

Goniothalamus roseipetalus and *G. sukhirinensis* (Annonaceae): Two new species from Peninsular Thailand

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Abstract

Two new *Goniothalamus* species (Annonaceae), *G. roseipetalus* **sp. nov.** and *G. sukhirinensis* **sp. nov.**, are described from the southern limits of Peninsular Thailand (Narathiwat and Yala Provinces). Both new species resemble *G. macrophyllus*, *G. scortechinii* and *G. uvarioides*. The addition of these two new species brings the total number of *Goniothalamus* species in Thailand to 27. Separate identification keys are provided for flowering and fruiting specimens of the Thai species.

Keywords

Annonaceae, *Goniothalamus roseipetalus*, *Goniothalamus sukhirinensis*, new species, Thailand

Introduction

The genus *Goniothalamus* (Blume) Hook.f. & Thomson (Annonaceae subfam. Annonoideae tribe Annoneae: Chatrou et al. 2012; Guo et al. 2017) is widely distributed in lowland and submontane tropical forests across Southeast Asia (Thomas et al. 2017). It is characterised by pendent, protogynous flowers with two trimerous petal whorls, with the inner whorl forming a mitriform dome over the reproductive organs (a ‘type

III' chamber *sensu* Saunders 2010). The outer petals are typically larger than the inner and periodically block the apertures between the inner petals, thereby controlling pollinator access and enabling the flower to temporarily trap the pollinating beetles (Lau et al. 2016). The timing of the petal movements that regulate pollinator trapping and release are synchronised with the circadian rhythms of the beetles (Lau et al. 2017; Saunders 2020); this allows the plant to utilise beetles with diverse circadian activities, and also allows the staminate floral phase to be extended to promote pollen deposition and enhance interfloral movement of beetles. These floral characteristics provide a possible biotic explanation for the statistically significant increase in the evolutionary diversification rate recently reported for the genus (Xue et al. 2020).

Goniothalamus fruits are apocarpous, with distinct fleshy 'monocarps' that develop from individual carpels after fertilisation. Two contrasting seed dispersal systems have been inferred, correlated with differences in fruit and seed morphology (Tang et al. 2015a): the species that are dispersed by non-volant mammals typically have ramiflorous or cauliflorous fruits with large (often sessile) monocarps and hairy seeds; whereas the species that are bird-dispersed have fruits that are borne on young growth and have small stipitate monocarps with glabrous seeds.

Goniothalamus is comparatively species-rich, with over 130 species. Although the genus has never been comprehensively revised, there are several recent regional taxonomic studies, including Thailand (Saunders and Chalermglin 2008), Peninsular Malaysia (Saunders 2003), Sumatra (Saunders 2002) and Borneo (Turner 2014). Twenty-five *Goniothalamus* species have been recorded from Thailand (Saunders and Chalermglin 2008), with the majority (14 species) occurring in Peninsular Thailand, viz. *G. expansus* Craib, *G. giganteus* (Wall. ex) Hook.f. & Thomson, *G. latestigma* C.E.C.Fisch., *G. macrophyllus* (Blume) Hook.f. & Thomson, *G. malayanus* Hook.f. & Thomson, *G. ridleyi* King, *G. rotundisepalus* M.R.Hend., *G. scortechinii* King, *G. tapis* Miq., *G. tavoyensis* Chatterjee, *G. tenuifolius* King, *G. tortilipetalus* M.R.Hend., *G. undulatus* Ridl. and *G. uvarioides* King. Several other *Goniothalamus* species are recorded from Peninsular Malaysia, close to the Thai border (Saunders 2003), viz. *G. curtisii* King, *G. montanus* J.Sinclair and *G. subevenius* King. Recent fieldwork in Narathiwat and Yala Provinces of Peninsular Thailand has resulted in collections of two new species that are described here as *G. roseipetalus* and *G. sukhirinensis*. The species descriptions provided here are based on observations and measurements from living material.

New species descriptions

***Goniothalamus roseipetalus* Leerat., Chalermglin & R.M.K.Saunders, sp. nov.**

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Figs 1–3

Diagnosis. *Goniothalamus roseipetalus* resembles *G. scortechinii* and *G. uvarioides* but is distinguished by its leaves with generally fewer secondary veins (15–22 pairs), wider sepals (24–35 mm), and wider inner petals (8–11 mm). It is also distinguished

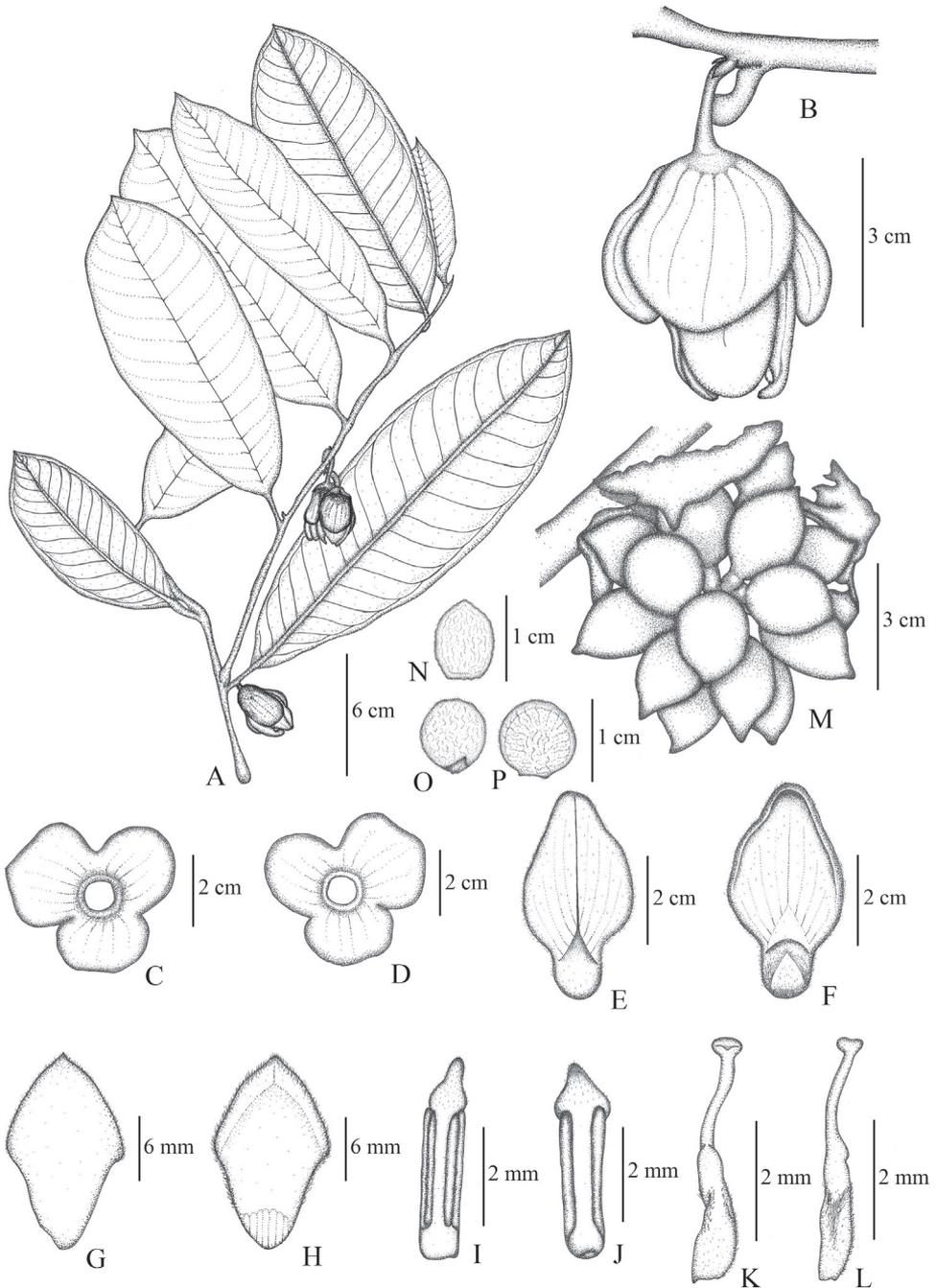


Figure 1. *Goniothalamus roseipetalus* sp. nov. **A** flowering branch **B** flower **C** calyx of fused sepals (abaxial) **D** calyx of fused sepals (adaxial) **E** outer petal (abaxial) **F** outer petals (adaxial) **G** inner petal (abaxial). **H** inner petal (adaxial) **I** stamen (abaxial) **J** stamen (adaxial) **K** carpel (abaxial) **L** carpel (adaxial) **M** fruit, composed of separate monocarps. **N–P** seeds (different orientations). Drawn by A. Somphrom **A–L** from *C. Leeratiwong 21–1708* (PSU) **M–P** from *C. Leeratiwong 21–1707* (PSU).

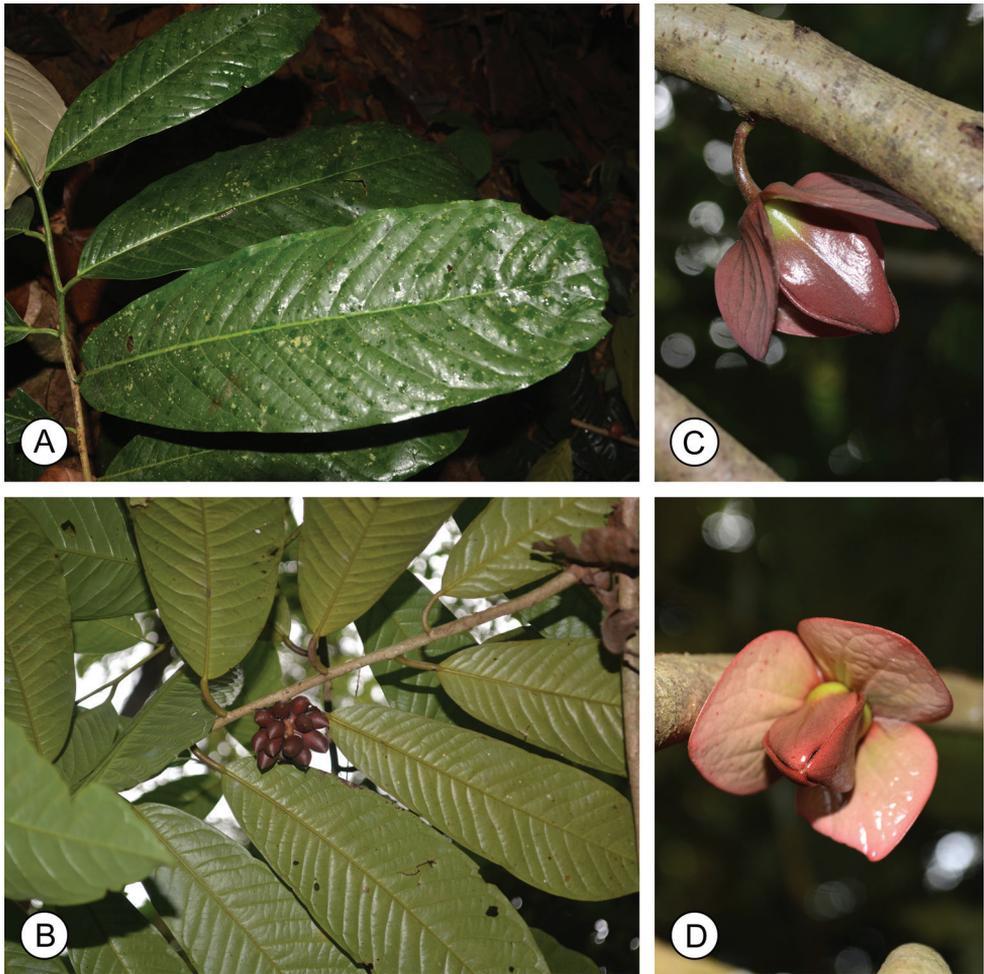


Figure 2. *Goniothalamus roseipetalus* sp. nov. **A** branch with leaves (adaxial) **B** fruiting branch with leaves (abaxial) **C, D** flowers. Photos by P. Chalermglin.

from *G. scortechinii* by its wider outer petals (14–25 mm), and is distinguished from *G. uvarioides* by its smaller, single-seeded monocarps (8–15 by 7–9 mm), borne on shorter stipes (3–5 mm).

