)	Ganluo, Sichuan, China	PQ505691	PQ505693	PQ505695	PQ496498	PQ505697	Li et al. 2024
S. ganluoensis	Zhao et al. YUS13920 (YUKU)	Kangding, Sichuan, China	PQ505692	PQ505694	PQ505696	PQ496499	PQ505698	Li et al. 2024
S. holotricha	Zhao et al. YUS13475 (YUKU)	Jiulong, Sichuan, China	PQ629034	PQ629056	PQ629042	PQ611192	PQ629050	Xu et al. 2025
S. holotricha	Zhao et al. YUS12867 (YUKU)	Yuexi, Sichuan, China	PQ629031	PQ629057	PQ629043	PQ611193	PQ629051	Xu et al. 2025
S. indica	zjq20160061 (SANU)	Xizang, China	MN794334	MN794334	MN794334	–	MN794334	Zhao et al. 2020
S. indica var. *obtusifolia*	Wang et al. YUS13936 (YUKU)	Deqin, Yunnan, China	PQ505699	PQ505701	PQ505703	PQ496500	PQ505705	Li et al. 2024
S. indica var. *obtusifolia*	Wang et al. YUS13959 (YUKU)	Deqin, Yunnan, China	PQ505700	PQ505702	PQ505704	PQ496501	PQ505706	Li et al. 2024
S. jiaozishanensis	Chen et al. JZS001 (YUKU)	Luquan, Yunnan, China	MZ343264	MZ343262	MZ343263	–	MZ343261	Wang et al. 2022
S. jiaozishanensis	Chen et al. JZS002 (YUKU)	Luquan, Yunnan, China	MZ343269	MZ343267	MZ343268	–	MZ343266	Wang et al. 2022
S. jiaozishanensis	Chen et al. YUS5900 (YUKU)	Luquan, Yunnan, China	PQ629036	PQ629058	PQ629044	PQ611194	PQ629052	Xu et al. 2025
S. obliquifolia	Zhao et al. YUS9064 (YUKU)	Hanyuan, Sichuan, China	**PQ629072**	**PQ629076**	**PQ629068**	**PQ623398**	**PQ629064**	This study
S. obliquifolia	Huang et al. YUS9366 (YUKU)	Shimian, Sichuan, China	**PQ629073**	**PQ629077**	**PQ629069**	**PQ623399**	**PQ629065**	This study
S. yunnanensis	Chen s.n. (HIB)	Yunnan, China	–	–	–	KC988288	KC988295	Chen et al. 2014
S. yunnanensis	Mayuzumi C00115 (TI)	Yunnan, China	AB480669	–	–	AB088582	–	Mayuzumi and Ohba 2004
S. yunnanensis	Chen et al. YUS6697 (YUKU)	Heqing, Yunnan, China	PQ629037	PQ629060	PQ629045	PQ611195	PQ629053	Xu et al. 2025
S. yunnanensis	Chen et al. YUS13776 (YUKU)	Heqing, Yunnan, China	PQ629033	PQ629061	PQ629041	PQ611191	PQ629049	Xu et al. 2025

Note. Accession numbers in bold indicates newly generated data for this study.

to select the best-fitting likelihood model for Maximum Likelihood (ML) and Bayesian Inference (BI) using the bias-corrected Akaike information criterion (AICc). Maximum likelihood bootstrapping was performed with 5,000 rapid bootstrap (BS) analyses followed by a search for the best-scoring tree in a single run through IQ-tree v2.1.2 (Nguyen et al. 2015). Bayesian inference was conducted for the combined dataset using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) with two runs of four Markov chain Monte Carlo (MCMC) chains, each beginning with a random tree and sampling every 1000 generations for 20,000,000 generations. Finally, the concatenated trees were generated and visualized with their Maximum-Likelihood Bootstrap Support values (ML-BS) and Bayesian Inference Posterior Probability (BI-PP) in Figtree v1.4.3 (Rambaut 2017).

Results and discussion

A total of 23 accessions representing 12 species from the genus *Sinocrassula*, along with one outgroup (*Kungia aliciae*), were included in the phylogenetic analysis. The concatenated dataset was 6,433 bp in length and GTR + F + I + G4 was selected as the best evolutionary model of nucleotide substitutions. The inferred phylogenetic trees from the ML and BI analyses revealed identical topologies. Two samples of the new species formed a highly supported clade (ML-BS = 99; BI-PP = 1.00, Fig. 1), and were found to be sister to *Sinocrassula ganluoensis* (ML-BS = 100, BI-PP = 1.00, Fig. 1). Then, they together are sister to *S. diversifolia* (ML-BS = 74, BI-PP = 0.94, Fig. 1). Although the new species is closely related to *S. ganluoensis* and *S. diversifolia*, it can be easily distinguished from these two species by its asymmetrical leaves and triangular petals, which are white at the base with dense purple-red stripes and spots on the both surfaces upward. Morphologically, the new species is most similar to *S. indica* (Table 2). However, the phylogenetic analysis showed that they have a distant relationship (Fig. 1).

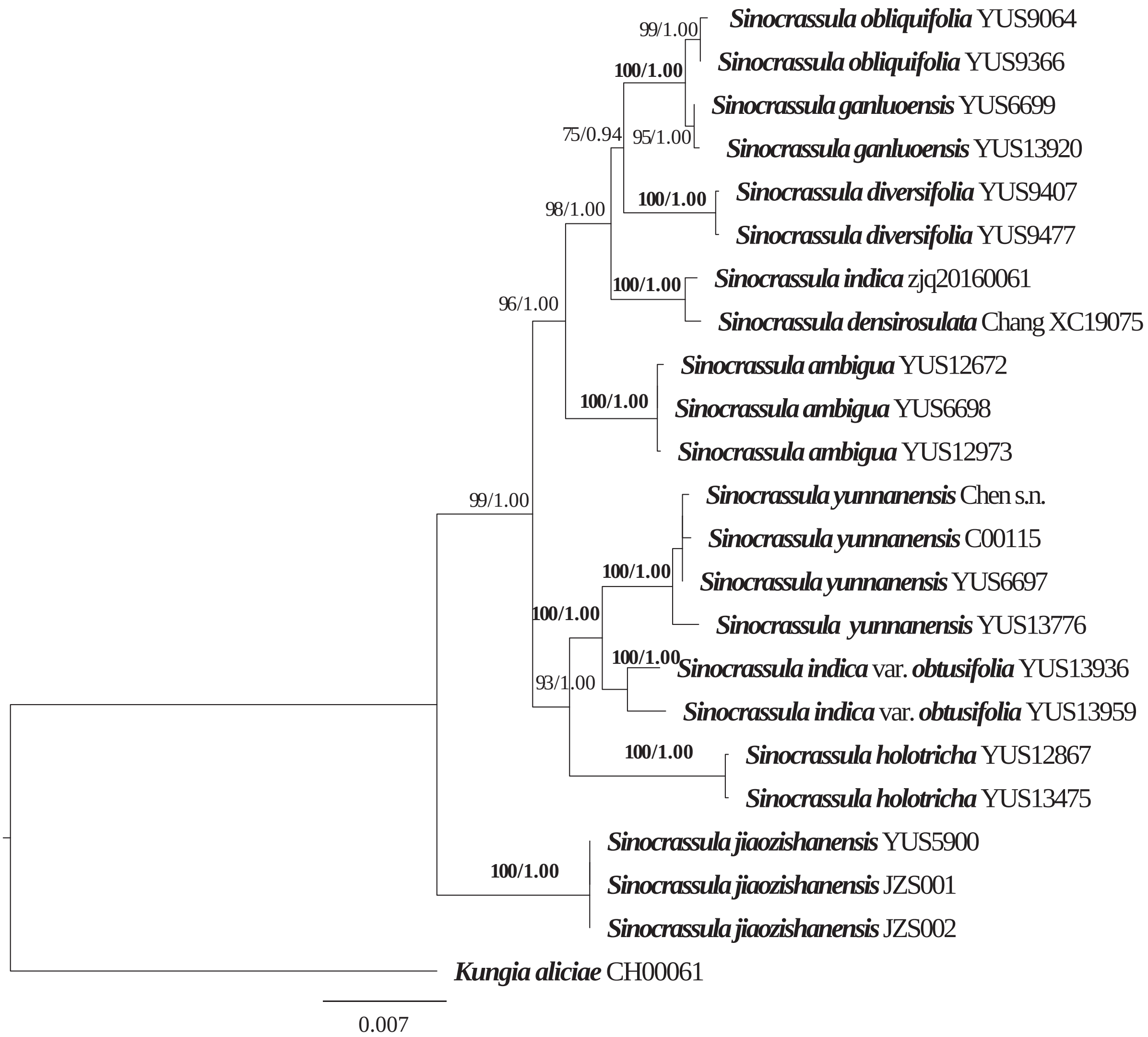

Figure 1. The maximum likelihood phylogeny of *Sinocrassula* and its allies based on four chloroplast markers (*psb*A-*trn*H, *trn*L-F, *rbc*L, and *mat*K) and one nuclear marker (ITS). Values associated with branches are Maximum Likelihood Bootstrap Support (ML-BS) and Bayesian Inference Posterior Probability (BI-PP). Bold indicated it received full ML-BS and BI-PP support.

Table 2. Morphological comparison of *Sinocrassula diversifolia*, *S. ganluoensis*, *S. indica*, and *S. obliquifolia*.

Character	*S. diversifolia*	*S. ganluoensis*	*S. indica*	*S. obliquifolia*
Life cycle	—	Perennial	Biennial	Biennial
Plant surface	Glabrous, many parts purple-spotted	Glabrous	Glabrous	Glabrous, many parts purple-spotted
Basal leaves	Rosette lax, many parts brown-spotted, broadly obovate	Rosette compact, orbicular-lanceolate	Rosette, spatulate-oblong	Rosette, asymmetrical leaves ovoid to lanceolate
Stem leaves	Alternate, dimorphic	Alternate, Linear, lanceolate	Alternate, broadly oblanceolate, subobovate, ovate-orbicula	Alternate alternate, lanceolate, many parts with purple spots
Sepals	Triangular-lanceolate	Ovate-lanceolate	Broadly triangular	Broadly triangular
Bracts	Resembling distal stem leaves but smaller	Lanceolate	Resembling distal stem leaves but smaller	Lanceolate
Inflorescences	Corymbiform	Corymbiform	Paniculate, often corymbiform	Corymbiform
Length of flowering stems	—	5–11.5 cm	5–60 cm or shorter	10–18 cm
Petals	Lanceolate, yellow, spotted with purple	Broad triangular, yellowish, deeply purplish red upward	Lanceolate to ovate, red, reddish, yellow or greenish yellow	Triangular, base white, spotted with purple upward
Nectar scales	Broadly quadrate	Quadrate	Quadrate, apex emarginate	Rectangler
Nectar scales size	0.3–0.5 × 0.5–0.7 mm	0.5 × 0.9 mm	—	0.3–0.5 × 0.2–0.3 mm
Length of styles	0.5–1.0 mm	0.6–1 mm	Less than 1.0 mm	1.0–1.5 mm

The new species exhibits some typical characteristics of *Sinocrassula* including compact rosette, stem leaves alternate and lanceolate, bracts resembling distal stem leaves but smaller, flowers 5-merous, broadly triangular sepals, and ovoid carpels (Fu and Ohba 2013). A comparison of morphological characters among the new species and its morphologically similar species is shown in Table 2. The new species can easily distinguished from other species by its unique combination of asymmetrical leaves, rectangler nectar scales, and triangular petals which are white at the base and gradually transition to purple toward the tips (Fig. 2).

Taxonomic treatment

Sinocrassula obliquifolia **Jing Zhao, J.Guan Wang & X.M.Zhou, sp. nov.**
urn:lsid:ipni.org:names:77360183-1
Figs 2, 3

Type. China • Sichuan: Hanyuan County, Yaan City, elev. ca. 837 m, 29.333262°N, 102.571721°E, on the granite crevices, 6 July 2023, *Jing Zhao et al. YUS9064* (holotype YUKU!; isotype YUKU!).

Diagnosis. *Sinocrassula obliquifolia* is similar to *S. indica* in having rosette, ovoid carpels, and broadly triangular sepals. However, this new species has shorter flowering stem (10–18 cm) (vs. 5–60 cm in *S. indica*), lanceolate stem leaves (vs. oblanceolate to ovate-orbicular stem leaves in *S. indica*), triangular petals (vs. lanceolate to ovate petals in *S. indica*), petals with a white base adorned with dense purple-red stripes and spots on the surfaces upward (vs. red, reddish, yellow, or greenish-yellow in *S. indica*) and rectangle nectar scales (vs. quadrate in *S. indica*). *S. obliquifolia* is also similar to *S. diversifolia* in having corymbiform inflorescences, petals, and stamens with purple spots. However, *S. obliquifolia* has a well-defined rosette (vs. less defined rosette in *S. diversifolia*), monomorphic stem leaves (vs. dimorphic stem leaves in *S. diversifolia*), triangular petals with dense purple-red stripes and spots on the surfaces upward

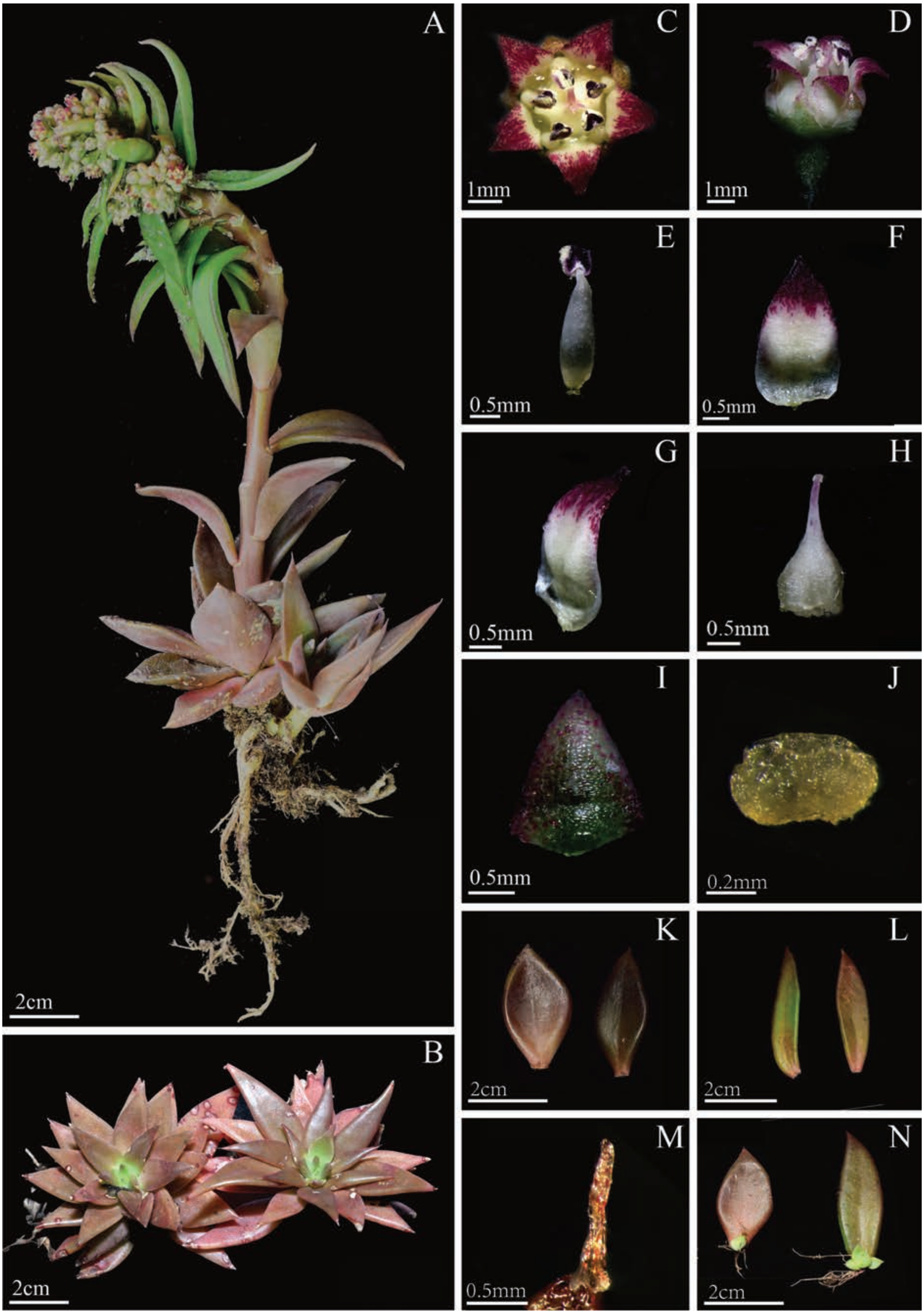

Figure 2. *Sinocrassula obliquifolia* **A, B** habit **C, D** flowers **E** stamen **F, G** petals **H** carpel **I** sepal **J** nectar scale **K** basal leaves **L** stem leaves **M** gland of basal leaf **N** leaves that abscise and bud during the flowering period, left: basal leaf, right: stem leaf.

(vs. lanceolate petals that are yellow with purple spots in *S. diversifolia*) and rectangle nectar scales (vs. broadly quadrate in *S. diversifolia*).

Description. Perennial herbs, terrestrial or lithophytic, 5.0–20.0 cm tall, rosette 5.0–8.0 × 6.0–8.0 cm. Roots fibrous. Basal leaves rosette, spirally arranged, asymmetrically ovoid to lanceolate, apex with glands, 2.0–3.0 × 1.0–2.0 cm. Flowering stems terminal, 10.0–18.0 cm, glabrous with purple spots. Stem leaves alternate, lanceolate, many parts with purple spots. Bract resembling distal stem leaves but smaller, lanceolate, 3.0–3.5 × 0.5–1.0 cm. Inflorescences corymbiform, ca. 2.0–3.0 cm in diameter. Flowers small, ca. 3–6 mm in diameter. Sepals broadly triangular, purple with red spots, 1.5–2.0 × 0.5–1.2 mm. Petals triangular, base white, with dense purple-red stripes and spots on the surfaces upward, 2.0–4.0 × 1.0–2.0 mm. Stamens ca. 2–3 mm, white, anthers oblong-cordate, ca. 0.5 mm, pollen yellow. Nectar scales broadly

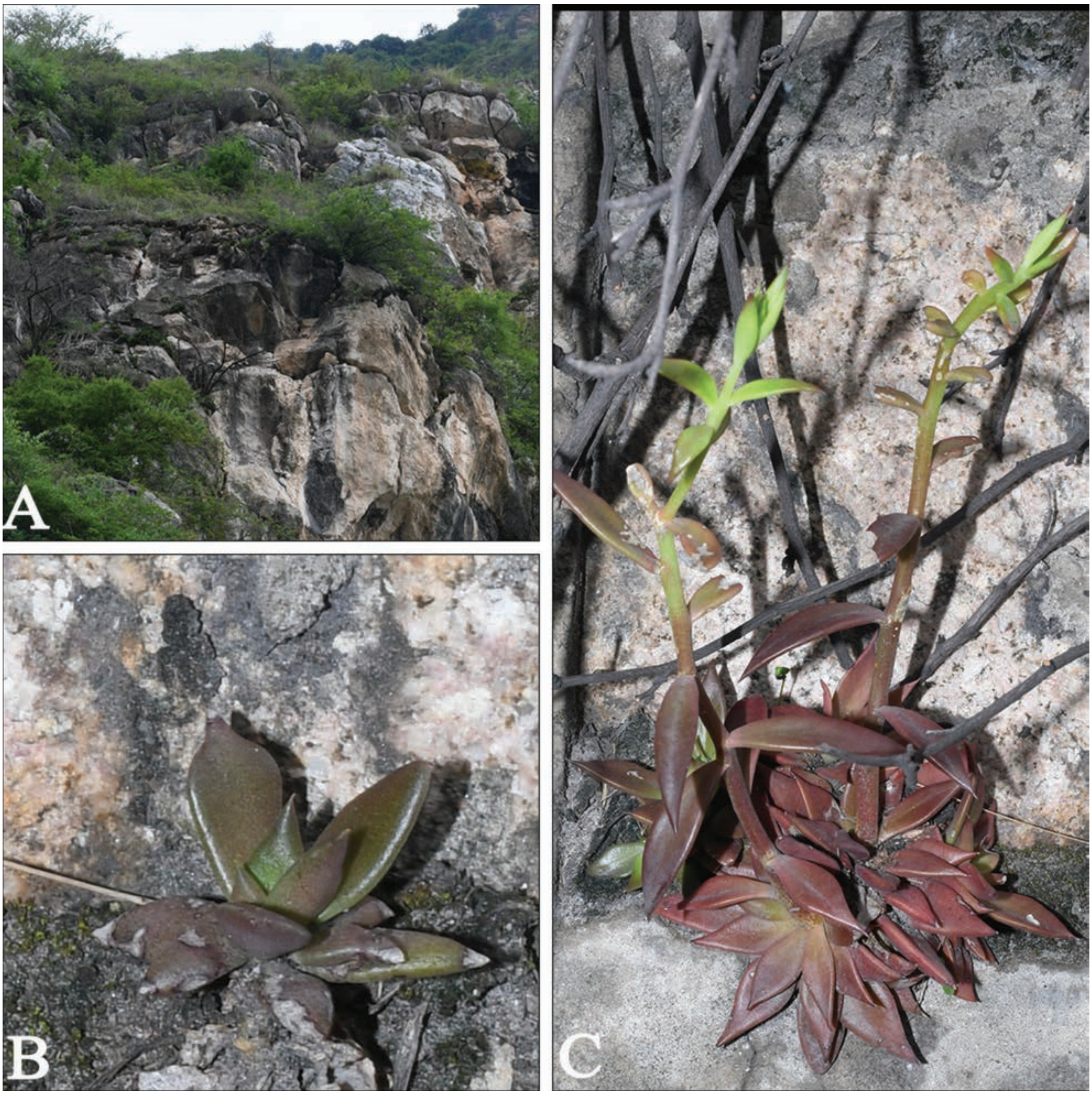

Figure 3. *Sinocrassula obliquifolia* **A** habitat **B, C** habit.

rectangular, ca. 0.3–0.5 × 0.2–0.3 mm. Carpels 5, ovoid, clockwise rotation, 1.0–2.0 × 0.5–1 mm, styles 1.0–1.5 mm. Flowering June–October which coincides with the rainy season and the shedding of the leaves and the emergence of new buds.

Distribution and habitat. *Sinocrassula obliquifolia* is currently known in central Sichuan Province, China. Two populations were found in granite crevices, as well as on dry stony or gravelly slopes at elevations ranging from 837 to 1140 m.

Additional specimens examined (paratypes). CHINA • Sichuan: Shimian County, Yaan City, elev. ca. 1140 m, 29.258594°N, 102.371583°E, on the granite crevices, 12 May 2023, *Chuan-Jie Huang et al. YUS9366* (YUKU!).

Etymology. The epithet *obliquifolia* refers to the asymmetrical leaves of the basal leaves, a unique characteristic for this species within *Sinocrassula*. Its Chinese name is suggested as ‘斜叶石莲(xie ye shi lian)’.

Acknowledgements

We thank Yi Liu for help in the field. Three anonymous reviewers and an editor are thanked for their helpful suggestions.

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 Science & Technology Fundamental Resources Investigation Program of China (Grant No. 2022FY100201) and the Postdoctoral Research Funds of Yunnan University (Grant No. W8163003).

Author contributions

Data curation: ZRH. Formal analysis: SLF. Resources: CJH, ML. Writing – original draft: RJL, JZ. Writing – review and editing: XMZ, JGW.

Author ORCIDs

Rong-Juan Li https://orcid.org/0009-0008-2667-398X
Jing Zhao https://orcid.org/0000-0001-7871-2209
Shao-Li Fang https://orcid.org/0000-0001-5942-0366
Chuan-Jie Huang https://orcid.org/0009-0000-3507-2766
Miao Luo https://orcid.org/0009-0006-9317-9151
Zhao-Rong He https://orcid.org/0000-0001-6492-3689
Xin-Mao Zhou https://orcid.org/0000-0003-3555-7784
Jia-Guan Wang https://orcid.org/0000-0003-0264-9180

Data availability

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

References

Averyanov LV, Byalt VV, Vinh NT, Loc PK, Hieu NQ (2014) *Sinocrassula vietnamenis* (Crassulaceae), new species and new generic record in the flora of Vietnam. Taiwania 59(4): 348–352. https://doi.org/10.6165/tai.2014.59.4.348

Berger A (1930) Crassulaceae. In: Engler A, Prantl K (Eds) Die natürlichen Pflanzenfamilien 2(18): 352.

Chen L-Y, Zhao S-Y, Mao K-S, Les DH, Wang Q-F, Moody ML (2014) Historical biogeography of Haloragaceae: An out-of-Australia hypothesis with multiple intercontinental dispersals. Molecular Phylogenetics and Evolution 78: 87–95. https://doi.org/10.1016/j.ympev.2014.04.030

Eggli U (2003) Illustrated handbook of succulent plants: Crassulaceae. Springer, Berlin Heidelberg. https://doi.org/10.1007/978-3-642-55874-0

Fu K-J, Ohba H (2013) Crassulaceae. In: Wu Z-Y, Raven PH (Eds) Flora of China (Vol. 8). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 202–268.

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/nt. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008

Huelsenbeck JP, Ronquist F (2001) Mrbayes: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kalyaanamoorthy S, Minh BQ, Wong TKF, 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

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

Li R-J, Zhao J, Fang S-L, Chen C, Luo M, Zhou X-M, Wang J-G (2024) *Sinocrassula ganluoensis* (Crassulaceae), a new species from China. Phytotaxa 675(1): 079–086. https://doi.org/10.11646/phytotaxa.675.1.7

Mayuzumi S, Ohba H (2009) Molecular phylogeny and taxonomic revision of Hylotelephium (Crassulaceae) and its allied genera based on ITS (nrDNA) and *trn*T-L-F (cpDNA) sequences and seed testa morphology. [Unpublished]

Messerschmid TFE, Klein JT, Kadereit G, Kadereit JW (2020) Linnaeus' folly phylogeny, evolution and classification of *Sedum* (Crassulaceae) and Crassulaceae subfamily Sempervivoideae. Taxon 69(5): 892–926. https://doi.org/10.1002/tax.12316

Nguyen LT, Schmidt HA, von 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

Rambaut A (2017) FigTree-version 1.4.3, a graphical viewer of phylogenetic trees.

Thiede J, Eggli U (2007) Crassulaceae. In: Kubitzki K (Ed.) The families and genera of vascular plants. Springer, Berlin, Heidelberg, 83–118. https://doi.org/10.1007/978-3-540-32219-1_12

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/science/ih/ [Accessed 15 Jan. 2024]

Wang J, Du F, Liu Y (2012) *Sinocrassula stenosquamata* (Crassulaceae), a new species from Yunnan, China. Acta Botanica Boreal-Occidentalia Sinica 32(12): 2563–2565. https://doi.org/10.3969/j.issn.1000-4025.2012.12.030

Wang J-G, Chen C, Wang Y, He Z-R (2022) *Sinocrassula jiaozishanensis* (Crassulaceae), a new species from Yunnan Province, China. Phytotaxa 541(1): 010–020. https://doi.org/10.11646/phytotaxa.541.1.2

Xie H-H, Yoshikawa M (2012) Grasshopper ketone 3-O-primveroside from *Sinocrassula indica*. Journal of Asian Natural Products Research 14(5): 503–507. https://doi.org/10.1080/10286020.2012.669376

Xu Z-B, Zhao J, Li R-J, Jiang H, Wang J-G, Chen C (2025) *Sinocrassula holotricha* (Crassulaceae), a new species from Sichuan, China. PhytoKeys 251: 241–250. https://doi.org/10.3897/phytokeys.251.134633

Zhang D, Gao F, Jakovli'c I, Zou H, Zhang J, Li W-X, Wang G-T (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

Zhao D-N, Ren Y, Zhang J-Q (2020) Conservation and innovation: Plastome evolution during rapid radiation of *Rhodiola* on the Qinghai-Tibetan Plateau. Molecular Phylogenetics and Evolution 144: 106713. https://doi.org/10.1016/j.ympev.2019.106713

Zhao J, Nakamura N, Hattori M, Yang X-W, Komatsu K, Qiu M-H (2004) New triterpenoid saponins from the roots of *Siocrassula asclepiadea*. Chemical and Pharmaceutical Bulletin 52(2): 230–237. https://doi.org/10.1248/cpb.52.230

Zhu C-M (2015) A discussion on the application of Crassulaceae plants in urban vertical greening. Jiangxi Building Materials 8: 214–216.

PhytoKeys 255: 113–122 (2025)
DOI: 10.3897/phytokeys.255.145664

Research Article

Caloneis nanyiensis sp. nov. (Bacillariophyta) from Nanyi Lake of Anhui Province, China

Yong Fang[1], Pan Yu[2,3], Qing-Min You[1], John Patrick Kociolek[4], Yue Cao[5], Quan-Xi Wang[1], Zhi-Ping Qian[1]

1 *College of Life Sciences, Shanghai Normal University, Shanghai 200234, China*
2 *School of Environment and Geographical Sciences, Shanghai Normal University, Shanghai 200234, China*
3 *Yangtze River Delta Urban Wetland Ecosystem National Field Scientific Observation and Research Station, Shanghai 200234, China*
4 *Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, UCB 218, Boulder CO80309, USA*
5 *Institute of Science and Technology, Shanghai Institute of Technology, Shanghai 201418, China*
Corresponding author: Zhi-Ping Qian (qzp0313@shnu.edu.cn)

Abstract

A new species, *Caloneis nanyiensis* **sp. nov.**, is described from Nanyi Lake, the largest lake in southern Anhui Province, China. Observations were made using a light and scanning electron microscope documenting the size, shape and ultrastructure of the new species. *Caloneis nanyiensis* **sp. nov.** has rhombic valves with acutely rounded apices. The raphe is narrow and arched. An internal axial plate covers the alveoli, leaving small marginal openings bordered by costa, thickened and raised from the valve face. The striae are slightly radiate to parallel in the central area, becoming radiate towards the apices. By comparing the new species with similar *Caloneis* species, *C. nanyiensis* **sp. nov.** was confirmed to be sufficiently different with respect to valve size and striae density to be recognised as new to science. The new species lives in freshwater habitats and epiphytic on *Cladophora*. The discovery enhances our knowledge of the diversity of freshwater diatoms in China.

Key words: Axial area, *Caloneis*, girdle, raphe, rhombic valve

Academic editor: Bing Liu
Received: 31 December 2024
Accepted: 10 March 2025
Published: 17 April 2025

Citation: Fang Y, Yu P, You Q-M, Kociolek JP, Cao Y, Wang Q-X, Qian Z-P (2025) *Caloneis nanyiensis* sp. nov. (Bacillariophyta) from Nanyi Lake of Anhui Province, China. PhytoKeys 255: 113–122. https://doi.org/10.3897/phytokeys.255.145664

Introduction

Cleve and Grove initially described the genus *Caloneis* at the subgroup level, originally within the genus *Navicula* (Cleve and Grove 1891). Unfortunately, they did not provide a valid description; however, a few years later, Cleve provided a valid generic description in the *Synopsis of the Naviculoid Diatoms* (Cleve 1894). The type species of the genus *Caloneis* was indicated by Boyer (1927) to be *Caloneis amphisbaena* (Bory) Cleve (1894). The primary diagnostic features of this genus include valves that are linear-lanceolate to elliptical with capitate or rostrate ends. The shapes of axial and central areas are variable. Distal raphe fissures are usually distinct. The chambered striae give the appearance of one to two longitudinal lines. The striae of *Caloneis* are composed of fine alveoli. The outer wall of the alveoli is perforated by many rows of small, round poroids occluded by hymens and the inner wall of each alveolus typically opens to the inside of the valve by one fairly large, transapically elongate areola (Round et al. 1990). *Caloneis* is considered a widely distributed genus with a very broad ecological range, including freshwater, brackish and marine environments (Levkov and Williams 2014). To date, 392 taxonomically accepted species of *Caloneis* are listed in AlgaeBase (Guiry and Guiry 2025). After 2000, several new species have been reported from various localities from around the world (Lange-Bertalot et al. 2004; Metzeltin et al. 2005; Metzeltin and Lange-Bertalot 2007; Levkov and Williams 2014).

Traditionally, *Caloneis* and *Pinnularia* Ehrenberg have been considered distinct genera, the diagnostic features between the two genera mainly including the genus *Caloneis* having alveolate striae that are usually thinner and denser than those of *Pinnularia* (Hustedt 1935; Round et al. 1990; Mann 2001; Levkov and Williams 2014; Kulikovskiy et al. 2023). Molecular studies conducted thus far indicate that both the genus *Pinnularia* and the genus *Caloneis* are not monophyletic (Bruder et al. 2008; Souffreau et al. 2011; Kulikovskiy et al. 2023). These findings suggest that the genus *Pinnularia* may require further subdivision, which remains unresolved. With the in-depth study of molecular systematics, we believe that the taxonomic status of the genera *Pinnularia* and *Caloneis* will be more accurately and clearly divided.

In China, the vast majority of new *Caloneis* species were published before 2000, including *Caloneis bacillum* f. *latilanceolatum* Zhu and Chen (1995), *Caloneis chansiensis* Skvortzov (1935), *C. elongata* var. *constricta* Cheng and Chin (1980), *Caloneis fasciata* var. *pekinensis* Skvortzov (1928), *Caloneis holstii* var. *tibetica* Jao (1964), *Caloneis hunanensis* Chen and Zhu (Zhu and Chen 1989), *Caloneis lepidula* var. *angustata* Skvortzov (1976), *Caloneis patagonica* var. *sinica* Skvortzov (1938a), *C. platycephala* Cheng and Chin (1980), *Caloneis schroderi* var. *densestriata* Skvortzov (1976), *Caloneis schumanniana* var. *biconstricta* f. *minor* Zhu and Chen (1995), *Caloneis silicula* var. *hankensis* Skvortzov (1929), *Caloneis schumanniana* f. *gracilis* Skvortzov (1935), *Caloneis silicula* var. *hinganica* Skvortzov (1976) and *Caloneis sphagnicola* Skvortzov (1938b). After 2000, only one taxon has been reported in this genus: *Caloneis clevei* var. *parallela* Skvortzov ex Gololobova and Kulikovskiy (Skvortzov 2012).

During the investigation of freshwater diatom diversity in Nanyi Lake, we discovered a new species, which is described here as *Caloneis nanyiensis* sp. nov. The purpose of this study was to document and formally describe the species, based on both light microscope (LM) and scanning electron microscope (SEM) observations and to compare it with other morphologically similar species of the genus.

Material and methods

Diatom samples were collected from Nanyi Lake (31°01'–31°10'N, 118°50'–119°3'E), Anhui Province, China, in August 2018. The lake area is 210 km^2. Nanyi Lake was formed by the differentiation of the ancient Danyang Lake. The ancient Danyang Lake system was a stagnant lake formed by long-term siltation and water accumulation in a newly-constructed fault depression. The climate of the Nanyi Lake area, as reported by the Langxi County meteorological station, belongs to the North subtropical monsoon humid climate zone. The main features are: mild climate, four distinct seasons, hot and rainy season, abundant rainfall and ample sunshine (Jia et al. 2021).

In the field, several water chemistry characteristics were recorded, including: pH, temperature, salinity, total dissolved solids (TDS) and conductivity. These parameters were measured using a YSIPro Plus multiparameter meter (YSI, Ohio, USA). In the field, collections of attached algae were scraped from the surfaces of stones using (sterilised?) toothbrushes and/or a knife and the samples were placed in a bottle, preserved with formalin (4% final concentration) and sealed.

In the laboratory, the diatom valves were cleaned of organic matter using the Microwave Accelerated Reaction System (Model MARS, CEM Corporation, USA) (Parr et al. 2004). The digestion followed a pre-programmed digestion scheme (temperature: 180 °C, ramp: 15 min, hold: 15 min) (Yu et al. 2019). After digestion, samples were alternatively centrifuged (5 minutes at 3500 rpm) and washed with distilled water (approximately five times) until the pH of the sample was approximately neutral. The cleaned material was kept in 95% ethanol. Cleaned diatom specimens were mounted on glass slides in Naphrax for light microscopy (LM) or air-dried on to coverslips and mounted on to Cu stubs for observation with a scanning electron microscope (SEM). LM studies were made with a ZEISS AXIO Imager A2 microscope fitted with DIC optics and a 1.4 numerical aperture, 100× oil immersion objective. SEM examination was conducted using a Hitachi SU-8010 (2 kV, working distance less than 6 mm). Images were compiled with Adobe Photoshop CS6. Morphological terminology follows Round et al. (1990). Both unprepared (field) samples and prepared slides of mounted material are housed in the Laboratory of Algae and Environment, Department of Biology, Shanghai Normal University (**SHTU**).

Results

Caloneis nanyiensis **Pan Yu & Qing-Min You, sp. nov.**

Figs 1–3

Type material. ***Holotype.*** Specimen circled on slide NYH-20180801 (= Fig. 1A), deposited in the Herbarium of Shanghai Normal University (SHTU), China.

Isotype. Specimen circled on slide 652047 (= Fig. 1D), deposited in the Herbarium of University of Colorado, Boulder, USA.

Type locality. China. Anhui Province: Nanyi Lake, sampling site with the coordinates 31°01'N, 118°50'E. Diatom samples collected by Pan Yu, 19 August 2018.

Description. ***LM*** (Fig. 1A–M): Valve length 14.5–28.5 μm, width 8.0–10.2 μm. Valves rhombical in shape with acutely rounded apices. Raphe narrow and arched, with slightly unilaterally bent central pores and slightly curved terminal fissures. Axial area linear-lanceolate, becoming wider at the central area. Striae are slightly radiate to parallel in the central area, becoming more radiate towards the apices, 16–18 in 10 μm.

SEM valve exterior (Fig. 2A–E): Valve face usually uneven, with slightly raised ends and a slightly concave middle (Fig. 2A, B). Central area nearly rhombic, with a width accounting for 1/4–1/3 of the valve, the striae pattern in the central area is obviously shorter. (Fig. 2A, B, E). Raphe branches arched with proximal raphe ends weakly unilaterally deflected and dilated, drop-like in shape (Fig. 2A, B, E). Distal raphe fissures hooked and continuing on to the mantle (Fig. 2C, D). Striae multiseriate, composed of 4–5 rows of small, rounded areolae, which

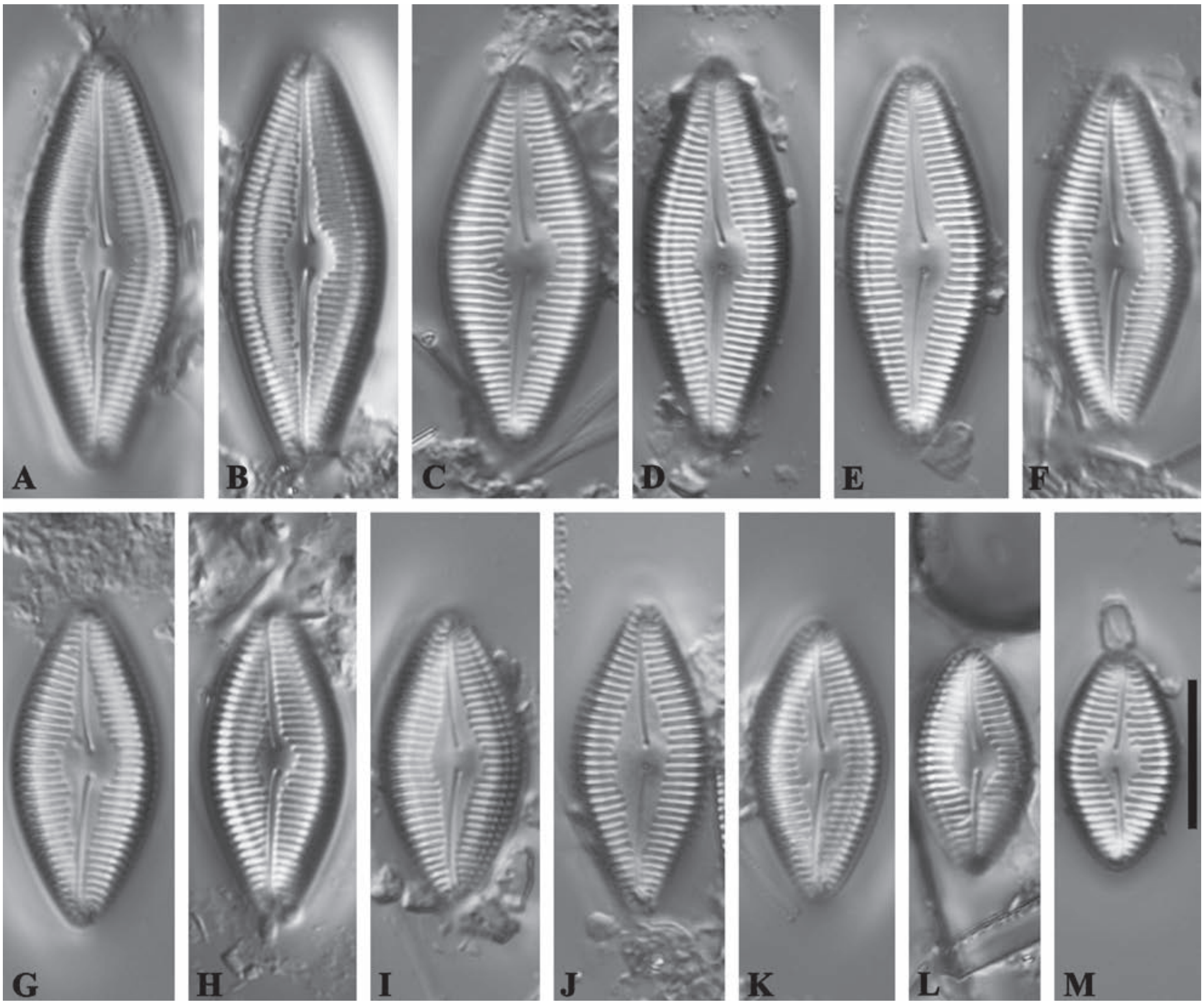

Figure 1. *Caloneis nanyiensis* sp. nov. LM ×1000 **A–M** thirteen valves showing a size diminution series; note that the valves rhombic and raphe narrow and arched. **A** Illustration of holotype specimen **D** illustration of isotype specimen. Scale bars: 10 μm (**M**).

are occluded by heavy silicified, perforated hymenes (Fig. 2D, E). Girdle with a single row of linear poroids (Fig. 2A).

SEM valve interior (Fig. 3A–E): The raphe is filiform and slightly arched (Fig. 3A). The proximal raphe endings are deflected to the same sides (Fig. 3B, C) and curve towards a central nodule that is positioned to one side of the central area. The raphe terminates distally as an elevated helictoglossa (Fig. 3D, E). An axial plate covers the alveoli, leaving small marginal openings bordered by costae thickened and raised from the valve face (Fig. 3A, B). Striae composed of 2–4 areolae are present on the mantle (Fig. 3D, E).

Etymology. The new species is named after the type locality, Nanyi Lake.

Distribution and ecology. So far, the new species has only been collected at the type locality in Nanyi Lake. The habitat of the new species is characterised by pH 8.1, water temperature 30.2 °C, TDS 0.204 $g.l^{-1}$, conductivity 175.7 $μs.cm^{-1}$, collected in one sample (NYH–20180801) on *Cladophora*. In the type sample, this new species occurred at less than 2% relative abundance, established from a total count of 400 valves. Amongst the species co-occurring with *Caloneis nanyiensis* sp. nov., only four species accounted for more than 5% of

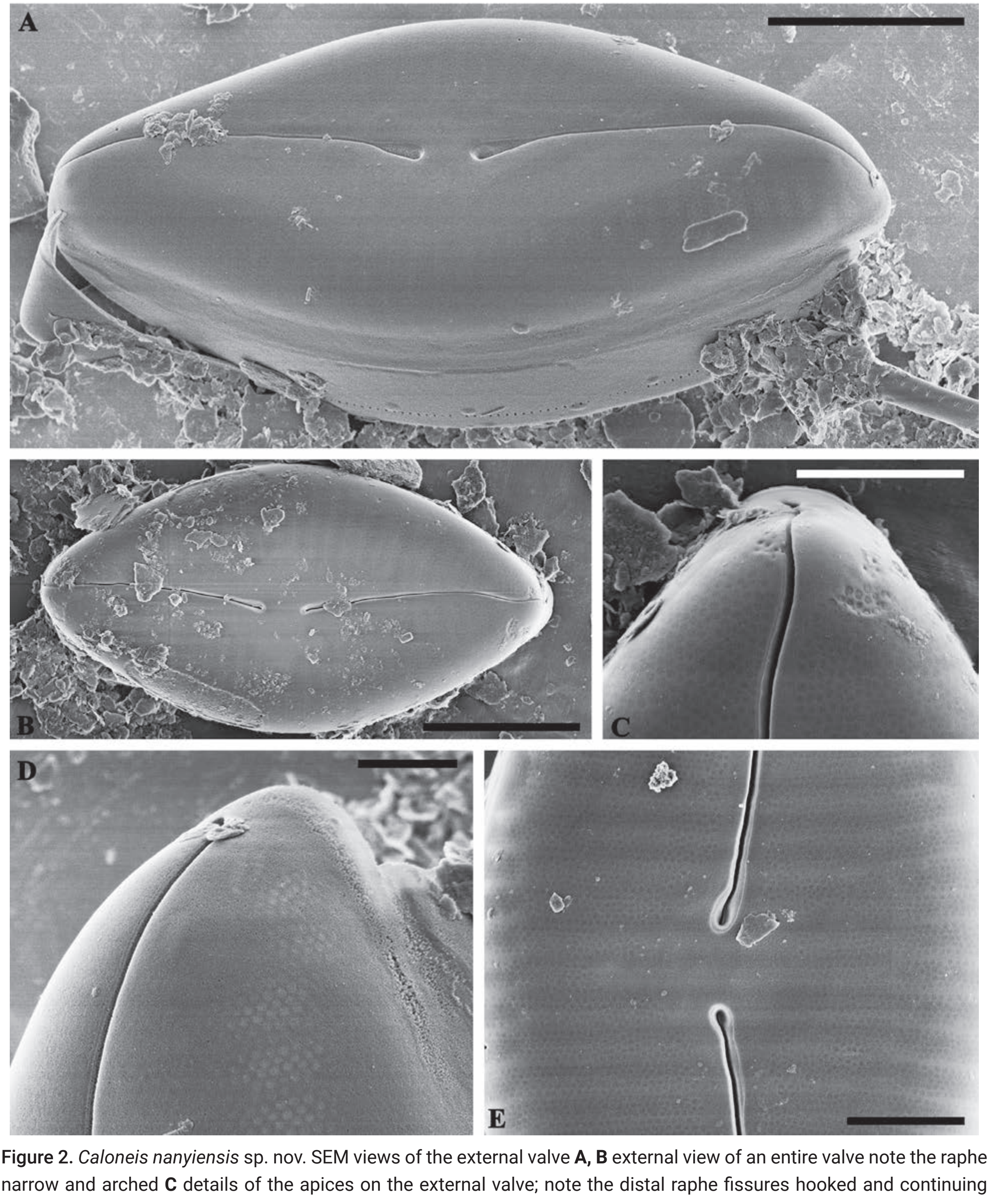

Figure 2. *Caloneis nanyiensis* sp. nov. SEM views of the external valve **A, B** external view of an entire valve note the raphe narrow and arched **C** details of the apices on the external valve; note the distal raphe fissures hooked and continuing on to the mantle **D** details of the striae on the external valve; note the Striae multiseriate, composed of small rounded areolae and areolae are occluded by heavy silicified, perforated hymenes **E** showing the central area. Scale bars: 5 μm (**A, B**); 2 μm (**C, E**); 1 μm (**D**).

the assemblage: *Fragilaria pararumpens* Lange-Bertalot, Hofmann & Werum (Hofmann et al. 2013) (25.75%), *Achnanthidium jackii* Rabenhorst (Rabenhorst 1861) (18.75%), *Aulacoseira granulata* (Ehrenberg) Simonsen (Simonsen 1979) (11.5%) and *Encyonopsis microcephala* (Grunow) Krammer (Krammer 1997) (%).

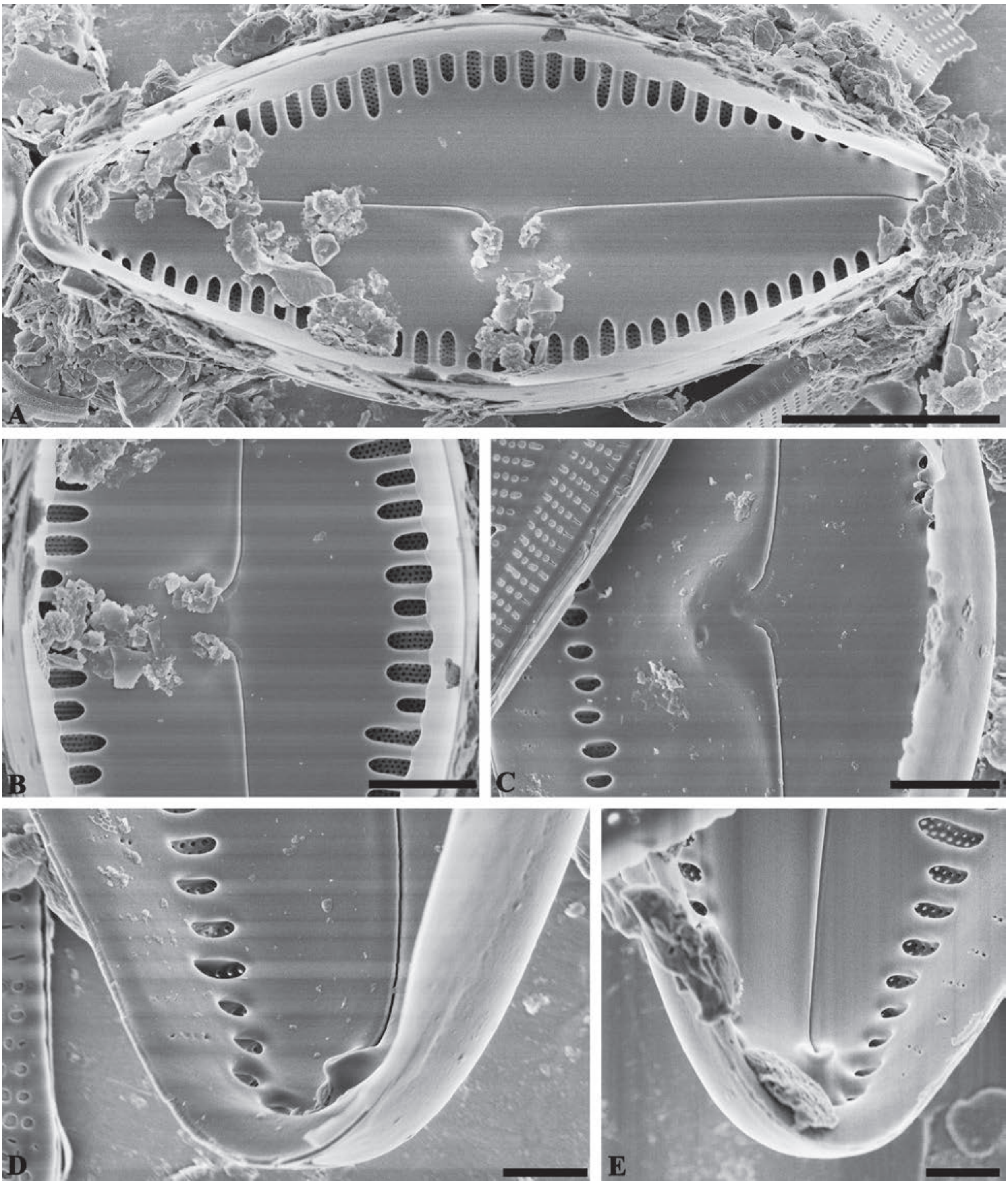

Figure 3. *Caloneis nanyiensis* sp. nov. SEM views of valve interior. **A** Internal view of an entire valve; note the raphe is filiform and slightly arched **B, C** details of the proximal raphe endings which are deflected to the same side **D, E** details of the distal raphe ends terminating as helictoglossae and the striae are composed of 2–4 areolae on the mantle. Scale bars: 5 µm (**A**); 2 µm (**B, C**); 1 µm (**D, E**).

Discussion

The new species described in this study possesses all the ultrastructural features of the genus *Caloneis* (Round et al. 1990), including having a narrow, slightly arched raphe with slightly unilaterally bent central pores and slightly curved terminal fissures, an axial plate covering the alveoli and striae parallel in the central area, becoming radiate towards the apices.

Caloneis nanyiensis sp. nov. can be compared to several species in the same genus, based on similarities in the outline and structure of the valve, including *C. caribeana* Metzeltin & Lange-Bertalot (Metzeltin and Lange-Bertalot 2007), *C. permagna* (Bailey) Cleve (Cleve 1894) and *Caloneis schumanniana* var. *lancettula* Hustedt (Hustedt 1930). The morphological characteristics of *C. nanyiensis* and these similar species are summarised in Table 1 to facilitate their comparison. The outline of the valves of *C. nanyiensis* is rhombical with acutely rounded ends, while that of *C. caribeana* is rhombical to rhombic-lanceolate with acutely rounded ends, *C. permagna* is rhombic-lanceolate with acutely rounded ends and *C. schumanniana* var. *lancettula* is narrow-lanceolate with broadly rounded ends. Additionally, the valves of *C. nanyiensis* are shorter (14.5–28.5 μm) than *C. permagna* (85–220 μm), *C. caribeana* (32–57 μm) and *C. schumanniana* var. *lancettula* (35–40 μm), as well as narrower (8.0–10.2 μm in *C. nanyiensis*) versus the breadth of valves in *C. permagna* (35–55 μm) and *C. caribeana* (14–16 μm). Furthermore, no central area was observed in the new species, but *C. caribeana* has a narrow fascia central area, *C. permagna* has an irregularly lanceolate central area and *C. schumanniana* var. *lancettula* has a transapically rectangular central area. The stria density of *C. nanyiensis* is higher (16–18/10 μm) than that of *C. permagna* (9–12/10 μm), while being lower than those of the other similar species. Individuals in the populations of *C. nanyiensis* examined here have a valve outline that is rhombical in shape, which, together with the slightly curved raphe, help to differentiate it from other species in the genus.

We also compared our new species with the smaller valves of *C. distinguenda* Levkov & Williams (Levkov and Williams 2014). However, the latter taxon is characterised by elliptic-lanceolate valves being 36 μm long and 15 μm wide. Additionally, the central area in smaller specimens of *C. distinguenda* is distinctly separated from axial area and transversally elliptic in shape. The density of the striae in smaller specimens of *C. distinguenda* is lower (14–16/10 μm) than that of *C. nanyiensis*.

Caloneis are common in alkaline, brackish and marine habitats, species of the genus *Caloneis* having a broad ecological niche (Levkov and Williams 2014).

Table1. Comparison of morphological characteristics of *Caloneis nanyiensis* sp. nov. and closely-related taxa.

Species/Feature	***C. nanyiensis* sp. nov.**	***C. caribeana* Metzeltin & Lange-Bertalot**	***C. permagna* (Bailey) Cleve**	***C. schumanniana* var. *lancettula* Hustedt**
Valve length (μm)	14.5–28.5	32–57	85–220	35–40
Valve width (μm)	8.0–10.2	14–16	35–55	8
Valve outline	Rhombical	Rhombical to rhombic-lanceolate	Rhombical-lanceolate	Narrow-lanceolate
Valve apices	Acutely round	Acutely round	Acutely round	Broadly rounded
Axial area	Linear to lanceolate	Narrow, linear	Irregularly lanceolate	Narrow-lanceolate
Central area	Absent	Very narrow fascia	Irregularly lanceolate	Transapically rectangular
Raphe	Narrow and arched	Distinctly curved	Straight	Straight
Density of striae (10 μm)	16–18	17–20	9–12	17–20
References	Current study	Metzeltin and Lange-Bertalot (2007)	Cleve (1894), Krammer and Lange-Bertalot (1986)	Hustedt (1930), Krammer and Lange-Bertalot (1986)

For example, *Caloneis* is diverse in cold-water oligotrophic habitats, *C. limosa* prefers to inhabit alkaliphilic, oligotrophic and up to β-mesosaprobic water bodies, and *C. schumanniana* is classified as a planktonic-benthic, oligo-xenosaprobiontic and alkaliphilic species (Denys 1991; Stancheva et al. 2009; Levkov and Williams 2014). In the present study, *Caloneis nanyiensis* has only been found on *Cladophora* in Nanyi Lake. The water quality of Nanyi Lake reflects a moderate nutrient level. This investigation did not detect the presence of this novel species in any other location. It is postulated that this species may be a good indicator of water bodies with moderate nutrient levels. However, further ecological studies are needed to confirm this hypothesis.

Acknowledgements

We would like to thank Lixuan Zhang, Linxin Lu, Bingwei Xing and Yang Yu for help in the field and in the preparation of samples for microscopy. We extend our heartfelt gratitude to the reviewer for the invaluable guidance and support provided. The insightful recommendations have been instrumental in enhancing the quality of this manuscript and we are deeply appreciative of your professional expertise and dedication.

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 and supported by National Natural Science Foundation of China (No. 32100165, 32170205).

Author contributions

Formal analysis: YF, PY. Investigation: YC, QMY. Project administration: QXW, Writing – original draft: YF. Writing – review and editing: ZPQ, JPK, PY.

Author ORCIDs

Pan Yu https://orcid.org/0000-0001-7937-2381
John Patrick Kociolek https://orcid.org/0000-0001-9824-7164
Zhi-Ping Qian https://orcid.org/0009-0008-7514-8073

Data availability

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

References

Boyer CS (1927) Synopsis of North American Diatomaceae, Supplement, Part 2. Naviculatae, Surirellatae. Proceedings. Academy of Natural Sciences of Philadelphia 79: 229–583.

Bruder K, Sato S, Medlin LK (2008) Morphological and molecular investigations of naviculoid diatoms IV. Pinnularia vs Caloneis. Diatom 24: 8–24.

Cheng Z, Chin TG (1980) Notes on some new species and new records of diatoms from the Strait of Taiwan (Fujian Coast), China. V. *Coscinodiscus*, *Caloneis*. Universitatis Amoiensis, Acta Scientiarum Naturalium 19(3): 109–116, 126.

Cleve PT (1894) Synopsis of the naviculoid diatoms. Part I. Kongliga Svenska Vetenskapsakademiens Handlingar Series 4 26(2): 1–194.

Cleve PT, Grove E (1891) Sur quelques Diatomées nouvelles ou peu connues. Le Diatomiste 1: 64–68.

Denys L (1991) A check-list of the diatoms in the Holocene deposits of the western Belgian coastal plain with a survey of their apparent ecological requirements. Professional Paper Belgische Dienst 246: 1–41.

Guiry MD, Guiry GM (2025) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org [accessed 04 March 2025]

Hofmann G, Werum M, Lange-Bertalot H (2013) Diatomeen im Süßwasser-Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. Koeltz Scientific Books, Königstein, 908 pp.

Hustedt F (1930) Bacillariophyta (Diatomeae) Zweite Auflage. In: Pascher A (Ed.) Die Süsswasser-Flora Mitteleuropas. Heft 10. Verlag von Gustav Fischer, Jena, 466 pp.

Hustedt F (1935) Untersuchungen über den Bau der Diatomeen, X und XI. Berichte der Deutschen Botanischen Gesellschaft 53(1): 3–41. https://doi.org/10.1111/j.1438-8677.1935.tb01863.x

Jao C (1964) Some fresh-water algae from southern Tibet. Oceanologia et Limnologia Sinica 6(2): 169–192.

Jia RY, Wang H, Li XX, Yang YY, Zou ZH, Wu LC, Liang MD, Yi R, Zhou Z, Xia QP (2021) Plant Investigation of Nanyi Lake Wetland in Xuancheng District, Anhui Province. Bulletin of Biology 56(5): 10–13.

Krammer K (1997) Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 2. *Encyonema* Part., *Encyonopsis* und *Cymbellopsis*. Bibliotheca Diatomologica 37: 1–469.

Krammer K, Lange-Bertalot H (1986). Bacillariophyceae. 1. Teil: Naviculaceae In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) Süsswasserflora von Mitteleuropa, Band 2/1. Gustav Fisher Verlag, Jena, 876 pp.

Kulikovskiy M, Glushchenko A, Kezlya E, Kuznetsova I, Kociolek JP, Maltsev Y (2023) The genus *Pinnularia* Ehrenberg (Bacillariophyta) from the Transbaikal Area (Russia, Siberia): Description of seven new species on the basis of morphology and molecular data with discussion of the phylogenetic position of *Caloneis*. Plants 12(20): 3552. https://doi.org/10.3390/plants12203552

Lange-Bertalot H, Genkal SI, Vekhov NV (2004) New freshwater species of Bacillariophyta. Biologia Vnutrennikh 4: 12–17.

Levkov Z, Williams DM (2014) Observations on *Caloneis* Cleve (Bacillariophyceae) species from the ancient lakes Ohrid and Prespa. Nova Hedwigia. Beiheft 143: 141–158. https://doi.org/10.1127/1438-9134/2014/008

Mann DG (2001) A discussion of *Caloneis* and related genera. Diatom 17: 29–36. https://doi.org/10.11464/diatom1985.17.0_29

Metzeltin D, Lange-Bertalot H (2007) Tropical diatoms of South America II. Special remarks on biogeography disjunction. Iconographia Diatomologica 18: 1–877.

Metzeltin D, Lange-Bertalot H, García-Rodríguez F (2005) Diatoms of Uruguay. Compared with other taxa from South America and elsewhere. Iconographia Diatomologica 15: 1–736.

Parr JF, Taffs KH, Lane CM (2004) A microwave digestion technique for the extraction of fossil diatoms from coastal lake and swamp sediments. Journal of Paleolimnology 31(3): 383–390. https://doi.org/10.1023/B:JOPL.0000021857.32734.c6

Rabenhorst L (1861) Die Algen Europas, Fortsetzung der Algen Sachsens, resp. Mittel-Europas. Dresden Decades I-CIX.

Round FE, Crawford RM, Mann DG (1990) The Diatoms: Biology and Morphology of the Genera. Cambridge University Press, Cambridge, 747 pp.

Simonsen R (1979) The diatom system: Ideas on phylogeny. Bacillaria 2: 9–71.

Skvortzov BV (1935) Diatomées récoltées par le Père E. Licent au cours de ses voyages dans le Nord de la Chine au bas Tibet, en Mongolie et en Mandjourie. Publications du Musée Hoangho Paiho de Tien Tsin 36: 1–43.

Skvortzov BV (2012) New and little known fresh-and brackish water diatoms chiefly from the Eastern part of Asia and their geographical distribution. In: Kulikovskiy MS, Lange-Bertalot H, Metzeltin D, Witkowski A (Eds) Lake Baikal: hotspot of endemic diatoms I. Iconographia Diatomologica vol. 23, A.R.G. Gantner Verlag K.G., Ruggell, 749–861.

Skvortzov BV (1928) Diatoms from ponds of Peking. The Peking Society of Natural History Bulletin 3: 43–48.

Skvortzov BV (1929) A Contribution to the Algae, Primorsk District of Far East, U.S.S.R. Diatoms of Hanka Lake. Memoirs of the Southern Ussuri Branch of the State Russian Geographical Society, Vladivostok, 66 pp.

Skvortzov BV (1938a) Diatoms from Chengtu, Szechwan, Western China. Philippine Journal of Science 66(4): 479–496.

Skvortzov BV (1976) Moss diatom flora from River Gan in the northern part or Great Khingan Mountains, China, with description of a new genera *Porosularia* gen. nov. from Northern and Southern China. (First Part). Quarterly Journal of the Taiwan Museum 29(1–2): 111–152.

Skvortzov BV (1938b) Diatoms from a mountain bog, Kaolingtze, Pinchiang-Sheng Province, Manchoukuo. Philippine Journal of Science 66(3): 343–362.

Souffreau C, Verbruggen H, Wolfe AP, Vanormelingen P, Siver PA, Cox EJ, Mann DG, Van de Vijver B, Sabbe K, Vyverman W (2011) A time-calibrated multi-gene phylogeny of the diatom genus *Pinnularia*. Molecular Phylogenetics and Evolution 61(3): 866–879. https://doi.org/10.1016/j.ympev.2011.08.031

Stancheva R, Manoylov K, Gillett N (2009) Morphological variation of the *Caloneis schumanniana* species complex (Bacillariophyceae) from different environmental conditions in North American streams. Hydrobiologia 635(1): 157–170. https://doi.org/10.1007/s10750-009-9908-4

Yu P, You QM, Kociolek JP, Wang QX (2019) Three new freshwater species of the genus *Achnanthidium* (Bacillariophyta, Achnanthidiaceae) from Taiping Lake, China. Fottea 19(1): 33–49. https://doi.org/10.5507/fot.2018.015

Zhu HZ, Chen JY (1989) New species and varieties of diatom from Suoxiyu. Science Press, Beijing, 33–37.

Zhu HZ, Chen JY (1995) New taxa of diatom (Bacillariophyta) from Xizang (Tibet) (I). Zhiwu Fenlei Xuebao 33: 516–519.

PhytoKeys

PhytoKeys 255: 123–141 (2025)
DOI: 10.3897/phytokeys.255.146179

Research Article

Cardamine tangutorum O.E.Schulz (Brassicaceae), a new synonym of *Cardamine macrophylla* Willd

Jia-lu Li[1], Yi He[1], Quan-ru Liu[1]

1 *Key Laboratory of Biodiversity Science and Ecological Engineering, Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, China*
Corresponding author: Quan-ru Liu (liuquanru@bnu.edu.cn)

Abstract

Cardamine macrophylla Willd. and *Cardamine tangutorum* O.E.Schulz are both widely distributed plants of the genus *Cardamine* L. in East Asia, and they are regarded as the same species in *Flora of China*. In this study, through literature and specimen research, morphological measurement and phylogenetic analyses, the results show that the traditional distinguishing characters cannot distinguish the two species. *Cardamine tangutorum* O.E.Schulz inserted into the branch of *Cardamine macrophylla* Willd. in the molecular phylogenetic tree. Therefore, based on the results of this study, *Cardamine tangutorum* O.E.Schulz was treated as the synonym of *Cardamine macrophylla* Willd.

