

Four new species of *Perilimnastes* (Sonerileae, Melastomataceae) from Vietnam

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Abstract

Perilimnastes is a genus currently treated in the polyphyletic *Phyllagathis*. Recent phylogenomic analyses have identified a morphologically cohesive lineage referred to as the *Phyllagathis* (raphides) clade, which should be excluded from *Phyllagathis* and treated as a distinct genus under the name *Perilimnastes*. Morphological and phylogenomic data have confirmed that four new species collected from Vietnam are part of the *Phyllagathis* (raphides) clade. They are described herein as *Perilimnastes multisejala*, *P. setipetiola*, *P. uniflora*, and *P. banaensis*. *Perilimnastes multisejala* is phylogenetically closest to *Phyllagathis setotheca*, and morphologically to *P. fruticosa* and *P. stenophylla*, but is distinct in the 4- to 8-lobed calyx, 28 × 9 mm, apically long acuminate petals, and 1–2 mm pedicel at fruiting stage. *Perilimnastes setipetiola*, *P. uniflora*, and *P. banaensis* are phylogenetically most closely related. *Perilimnastes uniflora* is characterized by its prostrate habit, small size, glabrous, obovate to obovate-lanceolate leaf blade, and solitary flower. *Perilimnastes setipetiola* and *P. banaensis* resemble each other in habit, leaf size and shape, and sessile or near sessile inflorescences but can be easily distinguished by the indumentum of the stems and leaves.

Key words: Melastomataceae, *Perilimnastes*, *Phyllagathis*, taxonomy



Academic editor: Marcelo Reginato

Received: 6 September 2023

Accepted: 19 October 2023

Published: 3 November 2023

Citation: Dai J-H, Van Do T, Liu Y (2023) Four new species of *Perilimnastes* (Sonerileae, Melastomataceae) from Vietnam. *PhytoKeys* 235: 1–19. <https://doi.org/10.3897/phytokeys.235.112133>

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Introduction

Perilimnastes Ridl. was established based on *P. fruticosa* (Ridl.) Ridl. (Ridley 1918, 1922), a species originally published in *Anerinckleistus* Korth. as *A. fruticosus* Ridl. (Ridley 1908). *Perilimnastes fruticosa* is characterized by its shrubby habit, subcoriaceous 3-veined leaves, few-flowered cymes, isomorphic stamens, and crowned capsules. It grows on rocks along streams in forests. Ridley (1918) noted that the fruit of *P. fruticosa* (= *A. fruticosus*) did not fit into any existing genera and described it as a distinct genus named *Perilimnastes*. Nayar (1974) accepted Ridley's concept of *Perilimnastes* and described a second species, *P. rupicola* M.P.Nayar, which resembles *P. fruticosa* in habit and morphology

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of leaves, calyx lobes, stamens, and capsules. However, subsequent authors didn't recognize *Perilimnastes*. Maxwell (1982, 1989) synonymized *Perilimnastes* and accommodated its two members in *Anerinacleistus* sect. *Coriaceae* Ridl. (as *A. fruticosus*) and sect. *Anerinacleistus* [as *A. rupicola* (Nayar) J.F.Maxwell], respectively. On the other hand, Cellinese (2002, 2003) placed both species within the broadly defined *Phyllagathis* Blume [as *P. fruticosa* (Ridl.) C.Hansen ex Cellin. and *P. rupicola* (M.P.Nayar) C.Hansen ex Cellin., respectively].

The classification of Asian Sonerileae at generic level has been a topic of ongoing controversy, particularly regarding the delimitation of *Phyllagathis* and various genera morphologically related to it (Diels 1932; Li 1944; Chen 1984a, b; Hansen 1992; Cellinese and Renner 1997; Cellinese 2002, 2003; Chen and Renner 2007). A series of molecular phylogenetic analyses consistently demonstrated the polyphyletic nature of *Phyllagathis* (Zeng et al. 2016; Zhou et al. 2018; Zhou et al. 2019a, b, c; Liu et al. 2022; Zhou et al. 2022). Zhou et al. (2022) presented the first well-resolved phylogeny of Asian Sonerileae and identified 34 major clades based on genome-scale data. Species currently treated in *Phyllagathis* were found in 17 lineages scattered across Asian Sonerileae. The type of *Phyllagathis* showed no close relationships with other members and the genus may have to be redefined as monotypic. Samples of *Anerinacleistus* formed a strongly supported clade with certain Bornean species of *Phyllagathis*. The generic type of *Perilimnastes*, namely *P. fruticosa* (also known as *Phyllagathis fruticosa* and *Anerinacleistus fruticosus*), was not included in the analyses. Nonetheless, species closely resembling *P. fruticosa*, such as *Phyllagathis stenophylla* (Merr. & Chun) H.L.Li and *Phyllagathis suberalata* C.Hansen, were recovered as part of the *Phyllagathis* (raphides) clade, which consists of members characterized by a fruticose/suffruticose growth habit, cuneate to rounded leaf base, umbellate or cymose inflorescences sometimes sessile or reduced to a single flower, isomorphic stamens, crowned capsules, horned placental column, thready placentas, as well as the presence of raphide crystals in some species. Based on these diagnostic characteristics and notable similarity observed between sampled and unsampled species, Zhou et al. (2022) concluded that the *Phyllagathis* (raphides) clade should contain approximately 20 species distributed in southernmost China, Vietnam, the Malay Peninsula, and Borneo. Given its distant relationship with the generic type of *Phyllagathis*, this clade justifies recognition as a distinct genus. As compelling morphological evidence indicates that the type of *Perilimnastes* (*P. fruticosa*) is a member of the *Phyllagathis* (raphides) clade, *Perilimnastes* should be resurrected as its generic name (Zhou et al. 2022).

During a field expedition in Vietnam, four species that were previously unrecorded in the Flora of Vietnam were collected from Đại Lộc, Quảng Nam Province (1 sp.), Đà Lạt, Lâm Đồng Province (1 sp.), and Hòa Ninh, Đà Nẵng (2 spp.) (Fig. 1). These plants share strong morphological resemblance to *Perilimnastes* [= the *Phyllagathis* (raphides) clade] and their placement within this clade was later confirmed through phylogenomic analyses conducted by Zhou et al. (2022). Morphological comparison between the four plants and their possible relatives revealed that they represent species new to science, which we described below as *Perilimnastes multisejala* J.H.Dai, T.V.Do & Ying Liu (Figs 2–4), *P. setipetiola* J.H.Dai, T.V.Do & Ying Liu (Figs 5, 6), *P. uniflora* J.H.Dai, T.V.Do & Ying Liu (Figs 7, 8), and *P. banaensis* J.H.Dai, T.V.Do & Ying Liu (Figs 9, 10).

Morphological comparison

Morphological and distribution data were obtained through field, herbarium and literature surveys as well as observation of living plants in the facilities of Sun Yat-sen University. Specimens of the species concerned (GXMI, IBSC, IBK, KUN, PE, SYS) or their high-resolution photos (A, BM, C, E, G, K, NY, P, US) were examined. Species delimitation followed Chen (1984b), Hansen (1992), and Cellinese (2002, 2003).

According to previous phylogenomic analyses, *P. multisejala* is closest to *Phyllagathis setotheca* H.L.Li from China (Zhou et al. 2022). However, the two species are quite distinct in leaf size (2.4–8 × 0.7–2.4 cm vs. 10–20 × 3–8 cm), number of flowers per inflorescence (1 or 2, rarely 3 vs. 3 to more than 20), number and shape of calyx lobes (4–8, linear vs. 4, long triangular) and length of pedicels (1–2 mm vs. 8–18 mm). In terms of morphology, *P. multisejala* closely resembles *P. fruticosa* and *P. stenophylla*. All three species are shrubs with druses, somewhat oblong-lanceolate, coriaceous, 3-veined leaf blade, few-flowered inflorescences, and narrow calyx lobes. Nevertheless, *P. multisejala* can be easily distinguished from the latter two species in the petals 28 × 9 mm, apically long acuminate (vs. 8.5–16 × 3.5–5 mm, acuminate, and 12 × 6 mm, short acuminate), calyx lobes 4–8 (vs. 4) and pedicels 1–2 mm at fruiting stage (vs. 10–15 mm). A comparison of the four species can be found in Suppl. material 1: table S1.

Perilimnastes setipetiola is resolved as sister to *P. uniflora*, and *P. setipetiola*-*P. uniflora* to *P. banaensis* (Zhou et al. 2022). Despite their close relationship, these species are morphologically quite different from one another. *Perilimnastes uniflora* is characterized by its small size (to 30 cm tall), the whole plant glabrous except for sparse minute brown glands when young, stems prostrate at middle and lower parts, leaf blade obovate to obovate-lanceolate, and solitary flower. *Perilimnastes setipetiola* and *P. banaensis* resemble each other in the shrubby habit, leaf size, somewhat elliptic leaf blade, and sessile or subsessile inflorescences with multiple flowers, however, the two species differ markedly in the indumentum of the stems and petioles (stems and petioles pubescent with stellate hairs when young, petioles hispid with stout, 2–4 mm long bristles vs. densely villous with appressed brown hyaline uniseriate hairs). According to Zhou et al. (2022), *P. setipetiola*, *P. uniflora*, and *P. banaensis* formed the sister clade of a Bornean lineage containing *Phyllagathis elliptica* Stapf and *P. dispar* (Cogn.) C.Hansen. The former three species are linked to the Bornean lineage by the presence of raphide crystals, somewhat elliptic leaf blade, and terminal and axillary umbels with very short or no peduncles in some of the species, however, they can be distinguished from the latter based on a combination of height, habit, indumentum, and anther morphology. In addition to the Bornean lineage, *P. setipetiola* shares similarities in habit, leaf size and shape with *P. setotheca* and *P. ovalifolia* H.L.Li. However, it differs from the latter species in terms of the indumentum of leaf petiole and the length of peduncles. *Perilimnastes uniflora* also resembles *Phyllagathis guillauminii* H.L.Li and *P. rupicola*, two species not sampled in previous phylogenetic studies, in crystal type and leaf shape, but differs in indumentum and the length of the pedicel at fruiting stage. A comparison of the species discussed above is provided in Suppl. material 1: table S2.

Phylogenetic data and morphological comparison justify the recognition of *P. multisejala*, *P. setipetiola*, *P. uniflora*, and *P. banaensis* as distinct species in *Perilimnastes*. The formal taxonomic treatment of other species in the *Phyllagathis* (raphides) clade will be dealt with in another study.

Geographical distribution

The four new species are geographically quite isolated from related species previously discussed (Suppl. material 1: tables S1, S2). *Perilimnastes multisejala* is located in central Vietnam (Fig. 1), whereas its related species, *P. setothesca* and *P. stenophylla* are documented in southernmost China and northern Vietnam, and *P. fruticosa* in the Malay Peninsula. *Perilimnastes setipetiola* is distributed in Đà Lạt, southern Vietnam and *P. banaensis* and *P. uniflora* are found in central Vietnam (Fig. 1). The three species are morphologically/phylogenetically related to *P. elliptica*, *P. dispar*, *P. rupicola*, *P. setothesca*, *P. ovalifolia*, and *P. guillauminii*. *Phyllagathis elliptica*, *P. dispar*, and *P. rupicola* are endemic species of Borneo, *P. setothesca* and *P. ovalifolia* are found in southernmost regions of China and northern Vietnam, while *P. guillauminii* has been documented in Bien Hoa, southern Vietnam. Members of the *Phyllagathis* (raphides) clade typically inhabit moist and shady environments in forests, such as damp slopes or rocky areas along or near streams and waterfalls. However, it is uncommon for multiple species of this clade to coexist in the same location. In this particular case, only *P. banaensis* and *P. uniflora* were observed together at an elevation of 1,360 m near the summit of Ba Na Hills in central Vietnam. Nevertheless, the two species prefer somewhat different microhabitats. Individuals of *P. banaensis* occupy damp slopes alongside other shrubs and lianas, whereas those of *P. uniflora* typically inhabit moist exposed rocks with fewer shrubs and lianas around.

Taxonomic treatment

Perilimnastes multisejala J.H.Dai, T.V.Do & Ying Liu, sp. nov.

urn:lsid:ipni.org:names:77329901-1

Figs 2–4

Type. VIETNAM. Quảng Nam Province: Đại Lộc, about 400 m south of Khu Du Lịch Sinh Thái Khe Lim, along newly opened road, 574 m elevation, on rocks along a stream, 23 Nov 2019, Jin-hong Dai and Ying Liu 821 (holotype: PE; isotypes: A, SYS, VNMN).

Diagnosis. Resembles *P. fruticosa* and *P. stenophylla* in the habitat preference, habit, leaf and inflorescence morphology but differs from these species in the petals 28×9 mm, apex long acuminate (vs. $8.5\text{--}16 \times 3.5\text{--}5$ mm, acuminate, and 12×6 mm, short acuminate), calyx lobes 4–8 (vs. 4) and pedicels only 1–2 mm at fruiting stage (vs. 10–15 mm).

Description. Shrubs, much-branched, up to 0.8 m tall, with druses in many parts. Stems obtusely 4-sided, slightly compressed when young; branchlets glabrous, sulcate, nodes only pubescent with uniseriate hairs when young. Leaves opposite, equal to distinctly unequal in a pair, pubescent with brownish-yellow stellate hairs only when young, glabrous when mature; petiole 0–10 mm; leaf blade obovate-lanceolate, oblong-lanceolate to oblanceolate, $2.4\text{--}8 \times$

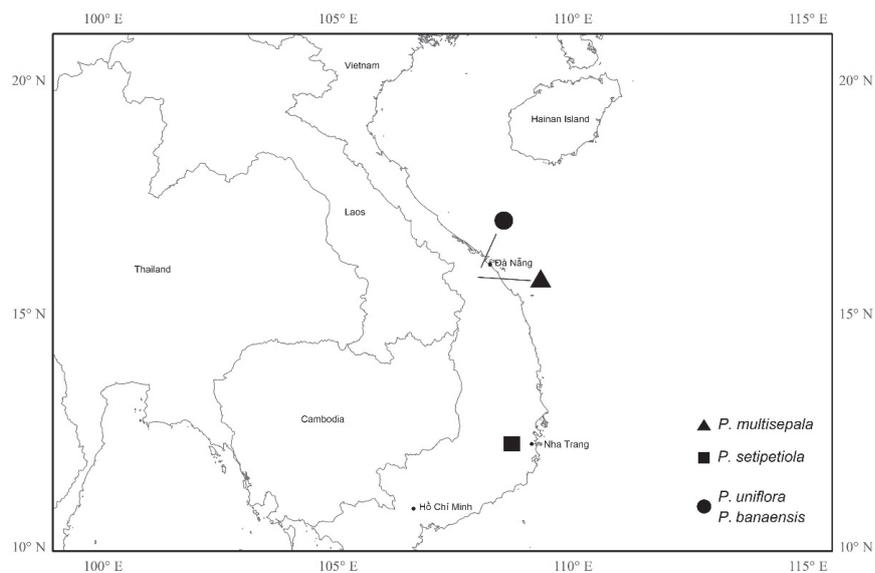


Figure 1. Distribution of *Perilimnastes multisejala* (triangle), *P. setipetiola* (square), and *P. uniflora* and *P. banaensis* (solid circle).

0.7–2.4 cm, subcoriaceous, 3-veined with the lateral two veins often diverged from the midvein above the base, dark green adaxially, pale green abaxially, base cuneate, margin entire, apex obtuse, acute, rarely shortly acuminate. Inflorescences terminal, cymose contracted to umbellate, solitary or 2-flowered, rarely 3-flowered; peduncle ca. 1 mm long, sometimes sessile, subtended by a pair of bracts to 5 mm long. Flowers 4-merous; pedicel 1–2 mm long, glabrous; hypanthium funnel-shaped, 7–8 mm long, sparsely pubescent with stellate hairs; calyx lobes linear, laterally compressed, alternipetalous 4, 8–10 mm long, antepetalous 0–4, 3–8 mm long, sparsely pubescent with stellate hairs; petals pinkish purple, 28 × 9 mm, ovate, slightly oblique, apex long acuminate, abaxially very sparsely pubescent with stellate hairs; stamens 8, isomorphic, filaments 7–9 mm long, glabrous, anthers lanceolate, yellow, 9 mm long, connective decurrent, tuberculate ventrally, forming a spur dorsally; ovary half as long as hypanthium (crown excluded), ovary crown wedge-like, 4-lobed; style 22 mm long. Capsule cup-shaped, 7–8 × 7 mm, 4-sided; hypanthium 8-ribbed; crown enlarged enclosing an obpyramidal space; placental column unbeaked, 4-horned; placenta thready.

Phenology. Flowers, young fruits and old fruits in November.

Etymology. The specific epithet is based on the 4–8 calyx lobes of this species.

Distribution. *Perilimnastes multisejala* is currently known from Đại Lộc, Quảng Nam Province, Vietnam (Fig. 1). It grows on rocks along streams in the forest, at 574 m elevation.

***Perilimnastes setipetiola* J.H.Dai, T.V.Do & Ying Liu, sp. nov.**

urn:lsid:ipni.org:names:77329904-1

Figs 5, 6

Type. VIETNAM. Lâm Đồng Province: Đà Lạt, Bidoup Nui Ba National Park, 1,500–1,700 m elevation, at damp places under forest, 29 Nov 2019, Jin-hong Dai and Ying Liu 836 (holotype: PE; isotypes: A, SYS, VNMN).



Figure 2. Holotype of *Perilimnastes multisepala*, Jin-hong Dai and Ying Liu 821 (PE). The inset shows druses (as white spots) on adaxial leaf surface under stereoscope. Scale bars: 5 cm, 1 mm (inset).



Figure 3. *Perilimnastes multisejala* **A** habit **B** adaxial (top) and abaxial (bottom) leaf surfaces **C** close-up of a branchlet showing a 2-flowered cyme. All from Jin-hong Dai and Ying Liu 821 (A, PE, SYS).

Diagnosis. Resembles *P. banaensis*, *P. elliptica* and *P. dispar* in having hyaline hairs, raphide crystals, somewhat elliptic leaf blade and umbels with very short or no peduncles but differs markedly from *P. banaensis* in the indumentum of the stems and petioles (both pubescent with stellate hairs when young, petioles hispid with long bristles vs. densely villous with appressed hyaline uniseriate hairs), and from the latter two species in height (40–120 cm vs. up to 45 cm), habit (shrubby vs. herbal), anther color (pink vs. yellow) and the morphology of connectives (prolonged below anthers vs. not prolonged). Also resembles *P. setotheca* and *P. ovalifolia* in habit, leaf size and shape but differs in petiole his-

pid with stout, 2–4 mm long bristles (vs. glabrous in *P. setothesca* and densely hirsute with soft hairs in *P. ovalifolia*) and umbels with 0–2 mm peduncles (vs. peduncles 8–18 mm long in *P. setothesca* and 10–30 mm long in *P. ovalifolia*).



Figure 4. *Perilimnastes multisejala* **A** top view of a flower **B** longitudinal section of a flower showing the isomorphic stamens **C** top view of a young capsule **D** lateral view of a young capsule **E** top view of an old capsule **F** longitudinal section of an old capsule showing enlarged ovary crown and morphology of the placental column and placentas. Scale bars: 5 mm (**B**); 3 mm (**F**). All from Jin-hong Dai and Ying Liu 821 (A, PE, SYS).



Figure 5. Holotype of *Perilimnastes setipetiola*, Jin-hong Dai and Ying Liu 836 (PE). The inset shows raphides (as white oblong spots) on adaxial leaf surface under stereoscope. Scale bars: 5 cm, 1 mm (inset).

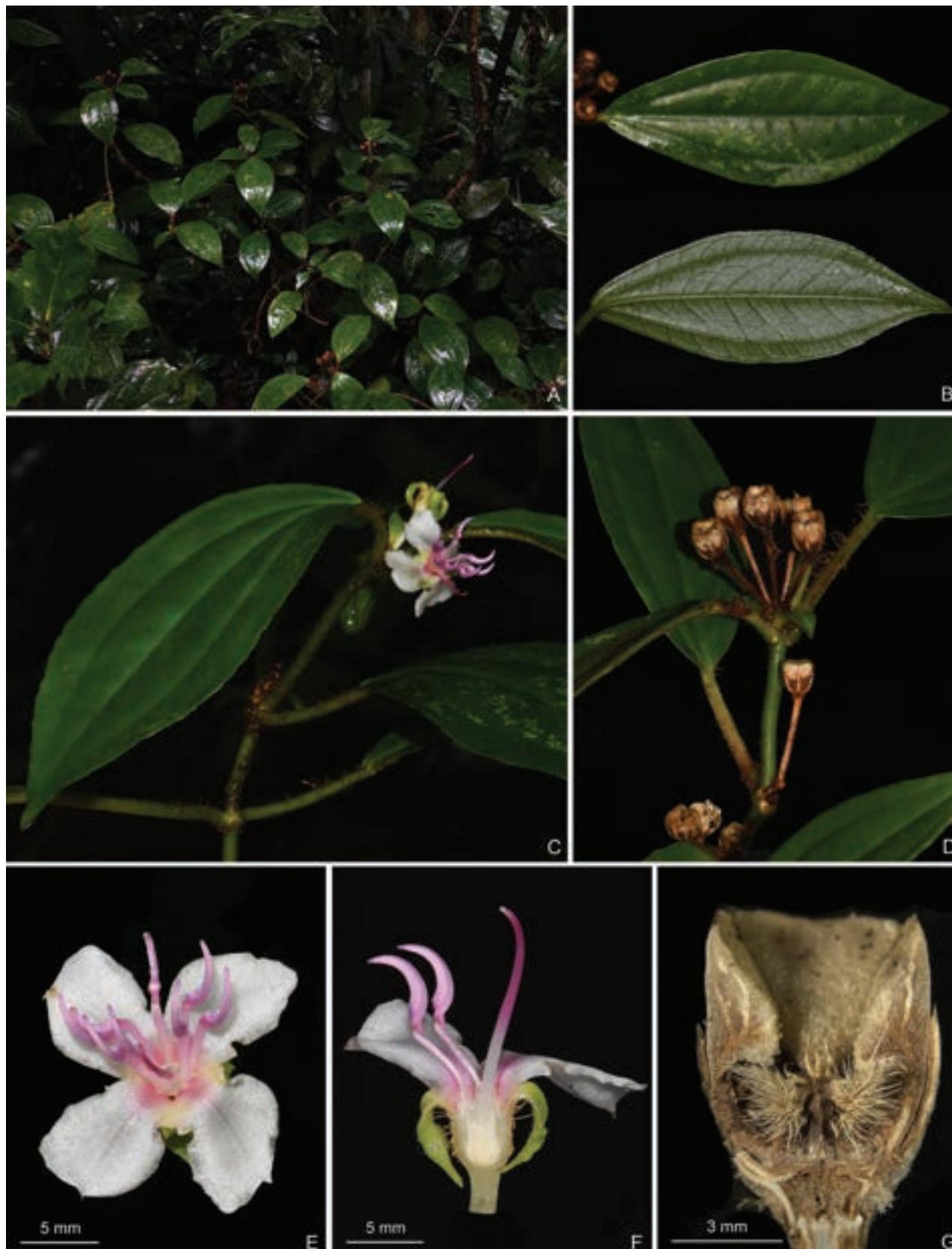


Figure 6. *Perilimnastes setipetiola* **A** habit **B** adaxial (top) and abaxial (bottom) leaf surfaces **C** a flowering branch **D** a branch showing hispid petioles and terminal and axillary infructescences **E** top view of a flower **F** longitudinal section of a flower showing the isomorphic stamens **G** longitudinal section of an old capsule showing enlarged ovary crown and morphology of the placental column and placentas. Scale bars: 5 mm (**E**, **F**); 3 mm (**G**). All from Jin-hong Dai and Ying Liu 836 (A, PE, SYS).

Description. Shrubs, 40–120 cm tall, branched, with raphides in all parts. Stems obtusely 4-sided when young, pubescent with brownish-yellow stellate hairs and rarely uniseriate hyaline hairs (both composed of elongated cells) when young, glabrescent when mature. Leaves opposite, equal or subequal in a pair; petiole 1.2–6 cm long, pubescent with brownish-yellow stellate hairs when young, hispid with stout, 2–4 mm long bristles; leaf blade broadly elliptic to elliptic, 5.6–15 × 1.9–6.4 cm, papery to stiffly papery, pubescent with brownish-yellow stellate hairs when young, glabrous on the upper surface and sparsely pubescent along veins on lower surface when mature, often 5-veined with the marginal two slightly inconspicuous and the inner two diverged from the midvein above the base, base cuneate, margin entire, apex acuminate, short acuminate, rarely acute. Inflorescences terminal and axillary, umbellate, 2–11-flowered, subtended by two sessile bracts; sessile or with peduncle up to 2 mm long. Flowers 4-merous; pedicel 8–13 mm long (16–25 mm in fruit), glabrous; hypanthium funnel-shaped, 5–7 mm long, pubescent with multiseriate hairs and sparsely so with stellate hairs; calyx lobes triangular-ovate, 6 mm long, glabrescent; petals pinkish-white, broadly ovate, oblique, ca. 10 mm long, apex acute; stamens isomorphic, filaments ca. 6 mm, anthers pink, lanceolate, ca. 6 mm, connective decurrent, prolonged below anther, forming a spur dorsally; ovary half as long as hypanthium (crown excluded), ovary crown wedge-like, 4-lobed; style 15 mm long. Capsule cup-shaped, ca. 7 × 6 mm, 4-sided; hypanthium 8-ribbed; crown enlarged enclosing an obpyramidal space; placental column unbeaked, 4-horned; placenta thready.

Phenology. Flowers and old fruits in November.

Etymology. The specific epithet is based on the stout long bristles on the petiole of this species.

Distribution. *Perilimnastes setipetiola* is currently known from Đà Lạt, Lâm Đồng Province, Vietnam (Fig. 1). It occurs at damp places in forests, at 1,500–1,700 m elevation.

Additional specimen examined. VIETNAM. Lâm Đồng Province: Lạc Dương district, 40 km to northeast from Đà Lạt city. Closed primary wet broadleaved cloud forest on southwest macroslope of Hon Giao mountain ridge at 1,600–1,700 m elevation, 21 Apr 1997, L.Averyanov, N.Q.Binh, N.T.Hiep, VH 4133 [P (P05200269)].

***Perilimnastes uniflora* J.H.Dai, T.V.Do & Ying Liu, sp. nov.**

urn:lsid:ipni.org:names:77329905-1

Figs 7, 8

Type. VIETNAM. Đà Nẵng: Hòa Ninh, Ba Na Hills, 1,360 m elevation, in forests on damp rocks along steam, 22 Nov 2019, Jin-hong Dai and Ying Liu 814 (holotype: PE; isotypes: A, SYS, VNMN).

Diagnosis. Resembles *P. guillauminii* and *P. rupicola* in having raphide crystals, 3-veined leaves with cuneate base and somewhat acuminate apex, and narrow calyx lobes, but differs from *P. rupicola* in its pink anthers (vs. yellow) and from both in the stems glabrous except for minute brown glands when young (vs. covered with long bristles in *P. guillauminii* and hyaline uniseriate hairs in *P. rupicola*) and pedicel 4 mm long at fruiting stage (vs. 16 mm long in *P. guillauminii* and 22 mm long in *P. rupicola*).



Figure 7. Holotype of *Perilimnastes uniflora*, Jin-hong Dai and Ying Liu 814 (PE). The inset shows raphides (as white oblong spots) on adaxial leaf surface under stereoscope. Scale bars: 5 cm, 1 mm (inset).



Figure 8. *Perilimnastes uniflora* **A** habit **B** a flowering individual **C** close-up of a branchlet **D** adaxial (left) and abaxial (right) leaf surfaces **E** a flowering branch **F** close-up of an inflorescence showing a solitary flower **G** top view of a flower **H** longitudinal section of a flower showing the isomorphic stamens **I** longitudinal section of an old capsule showing enlarged ovary crown and morphology of the placental column and placentas. Scale bars: 5 mm (**D**, **G**, **H**); 2 mm (**I**). All from Jin-hong Dai and Ying Liu 814 (A, PE, SYS).

Description. Shrublets or somewhat woody herbs, to 30 cm tall, with raphides in all parts. Stems prostrate at middle and lower parts, branched, with adventitious roots at lower nodes; branchlets quadrangular and with sparse minute brown glands when young, glabrescent; older branches obtusely 4-sided; leafy distally and leafless proximally. Leaves opposite, equal to unequal in a pair, with minute brown glands only when young, glabrescent when mature; petiole 0.5–2 cm long; leaf blade obovate to obovate-lanceolate, sometimes elliptic, 4.2–9.5 × 1.3–3.4 cm, papery, 3-veined with the lateral two veins diverged from the midvein at or above the base, green to dark green adaxially, pale green abaxially, base cuneate to narrowly cuneate, margin entire or inconspicuously minutely repand, apex acuminate to long acuminate, sometimes caudate. Inflorescences terminal, flower solitary, subtended by a pair of bracts ca. 4 mm long. Flowers 4-merous; pedicel ca. 2 mm long, 4 mm at fruiting stage, glabrous; hypanthium funnel-shaped, 5–6 mm long, glabrous except for minute glands; calyx lobes 4, linear, 6–9 mm long, with minute glands; petals pinkish-purple, 11–13 × 4–6 mm, ovate, minutely oblique, apex acute to short acuminate, glabrous on both sides; stamens 8, isomorphic, filaments pink, ca. 6 mm long, glabrous, anthers lanceolate, pink, 5–7 mm long, connective decurrent, forming two ventral lobes and a dorsal spur; ovary half as long as hypanthium (crown excluded), ovary crown wedge-like, 4-lobed; style 13–15 mm long. Capsule cup-shaped, ca. 5 × 5 mm, 4-sided; hypanthium 8-ribbed; crown enlarged enclosing an obpyramidal space; placental column unbeaked, 4-horned; placenta thready.

Phenology. Flowers in June and produces old fruits in November.

Etymology. The specific epithet is based on the solitary flowers of this species.

Distribution. *Perilimnastes uniflora* is currently only known from Ba Na Hills, Hòa Ninh, Đà Nẵng, Vietnam (Fig. 1). It occurs on damp rocks along streams in forests, at 1,360 m elevation.

***Perilimnastes banaensis* J.H.Dai, T.V.Do & Ying Liu, sp. nov.**

urn:lsid:ipni.org:names:77329906-1

Figs 9, 10

Type. VIETNAM. Đà Nẵng: Hòa Ninh, Ba Na Hills, 1,360 m elevation, in forests on damp slopes near steam, 22 Nov 2019, Jin-hong Dai and Ying Liu 813 (holotype: PE; isotypes: A, SYS, VNMN).

Diagnosis. Resembles *P. ovalifolia* and *P. setipetiola* in having raphide crystals, hyaline hairs, somewhat elliptic leaf blade, and umbels with very short or no peduncle, but differs in the stems and petioles densely villous with brown hyaline uniseriate hairs (vs. stems densely retrorse hirsute, glabrescent, and petioles densely hirsute to setose in the former species, and stems pubescent with brownish-yellow stellate hairs and rarely also hyaline hairs, petioles with brownish-yellow stellate hairs when young and hispid with long bristles in the latter).

Description. Shrubs to 60 cm tall, with raphides in all parts. Stems branched, prostrate at lower parts; branchlets obtusely 4-sided and densely villous with appressed, brown hyaline uniseriate hairs composed of much elongated cells and tipped with a brown glandular cell; older branches near terete and glabrescent; leafy distally and leafless proximally. Leaves opposite, equal to unequal in a pair;



Figure 9. Holotype of *Perilimnastes banaensis*, Jin-hong Dai and Ying Liu 813 (PE). The inset shows raphides (as white oblong spots) on adaxial leaf surface under stereoscope. Scale bars: 5 cm, 1 mm (inset).

petiole 1.5–4.6 cm long, densely villous with appressed, brown hyaline hairs; leaf blade elliptic, 5.5–13 × 2.5–6.5 cm, thick papery, with minute brown glands when young on both surfaces, abaxially sparsely pubescent with appressed brown hyaline hairs, densely so along the veins, 3 or 5-veined, dark green adax-

ially, pale green abaxially, base acute to rounded, margin entire or sometimes inconspicuously minutely repand, apex short acuminate to acute. Inflorescences and flowers unknown. Infructescences terminal, umbellate, sessile, capsules 2–7, pedicel 5–13 mm long. Old capsules cup-shaped, ca. 5 × 5 mm, 4-sided;



Figure 10. *Perilimnastes banaensis* **A** habit **B** a branch with old capsules **C** close-up of a branchlet **D** adaxial (left) and abaxial (right) leaf surfaces **E** a sessile infructescence **F** longitudinal section of an old capsule showing enlarged ovary crown and morphology of the placental column and placentas. Scale bars: 2 mm (**F**). All from Jin-hong Dai and Ying Liu 813 (A, PE, SYS).

hypanthium 8-ribbed; crown enlarged enclosing an obpyramidal space; placental column unbeaked, 4-horned; placenta thready.

Phenology. Old fruits in November.

Etymology. The specific epithet is based on Ba Na hills, the type locality of this species.

Distribution. *Perilimnastes banaensis* is currently only known from Ba Na Hills, Hòa Ninh, Đà Nẵng, Vietnam (Fig. 1). It occurs on damp slopes in forests often near streams, at 1,360 m elevation.

Acknowledgements

We are grateful to Dr. David E. Boufford and the anonymous reviewer for their valuable comments on the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (grants 32170220), Natural Science Foundation of Guangdong Province (grant 2021A1515011214), and partly by the Ministry of Planning and Investment, Vietnam, and the Vietnam Academy of Science and Technology under the project code UQĐTCB.06/22–23 to TVD.

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Data availability

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

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Supplementary material 1

Supplementary data

Authors: Jin-Hong Dai, Truong Van Do, Ying Liu

Data type: docx

Explanation note: **table S1**. Comparison of *Perilimnastes multisejala* and related species. The name of the new species is indicated in bold; **table S2**. Comparison of *Perilimnastes banaensis*, *P. setipetiola*, *P. uniflora*, and related species. The names of the new species are indicated in bold.

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Link: <https://doi.org/10.3897/phytokeys.235.112133.suppl1>

Paraboea zunyiensis (Gesneriaceae), a new species from north Guizhou, China

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Abstract

A new lithophytic species, *Paraboea zunyiensis* T.Deng, F.Wen & R.B.Zhang (Gesneriaceae), inhabiting Karst rocks in northern Guizhou, China, is introduced and depicted in this study. It bears a resemblance to *P. crassifolia* (Hemsl.) B.L. Burt, yet is distinguishable by its shorter filaments and staminodes, triangular ovate calyx segments, and ovaries surpassing the styles in length. Moreover, the phylogenetic tree constructed from nuclear DNA (ITS) and plastid DNA (*trnL-F*) data firmly support the differentiation of this novel species from *P. crassifolia*.

Key words: Flora of Guizhou, lithophyte, new taxon, *Paraboea crassifolia*, taxonomy



Academic editor: Bo Li

Received: 21 August 2023

Accepted: 25 October 2023

Published: 6 November 2023

Citation: Deng T, Wen F, Xie D-J, Wei R-X, He L, Dou Q-L, Qian Z-M, Zhang R-B (2023) *Paraboea zunyiensis* (Gesneriaceae), a new species from north Guizhou, China. *PhytoKeys* 235: 21–29. <https://doi.org/10.3897/phytokeys.235.111412>

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Introduction

Paraboea (C.B. Clarke) Ridl. was first published by Clarke as a section, *Didymocarpus* sect. *Paraboea* Clarke (1883) (Xu et al. 2008), and was elevated to generic level by Ridley (1905). Burt (1984) defined *Boea* and *Paraboea* based on differences in indumentum (simple straight hairs in *Boea* and interwoven arachnoid-like hairs in *Paraboea*). *Paraboea* comprises ca. 130 species characterized by abaxially matted leaves with densely interwoven indumentum and flowers featuring flat-faced to shortly campanulate corolla and non-erect anthers (Guo 2016; Xu et al. 2017). At present, this genus is a member of the tribe Didymoeapeae, subfamily Cyrtandroideae, family Gesneriaceae (Wang et al. 1990), and is primarily distributed in Karst habitats in China. Exceptions include *Paraboea crassifolia* W.B. Xu & J. Guo (exclusively found in the Danxia landscape of Rong County, Guangxi, China) (Guo 2016) and *P. sinensis* (Oliv.) B.L. Burt (found in both the Karst and Danxia landscapes) (Wei 2018; Wei et al. 2022).

Firstly, in China, *Paraboea* was initially recognized with 18 species, most of which exhibit narrow endemism (Wang et al. 1998; Fu et al. 2004). The southern region of China hosts a rich diversity of *Paraboea* species. Over ten new species have been reported since 2004 across various provinces: Guangxi (Xu

and Wei 2004; Chen et al. 2008; Xu et al. 2012; Wen and Wei 2016), Guangdong (Wen et al. 2013; Wen and Wei 2016), Yunnan (Chen et al. 2012; He et al. 2018; Zhang et al. 2020), Hunan (Wen and Wei 2016), and Guizhou Province (Wen and Wei 2016; Guo et al. 2020).

In April 2023, a *Paraboea*-like species that was morphologically similar to *P. crassifolia* (Hemsl.) B.L.Burtt. was discovered in Guizhou Province, China. Through meticulous analysis of flowering specimens in the laboratory and detailed observation of live plants to compare vegetative and reproductive organs, significant distinctions between the two species became evident. The application of ITS and *trnL-F* for phylogenetic analysis further validated the distinctness of the new species from *P. crassifolia*. As a result, a conclusion was reached, designating it as a novel species within the realm of scientific understanding.

Materials and methods

Taxonomic revision

The studied specimens were obtained from the type locality and deposited in the Botany Herbarium at Zunyi Normal College (ZY) and the Guangxi Institute of Botany Herbarium (IBK). Using a stereomicroscope (Olympus Optical Microscope SZ61, Olympus Corporation, Japan), we conducted micromorphological analyses and photography. We compared the morphological traits with the protologue and type specimens of previously described *Paraboea* species, especially new *Paraboea* taxa from Guizhou and nearby provinces, along with herbarium specimens at relevant herbaria (e.g., IBK, IBSC, KUN, PE, and ZY).

Phylogenetic analysis

Leaf material of the undescribed species was collected in Maoli Town, Zunyi City (Guizhou, China) and promptly silica-dried for DNA extraction. The nuclear ribosomal internal transcribed spacer (ITS) region and plastid *trnL-F* intron spacer region (*trnL-F*) were utilized in the study. Following Weber et al. (2011), we employed primers, conducted DNA extraction, PCR amplification and sequencing. To elucidate the genus's phylogenetic affinities, we integrated 36 *Paraboea* species (Table 1). Three former *Boea* species, *Damrongia clarkeana* (Hemsl.) C.Puglisi, *Dorcoceras hygrometrica* Bunge and *Dorcoceras philippinense* Schltr., were selected as outgroups based on prior phylogenetic analyses (Möller et al. 2011; Guo 2016).

Bayesian inference was implemented using MrBayes v3.2.6. Prior to the Bayesian analysis, the mrModelTest v1.0 incorporating the Akaike information criterion (AIC) was used for selecting the best-fit molecular evolution model (GTR+I+G for the ITS and GTR for the *trnL-F*). Homogeneity testing was conducted via PAUP4 software (<https://paup.phylosolutions.com/>) yielding a *p* value < 0.05, thus prompting the merger of the two regions for subsequent analyses. The BI analyses entailed four Markov Chain Monte Carlo (MCMC) chains, with tree sampling every 100 generations for 2,000,000 generations from a random tree. Upon stabilizing log-likelihood scores, a consensus tree

was computed, excluding 5,000 sampled trees as burn-in (Xie et al. 2014). Tree visualization was carried out in FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Table 1. The GenBank accession numbers used in this study.

Species name	ITS	<i>trnL-F</i>
<i>Damrongia clarkeana</i>	KJ475430	KM232645
<i>Dorcoceras hygrometrica</i>	FJ501319	FJ501476
<i>Dorcoceras philippensis</i>	HQ632953	HQ632862
<i>Paraboea acutifolia</i>	JN934753	FJ501464
<i>Paraboea amplifolia</i>	JN934754	JN934712
<i>Paraboea burttii</i>	JN934756	JN934714
<i>Paraboea capitata</i>	FJ501315	AJ492298
<i>Paraboea clarkei</i>	JN934757	JN934715
<i>Paraboea crassifolia</i>	KU203970	FJ501472
<i>Paraboea dictyoneura</i>	KJ475415	FJ501463
<i>Paraboea divaricata</i>	JN934759	JN934717
<i>Paraboea effusa</i>	JN934760	JN934718
<i>Paraboea glabra</i>	JN934761	JN934719
<i>Paraboea glabrescens</i>	JN934785	JN934743
<i>Paraboea glabrisepala</i>	JN934762	JN934720
<i>Paraboea glanduliflora</i>	JN934763	JN934721
<i>Paraboea glandulosa</i>	JN934784	JN934742
<i>Paraboea glutinosa</i>	JN934764	JN934722
<i>Paraboea hainanensis</i>	MF315101	MF315107
<i>Paraboea harroviana</i> var. <i>ovata</i>	JN934765	JN934723
<i>Paraboea havilandii</i>	JN934766	JN934724
<i>Paraboea incudicarpa</i>	JN934767	JN934725
<i>Paraboea martinii</i>	MF358702	MF358718
<i>Paraboea neurophylla</i>	JN934769	JN934727
<i>Paraboea paniculata</i>	JN934770	JN934728
<i>Paraboea paramartinii</i>	JN934771	JN934729
<i>Paraboea rufescens</i>	JN934772	FJ501469
<i>Paraboea sinensis</i>	JN934773	FJ501474
<i>Paraboea subplana</i>	JN934786	JN934744
<i>Paraboea suffruticosa</i>	JN934774	JN934732
<i>Paraboea swinhoei</i>	JN934775	JN934733
<i>Paraboea tarutaoensis</i>	JN934776	JN934734
<i>Paraboea trachyphylla</i>	JN934777	JN934735
<i>Paraboea trisepala</i>	JN934778	JN934736
<i>Paraboea umbellata</i>	JN934779	JN934737
<i>Paraboea velutina</i>	JN934780	MF358725
<i>Paraboea verticillata</i>	JN934781	JN934739
<i>Paraboea vulpina</i>	JN934782	JN934740
<i>Paraboea zunyiensis</i> sp. nov.	OR125066	OR123588

Taxonomic treatment

Paraboea zunyiensis T.Deng, F.Wen & R.B.Zhang, sp. nov.

urn:lsid:ipni.org:names:77330011-1

Figs 1, 2

Diagnosis. *Paraboea zunyiensis* morphologically resembles *P. crassifolia*, but can be distinguished by the shorter staminodes (< 1 mm vs. 2–2.5 mm in *P. crassifolia*, following the same order), filaments (ca. 1 mm vs. (3–) 5.5–7 mm) and anthers (1.5–2.3 mm vs. 2.5–3 mm), calyx lobe shape (triangular ovate vs. narrowly triangular to linear), the outer calyx indumentum (tomentose vs. puberulent or velutinous), and the ovary length (ovaries longer than the styles vs. ovaries shorter than the styles).

Type. CHINA, Guizhou Province, Zunyi City, Maoli Town, Xiazhai Valley, elev. ca. 1000 m, 27.36986425°N, 107.05679454°E, growing on the Karst rocks alongside the stream. 15 April 2023, Ren-Bo Zhang ZRB2493 (fl.) (**holotype**: IBK!, **isotypes**: ZY!) and 27 May 2023 Ren-Bo Zhang ZRB2498 (fr.) (**paratype**: ZY!).

Description. **Herbs**, stemless. **Leaves** basal, petiole 0.8–1.5 cm long; leaf blade spatulate or oboval-oblong, 3.5–12 (–25) × 1–3.5 (–8) cm, thick papery to leathery, adaxially cobwebby-woolly, glabrescent, abaxially densely cobwebby-woolly to pannose, base attenuate to cuneate, margin crenate or subentire, involute, apex rounded or acute; lateral veins 4–7 on each side of midrib. **Cymes** 1–2, axillary, each cyme 3–7 branched and 9–22 flowered; peduncle 2–6 cm long, ca. 1.5 mm in diam, cobwebby-woolly, glabrescent from upper part; bracts 2, opposite, linear, 2–3 × 0.6–0.9 mm, outside cobwebby-woolly. **Pedicel** 1–2 cm long, cobwebby-woolly. **Calyx** ca. 3 mm long, 5-sect from near base; segments triangular ovate, 1–2 × ca. 0.5 mm, outside brown tomentose. **Corolla** blue-purplish, ca. 1.5 cm long, subglabrous on both sides; tube 7–8 mm long; adaxial lip ca. 3 mm long, lobes 2–3 × ca. 5 mm; abaxial lip ca. 7 mm long, lobes 3–4 × 5–6 mm. **Stamens** 2, filaments ca. 1 mm long, glabrous; anthers 1.5–2.5 mm long; staminodes 2, ca. 0.8 mm long. **Pistil** glabrous; ovary 4–6 mm long; style 3–5 mm long; stigma capitate. **Capsule** spirally twisted, 2–4 cm long, glabrous. **Seeds** 0.5–0.7 × 0.2–0.3 mm, reticulate, apiculate or cuspidate at both ends. **Fl.** Apr–May. **Fr.** May–Jun.

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

Etymology. The specific epithet is derived from the type locality, Zunyi City, Guizhou Province, China.

Vernacular name. The Chinese name proposed here is “遵义蛛毛苣苔”. Phonetically, it is “Zūn yì zhū máo jù tái”.

Distribution and ecology. The new species is endemic to Guizhou Province and is known only from the type locality, Xiazhai Valley in Zunyi City. It grows on the steep Karst cliff in a valley, at an altitude ca. 1000 m.

Conservation status. *Paraboea zunyiensis* is known only from the type locality, with the individuals estimated to be over thousands of plants. Considering the narrow distribution area, we proposed it as “NT” (near threatened) according to the guidelines for using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

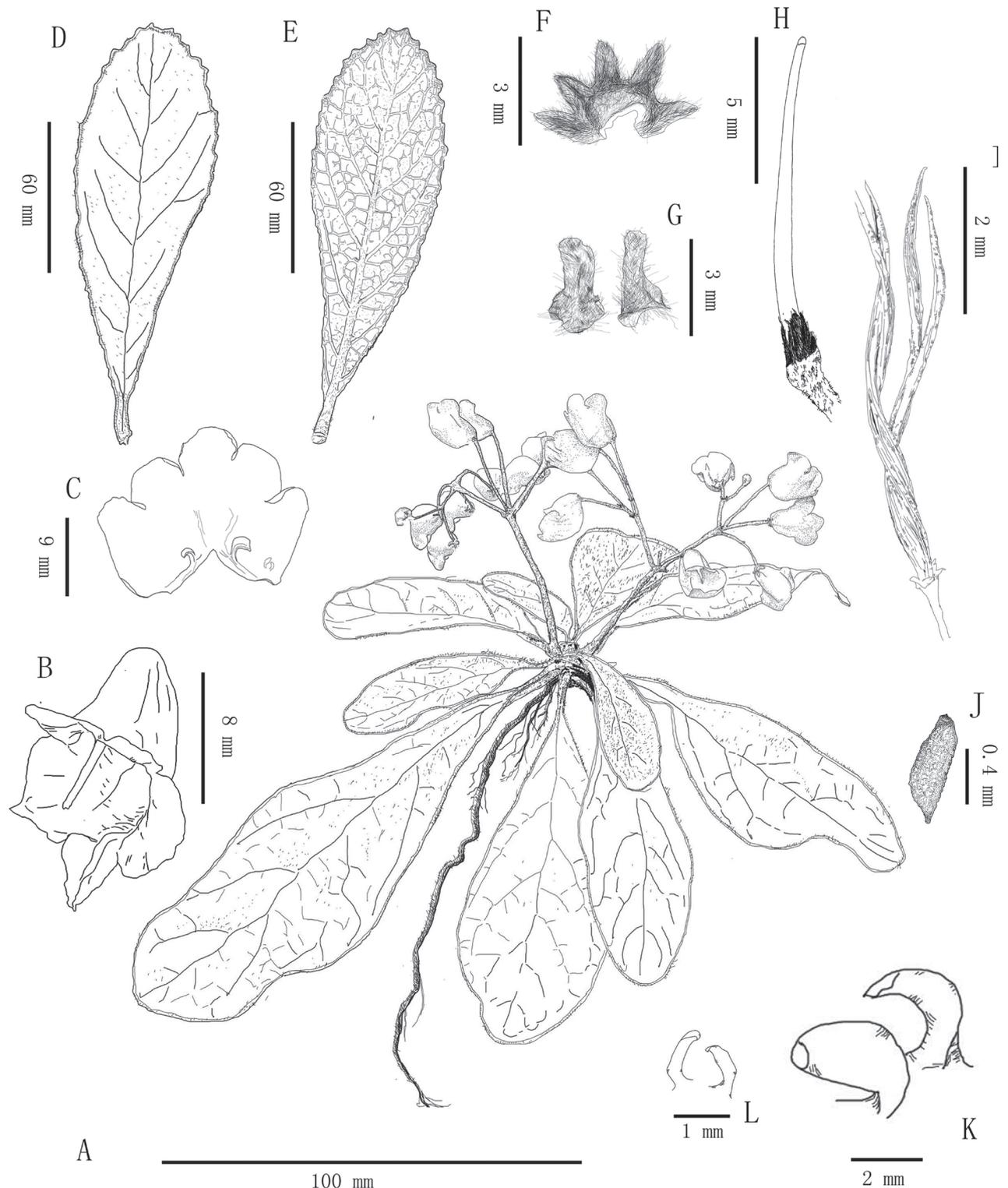


Figure 1. Line drawing of *Paraboea zunyiensis* T.Deng, F.Wen & R.B.Zhang, sp. nov. **A** flowering plant **B** corolla **C** opened corolla **D** adaxial leaf surface **E** abaxial leaf surface **F** calyx **G** bracts **H** pistil **I** capsules **J** seed **K** fertile stamens **L** staminodes. Drawings by Tan Deng from the type specimens.

Taxonomic and phylogenetic notes. The aligned matrix of ITS and *trnL-F* sequences comprised 1562 characters. The three outgroup species are clearly distinguishable from the *Paraboea* species (Fig. 3). *P. hainanensis* (Chun) B.L.Burt is quite different and other *Paraboea* species form two big clades, which matches a

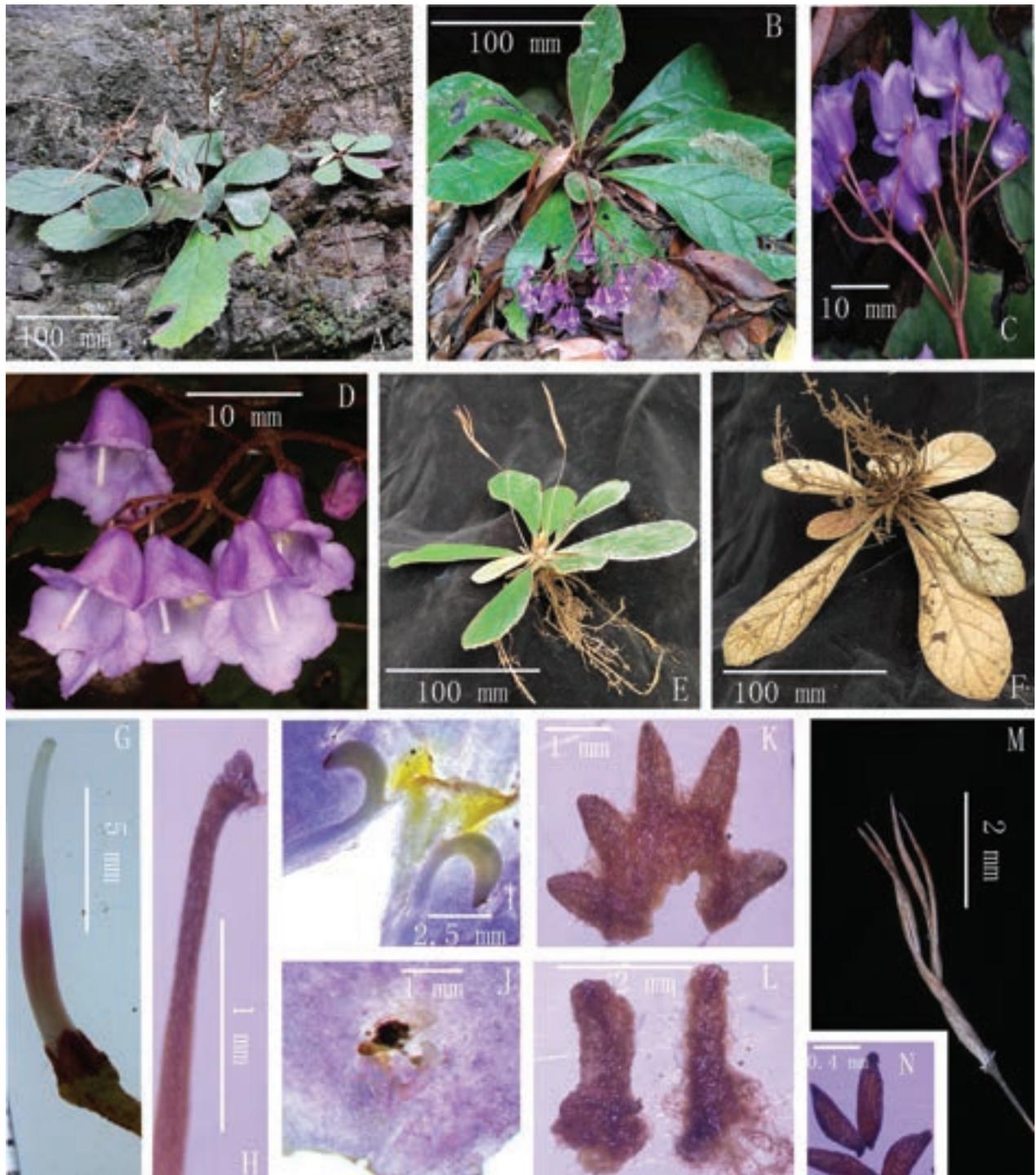


Figure 2. Living or re-watered photographs of *Paraboea zunyiensis* T.Deng, F.Wen & R.B.Zhang, sp. nov. **A** habitat **B** flowering plant **C** cyme **D** flowers **E** fruiting plant **F** upward view of the plant **G** pistil **H** stigma and style **I** fertile stamens **J** staminodes **K** calyx **L** bracts **M** capsules **N** seeds (Photographed by T. Deng and R.B. Zhang)

previous study (Guo 2016). Although *P. zunyiensis* and *P. crassifolia* are in the same branch (BI = 100%), they are not clustered together and they can be morphologically distinguished from the traits presented in Table 2. *P. zunyiensis* is clustered with *P. neurophylla* (Hance) B.L.Burtt and *P. trisepala* W.H.Chen & Y.M.Shui (BI = 100%). *P. crassifolia* and *P. velutina* (W.T.Wang & C.Z.Gao) B.L.Burtt are clustered as a sister group (BI = 95%) and then clustered with *P. dictyoneura* (Hance) B.L.Burtt.

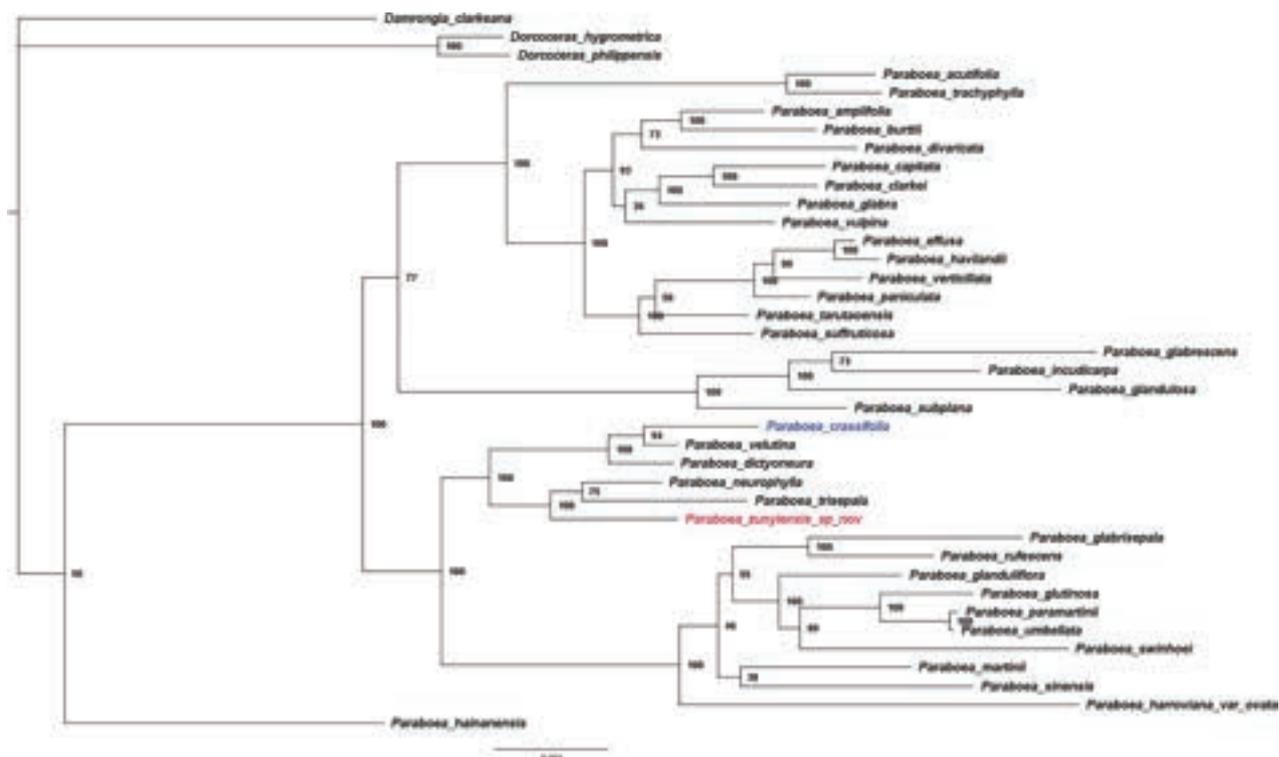


Figure 3. Bayesian phylogenetic tree of *Paraboea* including *P. zunyiensis* based on the combined data from ITS and *trnL-F*, with the branch lengths indicating the nucleotide substitution rates and the posterior probabilities are shown beside the branches.

Table 2. Morphological and phenological comparison of *Paraboea zunyiensis* and *P. crassifolia*.

Characters	<i>Paraboea zunyiensis</i>	<i>Paraboea crassifolia</i>
Bract width (mm)	0.6–0.9	ca. 0.5
Calyx lobes	triangular ovate	narrowly triangular to linear
Calyx outer indumentum	tomentose	puberulent or velutinous
Filament length (mm)	ca. 1	(3–) 5.5–7
Anther length (mm)	1.5–2.3	2.5–3
Staminode length (mm)	< 1	2–2.5
Ovary (mm)	4–6 (longer than styles)	3–4 (shorter than styles)
Style (mm)	3–5	5.5–6
Fl.	Apr–May	Mar–Jul
Fr.	May–Jun	Sep

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was financially supported by the National Natural Science Foundation of China (31860162) and the Doctor Foundation of Zunyi Normal College (BS[2018]17).

Author contributions

Conceptualization: FW. Data curation: QLD, DJX. Formal analysis: QLD. Investigation: TD, RBZ. Resources: LH. Software: ZMQ, RXW. Supervision: FW. Visualization: RXW, TD. Writing - original draft: LH, RBZ, TD. Writing - review and editing: FW.

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Data availability

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

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Bomarea pastazensis (Alstroemeriaceae), an exceptionally small new species from the eastern Andean slopes of Ecuador

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Abstract

Recent field research on the eastern slopes of the Andes resulted in the discovery of a new species of *Bomarea* from the Cerro Candelaria Reserve in the Tungurahua province of Ecuador. *Bomarea pastazensis* is the second smallest species in the genus and differs from the smallest by the presence of glutinous trichomes on the ovary, glabrous sepals, and greenish-yellow petals with purple spots. Based on IUCN guidelines, a preliminary conservation status is assigned as Vulnerable (VU).

Resumen

Recientes investigaciones de campo en las estribaciones orientales de los Andes dieron como resultado el descubrimiento de una nueva especie de *Bomarea* en la Reserva Cerro Candelaria en la provincia de Tungurahua en Ecuador. *Bomarea pastazensis* es la segunda especie más pequeña del género y se diferencia por la presencia de tricomas glutinosos en el ovario, sépalos glabros y pétalos verdes con amarillo y manchas moradas. Con base en los lineamientos de la IUCN, se le asigna un estado preliminar de conservación de Vulnerable (VU).

Key words: Alstroemeriaceae, Andes, *Bomarea*, Ecuador, endemism, taxonomy



Academic editor: M. Alejandra Jaramillo

Received: 4 August 2023

Accepted: 10 October 2023

Published: 10 November 2023

Citation: Clark JL, Fierro-Minda

A, Exe N, Johnson M, Tribble CM,

Jost L (2023) *Bomarea pastazensis*

(Alstroemeriaceae), an exceptionally

small new species from the eastern

Andean slopes of Ecuador. *PhytoKeys*

235: 31–42. [https://doi.org/10.3897/](https://doi.org/10.3897/phytokeys.235.110525)

[phytokeys.235.110525](https://doi.org/10.3897/phytokeys.235.110525)

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Introduction

There are more than 100 species of *Bomarea* Mirb. in South America (Hofreiter 2006) and 39 species in Ecuador (Harling and Neuendorf 2003). The description of *Bomarea pastazensis* brings the total diversity to 40 species in Ecuador. The most recent monograph of *Bomarea* was provided by Hofreiter and Tillich (2002) and included an updated classification system based on Baker (1888) that recognized the following four subgenera: *Baccata* Hofr., *Bomarea* Baker, *Sphaerine* (Herb.) Baker, and *Wichuraea* (M. Roemer) Baker. More recently, molecular phylogenetic studies have failed to support the monophyly of these subgenera (Alzate et al. 2008b; Tribble et al. 2022), suggesting that many of the

morphological characters that previously defined infrageneric classification are homoplastic or have evolved convergently. Alzate et al. (2008b) showed that traditionally recognized subgeneric ranks proposed by Baker (1888) and Hofreiter and Tillich (2002) are polyphyletic. Tribble et al. (2022) described three main clades within *Bomarea*, but no updated classification system has yet been proposed. In the present study we recognize the lack of strongly supported subgeneric ranks (Alzate et al. 2008b; Tribble et al. 2022) and discuss the traditional subgenus that most resembles *Bomarea pastazensis*.

Bomarea pastazensis most closely resembles the morphologies associated with the subgenus *Sphaerine* because of the following characters (Hofreiter 2005): erect or hanging habit [mostly non-twining except for *B. coccinea* (Ruiz & Pav.) Baker] (Fig. 1E), resupinate leaves (Figs 1D, 2E), and inferior ovaries (Figs 1A–C, 2A). Recent molecular work suggests that *Sphaerine* is non-monophyletic and some morphological characteristics of this group are likely adaptations to environmental conditions (Alzate et al. 2008b; Tribble et al. 2022). The subgenus *Sphaerine* ranges from the northern Andes of Colombia and Venezuela to Bolivia. In Ecuador, the subgenus is distributed mainly on the eastern Andean slopes. A detailed taxonomic history and overview of *Sphaerine* was provided by Hofreiter (2006), including updated circumscriptions and two new species. The addition of *Bomarea pastazensis* brings the total diversity of subgenus *Sphaerine* to 13 species.

Ecuador is the center of diversity for *Bomarea* (Hofreiter and Tillich 2002; Harling and Neuendorf 2003; Hofreiter 2005; Hofreiter and Rodríguez 2006; Alzate et al. 2008a), which is supported by the presence of 40 of the 120+ currently known species. The other species of *Bomarea* subgenus *Sphaerine* occurring in Ecuador (Hofreiter 2005; Hofreiter and Rodríguez 2006) are: *B. brachysepala* Benth. (Fig. 1A), *B. distichifolia* (Ruiz & Pav.) Baker (Fig. 1B), *B. hieronymi* Pax (Fig. 1), *B. linifolia* (Kunth) Baker, and *B. nervosa* (Herb.) Baker.

The Cerro Candelaria Reserve, owned by the Ecuadorian NGO Fundación EcoMinga, is within the upper Pastaza watershed, an area recently documented for high-levels of local endemism because of the presence of microclimates created by Amazon-Andean airflow currents and the irregular topography (Jost 2004). The Cerro Candelaria Reserve comprises 2800+ hectares, ranging in altitude from 1700 to 3860 m (Reyes-Puig et al. 2013). The private reserve is located within the Llanganates-Sangay corridor, which facilitates a protected corridor for biodiversity between the Llanganates and Sangay National Parks (Fig. 3).

Materials and methods

Plants were photographed and collected during three separate field expeditions to the Cerro Candelaria Reserve by Clark in 2016 (Clark 2016), Exe and Johnson in January 2022, and Exe, Johnson, and Fierro-Minda in November 2022. Specimens were deposited at Pontificia Universidad Católica del Ecuador (QCA), Marie Selby Botanical Gardens (SEL), and Universidad de Guayaquil (GUAY). Digital images were taken of live specimens in the field using an Olympus TG5 and a Nikon D100 DSLR with a Nikon 105 mm lens and a Nikon SB-29s ring flash. Morphological observations and measurements were made from live collections, herbarium specimens, and digital images using the program ImageJ (Schneider et al. 2012).

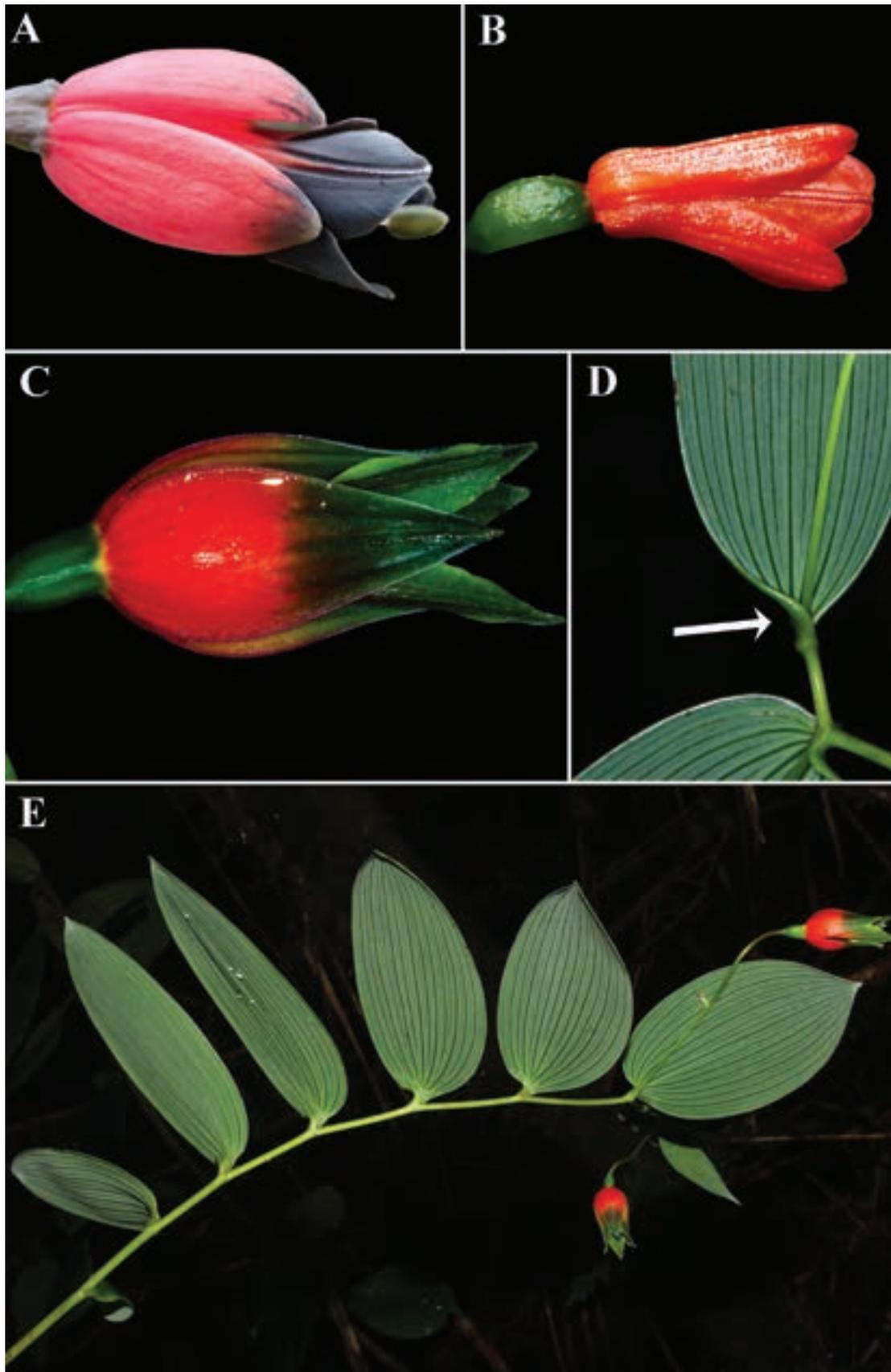


Figure 1. Some species of Ecuadorian *Bomarea* subgenus *Sphaerine* **A** *Bomarea brachysepala* **B** *Bomarea distichifolia* **C** *Bomarea hieronymi* **D** resupinate leaf (rotated petiole indicated with white arrow) of *Bomarea hieronymi* **E** terrestrial habit (non-twining) of *Bomarea hieronymi*. Photos **A**, **B** by N. Exe, **C–E** by J.L. Clark (J.L. Clark 17350).

We assessed the extinction risk of *Bomarea pastazensis* following the IUCN Red List Categories and Criteria (IUCN 2012) and guidelines of the IUCN Standards and Petitions Committee (2022). We considered observations, collection localities, and population estimate from fieldwork. Species extent of occurrence (EEO) and area of occupancy (AOO) were calculated using GeoCAT (Bachman et al. 2011) with the default setting of 2 km² grid.

A note on terminology

Some botanical terminology has been used inconsistently in previous descriptions of *Bomarea*, and other terminology is specific to *Bomarea*. Here, we clarify our use of potentially confusing terms to ensure that our definitions are unambiguous while linking the description to other literature. Botanical glossaries usually refer to sepals as the outer whorl of the perianth and petals as the inner whorl of the perianth (e.g., Harris and Harris 2006; Pell and Angell 2016). In contrast, ‘tepal’ is used when describing cases where the outer and inner perianth whorls are identical or when perianth whorls are not readily differentiated. Some *Bomarea* taxonomists use the terms inner and outer tepals in their descriptions of the perianth (Hofreiter 2005; 2006; Hofreiter and Rodríguez 2006) and others use the terms sepals and petals (Harling and Neuendorf 2003). The description of *Bomarea pastazensis* uses sepals and petals to provide greater specificity because the two perianth layers are easily differentiated. Furthermore, we use the terms adaxial and abaxial following the definition of these terms to refer to the portion of the leaf facing towards or away from, respectively, the structure-bearing organ (the stem) during development (Eckel 2011). When referring to the surface of resupinate leaves (rotating 180 degrees) of *Bomarea pastazensis*, abaxial is the upper surface of the leaf, and adaxial is the lower surface of the leaf, terminology that is consistent with other *Bomarea* descriptions (Hofreiter and Lysheide 2006). Following Hofreiter (2005), we use the term “claw” to refer to the horn-like thickened sepal apex in many *Bomarea* species.

Taxonomic treatment

***Bomarea pastazensis* J.L.Clark, Fierro-Minda & N.Exe, sp. nov.**

urn:lsid:ipni.org:names:77330582-1

Figs 2, 4

Diagnosis. Similar to *Bomarea pumila* Griseb. ex Baker, differing in plant height reaching 10–14 cm (vs. 5–8 cm high in *B. pumila*), pubescent stem (vs. glabrous stem in *B. pumila*), the presence of ciliate leaf margin (vs. glabrous leaf margin in *B. pumila*), and the presence of dark spots on the petals (vs. no spotting on the petals in *B. pumila*).

Type. ECUADOR. Tungurahua: Cantón Baños, Parroquia Río Verde, Cerro Candelaria Reserve (Fundación EcoMinga), upper Pastaza watershed, 1°28'39.33"S, 78°17'53.61"W, 3642 m, 10 Mar 2016, J.L. Clark 14839 (holotype: QCA! [245371]; isotype: SEL! [079072]).

Description. Terrestrial or epiphytic herb. **Rhizome** short with multiple underground fusiform to globose root tubers, pale and heavily stippled with dull reddish-purple spots, 5–9 × 3–4 mm, surface striate, yellowish-brown with red spots

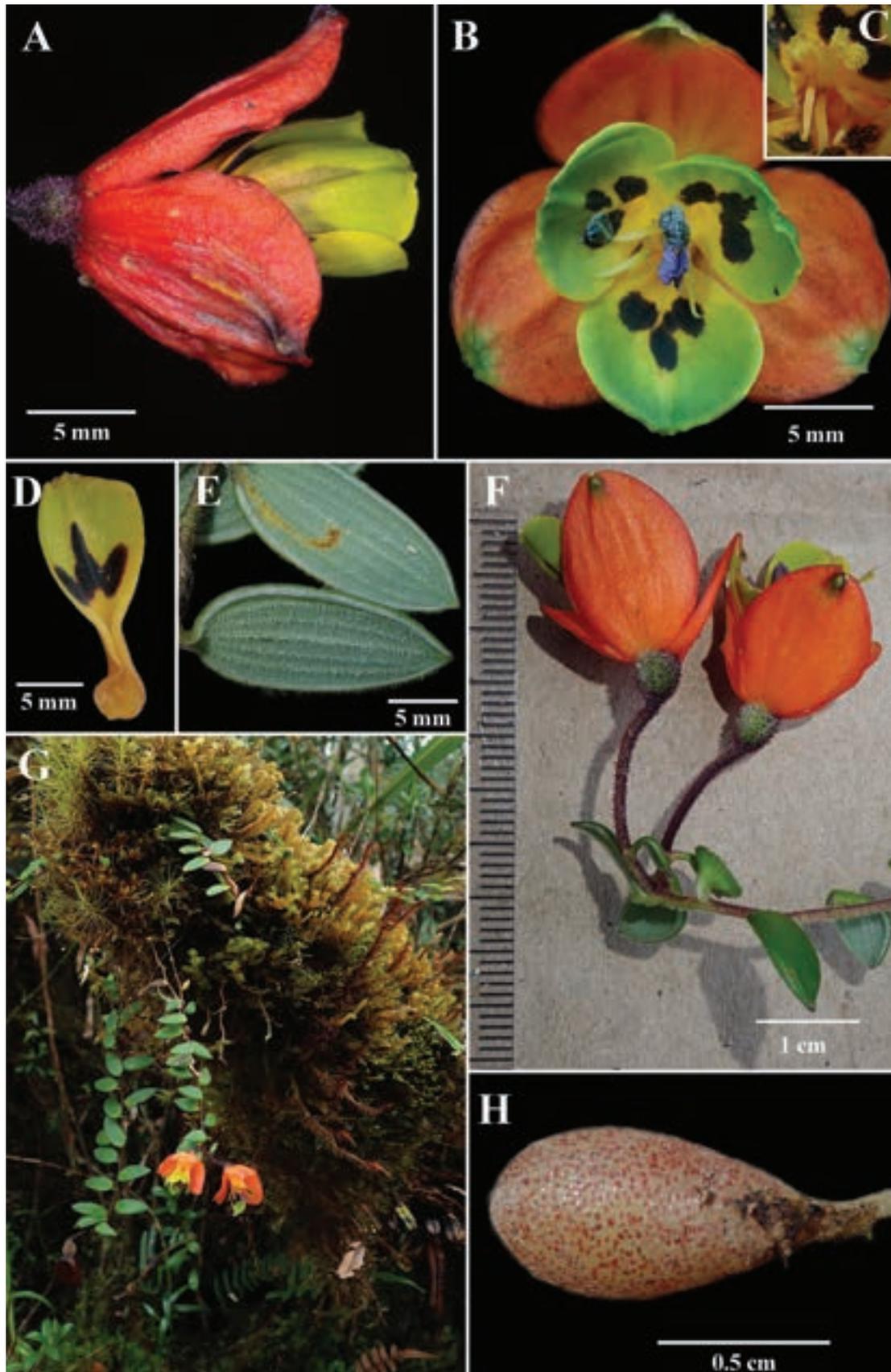


Figure 2. *Bomarea pastazensis* **A** lateral view of flower **B** front view of flower featuring mature androecium **C** mature gynoecium **D** petal **E** adaxial leaf surface **F** mature flowers with foliage **G** habitat **H** tuberous roots. Photos **A** by J.L. Clark (J.L. Clark 14839), **B–H** by N. Exe (N. Exe et al. 2).

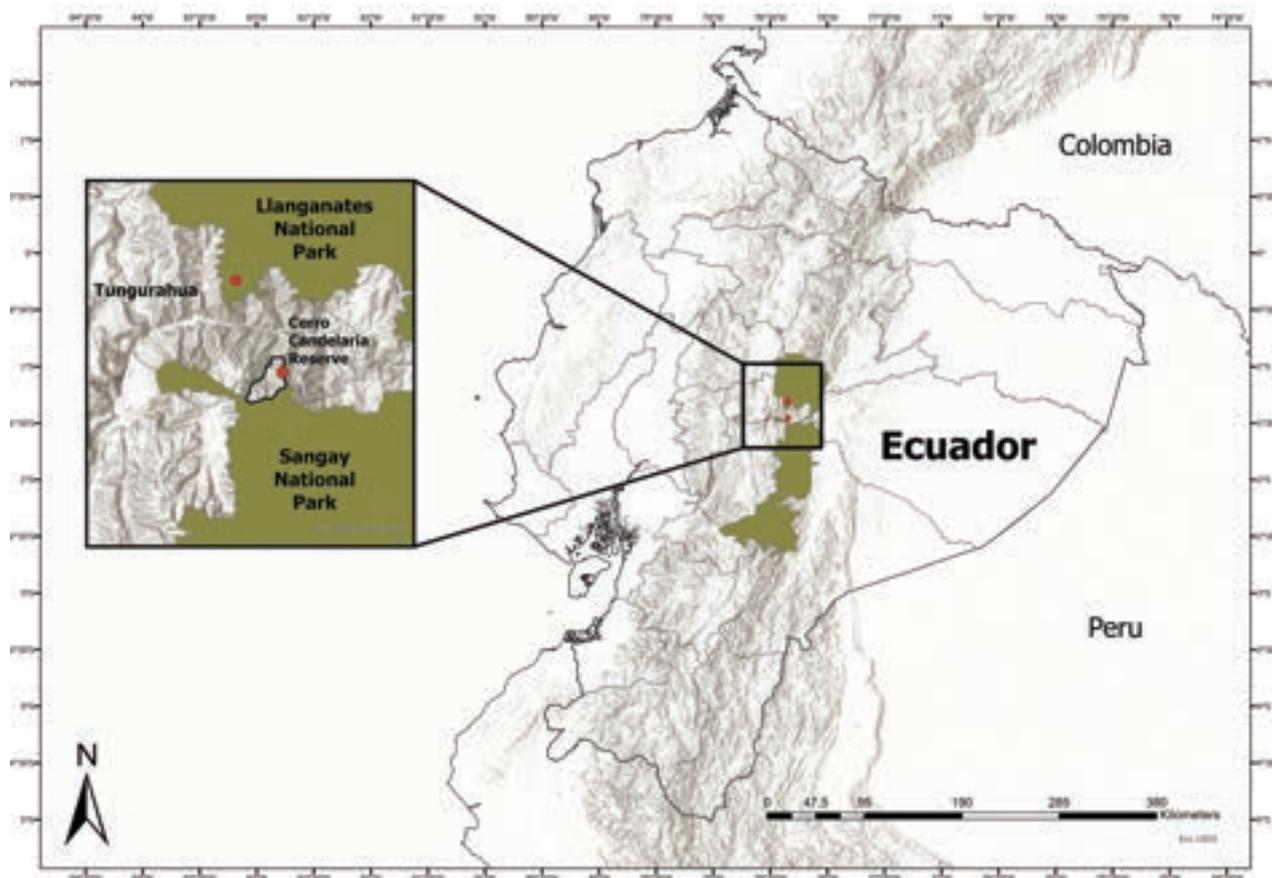


Figure 3. Distribution map of *Bomarea pastazensis*. Red dots indicate collections or observations.

(Fig. 2H). **Stem** erect, 10–14 cm long, ca. 0.13 cm in diameter, slender, terete, base pubescent, apex puberulous, internodes 0.6 cm long. **Leaves** alternate; blade ovate, 1.4–1.8 × 0.4–0.7 cm, base rounded to obtuse, apex acute, abaxially glabrous, adaxially light green, suffused with whitish-translucent trichomes, ca., 0.2 mm long, clustered along veins; blades with 5–7 prominent parallel veins, raised below and slightly raised above; margin hyaline, slightly revolute with unicellular trichomes to 0.2 mm long; petioles resupinate and canaliculate, 0.1–0.4 cm long, basal leaves reduced to scales. **Flowers** produced from the stem apex, usually one (rarely two) apical flower(s) per stem.; pedicels 1–1.4 cm long, terete, with brownish red to dark purple trichomes, bisexual, actinomorphic, epigynous. **Sepals** 3, 1.0–1.3 × 1.0–1.2 cm, each sepal with 6 parallel veins, broadly ovate, apically rounded, bright red to orange-red with a 1 mm green to black claw, inner and outer surfaces glabrous. **Petals** 3, basally constricted and caniculate, distally obovate to broadly spatulate, 1.5–1.6 × 0.6–0.7 cm, greenish yellow, puberulous at base, inner surface with 3 large reddish-brown spots, outer surface uniformly yellow (occasionally yellow suffused with red from the inner spots). **Androecium** of 6 free stamens, 0.8–0.9 cm long, thickened near center of filaments; anthers pseudo-basifixed, 0.3 × 0.1 cm, fusiform; pollen grains lilac. **Gynoecium** comprised of three fused carpels, ovary 0.5 × 0.4 cm with surface covered with glutinous trichomes, style ca. 1 cm long, stigma with three circinate lobes. **Fruits** not observed.

Ecology. Found growing on *Sphagnum* and mossy cushions at ground level and epiphytically (up to 2.5 m high) in high elevation cloud forest and paramo



Figure 4. Holotype of *Bomarea pastazensis*, J.L. Clark 14839 (QCA).

(observed from 3235 to 3700 m). Plants growing in partially shaded areas to full sunlight. Stems erect to hanging, with flowers often found on or slightly above ground level. Flowers protandrous, commonly with one flower per stem but occasionally two.

Phenology. Observed in flower in January, March, July, and November. Approximately 20 individual plants with mature flowers were located during an expedition in November of 2022 and fewer individuals with mature flowers were observed in January of 2022. Fruits not documented.

Etymology. The specific epithet, *pastazensis*, reflects the watershed of the type locality that includes Río Pastaza and adjacent tributaries.

Distribution and preliminary assessment of conservation status. *Bomarea pastazensis* is endemic to the upper Pastaza watershed, located in the eastern Andean slopes of Ecuador. The first documented population was inside Cerro Candelaria Reserve in Tungurahua province (Fig. 3). The Cerro Candelaria Reserve is a private reserve of 2800+ hectares managed by Fundación EcoMinga. It is bordered on the north by EcoMinga's Naturetrek Reserve, and the south by Parque Nacional Sangay. Along with Fundación EcoMinga's Machay and Naturetrek Reserves to the north, it forms a protected corridor between Parque Nacional Sangay and Parque Nacional Llanganates. Cerro Candelaria Reserve was founded by Fundación EcoMinga in 2007 and financed by the World Land Trust (UK); the majority of the reserve is undisturbed by anthropogenic activity. A diverse flora and fauna has been recorded here, including many endemic species and species of high conservation priority (Jost 2004; Reyes-Puig et al. 2013), such as *Blakea attenboroughii* Penneys & L.Jost (Melastomataceae) which is endemic to the lower elevation forests of Bosque Protector Cerro Candelaria. Several recently described species in the Orchidaceae share a similar geographic distribution to *Bomarea pastazensis* (Fig. 3), include *Teagueia barbeliana* L.Jost & Shepard, *T. puroana* L.Jost & Shepard, *T. kostoglouana* L.Jost & Shepard, *T. lizziefinchiana* L.Jost & Shepard, and *T. anitana* L.Jost & Shepard (Jost and Shepard 2011; 2017). Additional surveys in Cerro Candelaria and in the surrounding upper Rio Pastaza watershed are likely to yield many more undescribed species, and potentially additional populations of *Bomarea pastazensis*. The distribution of this species highlights the importance of the Llanganates-Sangay corridor for allowing species gene flow and connectivity among populations (Ríos Alvear and Reyes-Puig 2015). A second documented population of *Bomarea pastazensis* was provided by digital images from EcoMinga's park guard Eduardo Peña (July 2023) in the Llanganates National Park, north of Río Pastaza (Fig. 3). We calculated the AOO=12 km² from Peña's observation and the type collection (Fig. 3). Based on the available information and according to the IUCN Red List criteria (IUCN 2012; IUCN Standards and Petitions Committee 2022), *Bomarea pastazensis* is preliminarily assessed as Vulnerable (VU) based on a limited area of occupancy (IUCN criterion D2 where AOO <20 km²) and dependence on conservation efforts for its continued survival. The only documented populations of *Bomarea pastazensis* are located inside protected areas, at elevations that are used for agriculture in unprotected parts of the Pastaza province. Effective conservation of this and the other unique species of the Pastaza watershed will require constant vigilance.

Comments. *Bomarea pastazensis* differs from other congeners by a distinctive pattern of three dark spots on the petals (Fig. 2D), widely opened flowers,

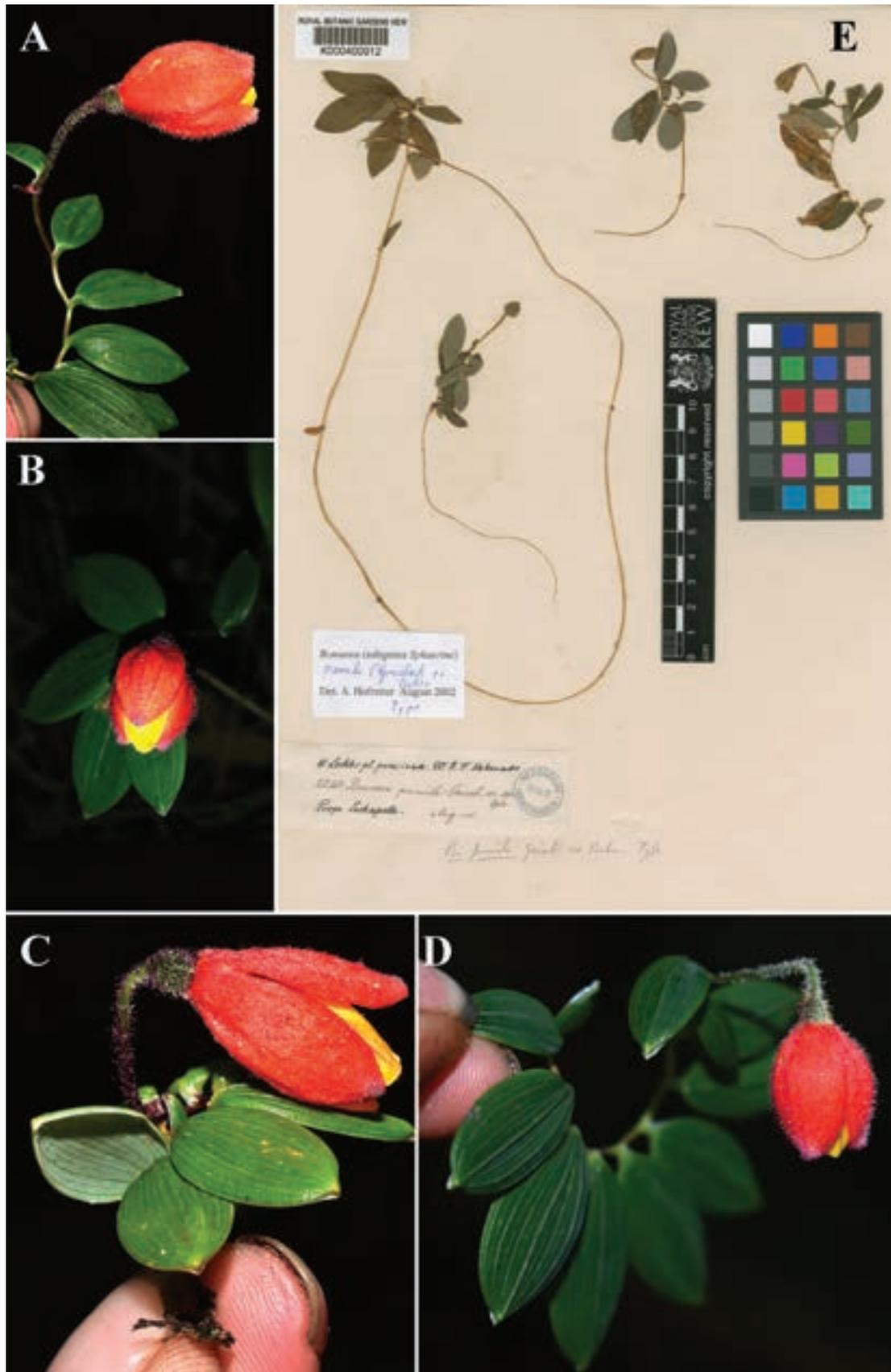


Figure 5. *Bomarea pumila* **A–D** field images from Peru **E** syntype (K! [K000400912]) of *Bomarea pumila* of W. Lechler 2240 from Sachapata, Peru. Photos **A–D** from Lucely L. Vilca Bustamente **E** from The Herbarium Catalogue, Royal Botanic Gardens, Kew. The Board of Trustees of the RBG, Kew.

Table 1. General geographic distribution (names in parentheses indicate Ecuadorian province) and comparison of morphological characters between *Bomarea pastazensis* and *B. pumila*.

	<i>Bomarea pumila</i>	<i>Bomarea pastazensis</i>
Habit	erect	erect to hanging
Plant height	5–9 cm long	10–14 cm long
Stem surface	glabrous	pubescent
Leaf – relative size	leaves not uniform on stem; central leaves relatively larger compared with basal and apical leaves	leaves relatively uniform on stem
Leaf margin	glabrous	ciliate
Pedicle length	1.5–4 cm long	1–1.4 cm long
Flower length	0.8–1.5 cm long	1.5–1.6 cm long
Flower width during anthesis	2–3 cm wide	1.5–2 cm wide
Sepal surface	conspicuously pubescent	sparsely pubescent at base only
Sepal horn	white to reddish horn at apex	green to black horn at apex
Petal coloration (=inner tepals)	yellow with a red stripe and green tip.	green suffused with yellow with three prominent red spots at the base
Distribution	Peru and Bolivia	Ecuador (Tungurahua)

and small size. *Bomarea pumila* and *B. pastazensis* share similar traits such as their small size in comparison with the rest of *Bomarea* species, the presence of pubescence throughout their above-surface organs, and the color patterns of the flowers (Figs 2, 5; Table 1). *B. pastazensis* is slightly bigger in size (10–14 cm tall) than *B. pumila* (5–9 cm tall). In contrast, *B. pastazensis* is sparsely pubescent at the base of the sepals, while the sepals of *B. pumila* are uniformly densely pubescent (Fig. 5). The petals of *B. pumila* are not spotted, which differentiates it from *B. pastazensis* which has a three-spotted pattern on the petals (Fig. 2B). The two species are geographically isolated with *B. pastazensis* endemic to the Pastaza watershed on the eastern Andean slopes in central Ecuador (Tungurahua province) and *B. pumila* from Central Peru to northern Bolivia. Table 1 provides a summary of distribution and useful characters for differentiating *B. pastazensis* and *B. pumila*.