Types. Thailand: Narathiwat: Cha Nae, Du Son Yo subdistrict, 400 m alt., 15 April 2021, *C. Leeratiwong 21–1706* (holotype PSU; isotypes BKF, KKU).

Description. Shrubs to small trees, to 4 m. Young branches glabrous. Leaf laminae 15–40 by 3–13 cm, length/width ratio 2.8–5, elliptic to oblanceolate, apex generally acuminate (rarely acute to obtuse), acumen 3–10 mm long, base broadly cuneate, chartaceous, glabrous ab- and adaxially (sometimes sparsely pubescent over midrib); midrib strongly prominent abaxially, sunken adaxially; secondary veins 15–22 pairs, plane adaxially; tertiary veins percurrent, slightly distinct, lacking a ‘granular’ appearance abaxially; petioles 12–22 mm by 1.5–2.5 mm, glabrous to sparsely pubescent. Flowers solitary, of-

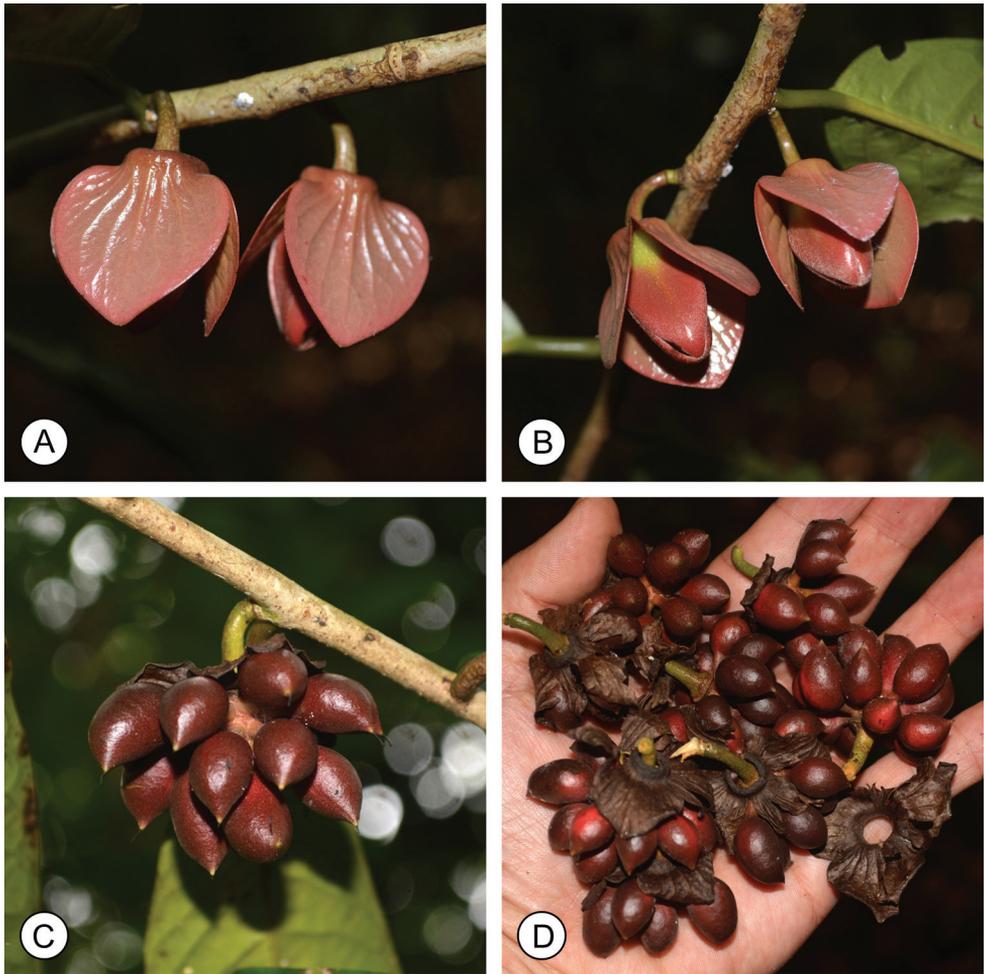


Figure 3. *Goniothalamus roseipetalus* sp. nov. **A, B** flowers **C, D** fruits showing persistent calyx. Photos by P. Chalermglin.

ten on main trunk (cauliflorous), rarely on older branches (ramiflorous), pendent; flowering pedicels 10–17 mm long, sparsely hairy; pedicel bracts ovate to broadly lanceolate, 2–4 by 2–3 mm. Sepals (violet-)pink, broadly ovate, 20–30 by 24–35 mm, basally connate (10–17 mm from base), apex rounded, glabrous ab- and adaxially, with sparsely hairy margins, venation distinct, 5–7-veined. Outer petals greenish-pink when young, (violet-)pink (green at claw) when mature, 25–45 by 14–25 mm with 4–10 mm-long claw, length/width ratio 1.7–2.2, fleshy, (lanceolate-)ovate, apex obtuse to mucronate, reflexed, sparsely hairy abaxially (more densely along margins basally), sparsely hairy (more densely apically) adaxially with velutinous basal region facing apertures between inner petals, midrib and venation indistinct ab- and adaxially. Inner petals 12–20 by 8–11 mm with 2–5 mm-long claw, length/width ratio 1.5–1.8, oblanceolate, densely hairy ab- and adaxially, greenish-pink when young, pale pink when mature, apex acute, lacking

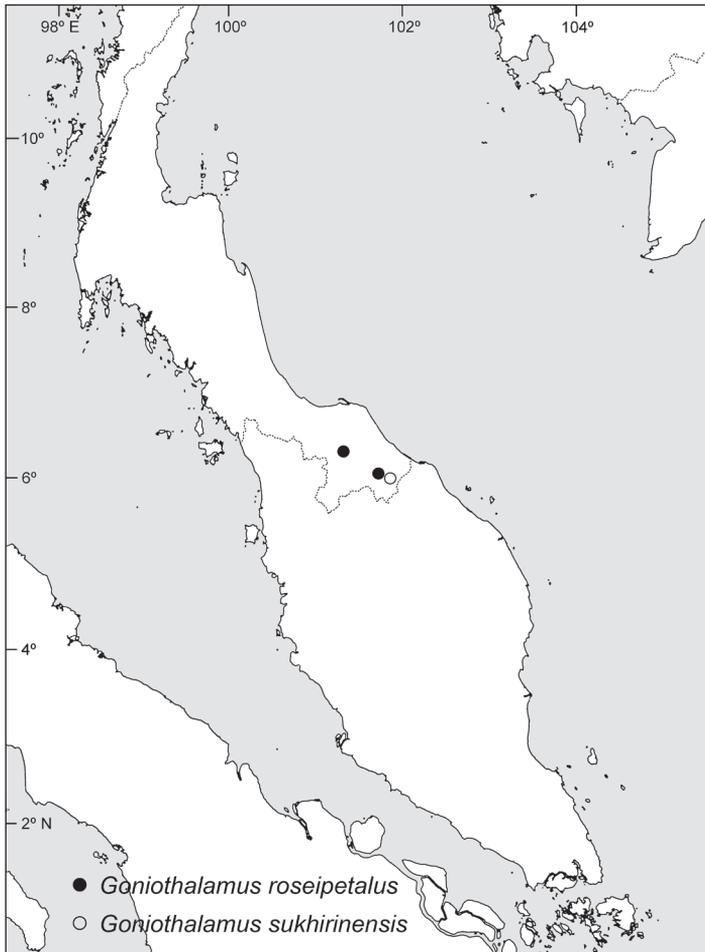


Figure 4. Distributions of *Goniotalamus roseipetalus* and *G. sukhirinensis*.

a glabrous lateral flange on the inner petal claws. Stamens numerous, narrowly oblong, 3–4 mm long; connectives apiculate, papillate. Carpels 20–35 per flower, ovary oblong, 2–2.5 mm long, with white hairs; stigma and pseudostyle 2–3 mm long, stigma subulate, glabrous. Fruits with persistent calyx, immature fruits greenish-pink, mature fruits (pinkish-)red; fruiting pedicels 10–20 by 2–2.5 mm, sparsely hairy to glabrous. Monocarps 5–20 per fruit, 1–2-seeded, 8–17 by 7–10 mm, length/width ratio 1.1–1.7, ellipsoid to ovoid, apex apiculate, apicule 0.5–1.5 mm long, smooth, sparsely hairy, glossy, pericarp 1–2 mm thick, stipes 3–6 by 1.5–2 mm, moderately hairy. Seeds with mucilage, 9–11 by 8–9 mm, length/width ratio 1.1–1.6, ovoid, testa sparsely pubescent, rugose.

Phenology. Flowering in March and April; fruiting in August (based on limited data).

Distribution and habitat. Endemic to Peninsular Thailand, where it occurs in Narathiwat and Yala Provinces (Fig. 4). Growing in shady and moist areas of tropical rainforests and forest margins between para-rubber plantations and remnant rainforests; 100–400 m alt.

Etymology. In reference to the red pigmentation of the petals.

Local name. Panan klip muang (ปานันกลีบม่วง) (general).

Additional specimens examined (paratypes). **Thailand:** Yala Province, Bannang Sata, 350 m alt., 1 August 2020, *C. Leeratiwong 20–1684* (PSU); Narathiwat Province: Cha Nae District, Du Son Yo subdistrict, 100 m alt., 6 March 2021, *C. Leeratiwong 21–1705* (PSU).

Discussion. Although *G. roseipetalus* is yet to be included in a molecular phylogenetic analysis, it shares several morphological similarities with species in a clade (nested within clade 'A1a' *sensu* Tang et al. 2015a, b) that comprises *G. loerzingii* R.M.K.Saunders, *G. macrophyllus*, *G. scortechinii*, *G. uvarioides* and *G. wrayi* King. These species were previously classified by Bân (1974) within *Goniothalamus* subgen. *Goniothalamus* sect. *Goniothalamus*, and are characterised by their essentially glabrous vegetative shoots and petioles, percurrent tertiary leaf venation, generally fused sepals with distinct venation, short inner petals, apiculate staminal connectives, relatively few carpels per flower, thick-cylindrical pseudostyles with a broad, hairy stigma, and seeds with a hairy testa. Although *G. roseipetalus* shares most of these diagnostic characters, its stigmas are glabrous.