Key words: *Cardamine*, China, morphology, phylogenetics, taxonomy

Introduction

The genus *Cardamine* L. belongs to the tribe Cardamineae within the Brassicaceae family. It is a cosmopolitan group with approximately 280 species distributed worldwide (Marhold et al. 2021). As one of the global centers of diversity, *Flora of China* records 48 species of *Cardamine* L. in China, 24 of which are endemic. New species and distribution records of *Cardamine* L. in China continue to be reported (Al-Shehbaz 2015a, 2015b; Diao et al. 2023)(Marhold et al. 2007; Al-Shehbaz and Boufford 2008; Chen et al. 2011; An et al. 2016; Marhold et al. 2016; Šlenker et al. 2018; Wu et al. 2021; Guo et al. 2024; Li et al. 2024). To date, there are 61 *Cardamine* L. species in China, 31 of which are endemic.

In 1753, Linnaeus published the genus *Cardamine* L. in Species Plantarum, which included 15 plant species. The type species of the genus is *Cardamine pratensis* L. Additionally, Linnaeus also established the genus *Dentaria* L. (Linnaeus 1753). In 1769, Crantz merged *Dentaria* L. into *Cardamine* L. (Crantz 1769). O. E. Schulz classified *Cardamine* L. into 13 sections based on the presence or absence of scales on the rhizome surface, ovule number, funiculus morphology, and the relative position of cotyledons and radicles (Schulz 1903) (Schulz 1931, 1936). This system was adopted by major floras such as FRPS and Flora of the USSR (Komarov 1939; Zhou. et al. 1987). In FRPS, Chinese *Cardamine* L. species were divided into seven sections based on Schulz's classification system, with most species assigned to the section *Cardamine* L.

Academic editor: Peter de Lange
Received: 13 January 2025
Accepted: 21 March 2025
Published: 17 April 2025

Citation: Li J, He Y, Liu Q (2025) *Cardamine tangutorum* O.E.Schulz (Brassicaceae), a new synonym of *Cardamine macrophylla* Willd. PhytoKeys 255: 123–141. https://doi.org/10.3897/phytokeys.255.146179

Al-Shehbaz proposed the concept of a broadly circumscribed *Cardamine* L., defining the genus as a cruciferous group characterized by “flattened and winged seeds, and dehiscent siliques that split elastically through torsion.” This treatment incorporates *Dentaria* into the expanded *Cardamine s. l.* and abolishes infrageneric classifications (Al-Shehbaz 1988, 2000, 2015c) Al-Shehbaz and Guang 1998; Zhou et al. 2001). These taxonomic revisions have subsequently received support from molecular phylogenetic evidence (Franzke et al. 1998; Sweeney and Price 2000). Major floristic works, including Flora of China and Flora of Pan-Himalaya, have adopted Al-Shehbaz’s taxonomic treatment of *Cardamine* L. (Zhou et al. 2001; Al-Shehbaz 2015c).

During the nomenclatural verification and taxonomic studies of *Cardamine* L. species in China, we found that the identification of specimens for the widely distributed *Cardamine macrophylla* and *Cardamine tangutorum* in East Asia was inconsistent, leading to controversies in their species delimitation. Flora of China suggested that these two taxa might represent the same species, requiring further verification (Zhou et al. 2001).

Cardamine macrophylla Willd. was first described by Willdenow, based on specimens collected from the Taz River estuary in the Mangazeya region of northern Siberia, USSR (as shown in Fig. 2). The distribution of *Cardamine macrophylla* spans a wide latitudinal range, occurring in Siberia, the Qinling Mountains, and the Hengduan Mountains. Due to its significant morphological variation, numerous subspecies and varieties have been described under this species. In 1825, D. Don described *Cardamine polyphylla* D. Don (Don and Hamilton 1825). In 1872, several varieties of *Cardamine macrophylla* were published in Flora of British India: *Cardamine macrophylla* var. *dentariifolia* Hook. f.& T. Anderson, *Cardamine macrophylla* var. *lobata* Hook. f.& T. Anderson, and *Cardamine macrophylla* var. *sikkimensis* Hook. f.& T. Anderson. Additionally, the nomen nudum *Cardamine foliosa* Wall. was treated as *Cardamine macrophylla* var. *foliosa* Hook. f.& T. Anderson (Wallich and East 1828; Hooker 1875). In Schulz’s 1903 system, *Cardamine macrophylla* was placed in the *Macrophyllum* section (Schulz 1903).

In 1980, Tai Yien Cheo and colleagues treated *Cardamine polyphylla* D. Don as a variety of *Cardamine macrophylla* Willd., naming it *Cardamine macrophylla* Willd. var. *polyphylla* (D. Don) T. Y. Cheo et Fang. They also described a new variety, *Cardamine macrophylla* Willd. var. *diplodonta* T. Y. Cheo, based on variations in leaf margin serration and the number of cauline leaves (Cheo et al. 1980). In the FRPS, three varieties of *C. macrophylla* were recognized: *C. macrophylla* Willd. var. *polyphylla* (D. Don) T. Y. Cheo et Fang, *C. macrophylla* Willd. var. *diplodonta* T. Y. Cheo, and *Cardamine macrophylla* var. *crenata* Trautv. (Trautvetter 1887). However, the 2001 edition of Flora of China synonymized these varieties under *C. macrophylla*. Additionally, it synonymized *Dentaria sino-manshurica* Kitag (Kitagawa 1937) and *Cardamine urbaniana* O.E. Schulz (Schulz 1903) with *C. macrophylla* (Zhou et al. 2001).

Cardamine tangutorum O.E. Schulz was described by Schulz in 1903. According to the protologue, this species is distinguished by its purplish-red, prominently veined sepals with translucent narrow margins and its triangularly swollen ovule stalks measuring 0.5–4.0 mm in length. Schulz cited multiple specimens as syntypes for this species: Gansu 1872 *N. M. Przewalski #s.n.,*1879 *N. M. Przewalski #s.n.,*1880 *N. M. Przewalski #s.n.*; *Gansu* 1885

G. N. Potanin #s.n.; *Shaanxi*, Miaowang Mountain 1899 *J. Giraldi No. 3379 No. 3378* ;Shaanxi 1884 *Potanin #s.n.*; Beijing Xiaowutai Mountain 1879 *Möllendorff #s.n.*; Sichuan 1894 *Rosthorn No. 2583* (see Fig. 1). In Schulz's classification system for *Cardamine* L., *C. tangutorum* was placed in the section *Dentaria* (Schulz 1903). Both the FRPS and Flora of China recognized *C. tangutorum*. However, the editors of Flora of China noted the morphological similarity between *C. tangutorum* and *C. macrophylla*, suggesting that they might represent the same species (Zhou. et al. 1987; Zhou et al. 2001), with a common species distributed in subalpine and alpine zones in the Himalayas, North China, Northeast China, Central China, and Hengduan Mountains.

According to records from Flora Reipublicae Popularis Sinicae, Flora of China, and Flora of Pan-Himalaya, the morphological distinctions between *Cardamine macrophylla* and *C. tangutorum* are primarily as follows: *C. macrophylla* exhibits a conspicuously thickened rhizome lacking scale-like appendages, a plant height of (20–) 30–95 (–115) cm, 3–12 cauline leaves scattered along the stem, and uppermost leaflets of the cauline leaves decurrent into winged extensions. *C. tangutorum* is characterized by a slender, whip-like rhizome with scale-like appendages, a plant height of (8–) 15–30 (–40) cm, 1–3 cauline leaves clustered at the upper stem, and comparatively narrower and smaller cauline leaves with fewer lateral leaflets and non-decurrent uppermost leaflets.

Based on the above understanding, we conducted morphological and molecular phylogenetic studies, supported by a thorough review of the literature and examination of specimens, to clarify the taxonomic status of these two species.

Method

Sampling and morphological analyses

Specimens with complete rhizoid and multiple copies were selected from PE, BNU, KUN and other herbaria, as well as the digital HD and other type specimen photos on the website of LE Herbarium. Specimens' collection are shown in Table 1. A total of 46 population specimens were collected for morphological analyses. Re-identified and grouped according to the retrieval characters described above. Ten traits were selected according to FRPS, Flora of China, and protologue, and measured by ImageJ (Schindelin et al. 2012), and two ratios were calculated. The characters and coding are listed in Table 2. PCA was performed by Past 4.13 after standardization by IBM SPSS Statistics (v.27) (Hammer et al. 2022; IBMCorp 2020).

Phylogenetic analyses

Fresh plant leaves were collected in the field and quickly dried with silica gel. Plant samples were sent to Beijing Novogene Corporation for quality testing and re-sequencing. The sequencing platform, Illumina HiSeq X Ten and BGI, was used to generate approximately 6 GB of data for each sample. The chloroplast genome was assembled from the clean data using Get Organelle (Jin et al. 2020). Plastid Genome Annotator (PGA) was used to annotate the chloroplast genome with *Amborella trichopoda* Baill. from software as references (Qu et al. 2019). Then, 26 plastid genome sequences were downloaded from

Figure 1. Syntype specimens of *Cardamine tangutorum* including 1880 N. M. Przewalski #s.n. (top left), 1885 G. N. Potanin #s.n. (top right), 1879 N. M. Przewalski #s.n. (bottom left), and Rosthorn #2583 (bottom right).

Table 1. Taxa and sample sites, bold specimen representation was used for molecular phylogenetic analysis.

Taxa and sample sites
***C.tangutorum* O.E. Schulz**
China Sichuan, Zhongsu Team, *7165*
China Sichuan: Wenchuan, K.Y.Lang ,*L.Q.Li, Y. Fei, 1136*
China Sichuan: Aba, K.Y.Lang ,*L.Q.Li, Y. Fei, 2048*
China Qinghai: *K.M.Liou, 6185*
China Qinghai, Z.H.Zhang etc, *4484*
China Gansu, *T.P.Wang 7275*
China Gansu, *T.P.Wang 6979*
China Gansu, *Tsi-Tang Li198*
China Hebei: Tuoliang, *TL006*
China Hebei: Tuoliang, *TL008*
China Hebei: Tuoliang, *TL023*
China Hebei: Tuoliang, TL007
China Beijing: Donglingshan, *C. Wang, 50611006*
China: Eastern Gansu, *G. H. Potani, 1885*
China Gansu: Tanggulashan, *N. M. Przewalski, 1872*
China Hebei: Xiaowutaishan, *62079 CC. W. Wang*
China Gansu: 1879, *N. M. Przewalski #s.n.*(syntype of *C. tangutorum*)
China Gansu: 1880, *N. M. Przewalski #s.n.*(syntype of *C. tangutorum*)
***C.macrophylla* Willd.**
China Chongqing, Chengkou, G212 Road, *zhang698*
China Sichuan, Aba, *zhang801*
China Sichuan: Bazhong, *GSL2015050209*
China Hunan, longshan, Bamianshan, *zhang667*
China Hubei, Yanzi Zhen, *zhang676*
China Yunnan, *T.T.yu 9780*
China Sichuan, *S.X, Yu, Y.T.Hou, X.X.Zhang, Y.M.Zhao, 4812*
China Sichuan, *K.Y.Lang, L.Q.Li, Y.Li, 1917*
China Sichuan: Batang, *K.Y.Lang, L.Q.Li, Y.Li, 2471*
China Sichuan: Nanping, *K.Y.Lang, L.Q.Li, Y.Li, 1608*
China Hebei: Xilingshan, *J.X.Duan, 239*
China Shanxi: Nuanshuihe, *Shanxi Investigation Team, 530*
China Shanxi: Shiziping, *Shanxi Investigation Team, 592*
China Shanxi: Yuwu, *J.M.Liu, 1662*
China Henan: Neixiang, *D.E.Boufford,C.Y.Xi,T.S.Ying etc.26303*
China Hubei: Shennongjia, *Hubei Shennongjia Planting Research Team, 10006*
China Anhui: Yuexi, *X.L.Liu, 492*
China Hunan: Sangzhi, *B. Zhang & X. Xiang, 090425022*
China Sichuan: Kangding, *Chuanxi Team, K.J.Guan, W.C.Wang etc,772*
China Sichuan: Kangding, *Chuanxi Team, K.J.Guan, W.C.Wang etc,387*
China Sichuan: Kangding, *Y.T.Zhang & K.Y.Lang, 37*
China Sichuan: Ganzi, *Xizang Team, 73-03*
China Sichuan: Ganzi, *Y.T.Zhang & K.Y.Lang, 121*
China Sichuan, E.H.Li,*Y.F.Han, J.G.Liao, Y. Hu, H82-335*
China Sichuan: Muli, *Qingzang Team, 13042*
China Sichuan: Xiangcheng, *Qingzang Team & Hengduanshan Team, 003712*
China Sichuan: Yanyuan, *Qingzang Team, 12344*
China Sichuan: Maerkang, *X. Li, 70477*
China Sichuan: Jiulong, *Z.X.Tang, X.W.Tian, Q.G.Sun, 245*

NCBI (Table 7), including 23 species of *Cardamine* L.and 2 species, *Rorippa sylvestris* (L.) Besser, *Rorippa indica* (L.) Hiern as outgroup. The annotated sequences were imported into PhyloSuite (Zhang et al. 2020), the Mafft module

Table 2. List of morphological characters and their acronyms used in analyses.

Acronym	Description of character
Scaly*	Presence of scales on rhizome (with 1/without 0)
RW	Rhizome diameter
CW	Mid-stem diameter
H*	Height of stem
LH*	Height of the location of lowest stem leaves
No. SL*	Number of stem leaves
No. MLL*	Number of lateral leaflets on mid-stem leaves
LTL*	Length of the terminal leaflet on mid-stem leaves
WTL	Width of the terminal leaflet on mid-stem leaves
DL*	Length of the uppermost leaflet's lower edge on mid-stem leaves
Ratio	
LH/H*,LTL/WTL*,RW/CW*	

was used for sequence alignment (Katoh et al. 2019), and the ModelFinder module was used to calculate the nucleotide substitution model for the aligned sequences. The maximum likelihood (ML) tree was constructed using IQ-TREE (Minh et al. 2020), with the nucleotide substitution model set to GTR+R3+F and a standard bootstrap value of 1000.

Flow cytometric measurements and estimation of DNA ploidy levels

Methods referring to Marhold et al. (2010) and Kobrlová and Hroneš (2019) measured the nuclear DNA content using flow cytometry. Inferred the DNA ploidy levels within the studied populations based on *Cardamine* L.species with known ploidy. The relative nuclear DNA content was determined using PI, a DNA intercalating fluorescent dye, with arbitrary units (a.u.) as the unit of measurement. The buffer solution used was LB01. Dehydrated leaves, preserved by drying at 40 °C for 18–24 months, were used for the determination of chromosome ploidy. The sample sources and voucher specimens are presented in Table 3. In a pre-cooled culture dish, 1–2 mL of LB01 buffer solution and 2 cm^2 of dry leaves were added. After rapid chopping, the mixture was filtered through a 400-mesh gauze, centrifuged at 4 °C, 3000 rpm for 10 minutes, and the supernatant was discarded. The pellet was re-suspended in 600 µL of LB01 buffer solution, followed by the addition of 100 µL of PI solution (50 µg/mL), and stained in the dark for 15 minutes. Ploidy level of the stained cell suspension was determined by flow cytometry (ACEA NovoCyte 3130). Using 488 nm blue light excitation, 10,000 cells were collected at a time. The other samples were determined under the same voltage bar using C. scutata ($2n = 4x = 32$) as the reference for tetraploid.

Result

Principal component analysis results

Using SPSS software, a cluster analysis was conducted on the standardized data matrix, resulting in a character correlation matrix as shown in Table 4. The cluster analysis results indicate that none of the Pearson correlation coefficients

Table 3. List of taxa and sample sites used in flow cytometric measurements.

Taxa	Voucher information
C. tangutorum	BNU2023WLH077
	BNU2022xz
	BNU2023ZJK26
	BNU2022HLG 002
C. macrophylla	BNU2022YN070
	BNU2022YN004
	BNU2022mcs006
	BNU2022em002

Table 4. Correlation of morphological characters between *C. macrophylla* and *C. tangutorum*.

	H	LHH	No. SL	No. MLL	LTL	LTL/WTL	DL	RW/CW	SCALY
H	–	-0.16	0.209	0.402	0.597	0.209	0.509	0.295	0.037
LHH		–	-0.653	-0.136	-0.161	0	-0.08	-0.05	0.041
No. SL			–	0.114	0.162	0.171	0.201	0.049	-0.052
No. MLL				–	0.298	0.093	0.312	0.16	0.002
LTL					–	0.267	0.753	0.431	0.271
LTL/WTL						–	0.325	0.102	-0.03
DL							–	0.37	0.045
RW/CW								–	0.119
SCALY									–

between each pair of characters reached 0.8. The highest correlation was between the length of the apical leaflet of the stem leaves and the downwards extension length of the first pair of lateral leaflets, with a coefficient of 0.753. Thus, no significant correlations were observed among other traits, allowing all traits to be retained for subsequent multivariate principal component analysis.

Principal component analysis (PCA) was performed on the standardized data matrix using PAST software, and the contribution values of each principal component (PC) are shown in Table 5. The cumulative contribution of the first four principal components was 77.658%, indicating successful dimensionality reduction, making PCA suitable for this study. Based on previously identified results, the data was grouped and incorporated into the standardized data matrix, as shown in Fig. 3.

Based on the results of the PCA, the distribution ranges of *C. macrophylla* and *C. tangutorum* along the PC3 and PC2 axes are essentially identical. Along the PC1 axis, the two species exhibit a clear continuous transitional distribution, with significant overlap in their primary distribution ranges and their core distribution areas also show substantial overlap. In PC1, characteristics with larger contributions include the length of the downwards extension of the uppermost lateral leaflets of the stem leaves, the length of the apical leaflets of the stem leaves, and plant height. In PC2, the characteristic with the largest contribution is the position of leaf attachment. For PC3, the characteristics with the highest contributions are the leaf length-to-width ratio and the number of lateral leaflets on the stem leaves. Box plots of these high-contributing traits, as shown in Fig. 4, indicate that the distribution ranges of all these high-contributing characteristics also demonstrate significant overlap.

Figure 2. Holotype specimen of *Cardamine macrophylla*.

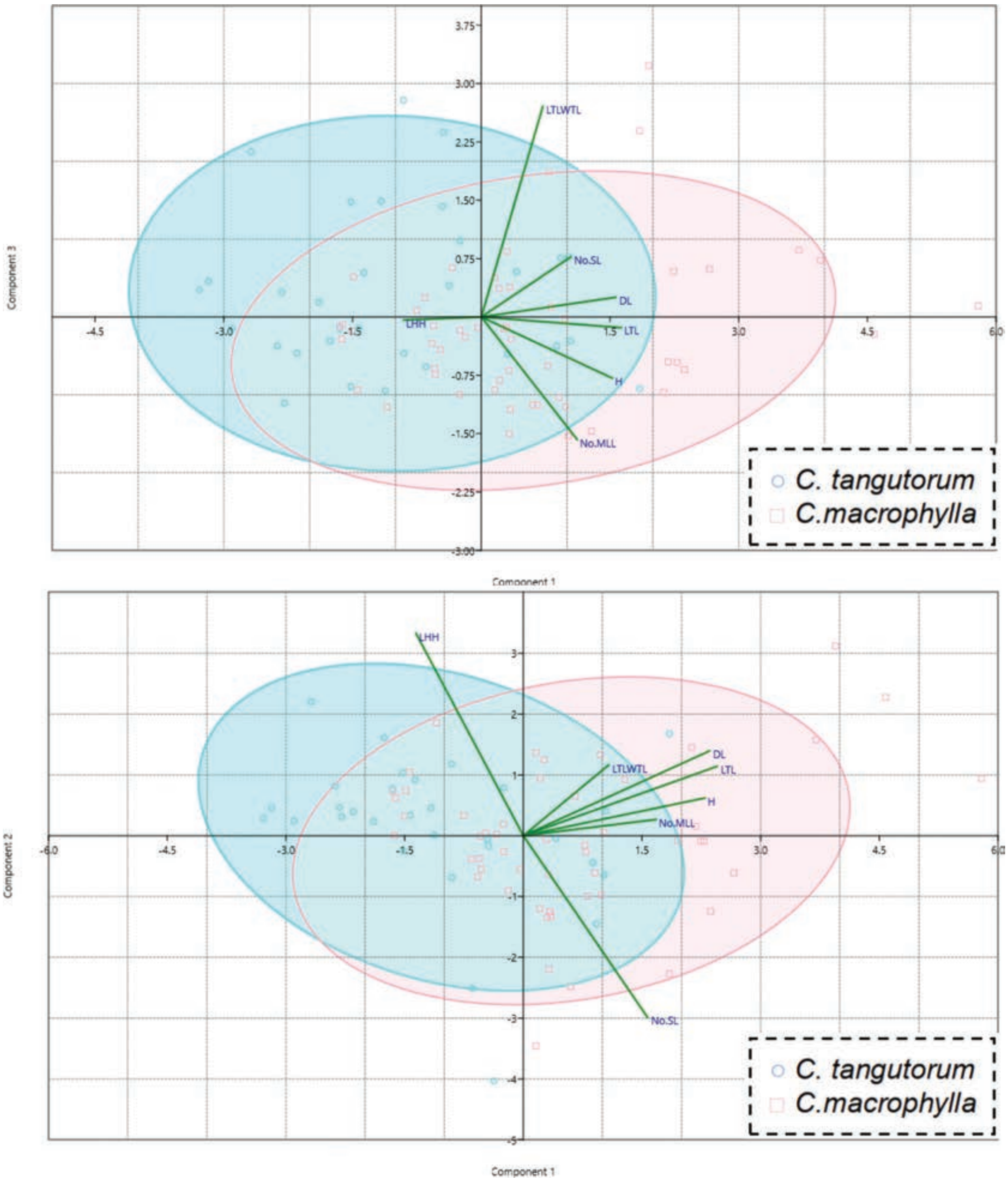

Figure 3. Ordination diagrams of principal component analyses, Grouping reference specimen identification.

Table 5. Contribution Values of Each Principal Component.

Principal component (PC)	Eigenvalue	Proportion (%)	Cumulative (%)
PC1	2.938	36.615	36.615
PC2	1.511	18.828	55.443
PC3	0.956	11.919	67.362
PC4	0.826	10.296	77.658
PC5	0.601	7.493	85.151
PC6	0.475	5.917	91.067
PC7	0.351	4.368	95.435
PC8	0.244	3.0354	98.471
PC9	0.123	1.5291	100.000

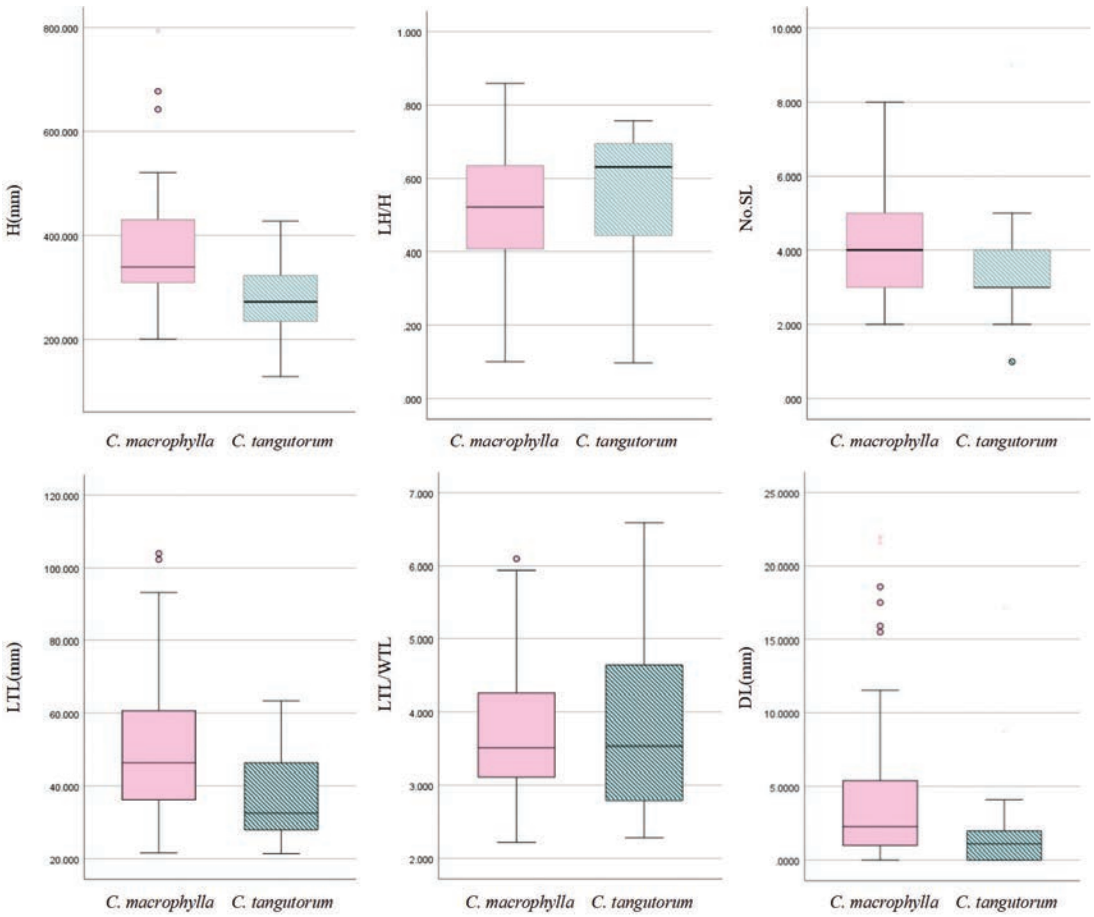

Figure 4. Box Plots of Traits with High Principal Component Contributions.

Molecular phylogenetic study

The chloroplast genome tree is illustrated in Fig. 5, with bootstrap support (BS) values indicated below the branches. The *Cardamine* L. species selected for this study form a well-supported monophyletic group, divided into three distinct clades. *C. tangutorum* is nested within the monophyletic group formed by *C. macrophylla*, both clustering together in clade 3. Besides *C. macrophylla* and *C. tangutorum*, morphologically similar species such as *C. leucantha* and *C. fragarifolia* were included in constructing the phylogenetic tree.*C. leucantha* clusters with *C. impatiens* at the base of clade 3. *C. leucantha* forms a monophyletic group with *C. glanduligera*, positioned within clade 3.

Flow cytometric measurements and estimations of relative DNA content

The relative DNA content between different populations of *C. macrophylla* and *C. tangutorum* was calculated using 12-to 24-month-old specimens. The results showed that for all the populations measured in this study, the coefficient of variation ranged from 0.49% to 6.89%, as shown in the Table 6, indicating that the results were credible and that the relative DNA content of *C. macrophylla* and *C. tangutorum* was stable within the populations. The relative DNA content of *C. macrophylla* and *C. tangutorum* is shown below; the relative DNA content of *C. macrophylla* and *C. tangutorum* varies greatly among populations, but the distribution range at the species level is basically the same (shown in Fig. 6).

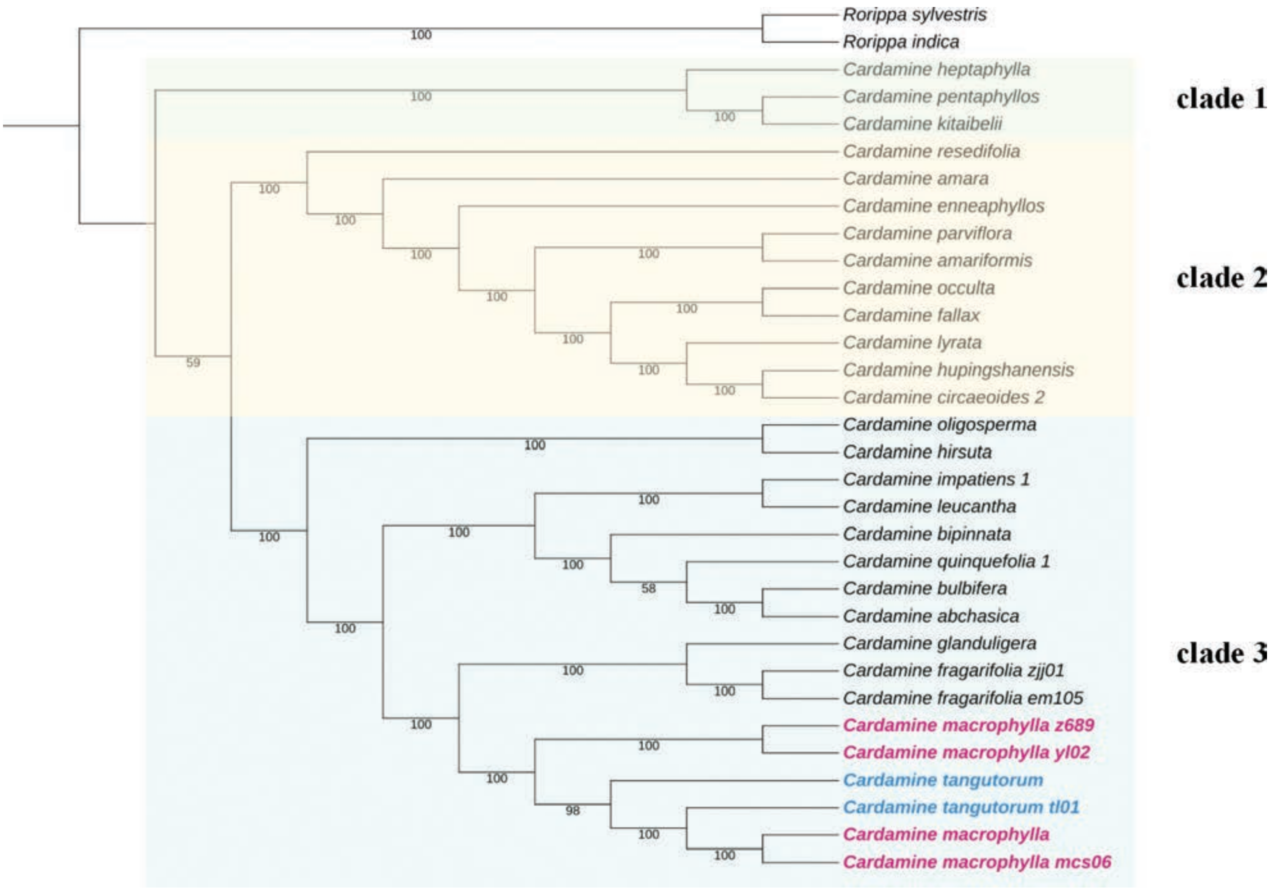

Figure 5. The strict consensus tree resulted from IQ-TREE analysis using plastid genome sequence. Bootstrap values (BS) are shown under branches. Red marking *C. macrophylla*, blue marking *C. tangutorum*.

Table 6. Relative genome sizes obtained for *C.macrophylla, C. tangutorum*.

Taxa	Voucher information	Relative genome size in a.u. (arbitrary units)	Variation (%)
C. tangutorum	BNU2023WLH077	1.316	1.89%
C. tangutorum	BNU2022xz	1.018	6.49%
C. tangutorum	BNU2023ZJK26	1.415	6.13%
C. macrophylla	BNU2022YN070	1.440	3.10%
C. macrophylla	BNU2022YN004	0.888	6.02%
C. macrophylla	BNU2022mcs006	1.333	4.33%
C. macrophylla	BNU2022em002	0.985	5.45%
C. tangutorum	BNU2022HLG 002	1.426	0.49%

Discussion

Taxonomic status of *C. tangutorum*

Through morphological analysis of 46 populations, it was found that the distinguishing features between *C. macrophylla* and *C. tangutorum* significantly overlap. The decurrence in leaflets of *C. macrophylla* and *C. tangutorum* significantly overlaps in their distribution ranges. Generally, the decurrence in leaflets of *C. macrophylla* tends to be longer. However, some populations, such as *Qingzang Expedition 12344* and *T.T.yu 9780*, exhibit nearly no decurrence in leaflets. Other related

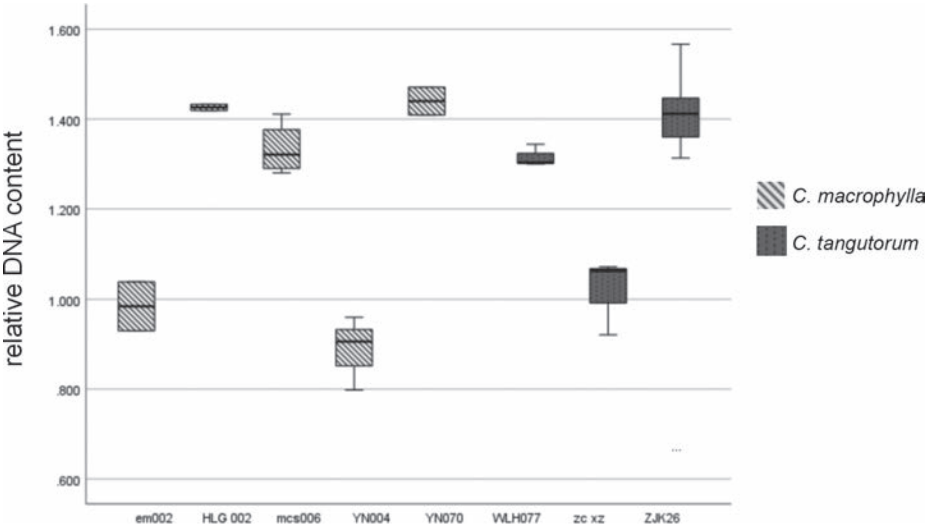

Figure 6. Relative genome sizes obtained for *C.macrophylla*, *C. tangutorum*.

Table 7. Taxa in phylogenetic analyses from GenBank.

GenBank accession numbers	Species name
NC026446	*C. resedifolia*
MT136871	*C. quinquefolia*
MK637691	*C. pentaphyllos*
NC036964	*C. parviflora*
NC036963	*C. oligosperma*
MZ043777	*C. occulta*
MF405340	*C. macrophylla*
MZ846206	*C. lyrata*
MK637684	*C. kitaibelii*
NC026445	*C. impatiens*
ON322745	*C. hupingshanensis*
MK637681	*C. hirsuta*
MN651504	*C. heptaphylla*
MK637680	*C. glanduligera*
MZ043778	*C. fallax*
NC049605	*C. enneaphyllos*
OL634846	*C. circaeoides*
NC049603	*C. bulbifera*
MN651509	*C. bipinnata*
MZ043776	*C. amariformis*
NC036962	*C. amara*
NC060863	*C. abchasica*
KJ136821	*C. impatiens*
NC069649	*Rorippa sylvestris*
NC065833	*Rorippa indica*

species within the genus, such as *C. lyrata* and *C. occulta*, exhibit continuous variability in decurrence in leaflets, making this trait unsuitable as a basis for species differentiation. The position of stem leaves is also a significant distinguishing feature in FRPS (Zhou. et al. 1987), but as shown in the boxplot, the lower stem leaf positions of both *C. macrophylla* and *C. tangutorum* are essentially the same, located in the upper part of the plant, cannot be used for distinguishing.

Previously, when *C. tangutorum* was described, Schulz cited many collections as syntype specimens. Comparing the syntype specimens of *C. tangutorum* with the holotype specimens of *C. macrophylla*, decurrence in leaflets cannot be observed at the leaflet bases in *C. macrophylla* holotypes. Furthermore, significant variations in leaf length-width ratios, sizes, and positions were observed between different isotype specimens of *C. tangutorum* (as shown in Fig. 1).

Upon examining the extant specimens of *C. macrophylla* and *C. tangutorum* in herbariums, we found that for both species, individuals with slender whip-like rhizomes have grooves on the rhizome surface but lack significant scales. However, some grayish-white triangular scales or leftover marks after their detachment were commonly present at the bases of leaf buds and branch buds (as shown in Fig. 7).

Considering that both *C. macrophylla* and *C. tangutorum* are perennial herbaceous plants with persistent rhizomes, these scaly appendages should be regarded as bud scales. In individuals with significantly fleshy rhizomes, these scales on the rhizome surface are less prominent. The presence or absence of scales on rhizomes, as emphasized in Schulz's classification system, was considered an important criterion for subgroup classification. In Schulz's system, a key feature of the *Cardamine* L. group is the prominent scales on rhizomes, whereas the macrophylla group lacks them. However, in The Families and Genera of Angiosperms in China it was suggested that the two groups in China should be merged, negating Schulz's classification viewpoint (Wu et al. 2003). Examining the specimens, it is evident that the prominence of rhizome scales is affected by the plant's age and the degree of rhizome fleshiness and does not serve as a basis for species differentiation. The quantitative taxonomy results indicate that none of the traditional differentiating traits effectively distinguish *C. macrophylla* from *C. tangutorum*. *C. macrophylla* shows significant morphological variability, within which *C. tangutorum* should be included.

Some species of *Cardamine* L., such as *C. yezoensis*, *C. pratensis*, etc., have variation in ploidy and relative DNA content within species (Marhold et al. 2010). The results of flow cytometry showed that the relative DNA content of *C. macrophylla* and *C. tangutorum* was stable within populations, but there was great variation among populations, showing polymorphism of ploidy and relative DNA content. However, the distribution range of DNA content of *C. macrophylla* and *C. tangutorum* were basically the same, which supported the combination of *C. macrophylla* and *C. tangutorum*.

Multiple populations of *C. macrophylla* and *C. tangutorum* were selected for the molecular phylogenetic study. The results indicate that both species share a high similarity in chloroplast genomes, with *C. tangutorum* nested within the monophyletic clade of *C. macrophylla*. This suggests close phylogenetic relationships at the molecular level, making it inappropriate to treat them as distinct species. *C. leucantha* and C. *fragarifolia*, with similar leaflet morphology to both *C. macrophylla* and *C. tangutorum*, all with long lanceolate leaflets and cuneate bases, are positioned within clade 3 in the molecular phylogenetic tree. This possibly suggests the single evolutionary origin of these morphological characters.

Combining molecular phylogenetic, cytological and morphological studies, it is concluded that the morphological range of *C. macrophylla* and *C. tangutorum* significantly overlaps, making them difficult to distinguish. Their molecular phylogenetic positions are nested within the same monophyletic group, rendering them indistinct. Considering the common species distributed, *C. macrophylla* and *C. tangutorum* should be treated as a single species, and *C. tangutorum* should be treated as a synonym of *C. macrophylla*.

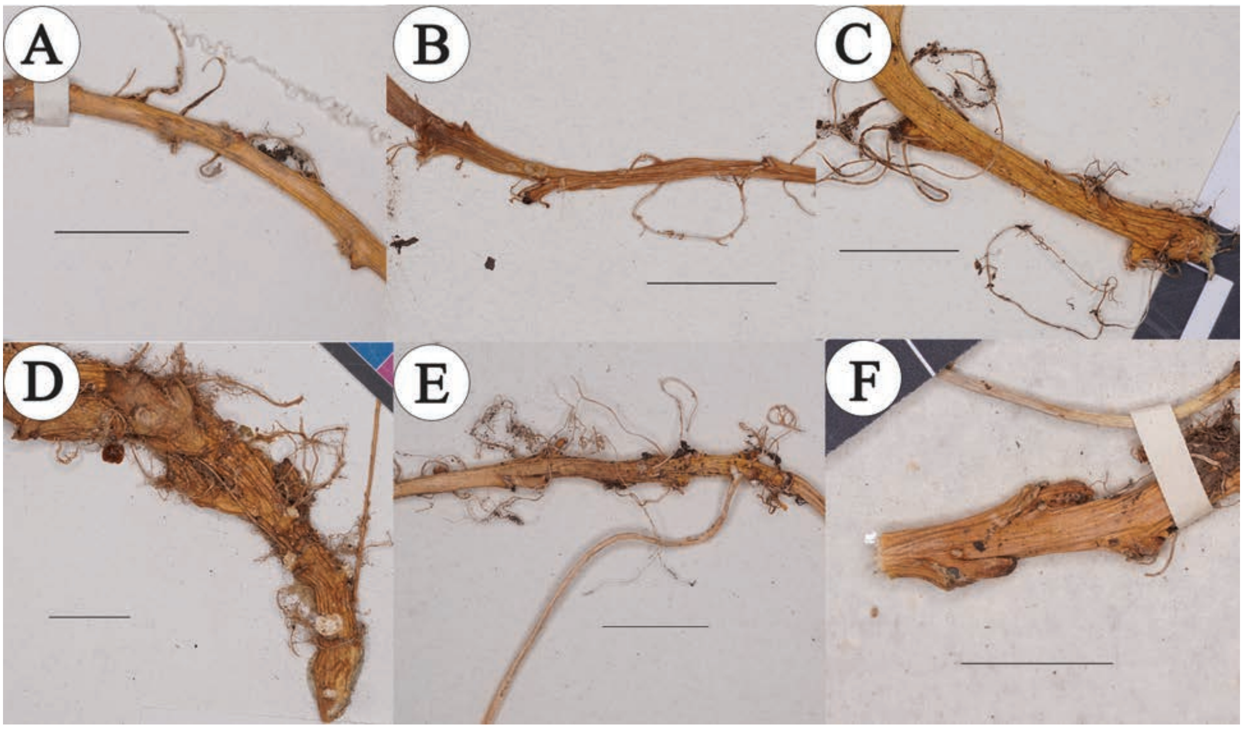

Figure 7. Rhizomes of *C. tangutorum* and *C. macrophylla* (**A, B** *C. tangutorum* **C–F** *C. macrophylla*). Scale bar: 1 cm.

Taxonomic treatment

Cardamine macrophylla Willd., Sp. Pl. 3 (1): 484. 1800.

=*Cardamine macrophylla* var. *crenata* Trautv., Trudy Imp. S.-Peterburgsk. Bot. Sada 5 (1): 18. 1877.

=*Cardamine macrophylla* var. *dentariifolia* Hook. f.& T. Anderson Fl. Brit. India [J. D. Hooker] 1 (1): 139 (1872). Type (designated by (Marhold et al. 2015)): India, Himal.Bor.Occ., *Thomson, T. s.n.*, (K000397478!)

=*Cardamine macrophylla* var. *diplodonta* T.Y. Cheo, Bull. Bot. Lab. N.-E. Forest. Inst., Harbin 6: 20. 1980.

=*Cardamine foliosa* Wall.,[Numer. List: 4779. 1831. nom. nud.]

≡*Cardamine macrophylla* var. *foliosa* Hook. f.& T. Anderson Fl. Brit. India [J. D. Hooker] 1 (1): 139 (1872). Type(designated by (Marhold et al. 2015)): India, Kumaon, Wallich, Cat.Wall.4779 (K000247365!, P00747537!, B_10_0241370!, B_10_0241369!, GH00549127!).

=*Cardamine macrophylla* var. *lobata* Hook. f.& T. Anderson, Fl. Brit. India [J. D. Hooker] 1 (1): 139 (1872). Type(designated by (Marhold et al. 2015)): [INDIA] [Label 1]: “marshy meadows, Nira Zanskar, 12,900 ft, 2 July 1849 [?]”, [Label 2]: “Hab. Himal. Bor. Occ., W. Tibet, Regio Temp., Alt. 12,900 ft, T. T. [T. Thomson] s.n.” (K000397477).

=*Cardamine macrophylla* var. *moupinensis* Franch., Pl. David. 2: 18. 1888. Type(designated by (Marhold et al. 2015)): [CHINA], [Label 1 (handwritten)]: “Moupin, Thibet oriental, lieux frais en montagne, Avril 1869”, [Label 2 (printed)] “Chine (Thibet Oriental), Province de Moupin, 1870 [sic!], David s.n.” (P00747519, Isolectotype: P00747518).

=*Cardamine macrophylla* var. *sikkimensis* Hook. f.& T. Anderson Fl. Brit. India [J. D. Hooker] 1 (1): 139 (1872). Type (designed by (Marhold et al.

2015)): India, Sikkim Lachung, 03 September 1849, *Hooker, J.D. s.n.* (K000397479!, isolectotype: K000397480!, K000397481!, GH00549128!)
=*Cardamine polyphylla* D. Don not O. E. Schulz, Prodr. Fl. Nepal.: 201. 1825.
=*Cardamine sachalinensis* Miyabe & T. Miyake, Fl. Saghalin No. 58, t. 3, fig. 1–3, 1915.
=*Cardamine sino-manshurica* Kitag., Rep. Inst. Sci. Res. Manchoukuo 4: 111, 1940.
≡*Dentaria sino-manshurica* Kitag.Rep. Inst. Sci. Res. Manchoukuo 4: 111, 1940.
=*Cardamine urbaniana* O.E. Schulz, Bot. Jahrb. Syst. 32 (2–3): 396. 1903.
Syntype: China, Sichuan, 1885–1888, *A. Henry 5635 (*B 10 0241328!); China, Shaanxi, Huangcaoping County, 20 June 1894, *G. Giraldi 447* (B 10 0241329!)
=*Dentaria gmelinii* Tausch, Flora 19 (2): 402, 1836.
=*Dentaria macrophylla* Bunge ex Maxim., Prim. Fl. Amur. 45, 1859.
=*Dentaria wallichii* G. Don, Gen. Hist. 1: 172, 1831.
=*Dentaria willdenowii* Tausch, Flora 19 (2): 403, 1836.
=*Cardamine tangutorum* O.E. Schulz, Bot. Jahrb. Syst. 32 (2–3): 360. 1903.

Holotype. Russia, Northern Sibiria, Mangezeya, at the mouth of Taz River (B-W11970-030!)

Syntype. China, Gansu, Terra Tangurorum, *N. M. Przewalski No. 1872 (*LE01014556!); China, Gansu, Terra Tangurorum, *N. M. Przewalski No. 1873 (*K000697742!); China, Gansu, Terra Tangurorum, *N. M. Przewalski No. 1880* (LE01014557!); China, Gansu, orient, *G. N. Potanin 1885* (LE01014555!, LE01014558!); China, Sichuan, “Tsakulao”,1891, *A. v. Rosthorn 2583.*, (B 10 0241334!); China, Shaanxi, Baoji County, Mountain Miaowangshan, 1899, *J. Giraldi No. 3379*; China, Hebei, Mountain Xiaowutaishan, 1879, *O. V. Möllendorff s. n.*

Habitat. Often in shady areas under forests, along ditches or in subalpine meadows.

Distribution. Widely distributed in Siberia, Mongolia, Himalayas, North China, Northeast China, Central China, Hengduan Mountains.

Phenological period. Flowering from May to July, fruit from June to September.

Description. Perennial herbs, 30 cm–70 cm tall, up to 1 m. Rhizomes creeping, sometimes tuberous; basal leaves pinnate, pedicled on creeping rhizomes, terminal leaflet long ovate to lanceolate, margin toothed, lateral leaflet similar to terminal leaflet. Cauline leaves are similar to basal leaves but slightly smaller, mostly in the middle and upper parts of the plant. Flowers lilac to purplish red, calyx margins white membranous, petals with long claws; the seeds are oblong. This species showed high diversity in the size of leaflets and morphology of leaf margins.

Specimens examined. • **Sichuan Province**: Yajiang County, Mountain Kazila, 08 September 2011, *He et al. SCU-11-360*, (KUN1235449!, KUN1235450!); Kangding County, *He et al. SCU-080340*, (KUN1235446!); • **Yunnan Province**: Shangri-La County, 24 July 2014, *Guo et al. 14CS9432*, (KUN1321027!); • **Heilongjiang Province**: Jinshantun County, Huilongwan National Forrest Park, *Hou et al. 389*, (QFNU0006577!, QFNU0006578!); • **Hebei**: Donggou Temple, 16 May 1951, *Wencai Wang 2122*, (PE 01004424!, PE 01004426!); Xiaowutai Mountain, Tielin Temple, 29 July 1906, Y. Yabe s.n., (NAS00326894!, NAS00326895!) • **Shanxi Province**: Mountain Guandishan, 23 June 1959, *Sai Ma 15030*, (WUK0321706!, WUK0324493!); Wutaishan Mountain, 17 July 1907, *Y. Yabe s. n.*, (NAS00326872); Lingchuan County, Fenghuang Valley, 14 April 2014, *Kong et al. k0087*, (SD00018202!, SD00016344!) • **Tibet Province**: Mangkang County, 318 Road, 20 July 2008, *Zhang et al. SunH-07ZX-0503* (KUN1300753!, KUN1300754!, KUN1300755!); Yadong County, Naiduila Mountain, 23 August 2013, *Y.S.Chen et al.13-1966*, (PE02000587!, PE02000588!); Dingqing County, 22 July 2016, *Shuai Li et al. 20167324*, (BNU0030842!); • **Gansu Province**: Tan County, Mountain Lougu, 15 June 1956, *Huanghe Exped 4911*, (WUK0085438!); Zhuoni County, Chebagou Valley, *Yin et al. LiuJQ-GN-2011-128*, (KUN1235443!); Maqu County, Xiuma, 03 September 2008, *Li et al. LiJ0061*, (KUN1235442!); Maqu County, Langmu Temple, 01 June 1999, *Bailong River Exped 1589*, (PE01556040!); Lianhua Mountain, 13 May 2002, *Xuegang Sun 2741*, (PE01998146!); Linxia County, Dalijia Mountain, 28 July 1988, *Ji Ma 88090*, (NAS00326893!, NAS00326896!, NAS00326897!); • **Beijing**: Baihua Mountain, August 1981, *Anonymous 81-0322*, (BJFC00019955!, BJFC00019954!, BJFC00019953!); Baihua Mountain, June 1990, *D. D. Lu et al. 272*, (BJFC00019961!, BJFC00019960!, BJFC00019959!, BJFC00019956!); Mentougou County, Lingshan Mountain, 01 May 2009, *G. M. Zhang 200923*, (BJFC00066271!, BJFC00066273, BJFC00066274!); • **Shaanxi**: Long County, 19 May 1983, Sujia River, J. X. Yang 4207, (WUK0438580!, WUK0438581!); • **Qinghai Province**: Ledu County, Qutan, 24 June 1970, *Benzhao Gou 7198*, (WUK0308377); Menyuan County, 26 July 2008, *Yuhu Wu LJQ-QLS-2008-0143*, (KUN1235444!, KUN1235445!); Maqin County, Dawu, 14 July 2014, *Xiaoyu Wu, Xiaolei Zhang s.n.*, QH2014006, (BNU0020082!); Huzhu County, Beishan Foresty Centre, 13 July 1982, B. *Z. Gou 25521*, (HNWP102005!); Guide County, Laji Mountain, 20 June 1992, *R. F. Huang 3710*, (HNWP169550!, HNWP169551!); • **Mongolia**: 17 August 1979, *Губанов 7845*, (MW0179858!, MW0179859!);17 August 1979, *Губанов 7830*, (MW0179855!, MW0179856!); 14 August 1979, *Губанов 7704*, (MW0179853!, MW0179854!); 9 August 1979, *Губанов 7610*,(MW0179857!, MW0179863!); • **Russia**, **Altai & Sayany Mountains**: 9 July 1984, Триль 4493, (MW0081495!); 27 July 1983, *Шауло 2019*, (MW0081478!); 17 July 1984, *Сонникова 1535*, (MW0081469!);18 June 1983, *Сонникова 1532*, (MW0081472!); 1 August 1982, *Сонникова 1529*, (MW0081468!); 8 August 1979, *Ухина 1518*, (MW0081474!); 25 June 1978, Ухина 1515, (MW0081471!); 13 June 1988, *Шауло 30*, (MW0081475!, MW0081494!); • **Russian Far East**: 16 August 1990, *Штрик 90-569*, (MW0081357!, MW0081364!); 23 August 1990, *Штрик 90-449*, (MW0081358!, MW0081372!); 8 August 1990, *Борисов 90-189*, (MW0081369!); 28 July 1988, *Кемниц 88-238*, (MW0081367!); 25 July 1998, *Кемниц 88-222*, (MW0081366!); • **Central Siberia**: 23 July 1977, *Куваев 85*,(MW0081457!) • **Baikal & Transbaikal region**: 9, August 1929, *Назаров 12.819*, (MW0081409!); 7 July 1929, *Назаров 12.118*, (MW0081404!) • **Kazakhstan**, **Western Altai Mountains**: 27, August, 1932, *Воронов 720*, (MW0081463!); 18 July 1930, *Смирнов 173*, (MW0081461!, MW0081462!).

Acknowledgments

We would like to express gratitude to the staff of PE, KUN for their support of this study.

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 financed by the National Natural Science Foundation of China (No. 210100203 & No. 32200170).

Author contributions

All authors have contributed equally.

Author ORCIDs

Jia-lu Li https://orcid.org/0009-0002-8147-5157
Yi He https://orcid.org/0000-0002-6925-7299
Quan-ru Liu https://orcid.org/0000-0003-4270-4746

Data availability

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

References

Al-Shehbaz IA (1988) The genera of Arabideae (Cruciferae, Brassicaceae) in the southeastern United-States. Journal of the Arnold Arboretum 69: 85–166. https://doi.org/10.5962/bhl.part.2391

Al-Shehbaz IA (2000) New or noteworthy species of Cardamine (Brassicaceae) from China. Novon 10: 323–328. https://doi.org/10.2307/3392976

Al-Shehbaz IA (2015a) *Cardamine xinfenii* (Brassicaceae), a New Species from Sichuan (China). Harvard Papers in Botany 20: 145–146. https://doi.org/10.3100/hpib.v20iss2.2015.n3

Al-Shehbaz IA (2015b) *Cardamine hongdeyuana* (Brassicaceae), a new species from Xizang, China. Kew Bulletin 70: 1–4. https://doi.org/10.1007/s12225-014-9556-3

Al-Shehbaz IA (2015c) Flora of Pan-Himalaya, Volume 30: Brassicaceae, Cambridge University Press and Science Press, Beijing.

Al-Shehbaz IA, Boufford DE (2008) *Cardamine tianqingiae* (Brassicaceae), a new species from Gansu Province, China. Harvard Papers in Botany 13: 89–91. https://doi.org/10.3100/1043-4534(2008)13[89:CTBANS]2.0.CO;2

Al-Shehbaz IA, Guang Y (1998) Notes on Chinese Cardamine(Brassicaceae). Harvard Papers in Botany 3: 73–77.

An M, Lin Y, Yu L, Yang Y, Cheng G, Li J (2016) *Cardamine kuankuoshuiense* (Brassicaceae), a new species from Guizhou, China. Phytotaxa 267: 233–236. https://doi.org/10.11646/phytotaxa.267.3.7

Chen W, Zhang S, Wang J, Liu J, Ivanovich SA (2011) Some new or noteworthy plant species for China found in North West Xinjiang. Turczaninowia 14: 75–80.

Cheo T, Lu L, Guo R (1980) Materiae an Floram Cruciferum Sinicarum. Bulletin of Botanical Research.

Crantz HJNV (1769) Henrici Ioan. Nepom. Cranz. Classis cruciformium emendata cum figuris aeneis in necessarium Instit. rei herbariae supplementum.

Diao Y, Zhao CF, Chen YB, Hu ZL (2023) Cardamine libagouensis, a new species from Hubei, China. Nordic Journal of Botany. https://doi.org/10.1111/njb.03914

Don D, Hamilton F (1825) Prodomus florae Nepalensis: sive Enumeratio vegetabilium quae in itinere per Nepaliam proprie dictam et regiones conterminas, ann. 1802–1803. Detexit atque legit dd Franciscus Hamilton,(olim Buchanan) Accedunt plantae ad Wallich nuperius missae, J. Gale. https://doi.org/10.5962/bhl.title.86

Franzke A, Pollmann K, Bleeker W, Kohrt R, Hurka H (1998) Molecular systematics of Cardamine and allied genera (Brassicaceae): Its and non-coding chloroplast DNA. Folia Geobotanica 33: 225–240. https://doi.org/10.1007/BF03216204

Guo YJ, Li JL, Al-Shehbaz IA, Qian LS, Feng MJ, Yui JP, Liu QR (2024) Cardamine sichuanensis (Brassicaceae), a new species from Sichuan, China. Phytotaxa 663: 15–25. https://doi.org/10.11646/phytotaxa.663.1.2

Hammer Ø, Harper TDA, Ryan PD (2022) PAST: Paleontological Statistics Software Package for Education and Data Analysis (Version 4.13).

Hooker JD (1875) The flora of British India. Vol. v.1 (1875), L. Reeve, London. https://doi.org/10.5962/bhl.title.678

IBMCorp (2020) IBM SPSS Statistics for Windows (Version 26). NY.

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. https://doi.org/10.1186/s13059-020-02154-5

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kitagawa T (1937) Report of the Institute of Scientific Research, Manchoukuo. Tairiku Kagakuin Kenkyu Hokoku 4: 111.

Kobrlová L, Hroneš M (2019) First insights into the evolution of genome size in the borage family: A complete data set for Boraginaceae from the Czech Republic. Botanical Journal of the Linnean Society 189: 115–131. https://doi.org/10.1093/botlinnean/boy079

Komarov VL (1939) Flora of the U.S.S.R. Vol. 8. Izdatel'stvo Akademii Nauk SSSR, 115–129.

Li J, Zhang C, He Y, Liu Q (2024) Cardamine zhangjiajieensis, a new species of Brassicaceae in China. PhytoKeys 248: 293–304. https://doi.org/10.3897/phytokeys.248.119678

Linnaeus C (1753) Species plantarum. Vol. 2, Impensis GC Nauk, 654 pp.

Marhold K, Lihova J, Al-Shehbaz IA, Kudoh H (2007) The correct interpretation and lectotypification of the name Cardamine fallax (Brassicaceae). Journal of Plant Research 120: 655–660. https://doi.org/10.1007/s10265-007-0107-y

Marhold K, Kudoh H, Pak JH, Watanabe K, Spaniel S, Lihova J (2010) Cytotype diversity and genome size variation in eastern Asian polyploid Cardamine (Brassicaceae) species. Annals of Botany 105: 249–264. https://doi.org/10.1093/aob/mcp282

Marhold K, Kempa M, Al-Shehbaz I (2015) Lectotypification of names of Himalayan Brassicaceae taxa currently placed in the genus Cardamine. PhytoKeys 50: 9–23. https://doi.org/10.3897/phytokeys.50.5080

Marhold K, Slenker M, Kudoh H, Zozomova-Lihova J (2016) *Cardamine occulta*, the correct species name for invasive Asian plants previously classified as *C. flexuosa*, and its occurrence in Europe. PhytoKeys: 57–72. https://doi.org/10.3897/phytokeys.62.7865

Marhold K, Kempa M, Kučera J, Skokanová K, Smatanová J, Šingliarová B, Šlenker M, Zozomová-Lihová J (2021) Datababase of names, chromosome numbers, ploidy levels and genome sizes of the tribe Cardamineae. https://cardamine.sav.sk

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

Qu XJ, Moore MJ, Li DZ, Yi TS (2019) PGA: A software package for rapid, accurate, and flexible batch annotation of plastomes. Plant Methods 15: 50. https://doi.org/10.1186/s13007-019-0435-7

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: An open-source platform for biological-image analysis. Nature Methods 9: 676–682. https://doi.org/10.1038/nmeth.2019

Schulz OE (1903) Monographie der Gattung Cardamine. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 32: 280. https://doi.org/10.5962/bhl.title.15567

Schulz OE (1931) Einige neue Cruciferen. Notizblatt Des Königl. Botanischen Gartens Und Museums Zu Berlin 11: 225–230. https://doi.org/10.2307/3994773

Schulz OE (1936) Cruciferae. Die Naturlichen Pflanzenfamilien 17B: 227–658.

Šlenker M, Zozomová-Lihová J, Mandáková T, Kudoh H, Zhao Y, Soejima A, Yahara T, Skokanová K, Španiel S, Marhold K (2018) Morphology and genome size of the widespread weed *Cardamine occulta*: How it differs from cleistogamic *C. kokaiensis* and other closely related taxa in Europe and Asia. Botanical Journal of the Linnean Society 187: 456–482. https://doi.org/10.1093/botlinnean/boy030

Sweeney PW, Price RA (2000) Polyphyly of the genus *Dentaria* (Brassicaceae): Evidence from *trnL* intron and *ndhF* sequence data. Systematic Botany 25: 468–478. https://doi.org/10.2307/2666690

Trautvetter ERV (1887) Trudy Imperatorskago S.-Peterburgskago botanicheskago sada. Acta Horti Petropolitani. v: 10. [Imperatorskiĭ S.-Peterburgskiĭ botanicheskiĭ sad, S.-Peterburg]

Wallich NN, East ICM (1828) Numerical list of dried specimens of plants in the Museum of the Honl. East India Company, [s.n.], London. https://doi.org/10.5962/bhl.title.1917

Wu Z, Lu A, Tang Y, Chen Z, Li D (2003) The Families and Genera of Angiosperms in China. A Comprehensive analysis, Science Press, Beijing.

Wu L, Liu W, Mu C, Al-shehbaz IA (2021) Cardamine hunanensis (Brassicaceae), a remarkable new species from Hunan (China) with fully bracteate racemes. Phytotaxa 512. https://doi.org/10.11646/phytotaxa.512.1.7

Zhang D, Gao FL, Jakovlic 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: 348–355. https://doi.org/10.1111/1755-0998.13096

Zhou TY, Guo XY, Lan YZ, Lou LL, Kuan KC, An ZX (1987) Cruciferae. In: Cheo TY (Ed.) Science Press, Beijing, 1–483. [in Chinese]

Zhou T, Lu L, Yang G, Al-Shehbaz IA (2001) Flora of China. 8. Brassicaceae through Saxifragaceae [Hauptbd.], Science Press.

PhytoKeys 255: 143–169 (2025)
DOI: 10.3897/phytokeys.255.141395

Research Article

Studies of Vietnamese Pteridophyte Flora 3

Cheng-Wei Chen[1,2,3], Van Truong Do[4], Quang Cuong Truong[5], Viet Dai Dang[6], Hong Truong Luu[6], Yi-Shan Chao[3], Yao-Moan Huang[7], Kuo-Fang Chung[2]

1 *Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei 115, Taiwan*
2 *Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan*
3 *Department of Life Science, National Taiwan Normal University, Taipei 106, Taiwan*
4 *Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam*
5 *International Center for Tropical Highlands Ecosystems Research of Bidoup - Nui Ba National Park, Lam Dong, Vietnam*
6 *Institute of Advanced Technology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam*
7 *Taiwan Forestry Research Institute, Taipei 100, Taiwan*
Corresponding authors: Cheng-Wei Chen (wade0504@gmail.com); Kuo-Fang Chung (bochung@gate.sinica.edu.tw)

Abstract

This is the third paper in a series dedicated to updating the knowledge of the Vietnamese pteridophyte flora. Based on recent collections, we first reported three new national records of ferns: *Haplopteris yakushimensis*, *Lindsaea kohkongensis*, and *Pteris pseudowulaiensis*. Secondly, we conducted phylogenetic analyses to investigate the placements of *Lindsaea kohkongensis* and *Leptochilus poilanei*, each based on three plastid DNA markers. Our results revealed that *Lindsaea kohkongensis* is sister to *L. ensifolia*, while *Leptochilus poilanei* is embedded within *L. cantoniensis*. We discussed these results in the context of systematics. Lastly, we reported chromosome numbers for 20 fern species in Vietnam. For seven of these species, including *Gymnosphaera salletii*, *Lepisorus spicatus*, *Leptochilus poilanei*, *Pteridrys costularis*, *Pteris latipinna*, *Pyrrosia eberhardtii*, and *Tectaria setulosa*, these counts were recorded for the first time. Additionally, three new cytotypes were identified for *Diplazium doederleinii*, *Pteris esquirolii*, and *Tectaria harlandii*. This study underscores the need for more diverse data, including DNA sequences, chromosome numbers, and reproductive modes, to be collected and integrated into systematic studies and taxonomic treatments to enhance our understanding of Vietnam's pteridophyte flora.

Key words: Chromosome number, cytology, Indochina, new records, phylogeny

Academic editor: Joel Nitta
Received: 9 November 2024
Accepted: 24 March 2025
Published: 17 April 2025

Citation: Chen C-W, Do VT, Truong QC, Dang VD, Luu HT, Chao Y-S, Huang Y-M, Chung K-F (2025) Studies of Vietnamese Pteridophyte Flora 3. PhytoKeys 255: 143–169. https://doi.org/10.3897/phytokeys.255.141395

Introduction

This marks the third paper in our series on Vietnam's pterido-flora (Chen et al. 2021; 2023). This series of studies aims to provide updated knowledge of the country's pteridophyte flora, based on recent expeditions as well as the studies of herbarium specimens and relevant literature. Here, we reported three new additions to Vietnam's flora: *Haplopteris yakushimensis* C.W.Chen & Ebihara (Fig. 1), *Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun (Fig. 2), and *Pteris pseudowulaiensis* Y.S.Chao (Fig. 3) based on our new collections.