Additional specimens examined. ECUADOR. Tungurahua: Cantón Baños, Parroquia Río Verde, Bosque Protector Cerro Candelaria (Fundación EcoMinga), upper Pastaza watershed, Cerro Candelaria summit trail, just below paramo, 1°28'39.33"S, 78°17'53.61"W, 3150–3827 m, 1 Jan 2022, N. Exe, M. Johnson & A. Fierro-Minda 2 (GUAY).

Acknowledgements

We thank the Ministry of the Environment of Ecuador for providing permits for specimen collection and transportation (Research permit Aves y Conservación N° 007-2018-IC-FLO-FAU and mobilization permit Aves y Conservación 005-FLO-2019-DPAP-MA). We thank Lucely L. Vilca Bustameante (Instituto Nacional de Investigación en Glaciares y Ecosistemas de Montaña, Cusco, Peru) for sharing her field images of *Bomarea pumila* (Fig. 5). We are grateful to Guillermo Peña for assisting with the expedition and collection of specimens, as well as providing

supplemental locality information. We thank the Science Collections Digitisation Project at the Royal Botanic Gardens, Kew for facilitating an image of the syntype for *Bomarea pumila* (Fig. 5). We are grateful to Alan W. Meerow and an anonymous reviewer for providing helpful feedback on an earlier version of the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

John Clark made the type collection in 2016. Nolan Exe, Mia Johnson, and Alisson Fierro-Minda conducted additional fieldwork in 2022. All authors contributed to writing the manuscript. Jost wrote the IUCN assessment. Tribble facilitated the use of terminology specific to Alstroemeriaceae.

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Data availability

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

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Gesneria tuberifera (Gesneriaceae), a new lithophytic species from the Sierra de Bahoruco, Barahona Peninsula of southern Hispaniola (Dominican Republic)

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Abstract

A narrowly endemic new species of *Gesneria* is described from the Sierra de Bahoruco in the Dominican Republic's Barahona Peninsula of southern Hispaniola. *Gesneria tuberifera* J.L.Clark & T.Clase, **sp. nov.** differs from all other congeners by the presence of a tuber and tubular red corollas with yellow lobes. Images and a discussion are provided to summarize the presence of tubers in other Gesneriaceae and differentiate *Gesneria tuberifera* from congeners that share a lithophytic habit. Based on IUCN guidelines, a preliminary conservation status of Endangered (EN) is assessed for *G. tuberifera*.

Resumen

Se describe una nueva especie de *Gesneria* endémica de la Sierra de Bahoruco en la península de Barahona en la República Dominicana, en el sur de La Española. *Gesneria tuberifera* J.L.Clark & T.Clase, **sp. nov.** se diferencia de todos los demás congéneres por la presencia de un tubérculo y corolas rojas tubulares con lóbulos amarillos. Se proporcionan imágenes y una discusión para resumir la presencia de tubérculos en otras Gesneriaceae y diferenciar a *Gesneria tuberifera* de otros congéneres que comparten un hábito litófito. Con base en las pautas de la UICN, se evalúa un estado de conservación preliminar de En Peligro (EN) para *G. tuberifera*.

Key words: Biodiversity, Gesneriaceae, Sierra de Bahoruco, systematics, taxonomy, tuber



Academic editor: Michael Moeller

Received: 3 August 2023

Accepted: 13 October 2023

Published: 10 November 2023

Citation: Clark JL, Clase T (2023) *Gesneria tuberifera* (Gesneriaceae), a new lithophytic species from the Sierra de Bahoruco, Barahona Peninsula of southern Hispaniola (Dominican Republic). *PhytoKeys* 235: 43–52. <https://doi.org/10.3897/phytokeys.235.110476>

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Introduction

The flowering plant family Gesneriaceae, with over 3400 species and 150+ genera (Weber 2004; Weber et al. 2013), is in the order Lamiales. The family is divided into three subfamilies and seven tribes (Weber et al. 2013, 2020), each of which represent monophyletic lineages (Ogutcen et al. 2021). Most Neotropical members are in the subfamily Gesnerioideae, which is represented by 1200+ species and 77 genera (Clark et al. 2020). *Gesneria* L. is classified in the tribe Gesnerieae and subtribe Gesneriinae Oerst. (Weber et al. 2013, 2020).

The subtribe Gesneriinae is strongly supported as a monophyletic clade that is mostly Caribbean and includes the following three genera: *Gesneria*,

Pheidonocarpa L.E.Skog, and *Rhytidophyllum* Mart. *Pheidonocarpa* has one species with a subspecies endemic to Cuba and a second subspecies endemic to Jamaica. *Rhytidophyllum* is mostly endemic to the Caribbean islands, with the exception of two species in northern South America. *Gesneria* is entirely endemic to the Caribbean region and was most recently monographed by Skog (1976). Updated circumscriptions to several broadly defined species in Skog (1976) were re-circumscribed in publications by Clark et al. (2019), Lambert et al. (2017), and Joly et al. (2023). The addition of *Gesneria tuberifera* brings the total species diversity of *Gesneria* to 63 or 73 taxa when including infraspecific ranks.

Gesneria is broadly characterized by alternate leaves, an inferior or sub-inferior ovary, and glabrous filaments that are substantially free (adnate at base only) from the corolla tube. The habit for *Gesneria* is variable, but most species are perennial subshrubs (lithophytes or terrestrial) or lithophytes with leaves in a rosette. The corolla shape in *Gesneria* ranges from tubular to campanulate. The base chromosome number for all members of the tribe Gesnerieae is $n = 14$ (Lee 1966, 1968), a character that supports the monophyly of *Gesneria*, *Pheidonocarpa* L.E.Skog, and *Rhytidophyllum*. The flowers of *Gesneria* are usually zygomorphic. *Gesneria radiata* J.L.Clark & Cinea is a recently described species (Joly et al. 2023) and is the only species in the genus with corollas that appear radially symmetrical.

Plants were photographed in the field and subsequently pressed and dried. Specimens were deposited at the Jardín Botánico Nacional "Dr. Rafael M. Moscoso" (JBSD), Marie Selby Botanical Gardens (SEL), United States National Herbarium (US), New York Botanical Garden (NY), Missouri Botanical Garden (MO), and other herbaria. Photographs were taken of live specimens in the field using a Nikon D7200 DSLR camera, Nikon 105mm lens, and Nikon SB-29s ring flash. Morphological observations and measurements were made from live collections, alcohol-preserved material, and digital images using the *ImageJ* program (Schneider et al. 2012).

The extinction risk for *Gesneria tuberifera* was assessed following the IUCN (2012) and guidelines of the IUCN Standards and Petitions Committee (2022). Observations, collection localities, and population estimates from fieldwork were considered when assessing the IUCN category. Species area of occupancy (AOO) was calculated using GeoCAT (Bachman et al. 2011) with the default setting of a 2 km² grid (extent of occurrence (EOO) was not calculated because of the limited number of known populations).

Taxonomic treatment

Gesneria tuberifera J.L.Clark & T.Clase, sp. nov.

urn:lsid:ipni.org:names:77330583-1

Fig. 1

Diagnosis. Differs from all other *Gesneria* by the presence of a tuber. Additional characters that differentiate *G. tuberifera* from congeners is a rosette of leaves, elongate red tubular corollas with yellow lobes, and a lithophytic habit.

Type. DOMINICAN REPUBLIC. Pedernales: Sierra de Bahoruco, Las Mercedes, km 28 on the road Cabo Rojo–Aceitillar, Cañada La U, 18°7'13.05"N, 71°37'25.09"W, 840 m, 26 Jan 2023, J.L. Clark & T. Clase 17279 (holotype: JBSD; isotypes: FLAS, MO, MT, NY, SEL, US).

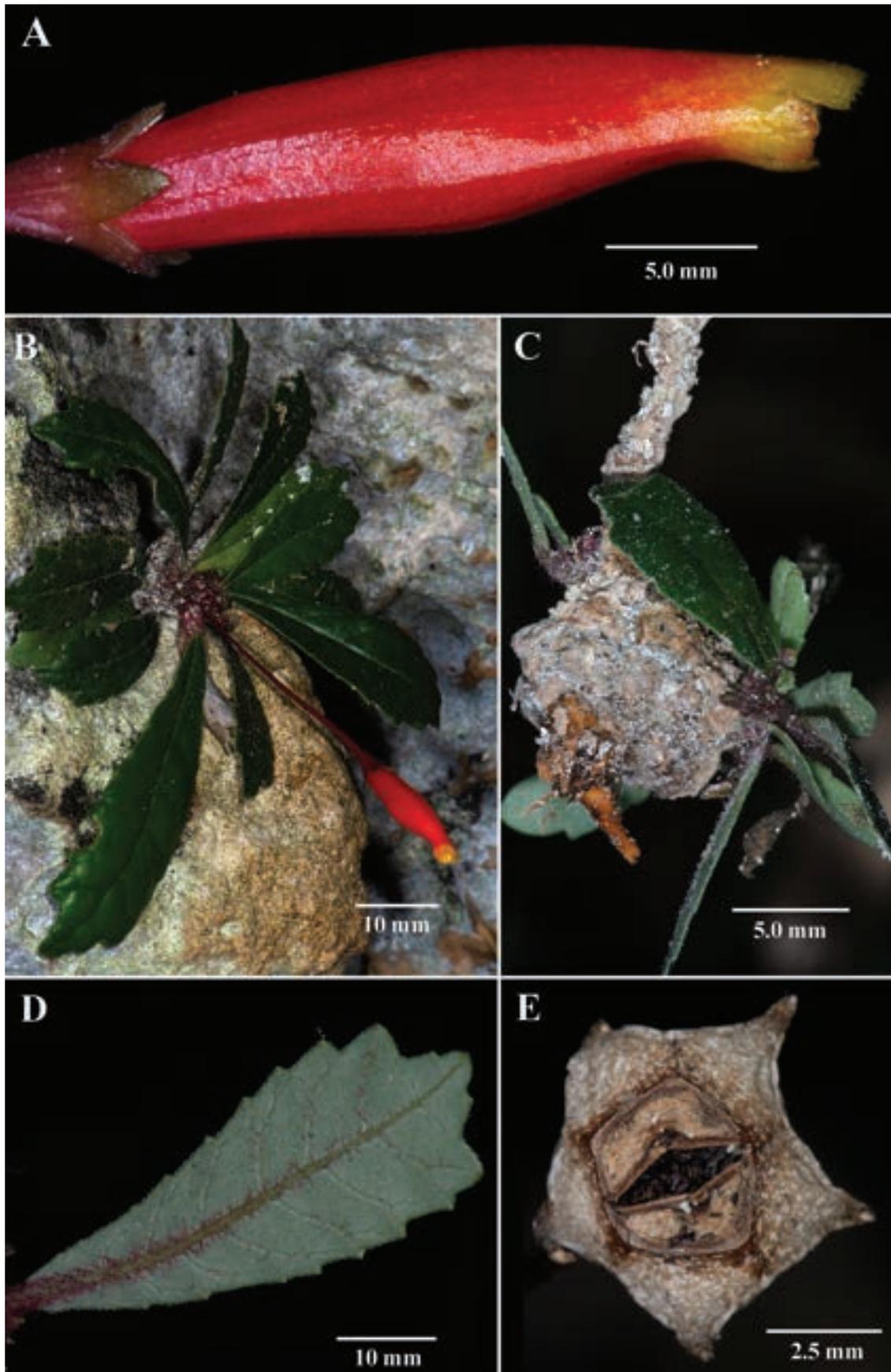


Figure 1. *Gesneria tuberifera* J.L.Clark & T.Clase **A** lateral view of mature flower **B** lithophytic habit **C** tuber with foliage **D** abaxial leaf surface **E** mature fruit (**A, B** J.L. Clark 17279 **C** J.L. Clark 17284 **E** J.L. Clark 17312). Photos by John L. Clark.

Description. Unbranched lithophyte with well-developed woody tubers to 3.5 cm in diameter, older individuals with elongate woody shoots to 30 cm long subtending a rosette of leaves, younger individuals with short shoots (<5 cm long) with leaves in a basal rosette, apex of shoots covered with dense red pilose trichomes, base of shoots glabrescent. **Leaves** alternate, always clustered (rosette), coriaceous, petioles 0.5–1.0 cm long, velutinous, reddish; blade cuneate to broadly obovate, 5–8.5 × 1.5–2.5 cm, base attenuate, apex acute, margin sparsely serrate with 6–10 shallow lobes that become more deeply lobed near apex, margin of leaf with evenly spaced white trichomes, abaxially light green, densely pilose along main vein, especially near base, sparsely pilose along main vein near leaf apex, adaxially dark green, glabrous, lateral veins 6–9 per side. **Inflorescence** reduced to a single axillary flower, pedicel uniformly red, erect to horizontal, 3–5 cm long, in the upper axils. **Floral** tube obconic, 2–4 × 2–3 mm, uniformly red, shallowly sulcate with five ridges. **Calyx** lobes five, erect, 3–5 mm long, 2 mm wide, triangular, uniformly red. **Corolla** zygomorphic, uniformly tubular to slightly constricted apically, 2.2–2.7 cm long, 1–1.5 cm wide, mostly red with yellow lobes, limb with five erect lobes, subequal, semi-orbiculate, 1–2 × 1.5–2.5 mm, entire. **Androecium** with four stamens, 1.9–2.5 cm long, briefly adnate to the base of the corolla tube, included; anthers oblong, 1.0–2.5 × 1.0–1.5 mm; staminode present. **Gynoecium** with inferior ovary, disc annular, white; immature ovary globose, mature ovary not observed (flowers protandrous). **Fruit** a sub-woody globose bivalved capsule, 4–6 × 3–5 mm. **Seeds** fusiform, striated, twisted, 0.5–1.0 × 0.3 mm, dark brown to black.

Phenology. Mature flowers were documented during January, February, June, and August. Mature fruits were documented during January, February, June, and August.

Etymology. The specific epithet means growing a tuber and is derived from the presence of a swollen stem base (Fig. 1C), a vegetative character consistent throughout the two populations observed during a 2023 field expedition to the Dominican Republic.

Distribution and preliminary assessment of conservation status. *Gesneria tuberifera* is endemic to the Sierra de Bahoruco or Bahoruco mountain range in the Dominican Republic's Barahona Peninsula of southern Hispaniola. The Sierra de Bahoruco is in the southwestern region of the Dominican Republic, adjacent to the southern border with Haiti, and encompasses the provinces of Pedernales and Barahona. Some parts of Sierra de Bahoruco comprise a national park, Sierra de Bahoruco National Park (Parque Nacional Sierra de Bahoruco). The park is recognized by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as a Biosphere reserve. The forest is classified as humid forest (bosque húmedo) transitioning to broadleaf forest to pine (bosque latifoliado al pinar) in a riverside forest of a ravine (bosque ribereño de una cañada). Common woody plants in the ravine include *Cassia spectabilis* DC. (Fabaceae), *Comocladia* P.Browne (Anacardiaceae), *Ficus* L. sp. (Moraceae), *Mastichodendron* sp. (Engl.) H.J.Lam (Sapotaceae), *Ocotea* sp. Aubl. (Laureaceae), *Oxandra* sp. A.Rich (Annonaceae), *Pinus occidentalis* Sw. (Pinaceae), and *Sloanea* L. (Elaeocarpaceae).

There are two documented populations of *Gesneria tuberifera*, and both are south of the Sierra de Bahoruco National Park. Thus, there are no known populations of *G. tuberifera* within the Sierra de Bahoruco National Park or other

protected areas. The two populations of *G. tuberifera* are within relatively easy access to major roads. The population from the type locality (Pedernales) includes approximately 100 individuals, covering a vertical limestone outcrop. The population from Enríquillo (Barahona) was more limited, with fewer than 50 individuals. The area of occupancy (AOO) was calculated as 12 km². Following the IUCN Red List Categories and Criteria (IUCN 2012) and guidelines of the IUCN Standards and Petitions Committee (2022), *Gesneria tuberifera* is preliminarily assessed as Endangered (EN), which is supported by a restricted population of less than 250 mature individuals (D).

Comments. Most *Gesneria* taxa are multibranched terrestrial or lithophytic shrubs, 1–2 m tall. There are fewer than 10 species of *Gesneria* with a lithophytic habit with leaves in a rosette. Rosette-forming here is broadly defined to include taxa where there is a basal rosette of leaves without a developed stem (Fig. 1B, E), a growth habit typical of many herbaceous annuals. In addition, rosette-forming can also describe perennial elongate shoots that might reach a length of 30 cm where an apical rosette of leaves is produced (Fig. 2E). It is common in older individuals of lithophytic *Gesneria* taxa to develop unbranched elongate shoots that subtend rosettes of leaves. In contrast, lithophytic shrubs are often branched, reach 2 meters in height, but never form rosettes or clusters of leaves. *Gesneria tuberifera* includes individuals with basal rosettes of leaves (Fig. 1E) and individuals with unbranched elongate shoots (ca. 30 cm long) subtending a terminal rosette of leaves.

A recent phylogenetic study by Joly et al. (2018) strongly supported the presence of several clades of shrubs (terrestrial or lithophytic) with hummingbird specialist pollination syndromes, but only one clade (fig. 6 in Joly et al. 2018) correlates with obligate lithophytes with leaves in a rosette. Many of the lithophytic rosette taxa are similar to *Gesneria tuberifera*, such as *G. acaulis* L. (Fig. 2B) from Jamaica, *G. cuneifolia* (DC.) Fritsch from Puerto Rico, *G. pedicellaris* Alain (Fig. 2G) from Dominican Republic, *G. purpurascens* Urb. (Fig. 2H) from Cuba, *G. reticulata* (Griseb.) Urb. (Fig. 2C, D) from Puerto Rico and Hispaniola, and *G. yamuriensis* Britton & P.Wilson from Cuba. Other *Gesneria* lithophytes with leaves in a rosette that were not represented in the phylogeny in Joly et al. (2018) include *Gesneria christii* Urb. (Fig. 2A) from Hispaniola and *G. libanensis* Linden ex C. Morren (Fig. 2E, F) from Cuba. *Gesneria tuberifera* differs from all other lithophytes with a rosette of leaves by the presence of tubers (Fig. 1C) and tubular red corollas with yellow lobes (Fig. 1A). In contrast, most lithophytic *Gesneria* with rosette leaves have uniformly red tubular corollas (Fig. 2) without contrasting coloration on the lobes.

The presence of tubers is relatively rare in Gesneriaceae. The only large genus (65+ species) where tubers are common is *Sinningia* Nees mainly from Brazil, which includes the commonly cultivated species *Sinningia speciosa* (Lodd.) Hiern (Fig. 3E, F). Several small genera have tubers such as the monotypic genus *Lembocarpus* Leeuwenb. and *Rhoogeton* Leeuwenb. (2 species). Tubers are also present in *Pachycaulos*, a genus that was recently expanded (Clark et al. 2023) to include two species, *Pachycaulos huancabambae* J.L.Clark & Moonlight and *P. nummularia* (Hanst.) J.L.Clark & J.F.Smith (Fig. 3G, H). The tribe Sphaerorrhizeae was established by Roalson and Boggan (Roalson et al. 2005) to accommodate a small clade of four species that are partly characterized by “stringy” rhizomes with tuber-like swellings.

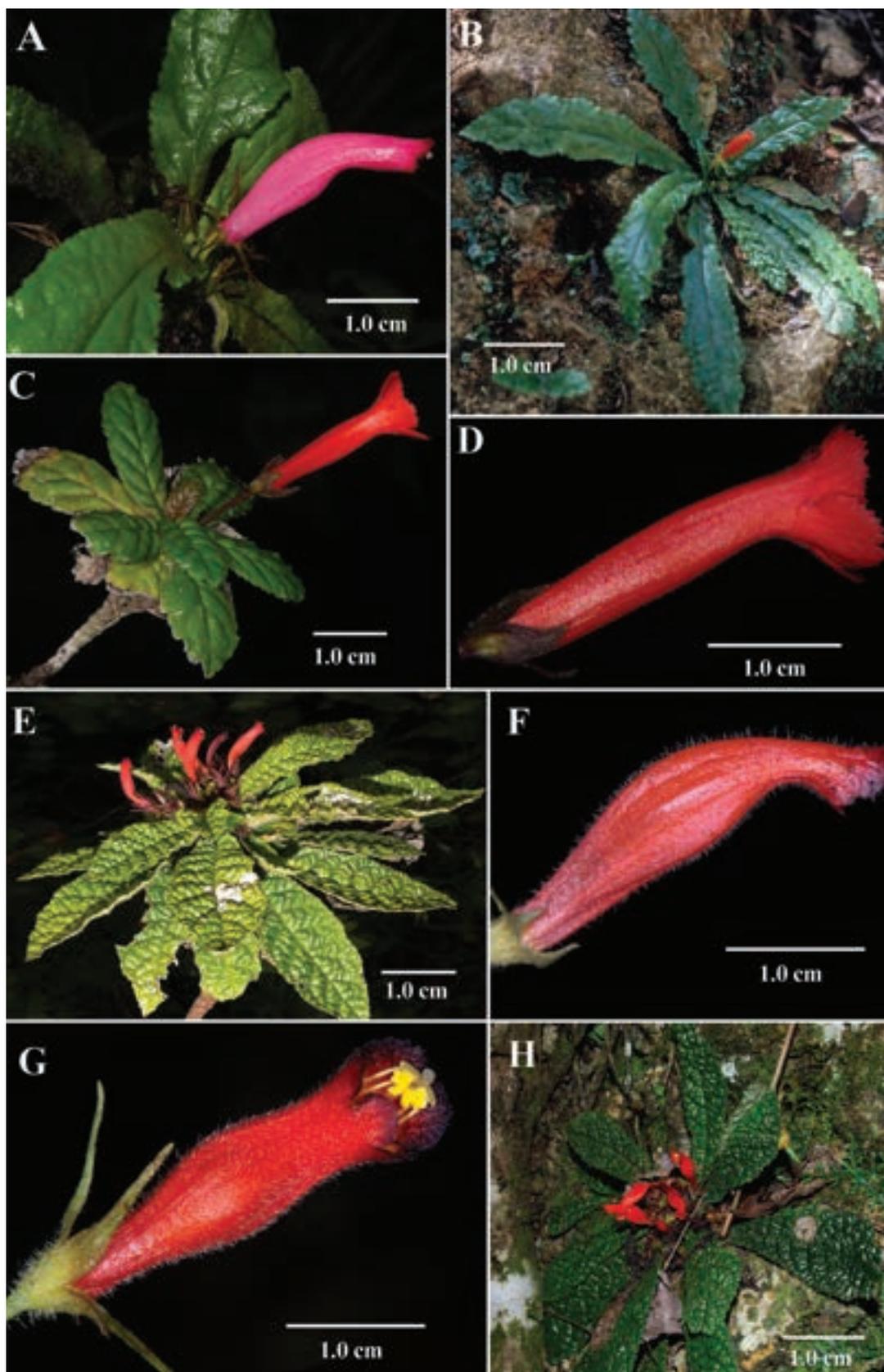


Figure 2. *Gesneria* taxa with lithophytic habits and elongate tubular red corollas **A** *Gesneria acaulis* L. **B** *Gesneria christii* Urb. **C, D** *Gesneria reticulata* (Griseb.) Urb. **E, F** *Gesneria libanensis* Linden ex C. Morren **G** *Gesneria pedicellaris* Alain **H** *Gesneria purpurascens* Urb. (**A** J.L. Clark 14532 **B** T. Talpey s.n. **C, D** J.L. Clark 17420 **E, F** J.L. Clark 15987 **G** J.L. Clark 17934 **H** J.L. Clark 12769). Photos by John L. Clark

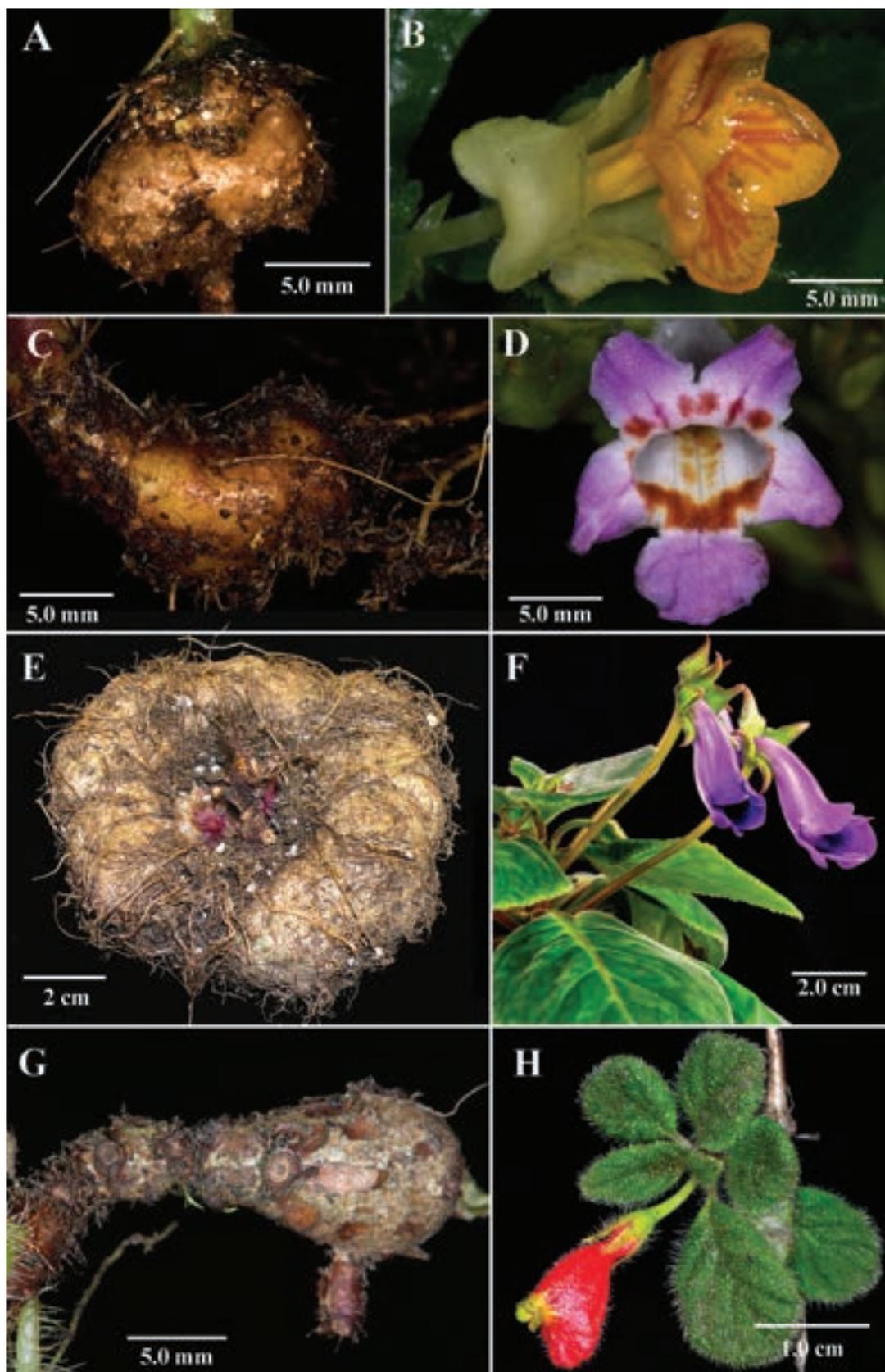


Figure 3. Gesneriaceae taxa with tubers **A, B** *Chrysothemis friedrichsthaliana* (Hanst.) H.E.Moore **C, D** *Trichodrymonia pedunculata* (L.E.Skog) M.M.Mora & J.L.Clark **E, F** *Sinningia speciosa* (Lodd.) Hiern 'Niemeyer' **G, H** *Pachycaulos nummularia* (Hanst.) J.L.Clark & J.F.Smith (**A, B** J.L. Clark 12699 **C, D** J.L. Clark 12680 **E, F** D. Zaitlin s.n. **G, H** J.L. Clark 16357). Photos **A, B, C** and **D** by John L. Clark and photos **E, F** by David Zaitlin.

Other examples of tubers in Gesneriaceae are limited to single taxa within genera that are not usually tuberous. For example, *Chrysothemis friedrichsthaliana* (Hanst.) H.E. Moore forms tubers (Fig. 2A, B), but their presence is inconsistent. Even when actively looking for tubers in wild populations of *Chrysothemis friedrichsthaliana*, their presence is sometimes completely absent or present in only a few individuals. Tubers are mostly absent in *Trichodrymonia*, but one exception is *Trichodrymonia pedunculata* (L.E.Skog) M.M.Mora & J.L.Clark where tubers are consistently present in cultivation and in the wild (Fig. 3C, D) in eastern Panama. The presence of tubers in *Gesneria tuberculata* is the first documented example of this unusual character in the genus and it represents the only member of Gesneriaceae with tubers from the Caribbean.

The presence of tubers is mostly unknown in the Old World members of Gesneriaceae. The recently described monotypic genus, whose single species, *Michaelmoelleria vietnamensis* F.Wen, Z.B.Xin & T.V.Do, was not reported to have tubers when it was described from field collections (Wen et al. 2020), but horticulturists have noticed tubers in cultivation (D. Zaitlin, pers. comm.). Weber (2004) provides a comprehensive survey on the range of morphological features documented throughout Gesneriaceae, and reports that tubers are confined to the New World Gesneriaceae. Thus, the presence of tubers in *Michaelmoelleria* F.Wen, Y.G.Wei & T.V.Do is the first and only known species of Old World Gesneriaceae with tubers.

Additional specimens examined. DOMINICAN REPUBLIC. Barahona: Sierra de Bahoruco, road from the coastal town of Enriquillo towards the community Blanco, area known locally as El Fondo Farallon, adjacent to Río Fondo, 17°57'39.04"N, 71°13'50.16"W, 407 m, 28 Jan 2023, J.L. Clark et al. 17312 (FLAS, JBSD, MO, MT, NY, SEL, US); Sierra de Bahoruco, Municipio Enriquillo, seccion Los Blancos, paraje El Fondo, subiendo hacia loma Materesa, 600 m, 11 Jun 2009, B. Peguero et al. 4717 (JBSD). **Pedernales:** Sierra de Bahoruco, road Cabo Rojo–Las Mercedes, Finca de Isabel, 18°06'50.09"N, 71°37'10.14"W, 738 m, 26 Jan 2023, J.L. Clark & T. Clase 17284 (FLAS, JBSD, MO, MT, NY, SEL, US); Sierra de Bahoruco, sección Las Mercedes, Aceitillar, subiendo en la carretera Cabo Rojo, 18°6'8.04"N, 71°37'14.3"W, 400–500 m, 10 Jun 2007, T. Clase et al. 4526 (JBSD); Sierra de Bahoruco, km 26 Norte desde el Puerto de Cabo Rojo (de la Alcoa Exploration Company) en el camino minero a Las Mercedes y Aceitillar, 18°06'N, 71°36'W, 610 m, 16 Feb 1982, T. Zanoni, M. Mejía, J. Pimentel & J.T. Mickel 19068 (JBSD, NY); Sierra de Bahoruco, entre los kms. 25–28 de la Carretera Puerto de Cabo Rojo hacia Aceitillar, 18°06'N, 71°37'W, 820 m, 1 Aug 1984, M. Mejía, J. Pimentel & R. García 1076 (JBSD).

Acknowledgments

We thank the Ministerio del Medio Ambiente (Dominican Republic) for permitting fieldwork. We are grateful to the following colleagues for logistical help during our 2023 expedition to Sierra de Bahoruco: Lucas Majure, Yommi Piña, Elizabeth Septimo, and Dichoso Felix. We thank Laurence E. Skog, David Zaitlin, and two anonymous reviewers for their feedback on an earlier version of the manuscript. Jeanne Katzenstein is acknowledged for her ongoing support of Gesneriaceae research at Marie Selby Botanical Gardens, including funding

our 2023 field expedition to the Dominican Republic. We are grateful to David Zaitlin for sharing his knowledge and images of *Sinningia speciosa* (Fig. 3E, F) and insights on the formation of tubers in *Michaelmoelleria*. We thank Alain Chautems, Larry E. Skog, and an anonymous reviewer for providing helpful feedback on an earlier version of the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

John Clark wrote the manuscript. Teodoro Clase and John Clark conducted a 2023 field expedition. Teodoro Clase provided essential feedback and edits to the manuscript.

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Data availability

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

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Pollen morphology of *Clerodendrum* L. (Lamiaceae) from China and its systematic implications

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Abstract

Pollen morphology of 26 taxa of *Clerodendrum*, as well as one species of *Volkameria* from China, was investigated through a scanning electron microscope (SEM). Pollen grains of *Clerodendrum* are monads, radiosymmetric and tricolpate, with medium or large size. The equatorial view of the pollen grains is spheroidal or subprolate and the polar view is (sub) circular or rounded triangular. The colpus membrane of the investigated taxa is sunken (rarely even). Five varying pollen types are delimited on the basis of exine sculpturing: (1) spine-tectum perforatum; (2) spine-tectum imperforatum; (3) microspine-tectum perforatum; (4) microspine-tectum imperforatum; and (5) obtuser spine. The results indicate that *Clerodendrum* is closely related to several genera in Lamiaceae, including *Aegiphila*, *Amasonia*, *Kalaharia*, *Tetraclea*, *Volkameria*, *Oxera*, *Faradaya*, and *Hosea*, as supported by previous phylogenetic studies. Additionally, the conventional infrageneric classification of *Clerodendrum* based on inflorescence and leaf characters is not supported by the results. However, the palynological data can be used to identify some closely related species with similar external characteristics. In conclusion, the investigation of pollen morphology not only contributes novel data from palynology for *Clerodendrum* but also provides a basis for future comprehensive classification of this genus.

Key words: *Clerodendrum*, pollen morphology, SEM, taxonomy



Academic editor: Chun-Lei Xiang
Received: 23 August 2023
Accepted: 28 October 2023
Published: 10 November 2023

Citation: Huang X, Wu R, Xiong Z, Ma Z (2023) Pollen morphology of *Clerodendrum* L. (Lamiaceae) from China and its systematic implications. *PhytoKeys* 235: 53–68. <https://doi.org/10.3897/phytokeys.235.111516>

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Introduction

The genus *Clerodendrum* L. (Lamiaceae), comprising approximately 400 species, is mainly distributed in tropical and subtropical Asia, Africa, and America (Li et al. 2016). It belongs to the subfamily Ajugoideae (Harley et al. 2004), a monophyletic group divided into four main clades recognized as tribes: Ajugeae, Rotheceae, Teucricae, and Clerodendreae. *Clerodendrum*, along with *Volkameria* L., *Kalaharia* Baill., *Amasonia* L.f., *Tetraclea* A. Gray, *Hosea* Ridl., *Aegiphila* Jacq., *Ovieda* L., and *Oxera* Labill., are members of Clerodendreae (Xiang et al. 2018; Zhao et al. 2021). However, generic relationships within Clerodendreae require further study (Zhao et al. 2021).

Previous infrageneric classification of *Clerodendrum* have been proposed using morphological characters. Based on inflorescence structure, Briquet (1895) divided the genus into three subgenera, whereas Thomas (1936) put forward a

different classification relying on calyx structure. Moldenke (1985) merged the work of Thomas and Briquet, and sequentially raised a new classification with two large subgenera: *Tridens* Roem. & Schult. and *Volkameria*. Both Cantino (1992) and Rimpler et al. (1992) suggested that *Clerodendrum* may be paraphyletic or polyphyletic, but infrageneric relationships of the genus proposed by them were different. Based on molecular and morphological phylogenetic analyses, *Ovieda*, *Rothea* Raf., and *Volkameria* were resurrected from *Clerodendrum* (Steane et al. 1997, 1999, 2004; Yuan et al. 2010).

In total, 34 species and 7 varieties of *Clerodendrum* are recorded from China and represented (Chen and Gilbert 1994). The most recent taxonomic treatment (Pei and Chen 1982) split the Chinese species into two sections based on corolla tube length and leaves' arrangement. Section *Siphonanthus* Schauer comprised only one taxon that was characterized by corolla tube 5 cm long or longer, and leaves in whorls of 3–5, while section *Clerodendrum* included remaining species characterized by corolla tube less than 5 cm long, and opposite leaves or in whorls of three. However, throughout the field investigation and specimen examination, we have found that those quantitative characters are quite variable and can easily lead to misidentification. There is an urgent need to explore more morphological characters to build a more predictable classification system.

Palynological characters were considered as important characters for the taxonomy of Lamiaceae (Erdtman 1952; Abu-Asab and Cantino 1989, 1994; Abu-Asab 1991; Harley et al. 1992; Trudel and Morton 1992; Abu-Asab and Cantino 1994; Large and Mabberley 1995; Moon et al. 2008a, 2008b; Özler et al. 2011; Badamtsetseg et al. 2012; Ma et al. 2016). However, pollen morphology of the genus *Clerodendrum* is poorly known. Raj (1983) analyzed pollen morphology of 10 species of *Clerodendrum* using light microscope (LM), and only *C. bungei* Steud. was observed by scanning electron microscope (SEM). Perveen and Qaiser (2007) described the pollen structure of *C. phlomidis* L.f. in the palynology study of Verbenaceae in Pakistan. Liu (1985), in China, inspected the pollen morphology of 17 species and one variety of *Clerodendrum* comprising by light microscope, and only *C. cyrtophyllum* Turcz. was observed by SEM. Summarized from the studies mentioned above, it indicates that palynological characters such as variation in size, shape, and exine sculpturing are taxonomically valuable in the identification of closely related species in *Clerodendrum*.

In this study, we investigate the pollen morphology of 25 species and one variety of *Clerodendrum*, one species of *Volkameria* L. (*V. inermis* L.) which was traditionally placed within *Clerodendrum*. The objectives of this study are: (1) to provide extra palynological data to establish a more precise infrageneric classification for the genus; (2) further contribute to a comprehensive systematic study for *Clerodendrum* and clarify its relationship with other taxa of subfamily Ajugoideae.

Materials and methods

Pollen grains of 26 taxa (Chen and Gilbert 1994) of *Clerodendrum* and one species *Volkameria* were sampled. Pollen material was mainly collected from herbarium specimens deposited at herbaria IBSC and KUN, and the rest were collected in the field (see Table 1).

Table 1. Specimens investigated.

Taxa	Collection localities	Collecting date	Collector	Number	Herbarium
<i>C. brachystemon</i> C.Y.Wu & R.C.Fang	Xizang, Motuo, 793 m	2019.8.24	Z. Xie	693	GAUA
<i>C. bracteatum</i> Wall. ex Walp.	Yunnan, Gongshan, 1889 m	1982.8.5	Gongshan Team	8889	KUN
<i>C. bungei</i> Steud.	Guangxi, Jinxiu, 820 m	1981.11.21	Dayaoshan Team	13341	IBSC
<i>C. chinense</i> var. <i>simplex</i> (Moldenke) S. L. Chen	Guangxi, Baise, 646 m	2022.6.2	Z. Xiong et al.	JX001	GAUA
<i>C. colebrookianum</i> Walp.	Xizang, Motuo, 848 m	2019.8.24	Z. Xie	GXU0020	GAUA
<i>C. cyrtophyllum</i> Turcz.	Guangxi, Guigang, 110 m	2019.7.10	Z. H. Ma et al.	GXU0016	GAUA
<i>C. fortunatum</i> L.	Guangdong, Huizhou, 160 m	2013.9.07	H. G. Ye et al.	21973	IBSC
<i>C. garrettianum</i> Craib	Yunnan, 900 m	1936.9	C. W. Wong	78761	IBSC
<i>C. griffithianum</i> C.B.Clarke	Yunnan, Yingjiang, 830 m	1981.2.24	S. W. Yu, Q. T. Zhang	602	KUN
<i>C. hainanense</i> Hand.-Mazz.	Hannan, Lingshui, 600 m	1956.10.30	L. Tang	2878	IBSC
<i>C. henryi</i> Pei	Guangdong, Guangzhou, 14 m	2021.5.23	R. Wu	GZ002	GAUA
<i>C. indicum</i> (L.) Kuntze.	Yunnan, Mengla, 580 m	2002.11.24	S. S. Zhou	570	IBSC
<i>C. japonicum</i> (Thunb.) Sweet	Guangdong, Guangzhou, 14 m	2021.5.23	R. Wu	GZ001	GAUA
<i>C. kaichianum</i> Hsu	Henan, Neixiang	2005.8.5	C. S. Zhu	2005095	IBSC
<i>C. kwangtungense</i> Hand.-Mazz.	Guangxi, Rongshui	1958.9.3	S. Q. Chen	16653	IBSC
<i>C. lindleyi</i> Decne. ex Planch.	Guangxi, Baise, 321 m	2019.10.12	Q. B. Zeng, Z. Xie	763	GAUA
<i>C. longilimbium</i> Pei	Yunnan Lingcang, 1500 m	1958.9.27	T. P. Zhu	0011	IBSC
<i>C. mandarinorum</i> Diels	Guangdong, Huaiji	2000.9	W. M. Yi, Z. F. Huang	15985	IBSC
<i>C. paniculatum</i> L.	Taiwan	1988.11.27	S. Z. Yang	11431	IBSC
<i>C. speciosum</i> Dombroin	Yunnan, Mengla, 570 m	2004.1.1	H. Wang.	7444	IBSC
<i>C. splendens</i> G. Don	Guangxi, Nanning, 79 m	2021.11.29	Z. Xiong	NN008	GAUA
<i>C. sylvestre</i> Moldenke	Guangxi, Guilin	1950.6.13	Z. S. Chung	808315	IBSC
<i>C. trichotomum</i> Thunb.	Hubei, Badong	1957.7.15	G. X. Fu	740	IBSC
<i>C. trichotomum</i> var. <i>fargesii</i> (Dode) Rehder	Sichuan, Leibo, 1600 m	1989.8.13	Q. S. Zhao	517	IBSC
<i>C. villosum</i> Blume	Yunnan, Yingjiang	1986.11.18	86 Team	01033	KUN
<i>C. wallichii</i> Merr.	Yunnan, Mengla, 570 m	2004.3.22	H. wang	6427	IBSC
<i>V. inermis</i> L.	Guangdong, Lianjiang, -1.2 m	2019.10.1	Z. H. Ma et al.	750	GAUA

Flowers were dipped in glacial acetic acid (Reitsma 1969), and pollen was released using tweezers. Due to the friable nature of *Clerodendrum* pollen, the pollen samples for SEM were acetolysed for 10 min at indoor temperature that were adjusted from the method of Erdtman (1969). After acetolysis, pollen grains were progressively dehydrated in ethanol solutions of different concentrations (30%, 50%, and 70%) which were then bathed ultrasonically. Thereafter, the dehydrated pollen grains were dropped to a copper platform with double-side adhesive tape, air-dried and coated with gold. Pollen grains were observed and photographed using an electron microscope (Hitachi-S3400) at 10 kV. The measurements were based on randomly selected 20 pollen grains from each specimen using SEM, including the equatorial diameter (E), axis diameter (P) and the respective maximum, minimum and mean values.

The terminology used was according to Punt et al. (2007). Shape classes (P/E) were in accordance with Erdtman et al. (1993). Pollen size classes were plotted following Hesse et al. (2009). The pollen morphology of *Clerodendrum* is primarily described by qualitative and quantitative characteristics including shape, aperture type, colpus features and sexine ornamentation.

Results

Palynological characteristics of all the investigated samples are given in Table 2 and illustrated in Figs 1–5.

Table 2. Pollen morphology of *Clerodendrum*.

Taxa	P value(μm)	E value(μm)	P/E	Size	Shape	Amb	Aperture type	Colpus membrane	Exine type	Figures
<i>C. brachystemon</i>	47.98 (44.44–52.93)	42.27 (37.40–46.12)	1.14	Medium	Subprolate	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	3A–C
<i>C. bracteatum</i>	45.74 (40.95–52.14)	41.08 (36.52–43.93)	1.11	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	2M–O
<i>C. bungei</i>	48.92 (42.70–54.18)	36.20 (29.85–42.04)	1.35	Medium	Subprolate	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	2G–I
<i>C. chinense</i>	51.59 (44.50–59.02)	40.76 (37.23–44.57)	1.27	Large	Subprolate	Subcircular	Tricolpate	Sunken	spine-tectum imperforatum	1A–C
<i>C. colebrookianum</i>	47.78 (42.03–53.44)	44.27 (40.72–49.44)	1.08	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum imperforatum	1P–R
<i>C. cyrtophyllum</i>	42.27 (38.35–45.75)	40.65 (37.57–43.41)	1.04	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	microspine-tectum perforatum	4M–O
<i>C. fortunatum</i>	47.37 (40.46–53.29)	36.16 (31.56–40.61)	1.31	Medium	Subprolate	Subcircular	Tricolpate	Sunken	microspine-tectum perforatum	4A–C
<i>C. garrettianum</i>	57.83 (49.63–65.89)	41.93 (36.11–46.69)	1.38	Large	Subprolate	Rounded triangular	Tricolpate	Sunken	microspine-tectum perforatum	4D–F
<i>C. griffithianum</i>	56.08 (49.86–64.19)	41.03 (36.42–44.96)	1.37	Large	Subprolate	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	2P–R
<i>C. hainanense</i>	49.43 (43.11–56.79)	44.13 (35.78–49.33)	1.12	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	2J–L
<i>C. henryi</i>	46.54 (41.22–52.40)	40.10 (33.45–46.58)	1.16	Medium	Spheroidal	Circular	Tricolpate	Sunken	microspine-tectum imperforatum	3J–L
<i>C. indicum</i>	70.05 (45.45–83.76)	50.93 (38.68–59.63)	1.38	Large	Subprolate	Subcircular	Tricolpate	Sunken	obtuser spine	5M–O
<i>C. japonicum</i>	56.59 (52.75–63.39)	53.59 (49.43–58.10)	1.06	Large	Spheroidal	Circular	Tricolpate	Sunken	microspine-tectum perforatum	4G–I
<i>C. kaichianum</i>	49.21 (43.03–54.29)	37.05 (31.89–42.65)	1.33	Medium	Subprolate	Circular	Tricolpate	Sunken	microspine-tectum perforatum	4J–L
<i>C. kwangtungense</i>	48.20 (45.40–51.57)	42.80 (36.95–45.69)	1.13	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	3D–F
<i>C. lindleyi</i>	46.68 (39.81–53.88)	36.49 (32.23–39.91)	1.28	Medium	Subprolate	Rounded triangular	Tricolpate	Sunken	spine-tectum imperforatum	1M–O
<i>C. longilimbum</i>	53.75 (48.57–61.53)	42.66 (35.05–47.82)	1.26	Large	Subprolate	Rounded triangular	Tricolpate	Sunken	microspine-tectum perforatum	5A–C
<i>C. mandarinorum</i>	41.95 (39.77–45.27)	39.96 (37.51–42.30)	1.05	Medium	Spheroidal	Circular	Tricolpate	Sunken	spine-tectum imperforatum	1J–K
<i>C. paniculatum</i>	49.08 (44.87–56.81)	45.44 (38.50–50.13)	1.08	Medium	Spheroidal	Circular	Tricolpate	Sunken	microspine-tectum imperforatum	3M–O
<i>C. speciosum</i>	47.68 (44.18–50.53)	45.20 (41.77–50.77)	1.05	Medium	Spheroidal	Circular	Tricolpate	Sunken	microspine-tectum perforatum	5D–F
<i>C. splendens</i>	45.46 (38.36–51.00)	41.63 (38.95–46.02)	1.09	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	microspine-tectum perforatum	5G–I
<i>C. sylvestre</i>	44.87 (42.39–49.00)	42.60 (38.17–45.67)	1.05	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	3G–I
<i>C. trichotomum</i>	42.81 (38.50–47.65)	40.89 (38.25–43.78)	1.05	Medium	Spheroidal	Subcircular	Tricolpate	Sunken	spine-tectum imperforatum	1G–I
<i>C. trichotomum</i> var. <i>fargesii</i>	46.71 (43.60–49.45)	43.35 (40.45–44.92)	1.08	Medium	Spheroidal	Rounded triangular	Tricolpate	Sunken	spine-tectum perforatum	2D–F
<i>C. villosum</i>	44.95 (38.62–52.91)	38.24 (33.53–42.20)	1.18	Medium	Subprolate	Subcircular	Tricolpate	Sunken	spine-tectum imperforatum	1D–F
<i>C. wallichii</i>	51.26 (44.92–60.28)	39.62 (30.71–43.80)	1.29	Large	Subprolate	Rounded triangular	Tricolpate	Sunken	microspine-tectum perforatum	5J–L
<i>V. inermis</i>	55.31 (44.03–62.04)	42.02 (36.15–45.43)	1.32	Large	Subprolate	Rounded triangular	Tricolpate	Sunken	spine-tectum imperforatum	2A–C

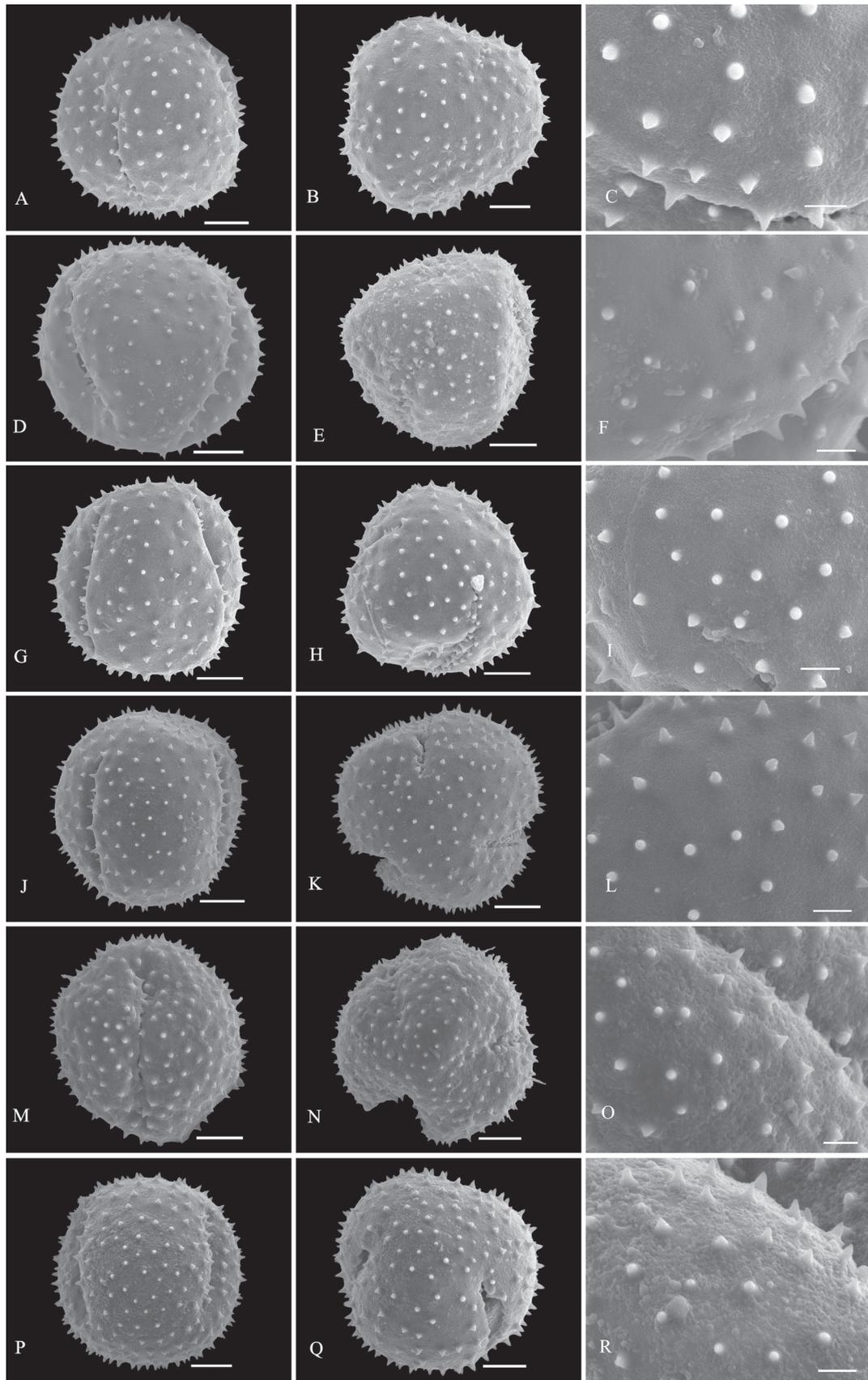


Figure 1. SEM micrographs of pollen grains of *Clerodendrum* **A–C** *C. chinense* **D–F** *C. villosum* **G–I** *C. trichotomum* **J–L** *C. mandarinorum* **M–O** *C. lindleyi* **P–R** *C. colebrookianum*. Equatorial view: **A, D, G, J, M, P**; polar view: **B, E, H, K, N, Q**; exine ornamentation: **C, F, I, L, O, R**. Scale bars: 10 μm (**A, B, D, E, G, H, J, K, M, N, P, Q**); 3 μm (**C, F, I, L, O, R**).

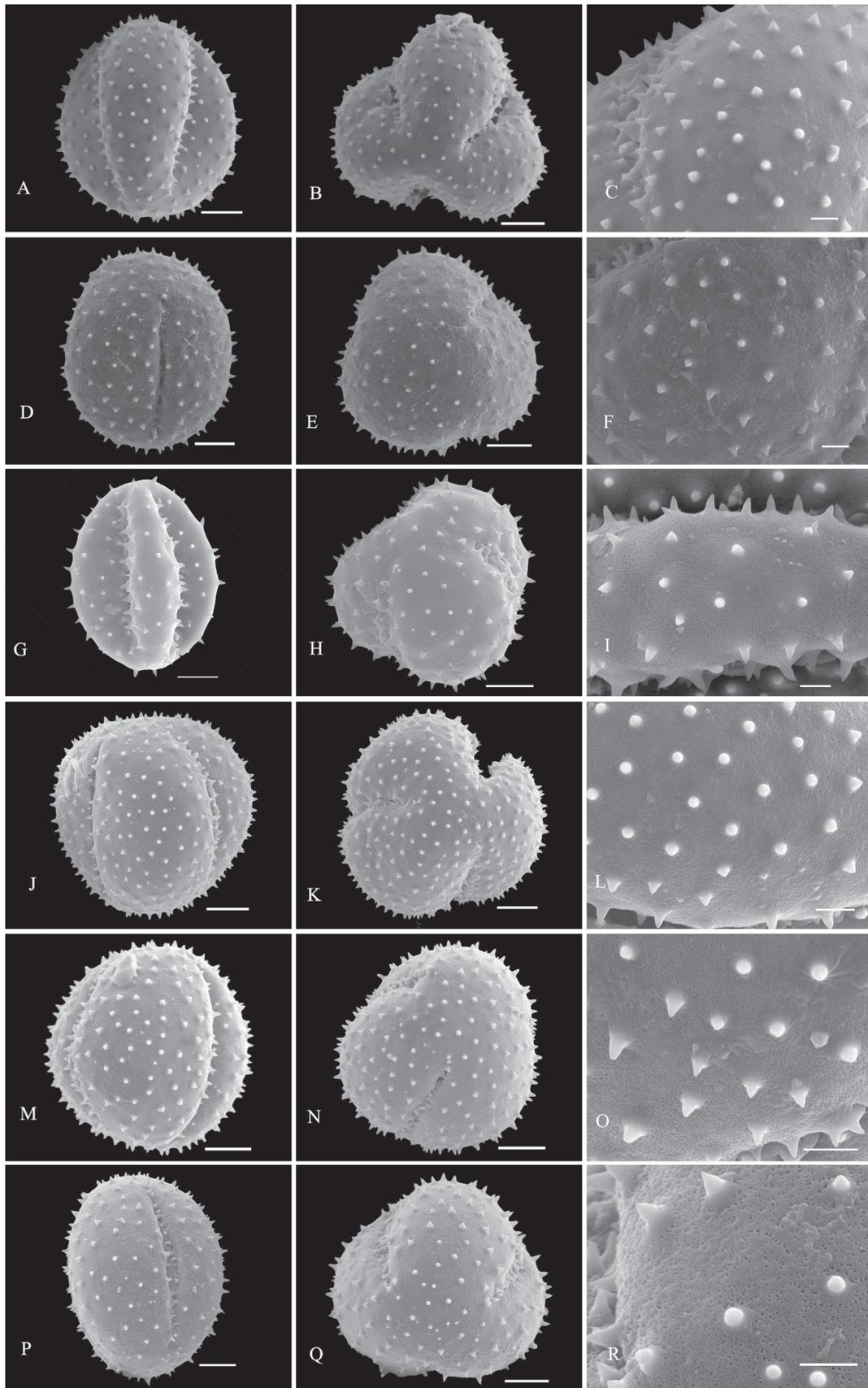


Figure 2. SEM micrographs of pollen grains of *Clerodendrum* **A–C** *Volkameria inermis* **D–F** *C. trichotomum* var. *fargesii* **G–I** *C. bungei* **J–L** *C. hainanense* **M–O** *C. bracteatum* **P–R** *C. griffithianum*. Equatorial view: **A, D, G, J, M, P**; polar view: **B, E, H, K, N, Q**; exine ornamentation: **C, F, I, L, O, R**. Scale bars: 10 μm (**A, B, D, E, G, H, J, K, M, N, P, Q**); 3 μm (**C, F, I, L, O, R**).

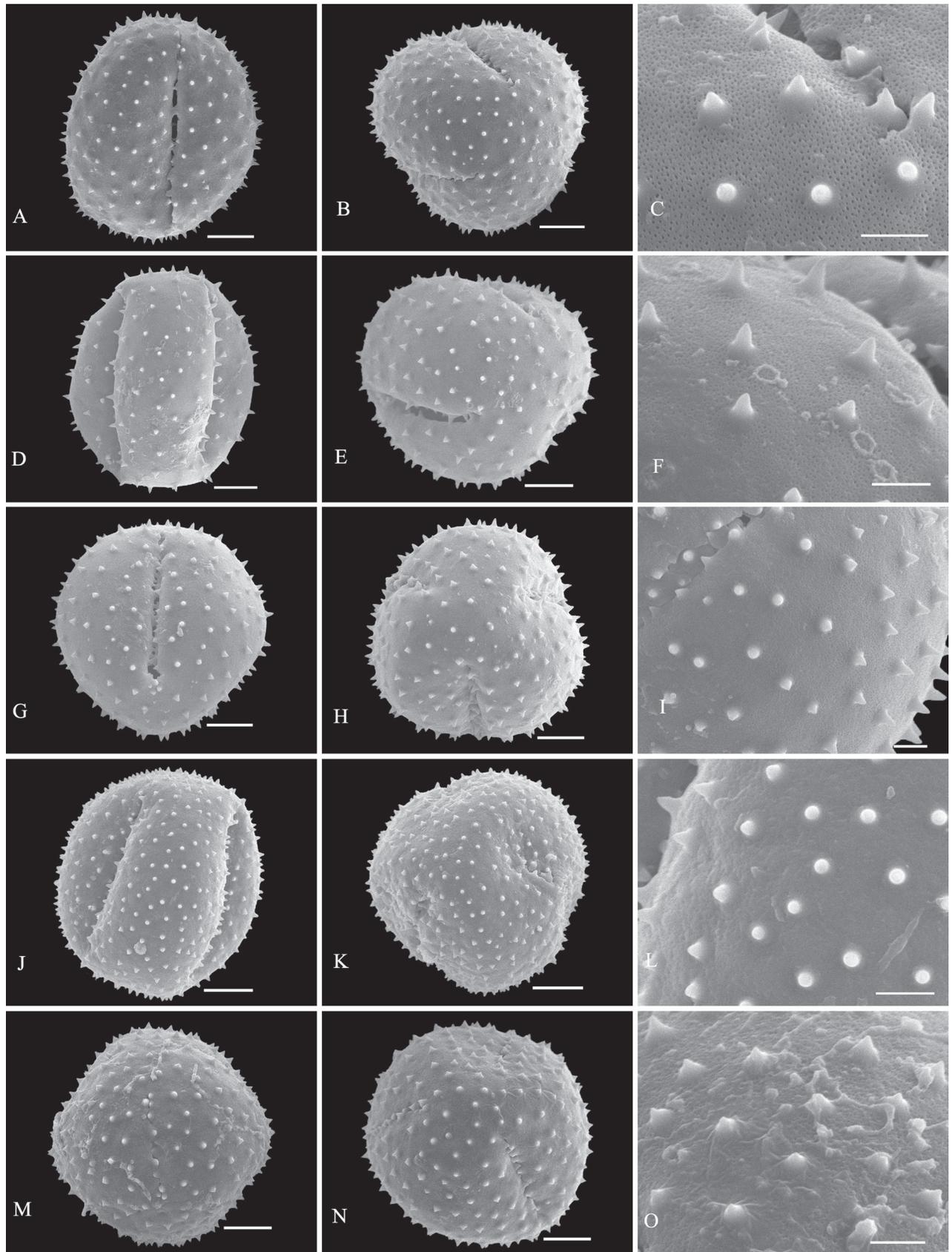


Figure 3. SEM micrographs of pollen grains of *Clerodendrum* **A–C** *C. brachystemon* **D–F** *C. kwangtungense* **G–I** *C. sylvestre* **J–L** *C. henryi* **M–O** *C. paniculatum*. Equatorial view: **A, D, G, J, M**; polar view: **B, E, H, K, N**; exine ornamentation: **C, F, I, L, O**. Scale bars: 10 μm (**A, B, D, E, G, H, J, K, M, N**); 3 μm (**C, F, I, L, O**).

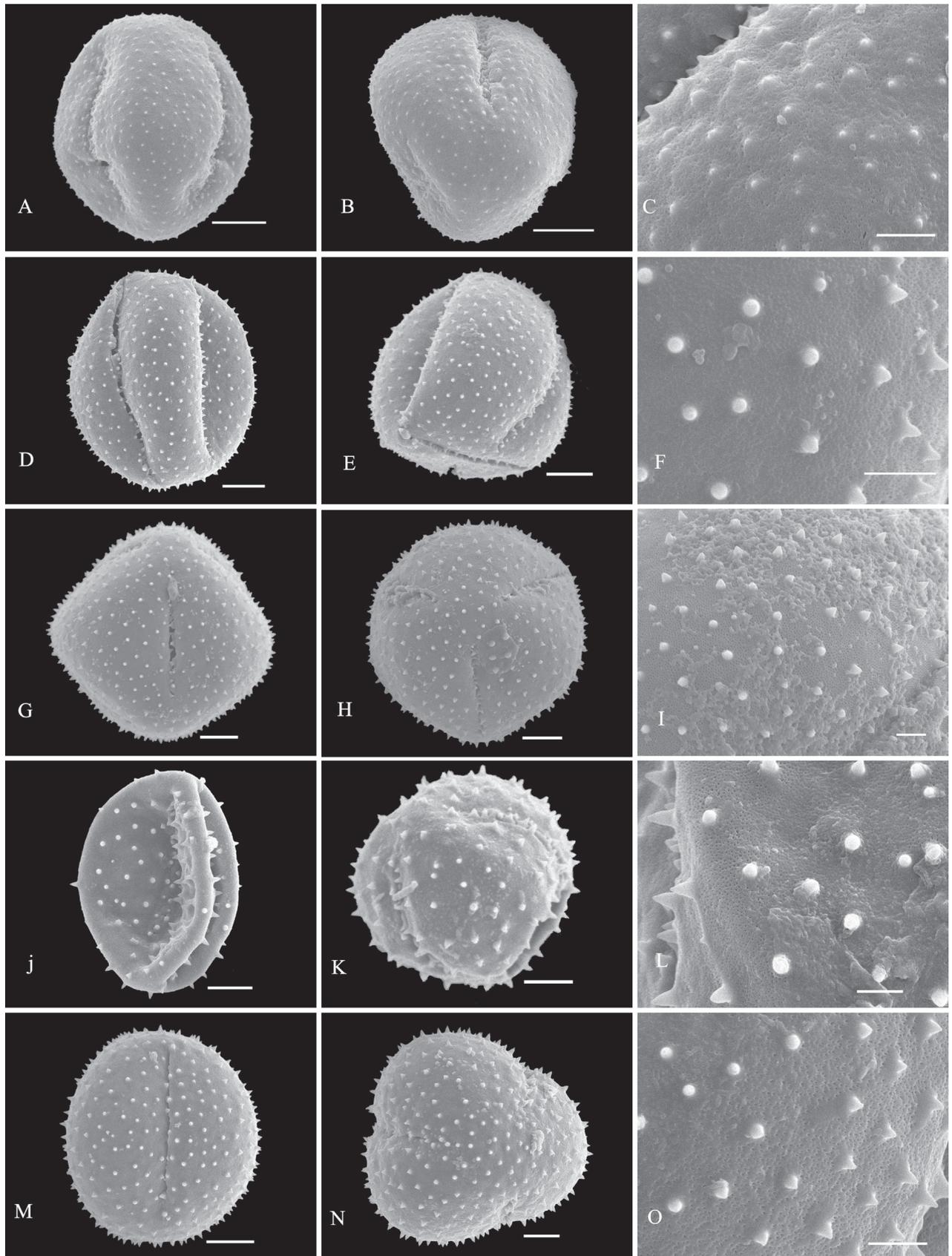


Figure 4. SEM micrographs of pollen grains of *Clerodendrum* **A–C** *C. fortunatum* **D–F** *C. garrettianum* **G–I** *C. japonicum* **J–L** *C. kaichianum* **M–O** *C. cyrtophyllum*. Equatorial view: **A, D, G, J, M**; polar view: **B, E, H, K, N**; exine ornamentation: **C, F, I, L, O**. Scale bars: 10 μm (**A, B, D, E, G, H, J, K, M, N**); 3 μm (**C, F, I, L, O**).

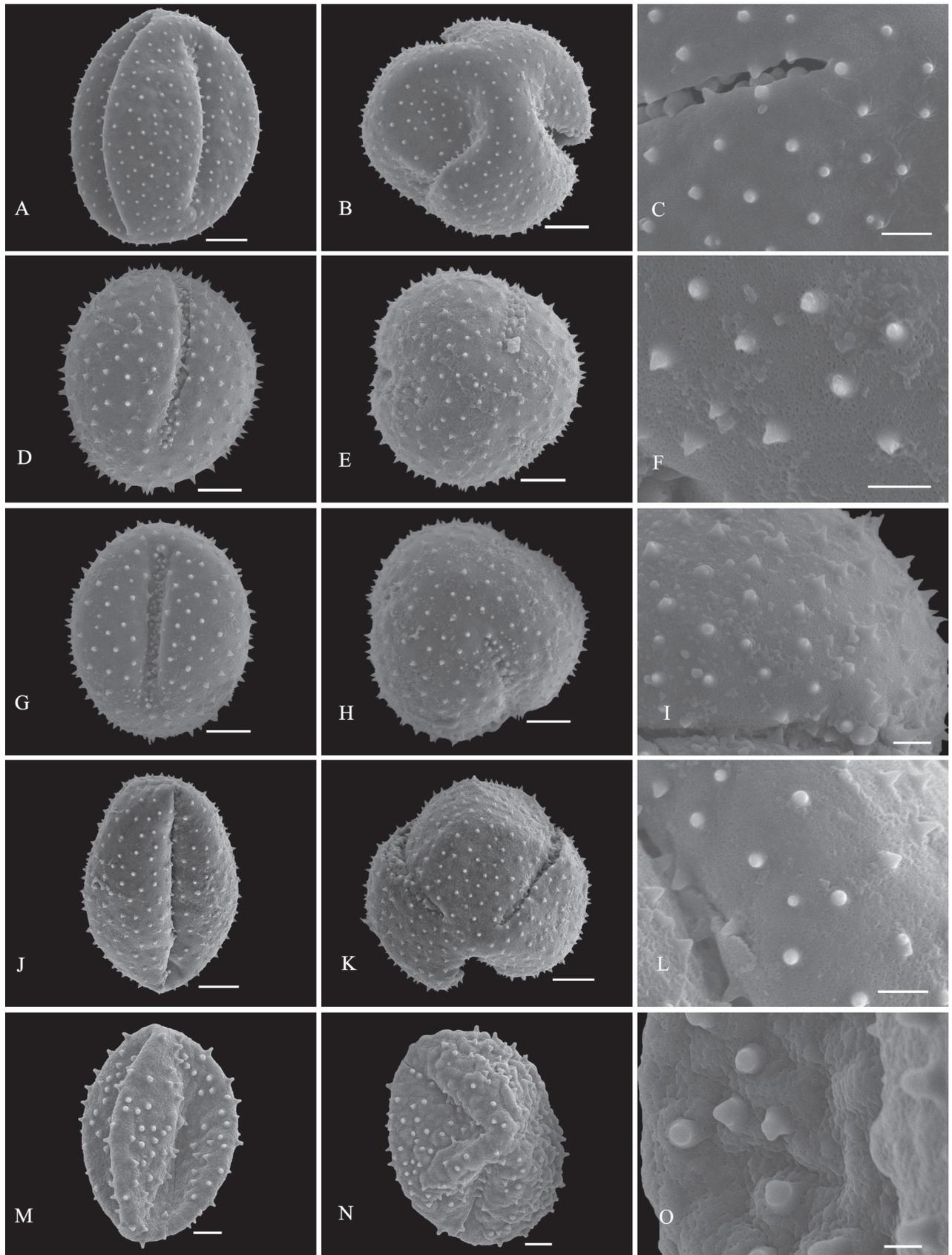


Figure 5. SEM micrographs of pollen grains of *Clerodendrum* **A–C** *C. longilimbum* **D–F** *C. speciosum* **G–I** *C. splendens* **J–L** *C. wallichii* **M–O** *C. indicum*. Equatorial view: **A, D, G, J, M**; polar view: **B, E, H, K, N**; exine ornamentation: **C, F, I, L, O**. Scale bars: 10 μm (**A, B, D, E, G, H, J, K, M, N**); 3 μm (**C, F, I, L, O**).

Pollen grains of *Clerodendrum* (including *Volkameria inermis*) are radiosymmetric, tricolpate, and monads. The size of pollen grains is medium (26–50 µm) or large (50–100 µm). The average value of the polar axis (P) is measured as 49.48 µm (41.95–70.05 µm) and that of equatorial diameter (E) is 41.89 µm (36.16–53.59 µm). The P/E ratio varies from 1.04 (*C. cyrtophyllum*) to 1.38 (*C. garrettianum* Craib). The pollen shape class is mainly spheroidal (0.88–1.14) or subprolate (1.14–1.33).

Exine sculpture can be divided into five different types: (1) spine-tectum perforatum, (2) spine-tectum imperforatum, (3) microspine-tectum perforatum, (4) microspine-tectum imperforatum, and (5) obtuser spine. The grains of type (1) are the largest groups, accounting for over a quarter of all the investigated species (Figs 2D–R, 3A–I). The type (2) with a rounded tectal perforation, less than 1 µm in diameter, can be distinguished from the spine-tectum perforatum (Figs 1A–R, 2A–C). Compared with type (1) and type (2), the type (3) (Figs 4A–O, 5A–L) and the type (4) (Fig. 3J–O) have more spines and are less than 1 µm in length. The type (5) is only found in one taxon (*C. indicum* (L.) Kunze.) (Fig. 5M–O), which happens to be the only species of sect. *Siphonanthus*.

Discussion

Taxonomic implications of pollen morphology of *Clerodendrum*

Most pollen grains of *Clerodendrum* species investigated here are spheroidal or subprolate in equatorial view. Subprolate or prolate pollen grains observed in some species (*C. bungei*, *C. intermedium* Chamisso, *C. phlomidis*) and reported in previous studies (Raj 1983; Perveen and Qaiser 2007) were probably affected by the acetolysis treatment, as the pollen shape of Lamiaceae is easily affected during hydration and/or fixation (Sebsebe and Harley 1992). On account of the broken colpus membranes, oblate or suboblate pollen grains are easily incorrectly considered as subprolate or prolate (Harley 1992). Raj (1983) described pollen of *C. speciosissimum* C. Morren as distinct with three to four to six apertures, which was the first report of non-triaperturate pollen in *Clerodendrum* (without illustration). In our study, in contrast, only triaperturate pollens were observed from *Clerodendrum* (Figs 1–5). Based on the numbers of apertures and the characters of exine sculpture, Liu (1985) considered that pollen grains of most species of *Clerodendrum* (excepting *C. fortunatum* L., *C. paniculatum* L., *C. trichotomum* Thunb., and *C. yunnanense* Hu ex Hand.-Mazz.) are tricolpate-spiny, characterized by large or very large size, tricolpate, dense or sparse spine over the microreticulate exine ornamentation. Overall, our results are mostly consistent with the finding of Liu (1985). However, *C. fortunatum*, *C. paniculatum*, *C. trichotomum* and *C. yunnanense* pantocolpate pollen grains are as described by Liu (1985). This type is characterized by spheroidal grains of large size, with pantocolpate (6 to 8) and spiny exine. In contrast, all pollens grains of *Clerodendrum* (including *C. fortunatum*, *C. paniculatum* and *C. trichotomum*) observed in this study are tricolpate with dense or sparse spine in the exine (Figs 1–5).

The infrageneric classification system applied in Flora of China (Chen and Gilbert 1994) is not fully supported by our results. In comparison to the pollen shape and size, the exine sculpture appears to be a more taxonomically

valuable and steady character. There is a clear distinction in pollen exine sculpture between sect. *Siphonanthus* and sect. *Clerodendrum*. The obtuser spine exine sculpture is only found in *C. indicum* (Fig. 5M–O), the sole species of sect. *Siphonanthus*. However, consistency between morphology and exine sculpture is hardly found in taxa belonging to sect. *Clerodendrum*. For example, species in Ser. *Axilliflorae* Schauer have different exine sculpture types: *C. griffithianum* C. B. Clarke is spine-tectum perforatum (Fig. 2P–R); *C. fortunatum* is microspine-tectum perforatum (Fig. 4A–C) and *Volkameria inermis* (= *C. inerme* (L.) Gaertn.) belongs to the spine-tectum imperforatum type (Fig. 2A–C). A similar phenomenon is observed in Ser. *Densiflora* Schauer: *C. bracteatum* Walp. ex Walp. and *C. bungei* belong to spine-tectum perforatum type, while *C. lindleyi* Decne. ex Planch. and *C. chinense* var. *simplex* (Moldenke) S. L. Chen. belong to spine-tectum imperforatum type (Table 2); In Ser. *Penduliflorae* Schauer: *C. cyrtophyllum*, *C. garrettianum*, *C. wallichii* Merr. and *C. longilimbium* Pei belong to the microspine-tectum perforatum type, *C. hainanense* Hand.-Mazz. and *C. kwangtungense* Hand.-Mazz. belong to the spine-tectum perforatum type and *C. henryi* Pei belongs to microspine-tectum imperforatum type (Table 2); In Ser. *Paniculata* Schauer: *C. colebrookianum* Walp., *C. mandarinorum* Diels, *C. trichotomum* and *C. villosum* Blume belong to the spine-tectum imperforatum type (Table 2), *C. brachystemon* C. Y. Wu et R. C. Fang belongs to the spine-tectum perforatum type and *C. kaichianum* Hsu belongs to the microspine-tectum perforatum type (Table 2); In Ser. *Squamata* Schauer: *C. japonicum* (Thunb.) Sweet belongs to the microspine-tectum perforatum type (Table 2), whereas *C. paniculatum* belongs to the microspine-tectum imperforatum type (Table 2). In conclusion, the pollen morphology of Chinese *Clerodendrum* species investigated in this study does not provide obvious evidence for infra-sectional classification.

Pollen characteristics have been proven to be useful in species delimitation in some genera of Lamiaceae (Erdtman 1952; Abu-Asab and Cantino 1989, 1994; Abu-Asab 1991; Trudel and Morton 1992; Large and Mabberley 1995; Moon et al. 2008a, 2008b; Özler et al. 2011; Badamtsetseg et al. 2012; Ma et al. 2016). According to our results, the varieties could be easily distinguished from the original variety. For instance, the pollen tectum of *Clerodendrum trichotomum* is imperforated, whereas that of *C. trichotomum* var. *fargesii* (Dode) Rehder is perforated. In addition, species that are difficult to distinguish from one another based on external morphology can be discerned at the pollen level. For example, *C. wallichii* and *C. henryi* share many similarities such as 4-angled branchlets, white corolla, ovate lobes, exerted stamens, and style (Chen and Gilbert 1994). The two species are challenging to differentiate at first glance due to their overlapping habitats, similar plant height, and leaf shape. The primary identification characteristic relies on the length of the petiole: which in the case of *C. wallichii* is typically about 1 cm, while *C. henryi* generally exceeds 1 cm in length. Additionally, young branches of *C. wallichii* may exhibit winged branchlets, whereas this characteristic is absent in *C. henryi* (Chen and Gilbert 1994). Our study suggests that morphology of pollen grains can help to distinguish *C. wallichii* (with perforatum tectum; Fig. 5J–L), from *C. henryi* (imperforated tectum; Fig. 3J–L). Morphologically, *Clerodendrum lindleyi* is very similar to *C. bungei* and shares some characteristics: leaf blade broadly ovate to cordate; terminal inflorescences, dense, capitate, corymbose cymes; corolla pinkish to purple, lobes obovate; drupes blue-black and subglobose (Chen and Gilbert 1994). However, our study

indicates that they are easily distinguished from each other because the exine sculpture type pollen of *C. bungei* has a perforated tectum (Fig. 2G–I), whereas that of *C. lindleyi* is imperforated (Fig. 1M–O).

Comparison of pollen morphology of *Clerodendrum* and related genera within Lamiaceae

Pollen grains of Lamiaceae are commonly monad, isopolar, and there is a significant relationship between the number of pollen colpi in the subfamilies of Lamiaceae (Abu-Asab and Cantino 1989; Large and Mabberley 1995; Ma et al. 2016). The tectum of the pollen grains in Ajugoideae usually exhibits projections and spinules. For example, supratectal projections are conical in *Cardioteucris* C.Y. Wu and *Amethystea* L. (Abu-Asab and Cantino 1989); spinose in *Tripora* P.D. Cantino et al. (1998) and *Trichostema* Gronov. (Harley et al. 2004); spinulose in *Caryopteris* Bunge (Abu-Asab et al. 1993) and *Teucrium* L. (Harley et al. 2004). Perveen and Qaiser (2007) described the ornamentation of *Clerodendrum phlomidis* (*C. phlomidis*) as *Clerodendrum phlomidis*-type (Tectum reticulate with spinules or reticulate-rugulate). Our results complement these previous findings.

Clerodendrum is currently placed within Ajugoideae of Lamiaceae based on molecular phylogenetic evidence (Wagstaff and Olmstead 1997; Wagstaff et al. 1998). Recently, Zhao et al. (2021) further divided Ajugoideae into four tribes (Ajugeae, Clerodendreae, Teucriae and Rotheceae) and assigned *Clerodendrum* and *Volkameria* in Clerodendreae. In our palynological study, the pollen grains of *V. inermis* (Fig. 2) are spine-tectum imperforatum, which is the same as found in several species of *Clerodendrum* (*C. villosum*, *C. trichotomum*, *C. mandarinorum*, *C. colebrookianum* and *C. lindleyi*; Fig. 1; Table 2). Therefore, our palynological data support the close relationship between *Clerodendrum* and *Volkameria* (Zhao et al. 2021). Barrabe et al. (2015) suggested that *Clerodendrum* has a close relationship with *Amasonia*, *Kalaharia*, *Tetraclea*, and *Volkameria*, and that they are sister taxa to the alliance encompassing *Hosea*, *Aegiphila*, *Oxera*, and *Faradaya*. Zhao et al. (2021) found that *Clerodendrum* was grouped together with *Oxera* and *Volkameria*. Here, pollen morphology supports Barrabe et al.'s (2015) and Zhao et al.'s (2021) findings. Palynological evidence has revealed that pollen characteristics of *Clerodendrum* (Figs 1–5; Table 2) and *Volkameria* (Fig. 2; Table 2), are similar to those of *Aegiphila*, *Amasonia*, *Faradaya*, *Hosea*, *Kalaharia*, *Tetraclea* and *Oxera* (Raj 1983). Most species exhibit radiosymmetric, tricolpate, monads, spiny ornamentation and spheroidal or subprolate shapes. Although palynological evidence supports that *Clerodendrum* is closely related to other eight genera, accurate relationships among those genera require a more comprehensive study.

Conclusions

The pollen morphology of *Clerodendrum* from China was systematically reported for the first time in this study. Pollen morphology supports that *Clerodendrum* is a member of Ajugoideae, and some characteristics have significant taxonomic value for infraspecific classification and the identification of morphologically closely related taxa within *Clerodendrum*.

Acknowledgments

We thank Pei-duo Tang (Nanning) for technical assistance with SEM observations. We are most grateful to herbarium IBSC, KUN and GAUA for providing pollen materials.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work is supported by the National Natural Science Foundation of China (Grant No. 31970220 and 32260047), the Natural Science Foundation of Guangxi Province (Grant No. 2018GXNSFAA281132 and 2023GXNSFAA026346), and the Foundation of Guangxi Key Laboratory of Sugarcane Biology (GXKLSCB-202004).

Author contributions

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Data availability

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

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Mazus motuoensis (Mazaceae), a new species from Xizang, China

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Abstract

Mazus motuoensis W.B.Ju, Bo Xu bis & X.F.Gao is a newly described species found in Xizang Autonomous Region, China. Morphologically, this species differs from all the other known *Mazus* species by having erect perennial herb form with a rhizome, presence of multicellular hairs, without basal leaves, opposite arrangement of stem leaves, and corolla lobes with erose-toothed margins. Molecular phylogenetic analysis using nuclear and cpDNA genes suggests that this new species occupies a basal position within *Mazus*. In conclusion, both morphological evidence and molecular phylogenetic analyses support that this species belongs to *Mazus* and represents an as-yet-unreported new species with distinct differences from other species within the genus.

Key words: *Mazus*, molecular phylogenetics, morphology, taxonomy

Introduction

Mazus Loureiro is the largest genus within the family Mazaceae Reveal (2011), comprising 38 accepted species (POWO 2022). Most of these species are found in eastern and southeastern Asia, Australia, and New Zealand (Li 1954; Hsieh 2000). The genus is characterized by a distinct two-lipped corolla (3/2-bilabiate), a palate with two longitudinal plaits, and a capsule enclosed in a persistent calyx (Fischer 2004; Deng et al. 2019). In China, there are approximately 31 species and three varieties have been recognized (Hong et al. 1998; Hsieh 2000; Deng et al. 2016; Ying 2019; Xiang et al. 2021; Li et al. 2022), which is the distribution and diversity center of the genus (Hsieh 2000). Originally categorized under Scrophulariaceae through morphological studies (Von Wettstein 1891; Thieret 1954, 1967; Hong et al. 1998), molecular phylogenetic analyses unveiled a robustly supported clade uniting *Mazus* and *Lancea* Hook.f. & Thomson, recognized as the subfamily Mazoideae within Phrymaceae (Beardsley and Olmstead 2002). Nevertheless, subsequent phylogenetic studies confirmed that *Mazus* should be separated from Phrymaceae (Oxelman et al. 2005; Albach et al. 2009; Xia et al. 2009; Schäferhoff et al. 2010), leading to the establishment of a new family called Mazaceae within the Lamiales. The latest



Academic editor: Eberhard Fischer

Received: 13 August 2023

Accepted: 23 October 2023

Published: 14 November 2023

Citation: Ju W-B, Li X, Deng H-N, Li M, He X-J, Gao X-F, Xu B (2023) *Mazus motuoensis* (Mazaceae), a new species from Xizang, China. PhytoKeys 235: 69–79. <https://doi.org/10.3897/phytokeys.235.111092>

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phylogenetic studies and morphological evidence indicate that *M. lanceifolius* Hemsly is a distinct species, positioned at the most basal branch within the Mazaceae family, and is the sister genus to the three recognized genera *Dodartia*, *Lancea*, and *Mazus* (Xia et al. 2012; Deng et al. 2019; Xiang et al. 2021).

In 2022, a field survey was conducted in Motuo County, located within the Xizang Autonomous Region of southwest China, the authors discovered an unknown species of Mazaceae in an evergreen broad-leaved forest. Through careful comparison with specimens, related literature, and phylogenetic analysis of Mazaceae, it was concluded that this species represents a new addition to the *Mazus*.

Materials and methods

Morphological analysis

One population of this new species was rediscovered in Mar 2022 in Xizang Autonomous Region, China. Morphological observations of the new species were conducted using living plants collected from the type locality, as well as type specimens deposited at CDBI. Detailed photographs of morphological features, such as rhizomes, multicellular hairs, stems, leaves, inflorescences, and flowers, were taken using a digital camera and stereoscope. Measurements were carried out on both wild plants and pressed specimens using a ruler and a metric vernier caliper. Digital herbarium images of *Mazus* specimens were sourced from diverse outlets, including JSTOR Global Plants (<http://plants.jstor.org/>), the Global Biodiversity Information Facility (<https://www.gbif.org/zh/>), the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>), and Europeana (<https://www.europeana.eu/en/search>). A thorough examination and comparison of these images with the new species ensued. Subsequently, the morphological attributes of the species were meticulously described in accordance with the guidelines provided by the Flora of China (Hong et al. 1998).

Assessment of conservation status

In the field, we conducted an estimation of the population size of the new species and evaluated the factors posing threats to its existence. In order to determine the conservation status of the new species, we applied the established criteria as outlined by the International Union for Conservation of Nature (IUCN 2019) Red List.

DNA sequencing and outgroup selection

We extracted total DNA from silica gel-dried leaves of the new species using a modified CTAB protocol (Doyle and Doyle 1987). To determine the phylogenetic position of the new species within the *Mazus* genus, we employed two datasets for our analysis. The first dataset consisted of a combined matrix of two cpDNA regions (*rbcL*, *trnL-trnF*), while the second dataset was nrITS. The DNA sequences were amplified and sequenced following the methods described by Deng et al. (2019), using the primers specified in their study. Based on previous phylogenies (Deng et al. 2019; Xiang et al. 2021), 20 species with

28 accession of the relatives of *M. motuoensis* were selected as ingroups. Additionally, we chose five species from three different genera as outgroups. The related sequences were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>). The GenBank accession numbers for the new species are OQ383430 (*trnL-F*), OQ383431 (*rbcL*) and OP720888 (*ITS*). A comprehensive list of all species included in the phylogenetic analysis, along with their respective accession numbers, can be found in Table 1.

Phylogenetic analysis

The sequence chromatograms were visually inspected on Sequencher 5.2.4 (Gene Codes Corporation) and integrated into a single sequence. All sequences were then aligned with MUSCLE in MEGA 7.0.14 (Kumar et al. 2016) and manually adjusted. Phylogenetic analyses were performed based on the combined cpDNA dataset (*rbcL* and *trnL-trnF*) and the nrITS dataset using both the maximum likelihood (ML) and Bayesian inference (BI), respectively. We did not combine the cpDNA and nrITS datasets for analysis because of the different sampling of taxa in the datasets. Settings of parameters during analysis follow those presented in Deng et al. (2019) and Xiang et al. (2021).

Results and discussion

Morphological analysis

Morphologically, the new species has intermediate characteristics of *Mazus* and *Puchiumazus*. The new species has characteristics such as rhizomes, erect stems, and stem leaves opposite similar to *Puchiumazus*. However, the plant of this new species is covered with multicellular hair and has stems that are not quadrangular and leaf blade elliptic-ovate, which distinguishes it from the only known species, *Puchumazus lancefolius* (Hemsley) Bo Li, D.G.Zhang, and C.L.Xiang (Xiang et al. 2021). There are also perennial herb species with erect stems and opposite cauline leaves in the genus of *Mazus*, such as *M. caducifer* Hance (1882). But the new species has a series of ray characteristics not commonly seen in *Mazus*, including single erect unbranched stems without basal leaves, stem leaves many and opposite, petioles nearly absent, lobes margin erose-toothed. In conclusion, based on the morphological key provided by Hong et al. (1998), the new species is classified morphologically within the *Mazus* and represents an anomalous existence.

Phylogenetic analysis

The phylogenetic tree was generated using a combined cpDNA matrix, consisting of 34 aligned sequences and comprising 2197 characters (*rbcL*: 1318 bp; *trnL-trnF*: 879 bp). Additionally, the nrITS matrix included 28 aligned sequences and comprised 703 characters. Due to differences in taxon sampling between the cpDNA and nrITS datasets, they were not combined for analysis. Both maximum likelihood (ML) and Bayesian inference (BI) methods yielded congruent topologies. Therefore, only the results of the ML trees are presented (Figs 1, 2: MLBS: 100%, BIPP: 1.00; all support values follow this order hereafter).

Table 1. Information of samples used for phylogenetic inference in this study.

Taxa	rbcl	trnL-F	ITS
<i>Mazus alpinus</i> Masamune 1	KX783481	KX783520	MK192641
<i>Mazus alpinus</i> Masamune 2	KX783480	KX783519	MK192642
<i>Mazus caducifer</i> Hance 1	KX783477	KX783516	MK192664
<i>Mazus caducifer</i> Hance 2	KX783487	KX783526	MK192659
<i>Mazus celsioides</i> Handel-Mazzetti	KX783486	KX783525	●
<i>Mazus fruticosus</i> Bo Li, D.G.Zhang & C.L.Xiang 1	KX783470	KX783509	MK192660
<i>Mazus fruticosus</i> Bo Li, D.G.Zhang & C.L.Xiang 2	KX783471	KX783510	MK192649
<i>Mazus gracilis</i> Hemsley	FJ172729	FJ172687	FJ172738
<i>Mazus humilis</i> Handel-Mazzetti	●	MK266421	MK192667
<i>Mazus japonicus</i> (Thunburg) O. Kuntze	FJ172728	FJ172686	●
<i>Mazus japonicus</i> var. <i>delavayi</i> (Bonati) Tsoong	KX783482	KX783521	●
<i>Mazus longipes</i> Bonati	KX783474	KX783513	MK192652
<i>Mazus miquelii</i> Makino 1	KX783475	KX783514	MK192637
<i>Mazus miquelii</i> Makino 2	KX783476	KX783515	MK192655
<i>Mazus miquelii</i> Makino 3	KX783483	KX783522	MK192656
<i>Mazus motuoensis</i> W.B.Ju, Bo Xu bis & X.F.Gao	OQ383431	OQ383430	OP720888
<i>Mazus novaezeelandiae</i> W.R.Barker	KX783469	KX783508	MK192676
<i>Mazus omeiensis</i> H. L. Li 1	KX807209	KX807208	MK192636
<i>Mazus omeiensis</i> H. L. Li 2	FJ172731	FJ172688	MK192663
<i>Mazus procumbens</i> Hemsley	KX783478	KX783517	MK192647
<i>Mazus pulchellus</i> Hemsley	KX783472	KX783511	MK192638
<i>Mazus pumilio</i> R.Brown	KX783468	KX783507	MK192671
<i>Mazus pumilus</i> (N. L. Burman) Steenis 1	MK266346	KX807206	MH711724
<i>Mazus pumilus</i> (N. L. Burman) Steenis 2	HM850162	KX807207	FJ172737
<i>Mazus reptans</i> N.E. Brown	HQ384872	AF479004	AF478940
<i>Mazus spicatus</i> Vaniot	FJ172730	FJ172689	FJ172740
<i>Mazus sunhangii</i> D. G. Zhang & T. Deng 1	KX783485	KX783524	●
<i>Mazus sunhangii</i> D. G. Zhang & T. Deng 2	KX783484	KX783523	●
<i>Mazus xiuningensis</i> X. H. Guo & X. L. Liu	MK266349	MK266430	●
OUTgroup			
<i>Puchiumazus lanceifolius</i> (Hemsly) Bo Li, D. G. Zhang & C. L. Xiang 1	MW373737	MW373741	MW364623
<i>Puchiumazus lanceifolius</i> (Hemsly) Bo Li, D. G. Zhang & C. L. Xiang 2	MW373738	MW373742	MW364624
<i>Dodartia orientalis</i> Linnaeus	JQ342984	JQ342981	JQ342980
<i>Lancea tibetica</i> J. D. Hooker & Thomson 1	KX783467	KX807205	MK192678
<i>Lancea tibetica</i> J. D. Hooker & Thomson 2	MF786661	FJ172685	FJ172736

● refers to a missing sequence.

