

Thismia limkokthayi (Thismiaceae): A new mycoheterotrophic species from Genting Highlands in Pahang, Malaysia

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Abstract

Thismia limkokthayi, a distinct mitriform species of the mycoheterotrophic genus *Thismia*, is described and illustrated. It was found at a locality in the upland areas of Genting Highlands, Pahang, Malaysia. This new species is morphologically similar to members of *Thismia* sect. *Geomitra*, but differs in several characteristics, including the colour of the floral tube, the inner surface of the floral tube with longitudinal ribs and absent transverse bars, a stamen apex with a central lobe (prolongation of the rib) and two lateral lobes (the tips of each are recurved) and a black-purplish stigma. *Thismia limkokthayi* is provisionally classified as Critically Endangered according to the IUCN Red List Categories and Criteria.

Keywords

fairy lantern, Genting Highlands, taxonomy, upper hill dipterocarp forest

Introduction

Fairy lanterns, *Thismia* Griff. (1844) (Thismiaceae), are non-photosynthetic mycoheterotrophic herbs distributed mainly in the tropics. The number of known species of this genus has increased rapidly in recent years. Currently, approximately 96 species

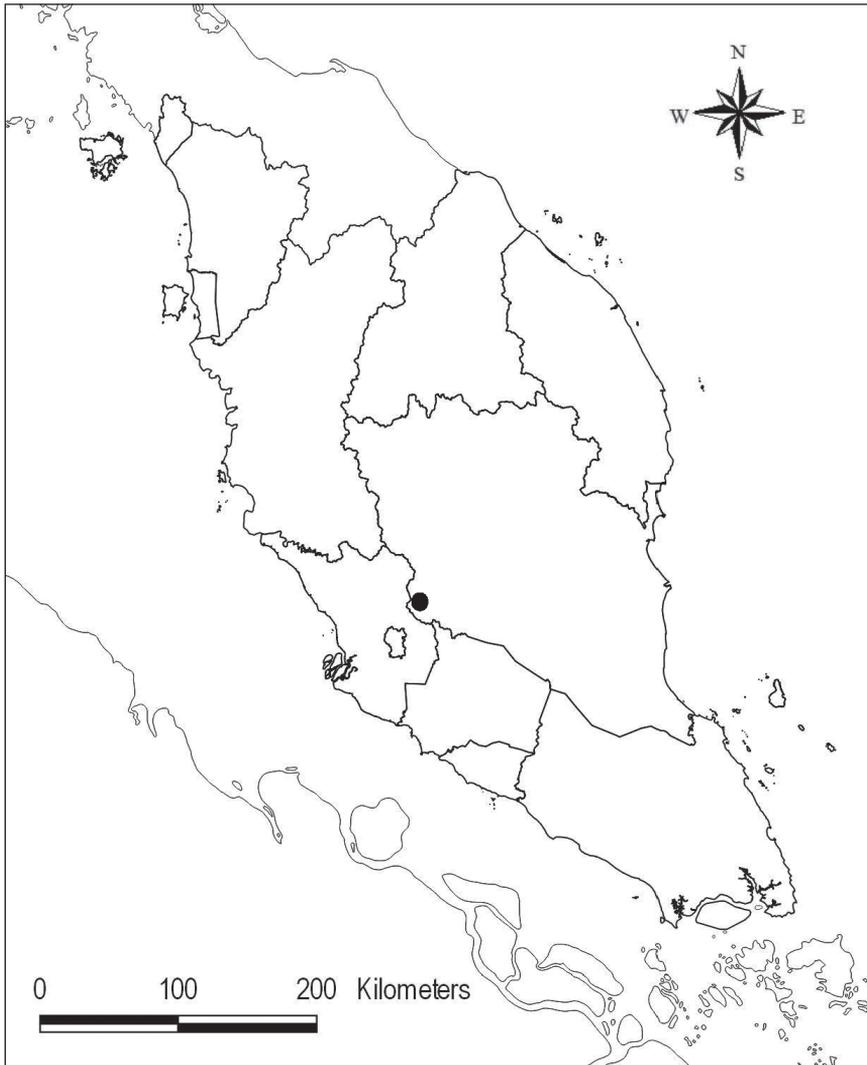
are known (Imhof 2010 onwards; Siti-Munirah et al. 2021; Chantanaorrapint and Seelanan 2021; Siti-Munirah and Dome 2022). To date, three species of *Thismia* are known in Pahang State: *T. alba* Holttum & Jonker, *T. aseroe* Becc. and *T. racemosa* Ridl. (Jonker 1948; Chua and Saw 2006). Morphologically, all of these species belong to *Thismia* sect. *Thismia* subsect. *Odoardoa*, in which all the tepals are fully open, spreading and not forming any mitre as in sect. *Geomitra*.

During a routine assessment of work progress near the Clearwater Way trail, located in a private forest not far from the Resorts World Genting Awana Hotel (Genting Highlands, Bentong District, Pahang), an unknown plant was discovered. The discovery was made on 1 April 2022, by the third author, who observed the unknown plant in a population growing on the nature trail. Based on images sent to the first author for identification, it was suspected to be a new taxon of *Thismia*. Later (7 April 2022), we visited the site together and were able to find additional plants within the same population. Several plants were collected for the herbarium and for taxonomic study. After careful examination, the specimens were found to have some novel features in terms of flower tube and tepal morphology. These features formed a unique combination of characteristics that were not matched with any of the described species of *Thismia*. Therefore, we now describe a new species that we have named *Thismia limkokthayi* Siti-Munirah & E.Chan. This additional new species brings the number of currently known species of *Thismia* in Pahang to four. This discovery has also resulted in *T. limkokthayi* becoming the first mitriform (*Geomitra*) species reported in the State of Pahang.

Materials and methods

This study is based on material collected on 7 April 2022 from Genting Highlands Forest, Bentong District, Pahang (Map 1), on private land near the Resorts World Genting Awana Hotel, which is one of the flagships for tourism in the mountains of this region. Genting Highlands is a famous mountainous region in Bentong District, Pahang, located approximately 50 km from the capital of Malaysia, Kuala Lumpur. Genting Highlands is renowned for entertainment, hospitality and tourism, as well as nature. As an area with an exceptional diversity of flora and fauna, it is home to a significant reservoir of primary rainforest (more than 15,000 acres); however, part of the area is fully developed.

One of the collected individuals was observed during its flowering growth (in the office at the Forest Research Institute Malaysia (FRIM)) and was selected as holotype (FRI91138c) due to its perfect condition. Morphological characteristics were examined by stereomicroscopy and high-resolution macrophotography. Measurements were made on fresh and liquid preserved material. The specimen was thoroughly compared with original drawings and descriptions in the protologues of *Thismia* sect. *Geomitra*.



Map 1. Map of the Malay Peninsula indicating the location of the Genting Highlands (dot), the type locality of *Thismia limkokthayi*.

Taxonomic account

Thismia limkokthayi Siti-Munirah & E.Chan, sp. nov.

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Figs 1–3

Diagnosis. *Thismia limkokthayi* is distinguished from closely-related species by the following combination of characteristics: black to dark brown floral tube and appendages,



Figure 1. *Thismia limkokthayi* with scale (all in centimetres (cm)) **A** plants with mature (FRI91138c) and young flowers with long stems **B** mature flower with a very short stem (FRI91138b) **C** stamens (FRI91138a). Photos by Siti-Munirah MY.

the presence of mitre fovea between the bases of claviform appendages, the presence of longitudinal ribs and absent transverse bars on the inner side of the floral tube, the apex of stamens with a central lobe (prolongation of the rib) and two lateral lobes (the tips of each are recurved) and dark purple stigma.

Type. MALAYSIA. Peninsular Malaysia: Pahang, Bentong District, Genting Highlands, ca. 1137 m elev., 28 April 2022, Siti-Munirah MY, FRI91138c (holotype KEP!, spirit collection, barcode no. SC12022).

Achlorophyllous herbs up to ca. 14 cm. **Roots** coralliform, surface hairy, apices brownish-white. **Stems** up to ca. 4.5 cm tall or very short (possibly for young plants), ca. 2–3 mm in diameter, erect, ascending, white and becoming brownish with age, glabrous, terete. **Leaves** glabrous, pale brown, dark brown in the upper part or towards the apex, scale-like, triangular-ovate to lanceolate, up to 12 mm long, 3 mm wide at the base, apex acute to acuminate, spirally arranged, more crowded in the upper part of the stem. **Floral bracts** 3, pale brown to dark brown towards the apex, similar to upper leaves, but slightly larger, 10–12 mm long, apex acute to acuminate, 2 mm wide at the base. **Pedicels** lengthen up to 5 mm during flower growth, 2–3 mm wide. **Flowers** solitary or in clusters of 2, opening in succession in the latter case and forming loose monochasial inflorescences, lateral flower located in the axil of one of the floral bracts of the terminal flower and also bearing its own floral bracts; each flower up to 8 cm long (including ovary and appendages); **perianth** actinomorphic with 6 tepals fused to form a floral tube with a dome-shaped mitre with 3 slender, claviform appendages on its top; **floral tube** black brownish/brown blackish, urceolate, ca. 20–25 mm long, ca. 5–10 mm wide, constricted just above the ovary, widest in the upper part; **outer surface** with 6 longitudinal ribs, glabrous, rough, black to dark brown; **inner surface** with 6 greenish longitudinal ribs, without transverse bars, black to dark brown; **outer tepal lobes** 3, brownish orange, minute, ca. 1 mm long, 7 mm wide at base, broadly triangular, erect; **inner tepal lobes** 3, black to dark brown, thick, cuneate, surface glabrous, apically adnate to form a dome-shaped mitre; **mitre** with 3 lateral, round-shaped, ca. 8–10 mm wide apertures, 3 hood-like accessory lobes, more curved during the early stages of flowering and flattening when the flower is older and matured; **mitre appendages** each ca. 27–30 mm long, their base wide and flattened, forming a fovea in the centre of the mitre, becoming narrower above, claviform at apex, glabrous, dark brown to pale orange towards tip. **Stamens** 6, pendulous from the apical margin of the floral tube; **annulus** absent; **filaments** orange and white, curved downwards, with bases slightly emerged above floral tube apex, not connate, forming 6 apertures apparent when viewed from above; **connectives** broad, orange-yellowish in lower half and black/dark brown in upper half, laterally connate to form a tube, ca. 12 mm long, each with prominent longitudinal rib extending along the entire length of the inner surface of the connective; supraconnective apex with one central lobe (extension of the rib) and two smaller side lobes with tips recurved inwards/truncate, glabrous; **lateral appendage** skirt-like, black, protruding towards the floral tube, not reaching connective apex, glabrous including on margins, only sparsely hairy on each horn-like corner; individual **stamens** with 2 thecae (abaxial, dehiscing towards the inner surface of the floral tube),

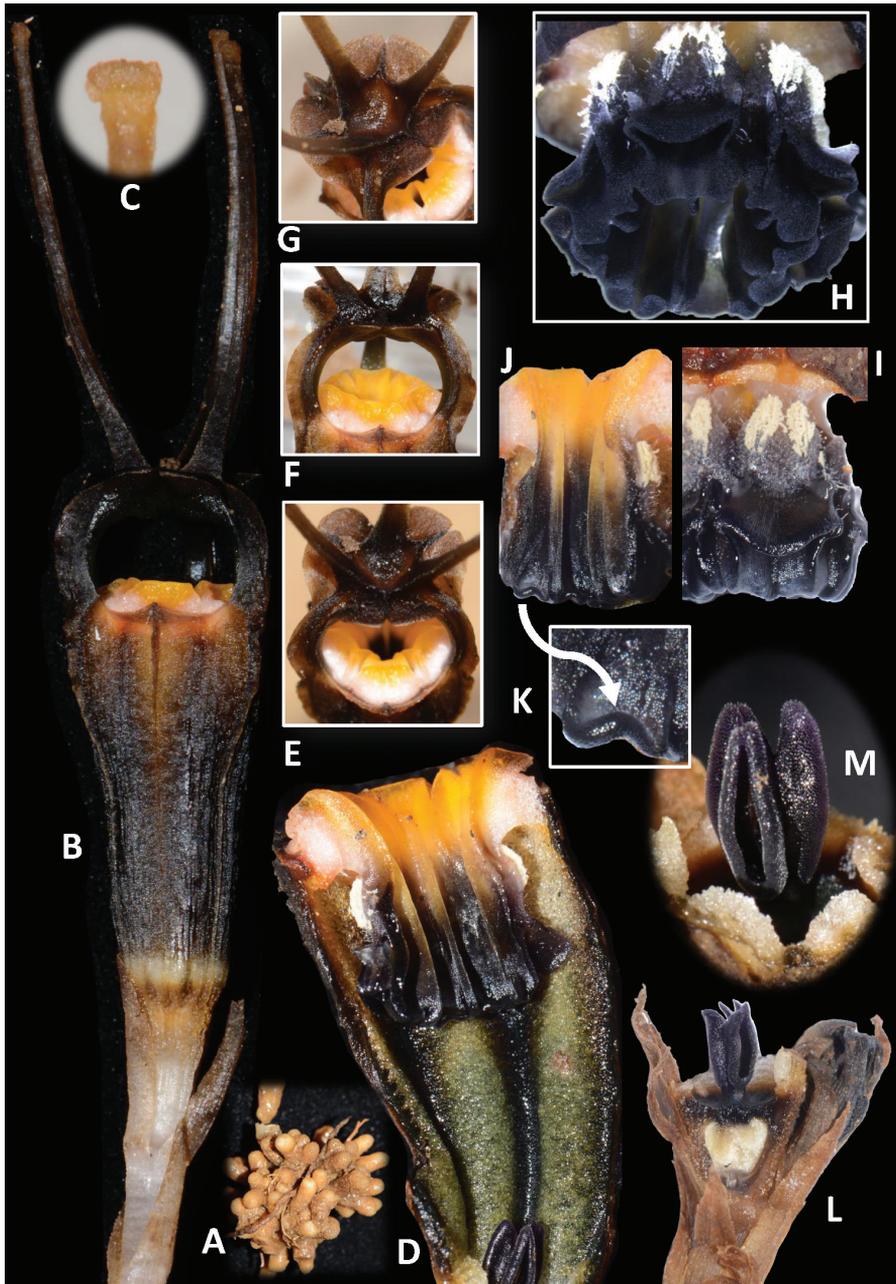


Figure 2. *Thysmia limkokthayi* **A** coralliform roots **B** full flower **C** claviform tip of mitre appendage **D** longitudinal section of floral tube, showing inner surface **E** outer tepal and inner tepal **F** side view of mitre **G** mitre viewed from above **H** six stamens, viewed from below **I** outer view of a stamen **J** inner view of two pendulous stamens **K** detail of the side lobe on the supraconnective apex (side lobe with truncate tips that are recurved inwards) **L** ovary with style, stigma and flower bud surrounded by bracts **M** stigmas. All from FRI FRI91138a (**A, D, I, J, K, L, M**), FRI91138b (**H**) and FRI91138c (**B, C, E, F, G**). Photos by Siti-Munirah MY; images not to scale (see dimensions in description and Fig. 1).

each **theca** oblong, 2 mm long; **interstaminal glands** elliptic-oblong, translucent, inserted on the line of fusion between connectives. **Ovary** inferior, obconical, ca. 4–5 mm long, brown to dark, with 6 longitudinal ribs, unilocular; **placentas** 3; **style** short, ca. 0.3 mm long, black/purplish; **stigma** 3-lobed, black-purplish, **stigma lobe** oblong, ca. 3 mm long, folded, bifid at apex, surface slightly papillose. **Fruit** not observed.

Additional specimens examined (paratypes). Peninsular Malaysia: Pahang, Bentong, Genting Highlands, ca. 1137 m elev., 7 April 2022, Siti-Munirah MY, Eddie C & Suhaida M, FRI91138a (KEP, spirit collection, barcode no. SC12021); 7 April 2022, Siti-Munirah MY, Eddie C & Suhaida M, FRI91138b (KEP, spirit collection, barcode no. SC12020)

Distribution. Endemic to Peninsular Malaysia, Pahang. Currently known only from the type locality.

Ecology and habitat. In lower montane forest and upper dipterocarp forest, on moist soil in shade, near an open space (hiking trail; Fig. 3A) and sloping area. This species occurs in a healthy undisturbed forest at an altitude of about 1137 m. The site is in a private forest and within a watershed. The forest area remains intact, apart from where it was affected by recent flooding and water surge (19 December 2021). The impact of this natural disaster completely reshaped part of the river area. However, this area is currently recovering. Fortunately, the only known population of *Thismia limkokthayi* is located away from the riverbank, on the slope of the main trail. As a result of this discovery, the trail was moved to another part of the forest. The flowering period is from March to April.

Etymology. *Thismia limkokthayi* was named in honour of Tan Sri Lim Kok Thay, Chairman of the Genting Group, who is closely involved in efforts to develop eco-tourism facilities and amenities supporting the preservation and sustainability of important biodiversity assets and sites in Genting Highlands.

Preliminary conservation status. Following the IUCN Red List Categories and Criteria (IUCN 2019), this species has been assessed as Critically Endangered (CR B2ab (iii); D) due to its small population and threats to its microhabitat. It is currently known from only one locality (the type locality) and is certainly a very rare species since only three individuals have been observed. Although the type locality is in a private forest, it may be exposed to future tourist activities in the Resorts World Genting Awana area. The habitat of the species is located on the original trail leading from the entrance of Clearwater Way to Chin Swee Caves Temple. However, since this is private land, the protection of this area from disturbance remains possible. As such, efforts must be made to locate this species in the surrounding area.

Notes. *Thismia* species are characterised by their peculiar appearance and flower morphology, which are distinctive and simultaneously very diverse (Shepeleva et al. 2020). Most *Thismia* species have various unique structural combinations. Amongst them, there are species belonging to the *Thismia* group of which inner tepals are fused together in their upper part and form a roof-like or hat-like structure called a mitre. In Peninsular Malaysia, there are two groups of mitriform *Thismia*: first including *Thismia* species with mitre appendages (e.g. *Thismia clavigera*, *Thismia kelantanensis* and *T. clavigeroides*) and



Figure 3. *Thysmia limkokthayi* Siti-Munirah & Eddie Chan **A** group photo with the *T. limkokthayi* (white arrow) in its habitat (from left; Angan A, Siti-Munirah MY, Eddie C, Ahmad Norsidar AH and Suhaida M) **B–C** different stages of anthesis of *T. limkokthayi* in its habitat (in situ) **B** two mature flowers from one individual **C** a fresh flower (on the left) and a new young flower starting to bloom (on the right). Photos by Siti-Munirah MY.

second including *Thismia* species without appendages (e.g. *T. latiffiana* Siti-Munirah & Dome and *T. sitimeriamiae* Siti-Munirah, Dome & Thorogood).

Thismia limkokthayi is easily recognised by a combination of the following characteristics: coralliform roots, erect outer perianth lobes, mitriform inner perianth lobes with three erect, slender, claviform appendages that form a mitre fovea with their flattened bases in the centre, absence of the annulus, presence of longitudinal ribs on the inner side of the floral tube (or absence of the reticulate inner side), the apex of stamens with a central lobe (prolongation of the rib) and two lateral lobes (the tips of each are recurved) and black-purple stigma.

Based on the infrageneric classification of Kumar et al. (2017), *T. limkokthayi* superficially resembles *Thismia* species in the *Thismia* subgenus *Thismia* section *Geomitra* (Becc.) Kumar & S.W. Gale, mainly based on the presence of inner tepals forming a mitre, each with filiform appendage and central mitral appendages that are free from each other, with the outer tepals always being short (less than 2 mm long). Two species were included in section *Geomitra*: *T. clavigera* (Becc.) F. Muell. and *T. betung-kerihunensis* Tsukaya & H. Okada.

To date, *T. limkokthayi* has not been included in any DNA-based analysis. According to the classification proposed by Shepeleva et al. (2020), *T. limkokthayi* is possibly related to species in clade 3 because it is characterised by coralliform roots, inner tepals fused into a mitre and free mitre appendages extending from a central point. Within clade 3 (i.e. section *Sarcosiphon* sensu Jonker 1948), there are two morphologically distinct groups characterised by the presence of a prominent central rib along the inner side of the connective and the absence of an annulus (Dančák et al. 2020). They can be distinguished by the presence of distinct appendages at the top of the mitre (*T. clavigera* group) or their absence (*T. goodii* group). However, their exact relationship cannot be clarified until more species in both groups are sequenced.

Currently, there are five species known in the *T. clavigera* group: *T. betung-kerihunensis*, *T. clavigera*, *T. clavigeroides* Chantanaorr & Seelanan, *T. kelantanensis* Siti-Munirah and *T. sumatrana* Suetsugu & Tsukaya (Tsukaya and Okada 2012; Siti-Munirah 2018; Suetsugu et al. 2018; Chantanaorrapint and Seelanan 2021). *Thismia limkokthayi* clearly differs from all these species by the absence of transverse bars on the inner surface of the floral tube. Notably, *T. limkokthayi* clearly resembles *T. kelantanensis*, an endemic species from Kelantan (Malay Peninsula), which also has three slender, claviform appendages at the tip of the mitre and erect outer tepals. However, *T. kelantanensis* is easily distinguished from *T. limkokthayi* by its bluish filaments and stamens, as well as the six-part cap on the mitre. Additionally, it also resembles *T. clavigeroides* Chantanaorr & Seelanan from Thailand and *T. sumatrana* Suetsugu & Tsukaya from Sumatra in its general appearance. However, *T. limkokthayi* differs from both of these species by the curved outer lobes on the supraconnective apex. Moreover, the connective appendage of *T. sumatrana* is hairy, while it is glabrous in *T. limkokthayi* and the flower colour of *T. clavigeroides* (including the stamens) is almost whitish, while that of *T. limkokthayi* is blackish-brown. A comparison of morphological characteristics between *T. limkokthayi* and other related species is presented in Table 1.

Table 1. Morphological differences between *Thismia limkokthayi* and related species. The characters of previously-described species are taken from the protologues and recent publications on *T. betungkerihunensis* (Tsukaya and Okada 2012), *T. clavigera* (Chantanaorrapint and Chantanaorrapint 2009), *T. kelantanensis* (Siti-Munirah 2018) and *T. sumatrana* (Suetsugu et al. 2018).

Characters	<i>T. limkokthayi</i>	<i>T. betungkerihunensis</i>	<i>T. clavigera</i>	<i>T. clavigeroides</i>	<i>T. kelantanensis</i>	<i>T. sumatrana</i>
Colour of floral tube	Black-brownish/ brown-blackish	White with indigo and brown to pale brown with purple-dark blue	White-orangish	Whitish	Pale to bright and dark blue-purplish translucent,	Unknown
Colour of mitre	Black-brownish/ brown-blackish	Blue-green	Yellowish-orange	Pale brown or grey	Yellow to bright orange	Unknown
Colour of inner tepal lobes	Brown-blackish	Blue-green	Orange	Pale brown or grey	Bright orange	Unknown
Outer tepal	Erect	Erect	Erect	Reflexed	Erect	Reflexed
Colour of filament	Orange and white	Blue-green	Orange	White	Bright blue	Unknown
Colour of appendages	Dark brown to pale orange	Pale blue tinged with orange	Orange	Pale brown or grey below, blue-green at tip	Orange	Unknown
Fovea	Present	Absent	Absent	Absent	Absent	Absent
Apex of supraconnective	One central lobe (extension of the rib) and two smaller side lobes with tips recurved inwards/ truncate, glabrous	One central lobe (extension of the rib) and two smaller side lobes, hairy	Acute	One central lobe (extension of the rib) and two smaller side lobes	Acuminate	Acute, hairy
Presence of transverse bars	Absent	Present	Present	Present	Present	Present

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References

- Chantanaorrapint S, Chantanaorrapint A (2009) *Thismia clavigera* (Thismiaceae), a new record for Thailand. Thai Forest Bulletin (Botany) 37: 27–31.
- Chantanaorrapint S, Seelanan T (2021) *Thismia clavigeroides* (Thismiaceae), a new mycoheterotrophic species from Thailand. Systematic Botany 46(1): 18–23. <https://doi.org/10.1600/036364421X16128061189468>
- Chua LSL, Saw LG (2006) Plants of Krau. FRIM Research Pamphlet No.126. Forest Research Institute Malaysia, Kepong, 227 pp.
- Dančák M, Hroneš M, Sochor M (2020) *Thismia ornata* and *T. coronata* (Thismiaceae), two new species from Sarawak, Borneo. Willdenowia 50(1): 65–76. <https://doi.org/10.3372/wi.50.50106>
- Griffith W (1844) On the root parasites referred by authors to Rhizanthæe and their allies. Proceedings of the Linnean Society of London 1: 216–221.
- Imhof S (2010 onwards) Mycoheterotrophic plants - How many of them are there? <http://mhp.myspecies.info> [accessed 10.4.2022]
- IUCN (2019) Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Jonker FP (1948) Burmanniaceae. In: van Steenis CGGJ (Ed.) Flora Malesiana. Noordhoff-Kolf, Leiden, series 1(4): 13–26.
- Kumar P, Gale SW, Li JH, Bouamanivong S, Fischer GA (2017) *Thismia nigricoronata*, a new species of Burmanniaceae (Thismieae, Dioscoreales) from Vang Vieng, Vientiane Province, Laos, and a key to subgeneric classification. Phytotaxa 319(3): 225–240. <https://doi.org/10.11646/phytotaxa.319.3.2>
- Shepeleva EA, Schelkunov MI, Hroneš M, Sochor M, Dančák M, Merckx VSFT, Kikuchi IABS, Chantanaorrapint S, Suetsugu K, Tsukaya H, Mar SS, Luu HT, Li H-Q, Logacheva MD, Nuraliev MS (2020) Phylogenetics of the mycoheterotrophic genus *Thismia* (Thismiaceae: Dioscoreales) with a focus on the Old World taxa: Delineation of novel natural groups and insights into the evolution of morphological traits. Botanical Journal of the Linnean Society 193(3): 287–315. <https://doi.org/10.1093/botlinnean/boaa017>
- Siti-Munirah MY (2018) *Thismia kelantanensis* (Thismiaceae), a new species from Kelantan, Peninsular Malaysia. Kew Bulletin 73(3): e42. <https://doi.org/10.1007/s12225-018-9767-0>
- Siti-Munirah MY, Dome N (2022) *Thismia latiffiana* (Thismiaceae), an unusual new species from Terengganu, Peninsular Malaysia. PhytoKeys 188: 105–114. <https://doi.org/10.3897/phytokeys.188.77061>
- Siti-Munirah MY, Dome N, Thorogood CJ (2021) *Thismia sitimeriamiae* (Thismiaceae), an extraordinary new species from Terengganu, Peninsular Malaysia. PhytoKeys 179: 75–89. <https://doi.org/10.3897/phytokeys.179.68300>
- Suetsugu K, Tsukaya H, Nurainas N, Okada H (2018) *Thismia sumatrana* (Thismiaceae), a new species from West Sumatra, Indonesia, with discussions on the taxonomic identity of *Thismia clavigera*. PhytoKeys 113: 59–67. <https://doi.org/10.3897/phytokeys.113.29103>
- Tsukaya H, Okada H (2012) A new species of *Thismia* (Thismiaceae) from West Kalimantan, Borneo. Systematic Botany 37(1): 53–57. <https://doi.org/10.1600/036364412X616639>

Nomenclatural and taxonomic notes on *Rubus davidianus* Kuntze and *R. viburnifolius* Franch

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Abstract

Critical examinations of specimens, with literature reviews, have shown that *Rubus davidianus* is conspecific with *R. lambertianus*. Therefore, we treat *R. davidianus* as a new synonym within *Rubus*. We propose a new name, *Rubus loirensis* Tiran Huang nom. nov. to replace the later homonym of *R. pycnanthus* Genev. Additionally, lectotypification of three names, *R. davidianus* Kuntze, *R. malifolius* Focke and *R. viburnifolius* Franch., are designated here after examination of previous works.

Keywords

new synonyms, *Rubus davidianus*, *R. lambertianus*, *R. malifolius*, *R. viburnifolius*, species identity

Introduction

Rubus L. is one of the most complicated taxonomic groups in the plant kingdom and is distributed worldwide from the lowland tropics to the subarctic region (Thompson 1995). Intraspecific/interspecific morphology and ploidy variability, apomictic

tendencies and the capability of many species to hybridise widely across multiple ploidy levels, complicate *Rubus* taxonomy (Bammi and Olmo 1966; Alice et al. 2001; Mimura et al. 2014; Carter et al. 2019). In response to this, taxonomists disagree broadly about the number of species in the genus, with different estimates ranging from 250 (Mabberley 2017), 700 (Robertson 1974; Lu and Boufford 2003), 750 (Lu 1985), 600–800 (Thompson 1995) to more than 1000 (Jennings 1988). The most recent global taxonomic treatment of this genus was conducted by Focke in 1910, 1911 and 1914 and 12 subgenera were defined. Phylogenetic results over the past 25 years suggest that Focke's subdivisions of *Rubus* are not monophyletic and large-scale taxonomic revisions are necessary. While working on the infrageneric re-classification of *Rubus*, we found that the taxonomic status of *R. davidianus* Kuntze and *R. viburnifolius* Franch. should be verified, especially in China (Lu and Boufford 2003). The names related to these two species, *Batidaea viburnifolia* Greene, *R. pycnanthus* Genev. and *R. viburnifolius* Focke, were also checked.

Rubus malifolius Focke (1890) was published, based on the collection from Chiensih, Hubei, China, A. Henry, 1885, no. 5794 (Syntypes BM000622260!; GH00040667!; K000737665!; US00097945!; Fig. 1A–D). Its critical characteristics were described as “Shrubs scandent. Leaves simple, elliptic or oblong-elliptic, base subrounded, margin inconspicuously shallowly serrate, apex acuminate, rarely acute, abaxial surface of leaves tomentose. Inflorescences terminal, racemes, bracts caducous, linear-oblong, pubescent initially, apex acute to shortly acuminate. Calyx abaxially densely tomentose-villous; sepals ovate to triangular-ovate or lanceolate. Petals white or white with pink spots, round, both surfaces thinly pubescent, base shortly clawed. Stamens many, slightly villous, anthers hirtose. Pistils much longer than stamens, ovary glabrous, styles glabrous, apex clavate. Aggregate fruit purplish-black at maturity, compressed globose, glabrous”.

Léveillé and Vaniot (1904) described *R. arbor* H. Lév. & Vaniot, based on the collection from Kouy-Tchéou Siao-tchang, Pin-fa, China, J. Cavaleri, May 1903, no. 1003 (Holotype E00010623!; Isotypes A00040529 (fragment with image of E00010623)!; E00313554!; K000737664!; Fig. 2A–D). Pax and Hoffmann (1922) described *R. limprichtii* Pax & K. Hoffm., based on the collection from Yatschou fu, Taldes Ya ho oberhalb Tschu schi ping, Hänge des Passes Tsiu gang schan, China, H.W. Limpricht, Jun 1914, no. 1564 (Syntype A00040666!; Fig. 3A). These two species were treated as synonyms of *R. malifolius* by Lu and Boufford (2003: 274) in Flora of China.

Franchet (1895) described *R. viburnifolius* Franch. in Bull. Mus. Hist. Nat. (Paris), based on the collection from les bois à Tchen-fong-chan, Yunnan, China, J.M. Delavay, Sep 1894, s. n. (Syntypes P00746144!, P00746145!; Fig. 3B–C). Greene (1906: 242) described *Batidea viburnifolia* Greene, based on the collection from Selkirk Mountains, US, C.H. Shaw, Aug 1904, no. 472 (Holotype US01106201!; Isotypes MIN1002232!; NY00418578!; S-G-8589 (fragment with image of NY00418578)!; Figs 3D and 4A–C). Then P.A. Rydberg (1913) merged the species into *Rubus* and

proposed a new combination, *R. viburnifolius* (Greene) Rydb. However, it is a later homonym of *R. viburnifolius* Franch. and is, therefore, illegitimate under Art. 53.1 (Turland et al. 2018). In view of this, Berger (1925) treated it as a variety of *R. idaeus* L., which was named as *R. idaeus* var. *viburnifolius* (Greene) A. Berger. Focke (1910) described *R. viburnifolius* Focke, based on the collection from Sze-mao, Yunnan, China, A. Henry, no. 11714, 11714A & B and 11714C (Holotype B101154586!; Isotypes A00040762!, A00132848!, A00132850!, A00132854!; MO-255250!; Figs 4D, 5A–D and 6A). Later, Focke rejected this *R. viburnifolius* (1910: 117; non-Franchet 1895, non-Rydberg 1913) and replaced it with *R. evadens* Focke (Isotypes E00010593!, E00317755!, E00317756!; IBSC0004402!; K000737732!, K000737733!, K000737734!; US00095499!, US00996968!; Figs 6B–D, 7A–D and 8A–B). Although *R. viburnifolius* Franch. is the legitimate name amongst these three names, the identification of their taxonomic status is still necessary.

Rubus lambertianus Ser. (1825) was published, based on the collection from China, Staunton, G. L., s. n. (Holotype G00316024!; Fig. 8C). Critical characteristics of the species were described as “Branchlets terete, thinly pubescent or subglabrous, with sparse, curved minute prickles. Leaves simple, cordate, base cordate, margin distinctly 3–5 lobed or undulate, serrulate. Stipules caducous, free. Inflorescences terminal usually cymose panicles, rachis and pedicels thinly pubescent, subglabrous, or glabrous. Calyx abaxially thinly pubescent, sepals ovate-lanceolate or triangular-lanceolate, margin entire, apex acuminate, margin of inner sepals grey tomentose. Petals white, obovate, glabrous, slightly shorter than or nearly as long as sepals, base clawed. Stamens many, somewhat shorter than petals; filaments broad, complanate. Pistils slightly shorter than or ca. as long as stamens, glabrous. Aggregate fruit red at maturity, subglobose, glabrous, with many drupelets, pyrenes small, prominently rugose”.

Hance (1882) described *R. ochlanthus* Hance, based on the collection from ad pagum Sai-ngau, secus fl. Lien-chau, Cantonensis, China, B.C. Henry, Oct 1881, no. 22021 (Holotype BM000885437!; Fig. 8D). According to the protologue, it was closely allied to *R. paniculatus* Sm., but was entirely distinct by the want of coloured indumentum, the much denser and more copious-flowered inflorescence and the smaller flowers. Focke (1874) described *R. pycnanthus* Focke, based on the collection from China, Duus, no. 11 (Holotype C10018009!; Fig. 9A). In the protologue, Focke stated that *R. lambertianus* was different from *R. pycnanthus* by its lanceolate-acuminate sepals. However, these two species were treated as synonyms of *R. lambertianus* by Lu and Boufford (2003) in Flora of China.

Kuntze (1879) described *R. davidianus* Kuntze, based on the collection from Moupin, Su-Tchuen, China, A. David, Aug 1869, s. n. (Syntypes P00755281!, P00755282!, P00755283!; Fig. 9B–D). In Flora of China, Lu and Boufford (2003) considered it as a synonym of *R. crataegifolius* Bunge. However, its characters of terminal cymose panicles or axillary subracemes are different from those of *R. crataegifolius*. Thus, the taxonomic status of *R. davidianus* needs further research and its taxonomic treatment remains ambiguous.



Figure 1. A–D Syntypes of *R. malifolius* Focke. Specimen barcodes: BM000622260, GH00040667, K000737665 and US00097945, respectively.

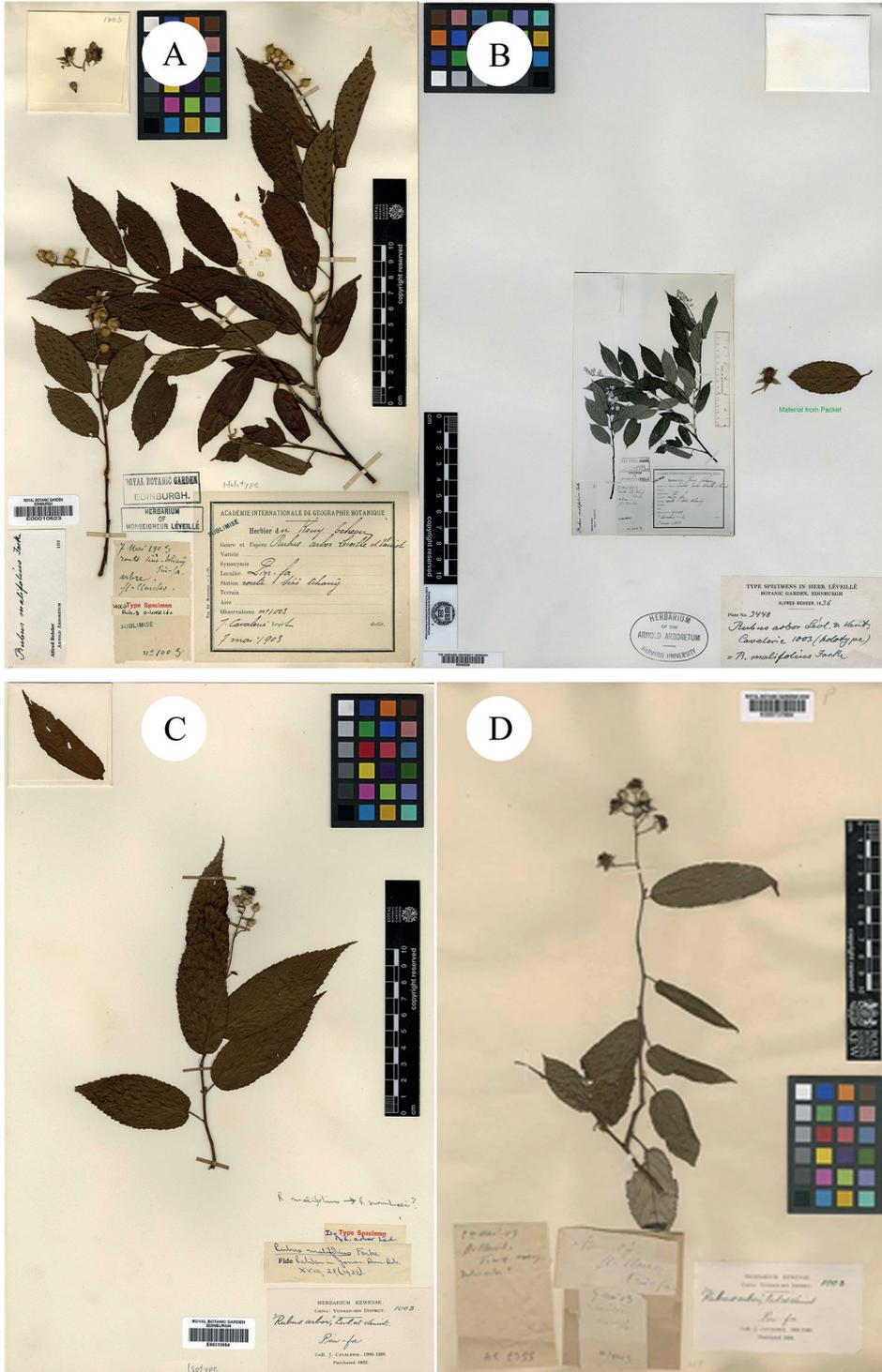


Figure 2. **A** Holotype of *R. arbor* H. Lév. & Vaniot **B–D** Isotypes of *R. arbor* H. Lév. & Vaniot. Specimen barcodes: E00010623, A00040529, E00313554 and K000737664, respectively.

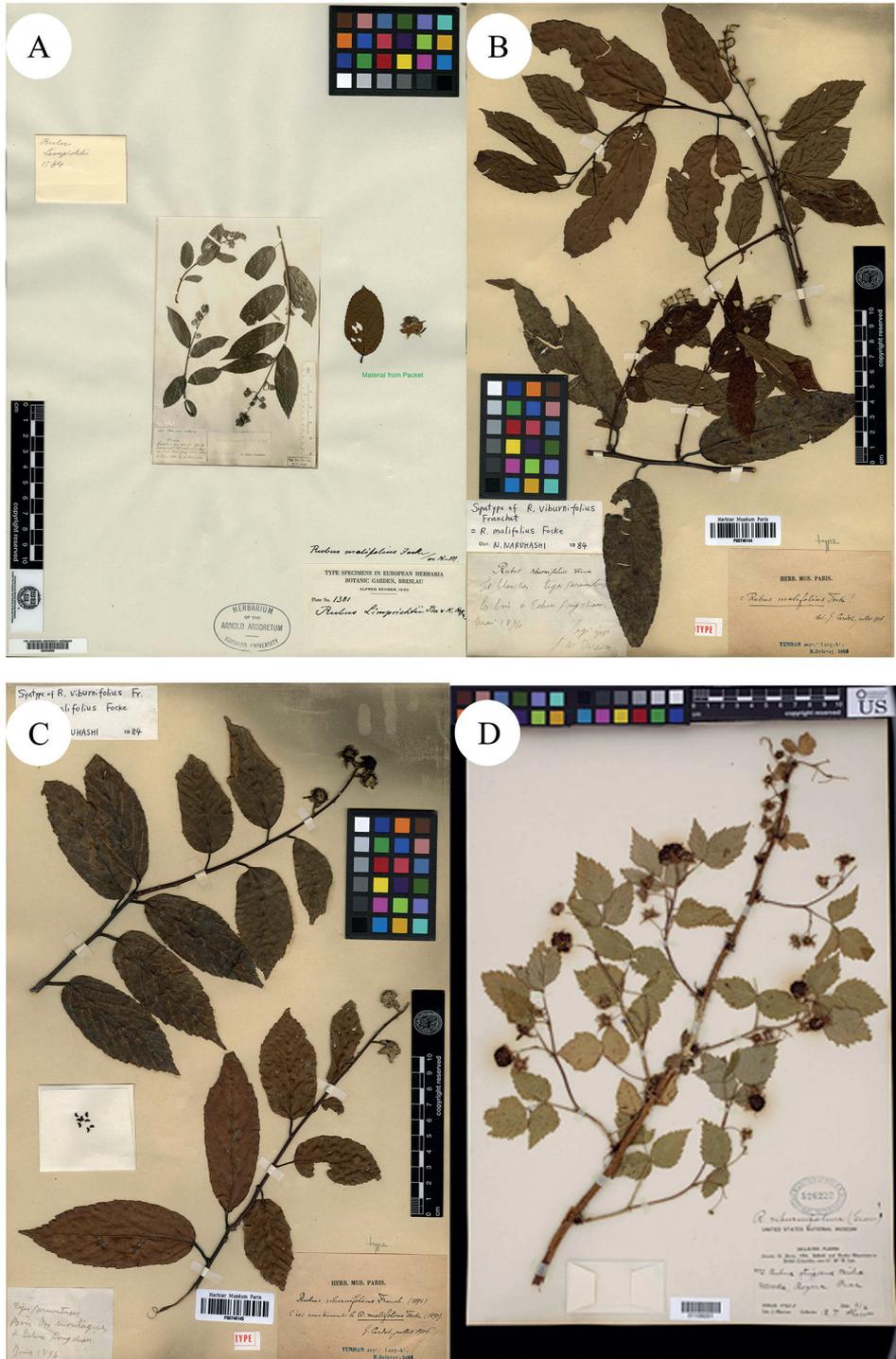


Figure 3. **A** Syntype of *R. limprichtii* Pax & K. Hoffm. **B–C** Syntypes of *R. viburnifolius* Franch. **D** Holotype of *B. viburnifolia* Greene. Specimen barcodes: A00040666, P00746144, P00746145 and US01106201, respectively.

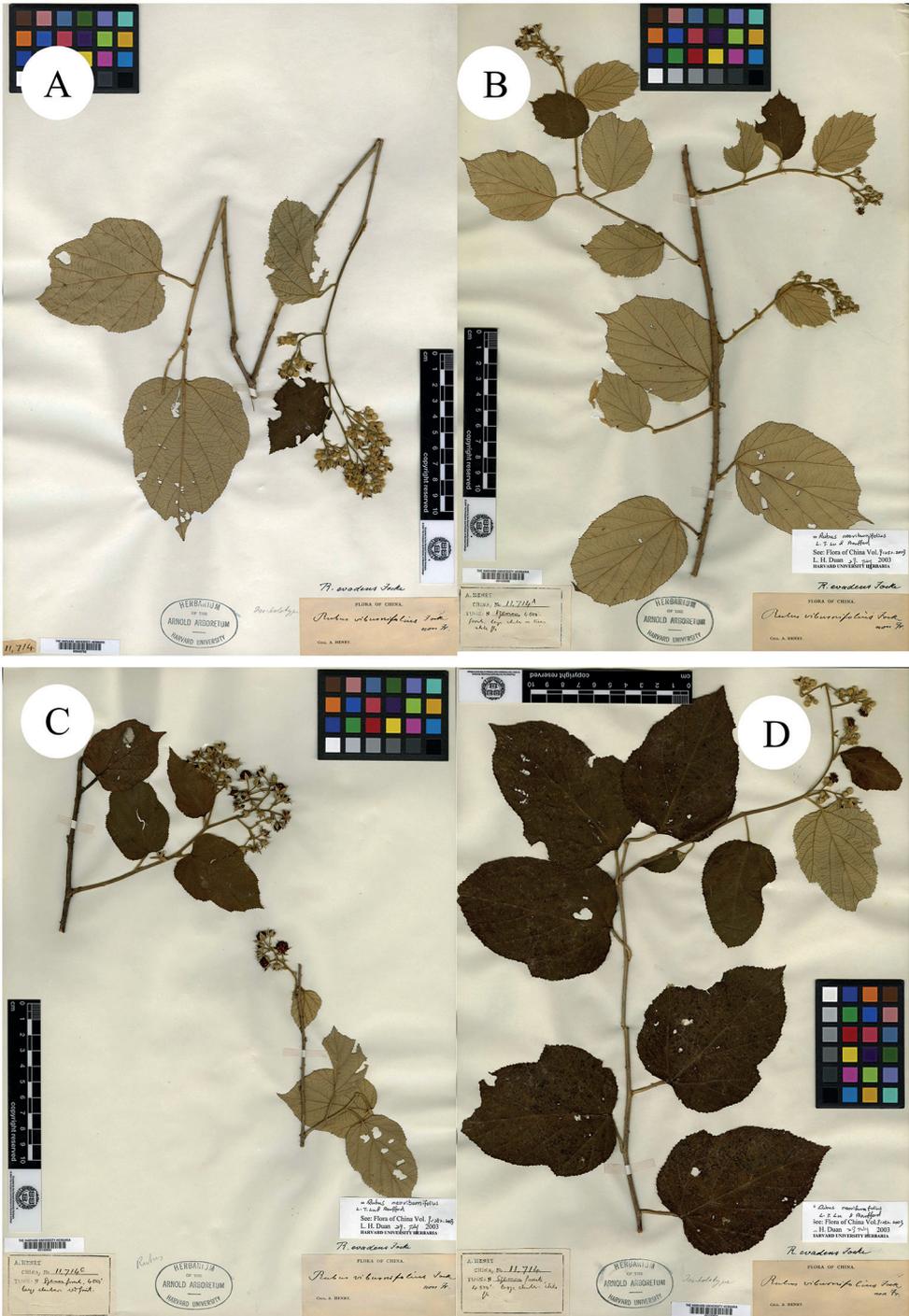


Figure 5. A–D Isotypes of *R. viburnifolius* Focke. Specimen barcodes: A00040762, A00132848, A00132850 and A00132854, respectively.

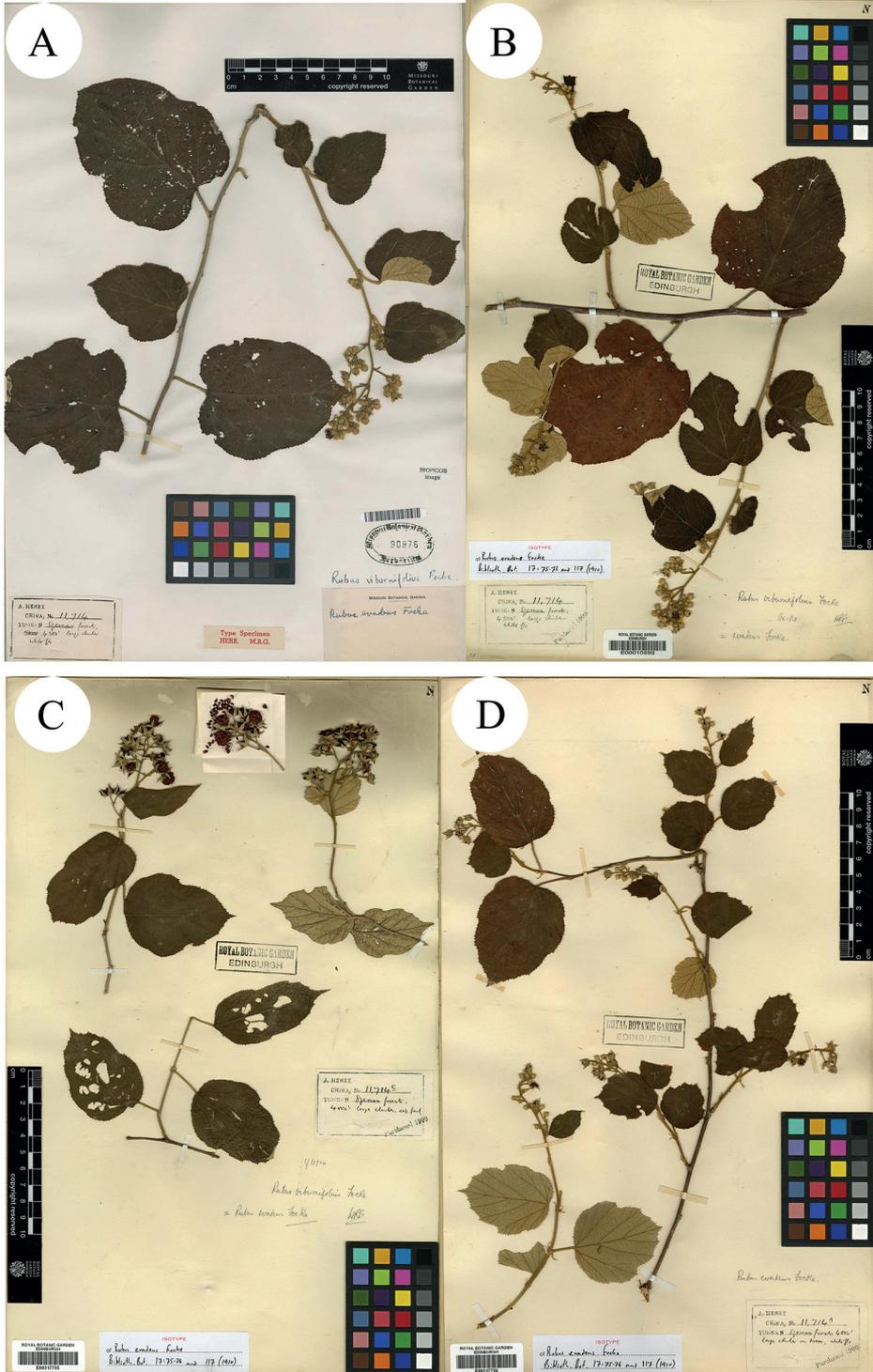


Figure 6. A Isotype of *R. viburnifolius* Focke B–D Isotypes of *R. evadens* Focke. Specimen barcodes: MO255250, E00010593, E00317755 and E00317756, respectively.

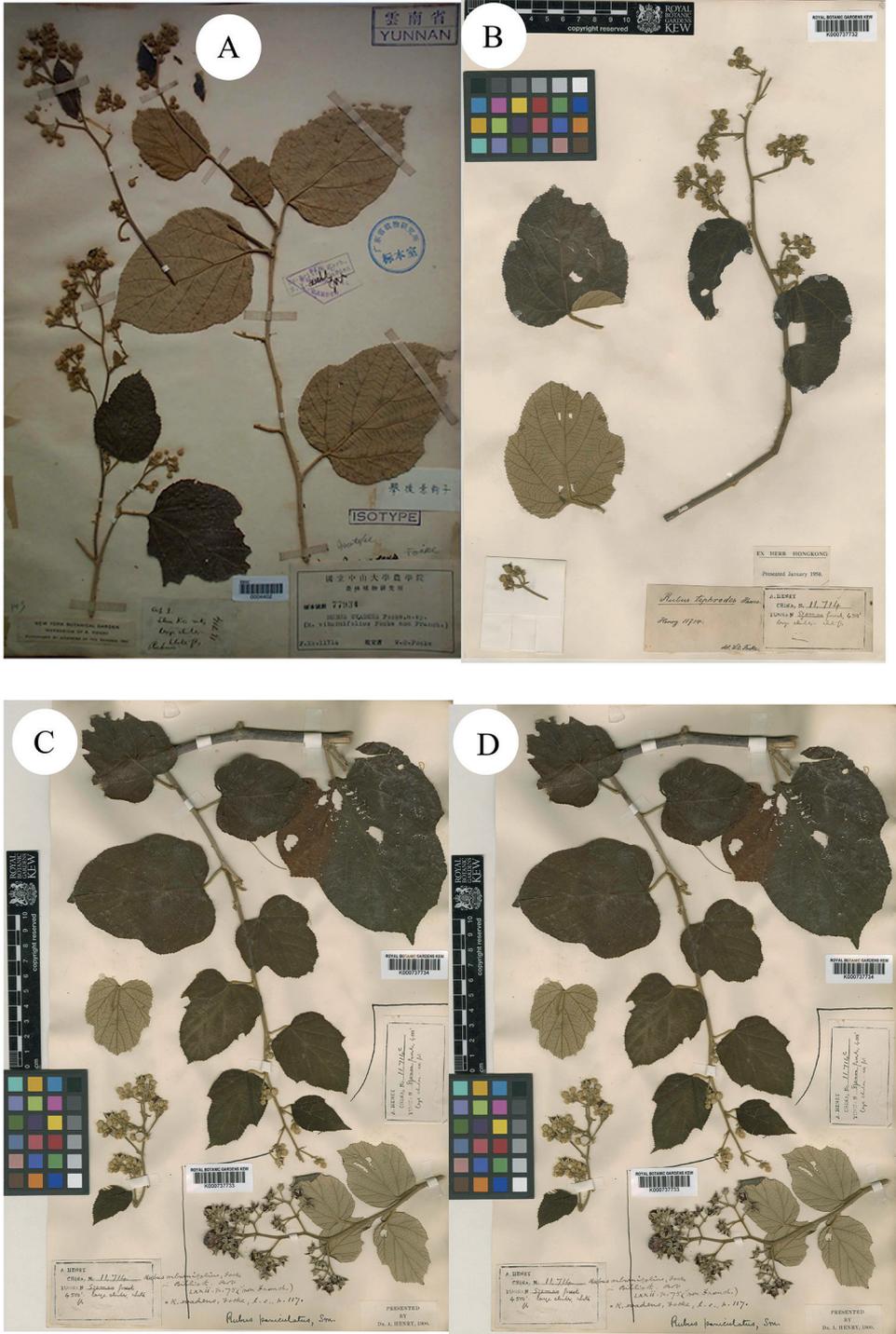


Figure 7. A–C Isotypes of *R. evadens* Focke. D Isotype of *R. viburnifolius* Focke. Specimen barcodes: IBSC0004402, K000737732, K000737733 (lower part of C), K000737734 (top part of C) and PE00020807, respectively.