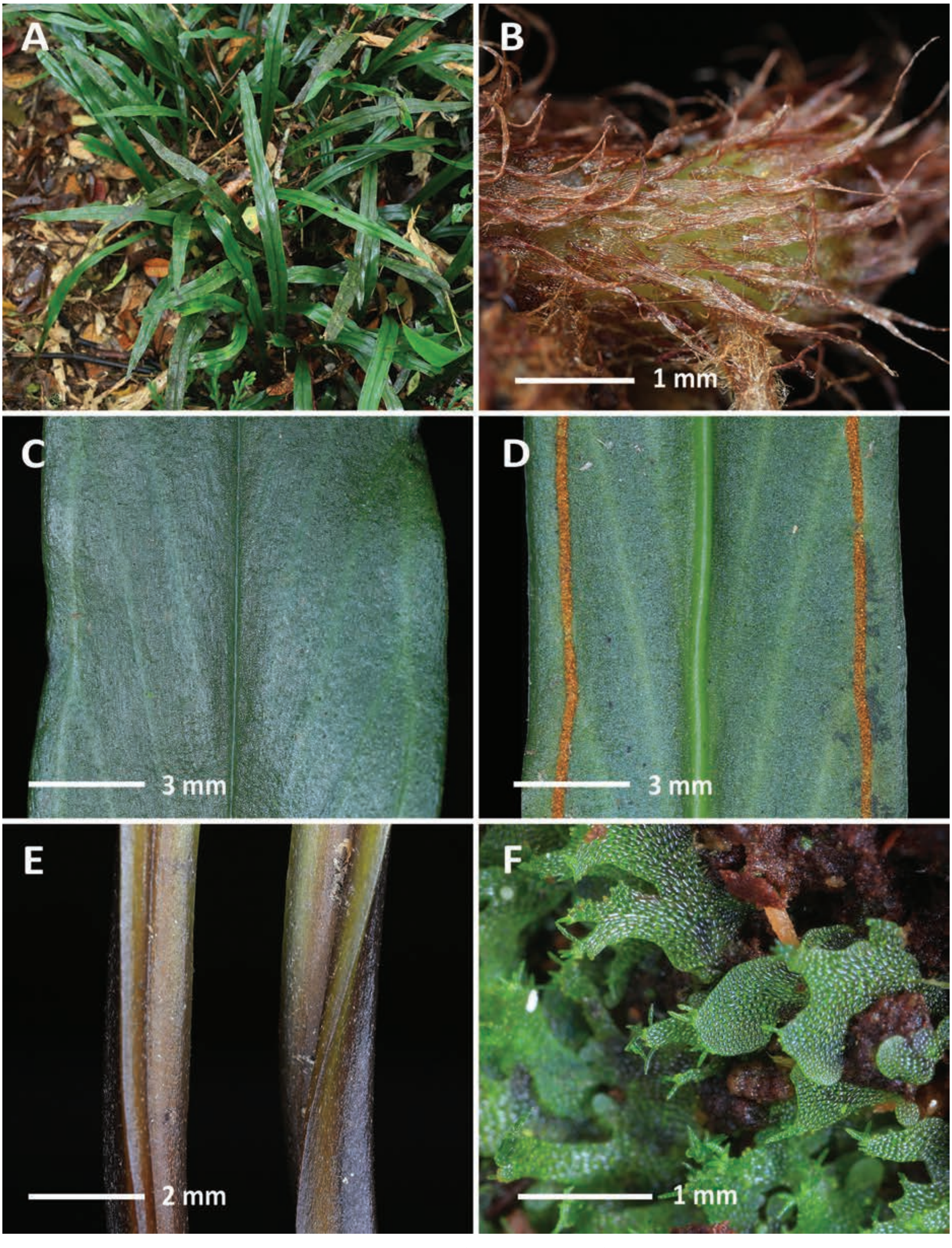

Figure 1. *Haplopteris yakushimensis* C.W.Chen & Ebihara (*Chen Wade6952*) **A** habit **B** rhizome scales **C** adaxial lamina **D** abaxial lamina **E** stipes **F** gametophytes. Photographed by C.-W. Chen.

Additionally, we performed phylogenetic analyses to elucidate the placements of *Lindsaea kohkongensis* (Fig. 2) and *Leptochilus poilanei* (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang (Fig. 4). In 2018, we collected an unknown *Lindsaea* specimen from Phu Quoc Island of southern Vietnam. This specimen closely

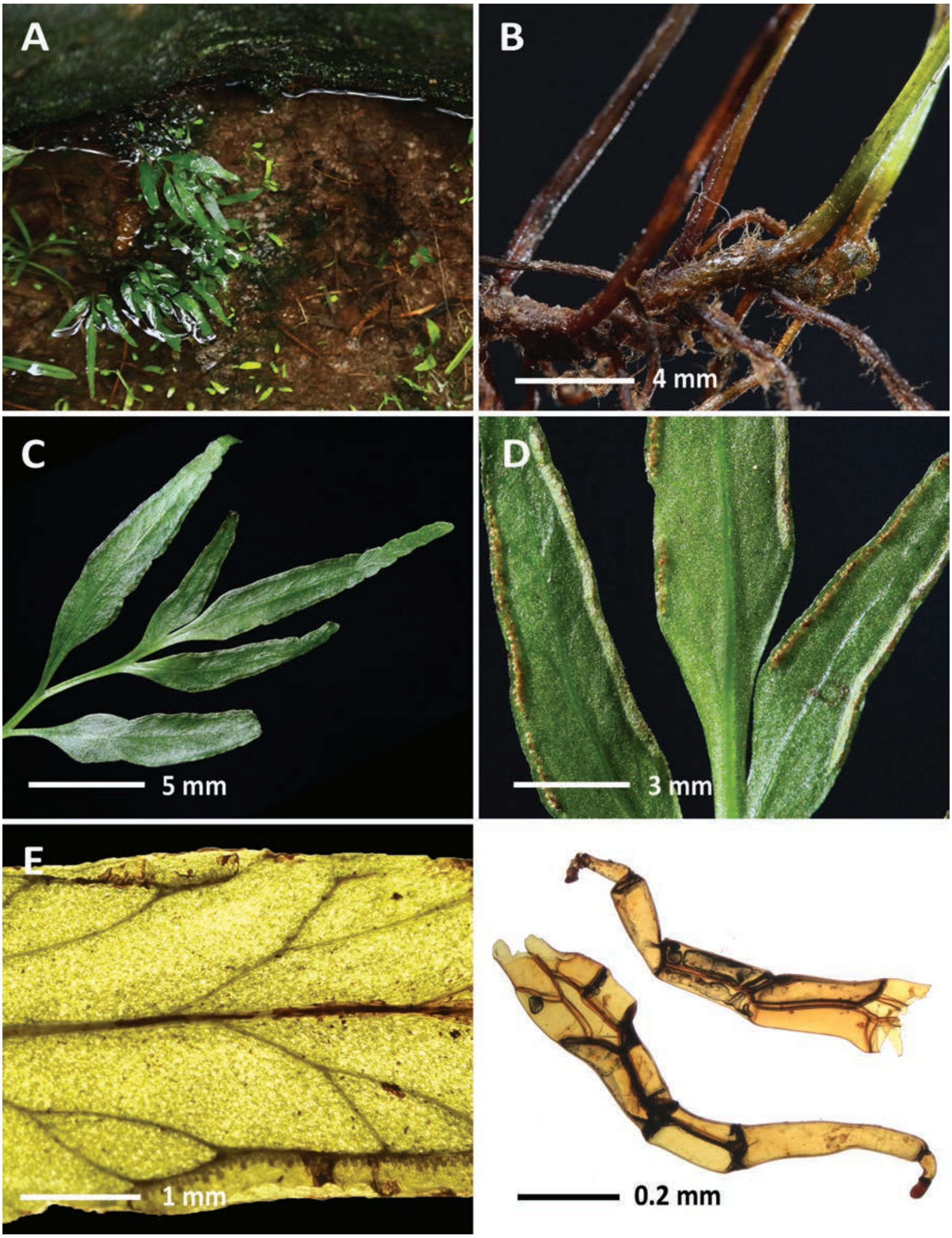

Figure 2. *Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun (*Chen Wade5034*) **A** habit **B** rhizome **C** adaxial lamina **D** abaxial lamina **E** venation **F** rhizome scales. Photographed by C.-W. Chen.

resembles *L. ensifolia* Sw. but being much smaller and with an unusual subaquatic habit. Specimens from Cambodia and Malaysia with the same morphology had recently been described as *Lindsaea kohkongensis* (Yun et al. 2023) but without molecular data. Here, we conducted a phylogenetic analysis to test whether *L. kohkongensis* is a distinct species or an eco-form of *L. ensifolia*.

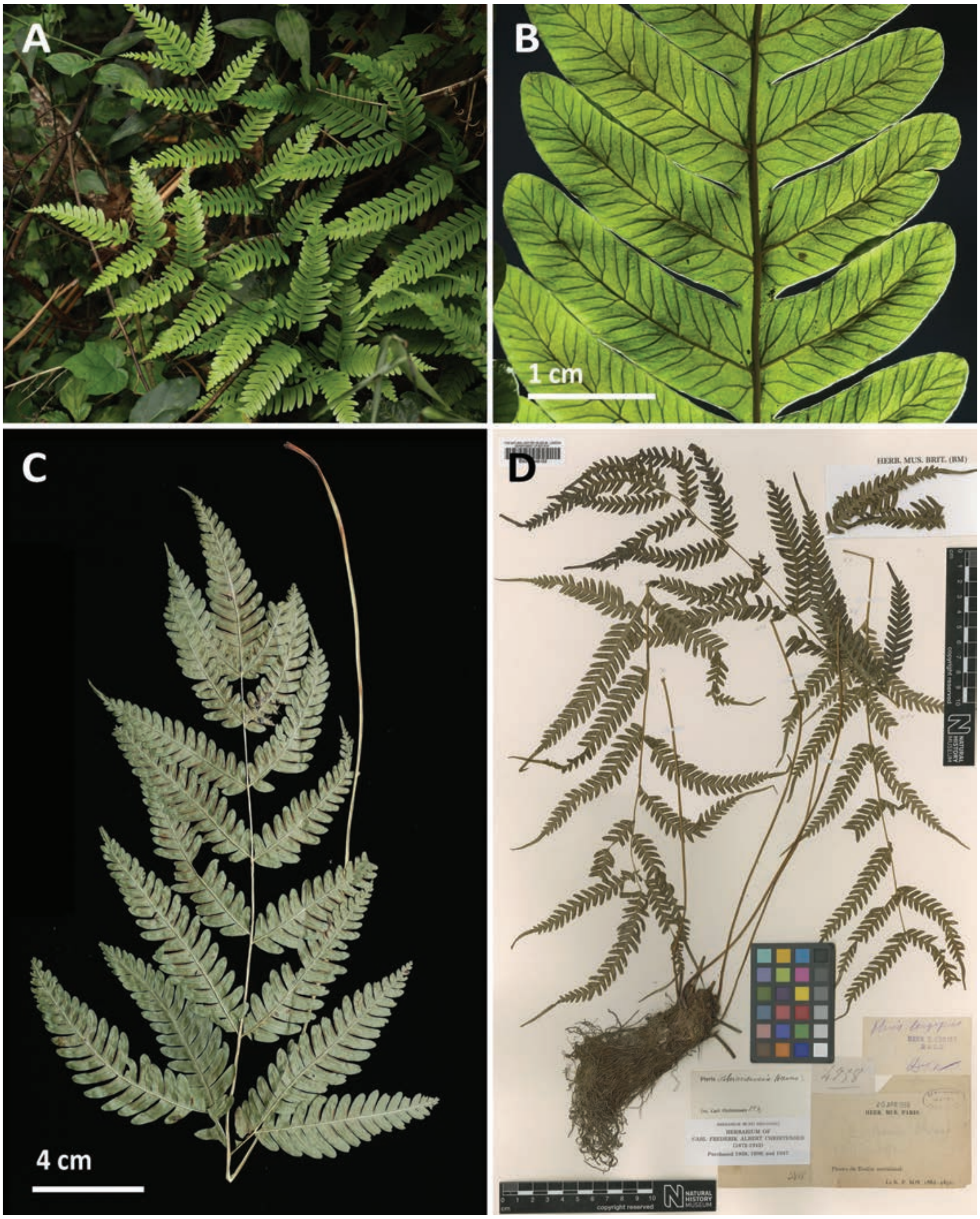

Figure 3. Comparison of *Pteris pseudowulaiensis* C.M.Kuo (A, B, C, *Chao 3509 and 3517*) and *P. oshimensis* Hieron. (D, *Bon 4758*) **A** habit **B** lamina (backlight) **C** specimen **D** specimen, CC BY © The Trustees of the Natural History Museum, London. Photographed by C.-W. Chen and Y.-S. Chao.

Leptochilus poilanei was described by Christensen and Tardieu-Blot (1939) under the genus *Colysis* (≡ *Leptochilus*), based on specimens collected from Annam, which is now part of central Vietnam. Following its initial description, the species remained largely unstudied until Zhang et al. (2018) reclassified it under *Leptochilus*. To our knowledge, this species is only known from its type collection, and our new collection (Fig. 4) from Nui Chua National Park represents the second collection of this species. It can be readily distinguished from other congeneric species in Vietnam by its strongly dimorphic fronds: fertile fronds are mostly linear and simple (rarely tripartite), while sterile ones are simple to pinnatifid.

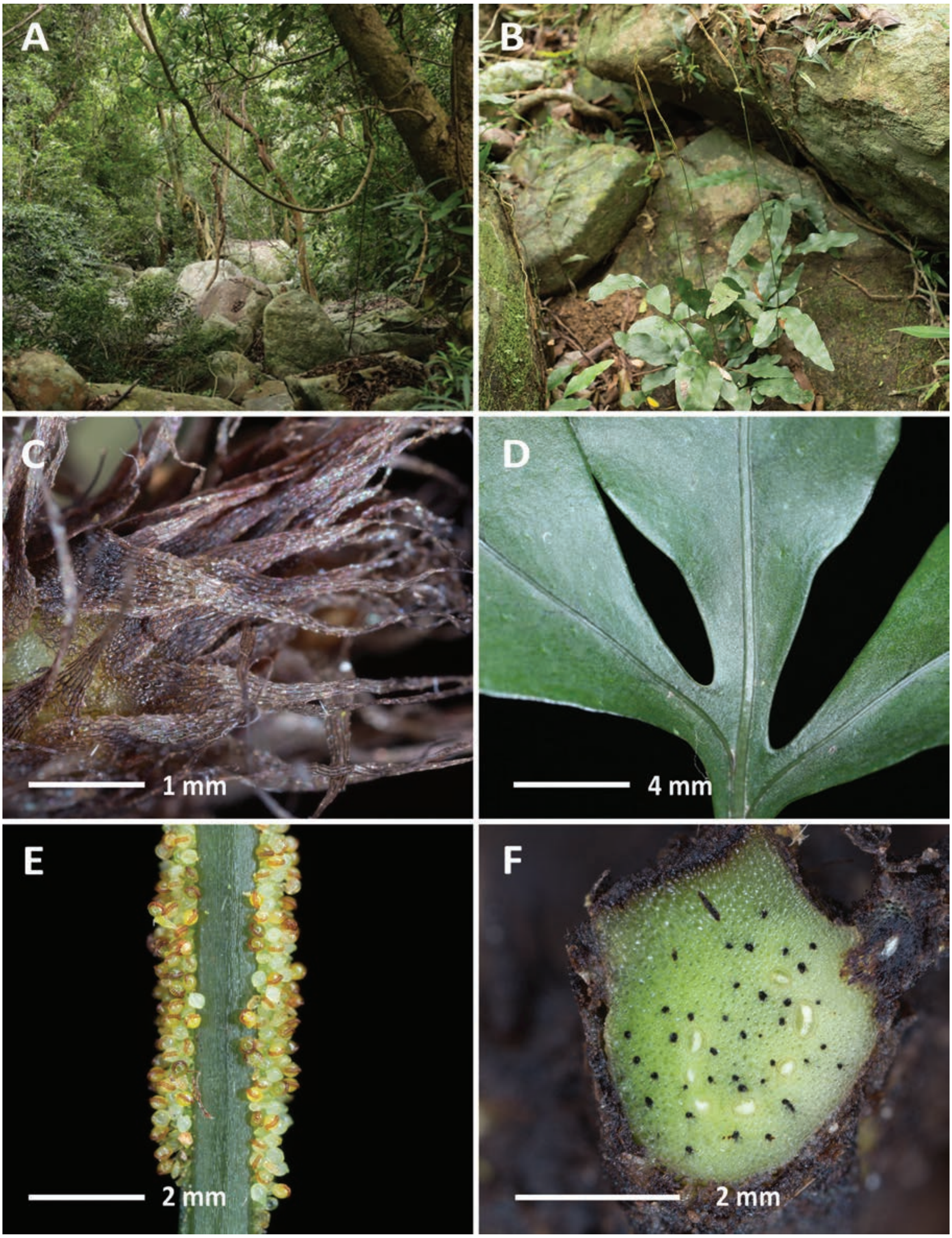

Figure 4. *Leptochilus poilanei* (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang (*Chen Wade6804*) **A** habitat **B** habit **C** rhizome scales **D** sterile frond **E** fertile frond **F** cross section of rhizome. Photographed by C.-W. Chen.

Lastly, we presented new chromosome counts of Vietnamese fern species and explored the implications of our findings in the context of systematics. Chromosome numbers have long been recognized as important information in plant systematics (Rice et al. 2015). This significance is particularly pronounced for ferns, as they exhibit the highest frequency of polyploid speciation among vascular plants (Wood et al. 2009). Despite the importance of chromosome number data, research on Vietnamese ferns in this regard has been limited. To date, no systematic survey has been conducted. Instead, available data remains sporadic and dispersed throughout various articles, often in the context of new species' descriptions (e.g., Chen et al. 2023), new species records (e.g., Ding et al. 2013), or cytological studies focused on specific taxa, including specimens collected from Vietnam (e.g., Nayak and Singh 1989). By reporting the chromosome numbers of Vietnamese fern species and demonstrating how these data can be integrated into systematic studies, we aim to raise awareness of chromosome studies among local botanists, thereby encouraging further related research in the future.

Materials and methods

Specimen identification and distribution

We conducted field expeditions in Bidoup Nui-Ba National Park, Cuc Phuong National Park, Phia Oac-Phia Den National Park, Phu Quoc National park, and Nui Chua National Park during 2018–2023. To ensure the correct identifications of our newly collected specimens, we compared them with herbarium specimens, relevant literature, including published papers, checklist, and flora from neighboring countries (see notes under each species), and original protologues and types through the Biodiversity Heritage Library (https://www.biodiversitylibrary.org/) and JSTOR Global Plants (https://plants.jstor.org/). To confirm the known distribution of each species, we searched the names against World Ferns (Hassler 1994–2024) and GBIF (https://www.gbif.org/) and manually examined the available specimen images.

Cytological observations

To determine chromosome numbers, root tips were collected either from the field or transplanted plants in greenhouses. These root tips were treated with a 1:1 mixture of hydroxyquinoline and cycloheximide (Sigma-Aldrich, USA) for approximately 16 hours at 18 °C. Following this, the root tips were fixed in a 3:1 mixture of 95% ethanol and 45% acetic acid for about 12 hours at room temperature. Subsequently, the root tips were macerated using a 1:1 mixture of cellulase (Yakult, Japan) and pectolyase (Sigma-Aldrich, USA) for 1 hour at 37 °C. Finally, the treated root tips were squashed in 2% acetocarmine and observed under a microscope (Zeiss Axio Imager A1, Germany). To determine the ploidy of our observed chromosome numbers, we consulted two online databases, Chromosome Counts Database (CCDB, Rice et al. 2015) and Index to Plant Chromosome Numbers (IPCN, Goldblatt and Lowry 2011), and considered the lowest sporophytic counts known for a genus as the diploid.

Furthermore, we counted the spore numbers per sporangium and observed the spore shape regularity to determine the reproductive modes whenever possible. We also compared the spore sizes of *Leptochilus cantoniensis* (Baker) Ching and *L. poilanei* by measuring 30 spores from each species.

Molecular phylogenetic analysis

To elucidate the phylogenetic placement of *Lindsaea kohkongensis* and *Leptochilus poilanei*, we conducted two phylogenetic analyses each based on three chloroplast markers including *matK*, *trnH-psbA*, and *trnL-F* for *Lindsaea* and *rbcL*, *rps4-trnS*, and *trnL-F* for *Leptochilus*. These markers were chosen to integrate our newly generated sequences into previous studies (Lehtonen et al. 2010; Zhang et al. 2019; Chen et al. 2020a; Fujiwara et al. 2023). We extracted genomic DNA from fresh fronds using Qiagen DNeasy Plant Mini Kit (Hilden, Germany), following the manufacturer's protocol. We conducted PCR to amplify the five DNA markers using the primers listed in Table 1.

For *Lindsaea*, we included the sequences of 26 specimens as listed in Appendix 1: Table A1. Among these, the sequences of 14 specimens were downloaded from GenBank. To test the monophyly of *L. ensifolia*, this sampling included 1) *L. ensifolia* from a broad geographic range, including Bangladesh, Brunei, Cambodia, India, Malaysia, Nepal, Solomon Islands, Thailand, and Vietnam; and 2) closely related species identified by Lehtonen et al. (2010). For *Leptochilus*, in addition to our newly sequenced *L. poilanei*, we download the sequences of another 21 specimens from GenBank. In total, 22 specimens representing 19 species were included in the phylogenetic analysis as listed in Appendix 1: Table A2. This sampling covered all the major clades found in previous studies (Zhang et al. 2019; Chen et al. 2020a).

For our newly generated sequences, we first manually inspected raw reads, removing any ambiguous bases using BioEdit (Hall 1999). Subsequently, we aligned the sequences of each marker using MUSCLE (Edgar 2004) with default settings. These individual marker alignments were then concatenated into a single alignment, because chloroplast genome is non-recombining and therefore has a single evolutionary history. We then conducted maximum likelihood (ML) analyses using IQTREE (Minh et al. 2020) each with five partitions (three codon positions for *matK* and *rbcL*, and *rps4-trnS*, *trnH-psbA* and *trnL-F*, each treated as a single partition for simplification although they contain partly coding regions). The best-fit model for each partition and the best-fit partition scheme were determined by ModelFinder (Lanfear et al. 2012; Kalyaanamoorthy et al. 2017) as implemented in IQTREE (Minh et al. 2020). To assess branch support, we performed 1000 ultrafast bootstrap replicates using UFBoot (Hoang et al. 2018). The two concatenated alignments and the resulting phylogenetic trees are available on the Dryad Digital Repository (Chen et al. 2024).

Table 1. PCR Primers used in this study.

Region	Name	Sequence 5' to 3'	Reference
matK	FERN matK fEDR	ATTCATTCRATRTTTTTATTTHTGGARGAYAGATT	Kuo et al. 2011
matK	DeLin matK rNRD	CTACGCAAYSCATCYCGATTT	Kuo et al. 2011
rbcL	1F	ATGTCACCACAAACAGAAAC	Fay et al. 1997
rbcL	1379R	TCACAAGCAGCAGCTAGTTCAGGACTC	Wolf et al. 1999
rps4-trnS	rps5	ATGTCCCGTTATCGAGGACCT	Nadot et al. 1995
rps4-trnS	trnSR	TACCGAGGGTTCGAATC	Smith and Cranfill 2002
trnH-psbA	trnH	CGCGCATGGTGGATTCACAATCC	Tate and Simpson 2003
trnH-psbA	psbA3'f	GTTATGCATGAACGTAATGCTC	Sang et al. 1997
trnL-F	FernL1Ir1	GGYAATCCTGAGCCAAATC	Li et al. 2009
trnL-F	F	ATTTGAACTGGTGACACGAG	Taberlet et al. 1991

Results

Phylogenetic placement of *Lindsaea kohkongensis*

The concatenated three-marker alignment contains 1832 bp including 85 parsimony informative sites and 39.2% missing data (17,973 bp). ModelFinder merged all the five partitions into one alignment with the best-fit model identified as K3Pu+F+R2. The phylogram generated by the ML analysis based on the concatenated alignment is shown in Fig. 5. The inferred species relationships closely align with the phylogeny reconstructed by Lehtonen et al. (2010) although the nodes are poorly supported in general. However, *Lindsaea kohkongensis* is strongly supported as the sister group to a clade comprising all the *L. ensifolia* specimens included in this analysis.

Phylogenetic placement of *Leptochilus poilanei*

The concatenated three-marker alignment contains 3331 bp including 248 parsimony informative sites and 17.8% missing data (13077 bp). ModelFinder subset the alignment into two partitions, the first including first and third codon position of *rbcL*, and the second including the second codon position of *rbcL* along with

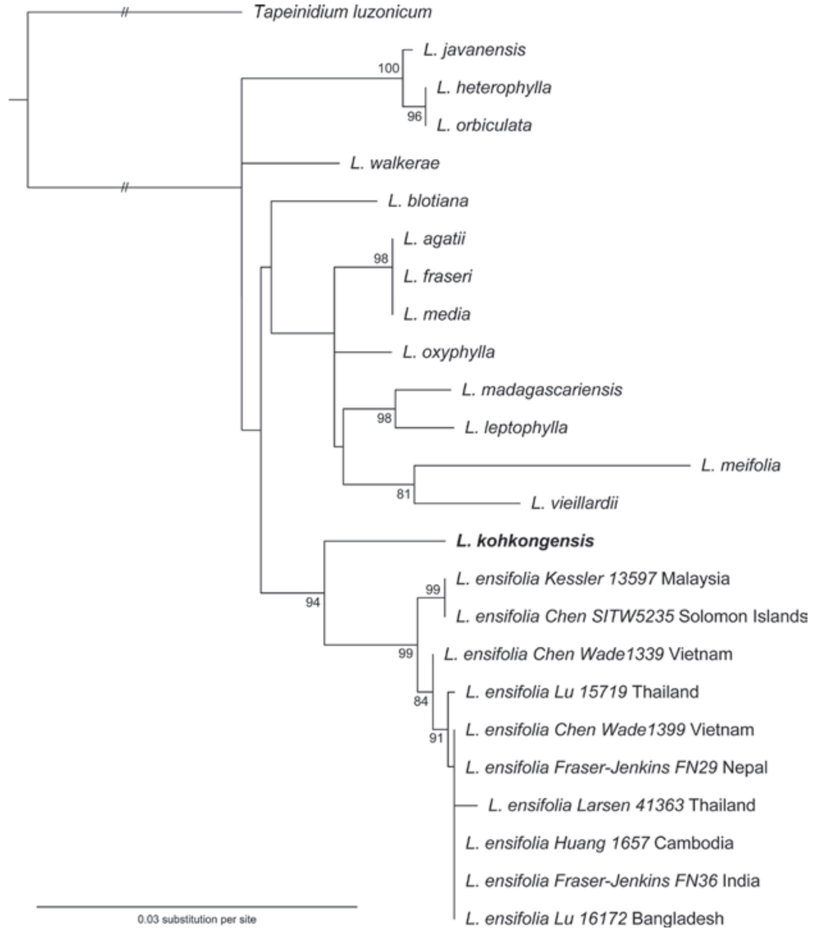

Figure 5. Phylogram of *Lindsaea* reconstructed from the maximum likelihood analysis of the concatenated plastid dataset (*matK*, *trnH-psbA*, and *trnL-F*). The branch length to the outgroup *Tapeinidium* is not to scale for better visualization. Support values below 80 are not shown on the nodes. *L. kohkongensis* is indicated in bold.

rps4-trnS and *trnL-F*. The best-fit models determined for the two partitions were TNe+I+G4 and K3Pu+F+R2, respectively. The phylogram generated by the ML analysis based on the concatenated alignment is shown in Fig. 6. The inferred species relationships are similar to previous studies (Zhang et al. 2019; Chen et al. 2020a) as expected, although poorly supported in general. *Leptochilus poilanei* is nested in a highly supported clade containing three specimens of *L. cantoniensis*.

Both *L. cantoniensis* (based on *Hsu 3399*) and *L. poilanei* (based on *Chen Wade6804*) produce 64 spores in each sporangium with normal morphology. The spore size of *L. cantoniensis* and *L. poilanei* is 44.3 ± 3.4 and 63.0 ± 4.9 μm, respectively. The spores of *L. poilanei* are significantly larger than those of *L. cantoniensis* (t-test, $p < 0.001$).

Chromosome number of 20 species

We successfully counted the chromosome numbers of 20 species, each determined by multiple cells. Their chromosome numbers and ploidy levels inferred from the known lowest base number are shown in Table 2. Figs 7–13 illustrated representative cells for all the 20 species. Among these, chromosome numbers were recorded for the first time for seven species: *Gymnosphaera salletii* (Tardieu & C.Chr.) S.Y.Dong, *Lepisorus spicatus* (L.f.) Li Wang, *Leptochilus poilanei*, *Pteridrys costularis* Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou, *Pteris latipinna* Y.S.Chao & W.L.Chiou, *Pyrrosia eberhardtii* (Christ) Ching, and *Tectaria setulosa* (Baker) Holttum. Furthermore, we reported the new cytotypes for three species: *Diplazium doederleinii* (Luerss.) Makino, *Pteris esquirolii* H.Christ, and *Tectaria harlandii* (Hook.) C.M.Kuo.

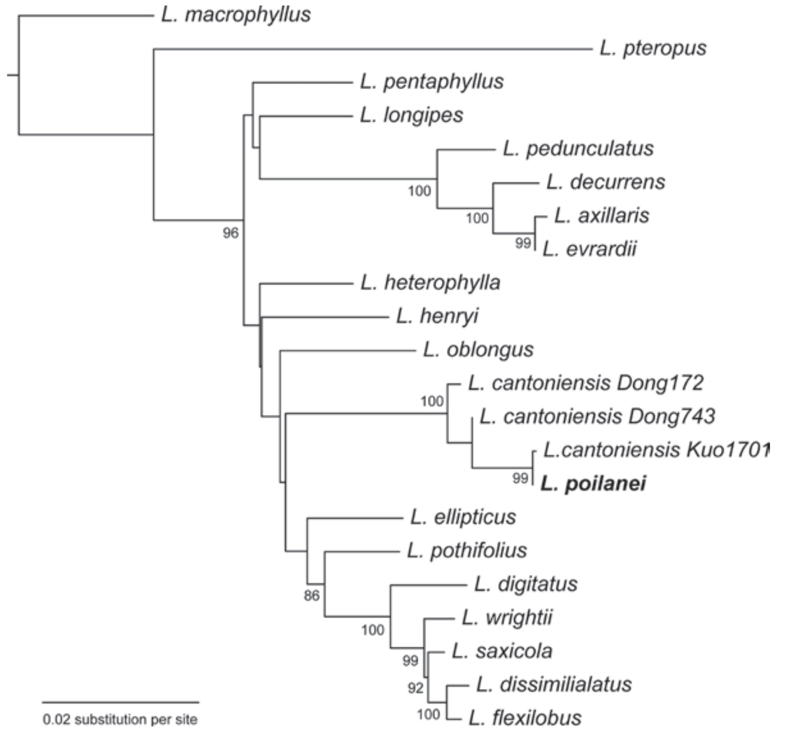

Figure 6. Phylogram of *Leptochilus* reconstructed from the maximum likelihood analysis of the concatenated plastid dataset (*rbcL*, *rps4-trnS*, and *trnL-F*). Support values below 80 are not shown on the nodes. *L. poilanei* is indicated in bold.

Table 2. Plant materials used in this study for chromosome counting, their inferred ploidy, spores number in each sporangium (s/s), and corresponding figures. Asterisks "*" indicate newly reported species. Hash marks "#" indicate newly reported cytotypes. Em-dashes "—" indicate missing data.

Taxa	Voucher	Chromosome no.	Ploidy	s/s	Figure
Asplenium normale D.Don	*Chen Wade5121*	2*n* = 72	2x	64	7A
Asplenium tenerum G.Forst.	*Chen Wade5342*	2*n* = 144	4x	64	7B
Ctenitis eatonii (Baker) Ching	*Chen Wade6815*	2*n* = 82	2x	—	7C
Didymochlaena truncatula (Sw.) J.Sm	*Chen Wade5817*	2*n* = 82	2x	64	8A
Diplazium doederleinii (Luerss.) Makino#	*Chao 3524*	2*n* = 82	2x	64	8B
Diplazium donianum (Mett.) Tardieu	*Chen Wade6853*	2*n* = 123	3x	—	8C
Grypothrix simplex (Hook.) S.E.Fawc. & A.R.Sm	*Chen Wade6879*	2*n* = 72	2x	64	9A
Gymnosphaera salletii (Tardieu & C.Chr.) S.Y.Dong*	*Chen Wade6586*	2*n* = 138	2x	64	9B
Lepisorus spicatus (L.f.) Li Wang*	*Chen Wade6598*	2*n* = 70	2x	—	9C
Leptochilus poilanei (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang*	*Chen Wade6804*	2*n* = 144	4x	64	10A
Pleocnemia winitii Holttum	*Chao 3534*	2*n* = 82	2x	64	10B
Polystichum biaristatum (Blume) T.Moore	*Chen Wade5070*	2*n* = 82	2x	64	10C
Pteridrys costularis Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou*	*Chen Wade6819*	2*n* = 82	2x	—	11A
Pteris cadieri H.Christ	*Chen Wade5737*, *Chao 3501*	2*n* = 87	3x	32	11B, 11C
Pteris esquirolii H.Christ#	*Chao 3511*	2*n* = 58	2x	64	12A
Pteris latipinna Y.S.Chao & W.L.Chiou*	*Chao 3526*	2*n* = 58	2x	32	12B
Pteris pseudowulaiensis C.M.Kuo	*Chao 3515*	2*n* = 58	2x	32	12C
Pyrrosia eberhardtii (Christ) Ching*	*Chen Wade5607*	2*n* = 74	2x	—	13A
Tectaria harlandii (Hook.) C.M.Kuo#	*Chen Wade6874*	2*n* = 80	2x	—	13B
Tectaria setulosa (Baker) Holttum *	*Chen Wade6852*	2*n* = 80	2x	64	13C

Discussion

Systematic uniqueness of *Lindsaea kohkongensis*

When we collected this species from Phu Quoc island in 2018, we were hesitant to describe it. Morphologically, it closely resembles *L. ensifolia*, leading us to question if it might be an eco-form of the latter. In this study, we conducted a phylogenetic analysis based on three chloroplast regions, sampling *L. ensifolia* from a wide geographic distribution throughout Asia and the Pacific Islands. The results indicate that *L. kohkongensis* is sister to *L. ensifolia* rather than being embedded within it (Fig. 6). On one hand, this suggests that *L. kohkongensis* can be recognized as a distinct species based on the principle of monophyly. On the other hand, it could also be classified within a broadly circumscribed *L. ensifolia*. To our knowledge, *L. ensifolia* typically grows terrestrially in a diverse habitat from swampy forests, dipterocarp forests, to exposed grasslands (Kramer 1971; personal observation). In contrast, *L. kohkongensis* is exclusively found in shaded valleys as lithophytes and is frequently submerged in water (Yun et al. 2023; personal observation). This distinction suggests an ecological speciation event that needs to be tested in the future.

While identifying our specimen from Phu Quoc island, we came across a rheophyte form of *L. ensifolia* described as *L. ensifolia* var. *rheophila* K.Iwats from Sumatra (Iwatsuki 1977). After examining the type materials deposited in KYO, we concluded that this is the same species as *L. kohkongensis* and should be treated as a synonym of it. As noted by Kramer (1967), *L. ensifolia* is one of the most widespread and variable species of the genus. Apart from var. *rheophila*, few other

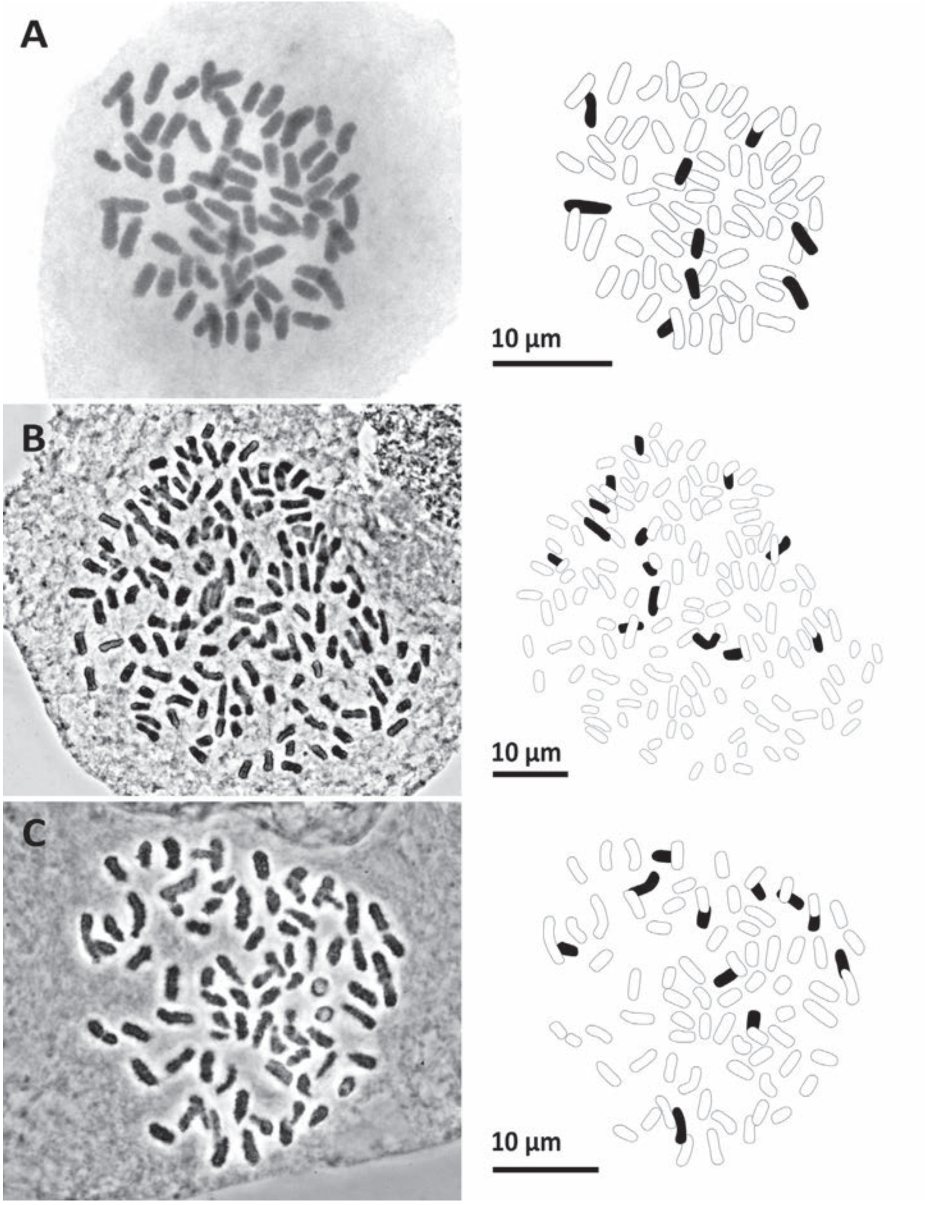

Figure 7. Mitotic chromosomes with explanatory illustrations **A** *Asplenium normale* (2*n* = 72) **B** *Asplenium tenerum* (2*n* = 144) **C** *Ctenitis eatonii* (2*n* = 82).

subspecies or varieties have been published, such as subsp. *coriacea* (Alderw.) K.U.Kramer from Bornean swamp forests. Furthermore, ploidy variations (diploid, triploid, and tetraploid) and different reproductive modes (sexual and apomixis) have been observed in *L. ensifolia* (e.g., Lin et al. 1996). Although we here tentatively recognize *L. kohkongensis* as a distinct species, the variability observed in *L. ensifolia* underscores the need for a systematic study of this complex species.

Hybrid origin hypothesis of *Leptochilus poilanei*

Our phylogenetic analysis based on three plastid markers unambiguously resolves *L. poilanei* within a clade that includes three specimens of *L. cantoniensis* (Fig. 6). This result is unexpected, considering the clear morphological distinctions between these two species. Specifically, fully developed sterile fronds of *L. poilanei* are pinnatifid, while those of *L. cantoniensis* remain consistently simple (Fig. 14).

Our examination of the reproductive mode indicates that both species undergo sexual reproduction, as evidenced by the production of 64 well-formed spores in each sporangium. The mitotic chromosome count for *L. poilanei* is $2n = 144$ (Fig. 10A), suggesting it is a tetraploid, given the base number of the genus is $x = 36$ (Rice et al. 2015). Although the chromosome number of *L. cantoniensis* is presently unknown, its spores are significantly smaller than those of *L. poilanei* (44.3 ± 3.4 vs. 63.0 ± 4.9 μm). Considering the widely found correlation between ploidy and spore size in ferns (Barrington et al. 1986), we propose that *L. cantoniensis* is likely a diploid species.

Building on the evidence from our study, we propose that *L. poilanei* has a hybrid origin. We suggest that *L. poilanei* originated from hybridization between *L. cantoniensis* (as the maternal parent, considering chloroplast inheritance in ferns, e.g., Gastony and Yatskievych 1992) and an unidentified diploid species, followed by polyploidization. The close similarity in chloroplast DNA markers between *L. cantoniensis* and *L. poilanei* suggests a relatively recent hybridization event. This hypothesis gains further support from the overlapping distribution of *L. cantoniensis* and *L. poilanei* in central Vietnam. While the identity of the other parent remains elusive, we anticipate it is likely a species with pinnatifid fronds, given that *L. poilanei* exhibits this characteristic. Future studies employing bi-parentally inherited nuclear markers and comprehensive sampling of the genus in Vietnam are essential to test our hypothesis.

New findings of chromosome number

In the following paragraphs, we discussed the systematic implications of our new finding (i.e., new species counts or new cytotypes) species by species, following alphabetical order.

Diplazium doederleinii **(Luerss.) Makino—$2n = 82$, sexual diploid, Fig. 8B**

Previous studies reported triploid ($2n = 123$) and tetraploid ($2n = 164$) specimens from Japan (Takamiya et al. 2001). Our discovery of a diploid specimen from Vietnam provides further support for the long-observed cytogeographic pattern: diploids are typically found in warmer tropics, whereas polyploids are prevalent in cooler temperate regions (e.g. Chang et al. 2013). As demonstrated by Takamiya et al. (2001), there is a little genetic divergence between triploids and tetraploids. Future studies could incorporate the newly discovered diploid into genetic analyses to further investigate the origins of polyploids.

Gymnosphaera salletii **(Tardieu & C.Chr.) S.Y.Dong—$2n = 138$, sexual diploid, Fig. 9B**

The first chromosome count reported for the species. This Vietnam endemic species was recently reevaluated by Li et al. (2024), who described two new species previously been misidentified as *G. salletii*. *Gymnosphaera* is predominantly a paleotropical genus, comprising approximately 46 species (Dong and Zuo 2018). All four previously reported species with chromosome counts in the genus are diploid, namely *G. capensis* (L.f.) S.Y.Dong, *G. gigantea* (Wall. ex Hook.) S.Y.Dong, *G. khasyana* (T.Moore ex Kuhn) Ching, and *G. podophylla*

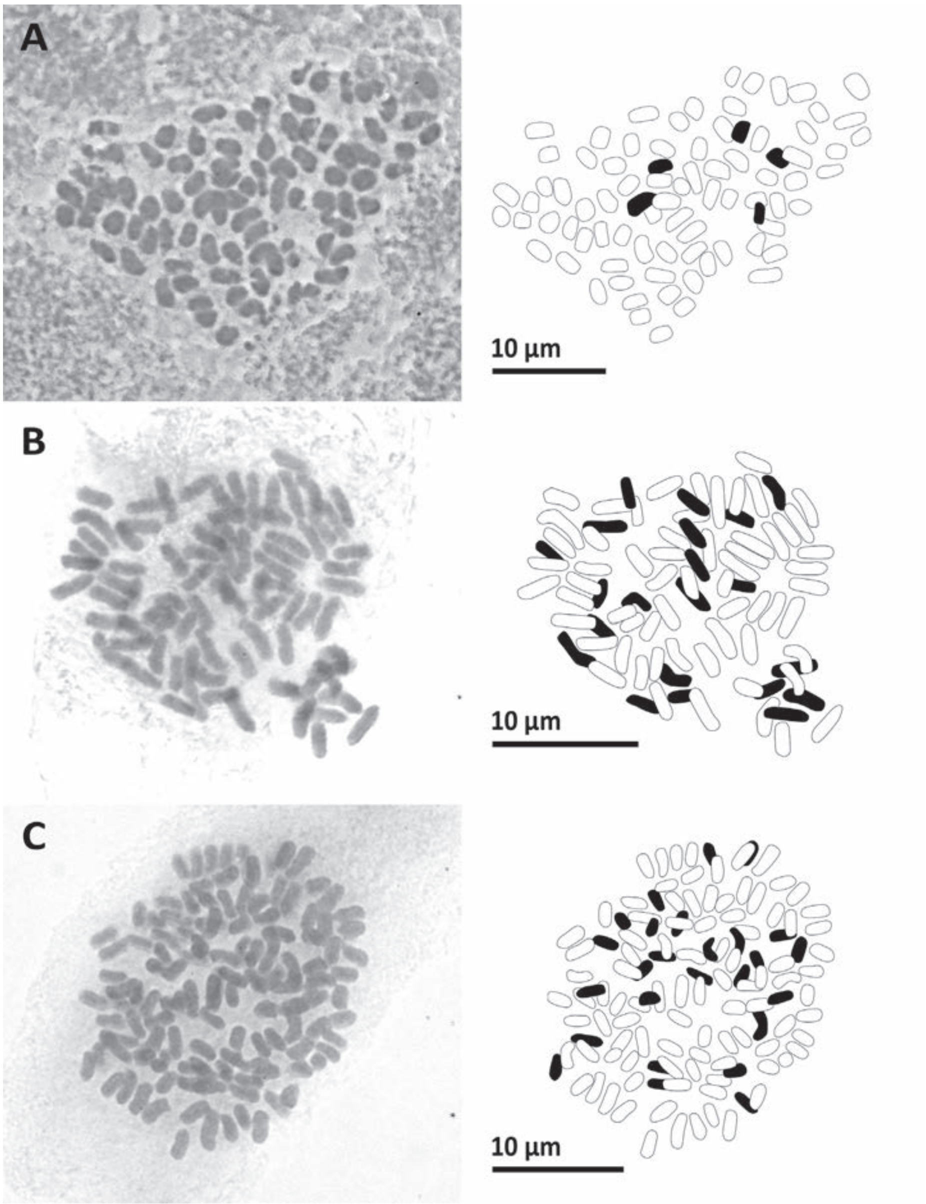

Figure 8. Mitotic chromosomes with explanatory illustrations **A** *Didymochlaena truncatula* (2*n* = 82) **B** *Diplazium doederleinii* (2*n* = 82) **C** *Diplazium donianum* (2*n* = 123).

(Hook.) Copel. (Manton and Sledge 1954; Löve et al. 1977; Kato 1999). Notably, there is a correlation between geographic distribution and the basic chromosome number: all Asian species exhibit $x = 69$, while African species have $x = 70$.

Lepisorus spicatus (L.f.) Li Wang—2*n* = 70, diploid, Fig. 9C

The first chromosome count reported for the species. Found across the paleotropics, this species has often been confused with *L. mucronatus* (Fée) Li Wang. Following Hovenkamp (1998), we identify the species by the presence of entire rhizome scales with hyaline margins. Following Zhao et al. (2020), this species belongs to section *Belvisia* (Mirb.) C.F. Zhao, R.Wei & X.C. Zhang, a small clade comprising eight species (Hovenkamp and Franken 1993). *Lepisorus mucronatus* is the only other species in this section with known chromosome numbers and has been determined as tetraploid (2*n* = 140) from Australia (Tindale and Roy 2002).

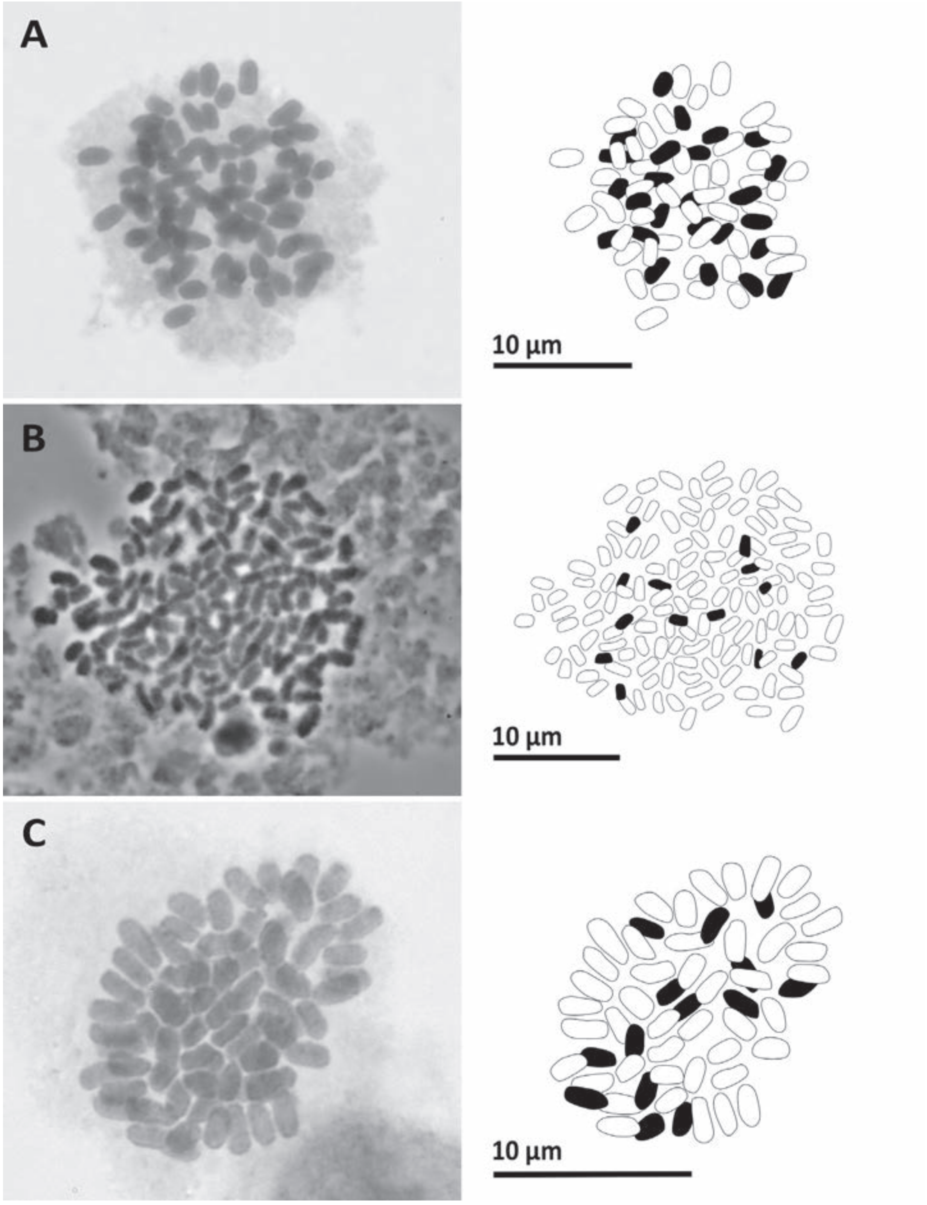

Figure 9. Mitotic chromosomes with explanatory illustrations **A** *Grypothrix simplex* (2*n* = 72) **B** *Gymnosphaera salletii* (2*n* = 138) **C** *Lepisorus spicatus* (2*n* = 70).

Leptochilus poilanei (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang—2*n* = 144, sexual tetraploid, Fig. 10A

As discussed in previous sections, we hypothesize that this Vietnam endemic species originated from a hybridization between *L. cantoniensis* and an unidentified diploid species, followed by polyploidization. *Leptochilus* is predominantly a tropical Asian genus with approximately 51 species (Zhang et al. 2024). Prior to this study, chromosome numbers were known for only four species including: *L. axillaris* (Cav.) Kaulf., *L. decurrens* Blume, *L. ellipticus* (Thunb.) Noot., and *L. pothifolius* (Buch.-Ham. ex D.Don) Fraser-Jenk. (e.g., Mitui 1968; Löve et al. 1977; Manickam and Irudayaraj 1989). Among these four species, *L. decurrens* is the only one reported to exist in both diploids and tetraploids, while the other three species are known only as diploids.

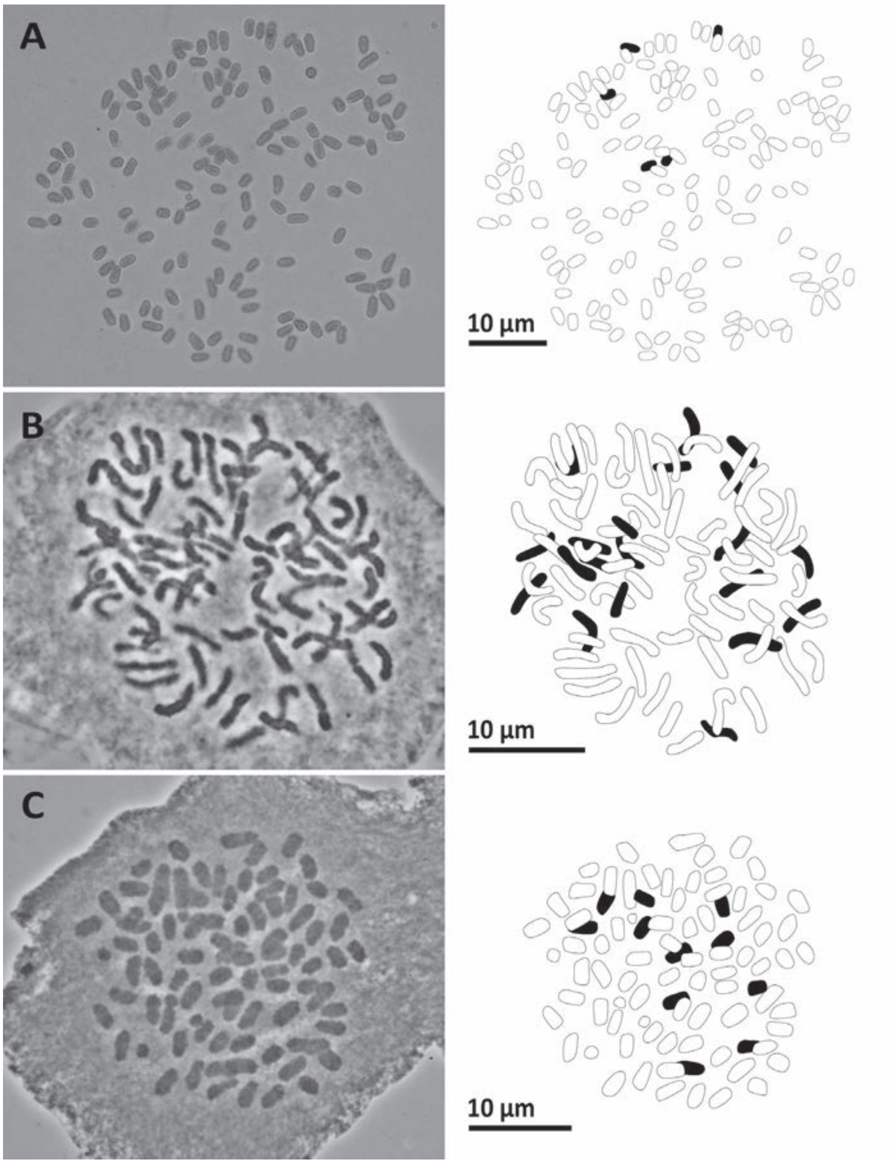

Figure 10. Mitotic chromosomes with explanatory illustrations **A** *Leptochilus poilanei* (2*n* = 144) **B** *Pleocnemia winitii* (2*n* = 82) **C** *Polystichum biaristatum* (2*n* = 82).

Pteridrys costularis Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou—2*n* = 82, diploid, Fig. 11A

The first chromosome count reported for the species. *Pteridrys* is a tropical Asian genus with ca. 22 species, and 11 of them are recorded in Vietnam (Zhou and Zhang 2019). So far, all the four species (the other three are *P. australis* Ching ex C.Chr. & Ching, *P. cnemidaria* (Christ) C.Chr.et.Ching, and *P. syrmatica* (Willd.) C.Chr. & Ching) with reported chromosome numbers are diploids (Manton 1954; Tsai and Shieh 1985; Bidin and Go 1995).

Pteris esquirolii H.Christ—*2n = 58*, sexual diploid, Fig. 12A

This species is reported from southern China, Taiwan, and Vietnam (Chao 2019). Prior to this study, the only reported chromosome count was 2*n* = ca. 90

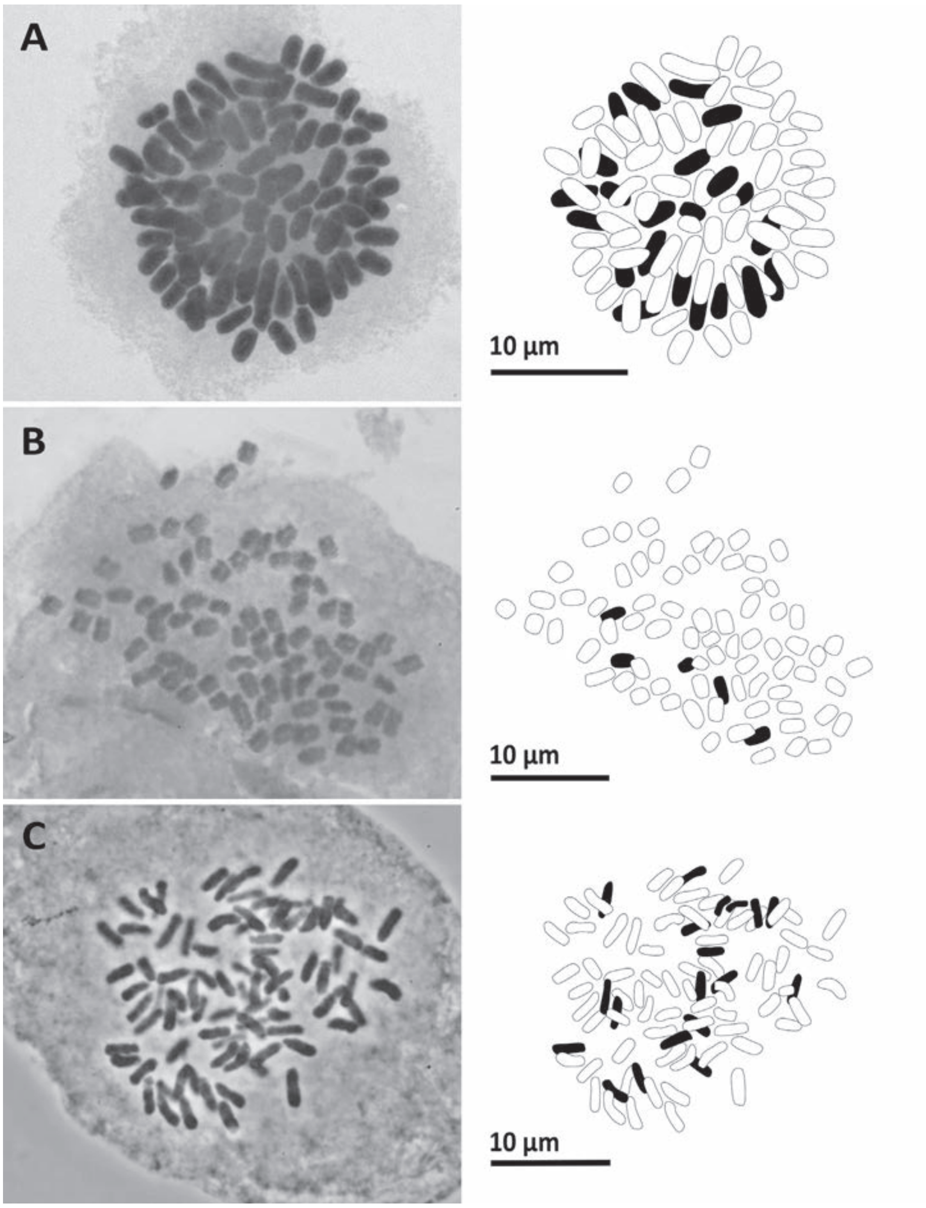

Figure 11. Mitotic chromosomes with explanatory illustrations **A** *Pteridrys costularis* (2*n* = 82) **B** *Pteris cadieri* (2*n* = 87) **C** *Pteris cadieri* (2*n* = 87).

(presumably an apogamous triploid due to the count of 32 spores per sporangium) from southern China (Lin et al. 2002). Although Chao (2019) found very little genetic divergence among populations from China and Taiwan, it may be worthwhile to include the Vietnamese population in future analyses, given their different ploidy level and reproductive mode. This is particularly important considering the prevalence of reticulate evolution observed in the genus (e.g., Chao et al. 2012, 2022).

Pteris latipinna **Y.S.Chao & W.L.Chiou—*2n = 58*, apogamous diploid, Fig. 12B**

This is the first chromosome count reported for this species. This species was firstly described from Taiwan (Chao et al. 2017) and later reported from China and Vietnam (Chen et al. 2020b). Our chromosome counts support the hypothesis proposed by Chao et al. (2022) that *P. latipinna* is a diploid,

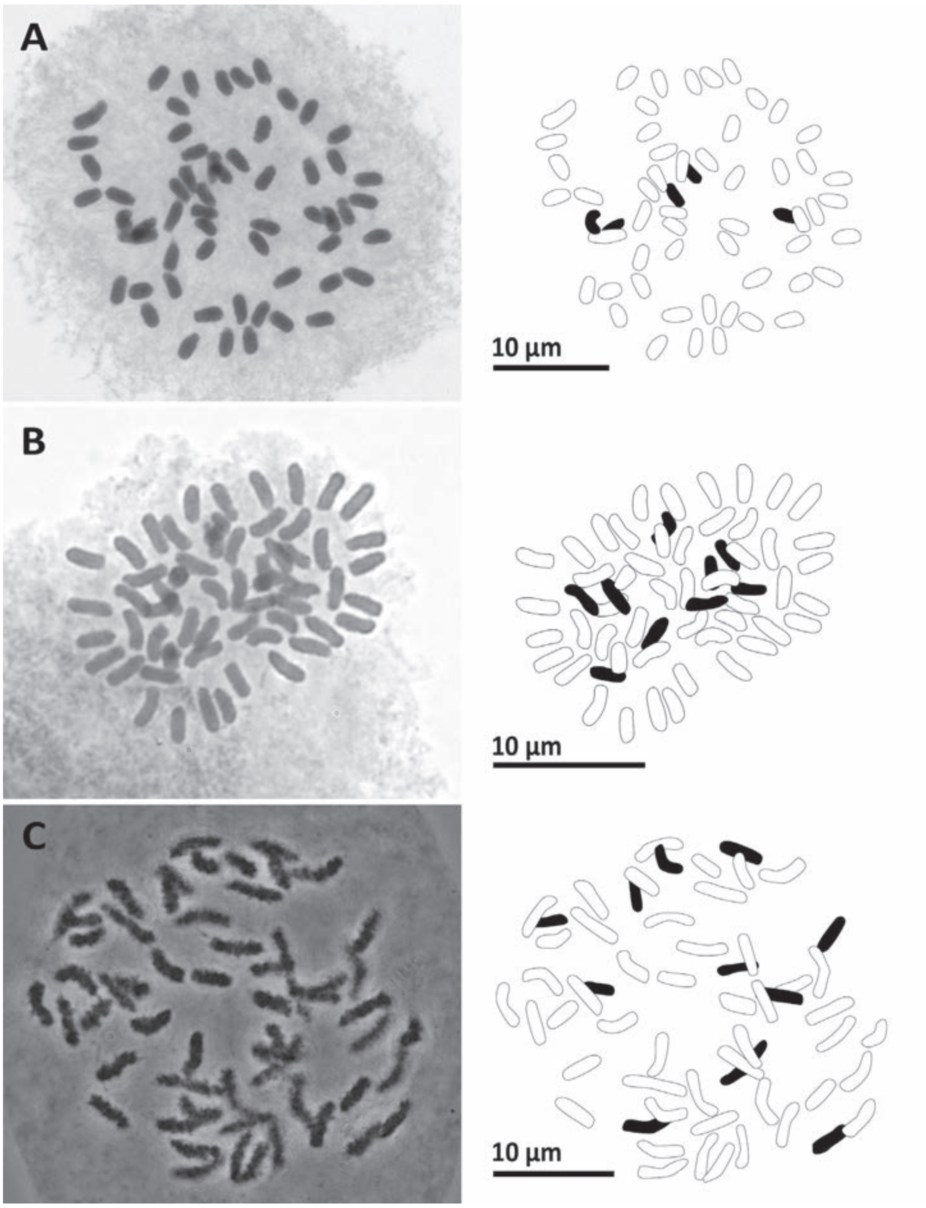

Figure 12. Mitotic chromosomes with explanatory illustrations **A** *Pteris esquirolii* (2*n* = 58) **B** *Pteris latipinna* (2*n* = 58) **C** *Pteris pseudowulaiensis* (2*n* = 58).

as indicated by flow cytometry. Our spore number counts also confirm that *P. latipinna* is reproduced through apogamous reproduction, which is common for the genus (Walker 1962).

Pyrrosia eberhardtii **(Christ) Ching—2*n* = 74, diploid, Fig. 13A**

The first chromosome count reported for the species. This species is recorded in southern China, Thailand, and Vietnam, with its type locality in Vietnam. It has sometimes been grouped under a broadly defined *P. lingua* (Thunb.) Farw. alongside other closely related species such as *P. oblonga* Ching and *P. heteractis* (Mett.) Ching. (Hovenkamp 1986). This broader classification of *P. lingua* is further supported by Zhou et al. (2017), showing a close relationship among these species. The chromosome numbers of *P. lingua* have been reported from China and Japan, and both are diploids (e.g., Takei 1969; Kato 1999).