The results of nrITS and cpDNA phylogenies (Figs 1, 2) show that *Dodartia-Lancea* formed the basal clade with *Puchiumazus* clade as sister to *Mazus* (100%, 0.99 in nrITS tree; 100%, 1 in cpDNA tree). The new species was located at the base of the genus *Mazus* with other sampled species of *Mazus* together

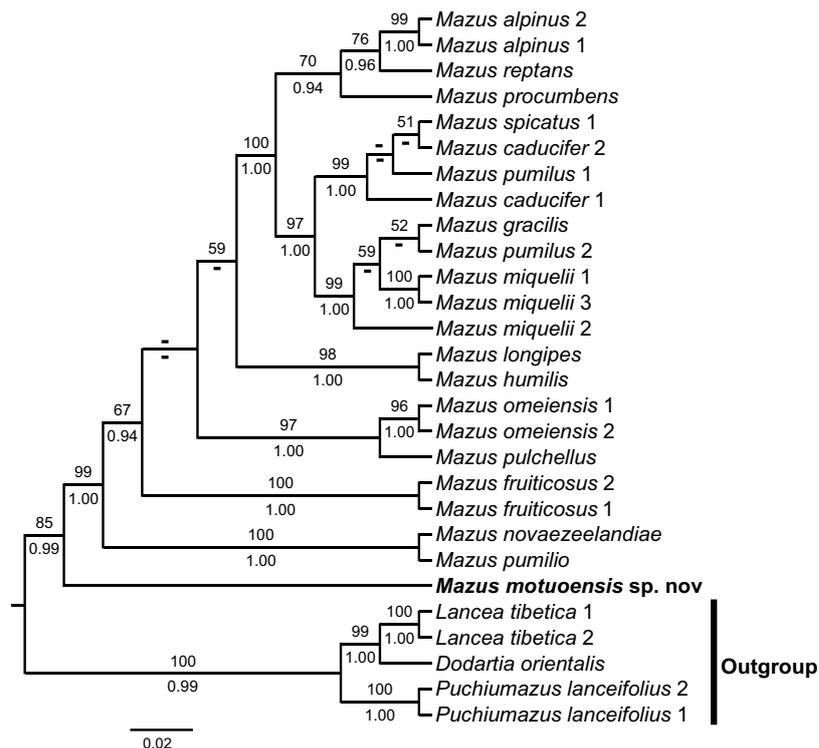


Figure 1. Phylogenetic relationships of *M. motuoensis* and related species inferred from ML and BI analyses based on the nrITS dataset. Numbers on the branches indicate the bootstrap support of the ML and the posterior probability of BI analyses.

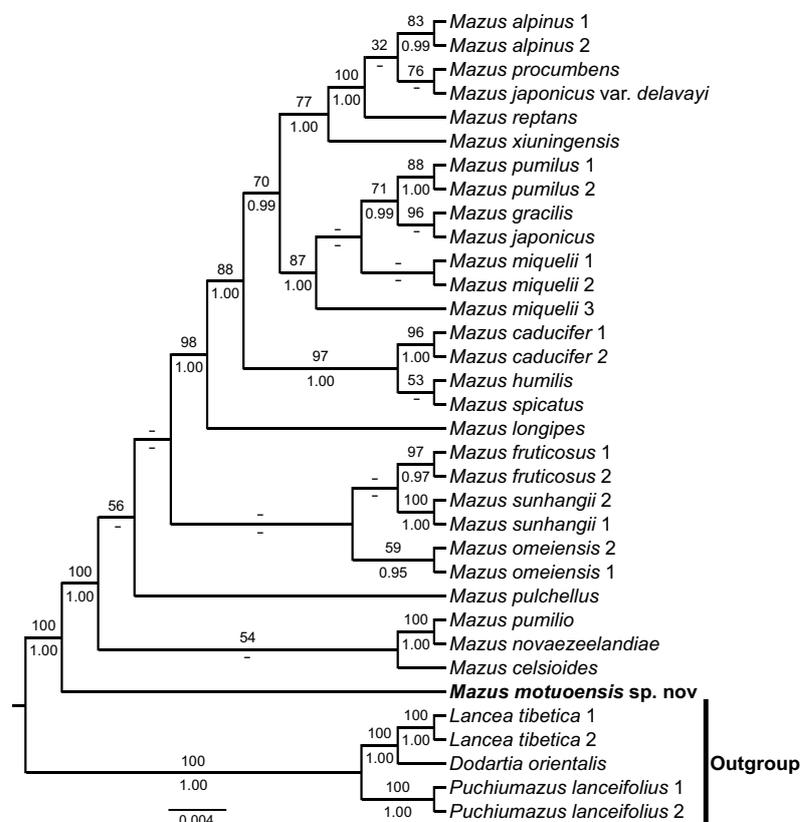


Figure 2. Phylogenetic relationships of *M. motuoensis* and related species inferred from ML and BI analyses based on the combined dataset of *rbcL* and *trnL-trnF*. Numbers on the branches indicate the bootstrap support of the ML and the posterior probability of BI analyses.

forming monophyletic clade with strong support (Fig. 1: 85%, 0.99; Fig. 2: 100%, 1). This phylogenetic result is broadly consistent with previous studies (Xiang et al. 2021), indicating that the new species belongs to the *Mazus* representing a unique presence of the genus.

Taxonomic treatment

***Mazus motuoensis* W.B.Ju, Bo Xu bis & X.F.Gao, sp. nov.**

urn:lsid:ipni.org:names:77330788-1

Figs 3–5

Diagnosis. The new species is distinguished from congeneric species by its rhizomes, perennial herb covered with multicellular white villus, erect and unbranched stems, having no basal leaves, stem leaves opposite, subsessile, lower lobes margins erose-toothed.

Type. CHINA, Xizang, Motuo County, DeXing town, Nibi Valley, ditch edge in the forest, 29°22'27.98"N, 95°10'0.88"E, alt. 2253 m. 31 Mar 2022, WenBin JU & XIONG LI, YLZB08519 (holotype: CDBI0279767; isotypes: CDBI0279765, CDBI0279766)

Description. Perennial herbs, 15–25 cm tall, the whole plant is covered with long white soft multicellular hairs. Rhizome white. **Stems** erect, unbranched. **Leaves** opposite, numerous, petiole inconspicuous to nearly absent; lower leaf blade scalelike and small, obovate-oblong, apex obtuse, middle and upper leaves with leaf blade elliptic to ovate, papery, 0.8–4.0 × 0.4–1.8 cm, adaxially clothed with multicellular hairs, abaxially subglabrous, multicellular hairs on veins, base cuneate, margin serrate, lateral veins 3–5 pairs. **Racemes** terminal, ascending to 5 cm long, lax, fewer than 5; pedicels 4–6 mm, glabrous or with a few multicellular hairs; bracts tiny, narrowly lanceolate to linear, glabrous. **Calyces** broadly campanulate, ca. 6 mm long, 5-veined, glabrous outside and inside, lobes 5, triangular-lanceolate, as long as tube, apex acute, midrib conspicuous, lateral veins inconspicuous. **Corolla** 1.2–1.5 cm long, white, but often purple on upper lobes, glabrous outside and inside apart from clavate hairs on palate; tube 0.3–0.5 cm long, shorter than calyx; limb 2-lipped, upper lip bilobed, slightly upwarp, lobes triangular ovate, apex subacute, sometimes weakly obtuse or retuse; lower lip trilobed, lobes margins erose-toothed, middle lobe usually rounded, smaller than lateral lobes, yellow palate comprising 2 longitudinal elevations extending from point of filament fusion to the base of lower lobes, with erect clavate hairs. **Stamens** 4, didynamous, glabrous, inserted at the same level in distal part of tube, inserted at the distal end of the tube at the same level, included; anterior pair longer, curved, appressed to corolla tube, posterior pair spreading; anthers bithecal, locules divergent, apically connivent, positioned adjacent to corolla tube on upper lip; filaments filiform, glabrous. **Ovary** ca. 2 mm long, glabrous, ovoid; styles ca. 7 mm long, included, glabrous, exerted beyond anthers, stigma bilobed. Fresh capsule and calyx light green, included by persistent calyx.

Distribution and habitat. *Mazus motuoensis* is currently known from Nibi Valley, Motuo County, Xizang, China. It can be found under evergreen broad-leaved forest at altitudes of 2253 m.

Phenology. Flowering was observed from May to June.

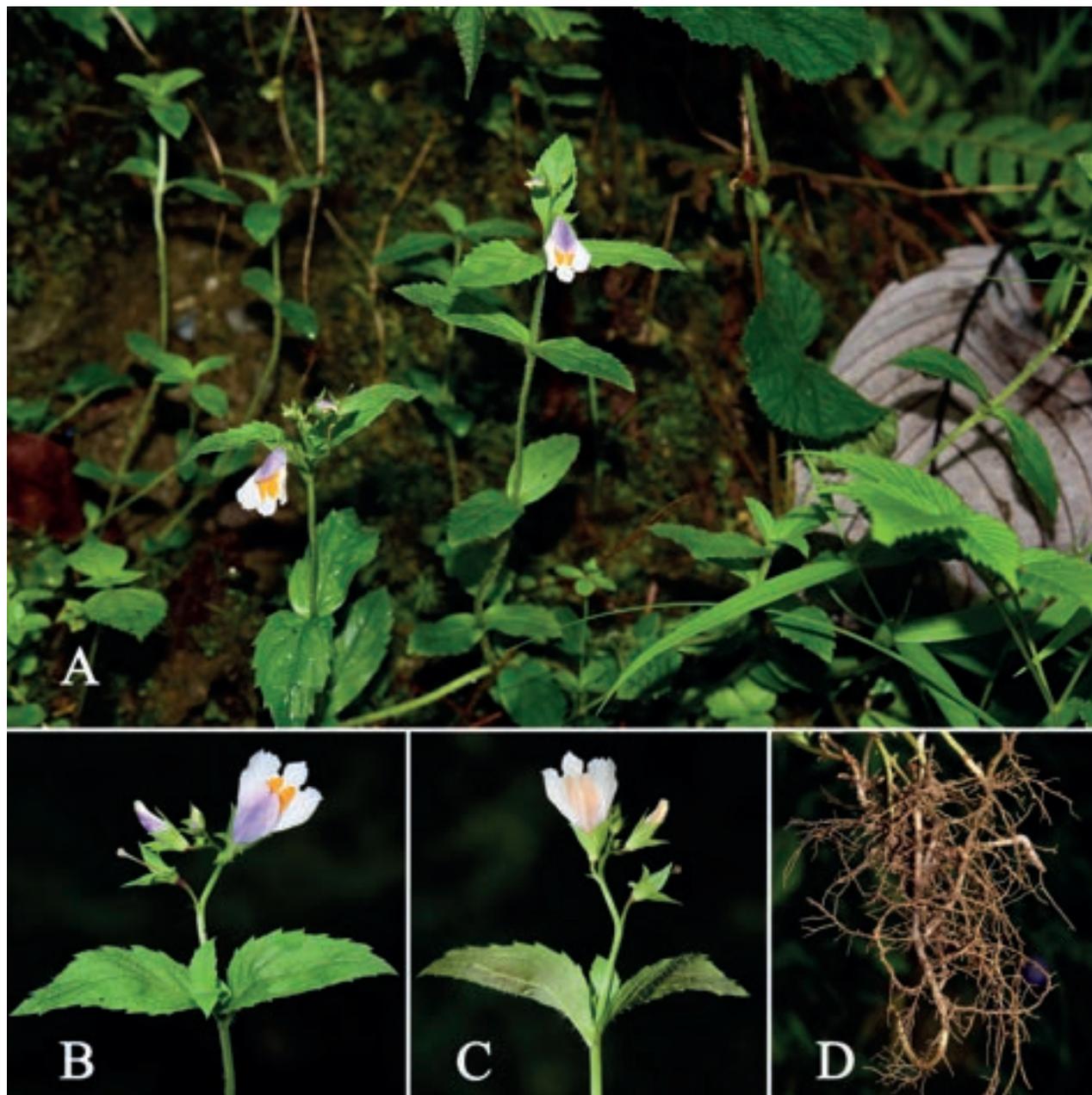


Figure 3. Living images of *M. motuoensis* A habit B inflorescence in frontal view C inflorescence in rear view D rhizome.

Etymology. The specific epithet “motuoensis” refers to the locality, Motuo County, Xizang, China.

Vernacular name. Simplified Chinese: 墨脱通泉草; Chinese pinyin: Mòtuō Tōngquáncǎo.

Conservation status. Currently, the authors have discovered only one population of *Mazus motuoensis* from one single locality in Nibi Valley of Motuo County in Xizang Province, China, and ca. 30 individuals from the type locality. Evergreen broad-leaved forests are widely distributed in this area, so we speculate that this new species has a relatively wide distribution range. Due to insufficient field survey, the natural distribution of this species in the wild is not clear. Following the IUCN Red List criteria (2019), we suggest this species placement in the Data Deficient.

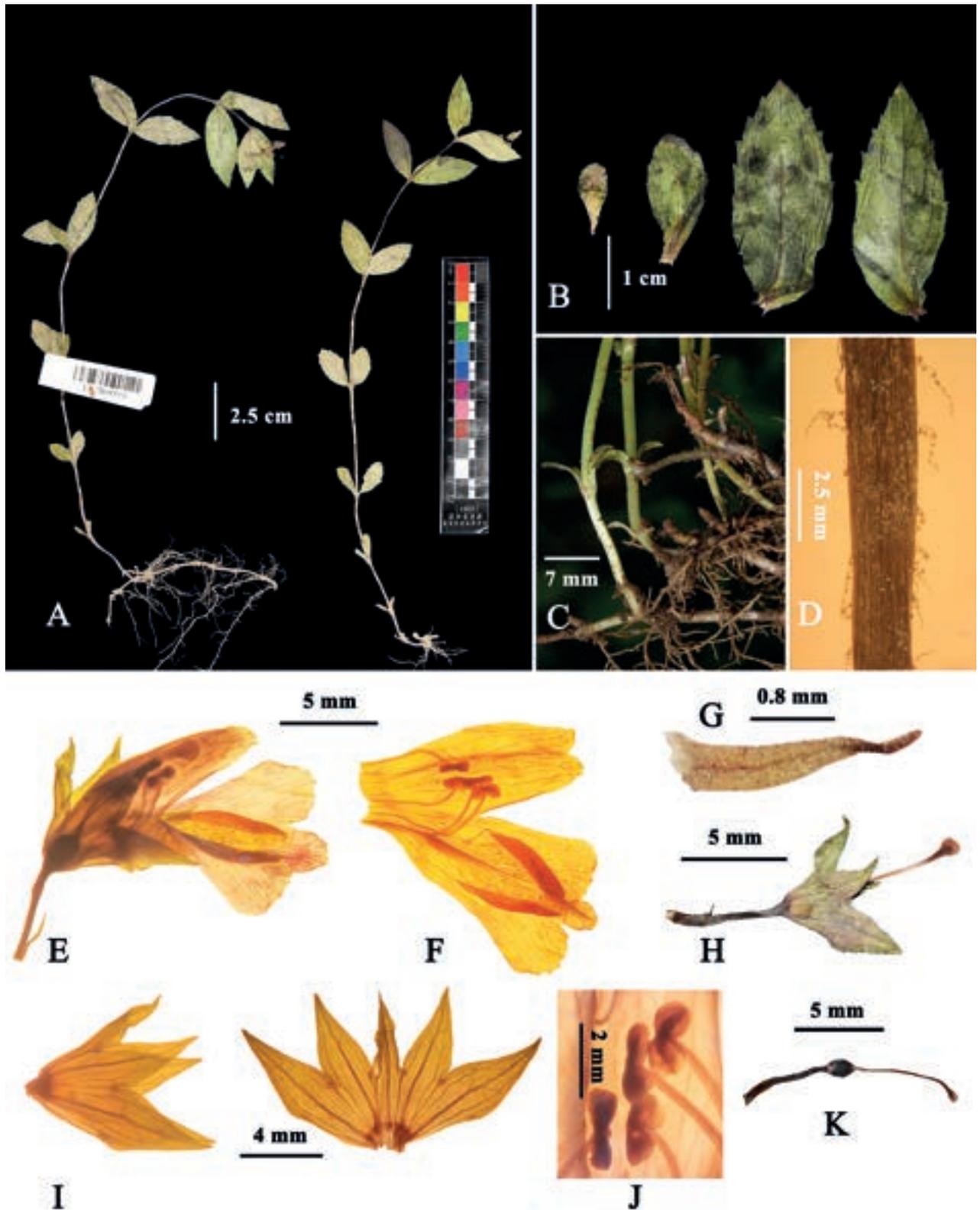


Figure 4. Morphology of *M. motuoensis* A plant B leaves C old stems D multicellular hairs attached to stem E flower F unfolded corolla, showing limb upper lip and lower lip G bract H, I calyx J anthers K ovary and style.

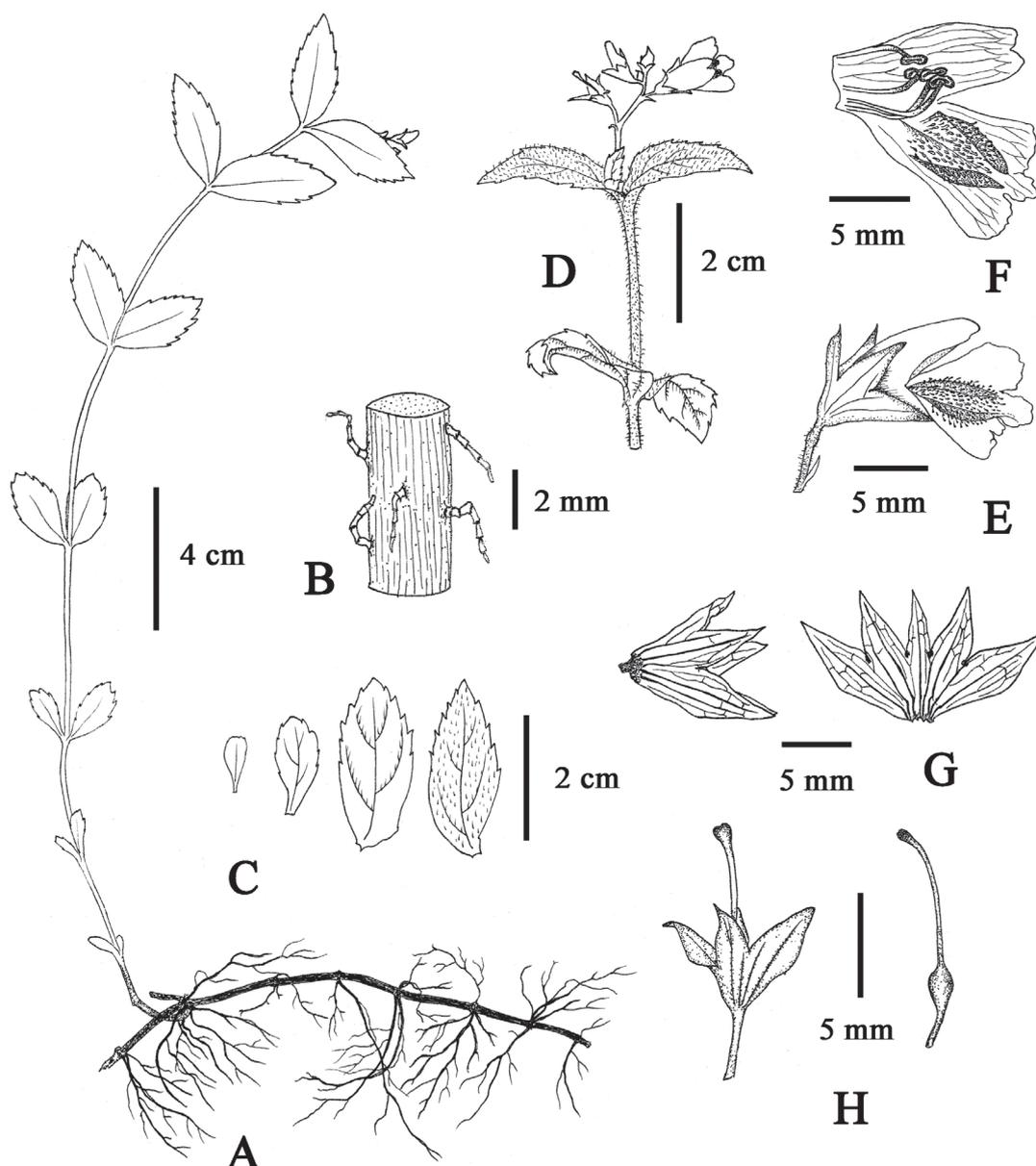


Figure 5. Line drawings of *M. motuoensis* **A** whole plant **B** multicellular hairs attached to stem **C** leaves **D** inflorescence **E** flower **F** unfolded corolla **G** calyx **H** ovary and style. Drawn by Mr. Zhen-long Liang.

Acknowledgements

We would like to express our sincere thanks to Mr. Zhenlong Liang (Chengdu Institute of Biology, Chinese Academy of Sciences) for his help with the DNA extraction, PCR amplification and the line drawing. We are grateful to Dr. Fei Zhao for providing valuable suggestions during the phylogenetic analysis process of the new species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was supported by the Biological Resources Programme, Chinese Academy of Sciences, BRP CAS (Grant No. KFJ-BRP-017-102), and the Wild Plants Sharing and Service Platform of Sichuan Province.

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Data availability

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

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Neottia maolanensis, a replacement name for *Neottia bifida* M.N.Wang (Orchidaceae)

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Abstract

According to Articles 53.1 of the International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code), *Neottia bifida* M.N.Wang (as 'bifidus'; PhytoKeys 229: 222, 2023) is an illegitimate name, and hence a new name *Neottia maolanensis* M. N. Wang is proposed here.

Key words: homonym, illegitimate, *Neottia maolanensis*, Orchidaceae, replacement name

A new mycoheterotrophic orchid species was named as *Neottia bifidus* M. N. Wang in our recent work (Wang et al. 2023). However, it was overlooked that the specific epithet spelled as “bifidus” is incorrect. The correct one should be “bifida”. Unfortunately, we found “*Neottia bifida*” is a later homonym (Blume 1825). According to Articles 6.11, 53.1 of the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al. 2018) that is currently in force, a new name is proposed here.



Academic editor: Vincent Droissart

Received: 4 October 2023

Accepted: 23 October 2023

Published: 14 November 2023

Citation: Wang M-N, Wu X-Y, Tan C-J, Yu P, Rao W-H, Chen J-S, Li J, Chen J-B (2023) *Neottia maolanensis*, a replacement name for *Neottia bifida* M.N.Wang (Orchidaceae). PhytoKeys 235: 81–82. <https://doi.org/10.3897/phytokeys.235.113651>

***Neottia maolanensis* M.N. Wang, nom. nov.**

urn:lsid:ipni.org:names:77330789-1

Replaced name. *Neottia bifida* M.N.Wang (as 'bifidus'; in PhytoKeys 229: 222, 2023), *nom. illeg.*, non. *Neottia bifida* Blume (in Bijdr. Fl. Ned. Ind. 8: 408, 1825).

Type. CHINA. Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo County, the Maolan National Nature Reserve, 825 m elev., 23 Apr 2021, J.B.Chen 00599 (holotype: NOCC!).

Chinese name. 茂兰鸟巢兰.

Etymology. The species epithet refers to the type locality of the new species.

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Acknowledgements

We thank Prof. Fu-Wu Xing for the constructive comments of the revise.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was financially supported by the National Natural Science Foundation of China (Grant No. 32001245) and the Science, Technology and Innovation Commission of Shenzhen Municipality (Grant No. KCXFZ20211020164200001).

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Data availability

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

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Taxonomic revision of the *Erigeron acris* group (Asteraceae) in Murmansk Region, Russia, reveals a complex pattern of native and alien taxa

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Abstract

Based on the evidence of morphology and a comprehensive revision of herbarium collections and field records, the taxonomy of the *Erigeron acris* group in Murmansk Region, European Russia, is completely revised. Its accepted diversity is increased from 2 to 8 taxa, including putative hybrids. The only native species, *E. politus*, is distributed in mountainous regions, along sea coasts and in the Kutsa River basin. Five species are alien: *E. rigidus* (previously confused with *E. acris* s.str.), *E. acris* s.str. (first recorded in the narrow taxonomic definition), *E. brachycephalus* (previously unrecorded), *E. droebachiensis* and *E. uralensis* (previously reported in error). Two major waves of the introduction of alien taxa are discovered, with different occurrences and species compositions. Regional and local dispersal by pomors (historical Russian settlers) occurred during their colonisation and traditional activities since the 12th century (archaeophytes or early neophytes); such alien taxa (*E. rigidus*, *E. brachycephalus*, and partly *E. acris*) are particularly common within the territory traditionally settled by Russian colonists but also found elsewhere along historical trade routes. Other alien species of the *E. acris* group (*E. droebachiensis*, *E. uralensis*, and partly *E. acris* and *E. brachycephalus*) colonised industrial areas in the 1960s–1990s as seed contaminants introduced during revegetation of slag dumps, stockyards, dams and channels. Putative hybrids between *E. politus* (native), *E. rigidus* and *E. acris* (aliens) are found in the places of co-occurrence. Updated nomenclature, synonymy and descriptions are provided for all accepted taxa.

Key words: Compositae, Kola Peninsula, Lapland, mapping, nomenclature, plant invasions, Pomors, taxonomy

Introduction

Although a modern comprehensive inventory of the flora of Murmansk Region (European Russia) is still lacking, its vascular plants are relatively well known due to the 200-years-long history of botanical studies in this territory (Kozhin et al. 2020a). However, many taxonomically critical taxa still require revision in this territory, in order to elucidate their diversity and distributions. Besides, alien plants of Murmansk Region have never been at the focus of a dedicated study



Academic editor: A. Sukhorukov

Received: 11 August 2023

Accepted: 28 October 2023

Published: 15 November 2023

Citation: Sennikov AN, Kozhin MN (2023) Taxonomic revision of the *Erigeron acris* group (Asteraceae) in Murmansk Region, Russia, reveals a complex pattern of native and alien taxa. *PhytoKeys* 235: 83–128. <https://doi.org/10.3897/phytokeys.235.111020>

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(Kozhin and Sennikov 2022), and require not only a special effort for their data mobilisation from the vast corpus of grey literature but also reassessment of their residence status (native vs. alien) because many archaeophytes or other long-term residents have been traditionally considered within the native component of the flora (Kozhin et al. in prep.).

One small, yet taxonomically unresolved group is a complex of *Erigeron acris* L. s.l. It belongs to *E.* sect. *Trimorpha* (Cass.) DC. This section is distinct due to the presence of three types of flowers in the capitula: tubular flowers in the centre, ray flowers without a lamina in the middle, and ray flowers with a short lamina at the margin (Nesom 2008). Among European taxa of this section, *E. acris* s.l. differs by its very short ligules and monocarpic life form (Halliday 1976).

The taxonomic diversity in the *E. acris* group seems to be maintained by self-pollination (Noyes 2000); the resulting taxa are stable in largely sympatric areas (Olander and Tyler 2017) and may therefore be treated at the rank of species (e.g. Tzvelev 1994). However, since diagnostic characters are very meagre in this taxonomic group, whose diversity has probably resulted from extensive hybridization (Tzvelev 1994; Olander and Tyler 2017), the rank of subspecies was also employed (e.g. Kurtto and Väre 1998; Olander and Tyler 2017).

The taxonomy of *E. acris* L. s.l. in Murmansk Region and neighbouring territories has been controversially treated. Orlova (1966) accepted two taxa, i.e. *E. acris* with hairy phyllaries, leaves and stems, and the nearly glabrous *E. politus* Fr. Kurtto and Väre (1998) recognized only one taxon in Finnish Lapland, *E. acris* subsp. *politus* (Fr.) H.Lindb., a less hairy plant with rather few capitula and pinkish phyllaries. Tzvelev (1990, 1994) revised the taxonomy of *E. acris* s.l. in Eastern Europe; he accepted three taxa in Murmansk Region: *E. acris* s.str., whose synflorescence branches are densely covered by long simple hairs, *E. uralensis* Less. (syn. *E. brachycephalus* H.Lindb.), whose synflorescence branches are subglabrous or covered by short simple hairs, and *E. politus* Fr. (syn. *E. elongatus* Ledeb.), which embraces lower-sized subglabrous plants with longer branches and fewer capitula. Tzvelev also suggested that the name *E. decoloratus* may belong to populations intermediate between *E. politus* and *E. uralensis*, which do not deserve taxonomic separation from the latter. Olander and Tyler (2017) revised this group in Sweden and accepted three taxa, of which *E. acris* subsp. *acris* is ubiquitous but more abundant in the south, with involucre bracts almost completely covered by simple hairs, *E. acris* subsp. *droebachiensis* (O.F.Müll.) Mela (central Sweden) with pale phyllaries up to 5 mm long, numerous capitula and inconspicuous ray flowers, and *E. acris* subsp. *politus* (northern Sweden) with darker phyllaries over 5 mm long, fewer capitula and well exerted ray flowers. They also indicated that the name *E. acris* subsp. *decoloratus* (H.Lindb.) Hiitonen may belong to hybrids between *E. acris* subsp. *acris* and the other two subspecies.

Hybrids in the *E. acris* group have long been reported or suspected, including those between hairy and glabrous taxa (e.g. Blytt 1906; Thellung 1923; Šída 1998, 2000, 2004), although some of these tentative reports (Botschantzev 1959; Tzvelev 1994) appeared to have mistaken unrecognised or synonymised taxa for hybrids.

Taxonomic opinions about the subdivision of *E. acris* s.l. differed widely to the extent that the treatments covering the same territory or closely neighbouring areas may be largely incongruent in the number of accepted taxa and their

delimitation and diagnostic characters. This discrepancy urged us to revise the taxonomy of this group in Murmansk Region, in order to bridge together the existing treatments and to uncover the diversity and distribution patterns of the taxa involved. We also wanted to evaluate the resident status of these taxa in Murmansk Region because of their strong association with human dispersal (Kurtto and Väre 1998).

The present work provides a detailed treatment of the *E. acris* group for Murmansk Region but includes the whole history of its studies and involves comparisons with all the relevant taxa recognised in Fennoscandia, and places this study in the European context. It is considered a step towards a new revision of this difficult taxonomic group in Eastern Fennoscandia, which is a long and complicated process.

Materials and methods

Study area

Murmansk Region is a top-level federal subject of the Russian Federation, situated in the north-western part of European Russia; this territory is also known as Russian Lapland in historical literature. It is largely situated on the Kola Peninsula, surrounded by the Barents Sea in the north and by the White Sea in the south. Its total area constitutes 144,902 km². This territory is part of the Subarctic Zone; its vegetation is represented by tundra in the north, forest tundra in the major part of the mainland, and northern taiga in the south-west, next to the borders with Finland and Russian Karelia (Chernov 1971). The relief is largely flat and nearly monotonous, except for rocky mountain groups in the western part of the territory, among which the Khibiny and Lovozero Mts. are the highest to reach the maximum of 1191 m above sea level (Fig. 1A). Smaller but important hills and isolated outcrops are situated in the Kovdor and Kandalaksha Districts, whereas the north-western coast is incised by fjords and the other coastal areas are traversed by deeper river valleys. Forested areas are extensive in the western part, and forests are significantly present in the basins of the Ponoï River and the rivers flowing into the southern coastal waters of the White Sea (Fig. 1B).

Material examined

This study was based on a comprehensive sampling of all herbarium specimens available from the study area and kept at H, INEP, KAND, KPABG, LE, LECB, MW, OULU, PTZ, TROM (herbarium acronyms according to Index Herbariorum (Thiers 2023)) and the Herbarium of the Apatity Branch of the Murmansk Arctic University (unregistered, provisional acronym ARCT). Documented observations (iNaturalist 2023) were also used. The specimens (258) and observations (3) were georeferenced and databased, and their data were made available as a taxonomic dataset (Sennikov and Kozhin 2023), which includes 261 herbarium specimens and documented observations altogether. Point distribution maps were generated in ArcGis 10.3.1 (<https://www.esri.com>) from the database, taking into account the residence status and period of introduction of the species in every locality; we distinguished three categories of species

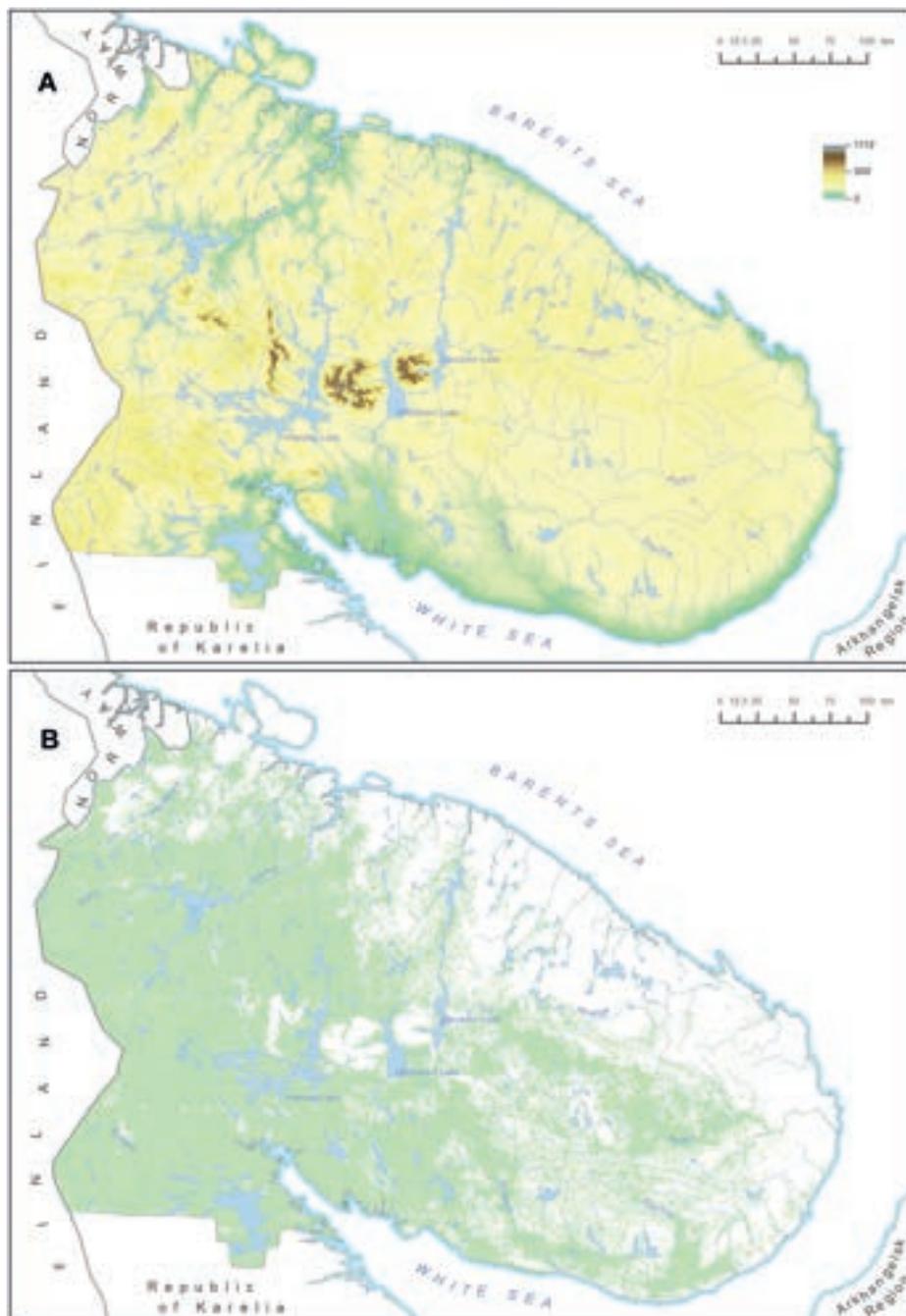


Figure 1. Study area: Murmansk Region, Russia **A** hypsometric map, major rivers and lakes **B** distribution of forested landscapes (denoted by green colour). Maps were created using ArcGIS software by Esri. ArcGIS is the intellectual property of Esri and is used herein under license. Copyright Esri. All rights reserved.

residence: native, pre-industrial alien (introduced prior to the industrialisation in the USSR, i.e. before the 1930s) and industrial alien (introduced with or after the industrialisation in the USSR, sometimes after 1930 but usually after 1960).

The herbarium specimens were examined taxonomically by A. Sennikov either *de visu* (H, OULU), or as high-quality scanned images (ARCT, INEP, KAND, KPABG, LE, MW), or as low-quality scanned images (TROM), or as photographs (LECB, PTZ). Scanned images were also used as illustrations.

Nomenclature and bibliography

Special effort was used to trace validly published names at the level of species and subspecies, in order to produce a stable synonymy whenever the species or subspecies rank is preferred. More precise publication dates were traced from a variety of bibliographic sources, which helped to establish the sequence of publication and to ensure priority in difficult cases.

Diagnostic characters

As in the previous revisions (Tzvelev 1994; Kurtto and Väre 1998; Šída 1998, 2000), we examined the following diagnostic characters: stem and phyllary colouration; stem, leaf and phyllary pubescence; length and density of pubescence; number, shape and density of cauline leaves; shape of synflorescence; ligulate flower colour. The value of individual characters may be limited due to high genetic variability or phenotypic plasticity, whereas a complex of characters may unambiguously characterise the taxa.

In this work, we used a classical method of morphological comparisons, observing discontinuities in plant variability. We did not use statistical methods (like employed by Olander and Tyler (2017)) because, in order to distinguish between very closely related taxa, infra- and interpopulational variability must be considered separately, whereas our material was represented by previously collected herbarium specimens, i.e. a random selection from multiple populations that do not allow to assess their interpopulational variability.

Taxonomic concept and ranking

We follow the concept of narrowly defined taxa in the *Erigeron acris* group, which is universally adopted nowadays (Halliday 1976; Tzvelev 1994; Kurtto and Väre 1998; Šída 1998, 2000, 2004; Greuter 2006; Olander and Tyler 2017; PoWO 2023). This concept is justified because of the limited variability and morphological distinction of the accepted taxa. Ranking in this group is disputable; species level is currently accepted in the Czech Republic (Šída 1998, 2000, 2004) and Eastern Europe (Tzvelev 1994), whereas subspecies level is preferred in Finland (Kurtto and Väre 1998) and Sweden (Olander and Tyler 2017), and in European (Halliday 1976; Greuter 2006) and global (PoWO 2023) compilations. We accept species rank because the ranked taxa are clearly defined by morphology and commonly co-occur in the same territory with limited hybridisation, thus complying with the biological species concept due to apparent reproductive isolation (Gao and Rieseberg 2020).

Classification of alien occurrences

In agreement with Pyšek et al. (2002), origin status and invasion status were determined for each accepted species, and residence status was determined for each recorded locality. We determined the origin status as native or alien; a taxon was treated as alien if it arrived to the territory with human assistance at any time period, including the remote past from which no historical plant records or other direct evidence are available. Each non-native species was

classified according to its invasion status either as casual or naturalised, with further estimation of invasiveness (Richardson et al. 2000). Records of alien plants were classified according to their period of introduction (residence status), using the major subdivision between archaeophytes and neophytes as in Pyšek et al. (2002) and major periods of the recent political history as in Sennikov and Lazkov (2021). For temporal classification of neophyte alien records of the *Erigeron acris* group in Murmansk Region, we used the 1930s as a temporal limit; this limit reflects a major change in economic activities, transportation and human migrations, which was linked with the beginning of industrialisation in the USSR (Lewis 1979).

The history of introduction was determined based on the history of human activities in a certain locality and in the territory as a whole. The local history was obtained from historical accounts in cases of the distant past, or from technical reports and local knowledge in cases of the recent past. Local plant introductions were linked to the local human activities and their time periods. We cross-checked our information against the knowledge available from the neighbouring territories, i.e. Finland and Russian Karelia.

We inferred pathways of introduction for alien taxa, based on direct evidence as recorded by field collectors or on indirect evidence as derived from the local history. The pathways were categorised according to Hulme et al. (2008) and interpreted as recommended by Harrower et al. (2018).

Results

Overview of historical herbarium collections

The examined collections are comprehensive historical materials and include all periods of the botanical history in the present-day territory of Murmansk Region. Many of these collections were taken into account in various botanical publications. So far, no proper overview of botanical collections and their corresponding publications exist for Murmansk Region; for this reason, we provide a more detailed description of the *Erigeron* collections in order to uncover their link with historical publications and major events of the botanical exploration.

The first record of *Erigeron acris* s.l. from the Kola Peninsula was published by Jacob Fellman (1831), who reported this group from the south-western part of the territory. Nowadays, Jacob Fellman's herbarium collection is fragmentary; its *reliquiae* are preserved at the University of Helsinki (Väre 2011). However, no specimens of *E. acris* s.l. survived in this collection.

The earliest historical specimens are available from Russian academic expeditions and the Finnish botanical exploration of the Kola Peninsula. The first extant specimens were collected by A.F. Middendorf in 1840 during his academic expedition along the Barents Sea coast (Sukhova and Tammiksaar 2015). Further collections were exclusively Finnish, linked with botanical explorations of Russian Lapland (Uotila 2013; Väre 2017). During 1861 and 1863, N.I. Fellman and his team made very important collections along the southern and eastern sea coasts and in Kola Town (Sennikov and Kozhin 2018), thus bringing evidence for an early introduction of alien plants by the Pomors. A.J. Malmberg in 1870 (Malmberg 1926; Lappalainen 1959), R.B. Enwald and C.A. Knabe in 1880 (Uotila 2013) acted as commercial botanical collectors and brought

many well-documented specimens from the coastal areas. At the same time, geologist A. Göbel, who was dispatched by the Russian Academy of Sciences to Russian Lapland in 1868–1870, made some collections along the northern and eastern coasts but his botanical collections were very poorly prepared and extremely inaccurately documented. V.F. Brotherus (1886) collected in the western parts of the territory in 1885, and made the first good collections from the Rybachii Peninsula. These early explorations, which aimed at the primary floristic knowledge and focused largely on coastal areas with an emphasis on its western (Kandalaksha) and eastern (Ponoi) extremities, culminated with the Great Kola Expedition in 1887, 1889 and 1892, which was organised by the University of Helsinki and the Societas pro Fauna et Flora Fennica with the major aim to cover the interior parts of the Kola Peninsula (Rikkinen 1980; Uotila 2013). With these expeditions, the basic knowledge about native and archaeophytic populations of *E. acris* s.l. was obtained.

Further botanical expeditions focused on the “white spot” areas from which no botanical knowledge had been available. V. Borg and A. Rantaniemi extensively collected in Kuusamo, covering the territory of the Kutsa River and neighbouring villages (Uotila 2013). This important territory became a nature reserve and had been subsequently visited several times by various Finnish researchers before it was ceded to the USSR after the Second World War. More recently, it was revisited by T. Ulvinen who published a synopsis of the flora of the Kutsa Nature Reserve and its vicinities (Ulvinen 1996).

K. Regel made extensive explorations of plant communities in the Kola Peninsula but collected rather few specimens. We traced only two specimens of *E. acris* s.l. which he collected along the Ponoi River (Regel 1914, 1927). Another great early expedition focused on plant communities was made in 1927 by G. Zinserling, who collected many important specimens along the southern coast of the Kola Peninsula (Zinserling 1935).

The Polar-Alpine Botanical Garden-Institute was established in 1931 in the Khibiny Mts. This botanical institution triggered a new period of regular botanical studies of Murmansk Region. Eventually, these academic activities led to the five-volume book “Flora of Murmansk Region” (Gorodkov 1953; Poyarkova 1954, 1956, 1959, 1966), which was considered among the best regional synopses in the USSR. Distribution maps based on point occurrence data, which were provided for each species treated in this book, were digitised and made available online, including two accepted species of *E. acris* s.l. (Kozhin et al. 2020a).

Higher mountains of the western part of the Kola Peninsula were in focus of botanical studies in the 1930s, when their exploration for mining of natural resources had been initiated. This study was summarised by B. Mishkin in his monograph on the flora of the Khibiny Mts. (Mishkin 1953). The neighbouring Lovozero Mts. were studied in detail much later, during the 1970s–1980s (Belkina et al. 1991).

The Kandalaksha Bay, with its many islands, has been thoroughly explored for 75 years due to the existence of the Kandalaksha Nature Reserve (Kozhin and Sennikov 2020). The herbarium collections from its territory are kept also in a dedicated herbarium repository in the reserve.

Another nature reserve with a long-standing record of botanical explorations is Pasvik, situated at the border with Norway. Despite its tiny territory, its vascular plants were completely inventoried three times, but only the latest revision included records of *E. acris* s.l. (Kravchenko 2020). Alien plants of this territory

were studied in the course of a transborder project that involved botanists from Norway, Finland and Russia (Alm et al. 1997).

As native vascular plants of Murmansk Region were considered rather sufficiently studied, their alien counterparts remained largely neglected (Kozhin et al. in prep.). During the latest 20 years, the effect of the revegetation of slag dumps in electric power stations and stockyards in mining factories was examined (Evdokimova et al. 2005; Kapelkina 2014; Timofeeva et al. 2016), however, without paying a proper attention to the introduction of alien plants. Their inventory in Murmansk Region has been started recently, with a few minor contributions published to date (Kozhin et al. 2020b; Kozhin and Sennikov 2022).

Taxonomic synopsis

1. *Erigeron politus* Fr. in Bot. Not. 1843: 120 (1843)

Fig. 3

- *Erigeron acris* var. *politus* (Fr.) Mela, Lyhyk. Kasvioppi Kasvio, ed. 1: 66 (1877)
- *Erigeron acris* subvar. *politus* (Fr.) Mela, Lyhyk. Kasvioppi Kasvio, ed. 2: 79 (1884)
- *Erigeron droebachiensis* var. *politus* (Fr.) Mela, Suomen Kasvio, ed. 3: 174 (1895)
- *Erigeron acris* subsp. *politus* (Fr.) H.Lindb., Enum. Pl. Fennoscand. Orient.: 56 (1901).
- = *Erigeron elongatus* Ledeb., Icon. Pl. Fl. Ross. 1: 9 (1829), nom. illeg., non Moench (1802)
- *Erigeron acris* var. *elongatus* Herder in Bull. Soc. Imp. Naturalistes Moscou 38(2): 391 (1865)
- *Erigeron acris* subsp. *elongatus* (Herder) Kindb., Svensk Fl.: 296 (1877)
- *Erigeron acris* f. *elongatus* (Herder) Mela, Lyhyk. Kasvioppi Kasvio, ed. 1: 66 (1877)
- *Erigeron droebachiensis* subsp. *elongatus* (Herder) Mela, Suomen Kasvio, ed. 3: 174 (1895). Type. RUSSIA. “Altai”, 1826, Herb. Ledebour 1308 (lectotype LE 1043841, designated here; isolectotype LE 1043843).

Type. NORWAY. “Norvegia austr. fr.,” *M. Blytt* [E.Fries, *Herbarium Normale* VIII: Suppl. no. 1b] (lectotype H 1642416, designated here). Fig. 2. Superseded neotype: SWEDEN. “Jmt. Duved,” 23.07.1931, *Th. Brandt* (LD 1367491, designated by Olander and Tyler (2017: 46)).

Description. Stems 25–40 cm tall, branched in the upper third, intensely purple-coloured to nearly green, completely glabrous or covered by scattered hairs 0.5–0.8 mm long. Cauline leaves 3–8 under the synflorescence, spaced, gradually decreasing towards the stem top, nearly glabrous on both sides, hairy mostly along margins. Synflorescence with long branches carrying solitary to 2–3 capitula, nearly corymbose at the top, glabrous or with solitary hairs. Phyllaries 6–7.5 mm long, purple-coloured completely or near the apex, covered by sparse hairs in the lower part or near the base, or nearly glabrous. Ray flowers dark-lilac to pale-pinkish. Pappus greyish-white.

Flowers in July, fruits in August.

Distribution in Murmansk Region. Khibiny Mtrs., Lovozero Mts., Turii Mys, Kutsa River, Ponoï River, Orlov Cape, Kandalaksha Gulf, Rybachii Peninsula, Ambarnaya (Pikku Maattivuono) Bay, Drozdovka Village, Chapoma Village (Fig. 4A).

Global distribution. Subarctic and Northern Boreal zones of Fennoscandia, Eastern Europe and Asia, mountains of southern Siberia (Altai).



Figure 3. Lectotype of *Erigeron elongatus* Ledeb. (LE 1043841). Courtesy of the Komarov Botanical Institute, Russian Academy of Sciences.

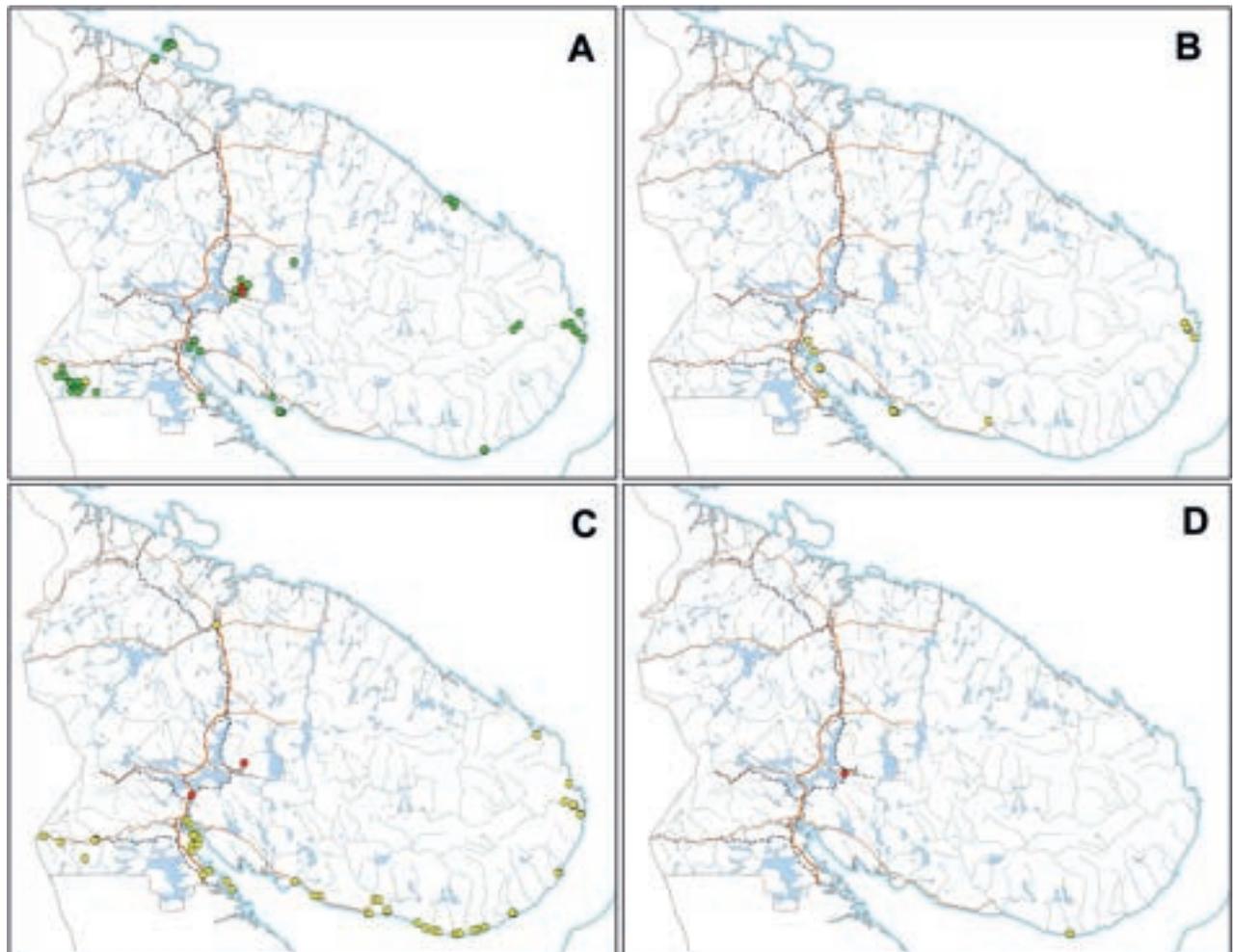


Figure 4. Distribution of the *Erigeron acris* group in Murmansk Region, Russia **A** *E. politus* F. **B** *E. xpirosiusculus* Sennikov **C** *E. rigidus* Fr. **D** *E. xintercalaris* Sennikov. Origin and residence status: green – native; yellow – pre-industrial alien; red – industrial alien. Maps were created using ArcGIS software by Esri. ArcGIS is the intellectual property of Esri and is used herein under license. Copyright Esri. All rights reserved.

Nomenclatural note. The circumstances of the valid publication of *Erigeron politus* are rather peculiar. Fries (1843a) mentioned two variants of his *E. elongatus*, which included subglabrous, presumably perennial plants of Norwegian mountains and Lapland, of which the first one corresponded to *E. droebachiensis* and the second one was deemed to be the same as *E. glabratus* in Hooker (1834) and *E. villarsii* Bellardi in Hartman (1838). A plant of the first variant was distributed by him in his exsiccatae, “Herbarium Normale” (Fries 1842). Shortly thereafter, Fries (1843b) changed his mind and decided that the first variant (*E. droebachiensis*, which he considered the same as *E. elongatus*), was annual and the second variant was perennial, and described the latter as a new species, *E. politus*. To complement the plant distributed in “Herbarium Normale” (Fries 1842), he issued a supplement to this fascicle, which contained the only number (Fries 1843c), most probably distributed along with the protologue in August 1843. This supplement seems to be very rare in collections. Olander and Tyler (2017) were unable to trace a specimen of that gathering at S and UPS, but a good specimen is available at H. This specimen is designated here as lectotype, thus superseding the neotype des-

ignated by Olander and Tyler (2017). The lectotype has dark glabrous phyllaries 6–7 mm long, 7 capitula on long branches and much exserted ray flowers (extending the pappus up to 3 mm), and thus fully corresponds to *E. politus* as accepted in Olander and Tyler (2017).

Taxonomic note. The main distribution area of *E. politus* in Murmansk Region consists of a few separate areas. Plants occurring in these areas are characterised by small but noticeable differences. Plants from the Kutsa River are exceedingly glabrous, with regularly glabrous stems and almost totally glabrous leaves, which are hairy largely along the margins; their involucre are hairy mostly at the base but forms with sparingly hairy involucre surfaces are also known. In Petsamo, plants have their involucre more regularly hairy in the basal half, and their stems are regularly but sparsely hairy. Similar plants are found in the Khibiny and Lovozero Mts., and along the Barents Sea coast. However, the plant hairiness is not completely constant, and deviating individuals can be found in all populations.

2. *Erigeron xpilosiusculus* Sennikov, sp. hybr. nov.

urn:lsid:ipni.org:names:77331060-1

Fig. 5

Type. RUSSIA. Karelian Republic: Paanajärvi, Kauppila, torr mark nära gården [= in colle sicco], 29.07.1936, H. Lindberg [Plantae Finlandiae Exsiccatae no. 1369] (holotype H 039503 pro parte [plant 1]; isotypes H 339935 pro parte, OULU 059259).

Description. Stems 25–50 cm tall, branched in the upper third, intensely to slightly purple-coloured, covered by sparse to numerous hairs 0.5–1 mm long mostly in the basal half. Cauline leaves 4–8 under the synflorescence, spaced, gradually decreasing towards the stem top, unevenly covered by sparse hairs 0.5–1 mm long on both sides. Synflorescence with long branches carrying solitary to 2–3 capitula, nearly corymbose at the top, branches subglabrous or with sparse hairs. Phyllaries 6–7.5 mm long, purple-coloured completely or near the apex, covered by sparse hairs in the basal part or up to the apex. Ray flowers dark-lilac to pale-pinkish. Pappus greyish-white.

Flowers in July, fruits in July to August. As evident from the plants collected in mixed populations, the flowering and fruiting of the hybrid occur earlier than in its native parent, *Erigeron politus*. When plants of *E. politus* start to blossom, the hybrid is already in the last flowers. Distribution in Murmansk Region. Kandalaksha Gulf, Turii Mys, Varzuga River (lower course), Ponoï River (lower course) (Fig. 4B).

Global distribution. Subarctic and Northern Boreal zones of Fennoscandia.

Etymology. The species epithet, meaning 'slightly more hairy' (*pilosior*, Lat.: more hairy; *-usculus*, Lat.: diminutive suffix), was selected to reflect a slightly greater hairiness of the hybrid in comparison to its more glabrous parent, *E. politus*.

Nomenclature note. The type collection is taxonomically mixed. It contains typical plants of *E. rigidus* and the hybrid, which is less hairy and slightly less vigorous. This collection was distributed by Lindberg (1944) in his exsiccatae but its specimens were formed by chance: some appear to contain plants



Figure 5. Holotype of *Erigeron pilosiusculus* Sennikov (H 039503, plant 1). Courtesy of the Finnish Museum of Natural History, University of Helsinki.

of *E. rigidus* only (H 039491), some belong only to the hybrid (OULU 059259), whereas the others may be mixed on the same sheet (H 339935).

Taxonomic note. The morphology of this taxon is intermediate between *E. rigidus* and *E. politus*. Such plants typically have stems and leaves rather hairy, sometimes close to the pubescence of *E. rigidus* but never as dense and abundant as in the latter. On the other hand, its involucre highly resemble those of *E. politus* but are very sparsely covered by hairs. Because of this intermediacy, such plants were identified either as *E. politus* or as *E. rigidus*, likely depending on which part of the plant was more closely observed. We cannot refer these intermediate plants to any of the species, and therefore assume their hybrid origin, which requires a separate taxonomic placement as proposed here.

The distribution of the alleged hybrids lies completely within the area of intense anthropogenic influence, whereas only typical plants of *E. politus* were observed in the areas of its presumably native distribution (higher mountains in the centre of the Kola Peninsula and the Kutsa River basin). We consider this distribution pattern as a strong evidence for the anthropogenic origin of the presumed hybrid, which was formed within the area to which both native and alien taxa of the *E. acris* group were transported by humans.

3. *Erigeron rigidus* Fr., Novit. Fl. Suec. Mant. III: 107 (1843)

Fig. 6

- *Erigeron acris* var. *rigidus* (Fr.) A. Blytt, Norges Fl. 2: 562 (1874) – *Erigeron politus* subsp. *rigidus* (Fr.) Jørg. in Forh. Vidensk.-Selsk. Kristiania 1894(8): 27 (1894).
- = *Erigeron acris* var. *ruber* Hartm., Handb. Skand. Fl., ed. 1: 315 (1820). Type. SWEDEN. Lule lappmark, S.N. Casström (holotype S, not traced).

Type. NORWAY. Filefjell: Nystuen, M. Blytt (lectotype UPS, designated here).

Description. Stems 25–50 cm tall, branched in the upper third, intensely to slightly purple-coloured, evenly covered by numerous hairs 0.5–1 mm long. Cauline leaves 4–8(12) under the synflorescence, spaced, gradually decreasing towards the stem top, completely covered by numerous hairs 0.5–1 mm long on both sides but subglabrous at the base below. Synflorescence with long branches carrying solitary to 2–3 capitula, nearly corymbose at the top, with numerous hairs 0.4–0.7 mm long. Phyllaries 6–7.5 mm long, purple-coloured completely or in the apical part, rather densely covered by hairs up to 0.5–0.8 mm long. Ray flowers intensely lilac. Pappus greyish-white.

Flowers in July to August, fruits in August.

Distribution in Murmansk Region. Coastal area of the White Sea, road from Alakurtti to Salla and Vuorijarvi Village, isolated at Kirovsk Town, Zasheyek Village and Kola Town (Fig. 4C).

Global distribution. Boreal zone of Fennoscandia and Eastern Europe, southern limit unknown.

Nomenclature note. Fries (1843a) mentioned two areas from which his new species was described, Filefjeld in Norway and Norrland in Sweden. The Norwegian report was based on a single specimen collected by M. Blytt in Nystuen and cited in the protologue, which is a syntype. The basis for the Swedish part



Figure 6. Lectotype of *Erigeron rigidus* Fr. (UPS). Courtesy of the Museum of Evolution, Uppsala University.

of the distribution area was not specified in the protologue but Fries indicated by an exclamation mark that he had seen some (otherwise uncited) material. The specimen collected by Blytt has been traced at UPS (Hjertson, pers. comm.) and is designated as lectotype here. Hartman (1820) described *Erigeron acris* var. *ruber* Hartm. from Swedish Lapland, which was briefly characterised by “dark-red” ligulate flowers. This character indicates that the plant was intensely purple-coloured; together with its occurrence in Lapland, this character unambiguously points at *E. rigidus*. Quite exceptionally in those times, the protologue of *E. acris* var. *ruber* Hartm. (Hartman 1820) included citation of a single specimen collected by Samuel Niclas Casström, which is apparently the holotype. The collections of Casström were bequeathed after his death to the Swedish Museum of Natural History (Lindman 1916), where the holotype should be currently kept (not traced).

Taxonomic note. This species is most similar to *Erigeron acris* s.str., from which it differs in typically red stems and phyllaries, and in sparser and shorter pubescence on stems, leaves and phyllaries. Its distribution area remains unknown due to the ongoing confusion with *E. acris* s.str.; so far, we feel certain to state that *E. rigidus* is common in southern Finland and Karelia, together with *E. acris* s.str., but goes farther northwards than the latter species. In Central and Southern Europe there is another similar taxon, *E. muralis* Lapeyr. (= *E. serotinus* Weihe), which apparently differs in its habit and much denser foliage ((10)17–27(40) stem leaves in *E. muralis* vs. 4–8(12) stem leaves in *E. rigidus*) (Šída 2004). Besides, *E. rigidus* flowers together with *E. acris*, whereas the flowering of *E. muralis* occurs much later (Šída 2001)

4. *Erigeron* ×*intercalaris* Sennikov, sp. hybr. nov.

urn:lsid:ipni.org:names:77331061-1

Fig. 7

Type. RUSSIA. Karelian Republic: Louhi District, “Paanajärvi, Rajala, vägkant vid Mäntyjoki” [= northern side of Paanajärvi Lake, roadside between formerly populated places], 22.07.1936, H. Lindberg (holotype H 039504).

Description. Stems 30–50 cm tall, branched in the upper third or half, intensely to slightly purple-coloured, evenly covered by numerous hairs 1–2 mm long. Cauline leaves 4–8 under the synflorescence, spaced, gradually decreasing towards the stem top, completely covered by numerous hairs 0.5–1(1.5) mm long on both sides or subglabrous at the base below. Synflorescence with long branches carrying solitary to 2–3 capitula, nearly corymbose at the top, with numerous hairs 0.8–1 mm long. Phyllaries 6–7.5 mm long, purple-coloured completely or in the apical part, abundantly covered by hairs 1–2 mm long. Ray flowers intensely lilac. Pappus greyish-white.

Flowers in July to August, fruits in August.

Distribution in Murmansk Region. Tetrino Village, Apatity Town (Fig. 4D).

Global distribution. Expected in the Boreal zone of Fennoscandia and Eastern Europe.

Etymology. The species epithet (*intercalaris*, Lat.: intercalary) reflects the intermediate morphology of the hybrid between its presumed parents. Nomenclatural note. The type locality has been extensively sampled for *Erigeron*



Figure 7. Holotype of *Erigeron xintercalaris* Sennikov (H 039504). Courtesy of the Finnish Museum of Natural History, University of Helsinki.

plants, which were distributed by Lindberg (1944) in his exsiccatae. The material from the Mäntyjoki River distributed as *Plantae Finlandiae Exsiccatae* 1370 is taxonomically heterogeneous: a few plants of *E. acris* s.str. (H 339936 pro parte) were mixed with abundant collections of its hybrid with *E. rigidus* (H 339936 pro parte, H 039487, OULU 059260). The latter specimens are paratypes of our *E. xintercalaris*. The hybrid plants look very slender and depressed, much less vigorous than the specimens of *E. acris* s.str. collected in the same locality, thus probably indicating outbreeding depression (Bleeker et al. 2007).

Taxonomic note. The hybrid differs from *E. acris* in a regular purple colouration of its stems and phyllaries, and in a shorter and sparser pubescence on its leaves and stems. It differs from *E. rigidus* in a denser and longer hairiness of its stems, leaves and phyllaries, and in a lesser purple colouration of its stems and phyllaries.

5. *Erigeron acris* L., Sp. Pl. 2: 863 (1753).

Type. Probably southern Sweden. Herb. Linnaeus 994.16 (lectotype LINN, designated by Huber (1993: 44)).

Description. Stems 25–40 cm tall, branched in the upper half, green or slightly to rather intensely purple-coloured, evenly covered by abundant hairs 1–1.3(1.5) mm long. Cauline leaves 5–10 under the synflorescence, spaced, gradually decreasing towards the stem top, completely covered by numerous hairs 0.5–1 mm long on both sides. Synflorescence with long branches carrying solitary to 2–3 capitula, nearly corymbose at the top, with numerous hairs 0.4–0.7(1) mm long. Phyllaries 6–7.5 mm long, green or purple-coloured on the tips, completely covered by hairs up to 0.7–1 mm long. Ray flowers pale-pink. Pappus greyish-white.

Flowers in July to August, fruits in August.

Distribution in Murmansk Region. Kandalaksha Town, Nivsky Village, Kandalaksha and Apatity industrial areas, Apatity Town, Pasvik, Tetrino Village (Fig. 8A).

Global distribution. Boreal, Hemiboreal and Temperate zones of Europe and Siberia. Nomenclatural note. The lectotype specimen at LINN was not labelled but most likely was collected by C. Linnaeus himself in Uppsala, Sweden. This specimen is a very typical representative of the species, being a greenish plant with abundant long hairs.

Taxonomic note. This species is characteristic for its overall green colour of stems, leaves and phyllaries, with a red tint being present mostly at the stem base and on the tips of the phyllaries. The plant habit is the same as in *E. politus* and *E. rigidus*, with rather few sparse leaves on the stem. Another typical feature of this species is a long and dense pubescence, covering all parts of the plant (stems, leaves and phyllaries).

6. *Erigeron droebachiensis* O.F.Müll., Fl. Dan. 5(15): 4, tab. 874 (1782)

– *Erigeron acris* var. *droebachiensis* (O.F.Müll.) Willd., Sp. Pl., ed. 3, 3(3): 1959 (1803) – *Erigeron acris* subsp. *droebachiensis* (O.F.Müll.) Mela, Lyhyk. Kasvioppi Kasvio, ed. 1: 66 (1877).

= *Erigeron acris* var. *angustatus* Hartm., Handb. Skand. Fl., ed. 1: 315 (1820) – *Erigeron acris* subsp. *angustatus* (Hartm.) Fr., Novit. Fl. Suec. Mant. III: 107 (1843) – *Erigeron acris* f. *angustatus* (Hartm.) Fr., Summa Veg. Skand. 1: 183 (1846). Type. [icon] Flora Danica, tab. 874 (1782) (lectotype designated here).

Type. [icon] Flora Danica, tab. 874 (1782) (lectotype designated here). Fig. 9. Epitype (designated here): NORWAY. Ringerike, 05.07.1892, J. Dyring (H 1642568). Fig. 10.

Description. Stems 30–70 cm tall, branched in the upper third, green or slightly purple-coloured, sparsely covered by numerous hairs 0.5–1 mm long in the basal third or nearly glabrous. Cauline leaves 12–20 under the synflorescence, sparse or slightly congested, gradually reduced towards the stem top, middle and lower ones covered by numerous hairs 0.3–0.8(1) mm long on both sides or along margins only. Synflorescence with rather short branches carrying few to several capitula, racemose in shape, branches glabrous or with solitary hairs 0.3–0.4 mm long. Phyllaries 5.5–6 mm long, slightly or moderate-

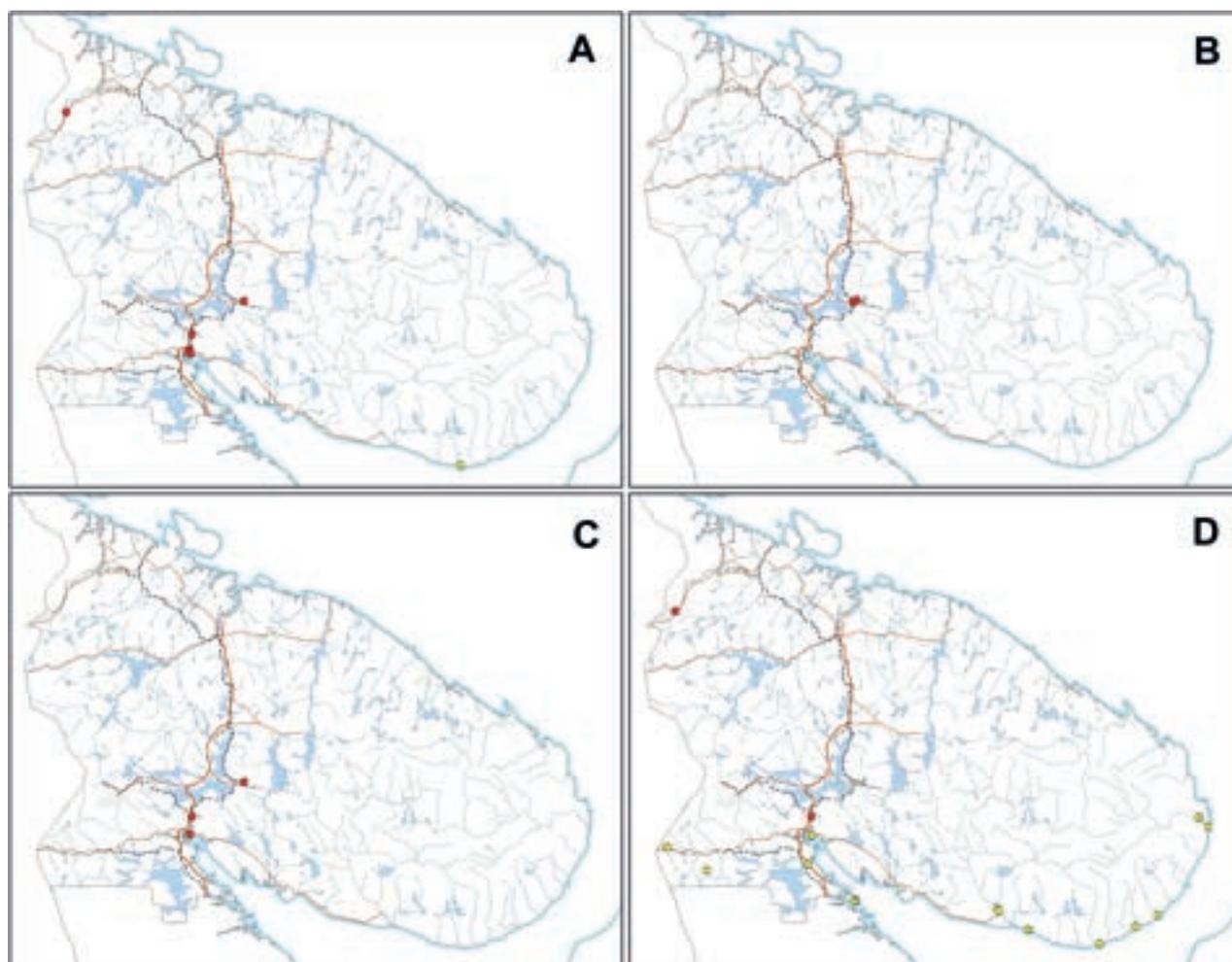


Figure 8. Distribution of the *Erigeron acris* group in Murmansk Region, Russia **A** *E. acris* L. **B** *E. droebachiensis* O.F.Müll. **C** *E. uralensis* Less. **D** *E. brachycephalus* H.Lindb. Origin and residence status: yellow – pre-industrial alien; red – industrial alien. Maps were created using ArcGIS software by Esri. ArcGIS is the intellectual property of Esri and is used herein under license. Copyright Esri. All rights reserved.



Figure 9. The original illustration (lectotype) of *Erigeron droebachiensis* O.F.Müll. Reproduced from Müller (1782: tab. 874).



Figure 10. Epitype of *Erigeron droebachiensis* O.F.Müll. (H 1642568). Courtesy of the Finnish Museum of Natural History, University of Helsinki.

ly purple-coloured, outer and middle ones sparsely covered by hairs 0.5–1 mm long at base or on the basal half, innermost ones glabrous. Ray flowers pink. Pappus greyish-white.

Flowers in July, fruits in August.

Distribution in Murmansk Region. Apatity industrial area (Fig. 8B).

Global distribution. Boreal and Hemiboreal zones of Fennoscandia and Eastern Europe, southern limit unknown.

Nomenclatural note. The species name is derived from Drøbak, now a town in Viken County, Norway, which is the original locality of the species (Müller 1782). This derivation implied the Latinisation of this place name as “Droebachia”, from which “droebachiensis” is produced by analogy with e.g. “hafniensis” that was derived from “Hafnia”, i.e. Copenhagen (Stearn 1966). The species epithet “droebachiensis” is therefore grammatically correct and cannot be changed to “droebachensis” as used in PoWO (2023), which would imply a different Latinisation as “Droebachum”. No original herbarium collections of *Erigeron droebachiensis* have been traced in Denmark (Ryding, pers. comm.) and Norway (Salvesen, pers. comm.). The only extant original element on which the species name was based is the illustration published in the protologue (Müller 1782). We agree that the original plant described by Müller was a glabrous taxon with corymbose synflorescences occurring as native in Fennoscandia, which was recognised in a similar way by other modern researchers (Tzvelev 1994; Kurtto and Väre 1998). Tzvelev (2001) attempted to radically change the application of the name *E. droebachiensis*, which he suggested to apply to a hybrid between *E. acris* s.l. and *E. canadensis* L., otherwise known as *E. xhuelsenii* Vatke (Seregin 2015b). This erroneous application affected some Russian collections and literature (Seregin 2005, 2010, 2015a) but gained no recognition elsewhere. Although we agree with Olander and Tyler (2017) that the original illustration of *E. droebachiensis* unambiguously represents the species, its identity is far from apparent to those who are not familiar with the *Erigeron acris* group in Scandinavia. This is evident by the gross misinterpretation of this illustration by Tzvelev (2001), and by the uncertainty expressed by Šída (1998). To avoid further doubts and debates, we formally designate the illustration as a lectotype of *E. droebachiensis*, and support this illustration by an epitype collected in Ringerike, a traditional district situated at the distance of 50 km from Drøbak. The epitype specimen is nearly glabrous, except for the basal part of stems and capitula, and also leaf margins. A larger plant of this specimen agrees with the original illustration in a branched paniculate synflorescence, long leaves and long-exserted ligules. A smaller plant attached to the same sheet agrees with the larger plant in the pubescence and represents its reduced variant with unbranched stems, shorter leaves and a raceme-like synflorescence. *Erigeron acris* var. *angustatus* Hartm. was described (Hartman 1820) without any original locality indicated in the protologue. One diagnostic character of this variety (small stalked flowering heads) indicated the racemose synflorescence; the second character (larger apical capitulum) was derived from the diagnosis of *E. droebachiensis*. Subsequently Hartman (1838) explicitly noted that this variety corresponds to *E. droebachiensis*, whose illustration (but not the name itself) was cited in the protologue, and we designate this illustration as the lectotype of Hartman’s

variety. Fries (1843b) elevated this variety to the subspecies level, thus creating the earliest available name at this rank.

Taxonomic note. The distribution of *Erigeron droebachiensis* outside Fennoscandia is partly obscured due to its common confusion with other taxa of the *E. acris* group. Šída (1998) presumed that this species may turn to be identical to *E. macrophyllus* Herbich, which occurs in Central and Southern Europe, although the latter is characterised by more numerous and dense cauline leaves, which are 20–45 in number (Tzvelev 1994; Šída 2001).

7. *Erigeron uralensis* Less. in *Linnaea* 9: 186 (1834)

Fig. 11

– *Erigeron acris* var. *microcephalus* Ledeb., Fl. Ross. 2(2,6): 489 (1845).

Type. RUSSIA. Chelyabinsk Region: “Zlatoust”, 07.1832, C.F. Lessing (lectotype LE 01043675, designated here; isolectotype LE 01043674).

Description. Stems 30–50 cm tall, branched in the upper third, intensely to slightly purple-coloured, sparsely covered by numerous hairs 0.5–0.8 mm long. Cauline leaves 8–12 under the synflorescence, sparse or slightly congested, noticeably reduced towards the stem top, very sparsely covered by numerous hairs 0.3–0.5 mm long on both sides (nearly glabrous in the middle part). Synflorescence with rather short branches carrying few to several capitula, racemose in shape, with rather sparse hairs 0.3–0.4 mm long. Phyllaries 5.5–6 mm long, slightly or moderately purple-coloured, outer and middle ones sparsely covered by hairs up to 0.5–1 mm long, innermost ones with solitary hairs. Ray flowers pink. Pappus greyish-white.

Flowers in July to August, fruits in August.

Distribution in Murmansk Region. Kandalaksha and Apatity industrial areas (Fig. 8C).

Global distribution. Boreal and Hemiboreal zones of Fennoscandia and Eastern Europe, Ural Mts.

Nomenclatural note. The species was described on the basis of a single herbarium collection from Zlatoust Town, Chelyabinsk Region, Russia (Lessing 1834). Ledebour (1845) cited a specimen of the original collection at the Berlin Botanical Garden, which is no longer extant. Two other specimens are preserved at the Komarov Botanical Institute in Saint-Petersburg, of which one is selected here as lectotype.

Taxonomic note. Tzvelev (1994) recognised a single species with numerous capitula on short branches in the Russian North, which he named *E. uralensis* and considered to include a few other previously described species. Among these synonyms, *E. decoloratus* H.Lindb. and *E. elongatiformis* Novopokr. ex Serg. were apparently added in error because they belong to the group with corymbose synflorescences (few larger heads on longer branches), whereas *E. brachycephalus* shares all essential characters with the type collection of *E. uralensis* (paniculate synflorescence with numerous heads on shorter branches, sparse pubescence on involucre and synflorescence branches). This species is seemingly distributed from Eastern Finland (Mäkelä 1980) to the



Figure 11. Lectotype of *Erigeron uralensis* Less. (LE 01043675). Courtesy of the Komarov Botanical Institute, Russian Academy of Sciences.

Ural Mountains (Lessing 1834) and Siberia (Tzvelev 1994). Although the original material of *E. brachycephalus* largely includes specimens of *E. uralensis*, its designated lectotype (Väre 2012) differs in the density of pubescence and should be referred to another taxon. These species names are therefore not synonyms.

8. *Erigeron brachycephalus* H.Lindb., Sched. Pl. Finland. Exsicc. Fasc. 21–42: 88 (1944)

– *Erigeron acris* subsp. *brachycephalus* (H.Lindb.) Hiitonen in Ann. Bot. Fenn. 8(1): 78 (1971).

Type. RUSSIA. Leningrad Region: “Isthmus Karelicus, par. Metsäpirtti [now Priozersk District], Taipale [now Solovievo], in campo sicco una cum *E. acris* (n. 1371) crescens”, 26.06.1934, H. Lindberg [Plantae Finlandiae Exsiccatae no. 1372] (lectotype H 340008, designated by Väre (2012: 41); isolectotype H 758234 pro parte).

Description. Stems 30–50 cm tall, branched in the upper third, intensely to slightly purple-coloured, rather densely covered by numerous hairs 0.6–1 mm long. Cauline leaves 8–14 under the synflorescence, rather congested, noticeably reduced towards the stem top, completely covered by numerous hairs ca. 0.5 mm long on both sides. Synflorescence with rather short branches carrying few to several capitula, racemose in shape, with abundant hairs 0.2–0.4(0.5) mm long. Phyllaries 5.5–6 mm long, slightly or moderately purple-coloured, outer and middle ones moderately covered by hairs up to 0.5–0.8 mm long, innermost ones with sparse to rare hairs. Ray flowers bright-pink. Pappus greyish-white.

Flowers in July to August, fruits in August.

Distribution in Murmansk Region. Coastal area of the White Sea, Vuorijarvi and Kuolajarvi Villages, Nivsky Village, isolated in Pasvik (Fig. 8D).

Global distribution. Boreal zone of Fennoscandia and Eastern Europe, southern limit unknown.

Nomenclatural note. The lectotype collection of *Erigeron brachycephalus* is taxonomically mixed. The designated lectotype at H (Väre 2012) belongs to the more hairy taxon (*E. brachycephalus* s.str. as defined in our work), whereas its presumed duplicates at OULU and S belong to the less hairy taxon, *E. uralensis*. A duplicate at H is mixed, with both taxa mounted together. Although Lindberg (1944) described his new species as “usually” less hairy than *E. acris* s.str., by adding the word “usually” he apparently included also more hairy plants as casual variants. Further collections included into the original circumscription of *E. brachycephalus* as other syntypes belong to even more deviating taxa, e.g. *E. droebachiensis*. Although the lectotype specimen of *E. brachycephalus* is different from the other parts of this collection examined by us, it cannot be treated as incongruent with the protologue because of a broader taxonomic circumscription used by Lindberg (1944).

Taxonomic note. This species is most similar to *E. uralensis*, into which it has been recently included (Tzvelev 1994). It differs from the latter in a constantly much denser and more regular pubescence on stems, synflorescence branches, leaves and involucre, and by a regular red colouration of the whole plant.

Prior to its scientific recognition, this taxon went under the collective name *E. droebachiensis* in Finland (Lindberg 1938). It was originally collected with the co-occurring *E. acris* s.str., from which it was distinguished by the paniculate synflorescence, a greater number of smaller heads, a much lesser development of pubescence and a later flowering period (Lindberg 1944).

Identification key

- 1 Well-developed synflorescences paniculate (compound raceme), lower leaf axils with compact raceme-like branches; phyllaries 5.5–6 mm long..... **2**
 - Well-developed synflorescences corymbose, lower leaf axils with single or few capitula on long stalks; synflorescence branches glabrous or with abundant hairs; phyllaries 6–7.5 mm long **4**
- 2 Synflorescence branches glabrous or with solitary hairs; outer and middle phyllaries basally or in the basal half with sparse hairs ... ***E. droebachiensis***
 - Synflorescence branches with sparse to abundant hairs; outer and middle phyllaries hairy up to their top **3**
- 3 Synflorescence branches with abundant hairs; outer and middle phyllaries moderately covered by hairs up to 0.5–0.8 mm long..... ***E. brachycephalus***
 - Synflorescence branches with sparse hairs; outer and middle phyllaries sparsely covered by hairs up to 0.5–1 mm long ***E. uralensis***
- 4 Outer and middle phyllaries glabrous or with few hairs scattered in the basal part, inner ones without hairs; synflorescence branches glabrous or with solitary hairs; cauline leaves usually subglabrous, with hairs confined to leaf margins..... ***E. politus***
 - Outer and middle phyllaries with numerous hairs covering at least their basal half; synflorescence branches with numerous or abundant short hairs; cauline leaves with abundant short hairs along the whole surfaces..... **5**
- 5 Phyllaries usually violet; outer and middle phyllaries rather densely covered by hairs up to 0.5–0.8(1) mm long; stems completely violet, with numerous hairs 0.5–1(1.5) mm long ***E. rigidus***
 - Phyllaries green, apically violet; outer and middle phyllaries completely covered by abundant hairs up to 0.7–1(1.5) mm long; stems violet at the base or in the lower half, with abundant hairs 1–1.5(2) mm long..... ***E. acris***

Excluded taxa

During the whole history of botanical studies, some populations of the *Erigeron acris* group occurring in Murmansk Region were reported under wrong names.

Quite commonly taxa were treated in very broad circumscriptions; such examples are *E. acris* s.l. that included either the whole complex or its hairy representatives, or *E. politus* that included its hybrids with *E. rigidus*. Such misidentifications are too impractical to mention because of their exceedingly high number.

Sometimes, more precise identifications were published, which were mostly wrong due to vague taxonomic concepts of the past. Such identifications were rather few, and such species names are in current use for narrowly defined taxa. We traced the background for these wrong records in order to provide their correct identity (Table 1).

Table 1. Rejected historical records in the *Erigeron acris* group, their background and accepted identity.

Published name	Source	Basis of records	Our identification	Comments
<i>E. acris</i> L.	Botschantzev 1959	LE 01102450, LE 01102451	<i>E. ×intercalaris</i>	these specimens are very similar to <i>E. acris</i> s.str.
<i>E. acris</i> L.	Orlova 1966	many specimens at KPABG	mostly <i>E. rigidus</i> and its hybrids, <i>E. brachycephalus</i> , one specimen of <i>E. acris</i> s.str.	including all hairy taxa of <i>E. acris</i> s.l.
<i>E. acris</i> L.	Tzvelev 1994	LE 01102456	<i>E. rigidus</i>	also the material used in Botschantzev (1959)
<i>E. acris</i> L.	Ulvinen 1996	OULU 158562	<i>E. rigidus</i>	<i>E. acris</i> s.str. is absent in this territory
<i>E. acris</i> L.	Kravchenko 2020	TROM 54773	<i>E. brachycephalus</i>	his second record belongs to <i>E. acris</i> s.str.
<i>E. decoloratus</i> H.Lindb.	Ulvinen 1996	H 039483 H 039484	<i>E. politus</i>	mere misidentification
<i>E. droebachiensis</i> O.F.Müll.	Fellman 1864	H 846840 LE 01102478	<i>E. brachycephalus</i>	plants with racemose synflorescences
<i>E. droebachiensis</i> O.F.Müll.	Mäkelä 1980	H 340138 H 340141 H 340142 H 340146	<i>E. rigidus</i>	also the material at H used by Fellman (1864)
<i>E. uralensis</i> Less.	Tzvelev 1994	LE 01102459	<i>E. politus</i>	slender plant with longer branches

Discussion

Diagnostic characters, their value and variability

Although we relied upon diagnostic characters on which the previous works (Tzvelev 1994; Kurtto and Väre 1998; Šída 1998, 2000; Olander and Tyler 2017) have been based, every character was reassessed in the course of our revision.

Easy to catch is the feature of purple colouration, which may affect all vegetative parts of the plant: stems, leaves and phyllaries. We found this character to be of good subsidiary value: although it may quite widely vary in plants of the same taxon being nearly green to completely purplish (e.g. *E. politus*), it may reliably serve for primary diagnostics between *E. rigidus* (purplish) and *E. acris* s.str. (green).

Synflorescence shape (corymbose vs. racemose) is found to be a strong and highly reliable character, in agreement with the work of Šída (1998, 2000, 2004). In weak plants, synflorescences may be reduced and their shape may appear uncertain; in such cases, this character can be inferred from the length of pedicels (synflorescence branches) and the number of capitula, as already used by some researchers (Tzvelev 1994; Kurtto and Väre 1998).

Size of capitula, measured as length of phyllaries, is an important character apparently correlating with the synflorescence shape. It can be used as a proxy for the latter, too.

Number, shape and density of cauline leaves have been commonly used to distinguish between some taxa in Central and southern Eastern Europe (Tz-

velev 1994; Šída 1998, 2000, 2004), which may differ in the density of foliage and ultimately the absolute number of stem leaves below the synflorescence. Although these characters are undoubtedly useful in the *E. acris* group, their use in the European North is rather limited because of the lack of the densely leaved taxa. In general, our plants with corymbose and racemose synflorescences may differ in the number of cauline leaves and their density, but this difference is not so prominent and this character can be used as auxiliary here.

Flower colour (ligulate flowers) varies between pale and dark lilac, rarely (in *E. decoloratus*) white ligules were observed in plants outside the study area. We noticed that this character correlates with the purple colouration of stems and phyllaries and is therefore similarly variable, and sometimes may vary within a single plant when one branch is purplish and the other is greenish. For this reason we do not give a separate diagnostic value to this character.

Length of ligulate flowers was sometimes used (Fries 1846; Olander and Tyler 2017) but we found this character variable within the same taxon. Its value is rather uncertain.

Pubescence (presence or absence of simple hairs) is considered another primary taxonomic character (Tzvelev 1994; Kurtto and Väre 1998; Šída 1998, 2000; Olander and Tyler 2017). Plants in the *E. acris* group differ remarkably in the density and length of pubescence on stems, leaves and phyllaries. Despite a certain level of variability, we find this character reliable in distinguishing taxa within the groups with different types of synflorescence, with the hairiness ranging from extremely sparse (*E. politus*, *E. droebachiensis*) through moderate (*E. rigidus*, *E. uralensis*) to abundant (*E. acris* s.str., *E. brachycephalus*). The length of pubescence is important to help distinguishing between some taxa with shorter (*E. rigidus*, *E. brachycephalus*) and longer (*E. acris* s.str.) hairs, and



Figure 12. Flowering heads of the *Erigeron acris* group in Murmansk Region **A** *E. politus* Fr. (KPABG 040062) **B** *E. xpilosusculus* Sennikov (KPABG 039993) **C** *E. rigidus* Fr. (KPABG 040017) **D** *E. xintercalaris* Sennikov (KPABG 039998) **E** *E. acris* L. (KPABG 043965) **F** *E. droebachiensis* O.F.Müll. (KPABG 047662) **G** *E. uralensis* Less. (KPABG 043994) **H** *E. brachycephalus* H.Lindb. (KPABG 040025).

can be used when the other characters are expressed ambiguously. The differences in pubescence are most easily observable on the involucre (Fig. 12).

Achene characters are difficult to use in herbarium specimens, which are collected mostly in flower (Olander and Tyler 2017). However, we noticed that achene hairiness may vary within the same narrowly defined taxon, and the diagnostic value of this character is therefore doubtful.

Comparisons of taxonomic concepts

The first attempt to classify the diversity of the *Erigeron acris* group was made by Müller (1782) who described *E. droebachiensis* from a single locality in Viken County, Norway. This species was separated not because of the advanced knowledge in this taxonomic group; instead, it was compared with *E. canadensis* L., a distantly related species which appears to be only superficially similar because of its racemose synflorescence.

Early botanical works recognised only a single species in the *E. acris* group, but its apparent morphological variability was reflected in varieties. Hartman (1820) noted some differences in the size of flowering capitula and the colour of ligulate flowers as taxonomically significant characters; at the same time, he merged the previously described *E. droebachiensis* even without a note on its name.

Elias Fries (1843a, 1843b, 1846) recognised several taxa in this group, at the level of species and below. He used several characters to ground his taxonomy, including synflorescence shape, size of capitula, features of foliage, pubescence of all parts of the plants, ligulate flower colour and length. Most notably, he distinguished between the southern *E. acris* s.str. with an abundant soft pubescence and the northern *E. rigidus* with sparser stiff hairs, the distinction being of a wide phylogeographic importance but commonly neglected in later taxonomic works.