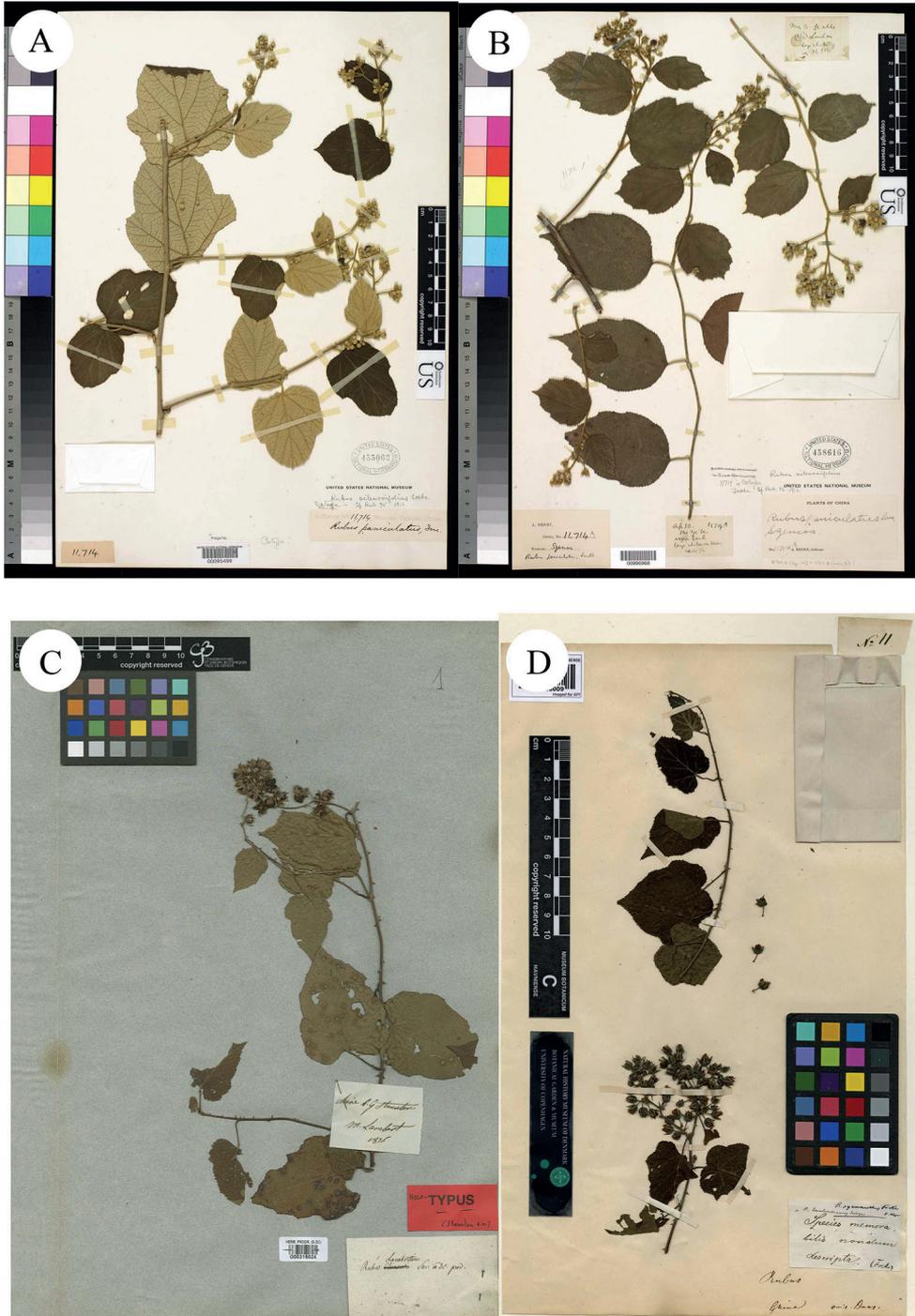


Figure 8. A–B Isotypes of *R. evadens* Focke; C Holotype of *R. lambertianus* Ser. D Holotype of *R. pycnanthus* Focke. Specimen barcodes: US00095499, US00996968, G00316024 and C10018009, respectively.

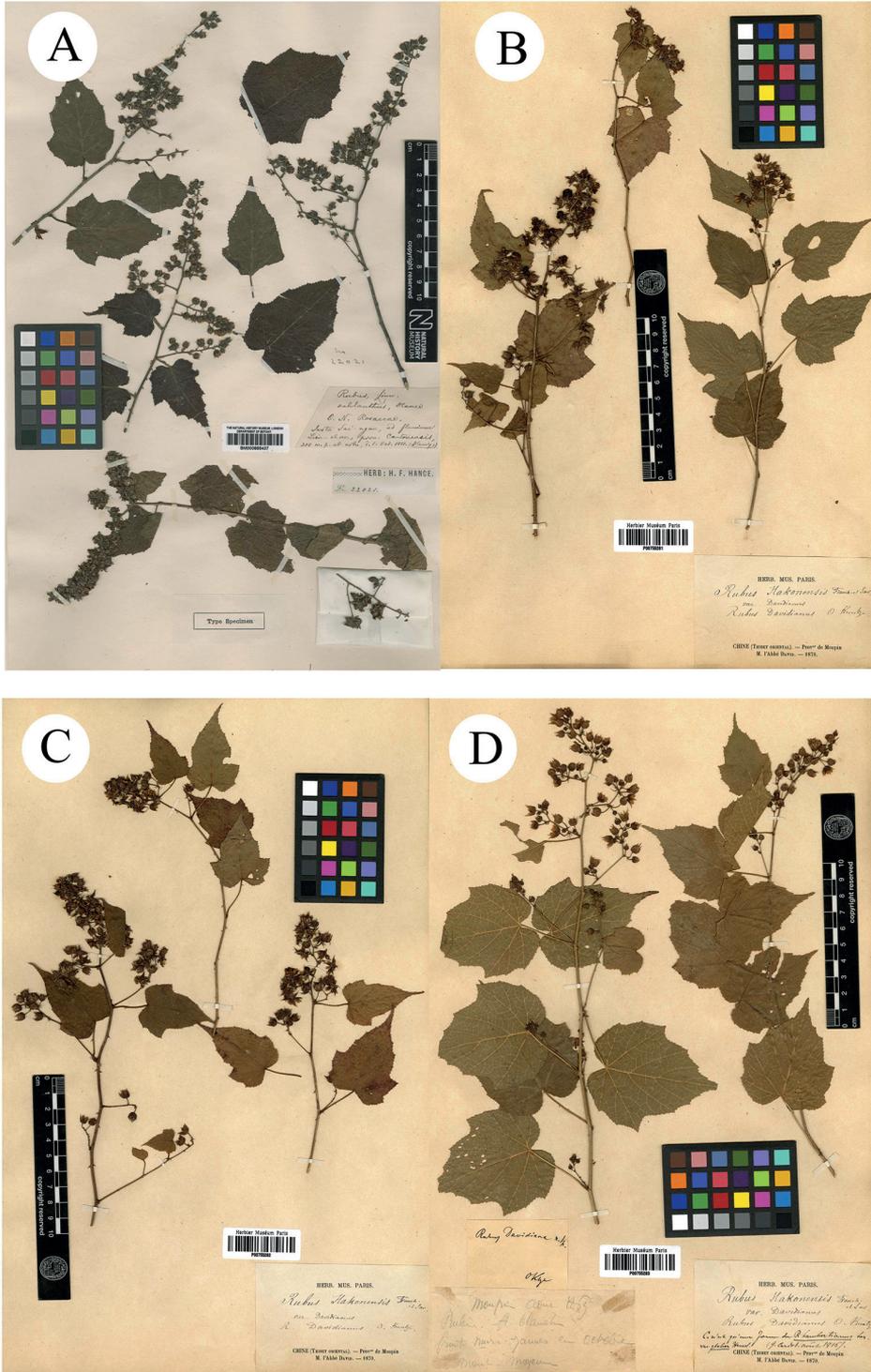


Figure 9. A Holotype of *R. ochlanthus* Hance C–D Syntypes of *R. davidianus* Kuntze. Specimen barcodes: BM000885437, P00755281, P00755282 and P00755283, respectively.

Materials and methods

We critically examined herbarium specimens of each species above, including all kinds of type specimens in A, BM, C, E, G, GH, IBS, K, MIN, MO, NY, P and US and checked them with protologues of each species.

Results

The examination of herbarium specimens, identified as *R. arbor*, *R. limprichtii* and *R. malifolius*, indicated that they represented one species. According to Art. 11.4 of the “International Code of Nomenclature for Algae, Fungi and Plants (Shenzhen Code)” (Turland et al. 2018), *R. malifolius* Focke is the correct name of this species. In the same way, both *R. viburnifolius* (Greene) Rydb. and *R. viburnifolius* Focke are two later homonyms of *R. viburnifolius* Franch. and are, therefore, illegitimate under Art. 53.1 (Turland et al. 2018). Amongst these names with epithets such as “*viburnifolius*” and *R. viburnifolius* (Greene) Rydb., once described as *Batidea viburnifolia* Greene, this is characterised by “Leaves imparipinnate, 3–5-foliolate, terminal leaflet prominently petiolulate, petioles bristly, glandular-hispid and puberulent; abaxial surface of leaflets densely tomentose; stipules and bracts linear; terminal inflorescences short racemes, rarely several flower clusters in leaf axils; abaxial surface of calyx ± with needle-like prickles; pedicels densely glandular-hispid and somewhat bristly; petals white; fruit hemispherical, broad, red or yellowish, drupelets very numerous, comparatively small, falling together from the dry receptacle, pubescent”. These characters indicate that it is very closely related to *R. idaeus* and the differences are that the petiole, pedicel and abaxial surface of the calyx of *R. idaeus* have no glandular hairs. Thus, A. Berger (1925: 51) proposed *R. idaeus* var. *viburnifolius* (Greene) A. Berger. as its correct name. On the other hand, Focke (1910: 75) proposed the name *R. viburnifolius* Focke to represent one Chinese *Rubus* species, but later he noticed his error and replaced it with *R. evadens* Focke (1910: 117). As the specimens of *R. viburnifolius* Franch. which is described from China, were unable to be viewed, this was treated as a suspicious species in Flora of China (Lu and Boufford 2003). We identified two specimens of *R. viburnifolius* Franch. in P and describe the characters of them as: “leaves simple, elliptic or oblong-elliptic, coarsely sharply serrate, base subrounded; stipules caducous, linear-oblong to ovate-lanceolate; terminal inflorescences racemes; rachis and pedicel densely tomentose-villous, gradually glabrescent, finally glabrous; bracts caducous, linear-oblong, apex acute to shortly acuminate; calyx abaxially densely tomentose-villous, sepals entire”. All of these characters indicate that *R. viburnifolius* Franch. is conspecific with *R. malifolius* and, therefore, *R. viburnifolius* Franch. is a later synonym of *R. malifolius*.

The examination of herbarium specimens, identified as *R. lambertianus*, *R. ochlanthus* and *R. pycnanthus* Focke, indicated that they represent the same species and, therefore, *R. lambertianus* is the correct name of this species. *R. davidianus* is a Chinese *Rubus* species described by Kuntze and three specimens of it have been

identified in P, characters of them being described as: “shrubs; leaves simple, broadly ovate, rarely oblong-ovate, abaxially pilose, more densely so along veins, rarely glabrous, with sparse, minute prickles along mid-vein, adaxially pilose or hairy only along veins, cordate at base, margin distinctly 3–5-lobed or undulate, serrulate, apex acuminate; stipules and bracts narrower, less than 2×1 cm, linearly lobed; terminal inflorescences cymose panicles, axillary ones often subracemes, shorter, sometimes flowers few in clusters in leaf axils; pedicel 0.5–1 cm long; calyx abaxially thinly pubescent, sepals ovate-lanceolate or triangular-lanceolate, undivided; petals obovate, glabrous, slightly shorter than or nearly as long as sepals”. These characteristics are consistent with those of *R. lambertianus*, which indicate that *R. davidianus* is a later synonym of *R. lambertianus*, not *R. crataegifolius*.

In the process of *R. pycnanthus* Focke identification, we found that another plant, occurring in Haute-Vienne, Saint-Sulpice-les-Feuilles, Thias, Lamy, Angers, Maine-et-Loire, France, was also named as *R. pycnanthus* Genev. (Genevier 1880). Actually, Genevier (1868) firstly published it as *R. pyramidatus* Genev. Then he rejected it because Müller (1859) had published a name with the same epithet “*pyramidatus*” for a German plant. According to Art. 53.1 (Turland et al. 2018), *R. pycnanthus* Genev. is also a later homonym of *R. pycnanthus* Focke and, therefore, a new name, *R. loirensis* Ti R. Huang nom. nov., is proposed.

Taxonomic treatment

1. *Rubus lambertianus* Ser. Prodr. [A. P. de Candolle] 2: 567. 1825.

R. davidianus Kuntze Meth. Sp.-Besch. *Rubus* 58. 1879. syn. nov. Type: China, Moupin, Su-Tchuen, A. David, Aug 1869, s. n. (lectotype designated here by Ti R. Huang: P [P00755283]!; isolectotypes: P [P00755281, P00755282]!).

R. ochlanthus Hance J. Bot. 20: 260. 1882. Type: China, ad pagum Sai-ngau, secus fl. Lien-chau, Cantonensis, B.C. Henry, Oct 1881, no. 22021 (holotype: BM000885437]!).

R. pycnanthus Focke Abh. Naturwiss. Vereins Bremen 4: 196. 1874. non Genevier (1880: 210). Type: China, Duus, no.11 (holotype: C [C10018009]!).

Type. CHINA, Staunton, G. L., s. n. (holotype: G [G00316024]!).

Distribution and habitat. *Rubus lambertianus* grows in slopes, roadsides, montane valleys, grasslands, thickets and forest margins. Its elevation ranges from low to medium. In China, it is distributed in Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Taiwan, Yunnan and Zhejiang Provinces and overseas in Japan.

Phenology. Flowering from July to August and fruiting from September to November.

Taxonomic notes. *Rubus lambertianus* is similar to *R. laxus* Focke, the differences being: the latter has leaves narrowly ovate; pedicel 1–2 cm long; sepals ovate or ovate-triangular, outer sepals pinnately lacinate, petals slightly pubescent.

Additional specimens examined. CHINA. Sichuan. 1934, T.H.Tu, no. 1604 (IBSC0324688); 15 October 1935, Xianyu, no. 6908 (NAS00366117); 15 October 1935, Xianyu He, no. 6908 (NAS00366117); 20 August 1963, Chuanxi Expedition Kechien Kuan Wentsai Wang et al., no. 2437 (PE02092824); 12 June 2014, Shuren Zhang et al., no. 1833 (PE01918855); 22 September 1978, Ya'an Expedition, s.n. (SM707005133, SM707005134). **Yunnan.** Shen'e Liu, no. 14014 (IBSC0324680); 25 June 1946, Shen'e Liu, no. 15383 (IBSC0324683); 8 August 1938, Tetsun Yu, no. 17291 (KUN711083); 28 July 1985, Zhanhe Ji, no. 306 (PE01828470); 24 May 1998, TianGang Gao, no. 1681 (PE01828469); 25 August 2002, Hong Wang, no. 6120 (PE01813595).

2. *Rubus malifolius* Focke Hooker's Icon. Pl. 20: t. 1947. 1890.

- R. viburnifolius* Franch. Bull. Mus. Hist. Nat. (Paris) 1: 63. 1895. Non Focke (1910: 75) nec Rydberg (1913: 446) Type: China, les bois à Tchen-fong-chan. Yunnan, J.M. Delavay, Sep 1894, s. n. (lectotype P00746144! (designated here by Ti R. Huang); isolectotype P00746145!).
- R. arbor* H. Lév. & Vaniot Bull. Soc. Bot. France 51: 217. 1904. Type: China, Kouy-Tchéou Siao-tchang, Pin-fa, J. Cavaleri, May. 1903, no. 1003 (holotype: E [E00010623]!; isotypes: A [A00040529] (with an image of E00010623)!, E [E00313554]!, K [K000737664]!).
- R. limprichtii* Pax & K. Hoffm. Repert. Spec. Nov. Regni Veg. Beih. 12: 406. 1922. Type: China, Yatschou fu, Taldes Ya ho oberhalb Tschu schi ping, Hänge des Passes Tsiu gang schan, H.W. Limprich, Jun. 1914, no. 1564 (holotype: A [A00040666]!).

Type. CHINA, Chienshih, Hubei, A. Henry, 1885, no. 5794 (lectotype designated here by Ti R. Huang: K [K000737665]!; isolectotypes: BM [BM000622260]!, GH [GH00040667]!, US [US00097945]!).

Distribution and habitat. *Rubus malifolius* grows in slopes, ravines, stream sides, montane valleys, forests and thickets. Its elevation ranges from 400–2200 m. It is endemic to China and is distributed in Guangdong, Guangxi, Guizhou, Hubei, Hunan, Sichuan and Yunnan Provinces.

Phenology. Flowering from May to June and fruiting from July to August.

Taxonomic notes. *Rubus malifolius* is similar to *R. prepanthus* Focke, the differences being: the latter has leaves narrowly obovate or broadly ovate-lanceolate to narrowly lanceolate, base rounded to subtruncate; stamens glabrous or anthers slightly villous; styles ca. as long as or slightly longer than stamens.

Additional specimens examined. CHINA. Yunnan. E.E. Maire, no. 104 (IBSC0340297); 15 July 1934, H.T.Tsai, no. 62641 (IBSC0340298); 16 May 1973, Zhihao Hu, no. 1382 (IBSC0340299; PE01833218); 24 May 1973, Bixing Sun et al., no. 401 (IBSC0340301; PE01833217); 14 August 1934, H.T.Tsai, no. 62641 (NAS00366395); 8 May 1964, Wang Shouzheng, no. 205 (KUN711739);

4 June 1959, Anquan Wu, no. 8240 (KUN711742); 15 July 1934, H.T.Tsai, no. 62641 (KUN757822; PE01833216, PE00252217); 12 August 1947, K.M. Feng, no. 11103 (PE00252220); 13 April 1940, C.W.Wang, no. 88450 (PE00252221); 20 August 1985, Zhanhe Ji Shunying Song & Xintang Ma, no. 601 (PE01833194, PE01833216); 6 April 1993, Yumin Shui, no. 2131 (PE01840835). **Sichuan:** 1932, T.T. Yu, no. 848 (IBSC0340264; PE00252196); 12 May 1941, Wenpei Fang, no. 16617 (IBSC0340273; PE00252199); 12 May 1941, Wenpei Fang, no. 16619 (IBSC0340277; PE00252200); Jinguiyuan, Huangjing, Gulin County, 29 May 2010, PE-GulinExpedition Team, no.40 (PE01864955); Xixi, Shuiwei, Xuyong County, Liang Zhang Xinmao Zhou & Wenbin Ju, no. HGX14303 (CDBI0226242; CDBI0226243). **Guizhou:** 22 June 1935, S.W.Teng, no. 640 (IBSC0340289); 3 July 1936, S.W.Teng, no. 90506 (IBK00065627, IBK00065634; IBSC0340283; NAS00366394; KUN711716; PE00252176); 14 July 1931, S.S.Sin, no. 51134 (IBSC0340287; IBSC0340291); 22 May 1930, Y.Tsiang, no. 5030 (IBSC0340288); 13 June 2003, Ye He, no.1-197 (PE01833201); 29 May 2016, Xinyun Lu, no. KKS1602173 (ZY0000066).

3. *Rubus loirensis* T.Huang, nom. nov.

urn:lsid:ipni.org:names:77306480-1

Replaced synonym. *R. pycnanthus* Genev. (1880: 210), non Focke (1874: 196).

Type. France, Haute-Vienne, Saint-Sulpice-les-Feuilles, Thias, Lamy, Angers, Maine-et-Loire.

Distribution and habitat. *Rubus loirensis* grows in woods, hedges, shale and granite. In France, it is distributed in Haute-Vienne, Maine-et-Loire and Loire-Inférieure.

Phenology. Flowering from June to July.

Taxonomic notes. L.G. Genevier (1868: 192) wrongly reported this species as *R. pyramidatus* P.J. Müll. in the Mém. Soc. Acad. Maine Loire. Later, L.G. Genevier corrected the error and proposed a replacement name *R. pycnanthus*.

R. loirensis is similar to *R. anadenes* P.J.Müll. ex Genev., the differences being: the former has petals wider, the stamens exceeding the styles and erect peduncles. It is also similar to *R. atrocaulis* P.J.Müll., the differences being: the former petals white and it is different from *R. stereacanthos* P.J.Müll. ex Genev. by its narrow panicles.

4. *Rubus evadens* Focke, **Biblioth. Bot. 17 (Heft 72 part I): 117 (75–76; fig. 27). 1910).**

R. nanopetalus Cardot, Notul. Syst. (Paris) 3: 300. 1917. Type: China, Lao-tsou-te-outze, Yunnan, Bons d'Anty, s. n. (holotype: P [P00746126]!).

R. viburnifolius Focke var. *apetalus* Y. Gu & W.L. Li, Bull. Bot. Res., Harbin 20(2): 122. 2000. Type: China, Yuanyang County, Yunnan, 1996, Yin Gu et al., no. 018 (holotype: NAS (JSBI); Jing dong, 1996, Yin Gu et al., no. 240, 241 (paratypes: NAS (JSBI)); Yuanyang-Lüchun divide (元阳绿春分水岭), 1996, Yin Gu et al., no. 030, 033 (paratypes: NAS (JSBI)).

Replaced synonym. *R. viburnifolius* Focke (1910: 75), non Franchet (1895: 63) nec Rydberg (1913: 446). — *Rubus neoviburnifolius* Lu & Boufford (2003: 252).

Type. CHINA, Szemao, Yunnan, A. Henry, no. 11714, 11714A & B and 11714C (holotype: B [B101154586]!; isotypes: A [A00040762, A00132848, A00132850, A00132854]!, E [E00010593, E00317755, E00317756]!, IBSC [IBSC0004402]!, K [K000737732, K000737733, K000737734]!, MO [MO-255250]!, NY [NY00429679]!, PE [PE00020807]!, SYS [SYS00076267]!, US [US00996968, US00095499]!).

Distribution and habitat. *Rubus evadens* grows in dry slopes and mixed forests. Its elevation ranges from 1200 to 3000 m. It is endemic to southern Yunnan.

Phenology. Flowering from June to July and fruiting from August to October.

Taxonomic notes. *Rubus evadens* is similar to *R. paniculatus* Smith, the differences being: the latter has leaves ovate to narrowly ovate, apically acuminate; petioles 2–4 cm long; flowers to 18 mm in diam.; terminal cymose panicles broad, lax.

5. *Rubus idaeus* L. var. *viburnifolius* (Greene) Greene ex A. Berger, New York Agric. Exp. Sta. Bull. 2: 51. 1925.

R. viburnifolius (Greene) Rydb. (1913: 446) ≡ *Batidea viburnifolia* Greene (1906: 242) Type: US, Selkirk Mountains, C.H. Shaw, Aug 1904, no. 472 (holotype: US [US01106201]!; isotypes: MIN [MIN1002232]!, NY [NY00418578]!, S [S-G-8589]! (with image of NY00418578).

Distribution and habitat. *Rubus idaeus* var. *viburnifolius* (Greene) Greene ex A. Berger grows in woods. It is distributed in western North America, Alaska to Mackenzie, Montana and south to British Columbia and perhaps to Wyoming and Utah.

Phenology. Unknown.

Taxonomic notes. *Rubus idaeus* var. *viburnifolius* (Greene) Greene ex A. Berger is treated as a variety of *R. idaeus* and is similar to *R. idaeus* var. *peramoenus* (Greene ex Fedde) Fernald. The differences are: canes glabrous or puberulent and more or less densely bristly; leaflets also green on both sides or somewhat tomentose underneath when young, but strongly veined beneath and more or less plicate; the former has inflorescence rachis and pedicels with glandular hairs; abaxial surface of calyx without glandular hairs; branchlets, petioles and pedicel with sparse prickles or nearly unarmed.

Discussion

Lu and Boufford (2003: 285) listed *R. viburnifolius* Franch. at the end of Flora of China to indicate that it had been described from Yunnan, China, but they have not seen any specimens and are, therefore, unable to treat it. Meanwhile, they stated that further revision of this species was necessary. In this paper, we carried out critical examinations of herbarium specimens, from which morphological characters of *R. arbor*, *R. limprichtii*, *R. malifolius* and *R. viburnifolius* were studied. Morphological characters of *R. arbor*, *R. limprichtii*, *R. malifolius* and *R. viburnifolius* Franch. indicate that they represent the same species and, therefore, *R. malifolius* is the correct name according to the “International Code of Nomenclature for Algae, Fungi and Plants (Shenzhen Code)” (Turland et al. 2018). Additionally, both *R. viburnifolius* (Greene) Rydb. and *R. viburnifolius* Focke are later homonyms of *R. viburnifolius* Franch., in which *R. viburnifolius* (Greene) Rydb. was replaced by *R. idaeus* var. *viburnifolius* and *R. viburnifolius* Focke was replaced by *R. evadens*.

Rubus davidianus was treated as a synonym of *R. crataegifolius* Bunge by Lu and Boufford (2003: 236) in Flora of China. However, examination of herbarium specimens indicates that there are distinct differences between the two species. The differences are: the former has inflorescences with terminal cymose panicles, axillary ones often subracemes, shorter, sometimes flowers few in clusters in leaf axils; stipules and bracts narrower, less than 2 × 1 cm, linearly lobed; the latter has inflorescences terminal, rarely axillary, short racemes or flowers several in cluster; stipules and bracts linear, entire. Thus, we conclude that *R. davidianus* and *R. crataegifolius* should represent two different species of *Rubus* and *R. davidianus* should be a synonym of *R. lambertianus*. Three specimens stored under *R. pyramidatus* P.J. Müll. in P were found; however, the specimens stored under either *R. pycnanthus* Genev. or *R. pyramidatus* Genev. could not be traced. Based on the existing characters of *R. pyramidatus* P.J. Müll. and *R. pycnanthus* Genev., we can identify that these two species are different from that of *R. pycnanthus* Focke. Though there are old attempts to synonymise *R. pycnanthus* Genev., the taxonomic status of *R. pycnanthus* Genev. should still be studied.

Species identification of *Rubus* species indicates that many homonyms and synonyms still exist in the genus *Rubus*, especially when they were more common in the 18th, 19th and 20th century. This could be interpreted in three ways. First, because of the propensity for interspecific hybridisation, polyploidy and apomixis, morphological characters of the species under this genus are highly variable and diverse. This makes species division and identification very difficult. Second, the original publications of species are often kept in the libraries of various scientific research institutions and some original publications are even kept in private collections. Objectively, this increases the difficulty for people to obtain and read the information of species publications. Third, examination of type specimens could not be easily accessed since digitisation of specimens was not yet widespread. Therefore, species names of *Rubus*, once not given sufficient attention or had not been discovered, should be emphasised in further taxonomic studies, using the integrative morphological characters and integrative systematics.

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References

- Alice LA, Eriksson T, Eriksen B, Campbell CS (2001) Hybridization and gene flow between distantly related species of *Rubus* (Rosaceae), evidence from nuclear ribosomal DNA internal transcribed spacer region sequences. *Systematic Botany* 26: 769–778. <http://www.bioone.org/doi/full/10.1043/0363-6445-26.4.769>
- Bammi RK, Olmo HP (1966) Cytogenetics of *Rubus* V. natural hybridization between *R. procerus* P.J. Müell. and *R. laciniatus* Willd. *Evolution; International Journal of Organic Evolution* 20(4): 617–633. <https://doi.org/10.1111/j.1558-5646.1966.tb03392.x>
- Berger A (1925) The systematic botany of edible brambles. The small fruits of New York. New York (State) Agricultural Experiment Station Bulletin 33(2): 51.
- Cardot J (1917) Rosacées Nouvelles D'Extrême-Orient (suile). *Notulae Systematicae. Herbarium de Muséum de Paris. Phanogramie. Paris* 3: 300.
- Carter KA, Liston A, Bassil N, Alice LA, Bushakra JM, Sutherland BL, Mockler TC, Bryant DW, Hummer KE (2019) Target capture sequencing unravels *Rubus* evolution. *Frontiers in Plant Science* 10: e01615. <https://doi.org/10.3389/fpls.2019.01615>
- Focke WO (1874) *Batographische Abhandlungen. Abhandlungen Herausgegeben vom Naturwissenschaftlichen Verein zu Bremen* 4: 196.
- Focke WO (1890) *Rubus malifolius* Focke. In: Hooker's *Icones plantarum*. Longman Rees Orme Brown Green, & Longman, London, 20(2): 10 [pl. 1947]. <https://doi.org/10.5962/bhl.title.16059>
- Focke WO (1910) *Species Ruborum. Monographiae generis Rubi Prodrromus. Pars I. Biblioth. Bot.* 17 Helft 72(1): 75–76 [+ 117]. <https://doi.org/10.5962/bhl.title.15533>
- Focke WO (1911) *Species Ruborum. Monographiae generis Rubi Prodrromus. Pars II. Biblioth. Bot.* 17 Helft 72(2): 121–223. <https://doi.org/10.5962/bhl.title.15533>
- Focke WO (1914) *Species Ruborum. Monographiae generis Rubi Prodrromus. Pars III. Biblioth. Bot.* 17 Heft 83: 224–498. <https://doi.org/10.5962/bhl.title.15533>
- Franchet A (1895) Sur quelques plantes de la Chine occidentale. *Bulletin du Muséum d'Histoire Naturelle* 1: 63. <https://doi.org/10.1080/00378941.1896.10828845>
- Genevier LG (1868) Essai monographique sur les *Rubus* du bassin de la Loire. *Mémoires de la Société Académique de Maine-et-Loire* 24: 192. <https://doi.org/10.5962/bhl.title.15277>
- Genevier LG (1880) Essai monographie des *Rubus* du bassin de la Loire ed. 2. Paris: Librairie Savy, 77 Boulevard Saint-Germain; Nantes: Chez l'auteur, 2 bis, Rue Franklin 210.
- Greene EL (1906) Certain Rosaceous Genera. *Leaflets of Botanical Observation and Criticism* 1(4): 242. <https://doi.org/10.5962/bhl.title.57566>

- Gu Y, Li WL, Wang CY, Yu H, Shi ZM, Peng LJ (2000) New taxa of Chinese *Rubus* L. (Rosaceae). *Bulletin of Botanical Research* 20(2): 122.
- Hance (1882) *Specilegia flore sinensis*: diagnoses of new and habitats of rare or hitherto unrecorded Chinese plants. *Journal of Botany, British and Foreign* 20: 260.
- Jennings DL (1988) *Raspberries and blackberries: their breeding, diseases and growth*. Academic Press, London.
- Kuntze CEO (1879) *Methodik der Speciesbeschreibung und Rubus*: Monographie der einfachblättrigen und krautigen brombeeren verbunden mit betrachtungen über die fehler der jetzigen. Verlag Von Arthur Felix, Leipzig, 58.
- Léveillé AAH (1904) Nouveautés Chinoises, Coreennes et Japonaises. *Bulletin de la Société Botanique de France* 51: 217 [pl. 3]. <https://doi.org/10.1080/00378941.1904.10829119>
- Lu LT (1985) *Rubus* L. Rosaceae. In: Yu TT, Lu LT, Ku TC, Kuan KC, Li CL (Eds) *Flora Reipublicae Popularis Sinicae*, vol. 37. Science Press, Beijing, 10–218.
- Lu LT, Boufford DE (2003) *Rubus* L. In: *Flora of China*. Vol. 9. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 195–285.
- Mabberley DJ (2017) *Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classification and Uses*. Cambridge University Press, Cambridge, 808–810. <https://doi.org/10.1017/9781316335581>
- Mimura M, Mishima M, Lascoux M, Yahara T (2014) Range shift and introgression of the rear and leading populations in two ecologically distinct *Rubus* species. *BMC Evolutionary Biology* 14(1): e209. <https://doi.org/10.1186/s12862-014-0209-9>
- Müller PJ (1859) Versuch einer monographischen darstellung der gallo-germanischen arten der gattung *Rubus*. *Jahresbericht der Pollichia, eines Naturwissenschaftlichen Vereins der bayerischen Pfalz* 26–27: 291.
- Pax F (1922) Aufzählung der von Dr. Limpricht in Ostasien gesammelten Pflanzen. *Repertorium Specierum Novarum Regni Vegetabilis* 12: 406.
- Robertson RK (1974) The genera of Rosaceae in the southeastern United States. *Journal of the Arnold Arboretum* 55(3): 352–360. <https://doi.org/10.5962/p.67289>
- Rydberg PA (1913) *Rubus* L. in North America *Flora*. New York Botanical Garden [Reprinted in 1963] 22(5): 446.
- Seringe NC (1825) *Rubus lambertianus* Ser. In: *Prodromus Systematis Naturalis Regni Vegetabilis* 2: 567.
- Thompson MM (1995) Chromosome numbers of *Rubus* species at the national clonal germplasm repository. *HortScience* 30(7): 1447–1452. <https://doi.org/10.21273/HORTSCI.30.7.1447>
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF [Eds] (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. *Regnum Vegetabile* 159. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>

Hydrangea marunoi (Hydrangeaceae), a new species from Osumi Peninsula, southern Japan

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Abstract

Hydrangea marunoi Tagane & S. Fujii, from the Kimotsuki Mountains in the Osumi Peninsula, southern Japan, is described and illustrated. It is morphologically similar to *H. alternifolia* in having three-petaloid calyx lobes in marginal flowers, but is distinguished by the larger stamen number, and longer styles and seeds. Multiplex ISSR genotyping by sequencing (MIG-seq) demonstrated that the new species is monophyletic and closely related to *H. amamiobsimensis* and *H. moellendorffii* rather than *H. alternifolia*.

Keywords

Cardiandra, endemic species, flora, MIG-seq, plant taxonomy, Saxifragales

Introduction

Hydrangea L., including approximately 270 natural species (De Smet et al. 2015) and four widely cultivated species (Fulcher et al. 2016), is a well-known genus in Hydrangeaceae. Based on phylogenetic analysis, De Smet et al. (2015) proposed a broad circumscription of *Hydrangea* comprising approximately 200 species distributed across East and Southeast Asia and the Americas. Most *Hydrangea* species are shrubs or lianas. However, the species of *Hydrangea* L. sect. *Cardiandra* (Siebold & Zucc.) Y.De Smet

& Samain are herbs that have been treated as members of the genus *Cardiandra* (Ohba 1985a, b, 2001; Wei and Bartholomew 2001; De Smet et al. 2015). In the current broad circumscription of *Hydrangea*, it is treated as a section of the genus *Hydrangea*, which additionally also includes eight groups previously known as genera i.e. *Broussaisia* Gaudich, *Decumaria* L., *Deinathe* Maxim., *Hydrangea s.str.*, and *Pileostegia* Hook. f. & Thomson, *Platycrater* Siebold & Zucc., and *Schizophragma* Siebold & Zucc. In the phylogeny by De Smet et al. (2015), *Hydrangea* sect. *Cardiandra* is monophyletic and a sister to the sect. *Deinathe*, which comprises two known herbaceous species from China to Japan.

In *Hydrangea* sect. *Cardiandra*, four species, *H. alternifolia* L., *H. amamiohsimensis* (Koidz.) Y. De Smet et Granados, *H. moellendorffii* Hance, and *H. densifolia* (C. F. Wei) Y. De Smet & Granados are known from Japan, Taiwan and China (Ohba 1985a, b, 2001; Wei and Bartholomew 2001; Ohashi 2017), all but *H. densifolia* are known from Japan.

During our floristic survey in Kagoshima Prefecture, southern Japan in 2021, we collected an unknown flowering species of *Hydrangea* sect. *Cardiandra*. It is similar to *H. alternifolia* in appearance, but it differs from it in its habitat and some floral characters. To clarify the relationship between the unknown species and the other species of *Hydrangea* sect. *Cardiandra* in Japan, we examined the phylogenetic relationships of 52 samples of *H. alternifolia*, *H. amamiohsimensis*, *H. moellendorffii*, and the unknown species using multiplex ISSR genotyping by sequencing (MIG-seq, Suyama and Matsuki 2015) and compared this with our observations of morphological characteristics. Multiplexed inter-simple sequence repeats (ISSR) genotyping by sequencing (MIG-seq) is a technique used to obtain many single nucleotide polymorphisms (SNPs) throughout a genome, which is valuable for determining molecular phylogenetic trees. It has been successfully applied to resolve the taxonomy of closely related taxa, including in *Hydrangea* (Hirota et al. 2022). Based on the phylogenetic hypotheses resulting from MIG-seq analysis and subsequent morphological observations, we describe *Hydrangea marunoi*, sp. nov.

Materials and methods

Morphological observation and assessment of conservation status

To assess the novelty of the unknown species, we consulted the taxonomic literature (Ohba 1985a, b, 2001; Wei and Bartholomew 2001; Ohashi 2017) and herbarium specimens at FU, KAG, KAP, and TI, as well as the digitized specimen images of FKSE, TRPM, and those available at the Shimane Nature Museum of Mt. Sanbe available on the web (Digital herbarium of Shimane University Faculty of Life and Environment Sciences <http://tayousei.life.shimane-u.ac.jp/herbarium/>).

The conservation status was calculated following the IUCN Red List categories and criteria v3.1 (IUCN 2012) and IUCN guideline (IUCN 2019). The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were calculated using the GeoCAT software (Bachman et al. 2011).

Taxon sampling for phylogenetic analysis

To perform the phylogenetic analysis, 52 samples of the *Hydrangea* sect. *Cardiandra* were gathered from both our field surveys in Japan and herbarium specimens deposited at the Kagoshima University Museum (KAG): 10 *H. marunoi* samples, two *H. amamiohsimensis* samples, four *H. moellendorffii* samples, and 36 *H. alternifolia* samples (Suppl. material 1: Table S1; Fig. 1). Additionally, one sample of *H. bifida* (Maxim) Y.De Smet & C.Granados of *Hydrangea* sect. *Deinanthe* was used as the outgroup. During the field survey, a small piece of leaf was cut, placed in a tea bag, and dried with silica gel in a zip-lock bag.

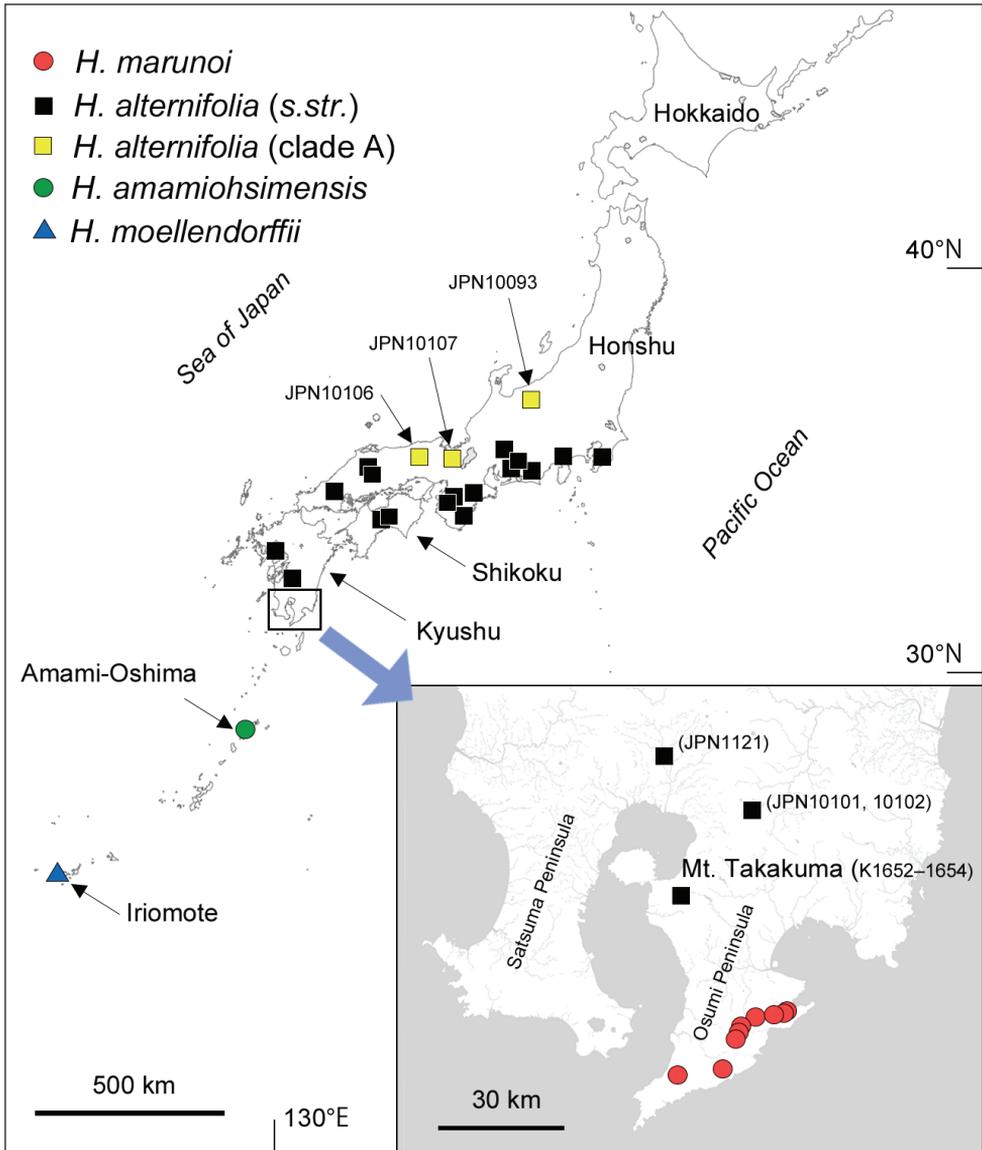


Figure 1. Collection localities of the four *Hydrangea* sect. *Cardiandra* species in this study.

MIG-seq analysis

Total DNA was extracted from dried leaves using the cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). For *de novo* SNP detection, MIG-seq (Suyama and Matsuki 2015) was performed according to the protocol described by Suyama et al. (2022). To prepare the MIG-seq library, a two-step PCR amplification process was performed: ISSR regions were amplified using the first PCR, and Illumina sequencing adaptors and indices were added to the first PCR products during the second PCR. Sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using a MiSeq Reagent Kit v3 (150 cycles; Illumina). We skipped the sequencing of the first 17 bases of reads 1 and 2 (SSR primer regions and anchors) using “DarkCycle.” Low-quality and extremely short reads containing adapter sequences were removed using Trimmomatic 0.39 (Bolger et al. 2014). The Stacks 2.60 pipeline (Rochette et al. 2019) was used for *de novo* SNP genotyping with the following parameters: the minimum depth of coverage required to create a stack (m) = 3, the maximum distance between stacks (M) = 2, and the maximum mismatches between loci when building the catalog (n) = 2. Three criteria were used for the SNP filtering. First, any SNP site where one of the two alleles had less than three counts was filtered out owing to the difficulty in distinguishing polymorphisms from sequencing errors when the minor allele count of SNPs is extremely low (Roesti et al. 2012). Second, SNPs with high heterozygosity ($H_o \geq 0.6$) were removed because excess heterozygosity may have resulted from artifactual loci built from several paralogous genomic regions. Third, SNPs with a genotyping rate of < 30% were eliminated. Using the third criterion, the SNPs retained by at least 16 samples were included in the SNP dataset.

Maximum likelihood phylogeny based on SNPs was inferred using the RAxML 8.2.10 software (Stamatakis 2014). We used a GTRCAT model with an ascertainment bias correction using the Lewis method and performed 1,000 replicates of parallelized tree search bootstrapping.

Results

Among the 17,753,114 raw reads ($334,964 \pm 34,812$ reads per sample) obtained, 13,254,044 reads ($250,076 \pm 28,521$ reads per sample) remained after quality control. After *de novo* SNP detection and filtering, 1875 loci and 4506 SNPs were identified. *Hydrangea bifida* (JPN4970) was removed from the SNP dataset because of its high proportion of missing data (0.982). The ten *H. marunoi* samples were monophyletic and formed two geographically defined groups; populations from Mt. Nokubi (K1658–1661) and that from the Oda River (JPN9950, JPN10103, K1633, K1637, K1638, KAG088891) (Fig. 2). *Hydrangea marunoi* was sister to a clade that included *H. amamiohsimensis* and *H. moellendorffii*. *Hydrangea marunoi*, *H. amamiohsimensis*, and *H. moellendorffii* were all supported as monophyletic by bootstrap values of 100%. *Hydrangea alternifolia* was sister to a clade that included these three species.

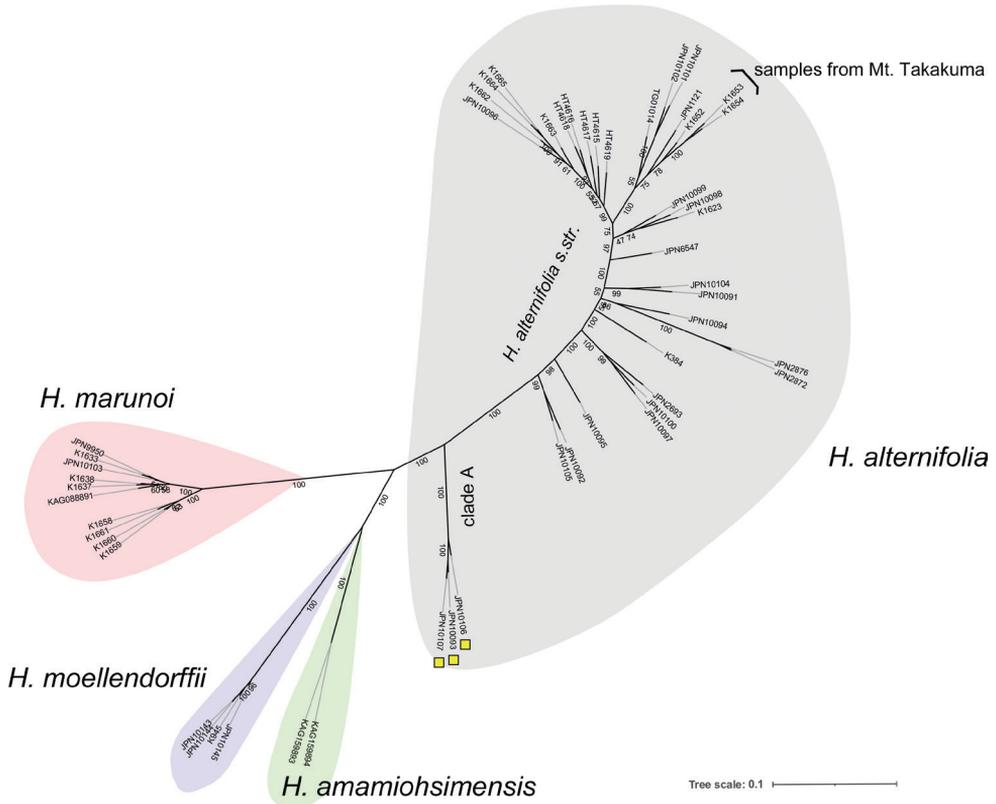


Figure 2. Molecular phylogenetic tree using MIG-seq data of 52 samples of *Hydrangea* sect. *Cardiandra*. Bootstrap values are shown on the internodes.

Three samples of *H. alternifolia* collected from Tottori, Kyoto, and Niigata prefectures (JPN10093, 10106, 10107, respectively, Fig. 1), located on the Sea of Japan (western) side of Honshu Island, were clustered at the base of *H. alternifolia*; this clade, supported by a 100% bootstrap value, was closer to the clade including *H. marunoi*, *H. amamiohsimensis*, and *H. moellendorffii* (Fig. 2, clade A).

Taxonomic treatment

***Hydrangea marunoi* Tagane & S.Fujii, sp. nov.**

urn:lsid:ipni.org:names:77306481-1

Figs 3, 4

Diagnosis. *Hydrangea marunoi* is similar to *H. alternifolia* in having three-petaloid calyx lobes in marginal flowers but is distinguished by its higher number of stamens (28–32 stamens in *H. marunoi* vs. 15–26 stamens in *H. alternifolia*), longer styles (1.2–1.8 mm long in fruiting vs. to 1.2 mm long), and longer seeds (1.2–1.5 mm long vs. 0.7–1 mm long).

Type. JAPAN. Kagoshima Prefecture, Kimotsuki Town, along the Oda River, 8 Aug. 2021, *S. Tagane K1637* (holotype: KAG 153198!; isotype: KYO!).

Description. Rhizomatous perennial herb, 31–103 cm tall. Stems green *in vivo*, grayish-brown when dry, 3.5–6 mm in diameter near the base, puberulous when young, and subsequently glabrous. Leaves alternate, 7–11 per stem, petiolate; blades ovate, ovate-elliptic, elliptic-oblong, obovate-elliptic, (6.5–)10–28.4 × (2.5–)3.3–10.5 cm, chartaceous, sparsely pubescent to subglabrous on both surfaces, grayish-green adaxially, light pale green abaxially, apex acuminate, acumen to 2.5 cm long, base cuneate, decurrent, margin serrate, midrib slightly prominent adaxially, prominent abaxially, secondary veins 8–13 pairs, prominent abaxially, tertiary veins scalariform-reticulate, distinct abaxially; petioles 1–5 cm long, glabrous. Inflorescences terminal, or occasionally terminal and axillary on the upper stem, a corymbose cyme, 6–18 cm in diameter; bract and bracteoles leafy or narrowly lanceolate to linear, persistent. Sterile flowers (functional male flowers) with sepals 3, rarely 2 or 4, white, rarely shallowly tinged with pink, ovate, broadly ovate, suborbicular, 0.4–1.4 × 0.4–1.4 cm, apex obtuse to rounded. Fertile flowers hermaphrodite, pedicellate; pedicels 0.3–1.3 cm long, puberulous. Calyx tube cupular, 1.2–1.8 mm long, puberulous; lobes broadly triangular-ovate, 0.8–1.2 mm long, puberulous, apex rounded, margin ciliolate. Petals white, rarely shallowly tinged with pink, elliptic to suborbicular, 3.7–4.5 mm long. Stamens 28–32, 2.8–5.2 mm long, anthers 0.6–0.8 mm long, filaments 2.2–4.4 mm long, white, glabrous, flattened. Ovary fused with calyx tube, 3-locular, 22–31 ovules per locule. Styles 3, 0.9–1.1 mm long in anthesis, elongate to 1.2–1.8 mm in fruiting. Capsules ellipsoid to subglobose, 2.5–3.8 mm long, 2.2–3.5 mm in diameter. Seeds brown, 1.1–1.5 mm long (including wings); wings translucent and lighter than the seed body color.

Distribution. Japan, Kagoshima Prefecture, Kimotsuki Mountains in the Osumi Peninsula (Fig. 1).

Habitat and ecology. *Hydrangea marunoi* usually grows on semi-shaded wet rocks along streams (Fig. 3A, C), where it grows with *Hymenasplenium murakami-batanakae* Nakaike (Aspleniaceae), *Leptochilus neopothifolius* Nakaike (Polypodiaceae), *Thelypteris esquirolii* (Christ) Ching (Thelypteridaceae), *T. pozoi* (Lag.) C.V.Morton subsp. *mollissima* (Fisch. ex Kunze) C.V.Morton, *Tricyrtis affinis* Makino (Liliaceae), *Ophiorrhiza japonica* Blume (Rubiaceae), and *Pilea hamaoi* Makino (Urticaceae). Only one soil-growing population was identified on the steep slope of the valley near the Mt. Nokubidake summit (897 m elevation) (Fig. 3B). Flowering specimens were collected from August to September, and fruiting specimens were collected from late September to December.

Etymology. The species epithet *marunoi* is named after Mr. Katsutoshi Maruno, a local botanist who made significant contributions, including elucidating the flora of Kagoshima Prefecture and collecting specimens of this species, as cited earlier.

Vernacular name. Kimotsuki kusa-ajisai (suggested here). ‘Kimotsuki’ named after the Kimotsuki Mountains in Osumi Peninsula where the species occur and ‘kusa-ajisai’ is the common Japanese name for the species of *Hydrangea* sect. *Cardiandra*.

