

A new species of *Hibiscus* (Malvaceae, Malvoideae) from Guyana

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Abstract

Hibiscus marioniae Dorr, **sp. nov.** is described and illustrated. It evidently is restricted to central Guyana, northeast of the Kanuku Mountains near the Rewa River, a tributary of the Rupununi River. The new species is most similar morphologically to *H. amazonicus* Fryxell, which was described from Amazonas, Brazil.

Keywords

Guyana, *Hibiscus*, Malvaceae, Malvoideae

Introduction

A collection of *Hibiscus* L. (Malvaceae, Malvoideae) made by Marion J. Jansen-Jacobs in central Guyana northeast of the Kanuku Mountains near the Rewa River in 1999 was identified by the late Paul A. Fryxell as “*Hibiscus* aff. *verbasciformis* Klotzsch ex Hochr. vel sp. nov.” No additional material of this taxon has been collected since then, nor have additional specimens been found in searches of herbaria (CAY, K, Naturalis, NY, P, US, W) with rich collections of specimens from the Guianas. Likewise, nothing that matches the Jansen-Jacobs collection has been reported from Brazil (Fernandes Júnior and Coutinho 2022) or the adjacent Venezuelan Guyana (Fryxell 2001). This Guyanese collection does not match *H. verbasciformis*, which is an illegitimate

superfluous name for *H. spathulatus* Garcke, as closely as it matches *H. amazonicus* Fryxell. In French Guiana especially, *H. amazonicus* has been confused with and misidentified as *H. spathulatus*. Although similar to *H. amazonicus*, the *Hibiscus* collection made by Jansen-Jacobs in Guyana is distinct and is described and illustrated below.

Taxonomic treatment

Hibiscus marioniae Dorr, sp. nov.

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Fig. 1

Diagnosis. *Hibiscus marioniae* Dorr differs from *H. amazonicus* Fryxell in having elliptic (versus ovate) leaf blades with cuneate (versus cordate to truncate and often asymmetrical) bases, a crenulate (versus coarsely toothed) margin, and long acuminate (versus acute) apices; more numerous (12 versus 8) and narrower (ca. 1.0 versus (2.0–)3.0–6.0 mm broad) involucellar bracts that are very slightly spathulate (versus distinctly spathulate, broadly lanceolate or imperfectly stipitate-peltate); and glabrous (versus minutely scaberrulous) seeds.

Type. GUYANA. [Upper Takutu-Upper Essequibo]: Upper Essequibo Region, Rewa River, Spider Mountains, 03°08'N, 058°32'W, 400–500 m alt., 20 Sep 1999 (fl, fr), *M.J. Jansen-Jacobs, B.J.H. ter Welle, P.P. Haripersaud, O. Muller & M. van der Zee* 6011 (holotype: U barcode 0067247!; isotypes: NY!, TEX barcode 00568796 as image!).

Description. Suffrutescent herbs, to 50 cm tall; stems woody at base, unarmed, sparingly to moderately pubescent, with appressed 4–8-armed stellate hairs ca. 1 mm in diameter. Leaves simple, elliptic, 8.5–13.0 × 2.5–4.0 cm, base cuneate, palmately 3-nerved at base, midrib and 2° nerves slightly raised above, prominent below, margin crenulate, apex long acuminate, leaf blades sparingly pubescent above and below with yellowish, bifurcate and stellate hairs, stellate hairs 4–8-armed, arms ca. 1.0 mm long, erect, bifurcate hairs more frequent below than above; petioles 1.5–2.5 cm long, with a ventral line of short whitish stellate-hairs and more conspicuous and abundant yellowish stellate hairs, the latter hairs not in a line and denser distally; stipules almost linear, ca. 1.0–2.0 mm long, caducous. Flowers solitary or paired in leaf axils toward apices of stems; pedicels 3.5–7.0 cm long, not articulated, pubescent with ± appressed stellate hairs and more conspicuous, 4–8-armed stellate hairs with arms to 2.0 mm long. Bracts of involucre 12, distinct, 10.0–15.0 × ca. 0.75–1.0 mm, not or scarcely exceeding united portion of calyx at anthesis, very slightly spathulate apically, plane, with simple, bifurcate, and stellate hairs, hairs or arms of stellate hairs to 2.0 mm long. Calyx 5-lobed, united ca. half way, lobes 1.0–1.2 × 0.5–0.6 cm at anthesis, broadly triangular, apices acute, papery, light green with 3 darker but not thickened veins, nectaries absent, outer surface with yellowish simple and mostly 4-armed stellate hairs, arms to 2.0 mm long, inner surface with a few scattered simple hairs, accrescent in fruit, turning blackish (on herbarium specimens, at least), lobes expanding to 2.0–2.5 × 1.2 cm. Petals ca. 2.5 cm

long (only one flower seen and not dissected), white (fide *Jansen-Jacobs et al. 6011*), corolla shape unknown. Staminal column shorter than the petals; anthers purple (fide *Jansen-Jacobs et al. 6011*). Styles and stigmas not seen. Capsules enclosed in accrescent calyces, 5-locular, chartaceous, capsule walls undulate and molded around individual seeds, walls covered with minute whitish hairs and more conspicuous yellowish simple hairs to 2.0 mm long. Seeds ca. 2.0×2.0 mm, globose-reniform, brownish (the hilum blackish), glabrous.

Etymology. The species epithet honors Marion J. Jansen-Jacobs who has contributed greatly to our understanding of the flora of the Guianas as collector, herbarium curator, author, and executive director of the Flora of the Guianas project.

Distribution and ecology. Known only from the type collection, which was made in central Guyana, northeast of the Kanuku Mountains; 400–500 m alt. According to information on the specimen label, the plant was found in an open spot in forest on rock in the “Spider Mountains.” The name of these mountains does not appear in standard gazetteers (Stephens and Traylor 1985; Anonymous 1993; Guyana Lands and Surveys Commission 2019) and it may have been a name created by the collectors of the type.

Discussion. *Hibiscus* as traditionally treated is a species-rich genus of ca. 200 (Hochreutiner 1900) to over 400 species (POWO 2022). It is included in the Hibisceae, a tribe defined by loculicidally dehiscent (“capsular”) fruit, lack of gossypol glands, 5-toothed staminal column apex, styles usually apically branching, stigmas usually terminal, and style branches equal in number to the carpels (Pfeil et al. 2002). Molecular data, however, suggest that the traditional concept of *Hibiscus* that is based on morphology created a “severely” paraphyletic genus (Pfeil et al. 2002; Pfeil and Crisp 2005; Koopman and Baum 2008), which has other tribes (Decaschistieae and Malvavisceae) and other genera of Hibisceae nested within it. Infrageneric classification of *Hibiscus* also has been problematic (Pfeil and Crisp 2005) with unresolved conflicts between the sectional classifications based on morphology proposed by de Candolle (1824), Grisebach (1859), Gürke (1892), Hochreutiner (1900), Ulbrich (1921), van Borssum Waalkes (1966), and Fryxell (1988). This makes assigning *H. marioniae* to a section problematic.

Morphologically, *Hibiscus marioniae* is most similar to *H. amazonicus*. The two species share unarmed stems, an involucler comprised of distinct bracts, a papery or chartaceous 5-fid calyx that is ca. half-divided and accrescent in fruit, and a staminal column shorter than the petals. When Fryxell (1984) described *H. amazonicus*, he compared it to three other species found in South America (*H. dimidiatus* Schrank, *H. sororius* L., and *H. spathulatus* Garcke) and stated they formed a natural grouping or alliance, but he refrained from naming this group. It appears that Fryxell made the connection to this group because as stated in his protologue, *H. amazonicus* keyed out (somewhat ambiguously) in Gürke (1892) and Kearney (1957) either to *H. sororius* or *H. verbasciformis* ($\equiv$ *H. spathulatus*). Morphologically, especially in vestiture, these three species are very similar to each other, and they only can be distinguished easily by the shape of their involucellar bracts.



Figure 1. *Hibiscus marioniae* Dorr, sp. nov. **A** habit **B** detail of calyx showing vestiture **C** flower showing corolla, calyx, and involucellar bracts **D** seeds lateral views **E** seed showing hilum (**A–E** from Jansen-Jacobs *et al.* 6011).

Earlier, Grisebach (1859) had made *Hibiscus sororius*, which also occurs in the West Indies and Mexico, the type and sole member of *H.* sect. *Trionastrum* Griseb. He categorized this section as having distinct involucellar bracts that are apically enlarged,

a 5-fid ventricose calyx, and glabrous seeds. While *H. amazonicus*, *H. marioniae*, and *H. spathulatus* could be placed in this section, the cordate-ovate involucellar bracts of the closely allied *H. dimidiatus* and the puberulous or hirtellous seeds of *H. spathulatus* and *H. dimidiatus*, respectively, would require modification of Grisebach's circumscription of his monotypic section.

Gürke (1892) overlooked *Hibiscus* sect. *Trionastrum* when he revised the Brazilian species of *Hibiscus* and he placed *H. sororius*, *H. dimidiatus*, and *H. spathulatus* in sect. *Ketmia* (Mill.) DC. ($\equiv$ sect. *Hibiscus*), which he defined by involucellar bracts subulate-falciform, setaceous, linear, lanceolate, ovate, spathulate, or dilated apically but not bifurcate, and calyx not inflated with eglandular lobes. These characters, especially the negative or absent ones, seem to have been selected to set this section apart from sect. *Furcaria* DC. Nonetheless, little seems to separate sect. *Trionastrum* from sect. *Hibiscus* apart from the calyx being ventricose (i.e., swollen or distended) in the former and not inflated in the latter. Also, the seeds of sect. *Hibiscus* are either glabrous or pubescent but without the distinctive cotton-like hairs found in sect. *Bombicella*. Fryxell (1988) later recognized both sect. *Hibiscus* and sect. *Trionastrum* in his treatment of the Mexican species of *Hibiscus* and placed *H. sororius* in sect. *Trionastrum* but said nothing about the other South American allies of this species, presumably because they do not occur in Mexico.

Hochreutiner (1900) in his revision of *Hibiscus* had a very different assessment of the relationships of the three species that Fryxell (1984) later thought to be allied with *H. amazonicus*. Hochreutiner (1900), who also overlooked *H. sect. Trionastrum*, placed *H. sororius* in sect. *Spatula* Hochr., which he defined by its apically dilated involucellar bracts and glabrous seeds; *H. dimidiatus* in sect. *Trichospermum* Hochr. (= sect. *Hibiscus*); and *H. spathulatus* (as *H. verbasciformis*) in sect. *Trionum* DC. He clearly was unhappy with his sectional placement of *H. spathulatus* since he acknowledged that it was the only species that he included in sect. *Trionum* with lanceolate bracts and deeply lobed calyces.

No members of *Hibiscus* sect. *Trionastrum* (or sect. *Spatula*) were included in the analysis of Pfeil and Crisp (2005: table 2). However, given that *H. marioniae* and its presumed relatives have distinct involucellar bracts and non-inflated calyces, these species will probably be found to belong to the “/Euhibiscus” clade, a rank free clade recognized by Pfeil and Crisp (2005) that contains sect. *Hibiscus*. Thus, irrespective of how the paraphyly in *Hibiscus* and the Hibisceae eventually is resolved taxonomically, *H. marioniae* likely will remain in or closely allied to *Hibiscus* s.str.

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Oreocharis polyneura, a new species of Gesneriaceae from southern Yunnan, China

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Abstract

Oreocharis polyneura, a new species from southern Yunnan, China, is described and illustrated. It is morphologically similar to *O. rhytidophylla* by having more obvious lateral veins forming a crosslinked network on the adaxial surface of leaf blades, but can be distinguished by having more lateral veins (12–15 pairs vs. 7–9 pairs) of leaf blades, shorter corolla tubes (the length ratio of corolla tube to corolla lobes = 1.2–2.9 vs. 3.4–6) and shorter pistils (6–8 mm long vs. 27–30 mm long). In addition, a detailed morphological description, a photographic illustration, the distribution and phenology of the new species are presented.

Keywords

flora of Yunnan, morphology, new taxon, *Oreocharis rhytidophylla*, taxonomy

Introduction

In recent years, the genus *Oreocharis* Benth. has been a hotspot for the research of the family Gesneriaceae. The newly circumscribed genus *Oreocharis* is recently enlarged

by incorporating ten other genera (*Ancylostemon* Craib, *Bournea* Oliv., *Dayaoshania* W.T.Wang, *Deinocheilos* W.T.Wang, *Isometrum* Craib, *Opithandra* B.L.Burt, *Paraisometrum* W.T.Wang, *Thamnocharis* W.T.Wang, *Tremacron* Craib, *Briggsia* Craib s.str.) with high floral diversity (Möller et al. 2011; Weber et al. 2013), although *Bournea* was later reinstated as an independent genus (Chen et al. 2020a). Hitherto, *Oreocharis* s.l. contains ca. 150 taxa mainly distributed in southern and southwestern China (Wen et al. 2021), with a few of them (ca. 15 species) expanding to Bhutan, India, Japan, Myanmar, Thailand, and northern and central Vietnam (Möller et al. 2016, 2017, 2018; Cai et al. 2020a; Le et al. 2022). In China, southern Yunnan is rich in species diversity of the genus, with about 38 species recorded in this area (Li and Wang 2004; Möller et al. 2011; Chen et al. 2012, 2013, 2020b; Tan et al. 2013, 2015; Rossini and Freitas 2014; Cai et al. 2015, 2020; Cai and Dao 2020).

In 2021, during the expedition of the plant resources of tropical Yunnan in China, we collected an interesting plant of Gesneriaceae from Lancang county at anthesis (Figs 1, 2). Judging from the vegetative habit and floral characteristics, we considered it a member of *Oreocharis*. Upon careful comparisons of diagnostic morphological and anatomical features from the closely related species from China and adjacent regions, we determined that the plant is new to science and thus describe it herein. Its morphological characters are compared with the closely related species *Oreocharis rhytidophylla* C.Y. Wu ex H.W. Li (Li 1983; Zhang et al. 2019) (Fig. 3).

Materials and methods

Vouchers of the new species were collected from Lancang County, Yunnan Province, China. Photographs and phenology data were obtained during field expeditions. Morphological examinations and measurements of the new species were conducted on both living plants in the wild and herbarium specimens. All morphological characters are described according to the terminology presented by Wang et al. (1990, 1998). The preliminary conservation assessment for the new species was evaluated according to the guidelines of the IUCN Red List Categories and Criteria (IUCN 2019).

Taxonomic treatment

***Oreocharis polyneura* Y.H.Tan, F.Wen & Y.X.Gong, sp. nov.**

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Figs 1, 2

Diagnosis. *Oreocharis polyneura* is similar to *O. rhytidophylla* in having more obvious lateral veins forming crosslinked network on the adaxial surface of leaf blades, but can be distinguished by having more lateral veins (12–15 pairs vs. 7–9 pairs) of leaf blades, shorter corolla tubes (the length ratio of corolla tube to corolla lobes = 1.2–2.9 vs. 3.4–6) and shorter pistils (6–8 mm long vs. 27–30 mm long).

Type. CHINA. Yunnan Province, Puer City, Lancang County, Zhutang Town, on rocks or cliffs in evergreen broad-leaved forests in ravines, 22°45'44.28"N, 99°43'26.81"E, elev. 2137 m, 29 July 2021, *Y.X. Gong G344* (holotype: HITBC0079099; isotypes: HITBC, IBK). Fig. 2.

Description. Perennial herbs. *Rhizomes* subterete, short, straight, with numerous fibrous roots. *Leaves* basally forming a rosette, 4–8, petiolate; petioles terete, 1.2–7.0 cm long, 3–5 mm in diameter, densely rust-brown pannose, leaf blade thickly chartaceous, elliptic to ovate-elliptic, 4–13 × 2–7 cm, apex rounded, base rounded to subcordate, margin serrate to crenate, adaxially green, glabrous, abaxially whitish-green, densely rust-brown pannose along veins, lateral veins 12–15 on each side of the midrib, adaxially conspicuously sunken, abaxially conspicuously prominent, anastomosed forming on both surfaces. *Inflorescences* cymose, axillary, 2–4, 2–4-branched, 4–10-flowered; peduncles 4.5–9.0 cm long, ca. 1–3 mm in diameter, rust-brown lanate; bracts 3, verticillate, each 8.5–9.5 × 3.2–4.2 mm, lanceolate to elliptic, margins usually entire, occasionally 2–3-denticulate, adaxially glabrous, abaxially rust-brown pannose; pedicels 1.0–3.5 cm long, ca. 1 mm in diameter, sparsely puberulent. *Calyces* actinomorphic, 5-parted from the base, segments equal, linear-lanceolate, 7.5–9.0 × 1.5–2.5 mm, margin dentate, adaxially glabrous, abaxially rust-brown pannose. *Corollas* yellow, 1.8–2.2 cm long, outside glabrous, inside sparsely glandular-puberulent in the throat on adaxial lobes; tube narrowly infundibuliform, slightly bent near the throat, 1–1.5 cm long, 4–5.5 mm in diameter, proximally and distally almost equal in width; limb indistinctively bilabiate, adaxial lip 2-lobed, lobes obovate to elliptic, 5.8–6.2 × 3.8–4.8 mm, apex obtuse, margin entire, abaxial lip 3-lobed, lobes obovate to elliptic, almost equal, 6.2–8.2 × 5.2–6.2 mm, apex rounded, margin entire. *Stamens* 4, included, two pairs of stamens cohering at the anther tips, adnate to corolla tube 4–6 mm from base; adaxial stamens 3.5–4.5 mm long, abaxial stamens 5–6 mm long; filaments yellow, linear, glabrous; anthers basifixed, reniform, 2-locular, dehiscent transversely; staminode 1, 1.4 mm long, adnate to corolla tube 1.8 mm from the base. *Pistils* 6–8 mm long, glabrous; ovary 5–6.5 mm long, ca. 1.5 mm in diameter, glabrous, with two parietal placentae; style 1–1.5 mm long; stigma 1, disciform, retuse, edge flipped outwardly, 1.5–2.0 mm in diameter. *Discs* ring-shaped, ca. 2.3 mm high, margin slightly undulate with 5 irregularly and shallow lobes. *Capsules* indistinctively 4-angled to subterete, ca. 4 cm long, ca. 3 mm in diameter, glabrous.

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

Etymology. Greek *polys*, many, and *neuron*, nerve, alluding to abaxially conspicuous lateral leaf veins.

Habitat and distribution. Endemic to Zhutang town, Lancang county, Puer city, Yunnan Province, China (Fig. 4). It grows on rocks or cliffs under evergreen broad-leaved forests in ravines at elevations of 1900–2200 m.

Conservation status. *Oreocharis polyneura* is currently known only from its type locality. Based on our present knowledge and available data, its conservation status is assessed as “Data Deficient” (DD; IUCN 2019).

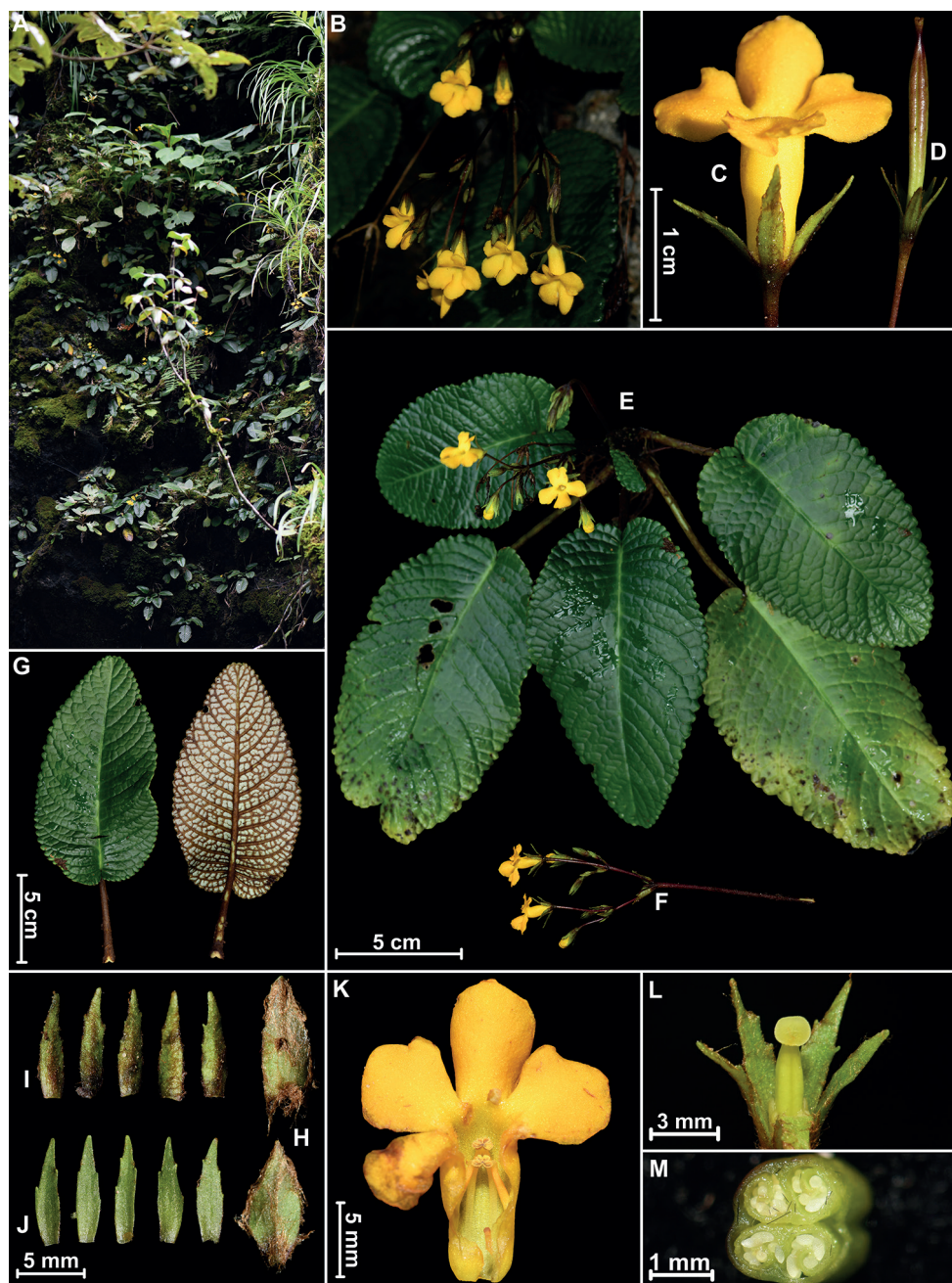


Figure 1. *Oreocharis polyneura* Y.H.Tan, F.Wen & Y.X.Gong, sp. nov. **A** habit **B** inflorescence **C** behind view of flower **D** fruit **E** whole plant **F** inflorescence with scale **G** adaxial and abaxial leaf surfaces **H** bracts **I, J** calyces **K** opened corolla showing stamens and staminode **L** pistil with disc and calyces **M** cross section of fruit. Photographed by Yan-Xiong Gong.



Figure 2. Type sheet of *Oreocharis polyneura* Y.H.Tan, F.Wen & Y.X.Gong, sp. nov.



Figure 3. Type sheet of *Oreocharis rhytidophylla* C.Y. Wu ex H.W. Li.

Vernacular name. The Chinese name of the new species is “Duō Mài Mǎ Líng Jù Tái” (多脉马铃薯苔). The first two words, Duō Mài mean the numerous veins of the leaf blades, and the rest four words mean the genus *Oreocharis* in mandarin.

Notes. Morphologically, *Oreocharis polyneura* is most similar to *O. rhytidophylla* in its leaves and inflorescences but is readily distinguishable by an array of characters (see Table 1).

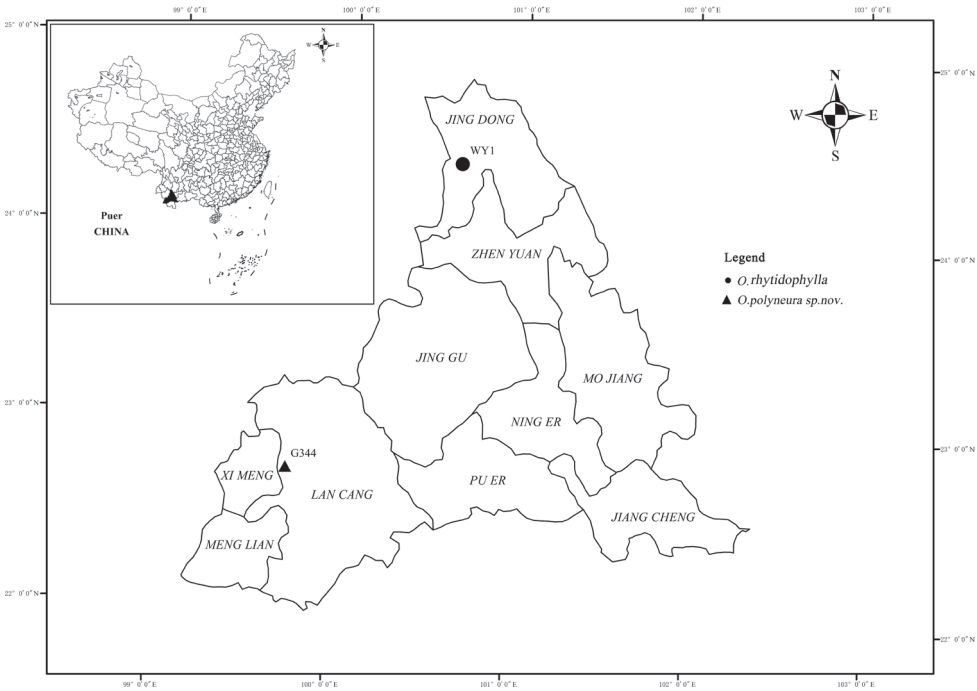


Figure 4. Distribution of *Oreocharis polyneura* (black triangle) and *O. rhytidophylla* (black circle).

Table 1. Morphological comparison between *Oreocharis polyneura* and *O. rhytidophylla*.

Character	<i>Oreocharis polyneura</i>	<i>Oreocharis rhytidophylla</i>
Petiole	terete	plane
Leaf	lateral veins 12–15 pairs	lateral veins 7–9 pairs
Peduncle	4.5–9 cm long	up to 14 cm long
Corolla	1.8–2.2 cm long; tube narrowly infundibuliform	3.5–3.7 cm long; tube cylindric
Length ratio of corolla tube to corolla lobes	1.2–2.9	3.4–6
Stamen	two pairs cohersed at the anther tips, adnate to corolla tube 4–6 mm from base	adaxial stamens cohersed in pair, abaxial stamens free, adnate to corolla tube 8–9 mm from base
Pistil	6.0–8.0 mm long; ovary 5.0–6.5 mm long	27–30 mm long; ovary 17–18 mm long

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Eriobotrya crassifolia (Rosaceae), a new species from Yunnan Province, China

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Abstract

The new species *Eriobotrya crassifolia*, collected from Yunnan Province, China, is characterised and illustrated. A phylogeny based on chloroplast genomes supported its closest affinity with *E. tengyuehensis*, while a phylogeny based on 197 single-copy nuclear genes supported its closest affinity with *E. fragrans* and *E. deflexa*. Morphologically, however, it resembles *E. angustissima*. Nevertheless, it can be easily distinguished from *E. angustissima* by its abaxially retroflexed and sharply serrate leaf margins, densely rusty tomentose inflorescences, and oblong or elliptic leaves.

Keywords

Eriobotrya, new species, molecular evidence, morphological traits

Introduction

The genus *Eriobotrya* Lindley (Rosaceae, tribe Maleae) is an economically important genus, widely distributed across the tropical and subtropical regions of East Asia (Chen et al. 2022). It consists of around 30 species, with 14 species distributed in China (Yü et al. 1974; Gu and Spongberg 2003). During the past decade, a total of four new *Eriobotrya* species have been reported and described, including *E. fulvicoma* W.Y. Chun

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ex W.B. Liao, F.F. Li & D.F. Cui (Li et al. 2012), *E. condaoensis* X.F. Gao, M. Idrees & T.V. Do (Idrees et al. 2018), *E. laoshanica* W.B. Liao, Q. Fan & S.F. Chen (Chen et al. 2020) and *E. shanense* D.H. Kang, H.G. Ong & Y.D. Kim (Kang et al. 2021).

Recently, intergeneric delimitation between *Eriobotrya* and *Rhaphiolepis* has been greatly disputed, based on the questions of whether they are both monophyletic or whether the latter is nested within the former. Liu et al. (2020a) argued that *Eriobotrya* and *Rhaphiolepis* should be merged into one genus, based on the evidence from chloroplast genomes, as well as the entire nrITS, which strongly supported the paraphyly of *Eriobotrya*, with *Rhaphiolepis* nested within it. However, based on the partial ITS region, the phylogenetic trees inferred from Bayesian Inference (BI) and Maximum Likelihood (ML) demonstrated that *Eriobotrya* is monophyletic with robust support and could be distinct from *Rhaphiolepis* (Kang et al. 2021). In the latest research (Dong et al. 2022), with increased sampling and well-supported phylogenies, based on nrITS datasets, the monophyly of *Eriobotrya*, as well as *Rhaphiolepis*, was also supported. Based on our recent work (Chen et al. 2022), the plastome data, indeed, agree with the paraphyly of *Eriobotrya*, while simplified haplotype networks of three nuclear genes of *PXMP2*, *TPP2* and *C23H* indicated that all haplotypes of *Rhaphiolepis* formed one distinct clade and the phylogenetic tree constructed from 197 nuclear genes revealed that *R. philippinensis*, as a representative of *Rhaphiolepis*, was placed outside all *Eriobotrya* species with high support. Besides, morphologically, the (circular) annular ring after sepal senescence could only be observed in *Rhaphiolepis* regardless of the difference in persistent sepals of *Eriobotrya* (Robertson et al. 1991; Gu and Spongberg 2003). Interspecific and intergeneric chloroplast capture might be frequent in Maleae (Liu et al. 2020b; Chen et al. 2022). Therefore, we suggest that ancient chloroplast capture once occurred in the ancestor of *Rhaphiolepis*, which captured the chloroplast of *Eriobotrya* species, thus causing the cyto-nuclear discordance. Here, we propose that both *Eriobotrya* and *Rhaphiolepis* are monophyletic and these two genera should not be merged.

Previously, we described a new species of *Eriobotrya laoshanica* that flowers in autumn in the Laoshan Natural Reserve, Malipo County, south-eastern Yunnan Province, China (Chen et al. 2020). Afterwards, through our numerous field investigations, we identified another new sympatric *Eriobotrya* species that flowers in spring. After careful comparison and verification, we confirmed it as a new species, which is described and illustrated here.

Materials and methods

Field investigations and observations were conducted during the flowering and fruiting periods of the putative new species. Fresh leaves were collected and stored in silica gel for molecular experiments. Herbarium specimens were used for further morphological comparisons. Voucher specimens were deposited in the herbarium of Sun Yat-sen University (SYS). Total DNA extraction, sequencing and data analyses were conducted following procedures described by Chen et al. (2022).

We retrieved all available chloroplast genomes of *Eriobotrya* and *Raphiolepis* from the NCBI public database (<https://www.ncbi.nlm.nih.gov/>). In total, we downloaded 58 accessions representing 24 *Eriobotrya* species, eight *Raphiolepis* species and five related species as outgroups. Chloroplast genome sequences were aligned using MAFFT v.7 (Kato and Standley 2013). The alignment was then trimmed using trimAl v.1.2 by setting the “gappyout” model (Capella-Gutiérrez et al. 2009). The Maximum Likelihood (ML) tree was constructed using IQ-TREE v.1.6.8 under 5,000 replicates of the SH approximate likelihood ratio test (SH-aLRT) and 10,000 replicates of ultrafast bootstraps (UFBS) (Nguyen et al. 2015). For the phylogenetic tree based on nuclear genes, we referred to the results of Chen et al. (2022), in which 197 single copy nuclear genes were used and one accession of the new species, 35 accessions of other *Eriobotrya* species, one accession of *Raphiolepis* and five accessions of outgroups were included.

Results

The alignment length of the chloroplast genome was 156,859 bp, amongst which 1,736 sites were parsimony-informative. According to the Bayesian Information Criterion (BIC), the best-fit nucleotide substitution model was GTR+F+I+G4.

The phylogenetic tree inferred from chloroplast genomes demonstrated that this new species is sister to *E. tengyuehensis* with high support (SH-aLRT = 98; UFBS = 100, Fig. 1), but distantly related to *E. fragrans* and *E. deflexa*. However, based on the 197 single copy nuclear genes, the species tree showed that this new species was closely related to *E. fragrans* and *E. deflexa* with low support (quartet score, QS = 38; local posterior probabilities, Astral-PP = 0.74) (fig. 5b in Chen et al. 2022), while the divergence time estimation indicated that it was sister to the sympatric species of *E. laoshanica* (fig. 8b in Chen et al. 2022), in which this new species was marked with *E. sp1*. All this molecular evidence identified that *E. angustissima* was distantly related with the new species although they were similar in morphology.