Goniothalamus roseipetalus is morphologically most similar to *G. scortechinii* and *G. uvarioides*. It differs from these species, however, as it generally has fewer secondary veins in its leaves (15–22 pairs, vs [18–]21–26[–32] in *G. scortechinii* and 24–35 in *G. uvarioides*), larger sepals (20–30 by 24–35 mm, vs 8–24 by 8–23 mm in *G. scortechinii* and 12–16 by 5–13 mm in *G. uvarioides*), and wider inner petals (8–11 mm, vs 5–8 mm in *G. scortechinii* and 7–8.5 mm in *G. uvarioides*). It also has wider outer petals (14–25 mm) than *G. scortechinii* (8–14 mm), and can be distinguished from *G. uvarioides* by reference to its smaller monocarps (8–15 by 7–9 mm, vs 31–44 by 15–18 mm) with a single seed (vs four or five seeds per monocarp) and shorter stipes (3–5 mm, vs 12.5–17.5 mm). *Goniothalamus roseipetalus* also resembles the widespread species *G. macrophyllus*, although the latter species has creamy-white petals.

Goniothalamus roseipetalus also resembles *G. calycinus* J. Sinclair, a species that is endemic to Terengganu in Peninsular Malaysia (Saunders 2003). *Goniothalamus roseipetalus* differs, however, in its flower position (with flowers borne on young or older branches in *G. calycinus*), larger sepals (only 4.5–17 by 4–17 mm in *G. calycinus*), wider outer petals (only 7–14 mm wide in *G. calycinus*), larger inner petals (only 8–14 by 5–7.5 mm in *G. calycinus*), and by the absence of a persistent calyx in fruits of *G. calycinus*.

***Goniothalamus sukhirinensis* Leerat., Chalermglin & R.M.K.Saunders, sp. nov.**

urn:lsid:ipni.org:names:77221294-1

Figs 5, 6

Diagnosis. *Goniothalamus sukhirinensis* resembles *G. macrophyllus* and *G. scortechinii*, but is distinguished by its densely hairy shoots, numerous secondary veins (32–40 pairs per leaf), generally longer pedicels (flowering: 12–18 mm; fruiting: 20–25 mm), larger outer petals (34–37 by 18–22 mm), larger monocarps (20–27 by 9–13 mm) that are densely hairy, and longer seeds (13–17 mm).

Types. Thailand: Narathiwat: Sukhirin, Ban Yade village, Ma Mong subdistrict, 167 m alt., 6 March 2021, *C. Leeratiwong 21–1708* (holotype PSU; isotypes BKF, KKU).

Description. Shrubs to small trees, to 4 m. Young branches densely appressed-pubescent. Leaf laminas 28–50 by 7–16 cm, length/width ratio 3.1–4, (lanceolate-) oblong, apex generally acuminate to caudate (rarely acute to obtuse), acumen 7–20 mm long, base broadly cuneate, subcoriaceous, glabrous abaxially (sparsely hairy over midrib), sparsely pubescent adaxially (densely hairy over veins); midrib strongly prominent abaxially, sunken adaxially; secondary veins 32–40 pairs, plane adaxially; tertiary veins percurrent, distinct, lacking a ‘granular’ appearance abaxially; petioles 20–30 mm by 4–6 mm, densely pubescent. Flowers solitary or paired, often on main trunk (cauliflorous), rarely on older branches (ramiflorous), pendent; flowering pedicels 12–18 mm long, densely hairy; pedicel bracts ovate-triangular, 2.5–3 by 1–1.5 mm. Sepals greenish-pink, broadly ovate, 7–9.5 by 7.5–10 mm, basally connate (2.5–3 mm from base), apex acute, moderately hairy abaxially, sparsely hairy adaxially, venation indistinct. Outer petals greenish-yellow when young, whitish-yellow (green at claw) when mature, 34–37 by 18–22 mm with 3–5 mm-long claw, length/width ratio 1.6–1.9, fleshy, (lanceolate-)ovate, apex acuminate, densely hairy abaxially, moderately hairy adaxially with velutinous basal region facing apertures between inner petals, midrib raised adaxially, venation indistinct ab- and adaxially. Inner petals 13–15 by 7–8 mm with 2–3 mm long claw, length/width ratio 1.8–1.9, ovate-lanceolate, densely hairy abaxially, sparsely hairy distally adaxially, yellowish-green when young, pinkish-orange to reddish-brown when mature, apex acuminate, lacking a glabrous lateral flange on the inner petal claws. Stamens numerous, oblong, 2.5–3.7 mm long; connectives apiculate, papillate. Carpels 11–20 per flower, ovary oblong, 2–3 mm long, with white hairs; stigma and pseudostyle 2–2.5 mm long, stigma funnel-shaped, hairy. Fruits sometimes with persistent calyx, immature fruits brownish-green, mature fruits not seen; fruiting pedicels 20–25 by 2–3.5 mm, sparsely hairy. Monocarps 5–14 per fruit, single-seeded, 20–27 by 9–13 mm, length/width ratio 2–2.7, (obovoid-)ellipsoid, apex apiculate, apicule 5–8 mm long, smooth, densely hairy, glossy, pericarp 1–2 mm thick, stipes 7–15 by 2–3 mm, densely hairy. Seeds 13–17 by 8–10 mm, length/width ratio 1.6–1.7, ellipsoid, testa densely villose, slightly rugose.

Phenology. Flowering and fruiting in February and March (based on limited data).

Distribution and habitat. Endemic to Narathiwat Province, Peninsular Thailand (Fig. 4). Growing in shady and moist areas of tropical rainforests; 167–200 m alt.

Etymology. From the name Sukhirin, Narathiwat Province.

Local name. Ratchakhru khao (ราชครุขาว) (Narathiwat).

Additional specimen examined (paratype). Thailand: Narathiwat Province: Sukhirin District, Ban Yade village, Ma Mong subdistrict, 200 m alt., 28 February 2021, *C. Leeratiwong 21–1707* (PSU).

Discussion. As with the previous species, *G. sukhirinensis* is yet to be included in a molecular phylogenetic analysis but has strong morphological affiliations with a clade that comprises *G. loerzingii* R.M.K.Saunders, *G. macrophyllus*, *G. scortechinii*, *G. uvarioides* and *G. wrayi* King (nested within clade ‘A1a’ *sensu* Tang et al. 2015a, b). The morphological characteristics of this clade are detailed under *G. roseipetalus*, above.

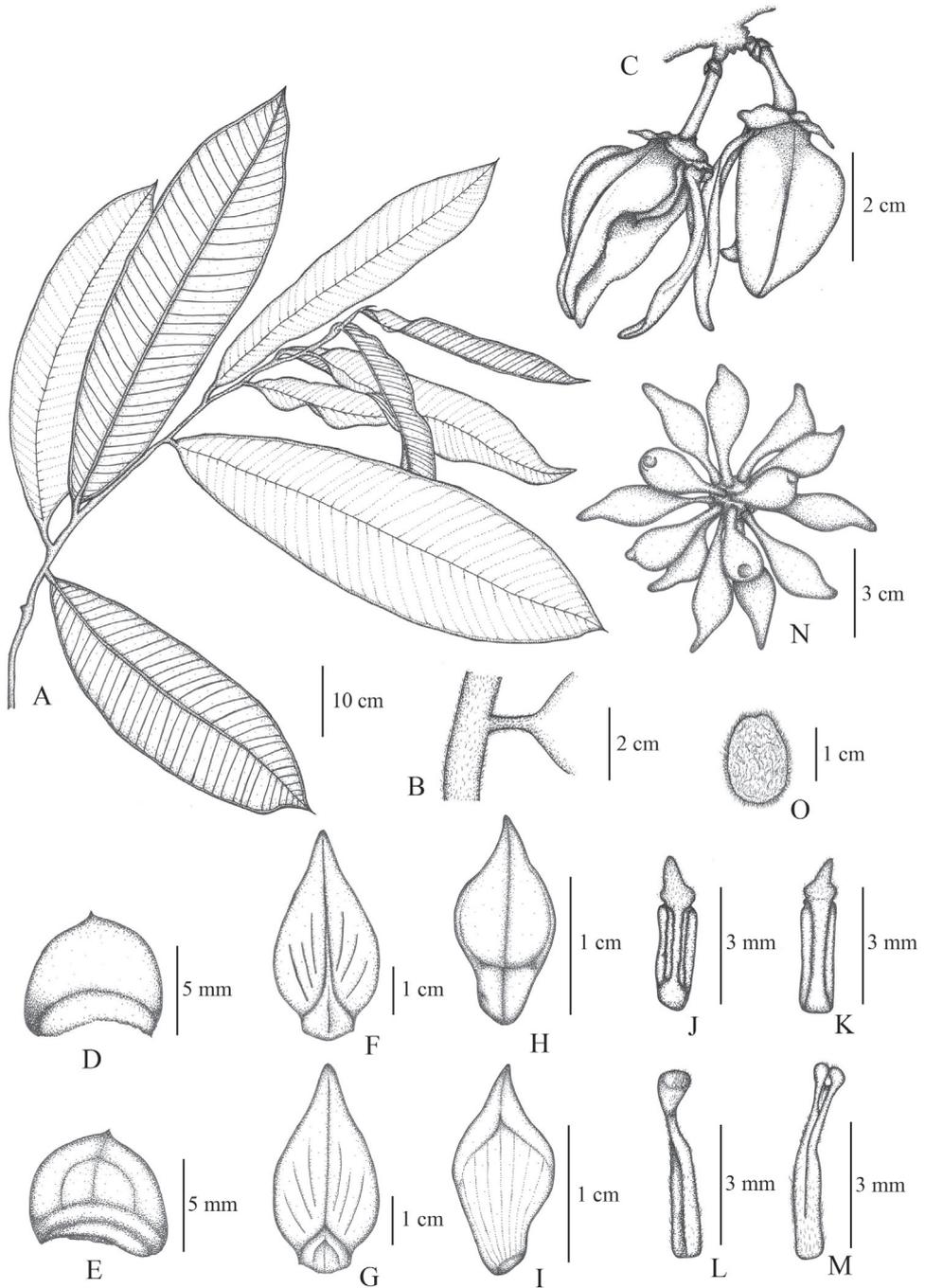


Figure 5. *Goniothalamus sukhirinensis* sp. nov. **A** vegetative branch **B** petiole, showing base of leaf lamina **C** flowers **D** sepal (abaxial) **E** sepal (adaxial) **F** outer petal (abaxial) **G** outer petals (adaxial) **H** inner petal (abaxial) **I** inner petal (adaxial) **J** stamen (abaxial) **K** stamen (adaxial) **L** carpel (abaxial) **M** carpel (adaxial) **N** fruit, composed of separate monocarps **O** seed with hairy surface. Drawn by A. Somphrom from C. Leeratiwong 21–1708 (PSU).



Figure 6. *Goniiothalamus sukhirinensis* sp. nov. **A** leaf (abaxial) **B, C** flowers **D** fruit. Photos by P. Chalermglin.