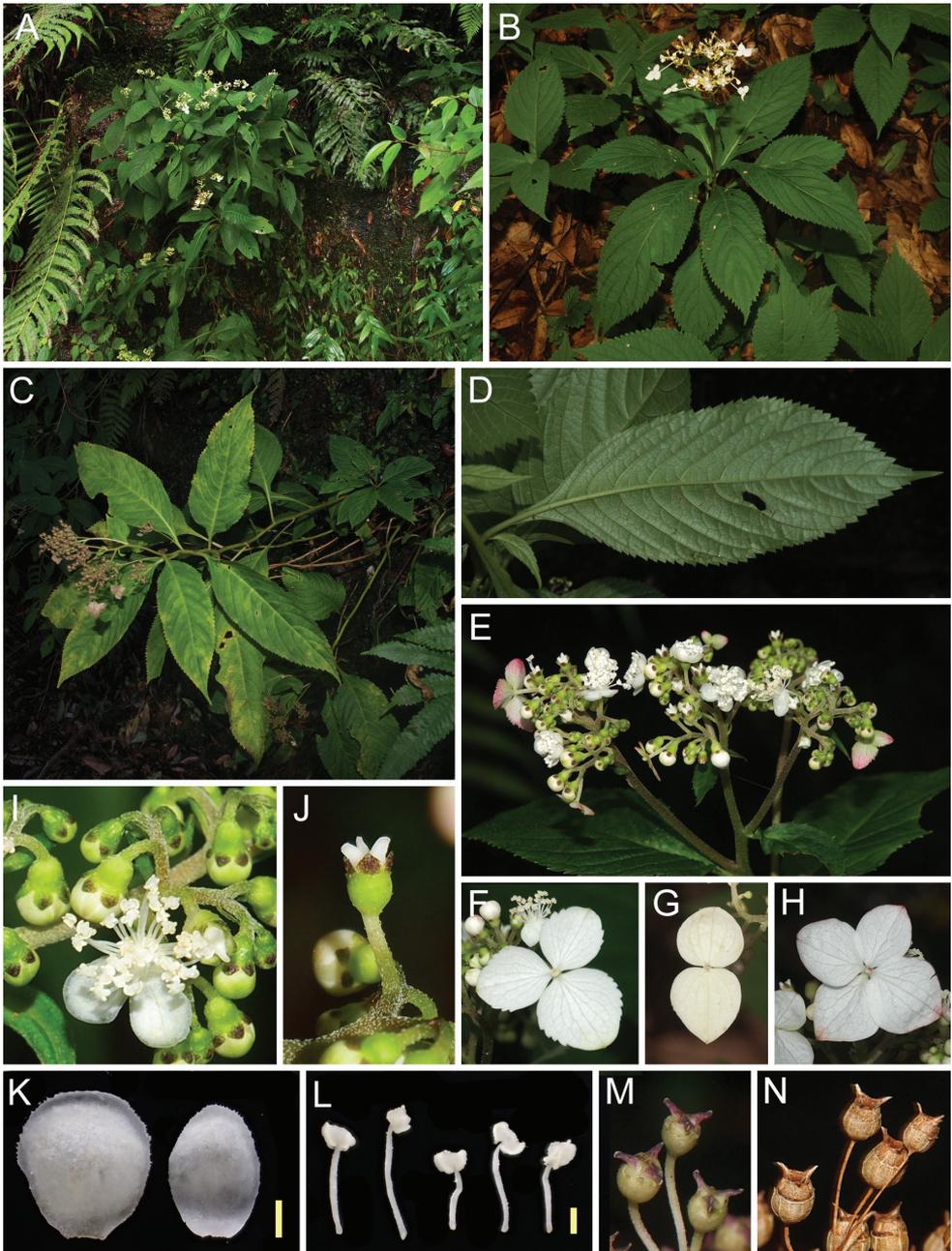


Figure 3. *Hydrangea marunoi* Tagane & S. Fujii, sp. nov. **A–C** habit **D** abaxial lower leaf surface **E** inflorescence **F–H** petaloid calyx lobes in marginal flower **I** flower and flower buds **J** calyx and styles after anthesis (petals and anthers fallen) **K** petals **L** stamens **M** fruits **N** dried fruits. Scale bars: 1 mm (**K**, **L**).

Conservation status. Vulnerable (VU). *Hydrangea marunoi* is known from several populations in Osumi Peninsula (Fig. 1) and the total number of individuals is estimated to be fewer than 1000. Based on the specimen records, the extent of occurrence (EOO) is calculated to be 162 km² by GeoCAT (Bachman et al. 2011) and the area of occupancy (AOO) is 40 km². Some of the habitats are located within the protected area of the Kirishima-Kinkowan National Park and the Inaodake Nature Conservation Area, and the habitat has not been disturbed. Given this situation, it is assessed here as Vulnerable according to the IUCN criterion D (IUCN 2012, 2019).

Notes. The style length is one of the critical characteristics in delimiting the taxa of the Japanese *Hydrangea* sect. *Cardiandra* (Ohba 1985a, b, 2001; Ohashi 2017). Ohashi (2017) described the *Hydrangea alternifolia* style length as 1–1.5 mm (fruiting), whereas Ohba (1985b, 2001) described it as 0.5–1(–1.2) mm. Our examination of the *H. alternifolia* specimens resulted in the style length varied from 0.6–1.2 mm, supporting Ohba's description. One possible explanation is that Ohashi (2017) regarded *H. marunoi* as an infraspecific variation of *H. alternifolia*, and the length of 1–1.5 mm might include the range of *H. marunoi*.

Hydrangea marunoi is endemic to the Kimotsuki Mountains of the Osumi Peninsula, located in the southernmost part of Kyushu Island. Other taxa endemic to this area include *Rhododendron mayebarae* Nakai et H. Hara var. *ohsumiense* T. Yamaz. (Ericaceae; Yamazaki 1984), *R. yakumontanum* (T. Yamaz.) T. Yamaz. var. *katsumarunoanum* Minamitani (Ericaceae; Minamitani et al. 2018), and *Lysimachia ohsumiensis* H. Hara (Primulaceae Hara & Kurosawa, 1959). Further research in this region may reveal new taxa.

Additional specimens examined. JAPAN. Kagoshima Pref. Kimotsuki Town: Kishiragoe, 12 Aug. 1916, fl., *Z. Tashiro s.n.* (TNS 28658); Mankuro, 9 Sept. 2008, fl., *K. Maruno s.n.* (KAG088530); *ibid.*, 10 Sept. 2008, fl., *K. Maruno s.n.* (KAG 088557); Mt. Hoyoshi, 10 Sept. 2008, fl., *K. Maruno s.n.* (KAG 088561); *ibid.*, 27 Oct. 2008, fl., *K. Maruno s.n.* (KAG 088641); Uchinoura, Samuta Forest Road, 6 Sept. 2009, ster. with last year's infr., *K. Maruno s.n.* (KAG 088825); Uchinoura, 3 Aug. 1946, fl., *I. Furusawa s.n.* (TI); along Oda River, 15 Sept. 2009, fl., *K. Maruno s.n.* (KAG 088889, KAG 088890, KAG 088891, KAG 088892, KAG 088893, KAG 088894); *ibid.*, 8 Aug. 2021, fl., *S. Fujii 19274* (KYO, TI, TNS), *19280* (KYO, TI, TNS); *ibid.*, 8 Aug. 2021, fl., *S. Tagane K1633* (KAG 153194), *K1638* (KAG 153199); *ibid.*, 4 Dec. 2021, fr., *S. Tagane & K. Fuse K1828* (KAG 153605). Kinko Town: Mt. Uodake (Mt. Hassan), 15 Sept. 1988, fl., *K. Maruno s.n.* (KAG 156990); *ibid.*, 25 Sept. 1988, young fr., *K. Maruno s.n.* (KAG 082268); Mt. Karekidake, 16 Sept. 2009, fl., *K. Maruno s.n.* (KAG088911); Mt. Aranishi, 5 Nov. 2009, young infl., *K. Maruno s.n.* (KAG 088956); Minamiosumi Town: Satahetsuka, 2 Sept. 2004, fl., *Y. Morita & K. Maruno s.n.* (KAP 00400106s, KAP 00400107s); Mt. Nokubidake, fl. bud, 9 Aug. 2008, *K. Maruno s.n.* (KAG 088497); *ibid.*, fl. bud, 9 Aug. 2008, *T. Ohya s.n.* (KAP 00800421s, KAP00800443s); *ibid.*, 29 Sept. 2021, fl., *S. Tagane K1658* (KYO), *K1659* (TI), *K1660* (KAG 153218) & *K1661* (TNS).



Figure 4. Holotype of *Hydrangea marunoi* Tagane & S. Fujii, sp. nov. (*S. Tagane* K1637 [KAG 153198]).

Discussion

In appearance, *H. marunoi* is more similar to *H. alternifolia*, typically having three-petaloid calyx lobes in marginal flowers, than to *H. moellendorffii*, which has two-petaloid calyx lobes or to *H. amamiohsimensis* without petaloid calyx lobes. However, the MIG-seq tree (Fig. 2) clearly exhibited that *H. marunoi* is more closely related to the clade consisting of *H. amamiohsimensis* endemic to Amami-Oshima, an island located 583 km south of Kyushu Island, and *H. moellendorffii* of Iriomote Island, located 282 km east of Taiwan, than to *H. alternifolia* widely distributed on Honshu, Shikoku, and Kyushu islands (Fig. 2). The three samples K1652–1654 collected from Mt. Takakuma in the northern Osumi Peninsula, just 30 km north and the closest to the collection site of *H. marunoi* among the collection sites of *H. alternifolia* in this study, are genetically divergent from *H. marunoi*. The flowers of individuals from Mt. Takakuma showed 26 stamens and 1.1–1.2 mm long styles, which are typical characters of *H. alternifolia*. The habitat preference also supported this relationship; *H. marunoi* mostly grows on wet rocks by the stream, which is the typical habitat of *H. amamiohsimensis* on Amami-Oshima and *H. moellendorffii* on Iriomote Island, whereas *H. alternifolia* usually grows on the soil in the forest.

The MIG-seq tree also revealed that a clade *H. alternifolia* consisting of three samples JPN10093, 10106 and 10107, collected from the Sea of Japan (western) side of Honshu Island (yellow squares in Fig. 1, designated as clade A) is highly differentiated from the rest of *H. alternifolia* samples (designated as *H. alternifolia s.str.*) (Fig. 2). Further morphological studies based on additional materials are required to characterize this clade.

Key to the species of *Hydrangea* sect. *Cardiandra* (partly based on Ohba 1985b; Wei and Bartholomew 2001; Ohashi 2017)

- 1 Leaves sparsely scattered along stem, alternate..... 2
- Leaves distally on stem, usually 4–8 fascicled [China (Zhejiang), Taiwan].....
..... ***H. densifolia***
- 2 Petaloid calyx lobes in marginal flowers present 3
- Petaloid calyx lobes in marginal flowers absent [Japan (Amami-Oshima Island)] ***H. amamiohsimensis***
- 3 Petaloid calyx lobes in marginal flowers 3 (rarely 2 or 4) [Japan (Honshu, Shikoku, Kyushu)] 4
- Petaloid calyx lobes in marginal flowers 2 (very rarely 3) [China, Japan (Iriomote Island)] ***H. moellendorffii***
- 4 Stamens 15–26; styles to 1.2 mm long in fruiting; seeds 0.7–1 mm long [Japan (Honshu, Shikoku, Kyushu)] ***H. alternifolia***
- Stamens 28–32; styles 1.2–1.8 mm long in fruiting; seeds 1.2–1.5 mm long [Japan (Kyushu: Kimotsuki Mountains)] ***H. marunoi***

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References

- Bachman SP, Moat J, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15): 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- De Smet Y, Granados Mendoza C, Stefan Wanke S, Goetghebeur P, Samain MS (2015) Molecular phylogenetics and new (infra)generic classification to alleviate polyphyly in tribe Hydrangeae (Cornales: Hydrangeaceae). *Taxon* 64(4): 741–753. <https://doi.org/10.12705/644.6>
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* (San Francisco, Calif.) 12: 12–15.
- Fulcher A, Owen JS, LeBude A (2016) *Hydrangea* production: Species-specific production guide. University of Tennessee Extension Publication, PB 1840-B: 1–12.
- Hara H, Kurosawa Y (1959) Notes on Japanese species of *Lysimachia* Sect. *Nummularia*. *Shokubutsu Kenkyu Zasshi* 34: 8–10.
- Hirota SK, Yahara T, Fuse K, Sato H, Tagane S, Fujii S, Minamitani T, Suyama Y (2022) Molecular phylogeny and taxonomy of the *Hydrangea serrata* complex (Hydrangeaceae) in western Japan, including a new subspecies of *H. acuminata* from Yakushima. *PhytoKeys* 188: 49–71. <https://doi.org/10.3897/phytokeys.188.64259>
- IUCN (2012) IUCN Red List Categories and Criteria, Version 3.1. Gland and Cambridge.
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee.
- Minamitani T, Kadota Y, Yonekura K (2018) A classification of the genus *Rhododendron* Sect. *Brachycalyx* (Ericaceae) in Japan (1). *Shokubutsu Kenkyu Zasshi* 93: 75–103.
- Ohashi H (2017) Hydrangeaceae. In: Ohashi H, Kadota Y, Murata J, Yonekura K (Eds) *Wild Flowers of Japan*, revised edition 4. Heibonsha, Tokyo, 157–172. [In Japanese]

- Ohba H (1985a) A systematic revision of the genus *Cardiandra* (Saxifragaceae-Hydrangeoideae) (1). Shokubutsu Kenkyu Zasshi 60: 139–147.
- Ohba H (1985b) A systematic revision of the genus *Cardiandra* (Saxifragaceae-Hydrangeoideae) (2). Shokubutsu Kenkyu Zasshi 60: 161–171.
- Ohba H (2001) *Cardiandra*. In: Iwatsuki K, Boufford DE, Ohba H (Eds) Flora of Japan IIB, Kodansha, Tokyo, 94–95.
- Rochette NC, Rivera-Colón AG, Catchen JM (2019) Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28(21): 4737–4754. <https://doi.org/10.1111/mec.15253>
- Roesti M, Salzburger W, Berner D (2012) Uninformative polymorphisms bias genome scans for signatures of selection. BMC Evolutionary Biology 12(1): e94. <https://doi.org/10.1186/1471-2148-12-94>
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Suyama Y, Matsuki Y (2015) MIG-seq: An effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. Scientific Reports 5(1): e16963. <https://doi.org/10.1038/srep16963>
- Suyama Y, Hirota SK, Matsuo A, Tsunamoto Y, Mitsuyuki C, Shimura A, Okano K (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. Ecological Research 37(1): 171–181. <https://doi.org/10.1111/1440-1703.12270>
- Wei ZF, Bartholomew B (2001) *Cardiandra*. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China 8. Beijing, Science Press and Missouri Botanical Garden Press, St. Louis, 406–407. <http://www.efloras.org>
- Yamazaki T (1984) Some new taxa of *Rhododendron* sect. *Braohycalyx* in Kyushu and Shikoku, Japan. Shokubutsu Kenkyu Zasshi 59: 204–210.

Supplementary material I

Table S1

Authors: Shuichiro Tagane, Shinji Fujii, Shun K. Hirota, Akiyo Naiki, Tetsukazu Yahara

Data type: sample list

Explanation note: Sample list used for phylogenetic analysis in this study.

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

Link: <https://doi.org/10.3897/phytokeys.211.89452.suppl1>

***Akhania*, a new genus for *Salsola daghestanica*, *Caroxylon canescens* and *C. carpathum* (Salsoloideae, Chenopodiaceae, Amaranthaceae)**

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Abstract

Genus *Salsola* s.l. was recently split into several genera of different phylogenetic placements within Salsoloideae, but both taxonomic and phylogenetic relationships of some parts of the former broadly defined *Salsola* still need to be clarified. A remarkable example is *Salsola canescens* nom. illegit. ≡ *Salsola boissieri*, a taxon with tricky taxonomic history that was only recently transferred to the genus *Caroxylon* (tribe Caroxyleae). *Salsola daghestanica*, a narrow endemic of Central Dagestan (Russian Federation), was not even included in previous molecular studies of Salsoloideae and therefore still lacks an appropriate estimation of its relationships. Molecular phylogeny constructed here using nuclear and plastid DNA sequence data clearly placed *Salsola daghestanica* and *Caroxylon carpathum* as sister taxa and the clade *S. daghestanica*, *Caroxylon canescens* (*Salsola boissieri*), *C. carpathum* (*Salsola carpatha*) as a sister of the monophyletic *Caroxylon*. All three species are distinct from *Caroxylon* from a morphological standpoint. In conclusion, a new genus, *Akhania*, was established for these taxa. The detailed distribution of *Akhania daghestanica* is presented for the first time.

Keywords

Akhania, Amaranthaceae, Chenopodiaceae, molecular phylogeny, new genus

Introduction

If circumscribed broadly, genus *Salsola* L. encompasses a large number of species, mostly distributed in the steppes, deserts and mountains of Eurasia, northern and southern Africa (e.g., Freitag and Rilke 1997). Comprehensive molecular phylogeny of subfamily Salsoloideae (Akhani et al. 2007) clearly revealed that *Salsola* is widely polyphyletic, and thus the broad circumscription of the genus is of purely historic interest. The members of *Salsola* s.l. must be transferred to numerous reinstated or newly established genera (Akhani et al. 2007, 2016; Rudov et al. 2020). The current system of Salsoloideae (Akhani et al. 2007) is in good congruence with both morphological and biochemical data (Akhani et al. 2007), and today it appears as widely accepted (e.g., Wen et al. 2010; Feodorova and Samigullin 2014; Sukhorukov 2014; Hernández-Ledesma et al. 2015; Sukhorukov et al. 2016, 2019; Mucina 2017, among others).

However, both taxonomic and phylogenetic relationships of some parts of the former broadly defined *Salsola* still need to be clarified. A remarkable example is *Salsola canescens* (Moq.) Boiss. [nom. illegit., non Pers.], a Western and Central Asian taxon that was recently transferred to the genus *Caroxylon* Moq. (tribe Caroxyleae) (Akhani et al. 2007) but previously was included in *Salsola* sect. *Belanthera* Iljin under the name *S. boissieri* Botsch. (Bochantsev 1968). The latter binomial was substituted by the names *Caroxylon canescens* (Moq.) Akhani (Akhani et al. 2007), *C. boissieri* (Botsch.) Freitag nom. superfl. (Breckle et al. 2013) and *Climacoptera canescens* (Moq.) G.L.Chu (Zhu and Sanderson 2017). However, Sukhorukov et al. (2016) have pointed out that *Caroxylon canescens* is morphologically different from all other members of *Caroxylon* Thunb. as well as of *Climacoptera* Botsch., and thus, its circumscription with either of these two genera is problematic. So, despite the phylogenetic results of Akhani et al. (2007), the taxonomic placement of *S. boissieri* (former *S. canescens*) remains ambiguous. This ambiguity also applies to *Caroxylon carpathum* (P.H.Davis) Akhani & Roalson (*Salsola carpatha* P.H.Davis), a narrow endemic of the Greek Islands that are situated in the Aegean Sea. This species is morphologically very close to *C. canescens*, which was already mentioned by Davis (1953), who considered both taxa within broadly defined *Salsola* (Davis 1953).

Whereas *Salsola canescens* and *S. carpatha* are eventually considered to be a part of *Caroxylon* (Akhani et al. 2007), *Salsola daghestanica* (Turcz.) Turcz., a remarkable narrow endemic of Central Dagestan (Eastern Caucasus, Russian Federation), was not even included in the previous molecular studies of Salsoloideae Raf. and therefore still lacks an appropriate estimation of its relationships. This mysterious species grows in the foothills and mountains at elevations of up to 1,200 m a.s.l. It is morphologically well-recognizable due to the striking unique combination of bushy habit, long linear leaves that are not gibbous at base, bracts that are longer than flowers, small wing-like perianth appendages, and anther thecae divided almost to the top. Because of the latter character, *S. daghestanica* was also included in *Salsola* sect. *Belanthera* (e.g., Iljin 1936; Bochantsev 1980) and currently this species is preliminarily considered within *Salsola* (Sukhorukov and Akopian 2013). However, this species differs from other *Salsola* in having shrubby vs. annual (rarely subshrubby in *Salsola griffithii* (Bunge) Freitag and Khani only) life history, soft simple hairs vs. papillae, obtuse vs. mucronate leaf tips,

and large vs. inconspicuous anther appendages. Based on the evidence from the external morphology, *Salsola daghestanica* must undoubtedly be placed within the tribe *Caroxyleae*, not *Salsoleae* (Akhani et al. 2007).

Salsola daghestanica is not mentioned in the study of Tzvelev (1993) who considered sect. *Belanthera* as a part of the restored genus *Caroxylon* even before the widespread use of molecular methods. Elenevsky (1966) proposed that there is a close relationship between *S. daghestanica* and *S. canescens*, but this proposition still needs to be confirmed and clarified.

Due to the pending taxonomic positions of *Caroxylon canescens*, *C. carpathum* and *Salsola daghestanica*, the estimation of the correct phylogenetic and taxonomic relationships of these three taxa within the frameworks of molecular phylogenetics, conventional comparative morphology, and biogeography is the main goal of our study.

Materials and methods

Taxon sampling

Specimens of *Salsola daghestanica* were studied in eight herbaria (LE, LECB, MW, MHA, MOSP, MSK, MSKU, RV, RWBG, and WIR). The distribution map (Fig. 3) is based on the original summary of all analyzed herbarium specimens (Appendix 1), as well as on additional data from the GBIF database (GBIF Secretariat 2021), and the studies of Grossheim (1945), Murtazaliev (2009, 2016), and Magomedova et al. (2016). This map was prepared using SimpleMappr online tool (Shorthouse 2010).

DNA extraction, amplification and sequencing

The total DNA was extracted from the herbarium leaf tissues using the DNeasy Plant Pro Kit (Qiagen, Germany, <https://www.qiagen.com>) according to the manufacturer's protocol. Following Akhani et al. (2007), we used two molecular markers for the molecular phylogenetic analysis: nuclear ribosomal internal transcribed spacer (ITS) and plastid intergenic spacer (IGS) *psbB-psbH*. For the amplification of the ITS region we utilized primers NNC-18S10 and C26A (Wen and Zimmer 1996). The *psbB-psbH* IGS was amplified utilizing primer *psbB-psbH-f* and *psbB-psbH-r* (Xu et al. 2000). The PCR cocktail (20 μ L) contained 1.5–2 ng of the total DNA, 5 pmol of each primer, 4 μ L of Ready-to-Use PCR Master mix 5 \times MasDDTaqMIX-2025 containing a “hot-start” SmarTaq DNA polymerase (Dialat Ltd., Moscow, Russia, <http://en.dialat.ru>), and 13 μ L of deionized water. PCR reaction was performed using a MJ Research PTC220 DNA Engine Dyad Thermal Cycler (BioRad, Foster City, CA, United States, <https://www.bio-rad.com>). For the ITS loci, the PCR profile included the initial DNA denaturation at 94 °C for 3 min and 34 reaction cycles of DNA denaturation at 94 °C for 20 s, annealing primers at 50 °C for 30 s, and the extension of the new strands of the DNA at 72 °C for 40 s, with the final 8 min of the extension at 72 °C. For *psbB-psbH* IGS, the PCR profile included the initial DNA denaturation at 94 °C for 3 min

and 33 reaction cycles of DNA denaturation at 94 °C for 30 s, annealing primers at 53 °C for 30 s, and the extension of the new strands of the DNA at 72 °C for 90 s with the final 8 min of the extension at 72 °C. The PCR products were purified by precipitation in 0.125 M ammonium acetate solution in 70% ethanol (Daniels 2003) and visualized on the 1% agarose gel in 0.5× TBE buffer containing ethidium bromide. All PCR products were sequenced on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA, <https://www.thermofisher.com>) at the LLC Syntol, Moscow, Russia (<https://www.syntol.ru>) using the same primers that were used to amplify both loci.

All sequences were deposited in the GenBank database; the accession numbers of the newly obtained sequences are presented in the Table 1.

Molecular alignment and phylogenetic analysis

The core dataset was reconstructed utilizing the Genbank numbers first published by Akhani et al. (2007); the new sequences of ITS and *psbB-psbH* IGS loci of *Salsola daghestanica* and *Caroxylon canescens* (Table 1) were added to the analyses. In total, 141 ITS and 118 *psbB-psbH* IGS sequences were analyzed in the present study. The concatenated alignment contained 117 nucleotide sequences.

The ITS and *psbB-psbH* IGS sequences were first aligned separately using MAFFT v. 7 with the strategy L-INS-I (Katoh et al. 2002; Katoh and Standley 2013), manually corrected and concatenated with BioEdit v. 7.0 (Hall 1999). The alignments of the ITS and plastid sequence data were analyzed individually, as well as the combined Supermatrix.

The Maximum Likelihood (ML) analyses of all molecular alignments (Felsenstein 1981) were conducted with RAxML v. 8.2.10 using raxmlGUI beta version 2.0 (Stamatakis 2014; Kozlov et al. 2019; Edler et al. 2021) under the assumptions of GTR + GAMMA model (Stamatakis 2014). ML bootstrap values (Sauermaun 1989) were based on 1000 fast replicates (Stamatakis 2014). We visualized the resulting trees in FigTree v.1.4.3 (Rambaut and Drummond 2012) and finally prepared all the figures using the InkScape v.0.48.2 (<https://inkscape.org/release/inkscape-0.48/>).

Table 1. Collection data, collectors, the numbers of isolates and the GenBank accession numbers of newly analyzed samples of *Salsola daghestanica* and *Caroxylon canescens*.

Species, isolate	Specimens	GenBank	GenBank acces-
		accession numbers: ITS	sion numbers: <i>psbB-psbH</i> IGS
<i>Caroxylon canescens</i> , isolate CARC1	Israel, Mt. Hermon, September 2017, O. Fragman-Sapir <i>s.n.</i> (MW)	OP364980	OP493534
<i>Salsola daghestanica</i> , isolate SD1	Dagestan, Laksky distr., nr Kamasha vill., 8 Aug 1953; Magomedov <i>s.n.</i> (MHA0233906)	ON502720	ON512444
<i>S. daghestanica</i> , isolate SD2	Dagestan, Laksky distr., 18 Oct 1953; Magomedov <i>s.n.</i> (MHA0233907)	ON502721	ON512445
<i>S. daghestanica</i> , isolate SD3	Dagestan, Laksky distr., nr Kamasha vill., 18 Aug 1953; Magomedov <i>s.n.</i> (MHA0233905)	ON502722	ON512446

As in Akhani et al. (2007), the ITS ML tree was rooted relative to *Bienertia sinuspersici* Akhani (DQ499349), *Suaeda maritima* (L.) Dumort. (EF453508), *Suaeda cucullata* Aellen (EF453509) [all – Suaedoideae], *Kalidium caspicum* (L.) Ung.-Sternb. (EF453444), *Microcnemum coralloides* (Loscos & J.Pardo) Font Quer (EF453448), and *Salicornia persica* Akhani (EF453460) [all – Salicornioideae]. To root the ML trees that were based on (a) *psbB-psbH* IGS and (b) concatenated alignments we used *Bienertia sinuspersici*, *Microcnemum coralloides*, and *Suaeda maritima* (Akhani et al. 2007).

Results

Molecular phylogeny

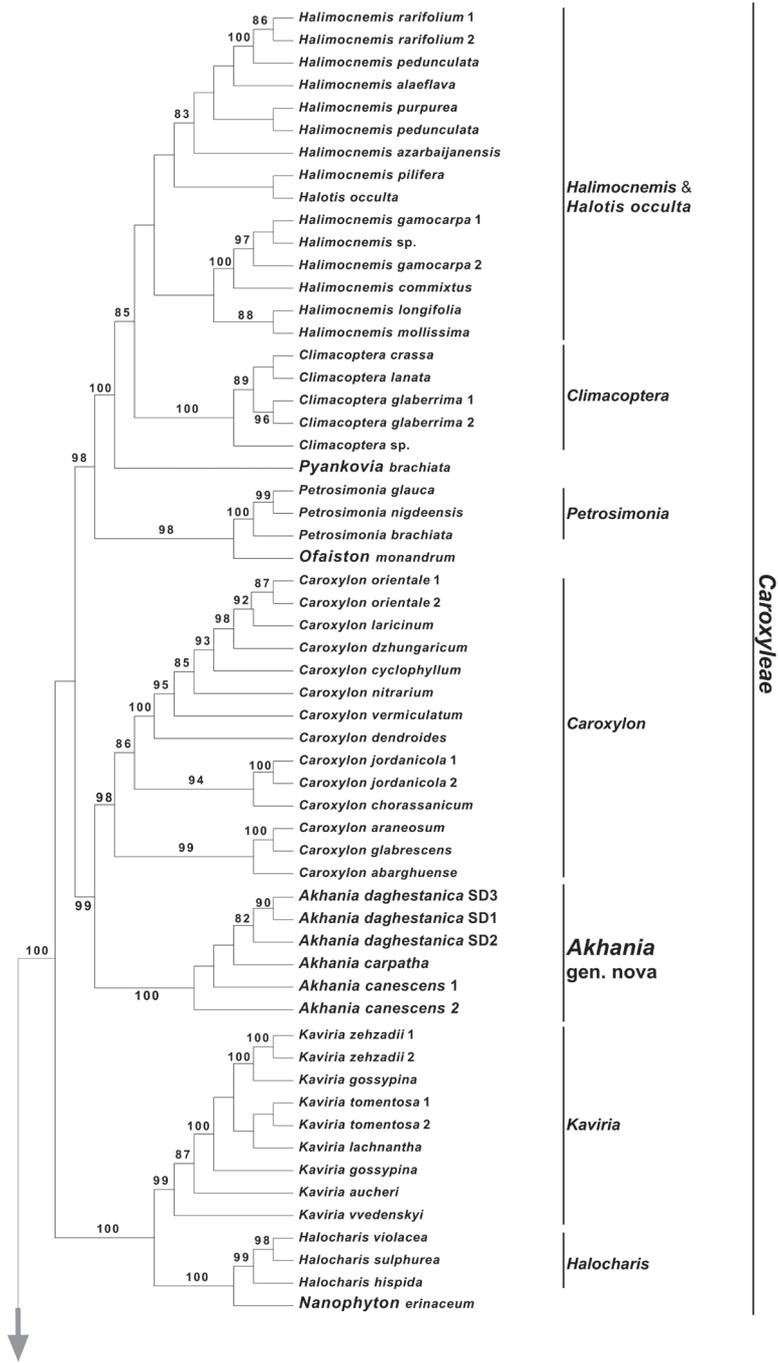
The total number of characters in the final ITS alignment was 781, consisting of 379 invariant characters (proportion = 0.485) and 484 variable characters. The total number of characters in the final *psbB-psbH* IGS (plastid) alignment was 751, consisting of 159 invariant characters (proportion = 0.211) and 281 variable characters. The total number of characters in the final concatenated alignment was 1,532, consisting of 517 invariant characters (proportion = 0.337) and 741 variable characters.

The ML analysis of ITS dataset resulted in a tree with $- \ln L = 15718.422393$ (Suppl. material 1: Fig. S1). The ML analysis of *psbB-psbH* IGS dataset resulted in a tree with $- \ln L = 5091.478154$ (Suppl. material 2: Fig. S2). The shapes of the obtained trees were softly incongruent, clearly showing the lack of significant character conflict between nuclear and plastid sequence data. The ML analysis of combined dataset resulted a tree with $- \ln L = 19360.032213$ (Fig. 1).

The shape of the ML tree that resulted from the analysis of the concatenated matrix is identical to the tree in Akhani et al. (2007), as was expected. Because of this, we limit the description of the results only to the key findings that are related to the major goal of this study: our analysis clearly revealed that *Salsola daghestanica*, *Caroxylon canescens* and *Caroxylon carpathum* are sister taxa forming a clade 'Akhania', which appeared to be a highly supported sister (ML BS = 100% and 99%) of the monophyletic genus *Caroxylon* (Fig. 1).

Discussion

Salsola sect. *Belanthera* s.l. included species with the anther's thecae divided up to their top (Iljin 1936). This character unites the species from the reinstated genus *Caroxylon* Moq. (Tzvelev 1993; Akhani et al. 2007) and a newly established genus *Kaviria* Akhani & E.H.Roalson (Akhani et al. 2007). Bochantsev (1968) selected *Salsola tomentosa* (Moq.) Spach (now *Kaviria tomentosa* (Moq.) Akhani) as a lectotype of this section and excluded from its circumscription all members of the current genus



Camphorosmeae, Salsoleae, and Outgroups

Figure 1. The best tree (–InL= 19360.032213) recovered from the ML analysis (RAxML with GTR + GAMMA) of the ITS plus *psbB-psbH* IGS Supermatrix of Caroxyleae, Salsoleae, Camphorosmeae and outgroups. Numbers above branches indicate ML Bootstrap support values that are equal to or more than 80%. Image: Evgeny V. Mavrodiev.

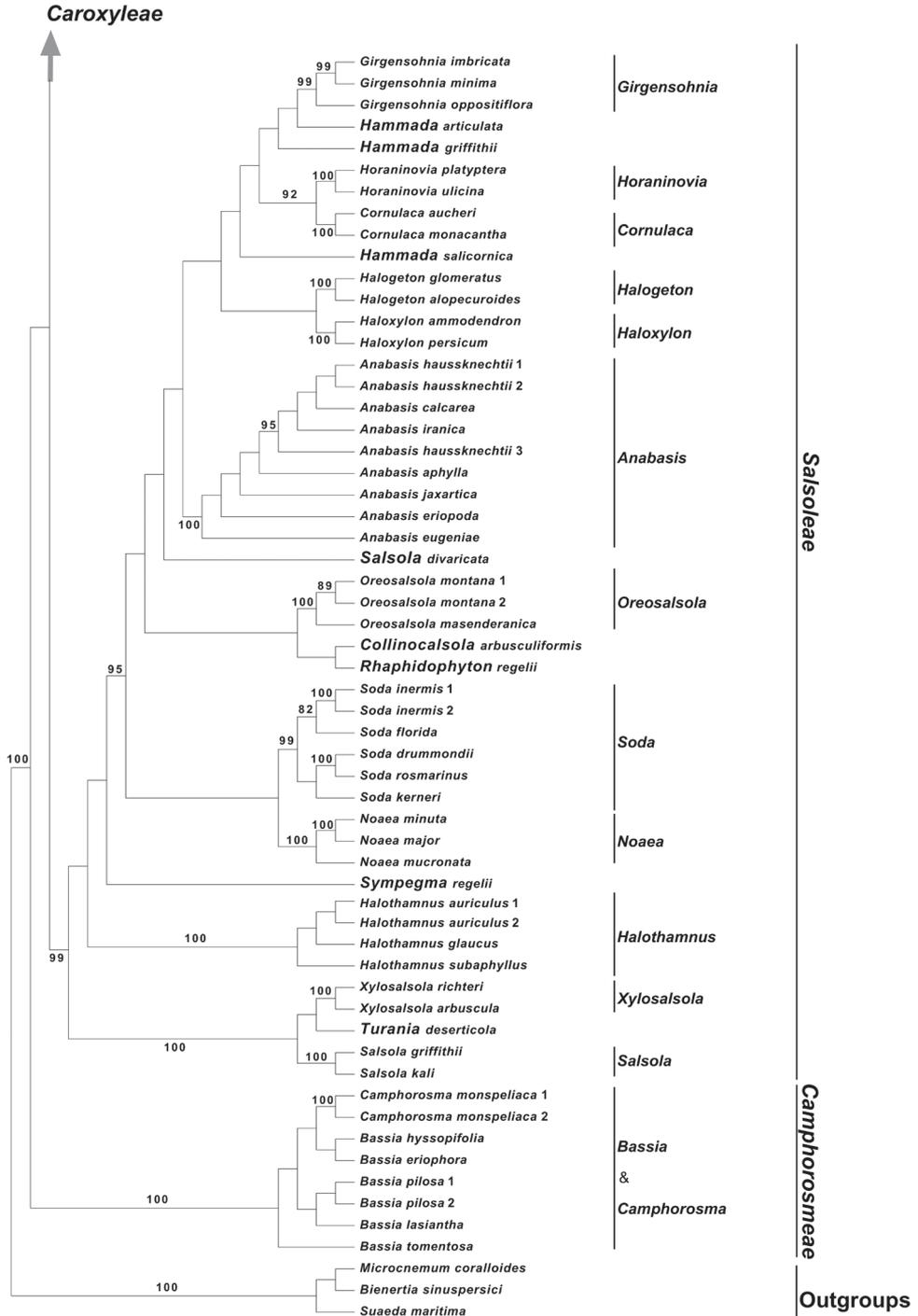


Figure 1. Continued.

Caroxylon (e.g., species with gibbous leaf base). In his later study, Bochantsev (1980) clearly states that sect. *Belanthera* encompassed the species with non-gibbous leaf base, divided thecae and noticeable anther's vesicles — the diagnostic characters of the current genus *Kaviria*. However, species that have been described by Bochantsev (1941, 1980) from the relationship of the variable *Salsola boissieri* (e.g., *S. titovii* Botsch., *S. podlechii* Botsch.) were not accepted in subsequent accounts (Freitag and Rilke 1997; Breckle et al. 2013) and because of this were not included in recent molecular phylogenetic analyses. Similarly, phylogenetic relationships of some taxa that were proposed to be closely related to *Caroxylon canescens*, namely of *S. canescens* subsp. *serpenticola* Freitag & E.Özhatay (Freitag and Özhatay 1997), and *Salsola turcica* Yild., remain unknown. Because of this, the actual taxonomic composition of the clades *Caroxylon* and *Kaviria*, as well as other related groups, still requires further investigation.

An example of these kinds of ambiguous generic placements are two members of a newly described *Akhania* Sukhor. (below), namely *Caroxylon carpathum* and *C. canescens*. Both of these taxa were previously considered under *Salsola* (as *Salsola carpatha* and *S. canescens* \equiv *S. boissieri*).

Also, as we have already mentioned above, *Caroxylon canescens* was recently transferred to *Climacoptera* (as *C. canescens* (Moq.) G.L.Chu). The latter *ad hoc* combination, however, was created without any explanation (Zhu and Sanderson 2017), but all species of *Climacoptera* in its recent circumscription are annuals and also have a decurrent leaf base. This combination of characters is absent in all members of *Akhania*, incl. *A. canescens*. Also, from the molecular phylogenetic standpoint, *Caroxylon canescens* (*Salsola boissieri*) is not closely related to *Climacoptera* (Fig. 1).

Despite the fact that, based on the evidence from its external morphology, both *Salsola boissieri* and *Salsola carpatha* can be included in the genus *Caroxylon* (Akhani et al. 2007), and *S. daghestanica* resembles the species of *Kaviria*, the unique combination of morphological characters (Table 2) strongly supports the separate placement of all three taxa from either *Kaviria* and *Caroxylon*. A new genus, *Akhania* Sukhor. is established in this study for these three members of the same-name clade. On the molecular tree, the latter is a sister of monophyletic *Caroxylon* (Fig. 1, Suppl. materials 1, 2: Figs S1, S2),

Table 2. Morphological differences between *Akhania* and *Caroxylon*.

Character / Taxon	<i>Akhania</i>	<i>Caroxylon</i> spp.
Hairs	not denticulate	denticulate
Leaf base	neither gibbous nor broadened	\pm gibbous and broadened
Leaf shape and size	linear, lanceolate or broadly lanceolate, flattened, up to 35 mm long	subulate, not flattened, up to 20 mm or scale-like
Bracts	large, not gibbous, orbicular at base, abruptly (in <i>A. daghestanica</i>) diminishing above the base	small, gibbous, orbicular but not abruptly diminishing above the base
Anther appendages	conspicuous	unnoticeable or small
Wing-like appendages on the perianth segments	below the middle of the segments	in the middle part of the segments or absent

Taxonomic conclusions

Akhania Sukhor., gen. nov. (Caroxyleae, Salsoloideae).

urn:lsid:ipni.org:names:77306620-1

Type species. *Akhania daghestanica* (Bunge) Sukhor. (Fig. 2).

Description. Subshrubs or small shrubs 20–100 cm tall, with several or numerous stems forming \pm bushy habit, glabrous or covered with papillae and tiny caducous simple and smooth (not denticulate) hairs; leaves linear to broadly lanceolate, 5–35 \times 1.0–3.0 mm, bright green, glaucous or greyish, covered with appressed simple (partially caducous) hairs, basally not gibbous and not broadened; bracts leaf-like, usually exceeding flowers or equaling, basally orbicular, abruptly (*C. daghestanica*) or continuously (*C. canescens*, *C. carpatha*) diminishing above the base; flowers with two bracteoles smaller than bract; perianth segments 5, glabrous or pubescent, apically obtuse, at fruiting each segment bears wings originated below the middle of each segment; anthers 5, 1.3–3.0 mm long, thecae divided almost to the top, apically with a large (0.8–2.0 mm long) vesicle that is not clearly separated from the thecae; styles shorter than the stigma; seeds with horizontal or vertical embryo position.

Etymology. The new genus is named after Iranian botanist Hossein Akhani.

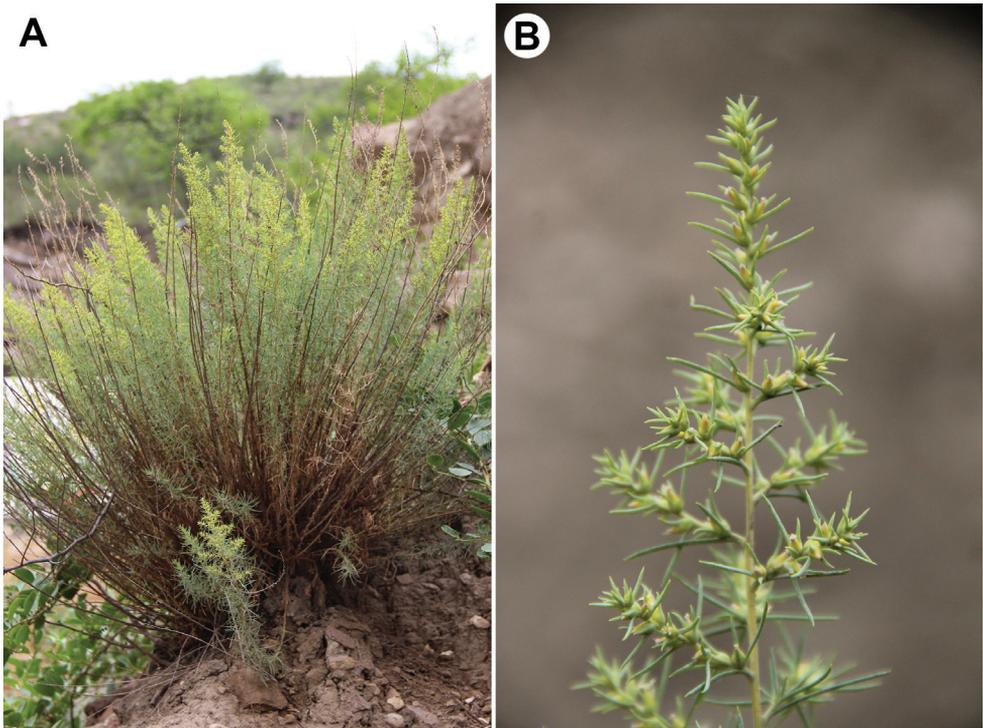


Figure 2. *Akhania daghestanica* **A** adult plant **B** the upper part of the inflorescence. Photographs by D.S. Shilnikov (Russia, Dagestan, Shamilsky distr., Hebda vill., 14 Jul 2020).

The genus consists of three species. The distribution areas of *A. daghestanica* (Dagestan) (Fig. 3), *A. canescens* (Turkey, Iraq, Iran, and Afghanistan) and *A. carpatha* (Aegean Islands) are remarkably disjunctive.

Akhania differs from the related *Caroxylon* by several remarkable characters or their combinations (Table 2, Fig. 4).

1. *Akhania daghestanica* (Bunge) Sukhor., comb. nov.

urn:lsid:ipni.org:names:77306622-1

≡ *Noaea daghestanica* Bunge, Anabas. Rev. [Mèm. Acad. Sci. Pétersb., sér. 7, 4(11)]: 26 (1862).

≡ *Salsola daghestanica* (Bunge) Turcz. ex Trautv., Increm. Fl. Phaenog. Ross. 649. 1883; Trautv., Trudy Imp. S.-Peterburgsk. Bot. Sada 9(1): 133 (1884).

≡ *Salsola daghestanica* (Bunge) Czern. ex Lipsky, Trudy Imp. S.-Peterburgsk. Bot. Sada 14(2): 295 (1897) isonym.

Holotype. Caucasus orientalis, provincia Daghestan, fl., Patritzky s.n. (KW, isotype – LE!).

Distribution. A local endemic to Dagestan Republic, Russia (Fig. 3).

Habitat. The species is found in open undisturbed habitats, primarily on grassy hills and mountain slopes and screes, at altitudes 500–1200 m a.s.l. It prefers lightly saline and gypsum-enriched soils.

Phenology. Flowering – June–July, fruiting – October.

Conservation status. *Akhania daghestanica* has an estimated extent of occurrence of 8,403 km² (which would place the species in the Vulnerable (VU) category according to IUCN (2021)), and an area of occupancy of 112 km² (which

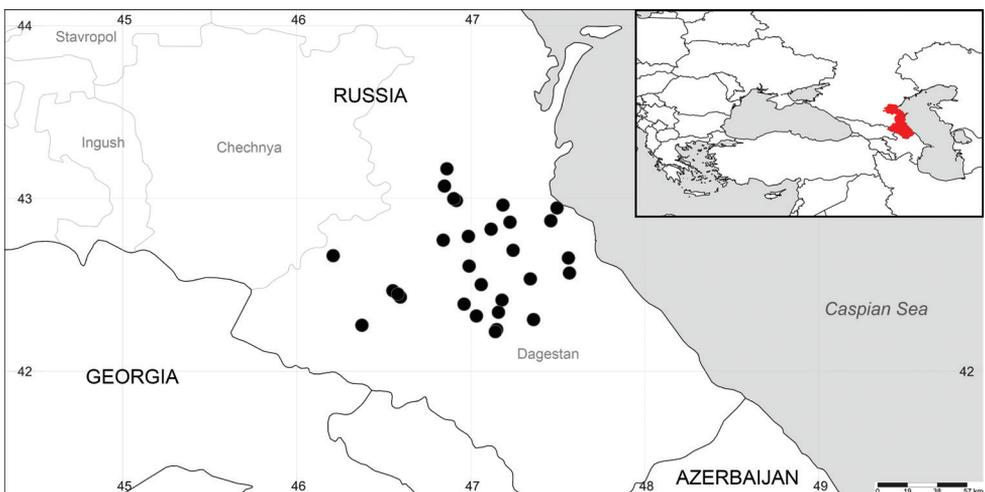


Figure 3. The distribution area of *Akhania daghestanica*. The insert map shows the location of Dagestan Republic (colored in red).

would place it in Endangered, EN). Approximately half of the records are dated later than the 1980s (Murtazaliev 2009, 2016; Magomedova et al. 2016; see also Fig. 4, Appendix 1), and the species is likely undercollected, as many Chenopodiaceae are. Therefore, there is no direct evidence of declining population size and fluctuations. However, since the species is found only in natural habitats, it can be threatened by cattle grazing and agriculture. Due to its restricted distribution and possible habitat loss in the future, the species qualifies to be assigned preliminary conservation status of Vulnerable (VU), based on criterion B1 b(iii) of the IUCN Red List categories and criteria (IUCN 2021).

2. *Akbania canescens* (Moq.) Sukhor., comb. nov.

urn:lsid:ipni.org:names:77306623-1

- ≡ *Noaea canescens* Moq. in DC., Prodr. 13(2): 208 (1849).
- ≡ *Caroxylon canescens* (Moq.) Akhani & Roalson, Int. J. Pl. Sci. 168(6): 947 (2007).
- ≡ *Salsola canescens* (Moq.) Boiss. Fl. Orient. [Boissier] 4: 963 (1879), nom. illegit., non Pers. (1805).
- ≡ *Salsola boissieri* Botsch., Bot. Zhurn. 53: 1442 (1968).
- ≡ *Caroxylon boissieri* (Botsch.) Freitag, Vasc. Pl. Afghanistan: 264 (2013), nom. superfl.
- ≡ *Climacoptera canescens* (Moq.) G.L.Chu in Chu & Sanderson, Gen. New Evol. System World Chenopod.: 312 (2017).

Lectotype (Bochantsev 1968): [Iraq] In cacumine m. Gara Kurdist. orientem versus frequens, 1843, *Th. Kotschy* 346 (LE! isoelectotypes G! H, JE, K000899548! P, W0046462!).

Distribution. This species is distributed across Irano-Turanian Region (Freitag and Rilke 1997 as *Salsola canescens*).

Habitat. The slopes of hills, frequently screes.

Phenology. Flowering – July–August, fruiting – October–November.

Conservation status. The taxonomic composition of this species is still insufficiently studied. Therefore, its current conservation status cannot be properly evaluated.

3. *Akbania carpatha* (P.H.Davis) Sukhor., comb. nov.

urn:lsid:ipni.org:names:77306624-1

- ≡ *Salsola carpatha* P.H.Davis, Notes Roy. Bot. Gard. Edinburgh 21: 139 (1953).
- ≡ *Caroxylon carpathum* (P.H.Davis) Akhani & Roalson, Int. J. Pl. Sci. 168(6): 947 (2007).

Holotype. GREECE, Karpathos [Island], Vurgunda (NW of Olymbos), at 5–20 m alt., on calcareous sea rocks with *Galium canum*, 24 Jul 1950, P.H. Davis 18025 (sheet I – K000899552! Sheet II – K000899553! isotype – E00279875!).

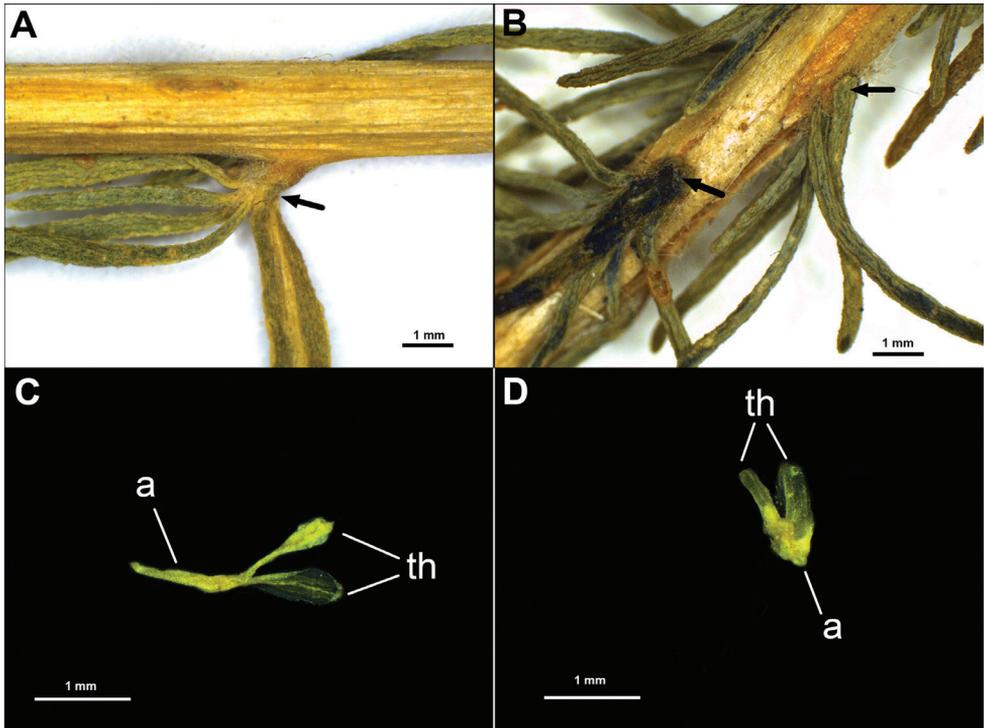


Figure 4. Morphological differences between *Akhania* and *Caroxylon* **A** non-gibbous leaf base in *Akhania daghestanica* **B** gibbous leaf base in *Caroxylon laricinum* **C** anther of *Akhania daghestanica* with a large appendage (vesicle) **D** anther of *Caroxylon laricinum* with a small appendage. Abbreviations: a – anther appendage, th – thecae. Scale bar: 1 mm. Black arrows indicate the leaf base.

Distribution. This species is localized in three Greek islands situated in the Aegean Sea: Crete, Karpathos, Kyklides and adjacent islets (Davis 1953; Christodoulakis et al. 1990; Strid and Tan 1997).

Habitat. Rocks, usually calcareous.

Phenology. Flowering – July–August, fruiting – October–November.

Conservation status. Not evaluated yet, but likely Vulnerable VU (IUCN 2021).