Discussion

Based on the PhyloNet analysis of Chen et al. (2022), we considered that *E. tengyuehensis* might originate from the hybridisation of *E. obovata* and this new species, with the latter as its female parent. It is for this reason that *E. tengyuehensis* and the new species formed a well-supported sister clade in the chloroplast phylogeny. Based on the species tree, this new species was closely related with *E. fragrans* and *E. deflexa*, but the support values were very low. As concluded by Chen et al. (2022), extensive ancient hybridisation events occurred in this genus during species diversification. Hence, this new species might also be involved in ancient hybridisation events and its parents were not found in extant species. Morphological traits of the parents were also unknown and we supposed morphological similarity between this new species and *E. angustissima*

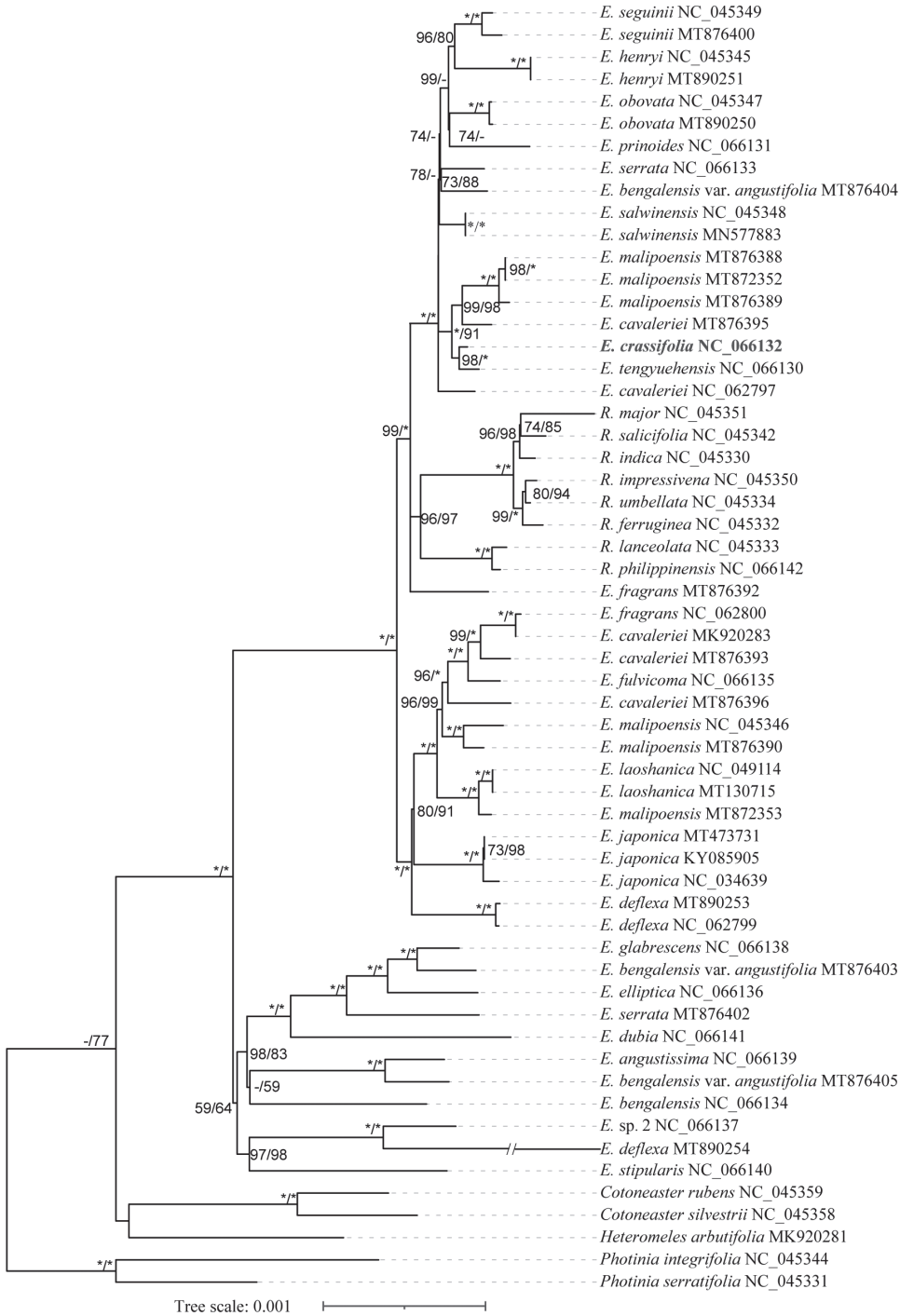


Figure 1. The phylogenetic tree, based on 58 complete chloroplast genomes. Numbers near nodes indicated SH approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap support values (UFBS), respectively. The symbols “*” imply that SH-aLRT $\geq$ 95% or UFBS $\geq$ 95%. The symbols “-” imply that SH-aLRT $<$ 50% or UFBS $<$ 50%. The new species was highlighted in bold.

was just a coincidence. Besides, population genomics of *Eriobotrya* species, especially for those distributed in Yunnan Province, need to be further conducted to explore the potential causes of those discordances.

Geographically, *E. fragrans* is distributed in Guangdong and Guangxi Provinces, China and *E. deflexa* is distributed in Guangdong and Taiwan Provinces, China. Additionally, they are also significantly different from this new species in morphology. Though this new species shares the same distribution regions with *E. laoshanica*, they are distinctly different, both in phenology and morphology. The new species is morphologically similar to *E. angustissima* and *E. tengyuehensis*. However, by our comprehensive comparisons, it could be easily distinguished from *E. angustissima* by its oblong or elliptic (vs. narrowly oblong) and thickly (vs. thinly) coriaceous leaves; inflorescence densely rusty tomentose (vs. glabrous or glabrescent) and 10–17 lateral veins (vs. 8–10). Though this new species shares a few characteristics with *E. tengyuehensis*, for example, thickly coriaceous leaves, similar number of lateral veins and styles, it could be easily distinguished from the latter by its abaxially retroflexed and sharply serrate margin (vs. not retroflexed and entire basally, serrate apically) and much smaller leaves (9–11 × 2.5–3.5 cm vs. 12–17 × 5–7 cm) (Table 1).

Table 1. Morphological comparisons amongst *Eriobotrya crassifolia*, *E. tengyuehensis* and *E. angustissima*.

Characters	<i>E. crassifolia</i>	<i>E. angustissima</i>	<i>E. tengyuehensis</i>
Leaf shape and size	oblong or elliptic, 9–11 × 2.5–3.5 cm	narrowly oblong or very narrow linear-lanceolate, 5–10 × 1–2 cm	oblong, elliptic or nearly obovate, 12–17 × 5–7 cm
Leave margin	abaxially retroflexed, sharply serrate	abaxially slightly retroflexed, entire basally, serrate apically	abaxially not retroflexed, entire basally, serrate apically
Texture of leaves	thickly coriaceous	thinly coriaceous	thickly coriaceous
Lateral veins	10–17 pairs	8–10 pairs	10–15 pairs
Inflorescences	densely rusty tomentose	glabrous or glabrescent	brownish-yellow tomentose
Petal shape	obovate-triangular or suborbicular	obovate or obcordate	obovate
Fruit shape and size (diameter)	oblong or subglobose, 6–7 mm	subglobose, 10 mm	subglobose, 7 mm
Styles	2–4	3–5	2–3

Taxonomic treatment

***Eriobotrya crassifolia* Q.Fan, S.F.Chen & K.K.Meng, sp. nov.**

urn:lsid:ipni.org:names:77308552-1

厚叶枇杷

Type. CHINA. Yunnan Province, Malipo County, Xiajinchang Town, Mount Laoshan, in thick forests on the slopes of limestone hills, 23°07'N, 104°50'E, 1684 m a.s.l., 21 March 2022, Q. Fan 19220 (holotype: SYS; isotype: IBSC, SYS) (Figs 2, 3).

Diagnosis. The new species resembles *E. angustissima*, but differs from the latter by its leaf shape and texture, the number of lateral veins and the indumentum of inflorescence.

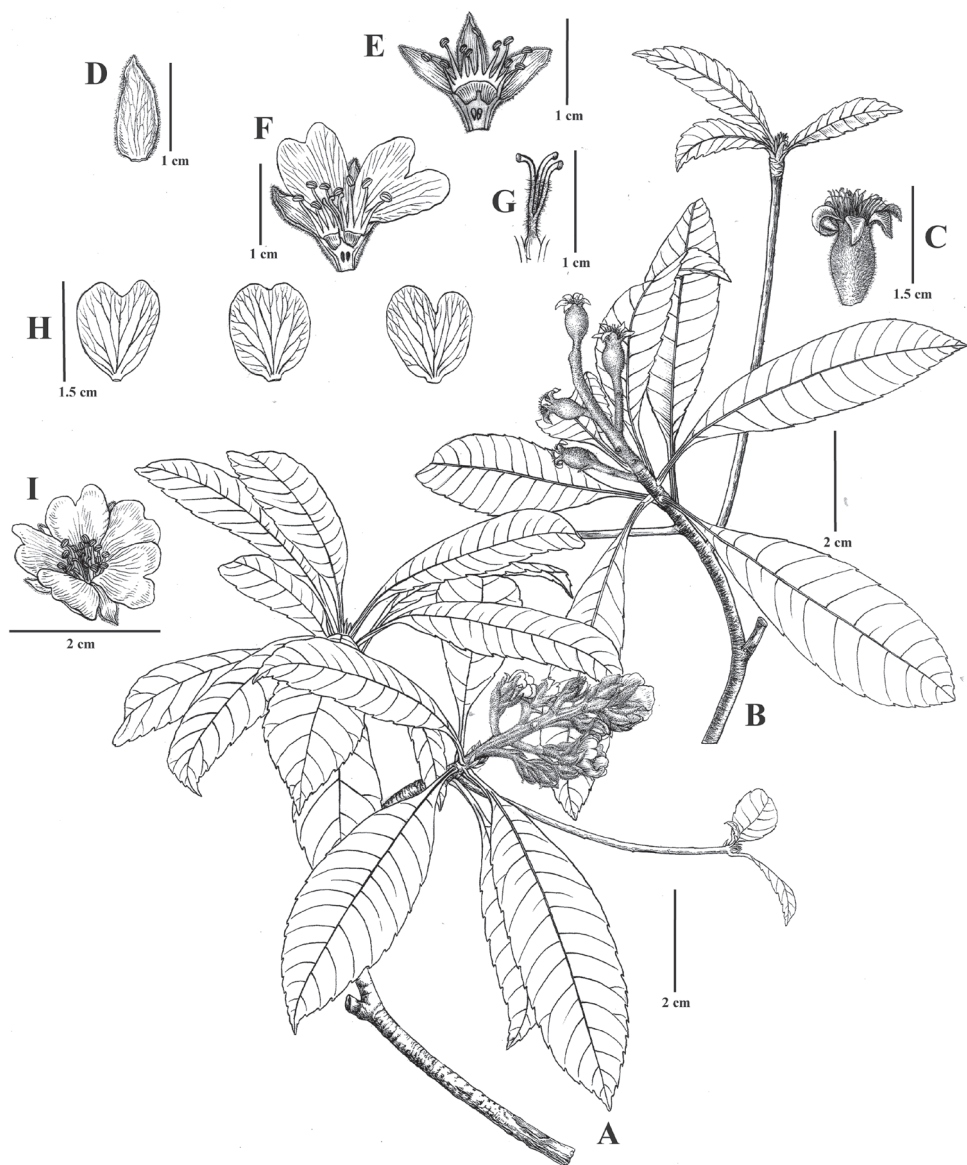


Figure 2. *Eriobotrya crassifolia* sp. nov. **A** flowering branch **B** fruiting branch **C** young fruits with persistent calyx lobes **D** calyx lobe **E** flower in longitudinal section, showing the ovary, stamen and calyx lobes **F** flower in longitudinal section, showing the pistil, stamens and petals **G** styles **H** petals **I** flower in front view. Illustrated by Yun-Xiao Liu.

Description. Evergreen trees, 15–25 m tall; stems 10–30 cm in diameter; branchlets grey-white, terete, glabrous, 4–8 mm in diameter. Leaves spirally inserted on branches and often crowded at tips of branchlets, margin sparsely serrate; petioles 1–1.5 cm long, glabrous; stipules linear-lanceolate, 10–12 × 1–2 mm, glabrous; leaf blades oblong or elliptic, 9–11 × 2.5–3.5 cm, thickly coriaceous, glabrous, mid-vein

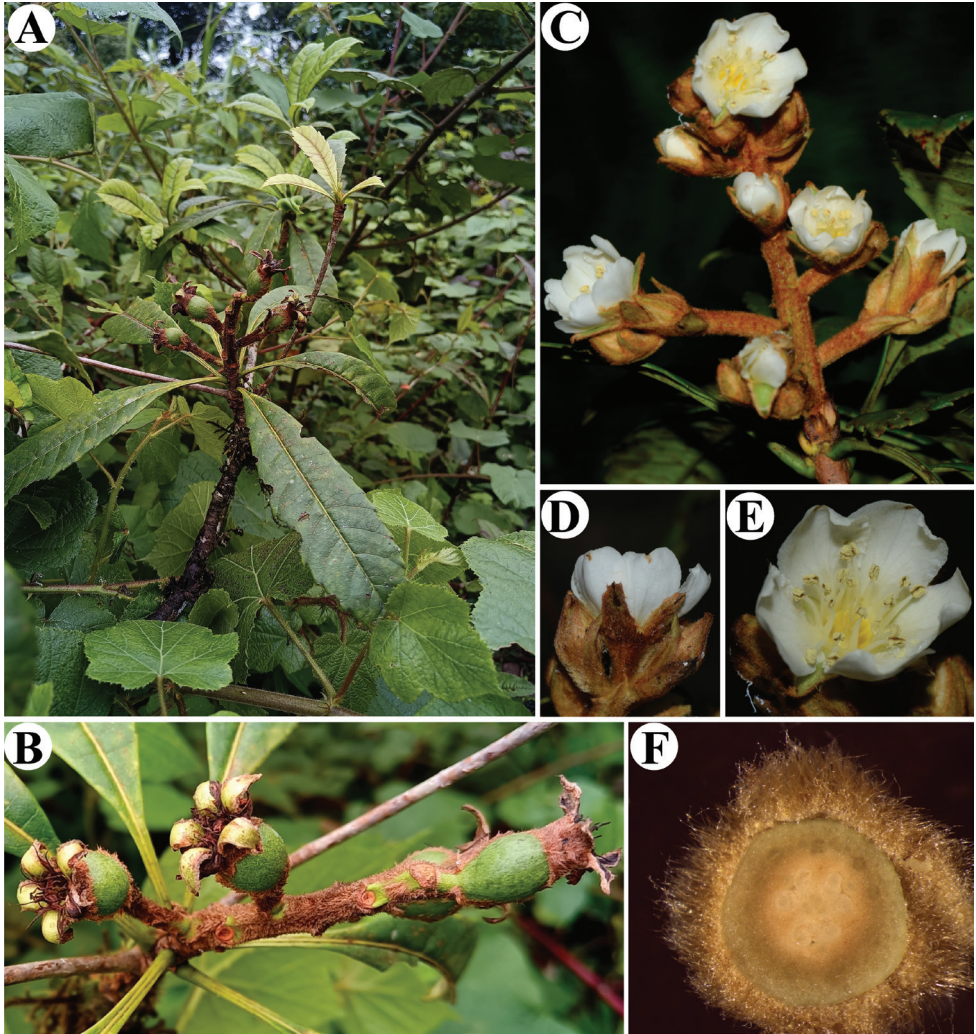


Figure 3. *Eriobotrya crassifolia* sp. nov. **A, B** fruiting branch **C** flowering branch **D** flower in side view **E** flower in front view **F** ovary in transverse section, showing the 3-loculed ovary. Photographed by Qiang Fan.

prominent, raised abaxially, lateral veins 10–17 pairs, sporadically dichotomous before terminating at margin, apex acute to acuminate, base cuneate, margin deflexed with sharply serrate. Inflorescence in terminal panicles, 17- to 30-flowered, 6.7–9.8 × 4–7.7 cm, with 4–7 lateral racemes, peduncle and pedicels densely rusty tomentose, pedicels 3–4 mm; bracts and bracteoles ovate-triangular, 2–4 × 8–10 mm, abaxially densely tomentose, adaxially glabrous; petals white, quincuncial, obovate-triangular or suborbicular, 9–10 × 6–8 mm, apex emarginate; stamens 20; filaments 5.3–8.1 mm long, glabrous; anthers 1.4–1.5 mm long; styles 6.2–9 mm long; ovary inferior; hypanthium shallow-cupular, 9–10 × 6–8 mm, abaxially densely rusty tomentose, 5-lobed, the calyx lobes triangular-ovate, 9–10 × 5–6 mm, abaxially densely rusty tomentose; ovary

2–4-loculed, with 2 ovules per locule; styles 2–4, mostly 3, densely yellowish villous in the lower part, connate at base or fused at one fourth of the base; fruits ellipsoid or subglobose, 6–7 × 7–9 mm, glabrescent, capsules crowned by five persistent calyx lobes; seeds 2–3 per fruit.

Phenology. Flowering from March to April, fruiting from June to August.

Etymology. Latin *crassus*, thick, and *folia*, leaved, alluding to leaf thickness

Distribution and habitat. Presently, *Eriobotrya crassifolia* is known from a single locality, Laoshan Natural Reserve, Malipo County, south-eastern Yunnan Province, China. It is distributed in thick forests on the slopes of limestone hills at altitudes of 1502–1684 m a.s.l.

Conservation status. Only two populations were found with no more than 200 mature individuals. Thus, the species status could be considered as Endangered (EN; D), according to the IUCN Red List Criteria (IUCN Standards and Petitions Subcommittee 2022).

Additional specimens examined (paratypes). CHINA. Yunnan: Malipo County, Xiajinchang Township, Laoshan Natural Reserve, 23°07'N, 104°50'E, 1684 m a.s.l., 2 Dec 2015 (no fl. and no fr.), *Q. Fan 13941* (SYS); the same locality, 27 June 2022 (young fr.), *Q. Fan 19580* (SYS); Malipo County, Tianbao Township, Laoshan Natural Reserve, in thick forests on the slopes of limestone hills, 23°11'N, 104°48'E, 1502 m a.s.l., 16 September 2015 (no fl. and no fr.), *Q. Fan 13713* (SYS).

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***Begonia parvibracteata*, a new species in *Begonia* sect. *Platycentrum* (Begoniaceae) from Guangxi of China, based on morphological and molecular evidence**

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Abstract

The previously reported begonias in a limestone forest of Guangxi mainly belong to *Begonia* sect. *Coelocentrum* Irmscher. In this article, we described and illustrated a new species in sect. *Platycentrum* (Klotzsch) A.DC., *Begonia parvibracteata* X.X.Feng, R.K.Li & Z.X.Liu, which was discovered in a karst forest of south-western Guangxi. The begonia shows high morphological similarity to *B. subhowii* S.H. Huang and *B. psilophylla* Irmscher, but differs from the latter two in its narrower oblique-ovate asymmetric leaf blade, 4 (occasionally 6) tepals of pistillate flower and smaller membranous inflorescence bracts. Molecular phylogenetic analysis, based on ITS sequence data, supports the new species as monophyletic and distinct from *B. subhowii* and *B. psilophylla*. Considering its narrow distribution and the disturbance of human activities, the conservation status of new taxon is evaluated as “Vulnerable” (VU B1, B2 ab (i, iv, v), D2) according to the IUCN Red List Categories and Criteria.

Keywords

ITS, morphology, new taxon, southern China, taxonomy

* These authors contributed equally.

Introduction

According to the latest report, China's wild begonias have already increased to 239 species (iBegonia 2021). The total number of species could reach 300 in the coming years, meeting the prediction of Tian et al. (2018). Consisting of 122 species, Sect. *Platycentrum* (Klotzsch) A.DC. represents the largest section of *Begonia* in China, with 66 species mainly distributed in Yunnan. Eighty-seven species of *Begonia* have so far been found in Guangxi (iBegonia 2021), most of which (60 species) are distributed in the karst forest and belong to *B.* sect. *Coelocentrum* Irmscher.

In Guangxi, only 11 species belong to *B.* sect. *Platycentrum* (Klotzsch) A.DC., and six of which are widespread in south and southeast China: *B. circumlobata* Hance, *B. edulis* Lévl., *B. handelii* Irmsch., *B. hemsleyana* Hook.f., *B. longiciliata* C.Y.Wu and *B. longifolia* Blume. Only five species of *B.* sect. *Platycentrum* are endemic to Guangxi, including *B. tsoongii* C.Y.Wu (Wu and Ku 1995), *B. longanensis* C.Y.Wu (Wu and Ku 1997), *B. aurora* C.I Peng, Yan Liu & W.B. Xu (Liu et al. 2020), *B. scorpiuroloba* D.K.Tian & Q.Tian (Tian et al. 2021) and *B. pseudoedulis* D.K.Tian, X.X.Feng & R.K.Li (Feng et al. 2021).

An unknown *Begonia* taxon with reproductive organs was collected from Guangxi during our field survey and plant collection in May 2020 and October 2021, respectively. The begonia definitely belongs to *B.* sect. *Platycentrum*, but differs markedly from the reported 11 begonias of the same section in Guangxi. After further detailed morphological observation, morphological comparison with similar species and molecular analysis, it is confirmed as a new species in *Begonia* sect. *Platycentrum*.

Materials and methods

Taxonomic observation

Morphological characters were observed and measured from fresh samples in the field. Morphological comparisons with similar taxa were undertaken by consulting the literature, examining herbarium (IBK and IBSC) specimens and observing living collections cultivated in the nursery of Enshi Dongsheng Plant Development Co. Ltd. The specimens were deposited at the Herbarium of South China Botanical Garden (IBSC), CAS and Chenshan Herbarium (CSH) of Shanghai Chenshan Botanical Garden.

DNA sequencing and molecular analysis

The fresh leaves of the putative new species and the morphologically similar allied species, *B. subhowiii* and *B. psilophylla*, were collected in the field and the nursery, respectively. Total DNA was extracted from nine individuals of the three species (*B. parvibracteata*, *B. subhowiii* and *B. psilophylla*) with the CTAB method (Doyle and Doyle 1987). The internal transcribed spacers 1 and 2 (ITS) and the 5.8S gene were amplified using the primers 17SE and 26SE (Forrest and Hollingsworth 2003).

PCR amplification and Sanger sequencing were performed according to Fan et al. (2014), with an annealing temperature of 53 °C. The ITS sequences of the three species were uploaded to GenBank (<http://www.ncbi.nlm.nih.gov/>) with the accession numbers OL892048, OL892049 and OL892050 for *B. parvibracteata* sp. nov., OL871361, OL871362 and OL871363 for *B. subhowii* and OL851701, OL851702 and OL851703 for *B. psilophylla* (Table 1).

We chose 17 species from sect. *Platycentrum* and 8 species from other sections of *Begonia* native to mainland China to place the new species in a phylogenetic context (Table 1). *Begonia socotrana* Hook.f in sect. *Peltaugustia* (Warb.) Barkley from Socotra (for the coast of Africa) was selected as an out-group in the phylogenetic analysis (Moonlight et al. 2018). Except for the nine individuals of the putative new species and its two allied species, the additional ITS sequences for 23 *Begonia* species were downloaded from the NR database of NCBI. All these sequences were aligned using BioEdit v.7.2.5 (Hall 1999) and a phylogenetic analysis using Bayesian Inference (BI) was undertaken in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). The GTR+G model was chosen as the optimal model of nucleotide substitution according to the Akaike Information Criterion (AIC; Burnham and Anderson 2002) as implemented in MrModeltest 2.3 (Nylander 2004). The Markov chains were run for 1,000,000 generations and sampled at each 100 generations, with the first 25% discarded as burn-in.

Table 1. *Begonia* species and populations included in the phylogenetic analysis (Sectional placement follows Moonlight et al. 2018).

Taxon	Origin	GenBank accession no.	Section	Collector, voucher (Herbarium)
<i>Begonia acetosella</i> Craib.	Mengla, Yunnan, China	MW690106	<i>Platycentrum</i>	Wang, W.G., WWG005 (HITBC)
<i>Begonia biflora</i> Ku	Malipo, Yunnan, China	JF975965	<i>Coelocentrum</i>	Shui, Y.M. et al. 20484 (KUN)
<i>Begonia chingii</i> Irmsch.	Napo, Guangxi, China	KP710820	<i>Reichenheimia</i>	Tian, D.K., Li, C. TDK785 (CSH)
<i>Begonia circumlobata</i> Hance	Xinyi, Guangdong, China	KP710815	<i>Platycentrum</i>	Tian, D.K., Li, X.P. TDK866 (CSH)
<i>Begonia cucurbitifolia</i> C. Y. Wu	Yunnan, China	JF975969	<i>Platycentrum</i>	Y.M., Shui et al. GBOWS1284 (KUN)
	—	JF975968		—
<i>Begonia edulis</i> Lévl.	Bama, Guangxi, China	KP710813	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK757 (CSH)
<i>Begonia grandis</i> Dry.	Yongshun, Huhan, China	KP710828	<i>Diploclinium</i>	Li, X.P. Li, X.J. LXJ022 (CSH)
<i>Begonia handelii</i> Irmsch.	Fengshan, Guangxi, China	KP710818	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK763 (CSH)
<i>Begonia hatacoa</i> Buch.-Ham. ex D. Don	—	AF485111	<i>Platycentrum</i>	—
<i>Begonia hemsleyana</i> Hook. f.	—	KP710806	<i>Platycentrum</i>	—
<i>Begonia henryi</i> Hemsl.	Leshan, Sichuan, China	KP710822	<i>Reichenheimia</i>	Tian, D.K., Tian, L.Z. TDK2249 (CSH)
<i>Begonia huangii</i> Y. M. Shui & W. H. Chen	Gejiu, Yunnan, China	JF976001	<i>Coelocentrum</i>	Shui, Y.M. et al. 40782 (KUN)

Taxon	Origin	GenBank accession no.	Section	Collector, voucher (Herbarium)
<i>Begonia jinyunensis</i> C. I Peng, B. Ding & Q. Wang	Jinyunshan, Chongqing, China	MZ145345	<i>Platycentrum</i>	–
<i>Begonia labordei</i> Lévl.	Yunnan, China	KF636452	<i>Diploclinium</i>	Peng 20520 (HAST)
<i>Begonia longifolia</i> Blume	Mengla, Yunnan, China	MW690102	<i>Platycentrum</i>	Wang, W.G., WW/G001 (HITBC)
<i>Begonia longistyla</i> Y. M. Shui & W. H. Chen	Hekou, Yunnan, China	JF976018	<i>Coelocentrum</i>	Shui, Y.M. et al. 40778 (KUN)
<i>Begonia megalophyllaria</i> C. Y. Wu	Yunnan, China	JF976026	<i>Platycentrum</i>	Y,M,Shui et al.D-33(KUN)
<i>Begonia multangula</i> Blume	Jawa, Lesser Sunda Is.	MN453434	<i>Platycentrum</i>	–
<i>Begonia ornithophylla</i> Irmsch.	Guangxi, China	JF976032	<i>Coelocentrum</i>	Y,M,Shui et al. B2005- 061(KUN)
<i>Begonia parvibracteata</i> X.X.Feng, R.K.Li & Z.X.Liu	Longzhou, Guangxi, China	OL892048 OL892049 OL892050	<i>Platycentrum</i>	Xin Xin Feng, et al. 835307 (IBSC)
<i>Begonia pedatifida</i> Lévl.	Tianlin, Guangxi, China	KP710810	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK774 (CSH)
<i>Begonia psilophylla</i> Irmsch.	Hekou, Yunnan, China	OL851701 OL851702 OL851703	<i>Platycentrum</i>	– – –
<i>Begonia pulchrifolia</i> D.K.Tian & C.H.Li	Meinfeng, Leshan, Sichuan, China	KP710811	<i>Platycentrum</i>	Tian, D.K., et al. TDK2243 (CSH)
<i>Begonia scorpiuroloba</i> D.K.Tian & Q.Tian	Fanchenggang, Guangxi, China	MZ145351	<i>Platycentrum</i>	Tian, D.K., et al.TDK2269(CSH)
<i>Begonia socotrana</i> Hook.f.	Socotra	AF469121	<i>Peltaugustia</i>	–
<i>Begonia subhowii</i> S. H. Huang	Malipo, Yunnan, China	OL871361 OL871362 OL871363	<i>Platycentrum</i>	– – –

Taxonomy

Begonia parvibracteata X.X.Feng, R.K.Li & Z.X.Liu, sp. nov.

urn:lsid:ipni.org:names:77308553-1

Figs 1, 2

Chinese name: 小苞秋海棠

Type. CHINA Guangxi, Longzhou County (龙州县), Zhubu Town (逐卜乡), Bannong Village (板弄屯), Yinghuagu Tourist Resort, 22°33'51"N, 106°57'03"E, (Fig. 3), 263 m alt., in shaded environment of limestone forest, October, 2021, *Xin-Xin Feng, Ren-Kun Li & Zhi-Xian Liu* (holotype: 835307, IBSC!; isotype: SYS!). Longzhou County (龙州县), Zhubu Town (逐卜乡), Pona Village (坡那屯), Nonggang National Nature Reserve, 22°39'03"N, 106°57'18"E, 190 m alt., on rock under limestone forest, 18 May 2020, *Dai-Ke Tian & JinYE Zhou* TDK4119 (CSH!).

Diagnosis. *Begonia parvibracteata* morphologically resembles *B. subhowii* and *B. psilophylla* in rhizome and leaf characters. However, it has narrowly oblique-ovate asymmetric leaf blades, 4 (rarely 6) tepals in pistillate flowers and small (6–8 × 3–5 mm)

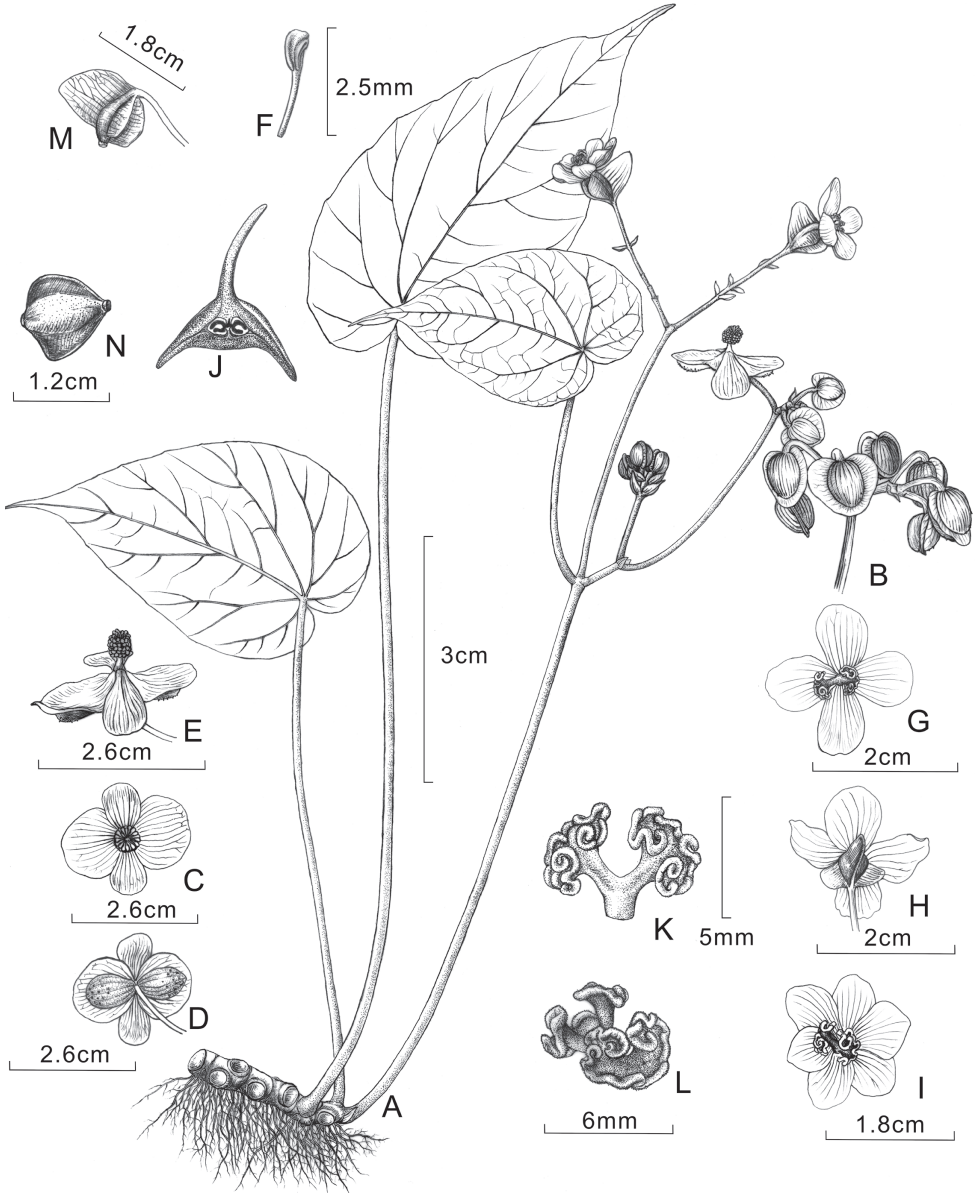


Figure 1. *Begonia parvibracteata* drawn by Yunxiao Liu **A** habit **B** staminate inflorescence **C, D** front and back views of staminate flower **E** lateral view of staminate flower **F** stamen **G, H** front and back views of pistillate flower with 4 tepals **I** front view of pistillate flower with 6 tepals **J** cross section of ovary **K** styles and stigmas **L** stigmas **M** abaxial view of capsule showing wings **N** lateral view of capsule showing two shorter wings.

bracts in inflorescences. These characters differ from the widely ovate leaf blades, 5 (rarely 6) tepals of pistillate flowers, and distinctly large bracts in inflorescences of *B. subhowii*. *B. parvibracteata* is dissimilar to *B. psilophylla* in its 4 (rarely 6) tepals in pistillate flowers and asymmetric, narrowly oblique-ovate leaf blade.

Begonia parvibracteata forms a monophyletic group clustered with *B. cucurbitifolia* in the phylogenetic tree, but the latter has nearly symmetric, 3–4 lobed leaf blade, 5-tepaled pistillate flower, definitely differing from the new species.