Goniiothalamus sukhirinensis resembles *G. macrophyllus* and *G. scortechinii*, but differs in several key characters: densely hairy shoots (vs glabrous to medium-hairy); numerous secondary veins (32–40 pairs per leaf, vs 12–23 in *G. macrophyllus* and [18–]21–26[–32] in *G. scortechinii*); generally longer flowering pedicels (12–18 mm, vs 5–11.5 mm in *G. macrophyllus* and 8–13 mm in *G. scortechinii*); larger outer petals (34–37 by 18–22 mm, vs 10–28 by 4.5–11.5 mm in *G. macrophyllus* and 20–33 by 8–14 mm in *G. scortechinii*); longer fruiting pedicels (20–25 mm, vs 7–19 in *G. macrophyllus* and 8–20 mm in *G. scortechinii*); larger monocarps (20–27 by 9–13 mm, vs 8–15 by 7.5–10 mm in *G. macrophyllus* and 9–18 by 6–10 mm in *G. scortechinii*) that are densely hairy (vs subglabrous to medium-hairy); and longer seeds (13–17 mm, vs 8.5–12 mm in *G. macrophyllus* and 8–11 mm in *G. scortechinii*). *Goniiothalamus*

sukhirinensis also differs from *G. macrophyllus* as its leaves lack the fine ‘granular’ appearance of the latter species (due to the immersion of tertiary and higher-order veins: Saunders, 2002), and has longer monocarp stipes (7–15 mm, vs up to 1.8 mm in *G. macrophyllus*).

Key to *Goniothalamus* species in Thailand (flowering specimens)

- 1a Stamen connective apex apiculate 2
 2a Young branches densely hairy to velutinous 3
 3a Leaf laminas 28–50 cm long, with 32–40 pairs of secondary veins; sepals 7–9.5 by 7.5–10 mm; outer petals 34–37 by 18–22 mm; inner petals 13–15 by 7–8 mm ***G. sukhirinensis* sp. nov.**
 3b Leaf laminas 50–76 cm long, with 24–32 pairs of secondary veins; sepals 30–40 by 28–30 mm; outer petals 60–80 by 30–40 mm; inner petals ca. 35 by ca. 17 mm ***G. cheliensis* H.H.Hu**
 2b Young branches glabrous to hairy 4
 4a Flowers in large fascicles, exclusively from woody tubercles at base of trunk ***G. ridleyi* King**
 4b Flowers solitary or in pairs, not exclusively from base of trunk 5
 5a Adaxial surface of outer petals with glabrous or sparsely hairy region facing apertures between inner petals 6
 6a Leaves with 24–35 pairs of secondary veins ***G. uvarioides* King**
 6b Leaves with 11–22 pairs of secondary veins ***G. tapis* Miq.**
 5b Adaxial surface of outer petals with velutinous region facing apertures between inner petals 7
 7a Flowering pedicels 20–37 mm long; carpels 50–100 per flower ***G. tortilipetalus* M.R.Hend.**
 7b Flowering pedicels 5–19 mm long; carpels 8–50 per flower 8
 8a Leaves with 9–12 pairs of secondary veins; stamen connective apex distinctly tapered; carpels 8–10 per flower ***G. tavoyensis* Chatterjee**
 8b Leaves with 12–26(–32) pairs of secondary veins; stamen connective apex not distinctly tapered; carpels 11–50 per flower 9
 9a Tertiary venation reticulate 10
 10a Sepals 11–18.5 mm long, 8–15.5 mm wide; outer petals 21–46 mm long, 4.5–18 mm wide, yellow; inner petal length/width ratio 3–6.5; stamens 95–120 per flower ***G. calvicarpus* Craib**
 10b Sepals 14–29 mm long, 12–26 mm wide; outer petals 36–104 mm long, 14–24 mm wide, green; inner petals length/width ratio 1.8–3.6; stamens 100–200 per flower ***G. griffithii* Hook.f. & Thomson**
 9b Tertiary venation percurrent 11
 11a Sepals 24–35 mm wide; outer petals 14–25 mm wide ***G. roseipetalus* sp. nov.**
 11b Sepals 4–23 mm wide; outer petals 4.5–14 mm wide 12

- 12a Leaf laminas (sub-)coriaceous, with fine “granular” texture abaxially (due to immersion of tertiary and lower order veins); leaves with 12–23 pairs of secondary veins ***G. macrophyllus* (Blume) Hook.f. & Thomson**
- 12b Leaf laminas papyraceous, without fine ‘granular’ texture abaxially; leaves with (18–)21–26(–32) pairs of secondary veins..... ***G. scortechinii* King**
- 1b Stamen connective apex truncate **13**
- 13a Inner petal claws with distinct glabrous lateral flange **14**
- 14a Flowering pedicels 7–23 mm long; stigma subulate..... **15**
- 15a Outer petals 23–43 mm long, 12–23 mm wide; carpels 40–100 per flower ***G. sawtehii* C.E.C.Fisch.**
- 15b Outer petals 10.5–32 mm long, 5.5–17.5 mm wide; carpels 10–54 per flower ***G. undulatus* Ridl.**
- 14b Flowering pedicels 2–11.5 mm long; stigma fusiform or funnel-shaped **16**
- 16a Young branches densely hairy to velutinous ***G. tamirensis* Pierre ex Finet & Gagnep.**
- 16b Young branches glabrous to hairy **17**
- 17a Flowering pedicels 5–11.5 mm long; sepal venation generally indistinct; outer petals 12.5–73 mm long; stigma fusiform.... ***G. laoticus* (Finet & Gagnep.) Bân**
- 17b Flowering pedicels 2–6 mm long; sepal venation distinct; outer petals 8.5–39 mm long; stigma funnel-shaped **18**
- 18a Leaf laminas 8–14 cm long, 1.5–4 cm wide; petioles 3.5–7 mm long; sepals 3–9 mm long, 3.5–6 mm wide; outer petals 8.5–15 mm long, 3.5–8 mm wide, very densely hairy ab- and adaxially; inner petals 6.5–10 mm long, 3–4.5 mm wide; ovary glabrous ***G. elegans* Ast**
- 18b Leaf laminas 12.5–24.5 cm long, 4–8.5 cm wide; petioles 5–15 mm long; sepals 7.5–12.5 mm long, 5.5–11 mm wide; outer petals 23–39 mm long, 7–15 mm wide, glabrous to hairy ab- and adaxially; inner petals 10–16 mm long, 5.5–9 mm wide; ovary sparsely hairy ***G. latestigma* C.E.C.Fisch.**
- 13b Inner petal claws without glabrous lateral flange **19**
- 19a Tertiary leaf venation generally reticulate; outer petals with velutinous region at base of adaxial surface (facing aperture between inner petals); inner petals velutinous adaxially **20**
- 20a Sepals 2.5–3.5 mm long, basally connate, venation indistinct; outer petal venation distinct; stigma fusiform ***G. repevensis* Pierre ex Finet & Gagnep.**
- 20b Sepals 4.5–19 mm long, free, venation distinct; outer petal venation indistinct; stigma subulate or funnel-shaped **21**
- 21a Young branches glabrous; outer petal length/width ratio 3.4–5.2 ***G. expansus* Craib**
- 21b Young branches very sparsely to densely hairy; outer petal length/width ratio 1.6–3.8 **22**
- 22a Leaves with fine “granular” texture abaxially (due to immersion of tertiary and lower order veins); flowers slightly supra-axillary... ***G. rotundisepalus* M.R.Hend.**
- 22b Leaves without fine “granular” texture abaxially; flowers axillary ***G. tenuifolius* King**

- 19b Tertiary leaf venation percurrent; outer petals with glabrous or sparsely hairy region at base of adaxial surface (facing aperture between inner petals); inner petals glabrous to densely hairy adaxially.....**23**
- 23a Flowering pedicels 20–48 mm long; sepals 7–15 mm long; outer petals 68–113 mm long, 28–63 mm wide ***G. giganteus* (Wall. ex) Hook.f. & Thomson**
- 23b Flowering pedicels 8–16(–21) mm long; sepals 2–8 mm long; outer petals 16–50(–62) mm long, 7–22(–32) mm wide **24**
- 24a Inner petals glabrous adaxially; ovaries densely hairy
..... ***G. malayanus* Hook.f. & Thomson**
- 24b Inner petals (densely) hairy adaxially (sometimes glabrous towards base); ovaries glabrous to sparsely hairy **25**
- 25a Leaf laminas 23–32 cm long, with 14–21 pairs of secondary veins; stamens 50–160 per flower; carpels 4–11 per flower.....
..... ***G. aurantiacus* R.M.K.Saunders & Chalermglin**
- 25b Leaf laminas 17–25.5 cm long, with 13–16 pairs of secondary veins; stamens ca. 180–200 per flower; carpels ca. 18–20 per flower..... **26**
- 26a Flowering pedicels densely hairy; outer petals densely hairy abaxially, very densely hairy adaxially, venation indistinct; inner petals very densely hairy abaxially..
..... ***G. maewongensis* R.M.K.Saunders & Chalermglin**
- 26b Flowering pedicels very sparsely hairy; outer petals subglabrous abaxially, glabrous adaxially, venation distinct; inner petals sparsely hairy abaxially.....
..... ***G. rongkhanus* R.M.K.Saunders & Chalermglin**

Key to *Goniothalamus* species in Thailand (fruiting specimens)

- 1a Adaxial surface of leaves with very prominent secondary veins **2**
- 2a Leaf laminas 50–76 cm long, 13–22 cm wide, with 24–32 pairs of secondary veins; leaf midrib densely hairy to velutinous; petioles 17–30 mm long, velutinous; monocarps densely hairy..... ***G. cheliensis* H.H.Hu**
- 2b Leaf laminas 12.5–39.5 cm long, 3.5–9.5(–11.5) cm wide, with 10–22 pairs of secondary veins; leaf midrib glabrous to sparsely hairy; petioles 4–16 mm long, glabrous to hairy; monocarps glabrous to hairy **3**
- 3a Monocarps distinctly warty ***G. giganteus* (Wall. ex) Hook.f. & Thomson**
- 3b Monocarps smooth or finely rugulose **4**
- 4a Fruits restricted to trunk; fruiting pedicels 19–36 mm long
..... ***G. tortilipetalus* M.R.Hend.**
- 4b Fruits not restricted to trunk; fruiting pedicels 10–19 mm long..... **5**
- 5a Tertiary leaf venation percurrent; fruits without persistent calyx; monocarps 16–40 mm long, 8–13(–17) mm wide; seeds 13–20 mm long, with (sparsely) hairy testa ***G. malayanus* Hook.f. & Thomson**
- 5b Tertiary leaf venation reticulate; fruits with persistent calyx; monocarps 10–14 mm long, 7–8 mm wide; seeds 10–12 mm long, with glabrous testa..... **6**
- 6a Monocarps red; seeds slightly rugose..... ***G. calvicarpus* Craib**
- 6b Monocarps yellow-brown; seeds smooth ***G. griffithii* Hook.f. & Thomson**