Key to the species

- 1 Shrub up to 1 m high; bracts abruptly diminishing from the orbicular base, leaves linear, perianth glabrous, wings at fruiting white or yellowish. Endemic of E Caucasus (Dagestan, Russian Federation) *A. daghestanica*
- Subshrubs up to 50 cm; bracts continuously diminishing from the orbicular base, leaves ± flattened, perianth pubescent, wings reddish, but turning into brown at dissemination. Species distributed outside of E Caucasus 2

- 2 Leaves linear to lanceolate. Irano-Turanian Region (Turkey, Iraq, Iran, and Afghanistan) *A. canescens*
- Leaves broadly lanceolate or narrowly oblong. Islands of the Aegean Sea.....
..... *A. carpatha*

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References

- Akhani H, Edwards G, Roalson EH (2007) Diversification of the Old World Salsoleae s.l. (Chenopodiaceae): Molecular phylogenetic analysis of nuclear and chloroplast data sets and a revised classification. *International Journal of Plant Sciences* 168(6): 931–956. <https://doi.org/10.1086/518263>
- Akhani H, Khoshravesh R, Malekmohammadi R (2016) Taxonomic novelties from Irano-Turanian region and NE Iran: *Oreosalsola*, a new segregate from *Salsola* s.l., two new species in *Anabasis* and *Salvia*, and two new combinations in *Caroxylon* and *Seseli*. *Phytotaxa* 249(1): 159–180. <https://doi.org/10.11646/phytotaxa.249.1.7>
- Bochantsev [Botschantzev] VP (1968) Obzor vidov sektiï *Belanthera* Iljin roda *Salsola* L. [Overview of the section *Belanthera* Iljin of the genus *Salsola* L.]. *Botanichesky Zhurnal* 53(10): 1440–1450. [In Russian]
- Bochantsev [Botschantzev] VP (1980) Vidy sektiï *Belanthera* Iljin roda *Salsola* L. [Species of the section *Belanthera* Iljin of the genus *Salsola* L.]. *Novosti Sistematiki Vysshikh Rasteniy* 17: 112–135. [In Russian]
- Bochantsev [Botschantzev] VP, Vvedensky AI (1941) Novye vidy rasteniy [New plant species]. *Botanicheskie Materialy Gerbariya Botanicheskogo Instituta Uzbekistanskogo Filiala AN SSSR* 3: 3–20.
- Breckle SW, Hedge IA, Rafiqpoor MD (2013) Vascular plants of Afghanistan. An augmented checklist. *Scientia Bonnensis*, Bonn, 598 pp.
- Christodoulakis D, Georgiadis Th, Economidou E, Iatrou D, Tzanoudakis D (1990) Flora und Vegetation Dionysaden-Inseln (Südägäis, Griechenland). *Willdenowia* 19(2): 425–443. <https://www.jstor.org/stable/3996651>

- Daniels SE (2003) Preparation and direct automated cycle sequencing of PCR products. In: Bartlett JMS, Stirling D (Eds) PCR Protocols. Methods in Molecular Biology, Vol. 226. Humana Press, Totowa, 341–346. <https://doi.org/10.1385/1-59259-384-4:341>
- Davis PH (1953) Notes on the summer flora of the Aegean. Notes from the Royal Botanic Garden Edinburgh 21: 101–142.
- Edler D, Klein J, Antonelli A, Silvestro D (2021) RaxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12(2): 373–377. <https://doi.org/10.1111/2041-210X.13512>
- Elenevsky AG (1966) On some floristic peculiarities of the inland Dagestan. Bulletin of the Moscow Society of Naturalists, sect. biol. 71(5): 107–117. [In Russian]
- Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17(6): 368–376. <https://doi.org/10.1007/BF01734359>
- Fedorova TA, Samigullin TH (2014) The taxonomic position of subsection *Vermiculatae* Botsch. of section *Caroxylon* (Thunb.) Fenzl, genus *Salsola* L. (Chenopodiaceae Juss.). Voprosy Soveremennoy Algologii 3: 1–36. [In Russian]
- Freitag H, Özhatay E (1997) A new subspecies of *Salsola canescens* (Chenopodiaceae) from SW Anatolia, Turkey. Willdenowia 27(1–2): 185–190. <https://doi.org/10.3372/wi.27.2717>
- Freitag H, Rilke S (1997) *Salsola* [s.l.]. In: Rechinger KH (Ed.) Flora Iranica, Vol. 172. Akademische Druck- und Verlagsanstalt, Graz, 154–255.
- Grossheim AA (1945) Flora Kavkaza [Flora of the Caucasus], 2nd edn., Vol. 3. Akademiya Nauk, Baku, 1–321. [In Russian]
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Hernández-Ledesma P, Berendsohn WG, Borsch Th, von Mering S, Akhani H, Arias S, Castañeda-Noa I, Eggli U, Eriksson R, Flores-Olvera H, Fuentes-Bazán S, Kadereit G, Klak C, Korotkova N, Nyffeler R, Ocampo G, Ochoterena H, Oxelman B, Rabeler RK, Sanchez A, Schlumpberger BO, Uotila P (2015) A taxonomic backbone for the global synthesis of species diversity in the angiosperm order *Caryophyllales*. Willdenowia 45(3): 281–383. <https://doi.org/10.3372/wi.45.45301>
- Iljin MM (1936) Chenopodiaceae. In: Shishkin BK (Ed.) Flora of USSR, Vol. 6. Akademiya Nauk, Moscow-Leningrad, 2–354. [In Russian]
- IUCN (2021) The IUCN Red List of Threatened Species. Version 2021-3. <https://www.iucn-redlist.org> [accessed 10.06.2022]
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35(21): 4453–4455. <https://doi.org/10.1093/bioinformatics/btz305>

- Magomedova BM, Mingazhova MM, Shamanova FH (2016) Izmenchivost' priznakov pobe-ga *Salsola daghestanica* (Turcz.) Turcz. v Dagestane [Variability of the shoot characters of *Salsola daghestanica* (Turcz.) Turcz. in Dagestan]. Yug Rossii: Ecologiya & Development 11(4): 194–200. [In Russian]
- Mucina L (2017) *Caroxylon* (Chenopodiaceae s.str.) in continental Southern Africa and Mada-gascar: A preliminary nomenclatural synopsis and biogeographical considerations. Phyto-taxa 312(2): 151–178. <https://doi.org/10.11646/phytotaxa.312.2.1>
- Murtazaliev RA (2009) Konspekt flory Dagestana [A compendium of the flora of Dagestan], Vol. 1. Epokha, Makhachkala, 1–319. [In Russian]
- Murtazaliev RA (2016) Analysis of species distribution in the Dagestan flora. Botanicheskii Zhurnal 101(9): 1056–1074. [In Russian]
- Rambaut A, Drummond AJ (2012) FigTree Version 1.4.4. University of Edinburgh, Institute of Evolutionary Biology. <http://tree.bio.ed.ac.uk/software/figtree/> [accessed 10.05.2019]
- Rudov A, Mashkour M, Djamali M, Akhani H (2020) A review of C₄ plants in Southwest Asia: An ecological, geographical and taxonomical analysis of a region with high diversity of C₄ Eudicots. Frontiers in Plant Science 11: 546518. <https://doi.org/10.3389/fpls.2020.546518>
- Sauerermann W (1989) Bootstrapping the Maximum Likelihood estimator in high-dimension-al Log-linear models. Annals of Statistics 17(3): 1198–1216. <https://doi.org/10.1214/aos/1176347264>
- Secretariat GBIF (2021) *Salsola daghestanica* (Turcz.) Turcz. In: GBIF Backbone Taxonomy. Checklist dataset. <https://doi.org/10.15468/39omei> [accessed via GBIF.org on 10.06.2022]
- Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. <http://www.simplemappr.net> [accessed 15.06.2022]
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. <https://doi.org/10.1093/bioinfor-matics/btu033>
- Strid A, Tan K (1997) Flora Hellenica, Vol. 1. Koelz Scientific Books, Königstein xxxvi + 547 pp.
- Sukhorukov AP (2014) The carpology of the Chenopodiaceae with reference to the phylogeny, systematics and diagnostics of its representatives. Grif & Co., Tula, 397 pp. [In Russian with English summary]
- Sukhorukov AP, Akopian JA (2013) A compendium of the Chenopodiaceae in the Caucasus. Maks Press, Moscow, 76 pp. [In Russian]
- Sukhorukov AP, Aellen P, Edmondson JR, Townsend CC (2016) Chenopodiaceae. In: Ghazan-far SA, Edmondson JR (Eds) Flora of Iraq, Vol. 5(1). Bell and Bain Ltd, Glasgow, 164–256.
- Sukhorukov AP, Kushunina M, El Mokni R, Ardenghi NMG, Verloove F, Uotila P, Baider C, Bruyns PV, Klak C (2019) Chorological and taxonomic notes on African plants, 4: Caryophyllales. Botany Letters 166(4): 401–416. <https://doi.org/10.1080/23818107.2019.1652848>
- Tzvelev NN (1993) Notes on Chenopodiaceae of Eastern Europe. Ukrainsky Botanicheskyy Zhurnal 50: 78–85. [In Russian]

- Wen J, Zimmer EA (1996) Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6(2): 167–177. <https://doi.org/10.1006/mpev.1996.0069>
- Wen ZB, Zhang ML, Zhu GL, Sanderson SC (2010) Phylogeny of Salsoleae s.l. (Chenopodiaceae) based on DNA sequence data from ITS, psbB-psbH, and rbcL, with emphasis on taxa of northwestern China. *Plant Systematics and Evolution* 288(1–2): 25–42. <https://doi.org/10.1007/s00606-010-0310-5>
- Xu DH, Abe J, Sakai M, Kanazawa A, Shimamoto Y (2000) Sequence variation of non-coding regions of chloroplast DNA of soybean and related wild species and its implications for the evolution of different chloroplast haplotypes. *Theoretical and Applied Genetics* 101(5–6): 724–732. <https://doi.org/10.1007/s001220051537>
- Zhu (Chu) GL, Sanderson SC (2017) Genera and a new evolutionary system of World Chenopodiaceae. Science Press, Beijing, 1–150.

Appendix I

Specimens used for the mapping of *Akhania daghestanica* (other records were obtained from Grossheim 1945; Murtazaliev 2009; Magomedova et al. 2016; GBIF Secretariat 2021).

Russia. Dagestan Rep.: [Kizilyurtovsky distr.] Chiryurt vill., 4 Jul 1891, *V. Lipsky s.n.* (LE); [Laksky / Levashinsky distr.] between Kumukh & Tsudakhar vill., 3800 ft, 23 Aug 1898, *Th. Alexeenko s.n.* (LE); [Untsukul'sky distr.] nr Gumry vill., 25 May 1901, *Th. Alexeenko 13056* (LE); Temirkhan-Shura [Buynak'sky], 3 Oct 1913, *N. Pastukhov exs. 170* (LE, MW0663693); [Gunib'sky distr.] nr Choh vill., 16 Oct 1914, *D. Bubaev s.n.* (LE); [Untsukul'sky distr.] Arakani gorge, 28 Aug 1927, *A. Poretsky s.n.* (LE); Kakhib'sky distr., 26 Jul 1931, *G. Stupnikov 660* (MW0663694); [Buynak'sky distr.] nr Nizhny Dzhengutay vill., 2 Aug 1935, *E. Schiffers s.n.* (LE); [Buynak'sky distr.] nr Kapchugay vill., 1931, *E. Schiffers 42* (LE); [Kizilyurtovsky distr.] nr Chiryurt vill., 9 Sep 1936, *M. Iljin & E. Iljina 90* (LE); nr Makhachkala town, Tarki vill., 22 Jul 1953, *Ya. Prokhanov 1146* (LE); Laksky distr., nr Kamasha vill., 18 Aug 1953, *Magomedov s.n.* (MHA0233905); Untsukul'sky distr., 29 Aug 1953, *Ya. Prokhanov & N. Cheldyshev 372* (LE); [nr Makhachkala town] Kukurtau gorge, 10 Aug 1956, *Ya. Prokhanov 137* (LE); Buynak'sky distr., nr Dubki vill., 8 Aug 1981, *Yu. Menitsky & al. 437* (LE); [Buynak'sky distr.] nr Chirkey Water Reservoir, 10 km SE of Dubki vill., 11 Jul 2013, *A.S. Zernov 8135* (MW0663692); Shamil'sky distr., Khebda vill., 42.451562 N 46.560036 E, 14 Jul 2020, *D. Shilnikov* (pers. obs., see also Fig. 2).

Supplementary material 1

Figure S1

Authors: Alexander P. Sukhorukov, Alina V. Fedorova, Maria Kushunina, Evgeny V. Mavrodiev

Data type: Image

Explanation note: The best ML tree ($-\ln L = 15718.422393$) recovered from the ML analysis (RAxML with GTR + GAMMA) of the ITS matrix of Caroxyleae, Salsoleae, Camphorosmeae and outgroups. Image: Alina V. Fedorova.

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Link: <https://doi.org/10.3897/phytokeys.211.89408.suppl1>

Supplementary material 2

Figure S2

Authors: Alexander P. Sukhorukov, Alina V. Fedorova, Maria Kushunina, Evgeny V. Mavrodiev

Data type: Image

Explanation note: The best ML tree ($-\ln L = 5091.478154$) recovered from the ML analysis (RAxML with GTR + GAMMA) of the *psbB-psbH* IGS matrix of Caroxyleae, Salsoleae, Camphorosmeae and outgroups. Image: Alina V. Fedorova.

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Link: <https://doi.org/10.3897/phytokeys.211.89408.suppl2>

Hemiboea kaiyangensis, a new species of Gesneriaceae endemic to Guizhou, China

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Abstract

A new species of Gesneriaceae from Guizhou, China, *Hemiboea kaiyangensis* **sp. nov.**, is described and illustrated. We investigated its phylogenetic position and relationships with 13 other species of *Hemiboea* C.B.Clarke, which present large morphological diversity in the genus, based on molecular analyses of the nuclear ribosomal internal transcribed spacer (ITS) and the chloroplast *trnL-F* intron-spacer sequences. The molecular phylogenetic analyses revealed that the new species is most closely related to *H. ovalifolia*. A diagnostic table and discussion of morphological characters are provided to differentiate the new species from *H. longisepala*, *H. flaccida* and *H. ovalifolia*.

Keywords

Gesneriad, ITS, morphology, phylogeny, taxonomy, *trnL-F*

Introduction

The genus *Hemiboea* C.B.Clarke has traditionally been divided into two sections, sect. *Subcapitatae* C.B.Clarke and sect. *Hemiboea* (Li 1987a, 1987b). Many species of this genus can be found on limestone hills in tropical and subtropical evergreen broadleaved forests. Most species of *Hemiboea* are located in southern and southwestern China, while only a few are located outside China. For instance, *H. subcapitata* C.B.Clarke and *H. cavaleriei* Lév. var. *paucinervis* W.T.Wang & Z.Y.Li ex Z.Y.Li are also found in northern Vietnam; *H. bicornuta* (Hayata) Ohwi is distributed in Taiwan Island of China and the Ryukyu Islands of Japan (Li and Wang 2005).

So far, a total of 38 species and six varieties have been described in *Hemiboea* (Li and Liu 2004; Xu et al. 2010; Huang et al. 2011; Wen et al. 2011; Pan et al. 2012; Xu et al. 2012; Wen et al. 2013; Zhou et al. 2013; Zhang et al. 2014; Huang et al. 2017; Chen et al. 2018; Li et al. 2018; Wei 2018; Li et al. 2019; Nguyen et al. 2019; Wu et al. 2019; Huang et al. 2020; Nguyen et al. 2021). The small genus *Metabriggsia* W.T.Wang was established based on two species, *M. ovalifolia* W.T.Wang and *M. purpureotincta* W.T.Wang from Guangxi, China (Wang 1983). Some similarities in corolla morphology with certain species of the former *Briggsia* Craib are reflected in the generic name (Wang 1983), though *Metabriggsia* is morphologically similar to *Hemiboea*, and in recent molecular-based taxonomic studies, the two species of *Metabriggsia* were sunk into *Hemiboea* as *H. ovalifolia* (W.T.Wang) A.Weber & Mich.Möller and *H. purpureotincta* (W.T.Wang) A.Weber & Mich.Möller (Weber et al. 2011).

During the course of a 2009 floristic study in Guizhou Province, China, we collected specimens of an unidentified *Hemiboea* from a limestone area in Kaiyang County. It seemed a species of the sect. *Subcapitatae* of the genus *Hemiboea* because of the free calyx lobes and elongate corolla. However, it differed from all known species in this genus. We also observed that its vegetative morphology was similar to that of *H. ovalifolia* because of the stems covered with long white pubescent hairs, and the leaf blades herbaceous and ovate or ovate-oblong. The inflorescence and flower morphology indicated that this species belongs to *Hemiboea* as previously circumscribed; for example, it possesses involucre, its corolla is infundibular-tubular, and has a ring of hairs on the interior above the corolla base. After consulting national floras and the relevant literature (Li 1983, 1987a, b; Wei and Wen 1995; Weitzman et al. 1997; Wang et al. 1998; Li and Liu 2004; Li and Wang 2005; Wei et al. 2010; Xu et al. 2010), as well as herbarium specimens, we confirmed that it is an undescribed species. We described and illustrated the new species herein. We used molecular data of the nuclear ribosomal internal transcribed spacers (ITS) and the chloroplast *trnL-F* intron-spacer (*trnL-F*) to confirm the placement of the newly collected material in *Hemiboea* and to infer its phylogenetic relationships with other species in the genus.

Methods

Taxon sampling

To investigate the phylogenetic placement of the newly collected taxon, our sampling focused on species of *Hemiboea* and its closest related genera according to Möller et al. (2011) and Weber et al. (2011). The ITS and *trnL-F* sequences used in Weber et al. (2011) included 13 species of *Hemiboea* and four outgroup samples (one species of *Ornithoboea*, one of *Paraboea* and two of *Glabrella*) were downloaded from Genbank (Table 1). Leaf material of the new species was collected from living plants in the type locality and rapidly dried in silica gel.

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

Species	Voucher number	<i>trnL</i> -F	ITS
Outgroup samples			
<i>Briggsia longipes</i> (Hemsl. ex Oliv.) Craib	MMO 01-122	FJ501545	AF055052/ AF055053
<i>Briggsia mihieri</i> (Franch.) Craib	Y.Z.Wang 11315B	FJ501544	FJ501363
<i>Ornithoboea wildeana</i> Craib	Middleton & al. 4531	JN934710	JN934752
<i>Paraboea rufescens</i> (Franchet) B.L.Burtt	Möller MMO 01-108/3	JN934730	JN934772
Ingroup samples			
<i>Hemiboea ovalifolia</i> W.T.Wang (W.T.Wang) A.Weber & Mich. Möller	B.M.Nong 06-1	HQ632883	HQ632980
<i>Hemiboea purpureotincta</i> (W.T.Wang) A.Weber & Mich.Möller	MMO 06-813	HQ632884	HQ632981
<i>Hemiboea bicornuta</i> (Hayata) Ohwi	RBGE cult. 19951207	FJ501534	FJ501356
<i>Hemiboea cavaleriei</i> Lévl.	Z.J.Gu G3	FJ501533	FJ501355
<i>Hemiboea fangii</i> Chun ex Z.Yu Li	MMO 08-1284	HQ632882	HQ632979
<i>Hemiboea follicularis</i> C.B.Clarke	Y.G.Weï G03	HQ632885	HQ632982
<i>Hemiboea gracilis</i> Franchet	Y.Z.Wang 11317	FJ501536	*
<i>Hemiboea longgangensis</i> Z.Yu Li	Y.G.Weï 07-550	HQ632889	HQ632986
<i>Hemiboea longzhouensis</i> W.T.Wang	MMO 07-1127	HQ632888	HQ632985
<i>Hemiboea omeiensis</i> W.T. Wang	MMO 08-1271	HQ632886	HQ632983
<i>Hemiboea kaiyangensis</i> T. Peng & S.Z.He, sp. nov.	Shun-Zhi He, 090819	JN644339	JN644335
<i>Hemiboea rubribracteata</i> Z.Yu Li & Yan Liu	MMO 07-1093	HQ632890	HQ632987
<i>Hemiboea subcapitata</i> C.B.Clarke	Y.Z.Wang 11306	FJ501535	FJ501357

* – Wei et al. 2010 *Litostigma* paper.

DNA extraction, PCR and direct sequencing

Molecular methods and protocols followed Möller et al. (2009) and Weber et al. (2011). The Genbank accession numbers for ITS and *trnL*-F of the new species are JN644339 and JN644335, respectively (Table 1).

Phylogenetic analysis

Sequences of ITS and *trnL*-F of the new species were aligned with the existing matrices of Weber et al. (2011), and the combined data analyzed by Möller et al. (2009, 2011) and Weber et al. (2011). All characters were unordered and equally weighted. Heuristic searches were implemented as 1000 random taxon-addition sequences, with tree bisection–reconnection (TBR) branch swapping, the MULTRESS and STEEPEST DESCENT option in effect. Branch support was obtained by bootstrapping (Felsenstein, 1985ab) with 1000 random resamplings and TBR on and MULTREES off (Möller et al. 2009).

Results

The combined dataset included 1490 characters and contained 1125 (75.5%) constant sites, 333 (22.3%) variable sites and 154 (10.3%) parsimony-informative sites. The heuristic analysis resulted in three most parsimonious trees with a length of 553 steps,

a consistency index (CI) of 0.7776, and a retention index (RI) of 0.6295. The strict consensus tree supports that the new species nests in a strongly supported clade of *Hemiboea* (BS = 100%), and it is the sister taxon to *H. ovalifolia* (BS = 99%) (Fig. 3).

Discussion

The molecular phylogenetic analysis revealed that the new collection *Hemiboea kaiyangensis* fell into the clade of *Hemiboea*, and its most closely related species was *H. ovalifolia*, which is congruent to the morphological evidence. Morphologically, this species is most similar to *H. longisepala*, *H. flaccida*, and *H. ovalifolia*, and it can be easily distinguished by the characters summarized in Table 2.

Table 2. Diagnostic characters used to differentiate *Hemiboea kaiyangensis* from most similar taxa.

Taxon	<i>Hemiboea kaiyangensis</i>	<i>H. longisepala</i>	<i>H. flaccida</i>	<i>H. ovalifolia</i>	
Stem	densely pubescent	Glabrous	densely brown puberulent to villous	brown villous	
Leaf blade	Texture	herbaceous	Papery	slightly fleshy	
	Shape	ovate or ovate-oblong	ovate-lanceolate to elliptic-lanceolate	elliptic to ovate	
	Hairs	appressed pubescent on adaxially and abaxially surface	adaxially sparsely pubescent, abaxially glabrous	pubescent to densely puberulent on adaxially and abaxially surface	appressed pubescent on adaxially and abaxially surface
	Lateral veins	7–10 on each side	10–12 on each side	5–8 on each side	5–10 on each side
Peduncle(cm)	10–18	3–3.6	0.4–1.9	7.5–12.5	
Cyme	Hairs on peduncle	densely white long glandular pubescent	glabrous	sparsely glandular puberulent to pilose	brown glandular pubescent
	Involucre	cordate, early deciduous	spheroidal, ca. 1.7 cm in diam.	nearly spheroidal, 1–2.5 cm in diam., outside sparsely glandular puberulent	nearly spheroidal, early deciduous
	Calyx lobes	oblong-lanceolate, apex obtuse or slightly obtuse, 12–13 × 2.5–3 mm, outside densely glandular pubescent, glabrous inner, 3 veins on one lobe	linear-lanceolate, 19–20 × ca. 2.5 mm, outside and margin glabrous	linear, 5–9 × 2.5–3 mm, outside and margin sparsely glandular puberulent	lanceolate-linear, 9–10 × 1.5–2 mm, pubescent outer, glabrous inner, 3–5 veins on one lobe
	Colour	outside pale yellowish-green to pale yellowish-white	outside white	outside white, inside purple spotted	white, suffused yellow-green
	Hairs	outside densely glandular puberulent, inside glabrous	outside glabrous	outside sparsely glandular puberulent, inside glabrous	outside sparsely pubescent near apex, inside glabrous
	Size	4.5–5 cm long; tube 3.5–4 cm; adaxial lip 6–7 mm, abaxial lip 6–7 mm	ca. 3.4 cm long, tube ca. 2.6 cm; adaxial lip ca. 6 mm; abaxial lip ca. 8 mm	3–3.4 cm long, tube 2.3–2.5 cm; adaxial lip 4–5 mm; abaxial lip 7–9 mm	ca. 3.6 cm long, tube ca. 2.7 cm; adaxial lip ca. 2.8 mm; abaxial lip ca. 1 cm
Flower	Staminodes	2, ca. 7 mm long	3, central 1 ca 1.5 mm long, lateral 5.5 mm	2, 6–8 mm long	3, central 1 ca. 1.5 mm long, lateral 2, 9–10 mm
	Pistil	glabrous	glabrous	sparsely glandular puberulent	sparsely puberulent

Taxonomic treatment

Hemiboea kaiyangensis T.Peng & S.Z.He, sp. nov.

urn:lsid:ipni.org:names:77306628-1

Figs 1, 2, 3

Type. CHINA; center of Guizhou Province, Zijiang gorge, Kaiyang County, grows on cliffs under forests along the road; alt. 1000–1020 m. 2009-08-18, *Shun-Zhi He* 90819 (Holotype: HGCM!, isotype: GNUB!, IBK!) (Figs 1, 2).

Diagnosis. *Hemiboea kaiyangensis* is most similar to *H. longisepala* Z.Y.Li, *H. flaccida* Chun ex Z.Y.Li and *H. ovalifolia* (W.T.Wang) A.Weber & Mich.Möller in the glabrous pistil, but it differs in the cordate involucre bracts that are early deciduous, the corolla densely glandular puberulent outside and glabrous inside, and pale yellowish-green to pale yellowish-white outside.

Description. Perennial herb. Rhizomatous. Stems 25–60 cm long, 5–7 mm in diam., densely pubescent. Petiole 0.5–4.5 cm long, densely pubescent. Leaf blade herbaceous, oblique, iso- to distinctly anisophyllous, ovate or ovate-oblong, 13–26 × 5–8 cm, apex acute, base oblique cuneate, margin nearly entire or unapparent sinuous dentate, appressed pubescent on both sides, lateral veins 7–10 on each side. Cymes 2–3, terminal or subterminal, 6–12 flowers per cyme; peduncle 10–18 cm long, densely pubescent with white long glandular hairs; involucre cordate, apex cuspidate, early deciduous; pedicel 0.7–1.5 cm long, pubescent with white long glandular hairs. Calyx lobes 5; lobes oblong-lanceolate, apex obtuse or slightly obtuse, 12–13 × 2.5–3 mm, outside densely pubescent with glandular hairs of 3–4 mm long, inside glabrous, 3 veins per lobe. Corolla pale yellowish-green to pale yellowish-white outside, small purplish-brown spotted inside, 4.5–5 cm long, densely glandular-puberulent outside, glabrous with a ring of white hairs ca. 4 mm above the corolla base inside; tube 3.5–4 cm long, mouth 1–1.3 cm in diam.; limb distinctly 2-lipped, adaxial lip 2-lobed, lobes obliquely semicircular, apex obtuse to rounded, 6–7 mm long, 8–9 mm in diam. at the base of lobes; abaxial lip tripartite, lobes margin ciliolate, the central broadly ovate to ovate-elliptic, 6–7 mm long, two lateral oblique triangle, 7–8 cm long. Stamens 2, glabrous, adnate to 1.8 cm above the corolla base, filament ca. 1.3 cm long, geniculate at the middle; anthers slightly oblong, dark purple, apex coherent. Staminodes 2, glabrous, adnate to 1.7 cm above the corolla base, ca. 7 mm long. Nectary disc ring-like, 1.1–1.2 mm high, atop with ca. 6 obviously erose crena. Pistil 2–2.8 cm long, ovary 7–9 mm long, glabrous, style 1.4–2 cm long, glabrous, stigma 1, terminal truncate, slightly 2-lobed. Capsule obliquely linear-lanceolate, 2–2.4 cm long, 3–3.3 mm in diam., glabrous, slightly curved.

Pollen description. Pollen grains of *Hemiboea kaiyangensis* are prolate-spheroidal, long or oblate, 3-colporoidate grains. In polar view, the outline is close to triangular-circular. The ectocolpi measures 33.05–33.57 × 12.15–14.23 μm and the endoapertures are laterally fused to form an endocingulum. Exine reticulate, muri smooth. The

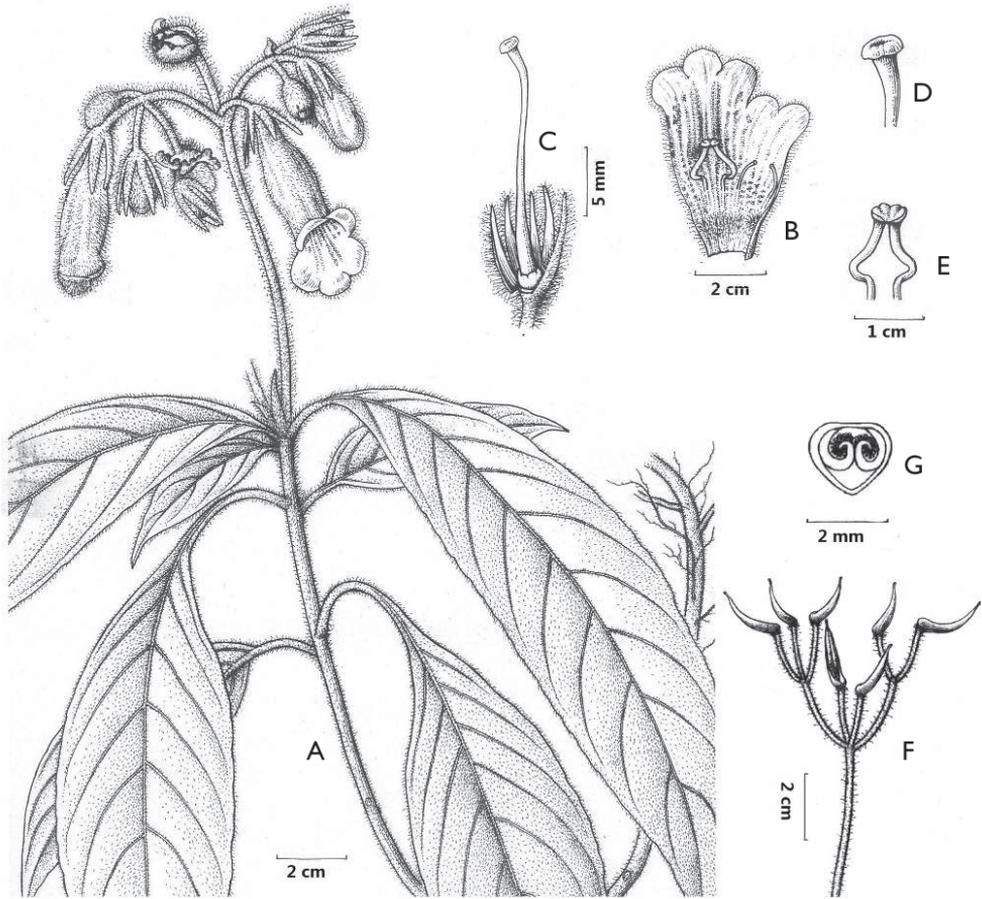


Figure 1. *Hemiboea kaiyangensis* T.Peng & S.Z.He **A** habit, showing flowering branch **B** opened corolla, showing stamens, staminodes and ring of hairs at base of tube **C** calyx, pistil and disc **D** stigma **E** stamens **F** inflorescence **G** cross section of ovary, showing parietal placentation. Drawing by S.Q.He and Y.X.Zhu.

width of muri is unequal in size. The sizes and shapes of perforations are irregular, and vary in size from $0.14\text{--}0.67 \times 0.11\text{--}0.61 \mu\text{m}$.

Distribution and ecology. Known only from a single limestone gorge in Kaiyang county, Guizhou Province, China. Only five populations were found, growing on the mouth of caves in shady and damp forests, close to a road, between 900 and 930 m in elevation.

Etymology. The name of the new species, *kaiyangensis*, refers to the type locality, Kaiyang County, Guiyang, Guizhou Province, China.

Conservation status. The populations of *Hemiboea kaiyangensis* are endemic to Kaiyang county, center of Guizhou Province, China, and the species only known from the type locality at present. The five detected populations grow dispersed in a limestone gorge, and cover only an area of about 1.25 km^2 and include a total of 75–120 indi-

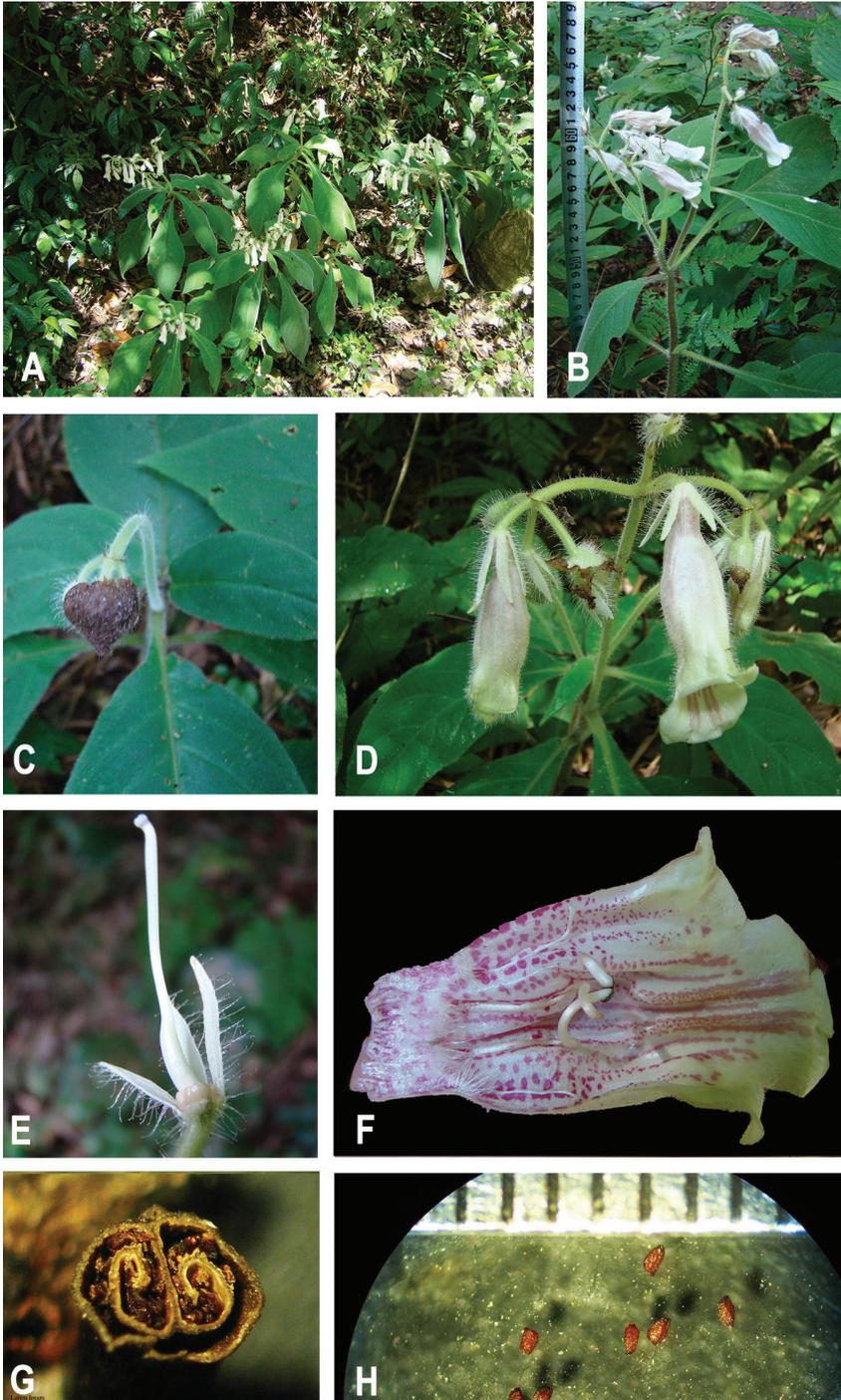


Figure 2. *Hemiboea kaiyangensis* T.Peng & S.Z.He **A** habitat **B** plant with flowering branches **C** involucre, early deciduous, before flower opening **D** pair-flowered cymes **E** calyx, disc and pistil **F** opened corolla, showing stamens, staminodes and ring of hairs at base of tube **G** cross section of capsule, showing two parietal placentation **H** seeds. Scale bar: 0.5 mm (Based on the holotype *Shun-Zhi He 90819*).

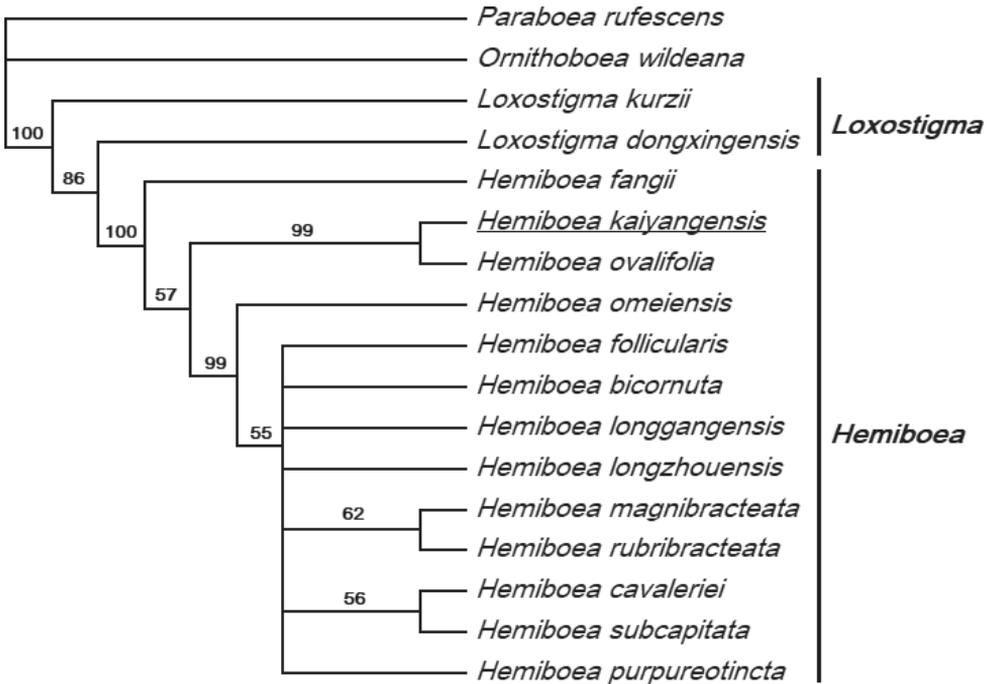


Figure 3. Strict consensus tree of three parsimony trees based on combined ITS and *trnL-F* data. Numbers above branches are bootstrap values. Underline indicates the new species.

viduals. However, until further investigation, the species should be designated as “Data Deficient” (DD) according to the IUCN standards (IUCN 2019).

Notes. As previously mentioned, *Hemiboea kaiyangensis* is most morphologically similar to *H. longisepala*, *H. flaccida* and *H. ovalifolia* in their glabrous pistil, but some characters, such as the early deciduous and cordate involucre bracts, the corolla indumentum (outside densely glandular puberulent and inside glabrous), and the corolla color (outside pale yellowish-green to pale yellowish-white), help us to easily distinguish them. Specifically, *H. kaiyangensis* is similar to *H. flaccida* in having two staminodes, but it differs in the longer peduncle (10–18 cm), the involucre cordate but early deciduous, the bigger calyx lobes, oblong-lanceolate (12–13 × 2.5–3 mm), and the glabrous pistil. This new species is also similar to *H. ovalifolia* in the texture and shape of leaf blade, but it can be distinguished in the peduncle indument, with dense white long glandular hairs, the involucre cordate, the calyx lobes outside densely glandular pubescent, the corolla outside densely glandular puberulent, 2 staminodes, and the pistil glabrous. Lastly, *H. kaiyangensis* is similar to *H. longisepala*, but it differ from the latter in the stem densely pubescent, the longer peduncle (10–18 cm), the peduncle with dense white long glandular hairs, the corolla outside densely glandular puberulent and 2 staminodes. All compared details of four congeners were listed in Table 2.

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References

- Chen WH, Zhang YM, Li ZY, Nguyen QH, Nguyen TH, Shui YM (2018) *Hemiboea crystallina*, a new species of Gesneriaceae from karst regions of China and Vietnam. *Phytotaxa* 336(1): 095–099. <https://doi.org/10.11646/phytotaxa.336.1.8>
- Huang YS, Xu WB, Peng RC, Liu Y (2011) A New Variety of *Hemiboea* (Gesneriaceae) from Limestone Areas in Guangxi, China. *Taiwania* 56(3): 240–243.
- Huang J, Xiang XG, Lu YB, Pan B, Zhang Q (2017) *Hemiboea pterocaulis* comb. et stat. nov. (Gesneriaceae), a new critically endangered species segregated from *H. subcapitata*. *Nordic Journal of Botany* 36(1–2): 1–10. <https://doi.org/10.1111/njb.01468>
- Huang ZP, Li JH, Pan B, Qin XM, Zhang Q, Lu YB (2020) *Hemiboea yongfuensis* (Gesneriaceae): A cryptic and critically endangered new species from North Guangxi, China. *Nordic Journal of Botany* 38(3): 1–8. <https://doi.org/10.1111/njb.02435>
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. <http://cmsdocs.s3.amazonaws.com/RedListGuidelines.pdf>
- Li ZY (1983) Taxa nova *Hemiboeae* (Gesneriaceae). *Acta Phytotaxonomica Sinica* 21(2): 194–203. [in Chinese]
- Li ZY (1987a) A study of the genus *Hemiboeae* (Gesneriaceae). *Acta Phytotaxonomica Sinica* 25(2): 220–230. [in Chinese]
- Li ZY (1987b) A study of the genus *Hemiboea* (Gesneriaceae) (Cont.). *Acta Phytotaxonomica Sinica* 25(3): 91–92. [in Chinese]
- Li ZY, Liu Y (2004) *Hemiboea rubribracteata* Z. Y. Li & Yan Liu, a new species of *Hemiboeae* (Gesneriaceae) from Guangxi, China. *Acta Phytotaxonomica Sinica* 42(6): 537–540. [in Chinese]
- Li ZY, Wang YZ (2005) Plants of Gesneriaceae in China. Henan Science and Technology Publishing House, 123–153. [in Chinese]
- Li SY, Han MQ, Li XJ, Li ZY, Xiang XG (2018) *Hemiboea suiyangensis* (Gesneriaceae): A new species from Guizhou, China. *PhytoKeys* 99: 99–106. <https://doi.org/10.3897/phytokeys.99.25265>
- Li XQ, Guo ZY, Li Y, Zhou P, Chen XH, Li ZY, Xiang X (2019) *Hemiboea guangdongensis* comb. & stat. nov., a cryptic species segregated from *H. subcapitata* (Gesneriaceae) based

- on morphological and molecular data. *Nordic Journal of Botany* 2019: e02574 1–7. <https://doi.org/10.1111/njb.02574>
- Möller M, Pfosser M, Jang CG, Mayer V, Clark A, Hollingsworth ML, Barfuß ZJM, Kiehn M, Weber A (2009) A preliminary phylogeny of the didymocarpoid Gesneriaceae based on three molecular data sets: Incongruence with available tribal classifications. *American Journal of Botany* 96(5): 989–1010. <https://doi.org/10.3732/ajb.0800291>
- Möller M, Forrest A, Wei YG, Weber A (2011) A molecular phylogenetic assessment of the advanced Asian and Malesian didymocarpoid Gesneriaceae with focus on non-monophyletic and monotypic genera. *Plant Systematics and Evolution* 292(3): 223–248. <https://doi.org/10.1007/s00606-010-0413-z>
- Nguyen HG, Averyanov L, Wen F (2019) *Hemiboea thanhhoensis* (Gesneriaceae), a new species from northern Vietnam. *Phytotaxa* 414(3): 146–150. <https://doi.org/10.11646/phytotaxa.414.3.3>
- Nguyen CH, Nguyen LV, Nguyen KS, Egorov A, Averyanov L (2021) *Hemiboea chanii* (Gesneriaceae), a new species from limestone areas of northern Vietnam. *PhytoKeys* 183(1): 108–114. <https://doi.org/10.3897/phytokeys.183.69180>
- Pan B, Wu WH, Xu WB (2012) *Hemiboea pseudomagnibracteata* (Gesneriaceae), a New Species from Guangxi, China. *Taiwania* 57(2): 188–192.
- Wang WT (1983) Genus novum Gesneriacearum e Guangxi. *Guahaia* 3(1): 1–6.
- Wang WT, Pan KY, Li ZY, Weitzman AL, Skog LE (1998) Gesneriaceae. In: Wu ZH, Raven PH (Eds) *Flora of China* vol. 18. Science Press, Beijing; Missouri Botanical Garden Press, Saint Louis, 244–401.
- Weber A, Wei YG, Sontag S, Möller M (2011) Inclusion of *Metabriggsia* into *Hemiboea* (Gesneriaceae). *Phytotaxa* 23(1): 37–48. <https://doi.org/10.11646/phytotaxa.23.1.2>
- Wei YG (2018) *The Distribution and Conservation Status of Native Plants in Guangxi, China*. China Forestry Publishing House, Beijing, 876 pp.
- Wei YG, Wen HQ (1995) Two new species from Guangxi. *Guihaia* 15(3): 216–219. [in Chinese]
- Wei YG, Wen F, Möller M, Monro Cui C (2010) *Metabriggsia* and *Hemiboea*. In: Wei YG, Wen F, Möller M, Monro A, Zhang Q, Gao Q, Mou HF, Zhong SH, Cui C (Eds) *Gesneriaceae of South China*. Guangxi Science and Technology Publishing House, Nanning, 174–216.
- Weitzman AL, Skog LE, Wang WT, Pan KY, Li ZY (1997) New taxa, new combination, and notes on Chinese Gesneriaceae. *Novon* 7(4): 423–435. <https://doi.org/10.2307/3391777>
- Wen F, Tang WX, Wei YG (2011) *Hemiboea angustifolia* (Gesneriaceae), a new species endemic to a tropical limestone area of Guangxi, China. *Phytotaxa* 30(1): 53–59. <https://doi.org/10.11646/phytotaxa.30.1.4>
- Wen F, Zhao B, Liang GY, Wei YG (2013) *Hemiboea lutea* sp. nov. (Gesneriaceae) from Guangxi, China. *Nordic Journal of Botany* 31(6): 720–723. <https://doi.org/10.1111/j.1756-1051.2013.01697.x>
- Wu ZW, Guo ZY, Deng CY, Li ZY, Xiang XG (2019) *Hemiboea albiflora*, a new species of Gesneriaceae from Guizhou, China. *PhytoKeys* 122: 79–86. <https://doi.org/10.3897/phytokeys.122.33783>

- Xu WB, Wu WH, Nong DX, Liu Y (2010) *Hemiboea purpurea* sp. nov. (Gesneriaceae) from a limestone area in Guangxi, China. *Nordic Journal of Botany* 28(3): 313–315. <https://doi.org/10.1111/j.1756-1051.2009.00722.x>
- Xu WB, Haung YS, Peng RC, Zhuang XY (2012) *Hemiboea sinovietnamica* sp. nov. (Gesneriaceae) from a limestone area along the boundary of Sino-Vietnam. *Nordic Journal of Botany* 30(6): 691–695. <https://doi.org/10.1111/j.1756-1051.2012.01340.x>
- Zhang LX, Tan YH, Li JW, Wen B, Yin JT, Lan QY (2014) *Hemiboea malipoensis*, a new species of Gesneriaceae from southeastern Yunnan, China. *Phytotaxa* 174(3): 165–172. <https://doi.org/10.11646/phytotaxa.174.3.5>
- Zhou SB, Hong X, Wen F, Xiao HM (2013) *Hemiboea roseoalba* S.B. Zhou, X. Hong & F. Wen (Gesneriaceae), A new species from Guangdong, China. *Bangladesh Journal of Plant Taxonomy* 20(2): 171–177. <https://doi.org/10.3329/bjpt.v20i2.17391>

Lectotypification of *Cycas debaoensis* (Cycadaceae)

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Abstract

The type of *Cycas debaoensis* Y.C.Zhong & C.J.Chen (*Y.C.Zhong* 8762) consists of nine duplicates in PE. Our new investigation of the type collection suggests that the type includes at least two different gatherings which should be considered as syntypes. For nomenclatural purposes, we lectotypify the name *Cycas debaoensis* with the herbarium sheet PE00047578 and consider other duplicates in PE and GXMI as isolectotypes. The seeds in the capsule (*Y.C.Zhong* s.n. collected in Oct 1998) are considered as a syntype.

Keywords

Cycadaceae, *Cycas debaoensis*, gathering, gymnosperm, lectotypification, nomenclature, Shenzhen Code

Introduction

Cycas debaoensis Y.C.Zhong & C.J.Chen belongs to the gymnosperm family Cycadaceae. This species is similar to *C. multipinnata* C.J.Chen & S.Y.Yang in the bi- or tri-pinnate compound leaves, but differs from the latter by its shorter and more numerous leaves with narrower, thicker leaflets and the longer megasporophylls with a larger lamina (Hill 2008). It has extremely small populations occurring in Guangxi and Yunnan of China (Chen and Zhong 1997; Chen and Stevenson 1999; Xi et al. 2022) and is considered as Critically Endangered (CR), (Hill 2008; Yang 2021). The species is listed as a first class species in the recently released National Key Protected Wild Plant Species of China (<http://www.forestry.gov.cn/main/3954/20210908/163949170374051.html>).

Cycas debaoensis was formally described by Chen and Zhong (1997) and *Y.C.Zhong* 8762 deposited in PE and was designated as the type specimen. We made an investigation of the type specimen and discovered that the situation is far from straightforward. There are nine herbarium sheets of *Y.C.Zhong* 8762 in PE. Of these nine sheets, three have labels in Y.C.Zhong & C.J.Chen's handwriting (PE00047578, PE00047579, PE00031036), while the other six possess only printed labels (PE01458925, PE01458924, PE00934025, PE00934026, PE00934027 and PE00934028). The labels of these duplicates indicate the type status (holotype or isotype): seven of them are marked as holotype/holotypus, while two of them are labelled as isotypus. Although Lin (2014) considered that the duplicate (PE00934026) is the holotype, this is not the case.

The nine sheets cannot be considered as a gathering. There are no serial numbers showing that all of them belong to a single gathering: six of them have information on the collection labels indicating that they are parts of a gathering, i.e. 1/9 (PE01458925), 2/9 (PE01458924), 3/9 (PE00934025), 4/9 (PE00934026), 5/9 (PE00934027), 9/9 (PE00934028), but three others only possess handwritten collection notes and are lacking such information. Moreover, one of the nine duplicates (PE00031036), in fact, consists of two different parts: the leaves and the megasporophylls were collected in Aug 1997 (*Y.C.Zhong* 8762), whereas the seeds in the capsule were collected in Oct 1998 (*Y.C.Zhong* s.n.). Under Art. 8.2 of the Shenzhen Code (Turland et al. 2018), the term “gathering” is used for a collection presumed to be of a single taxon made by the same collector(s) at the same time from a single locality. The type of *C. debaoensis* clearly includes two different gatherings that constitute syntypes. Under Art. 8.1, a type should be a specimen, namely a gathering or part of a gathering; we thus think that the name *C. debaoensis* requires to be lectotypified.

The duplicate (PE00047578) bears the authors' handwriting indicating “*Cycas debaoensis* Y.C.Zhong & C.J.Chen sp. nov.” and “holotypus”, suggesting that the specimen was studied by the original authors. Two other duplicates (PE00047579, PE00031036) also bear handwritten identification labels which, however, are marked “isotypus”. Moreover, the specimen PE00047578 is well preserved and includes both leaf characters and female reproductive characters (three megasporophylls with five young seeds attached to one of the megasporophylls). We thus designate *Y.C.Zhong* 8762 (PE00047578) as the lectotype here and consider the other duplicates as isolectotypes.

Typification

Cycas debaoensis Y.C.Zhong & C.J.Chen, *Acta Phytotax. Sin.* 35(6): 571 (1997).

Figs 1, 2

Type. CHINA (中国). Guangxi (广西), Debao Co. (德保县), rocky mountains, alt. 850 m, brown calcareous soil, near huge rocks, height 2.9 m, leaves compound 3-pinnate, female with young seeds, 27 Aug 1997, *Y.C.Zhong* (钟业聪) 8762 (lectotype: PE00047578, here designated; isolectotypes: PE00047579, PE00031036 excl. seeds



Figure 1. Lectotype of *Cycas debaoensis* Y.C.Zhong & C.J.Chen: Y.C.Zhong 8762 (PE00047578).



Figure 2. Two different gatherings mounted on a single herbarium sheet (PE00031036): leaves and megasporophylls (*Y.C.Zhong 8762* collected in Aug 1997) and seeds in the capsule (*Y.C.Zhong s.n.* collected in Oct 1998).

in the capsule collected in Oct 1998 by Y.C.Zhong, PE00934025, PE00934026, PE00934027, PE00934028, PE01458924, PE01458925, GXMI050022).

Note. The seeds in the capsule of the duplicate (*Y.C.Zhong s.n.*: PE00031036) should be considered as a syntype. The protologue and type specimen images are available in Suppl. material 1.

Acknowledgements

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References

- Chen CJ, Stevenson DW (1999) Cycadaceae. In: Raven PR, Wu ZY (Eds) Flora of China (Vol. 4). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 1–7.
- Chen CJ, Zhong YC (1997) *Cycas debaoensis* Y.C.Zhong et C.J.Chen - a new cycad from China. Acta Phytotaxonomica Sinica 35: 571.
- Hill KD (2008) The genus *Cycas* (Cycadaceae) in China. Telopea 12: 71–118. <https://doi.org/10.7751/telopea20085804>
- Lin Q (2014) Type Specimens in China National Herbarium (PE) (Vol. 3). Henan Science & Technology Press, Zhengzhou, 487 pp.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>
- Xi HH, Wang YQ, Pan YZ, Xu T, Zhan QQ, Liu J, Feng XY, Gong X (2022) Resources and protection of *Cycas* plants in China. Biodiversity Science 30(7): e21495. <https://doi.org/10.17520/biods.2021495>
- Yang Y (2021) An updated red list assessment of gymnosperms from China (Version 2021). Biodiversity Science 29(12): 1599–1606. <https://doi.org/10.17520/biods.2021342>

Supplementary material I

Figures S1–S11

Authors: Yong Yang, David K. Ferguson

Data type: Figures (in rar. archive)

Explanation note: Isolectotypes and protologue of *Cycas debaoensis*.