Fellman (1864) reported *E. droebachiensis* from Russian Lapland; however, this was not the taxon in our current understanding.

Mela (1877, 1884, 1895, 1899) developed a complicated taxonomic classification for the *E. acris* group in Finland, in which he recognised 10 infraspecific taxa at the level of subspecies, variety, subvariety or forma. His primary distinction was laid between *E. acris* s.l. and *E. droebachiensis* s.l., which he distinguished on the basis of hairiness and subdivided further for the size and colour of capitula and minor details of pubescence. The two-taxon system in the *E. acris* group developed by Mela was used in contemporary Finnish publications (e.g., Saelan et al. 1889). His elevation of taxonomic ranks in this group (Mela 1895, 1899), giving the species status to *E. droebachiensis*, was not supported by other botanists.

Botschantzev (1959) attempted to revise the *E. acris* group in the USSR. He recognised only two taxa in the European North, the glabrous *E. politus* and the hairy *E. acris*, between which abundant hybrids were allegedly found. His treatment was based on the collections at LE. The first detailed taxonomic revision of this group in Murmansk Region was provided by Orlova (1966), who followed Botschantzev (1959) in the taxonomy. Orlova revised the collections at KPABG and provided point distribution maps of both taxa accepted in the territory.

Tzvelev (1994) provided a new taxonomic treatment of the *E. acris* group in the European part of the USSR. In the North, he maintained the two taxa rec-

ognised by his predecessors in the same circumscription but added the ill-defined collective species *E. uralensis*, which he distinguished by its numerous flowering heads (paniculate synflorescence) and believed to have originated from interspecific hybridisation between *E. politus* and *E. acris*. He collected three synonyms under this species name: *E. brachycephalus*, *E. decoloratus* and *E. elongatiformis*. By doing so, Tzvelev disregarded the character of paniculate vs. corymbose synflorescences (*E. brachycephalus* and *E. uralensis* have paniculate synflorescences, whereas *E. decoloratus* and *E. elongatiformis* have corymbose synflorescences) and apparent differences in pubescence. He used many diagnostic characters to distinguish between his species, and the number of taxa that he accepted was the highest among all taxonomic revisions to date. Nevertheless, the inconsistent use of morphological characters in his treatment did not allow him to achieve a clear picture of plant taxonomy and distributions.

The latest Finnish synopsis of the *E. acris* group (Kurtto and Väre 1998) was most detailed in Eastern Europe. It accepted five subspecies of *E. acris* s.l. on the basis of pubescence, presence or absence of a purple tint, and number of flowering heads. The subspecies were assessed according to their residence status, as native or alien (archaeophyte), and mapped according to traditional biogeographic provinces of East Fennoscandia, thus revealing their distribution patterns. This treatment was quite fairly and accurately set, except for the broad treatment of *E. acris* subsp. *acris* (which included *E. rigidus*) and the lack of recognition of hybrids.

Synflorescence shape was considered a primary character by Šída (1998, 2000, 2004), who based the uppermost-level division in his classification (ranked as series: *E. ser. Trimorpha* (Cass.) Šída, *E. ser. Macrophylli* Šída) on this character but also added a separate series (*E. ser. Politi* Šída) to accommodate completely glabrous plants. The main classification rank in this system was species. Šída provided a brief overview of the *E. acris* group in the whole of Eurasia (Šída 1998) and a detailed taxonomic treatment for the Czech Republic (Šída 2000, 2004). He precisely defined the species and mentioned the presence of hybrids. His work provided highly useful insights for our treatment but could not be used at the species level.

The latest taxonomic revision of the *E. acris* group in Sweden, with taxonomic implications for Fennoscandia (Olander and Tyler 2017), was based on a complex statistical analysis of numerous measures rather than traditional observations. Olander and Tyler (2017) believed that their accepted taxa (ranked as subspecies) may be distinguished by a combination of the characters of pubescence and dimensions (of stems, leaves and phyllaries, also including leaf and capitula number). However, they failed to observe minute differences in pubescence between *E. rigidus* and *E. acris*, and also reduced *E. brachycephalus* to a synonym of *E. droebachiensis* despite their apparently different types of pubescence, probably because they had insufficient material for examination.

Phenology

The available herbarium collections indicate that flowering period may be highly variable due to the differences in particular season, vegetation zone or even local conditions. In the same locality but in different years, plants of the same species may start flowering with a difference as high as a month. At the same

time, plants collected within one day in the same place may show differences corresponding to one week of observations within a single population. Plants collected in different vegetation zones (tundra vs. taiga) may start flowering with a delay of two weeks or even greater, whereas the flowering period may be quite short and limited to two or three weeks. This makes summarily observations within the whole territory of Murmansk Region practically meaningless.

At the same time, plants of different taxa may develop in clearly different periods when observed as co-occurring within the same locality. In this case, plants of *E. rigidus* and its hybrids may develop significantly earlier than those of *E. politus*, whereas plants of *E. brachycephalus* start flowering apparently later than those of *E. rigidus*. Since in such cases the difference in flowering periods is approximately a week or less, the summary observations may practically coincide.

Geographical distributions

Distribution patterns of each species in the *Erigeron acris* group are individual. Although some species may co-occur in the same locality and may share some part of their history of dispersal, their main sources and drivers seem to be different.

Due to a considerable confusion between the segregate taxa in this group even in the most detailed treatments (Mäkelä 1980; Tzvelev 1994), their major distribution areas remain partly obscure. The lack of separation between the native and secondary parts of distribution areas in these works makes the identification of their origin even more difficult.

Erigeron politus is a native taxon, which occurs in mountains and uplands, and in some fjords and river ravines along sea coasts in Murmansk Region. This species is a largely subarctic (oroarctic) plant in Fennoscandia (Mossberg and Stenberg 2018). Its secondary dispersal is very minor and limited to short-distance transfer of diaspores from native populations to neighbouring anthropogenous habitats (roadsides, waste lands, populated places). The species is favoured by disturbance and can be found growing rather abundantly in industrial waste lands.

The present-day distribution of *E. rigidus* in Murmansk Region seems to be a fair reflection of its historical dispersal. The species distribution is limited to two major areas: the entire White Sea coast with adjacent islands, which was a traditional area of the Pomors economy, and the Alakurtti–Salla road, which was a historical traffic route between northern Russia and Finland (Fig. 13A). Two isolated localities can be explained by further dispersal: Kola (by historical trade) and Apatity (more recent dispersal in the early industrial times, but most likely with some local and traditional-like traffic). In undoubtedly native habitats (tundra and remote river ravines, in which *E. politus* typically occurs) this species is always lacking.

The distribution of *E. brachycephalus* follows the same pattern but is much sparser, also suggesting its connection with the pomors. However, two of its known localities have a recent origin. In the Pasvik area, the species was collected from a recently abandoned Russian military camp (erroneously reported as *E. acris*: Kravchenko 2020), which had a post-war origin, whereas its presence along the channel of a hydroelectric power station may be linked with revegetation activities during the late Soviet times.

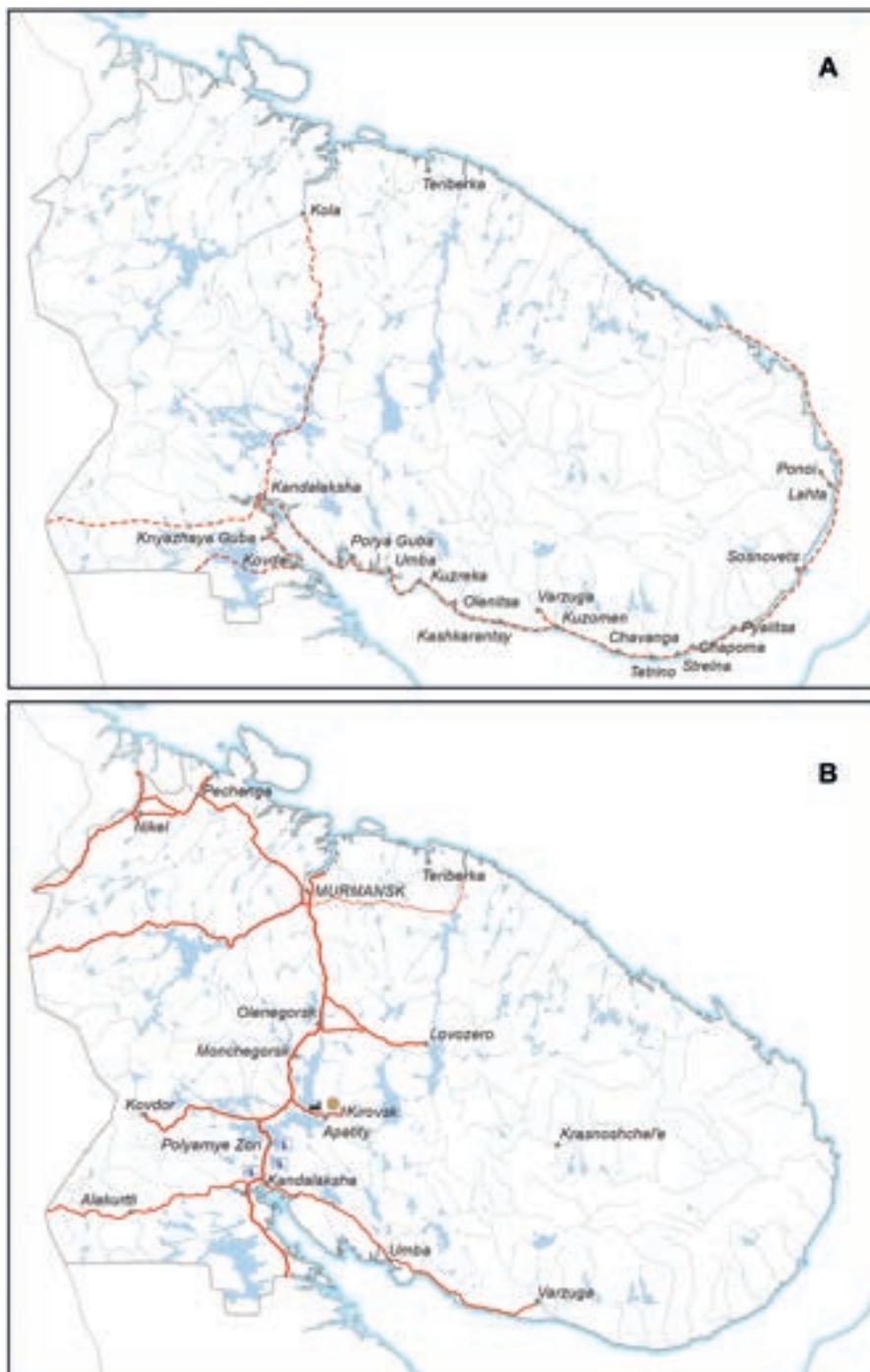


Figure 13. Main transport networks and their major populated places in Murmansk Region **A** pre-industrial period **B** industrial period. Red dashed lines (**A**) show the Pomors transport route along the White Sea, the Kola road to the north, and two trade communication roads between the White Sea and northern Finland. Red solid lines (**B**) indicate main roads, black dashed lines (**B**) are railways. Special signs (**B**) denote electric thermal (black) and hydro (blue) power stations, and nepheline mining area (brown) mentioned in the text. Maps were created using ArcGIS software by Esri. ArcGIS is the intellectual property of Esri and is used herein under license. Copyright Esri. All rights reserved.

Despite the broad occurrence erroneously reported in the past, *E. acris* s.str. was found only in a few scattered localities, mostly very recently. The only old locality of this species is known at Tetrino, along the southern coast of the Kola

Peninsula. This population is part of a local hybrid swarm, found together with *E. rigidus* and their hybrids in 1937, and therefore can also be linked with traditional activities of the pomors.

A large area of the recent introduction of *E. acris* is situated along the Niva cascade of hydroelectric power stations (Fig. 13B). This cascade continues from Kandalaksha Town in the south to Lake Imandra in the north. To date, plants of *E. acris* were recorded in connection with the second and third power stations, growing in large populations. Another isolated population was found on abandoned stockyards of an apatite-nepheline processing plant (concentrating mill) (Fig. 13B). One more locality, in Pasvik (Kravchenko 2020), is linked with another abandoned military place.

Erigeron uralensis was newly discovered in three localities along the Niva cascade of hydroelectric power stations and on abandoned stockyards of an apatite-nepheline processing plant. It was found in mixed populations together with *E. acris*.

Erigeron droebachiensis was collected twice in a single locality, an abandoned slag-dump at the Kirovsk thermal electric power station (Fig. 13B). This place of introduction is not connected with any other taxon of the *E. acris* group, thus showing its independent origin.

Pathways and periods of introduction of alien species

It may be exceedingly difficult to establish pathways and periods of introduction of particular alien plants in the territories with a long and complicated history of introductions, or even to distinguish between native and alien plants. However, the low natural floristic richness of the Arctic is particularly helpful in revealing alien plants, and its harsh climatic conditions efficiently limit the introduction and further spread of alien plants in this territory; for this reason, the diversity of non-native alien plants in the Arctic is still considerably lower than at the southern latitudes (Wasowicz et al. 2019).

The territory of Murmansk Region belongs partly to the Subarctic tundra, partly to the Northern Boreal forest (Chernov 1971). These species-poor landscapes clearly allow for detecting alien plants when present outside seminatural or human-transformed landscapes near inhabited places or places of economic activities. We considered habitats and distribution patterns of taxa of the *E. acris* group in Murmansk Region and determined that only one species, *E. politus*, is undoubtedly native in the territory because its occurrence is predominantly linked to native landscapes, whereas the other taxa are clearly alien because they are confined to the areas of historical or modern human activities. This conclusion provides a major correction to all previous treatments of this group in Murmansk Region (Botschantzev 1959; Orlova 1966; Tzvelev 1994), which were based on the belief that '*E. acris*' (i.e. the *E. acris* group excluding *E. politus*) is also native to this territory due to its wide distribution and common occurrence in seminatural habitats. However, the occurrence of *E. rigidus* and *E. brachycephalus* is confined to the areas with strong anthropogenous influence; their localities are typically situated nearby old human settlements or places of traditional occupational activities (fishing, saltmaking, mining). The earliest records of *E. rigidus*, documented by herbarium specimens at H, were made in the 19th century near old Russian villages along the southern coast of the Kola

Peninsula and along the lower course of the Ponoï River and Kola River, as well as in several Finnish villages along the road from the White Sea to Salla via Alakurtti (formerly in Oulu or Lapland Region, Finland) which was situated on a major communication road between the Russian and Finnish North (Ahti and Hämet-Ahti 1971). These records evidenced that in the 19th century the species had been firmly established and widespread in the territory, and already connected with the areas of traditional Russian economic activities. Its more precise period of introduction to the territory can be only inferred from its historical occurrences: some older Russian villages along the southern coasts were established well before the 16th century, which is the accepted chronological limit between archaeophytes and neophytes (Pyšek et al. 2002), and the traditional Russian economy along the southern coast of the Kola Peninsula has continued since the 12th century (Bernstam 1978). As the factors that can be linked to the present-day distribution of *E. rigidus* in the Kola Peninsula (economic activities of the Pomors) emerged and became strong so early in the history, we conclude that this species is most likely an archaeophyte in Murmansk Region.

The pathways of introduction of *E. rigidus* are linked to the economic activities of the Pomors. The taxa of the *E. acris* group are very minor and insignificant weeds of field crops (Krascheninnikov et al. 1935), and their effective dispersal as contaminants is unknown and therefore unlikely. Furthermore, the species occurrence in Murmansk Region strongly suggests that its local dispersal was not directly linked with transport of goods or commodities like grain; on the contrary, the species is very commonly found on fishing and sailing places in which no cargo had been discharged, with the continuous occurrence along the coasts. For this reason, we assume that the species was introduced and further dispersed adhering to clothes, footwear and other items possessed and moved by travellers, who may be trading, fishing or performing any other traditional occupation. To the Kuusamo area, it may have been introduced by Russian peddlers who were known to trade regularly in the area (Ahti and Hämet-Ahti 1971). This pathway can be classified as Transport-Stowaway: People and their luggage/equipment (Harrower et al. 2018). Local colonisation occurred not only by wind; it was apparently aided by cattle (lambs and cows), as evident from historical records on pastures and along small watercourses that are known to have been used for grazing (Zaitseva 2000).

The historical occurrence of *E. brachycephalus*, which is similar to the distribution of *E. rigidus* but much sparser, suggests that this species was introduced and dispersed using the same agents and factors but likely in later times, probably in the 16th–17th centuries when further large villages were established in the Kandalaksha Gulf and along the lower course of the Ponoï River (Bernstam 1978). This species can therefore be classified as an early neophyte. Its distribution was formed using the same pathway and source of introduction as those that shaped the distribution of *E. rigidus*.

Bernstam (1978) suggested that the Pomors originated in the Ladoga area, southern Karelia, from which they gradually colonised the coasts of the White Sea. Their origin and vector of colonisation agrees with the nearest native distribution area of *E. rigidus* and *E. brachycephalus*, which we define as central and southern Karelia. In northern Karelia, these two species were introduced by humans many hundred years ago.

Since historical records of *E. acris* are limited to a single village (Tetrino) without further localities along the sea coast, we assume this occurrence to have originated from old long-distance dispersal. Tetrino is an early village on the coast, dated from the 17th century, and its history was connected with the Resurrection Monastery at Istra (now Moscow Region) that established the village (Bernstam 1978) and the Solovetsky Monastery that, before the revolution, in the 15th–18th centuries possessed and in the 18th–20th centuries supervised territories of the former Tetrino District (Dositheos 1836). The monastery traffic from the Solovetsky Archipelago (now Archangelsk Region) may have been responsible for the long-distance dispersal of *E. acris* and its introduction to the territory as an early neophyte.

The modern introduction of *E. brachycephalus* and *E. acris* to military camps is linked with longer-distance transportation but probably the same pathway of introduction; it occurred in the late Soviet period after the Second World War. Similarly, cargo traffic was responsible for the recent occurrence of *E. acris* at the Apatity railway station. So far, it is uncertain what kind of item was contaminated with the *Erigeron* seeds.

Erigeron acris and *E. uralensis* were collected on abandoned stockyards of the First apatite-nepheline processing plant (concentrating mill) of the Apatite mining and processing enterprise. These stockyards functioned during 1956–1963 (Gershenkop et al. 2010; Mazukhina 2019) to accumulate and dump tailings produced while processing apatite-nepheline ore. After this period, the stockyards were closed and their territory was revegetated (covered by ground and plant cover), in agreement with the standards (Smetanin 2000; Chibrik 2002). To achieve a higher density of the restored plant cover, seeds of rhizomatous perennial herbs and grasses have been used since 1964 (Kryuchkov 1985; Druzhinina and Mialo 1990; Timofeeva et al. 2016). Such seeds were commercially produced in the USSR during the revegetation period rather than imported from abroad (Zolotarev et al. 2017). This type of revegetation was standard for all types of industrial lands, including slag-dumps (Smetanin 2000; Belozerova 2006). When similarly revegetated slag-dumps of the Ural Region were examined botanically, the high presence of *E. acris* s.l. was noted as a weed, especially on bare ground patches (Glazyrina et al. 2009; Chaschikhina 2021). This presence can be explained by contamination of the seed used in revegetation, also in Murmansk Region. This pathway can be classified as Transport-Contaminant: Seed contaminant. The source territory of introduction should be situated at least at Saint-Petersburg or farther southwards, where both alien species may co-occur. A more remote location of the source area may be indicated by the fact that *E. rigidus*, which belongs to the most common taxa in southern Karelia and Leningrad Region, has never been found on revegetated grounds.

We also considered a possibility for the seeds of the *E. acris* group to arrive with contaminated soils and found this pathway practically impossible. The topsoil used for revegetation in Murmansk Region was locally excavated peat rather than any substrate imported from previously vegetated places (Kryuchkov 1985). Although many ruderal plants, including the *E. acris* group, were observed on revegetated grounds in Krasnoyarsk Region where fertile soil had been used in revegetation (Efimov and Shishikin 2014), local peat can be considered completely free of any unwanted botanical contamination.

An abandoned slag-dump at the Kirovsk thermal electric power station, on which the only locality of *E. droebachiensis* was found, has been revegetated in a similar way but after 1990 (Davydov and Redkina 2021). The nearest distribution area of this alien taxon is Southern Karelia, where it occurs together with *E. uralensis* and *E. brachycephalus*.

The same two taxa, *E. acris* and *E. uralensis* (together with *E. brachycephalus*), were found along the Niva cascade of hydroelectric power stations (HPS). The last power station in this cascade was completed in 1954. This construction suggests that revegetation on the channels and dams of this cascade should have occurred no later than in the 1960s. The alien species composition and their presumed period of introduction essentially coincide for the cascade of HPSs and the mining stockyards, thus indicating a likely similar origin of the seed used in revegetation in both cases.

The occurrence of *E. acris* and *E. uralensis* at the mouth of the Niva River suggests their self-dispersal downstream from the places of their original introduction. This possibility is confirmed by the experiments indicating the ability of seeds of the *E. acris* group to drift along watercourses and remain viable in the end (Bill et al. 1999).

Naturalisation and further spread of alien taxa

All the non-native taxa of the *Erigeron acris* group found in Murmansk Region can be considered naturalised aliens. These plants are biennial or short-lived perennial, reproducing by seed, and self-sustaining populations are essential for their continuous presence in the territory, which has been repeatedly observed by numerous collectors in various localities.

From the sparsely scattered pattern of historical records of *E. acris* s.str. and *E. brachycephalus* and the continuous distribution of *E. rigidus*, all having been introduced to Murmansk Region with travelling humans but in different time periods, we infer that some kind of longer-distance dispersal may have occurred to deliver the first propagules of these species to the territory. This introduction was subsequently complemented by short-distance dispersal with the same agents and by local dispersal with cattle.

In spite of the long period of introduction, none of these tree taxa became truly invasive. All these species formed stable local populations in semi-natural landscapes or near populated places, but none of them shows a tendency to expand from their locally restricted refugia further into native landscapes.

The alien populations introduced in the post-industrialisation period (after the Second World War) were recorded in technogenic landscapes or in military areas. In most cases these alien plants were not observed outside the area of their original introduction. However, the occurrence of *E. acris* and *E. uralensis* along the Niva cascade of hydroelectric power stations demonstrates their potential for further dispersal by running water and by wind along the river corridor, when new populations successfully established downstream from the places of their original introduction.

It is commonly considered that populations of native plant species should be used in revegetation (Smetanin 2000; Chibrik 2002; Timofeeva et al. 2016). However, the list of species tried and recommended for this purpose in Murmansk Region (Kryuchkov 1985; Druzhinina and Mialo 1990; Timofeeva et al.

2016) contains plants (*Bromus inermis*, *Lolium pratense*, *Phleum pratense*, *Alopecurus pratensis*) which are not native to Murmansk Region, and therefore their introduction contributes to a further pollution of this territory by alien plants. Moreover, these seeds are produced commercially by agricultural enterprises (Zolotarev et al. 2017) far away from the area of introduction. Use of commercial seeds collected in remote territories brings seed contaminants which may appear either as easily recognisable exotic aliens or as cryptic invaders masquerading as their native relatives. The latter case is exemplified here by the *E. acris* group, which provides an alarming example of new invasions that can be started even when the technical standards in revegetation have been duly followed, and then completely neglected because of the lack of knowledge in this taxonomically difficult group. In order to prevent such situations, we suggest that protection from invasive non-native plants should be more efficient at the stage of seed import, and monitoring and management of invasive non-native plants should be established in places of revegetation works.

Putative hybridisation

So far, there is no direct confirmation of interspecific hybridisation in the *Erigeron acris* group which is based on genetic studies or experimental crosses. However, the existence of putative hybrids has been noted by a number of researchers who attempted to define taxa more precisely in this group.

Botschantzev (1959), who accepted only two taxa of the *E. acris* group in the Russian North, the hairy *E. acris* and the glabrous *E. politus* (which he named *E. elongatus*), speculated that individuals with presumably intermediate morphology originated from hybridisation between these two species. He also suggested that *E. brachycephalus*, *E. droebachiensis*, *E. elongatiformis* and *E. uralensis* may have the same hybrid origin. However, the specimens at LE collected from Murmansk Region, which Botschantzev identified as hybrids, mostly belong to *E. politus*, except for two specimens of *E. rigidus*, one specimen of *E. x pilosiusculus* and one specimen of *E. brachycephalus* that do not show any regular pattern.

Tzvelev (1990, 1994) agreed with Botschantzev's hypothesis of intermediate origin of these taxa, except for *E. droebachiensis*. He lumped these taxa together as a single species, which he named *E. uralensis* by priority, and expanded it by adding *E. decoloratus* as a further synonym. Like Botschantzev (1959), Tzvelev (1990) also noticed presumably hybridogenous individuals between his accepted taxa.

As *E. rigidus* possesses a seemingly intermediate morphology between *E. acris* and *E. politus* and its main distribution area has an altitudinal character and lies between the areas of these two species, we speculate that this species originated from ancient interspecific hybridisation. This idea may also explain the more recent hybridisation between *E. acris* and *E. rigidus* and between *E. politus* and *E. rigidus* in the places of their current co-occurrence. This hybridisation is also inferred from intermediate morphology of co-occurring individuals, which are regularly collected together and placed on the same herbarium sheets by collectors.

So far, we detected several localities with hybridisation between *E. politus* and *E. rigidus* (Kandalaksha Gulf, Turii Mys, Varzuga, the mouth and lower course of Ponoï River) and two local areas of hybridisation between *E. acris* and *E. rigidus* (Tetrino Village, Apatity Town). We expect that the hybridisation

may be even more widespread and complicated but it cannot be studied in full on the basis of the morphology of historical herbarium specimens.

Conclusions

Our treatment is a pilot study on the *Erigeron acris* group in Eastern Europe that covers a single first-level administrative subdivision of Russia, which was selected due to its extreme northern position that allows easier detection of introduced plants. The territory of Murmansk Region is a fair representative of the Lapland flora; it makes possible to decipher the taxonomic composition in the Fennoscandian North with this territorial example. Murmansk Region is a meeting point for the western (Atlantic) and eastern (Siberian) flora (e.g. Kremenetski et al. 1999) and southern (Hemiboreal) flora (e.g. Korsakova et al. 2021), with a rich and diverse alien component which has arrived mostly through southern pathways (Kozhin et al. 2020b).

The present contribution puts forward a morphology-based hypothesis about the taxonomic structure, distribution and history of the *E. acris* group in the Russian North. We provide the following major conclusions:

1. The taxonomic diversity can be classified into two main groups, which are characterised by their synflorescence structure: plants with corymbose synflorescences (corresponding to *E. ser. Trimorpha*) and plants with paniculate synflorescences (corresponding to *E. ser. Macrophylli*).
2. Plants sharing the same type of synflorescence but characterised by a various density of pubescence (ranging from nearly glabrous to moderately pilose and, ultimately, to densely hairy) are closely related and may be connected by hybridisation, and for this reason should be classified in the same group. The taxonomic separation of subglabrous taxa (corresponding to *E. ser. Politi*) is not supported.
3. The regional taxonomic diversity has been dramatically underestimated. This was caused by taxonomic confusions and the lack of taxonomic expertise, as well as by numerous recent introductions. Some taxa previously treated as synonyms should be restored (*E. brachycephalus*, *E. rigidus*). On the other hand, a number of previous new records were based on misidentifications (*E. decoloratus* should be excluded as reported in error, earlier records of *E. acris*, *E. droebachiensis* and *E. uralensis* were erroneous).
4. *Erigeron acris* of the current treatments in Eastern Europe and Fennoscandia contains two distinct taxa: *E. acris* s.str., which is more hairy and green, common in the southern part of Fennoscandia, and *E. rigidus*, which is less hairy and purplish, reaching the northern part of Fennoscandia. This morphological and biogeographical distinction is very clear and unambiguous.
5. The distribution of the only native species in the area, *E. politus*, is limited to the mountainous or hilly areas, or to the territories with deeper river valleys. The distribution of old introduced taxa (archaeophytes and old neophytes) is restricted to the area which has been traditionally inhabited or used by Russian settlers. The distribution of new introduced taxa (recent neophytes) is limited to industrial areas.
6. The present-day picture of a high taxonomic diversity and extensive distribution of alien taxa was caused by a combination of long-distance and

local dispersal events. The first major cause of introduction and further dispersal of alien taxa were Russian seashore settlers, who have inhabited the territory for several centuries and carried the diaspores along their fishing and trade routes. Among the taxa introduced and dispersed in this way, the naturalisation of *E. rigidus* is the earliest, following by *E. brachycephalus* and *E. acris*. The introduction of *E. acris*, *E. brachycephalus*, *E. droebachiensis* and *E. uralensis* occurred from remote territories with revegetation of industrial areas (dams, tailings and slag-dumps).

7. Considering the means of introduction, we assume that the main historical pathway was dispersal of diaspores by transport (by vehicles and then by feet) rather than with contaminated items. This conclusion agrees with the transportation and dispersal of the diaspores by fishermen who were not known to carry any significant cargo during their activities. The observed historical introduction was not connected with agriculture or hay-making either. The main modern pathway was seed contamination, coupled with long-distance dispersal by transport.
8. When native and introduced taxa of *E. ser. Trimorpha* come into contact, individuals with intermediate morphology may be observed in the same localities. These intermediates presumably originate from hybridisation, which may cause gene pollution in native (*E. politus*) and introduced (*E. rigidus* and *E. acris*) taxa, posing another threat to the native biodiversity (Bleeker et al. 2007). Besides, alien species may outcompete their native close relatives due to shared ecological niches (Pouteau et al. 2023).
9. This morphology-based taxonomic hypothesis provides a background to future phylogenetic studies on these groups, which should also take into account the variability (both infraspecific variability and introgressive hybridisation) and complicated history of human-mediated dispersal.

In comparison to the previous treatments, our taxonomic concept most closely corresponds to the ideas of Šida (1998). Like Mela (1877, 1884, 1895, 1899), we accept a single major subdivision of the *E. acris* group but use the synflorescence structure as the main character (Šida 1998). Our taxonomic revision closely corresponds to the latest Finnish synopsis (Kurtto and Väre 1998) in the species circumscriptions (with the additional separation of *E. rigidus*) but differs in the nomenclature; it can be considered an expansion of the Finnish treatment northwards. Our revision is also a development of the treatment for Eastern Europe (Tzvelev 1994), which differs in a coarser taxonomic resolution. The taxonomic revision of Olander and Tyler (2017), which was based on a statistical analysis of morphological characters, differs from any previous treatment and is largely incongruent with our conclusions due to its lumping approach.

Although taxonomic treatments produced for smaller territories (like Murmansk Region) seem to be limited in their scope, they can achieve very detailed, reliable and therefore useful results when placed in a broader context. For example, our revision of *Erigeron annuus* L. s.l. in Eastern Fennoscandia (Sennikov and Kurtto 2019) covered a small portion of its global distribution area but took the global studies into account, which facilitated further revisions in Europe (Otto and Ferloove 2019; Gudžinskas and Taura 2020; Sennikov and Galasso 2021) and Asia (Sennikov et al. 2020; Sennikov and Lazkov 2021). We

hope that the results of our present study pave a path to further detailed revisions of the *E. acris* group in Fennoscandia and Eastern Europe.

As the next step, we welcome further cooperation to confirm the taxonomic structure proposed in this work by phylogenetic methods. As long as reliable phylogenies are not available, the validity of our conclusions is confirmed by the match between plant morphology and historical processes uncovered in our work.

Acknowledgements

We are deeply obliged to Mats Hjertson (Uppsala) for providing photographs of the original material of *Erigeron rigidus*. We are highly grateful to Per Harald Salvesen (Bergen) and Olof Ryding (Copenhagen) for their kind, albeit futile searches for the missing original specimens of *E. droebachiensis* in Norwegian collections and in the collections of C. Denis Melnikov and Peter Efimov (Saint-Petersburg) who kindly provided scanned images and photographs of type specimens from LE, Galina Konechnaya (Saint-Petersburg) who communicated specimen images from LECB, Xenia Popova (Moscow) who provided specimens for scanning from MW, and Alexei Kravchenko (Petrozavodsk) who supplied specimen images from PTZ. We wish to dedicate this paper to Teuvo Ahti and Leena Hämet-Ahti (Helsinki), whose exemplary study on synanthropic plants of Kuusamo, northern Finland, has inspired and guided our work.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research received no external funding to ANS. The work of MNK was funded by the Russian Science Foundation, grant number 22-27-20009, <https://rscf.ru/project/22-27-20009/>.

Author contributions

ANS developed the taxonomic concept and revised the nomenclature. ANS and MNK collected the material. ANS identified the material with the input from MNK. ANS analysed the status of plant records and pathways of introduction. ANS wrote the manuscript. ANS and MNK revised the manuscript. Both authors agreed to the final version of the manuscript.

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Data availability

The dataset of distributional records collected for the present work was published through the Global Biodiversity Information Facility (available online: <https://doi.org/10.15468/een8vj>).

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First record of the genus *Camptotheca* (Nyssaceae) in Vietnam and the lectotypification of *C. acuminata*

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Abstract

As a primary source of anticancer camptothecin, *Camptotheca* (Nyssaceae) is an economically valuable genus and has long been recorded as endemic to China. Here, *Camptotheca* is reported as a new record to the flora of Vietnam with the discovery of a wild population of *C. acuminata* from Lai Chau Province of northern Vietnam. Based on the consultation of relevant literature and type specimens of *C. acuminata*, a lectotype of the species is designated. Photographic illustrations, morphological description and a distribution map of *C. acuminata* is provided, and a key to all known species of Nyssaceae in Vietnam is presented, too.

Key words: *Camptotheca*, lectotype, Northern Vietnam, Nyssaceae

Introduction

Nyssaceae is a small angiosperm family phylogenetically placed in the order Cornales (Angiosperm Phylogeny Group 2016). It has been reduced to be a subfamily (namely Nyssioideae) of Cornaceae (Angiosperm Phylogeny Group 2009; Reveal and Chase 2011) or divided into three separated smaller families (Mastixiaceae, Davidiaceae, and Nyssaceae) (Thomas et al. 2021). Within Cornales, the phylogenetic position of Nyssaceae has been controversial. It was supported to be a sister of either Curtisiaceae in the analysis of nuclear genomes (Zhang et al. 2020), a clade comprised of Grubbiaceae and Curtisiaceae using an Angiosperms353 dataset (Thomas et al. 2021), or another clade formed by Hydrostachyaceae, Hydrangeaceae, and Loasaceae in chloroplast phylogenies (Schenk and Hufford 2010; Fu et al. 2019; Li et al. 2021). As currently circumscribed, five genera are recognized in Nyssaceae, viz., *Mastixia* Blume, *Davidia* Baill., *Nyssa* L., *Diplopanax* Hand.-Mazz., and *Camptotheca* Decne (Stevens 2001 onwards).

Within Nyssaceae, *Camptotheca* is a distinct genus and could be readily distinguished from other genera by its samaralike fruits clustered in a globose head (Qin and Chamlong 2007). In previous molecular phylogenetic analyses, a sister relationship between *Camptotheca* and *Nyssa* was solidly supported no matter using nuclear or plastid data (Xiang et al. 2011; Chen et al. 2016; Fu et al. 2017, 2019; Li et al. 2019, 2021; Thomas et al. 2021). *Camptotheca* has long been recorded as an endemic genus of seed plants in China (Fang et al. 1983;



Academic editor: Hugo de Boer

Received: 25 September 2023

Accepted: 31 October 2023

Published: 15 November 2023

Citation: Zhu Z-H, Trinh NB, Hoang TS, Li B (2023) First record of the genus *Camptotheca* (Nyssaceae) in Vietnam and the lectotypification of *C. acuminata*. *PhytoKeys* 235: 129–136. <https://doi.org/10.3897/phytokeys.235.113267>

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Ying and Zhang 1994; Qin and Chamlong 2007). Since the publication of the type species, *C. acuminata* Decne., four additional taxa have been described in the genus, viz., *C. acuminata* var. *tenuifolia* W.P. Fang & Soong, *C. acuminata* var. *rotundifolia* B.M. Yang & L.D. Duan, *C. yunnanensis* Dode, and *C. lowreyana* S.Y. Li. However, the first three names have been treated as synonyms of *C. acuminata*. As currently recognized in Flora of China (Qin and Chamlong 2007), the two species, *C. acuminata* and *C. lowreyana*, can be distinguished by the shapes and number of lateral veins of leaves (Li 1997; Qin and Chamlong 2007).

Camptotheca is an ecologically and economically important genus, which do not only play a great role in landscaping (Zhang et al. 2004; Yang et al. 2012; Du 2013) but also is one of the most valuable woody medicine resources (Feng et al. 2000; Li et al. 2002). Ever since a special alkaloid, camptothecin, was successfully isolated from *C. acuminata* (Wall 1966), many studies have focused on its powerful anticancer effects (Venditto and Simanek 2010; Martino et al. 2017; Wang et al. 2023), and related drugs have been developed and approved for treating various cancers (Khaiwa et al. 2021; Jiao et al. 2023). Because of its great value and potential uses, *C. acuminata* was included in the List of National Key Protected Wild Plants of China (National Forestry Administration 1999).

In 2022, we encountered a small population of unknown trees without flowers and fruits in Phong Tho District of Lai Chau Province, northern Vietnam. When revisiting the locality from May to July 2023, we successfully collected flowering and fruiting specimens of this tree. After the consultation of relevant literature (Li 1997; Qin and Chamlong 2007) and comparison of type as well as other herbarium specimens, we confidently confirmed its identity as *C. acuminata* based on its morphology (Fig. 1), which appears to be the first record of the species and the genus *Camptotheca* for the flora of Vietnam.

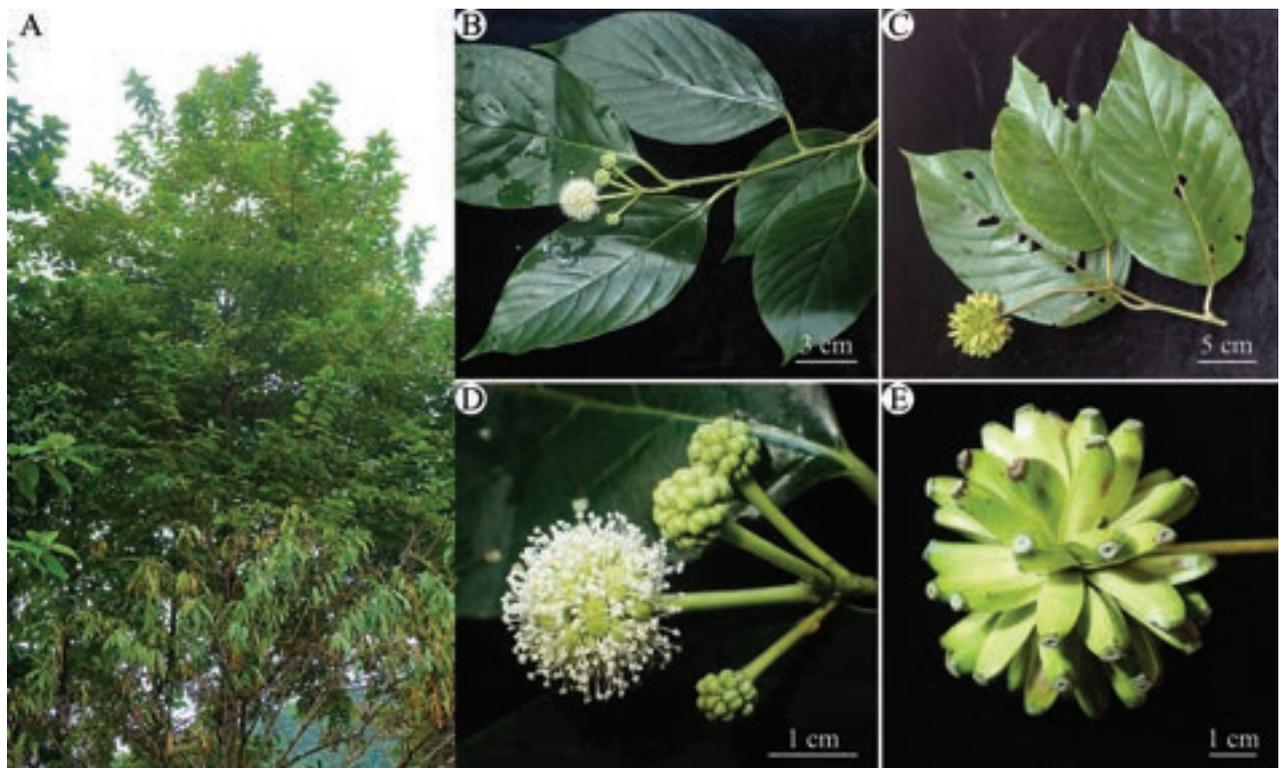


Figure 1. *Camptotheca acuminata* **A** habitat and habit **B** flowering branch **C** fruiting branch **D** inflorescences **E** fruits.

Materials and methods

Field surveys were carried out in northern Vietnam from 2022 and 2023. Voucher specimens of *Camptotheca acuminata* were collected from Phong Tho District, Lai Chau Province, and deposited in the herbarium of the Vietnamese Academy of Forest Sciences (VAFS).

High-resolution images of the type materials of *C. acuminata* held at the Muséum National d'Histoire Naturelle (P, acronyms according to Thiers 2023+) and the herbarium of Royal Botanic Gardens, Kew (K) were examined via the JSTOR Global Plants website (<https://plants.jstor.org/>). Other herbarium specimens (mainly from CAF, GXMI, HITBC, IBK, IBSC, KUN, NAS, and PE) were consulted via the Chinese Virtual Herbarium platform (<https://www.cvh.ac.cn/index.php>). The distribution map is prepared using data obtained from herbaria records as well as our field observations.

Taxonomic treatment

***Camptotheca acuminata* Decaisne Bull. Soc. Bot. France. 20: 157 (1873).**

Lectotype. (designated here):—CHINA. Kiang-si (Jiangxi Province): Vallée du Ly-Chan (Lushan Mountain), 1868, *A. David* 866 (lectotype P00545522! [image available at <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00545522>]; isolectotypes: K000704811! [image available at <https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000704811>] P00545523! [image available at <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00545523>], P00545524! [image available at <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00545524>], P00545525! [image available at <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00545525>]).

Description. Trees deciduous, to 20 m high; bark light gray, deeply furrowed; young branchlets cylindrical, purplish, with gray pubescence, villous; old branchlets glabrous, sparse round or oval lenticels. Leaves alternative; petiole 1.5–3 cm, flat or slightly grooved above, round below, puberulent when young, and almost glabrous mature, blackish when dry; leaf blade papery, 12–28 × 6–12 cm, oblong-ovate, oblong-elliptic or orbicular, base subrounded, margin entire, apex acute, slightly pubescent and pale green adaxially, greenish and lucid abaxially; pinnate veins both surfaces sparsely pubescent, midrib prominent both surfaces, lateral veins (4–)8–11(–15) pairs, slightly prominent adaxially or slightly prominent only near base abaxially. Inflorescence head, terminal or axillary, 1.5–2 in diam.; peduncle 4–6 cm, cylindrical, puberulent when young, then glabrous. Flowers polygamous; bracts 3, triangular, 2.5–3 mm, both surfaces pubescent; calyx cup-shaped, shallowly 5 lobed; lobes toothed; petals 5, caducous, light green, ca. 2 mm; disk conspicuous; stamens 10, outer 5 longer than, glabrous; filaments slender; anthers tetradymous; style ca. 4 mm, glabrous; stigmas 2. Fruit thinly winged, clustered in a globose head, green when young, yellowish brown after drying, 2.5–3.5 cm × 5–7 mm. Seed 1. Cotyledons lanceolate, 2–4 × ca. 1 cm, pinniveined, with 6–8 lateral veins on each side.

Illustrations. Fang and Su (1981: 316, fig. 120: 1–7); Yu (1993: 277, fig. 4: 351); Li (1997: 351–352, fig. 1–2); Wu et al. (2007: 322, fig. 322: 1–3).

Phenology. Flower: May–July, fruit: September.

Distribution and habitat. *Camptotheca acuminata* is widely distributed in southern China provinces (Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Yunnan, Zhejiang), and always grows near riverbanks and forest margins below alt. 1000 m. The newly discovered population of *C. acuminata* is located in northern Vietnam and near the China-Vietnam borders (Fig. 2).

Additional specimens examined. CHINA, Fujian Province: Xiamen City, Siming District, Wanshi Botanical Garden, roadside, elev. 56 m, 24°27'20.2"N, 118°5'38.1"E, 24 June 2020, fr. *T.Wang* 402 (AU!); Hubei Province: Xiaogan City, Dawu County, elev. 445 m, 31°43'5.8"N, 114°19'8.9"E, 03 Nov. 2020, fr. *C. Dai* DC78 (HIB!); Sichuan Province: Leshan City, E'bian Yi Autonomous County, roadsides under forests, elev. 961 m, 29°18'53"N, 103°16'56"E, 25 Nov. 2017, fr. *X.J.Li LiXJ830* (KUN!); Anhui Province: Xuancheng City, Jingde County, Hui River Protection Area, riversides, elev. 169 m, 30°22'37.2"N, 118°22'55.2"E, 18 Oct. 2016, fr. *W.Zhang and H.F.Wang ANUB02040* (ANUB!); Guizhou Province: Bijie City, Zhijin County, Qimo Town, Sanjiashan Village, broadleaf forests, elev. 1324 m, 26°41'55.2"N, 105°46'58.4"E, 07 Aug. 2015, fr. *L.Chen* 522425150807009LY (GZTM!); Jiangxi Province: Ganzhou City, Xinfeng County, Jinpen Mountain, valley, elev. 375 m, 25°13'32"N, 115°12'44"E, 03 Oct. 2014, fr. *R.P.Kuang LXP03-04734* (HNNU!); Hunan Province: Xiangxi Autonomous Prefecture, Baojing County, Fuxing Town, elev. 397 m, 28°38'53.9"N, 109°44'58.6"E, 12 Aug. 2012, fr. *X.J.*

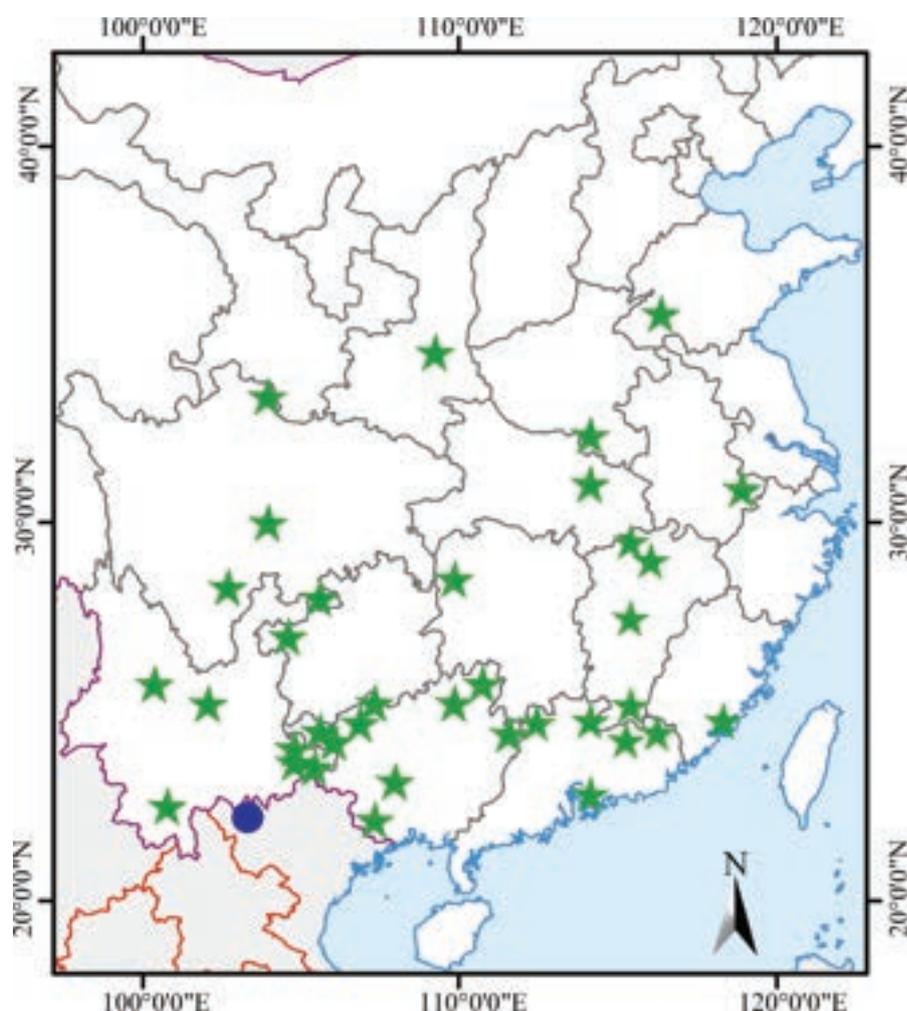


Figure 2. Distribution of *Camptotheca acuminata* in China (green stars) and Vietnam (blue circle).

Su and H.B.Liu 433125D00090811017 (JIU!). Vietnam. Lai Chau Province: Phong Tho District, Pa Ve Su commune, Mu Sang, Vang Ma Chai, in forests, elev. 1150 m, 22°39'38.48"N, 103°15'29.56"E, 11 June 2023, fr. *T.S.Hoang 22039* (VAFS).

Note. In the protologue of *Camptotheca acuminata*, Decaisne (1873) noted its type locality as “Thibet orientale, prov. Moupin, Ly-chan valley” which was proved to be erroneous according to the examination of David’s original collection labels (Franchet 1884). In fact, the type gathering (*A.David 866*) was collected by Father Armand David in 1868 from Lushan Mountain of Jiangxi Province, eastern China (Franchet 1884). When tracing the gathering, we sorted out four separate specimens held at the Muséum National d’Histoire Naturelle (P) and one deposited in the herbarium of Royal Botanic Gardens, Kew (K), and confirmed that not a single specimen has been designated as the type. Thus, we here propose the best preserved one simultaneously having flowers and fruits (barcode no. P00545522) as the lectotype of *C. acuminata* in accordance with the Article 9.3 of the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) (Turland et al. 2018).

In Vietnam, only one genus of Nyssaceae was previously recorded, i.e., *Nyssa* L. As currently known, five species of the genus have been discovered in the country (Fang et al. 1983; Qin and Chamlong 2007; Tan and Deng 2016; Tagane et al. 2020), viz., *Nyssa javanica* (Blume) Wangerin, *Nyssa sinensis* Oliv., *Nyssa bifida* Craib, *Nyssa bidouensis* Tagane & Yahara and *Nyssa hongiaoensis* Tagane & Komada. With the supplement of *Camptotheca* and *C. acuminata* to the Vietnamese flora, the Nyssaceae is now represented by two genera and six species. Thus, a key to all species of Nyssaceae in Vietnam is provided below.

Key to the genera and species of Nyssaceae in Vietnam

- 1 Fruit thinly winged, clustered in a globose head ... ***Camptotheca* (*C. acuminata*)**
- Fruit drupaceous, laterally flattened, solitary or several in a cluster **2 (*Nyssa*)**
- 2 Trees evergreen ***N. hongiaoensis***
- Trees deciduous **3**
- 3 Flowers pedicellate, in umbels or racemes ***N. sinensis***
- Flowers sessile or male ones shortly pedicellate, in capitates **4**
- 4 Branchlets glabrous ***N. bidouensis***
- Branchlets densely tomentose **5**
- 5 Branches and leaves glabrescent to subglabrous when mature ***N. javanica***
- Branches and leaves persistently densely tomentose ***N. bifida***

Acknowledgements

The authors are grateful to the keepers of CAF, GXMI, HITBC, IBK, IBSC, K, KUN, NAS, P, and PE for their assistance during specimens’ examination and to Dr. Shuai Liao (South China Botanical Garden, the Chinese Academy of Sciences) for sharing relevant taxonomic literature.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 32160047).

Author contributions

Data curation: ZHZ. Funding acquisition: BL. Investigation: TSTH, NBT, ZHZ. Resources: TSTH, NBT. Supervision: BL. Validation: BL. Writing - original draft: ZHZ. Writing - review and editing: BL.

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Data availability

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

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Molecular phylogeny and taxonomy of *Hosta* (Asparagaceae) on Shikoku Island, Japan, including five new species, one new subspecies, and two new status assignments

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Abstract

Japan has 16 native species of the genus *Hosta* Tratt. (Asparagaceae). A recent study on *Hosta* based on field surveys and molecular phylogenetic analyses resulted in the discovery of six unknown taxa in Kochi Prefecture, Shikoku Island, southwestern Japan. We aimed to identify these unknown taxa. Therefore, we constructed a finely resolved phylogeny for 320 *Hosta* samples collected from the Honshu, Shikoku, and Kyushu Islands using multiplex inter-simple sequence repeat genotyping by sequencing (MIG-seq). Based on this phylogenetic analysis and related morphological observations, we describe five new species, *H. longipedicellata* **sp. nov.**, *H. minazukiflora* **sp. nov.**, *H. polyneuronoides* **sp. nov.**, *H. samukazemontana* **sp. nov.**, and *H. takiminazukiflora* **sp. nov.** and one new subspecies, *H. takiminazukiflora* subsp. *grandis* **subsp. nov.** In addition, we propose two new status assignments, *H. tardiva* subsp. *densinervia* **comb. and stat. nov.** and *H. scabrinervia* **stat. nov.** We also propose classifying *H. kikutii* var. *tosana* as a species, *H. tosana*. Further studies that combine MIG-seq with careful morphological observations are needed for *Hosta* plants on all Japanese islands, which may result in the discovery of even more undescribed species.

Key words: anacladogenetic speciation, flowering season, MIG-seq, next generation sequencing, reproductive isolation, threatened species

Introduction

The genus *Hosta* Tratt. (Asparagaceae) comprises 22–25 species endemic to East Asia and Russia (Jones 1989). Japan has 16 native species of *Hosta* (Fujita 1976; Tamura 2015; Tamura and Fujita 2016; Yahara et al. 2021); this is a higher species richness than is present in China (four species), Korea (six species), and Russia (one species) (Chen and Boufford 2000; Lee et al. 2019). Recently, Yahara et al. (2021) conducted a molecular phylogenetic



Academic editor: Clifford Morden
Received: 22 December 2022
Accepted: 4 October 2023
Published: 17 November 2023

Citation: Yahara T, Hirota SK, Fujii S, Kokami Y, Fuse K, Sato H, Tagane S, Suyama Y (2023) Molecular phylogeny and taxonomy of *Hosta* (Asparagaceae) on Shikoku Island, Japan, including five new species, one new subspecies, and two new status assignments. *PhytoKeys* 235: 137–187. <https://doi.org/10.3897/phytokeys.235.99140>

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study of *Hosta* species on Kyushu Island, Japan, and described a new species, *Hosta alata* Hatus. ex Yahara, based on a finely resolved phylogenetic tree reconstructed using multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq) as well as morphological and ecological observations.

Here, we examined the molecular phylogeny and taxonomy of *Hosta* on Shikoku Island, located east of Kyushu Island, which has the highest diversity of *Hosta* in Japan. According to Fujita (1976) and Kochi Prefecture & Makino Memorial Foundation of Kochi Prefecture (2009), the following nine species (11 taxa) were recorded in Shikoku: *H. alismifolia* F. Maek., *H. capitata* (Koidz.) Nakai, *H. kikutii* F. Maek. var. *caput-avis* (F. Maek.) F. Maek. and var. *polyneuron* (F. Maek.) N. Fujita, *H. longipes* (Franch. & Sav.) Matsum. var. *caduca* N. Fujita and var. *gracillima* (F. Maek.) N. Fujita, *H. longissima* F. Maek., *H. shikokiana* N. Fujita, *H. sieboldiana* (Hook.) Engl., *H. sieboldii* (Paxton) J. W. Ingram, and *H. tardiva* Nakai. Among these species, *H. kikutii* in Shikoku is highly polymorphic. Fujita (1976) noted that eight *H. kikutii* populations distributed in different river basins of Shikoku showed remarkable variation in leaf and flower morphologies and flowering phenology. Fujita and Tamura (2008) described two new varieties of *H. kikutii* from Shikoku: *H. kikutii* var. *densinervia* N. Fujita & M. N. Tamura and var. *scabrinervia* N. Fujita & M. N. Tamura. These two varieties were described as having different leaf vein characteristics: abaxially slightly papillose (var. *densinervia*) and prominently papillose (var. *scabrinervia*). These two varieties were distinguished from *H. kikutii* var. *tosana* (F. Maek.) F. Maek. distributed in eastern Shikoku by their straight flowering stems (Fujita and Tamura 2008 treating this variety as *H. kikutii* var. *caput-avis* (F. Maek.) F. Maek., Tamura and Fujita 2016 as var. *tosana*).

This study was initiated based on the findings of our previous research, which identified *Hosta alata* Hatus. ex Yahara of Kyushu Island as a new species (Yahara et al. 2021) as well as preliminary studies on plants identified as *H. kikutii* on Shikoku Island. These findings led to the development of the following hypotheses. First, we hypothesized that three varieties of *H. kikutii* in Shikoku are not closely related to *H. kikutii* var. *kikutii* collected from Kyushu Island, which is known to be related to *H. longipes* var. *caduca* (Yahara et al. 2021). Second, we hypothesized that *H. kikutii* var. *tosana* is not closely related to *H. kikutii* var. *densinervia* and var. *scabrinervia*. Third, because we found that two neighboring populations of "*H. kikutii* var. *densinervia*" distributed along the Asemi River on Shikoku Island are morphologically distinct and have different flowering seasons (one population flowering in June and fruiting in August, whereas the other flowering in August and fruiting in September), we hypothesized that the two populations have diverged into two distinct biological species.

To elucidate the taxonomy of *Hosta* plants previously identified as *H. kikutii* in Shikoku, we collected DNA samples of *Hosta* plants from Shikoku, Kyushu and Honshu. Following a previous study on *H. alata* (Yahara et al. 2021), we utilized MIG-seq to obtain a finely resolved phylogeny. The objective of this study is to present the results of molecular phylogenetic analyses and subsequent morphological observations, and revise the taxonomy of *Hosta* spp. on Shikoku.

Materials and methods

Field surveys and samples examined

We collected 320 DNA samples and voucher specimens from 70 localities for 30 taxa of *Hosta* in Japan (Suppl. material 1). These collections include specimens of three varieties distinguished by Tamura and Fujita (2016), *H. kikutii* var. *densinervia*, var. *scabrinervia*, and var. *tosana*, from their respective type localities. We attempted to identify our specimens based on the diagnostic traits described by Fujita and Tamura (2008) and Tamura and Fujita (2016), and the observation of the type localities for these varieties. However, we encountered many specimens that could not be definitively classified into any of the three varieties. As a result, we assigned tentative names to these specimens during our field surveys and subsequent molecular analyses. The MIG-seq data for the *H. alata* group determined in a previous study (Yahara et al. 2021; accession number DRA011465) were also included in the phylogenetic analysis.

DNA isolation, sequencing, and construction of SNP-based phylogenetic trees

Total DNA was extracted from the dried leaves using the CTAB method (Doyle and Doyle 1990). *De novo* single nucleotide polymorphism (SNP) discovery was performed using MIG-seq (Suyama and Matsuki 2015). A MIG-seq library was prepared using a two-step PCR amplification process based on the method described by Suyama et al. (2022). Sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) and MiSeq Reagent Kit v3 (150 cycles, Illumina). Low-quality and extremely short reads containing adapter sequences were removed using Trimmomatic 0.39 (Bolger et al. 2014). The Stacks 2.62 pipeline software (Catchen et al. 2013; Rochette et al. 2019) was used to obtain individual genotypes with the following parameters: minimum depth of coverage required to create a stack (m) = 3, the maximum distance between stacks (M) = 2, and maximum mismatches between loci when building the catalog (n) = 2. Three different filtering criteria were considered for quality control of the SNP data. First, any SNP site where one of two alleles had less than three counts was filtered out because it was difficult to distinguish polymorphisms from sequencing errors when the minor allele count of SNPs was too 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, a SNP was excluded if the number of samples shared by the SNP was below the reference value R , the minimum percentage of samples that retained an SNP. Although phylogenetic reconstructions using datasets with high R values tend to neglect the presence of SNPs unique to each lineage, phylogenetic reconstructions using datasets with low R values can contain noise with artifacts. Yahara et al. (2021) compared the performance of phylogenetic reconstructions for *Hosta alata* and its allies using datasets with $R=0.1$, 0.3, 0.5, and 0.8 and showed that the most reliable result was obtained when $R=0.5$. In this study, we used $R=0.3$ for phylogenetic reconstructions because the performance was higher at $R=0.3$ than at $R=0.5$. The major differences between the results at $R=0.3$ and $R=0.5$ are described in the Results section. The SNP detection using $R=0.3$ was repeated hierarchically: the first detection for all samples,

the second detection for 159 samples of Clade 1 (see Fig. 1), and the third detection for 45 samples of Clade 4. Pairwise F_{ST} between taxa of Clades 1 and 4 was calculated using the SNPs with $R=0.3$ for all samples by the populations program of the Stacks pipeline. In population genetic analysis using STRUCTURE 2.3.4 (Pritchard et al. 2000), SNP detections were executed using $R=0.5$ to reduce SNPs with defects in some samples as much as possible. If a locus had two or more SNP sites, only one SNP site was used to avoid the inclusion of linked SNPs.

The Maximum Likelihood phylogeny of the Japanese species of *Hosta* based on SNPs was inferred for all samples using RAxML 8.2.10 (Stamatakis 2014), as well as for two subsets of samples: Clade 1 and Clade 4. We used a GTRCAT model with an ascertainment bias correction using the Lewis method and performed 1,000 replicates of parallelized tree search bootstrapping. Phylogenetic networks using the Neighbor-Net method were performed by SplitsTree4 4.14.6 (Huson and Bryant 2006) using the uncorrelated P distance matrix calculated from the SNP matrix with $R=0.3$ for Clades 1 and 4. *Hosta gracillima*, which was used as an outgroup in the phylogenetic reconstruction, was not included in the phylogenetic networks of Clade 1. Population genetic structures of Clades 1 and 4 were examined using STRUCTURE 2.3.4 (Pritchard et al. 2000). Furthermore, hierarchical STRUCTURE analyses were performed for Clade 1 to detect more detailed population structure. We performed 30 independent runs with a burn-in of 100,000 steps and an additional 100,000 steps with the admixture model and estimated log-likelihoods for each number of clusters ($K = 1-10$). Optimal K values were determined using the ΔK method of Evanno et al. (2005) in the STRUCTURE Harvester (Earl and von Holdt 2012). Graphical results were obtained using CLUMPAK (Cluster Markov Packager Across K ; Kopelman et al. 2015).

Morphological observations

Based on the results of phylogenetic analyses using MIG-seq, we reassessed the morphological traits utilized for classifying *Hosta* taxa in previous studies (Maekawa 1940, 1948, 1952; Fujita 1976; Fujita and Tamura 2008; Tamura and Fujita 2016) and identified morphological traits that can serve as diagnostic markers for newly discovered or revised taxa in this study. Using specimens subjected to phylogenetic analyses, we measured the following traits: the number of flowers per scape, length and width of the flower bract (a bract located in the axil from which a pedicel arises), pedicel length, perianth length, length of the narrowed part of the perianth tube, length and width of perianth lobes, stamen length, pistil length, anther-sac length, scape length, raceme length, length and width of leaf blades, length and width of petioles, and the number of lateral veins on leaves. We also measured the length of the peduncle bract (a bract located on a peduncle, not subtending a flower), if it is present. To complement these measurements, the same sets of traits were measured on confidently identified specimens deposited in KYO and MKB. All measurements were made to the nearest 1 mm, except for anther-sac length measured to the nearest 0.5 mm.

Data resources

All raw MIG-seq data were deposited in the DDBJ Sequence Read Archive (DRA) under accession numbers DRA011621, DRA013286, and DRA015301.

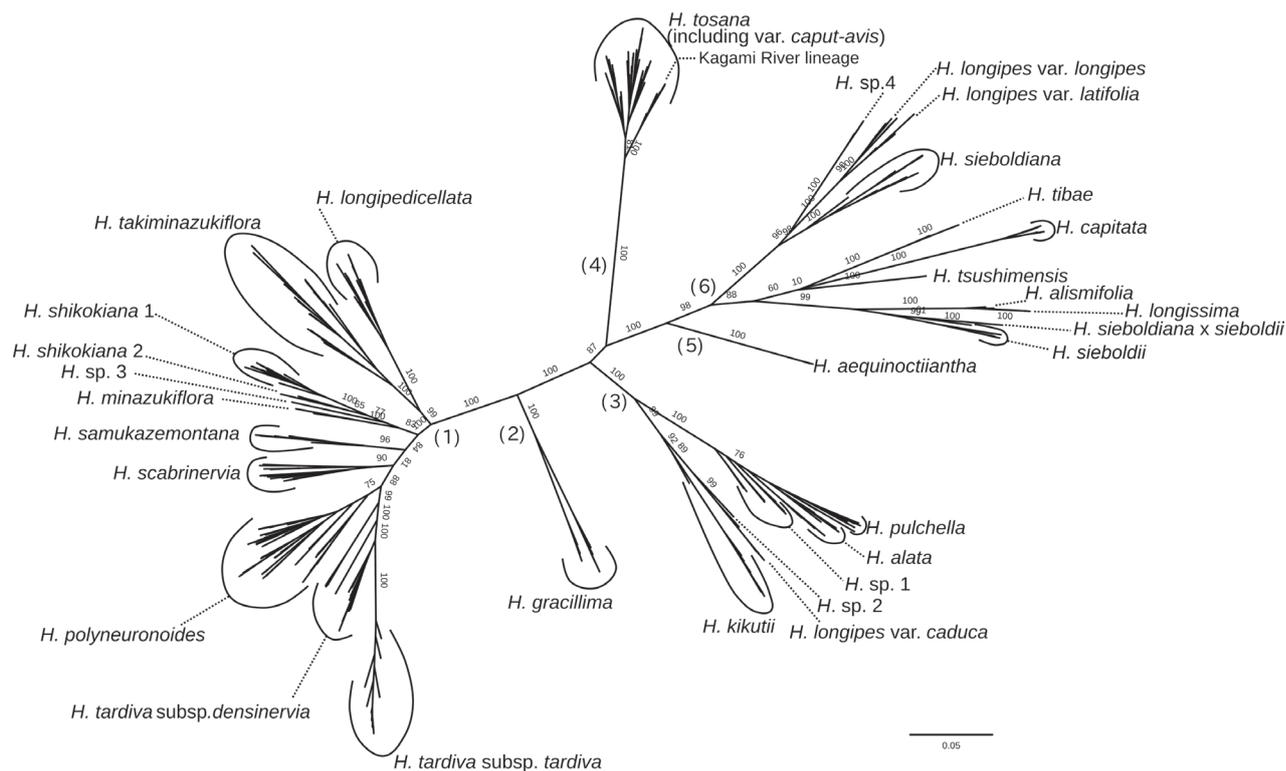


Figure 1. An unrooted Maximum Likelihood tree of 28 Japanese species of *Hosta*, reconstructed using MIG-seq data. Bootstrap values (50% or larger) are indicated on internodes in or above the species level. Numbers in parentheses indicate the IDs of 6 clades.

Results

Phylogenetic tree reconstructed using MIG-seq

A total of 96,556,838 raw reads ($301,740 \pm 10,362$ reads per sample) were obtained from MIG-seq, and 79,861,049 reads ($249,566 \pm 8,620$ reads per sample) were used for further analysis. After *de novo* SNP detection and filtering, 13,328 SNPs on 2,060 loci selected by $R=0.3$ were used for the phylogenetic reconstruction of all samples. Phylogenetic analyses of infrgeneric groups and STRUCTURE analyses were performed using different SNP datasets selected by $R=0.3$ and $R=0.5$, respectively (see Suppl. material 2).

In the following descriptions of the results obtained from phylogenetic analyses, we employ new names as determined by the conclusions of this study for five new species (*H. longipedicellata* sp. nov., *H. minazukiflora* sp. nov., *H. polyneuronooides* sp. nov., *H. samukazemontana* sp. nov., and *H. takiminazukiflora* sp. nov.), one new subspecies (*H. takiminazukiflora* subsp. *grandis* subsp. nov.), and two new taxonomic status assignments (*H. tardiva* subsp. *densinervia* comb. & stat. nov. and *H. scabrinervia* stat. nov.) (See the Taxonomy section for authorities of these new names). *Hosta tardiva* subsp. *densinervia* and *H. scabrinervia* are based on *H. kikutii* var. *densinervia* and var. *scabrinervia*, respectively (Fujita and Tamura 2008; also see Tamura 2015, Tamura and Fujita 2016). On the other hand, concerning *H. tosana* previously treated as *H. kikutii* var. *caput-avis* by Fujita and Tamura (2008), and *H. kikutii* var. *tosana* by Tamura and Fujita (2016), we adhere to the earlier classification proposed by Maekawa (1948), who differentiated between *H. tosana* F. Maek.

var. *tosana* and *H. tosana* var. *caput-avis* F. Maek. This is done to facilitate a comparison of the results of the present phylogenetic analysis with the earlier classification, concluding that these two varieties were indistinguishable. Furthermore, the results include two unknown species, designated as *H. sp. 3* and *H. sp. 4*, in addition to two other unknown species, *H. sp. 1* and *H. sp. 2*, reported by Yahara et al. (2021).

In the Maximum Likelihood tree reconstructed using the MIG-seq dataset at $R=0.3$ (Fig. 1), 28 Japanese *Hosta* species were clustered into the following six clades. The monophyly of Clade 1 was supported by a 100% bootstrap value and Clade 1 included *H. tardiva* subsp. *tardiva*, *H. tardiva* subsp. *densinervia* (previously classified as *H. kikutii* var. *densinervia*), *H. scabrinervia* (previously classified as *H. kikutii* var. *scabrinervia*), *H. shikokiana*, and six undescribed species (*H. longipedicellata*, *H. takiminazukiflora*, *H. samukazemontana*, *H. minazukiflora*, *H. polyneuronoides*, and *H. sp. 3*). The monophyly of Clade 2 was supported by a 100% bootstrap value with *H. gracillima* F. Maek. (= *H. longipes* var. *gracillima*). The monophyly of Clade 3 was supported by a 100% bootstrap value; Clade 3 comprised *H. alata*, *H. pulchella* N. Fujita, *H. kikutii*, *H. longipes* var. *caduca*, and two unknown species designated as *H. sp. 1* and *H. sp. 2* by Yahara et al. (2021). The monophyly of Clade 4 was supported by a 100% bootstrap value and Clade 4 comprised *H. tosana*. Monophyly of Clade 5 was supported by a 100% bootstrap value and Clade 5 comprised *H. aequinoctiantha* Koidz. ex Araki (= *H. longipes* var. *aequinoctiantha* (Koidz. ex Araki) Kitam.). Finally, monophyly of Clade 6 was supported by a 98% bootstrap value and Clade 6 comprised *H. longipes* (var. *latifolia* and var. *longipes*), *H. sieboldiana*, *H. capitata*, *H. tibae* F. Maek., *H. tsushimensis* N. Fujita, *H. sieboldii*, *H. alismifolia*, *H. longissima*, and an unknown species from Kii Peninsula, Honshu Island, designated as *H. sp. 4*, which had been identified as *H. kikutii* var. *caput-avis* (Maekawa 1948, 1952; Fujita 1976).

In the full data set tree (Fig. 1), a clade with a bootstrap support of 99%, comprising *H. takiminazukiflora* and *H. longipedicellata* was sister to the other species of Clade 1. To obtain a better resolution of phylogenetic relationships within Clade 1, we reconstructed the Maximum Likelihood tree for Clade 1 using *H. gracillima* (the closest related species) as an outgroup (Fig. 2). In this tree, the monophyly of conspecific samples was supported by a 100% bootstrap value for each of six species (*H. tardiva*, *H. samukazemontana*, *H. minazukiflora*, *H. shikokiana*, *H. longipedicellata*, and *H. takiminazukiflora*, arranged in order from the top of Fig. 2), and by a bootstrap value of 82% for *H. scabrinervia*. The clade including *H. minazukiflora* and *H. shikokiana* was supported by a 99% bootstrap value. *Hosta shikokiana* comprised two distinct clades, designated as *H. shikokiana* 1 and *H. shikokiana* 2, which were supported by bootstrap values of 100% and 99%, respectively. The samples of *H. shikokiana* 2 were placed between *H. shikokiana* 1 and *H. minazukiflora* in the SplitsTree (Fig. 3). *Hosta sp. 3* from Honshu (FJI12268, Wakayama Prefecture in the Kii Peninsula) was sister to a clade with a bootstrap support of 99%, comprising *H. shikokiana* and *H. minazukiflora*. In the full data set tree (Fig. 1), *H. minazukiflora* was sister to a subgroup including *H. sp. 3* and *H. shikokiana*, but the resolution was low; the bootstrap support for the latter subgroup was as low as 46%.

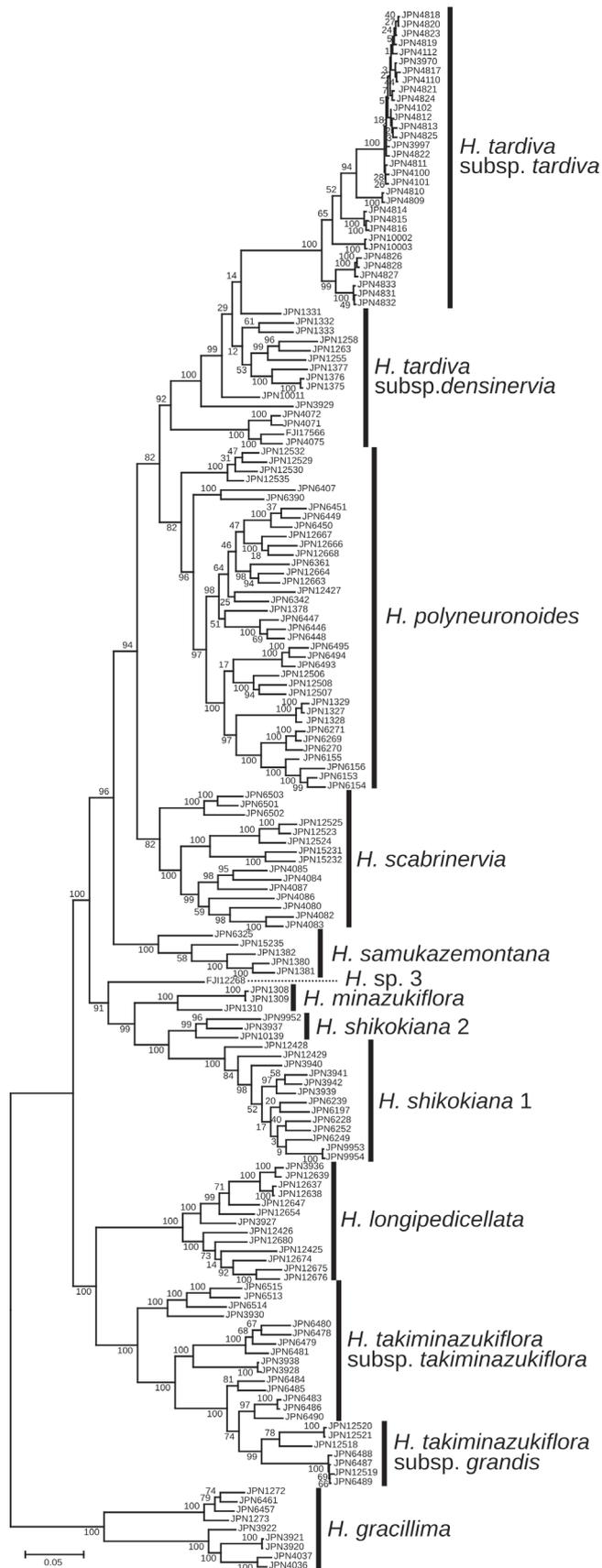


Figure 2. A rooted Maximum Likelihood tree for *Hosta* Clade 1 reconstructed using MIG-seq data. Bootstrap values are indicated on internodes. Based on the Maximum Likelihood tree of 28 Japanese species (Fig. 1), *H. gracillima* is used as an outgroup.

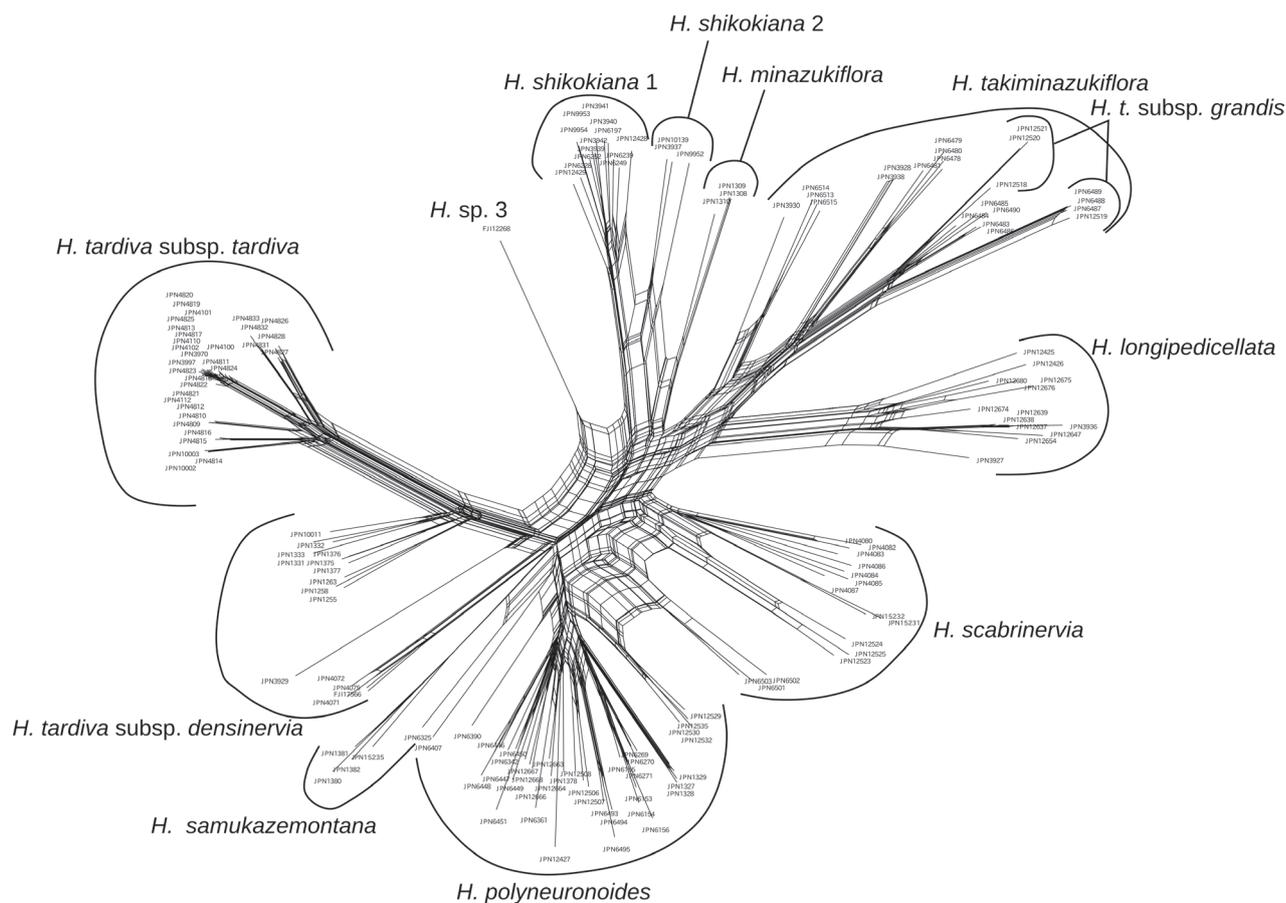


Figure 3. A SplitsTree for *Hosta* Clade 1 reconstructed using MIG-seq data.

The overall topology of the Maximum Likelihood tree reconstructed using the MIG-seq dataset at $R=0.5$ (not shown) was identical to Fig. 1, where the 28 Japanese *Hosta* species were clustered into six clades. The topology of Clade 1 in the Maximum Likelihood tree at $R=0.5$ was also identical to Fig. 1, except for *H. scabrinervia*. In this species, the samples did not form a cluster but instead scattered between a clade comprising *H. longipedicellata* and *H. takiminzakiflora* and another clade comprising *H. shikokiana*, *H. minazukiflora*, and *H. sp. 3*.

The monophyletic relationship between *H. tardiva* subsp. *tardiva* and subsp. *densinervia* was supported by a 92% bootstrap value (Fig. 2). The monophyly of *H. tardiva* subsp. *tardiva* was supported by a 100% bootstrap value, but *H. tardiva* subsp. *densinervia* was not monophyletic; a cluster including three samples from the type locality of *H. tardiva* subsp. *densinervia* (published as *H. kikutii* var. *densinervia*) in Tokushima Prefecture (JPN4071, 4072, 4075) and an additional sample from its vicinity (FJI17566) was sister to a clade including both the other samples of *H. tardiva* subsp. *densinervia* from Kochi Prefecture and *H. tardiva* subsp. *tardiva*, and the monophyly of the latter clade was supported by a 100% bootstrap value (Fig. 2). The closer relationship of *H. tardiva* subsp. *tardiva* with the ten samples of subsp. *densinervia* was also supported by SplitsTree (Fig. 3).

STRUCTURE analysis for Clade 1 indicated that ΔK was highest at $K=3$ (Fig. 4A). At $K=3$, *H. tardiva* subsp. *tardiva* exhibited a distinct genetic identity represented by light blue, while *H. polyneuronoides* was primarily associated with the second genetic identity depicted by dark purple. *Hosta tardiva* subsp.

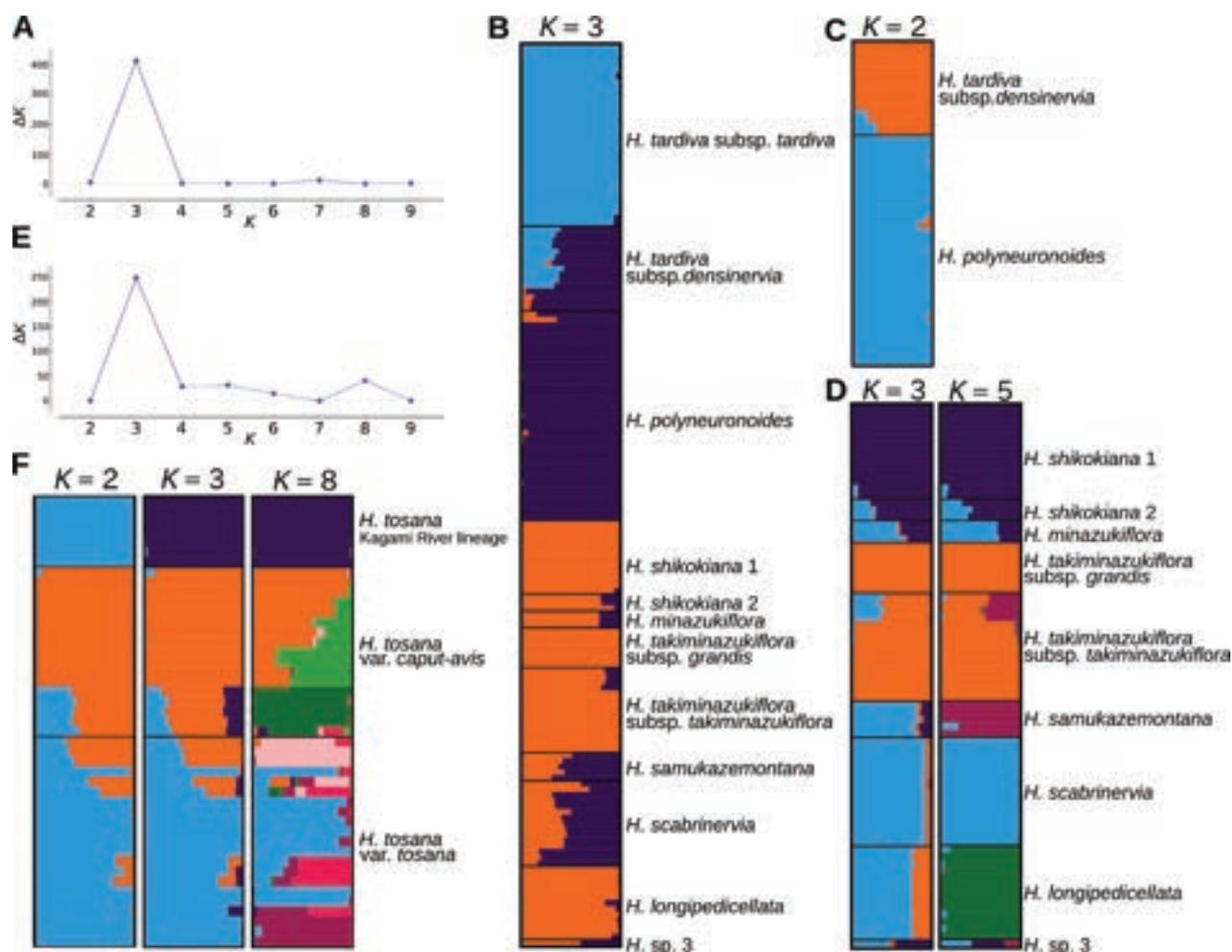


Figure 4. Population genetic structure of *Hosta* Clade 1 and Clade 4 **A**, **E** changes of ΔK with K in Clades 1 and 4, respectively **B** a diagram showing the result of STRUCTURE analysis for Clade 1 at $K=3$ **C** a diagram showing the result of hierarchical STRUCTURE analysis for *H. polyneuronoides* and *H. tardiva* subsp. *densinervia* **D** diagrams showing the results of hierarchical STRUCTURE analysis for *H. shikokiana* and six related species at $K=3$ and 5 **F** diagrams showing the results of STRUCTURE analysis for Clade 4 at $K=2, 3$, and 8.

densinervia exhibited a mixture of the first and second identities. The remaining seven species showed higher probabilities of a genetic component derived from the third identity illustrated by orange (Fig. 4B). Considering that *H. tardiva* subsp. *tardiva* is a highly sterile taxon (Fujita 1976), hierarchical STRUCTURE analysis was performed for *H. tardiva* subsp. *densinervia* and *H. polyneuronoides*, excluding *H. tardiva* subsp. *tardiva*. This analysis revealed that ΔK was highest at $K=2$ (Suppl. material 3). At $K=2$, *H. tardiva* subsp. *densinervia* was predominantly characterized by the first identity depicted by orange, while *H. polyneuronoides* was dominated by the second identity represented by light blue (Fig. 4C). Four samples of *H. tardiva* subsp. *densinervia* collected from Miyoshi city (JPN4071, 4072, 4075, FJI17566) exhibited admixture with *H. polyneuronoides*. Hierarchical STRUCTURE analysis for the other seven species showed that ΔK was highest at $K=3$ and second highest at $K=5$ (Suppl. material 3). At $K=3$, *H. shikokiana* was primarily associated with the first identity depicted by dark purple, *H. takiminazukiflora* was dominated by the second identity represented by orange, and *H. samukazemontana*, *H. scabrinervia*, and *H. longipedicellata* were

primarily associated with the third identity represented by light blue (Fig. 4D). At $K=5$, *H. samukazemontana*, *H. scabrinervia*, and *H. longipedicellata* were predominantly characterized by different identities represented by red-purple, light blue, and green, respectively. Four samples of *H. takiminazukiflora* subsp. *takiminazukiflora* collected at Mt. Inamura (JPN3930, 6513–6515) exhibited a mixture of two identities, one identity predominantly found in *H. takiminazukiflora* (orange) and another identity dominant in *H. samukazemontana* (dark purple). *Hosta minazukiflora* exhibited a mixture of two identities, predominantly the ones found in *H. shikokiana* (dark purple) and *H. scabrinervia* (light blue). *Hosta* sp. 3 showed a mixture of three identities at both $K=3$ and $K=5$.

Clade 4 consisting of *H. tosana* was more fully examined (Fig. 5). Seven samples collected from the Kagami River basin formed a distinct clade with a bootstrap support of 100% in the Maximum Likelihood tree. This clade, designated as the Kagami River lineage, formed the basal-most position of the 38 samples in Clade 4 (Fig. 1) and also formed a distinct cluster in the SplitsTree analysis (Fig. 6). The other samples of *H. tosana* var. *tosana* and var. *caput-avis* formed a cluster, but neither var. *tosana* nor var. *caput-avis* was monophyletic (Fig. 5). A clade including four samples collected from the type locality of *H. tosana* var. *caput-avis* (JPN12536–12539) and another sample from its vicinity (JPN12653) was sister to a clade including 12 samples of *H. tosana* var. *caput-avis* from Kami City, ca. 24 km NW of the type locality, and 21 samples of *H. tosana* var. *tosana*.

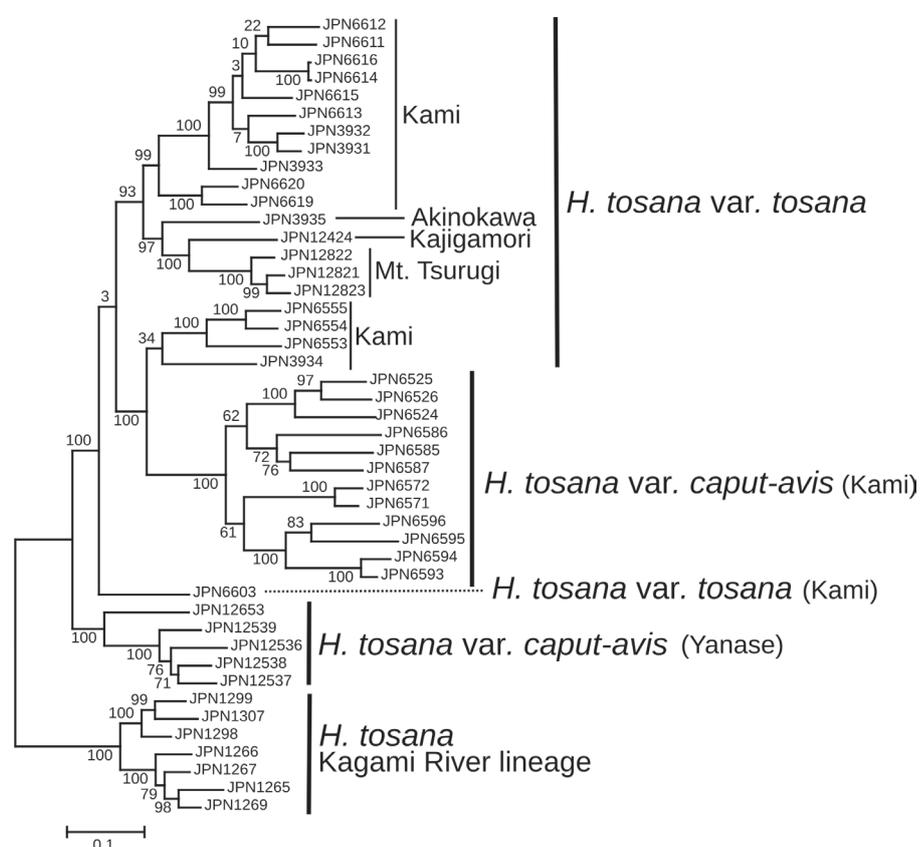


Figure 5. A rooted Maximum Likelihood tree for *Hosta* Clade 4 reconstructed using MIG-seq data. Bootstrap values are indicated on internodes. Based on the Maximum Likelihood tree of 28 Japanese species (Fig. 1), the tree is rooted by the Kagami River lineage of *H. tosana*.