Description. Perennial evergreen herb, monoecious, 15–30 cm tall. **Rhizome** creeping, ca. 8.5–12 cm long and 6–10 mm thick, internode short or not obvious; erect stem only seen at anthesis, ca. 7.5–10 cm long, internodes 1–2 (3), green to reddish-green, glabrous. **Stipules** brownish-red, translucent, ovate-triangular, ca. 8–10 × 4–5 mm, glabrous. **Leaves** 3–6 basal and 2–3 aerial, petiole yellowish-green, 18–22 cm long, glabrous; blade asymmetric, ovate to narrowly ovate, 14–16 × 7.5–9 cm; apex acuminate to caudate, base oblique-cordate; leaf margin usually almost entire or occasionally crenate; venation palmate with 6–8 primary veins, adaxially slightly concave, abaxially convex; leaf blade fleshy, abaxially pale-green, glabrous; adaxially green, glabrous. **Inflorescences** arising from erect stem; dichasial cymes branching one to two times, peduncle 10–12 cm long, glabrous; flowers unisexual, 3–7 flowers per inflorescences; bracts membranous, triangular to widely ovate, brownish-red, 6–8 × 3–5 mm, glabrous. **Staminate flower:** pedicels pale green, ca. 2–2.3 cm long, glabrous; tepals 4, outer 2 tepals ovate to circular, 13–21 × 12–20 mm, adaxially concave, pinkish-white, abaxially convex, pinkish-white, red hispid; inner 2 obovate, 10–18 × 8–12 mm, margin irregularly entire, white, glabrous; androecium cylindrical, ca. 6.6–7.5 mm across; stamens numerous, ca. 2–2.5 mm long, anthers yellow, clavate, base cuneate, ca. 1.5 mm long. **Pistillate flower:** pedicels pale green, ca. 2–2.6 cm long, glabrous; tepals 4, occasionally 6, white, irregularly ovate, obovate or cuneate, sub-equal, 18–20 × 10–15 mm, glabrous; ovary yellowish-green, trigonous-ellipsoid, 11–12 × 5–6 mm (wings excluded), glabrous; 2-loculed, placentae axillary, placentae bifid per locule; styles 2, fused at base, yellow, ca. 5–6 mm long, apically Y-shaped, stigma U-shaped, spirally twisted. **Capsules** nodding, trigonous-ellipsoid, ca. 11–13 × 5–6.5 mm (wings excluded), yellowish-green, glabrous, unequally 3-winged, abaxial wing triangular to ligulate, ca. 10–13 mm long; lateral wings lunate, 3–6 mm long.

Distribution and habitat. Currently known from four localities in Longzhou County and Daxin County, Guangxi, China. It usually grows on rocks or rock cracks in limestone under forest.

Phenology. Flowering September–October, fruiting October–December.

Etymology. The specific epithet “*parvibracteata*” refers to the short small bracts of the new species. The Chinese name is given as “小苞秋海棠” (*Begonia* with small inflorescence bracts).

Conservation status. There are three populations with approximately 1000 individuals found in Longzhou County. Another one population with approximately 500 individuals is distributed in the Encheng National Nature Reserve, Daxin County. Some plants of this *Begonia* are over-collected and sold in the local medicinal herb market of Longzhou County. According to the IUCN Red List Categories and Criteria (IUCN 2022), *B. parvibracteata* should be assessed as “Vulnerable (VU B1, B2 ab (i, iv, v), D2)” due to its narrow distribution and the disturbance by human activities.

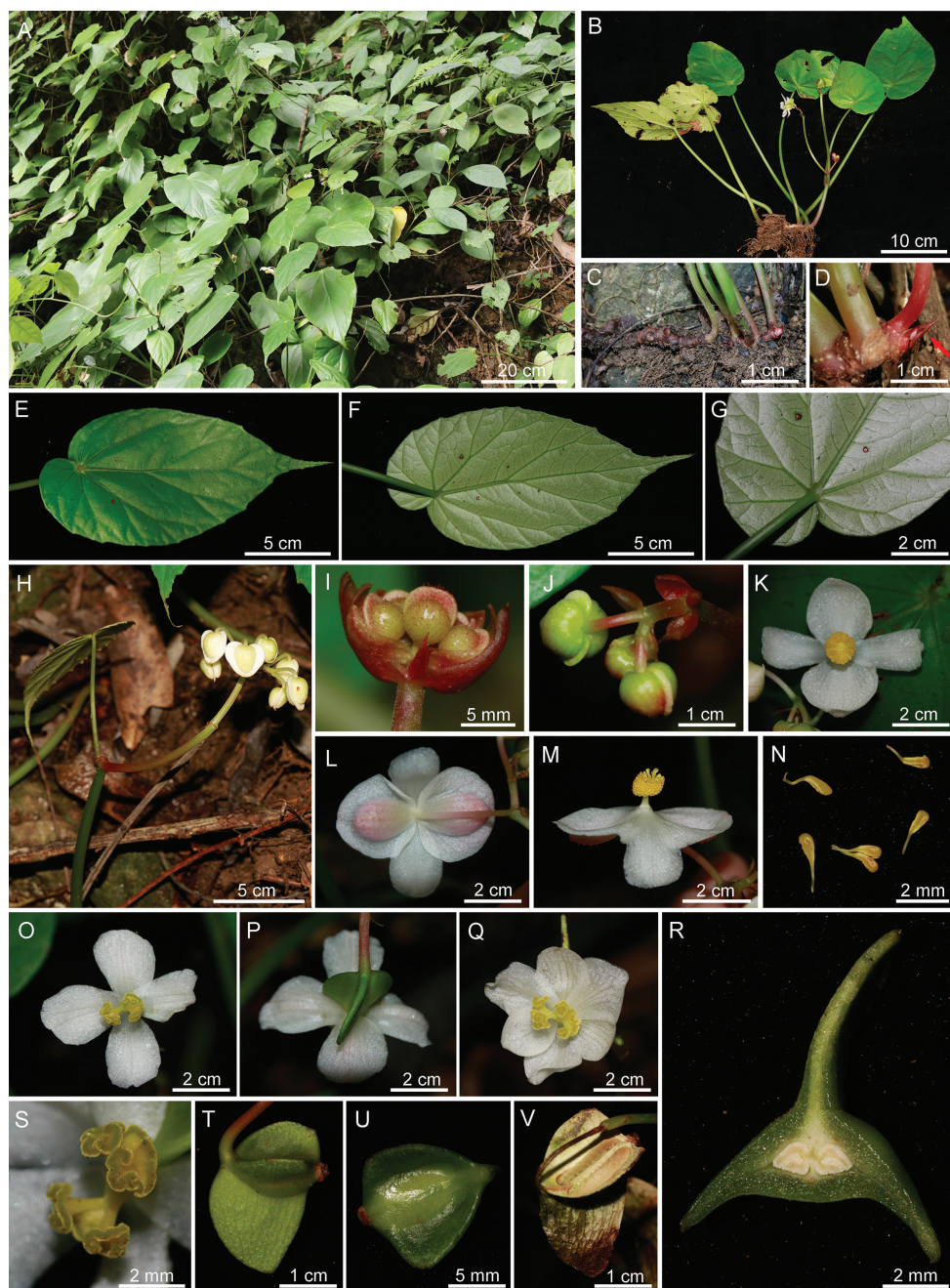


Figure 2. Habitat and morphology of *Begonia parvibracteata* **A** habitat **B** flowering plant **C** creeping rhizome **D** shoot top with stipule **E** leaf blade (adaxial) **F, G** leaf blade (abaxial) **H** erect stem with inflorescence **I, J** young inflorescence and bract; **K, L** front and back views of staminate flower with 4 tepals **M** lateral view of staminate flower **N** stamens **O, P** front and back views of pistillate flower with 4 tepals **Q** front view of pistillate flower with 6 tepals **R** cross section of ovary **S** stigmas **T, U** immature capsule **V** dried mature capsule (Photos by Z.X. Liu).

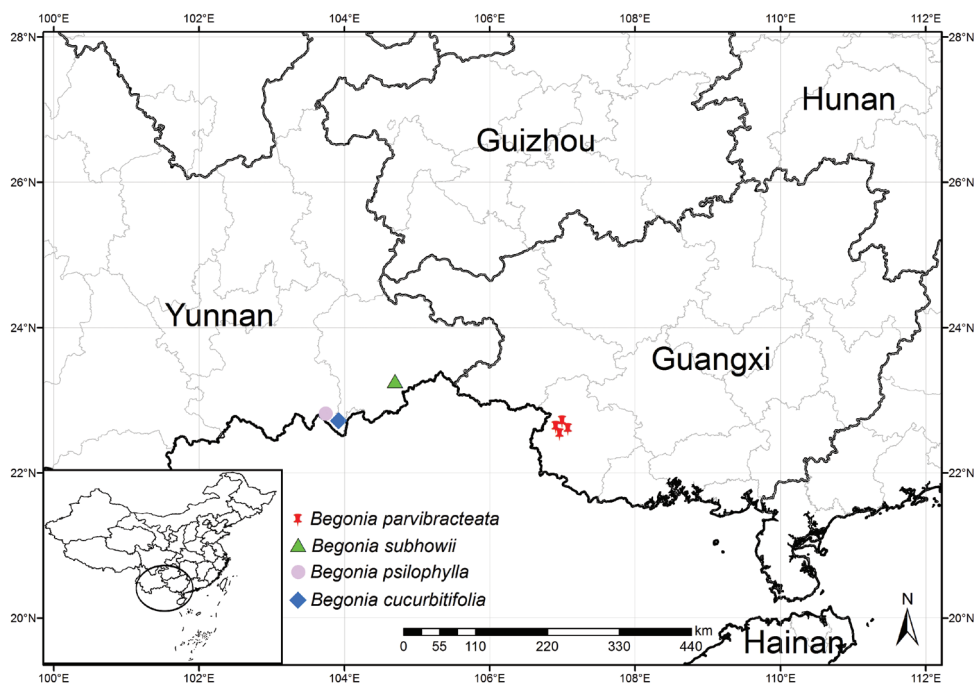


Figure 3. Distribution of *B. parvibracteata*, *B. subhowii*, *B. psilophylla* and *B. cucurbitifolia*.

Molecular analysis

The aligned matrix of the ITS sequence data was 727 bp long. The result of Bayesian Inference analysis is shown in Fig. 4. *Begonia* sect. *Platycentrum* appears monophyletic with a high Bayesian posterior probability (bpp = 1) (Fig. 4). The three samples of the putative new species form a monophyletic group clustered with *B. cucurbitifolia* (bpp = 0.89). *Begonia subhowii* with the highest morphological similarity to *B. parvibracteata* formed another subclade.

The nodes with bpp < 0.50 have been collapsed. Sectional placement of taxa is indicated by the following abbreviations: *Coe* (*Coelocentrum*), *Dip* (*Diplocinium*), *Pla* (*Platycentrum*), *Rei* (*Reichenheimia*) and *Pel* (*Peltaugustia*). The numbers after the species names indicate different populations. The samples of the new species are marked with stars.

Discussion

The new begonia with 2-loculed ovary, axillary placentation and bifid placenta can be clearly assigned to *B. sect. Platycentrum* (Gu et al. 2007). Within this largest section for *Begonia* in China, *B. parvibracteata* shows high morphological resemblance to *B. subhowii* and *B. psilophylla*, both of which are distributed in south-eastern Yunnan (Shui and Huang 1999; Shui and Chen 2018), including creeping rhizome, glabrous

plants with both basal and cauline leaves at anthesis, usually almost entire leaf margin, palmate venation, fleshy green leaf blade and 4 tepals of staminate flower (Table 2).

In *B. subhowii*, there are 5 (occasionally 6) tepals in the pistillate flower and the bracts of inflorescence is distinctly long and large (5 × 3 cm) (Fig. 5). These characters differ from 4 (occasionally 6) tepals and triangular to widely ovate (6–8 × 3–5 mm) bracts in *B. parvibracteata*. In addition, the leaf blade is wider ovate in *B. subhowii*, compared with the narrower oblique-ovate shape of *B. parvibracteata*.

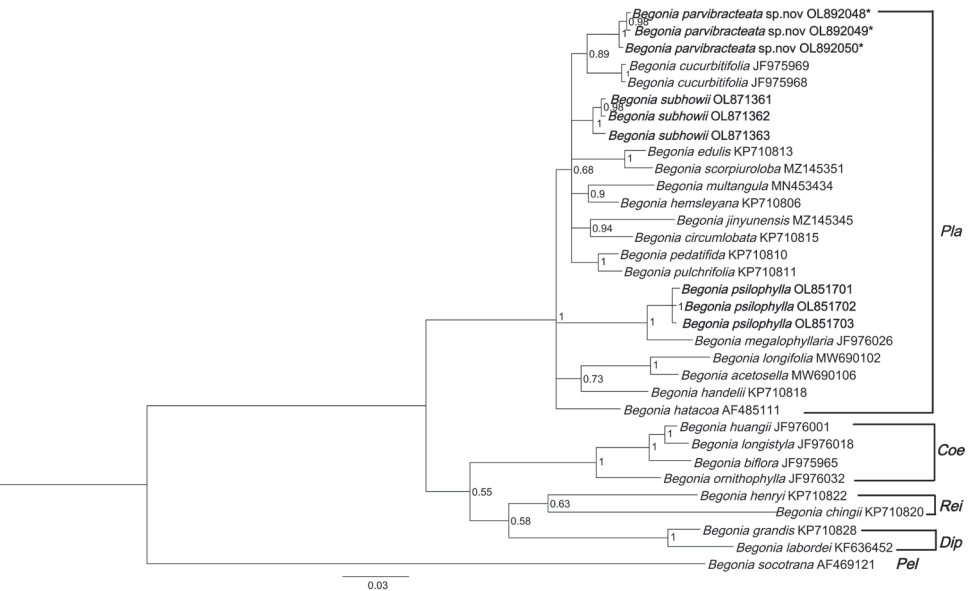


Figure 4. Bayesian Inference of the phylogenetic position of the newly-described *B. parvibracteata* within sect. *Platycentrum*, based on nuclear ITS sequences.

Table 2. Morphological comparison of *B. parvibracteata* and relevant taxa.

Character	<i>B. subhowii</i>	<i>B. psilophylla</i>	<i>B. cucurbitifolia</i>	<i>B. parvibracteata</i>
Leaf blade shape	asymmetric, widely ovate	nearly symmetric, widely ovate to cordate	nearly symmetric, orbicular, 3–4 lobed	asymmetric, narrowly oblique-ovate
Leaf blade base	asymmetric, oblique-cordate	nearly symmetric, obtuse	nearly symmetric, cordate	asymmetric, oblique-cordate
Leaf margin	irregularly serrulate	minutely serrulate	minutely serrulate	usually almost entire or occasionally crenate
Inflorescence bracts	ovate-oblong, 50 × 30 mm, glabrous, apex acuminate	elliptic to ovate-oblong, 7–9 × 4–5 mm, glabrous	subglabrous, caduceus	triangular, 6–8 × 3–5 mm, glabrous
Tepal number of pistillate flowers	usually 5, rarely 6	usually 5, rarely 6	5	usually 4, rarely 6
Phenology (flowering; fruiting)	April–May; May–July	February–March; March–May	July–August; August–October	September–October; October–December
Habitat	700–1500 m alt., limestone, Yunnan; Vietnam	100–700 m alt., limestone, Yunnan	430 m alt., limestone, Yunnan	263 m alt., limestone, Guangxi

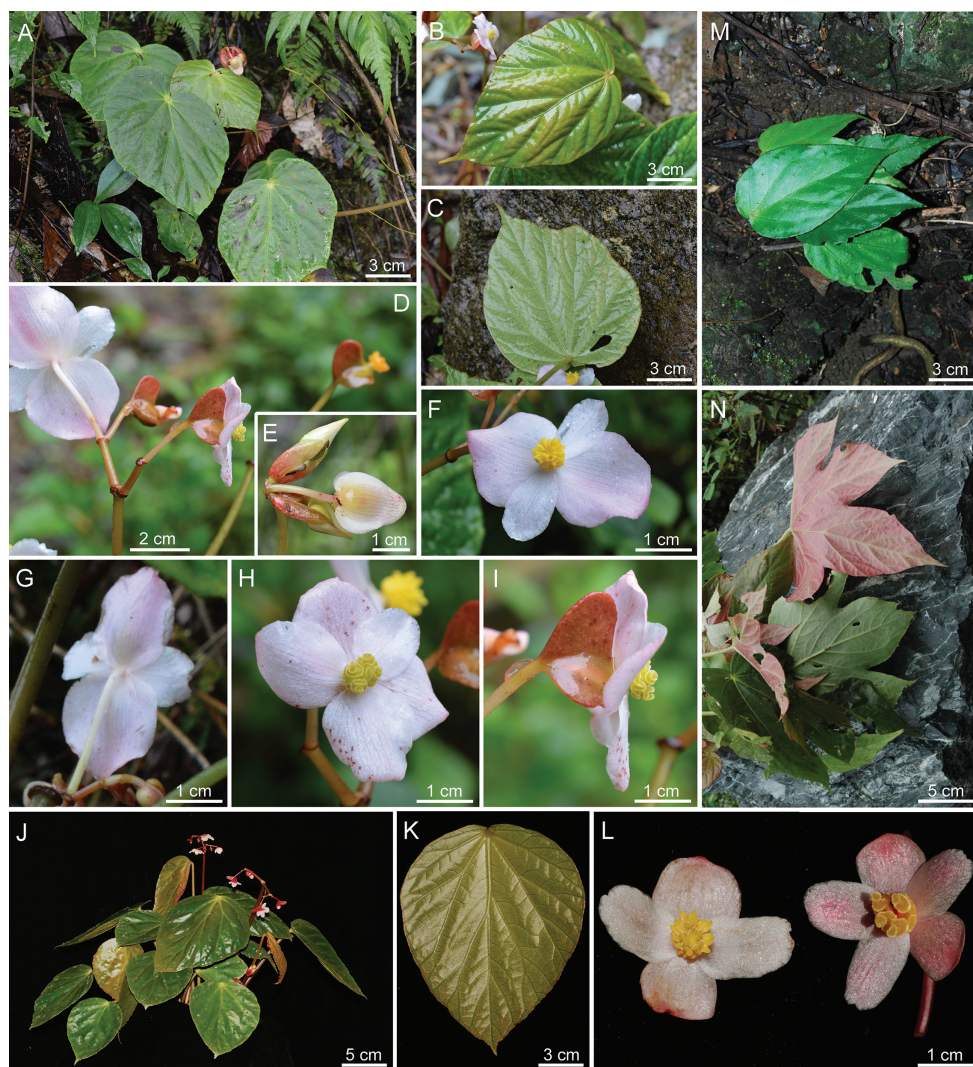


Figure 5. *B. subhowii* **A–I** *B. psilophylla* **J–L** *B. cucurbitifolia* **M, N** showing similar features comparable to *B. parvibracteata* **A** habitat **B** young leaf blade (adaxial) **C** leaf blade (abaxial) **D** inflorescence **E** bracts **F, G** front and back views of staminate flower **H** front view of pistillate flower with 5 tepals **I** lateral view of pistillate flower **J** flowering plant **K** symmetric leaf blade **L** front view of flower **M** leaf blade of juvenile plant **N** mature leaf blade (Photos **A–I** by R.K. Li **J–L** by Z.X. Liu **M, N** by D.K. Tian).

In *B. psilophylla*, the leaf blade is nearly symmetric, widely ovate with symmetric cordate base, in contrast with the narrower ovate leaf blade and oblique-cordate base of the new species. Furthermore, there are 5 (occasionally 6) tepals in the pistillate flower of *B. psilophylla*, being different from the 4 (occasionally 6) tepals of *B. parvibracteata*.

According to the phylogenetic tree, based on ITS sequences, the closest relative of *B. parvibracteata* is *B. cucurbitifolia* which is also distributed in south-eastern Yunnan.

However, *B. cucurbitifolia* is remarkably distinct from *B. parvibracteata* in morphology, including nearly symmetric, 3–4 lobed leaf blade and 5-tepaled pistillate flower.

For the flowering time, *B. parvibracteata* blooms later compared with its three allied species mentioned above.

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Phalaenopsis medogensis (Orchidaceae, Epidendroideae, Vandaeae), a new species from Xizang, China

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Abstract

A new species of Orchidaceae, *Phalaenopsis medogensis*, from Motuo, Xizang, is described and illustrated based on morphological characters and molecular phylogenetics analysis. Molecular phylogenetic analysis and morphological characters indicate that *P. medogensis* is close to *P. deliciosa*, *P. gibbosa* and *P. lobbii*, but differs from them by having triangular wings on the column foot, rhombic lip mid-lobe with a fleshy-horned appendage at the base, and concave lip lateral lobes, the lower part white with a deep purplish-red spot and hairy, the upper part pale yellow with dense rust spots.

Keywords

Flora, Motuo County, orchid taxonomy, Yarlungzangbo River

Introduction

The genus *Phalaenopsis* Bl. comprises 75 accepted names (<https://powo.science.kew.org/>), distributed from India and Southeast Asia to Australia and New Guinea with centres of diversity in Indonesia and the Philippines (Pridgeon et al. 2014; Deng et al. 2015). Based on molecular and morphological evidence, seven genera, including

Doritis, *Hygrochilus*, *Kingidium*, *Lesliea*, *Nothodoritis*, *Ornithochilus* and *Sedirea*, have been merged into *Phalaenopsis* s.l. (Tsai et al. 2010; Cribb and Schuiteman 2012; Kocyan and Schuiteman 2014). *Phalaenopsis* s.l. was redefined and a new infrageneric classification was proposed, comprising four subgenera, namely subgen. *Parishianae* (Sweet) Christenson, subgen. *Phalaenopsis* Blume, subgen. *Hygrochilus* (Pfitzer) Kocyan & Schuit, and subgen. *Ornithochilus* (Lindl.) Kocyan & Schuit (Kocyan and Schuiteman 2014; Pridgeon et al. 2014; Li et al. 2016; Aung and Jin 2021).

There are about 24 species of *Phalaenopsis* in China (Zhou et al. 2021). During our fieldwork in Motuo County, Nyingchi City, Xizang Autonomous Region, China in 2022, an unknown species of *Phalaenopsis* was found in the evergreen broad-leaved forest along the Yarlungzangbo River. Based on morphological characters and molecular evidence, it was identified as a new species of *Phalaenopsis* and is described below.

Materials and methods

Morphological characters of the new species were observed, measured and photographed, based on living plants in Motuo, Xizang. Four markers, including one nuclear marker (nrITS) and three plastid markers (*matK*, *trnL* and *trnL-F*), were used in molecular systematics. Primers and amplification procedures of the four markers followed Deng et al. (2015). In total, 40 species in *Phalaenopsis* and seven species of *Aeridinae* (Suppl. material 1) were included in the molecular analysis. The conflict between nrDNA and plastid DNA data was assessed in PAUA using the length difference test (ILD) (Darlu and Lecointre 2002). Two species, *Aeranthus grandiflora* Lindley and *Podangis dactyloceras* (Reichenbach) Schltr., were used as the outgroup, based on previous results (Chase et al. 2015; Li et al. 2019). Sequences were edited independently and assembled using SeqMan (<https://www.dnastar.com/>). Sequence alignment, model selection and super matrix construction were performed in the Phylosuite (Zhang et al. 2020). Bayesian Inference was inferred with MrBayes v. 3.2.7a on XSEDE in the CIPRES Science Gateway online web server (Miller et al. 2010). The model of partition selection was found in ModelFinder (Kalyaanamoorthy et al. 2017) with Corrected Akaike Information Criterion (AICc). GTR+F+I+G4 was selected as the best model for ITS and GTR+F+G4 for the three plastid markers. Two separate Markov Chain Monte Carlo (MCMC) analyses were performed, proceeding for 1,000,000 generations and sampling every 1000 generations. Maximum Likelihood (ML) analyses and model selection were performed in IQ-Tree 2 (Minh et al. 2020). Support values for the clade were estimated using 1,000,000 bootstrap replicates.

Results

The ILD test indicated that plastid markers and nrITS were not suitable for combined analysis. *Phalaenopsis medogensis* belongs to subgen. *Parishianae* and is close to *P. deliciosa*, *P. gibbosa* and *P. lobbii*, based on molecular phylogenetic analyses. The

Bayesian Inference and ML analyses of ITS showed that *P. medogensis* belongs to subgen. *Parishianae* with moderate to high support (Fig. 1, PP = 1, BS_{ML} = 93.5). The Bayesian Inference of the three plastid markers showed that *P. medogensis* is sister to the group that includes *P. deliciosa*-*P. lobbii* with high support (Fig. 2, PP = 1).

Taxonomy

Phalaenopsis medogensis X.H.Jin & C.B.Ma, sp. nov.

urn:lsid:ipni.org:names:77308554-1

Figs 3, 4

墨脱蝴蝶兰

Type. CHINA. Xizang Autonomous Region, Nyingchi City, Motuo County, elev. 800 m, 18 Apr 2022, Xiaohua Jin, Chongbo Ma & Tiankai Zhang 38519 (holotype PE!, isotype, PE!).

Diagnosis. *Phalaenopsis medogensis* is morphologically close to *P. deliciosa*, *P. gibbosa* and *P. lobbii*, but readily distinguished from them by its column foot having a pair of triangular wings, and a lip with concave subrectangular lateral lobes, that are white

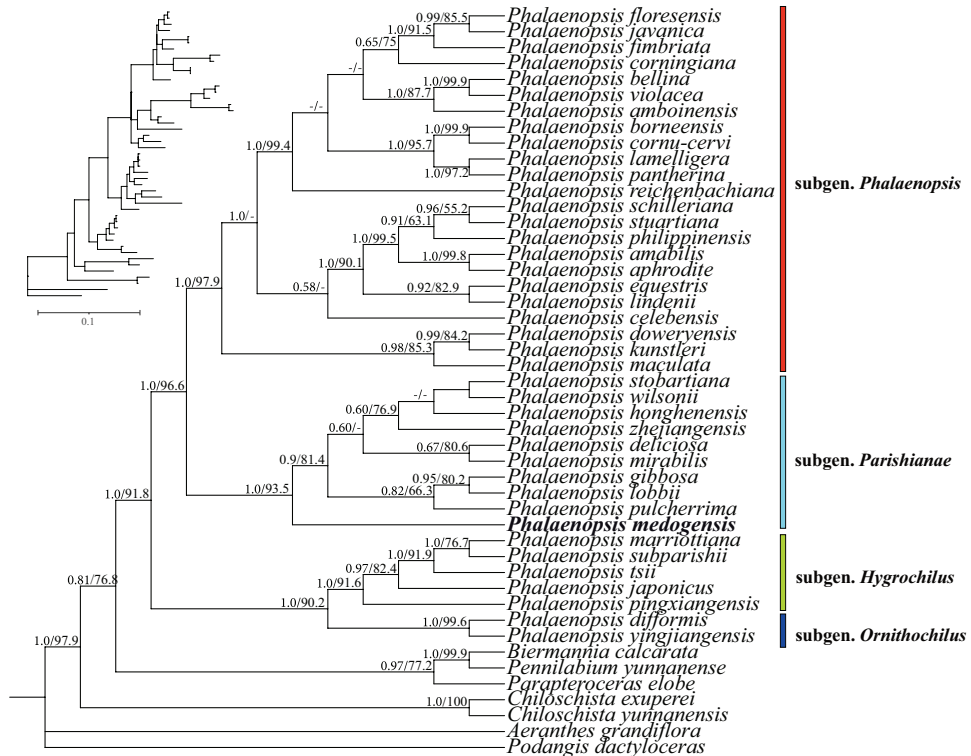


Figure 1. Phylogram of Bayesian Inference based on nrDNA ITS. Numbers above branches indicate posterior probabilities (PP) for BI analysis and bootstrap percentages (BS) for ML, respectively. A dash “-” indicates that support at a node < 50%.

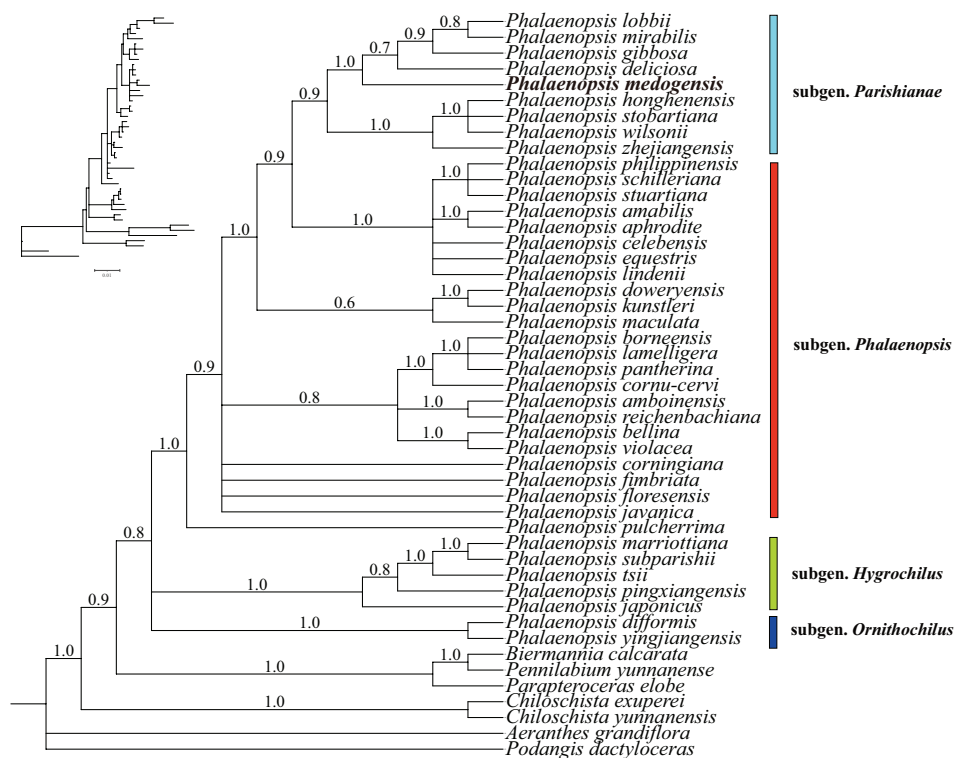


Figure 2. Phylogram of Bayesian Inference, based on plastid sequences (*matK*, *trnL* and *trnL-F*). Numbers above branches indicate posterior probabilities (PP).



Figure 3. Habitat and plants of *Phalaenopsis medogensis*.

with a deep purplish-red spot in their lower part and hairy and pale yellow with dense rust-coloured spots in their upper part, and a rhombic mid-lobe with a fleshy horned appendage at base and grooved in the centre (Table 1).

Table 1. Morphological comparison of *Phalaenopsis medogensis* and similar species.

	<i>P. medogensis</i>	<i>P. deliciosa</i>	<i>P. gibbosa</i>	<i>P. lobbii</i>
Flower colour	yellow	white with pale purple stripes or markings	white tinged with orange	White
Lateral sepals	broadly triangular, ca. 10 × 6 mm transversely spreading	obliquely ovate, 5.5–6 × 3.5–4 mm curving backwards	obliquely ovate or ovate-elliptic, about 6.5 × 4.5 mm	obliquely ovate to suborbicular, 8 × 7 mm, somewhat reflexed
Mid-lobe of lip	rhombic, 10 × 7 mm; disc grooved with a fleshy protuberant appendage; appendage 2–3 teeth on either side and apex with two long horns	obovate-cuneate, 6 × 5 mm, apex emarginate, with a central ridge; disc with a flattened and Y-shaped appendage	broadly triangular, concave, apex rounded 5–6 × 6–8 mm; disc with a denticulate free callus	reniform, 6 × 10 mm, concave with lateral margins shallowly incurved; disc with a callus of four filiform appendages
Lateral lobes of lip	subrectangular, concave, almost boat-shaped, lower part white with a deep purplish-red spot and hairy, upper part pale yellow with dense rust-coloured spots	obliquely elliptic-obovate, rounded, with tooth-like flaps, base decurrent and forming a broadly conic spur	erect, linear, falcate, acuminate	erect, falcate, parallel to middle and then diverging and forming a U-shaped structure
Column foot	with triangular wings on both sides	without wings	without wings	without wings



Figure 4. Flowers of *Phalaenopsis medogensis* X.H. Jin & C.B. Ma, sp. nov. **A** front view of flower **B** lateral view of flower **C** front view of flower, showing basal part of lip **D** lateral view of column, ovary and pedicel **E** lip, side view **F** lip, dorsal view **G** column and column foot **H** dorsal sepal **I** petal **J** lateral sepal **K** anther cap. Photographed by Xiaohua Jin and Chongbo Ma.

Epiphytic plant. Roots greenish, elongate, flattened, densely verrucose and prostrate along the twigs or trunk. Stem very short. Leaf 1, oblong-elliptic, 7–10 × 3–4 cm. Inflorescences 1 or 2, suberect or arching, ca. 12 cm long, unbranched, laxly 5–6 flowered; floral bracts ovate-triangular, 5–6 mm long. Flowers 1–1.2 cm in diameter,

yellow; sepals and petals pure yellow, lacking spots or other colouration. Dorsal sepal similar to petals, elliptic, ca. 7–8 × 5 mm; lateral sepals broadly triangular, ca. 10 × 6 mm. Petals long elliptic, ca. 9 × 5 mm. Lip 3-lobed; lateral lobes subrectangular, concave, almost boat-shaped, 2 × 4 mm, lower part white with a deep purplish-red spot and hairy, upper part pale yellow with dense rust-coloured spots; mid-lobe rhombic, ca. 10 × 7 mm, disc grooved, base with a fleshy protuberant appendage; appendage with 2–3 teeth on either side, apex with two long horns. Column subparallel to mid-lobe, ca. 5 mm long; column foot ca. 4 mm long, with triangular wings on both sides; anther cap yellow, hemi-spherical.

Etymology. The epithet “*medogensis*” refers to the type locality of the new species, Medog County, Nyingchi City, Xizang Autonomous Region, China.

Distribution and habitat. *Phalaenopsis medogensis* is currently known only from the type locality in Motuo, Xizang, China. It is epiphytic on trunks and twigs at elevations of 700–900m along the hot valley of the Yarlungzangbo River.

Phenology. Flowering in March to April.

Conservation status. *Phalaenopsis medogensis* grows in the tropical rain forest in Motuo County. At least one population of about 30 plants was discovered during our fieldwork. The habitat has been significantly damaged due to the development of agriculture and road construction. We tentatively assessed the risk of extinction of the *Phalaenopsis medogensis* as Critically Endangered (CR) under criteria B2ab(i, ii, iii, iv, v) according to the IUCN criteria version 15.1 (IUCN 2022).