- 1b Adaxial surface of leaves with impressed or only slightly prominent secondary veins 7
- 7a Tertiary leaf venation percurrent 8
- 8a Monocarps apiculate 9
- 9a Young branches densely hairy; leaves with 32–40 pairs of secondary veins; fruiting pedicels 20–25 mm long; monocarps 20–27 mm long, densely hairy; monocarp stipes 7–15 mm long ***G. sukhirinensis* sp. nov.**
- 9b Young branches glabrous to hairy; leaves with 12–23 pairs of secondary veins; fruiting pedicels 7–20 mm long; monocarps 8–15 mm long (very) sparsely hairy; monocarp stipes up to 5 mm long 10
- 10a Fruits without persistent calyx
..... ***G. macrophyllus* (Blume) Hook.f. & Thomson**
- 10b Fruits with persistent calyx ***G. roseipetalus* sp. nov.**
- 8b Monocarps not apiculate 11
- 11a Monocarps 7–18 mm long, 6–10 mm wide 12
- 12a Leaf laminas 22–40(–50) cm long, 5.5–12(–19.5) cm wide, with (18–)21–26(–32) pairs of secondary veins ***G. scortechinii* King**
- 12b Leaf laminas 8–20 cm long, 2.5–6 cm wide, with 8–14 pairs of secondary veins ***G. tenuifolius* King**
- 11b Monocarps 16–56 mm long, 13–29 mm wide 13
- 13a Leaf laminas 32–48 cm long, 7–15.5 cm wide, with 24–35 pairs of secondary veins; fruits without persistent calyx ***G. uvarioides* King**
- 13b Leaf laminas 14–32 cm long, 3.5–9.5 cm wide, with 11–21 pairs of secondary veins; fruits with persistent calyx 14
- 14a Fruits restricted to base of trunk; fruiting pedicels 30–130 mm long
..... ***G. ridleyi* King**
- 14b Fruits not restricted to base of trunk; fruiting pedicels 10–22 mm long 15
- 15a Monocarps subsessile or with stipe ≤ 1 mm long; seeds 18–19 mm wide, rugose ***G. maewongensis* R.M.K.Saunders & Chalermglin**
- 15b Monocarp stipes 2–8 mm long; seeds 13–17 mm wide; smooth to slightly rugulose 16
- 16a Leaf laminas 23–32 cm long, with 14–21 pairs of secondary veins; fruiting pedicels ca. 14 mm long; seeds 19–27 mm long, length/width ratio 1.5–1.7
..... ***G. aurantiacus* R.M.K.Saunders & Chalermglin**
- 16b Leaf laminas 17–25 cm long, with 13–16 pairs of secondary veins; fruiting pedicels 16–22 mm long; seeds 15–22 mm long, length/width ratio 1.1–1.5
..... ***G. rongkalanus* R.M.K.Saunders & Chalermglin**
- 7b Tertiary leaf venation reticulate 17
- 17a Monocarps 22–56 mm long, 14–21 mm wide, with prominent longitudinal ridge; up to 7 seeds per monocarp ***G. laoticus* (Finet & Gagnep.) Bân**
- 17b Monocarps 8.5–26 mm long, 6–11 mm wide, without longitudinal ridge; 1 or 2 seeds per monocarp 18
- 18a Fruiting pedicels 8–20 mm long 19
- 19a Young branches (very densely) hairy 20

- 20a Leaf laminas glossy adaxially; monocarp stipes 6.5–16.5 mm long; seeds 9–11.5 mm long..... *G. undulatus* Ridl.
- 20b Leaf laminas matt adaxially; monocarp stipes 3–6 mm long; seeds 11–14 mm long *G. sawtehii* C.E.C.Fisch.
- 19b Young branches glabrous to hairy.....21
- 21a Leaf lamina tertiary venation (clearly) distinct..... *G. expansus* Craib
- 21b Leaf lamina tertiary venation indistinct to \pm distinct22
- 22a Leaf laminas with 9–12 pairs of secondary veins; monocarps greenish-yellow, ca. 19 mm long; seeds 17–18 mm long *G. tavoyensis* Chatterjee
- 22b Leaf laminas with 11–16 pairs of secondary veins; monocarps (dark) red, 10–14 mm long; seeds 9–12 mm long *G. tapis* Miq.
- 18b Fruiting pedicels 4–8 mm long23
- 23a Monocarps greenish-yellow24
- 24a Young branches hairy; leaf laminas 12.5–24.5 cm long, 4–8.5 cm wide; monocarps smooth; seeds ca. 17.5 mm long..... *G. latestigma* C.E.C.Fisch.
- 24b Young branches (very) sparsely hairy; leaf laminas 10.5–13.5(–16) cm long, 3–5 cm wide; monocarps very finely rugulose; seeds ca. 9.5 mm long.....
..... *G. repevensis* Pierre ex Finet & Gagnep.
- 23b Monocarps red.....25
- 25a Fruits without persistent calyx; seeds subglabrous to hairy *G. tapis* Miq.
- 25b Fruits with persistent calyx; seeds glabrous26
- 26a Leaf laminas 1.5–4 cm wide, \pm glossy adaxially..... *G. elegans* Ast
- 26b Leaf laminas 3.5–8.5 cm wide, (\pm) matt adaxially27
- 27a Monocarps glabrous; monocarp stipes 4.5–13 mm long
..... *G. tamirensis* Pierre ex Finet & Gagnep.
- 27b Monocarps very sparsely hairy; monocarp stipes 2–3 mm long.....
..... *G. rotundisepalus* M.R.Hend.

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Saxifraga viridiflora (Saxifragaceae), an unusual new species from Guangxi, China

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Abstract

Saxifraga viridiflora, a remarkable new species of the genus *Saxifraga* sect. *Irregulares* (Saxifragaceae) from Guangxi, is described and illustrated herein. This new species morphologically differs from all known *S. sect. Irregulares* taxa by its greenish petals, verruculose sepals, and thick leathery leaf blade abaxially scarlet with white spots.

Keywords

China, Guangxi, new species, Saxifragaceae, taxonomy

Introduction

Saxifraga Linnaeus, the largest genus of Saxifragaceae, comprises more than 440 species widely distributed throughout the Northern Hemisphere (Pan et al. 2001; Tkach et al. 2015a, b). Previous molecular phylogenetic studies suggested that *Saxifraga* is monophyletic, providing that *S. sect. Micranthes* (Haw.) D. Don is excluded

* The authors contributed equally to this study.

(Soltis et al. 1996; Prieto et al. 2013; Deng et al. 2015; Tkach et al. 2015a, b). Recent molecular phylogenetic research covered at least 13 sections and 9 subsections within the genus (Tkach et al. 2015b). *S.* sect. *Irregulares* Haw., characterized by zygomorphic flowers with two elongated petals and stamens with club-shaped filaments (Tkach et al. 2015b), is the ancestral clade of *Saxifraga* first described by Haworth (Haworth 1803; Soltis et al. 2001; Zhang et al. 2015; Tkach et al. 2015b; Zhang et al. 2019b). This section currently comprises 16 species mainly distributed in East Asia (Magota et al. 2021).

China has a vast territory with a wide range of complex and diverse topographies and soils and covering several climate types, which contribute to the wealth of Chinese botanical diversity (Sun et al. 2017; Chen et al. 2018). Twelve species of *Saxifraga* sect. *Irregulares* are native to China, including the recently reported new species, *S. daqiaoensis* F.G.Wang & F.W.Xing (Wang et al. 2008), *S. kegangii* D.G.Zhang, Y.Meng & M.H.Zhang (Zhang et al. 2017), *S. luoxiaoensis* W.B.Liao, L.Wang & X.J.Zhang (Zhang et al. 2018), *S. shennongji* L.Wang, W.B.Liao & J.J.Zhang (Zhang et al. 2019a), and *S. damingshanensis* W.B.Liao, W.Y.Zhao & J.H.Jin (Zhao et al. 2019).

In 2021, we inadvertently found a peculiar plant photograph posted on Baidu Tieba (<https://tieba.baidu.com/>), one of the most used Chinese communication platforms, and immediately deemed it to be a new species of *Saxifraga* sect. *Irregulares*, as it possesses zygomorphic flowers with two elongated petals and stamens with club-shaped filaments, but its petals are greenish, which cannot be found in any existing species of *Saxifraga* sect. *Irregulares*. We contacted the author of this photograph, Mr. Luo Dexuan, for phenological and geographical information regarding this specimen, and conducted fieldwork for this undescribed specimen. Subsequent morphological comparisons supported the status of the taxon as a new species, and it is described herein.

Taxonomy treatment

Saxifraga viridiflora X.J.Zhang, T.Deng, J.T.Chen & H.Sun, sp. nov.

urn:lsid:ipni.org:names:77221295-1

Figs 1, 2

Type. CHINA. Guangxi: Guilin City, Yongfu County, Baishou Town, 109°46'58.99"E, 25°5'15.5"N, 586 m alt., 27 June 2021, X.J. Zhang, D.X. Luo Zhangxj98 (Holotype: KUN!; Isotypes: JIU!, SYS!).

Diagnosis. *Saxifraga viridiflora* is easily distinguished from any other species of *Saxifraga* sect. *Irregulares* by having greenish petals (vs. white petals). *S. viridiflora* morphologically resembles *S. epiphylla* and *S. kegangii*, but is distinct from the latter two in its leaf blade abaxially scarlet with white spots (vs. abaxially greenish/reddish with brown or yellow-green spots), and sepals with verruculose surface (vs. sepals without verruculose surface) (Table 1).

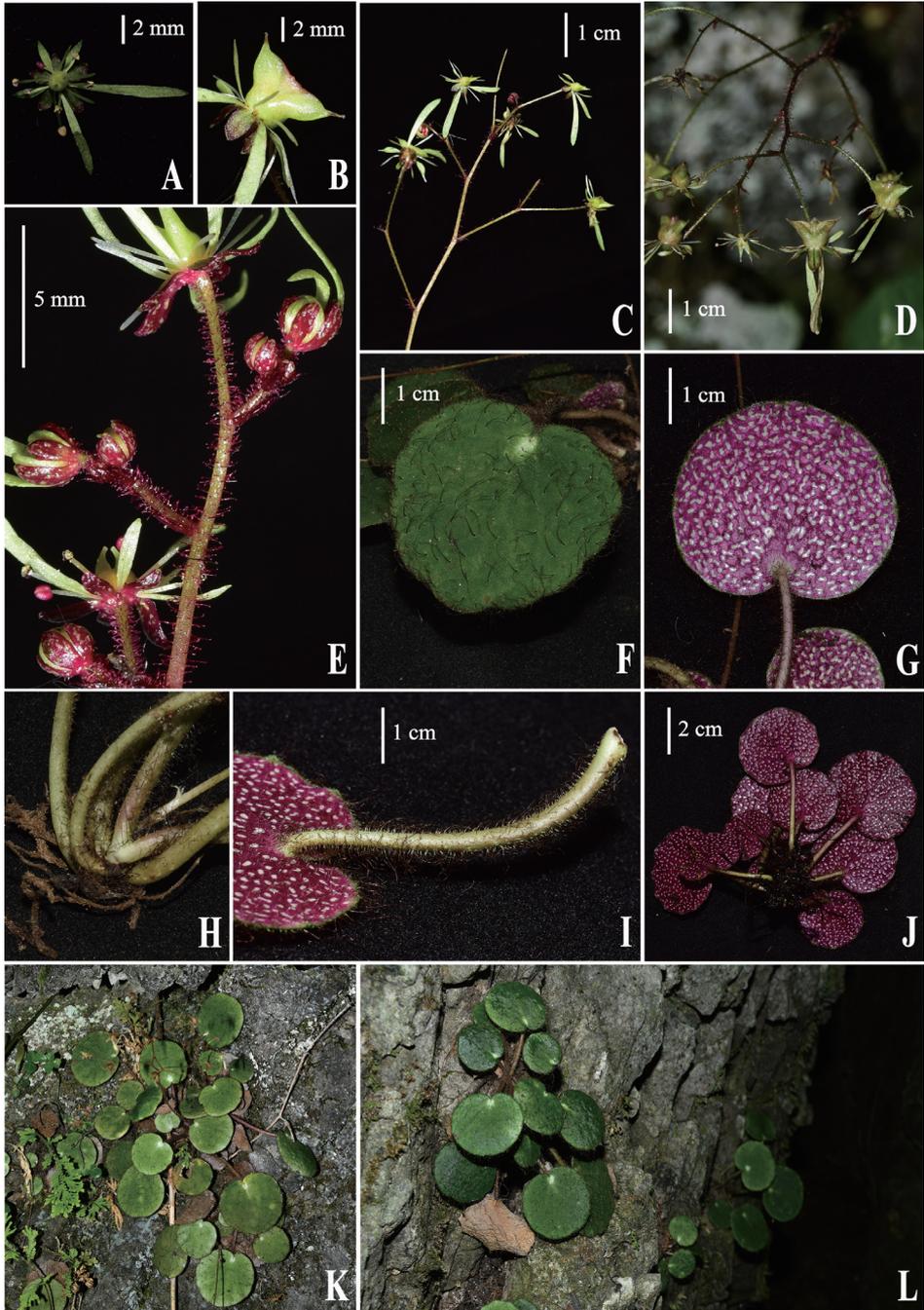


Figure 1. *Saxifraga viridiflora* X.J.Zhang, T.Deng, J.T.Chen & H.Sun, sp. nov. **A** flower, petals 5, greenish **B** fruit, capsule winged when mature **C** inflorescence **D** infructescence **E** pedicels glandular pubescent; sepals red, glabrous, abaxially white verruculose **F** adaxial leaf surface dark green, crisped villous **G**, **J** abaxial leaf surface scarlet, with white spotted, crisped villous **H** rhizomes crisped villous, petiole base unsheathed **I** petiole with crisped villous **K**, **L** plants and habitat.