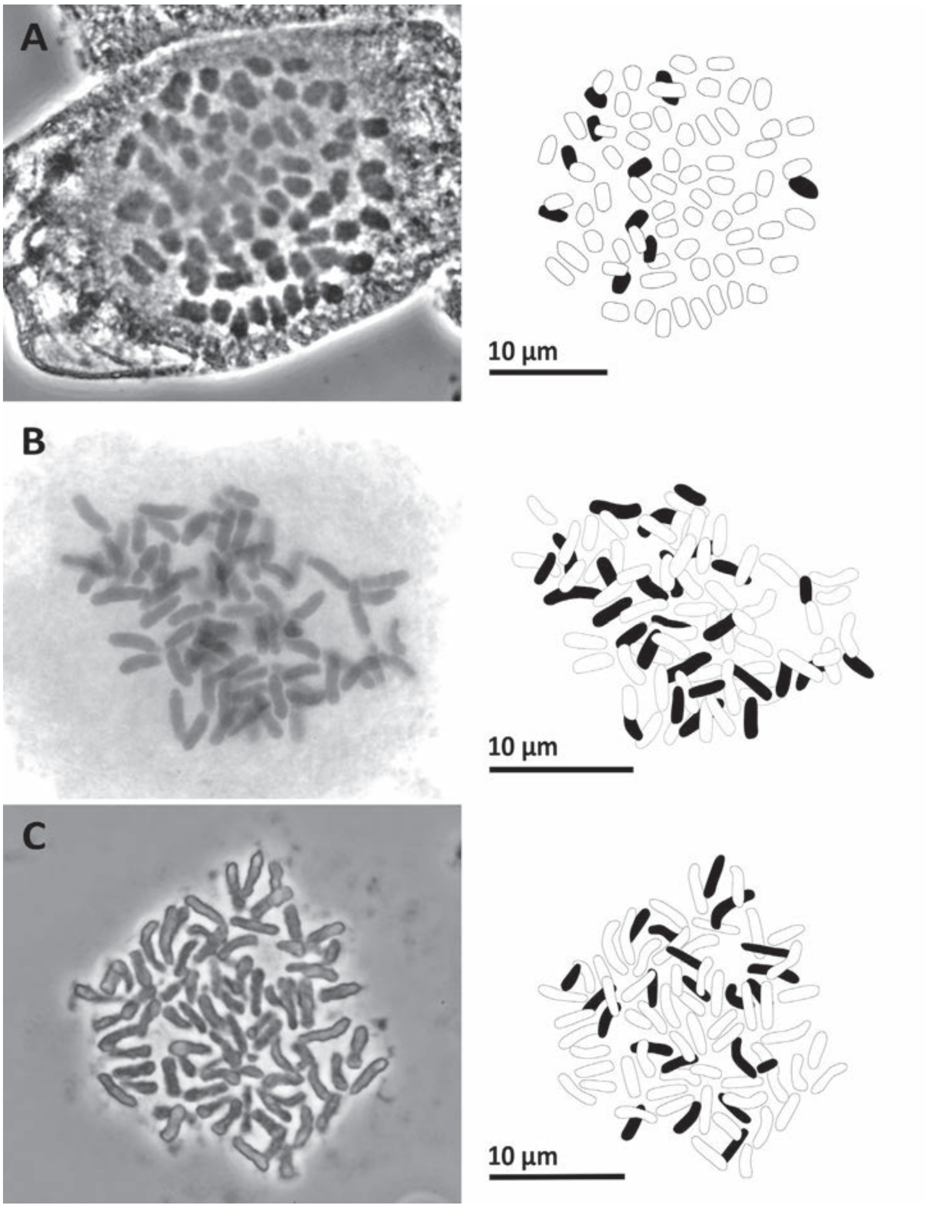

Figure 13. Mitotic chromosomes with explanatory illustrations **A** *Pyrrosia eberhardtii* (2*n* = 74) **B** *Tectaria harlandii* (2*n* = 80) **C** *Tectaria setulosa* (2*n* = 80).

Tectaria harlandii (Hook.) C.M.Kuo—2*n* = 80, diploid, Fig. 13B

This species has been documented in the Ryukyu Islands, southern China, northern Vietnam, and Taiwan. Tetraploid populations (*n* = 80) have been reported in the Ryukyu Islands (as *Hemigramma decurrens* (Hook.) Copel., Mitui 1976) and Taiwan (Tsai and Shieh 1985). In our study, we provide the first record of a diploid form Vietnam. Notably, this species has been proposed as the maternal parent of the triploid (2*n* = 120) sterile hybrid species *T. × hongkongensis* (Zhao and Dong 2016), with *T. zeilanica* (Houtt.) Sledge as the paternal parent. It is reasonable to assume that *T. × hongkongensis* originated from the hybridization between diploid and tetraploid parents. Given that both diploids and tetraploids are now confirmed in these two parental species (Manton and Sledge 1954; Tsai and Shieh 1985), it would be interesting to investigate whether there is a bias in the direction of hybridization based on ploidy level.

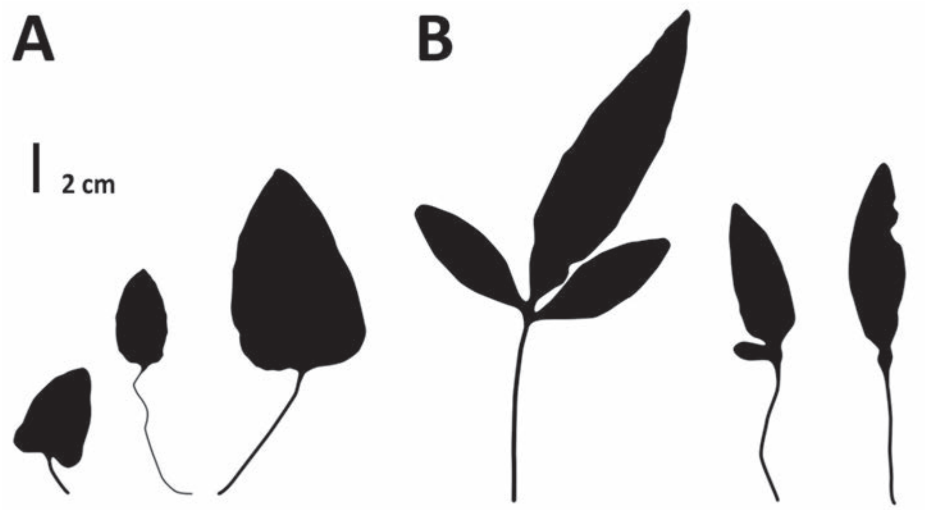

Figure 14. Comparison of the sterile fronds between *Leptochilus cantoniensis* and *L. poilanei* **A** *L. cantoniensis*, based on *Kuo 1701* (TAIF [509328], left), *Cadière 158* (MICH [1191289], middle), and *s.c.*, *s.n.* (K [000959730], right) **B** *L. poilanei*, based on *Poilane 5373* (BM [000036782], all the three fronds).

Tectaria setulosa **(Baker) Holttum—2*n* = 80, sexual diploid, Fig. 13C**

The first chromosome count reported for the species. Initially described from Ba Vi mountain range in northern Vietnam, this species has since been documented in southern China, Indochina, and Peninsular Malaysia. Additionally, a variety *raciborskii* (Alderw.) Holttum has been further identified extending to Java without known ploidy.

New records from Vietnam

Haplopteris yakushimensis **C.W.Chen & Ebihara, Phytotaxa 156(4): 232. 2014.** Fig. 1

Type. Japan. • Kagoshima Pref., Yakushima Island, Nakabase River, 20 Aug 1982, *Nakaike s.n.* (holotype TNS [VS-456666!]).

Distribution and ecology. This species was previously recorded only in Japan and Taiwan (Chen et al. 2014, TPG 2019). In Vietnam, *H. yakushimensis* is found in damp evergreen broadleaf forests at elevations of 1200–1360 m, where it grows on rocks with thick compost near streams.

Specimens examined. Vietnam. • Cao Bang Province: Phia-Oac Phia-Den National Park, 6 Dec 2013, *Zhang et al. 6755* (MO, TAIF [499240!], VNMN). Phia-Oac Phia-Den National Park, Nguyen Binh District, Thanh Cong Ward, 22.589539°N, 105.880403°E, 1353 m, 8 Nov 2023, *Chen Wade6952* (TAIF!, VNMN!). • Ha Giang Province: Vi Xuyen District, Cao Bo Ward, 22.767500°N, 104.880394°E, 1200–1360 m, 12 Sep 2000, *Harder et al. 5514* (UC [1763099!]).

Note. *Haplopteris*, a fern genus comprising approximately 40 species, is primarily found in tropical Africa, Asia, and the Pacific Islands (Schuettpelz et al. 2016). In Vietnam, eight *Haplopteris* species were previously recorded: *H. angustifolia* (Blume) E.H.Crane, *H. doniana* (Mett. ex Hieron.) E.H.Crane, *H. elongata* (Sw.) E.H.Crane, *H. ensata* (Christ) C.W.Chen & S.Linds., *H. ensiformis* (Sw.) E.H.Crane, *H. flexuosa* (Fée) E.H.Crane, *H. hainanensis* (C.Chr. ex Ching) E.H.Crane, and

H. sikkimensis (Kuhn) E.H.Crane (Chen et al. 2023). *Haplopteris yakushimensis* can be distinguished from these species by having fronds broader than 1 cm wide, costae that are grooved on the adaxial side and raised on the abaxial side, and the submarginal (ca. 1–2 mm away from the margins) sori lines.

Recent studies have identified two genetically distinct yet morphologically indistinguishable lineages in *H. yakushimensis* (Kuo et al. 2017; TPG 2019). With the discovery of new populations in Vietnam, we are currently working to clarify these lineages using an integrated approach that includes reproductive biology, genome size estimation, and nuclear markers. The results of this research will be published in a separate paper.

***Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun, Korean J. Pl. Taxon. 53(4): 289. 2023.**

Fig. 2

Type. Cambodia • Koh Kong, Thma Bang District, near Chamnar village, 22 Dec 2013, *Sun et al. C5520* (holotype HIBR; isotypes HIBR, KB).

Distribution and ecology. This species was recently described from Cambodia and Malaysia. In Vietnam, it grows along a valley in damp tropical forests as lithophytes and is frequently submerged in water.

Specimens examined. Vietnam. • Kien Giang Province: Phu Quoc district, Phu Quoc National Park, 6 Mar 2018, *Chen Wade5034* (TAIF [514046!]).

Note. Morphologically, this species closely resembles *Lindsaea ensifolia*. Although *L. kohkongensis* is usually a much smaller species than *L. ensifolia*, we did not identify any qualitative trait to distinguish them. Currently, subaquatic habitat preference appears to be the most reliable characteristic for differentiating this species from *L. ensifolia*. Yun et al. (2023) described *L. kohkongensis* as having free venation, in contrast to the anastomosing venation found in *L. ensifolia*. However, this is not true. As demonstrated in Fig. 3G of their paper and Fig. 2F of the current study, the veins of *L. kohkongensis* are connected near the lamina margin, forming areoles in fertile fronds.

***Pteris pseudowulaiensis* Y.S.Chao, Taiwania 66(3): 314. 2021.**

Fig. 3

Type. Taiwan • New Taipei, Mt. Pataoerh, 600–700 m, 29 Apr 2016, *Hsu 8437* (holotype TAIF [497137!]; isotype TAIF [497138!]).

Distribution and ecology. This species, recently described from Taiwan, was initially reported from southern China and Taiwan (Chao et al. 2021). In Vietnam, *P. peudowulaiensis* was discovered in relatively dry evergreen broadleaf forests at lowland areas of Cuc Phuong National Park.

Specimens examined. Vietnam • Ninh Binh Province: Cuc Phuong National Park, Muong Khu trail, *Y.-S. Chao 3509*, *3515*, *3517*, *3531* (TAIF!, VNMN!).

Note. This species belongs to the *Pteris fauriei* Hieron. complex, a taxonomically challenging group due to its reticulate evolution involving hybridization, polyploidization, and apomixis (Chao et al. 2022). In Vietnam, five species from this complex have been recorded, namely *P. arisanensis* Tagawa (Chao et al. 2022), *P. kawabatae* Sa.Kurata (Chao et al. 2021), *P. latipinna* Y.S.Chao & W.L.Chiou (Chen et al. 2020b), *P. oshimensis* Hieron. (Liao et al. 2013), and *P. pseudowulaiensis*. Morphologically, *P. pseudowulaiensis* closely resembles *P. oshimensis* but differs by having broader pinnae, measuring 2–3.5 cm compared to less than 2 cm in *P. oshimensis*. According to Chao et al. (2022), *P. pseudowulaiensis* is an apogamous diploid originating from hybridization of *P. wulaiensis* C.M.Kuo (a species reported from Japan and Taiwan) and another unknown species.

Acknowledgements

We extend our gratitude to Hoang Thi Nha, Nong Minh Hoan, Ly Ton Senh, Trong Tin Tran Vo, and Cong Ty Tu for their assistance during fieldwork. Our sincere thanks also go to the directors of Bidoup Nui-Ba National Park, Cuc Phuong National Park, Phia Oac-Phia Den National Park, Phu Quoc National Park, and Nui Chua National Park, as well as the Vietnam Academy of Science and Technology, for granting us the collection permits. We thank Li-Bing Zhang for sharing his duplicated specimen of *Haplopteris yakushimensis* with us. We are indebted to the curator and staff of the BM herbarium for granting us access to their specimen.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Field work in Nui Chua National Park was supported by the Vietnam Academy of Science and Technology (UDNGDP.01/24-25). Funding for this study was provided by the National Science and Technology Council of Taiwan (NSTC 112-2621-B-054-001-MY3).

Author contributions

CWC conceptualizes the study, conducts the analysis, and writes the original draft. CWC, YMH, YSC collect the data. YMH and KFC provide lab equipment. All authors conduct field work and revise the manuscript.

Author ORCIDs

Cheng-Wei Chen https://orcid.org/0000-0001-9709-6739
Van Truong Do https://orcid.org/0000-0002-0585-5513
Hong Truong Luu https://orcid.org/0000-0002-7036-7081
Yi-Shan Chao https://orcid.org/0000-0001-7433-5987
Kuo-Fang Chung https://orcid.org/0000-0003-3628-2567

Data availability

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

References

Barrington DS, Paris CA, Ranker TA (1986) Systematic inferences from spore and stomate size in the ferns. American Fern Journal 76: 149–159. https://doi.org/10.2307/1547723

Bidin AA, Go R (1995) Chromosome base numbers for Tectaria and allied genera in peninsular Malaysia. Natural History Research (Chiba) 3: 115–122.

Chang Y, Li J, Lu S, Schneider H (2013) Species diversity and reticulate evolution in the *Asplenium normale* complex (Aspleniaceae) in China and adjacent areas. Taxon 62: 673–687. https://doi.org/10.12705/624.6

Chao YS (2019) New record of *Pteris esquirolii* in Taiwan, with two similar new species, *Pteris rugosifolia* and *Pteris subesquirolii*. Systematic Botany 44: 274–281. https://doi.org/10.1600/036364419X15562052252072

Chao YS, Dong SY, Chiang YC, Liu HY, Chiou WL (2012) Extreme multiple reticulate origins of the *Pteris cadieri* complex (Pteridaceae). International Journal of Molecular Sciences 13: 4523–4544. https://doi.org/10.3390/ijms13044523

Chao YS, Ebihara A, Chiou WL, Huang YM (2017) *Pteris latipinna* sp. nov. (Pteridaceae), a new species segregated from *Pteris fauriei*. PhytoKeys 85: 95–108. https://doi.org/10.3897/phytokeys.85.14884

Chao YS, Chiou WL, Ebihara A, Hsu TC, Chang YH, Lin CY (2021) Taxonomic and nomenclatural novelties in the *Pteris fauriei* group (Pteridaceae). Taiwania 66: 307–316.

Chao YS, Ebihara A, Chiou WL, Tsai JM, Huang YW, Ranker TA (2022) Reticulate evolution in the *Pteris fauriei* group (Pteridaceae). Scientific Reports 12: 9145. https://doi.org/10.1038/s41598-022-11390-7

Chen CW, Ebihara A, Chiou WL, Li CW (2014) *Haplopteris yakushimensis* (Pteridaceae, Vittarioideae), a new species from Yakushima Island, Japan. Phytotaxa 156: 229–234. https://doi.org/10.11646/phytotaxa.156.4.5

Chen CC, Hyvönen J, Schneider H (2020a) Exploring phylogeny of the microsoroid ferns (Polypodiaceae) based on six plastid DNA markers. Molecular Phylogenetics and Evolution 143: 106665. https://doi.org/10.1016/j.ympev.2019.106665

Chen CW, Hsu TC, Chao YS, Lu PF, Li CW, Tram NKT, Nguyen ILPT, Dang MT, Luu HT, Parris B (2020b) A newly recorded genus and twelve newly recorded species of ferns for Vietnam from Lang Biang Plateau. Phytotaxa 443: 121–143. https://doi.org/10.11646/phytotaxa.443.2.1

Chen CW, Ebihara A, Cheng KY, Hsu TC, Huang YM, Dang VD, Luu HT, Le VS, Li CW (2021) Studies of Vietnamese pteridophyte flora 1. Systematic Botany 46: 573–581. https://doi.org/10.1600/036364421X16312067913507

Chen CW, Hsu TC, Doan HS, Do VH, Luu HT, Le VS, Liu YC, Li CW, Huang YM, Chung KF (2023) Studies of Vietnamese pteridophyte flora 2. Systematic Botany 48: 159–172. https://doi.org/10.1600/036364423X16847773873161

Chen CW, Do VT, Truong QC, Dang VD, Luu HT, Chao YS, Huang YM, Chung KF (2024) Studies of Vietnamese Pteridophyte Flora 3 [Dataset]. Dryad. https://doi.org/10.5061/dryad.4j0zpc8mz

Christensen C, Tardieu-Blot ML (1939) Les fougeres d'Indochine. XV. Dipteroideae, XVI. Polypodioideae, XVII. Elaphoglossoideae. Notulae Systematicae 8: 175–210.

Ding HH, Wang P, Dong SY (2013) *Dryopsis* (Dryopteridaceae), a fern genus new to Vietnam. Taiwania 58: 80–84.

Dong SY, Zuo ZY (2018) On the recognition of *Gymnosphaera* as a distinct genus in Cyatheaceae. Annals of the Missouri Botanical Garden 103: 1–23. https://doi.org/10.3417/2017049

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). Kew Bulletin 52: 111–120. https://doi.org/10.2307/4117844

Fujiwara T, Quang BH, Tagane S, Murakami N, Oguri E (2023) *Leptochilus ornithopus* (Polypodiaceae), a new hemiepiphytic fern species from central highlands of Vietnam. Phytotaxa 584: 149–160. https://doi.org/10.11646/phytotaxa.584.3.2

Gastony GJ, Yatskievych G (1992) Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. American Journal of Botany 79: 716–722. https://doi.org/10.1002/j.1537-2197.1992.tb14613.x

Goldblatt P, Lowry PP (2011) The Index to Plant Chromosome Numbers (IPCN): Three decades of publication by the Missouri Botanical Garden come to an end. Annals of the Missouri Botanical Garden 98: 226–227. https://doi.org/10.3417/2011027

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hassler M (1994–2024) World ferns. Synonymic checklist and distribution of ferns and lycophytes of the world. https://www.worldplants.de/ferns/ [last accessed 9 Nov 2024]

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281

Hovenkamp PH (1986) A monograph of the fern genus *Pyrrosia* (Polypodiaceae). Leiden Botanical Series 9: 1–280. https://doi.org/10.1163/9789004628090_003

Hovenkamp PH (1998) Polypodiaceae. Flora Malesiana-Series 2. Pteridophyta 3: 1–234.

Hovenkamp PH, Franken NAP (1993) An account of the fern genus *Belvisia* Mirbel (Polypodiaceae). Blumea 37: 511–527.

Iwatsuki K (1977) Notes on Asiatic pteridophytes 1. Acta Phytotaxonomica et Geobotanica 28: 160–166.

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

Kato M (1999) A cytotaxonomic study of Hainan (S. China) pteridophytes with notes on polyploidy and apogamy of Chinese species. In: Zhang XC, Shing KH (Eds) Ching Memorial Volume. China Forestry Publishing House, Beijing, 1–19.

Kramer KU (1967) The lindsaeoid ferns of the Old World III. Notes on *Lindsaea* and *Sphenomeris* in the Flora Malesiana area. Blumea 15: 557–574.

Kramer KU (1971) Lindsaea-group. Flora Malesiana-Series 2. Pteridophyta 1: 177–254.

Kuo LY, Li FW, Chiou WL, Wang CN (2011) First insights into fern *matK* phylogeny. Molecular Phylogenetics and Evolution 59: 556–566. https://doi.org/10.1016/j.ympev.2011.03.010

Kuo LY, Chen CW, Shinohara W, Ebihara A, Kudoh H, Sato H, Huang YM, Chiou WL (2017) Not only in the temperate zone: Independent gametophytes of two vittarioid ferns (Pteridaceae, Polypodiales) in East Asian subtropics. Journal of Plant Research 130: 255–262. https://doi.org/10.1007/s10265-016-0897-x

Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Lehtonen S, Tuomisto H, Rouhan G, Christenhusz MJ (2010) Phylogenetics and classification of the pantropical fern family Lindsaeaceae. Botanical Journal of the Linnean Society 163: 305–359. https://doi.org/10.1111/j.1095-8339.2010.01063.x

Li FW, Tan BC, Buchbender V, Moran RC, Rouhan G, Wang CN, Quandt D (2009) Identifying a mysterious aquatic fern gametophyte. Plant Systematics and Evolution 281: 77–86. https://doi.org/10.1007/s00606-009-0188-2

Li SH, Zuo ZY, Chen CW, Pham VT, Luu HT, Dong SY (2024) Three out of one: Revising the species delimitation of the tree fern *Gymnosphaera salletii* (Cyatheaceae), with particular reference to the foliar nectary. Botanical Journal of the Linnean Society 204: 63–75. https://doi.org/10.1093/botlinnean/boad036

Liao W, Ding M, Wu ZY, Prado J, Gilbert MG (2013) Pteris. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China. Science Press, Missouri Botanical Garden Press, Beijing, St. Louis, 181–256.

Lin SJ, Iwatsuki K, Kato M (1996) Cytotaxonomic study of ferns from China, I. Species of Yunnan. Shokubutsu Kenkyu Zasshi 71: 214–222.

Lin SJ, Zhuang HR, Iwatsuki K, Lu HS (2002) Cytotaxonomic study of ferns from China II. Species of Fujian. Shokubutsu Kenkyu Zasshi 77: 129–138.

Löve A, Löve D, Pichi Sermolli REG (1977) Cytotaxonomical Atlas of the Pteridophyta. Cramer, Liechtenstein.

Manickam VS, Irudayaraj V (1989) Corrections to the cytology of ferns of the western Ghats. Indian Fern Journal 6: 131–132.

Manton I (1954) Cytology of One Hundred Ferns. Appendix in Revised Flora of Malaya, vol. 2: Ferns, Holttum RE. Government Printing Office, Singapore.

Manton I, Sledge WA (1954) Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 238: 127–185. https://doi.org/10.1098/rstb.1954.0008

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

Mitui K (1968) Chromosomes and speciation in ferns. Science Reports of the Tokyo Kyoiku Daigaku B 13: 185–333.

Mitui K (1976) Chromosome numbers of some ferns in the Rykyu Islands. Shokubutsu Kenkyu Zasshi 51: 33–41.

Nadot S, Bittar G, Carter L, Lacroix R, Lejeune B (1995) A phylogenetic analysis of monocotyledons based on the chloroplast gene *rps4*, using parsimony and a new numerical phenetics method. Molecular Phylogenetics and Evolution 4: 257–282. https://doi.org/10.1006/mpev.1995.1024

Nayak SK, Singh PK (1989) Cytological studies in the genus *Azolla*. Cytologia 54: 275–286. https://doi.org/10.1508/cytologia.54.275

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. The New Phytologist 206: 19–26. https://doi.org/10.1111/nph.13191 [last accessed 4 Nov 2024]

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). American Journal of Botany 84: 1120–1136. https://doi.org/10.2307/2446155

Schuettpelz E, Chen CW, Kessler M, Pinson JB, Johnson B, Davila A, Cochran AT, Huiet L, Pryer KM (2016) A revised generic classification of vittarioid ferns (Pteridaceae) based on molecular, micromorphological, and geographic data. Taxon 65: 708–722. https://doi.org/10.12705/654.2

Smith AR, Cranfill R (2002) Intrafamilial relationships of the thelypteroid ferns (Thelypteridaceae). American Fern Journal 92: 131–149. https://doi.org/10.1640/0002-8444(2002)092[0131:IROTTF]2.0.CO;2

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109. https://doi.org/10.1007/BF00037152

Takamiya M, Ohta N, Yatabe Y, Murakami N (2001) Cytological, morphological, genetic, and molecular phylogenetic studies on intraspecific differentiations within *Diplazium doederleinii* (Woodsiaceae: Pteridophyta). International Journal of Plant Sciences 162: 625–636. https://doi.org/10.1086/320144

Takei M (1969) Karyological studies in Polypodiaceae I. Karyotypes of a few species of the genus *Lemmaphyllum* and *Pyrrosia* in Japan. The Botanical Magazine 82: 482–487. https://doi.org/10.15281/jplantres1887.82.482

Tate JA, Simpson BB (2003) Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Systematic Botany 28: 723–737.

Tindale MD, Roy SK (2002) A cytotaxonomic survey of the Pteridophyta of Australia. Australian Systematic Botany 15: 839–937. https://doi.org/10.1071/SB00034

TPG (2019) Updating Taiwanese pteridophyte checklist: A new phylogenetic classification. Taiwania 64: 367–395.

Tsai JL, Shieh WC (1985) A cytotaxonomic survey of the fern family Aspidiaceae (sensu Copeland) in Taiwan. Journal of Science and Engineering 22: 121–144.

Walker TG (1962) Cytology and evolution in the fern genus *Pteris* L. Evolution; International Journal of Organic Evolution 16: 27–43. https://doi.org/10.2307/2406264

Wolf PG, Sipes SD, White MR, Martines ML, Pryer KM, Smith AR, Ueda K (1999) Phylogenetic relationships of the enigmatic fern families Hymenophyllopsidaceae and Lophosoriaceae: Evidence from *rbcL* nucleotide sequences. Plant Systematics and Evolution 219: 263–270. https://doi.org/10.1007/BF00985583

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH (2009) The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences of the United States of America 106: 13875–13879. https://doi.org/10.1073/pnas.0811575106

Yun N, Moon MO, Sun BY, Hwang IC (2023) *Lindsaea kohkongensis* (Lindsaeaceae), a new species from Cambodia and Malaysia. Korean Journal of Plant Taxonomy 53: 288–293. https://doi.org/10.11110/kjpt.2023.53.4.288

Zhang L, Guo L, Zhang LB (2018) New combinations in the fern genus *Leptochilus* (Polypodiaceae). Phytotaxa 374: 172–176. https://doi.org/10.11646/phytotaxa.374.2.10

Zhang L, Lu TN, Zhou XM, Chen DK, Knapp R, Zhou L, Gao XF, Zhang LB (2019) A plastid phylogeny of the Old World fern genus *Leptochilus* (Polypodiaceae): Implications for cryptic speciation and progressive colonization from lower to higher latitudes. Molecular Phylogenetics and Evolution 134: 311–322. https://doi.org/10.1016/j.ympev.2019.01.013

Zhang L, Lu TN, Zhou XM, Zhou Z, Thepkayson K, Luong TT, Zhang LB (2024) Exploring the diversity of the java fern genus *Leptochilus* (Polypodiaceae) in the Indo-Burma biodiversity hotspot. Phytotaxa 659: 213–235. https://doi.org/10.11646/phytotaxa.659.3.1

Zhao HG, Dong SY (2016) A new hybrid of *Tectaria* (Tectariaceae) from southern China. Phytotaxa 266: 213–218. https://doi.org/10.11646/phytotaxa.266.3.5

Zhao CF, Wei R, Zhang XC, Xiang QP (2020) Backbone phylogeny of *Lepisorus* (Polypodiaceae) and a novel infrageneric classification based on the total evidence from plastid and morphological data. Cladistics 36: 235–258. https://doi.org/10.1111/cla.12403

Zhou XM, Zhang LB (2019) A monograph of the fern genus *Pteridrys* (Pteridryaceae). Systematic Botany 44: 243–273. https://doi.org/10.1600/036364419X15562052252153

Zhou XM, Zhang L, Chen CW, Li CX, Huang YM, Chen DK, Lu TN, Cicuzza D, Knapp R, Luong TT, Nitta JH (2017) A plastid phylogeny and character evolution of the Old World fern genus *Pyrrosia* (Polypodiaceae) with the description of a new genus: *Hovenkampia* (Polypodiaceae). Molecular Phylogenetics and Evolution 114: 271–294. https://doi.org/10.1016/j.ympev.2017.06.020

Appendix 1

Table A1. Specimens of *Lindsaea* used for molecular phylogenetic analysis in this study. New sequences generated in this study are indicated in bold. En-dashes "–" indicate missing data.

Taxa	Voucher	Herbarium	Country	Matk	Trnh-Psba	Trnl-F
Lindsaea agatii (Brack.) Lehtonen & Tuomisto	Chen SITW5698	TAIF [481048]	Solomon Islands	**PQ149394**	**PQ149410**	**PQ149412**
Lindsaea blotiana K.U.Kramer	Rakotondrainibe 6350	P	Madagascar	–	GU478510	GU478813
Lindsaea ensifolia Sw.	Fraser-Jenkins FN29	TAIF [357318]	Nepal	–	**PQ149405**	–
Lindsaea ensifolia Sw.	Fraser-Jenkins FN36	TAIF [357830]	India	–	**PQ149404**	–
Lindsaea ensifolia Sw.	Huang 1657	TAIF [307881]	Cambodia	–	**PQ149407**	–
Lindsaea ensifolia Sw.	Kessler 13597	UC [1951608]	Malaysia	–	GU478521	–
Lindsaea ensifolia Sw.	Larsen 41363	AAU	Thailand	–	GU478522	GU478853
Lindsaea ensifolia Sw.	Lu 15719	TAIF [398238]	Thailand	**PQ149396**	**PQ149403**	**PQ149416**
Lindsaea ensifolia Sw.	Lu 16172	TAIF [296864]	Bangladesh	–	**PQ149408**	–
Lindsaea ensifolia Sw.	Chen SITW5235	TAIF [480899]	Solomon Islands	**PQ149399**	**PQ149402**	**PQ149415**
Lindsaea ensifolia Sw.	Chen Wade1339	TAIF [411090]	Vietnam	**PQ149398**	**PQ149401**	**PQ149414**
Lindsaea ensifolia Sw.	Chen Wade1399	TAIF [411162]	Vietnam	**PQ149397**	**PQ149406**	**PQ149417**
Lindsaea fraseri Hook.	Streimann 8951	L	Australia	–	FJ360908	FJ360998
Lindsaea heterophylla Dryand.	Kramer 8285	Z	China	–	KF652043	KF652056
Lindsaea javanensis Blume	Chen Wade4151	TAIF [458804]	Vietnam	**PQ149393**	**PQ149409**	**PQ149411**
Lindsaea kohkongensis I.C.Hwang, M.O.Moon & B.Y.Sun	Chen Wade5034	TAIF [514046]	Vietnam	**PQ149395**	**PQ149400**	**PQ149413**
Lindsaea leptophylla Baker	Raharimalala 2017	MO	Madagascar	–	GU478509	GU478819
Lindsaea madagascariensis Baker	Rakotondrainibe 6349	P	Madagascar	–	GU478512	GU478815
Lindsaea media R.Br.	van der Werff 11655	MO	Australia	–	GU478516	GU478843
Lindsaea meifolia (Kunth) Mett. ex Kuhn	Liesner 7062	MO	Venezuela	–	GU478478	GU478783
Lindsaea orbiculata (Lam.) Mett. ex Kuhn	Averyanov et al. VH4814	AAU	Vietnam	–	FJ360922	FJ361013
Lindsaea oxyphylla Baker	Gautier 2662	P	Madagascar	–	GU478508	GU478816
Lindsaea vieillardii Mett.	McKee 5304	U	New Caledonia	–	GU478496	GU478844
Lindsaea walkerae Hook.	Bostock 638	Z	Australia	–	GU478517	GU478841
Tapeinidium luzonicum (Hook.) K.U.Kramer	Kessler 13610	GOET	Malaysia	–	GU478444	GU478751

Table A2. Specimens of *Leptochilus* used for molecular phylogenetic analysis in this study. New sequences generated in this study are indicated in bold.

Taxa	Voucher	Herbarium	Country	Rbcl	Rps4-Trns	Trnl-F
Leptochilus axillaris (Cav.) Kaulf.	Wu 2462	KUN	Laos	JX103701	JX103743	JX103785
Leptochilus cantoniensis (Baker) Ching	Dong 743	PE	China	EU482945	EU482995	EU483041
Leptochilus cantoniensis (Baker) Ching	Dong 172	PE	China	EU482946	EU482996	EU483042
Leptochilus cantoniensis (Baker) Ching	Kuo 1701	TAIF [509328]	China	MT137055	MH665095	MH665162
Leptochilus decurrens Blume	Chen Wade1769	TAIF [388951]	Indonesia	MH768462	MH768527	MH768586
Leptochilus digitatus (Baker) Noot.	Chao 1556	TAIF (432480)	Vietnam	MH665034	MH665097	MH665164
Leptochilus dissimilialatus (Bonap.) Liang Zhang & Li Bing Zhang	Zhang et al. 6362	CDBI, MO, VNMN	Vietnam	MH768419	MH768481	MH768547
Leptochilus ellipticus (Thunb.) Noot.	Chen Wade3656	TAIF [440115]	Japan	MH768444	MH768508	MH768572
Leptochilus evrardii (Tardieu) Liang Zhang & Li Bing Zhang	Zhang et al. 8633	CDBI, MO	Vietnam	MH768461	MH768526	MH768585
Leptochilus flexilobus (Christ) Liang Zhang & Li Bing Zhang	Chen Wade2518	TAIF [440142]	China	MH665042	MH665106	MH665173
Leptochilus henryi (Baker) X.C.Zhang	Zhang et al. 9289	CDBI	China	MH768459	MH768524	MH768583
Leptochilus heterophyllus (S.K.Wu & P.K.Lôc) comb. ined.	Wu WP-135	KUN	Vietnam	JX103688	JX103730	JX103772
Leptochilus longipes (Ching) X.C.Zhang	Chen Wade4102	TAIF [458794]	Vietnam	MH665049	MH665114	MH665181
Leptochilus macrophyllus (Blume) Noot.	Chen Wade1962	TAIF [388897]	Indonesia	MH768449	MH768513	MH768575
Leptochilus oblongus Li Bing Zhang, Liang Zhang & N.T.Lu	Zhang et al. 6299	CDBI, MO, VNMN	Vietnam	MH768429	MH768491	MH768557
Leptochilus pedunculatus (Hook. & Grev.) Fraser-Jenk.	Chen Wade1334	TAIF [410768]	Vietnam	MH051168	MH113467	MH113500
Leptochilus pentaphyllus (Baker) Liang Zhang & Li Bing Zhang	Zhang 1777	KUN	China	MH768474	MH768539	MH768599
Leptochilus poilanei (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang	Chen Wade6804	TAIF	Vietnam	**PQ149390**	**PQ149391**	**PQ149392**
Leptochilus pothifolius (Buch.-Ham. ex D.Don) Fraser-Jenk.	Chen Wade2519	TAIF [440141]	China	MH665056	MH665122	MH665189
Leptochilus pteropus (Blume) Fraser-Jenk.	Chen 1010	H	Taiwan	MH051176	MH113475	MH113508
Leptochilus saxicola (H.G.Zhou & Hua Li) Liang Zhang & Li Bing Zhang	Wei 2017	KUN	China	MH768471	MH768536	MH768595
Leptochilus wrightii (Hook.) X.C.Zhang	Chen 1087	H	Taiwan	MH051170	MH113469	MH113502

PhytoKeys 255: 171–187 (2025)
DOI: 10.3897/phytokeys.255.143020

Research Article

Morphological and molecular evidence suggests that *Indosasa gigantea* and *Acidosasa glauca* (Poaceae, Bambusoideae, Arundinarieae) are conspecific

Zhengyang Niu[1,2,3], Zhuoyu Cai[1,4,5], Jun Yin[6], Yihua Tong[1,3], Nianhe Xia[1,3]

1 *Laboratory of Plant Resources Conservation and Sustainable Utilization & Key Laboratory of National Forestry and Grassland Administration on Plant Conservation and Utilization in Southern China, South China Botanical Garden, Chinese Academy of Sciences, 510650, Guangzhou, China*
2 *University of Chinese Academy of Sciences, 100049, Beijing, China*
3 *South China National Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China*
4 *Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, 210037, Nanjing, China*
5 *Bamboo Research Institute, Nanjing Forestry University, 210037, Nanjing, China*
6 *Forestry Workstation, Baoxi County, 323724, Longquan, China*
Corresponding author: Yihua Tong (yh-tong@scbg.ac.cn)

Abstract

Indosasa gigantea is a bamboo with great economical value, but its generic designation has been controversial for a long time. This study aims to ascertain whether *I. gigantea* belongs to *Indosasa* or *Acidosasa*, based on morphological and molecular evidence from both plastome and single-copy nuclear orthologous genes. The results of phylogenetic analyses, based on plastid genomes and nuclear gene sequences, both strongly supported that *I. gigantea* is distantly related to other members of *Indosasa*, but clustered with *Acidosasa glauca*, the type species of *Acidosasa*. Further morphological studies demonstrated that *I. gigantea* is conspecific with *A. glauca*. Thus, *I. gigantea* was proposed as a synonym of *A. glauca*. Colour plates as well as a detailed description of this species are also provided.

Key words: Arundinarieae, morphology, new synonym, plastomes, single-copy orthologous genes, phylogeny

Academic editor: Wei Lim Goh
Received: 29 November 2024
Accepted: 10 March 2025
Published: 24 April 2025

Citation: Niu Z, Cai Z, Yin J, Tong Y, Xia N (2025) Morphological and molecular evidence suggests that *Indosasa gigantea* and *Acidosasa glauca* (Poaceae, Bambusoideae, Arundinarieae) are conspecific. PhytoKeys 255: 171–187. https://doi.org/10.3897/phytokeys.255.143020

Introduction

Indosasa gigantea (T. H. Wen) T. H. Wen (1991) is a bamboo native to Fujian Province of China, with great economical value for its tasty bamboo shoots and beautiful figure with erect culms that are 7–17 m tall (Wen 1991; Zheng and Lin 1995; Zhu and Zhao 1996; Liao et al. 2003, 2016; Zhu and Stapleton 2006; Wang 2010). This species has been introduced to neighbouring Zhejiang and Guangdong Provinces (often called 江南笋 jiāng nán sǔn) in recent years according to the historical documents (Wen 1991) and our field investigations. However, the generic designation of *I. gigantea* has been controversial for a long time. Initially, Wen (1983) described this species as a new species of *Sinobambusa* Makino ex Nakai (Nakai 1925), namely *S. gigantea* T. H. Wen, based on vegetative materials. Eight years later, he stated that some newly-collected reproductive materials of this species showed the diagnostic characters for *Indosasa*, i.e. iterauctant or indeterminate inflorescence and six stamens per floret, so he transferred it to *Indosasa* McClure (Wen 1991). However, after re-examining the reproductive materials of *I. gigantea*, Xie and Chen (1993) argued that this species actually had semelauctant and raceme-like inflorescence with true pedicellate spikelets, which matched well with the diagnostic characters of *Acidosasa* B. M. Yang (1981). Thus, they made a new combination for this species as *Acidosasa gigantea* (T. H. Wen) Q. Z. Xie & W. Y. Zhang. It seems that Xie and Chen's opinion was ignored, since many later important floral works and databases adopted *Indosasa gigantea* (T. H. Wen) T. H. Wen (Wen et al. 1993; Zhu and Stapleton 2006; Vorontsova et al. 2016; IPNI 2024). However, Zhu and Stapleton (2006) noted that "a different interpretation of the inflorescence can place this species in *Acidosasa*" in their account of *Indosasa* of "Flora of China".

Neither Wen (1991) or Xie and Chen (1993) provided any information of the flowering specimens they examined. As we known, the spikelet type (true spikelet or pseudo-spikelet) is the key character to differentiate *Indosasa* and *Acidosasa*. To ascertain the spikelet type of *I. gigantea*, we visited the herbarium of Zhejiang Forestry Institute (ZJFI) where Wen worked and tried to find the flowering materials of this species. However, despite of an exhaustive search, we could not find any reproductive material of this species, other than the holotype with vegetative organs. Surprisingly to us, the holotype of *I. gigantea* shows a number of characters that are very similar to the type species of *Acidosasa*, i.e. *A. glauca* B. M. Yang, especially the morphology of culm leaf, such as the powdery and setose sheath, arcuate ligule and auricles with well-developed oral setae. To clear the confusions of the taxonomic identity of *Indosasa gigantea*, we conducted morphological and phylogenetic studies as below.

Materials and methods

Morphological study

Voucher specimens were collected during several field trips from 2019–2022 mainly to the type localities of many bamboos including Longquan County of Zhejiang Province (type locality of *Indosasa gigantea*) and Jianghua County of Hunan Province (type locality of *Acidosasa glauca*) and were kept in the Herbarium of South China Botanical Garden (IBSC). Some flowering materials (*BH225*, IBSC) were collected at Shaoguan City of Guangdong Province which were named as "江南笋" and we identified them as *Indosasa gigantea*. Specimens from the Herbarium of Hunan Normal University (HNNU), IBSC and ZJFI were examined. Herbarium acronyms follow Thiers (2024, updated continuously). Flowering materials were dissected under a stereomicroscope (Mshot-MZ101) and small parts were measured and photographed with the camera attachment (Mshot-MSX2). Terminology follows McClure (1940), Li et al. (2006) and Beentje (2016).

Taxon sampling, DNA extraction and sequencing

To ascertain the phylogenetic position of *Indosasa gigantea*, phylogenetic analyses, based on sequences of plastomes and single-copy orthologous genes, were conducted. The taxon sampling referred to a previous study of the tribe

Arundinarieae by Guo et al. (2021). For plastome-based phylogenetic inference, the ingroup contains two samples of *I. gigantea* (*NZY177* from the type locality and *BH225* from the flowering population at Shaoguan City of Guangdong Province), two samples of the type species of *Acidosasa*, i.e. *Acidosasa glauca* B. M. Yang (*CZY56* from the type locality of *A. glauca* and *NZY152* from the type locality of *A. chienouensis*, a synonym of *A. glauca*), the type species of *Indosasa*, i.e. *Indosasa crassiflora* McClure and representatives from other genera in Arundinarieae. *Bambusa vulgaris* Nees from the tribe Bambuseae was chosen as the outgroup. In total, 31 samples representing 29 species from 19 genera were included with eight newly-sequenced and 23 downloaded from GenBank. For nuclear gene-based phylogenetic inference, a total of 26 samples representing 24 species from 14 genera in the tribe Arundinarieae were included with data of five species unavailable. Voucher information and GenBank accession numbers of plastomes were listed in Suppl. material 1: table S1.

For DNA extractions, young leaves were collected in the field and dried with silica gel. Genomic DNAs were extracted from the dried leaves using the TIANGEN Genomic DNA Extraction Kit (TIANGEN, Beijing, China), following the manufacturer's instructions and 1 μg DNA per sample was sheared using a Covaris M220 ultrasonicator (Covaris, Woburn, MA). We enriched the resulting 350-bp fragments using PCR and prepared a paired-end library using the NEBNext® UltraTM DNA Library Prep Kit which we sequenced on a NovaSeq 6000 platform. After filtration of adapters and low-quality reads using Fastp software v. 0.23.2 (Chen et al. 2018), at least 40 Gb deep genome skimming (DGS) data were generated.

Plastome assembly and nuclear single-copy orthologous genes recovery

We used filtered clean reads to *de novo* assemble complete plastid genomes using the GetOrganelle v. 1.7.6.1 pipeline (Jin et al. 2020), with the plastome of *Phyllostachys edulis* (Carrière) J. Houz. (GenBank accession No. HQ337796) as reference. We set six k-mer values, viz. 21, 45, 65, 85, 105 and 125, for plastid contig assembly. Following assembly, we aligned two generated plastid sequences with opposite short single-copy (SSC) region directions to the reference sequence using Mauve v. 2.4.0 (Darling et al. 2004). We visualised and selected the sequence with the same SSC direction as the reference as the final plastome in the software Geneious v. 9.1.4 (Kearse et al. 2012).

For nuclear genes recovery, we used the protein-coding sequences of six previously published bamboo genomes—*Dendrocalamus latiflorus* Munro (Zheng et al. 2022), *Phyllostachys edulis* (Carrière) J. Houz. (Zhao et al. 2018), *Bonia amplexicaulis* (L. C. Chia, H. L. Fung & Y. L. Yang) N. H. Xia, *Guadua angustifolia* Kunth, *Olyra latifolia* L. and *Raddia guianensis* (Brongn.) Hitchc. (Guo et al. 2019), to identify 737 common nuclear single-copy orthologous genes (SOGs) using Orthofinder v. 2.5.4 (Emms and Kelly 2019). We assembled putative SOGs using HybPiper v. 2.0.1 (Johnson et al. 2016). We mapped filtered clean reads to each SOG using the BWA mapper function in HybPiper. We then *de novo* assembled reads mapped to each gene into contigs with the best k-mer automatically detected by SPAdes v. 3.15.0 (Bankevich et al. 2012). We aligned the assembled contigs to the reference SOG dataset and used a python script 'retrieve_sequences.py' to recover 737 putative orthologs for each sample.

However, because all of our samples are polyploid (Guo et al. 2019), some so-called SOGs might have multiple copies. We therefore used a python script 'paralog_retriever.py' to detect and disregard potential paralogs. After this step, we retained 439 SOGs.

Alignment construction and phylogenetic inference

We aligned the plastid genomes and 439 SOGs using MAFFT v. 7.505 (Katoh and Standley 2013) in the software Geneious. We trimmed each single-gene matrix using trimAl v.1.4 (Salvador et al. 2009) with default settings. We then removed those nuclear genes with lengths shorter than 300 bp or with > 25% missing data. The final nuclear dataset used for phylogenetic analyses included 433 conserved nuclear genes.

As the plastome is a linkage group without recombination (Doyle 2022), we thus performed Maximum Likelihood (ML) analysis for plastid DNA data. We ran the multispecies coalescent-based method for phylogenetic inference for the nuclear dataset as different nuclear genes possess heterogeneous nucleotide substitution rates (Doyle 1992; Maddison 1997).

For plastome-based phylogenetic inference, we used RAxML v. 8.2.12 (Stamatakis 2014) to perform 20 addition replicates under the GTR+Γ model. We chose the GTR+Γ model because it accommodates rate heterogeneity amongst sites, while the other available GTR models in RAxML are less appropriate due to the small taxon sampling size (Stamatakis 2014; Cai et al. 2021). We estimated branch support using a rapid bootstrap algorithm with 1000 bootstrap replicates. For nuclear gene-based phylogenetic inference, we inferred individual ML trees using RAxML for each nuclear gene and estimated branch support using bootstrapping analysis with 500 replicates, all using the GTR+Γ model. We tested different thresholds by collapsing branches with support < 30% and 50% and compared the resulting trees to the tree without collapsed branches. This procedure was applied to each ML bifurcation locus tree by Newick Utilities (Junier and Zdobnov 2010). We combined all the generated bifurcation trees to infer the species tree using ASTRAL-III (Zhang et al. 2018). The local posterior probability was calculated with the parameter '-t 3'.

Results

Morphological study

A detailed morphological comparison between *Indosasa gigantea* and *Acidosasa glauca* was conducted, based on examination of type specimens, critical analysis of descriptions in the protologues and observations in the field. The results showed that the two species share exactly the same key characters, such as the thickly powdery young culms, branch complement with three branches at each mid-culm node, sparsely brown setose and white-powdery abaxial surface of culm leaf sheaths with a densely brown setose base, ovate to falcate culm leaf auricles with many radiating or sometimes curly oral setae, prominent culm leaf ligules, narrow triangular to lanceolate culm leaf blades, 3 or 4 foliage leaves per ultimate branch, glabrous foliage leaf sheaths, undeveloped foliage leaf auricles usually without oral setae or with several oral setae at the most basal leaf sheath apex and foliage leaf blades being glabrous adaxially and pubescent abaxially (Table 1, Figs 1–4). The only difference seems to be the colour of culm leaf sheaths: *I. gigantea* has pale red-brown abaxial surface of culm leaf sheaths (Fig. 3B5), while that of *A. glauca* is green to yellow-brown (Fig. 3A5). However, the colour of culm leaf sheaths seems to be unstable, which is easily affected by the light condition of the habitats according to our observations in the wild. Specifically, with a strong light condition, the culm leaf sheaths will often be redder than those within weak light conditions.

The flowering materials found at Shaoguan City of Guangdong Province have raceme-like inflorescence with (1–)2–5 pedicellate spikelets, two glumes, each spikelet with several to over ten florets, pubescent rachilla segments, glabrous and 11–13-veined lemma, palea with the ciliate upper parts of keels and acute apex, three lodicules, six stamens with 4–5 mm long anthers and ovary with one style and three stigmas (Fig. 5).

Phylogenetic study

The basic features of the plastomes of all the samples in our study are summarised in Suppl. material 1: table S2. The plastid genome sequence alignment, based on two samples of *Acidosasa glauca* and two samples of *Indosasa gigantea*, is green in all sites, which means that the four plastid genomes are identical (Fig. 6B). The size of the plastome is 139,677 bp, including a large single-copy (LSC) region with 83,261 bp, a short single-copy (SSC) region with 12,816 bp and one pair of inverted repeats with 21,795 bp (Suppl. material 1: table S2).

Table 1. Comparison of key morphological characters between *Indosasa gigantea* and *Acidosasa glauca*.

Characters	*I. gigantea*	*A. glauca*
Young culm	Glabrous, with thick white powder	Glabrous, with thick white powder
Number of branches at mid-culm node	three	three
Culm leaf		
Colour of sheath	Pale red-brown	Yellow-brown
Abaxial surface of sheath	Sparsely brown setose and white powdery, densely brown setose at base	Sparsely brown setose and white powdery, densely brown setose at base
Auricles	Ovate to falcate	Falcate
Oral setae	Radiating, ca. 1 cm long	Radiating or curly, ca. 0.8 cm long
Ligule	3–5 mm high, prominent	2–3 mm high, prominent
Shape of blade	Narrow triangular to lanceolate	Narrow triangular to lanceolate
Number of foliage leaves per ultimate branch	3 or 4	3 or 4
Foliage leaf		
Abaxial surface of sheath	Glabrous	Glabrous
Auricles	Absent	Absent
Oral setae	Usually absent or several at the most basal leaf sheath apex	Usually absent or several at the most basal leaf sheath apex
Abaxial surface of blade	Pubescent	Pubescent
Adaxial surface of blade	Glabrous	Glabrous

Figure 1. Isotypes of *A. glauca* B. M. Yang (*B. M. Yang 06431*, HNNU) **A, B** sheets with vegetative part **C** sheet with flowering branches and a culm leaf **D** spikelet pedicels **E** flowering branch.

Both *Acidosasa* and *Indosasa* are resolved as polyphyletic in the plastome-based tree (Fig. 6) and nuclear SOG-based species tree (Fig. 7). In the plastome-based tree, the two samples of *I. gigantea* and the two samples of *A. glauca* are clustered into a monophyletic clade with high support values (BS = 100) and without any branch length in their interiors (BS < 70). The nuclear SOG-based species tree also strongly supported (PP = 1.0) that *I. gigantea* is clustered with *A. glauca*, the type species of *Acidosasa*, but distantly related to *I. crassiflora*, the type species of *Indosasa*.

竹类标本野外记录

野外采集号数 80556 采集日期 1980年5月23日

采集地点 龙泉宝溪公社溪头大队 海拔高

栖地 梧桐口溪边 土壤

高度 16.67 M 胸高直径 9.9 CM

秆 秆重58公斤，枝叶重8.7斤 节间长77cm，(2.33尺)
幼秆具白粉，绿色节下尤甚，无毛，老秆绿黄色
节内长1cm，节内与节下被白粉

枝、叶 分枝3

竹箨 笋期4月底至5月底，金黄色被白粉与紫刺毛，
箨基有长密毛，毛刺人，箨耳卵状至镰刀状，
黄褐色，燧毛长1cm，黄褐色，箨舌山峰状纤

花、果 毛，长2mm，箨叶绿色，无粉

用途及其它 笋面到红色，笋苦涩，竹材软，较脆
当地作风景竹

土名 江南竹

采集人 温、金登华

80556

模式标本

学名：Sinobambusa gigantea Wen sp. nov

定名人：Wen 检定时间：1981.12

Figure 2. Holotype of *Indosasa gigantea* (T. H. Wen) T. H. Wen (*T. H. Wen & D. H. Jin Wen80556*, ZJFI).

Discussion

The morphological characters of the specimens collected in the field (vouchers: *CZY56* and *NZY177*) matched well with the description of *Indosasa gigantea* made by Wen (1991) (except the type of spikelet) and the holotype of *Acidosasa glauca* (Table 1, Figs 1–4). Our phylogenetic study also suggested that *I. gigantea* is a member of *Acidosasa* rather than *Indosasa*. Wen (1991)

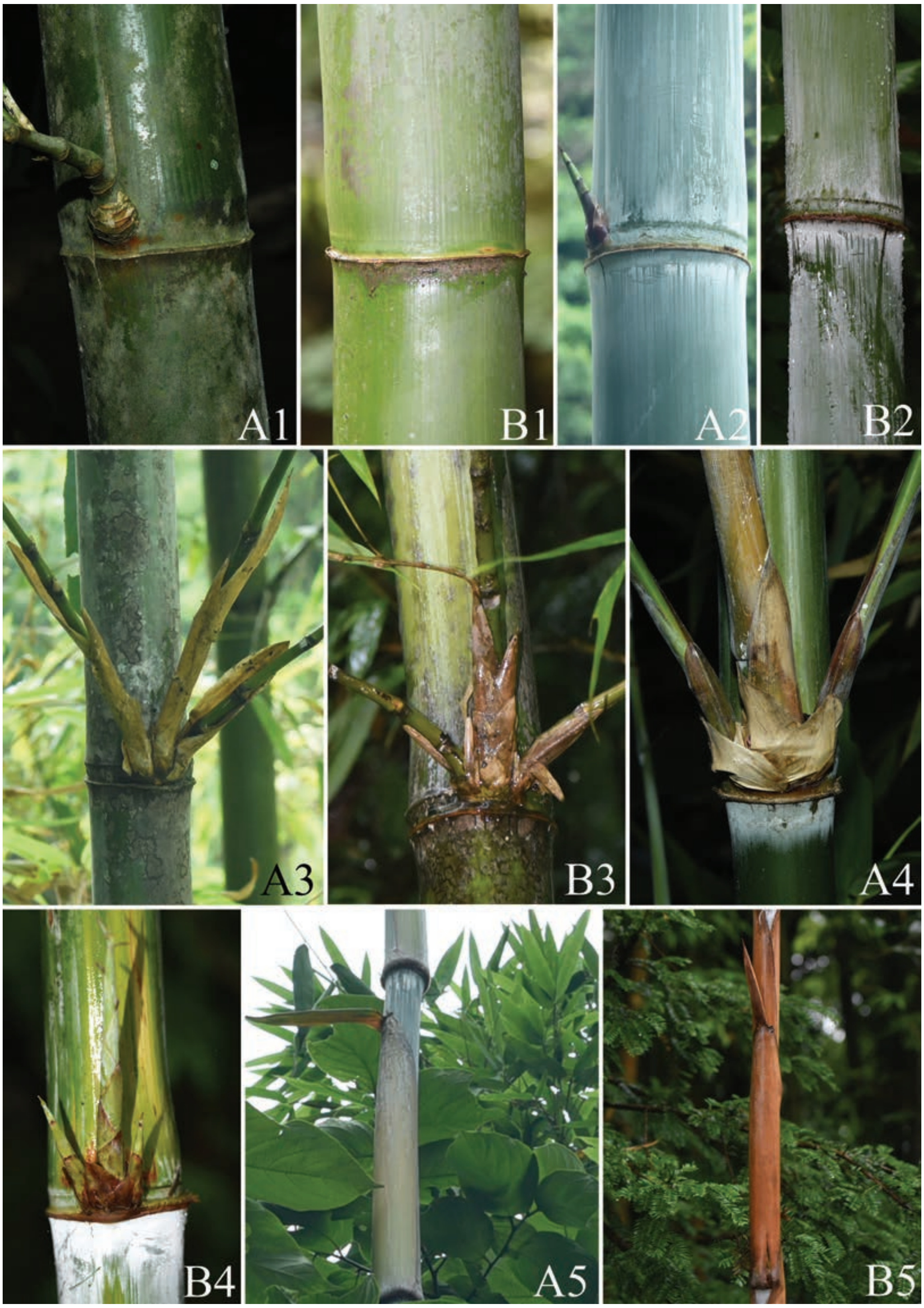

Figure 3. Morphological comparison of *Acidosasa glauca* (**A**) and *Indosasa gigantea* (**B**). **A1**–**B1** old culms **A2**–**B2** young culms **A3**–**B3** three branches at old mid-culm node **A4**–**B4** three branches at young mid-culm node **A5**–**B5** culm leaves.

did describe "indeterminate inflorescence" for *Indosasa gigantea*, which is a key character of *Indosasa*, but its flowering materials found at IBSC (voucher: *BH225*) are diagnosed by the possession of spikelet pedicels. Thus, we argue that the transfer of this species from *Indosasa* to *Acidosasa* by Xie and Chen is correct, although we did not find the flowering specimen mentioned by them.

Although there are multiple instances of conflict between the plastid and nuclear SOG-based trees, the plastid and nuclear sequences both strongly

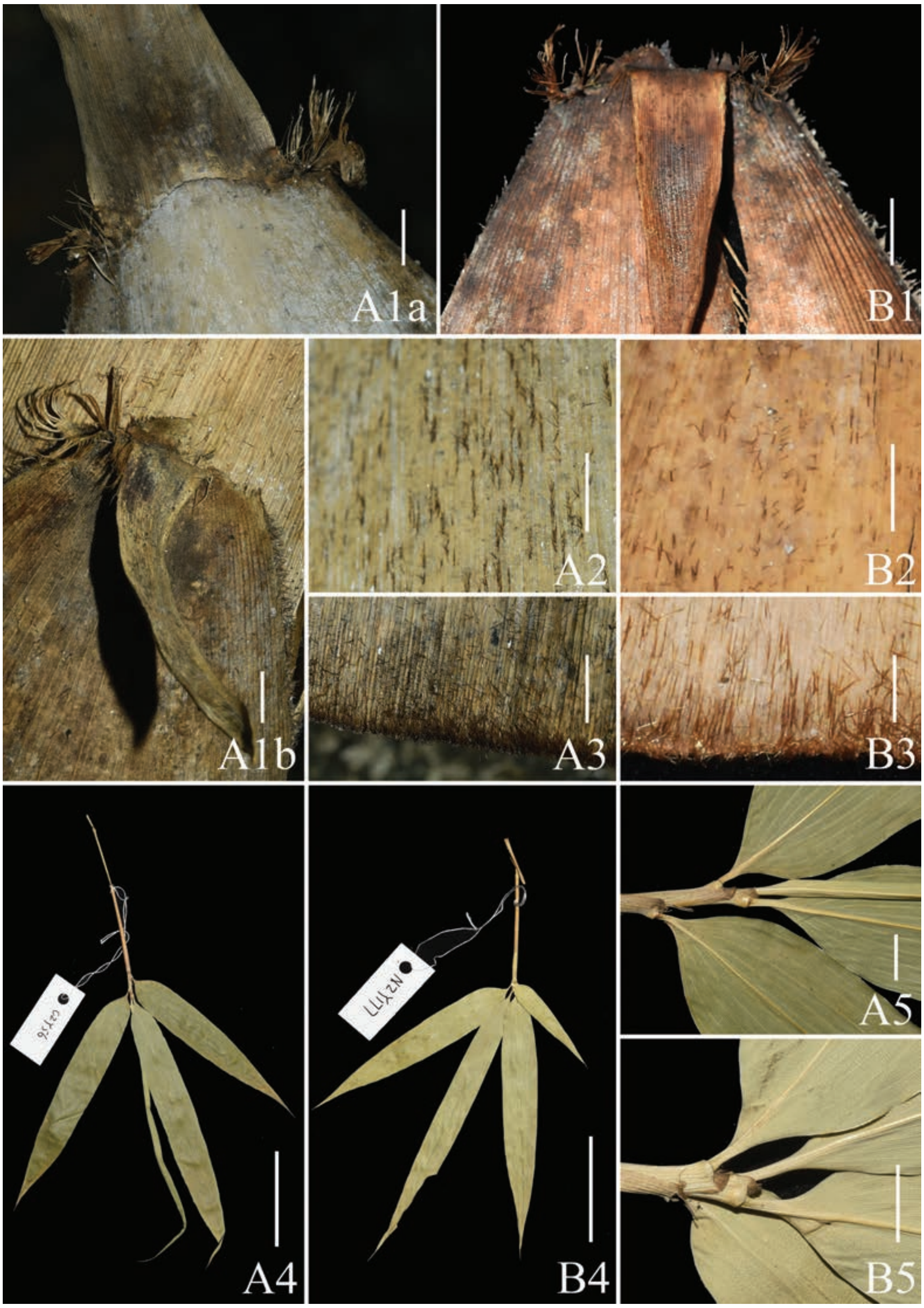

Figure 4. Morphological comparison of *Acidosasa glauca* (**A**) and *Indosasa gigantea* (**B**). **A1**–**B1** culm leaf apex, showing auricles, oral setae, ligules and blades **A2**–**B2** abaxial surface of culm leaf sheaths covered with sparse brown setae and white powder **A3**–**B3** bases of abaxial surface of culm leaf sheaths covered with dense brown setae **A4**–**B4** ultimate foliage leafy branches **A5**–**B5** foliage leaf sheath and ligules. Scale bars: 1 cm (**A1**–**A3**, **B1**–**B3**); 5 cm (**A4**–**A5**); 5 mm (**A5**–**B5**).

support that two samples *Acidosasa glauca* and two samples of *Indosasa gigantea* intermingle together (Figs 6, 7). We also noted that *A. chienouensis*, which is the synonym of *A. glauca*, is always clustered with *I. gigantea*, whether based on several plastid molecular markers (Zeng et al. 2010) or single nuclear gene (Zhang et al. 2012). Hence, our phylogenetic studies are congruent with previous studies on the relationships between *A. glauca* and *I. gigantea*.

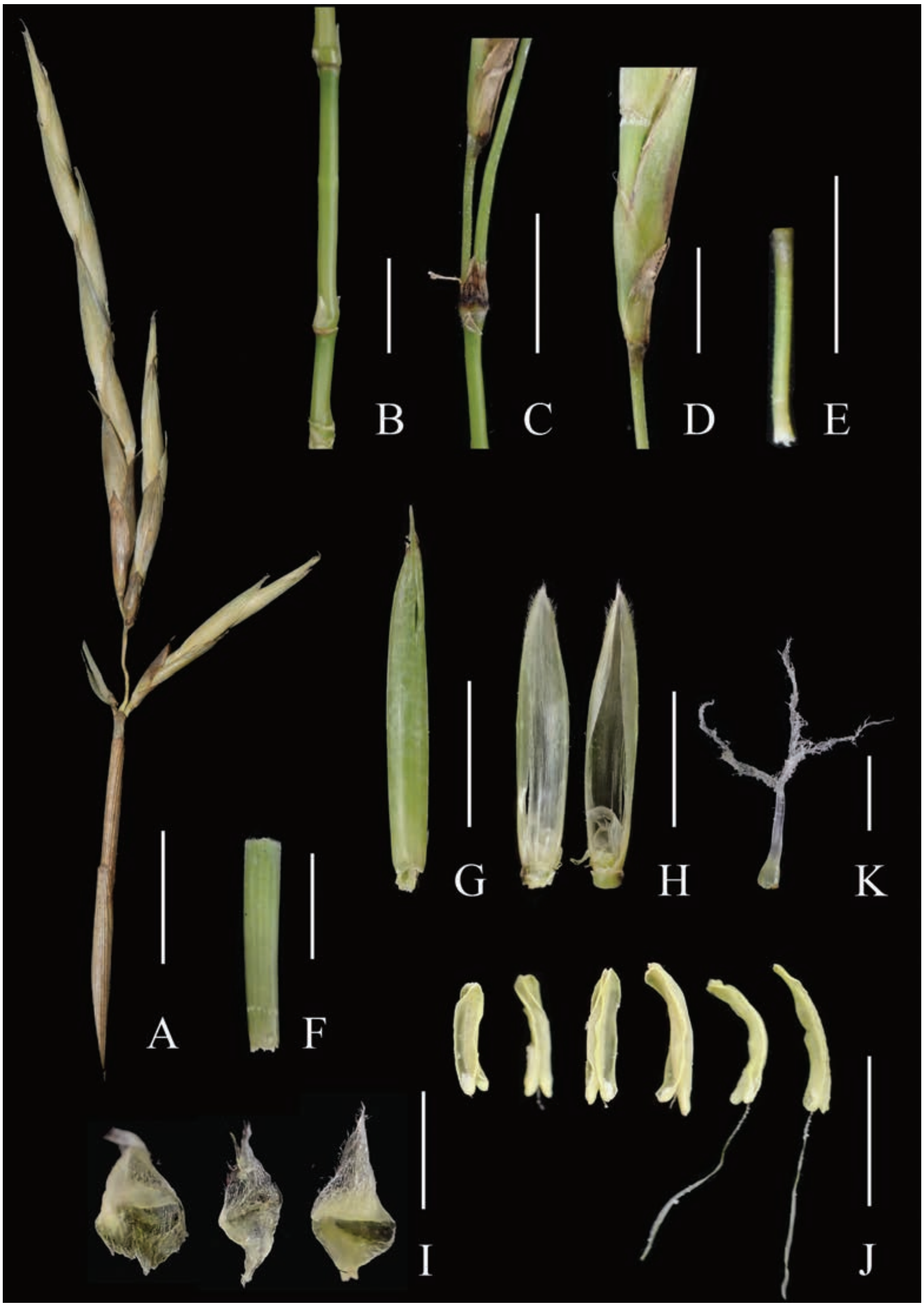

Figure 5. Dissection of inflorescence of *Indosasa gigantea* (voucher: *BH225*, IBSC) **A** flowering branch **B** internodes of lower part of flowering branch **C** small membranous bract at the base of spikelet pedicel **D** base of spikelet, showing two glumes and a floret **E** spikelet pedicel **F** rachilla segment **G** lemma, abaxial view **H** palea, adaxial (left) and abaxial (right) view **I** lodicules **J** stamens, with two possessing filaments **K** pistil. Scale bars: 1 cm (**A**); 5 mm (**B–H**, **J**); 2 mm (**K–I**).