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Link: <https://doi.org/10.3897/phytokeys.211.93650.suppl1>

Two new species of *Boesenbergia* (Zingiberaceae), from Sabah, Malaysia

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Abstract

Two new species of *Boesenbergia*, *B. sugudensis* **sp. nov.** and *B. truncata* **sp. nov.** were discovered in Sabah, Malaysian Borneo. *Boesenbergia sugudensis* resembles *B. imbakensis* in that the leaf sheath of the plant is not thickened and in the anther thecae dehiscing by longitudinal slits, but differs in having a longer petiole and tubular calyx. *Boesenbergia truncata* resembles *B. orbiculata* by the short petiole and a bilobed calyx, but differs by the truncate leaf base, the acute leaf apex, opposite leaves with a narrower lamina parallel to the ground and anther thecae dehiscing by pores. The new species are described and illustrated in detail.

Keywords

Boesenbergia, Borneo, hill slope, Sabah

Introduction

Boesenbergia Kuntze is one of the genera in the family Zingiberaceae with small-size species. The genus is placed in the subtribe Zingiberae of the tribe Zingiberoideae (Kress et al. 2002) and harbours approximately 99 species distributed in tropical Asia with the centre of diversity in Thailand (28 species) and Borneo (36 species) (Smith 1987; Ibrahim 1992; Siriruga 1992; Poulsen 1993; Larsen 1997; Larsen et al. 1999; Cowley 2000; Saensouk and Larsen 2001; Lamb et al. 2013). There were 8 species in Sabah with one variety. The discovery of the two species described below elevates the number of *Boesenbergia* species in Borneo to 38 species.

Smith (1987) pointed out that the important diagnostic characters for Bornean *Boesenbergia* are patterns of anther dehiscence, characteristics of seeds, variegation of leaf and character of leaf-shoots. Sakai and Nagamasu (2006, 2009) added arrangement of flowers in inflorescence as another character. Meanwhile, different characters were used for the diagnosis of *Boesenbergia longiflora* complex from Indochina, namely, flowers per inflorescences, flower colour, labellum pattern colour, shape and measurement, floral tube length, androecial tube length, anther length and underground architecture (Mood et al. 2013).

Materials and methods

The morphology of the two new species was analyzed using herbarium materials (AAU, C, E, K, KEP, KUL, L, P, SAN, SING, and SNP) and living plants. During fieldwork, careful observations and measurements of morphological characters were recorded prior to preparation of herbarium specimens in the field. When possible, up to four duplicates of each collection were made, and a sample of a fresh leaf was preserved in silica gel for genetic studies. Voucher specimens were deposited at BORH and SAN. Floral bracts and flowers were immediately fixed in FAA (formaldehyde: glacial acetic acid: alcohol).

Field observations included habit; rhizome (diameter, shape, colour, indumentum); leafy shoot (height and distance between adjacent shoots); leaf sheath (number, length, colour, indumentum); ligule (length, shape of apex, colour, indumentum); petiole (length, shape (whether channelled or rounded in cross section), colour, indumentum); number of leaves per leafy-shoot; lamina (size, shape, aspect of lamina (whether held at a certain angle), venation, texture, colour (on both surfaces), indumentum, base, margin, apex); inflorescence size, floral bracts arrangement, and size, shape, texture, colour, indumentum, bracteoles (colour, hairiness, texture, shape); calyx, corolla, labellum, staminode, stamen, ovary, fruit, seed and aril.

Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility, and are available at <https://doi.org/10.15468/4c4gag>.

Key to species of Bornean *Boesenbergia* (modified from Sakai and Nagamasu 2009)

- 1 Creeping herbs; shoots normally single-leaved; inflorescence more or less sessile; anther dehiscing by slits 2
- Erect herbs; shoots with one, to many leaves; inflorescence sessile or long pedunculate; anther dehiscing by slits or pores..... 6
- 2 Leaves more or less circular, obtuse or obscurely emarginate at apex.....
..... *B. orbiculata* R.M. Sm
- Leaves elliptic or lanceolate, acute at apex 3
- 3 Leaves plain green 4
- Leaves variegated..... 5
- 4 Corolla tube pubescent outside; flowers not red at throat; labellum entire
..... *B. flavoalba* R.M. Sm
- Corolla tube glabrous outside; flowers red at throat labellum bilobed
..... *B. flavorubra* R.M. Sm
- 5 Petioles 2–3 cm; lamina 7–12 by 2.5–7 cm, dark green with a band of lighter green up the midrib, variegation sometimes extending to the main lateral veins *B. variegata* R.M. Sm
- Petiole to 0.5 cm, lamina 4–8 by 1.5–2 cm, mid green with a broad silver band on either side of the midrib above *B. kerbyi* R.M. Sm
- 6 Fertile shoots single-leaved, rarely bladeless or 2- or 3-leaved..... 7
- Fertile shoots with two or more leaves..... 11
- 7 Lamina 50 by 12 cm or larger..... *B. grandifolia* (Valeton) Merr
- Lamina much smaller, not exceeding 30 cm long..... 8
- 8 Lamina deeply cordate at the base..... *B. cordata* R.M. Sm
- Base of the lamina more or less attenuate not cordate 9
- 9 Petiole 17–34 cm long..... *B. bruneiana* Cowley
- Petiole not exceeding 17 cm..... 10
- 10 Lamina 7–12 cm wide; petiole robust ca. 5 mm thick; lamina with appressed hairs especially around midrib below *B. lambirensis* S. Sakai & Nagam
- Lamina less than 7 cm wide; petiole slender, 2 mm or less thick; leaves glabrous *B. ischonosphon* S. Sakai & Nagam
- 11 Outermost bract forming a bucket or vase-like structure enclosing inflorescence sometimes together with sheaths of upper leaves; lamina large, much longer than 30 cm..... 12
- Leaf base or sheaths not thickened as above, or if thickened, leaves are much shorter 14
- 12 Petiole 42–50 cm long *B. jangarunii* Cowley
- Leaf base long-attenuate forming a winged petiole less than 25 cm long ... 13
- 13 Inflorescence densely pubescent; anther ca. 3 mm long, dehiscing by subapical pores..... *B. hosensis* Cowley
- Plant almost glabrous, anther ca. 10 mm long, dehiscing for ca. 6 mm long (probably dehiscing by slits)..... *B. armeniaca* Cowley

- 14 Leaves larger than 20 by 7 cm; anthers dehiscing by pores **15**
 – Leaves shorter than 20 cm, if longer narrower than 7 cm; anthers dehiscing
 by slits or pores **16**
- 15 Leaf sheath sparsely hairy or glabrous; bracts 5–8 cm; corolla tube 8–10 cm;
 ovary glabrous ***B. grandis* R.M. Sm**
 – Leaf sheath densely hairy; bracts 2–3.5 cm long; corolla tube ca. 5.5 cm long;
 ovary densely hairy in upper half ***B. lysichitoides* S. Sakai & Nagam**
- 16 Inflorescence long-exserted from the leaf sheaths when fully grown, spindle-
 shaped; flowers red and white ***B. pulchella* (Ridl.) Merr**
 – Inflorescence never long-exserted or spindle-shaped; flower colours variable .
 **17**
- 17 Leaves linear, arrangement of blades strongly flabellate **18**
 – Leaves elliptic, lanceolate or rarely linear-lanceolate, arrangement of blades
 never flabellate **19**
- 18 Flowers plain yellow; anther dehiscing by apical pores; bracts 3.5–6.5 cm.....
 ***B. flabellata* S. Sakai & Nagam**
 – Flowers white, yellow in the centre, pink at the base; anther dehiscing by slits;
 bracts up to 3 cm ***B. burttiana* R.M. Sm**
- 19 Leaves variegated **20**
 – Leaves plain green **24**
- 20 Leaves bullate, dark green around main veins and almost silvery on raised
 areas ***B. hutchinsoniana* B.L. Burtt & R.M. Sm**
 – Leaves smooth with a silverish or light green central cloud **21**
- 21 Petiole never exceeding 3 cm, lamina oblanceolate with attenuate base
 ***B. hirta* (Ridl.) Merr**
 – Petiole usually much longer than 3 cm, lamina lanceolate to elliptic with
 cuneate base **22**
- 22 Leaves with a silver cloud; flowers yellow, labellum orange spotted
 ***B. ornata* (N.E. Br.) R.M. Sm**
 – Leaves with yellow cloud, flowers orange or white with some yellow and red-
 dish purple **23**
- 23 Leaves 5–12 by 3–4 cm; flowers orange, darker at base of labellum; anther
 dehiscing throughout its length ***B. aurantiaca* R.M. Sm**
 – Leaves 18–23 by 4–6 cm; flowers white with some yellow and reddish purple;
 anther dehiscing by apical pores, or anther dehiscent only in upper 2/3
 ***B. belalongensis* A.D. Poulsen**
- 24 At least a few uppermost leaf sheaths thickened and forming a cup-shaped
 structure **25**
 – Leaf sheath not thickened as above **26**
- 25 Innermost leaf sheaths enclosing inflorescence much shorter and wider than
 outer ones; leaves drying darkish brown
 ***B. laevivaginata* S. Sakai & Nagam**
 – All leaves with more or less equal laminae; leaves green or grey-green when
 dry ***B. urceoligena* A. D. Poulsen**

- 26 Anther dehiscing by slits throughout their length27
 – Anther dehiscing by pores.....28
 27 Petiole less than 8 cm; bracts ca. 2.6 × 0.4 cm; calyx 3-lobed
*B. imbakensis* S. Sakai & Nagam
 – Petiole more than 10 cm, Calyx tubular.... *B. sugudensis* N.F. Lam, sp. nov.
 28 Lamina wider than 4 cm, petiole 2 cm, lamina 5.2–6.5 × 3.4–3.6 cm
*B. truncata* N.F. Lam, sp. nov.
 – Width of lamina less than 4 cm29
 29 Lamina narrowly lanceolate, lamina 12–20 by 1.5–3 cm; petiole usually
 7–8 cm*B. stenophylla* R.M. Sm
 – Lamina much shorter, up to 12 cm long, if longer, petiole much shorter than
 7 cm30
 30 Leaf sheath and ligule long pubescent *B. parva* (Ridl.) Merr
 – Leaf sheath and ligule almost glabrous31
 31 Flowers yellow-orange.....*B. oligosperma* (K. Schum.) R.M. Sm
 – Flowers white and yellow occasionally with red in throat
*B. subulata* S. Sakai & Nagam

Taxonomy

Boesenbergia sugudensis N.F.Lam, sp. nov.

urn:lsid:ipni.org:names:77306651-1

Figs 1, 2

Diagnosis. The new species resembles *B. imbakensis* S. Sakai & Nagam. in that the leaf sheaths are not thickened and in the anther thecae dehiscing longitudinally, but differs in having a longer petiole (>10 cm vs. 4–7.5 cm) and bilobed apex of calyx (vs. trilobed) (Table 1).

Type. MALAYSIA. Borneo. Sabah. cult. at Kipandi Park, Moyog, 05°54.68'N, 116°06.27'E, 700 m elevation, 12 October 2016, *Lam Nyee Fan 356* (holotype BORH!, isotype SAN). Original material collected by Linus Gokusing (BS-23) at Kampung (Kg.) Sugud, Penampang, Sabah, 05°50.23'N, 116°06.60'E, 50–100 m elevation.

Description. Terrestrial, evergreen, herb. *Rhizome* fibrous, subterranean, ca. 0.8 cm in diameter, base ca. 1.5 cm in diameter, roots white. *Leafy shoots* 44 cm tall, with 2–3 leaves forming a loose pseudostem, erect, ca. 13 cm long, with 2–3 outer leafless sheaths, 3.9–12.5 × 0.8–1.25 cm, green, pubescent on outer surface and glabrous on inner surface, veins 1 mm apart. *Ligule* ca. 0.4 cm long, caudate, brownish green, glabrous. *Petiole* 12–22.5 cm long, canaliculate, green, reddish in lower half. *Lamina* elliptic, 20–22 × 6.5–7.5 cm, erect, dark green above, pale green beneath, glabrous above, pubescent beneath, base rounded, margin entire, glabrous, apex acuminate, with acumen ca. 3 mm. *Inflorescence* ca. 4.7 cm, peduncle 0.8 cm, spike ca. 7.2 × 3 cm. flowers arranged in one-sided spiral, 18 flowers including 5 new buds and 4 old buds, one flower open at a time. *Fertile bracts* linear elliptic, ca. 6.5 cm long, translucent

pubescent on outer surface and glabrous on inner surface, margin entire, apex attenuate. *Bracteoles* elliptic, ca. 3.8×0.8 cm, translucent, pubescent on outer surface and glabrous on inner surface, margin entire, apex acute. *Flower* white, born singly from each bract and bracteole; calyx 1 cm long, tubular, 2-lobed, translucent, pubescent on both surfaces; corolla tube ca. 4.6 cm long, ca. 1.2 mm wide at base, lobes white, glabrous throughout, dorsal lobe ovate-oblong, ca. 1.7×0.45 cm, concave, erect, apex acute, lateral lobes ovate, ca. 1.7×0.3 cm, clasping the labellum and extending 4 mm beyond, apex rounded; labellum, obovate-elliptic, ca. 1.3 cm \times 1 cm curved-backward, with a narrow light red band in the centre lower half, yellow towards apex, glabrous; lateral staminodes white, narrowly obovate, ca. 1.5×0.5 cm, glabrous; stamen white throughout, ca. 5.7 cm long, filament ca. 3.5×1.2 mm (widest at base), pubescent adaxially and abaxially, anther ca. 0.3×0.2 cm, glabrous, anther crest ca. 2×3 mm, bilobed, glabrous, thecae oblong, ca. 0.2×0.1 cm, glabrous, dehiscing longitudinally for its entire length; ovary ca. 5×2 mm, 8.6 cm, stigma cup-shaped, glabrous; epigynous glands two, ca. 0.45 cm long, linear, apex truncate, white. *Fruit* not seen.

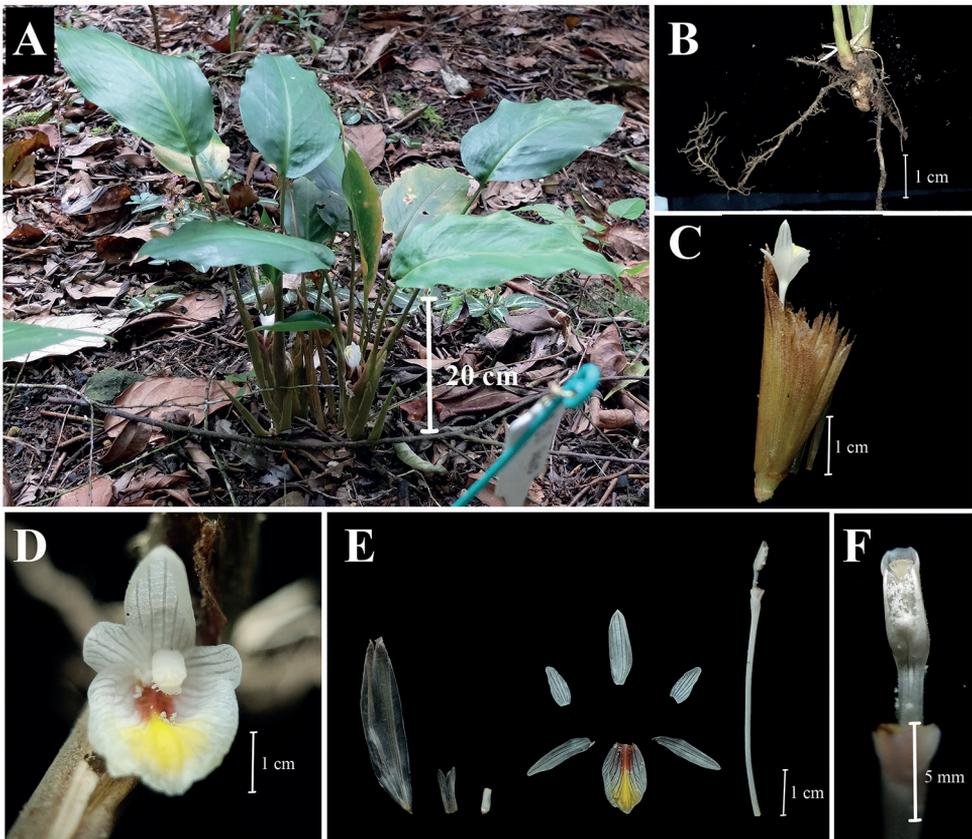


Figure 1. *Boesenbergia sugudensis* **A** habit **B** rhizome and roots **C** spike with one open flower **D** flower **E** bracteole, calyx, corolla lobes, staminodes, labellum, floral tube with stamen **F** stamen, ventral view (Photographed by Lam Nyee Fan).

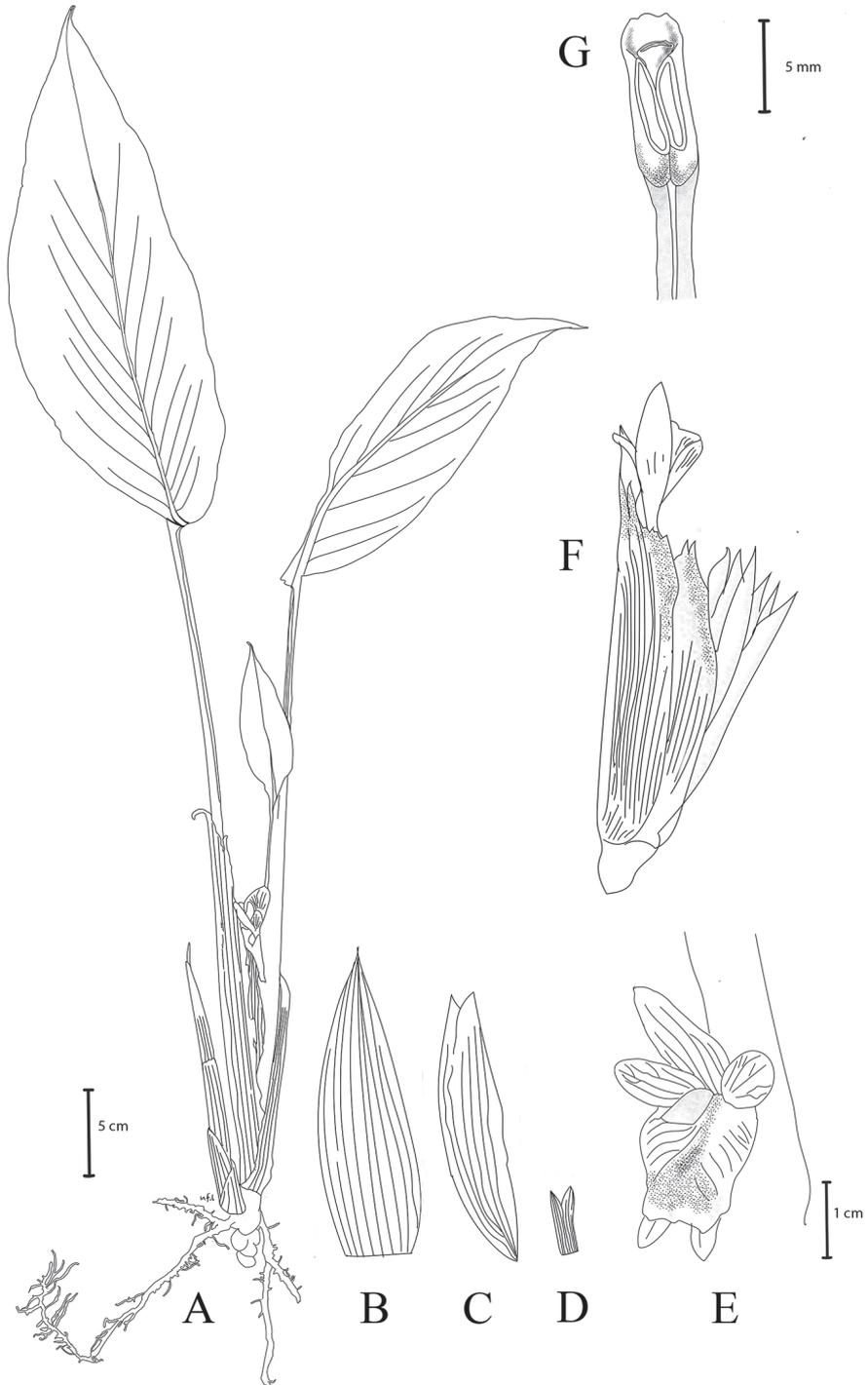


Figure 2. *Boesenbergia sugudensis* Lam N.F., sp. nov. **A** habit **B** bract **C** bracteole **D** calyx **E** flower **F** spike with one open flower **G** stamen, ventral view (Drawing by Lam Nyee Fan). Scale bars: 5 cm (**A**); 1 cm (**B, C, D, E, F**); 5 mm (**G**).

Table 1. Distinguishing morphological characters of *Boesenbergia sugudensis*, *B. imbakensis*, *B. truncata* and *B. orbiculata*.

Characters	Species			
	<i>B. sugudensis</i>	<i>B. imbakensis</i>	<i>B. truncata</i>	<i>B. orbiculata</i>
Plant height (cm)	44	30	11.5	8
Rhizome	Fibrous	Small	Fibrous, sections of rhizome with 1–2 cm long and papery texture bracts	Unknown
Ligule	0.4 cm, caudate, brownish green, glabrous	1 cm, triangular, green, glabrous	0.3 cm, entire, light brown, glabrous	2 cm, bilobed, lobes rounded, glabrous
Petiole	22.5 cm, green, base reddish up to middle part	4–7.5 cm, green, base reddish up to middle	2 cm long, green, base reddish up to middle part	2 cm
Lamina	Elliptic, upper surface dark green, lower surface paler	Narrowly ovate to obovate, plain green	Elliptic, unequal/oblique?, upper surface dark green, lower surface lighter green	sub-orbiculate, upper surface pale green, lower surface light green
Leaf size (cm)	21 × 7.3	11–16 × 3–4	5.2–6.5 × 3.4–3.6	5–8 × 4–7
Leaf base	Rounded	Attenuate	Truncated to sub-cordate	Sub-cordate
Leaf apex	Acuminate, acumen ca. 3 mm	Slightly acuminate, acumen ca. 1–2 mm	Acute, acumen ca. 1 mm	Sub-obicular, obtuse or occasionally shallowly emarginate
Bracts	6.5 cm, translucent white, linear elliptic, glabrous	2.6 × 0.4 cm, narrowly ovate, membranous	1.8 × 3 cm, white, narrowly ovate, pubescent	2.5 cm, boat-shaped, whitish brown, sparsely pubescent,
Calyx	Tubular, apex bilobed, glabrous	Tubular, apex unequally and shallowly 3-lobed, glabrous	Tubular, apex bilobed, pubescent	Unilaterally split, apex bilobed
Labellum	White with narrow light red band from base until the middle, yellow spread towards the lip, 1.3 × 1 cm	White with yellow on the centre and red at the throat, 1.8 × 1.4 cm	White with yellow band at base in the middle, spread towards lip, 0.6 × 0.5 cm	White with deep yellow in the centre and a red mark at the base, 1 × 1 cm
Lateral corolla lobe	Glabrous, white, 1.7 × 0.3 cm, ovate, apex rounded, longer than labellum	Glabrous, white, 1.4 × 0.4 cm	Pubescent, white, 0.3 × 0.1 cm	1 cm long
Anther	Upper and lower surfaces pubescent, 0.6 cm	Glabrous on ventral, shortly pubescent on the dorsal surface, 0.6 cm	Glabrous, 0.4 cm long	Slightly pubescent, 0.4–0.5 cm
Anther dehiscent	Slit	Slit	Pore	Slit
Stigma	Cup-shaped, white, glabrous	Unknown	Emarginate, white, glabrous	Shape and colour unknown, glabrous

Distribution. Endemic in Borneo, Sabah; known only from the type locality, Kg. Sugud.

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

Ecology. Primary forest in lowlands, hill slope at 50–100 m elevation.

Conservation status. Data Deficient (DD). The taxon was assessed using the criteria described in IUCN (2001). The taxon is endemic to Sabah and only found at Kg. Sugud, Penampang, Sabah, Malaysia. One population was observed at the site where specimens were collected.

***Boesenbergia truncata* N.F.Lam, sp. nov.**

urn:lsid:ipni.org:names:77306652-1

Figs 3, 4, 5

Diagnosis. The new species resembles *B. orbiculata* by the short petiole (c. 2 cm long) and the bilobed calyx, but differs in having truncate leaf base, an acute leaf apex (vs.

sub-obicular, obtuse, or occasionally retuse), paired opposite leaves, and lamina parallel to the ground (vs. a single shoot), anther thecae dehiscing by pore (vs. slit), and the lamina slightly narrow (3.4–3.6 cm vs. 4–7 cm) (Table 1).

Type. MALAYSIA. Borneo. Sabah. cult. at Kipandi Park, Moyog, 05°54.68'N, 116°06.27'E, 700 m elevation. 12 October 2016, *Lam Nyee Fan 342* (holotype BORH!, isotype SAN). Original material collected near the park, by Linus Gokusung (BS-09), 100 m west of Kipandi Park, Sabah, 05°52.28'N, 116°14.95'E, 700 m elevation.

Description. Terrestrial, evergreen, herb. *Rhizome* fibrous, subterranean, ca. 2 cm long internodes, base ca. 0.4 cm in diameter, light brown, roots white, ca. 5 cm long. *Leafy shoots* ca. 11.5 cm tall, with erect pseudostem ca. 1.5 cm long, with 1 sheath, ca. 1.4 × 0.6 cm, glabrous, veins 1 mm apart, green with light purple at base, margins entire. *Ligule* 0.3 cm long, entire, light brown, glabrous. *Petiole* 1.4–2 cm long, canaliculate, green, base reddish up to middle. Leafy shoots with two leaves opposite to each other and parallel to the ground. *Lamina* unequal elliptic, 5.2–6.5 × 3.4–3.6 cm, dark green above, lighter green beneath, glabrous, margin entire; base truncated, apex acute with acumen ca. 1 mm. *Inflorescence* ca. 2.5 × 1.5 cm, peduncle ca. 0.45 cm, flowers arranged in one-sided spiral, 8 flowers including one new bud and 1 old bud, one flower open at a time. *Fertile bracts* narrowly lanceolate, ca. 1.8 × 3 cm, white, outer and inner

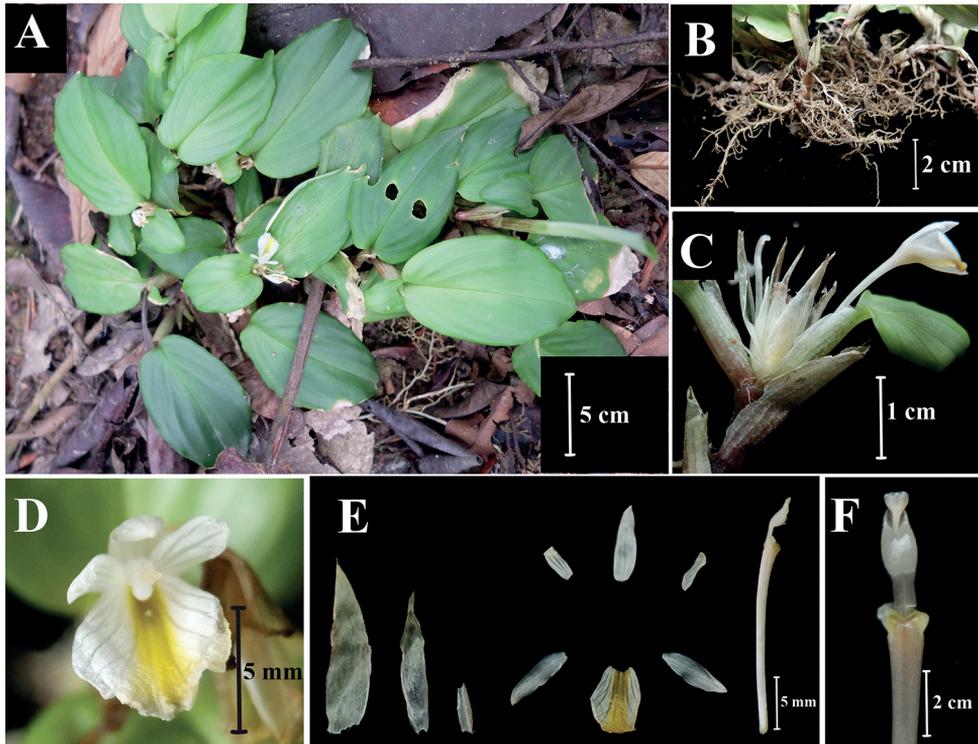


Figure 3. *Boesenbergia truncata* **A** habit **B** rhizome and roots **C** spike with one open flower **D** flower **E** ract, bracteole, calyx, corolla lobes, staminodes, labellum, floral tube with stamen **F** stamen, ventral view (Photographed by Lam Nyee Fan).

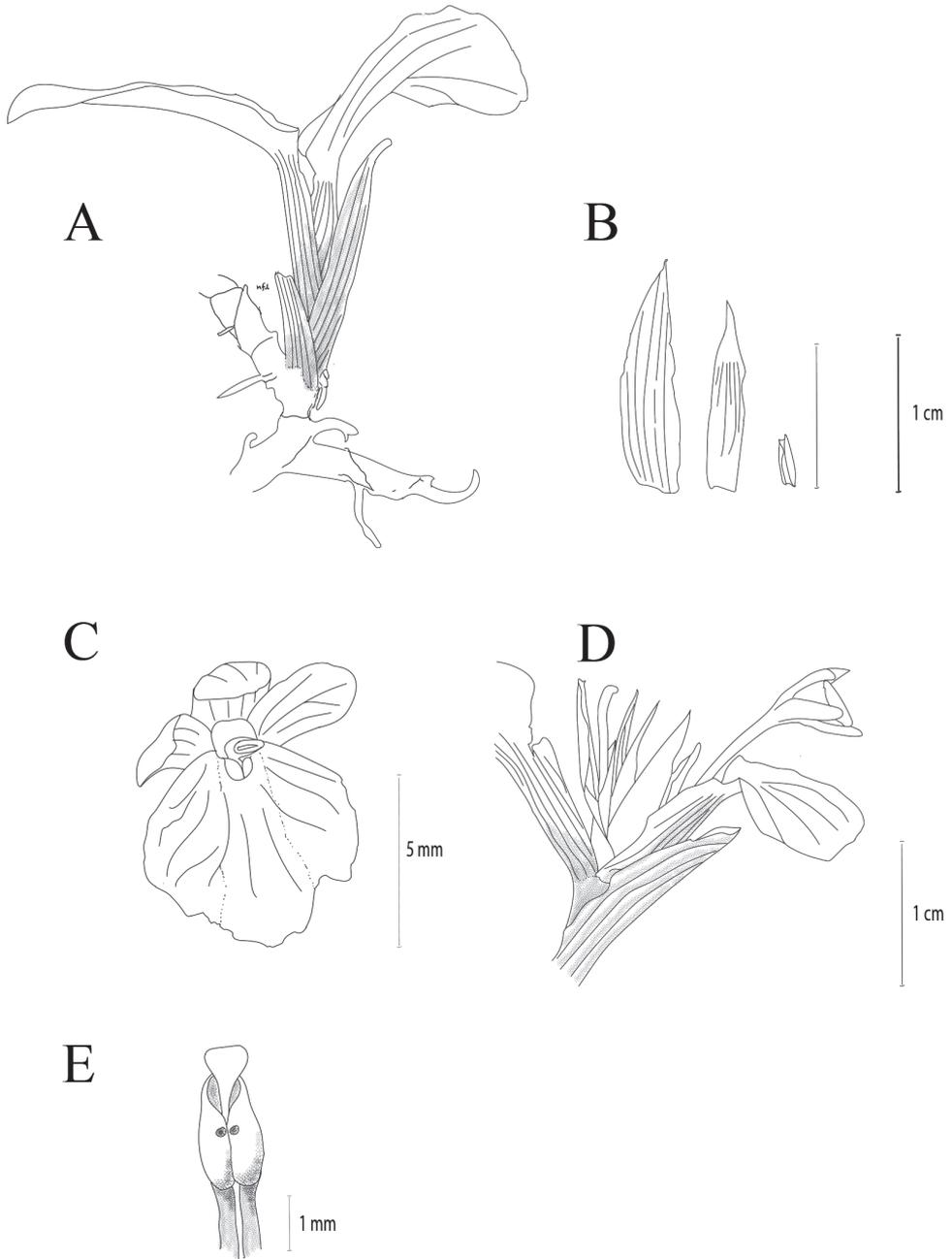


Figure 4. *Boesenbergia truncata* Lam N.F., sp. nov. **A** habit, lateral view **B** bract **C** bracteole **D** calyx **E** flower **F** spike with one open flower **G** stamen, ventral view (Drawing by Lam Nyee Fan). Scale bars: 1 cm (**A**, **B**); 5 mm (**C**); 1 cm (**D**); 1 mm (**E**).



Figure 5. Shape of base of leaf of *Boesenbergia truncata*.

surfaces pubescent, margin entire, apex caudate. *Bracteoles* linear elliptic, ca. 1.5×2 cm, white, outer and inner surfaces pubescent, margin entire, apex acuminate. *Flower* white, born singly from each bract and bracteole, calyx 0.4 cm long, tubular, white, pubescent, corolla tube-white, pubescent, apex acute, dorsal lobe lanceolate, ca. 0.8×0.2 cm, concave, lateral lobes elliptic, ca. 0.6×0.2 cm, labellum obovate, ca. 0.6 cm \times 0.5 cm, yellow band at base in the centre until the apex, curved-backward, lateral staminodes oblong, ca. 0.3×0.1 cm, white, tip rounded, pubescent, stamen ca. 2.3 cm long; filament ca. 4×1 mm (widest at base), glabrous adaxially and abaxially, anther ca. 2 mm long, glabrous; anther crest bilobed, glabrous; thecae oblong, ca. 0.2×0.1 cm, white, pubescent, dehiscing by pore, stigma emarginate, white, glabrous. Fruit not seen.

Distribution. Endemic in Borneo, Sabah; known only from Kipandi Park of Crocker Range.

Etymology. The species epithet refers to truncated leaf base.

Ecology. Primary forest, hill slope at 500–800 m elevation.

Conservation status. Vulnerable (VU D2). The taxon was assessed using criteria described in IUCN (2001). The taxon is endemic to Sabah and only found at Crocker Range, Sabah, Malaysia. There were only 3 populations found at the site of collection. This taxon is not found outside the type locality.

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References

- Cowley J (2000) Three new gingers from Borneo. *Kew Bulletin* 55(3): 669–678. <https://doi.org/10.2307/4118783>
- Ibrahim H (1992) Zingiberaceous species of Tawau Hills Park, Sabah. *A Scientific Journey Through Borneo: Tawau Hills Park, Sabah*. Pelanduk Publications, Selangor, 95–106.
- Kress WJ, Prince LM, Williams KJ (2002) The phylogeny and new classification of the gingers (Zingiberaceae): Evidence from molecular data. *American Journal of Botany* 89(11): 1682–1696. <https://doi.org/10.3732/ajb.89.10.1682>
- Lamb A, Gobilik J, Ardiyani M, Poulsen AD (2013) *A Guide to Gingers of Borneo*. Natural History Publications, Borneo, 144 pp.
- Larsen K (1997) Further studies in the genus *Boesenbergia* (Zingiberaceae). *Nordic Journal of Botany* 17(4): 361–366. <https://doi.org/10.1111/j.1756-1051.1997.tb00330.x>
- Larsen K, Ibrahim H, Khaw SH, Saw LG (1999) *Gingers of Peninsular Malaysia and Singapore*. Natural History Publications, Borneo, 135 pp.
- Mood J, Prince L, Veldkamp J, Dey S (2013). The history and identity of *Boesenbergia longiflora* (Zingiberaceae) and descriptions of five related new taxa. *Gard. Bull. Singapore* 65: 47–95.
- Poulsen AD (1993) Two new species of *Boesenbergia* (Zingiberaceae) from Borneo. *Nordic Journal of Botany* 13(3): 289–294. <https://doi.org/10.1111/j.1756-1051.1993.tb00051.x>
- Saensouk S, Larsen K (2001) *Boesenbergia baimaii*, a new species of Zingiberaceae from Thailand. *Nordic Journal of Botany* 21(6): 595–598. <https://doi.org/10.1111/j.1756-1051.2001.tb00818.x>
- Sakai S, Nagamasu H (2006) Notes on inflorescence structure of *Boesenbergia* (Zingiberaceae). *APG. Acta Phytotaxonomica et Geobotanica* 57(1): 107–111.
- Sakai S, Nagamasu H (2009) Systematic Studies of Bornean Zingiberaceae VI: Three New Species of *Boesenbergia* (Zingiberaceae). *Acta Phytotaxonomica et Geobotanica APG* 60(1): 47–55.
- Sirirugsa P (1992) A revision of the genus *Boesenbergia* Kuntze (Zingiberaceae) in Thailand. *The Natural History Bulletin of the Siam Society* 40: 67–90.
- Smith RM (1987) Review of Bornean Zingiberaceae. III. (Hedychieae). *Notes from the Royal Botanic Garden Edinburgh* 44(2): 203–232.

Molecular and morphological evidence reveals a new fern species of *Hymenasplenium* (Aspleniaceae) from south and southwestern China

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Abstract

Hymenasplenium obtusidentatum, a new fern species of the *H. excisum* subclade of *Hymenasplenium* (Aspleniaceae) from south and southwestern China was described. Molecular phylogenetic analyses and morphological observations of *H. obtusidentatum* and related species clearly indicated that this is a distinct taxonomic entity. Phylogenetically, *H. obtusidentatum* was confirmed to represent a diverging lineage in the *H. excisum* subclade of *Hymenasplenium* and was closely related to one lineage that includes accessions identified as *H. obscurum*, *H. pseudobscurum* and *H. tholiformis*. Morphologically, *H. obtusidentatum* can be distinguished by the combination of its lamina base truncate, stipe not shiny and with color of reddish brown to dark brown, and pinna marginal teeth that are not sharp, but blunt or rounded. A complete species description and comparison with related species in the *H. excisum* subclade were provided. The holotype of *H. obtusidentatum* was designated.

Keywords

Hymenasplenium, *H. excisum* subclade, new taxon, species complex, taxonomy

Introduction

Hymenasplenium Hayata (1927: 712) is one of the two genera in the nearly globally distributed and species-rich fern family Aspleniaceae (Murakami 1995; Murakami et al. 1999; Schneider et al. 2004; PPG I 2016; Xu et al. 2018a, 2020). Compared with

the large genus *Asplenium* L., which is estimated to contain more than 700 species (PPG I 2016; Xu et al. 2020), *Hymenasplenium* consists of only about 60 species (Xu et al. 2018a). Three major clades and several subclades have been recognized in recent phylogenetic studies of this genus (Chang et al. 2018; Xu et al. 2018a). An exploration of species diversity and taxonomy within *Hymenasplenium* is still in progress because of the existence of numerous species complexes (Lin and Viane 2013) and extensive cryptic speciation in this genus (Ching 1965; Xu et al. 2018a; Zhang et al. 2021). Recently, our understanding of *Hymenasplenium* has increased due to the discovery of many new species and taxonomic research in some complex groups, such as *H. unilateral* s. l. and the *H. retusulum*-*H. latidens* groups (Chang et al. 2018; Xu et al. 2018b, c, d, e, 2019a, b; Zhang et al. 2021). However, many species in *Hymenasplenium* are still poorly understood and awaiting further study (Lin and Viane 2013; Chang et al. 2018; Xu et al. 2018a).

The *H. excisum* subclade belongs to one of the six subclades in the Old World clade of *Hymenasplenium* (Xu et al. 2018a). In this subclade, several distinct lineages have been discovered, but only four species have been described and widely accepted: *H. excisum* (C. Presl) S. Lindsay, *H. obscurum* (Blume) Tagawa, *H. pseudobscurum* Viane, and the recently published *H. tholiformis* Liang Zhang, W.B. Ju & K.W. Xu (Lin and Viane 2013; Xu et al. 2018a; Qiu et al. 2022). Xu et al. (2018a) estimated that at least six undescribed species exist in this subclade and numbered four of them from sp8 to sp11, but without taxonomic description and treatment. Of these four described species and undescribed taxa, *H. excisum* and *H. obscurum* were reported to have a large distribution area from south to southwestern China, and tropical Asian to tropical Africa, but the occurrence of the true *H. obscurum* in China has been doubted (Lin and Viane 2013). *Hymenasplenium pseudobscurum* distributes in south and southwestern China, and parts of tropical Asia, such as northern Thailand and Vietnam. *Hymenasplenium tholiformis* is found to be endemic to southeastern Xizang of China. The undescribed sp8 and sp9 have been found only in Yunnan province in southwestern China, and sp10 and sp11 have been found in Hainan province in southern China. This indicates that south to southwestern China is one of the diversity and distribution centers of the *H. excisum* subclade (Lin and Viane 2013). Thus, further exploration of the species diversity and speciation of this group in south and southwestern China is necessary.

As a continued effort to clarify the species diversity and taxonomy of *Hymenasplenium*, we conducted fieldworks in south and southwestern China. During these trips, we collected some specimens of a new taxon of *H. excisum* subclade that were obviously different from all other species of the subclade in both morphology and phylogeny analyses. To clarify its taxonomic status, we studied the morphological differences between this taxon and related species and investigated the distinction of this putative new species by analyzing chloroplast DNA sequences for members of the monophyletic paleotropical clade of *Hymenasplenium* (Murakami and Moran 1993; Murakami and Schaal 1994; Murakami 1995; Gabancho and Prada 2011; Xu et al. 2018a). Here, we present our results, provide a description of this new taxon, and compare it with related species in the *H. excisum* subclade.

Materials and methods

Morphological studies

The morphological characteristics of the new species were observed in the field. Herbarium specimens of *Hymenasplenium* at PE, KUN, HITBC and PYU were studied. Digital specimens of related species of the new taxon were examined from the online databases of CVH (<https://www.cvh.ac.cn/>) and JSTOR Global Plants (<https://plants.jstor.org/>).

Spore counting and imaging

Spores were obtained from newly collected specimens and examined with an Olympus BX-51 light microscope to examine aborted spores and determine the number of spores per sporangium. Mature sporangia from each specimen were removed and ruptured with a needle tip. The number of spores per sporangium was counted. The presence of 64 spores per sporangium was considered an indicator of the absence of apomictic reproduction (Wagner and Chen 1965; Dyer et al. 2012). Spore ornamentation was examined using a tabletop scanning electron microscope (ZEISS EVO LS 10) with spores sputter-coated with gold particles.

Chloroplast DNA sequencing and phylogenetic analyses

To clarify the phylogenetic position of the new species, the taxa were sampled to include representatives of all the six subclades in the Old World clade of *Hymenasplenium* (Xu et al. 2018a). DNA sequences of plastid markers of 72 accessions representing 29 species of *Hymenasplenium* were sampled. Three species of the New World clades were used as outgroups. Voucher information and GenBank accession numbers for each sampled taxon were provided in Appendix 1.

Genomic DNA was extracted from the silica gel-dried leaf material of each sampled individual using the modified CTAB method (Doyle and Doyle 1987). Three regions of the chloroplast genome were amplified and sequenced using established primers and protocols, including the *rbcL* gene (Taberlet et al. 1991), *rps4-trnS* region (partial *rps4* gene and *rps4-trnS* intergenic spacer) (Schneider et al. 2005), and *trnH-psbA* intergenic spacer (Ebihara et al. 2010). These chloroplast regions were selected because they have previously been successfully used to assess relationships in *Hymenasplenium* (Murakami et al. 1999; Schneider et al. 2004; Xu et al. 2018a). For each of the three chloroplast regions, identical sequences of specimens from the same location were reduced to a single exemplar sequence and deposited in GenBank (see Appendix 1 for accession numbers).

Sequences were edited using the Staden Package (Staden et al. 2000), automatically aligned with Clustal X (Thompson et al. 1997), and manually corrected in BioEdit v.7.0.1 (Hall 2004). Special attention was given to the detection of ambiguously aligned regions and putative sequencing errors. A combined *rbcL*, *rps4-trnS*, and *trnH-psbA* chloroplast

alignment was constructed. The sequences of the three cpDNA fragments were combined because they were inherited together. Ambiguous indels were excluded, and unambiguous indels were coded and scored using GapCoder (Young and Healy 2003). The datasets comprising sequences from the three chloroplast regions were analyzed using maximum parsimony (**MP**), maximum likelihood (**ML**) and Bayesian inference (**BI**). Maximum parsimony analyses were carried out in PAUP* 4.0b10 (Swofford 2002) using the heuristic search mode, 1,000 random starting replicates, and TBR branch swapping, with MULTREES and Collapse on. Bootstrap values were estimated using 1,000 bootstrap replicates under the heuristic search mode, each with 100 random starting replicates. Maximum likelihood analyses were carried out in PhyML 3.0 (Guindon et al. 2010) using default settings, and the best fit models for the parameter-based analyses were selected using jModelTest (Posada 2008) with the Akaike information criterion (Akaike 1974). Parameter values were estimated simultaneously with the analyses. Bayesian inference was carried out in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with four chains and the model selected by jModelTest with the Akaike information criterion (Akaike 1974). Chains were run for two million generations, and trees were sampled every 100 generations. Convergence was evaluated by examining the standard deviation of split frequencies among runs and by plotting the log-likelihood values from each run using TRACER v.1.4 (Rambaut and Drummond 2007). The remaining trees were used to calculate a 50% majority-rule consensus topology and posterior probabilities (**PP**).

Results

Morphological comparison and spore counting

Like most species in *Hymenasplenium*, the new species has a long-creeping rhizome, once-pinnate laminae, asymmetrical pinnae, and elliptic to reniform spores. However, the new species can be distinguished from other species in the genus by the combined characteristics of truncate lamina base, reddish brown to dark brown stipe and rachis, stipe not shiny and with scale or subglabrous, and pinna marginal teeth not sharp, but blunt or rounded (Figs 1, 2). In contrast, the closely related species *H. obscurum* and *H. pseudobscurum* have not shiny but dull green to grayish green stipe, whereas *H. excisum* and *H. tholiformis* have shiny and dark purple to black stipe. The comparison of morphological characteristics to differentiate the described species in the *H. excisum* subclade is shown in Table 1. The materials of the new taxon examined in this study contained 64 spores in each sporangium. Thus, *H. obtusidentatum* may not be an apogamous species.

Chloroplast DNA phylogeny

The total length of the concatenated *rbcL*, *rps4-trnS*, and *trnH-psbA* alignment was 2527 bp. The alignment contained 138 variable characters, 98 of which were



Figure 1. *Hymenasplenium obtusidentatum* **A** habit **B, C** plant **D** pinnae in middle part of leaf **E** scale **F** spore.

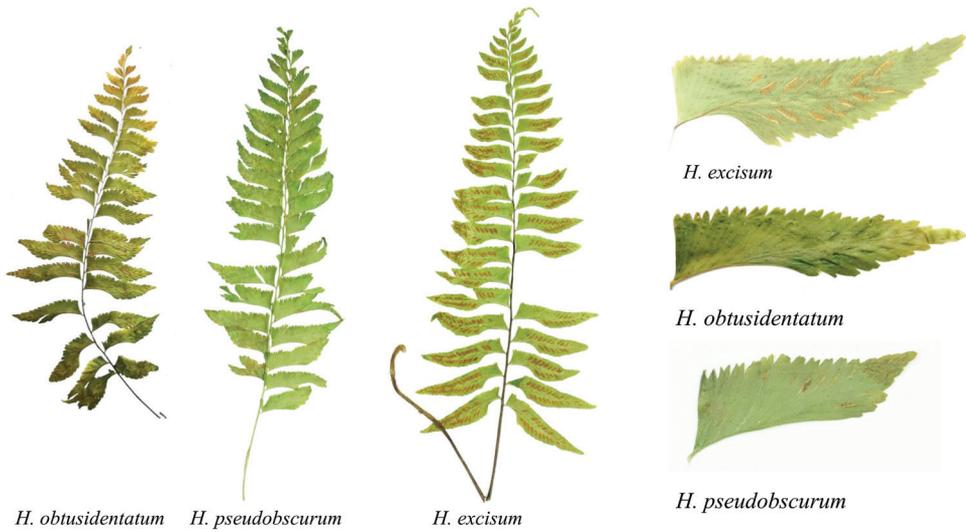


Figure 2. Comparison of frond sketches of representative specimens available (*H. obtusidentatum*: Chang1258, *H. pseudobscurum*: Chang1010, *H. excisum*: Chang992) for species of the *Hymenasplenium excisum* subclade.

Table 1. Comparison of morphological characters to differentiate the new taxon (*Hymenasplenium obtusidentatum*) and the four described species in the *H. excisum* subclade.

Characters	<i>H. obtusidentatum</i>	<i>H. excisum</i>	<i>H. obscurum</i>	<i>H. pseudobscurum</i>	<i>H. tholiformis</i>
Size of lamina	15–30 × 8–15 cm	20–40 × 10–15 cm	20–30 × 5–10 cm	20–25 × 5–10 cm	13–16 × 3–5 cm
Lamina base	truncate	truncate and widest	truncate	truncate	truncate
Rhizome size	2–4 mm in diam.	3–5 mm in diam.	3–5 mm in diam.	3–5 mm in diam.	2 mm in diam.
Stipe color	not shiny, reddish brown to dark brown	shiny, dark purple to black	not shiny, dull green to grayish green	not shiny, dull green to grayish green	shiny, black purple
Pinna shape	trapeziform to falcate	trapeziform to falcate	trapeziform to falcate	trapeziform to falcate	trapeziform
Size of middle pinnae	3.5–8 × 1–1.8 cm	5–10 × 1.3–2 cm	3.5–7 × 0.8–1.3 cm	2.5–4 × 0.8–1.8 cm	2.5–8 × 0.6–1 cm
Shape of pinna apex	acute or rarely obtuse	acute to obtuse	obtuse to subacute	obtuse to subacute	round
Pinnae marginal teeth	blunt or rounded	sharp	sharp to blunt	sharp to blunt	sharp
Number of basicopic veins lacking	4–6	(3 or) 4–6	3–5	3–5	3–4
Sori position	inframedial to medial	medial	medial	medial to supramedial	medial
Indusium	single	single	single	double	single

parsimony informative. The three phylogenetic analyses, MP, ML, and BI, of the combined chloroplast dataset recovered similar topologies (Fig. 3). No significant conflict (bootstrap value > 70%) was detected among the topologies obtained via the separate phylogenetic analyses.

The *H. excisum* subclade recovered in paleotropical *Hymenasplenium* included several well-supported lineages but represented only four described species (Fig. 3). One lineage (BS = 92%, BS = 98%, BI p = 1) was comprised of accessions identified as *H. obscurum* and *H. pseudobscurum*. This lineage formed a separate clade with

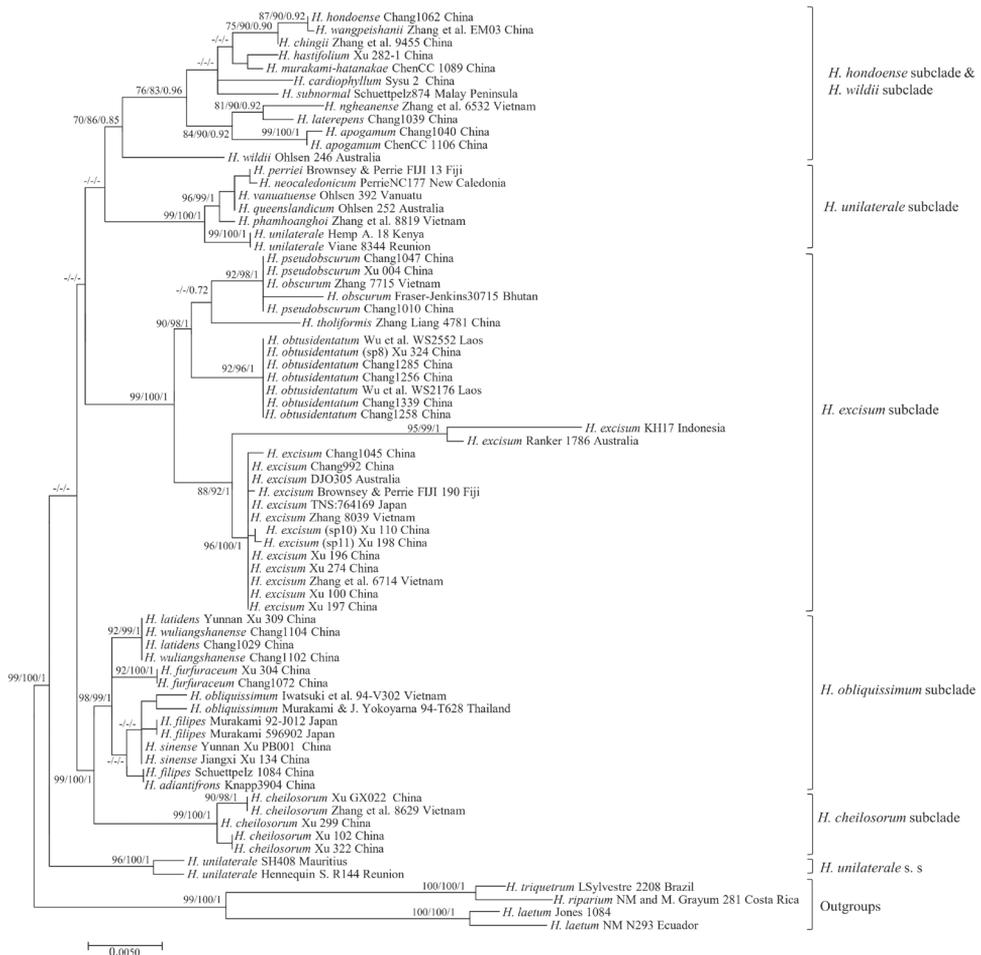


Figure 3. Maximum likelihood phylogeny based on the concatenated plastid DNA sequence dataset. Maximum parsimony and Bayesian analyses recovered identical topologies with respect to the relationships among the main clades of the paleotropical *Hymenasplenium*. For each node, the following values are provided: maximum parsimony bootstrap (%) / maximum likelihood bootstrap (%) / and posterior confidence (p-value). Columns on the right refer to the subclades described in Xu et al. (2018a). Outgroup taxa are shown as a sister to the paleotropical *Hymenasplenium*.