Figure 2. Photograph of the holotype of *Saxifraga viridiflora* X.J.Zhang, T.Deng, J.T.Chen & H.Sun, sp. nov. (Zhangx98, KUN1519096).

Table 1. Diagnostic characters of *Saxifraga viridiflora* and comparison with other related species of *S.* sect. *Irregulares*.

Characters	<i>S. viridiflora</i> sp. nov.	<i>S. epiphylla</i>	<i>S. kegangii</i>
Foliar embryo	absent	present	absent
Leaf shape	reniform to orbicular	ovate	fan-shaped to orbicular
Leaf margin	shallowly crenate to subentire	undulate, thickly dentate	entire or 8–10-crenate
Leaf texture	thick leathery to fleshy	leathery	leathery
Abaxial surface of leaf blade	scarlet, with white spotted	gray-green to red, with brown or yellow-green spotted	gray-green, with yellow-green spotted
Trichomes on leaf	both surfaces crisped villous	both surfaces glandular hispid	adaxially glabrous, abaxially glabrous or sparsely hispid
Petals	greenish	white, the base of three smallest petal with yellow spot	white, the base of three smallest petal with yellow spot
Sepals	red, glabrous, abaxially white verruculose	greenish, abaxially and marginally glandular hairy, without verruculose	greenish, abaxially and marginally glandular hairy, without verruculose

Description. Perennial herbs, 12–30 cm tall. Stolons absent. Rhizomes rather short. Leaves all basal; petiole 5–12 cm long, crisped villous dark-purple (ca. 6 mm); leaf blade reniform, thick leathery, 2.5–4.0 cm long × 3.5–5.3 cm wide, base cordate, margin undulate, apex obtuse, both surfaces crisped villous dark-purple (5.0–9.0 mm long), adaxially greenish, abaxially purple or dark red, with white spots. Inflorescence paniculate, ca. 20 cm long. 5–10-flowered; branches 2.0–3.0 cm long, glandular pubescent, 1–2-flowered; pedicels slender, 1.0–2.0 cm long, glandular pubescent. Flowers zygomorphic; sepals 5, spreading to reflexed, narrowly ovate, 2.5–3.5 mm long × 1.5–2 mm wide, glabrous, abaxially red, with white verruculose, adaxially greenish, 3–5-veined, apex obtuse. Petals 5, greenish, margin entire, glabrous, apex acute; the three smallest lanceolate, 3.0–4.0 mm long × 1.0–1.2 mm wide, 3-veined; the two largest lanceolate oblong, 0.7–1.4 cm long × 1.0–1.2 mm wide, 3-veined. Stamens 10, 3.2–4.0 mm long. Ovary ovoid, 1.5–2.0 mm long, disc obscure; styles divergent ca. 1.0–1.8 mm long. Capsule beaks winged when mature, carpels 5–7 mm long × 3–4 mm wide. Seeds elliptic, the two sides slightly bent, ca. 0.6 mm long.

Etymology. The specific epithet refers to the flowers of this new species that are green throughout the flowering period, differing from those of all other known *Saxifraga* sect. *Irregulares* species. The Chinese name is given as “绿花虎耳草” (lǜ huā hǔ ěr cǎo), referring to the greenish petals of the new species.

Phenology. In a two year personal observation of this new species in its native range, Guangxi. Guilin City (Luo Dexuan, pers. comm.), *S. viridiflora* was flowering from April to July and fruiting from June to August.

Distribution and ecology. The new species, *Saxifraga viridiflora*, is currently known only from Yongfu County, Guangxi Province, China. It was observed to grow on dry limestone under dense jungles at altitudes between 500 and 600 m.

Paratypes. CHINA. Guangxi. Guilin City, Yongfu County, Baishou Town, 109°46'49.3"E, 25°5'16.1"N, 547 m alt., 10 July 2021, X.J. Zhang, L.J. Li, J.Y. Peng, P.R. Luo Deng12030 (KUN); same locality, 575 m alt., 27 June 2021, X.J. Zhang, D.X. Luo Zhangxj99 (KUN).

Discussion

The new species *Saxifraga viridiflora* has zygomorphic flowers and stolons absent, which indicate a position in *S.* sect. *Irregulares* ser. *Rufescentes*. *Saxifraga viridiflora* is distinct from all known sect. *Irregulares* taxa by its greenish petals, verruculose sepals, and thick leathery leaf blade abaxially scarlet with white spots.

Geographically, *Saxifraga viridiflora* was only found in Yongfu County of Guangxi Province, China. It grows only on dry rocks under dense jungles in limestone area, whereas other related species of sect. *Irregulares* usually grow on damp cliffs and rocks nearby valleys. Here we argue that the environmental heterogeneity plays an important role in the differentiation of the species in sect. *Irregulares*, given the leaf blade of *Saxifraga viridiflora* is thick leathery or fleshy (grow on dry rocks), while the leaf blade of other related species of sect. *Irregulares* are mainly papery or leathery (grow on damp rocks).

Notably, only seven species of *Saxifraga* sect. *Irregulares* were recorded in “Flora of China” (Pan et al. 2001). However, six new species of *S.* sect. *Irregulares* were discovered in China in recent years, provided that *Saxifraga viridiflora* is counted. Furthermore, most of these new species were confined to a narrow geographical range. Species richness of *S.* sect. *Irregulares* has been quite underrated, and more field investigations and phylogenetic analyses are needed to infer its biodiversity and speciation history.

Since several new species of *S.* sect. *Irregulares* have been published in recent years, we include here an identification key to include all species known so far for this section.

Identification key to *Saxifraga* sect. *Irregulares*

- | | | |
|----|--|---------------------------|
| 1 | Stolons arising from axils of basal leaves, filiform | <i>S. stolonifera</i> |
| – | Stolons absent..... | 2 |
| 2 | Aerial stems developed; leaves cauligenous | <i>S. sendaica</i> |
| – | Aerial stems not developed; leaves radical..... | 3 |
| 3 | Leaf blade abaxially spotted..... | 4 |
| – | Leaf blade abaxially usually concolorous | 12 |
| 4 | Leaf blade elliptic to oblong, base cuneate..... | <i>S. kwangsiensis</i> |
| – | Leaf blade fan-shaped or ovate to broadly so, base cordate or peltate..... | 5 |
| 5 | Leaf blade with foliar embryos in sinus adaxially | <i>S. epiphylla</i> |
| – | Leaf blade without foliar embryos | 6 |
| 6 | Leaf base peltate..... | 7 |
| – | Leaf base cordate..... | 8 |
| 7 | Leaf blade papyraceous, apex acute | <i>S. mengtzeana</i> |
| – | Leaf blade thickly coriaceous, apex obtuse..... | <i>S. daqiaoensis</i> |
| 8 | Leaf margin lobed | 9 |
| – | Leaf margin shallowly crenate to subentire..... | 11 |
| 9 | Capsule beaks winged; leaf lobes margin irregularly dentate.... | <i>S. luoxiaoensis</i> |
| – | Capsule beaks divergent; leaf lobes margin entire..... | 10 |
| 10 | Abaxial surface of leaf blade purple spotted..... | <i>S. damingshanensis</i> |
| – | Abaxial surface of leaf blade yellow spotted | <i>S. shennongii</i> |

11	Leaf blade abaxially gray-green with yellow-green spots.....	<i>S. kegangii</i>
–	Leaf blade abaxially scarlet with white spots.....	<i>S. viridiflora</i>
12	Longest petal serrate at margin.....	<i>S. fortunei</i>
–	Longest petal entire at margin.....	13
13	Leaf cleft.....	14
–	Leaf shallowly lobed.....	15
14	Upper petals nearly lanceolate, not spotted.....	<i>S. acerifolia</i>
–	Upper petals widely ovate, spotted.....	<i>S. cortusifolia</i>
15	Bracts leafy.....	<i>S. nipponica</i>
–	Bracts linear.....	16
16	Flowering stem and inflorescence reddish long glandular villous.....	
	<i>S. rufescens</i>
–	Flowering stem and inflorescence shortly glandular pubescent....	<i>S. imparilis</i>

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Notes on the systematics of *Cuscuta* sect. *Subulatae* (subg. *Grammica*) with the description of *Cuscuta mantiqueirana*, a new species from Brazil

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Abstract

Cuscuta mantiqueirana Costea, S.S. Silva & Sim.-Bianch. a new species from montane cloud forests of the Serra da Mantiqueira, Brazil, is described and illustrated. The morphological and phylogenetic analyses revealed that the new species belongs to sect. *Subulatae* of subg. *Grammica*. The new species is related to *C. odorata* var. *botryoides*, *C. rotundiflora* and *C. globiflora* from which it differs in narrower calyx lobes and the presence of four stomatiferous lobes or projections at the distal part of the ovary. A detailed morphological comparison with *C. odorata* var. *botryoides*, morphologically the most similar taxon, is provided along with the geographical distribution, ecology and host range of the species. The morphological and phylogenetic relationships of the new species, as well as the diversity of stomatiferous projections, are discussed in the broader context of sect. *Subulatae* and subg. *Grammica*. *Cuscuta boliviana* var. *paranensis* is considered a synonym of *C. odorata* var. *botryoides*.