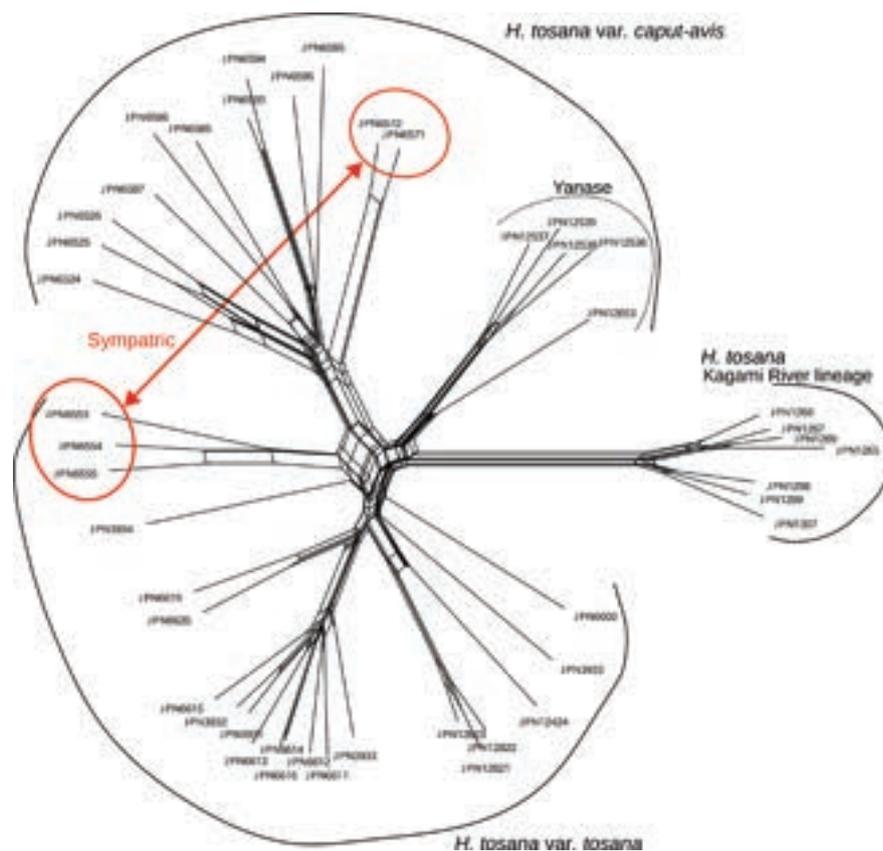


Figure 6. A SplitsTree for *Hosta* Clade 4 reconstructed using MIG-seq data.

STRUCTURE analysis for Clade 4 showed that ΔK was highest at $K=3$ and second highest at $K=8$ (Fig. 4E). At $K=3$, the Kagami River lineage of *H. tosana* was dominated by the first identity depicted by dark purple. For *H. tosana* var. *caput-avis*, out of the 12 samples collected from Kami City (depicted in the upper section), the second identity, represented by the orange color, was predominant. However, among the five samples taken from the vicinity of the type locality (depicted in the lower section), a mixture of three identities was observed, indicated by dark purple, orange, and light blue colors. For *H. tosana* var. *tosana*, the third identity (light blue) was predominant. However, three samples (depicted in the uppermost section; from Befu Valley, Kami City, JPN6553–55) were mixed with the second (orange) and third (light blue) identities, and two samples from Kami City (other than Befu Valley, JPN3934 and 6603) showed a mixture of three identities. At $K=8$, the Kagami River lineage of *H. tosana* was dominated by the first identity (dark purple), but *H. tosana* var. *caput-avis* and var. *tosana* showed a complicated mixture of five or six identities.

Morphological and phenological divergence between taxa with adjacent distribution ranges

(1) *Hosta tardiva* subsp. *densinervia* and *H. polyneuronoides*

We observed five populations of *H. tardiva* subsp. *densinervia* at elevations from 90 m to 900 m (Fig. 7B). Although the populations are geographically isolated in three river basins, the plants in these populations are morphologi-

cally indistinguishable. We collected *H. polyneuronoides* at elevations from 237 m to 1980 m (Fig. 7C). These populations are located in the upper reaches and headwaters of the Yoshino River and on the ridgeline from Mt. Ishizuchi to Mt. Komochi-gongen and its vicinity where *H. tardiva* subsp. *densinervia* is not distributed (see a dotted quadrilateral in Fig. 7B). The specimens of *H. polyneuronoides* collected from Mt. Ishizuchi were morphologically identical to another specimen collected from the same location (Takahashi & Fujita 226; see the Taxonomy section for details of this specimen record). This specimen was identified as *H. shikokiana* in its original description by Fujita (1976).

Hosta polyneuronoides is morphologically similar to *H. tardiva* subsp. *densinervia*, but is distinguished by smaller inflorescence (3–10 flowers per inflorescence vs. more than 20 per inflorescence in *H. tardiva* subsp. *densinervia*) and shorter anther sacs (3.5–4 mm long vs. 5 mm long). *Hosta polyneuronoides* tends to flower earlier than *H. tardiva* subsp. *densinervia*: flowering specimens of *H. tardiva* subsp. *densinervia* were collected between August 13 and September 27, whereas flowering specimens of *H. polyneuronoides* were collected between July 25 and August 20 (see specimen records cited in the Taxonomy section). While *H. tardiva* subsp. *densinervia* was observed to grow on soil or rocks in open habitats along riverbanks, *H. polyneuronoides* was found to grow on wet rocks in shaded habitats along streams.

(2) *Hosta tardiva* subsp. *densinervia*, *H. polyneuronoides*, and *H. scabrinervia*

The type locality of *H. scabrinervia* (Fig. 7, open rectangular) is located 15.5 km south of the type locality of *H. tardiva* subsp. *densinervia*, both of which are located along the Yoshino River, at elevations of 90 m (*H. tardiva* subsp. *densinervia*) and 180 m (*H. scabrinervia*). Fujita and Tamura (2008) distinguished *H. kikutii* var. *densinervia* (i.e., *H. densinervia* subsp. *densinervia*) from *H. kikutii* var. *scabrinervia* (i.e., *H. scabrinervia*) by the slightly papillose abaxial surface of the leaf nerves (vs. prominently papillose in var. *scabrinervia*). However, in the type locality population of *H. scabrinervia*, *Hosta* plants are highly variable in the state of abaxial nerve surface; some plants have leaf nerves prominently papillose adaxially (e.g., JPN4080, 4084–85), but other plants have leaf nerves almost smooth adaxially (e.g., JPN4082–83, 4086) or intermediately papillose adaxially (e.g., JPN 4087). All seven samples clustered into a single clade in both the Maximum Likelihood tree and SplitsTree (Figs 2, 3). While *H. tardiva* subsp. *densinervia* is closely related to *H. tardiva* subsp. *tardiva*, *H. scabrinervia* is positioned between *H. samukazemontana* and a clade comprising *H. minazukiflora*, *H. shikokiana*, and *H. sp. 3* (Fig. 2). The flowering specimens of *H. scabrinervia* and *H. tardiva* subsp. *densinervia* were collected from July 13 to July 25 and from August 13 to September 27, respectively. Morphologically, *H. tardiva* subsp. *densinervia* and *H. scabrinervia* are very similar, but distinguished by their anther sac length (5 mm in the former vs. 3 mm in the latter). Additionally, *H. tardiva* subsp. *densinervia* has more than 20 flowers per scape (except for small plants growing on rocks), while *H. scabrinervia* has 15–20 flowers per scape.

Two specimens (MBK0319724 and MBK0179549) collected at an elevation of 200 m along the middle reach of the Yoshino River, at 7.5–9 km south of the type locality of *H. scabrinervia*, respectively, were morphologically

identical to *H. scabrinervia* in the type locality (Fig. 7B). Five other specimens of *H. scabrinervia* (JPN12523–12525, 15231, 15232) were collected at an elevation of 550 m, 33 km WSW of the type locality of *H. scabrinervia*, within the range of *H. polyneuronoides* (Fig. 7B, C). At 5–6 km W of the locality of the five specimens, the specimens of *H. polyneuronoides* (JPN12529, 12530, 12532, and 12535) were collected. In this area, *H. scabrinervia* was found to grow on wet cliffs, whereas *H. polyneuronoides* was found on rocks along the stream.

(3) *Hosta polyneuronoides* and *H. shikokiana*

In addition to *H. scabrinervia*, five other species (*H. minazukiflora*, *H. longipedicellata*, *H. shikokiana*, *H. samukazemontana*, and *H. takiminazukiflora*) are densely distributed within or near the range of *Hosta polyneuronoides* (Fig. 7C). Among them, *H. shikokiana*, endemic to the serpentine area of Mt. Higashi-akaishi (Sh in Fig. 7) and its surroundings, is most distinct in having leaf blades strongly undulating along the margin, lustrous below when dried, each leaf blade decurrent to a winged petiole, purple perianths with three dark purple veins inside each perianth lobe, and usually purplish scapes and flower buds (Fig. 11). At Mt. Higashi-akaishi, we collected *H. shikokiana* in open habitats at an elevation of 1660 m along the rocky ridgeline (JPN6197, 6228, 6239), and *H. polyneuronoides* in shaded habitats on wet rocks along streams at elevations of 970 m (JPN 6269–71) and 1040 m (JPN6153–56).

(4) *Hosta minazukiflora* and *H. shikokiana*

The type locality of *H. minazukiflora* (M in Fig. 7) is located 20 km SE of the habitat of *H. shikokiana* at the ridgeline of Mt. Higashi-akaishi. *Hosta minazukiflora* was observed to have flower buds on May 23 and flowers on June 13 and 25. In contrast, *H. shikokiana* was observed to have flower buds on June 9, and flowers from July 14 to 21 (see specimens cited in the Taxonomy section). Morphologically, *H. shikokiana* has leaf blades strongly undulate along margin, usually purplish scapes, and purple perianths with dark purple inside veins (Fig. 11), whereas *H. minazukiflora* has leaf blades plain or only weakly undulate along margin, usually green scapes, and lavender perianths with darker colored inside veins (Figs 12, 13). Phylogenetic analyses revealed that *H. shikokiana* consisted of two distinct clades: *H. shikokiana* 1, collected from the mountain range of Mt. Higashi-akaishi, and *H. shikokiana* 2, collected from the other mountains located northeast of the type locality of *H. minazukiflora* (JPN3937, 9952). Both *H. shikokiana* 1 and *H. shikokiana* 2 exhibited the diagnostic traits mentioned above. While the SplitsTree analysis (Fig. 3) indicated that *H. shikokiana* 2 was positioned between *H. minazukiflora* and *H. shikokiana* 1, the Maximum Likelihood tree (Fig. 2) supported a sister relationship between *H. shikokiana* 1 and *H. shikokiana* 2 with a bootstrap value of 100%.

(5) *Hosta polyneuronoides* and *H. minazukiflora*

A population of *H. polyneuronoides* is located at an elevation of 480 m at 7.5 km north of the type locality of *H. minazukiflora* (at an elevation of 280 m), both growing on rocks along the stream of a tributary of the Yoshino River (see M in Fig. 7C).

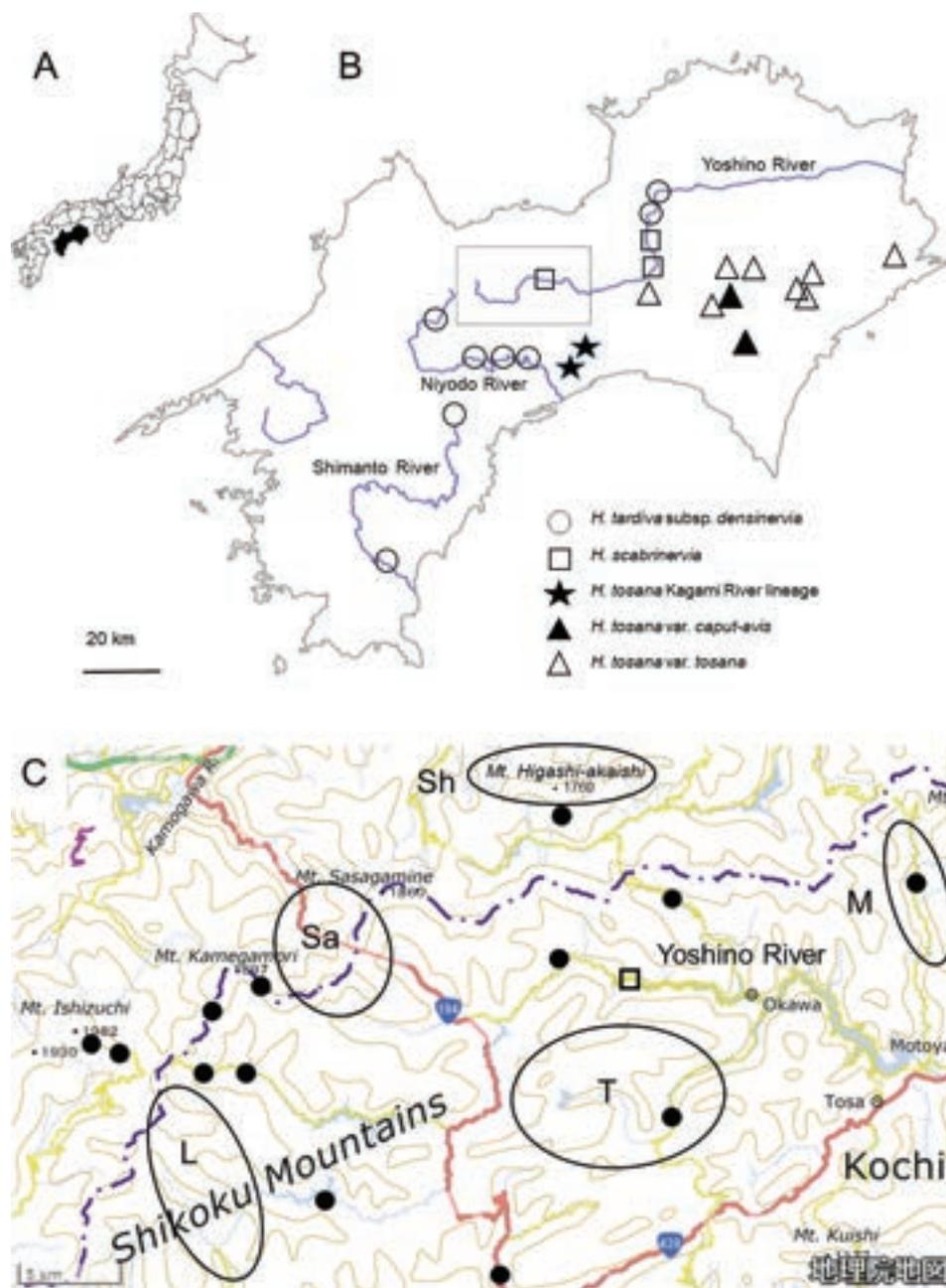


Figure 7. Maps of collection locations for *Hosta* species **A** Shikoku Island (black) in relation to Japan **B** detail of Shikoku Island with population locations for some species and varieties **C** details of collection locations for *H. polyneuronoides* (solid circles) and *H. scabrinervia* (an open square), and approximate ranges (circles) of *H. minazukiflora* (M), *H. longipedicellata* (L), *H. samukazemontana* (Sa), *H. shikokiana* (Sh), and *H. takimnazukiflora* (T). Considering conservation concerns, we refrained from disclosing the precise collection locations of these five species. The contour map was reproduced from a webpage. (<https://maps.gsi.go.jp/#11/33.726624/133.351364/&base=english&ls=english&disp=1&vs=c1g1j0h-0k0l0u0t0z0r0s0m0f1&d=m>) in accordance with the usage regulations of the Geospatial Information Authority of Japan.

This is one of the two neighboring populations of “*H. kikutii* var. *densinervia*’ along the Asemi River mentioned in the Introduction, and the other is *H. minazukiflora*. While *H. polyneuronoides* grows on wet rock, *H. minazukiflora* grows on a vertical cliff. While flowering specimens of *H. polyneuronoides* were collected from July 28 to August 20 (see specimens cited in the Taxonomy section), flowering specimens of *H. minazukiflora* along the Asemi River were col-

lected on June 23 and 25. On August 19, 2020, we collected *H. polyneuronoides* specimens with flower buds on scapes (JPN1327–29, Fig. 9A) and a specimen of *H. minazukiflora* with fruits (JPN1310, Fig. 13). In late August 2020, we also observed and collected mature plants of *H. polyneuronoides* that had just begun to bloom in three other localities: Niyodogawa-cho, Ino-cho, and Ochi-cho in Kochi City (Fig. 7). We compared morphological traits between these specimens and the type specimens of *H. minazukiflora* deposited in MBK (Table 1). *Hosta minazukiflora* had shorter leaf blade length, narrower leaf blade width, and shorter petiole lengths than *H. polyneuronoides*. For the number of flowers and length of flower bracts, *H. minazukiflora* had distinctly smaller values than *H. polyneuronoides*. The two species were also distinguished by their anther sac length (3.5–4 mm in *H. polyneuronoides* and 3 mm *H. minazukiflora*).

(6) *Hosta takiminazukiflora* and *H. minazukiflora*

The type locality of *Hosta takiminazukiflora* was only 14 km west of that of *H. minazukiflora* (T and M in Fig. 7C, respectively). *Hosta takiminazukiflora* was observed to grow on wet cliff faces. Flowering specimens of *H. takiminazukiflora* were collected from June 24 to August 8. Morphologically, *H. minazukiflora* (Figs 12, 13) has petioles and scapes not or only sparsely dotted and lavender perianths with three darker colored inside veins per lobe, whereas *H. takiminazukiflora* (Figs 14, 15) has petioles and scapes densely dotted with purple and whitish perianths with a darker colored midvein. The two species are similar in appearance, but are not closely related: *H. takiminazukiflora* is sister to *H. longipedicellata* and *H. minazukiflora* is sister to *H. shikokiana* (Figs 1, 2).

Table 1. Measurements of 14 morphological traits in nine taxa of *Hosta* in Shikoku. Aspect ratio is defined as leaf length divided by leaf width.

	<i>H. t. densinervia</i>	<i>H. polyneuronoides</i>	<i>H. scabrinervia</i>	<i>H. minazukiflora</i>	<i>H. shikokiana</i>	<i>H. t. takiminazukiflora</i>	<i>H. t. grandis</i>	<i>H. longipedicellata</i>	<i>H. samukazemontana</i>
Leaf blade length	12–23 cm	7.5–28.5	16.6–32	11.8–17	8.1–9.3	11–26.5	22–25	13.4–30.2	7–22
Leaf blade width	3.2–11.5 cm	3.3–15.3	7.5–14.8	2.9–6.2	2.9–5.0	3.6–10.5	13–14	5.4–11.8	3.6–10.7
Aspect ratio	2–3.6	0.9–2.9	1.6–3	2.4–4.3	1.9–2.9	2.1–3.3	1.7–1.8	2.2–3	1.8–2.9
Lateral veins	6–11 pairs	8–9	7–15	6–9	5–7	6–10	12–13	7–10	6–12
Petiole length	9–36 cm	5–40	5.5–47	8–19	6.5–8.5	3.4–22	35–38	15–34.3	3.2–14.5
Scape length	27–44 cm	15–51	28.5–45	18–37	20.5–29	8.6–28.5	31–36	26.5–32.5	20–43
Raceme length	6–9 cm	4.3–9	5–12	10–15	5.3–13	5.9–18.5	12–15.5	7.6–9	7–9
Flower number	15–25	3–10	15–17	3–6	2–10	4–18	10–12	7–15	5–18
Floral bract length	2–3 cm	1.2–3.1	2–4.6	1.3–1.6	1.3–2.2	2–4.7	2.8–4.3	2–2.5	1.7–2.1
Floral bract width	0.4 cm	0.1–0.8	0.4–1.3	0.2–0.4	0.1–0.3	0.2–0.7	0.4–0.5	0.3–0.4	0.3–0.5
Pedicle length	0.8–1.8 cm	0.6–1.3	1.5–3.4	1.1–1.2	0.6–1.5	1.4–2.5	1.9–2.0	2.5–3.3	1–1.8
Perianth length	3.9–5.3 cm	4.5	5–6.8	4.1–4.7	4.4–5.0	4.0–5.7	3.8–4.9	4.4–6.4	4.5–5
Perianth lobe length	1.1–2.0 cm	0.7–1.2	0.8–1.6	1.2–1.4	1.4–1.7	1.2–1.8	1.2–1.3	1–1.5	1–1.3
Perianth lobe width	0.4–0.8 cm	0.4–1	0.5–1	0.6–0.8	0.8–1.0	0.5–1.0	0.7–0.8	0.6–0.9	0.7–0.8
Anther-sac length	5 mm	3.5–4	3	3	2.5–3	2–3	3	3	3

(7) *Hosta takiminazukiflora* subsp. *takiminazukiflora* and subsp. *grandis*

A small population of *H. takiminazukiflora* subsp. *grandis* (JPN6487–89, JPN12518–12521) was found alongside a larger population of *H. takiminazukiflora* subsp. *takiminazukiflora* (JPN6483–86 and JPN6490), with the former growing upright from the soil between the rocks under the waterfall and the latter deflected from the wet cliff of the waterfall. Both subspecies were found in flower on June 24, 2021.

Hosta takiminazukiflora subsp. *grandis* (Fig. 16) is easily distinguished from subsp. *takiminazukiflora* in having erect scapes 31–36 cm in height (vs. deflected scapes less than 30 cm long in subsp. *takiminazukiflora*), wider leaves (13–14 cm wide vs. 3.6–10.5 cm wide), cordate at the base (vs. cuneate), glaucous abaxially (vs. not glaucous but light green), lateral veins 12–13 pairs (vs. usually 6–10 pairs), and petioles green (vs. dotted with purple). These differences are maintained under fertilization and cultivation conditions in the Kochi Prefectural Makino Botanical Garden. Despite its morphological distinctiveness, subsp. *grandis* is phylogenetically included within a clade of *H. takiminazukiflora* (Figs 2, 3) because it is sister to the sympatric population of subsp. *takiminazukiflora* and is more remotely related to the other populations of subsp. *takiminazukiflora*. In the SplitsTree analysis of Clade 1 (Fig. 3), four samples of *H. takiminazukiflora* subsp. *grandis* formed a cluster, while the other three samples clustered with five samples of *H. takiminazukiflora* subsp. *takiminazukiflora* collected from the same locality. However, in the further SplitsTree analysis of a clade comprising *H. takiminazukiflora* and *H. longipedicellata*, seven samples of *H. takiminazukiflora* subsp. *grandis* formed a separate cluster from the cluster containing the five samples of *H. takiminazukiflora* subsp. *takiminazukiflora* from the same locality (not shown). Furthermore, in the Maximum Likelihood tree, all seven samples of *H. takiminazukiflora* subsp. *grandis* formed a clade with a bootstrap support of 99%, which is significantly differentiated from the sympatric population of subsp. *takiminazukiflora* (JPN6483–86, 6490) (Fig. 2). *Hosta takiminazukiflora* subsp. *takiminazukiflora*, collected from three other localities (Mt. Inamura: JPN3930, 6513–15; along Road 6: JPN6478–81; Mt. Higashikado: JPN3928, 3938), clustered into three separate subclades. However, these subclades were morphologically indistinguishable from the lineage of *Hosta takiminazukiflora* subsp. *takiminazukiflora* that is sympatric with *H. takiminazukiflora* subsp. *grandis*.

(8) *Hosta longipedicellata* and *H. takiminazukiflora*

The type locality of *H. longipedicellata* is located at 22.5 km west of that of *H. takiminazukiflora* and the two species are distributed in two mountain regions separated by an upper reach of the Yoshino River (L and T in Fig. 7C, respectively). The flowering type specimens of *H. longipedicellata* and *H. takiminazukiflora* were collected on August 1, 2006 and July 25, 1983, respectively. Both *H. takiminazukiflora* subsp. *takiminazukiflora* and *H. longipedicellata* grows on wet cliffs. Morphologically, *H. longipedicellata* (Figs 17, 18) is similar to *H. takiminazukiflora* subsp. *takiminazukiflora*, but it can be distinguished by longer pedicels (2.5–3.3 cm long in *H. longipedicellata* compared to 1.4–2.5 cm long in *H. takiminazukiflora*), shorter flower bracts (1.5–2.5 cm long compared to (2.2–)2.5–3.7 cm long) that

wither during flowering (as opposed to being fresh), and leaf veins papillose on the abaxial surface (as opposed to being smooth). Phylogenetically, the sister relationship between *H. longipedicellata* and *H. takiminazukiflora* was supported by a bootstrap value of 99% (Fig. 1) or 100% (Fig. 2). However, in SplitsTree (Fig. 3), *H. longipedicellata* was distinct from *H. scabrinervia* and *H. takiminazukiflora*.

(9) *Hosta samukazemontana*, *H. shikokiana*, and *H. polyneuronoides*

The type locality of *H. samukazemontana* (Sa in Fig. 7C) is located 13 km SW of the type locality of *H. shikokiana* (Sh in Fig. 7C) to which *H. samukazemontana* was included by Fujita (1976). *Hosta samukazemontana* was observed growing on wet cliffs of Mt. Kanpu (called Mt. Samukaze in the old days), while *H. shikokiana* was found on rocky slopes along the ridgeline of Mt. Higashi-akaishi and its vicinity. Morphologically, *H. samukazemontana* is similar to *H. shikokiana* with leaves shorter than 20 cm and scapes shorter than 40 cm, but distinguished by deflected scapes upwardly curved at the tip when flowering and fruiting (in contrast to being straight when flowering and fruiting in *H. shikokiana*), green scape color (as opposed to the usual purplish color, dotted with purple in the lower part), whitish perianths (vs. purple), narrowly winged petioles (instead of widely winged, particularly in the upper part), and a weakly undulate leaf margin (in contrast to a strongly undulate margin).

Along the Kamegamori forest road, we collected both *H. samukazemontana* (JPN6325, with flower buds on June 22, 2021) and *H. polyneuronoides* (JPN6342 and 6361, without flower buds on June 22, 2021). The two populations were approximately 500 m apart along the road. Flowering specimens of *H. samukazemontana* were collected from Mt. Kanpu on June 19, July 24 and July 25 (see specimens cited in the Taxonomy section) and flowering specimens of *H. polyneuronoides* from Mt. Ishizuchi, located in the vicinity of Mt. Kanpu and the Kamegamori forest road, were collected on July 28 and August 7. Morphologically, *H. samukazemontana* is distinguished from *H. polyneuronoides* by deflected scapes upwardly curved at the tip (vs. straight) and anther sacs 3 mm long (vs. 3.5–4 mm long).

Morphological and phenological observations on *H. tosana*

Two populations of the Kagami River lineage of *H. tosana* were observed on cliffs along the Kagami River in Kochi City. This location is 50 km SSW of the population of *H. tosana* var. *tosana* along the Monobe River. The specimen of the Kagami River lineage with flowers (Fig. 21A) was collected on July 14, 2013 at 130 m elevation. Near this locality, three additional flowering specimens were collected in mid-July (MBK0104375, MBK0247212, MBK0247214), two specimens with young fruits were collected in late July (MBK0247386, MBK0247387), and a fruiting specimen was collected in early October (MBK0208327). These populations were found below 300 m elevation, whereas the localities of typical *H. tosana* var. *tosana* were above 1000 m elevation. Morphologically, the Kagami River lineage of *H. tosana* is distinguished from typical *H. tosana* var. *tosana* (Fig. 22) by upwardly curving scapes at the apex when flowering and fruiting (vs. curved downward at apex) and leaves with fewer lateral veins (5–10(–11) compared to 10–13) running at wider intervals (usually 1 cm in contrast to usually 0.7 cm).

In the Befu Valley of Kami City, we collected *H. tosana* var. *caput-avis* from two populations: the upstream population (JPN6524–6526; Fig. 23A) and the downstream population (JPN6571, 6572), which were 1 km apart along the upper reach of the Monobe River. We also collected *H. tosana* var. *tosana* (JPN6553–55; Fig. 23B) within a distance of 100 m from the downstream population. These samples of two varieties in sympatry are depicted by red circles in the Splits Tree (Fig. 6). On June 25, 2021, plants of var. *caput-avis* were flowering in the downstream population and just before flowering (in flower buds) in the upstream population, whereas var. *tosana* had young scapes before flowerings. The two varieties were distinguished by scapes (strongly bent at the base and strongly curved at the apex in var. *caput-avis* vs. usually gently bent like a bow in var. *tosana*; Figs 23, 24) and peduncle bracts enclosing young flower buds (upward curved in var. *caput-avis* vs. straight in var. *tosana*; see enlarged photographs in Fig. 23). Additional specimens of var. *caput-avis* (JPN6586, 6587, 6793–6796) were collected from the higher elevations of Mt. Ishidate, located east of the above locality, whereas additional specimens of var. *tosana* (JPN6603, 6611–6616, 6619, and 6620) were collected at lower elevations located west of the above locality. Some plants of var. *tosana* (JPN6614, 6616) were flowering at a lower elevation. In flowering specimens, var. *caput-avis* (JPN6571–6572) and var. *tosana* (JPN6614, 6616) were distinguished by the length of the flower bracts (3–4.7 cm in var. *caput-avis* vs. 1.5–2 cm in var. *tosana*), the length of flowers (5–6.5 cm vs. 4.3–5 cm), and the length of pedicels (1.5–2.3 cm vs. 0.9–1.3 cm).

In Yanase, Aki-gun, the type locality of *H. tosana* var. *caput-avis*, we only found *H. tosana* var. *caput-avis*. Four samples from Yanase and an additional sample from its vicinity formed a clade with a bootstrap support of 100%. This clade was sister to another clade with a bootstrap support of 100%, comprising *H. tosana* var. *caput-avis* from Kami City and var. *tosana* from Kami City and three other localities including its type locality at Kajigamori (Fig. 5). The samples from Yanase and its vicinity also formed a distinct cluster in the Splits Tree analysis (Fig. 6).

Genetic divergence between taxa

Genetic divergence between taxa, measured by F_{ST} (Table 2), varied from 0.07 to 0.52. Specifically, F_{ST} values between infraspecific taxa were observed as 0.10 and 0.14 between *H. tosana* var. *tosana* and var. *caput-avis*, and *H. takiminazukiflora* subsp. *takiminazukiflora* and subsp. *grandis*, respectively. Additionally, F_{ST} values were found to be 0.15 and 0.16 between the Kagami River lineage and either *H. tosana* var. *tosana* or var. *caput-avis*, respectively. Between species within Clade 1, F_{ST} values exhibited variability, ranging from 0.07 (between *H. polyneuronoides* and *H. tardiva* subsp. *densiflora*) to 0.33 (between *H. minazukiflora* and either *H. tardiva* subsp. *tardiva* or *H. samukazemontana*), and 0.38 (between *H. takiminazukiflora* subsp. *grandis* and either *H. tardiva* subsp. *tardiva*, *H. shikokiana* 2, or *H. minazukiflora*). Notably, F_{ST} values between *H. takiminazukiflora* subsp. *grandis* and other species within Clade 1 (ranging from 0.18 to 0.38) tended to be larger than values observed between other species (ranging from 0.10 to 0.33).

Table 2. Genetic divergence between species or subspecies measured by F_{ST} . The abbreviations in the first line represent taxon names from *H. t. densiflora* (*H. tardiva* subsp. *densiflora*) to *H. tosana* var. *tosana*, and the last *capu* represents *H. tosana* var. *caput-avis*.

	<i>dens</i>	<i>poly</i>	<i>shi1</i>	<i>shi2</i>	<i>mina</i>	<i>grand</i>	<i>taki</i>	<i>samu</i>	<i>scab</i>	<i>long</i>	<i>Kaga</i>	<i>tosa</i>	<i>capu</i>
<i>H. t. tardiva</i>	0.16	0.15	0.29	0.32	0.33	0.38	0.27	0.30	0.23	0.31	0.44	0.40	0.40
<i>H. t. densinervia</i>		0.07	0.18	0.18	0.18	0.25	0.16	0.16	0.12	0.19	0.37	0.34	0.33
<i>H. polyneuronoides</i>			0.14	0.12	0.12	0.18	0.14	0.10	0.09	0.15	0.30	0.29	0.28
<i>H. shikokiana</i> 1				0.17	0.22	0.30	0.19	0.24	0.18	0.24	0.41	0.36	0.36
<i>H. shikokiana</i> 2					0.27	0.38	0.17	0.31	0.17	0.24	0.50	0.40	0.41
<i>H. minazukiflora</i>						0.38	0.18	0.33	0.16	0.25	0.52	0.43	0.42
<i>H. t. grandis</i>							0.14	0.37	0.22	0.28	0.51	0.42	0.43
<i>H. t. takiminazukiflora</i>								0.18	0.14	0.17	0.37	0.33	0.32
<i>H. samukazemontana</i>									0.17	0.25	0.49	0.41	0.41
<i>H. scabrinervia</i>										0.17	0.38	0.34	0.33
<i>H. longipedicellata</i>											0.42	0.37	0.36
<i>H. tosana</i> Kagami River lineage												0.15	0.16
<i>H. tosana</i> var. <i>tosana</i>													0.10

Discussion

Our phylogenetic analysis of Japanese *Hosta* species showed that *H. tardiva* subsp. *densinervia* and *H. scabrinervia*, previously classified as *H. kikutii* var. *densinervia* and var. *scabrinervia* by Tamura and Fujita (2016), belong to Clade 1 (Fig. 1). In contrast, *H. kikutii*, previously classified as *H. kikutii* var. *kikutii* belongs to Clade 3, while *H. tosana*, previously classified as *H. kikutii* var. *tosana*, belongs to Clade 4. Therefore, “*H. kikutii*” in the sense of Tamura and Fujita (2016) are polymorphic.

Among taxa previously treated as varieties of *H. kikutii*, *H. tardiva* subsp. *densinervia* formed a clade with *H. tardiva* subsp. *tardiva*, supported by a bootstrap value as high as 99% (Fig. 1) or 92% (Fig. 2). This result was unexpected because they are placed in different sections due to their significant morphological differences. According to Fujita’s key (1976), Japanese *Hosta* species can be classified into two main groups based on the presence or absence of darker colored veins inside the perianths. *Hosta tardiva* subsp. *tardiva* belongs to Sect. *Tardanthae* of the former group, while *H. tardiva* subsp. *densinervia* belongs to Sect. *Helipteroides* of the latter group. *Hosta tardiva* subsp. *tardiva* has attractive purple perianths with darker colored inside veins and is widely cultivated in gardens (Fujita 1976). This subspecies shows high sterility in flowers (Fujita 1976), and is suggested to be of hybrid origin (Tamura and Fujita 2016). It has a diploid chromosome number of $2n=60$, but has two pairs of homologous chromosomes that are unequal in size and morphology which is believed to be the cause of the species high sterility (Kaneko 1970).

Considering significant morphological differences, it is puzzling that the cluster of *H. tardiva* subsp. *tardiva* is a single offshoot of *H. tardiva*. Consequently, if *H. tardiva* subsp. *tardiva* is separated, *H. tardiva* subsp. *densinervia* cannot be considered monophyletic. The result of the STRUCTURE analysis provides a clue to explain this puzzling result. Ten samples of *H. tardiva* subsp. *densinervia* col-

lected from the lower reach of the Niyodo River exhibit a mixture of two genetic identities: one identity is dominant in *H. tardiva* subsp. *tardiva*, and another identity is dominant in *H. polyneuronoides*, as well as in other samples of *H. tardiva* subsp. *densinervia*, including three samples from the type locality population in Tokushima Prefecture. In the Maximum Likelihood tree (Fig. 2) and the SplitsTree (Fig. 3), these ten samples are positioned between *H. tardiva* subsp. *tardiva* and five other samples of *H. tardiva* subsp. *densinervia*. Although the flowers of *H. tardiva* subsp. *tardiva* are highly sterile (Fujita 1976), Fujii (2018) reported that *H. tardiva* subsp. *tardiva* can cross with other species, producing fertile pollen grains. This finding suggests that the ten samples of *H. tardiva* subsp. *densinervia* from the Niyodo River originated through hybridization events between *H. tardiva* subsp. *tardiva* and ancestral lineages of *H. tardiva* subsp. *densinervia*. Despite this hybrid origin, these ten samples are morphologically identical to other samples of *H. tardiva* subsp. *densinervia* and lack morphological traits indicative of their hybridization with *H. tardiva* subsp. *tardiva*. Therefore, we propose that the Niyodo River population of *H. tardiva* subsp. *densinervia* has become stabilized through repeated backcrossing with ancestral lineages of *H. tardiva* subsp. *densinervia*.

Hosta tardiva is likely an instance of anacladogenetic speciation, wherein a new species originates through budding, initially rendering the ancestral taxon paraphyletic. In such instances, it becomes necessary to acknowledge a paraphyletic subspecies when both derived and ancestral lineages display distinct diagnostic traits that set them apart (Carnicero et al. 2019). Theoretically, these subspecies are referred to as diachronic subspecies, constituting segments of species-level clades that are differentiated from other parts of the clade by evolutionarily significant characteristics (Reydon and Kunz 2021). Our classification of *H. tardiva* subsp. *tardiva* and *H. tardiva* subsp. *densinervia* is grounded in this subspecies concept.

Hosta tardiva subsp. *densinervia* and *H. scabrinervia* were initially described as *H. kikutii* var. *densinervia* and var. *scabrinervia* by Fujita and Tamura (2008). Morphologically, *H. scabrinervia* is similar to *H. tardiva* subsp. *densinervia* but distinguished by anther length (3 mm long compared to 5 mm long). These taxa are also distinct in their phylogenetic positions within Clade 1 and their flowering seasons. Flowering specimens of *H. tardiva* subsp. *densinervia* were collected between August 13 and September 27. On the other hand, the flowering specimens of *H. scabrinervia* were collected from July 13 to July 25. Therefore, *H. tardiva* subsp. *densinervia* and *H. scabrinervia* are considered to be reproductively isolated, and we propose treating *H. scabrinervia* as a separate species from *H. tardiva*.

In the STRUCTURE analysis, *H. scabrinervia* exhibited a mixture of two genetic identities, one identity dominated in *H. tardiva* subsp. *densinervia* and another identity shared by seven species. This result seems to suggest a hybrid origin of *H. scabrinervia*. Among the seven species, *H. takiminazukiflora* subsp. *takiminazukiflora* is most similar to *H. scabrinervia*; however, it can be distinguished by several characteristics. It has purplish green or purple flower bracts instead of white or purplish white in *H. scabrinervia*, and its petioles are shorter (3–22 cm long as opposed to (20–)22–40 cm long). *Hosta polyneuronoides* is also similar to *H. scabrinervia* but can be distinguished by longer anther-sacs (3.5–4 mm long vs. 3 mm in *H. scabrinervia*). If *H. scabrinervia* originated from a hybridization, *H. polyneuronoides* and *H. takiminazukiflora* subsp. *takiminazukiflora* are

most likely candidates of parental species. While available specimen records showed that *H. polyneuronoides* flowers earlier (July 25 to August 20) than *H. scabrinervia* (July 13 to July 25), these flowering records are close and two taxa may be able to hybridize in late July. However, at $K=5$ in the hierarchical STRUCTURE analysis, *H. scabrinervia* exhibited a unique genetic identity depicted by light blue, and did not exhibit another identity depicted by orange, which is dominant in *H. takiminazukiflora* subsp. *takiminazukiflora*. This result suggests that genetic variation accumulated after the origin of *H. scabrinervia*, even if it originated through a hybridization event between *H. polyneuronoides* and *H. takiminazukiflora* subsp. *takiminazukiflora*. In the SplitsTree, *H. scabrinervia* was separated from *H. takiminazukiflora*, which is closely related to *H. longipedicellata*, but connected with *H. polyneuronoides*. This relationship as well as the result of STRUCTURE analysis at $K=3$ suggest that *H. scabrinervia* is a species separated from *H. takiminazukiflora* and *H. polyneuronoides*, but could have a history of introgression with *H. polyneuronoides*.

Hosta polyneuronoides is closely related to *H. tardiva*, but its monophyly was supported by a bootstrap value of 82% (Fig. 2). It is morphologically similar to *H. tardiva* subsp. *densiflora*, having whitish perianths without distinct inside veins. However, *H. polyneuronoides* is distinguished by having 10 or fewer flowers per scape (compared to 20 or more in *H. tardiva* subsp. *densiflora*), and anther lengths of 3.5–4 mm (as opposed to 5 mm). Two taxa grow in different habitats: *H. tardiva* subsp. *densinervia* thrives in more open habitats on soil or crevices of dry rocks along riverbanks at elevations ranging from 90 m to 900 m, while *H. polyneuronoides* prefers shaded habitats on wet rocks along streams at elevations ranging from 237 m to 1980 m. While *H. tardiva* subsp. *densinervia* is widely distributed in the lower reaches of the Yoshino River, Niyo-do River, and Shimanto River, *H. polyneuronoides* is more restricted to a narrow area at the headwaters of the Yoshino River (Fig. 7). These two taxa may have differentiated due to geographical isolation and disruptive selection in distinct habitats. Despite an F_{ST} value of 0.07 between *H. tardiva* subsp. *densinervia* and *H. polyneuronoides*, which is lower when compared to the F_{ST} values between other species of Clade 1, the two taxa exhibited distinct identities in the hierarchical STRUCTURE analysis (Fig. 4C). Considering these findings, we propose distinguishing *H. polyneuronoides* as a separate species from *H. tardiva*.

Our phylogenetic analysis also indicated that another morphologically distinct species, *H. shikokiana*, belonged to Clade 1. Due to its morphological distinctiveness, Fujita (1976) placed *H. shikokiana* in Section *Eubryocles* F. Maek. together with a Chinese species *H. ventricosa* Stearn. However, *H. shikokiana* is closely related to a newly discovered species, *H. minazukiflora*. In addition to molecular phylogenetic analyses, the STRUCTURE analysis revealed a unique genetic identity shared between *H. shikokiana* and *H. minazukiflora* (Fig. 4). Thus, there is some justification for considering these two as intraspecific taxa within a single species. However, it is challenging to identify a diagnostic trait that categorizes *H. shikokiana* and *H. minazukiflora* as a single species. Considering the morphological differences between *H. minazukiflora* and *H. shikokiana*, we propose treating them as separate species. This proposition is also supported by a relatively high F_{ST} value of 0.22, indicating limited gene flow between *H. shikokiana* and *H. minazukiflora*. The clade comprising *H. shikokiana* and *H. minazukiflora* was found to be the sister group to *Hosta* sp. 3 collected from Wakayama Prefecture,

Honshu. This result indicates that the divergence of *H. shikokiana* and its related species occurred in a larger geographic area, including regions beyond Shikoku. Further studies with more extensive sampling across a broader area are needed to fully understand the diversity of *H. shikokiana* and its related species.

Our phylogenetic analyses, morphological observations, and field investigations have led to the discovery of three additional new species: *H. samukazemontana*, *H. longipedicellata*, and *H. takiminazukiflora*. Among them, *H. samukazemontana* occupies an intermediate position between a clade consisting of *H. shikokiana*, *H. minazukiflora*, and *H. sp. 3*, and another clade comprising *H. tardiva* subsp. *tardiva*, *H. tardiva* subsp. *densinervia*, and *H. scabrinervia* (Fig. 2). This separate position supports the notion that *H. samukazemontana* is a distinct species. *Hosta samukazemontana* is endemic to Mt. Kanpu and its surrounding area where it thrives on cliffs. Morphologically, *H. samukazemontana* is characterized by its scapes deflected from cliffs and curving upwards at the tip. In the STRUCTURE analysis, *H. samukazemontana* as well as *H. scabrinervia* exhibited a mixture of two genetic identities, one identity dominated in *H. tardiva* subsp. *densinervia* and another identity shared among seven species. However, at $K=5$ in the hierarchical STRUCTURE analysis, *H. samukazemontana* exhibited a unique genetic identity. These findings, along with the results of phylogenetic analyses and morphological observations, provide support for considering *H. samukazemontana* as a distinct species.

Hosta longipedicellata and *H. takiminazukiflora* formed a clade with a bootstrap support of 100%, and this clade, along with another clade comprising seven other species, originated at the base of Clade 1. This phylogenetic relationship supports the differentiation of *H. longipedicellata* and *H. takiminazukiflora* from the seven other species. We propose treating them as two distinct species, taking into account their genetic differences (Fig. 4, Table 2) and morphological characteristics (Figs 14, 15, 18, 19). The distribution ranges of *H. longipedicellata* and *H. takiminazukiflora* are isolated by an upstream reach of the Yoshino River (L and T in Fig. 7C) and their geological substrates are mafic schist and pelitic schist, respectively (Geological Survey of Japan 2023). These two species may have differentiated due to geographical isolation and disruptive selection on distinct geological substrates.

The divergence between the two sympatric subspecies of *H. takiminazukiflora* is intriguing. *Hosta takiminazukiflora* subsp. *grandis* is characterized by several distinct features including a cordate leaf base, a glaucous lower surface, green petioles and erect scapes without purple dots. These characteristics remain consistent under fertilized cultivation. In the Maximum Likelihood tree (Fig. 2), seven samples of *H. takiminazukiflora* subsp. *grandis* and three samples of *H. takiminazukiflora* subsp. *takiminazukiflora* formed two monophyletic groups with bootstrap support of 99% and 97%, respectively, even though they were collected at the same location. Consequently, considering these samples as variations within a single population is challenging. The observed genetic differentiation, coupled with distinct morphological variations, is likely sustained by strong disruptive selection and some degree of reproductive isolation. Based on this evidence, we treat them as two separate subspecies. This is likely another instance of anacladogenetic speciation, which initially resulted in the ancestral taxon, *H. takiminazukiflora* subsp. *takiminazukiflora*, being paraphyletic.

Notably, the F_{ST} values between *H. takiminazukiflora* subsp. *grandis* and the other species were relatively high, ranging from 0.22 to 0.51, while F_{ST} values

between the two subspecies were as low as 0.09. This suggests that *H. takiminazukiflora* subsp. *grandis* may have originated through hybridization between an unknown species and a lineage of *H. takiminazukiflora* subsp. *takiminazukiflora*. Further investigations are necessary to test this hypothesis and elucidate the origin and taxonomic status of *H. takiminazukiflora* subsp. *grandis*.

While Clade 1 was classified into nine separate species, Clade 4 was considered to be a single species, *H. tosana*. Following the earlier classification proposed by Maekawa (1948), we morphologically identified two varieties, *H. tosana* var. *tosana* and var. *caput-avis*, but both varieties were not monophyletic (Fig. 5). The F_{ST} value between *H. tosana* var. *caput-avis* and var. *tosana* was of 0.10, the second smallest following the 0.07 F_{ST} value between *H. polyneuronoides* and *H. tardiva* subsp. *densinervia*. This suggests a relatively weak differentiation between these two “varieties”. Furthermore, the results of the STRUCTURE analysis (Fig. 4F) showed that the JPN6553–55 samples identified as *H. tosana* var. *tosana* exhibited a mixture of genetic identities from *H. tosana* var. *caput-avis* and var. *tosana*, suggesting a possible origin through hybridization. The SplitTree analysis (Fig. 6) placed the JPN6553–55 samples between other samples of *H. tosana* var. *tosana* and *H. tosana* var. *caput-avis*, supporting a hybrid origin. These findings suggest that *H. tosana* var. *caput-avis* and *H. tosana* var. *tosana* hybridize in the sympatric population along the Befu Valley. In contrast, *H. tosana* var. *caput-avis* in Yanase, the type locality (JPN12536-12539) and its vicinity (JPN12653) showed clear differentiation from *H. tosana* var. *caput-avis* in Kami City (Figs 5, 6), suggesting that “*H. tosana* var. *caput-avis*” is polyphyletic. The available evidence indicates that var. *caput-avis* is not a distinct variety.

Additionally, we discovered another lineage designated as the Kagami River lineage, which was monophyletic and distinct from other lineages. The F_{ST} values between the Kagami River lineage and the two “varieties” were 0.15 and 0.16, respectively, indicating significant differentiation of the Kagami River lineage from the two “varieties”. It is likely that the Kagami River lineage can be distinguished as an infraspecific taxon. However, since *H. tosana* is widely recorded in the eastern part of Shikoku, further studies encompassing the entire range of *H. tosana* are needed to elucidate the taxonomic status of the Kagami River lineage.

Remarkably, seven species within Clade 1 (*H. longipedicellata*, *H. minazukiflora*, *H. samukazemontana*, *H. scabrinervia*, *H. shikokiana*, *H. takiminazukiflora*, and *H. polyneuronoides*) are densely distributed in the upper reaches and headwaters of the Yoshino River (Fig. 7B, C). This pattern suggests that speciation within *Hosta* Clade 1 was uniquely accelerated by factors specific to this region. Within Clade 1, *H. polyneuronoides* occupies a broader elevation range from 237 m to 1980 m. While *H. polyneuronoides* grows on rocks along streams and *H. shikokiana* thrives on the soil on the ridgeline of Mt. Ishizuchi, five other species grow on cliffs in geographically isolated localities (L, Sa, T, M, and an open square in Fig. 7C). Thus, spatial isolation between cliff habitats is likely a driving force behind the speciation of these five species. In addition to spatial isolation, the geological diversity of this region could also contribute to the speciation of Clade 1. For instance, *H. shikokiana* is restricted to Mt. Higashi-akaishi and its surroundings, where serpentine rock is exposed. Some endemic taxa associated with serpentine soil, such as *Adenophora triphylla* (Thunb.) A. DC. var. *puellaris* (Honda) H. Hara and *Euphrasia microphylla* Koidz., are known in this area (Yamanaka 1952, 1958). Furthermore, *H. longipedicellata* and *H. takiminazukiflora* are found

on different geological substrates: mafic schist and pelitic schist, respectively (Geological Survey of Japan 2023). Studying how these species have adapted to cliff habitats and different substrates will yield a deeper understanding of the factors propelling the diversification of these species.

In the Taxonomy section, we provide a key to the taxa and update the taxonomy of *Hosta* species found on Shikoku Island, belonging to Clades 1 and 4. This update includes descriptions of six new taxa, encompassing five new species and one new subspecies, along with revised status for two existing taxa. However, we have refrained from revising the taxonomy of Clade 2, Clade 3, and Clade 6, pending further studies. Further comprehensive studies combining MIG-seq with meticulous morphological observations are necessary for these clades.

Below, we first treat *H. tardiva* subsp. *tardiva* and *H. tardiva* subsp. *densinervia* comb. & stat. nov., located on the top of the Maximum Likelihood tree for Clade 1 (Fig. 2). Secondly, we treat *H. scabrinervia* stat. nov. because it was compared with *H. tardiva* subsp. *densinervia* in the original description (Fujita and Tamura 2008). Thirdly, we treat two closely related species of Clade 1, *H. shikokiana* and *H. minazukiflora* sp. nov. Fourthly, we describe three species of Clade 1, *H. takiminazukiflora* sp. nov., *H. longipedicellata* sp. nov., and *H. samukazemontana* sp. nov. Fifthly, we treat a species of Clade 4, *H. tosana*.

Taxonomy

Key to the species of *Hosta* from Shikoku Island

- 1 Bracts ascending or spreading before flowering **Clade 2, 3, and 6; not treated below**
- Bracts tightly closed before flowering **2**
- 2 Scapes deflected and gently bent like a bow, curved upwards at the tip **3**
- Scapes straight **4**
- 3 Perianths 4.5–5 cm long, whitish. Flower bracts whitish purple, 1.7–2.1 cm long **8. *Hosta samukazemontana***
- Perianths 6.1–6.9 cm long, purple. Flower bracts whitish green, 2.2–5.2 cm long **9. *Hosta tosana***
- 4 Leaf margin strongly undulated. Leaves with decurrent to winged petioles, lustrous below when dried **4. *Hosta shikokiana***
- Leaf margin either not undulated or weakly undulated. Leaves not or only slightly decurrent to petioles, not lustrous below when dried **5**
- 5 Perianths purple or lavender; inside veins distinct **6**
- Perianths whitish; inside veins indistinct **7**
- 6 Perianths purple; five distinct veins inside each lobe **1A. *Hosta tardiva* subsp. *tardiva***
- Perianths lavender; three distinct veins inside each lobe **5. *Hosta minazukiflora***
- 7 Anthers 3.5–5 mm long **8**
- Anthers 2.5–3 mm long **9**
- 8 Flowers more than 20 per scape. Anthers 5 mm long **1B. *Hosta tardiva* subsp. *densinervia***
- Flowers 3–10 per scape. Anthers 3.5–4 mm long **2. *Hosta polyneuronoides***

- 9 Leaf blades glaucous abaxially, broadly ovate **6A. *Hosta takiminazukiflora* subsp. *grandis***
– Leaf blades light green abaxially, not glaucous, ovate or oblong-ovate..... **10**
10 Flower bracts 2–2.5 cm long, withering during flowering. Leaf veins papillose on abaxial surface **7. *Hosta longipedicellata***
– Flower bracts (2–)2.5–4.6 cm long, fresh during flowering..... **11**
11 Flowers 15–20 per inflorescence. Flower bracts white or purplish white. Petioles (20–)22–40 cm long. Leaf veins papillose or smooth on abaxial surface **3. *Hosta scabrinervia***
– Flowers 4–13 per inflorescence. Flower bracts purplish green or purple at anthesis. Petioles 3–22 cm long. Leaf veins smooth on abaxial surface... **6. *Hosta takiminazukiflora* subsp. *takiminazukiflora***

1. *Hosta tardiva* Nakai, Bot. Mag. (Tokyo) 44: 513. 1930.

Type. JAPAN. Prov. Awa (Ehime Pref.): tractu Myōtō, in oppido Kamomyō, n.d., *J. Nikai* 1377 (holotype TI, n.v.).

1A. *Hosta tardiva* subsp. *tardiva*

Phenology. August to September.

Distribution and habitat. Japan (Shikoku Island). This species is widely distributed in Shikoku and typically thrives on herbaceous slopes near villages. It is cultivated in Honshu but often naturalized when it escapes cultivation.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be qualified as LC (Least Concern) because its population is estimated to exceed 1000.

Japanese name. Nankai-giboshi (Nakai 1930).

Specimens examined. JAPAN. Kochi Pref.: Takaoka-gun, Kubokawa-cho, 8 Aug. 2001, *Se. Fujii* 8725 (JPN10003, FU!), ditto, 23 Aug. 2002, *Se. Fujii* 9271 (JPN10002, FU!); Cultivated at Makino Botanical Garden (32 specimens listed in the Suppl. material 1; FU!).

Note. In Shikoku, it usually grows near mountain villages, suggesting that its wild populations escaped from cultivation.

1B. *Hosta tardiva* subsp. *densinervia* (N. Fujita & M.N. Tamura) Yahara & Se.Fujii, comb. & stat. nov.

urn:lsid:ipni.org:names:77331104-1

Fig. 8

Hosta kikutii var. *densinervia* N. Fujita & M. N. Tamura, Acta Phytotax. Geobot. 59: 34 (2008); Tamura & Fujita in Iwatsuki et al., Fl. Jap. IVb: 143 (2016).
Type. JAPAN, Shikoku: Tokushima Pref, Miyoshi-gun, Ikeda-cho, Shikino-kami Ferry, 27 Sep. 1965, with flowers, *S. Takafuji* 253 (holotype KYO!, isotype KYO!).

Hosta kikutii var. *polyneuron* sensu Fujita, Acta Phytotax. Geobot. 27: 80. 1976, p.p.

Phenology. Mid-August to September.

Distribution and habitat. Japan (Ehime, Kagawa, Kochi, and Tokushima Prefectures). This subspecies is distributed in the lower reaches of the Yoshino River, the middle and lower reaches of the Niyodo River, the headwater of the Shinjyo River (near the headwaters of the Shimanto River), and the lower reaches of the Shimanto River (Fig. 7). It grows in open habitats on rocks and soil along the riverbanks.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as LC (Least Concern) because its population is estimated to exceed 1000.

Japanese name. Sudare-giboshi (Maekawa 1952, excluding Yakushima-giboshi), Awa-giboshi (Fujita and Tamura 2008).

Specimens examined. JAPAN. Ehime Pref.: Kamiukena-gun, Omogokei, 13 Aug. 1970, *N. Fujita* 269, with flowers (KYO!); Saijyo City, 450 m elev., 6 Aug. 1971, *H. Takahashi & N. Fujita* 273 with young flower buds (KYO!); Nii-gun, 500 m elev., 30 Aug. 1955, *G. Murata* 9233 with flowers (KYO!). **Kagawa Pref.:** Mt. Otake, 24 Sep. 1961, *S. Sakaguchi* s.n. with flowers (KYO!). **Kochi Pref.:** Agawa-gun, Niyodogawa-cho, Kuki, 80 m elev., 18 Aug. 2020, *T. Yahara et al.* JPN1255, 1258, 1263 with flowers (FU!); ditto, in the headwater of a tributary of Shinjyo River, 900 m elev., cultivated in Makino Botanical Garden, 8 Apr. 2021, *T. Yahara et al.* JPN3929 sterile (FU!); Agawa-gun, Ino-cho, Kashiki, 40 m elev., 20 Aug. 2020, *T. Yahara et al.* JPN1331–1333 with flowers (FU!); Takaoka-gun, Ochi-cho, Kataoka, 50 m elev., 20 Aug. 2020, *T. Yahara et al.* JPN1375–1377 with flowers (FU!); ditto, Tokoroyama, 13 Sep. 1962, *G. Murata* 17106 and 17107 with flowers

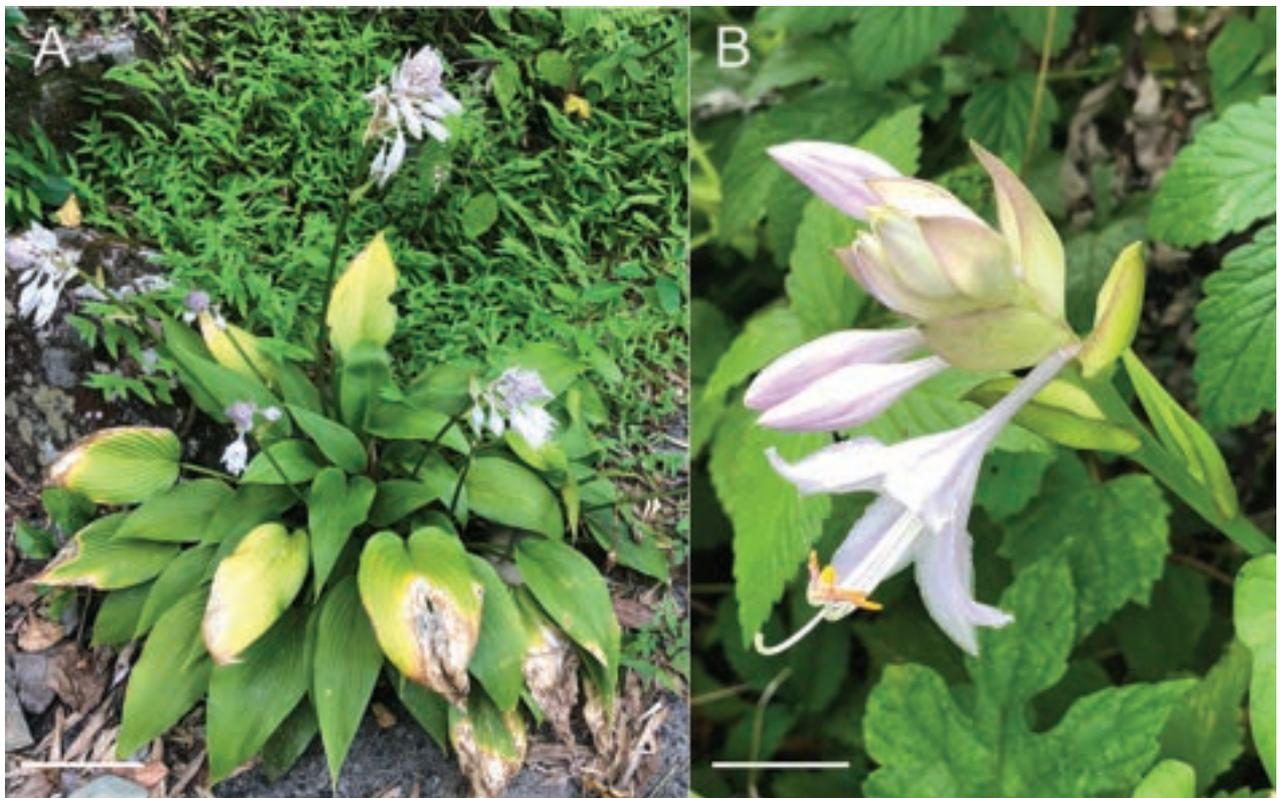


Figure 8. Flowering plants of *H. tardiva* subsp. *densinervia* **A** JPN1263, 18 Aug. 2020 **B** JPN1332, 20 Aug. 2020. Scale bars: 20 cm (**A**); 2 cm (**B**).

(KYO!); Hata-gun, Nakamura-cho, along the Shimanto River, 20 Aug. 1913, Z. Tashiro s.n. with flowers (KYO!). **Tokushima Pref.:** Miyoshi City, Shikino-kami, 90 m elev., 11 Apr. 2021, sterile, T. Yahara et al. JPN4071, 4072, and 4075 (FU!); Ikeda-cho, along the Yoshino River, 16 Sep. 1971, C. Abe 43049 with flowers (KYO!); Miyoshi-gun, Minawa-mura, Kawasaki, along the Iya River, 8 Sep. 1958, T. Yamanaka 26270 with flowers (KYO!).

Note. This subspecies is morphologically more similar to *H. polyneuronoides*, rather than to *H. tardiva* subsp. *tardiva*.

2. *Hosta polyneuronoides* Yahara & Se.Fujii, sp. nov.

urn:lsid:ipni.org:names:77331106-1

Fig. 9

Diagnosis. *Hosta polyneuronoides* is distinguished from *H. tardiva* subsp. *densinervia* in having 3–10 flowers per inflorescence (in contrast to 15–25 in subsp. *densinervia*) and anthers 3.5–4 mm long (compared to 5 mm long).

Type. JAPAN. Kochi Pref.: Agawa-gun, Ino-cho, near Nitaki Bridge, 237 m elev., 20 Aug. 2020, T. Yahara, K. Fuse & H. Sato JPN1378 with flowers (holotype FU!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 3–15 per ramet; blades ovate or oblong-ovate, 7.5–28.5 cm long, 3.3–15.3 cm wide, 0.9–2.9 times longer than width, thinly papery, glabrous on both surfaces, base cuneate to subcordate, apex acute to short acuminate, margin entire, veins 8–9 pairs, smooth on the lower surface; petioles 5–40 cm long, narrowly winged, 0.3–0.5 cm wide, glabrous. Scape 15–51 cm long, rachis terete, bract lanceolate, 4 cm long, 0.3–0.5 cm wide, light green, glabrous. Raceme 4.3–9 cm long, 3–10-flowered; flower bracts vivid (not withering) in anthesis, erect or diagonally spreading, whitish green, oblong-lanceolate, boat-shaped, 1.2–3.1 cm long, 0.1–0.8 cm wide, membranous, glabrous, apex acuminate. Flowers not fragrant, 5.7–6.0 cm long; pedicels 0.6–1.3 cm long, glabrous. Perianths white or very pale purple-white, funnel-form, 2.1–4.9 cm long, glabrous, 6-lobed; tube abruptly dilated from apical 2/3, lobes narrowly triangular, 0.7–1.2 cm long, 0.4–1 cm wide, apex acute. Stamens 6, 0.1–0.4 cm exerted from perianth; filaments white, free, 4.5–4.8 cm long, glabrous, anthers purple to yellow when fresh, dark blue-grey to light yellow when dried, 3.5–4 mm long. Ovary ellipsoid, 0.5 cm long, glabrous style 5.5–5.7 cm long, upwardly curved at the distal part, subequal to 1.5 cm exerted from perianth, glabrous, stigma capitate. Capsules or seeds not observed.

Phenology. Flowering from July to August.

Distribution and habitat. Japan (Kochi and Ehime Prefectures). *Hosta polyneuronoides* grows on open or shaded rocks and rock cliffs along rivers in the upper reaches and headwaters of the Yoshino River, and on the rocky ridgeline from Mt. Ishizuchi to Mt. Komochi-gongen and its vicinity.

Etymology. This subspecies was named for its resemblance to *H. tardiva* subsp. *densinervia* identified as *Hosta kikutii* var. *polyneuron* by Fujita (1976).

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as NT (Near threatened) because its population is estimated to exceed 1000 but may be declining under the influence of river bank construction and collection for horticulture.

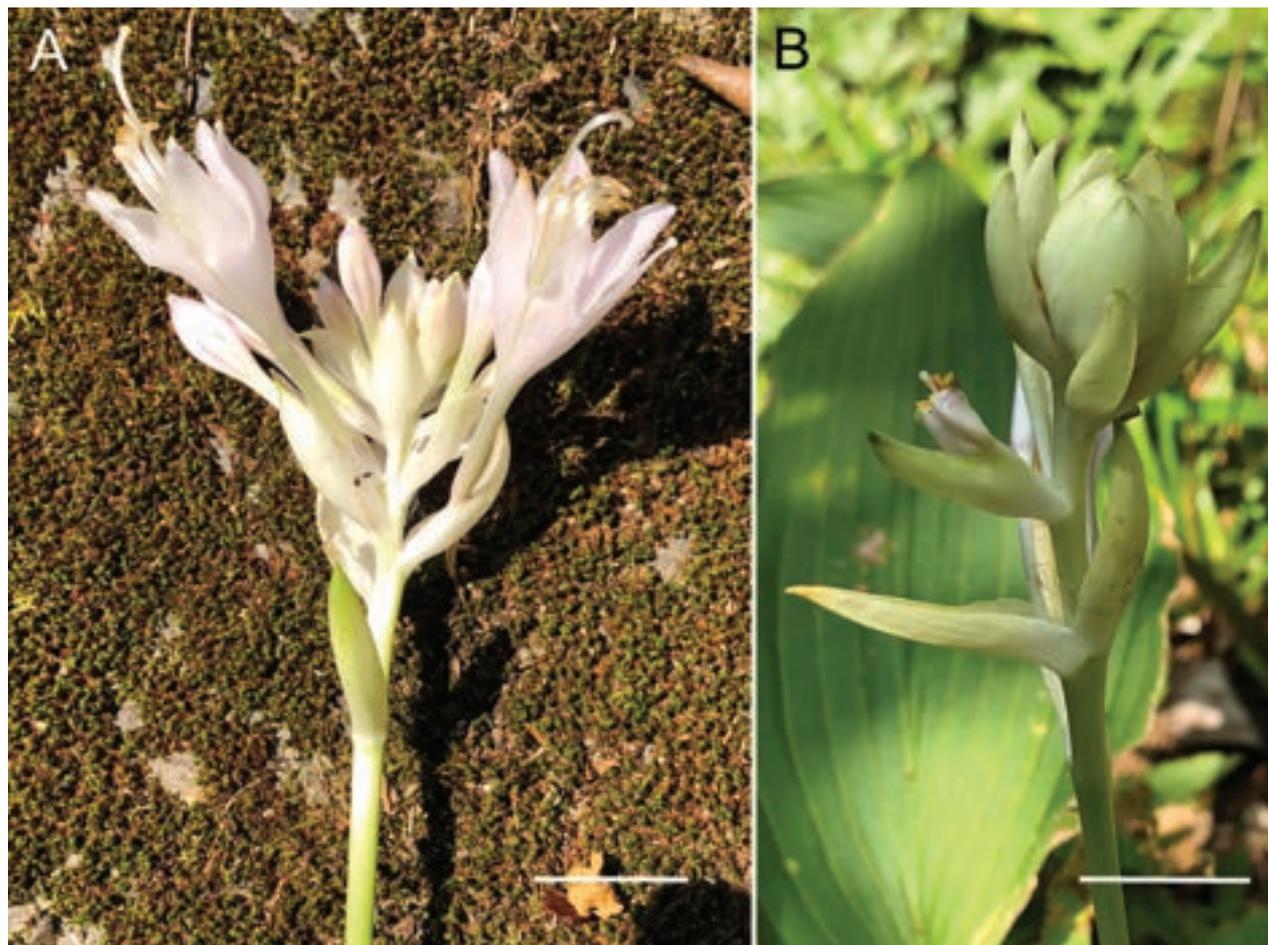


Figure 9. Flowering plants of *H. polyneuronoides* **A** JPN1378, 20 Aug. 2020 **B** JPN1327, 20 Aug. 2020. Scale bars: 2 cm (**A, B**).

Japanese name. Oku-sudare giboshi (new).

Additional specimens examined. JAPAN. Ehime Pref.: Niihama City, Mt. Higashi-akaishi, 970 m elev., 21 Jun. 2021, *T. Yahara et al.* JPN6269–6271 sterile (FU!); ditto, 1040 m elev., 21 Jun. 2021, *T. Yahara et al.* JPN6153–6156 sterile (FU!); Mt. Ishizuchi, 28 Jul. 1972, *S. Takafuji* 799 with flowers (KYO!); Kamiukena-gun, Mt. Ishizuchi, 1600 m elev., 23 Jun. 2021, *T. Yahara et al.* JPN6390 sterile (FU!); ditto, 1980 m elev., 23 Jun. 2021, *T. Yahara et al.* JPN6407 sterile (FU!); Saijyo City, Mts. Ishizuchi, from Yoake-toge to Mt. Nishinokanmuri-dake, 1600 m elev., 7 Aug. 1971, *H. Takahashi & N. Fujita* 226 with flowers (KYO!). **Kochi Pref.:** Nagaoka-gun, Motoyama-cho, Along Asemi River, 480 m elev., 19 Aug. 2020, *T. Yahara et al.* JPN1327–1329 with flower buds (FU!); Tosa-gun, Okawa-mura, 570 m elev., 24 Jun. 2021, *T. Yahara et al.* JPN6493–6495 sterile (FU!); Tosa-gun, Ohkawa-mura, Kawasaki, 400 m elev., 24 Jun. 2021, *T. Yahara et al.* JPN6501–6503 with flower buds (FU!); Tosa-gun, Ohkawa-mura, Takano, 24 Jun. 2022, *K. Fuse et al.* JPN12529, 12530, 12532, 12535 sterile (FU!); Tosa-gun, Ohkawa-mura, near Okina Waterfall, 443 m elev., Jul. 25, 2023, *Se. Fujii*, FJW49-1, 2 with flowers (MBK!); Tosa-gun, Tosa-cho, 24 Jun. 2022, *K. Fuse et al.* JPN12506–12508 sterile (FU!); Agawa-gun, Ino-cho, 750 m elevation, 23 Jun. 2021, *T. Yahara et al.* JPN6449–6451 sterile (FU!); Agawa-gun, Ino-cho, 900 m elevation, 23 Jun. 2021, *T. Yahara et al.* JPN6446–6448 sterile (FU!); Agawa-gun,

Ino-cho, Kamegamori Forest Road, 1650 m elev., 22 Jun. 2021, *T. Yahara et al. JPN6342, 6361* sterile (FU!); ditto, Mt. Komochi-gongen, 29 Jun. 2022, *K. Fuse et al. JPN12663, 12664* sterile (FU!); Agawa-gun, Ino-cho, Ohmori-gawa dam, 755 m elev., 3 May 2022, *Se. Fujii JPN12427* sterile (FU!); Agawa-gun, Ino-cho, Terakawa-Ohdaru, 29 Jun. 2022, *K. Fuse et al. JPN12666–12668* sterile (FU!). The sterile specimens were identified using MIG-seq.

Note. Two specimens collected from Mt. Ishizuchi (*S. Takafuji 799* and *H. Takahashi & N. Fujita 226*) were cited in the original description of *H. shikokiana* (Fujita 1976), but were identified as *H. polyneuronoides* by molecular evidence and also morphologically in having leaves not undulate along the margin and not lustrous below, perianths not purple and without dark purple veins, and anthers 0.4 mm long.

3. *Hosta scabrinervia* (N. Fujita & M.N. Tamura) Yahara & Se.Fujii, stat. nov.

urn:lsid:ipni.org:names:77331107-1

Fig. 10

Hosta kikutii var. *scabrinervia* N. Fujita & M. N. Tamura, Acta Phytotax. Geobot. 59: 34. 2008. Type: JAPAN. Tokushima Pref., Miyoshi-gun, Ohboke, along the river, 13 Jul. 1968, *C. Abe 33197* (holotype KYO!).

Hosta kikutii var. *polyneuron* sensu Fujita, Acta Phytotax. Geobot. 27: 80. 1976, p.p.

Phenology. Flowering in July.

Distribution and habitat. Japan (Tokushima and Kochi Prefectures). The typical lineage of this species grows in rock crevices in the open habitats of riverbanks along the middle reach of Yoshino River. The upstream lineage (JPN12523-12525, FJS00006, FJS00007) grows on the wet cliffs in the upper reaches of the Yoshino River.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as VU (Vulnerable) because its population is estimated to be between 250 and 1000.

Japanese name. Zaratsuki-giboshi (Fujita and Tamura 2008).

Additional specimens examined. **JAPAN. Kochi Pref.:** Nagaoka-gun, Ohtoyo-mura, Isodani, along Yoshino River, 8 Sep. 1958, *T. Yamanaka 26222* with fruit (KYO!); Nagaoka-gun, Ohtoyo-cho, Higashidoi, Yoshino River, 4 Jul. 2002, *H. Sasaoka FOK-603540* with flowers (MBK0170549!); left bank of Yoshino River, Okubo, Ohtoyo-cho, Nagaoka-gun, 26 Nov. 2017, *A. Sakamoto et al. FOS-017749* with fruit (MBK0319724!); ditto, 18 Jul. 2004, *M. Matsumoto et al. FOK-067597* with flowers (MBK0087490!); Tosa-gun, Ohkawa-mura, Takano, 24 Jun. 2022, *K. Fuse et al. JPN12523–12525* (FU!); Tosa-gun, Ohkawa-mura, Kawasaki, Jul. 25, 2023, *Se. Fujii FJW-50-1, 2* with flowers (MBK0342373!, MBK0342374!); Tosa-gun, Ohkawa-mura, Kogane Waterfall, 23 Jul. 2022, *Se. Fujii JPN15231, 15232*, with flowers (FU!); ditto, Jul. 25, 2023, *Se. Fujii FJW-51-1–4* with flowers (MBK!); Nagaoka-gun, Motoyama-cho, in front of Kizenzan Park, 8 Aug. 1971, *H. Takahashi & N. Fujita 214* with flowers and fruit (KYO!); . **Tokushima Pref.:** Miyoshi-gun, Oboke, 11 Apr. 2011, *T. Yahara et al. JPN4080, 4082–4087* sterile (FU!); ditto, 22 Jul. 1967, *C. Abe 33157* with fruits (KYO!).



Figure 10. Flowering plants of *H. scabrinervia*. **A** Abe 33197 (holotype KYO) **B, C** JPN15231, 23 Jul. 2022. Scale bars: 2 cm (**A, B**).

Note. Fujita and Tamura (2008) distinguished var. *scabrinervia* from var. *densinervia* by the papillose lower surface of the lateral veins, but some plants of the type locality population have smooth lower lateral vein surfaces. Morphologically, *H. scabrinervia* is similar to *H. tardiva* subsp. *densinervia* but distinguished by the flowering season (July in contrast to August to September in the latter) and anther length (3 mm long vs. 5 mm long). The specimens collected from Kogane Waterfall (JPN15231, etc.) have exceptionally long pedicels (2.9–3.4 cm long), compared to 2–2.7 cm in other specimens. In this aspect, they are similar to *H. longipedicellata*, but can be distinguished by their flower bracts which remain fresh during flowering (unlike the withering flower bracts of *H. longipedicellata*). In the original description of *H. kikutii* var. *scabrinervia*, Fujita and Tamura (2008) cited specimens from Ehime and Kochi Prefectures, but further studies combining molecular analyses with subsequent morphological observations are required to confirm their identities. As far as examined, the range of *H. scabrinervia* is restricted to a narrow region in the middle reach of Yoshino River (Fig. 7).

4. *Hosta shikokiana* N. Fujita, Acta Phytotax. Geobot. 27: 93. 1976. Plants on Mt. Higashi-akaishi, Mt. Akaishi, Mt. Nishi-akaishi, and Mt. Shiragayama only. Fig. 11

Type. JAPAN. Ehime Pref., Mt. Higashi-akaishi, 7 Aug. 1957, T. Yamanaka 22475 (holotype KYO!).



Figure 11. Plants of *H. shikokiana* **A** JPN5249 **B** a flowering plant (photo taken on 12 Jul. 2018 by Se. Fujii; not collected). Scale bars: 10 cm (**A**); 5 cm (**B**).

Phenology. Flowering in July and fruiting in August.

Distribution and habitat. Japan (Ehime and Kochi Prefectures) (endemic). This species grows in open habitats on the rocky ridgeline of Mt. Higashi-akaishi and its surrounding areas on Shikoku Island.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as VU (Vulnerable) because its population is estimated to be between 250 and 1000.

Japanese name. Shikoku-giboshi (Fujita 1976).

Additional specimens examined. **JAPAN. Ehime Pref.:** Mt. Higashi-akaishi, 15 Jul. 1952, *T. Yamanaka 8901* with flowers (KYO!); ditto (as Mt. Akaishi), 9 Jul. 1928, *G. Koidzumi s.n.* with flower buds (KYO!); ditto, 1650 m elev., 21 Jun. 2021, *T. Yahara et al. JPN6197, 6228, 6239, 6249, and 6252* with flower buds (FU!); ditto, 1640 m elev., 14 Jul. 1960, *K. Tsuchiya 500* with flowers (KYO!); ditto, Mt. Hachimaki (a peak of Mts. Higashi-akaishi), 22 Jul. 2022, *Se. Fujii JPN9953, 31 Jul. 2022, Se. Fujii JPN9954* with flowers (FU!); derived from Mt. Higashi-akaishi, cultivated in Makino Botanical Garden, 8 Apr. 2021, *T. Yahara & Se. Fujii JPN3939–3942* sterile (FU!); between Mt. Higashi-akaishi and Mt. Hutatsudake, 1600 m elev., 8 Sep. 1961, *G. Murata 14981* with fruit (KYO!); ditto, cultivated stock of *G. Murata 14981, G. Murata s.n.* with flowers (KYO!). **Kochi Pref.:** Mt. Shiraga (cultivated stock), *T. Yahara & Se. Fujii JPN3937, 8 Apr. 2021, sterile (FU!); ditto, Se. Fujii JPN9955, 23 Jul. 2021* with flowers (FU!).

Note. The following specimens cited by Fujita (1976) are not this species; the specimen from Mt. Kanpu (*Yamawaki s.n., TI*) is *H. samukazemontana*, and the specimens from Mt. Ishizuchi (*Takahashi & Fujita 226, KYO; Takafuji s.n., KYO*) are *H. polyneuronoides*.

5. *Hosta minazukiflora* Se. Fujii & Yahara, sp. nov.

urn:lsid:ipni.org:names:77331108-1

Figs 12, 13

Diagnosis. *Hosta minazukiflora* is similar to *H. longipedicellata* in having leaves shorter than 20 cm, straight scapes shorter than 43 cm, and perianth lobes 0.6–0.8 cm wide. However, *H. minazukiflora* is distinguished from *H. longipedicellata* by its lavender flowers (vs. whitish), shorter pedicels (1.1–1.2 cm vs. 2.5–3.3 cm), narrower leaves (2.9–6.2 cm wide vs. (5.4–)9–12 cm wide) smooth on the lower surface (vs. papillose), and the occurrence at elevations of 270–280 m (vs. 1700–1750 m).

Type. JAPAN. Kochi Pref.: Nagaoka-gun, Motoyama-cho, Asemi River, 25 Jun. 2017, Se. Fujii & K. Yabe FOS-012016 with flowers (holotype MBK0318414!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 4–6 per ramet; blades oblong-ovate to oblong-lanceolate, 11.8–17.0 cm long, 2.9–6.2 cm wide, 2.4–4.3 times longer than width, thinly papery, glabrous on both surfaces, base cuneate, decurrent, slightly folded, apex acuminate, margin entire, veins in 6–9 pairs, smooth on the lower surface; petioles 8–19 cm long, narrowly winged, wing 0.1–0.3 cm wide, glabrous. Scape 18–43 cm long, terete. Raceme 10–15 cm long, 3–6-flowered; flower bracts vivid (not withering) in anthesis, erect, whitish purple, oblong-lanceolate, boat-shaped, 1.3–1.6 cm long, 0.2–0.4 cm wide, membranous, glabrous, apex acute. Flowers not fragrant, 5.2–5.9 cm long; pedicels 1.1–1.2 cm long, glabrous. Perianth whitish purple, funnel-form, 4.1–4.7 cm long, glabrous, 6-lobed; abruptly dilated from apical 2/3, lobes narrowly triangular, 1.2–1.4 cm long, 0.6–0.8 cm wide, apex acute. Stamens 6; filaments 4.6–5.4 cm long, white, free, glabrous, 0.5–0.7 cm exerted from perianth, upwardly curved at the distal part, anthers purple when fresh, dark blue-grey when dried, 3 mm long. Style 5.2–6.1 cm long, upwardly curved at the distal part, 1.1–1.4 cm exerted from the perianth, glabrous, stigma capitate. Ovary ellipsoid, 0.6 cm long, glabrous. Capsule green, cylindrical, 1.8–2.3 cm long, 0.3–0.4 cm wide, shallowly 3-angled. Seeds ellipsoid-ovoid, 2 mm long, with wings 4 mm long, black when dry.

Phenology. Flowering from mid to late June and fruiting in August.

Distribution and habitat. Japan (Kochi Prefecture). This species grows on rock cliffs along the Asemi River, a branch of the Yoshino River, in the central part of the Kochi Prefecture on Shikoku Island.

Etymology. The specific epithet was derived from the flowering season in June. Minazuki refers to June in Japanese.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as EN (Endangered) because its population is estimated to be between 50 and 250.

Japanese name. Minazuki-giboshi (new).

Additional specimens examined. JAPAN. Kochi Pref.: Nagaoka-gun, Motoyama-cho, Asemi River, 280 m elev., 19 Aug. 2020, T. Yahara et al. JPN1308 sterile, JPN1309 sterile, and JPN1310 with fruit (FU!); ditto, 13 Jun. 2004, Y. Yamashita et al. FOK-066868 with flowers (MBK0083845!); ditto, 23 May 2002, Y. Kokami et al. FOK-055767 with flower buds (MBK0146148!); ditto, 270 m elev., 25 Jun. 2017, Se. Fujii & K. Yabe FOS-012017 with flowers (MBK0318415!).



Figure 12. The type specimen of *H. minazukiflora*: Se. Fujii & K. Yabe FOS-012016 (holotype MBK).



Figure 13. Flowering plants of *H. minazukiflora*. Photos taken in the type locality (on 25 Jun. 2017 by Se. Fujii; not collected). Scale bars: 20 cm (A); 2 cm (B).

Note. *Hosta minazukiflora* is sister to *H. shikokiana* (Figs 2, 3), but morphologically more similar to *H. longipedicellata* (see diagnosis) and *H. scabrinervia*. The type specimen Se. Fujii & K. Yabe FOS-012016 was first identified as *H. kikutii* var. *scabrinervia*. While the two species are distributed in the Yoshino River system, *H. minazukiflora* is easily distinguished from *H. scabrinervia* by its smaller leaves (12–17 cm long and 3–6 cm wide compared to 17–32 cm long and 7.5–15 cm wide in *H. scabrinervia*), lavender perianths (in contrast to whitish perianths), and earlier flowering season in June (rather than July).

6. *Hosta takiminazukiflora* Se.Fujii & Yahara, sp. nov.

urn:lsid:ipni.org:names:77331109-1

Figs 14, 15

Diagnosis. *Hosta takiminazukiflora* is similar to *H. longipedicellata* and *H. minazukiflora*. It is distinguished from *H. longipedicellata* by leaves smooth on the lower surface (vs. papillose), pedicels 1.4–2.5 cm long (compared to 2.5–3.3 cm long), and flower bracts being fresh during flowering (in contrast to being withering), and from *H. minazukiflora* by perianths with a distinct midvein (as opposed to three distinct veins) and pedicels 1.4–2.5 cm long (compared to 1.1–1.2 cm long).

Type. JAPAN. Kochi Pref.: Tosa County, Ookawa village, Mt. Higashikado-yama, 22 Jul. 2007, with flowers, N. Inagaki et al. FOK-080097 with flowers (holotype MBK0189902!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 3–12 per ramet; blades oblong-ovate, 11–26.5 cm long, 3.6–10.5 cm, 2.1–3.3 times longer than wide, thinly papery, glabrous on both surfaces, base cuneate to obtuse, often decurrent, apex long acuminate, margin entire, veins in 6–10 pairs, smooth



Figure 14. The type specimen of *H. takiminzukiflora*: N. Inagaki et al. FOK-080097 (holotype MBK).

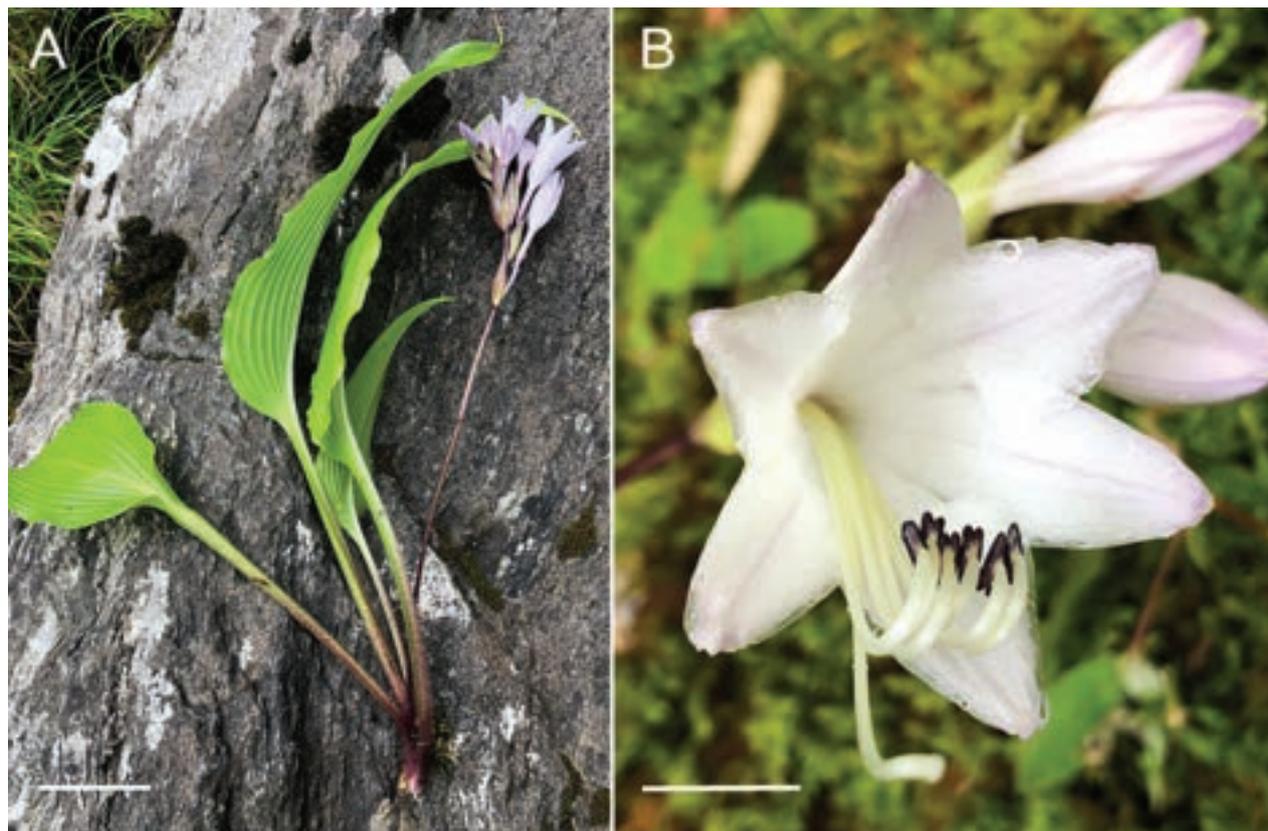


Figure 15. A flowering plant of *H. takiminazukiflora* subsp. *takiminazukiflora* (JPN6481, 24 Jun. 2021). Scale bars: 5 cm (A); 2 cm (B).

on the lower surface; petioles 3.4–22 cm long, narrowly winged, wing 0.1–0.6 cm wide, glabrous. Scape 8.6–28.5 cm long, terete. Raceme 5.9–18.5 cm long, 4–13-flowered; flower bracts vivid (not withering) in anthesis, erect, purplish light green, light bluish purple, ovate oblong-lanceolate, boat-shaped, 2–4.7 cm long, 0.2–0.7 cm wide, papery, glabrous, apex acute to acuminate. Flowers not fragrant; pedicels 1.4–2.5 cm long, glabrous; perianths 4–5.7 cm long, funnel-form, pale white-purple to light bluish-purple outside, almost white to light pale purple inside, midveins more or less purplish, glabrous, 6-lobed; tube dilated from apical 1/2, lobes triangular, 1.2–1.8 cm long, 0.5–1 cm wide, apex acute. Stamens 6, same or ca. 0.5 cm exerted from perianths; filaments 3.9–5.8 cm long, upwardly curved at the distal part, white, free, glabrous, anthers purple when fresh, dark blue-grey when dried, 3 mm long. Ovary ellipsoid, 0.6–0.7 cm long, style 4.5–6.5 cm long, upwardly curved at the distal part, up to 1 cm exerted from perianth, glabrous, stigma capitate. Young capsules 2.8 cm long (for MBK0087737).

Phenology. Flowering in late June to early August.

Distribution and habitat. Japan (Kochi Prefecture: Tosa County, endemic to Mt. Inamura, Mt. Higashikado, and the surrounding area). It grows on cliffs.

Etymology. A specific epithet is derived from its habit of growing on rock cliffs (called 'taki' in Kochi dialect) and flowering in June (Minazuki).

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as VU (Vulnerable) because its population is estimated to be between 250 and 1000.

Japanese name. Taki-minazuki-giboshi (new).

Additional specimens examined. JAPAN. Kochi Pref.: Tosa County, Ooka-wa village, 900 m, 27 Jul. 2004, *N. Inagaki et al.* FOK-067742 with flowers and young fruits (MBK0087737!); Tosa County, Tosa Town, 620 m elev., 24 Jun. 2021, *T. Yahara et al.* JPN6478–6486, 6490 with flowers (FU!); Agawa-gun, Ino-cho, Mt. Inamura, 1390 m elev., 2 Aug. 2019, *Y. Oohira* 14695 with flowers (MBK0314655!); Agawa-gun, Ino-cho, north cliff of Mt. Inamura, 23 Jul. 2022, *Se. Fujii* JPN15229, 15230 with flowers (FU!).

Note. The clade comprising this species and *H. longipedicellata* is sister to all the other species in Clade 1 (Fig. 2), and its morphological similarities to *H. longipedicellata* and *H. minazukiflora* are considered to be derived from a common ancestor.

6A. *Hosta takiminazukiflora* subsp. *grandis* Se.Fujii & Yahara, subsp. nov.

urn:lsid:ipni.org:names:77331110-1

Fig. 16

Diagnosis. *Hosta takiminazukiflora* subsp. *grandis* is distinguished from subsp. *takiminazukiflora* in having scapes 31–36 cm long (compared to 8.6–28.5 cm long in subsp. *takiminazukiflora*), broader leaf blades (13–14 cm wide compared to 3.6–10.5 cm wide), cordate leaf base (vs. cuneate to obtuse), glaucous lower leaf surface, lateral veins 12–13 pairs (as opposed to 6–10 pairs), and petioles green, not dotted with purple.