H. tholiformis, but lacked support. Another lineage (BS = 92%, BS = 96%, BI $p = 1$) was comprised of accessions of the new taxon in this study including collections identified as a proposed new species (sp8, Zhang Liang 4781) and *H. obscurum* (Wu et al. WS2552; Wu et al. WS2176) in former studies (Xu et al. 2018a; Qiu et al. 2022). There was one more lineage (BS = 88%, BS = 92%, BI $p = 1$) that was comprised of several divergent lineages, but included accessions all identified as *H. excisum*.

Discussion

In the present study, the *Hymenasplenium excisum* subclade was found to be polyphyletic (Fig. 3), as previously suspected (Murakami 1995; Chang et al. 2018; Xu et al. 2018a). Except for described species, such as *H. excisum*, *H. obscurum*, *H. pseudobscurum*, and *H. tholiformis*, undescribed species are supposed to exist in this subclade (Xu et al. 2018a). The new taxon presented in this study referred to one of the four numbered species (sp8–sp11) in the *H. excisum* subclade proposed by Xu et al. (2018a) in a phylogenetic study of *Hymenasplenium*. The founding of this new species is consistent with the results of the previous studies that cryptic speciation exists and reinforces that urgent study is necessary for species diversity and delimitation within *Hymenasplenium* (Chang et al. 2018; Xu et al. 2018a).

Phylogenetically, the monophyly of *H. obtusidentatum* is strongly supported by the chloroplast sequences analyses (Fig. 3). Morphologically, the distinction of *H. obtusidentatum* is also well reflected. The gross morphology of *H. obtusidentatum* is similar to *H. excisum*, with regard to the laminae and pinna shape, as well as sori arrangement, but differs in the truncate lamina base, not shiny and thinner stipe with color of reddish brown to dark brown, and blunt or rounded pinna marginal teeth (Table 1; Figs 1, 2). Within the *H. excisum* subclade, *H. obtusidentatum* can also be distinguished from *H. obscurum* and *H. pseudobscurum* by not having dull green to grayish green stipe and double indusium. Another species, *H. bivalvatum* (B. K. Nayar & Geevarghese) Viane, was reported to be morphologically similar to *H. pseudobscurum* which also has double indusium but with shiny and dark purple to black stipe, but the occurrence of this taxon in China still needs to be verified (Lin and Viane 2013). The other recently published new species, *H. tholiformis*, has also shiny and dark purple to black stipe but with single indusium. *Hymenasplenium obtusidentatum* can also be easily distinguished from these two species by having reddish brown to dark brown but not shiny stipe. The distribution of this newly described taxon is currently known in south and southwestern China, and extending to adjacent areas such as Laos, commonly growing together with *H. excisum* on slopes near streams in half-shaded forests.

Though *H. obtusidentatum* is distributed together with *H. excisum* and is morphologically similar to *H. excisum*, this new taxon was found to be closely related to *H. obscurum* and *H. pseudobscurum* in the chloroplast phylogenetic analyses. Different ploidy levels, including diploid, tetraploid, and even hexaploid, are found in *H. excisum*, *H. obscurum* and *H. pseudobscurum*. Besides, reticulations and allopolyploidizations were assumed to occur in *Hymenasplenium* (Chang et al. 2018; Zhang et al. 2021). Thus, the

relationships between the new taxon and the remaining species in the *H. excisum* subclade are worth further research. Future studies of multiple biparentally inherited nuclear DNA markers to enhance our ability to trace the evolutionary history of these ferns using statistically consistent methodology, such as coalescence analyses, are warranted.

Taxonomic treatment

Hymenasplenium obtusidentatum Y.Fen Chang & G.Cheng Zhang, sp. nov.

urn:lsid:ipni.org:names:77306994-1

Type. CHINA. Yunnan Province, Xishuangbanna, Menghai: Chang1258. 2019. (holotype, HITBC; isotype, KUN) (Figs 2, 3).

Description. Plants 25–40 cm tall. Rhizomes long creeping, 2–4 mm in diameter, apex scaly; scales brown, lanceolate to triangular, entire. Fronds remote, up to 6 mm apart, grayish green when dry, herbaceous; stipe reddish brown to dark brown, 10–20 cm long, base ca. 1–1.5 mm in diam., subglabrous, base with sparse scales similar to those on rhizome; lamina one-pinnate, narrowly triangular to triangular, 15–30 × 8–15 cm, base truncate and reduced, apex acuminate to caudate; rachis reddish brown to dark brown, subglabrous; pinnae 15–21 pairs, trapeziform to falcate, basal pinnae nearly opposite, middle pinnae 3.5–8 × 1–1.8 cm, dimidiate, apex obtuse to acute, base asymmetrical, acroscopic side truncate and often almost parallel to rachis, basiscopic side of basal pinnae excavate, in middle pinnae narrowly cuneate and entire, acroscopic margin serrate, teeth blunt or rounded; pinnae spreading to ascending. Veins forking and terminating in marginal teeth, basiscopic side with 4–6 veins lacking. Sori inframedial to medial, linear, 3–5 mm, indusia persistent, pale brown, membranous, entire, opening toward the costa. Spores elliptic to reniform, perispore fimbriate-alate, 38–43 µm in diam.; 64 spores per sporangium.

Diagnosis. *Hymenasplenium obtusidentatum* is similar to *H. excisum*. However, *H. obtusidentatum* has a thinner rhizome and stipe, not shiny and reddish brown to dark brown stipe and rachis, blunt or rounded pinna marginal teeth, and truncate lamina base.

Distribution and habitat. *Hymenasplenium obtusidentatum* is currently known to coexist with *H. excisum* in south and southwestern China, and adjacent areas. It occurs in soil or on rocks near streams in half-shaded forests at alt. 1000–1500 m.

Additional specimens examined. China. Yunnan Province, Menghai County, 2019; Chang1256, Chang1260; Chang1261, Chang1262; Chang1339 (HITBC); Pu'er City, Pu'er Sun River National Forest Park, 2018; Xu 324 (SYS). Hainan Province, Ledong County, 2019; Chang1284, Chang1285; Chang1286 (HITBC).

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References

- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716–723. <https://doi.org/10.1109/TAC.1974.1100705>
- Chang YF, Hori K, Murakami N, Cao LM, Lu SG, Schneider H (2018) Validation of *Hymenasplenium laterepens* (Aspleniaceae): Evidence from morphology and molecular analyses. *Phytotaxa* 374(4): 277–290. <https://doi.org/10.11646/phytotaxa.374.4.1>
- Ching RC (1965) *Asplenium unilaterale* Lam. and its confused species in China. *Journal of Systematics and Evolution* 10(3): 183–191.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* 19: 11–15.
- Dyer RJ, Savolainen V, Schneider H (2012) Apomixis and reticulate evolution in the *Asplenium monanthes* fern complex. *Annals of Botany* 110(8): 1515–1529. <https://doi.org/10.1093/aob/mcs202>
- Ebihara A, Nitta JH, Ito M (2010) Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. *PLoS ONE* 5(12): e15136. <https://doi.org/10.1371/journal.pone.0015136>
- Gabancho LR, Prada C (2011) The genus *Hymenasplenium* (Aspleniaceae) in Cuba, including new combinations for the Neotropical species. *American Fern Journal* 101(4): 265–281. <https://doi.org/10.1640/0002-8444-101.4.265>
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59(3): 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Hall T (2004) BioEdit 7.0.1. Department of Microbiology, North Carolina State University. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* (Oxford, England) 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Lin YX, Viane R (2013) Aspleniaceae. In: Wu ZY, Raven PH, Hong DY (Eds) *Flora of China*, vol. 2–3. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 267–316.
- Murakami N (1995) Systematics and evolutionary biology of the fern genus *Hymenasplenium* (Aspleniaceae). *Journal of Plant Research* 108(2): 257–268. <https://doi.org/10.1007/BF02344351>
- Murakami N, Moran RC (1993) Monograph of the neotropical species of *Asplenium* sect. *Hymenasplenium* (Aspleniaceae). *Annals of the Missouri Botanical Garden* 80(1): 1–38. <https://doi.org/10.2307/2399820>
- Murakami N, Schaal BA (1994) Chloroplast DNA variation and the phylogeny of *Asplenium* sect. *Hymenasplenium* (Aspleniaceae) in the new world tropics. *Journal of Plant Research* 107(3): 245–251. <https://doi.org/10.1007/BF02344251>

- Murakami N, Nogami S, Watanabe M, Iwatsuki K (1999) Phylogeny of Aspleniaceae inferred from *rbcL* nucleotide sequences. *American Fern Journal* 89(4): 232–343. <https://doi.org/10.2307/1547233>
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- PPG I (2016) A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54(6): 563–603. <https://doi.org/10.1111/jse.12229>
- Qiu YL, Xu KW, Ju WB, Zhao WL, Zhang L (2022) *Hymenasplenium tholiformis* (Aspleniaceae), a new fern species from southeastern Xizang, China based on morphological and molecular evidence. *PhytoKeys* 204: 43–56. <https://doi.org/10.3897/phytokeys.204.85746>
- Rambaut A, Drummond AJ (2007) Tracer 1.4. <http://beast.bio.ed.ac.uk/Tracer>
- Schneider H, Russell SJ, Cox CJ, Bakker F, Henderson S, Rumsey F, Barrett J, Gibby M, Vogel J (2004) Chloroplast phylogeny of Asplenioid ferns based on *rbcL* and *trnL-F* spacer sequences (Polypodiidae, Aspleniaceae) and its implications for biogeography. *Systematic Botany* 29(2): 260–274. <https://doi.org/10.1600/036364404774195476>
- Schneider H, Ranker TA, Russell SJ, Cranfill R, Geiger JMO, Agurauja R, Wood KR, Grundmann M, Kloberdanz K, Vogel JC (2005) Origin of the endemic fern genus *Diellia* coincides with the renewal of Hawaiian terrestrial life in the Miocene. *Proceedings of the Royal Society of London: Series B, Biological Sciences* 272: 455–460. <https://doi.org/10.1098/rspb.2004.2965>
- Staden R, Beal KF, Bonfield JK (2000) The staden package, 1998. *Bioinformatics Methods and Protocols* 132: 115–130. <https://doi.org/10.1385/1-59259-192-2:115>
- Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17(5): 1105–1109. <https://doi.org/10.1007/BF00037152>
- Thiers B (2016) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <https://doi.org/10.1007/s12228-016-9423-7>
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25(24): 4876–4882. <https://doi.org/10.1093/nar/25.24.4876>
- Wagner WH, Chen KL (1965) Abortion of spores and sporangia as a tool in the detection of *Dryopteris* Hybrids. *American Fern Journal* 55(1): 9–29. <https://doi.org/10.2307/1546429>
- Xu KW, Zhou XM, Yin QY, Zhang L, Lu NT, Knapp R, Luong TT, He H, Fan Q, Zhao WY, Gao XF, Liao WB, Zhang LB (2018a) A global plastid phylogeny uncovers extensive cryptic speciation in the fern genus *Hymenasplenium* (Aspleniaceae). *Molecular Phylogenetics and Evolution* 127: 203–216. <https://doi.org/10.1016/j.ympev.2018.05.021>
- Xu KW, Zhang L, Lu NT, Zhou XM, He H, Luong TT, Knapp R, Liao WB, Zhang LB (2018b) Nine new species of *Hymenasplenium* (Aspleniaceae) from Asia. *Phytotaxa* 358(1): 1–25. <https://doi.org/10.11646/phytotaxa.358.1.1>

- Xu KW, Zhou XM, Zhang LB, Liao WB (2018c) *Hymenasplenium hastifolium* sp. nov. (Aspleniaceae) from a karst cave in western Guangxi, China. *Phytotaxa* 333(2): 281–286. <https://doi.org/10.11646/phytotaxa.333.2.13>
- Xu KW, Zhou XM, Liao WB, Zhang LB (2018d) *Hymenasplenium perriei* (Aspleniaceae), a new fern species from Fiji. *Phytotaxa* 356(2): 177–180. <https://doi.org/10.11646/phytotaxa.356.2.8>
- Xu KW, Lu JM, Zhang X, Zhang LB (2018e) *Hymenasplenium pubirhizoma* comb. nov. (Aspleniaceae) from China. *Phytotaxa* 351(2): 186–188. <https://doi.org/10.11646/phytotaxa.351.2.8>
- Xu KW, Chen CW, Kamau P, Liao WB, Zhang LB (2019a) Four new species of the fern genus *Hymenasplenium* (Aspleniaceae) from Africa and Asia. *Phytotaxa* 416(1): 34–42. <https://doi.org/10.11646/phytotaxa.416.1.4>
- Xu KW, Lorence D, Wood KR, Liao WB, Zhang LB (2019b) A revision of the *Hymenasplenium unilaterale* subclade (Aspleniaceae; Pteridophyta) with the description of nine new species. *Phytotaxa* 419(1): 1–27. <https://doi.org/10.11646/phytotaxa.419.1.1>
- Xu KW, Zhang L, Rothfels C, Smith A, Viane R, Wood K, Chen CW, Knapp R, Zhou L (2020) A global plastid phylogeny of the fern genus *Asplenium* (Aspleniaceae). *Cladistics* 36(1): 22–71. <https://doi.org/10.1111/cla.12384>
- Young ND, Healy J (2003) GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4(1): 1–6. <https://doi.org/10.1186/1471-2105-4-6>
- Zhang GC, Hong HF, Chen GH, Lu SG, Chang YF (2021) Species delimitation of *Hymenasplenium obliquissimum* group (Aspleniaceae) in southwestern China. *Phytotaxa* 480(1): 29–44. <https://doi.org/10.11646/phytotaxa.480.1.3>

Appendix I

Voucher specimens and GenBank accession numbers for the DNA sequences used in this study. Information is presented in the following order: species, voucher, locality, GenBank numbers for *rbcL*, *rps4* & *rps4-trnS*, *trnH-psbA*. “*” represents the newly published sequences in this study; “–” represents no data. Herbaria acronyms follow Index Herbariorum (Thiers 2016).

Hymenasplenium adiantifrons (Hayata) Viane & S. Y. Dong; Knapp 3904 (P); Taiwan, China; –, MH065323, –. *H. apogamum* (N. Murak. & Hatan.) N. Murak. & Hatan.; Chang1040 (HITBC); xishuangbanna, Yunnan, China; MH884808, MH884830, MH884838. *H. apogamum*; ChenCC1106 (HITBC); Taiwan, China; MH884813, MH884832, MH884839. *H. cardiophyllum* (Hance) Nakaike; Sysu 2; Cult. in Sun-yat Sen University; MH065387, MH065306, –. *H. cheilosorum* (Kunze ex Mettenius) Tagawa; Xu 102 (SYS); Hainan, China; MH065385, MH065346, –. *H. cheilosorum*; Xu 299 (SYS); Guangxi, China; MH065402, MH065350, –. *H. cheilosorum*; Xu 322 (SYS); Yunnan, China; MH065405, MH065352, –. *H. cheilosorum*; Xu GX022 (SYS); Guangxi, China; MH065381, MH065343, –. *H. cheilosorum*; Zhang et al. 8629 (CDBI, MO, PHH); Lam Dong, Vietnam;

MH065415, MH065357, –. *H. chingii* K. W. Xu, Li Bing Zhang & W. B. Liao; Zhang et al. 9455 (CDBI); Guizhou, China; MH065430, MH065333, –. *H. excisum* (C. Presl) S. Lindsay; Brownsey & Perrie FIJI 190 (WELT); Fiji; KP774884, KP851882, –. *H. excisum*; Chang1045 (HITBC); Yunnan, China; OP375802*, OP375808*, –. *H. excisum*; Chang992 (HITBC); Yunnan, China; OP375801*, OP375807*, –. *H. excisum*; DJ0305; Kaaruu Creek, Queensland, Australia; KP774930, –, KP851883. *H. excisum*; Xu 274 (SYS); Guangdong, China; MH065389, –, –. *H. excisum*; KH17; Indonesia; GU586828, –, –. *H. excisum*; Ranker 1786 (COLO); Hawaii; AY549728, –, –. *H. excisum*; TNS:764169; Japan; AB574888, –, –. *H. excisum*; Xu 100 (SYS); Hainan, China; MH065383, MH065344, –. *H. excisum*; Xu 110 (SYS); Hainan, China; MH065384, MH065345, –. *H. excisum*; Xu 198 (SYS); Hainan, China; MH065394, MH065341, –. *H. excisum*; Xu 196 (SYS); Hainan, China; MH065390, –, –. *H. excisum*; Xu 197 (SYS); Hainan, China; MH065391, –, –. *H. excisum*; Zhang et al. 6714 (CDBI, MO, VNMN); Bac Kan, Vietnam; MH065436, MH065375, –. *H. excisum*; Zhang et al. 8039 (CDBI, MO, VNMN); Quang Nam, Vietnam; MH065419, MH065361, –. *H. filipes* (Copeland) Sugimoto, Murakami 596902 (TI); Kagoshima, Japan; U30605, –, –. *H. filipes*; Murakami 92-J012; Kagoshima, Japan; AB016176, –, –. *H. filipes*; Schuettpelz_1084 (HITBC); Taiwan, China; MW194197, –, –. *H. furfuraceum* (Ching) Viane & S. Y. Dong; Chang1072 (HITBC); Yunnan, China; MW194198, MW194221, MW194182. *H. furfuraceum*; Xu 304 (SYS); Yunnan, China; MH065406, MH065353, –. *H. hastifolium* Ke Wang Xu, Li Bing Zhang & W. B. Liao; Xu 282-1 (SYS); Guangxi, China; MH065398, MH065313, –. *H. hondoense* (N. Murak. & Hatan.) Nakaike; Chang1062 (HITBC); zhaotong, Yunnan, China; MH884814, MH884833, MH884840. *H. laetum* (Sw.) L. Regalado & Prada; Jones 1084 (TUR); KJ628715, –, –. *H. laetum*; NM N293; Ecuador; AB014707, –, –. *H. laterepens* N. Murak. & X. Cheng ex Y.Fen Chang & K. Hori; Chang1039 (HITBC); xishuangbanna, Yunnan, China; MH884807, MH884829; MH884837. *H. latidens* (Ching) Viane & S. Y. Dong; Chang1029 (HITBC); Yunnan, China; MW194204, MW194219, MW194180. *H. latidens* (Ching) Viane & S. Y. Dong; Xu 309 (SYS); Yunnan, China; MH065407, MH065318, –. *H. murakami-batanakae* Nakaike; ChenCC1089 (HITBC); Taiwan, China; MH884823, –, –. *H. neocaledonicum* Li Bing Zhang & K. W. Xu; PerrieNC177 (WELT); New Caledonia; KP774896, KP851878, –. *H. ngbeanense* Li Bing Zhang, K. W. Xu & N. T. Lu; Zhang et al. 6532 (CDBI, MO, VNMN); Phu Tho, Vietnam; MH065426, MH065331, –. *H. obliquissimum* (Hayata) Sugimoto; Iwatsuki et al. 94-V302; Hoang Lien Son, Vietnam; AB016187, –, –. *H. obliquissimum*; Murakami & J. Yokoyarna 94-T628; Chiang Mai, Thailand; AB016178, –, –. *H. obscurum* (Blume) Tagawa; Fraser-Jenkins30715; Bhutan; MH884826, MH884834, –. *H. obscurum*; Zhang et al. 7715 (CDBI, MO, VNMN); Thanh Hoa, Vietnam; MH065411, MH065354, –. *H. obscurum*; Wu et al. WS2176 (MO); Xiangkhoang, Laos; ON85986, ON859875, –. *H. obscurum*; Wu et al. WS2552 (MO); Louangphrabang, Laos; ON859870, ON859876, –. *H. obtusidentatum* Y.Fen Chang & G.Cheng Zhang; Chang1256 (HITBC); Yunnan, China; OP375803*, OP375809*,

OP375813*. *H. obtusidentatum*; Chang1258 (HITBC); Yunnan, China; OP375804*, OP375810*, OP375814*. *H. obtusidentatum*; Chang1285 (HITBC); Hainan, China; OP375805*, OP375811*, OP375815*. *H. obtusidentatum*; Chang1339 (HITBC); Yunnan, China; OP375806*, OP375812*, OP375816*. *H. perriei* Li Bing Zhang & K. W. Xu; Brownsey & Perrie FIJI 13 (WELT); Fiji; KP774885, KP851880, –. *H. phamboanghoi* Li Bing Zhang, K. W. Xu & T. T. Luong; Zhang et al. 8819 (CDBI, MO, PHH); Khanh Hoa, Vietnam; MH065432, MH065334, –. *H. pseudobscurum* Viane; Chang1010 (HITBC); Yunnan, China; MH884827, MH884835, MH884841. *H. pseudobscurum*; Chang1047 (HITBC); Yunnan, China; MH884828, MH884836, MH884842. *H. pseudobscurum*; Xu 004 (SYS); Hong Kong, China; MH065380, MH065342, –. *H. queenslandicum* Li Bing Zhang & K. W. Xu; Ohlsen 252 (MELU); Queensland, Australia; KP774849, KP851879, –. *H. riparium* (Liebm.) L. Regalado & Prada; NM and M. Grayum 281; Virgen del Socorro, Costa Rica; AB014708, –, –. *H. sinense* K. W. Xu, Li Bing Zhang & W. B. Liao; Xu 134 (SYS); Jiangxi, China; MH065388, MH065348, –. *H. sinense*; Xu PB001 (SYS); Yunnan, China; MH065386, MH065347, –. *H. sp8*; Xu 324 (SYS); Yunnan, China; MH065404, MH065351, –. *H. subnormale* (Copel.) Nakaïke; Schuettpelz 874; Malay Peninsula; MH884824, –, –. *H. tholiformis* Liang Zhang, K. W. Xu & W. B. Ju; Zhang Liang 4781 (KUN); Tibet, China; ON859868, –, ON859874. *H. triquetrum* (N.Murak. & R.C.Moran) L.Regalado & Prada; L.Sylvestre 2208 (RB); Brazil; KT329398, –, –. *H. unilaterale* (Lam.) Hayata; Hemp A. 18 (BM); Kenya; AF240652, –, –. *H. unilaterale*; Hennequin S. R144 (BM, REU); Reunion; KF992497, –, –. *H. unilaterale*; RV8344; Reunion; GU929873, –, –. *H. unilaterale*; SH408; Mauritius; MH884825, –, –. *H. vanuatuense* Li Bing Zhang & K. W. Xu; Ohlsen 392 (MELU); Tanna, Vanuatu; KP774898, KP85188, –. *H. wangpeishanii* Li Bing Zhang & K. W. Xu; Zhang et al. EM03 (CDBI); Sichuan, China; MH065410, MH065333, –. *H. wildii* (F. M. Bailey) D. Ohlsen; DJO246 (MELU); Queensland, Australia; KP774927, KP851877, –. *H. wuliangshanense* (Ching) Viane & S. Y. Dong; Chang1102 (HITBC); Yunnan, China; MW194208, MW194224, MW194184. *H. wuliangshanense*; Chang1104 (HITBC); Yunnan, China; MW194209, MW194225, MW194185.

Two new species of *Miersia* and their phylogenetic placements alongside the recently described *M. putaendensis* (Gilliesieae, Allioideae, Amaryllidaceae)

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Abstract

Two new species of the Chilean endemic genus *Miersia* (Gilliesieae, Allioideae, Amaryllidaceae) are described, *M. stellata* and *M. raucoana*, alongside morphological descriptions, a distribution map, illustrations, conservation status assessments, and an updated key to all species of *Miersia*. Additionally, phylogenetic analyses of DNA sequences were performed to inquire into the evolutionary affinities of both new species and the recently described, *M. putaendensis*, within the tribe Gilliesieae.

Keywords

Alliaceae, Central Chile, IUCN, phylogeny, taxonomy

Introduction

Amaryllidaceae J.St.-Hil. consists of three subfamilies in its modern circumscription: Amaryllidoideae Burnett, Agapanthoideae Endl., and Allioideae Herb. (Chase et al. 2009; Pellicer et al. 2017). Allioideae includes several bulbous or rhizomatous species of agricultural and ornamental importance divided into four tribes: Allieae Dumort is distributed in the Northern Hemisphere, Tulbaghieae Endl. ex Meisn. is restricted to South Africa, and Leucocoryneae Ravenna and Gilliesiae Baker, are both endemic to South America (Sassone and Giussani 2018; Escobar et al. 2020). Recent phylogenetic and karyotypic studies have determined that the crown group of Allioideae diversified ~62 Mya, and support a Gondwanan origin for Allioideae, with vicariant events as the cause of the intercontinental distribution of its four tribes (Costa et al. 2020).

Gilliesiae is a poorly known tribe comprising several threatened species (Torres-Mellado et al. 2012) characterized by zygomorphic flowers, a character state that is divergent from the rest of Allioideae, which typically have actinomorphic flowers (Rudall et al. 2002; Escobar et al. 2020; García et al. 2022). This tribe is composed of eight genera mainly distributed in the southern cone of South America: *Ancrumia* Harv. ex Baker, *Gethyum* Phil., *Gilliesia* Lindl., *Miersia* Lindl., *Speea* Loes. (Mediterranean Chile), *Solaria* Phil. (Chile and Argentina), *Trichlora* Baker (Peru) and *Schickendantziella* Speg. (Argentina and Bolivia) (Escobar 2012; Escobar et al. 2012). Phylogenetic studies of Gilliesiae suggested that its most recent common ancestor diverged during the Miocene ca. (29-) 18 (-7) Mya, a period characterized by a global increase in temperature, a retreat of glacial cover and great tectonic activity that produced the uplift of the Andes, a process directly related to the diversification of this clade (Sassone and Giussani 2018; Costa et al. 2020).

Miersia is endemic to central Chile and includes bulbous herbs with zygomorphic flowers, perigones formed by six free green-violaceous tepals, sometimes very reduced tepaliferous appendages, and in most species, a staminal tube formed by the fusion of 6 fertile stamens (Rudall et al. 2002; Escobar 2012; Cádiz-Véliz 2021). The latest taxonomic and phylogenetic treatment of *Miersia* comprised five accepted species and evidenced that the single species in *Speea*, *S. humilis* (Phil.) Loes. ex K.Krause, is embedded within *Miersia* (Escobar 2012; Escobar et al. 2020). Recently, Cádiz-Véliz (2021) described the new species *M. putaendensis* A.Cádiz-Véliz from the Valparaíso Region based on its morphological distinctiveness; however, its phylogenetic position is still unknown within *Miersia*.

As the result of two independent field explorations in central Chile during the winter (August) of 2020, two undescribed species of *Miersia* were discovered. The first species was found on a rocky outcrop near the town of Lampa (33°16'S, 70°51'W, 600 m a.s.l.), Metropolitan Region of Santiago, and the second, inhabiting a rocky slope close to the town of Rauco, Curicó (34°54'S, 71°22'W, 535 m a.s.l.), Maule Region. This study describes these new species and provides a distribution map, illustrations and conservation assessments for them, besides an updated identification key to all species of *Miersia*. Additionally, the recently described *Miersia putaendensis* and both species described here were placed in the phylogeny of Gilliesiae to evaluate their evolutionary affinities within this tribe.

Methods

Herbarium and fieldwork

Fieldwork to collect the type specimens and silica-dried leaves for DNA extractions was carried out in June 2021 in Cerro Quilhuica, Lampa, and in July 2021 in the hills of Rauco, Curico (Fig. 1). Specimens were collected and deposited in the collections of the EIF, CONC, JBN, and SGO herbaria (Thiers 2022, updated continuously). Additionally, flowers were collected in 70% ethanol for morphological measurements and descriptions. The main taxonomic literature on Gilliesieae was consulted for morphological descriptions of previously described species (Ravenna 2000; Escobar et al.

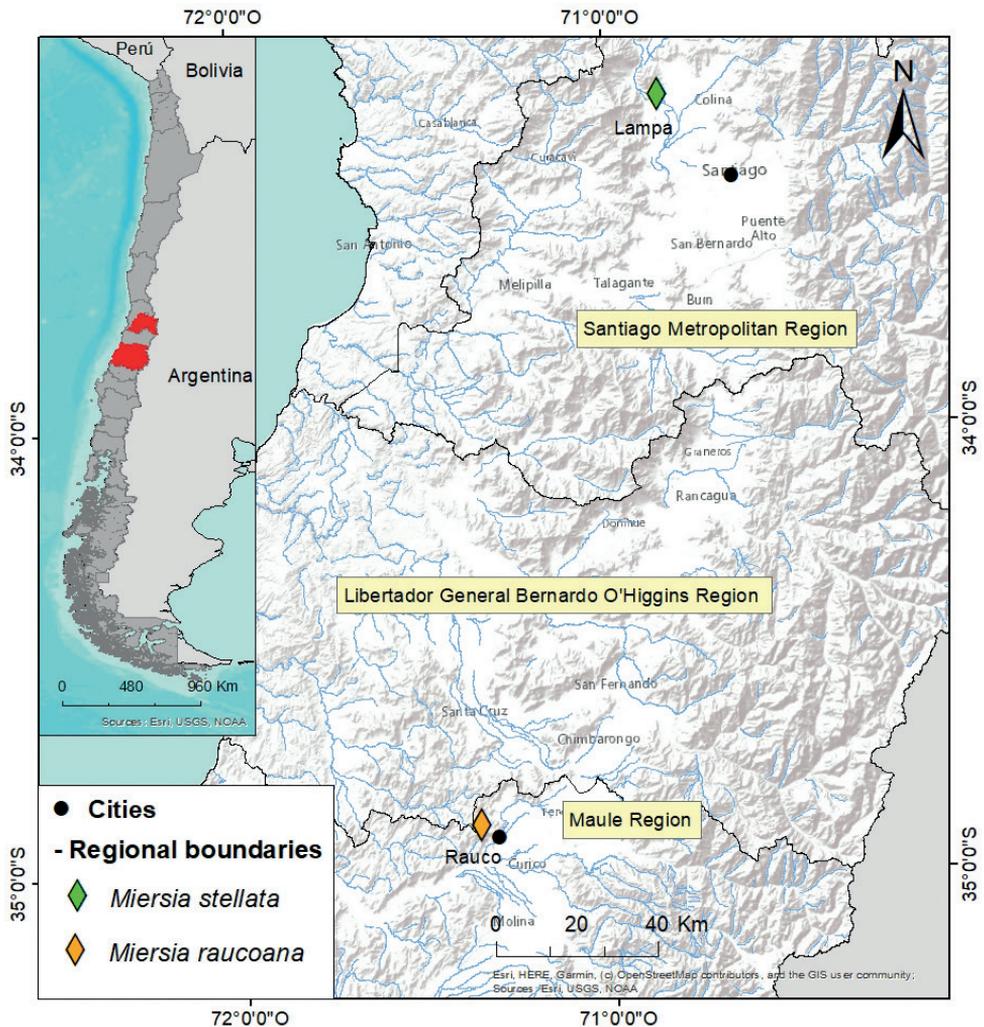


Figure 1. Distribution of *Miersia stellata* (green diamond) and *Miersia raucoana* (orange diamond). Map by Claudia Cuevas.

2010, 2020; Escobar 2012; Cádiz-Véliz 2021). Plant terminology follows Beentje (2012). Measurements were made using a Motic MZ-171 stereomicroscope for structures smaller than 1 cm or with the naked eye for larger structures. All widths were measured over the widest portion of the structure.

Taxon sampling and phylogenetic analyses

Genomic DNA was extracted from type specimens of the three species of *Miersia* (*Miersia putaendensis*: A. Cádiz-Véliz 548, EIF 14041, isotype; *Miersia raucoana*: N. García et al. 6139, EIF 14824, holotype; *Miersia stellata*: N. García & C. Cuevas 6132, EIF 14823, holotype) using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Based on previous studies and sequences available for Gilliesieae (Escobar et al. 2020), we amplified the *rbcL* gene and *trnL-F* intron and spacer, which together form our chloroplast DNA (cpDNA) matrix, and the nuclear ribosomal DNA internal transcribed spacer (nrITS). The amplification of DNA fragments followed the protocols described by Escobar et al. (2020). Sequencing was performed using the same amplification primers, by Macrogen, South Korea. We generated nine new sequences and deposited them in GenBank (Suppl. material 3: Table S1); the remaining sequences were obtained from datasets by Escobar et al. (2020), available in the Treebase repository (N 26352) and in Zenodo (doi: 10.5281/zenodo.6581791).

Preliminary examination of all sequence data revealed that several were identical; therefore, we kept a single sequence per species in general (two accessions for *Gethyum atropurpureum* Phil. and *Solaria miersioides* Phil.) and excluded accessions that were represented in a single locus dataset. Herbarium material of the accession Escobar 84 (CONC, EIF), which was treated as *Miersia* sp. by Escobar et al. (2020), was reassessed and is treated as *Miersia* cf. *chilensis* Lindl. in the present study. Hence, our taxon sampling was identical and complete for all loci (only *rbcL* missing from *Miersia cornuta* Phil. 152) and included 20 ingroup (Gilliesieae) and 6 outgroup accessions (Suppl. material 3: Table S1), that correspond to five Leucocoryneae taxa and one representative of tribe Tulbaghieae (*Tulbaghia capensis* L.). Editing and assembling of sequences were performed in Geneious Prime v.2022.1.1 (<https://www.geneious.com>). Sequences were aligned using MAFFT v.1.4.0 (Katoh and Standley 2013).

A maximum likelihood (ML) analysis was performed for the concatenated matrix of all loci using RAXML-NG v.1.1.0 (Kozlov et al. 2019), GTR+ Γ as the model of molecular evolution, and conducting 100 tree searches using 50 random and 50 parsimony-based starting trees to pick the best-scoring topology. Each cpDNA locus (*rbcL*, *trnL-F*) and nrITS were considered as separate partitions to increase model fit by accommodating locus-specific variation. Subsequently, likelihood bootstrap analyses (Felsenstein 1985) were conducted with 1,000 pseudoreplicates. Additionally, we performed separate analyses for the nrITS and cpDNA datasets following the same parameters as above, except for the tree searches which were performed using 25 random

and 25 parsimony-based starting trees for each. All trees were rooted using *Tulbaghia capensis*, following Escobar et al. (2020). Alignments of cpDNA and nrITS matrices, as well as maximum likelihood and bootstrap trees, are available in Zenodo (doi: 10.5281/zenodo.6581791).

Conservation assessment

The assessment of the conservation status of both species was conducted using the International Union for Conservation of Nature (IUCN 2017) criteria. The extent of occurrence (EOO) and area of occupancy (AOO) were calculated using Google Earth and threats were identified from field observations.

Results

Phylogenetic analyses

Our ML tree is overall congruent with the phylogeny reported in Escobar et al. (2020), and in particular, regarding the monophyly of Gilliesieae and the presence of two major clades: 1) *Gilliesia* + *Ancrumia* + *Gethyum* + *Solaria*, and 2) *Miersia* + *Speea* (= *Miersia humilis*) (Fig. 2). With respect to relationships within the *Miersia* clade, our phylogeny also agrees with Escobar et al. (2020) regarding the inference of two subclades: 1) *M. cornuta* + *M. leporina* (= Miersia I clade, Fig. 2), and 2) *M. chilensis* + *M. humilis* + *M. minor* + *M. tenuiseta* (= Miersia II clade, Fig. 2). *Miersia putaendensis* is retrieved with strong support (BS = 100) in the Miersia I clade, whereas *M. raucoana* and *M. stellata* are part of the Miersia II clade, albeit this clade has moderate support (BS = 85; see Fig. 2).

Taxonomic treatment

***Miersia stellata* C.Cuevas & Nic.García, sp. nov.**

urn:lsid:ipni.org:names:77306995-1

Figs 3, 4

Diagnosis. *Miersia stellata* differs from *Miersia humilis* (Phil.) M.F.Fay & Christenh. by a capitate stigma (vs. trilobed stigma), six bifid, rarely trifid, flat tepaliferous appendages (vs. tepaliferous appendages absent), and a cylindrical to urceolate staminal tube with a short apical reflexed rim (vs. staminal filaments fused in their basal half and covering the ovary, but not forming an urceolate tube).

Type. CHILE. Región Metropolitana de Santiago: Provincia de Chacabuco, Comuna de Lampa, cerro Quilhuica, 600 m a.s.l., 17 June 2021, *N. García & C. Cuevas 6132* (holotype: EIF 14823; isotypes: CONC, JBN, SGO).

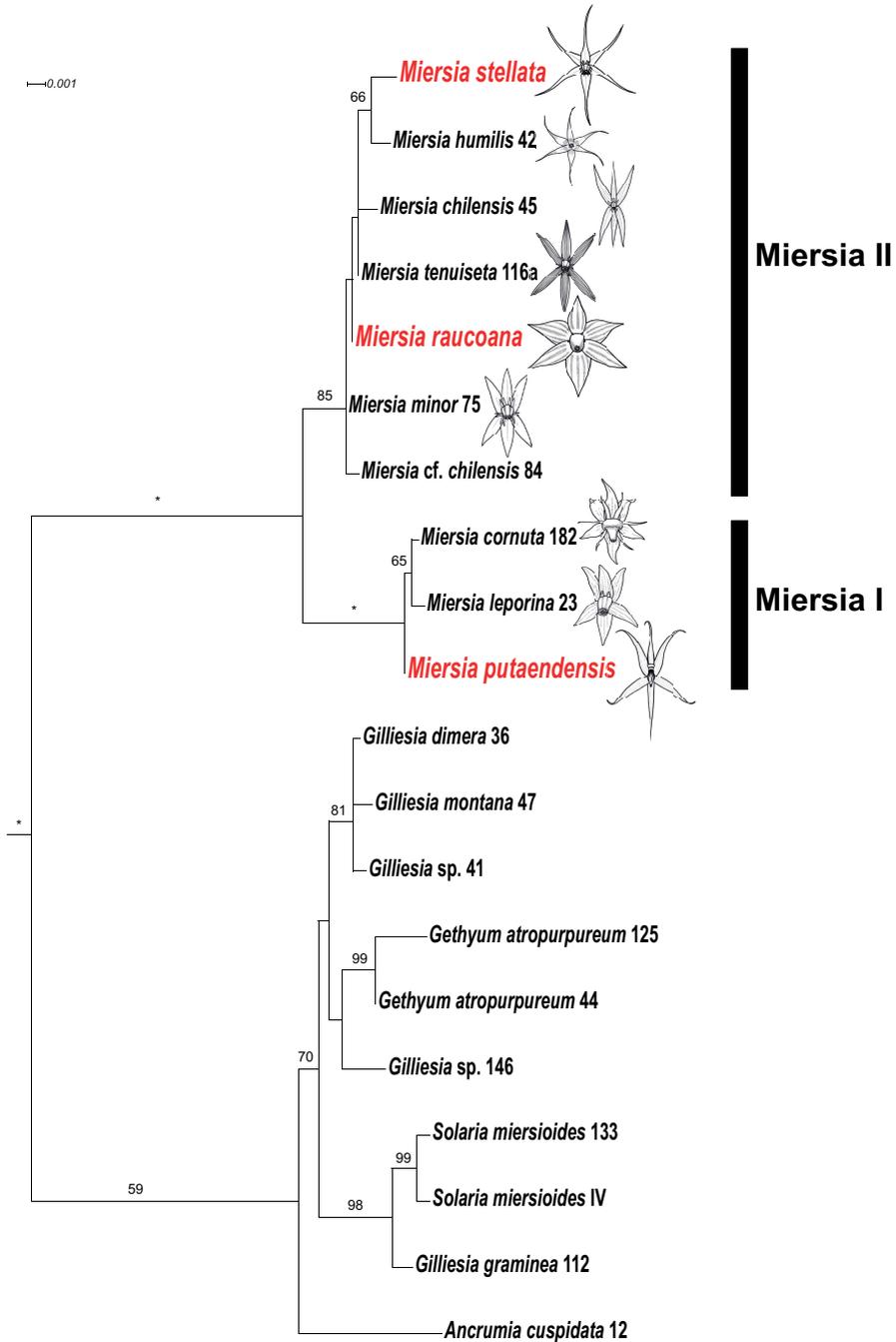


Figure 2. Maximum likelihood phylogram of Gilliesieae based on concatenated analysis of nrDNA ITS and cpDNA (*trnL-F*, *rbcL*). Numbers above branches represent bootstrap (BS) values > 50, asterisks indicate BS = 100. Numbers following species names correspond to accession numbers in Escobar et al. (2020). Novel *Miersia* species being placed within phylogeny are in red font. Two major *Miersia* clades (I, II) are denoted with black bars. Outgroups have been excluded from the figure and root branch is not to scale. Illustrations by Arón Cádiz-Véliz.

Description. Terrestrial saxicolous herbs. **Bulbs** ovoid, usually flattened due to growth in rock crevices, external cataphylls light brown, 11–15 × 5–10 mm. **Leaves** 2–3, linear, hanging, 7–20 × 0.09–0.2 cm. **Scapes** 1–2, cylindrical, hollow, 20–70 × 1–1.3 mm. **Spathe** 2-valvate, herbaceous, lanceolate, 7–12 × 1.5–2 mm, fused on their basal ¼ (~2.5 mm), whitish with veins inconspicuous or purple spotted. **Inflorescences** a pseudo-umbel with 1–2 (–3) slightly zygomorphic, star-shaped flowers; **pedicels** unequal, 1.4–2.7 cm long, apex curved in a right angle (~90°). **Tepals** 6, free, membranous, light green, rarely purplish, lanceolate, caudate, straight, **outer** 12 × 2–2.5 mm, 5 acrodromous veins, **inner** 11–11.5 × 1.5–1.8 mm, 3 acrodromous veins, on both whorls only the central is well marked and runs throughout the complete length, cauda 0.4–0.5 mm wide comprising ~2/3 of the tepal's length. **Tepaliferous appendages** 6, green, deeply bifid, rarely trifid, flat, upper pair with lanceolate segments, each segment sometimes shortly bifid, fused at base ~0.6 mm, 2.0–2.5 × 0.4–0.5 mm, lateral appendages one pair on each side, with linear to linear-lanceolate segments, attached to the base of inner tepals, segments fused at base ~0.1 mm long, 2.0–2.5 × ca. 0.2 mm. **Stamens** 6, filaments 0.2–0.3 mm long, adnate internally to the staminal tube; **staminal tube** cylindrical to urceolate, whitish with two purple longitudinal stripes and three longitudinal folds on its upper side, single longitudinal fold on the lower side, apex with a short reflexed rim, papillose, 2.0–2.5 × 1.5–2.5 mm; **anthers** yellow (purple when dry), 0.8–1.0 mm long, exerted. **Ovary** superior, spherical to obovoid, 1.0–1.3 mm long, trilocular, 12 ovules per locule, biseriate; **style** nodding, exerted, 1.7–2.0 mm long; **stigma** capitate. **Capsules** obovoid to spherical, 3-valved, 4–8 × 4–6 mm. **Seeds** not seen.

Distribution and habitat. *Miersia stellata* has been recorded in a single rocky outcrop in the Quilhuica hill, Lampa (~33.3° S), which is an isolated hill, between the main coastal mountain range (*cordillera de la Costa*) and the basin of Santiago. This south-facing rocky outcrop is at the bottom of a creek at 600 m a.s.l. The new species grows exclusively in rock crevices along with *Tristagma graminifolium* (Phil.) Ravena. The surrounding vegetation corresponds to a degraded sclerophyllous arborescent scrub composed of *Lithraea caustica* (Molina) Hook. & Arn., *Quillaja saponaria* Molina and *Porlieria chilensis* I.M. Johnst.

Phenology. This species has been seen in flowers between May and August. Immature fruits have been recorded during August and September; in general, fructification is low in the population.

Etymology. The specific epithet *stellata* refers to star-shaped form of flowers.

Vernacular name. Although no popular common name is known for *Miersia stellata*, we propose to name it “*estrella de Lampa*” or “Lampa star”.

Conservation status. *Miersia stellata* can be considered Critically Endangered (CR) according to criteria B2ab(iii). Its area of occupancy is < 10 km², with an estimate of 120 m² (~0.0001 km²). Only a single population of < 100 individuals has been recorded despite sampling efforts in surrounding areas in suitable seasons and habitats. In addition, it inhabits an area intensely degraded by human activities and is close to a highway with heavy traffic and to populated locations (Batuco, Lampa).

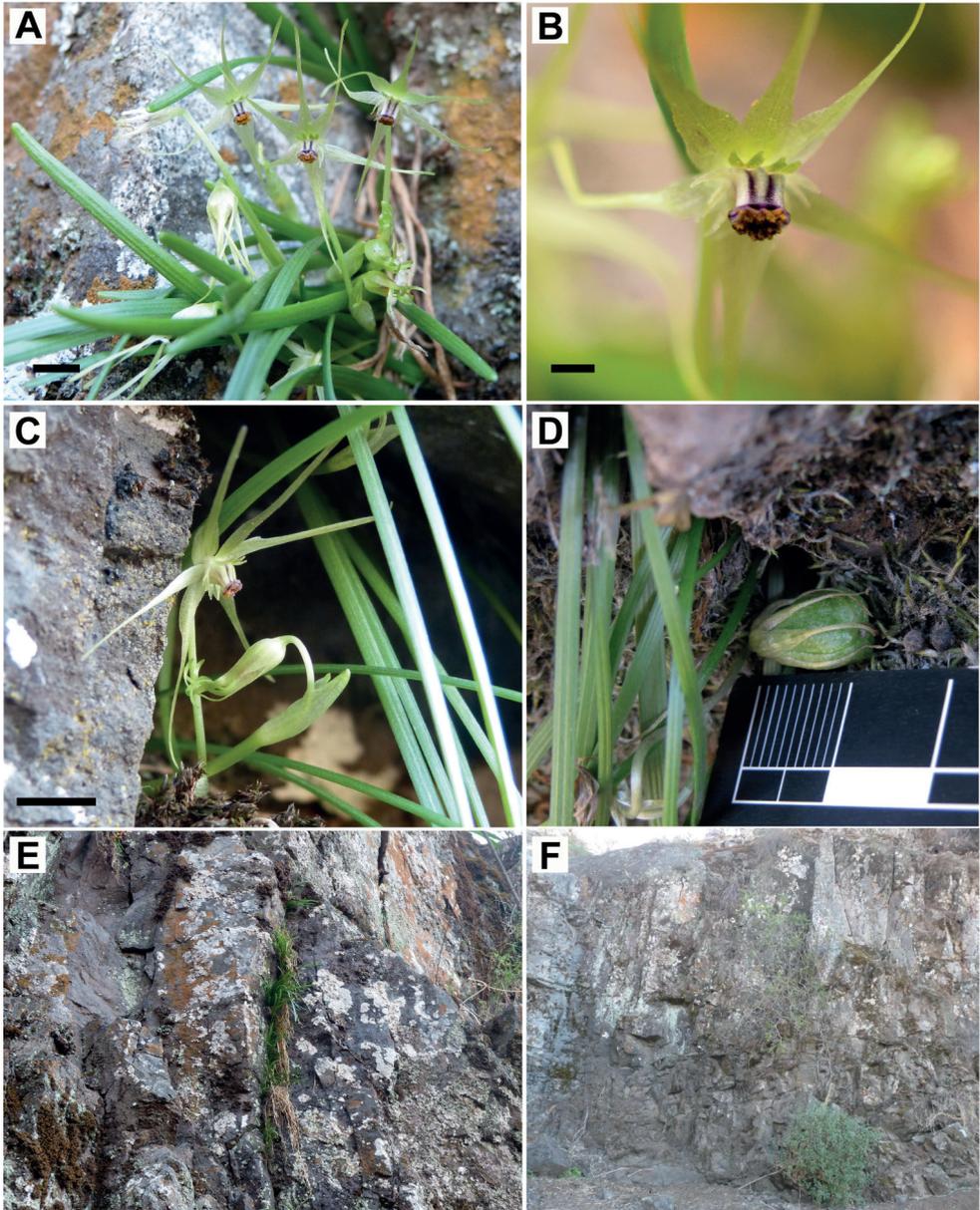


Figure 3. *Miersia stellata* C.Cuevas & Nic.García **A** front view of flowers **B** detail of flower showing tepaliferous appendages and staminal tube **C** lateral view of flower **D** immature fruit **E** habit **F** habitat. Scale bars: 5 mm (**A, C**); 2 mm (**B**). Photos by Nicolás García (**A, C, E, F**), Nicolás Villaseca (**B**), Claudia Cuevas (**D**).

Additional specimens examined (paratypes). CHILE. **Región Metropolitana de Santiago:** Provincia de Chacabuco, Comuna de Lampa, cerro Quilhuica, 600 m a.s.l., 5 August 2020, C. Cuevas s.n. (EIF); 16 September 2020, N. García, C. Cuevas & M. Villalobos 5843 (EIF).

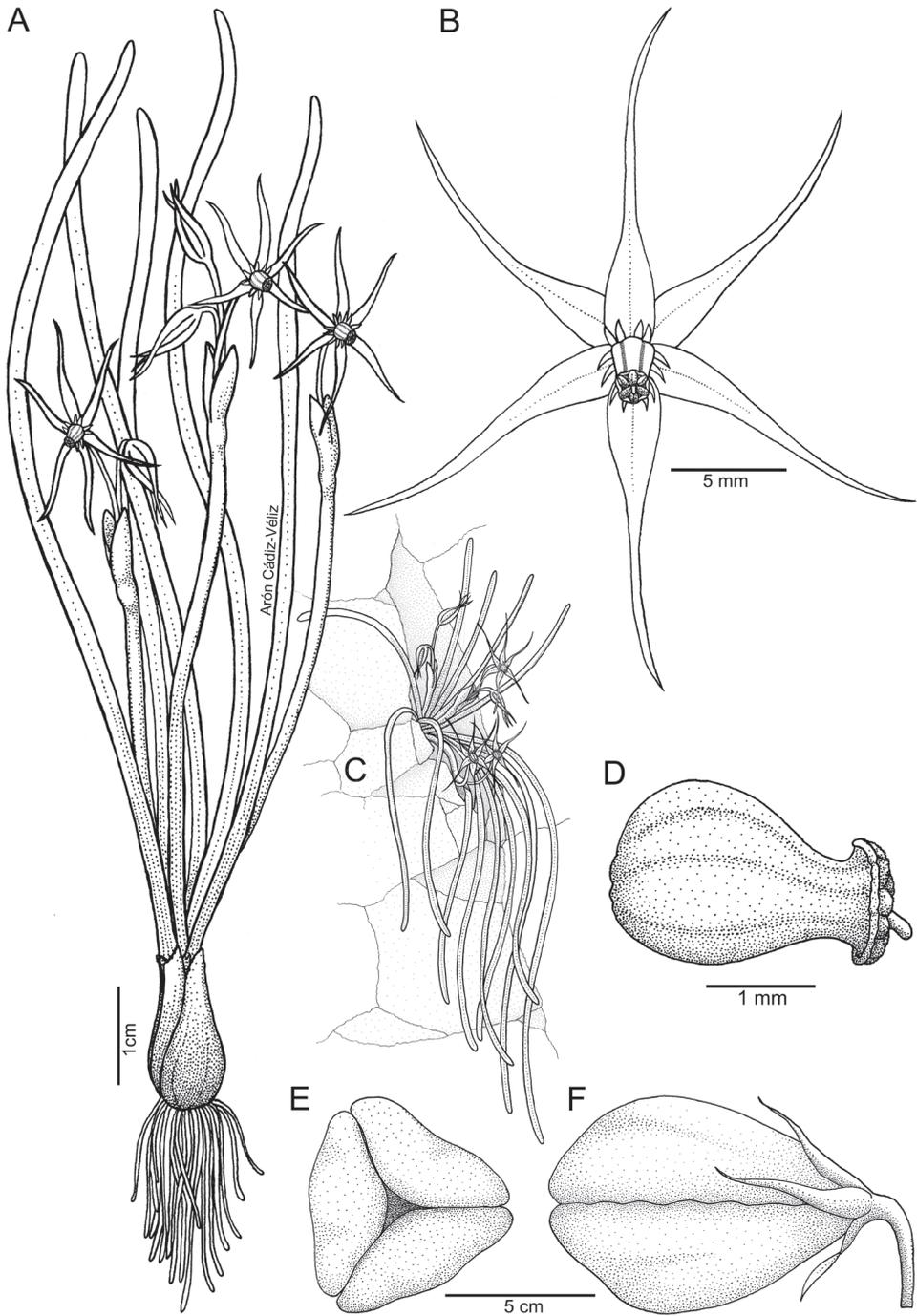


Figure 4. *Miersia stellata* C.Cuevas & Nic.García **A** habit **B** flower (frontal view) **C** plant growing in its natural habitat **D** staminal tube (lateral view) **E** fruit (apical view) **F** fruit (lateral view). Illustration by Arón Cádiz-Vélez.

***Miersia raucoana* J.E.Sepúlveda & Nic.García, sp. nov.**

urn:lsid:ipni.org:names:77306996-1

Figs 5, 6

Diagnosis. *Miersia raucoana* differs from *Miersia tenuiseta* Ravenna by the lack of tepaliferous appendages or tiny and awl-shaped, < 0.5 mm long, if present (vs. 6 conspicuous filiform, bifid tepaliferous appendages), a slightly zygomorphic conical staminal tube (vs. strongly zygomorphic urceolate staminal tube), and 3–5 purple longitudinal stripes in each tepal (vs. none or single broad central longitudinal stripe).

Type. CHILE. Región del Maule: Provincia de Curicó, Comuna de Rauco, quebrada Guayacán, 535 m a.s.l., 6 July 2021, *N. García, J. Sepúlveda, A. Cádiz-Véliz, C. Soto & M. Tobar 6139* (holotype: EIF 14824; isotypes: CONC, JBN, SGO).