Keywords

Convolvulaceae, dodders, ITS, morphology, parasitic plant, phylogeny, systematics

Introduction

With ca. 30 species, *Cuscuta* sect. *Subulatae* (Engelm.) Costea & Stefanović is the largest infrageneric group of subg. *Grammica* (Lour.) Peter, and of *Cuscuta* L. in general (Costea et al. 2015). The section was recently circumscribed (Costea et al. 2015) based on a core of species included by Engelm. (1859) in subsect. *Subulatae*. This infrageneric clade has diversified in South America (Yuncker 1922, 1932; Stefanović et al. 2007; García et al. 2014), but it also contains two African species, *C. kilimanjari* Oliv. (distributed across Tropical East Africa, Central Africa and Madagascar) and *C. blepharolepis* Welw. ex Hiern. (a more enigmatic taxon known only from two collections in Guinea and Angola, in Western Africa). Although *C. blepharolepis* has not yet been sampled, *C. kilimanjari* was found to be nested deeply within this clade, as sister to *C. cristata* Engelm. (S. Brazil to N. Argentina), strongly suggesting long-distance dispersal (Stefanović et al. 2007; García et al. 2014). Flowers of sect. *Subulatae* are among the largest in *Cuscuta*, often fleshy, and in many species apparently cross-pollinated (Wright et al. 2012). Infrastaminal scales, which are unique structures with defence role in *Cuscuta* flowers (Riviere et al. 2013), are very diverse in shape, size and number of fimbriae; in a few species they are entirely reduced while in others they possess densely papillate fimbriae (Riviere et al. 2013). Pollen is also more varied among species than in other sections of subg. *Grammica*; it can be 3, 4, 5 or 6–7-colpate, and tectum can be imperforate, perforate, microreticulate, or reticulate (Welsh et al. 2010). Styles are thick, cylindrical or subulate, and stigmas are large, convoluted and lobed (Yuncker 1932; Wright et al. 2011). The fruit is usually dehiscent (Ho and Costea 2018) with large seeds (Olszewski et al. 2020). Plastome evolution studies have revealed extensive losses of plastid genes, including the otherwise highly conserved small and large ribosomal subunits (Braukmann et al. 2013).

Engelm. (1859), Yuncker (1921, 1922, 1923, 1932) and Hunziker (1947, 1949, 1950) described the majority of taxa in sect. *Subulatae*, but this clade has not been revised at species level to date. Among the practical obstacles towards a taxonomic revision of this section are the scarcity of herbarium material available and notoriously difficult DNA extraction from herbarium specimens. After plants dry, they often become brown-blackish and more difficult to analyze morphologically. Plastid sequences, which have been used extensively to reconstruct phylogeny of the entire genus (García et al. 2014), subgenus *Grammica* (Stefanović et al. 2007), and multiple clades of the latter subgenus (e.g., Costea et al. 2008, 2011a, 2011b, 2013, 2020; Costea and Stefanović 2009), cannot be employed for this section because of the plastome reductions (Braukmann et al. 2013).

The objective of this study is to report a new species in sect. *Subulatae*, as well as to discuss its putative relationships with other taxa in this group. The new species has been discovered independently both among herbarium specimens and by doing field work.

Materials and methods

Cuscuta specimens from the following herbaria were examined and annotated: AAU, B, BAB, BM, BR, BRIT, CAS, CEN, CORD, CTES, DAO, DIAM, E, ESA, F, G, GH, HB, HRCB, HUEFS, HUFU, HUSC, IAC, JEPS, K, L, LIL, LP, LPB, LPS, MA, MBM, MEL, MERL, MEXU, MICH, MO, NY, OXF, P, PACA, PMSP, QCNE, R, RB, RSA, S, SGO, SI, SJRP, SP, SPF, SPSF, TEX, TRTE, UB, UCR, UEC, UPCB, UPRRP, UPS, US, W, and WLU (Herbaria acronyms from Thiers 2018-continuously updated). In addition, we conducted a series of targeted fieldtrips to Serra da Mantiqueira, which included Itatiaia National Park (Rio de Janeiro), São José dos Campos, São Francisco Xavier, Pindamonhangaba, Campos do Jordão (São Paulo) and Camanducaia (Minas Gerais) to observe the species in the field and collect additional samples for the molecular analysis. We also provisionally assessed the conservation status of the new species using georeferenced herbarium specimens mapped in GeoCAT (Bachman et al. 2011). This rapid geospatial analysis tool determines the extent of occurrence (EOO) and the area of occupancy (AOO) and assigns a conservation status based on criteria B1 and B2 established by IUCN Standards and Petitions Subcommittee (2019). The GeoCAT file is available at: <https://1drv.ms/u/s!Aj8HnxOfiCaFgpo6hJhTTuzBNhFV2w?e=BLBf0i>.

Molecular phylogenetic analyses

Of the 28 collections of individuals belonging to the new species *C. mantiqueirana*, three specimens (Appendix 1) were found to be of sufficient quality and quantity for molecular studies. To infer the phylogenetic affinities of this species within *Cuscuta* sect. *Subulatae*, we obtained sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA). DNA extractions, polymerase chain reaction (PCR) reagents and conditions, amplicon purifications, cloning, and sequencing procedures follow Stefanović et al. (2007) and Stefanović and Costea (2008). The sequences generated in this study have been submitted to GenBank (accession numbers MZ389688–MZ389691). Using Se-AL v.2.0a11 (Rambaut 2002), newly obtained sequences were incorporated into previously aligned nrITS matrix of accessions from *Cuscuta* sect. *Subulatae* (Stefanović et al. 2007; Stefanović and Costea 2008; deposited in TreeBASE under study number S1929). Based on these, more inclusive analyses, we selected *C. microstyla* Engelm. as a functional outgroup.

Phylogenetic analyses were conducted under parsimony and maximum likelihood using PAUP* v4.0b10 (Swofford 2002). Sequence data were treated as unordered and all changes were equally weighted. Gaps in the alignments were treated as missing data. Given the moderate number of terminal units, the parsimony searches were conducted with a Branch-and-Bound algorithm, ensuring recovery of all of the most parsimonious (MP) trees. The full heuristic searches for maximum likelihood (ML) trees were performed under the general time-reversible (GTR) model of DNA substitution

(Lanave et al. 1984), with the rate of variation among nucleotides following a discrete gamma distribution and allowing for invariable sites (GTR+G+I), involving 100 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping, and MULTREES option on. All model parameters were estimated from data, except the base composition, where empirical frequencies have been used. The support for clades was inferred by nonparametric bootstrapping (Felsenstein 1985), under parsimony, using 1,000 heuristic bootstrap pseudoreplicates, TBR branch swapping, and MULTREES option on. Support for a relationship was considered weak if bootstrap value was < 70%, moderate if between 70 and 90%, and strong if > 90%.

Microscopy

Flowers, fruits and seeds removed from herbarium specimens were steeped in gradually warmed 50% ethanol, which was then allowed to boil for a few seconds to rehydrate tissues. An ethanol solution is more suitable for rehydration than simple water because it removes some of the dark pigments that result after drying, and at the same time, it hardens the tissues, which are very delicate in the *Cuscuta* flowers. For basic morphology, flowers were dissected under a Nikon SMZ 1500 stereomicroscope and imaged with PaxCam Arc digital camera equipped with a PAX-IT 8.2 (MIS Inc. 2021, Villa Park, Illinois) imaging software. Numerous photographs illustrating details of the floral and fruit morphology for all taxa, including their type collections, are made available on the Digital Atlas of *Cuscuta* website (Costea 2007-onwards). To examine finer (micro)morphological features, rehydrated flowers, fruits and seed samples were dehydrated through an ethanol series (50%, 70%, 85%, 95%, and 100%; each step 1h) and then critically point dried with Tousimis Autosamdri-931. Samples were mounted on aluminum stubs and sputter-coated with 30 nm of gold using Emitech K550 sputter coater. Examination, imaging and measurements were made using a Hitachi SU1510 scanning electron microscope (SEM) at 5–10 kV.

Results

Molecular phylogeny

The parsimony analysis resulted in 12 MP trees [length = 161; consistency index (CI) = 0.907; retention index (RI) = 0.957]. The maximum likelihood analysis resulted in a single ML tree, topologically fully compatible with the strict consensus of the MP trees. The ML phylogeny was selected to illustrate the inferred relationships in this section, including the placement of *C. mantiqueirana*, as well as branch lengths (Fig. 1). Based on the strong support, as measured by bootstrap values, and the sequence divergence, as indicated by the branch lengths, molecular data revealed three major lineages within *Cuscuta* sect. *Subulatae* (Fig. 1). These results are fully consistent with our previous findings (Stefanović et al. 2007; Stefanović and Costea 2008).

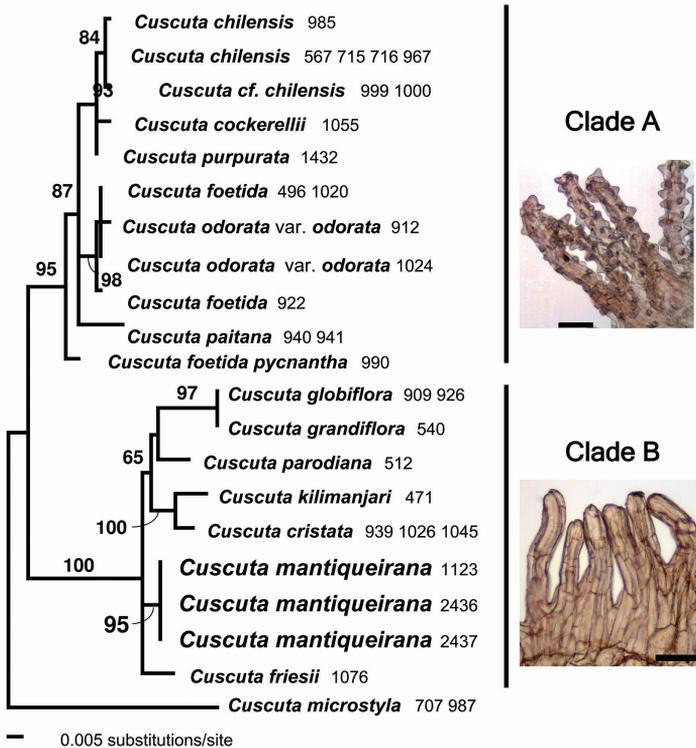


Figure 1. Phylogenetic relationships among species of *Cuscuta* sect. *Subulatae* shown as a phylogram obtained from maximum likelihood analysis of nrITS sequence data under the GTA+G+I model of DNA evolution. Tree is rooted using *C. microstyla* as a functional outgroup. Numbers following species names correspond to DNA accessions (Appendix 1). Bootstrap values are indicated. Photos represent fimbriae of infrastaminal scales: papillate in clade A and not papillate in clade B. Scale bars: 100 μm.

Cuscuta microstyla formed a distinct lineage within sect. *Subulatae* (Fig. 1). This Chilean-Argentinean species is restricted to high Andes, and represents an exception in the section in its relatively small flowers and indehiscent fruits. The second lineage (Clade A; 95% BS; Fig. 1) consisted of species (e.g., *C. chilensis* Ker Gawl., *C. cockerellii* Yunck., *C. foetida* Kunth, *C. odorata* Ruiz & Pav. var. *odorata*, *C. paitana* Yunck., *C. purpurata* Yunck.; Fig. 1) with tubular-campanulate flowers, papillate infrastaminal scales (Riviere et al. 2013), and 3-colpate pollen grains (Welsh et al. 2010) primarily distributed along the Andes (Ecuador, Peru, Bolivia, Chile). Finally, the third lineage (clade B; 100% BS; Fig. 1) that also included *C. mantiqueirana*, is characterized by flowers with rotate or globose to urceolate corollas, infrastaminal scales without or with only a few papillae (Riviere et al. 2013), and usually (although some exceptions are known) 4–6-colpate pollen grains (Welsh et al. 2010). This lineage includes *C. cristata* Engelm., *C. globiflora* Engelm., *C. friesii* Yunck., *C. grandiflora* Kunth, *C. kilimanjari*, *C. parodiana* Yunck. (Fig. 1; clade B), and most likely, based on their morphological

similarity, several other species that could not be included in the molecular analysis: *C. odorata* Ruiz & Pav. var. *botryoides* Engelm., *C. rotundiflora* Hunz., and *C. boliviana* Yunck. Members of this morphologically identified clade occur east of the Andes in Argentina, Uruguay and Brazil, but a few are found along the Andes (Colombia to Chile). An identification key for the taxa of clade B is included below. Among these taxa, *C. mantiqueirana* is morphologically most similar, and geographically closest to *C. odorata* var. *botryoides*, and a comparison between the two taxa is provided in Table 1. Based on our current sampling, *C. mantiqueirana* is reciprocally monophyletic and molecularly distinct from other members of this clade, as evidenced by the branch length subtending it and strong bootstrap support (Fig. 1). The molecular results agree with the morphological distinctiveness of all the species considered, suggesting that taxa for which DNA could not be extracted, but which are morphologically distinct, will also be validated as discrete lineages when molecular data become available.