Type. JAPAN. Kochi Pref.: Tosa-gun, Tosa-cho, along Seto River, 24 Jun. 2021, *T. Yahara et al.* JPN6487 with flowers (holotype FU!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 3–5 per ramet; blades oblong-ovate, 22–25 cm long, 13–14 cm wide, 1.7–1.8 times longer than width, thinly papery, glabrous on both surfaces, base usually cordate, often decurrent, apex acuminate, margin entire, veins in 12–13 pairs, smooth or slightly papillose on the lower surface; petioles 35–38 cm long, narrowly winged, wing 0.3 cm wide, glabrous. Scape 31–36 cm long, terete. Raceme 12–15.5 cm long, 4–18-flowered; flower bracts vivid (not withering) in anthesis, erect, purplish light green, oblong-lanceolate, boat-shaped, 2.8–4.3 cm long, 0.4–0.5 cm wide, papery, glabrous, apex acuminate. Flowers not fragrant; pedicels 1.9–2 cm long, pale purple, glabrous; perianths 3.8–4.9 cm long, funnel-form, glabrous, 6-lobed, pale blue-purple outside, almost white to pale purple inside, midveins faintly purplish adaxially; tube dilated from apical 2/3, lobes oblong-triangular, 1.2–1.3 cm long, 0.7–0.8 cm wide, apex acute. Stamens 6, ca. 0.5 cm exerted from perianth; filaments 4.3–4.5 cm long, upwardly curved at the distal part, white, free, glabrous, anthers purple when fresh, dark blue-grey when dried, 3 mm long. Ovary ellipsoid, 0.6–0.7 cm long, style 4.5–5.1 cm long, upwardly curved at the distal part, ca. 1 cm exerted from perianth, glabrous, stigma capitate. Capsules or seeds not observed.

Phenology. Flowering in late June.

Distribution and habitat. Japan (Kochi Prefecture) (endemic). This species grows on soil near waterfalls, whereas subsp. *takiminazukiflora* grows on rock cliffs.

Etymology. The specific epithet is derived from its larger plant size rather than that of the typical subspecies.

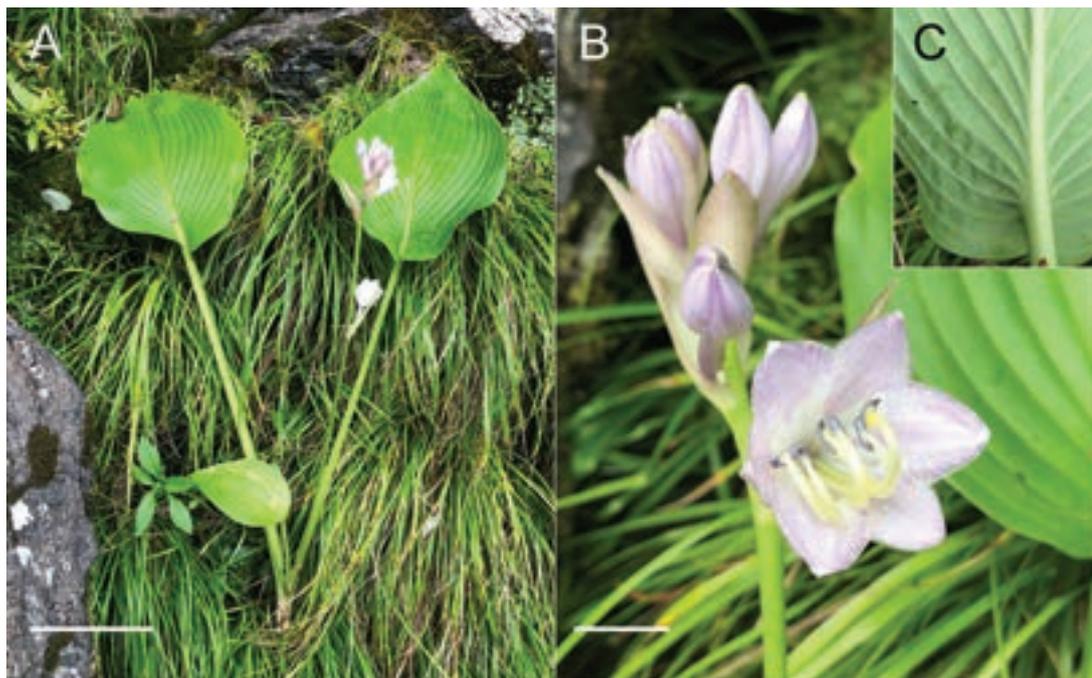


Figure 16. **A, B** flowering plant of *H. takiminazukiflora* subsp. *grandis* (JPN6487, 24 Jun. 2021) **C** its lower leaf surface. Scale bars: 10 cm (**A**); 1 cm (**B**).

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as CR (Critically endangered) because its population is estimated to be fewer than 50.

Japanese name. Setogawa-giboshi (new).

Additional specimens examined. **JAPAN. Kochi Pref.:** Tosa-gun, Tosa-cho, along Seto River, 24 Jun. 2021, *T. Yahara et al.* JPN6488–6489 with flowers (FU!); ditto, 24 Jun. 2022, *K. Fuse et al.* JPN12518–12521 with flowers (FU!).

Note. In the type locality, subsp. *grandis* grows side by side with subsp. *takiminazukiflora*, and both subspecies flower simultaneously in late June, but the two subspecies are morphologically distinct and subsp. *grandis* formed a monophyletic group significantly separated from the sympatric population of subsp. *takiminazukiflora*. Therefore, subsp. *grandis* appears to be an evolutionary distinct lineage. This lineage is of particular interest as a research material for studying rapid speciation under disruptive selection and also serves as a valuable resource for breeding *Hosta* cultivars. Taking into account these characteristics, as well as its morphological distinctiveness, we distinguish it as a subspecies. Due to its limited population size, conservation measures are urgently necessary.

7. *Hosta longipedicellata* Se.Fujii & Yahara, sp. nov.

urn:lsid:ipni.org:names:77331162-1

Figs 17, 18

Diagnosis. *Hosta longipedicellata* is similar to *H. scabrinervia*, but distinguished by its flower bracts 2–2.2 cm long, withering during flowering (compared to



Figure 17. The type specimen of *H. longipedicellata*: S. Kobayashi & Y. Kurahashi FOK-077809 (holotype MBK).

(2–)2.2–4.6 cm long, fresh during flowering), smaller and narrower leaf blades (compared to up to 25 × 15 cm), and the occurrence at elevations at 1700–1750 m (in contrast to at 90–550 m).

Type. JAPAN. Kochi Pref.: Tosa-gun, Hongawa-mura, Mt. Tsutsujo, 1750 m elev., 1 Aug. 2006, *S. Kobayashi & Y. Kurahashi FOK-077809* with flowers (holotype MBK0164413!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 3 per ramet; blades ovate or oblong-ovate, 13.4–30.2 cm long, 5.4–11.8 cm, 2.2–3 time longer than wide, thinly papery, glabrous on both surfaces, base rounded to subcordate, apex acute to short acuminate, margin entire, veins in 7–10 pairs, papillose on the lower surface; petioles 15–34.3 cm long, narrowly winged, wing 0.1–0.2 cm wide, glabrous. Scape 26.5–32.5 cm long, terete. Raceme 7.6–9 cm long, 7–15-flowered; flower bracts withering in anthesis, erect, purplish light green in the upper part, light green in the lower part, oblong-lanceolate, boat-shaped, 2–2.5 cm long, 0.3–0.4 cm wide, membranous, glabrous, apex acute. Flowers not fragrant; pedicels 2.5–3.3 cm long, glabrous; perianth 4.4–6.4 cm long, funnel-form, glabrous, 6-lobed, whitish purple outside in flower buds, almost white outside and inside when flowering, midveins purplish adaxially; tube dilated from apical 2/3, lobes triangular, 1.0–1.5 cm long, 0.6–0.9 cm wide, apex obtuse. Stamens 6, slightly shorter than perianth, not exerted; filaments 4.6–4.8 cm long, upwardly curved at the distal part, white, free, glabrous, anthers purple when fresh, dark blue-grey when dried, 3 mm long. Ovary ellipsoid, 0.6–0.7 cm long, style 5.1–5.6 cm long, upwardly curved at the distal part, 1.1 cm exerted from perianths, glabrous, stigma capitate. Capsules or seeds not observed.

Phenology. Flowering from late July to early August.

Distribution and habitat. Japan (Kochi Prefecture). This species grows on rock cliffs at Mt. Tsutsujo, Mt. Tebako, Yasui Valley, and its vicinity in Kochi Prefecture on Shikoku Island.

Etymology. The specific epithet is derived from the long pedicel.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as VU (Vulnerable) because its population is estimated to be between 250 and 1000.

Japanese name. Kamuro-giboshi (new).

Additional specimens examined. JAPAN. Kochi Pref.: Tosa-gun. Hongawa-mura, Mt. Tsutsujo, 28 Jun. 2022, *K. Fuse et al. JPN12637–12639, 12647, 12654* sterile (FU!); Agawa-gun, Niyodogawa-cho, 612 m elev., 3 May 2022, *Se. Fujii JPN12425, 12426* sterile (FU!); Agawa-gun, Niyodogawa-cho, Yasui Valley, 470 m elev., 30 Jun. 2022, *K. Fuse et al. JPN12674, 12676, 12680* sterile, *JPN12675* with flower buds (FU!); Agawa-gun, Niyodogawa-cho, Miyagahira, 612 m elev., Jul. 22, 2023, *Se. Fujii FJW48-1, 2, 3* with flowers (MBK!); cultivated at Makino Botanical Garden, derived from Mt. Tsutsujo, 1750 m elev., *T. Yahara et al. JPN3927* sterile (FU!); cultivated at Makino Botanical Garden, derived from Mt. Tebako, 1700 m elev., *T. Yahara et al. JPN3936* sterile (FU!).

Note. The plant shown in Fig. 18 is a clone of JPN3927 that grew larger than the wild state after fertilization and was not used for the above description.



Figure 18. Plants of *H. longipedicellata* cultivated at the Makino Botanical Garden (photos taken on 3 Jun. 2022 by Se. Fujii). Scale bars: 10 cm (A); 1 cm (B).

8. *Hosta samukazemontana* Se.Fujii & Yahara, sp. nov.

urn:lsid:ipni.org:names:77331111-1

Figs 19, 20

Diagnosis. *Hosta samukazemontana* is distinguished from *H. polyneuronioides* by anther size (3 mm vs. 3.5–4 mm), and inflorescences (deflected and curved upward at the top in contrast to erect and straight). *Hosta samukazemontana* is also similar to *H. tosana* in having inflorescences deflected and curved upward at the top, but distinguished from *H. tosana* by whitish perianths 4.5–5 cm long (in contrast to purple perianths 6.1–6.9 cm long) and whitish purple flower bracts 1.7–2.1 cm long (as opposed to whitish green perianths 2.2–5.2 cm long).

Type. JAPAN. Kochi Pref.: Tosa County, Hongawa Village, Mt. Kanpu, 25 Jul. 1983, Y. Kokami M83-255 with flowers (holotype MBK0175579!).

Description. Herbs perennial. Leaves basal, spiral, long petiolate, 2–6 per ramet; blades oblong-ovate, elliptic-ovate, ovate, 7–22 cm long, 3.6–10.7 cm wide, 1.8–2.9 time longer than wide, thinly papery, glabrous on both surfaces, base cuneate, rounded, cordate, decurrent, slightly folded, apex acuminate, margin entire, veins in 6–12 pairs, smooth on the lower surface; petioles 3.2–14.5 cm long, narrowly winged, wings 0.1–0.2 cm wide, glabrous. Scape 20–43 cm long, terete, deflected and curved upward at the top. Raceme 7–9 cm long, 5–18-flowered; flower bracts vivid (not withering) in anthesis, erect, whitish purple, oblong-lanceolate, boat-shaped, 1.7–2.1 long, 0.3–0.5 cm wide, mem-



Figure 19. The type specimen of *H. samukazemontana*: Y. Kokami M83-255 (holotype MBK).



Figure 20. Plants of *H. samukazemontana* **A** JPN1381 **B** JPN6325 **C** photo taken on 17 Jul. 2013 by Y. Kokami; not collected. Scale bars: 10 cm (**A, B**); 2 cm (**C**).

branous, glabrous, apex acuminate. Flowers 5–6 cm long; pedicels 1–1.8 cm long, glabrous. Perianths 4.5–5 cm long, purple-white outside, almost white inside, funnel-form, glabrous, 6-lobed, tube abruptly dilated from apical 2/3, lobes narrowly triangular, 1–1.3 cm long, 0.7–0.8 cm wide, apex acute, three veins distinct inside. Stamens 6; filaments 4.8 cm long, white, free, glabrous, almost as long as perianth, upwardly curved at the distal part, anthers purple when fresh, dark blue-grey when dried, 3 mm long. Ovary ellipsoid, 1 cm long, style 5 cm long, upwardly curved at the distal part, 0.5 cm exerted from perianth, glabrous, stigma capitate. Capsules green, cylindrical, 2.3–2.4 cm long, 0.4 cm wide, 3-angled. Seeds 2.2 mm long, with wings ca. 4 mm long, blackish brown.

Phenology. Flowering in July, and fruiting in August.

Distribution and habitat. Japan (Ehime and Kochi Prefectures) (endemic). This species grows on rock cliffs in the vicinity of Mt. Kanpu in the central part of the Kochi Prefecture on Shikoku Island.

Etymology. The specific epithet was derived from the old name of the type locality (Mt. Samukaze).

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as VU (Vulnerable) because its population is estimated to be between 250 and 1000.

Japanese name. Samukaze-giboshi (new).

Additional specimens examined. JAPAN. Kochi Pref.: Agawa-gun, Ino-cho, along Kamegamori Forest Road, 1660 m elev., 22 Jun. 2021, *T. Yahara et al.* JPN6325 with flower buds (FU!). **Ehime Pref.:** Saijyo City, W slope of Mt. Kanpu, 1050 m elev., 20 Aug. 2020, *T. Yahara et al.* JPN1380–1382 with fruit (FU!);

Saijyo City, Mt. Kanpu 1600 m, 24 Jul. 2022, Se. *Fujii JPN15235* with flowers (FU!); Mt. Kanpu, June 19, 1943, S. *Yamawaki s.n.* with flowers (TI!).

Note. This species was identified as *H. shikokiana* by Fujita (1976), but is distinguished from *H. shikokiana* in its whitish perianth color and scapes deflected and curved at the top when flowering and fruiting (Fig. 20). The distribution record of "*H. kikutii* var. *caput-avis*" from Mt. Kanpu in the Flora of Kochi Prefecture (Kochi Prefecture & Makino Memorial Foundation of Kochi Prefecture 2009) is a misidentification of this species.

9. *Hosta tosana* F. Maek., J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 5: 376. 1940.

Fig. 21–24

Hosta tosana F. Maek., J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 5: 376. 1940. Type. Japan, Shikoku: Kochi Prefecture, Mt. Kajigamine (now called Kajigamori), T. *Yoshinaga s.n.* (unknown; not deposited in TI).

Hosta kikutii var. *tosana* (F. Maek.) F. Maek., *Engei-daijiten* 2:633. 1950; Tamura & Fujita in Iwatsuki et al. *Fl. Jap. IVb*: 143. 2016.

Hosta tosana var. *caput-avis* F. Maek., *J. Jap. Bot.* 22: 64. 1948. Type. JAPAN. Kochi Pref., Yanase, K. *Yasui* 332 (unknown; not deposited in TI).

Hosta caput-avis (F. Maek.) F. Maek. in Nakai, *Iconogr. Pl. Asiae Orient.* 5: 495. 1952.

Hosta kikutii var. *caput-avis* (F. Maek.) F. Maek., *Engei-daijiten (Encycl. Hort.)* 2: 633. 1950; Fujita, *Acta Phytotax. Geobot.* 27: 79. 1976.

Phenology. Flowering in June to July.

Distribution and habitat. Japan (Kochi and Tokushima Prefectures). This species grows on wet slopes, rocky cliffs, rocky riverbanks, and tree trunks in the elevations from 77 m to 1420 m.

Conservation status. Using criterion D1 for IUCN Red List categories (IUCN 2012, IUCN 2022), we recommend that this species be classified as NT (Near threatened) because its population is estimated to exceed 1000 but may be declining under the influence of deer browsing, river bank construction, and collection for horticulture.

Japanese name. Tosa-no-giboshi (Maekawa 1940), Unazuki-giboshi (Maekawa 1948).

Specimens examined.

Morphs corresponding to "var. *tosana*":

Kochi Pref.: Locality not specified, cultivated in Tokyo, 1 Aug. 1943, *Maekawa 7043* with flowers (TI; the cultivated plant may have been collected by T. Yoshinaga from the type locality, as this is the only specimen of this taxon deposited in TI, where Maekawa studied *Hosta*); Nagaoka-gun, Otoyo-cho, Mt. Kajigamori, 30 Apr. 2022, Se. *Fujii JPN12424* sterile (FU!); Kami City, Monobe Town, O-dochi, 500 m elev., 25 Jun. 2021, T. *Yahara et al. JPN6619–6620* with flowers (FU!); ditto, Go-o-do, 553 m elev., cultivated at Makino Botanical Garden, 8 Apr. 2021, T. *Yahara & Se. Fujii JPN3931–3933* sterile (FU!); ditto, 600 m elev., 25 Jun. 2021, T. *Yahara et al. JPN6614 & 6616* with flowers, *JPN6611–6613, 6615* sterile (FU!); Miyanose, 400 m elev., T. *Yahara et al. JPN6603* sterile (FU!); Befu, 550 m elev., cultivated at Makino Botanical Garden, 8 Apr. 2021, T. *Yahara & Se. Fujii JPN3934* sterile (FU!); Befu Valley, 700 m elev., 25 Jun. 2021,



Figure 21. Plants of *H. tosana* Kagami River lineage **A** photo taken on 7 Jul. 2021 by Se. Fujii, not collected **B** JPN1307. Scale bars: 5 cm (**A**, **B**).

T. Yahara et al. JPN6553–6555 with flowers (FU!); Aki City, 77 m elev., cultivated at Makino Botanical Garden, 8 Apr. 2021, *T. Yahara & Se. Fujii JPN3935* sterile (FU!); Aki-gun, Kitagawa-mura, Shima, along the Nahari River, 28 May 2022, *K. Fuse et al. JPN12653* sterile (FU!). **Tokushima Pref.:** Mt. Tsurugi, 14 Aug. 1931, *Z. Tashiro s.n.* with flowers (KYO!); ditto, below Minokoshi, 1400 m elev., 14 Aug. 1954, *G. Murata 7977* with flowers (KYO!); ditto, 1420 m elev., 23 Jun. 2022, *T. Yahara et al. JPN12821–12823* sterile (FU!); Higashiyayama-mura, Inter Sugeoi et Minokoshi, 800 m elev., 12 Aug. 1954, *G. Murata 7814 & 7816* with flowers and young fruits (KYO!); Naka County, Wajiki Town, 14 Jul. 1965, *S. Takafuji 216* with flowers (KYO!); ditto, Kizawa Village, 24 Jul. 1980, *S. Takafuji 1442* with flowers (KYO!); ditto, Riu-toge in Miyahama-mura, 2 Jul. 1952, *G. Murata 5752* with flowers (KYO!); ditto, Kizu Village, 24 Jul. 1974, *S. Takafuji 950* with flowers (KYO!).

Morphs corresponding to “var. caput-avis”:

Kochi Pref.: Aki-gun, Yanase, 25 Jun. 2020, *K. Fuse et al. 12536–12539* sterile (FU!); Kami City, Monobe Town, Befu Valley, 25 Jun. 2021, *T. Yahara et al. JPN6524–6526* with flower buds, 6571 with flowers, 6572 with flower buds (FU!); Mt. Ishidate, 25 Jun. 2021, *T. Yahara et al. JPN6585–6587, 6593–6596* with flower buds (FU!); on the river bank of Yanase, 1000 m elev., 20 Jul. 1958, *S. Hatusima 22009A* with wilted flowers (KAG056576!); transplanted from Yanase, 29 Jun. 1960, *S. Hatusima s.n.* with flowers (KAG056577!); ditto, 10 Jun. 1967, *Hatusima s.n.* with flowers (KAG056578!).



Figure 22. A flowering plant of *H. tosana* (a form corresponding to var. *tosana*, JPN6616). Scale bars: 5 cm (A); 1 cm (B).

The Kagami River lineage:

Kochi Pref.: Kochi City, Tosayama, Hirose, 85 m elev., 3 Oct. 2008, *Hamaguchi & N. Shintani* PRC-00118 with fruits (MBK0208327!); ditto, 14 Jul. 2001, *K. Hosokawa et al.* FOK-001722 with flowers (MBK0104375!); Kuwao, 130 m elev., 14 Jul. 2013, *A. Sakamoto* FOS-004959 with flowers (MBK0247214!, MBK0247215!); from Kuwao to Tsunami, 27 Jul. 1968, *N. Naruhashi & M. Wakabayashi* 223 with flowers (KYO!); Namekawa, 30 m elev., 19 Aug. 2020, *T. Yahara et al.* JPN1265 with fruit, JPN1266 sterile, JPN1267 sterile, JPN1269 with fruit (FU!); Kajitani, 160 m elev., 29 July 2013, *A. Sakamoto* FOS-005031 with young fruits (MBK0247386!) and FOS-005032 with young fruits (MBK0247387!); Miyanokubo, 160 m elev., 14 Jul. 2013, *A. Sakamoto* FOS-004958 with flowers (MBK0247213!); ditto, 170 m elev., 14 Jul. 2013, *A. Sakamoto* FOS-004957 with fruits (MBK0247212!); Oh-ana Valley, 110 m elev., 19 Aug. 2020, *T. Yahara et al.* JPN1298 sterile, JPN1299 sterile, JPN1307 with flowers (FU!); Kagami-mura, Kawaguchi, along the Kagami River, 9 Jul. 1958, *T. Yamanaka* 25548 with flowers (KYO!).

Note. The Kagami River lineage is sister to a clade comprising all other samples (Fig. 5). It is morphologically distinguished from other samples by having leaves with fewer lateral veins (5–11 compared to 10–13) that run at wider intervals (1 cm vs 0.7 cm), and it is distinct in its occurrence at elevations below 300 m (compared to elevations ranging from 77 m to 1420 m, usually above 300 m). It is likely that this lineage could be classified as a subspecies of *H. tosana* or a separate species. However, further studies are necessary to arrive at definitive conclusions regarding this lineage because *H. tosana* is a polymorphic species widely distributed in the eastern part of Shikoku. Nonetheless, our collections did not cover the entire range of this species.



Figure 23. Plants of *H. tosana* **A** a form corresponding to var. *caput-avis* (JPN6524) **B** a form corresponding to var. *tosana* (JPN6555). Scale bars: 5 cm (**A**, **B**).

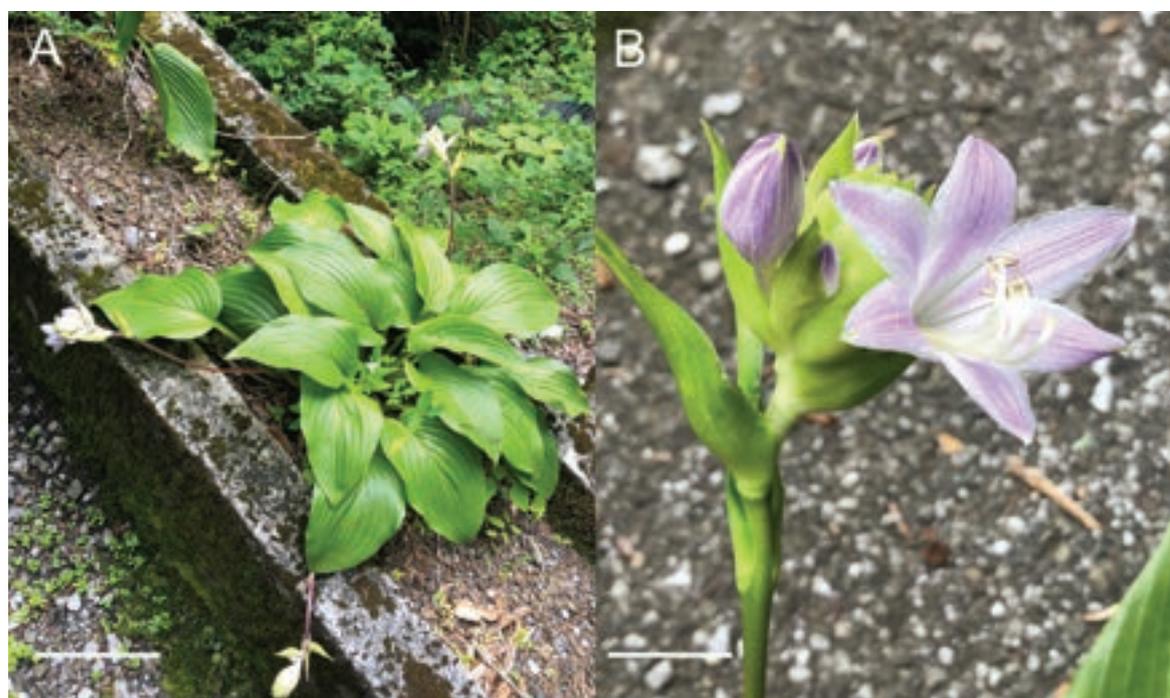


Figure 24. Plants of *H. tosana* (a form corresponding to var. *caput-avis*) **A** JPN6571 **B** JPN6572. Scale bars: 10 cm (**A**); 1 cm (**B**).

According to the MIG-seq tree, two genetically distinct lineages are distributed along the Monobe River in Kami City (Figs 5, 6). The lineages were identified as *H. tosana* var. *tosana* and *H. tosana* var. *caput-avis*. However, population ge-

netic evidence shows that the two varieties hybridize in the sympatric population along the Befu Valley. The available evidence indicates that var. *caput-avis* is difficult to distinguish from var. *tosana* in Kami City.

While the two varieties appeared to lack distinction in Kami City, the population of Yanase, which serves as the type locality for *H. tosana* var. *caput-avis*, exhibited clear differentiation from the populations in Kami City. However, just like in the case of the Kagami River lineage, further studies using a larger sample size are necessary to definitively conclude about the Yanase lineage.

A lineage distributed in SE Kinki, Honshu (*H. sp.* 4 in Fig. 1) was identified as *H. kikutii* var. *caput-avis* by Fujita (1976) and Fujita and Tamura (2008), and later treated as *H. kikutii* var. *tosana* by Tamura and Fujita (2016). However, it is not related to *H. tosana* of Clade 4, but to *H. longipes* of Clade 6 (Fig. 1).

Acknowledgements

DNA samples and voucher specimens of *Hosta* spp. were collected in the protected areas of Ashizuri-Uwaumi National Park, Ishizuchi National (Kokutei) Park, Tsurugi National (Kokutei) Park, and Akaishi Mountains Nature Reserve, with permission from the Ministry of Environment, Ehime Prefecture, and Tokushima Prefecture. We thank Satoru Kinoshita and Yasushi Ibaragi for identifying the location of *H. tosana* in Tokushima Prefecture, and Shinji Fujii for providing DNA samples of *H. densiflora* subsp. *densiflora* (FJI17566) and *H. sp.* 3 (FIJ12268). We thank the Ministry of the Environment's Rare Species Conservation Promotion Office and Saki Funamoto of Kyushu Open University for their assistance in obtaining collection permits. We are also grateful to the curators of FU, KAG, KYO, MBK, TI and VNMN for their help to access specimens for our studies.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by the Environment Research and Technology Development Fund (JPMEERF20204001 and JPMEERF20234001) of the Ministry of Environment, Japan.

Author contributions

Data curation: TY, SF. Formal analysis: YS, TY, SKH. Investigation: HS, KF. Resources: YK, KF, HS, SF. Writing - original draft: TY, ST. Writing - review and editing: TY.

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Data availability

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

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Supplementary material 1

20 DNA samples and voucher specimens from 70 localities for 30 taxa of *Hosta* in Japan

Authors: Tetsukazu Yahara, Shun K. Hirota, Seiko Fujii, Yasushi Kokami, Kengo Fuse, Hiroyuki Sato, Shuichiro Tagane, Yoshihisa Suyama

Data type: xlsx

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Link: <https://doi.org/10.3897/phytokeys.235.99140.suppl1>

Supplementary material 2

Sample sets

Authors: Tetsukazu Yahara, Shun K. Hirota, Seiko Fujii, Yasushi Kokami, Kengo Fuse, Hiroyuki Sato, Shuichiro Tagane, Yoshihisa Suyama

Data type: xlsx

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Link: <https://doi.org/10.3897/phytokeys.235.99140.suppl2>

Supplementary material 3

Changes of ΔK with K in the samples of *H. polyneuronoides* and *H. tardiva* subsp. *densinervia* (A) and the other species of group 1 (B)

Authors: Tetsukazu Yahara, Shun K. Hirota, Seiko Fujii, Yasushi Kokami, Kengo Fuse, Hiroyuki Sato, Shuichiro Tagane, Yoshihisa Suyama

Data type: docx

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Link: <https://doi.org/10.3897/phytokeys.235.99140.suppl3>

Lysimachia cavicola (Primulaceae), a new species from Guangxi, China

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Abstract

Lysimachia cavicola (Subgen. *Idiophyton*, Primulaceae), a new species from Guangxi, China, is here described and illustrated based on morphological data. Although it shares similarities with *L. microcarpa*, *L. fooningensis*, and *L. capillipes*, there are distinguishing characteristics that set it apart. These include erect stems either solitary or in clusters of 1 to 2, herbaceous, terete, and densely glandular hairy. The leaves are either ovate or elliptical lanceolate, with inconspicuously reticulate veins. The petiole measures 2–4 mm in length covered with minute glandular hairy. The corolla is deeply parted, measuring 6–8 mm in length, with narrowly elliptic or narrowly oblong lobes that are 1–2 mm wide. The capsule is globose, measuring 2–3 × 2–3 mm, and possesses a chalky, brittle texture, which splits into 5-valved segments. The calyx of the plant appears yellowish-white during fruiting. This newly discovered species is endemic to limestone areas in Fengshan County, Guangxi, China.

Key words: Conservation, dòng shēng xiāng cǎo, Fengshan, limestone, new species, taxonomy



Academic editor: Avelinah Julius

Received: 14 July 2023

Accepted: 30 October 2023

Published: 17 November 2023

Citation: Nong Y, Fang Y, Xu C-G, Wei G-Y, Yan K-J, Hu R-C, Wen Y-G (2023) *Lysimachia cavicola* (Primulaceae), a new species from Guangxi, China. PhytoKeys 235: 189–198. <https://doi.org/10.3897/phytokeys.235.109528>

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Introduction

The genus *Lysimachia* L. (1753: 146) was originally placed in Primulaceae (Cronquist 1981; Takhtajan 1997), but later transferred into Myrsinaceae, based on morphological and molecular evidence (Källersjö et al. 2000; Hao et al. 2004). Myrsinaceae was later merged into Primulaceae s.l., hence *Lysimachia* was replaced into Primulaceae (Chen et al. 2016). *Lysimachia* L. is the largest genus in the tribe Lysimachieae (Primulaceae), which consists of approximately 200 species worldwide (Hao et al. 2004; Julius et al. 2016). According to the flower and gland morphology, the genus is separated into five subgenera, viz. subgen. *Idiophyton* Hand.–Mazz., subgen. *Lysimachia*, subgen. *Palladia* (Moench) Hand.–Mazz., subgen. *Heterostylandra* (Hand.–Mazz.) F.H.Chen & C.M.Hu and subgen. *Naumburgia* (Moench) Klatt. (Chen et al. 1989). The majority of species within the genus are distributed in temperate and subtropical regions of the

Northern Hemisphere, with some species in Africa, Australia and South America. In China, the genus has 138 species and is highly diversified in south-western China, especially in limestone areas (Hu and Kelso 1996). Subsequently, more than 20 new species of *Lysimachia* have been discovered in the last two decades (e.g., Yan et al. 2017; Wang et al. 2018; Huang et al. 2019; Liu et al. 2020; Mou et al. 2020; Yi 2020; Ju et al. 2021; Lu et al. 2021; Yan et al. 2023).

The south-western limestone karst area is one of China's biodiversity hotspots. Multiple field works have been conducted in this area in recent years. During fieldwork in April 2021 to Fengshan County in Southwest of China, we discovered an unknown species glowed at the cave entrance. After several observations and consulting relevant literature (Hu and Kelso 1996), we confirm that the unusual plant is a species new to science of *Lysimachia* and is described below.

Materials and methods

Morphology

The new species were described based on field observations that were conducted between April and August and examination of herbarium specimens at IBK and GXMI. Other related *Lysimachia* species were examined based on online images from the Kew Herbarium Catalogue (<http://apps.kew.org/herbcat/gotoHomePage.do>) and JSTOR Global Plants (<http://plants.jstor.org/>) and KUN, PE, IBSC and HITBC. Morphological characteristics that differentiate it from all other species in the genus of *Lysimachia* were used. The following characteristics were observed: stems, leaves, pedicels, flowers, receptacles, petals, stamens, gynoecium, carpels, size of flowers, size and shape of petals, number of stamens, and the shape of gynoecium and fruit. We also observed living plants of the new species during the flowering and fruiting period (April to July).

Descriptions were written from herbarium specimens. Measurements were made with a tape-measure and calipers. The structure of the indumentum and its distribution was observed and described under a dissecting microscope at magnifications of more than 20×. Additional information on locality, habitat, ecology, plant form and fruits were collected in the field and taken from herbarium labels. Conservation threat assessment followed IUCN Categories and Criteria (IUCN 2022).

Results and discussion

Taxonomy

***Lysimachia cavicola* Y.Nong & Y.G.Wen, sp. nov.**

urn:lsid:ipni.org:names:77331112-1

Figs 1–4

Chinese name. dòng shēng xiāng cǎo (洞生香草).

Diagnosis. *Lysimachia cavicola* shares several similarities with *L. microcarpa* C.Y.Wu, *L. fooningensis* C.Y.Wu, and *L. capillipes* Hemsl., including leaves alternate, flowers solitary in leaf axils, corolla yellow, anthers longer than filaments



Figure 1. Habitat of *Lysimachia cavicola* at the entrance of a limestone cave. Photographed by YN and K-J Y.

with distinct basifixation, and typically opening by apical pores, along with capsules dehiscing by valves. However, the new species can be easily distinguished by the following characteristics: stems solitary or clustered in 1–2, herbaceous and densely glandular; leaves ovate or elliptical lanceolate with

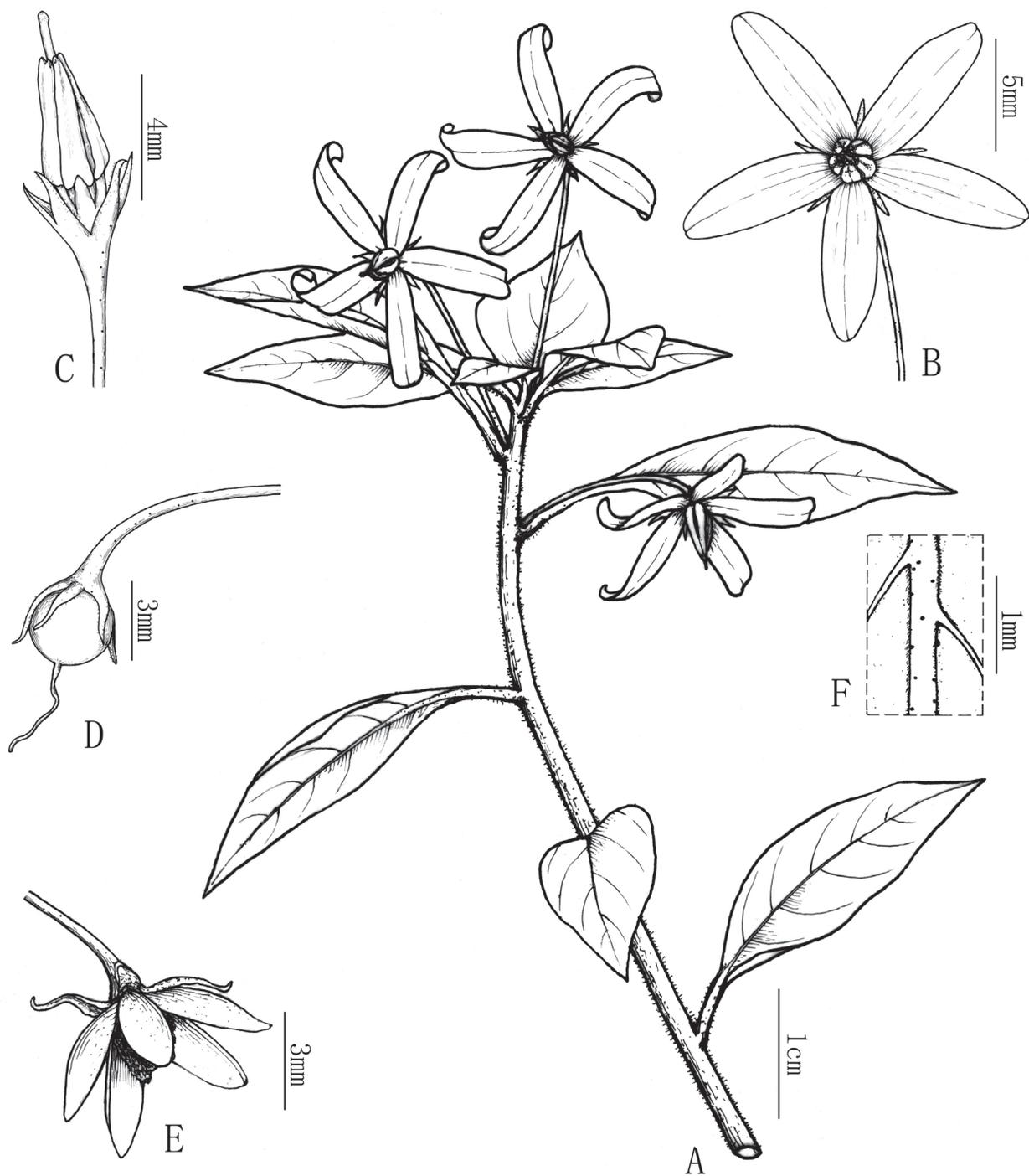


Figure 2. Line drawing of *Lysimachia cavicola* **A** flowering branch **B** flower **C** anthers **D** fruit **E** capsule **F** mid vein abaxially. Drawn by X-CQ.

inconspicuously reticulate veins; petioles 2–4 mm long that are minutely glandular hairy; capsules globose, 2–3 mm in diam., about the same length as the calyx. A more detailed morphological differences among the four species is presented in Table 1.

Type. CHINA. Guangxi: Fengshan, limestone cave entrance, 24°34'17"N, 106°50'31"E, alt. 794 m, 23 April 2021 (fl.), R.C. Hu HRC210423003 (holotype, GXMI!; isotypes, IBK!).

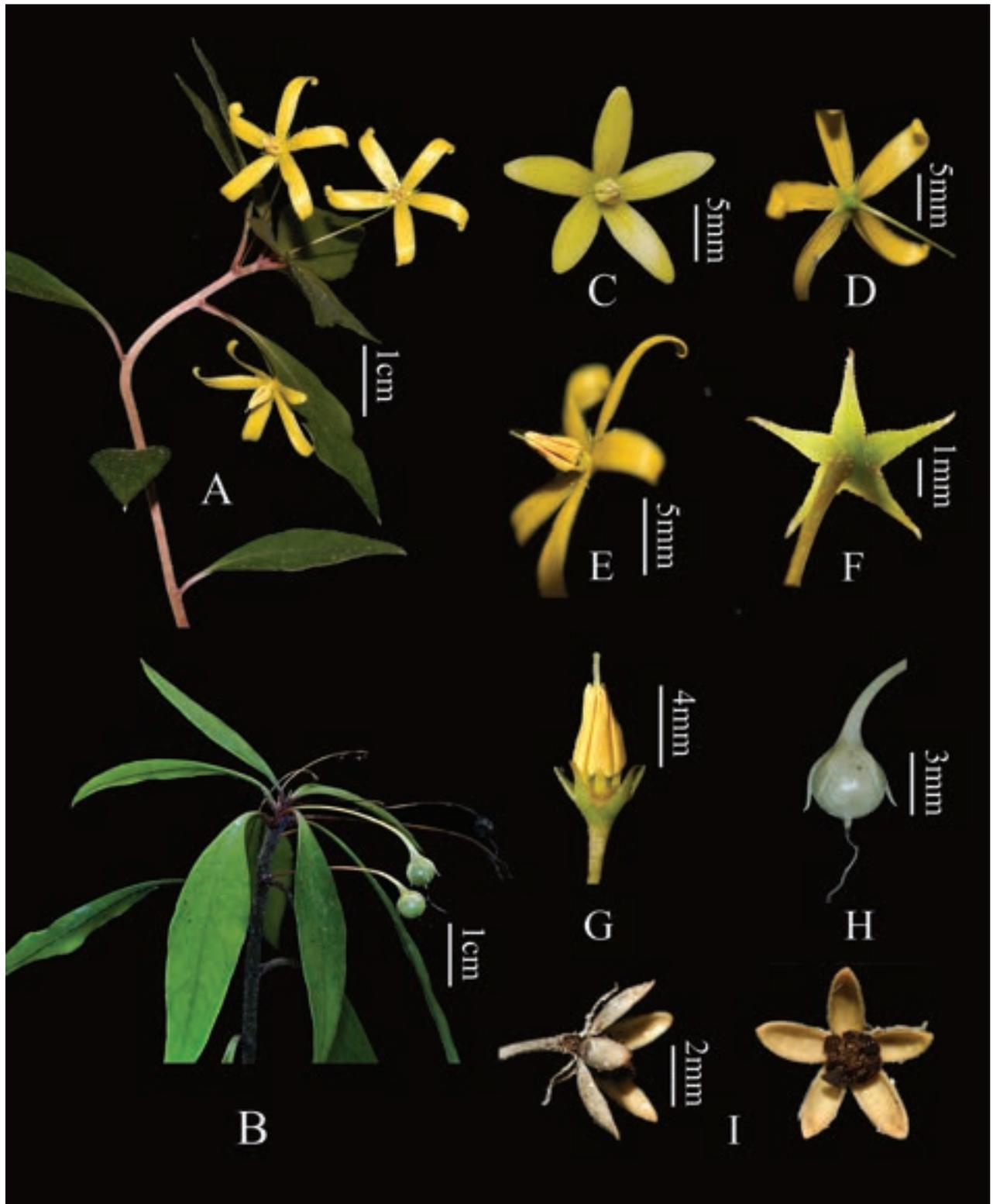


Figure 3. *Lysimachia cavicola* **A** flowering plant **B** fruiting plant **C** flower (front view) **D** flower (back view) **E** flower (lateral view) **F** calyx (back view) **G** anthers **H** fruit **I** lateral (left) and front view (right) of capsule. Photographed by YN and K-JY.

Description. A perennial, herbaceous, 4–15 cm tall, aromatic when dry. **Stems** erect, solitary or 1–2 in clusters, terete and densely glandular. **Leaves** alternate: lamina ovate or elliptical lanceolate, 1.5–3(–4) × 0.5–1.6 cm, base attenuate, apex acuminate, glabrous except densely glandular hairy on midrib abaxially, vein-



Figure 4. Digital images of type specimens **A** *Lysimachia cavicola* **B** *L. microcarpa* **C** *L. fooningensis* **D** *L. capillipes*.

Table 1. Main morphological differences amongst *Lysimachia cavicola*, *L. microcarpa*, *L. fooningensis*, *L. capillipes*.

Morphological traits	<i>L. cavicola</i>	<i>L. microcarpa</i>	<i>L. fooningensis</i>	<i>L. capillipes</i>
Height	4–15 cm	10–30 cm	20–50 cm	40–60 cm
Stems	solitary or 1–2 in clusters, erect, terete and densely glandular	multiple clusters, ascending to erect, terete or weakly angular, upper part minutely glandular	solitary or 2–3 in clusters, erect, woody at base, terete or weakly angular in upper part, densely glandular	2 to many, erect, angular or winged, branched from middle, glabrous
Leaf blade	ovate or elliptical lanceolate, 1.5–3(4) × 0.5–1.6 cm; veins inconspicuously reticulate	ovate to rhomboid-ovate or ovate-elliptic, 1.5–3(–6) × 0.7–3 cm; veins prominently reticulate	elliptic-lanceolate to narrowly lanceolate, 3–11 × 0.7–2.8 cm; veins prominently reticulate	ovate to lanceolate, 1.5–3 × 1–3 cm; veins 4 or 5 pairs; veins inconspicuously reticulate
Petiole	2–4 mm, minutely glandular	4–8 mm, sparsely glandular	5–15 mm, sparsely glandular	2–8 mm, glabrous
Corolla	yellow, 6–8 mm, deeply parted; lobes narrowly elliptic or narrowly oblong, 5–8 × 1–2 mm, apex obtuse	yellow, 7–10 mm, deeply parted; lobes narrowly oblong, 6–9 × 2.7–4 mm, apex obtuse	yellow, 9–11 mm, deeply parted; lobes linear, 8–10 × 2–3 mm, apex obtuse	yellow, 6–8 mm, deeply parted; lobes narrowly oblong to linear, 5–7 × 1.8–3 mm, apex subobtuse
Calyx	lobes ovate-lanceolate, apex acuminate, 2 mm, surface glabrous except glandular hairy abaxially, margins sparsely glandular hairy, calyx yellowish-white in fruiting	lobes ovate, apex acuminate, 3.5–4 mm, surface glabrous, margins sparsely glandular hairy, calyx green in fruiting	lobes triangular, apex acuminate, 2.5 mm, surface glabrous, margins sparsely glandular hairy, calyx green in fruiting	lobes ovate to lanceolate, apex acuminate to subulate, 2–4 mm, surface glabrous, margins minutely glandular hairy, calyx green in fruiting
Capsule	globose, 2–3 mm in diam., ca. as long as calyx	globose, 3–4 mm in diam., ca. as long as calyx	subglobose, 4 mm in diam., longer than calyx	globose, 3–4 mm in diam., longer than calyx

lets invisible; petiole 2–4 mm long, minutely glandular. **Flowers** 5-merous, solitary, axillary at upper part of leaf axil; pedicel filiform, 2–4 cm long, sparsely glandular hairy; calyx green at flowering, lobes deeply parted, ovate-lanceolate, c. 2 mm long, glabrous except sparsely glandular abaxially and margin; corolla yellow, lobes deeply parted, narrowly elliptic or narrowly oblong, 6–8 × 1–2 mm, apex recurved downward, glabrous on both surfaces; filaments short, not more than half the length of anthers, filaments connate basally into c. 1 mm high ring, free parts 1 mm; anthers 4 mm long, basifixed, opening by apical pores. **Capsule** globose, 2–3 × 2–3 mm, chalky, brittle, split into 5-valved, calyx yellowish-white in fruiting.

Phenology. Flowering in April–May; fruiting in June–July.

Etymology. Fengshan, situated in the southwest of Guangxi, China, is a biodiversity hotspot known for its remarkable discoveries of new species (Nong et al. 2010; Wen et al. 2012; Li et al. 2019). One such species, *Lysimachia cavicola*, was found at the cave entrance in Fengshan, and the specific epithet is named after its habitat.

Distribution and habit. The new species is currently known only from the type locality from the southwest of Guangxi, China (Fig. 5). The new species mainly occurs at elevations of 800 m and it grows at the entrance of caves.

IUCN Red list category. Data Deficient (DD). Data available for the new species are still insufficient to assess its conservation status. According to the IUCN criteria (IUCN 2022), it is considered Data Deficient until further information becomes available. Although *Lysimachia cavicola* currently has relatively good growth, further collecting and monitoring is necessary to allow more conclusive estimations about its rarity and vulnerability for future conservation planning of this species.

Additional specimen. CHINA. Guangxi: Fengshan, limestone cave entrance, 24°34'17"N, 106°50'31"E, alt. 794 m, 19 June 2023 (fr.), You Nong NY20230619001 (GXMI!).



Figure 5. The distribution of *Lysimachia cavicola* (black pentagram) in Guangxi, China.

Discussion

Based on the classification of *Lysimachia* by Handel-Mazzetti (1928) and Chen et al. (1989), the new species clearly belongs to *Lysimachia* subgenus *Idiophyton* sect. *Apodanthera* ser. *Evalues* Hand.–Mazz., which are characterised by corolla yellow, filaments connate into a thin ring and adnate at basal part of corolla tube, and the anthers longer than filaments, distinctly basifixed, opening by apical pores.

During our fieldwork, only one population with less than 50 individuals was discovered. All individuals were found growing at the cave entrance. More works such as conservation assessment and ex-situ conservation need to be done according to its limited populations, localities. Consequently, comprehensive surveys and studies on the phylogenetic evolution of *Lysimachia* within the limestone areas of southwest China will yield significant scientific insights into floristic geography and the phylogeny of *Lysimachia* in this particular region.

Acknowledgements

We are grateful to Lan Xiangchun for fieldwork assistance and Qu Xincheng for the line drawing (Guangxi Institute of Traditional Medical and Pharmaceutical Sciences, Nanning). The first author wants to thank Hua Chenyu (a singer from China) for some of his songs that had greatly inspired him to overcome his depression during these years.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (32000264), the Survey and Collection of Germplasm Resources of Woody & Herbaceous Plants in Guangxi, China (GXFS-2021-34).

Author contributions

Data curation: YN, RCH. Funding acquisition: YN, RCH, and GYW. Investigation: YN, CGX, GYW, KJY. Methodology: YN, KJY, YGW. Project administration: YN, RCH. Supervision: CGX, GYW, KJY, YGW. Visualization: YN, YF, KJY. Writing-original draft: YN, YGW. Writing-review and editing: YN.

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Data availability

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

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Synotis jinpingensis (Asteraceae, Senecioneae), a new species with white ray florets from southeastern Yunnan, China

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Abstract

Synotis jinpingensis (Asteraceae, Senecioneae), a new species from Jinping county in southeastern Yunnan province, China, is described and illustrated. This species is distinguished by having white ray florets in the genus *Synotis*, in which only species with yellow ray florets have been hitherto known. In habit and leaf shape *S. jinpingensis* is most closely similar to *S. duclouxii*, a species occurring in southwestern Guizhou, southern Sichuan and northeastern Yunnan, China, but differs, in addition to the color of ray florets, by having fewer lateral veins of leaves, obviously longer bracts of calyculus, and larger phyllaries. The membership of the new species within *Synotis* is strongly corroborated by evidence from floral micromorphology and phylogenetic analyses based on ITS sequence data. Color photographs of living plants, a distribution map, and provisional IUCN status of *S. jinpingensis* are provided.

Key words: Compositae, floral micromorphology, ITS sequence data, taxonomy



Academic editor: Peter de Lange

Received: 5 September 2023

Accepted: 22 October 2023

Published: 20 November 2023

Citation: Zhao L-C, Chen R, Yu Z-Y, Tang M, Yang Q-E (2023) *Synotis jinpingensis* (Asteraceae, Senecioneae), a new species with white ray florets from southeastern Yunnan, China. *PhytoKeys* 235: 199–210. <https://doi.org/10.3897/phytokeys.235.112230>

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Introduction

Synotis (Clarke) C. Jeffrey & Y.L. Chen (Asteraceae, Senecioneae), was segregated from *Senecio* L. based on its sub-shrubby habit and anther bases with sterile tailed auricles. *Synotis* is endemic to the Sino-Himalayan region, except for two species occurring in northwestern China and Kyrgyzstan (Jeffrey and Chen 1984; Li et al. 2018). Sixty species are currently recognized in the genus, with 48 recorded in China (Jeffrey and Chen 1984; Chen 1999; Chen et al. 2011; Tang et al. 2013a, b, c, 2014, 2017, 2022; Tong et al. 2017; Li et al. 2020; Liu et al. 2020, 2021; Tang and Chen 2021; Zhang et al. 2021; Fan et al. 2022a, b).

During a botanical trip in March 2022 to southeastern Yunnan, China, we discovered an unusual population of *Synotis* with white ray florets (Fig. 1) in the Fenshuiling National Nature Reserve in Jinping county (Fig. 2). All the previously known species in *Synotis* have yellow ray florets. The habit and the obovate-lanceolate or elliptic leaves of the plants are most closely similar to those of *S. duclouxii*, a species occurring in southwestern Guizhou, southern

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Sichuan, and northeastern Yunnan, China (Liu et al. 2020) (Fig. 2), but differ by having white florets and several other morphological characters, including fewer nerves on the leaves and longer bracts of calyculus. We conducted floral micromorphological studies and performed phylogenetic analyses based on ITS sequence data to further confirm the membership of the new species within the genus *Synotis*. We therefore determine that the population in question represents a hitherto undescribed species, which we describe below.

Material and methods

Gross morphology

We conducted careful observations of living plants in a population of the new species in Jinping county in southeastern Yunnan, China. All the major morphological characters, including the habit, the shape and size of leaves, involucre, ray and disk florets, and the type of synflorescence, which were observed and photographed on living plants by digital camera (OLYMPUS TG-6, Tokyo).

Floral micromorphology

For the study of three floral micromorphological characters of the new species (voucher: Z. Y. Yu et al. JXAU 01, JXAU), including papillae on style arms, filament collar, and anther endothelial cell thickenings in stamens [(for all these three micromorphological characters were considered taxonomic significance in the *Synotis* reported by Jeffrey and Chen (1984) and Tang (2014)], we followed the method of Tang et al. (2014), and all these characters were observed and photographed under microscope (ZEISS AXIO IMAGER A2M, Germany).

Phylogenetic analyses

Based on the results of previous phylogenetic analyses of the tribe Senecioneae (Pelser et al. 2002, 2003, 2007, 2010), we selected 82 samples representing 79 species in 38 genera within Senecioneae for our analyses. These included 30 species of *Senecio* and 13 species in *Synotis*. *Abrotanella emarginata* Cass. (subtribe Abrotanellinae) was selected as a root, according to the results of Pelser et al. (2007, 2010). We generated new sequences for our new species and its putative close ally *S. duclouxii*, and another species *S. cavaleriei* (H. Lév.) C. Jeffrey & Y.L. Chen, while the sequences of other species were retrieved from GenBank. Voucher information and GenBank accession numbers for the material used in this study are given in Appendix 1.

Total genomic DNA of *Synotis cavaleriei*, *S. duclouxii* and our new species were extracted from silica gel-dried leaves using the modified CTAB method of Doyle and Doyle (1987). The nuclear regions (ITS) were sequenced, with the primer pairs ITS4 and ITS5 (White et al. 1990). Amplification and sequencing reactions followed Tang (2014) and Ren et al. (2017).

All sequences were aligned with MAFFT 7.450 (Katoh and Standley 2013). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit model using BIC criterion. Maximum Likelihood (ML) analysis was generated

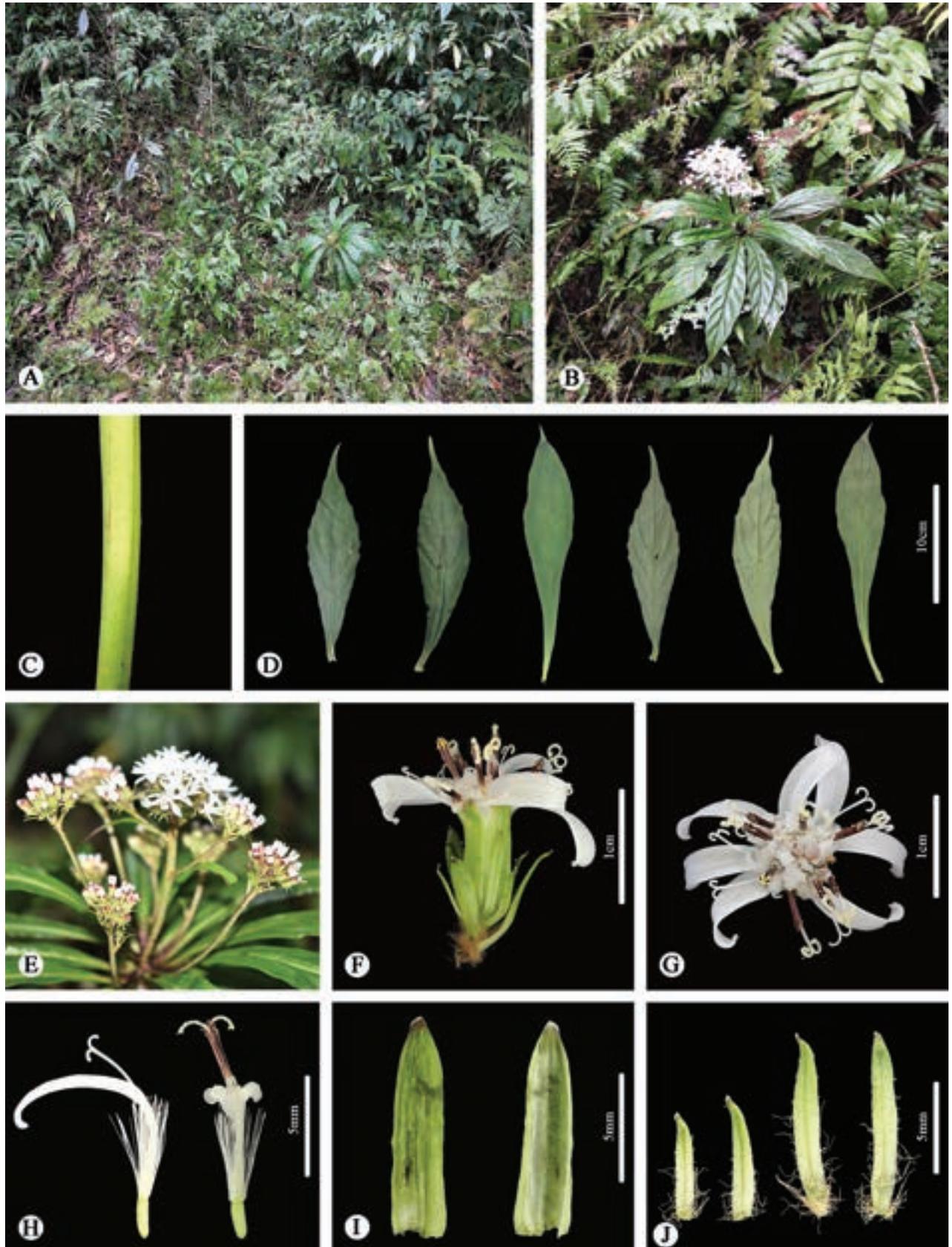


Figure 1. *Synotis jinpingensis* in the wild (Jinping, Yunnan, China) **A** habitat **B** habit **C** portion of stem **D** leaves (left three: adaxial side; right three: abaxial side) **E** synflorescence **F** capitulum (side view) **G** capitulum (top view) **H** florets (left: ray floret; right: disk floret) **I** phyllaries (left: adaxial side; right: abaxial side) **J** bracts of calyculus.

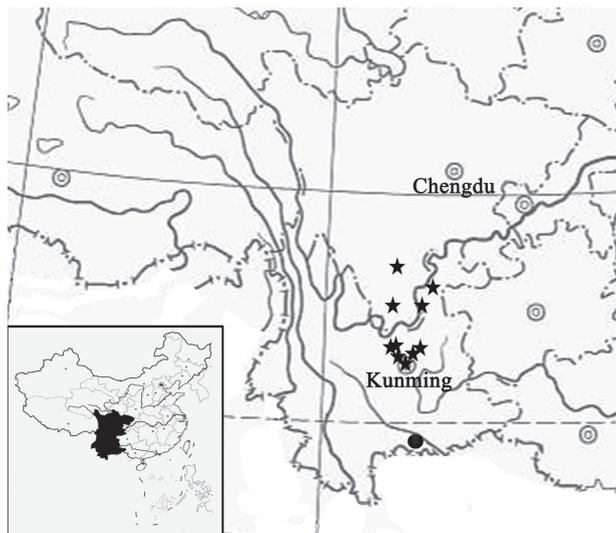


Figure 2. Distribution map of *Synotis jinpingensis* (black dot) and *S. duclouxii* (black star).

by IQ-TREE 2.1.3 (Nguyen et al. 2015), with 20 000 ultrafast bootstraps, under GTR+F+I+R3 model by ModelFinder. Bayesian Inference (BI) (Ronquist et al. 2012) analysis was carried out by MrBayes 3.2.6 (Ronquist et al. 2012), under GTR+F+I+G4 model, with 3 000 000 generations, sampling every 1 000 generations to ensure the convergence (average deviation of split frequencies less than 0.01 and the effective sample sizes over 200), in which the first 25% of sampled data treated were discarded as burn-in and the remaining trees were used to estimate the posterior probabilities (PP). Bootstrap percentage (MLBS) values ≥ 70 (Hillis and Bull 1993) and PP values ≥ 0.95 were regarded as strong support.

Results and discussion

Gross morphology

As shown in Figs 1, 2, our new species has a habit typical of *Synotis* ser. *Synotis* (Jeffrey and Chen 1984), with the leaves clustered at the bottom of the synflorescence, indicating that this species should belong to this series. It is readily distinguishable from all other species within the genus by its white ray florets. From its putative closest ally, *S. duclouxii*, the new species differs additionally by having fewer nerves on the leaves (10–14 vs. 18–20), longer bracts of calyculus (6–8 mm vs. 1–3 mm) and larger phyllaries (8–10 mm long, 2–3 mm broad vs. 5–7 mm long, 1–2 mm broad) [see Liu et al. (2020) for morphological characters of *S. duclouxii*].

Floral micromorphology

The central tuft of papillae on the style arms of our new species is prominent, much longer than laterals (Fig. 3A). The anther collars are balusterform (Fig. 3B), the anther tails are ca. 1.5 times as long as antheropodia (Fig. 3B), and the endothelial cell wall thickenings are radial (Fig. 3C). All these characters match well those reported previously in *Synotis* (Jeffrey and Chen 1984; Tang et al. 2013a, b, 2014; Tang 2014; Li et al. 2018) and further confirm the generic affiliation of our new species.

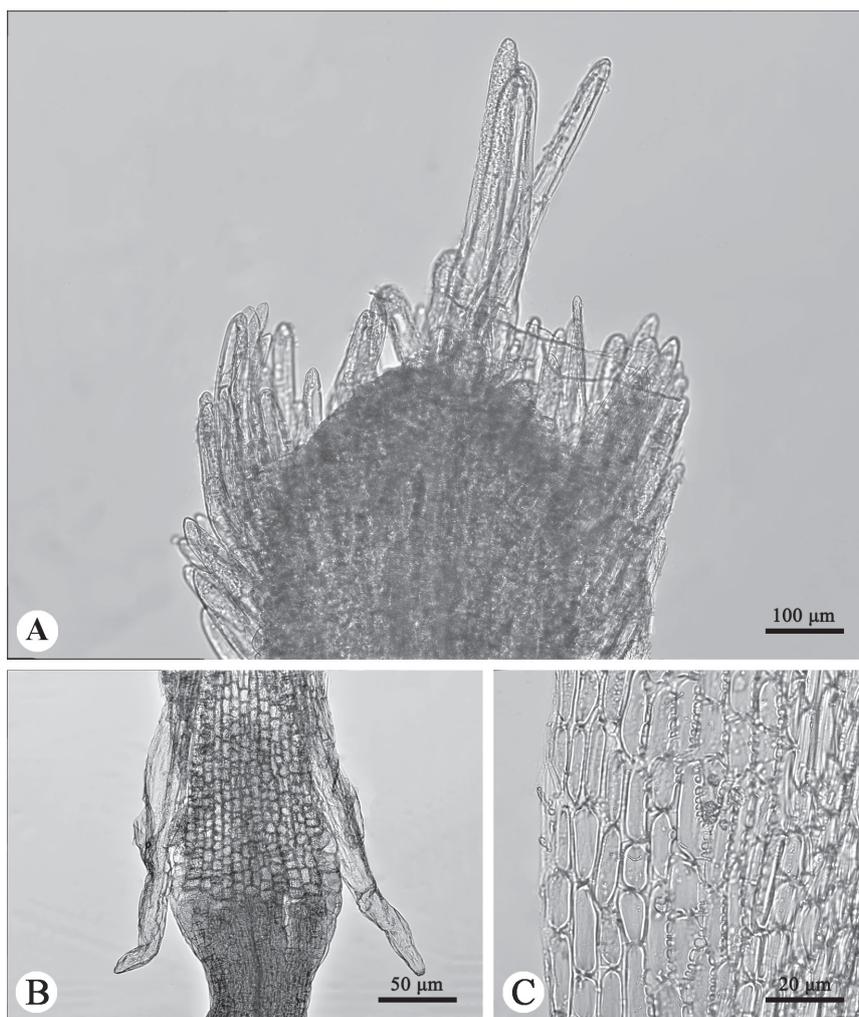


Figure 3. Three micromorphological characters of *Synotis jinpingensis* **A** papillae on style arms **B** anther collar and anther tails **C** anther endothelial cell wall thickenings.

Phylogenetic analyses

Our ML and BI analyses produce almost identical topologies, and they are also consistent with the results of previous studies (Pelser et al. 2007, 2010; Tang et al. 2014; Tong et al. 2017; Li et al. 2018). As shown in the ML tree (Fig. 5), all the sampled species of *Synotis* form a strongly supported clade (MLBS/PP = 99%/1.00) in subtribe Senecioninae and our new species is deeply nested within this clade. The membership of our new species within *Synotis* is thus strongly corroborated by ITS sequence data.

Taxonomic treatment

***Synotis jinpingensis* M.Tang, Z.Y.Yu & Q.E.Yang, sp. nov.**

urn:lsid:ipni.org:names:77331168-1

Figs 1, 4

Type. CHINA. Yunnan province: Jinping county, Fenshuiling National Nature Reserve, Guaitang village, in mixed forests, alt. ca. 2400 m, 22°45'36.87"N, 103°28'4.65"E, 30 March 2022, Z.Y. Yu et al. *JXAU 01* (holotype: JXAU; isotypes: IBSC, JXAU). Fig. 4.



Figure 4. Holotype (A) and isotype (B) sheets of *Synotis jinpingensis*.

Diagnosis. *Synotis jinpingensis* is most closely similar to *S. duclouxii* in habit and leaf shape, but differs by having white (vs. yellow) ray florets, fewer nerves on the leaves (10–14 vs. 18–20), longer bracts of calyculus (6–8 mm vs. 1–3 mm), and larger phyllaries (8–10 mm long, 2–3 mm broad vs. 5–7 mm long, 1–2 mm broad).

Description. Perennial herbs, erect, rhizomatous. Rhizome thick, horizontal. Vegetative stems solitary, simple, rarely branched, erect, 50–100 cm tall, median and lower parts subglabrous, upper part often densely yellowish setulose. Flowering stems solitary, erect, scapiform, 15–30 cm tall, few-branched, fulvous tomentose. Leaves always aggregate at base of fertile shoot; petioles 1–1.5 cm long; blades obovate-lanceolate or elliptic, 12–18 cm long, 2.5–4 cm broad, papyraceous, abaxially glabrous or subglabrous, adaxially sparsely pubescent, pinnately veined, lateral veins 10–14, arcuate-ascending, base cuneate, margin shallowly sinuate-apiculate, apex acute-acuminate. Stem leaves on reproductive shoots few, narrowly lanceolate, remote, much smaller. Capitula radiate, numerous, arranged in an attenuate broadly paniculoid corymb; peduncles 3–5 mm long, fulvous tomentose, 1- or 2-bracteate; bracts below capitula linear, 5–20 mm long. Involucres cylindrical-campanulate, 8–10 mm long, 2–3 mm broad, with 7–10 subulate bracteoles at base, bracts of calyculus linear, 6–8 mm long, green, apically purple; phyllaries usually 7 or 8, occasionally 5 or 6, oblong, 2–3 mm broad, green, herbaceous, glabrous, apically acute, purple. Ray florets 7 or 8; corolla tube 3.5–4.5 mm long, glabrous; lamina white, oblong-lanceolate, 6–9 mm long, 1.5–2.5 mm broad, 3–4-veined, apically obtuse, 3-denticulate. Disk florets 8–12, white; corolla 4–5 mm long, with ca. 4.5 mm long tube and funnellform limb; lobes ovate-oblong, 3–3.5 mm long, apically acute. Anthers ca. 3 mm, anther tails ca. 1.5 times as long as antheropodia;

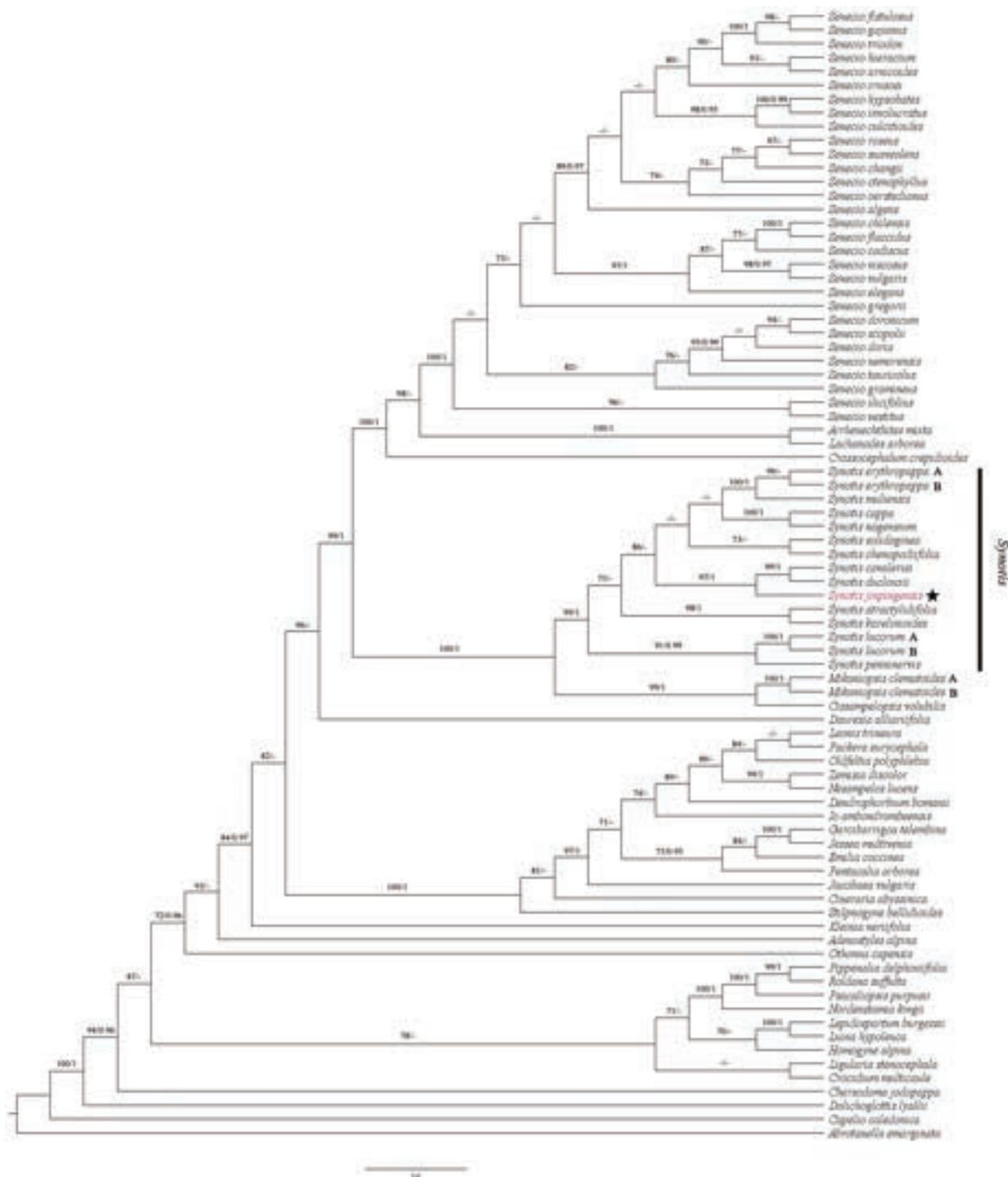


Figure 5. The maximum likelihood tree of tribe Senecioneae based on ITS dataset, with *Synotis jinpingensis* highlighted in red font. Bootstrap values (MLBS) and posterior probabilities (PP) are indicated above the branches. Dashes (-) indicate MLBS < 70% or PP < 0.95.

appendages ovate-oblong; antheropodia slightly dilated at base. Style branches ca. 2 mm long, fringed with long fine papillae, the central tuft prominent, much longer than laterals. Achenes 1.8–2 mm, glabrous. Pappus white, 6–7.5 mm long.

Phenology. Flowering from March to April; fruiting from April to July.

Etymology. The species is named after its type locality, i.e., Jinping county in southeastern Yunnan province, China.

Distribution and habitat. *Synotis jinpingensis* is currently known from its type locality, i.e., Jinping county in southeastern Yunnan province, China (Fig. 2). It grows in mixed forests at an altitude of ca. 2400 m above sea level.

Conservation status. *Synotis jinpingensis* seems currently known only from its type locality. Four small populations of this species, each with ca. 100 individuals, have been discovered there. The habitat of *S. jinpingensis* is now well preserved. The discovery of further populations of this species is to be expected as botanical exploration of southeastern Yunnan proceeds. According to the IUCN Red List Categories and Criteria (IUCN 2022), the new species may better be categorized as Data Deficient (DD).

Acknowledgements

We thank Dr. Patricio Saldivia Pérez and Dr. Rob Smissen for their helpful comments which helped to improve our manuscript. We also thank Mr. Zongli Liang, Mr. Jianhong Li and Mr. Hailong He with the Fenshuiling National Nature Reserve in Jinping county, Yunnan, China, for their help with the field work.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 31960043).

Author contributions

Data curation: ZYY. Formal analysis: RC, LCZ. Writing – original draft: MT. Writing – review and editing: QEY.

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Data availability

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

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Appendix 1

Table A1. Voucher information and GenBank accession of species used in our study.

GenBank	Species	Voucher information	Herbarium
MH371137	<i>Synotis atractylidifolia</i> (Y. Ling) C. Jeffrey & Y. L. Chen	M. Tang & L. Wang 361	IBSC
EF538402	<i>Synotis cappa</i> (Buch.-Ham. ex D. Don) C. Jeffrey & Y. L. Chen	Tessier-Yandell 86	IBSC
KJ851593	<i>Synotis chenopodiifolia</i> (DC.) M. Tang et al.	M. Tang & C. Ren 532	IBSC
KU696133	<i>Synotis erythropappa</i> (Bureau & Franch.) C. Jeffrey & Y. L. Chen A	C. Ren 150	IBSC
MH117782	<i>Synotis erythropappa</i> (Bureau & Franch.) C. Jeffrey & Y. L. Chen B	YLDP026D	IBSC
MH371138	<i>Synotis karelinioides</i> (C. Winkl.) C. Ren et al.	H.M. Li & Q.G. Mao 325	IBSC
AY723255	<i>Synotis lucorum</i> (Franch.) C. Jeffrey & Y. L. Chen A	J.Q. Liu 2177	IBSC
KU696134	<i>Synotis lucorum</i> (Franch.) C. Jeffrey & Y. L. Chen B	Q.E. Yang et al. 3128	IBSC
KU696135	<i>Synotis muliensis</i> Y. L. Chen	C. Ren 148	IBSC
AF459922	<i>Synotis nagensium</i> (C. B. Clarke) C. Jeffrey & Y. L. Chen	B. Bartholomew et al. 1991	IBSC
KY347902	<i>Synotis penninervis</i> (H. Koyama) T.J. Tong et al.	M. Tang & C. Ren 626	IBSC
KX549951	<i>Synotis solidaginea</i> (Hand.-Mazz.) C. Jeffrey & Y. L. Chen	2015XZ001-B	IBSC
–	<i>Synotis duclouxii</i> (Dunn) C. Jeffrey & Y. L. Chen	C. Ren & L.Y. Wang 485	IBSC
–	<i>Synotis cavaleriei</i> (H. Lév.) C. Jeffrey & Y. L. Chen	L.Y. Wang 72	IBSC
–	<i>Synotis jinpingensis</i> M. Tang et al.	Y. Yu et al. JXAU01	JXAU
EF538296	<i>Senecio algens</i> Wedd.	S.G. Beck 2879	S
EF538298	<i>Senecio arnicoides</i> Hook. & Arn.	O. Zoellner 3474	L
GU818506	<i>Senecio cadiscus</i> B. Nord. & Pelser	Rourke 1118	S
KU499905	<i>Senecio changii</i> C. Ren & Q. E. Yang	C. Ren et al. WL146	IBSC
EF538313	<i>Senecio chilensis</i> Less.	O. Zollner 2958	S
EF538290	<i>Senecio cruseoi</i> Pelser	T. Stussey 6560	H
EF538322	<i>Senecio ctenophyllus</i> Phil.	O. Zoellner 3959	U
EF538312	<i>Senecio culcitioides</i> Wedd.	B. Ollgaard & H. Balslev 8822	CHR
AF459946	<i>Senecio doria</i> L.	P.B. Pelser cult. 129	WIS
JX895355	<i>Senecio doronicum</i> (L.) L.	J. Calvo 4000	WIS
GU818642	<i>Senecio elegans</i> L.	Cron & Goodman 687	–
EF538335	<i>Senecio fistulosus</i> Poepp. ex Less.	S.G. Beck & M. Liberman 9672	MJG
EF538336	<i>Senecio flaccidus</i> Less.	J. Thuret s.n.	MJG
GU818649	<i>Senecio gayanus</i> DC.	M. Rosas 2157	–
GU818650	<i>Senecio gramineus</i> Harv.	F.K. Hoener 2104	–
GU818651	<i>Senecio gregorii</i> F. Muell.	D.E. Albrecht 7091	–
GU818652	<i>Senecio hieracium</i> J. Rémy	M. Baeza & L. Finot 3695	–
EF538348	<i>Senecio hypsobates</i> Wedd	B. Ollgaard & H. Balslev 9863	U
GU818662	<i>Senecio ilicifolius</i> L.	Cron & Goodman 686	
EF538150	<i>Senecio involucratus</i> (Kunth) DC.	B. Nordenstam 9438	L
AF459937	<i>Senecio nemorensis</i> L.	P.B. Pelser cult.102	L
EF538362	<i>Senecio oerstedianus</i> Benth.	B. Nordenstam 9160	S
EF538373	<i>Senecio roseus</i> Sch. Bip.	J. Garcia P. 250	U
JX895384	<i>Senecio scopolii</i> Hoppe & Hornsch. ex Bluff & Fingerh.	J. Calvo 4715	–
EF538222	<i>Senecio suaveolens</i> (L.) Elliott	D.C. Dister s.n.	MJG
GU817570	<i>Senecio tauricolus</i> V. A. Matthews	Budak et al. 1735	–

GenBank	Species	Voucher information	Herbarium
GU818707	<i>Senecio triodon</i> Phil.	F. Luebert & S. Teillier 2266	–
GU818708	<i>Senecio vestitus</i> P. J. Bergius	W. Greuter 21766	–
AF459925	<i>Senecio viscosus</i> L.	P.B. Pelser 300	TEX
AF459924	<i>Senecio vulgaris</i> L.	P.B. Pelser cult. 188	CHR
GU818721	<i>Stilpnogyne bellidioides</i> DC.	P. Goldblatt & L. Porter 11729	–
EF538416	<i>Zemisia discolor</i> (Sw.) B. Nord.	G.L. Webster et al. 8420	S
EF538143	<i>Abrotanella emarginata</i> (Gaudich.) Cass.	R.N.P. Goodall & J. Wood 3352	MU
EF538146	<i>Adenostyles alpina</i> Bluff & Fingerh.	C.H. Uthink 98-189a	MJG
EF538156	<i>Arrhenechthites mixta</i> (A. Rich.) Belcher	M.E. Lawrence 1308	S
GU818508	<i>Capelio caledonica</i> B. Nord.	B. Nordenstam 9644	–
EF538167	<i>Chersodoma jodopappa</i> (Sch. Bip.) Cabrera	I. Hensen 2617	S
GU818512	<i>Cineraria abyssinica</i> Sch. Bip. ex A. Rich.	P.B. Pelser cult. 208	L
EF538172	<i>Cissampelopsis volubilis</i> (Blume) Miq.	Carvalho 3175	US
AF459968	<i>Crassocephalum crepidioides</i> (Benth.) S. Moore	P.B. Pelser cult. 354	S
GU818541	<i>Crocidium multicaule</i> Hook.	B. Bartholomew 5749	–
AF457413	<i>Dauresia alliariifolia</i> (O. Hoffm.) B. Nord. & Pelser	Mueller & Tilson 907	–
EF538181	<i>Dendrophorbium bomanii</i> (R. E. Fr.) C. Jeffrey	M. Dematteis & G. Seijo 722	MU
GU818546	<i>Dolichoglottis lyallii</i> (Hook. f.) B. Nord.	A. Strid 22172	–
AF459966	<i>Emilia coccinea</i> (Sims) G. Don	P.B. Pelser cult. 126	MJG
EF538211	<i>Garcibarrigoa telembina</i> (Cuatrec.) Cuatrec.	L. Holm-Nielsen et al. 6211	S
KU570815	<i>Homogyne alpina</i> Cass.	Comes 11	MJG
GU818559	<i>Io ambondrombeensis</i> (Humbert) B. Nord.	S.T. Malcomber et al. 1380	–
AF459941	<i>Jacobaea vulgaris</i> Gaertn.	P.B. Pelser cult. 6	S
EF538246	<i>Jessea multivenia</i> (Benth.) H. Rob. & Cuatrec.	B. Nordenstam 9161	K
GU818568	<i>Kleinia neriifolia</i> Haw.	P.B. Pelser cult. 216	–
GU818574	<i>Lachanodes arborea</i> (Roxb.) B. Nord.	R. Cairns-Wicks s.n.	–
EF538249	<i>Leonis trineura</i> (Griseb.) B. Nord.	Smith et al. 3238	L
EF538250	<i>Lepidospartum burgessii</i> B.L. Turner	R.D. Worthington 12382	L
AF459961	<i>Ligularia stenocephala</i> (Maxim.) Matsum. & Koidz.	R.R. Kowal 3092	WIS
GU818593	<i>Luina hypoleuca</i> Benth.	W. Greuter 17706	–
GU818595	<i>Mikaniopsis clematoides</i> (Sch. Bip. ex A. Rich.) Milne-Redh. A	W.J.J.O. de Wilde & B.E.E. de Wilde-Duyfjes 9006	–
GU817581	<i>Mikaniopsis clematoides</i> (Sch. Bip. ex A. Rich.) Milne-Redh. B	I. Friis et al. 499	–
EF538266	<i>Nesampelos lucens</i> (Poir.) B. Nord.	Zanoni 45570	JBSD
EF538267	<i>Nordenstamia kingii</i> (H. Rob. & Cuatrec.) B. Nord.	B. Stahl 5572A	S
EF538271	<i>Oldfeltia polyphlebia</i> (Griseb.) B. Nord. & Lundin	B. Nordenstam & R. Lundin 340	S
AF459960	<i>Othonna capensis</i> L. H. Bailey	P.B. Pelser cult. 106	S
GU818608	<i>Packera eurycephala</i> (Torr. & A. Gray) W.A. Weber & Á. Löve	M.A. Vincent 8581	MU
EF538283	<i>Pentacalia arborea</i> (Kunth) H. Rob. & Cuatrec.	B. Ollgaard & H. Balslev 8298	MU
GU818627	<i>Pippenalia delphiniifolia</i> (Rydb.) McVaugh	Spellenberg & Bacon 11048	–
GU818629	<i>Psacaliopsis purpusii</i> (Greenm. ex Brandege) H. Rob. & Brettell	Panero et al. 2607	–
GU818631	<i>Roldana suffulta</i> (Greenm.) H. Rob. & Brettell	Rzedowski 36569	–

Five new and noteworthy species of Epidendroideae (Orchidaceae) from southwestern China based on morphological and phylogenetic evidence

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Academic editor: Vincent Droissart

Received: 16 August 2023

Accepted: 23 October 2023

Published: 20 November 2023

Citation: Ya J-D, Wang W-T, Liu Y-L, Jiang H, Han Z-D, Zhang T, Huang H, Cai J, Li D-Z (2023) Five new and noteworthy species of Epidendroideae (Orchidaceae) from southwestern China based on morphological and phylogenetic evidence. *PhytoKeys* 235: 211–236. <https://doi.org/10.3897/phytokeys.235.111230>

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Abstract

Five new orchid species from southwestern China's Yunnan Province and the Tibetan Autonomous Region, *Neottia lihengiae*, *Neottia chawalongensis*, *Papilionanthe motuoensis*, *Gastrochilus lihengiae*, and *Gastrochilus bernhardtianus*, are described and illustrated. To confirm their identities, and to resolve phylogenetic relationships, we sequenced the complete plastomes of these taxa with their congeneric species, adding new plastomes of three *Neottia* species, two *Papilionanthe* species and nine *Gastrochilus* species. Combined with published plastid sequences, our well-resolved phylogeny supported the alliance of *N. lihengiae* with the the *N. grandiflora* + *N. pinetorum* clade. *Neottia chawalongensis* is now sister to *N. alternifolia*, while *P. motuoensis* is closely related to *P. subulata* + *P. teres*. Conversely, phylogenetic analyses based on complete plastomes and plastid sequences showed inconsistent relationships among taxa in the genus *Gastrochilus*, but the two new species, *G. lihengiae* and *G. bernhardtianus* were supported by all datasets.

Key words: *Neottia*, *Gastrochilus*, *Papilionanthe*, Plastid phylogenomics, Taxonomy, Tibet, Yunnan

Introduction

The Himalaya and Hengduan Mountains of southwestern China are iconic biodiversity hotspots of global significance (Liu et al. 2022). However, the species diversity of this region remains inadequately understood. In recent years, a considerable number of new plant species were described from these regions (Cai et al. 2019). Taking the Orchidaceae as an example, early floristic accounts

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of Tibet (Xizang) in the “Flora Xizangica” included only 64 genera totaling 191 species (Wu 1987). In contrast, a number of new species and new geographic records were reported over the last three decades (Wang et al. 2018; Liu et al. 2020a; Li et al. 2022, 2023), increasing the number of orchids in Tibet to 110 genera with 491 species in the latest checklist (Wang et al. 2023). Long-term and in-depth field investigations are still required to meet the urgent challenge of conserving rare species in these mountainous regions as their habitats undergo rapid changes.

The Orchidaceae is one of the largest families of angiosperms in the world, with approximately 190 genera and 1600 species in China (Jin et al. 2019). The Hengduan Mountains and the Himalaya, particularly counties of Gongshan, Shangri-La, and Lijiang (Yunnan) and Motuo (Medog) in Tibet (Xizang) are particularly rich in orchids (Zhang et al. 2015).

The genus *Neottia* Guett. a member of the Neottieae, was first established in 1754 consisting of a few, small, mycoheterotrophic orchids (Chen 1999). Recent molecular studies indicate that the autotrophic genus *Listera* Brown should be submerged within *Neottia* s.l. The monophyletic tribe Neottieae becomes the sister to the majority of the remaining members of the largest subfamily, Epidendroideae in the Orchidaceae (Pridgeon et al. 2005; Chen et al. 2009; Chase et al. 2015; Zhou and Jin 2018). There are 73 accepted species in *Neottia* s.l. distributed widely across the temperate and subarctic regions of the Northern Hemisphere, but the genus extends to northwest Africa, with a few species native to alpine regions in subtropical eastern Asia (Pridgeon et al. 2005; Chen et al. 2009; POWO 2023). Morphologically, *Neottia* is readily distinguished from other terrestrial epidendroids by its two opposite to nearly opposite leaves (when present) or it is leafless. Each resupinate flower contains a curved column containing two sectile but naked pollinia (indicative of basal epidendroids).

The small genus *Papilionanthe* Schltr., a member of Vandeeae (subtribe Aeridinae), was first described by Schlechter in 1915 based on *Vanda teres* (Roxb.) Lindl. published previously in genus *Vanda* R.Br. The genus *Papilionanthe* s.s. is distinguished from other genera in subtribe Aeridinae by multiple characters. It has fleshy and terete leaves, and a short inflorescence arising from a node opposite the leaf. The trilobate labellum is spurred. Its mid-lobe is often dilated and 2- or 3-lobed at its apex. The subterete and short column has a short foot. Pollinia are attached to a broadly triangular or subquadrate stipe which, in turn, is attached to a large and cellular viscidium (Chen et al. 2009; Pridgeon et al. 2014). Ten *Papilionanthe* species are recognized currently distributed from India eastward to south-central China and Malesia (POWO 2023).

The genus *Gastrochilus* D. Don is also a member of the subtribe Aeridinae and was established in 1825. It is characterized by monopodial growth, erect or pendulous stems and short axillary inflorescences. The labellum has a saccate hypochile. Two porate and globose pollinia are borne on a slender stipe (Chen et al. 2009; Pridgeon et al. 2014; Liu et al. 2019). Recent molecular studies of *Gastrochilus* show that traditional classification based on morphological characters is well supported (Liu et al. 2019). The genus consists of ca. 70 species, distributed through subtropical Asia, from Sri Lanka and India into the Himalaya, eastwards to southern China, southern

Japan and southwards to the Philippines and Indonesian archipelago. Approximately 40 species are found in China (Chen et al. 2009; Liu et al. 2019; POWO 2023).

We collected specimens of five previously unidentified species during our field surveys in Yunnan and Tibet from 2016–2023. Following a review of the literature (see Pearce and Cribb 2002; Chen et al. 2009; Raskoti 2009; Jin and Pang 2016; Averyanov et al. 2018; Wu et al. 2019; Mu et al. 2020; Liao et al. 2022), morphological studies of herbarium specimens and plastid phylogenomic analyses, we concluded that these specimens are new to the genera *Neottia* (Orchidaceae: Epidendroideae, Neottieae), *Papilionanthe* and *Gastrochilus* (Orchidaceae: Epidendroideae, Vandeeae, Aeridinae) respectively. These species are analyzed and described below.

Materials and methods

Morphological studies

Living plants and herbarium specimens were collected in the field in the Hengduan Mountains of northwestern Yunnan and the Himalaya of southeastern Tibet. Morphological characters and measurements of the specimens described here were based on at least 5 living specimens first observed in the field then cultivated plants in the greenhouse. Voucher specimens are deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (**KUN**) and the Herbarium of the Yunnan Academy of Forestry and Grassland (**YAF**).

Taxon sampling, DNA extraction, sequencing, assembling and annotation

To clarify the phylogenetic relationships of five potentially new species with closely related species, we sampled and sequenced plastomes of 17 accessions representing three *Neottia* species, two *Papilionanthe* species and nine *Gastrochilus* species. Including those retrieved from the National Centre for Biotechnology Information (NCBI) database, our dataset comprises 412 plastid genes of a total of 83 accessions.