Note. *P. medogensis* is similar to three species in subgen. *Parishianae*, namely, *P. deliciosa*, *P. gibbosa* and *P. lobbii*, but is readily distinguished from them based on morphological characters given in Table1.

Key to species of subgen. *Parishianae* in China

- 1a Leaves deciduous; lip mid-lobe smaller than petals and sepals and with central protuberance **2**
- 1b Leaves more or less persistent; lip mid-lobe broad, lacking an obvious protuberance or concave U-shaped compound structure in centre..... **5**
- 2a Column obviously elongated, ca. 5 × 1.2 mm, near base with an appendage; rostellum apex hooked and slightly bilobed, petals wider than sepals *P. zhejiangensis*
- 2b Column not obviously elongated, without an appendage at base; petals and sepals similar in width..... **3**
- 3a Lip mid-lobe obcordate with a central apical fleshy knob..... *P. wilsonii*
- 3b Lip mid-lobe of lip not obcordate, lacking a terminal notch **4**
- 4a Flowers pink; lip mid-lobe with a conspicuous constriction . *P. honghenensis*
- 4b Flowers deep green; lip mid-lobe oblanceolate, lacking any conspicuous constriction *P. stobartiana*
- 5a Terrestrial or lithophytic; lip mid-lobe split into three lobelets *P. pulcherrima*
- 5b Epiphytic; lip mid-lobe entire, slightly concave..... **6**

- 6a Column base not protuberant; lip more or less spurred; lateral lobe margins dentate.....7
- 6b Column base distinctly protuberant; lip not spurred; lateral lobes entire.....8
- 7a Lip mid-lobe sagittate; spur sacculate..... *P. mirabilis*
- 7b Lip mid-lobe obovate-cuneate, apex deeply emarginate, with a thickened central longitudinal ridge; spur indistinct..... *P. deliciosa*
- 8a Lip mid-lobe rhombic, its base with a fleshy protuberant appendage; appendage with 2–3 teeth on either side, apex with two long horns; column foot with triangular wings on both sides..... *P. medogensis*
- 8b Lip mid-lobe non-rhombic, its base with a callus with filiform appendages.....9
- 9a Lip mid-lobe white with one broad longitudinal chestnut-brown stripe, the basal callus deeply forked, with a crescent-shaped appendage in middle, each arm of the callus divided into two filiform-linear antennae *P. malipoensis*
- 9b Lip mid-lobe white with two broad longitudinal chestnut-brown or yellow stripes, its basal callus of four filiform appendages.....10
- 10a Lateral sepals transversely spreading, ovate-elliptic*P. lobbii*
- 10b Lateral sepals curving backwards, ovate *P. gibbosa*

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Supplementary material I

Voucher and GenBank accession numbers

Authors: Chong-Bo Ma Wang Xi-Long, Chen Wen-Shuai, Liu Zheng, Xiao-Hua Jin
Data type: Docx file.

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Morphological and molecular evidence reject conspecificity of Malagasy and Mascarene *Parablechnum* (Polypodiopsida, Blechnaceae)

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Abstract

Under the current treatment of the Blechnaceae, only one species of the fern genus *Parablechnum* is recognised in the western Indian Ocean, often referred to as *P. marginatum*. Two varieties are currently recognised within it: a type variety present in the Mascarene Islands of Réunion and Mauritius and *P. marginatum* var. *humbertii* in Madagascar. Recent molecular evidence suggested that these two varieties are not closely related, questioning their conspecific status. To collect further evidence to support a taxonomic decision, we performed a morphological study based on 57 herbarium specimens comparing traits from general morphology, cross section of the fertile pinnae, sporangia and spores. As a result, Malagasy specimens can be distinguished morphologically from the Mascarene ones by pinna apex and pinna section, the presence of sporangiasters and spore ornamentation. Additionally, spore size analyses resulted in statistically significant differences between both varieties. Our results, aligned with the available phylogenetic data, support that these two taxa should be recognised as separate species and, hence, we propose the necessary new combination and provide full descriptions.

Keywords

biogeography, Madagascar, *Parablechnum humbertii*, *Parablechnum marginatum*, Réunion, sporangiasters

Introduction

Parablechnum C.Presl is the most diverse genus within the fern family Blechnaceae, with about 65 species (Gasper et al. 2016) whose range shows two major centres of diversity, one in Meso and South America and a second one centred in Eastern Australasia. Currently, only two species are considered native outside these centres (Rakotondrainibe et al. 2013; Gasper et al. 2016), one present in southern and south-eastern Africa, *Parablechnum capense* (Burm.f.) Gasper & Salino and a second one native to the western Indian Ocean (Madagascar and the Mascarenes), *Parablechnum marginatum* (Kuhn) Gasper & Salino. Two varieties are currently considered within the latter (Rakotondrainibe et al. 2013; Gasper et al. 2016). The type variety, *Parablechnum marginatum* var. *marginatum*, is present in Réunion, from where the type was originally collected and was also collected in Mauritius (Appendix 1). A second variety, *P. marginatum* var. *humbertii* (Tardieu) Gasper & Salino accommodates the specimens found in Madagascar, originally considered as a distinct species by Tardieu-Blot (1955). The taxonomic rank of these two varieties, however, needs a reconsideration following recent molecular studies (Bauret 2017), where the specimens from Madagascar and Réunion did not cluster together in a monophyletic group. Here, we reassess the taxonomic status of the western Indian Ocean *Parablechnum* after a morphological examination of herbarium specimens of the two taxa.

Materials and methods

We examined 57 herbarium specimens from Herb. P (Appendix 1). We observed the general morphology (i.e. shape of the frond, scales, axes etc.); anatomy of the fertile pinnae; morphology of the sporangia; and size and ornamentation of the spores. Microscopy work followed Gurr (1966) and Ruzin (1999).

For the anatomy of the fertile pinnae, we performed cross-sections in at least two individuals of both varieties. The samples were softened for approximately 5 minutes in water and then manually cross-sectioned in the middle area of the fertile pinnae. The sections were then rinsed by immersion in a 50% solution of sodium hypochlorite for 2–5 minutes. After several washes with water, the sections were stained with 0.1% aqueous toluidine blue (TBO). All microscopic pictures were taken with a Nikon Eclipse Ci microscope with a Nikon DS-Fi2 camera.

Sporangia analysis was carried out by scraping the sori of the previously softened and rinsed pinnae. The protocol and terminology followed Prada et al. (2016), Molino et al. (2020) and Wal et al. (2021). Spores and sporangia were mounted directly in water, imaged under the optical microscope and measured with the *Piximètre* software (Henriot and Cheype 2022). We measured at least 30 spores from three different individuals of the two varieties, excluding the perispore and at least three sporangia from three different individuals of the two taxa. With the spore data, we calculated the shape and volume of each spore following the formulae of Barrington et al. (1986, 2020).

Spore and sporangium measurements were used to perform descriptive statistics and mean comparisons in R using the R Commander package (Fox 2005). Data were tested for normality using the Shapiro-Wilks normality test (Shapiro and Wilk 1965). For those that fit a normal distribution (spore volume), mean comparison was performed with a one-factor ANOVA and for those data that did not fit a normal distribution (spore length, width and shape), we performed a Mann-Whitney U test (Wilcoxon 1945; Mann and Whitney 1947).

From two individuals of each variety, we studied spore ornamentation through scanning electron microscopy (SEM). The samples were mounted in a sample holder with carbon adhesive, metallised with gold and observed in a SEM JSM 6400 JEOL operating at 20 kV. The observations were made at the National Center of Electronic Microscopy (CNME) of Universidad Complutense de Madrid. Photographs of details at a more macromorphological level, such as fronds or scales, were taken with a Leica Stereozoom S9i with Swing Arm Stand stereomicroscope.

Results

Our morphological analysis shows qualitative and quantitative differences between the two taxa that are summarised in Table 1 and the full descriptions given in the Nomenclature section. There are some differences in the sizes of the two taxa and in some characters, such as the sometimes slightly creeping rhizomes in the case of the *P. marginatum* var. *marginatum* (vs. erect or suberect in var. *humbertii*). However, we believe that the most reliable characters that easily distinguish the two taxa are the obtuse or acute apices on both sterile and fertile pinnae of *P. marginatum* var. *marginatum* (Fig. 1A) vs. the long-acuminate apices in both sterile and fertile pinnae of var. *humbertii* (Fig. 1B) and the smooth petiole surface in *P. marginatum* var. *marginatum* (Fig. 1C) vs. petioles with scars left by the scales in var. *humbertii* (Fig. 1D).

Fertile pinnae of *P. marginatum* var. *marginatum* present a costa, grooved adaxially and prominent abaxially, with three vascular bundles, elongated receptacle in the sori, covered by a short, complex indusium (composed by more than one cell layers), which arises at approximately one third of the distance between the margin and the costa, leaving a sterile portion towards the margin. The margin of the pinna is thick (Fig. 1E). Variety *humbertii* presents a costa grooved adaxially and prominent abaxially, with five vascular bundles and elongated receptacle in the sori, covered by long complex indusium, which arises on the first third of the distance between the margin and the costa, leaving a very small sterile portion of the margin (Fig. 1F). We consider that the different number of vascular bundles in the cross section of the fertile pinnae is the most distinctive diagnostic character.

Both taxa present monolete spores, with an ellipsoid outline in the polar view and flat-convex to concave-convex (reniform) in the equatorial longitudinal view. The spores of each taxon are described below. Sizes are rounded values; the exact values with their standard deviation can be found in Table 2, together with shape (length/width ratio) and estimated volume.

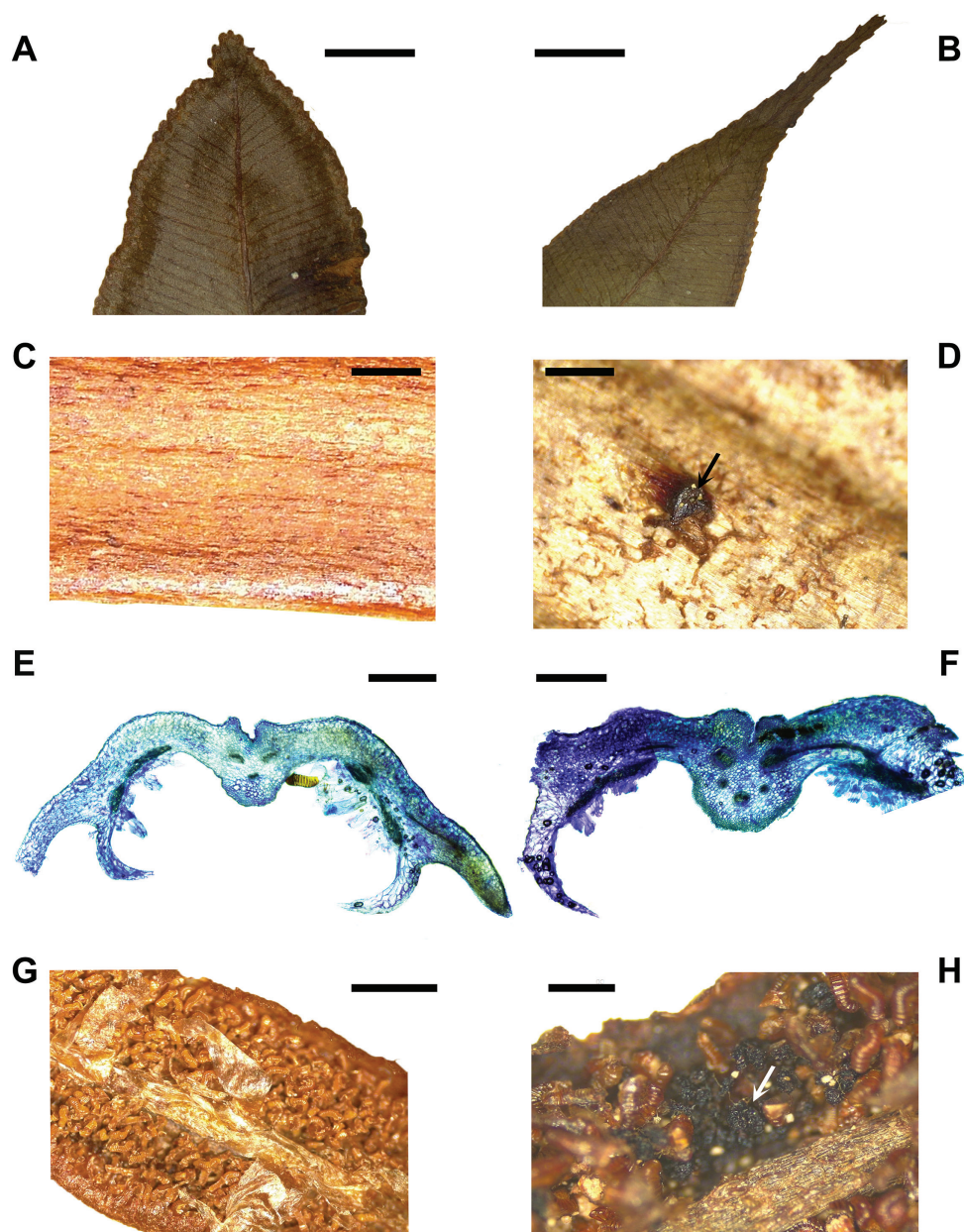


Figure 1. Details of the traits observed in *P. marginatum* var. *marginatum* (A, C, E, G) and *P. marginatum* var. *humbertii* (B, D, F, H) **A** apex of a sterile pinna in the var. *marginatum*, adaxial surface (Cowemoy s.n., P01462834) **B** apex of a sterile pinna in var. *humbertii*, adaxial surface (*Rakotondrainibe* 1673, P00100192) **C** petiole surface in the var. *marginatum* (*Cadet* 4050B2, P01462767) **D** petiole surface in var. *humbertii* with a scar left by a scale pointed with an arrow (*Rakotondrainibe* 1673, P00100193) **E** fertile pinna cross section of the var. *marginatum* (*Bradé* 958, P00917035) **F** fertile pinna cross section of var. *humbertii* (*Rakotondrainibe* & *Raharimalala* 2519, P00904704) **G** sorus in the var. *marginatum* (*Cadet* 4050B1, P01462768) **H** sori of var. *humbertii*, with sporangiasters pointed with an arrow (*Rakotondrainibe* 2743, P00059959). Scale bar: 5 mm (**A**); 2.5 mm (**B**); 1 mm (**C, D**); 800 μm (**E, F**); 2 mm (**G**); 500 μm (**H**).

Table 1. Summary of the most useful traits to distinguish *Parablechnum marginatum* var. *marginatum* from *P. marginatum* var. *humbertii*.

Taxon	Pinnae apices (Fig. 1A, B)	Petiole surface (Fig. 1C, D)	Number of bundles in the costa (Fig. 1G, H)	Sporangia (Fig. 1E, F)	Spore ornamentation (Fig. 2)
<i>P. marginatum</i> var. <i>marginatum</i>	Caudate	Smooth	5	Absent	Perisporium forming defined areolae, with filaments forming a net
<i>P. marginatum</i> var. <i>humbertii</i>	Long attenuate	With scars left by the scales	3	Present	Perisporium not forming defined areolae but a maze, filaments occasional

Table 2. Characterisation of the spores of both taxa. The mean $\pm$ standard deviation is presented.

Taxon	Spore length (μm)	Spore width (μm)	Shape	Volume (μm^3)
<i>P. marginatum</i> var. <i>marginatum</i>	64.32 $\pm$ 7.22	45.67 $\pm$ 6.83	1.42 $\pm$ 0.16	7.3435.74 $\pm$ 2.6210.03
<i>P. marginatum</i> var. <i>humbertii</i>	66.14 $\pm$ 5.42	48.74 $\pm$ 5.62	1.37 $\pm$ 0.12	8.4367.01 $\pm$ 2.3689.94

Spores of *P. marginatum* var. *marginatum*: (41–) 64 (–81) $\times$ (27–) 46 (–60) μm , perisporium folded cristate-reticulate, with protruding ridges and with large areas between them (areolae), measuring approximately 30 μm , covered with filamentous micro-ornamentation forming a kind of net that is arranged on a nearly smooth surface; internal structure of the perisporium of spongy appearance and irregularly granular exosporium (Fig. 2A, B).

Spores of *P. marginatum* var. *humbertii*: (49–) 66 (–78) $\times$ (35–) 49 (–62) μm , perisporium folded cristate-reticulate, with protruding ridges, but without large and regular areas between them, but rather irregular corridors, without filaments or with moderately abundant filaments distributed over the entire surface; internal structure of perisporium spongy in appearance and exosporium regularly granular (Fig. 2C, D).

After comparison of means using the tests specified above, we obtained significant differences for all characters between the two taxa (spore length $W = 6268.5$, $p\text{-value} = 0.047$; spore width $W = 5588.5$, $p\text{-value} = 0.0014$; shape $W = 8.959$, $p\text{-value} = 0.0057$; volume $F = 10.56$, $p\text{-value} = 0.001$), suggesting that the spores of *P. marginatum* var. *humbertii* are significantly larger than those of the var. *marginatum*. However, we believe that the best spore character to differentiate these taxa is the perisporium ornamentation, as, although there are significant differences in spore sizes, the ranges overlap (Fig. 2, Table 2). Spore ornamentation in *P. marginatum* var. *marginatum* form defined areolae and present a net of filamentous processes, while in var. *humbertii*, they do not form areolae, but corridors and filamentous processes are occasional.

The sporangia of both taxa are leptosporangiate, with pedicels of 2–3 rows of cells with a rosette joining them to a nearly spherical capsule with a vertical arc interrupted by a stoma. No posterior basal cells were observed. Table 3 summarises the morphometric variation of the sporangia. Additionally, the sporangia of *P. marginatum* var. *humbertii* are intermixed with shorter, darker, sterile sporangia that we interpret as

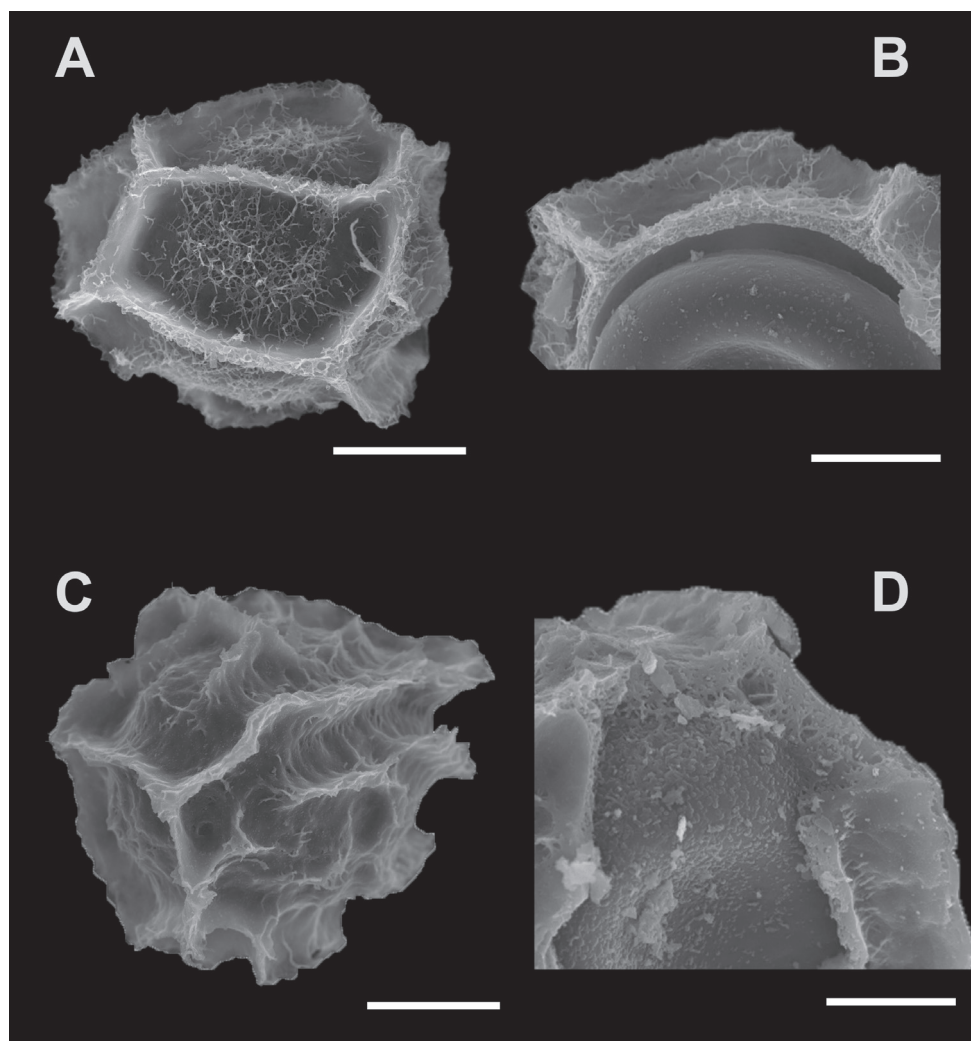


Figure 2. Spores of *Parablechnum marginatum* var. *marginatum* under SEM **A** spore (*Cowemoy s.n.*, P01462832) **B** detail of the internal structure of the perispore and the exospore (*Lorence s.n.*, MO2715099). Spore of *P. marginatum* var. *humbertii* under SEM **C** spore (*Rakotondrainibe 3571*, P0085125) **D** detail of the internal structure of the perispore and the exospore (*Rakotondrainibe 3571*, P00085125). Scale bar: 25 µm (**A**, **C**); 14 µm (**B**); 12 µm (**D**).

sporangiasters (Fig. 1H). None of the sporangium characters showed significant differences between the two taxa (number of arc cells $F = 3.237$, $p\text{-value} = 0.084$; arc width $F = 2.307$, $p\text{-value} = 0.142$; capsule length $W = 93$, $p\text{-value} = 0.905$; capsule width $F = 3.457$, $p\text{-value} = 0.075$; number of lip cells $W = 45$, $p\text{-value} = 0.117$; upper lip width $F = 0.087$, $p\text{-value} = 0.771$; lower lip width $F = 1.493$, $p\text{-value} = 0.237$; number of epistomium cells $W = 92$, $p\text{-value} = 0.3$; number of hypostomium cells $F = 0$, $p\text{-value} = 0.983$; pedicel length $F = 1.019$, $p\text{-value} = 0.319$; rosette length $F = 0.567$, $p\text{-value} = 0.455$).

Table 3. Characterisation of the sporangia of both taxa. The mean $\pm$ standard deviation is presented, all the values are in μm . Arc = number of cells in the arch; Arc wd = thickness of the arch; Cap = size of the sporangia capsule (length x width); Lip = number of cells forming the lip (stomium); Sup = upper lip cells width; Inf = lower lip cells width; Epi = number of cells in the epistomium; Hyp = number of cells in the hypostomium; Ros = rosette length; Ped = pedicel length.

Taxon	Arc	Arc wd	Cap	Lip	Sup	Inf	Epi	Hyp	Ros	Ped
<i>P. marginatum</i>	22.9 $\pm$	79.8 $\pm$	441 $\pm$ 71.3	4 $\pm$	52.4 $\pm$	54.6 $\pm$	3.5 $\pm$	2.5 $\pm$	59.3 $\pm$	561.7 $\pm$
var. <i>marginatum</i>	2.4	9.9	$\times$ 258.5 $\pm$ 26.6	1.1	16.5	17.3	1.1	0.8	17.7	110.7
<i>P. marginatum</i>	21.4	74.4 $\pm$	414.8 $\pm$ 73.4	4.7 $\pm$	54.5 $\pm$	46 $\pm$	3.1 $\pm$	2.5 $\pm$	55.4 $\pm$	514.3 $\pm$
var. <i>humbertii</i>	$\pm$ 2	7.7	$\times$ 278.1 $\pm$ 27.9	1.1	17.3	12.3	0.9	0.8	18.8	189

Discussion

To resolve the conspecificity hypothesis of *Parablechnum marginatum* var. *marginatum* and var. *humbertii*, we have performed a morphological analysis using traits usually showing systematic value within the family Blechnaceae. Regarding the anatomy of fertile pinnae, the study by Prada et al. (2016) defined the characters observable in pinnae cross-sections and showed how these have a high taxonomic value at the generic and specific level, which has been demonstrated in subsequent publications (Molino et al. 2019a, b; Bauret et al. 2020). Spores are a widely used character in fern taxonomy (Tryon and Tryon 1982; Barrington et al. 1986, 2020; Tryon and Lugardon 1990). Spore size, perisporium ornamentation and internal structure are known to be reasonably constant within species, but with considerable variation between species (Lugardon 1974; Tryon and Lugardon 1990). There are numerous studies on the spores of the family Blechnaceae and some of these have been used to successfully discriminate genera and species (Passarelli 2007; Passarelli et al. 2010; Moran et al. 2018; Silva et al. 2019, 2021; Molino et al. 2020; Wal et al. 2021). The genus *Parablechnum* is particularly complicated and the study of spores could be an important element in the delimitation of infrageneric taxa, as has been shown in other studies (Wal et al. 2021). The spores observed for these two taxa present typical ornamentation of the genus: folded cristate-reticulate perispore with or without filamentous processes (Moran et al. 2018). In this case, the ornamentation of the spores serves to distinguish the two taxa.

Sporangia are structures whose ontogeny and variation in characters have been studied for many groups of leptosporangiate ferns (e.g. Bower 1925; Copeland 1947; Wilson 1959). In particular, there have been specific studies in Blechnaceae where sporangia have been key in the separation of genera and species (Prada et al. 2016; Molino et al. 2020; Wal et al. 2021). Although we did not find differences in sporangial characters, their characterisation is novel and may be useful in future studies of the genus *Parablechnum* on a larger scale.

The presence of sporangiasters as a trait with taxonomic value in Blechnaceae was recently observed for the first time in *Parablechnum nesophilum* (T.C.Chambers & P.A.Farrant) Gasper & Salino, a species from Papua New Guinea (Molino et al. 2021).

Their occurrence in *P. marginatum* var. *humbertii* seems to also be a useful character to distinguish this taxon from the var. *marginatum*, suggesting that sporangiasters may be more widespread within the genus than previously thought.

In line with the phylogenetic tree topology obtained by Bauret (2017), this morphological comparison suggests that, as stated for the first time by Tardieu-Blot (1955), Madagascar specimens are not conspecific with those of the Mascarene Islands. In said analysis, *P. marginatum* var. *humbertii* is represented by two accessions and *P. marginatum* var. *marginatum* by another one. The latter is resolved in a clade with 16 American species that is sister to the former. Three maximally supported internal nodes (posterior probability ≥ 0.95 , bootstrap ≥ 95) segregate the Mascarene and Malagasy taxa.

Ferns in Madagascar and the archipelagos of the western Indian Ocean may be closely related to lineages from different biogeographic regions (Bauret et al. 2017a, b, 2018; Hennequin et al. 2017; Rouhan and Gaudeul 2021). To date, phylogenetic affinities suggest that *Parablechnum* species of the western Indian Ocean Islands are nested within a clade with many Neotropical taxa, in contrast with *P. capense*, the single continental African species, nested in an Austro-Pacific clade (Gasper et al. 2016, 2017).

Given the relatively recent age of the Blechnaceae (Testo and Sundue 2016) compared to the isolation of Madagascar and the origin of the Mascarenes, long-distance dispersal is the most likely hypothesis for explaining the presence of these taxa in these Islands (Bauret 2017; Bauret et al. 2017a, b, 2018; Rouhan and Gaudeul 2021). Given the topology of the phylogenetic tree (Bauret 2017), a single dispersal event cannot explain the occurrence of the two species in the Malagasy region and, so far, the most likely hypothesis includes two independent events. A richer sampling in an expanded phylogenetic analysis will be critical to resolving the number, origin and timing of the dispersion events to the region.

From the systematic point of view, available information rejects the conspecificity of the two taxa and, hence, we propose that the Malagasy taxon should recover the species rank within *Parablechnum*.

Nomenclature and full descriptions

***Parablechnum marginatum* (Kuhn) Gasper & Salino Phytotaxa 275(3): 191–227, 2016.**

≡ *Blechnum marginatum* Kuhn, Filic. Afr.: 92, 1868; *Blechnum montbrisonis* C.Chr. Index Filic. 157, 1905, nom. nov. for *Lomaria marginata* Fée, Mém. Foug., 5. Gen. Filic.: 71, 1852, nom. illeg. hom., non *L. marginata* Schrad., Gött. Gel. Anz. 871. 1824 [≡ *Lomariopsis marginata* (Schrad.) Kuhn].

Type. Habitat in insulâ Borboniâ, no date, de Montbrison s.n. (not found).

Description. *Plants* terrestrial; *rhizomes* erect, sub-erect or slightly creeping, non-stoloniferous, with ovate to lanceolate scales with elongated apex, more or less filiform, concolorous, brownish, membranaceous, up to 2 cm long; *fronds* dimorphic; *sterile*

fronds with **petioles** light brown, grooved adaxially, smooth, up to 50 cm long, with scales in basal zone decreasing in density distally, similar to those of the rhizome, **laminae** 1-pinnate, elliptic-acuminate, up to 1 m long, sometimes longer, **rachises** light brown, smooth, adaxially grooved, scales similar to those of petiole, more abundant on adaxial side, **pinnae** up to 30 pairs, alternate or subopposite, slightly smaller at base, lanceolate to oblong, stalked, becoming basiscopically adnate towards apex of frond, ca. 11 × 2 cm, base asymmetric, subcordate to truncate, apex acute or obtuse, margins slightly serrate, with conforming terminal pinna similar to lateral ones, **costae** light brown, grooved adaxially, prominent abaxially, with scales at base similar to those of rachis; **veinlet** simple or 1-furcate, patent, catadromous; **fertile fronds** larger than sterile ones and more erect, **petioles** similar to sterile fronds, **laminae** usually up to 50 cm long, lanceolate to oblong, **rachises** similar to sterile fronds, **pinnae** usually in more pairs than in sterile ones, linear, narrow, ca. 3.0 × 0.2 cm, slightly broader-based, asymmetrical, cordate, apex acute; **aerophores** present in both sterile and fertile fronds, tuberculiform, atropurpureus; **hydathodes** present in both sterile and fertile fronds, rounded or ovate; **sori** linear, continuous, on both sides of costa forming coenosori; **indusia** linear, continuous, opening towards costa, dark brown, membranaceous, usually lacerate.

Taxonomical notes. Christensen (1905) proposed *Blechnum montbrisonis* as a replacement name, as the name *Blechnum marginatum* proposed by Kuhn (1868) was based on an illegitimate basionym, *Lomaria marginata* Feé. This name was proposed by Feé (1852) after this combination had already been used by Scharder (1824) for what is now known as *Lomariopsis marginagta* (Schard.) Kuhn. However, according to the rules of the current Code (see Art. 6.14 Ex. 18; Turland et al. 2018), *Blechnum marginatum* would be a validly published replacement name for *Lomaria marginata* Feé and, therefore, *Blechnum montbrisonis* would be a superfluous name.

***Parablechnum humbertii* (Tardieu) S.Molino & Lafuente, comb. nov.**

urn:lsid:ipni.org:names:77308765-1

≡ *Blechnum humbertii* Tardieu Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 6: 232, f.5, 1955; *Blechnum montbrisonis* C. Chr. var. *humbertii* (Tardieu) Rakontondr. Adansonia, série 3, 35(2): 178, 2013. *Parablechnum marginatum* var. *humbertii* (Tardieu) Gasper & Salino Phytotaxa 275(3): 216, 2016.

Type. MADAGASCAR. ‘Vallée de la Lokoho, mont Beondroka, au Nord de Maroambihy, sylve à Lichens, sur gneiss et quartzite’, no date, Humbert 23554 (Holotype: P00483200).

Description. *Plants* terrestrial; *rhizomes* erect or sub-erect, non-stoloniferous, with ovate-lanceolate scales with elongated apex, more or less filiform, concolorous, brownish, membranaceous, with entire margins, up to 20 mm long; *fronds* dimorphic, sterile fronds with *petioles* 20–30 cm long, dark brown at base, straw-greyish distally, smooth, grooved adaxially, with scales in basal zone decreasing in density distally, similar to those of rhizome, leaving a black scar after falling off; *laminae* 1-pinnate, ellip-

tic-acuminate, up to 30 cm, sometimes longer, **rachises** light brown, smooth, grooved adaxially, scales similar to those of petiole but narrower, more abundant in the adaxial side, **pinnae** in up to 20 pairs, alternate or subopposite, slightly smaller at the base, lanceolate-oblong, stalked, becoming basiscopically adnate towards apex of the frond, 10 × 1.5 cm, base asymmetric, subcordate to truncated, apex long acuminate, margins serrate, with a conforming terminal pinnae similar to lateral ones; **veinlet** free, simple or 1-furcate, catadromous; **fertile fronds** longer than sterile and more erect, **petioles** similar to sterile fronds, **laminae** 30 cm long, sometimes longer, lanceolate-oblong, **rachises** similar to sterile fronds, **pinnae** usually in more pairs than in the sterile ones, linear, narrow, 12.0 × 0.2 cm, slightly broader-based, asymmetrical, cordate, apex long acuminate; **aerophores** present in both sterile and fertile fronds, tuberculiform, atropurpureus; **hydathodes** present in both sterile and fertile fronds, rounded or ovate; **sori** linear, continuous, forming coenosori on both sides of the costa; **indusia** linear, continuous, open towards the costa, dark brown, membranaceous, sometimes lacerate.