Our morphological and phylogenetic evidence further supported that *Indosasa gigantea* is conspecific with *Acidosasa glauca* (Figs 1–7, Table 1). As *Acidosasa glauca* B. M. Yang (1981) predates the basionym of *I. gigantea*, i.e. *Sinobambusa gigantea* T. H. Wen (1983), the latter is thus proposed as a synonym of *A. glauca* here according to Art. 11.4 of International Code of Nomenclature for algae, fungi and plants (Shenzhen Code) (Turland et al. 2018).

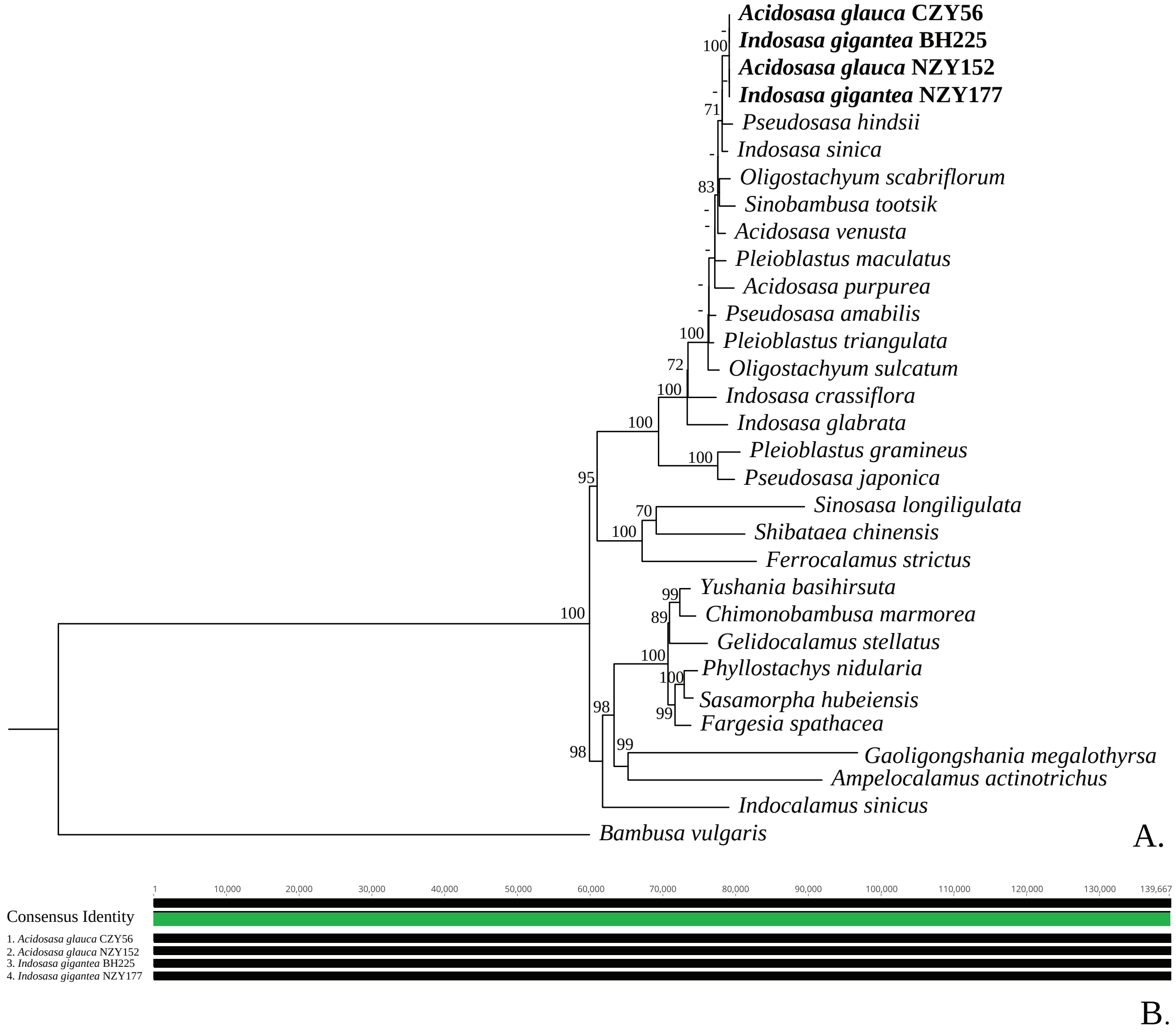

Figure 6. A The phylogram of 28 species belonging to 18 genera from Arundinarieae, based on plastome sequences. The bootstrap values ≥ 70% are shown around the branches, while those values < 70% are represented by the hyphens **B** the plastid genome sequence alignment of two samples of *A. glauca* and two samples of *I. gigantea*, showing that the four plastomes are totally identical.

Taxonomic treatment

Acidosasa glauca B. M. Yang, J. Hunan Teachers' Coll. (Nat. Sci. Ed.) 1981(2): 54 (1981)

= *Indosasa gigantea* (T. H. Wen) T. H. Wen, J. Bamboo Res. 10(1): 22 (1991). syn. nov. ≡ *Sinobambusa gigantea* T. H. Wen, J. Bamboo Res. 2(1): 57, fig. 10 (1983). ≡ *Acidosasa gigantea* (T. H. Wen) Q. Z. Xie & W. Y. Zhang, Bull. Bot. Res., Harbin 13(1): 74 (1993). Type: CHINA • Zhejiang: Longquan City, Baoxi Town, Wuling Village, 23 May 1980, *T. H. Wen & D. H. Jin Wen80556* (holotype: ZJFI!).

= *Acidosasa chienouensis* (T. H. Wen) C. S. Chao & T. H. Wen, J. Bamboo Res. 7(1): 31 (1988). ≡ *Indosasa chienouensis* T. H. Wen, J. Bamboo Res. 2(1): 67, fig. 19 (1983). Type: CHINA • Fujian: Chien'ou [Jian'ou] County, Wanmulin, 2 June 1981, *X. Q. Hua & P. X. Zhang FJ81607* (holotype: ZJFI!).

Type. China • Hunan: Jianghua County, Weizhuchong Town, Weizhuchong Village, 10 June 1981, *B. M. Yang 06431* (holotype: HNNU; isotypes: HNNU!).

Description. Running bamboo. Rhizomes leptomorph. Culms diffuse, erect, 7–17 m tall and 4–10 cm in diameter; internodes terete, 30–70 cm long, green, thickly white powdery when young, glabrous; supra-nodal ridges weakly prominent or flattened; sheath scars prominent, with a ring of dense brown setae when young, glabrescent when old. Mid-culm branch complement with three branches. Culm leaf sheaths initially pale green, turning to gold-yellow, yellow-brown or pale red-brown when old, caducous, triangular, thickly leathery, abaxially sparsely brown setose and white powdery, base densely brown setose, margins densely ciliate; auricles ovate to falcate, 3.5–10 × 2–6 mm; oral setae well-developed, many, scabrid, radiating or curly, 0.5–1 cm long; ligules fragile, arcuate, 2–4 mm tall, abaxially pubescent, apex with deciduous short cilia; blades erect or reflexed, easily deciduous, narrowly triangular to lanceolate, 2–12.5 × 0.7–3 cm, apex acuminate, base broadened or slightly narrowed. Foliage leaves 3 or 4 per ultimate branch; sheaths 4–6.5 cm long, glabrous, longitudinal ribs conspicuous; auricles absent; oral setae usually absent or several at the lowest one or two sheath apex; ligules truncate, 1–2 mm tall, abaxially pubescent; blades lanceolate, papery, 5–15 × 0.8–2 cm, base

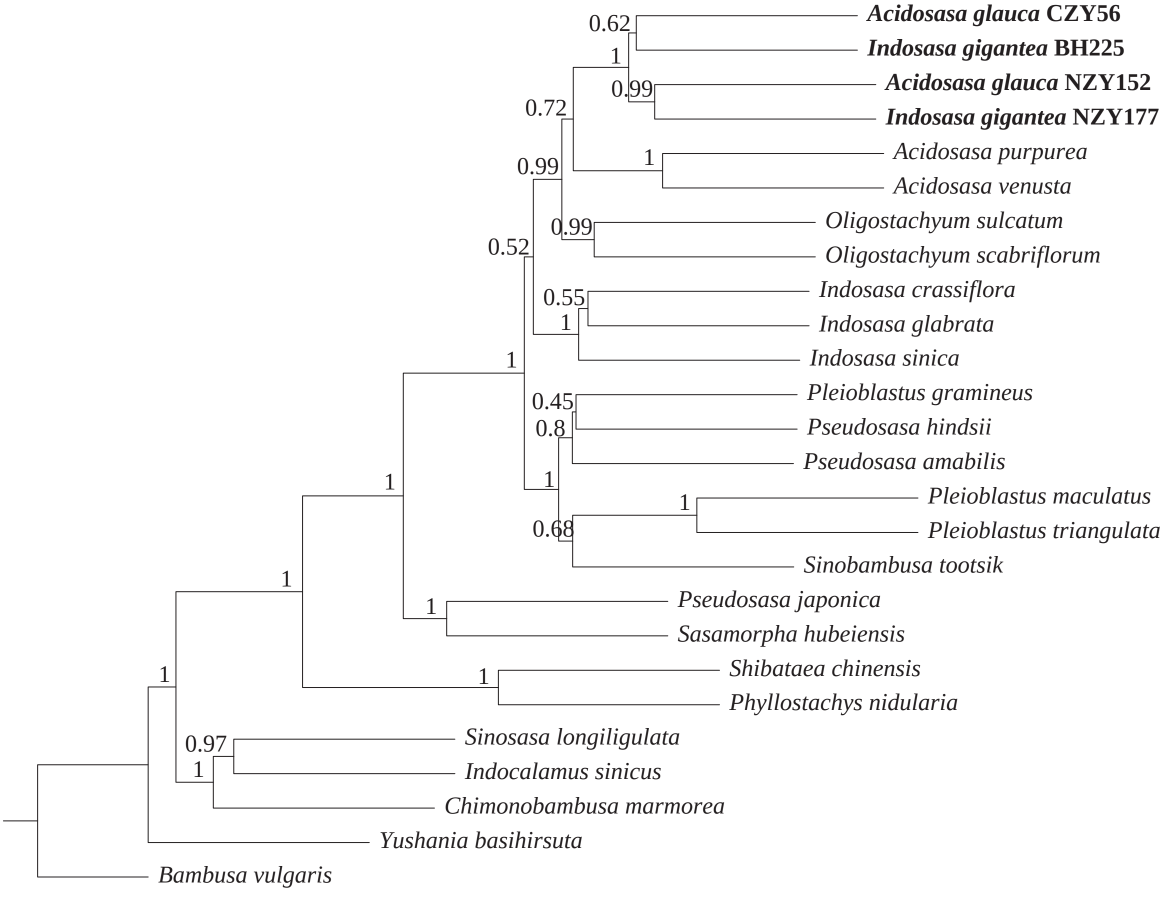

Figure 7. The ASTRAL species tree of 23 species belonging to 15 genera of the tribe Arundinarieae, which is reconciled by coalescence of 433 single-copy orthologous nuclear gene trees after collapsing branches with support < 30%. The posterior probabilities are shown around the branches.

subrounded or cuneate, abaxially pubescent, adaxially glabrous, both margins serrulate, secondary veins 4–6 pairs, transverse veins conspicuous, margins serrulate. Inflorescence raceme-like, with (1–)2–4 spikelets, axis glabrous, without white powder, basal internodes 6–8 mm long, glabrous; spikelet pedicels 3–12 mm long, glabrous, without white powder, basally subtended by a small membranous bract. Spikelets slightly laterally compressed, (1.5–)5–7.5 cm long, fertile florets 3–12(–15), uppermost one not fully developed; rachilla segments compressed, 5–10 mm long, pubescent, with several longitudinal ridges; glumes 2, first glume narrowly triangular, ca. 8 mm long, apically pubescent or glabrous, 1–3-veined, apex acute; second glume ovate to lanceolate, ca. 11 mm long, indumentum the same as the first glume, 7–9-veined; lemma lanceolate, ca. 13 mm long, abaxially sparsely pubescent at the upper parts, while glabrous at other parts or glabrous wholly, white powdery, 11–15-veined, apex acuminate; palea shorter than or equal to lemma, 9–13 mm long, 2-keeled, keels white ciliate on the upper parts, 4 or 5-veined between keels, 3-veined outside keels each side, apex acute; lodicules 3, 2.5–4 × 1–1.4 mm, ovate, the upper parts membranous, while middle and lower parts fleshy, margins sparsely ciliate; stamens 6, anthers initially yellow, brown when old, 4–5 mm long, filaments ca. 4 mm long; ovary ovate, ca. 1 mm long; style 1, 2–2.5 mm long; stigmas 3, plumose. Caryopsis unknown.

Distribution and habitat. Up to now, this species has been known native to north Fujian and widely cultivated in Zhejiang, Guangdong and Hunan. It prefers sunny environments and often grows well on mountain slopes near roadsides, creeks and farmlands.

Phenology. New shoots March to April. Flowers April to June.

Chinese names. Chinese name 粉酸竹 [fěn suān zhú]; “橄榄竹” [gǎn lǎn zhú].

Additional specimens examined. **China** • **Fujian:** Chien’ou City, Jiubao Village, Jiangdangping, 1 June 1981, *P. X. Zhang & X. Q. Hua FJ81606* (ZJFI); • Chien’ou City, the nursery of Wanmulin, 27°2'54"N, 118°8'33"E, 2 June 2022, *Z. Y. Niu NZY152* (IBSC); • Jiangle County, Longqishan, 24 May 1981, *P. X. Zhang & X. Q. Hua FJ81536* (ZJFI). **Guangdong:** • Fengkai County, Heishiding, 12 April 1982, *M. Y. Xiao 31853* (CANT); • Shaoguan City, Zhangshi Town, Luxi Village, 24°28'55"N, 113°27'27"E, 16 April 2023, *Y. H. Tong, J. B. Ni & D. H. Cui BH225* (IBSC); Shixing County, Chebaling, 18 April 2023, 24°46'3"N, 114°18'3"E, *Y. H. Tong, J. B. Ni & D. H. Cui BH238* (IBSC); • Yunan County, Gaoliang Service Area, 13 April 2023, 23°14'30"N, 111°54'16"E, *Y. H. Tong, J. B. Ni & D. H. Cui BH209* (IBSC). **Hunan:** • Jianghua County, Weizhuchong Town, roadside near Xiaoluguikou County Road 085, 24°53'10"N, 111°48'37"E, 19 May 2019, *Z.Y. Cai CZY56* (IBSC). **Zhejiang:** • Longquan City, Baoxi Town, Wuling Village, 28°0'27"N, 118°46'29"E, 20 June 2022, *Z. Y. Niu NZY177* (IBSC).

Local usage. Its culms can be used for construction. Its shoots are edible and tasty. It is very suitable for landscape due to the elegant architecture and appearance.

Acknowledgements

The authors thank Dr. Jian-Cheng Zhao for hosting our visits to the Herbarium of Zhejiang Forestry Institution (ZJFI).

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 National Natural Science Foundation of China (Grant nos. 32270227 and 31670196) and the Chinese Academy of Sciences (Biotaxonomic Scientist Post Grant No. CAS-TAX-24-049).

Author contributions

Zhengyang Niu: writing original manuscript, field investigation, data analysis. Zhuoyu Cai: field investigation. Jin Yun: field investigation. Yihua Tong: funding support, reviewing and editing the manuscript. Nianhe Xia: supervision, funding support, reviewing and editing the manuscript.

Author ORCIDs

Zhengyang Niu https://orcid.org/0000-0003-0281-1504
Zhuoyu Cai https://orcid.org/0000-0001-9288-0882
Yihua Tong https://orcid.org/0000-0002-5034-005X
Nianhe Xia https://orcid.org/0000-0001-9852-7393

Data availability

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

References

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477. https://doi.org/10.1089/cmb.2012.0021

Beentje H (2016) The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms (Second Edition). Royal Botanic Gardens Kew Press, 1–184.

Cai LM, Xi ZX, Lemmon EM, Lemmon AR, Mast A, Buddenhagen E, Liu L, Davis CC (2021) The Perfect Storm: Gene Tree Estimation Error, Incomplete Lineage Sorting, and Ancient Gene Flow Explain the Most Recalcitrant Ancient Angiosperm Clade, Malpighiales. Systematic Biology 70(3): 491–507. https://doi.org/10.1093/sysbio/syaa083

Chen SF, Zhou YQ, Chen YR, Gu J (2018) fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics (Oxford, England) 34(17): 884–890. https://doi.org/10.1093/bioinformatics/bty560

Darling ACE, Mau B, Blatter FR, Perna NT (2004) Mauve: Multiple alignment of conserved genomic sequence with rearrangements. Genome Research 14(7): 1394–1403. https://doi.org/10.1101/gr.2289704

Doyle JJ (1992) Gene trees and Species Trees. Systematic Botany 17(1): 144–163. https://doi.org/10.2307/2419070

Doyle JJ (2022) Defining Coalescent Genes: Theory Meets Practice in Organelle Phylogenomics. Systematic Biology 71(2): 476–489. https://doi.org/10.1093/sysbio/syab053

Emms DM, Kelly S (2019) OrthoFinder: Phylogenetic orthology inference for comparative genomics. Genome Biology 20(1): 238. https://doi.org/10.1186/s13059-019-1832-y

Guo ZH, Ma PF, Yang GQ, Hu JY, Liu YL, Xia EH, Zhong MC, Zhao L, Sun GL, Xu YX, Zhao YJ, Zhang YC, Zhang YX, Zhang XM, Zhou MY, Guo Y, Guo C, Liu JX, Ye XY, Chen YM, Li DZ (2019) Genome sequences provide insights into the reticulate origin and unique traits of woody bamboos. Molecular Plant 12(10): 1353–1365. https://doi.org/10.1016/j.molp.2019.05.009

Guo C, Ma PF, Yang GQ, Ye XY, Guo Y, Liu JX, Liu YL, Eaton DAR, Guo ZH, Li DZ (2021) Parallel ddRAD and genome skimming analyses reveal a radiative and reticulate evolutionary history of the temperate bamboos. Systematic Biology 70(4): 756–773. https://doi.org/10.1093/sysbio/syaa076

IPNI (2024) International Plant Names Index. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. http://www.ipni.org

Jin JJ, Yu WB, Yang JB, Song Y, 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

Johnson MG, Gardner EM, Liu Y, Medina R, Goffinet B, Shaw AJ, Zerega NJC, Wickett NJ (2016) HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. Applications in Plant Sciences 4(7): 1600016. https://doi.org/10.3732/apps.1600016

Junier T, Zdobnov EM (2010) The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. Bioinformatics (Oxford) 26: 1669–1670. https://doi.org/10.1093/bioinformatics/btq243

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

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford) 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Li DZ, Wang ZP, Zhu ZD, Xia NH, Jia LZ, Guo ZH, Yang GY, Stapleton CMA (2006) Bambuseae (Poaceae). In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, vol. 22. Science Press, Beijing, Missouri Botanical Garden Press, St. Louis, 7–180.

Liao GH, Zhou DX, Liu GW, Lin PY, Li MB (2003) Determination and analysis of nutritional components of *Indosasa gigantea*. Linye Gongcheng Xuebao S1: 1111–1112.

Liao PH, Zhen R, Lin YF, Lian HP, Wen XY (2016) Structure analysis on aboveground biomass of individual *Indosasa gigantea*. Journal of Fujian Forestry Science and Technology 43(4): 76–79.

Maddison WP (1997) Gene Trees in Species Trees. Systematic Biology 46(3): 523–536. https://doi.org/10.1093/sysbio/46.3.523

McClure FA (1940) New genera and species of Bambusaceae from eastern Asia. Lingnan University Science Bulletin 9: 1–67.

Nakai T (1925) Two new genera of Bambusaceae, with special remarks on the related genera growing in eastern Asia. Journal of the Arnold Arboretum 6(3): 152. https://doi.org/10.5962/bhl.part.24130

Salvador C, José MS, Toni G (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics (Oxford) 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Stamatakis A (2014) RaxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Thiers BM (2024) [updated continuously] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. https://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, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. https://doi.org/10.12705/Code.2018

Vorontsova MS, Clark LG, Dransfield J, Govaerts R, Baker WJ (2016) World checklist of bamboos and rattans. INBAR Technical Report, No. 37. International Network of Bamboo & Rattan, Beijing, 1–454.

Wang QL (2010) Study on shoot emergence and nursing technologies of *Acidosasa gigantea*. Linye Gongcheng Xuebao 24(1): 98–100.

Wen TH (1983) Studies of bamboo genus *Sinobambusa* from China and some other species. Journal of Bamboo Research 2(1): 57–86.

Wen TH (1988) Four new species and some combinations of bamboos. Journal of Bamboo Research 7(1): 23–31.

Wen TH (1991) Some ideas on the taxonomy of several Bambusoideae taxa. Journal of Bamboo Research 10(1): 11–25.

Wen TH, Chen SY, Fang W, Yao CY, Ceng XY (1993) Graminae (Bambusoideae). In: Lin Q (Ed.) Flora of Zhejiang (Vol. 7). Zhejiang Science and Technology Publishing House, Hangzhou, 28–109.

Xie QZ, Chen XY (1993) Two new combinations of Bambusoideae. Bulletin of Botanical Research 13(1): 73–75.

Yang BM (1981) A new species of *Acidosasa* from Hunan. Journal of Hunan Teacher's College, Nature Science Editions 2: 53–55.

Zeng CX, Zhang YX, Triplett JK, Yang JB, Li DZ (2010) Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of molecular divergence. Molecular Phylogenetics and Evolution 56(2): 821–839. https://doi.org/10.1016/j.ympev.2010.03.041

Zhang YX, Zeng CX, Li DZ (2012) Complex evolution in Arundinarieae (Poaceae: Bambusoideae): Incongruence between plastid and nuclear GBSSI gene phylogenies. Molecular Phylogenetics and Evolution 63(3): 777–797. https://doi.org/10.1016/j.ympev.2012.02.023

Zhang C, Rabiee M, Sayyari E, Mirarab S (2018) Astral-III: Polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinformatics 19(S6): 153. https://doi.org/10.1186/s12859-018-2129-y

Zhao HS, Gao ZM, Wang L, Wang JL, Wang SB, Fei BH, Chen CH, Shi CC, Liu XC, Zhang HL, Lou YF, Chen LF, Sun HY, Zhou XQ, Wang SN, Zhang C, Xu H, Li LC, Yang YH, Wei YL, Yang W, Gao Q, Yang HM, Zhao SC, Jiang ZH (2018) Chromosome-level reference genome and alternative splicing atlas of moso bamboo (*Phyllostachys edulis*). GigaScience 7(10): giy115. https://doi.org/10.1093/gigascience/giy115

Zheng QF, Lin YM (1995)) Graminae-Bambusoideae. In: Ling LK, Chang YT (Eds) Flora Fujianica (Vol. 6). Fuzhou: Fujian Science and Technology Press, 29–95.

Zheng YS, Yang DM, Rong JD, Chen LG, Zhu Q, He TY, Chen LY, Ye J, Fan LL, Gao YB, Zhang HX, Gu LF (2022) Allele-aware chromosome-scale assembly of the allopolyploid genome of hexaploid Ma bamboo (*Dendrocalamus latiflorus* Munro). Journal of Integrative Plant Biology 64(3): 649–670. https://doi.org/10.1111/jipb.13217

Zhu ZD, Stapleton CMA (2006) *Indosasa*. In: Wu ZY, Raven P (Eds) Flora of China (Vol. 22). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 143–147.

Zhu ZD, Zhao QS (1996) *Indosasa*. In: Geng BJ, Wang ZP (Eds) Flora Reipublicae Popularis Sinicae (Vol. 9). Science Press, Beijing, 204–224.

Supplementary material 1

433 single_copy orthologous gene alignments used for the reconstruction of ASTRAL species tree and two supplementary tables

Authors: Zhengyang Niu

Data type: zip

Explanation note: Here are 433 single_copy orthologous gene alignments used for the reconstruction of ASTRAL species tree, the basic features of a total of 31 plastomes and plastome and nuclear datasets and its corresponding specimen voucher information used in our study.

Link: https://doi.org/10.3897/phytokeys.255.143020.suppl1

PhytoKeys

PhytoKeys 255: 189–201 (2025)
DOI: 10.3897/phytokeys.255.147252

Research Article

Yushania rubrovaginata (Poaceae, Bambusoideae), a new combination for *Sasa rubrovaginata*

Xing Li[1,2,3], Jing-Bo Ni[1,2], Mian Liang[4], Yi-Hua Tong[1,2], Nian-He Xia[1,2]

1 *State Key Laboratory of Plant Diversity and Specialty Crops/Key Laboratory of Digital Botanical Garden of Guangdong Province, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China*
2 *South China National Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China*
3 *Key Laboratory of State Forestry Administration on Tropical Forestry Research, Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, China*
4 *The Administration Center of Guangxi Cenwanglaoshan National Nature Reserve, Baise 530000, China*
Corresponding author: Yi-Hua Tong (yh-tong@scbg.ac.cn)

Academic editor: Wei Lim Goh
Received: 20 January 2025
Accepted: 16 March 2025
Published: 24 April 2025

Citation: Li X, Ni J-B, Liang M, Tong Y-H, Xia N-H (2025) *Yushania rubrovaginata* (Poaceae, Bambusoideae), a new combination for *Sasa rubrovaginata*. PhytoKeys 255: 189–201. https://doi.org/10.3897/phytokeys.255.147252

Abstract

Sasa rubrovaginata is transferred to *Yushania* based on morphological and molecular evidence. The lectotype for *S. rubrovaginata* is designated. Besides, a revised description of this species and a key to the four *Yushania* species distributed in Cenwanlaoshan Mountain are also provided.

Key words: Bamboo, Cenwanlaoshan Mountain, *Sasa*, taxonomy, *Yushania*

Introduction

Sasa Makino & Shibata (1901) is a medium genus of Arundinarieae, Bambusoideae (Zhang et al. 2020a), and characterized by having a shrubby habit, leptomorph rhizomes, single branch per culm node, spikelets arranged into a panicle-like inflorescence, and six stamens and three stigmas per floret (Hu 1996; Wang and Stapleton 2006; Clayton et al. 2016; Yi et al. 2008; Shi et al. 2022). About 43 species are recognized in *Sasa* genus at present, which are mainly distributed in East Asia (Vorontsova et al. 2016; Soreng et al. 2022; Li et al. 2023a; POWO 2025). Previous phylogenetic studies indicated that *Sasa* is polyphyletic (Peng et al. 2008; Sungkaew et al. 2009; Triplett and Clark 2010; Zeng et al. 2010; Zhang et al. 2012; Qin et al. 2021), and most Chinese *Sasa* species were successively revised and transferred to *Sinosasa* L. C. Chia ex N. H. Xia et al., *Yushania* Keng f., or *Pseudosasa* Makino ex Nakai in recent studies (Qin et al. 2021; Li et al. 2022, 2023a, 2023b, 2023c). For now, there are only three accepted names under *Sasa* from China (POWO 2025), viz., *S. subglabra* McClure (1940), *S. hainanensis* C. D. Chu & C. S. Chao (Chao et al. 1980) and *S. rubrovaginata* C. H. Hu (1985).

Sasa rubrovaginata was described based on the only collection, *Nanzhidi* (Phytogeography expeditions of South China Institute of Botany) 5102, from Cenwanglaoshan Mountain in Guangxi, China. Only two duplicates of the type were found in N (Fig. 1). Both duplicates (N019023159 and N019023168) only constitute a leafy branch. No other specimens can be located in the Chinese herbaria. Without any information of rhizome and branch complement, which are very important for the placement of generic position, it was designated as a member of *Sasa*. Thus, in order to ascertain the taxonomic position of *S. rubrovaginata*, it is necessary to recollect complete specimens with rhizome and branch complement from the type locality.

Materials and methods

The complete specimens of *Sasa rubrovaginata* were collected from the type locality, i.e., Cenwanlaoshan Mountain, Langping Town, Tianlin County, Guangxi Zhuang Autonomous Region, China, during a field trip in September 2022. Fresh leaf samples were stored in sealed bags with silica gel for DNA extraction. Magnifier and ruler (0.5 mm scale) were used for observations and measurements. Some minor characters were observed with a stereo microscope (Mshot-MZ101, Guangzhou Micro-shot Technology Co., Ltd, Guangzhou, China). Photos of type specimens of *S. rubrovaginata* were downloaded from the Chinese Virtual Herbarium (https://www.cvh.ac.cn/) for comparison. The description was conducted based on both living and dried materials, and the descriptive terms follow McClure (1966) and Beentje (2016). Herbarium acronyms follow Thiers (2025).

To study the phylogenetic position of *S. rubrovaginata* within the tribe Arundinarieae, phylogenetic analyses were carried out by using the complete chloroplast genome data. A total of 30 representatives from all the five subtribes of the tribe Arundinarieae (Zhang et al. 2020a) were sampled with *Dendrocalamus strictus* (Roxburgh) Nees from the tribe Bambuseae as an outgroup. All the sampled taxa, information of voucher specimens and GenBank accession numbers were listed in Table 1.

DNA extraction, sequencing, assembly and annotation

Total genomic DNA was extracted from dried leaves using the modified CTAB method (Li et al. 2013) and sent to Novo Gene Company (Beijing, China) for DNA assessment. The genomic DNA was then cut up to 350 bp-sized fragments for the construction of the library, and paired-end sequencing was performed on the Illumina Hiseq 4000 platform. A total of 20 G clean data (150 bp read length) were generated from each sample. These clean data were utilized to assemble the plastome by GetOrganelle v.1.7.7 pipeline (Jin et al. 2020) using the plastome of *Phyllostachys edulis* (Carriere) J. Houzeau (accession number: HQ337796) as the reference, with k-mer values of 45, 65, 85, 105, 125, word size of 102, and extension rounds of 20. Bandage software (Wick et al. 2015) was used to visually check if the final result of the assembled genome was circular or not. Finally, the assembled plastome sequence with the same direction as the reference sequence was kept and manually operated in Geneious v. 9.1.4 (Kearse et al. 2012) with the structure of LSC-IRa-SSC-IRb.

Phylogenetic analysis

By using MAFFT v. 7.490, all of the complete chloroplast genomes were concatenated into a data matrix after being aligned. Maximum Likelihood (ML) and

Table 1. A list of the vouchers and GenBank accession numbers for the sampled species in this study.

Taxon	Voucher information	Accession number
Ingroup		
Acidosasa glauca B.M.Yang	CZY56 (IBSC)	OP850353
Ampelocalamus actinotrichus (Merr. & Chun) S.L.Chen, T.H.Wen & G.Y.Sheng	MPF10003 (KUN)	MF066245
Chimonobambusa tumidissinoda Ohrnb.	MPF10083 (KUN)	MF066244
Fargesia albocerea J.R.Xue & T.P.Yi	ZhangYu-QuD588 (SANU)	NC_043891
Fargesia communis T.P.Yi	ZhangYu-QuD540 (SANU)	NC_043934
Fargesia daminiu T.P.Yi & J.Y.Shi	ZhangYu-QuG660 (SANU)	NC_043942
Fargesia hygrophila J.R.Xue & T.P.Yi	ZhangYu-QuD552 (SANU)	NC_043938
Fargesia setosa T.P.Yi	ZhangYu-QuG695 (SANU)	NC_043939
Gaoligongshania megalothyrsa (Hand.-Mazz.) D.Z.Li, Hsueh & N.H.Xia	MPF10056 (KUN)	JX513419
Gelidocalamus stellatus T.H.Wen	BH102 (IBSC)	OP850347
Hsuehochloa calcareus (C.D.Chu & C.S.Chao) D.Z.Li & Y.X.Zhang	MPF10050 (KUN)	KJ496369
Indocalamus longiauritus Hand.-Mazz.	MPF10168 (KUN)	HQ337795
Indocalamus sinicus (Hance) Nakai	ZMY037 (KUN)	MF066250
Indosasa crassiflora McClure	BH58 (IBSC)	OK558536
Oligostachyum sulcatum Z.P.Wang & G.H.Ye	Not provided by the author	MW190089
Phyllostachys edulis (Carriere) J.Houzeau	MPF10163 (KUN)	HQ337796
Pleioblastus maculatus (McClure) C.D.Chu & C.S.Chao	CZY56 (IBSC)	JX513424
Pseudosasa cantorii (Munro) Keng f.	MPF10006 (KUN)	MF066255
Pseudosasa japonica (Siebold & Zucc. ex Steud.) Makino ex Nakai	Pjc-1 (ZJFC)	KT428377
Ravenochloa wilsonii (Rendle) D.Z.Li & Y.X.Zhang	MPF10146 (KUN)	JX513421
Sasa rubrovaginata C.H.Hu	LX178 (IBSC)	PQ010623
Sasa veitchii (Carriere) Rehder	LC1325 (ISC)	KU569975
Shibataea chiangshanensis T.H.Wen	ZLN-2011080 (KUN)	MF066257
Sinosasa fanjingshanensis N.H.Xia, Q.M.Qin & J.B.Ni	BH124 (IBSC)	OP850348
Sinosasa longiligulata (McClure) N.H.Xia, Q.M.Qin & J.B.Ni	CZY163 (IBSC)	OP850351
Sinobambusa tootsik (Makino) Makino ex Nakai	NH031 (IBSC)	OP850357
Yushania confusa (McClure) Z.P.Wang & G.H.Ye	ZhangYu-QuF642 (SANU)	NC_043893
Yushania maculata T.P.Yi	Not provided by the author	OR750784
Yushania niitakayamensis (Hayata) Keng f.	Not provided by the author	MN310560
Yushania shuichengensis T.P.Yi & L.Yang	Not provided by the author	OR750781
Outgroup		
Dendrocalamus strictus (Roxburgh) Nees	zmy018 (KUN)	MK679802

Bayesian Inference (BI) tools in the PhyloSuite v.1.2.3 platform (Xiang et al. 2023; Zhang et al. 2020b) were utilized for phylogenetic reconstructions. The Bayesian Information Criterion (BIC) in ModelFinder (Kalyaanamoorthy et al. 2017) was used to identify the optimal substitution model for ML and BI methods. Maximum likelihood phylogenies were inferred by using IQ-TREE v 2.2.0 (Nguyen et al. 2015) under the best-fit K81u+R4+F model. We then performed 1000 ultrafast bootstrap replicates and 1000 approximate likelihood ratio (SH-aLRT) tests to assess branch supports (Guindon et al. 2010). Bayesian Inference phylogenies were inferred using MrBayes v 3.2.7 (Ronquist et al. 2012) under the GTR+I+G+F model (2 parallel runs, 40,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in. ML and BI trees were visualized in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The real identity of *Sasa rubrovaginata*

According to the protologue and field note of the type specimens of *Sasa rubrovaginata*, the gathering was collected at an elevation of 2000 m at Cenwangling (岑王岭) (the main peak of Cenwanlaoshan), Langping (浪平), Tianlin County (田林县). During our field trip to Cenwanlaoshan Mountain, only one bamboo species that has a long-necked pachymorph rhizome and two or three branches on the mid or upper culm nodes were found at the elevation from 1968 m to 2062.5 m (the peak's elevation). The young and unbranched culms with foliage leaves at the apex (Fig. 2) fully matched with the protologue and the type specimens (Fig. 1) of *S. rubrovaginata* as well as the glabrous internodes, white powdery infranodal region, purple-red culm leaf sheath with a hispid base and ciliate margins, caducous culm leaf blades, truncate culm leaf ligules, 5–6 foliage leaves per ultimate branch with the glabrous sheath being ciliate on margins, falcate foliage leaf auricles with developed oral setae, truncate foliage leaf inner ligules, and glabrous foliage leaf blades with conspicuous transverse veins and 5–6 pairs secondary veins. Thus, we are very sure that the specimens we collected are *S. rubrovaginata*. Hu (1985) should take the young and unbranched culm with foliage leaves at the apex as a leafy branch, and supposed that the bamboo possessed solitary branch complement and leptomorph rhizome, just like the case of *S. tomentosa* C. D. Chu & C. S. Chao (≡ *Yushania tomentosa* (C. D. Chao & C. S. Chao) N. H. Xia et al.; see Li et al. 2023a for nomenclatural revision). Owing to the long-necked rhizome (Fig. 3G) and branch complement with mostly solitary branch at lower culm nodes and two to three (Fig. 3C) branches at mid and upper culm nodes, it should be a member of *Yushania* rather than *Sasa*.

After examining the type specimens and referring to the related literature (Yi et al. 2008; Shi et al. 2022; Li et al. 2023a), *S. rubrovaginata* is mostly similar to *Y. tomentosa* by sharing the branch complement with usually solitary branch at lower culm nodes and two to three branches at mid and upper culm nodes (Fig. 3C), the glabrous internodes with purple spots, the culm leaf with sheath being ciliate on margins and leaving persistent remains on internode when falling off, truncate ligule, falcate auricles with radiate oral setae (Fig. 3F), and foliage leaf with sheath being ciliate on margins, falcate auricles with radiate oral setae (Fig. 3D), truncate ligule and glabrous blades, but differs in having purple-red (vs. green to brown) and densely brown hispid (Fig. 3E) (vs. white to yellowish-brown hirsute) culm leaf sheath, and glabrous culm leaf ligules margin (vs. white ciliolate), foliage leaf sheath (vs. densely white hirsute), outer ligule margin (vs. white ciliate) and pseudopetiole (vs. white puberulous). A more detailed comparison of the two species is presented in Table 2.

Phylogenetic analysis

The chloroplast genomes of the sampled species vary from 139,404 bp (*Dendrocalamus strictus*) to 140,064 bp (*Gaoligongshania megalothyrsa* (Hand.-Mazz.) D. Z. Li, Hsueh & N. H. Xia) with an alignment of 144,047 bp. Sequence divergence was observed in this data matrix with 3,903 variable sites (2.71%) comprising 3,087 singleton variable sites (2.14%) and 816 parsimony informative

Table 2. Comparison of *Sasa rubrovaginata* and *Yushania tomentosa*.

Characters	***Sasa rubrovaginata***	***Yushania tomentosa***
Culm leaf		
Sheath	Purple-red when fresh, densely brown hispid	Green to brown when fresh, densely white to yellowish-brown hirsute
Ligule	Glabrous	White ciliolate on the margin
Foliage leaf		
Sheath	Glabrous, initially white powdery	Densely white hirsute
Pseudopetiole	Glabrous	White puberulous
Outer ligule	Glabrous	White ciliate on the margin

sites (0.57%). Only the ML tree was displayed (Fig. 4) with nodal support values from both ML and BI methods labeled on each node. As shown in the phylogenetic tree, *S. rubrovaginata* is distantly related to *S. veitchii* (Carriere) Rehder (= *S. albomarginata* (Miq.) Makino & Shibata, the type of *Sasa*) but forms a monophyletic clade with four *Yushania* species with strong nodal support (BS = 99.6% & PP = 1.00), which also supports that *S. rubrovaginata* should be a member of *Yushania*, rather than *Sasa*.

Discussion

Based on the morphological and molecular evidence, it is concluded that *Sasa rubrovaginata* represents a distinct species of *Yushania*. Accordingly, a new combination of *S. rubrovaginata* under *Yushania* should be made. Hu (1985) designated the collection *Nanzhidi 5102* (N) as the type of *S. rubrovaginata*, but there are two duplicates in N, which should be recognized as syntypes. We designated N019023168 (Fig. 1A) which bears some pencil annotations in Chinese and “Typus” in the identification slip by the author and has relatively better developed culm and foliage leaves as the lectotype of *S. rubrovaginata*.

Taxonomic treatment

Yushania rubrovaginata **(C. H. Hu) N. H. Xia, Y. H. Tong, J. B. Ni & X. Li, comb. nov.**
urn:lsid:ipni.org:names:77360579-1
Figs 1–3

Basionym. *Sasa rubrovaginata* C. H. Hu, Bamboo Research 2(2): 59 (1985)

Type. China. • Guangxi: Tianlin County, Langping Town, Cenwanlaoshan Mountain, elev. 2000 m, 26 Nov. 1957, *Nanzhidi 5102* (lectotype N019023168!, Fig. 1A, designated here; isolectotype N019023159!, Fig. 1B)

Description. Shrubby bamboo. Rhizomes pachymorph, necks 15–30 cm long, 3–5 mm in diameter, solid. Culms 1–3.5 m tall, 5–8 mm in diameter, diffuse; branches usually solitary at lower culm nodes, 2–3 at mid and upper culm nodes; internodes terete, 10–30 cm long, glabrous, densely purple-spotted, thickly white powdery below nodes, hollow; supranodal ridges raised. Culm buds solitary, long-ovate, yellow to light green, ciliate on the margin. Culm leaf sheaths persistent or tardily deciduous, ca. 1/2 as long as internodes, purple-red, densely brown hispid

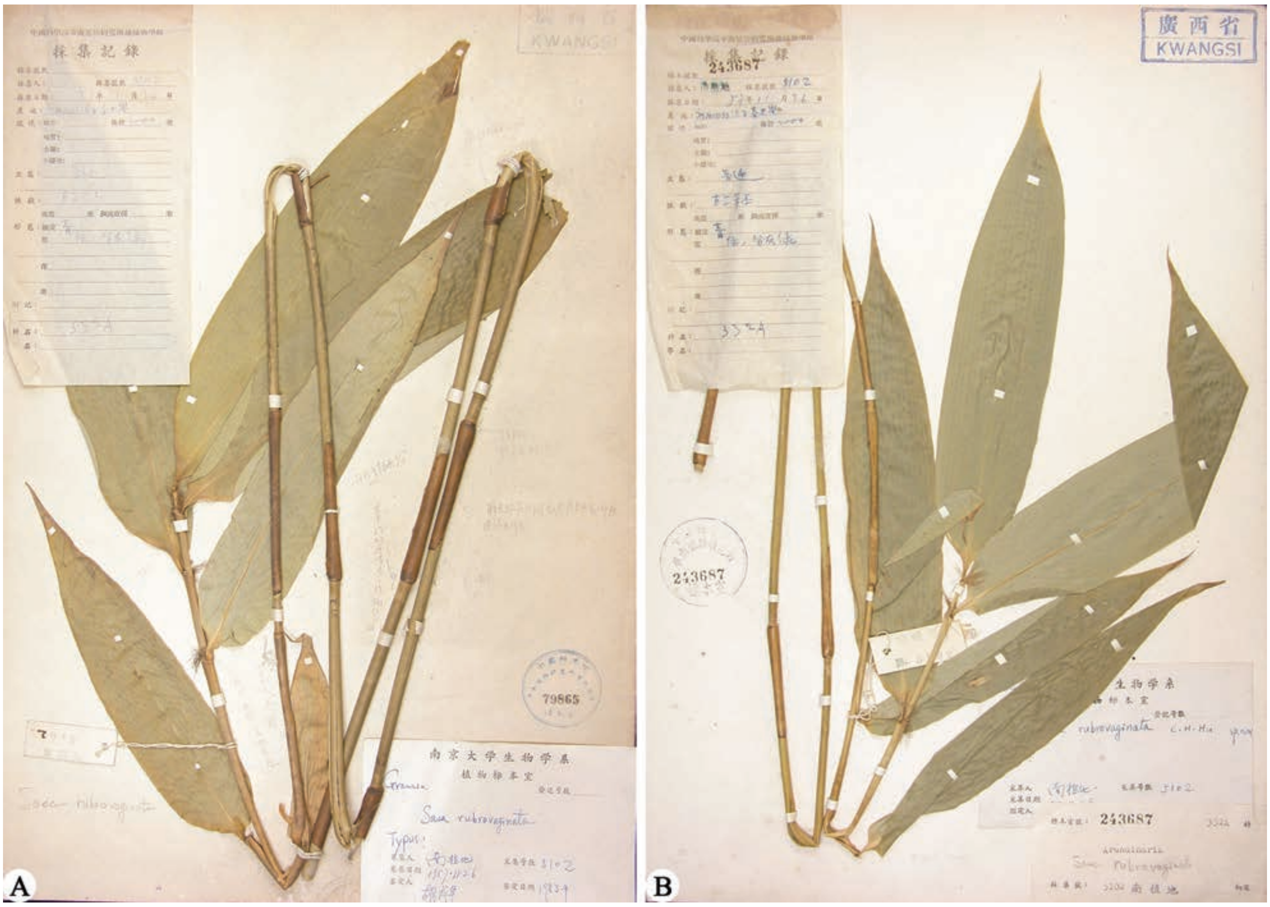

Figure 1. Lectotype (**A**) and isolectotype (**B**) of *Sasa rubrovaginata* C. H. Hu (*Nanzhi 5102*, N **A** N019023168 **B** N019023159). Photos downloaded from Chinese Virtual Herbarium (https://www.cvh.ac.cn/).

abaxially, densely ciliate on the margin; sheath scar prominent, with persistent remains of sheath base; auricles falcate, 3–5 × 1–2 mm; oral setae developed, radiate; ligule truncate, ca. 0.5 mm high; blades linear-lanceolate to lanceolate, reflexed, easily deciduous, margin serrulate. Foliage leaves 5–13 per ultimate branch; sheath glabrous, margin ciliate; auricles falcate, 1–3 × 0.5–1 mm; oral setae radiate, ca. 1 cm long; inner ligule truncate, ca. 1 mm high; outer ligule and pseudopetioles glabrous; blades broad-lanceolate to lanceolate, 17–26 × 3.5–6 cm, wavy when dry, glabrous, apex acuminate, base cuneate to obtuse; secondary veins 9–10 pairs, transverse veins conspicuous. Inflorescence unknown.

Distribution and habitat. It is only found in Cenwang Mountain, Tianlin County, Guangxi, China. It grows on top of mountains at an altitude of 1968 to 2062.5 meters (the peak's elevation).

Phenology. New shoots from August to September.

Chinese name. 红壳玉山竹 (Chinese pronunciation: hóng ké yù shān zhú).

Notes. Hu (1985) described that the culm leaf auricles and oral setae of *Yushania rubrovaginata* are undeveloped (see Fig. 1). In fact, this bamboo does have developed culm leaf auricles and oral setae (Fig. 3E, F). But the culm leaf auricles and oral setae are easy to fall off, which exactly happens to the type specimens of *Y. rubrovaginata*. Similarly, the culm leaf sheaths of the type specimens are glabrous on the mid and upper parts, and only the basal part is sparsely hispid. Actually, *Y. rubrovaginata* has a thoroughly hispid culm leaf sheath, and pits are left after the trichomes fall off (Fig. 3E).

Figure 2. A specimen of *Sasa rubrovaginata* C. H. Hu collected from the type locality, *X.Li & J.B.Ni LX178* (IBSC). Photo by Xing Li.

Wang and Stapleton (2006) treated *Sasa duplicata* W. T. Lin & Z. J. Feng (1992) as a synonym of *Y. rubrovaginata*. However, the former has a leptomorph rhizome, rather than pachymorph rhizome. Actually, *S. duplicata* owns some characters that are the same as *Pseudosasa cantorii* (Munro) P. C. Keng ex S. L. Chen et al. (Zhu et al. 2006), such as glabrous internodes, the white powdery infranodal region, branch complement with one to three branches at each culm node, culm leaf sheath with ciliate margin, arcuate to truncate culm leaf ligules and falcate auricles with developed oral setae, foliage leaf sheath with ciliate margin, truncate ligules, developed oral setae and lanceolate to oblong-lanceolate blades with conspicuous transverse veins. Thus, *S. duplicata* is probably a synonym of *P. cantorii*, but more work needs to be done to ascertain this.

There are another three *Yushania* species, viz., *Y. cartilaginea* T. H. Wen (1984), *Y. chingii* T. P. Yi and *Y. rugosa* T. P. Yi (1986), distributed in Cenwanglaoshan Mountain. *Yushania rubrovaginata* can be distinguishable from them by having branch

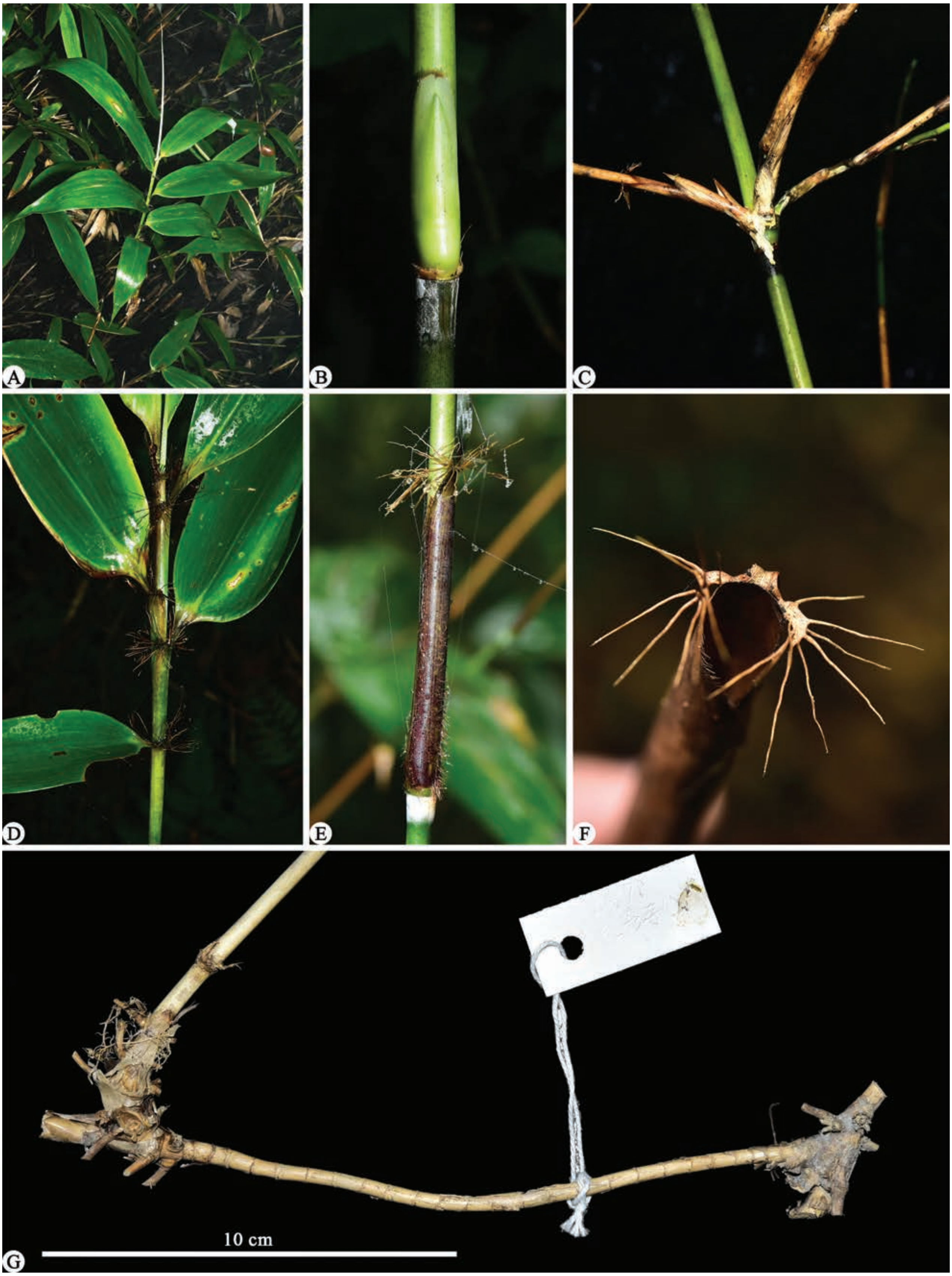

Figure 3. *Yushania rubrovaginata* **A** foliage leafy branch **B** culm bud **C** three branches at an upper culm node **D** partial foliage leafy branch, showing auricles and oral setae **E** culm leaf and white powdery infranodal region **F** apex of culm leaf sheath, showing ligules, auricles and oral setae **G** pachymorph rhizome with long neck. All photos by Xing Li.

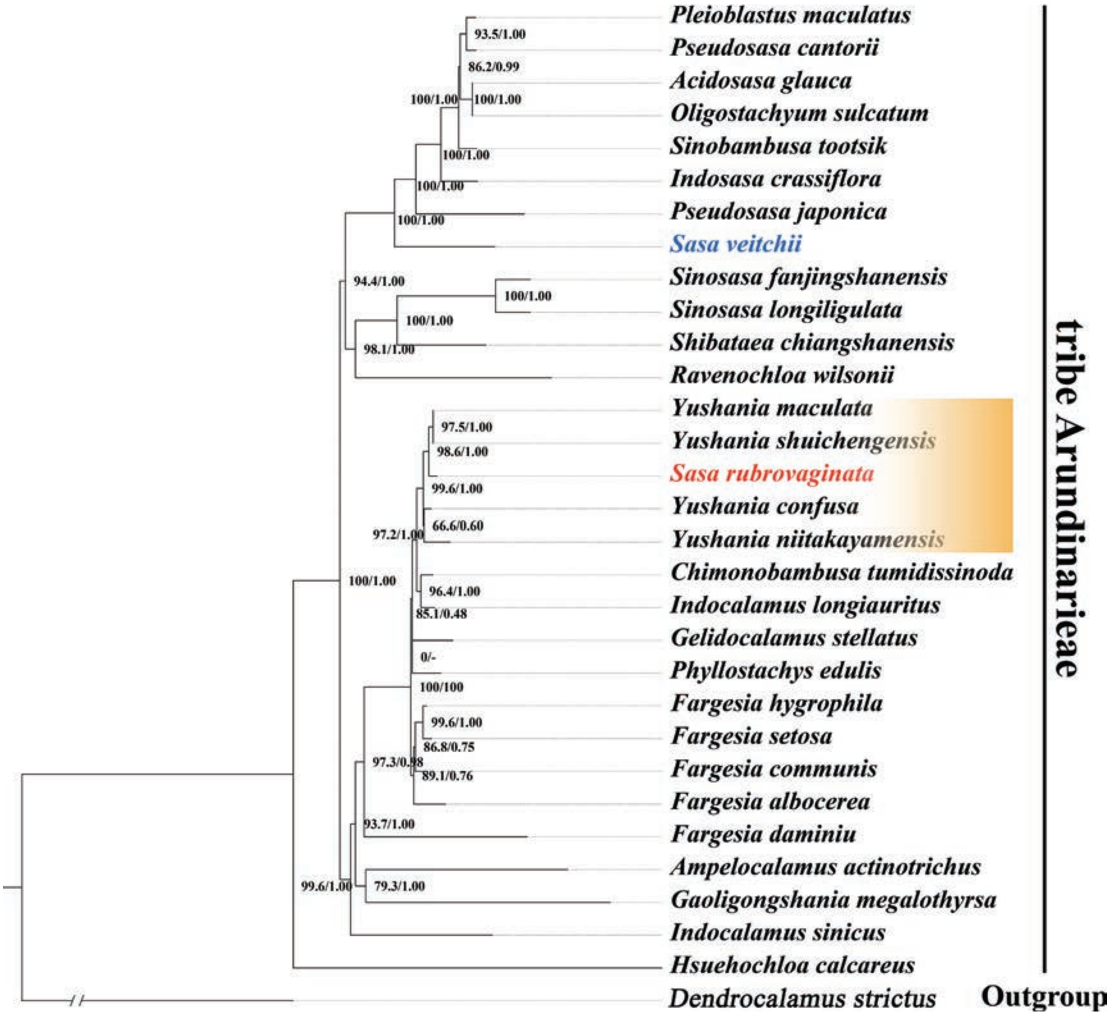

Figure 4. Phylogenetic tree derived from MI and BI methods based on the complete chloroplast sequences, showing the phylogenetic position of *Sasa rubrovaginata*. Bootstrap values and posterior probabilities are indicated at each node, and the type of *Sasa* is highlighted in blue.

complement with the solitary branch at lower culm nodes and two to three branches at mid and upper culm nodes, densely purple-spotted internodes, purple-red and densely brown hispid culm leaf sheath, and foliage leaf with developed and radiate oral setae. A key to these *Yushania* species is provided as follows.

Key to *Yushania* species distributed in Cenwanglaoshan Mountain

1a Branches solitary at each culm node **2**

2a Culm leaf sheath glabrous; auricles developed and falcate; oral setae radiate with trichomes 5–8 mm long .... ***Y. chingii***

2b Culm leaf sheath glabrous or sparsely purple-brown setose at base; auricles absent or small; oral setae absent or weak with trichomes 2–3 mm long... ***Y. rugosa***

1b Branches 1–3 at each culm node **3**

3a Internodes densely purple-spotted; culm leaf sheath purple-red, densely brown hispid; foliage leaf sheath with radiate oral setae .....***Y. rubrovaginata***

3b Internodes green, without purple spots; culm leaf sheath green to brown, glabrous; foliage leaf sheath with straight oral setae........... ***Y. cartilaginea***

Additional specimens examined

Yushania rubrovaginata: China. • Guangxi: Tianlin County, Langping Town, Cenwanglaoshan Mountain, Cenwangling, 25 September 2022, 24°29'22.4"N, 106°24'5.3"E, elev. 2062 m, *X. Li & J. B. Ni LX178* (IBSC).

Yushania tomentosa: China. • Guangxi: Rongshui County, Jiuwan Mountain, elev. 1400 m, 25 August 1958, *S. H. Chun 15320* (isotypes: NAS00070361, image; WUK0211330, image; N019023167, image; IFP15899999w0005, image); • Rongshui County, Wangdong Township, Jiuwan Mountain, Weilinjiang, 23 September 2022, 25°18'39.3"N, 108°38'13.2"E, elev. 1358 m, *X. Li & J. B. Ni LX168* (IBSC).

Yushania cartilaginea: China. • Guangxi: Baise City, [Tianlin County], Kashan [Laoshan = Cenwanlaoshan] forestry station, elev. 1700 m, 9 April 1982, *W. W. Chou L82433* (holotype: ZJFI).

Yushania chingii: China. • Guangxi: Tianlin County, Laoshan forestry station, elev. 1400 m, 14 January 1990, *J. P. Ruan 90005* (N 019025132, image; N 019025139, image; N 019025140, image).

Yushania rugosa: China. • Guizhou: Wangmo County, Maoping community, elev. 1500–1556 m, 26 August 1981, *T. P. Yi 81118* (holotype: SIFS).

Acknowledgments

We thank Mr. Fu-Hong Long from Langping protection station, Cenwanglaoshan Mountain National Nature Reserve for his assistance in the fieldwork.

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 Chinese Academy of Sciences (Biotaxonomic Scientist Post Grant No. CAS-TAX-24-049) and the National Natural Science Foundation of China (Grant No. 32270227).

Author contributions

Funding acquisition: NHX, YHT. Investigation: XL, ML, JBN. Methodology: XL. Supervision: YHT, NHX. Writing - original draft: XL. Writing - review and editing: NHX, YHT.

Author ORCIDs

Xing Li https://orcid.org/0000-0001-7146-0125
Yi-Hua Tong https://orcid.org/0000-0002-5034-005X
Nian-He Xia https://orcid.org/0000-0001-9852-7393

Data availability

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

References

Beentje H (2016) The Kew Plant Glossary, Second Edition. Royal Botanic Gardens, Kew, London, 1–184.

Chao CS, Chu CD, Hsiung WY (1980) A revision of some genera and species of Chinese bamboos. Acta Phytotaxonomica Sinica 18(1): 20–36.

Clayton WD, Vorontsova M, Harman KT, Williamson H (2016) GrassBase-The online world grass flora. https://www.kew.org/data/grassbase/index.html [Accessed 18 January 2025]

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–321. https://doi.org/10.1093/sysbio/syq010

Hu CH (1985) A revision of the genus *Sasa* from China. Bamboo Research 2(2): 56–62.

Hu CH (1996) *Sasa* Makino et Shibata. In: Keng PC, Wang ZP (Eds) Flora Reipublicae Popularis Sinicae (Vol. 9). Science Press, Beijing, 662–675.

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 T, 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

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford, England) 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Li JL, Wang S, Yi J, Wang L, Zhou SL (2013) A modified CTAB protocol for plant DNA extraction. Chinese Bulletin of Botany 48(1): 72–78. https://doi.org/10.3724/SP.J.1259.2013.00072

Li X, Tong YH, Xia NH (2022) *Sasa guangdongensis*, a new synonym of *Acidosasa carinata* (Poaceae: Bambusoideae). Phytotaxa 568(2): 204–212. https://doi.org/10.11646/phytotaxa.568.2.5

Li X, Ni JB, Tan F, Tong YH, Xia NH (2023a) *Yushania tomentosa* (Poaceae, Bambusoideae), a new combination from Guangxi. PhytoKeys 218: 11–18. https://doi.org/10.3897/phytokeys.218.97312

Li X, Ni JB, Xia NH (2023b) The identity of *Sasa oblongula* C. H. Hu (Poaceae, Bambusoideae, Arundinarieae): Evidence from morphology and molecular data. PhytoKeys 226: 17–32. https://doi.org/10.3897/phytokeys.226.101221

Li X, Ni JB, Cai ZY, Tong YH, Xia NH (2023c) *Sinosasa gracilis* (Poaceae: Bambusoideae), a new combination supported by morphological and phylogenetic evidence. PhytoKeys 226: 53–63. https://doi.org/10.3897/phytokeys.226.101164

Lin WT, Feng ZJ (1992) Six new bamboo species from Guangdong. Acta Phytotaxonomica Sinica 30(6): 561–562.

Makino T, Shibata K (1901) On *Sasa*, a new genus of Bambuseae, and its affinities. Botanical Magazine Tokyo 15(168): 18–31. https://doi.org/10.15281/jplantres1887.15.168_18

McClure FA (1940) New genera and species of Bambusaceae from eastern Asia. Lingnan University Science Bulletin 9: 1–67.

McClure FA (1966) The bamboos: A fresh perspective. Harvard University Press, Cambridge, 1–347. https://doi.org/10.4159/harvard.9780674428713

Nguyen LT, Schmidt HA, Haeseler V, 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

Peng S, Yang HQ, Li DZ (2008) Highly heterogeneous generic delimitation within the temperate bamboo clade (Poaceae: Bambusoideae): evidence from GBSSI and ITS sequences. Taxon 57(3): 799–810. https://doi.org/10.1002/tax.573011

POWO (2025) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:18942-1#children [accessed 9 Jan 2025]

Qin QM, Tong YH, Zheng XR, Ni JB, Xia NH (2021) *Sinosasa* (Poaceae: Bambusoideae), a new genus from China. Taxon 70(1): 27–47. https://doi.org/10.1002/tax.12422

Ronquist F, Teslenko M, Mark VDP, 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

Shi JY, Che BY, Zhang YX, Zhou DQ, Ma LS, Yao J, Zhang XZ (2022) *Yushania* Keng f. In: Shi JY, Zhang YX, Zhou DQ, Ma LS, Yao J (Eds) Illustrated Flora of Bambusoideae in China (Vol. 2). Science Press, Beijing & Springer Nature Singapore Pte Ltd., Singapore, 409–481. https://doi.org/10.1007/978-981-16-2758-3_43

Soreng RJ, Peterson PM, Zuloaga FO, Romaschenko K, Clark LG, Teisher JK, Gillespie LJ, Barberá P, Welker CAD, Kellogg EA, Li DZ, Davidse G (2022) A worldwide phylogenetic classification of the Poaceae (Gramineae) III: An update. Journal of Systematics and Evolution 60(3): 476–521. https://doi.org/10.1111/jse.12847

Sungkaew S, Stapleton C, Salamin N, Hodkinson TR (2009) Non-monophyly of the woody bamboos (Bambuseae; Poaceae): A multi-gene region phylogenetic analysis of Bambusoideae s.s. Journal of Plant Research 122(1): 95–108. https://doi.org/10.1007/s10265-008-0192-6

Thiers B (2025) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. http://sweetgum.nybg.org/ih [Accessed 8 January 2024]

Triplett JK, Clark LG (2010) Phylogeny of the temperate bamboos (Poaceae: Bambusoideae: Bambuseae) with an emphasis on Arundinaria and allies. Systematic Botany 35(1): 102–120. https://doi.org/10.1600/036364410790862678

Vorontsova MS, Clark LG, Dransfield J, Govaerts R, Baker WJ (2016) World checklist of bamboos and rattans. INBAR Technical Report No. 37. International Network of Bamboo & Rattan, Beijing, 1–454.

Wang ZP, Stapleton CMA (2006) *Sasa* Makino & Shibata. In: Wu ZY, Raven P (Eds) Flora of China (Vol. 22). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 109–112.

Wen TH (1984) New taxa of Bambusoideae in China (I). Journal of Bamboo Research 3(2): 28–29.

Wick RR, Schultz MB, Justin Z, Holt KE (2015) Bandage: Interactive visualization of de novo genome assemblies. Bioinformatics (Oxford, England) 31(20): 3350–3352. https://doi.org/10.1093/bioinformatics/btv383

Xiang CY, Gao FL, Jakovlić I, Lei HP, Hu Y, Zhang H, Zou H, Wang GT, Zhang D (2023) Using phyloSuite for molecular phylogeny and tree-based analyses. iMeta 2(1): e87. https://doi.org/10.1002/imt2.87

Yi TP (1986) Studies on the genus *Yushania*. Journal of Bamboo research 5(1): 45–64.

Yi TP, Shi JY, Ma LS, Wang HT, Yang L (2008) Iconographia Bambusoidearum Sinicarum. Science Press, Beijing, 1–766.