Total genomic DNA was extracted from silica-dried tissue using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China). Libraries for pair-end 150 bp sequencing with 200–400 bp insert size were conducted on a BGISEQ-T7 platform at BGI Shenzhen (China) for genome skimming, producing approximately 2Gbp high-quality reads per sample. The plastomes of *Neottia ovata* (L.) Hartm. (NC_030712) and *Gastrochilus formosanus* (Hayata) Hayata (MN124435) were used as references for the assembling of the clean reads (Feng et al. 2016; Liu et al. 2020b). Complete plastomes and the nuclear internal transcribed spacer (ITS) assembly were conducted using the Getorganelle toolkit (Jin et al. 2020). The parameters used were R = 15, k = 21,45,65,85,105,127, F = embplant_pt; R = 7, k = 21,45,65,85,105,127, F = embplant_nr, respectively. Assembled plastid genomes were annotated by PGA GENEIOUS R9.0.2 (Biomatters Ltd. Auckland, New Zealand) using the plastome of *Neottia ovata*, *Holcoglossum amesianum* (Rchb. F.) Christenson (NC_041511.1) and *Gastrochilus formosanus* (Li et al. 2019).

Phylogenetic analysis

For *Neottia*, a total of 22 taxa were included in the analysis of the data set comprising two plastid DNA (*matK*, *rbcl*) and nuclear ribosomal (nr) ITS sequences, *Cephalanthera longifolia* (L.) Fritsch was used as the outgroup based on Zhou and Jin (2018). For *Papilionanthe*, phylogenomic analysis was implemented based on nrITS and six plastid markers (*matK*, *trnL-trnF*, *psbA-trnH*, *atpI-atpH*, *trnS-trnFM* and *rbcl*) from 6 *Papilionanthe* species, including two newly sequenced species, and a total of 12 accessions representing four genera. They were all analyzed with *Ascocentrum ampullaceum* (Roxb.) Schltr. as the outgroup (Zhang et al. 2013). For *Gastrochilus*, eight publicly available plastome sequences of *Gastrochilus* species were obtained from GenBank (Suppl. material 1) along with nine newly sequenced *Gastrochilus* species ($n = 12$ plants). Therefore, a total of 17 *Gastrochilus* plastomes were included in this study with *Pomatocalpa spicatum* Breda as the outgroup (Liu et al. 2019). DNA sequences obtained from nrITS and chloroplast *matK*, *trnL-trnF*, *psbA-trnH*, *psbM-trnD* were combined as a data matrix. Voucher information and GenBank accession numbers are provided in Suppl. materials 1–4. The plastid genes were aligned individually using MAFFT v7.308 (Katoh and Standley 2013), and the alignment of ITS and plastid genes are available in ScienceDB, after which alignment columns used Gblocks 0.91 (Castresana 2000). Before phylogenetic analysis, the best-fit Akaike Information Criterion (AIC) model was selected in JModelTest v2.1.10 (Darriba et al. 2012). Phylogenetic trees were constructed by maximum likelihood (ML) in RaxML v8.2.11 (Stamatakis 2014) with 1,000 bootstrap replicates and Bayesian inference (BI) methods in MrBayes v3.2.6 (Ronquist et al. 2012). For Bayesian inference, two separate Markov chain Monte Carlo (MCMC) chains were run for 200,000 generations with mixed nucleotide substitution models and 25% (50,000) of the trees were deleted as burn-in, and the results of two independently run computations were merged to produce a Bayesian consistent tree and a posterior probability value (PP) for each branch.

Results and discussion

Characteristics of the plastomes

All newly sequenced plastomes were assembled completely and can be accessed from GenBank (Table 1). Their genome features were found to be nearly identical, and gene content is conserved with an identical set of 68 annotated unique protein-coding genes (except for *Neottia*) and 29–30 tRNA genes and 4 rRNA genes. While, all three newly sequenced *Neottia* plastome sizes ranged from 155,447–156,082 bp, their genomes were composed of an LSC region (84,270–84,930 bp), SSC region (17,875–18,113 bp) and two IR copies (26,367–26,682 bp), with 74–80 unique genes. Their overall G/C content was almost identical (37.5–37.6%). The total plastome lengths of *Papilionanthe teres* (Roxb.) Schltr. and the putative new species, *P. motuoensis* ranged from 147,829–148,619 bp. Among all *Gastrochilus* plastomes, plastome sizes ranged from 146,615 to 148,552 bp. The genomes were composed of a large single repeat region (LSC) (84,710–85,682 bp), a small single repeat region (SSC), region (10,357–11,173 bp) and two inverted repeat (IR) copies (25,767–26,007 bp). Their overall G/C content was nearly identical (36.6–36.8%).

Table 1. Summary of plastomic data and nrITS sequences for *Neottia*, *Papilionanthe* and *Gastrochilus* species.

Species	GenBank accession number	Raw data	Genome size (bp)	LSC	SSC	IR	Number of unique protein coding genes	Number of tRNAs	Number of rRNA	ITS GenBank accession number	Sequence length [bp]
<i>Neottia chawalongensis</i>	OR786306	1.40/1.38G	155447	84581	18113	26367	119	30	4	OR073413	625
<i>N. lihengiae</i>	OR002177	4.71/4.70G	155600	84270	17969	26682	114	30	4	OR073414	623
<i>N. sp.</i>	OR002178	4.05/3.81G	156082	84930	17875	26639	109	30	4		623
<i>Papilionanthe motuoensis</i>	OR772949	1.64/1.58G	148,619	84,574	12,055	25,945	107	30	4	OR073415	668
<i>P. teres</i>	OR772950	1.00/1.02G	147,829	85,680	11,445	25,352	101	29	4	OQ991258	662
<i>Gastrochilus bernhardtianus</i>	OR772951	1.17/1.10G	147,078	84,845	10,357	25,938	101	29	4	OR073405	398
<i>G. bernhardtianus</i>	OR002167	1.70/1.72G	146,615	84,710	10,371	25,767	101	29	4	OR073404	654
<i>G. fargesii</i>	OR002175	1.55/1.54G	148,552	85,682	11,132	25,951	110	30	4		656
<i>G. distichus</i>	OR002170	873/893MB	147,834	85,063	11,113	25,829	101	29	4	OR073407	409
<i>G. distichus</i>	OR002171	975/914MB	147,826	85,010	11,112	25,852	101	29	4	OR073406	654
<i>G. gongshanensis</i>	OR002173	1.46/1.48G	147,728	84,936	11,032	25,880	101	29	4	OR073411	410
<i>G. gongshanensis</i>	OR786306	1.83/1.88G	147,794	85,026	11,032	25,867	110	30	4	OR073412	655
<i>G. lihengiae</i>	OR002168	1.60/1.59G	147,940	84,863	11,165	25,956	101	29	4	OR073408	656
<i>G. lihengiae</i>	OR002169	2.01/2.00G	147,934	84,829	11,173	25,966	101	29	4		655
<i>G. nanchuanensis</i>	OR002176	1.56/1.46G	148,001	84,942	11,045	26,007	110	30	4	OR073410	89
<i>G. sp.</i>	OR002172	1.69/1.73G	147,706	84,938	11,032	25,867	110	30	4		621
<i>G. sp.</i>	OR002174	1.70/1.80G	147,708	84,938	11,032	25,869	101	29	4	OR073409	654

Phylogenetic relationships within *Neottia*

Phylogenetic relationships based on combined nrITS and plastid DNA (*matK*, *rbcL*) data indicated that *Neottia* s.l. is monophyletic with moderate support (BP = 84, PP = 0.9993). Within the sampled species, the widespread and temperate *N. ovata* diverged initially, which is consistent with the previous study by Zhou and Jin (2018), followed by the clade of *N. cordata* (L.) Rich. and *N. smallii* (Wiegand) Szlach. The newly discovered species *N. chawalongensis* (Fig. 1) is sister to *N. alternifolia* (King & Pantl.) Szlach. (PP = 1, BP = 97). Together, they constitute the sister clade of *N. meifongensis* (H.J.Su & C.Y.Hu) T. C. Hsu & S. W. Chung. The second new species, *N. lihengiae* (Fig. 1) is clustered with *N. pinctorum* (Lindl.) Szlach. the unidentified *Neottia* sp. and *N. wardii* (Rolfe) Szlach. (PP = 0.92, BP = 91).

Phylogenetic relationships of *Papilionanthe*

In the overall matrix of *Papilionanthe*, 75 sequences were obtained (13 nrITS sequences and 14 *matK*, 14 *trnL-trnF*, 11 *psbA-trnH*, 11 *atpI-atpH*, 9 *trnS-trnF*, and 3 *rbcL* sequences, respectively), and the combined dataset of 7 markers comprised 8558 aligned nucleotides, 790 bp from nrITS and 7768 bp from plastid regions, respectively.

The concatenated tree of nrITS and its plastid data show that *Papilionanthe* is monophyletic. The main clade of *Papilionanthe* is divided into two subclades (Fig. 2). In the first, *P. biswasiana* (Ghose & Mukerjee) Garay and *P. hookeriana* (Rchb.f.) Schltr. are sister species. In the second subclade, *P. uniflora* (Lindl.) Garay diverged first while the newly sequenced *P. teres* is well supported as

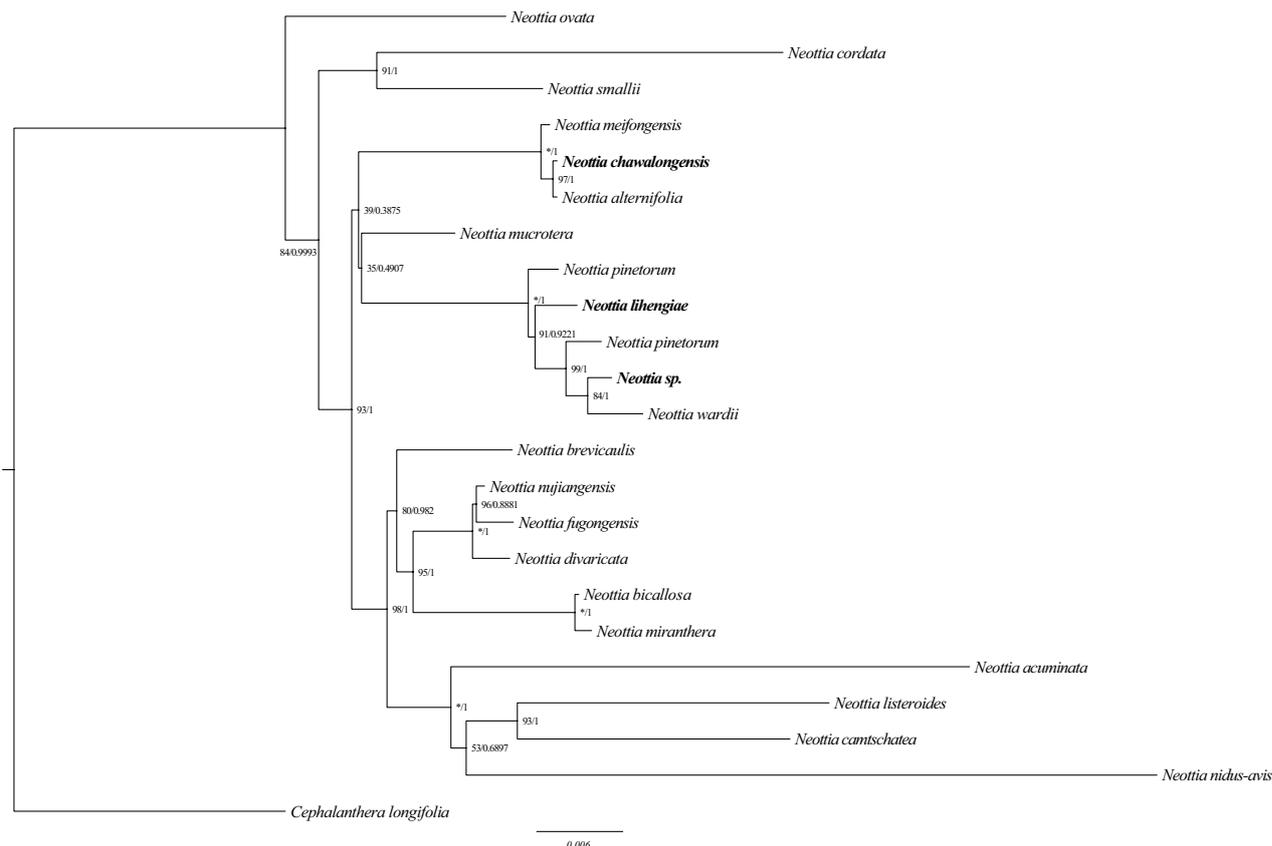


Figure 1. Phylogenetic relationships of *Neottia* species based on the nrITS, *matK* and *rbcl*. The ML and BI trees have the same topology. Numbers at nodes are Bayesian posterior probabilities and bootstrap percentages, respectively. "*" represents 100% support with newly sequenced species are shown in bold italics.

sister to *P. subulata* (Willd.) Garay (BP = 99, PP = 1). Collectively it is sister to the new species *P. motuoensis* (see below) (BP = 99, PP = 1). The BI and maximum likelihood (ML) trees yield the same topology. The posterior probabilities and bootstrap probabilities values are high, indicating a high degree of confidence for the result. However, this topology is inconsistent with the previous study by Zhang et al. (2013). The difference between these topologies was the position of *P. hookeriana* and *P. biswasiana*, now consisting of a sister group in this study.

Phylogenetic relationships of *Gastrochilus*

A total of 41 *Gastrochilus* species were included in this study to represent all six sections, 12 accessions representing nine species were newly generated in this study. Their relationships were confirmed using a combined dataset of nrITS and plastid *matK*, *trnL-trnF*, *psbA-trnH*, *psbM-trnD* sequences (Fig. 3a). The detailed sequence information is listed in Suppl. material 4, and the best-fit model selected by jModeltest is given in Table 2. Both RAxML and Bayesian inference (BI) analyses of the concatenated sequence supermatrix produced similar topologies for the *Gastrochilus* species. *Gastrochilus* s.l. is strongly supported as monophyletic with high posterior probabilities (PP) and bootstrap probabilities (BP) (Fig. 3a). The genus was subdivided into six well-supported clades, the earliest diverging clade is clade A (*G.* sect. *Pseudodistichi*), successively followed by clade B (*G.* sect. *Brachycaules*) (BP = 79, PP = 0.7916), clade C

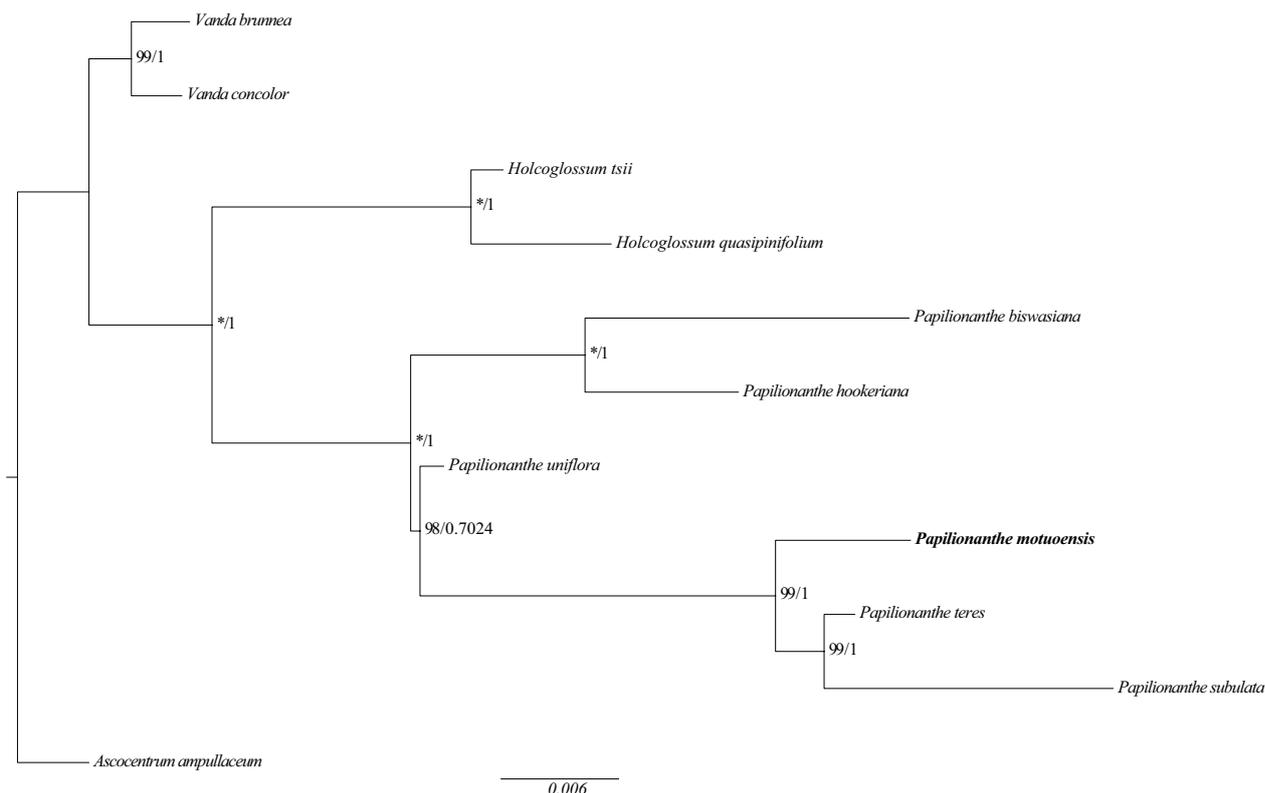


Figure 2. Phylogram of the genus *Papilionanthe* based on ML and BI analyses of the combined nrITS and plastid *matK*, *trnL-trnF*, *psbA-trnH*, *atpI-atpH*, *trnS-trnM*, *rbcL* sequences. The ML and BI trees are identical and the BP and PP values are given beside the nodes. “*” indicates 100% bootstrap support.

(*G.* sect. *Gastrochilus*) (BP = 100, PP = 0.9993) and clade D (*G.* sect. *Acinacifolii*) (BP = 99, PP = 1). Clades E and F are sister to each other and they together comprise a clade sister to clade D. Our two new species, *G. lihengiae* (see below) and *G. bernhardtianus* (see below) are resolved as distinct species in clade E (*G.* sect. *Microphylli*) in all data sets. *Gastrochilus distichus* (Lindl.) Kuntze + *G. prionophyllus* H. Jiang, D. P. Ye & Q. Liu is sister to *G. lihengiae* while *G. heminii* M. Liao, B. Xu & Yue.H. Cheng is sister to *G. bernhardtianus* and they consisting a sister group to *G. alatus*. Within clade F, two samples of *G. gongshanensis* Z.H.Tsi and two unidentified species from the Dali, Yunnan form a distinct sub-clade, which is sister to *G. yunlongensis* W. H. Rao, L. J. Chen & Z. J. Liu.

In the present study, analysis of 20 complete chloroplast genomes of *Gastrochilus* specimens provide a wealth of information to determine phylogenetic relationships within this genus, including a fully resolved phylogenetic tree with almost 100% bootstrap values and 1.00 posterior probabilities, and are better

Table 2. Best-fit models and parameters for each genus.

Genus	Region	AIC select model	Base frequencies				Base frequencies						p-inv (I)	Gamma shape (G)
			A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T		
<i>Neottia</i>	ITS, <i>matK</i> , <i>rbcL</i>	GTR+I+G	0.2907	0.1881	0.1987	0.3226	1.1924	1.7728	0.3011	0.2107	2.0924	1.0000	0.4320	0.9250
<i>Papilionanthe</i>	ITS, <i>matK</i> , <i>trnL-F</i> , <i>psbA-trnH</i> , <i>trnS-trnM</i>	GTR+G	0.3083	0.1866	0.1675	0.3376	1.1439	1.3935	0.4909	0.4617	1.3426	1.0000		0.3190
<i>Gastrochilus</i>	Plastome	GTR+I+G	0.3129	0.1844	0.1785	0.3242	1.0779	1.2742	0.3062	0.2105	1.1734	1.0000	0.7880	0.8410
<i>Gastrochilus</i>	ITS, <i>matK</i> , <i>trnL-F</i> , <i>psbA-trnH</i> , <i>psbM-trnD</i>	GTR+I+G	0.3207	0.1879	0.18	0.3114	0.8602	1.6575	0.3075	0.5360	1.8524	1.0000	0.1610	0.0210

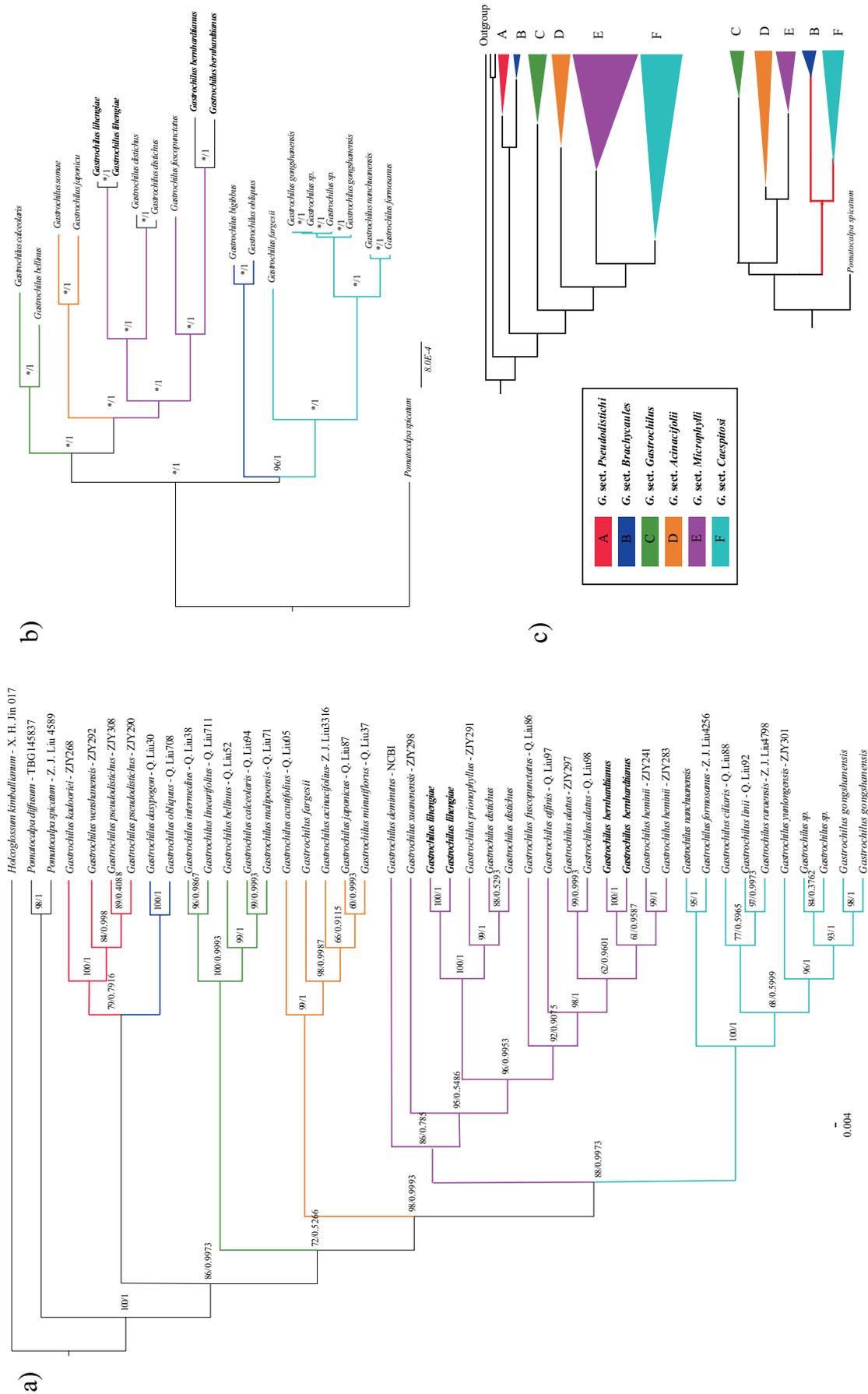


Figure 3. a Phylogenetic relationships in genus *Gastrochilus* based on nrDNA ITS and plastid *matK*, *trnL-trnF*, *psbA-trnH*, *psbM-trnD* sequences. **ML** and **BI** trees have the same topology and **BP** and **PP** are given beside the branches **b** phylogenetic relationships of *Gastrochilus* based on the complete plastomic sequences. All nodes are supported with a posterior probability (pp) of 1.0. “*” indicates 100% bootstrap support **c** phylogenetic tree based on combined nrITS and plastid DNA markers and conflicting topologies (clade B and clade F) are highlighted.

supported than in the studies of Liu et al. (2019) and Zhang et al. (2023) based on nrITS and plastid sequences (Fig. 3b). However, these phylogenetic relationships based on whole plastomes and chloroplast sequences suggest different topologies, particularly among the relationship between Clade B and subclade including *G. fargesii* (Fig. 3c). The analysis by Zhang et al. (2023) suggested that *G. obliquus* diverged early in the clade B with *G. formosanus* falling into clade F (Fig. 3a). In contrast, our plastome-based topology showed that species of clade B recovered as sister to clade F with high statistic support (Fig. 3c). One explanation is the difference in information sites, and the other possibility is sampling size. More samples with plastomic data should be used in future study to resolve the difference.

Conservation status

We preliminarily assessed the conservation status of the five new species using the IUCN Red List Categories and Criteria (IUCN 2022). *Neottia lihengiae* is known from two sites with a population of >2,000 individuals in an area of ca. 80 square kilometers scattered under a protected, evergreen broadleaved forest and a mossy dwarf forest in northwestern Yunnan, respectively. Based on population size and healthy habitats, the conservation status is proposed as Least Concern (LC). In contrast, the remaining four species, are known only from type localities and adjacent areas. For each of these species, only one or two populations with few individuals were detected during our two to three field surveys. More extensive fieldwork is needed to objectively assess their conservation status.. Therefore, the status of all remaining species are temporarily rated as Data Deficient (DD).

Taxonomic treatments

Neottia Guett.

1. *Neottia lihengiae* J.D.Ya, H.Jiang & D.Z.Li, sp. nov.

urn:lsid:ipni.org:names:77331171-1

(李恒对叶兰 Li Heng Dui Ye Lan)

Fig. 4

Diagnosis. *Neottia lihengiae* is morphologically similar to *N. biflora* (Schltr.) Szlach., but can be distinguished by its smaller plant size, ca. 5.5–9.0 cm tall (vs. 10–13 cm tall), its lax rachis of 2–5-flowered (vs. 1- or 2-flowered), floral bracts and sepals longer than their pedicel (vs. shorter than pedicel), smaller flowers with sepals and petals connivant and ca. 3.0 mm long (vs. spreading and ca. 6.0–7.0 mm long). The outer surfaces of the sepals are not carinate (vs. carinate). The labeulum is ligulate and its midvein is not thickened (vs. cuneate and midvein slightly thickened). The rostellum is almost equal to the anther (vs. distinctly shorter than the anther).

Type. CHINA. Yunnan Province, Diqing Prefecture, Shangri-La County, Tianbao mountain, 3800 m, under shrubs of a scree slope, 4 July 2020, J.-D. Ya et al. 20CS19095 (Holotype: KUN! isotype: KUN!)

Description. Terrestrial, autotrophic herbs, 5.5–9.0 cm tall. Rhizome with many elongate, filiform roots. Stem erect, slender, usually with 1 or 2 membranous ca. 8.0 mm long tubular sheaths at its base. Leaves 2, opposite, borne above the

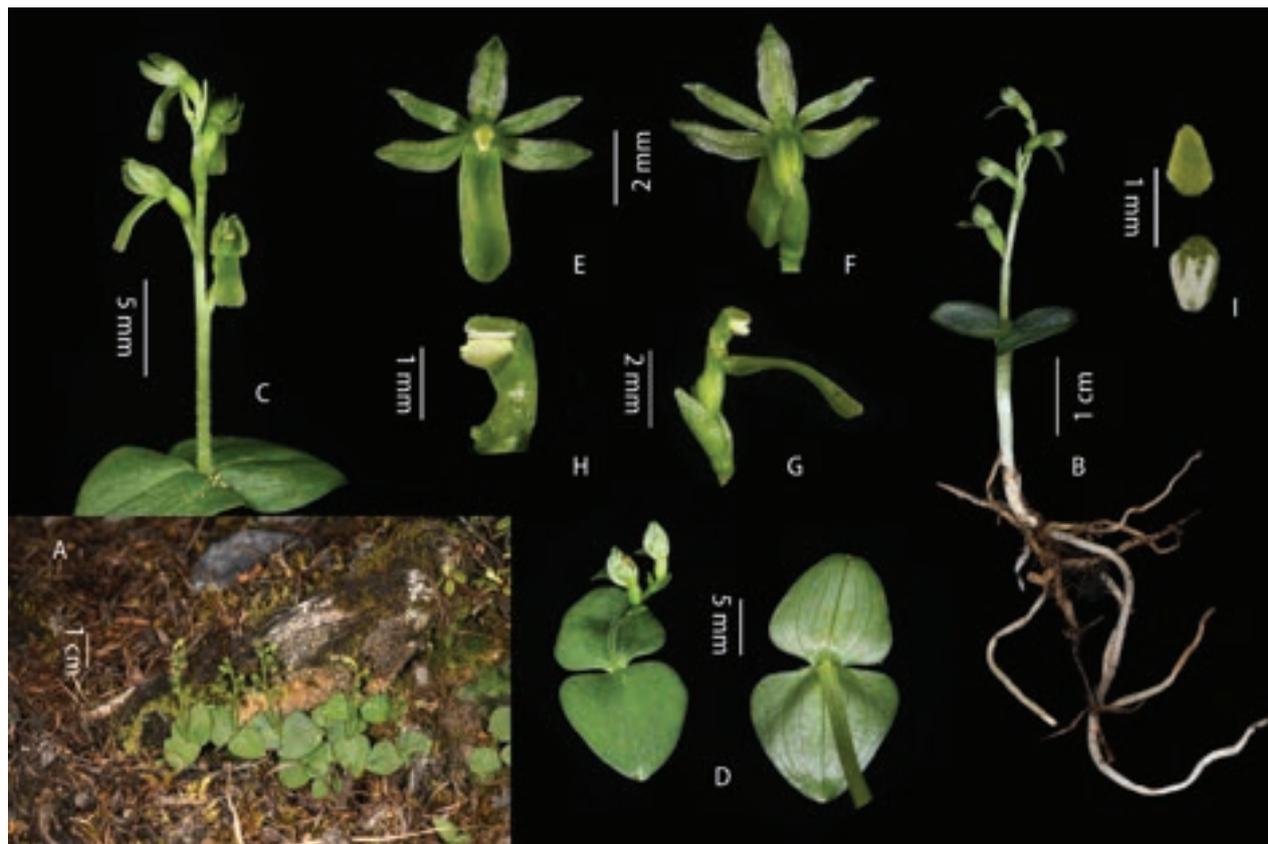


Figure 4. *Neottia lihengiae* J.D.Ya, H.Jiang & D.Z.Li, sp. nov. **A** habitat **B** plant **C** inflorescence **D** leaves **E** flower (front view) **F** flower (dorsal view) **G** column and labellum **H** column **I** anther cap. Photographed by J.-D. Ya.

middle of the plant, 7 veined from the base, subsessile, broadly ovate or broadly ovate-triangular, unequal in size, the larger leaf ca. 1.2×1.2 cm, the smaller one ca. 1.0×1.0 cm, with bases rounded and apices acute. Peduncle 0.7–1.2 cm, puberulous, rachis 1.2–1.8 cm, laxly 2–5-flowered; floral bracts ovate-lanceolate, concave, longer than the pedicel, 3–4 \times ca. 0.8 mm, apex acute to acuminate. Flowers resupinate, uniformly green; pedicel and ovary 2.0–3.0 mm long, glabrous; sepals and petals connivent. Dorsal sepal ovate-lanceolate, ca. 3.2×1.1 mm, 1-veined, apex subacute; lateral sepals lanceolate, slightly oblique, ca. 3.5×0.8 mm, 1-veined, apex acute. Lateral petals linear-lanceolate, ca. 3.0×0.6 mm, 1-veined, apices subacute; labellum ligulate, ca. 4.0×1.6 mm, entire to shallowly notched or emarginate at apex, usually with a minute tooth in the notch. Column slightly arcuate, ca. 1.7 mm long, anther inclined toward rostellum, ca. 0.9 mm; rostellum spreading forward, nearly as long as the anther.

Phenology. Flowers from June to July.

Etymology. Named in honor of late Prof. Li Heng, a Chinese botanist who made significant contributions to our understanding of plant diversity and phytogeography of the Gaoligong Mountains at the border between China and Myanmar (Guo et al. 2023).

Distribution and habitat. It is known from Northwest Yunnan including Lijiang and Diqing. It grows under shrubs colonizing scree slopes at elevations of 3700–3800 m.

Additional specimen examined. CHINA. Yunnan Province, Lijiang City, Gu-cheng District, Dadong Xiang, 3192 m, in the scree slope area under the forest

dominated by *Pinus densata* Mast. 17 June 2017, H. Jiang and W.P. Zhang 08835 (paratypes: YAF!); Yunnan Province, Diqing Prefecture, Shangri-La County, Tianbao mountain, 3719 m, under the shrub of scree slope, 15 Aug. 2018, C. Liu et al. 18CS17401 (paratypes: KUN!). *N. biflora*: CHINA. Sichuan, Dongrergo, K. A. H. Smith 3656 (isotypes, PE00027184!). *N. tianshanica*: CHINA. Xinjiang Uygur Autonomous Region, Tian-Shan, 18 July 1957, K.-Z. Guan 172 (holotype, LE 01012234!); CHINA. Xinjiang Uygur Autonomous Region, Urumqi, Houxia Zhen, 2161 m, J.D. Ya et al. 17CS16209 (KUN1437961!).

2. *Neottia chawalongensis* J.D.Ya & D.Z.Li, sp. nov.

urn:lsid:ipni.org:names:77331172-1

(察瓦龙对叶兰 Cha Wa Long Dui Ye Lan)

Fig. 5

Diagnosis. *Neottia chawalongensis* is similar to *N. pinetorum* (Lindl.) Szlach., but differs in having floral bracts longer than its pedicel (vs. shorter or as long as pedicel), a reduced pedicel ca. 1.9 mm (vs. 4–6 mm), and a shorter but pubescent ovary, ca. 2.8 mm (vs. glabrous, 3–4.5 mm). The labellum is lanceolate (vs. obovate-cuneate, oblong-cuneate, sublinear-cuneate, or oblanceolate), densely papillate (vs. slightly papillate), with labellum lobes narrowly lanceolate and apices acuminate (oblong-ovate and apices obtuse-rounded) while its sinus usually lacks a short tooth between the lobes.

Type. CHINA. Tibetan Autonomous Region, Linzhi City, Chayu County, Chawalong Township, 3757 m, under the shrub of scree slope, 21 July 2022, J.-D. Ya et al. 22CS22851 (KUN!)

Description. Terrestrial, autotrophic plants, 9.0–13.5 cm tall. Rhizome with many elongate, filiform roots. Stem 5.0–7.0 cm, erect, slender, ridged, usually with 1 or 2 membranous, long, tubular sheaths at its base. Leaves 2, opposite, borne in the middle of the plant, 5 veined from the base, subsessile, broadly ovate or broadly ovate-triangular, ca. 2.0 × 1.8 cm in diameter. Peduncle 1.8–2.4 cm, puberulous, rachis 2.7–4.3 cm, held laxly bearing 7–9-flowered; floral bracts ovate, concave, longer than the pedicel, 3–4 mm long with acute apices. Flowers resupinate, uniformly green; pedicel ca. 1.9 mm, glabrous to sparsely pubescent; ovary ca. 2.8 mm, pubescent with sepals and petals widely spreading. Dorsal sepal narrowly elliptical, ca. 3.5 × 1.3 mm, 1-veined, apex obtuse; lateral sepals narrowly elliptic-falcate, ca. 3.5 × 1.5 mm, with an obtuse apex. Lateral petals linear, ca. 3.0 × 0.4 mm, apices subacute; labellum pendulous, lanceolate, ca. 8.0 × 2.2 mm, margins densely papillate, apex deeply 2-lobed with lobes parallel, narrowly lanceolate, ca. 2.6 × 0.7 mm, and apices acuminate; disk with a thickened longitudinal channel extending from the base of the labellum almost to the sinus. Column slightly arcuate above the middle, 3.5 mm long; anther inclined towards the rostellum, ca. 0.8 mm; rostellum spreading forward.

Phenology. Flowers from July to August.

Etymology. The specific epithet “chawalongensis” refers to the type locality Chawalong (Cawarong) Township.

Distribution and habitat. At present, this new species is only found in Chawalong, Chayu, Tibet (Xizang), China. It is a predominantly terrestrial species growing on the scree slopes under the forest of *Abies* and *Picea* at an elevation



Figure 5. *Neottia chawalongensis* J.D.Ya & D.Z.Li, sp. nov. **A, B** plant **C** leaves **D** inflorescence **E** flower (front view) **F** adaxial sepals, petals and lip **G** abaxial sepals, petals and lip **H** column and lip **I** column **J** anther cap. Photographed by J.-D. Ya.

of 3757 m a.s.l. It appears to be locally abundant with other orchid species including *Ponerorchis chusua* (D. Don) Soó, *Galearis spathulate* (Lindl.) P. F. Hunt, *Cypripedium wardii* Rolfe, *C. bardolphianum* W.W.Sm. & Farrer and *C. flavum* P. F. Hunt & Summerh.

Additional specimen examined. *N. pinetorum*: INDIA. Sikkim, 10–11000 feet., J. D. Hooker 355 (holotype, K000974204!, isotype, AMES 00101020!); CHINA. Yunnan, upper Kiukiang valley, 2500 m, T.T.Yu 19644 (PE00027188!). *N. bambusetorum*: CHINA, Yunnan, Prope fines Tibeto-Birmanicas inter fluvios Lu-djiang (Salween) et Djiou-djiang (Irrawadi or. sup.), in jugi Tschiangschel, 27°52', lateris orientalis regione (frigide) temperata in bambusetis, 3275–3350 m, Hand.-Mazz.9238 (holorypus, WU0061594!)

Papilionanthe Schltr.

3. *Papilionanthe motuoensis* J.D.Ya & D.Z.Li, sp. nov.

urn:lsid:ipni.org:names:77331173-1

(墨脱凤蝶兰 Mo Tuo Feng Die Lan)

Figs 6, 7A–C

Diagnosis. *Papilionanthe motuoensis* is similar to *P. uniflora* (Lindl.) Garay but differs in having a glabrous pedicel and ovary (vs. glandular-pubescent). Its lateral petals are oblong-ovate (vs. oblong) with irregularly denticulate margins (vs. with undulating margins), truncate apices (vs. obtuse apices). Its labellum

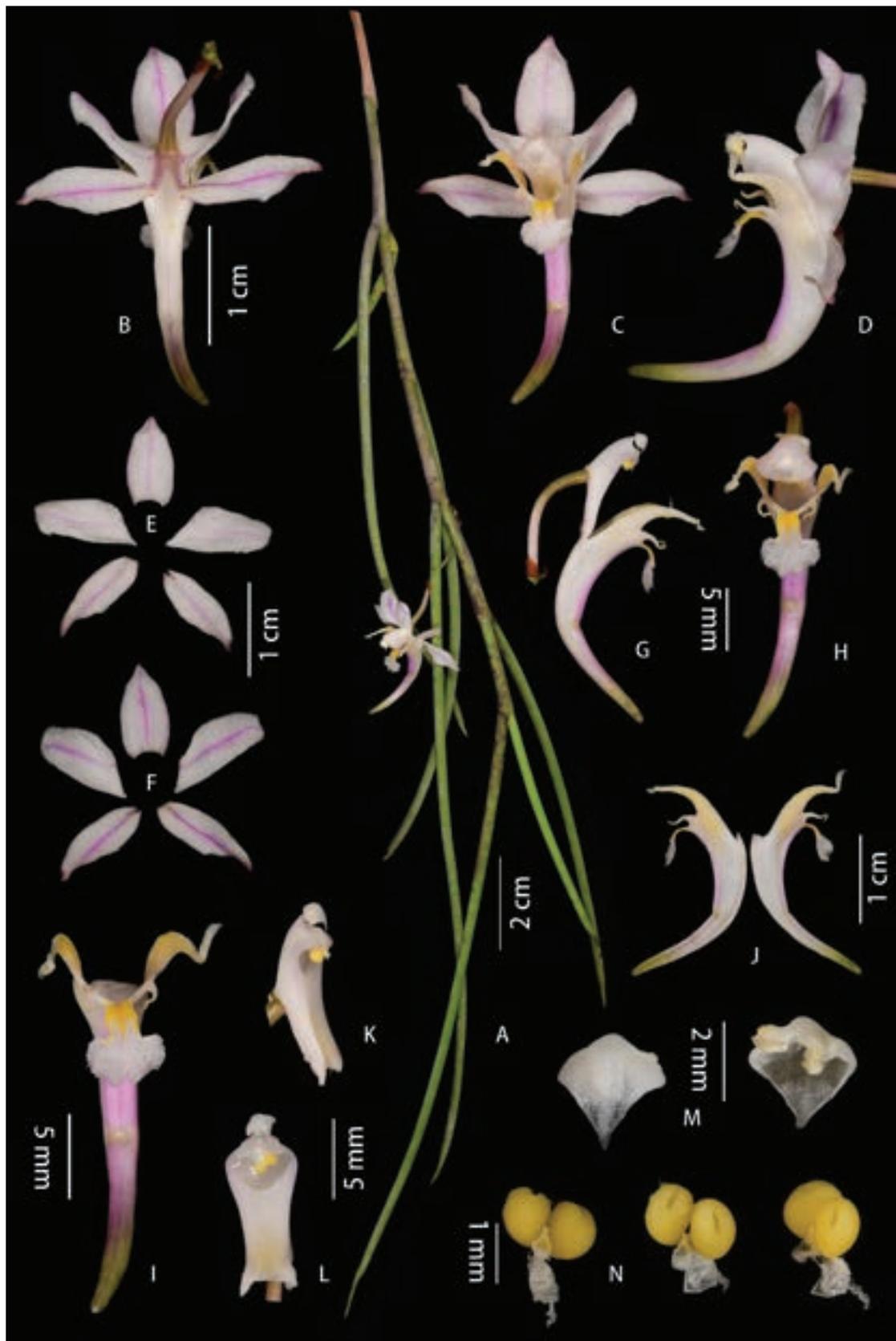


Figure 6. *Papilionanthe motuoensis* J.D.Ya & D.Z.Li, sp. nov. **A** plant **B** flower (dorsal view) **C** flower (front view) **D** flower (lateral view) **E** abaxial sepals and petals **F** adaxial sepals and petals **G** column and lip (lateral view) **H** column and lip (front view) **I** lip (front view) **J** lip (rip cutting) **K** column (lateral view) **L** column (front view) **M** anther cap **N** pollinarium. Photographed by J.-D. Ya.



Figure 7. A–C *Papilionanthe motuoensis* D–F *Papilionanthe uniflora* (Nepal). Photographed by: A–C J.-D. Ya D–F Bhakta Bahadur Raskoti.

is white tinged with yellow (vs. uniformly white), with a subflabellate mid-lobe and a labellum base with an apically dilate to reniform claw, its apex is emarginated with an irregularly denticulate margin (vs. mid-lobe simple, oblong, apex widely cuneate).

Type. CHINA. Yunnan, Kunming, voucher from cultivated plants at Kunming Institute of Botany, CAS, 20 Oct. 2020 (flowering), J.-D. Ya BC201015 (holotype: KUN!), plants originally collected from Tibet (Xizang), Linzhi City, Motuo County, 1625 m, at the edge of a subtropical, evergreen, broadleaved forest.

Description. Stems pendulous, terete, to 50 cm, 2.0 mm in diam. branched, enclosed in leaf sheaths. Leaves laxly alternate, terete, 9–16 × 0.2 cm, base with amplexicaul-sheathing, apex apiculate; sheaths tubular, 2.0–2.8 cm long, glabrous. Inflorescence ca. 1.5 cm, usually 1–2-flowered; peduncle slender, ca. 1.3 cm; floral bracts ovate-triangular, 1.5 × 1.2 mm. Flowers 2.5 cm in diam. sepals and lateral petals white, mid-vein pink, labellum white tinged with yellow, its spur with a whitish and/or pink tinge, apex yellowish green. Pedicel and ovary, ca. 1.2 cm long, glabrous. Dorsal sepal ovate, ca. 1.0 × 0.5 cm, acute, 5-veined; lateral sepals oblong, slightly falcate, ca. 1.1 × 0.4 cm, acuminate, 5-veined; lateral petals oblong-ovate, 1.1 × 0.6 cm, margin irregularly denticulate, apices truncate, 7-veined; labellum adnate to column foot, 3-lobed; lateral lobes deeply bifid, unequal, linear, acute, long lobule ca. 7.0 × 2.0 mm, short lobule ca. 4.0 × 1.0 mm; mid-lobe spreading, subflabellate, ca. 4.8 × 5.0 cm, base with a claw

ca. 2.2 × 2.0 mm, apical dilate to reniform, apex emarginate, margin irregularly denticulate; spur slightly curved forward, cylindrical, ca. 22.0 × 3.5 mm, narrowing towards the terminus, its interior pubescent. Column 7.0 × 3.0 mm, foot ca 5.2 mm, with narrowly and entire wings decurrent to foot; anther cap galeate with a acuminate apex, 2.0 × 2.5 mm; pollinia 2, subglobose, ca. 1.0 mm in diameter, waxy, porate, attached by a stipe to a broad cellular viscidium.

Phenology. Observed flowering in October.

Etymology. The specific epithet “*motuoensis*” refers to the type locality Motuo (Medog) County.

Additional specimens examined. Tibet (Xizang), Linzhi City, Motuo County, Bangxin Xiang, 1330 m, from subtropical, evergreen, broadleaved forest. Oct. 2019, M.-K. Li and W. Wang 2019343 (paratypes, Herbarium of Tibet Agricultural and Animal Husbandry University, No. 8 Xueyuan Road, Bayi District, Nyingchi, Tibet). *P. uniflora*: NEPAL. Gosain Than, N. Wallich no. 1993 (K001114863!); INDIA, Mao, C.B. Clarke 41790 (K000891405!)

Distribution and habitat.: The new epiphytic species was found only in Motuo County, Tibet (Xizang), China, growing on limbs in a subtropical, evergreen, broadleaved forest at elevations of 1300–1650 m.

***Gastrochilus* D.Don**

4. *Gastrochilus lihengiae* J.D.Ya, Ting Zhang & Z.D.Han, sp. nov.

urn:lsid:ipni.org:names:77331174-1

(纤细盆距兰 Xian Xi Pen Ju Lan)

Figs 8, 9

Diagnosis. The floral morphology of *Gastrochilus lihengiae* is similar to *G. distichus* (Lindl.) O. Kuntze and *G. prionophyllus* H. Jiang, D. P.Ye & Q. Liu, but can be distinguished from the former by its narrower leaves, blades 0.25–0.35 cm wide (vs. 0.4–0.6 cm), and distinctly serrate leaf margins (vs. entire), with acuminate and mucronate apices (vs. apex acute bearing 2 or 3 awns). The lateral petals are narrowly oblong (vs. subobovate). The labellum with a hypochile, ca 7.0 mm (vs. 4.0 mm). The outside of the hypochile with three ridges (vs. glabrous), and from the latter by its falcate-lanceolate (vs. ovate) leaves with mucronate apices (vs. apex with 2 unequally awns), the lateral petals are narrowly oblong (vs. subobovate), the central cushion on the epichile of the labellum is not thickened (vs. thickened), while the outer surface of its hypochile has three ridges (vs. glabrous).

Type. CHINA. Yunnan Province, Nujiang Prefecture, Gongshan County, Cikai Township, 1935 m, in the montane moist evergreen broad-leaved forest, 24 Apr. 2020, J.-D. Ya et al. 22CS21828 (Holotype: KUN! isotype: KUN!)

Description. Epiphytic herbs, stem pendulous, to 20 cm long, ca. 1.0–1.5 mm in diameter, slender, with 0.5–0.6 cm internodes, often branched with tiny red-purple spots. Leaves alternate, distichous, falcate-lanceolate, ca. 1.6–1.8 × 0.25–0.35 cm, the margin significantly serrate with an acuminate and mucronate apex. Inflorescences several, held opposite to nearly opposite the leaves, subumbellate, 1–3-flowered; peduncle 0.7–1.0 cm, slender, upper part enlarged, lower part with 2 cupular sheaths; floral bracts ovate, ca. 1.0 mm; pedicel and ovary 1.0–1.1 cm. Flowers yellow-green, with reddish brown spots.

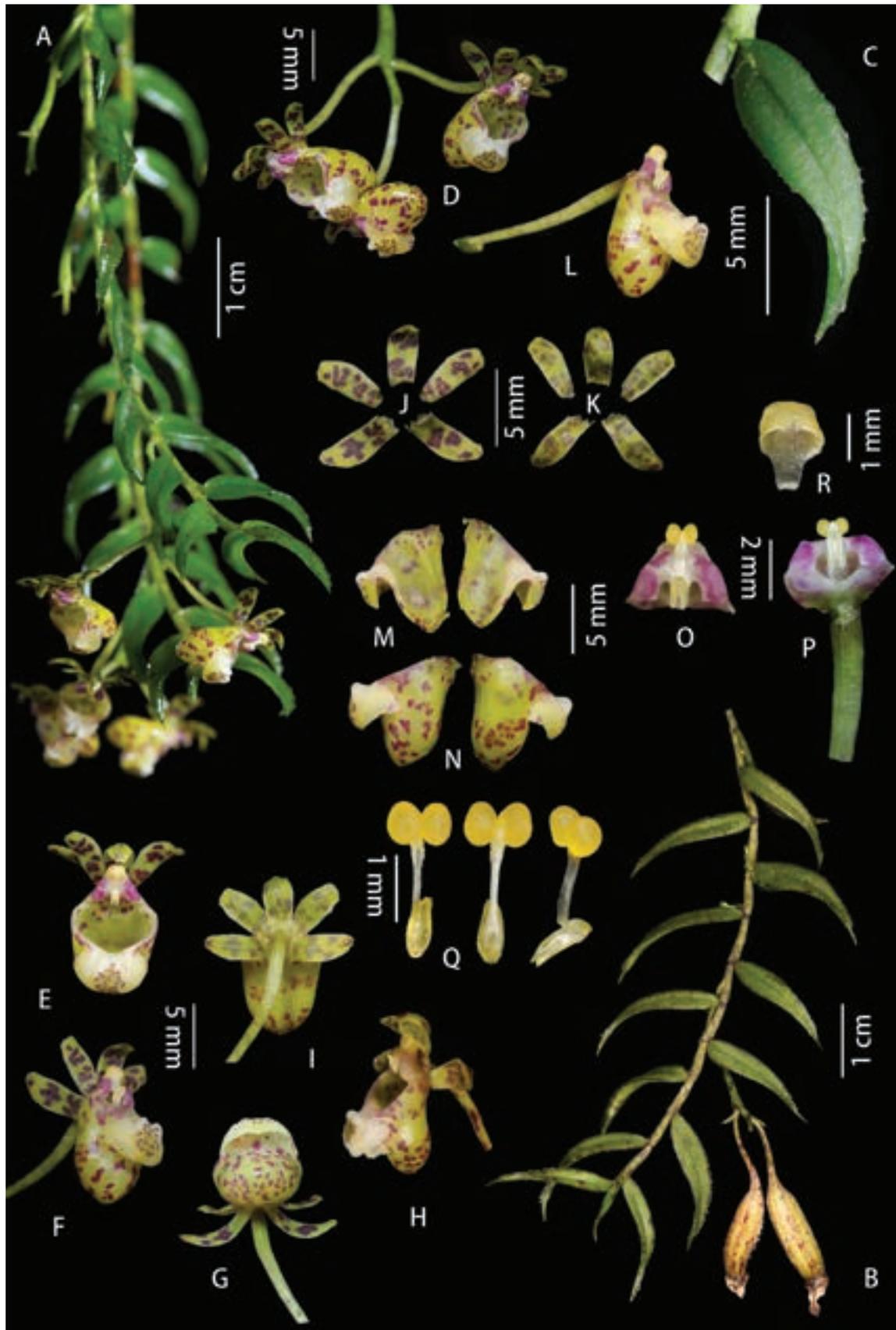


Figure 8. *Gastrochilus lihengiae* J.D.Ya, Ting Zhang & Z.D.Han, sp. nov. **A** flowering plant **B** fruiting plant; **C** Stem **C** leaf **D** inflorescence **E–I** flower (different view) **J** adaxial sepals and petals **K** abaxial sepals and petals **L** column and lip **M, N** lip (rip cutting) **O, P** column **Q** pollinarium **R** anther cap. Photographed by J.-D. Ya & Z.-D. Han.

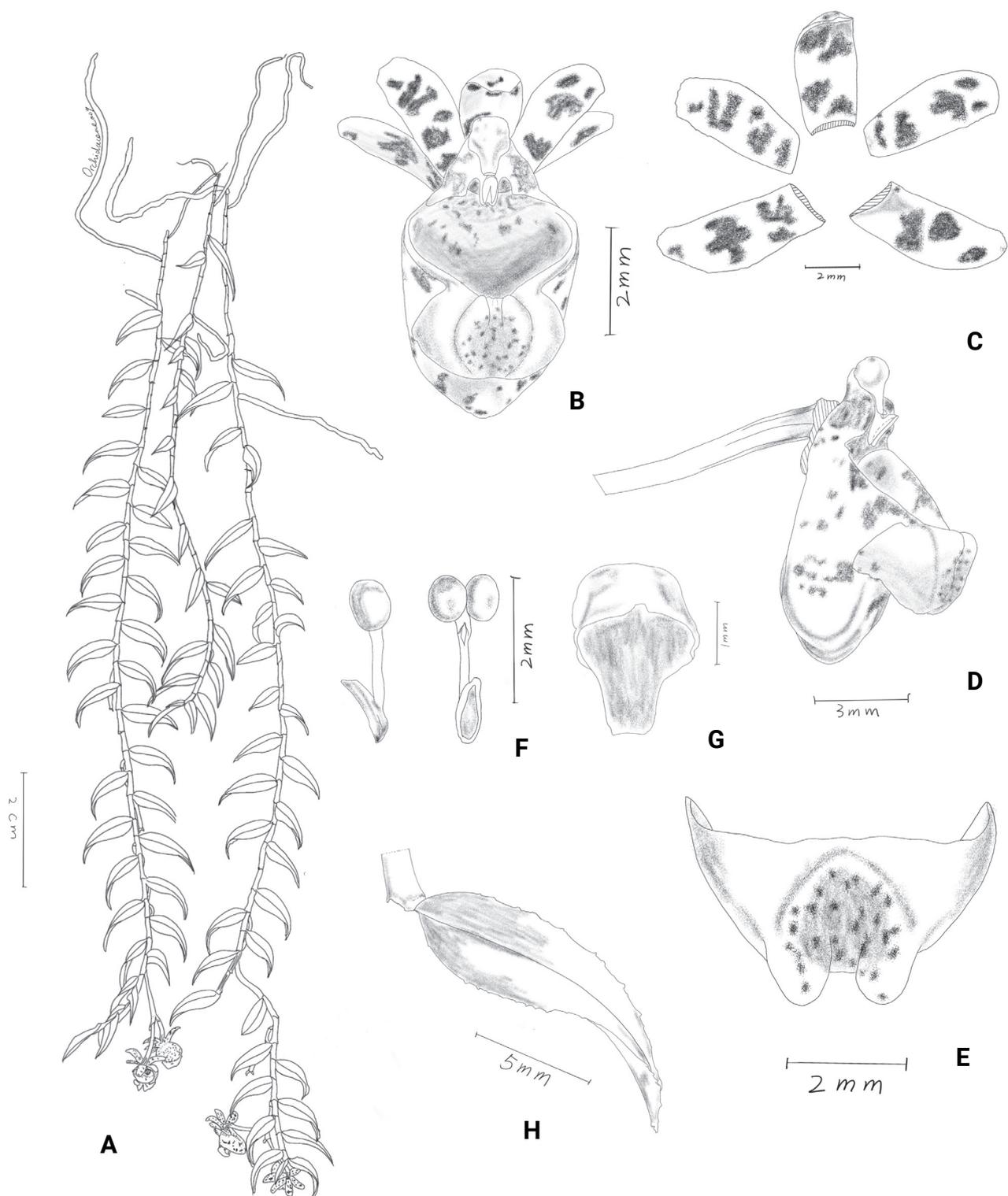


Figure 9. *Gastrochilus lihengiae* J.D.Ya, Ting Zhang & Z.D.Han, sp. nov. **A** plant **B** flower **C** sepals and petals **D** column and lip **E** epichile **F** pollinarium **G** anther cap **H** leaf. Drawn by Z.-D. Han.

Dorsal sepal concave, oblong-ovate, ca. 4.0×2.0 mm, apex obtuse; lateral sepals concave, narrowly oblong, ca. 5.5×1.8 mm, apex obtuse; lateral petals narrowly oblong, 4.0×1.8 mm, apices subtruncate. Labellum subdivided into an epichile and a saccate hypochile; the epichile subovate triangular, ca. 5.0×2.5 mm, adaxially glabrous, with a central cushion and 2 conic calli near its

base, the margin entire to irregularly denticulate, apex rounded; the hypochile subcupular, ca. 7.0 mm tall and ca. 5.2 mm in diam. outside with three ridges from the base of the column to its apex. Column stout, ca. 2.0 mm long, with rounded-auriculate wings at the base; anther cap narrowed into a beak towards its apex; the rostellum bilobed with an acuminate tip, and a horn-like awn arising from the center of each lobe; pollinarium ca 2.1 mm long; pollinia 2, yellow, 0.8 × 0.5 mm, almost hemispheric with a depression at the center; stipe elongate, obovate, ca.1.5 mm long; cellular viscidium elliptic, 1.0 × 0.5 mm. Capsules cylindrical, ca. 1.5 × 0.6 cm.

Phenology. Flowering from March to April, while the fruits matured in March in the following year.

Etymology. Named in honor of late Prof. Li Heng for her contributions to the orchid flora of Yunnan (Guo et al. 2023).

Distribution and habitat. At present, two populations of this new species were found in Gongshan County, Yunnan, China. It is epiphytic on tree trunks in the mixed evergreen broad-leaved forest or montane moist evergreen broad-leaved forest at an elevation of 1900–2100 m.

Additional specimens examined. CHINA. Yunnan Province, Nujiang Prefecture, Gongshan County, Dulongjiang Xiang, 2051 m, in the mixed evergreen broad-leaved forest, 4 Mar. 2023, Ting Zhang et al. 23CS24145 (paratypes, KUN!). *G. distichus*: INDIA. Skimm, J.D. Hooker 206. (holotype: K000873754!). *G. prionophyllus*: CHINA. Yunnan, Malipo County, Xia jinchang town, limestone forest, 1550–1650 m a.s.l., epiphytic on tree trunks or on rocks, 15 Mar. 2016, Qiang Liu 359 (holotype, HITBC!). *G. fargesii*: CHINA. Sichuan, Tschen-keou-tin, P.G. Farges 1236 (type, K00083803! isotype, AMES00271835!).

5. *Gastrochilus bernhardtianus* J.D.Ya & D.Z.Li, sp. nov.

urn:lsid:ipni.org:names:77331175-1

(丽江盆距兰 Li Jiang Pen Ju Lan)

Figs 10, 11, 12 A–C

Diagnosis. *Gastrochilus bernhardtianus* is similar to *G. affinis* (King & Pantl.) Schltr. in floral morphology, but can be distinguished by its shorter peduncle, ca. 0.3 cm (vs. 1.5–2.0 cm), pedicel and ovary ca. 4.5 mm (vs.0.6–1.3 cm). Sepals and lateral petals dark yellowish-green with densely purplish-red marks or spots flushed brown to purplish brown (vs. green flushed with brown to purplish brown). The dorsal sepal elliptic, ca 3.4 mm wide (vs. elliptic-oblong, 1.0–1.3 cm wide), lateral sepals narrowly ovate, ca. 5.5 × 2.8 mm (vs. elliptic-ovate, 3.5–4.0 × 0.7–1.3 mm). Lateral petals narrowly oblong, ca. 5.2 × 2.7 mm (vs. ovate-elliptic to elliptic, 3.0–4.0 × 1.0–1.3 cm). Labellum with purplish-red spots (vs. yellowish to greenish-yellow marks) and yellowish-green calli (vs. brown to purplish brown) with a transversely oblong epichile (vs. broadly subtriangular) and a green center (vs. deep purple to purplish-brown).

Type. CHINA. Yunnan Province: Lijiang Prefecture, Yulong County, Yunshanping, 3308 m, in cold-temperate, evergreen conifer forest, 20 May 2020, J.-D. Ya et al. 20CS19022 (Holotype: KUN!)

Description. Epiphytic herb, stem pendulous, with purplish spots, ca. 5.0 cm long, 1.5–2.0 mm in diameter. Leaves distichous, blade oblong-lanceolate, with

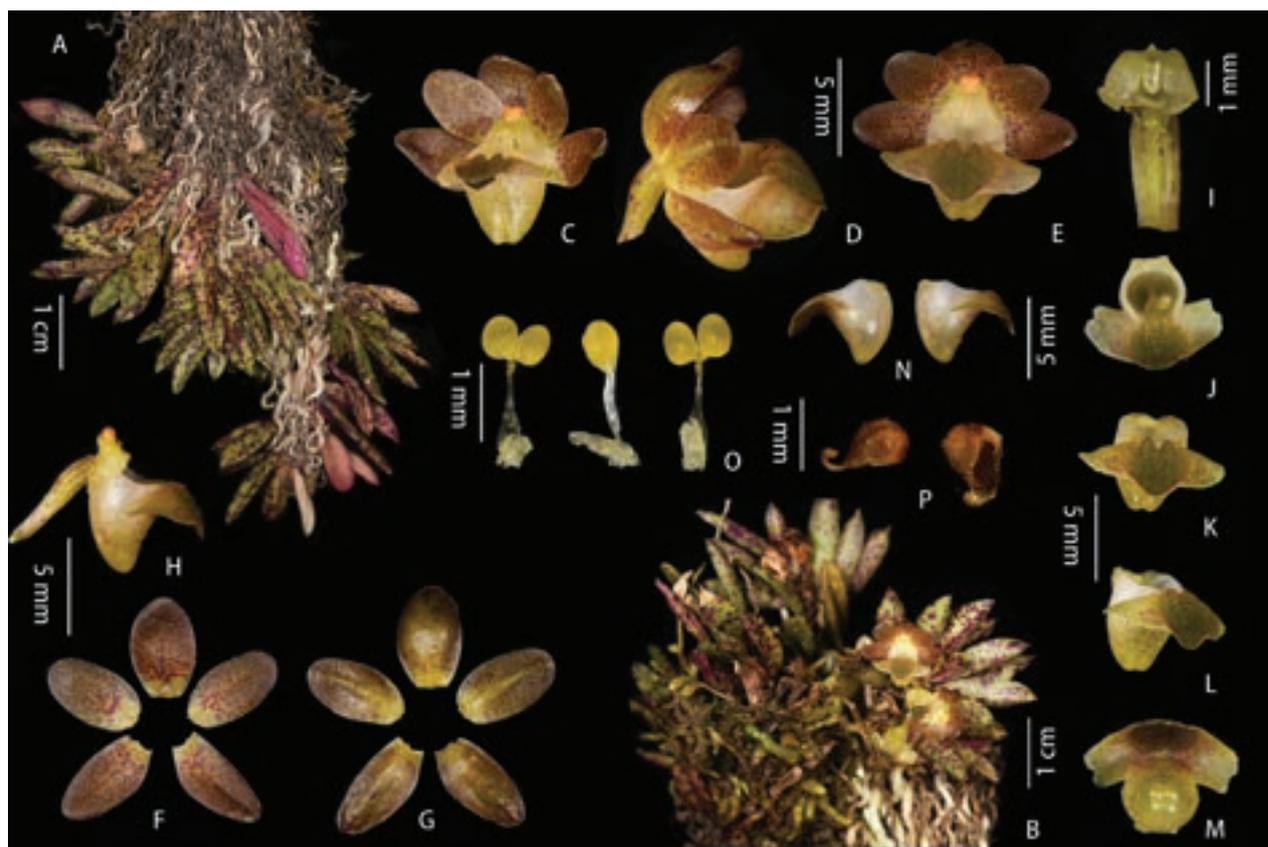


Figure 10. *Gastrochilus bernhardtianus* J.D.Ya & D.Z.Li, sp. nov. **A** plant **B** inflorescence **C–E** flower **F** adaxial sepals and petals **G** abaxial sepals and petals **H** column and lip **I** column **J–M** lip **N** lip (rip cutting) **O** pollinarium **P** anther cap. Photographed by J.-D. Ya.

purple-red spots on the abaxial leaf surface, 1.8–2.5 × 0.4–0.7 cm, base sheathing, apex acute and slightly trilobate. Racemes axillary, sub-umbellate, 1–2 flowered; peduncle ca. 0.3 cm, with purple-red spots; floral bracts ovate-triangular, ca. 1.0 mm; pedicel and ovary yellow-green with purple-red spots, ca. 4.5 mm. Flower densely marked with purplish-red spots flushed with brown to purplish brown, sepals and lateral petals dark yellowish-green. Dorsal sepal elliptical, ca. 5.2 × 3.4 mm, apex obtuse; lateral sepals narrowly ovate, ca. 5.5 × 2.8 mm, apices obtuse; lateral petals narrowly oblong, ca. 5.2 × 2.7 mm, apices obtuse. Labellum epichile with a green center and yellowish green margins, transversely oblong, ca. 8.0 × 2.8 mm, adaxially glabrous, with a central cushion and 2 conic calli near its base, margins erose, apex rounded; hypochile saccate, light yellowish green, subconical, ca. 5.1 mm tall and ca. 3.8 mm in diam. dorsally compressed, slightly bent outward, subacute to obtuse and shortly bifid at apex, with one internal ridge at the bottom. Column stout, ca. 2.0 mm, with rounded-auriculate wings at the base, anther cap galeate with recurved–acuminate apex, 1.2 × 0.9 mm; rostellum bilobed with an acuminate terminus; pollinarium ca 2.0 mm long; pollinia 2, yellow, 0.6 × 0.5 mm, almost hemispheric with a depression at the centre; stipe elongate, obovate, ca. 1.0 mm long; cellular viscidium elliptic, 0.8 × 0.3 mm.

Phenology. Flowering from May to June.

Etymology. The species is named after Peter Bernhardt, pollination biologist and orchidologist, for his contributions to pollination ecology of Chinese orchids in collaboration with botanists of China. Previously Professor of Biology at St.

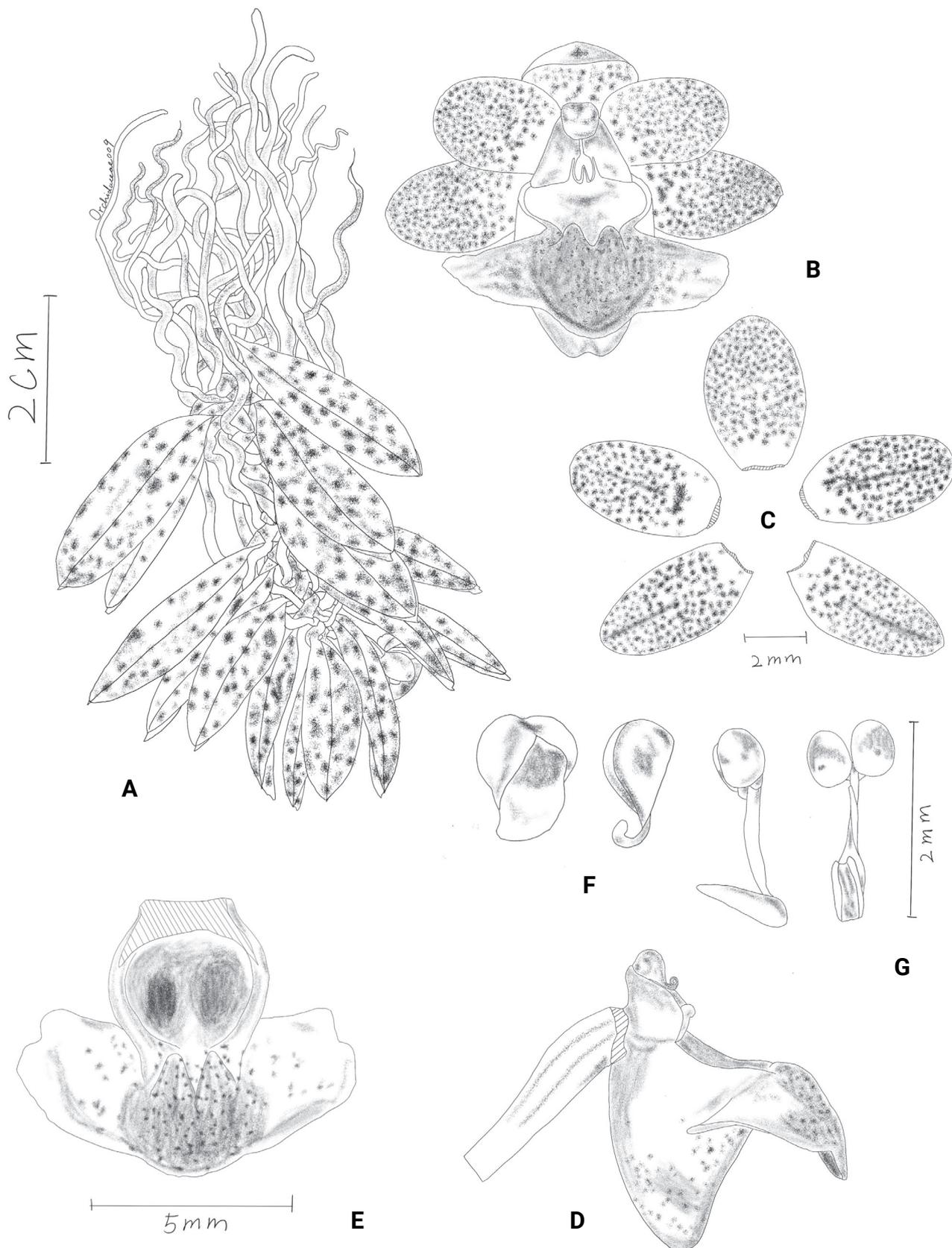


Figure 11. *Gastrochilus bernhardtianus* J.D.Ya & D.Z.Li, sp. nov. **A** plant **B** flower **C** sepals and petals **D** column and lip **E** lip **F** anther cap **G** pollinarium. Drawn by Z.-D. Han.



Figure 12. Comparison of two taxa of *Gastrochilus* **A–C** *G. bernhardtianus* with various colours in different areas **D, E** *G. affinis*. Photographed by J.-D. Ya.

Louis University, USA, Peter Bernhardt was the 2022 recipient of the Peter H. Raven Scientific Outreach Award (Raven 2023). Currently he works closely with colleagues in Yunnan as a research associate of the Missouri Botanical Garden, USA and as an adjunct professor at Curtin University, Perth, Western Australia.

Distribution and habitat. The new species is found only in Yulong County, Yunnan, China, and epiphytic on trees of the cold-temperate, evergreen needle-leaved forest dominated by *Picea likiangensis* (Franch.) E.Pritz. and *Abies forestii* Coltm.-Rog. at an elevation of 3300 m a.s.l.

Additional specimens examined. *G. affinis*: INDIA. Sikkim, Lachong Valey, R. Pantling 444 (K000891609!); CHINA. Yunnan, Fugong, Jiakedi, east slope of Gaoligongshan, epiphytic on trunk, alt., 2555 m, 16 May 2005, X. H. Jin 6984 (PE!); Yunnan, Tengchong, 2828 m, 31 Mar 2007, X.H. Jin 8936 (PE!). *G. alatus*: CHINA: Yunnan, Fugong, Zhuminglin, 2758 m, 16 May 2005, H.X. Jin 6998 (Holotype, PE!).

Acknowledgments

We are grateful to Prof. Xiao-hua Jin for his valuable discussions and suggestions, to Dr. Yan-Hui Zhao, Mr. Wei Zhang, Mr. Cheng Liu, Mr. Sheng-Ping Ming, Mr. Jin Li, and Mr. Chang-Hong Li for their kind assistance in the field, to Dr. Bhakta Bahadur Raskoti for his outstanding image. We convey our special thanks to the Gongshan Branch of the Gaoligong Mountains National Nature Reserve for their kind help in the field. This study was financially supported by the Science and Technology Basic Resources Investigation Program of China (grant No. 2021FY100200), the Key Basic Research Program of Yunnan Province, China (grant 202101BC070003), the National Wild Plant Germplasm Resource Center and a CAS Technology Talent Program to J. Cai, and National Forestry and Grassland Administration (grants 2019073017, 2019073019).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was financially supported by the Science and Technology Basic Resources Investigation Program of China (grant No. 2021FY100200), the Key Basic Research Program of Yunnan Province, China (grant 202101BC070003), the National Wild Plant Germplasm Resource Center and a CAS Technology Talent Program to J. Cai, and National Forestry and Grassland Administration (grants 2019073017, 2019073019).

Author contributions

J.D.Y. and D.Z.L. conceived of and designed the study. J.D.Y. J.H. Z.D.H. T.Z. and H.H. contributed plant materials. W.T.W. and Y.L.L. performed the plastome assembly, annotation, and phylogenetic analyses. J.D.Y. W.T.W. Y.L.L. and D.Z.L. wrote the manuscript. J.C. and D.Z.L. supported funding acquisition. All authors read and approved the final version of manuscript.

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Data availability

All relevant data are within the manuscript and its Additional files. The data that support the findings of this study are openly available in the Science Data Bank at <https://www.doi.org/10.57760/sciencedb.09591> or <http://resolve.pid21.cn/31253.11.sciencedb.09591>.

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Supplementary material 1

Voucher and GenBank accession numbers of *Neottia* samples.

Authors: Ji-Dong Ya, Wan-Ting Wang, Yun-Long Liu, Hong Jiang, Zhou-Dong Han, Ting Zhang, Hua Huang, Jie Cai, De-Zhu Li

Data type: xlsx

Explanation note: List of taxa and plant materials. “XXXX” indicates plastid sequences used in analysis without Genbank accession numbers. Alignment of sequences are available under the Science data bank.

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Link: <https://doi.org/10.3897/phytokeys.235.111230.suppl1>

Supplementary material 2

List of taxa, vouchers and GenBank accession numbers of *Papilionanthe* samples downloaded from NCBI (with newly sampled sequences added, below)

Authors: Ji-Dong Ya, Wan-Ting Wang, Yun-Long Liu, Hong Jiang, Zhou-Dong Han, Ting Zhang, Hua Huang, Jie Cai, De-Zhu Li

Data type: xlsx

Explanation note: List of taxa and plant materials. “XXXX” indicates plastid sequences used in analysis without Genbank accession numbers. Alignment of sequences are available under the Science data bank.

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Supplementary material 3

Summary of publicly available *Gastrochilus* plastomes sequences in this study

Authors: Ji-Dong Ya, Wan-Ting Wang, Yun-Long Liu, Hong Jiang, Zhou-Dong Han, Ting Zhang, Hua Huang, Jie Cai, De-Zhu Li

Data type: xlsx

Explanation note: List of taxa and plant materials. "XXXX" indicates plastid sequences used in analysis without Genbank accession numbers. Alignment of sequences are available under the Science data bank.

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Link: <https://doi.org/10.3897/phytokeys.235.111230.suppl3>

Supplementary material 4

Summary of publicly available *Gastrochilus* plastid sequences in this study

Authors: Ji-Dong Ya, Wan-Ting Wang, Yun-Long Liu, Hong Jiang, Zhou-Dong Han, Ting Zhang, Hua Huang, Jie Cai, De-Zhu Li

Data type: xlsx

Explanation note: List of taxa and plant materials. "XXXX" indicates plastid sequences used in analysis without Genbank accession numbers. Alignment of sequences are available under the Science data bank.

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Link: <https://doi.org/10.3897/phytokeys.235.111230.suppl4>

Curcuma maxwellii and *C. rubroaurantiaca* (Zingiberaceae, Zingiberoideae), two new species from Thailand

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Abstract

Curcuma maxwellii sp. nov. and *Curcuma rubroaurantiaca* sp. nov. (Zingiberaceae, Zingiberoideae, Zingibereae), two new red-orange-flowered species from Thailand, are described. They are compared to the morphologically closest species from the *Curcuma* subgen. *Ecomatae* and detailed descriptions, colour plates and information on their distribution, ecology, phenology and uses are provided. Preliminary IUCN conservation assessments for both of these species are proposed as Least Concern

Key words: *Curcuma flammae*, *Curcuma lindstromii*, *Curcuma rhomba*, gingers, subgenus *Ecomatae*, Least Concern, Zingibereae



Academic editor: Thomas Haevermans

Received: 20 August 2023

Accepted: 7 November 2023

Published: 20 November 2023

Citation: Leong-Škorničková J, Soonthornkalump S, Lindström AJ, Niwesrat S, Lim SQ, Suksathan P (2023) *Curcuma maxwellii* and *C. rubroaurantiaca* (Zingiberaceae, Zingiberoideae), two new species from Thailand. *PhytoKeys* 235: 237–248. <https://doi.org/10.3897/phytokeys.235.111400>

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Introduction

Curcuma L. (Zingiberaceae, Zingibereae) is one of the largest ginger genera widely distributed in South and Southeast Asia and South China, with a few species extending to northern Australia and the South Pacific (Záveská et al. 2012). The number of *Curcuma* species have been steadily rising in past 30 years from about 80 to the current estimate of more than 150 species (Leong-Škorničková et al. 2022). The genus is economically important with turmeric (*Curcuma longa* L.) being perhaps the best-known example, but very many other species are used as spices, condiments, vegetables, medicinal plants and several species are a prominent part of the tropical horticultural industry. The introduction about the genus and its subgenera was given in our recent works (Leong-Škorničková et al. 2015, 2020, 2021) and is, therefore, not repeated here.

Over 65 species from all three subgenera are present in Thailand (Leong-Škorničková et al. 2021), of which more than 20 were described in the last 10 years (e.g. Maknoi et al. (2011, 2019); Chen et al. (2015); Leong-Škorničková et al. (2017, 2020, 2021, 2022); Boonma and Saensouk (2019); Soonthornkalump

et al. (2020, 2021, 2022); Saensouk et al. (2021); Rakarcha et al. (2022); Ruchisansakun and Jenjittikul (2023)).

While working on the *Curcuma* account for the Flora of Thailand, we have realised that the herbarium material misidentified as *C. rhomba* Mood & K.Larsen or *Curcuma stenochila* Gagnep. in Thailand is heterogeneous. Although most material had similar shape of leaf blades and inflorescences and the flowers were mostly described as orange with red corolla lobes, the geographically distinct clusters, differences in plant indumentum and evidence from an existing photographic material confirmed the need to re-collect living flowering material from different parts of Thailand to make further conclusions on the taxonomic treatment of the taxa involved. Our work on this complex of species with bright orange flowers with red corolla lobes already led to description of *Curcuma lindstromii* from Chanthaburi and designating the lectotype of *C. stenochila* (Leong-Škorničková et al. 2022). Additional targeted fieldwork was done in 2023 in the following Provinces: Chiang Rai, Sakon Nakhon, Loei, Chayaphum and Phetchabun. The newly-re-collected material confirmed our suspicion that additional two taxa should be recognised and these are, therefore, described below as *Curcuma maxwellii* and *C. rubroaurantiaca*.

Although it remains unclear if *Curcuma stenochila* occurs in Thailand at all, it is clear that both of the two species described here are sufficiently distinct from it as elaborated in notes under each of the two species.

Material and methods

The description of these new species is based on measurements from living flowering material and examination of herbarium specimens including flowers preserved in spirit. All extant herbarium material of *Curcuma* was examined at AAU, BK, BKF, BM, CMU, E, K, L, P, PSU, QBG and SING. The style of description follows the recent works of Leong-Škorničková et al. (2013, 2014, 2017, 2020, 2021, 2022). The general plant terminology follows Beentje (2016). The preliminary conservation assessments are based on the guidelines of the IUCN (2022).

Taxonomic treatment

***Curcuma (Ecomatae) maxwellii* Škorničk. & Suksathan, sp. nov.**

urn:lsid:ipni.org:names:77331206-1

Figs 1–3

Diagnosis. Similar to *Curcuma rhomba* Mood & K.Larsen in general habit and flower colour, but differs by bracts green to green with slight reddish tinge, puberulent on both sides (versus solid dark red glossy bracts, glabrous on both sides), bracteoles present (vs. bracteoles absent), calyx puberulent throughout (vs. calyx glabrous, except few hairs on teeth), anther with 2–2.5 mm long narrowly conical spurs (vs. ca. 1 mm short broadly conical spurs with blunt apices).

Type. THAILAND, Chiang Rai Province, Chiang Khong District, Rim Khong Sub-district, 519 m a.s.l., 3 August 2023, Suksathan et al. JLS-4210 (**Holotype:** QBG! (including flowers in spirit as part of a single preparation); **Isotypes:** BKF!, E!, P!, SING! (BKF! & SING! including flowers in spirit as part of a single preparation)) Fig. 1.

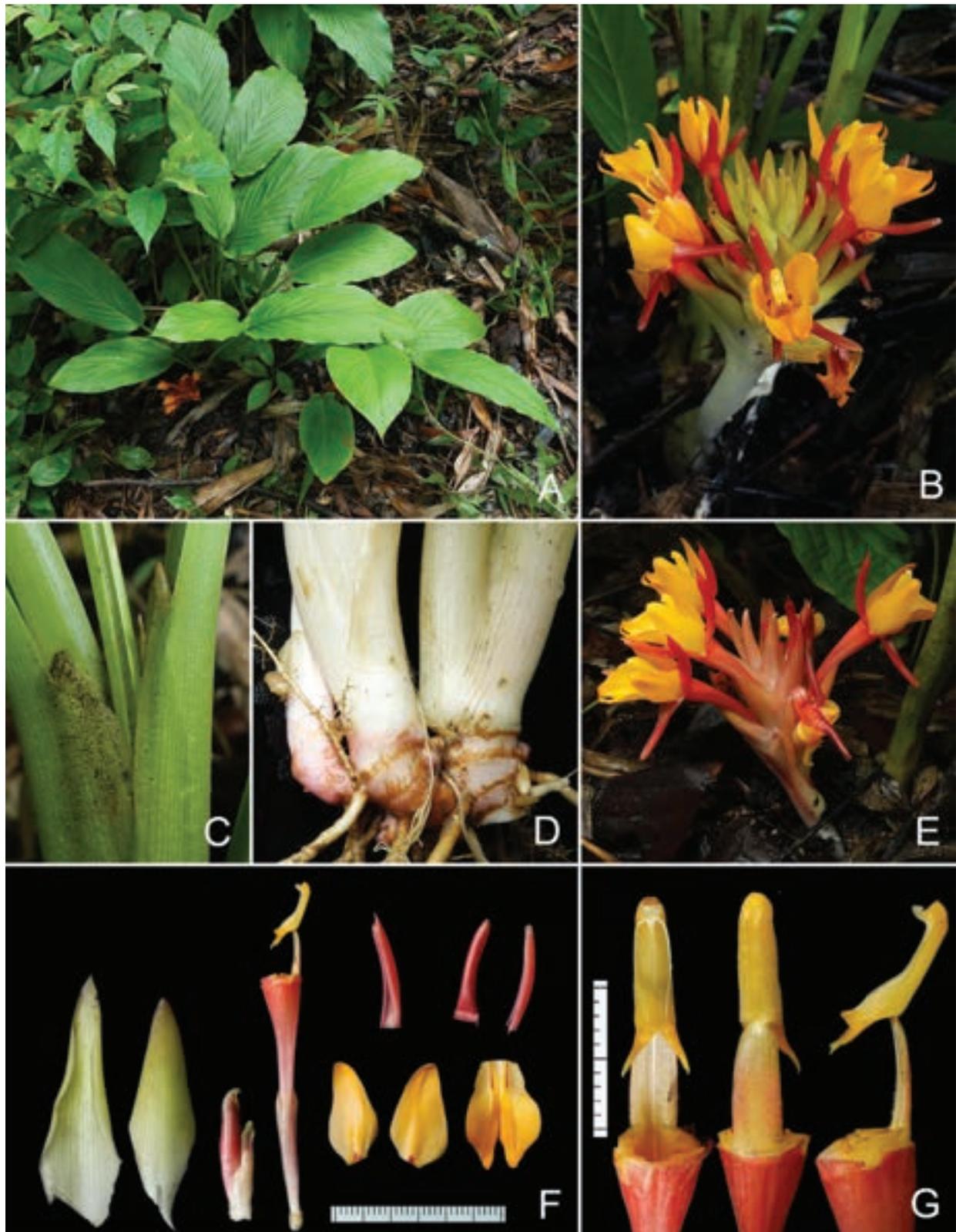


Figure 1. *Curcuma maxwellii* Škorničk. & Suksathan at the type locality **A** habit **B** inflorescence (top view) with detail of flower in front view **C** detail of leaf sheaths and ligules **D** rhizome **E** inflorescence with detail of flower in side view **F** two fertile bracts, three flower buds from the same cincinnus as the dissected flower, dissected flower (from left: floral tube with ovary, calyx and stamen attached, upper row dorsal and lateral corolla lobes, lower row lateral staminodes and labellum **G** stamen still attached to floral tube from front, back and side view. All from the type collection, *Suksathan et al. JLS-4210*. Photographed by Jana Leong-Škorničková.

Description. Rhizomatous herb to 0.8 m tall; **rhizome** ovoid, ca. 1–1.5 by 0.8–2 cm, with occasional thin branches ca. 4–5 mm diam., brown externally, yellow internally, aromatic with bitter smell; **root tubers** elliptic, ca. 2.5 by 1.1 cm, light brown externally, pure white internally with translucent white centre. **Leafy shoot** 95 cm long with 3–6 leaves when flowering; pseudostem 9–30 cm long, composed of leaf sheaths; **bladeless sheaths** decayed at anthesis; **leaf sheaths** white-green or with pale pink tinge at base turning green distally, glabrous, but pubescent towards the margins; ligule 4–6 mm long, bilobed, lobes round, hyaline, greenish-white, semi-translucent green, hairy; **petiole** 4.5–26 cm long (petiole of first leaf shortest, innermost leaves longer), canaliculate, green, glabrous; **leaf blade** elliptic to elliptic-ovate, 16–44 × 6.5–14 cm, prominently plicate, adaxially bright green, shortly hairy along main veins, abaxially lighter green, glabrous, mid-rib glabrous, green base cordate, apex acuminate, tip ca. 15–20 mm long, pubescent. **Inflorescence** central (often breaking through the pseudostem), many-flowered; **peduncle** 5–12 cm long, to 8 mm diam., white or with reddish tinge; **thyrses** 5–7.5 cm long, 4–6 cm diam. in the middle, without coma; **fertile bracts** 15–34 per inflorescence, 4–4.5 × 1.8–2.7 cm (larger at the base of the inflorescence), ovate to narrowly ovate, smaller at the apex, light green, sometimes with light reddish tinge throughout the bract, puberulent on both sides (slightly less so on the inside) connate in the lower 1/3; enclosing **cincinni** with 4 flowers at the base of the inflorescence, the number of flowers per bract gradually decreasing upwards; **bracteoles** small ca. 1–2 × 0.5–1 mm (outer ones larger, inner ones gradually smaller or totally absent), hyaline, translucent white, glabrous. **Flowers** 6–7 cm, much exserted from the bracts; **calyx** to 22 mm long, 3-toothed, unilaterally split 8–10 mm, semi-translucent white with pink tinge, distally cream to greenish, puberulent; **floral tube** ca. 4.5 cm long, externally pink at the base, gradually redder distally, pubescent, internally light orange, glabrous in basal and distal 1/3, pubescent in middle 1/3, with dorsally placed groove holding the style; **dorsal corolla lobe** 19–21 × 8–10 mm, triangular ovate, with sides rolled inwards, red outside, light orange inside, glabrous on both sides, apex mucronate, mucro ca. 2 mm; **lateral corolla lobes** 19–20 × 7–8 mm, narrowly triangular ovate with sides rolled inwards, glabrous, red on outside, light orange on inside; **labellum** ca. 20 mm long, 5–7 mm broad at basal 5 mm, then broadly ovate, 15–16 mm at widest point, apex bifid with an incision to 7 mm long, labellum orange with darker median bordered by maroon line at basal 4–5 mm; **lateral staminodes** 16–20 × 8–11 mm, narrowly ovate to bluntly rhomboid, orange with small triangular maroon patch at base (ca. 2 mm), glabrous on both sides. **Stamen** 16–18 mm long; **filament** 7–8 mm long, 3.5 mm broad at base, 2 mm broad at apex (the point of attachment to the connective), orange with reddish tinge dorsally, dorsally covered with glandular hairs; **anther** 13–14 mm long, spurred, connective orange, densely covered with short glandular hairs; **anther spurs** 2–2.5 mm long, narrowly triangular with sharp tips pointing outwards; **anther crest** thick, rounded, ca. 1 mm long and ca. 1.5 mm broad at base, orange; **anther thecae** 8–9 mm, forming narrowly obovate shape, dehiscing along their entire length, pollen white. **Epigynous glands** 2, ca. 3 mm long, ca. 0.6 mm diam., cream white. **Style** thin, white, glabrous, placed in a groove in dorsal side of floral tube; stigma ca. 1 mm long, 1 mm wide, white, ostiole ciliate, facing upwards. **Ovary** 2–3 × 2 mm, trilobular, densely hairy, hairs ca. 1 mm long. **Fruits** subglobose, ca. 10 mm in diam. (almost ripe), cream white

with very slight pink tinge, pubescent; **seeds** few per capsule (6–10), ca. 4 mm long (almost ripe), light brown, enclosed in semi-translucent white lacinate aril.

Habitat and phenology. Growing in semi-shade to shaded moist area, near streams, in mixed deciduous forest or primary evergreen hardwood forest, at 400–900 m a.s.l., on granite bedrock. The species flowers from June till September, with fruiting presumably extending into November.

Distribution. Only known from Chiang Mai and Chiang Rai Provinces, N Thailand.

Eponymy. We name this species after our late colleague and remarkable botanist James Franklin Maxwell (1945–2015), also known simply as Max, who collected this species in 1992 (Fig. 2). With more than 32,000 high quality collections, rich in flowers and/or fruits and carefully prepared with many duplicates and mostly with labels that contain much information, Max ranks amongst the best collectors of Thai plants (van Welzen 2023).

Vernacular name and uses. As the vernacular name Wan Pet Ma (ว่านเพชรมา) is used on several orange-flowered species with red corolla lobes including this species, we propose to use Wan Pet Ma Lanna (ว่านเพชรมาล้านนา) for this species. Based on the information from the local herbal specialist of the Hmong community, this species, which is locally abundant, has no medicinal uses and only has potential as an ornamental plant.

Provisional IUCN conservation assessment. During our extensive revision of all Thai *Curcuma* material in numerous herbaria (as listed in the Introduction), we have found an additional three herbarium collections, which could be confidently assigned to this species. We predict that the main threats to this species might include excessive collection from the habitat for horticultural purposes and trade, as well as conversion of unprotected areas into agricultural lands. However, the species tend to be locally abundant and at least one of the locations (Lam Nam Kok National Park, Khun Korn Waterfall) is in the legally-protected area. We, therefore, propose to treat this species as Least Concern (LC).