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Appendix I

Material examined

Parablechnum marginatum (Kuhn) Gasper & Salino

MAURITIUS. no date, Belanger 98C, (P01462824); no date, Bonpland s.n. (P01532252); ibidem (P01532253); 3 Dec 1909, Meller 6. **RÉUNION.** no date, no collector (P01462821); ibidem (P01462829); ibidem (P01462833); ibidem (P01557677); ibidem (P01557678); 1892, Cowemoy s.n. (P01462832); ibidem (P01462834); 1875, de Isle 606 (P01462825; P01462826; P01462827); 1842, Lépervanche-Mézière s.n. (P01462830); 1898, Lépervanche-Mézière 17 (P01462771); no date, Lépervanche-Mézière s.n. (P01462820; P01462823; P01462828); 8 Mar 1979, Lorence s.n. (MO3156135; MO3156136); Berge de la Rivière des Marsouins près Coserne

des Hirondelles Nébon, 22 Jul 1973, Cadet 4050B1 (P01462768); ibidem, Cadet 4050B2 (P01462767); Bourbon, no date, Richard 99 (P01462822); cirque de Salazie, sentier vers La Nouvelle, 29 Nov 1973, Badré 1052 bis (P01462769); ibidem, Badré 1055 (P01462836; P01462837); Fourré à Philippia, sentier de la Mare à Joseph au coteau Kerveguen, cirque de Cilaos, 16 Nov 1973, Badré 935 (P01462835, P00917036, P00917037); ibidem, Badré 958 (P00917032; P00917033; P00917034; P00917035); Nationale 3, Bord de route, entre le Col de Bellevue et la Plaine des Palmistes, 5 Nov 2004, Rakotondrainibe & Grangaud 6910 (P00411889); Sentier de Bélouve à la caverne Mussard, no date, Bosser 12208 (P01625974).

***Parablechnum humbertii* (Tardieu) S. Molino & Lafuente**

MADAGASCAR. Atsimo-Andrefana: Eboulis sableux Ambondrombe, 11 Apr 1941, Boiteau 4635 (P02284987; P01632222); **Diana:** Antsiranana, 24 Sep 2015, Baurer et al. 102 (P02435082); Antsiranana, Andapa, Parc National de Marojejy. Aux alentours du Camp 4, au bord de la rivière Andranomifototra, 25 Oct 2011, Rouhan et al. 1209 (P02432741; P02432742; P02432743; P02432744); Antsiranana, Andapa, Befingotra, RS d'Anjanaharibe-Sud, sur le versant Sud-Est, à 12.2 km à l'Ouest-Sud-Ouest de Befingra, 25 Nov 1994, Rakotondrainibe & Raharimalala 2519 (P00904704; P00046988); ibidem, 27 Nov 1994, Rakotondrainibe & Raharimalala 2534 (P00046998); Antsiranana, Andapa, RNI 12 du Marojejy. A 11 km au Nord-Ouest de Manantenina, 27 Oct 1996, Rakotondrainibe 3571 (P00085122; P00085123; P00085124; P00085125); Antsiranana, Ambanja, Massif du Manongarivo, Mt d'Antsatroto, berges de la rivière Ankaramihely, 19 May 1992, Rakotondrainibe 1673 (P00100192; P00100193; P00100194); Antsiranana, Iharana (Vohémar), Marojejy, route vers campement 3, 13 May 2015, Rabarijaona et al. ROM1053 (P00783078; P00783098); **Haute Matsiatra:** Fianarantsoa, Ambalavao, Ambatomboay, RNI d'Andringitra versant E; à environ 38 km au Sud d'Ambalavao, près de la source de la rivière Sahavatoy, 30 May 1995, Rakotondrainibe 2743 (P00059958; P00059959; P00059960; P00059961); **Sava:** Anjanaharibe, 19 Dec 1950, Cours 3772 (P01625801); Vallée de la Lokoho (nord-est), Mont Beondroka au Nord de Maroambihy, 17–22 March 1949 H. Humbert 23554, (P00483200, holotype).

A new synonym of *Enkianthus perulatus* (Ericaceae) in East Asia, based on morphological and molecular evidence

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Abstract

Enkianthus calophyllus was once treated as a synonym of *E. serrulatus*. However, field observations indicate that *E. calophyllus* is distinct from *E. serrulatus* but resembles *E. perulatus* in flowers, leaves, fruits and seeds. Hence, a taxonomic revision of these species was conducted based on morphological comparisons of flowers, leaves, fruits and seeds, as well as molecular analyses of nuclear ribosomal internal transcribed spacer (nrITS) and six plastid DNA markers (*psbA-trnH*, *rpl32-trnL*, *trnL-trnF*, *rps16-trnQ*, *psbJ-petA* and *matK*). The morphological and molecular results reject the synonymization of *E. calophyllus* with *E. serrulatus*, and instead show it to be placed in a clade with *E. perulatus*. Based on molecular evidence and a reassessment of the morphology we synonymize *E. calophyllus* with the older name *E. perulatus*.

Keywords

Enkianthus calophyllus, *Enkianthus perulatus*, morphology, phylogeny, synonym

Introduction

Enkianthus Lour. is a small genus in Ericaceae with about 12–17 species (Anderberg 1994; Kron et al. 2002; Fang and Stevens 2005). It is only distributed in East Asia, and most of its component species are in China and Japan. *Enkianthus* is ornamentally important for its elegant flowers and ecologically valuable because it always dominates in subtropical montane elfin forests (Hsu 1982). Accumulating molecular phylogenetic evidence suggests that *Enkianthus* is the first diverging lineage of Ericaceae (Kron and Chase 1993; Kron 1996; Morton et al. 1996; Anderberg et al. 2002; Kron et al. 2002), indicating that this genus is key to understand the evolution of Ericaceae.

Species in *Enkianthus* are shrubs or small trees, leaves blade serrate or subentire, inflorescences often umbels and racemes, corollas broadly campanulate to urceolate, capsule loculicidal, seeds often lamellate-winged (Fang and Stevens 2005). The species of *Enkianthus* vary in leaf texture, inflorescences structure, corolla shape, and anther morphology, pollen and seed (Cheng and Lai 1988; Anderberg 1994; Kron et al. 2002; Sarwar and Takahashi 2006). Infrageneric relationships of *Enkianthus* were studied by Anderberg (1994), who proposed a classification comprising four sections (sect. *Enkianthus*, sect. *Andromedina*, sect. *Enkiantella* and sect. *Meisteria*). Among them, sect. *Enkianthus* is monophyletic according to phylogenetic analyses (Tsutsumi and Hirayama 2012). However, due to the variable morphology in this genus (Hsu 1982), classification of some species, especially those with wide distribution range, remains controversial.

During the past years, we have found several unique *Enkianthus* populations in montane areas of Zhejiang and Jiangxi Province in China (Fig. 1). These plants are 1–3 m tall, white urceolate flowers with distinct basal gibbosities, rhombic-elliptic leaves and erect capsule. After scrutinizing the protologue and type specimens, we found that our collections matched the description of *E. calophyllus* T.Z. Hsu exactly (Fig. 2, Suppl. material 1: fig. S1B; Hsu 1985). When Fang and Stevens (2005) treated *E. calophyllus* as a synonym of *E. serrulatus* (E.H. Wils.) C.K. Schneid. (Fig. 3, Suppl. material 1: fig. S1C) in *Flora of China* (FOC), flowering specimens of *E. calophyllus* were lacking (Fang and Stevens 2005). However, our collections and *E. calophyllus* differ from *E. serrulatus* by urceolate corollas with distinct basal gibbosities, margin with ciliate, smaller fruits (0.5–0.7 cm × 0.3–0.4 cm) and seeds without distinct wings (Figs 2, 3; Schneider 1911; Hsu 1985), and such characters were described for *E. perulatus* C.K. Schneid. from Japan (Fig. 4, Suppl. material 1: fig. S1A; Schneider 1911). These observations raise a question about the taxonomic status of *E. calophyllus* and the identity of our collections. To identify our new collections and clarify the taxonomic status of *E. calophyllus*, morphological comparisons and molecular phylogenetic analyses were performed to study the taxonomic relationships amongst *E. calophyllus*, *E. perulatus*, *E. serrulatus* and our new collections.

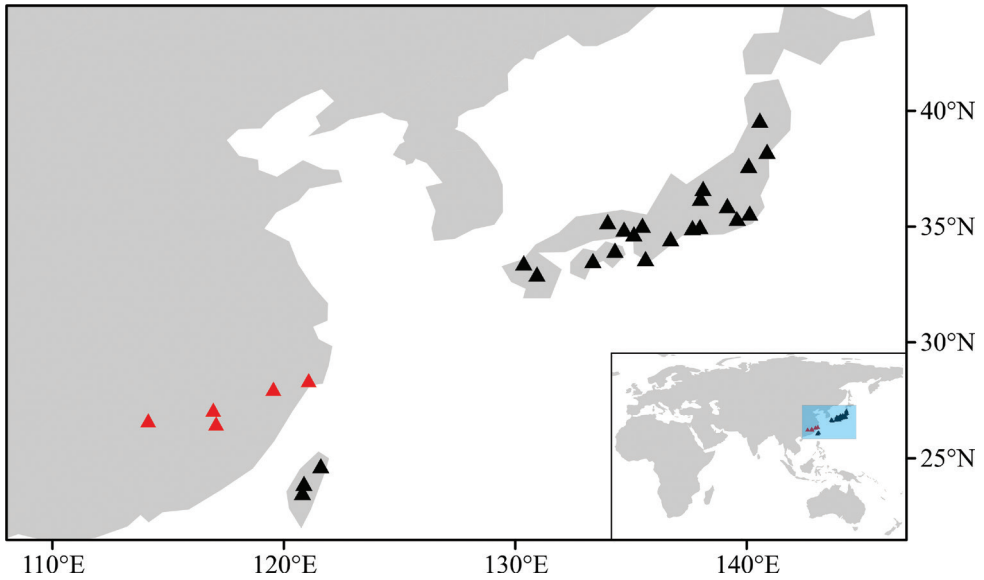


Figure 1. Distribution of *Enkianthus perulatus* based on specimen records and our field investigation. Black triangles indicate *E. perulatus*; red triangles indicate *E. calophyllus* (= *E. perulatus*).

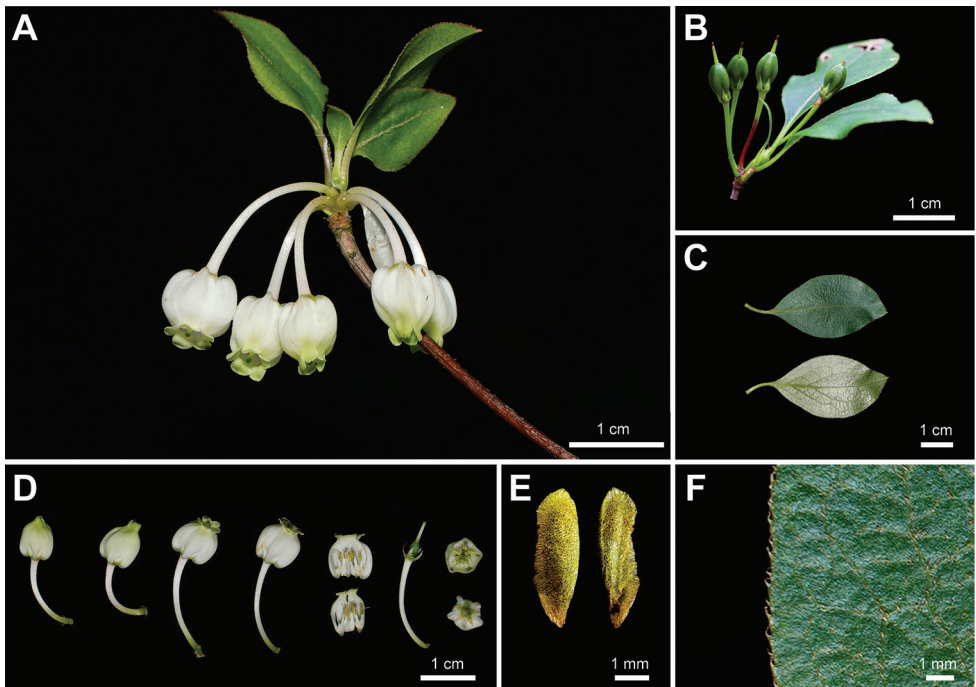


Figure 2. Morphology of *Enkianthus calophyllus* (= *E. perulatus*) **A** flowering branch **B** fruiting branch **C** leaves **D** flowers **E** seeds **F** leaf margin. **A–F** photographed by H. Liang.

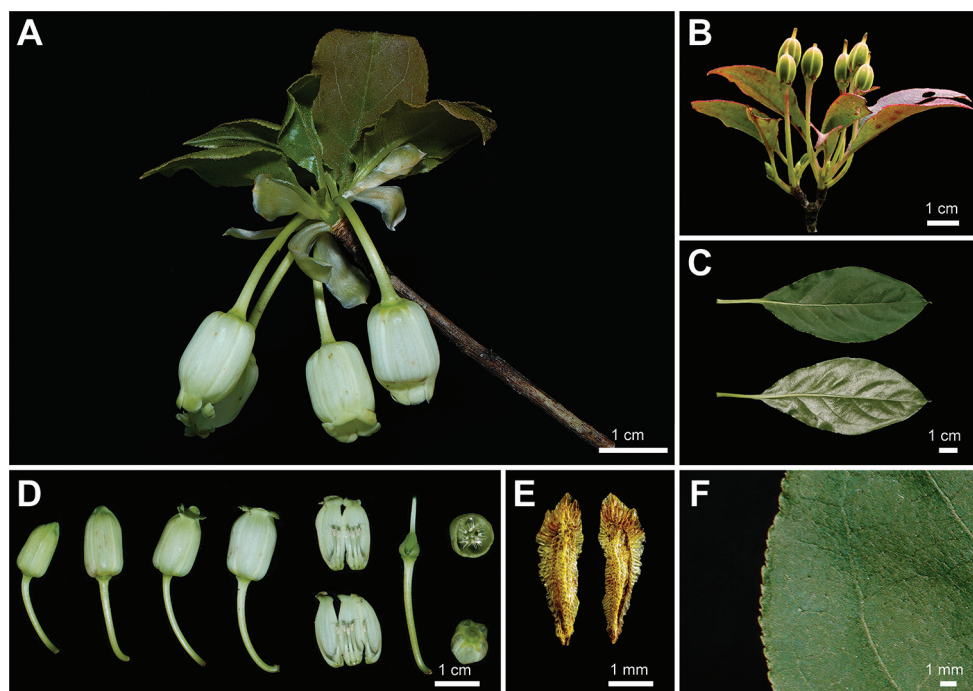


Figure 3. Morphology of *Enkianthus serrulatus* **A** flowering branch **B** fruiting branch **C** leaves **D** flowers **E** seeds **F** leaf margin. **A–F** photographed by H. Liang.

Materials and methods

Morphological studies

Our collections and digital images of *Enkianthus perulatus*, *E. calophyllus* and *E. serrulatus* from the Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>), the web of Plants of Taiwan (<http://tai2.ntu.edu.tw>), the Kingdonia (<http://kun.kingdonia.org/>) and the Global Biodiversity Information Facility (<https://www.gbif.org/>) were examined to test whether there are significant differences in leaves and fruits of the three species. The high-resolution images of those *Enkianthus* species were taken from specimens deposited at CDBI, CSFI, CSH, GA, GXMI, GZTM, JIU, JXAU, K, KUN, L, LGB, NAS, NTUF, P, PE, SYS, TAI, USF, and ZY. We randomly selected three well-preserved leaves and/or fruits from each specimen (28 specimens of *E. serrulatus*, 18 of *E. calophyllus* and 15 of *E. perulatus*). Among them, nine specimens of *E. calophyllus* and seven of *E. serrulatus* were collected by ourselves. Eight morphological characters, i.e., leaf length, leaf width, ratio of leaf length/width, pedicel length, fruit length, fruit width, ratio of fruit length/width and carpodium length, were measured in this study. ImageJ (Rasband 1997) was applied to the examination of the focal characters.

ANOVA was performed to test the significance of pairwise difference of eight characters using SPSS 26. Principal Component Analysis (PCA) was performed in

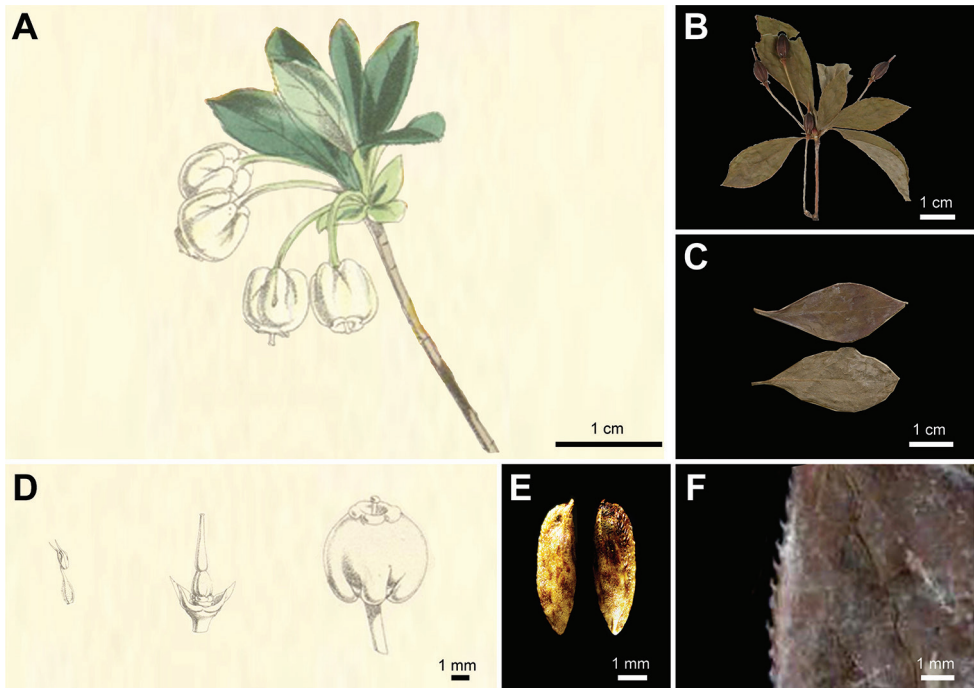


Figure 4. Morphology of *Enkianthus perulatus* **A** flowering branch (Hooker 1870) **B** fruiting branch (USF 121400) **C** leaves (K-000780276) **D** flowers (Hooker 1870) **E** seeds **F** leaf margin (K-000780276). **E** photographed by H. Liang.

ORIGIN 2021 to investigate the morphological variations among *E. perulatus*, *E. calophyllus* and *E. serrulatus*. Morphological analysis was not carried out for flowers and seeds, because there were only a few specimens available for analysis.

Sample collection, DNA extraction, PCR amplification, and sequencing

We collected 19 samples from 13 populations (1–3 individuals per population) of five *Enkianthus* species (Suppl. material 1: table S1). Of these, 17 samples belonged to species of sect. *Enkianthus*, i.e., six of *E. calophyllus*, four of *E. serrulatus*, four of *E. perulatus*, and three of *E. quinqueflorus* Lour. (Suppl. material 1: table S1). Two species of sect. *Enkiantella*, *E. chinensis* Franch. and *E. deflexus* (Griff.) Schneid. were also collected. Based on previous studies (Tsutsumi and Hirayama 2012), we downloaded nuclear ribosomal internal transcribed spacer (nrITS) and plastid DNA markers of other *Enkianthus* species from the National Center for Biotechnology Information's (NCBI; <http://www.ncbi.nlm.nih.gov/>) nucleotide database (Suppl. material 1: table S1). In addition, we selected species of genera *Rhododendron*, *Vaccinium* and *Clethra* as outgroups to carry out the phylogenetic analysis of sect. *Enkianthus* according to previous studies (Kron et al. 2002; Liu et al. 2014). Voucher specimens are deposited in the Herbarium of Jiangxi Agricultural University (JXAU). Total genomic

DNA were extracted from the silica-dried leaves using a modified cetyltrimethylammonium bromide method (Doyle and Doyle 1987). Six chloroplast DNA (cpDNA) regions (*psbA-trnH*, *rpL32-trnL*, *trnL-trnF*, *rps16-trnQ*, *psbJ-petA* and *matK*) (Taberlet et al. 1991; Sang et al. 1997; Shaw et al. 2007) and nrITS (Sun et al. 1994) in 19 individuals from 13 populations of *Enkianthus*, were PCR amplified and sequenced. The PCR amplification protocols followed Cheng et al. (2021), and primers are listed in supplementary (Suppl. material 1: table S2). Newly generated sequences in this study are deposited in GenBank (Suppl. material 1: table S1).

Phylogenetic analysis with cpDNA and nrDNA sequence data

The matrices of DNA sequences were aligned using MAFFT v.7 (Katoh and Standley 2013), and improved manually using BioEdit 7.0.9 (Hall 1999). Bayesian inference (BI) and maximum likelihood (ML) were used for phylogenetic analysis on the CIPRES Science Gateway 3.3 (www.phylo.org; Miller et al. 2015) with the best-fit model of DNA substitution estimated by jModelTest v.2.1.4 (Darriba et al. 2012). The alignments of nrITS, *matK* and the concatenated plastid DNA (*psbA-trnH* + *rpL32-trnL* + *trnL-trnF* + *rps16-trnQ* + *psbJ-petA* + *matK*) were analyzed with GTR + G, GTR and GTR + G + I model, separately. We reconstructed a *matK* phylogeny of *Enkianthus* because the other five chloroplast DNA regions were sequenced only in a subset of species. Bayesian analysis was constructed using MrBayes v.3.2.7 (Ronquist et al. 2012). We performed two independent BI runs with one cold and three heated chains for 10,000,000 Markov chain Monte Carlo generations. We sampled trees every 1,000 generations and discarded the first 25% generations as burn-in. ML analysis was conducted by RAxML-HPC (Stamatakis 2014) with 1000 bootstrap replications.

Results and discussion

Morphological analyses

Principal Component Analysis (PCA) showed that our collections of *Enkianthus calophyllus* clustered with their type specimens and the 95% confidence ellipse of *E. calophyllus* intersected marginally that of *E. serrulatus*; however, the ellipse of *E. calophyllus* almost overlapped with that of *E. perulatus* (Fig. 5). In addition, pairwise comparisons of the eight morphological traits among *E. perulatus*, *E. calophyllus* and *E. serrulatus* (ANOVA analysis) showed there were significant differences between *E. calophyllus* and *E. serrulatus* (Fig. 6), but no significant difference between *E. calophyllus* and *E. perulatus* in all compared traits except for leaf width and ratio of leaf length/width (Fig. 6). Last but not least, morphological observation also found that *E. calophyllus* was almost the same as *E. perulatus*, but differs from *E. serrulatus* in flowers and seeds (Table 1). These results suggest that *E. calophyllus* should be conspecific with *E. perulatus* rather than *E. serrulatus*. The morphological description and comparison are elaborated in Table 1.

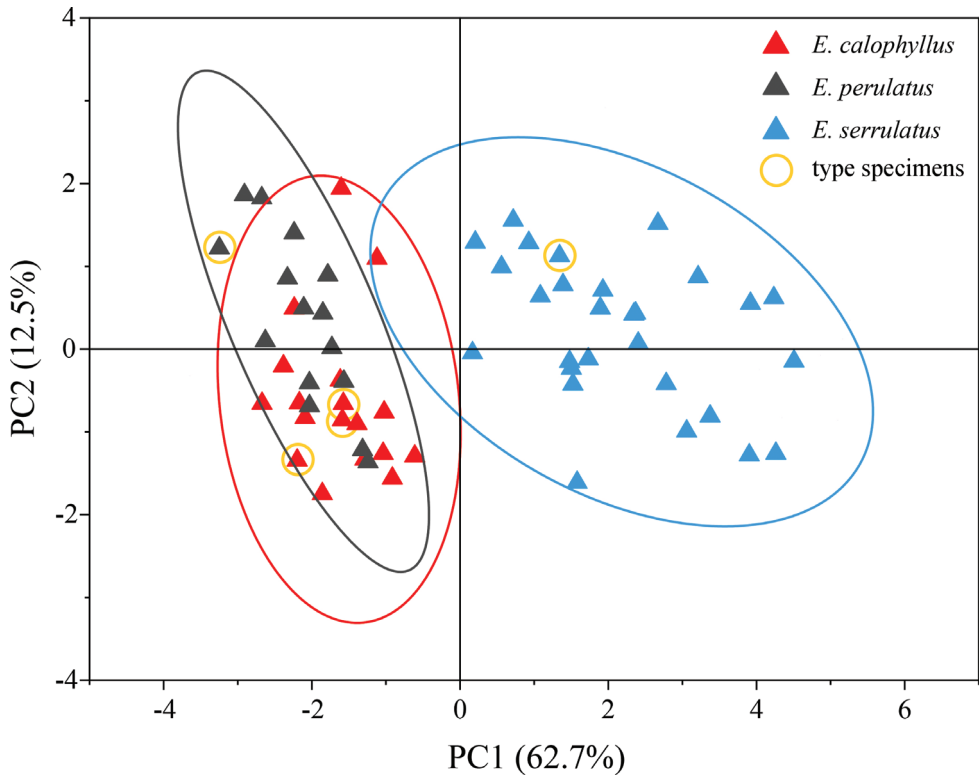


Figure 5. The Principal Component Analysis (PCA) plot for the morphological variations amongst *Enkianthus perulatus*, *E. calophyllus* (= *E. perulatus*) and *E. serrulatus*. Red, gray and blue triangles represent *E. perulatus*, *E. calophyllus* and *E. serrulatus*, respectively. Yellow circles indicate type specimens. The confidence ellipse level is 95%.

Phylogenetic relationships

Alignment length of nrITS sequences based on 10 species (*E. calophyllus* = *E. perulatus*) of *Enkianthus* (approx. 83% species of *Enkianthus*, Fang and Stevens 2005) is 595 bp, including 75 variable sites and 60 parsimony informative sites. Alignments of *matK* consisting of the same 10 species contain 755 constant sites, 24 variable sites and 9 parsimony informative sites. The concatenated length for six plastid DNA fragments based on five species of *Enkianthus* is 4,768 bp, and the matrix contains 124 variable sites and 85 parsimony informative sites in total. Phylogenetic analyses based on nrITS or *matK* supported that sect. *Enkianthus* was a monophyletic clade. Although the nrITS tree showed that six accessions of *E. calophyllus* form a monophyletic clade (bootstrap value, BS = 88, Bayesian posterior probability, PP = 0.86; Fig. 7A), this clade was nested within *E. perulatus* (BS = 100, PP = 1; Fig. 7A). Notably, *E. calophyllus* intermingled with *E. perulatus*, forming a highly supported clade in the *matK* tree (BS = 97, PP = 0.97; Suppl. material 1: fig. S2). Furthermore, the six plastid DNA tree supported that

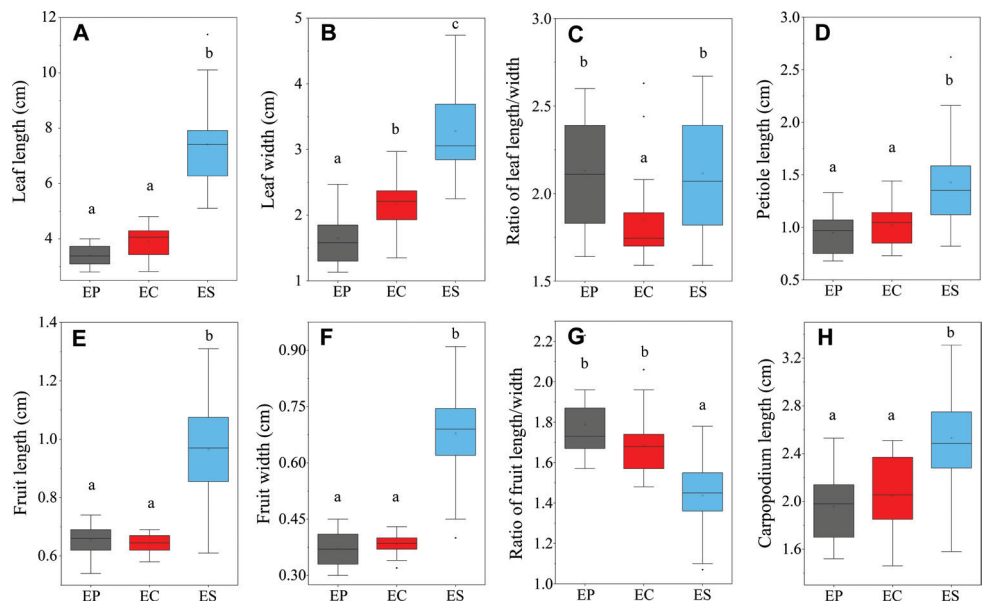


Figure 6. Box plots of the character comparisons amongst *Enkianthus perulatus* (EP), *E. calophyllus* (= *E. perulatus*; EC) and *E. serrulatus* (ES) **A** leaf length **B** leaf width **C** ratio of leaf length/width **D** pedicel length **E** fruit length **F** fruit width **G** ratio of fruit length/width **H** carpopodium length. The different lowercases on the top of the vertical line indicate significant differences ($P < 0.05$) between the three taxa.

Table 1. Comparisons of morphological characters amongst *Enkianthus perulatus*, *E. calophyllus* and *E. serrulatus*.

Characters	<i>E. perulatus</i>	<i>E. calophyllus</i>	<i>E. serrulatus</i>
Habit	shrubs deciduous, 1–2 m tall	shrubs deciduous, 1–3 m tall	shrubs or small tree, deciduous, 3–6 m tall
Texture of leaf	Papery	Papery	papery or thickly papery
Petiole	0.5–1.2 cm	0.5–1.4 cm	0.7–2.1 cm
Leaf blade	oblong, obovate-oblong; 2–4 (–5) cm × 0.8–2.0 cm	rhombic-elliptic, elliptic; 2.5–5.0 cm × 1.5–3.0 cm	elliptic, oblong-elliptic or obovate-elliptic; 6–9 (–11) cm × 3–4 (–5) cm
Leaf margin	margin with ciliate	margin with ciliate	margin without ciliate
Inflorescence	umbellate, 1–5-flowered	umbellate, 1–5-flowered	umbellate, 2–6 (–9)-flowered
Corolla	urceolate with distinct basal gibbosities; white; 0.6–0.7 cm × 0.5–0.7 cm	urceolate with distinct basal gibbosities; white; 0.6–0.8 cm × 0.5–0.8 cm	oblong-urceolate without distinct basal gibbosities; greenish-white; 1.3–1.6 cm × 0.8–1.0 cm
Length/width of Corolla	1.0–1.3	1.0–1.4	1.5–2.0
Pedicel	1–2 cm	1.4–2.2 cm	2–3 cm
Fruit	capsule erect, oblong, 0.6–0.7 cm × 0.3–0.4 cm	capsule erect, oblong, 0.5–0.7 cm × 0.3–0.4 cm	capsule erect, oblong, 0.8–1.2 cm × 0.5–0.8 cm
Seed	without distinct wings	without distinct wings	with distinct wings
Distribution	Taiwan China, Japan (Honshu, Shikoku and Kyushu)	China (Zhejiang, Fujian, Jiangxi)	China (Jiangxi, Hubei, Hunan, Guangdong, Guangxi, Guizhou, Chongqing, Sichuan, Yunnan)
Altitude	200–1600 m	600–1200 m	800–1800 m

the monophyly of *E. perulatus* and *E. calophyllus* was recovered again (BS = 100, PP = 1; Fig. 7B), and *E. calophyllus* is paraphyletic with respect to *E. perulatus* (Fig. 7B). In all the trees, *E. serrulatus* clustered with *E. quinqueflorus* rather than with *E. perulatus*.

Taken together, we propose that *E. calophyllus* should be recognized as a new synonym of *E. perulatus* rather than the synonym of *E. serrulatus* as suggested by Fang and Stevens (2005). In addition, *E. serrulatus* together with *E. quinqueflorus* may represent a well differentiated lineage relative to *E. perulatus*.

Taxonomic treatments

Enkianthus perulatus (Miq.) C.K. Schneid

Andromeda perulata Miq., Ann. Mus. Bot. Lugduno-Batavi 1: 31. 1863. Basionym.

Type: JAPAN. *W. Botanicus* 57 (holotype: L-0007044!, Suppl. material 1: fig. S1A).

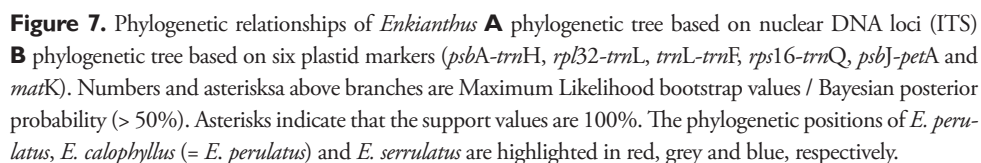
Enkianthus japonicus Hook. f., Bot. Mag. 96: 5822. 1870. Type: JAPAN. 1860, *R. Alcock s.n.* (holotype: K-000780276!). $\equiv$ *Enkianthus perulatus* var. *japonicus* (Hook. f.) Nakai, J. Jap. Bot. 12(12): 896. 1936. $\equiv$ *Enkianthus perulatus* f. *japonicus* (Hook. f.) Kitam., Acta Phytotax. Geobot. 25: 36. 1972.

—*Enkianthus taiwanianus* S.S. Ying, Quart. J. Chin. Forest. 9: 145. 1976. Type: CHINA. Taoyuan City, Peichiatienshan, 1976, *Ying 5301* (holotype: NTUF-F00006903!; isotype: NTUF-F00008372, NTUF-F00008372, NTUF-F00008372, NTUF-F00008372!). $\equiv$ *Enkianthus perulatus* var. *taiwanianus* (S.S. Ying) Y.C. Liu, Tr. Taiwan 514. 1988.

—*Enkianthus calophyllus* T.Z. Hsu, Acta Bot. Yunnan. 7(2): 151–152. 1985. syn. nov. Type: CHINA. Zhejiang: Jingning County, 16 May 1959, *S.Y. Zhang 5286* (holotype: PE-00005629!, Suppl. material 1: fig. S1B; isotype: NAS-00063024!, KUN-0001289!).