Zeng CX, Zhang YX, Triplettd JK, Yang JB, Li DZ (2010) Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of molecular divergence. Molecular Phylogenetics and Evolution 56(2): 821–839. https://doi.org/10.1016/j.ympev.2010.03.041

Zhang YX, Zeng CX, Li DZ (2012) Complex evolution in Arundinarieae (Poaceae: Bambusoideae): Incongruence between plastid and nuclear GBSSI gene phylogenies. Molecular Phylogenetics and Evolution 63(3): 777–797. https://doi.org/10.1016/j.ympev.2012.02.023

Zhang YX, Guo C, Li DZ (2020a) A new subtribal classification of Arundinarieae (Poaceae, Bambusoideae) with the description of a new genus. Plant Diversity 42(3): 127–134. https://doi.org/10.1016/j.pld.2020.03.004

Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020b) 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

Zhu ZD, Li DZ, Stapleton CMA (2006) *Pseudosasa* Makino ex Nakai. In: Wu ZY, Raven P (Eds) Flora of China (Vol. 22). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 115–121.

PhytoKeys 255: 203–213 (2025)
DOI: 10.3897/phytokeys.255.142428

Research Article

Prunus zhuxiensis (Rosaceae), a new species from Hubei, China

Qi-liang Gan[1], Wen-bin Xu[2], Xin-wei Li[2]

1 College of Pharmacy, Hubei University of Chinese Medicine, Wuhan 430065, Hubei, China
2 Wuhan Botanical Garden, the Chinese Academy of Sciences, Wuhan 430074, Hubei, China
Corresponding author: Xin-wei Li (lixinwei@wbgcas.cn)

Abstract

In the present paper, we describe a new species, *Prunus zhuxiensis* (*P.* subg. *Cerasus*), from Hubei, China, based on long-term field observations. This species closely resembles *P*. *serrulata* in having corymbose-racemose or subumbellate inflorescences, hairy petiole, pedicel, involucral bracts and black drupes. However, *P. zhuxiensis* differs distinctly from *P. serrulata* by its sweet edible drupes (versus bitter, inedible drupes in *P. serrulata*), stipules 4-lobed at the base (versus linear stipules in *P. serrulata*), smaller bracts, shorter pedicels and styles pilose at the base (versus glabrous styles in *P. serrulata*). Furthermore, molecular phylogenetic analyses indicate that *P. zhuxiensis* and *P. serrulata* are placed in separate clades, supporting their distinction.

Key words: China, Hubei, *Prunus zhuxiensis*, taxonomy

Introduction

Shi et al. (2013) re-defined the infrageneric relationships of *Prunus* L., based on molecular phylogenetic analyses. Currently, about 65 species are recognized within *P.* subg. *Cerasus* (Mill.) A.Gray, of which 44 species occur in China (Su et al. 2021; Yi et al. 2024b). Some new species within this subgenus have been reported from China in recent years (e.g. Xu et al. (2022); Liang et al. (2023); Yi et al. (2024a)). However, the phylogeny of *Prunus* subg. *Cerasus* inferred from chloroplast and/or nuclear genomic data (Hodel et al. 2021; Shen et al. 2023; Su et al. 2023) only partially corresponds with previously proposed morphological sectional classifications (Yu and Li 1986; Wang 2014) and several cases have also been detected in other lineages of Rosaceae, including the tribe Maleae (Liu et al. 2019, 2020, 2022, 2023; Jin et al. 2023, 2024; Wang et al. 2024) and the tribe Potentilleae (Xue et al. 2023).

During a botanical expedition conducted a few years ago in Zhuxi, Hubei, China, we encountered an interesting species of *Prunus* subg. *Cerasus*. After careful and detailed observations, we concluded that it represents a species new to botanical sciences, which we formally describe herein.

Academic editor: Bin-Bin Liu
Received: 23 November 2024
Accepted: 2 April 2025
Published: 24 April 2025

Citation: Gan Q-liang, Xu W-bin, Li X-wei (2025) *Prunus zhuxiensis* (Rosaceae), a new species from Hubei, China. PhytoKeys 255: 203–213. https://doi.org/10.3897/phytokeys.255.142428

Materials and methods

We collected specimens and took pictures of the new species in Zhuxi, Hubei, China. The specimens of the new species were deposited at the Herbarium of Wuhan Botanical Garden, CAS (HIB) and PE (China National Herbarium). We also checked the specimens at HIB, PE and CVH (Chinese Virtual Herbarium, https://www.cvh.ac.cn/) of *Prunus* subg. *Cerasus*. We carried out morphological comparisons in the field and herbaria. The specimens were observed with dissecting microscopes.

The complete chloroplast genome of the new species *Prunus zhuxiensis* (GenBank accession number: PV208095) was assembled using Getorganelle 1.7.7.0 (Jin et al. 2020) with Illumina genomic DNA sequencing data. We also downloaded chloroplast genomes of other *Prunus* species and two outgroup species (*Malus domestica* (Suckow) Borkh. and *Spiraea martini* H.Lév.). Before constructing phylogenetic trees, the chloroplast genomes were aligned with MAFFT 7.520 (Rozewicki et al. 2019) and the alignment was trimmed with Gblocks (Talavera and Castresana 2007) in Phylosuit 1.2.3 (Zhang et al. 2020). The Maximum Likelihood (ML) phylogenetic trees were generated with IQ-TREE 2.2.0 (ultrafast bootstrap 10000 replicates) (Nguyen et al. 2015) in Phylosuit 1.2.3 (Zhang et al. 2020). The nucleotide substitution model was determined with ModelFinder 2.2.0 (Kalyaanamoorthy et al. 2017) in Phylosuit 1.2.3 (Zhang et al. 2020) and then Bayesian Inference (BI) was performed using MrBayes 3.2.7 (10,000,000 generations; Ronquist et al. (2012)).

Taxonomic treatment

***Prunus zhuxiensis* Q.L.Gan, W.B.Xu & X.W.Li, sp. nov.**
urn:lsid:ipni.org:names:77360580-1
Figs 1–3

Diagnosis. *Prunus zhuxiensis* is similar to *P. serrulata* Lindl. (Li and Bartholomew 2003; Yi et al. 2024b) in its hairy pedicel and involucral bracts, corymbose-racemose or subumbellate inflorescences and black drupes, but the flowers of *P. zhuxiensis* appear before the leaves (at the same time as leaves in *P. serrulata*) and have reflexed sepals half as long as the hypanthium (spreading sepals up to as long as hypanthium in *P. serrulata*) and style pilose at the base (glabrous in *P. serrulata*), the fruits of *P. zhuxiensis* are sweet and edible, while those of *P. serrulata* bitter and inedible.

Type. China • Hubei Province, Zhuxi County, Quanxi Town, Baguashan Forest Farm, Hengduanshan, 32°3'50"N, 109°39'25"E, alt. 780 m, 15 March 2023, *Q.L. Gan 23-1-1* (***holotype*:** HIB [barcode 0342513!]; ***Isotypes***, PE [barcodes 02553525!, 02553526!]).

***Paratypes*:** • ibidem, alt. 780 m, 15 March 2023, *Q.L. Gan 23-1-2* (HIB [barcode 0346560!]), *Q.L. Gan 23-1-3* (HIB [barcode 0346559!]), *Q.L. Gan 23-1-4* (HIB [barcode 0346563!]); • ibidem, alt. 750 m, 26 April 2023, *Q.L. Gan 23-2-1* (HIB [barcode 0346561!]), *Q.L. Gan 23-2-2* (HIB [barcode 0346562!]), *Q.L. Gan 23-2-3* (HIB [barcode 0346564!]).

Description. Trees, deciduous, 8–12 m tall, bark grey, lenticels elliptic or long elliptic, sparsely transversely arranged. Young branchlets purple or green, densely grey pubescent. Leaf blades narrowly obovate, obovate or elliptic,

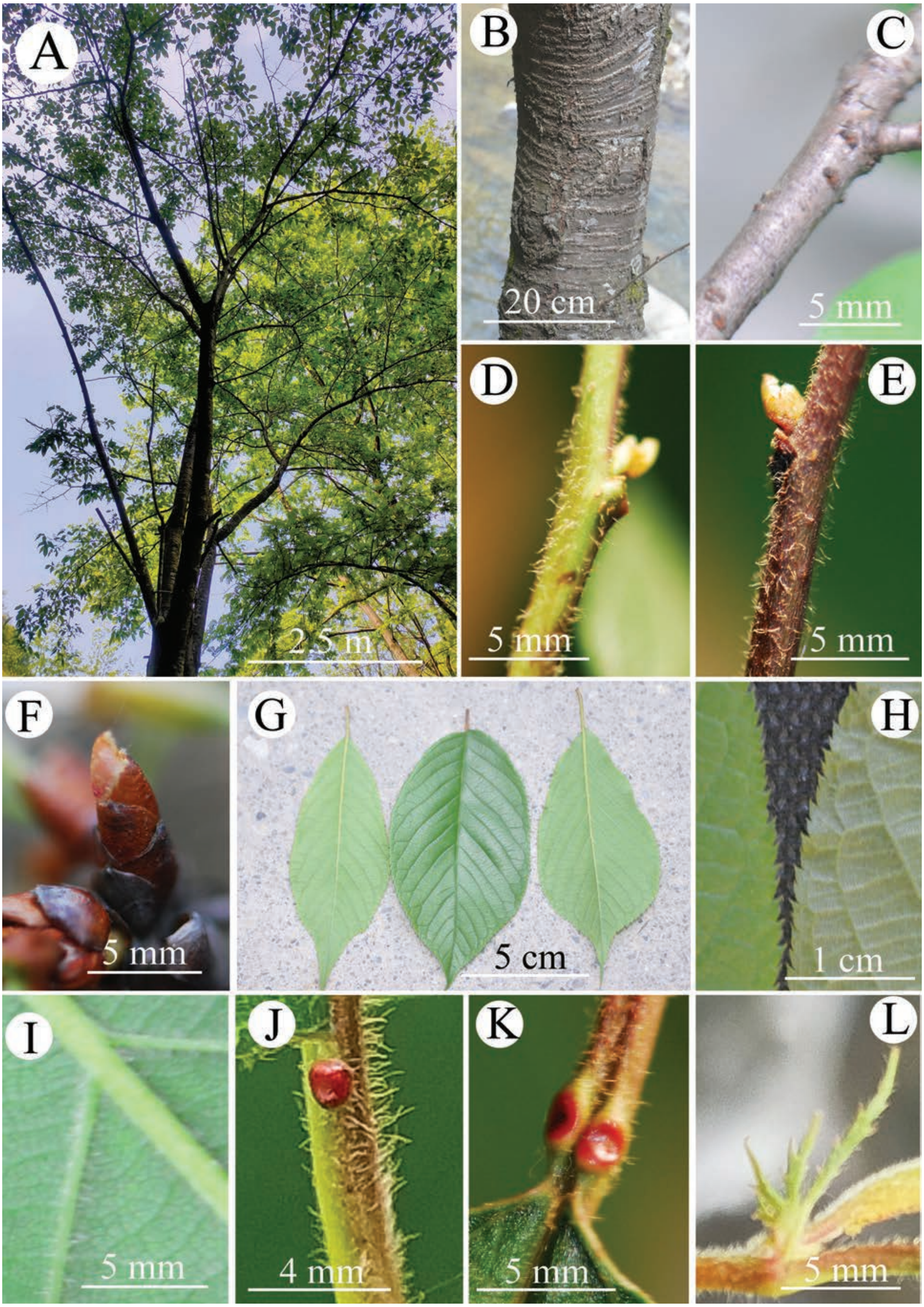

Figure 1. Vegetative characters of *P. zhuxiensis* **A** crown **B** trunk **C** old branch **D** branchlet **E** branchlet **F** winter bud **G** leaf blades **H** teeth at leaf margin **I** hairs on lower surface of a leaf **J** hairs on a petiole **K** glands at apex of a petiole **L** stipule.

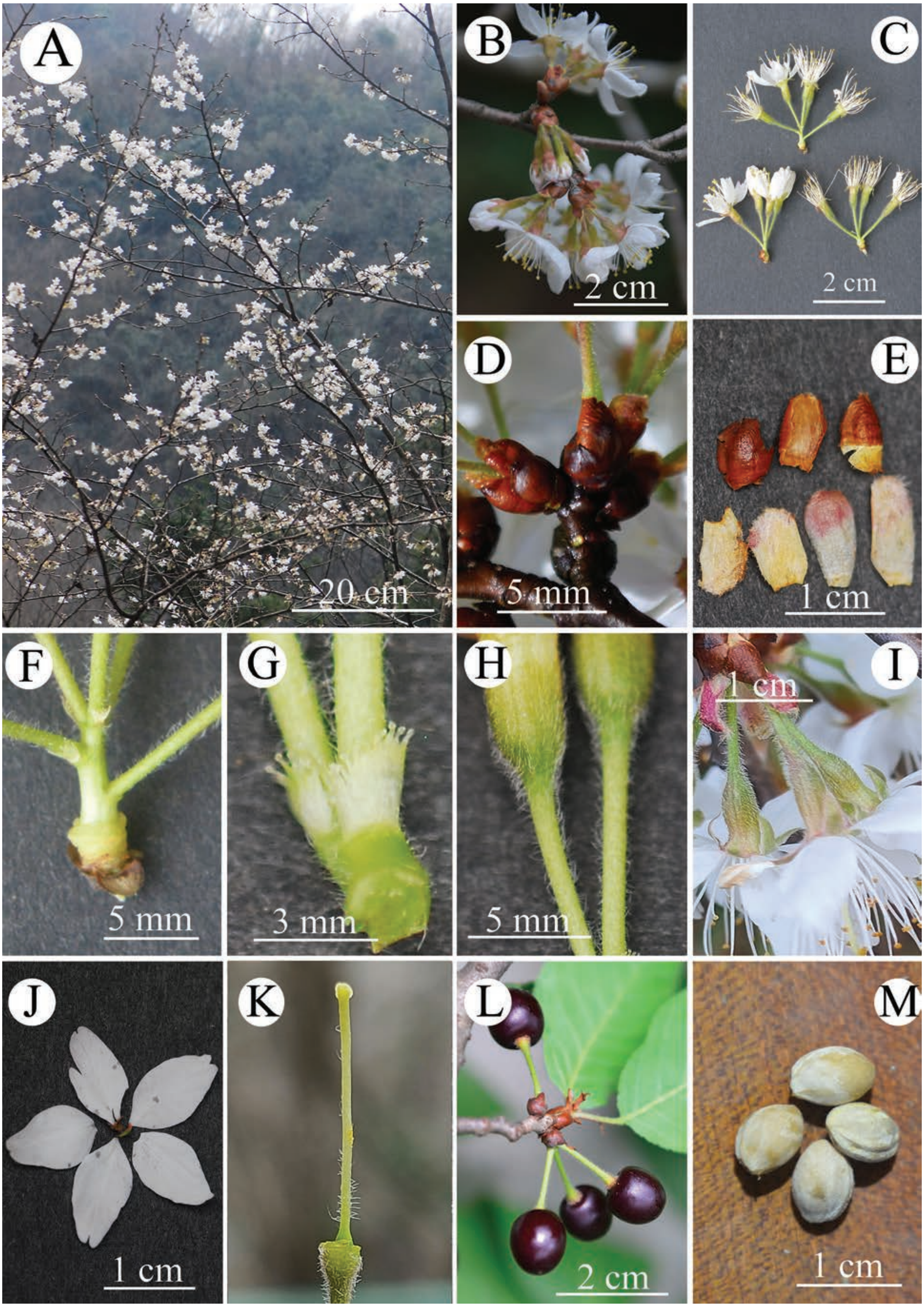

Figure 2. Reproductive characters of *P. zhuxiensis* **A** flowering branches **B** inflorescences **C** inflorescences **D** involucre **E** involucral bracts **F** peduncle **G** bracts **H** hypanthium **I** hypanthium and bracts **J** petals **K** ovary and style **L** drupes **M** endocarps.

Figure 3. The holotype of *P. zhuxiensis*.

3–12 × 1.5–5.5 cm, base cuneate or rounded, apex caudate or acuminate, margin crenately serrate or biserrate, teeth minute, tipped with apical glands, adaxially glabrous, abaxially white pilose on veins. Secondary veins 8–12 on each side. Petiole 10–14 mm, pubescent, with two purple disciform glands at upper part. Stipules 4-lobed at base, lobes margin capitate gland tipped, laciniato-fimbriate. Flowers emerging before leaves. Involucral bracts ovate, oblong, 4–7 × 3.5–4.5 mm, densely pubescent adaxially. Inflorescences umbellate or corymbose-racemose, 3–5-flowered. Peduncles short or absent. Pedicel 7–12 mm, pubescent. Bracts greenish-white, obovate, 1.5–2.5 × 1.5–2.5 mm, conical glandular serrate at apex. Hypanthium tabulate or campanulate, 6–7 × 2–3 mm, pubescent abaxially. Sepals ovate-lanceolate, 2–3 × 0.8–1 mm, reflexed. Styles 12–13 mm, pilose at base. Petals ovate or narrowly ovate, white, 10–13 × 6–7 mm, bifid at apex. Stamens 38–44, filaments up to 12 mm. Drupes globose or ovoid, black, 8–9 mm long, sweet, edible. Endocarp flat ovoid, 7–8 mm long, smooth.

Phenology. Flowering in March, fruiting in May.

Distribution and habitat. *P. zhuxiensis* is distributed sparsely in the mixed evergreen and deciduous broad-leaved forest in the mountains or along streams at altitudes 600–1500 m around the type locality. The main accompanying species of *P. zhuxiensis* are *Salix wilsonii* Seemen ex Diels, *Sycopsis sinensis* Oliv., *Camellia cuspidata* (Kochs) H.J.Veitch, *Phoebe zhennan* S.K.Lee & F.N.Wei, *Juglans mandshurica* Maxim., *Albizia julibrissin* Durazz., *Pterocarya stenoptera* C.DC., *Cornus kousa* subsp. *chinensis* (Osborn) Q.Y.Xiang, *Photinia beauverdiana* C.K.Schneid etc.

Etymology. The specific epithet *"zhuxiensis"* refers to the type locality, Zhuxi, Hubei, China. The Chinese name of this species is Zhuxiyingtao (Pinyin).

Notes. *P. zhuxiensis* co-occurs with *P. serrulata* in the same plant community. They are similar in their hairy pedicel and involucral bracts and black drupes. However, these two species can be distinguished by the pilose style base of *P. zhuxiensis* (which is glabrous in *P. serrulata*) and the sweet and edible drupes of *P. zhuxiensis* (compared to the bitter and inedible drupes of *P. serrulata*). The morphological differences are shown in Table 1, Suppl. material 1.

Molecular phylogeny. Both the BI and ML molecular trees (Fig. 4, Suppl. material 2) demonstrate the monophyly of *Prunus* subg. *Cerasus*, which is consistent with Shen et al. (2023). Shen et al. (2023) split the subg. *Cerasus* into seven lineages (Clades IIIa–IIIg) and the species of each of these lineages also cluster together in our phylogenetic trees. The new species *P. zhuxiensis* does not group with *P. serrulata* and it is nested in clade IIIc of Shen et al. (2023) and sister to a subclade consisting of *P. dolichadenia* Cardot, *P. tatsienensis* Batalin,

Table 1. Morphological comparison between *P. zhuxiensis* and *P. serrulata* (Li and Bartholomew 2003; Yi et al. 2024b).

Characters	*P. zhuxiensis*	*P. serrulata*
Petiole	apex with 2 disciform glands	apex with 1–3 rounded glands
Stipule	4-lobed at base	linear
Secondary veins	8–12 pairs	6–8 pairs
Phenology (in Zhuxi County)	flowering in March	flowering in April and May
Pedicel	0.7–1.2 cm	1.5–2.5 cm
Hypanthium	green or slightly purple	dark purple
Sepals	reflexed, 2–3 mm long, about half as long as hypanthium	spreading, 5 mm long, nearly as long as hypanthium
Petals	ovate or narrowly ovate	obovate
Style	pilose at base	glabrous
Bracts	greenish-white, 1.5–2.5 × 1.5–2.5 mm	brown or tinged greenish-brown, 5–8 × 2.5–4 mm
Fruits	black, sweet, edible	black, bitter, inedible

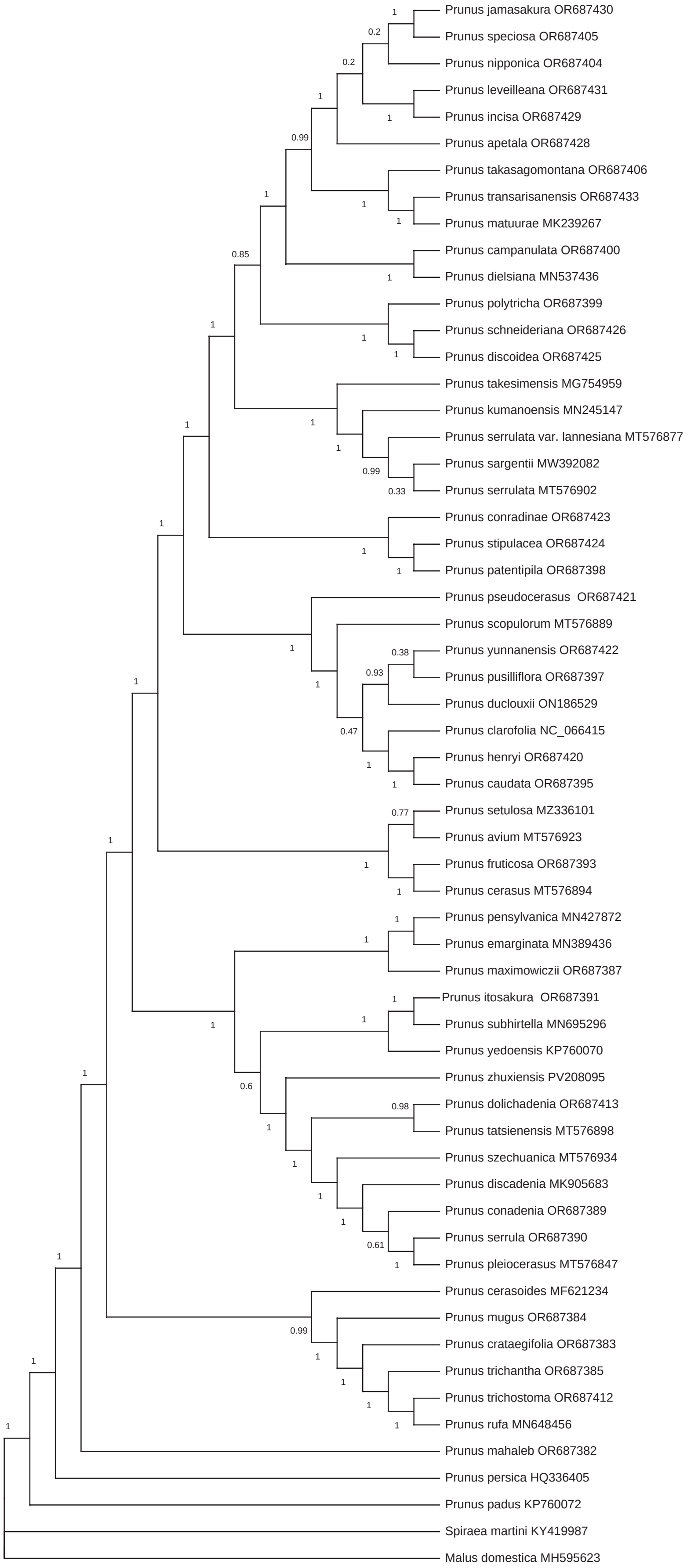

Figure 4. BI consensus tree of *Prunus* subg. *Cerasus*. GenBank accession number follows species name.

P. szechuanica Batalin, *P. discadenia* Koehne, *P. conadenia* Koehne, *P. serrula* Franch. and *P. pleiocerasus* Koehne. *P. serrulata* and *P. serrulata* var. *lannesiana* (Carrière) Makino and *P. sargentii* Rehder group together into a subclade as a part of clade IIIg of Shen et al. (2023). The present study shows that phylogenetic relatedness does not reflect the morphological resemblance of *P. zhuxiensis* and *P. serrulata*. While both *P. zhuxiensis* and *P. subhirtella* are placed in the same clade named IIIc of Shen et al. (2023), these two species share the characters of black drupes and hairy inflorescences and pilose style.

Key to species of *Prunus* subg. *Cerasus* in Zhuxi, Hubei, China (based on Li and Bartholomew (2003))

1a Bracts green, persistent **2**
2a Glands disciform or depressed at apex of teeth along bract margins **3**
3a Inflorescences subcorymbose-racemose or racemose .... ***Prunus szechuanica***
3b Inflorescences umbellate........ ***Prunus tatsienensis***
2b Glands not disciform or depressed at apex of teeth along bract margins **4**
4a Bracts 5–20 mm; sepals spreading .... ***Prunus setulosa***
4b Bracts 2–8 mm; sepals reflexed........ **5**
5a Hypanthium outside densely pilose .... ***Prunus wangii***
5b Hypanthium outside glabrous **6**
6a Stamens 20–30; drupe long ellipsoid .... ***Prunus clarofolia***
6b Stamens 32–54; drupe ovoid to subglobose........ ***Prunus conradinae***
1b Bracts brown or rarely greenish-white, rarely persistent........ **7**
7a Inflorescences more or less hairy or at least hairy when young **8**
8a Style glabrous **9**
9a Sepals about 1/2 as long as hypanthium .... ***Prunus pseudocerasus***
9b Sepals about 2 times as long as hypanthium .... ***Prunus dielsiana***
8b Style hairy........ **10**
10a Hypanthium tubular, base dilated........ ***Prunus subhirtella***
10b Hypanthium tabulate or campanulate, base not dilated ***Prunus zhuxiensis***
7b Inflorescences glabrous........ **11**
11a Sepals reflexed .... ***Prunus cyclamina***
11b Sepals straight or spreading........ **12**
12a Leaf blade margin serrulate or biserrate with acuminate to aristate teeth .... ***Prunus serrulata***
12b Leaf blade sharply serrate .... ***Prunus conradinae***

Acknowledgements

We are grateful to Prof. Zhenyu Li of Institute of Botany, CAS, for the assistance in identifying the new species.

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

Xin-wei Li and Qi-liang Gan carried out field exploration, Qi-liang Gan took the pictures, Xin-wei Li and Wen-bin Xu wrote the manuscript.

Author ORCIDs

Wen-bin Xu https://orcid.org/0009-0002-2645-0053
Xin-wei Li https://orcid.org/0000-0001-6317-1374

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information, or NCBI (https://www.ncbi.nlm.nih.gov/).

References

Hodel R, Zimmer E, Wen J (2021) A phylogenomic approach resolves the backbone of *Prunus* (Rosaceae) and identifies signals of hybridization and allopolyploidy. Molecular Phylogenetics and Evolution 160: 107118. https://doi.org/10.1016/j.ympev.2021.107118

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: 241. https://doi.org/10.1186/s13059-020-02154-5

Jin ZT, Hodel RGJ, Ma DK, Wang H, Liu GN, Ren C, Ge BJ, Fan Q, Jin SH, Xu C, Wu J, Liu BB (2023) Nightmare or delight: Taxonomic circumscription meets reticulate evolution in the phylogenomic era. Molecular Phylogenetics and Evolution 189: 107914. https://doi.org/10.1016/j.ympev.2023.107914

Jin ZT, Ma DK, Liu GN, Hodel RGJ, Jiang Y, Ge BJ, Liao S, Duan L, Ren C, Xu C, Wu J, Liu BB (2024) 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 73(3): 784–799. https://doi.org/10.1002/tax.13163

Kalyaanamoorthy S, Minh BQ, Wong TKF, 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 CL, Bartholomew B (2003) *Cerasus* Miller. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China vol. 9. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 404–420.

Liang SH, Qin B, Huang YH, Tang LM, Zhang ZJ, Li C, He YH, Jiang RH, Li JL (2023) *Prunus quanzhouensis*: A newly discovered species of *Prunus* subgen. *Cerasus* (Rosaceae) from northeastern Guangxi, China. Phytotaxa 622: 252–259. https://doi.org/10.11646/phytotaxa.622.4.2

Liu BB, Hong DY, Zhou SL, Xu C, Dong WP, 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(6): 678–694. https://doi.org/10.1111/jse.12542

Liu BB, Campbell CS, Hong DY, Wen J (2020) 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 BB, Ren C, Kwak M, Hodel RGJ, Xu C, He J, Zhou WB, Huang C-H, Ma H, Qian GZ, Hong DY, 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(5): 1020–1043. https://doi.org/10.1111/jipb.13246

Liu GN, Ma DK, Zhang Y, Hodel RGJ, Xie SY, Wang H, Jin ZT, Li FX, Jin SH, Zhao L, Xu C, Wei Y, Liu BB (2023) Phylogenomic analyses support a new infrageneric classification of *Pourthiaea* (Maleae, Rosaceae) using multiple inference methods and extensive taxon sampling. Taxon 72(6): 1285–1302. https://doi.org/10.1002/tax.13083

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna 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

Rozewicki J, Li SL, Amada KM, Standley DM, Katoh K (2019) MAFFT-DASH: Integrated protein sequence and structural alignment. Nucleic Acids Research 47(1): 5–10. https://doi.org/10.1093/nar/gkz342

Shen X, Zong W, Li Y, Liu X, Zhuge F, Zhou Q, Zhou S, Jiang D (2023) Evolution of Cherries (*Prunus* Subgenus *Cerasus*) Based on Chloroplast Genomes. International Journal of Molecular Sciences 24: 15612. https://doi.org/10.3390/ijms242115612

Shi S, Li J, Sun J, Yu J, Zhou S (2013) Phylogeny and classification of *Prunus* sensu lato (Rosaceae). Journal of Integrative Plant Biology 55: 1069–1079. https://doi.org/10.1111/jipb.12095

Su N, Liu BB, Wang JR, Tong RC, Ren C, Chang ZY, Zhao L, Potter D, Wen J (2021) On the species delimitation of the *Maddenia* group of *Prunus* (Rosaceae): Evidence from plastome and nuclear sequences and morphology. Frontiers in Plant Science 12: 743643. https://doi.org/10.3389/fpls.2021.743643

Su N, Hodel R, Wang X, Wang JR, Xie SY, Gui CX, Zhang L, Yang C, Zhao L, Potter D, Wen J (2023) Molecular phylogeny and inflorescence evolution of *Prunus* (Rosaceae) based on RAD-seq and genome skimming analyses. Plant Diversity 45: 397–408. https://doi.org/10.1016/j.pld.2023.03.013

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577. https://doi.org/10.1080/10635150701472164

Wang XR (2014) An illustrated monograph of cherry cultivars in China. Science Press, Beijing.

Wang H, Li XY, Jiang Y, Jin ZT, Ma DK, Liu B, Xu C, Ge BJ, Wang T, Fan Q, Jin SH, Liu GN, Liu BB (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

Xu SZ, Gan QL, Li ZY (2022) A new species of *Prunus* subgen. *Cerasus* from Central China. PhytoKeys 199: 1–7. https://doi.org/10.3897/phytokeys.199.84354

Xue T-T, Janssens SB, Liu B-B, Yu S-X (2023) Phylogenomic conflict analyses of the plastid and mitochondrial genomes via deep genome skimming highlight their independent evolutionary histories: A case study in the cinquefoil genus *Potentilla* sensu lato (Potentilleae, Rosaceae). Molecular Phylogenetics and Evolution 107956. https://doi.org/10.1016/j.ympev.2023.107956

Yi XG, Dong JJ, Chen J, Zhou HJ, Wu T, Gao SC, Chen XZ, Li M, Wang XR (2024a) Molecular and morphological evidence support a new species of Rosaceae *Prunus* subg. *Cerasus* from Wuyishan National Park, southeast China. PhytoKeys 237: 269–279. https://doi.org/10.3897/phytokeys.237.115098

Yi XG, Li M, Wang XR (2024b) A review on the taxonomy study of *Prunus* Subgen. *Cerasus* (Mill.) A. Gray. Journal of Nanjing Forestry University 48: 46–57.

Yu TT, Li CL (1986) *Cerasus*. In: Yu TT (Ed.) Flora Reipublicae Popularis Sinicae Vol. 38. Science Press, Beijing, 46–87.

Zhang D, Gao FL, Jakovlic 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: 348–355. https://doi.org/10.1111/1755-0998.13096

Supplementary material 1

Comparison between *P. zhuxiensis* and *P. serrulata*

Authors: Qi-liang Gan, Wen-bin Xu, Xin-wei Li

Data type: jpg

Link: https://doi.org/10.3897/phytokeys.255.142428.suppl@1

Supplementary material 2

ML consensus tree of *Prunus* subg. *Cerasus*

Authors: Qi-liang Gan, Wen-bin Xu, Xin-wei Li

Data type: jpg

Link: https://doi.org/10.3897/phytokeys.255.142428.suppl@2

PhytoKeys 255: 215–234 (2025)
DOI: 10.3897/phytokeys.255.144697

Research Article

Diving into Diversity: *Haslea berepwari* (Bacillariophyceae, Naviculaceae), a new species of marine diatom from New Caledonia

Fiddy Semba Prasetiya[1], Thierry Jauffrais[2], Mustapha Mohamed-Benkada[3,4], Nolwenn Callac[2], Dominique Ansquer[2], Elif Yılmaz[5], Claude Lemieux[6], Monique Turmel[6], Jean-Luc Mouget[7], Danang Ambar Prabowo[1], Debora Christin Purbani[1], Diah Radini Noerdjito[1], Varian Fahmi[1], Sulastri Arsad[5], Romain Gastineau[5]

1 *Research Center for Biosystematics and Evolution, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Jalan Raya Bogor Km 46, Cibinong, West Java 16911, Indonesia*
2 *Ifremer, IRD, Univ Nouvelle-Calédonie, Univ La Réunion, CNRS, UMR 9220 ENTROPIE, BP 32078, 98800, Nouméa, New Caledonia*
3 *Laboratory of Aquaculture and Bioremediation, Faculty of Nature and Life Sciences, University Oran1-Ahmed Ben Bella P.B. 1524 El M'Naouer 31000 Oran, Algeria*
4 *Faculty of Nature and Life Sciences, University of Sciences and Technology- Mohamed Boudiaf, El Mnaouar BP 1505, Bir El Djir 31000, Oran, Algeria*
5 *Institute of Marine and Environmental Sciences, University of Szczecin, Mickiewicza 16a, 70-383 Szczecin, Poland*
6 *Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, Québec City, Canada*
7 *Laboratoire Biologie des Organismes, Stress, Santé, Environnement (BiOSSE), Le Mans Université, Avenue Olivier Messiaen, 72085 Le Mans, France*
Corresponding authors: Fiddy Semba Prasetiya (fidd001@brin.go.id); Romain Gastineau (romain.gastineau@usz.edu.pl)

Abstract

The current article introduces and describes *Haslea berepwari* **sp. nov.**, a new species of diatom discovered in the vicinity of Boulouparis, New Caledonia. Under light microscopy, *H. berepwari* **sp. nov.** strongly resembles *Haslea pseudostrearia*, but preliminary molecular barcoding conducted using partial 18S and *rbcL* genes suggested that it was a distinct species. This was confirmed first by scanning electron microscopy which showed the differences in stria densities between both species. A short-reads genome-skimming protocol applied on *H. berepwari* **sp. nov.** led us to obtain its complete mitochondrial and plastid genomes. The mitogenome is 36,572 bp in length and as already observed among other species of *Haslea* spp., the *nad6* and *nad2* genes are fused within a single open-reading frame. The plastome is 131,897 bp length, and unlike the mitogenome, it is not colinear with those of *H. pseudostrearia*. The results derived from the sequencing of the plastome allowed to perform a 123-gene multigene maximum likelihood phylogeny that associates *H. berepwari* **sp. nov.** to *H. pseudostrearia* with maximum support at the nodes but also strictly distinguishes them, suggesting a greater genetic distance between these species than what has been previously observed between other marennine-producing species.

Key words: Coral Sea, Naviculales, organellar genomes, tropical diatoms

Academic editor: Bing Liu
Received: 19 December 2024
Accepted: 10 March 2025
Published: 25 April 2025

Citation: Prasetiya FS, Jauffrais T, Mohamed-Benkada M, Callac N, Ansquer D, Yılmaz E, Lemieux C, Turmel M, Mouget J-L, Prabowo DA, Purbani DC, Noerdjito DR, Fahmi V, Arsad S, Gastineau R (2025) Diving into Diversity: *Haslea berepwari* (Bacillariophyceae, Naviculaceae), a new species of marine diatom from New Caledonia. PhytoKeys 255: 215–234. https://doi.org/10.3897/phytokeys.255.144697

Introduction

The genus *Haslea* comprises a group of morphologically diverse pennate diatoms, currently including 36 taxonomically accepted species (Guiry and Guiry 2011). The holotype species of the genus is *Haslea ostrearia* (Gaillon) Simonsen 1974, also known as the 'blue diatom' due to its capacity to produce blue pigment commonly known as marennine (Simonsen 1974). Marennine is responsible for the green coloration observed in the gills of oysters along the Atlantic coast of France (Gaillon 1820; Gastineau et al. 2014). Green oysters, which are distinguished by their specific flavor attributed to modifications in fatty acids and their emerald hue, are less common and command a higher price in the French oyster industry (Prasetiya et al. 2017a). While the chemical structure of Marennine remains unknown, it has been shown to display several biological properties (Pouvreau et al. 2007; Pouvreau et al. 2008; Gastineau et al. 2012b; Prasetiya et al. 2016; Prasetiya et al. 2017b; Prasetiya et al. 2019a; Falaise et al. 2019a, 2019b; Permatasari et al. 2019; Prasetiya et al. 2020a; 2020b; Prasetiya et al. 2021a, 2021b; Seveno et al. 2024). Latest research and development efforts have focused on scaling up the production of the diatom and its pigment (Gargouch et al. 2022; Nghiem Xuan et al. 2021; Adjout et al. 2022; Prasetiya et al. 2022).

In recent years, several new species of *Haslea* have been described worldwide, some of which also produce blue pigments, while others exhibit shapes unusual for this genus (Poulin et al. 2004; Gastineau et al. 2012a, 2016, 2021b; Talgatti et al. 2014; Sterrenburg et al. 2015; Li et al. 2017; Prasetiya et al. 2019b; Lobban et al. 2020). Among the species that do not produce a blue pigment, *Haslea pseudostrearia* Massé, Rincé & E.J. Cox 2001 is of particular relevance to the present article. This species was first described by Massé et al. (2001) based on material from the Kingsbridge estuary in southern UK and received its species name due to its morphological similarities with *H. ostrearia*. Following this initial description, strains of non-blue *Haslea* were assigned to this species after being reported in various and distant parts of the world, including South Africa (GenBank: OK729589 and OK729583) and the Yellow Sea (An et al. 2017) (GenBank: KY320350 and KY320289).

The present study aims to describe a new species of pennate non-blue diatom from the genus *Haslea* originating from New Caledonia. New Caledonia, a territorial French collectivity, comprises several archipelagos and isolated islands, some of which are remnants of the Zealandia submerged continent. New Caledonia is situated approximately 1,500 km east of Australia, in the Southwestern Pacific Ocean and its largest island is named Grande Terre ('great land'). On the west coast of Grande Terre, in Boulouparis, the French national institute for ocean science and technology (Ifremer) has operated a station for experimental aquaculture for approximately 50 years (Galinie 1989) (currently co-operated with the Adecal Technopole) with decades of expertise in shrimps and other crustaceans' cultivation (e.g. for recent developments Lemonnier et al. 2021; Giraud et al. 2021, 2022; Colette et al. 2022, 2023; Nguyen et al. 2022; Callac et al. 2022, 2023, 2024), including experiments on the co-cultivation of shrimps with holothurians (Purcell et al. 2006; Bell et al. 2007). In 2020, a strain of non-blue *Haslea* has been isolated from one of the earthen ponds used in these studies. Under light microscopy (LM), the strain looked very similar to *H. pseudostrearia*, and if it were not for the contradictory results of molecular barcoding, it could have been assigned to this species.

In this article, we describe the new species *Haslea berepwari* sp. nov. This description is based on LM and scanning electron microscopy (SEM) observations combined with two multigene phylogenies derived from the sequencing of the mitochondrial and plastid genome of this new species. The interest of New Caledonia as a hotspot for the discovery of new diatom species, as illustrated by this article, will be also discussed.

Material and methods

Sampling site, isolation and culture condition

Samplings were done in August 2020 in shrimp earthen ponds use for the co-breeding of *Penaeus stylirostris* Stimpson, 1871 and *Holothuria scabra* Jaeger, 1833 at the experimental aquaculture station of Saint Vincent (Boulouparis), located on the west coast of Grande Terre (coordinates: 21°55'36.9"S, 166°05'00.9"E, sampling authorization 15569-2019/4-ISP-DENV) (Fig. 1). Monoclonal cultures were obtained by single cell isolations performed with micropipettes under an inverted microscope (Zeiss, Primovert). Subsequently, it was carefully rinsed in several drops of site-filtered seawater (0.2 µm). The isolated strain was then transferred to cell culture multidishes filled with filtered (0.2 µm), autoclaved, and ES1/3-enriched seawater (Lebeau et al. 1999). The isolates were then cultured in a thermo-controlled incubator at a temperature of 24 °C, with an illumination of 50 µmol photons m^{-2} s^{-1}. Finally, the isolates were transferred to 150 mL Erlenmeyer flasks containing 50 mL of ES1/3 medium and maintained under similar conditions in our culture collection. The strain was registered in the collections of Ifremer as P05.

Microscopic observations

Live LM images were obtained using a Leica microscope (DM750, Leica Microsystems) equipped with ICC50 camera. The morphometry analysis was conducted using the Image J software (Schneider et al. 2012). All LM pictures were taken within two weeks after isolation, isolation and come as close as possible to wild or natural material.

For cleaned LM and SEM observations, cells were subsequently rinsed in two consecutive baths of milliQ water and left to oxidize overnight in H_2O_2. The following day, a drop of the sample was deposited on a glass cover slide, air-dried, and

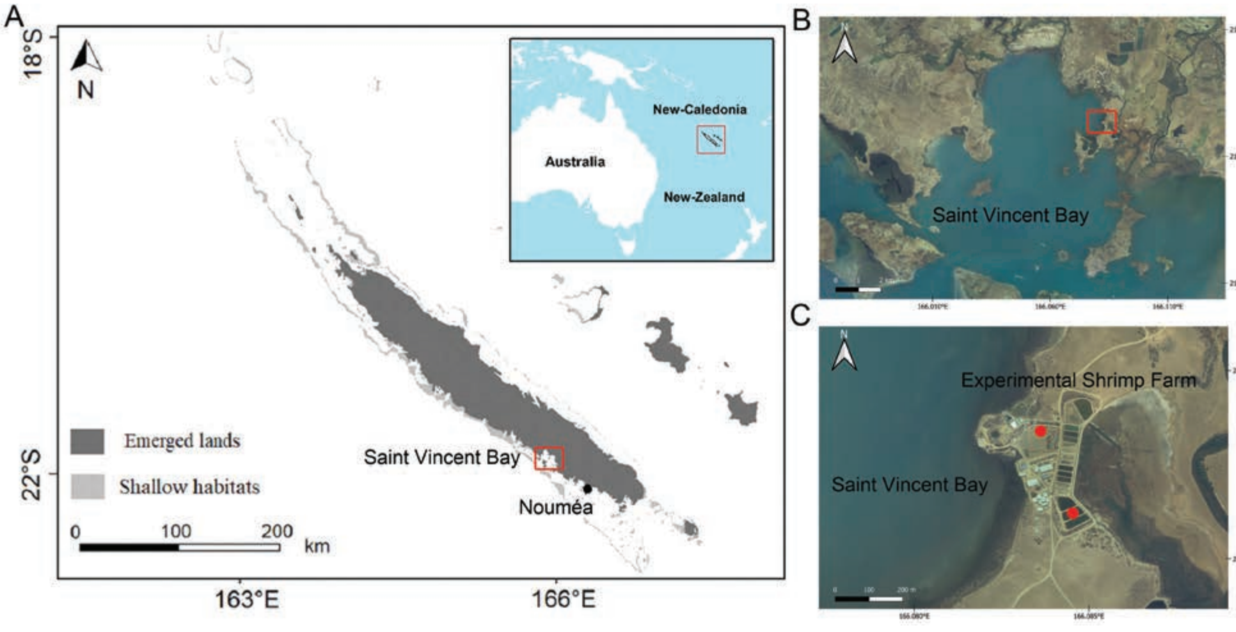

Figure 1. Sampling location of *Haslea berepwari* in the experimental aquaculture station of Saint Vincent (21°55'36.9"S, 166°05'00.9"E), the West Coast of Grande Terre, New Caledonia, during summer 2020. Sampling point is indicated with the red dots sign. Map produced with QGIS software version 3.43.3 (QGIS.org 2023).

placed in a furnace (2 hours at 450 °C) for complete removal of organic matter. Pictures were taken with the same Leica DM750 microscope mentioned above.

For scanning electron microscopy (SEM) observations, cleaned samples were coated with an 8 nm layer of platinum (LEICA, EM ACE 600) and imaged using an environmental SEM equipped with a secondary electron detector (JSM-IT300 LV, 20 kV, 15 mm working distance, JEOL) at the University of New Caledonia.

DNA extraction and preliminary molecular barcoding

A culture of *H. berepwari* sp. nov. was maintained in exponential growth phase by frequent dilutions with fresh medium. A volume of 10 mL was harvested by centrifugation at 4500g and 5 °C during 10 min (Firlabo, SW9R, Meyzieu, France). Pellets of cells were then stored at -20 °C until analysis. The PowerSoil® DNA Isolation Kit was then used to extract the DNA and lyse the frustules. The DNA amplification and sequencing protocols were adapted from Gastineau et al. (2016). In brief, PCR reactions were conducted in a final volume of 20 µl using hot-start Taq polymerase (1X) (Qiagen) with its buffer: 10X PCR buffer (Qiagen), 200 µM of a dNTPs mix (Promega). Two sets of primers were employed: one targeting the 18S rRNA gene (18SHASLEAF: 5'_CTGCCCTATCAGCTTTGGATGG_3', 18SHASLEAR: 5'_CCATTCAATCGGTAGGTGCG_3') and the *rbcl* gene (RBCLF: 5'_GTCTCAATCTGTATCAGAACGG_3', RBCLR: 5'_CGGTTAGCTGTTGGTGTTTCAGCG_3') at a final concentration of 0.2 µM for each primer. All amplifications were performed in a Veriti™ thermocycler (Applied Biosystems, USA) for 35 cycles, as follows: 1 min denaturation at 95 °C, 1 min annealing at 60 °C for the 18S rRNA gene and 59 °C for the *rbcL* gene, 1 min 30 s elongation at 72 °C, followed by a final elongation step at 72 °C for 7 min. The size of all amplicons was verified using agarose gel electrophoresis. The PCR products that matched the expected size (around 1300 bp for the 18S rRNA gene and 1400 bp for the *rbcL* gene) were sent to GenoScreen (Lille, France) for Sanger sequencing. The raw sequence chromatograms were checked using Geneious Prime software and the reverse and forward sequences were combined. The consensus sequences were pairwise aligned using MEGA 11 (Tamura et al. 2021) and compared with sequences of *Haslea* spp. obtained from GenBank, which suggested that the species was different from these references. Consensus sequences are available as supplementary files as explained below.

Next generation sequencing, assembly and annotation

The pool of DNA that remained from the PCR and Sanger protocol described above was sent to the Beijing Genomics Institute (BGI, Shenzhen, China) to be sequenced on a DNBSEQ platform for a total of ca. 100M clean 150 bp paired-end reads. Reads were assembled using SPAdes 4 (Bankevich et al. 2012) with a k-mer of 125. The different subunits of the plastome were joined using Consed (Gordon and Green 2013). Genes were identified and annotated as explained in Gastineau et al. (2021a). The maps of the mitochondrial and plastid genomes were drawn on the OGDRAW online portal (Lohse et al. 2013).

Multigene phylogeny

Two phylogenies were performed. The first one was based on the available plastid genomes. Protein-coding genes were extracted from the plastomes of *H. berepwari* sp. nov. and 17 other species of *Naviculaceae* downloaded from GenBank plus *Eunotia naegelii* Migula 1905 to be used as an outgroup. Genes that were not shared by all the selected taxa or were likely pseudogenes were removed from the dataset, leading to a total of 123 conserved genes, which were all independently aligned by MAFFT 7 (Katoh and Standley 2013) with the -auto option, then trimmed with trimAl (Capella-Gutiérrez et al. 2009) and the -automated1 option before being concatenated by Phyutility 2.7.1 (Smith and Dunn 2008). The best model of evolution was verified on the concatenated alignment using ModelTest-NG (Darriba et al. 2020), which returned the GTR+I+G as best model with the three modes (BIC, AIC and AICc). The maximum likelihood phylogeny was performed using IQ-TREE 2.2.0 (Minh et al. 2020) with 1000 ultrafast bootstrap replicates. The second multigene phylogeny was performed by appending recently published datasets (Yılmaz et al. 2024a, 2024b) with sequences from *H. berepwari* and other *Haslea* spp. The dataset contains three genes, namely *psbC*, *rbcL* and *18S*. The phylogeny was conducted using the same software as above, but the best model of evolution was evaluated on each gene alignment separately prior to concatenation, and was chosen based on the BIC mode. The model chosen were GTR+I+G4 (*psbC*), TIM3+I+G4 (*rbcL*) and TrN+I+G4 (*18S*). The dataset was partitioned based on these models, with 1000 ultrafast boostrap replicates. The dataset, partition file and complete tree are available as described in the data availability statement.

Results

Taxonomy

Haslea berepwari **Mouget, Gastineau & Jauffrais, sp. nov.**

Type material. ***Holotype*****:** The strain P05 was acid cleaned and mounted on a glass slide and is deposited in the herbarium “Paris Cryptogamie” (PC) at The French National Museum of Natural History under accession number PC0643624 (LM slide) and PC0643625 (SEM slide). The cell representative of the type is presented in Fig. 2.

Isotypes**:** SEM and LM slides with acid cleaned valves of strain P05 are kept at the Ifremer culture collection in New Caledonia under the accession number P05.

Type locality. Boulouparis, New Caledonia. *Haslea berepwari* was isolated from shrimp earthen ponds (coordinates: 21°55'36.9"S, 166°05'00.9"E, Fig. 1) by Thierry Jauffrais in August 2020 in Boulouparis during a co-culture experiment of *Penaeus stylirostris* and *Holothuria scabra*.

Etymology. The species designation is derived from the term “Boulouparis”, which is the one of the main cities on the west coast of New Caledonia. The name “Berepwari” is the translation of Boulouparis in xârâcùù, one of the main Melanesian languages spoken in New Caledonia.

Description. *LM* Living cells solitary, motile and lanceolate, equipped with two parietal, narrow band-like chloroplasts appressed to the girdle of the cell (Fig. 2).

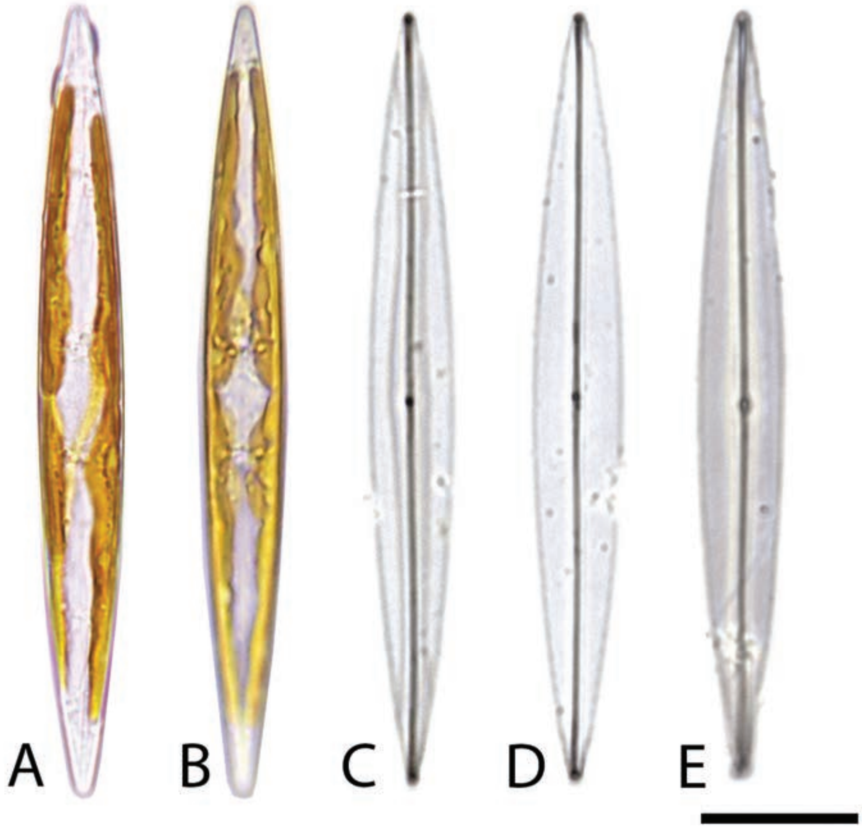

Figure 2. *Haslea berepwari* sp. nov. *in vivo* pictures with two parietal chloroplasts and apices without the presence blue pigment in light microscopy (**A, B**) and LM image of a cleaned valve (**C–E**), scale bar 20μm.

Valves narrow and lanceolate with acute apices. The maximum and minimum length of the monoclonal culture of *H. berepwari* was 101.0 μm and 95.4 μm, respectively (average 98.0 ± 1.5 μm, n = 30), while the maximum and minimum width was 15.0 μm and 9.7 μm (average 12.2 ± 1.1 μm, n = 30). On clean frustules, raphe straight with non-distinct central endings. Cell wall exceedingly delicate, with longitudinal and transapical striations not discernible under LM. In general, LM provides minimal visibility into the specifics of the valve characteristics and is not sufficient to distinguish between this species and *H. pseudostrearia*.

SEM In external valve view, the exterior is covered with long, continuous, and apical-oriented siliceous stripes (top layer), proximal raphe endings straight and slightly widened, slightly deflected dorsally, apical raphe endings ventrally hook shaped (Fig. 3A, D). The interior is composed of a grate-like layer of small areolae, separated by short bars arranged crosswise. Transverse bars of this layer are almost equal in the transapical and longitudinal bars. The areolae are occluded externally by hymens and remnants of this membrane are visible in Fig. 3D. The central area lacks a lateral extension (Fig. 3A, D). Internally, the raphe is slightly elevated and straight, with well-developed helictoglossae at the poles (Fig. 3E). Internal openings of the raphe fissures directed towards one side of the raphe sternum, except at the center and near the tips. Thin bar near the central ending of the raphe on one side of the valve only (Fig. 3C). A supplementary ridge runs alongside the raphe sternum across most of the valve. Internally, square-shaped areolae organized in orderly rows (Fig. 3B, C, E).

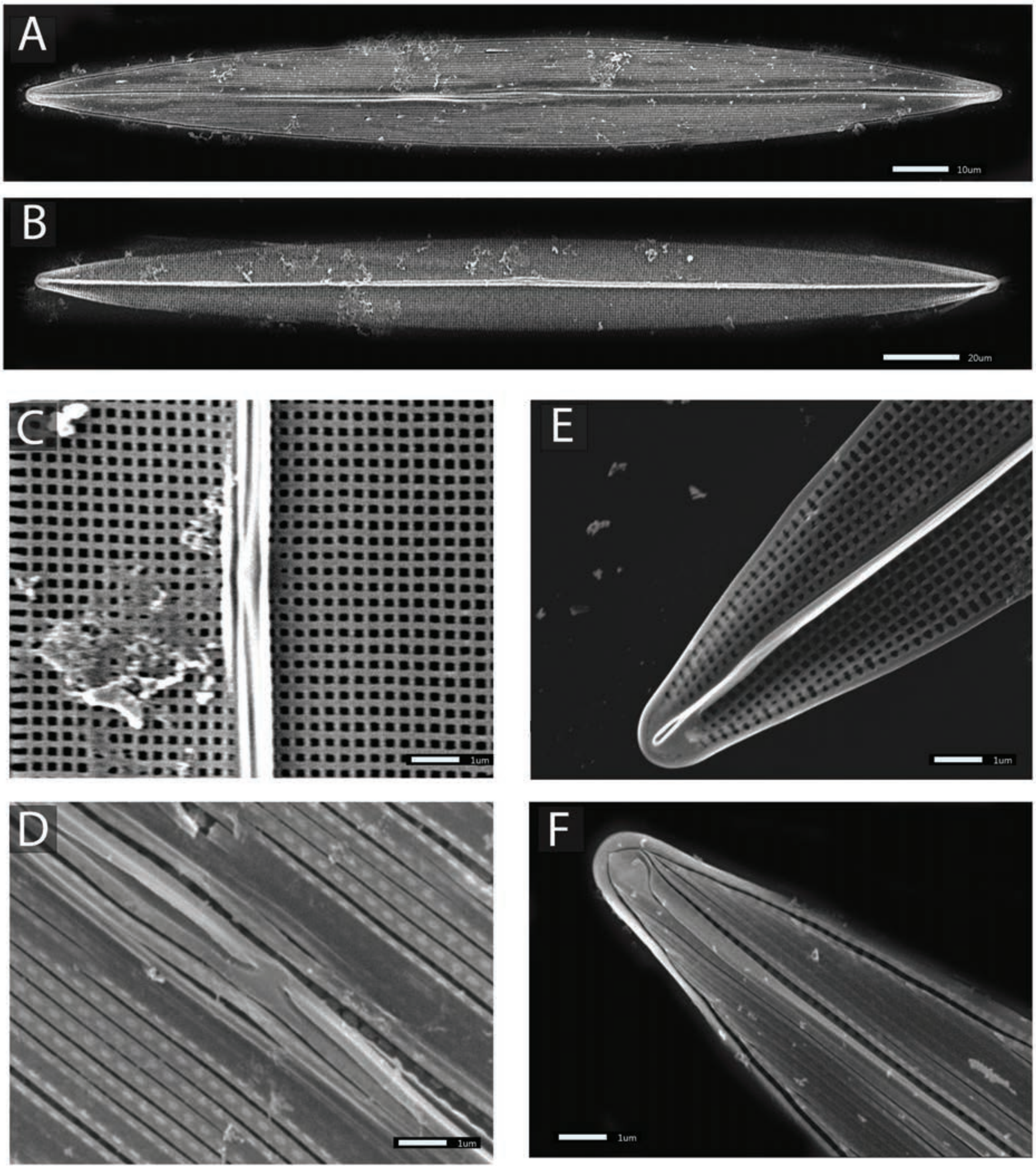

Figure 3. SEM micrographs of *Haslea berepwari* sp. nov. strain P05. **A** Whole valve in external view **B** whole valve in internal view **C** internal view of the center of the valve demonstrating accessory ribs and the presence of a thin bar on the left side of the raphe **D** external view of the valve centre showing approximate raphe endings and continuous longitudinal fissures **E** internal view of apex with the helictoglossa **F** external view of apex showing the curved distal raphe fissure and part of the parallel and longitudinal slits adjoining the peripheral one. Scale bars: 10 µm (**A**); 20 µm (**B**); 1 µm (**C–F**).

Externally, the valve seems covered with longitudinal bands, separated by slits running parallel to the raphe and converging into a single peripheral slit near the tips (Fig. 3A, F). The striation displays a transapical pattern of 37–38 striae per 10 µm intersected by a longitudinal pattern of 36 striae per 10 µm.

Differential diagnosis. A comparative analysis of morphological features between *H. berepwari*, *Haslea nusantara* (Mouget, Gastineau and Syakti) and *H. pseudostrearia* is detailed in Table 1. *Haslea berepwari* sp. nov. shares

Table 1. Comparison between *H. berepwari*, the similar species *H. pseudostrearia* and the tropical species.

Features	*H. nusantara*	*H. pseudostrearia*	*H. pseudostrearia*	*H. berepwari*
Length (µm)	73.9 ± 1.7	55.5 ± 0.2	37–43	98.0 ± 1.5
Width (µm)	6.8 ± 0.1	8.8 ± 0.1	6–7	12.2 ± 1.1
Transapical striae in 10 µm	36.0 ± 1.0	38.6 ± 0.2	34–36	36
Longitudinal striae in 10 µm	52.0 ± 2.0	42.8 ± 0.2	42	37–38
Pseudostauros	Not present	Not present	Not present	Not present
Axial costa	Present	Present	Present	Present
Central bar	Present	Present	Present	Present
Central raphe endings	Straight	Straight	Straight	Straight
Polar raphe endings	Straight	Deflected	Deflected	Deflected
Presence of blue pigment	Yes	No	No	No
References	Prasetiya et al. (2019b)	Prasetiya et al. (2019b)	Massé et al. (2001)	in this study

strong similarities with *H. pseudostrearia* but is distinguished from it by the density of striae, both transapical and longitudinal.

Genomics and phylogeny. *The nuclear rRNA gene cluster:* For reasons unknown, we failed to assemble the complete cluster of nuclear rRNA, even after adjusting the k-mer parameter for assembly. However, we successfully retrieved the complete 18S gene and submitted it to GenBank (PP725422). This sequence completely validated the results obtained previously from Sanger sequencing. The sequence was aligned using Clustal Omega (Sievers et al. 2011) with references ascribed to *H. pseudostrearia* (AY485524 and KY320350) and identity was respectively 95.12% and 95.30%, while these two references were 99.81% identical with each other.

Mitochondrial genome: The mitochondrial genome of *H. berepwari* was retrieved from the contigs file with redundant endings. After trimming and circularization (Fig. 4), its length is 36,572 bp (GenBank: PP728232). The mitogenome encodes for 34 proteins, considering that *nad11* is split into two distinct subunits. As it was noticed with other species of *Haslea* spp., *nad6* and *nad2* are merged into a single open reading frame (ORF), for a total size of 753 amino acids (Gastineau et al. 2021b; Dąbek et al. 2022). The mitogenome also encodes for three ORFs. The first one, *orf162*, corresponds to the conserved ORF generally found *mttB* and *rps11* (Pogoda et al. 2019; Dąbek et al. 2022). We note that our annotation software (Gagnon 2004) ascribed it to *rpl10*, a function suggested for this ORF in *Pleurosigma* sp. (QYJ09263) (Wang et al. 2022). However, in the absence of more evidence of the function of this gene, we will keep labelling it as orf162, nothing also that the size of the putative protein encoded is identical among all the species of *Haslea* spp. for whom a mitogenome is available. The two other ORF, namely orf171 and orf235, are interspersed between the two subunits of *nad11* and *cox3* and are similar to ORFs found in the same position among other species of *Haslea* spp. (Gastineau et al. 2021b; Dąbek et al. 2022). InterProScan queries returned no results for orf235. For orf171, four transmembrane domains, three cytoplasmic regions and two non-cytoplasmic domains were found. The mitogenome also encodes 22 tRNA and two ribosomal rRNA.

Plastid genome: The plastid genome is 131,897 bp long (GenBank: PP728231) and exhibits the usual quadripartite structure (Fig. 5). The LSC is 65,599 bp long and contains 74 protein-coding genes and 17 tRNA. The SSC is 48,934 bp long and contains 52 protein-coding genes, a single non-conserved ORF and seven tRNA. The inverted repeats are 8,682 bp long and contains two protein-coding genes, a

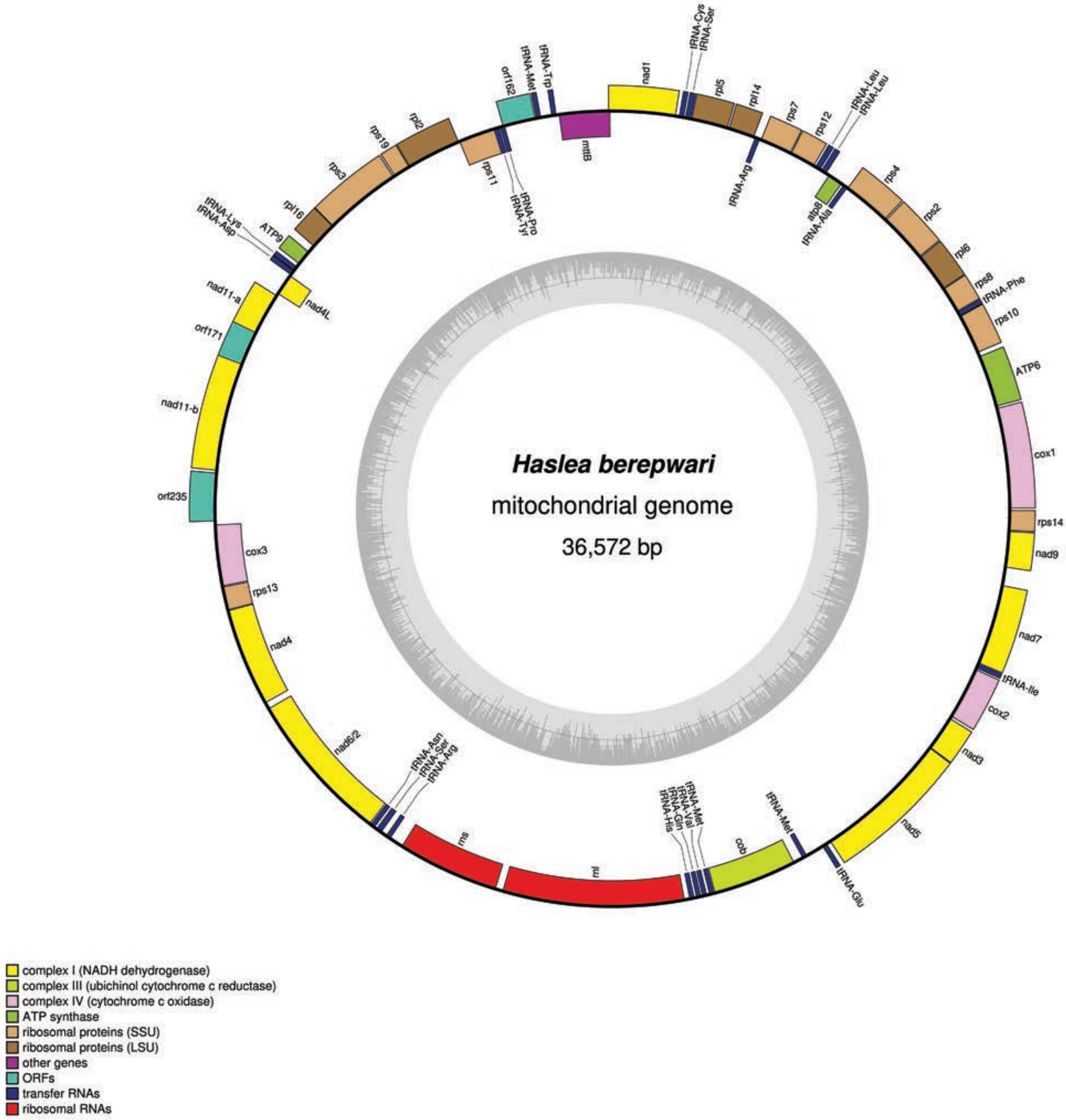

Figure 4. Mitochondrial genome of *Haslea berepwari* sp. nov. Coloured boxes represent genes, with colours denoting their categories as indicated in the bottom left legend. The inner grey ring shows variations in G+C content.

non-conserved ORF (orf118), three rRNA genes and three tRNA. The noticeable differences when compared to *H. pseudostrearia* are the position of *cplC* (between *psbA* and *ycf35*) and the absence of overlap between *ycf45* and the IRB.