Description. Terrestrial saxicolous herbs. **Bulbs** subglobose to ovoid, 10–12 × 7–10 mm, external cataphylls light brown. **Leaves** 3–4, linear, 8–23 × 0.08–0.15 cm. **Scapes** 2–3, cylindrical, hollow, 10–40 × ca. 0.8 mm. **Spathe** 2-valvate, herbaceous, lanceolate, 10–10.5 × 3–3.5 mm, fused on their basal ¼ (–2.5 mm), veins purple. **Inflorescences** a pseudo-umbel with 2–5 slightly zygomorphic flowers; **pedicels** unequal, 1.5–2.0 cm long. **Tepals** 6, free, membranous, creamy white to yellowish (in dry specimens) with 3–5 purple longitudinal stripes each (exceptionally without stripes), lanceolate to obovate, acute, straight to slightly reflexed apically, **outer** 8–9 × 3.5 mm, **inner** 7–8 × 3.0–3.5 mm. **Tepaliferous appendages** absent or inconspicuous, awl-shaped, purple, < 0.5 mm long. **Stamens** 6, filaments 0.5–0.8 mm long, diminishing in length towards the downward side of the flower, adnate internally to the staminal tube; **staminal tube** conical, apex narrowly tubular (ca. 0.8 × 0.5 mm), slightly zygomorphic, purple, papillose, 3–4 × 2.5–3.0 mm; **anthers** 6, yellow (purple when dry), ca. 0.3–0.4 mm long, exerted. **Ovary** superior, spherical to obovoid, 1.0–1.5 mm long, trilocular, 10 ovules per locule, biseriate; **style** straight to ascending, 1.5–2.0 mm long, reaching the anthers or exerted in mature flowers; **stigma** capitate. **Capsules** obovoid to spherical, 3-valved, 8 × 6.5 mm, 13–14. **Seeds** obovoid, 1.6–2.0 × 1.0–1.4 mm, testa reticulate, 13–14 per capsule.

Distribution and habitat. *Miersia raucoana* was originally recorded in a rocky east- to northeast-facing slope in the coastal mountain range of Rauco (–34.9°S), Maule Region. During the review process of this article, it was also recorded around the La Palmilla dam, located ca. 3 km north of the typical locality. It can be found growing in rock crevices or in the base of rocky outcrops, between 240 and 540 m a.s.l. The surrounding vegetation corresponds to a sclerophyllous arborescent scrub, where the most abundant species are *Lithraea caustica* (Molina) Hook. & Arn., *Peumus boldus* Molina, *Vachellia caven* (Molina) Seigler & Ebinger, *Retanilla trinervia* Hook. & Arn., *Chusquea cumingii* Nees, and *Leucostele chiloensis* (Colla) Schlumpb.

Phenology. *Miersia raucoana* has been recorded in flowers from May to early August. Fruits have been recorded in late July and throughout August.

Etymology. The specific epithet refers to Rauco, a municipality located to the west of the city of Curicó in the Maule region of Chile.

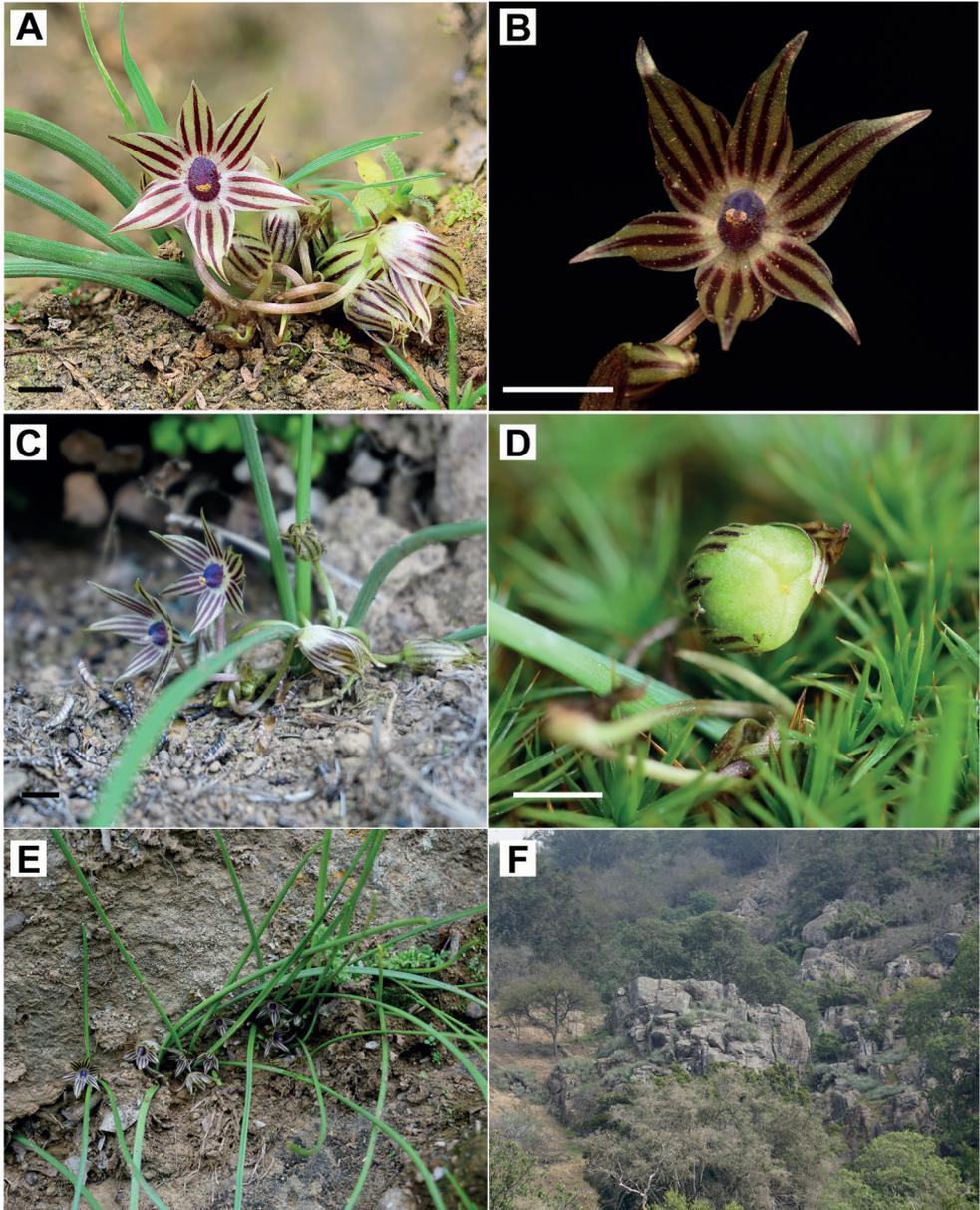


Figure 5. *Miersia raucoana* J.E.Sepúlveda & Nic.García **A** front view of flower **B** detail of flower lacking appendages **C** lateral view of flowers showing tiny tepaliferous appendages **D** immature fruit **E** habit **F** habitat. Scale bars: 5 mm. Photos by José Luis Inostroza (**A, D**), Matías Tobar (**B**), Joaquín Sepúlveda (**C, E, F**).

Vernacular name. We propose to name this species as “*miersia de Rauco*”.

Conservation status. *Miersia raucoana* can be considered Critically Endangered (CR) under criteria B2ab(iii), because its area of occupancy is < 10 km², with an estimated 1.8 km². Only a single population scattered throughout the latter area, with < 1,000 mature

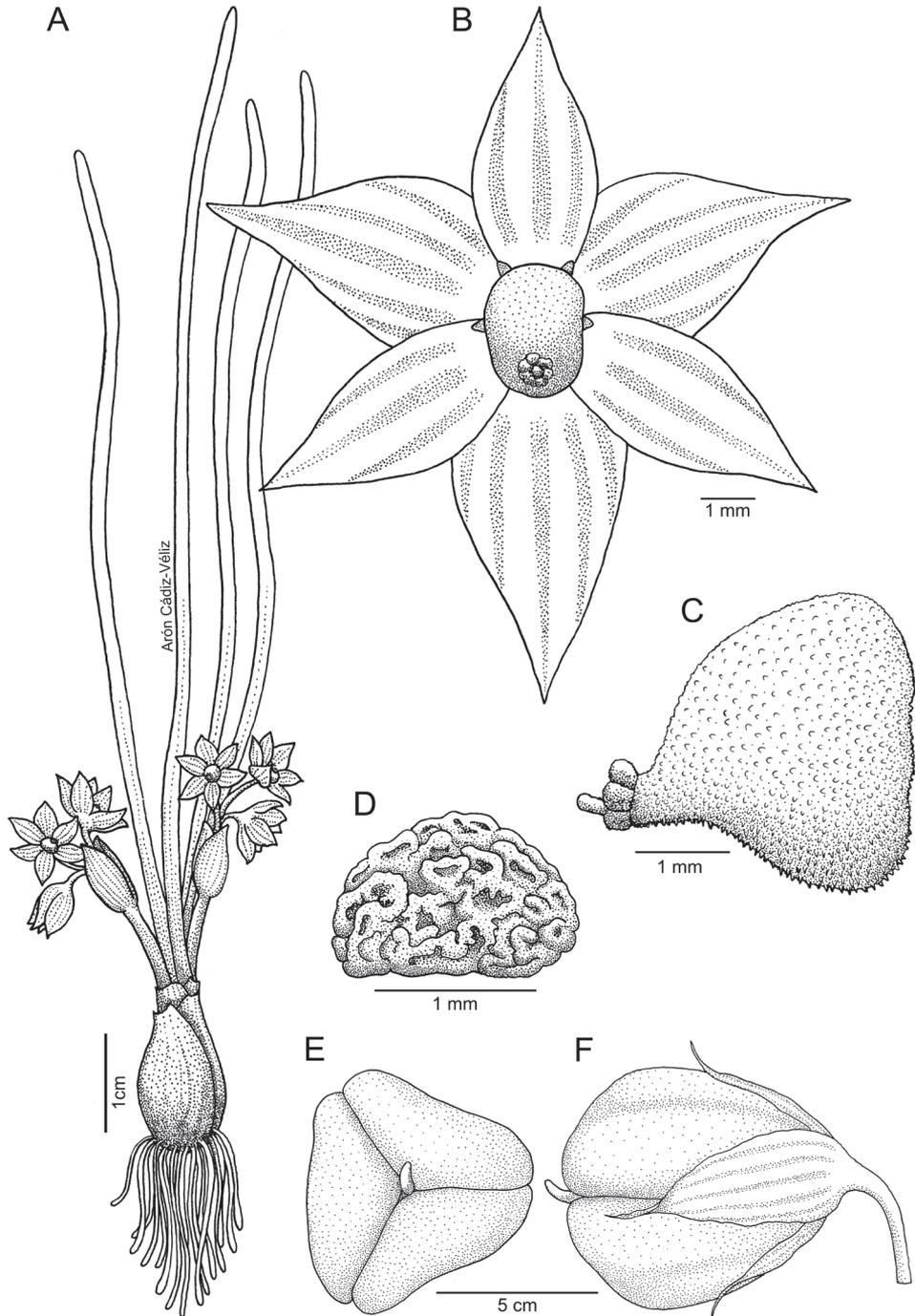


Figure 6. *Miersia raucoana* J.E.Sepúlveda & Nic.García **A** habit **B** flower (frontal view) **C** staminal tube (lateral view) **D** seed **E** fruit (apical view) **F** fruit (lateral view). Illustration by Arón Cádiz-Véliz.

individuals, has been recorded despite sampling efforts in surrounding areas in suitable seasons and habitats. In addition, the area is at risk of forest fires and is subject to land use change for agricultural crops, motorized sporting activities, and goat and cattle ranching.

Additional specimens examined (paratypes). CHILE. **Región del Maule:** Provincia de Curicó, Comuna de Rauco, quebrada Guayacán, 535 m a.s.l., 12 August 2020, *J. Sepúlveda s.n.* (EIF).

Key to the species of *Miersia* [modified from Cádiz-Véliz (2021)]

- 1 Flowers with 2 tepaliferous appendages above the staminal tube 2
- Flowers with 6 tepaliferous appendages around the staminal tube or appendages absent 3
- 2 Tepaliferous appendages lorate to cuneiform, apex truncate, erose and deflected, oriented frontward; white staminal tube featuring an elongated frontal lobe with a purple apical spot..... *M. putaendensis*
- Tepaliferous appendages oblong to subulate, apex entire, obtuse and straight, oriented upward; bluish-green staminal tube with an erect, short, upper lobe without a purple spot..... *M. leporina*
- 3 Tepals clearly reflexed on their distal half. Staminal tube with a globose deflected base. Tepaliferous appendages entire and filiform to narrowly lanceolate..... *M. cornuta*
- Tepals generally straight throughout or slightly reflexed. Staminal tube not globose at base. Tepaliferous appendages divided or absent 4
- 4 Tepals caudate over 2/3 of their length, longer than 11 mm..... 5
- Tepals acute to acuminate, shorter than 11 mm 6
- 5 Tepaliferous appendages absent. Staminal tube without an apical reflexed rim. Filaments born apically, conspicuous and seeming a continuation of the tube. Stigma trilobed *M. humilis* (= *Speea humilis*)
- Tepaliferous appendages present. Staminal tube with a short apical reflexed rim. Filaments inserted and born laterally on the inner face of the tube. Stigma capitate *M. stellata*
- 6 Tepals creamy white with 2 to 3 purple longitudinal stripes, rarely plain creamy white, perigone actinomorphic. Tepaliferous appendages absent or awl-shaped and shorter than 0.5 mm. Staminal tube conical, purplish; opening central and pointing towards the front of the flower..... *M. raucoana*
- Tepals plain light green to purplish or sometimes with a single central and broad purple longitudinal stripe (in *M. tenuiseta*), perigone zygomorphic. Tepaliferous appendages filiform or flat, bifid to trifid, longer than 0.5 mm. Staminal tube urceolate, whitish to greenish or with a wide purple stripe on upper face; opening lateral, placed towards the lower side of the flower 7
- 7 Tepals acuminate, apex generally reflexed..... *M. chilensis*
- Tepals acute, apex straight or inflexed 8

- 8 Outer tepals lanceolate to linear-lanceolate. Appendages filiform, upper and lateral similar *M. tenuiseta*
- Outer tepals ovate to oval-lanceolate. Appendages flat, upper and lateral different *M. minor*

Discussion

The present phylogenetic analysis of tribe Gilliesieae coincides with the results of Escobar et al. (2020), which was expected considering that we used the same molecular markers and some of the sequences produced by that work. Therefore, we also identified the same taxonomic issues, for instance, the paraphyly of *Gilliesia* with respect to *Solaria* and *Gethyum*, and the putative sister relationship of *Ancrumia* to the rest of that clade. On the other hand, the single species in *Speea* (i.e., *Speea humilis*) is well embedded within the *Miersia* clade (Fig. 2), with which it shares several putative synapomorphies, including oblong capsules, 12 chromosomes and filaments fused at least covering the ovary (Escobar et al. 2020); therefore, this species has been treated as *Miersia humilis* (Phil.) M.F.Fay & Christenh. in the present work. A generic circumscription of *Miersia* including *Speea* renders the former genus monophyletic according to currently available phylogenetic information. In this sense, *Miersia* s.l. is composed of nine species and is well diagnosed in the context of Gilliesieae taxonomy by the presence of six functional stamens and the lack of staminodes (Escobar et al. 2020).

Despite low resolution within the *Miersia* subclades, the phylogenetic position of the recently described species is well supported within Miersia I in the case of *M. putaendensis* and within Miersia II for *M. raucoana* and *M. stellata*. Given these subclade circumscriptions, Miersia I is composed of three species that have the northernmost distributions within the genus between the basins of the Choapa and Aconcagua rivers (–32°–33°S; Escobar 2012; Cádiz-Véliz 2021). Separate analyses of the nrITS and cpDNA datasets show a single strong cytonuclear discordance in *Miersia* regarding the position of *M. putaendensis* within Miersia I (Suppl. materials 1, 2: Figs S1 and S2, also available in Zenodo, doi: 10.5281/zenodo.6581791), which could be indicating a hybrid origin for one of the species involved in this clade. This mechanism of speciation was suggested as putatively present within *Miersia* by Escobar et al. (2020). No clear synapomorphy or diagnostic character has been detected for this subclade; however, it is noteworthy to mention that the only two species with only two upper appendages in *Miersia*, *M. leporina* Ravenna and *M. putaendensis*, belong to this group.

On the other hand, Miersia II contains at least six species with their distributions concentrated between the Maipo and Maule river basins (–33–36°S; Escobar 2012). However, the consistently low phylogenetic resolution and short branches in the Miersia II clade make it impossible to confidently ascertain relationships among species given the available data and suggest a rapid diversification within this clade (Escobar et al. 2020). As in Miersia I, no synapomorphy or diagnostic character has been suggested

for this clade (Escobar et al. 2020), which in turn shows considerable floral variation and diversification, exemplified by the outstanding divergence of *M. humilis* in characters such as stigma type and degree of fusion of staminal filaments, which otherwise are constant within *Miersia*.

Generic recircumscriptions within the *Gilliesia* clade are also desirable to comply with the primary principle of monophyly given a phylogenetic approach to biological classification (e.g., Wiley and Lieberman 2011; Judd et al. 2016); however, this issue is out of the scope of the present work. A comprehensive evolutionary study and proposal of generic classification for Gilliesieae must also include sequences and morphological considerations for *Trichlora* from Peru and *Schickendantziella* from Argentina and Bolivia, which are the only non-Chilean taxa in the tribe and have not been included in previous molecular phylogenetic research of the group (Escobar et al. 2020). Future studies should also include data from multiple low-copy nuclear genes to clarify the phylogeny of Gilliesieae, providing a robust framework to reassess the generic taxonomy and evaluate the diversification of this clade.

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References

- Beentje H (2012) The Kew Plant Glossary. An illustrated dictionary of plant terms. Kew Publishing, 1–164.
- Cádiz-Véliz A (2021) *Miersia putaendensis* sp. nov. (Gilliesieae, Amaryllidaceae), a new species endemic to Central Chile. *Phytotaxa* 502(3): 230–236. <https://doi.org/10.11646/phytotaxa.502.3.2>
- Chase MW, Reveal JL, Fay MF (2009) A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* 161(2): 132–136. <https://doi.org/10.1111/j.1095-8339.2009.00999.x>
- Costa L, Jimenez H, Carvalho R, Carvalho-Sobrinho J, Escobar I, Souza G (2020) Divide to conquer: Evolutionary history of Allioideae Tribes (Amaryllidaceae) is linked to distinct trends of karyotype evolution. *Frontiers in Plant Science* 11: e320. <https://doi.org/10.3389/fpls.2020.00320>

- Escobar I (2012) Sistemática de la tribu Gilliesieae Lindl. (Alliaceae), sobre la base de evidencias morfoanatómicas, citológicas y moleculares. Tesis Doctoral. Universidad de Concepción, Chile. http://repositorio.udec.cl/bitstream/11594/4136/1/Tesis%20Escobar_j_i.pdf
- Escobar I, Novoa P, Ruiz E, Negritto M, Baeza C (2010) Nuevo hallazgo de *Miersia cornuta* Phil. (Gilliesieae-Alliaceae). Gayana. Botánica 67(1): 130–134. <https://doi.org/10.4067/S0717-66432010000100012>
- Escobar I, Ruiz E, Baeza C (2012) Estudios cariotípicos en especies de Gilliesieae Lindl. (Gilliesioideae-Alliaceae) de Chile central. Gayana. Botánica 69(2): 240–250. <https://doi.org/10.4067/S0717-66432012000200003>
- Escobar I, Ruiz-Ponce E, Rudall PJ, Fay MF, Toro-Núñez O, Villalobos-Barrantes HM, Baeza CM (2020) Phylogenetic relationships based on nuclear and plastid DNA sequences reveal recent diversification and discordant patterns of morphological evolution of the Chilean genera of Gilliesieae (Amaryllidaceae: Allioideae). Botanical Journal of the Linnean Society 194(1): 84–99. <https://doi.org/10.1093/botlinnean/boaa035>
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution; International Journal of Organic Evolution 39(4): 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- García N, Sassone AB, Pinto R, Román MJ (2022) *Atacamallium minutiflorum* (Amaryllidaceae, Allioideae), new genus and species from the coastal desert of northern Chile. Taxon 71(3): 552–562. <https://doi.org/10.1002/tax.12684>
- IUCN (2017) Guidelines for using the IUCN red list categories and criteria, version 13. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ (2016) Plant Systematics: A Phylogenetic Approach, 4th edn. Sinauer Associates, 677 pp.
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kozlov AM, Darrriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35(21): 4453–4455. <https://doi.org/10.1093/bioinformatics/btz305>
- Pellicer J, Hidalgo O, Walker J, Chase M, Christenhusz M, Shackelford G, Leitch I, Fay M (2017) Genome size dynamics in tribe Gilliesieae (Amaryllidaceae, subfamily Allioideae) in the context of polyploidy and unusual incidence of Robertsonian translocations. Botanical Journal of the Linnean Society 184(1): 16–31. <https://doi.org/10.1093/botlinnean/box016>
- Ravenna P (2000) New or noteworthy *Miersia* species (Gilliesiaceae). Onira 5(7): 31–34.
- Rudall PJ, Bateman RM, Fay MF, Eastman A (2002) Floral anatomy and systematics of Alliaceae with particular reference to *Gilliesia*, a presumed insect mimic with strongly zygomorphic flowers. American Journal of Botany 89(12): 1867–1883. <https://doi.org/10.3732/ajb.89.12.1867>
- Sassone AB, Giussani LM (2018) Reconstructing the phylogenetic history of the tribe Leucocoryneae (Allioideae): Reticulate evolution and diversification in South America. Molecular Phylogenetics and Evolution 127: 437–448. <https://doi.org/10.1016/j.ympev.2018.04.034>

- Thiers BM (2022[, updated continuously]) Index Herbariorum. <http://sweetgum.nybg.org/science/ih/>
- Torres-Mellado GA, Escobar I, Palfner G, Casanova-Katny MA (2012) Micotrofia en Gilliesieae, una tribu amenazada y poco conocida de Alliaceae de Chile central. *Revista Chilena de Historia Natural* 85(2): 179–186. <https://doi.org/10.4067/S0716-078X2012000200004>
- Wiley EO, Lieberman BS (2011) *Phylogenetics: Theory and Practice of Phylogenetic Systematics*, 2nd edn. Wiley-Blackwell, 406 pp. <https://doi.org/10.1002/9781118017883>

Supplementary material I

Figure S1

Authors: Nicolás García, Claudia Cuevas, Joaquín E. Sepúlveda, Arón Cádiz-Véliz, María José Román

Data type: Eps file.

Explanation note: Maximum likelihood cladogram of Gilliesieae and outgroups based on nrDNA ITS. Numbers above branches represent bootstrap (BS) values > 50, asterisks indicate BS = 100. Numbers following species names correspond to accession numbers in Escobar et al. (2020).

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Link: <https://doi.org/10.3897/phytokeys.211.87842.suppl1>

Supplementary material 2

Figure S2

Authors: Nicolás García, Claudia Cuevas, Joaquín E. Sepúlveda, Arón Cádiz-Véliz, María José Román

Data type: Eps file.

Explanation note: Maximum likelihood cladogram of Gilliesieae and outgroups based on concatenated analysis of cpDNA (*trnL-F*, *rbcL*). Numbers above branches represent bootstrap (BS) values > 50, asterisks indicate BS = 100. Numbers following species names correspond to accession numbers in Escobar et al. (2020).

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Link: <https://doi.org/10.3897/phytokeys.211.87842.suppl2>

Supplementary material 3

Table S1

Authors: Nicolás García, Claudia Cuevas, Joaquín E. Sepúlveda, Arón Cádiz-Véliz, María José Román

Data type: Docx file.

Explanation note: **Table S1.** GenBank accession numbers of DNA sequences used in this study. Previously unpublished sequences are denoted with an asterisk (*).

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Link: <https://doi.org/10.3897/phytokeys.211.87842.suppl3>

Polygonatum praecox (Asparagaceae), a new species from mid-eastern China revealed by morphological and molecular evidence

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Abstract

A new species, *Polygonatum praecox* Y.F.Hu & J.W.Shao (Asparagaceae), is described and illustrated. This species is similar to *P. cyrtonema*, *P. odoratum* and *P. caulialatum*, but can be distinguished from *P. cyrtonema* by its racemose inflorescence, cylindrical hairless filaments and apex without a retrorse spur; from *P. odoratum* by its stout moniliform rhizome, straight stem and longer (1.7–2.2 cm long) floral tube; and from *P. caulialatum* by its upper part straight stem, yellowish-green corolla, lobes excurved and earlier flowering. The complete chloroplast genome of this new species is 155,115–155,256 bp in length. Phylogenetic analysis revealed that *P. praecox* is not genetically related to the above three morphological similar species, but is closely related to the two European species (*P. multiflorum* and *P. latifolium*). This species is relatively common in mid-eastern China and has previously been confused with *P. cyrtonema*. As its wild resources have decreased in recent years due to over-exploitation for medicinal or edible purposes, we classify it as Near Threatened (NT) according to the IUCN Red List Criteria.

Keywords

flowering phenology, medicinal plant, *Polygonatum caulialatum*, *P. cyrtonema*, *P. odoratum*

* These authors contributed equally to this work.

Introduction

Polygonatum Mill., the largest genus of Tribe Polygonateae in Asparagaceae, contains more than 70 species that are widely distributed in the warm temperate, subtropical and boreal zones of the Northern Hemisphere (Tang 1978; Chen and Tamura 2000; Meng et al. 2014; Wang et al. 2016; Xia et al. 2022). The eastern Himalaya and Hengduan Mountains and also North East Asia are the centres of diversity where ca. 50 of the species occur (Floden 2017; Zhao et al. 2019; Xia et al. 2022). *Polygonatum* is one of the most important medicinal taxa in Asia, with some species being widely used in traditional Chinese medicine, such as *P. cyrtonema* Hua, *P. sibiricum* Redouté and *P. kingianum* Coll. & Hemsl. (Zhao et al. 2018; Chinese Pharmacopoeia Commission 2020; Fan et al. 2020; Li et al. 2021). Most species in this genus are edible and can be cultivated in forests without occupying farmland; thus, some of them are emerging as a new generation crop that offers high yield and nutrition, but do not require fertile land for growth (Si and Zhu 2021).

Accurate species delimitation has become of practical importance in conservation and utilisation of plant resources (Isaac et al. 2004). Flower features, especially filament shape and vestiture and its position in the perianth tube, are vital in *Polygonatum* species identification (Tamura 1991, 1993; Tamura et al. 1997; Floden 2012). However, the observation of these flower features is relatively difficult due to the short flowering period of most species, while the easily observed vegetative organ features show high plasticity in different habitats. The systematics and species classification of *Polygonatum* still requires study to understand the diversity as shown by the synonym lists for some species according to the Flora of China, such as *P. cyrtonema*, *P. odoratum* (Mill.) Druce and *P. kingianum* (Chen and Tamura 2000) and by the recent description of distinctive new species which have been published in recent years (e.g. Cai et al. 2015; Floden 2015; Yang et al. 2020; Chen et al. 2021).

During an investigation of wild germplasm resources of *Polygonatum* in eastern China, we made several collections of a possibly unknown plant with alternate leaves, thick moniliform rhizome and large yellow-green flowers (1.7–2.2 cm long). This plant is in appearance similar to and has usually been identified as *P. cyrtonema*. However, we found that this plant differs from *P. cyrtonema* in its filaments (inserted near the distal 1/3 of the perianth tube, hairless and apical part without saccate-convex), flowering phenology (mid-March to early April) and inflorescence type (racemose). After further observation of its morphology and flowering phenology, chloroplast sequencing and phylogenetic analysis, we identified it as a new species which has been overlooked. Thus, we report the results of our investigation and the new species, named as *Polygonatum praecox* Y.F.Hu & J.W.Shao, sp. nov., is described and illustrated here.

Materials and methods

Morphological assessment

Six populations of the putative novel species (*Polygonatum praecox*) were found in Anhui, Zhejiang and Shaanxi Provinces (Fig. 1, Table 1) and some individuals from three populations (JZ, LY and QY) were transplanted to the Botanical Garden in Anhui Normal University for further observation of their morphology and flowering phenology. Some populations of *P. cyrtoneuma* (TTZ, QLF and JH, Table 1), *P. odoratum* (QS and CZ, Table 1) and *P. caulialatum* (KZ and TB, Table 1) were also collected and transplanted to the Garden for further morphological assessment. In the field, more than five living plants in each population were randomly selected for rhizomes, leaves, flowers morphological observations. The stability and variation patterns of these morphological traits (especially the filaments characteristics) and the flowering period were further observed in transplanted populations. All voucher specimens were deposited at the Herbarium of Anhui Normal University (ANUB). The specimens of *Polygonatum* in Herbaria PE, CSH, K, KUN, WU, JSPC, XBGH and NAS were also examined through digital platforms (CVH, GBIF, NSII), with special attention on the type specimens of *P. cyrtoneuma* and its synonyms and morphological similar species.

Genome sequencing, assembly and annotation

Fresh leaves of one individual per population in five populations (SY, JZ, LY, LA of *P. praecox* and KZ of *P. caulialatum*, Table 1) were collected and dried in silica for

Table 1. The information of the sampled and investigated populations.

<i>Polygonatum praecox</i> (putative new species)				
LY	Langya Mountain Scenic Spot, Langya, Chuzhou City, Anhui Province	32.2777	118.2866	ON736440
LA	Qingliangfeng Mountain, Linan, Hangzhou City, Zhejiang Province	30.1451	118.8705	ON943064
JZ	Tiantangzhai Scenic Spot, Jinzhai, Liuan City, Anhui Province	31.1256	115.7718	ON736439
SY	Jiashi Gorge, Banyan Town, Shanyang, Shangluo City, Shaanxi Province	33.3181	109.7701	ON736441
QY	Wumei Village, Yangtian Town, Qingyang, Chizhou City, Anhui Province	30.5829	117.9702	
HS	Bancang Nature Reserve, Huoshan, Anqing City, Anhui Province	31.1147	116.1091	
<i>P. caulialatum</i>				
KZ	Bashan Grand Canyon Scenic Area, Kaizhou, Chongqing City	31.6505	108.4345	ON943065
TB	Qingfengxia Forest Park, Taibai, Baoji City, Shaanxi Province	34.0099	107.4407	
<i>P. odoratum</i>				
QS	Tianzhu Mountain Scenic Spot, Qianshan, Anqing City, Anhui Province	30.7392	116.4663	
CZ	Langya Mountain, Langya, Chuzhou City, Anhui Province	32.2792	118.2811	
<i>P. cyrtoneuma</i>				
TTZ	Tiantangzhai Scenic Spot, Jinzhai, Liuan City, Anhui Province	31.1256	115.7718	
QLF	Qingliangfeng Mountain, Linan, Hangzhou City, Zhejiang Province	30.1451	118.8706	
JH	Jiuhua Mountain Scenic Spot, Qingyang, Chizhou City, Anhui Province	30.5112	117.8448	

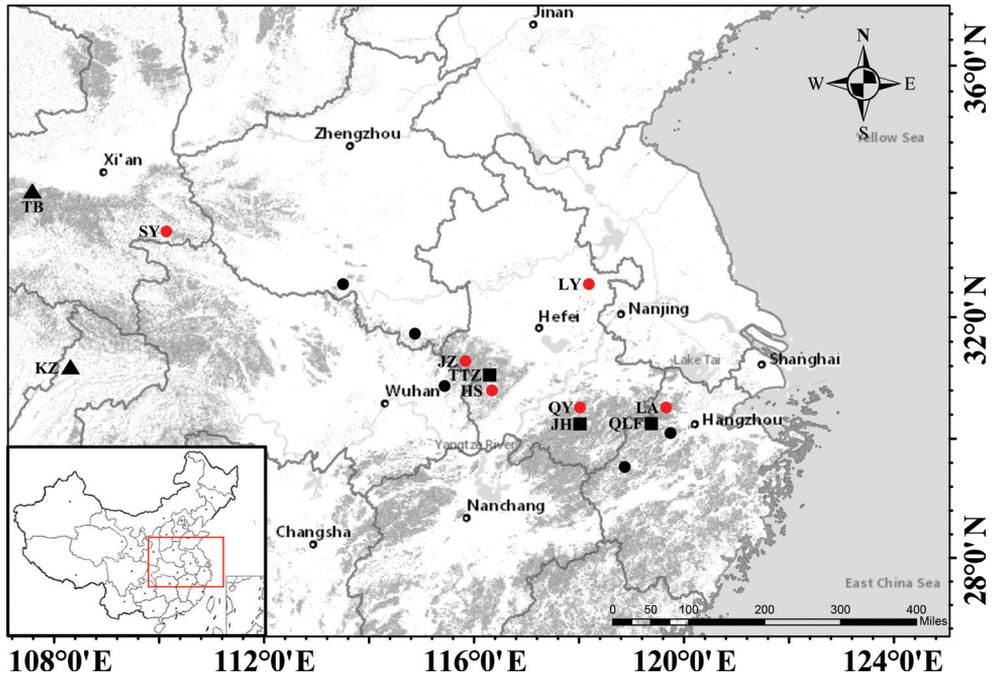


Figure 1. Distribution map of *Polygonatum praecox* (red dots showing locations found in this study; black dots showing locations identified by specimen examination), *P. caulialatum* (black triangle) and *P. cyrtonema* (black square).

molecular analysis. Genomic DNA was extracted using Tiangen DNasecure Plant Kit (DP320). All libraries were built through optimisation of the construction process and sent to the Germplasm Bank of Wild Species in Southwest China (GBOWS, Kunming, China) for Illumina sequencing. Approximately 3 GB of raw data were generated for each sample. Raw data were trimmed by removing adapters and low-quality reads by FastQC/ Trimmomatic. The complete chloroplast genome was assembled using Getorganelle v.1.7.5.2, through the original data reads (fastq / FQ file) with K-mer = 21, 65 and 105 (Jin et al. 2020). The plastome gene sequences of *P. odoratum* (MZ150858) were adopted as reference and seed sequences. PGA (Qu et al. 2019) was used for plastome annotation with manually checking the start/stop codons in Geneious 10.2.3 (<http://www.geneious.com>). The plastid genome map was drawn using OGDRAW (Greiner et al. 2019). Basic characteristics of chloroplast genomes were read in Geneious (Table 2).

Phylogenetic analyses

In order to reveal the phylogenetic relationship of the putative novel species and its related species, plastome sequence data of *Polygonatum* and outgroup (*Heteropolygonatum ogisui*) from GenBank were downloaded (Floden and Schilling 2018; Xia et al. 2021, 2022; Wang et al. 2022). All sequences were aligned using MACSE v.2 and one of the inverted repeats (IRa) was deleted using Geneious (e.g. Ranwez et al. 2018) before further analysis.

Table 2. Basic characteristics of chloroplast genomes of *Polygonatum praecox*, sp. nov.

Characteristic	<i>Polygonatum praecox</i>	<i>P. caulialatum</i>
Total length (bp)	155,115–155,256	155,318
GC%	37.7%–37.7%	37.7%
LSC length (bp)	84,252–85,225	84,252
SSC length (bp)	18,450–18,474	18,462
IR length (bp)	26,318–26,323	26,302
Total genes	112	112
Protein-coding genes	76	76
rRNA genes	4	4
tRNA genes	32	32

The phylogenetic tree was constructed using Maximum Likelihood (ML) methods and Bayesian Inference (BI) methods. The best substitution model was determined by ModelFinder in PhyloSuite (Kalyanamoorthy et al. 2017; Zhang et al. 2020). The ML analysis was performed using IQ-TREE v.1.6.12 with 1000 bootstrap replicates and (GTR) + G + I (Nguyen et al. 2015). The BI analysis was conducted in MrBayes v.3.2 (Ronquist et al. 2012). The Markov Chain Monte Carlo (MCMC) algorithm was run for 20 million generations and the trees were sampled every 1000 generations. Convergence was determined by examining the average standard deviation of the split frequencies (< 0.01). The first 25% of calculated trees were discarded as burn-in and the remaining trees were used to construct a consensus tree to estimate the posterior probability (PP).

Results and discussion

Characteristics of the complete plastid genome

The length of chloroplast complete genome of *Polygonatum praecox* samples was 155,115–155,256 bp (Fig. 2) and *P. caulialatum* was 155,318 bp; both possessed typical quadripartite structure (IRa, IRb, LSC and SSC). The characteristics and statistics of their plastid genomes are summarised in Table 2.

Phylogenetic relationship

As in previous phylogenetic analyses of *Polygonatum* (Meng et al. 2014; Xia et al. 2022), three well-supported clades corresponding to monophyletic sections were also resolved, i.e. *Verticillata*, *Sibirica* and *Polygonatum* (Fig. 3). The four individuals of the putative novel species from different geographic populations grouped into a monophyletic clade (Bootstrap Support (BS) = 100%, Bayesian Inference (BI) = 1), which were not sister to the three morphologically similar species (*P. cyrtoneuma*, *P. odoratum* and *P. caulialatum*, Fig. 3), although they are all in section *Polygonatum*. Unexpectedly, the putative novel species is closely related to the two European species (*P. multiflorum* Kunth and *P. latifolium* (Jacq.) Desf.) (Fig. 3).

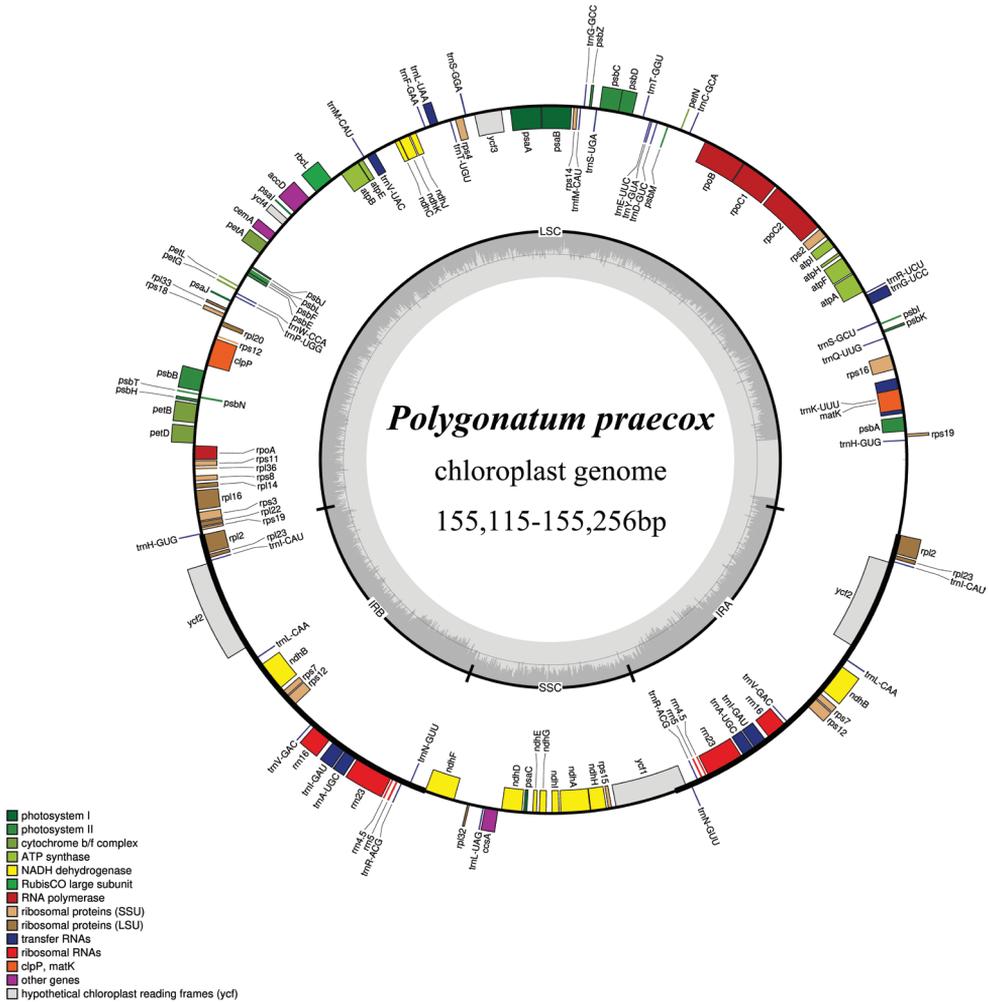


Figure 2. Plastid genome map of *Polygonatum praecox* Y.F.Hu & J.W.Shao, sp. nov.

Morphological assessment

The new species is morphologically similar to *Polygonatum cyrtonema* and *P. caulialatum* in its alternate oblong leaves, thick moniliform rhizome and large flowers (corolla about 1.7–2.2 cm long) (Figs 4, 5, Table 3), but it differs from *P. cyrtonema* in racemose inflorescence (vs. umbel-like), cylindrical and hairless filaments and its apex without saccate-convex (vs. papillose or shortly cottony, apex slightly dilated or with saccate-convex) and flowering from mid-March to early April (vs. from late April to late May); it differs from *P. caulialatum* in its terete stem (vs. obviously angled in upper part), yellowish-green corolla and lobes excurved (vs. green-white and lobes not excurved) and earlier flowering period (mid-March to early April vs. May to June). As to inflorescence type and flowering phenology, *Polygonatum praecox* is also similar to *P. odoratum* (raceme inflorescence and flowering from mid-March to early April), but they are very

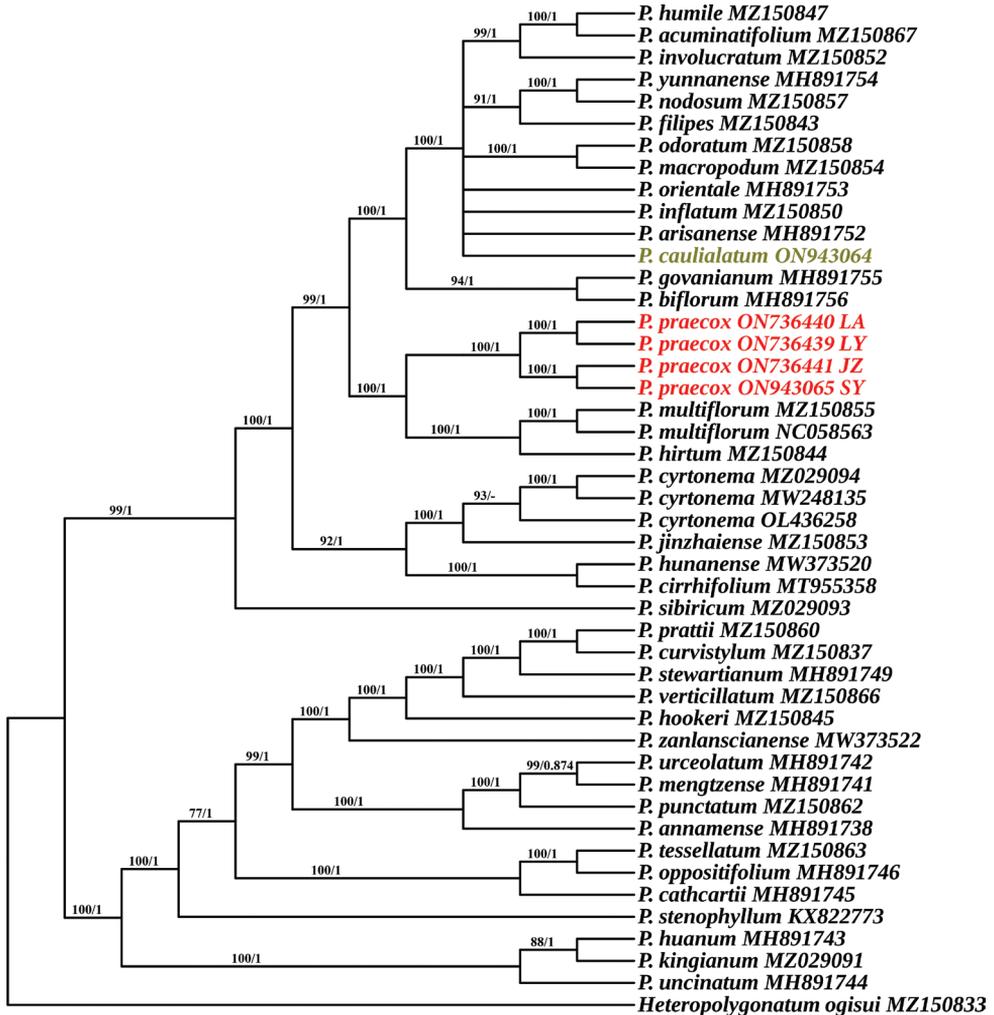


Figure 3. Phylogenetic relationships of the new species and its related species in *Polygonatum*, inferred from Maximum Likelihood (ML) and Bayesian Inference (BI) methods, based on the plastid genomes without one of the inverted repeats (IRa). Numbers on the branches indicate the bootstrap support of the ML and the posterior probability of BI analyses. The phylogenetic position of *P. praecox* is highlighted in red and *P. caulialatum* in brown. GenBank accession numbers are displayed after the species name.

different in rhizome morphology (moniliform vs. terete) and stem (terete vs. angled). In morphology, this new species is also easily distinguished from its genetically related species *P. multiflorum* and *P. latifolium* by its moniliform rhizome (vs. terete) and campanulate-cylindrical yellowish-green floral tube (vs. cylindrical, but somewhat contracted in the middle and whitish). In summary, there are obvious differences between the new species and its related species in morphology, especially in filament traits. However, because of the short flowering period, most previously collected specimens of *Polygonatum* were without blooming flowers and the stamen morphology is not easy to observe on dry specimens, which may be the reason for this new species being neglected for a long term.

Table 3. Morphological differences amongst *Polygonatum cyrtonema*, *P. odoratum*, *P. caulialatum* and *P. praecox*.

	<i>P. cyrtonema</i>	<i>P. odoratum</i>	<i>P. caulialatum</i>	<i>P. praecox</i>
Rhizome	usually gingeriform, 1–2.5 cm thick	terete, 0.5–1.0 cm thick	moniliform, 1.5–2.5 cm thick	moniliform, 1.5–2.5 cm thick
Stem	50–100 cm, terete	20–60 cm, upper part angled	40–80 cm, upper part angled	40–80 cm, terete
Inflorescence	umbel-like, 2–7(–14) flowered	raceme, 1–3(–5) flowered	raceme, 1–2(–3) flowered	raceme, 1–3(–4) flowered
Filament	papillose or shortly cottony, apex slightly dilated or saccate-convex	cylindrical and extending inwardly, smooth or verruculose	cylindrical and extending inwardly, smooth and glabrous	cylindrical and extending inwardly, smooth and glabrous
Corolla	yellowish-green, lobes excurved	green-white, lobes slightly excurved	green-white, lobes not excurved, crown slightly constricted	yellowish-green, lobes excurved
Flower phenology	late April to late May	mid-March to early April	May to June	mid-March to early April

Taxonomic treatment

Polygonatum praecox Y.F.Hu & J.W.Shao, sp. nov.

urn:lsid:ipni.org:names:77306998-1

Figs 4–6

Type. CHINA. Anhui: Chuzhou City, Langya District, Langya Mountain, 32°16'39"N, 118°17'12"E, Altitude: 147 m, 10 Apr 2020, *Yin Feng Hu & Jian Wen Shao* HYF20041003 (holotype: ANUB, 008492, Fig. 6; isotypes: ANUB, 008491, 008493).

Diagnosis. Most similar to *P. cyrtonema*, but differs in racemose inflorescence, cylindrical and glabrous filaments and apex not saccate convex.

Description. Rhizome moniliform, rarely tuberous moniliform, 1.5–2.5 cm thick. Stem arching, 40–80 cm, glabrous and not angled. Leaves 14–22, alternate; petiole short or nearly sessile; leaf blade elliptic to oblong-lanceolate, 8–13 × 4–6 cm, apex usually acuminate. Inflorescences raceme, (1)2–3(4)-flowered; peduncle 1–2 cm; bracteoles borne on the middle part of pedicel, subulate, < 2 mm or absent. Flowers pendulous, pedicel 0.5–1.5 cm long. Perianth yellowish-green, campanulate-cylindrical, 1.7–2.2 cm long; lobes ca. 3 mm long, excurved. Filaments inserted near the distal 1/3 of the perianth tube, cylindrical and extending inwardly, 3–6 mm long, smooth, apex without saccate-convex. Anthers 3.5–4.0 mm long. Ovary 4–6 mm in diam.; style 1.2–1.5 cm long. Berries black, ca. 1.2–1.5 cm in diam., 9–15 seeded.

Phenology. Flowering from mid-March to early April and fruiting from May to September.

Etymology. The specific epithet *praecox* alludes to early flowering of the new species as compared to *Polygonatum cyrtonema*, a morphologically similar species. The Chinese name of the new species is here given as 早花黄精 (Zǎo huā huáng jīng).

Distribution and habitat. Currently, *Polygonatum praecox* is known from more than 10 populations and it is fairly widely distributed in middle-eastern China (Fig. 1).

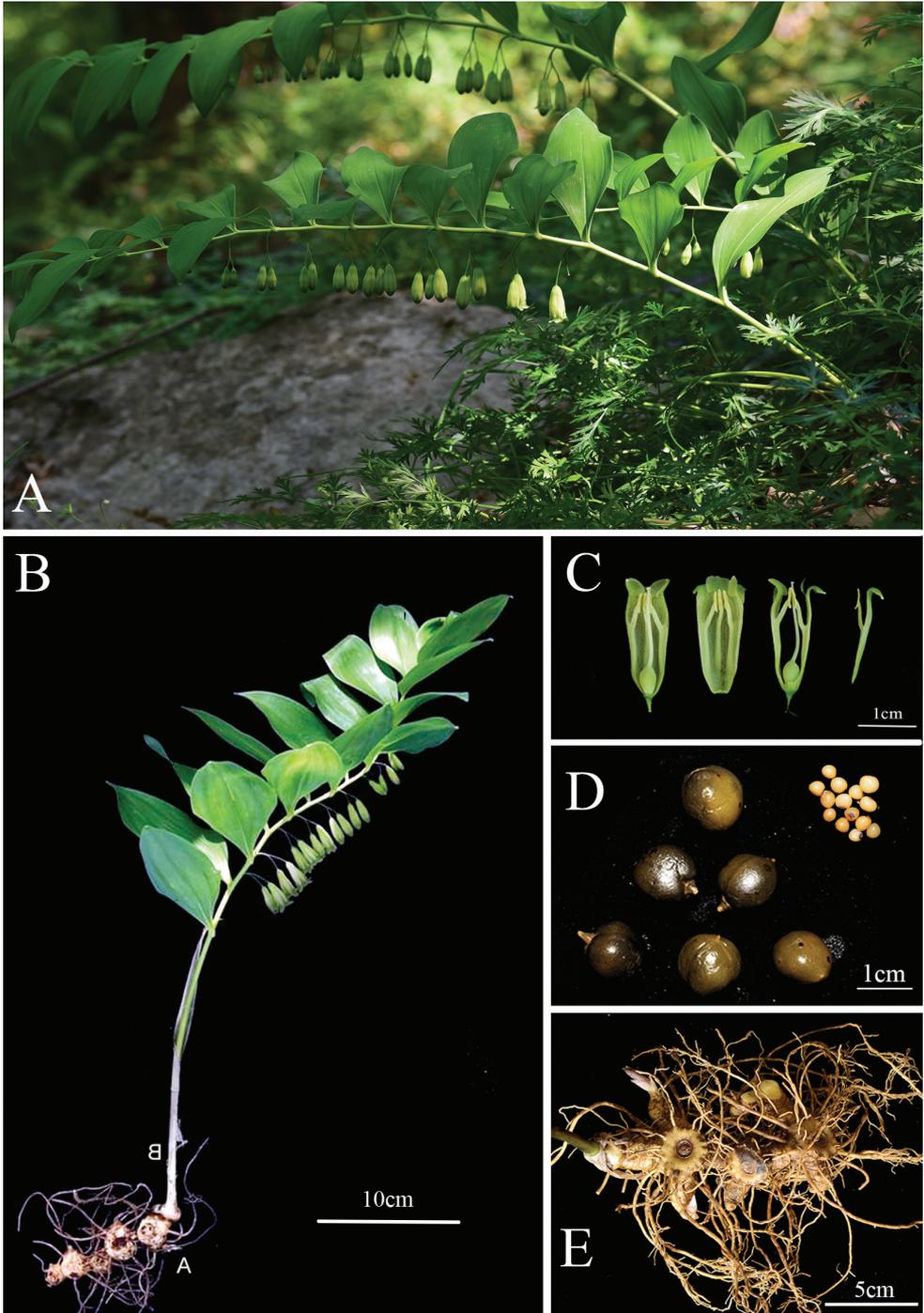


Figure 4. *Polygonatum praecox* Y.F.Hu & J.W.Shao, sp. nov. **A** habitat **B** general habit **C** longitudinal section of floral tube, showing stamens and pistil **D** seeds and fruits, soaked in alcohol **E** rhizome with roots. All Photos by Yingfeng Hu.