Description. Deciduous shrubs up to 3 m tall. Branchlets terete, glabrous. Leaves alternate on young shoots, generally 4–6 crowded at apex of branchlets; petioles 0.5–1.4 cm long; blades papery, oblong, obovate-oblong, rhombic-elliptic or elliptic, 2–5 cm long, 0.8–3.0 cm wide, apex acuminate, mucronate, base attenuate-cuneate or cuneate, margin ciliate, upper surface glabrous except sparsely pubescent on midrib, lower surface villous along lower part of midrib. Inflorescences terminal, umbellate, 1–5-flowered, pendulous. Pedicels 1.0–2.2 cm long, glabrous, reflexed at flowering, straight at fruiting. Bracts lanceolate, white or green, 0.8–1.8 cm long, caducous. Calyx lobes triangular, 2–4 mm long, glabrous, deeply 5-lobed. Corollas urceolate, with distinct basal gibbositities, white, 6–8 mm long, 5–8 mm wide, shallowly 5-lobed; lobes widely ovate, obtuse, much reflexed. Stamens 10, ca. $2/3$ as long as corolla; filaments villous at base, anthers with 2 awns on upper dorsal side. Ovaries glabrous. Capsules erect, oblong, 5-ridged, 5–7 mm long, 3–4 mm wide. Seeds compressed, narrowly oblong, ca. 4 mm long, 1 mm wide, with marginal-like ridges, without distinct wings.

Phenology. Flowering from April to May; fruiting from May to November.



Additional specimens examined. CHINA. **Zhejiang:** Yueqing City, Yandangshan, 6 April 2015, *X.Y. Ye* 2015040609 (CSH); *ibidem*, 20 May 2019, *H. Liang* LSBZ-259

(JXAU). **Fujian:** Taining County, Xinqiao Town, 16 June 1978, *G.L. Cai* 445 (KUN). **Jiangxi:** Jinggangshan City, Jinggang Mountains, 15 July 1965, *S.K. Lai et al.* 4466 (LBG); Lichuan County, Huixianfeng, 20 October 1985, *S.K. Lai & D.F. Huang* 473 (LBG); *ibidem*, 16 November 2021, *H. Liang* 088 (JXAU). **TAIWAN:** Taipei City, Tunlu, 14 April 1935, *T. Suzuki* 19235 (TAI); Taipei City, Lupeishan, 16 April 1991, *Y.B. Cheng & T.S. Hsieh* 1202 (TAI); Taoyuan City, Peichatienshan, 28 September 1984, *R.T. Li* 3532 (TAI); Chiayi City, Alishan, 5 April 1982, *Y.F. Chen* 4604 (TAI), *ibidem*, 17 May 1982, *Y.F. Chen* 4902 (TAI). **JAPAN. Honshu:** Wakayama-Prefecture, Ohdaigahara Mountains, 23 May 1925, *S. Saito* (PE); Shizuoka-Prefecture, Tagata-gun, Sanagi Mountains, 24 April 1952, *M. Furuse* 24733 (PE); Nagano-Prefecture, Iida-shi, 27 April 1962, *M. Furuse* 39658 (PE); Aichi-Prefecture, Minami-shitara-gun, 22 April 1978, *M. Furuse* 12532 (PE); Aichi-Prefecture, Shinshiro-shi, 6 August 1978, *M. Furuse* 13039 (PE).

Additional specimens of *Enkianthus serrulatus* examined. **CHINA. Guangxi:** Debao County, 25 April 1977, *D. Fang et al.* 3-219 (GXMI); Longsheng County, 24 August 2018, *H. Liang* LSBZ-218 (JXAU); Longlin County, 17 May 1977, *T.H. Wei* 3-0606 (GXMI); Xingan County, 26 July 1997, *G.Z. Li* 15137 (PE). **Guizhou:** Chishui City, 24 May 2020, *H. Liang* LSBZ-297 (JXAU); Leishan County, 13 June 2020, *H. Liang* LSBZ-323 (JXAU); Songtao County, 22 July 1959, *T.P. Zhu et al.* 1592 (KUN); Suiyang County, 11 May 2010, *Y.F. Zhou* KKS101197 (ZY); Zhengnan County, 14 October 2014, *H.W. Zhang* 520324141014031LY (GZTM). **Hubei:** Lichuan County, 7 October 1980, *B. Bartholomew et al.* 2014 (PE); Tongshanx County, 14 May 2017, *H.Y. Zhan et al.* LXP5905 (LBG); Yichang City, 19 March 2017, *D.G. Zhang et al.* ZCJ170319117 (JIU). **Hunan:** Sangzhi County, 4 August 2017, *Z.Y. Zhang et al.* LSBZ-142 (JXAU); Xinning City, 5 July 2017, *Z.K. Liu* LSBZ-135 (JXAU); Yongshun County, 3 August 2017, *Z.Y. Zhang et al.* LSBZ-138 (JXAU); Zhangjiajie City, 11 September 2015, *H. Zhou & D.S. Zhou* 15091113 (CSFI). **Jiangxi:** Jinggangshan City, 27 August 2020, *Y.F. Liu* LSBZ-365 (JXAU); Luxi County, 23 June 1984, *M.X. Nie* 113 (LBG); Suichuan County, 24 June 2016, *Z.C. Liu et al.* Lxp-13-18312 (SYS); Wuning County, 22 May 2014, *Y.H. Zhan et al.* LXP0912 (LBG). **Sichuan:** Hechuan City, 1934, *D.J. Yu* 3112 (PE); Leibo County, 3 July 1983, *Q.S. Zhao & Z.J. Zhao* 121212 (PE); Xuyong County, 17 September 2013, *W.B. Ju & H.N. Deng* HGX13668 (CDBI). **Yunnan:** Maguan County, 31 July 1961, *S.G. Wu* 3597 (KUN); Suijiang County, 4 May 1973, *B.X. Sun* 0112 (KUN). **Chongqing:** Fengjie County, 28 June 1958, *M.Y. Fang* 24515 (NAS); Shizhu County, 2 June 1978, *W.H. Wang* 1571 (CDBI).

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Supplementary material I

Supplementary data

Authors: Hua Liang, Lu Jiang, Danqi Li, Yi Yang, Dengmei Fan, Zhiyong Zhang

Data type: figures and tables (word document)

Explanation note: Type specimen of *Enkianthus perulatus*, *E. calophyllus* and *E. serrulatus*. Phylogenetic relationships of *Enkianthus* based on *matK*. Sample collection information and GenBank accessions in this study. Sequences of primers used for PCR amplification and sequencing.

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Link: <https://doi.org/10.3897/phytokeys.214.94294.suppl1>

Argostemma ehuangzhangense (Rubiaceae), a new species from Guangdong, China

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Abstract

Argostemma ehuangzhangense, a new Rubiaceae species from E'huangzhang Nature Reserve, Guangdong Province, China, is here described and illustrated. A morphological comparison between the new species and its putative relatives, *A. lamxayanum*, *A. laotica* and *A. verticillatum*, is presented. The new species is mostly similar to *A. laotica*, but they can be distinguished from each other since *Argostemma ehuangzhangense* presents solitary flower (vs. 2-flowered inflorescences), flower lobes 4 (vs. 5) and anthers opening by longitudinal slits (vs. apical pores). In a preliminary IUCN Red List status of *Argostemma ehuangzhangense* this species is assigned as Vulnerable (VU).

Keywords

Argostemma ehuangzhangense, China, Guangdong, new species, Rubiaceae

Introduction

The genus *Argostemma* Wall. (Wallich 1824) belongs to the coffee family, Rubiaceae (subfamily: Rubioideae), and in its own tribe Argostemmataceae (Bremer 1987; Bremer and Manen 2000). *Argostemma* is a large genus of more than 160 species and is widely distributed in the Old World tropics with most species occurring in SE Asia and two species in west tropical Africa (Bremer 1989; Sridith and Puff 2000; Mabberley 2017;

Lanorsavanh et al. 2020). In China, six species of *Argostemma* were recorded (Chen and Taylor 2011). Key morphological characters of *Argostemma* are (i) opposite or verticillate leaves that are slightly to markedly anisophyllous, (ii) 4- or 5-merous flowers without nectaries, (iii) white and rotate corollas, (iv) Inner surface of corolla tube glabrous, (v) free anthers or coherent into a tube, (vi) anthers with opening by longitudinal slits or apical pore, and (vii) sometimes connective prolonged at the apex (Puff et al. 1995; Chen and Taylor 2011).

An unknown *Argostemma* species was discovered during recent field surveys conducted between April 2017 and May 2018 at the E'huangzhang Nature Reserve, Yangchun City, Guangdong Province. The flowers of the unknown *Argostemma* species are clearly 4-merous and it differs from all known *Argostemma* species in China. In addition, we compared the unknown *Argostemma* from E'huangzhang Nature Reserve against *Argostemma* species occurring in Southeastern Asian which presented morphological characters divergent from those in our specimen. Thus, it was concluded that the unknown *Argostemma* from E'huangzhang Nature Reserve is a new species, which is hereby described and illustrated here.

Materials and methods

This study was conducted based on living and dried herbarium specimens collected from nearby Wufu Waterfall in the E'huangzhang Nature Reserve. Herbarium specimens at IBSC, KEP and KUN, as well as types at IBSC, K and NY, were examined for morphological comparisons (acronyms follow Thiers 2022). Taxonomic literature of the genus for Thailand (Sridith and Puff 2000; Sridith and Larsen 2004; Sridith 2007, 2009, 2012), China (Chen and Taylor 2011), Vietnam (Vu et al. 2020), Laos (Lanorsavanh and Chantaranothai 2013, 2016, 2019; Lanorsavanh et al. 2020), and Myanmar (Kress et al. 2003; Tanaka et al. 2010) were consulted.

Results

Argostemma ehuangzhangense is similar to *A. lamxayanum* and *A. laotica* by sharing the following morphological features: leaves isophyllous, verticillate or pseudo-verticillate; and leaves, pedicels, calyx and petals pubescent (Table 1). However, *Argostemma ehuangzhangense* is distinct from these species by its flower solitary (vs. flower (1-)2–10); peduncles absent (vs. peduncles short or 1–3.5 cm); flower 4-merous (vs. 5-merous); anther length 1.2–1.5 mm (vs. longer than 2 mm) and anthers longitudinal slits (vs. apical pore) (Table 1).

Amongst the *Argostemma* species known in China, *A. verticillatum* is morphologically similar to *Argostemma ehuangzhangense*. *Argostemma verticillatum* differs from the latter by its (i) glabrous stem, pedicels and calyx, (ii) inflorescence cymose and comprised of 1–3 umbelliform, (iii) flowers 5-merous, and (iv) filaments short (Table 1).

Table 1. Comparison of morphological characters between *Argostemma ehuangzhangense*, *A. lamxayanum*, *A. laotica* and *A. verticillatum*.

Characters	<i>A. ehuangzhangense</i>	<i>A. lamxayanum</i>	<i>A. laotica</i>	<i>A. verticillatum</i>
Plant height (cm)	1–4	2–12	1–2.5	2–10
Leaf blade shape	ovate to elliptic	elliptic, oblong or ovate	elliptic or oblanceolate	lanceolate or ovate-lanceolate
Leaf blade size (cm)	0.5–2.5×0.3–1.2	0.7–4×0.4–2.0	1–1.7×0.4–0.7	1–7×0.7–2.5
Lateral leaf veins	3–4-paired	4–8-paired	3–4-paired	4–7-paired
Leaf indumentum	both surfaces antrorse strigose (abaxial sparsely pubescent on the vein)	both surfaces hirsute	both surfaces pubescent	both surfaces glabrous or sparsely pubescent
Inflorescences	flowers solitary; peduncles absent	umbelliform, 1–10-flowered; peduncles 1–3.5 cm, glabrous	2-flowered; peduncles very short, pubescent	umbelliform, 1–3-flowered, composed cymose; peduncles 1–3 cm long, glabrous
Pedicel	8–18 mm, pubescent	4–12 mm, pubescent	6–7 mm, pubescent	5–10 mm, glabrous
Calyx lobes	4, pubescent outside	5, pubescent outside	5, pubescent outside	5, glabrous both side
Corolla lobes	4, pubescent outside	5, pubescent outside	5, pubescent both side	5, glabrous both side
Filament length (mm)	3.5–4	2.5–3	2.5–3.2	1–1.2 (–2.5)
Anthers coherence	connivent	free	connivent	free
Anther length (mm)	1.2–1.5	2.5–3	2.2–2.5 mm	2–3
Anther dehiscence	longitudinal slits	apical pores	apical pores	apical pores

Taxonomic treatment

Argostemma ehuangzhangense H.G.Ye, Jia Liu & W.B.Liao, sp. nov.

urn:lsid:ipni.org:names:77309064-1

粤西雪花

Type. CHINA. Guangdong Province: Yangchun City, Bajia Town, E'huangzhang Nature Reserve, near the Wufu Waterfall, 21°52'N, 111°25'E, a.s.l. 720 m, 3 May 2018, *Wan-Yi Zhao, Jia Liu, Qiao-Ling Ding, Fan Ye YC-2018-02* (holotype: SYS!, Barcode SYS00236851; isotype: SYS!, Barcode SYS00236852) (Figs 1, 2).

Diagnosis. *Argostemma ehuangzhangense* is similar to *A. lamxayanum* and *A. laotica* in its habit and pseudo-verticillate leaves, but differs in having terminal solitary 4-merous flowers, short anthers (1.2–1.5 mm long) opening by longitudinal slits.

Description. *Terrestrial* perennial herbs, 1–4 cm tall, attached to the substrate by tubers flattened globose, with a few roots. **Stems** erect, densely pubescent, with one pair of scale-like leaves at the lower middle portion. **Leaves** four per individual, clustered at the stem apex, verticillate, anisophyllous, petiole 0.5–2 long mm; blades membranous or thinly papery when dried, ovate to elliptic, 5–25 × 3–12 mm, cuneate at base, acute to obtuse at apex, margins entire; adaxial surface green, densely antrorse strigose; abaxial surface grey-white with white particles, sparsely pubescent on the midrib vein and lateral veins; lateral veins 3- or 4-pairs; stipules deciduous. **Flowers** solitary, terminal;

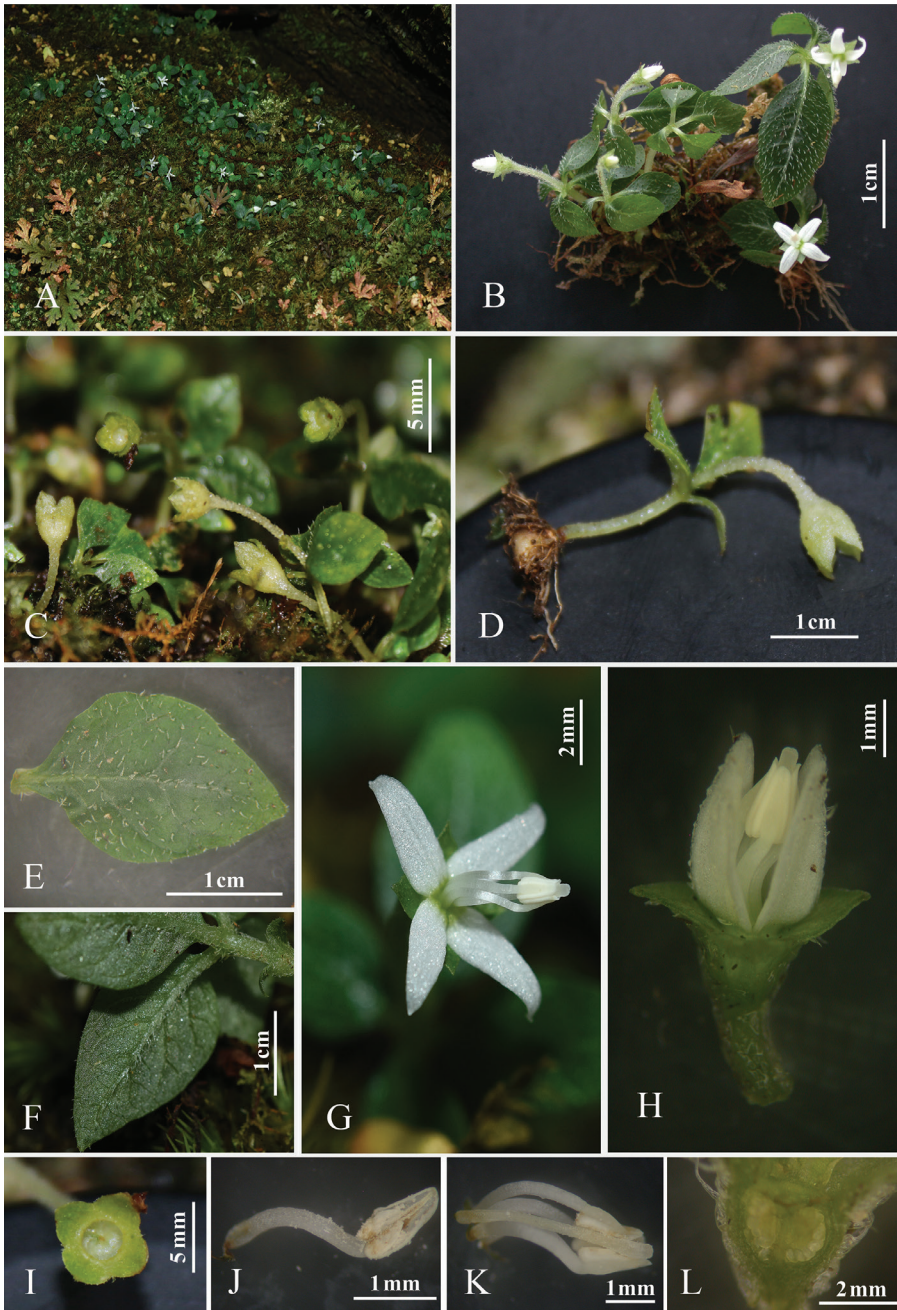


Figure 1. *Argostemma ehuangzhangense* **A** individuals in their natural habitat **B** flowering individuals **C** fruiting individuals **D** side view of an individuals with tuber **E** leaf blade adaxial surface view **F** leaf blade abaxial surface view **G** flower, internal corolla surface view **H** folwer, external corolla surface view **I** capsule, top view **J** stamen **K** style and stigma tightly enclosed by stamens **L** ovary longitudinal section view. (photographs **(A–G)** were taken by Wan-Yi Zhao in the original habitat area of E’huangzhang and photographs **(H–L)** were taken by Jia Liu in SYS Herbarium in May 2018).

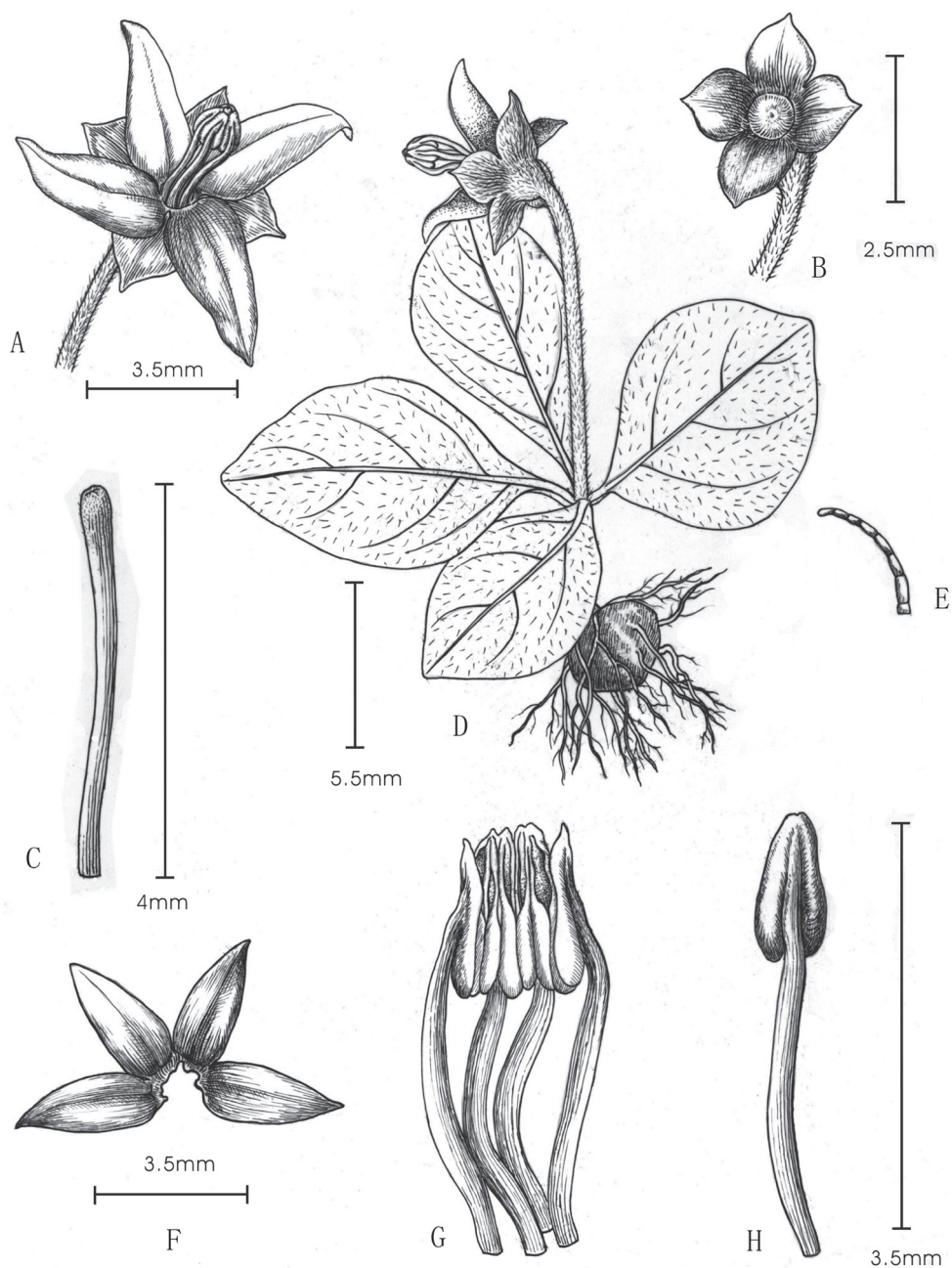


Figure 2. *Argostemma ehuanzhangense* **A** flower **B** fruit **C** style and stigma **D** habit of a mature plant **E** multicellular trichomes present on the stem, pedicel and ovary **F** corolla **G** organization of stamens **H** stamen (Drawn from living plants by Yun-Xiao Liu).

pedicels 0.8–1.8 cm, with densely pubescent, trichomes multicellular. **Calyx** densely pubescent, trichomes multicellular, hypanthium portion obovoid; lobes 4, subtriangular, 1–1.3 × 1.1–1.4 mm, abaxially pubescent, adaxially glabrous. **Corolla** white, rotate, external surface sparsely pubescent, internal surface glabrous, corolla tube 0.3–0.6 mm long; corolla lobes 4, oblong-lanceolate, 3–4 × 1.5–2 mm. **Stamens** 4; filaments free, 3.5–4 mm, exserted; anthers 1.2–1.5 mm long, coherent into a tube, dehiscent longitudinally. **Ovary** 2-locular, ovules numerous in each locule; style filiform, ca. 4 mm, with short pubescence, stigma capitate, exserted. **Capsule** obovoid, 2–5 mm in diameter, 1–3 mm long, pubescent, crowned by a persistent calyx, without ribs or furrows.

Phenology. This species is recorded flowering in March–May and fruiting in May–September.

Distribution. *Argostemma ehuanzhangense* is endemic to E'huangzhang Nature Reserve, southwestern Guangdong Province. It is currently known only from two populations recorded in county of Dianbai and city of Yangchun.

Habitat. Growing along river on wet sandstone under the evergreen broad-leaf forest at 400–750 m a.s.l.

Etymology. The specific epithet 'ehuanzhangense' is derived from the type locality, E'huangzhang Nature Reserve of the Guangdong Province, in China. This area is the oldest geological platform in the Guangdong Province, in which many endemic species occur (Wang et al. 2003; 2004; Ding et al. 2018). The new species is also expected to occur in the Yunkaishan National Nature Reserve, Maoming City, because this area shares a similar tectonic history with E'huangzhang. Therefore, we proposed for the vernacular name of the species as yuèxixuěhuā (粤西雪花).

Preliminary conservation status. The species is endemic to the Guangdong Province. According to our field survey, there are only two localities in which *Argostemma ehuanzhangense* is recorded and each population consists of 100–250 individuals. The number of mature individuals of *Argostemma ehuanzhangense* is more than 400, but less than 1000. Thus, we believe that *Argostemma ehuanzhangense* would be considered VU (Vulnerable) in an official IUCN Red List assessment (IUCN Standards and Petitions Subcommittee 2022) according to the D criterion.

Paratypes. CHINA. Guangdong Province: Yangchun City, Bajia Town, E'huangzhang Nature Reserve, 21°52'N, 111°25'E, a.s.l. 704 m, 29 Apr. 2017, *Hua-Gu Ye*, *Zhong-Cheng Liu* YHG-06 (SYS); same locality, 5 Aug. 2017, *Hua-Gu Ye*, *Wan-Yi Zhao*, *Zhong-Cheng Liu* YC2017-35 (SYS); Yangchun City, Bajia Town, 21°52'N, 111°25'E, a.s.l. 750 m, 1 Aug. 2001, *Hua-Gu Ye* 6119 (IBSC); Dianbai County, Luokeng Town, Shuangjifeng, 21°52'N, 111°21'E, a.s.l. 400 m, 8 Aug. 2001, *Hua-Gu Ye* 6427 (IBSC).

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Impatiens chenmoui (Balsaminaceae), a new species from southern Yunnan, China

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Abstract

Impatiens chenmoui (Balsaminaceae), a new species from southern Yunnan, China, was described and illustrated based on morphological and molecular evidence. This new species is morphologically most similar to *Impatiens oblongata* Ruchis. & Niet, but can be distinguished by 7–9 pairs of leaf veins, glabrous perianth, obovate upper petal, and capsule with trichome.

Keywords

China, *Impatiens chenmoui*, morphology, phylogeny, sect. *Uniflorae*

Introduction

The family Balsaminaceae contains two genera, the monotypic *Hydrocera* Blume (1825:241) and *Impatiens* Linnaeus (1753: 937) (APG Website, <http://www.mobot.org/MOBOT/research/APweb/>) *Impatiens* L. is a large genus of over 1000 species (Grey-Wilson 1980; Fischer 2004), mainly distributed in tropical and subtropical regions, with tropical Africa, Madagascar, southern India and Sri Lanka, eastern Himalayas (including SW China) and Southeast Asia as its five diversity centers (Song et al. 2003; Yuan et al. 2004; Yu et al. 2015). More than 270 species are currently known from China (Yu 2012), in which more than 200 species occurred in SW China (Chen et al. 2007), mainly distributed in Yunnan, Sichuan, Guangxi, Guizhou, and Xizang. *Impatiens* was divided into two subgenera, subgen. *Clavicularpa* S.X. Yu ex S.X. Yu & Wei Wang and subgen. *Impatiens* L. according to the latest phylogenetic studies. The

latter was further subdivided into seven sections (sect. *Fasciculatae*, sect. *Impatiens*, sect. *Racemosae*, sect. *Scorpioidae*, sect. *Semeiocardium*, sect. *Tuberosae*, and sect. *Uniflorae*) (Yu et al. 2015). Several new species of sect. *Uniflorae* have been described from India, Myanmar, Cambodia, Vietnam, and China. (e.g. Cho et al. 2017; Yang et al. 2017; Ruchisansakun et al. 2018; Kim et al. 2019; Zhang et al. 2020) in recent years.

In September 2019, during fieldwork in Mengla County, Yunnan, an unfamiliar *Impatiens* species was collected and transplanted to Shanghai Chenshan Botanical Garden. The flower blossomed in December 2020, indicating its unusual identity which may be new to science. In November 2021, we made a botanical trip back to Mengla County to collect flowers and fruit specimens. After careful comparison of relevant species from the adjacent area, we finally concluded that these specimens represent a species new to science, and described it here.

Methods

Morphology study

Morphological characters of the new species and related ones were compared based on living plants and herbarium specimens, including the digital resource of type specimens from JSTOR Global Plants (<https://plants.jstor.org/>). Herbarium specimens were examined in Chenshan Botanical Herbarium (CSH, index herbarium, <http://sweetgum.nybg.org/science/ih/herbarium-list/?NamOrganisationAcronym=CSH>), original protologues and relevant literature were also investigated.

Datasets preparation

To resolve the phylogenetic position of the putative new species, two molecular markers ITS (ITS1–5.8S–ITS2) and *atpB-rbcL* were used in this study. Leaf material of the putative new species was collected from the field and stored with silica. Total genomic DNA was extracted with the modified CTAB method (Doyle and Doyle 1987) for library construction at Benagen (<https://www.benagen.com>). Paired-end sequencing of the whole sequences from both ends of 150 bp fragments was performed on the DNBSEQ T7, and about 2 Gb clean data were produced. The plastome and nrDNA were de novo assembled using the GetOrganelle pipeline 1.7.6.1 (Jin et al. 2020). Sequences of *atpB-rbcL* were extracted from the plastome annotated in Geneious Prime 2021.2.2 (<https://www.geneious.com>) with comparison to the published plastome of *Impatiens balsamina* L. (GenBank accession: MW411292) as reference. Sequences of ITS1–5.8s–ITS2 were extracted with ITSx 1.1.3 (Bengtsson-Palme et al. 2013). The ITS dataset and the *atpB-rbcL* dataset were respectively aligned using MAFFT v7.450 by default setting. (Katoh and Standley 2013) and concatenated for phylogenetic analysis (Chen et al. 2020). Species sampling was based on previous studies (Yu et al. 2015; Ruchisansakun et al. 2018). All the sequence GenBank accession numbers were listed in Appendix 1.

Phylogenetic analysis

Maximum Likelihood estimation (ML) and Bayesian inference analysis (BI) were performed on Phylosuite v1.2.2 (Zhang et al. 2020). For ML, GTR+F+R4 was selected as the best fit model for the ITS dataset, and GTR+F+R5 was selected as the best fit model for the *atpB-rbcL* dataset according to AICc by Modelfinder (Kalyaanamoorthy et al. 2017). Maximum likelihood was estimated using IQ-TREE (Nguyen et al. 2015) under the Edge-linked partition model for 2000 ultrafast (Minh et al. 2013) bootstraps. For BI, GTR+I+G was selected as the best fit model for both datasets according to AICc by PartitionFinder2 (Lanfear et al. 2017). Bayesian Inference phylogeny analysis was inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under the partition model (2 parallel runs, 10,000,000 generations), in which the initial 25000 sampled data were discarded as burn-in. Tree files were visualized and annotated in Figtree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bootstrap (BS) and Posterior Probability (PP) values were used as an estimate of nodal robustness.

Result

The combined dataset was 1934bp in total, comprising 107 accessions/107 species, with *Hydrocera triflora* (L.) Wight. et Arn. selected as outgroup. Phylogenetic reconstruction of BI and ML produced similar topological structures (Fig. 1). The putative new species (marked in red) was resolved in the subgen. *Impatiens* sect. *Uniflorae*, forming a sister relationship with Myanmar species *I. oblongata* Ruchis. & Niet (PP = 0.957, BS = 94). Based on the morphological characters and phylogenetic result, we recognized this *Impatiens* species as a new species and described it here as *Impatiens chenmoui* Zheng W. Wang, Xiao C. Li & Qi Wang, sp. nov.

Taxonomic treatment

***Impatiens chenmoui* Zheng W. Wang, Xiao C. Li & Q. Wang ter, sp. nov.**

urn:lsid:ipni.org:names:77309066-1

Figs 2, 3, Appendix 2

Type. CHINA. Yunnan province, Mengla county (勐腊县) Xiangming Yi nationality township (象明彝族自治县) Kongming Mountain (孔明山) alt.1639m, 22°8'9.73"N, 101°8'48.86"E, 23 November 2021, *Zhengwei Wang and Xiaochen Li*, WZW04250 (Holotype: CSH0189505, CSH!; isotypes: CSH0192380, ZJFC!; CSH0189507, HZU!; CSH0189506, JJF!).

Diagnosis. *Impatiens chenmoui* is most similar to *I. oblongata* Ruchis. & Niet, but is distinguished by the glabrous dorsal petal, pedicel, and bracts, longer pedicel and spur, and fewer lateral sepals (Table 1).



Figure 1. Phylogenetic tree based on combined datasets of the nuclear ITS and plastid *atpB-rbcL* DNA sequences. The topological structure comes from Bayesian inference. Numbers near nodes are PP/BS, a dash ‘-’ indicates nodes not supported, subgen. *Clavicarpa* was collapsed.

Description. Herb annual. Stem erect, fleshy, glabrous, 12–35 cm tall. Leaves alternate, petioles 1–5 cm, leaf blade 9.5–2.5×1.6–3.5 cm, narrowly elliptic or oblong-lanceolate, apex acuminate or long acuminate, base cuneate, margin roughly crenate; adaxially dark green, pilose along veins, abaxially gray-green, glabrous, lateral veins 7–9 pairs. Inflorescences in upper leaf axils, 1-flowered. Pedicels green, glabrous, 2.5

–2.7 cm long. Bracts linear, persistent, 2–3 mm long. Flowers solitary, axillary, pink, or lavender, with pair of darker pink and yellow dots at the base. Lateral sepals 2, inversely coiled, glabrous, green, 2 mm long. Lower sepal funnelform, 3–4×2–3mm long, 2–3mm in depth, eaves navicular, base gradually constricted into a spur, variable, usually 1.4–1.7 cm long, rarely absent, mouth oblique, ca. 5mm wide, with ca. 2 mm long narrowly triangular tip. Dorsal petal circular, pink or mauve, 5–6×4–5mm, apex acuminate, glabrous, ca. 2mm long. United lateral petals sessile, 2-lobed, 6–8mm long. Upper petal large, obovate, 6–7×2–5mm, apex often concave. Lower petal small, axe-shaped, 7–8×1–3mm, apex rounded, without auriculus at back. Stamens 5, filaments linear, subulate, pale pink, ca. 2–3mm long, distally enlarged, anthers obtuse. Ovary fusiform, 5–carpellate, purple, 2–3 mm long, dorsal suture ridges with trichome. Capsule short fusiform, 12–18mm long, 4–5 mm in diam, with trichome along ridges. Seeds obovoid, brown, ca. 2 mm long, slightly compressed, pubescent with spirally sculptured hairs.