Multigene phylogeny: The 123-genes ML phylogeny led to a highly supported tree in which all nodes display maximum support (Fig. 6). For the genus *Haslea*, the tree distinguishes between a highly supported clade of marennine-like producing species and a second clade that contains *H. berepwari* sp. nov. and *H. pseudostrearia*. It is noteworthy that the genetic distance between both species is rather important when compared to the distance between 'blue' species. Other taxa registered as *Haslea* on GenBank are nested within *Navicula* spp., but their belonging to the genus *Haslea* has been invalidated in Li et al. (2017) and thus should be in-

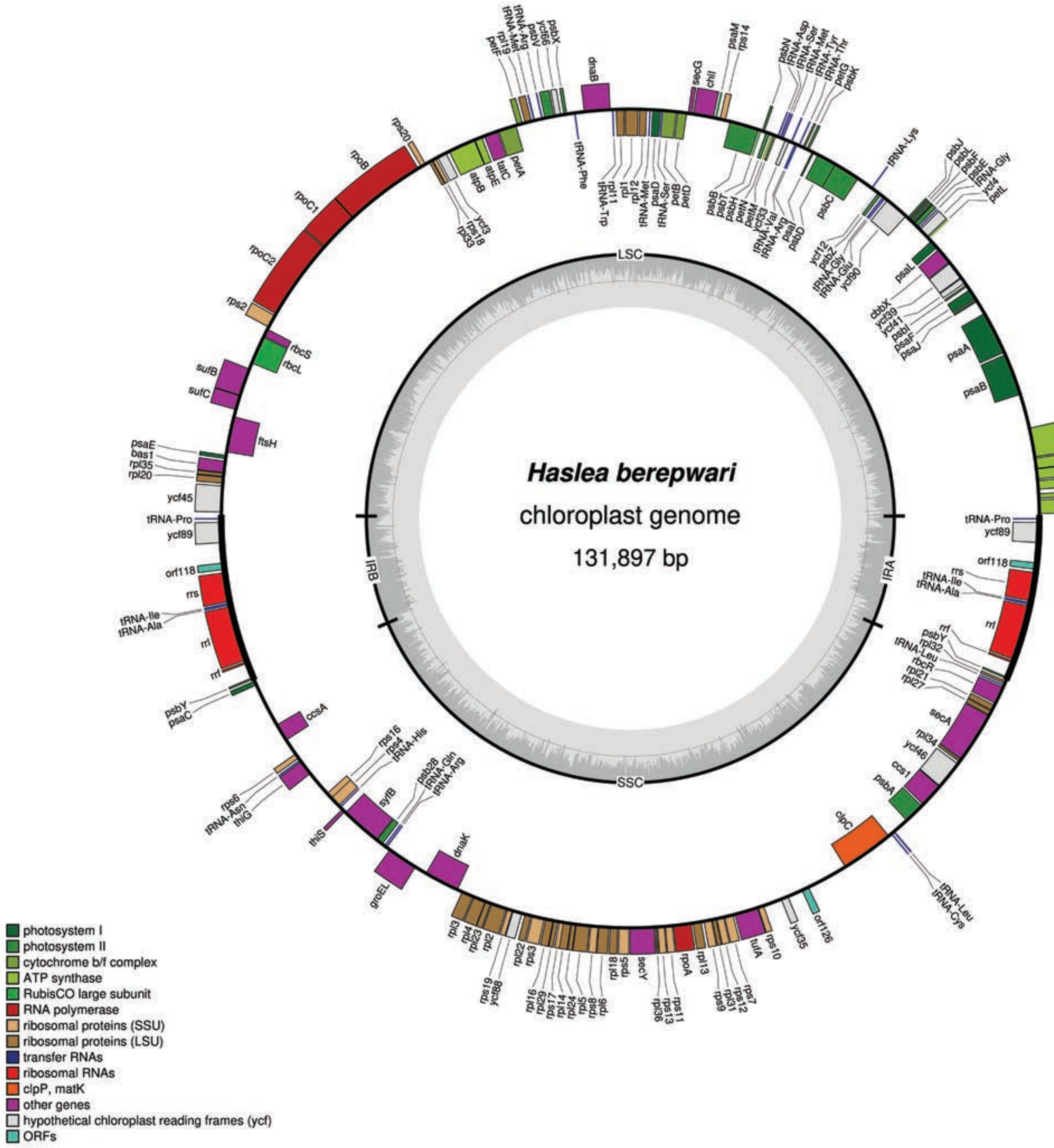

Figure 5. Plastid genome of *Haslea berepwari* sp. nov. Coloured boxes represent genes, with colours denoting their categories as indicated in the bottom left legend. The inner grey ring shows variations in G+C content.

stead regarded as *Navicula* spp. It is to note that *Seminavis robusta* D.B.Danielidis & D.G.Mann 2002 appears inside the *Navicula* clade, a position already observed in the 3-genes ML phylogeny recently published in Yılmaz et al. (2024a). The three-genes ML phylogeny (Fig. 7) also associated *H. berepwari* to a clade formed by two strains ascribed to *H. pseudostrearia* with high support. Sister to this clade is *Haslea arculata* Lobban & Ashworth, 2020, a species found in the Island of Guam and which is characterized by the curved shape of its frustule (Lobban et al. 2020). This large clade is sister to the sigmoid species *Haslea nipkowii* (Meister) M.Poulin & G.Massé 2004 (Poulin et al. 2004) and *Haslea feriarum* M.A. Tiffany & F.A.S. Sterrenburg 2015, a species with dorsoventral valve shape (Sterrenburg et al. 2015; Li et al. 2017). The tree strictly separates ‘blue’ and ‘non-blue’ taxa.

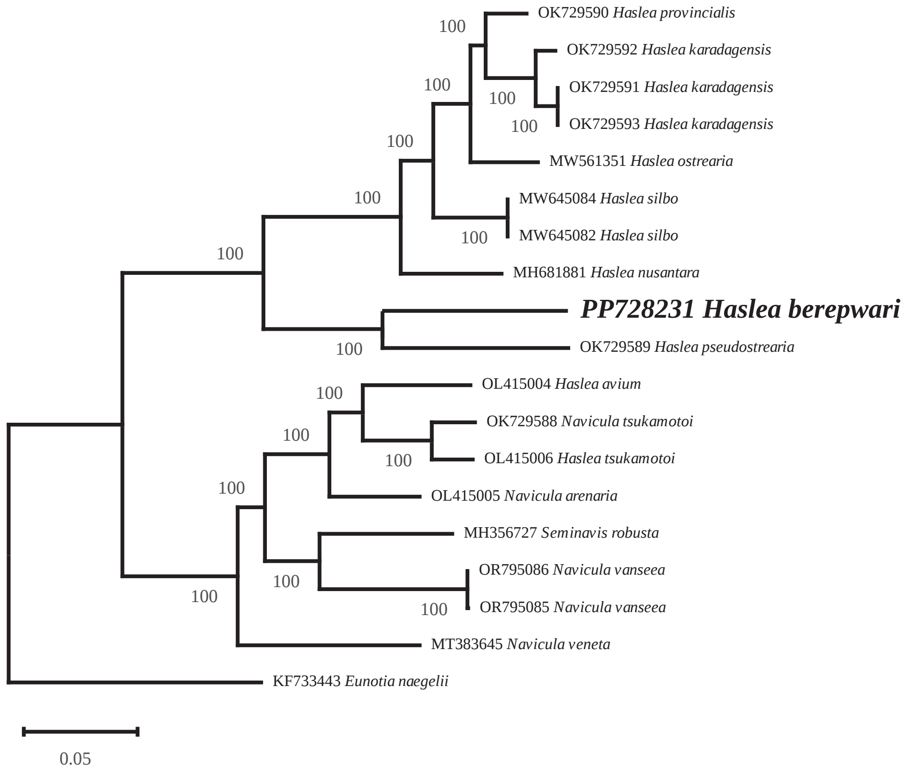

Figure 6. Maximum Likelihood phylogenetic tree obtained from concatenated alignments of 123 protein coding genes from 19 species of diatoms. The tree is rooted with *Eunotia naegelii*.

Discussion

Comparison between *H. berepwari* and similar species

Morphologically as well as phylogenetically, *H. berepwari* is very similar to *H. pseudostrearia*. We can cite the shape of the external distal raphe ending, which is curved in both taxa. This characteristic is also present among other species such as, for example, *Haslea salstonica* Massé, Rincé & E.J.Cox 2000 or *Haslea crucigera* (W.Smith) Simonsen 1974 (Massé et al. 2001). However, it has never been observed among any of the 'blue' species so far, and for this reason, it can be regarded at least as a relevant criteria to discriminate between *H. berepwari* and the 'blue' species *H. nusantara*, for example. The presence of a central thin bar close to the internal proximal raphe ending is an interesting character too, but that could also be misleading. This thin bar is exhibited by *H. berepwari* and *H. pseudostrearia*, but also by the 'blue' species *Haslea silbo* Gastineau, Hansen & Mouget, 2021 and *H. nusantara*, while it is completely absent in *H. ostrearia*, *H. karadagensis* and *Haslea provincialis* Gastineau, Hansen & Mouget, 2016 (Gastineau et al. 2012a, 2016, 2021a; Prasetiya et al. 2019b). So far, the difference in the density of longitudinal striae between *H. berepwa*ri and *H. pseudostrearia* seems to be the most reliable morphological character to distinguish between both species.

Evidences for a '*pseudostrearia*-like' clade among the genus *Haslea*

When they described *H. pseudostrearia*, Massé et al. (2001) noted its similarities to *H. ostrearia*, which influenced their choice of the name. At that time, their

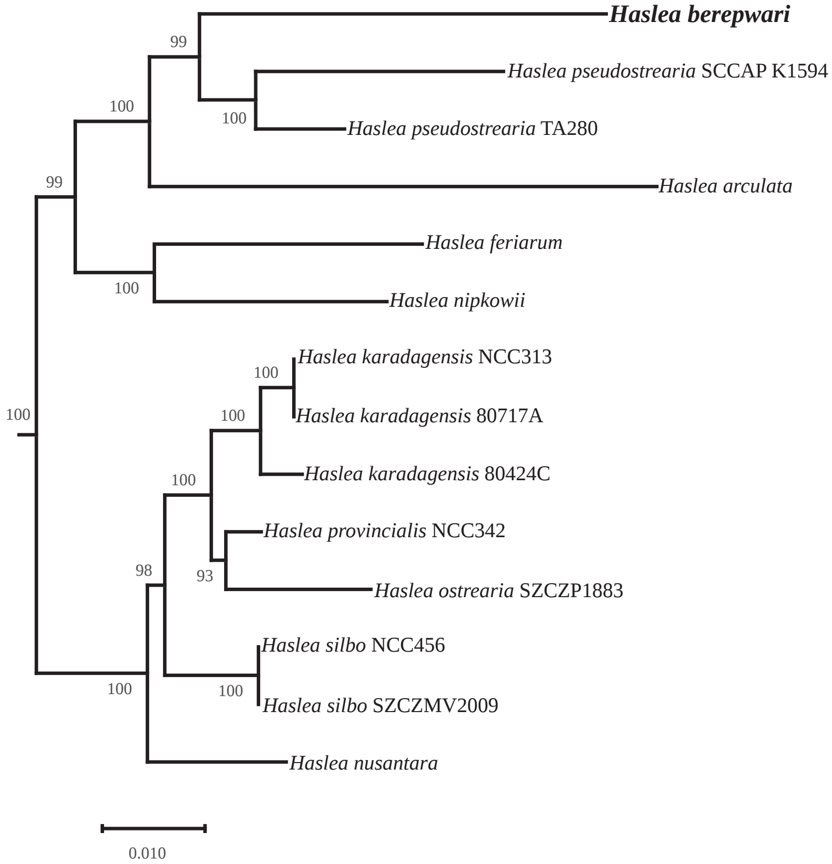

Figure 7. Maximum Likelihood phylogenetic tree obtained from concatenated alignments of three protein coding genes from 133 species of diatoms. The tree was rooted with *Triparma pacifica*. The subtree containing the 14 species of *Haslea* spp. is shown.

conclusions were based solely on morphology, as no molecular analyses were conducted. Later, the genetic proximity between these two species was assessed by the nuclear 18S-inferred phylogeny of Damsté et al. (2004) and, to some extent the plastidic 16S-inferred phylogeny of Poulin et al. (2004). However, two points need to be underlined. First, the sampling of properly identified *Haslea* spp. was of four taxa in Damsté et al. (2004). Poulin et al. (2004) identified six taxa at the species level plus one labelled as '*Haslea* sp.'. None of these studies included more than one single blue species, as it predates the description of *Haslea karadagensis* Davidovich, Gastineau & Mouget in 2012 (Gastineau et al. 2012a). It should be noted that in Poulin et al. (2004), with an increased sample of species, *H. pseudostrearia* clusters with the aforementioned *Haslea* sp. As a molecular marker, 16S has not been widely employed among diatoms, so comparisons were limited until complete plastid genomes had been published. Out of curiosity, the 16S gene of this *Haslea* sp. (AF514851) was submitted to a megablast query. It appears that after itself, the best result returned is *H. berepwari* (1324/1332 bp identical), before *H. pseudostrearia* SCCAP K-1594 (1321/1332 identical) or *H. ostrearia* (1320/1332 bp identical). There is limited information available regarding this *Haslea* sp., except that it originates from the Bay of Bourgneuf, France (Poulin et al. 2004). In the absence of further data on this strain, we can only hypothesize that there may be several species, worldwide and from very distinct environments, that could belong to a '*pseudostrearia*-like' clade, warranting further investigation. The three-genes tree provides additional support to this hypothesis. Indeed, the two strains ascribed to *H. pseudostrearia*, although clustering together, are characterized by an important genetic distance between them, consequently larger than what can be observed between the two strains of *H. silbo* or the three strains of *H. karadagensis* and even more important than the distance separating *H. ostrearia* from *H. provincialis*. We hope that in the future, we will be able to investigate more '*pseudostrearia*-like' taxa, with the same protocol as employed here.

Biodiversity of New Caledonian diatoms

The study of freshwater diatoms in New Caledonia can be traced back to the early 20th century (Pearson et al. 1922), at a time where the only available tool would be LM. With the development of SEM, the flora had been reinvestigated by Pr. René Le Cohu (1985), who, along with colleagues, continued to explorer the freshwater taxa (Le Cohu et al. 2018, 2020a, 2020b; Marquie et al. 2018). In contrast, research on marine diatoms has been more recent, relatively limited and was conducted by Dr. Catherine Riaux-Gobin and colleagues. Similar to the current article, their studies have highlighted the biodiversity of this region, sometimes leading to the description of new species and genera (Riaux-Gobin et al. 2022a, 2022b). New Caledonia also possess a coral reef, and such environments are known to host very diverse diatom assemblages (Risjani et al. 2021). For all these reasons, we might advocate for a more systematic investigation of New Caledonian diatom biodiversity. Such investigations should employ a protocol that integrates morphological and molecular (if not genomic) analyses. This approach would enhance our general knowledge on South Pacific diatoms and contribute to the development of accurate and reliable databases for coastal biomonitoring. Additionally, it is worth noting that this diatom was isolated from an aquaculture facility, and such facilities could also benefit from the bioprospection of local diatoms, for example, as feed sources for larval stages of artificially grown organisms

Acknowledgments

For the SEM images, this work has benefited from the facilities of P2M with the technical assistance of Aurélie Monnin and Olivia Barthelemy, Université de la Nouvelle Calédonie. The authors acknowledge the South Province of New Caledonia who delivered the sampling authorization (RECEP 15569-2019/4-ISP-DENV) for the GHANA/H2020-MSCA-RISE project in New Caledonia and Noémie Coulombier from the Adecal Technopole of New Caledonia for technical assistance.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This publication benefited from funding from the International research collaboration-scientific publication (Indonesia – France) 'Séjour Scientifique d'Haut Niveau Programme

2022' (SSHN 2022, granted to FSP) and the Science Impact Programme 2023–2024 (granted to FSP) from l'Institut Français d'Indonésie and the French Embassy in Indonesia. This work was co-financed by the Minister of Science under the "Regional Excellence Initiative" Program for 2024–2027 (RID/SP/0045/2024/01) and by the Horizon 2020 Research and Innovation Programme GHaNA (The Genus Haslea, New marine resources for blue biotechnology and Aquaculture) under Grant Agreement No 734708/GHANA/H2020-MSCA-RISE-2016 (granted to JLM). Claude Lemieux and Monique Turmel were supported by grant RGPIN-2017-04506 from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Author contributions

Conceptualisation: FSP, TJ, RG. Funding acquisition: JLM. Investigation: FSP, MMB, TJ, NC, DA, EY, CL, MT, JLM, DAP, DCP, DRN, VF, SA, RG. Project administration: TJ, JLM. Supervision: TJ, RG. Writing – original draft: FSP, TJ, NC, RG. Writing – review and editing: MMB, DA, EY, CL, MT, JLM, DAP, DCP, DRN, VF, SA.

Author ORCIDs

Fiddy Semba Prasetiya https://orcid.org/0000-0001-6474-2319
Thierry Jauffrais https://orcid.org/0000-0001-9681-6239
Mustapha Mohamed-Benkada https://orcid.org/0000-0003-1865-3854
Nolwenn Callac https://orcid.org/0000-0002-7578-3455
Elif Yılmaz https://orcid.org/0000-0001-7939-1814
Claude Lemieux https://orcid.org/0000-0001-9580-8042
Monique Turmel https://orcid.org/0000-0001-7060-035X
Jean-Luc Mouget https://orcid.org/0000-0003-0154-7289
Danang Ambar Prabowo https://orcid.org/0000-0001-8200-1266
Debora Christin Purbani https://orcid.org/0000-0001-9305-2835
Diah Radini Noerdjito https://orcid.org/0000-0002-3274-7307
Varian Fahmi https://orcid.org/0000-0002-4273-9087
Sulastri Arsad https://orcid.org/0000-0002-7322-7834
Romain Gastineau https://orcid.org/0000-0001-8661-5118

Data availability

All of the data that support the findings of this study are available in the main text. All the sequences derived from next generation sequencing are available on GenBank with accession numbers PP725422, PP728232 and PP728231. Sequencing reads have been deposited on the Sequence Read Archive (SRA) with accession number SRR31857227, BioProject PRJNA1199224 and BioSample SAMN45869835. Original LM and SEM pictures plus the sequences obtained by PCR and Sanger sequencing are available on Zenodo following this link: https://doi.org/10.5281/zenodo.14506379. The dataset for the three-genes phylogeny, the partition file used and the complete tree obtained are all available following this link: https://doi.org/10.5281/zenodo.14931226.

References

Adjout R, Mouget JL, Pruvost J, Chentir I, Loiseau C, Baba Hamed MB (2022) Effects of temperature, irradiance, and pH on the growth and biochemical composition of *Haslea ostrearia* batch-cultured in an airlift plan-photobioreactor. Applied Microbiology and Biotechnology 106(13–16): 5233–5247. https://doi.org/10.1007/s00253-022-12055-1

An SM, Choi DH, Lee JH, Lee H, Noh JH (2017) Identification of benthic diatoms isolated from the eastern tidal flats of the Yellow Sea: Comparison between morphological and molecular approaches. PLoS One 12(6): e0179422. https://doi.org/10.1371/journal.pone.0179422

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov A, Lesin VM, Nikolenko SI, Pham S, Prjibelski A, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19: 455–477. https://doi.org/10.1089/cmb.2012.0021

Bell JD, Agudo NN, Purcell SW, Blazer P, Simutoga M, Pham D, Della Patrona L (2007) Grow-out of sandfish *Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. Aquaculture (Amsterdam, Netherlands) 273(4): 509–519. https://doi.org/10.1016/j.aquaculture.2007.07.015

Callac N, Boulo V, Giraud C, Beauvais M, Ansquer D, Ballan V, Maillez JR, Wabete N, Pham D (2022) Microbiota of the rearing water of *Penaeus stylirostris* larvae influenced by lagoon seawater and specific key microbial lineages of larval stage and survival. Microbiology Spectrum 10(6): e0424122. https://doi.org/10.1128/spectrum.04241-22

Callac N, Giraud C, Boulo V, Wabete N, Pham D (2023) Microbial biomarker detection in shrimp larvae rearing water as putative bio-surveillance proxies in shrimp aquaculture. PeerJ 11: e15201. https://doi.org/10.7717/peerj.15201

Callac N, Giraud C, Pham D, Ansquer D, Wabete N, Boulo V (2024) Active microbiota of *Penaeus stylirostris* larvae: Partially shaped via vertical and horizontal transmissions and larval ontogeny. Microorganisms 12(3): 608. https://doi.org/10.3390/microorganisms12030608

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics (Oxford, England) 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Colette M, Guentas L, Gunkel-Grillon P, Callac N, Della Patrona L (2022) Is halophyte species growing in the vicinity of the shrimp ponds a promising agri-aquaculture system for shrimp ponds remediation in New Caledonia? Marine Pollution Bulletin 177: 113563. https://doi.org/10.1016/j.marpolbul.2022.113563

Colette M, Guentas L, Della Patrona L, Ansquer D, Callac N (2023) *Suaeda australis* and its associated rhizosphere microbiota: A comparison of the nutrient removal potential between different shrimp farm sediments in New Caledonia. Frontiers in Microbiology 14: 1260585. https://doi.org/10.3389/fmicb.2023.1260585

Dąbek P, Gastineau R, Bornman TG, Lemieux C, Turmel M, Hallegraeff G, Mouget J-L, Witkowski A (2022) The blue diatom *Haslea ostrearia* from the Indian Ocean coast of South Africa, with comparative analysis of *Haslea* organellar genomes. Frontiers in Marine Science 9: 950716. https://doi.org/10.3389/fmars.2022.950716

Damsté JS, Muyzer G, Abbas B, Rampen SW, Massé G, Allard WG, Belt ST, Robert JM, Rowland SJ, Moldowan JM, Barbanti SM, Fago FJ, Denisevich P, Dahl J, Trindade LA, Schouten S (2004) The rise of the rhizosolenid diatoms. Science 304(5670): 584–587. https://doi.org/10.1126/science.1096806

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. Molecular Biology and Evolution 37(1): 291–294. https://doi.org/10.1093/molbev/msz189

Falaise C, James A, Travers MA, Zanella M, Badawi M, Mouget JL (2019a) Complex relationships between the Blue Pigment Marennine and Marine Bacteria of the genus *Vibrio*. Marine Drugs 17(3): 160. https://doi.org/10.3390/md17030160

Falaise C, Cormier P, Tremblay R, Audet C, Deschênes JS, Turcotte F, François C, Seger A, Hallgraeff G, Lindquist N, Sirjacobs D, Gobert S, Lejeune P, Demoulin V, Mouget JL (2019b) Harmful or harmless: Biological effects of marennine on marine organisms. Aquatic Toxicology (Amsterdam, Netherlands) 209: 13–25. https://doi.org/10.1016/j.aquatox.2019.01.016

Gagnon J (2004) Création d'outils pour l'automatisation d'analyses phylogénétiques de génomes d'organites [Development of metagenomics tools for the phylogenetic analyzes of organellar genomes]. M.Sc. Dissertation, University of Laval, Québec, Canada. https://www.collectionscanada.gc.ca/obj/s4/f2/dsk3/QQLA/TC-QQLA-21635.pdf

Gaillon B (1820) Des huîtres vertes, et des causes de cette coloration. Journal de Physique, de Chimie, d'Histoire Naturelle et des Arts 91, 222–225.

Galinie C (1989) Shrimp aquaculture in New Caledonia. Actes de colloques Ifremer, Tahiti, French Polynesia, 20 Feb – 4 Mar 1989, n°9, chap. 5, 45–50. https://archimer.ifremer.fr/doc/00000/1496/

Gargouch N, Touchard R, Marec H, Mouget JL, Pruvost J, Massé A (2022) Submerged membrane photobioreactor for the cultivation of *Haslea ostrearia* and the continuous extraction of extracellular marennine. Bioresource Technology 350: 126922. https://doi.org/10.1016/j.biortech.2022.126922

Gastineau R, Davidovich NA, Bardeau JF, Caruso A, Leignel V, Hardivillier Y, Jacquette B, Davidovich OI, Rince Y, Gaudin P, Cox EJ, Mouget JL (2012a) *Haslea karadagensis* (Bacillariophyta): A second blue diatom, recorded from the Black Sea and producing a novel blue pigment. European Journal of Phycology 47: 469–479. https://doi.org/10.1080/09670262.2012.741713

Gastineau R, Hardivillier Y, Leignel V, Tekaya N, Morançais M, Fleurence J, Davidovich N, Jacquette B, Gaudin P, Hellio C, Bourgougnon N, Mouget JL (2012b) Greening effect on oysters and biological activities of the blue pigments produced by the diatom *Haslea karadagensis* (Naviculaceae). Aquaculture (Amsterdam, Netherlands) 368–369: 61–67. https://doi.org/10.1016/j.aquaculture.2012.09.016

Gastineau R, Turcotte F, Pouvreau J-B, Morançais M, Fleurence J, Windarto E, Prasetiya FS, Arsad S, Jaouen P, Babin M, Coiffard L, Couteau C, Bardeau JF, Jacquette B, Leignel V, Hardivillier Y, Marcotte I, Bourgougnon N, Tremblay R, Deschênes JS, Badawy H, Pasetto P, Davidovich N, Hansen G, Dittmer J, Mouget JL (2014) Marennine, promising blue pigments from a widespread *Haslea* diatom species complex. Marine Drugs 12: 3161–3189. https://doi.org/10.3390/md12063161

Gastineau R, Hansen G, Davidovich NA, Davidovich O, Bardeau J-F, Kaczmarska I, Ehrman JM, Leignel V, Hardivillier Y, Jacquette B, Poulin M, Morançais M, Fleurence J, Mouget J-L (2016) A new blue-pigmented hasleoid diatom, *Haslea provincialis*, from the Mediterranean Sea. European Journal of Phycology 51: 156–170. https://doi.org/10.1080/09670262.2015.1110861

Gastineau R, Hamedi C, Baba Hamed MB, Abi-Ayad S-ME-A, Bąk M, Lemieux C, Turmel M, Dobosz S, Wróbel RJ, Kierzek A, Lange-Bertalot H, Witkowski A (2021a) Morphological and molecular identification reveals that waters from an isolated oasis in Tamanrasset (extreme South of Algerian Sahara) are colonized by opportunistic and pollution-tolerant diatom species. Ecological Indicators 121(6): 107104. https://doi.org/10.1016/j.ecolind.2020.107104

Gastineau R, Hansen G, Poulin M, Lemieux C, Turmel M, Bardeau JF, Leignel V, Hardivillier Y, Morançais M, Fleurence J, Gaudin P, Méléder V, Cox EJ, Davidovich NA, Davidovich OI, Witkowski A, Kaczmarska I, Ehrman JM, Soler Onís E, Quintana AM, Mucko M, Mordret S, Sarno D, Jacquette B, Falaise C, Séveno J, Lindquist NL, Kemp

Jr PS, Eker-Develi E, Konucu M, Mouget JL (2021b) *Haslea silbo*, a novel cosmopolitan species of blue diatoms. Biology (Basel) 10: 328. https://doi.org/10.3390/biology10040328

Giraud C, Callac N, Beauvais M, Mailliez J, Ansquer D, Selmaoui-Folcher N, Pham D, Wabete N, Boulo V (2021) Potential lineage transmission within the active microbiota of the eggs and the nauplii of the shrimp *Litopenaeus stylirostris*: Possible influence of the rearing water and more. PeerJ 9: e12241. https://doi.org/10.7717/peerj.12241

Giraud C, Callac N, Boulo V, Lam JS, Pham D, Selmaoui-Folcher N, Wabete N (2022) The active microbiota of the eggs and the Nauplii of the Pacific Blue Shrimp *Litopenaeus stylirostris* partially shaped by a potential vertical transmission. Frontiers in Microbiology 13: 886752. https://doi.org/10.3389/fmicb.2022.886752

Gordon D, Green P (2013) Consed: A graphical editor for next-generation sequencing. Bioinformatics (Oxford, England) 29: 2936–2937. https://doi.org/10.1093/bioinformatics/btt515

Guiry MD, Guiry GM 21 (2011) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org [searched on 18 March 2025]

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

Le Cohu R (1985) Ultrastructure Des Diatomées de Nouvelle-Calédonie. Première Partie. International Journal of Limnology 21: 3–12. https://doi.org/10.1051/limn/1985004

Le Cohu R, Marquié J, Tudesque L (2018) Three new species of *Delicata* (Bacillariophyceae – Cymbellales) from New Caledonia and further observations on *Delicata nepouiana* Krammer and *D. neocaledonica* Krammer. Diatom Research 33(1): 39–53. https://doi.org/10.1080/0269249X.2017.1407827

Le Cohu R, Lange-Bertalot H, Van de Viver B, Tudesque L (2020a) Analysis and critical evaluation of structural features in four *Cymbellaceae* taxa from New Caledonia. Fottea 20(1): 75–85. https://doi.org/10.5507/fot.2019.017

Le Cohu R, Marquié J, Tudesque L (2020b) *Adlafia diahotensis*, sp. nov. (Bacillariophyceae), a new species from New Caledonia. Cryptogamie. Algologie 41(1): 1–6. https://doi.org/10.5252/cryptogamie-algologie2020v41a1

Lebeau T, Junter GA, Jouenne T, Robert JM (1999) Marennine production by agar-entrapped *Haslea ostrearia* Simonsen. Bioresource Technology 67: 13–17. https://doi.org/10.1016/S0960-8524(99)00096-6

Lemonnier H, Wabete N, Pham D, Lignot J-H, Barri K, Mermoud I, Royer F, Boulo V, Laugier T (2021) Iron deposits turn blue shrimp gills to orange. Aquaculture (Amsterdam, Netherlands) 540: 736697. https://doi.org/10.1016/j.aquaculture.2021.736697

Li Y, Chen X, Sun Z, Xu K (2017) Taxonomy and molecular phylogeny of three marine benthic species of *Haslea* (Bacillariophyceae), with transfer of two species to *Navicula*. Diatom Research 8347: 1–13. https://doi.org/10.1080/0269249X.2017.1401008

Lobban CS, Perez CO, Ashworth MP (2020) Non-blue *Haslea* species (Bacillariophyceae: Naviculaceae) in the benthic marine flora of Guam (Mariana Islands, Western Pacific Ocean). Diatom Research 35(2): 163–183. https://doi.org/10.1080/0269249X.2020.1761887

Lohse M, Drechsel O, Kahlau S, Bock R (2013) OrganellarGenomeDRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic acids research 41(Web Server issue): W575–W581. https://doi.org/https://doi.org/10.1093/nar/gkt289

Marquie J, Le Cohu R, Coste M (2018) *Adlafia moseri* and *A. tjibaoui* two new diatom species (Bacillariophyta) from New Caledonia with further observations on *Adlafia muscora* and *Kobayasiella saxicola.* Phytotaxa 357(1): 41–51. https://doi.org/10.11646/phytotaxa.357.1.4

Massé G, Rincé Y, Cox EJ, Allard G, Belt ST, Rowland SJ (2001) *Haslea salstonica* sp. nov. and *Haslea pseudostrearia* sp. nov. (Bacillariophyta), two new epibenthic diatoms from the Kingsbridge estuary, United Kingdom. Comptes Rendus de l'Académie des Sciences. Série III, Sciences de la Vie 324: 617–626. https://doi.org/10.1016/S0764-4469(01)01330-0

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(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nghiem Xuan R, Mouget JL, Turpin V, Jaouen P, Pruvost J (2021) Optimization of the growth and marennine production by the diatom *Haslea ostrearia* in photobioreactor. Algal Research-Biomass Biofuels and Bioproducts 55: 102251. https://doi.org/10.1016/j.algal.2021.102251

Nguyen NTB, Wantiez L, Lemaire P, Chim L (2022) Feed efficiency, tissue growth and energy budget changes during the molting cycle of Juvenile Mud Crab, *Scylla serrata*: Effects of dietary proteins, fishmeal versus soy protein concentrate. Fishes 7(6): 334. https://doi.org/10.3390/fishes7060334

Pearson WH, Gepp A, Carter N, Groves J, Smith AL, Wakefield EM, Lister G (1922) A systematic account of the plants collected in New Caledonia and the Isle of Pines by Mr. R. H. Compton, M.A., in 1914.-Part III. Cryptogams (Hepaticae-Fungi). Journal of the Linnean Society of London, Botany 46(305): 13–96. https://doi.org/10.1111/j.1095-8339.1922.tb00474.x

Permatasari I, Prasetiya F, Arsad S, Agung M, Mouget JL (2019) Antibacterial activity of *Haslea ostrearia* supernatant adapted in Indonesia against pathogenic bacteria relevant to mariculture (in-vitro study). Omni-Akuatika 15(1): 30–38. https://doi.org/10.20884/1.oa.2019.15.1.626

Pogoda CS, Keepers KG, Hamsher SE, Stepanek JG, Kane NC, Kociolek JP (2019) Comparative analysis of the mitochondrial genomes of six newly sequenced diatoms reveals group II introns in the barcoding region of *cox1*. Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis 30: 43–51. https://doi.org/10.1080/24701394.2018.1450397

Poulin M, Massé G, Belt ST, Delavault P, Rousseau F, Robert JM, Rowland SJ (2004) Morphological, biochemical and molecular evidence for the transfer of *Gyrosigma nipkowii* Meister to the genus *Haslea* (Bacillariophyta). European Journal of Phycology 39: 181–195. https://doi.org/10.1080/0967026042000202136

Pouvreau J, Housson E, Tallec LL, Morançais M, Rincé Y, Fleurence J, Pondaven P (2007) Growth inhibition of several marine diatom species induced by the shading effect and allelopathic activity of marennine, a blue-green polyphenolic pigment of the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen. Journal of Experimental Marine Biology and Ecology 352: 212–225. https://doi.org/10.1016/j.jembe.2007.07.011

Pouvreau JB, Morançais M, Taran F, Rosa P, Dufossé L, Guérard F, Pin S, Fleurence J, Pondaven P (2008) Antioxidant and free radical scavenging properties of marennine, a blue-green polyphenolic pigment from the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen responsible for the natural greening of cultured oysters. Journal of Agricultural and Food Chemistry 56(15): 6278–6286. https://doi.org/10.1021/jf073187n

Prasetiya FS, Safitri I, Widowati I, Cognie B, Decottignies P, Gastineau R, Morançais M, Windarto E, Tremblay R, Mouget JL (2016) Does allelopathy affect co-culturing

Haslea ostrearia with other microalgae relevant to aquaculture? Journal of Applied Phycology 1–14. https://doi.org/10.1007/s10811-015-0779-y

Prasetiya FS, Comeau L, Gastineau R, Decottignies P, Cognie B, Morançais M, Turcotte F, Mouget JL, Tremblay R (2017a) Effect of marennine produced by the blue diatom *Haslea ostrearia* on behavioral, physiological and biochemical traits of juvenile *Mytilus edulis* and *Crassostrea virginica*. Aquaculture (Amsterdam, Netherlands) 467: 138–148. https://doi.org/10.1016/j.aquaculture.2016.08.029

Prasetiya FS, Decottignies P, Barillé L, Gastineau R, Jacquette B, Figiel A, Morançais M, Tremblay R, Mouget JL, Cognie B (2017b) Cell size-based, passive selection of the blue diatom *Haslea ostrearia* by the oyster *Crassostrea gigas*. The Journal of Molluscan Studies 83: 145–152. https://doi.org/10.1093/mollus/eyx012

Prasetiya FS, Decottignies P, Tremblay R, Mouget JL, Cognie B (2019a) Does culture supernatant of *Haslea ostrearia* containing marennine a ff ect short-term physiological traits in the adult blue mussel *Mytilus edulis?* Aquaculture Reports 15: 100228–100228. https://doi.org/10.1016/j.aqrep.2019.100228

Prasetiya FS, Gastineau R, Poulin M, Lemieux C, Turmel M, Syakti AD, Hardivillier Y, Widowati I, Risjani Y, Iskandar I, Subroto T, Falaise C, Arsad S, Safitri I, Mouget JL, Leignel V (2019b) *Haslea nusantara* (Bacillariophyceae), a new blue diatom from the Java Sea, Indonesia: Morphology, biometry and molecular characterization. Plant Ecology and Evolution 152: 188–202. https://doi.org/10.5091/plecevo.2019.1623

Prasetiya FS, Decottignies P, Tremblay R, Mouget JL, Sunarto S, Iskandar I, Dhahiyat Y, Cognie B (2020a) Not only greening: The effects of marennine produced by *Haslea ostrearia* on physiological traits of three bivalve species. Aquaculture Reports 18: 100546–100546. https://doi.org/10.1016/j.aqrep.2020.100546

Prasetiya FS, Sunarto S, Bachtiar E, Agung MUK, Nathanael B, Pambudi AC, Lestari AD, Astuty S, Mouget JL (2020b) Effect of the blue pigment produced by the tropical diatom *Haslea nusantara* on marine organisms from different trophic levels and its bioactivity. Aquaculture Reports 17: 100389–100389. https://doi.org/10.1016/j.aqrep.2020.100389

Prasetiya FS, Arsad S, Astuty S, Mochamad U, Agung K, Stavrakakis C, Mouget JL (2021a) Effect of culture supernatant from *Haslea ostrearia* on the clearance rate and survival rate of adult Pacific oyster *Crassostrea gigas* infected with *Vibrio aestuarianus*. Aquaculture, Aquarium, Conservation & Legislation 14: 2050–2060. http://www.bioflux.com.ro/docs/2021.2050-2060.pdf

Prasetiya FS, Ramdhani DS, Mulyani Y, Mochamad U, Agung K, Arsad S, Mouget JL (2021b) Antioxidant activities of culture supernatant *Haslea ostrearia* adapted in Indonesia. Aquaculture, Aquarium, Conservation & Legislation 14: 111–119. http://bioflux.com.ro/docs/2021.111-119.pdf

Prasetiya FS, Foret M, Deschênes JS, Gastineau R, Mouget JL, Tremblay R (2022) Semi-continuous system for benthic diatom cultivation and marennine production. Algal Research 62: 102633. https://doi.org/10.1016/j.algal.2022.102633

Purcell SW, Patrois J, Fraisse N (2006) Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquaculture Research 37: 515–522. https://doi.org/10.1111/j.1365-2109.2006.01458.x

QGIS.org (2023) QGIS Geographic Information System. QGIS Association. http://www.qgis.org

Riaux-Gobin C, Witkowski A, Coste M, Berteaux-Lecellier V, Daniszewska-Kowalczyk G (2022a) Marine Achnanthales (Bacillariophyceae) from New Caledonia (Melanesia):

Assemblage specificities, ultramafic environment. Phytotaxa 552(1): 1–21. https://doi.org/10.11646/phytotaxa.552.1.1

Riaux-Gobin C, Witkowski A, Risjani Y, Yunianta Y, Berteaux-Lecellier V, Peszek Ł, Daniszewska-Kowalczyk G (2022b) *Upsilococconeis dapalistriata* gen. nov. & comb. nov. (Bacillariophyta) – a pantropical marine member of Cocconeidaceae. Oceanological and Hydrobiological Studies 51(1): 23–31. https://doi.org/10.26881/oahs-2022.1.03

Risjani Y, Witkowski A, Kryk A, Yunianta, Górecka E, Krzywda M, Safitri I, Sapar A, Dąbek P, Arsad S, Gusev E, Rudiyansyah, Peszek Ł, Wróbel RJ (2021) Indonesian coral reef habitats reveal exceptionally high species richness and biodiversity of diatom assemblages. Estuarine, Coastal and Shelf Science 261: 107551. https://doi.org/10.1016/j.ecss.2021.107551

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675. https://doi.org/10.1038/nmeth.2089

Seveno J, Witkowski A, Car A, Gastineau R, Sirjacobs D, Leignel V, Mouget JL (2024) Impact of blue *Haslea* spp. blooms on benthic diatom and bacterial communities. Phycology 4(3): 465–507. https://doi.org/10.3390/phycology4030027

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology 7: 539. https://doi.org/10.1038/msb.2011.75

Simonsen R (1974) The diatom plankton of the Indian Ocean Expedition of R/V Meteor 1964-5, “Meteor” Forschungsergebnisse. Reihe D: Biologie 19, 1–107.

Smith SA, Dunn CW (2008) Phyutility: A phyloinformatics tool for trees, alignments and molecular data. Bioinformatics (Oxford, England) 24(5): 715–716. https://doi.org/10.1093/bioinformatics/btm619

Sterrenburg FAS, Tiffany MA, Hinz F, Herwig WE, Hargraves PE (2015) Seven new species expand the morphological spectrum of *Haslea*. A comparison with *Gyrosigma* and *Pleurosigma* (Bacillariophyta). Phytotaxa 207: 143–162. https://doi.org/10.11646/phytotaxa.207.2.1

Talgatti D, Sar EA, Torgan LC (2014) *Haslea sigma* (Naviculaceae, Bacillariophyta) a new sigmoid benthic species from salt marshes of Southern Brazil. Phytotaxa 177: 231–238. https://doi.org/10.11646/phytotaxa.177.4.4

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Wang Y, Liu S, Wang J, Yao Y, Chen Y, Xu Q, Zhao Z, Chen N (2022) Diatom biodiversity and speciation revealed by comparative analysis of mitochondrial genomes. Frontiers in Plant Science 13: 749982. https://doi.org/10.3389/fpls.2022.749982

Yılmaz E, Mann DG, Gastineau R, Trobajo R, Solak CN, Górecka E, Turmel M, Lemieux C, Ertorun N, Witkowski A (2024a) Description of *Navicula vanseea* sp. nov. (Naviculales, Naviculaceae), a new species of diatom from the highly alkaline Lake Van (Republic of Türkiye) with complete characterisation of its organellar genomes and multigene phylogeny. PhytoKeys 241: 27–48. https://doi.org/10.3897/phytokeys.241.118903

Yılmaz E, Gastineau R, Solak CN, Górecka E, Trobajo R, Turmel M, Lemieux C, Otis C, Witkowski A, Mann DG (2024b) Morphological and molecular characterization of *Halamphora vantushpaensis* (Bacillariophyceae, Amphipleuraceae), a new diatom species widely dispersed on the shores of the soda Lake Van (Türkiye). PhytoKeys 249: 95–114. https://doi.org/10.3897/phytokeys.249.133205

PhytoKeys 255: 235–245 (2025)
DOI: 10.3897/phytokeys.255.143380

Research Article

Eumachia brevipedunculata (Rubiaceae, Palicoureeae), a new species from Yunnan, China

Dong-Li Quan[1,2], Yun-Hong Tan[1,2]

1 *Southeast Asia Biodiversity Research Institute & Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, 666303, Yunnan, China*
2 *Yunnan International Joint Laboratory of Southeast Asia Biodiversity Conservation & Yunnan Key Laboratory for Conservation of Tropical Rainforests and Asian Elephants, Menglun, Mengla, 666303, Yunnan, China*
Corresponding author: Yun-Hong Tan (tyh@xtbg.org.cn)

Abstract

Eumachia brevipedunculata is a newly-identified species of the tribe Palicoureeae of Rubiaceae from Yunnan, China. It is morphologically similar to *E. straminea*, but differs by its smaller stipules, shorter petioles, smaller leaf blades, reduced inflorescences with shorter peduncles, axes and pedicels, longer calyx lobes, yellow-green flowers with shorter corolla tubes and shorter anthers. This species is widely found in southern Yunnan, but has long been misidentified as *Psychotria*. We clarify its taxonomic status and provide a description and illustration here.

Key words: Flora, Indo-China, new taxon, *Psychotria*, Psychotrieae

Introduction

The pantropical genus *Eumachia* DC. (Rubiaceae, Palicoureeae) is characterised by the following characteristics: raphides in the tissues; shrub or small tree habit; smooth branchlets; flattened young shoots and young internodes; opposite leaves, drying yellowish-green; interpetiolar stipules that are persistent or fall by fragmentation, usually united around the stem, generally glandular when young, but becoming indurated and yellowed to ochre when old; terminal inflorescences with green to white axes and typically short bracts; white to cream or yellowish-green corollas with valvate aestivation; usually barbate throats; pyrenes adaxially plane to concave, with marginal preformed germination slits and lacking ethanol-soluble pigments; non-ruminate endosperm (Andersson 2001; Taylor 2005; Barrabé et al. 2012; Taylor et al. 2017). In contrast, its close relative *Psychotria* L. features vegetative parts drying reddish-brown to grey, deciduous stipules and pyrenes containing ethanol-soluble pigment (Andersson 2001; Davis et al. 2001; Barrabé et al. 2012; Taylor et al. 2017; Razafimandimbison and Rydin 2024).

The genus *Eumachia* was established in 1830, with the type species being *E. carnea* (G.Forst) DC. (de Candolle 1830). Since then, the genus has been treated as a synonym of *Psychotria* (Robbrecht 1988, 1993), though the characteristics of *E. carnea* do not match the latter. More recently, studies based on molecular phylogeny and morphology resurrected the genus *Eumachia* and transferred

Academic editor: Petra De Block
Received: 3 December 2024
Accepted: 24 March 2025
Published: 25 April 2025

Citation: Quan D-L, Tan Y-H (2025) *Eumachia brevipedunculata* (Rubiaceae, Palicoureeae), a new species from Yunnan, China. PhytoKeys 255: 235–246. https://doi.org/10.3897/phytokeys.255.143380

it from the tribe Psychotrieae to Palicoureeae (Barrabé et al. 2012; Barrabé and Davis 2013; Applequist 2014; Razafimandimbison et al. 2014). Some comprehensive combinations and treatments were subsequently made, expanding the circumscription of *Eumachia* (Barrabé et al. 2013; Delprete and Kirkbride 2015; Taylor et al. 2017). So far, 86 species have been reported in the genus, ranging from Palaeotropical to Neotropical (POWO 2025). Only four species are known from continental Asia (Taylor et al. 2017; Turner 2018). Two species *E. montana* (Blume) I.M.Turner and *E. straminea* (Hutch.) Barrabé are currently known in China (Wilson 1916; Chen and Taylor 2011; Taylor et al. 2017; Bijmoer et al. 2024).

Through our study of *Psychotria* in China, we identified a species extensively collected across southern Yunnan and Laos. However, it does not correspond to any previously recognised taxa. A thorough morphological analysis and consultations of relevant literature and specimens indicate that this species shares characteristics with *Eumachia* and is new to science. While it is similar to *E. straminea* in features such as its glabrous stems, papery (when dried) and elliptic-lanceolate leaf blades with 6–8 pairs of secondary veins and terminal inflorescences, it can be distinguished by its smaller stipules, leaf blades and inflorescences and by differences in the floral parts, as outlined in the conclusions below.

We described and illustrated the new *Eumachia* species here, including formal nomenclatural and morphological details, an explanation of the epithet, selected photographs, an overview of its habitat and phenology and a comparison with similar species that may be confused. Additionally, we included data on studied specimens, a preliminary conservation assessment following the IUCN Red List Categories and Criteria (IUCN 2012; IUCN Standards and Petitions Committee 2024) and notes on its distinctive morphological characteristics and ecological traits.

Materials and methods

The materials studied were derived from living plants collected in the field and herbarium specimens deposited at Xishuangbanna Tropical Botanical Garden (HITBC). To identify the morphological differences with related species, we examined specimens from the Herbaria K, MO, NY, P, IBK, IBSC, LE, A, E etc., through online databases including JSTOR Global Plants (https://plants.jstor.org/), CVH (https://www.cvh.ac.cn/), NSII (http://www.nsii.org.cn/) and GBIF (https://www.gbif.org/). Descriptions were based on morphological observations and measurements of living plants and herbarium specimens.

Taxonomy

Eumachia brevipedunculata **Y.H.Tan & D.L.Quan, sp. nov.**
urn:lsid:ipni.org:names:77360587-1
Fig. 1

Type. China • Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Township, Xishuangbanna Tropical Botanical Garden, tropical rain forest, 21°54'52.51"N, 101°15'25.92"E, 579 m a.s.l., 13 May 2024, flowering, *D.L.Quan & Y.H.Tan TYH3288* (holotype, HITBC0115875!; isotypes, HITBC0115876!, HITBC0115877!).

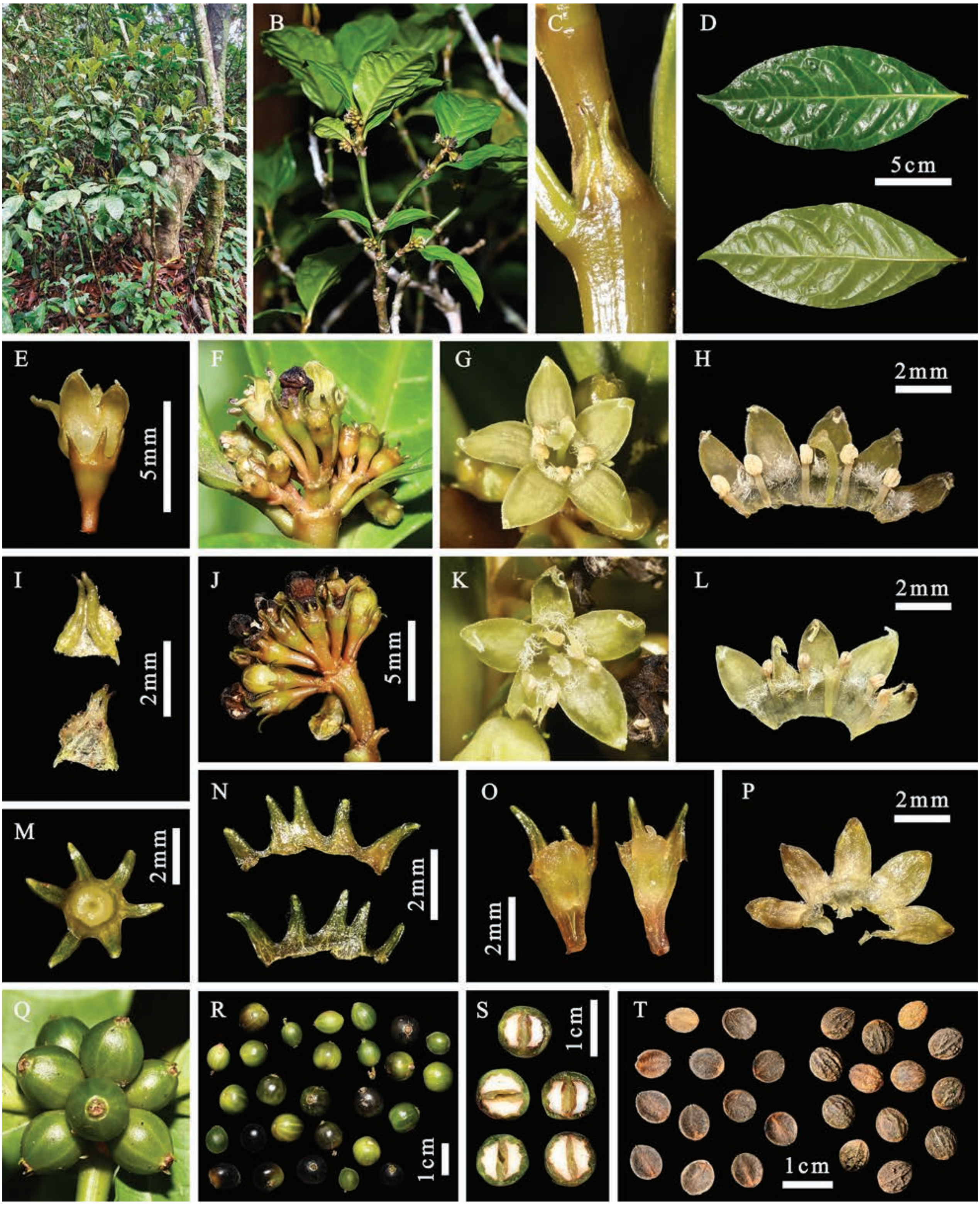

Figure 1. *Eumachia brevipedunculata* Y.H.Tan & D.L.Quan **A** habitat **B** habit **C** stipules **D** leaves **E** flower showing calyx, hypanthium and corolla **F** inflorescence **G** front view of long-styled flower **H** interior view of longitudinally opened long-styled flower **I** abaxial and adaxial surface of stipule **J** inflorescence **K** front view of short-styled flower **L** interior view of longitudinally opened short-styled flower **M** front view of calyx lobes and disc **N** abaxial and adaxial view of longitudinally opened calyx **O** longitudinal section through hypanthium, disc and calyx **P** abaxial view of opened corolla **Q** infructescence **R** fruits at different developmental stages **S** cross-section of fruits, showing pyrenes and seeds **T** ventral (left) and dorsal (right) view of pyrenes. Photographs by Dong-Li Quan.

Diagnosis. *Eumachia brevipedunculata* is morphologically similar to *E. straminea*, but can be distinguished by its smaller stipules (ca. 2 mm vs. 2.5–6 mm long), shorter petioles (0.5–1.2 cm vs. 1–2 cm long), smaller leaf blades (5–18 × 2–8 cm vs. 10–25 × 4–10 cm); smaller inflorescences (1–1.2 × 1–1.2 cm vs. 1–4 × 1–2.5 cm) without developed axes (vs. axes 3–10 mm long), shorter peduncles (up to 0.5 cm vs. 1–1.5 cm long), shorter pedicels (up to 1.5 mm vs. 1.5–4 mm long); longer calyx lobes (0.5–1.5 mm vs. up to 0.5 mm long), linear-lanceolate to narrowly ligulate calyx lobes (vs. denticulate), shorter corolla tubes (1–1.5 mm vs. 1.5–2 mm long), yellowish-green corolla (vs. white to cream) and shorter anthers (ca. 0.5 mm vs. ca. 1 mm long).

Description. Shrubs, 0.5–2.5 m tall, branched; ***stems*** terete, glabrous; ***internodes*** flattened. ***Leaves*** opposite, without domatia; ***petiole*** 0.5–1.2 cm long, glabrous; ***leaf blade*** concavo-convex, elliptic to lanceolate, 5–18 × 2–8 cm, green, often paler abaxially, adaxially somewhat shiny in life, drying papery, glabrous on both surfaces, base cuneate to attenuate, apex acute to acuminate, margins flat or slightly undulant, usually thinly revolute; ***secondary veins*** 6–8 at each side of the mid-rib, free or forming a weakly- to a well-developed looping submarginal vein, adaxially costa thickened to prominent and secondary veins sometimes prominent, abaxially costa and secondary veins prominent, remaining venation flat; ***stipules*** persistent or falling by fragmentation with persistent portion becoming indurated and yellow to ochre, interpetiolar to shortly fused around stem or forming a sheath, ca. 2 mm long, triangular to ovate, with 2 costae bearing 2 lobes, lobed to 1/4–1/2, lobes subulate with glandular tip, abaxially glabrous, adaxially at base with well-developed drying red-brown colleters. ***Inflorescences*** terminal, cymose, congested, subglobose to corymbiform, 1–1.2 × 1–1.2 cm, branched to 1–3 orders without developed secondary axes, generally 3- to 15-flowered, glabrous, subsessile to shortly pedunculate with ***peduncle*** up to 5 mm long; ***bracts*** narrowly triangular, ca. 0.5 mm long, pubescent to glabrescent outside, apex obtuse; ***pedicels*** up to 1.5 mm long. ***Flowers*** 5-merous, rarely 4- or 6-merous, usually bent down, distylous; **hypanthium** obconic, ca. 1.5 mm long, glabrous; ***calyx*** green to brown, glabrous, limb ca. 0.5 mm long, lobes 0.5–1.5 mm long, lobes linear-lanceolate to narrowly ligulate, apex obtuse; ***corolla*** yellowish-green, campanulate, tube 1.5–2.5 mm in diameter, 1–1.5 mm long and slightly shorter than half the corolla length, glabrous outside, inside white villous in throat, lobes valvate in bud, ovate, 2–2.5 × 1–1.5 mm, adaxially rostrate, abaxially smooth, apex obtuse, bent inwardly; ***stamens*** glabrous, in long-styled form 1.5–2 mm long, slightly exserted from corolla tube, in short-styled form 2.5–3 mm long, completely exserted from corolla tube; ***anthers*** elliptic to oblong, obtuse, dorsifixed, ca. 0.5 mm long, always exserted from corolla tube; ***filaments*** in long-styled form 1–1.5 mm long, in short-styled form 2–2.5 mm long; ***stigmas*** 2-lobed, lobes ca. 0.2 mm long; ***style*** erect, clavate; style and stigma in long-styled form 2–2.5 mm long, can exceed 1/2 of the corolla tube, but is never exserted from the corolla tube, in short-styled form 1.5–2 mm long, equal in length to or partially exserted from corolla tube; ***ovary*** inferior, 2-celled, ovules 1 in each cell, disc glabrous. ***Fruit*** drupaceous, fleshy, ellipsoid to subglobose, 0.6–1 cm in diameter, stipitate, glabrous, black at maturity, not reddening, ribbed when dried, calyx lobes persistent; ***pyrenes*** 2, hemispherical, bony, dorsally convex with granulose ornamentation or 1–5 shallowly ribbed, ventrally plano-concave, with marginal preformed germination slits, without ethanol-soluble pigments; ***seeds*** ellipsoid to hemispherical, 4.5–6 × 5–7 mm; ***endosperm*** fleshy, non-ruminate.

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

Etymology. The specific epithet '*brevipedunculata*' refers to the new species' distinguishing feature of reduced inflorescences with short peduncles, axes and pedicels. Its Chinese name is given as 短序肉沛木 (Pinyin: duǎn xù ròu pèi mù).

Habitat and distribution. This species thrives in tropical rainforests and humid evergreen broadleaf forests at 500–1200 m a.s.l. It has been collected from 23 locations in Yunnan and one site in Laos (Fig. 2).

Preliminary IUCN Conservation status. According to the current distribution of *Eumachia brevipedunculata*, the species is found in 23 locations in Yunnan and Laos. *Eumachia brevipedunculata* relies heavily on the formal conservation of primary habitat. More than 10 locations are situated within well-protected nature reserves or rainforests. However, some populations are located at the edge of these reserves or near roads, making them vulnerable to threats such as vegetation clearance, destruction by livestock or construction activities. The subpopulations are dispersed and relatively small in size and area, with an estimated total population

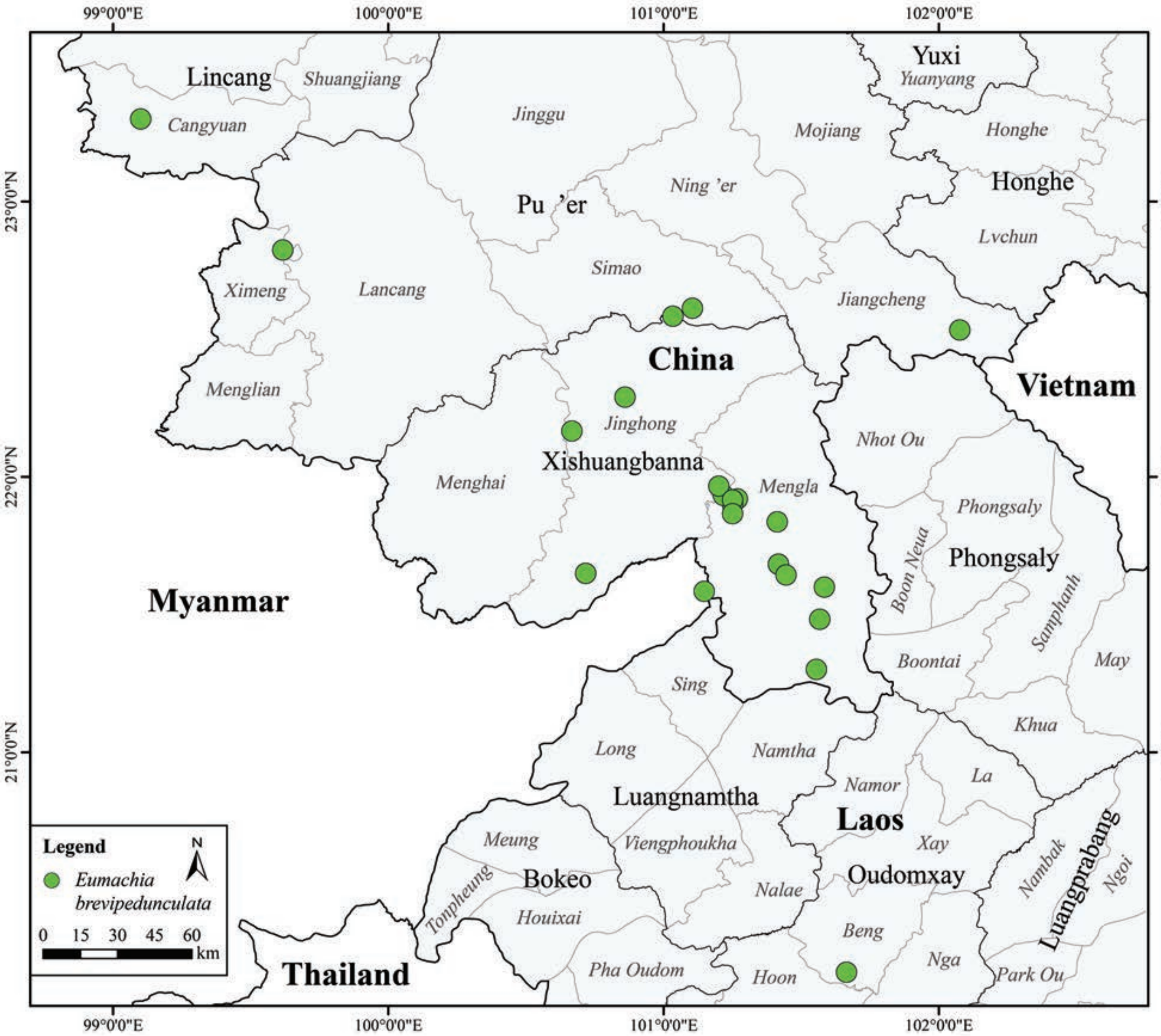

Figure 2. Distribution of *Eumachia brevipedunculata* Y.H.Tan & D.L.Quan.

of about 4,500 individuals. The extent of occurrence (EOO) for *E. brevipedunculata* is 41,638 km^2 and the area of occupancy (AOO) is 88 km^2, as calculated by Shiny-GeoCAT (Moat et al. 2023). Based on the IUCN Red List Categories and Criteria (IUCN 2012), notably on the number of locations and the EOO, this species qualifies for classification as Least Concern (LC).

Notes. *Eumachia brevipedunculata* features persistent or marcescent stipules, preformed germination slits, lacks ethanol-soluble pigments in the pyrenes and has a yellowish-green drying colour, which are general characteristics of the tribe Palicoureeae. In *Eumachia*, fruits are typically orange to red when ripe; however, the fruits of *E. brevipedunculata* and *E. straminea* are black. However, colourful fruits, for example, red, blue, white or black, are known to occur in Palicoureeae. *Eumachia* is not easy to diagnose morphologically because some characteristics were found to be widely variable, such as the stipule type (fused into a tube), the stem texture (smooth or corky), the structure of the inflorescences (lax to subcapitate), the shape of the pyrenes (abaxially smooth to ribbed and adaxially plano-concave to having one or two longitudinal grooves), the type of endosperm (entire to variously ruminate) (Andersson 2001, 2002; Razafimandimbison et al. 2014; Taylor et al. 2017; Taylor 2020; Santos et al. 2021). Moreover, the yellowish-green drying colour of the vegetative parts is generally regarded as a typical and diagnostic characteristic of *Eumachia* contrasted with reddish-brown to grey in *Psychotria* (Barrabé et al. 2012; Taylor et al. 2017). However, some studies considered that the colour of the dried leaves varies considerably with how the specimen was prepared and conserved (Taylor 2020; Taylor et al. 2023). All these variations increase the difficulty of intergeneric and interspecific identification of *Eumachia*. While these characteristics may be homoplasious and not necessarily diagnostic, they remain taxonomically useful (Taylor 2020). Additional taxonomically informative features of *Eumachia* include chemical constituents, the development of tertiary and quaternary leaf venation, leaf anatomy and the morphology of domatia and stomata (Taylor 2016; Berger et al. 2022).

Eumachia brevipedunculata is widely distributed in southern Yunnan, China. It has long been misidentified as a *Psychotria* species, for example, as *P. straminea* (synonym of *E. straminea*), *P. henryi* H.Lév., *P. asiatica* L., *P. siamica* Hutch. and so on. It is most similar to *E. straminea* (Fig. 3), but differs by its smaller stipules and leaf blades, shorter petioles, reduced inflorescences and differences in the floral parts such as longer calyx lobes, shorter corolla tubes and anthers and yellow-green (vs. white-cream) flowers (Table 1). The new *Eumachia* species is often confused with *P. henryi* because of the terminal inflorescences, the subcapitate or congested cymose and subsessile inflorescences, but it is distinct by the broader and glabrous leaf blades, the abaxially glabrous, persistent stipules, the undeveloped inflorescence axes, the smaller bracts, the glabrous calyx, the yellowish-green and campanulate corolla, the ovate and larger corolla lobes and the green-black and smaller fruits.