Specimens examined. Paratypes: THAILAND, Chiang Mai Province, along the road Fang to Chiang Mai; 27 July 1968; *Larsen, K., Santisuk, T. & Warncke, E.* 2766; AAU, BKF; Chiang Rai Province, Mueng District, Koon Gohn Falls [Khun Kon Waterfall], 900 m a.s.l., 20 August 1992, *Maxwell, J.F.* 92-440 (AAU, KUN, CMU); Khun Korn Fall [Khun Kon Waterfall], 680 m a.s.l., 22 June 2002, *Chamchumroon, V., Suphuntee, N., Koonkhunthod, N., Ngernsaengsaruy, C. & Tetsana, N.* 1601 (BKF, 2 sheets); Doi Tung, 26 Sep 1967, *Iwatsuki, K., Fukoka, N., Hutoh, M. & Chaiglom, D.* 13271 (BKF).

Notes. As already pointed out by Luru et al. (2017), all the material seen labelled as *C. rhomba* from Thailand in Mood and Larsen (2001) is distinct from the material from southern Vietnam and, in fact, represents several species. The specimen *Larsen & al.* 2766 represents *C. maxwellii* and is cited here amongst the paratypes. For this reason, we have compared *Curcuma maxwellii* to *C. rhomba* in the diagnosis. In northern Thailand, *C. maxwellii* might be confused with *Curcuma bicolor*, which also has red-orange flowers and is also known to occur in Chiang Mai and Mae Hong Son Provinces. The two species are easy to recognise when flowering as the flowers of *Curcuma bicolor* are much more open and the basal half of the staminodes is dark red (Fig. 3).

Outside of Thailand, *Curcuma maxwellii* is also similar to *Curcuma flammea* Škorničk. described from Laos, by general habit and shape of the inflorescence, but differs by adaxially glabrous leaf blades (vs. shortly puberulent), bracts light



Figure 2. James Franklin Maxwell in CMU Herbarium in 2013. Photographed by J. Leong-Škorničková.



Figure 3. Comparison of flower in front view of **A** *Curcuma bicolor* and **B** *C. maxwellii*. Photographed by Jana Leong-Škorničková.

green with more or less reddish tinge (vs. bracts white, pink to dark red), label-
lum without prominent basal claw, orange throughout with two thin red lines
bordering median band at base (vs. prominently violin-shaped labellum with a
prominent broad claw, bright orange with rich red shading and ornamentation),
staminodes orange with a small maroon triangular spot at base (vs. staminode
mostly bright red with distal part orange), anther with 2–2.5 mm long spurs, not
producing mucilage (vs. 3–4 mm long, producing a mucilage in *C. flammea*).

Curcuma maxwellii is distinct from *C. stenochila* by the shape of the labellum,
which has a narrow basal claw in *C. stenochila*, similar to that of *C. lindstromii*.

***Curcuma (Ecomatae) rubroaurantiaca* Škorničk. & Soonthornk., sp. nov.**

urn:lsid:ipni.org:names:77331207-1

Fig. 4

Diagnosis. Similar to *Curcuma maxwellii* by general habit and flower colour, but differs by leaf blades abaxially densely puberulent (vs. glabrous), inflorescence composed of up to 14 fertile bracts (vs. inflorescences composed of 15–34 bracts), bracteoles absent (vs. small bracteoles present), larger stamen 19–22 mm long (vs. 16–18 mm long), anther 16–17 mm long with flattened spurs, prominent anther crest 2–3 mm long with central longitudinal groove and anther thecae forming narrowly rhomboid shape (vs. anther 13–14 mm long with conical spurs, thick anther crest ca. 1 mm long without central longitudinal groove and anther thecae forming narrowly obovate shape).

Type. THAILAND, Sakon Nakhon Province, Phanna Nikhom District, Na Hua Bo subdistrict, ca. 200 m a.s.l., 15 July 2023, *Soonthornkalump Sutt-242* (**Holotype:** BKF! (including flowers in spirit as part of a single preparation); **Isotype:** SING! (including flowers in spirit as part of a single preparation)). Fig. 4.

Description. Rhizomatous herb to 0.6 m tall. **Rhizome** branched, main rhizome ovoid to obvoid, 1.5–2 × 1–2 cm, branches 1–4 cm long, up to 1.2 cm diam., cream-white to ochraceous externally, cream-white internally, slightly aromatic; **root tubers** globose to fusiform 2–4 × 1–2 cm. **Leafy shoot** with 2–5 leaves when flowering; pseudostem to ca. 20 cm long, composed of leaf sheaths; **bladeless sheaths** ca. 3–4, cream-white at base, tinged with rich brownish-red to red distally, fully decayed at anthesis; **leaf sheaths** brownish-red at base, gradually more green with reddish tinge, densely puberulent to puberulent; **ligule** 3–4 mm long, bilobed, lobes rounded to obtuse, brownish-red, densely puberulent; **petiole** (11–)18–30(–45) cm long (petiole of first leaf shortest, innermost leaves longer), canaliculate, green with brownish-red tinge (rarely plain green in distal part), puberulent to densely puberulent, less so in the groove; **leaf blade** broadly elliptic to elliptic-ovate, (12.5–)19–40 × (5–)9–17 cm, prominently plicate, adaxially green, pubescent on main raised veins, near mid-rib and margins, sometimes throughout entire leaf blade, abaxially lighter green, densely puberulent, mid-rib green, puberulent on both sides, base cordate, often slightly oblique, apex acute to acuminate. **Inflorescence** central, breaking through the pseudostem, many-flowered; **peduncle** to 7 mm diam., embedded within pseudostem; **thyrses** 4–8.5 cm long, 3–6 cm diam. in the middle, without coma; **fertile bracts** to 14 per inflorescence, 2–5 × 1–3.6 cm (largest at the base of the inflorescence), ovate to trullate with acute apex, gradually smaller and more ovate towards the apex, margin curved inwards, various colours from white to bright red, shortly puberulent to pubescent on both sides (longer, but sparser hair on the inside), connate in the lower 1/3 to 1/4, enclosing **cincinnus** with 4 flowers at the base of the inflorescence, the number of flowers per bract gradually decreasing to 2 or 1 upwards; bracteoles absent. **Flowers** (5–)6–7.4 cm long, much exerted from the bracts; **calyx** to 24 mm long, 3-toothed, unilaterally split 8–12 mm, semi-translucent with pink-red tinge, pubescent; **floral tube** (30–)40–48 mm long, externally white to pale yellow at the base, gradually with red tinge distally, sparsely shortly pubescent in the middle third, otherwise glabrous, internally pale yellow at the base, bright yellowish-orange distally, mostly glabrous, but pubescent at the point where

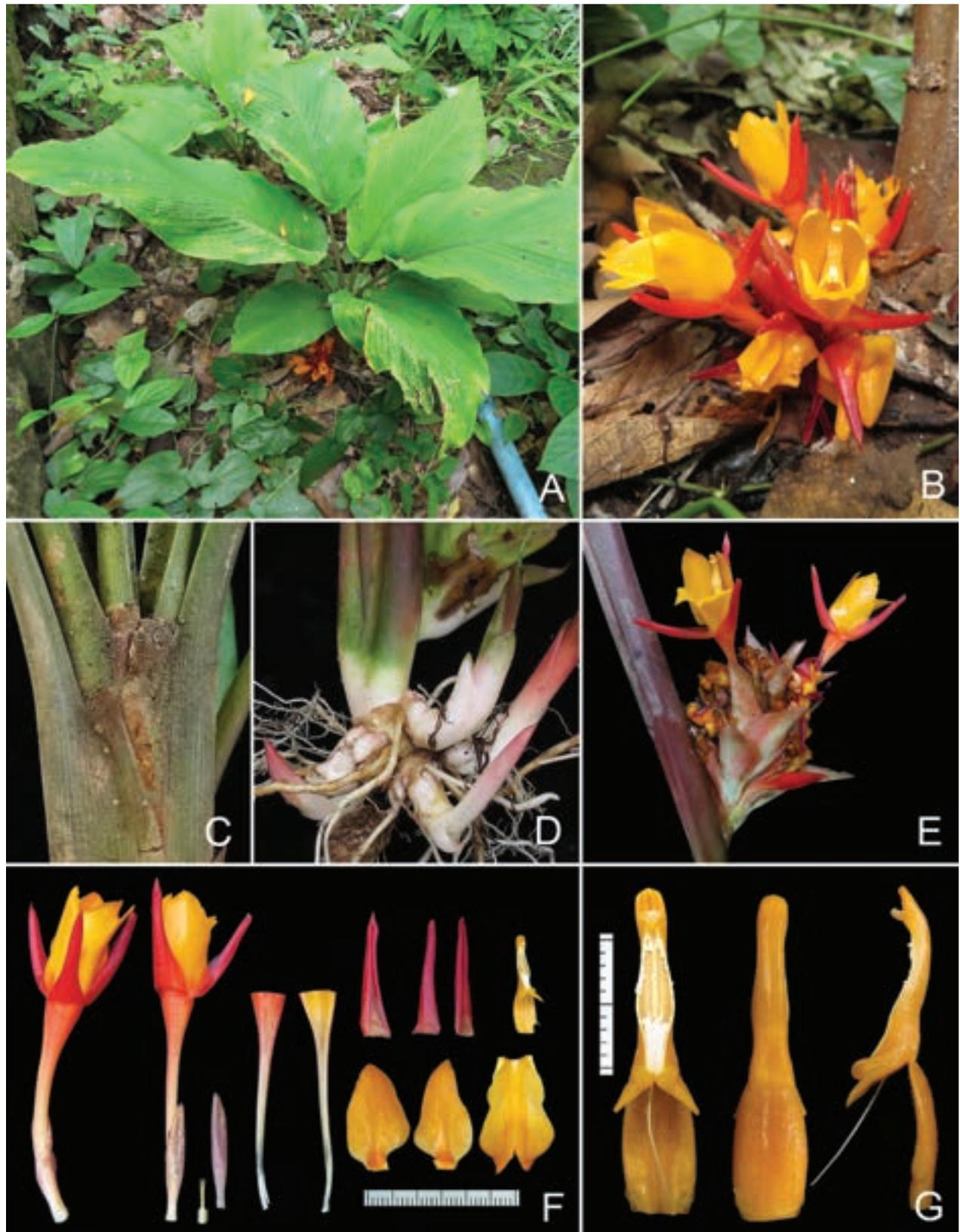


Figure 4. *Curcuma rubroaurantiaca* Škorničk. & Soonthornk. at the type locality **A** habit **B** inflorescence with detail of flower in front view **C** detail of leaf sheaths and ligules **D** rhizome **E** inflorescence with detail of flower in side view **F** two flowers and dissected flower (from left: ovary with epigynous glands, calyx, floral tube (dissected longitudinally), upper row dorsal and lateral corolla lobes and stamen, lower row lateral staminodes and labellum **G** stamen from front, back and side view. All from the type collection, *Soonthornkalump Sutt-242*. Photographed by Sutthinut Soonthornkalump.

the tube widens, with dorsally placed groove holding the style; **dorsal corolla lobe** 20–27 × 6–8 mm, triangular elliptic-oblong, with sides rolled inwards, red outside, light red to pale orange inside, glabrous on both sides, apex mucronate, mucro 2–3 mm, with a few sparse hairs; **lateral corolla lobes** 22–25 × 5–8 mm, triangular elliptic with hooded blunt apex, glabrous, red outside, light red to pale orange inside; **labellum** (20–)24–26 × 13–17 mm, 7–9 mm broad at basal 1/3, then bluntly rhomboid in distal 2/3, 13–17 mm at widest point, apex bifid with an incision 5–8 mm long, yellowish-orange, median band thick with central groove, darker orange, sides of the lamina yellow-orange, glabrous and shiny on both sides; **lateral staminodes** 20–22 × 11–13 mm, unequally bluntly rhomboid, bright orange, with pale reddish tinge at base, glabrous and somewhat shiny on both surfaces. **Stamen** ca. 19–22 mm long; **filament** 7–10 mm long, 3.5–4 mm broad at base, 1.5–2 mm broad at apex (the point of attachment to the connective), orange, sparsely covered with glandular hairs; **anther** 16–17 mm long, spurred, connective yellowish-orange, densely covered with short glandular hairs (especially dorsally, less so on the sides); **anther spurs** 2–3 mm long, triangular and flattened with sharp tips pointing outwards; **anther crest** thick ovate to oblong with rounded apex and central longitudinal groove, 1.5–3 mm long and 1.8–2 mm at base, darker orange; **anther thecae** 9–11 mm long, forming very narrowly rhomboid shape, dehiscing along their whole length, connective tissue at the base of the thecae forming blunt knob protruding forward; pollen white. **Epigynous glands** 2, 6–9 mm long, 0.5–1 mm in diam., apex acute, cream at base, yellowish distally. **Style** thin, white, glabrous, held in groove in dorsal side of floral tube; stigma ca. 1 mm long, 1–1.5 mm wide, cream to yellowish, ostiole ciliate, facing upwards. **Ovary** 2.5–3.5 × 2–2.5 mm, trilobular, cream, pubescent, hairs ca. 1 mm long. **Fruit** and **seeds** not seen.

Habitat and phenology. Growing in semi-shade and edges of evergreen forest mixed with bamboo, in moist places, at 200–800 m a.s.l., on sandstone as well as limestone bedrocks. The species flowers from July till September, with fruiting presumably extending into November.

Distribution. Known to occur in Loei, Sakon Nakhon, (NE Thailand), Chayaphum (E Thailand) and Phetchabun Provinces (N Thailand).

Etymology. The specific epithet refers to its bright red and orange flowers.

Vernacular name and uses. Similarly to the previous species, the vernacular name Wan Pet Ma (ว่านเพชรม้า) is used also on this species; we, therefore, propose refining the vernacular name to Wan Pet Ma Isan (ว่านเพชรม้าอีสาน) for this species. No uses were reported, but the species has a good potential as an ornamental plant.

Provisional IUCN conservation assessment. In addition to our collection, we have found an additional two herbarium collections from Loei Province (Phu Luang), two collections from Chayaphum Province (Chulabhorn Dam and Phu Kiew) and two specimens from Phetchabun, which could be confidently assigned to this species. Most of these areas are in National Parks under legal protection. The species was also sighted by us in an additional two legally-protected areas, namely Phu Pha Lom Forest Park (Loei Province) and Phu Phan National Park (Sakon Nakhon Province). We, therefore, propose to treat this species as Least Concern (LC).

Specimens examined. Paratypes: THAILAND, Loei Province, Phu Luang, s.d., *Bunchuai*, K. s.n. (BKF); Phu Luang, 1 September 1966, *Phusomsaeng*, S. 26 (BKF);

Mueang District, Wang Kan subdistrict, 13 October 2022, *Soonthornkalump*, S. Sutt 243 (QBG, including flowers in spirit as part of a single specimen).

Notes. Amongst the orange-red flowered species from this alliance in Thailand, this species is easy to recognise by its anther with well-developed and sometimes slightly recurved anther crest (observed, in particular, in populations in Loei), and thecae forming very narrowly rhomboid shape. This species can be recognised from *C. stenochila* by densely puberulent leaf blades and by the shape of the labellum, which does not have a narrow basal claw as in *C. stenochila*.

Key to red-orange flowered species of *Curcuma* subgen. *Ecomatae* occurring in Thailand

- 1 Lateral staminodes dark red in basal half ***C. bicolor***
- Lateral staminodes orange throughout, with small red to dark maroon patch either at the base or the tip **2**
- 2 Labellum with narrow basal claw and dark maroon almost black tips
..... ***C. lindstromii***
- Labellum with broad or no basal claw, tips of the same colour (orange) as the rest of the labellum **3**
- 3 Leaf blades densely puberulent abaxially, anther with prominent anther crest 2–3 mm long with central longitudinal groove and anther thecae forming narrowly rhomboid shape ***C. rubroaurantiaca***
- Leaf blades glabrous abaxially, anther with thick anther crest ca. 1 mm long without central longitudinal groove and anther thecae forming narrowly obovate shape ***C. maxwellii***

Acknowledgements

We thank curators of the following herbaria for allowing us to examine and photograph all *Curcuma* specimens in their care: AAU, BK, BKF, BM, CMU, E, K, L, P, PSU, QBG and SING. Research of JL-Š, and SQL is supported by the National Parks Board, Singapore. SS thanks the Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, for providing facilities and other support. We thank Dr. Varangrat Nguanchoo for sharing with us locality information in Chiang Rai enabling us to collect *Curcuma maxwellii* during the flowering period and local Hmong community at Rim Khong subdistrict for their warm hospitality and willingness to share their plant knowledge.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The work of all authors was funded by their respective institutions with no external funding received.

Author contributions

Jana Leong-Škorničková: conceptualisation, herbarium study, field survey, morphological descriptions, writing original draft, preparing figures, review and editing of the draft and approval of the final version. Sutthinut Soonthornkalump: field survey, morphological description, writing original draft, preparing figures, review and editing of the draft and approval of the final version. Anders Jan Lindström: field survey, review and editing of the draft and approval of the final version. Sira Niwesrat: review and editing of the draft and approval of the final version. Sarah Qing Lim: field survey, review and editing of the draft and approval of the final version. Piyakaset Suksathan: field survey, review and editing of the draft and approval of the final version.

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Data availability

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

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A taxonomic revision of Thai *Fernandoa* Welw. ex Seem. (Bignoniaceae)

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Abstract

A taxonomic revision of *Fernandoa* Welw. ex Seem. (Bignoniaceae) in Thailand is presented. Two species, *F. adenophylla* (Wall. ex G. Don) Steenis and *F. collignonii* (Dop) Steenis, are enumerated with updated morphological descriptions, illustrations and a taxonomic identification key, together with notes on distributions, distribution maps, habitats and ecology, phenology, conservation assessments, etymology, vernacular names, uses, and specimens examined. The collection of *Wallich Cat. 6502A* from Myanmar, Ava at G [G00133642] is designated here as the lectotype of *F. adenophylla* in a second step lectotypification. *F. collignonii* has a conservation status of Endangered [EN]. The leaf, stem, and wood anatomy and pollen morphology of *F. adenophylla* are also reported in this study.

Key words: Lamiales, morphology, palynology, second step lectotypification, Tecomeae, vegetative anatomy



Academic editor: Eberhard Fischer

Received: 16 September 2023

Accepted: 2 November 2023

Published: 22 November 2023

Citation: Ngernsaengsaruy C, Meeprom N, Boonthasak W, Attasook Y, Thunthawanich R (2023) A taxonomic revision of Thai *Fernandoa* Welw. ex Seem. (Bignoniaceae). *PhytoKeys* 235: 249–270. <https://doi.org/10.3897/phytokeys.235.112839>

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Introduction

Fernandoa Welw. ex Seem. is a small genus belonging to the tribe Tecomeae Endl. of the family Bignoniaceae (Fischer et al. 2004) and they are mostly trees occurring from tropical Africa to West Malesia. In total, the genus contains 15 species, of which five species dwell in Africa, three species in Madagascar (Bidgood 1994; Bidgood et al. 2006; POWO 2023) and seven species in Southeast Asia (Fischer et al. 2004; POWO 2023). These seven Southeast Asian species are *Fernandoa adenophylla* (Wall. ex G. Don) Steenis, *F. bracteata* (Dop) Steenis [native to Vietnam], *F. brilletii* (Dop) Steenis [native to Vietnam], *F. collignonii* (Dop) Steenis, *F. quangxiensis* D. D. Tao [native to Southern China], *F. macroloba* (Miq.) Steenis [Native to Sumatra] and *F. serrata* (Dop) Steenis [native to Vietnam] (POWO 2023). Seemann (1865: 330) when naming the new genus adopted Welwitsch's recommendation and dedicated the genus to Ferdinand II (Portuguese: Dom Fernando II), King of Portugal (1816–1885), but the name was erroneously published by the printers as *Ferdinandia* Welw. ex Seem.

Later, Seemann (1866: 123) corrected *Ferdinandia* to *Fernandoa* as Welsitsch intended. Subsequently, when Seemann (1870a: 280) named a new species from Africa, he treated *Fernandoa* as a typographical error and corrected it to *Ferdinandoa* Welw. ex Seem., in which he later reverted it to *Fernandoa* (Seemann 1871). Therefore, in botanical nomenclature, *Ferdinandia* and *Ferdinandoa* are orthographical variants (orth. var.) of *Fernandoa*. The Indomalaysian genera, *Haplophragma* Dop, *Spathodeopsis* Dop and *Hexaneurocarpon* Dop, and the Malagasy genus *Kigelianthe* Baill. were later synonymised under *Fernandoa*, otherwise an African genus when it was first established (van Steenis 1976).

Morphologically, *Fernandoa* is similar to *Radermachera* Zoll. & Moritzzi and *Stereospermum* Cham. in having decussate leaves, leaf rachises not keeled above, leaflets less than seven pairs, and septum of the ovary flat and without pseudoseptum. *Fernandoa* differs from *Radermachera* and *Stereospermum* based on lower surface of the leaflets with hairy domatia, fruits with longitudinal ribs, septum flat (vs lower surface of the leaflets without hairy domatia, fruits without longitudinal ribs, septum terete in *Radermachera* and *Stereospermum*) (Santisuk 1987; Fischer et al. 2004).

In Thailand, a taxonomic revision of the genus *Fernandoa* was published by Santisuk (1987) and two species were recognized, *F. adenophylla* and *F. collignonii*. In this paper, we had extensively examined Thai specimens of *Fernandoa* in various local and international herbaria including digital herbarium repositories. As a result, we hereby provide a comprehensive update to species descriptions, habitats and vernacular names, in addition to phenological observations, uses, IUCN conservation status, illustrations and distribution maps in Thailand for each species. Besides that, leaf, stem and wood anatomical characters and pollen morphology of *F. adenophylla* are presented, excluding *F. collignonii* because we did not collect specimens of this species.

Materials and methods

Herbarium specimens deposited in BK, BKF, QBG, and those included in the digital herbarium databases of G (G-DC) (<http://www.ville-ge.ch/musinfo/bd/cjb/chg/index.php?lang=en>), K (including K-W) (<http://www.kew.org/herbcat>), L (<https://bioportal.naturalis.nl/>), P (<https://science.mnhn.fr/institution/mnhn/collection/p/item/search>), and US (<https://collections.nmnh.si.edu/search/botany/>) were examined (all herbaria acronyms follow Thiers 2023, continuously updated). The taxonomic history of *Fernandoa* was compiled using the taxonomic literature (Don 1838; de Candolle 1845; Seemann 1865, 1866, 1870a, 1870b, 1871; Kurz 1877; Clarke 1884; Ridley 1923; Dop 1925, 1930a, 1930b; Santisuk 1973, 1974, 1987; van Steenis 1976, 1977; Kochummen 1978; Santisuk and Vidal 1985) and online databases (IPNI 2023; POWO 2023). The morphological characteristics, distributions, ecology, and phenology were described from historic and newly collected herbarium specimens and the author's observations during field work. The vernacular names were compiled from specimens examined and the literature (Santisuk 1974, 1987; Royal Institute 2013; Office of the Forest Herbarium, Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation 2014). The assessment of conservation status was performed following the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022) for a preliminary assessment

of the conservation category in combination with GeoCAT analysis (Bachman et al. 2011) and field information. The calculation of Extent of Occurrence (EOO) and Area of Occupancy (AOO) are based on GeoCAT (<https://www.kew.org/science/our-science/projects/geocat-geospatial-conservation-assessment-tool>). The lamina, rachis, and stem (branch) anatomical characters of *Fernandoa adenophylla* were investigated by transverse sectioning with a sliding microtome at 15–20 µm thickness. For the study of epidermal cells of leaves, they were peeled and mounted. The wood samples of *F. adenophylla* were sectioned with a sliding microtome at 20–30 µm thickness along the transverse, tangential, and radial planes. The permanent slides of leaves, stems (branches) and wood were made following the standard methods of Johansen (1940) and Kermanee (2008). The anatomical characteristics were investigated and recorded photographically with an Olympus BX53 microscope and an Olympus DP74 microscope digital camera at the Department of Botany, Faculty of Science, Kasetsart University (KU). The anatomical terminologies follow those in the study by Metcalfe and Chalk (1957). The samples of pollen grains of *F. adenophylla* were examined and recorded photographically with an Olympus BX53 microscope and an Olympus DP74 microscope digital camera. Materials were prepared for scanning electron microscopy (SEM) at the Scientific Equipment Centre, Faculty of Science, KU by mounting pollen grains on stubs using double-sided sellotape, sputter-coating them with gold and examining them using an FEI Quanta 450 SEM (Hillsboro, OR, USA) at 15.00 KV. The characteristics of pollen grains were examined and measured, following Erdtman (1945, 1952) and Simpson (2010). The pollen morphology terminologies follow those of Punt et al. (2007).

Results and discussion

Taxonomic treatment

***Fernandoa* Welw. ex Seem., J. Bot. 3: 330. 1865 (sphal. *Ferdinandia*), nom. illeg.; J. Bot. 4: 123. 1866.**

Kigelianthe Baill., Hist. Pl. 10: 50. 1891.

Haplophragma Dop, Bull. Soc. Bot. France 72: 889. 1925.

Spathodeopsis Dop, Compt. Rend. Hebd. Séances Acad. Sci. 189: 1096. 1929; et Bull. Mus. Natl. Hist. Nat., Sér. 2, 2: 151. 1930.

Hexaneurocarpon Dop, Compt. Rend. Hebd. Séances Acad. Sci. 189: 1097. 1929; et Bull. Mus. Natl. Hist. Nat., Sér. 2, 2: 153. 1930.

Tisserantodendron Sillans, Bull. Soc. Bot. France 98: 270. 1952.

Type species. *Fernandoa superba* Welw. ex Seem. = *Fernandoa ferdinandi* (Welw.) Baill. ex K. Schum.

Description. Trees. **Leaves** 1-pinnate, imparipinnate, decussate; rachises not keeled above; leaflets 5–9, opposite, chartaceous to subcoriaceous, with scattered glands on both surfaces or a few scattered glands and small hairy domatia in the axil of lateral veins below. **Inflorescence** a terminal thyrse or raceme, densely stellate tomentose, densely dendroid tomentose, sparsely haired or glabrous. **Flowers** nocturnal; calyx persistent, campanulate or tubular-campanulate, irregularly 2–5-lobed; corolla yellowish-green, creamy white to pale yellow,

corolla tube curved, constricted between basal and upper parts, basal tube short cylindrical, upper tube campanulate or infundibuliform-campanulate, bilabiate, 5-lobed, upper lobes 2 and lower lobes 3, subequal or unequal, crisped; stamens 4, didynamous, subexserted, anthers divaricate; staminode present; disc annular, surrounding the base of ovary; ovary superior, cylindrical, densely dendroid tomentose or glabrous, 2-celled, septum of the ovary flat without pseudoseptum, ovule numerous, axile placenta, style slender, stigma 2-lobed. **Fruit** a loculicidal capsule, cylindrical, twisted or straight to slightly arcuate, with longitudinal ridges, densely dendroid tomentose or glabrous, septum flat. **Seeds** numerous, flat, rather rectangular with a lateral hyaline-membranous wing.

A genus of fifteen species, distributed from Africa (5), Madagascar (3), and continental Southeast Asia to Sumatra (7); two species in Thailand.

A key to the species of *Fernandoa* in Thailand

- 1 Leaflets densely stellate and dendroid tomentose along midrib and basal part of lateral veins above, densely stellate and dendroid tomentose below; lowest pair of leaflets much reduced, resembling foliaceous pseudostipules; inflorescences (peduncles, axes and pedicels) densely stellate and dendroid tomentose; calyx campanulate, with 5 subequal or unequal lobes; upper tube of corolla campanulate; fruits twisted, with (6–)10 prominent longitudinal ridges; calyx, corolla, ovary and fruits densely dendroid tomentose..... **1. *F. adenophylla***
- Leaflets glabrous on both surfaces; lowest pair of leaflets not reduced to foliaceous pseudostipules; inflorescences (peduncles, axes and pedicels) sparsely hairy or glabrous; calyx tubular-campanulate, with 2–3 unequal lobes; upper tube of corolla infundibuliform-campanulate; fruits straight to slightly arcuate, with 6 prominent longitudinal ridges; calyx, corolla, ovary and fruits glabrous **2. *F. collignonii***

1. *Fernandoa adenophylla* (Wall. ex G. Don) Steenis, *Blumea* 23(1): 135. 1976; et *Fl. Males.*, Ser. 1, *Spermat.* 8(2): 158. 1977; Kochummen in *Ng, Tree Fl. Malaya* 3: 39. 1978; Santisuk & J. E. Vidal in J.-F. Leroy, *Fl. Cambodge Laos Vietnam* 22: 39. t. 5, fig. 1. 1985; Santisuk in Smitinand & K. Larsen, *Fl. Thailand* 5(1): 47. 1987.

Figs 1, 2

≡ *Bignonia adenophylla* Wall. [Numer. List: 221. *Wallich Cat.* 6502, nom. nud.] ex G. Don, *A Gen. Hist.* 4: 221. 1838.

≡ *Spathodea adenophylla* (Wall. ex G. Don) DC., *Prodr.* 9: 206. 1845.

≡ *Heterophragma adenophyllum* (Wall. ex G. Don) Seem., *J. Bot.* 8: 340. 1870.

≡ *Haplophragma adenophyllum* (Wall. ex G. Don) Dop, *Bull. Soc. Bot. France* 72: 890. 1925.

Type. Myanmar, Ava, 12 Oct 1826, *Wallich* 6502A (lectotype, first step designated by van Steenis (1976: 135), G [without barcode], second step designated here G [G00133642, photo seen]; isolectotypes K-W [K001124064, photo seen], G [G00134691, G00134695, photos seen], P [P00609736, photo seen].

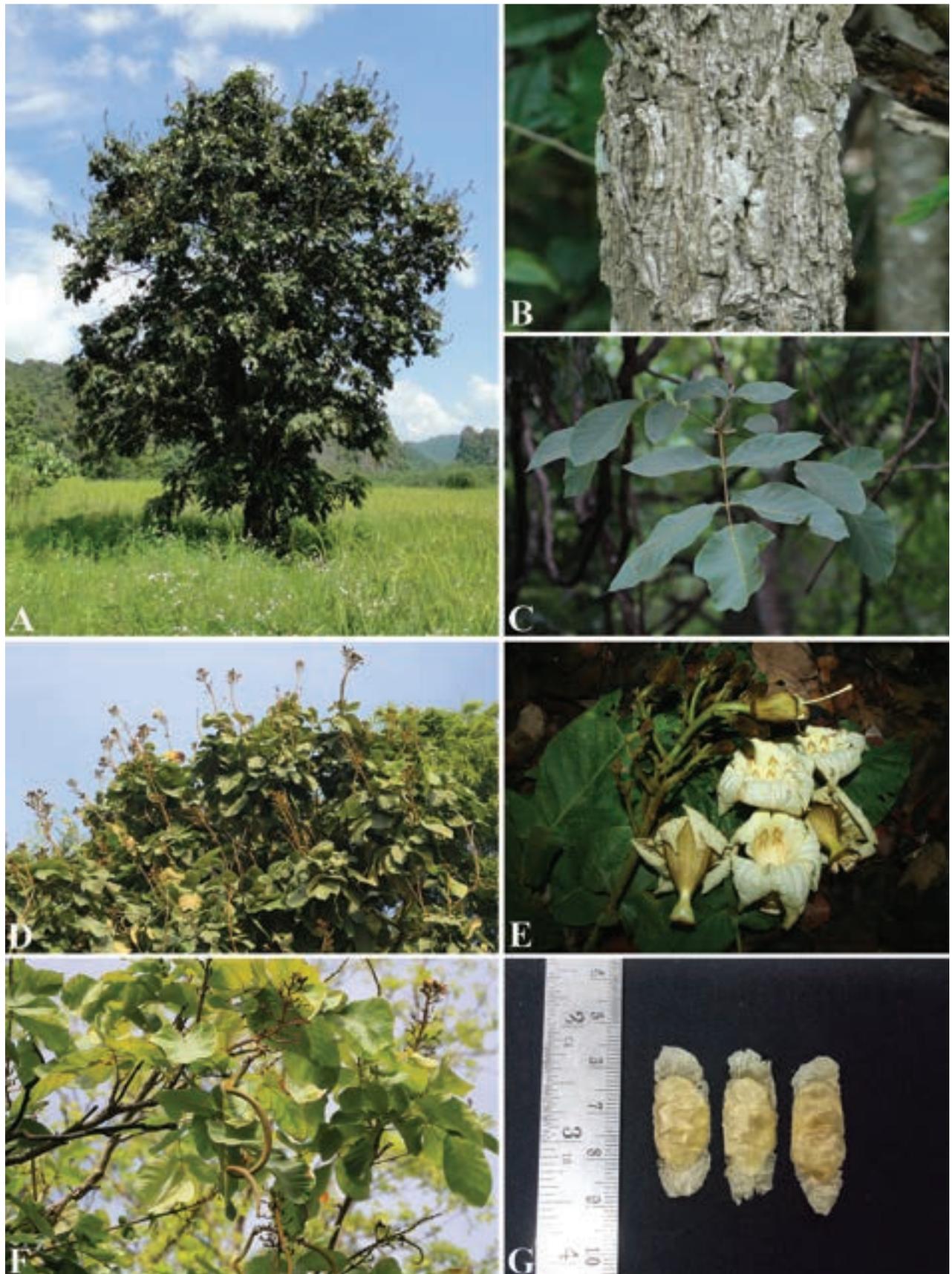


Figure 1. *Fernandoa adenophylla* **A** habitat and habit **B** bark **C** branch and leaves **D** branches, leaves and inflorescences **E** inflorescences and flowers **F** branches, leaves, inflorescences and fruits **G** winged seeds. Photos: Yanatshara Attasook (**A, B**) Chatchai Ngernsaengsaruy (**C–G**).

Description. Trees deciduous, 5–15(–20) m tall, up to 170 cm girth; bark irregularly cracked, corky, grey to greyish-brown; young branches densely stellate and dendroid tomentose. **Leaves** decussate; petioles very short or up to 1.5 cm long; rachises 6–41 cm long, 4-angular, channelled above; petioles and rachises densely stellate and dendroid tomentose; leaflets 5–9, opposite, laminas variable in shape, size, apex and base, obovate, elliptic, ovate, oblong, suborbicular or orbicular, the terminal leaflets largest, 10.5–46.5 × 6.5–28 cm, the lateral leaflets 3–33 × 3–21 cm, the lowest pair of leaflets near the base of petiole much reduced, resembling foliaceous pseudostipules, 0.5–8.5 × 0.5–7 cm, apex obtuse, rounded, acute, acuminate, cuspidate or emarginate, base cuneate, oblique, obtuse, truncate, cordate or subcordate, margin entire or repand, subcoriaceous, glabrous above, except densely stellate and dendroid tomentose along midrib and basal part of lateral veins above, densely stellate and dendroid tomentose below, with scattered glands on both surfaces, midrib and lateral veins raised below, lateral veins 4–11 pairs, curving and connected in loops near the margin, veinlets reticulate, with small hairy domatia in the axil of lateral veins below; petioles very short. **Inflorescence** a thyrse, 10–65 cm, erect, lax-flowered; peduncles 2–10 cm long; rachises 10–53 cm long; peduncles, axes and pedicels with dense stellate and dendroid tomentose. **Flowers:** calyx yellowish-green, thick, 5-ribbed, persistent, densely yellowish-brown dendroid tomentose outside, glabrous inside, campanulate, 2–4.5 × 1.5–3 cm, bilabiate, 5-lobed, upper lobes (posterior) 3 and lower lobes (anterior) 2, subequal or unequal, lobes triangular, 0.7–1.8 × 0.4–1.7 cm, apex acute; corolla yellowish-green, creamy white to pale yellow, thick, densely yellowish-brown dendroid tomentose outside, glabrous inside, corolla tube curved, constricted between basal and upper parts, basal tube short cylindrical, widened towards the base, 1.5–2.5 cm long, 1–2.5 cm wide at base, upper tube widened towards the mouth, campanulate, 3–5.5 cm long, 3.5–5 cm wide at mouth, bilabiate, 5-lobed, upper lobes 2 and lower lobes 3, subequal or unequal, lobes suborbicular or broadly obovate, 2.5–4 × 2.4–5.5 cm, apex rounded, crisped; stamens 4, didynamous, subexserted, longer pair 3.2–5.5 cm long, shorter pair 3–4.5 cm long, filaments arcuate, creamy white to pale yellow, glabrous, anthers 5–9 mm long; staminode 1, needle-like, 0.7–2.2 cm long; disc annular, surrounding the base of ovary, creamy white to pale yellow; ovary cylindrical, 0.6–1.5 cm long, with (6–)10 longitudinal ridges, densely dendroid tomentose, style slender, 3–5 cm long, creamy white to pale yellow, glabrous, stigma 2-lobed, 4–7 mm long. **Fruits** cylindrical, 34–85 × 1.5–3.5 cm, green turning brown when dry, twisted, with (6–)10 prominent longitudinal ridges, densely yellowish-brown dendroid tomentose, septum 2–3 mm thick, 1–1.5 cm wide. **Seeds** flat, rather rectangular with a lateral hyaline-membranous wing, 1.5–4 × 0.6–1.4 cm.

Distribution. India (Assam, Andaman and Nicobar Islands), Pakistan, Bangladesh, Myanmar, Vietnam, Laos, Cambodia, Thailand, Peninsular Malaysia.

Distribution in Thailand. NORTHERN: Mae Hong Son, Chiang Mai, Chiang Rai, Nan, Lamphun, Lampang, Tak, Sukhothai, Phitsanulok, Kamphaeng Phet, Nakhon Sawan; NORTH-EASTERN: Phetchabun, Loei, Sakon Nakhon, Khon Kaen; EASTERN: Nakhon Ratchasima, Ubon Ratchathani; SOUTH-WESTERN: Uthai Thani, Kanchanaburi, Phetchaburi, Prachuap Khiri Khan; CENTRAL: Suphan Buri, Saraburi, Bangkok (Queen Sirikit Park, cultivated); SOUTH-EASTERN: Chon Buri, Rayong, Chanthaburi, Trat; PENINSULAR: Chumphon, Ranong, Surat Thani, Phangnga, Nakhon Si Thammarat, Trang. (Fig. 3)



Figure 2. Lectotype of *Fernandoa adenophylla*, Wallich 6502A (G [G00133642]) from Ava, Myanmar. Photo: Conservatoire et Jardin botaniques de la Ville de Genève, Genève, Switzerland <https://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=55790&lang=en>.

Habitat and ecology. It is found in deciduous dipterocarp and mixed deciduous forests (with or without bamboo), mixed deciduous forests with bamboo on limestone hills, savannas, gaps or edge of dry evergreen and lower montane rain forests, transition between deciduous and evergreen forests, secondary forests, disturbed open areas, along roadsides, along riverbanks, at elevations of near above mean sea level (a.m.s.l.) up to 1,000 m.

Phenology. Flowering and fruiting nearly all year round.

Conservation status. *Fernandoa adenophylla* is widely distributed from India to Indochina and Peninsular Malaysia, and has a large extent of occurrence (EOO of 3,353,755.49 km²) and area of occupancy (AOO of 352 km²). Also,

considering it grows in secondary forests, disturbed open areas, and along roadsides, it is considered here as Least Concern (LC).

Etymology. The specific epithet of *Fernandoa adenophylla* is derived from the Greek compound words, "aden-", "adeno-" meaning gland (glandular-), and "-phylla" meaning -leaved, refers to the leaves of this species with a few scattered glands on both surfaces (Stearn 1992; Radcliffe-Smith 1998; Gledhill 2002).

Vernacular name. Khae khon (แคขน) (Northern); **Khae bit** (แคบิต) (Northern, North-Eastern); Khae phong (แคพอง) (Peninsular, Surat Thani); Khae rao (แคร้าว), Khae lao (แคลาว) (North-Eastern, Loei); Khae hua mu (แคหัวหมู) (Nakhon Ratchasima); Khae hang khang (แคหางค่าง) (General); Haeng pa (แฮงป่า) (Chanthaburi); Hong pa (โฮงป่า) (South-Western); Karen wood, Katsagon, Katsagon tree, Petthan (common name); Dhopa-paroli (Assam); Marodphali (Hindi).

Uses. Flowers and young fruits are consumed as boiled or grilled vegetables and required cooking. Cultivated as shade and ornamental trees (the author's observations). The wood is locally used in construction and used for making farming utensils. Bark, leaves, and seeds are used as medicinal purposes (Widodo 1998; Biodiversity-Based Economy Development Office (Public Organization) [BEDO] 2021).

Notes. *Bignonia adenophylla* was named by Wallich based on *Wallich Cat.* 6502 collected from Myanmar: 6502A from Irrawaddy River, Yenangheum (Yenangyaung), Prome, Sagaen (Sagaing), and Ava and 6502B from Taong Dong but unpublished, and then this name was described by Don (1838: 221). van Steenis (1976: 135) mentioned *Wallich Cat.* 6502 of Ava and Prome as type, when he transferred *B. adenophylla* under *Fernandoa adenophylla*. *Wallich Cat.* 6502 represents two gatherings (two different materials collected from two different cites, which are distinguished by *Wallich Cat.* 6502A and *Wallich Cat.* 6502B, respectively). *Wallich Cat.* 6502A is from Ava and Prome and *Wallich Cat.* 6502B is from Taong Dong. Thus, *Wallich Cat.* 6502A could be regarded as the true type specimen, and *Wallich Cat.* 6502B from Taong Dong is not type.

van Steenis (1976) cited *Wallich Cat.* 6502 from Ava and Prome at G as the holotype with isotypes in K and P. However, Wallich's collection numbers are known to be curated by species generally from multiple collections (Noltie and Watson 2021); therefore, his erroneous designation of holotype effectively selected a) a collection for the lectotype if the collection consists of multiple sheets or b) the lectotype if it is a unicate gathering. van Steenis (1976) did not mention the number of specimens, and following Art. 9.6 of the ICN (Turland et al. 2018), they constitute syntypes. Therefore, the name *Fernandoa adenophylla* has been lectotypified in a first step by van Steenis (1976) using specimen *Wallich Cat.* 6502A at G [without barcode] with isolectotypes at K [without barcode] and P [without barcode]. We located three sheets of the specimen *Wallich* 6502A from Ava at G [G00133642, G00134691, and G00134695] and two sheets of the specimen *Wallich* 6502A from Prome at G [G00133632 and G00134708]; The G [G00133642] specimen is better preserved and more complete than the others, and hence is selected here in a second step lectotypification. We also traced isolectotypes at K-W [K001124064] and P [P00609736].

Santisuk (1987) reported that the leaves, calyx, and ovary have a stellate tomentum, but in this study the calyx, corolla, ovary, and fruits only exhibited dendroid trichomes, whereas the leaves bear both stellate and dendroid trichomes.

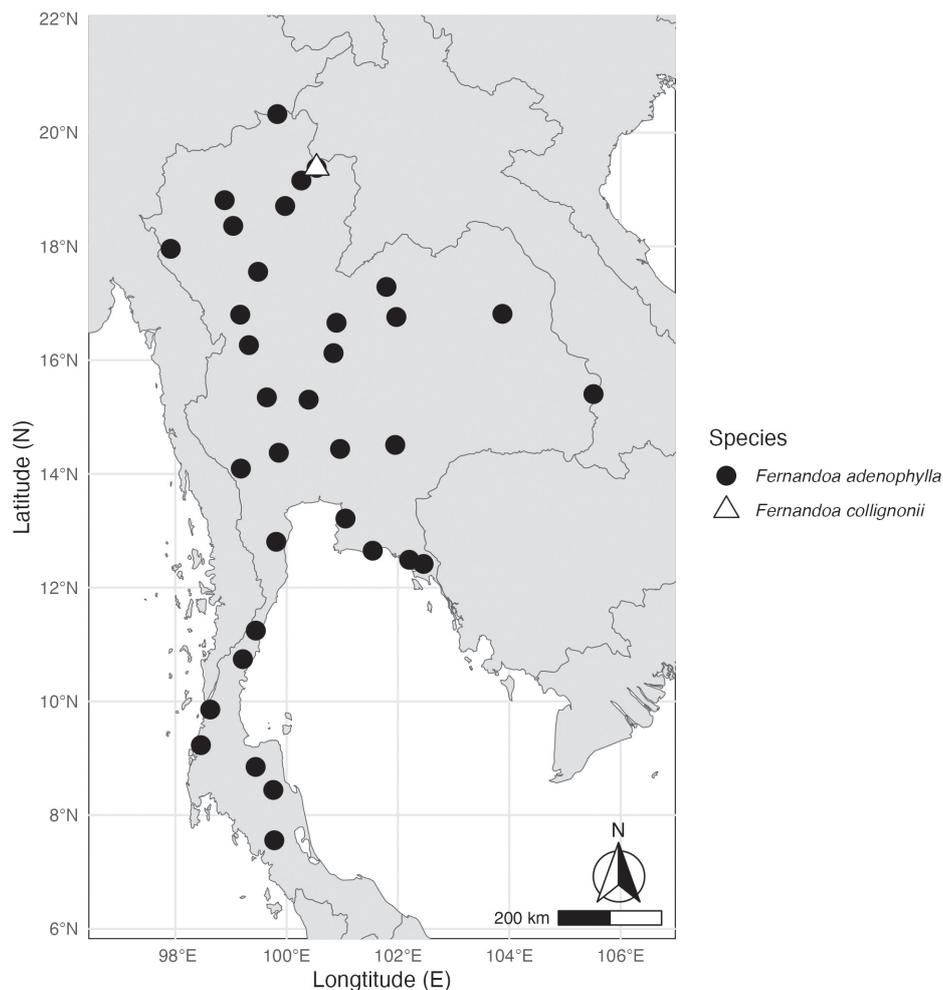


Figure 3. Distribution of *Fernandoa* in Thailand: *F. adenophylla* occurs in all floristic regions of Thailand and *F. collignonii* known only from Nan Province, Northern Thailand. [Thailand floristic regions follow *Flora of Thailand* Volume 16 Part 1 (The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation 2022)].

In addition to Santisuk (1987), the distribution in Mae Hong Son, Chiang Rai, Phayao, Nan, Lamphun, Sukhothai, Phitsanulok, Kamphaeng Phet, Nakhon Sawan, Sakon Nakhon, Khon Kaen, Ubon Ratchathani, Uthai Thani, Suphan Buri, Saraburi, Bangkok (cultivated), Rayong, Ranong, and Nakhon Si Thammarat Provinces were newly recorded in this study.

Santisuk (1987) reported that this species occurs in deciduous forests, savannas, and lowland secondary forests. Further, we confirmed it grows in more variable habitats (see habitat).

Additional specimens examined. THAILAND. Northern: Mae Hong Son [Sop Moei, 12 Jul 2013, *Pongamornkul* 3468 (QBG); Sop Moei, 1 Jan 2014, *Pongamornkul* 3928 (QBG); Ban Mueang Paeng, Mueang Paeng Subdistrict, Pai District; Salawin Forest Plantation, Mae Sariang District (Ngernsaengsaruy own observation)]; Chiang Mai [Locality not specified, 4 Oct 1921, *Nai Noi Mao* s.n. (BK) (Santisuk 1974); Chiang Dao District, fr., 27 Nov 1963, *Bunchuai* 1375 (BKF); Mae Rim District, Mae Sa valley, in cultivation area, 1,000 m alt., fl., 15 May 1974, *Jackson* 6039 (BKF); Hang Dong District, along the dirt road from Mae Heeyah Nai to Ban Bong, south side of Doi Suthep-Pui National Park,

425 m alt., fl., 15 Jul 1989, *Maxwell 89-903* (L [L3730390]); Doi Chiang Dao, SE foothills near Ban Yang Pong Luang, Chiang Dao District, 525 m alt., fl., 29 Oct 1989, *Maxwell 89-1326* (L [L3730538]); Doi Suthep-Pui National Park, Mae Rim District, in open place near Mae Sa stream, 650 m alt., fr., 9 Jan 1990, *Maxwell 90-37* (L [L3730536, L3730537]); Department of Biology, Faculty of Science, Chiang Mai University, 350 m alt., fl., 27 Jun 1992, *Palee 45* (L [L3730324]); Queen Sirikit Botanic Garden, Mae Rim District, 10 Aug 1994, *BGO. Staff 1294* (QBG); Maesa Elephant Camp, Mae Rim District, 25 Aug 1994, *BGO. Staff 1454* (QBG); Huai Gayo village area, San Kamphaeng District, 675 m alt., fr., 10 Oct 1995, *Palee 325* (BKF, L [L3730483, L3730484]); Mae Rim District, 5 Nov 1997, *Wattana & Siriphum 29* (QBG); Doi Suthep, *Kerr 3262* (ABD, BM, K) (Santisuk 1974); Chiang Rai [Doi Tung, base of the east side at Bah Kah (Akha) village, Mae Fa Luang District, 500 m alt., fl., 21 Jul 2006, *Maxwell 06-494* (L [L3730659]); Mueang Chiang Rai District (*Ngernsaengsaruy own observation*); Phayao [Rom Yen Subdistrict, Chiang Kham District, 18 Jun 2013, *La-ongsri et al. 2920* (QBG)]; Nan [Mueang Nan District, 14 Nov 2009, *Srithi 345* (QBG)]; Song Khwae District, 18 Oct 2019 (the authors own observation); Lamphun [Mae Tha District, 29 Jan 2010., *Romkham 132* (QBG)]; Lampang [Ngao, Mae Huat, 350 m alt., fl., 18 May 1954, *Smitinand 1579* (BKF)]; Tak [along Mae Ping River, Om Lu Rapids, fl., fr., 9 Dec 1920, *Rock 629* (US [US03206561, US03206562, US03206563]); Doi Pha Wo, trail from Nam Dip to Pang Luang, Mae Sot District, fl., 19 Dec 1920, *Rock 669* (US [US03206564]); between Palut and Nam Dip, on the trail from Raheng to Mae Sot, fl., 17 Dec 1920, *Rock 1080* (US [US03206556]); Lan Sang National Park, bamboo rich gallery forest along cascade, fr., 27 Dec 1974, *Geesink, Hiepko & Phengkklai 7941* (L [L2815249]); Lan Sang National Park, 300 m alt., fl., 5 Aug 1997, *Setbubpa 19* (BKF); Ban Tak District, 16 Oct 2014, *Tanming 715* (QBG); Mae Kor, *Winit 410* (ABD, K) (Santisuk 1974); Sukhothai [Si Satchanalai National Park, Si Satchanalai District, fl., 27 Jul 2015, *Maknoi 7946* (BKF, QBG)]; Phitsanulok [Thung Salaeng Luang National Park, c. 80 km east of Phitsanulok, c. 500 m alt., fl., 25 Jul 1973, *Murata, Fukuoka & Phengkklai T-17126* (BKF, L [L2815258]); Kamphaeng Phet [Mae Wong National Park, Khlong Lan District, 20 Oct 2001, *Wattana 1528* (QBG)]; Wang Khuang Subdistrict, Phran Kratai District (*Ngernsaengsaruy own observation*); Nakhon Sawan [Takhli District, 30 Jan 2013, *Maknoi 5210* (QBG)]; **North-Eastern:** Phetchabun [Khao Hin Pakarang Chon Daen District, 24 Nov 2009, *Maknoi & Tanaros 3425* (QBG); Chon Daen District, *Vacharapong 320* (BK) (Santisuk 1974)]; Loei [Wang Saphung District, 300 m alt., fl., 28 Apr 1946, *Nakkarn 66* (BKF); Wang Saphung District, 300 m alt., fl., y. fr., 28 Nov 1957, *Bunpheng 1030* (BKF, K) (Santisuk 1974)]; Pak Chom District, fl., 2 Sep 1968, *Phengnaren & Smitinand 583* (BKF); Pha Daeng, Wang Saphung District, *Suvarnakoses 1327* (BKF, K) (Santisuk 1974)]; Sakon Nakhon [Phu Phan National Park (*Ngernsaengsaruy own observation*)]; Khon Kaen [Ban Na Chan, Phu Pha Man National Park, 477.5 m alt., fr., 25 Feb 2011, *Norsaengsri, Tathana & Lakert 7616* (BKF, QBG)]; **Eastern:** Nakhon Ratchasima [Wang Nam Khiao District, Sakaerat Environmental Research Station, 420 m alt., fl., 17 Jul 1967, *Damrongsak 115* (BKF); Wang Nam Khiao District, fr., Dec 1967, *Damrongsak 418* (BKF); Khao Phayom, Wang Nam Khiao District, 430 m alt., fr., 21 Dec 1967, *Anan 15* (BKF); Pak Thong Chai District, Lam Phra Phloeng Dam, 400 m alt., fr., 29 Jan 1983, *Koyama, Terao & Wongprasert T-33101* (BKF); Nong Ra Wiang Subdistrict, fl., 3

Oct 2000, *Garcia & Phengklai* 385 (BKF); Ban Badan, Pak Thong Chai District, *Sono* 8 (BKF) (Santisuk 1974); Khao Phayom, Wang Nam Khiao District, *Nalam-poon* 15 (BKF) (Santisuk 1974); Ubon Ratchathani [Khong Chiam District (Ngernsaengsaruy own observation)]; **South-Western:** Uthai Thani [Khao Pla Ra, Lan Sak District, 19 Sep 2015, *Tanming* 892 (QBG); Mueang Ka Rung Sub-district, Ban Rai District; Phai Khiao Subdistrict, Sawang Arom District (Ngernsaengsaruy own observation)]; Kanchanaburi [Khwae Noi River Basin, near Neeckey (N. Wangka), 150 m alt., fr., 1 May 1946, *Bloembergen & Kostermans* 276 (BK, K, L [L2815284, L2815285], P [P02902332]); Khwae Noi River Basin, Kin Sai Yok, c. 120 km northwest of Kanchanaburi, along river bank, 100–150 m alt., fl., 2 Aug 1946, *Kostermans* 1436 (L [L2815280, L2815281], US [US03206558]); Ban Kao, fl., 14 Nov 1961, *Larsen* 8202 (L [L2815289]); Sai Yok District, sterile, 15 Dec 1961, *Larsen* 8733 (L [L2815287, L2815288]); Sai Yok District, 150 m alt., fl., 23 Nov 1971, *van Beusekom* et al. 3907 (BKF, L [L2815283]); Huai Ban Kao, 750 m alt., fr., 9 Nov 1971, *van Beusekom* et al. 3576 (BKF, L [L2815277]); Forest Research and Demonstration Center, Chalae Subdistrict, Thong Pha Phum District, 700 m alt., fr., 11 Dec 1995, *van Welzen* 2 (L [L2815250]); Bo Phloi, Lam I Su, fr., 13 Apr 2000, *Phengklai* et al. 12171, 12172 (BKF); Wang Pho, Lum Sum Subdistrict, Sai Yok District, 50 m alt., fl., 1 Jul 2006, *Chongko* 532 (L [L3731040, L3731041], QBG); Mahidol University, Kanchanaburi Campus, Sai Yok District, 21 Jul 2006, *Maxwell* 06-494 (QBG); Thipsukhontharam Temple, Huai Krachao District, 14 Oct 2014, *Tanming* 633 (QBG); Khao Thong, *Kerr* 19627 (ABD, BK, BM) (Santisuk 1974)]; Phetchaburi [Khao Phanoen Thung, Kaeng Krachan National Park, 23 Aug 1998, *Sasirat* 80 (QBG); Thung Luang, *Kerr* 20638 (ABD, BK, BM) (Santisuk 1974)]; Prachuap Khiri Khan [Bang Saphan District, *Put* 1383 (BK, BM) (Santisuk 1974)]; **Central:** Suphan Buri [Phu Hang Nak, U Thong District (Ngernsaengsaruy own observation)]; Saraburi [Sam Lan Forest, Mueang Saraburi District, 75 m alt., fl., 27 Jul 1975, *Maxwell* 75-722 (L [L2815259])]; Bangkok [Queen Sirikit Park, cultivated (Ngernsaengsaruy own observation)]; **South-Eastern:** Chon Buri [Si Racha District, sterile, 1 Dec 1927, *Collins* 1748 (BK, US [US03206557]); Khao Khiao, Si Racha District, 100 m alt., fl., 13 Jun 1976, *Maxwell* 76-393 (L [L2815252]); Khao Khiao Open Zoo, 9 Aug 2000, *Phengklai* 12610 (BKF); Si Racha District, *Collins* 223 (ABD) (Santisuk 1974)]; Rayong [Klaeng District, 19 Dec 2007, *Wes-sumritt* 55 (QBG); Chanthaburi [Khao Sa Bap, Makhm District, 11 May 1956, *Chit* 333 (BKF) (Santisuk 1974)]; Trat [wayside near sea level, fl., 1 Aug 1973, *Murata, Fukuoka & Phengklai* T-17348 (BKF, L [L2815254, L2815255], *Murata, Fukuoka & Phengklai* T-17389 (BKF, L [L2815251])]; **Peninsular:** Chumphon [Tha Sae District, *Jaray* 110 (BK) (Santisuk 1974); Kuring, *Kerr* 11609 (BK, K, L [L2815292]) (Santisuk 1974)]; Ranong [30–70 km south of Ranong, 50–100 m alt., fl., 27 Apr 1974, *Larsen & S. S. Larsen* 33441 (BKF, L [L2815256])]; Surat Thani [Ban Na San District, 100 m alt., fl., 14 Oct 1957, *Thaworn* 500 (BKF, K) (Santisuk 1974)]; Phangnga [Nang Yon, Takua Pa, District, *Kerr* 17047 (ABD, BK, BM) (Santisuk 1974)]; Nakhon Si Thammarat [Khiri Wong, Lan Saka District, fl., 18 Feb 1962, *Ploenchit* 1792 (BKF); Tha Sala District, Walailak University, 10 m alt., fl., 22 Dec 2006, *Pooma, Pattharahirantricin & Sirimongkol* 6528 (BKF); Trang [Khao Chong, Na Yong District, 80 m alt., fl., 29 Sep 1965, *Bunnab* 6 (BKF, L [L2815293]); Khao Chong, Na Yong District, 84 m alt., fl., y. fr., Sep 1965, *Bunnab* 193 (BKF); Huai Yot, 1 May 1916. *Vanpruk* 852 (BKF)].

2. *Fernandoa collignonii* (Dop) Steenis, *Blumea* 23(1): 136. 1976; Santisuk & J. E. Vidal in J.-F. Leroy, *Fl. Cambodge Laos Vietnam* 22: 42. t. 5, fig. 5–8. 1985; Santisuk in Smitinand & K. Larsen, *Fl. Thailand* 5(1): 48. fig. 20. 1987.
Figs 4, 5

≡ *Spathodeopsis collignonii* Dop, *Bull. Mus. Natl. Hist. Nat., Ser. 2*, 2: 152. 1930.

Type. Vietnam, Tonkin, Hoa Binh, Jul 1929, *Collignon s.n.* (holotype, P [P00609742, photo seen]).

Description. Trees, 5–12 (–20) m tall; bark irregularly cracked, corky, grey to greyish-brown; young branches glabrous. **Leaves** decussate; petioles 3.5–9 cm long; rachises 8.5–17.5 cm long, terete, channelled above; petioles and rachises sparsely hairy or glabrous; leaflets 7–9, opposite, laminas elliptic, elliptic-oblong, oblong or ovate, 6–17 × 2.5–7 cm, apex acuminate or caudate, base oblique, cuneate or obtuse, margin entire, chartaceous, glabrous on both surfaces, except small hairy domatia in the axil of lateral veins below, with a few scattered glands below, midrib and lateral veins raised below, lateral veins 4–10 pairs, curving and connected in loops near the margin, veinlets reticulate, the lowest pair smaller than the upper pair of leaflets, not reduced to foliaceous pseudostipules; petiolules very short or up to 4 mm long. **Inflorescence** a thyrses, 10–21 cm; peduncles 2–4 cm long; rachises 3.5–10 cm long; peduncles, axes and pedicels sparsely hairy or glabrous. **Flowers:** calyx thick, persistent, in flower buds 5-ribbed at least in the upper half, glabrous on both sides (sparse hairs outside in flower buds), tubular-campanulate, 2–3.5 × 1.5–2 cm, 2–3-lobed, unequal, apex acute (the posterior side with 2–3 lobes, halfway or more split towards the anterior base); corolla creamy white to pale yellow, glabrous on both sides, corolla tube curved, constricted between basal tube and upper tube, basal tube short cylindrical, widened towards the base, c. 2 cm long, 1–1.5 cm wide at base, upper tube widened towards the mouth, infundibuliform-campanulate, 4–4.5 cm long, 3.5–4 cm wide at mouth, bilabiate, 5-lobed, upper lobes 2 and lower lobes 3, subequal or unequal, lobes suborbicular or broadly obovate, 2–2.5 × 2–2.6 cm, apex rounded, crisped; stamens 4, didynamous, subexserted, longer pair c. 5 cm long, shorter pair c. 3 cm long, filaments arcuate, glabrous, anthers c. 6 mm long; staminode 1, needle-like, 5–6 mm long; disc annular, surrounding the base of ovary, c. 7 mm in diam.; ovary cylindrical, with 6 longitudinal ridges, glabrous, style slender, c. 4 cm long, glabrous, stigma 2-lobed. **Fruits** cylindrical, 33–70 × 3–6.5 cm, green turning brown when dry, straight to slightly arcuate, with 6 prominent longitudinal ridges, glabrous, septum 3–4 mm thick, 1.8–2.5 cm wide. **Seeds** flat, rather rectangular with a lateral hyaline-membranous wing, 4–4.5 × 1–1.8 cm.

Distribution. Vietnam, Laos, Thailand.

Distribution in Thailand. Northern: Nan. (Fig. 3).

Habitat and ecology. It is found in dry evergreen forest, limestone hills, lower montane rain forest, at elevations of 400–800 m a.m.s.l.

Phenology. Flowering April to July; fruiting July to December.

Conservation status. Endangered (EN) (Santisuk et al. 2006; Chamchumroon et al. 2017). This species is known only from Indochina (Vietnam, Laos, and Thailand), and has a small extent of occurrence (EOO of 14,250.11 km²) and area of occupancy (AOO of 20 km²). In Thailand it is known only from Northern Thailand, Nan Province, and has a small extent of occurrence (EOO

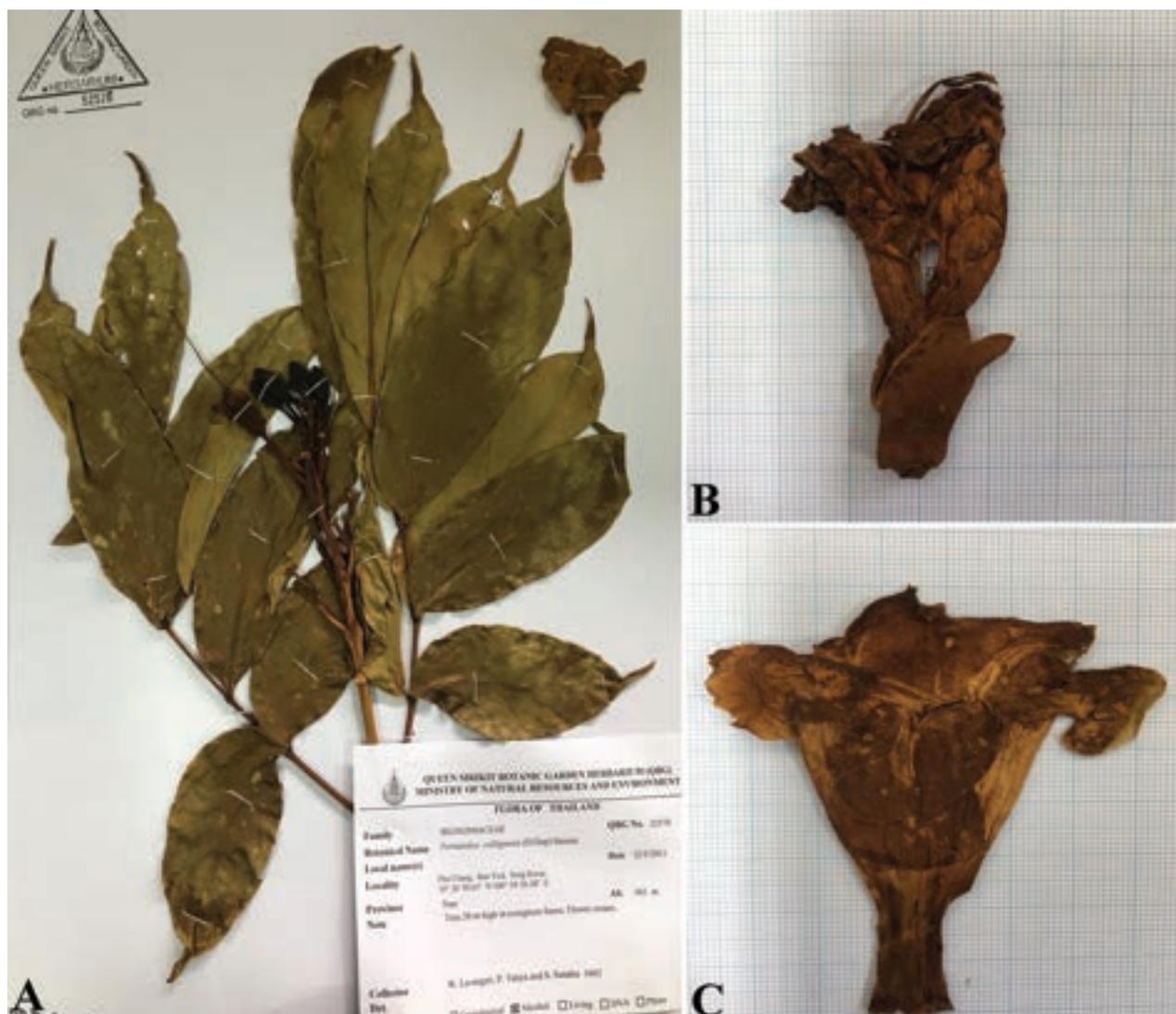


Figure 4. *Fernandoa collignonii* **A** branch, leaves, inflorescence and flowers [La-onsri, Tatiya & Satatha 1662 (QBG)] **B** flower **C** corolla [Srisanga, Maknoi, Panyachan & Tatiya 2884 (QBG)]. Photos: Pattarin Nunthamontree.

of 1,378.75 km²) and area of occupancy (AOO of 16 km²). It is appropriate to consider its status as Endangered [EN B2ab(ii, iv)].

Etymology. The specific epithet of *Fernandoa collignonii* honours L. Collignon, the collector of the type specimen.

Vernacular name. Khae dok lueang (แคดดอกเหลือง) (Nan) [Niyomdham & Puudjaa 7677 (BKF)]; **Khae hang khang san** (แคหางค่างสัน) (Northern); Dinh thoi (Tonkin); Dinh, Dinh vang, Dinh collignon (Vietnam).

Uses. No data recorded in Thailand. In Vietnam, Tonkin, Hoa Binh, the specimen Poilane 13012 (P [P02862885]) noted that its timber is good for all purposes, not being attacked by termites.

Notes. In addition to the key to the species, *Fernandoa collignonii* differs from *F. adenophylla* in its petioles 3.5–9 cm long (vs very short or up to 1.5 cm long because the lowest pair of leaflets near the base of petiole much reduced, resembling foliaceous pseudostipules), rachises terete (vs 4-angular), petioles and rachises sparsely hairy or glabrous (vs densely stellate and dendroid tomentose), the longest leaflets up to 17 cm (vs the longest terminal leaflets up to

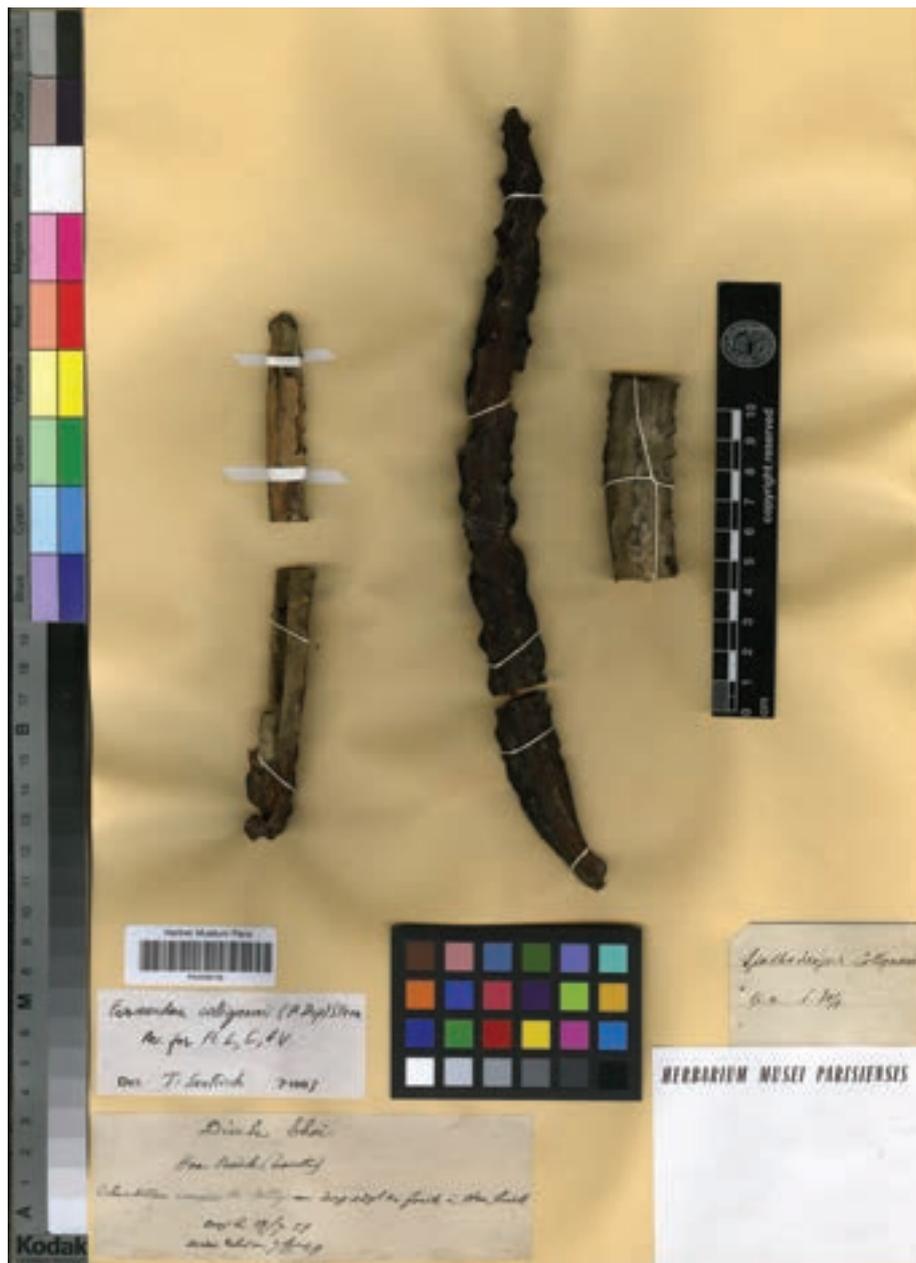


Figure 5. Holotype of *Fernandoa collignonii*, Collignon s.n. P [P00609742] from Hoa Binh, Tonkin, Vietnam, with immature fruits. Photo: Muséum National d'Histoire Naturelle (MNHN), Paris, France <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00609742>.

46.5 cm and lateral leaflets up to 33 cm), leaflets chartaceous, with a few scattered glands below (vs subcoriaceous, with scattered glands on both surfaces).

Santisuk (1987) reported that the height of this species ranges from 5–12 m tall, but the specimens *Srisanga* et al. 2884 (QBG), *La-onsri* et al. 1662 (QBG), and *La-onsri* et al. 1869 (QBG) collected from Nan Province mentioned 20 m tall. *Poilane* 13012 (P [P02862885]) collected from Vietnam was recorded to have trunk diameter to 1 m.

The flowers were mentioned by the specimens from Vietnam, *Evrard* 515 (L [L2815229]) to be reddish orange (rouge ochre), and *Poilane* 6055 (L [L2815228]) described them as white, but were recorded here as creamy white to pale yellow in this study.

Santisuk (1973, 1974) reported that this species is known only from Nan Province, noted as Mae Sanian, *Winit 1788* (BK, BKF, K). In addition, Tham Sakoen National Park and Pha Chang, Ban Yot, Yot Subdistrict, Song Khwae District, and Phu Huat, Sakat Subdistrict, Pua District were new localities recorded in this study.

Santisuk (1987) reported that this species is uncommon in evergreen forests. In addition, the specimen *Srisanga et al. 2884* (QBG) was collected in dry evergreen forest, at elevation of 800 m a.m.s.l., the specimen *La-onsri & Romkham 1311* (QBG) in limestone forest, and the specimen *Niyomdham & Puudjaa 7677* (BKF) in hill evergreen forest (= lower montane rain forest), at elevation of 800 m a.m.s.l. (see habitat).

Additional specimens examined. THAILAND. Northern: Nan [Sanian Subdistrict (noted Mae Sanian), in evergreen forest, 420 m alt., fr., 5 Aug 1926, *Winit 1788* (BK, BKF, K) (Santisuk 1973, 1974); Phu Huat, Sakat Subdistrict, Pua District, in hill evergreen forest, 800 m alt., fl., 19 May 2006, *Niyomdham & Puudjaa 7677* (BKF); Tham Sakoen National Park, Yot Subdistrict, Song Khwae District, in dry evergreen forest, 800 m alt., fl., 12 May 2006, *Srisanga et al. 2884* (QBG); Tham Sakoen National Park, Yot Subdistrict, Song Khwae District, in limestone forest, noted that fruits were green, 16 Dec 2010, *La-onsri & Romkham 1311* (QBG); Pha Chang, Ban Yot, Yot Subdistrict, Song Khwae District, in evergreen forest, 441 m alt., fl., 12 May 2011, *La-onsri, Tatiya & Satatha 1662* (QBG); Tham Sakoen National Park, near stream in evergreen forest, 600 m alt., fr., 27 Jul 2011, *La-onsri et al. 1869* (QBG)].

VIETNAM [Tonkin, Hoa Binh, 27 Aug 1926, *Poilane 13012* (P [P02862885]); Tonkin, Hoa Binh, fl., s.d., *Brillet 11* collected from the type locality (K [K000779292], P [P02862889]); Ninh Binh Province, Cuc Phuong National Park, noted with fruits, 16 Nov 2001, *Cuong 1548* (L [L3730433]); Annam, Nha Trang, noted that flowers white, 24 Apr 1923, *Poilane 6055* (L [L2815228]); Forêt sur le Song Cao de Song Trang à Binh Loi, près Nha Trang, fl., 16 Jul 1921, *Evrard 515* (L [L2815229]); Ninh Thuan Province, Ninh Hai District, Nui Chua National Park, fr., 16 Jan 2010, *Soejarto et al. DDS14712* (P [P03387600, P03387601]); **LAOS** [Xieng Khouang, 8 Nov 1920. *Poilane 2309* (BKF, L [L3730861], P [P02862887]); s.d., *Spire 228* (P [P02862886])].

Anatomical study

Leaves, stems (branches) and wood anatomy of *Fernandoa adenophylla*

This species has branched eglandular trichomes, and are uniseriate, unicellular. The unicellular trichomes have only one cell but are quite variable in length. Branched eglandular trichomes can be divided into two types: stellate (star-shaped, with many branches radiating outwards) and dendroid (have a tree-like branching form). Both trichomes are also found on the petioles, rachises, and inflorescences (peduncles, axes, and pedicels), except on the calyx, corolla, ovary, and fruits where is only found a dendroid trichome. (Fig. 6A–C) Peltate glandular trichomes that are sunken into epidermal cells on both surfaces. The cuticular ornamentation is deposited on the outer wall of the epidermal cells. The epidermal cells are arranged in a single layer on both surfaces, and are larger on the upper surface than on the lower one. The epidermal cells on the

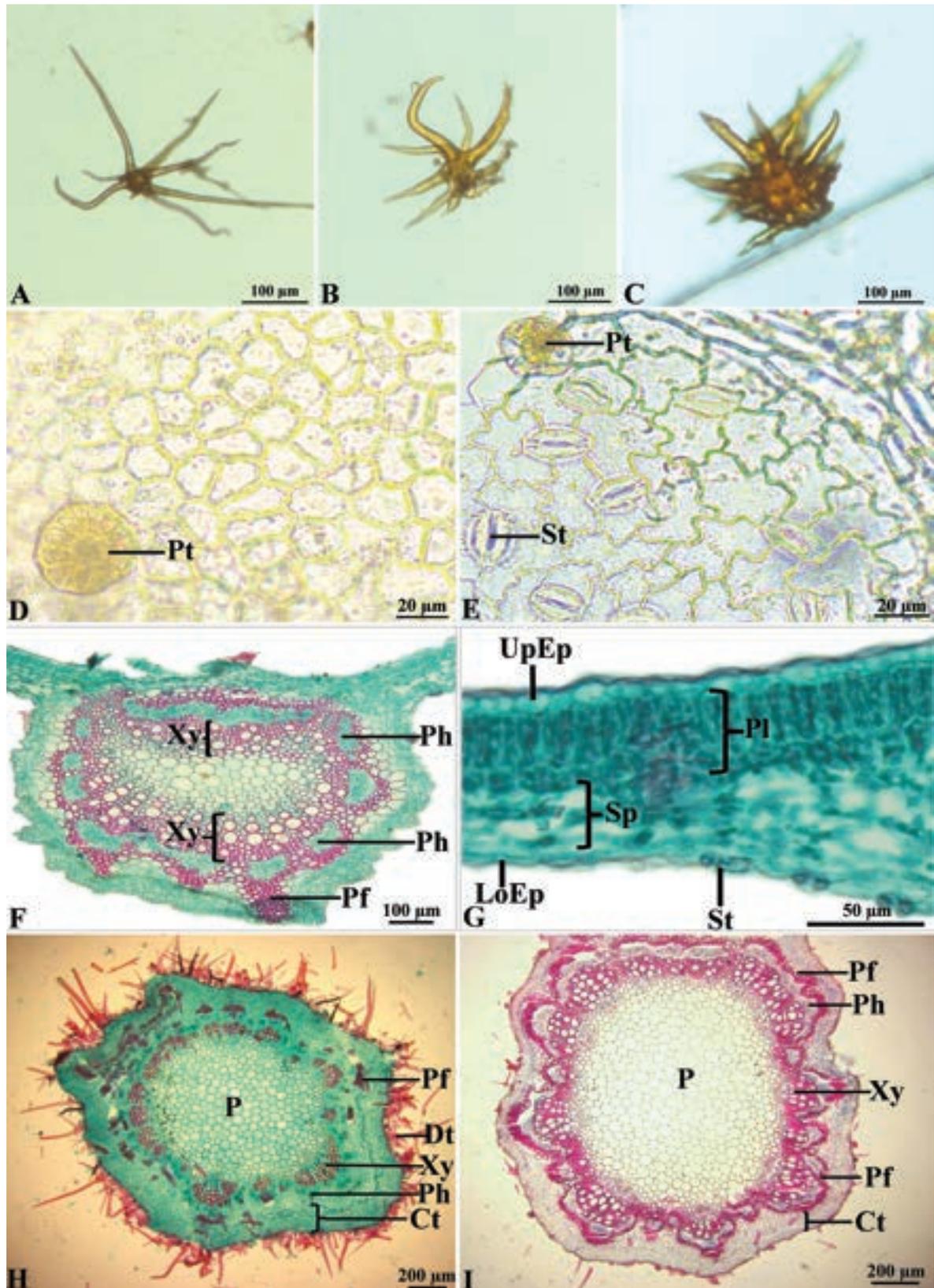


Figure 6. Leaf anatomy of *Fernandoa adenophylla* **A** stellate trichome **B, C** dendroid trichome **D** upper epidermis **E** lower epidermis **F, G** transverse section middle of leaflet **H** transverse section middle of immature rachis **I** transverse section middle of mature rachis. [Ct = cortex, Dt = dendroid trichome, LoEp = lower epidermis, P = pith, Pf = phloem fiber, Ph = phloem, Pl = palisade cells, Pt = peltate glandular trichome, Sp = spongy cells, St = stomata, UpEp = upper epidermis, Xy = xylem], the stain combination safranin and fast green.

upper surface are polygonal in shape with straight anticlinal walls, and on the lower surface are irregular in shape with undulate (wavy) anticlinal walls. The stomata are confined to the lower surface and are anomocytic. The mesophyll composed of palisade parenchyma (also called palisade mesophyll) underlying the upper epidermis and spongy parenchyma (also called spongy mesophyll) underlying the lower epidermis (bifacial leaf). The palisade parenchyma exhibits two layers: an upper tall one and a basal layer, about half in height, tightly packed cells and the spongy parenchyma comprised of loosely packed, irregularly shaped cells. In the midrib, the sclerenchymatous sheath of stele is made both by phloem fibers and lignified rays. Stele interpreted as two crescents, almost flat above and arched below, perhaps with two small bundles in the upper corners. The presence of sclerenchyma cells in the midrib is to provide support and protection for the leaf structure. (Fig. 6D–G).

The outline of the rachises in transverse section is 5-angular, it is channeled on the upper side. Stellate and dendroid trichomes are present as in the laminae. The epidermis in transverse section is circular or semicircular, and cells are usually smaller than cells in the ground tissue. The cortex of the young rachises is broader than the mature rachises. Parenchyma predominates in ground tissue, and fiber cells are present. The vascular bundles are completely ensheathed by sclerenchyma cells. The xylem is incompletely surrounded by phloem, interspaced with sclerenchyma cells. (Fig. 6H, I).

Secondary growth of stems (branches): The bark is made up of the periderm (also called outer bark), the cortex, and the phloem (also called inner bark). The periderm is 7–10 layered. The cortical parenchyma is 7–8 layered. The cells of the vascular cambium divide and supply secondary phloem and xylem. Fibers occur both in the primary and the secondary phloem. The pith is only parenchymatous. (Fig. 7A, B).

Fernandoa adenophylla has diffuse-porous wood. Vessels (pores) are solitary and form in groups of 2–4 cells or more, 20–100 µm in diam. Vessel density ranges from 4–20 vessels per mm². Axial parenchyma patterns are confluent. Rays are heterocellular, biseriate, sometimes uniseriate, with the procumbent cells 3–12 cells long, and with one row of the upright cells at both ends, and are sometimes homocellular with only the upright cells 2–3 cells long. The septate fibers are present. (Fig. 7C–E) The result of this study is consistent with Metcalfe and Chalk (1957) which reported wood anatomy of *Fernandoa*: rays are homocellular, sometimes heterocellular, with 4–11 cells long, and fibers are septate.

A comparison of wood anatomical characteristics of *Fernandoa adenophylla* with the previous studies of other two genera, *Dolichandrone* (Boonthasak and Ngernsaengsaruy 2021) and *Santisukia* (Meeprom et al. 2022) in the tribe Tecomeae of the family Bignoniaceae in Thailand is shown in Table 1.

Palynological study

Pollen morphology of *Fernandoa adenophylla*

The pollen grains of *Fernandoa adenophylla* are monads, isopolar, tricolpate, oblate, suboblate to oblate-spheroidal in shape. The size of the pollen grains is medium to large, the polar axis ranges between 29–55 µm, and the equatorial

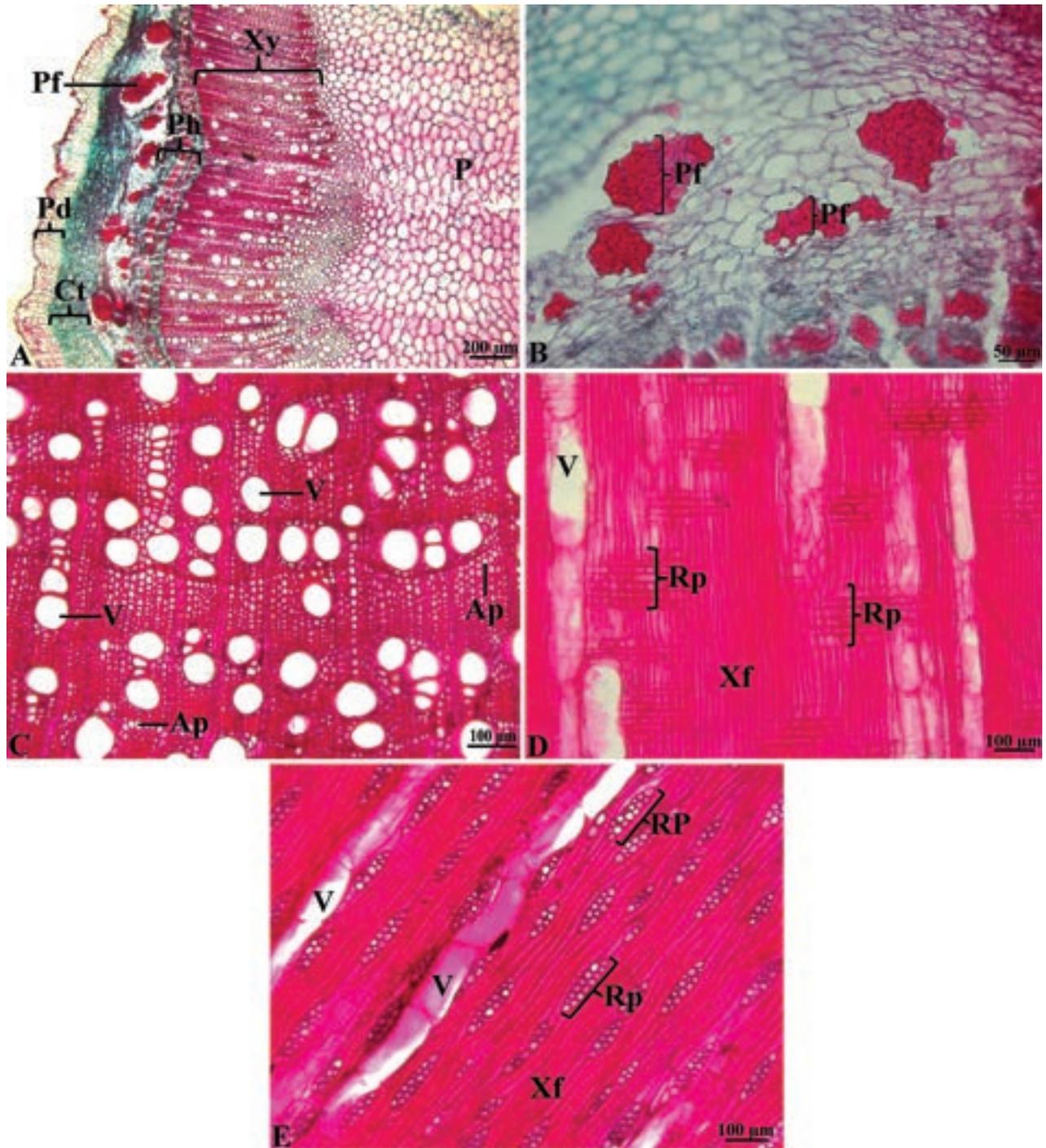


Figure 7. Stem (branch) and wood anatomy of *Fernandoa adenophylla* **A, B** transverse section secondary growth of stem **C–E** wood anatomy **C** transverse section **D** radial longitudinal section **E** tangential longitudinal section (shown in an oblique orientation) [Ap = axial parenchyma, Ct = cortex, P = pith, Pd = periderm, Pf = phloem fiber, Ph = phloem, Rp = ray parenchyma, V = vessel, Xf = xylem fiber, Xy = xylem], the stain combination safranin and fast green.

axis ranges between 27–54 μm . The exine sculpturing is reticulate. (Fig. 8) Santanachote (1981) reported that the pollen grains of this species are suboblate in shape, the polar axis ranges between 41–51 μm , and the equatorial axis ranges between 35–41 μm which shows slight differences in shape and size from this study.

Table 1. A comparison of wood anatomical characteristics of *Fernandoa adenophylla* with other two genera, *Dolichandrone* and *Santisukia* in the tribe Tecomeae of the family Bignoniaceae in Thailand.

Characters	<i>F. adenophylla</i>	<i>Dolichandrone</i>	<i>Santisukia</i>
Vessel arrangement	diffuse-porous	diffuse-porous	diffuse-porous
Vessel diameter (µm)	20–100	30–90	c. 100
Axial parenchyma	Confluent	banded, confluent	aliform, confluent
Ray parenchyma	biseriate, sometimes uniseriate heterocellular or uniseriate homocellular	uniseriate, sometimes biseriate heterocellular	biseriate, triseriate, tetraseriate heterocellular
Ray height (cells)	3–12	2–60	5–40
Ray width (rows of cells)	(1–)2	1(–2)	2–4
Fibers	Septate	septate	septate

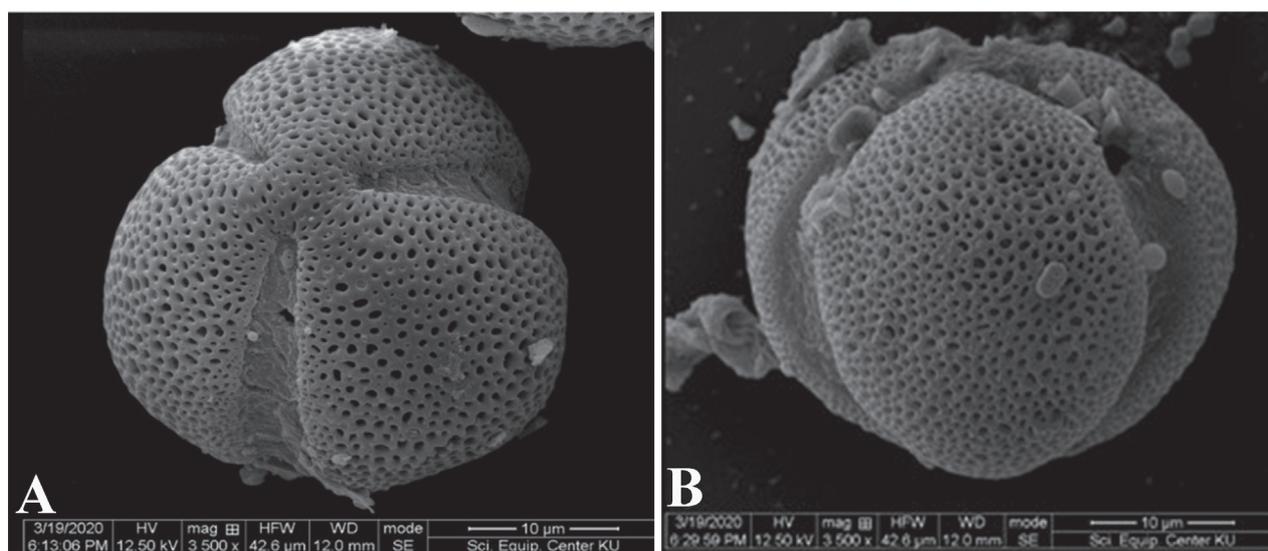


Figure 8. SEM micrographs of pollen grains of *Fernandoa adenophylla* **A** polar view **B** equatorial view.

Acknowledgements

We would like to thank the curators and staff of the BK, BKF and QBG for their assistance during visits and allowing access to the herbarium specimens, and those included in the digital herbarium databases of G, K (including K-W), L, P, and US. We are grateful to Dr Shuichiro Tagane for his kind and valuable advice about the type of *Fernandoa adenophylla*. We are grateful to the plant collectors of the genus *Fernandoa*. We also would like to thank Miss Pattarin Nunthamontree for the photographs of the herbarium specimens of *F. collignonii* deposited in QBG.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was funded by the International SciKU Branding (ISB), Faculty of Science, Kasetsart University.

Author contributions

Conceptualization: CN. Data curation: CN, NM. Formal analysis: CN. Funding acquisition: CN. Investigation: CN, NM, WB, YA, RT. Methodology: CN, WB, YA, RT. Project administration: CN. Resources: CN, YA. Supervision: CN. Writing – original draft: CN, YA. Writing – review and editing: CN, NM.

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Data availability

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

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