Phenology. Flowering and fruiting from October to December.

Distribution and ecology. This new species was found under evergreen broad-leaved forest at an elevation of 1500–1700 m on the limestone mountain ridge, and was currently known as only one population in Mengla County, Yunnan, China. This distribution area is very close to the border with Myanmar and Laos. We assume that this species should be also distributed in Myanmar and Laos due to their similar habitat.

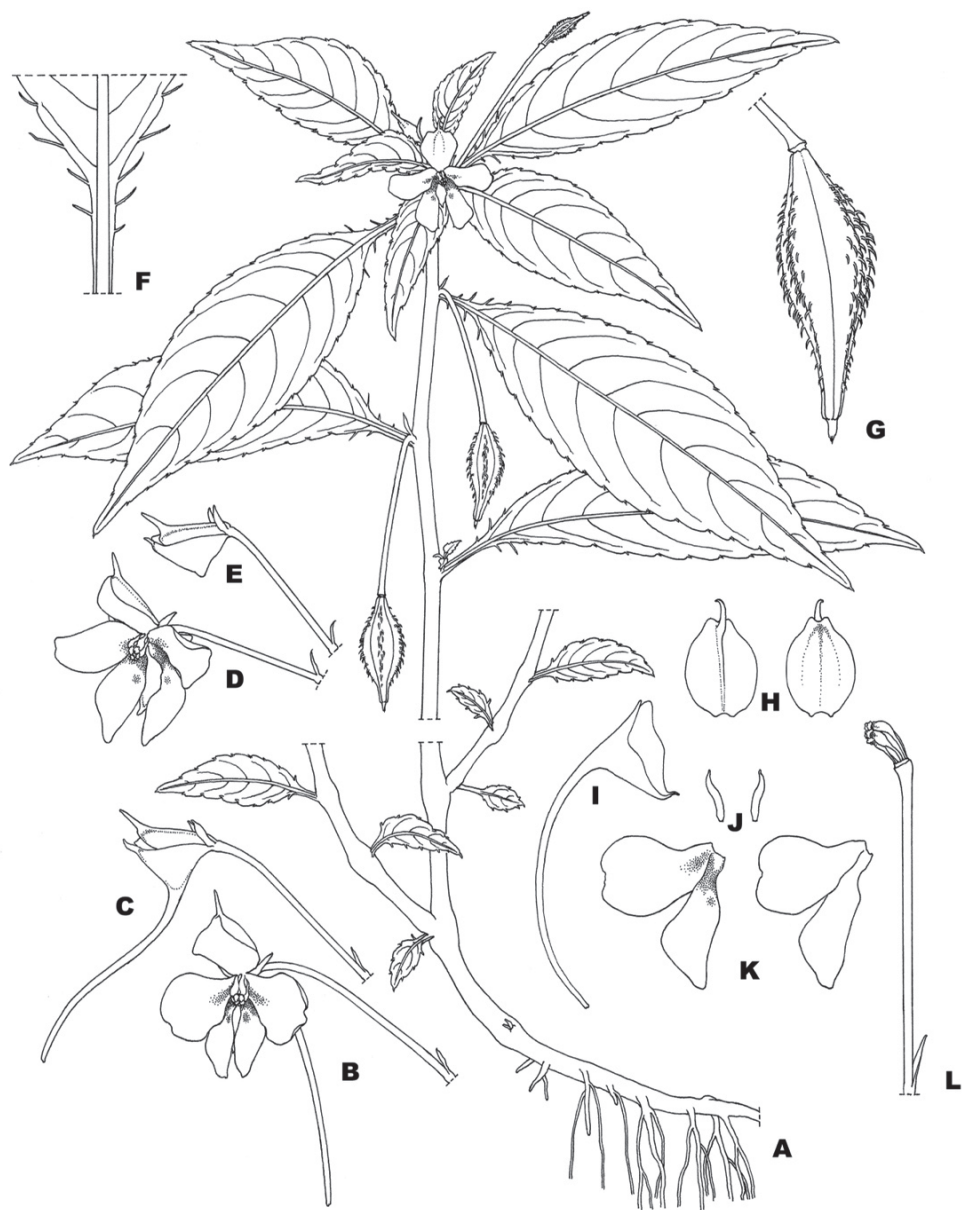
Etymology. The specific epithet “Chenmoui” was dedicated to the famous Chinese collector and botanist, Chen Mou (陈谋) (1903–1935) who was one of the founders of the first botanical garden cataloged by the Classification System of Plants in China, and died during the collection trip through southern Yunnan, China. The Chinese name was given as “陈谋凤仙花”.

Conservation status. This species is currently known only from one population in the type locality. The population is located in the tourist area of Kongming Mountain, where it could be easily disturbed by human activities, such as road construction and illegal mining. The IUCN status proposed is Vulnerable(VU) based on IUCN (2022) guidelines.

Additional specimens examined (Paratype). China, Yunnan province, Mengla county, Xiangming Yi nationality township, Kongming Mountain. 24 Oct. 2019, *Ruhua Xu and Yu Zhang*, *XRH001*(CSH!).

Table 1. Comparison of key features of *I. chenmoui* and *I. oblongata*.

Taxonomic traits	<i>I. chenmoui</i>	<i>I. oblongata</i>
Dorsal petal	Glabrous	midrib and tip pilose
Pedice	25–27 mm long, green, glabrous.	18–20 mm long, pink, pilose.
Ovary hair	Trichome	Pilose
Spur	14–17 mm long, glabrous, rarely absent.	8–12 mm long, pilose.
Bracts	Glabrous	Pilose
Lateral sepals	2, inversely coiled, glabrous	2–4, upper pair pilose; lower pair glabrous



Tianyi Yu 2022.9.14

Figure 2. *Impatiens chenmoui* sp. nov. **A** habit **B, C** flower with long spur **D, E** flower with spur nearly absent **F** leaf base **G** capsule **H** dorsal petal **I** spur **J** lateral sepals **K** united lateral petals **L** ovary surrounded by stamens.

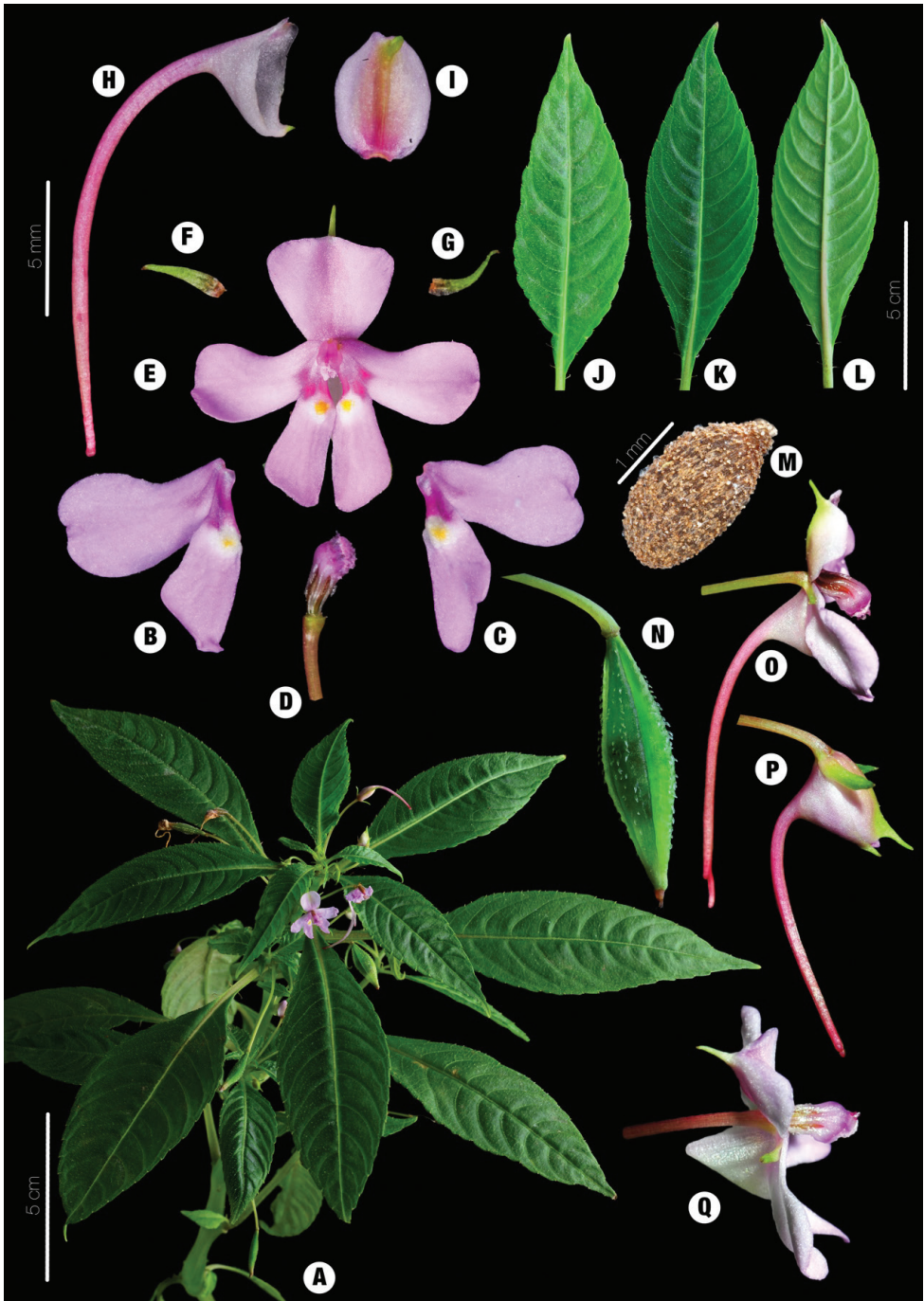


Figure 3. *Impatiens chenmou* sp. nov. **A** habit **B, C** united lateral petals **D** ovary surrounded by stamens **E** flower front view **F, G** lateral sepals **H** spur **I** dorsal petal **J–L** leaves **M** seed **N** capsule **O, P** long-spurred flower side view **Q** non-spurred flower side view.

Note. New species of sect. *Uniflorae* discovered from Southeast Asia in recent years were mostly found distributed on mountain summits in an evergreen forest, which indicated that the stone mountain in this area was likely to be one of the speciation centers of this section. *Impatiens* species exhibited interspecific and even intraspecific variation in spur length, at least from our observation of the same population of *I. davidii* Franchet, *I. platysepala* Y. L. Chen, and *I. chenmoui*, which may be considered as retaining of a bimodal pollinated system of bee and lepidopteran (Ruchisansakun et al. 2016). Floristic survey and pollination ecology study in these regions' *Impatiens* species is still insufficient, and more fieldwork is urgently needed.

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Appendix I

Table A1. Species and sequences sampling list with Genbank accession number.

Species	ITS	atpB–rbcL
<i>Hydrocera triflora</i>	AY348853	DQ147895
<i>Impatiens apalophylla</i>	KP776061	KP776011
<i>Impatiens aquatilis</i>	AY348745	DQ147811
<i>Impatiens arguta</i>	AY348746	DQ147812
<i>Impatiens aureliana</i>	AY348747	DQ147814
<i>Impatiens balansae</i>	KP776062	KP776012
<i>Impatiens balsamina</i>	AY348749	DQ147816
<i>Impatiens begoniifolia</i>	AY348752	DQ147819
<i>Impatiens bicornuta</i>	AY348754	DQ147821
<i>Impatiens blinii</i>	KP776063	KP776013
<i>Impatiens campanulata</i>	AY348758	DQ147822
<i>Impatiens capensis</i>	AY348759	DQ147823
<i>Impatiens chekiangensis</i>	KP776064	KP776014
<i>Impatiens chenmoui</i>	OP035808	OP095354
<i>Impatiens chinensis</i>	AY348761	DQ147825
<i>Impatiens chishuiensis</i>	KP776065	KP776015
<i>Impatiens chiulungensis</i>	KP776066	KP776016
<i>Impatiens chlorosepala</i>	KP776067	KP776017
<i>Impatiens clavigera</i>	KP776068	KP776018
<i>Impatiens conchibracteata</i>	AY348765	DQ147829

Species	ITS	<i>atpB-rbcL</i>
<i>Impatiens corchorifolia</i>	AY348767	DQ147831
<i>Impatiens cuspidata</i>	AY348769	DQ147832
<i>Impatiens cyanantha</i>	AY348770	DQ147833
<i>Impatiens cyathiflora</i>	AY348771	DQ147834
<i>Impatiens cymbifera</i>	KP776069	KP776019
<i>Impatiens davidii</i>	KP776070	KP776020
<i>Impatiens decurva</i>	MF979085	MF979082
<i>Impatiens delavayi</i>	AY348773	DQ147836
<i>Impatiens desmantha</i>	AY348774	DQ147837
<i>Impatiens drepanophora</i>	AY348776	DQ147838
<i>Impatiens duclouxii</i>	KP776071	KP776021
<i>Impatiens faberi</i>	AY348778	DQ147841
<i>Impatiens falcifer</i>	KP776072	KP776022
<i>Impatiens fischeri</i>	AY348781	DQ147843
<i>Impatiens fissicornis</i>	AY348782	DQ147844
<i>Impatiens flanaganiae</i>	AY348783	DQ147846
<i>Impatiens florulenta</i>	MF979087	MF979084
<i>Impatiens forrestii</i>	AY348784	DQ147847
<i>Impatiens fragicolor</i>	KP776073	KP776023
<i>Impatiens gongshanensis</i>	KP776074	KP776024
<i>Impatiens harae</i>	KP776075	KP776025
<i>Impatiens hians</i>	AY348791	DQ147849
<i>Impatiens hongkongensis</i>	KP776076	KP776027
<i>Impatiens hunanensis</i>	KP776077	KP776028
<i>Impatiens imbecilla</i>	AY348796	DQ147851
<i>Impatiens inaperta</i>	AY348797	DQ147852
<i>Impatiens lateristachys</i>	KP776078	KP776030
<i>Impatiens laxiflora</i>	KP776079	KP776031
<i>Impatiens lecomtei</i>	AY348802	DQ147855
<i>Impatiens leptocaulon</i>	KP776080	KP776032
<i>Impatiens macrovexilla</i>	KP776082	KP776034
<i>Impatiens malipoensis</i>	KP776083	KP776035
<i>Impatiens margaritifera</i>	KP776084	KP776036
<i>Impatiens mengtzeana</i>	AY348806	DQ147858
<i>Impatiens meruensis</i>	AY348807	DQ147859
<i>Impatiens monticola</i>	AY348810	DQ147860
<i>Impatiens muscicola</i>	KC905500	KC905547
<i>Impatiens napoensis</i>	AY348811	DQ147861
<i>Impatiens neglecta</i>	KP776087	KP776038
<i>Impatiens noei</i>	KC905504	KC905548
<i>Impatiens noli-tangere</i>	KP776088	KP776039
<i>Impatiens nubigena</i>	KP776089	KP776040
<i>Impatiens nyimana</i>	KP776090	KP776041
<i>Impatiens oblongata</i>	MF979086	MF979083
<i>Impatiens omeiana</i>	KP776092	DQ147864
<i>Impatiens oxyanthera</i>	AY348814	DQ147865
<i>Impatiens parviflora</i>	AY348816	DQ147866
<i>Impatiens patula</i>	KC905509	KC905549
<i>Impatiens phuluangensis</i>	KC905517	KC905554
<i>Impatiens platychlaena</i>	AY348818	DQ147867
<i>Impatiens platypetala</i>	AY348819	DQ147868
<i>Impatiens poculifer</i>	AY348820	DQ147870

Species	ITS	<i>atpB-rbcL</i>
<i>Impatiens principis</i>	KP776096	KP776026
<i>Impatiens pritzelii</i>	AY348821	KP776045
<i>Impatiens pseudoviola</i>	AY348822	DQ147871
<i>Impatiens pterosepala</i>	KP776097	KP776046
<i>Impatiens purpurea</i>	AY348823	DQ147872
<i>Impatiens racemosa</i>	KP776098	DQ147873
<i>Impatiens radiata</i>	AY348824	KP776047
<i>Impatiens rectangula</i>	AY348825	DQ147874
<i>Impatiens rubrostriata</i>	AY348828	DQ147876
<i>Impatiens santisukii</i>	KC905528	KC905550
<i>Impatiens scabrida</i>	KP776099	DQ147877
<i>Impatiens scullyi</i>	KP776100	KP776048
<i>Impatiens scutisepala</i>	AY348830	DQ147878
<i>Impatiens siculifer</i>	KP776101	KP776049
<i>Impatiens sodenii</i>	AY348832	DQ147879
<i>Impatiens soulieana</i>	AY348833	DQ147880
<i>Impatiens spathulata</i>	KP776102	KP776050
<i>Impatiens stenosepala</i>	AY348835	DQ147881
<i>Impatiens sulcata</i>	KP776103	KP776051
<i>Impatiens sunkoshiensis</i>	KP776104	KP776052
<i>Impatiens taronensis</i>	AY348838	DQ147882
<i>Impatiens teitensis</i>	AY348840	DQ147883
<i>Impatiens tienmushanica</i>	KP776105	KP776053
<i>Impatiens tortisepala</i>	KP776106	KP776054
<i>Impatiens trichosepala</i>	AY348843	DQ147885
<i>Impatiens tuberculata</i>	KP776107	KP776055
<i>Impatiens tubulosa</i>	KP776108	KP776056
<i>Impatiens uliginosa</i>	AY348845	DQ147887
<i>Impatiens usambarensis</i>	AY348847	DQ147890
<i>Impatiens violiflora</i>	KC905541	KC905551
<i>Impatiens walleriana</i>	AY348849	DQ147892
<i>Impatiens wenshanensis</i>	KP776110	KP776057
<i>Impatiens wilsonii</i>	KP776111	KP776058
<i>Impatiens xanthina</i>	AY348850	DQ147893
<i>Impatiens yaoshanensis</i>	KP776112	KP776059

Appendix 2



Figure A1. Holotype of *Impatiens chenmoui* sp. nov.

Centaurea lovricii, a new species of *C.* sect. *Centaurea* (Asteraceae) from Croatia

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Abstract

A new species, *Centaurea lovricii*, is described and illustrated from the island of Vis (Dalmatia, Croatia). It occurs on northwest-facing calcareous cliffs near the sea, where it grows with several other rare endemic species. *Centaurea lovricii* is morphologically similar to *C. glaberrima* and *C. divergens* of *C.* sect. *Centaurea*, from which it differs in having more succulent leaves with larger and less incised leaflets, bigger capitula, larger phyllaries with more developed appendages and denser and undulate fimbriae, larger florets, bigger achenes, and longer pappus. Its morphological features, distribution, ecology, conservation status and taxonomic affinities are examined. In addition, a new iconography and lectotypification for *C. glaberrima* and *C. divergens* is provided.

Keywords

Adriatic region, Balkan flora, *Centaurea*, Croatia, morphology, new species, taxonomy

Introduction

The genus *Centaurea* L. is one of the largest genera in the family Asteraceae. In its current circumscription as a natural group, it includes about 250 species (Susanna and Garcia-Jacas 2007, 2009). It is mainly distributed in the Euro-Mediterranean and

south-western Asian territories (Susanna et al. 1995, 2006; Susanna and Garcia-Jacas 2007, 2009; Font et al. 2009; López et al. 2011; Hilpold et al. 2014a, b). As emphasised by Hilpold et al. (2014b), three subgenera can be recognised, namely *C.* subgen. *Centaurea*, *C.* subgen. *Lopholoma* (Cass.) Dobrocz., and *C.* subgen. *Cyanus* (Mill.) Cass. ex Hayek, each represented by numerous sections and subsections.

According to the literature (Hilpold 2012; Boršić 2013; Hilpold et al. 2014a, b), within sect. *Centaurea* of subgen. *Centaurea* three subsections are recognised, i.e. subsect. *Centaurea*, subsect. *Phalolepis* (Cass.) Garcia-Jacas, Hilpold, Susanna & Vilatersana, and subsect. *Willkommia* (Blanca) Garcia-Jacas, Hilpold, Susanna & Vilatersana. In particular, subsect. *Centaurea* [formerly sect. *Acrolophus* (Cass.) DC.] is characterised by having triangular phyllary appendages that are regularly ciliate at the margin and often provided with a mucro at the apex. This subsection is widespread in the Mediterranean area (Hilpold 2012). Several of its species are distributed in Croatia, Montenegro, Serbia, Bosnia and Herzegovina, while some of them are exclusively known from Croatia and its islands (Lovrić 1976, 1990, 1995; Nikolić et al. 2015; Nikolić 2020, 2022). Within this subsection several endemic species are known from the western Balkan peninsula: *C. biokovensis* Teyber s.l., *C. crithmifolia* Vis., *C. cuspidata* Vis. s.l., *C. dalmatica* A.Kern., *C. derventana* Vis. & Pančić, *C. divergens* Vis., *C. fridericii* Vis., *C. glaberrima* Tausch, *C. gloriosa* Radić, *C. incompta* Vis., *C. kartschiana* Scop., *C. radichii* Plazibat, *C. spinosociliata* Seenus s.l. and *C. visianiana* Plazibat.

During field investigations focused on the flora of the Dalmatian islands (Croatia) a peculiar chasmophilous population of *Centaurea* growing on the cliffs of the island of Vis was found. Previously, it was examined by Lovrić (1982, 1983, 1990, 1995) and named *C. issaea* or *C. glaberrima* var. *issaea*. However, these names must be considered *nomina nuda*, because a description was not provided for them and no type material was indicated. According to Art. 38.1 and 40 of the ICN (Turland et al. 2018), the names were therefore not validly published. Later, these names were used by several authors (Van der Maarel and Van der Maarel-Versluys 1996; Bogdanović and Ruščić 2011; Boršić 2013; Terzi et al. 2019; Nikolić 2020, 2022), all confirming the occurrence of this taxon on the island of Vis. According to the results of molecular genetic investigations (nuclear and plastid DNA sequences, as well as Amplified Fragment Length Polymorphism data - AFLP) carried out by Boršić (2013) on the amph-Adriatic species of *Centaurea*, the population from Vis island is closely related to *C. glaberrima*, a species occurring in southern Croatia, Montenegro, Albania, Bosnia and Herzegovina. To examine the affinities of these two taxa, morphological studies of populations from the island of Vis and the type locality of *C. glaberrima* in Dalmatia were carried out, as well as of its type specimen kept in PRC. These analyses show that the population from the island of Vis is highly distinct from *C. glaberrima*, as well as from the allied *C. divergens* and the other species belonging to subsect. *Centaurea*. Therefore, based on morphological and ecological differences with other species in this subsection, the plants in question are treated here as a new species for science and named *C. lovricii*.

Materials and methods

For this morphological study, we examined 90 herbarium specimens and scanned images of specimens from several, also virtual, herbaria (BEO, BEOU, BP, BUNS, CAT, CNHM, K, MW, P, PAD, PI, PRC, SARA, U, W, WU, ZA, ZAGR and ZAHO). The herbarium acronyms follow Thiers (2022). In addition, we collected 20 plants of *C. lovricii* from the island of Vis (one population), and 50 plants of *C. glaberrima* (seven populations) and 40 plants of *C. divergens* (seven populations) from several other localities in Croatia, including *loci classici*. The investigations were carried out on material preserved in alcohol and glycerine solution, as well as on herbarium exsiccatae deposited at CAT and ZAGR. Information about distribution, habitat, ecology and phenology were obtained from field data and herbarium specimens.

Taxonomic treatment

1. *Centaurea lovricii* Bogdanović, Boršić, Ljubičić, Brullo & Giusso, sp. nov.

urn:lsid:ipni.org:names:77309067-1

Figs 1–3B, C, D

Centaurea glaberrima var. *issaea* Lovrić, Taxon 31(4): 763, 1982 (nom. nud.).

Centaurea issaea Lovrić, Pos. izd. Muz. grada Šibenika 10: 191, 1983 (nom. nud.).

Type. CROATIA. Island of Vis, Blišćevac bay, calcareous rocky slopes, 16 May 2021, 43°4'23.30"N, 16°5'51.59"E, S. Bogdanović & M. Temunović s.n. (holotype: ZAGR–68512; isotypes: CAT, CNHM, ZA, ZAGR).

Description. Perennial herb, with several stems, sterile leaf-rosettes and woody rootstock. Stems erect, glabrous, striate, shiny green, 25–50 cm long, not winged, laxly branched. Basal leaves fleshy, shiny green, glabrous, 1–2 pinnatisect, 7–20 cm × 5–40 mm; leaflets 5–15 per side, lanceolate, linear-lanceolate to linear, or oblong lanceolate, 5–30 × 0.8–5(–10) mm, often the terminal ones lanceolate and up to 10 mm wide; young leaves oblong lanceolate with entire margin or with few lateral teeth; petioles 5–55 mm long. Median and upper cauline leaves similar to basal ones, sessile or subsessile, 1(–2) pinnatisect, 1.5–12 × 0.5–3 cm; leaflets 1–12 per side, linear-lanceolate to linear, margins entire. Synflorescence laxly paniculate, with 7–30 solitary capitula. Peduncles 1–20 cm long, apex clavate, bracts 1–8, entire. Involucre ovoid, 13–15 × 8–12 mm. Phyllaries straw-coloured, glabrous, coriaceous, dorsally with 3–7 nerves; the outer ones ovate, 6.5–8 × 2.5–3.5 mm; the median ones ovate-oblong, 10–15 × 3.5–4.5 mm; the innermost linear, 12.5–15 × 2–2.8 mm; phyllary appendages well-developed, triangular to orbicular, dark brown, appressed, densely fimbriate with 7–10 pairs of undulate fimbriae, 0.3–1.7 mm long, ending with a terminal acute tooth. Florets pink-purplish. Outermost florets sterile; corolla tube glabrous, 7.5–9 mm long; corolla lobes linear, irregular, 7.5–11.5 mm long. Disc



Figure 1. Habit of *Centaurea lovricii* sp. nov. Drawn by Salvatore Brullo.

florets fertile; corolla tube 10–11.5 mm long; corolla lobes linear, equal, 5 mm long. Stamens 12.5–13.5 mm long; filaments 4.5–5 mm long; anthers 8–8.5 mm long, dark violet. Style 16–16.5 mm long, ciliate at apex; stigmas 1.5 mm long. Achenes oblong,

2.6–3 × 1.4–1.5 mm, glabrous, dark brown up to the apex, with several irregular straw-coloured longitudinal ridges. Pappus obscurely biseriate, whitish, with bristles 0.6–2.3 mm long.

Etymology. This species is dedicated to the Croatian botanist Andrija-Željko Lovrić (1943–2018), who was the first to collect it and consider it as a new species.

Phenology. *Centaurea lovricii* flowers from May to late June, and fruits from late June to July.

Distribution and ecology. This new species grows along the northern coast between Dragodid Bay and Oključina Bay of the island of Vis in Dalmatia, Croatia (Fig. 4). It grows on sea facing cliffs constituted of Triassic dolomites (Lozić et al. 2012) at 10–100 m a.s.l., in rocky crevices together with many other rare or endemic species (Fig. 3A). The most frequent chasmophytes occurring in this habitat are *Aurinia leucadea* subsp. *scopulorum* (Ginzb.) Plazibat, *Campanula teutana* Bogdanović & Brullo, *Centaurea ragusina* L., *Helichrysum italicum* subsp. *pseudolitoreum* (Fiori) Bacch., Brullo & Mossa, *Brassica incana* Ten., *Limonium issaeum* Bogdanović & Brullo, *Pimpinella tragium* subsp. *lithophila* (Schischk.) Tutin and *Sesleria interrupta* Vis. (Bacchetta et al. 2003; Bogdanović and Ruščić 2011; Bogdanović et al. 2014; Bogdanović and Brullo 2015; Terzi et al. 2019).

Conservation status. The single population of *C. lovricii* is composed of fewer than 1000 scattered mature individuals, so it can be considered Vulnerable (VU D1) according to the IUCN Red List Categories and Criteria (IUCN 2022). It is currently distributed in a very narrow coastal belt of ca. 0.5 km², so it has a restricted area of occupancy and only one location, but as its growing site is very steep and quite inaccessible, which makes the population unthreatened by any human disturbance, it does not qualify for subcritierion D2.

Chromosome number. According to Lovrić (1982, 1983) and Van Loon (1987) the ploidy level of *C. lovricii*, previously attributed to “*C. glaberrima* var. *issaea*”, is tetraploid with $2n = 4x = 36$ chromosomes, similarly to that of the allied species *C. glaberrima* and *C. divergens* (Siljak-Yakovlev et al. 2005). According to Boršić (2013) the estimated ploidy level for “*C. issaea*” is also tetraploid.

Discussion. Within *Centaurea* subsect. *Centaurea*, *C. lovricii* is most similar to *C. glaberrima*, particularly in the morphology of its capitula and phyllaries. *Centaurea glaberrima* is a species occurring in several localities in the north-western Balkans (Nikolić et al. 2015). However, these two species show some significant differences in habit and the morphology of their leaves, capitula and achenes (Table 1). In particular, *C. glaberrima* (Figs 5, 6) has a more robust and rigid habit, stems with up to 80 capitula, rigid basal leaves with leaflets linear-filiform (0.5–2 mm wide), involucre 6–10 mm long, with phyllaries up to 8 mm long, appendages straw-coloured, often tinged with pale-brown, with sparse fimbriae, outermost sterile florets with corolla tube 6–6.5 mm long and pappus bristles 0.2–0.8 mm long. In addition, the two species have different ecological requirements. *Centaurea glaberrima* usually behaves as a ruderal plant growing along roadsides, in dry rocky grasslands and occasionally also in rupestrian stands, whereas *C. lovricii* is a true chasmophyte in coastal stands. A population genetic study of some endemic Adriatic species of *Centaurea* using AFLPs was

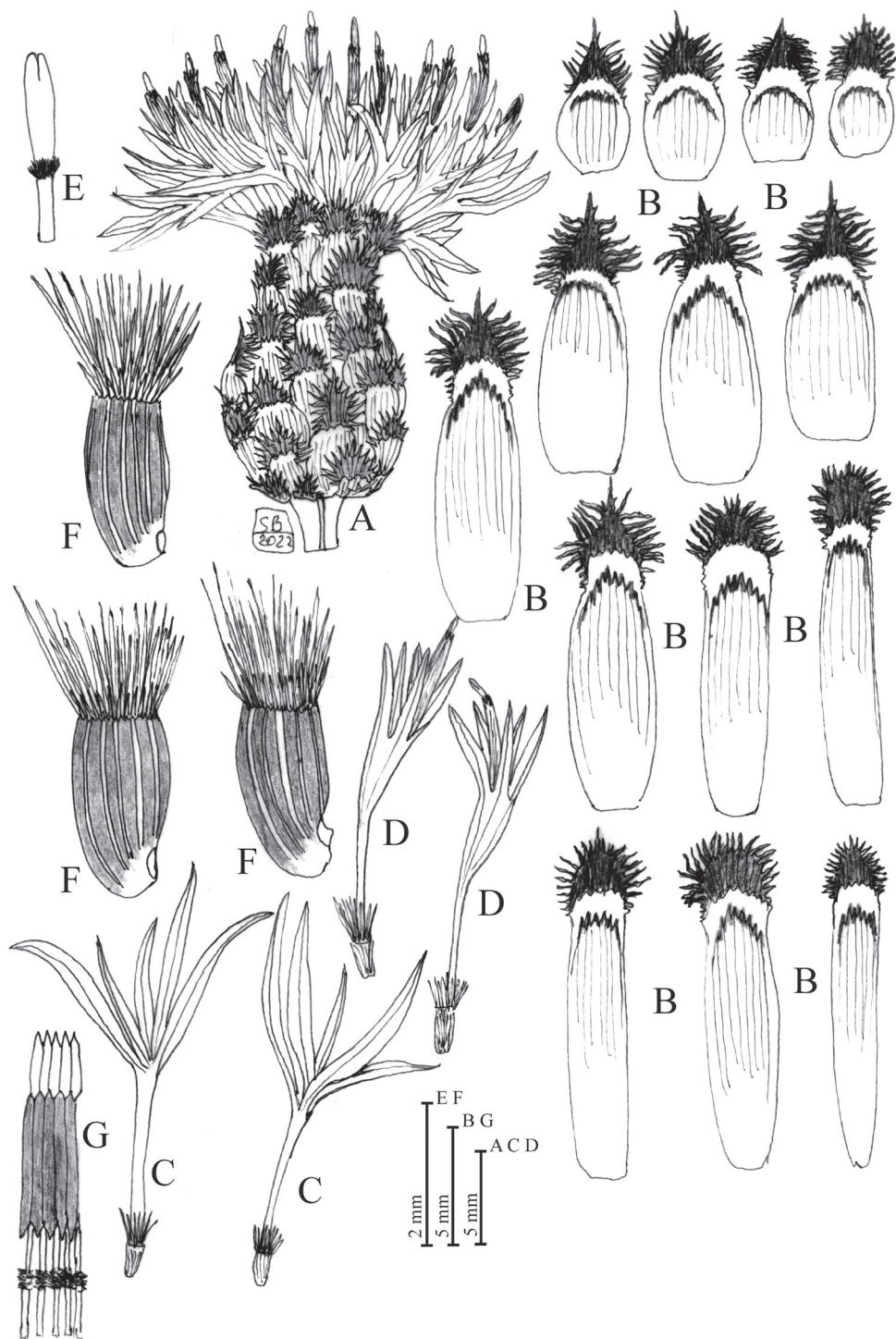


Figure 2. *Centaurea lovricii* sp. nov. **A** capitulum **B** phyllaries **C** outermost sterile florets **D** disc florets **E** stigma **F** achenes **G** stamens upper part. Drawn by Salvatore Brullo.