Additional specimens examined (paratypes). China • Yunnan Province: Xishuangbanna Dai Autonomous Prefecture: Mengla County: rainforest, 21°29'N, 101°34'E, 800 m a.s.l., 29 May 1982, flowering, *expedition team 32655* (HITBC0002822) • Mengla Township: Bubeng Village, 21°36'N, 101°35'E, 720 m a.s.l., 27 April 1982, flowering, *expedition team 31873* (HITBC0002825) • Menglun Township: forestry farm, 21°55'N, 101°15'E, 12 May 1975, flowering, *P.Z.Zhu*

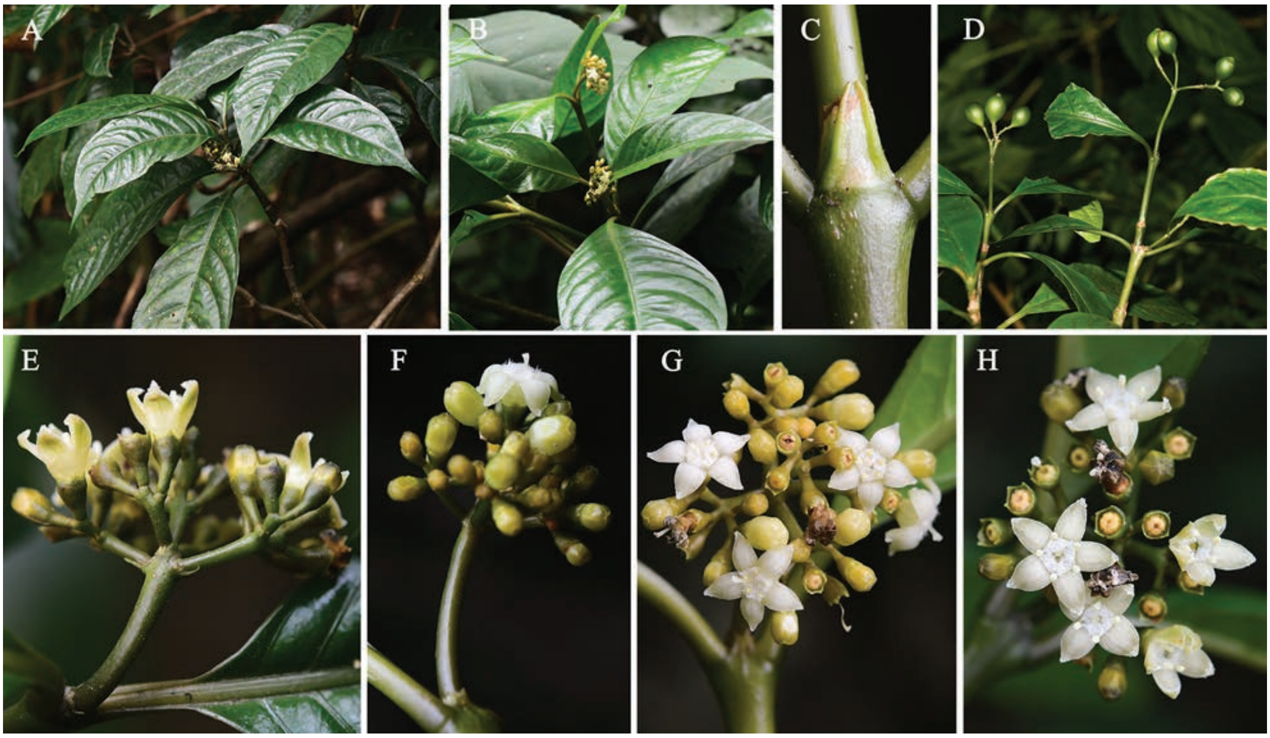

Figure 3. *Eumachia straminea* (Hutch.) Barrabé, C.M.Taylor & Razafim **A, B** habit **C** stipule **D** infructescence **E** inflorescence **F** inflorescence of long-styled flowers in lateral view **G** inflorescence of long-styled flowers in front view **H** inflorescence of short-styled flowers in front view. Photographs by Xiao-Wen Liao (**A–C, E–H**) and Xiao-Dong Zeng (**D**).

Table 1. Morphological comparison of *Eumachia brevipedunculata* and *E. straminea* (characters of *E. straminea* from Wilson (1916) and Chen and Taylor (2011) and the examined type specimens MO2536011 and MO2536013).

Characters	*E. brevipedunculata*	*E. straminea*
Stipule length	2 mm	2.5–6 mm
Petiole length	0.5–1.2 cm	1–2 cm
Leaf size	5–18 × 2–8 cm	10–25 × 4–10 cm
Inflorescence size	1–1.2 cm in diameter	1–4 × 1–2.5 cm
Length of inflorescence axes	ca. 0–0.3 cm	0.3–1 cm
Peduncle length	up to 5 mm	1–1.5 cm
Pedicel length	up to 1.5 mm	1.5–4 mm
Calyx lobe length	0.5–1.5 mm	up to 0.5 mm
Calyx lobe shape	linear-lanceolate to narrowly ligulate, apex obtuse	denticulate
Corolla colour	yellowish-green	white to cream
Corolla tube length	1–1.5 mm	1.5–2 mm
Anther length	ca. 0.5 mm	ca. 1 mm

12612 (HITBC0002833) • in the Menglun sub-reserve of Xishuangbanna National Nature Reserve, 21°56'N, 101°13'E, 650 m a.s.l., 11 August 1975, fruiting, *G.D.Tao 13730* (HITBC0002839) • Xiaola highway 53 km, dry open forest on the mountaintop, 21°58'N, 101°12'E, 900 m a.s.l., 22 October 1973, fruiting, *P.Z.Zhu 10402* (HITBC0002834) • Qixiang, dry forest, 21°55'N, 101°15'E, 1200 m a.s.l., 23 May 1961, flowering, *Y.H.Li 3278* (HITBC0002828) • Chengzi Village, 21°52'N, 101°15'E, 20

September 1972, fruiting, *G.D.Tao 7035* (HITBC037229, HITBC0002836) • Mengxing Village, ravine rainforest over limestone, 21°50'12.45"N, 101°24'45.25"E, 609 m a.s.l., 16 May 2021, flowering, *J.W.Li 6810* (HITBC0075681) • Xishuangbanna Tropical Botanical Garden: 21°54'52.69"N, 101°15'24.30"E, 576 m a.s.l., 24 January 2024, fruiting, *D.L.Quan & Y.H.Tan TYH3271* (HITBC0121858) • 21°55'11.86"N, 101°16'4.19"E, 553 m a.s.l., 28 April 2024, flowering, *D.L.Quan & Y.H.Tan TYH3280* (HITBC0121859) • 21°55'10.63"N, 101°16'7.14"E, 561 m a.s.l., 30 November 2024, fruiting, *D.L.Quan TYH3299* (HITBC0121860) • 21°54'N, 101°15'E, 540 m a.s.l., 2 January 1959, fruiting, *Y.H.Li 213* (HITBC0002841) • 21°54'N, 101°15'E, 570 m a.s.l., 13 July 1959, fruiting, *Y.H.Li 1602* (HITBC0002840) • 21°41'N, 101°25'E, 570 m a.s.l., 27 April 2011, flowering, *J.X.Hu C420152* (HITBC0022962) • 21°41'N, 101°25'E, 570 m a.s.l., 28 April 2011, fruiting, *J.X.Hu C420196* (HITBC0022961) • 21°41'N, 101°25'E, 570 m a.s.l., 24 June 2009, fruiting, *W.Q.Xiao C400518* (HITBC0022957, HITBC0034323, HITBC0034324) • Guanlei Township, near border, 21°35'5.88"N, 101°8'51.30"E, 596 m a.s.l., 29 April 2021, flowering, *J.W.Li 6636* (HITBC0075499) • on the way from Mengyuan Village to Longlin Village, 21°38'37.89"N, 101°26'39.59"E, 1005 m a.s.l., 16 April 2021, flowering, *S.K.Peng P1341* (HITBC0076112) • Shangyong Township, Longmen Village, 21°18'4.84"N, 101°33'16.03"E, 971 m a.s.l., 10 May 2019, flowering, *S.S.Zhou, J.H.Li & L.X.Wang G4-285* (HITBCXSBN002713) • Yiwu Township, beside the river, 1000 m a.s.l., 9 December 2010, fruiting, *J.T.Yin 1879* (HITBC0030358) • Jinghong: Nabanhe, beside the river, 22°10'N, 100°40'E, 700 m a.s.l., 7 November 1988, fruiting, *G.D.Tao 44896* (HITBC0002835) • Dadugang Township, Dahuangba Village, tropical lowland rainforest, 22°17'24.64"N, 100°51'34.40"E, 995 m a.s.l., 7 July 2019, flowering, *J.W.Li 4857* (HITBC0035650) • Menglong Township: Manyangguang Village, 21°39'N, 100°43'E, 16 October 1978, fruiting, *G.D.Tao 19704* (HITBC037215, HITBC037221, HITBC037231, HITBC0002838) • Guanglong Mountain, 21°39'N, 100°43'E, 800 m a.s.l., 6 November 1958, fruiting, *S.W.Zhao 0169* (HITBC0002814) • Mengyang Township, in the Mengyang sub-reserve of Xishuangbanna National Nature Reserve, 22°17'25"N, 100°51'35"E, 992 m a.s.l., 7 July 2019, flowering, *J.W.Li & L.Wang G3-788* (HITBC-XSBN001702) • Puwen, Dakaihe, 22°35'N, 101°2'E, 840 m a.s.l., 14 August 1977, fruiting, *G.D.Tao 16937* (HITBC0002837) • Lincang: Cangyuan County: Banhong Township, humid ravine rainforest, 23°18'N, 99°6'E, 750 m a.s.l., 19 May 1974, flowering, *Y.H.Li 11528* (HITBC0002827) • Pu ‘er City: Jiangcheng County: ravine rainforest beside the Tuka River, 4 August 2011, fruiting, *S.S.Zhou 10482* (HITBC0002897) • Simao District: Nanping Township, Taiyanghe Provincial Nature Reserve, forest by the ravine, 1 September 2011, fruiting, *S.S.Zhou 10760* (HITBC0002787) Ximeng County: Zhongke Township, near river, 22°49'28.47"N, 99°36'58.35"E, 854 m a.s.l., 26 June 2020, flowering, *D.P.Ye 1067* (HITBC0062765, HITBC0062766). Laos • Oudomxay Province: Beng Township: Nahom Village, sandy soil wet valley rain forest, 880 m a.s.l., 3 November 1996, flowering, *H.Wang 2392* (HITBC0002829).

Acknowledgements

The authors sincerely thank the Herbaria HITBC, KUN, PE, K, MO, NY, P, IBK, IBSC, LE, A, E etc., for providing answers to specimen queries and digital specimen information resources. We also thank Xiao-Wen Liao from Central South University of Forestry and Technology for providing the images of *E. straminea*.

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 National Natural Science Foundation of China (grant no. 31970223 awarded to Yun-Hong Tan), a Project of the Yunnan Province Science and Technology Department (Grant No. 202303AK140009, 202405AC350011, 202203AP140007), a Project of the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (Y4ZK111B04), a Project of Yunnan Wildlife Conservation (2023SJ09X-05) and the 14th Five-Year Plan of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Author contributions

Conceptualisation: YHT. Investigation: DLQ, YHT. Data curation: DLQ. Resources: YHT. Writing – original draft: DLQ. Writing – review and editing: YHT. All authors have read and approved the final manuscript.

Author ORCIDs

Dong-Li Quan https://orcid.org/0000-0002-0749-2007
Yun-Hong Tan https://orcid.org/0000-0001-6238-2743

Data availability

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

References

Andersson L (2001) *Margaritopsis* (Rubiaceae, Psychotrieae) is a pantropical genus. Systematics and Geography of Plants 71(1): 73–85. https://doi.org/10.2307/3668754

Andersson L (2002) Relationships and generic circumscriptions in the *Psychotria* complex (Rubiaceae, Psychotrieae). Systematics and Geography of Plants 72: 167–202.

Applequist WL (2014) Report of the nomenclature committee for vascular plants: 66. Taxon 63(6): 1358–1371. https://doi.org/10.12705/636.20

Barrabé L, Davis AP (2013) (2207) Proposal to conserve the name *Margaritopsis* against *Eumachia* (Rubiaceae). Taxon 62(5): 1069–1070. https://doi.org/10.12705/625.36

Barrabé L, Buerki S, Mouly A, Davis AP, Munzinger J, Maggia L (2012) Delimitation of the genus *Margaritopsis* (Rubiaceae) in the Asian, Australasian and Pacific region, based on molecular phylogenetic inference and morphology. Taxon 61(6): 1251–1268. https://doi.org/10.1002/tax.616007

Barrabé L, Mouly A, Florence J (2013) Psychotriae (Rubiaceae) neocaledonicarum specierum nomenclator. Adansonia 35(2): 281–357. https://doi.org/10.5252/a2013n2a6

Berger A, Valant-Vetschera K, Schinnerl J, Brecker L (2022) A revised classification of the sister tribes Palicoureeae and Psychotrieae (Rubiaceae) indicates genus-specific alkaloid accumulation. Phytochemistry Reviews 21: 941–986. https://doi.org/10.1007/s11101-021-09769-x

Bijmoer R, Arias Guerrero S, Creuwels J (2024) Naturalis Biodiversity Center (NL) – Botany. Naturalis Biodiversity Center. https://doi.org/10.15468/ib5ypt [accessed 1 March 2025]

Chen T, Taylor CM (2011) *Psychotria* (Rubiaceae) In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, Vol. 19. Science Press and Missouri Botanical Garden, Beijing and St. Louis, 57–368.

Davis AP, Bridson D, Jarvis C, Govaerts R (2001) The typification and characterization of the genus *Psychotria* L. (Rubiaceae). Botanical Journal of the Linnean Society 135(1): 35–42. https://doi.org/10.1111/j.1095-8339.2001.tb02366.x

de Candolle AP (1830) Prodromus systematis naturalis regni vegetabilis, sive, Enumeratio contracta ordinum generum specierumque plantarum huc usque cognitarium, juxta methodi naturalis, normas digesta. Sumptibus Sociorum Treuttel et Würtz 4: 478. https://doi.org/10.5962/bhl.title.286

Delprete PG, Kirkbride Jr JH (2015) New combinations in *Eumachia* (Rubiaceae) for species occurring on the Guiana Shield. Journal of the Botanical Research Institute of Texas 9(1): 75–79.

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. International Union for Conservation of Nature, Gland and Cambridge.

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

Moat J, Bachman S, Walker B (2023) ShinyGeoCAT – Geospatial Conservation Assessment Tools (BETA) [Software]. https://spbachman.shinyapps.io/geocat_staging/ [accessed 3 March 2025]

POWO (2025) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ [accessed 3 March 2025]

Razafimandimbison SG, Rydin C (2024) Phylogeny and classification of the coffee family (Rubiaceae, Gentianales): Overview and outlook. Taxon 73(3): 673–717. https://doi.org/10.1002/tax.13167

Razafimandimbison SG, Taylor CM, Wikström N, Pailler T, Khodabandeh A, Bremer B (2014) Phylogeny and generic limits in the sister tribes Psychotrieae and Palicoureeae (Rubiaceae): Evolution of schizocarps in *Psychotria* and origins of bacterial leaf nodules of the Malagasy species. American Journal of Botany 101(7): 1102–1126. https://doi.org/10.3732/ajb.1400076

Robbrecht E (1988) Tropical woody Rubiaceae. Opera Botanica Belgica 1: 1–271.

Robbrecht E (1993) Supplement to the 1988 outline of the classification of the Rubiaceae: Index to genera. Opera Botanica Belgica 6: 173–196.

Santos AF, do Carmo AAO, Harthman VC, Romagnolo MB, Souza LA (2021) Can fruit ontogenetic features prove to be an important tool in the circumscription of Psychotrieae alliance? Australian Systematic Botany 34(6): 527–540. https://doi.org/10.1071/SB20020

Taylor CM (2005) *Margaritopsis* (Rubiaceae, Psychotrieae) in the Neotropics. Systematics and Geography of Plants 75(2): 161–177. https://doi.org/10.2307/3668574

Taylor CM (2016) Rubiacearum americanarum magna hama pars XXXI: More new Neotropical species and morphological notes for *Psychotria* (Psychotrieae). Novon 24(4): 413–434. https://doi.org/10.3417/2015021

Taylor CM (2020) Overview of *Psychotria* in Madagascar (Rubiaceae, Psychotrieae), and of Bremekamp's foundational study of this group. Candollea 75(1): 51–70. https://doi.org/10.15553/c2020v751a5

Taylor CM, Razafimandimbison SG, Barrabé L, Jardim JG, Barbosa MRV (2017) *Eumachia* expanded, a pantropical genus distinct from *Psychotria* (Rubiaceae, Palicoureeae). Candollea 72(2): 289–318. https://doi.org/10.15553/c2017v722a6

Taylor CM, Schmidt HH, Gereau RE (2023) More new species of *Psychotria* from Madagascar (Rubiaceae, Psychotrieae). Novon 31(1): 10–32. https://doi.org/10.3417/2023772

Turner IM (2018) Three new combinations in *Eumachia* (Rubiaceae–Palicoureeae) from southeast Asia. Edinburgh Journal of Botany 76(1): 1–5. https://doi.org/10.1017/S0960428618000227

Wilson EH (1916) Plantae Wilsonianae: An enumeration of the woody plants collected in western China for the Arnold Arboretum of Harvard University during the years 1907, 1908, and 1910. The University Press 3(2): 416. https://doi.org/10.5962/bhl.title.191

PhytoKeys 255: 247–260 (2025)
DOI: 10.3897/phytokeys.255.149589

Research Article

Taxonomic studies on the genus *Hydrocotyle* (Apiales) from China: The distribution and morphology of *H. chiangdaoensis*, with *H. calcicola* reduced to a synonym

Jun Wen[1], Jun-Wen Zhu[1], Ren-Bin Zhu[2], Chun-Feng Song[1]

1 *Jiangsu Key Laboratory for the Research and Utilization of Plant Resources, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (Nanjing Botanical Garden Mem. Sun Yat-Sen), Nanjing, China*
2 *Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan, China*
Corresponding authors: Chun-Feng Song (cfsong79@cnbg.net); Ren-Bin Zhu (zhurenbin@xtbg.ac.cn)

Abstract

Based on observations of living plants of *Hydrocotyle calcicola* in the field, together with examination of herbarium specimens and descriptions of both *H. calcicola* and *H. chiangdaoensis* (including type material), we demonstrated that *H. calcicola* is a synonym of *H. chiangdaoensis*. The species was previously compared with *H. sibthorpioides*; our phylogenetic analysis revealed that *H. chiangdaoensis* and *H. sibthorpioides* belong to different lineages, the former being closely related to the larger-leaved clade.

Key words: *Hydrocotyle*, synonym, taxonomy, Yunnan

Academic editor: Alexander Sennikov
Received: 17 February 2025
Accepted: 10 April 2025
Published: 30 April 2025

Citation: Wen J, Zhu J-W, Zhu R-B, Song C-F (2025) Taxonomic studies on the genus *Hydrocotyle* (Apiales) from China: The distribution and morphology of *H. chiangdaoensis*, with *H. calcicola* reduced to a synonym. PhytoKeys 255: 247–260. https://doi.org/10.3897/phytokeys.255.149589

Introduction

The genus *Hydrocotyle* Tourn. ex L. contains approximately 180 species (Plants of the World Online, http://www.plantsoftheworldonline.org/). The native range of this genus is cosmopolitan, with Australia, South America, and China as three diversity distribution centers. *Hydrocotyle* was formerly a member of the family Apiaceae, but was later transferred to Araliaceae based on molecular phylogenetic studies (Chandler and Plunkett 2004; Plunkett et al. 2004; Nicolas and Plunkett 2009). Subsequently, Wen et al. (2024) highlighted the independent status of this genus within Apiales based on the analysis of chloroplast genome data. All these analyses have suggested that the genus *Hydrocotyle* is key to further study on the evolution of Apiales.

We have conducted a comprehensive study of *Hydrocotyle* in China and found that there are 16 species, 2 varieties, and 2 subspecies in the country, among which Yunnan Province is most species-rich (Sheh et al. 2005; Pimenov 2017). Some species occurring in Yunnan were also found in adjacent areas, such as *H. sibthorpioides* Lam., *H. javanica* Thunb., and *H. siamica* Craib, which were also found in Myanmar and Thailand (Watson and Smith 2004; Pimenov 2017). Studies on Myanmar's plant diversity have been conducted in recent years. A newly recorded species of *Hydrocotyle*, *H. chiangdaoensis* Murata, has been reported by Kang et al. (2018), which was formerly known to be endemic to Thailand. However, the photo of this species was very similar to *H. calcicola* Y.H.Li, which was endemic to Xishuangbanna, Yunnan Province, China (Sheh et al. 2005). Based on this, we conducted a comparative study between the two species.

Hydrocotyle chiangdaoensis was recognized as a new species by G. Murata in 1973 and was described based on *Murata G. et al.* T-15040 (holotype KYO 00028951, Fig. 1A; isotypes KYO 00028952, AUU, TI 00083127, and L 0008361, Fig. 2A–D), collected in Doi Chiang Dao, Northern Chiang Mai, Thailand (Murata 1973). In the protologue, Murata emphasized four distinguishing features of *H. chiangdaoensis* – palmate-dissected stipules, annual habit, not emitting any roots from the nodes, and limestone area endemic. Specifically, the annual habit was speculated because the stem and branches have terminal inflorescences (Murata 1973).

A collection of this species from Doi Chiang Dao (*M. Tagawa & K. Iwatsuki* T-4389, L.2583137) was reported by Hiroe as *H. siamensis* H. Wolff (1921) (Hiroe 1967). The latter name is a synonym of *H. siamica* Craib (Craib 1911), based on the same gatherings (*Hosseus* no. 192; E00000016, P00757659, L.2595136, and M0168575). *Hydrocotyle siamica* was described with long peduncled umbels (*Umbellae* axillarea, solitariae, pedunculo ad 10.5 cm. longo brunneo-puberulo suffultae) and large leaves (*Folia* pentagona, vix vel fere ad medium lobata, ad 9 cm. longa et 10 cm). The diagnostic characters of the specimen L.2583137 (subsequently identified as *H. chiangdaoensis*) are quite different from *H. siamica*, with short peduncled umbels and small leaves.

Figure 1. Holotype sheets of *Hydrocotyle chiangdaoensis* (**A**) and *H. calcicola* (**B**). The arrows indicate the roots of the species.

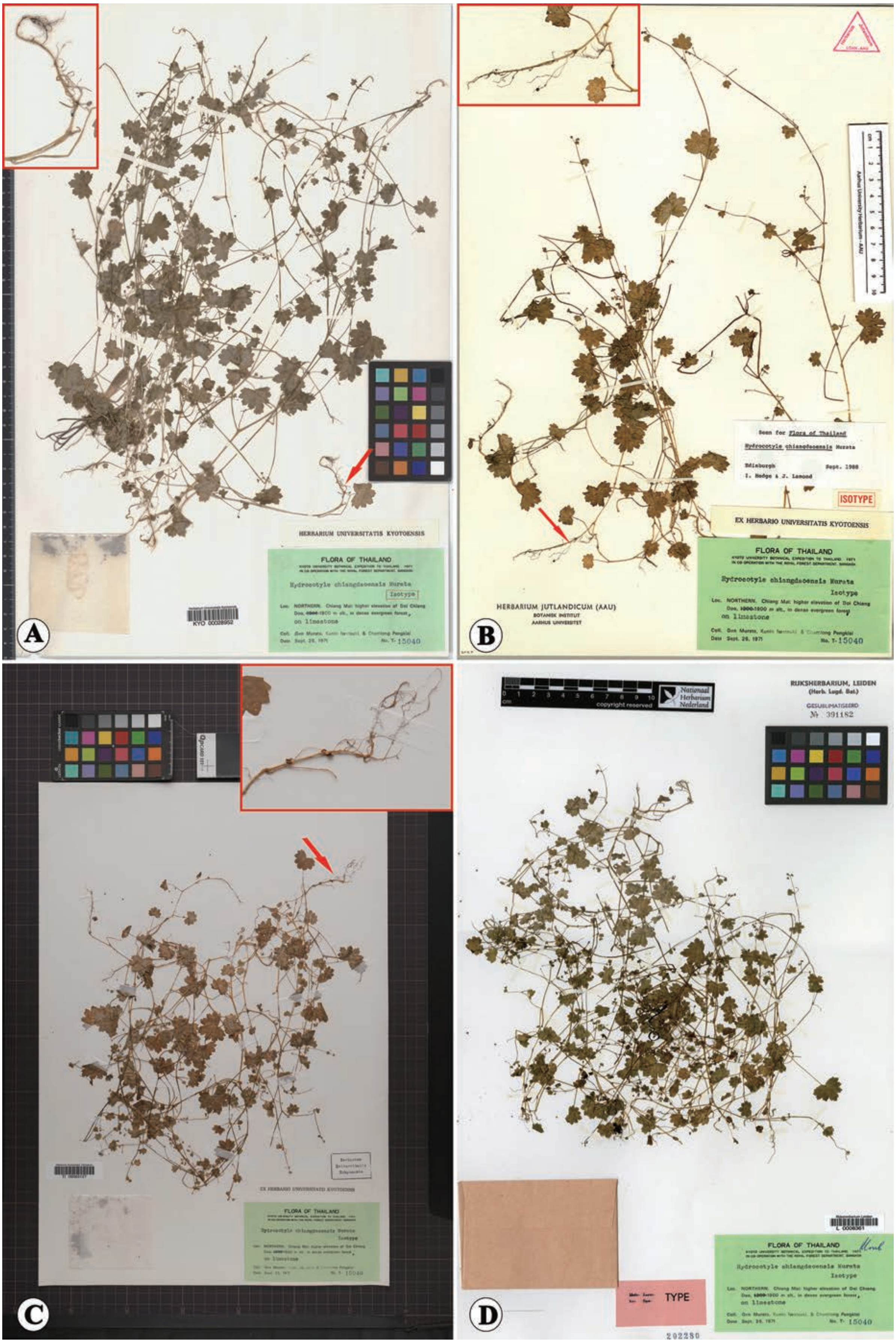

Figure 2. Isotype sheets of *Hydrocotyle chiangdaoensis*. The arrows indicate the roots of the species.

The oldest specimen of this species we found was collected by Kerr in 1922 (*Kerr* 6530, K005513556) from Doi Chiang Dao in Northern Chiang Mai, Thailand. For nearly 100 years since then, this species has only been seen in Doi Chiang Dao. Kang *et al.* (2018) made a new distribution record of this species in Ywangan Township, Southern Shan State, Myanmar, when they conducted joint floristic surveys of this area in 2017. Since then, it has not been seen anywhere else.

Hydrocotyle calcicola Y.H.Li was described on the basis of *G.D.Tao* 13671 (holotype HITBC0037397, Fig. 1B; and isotype KUN0467704, Fig. 3) from Jiluo Shan (according to textual research, it should be Jinuo Mountain), Jinghong City, Yunnan, China (Li and Zhang 1989). In the protologue, Li and Zhang highlighted the features that set this species apart from *H. sibthorpioides* Lam.: membranous leaves conspicuously covered with sparse spinous hairs, umbels of cymes usually terminal with opposite leaves, short and slender peduncles, umbels of 2–5 flowers, and mature fruits not purplish-spotted. Liou (1997) treated *H. calcicola* as a variety of *H. sibthorpioides* due to the weak difference only focused on flowers and fruit. This treatment has not been accepted by *Flora of China* (Sheh et al. 2005), because the habitat of *H. calcicola* was very special. It was noted in the protologue that the habitat of *H. calcicola* was also limestone, and the stipules were irregular, i.e. very similar to *H. chiangdaoensis*. Upon comparing the original descriptions of the two species, we found that many features of both were similar, with only minor differences in the descriptions of their life forms, roots, and fruit. *Hydrocotyle chiangdaoensis* was described as “not emitting any roots from the nodes”, with “annual habit” and “fruit broadly ovate, papillose-setulous, truncate or subcordate at the base”; but *H. calcicola* was described as “perennial herbs”, “rooting at nodes”, and “fruits subcordate, smooth on the outside, ribs conspicuously convex”. However, the annual habit of *H. chiangdaoensis* was speculated on the basis that the stem and branches have terminal inflorescences, which were also a feature of *H. calcicola* (Murata 1973; Li and Zhang 1989). We suggested that *H. calcicola* might be a synonym for *H. chiangdaoensis* based on their similar traits and the order of publication years.

This study aimed to determine the identity of *H. calcicola* and elucidate the distribution and morphological variation of *H. chiangdaoensis*. Our research was conducted mainly on observations of herbarium specimens (including the type material) and living plants in the field. The phylogenetic tree was reconstructed to determine the phylogenetic placement of *H. chiangdaoensis*.

Materials and methods

Morphological analysis

For morphological comparisons, we thoroughly examined specimens or high-resolution images of related *Hydrocotyle* Tourn. ex L. from the following herbaria: AAU, ANUB, AU, BC, BJM, BKF, BM, CAL, CDBI, CSFI, CSH, CZH, E, FJSI, G, GFS, GH, GNUG, GXMG, GZTM, HGAS, HITBC, HNWP, HZ, IBK, IBSC, IMC, IMDY, JIU, JJF, K, KUN, KYO, L, LBG, LE, M, MA, MPU, MW, NAS, NY, P, PE, PEY, QNUN, SANU, SM, SYS, SZ, SZG, TAI, TI, TNS, US, WCSBG, WU, WUK, XBGH, XZ, ZY. Two populations of *H. calcicola* were observed in the field, one from the type locality (Jinuo Mountain, Jinghong City, Yunnan) and another from

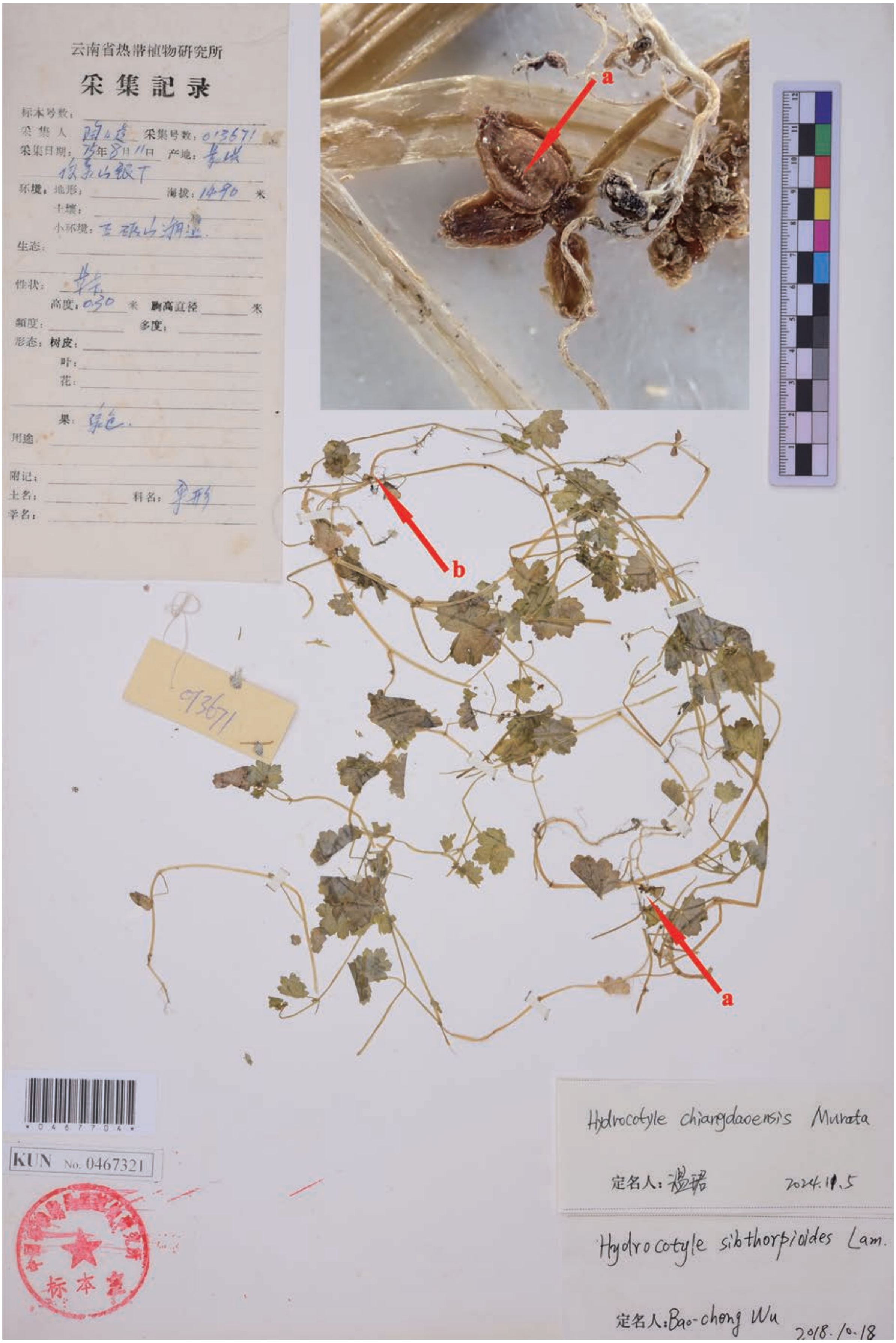

Figure 3. Isotype sheet of *Hydrocotyle calcicola*. The arrows indicate fruit (a) and root (b) of the species.

Ning’er County, Pu’er City, Yunnan. A comprehensive analysis of old herbarium specimens and fresh materials collected during our fieldwork was presented as the result of morphological comparisons.

Phylogenetic analysis

The complete chloroplast genomes were used to reconstruct the phylogenetic trees of the genus *Hydrocotyle* according to the analysis of Wen et al. (2024). A sample of *H. calcicola* from Pu’er was newly sequenced using the Illumina Novaseq 6000 platform at Novogene (Beijing, China), with paired-end reads 2 × 150 bp. The chloroplast genome was assembled using NOVOPlasty v4.3.3 (Dierckxsens et al. 2017). The assembled sequence was checked and annotated under Geneious Prime 2023.2.1 (created by the Biomatters development team, Ltd.), and was subsequently uploaded to the National Center for Biotechnology Information (NCBI) with accession number PV094900.

A total of twelve taxa from the genus *Hydrocotyle* have been sampled for phylogenetic analysis, including nine species. Two species from Apiaceae were selected as outgroups [*Dickinsia hydrocotyloides* Franch., and *Eryngium campestre* L.]. The whole genome sequence matrix was generated from MAFFT v7 (Katoh and Standley 2013). Two methods were employed to conduct phylogenetic analysis: Bayesian inference (BI) and maximum likelihood (ML). The best-fit model “GTR + I + G” was recommended by jModelTest v2.1.4 (Darriba et al. 2012). The ML analysis was performed by RAxML v8.2.4 (Stamatakis 2014). Rapid bootstrap analysis was implemented using 1000 bootstrap replicates to search for the best ML tree. MrBayes v3.2.7a (Ronquist et al. 2012) was employed to conduct the BI analysis. Two independent Markov chain Monte Carlo (MCMC) runs were performed, each with three heated chains and one cold chain for 10,000,000 generations. The average standard deviation of split frequencies should approach zero. Each run started with a random tree, sampling trees every 1000 generations, with the initial 25% discarded as burn-in. The posterior probability (PP) and bootstrap support (BS) were used to measure the supports of the phylogenetic tree implemented under BI and ML, respectively. The final trees were viewed and edited in FigTree v1.4 (Rambaut 2012).

Results and discussion

The type materials of *Hydrocotyle chiangdaoensis* (Figs 1A, 2) are small erect herbs, leaves cordate-orbiculate, gradually smaller above, slightly hairy above towards the veins, glabrous beneath, palmately 7-lobed, lobes usually 3-toothed, teeth obtuse, blades (0.6)1–2(2.5) cm long, (0.7)1.5–2.5(3) cm wide; petioles (0.7)1.5–3.5(5) cm long; stipules membranous flabellate-orbiculate, 1–5 mm wide, palmately dissected; umbels terminal or axillary of the branch or opposite the leaves of the branches, terminal cymose umbels 2–3, thin peduncles 0.5–6 mm long, umbel 2–4 flowers, pedicels very short, membranous bracts minute ovate-lanceolate; petals ovate-lanceolate; fruit broadly ovate, 1–1.2 mm long, papillose-setulous, base truncate or subcordate, stylopodia shortly conoid, short styles reflexed at the end. The root system of this species was described as “not emitting any roots from the nodes” in the protologue. However, we found that some individuals have roots growing from two or three nodes at the base (Fig. 2A–C).

Type specimens of *H. calcicola* (Figs 1B, 3) have shown that this species was rooting only at nodes of the basal stem and never elsewhere, the same as *H. chiangdaoensis*. The holotype of *H. calcicola* has membranous leaves subrounded or cordate, conspicuously covered with sparse spiny hairs above, glabrous beneath, cordate at the base, 5–7-lobed at the apex, lobes broadly obovate, crenate at the margin, 6–8 palmate nerves, 0.5–1.5 cm long, 0.7–2.5 cm wide; petiole 0.7–3 cm long, glabrous; stipules small, kidney-shaped, thinly branched, irregularly; cymose umbels usually terminal with opposite leaves, umbels 2–3, slender peduncles not quite equilong, laterals about 1 cm long, middle 1–2 mm long, umbel 2–5 flowers, flowers sessile, bracts ovate-lanceolate, about 1 mm long, membranous; petal ovate, about 0.5 mm long, white, filaments equal to or slightly shorter than the petal, anthers ovate; style about 0.2 mm long; fruits subcordate, 1–1.3 mm long, 0.8–1.2 mm wide, flat on both sides, smooth or with papillose setae on the outside, ribs conspicuously convex (Fig. 1B). Fruits with papillose setae were also discovered in the isotype (Fig. 3). These morphological features were corroborated through field observations of living plants in Jinuo Mountain, Jinghong City, Yunnan (Fig. 4), the type locality of *H. calcicola*. At the same time, after observing live plants in the type locality, we found that the young fruits are sometimes smooth, but most fruits have papillose setae in the furrow. The additional observations of living plants in Pu'er show that most of the traits of this population were consistent with the description of *H. calcicola*, including habitat, roots, leaves, stipules, terminal umbels, and fruits (Fig. 5). Our analysis added some additional information about this species. Two types of stipules in this population were observed, one irregularly divided and one palmately dissected (Fig. 5B). The latter form is the same as that of *H. chiangdaoensis*. Two petal numbers (4 and 5) have been observed in this population, marking the first time such numbers have been reported in *Hydrocotyle*.

In our examination of the type specimens (Figs 1B, 3) and field observation (Figs 4, 5) of *H. calcicola*, particularly concerning critical diagnostic characters, such as roots, leaves, stipules, inflorescence, and fruits, no significant differences were observed when compared to *H. chiangdaoensis*. In conclusion, *H. calcicola* is morphologically indistinguishable from *H. chiangdaoensis*. Therefore, we propose to synonymise *H. calcicola* with *H. chiangdaoensis*.

As previously noted, *H. calcicola* was compared with *H. sibthorpioides* by its discoverers (Li and Zhang 1989) and subsequently treated as a variety of the latter (Liou 1997). When we examined the specimens, we also found that the specimens of *H. calcicola* were often misidentified as *H. sibthorpioides* by various researchers. Thus, we here provide a detailed morphological comparison between the two species in Table 1, Fig. 6. The results show that there are clear morphological distinctions (especially in roots, inflorescences, and mericarps) between the two species. The phylogenetic analysis based on the chloroplast genome was implemented to clarify the relationship between these two species. Phylogenetic trees reconstructed by BI and ML methods both recovered a stable topology within the genus *Hydrocotyle* with strong support. Within the genus, *H. chiangdaoensis* and *H. sibthorpioides* were located in two different branches (Fig. 7). Two accessions of *H. chiangdaoensis* were gathered together, forming a sister branch of the larger-leaved clade (Clade I, Wen et al. 2024). The results of the morphological and phylogenetic analyses confirm that *H. chiangdaoensis* and *H. sibthorpioides* are different taxonomic entities.

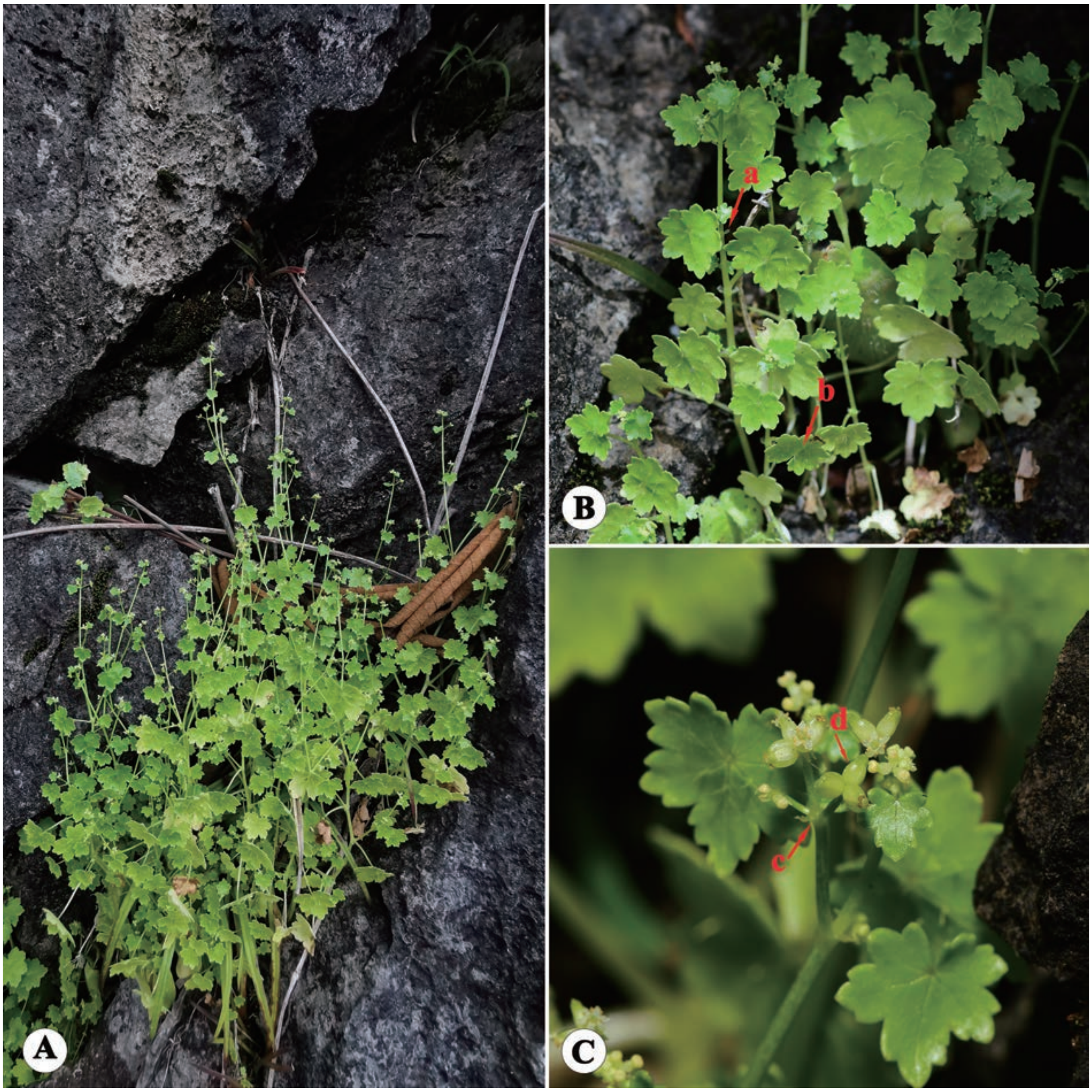

Figure 4. *Hydrocotyle chiangdaoensis* in the wild (China, Yunnan, Jinghong, Mountain Jinuo, the type locality of *H. calcicola*) **A** habitat **B** habit, with the arrows indicating stipule (a) and leaf epidermal spines (b) **C** the terminal inflorescence, with the arrows indicating cymose umbels (c) and fruit (d). Photographed by Ren-Bin Zhu.

Taxonomic treatments

Hydrocotyle chiangdaoensis **Murata, Acta Phytotax. Geobot. 25: 97. 1973.**
Common name. 石山天胡荽 [Pinyin: shí shān tiān hú suī]

= *Hydrocotyle calcicola* Y.H.Li, Guihaia 9: 25. 1989. = *Hydrocotyle sibthorpioides* var. *calcicola* (Y.H.Li) S.L.Liou, Fl. Yunnanica 7: 363. 1997. Type: China. Yunnan, Jinghong City, Jinuo Mountain, 11 August 1975, *G.D.Tao 13671* (holotype: HITBC0037397; isotype: KUN0467704).

Type. Thailand. Northern Chiang Mai, 26 September 1971, *Murata G. et al.* T-15040 (holotype: KYO00028951; isotype: KYO00028952, L0008361; TI00083127; AAU). Figs 1A, 2.

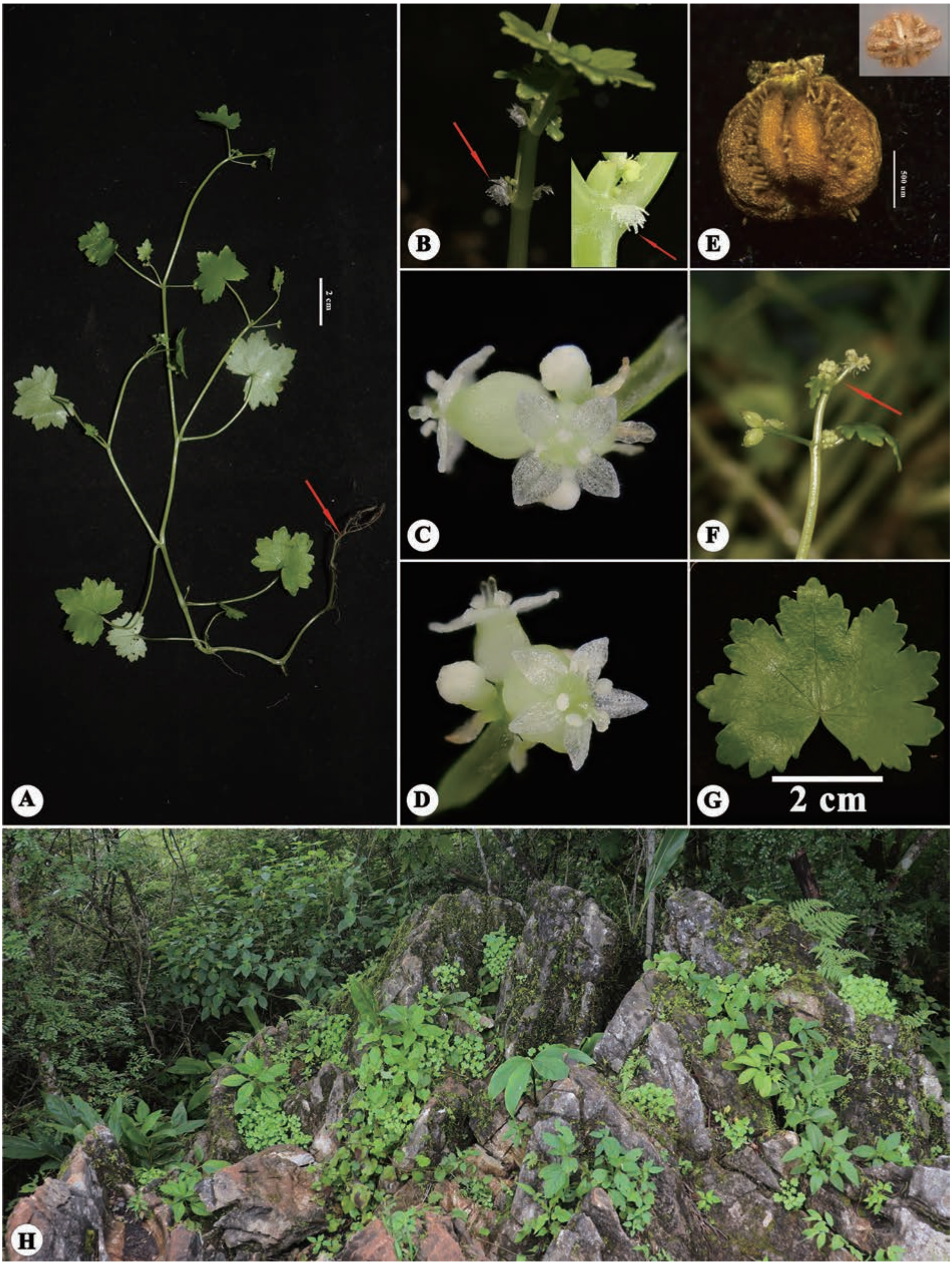

Figure 5. *Hydrocotyle chiangdaoensis* in the wild (China, Yunnan, Pu'er) **A** plant, with the arrow indicating root **B** stipules **C, D** flowers **E** fruit **F** the terminal inflorescence **G** leaf **H** habitat. Photographed by Jun Wen.

Etymology. This species is currently found only in limestone areas, so we have retained the Chinese name 石山天胡荽 [Pinyin: shí shān tiān hú suī] of *Hydrocotyle calcicola*.

Table 1. Morphological comparisons between *Hydrocotyle sibthorpioides* and *H. chiangdaoensis*.

	H. chiangdaoensis	***H. sibthorpioides***
Habitats	limestone areas, shady and moist places	forests, wet grassy places, stream banks
Roots	rooting only at nodes of the basal stem and never elsewhere	rooting at the nodes
Stems	erect or ascending, slender, branched, with ascending branches 2–20 cm long	weak, slender, filiform, creeping, diffusely branched
leaves	membranous leaves subrounded or cordate, palmately 5–9-lobed, conspicuously covered with sparse spiny hairs above towards the veins, glabrous beneath	membranous, leaf blade reniform-rounded, base cordate, crenate, shallowly 5–7-lobed or nearly entire, variably hairy, adaxially glabrous and abaxially sparsely strigose along veins, or sometimes both surfaces glabrous or densely puberulous
Stipules	membranous, thin, palmate-dissected or irregularly dissected, without spots	membranous, entire or irregularly lobed, with purplish stains
Inflorescences	umbels usually solitary at the nodes, with terminal cymose umbels	umbel solitary at the nodes
Rays of umbels	terminal 2–3, axillary 1	1
Bracteoles	membranous, ovate-lanceolate, minute	ovate to ovate-lanceolate, membranous, with bright yellow glands
Umbellules	2–5-flowered, sessile flowers	5–8-flowered
Flower	filaments equal to or slightly shorter than the petals, petals 4–5	filaments equal to or slightly longer than the petals, petals 5
Mericarps	broadly ovate or subcordate, papillose-setulous or sometimes smooth on the outside, ribs conspicuously convex; not easy to separate when mature	broadly globose, greenish-yellow when young, glabrous, covered with purplish stains when mature, intermediate ribs very prominent; easily separated when mature

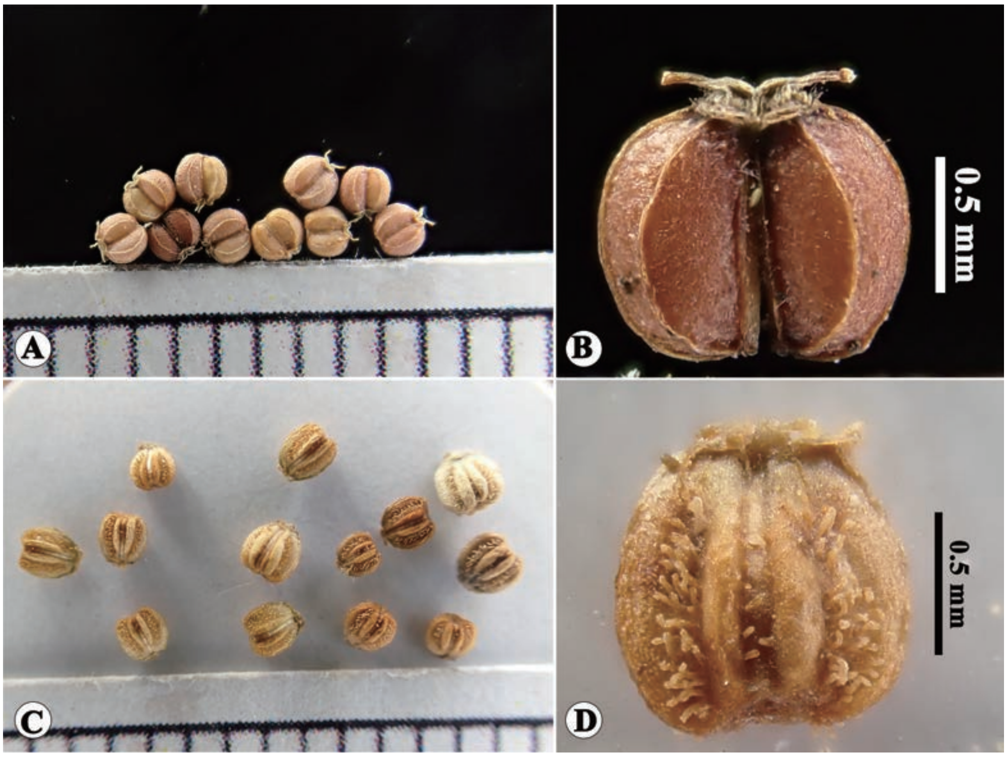

Figure 6. Fruits of *Hydrocotyle sibthorpioides* (China, Hunan, Zhangjiajie, NAS00638796) and *H. chiangdaoensis* (China, Yunnan, Pu'er) **A, B** fruits of *H. sibthorpioides* **C, D** fruits of *H. chiangdaoensis*.

Description. Herbs, 15–60 cm tall, glabrous. Stems erect or ascending, slender, branched, with ascending branches 2–20 cm long, rooting only at nodes of the basal stem and never elsewhere. Membranous leaves subrounded or

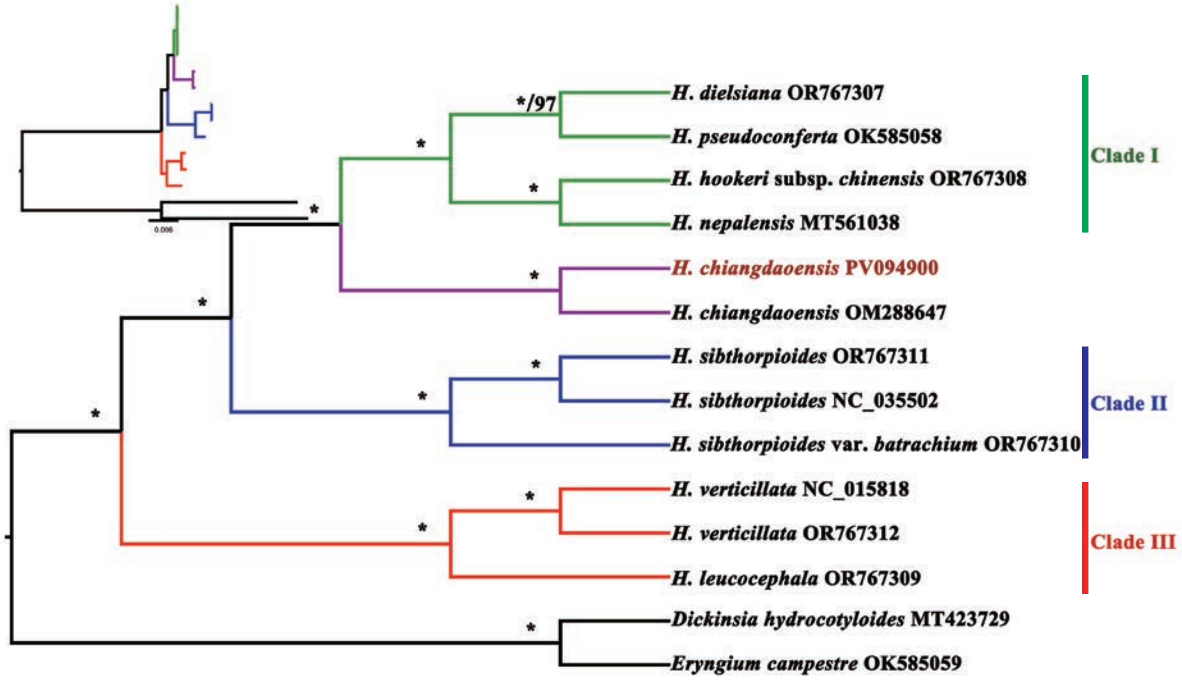

Figure 7. Phylogenetic tree inferred from 14 whole chloroplast genome sequences. Support values marked above the branches follow the order Bayesian inference (PP, posterior probability) / maximum likelihood (BS, bootstrap support), * represent the best support (100%).

cordate, gradually smaller above, palmately 5–9-lobed, lobes usually crenate at the margin, conspicuously covered with sparse spiny hairs above towards the veins, glabrous beneath, 0.5–4.5 cm long, 0.7–4 cm wide. Petiole 0.7–3.5 (5) cm long, glabrous. Stipules membranous flabellate-orbiculate, 1–5 mm wide, palmate-dissected or irregularly. Terminal umbels cymose, umbels 2–3, and usually solitary at the other nodes; slender peduncles not quite equaling, 0.5–10 mm long; umbel 2–5-flowered, sessile; bracts ovate-lanceolate, about 1 mm long; petals ovate-lanceolate, about 0.5 mm long, white, 4 or 5; membranous filaments equal to or slightly shorter than the petals; anthers ovate; style about 0.2 mm long. Mericarps broadly ovate or subcordate, 1–1.3 mm long, 0.8–1.2 mm wide, with papillose setae in the furrow or sometimes smooth on the outside, ribs conspicuously convex. The mericarps are not easy to separate when mature.

Distribution. China (Yunnan: Jinghong, Lincang, Pu'er), Myanmar (Southern Shan State: Ywangan Township), and Thailand (Chiang Mai).

Habitat. The species grows on limestone at elevations of 1300–2175 m above sea level, always in dense evergreen forests, shady and moist places.

Phenology. Flowering and fruiting from July to November.

Additional specimens examined. China. **Yunnan Province** • Jinghong City, Jinuo Mountain, 21°59'N, 101°05'E, alt. 1490 m, 11 August 1975, *G.D.Tao 13671* (HITBC081533, KUN0467704) • Lincang City, Yongde County, 24°09'27.3"N, 99°14'58"E, alt. 1923 m, October 2015, *LiYL1395* (KUN1372015) • Pu'er City, Lancang County, 26 September 1993, *Y.Y.Qian* 3024 (HITBC0122528) • Pu'er City, Ning'er County, 23°04'12.74"N, 101°01'43.3"E, alt. 1733–1840 m, 23 August 2023, *WJ2361* (NAS00714697–NAS00714702) • Pu'er City, Ning'er County, 23°04'22.6"N, 101°01'37.7"E, alt. 1798 m, 28 September 2020, *D.P.Ye 1994* (HITBC0063922, PE02521420) • Pu'er City, Ximeng County to Lancang County, 22°45'N, 99°40'E, alt. 1900 m, 24 October 1989, *G.D.Tao et al.* 39828 (HITBC0122527, KUN0462683).

Myanmar. **Southern Shan State** • Ywangan Township, 21°13'50.2"N, 96°31'03.7"E, alt. 1372 m, 6 October 2017, *Kim et al. MM-6405* (HHU).

Thailand. **Chiang Mai** • Doi Chiang Dao, alt. 1300–1900 m, 27 September 1971, *G. Murata et al. T-15147* (K005513596, L.2583778, P03259185) • Doi Chiang Dao, alt. 1900–2175 m, 14 September 1967, T. *Shimizu et al. T-10125* (K005513595, L.2583136) • Doi Chiang Dao, alt. 2000 m, 16 July 1958, *Th. Sørensen et al. 4172* • Doi Chiang Dao, alt. ca. 1700 m, 3 November 1922, *A.F.G. Kerr 6530* (K005513556) • Doi Chiang Dao, 18 October 1926, *no. 412* (K005513592) • Doi Chiang Dao, alt. 1400–1800 m, 5 January 1966, *M. Tagawa & K. Iwatsuki T-4389* (L.2583137) • Doi Chiang Dao, alt. 1975 m, 10 November 1995, *J.F. Maxwell 95-1157* (Topotype, L.4214318).

Note. *Hydrocotyle chiangdaoensis* has been recorded in China, Myanmar, and Thailand. This species is well characterized by its palmate-dissected or irregular stipules, cymose terminal umbels, and papillose-setulous fruits. This species is restricted to limestone areas. *Hydrocotyle chiangdaoensis* differs from *H. sibthorpioides* typically by its roots only growing at nodes of the basal stem and never elsewhere, with terminal cymose umbels and papillose-setulous fruits.

Acknowledgments

We are grateful to the reviewer Prof. Yan Yu and the editor Alexander Sennikov for their valuable comments on the manuscript. We thank Qi-Zhi Wang, Xu-Dong Ma, Xiao-Fang Chu, Shi-Kui Peng, Hong-Bo Ding, Jing Li, Qun Liu, and Prof. Yun-Hong Tan for their help during our fieldwork and the curators of AAU, HITBC, K, KUN, KYO, L, NAS, P, PE, and TI for allowing us to use their scanned images of specimens and for research facilities. Thanks are due to Dr. Wei Zhou for his kind help with the suggestions for improving 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 financially supported by the National Natural Science Foundation of China (Grant no. 32200191).

Author contributions

Investigation: RBZ, JWZ, and JW. Methodology: CFS. Writing – original draft: JW. Writing – review and editing: CFS and JW.

Author ORCIDs

Jun Wen https://orcid.org/0000-0002-1926-6517
Jun-Wen Zhu https://orcid.org/0009-0000-5554-445X
Ren-bin Zhu https://orcid.org/0000-0003-3925-1571
Chun-feng Song https://orcid.org/0000-0003-2077-0486

Data availability

All of the data that support the findings of this study are available in the main text. Voucher specimens were identified by Jun Wen and deposited in NAS (Herbarium, Institute of Botany, Chinese Academy of Sciences, Jiangsu Province) with deposition numbers NAS00714697–NAS00714702. Raw reads of the chloroplast genome were uploaded to NCBI and placed under project PRJNA1221964 with accession number SRR32321003.

References

Chandler GT, Plunkett GM (2004) Evolution in Apiales: Nuclear and chloroplast markers together in (almost) perfect harmony. Botanical Journal of the Linnean Society 144(2): 123–147. https://doi.org/10.1111/j.1095-8339.2003.00247.x

Craib G (1911) Umbelliferae. Bulletin of Miscellaneous Information, Royal Gardens, Kew, London 1911(1): 58. https://doi.org/10.2307/4115077

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Dierckxsens N, Mardulyn P, Smits G (2017) NOVOPlasty: De novo assembly of organelle genomes from whole genome data. Nucleic Acids Research 45(4): e18. https://doi.org/10.1093/nar/gkw955

Hiroe M (1967) Umbelliferae of Thailand, II. Acta Phytotaxonomica et Geobotanica 22(4–6): 141. https://doi.org/10.18942/bunruichiri.KJ00001078000

Kang D, Kyaw NO, Jung E, Shin J, Kim Y, Ong HG (2018) New records of flowering plants for the flora of Myanmar collected from southern Shan State. Korean Journal of Plant Taxonomy 48(3): 218–229. https://doi.org/10.11110/kjpt.2018.48.3.218

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

Li YH, Zhang Y (1989) New materials for flora of Xishuangbanna, Yunnan. Guihaia 9(1): 25–29.

Liou SL (1997) *Hydrocotyle sibthorpioides* var. *calcicola* (Y.H.Li) S.L.Liou. In: Wu ZY (Ed.) Flora Yunnanica, Vol. 7. Science Press, Beijing, 363 pp.

Murata G (1973) New or Interesting Plants from Southeast Asia 1. Acta Phytotaxonomica et Geobotanica 25(4–6): 97. https://doi.org/10.18942/bunruichiri.KJ00001078100

Nicolas AN, Plunkett GM (2009) The demise of subfamily Hydrocotyloideae (Apiaceae) and the re-alignment of its genera across the entire order Apiales. Molecular Phylogenetics and Evolution 53(1): 134–151. https://doi.org/10.1016/j.ympev.2009.06.010

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

Plunkett GM, Chandler GT, Lowry II PP, Pinney SM, Sprenkle TS (2004) Recent advances in understanding Apiales and a revised classification. South African Journal of Botany 70(3): 371–381. https://doi.org/10.1016/S0254-6299(15)30220-9

Rambaut A (2012) FigTree v1.4. University of Edinburgh, Edinburgh, UK. http://tree.bio.ed.ac.uk/software/gtree/

Ronquist F, Teslenko M, van der Mark P, Ayres D, 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

Sheh ML, Watson MF, Cannon JFM (2005) *Hydrocotyle* L. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, Vol. 14. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis, 14–17.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Watson MF, Smith A (2004) The Umbellifers of Myanmar: The last piece in the Asian puzzle. South African Journal of Botany 70(3): 427–432. https://doi.org/10.1016/S0254-6299(15)30226-X

Wen J, Wu BC, Li HM, Zhou W, Song CF (2024) Plastome structure and phylogenetic relationships of genus *Hydrocotyle* (Apiales): Provide insights into the plastome evolution of *Hydrocotyle*. BMC Plant Biology 24: 778. https://doi.org/10.1186/s12870-024-05483-w

Wolff H (1921) *Hydrocotyle siamensis* spec. nov. Repertorium Specierum Novarum Regni Vegetabilis 17: 155. https://doi.org/10.1002/fedr.19210170819