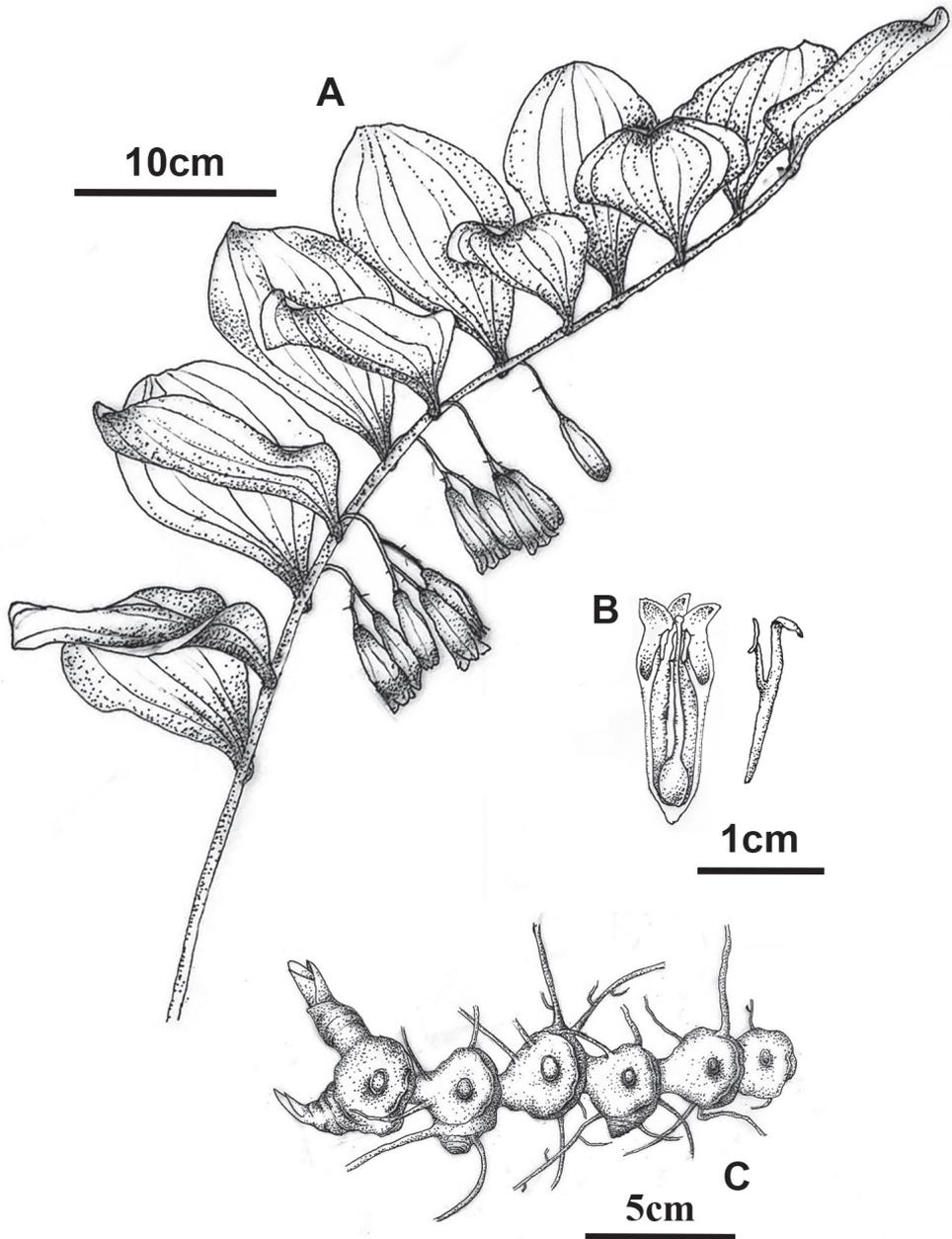


Figure 5. Illustration of *Polygonatum praecox* Y.F.Hu & J.W.Shao, sp. nov. **A** morphology of above-ground part **B** longitudinal section of floral tube, showing stamens and pistil **C** moniliform rhizome. Drawn according to the holotype by Wei Wu.

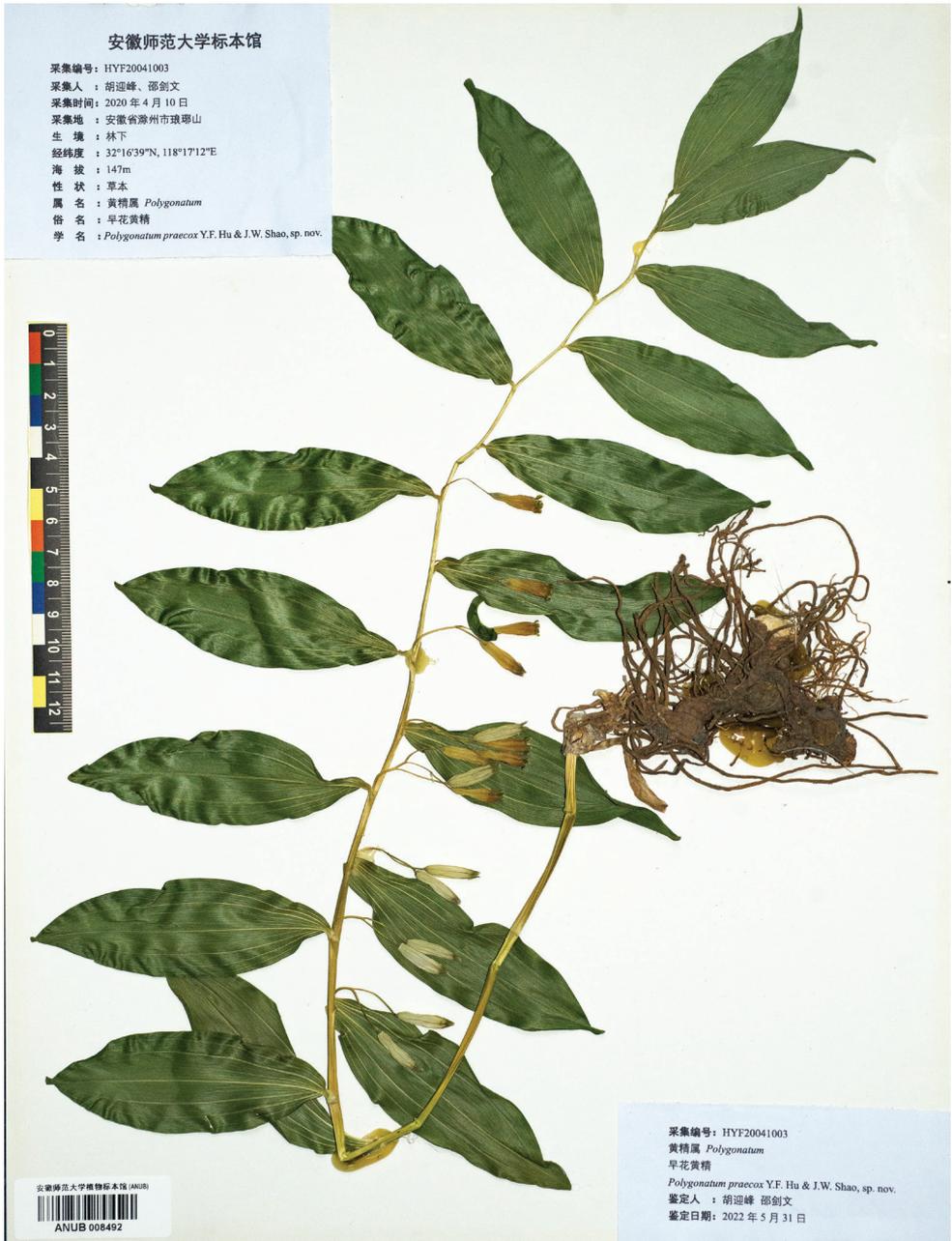


Figure 6. Holotype of *Polygonatum praecox* Y.F.Hu & J.W.Shao, sp. nov.

This species often occurs near valley streams under forest shade and on gravel or soil with good water permeability between elevations of 50 m to 1200 m.

Additional specimens examined (paratypes). CHINA. Anhui: Langya District, Langya Mountain, alt. 200 m, 3 Jul 1964, *Anonymous*, 101383 (JSPC); Langya

District, Langya Mountain, 4 May 1957, *Teng Yan Chang*, 0305591 (KUN). Zhejiang: Linan County, Changhua, alt. 1080 m, 17 Jun 1957, *Deng Lin Bing* 00223701 (NAS); Linan County, Tianmu Mountain, 18 May 1955, *Yuan Chang Qi* 00553413 (NAS). Hubei: Yinshan County, Wujiashan Forest Farm, alt. 1070 m, 06 Apr 2015, *Chen Bin* 0092527 (CSH); Yinshan County, alt. 815 m, 26 Apr 2015, *Ge Bin Jie* 0092551 (CSH). Henan: Song County, Xihe River, 8 May 1972, *Anonymous*, 00223667 (PE); Neixiang County, Baotianman Nature Reserve, 28 Aug 2008, *Liu Meng Ya* 0003911 (HEAC). Shaanxi: Shanyang County, Jiashi Gorge, Banyan Town, 26 July 2009, *Li En Feng* 010008 (XBGH).

Conservation status. Near Threatened. *Polygonatum praecox* is relatively common in middle-eastern China. As it is similar to *P. cyrtonema* in morphology and these two species occasionally co-exist in the wild, this new species is usually recognised as *P. cyrtonema* and has been exploited for medicinal or edible purposes. Its wild resources have clearly decreased in recent years. Therefore, we classify it as Near Threatened (NT) according to the IUCN Red List Criteria (IUCN 2019).

Acknowledgements

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References

- Cai XZ, Hu GW, Kamande EM, Ngumbau VM, Wei N (2015) *Polygonatum campanulatum* (Asparagaceae), a new species from Yunnan, China. *Phytotaxa* 236(1): 094–096. <https://doi.org/10.11646/phytotaxa.236.1.10>
- Chen XQ, Tamura MN (2000) *Polygonatum* Mill. In: Wu ZY, Raven PH (Eds) *Flora of China* (Vol. 24). Science Press, Beijing & Missouri Botanical Garden Press, Beijing/St. Louis, 225–235.
- Chen HY, Huang Y, Zhao XX, Yi SR (2021) *Polygonatum caulialatum*, a new species of medicinal plant of *Polygonatum* (Asparagaceae) from Sichuan, China. *Phytotaxa* 513(1): 055–061. <https://doi.org/10.11646/phytotaxa.513.1.3>
- Chinese Pharmacopoeia Commission (2020) *Pharmacopoeia of the People's Republic of China* (Part I), 2020 Ed. China Pharmaceutical Science and Technology Press, Beijing, 946 pp. [in Chinese]
- Fan B, Wei GL, Gan XF, Li TT, Qu ZY, Xu S, Liu C, Qian CQ (2020) Study on the varied content of *Polygonatum cyrtonema* polysaccharides in the processing of steaming and shining for nine times based on HPLC-MS/MS and chemometrics. *Microchemical Journal* 159: 105352. <https://doi.org/10.1016/j.microc.2020.105352>
- Floden A (2012) Reinstatement of *Polygonatum yunnanense* (Asparagaceae). *Phytotaxa* 58(1): 59–64. <https://doi.org/10.11646/phytotaxa.58.1.3>

- Floden A (2015) A new *Polygonatum* (Asparagaceae) endemic to the Trường Sơn of southern Vietnam. *Phytotaxa* 197(2): 125–131. <https://doi.org/10.11646/phytotaxa.197.2.5>
- Floden A (2017) Molecular phylogenetic studies of the genera of tribe Polygonateae (Asparagaceae: Nolinoideae): *Disporopsis*, *Heteropolygonatum*, and *Polygonatum*. The University of Tennessee, Knoxville, USA. Doctoral Dissertation. https://trace.tennessee.edu/utk_graddiss/4398
- Floden A, Schilling EE (2018) Using phylogenomics to reconstruct phylogenetic relationships within tribe Polygonateae (Asparagaceae), with a special focus on *Polygonatum*. *Molecular Phylogenetics and Evolution* 129: 202–213. <https://doi.org/10.1016/j.ympev.2018.08.017>
- Greiner S, Lehwark P, Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.1: Expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Research* 47(1): 59–64. <https://doi.org/10.1093/nar/gkz238>
- Isaac NJB, Mallet J, Mace GM (2004) Taxonomic inflation: Its influence on macroecology and conservation. *Trends in Ecology & Evolution* 19(9): 464–469. <https://doi.org/10.1016/j.tree.2004.06.004>
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [accessed 4 Sep 2019]
- Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ (2020) GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21(1): 241. <https://doi.org/10.1186/s13059-020-02154-5>
- Kalyaanamoorthy S, Minh BQ, Wong T, von Haeseler A, Jermini LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14(6): 587–589. <https://doi.org/10.1038/nmeth.4285>
- Li XL, Ma RH, Zhang F, Ni JZ, Thakur K, Wang SY, Zhang JG, Wei ZJ (2021) Evolutionary research trend of *Polygonatum* species: A comprehensive account of their transformation from traditional medicines to functional foods. *Critical Reviews in Food Science and Nutrition*, 1–18. <https://doi.org/10.1080/10408398.2021.1993783>
- Meng Y, Nie ZL, Deng T, Wen J, Yang YP (2014) Phylogenetics and evolution of phyllotaxy in the Solomon's seal genus *Polygonatum* (Asparagaceae: Polygonateae). *Botanical Journal of the Linnean Society* 176(4): 435–451. <https://doi.org/10.1111/boj.12218>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. <https://doi.org/10.1093/molbev/msu300>
- Qu XJ, Moore MJ, Li DZ, Yi TS (2019) PGA: A software package for rapid, accurate, and flexible batch annotation of plastomes. *Plant Methods* 15(1): 50. <https://doi.org/10.1186/s13007-019-0435-7>
- Ranwez V, Douzery EJP, Cambon C, Chantret N, Delsuc F (2018) MACSE v2: Toolkit for the Alignment of Coding Sequences Accounting for Frameshifts and Stop Codons. *Molecular Biology and Evolution* 35(10): 2582–2584. <https://doi.org/10.1093/molbev/msy159>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>

- Si JP, Zhu YX (2021) Polygonati rhizoma—a new high-quality crop with great potential and not occupying farmland (in Chinese). *SCIENTIA SINICA Vitae*, 51. <https://doi.org/10.1360/SSV-2020-0413>
- Tamura MN (1991) Biosystematic studies on the genus *Polygonatum* (Liliaceae) II. Morphology of staminal filaments of species indigenous to Japan and its adjacent regions. *Acta Phytotaxonomica et Geobotanica* 42: 1–18.
- Tamura MN (1993) Biosystematic studies on the genus *Polygonatum* (Liliaceae) III. Morphology of Staminal filaments and karyology of eleven Eurasian species. *Botanische Jahrbücher für Systematik* 115: 1–26.
- Tamura MN, Schwarzbach AE, Kruse S, Reski R (1997) Biosystematic studies on the genus *Polygonatum* (Convallariaceae) IV. Molecular phylogenetic analysis based on restriction site mapping of the chloroplast gene trnK. *Feddes Repertorium* 108(3–4): 159–168. <https://doi.org/10.1002/fedr.19971080306>
- Tang YC (1978) *Polygonatum* Mill. In: Wang FT, Tang T (Eds) *Flora Reipublicae Popularis Sinicae*. Science Press, Beijing, China.
- Wang JJ, Yang YP, Sun H, Wen J, Deng T, Nie ZL, Meng Y (2016) The biogeographic South-North divide of *Polygonatum* (Asparagaceae tribe Polygonateae) within Eastern Asia and its recent dispersals in the Northern Hemisphere. *PLoS ONE* 11(11): e0166134. <https://doi.org/10.1371/journal.pone.0166134>
- Wang J, Qian J, Jiang Y, Cheng XC, Zheng BJ, Chen SL, Yang FJ, Xu ZC, Duan BZ (2022) Comparative Analysis of Chloroplast Genome and New Insights Into Phylogenetic Relationships of *Polygonatum* and Tribe Polygonateae. *Frontiers in Plant Science* 13: e882189. <https://doi.org/10.3389/fpls.2022.882189>
- Xia MQ, Liu Y, Liu JJ, Chen DH, Shi Y, Bai ZC, Xiao Y, Peng C, Si JP, Li P, Qiu YX (2021) A new synonym of *Polygonatum* in China, based on morphological and molecular evidence. *PhytoKeys* 175: 137–149. <https://doi.org/10.3897/phytokeys.175.63383>
- Xia MQ, Liu Y, Liu JJ, Chen DH, Shi Y, Chen ZX, Chen DR, Jin RF, Chen HL, Zhu SS, Li P, Si JP, Qiu YX (2022) Out of the Himalaya-Hengduan Mountains: Phylogenomics, biogeography and diversification of *Polygonatum* Mill. (Asparagaceae) in the Northern Hemisphere. *Molecular Phylogenetics and Evolution* 169: 107431. <https://doi.org/10.1016/j.ympev.2022.107431>
- Yang HJ, Wu BH, Chen ZL, Wang P, Shi S, Li FF, Xu H, Cui DF (2020) *Polygonatum daminense* (Asparagaceae), a new species from China based on morphological and molecular evidence. *Phytotaxa* 449(3): 287–294. <https://doi.org/10.11646/phytotaxa.449.3.7>
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* 20(1): 348–355. <https://doi.org/10.1111/1755-0998.13096>
- Zhao P, Zhao CC, Li X, Gao QZ, Huang LQ, Xiao PG, Gao WY (2018) The genus *Polygonatum*: A review on ethnopharmacology, phytochemistry and pharmacology. *Journal of Ethnopharmacology* 214: 274–291. <https://doi.org/10.1016/j.jep.2017.12.006>
- Zhao LH, Zhou SD, He XJ (2019) A phylogenetic study of Chinese *Polygonatum* (Polygonateae, Asparagaceae). *Nordic Journal of Botany* 37(2): njb.02019. <https://doi.org/10.1111/njb.02019>

Impatiens hambaeksanensis (Balsaminaceae), a new species from South Korea

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Abstract

A new species *Impatiens hambaeksanensis* from Gangwon-do, South Korea, is described and illustrated, based on its morphology and distribution. *I. hambaeksanensis* is different from *I. furcillata*, another similar *Impatiens* species in South Korea, in some ways: *I. hambaeksanensis* possesses a serrate leaf margin with flat tooth tip, while *I. furcillata* possesses a crenate leaf margin with erect tooth tip; it has an erect inflorescence, while *I. furcillata* has a pendulous inflorescence; it has a smaller flower which is 2–2.6 cm long, while *I. furcillata* has a flower of 2.3–3.2 cm; the flower is white or pinkish-white with yellowish and purplish spots, while *I. furcillata* has a white lower sepal and pinkish-white lateral united petals with yellowish spots; the distal part of the lower sepal is mostly not coiled or rarely 1-coiled, while that of *I. furcillata* is never coiled; the spur tip is expanded, round and slightly biparted, while that of *I. furcillata* is expanded, ellipsoidal and clearly biparted. A taxonomic description, a holotype and photos of morphological characteristics of the new species are provided. A table which includes the morphological comparison and a geographical distribution map are presented as well.

Keywords

Gangwon-do, Korean endemic, morphology, new species, taxonomy

Introduction

Balsaminaceae are a family composed of two genera, which are *Impatiens* and *Hydrocera* (Yuan et al. 2004; Chen et al. 2007). In contrast to *Hydrocera* that is unispecific, *Impatiens* comprises more than 1000 species that are mainly distributed across tropical and subtropical regions (Grey-Wilson 1980; Chen et al. 2007; Xia et al. 2019;

Yuan et al. 2022). *Impatiens*, which is known to be taxonomically difficult to study, is distinguished from *Hydrocera* by lateral united petals, valvate fruit and explosive capsule (Grey-Wilson 1980; Chen et al. 2007; Xia et al. 2019).

The first study on Korean *Impatiens* (Balsaminaceae) reported three species, which were *I. textorii* Miq. (Mul-bong-seon in Korean), *I. noli-tangere* L. (No-rang-mul-bong-seon) and *I. furcillata* Hemsl. (San-mul-bong-seon) (Forbes and Hemsley 1886). Subsequently, four to eight *Impatiens* species were recorded in Korea (Nakai 1952; Chung 1956; Lee 1980; Lee 1996; Lee 2006). Most recently, four *Impatiens* species from Korea were reported (Chang et al. 2017). Amongst these recorded species, *I. furcillata* was reported as a new species by Forbes and Hemsley (1886), based on the type materials in the Royal Botanic Garden (K), which were collected in Port Hamilton (officially Geomundo Island in South Korea) and Gensan (Wonsan in South Korea). In previous studies on this species, either simple descriptions, such as, “The overall size is smaller compared to *I. textorii*”, “The spur is long and not coiled” and “The flower is white”, were recorded (Park 1974; Lee 1996) or only the plant list without a description or diagnosis was recorded (Chung et al. 1937; Park 1949; Chung 1956; Lee 1980; Lee 2006). Ji et al. (2010) later re-assessed the taxonomy and morphological characteristics of *I. furcillata*, based on the original description and type materials. They recognised many characteristics that distinguish this species from other Korean *Impatiens* species, including the glabrous stem, drooping peduncle, pinkish–white flower, ovate–oblong leaf blade and non-coiled, biparted spur tip. These characteristics were identical with those of *I. kojeensis* Y.N.Lee (Geo-jae-mul-bong-seon in Korean) and *I. hypophylla* var. *koreana* Nakai (Cheo-jin-mul-bong-seon). Consequently, *I. kojeensis* and *I. hypophylla* var. *koreana* were treated as synonyms of *I. furcillata* and the Korean name of *I. furcillata* was changed to “Cheo-jin-mul-bong-seon” (Ji et al. 2010).

Meanwhile, some morphological characteristics of “San-mul-bong-seon”, have been identified: the overall size is smaller compared to *I. textorii*, the spur is long and not coiled, the peduncle is erect above the leaf and the flower is white (Park 1974; Lee 1996). The present study was conducted to assign a new name to the natural population of “San-mul-bong-seon”, which has been falsely known as ‘*I. furcillata*’, in the Gangwon-do region of the Baekdudaegan Mountain range.

Materials and methods

The new species was examined using 20 individual plants (dried vouchers) which were collected in the type locality, the living plants and the immersion specimens in 70% ethyl alcohol collected in the type locality and other habitats (Talbot and White 2013) and the vouchers in the Herbarium of the National Institute of Biological Resources (**KB**) and the Herbarium of the Korean National Arboretum (**KH**) (acronyms after Thiers 2022). In particular, the macro-characteristics, such as the plant height and flower shape, colour and structure, were closely observed and photographed in the habitats. For the morphological observations of micro-characteristics, a light microscope (ECLIPSE E600, Nikon, Japan) and a stereoscopic microscope (LEICA MZ7₅, Leica, Germany) were used. The immersion

Table 1. Morphological differences between *I. hambaeksanensis* and *I. furcillata*. Abbreviations. L, Length; W, Width.

Characters	<i>I. hambaeksanensis</i>	<i>I. furcillata</i>
Leaf shape	elliptic to rhomboid–elliptic	narrowly elliptic to elliptic
Leaf margin shape	serrate	crenate
Leaf tooth tip direction	flat, forward	erect, upward
Inflorescence position	ascending, erect	descending, pendulous
Rachis length (cm)	4–10	0.9–2.2
Rachis trichome type	multicellular multiseriate glandular hair	none
Flower length (cm)	2–2.6	2.3–3.2
Lateral sepal size (L×W, mm)	6 × 4–5	3.5–5.3 × 2.4–4.2
Lateral sepal colour	brownish-white	greenish-white or rarely green
Lower sepal length (mm)	10–18	25–31
Lower sepal colour	white or pinkish-white with yellowish and purplish spots	white with yellowish spots
Lower sepal coiling state	non- to rarely 1-coiled	never coiled
Spur tip shape	round, expanded, slightly biparted	ellipsoidal, expanded, clearly biparted
Dorsal petal size (L×W, mm)	4.8–5.1 × 5.4–6	9–11 × 13–15
Dorsal petal colour	white or brownish-white	greenish-white
Lateral united petal length (mm)	9.5–13	17–24
Lateral united petal colour	white or rarely pinkish-white with yellowish and purplish spots	pinkish-white with yellowish spots
Lateral united petal basal lobe size (L×W, mm)	2.5–4 × 1–2	6.2–7.1 × 4.1–5.1
Lateral united petal distal lobe size (L×W, mm)	7–11 × 3.8–4.4	12–16 × 10–14
Filament length (mm)	ca. 3	3.1–4.5
Anther length (mm)	ca. 1	ca. 2.5
Ovary length (mm)	2.2–2.4	4–4.7
Fruit length (mm)	14–18	15–23

specimens for the small structures of the flower and seeds were photographed with scale bar using the stereoscopic microscope and measured, based on microscope magnification.

The morphological description was created using the collected immersion specimens and the vouchers. The figures, which clearly show the taxonomic characteristics of *I. hambaeksanensis*, are provided (Figs 1–4). In addition, we compared the newly-described species with the related taxon, *I. furcillata*, a species that is morphologically most similar to the new species (Table 1; Fig. 5).

Taxonomic treatment

Impatiens hambaeksanensis B.U.Oh, sp. nov.

urn:lsid:ipni.org:names:77306999-1

Figs 1–4

Type. SOUTH KOREA. Province Gangwon-do: Jeongseon-gun, Gohan-eup, Mt. Hambaeksan, shady valley near stream in mountainous area, 37°09'31.53"N, 128°53'16.90"E, 1171 m, 5 Sep 2021, B.U.Oh & J.O.Kim 210905-001 (holotype: KBI; isotypes: KBI, KEI) (Fig. 1).



Figure 1. Holotype of *Impatiens hambaeksanensis* B.U.Oh, B.U.Oh & J.O.Kim 210905-001 (KB).

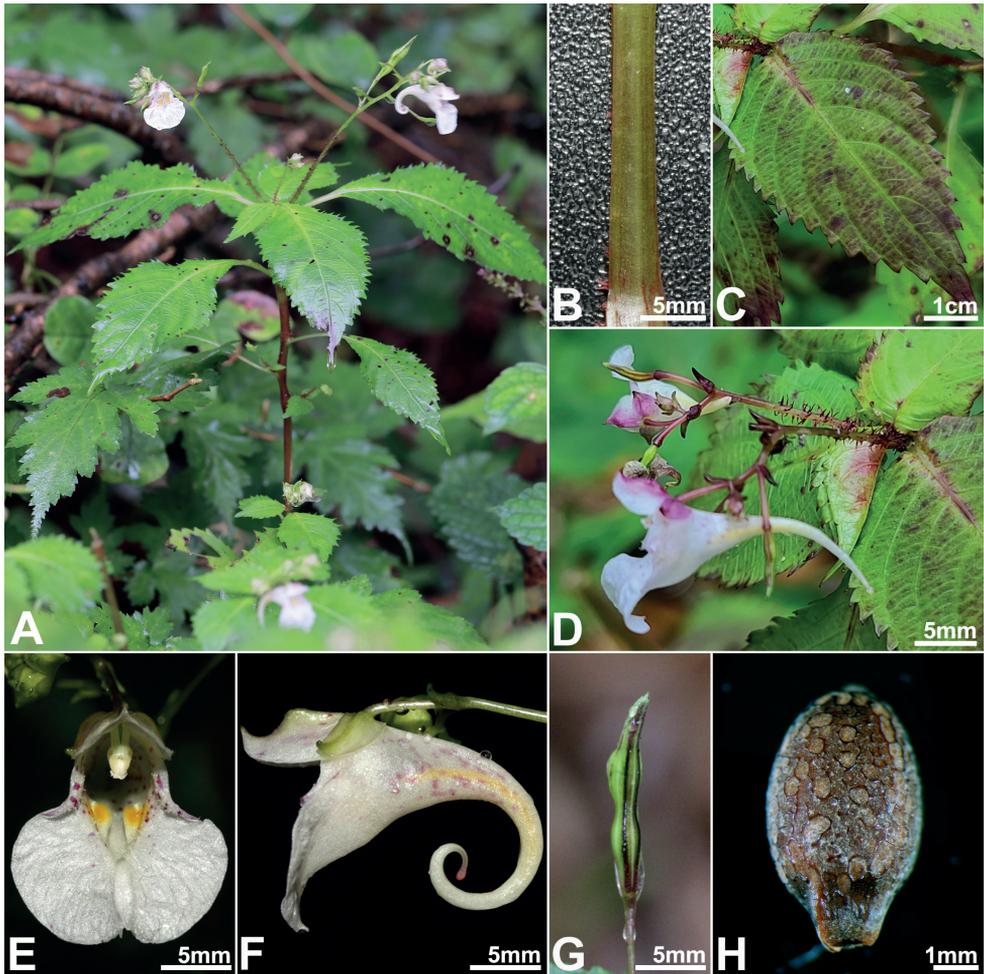


Figure 2. Morphological characteristics of *Impatiens hambaeksanensis* **A** habit **B** stem **C** leaf **D** inflorescence **E** frontal view of flower **F** lateral view of flower **G** fruit **H** seed. All photos by Byoung-Un Oh.

Diagnosis. *I. hambaeksanensis* is similar to *I. furcillata* in its overall characteristics, including taproot, alternate phyllotaxis and racemose inflorescence, but different from it in some ways: *I. hambaeksanensis* has serrate leaf margin with flat tooth tip; inflorescence is erect; flower is smaller and mostly white or rarely pinkish-white; the distal part of the lower sepal is mostly non-coiled or rarely 1-coiled; the spur tip is expanded, round and slightly biparted.

Description. Herb annual, 42–85 cm tall. Roots taproots. Stems erect, pale green to green or rarely purplish-green, branched, piliferous, with multicellular multiseriate glandular trichomes. Leaves alternate, usually glabrous or having scattered simple trichomes when immature; petioles 2–3.5 cm long; blade green, elliptic or rhomboid–elliptic, 6–11 cm long, 4–6 cm wide, apex acute, base acute or rounded, margin serrate.

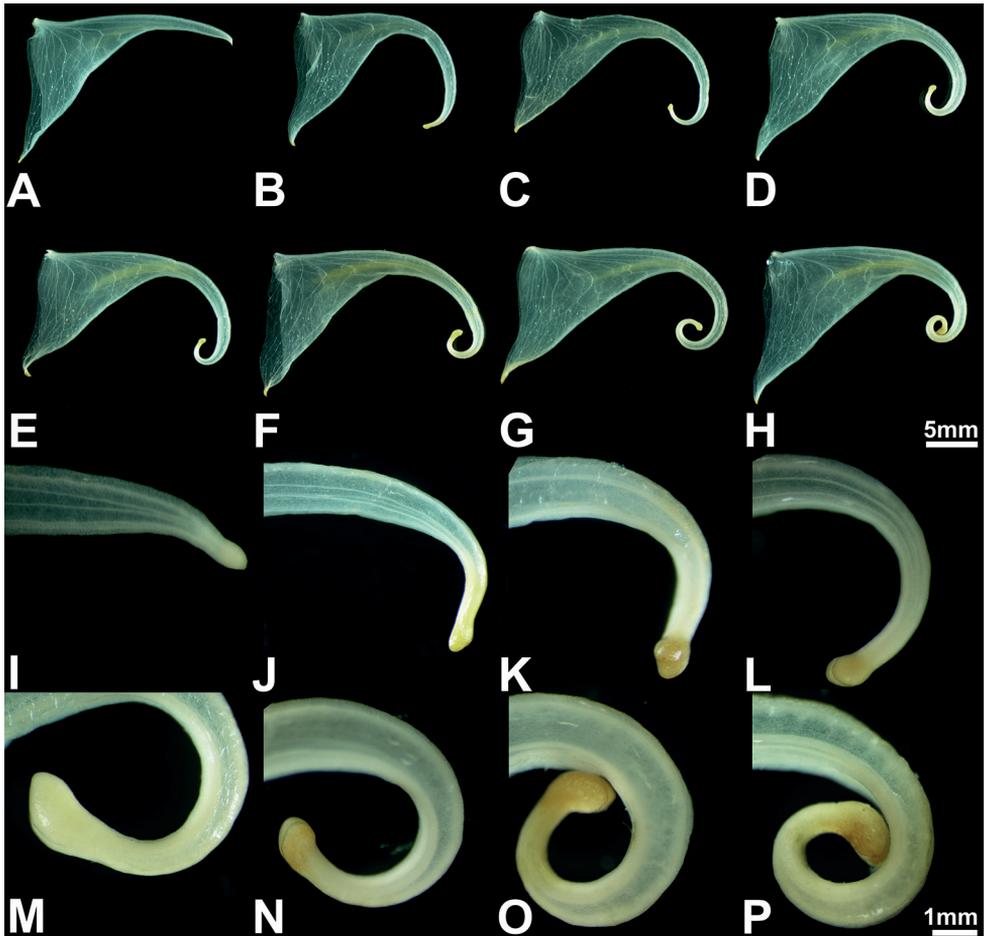


Figure 3. Flower variation ranges of *Impatiens hambaeksanensis* **A–H** lower sepals **I–P** distal part of lower sepals and spur tips. Scale bars: 5 mm (**A–H**); 1 mm (**I–P**).

Bracts triangular, 2.5–4 mm long, 1.5–2 mm wide, glabrous. Inflorescences racemose, axillary; rachises purplish-green, ascending, erect, 4–10 cm long, having dense multicellular multiseriate glandular trichomes; pedicels purplish-white, 0.7–1 cm long, glabrous. Flowers usually white or pinkish-white with yellowish and purplish spots, 2–2.6 cm long, 1.1–1.6 cm wide. Sepals 3; lateral sepals 2, brownish-white, ovate, ca. 6 mm long, 4–5 mm wide; lower sepal 1, white or pinkish-white with yellowish and purplish spots, funnel-form with slender spur, 10–18 mm long, 7–11 mm wide; spur usually not coiled, rarely 1-time coiled, 0.5–0.8 mm long, spur tip expanded, round, slightly biparted. Petals 3; dorsal petal 1, usually white or brownish-white, transversely elliptic, 4.8–5.1 mm long, 5.4–6 mm wide, apex emarginate, base truncate; lateral united petals 2, white or rarely pinkish-white with yellowish and purplish spots, 2-lobed, 9.5–13 mm long; basal lobe white, elliptic, 2.5–4 mm long, 1–2 mm wide; distal lobe white, obovate, 7–11 mm long, 3.8–4.4 mm wide. Stamens 5; filaments linear, upper

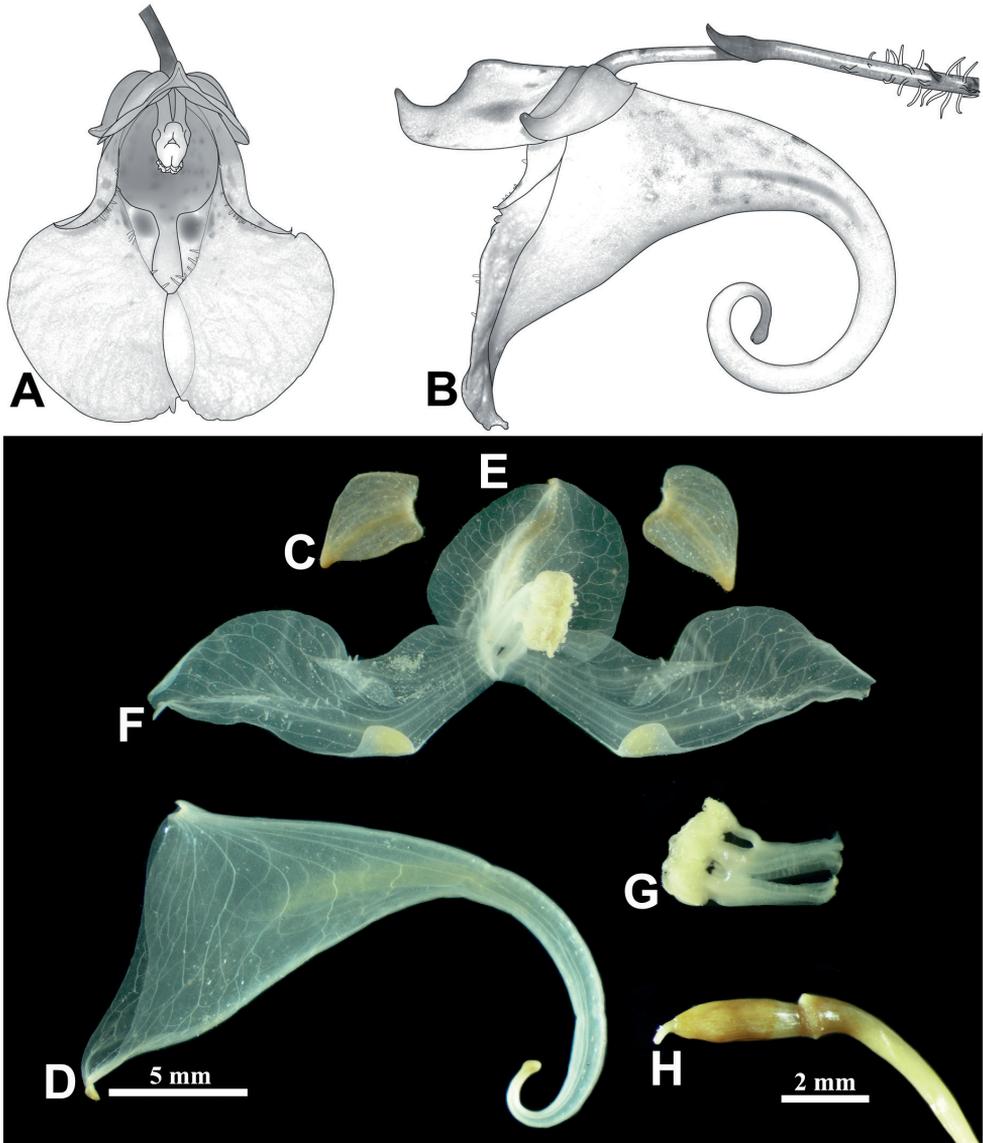


Figure 4. Reproductive organs and their vasculatures of *Impatiens hambaeksanensis* (White spots between veins are raphides) **A** frontal view of flower **B** lateral view of flower **C** lateral sepals **D** lower sepal **E** dorsal petal **F** lateral united petals **G** stamen **H** pistil. Scale bars: 5 mm (**D**); 2 mm (**C, E, F, G, H**).

part connate in a ring around the ovary apex, ca. 3 mm long; anthers white, ovoid, ca. 1 mm long. Pistil 1; ovary fusiform, 2.2–2.4 mm long, glabrous; style very short, ca. 0.5 mm long; stigma 5, beak-like. Fruits capsules, slender, fusiform, 14–18 mm long, glabrous. Seeds 2–5 per capsule, ellipsoidal, brown or dark brown, 4–4.6 mm long, 1.7–2.6 mm wide, surface irregularly reticulate with anticlinal wall. Pollen grains oblong with 4 apertures, 29.4–33.3 μm long, 15.7–21.6 μm wide (Figs 2–4).

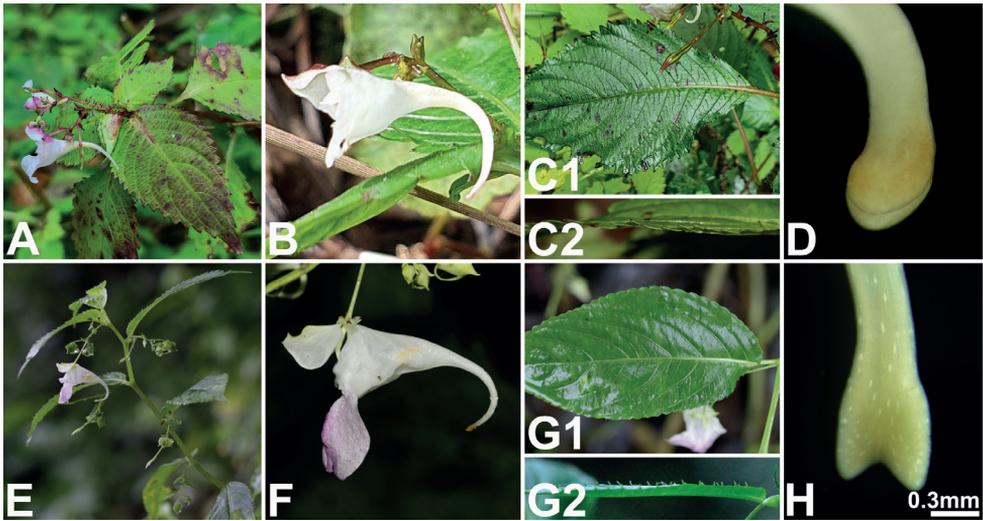


Figure 5. Major morphological differences between *Impatiens hambaeksanensis* (A–D) and *I. furcillata* (E–H) A, E inflorescences B, F lateral view of flowers and spur tip directions C, G leaves (C1, G1 bird's-eye view C2, G2 lateral view) D, H shape of spur tips. Scale bar: 0.3 mm (D, H). All photos by Byoung-Un Oh.

Distribution and habitat. In South Korea, *I. hambaeksanensis* is only observed in the central regions, especially in the Baekdudaegan Mountain range, including Gangwon-do (Jeongseon-gun and Yanggu-gun). *I. hambaeksanensis* is generally found in shady valleys or slopes near streams. In contrast, *I. furcillata* is distributed in the southern coastal regions of South Korea (Oh et al. 2016) (Fig. 6). This species is recorded from China and Russia, though it is possible that the plants in those regions were falsely identified as *I. furcillata*, considering their smaller flower which is 0.6–1.8 cm long and the northern limit of *I. furcillata* in South Korea.

Phenology. Flowering was observed from July to October. Fruiting was observed from late July to late October.

Conservation status. Currently, the known habitats of this new species are not legally protected. However, fortunately, many individuals of this species have been detected in the natural populations and the habitats are located in deep mountain valleys. Since the habitats are difficult to access, there may not be problems regarding habitat conservation within the near future. According to the IUCN (2019) Red List Criteria, we suggest that *I. hambaeksanensis* be treated as Data Deficient (DD).

Additional specimens examined. (paratypes): South Korea. Gangwon-do: Injae-gun, Mt. Daeamsan, 19 Sep 2021, *LJS21091901* (KH!); Jeongseon-gun, Mt. Hambaeksan, 5 Sep 2012, *KIMJH12157* (KB!), 2 Sep 2015, *Ji S-J et al., sn.* (KH!); Pyeongchang-gun, Mt. Gyeongbansan, 23 Aug 2012, *Nam C-H et al. Gyeongbansan-120823-035* (KH!); Taebaek-si, Mt. Taebaeksan, 16 Aug 2012, *Byeon J-G et al., sn.* (KH!), 10 Sep 2013, *Yang J-C et al., sn.* (KH!); Yangyang-gun, Mt. Seolaksan, 18 Sep 2018, *KIMJH18092* (KB!); Yanggu-gun, Dolsanryong, 17 Sep 2021, *B.U.Oh & J.O.Kim 210917-001* (KB!).

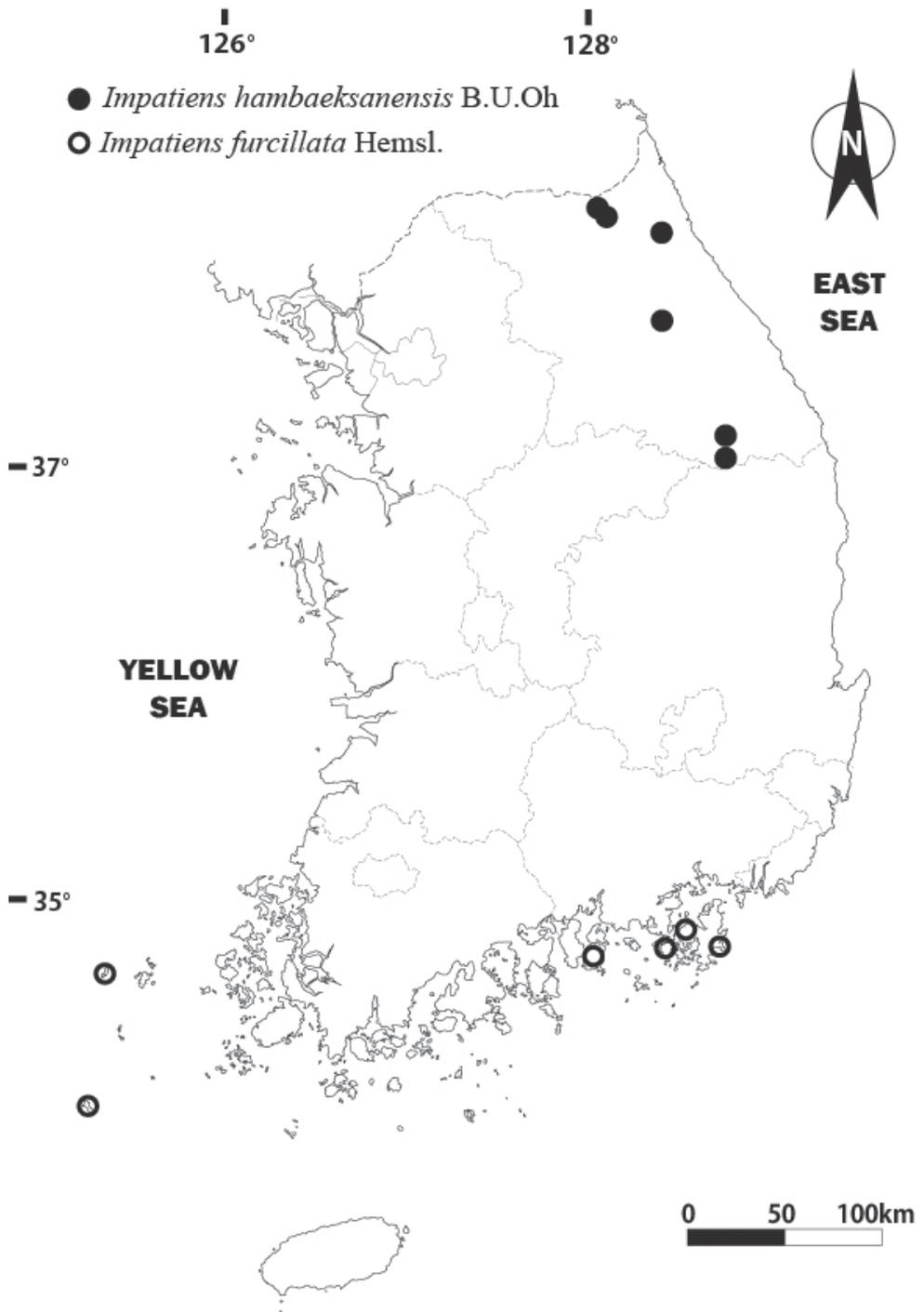


Figure 6. Geographical distribution of *Impatiens hambaeksanensis* (●) and *I. furcillata* (○).

Discussion

For the past 10 years, the authors have attempted to locate the habitats of *I. hambaeksanensis* (San-Mul-Bong-Seon), which has small, white flowers and the characteristic non-coiled spur (Park 1974). Recently, the authors discovered the natural populations, which correspond to *I. hambaeksanensis* in Mt. Hambaeksan and Dolsanryong in the north central region (Gangwon-do) of the Baekdudaegan Mountain range and they confirmed that these were what they had been looking for. In the habitat, most flowers were white and slightly pinkish flowers were seldom observed. In addition, the spur tips were not coiled in most flowers, while in some cases, the tips were coiled once. According to the new classification of the genus *Impatiens*, which was developed by Yu et al. (2016), *I. hambaeksanensis* belongs to the section *Impatiens* by having racemose inflorescence, 5-carpellate ovary, linear capsule and ellipsoid seed.

According to literature, *Impatiens koreana* Nakai (Nakai 1909) also has white flowers. However, after close examination of the holotype and the isotypes for *I. koreana* Nakai, it was confirmed that this taxon belonged to the natural populations of *I. textorii*. Therefore, *I. koreana* is considered a synonym of *I. textorii*.

In the Korean *Impatiens* species, the expanded spur tip is a taxonomically important characteristic in some cases. For example, *I. hambaeksanensis* has a spur tip that is expanded, round and slightly biparted. Meanwhile, *I. furcillata* has a spur tip that is ellipsoidal, expanded and clearly biparted, with each divided part having a pointed end. The biparted spur tip is considered to have been derived from the round, expanded and unparted tip. In addition, the clearly biparted spur tip of *I. furcillata* appears more evolutionarily advanced than that of *I. hambaeksanensis*.

The flowers of all the *Impatiens* species have coiled spur tips during the early stage of flower development, but as the flower matures, the lower sepal is stretched backwards, thus showing species specificity (Figs 4 and 5). In the case of *I. hambaeksanensis*, the shape of the lower sepal is highly variable and the extent to which the spur tip is coiled varies within a population. However, the spur tip is usually not coiled, except for uncommon cases where it is coiled once (Fig. 3).

Meanwhile, the two syntypes (*Oldham 123*, *Perry 98*) of *I. furcillata* which Hemsley W. previously cited were collected in Port Hamilton (officially Geomundo Island in Korea; *Oldham 123*) and Gensan (Wonsan, an old place name of Jindo Island in Korea; *Perry 98*) in Jeollanam-do (Forbes and Hemsley 1886; Choi 1995). It is also known that *I. furcillata* inhabits restricted southern-coastal regions of low altitude, including Gageodo Island in Shinan-gun, Mt. Cheonkwansan in Jangheung-gun of Jeollanam-do, Mt. Hogusan in Namhae-gun, Mireukdo Island and Mt. Byeokbansan in Tongyeong-si of Gyeongsangnam-do (Oh et al. 2016). Previously, a plant which is similar to *I. furcillata* was reported in China and Russia. The authors were aware of the existence of this plant in China and Russia and had the opportunity to observe and examine this plant in the field and from the vouchers available. However, this plant has smaller flowers than *I. furcillata*. In addition, from an ecological perspective, it is not probable that *I. furcillata* can exist in China and Russia, considering the northern limit of *I. furcillata*.

in South Korea, which is the southern coastal region. Therefore, the authors argue that maybe “*I. furcillata*” in China and Russia would have been falsely identified. This species in China and Russia has clearly biparted spur tip which is also observed in *I. furcillata* and this overlapping trait would have led to confusion and false identification.

In contrast, it is known that *I. hambaeksanensis* inhabits mountainous regions of central-northern Korea at elevations of 900–1200 m. Considering these distributional patterns of *I. furcillata* and *I. hambaeksanensis*, it can be concluded that these two species are geographically separated.

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References

- Chang KS, Son DC, Lee DH, Choi K, Oh SH (2017) Checklist of vascular plants in Korea. Korea National Arboretum, Pocheon.
- Chen YL, Akiyama S, Ohba H (2007) Balsaminaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China. Vol. 12. Science Press and Missouri Botanical Garden Press, Beijing & St. Louis.
- Choi CJ (1995) Gaheung. Encyclopedia of Korean Culture. The academy of Korean Studies. [in Korean]
- Chung TH (1956) Korean Flora (Herbaceous plants). Sinjisa, Seoul. [in Korean]
- Chung TH, Toh PS, Lee DB, Lee FJ (1937) Nomina Plantarum Koreanum. Chosen Natural History Society, Seoul. [in Korean]
- Forbes FB, Hemsley WB (1886) An enumeration of all the plants known from China Proper, Formosa, Hainan, Corea, the Luchu archipelago, and the island of Hongkong, together with their distribution and synonymy. The Journal of the Linnean Society 23: 1–162. <https://doi.org/10.1111/j.1095-8339.1886.tb00531.x>
- Grey-Wilson C (1980) *Impatiens* of Africa. Balkema, Rotterdam.
- IUCN (2019) Guidelines for using the IUCN red list categories and criteria. Version 14. Prepared by the Standards and Petitions Subcommittee. <http://www.iucnredlist.org/resources/redlistguidelines>
- Ji SJ, Kim YY, Oh BU (2010) Taxonomic review of *Impatiens furcillata* Hemsl. (Balsaminaceae). Korean Journal of Plant Taxonomy 40(1): 43–49. <https://doi.org/10.11110/kjpt.2010.40.1.043>
- Lee TB (1980) Illustrated Flora of Korea. Hyangmunsa, Seoul. [in Korean]
- Lee WT (1996) Standard Illustrations of Korean Plants. Academy Publishing Co., Seoul. [in Korean]

- Lee YN (2006) New Flora of Korea. Vol. 1. Kyo-Hak Publishing Co. Ltd., Seoul. [in Korean]
- Nakai T (1909) Flora Koreana. Pars Prima. The Journal of the College of Science. Imperial University of Tokyo 26: 1–304.
- Nakai T (1952) A synoptical sketch of Korean flora. Bulletin of the National Science Museum. Series B. Botany 31: 1–152.
- Oh BU, Ko SC, Kang SH, Paik WG, Yoo KO, Im HT, Jang CG, Chung GY, Choi BH, Choi HJ, Lee YM, Shin CH, Choi K, Han JH, Park SH, Kim HJ, Chang GS, Yang JC, Jeong SY, Lee CH, Oh SH, Jo DG (2016) Distribution Maps of Vascular Plants in Korea. Korea Forest Service and Korea National Arboretum, Pocheon.
- Park MK (1949) An Enumeration of Korean Plant. Ministry of Education Government of the Republic of Korea, Seoul. [in Korean]
- Park MK (1974) Key to the Herbaceous Plants of Korea (Dicotyledon). Jeongeum Co., Seoul. [in Korean]
- Talbot MJ, White RG (2013) Methanol fixation of plant tissue for scanning electron microscopy improves preservation of tissue morphology and dimensions. Plant Methods 9(1): 1–7. <https://doi.org/10.1186/1746-4811-9-36>
- Thiers B (2022) Index Herbariorum: a global directory of public 22 herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. [continuously updated] <http://sweetgum.nybg.org/science/ih>
- Xia CY, Sudhindra RG, Zhao XL, Do TV, Zhu XY, Qin Y, Deng HP, Yu SX (2019) *Impatiens maculifera* sp. nov. (Balsaminaceae) Yunnan, China. Nordic Journal of Botany 37(8): njb.02422. <https://doi.org/10.1111/njb.02422>
- Yu SX, Janssens SB, Zhu XY, Lidén M, Gao TG, Wang W (2016) Phylogeny of *Impatiens* (Balsaminaceae): Integrating molecular and morphological evidence into a new classification. Cladistics 32(2): 179–197. <https://doi.org/10.1111/cla.12119>
- Yuan YM, Song YI, Geuten K, Rahelivololona E, Wohlhauser S, Fischer E, Smets E, Küpfer P (2004) Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. Taxon 53(2): 391–404. <https://doi.org/10.2307/4135617>
- Yuan TH, Chen Y, Yu S, Ren LY, Huang RX, Li MJ, Bai XX (2022) *Impatiens liupanshuiensis* (Balsaminaceae), a new species from Guizhou, China. PhytoKeys 192(37): 37–44. <https://doi.org/10.3897/phytokeys.192.77269>