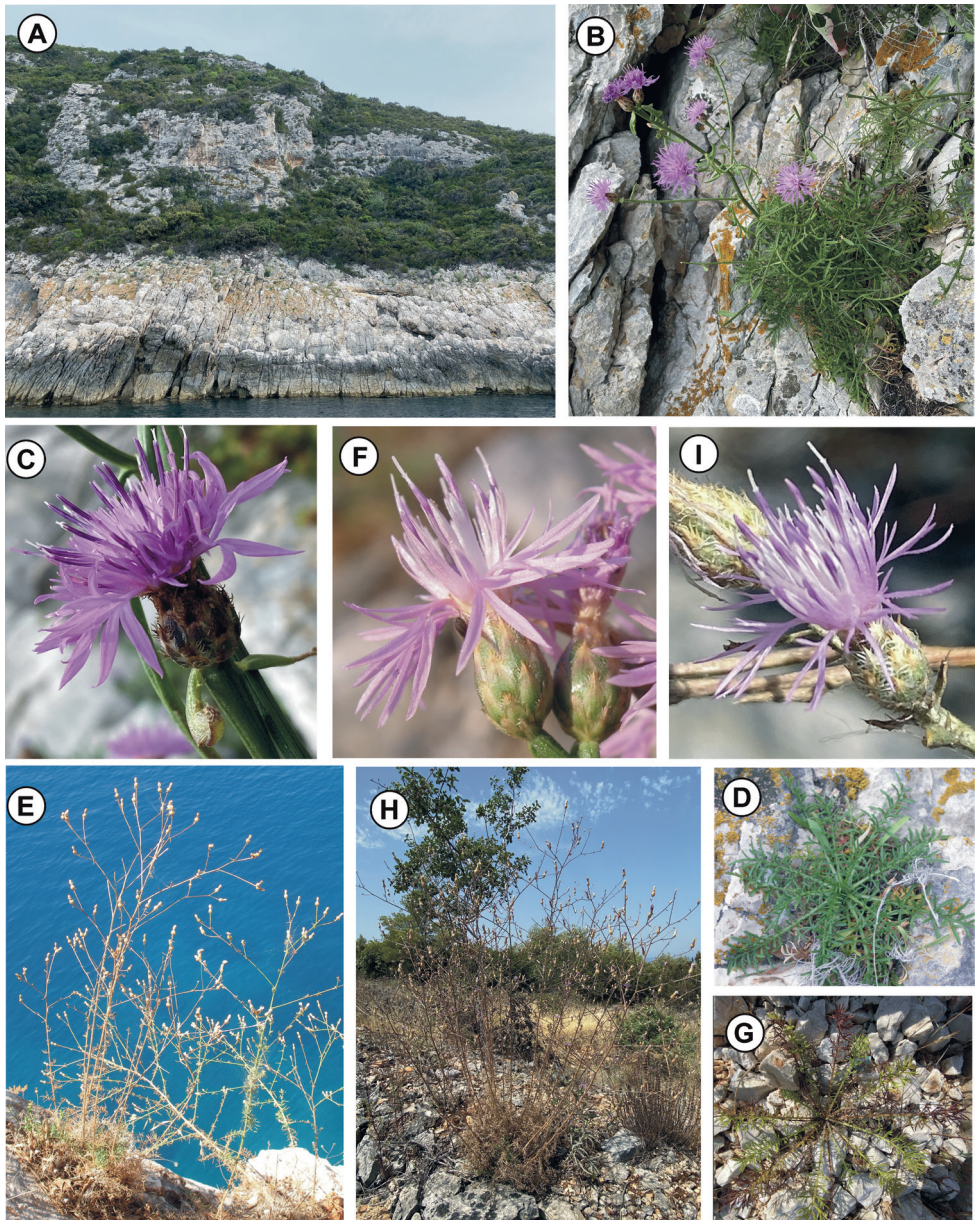


Figure 3. *Centaurea lovricii* sp. nov. **A** habitat **B** plant growing in natural habitat **C** capitulum **D** basal leaves; *C. glaberrima* **E** plant growing in natural habitat **F** capitula **G** basal leaves; *C. divergens* **H** plant growing in natural habitat **I** capitulum. Photographed by Sandro Bogdanović.

carried out by Boršić (2013), who showed the distinctness of the population from the island of Vis (sub *C. issaea*) from *C. glaberrima*, but also concluded that this population shows evidence of introgression with *C. glaberrima* and *C. spinosociliata*. A phylogeny

based on nrDNA internal transcribed spacer sequences revealed that *C. lovricii* still has an unclear phylogenetic position among the species of *C.* sect. *Centaurea* included in this study (Boršić 2013).

Another morphologically similar species is *C. divergens* (Fig. 6), which is often treated as a subspecies or variety of *C. glaberrima* (Malý 1928; Hayek 1931; Lovrić 1967–1968; Dostál 1976; Gavrilović and Janačković 2022), but its treatment as a distinct species, as proposed by Visiani (1847) and Hayek (1901), seems to be more appropriate (see Table 1). It differs from *C. lovricii* in having a hispid stem, rigid and dull green basal leaves with linear-filiform leaflets (0.5–2 mm wide), a 6–10 mm long involucre, phyllaries that are up to 8 mm long, with up to 2 mm long fimbriae, outermost sterile florets with a 5.5–6 mm long corolla tube, and up to 1.1 mm long pappus bristles. They are also ecologically well differentiated, because *C. divergens* is usually a typical ruderal species, while *C. lovricii* is a chasmophyte.

A nomenclatural overview and lectotype designation for *C. glaberrima* and *C. divergens*, species closely related to *C. lovricii*, are here provided:

Table 1. Main diagnostic features of *Centaurea lovricii*, *C. glaberrima* and *C. divergens*.

	<i>C. lovricii</i>	<i>C. glaberrima</i>	<i>C. divergens</i>
Stem	glabrous, shiny, 25–50 cm long,	glabrous, dull, 25–80 cm long,	hispid, dull, 25–100 cm long,
Basal leaves	fleshy, robust, glabrous, 1–2 pinnatisect, 7–20 cm long, with leaflets lanceolate to linear, 5–30 × 0.8–5 mm, the terminal one up to 10 mm wide	thin, rigid, glabrous, 1–3 pinnatisect, 8–15 cm long, with leaflets linear-filiform, 2–15 × 0.5–0.8(–2) mm	thin, rigid, hispid, 1–3 pinnatisect, 8–20 cm long, with leaflets linear-filiform, 2–15 × 0.5–0.8(–2) mm
Cauline leaves	1.5–12 cm long, 1–12 leaflets per side, linear lanceolate to linear	0.5–5 cm long, 1–15 leaflets per side, linear-filiform	0.5–7 cm long, 1–10 leaflets per side, linear-filiform
Synflorescence	laxly branched, with 7–30 capitula	laxly paniculate, very branched, with (15)50–80 capitula	densely paniculate, very branched, with 15–80 capitula
Capitula	solitary, involucre 13–15 × 8–12 mm, with peduncle 1–20 cm long	solitary to grouped, involucre 6–8.5(–10) × 4.5–5.5 mm, with peduncle 0.3–6 cm long	solitary to grouped, involucre 6–10 × 4–6.5 mm, with peduncle 0.2–7 cm long
Phyllaries	6.5–15 × 2–4.5 mm, with appendages triangular to orbicular, dark-brown, densely fimbriate with 7–10 pairs of undulate fimbriae 0.3–1.7 mm long	2.5–8 × 1.2–2.6 mm, with appendages triangular to orbicular, straw-coloured often tinged with pale-brown, 4–8 pairs of lax and more or less straight fimbriae, which are 0.3–1(–1.5) mm long	3–8 × 1.4–2.2 mm, with appendages triangular, straw-coloured, tinged with pale-brown, 3–6 pairs of lax and more or less straight fimbriae, which are 0.2–2 mm long
Corolla of outermost sterile florets	tube 7.5–9 mm long, lobes 7.5–11.5 mm long	tube 6–6.5 mm long, lobes 2–4.1 mm long	tube 5.5–6 mm long, lobes 3–6 mm long
Corolla of disc florets	tube 10–11.5 mm long, lobes 5 mm long	tube 5–6 mm long, lobes 2–2.5 mm long	tube 6.5–7.5 mm long, lobes 3–3.3 mm long
Stamens	12.5–13.5 mm long, with filament 4.5–5 mm long and anthers 8–8.5 mm long	7.5–9.8 mm long, with filaments 5.3–5.8 mm long and anthers 2.2–4 mm long	10–10.5 mm long, with filaments 5–5.5 mm long and anthers 5 mm long
Style	16–16.5 mm long	7.5–8.3 mm long	10 mm long
Stigma	1.5 mm long	1.25 mm long	1.2 mm long
Achenes	2.6–3 × 1.4–1.5 mm, glabrous, dark-brown up to the apex	2.5–2.7 × 1–1.2 mm, glabrous, dark-brown with straw-coloured strip above	2.3–2.5 × 0.9–1.1 mm, sparsely hairy, dark-brown with straw-coloured strip above
Pappus	obscurely 2-seriate, with bristles 0.6–2.3 mm long	2-seriate, with bristles 0.2–0.8 mm long	2-seriate, with bristles 0.2–1.1 mm long

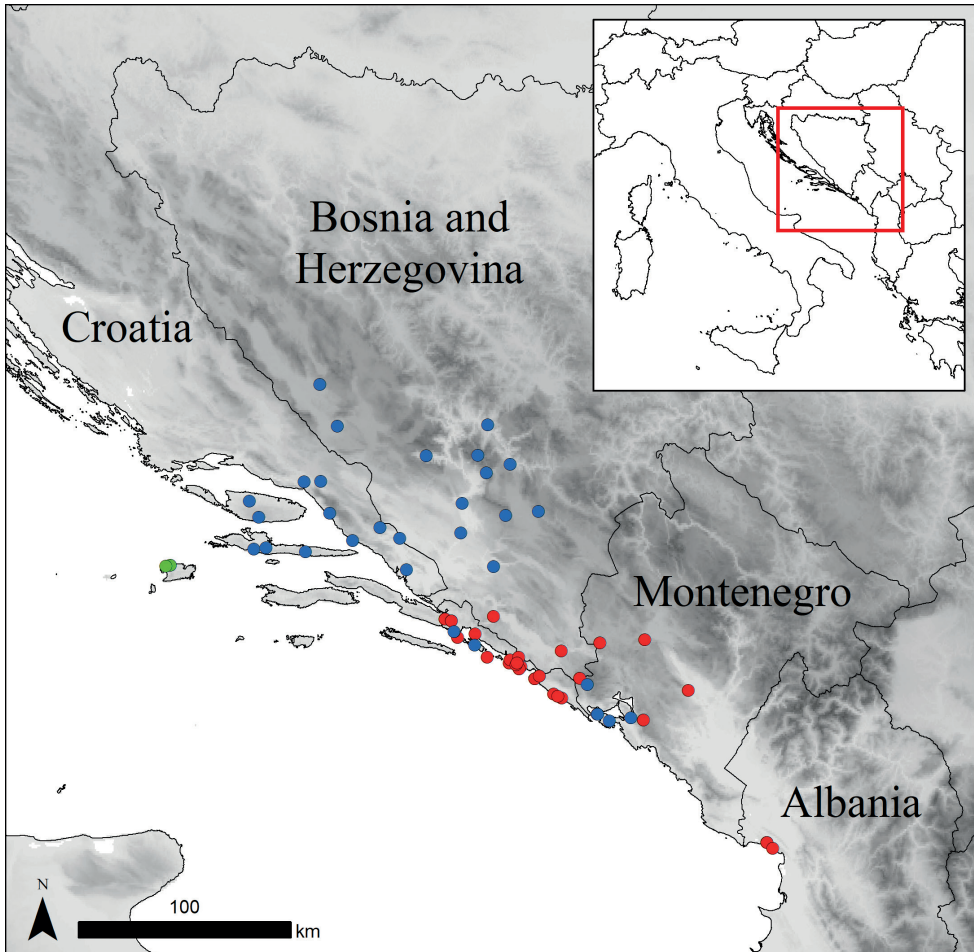


Figure 4. Distribution of *Centaurea lovricii* sp. nov. (green dots), *C. glaberrima* (red dots) and *C. divergens* (blue dots).

2. *Centaurea glaberrima* Tausch, Syll. Pl. Nov. 2: 249, 1828.

Figs 5, 6

Centaurea punctata Vis., Flora 12: 23, 1829. Type: Croatia. In agris sterilibus Dalm. [Dalmatia] montanae (prope Duare), *Visiani s.n.* (lectotype, designated here: PAD – HD02644).

Type. CROATIA. e Dalmat. [Dalmatia], s.d., *Sieber s.n.* (lectotype, central specimen designated here: PRC).

Nomenclatural note. Tausch (1828) described *C. glaberrima* from Dalmatia without indicating any precise locality. The herbarium sheet, with three specimens, that we found in PRC has an original herbarium label with Tausch's handwriting: "*Centaurea* – ?,



Figure 5. Habit of *Centaurea glaberrima*. Drawn by Salvatore Brullo.

e Dalmat. Sber. capit: parvus ovoideus divaricatum". Tausch usually shortened geographical names: "e Dalmat." refers to specimens collected in eastern Dalmatia, while "Sber." refers to the collector (i.e. Sieber). In addition, he provided a short morphological description. Later, to the same herbarium label, the identification [= *C. punctata* Vis.] was added. This was probably done by V. Kosteletzky (pers. observ. by curator P. Mraz). The fourth specimen on the sheet belongs to *C. spinosociliata* and it is correctly identified on a separate herbarium label by A. Hayek. Here we select the central specimen, which fits Tausch's description, as lectotype for the name *C. glaberrima*. One year later, Visiani (1829) described *C. punctata* from Duare [Zadvarje] in Dalmatia. We consulted the type specimen in PAD (PAD – HD02644), which fits Visiani's description given in the protologue. In fact, the morphology of the specimen perfectly corresponds to Tausch's description of *C. glaberrima*. Therefore, we here designate this specimen as a lectotype and include the name *C. punctata* as a synonym of *C. glaberrima*.

Iconography. Figs 5, 6; Tav. 11, Visiani, Fl. Dalmat. 2: 39, 1847, sub *C. punctata*; Tav. 47 (II, III, 4–14), Reichenbach, Icon. Fl. Germ. Helv. 15: 30, 1852, sub *C. punctata*; Tav. 155 (II, 10–11), Reichenbach, Icon. Fl. Germ. Helv. 15: 31, 1853, sub *C. punctata*.

Distribution. According to Greuter (2006), Barina et al. (2015) and Nikolić et al. (2015), *C. glaberrima* is distributed in Albania, Bosnia and Herzegovina, Croatia and Montenegro (Fig. 4).

3. *Centaurea divergens* Vis., Fl. Dalmat. 2: 37, 1847.

Fig. 6

Acosta divergens (Vis.) Soják, Čas. Nár. Muz. Odd. Přír. Prague 140: 134, 1972. Type.

Based on *Centaurea divergens* Vis.

Centaurea glaberrima subsp. *divergens* (Vis.) Hayek, Repert. Spec. Nov. Regni Veg. Beih. 30(2): 260, 1931. Type. Based on *Centaurea divergens* Vis.

Centaurea glaberrima var. *divergens* (Vis.) Malý, Glasn. Zemaljsk. Muz. Bosne Hercegovine Sarajevu. Priir. Nauke 40(1): 122, 1928 Type. Based on *Centaurea divergens* Vis.

Centaurea petteri Rchb.f., Icon. Fl. Germ. Helv. 15: 36, 1852. Type. Croatia. Auf dem Monte Mossor Dalm., June, *Reichenbach fil. s.n.* (W1889-0292916).

Type. CROATIA. In apricis montium Lesina, s.d., *Stalio* 448 (lectotype, designated here: PAD – HD02637).

Nomenclatural note. *Centaurea divergens* was described from the island of Hvar in central Dalmatia by Visiani (1847). In the protologue, Visiani clearly indicated "*in saxosis collium, et montium circa Lessina, unde misit prof. Stalio*" and we found original type material in the PAD herbarium that corresponds to description given in the protologue. Here we select a specimen that fits Visiani's description as lectotype for the name *C. divergens*.

Iconography. Fig. 6; Tav. 12(b)(41), Visiani, Fl. Dalmat. 2: 37, 1847; Tav. 51 (772) I, 1–7, Reichenbach, Icon. Fl. Germ. Helv. 15: 35, 1852; Tav. 52 (783) II, 9–16, Reichenbach, Icon. Fl. Germ. Helv. 15: 36, 1852, sub *Centaurea petteri*.

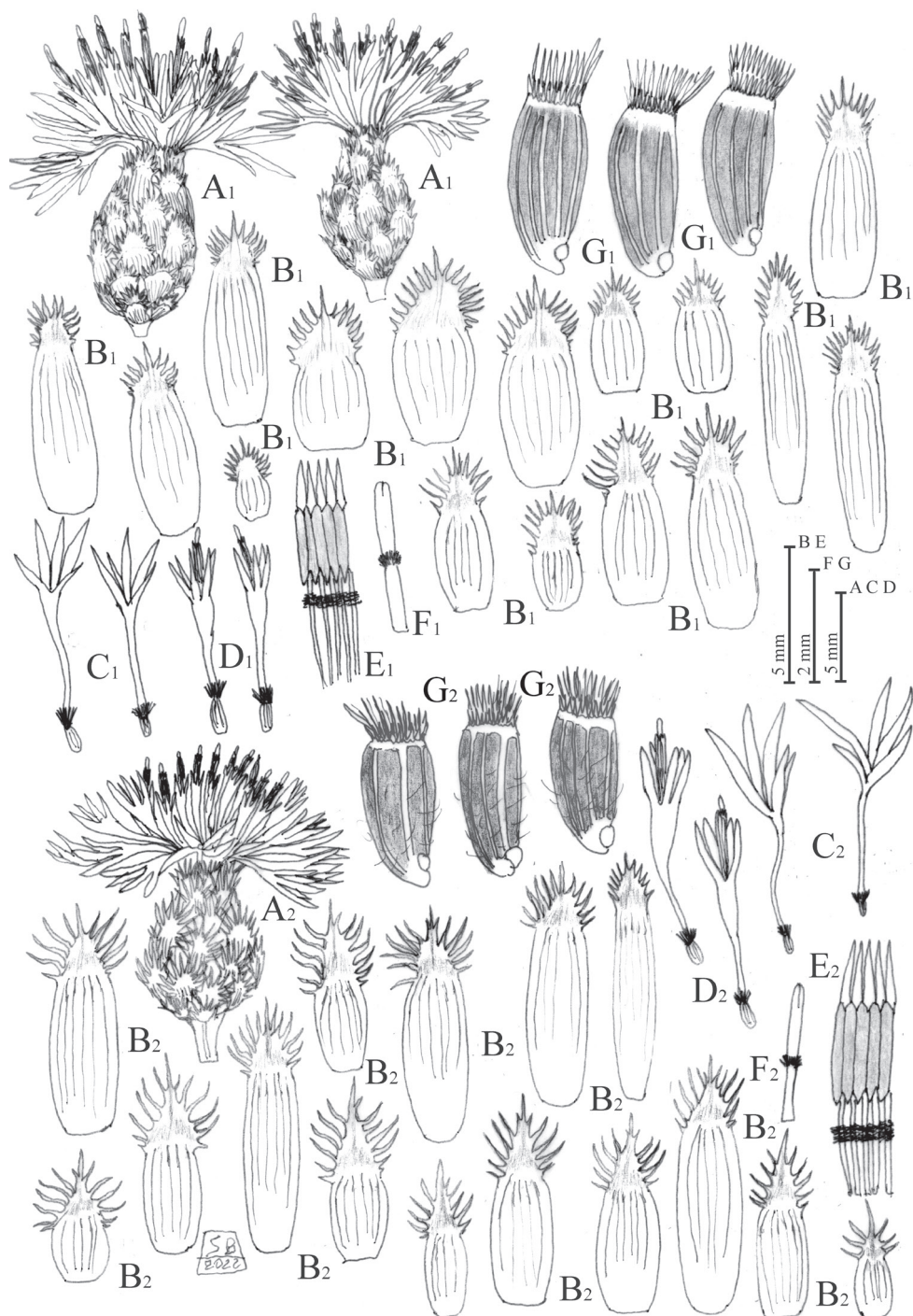


Figure 6. *Centaurea glaberrima* (1) and *C. divergens* (2) **A** capitula **B** phyllaries **C** outermost sterile florets **D** disc florets **E** stamens upper part **F** style.

Distribution. According to Greuter (2006), *C. divergens* is distributed in Bosnia and Herzegovina, Croatia, Montenegro (Fig. 4) and as an adventive plant in France.

Additional specimens examined. *Centaurea lovricii* (paratypes): CROATIA. Split-Dalmatia County, island of Vis, Kraljičina špilja, vertical rocks, 26 April 2010, *S. Bogdanović & Z. Liber s.n.* (ZAGR); Dalmazia, Isola di Vis, Oključina, pereti rocciosi sopra il mare, 25 May 2011, *S. Bogdanović, S. Brullo & G. Giusso s.n.* (CAT, ZAGR); Island of Vis, Oključina, vertical cliffs, 12 June 2010, *S. Bogdanović & I. Boršić s.n.* (ZAGR); Island of Vis, Oključina, vertical cliffs, 23 May 2010, *S. Bogdanović s.n.* (ZAGR). ***Centaurea glaberrima*: ALBANIA.** District of Shkodër, Rera e Hedhur E of village Baks-Rrjoll, on the slope of Mts Mali i Rencit, on limestone rocks, 41.83324°N, 19.53921°E, 76 m a.s.l., 5 August 2011, *Z. Barina & G. Somogyi 19743* (BP761381); District of Shkodër, in mountain Maja e Zezë above village Baks-Rrjoll, in dry grassland, on limestone, 41.85733°N, 19.50846°E, 248 m a.s.l.; 4 May 2014, *Z. Barina, D. Pifkó & G. Puskás 23227* (BP767834). **CROATIA.** Flora Dalmatica, In rupibus promontorii Punta Spezerea pr. Ragusam, 5 June 1906, *A. Degen s.n.* (PI012666); E Dalmat, s.d., *Tausch s.n.* (PRC); Dalmatia, 1832, *R. Visiani s.n.* (K000772946); Južna Hrvatska, Velji Do, obronci planina iznad Cavtata, 22 July 2013, *S. Bogdanović & I. Boršić s.n.* (ZAGR44365); Ragusa in Dalmatia, *R. Visiani s.n.*, sub *C. punctata* Vis. (P02815194); Ragusa, 15 June 1867, *R. Huter s.n.*, sub *C. punctata* Vis. (P02815195, P02815196, P02815197); Raguse, 14 June 1861, *R. Huter s.n.* (P04215180); Pelješac, Ston, uz cestu, 30 July 2021, *S. Bogdanović s.n.* (CAT, ZAGR); Pelješac, Ston, Majkovi, 11 July 2021, *S. Bogdanović s.n.* (CAT, ZAGR); Južna Dalmacija, otok Olipa, južna strana, uz obalu mora, 10 May 2018, *S. Bogdanović & I. Rešetnik s.n.* (ZAGR50465, ZAGR50466, ZAGR50462, ZAGR50463); Dalmacija, otok Lopud, stijene uz obalu mora, 12 May 2018, *I. Rešetnik & S. Bogdanović s.n.* (ZAGR53125); Dubrovnik, kod hotela Belvedere, stijene uz obalu mora, 12 May 2018, *I. Rešetnik & S. Bogdanović s.n.* (ZAGR53144); Otok Šipan: Kaludrica, u garigu, 25 August 1979, *M. Hečimović s.n.* (ZA13511); Na morskoj litici, Vrtac kod sela Popovići, ca 50 m, 19 July 1926, *V. Loschnigg s.n.* (ZA13510); In colle Gorizae et ad margines agrarum olivat. Lapad, 1868, *M. Vodopić s.n.* (ZA13508), sub *C. punctata* Vis.; Otok Lokrum: travnjak u masliniku kraj zgrade, 2 May 1959, *S. Horvatić s.n.* (ZA1307), sub *C. punctata* Vis.; In saxosis fruticetis sempervirentis ad Lapad (Gruž), 28 July 1928, *Th. Soška s.n.* (MW0794564); Slano, February 1972, *M. Obradović s.n.* (BUNS18767); Gradac, 9 May 1967, *M. Obradović s.n.* (BUNS18956); In rupibus calc. Ombla, pr. Dubrovnik, 15 August 1946, *O. Grebenščikov s.n.* (BEO26342); Lokrum, s.d., *S. Jovanović s.n.* (BEOU37269); Dalmatia. In rupestribus apricis prope urbem Dubrovnik (Ragusa), s.d., *A. Latzel s.n.* (BEOU37274); Dalmatia merid., in saxosis calcareis montis Ilinovo Brdo supra Gruda, 13 July 1928, *Soška s.n.* (BEOU37276); Dalmatia merid., in saxosis calcareis montis Srdj supra Dubrovnik, 20 July 1928, *Soška s.n.* (BEOU37277); Srdj (Dubrovnik), krečnjački kamenjari, 25 May 1990, *S. Stevanović & S. Jovanović s.n.* (BEOU502.90); Dubrovnik (okolina), Srdj, 25 May 1988, *S. Jovanović s.n.* (BEOU719); Padine brda Srdj, Dubrovnik, kamenjarski travnjaci, October 1993, *S. Maslo s.n.* (ZAGR); Konavoske stijene, Dubrovnik, pukotine krečnjačkih stijena uz morsku obalu, 16 July

2003, *S. Maslo s.n.* (ZAGR); Konavoske stijene (najzapadniji dio, kod Popovića), kamenjare, 1 August 1978, *I. Trinajstić s.n.* (CNHM4058:BOT); Dubrovnik, Lapad, 9 October 1977, *I. Trinajstić s.n.* (CNHM4050:BOT); Srđ iznad Dubrovnika, 29 November 1978, *I. Trinajstić s.n.* (CNHM4051:BOT). **BOSNIA AND HERZEGOVINA.** Weideplätze – V. Trebinje, n. Lastva, 7 August 1895, *Matulić s.n.* (SARA44076); In rupestribus montis Gliva prope Trebinje, July 1891, *K. Vandas s.n.* (SARA44075); Trebinje, 19 July 1892, *E. Brandis s.n.* (SARA44070); Am Wege von Meka gruda nach Dječ, 20 July 1925, *V. Hawelka s.n.* (SARA44054); In saxosis apricis prope Zavala, 18 October 1909, *K. Malý s.n.* (SARA44052). **MONTENEGRO.** Katunska nahija, 2 August 1991, *V. Stevanović s.n.* (BEOU1578.91); Ad Viluša, ca. 1.100 m, July 1904, *J. Rohlena s.n.* (PI012665); vidikovac iznad jezera Slano, 31 July 2021, *S. Bogdanović s.n.* (CAT, ZAGR); Gruda, Majden, 13 July 1928, *D. Petrović s.n.* (BEOU37275); Orjen, Poštirovnik, s. Prčanj cca 890 m s.m., s. dol., 2 August 1980, *Č. Šilić s.n.* (SARA44071, 44072, 44073, 44074). ***Centaurea divergens*: CROATIA.** Island of Hvar, St. Nikola, rocky grassland, 28 July 2021, *S. Bogdanović s.n.* (CAT, ZAGR); Zadvarje, along the road, 28 July 2021, *S. Bogdanović s.n.* (CAT, ZAGR); In apricis saxos. ins. Lesina, *Visiani s.n.* (P02815387); Dalmatia, in lapidos., pr. Macarsca, July 1880, *Th. illeg. s.n.* (P02815388); In collibus apricis Ins. Lessina, *D. Lagger s.n.* (P02815389); Lesina, *s.l., s.n.* (P02815389); Otok Šipan, ograde u garigu, 9 June 1979, *M. Hećimović s.n.* (ZA13512); Srednja Dalmacija, Grabovac, istočno od Šestanovca, uz cestu, 13 July 2014, *S. Bogdanović & U. Buzurović s.n.* (ZAGR38697, ZAGR38698); Grabovac, krečnjak, 13 July 2014, *U. Buzurović & S. Bogdanović s.n.* (BEO); Dalmacija, poluotok Pelješec, uvala Marčuleti, Rt Vrba, okomite stijene uz cestu, *M. Jeričević, N. Jeričević & S. Bogdanović s.n.* (ZAGR43796); Dalmatinska Zagora, Vrgorac, Prapatnice, Vegari, 20 July 20215, *M. Vukojević s.n.* (ZAGR40083); Neretva, okolica mjesta Rogotin, ruderalno, uz cestu, 20 July 2013, *S. Bogdanović & I. Boršić s.n.* (ZAGR44348); op. keinestrand b. Makarska, 26 July 1965, *T. Baretta 4* (U1112735); Dalmatia. In agris sterilibus ad Macarscam, *s.d., Pichler s.n.* (P02815392, P02815393, MW0794555, BEOU37201); Dalmatia, Lessina, *s.d., M. Botteri s.n.* (BEOU37200); Mala Duba, Živogošće, kamenite padine uz Jadransku magistralu, 12 July 2003, *S. Maslo s.n.* (ZAGR); Brač, Vidova gora, October 1976, *I. Trinajstić s.n.* (CNHM4054:BOT); Otok Brač, kod Bola, 28 March 1969, *I. Trinajstić s.n.* (CNHM4053:BOT); Otok Brač, uz cestu Supetar – Nerežišće, May 1968, *I. Trinajstić s.n.* (CNHM4052:BOT). **BOSNIA AND HERZEGOVINA.** Bosna, između Rilje i Kifinog sela, July 1969, *I. Trinajstić s.n.* (CNHM4055:BOT); Mostar, am Humberg, 100 m, July 1903, *E. Sagorski s.n.* (P04453688, P04453689); Mostar, in incultis, July 1907, *E. Sagorski s.n.* (P04132171); Mostar, in incultis ad m. Humberg, July 1905, *E. Sagorski s.n.* (SARA44056); Ad vias, July 1908, *E. Sagorski s.n.* (PI12696); Flora Hercegovine, Prenj, Porim, na izloženom grebenu, 29 July 1962, *I. Horvat s.n.* (ZAH0); Žitomislčići, Mostar, kamenite padine i šikare brda Osojnica, July 2001, *S. Maslo s.n.* (ZAGR); Hercegovina, Prenj pl., Prevoj, kamenite padine uz put za Rujište, July 2011, *S. Maslo s.n.* (ZAGR); Stolac, Križevac, 260 m alt., 43.06159N, 17.9839E, kamenjari, krečnjak, 1 July 2015, *M. Niketić, G. Tomović, K. Jakovljević & S. Đurović s.n.* (BEOU43550); In locis siccis agris Livanjsko polje, prope Prolog, 22 June 1970, *H. Ritter s.n.* (SARA48730); In locis siccis agris Livanjsko polje, prope Kablić, 30 July 1970, *H. Ritter s.n.* (SARA48729); In valle Drežanka

prope Drežnica, 4 August 1900, *K. Malý s.n.* (SARA 44058); In paucibus, Narontis, prope g. Grabovica, ca. 150 m, 5 October 1930, *K. Malý s.n.* (SARA44057); In paucibus, Narontis, prope g. Grabovica, 140 m, 7 September 1907, *K. Malý s.n.* (SARA44050); Konjic, in monte Vitaljica et Zlatar, 27 June 1955, *H. Ritter s.n.* (SARA44055); In valle Narontis prope Glavatičevo, solo dolomitico, 380 m, 17 July 1923, *K. Malý s.n.* (SARA44053); Inter Podgorjani-Lišani, 260 m, 18 August 1918, *K. Malý s.n.* (SARA44051); In saxosis apricis prope Bišina, ca. 1000 m, 7 August 1907, *K. Malý s.n.* (SARA44049); U dolini Rakitnice ispred Blaca, 10 August 1957, *H. Ritter s.n.* (SARA48725). **MONTENEGRO.** Boka Kotorska, Luštica iznad Rosa, kamenjari sa *Salvia officinalis*, krečnjak, 100 m, 3 August 1996, *D. Lakušić & B. Lakušić s.n.* (BEOU2150/96); Boka Kotorska, Luštica, prevoj između Rosa i Kračića, kamenjari, krečnjak, 350 m, 24 August 2002, *D. Lakušić & B. Lakušić s.n.* (BEOU15337); Kotor, put Prčanj-Rose, 29 June 2003, *P. Janačković s.n.* (BEOU38399); Iznad Vrbanja, kamenjari sa *Globularia cordifolia*, 16 June 1990, *V. Stevanović s.n.* (BEOU1198.90); Klinci, Sv. Tripun, Luštica, kamenje pored šoderskog put, 24 June 1995, *V. Karaman 501* (BEOU37273).

Key to *Centaurea lovricii* and allied species

- 1 Stem with 7–30 capitula; basal leaves fleshy, shiny, leaflets lanceolate to linear (0.8–10 mm wide); involucre 13–15 mm long, 8–12 mm wide; phyllaries up to 15 mm long, appendages dark-brown, fimbriae dense; outermost florets with lobes 7.5–11.5 mm long; pappus bristles up to 2.3 mm long ***C. lovricii***
- Stems with up to 80 capitula; basal leaves rigid, dull, leaflets linear-filiform (0.5–2 mm wide); involucre 6–10 mm long, 4–6.5 mm wide; phyllaries up to 8 mm long, appendages straw-coloured tinged with pale brown, fimbriae sparse; outermost florets with lobes 2–6 mm long; pappus up to 1.1 mm long **2**
- 2 Leaves glabrous; fimbriae on phyllaries up to 1(–1.5) mm long; disc floret tube 5–6 mm long and lobes 2–2.5 mm long; pappus bristles up to 0.8 mm long
..... ***C. glaberrima***
- Leaves hispid; fimbriae on phyllaries up to 2 mm long; disc floret tube 6.5–7.5 mm long and lobes 3–3.3 mm long; pappus bristles up to 1.1 mm long
..... ***C. divergens***

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According to Escobar (2012), there are two kinds of floral appendages around the staminal tube or urn in *Miersia*: 1) the upper pair of appendages are of staminal origin and 2) the four lateral appendages, one pair on each side of the staminal tube, are of tepaliferous origin. Consequently, mentions to “tepaliferous appendages” in diagnoses, descriptions, identification key and figure captions of our recent article (García et al. 2022) should be changed to “floral appendages”, to denote the mixed nature of those structures in most species of *Miersia*.

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