Identification key for taxa within clade B (see Fig. 1) of section *Subulatae*

- 1 Infrastaminal scales with densely papillate fimbriae..... **(Clade A)**
- Infrastaminal scales without papillae on fimbriae or only with a few distal papillae (scales sometimes absent in *C. kilimanjari*) **2 (Clade B)**
- 2 Corolla tubular-cylindrical becomes tubular-urceolate at fructification
..... ***C. parodiana***
- Corolla rotate or campanulate becomes globose to urceolate-globose at fructification **3**
- 3 Corolla membranous, rotate ***C. friesii*, *C. argentinana***
- Corolla fleshy, campanulate becomes globose to urceolate-globose at fructification..... **4**
- 4 Fruit indehiscent to late irregularly-dehiscent..... ***C. cristata***
- Fruit circumscissile dehiscent **5**
- 5 Infrastaminal scales absent or with a few fimbriae; Africa ***C. kilimanjari***
- Infrastaminal scales well developed with numerous fimbriae; South America **6**
- 6 Corolla lobes erect-connivent; stigmas conical ***C. globiflora***
- Corolla lobes spreading or reflexed; stigmas globose or depressed..... **7**
- 7 Stamen filaments and styles evidently subulate; stigmas 1–1.5 mm wide.....
..... ***C. rotundiflora***
- Stamen filaments and styles cylindrical or only slightly subulate; stigmas 0.3–0.7 mm wide **8**
- 8 Pedicels obconical; flowers (4–) 5–7 mm long; external calyx lobes usually not carinate; capsule with a collar around the interstylar aperture
..... ***C. mantiqueirana***
- Pedicels cylindrical; flowers 3.8–5 mm long; external calyx lobes carinate; capsule without a collar around the interstylar aperture
..... ***C. odorata* var. *botryoides* (= *C. boliviana* var. *paranensis*)**

Taxonomic treatment

Cuscuta mantiqueirana Costea, S.S.Silva, Sim.-Bianch., sp. nov.

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Figs 2, 3, 4; Table 1

Diagnosis. *Cuscuta mantiqueirana* is similar to *C. odorata* var. *botryoides*, *C. rotundiflora* and *C. globiflora* because of their corolla that becomes globose at fructification and their well-developed scales with numerous fimbriae, but it differs from all these taxa in narrower calyx lobes, the presence of four stomatiferous lobes or projections, 0.4–0.6 mm long at the ovary apex, which form a collar at the fruit stage, as well as 3-colpate pollen grains with reticulate tectum. It differs from *C. odorata* var. *botryoides* in the obconical pedicels and larger flowers, 4.5–7 mm long (pedicels are cylindrical and flowers 5.5–5 mm long in *C. odorata* var. *botryoides*). From *C. rotundiflora* it differs in the infrastaminal scales equalling or exceeding the corolla tube, cylindrical stamen filaments and styles, as well as stigmas 0.5–0.6 mm wide (in *C. rotundiflora* the scales are shorter than corolla tube, filaments and styles are subulate and stigmas 1–1.5 mm wide). From *C. globiflora* it differs in the spreading to reflexed corolla lobes and globose-depressed stigmas (in *C. globiflora*, corolla lobes are erect-connivent and stigmas conical).

Type. BRAZIL. Minas Gerais: Camanducaia, Monte Verde. Parasita com ramos desde alaranjados até vináceos, sempre em local sombreado e humido. Flores amarelas, 22 IV 1999, R. Simão-Bianchini 1241 (holotype: SP!; isotypes: K!, NY!, MBM!, R!, SPF!, UB n.v., UEC!, WLU!)

Description. *Stems* medium to coarse, yellow-orange, purple-tinged or entirely purple. *Inflorescences* paniculiform-racemiform, occasionally glomerulate, usually not confluent; *pedicels* 1.5–4 mm, thick, gradually tapering into the fleshy, conical receptacle; *bracts* 1 at the base of cymes, 1.1–2.5 mm long and 0.8–1.6 mm wide, fleshy, triangular-ovate, rounded, not carinate, margins entire. *Flowers* 5-merous, (4–) 5–7 mm long, fleshy, white when fresh, dark-brown when dried; papillae absent; *laticifers* ± visible, translucent in the calyx, corolla lobes, and more noticeable, dark-colored in the 1/3 distal half of the ovary; isolated, oblong-ovoid. A few stomata are present along the calyx mid-vein lobes, but stomatiferous carinas are usually absent; *calyx* 2.8–4 mm long, creamy-white to purple-tinged when fresh, dark-brown when dried, not reticulate, dull, cupulate, ca. 3/4 as long as corolla tube, divided 2/3–4/5 to the base, tube 0.5–1.5 mm long, lobes 2–2.5 mm long, ovate-oblong, longer than wide to as long as wide, the two external ones overlapping, usually not carinate, margins membranous, finely erose, not auriculate at base, apex rounded; *corolla* (4–) 4.5–7 mm long, tube 2.1–4.6 mm long, campanulate but becoming ± globose at fructification; lobes 1.8–2.5 mm long, initially erect, later reflexed, shorter than the tube, broadly ovate, overlapping, ± auriculate at base, margin entire to irregularly crenulate, apex rounded, straight; *stamens* exserted, shorter than the corolla lobes, anthers 0.8–1.2 mm long, elliptic to oblong, filaments 0.6–0.8 mm long; *pollen* 3-zonocolpate, 19–29.5 µm long

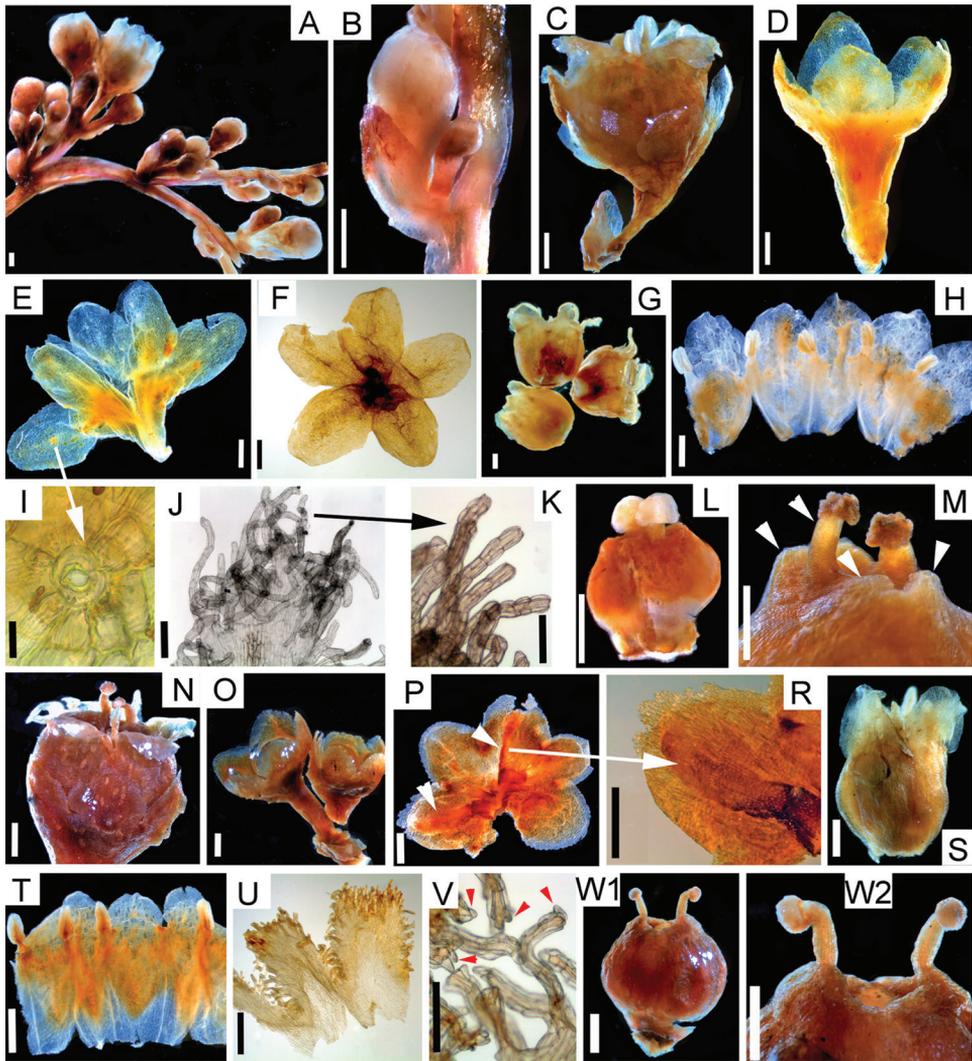


Figure 2. Comparative morphology of *Cuscuta mantiqueirana* and *C. odorata* var. *botryoides* using rehydrated herbarium material **A–M** *Cuscuta mantiqueirana* **A** inflorescence **B** incipient stage of inflorescence development showing bract and developing flowers **C** flower and bract **D** calyx 3D, note the receptacle prolonging into obconical pedicel **E, F** calyx dissected, variation **G** corolla 3D **H** corolla dissected to show infrastaminal scales and stamens **I** stoma on calyx lobe, note that large protuberances with stomata do not develop in this species **J** distal part of infrastaminal scale **K** detail of infrastaminal scale fimbriae, note that no papillae are present **L** gynoecium **M** detail of distal part of ovary showing 4 lobes with stomata flanking the two styles. All images were obtained from *G.F. Árbocz* et al. 2750 except A, B, H and L which are from the type, *Simão-Bianchini* 1241 **N–W** *Cuscuta odorata* var. *botryoides* **N** flower **O** calyxes 3D **P** calyx dissected, note the broader calyx lobes, two of them with prominent carinas that bear stomata (indicated by arrows) **R** detail of carinate calyx lobe **S** corolla 3D **T** fragment of dissected corolla **U** infrastaminal scales **V** detail of fimbriae showing 1–2 papillae at their tips (arrows) **W1** gynoecium **W2** detail of distal part of ovary, note the absence of lobes with stomata. All images from *Lobb* 49 except P, R = *Hatschbach* 22109 and V = *Hoehne* s.n. Scale bars: 1 mm (**A–H, L, N–Q, S–U, W1, W2**); 50 μ m (**I**); 0.5 mm (**J, M, R, K**); 0.25 mm (**V**).

Table 1. Morphological comparison between *C. mantiqueirana* and *C. odorata* var. *botryoides*.

Character	<i>Cuscuta mantiqueirana</i>	<i>Cuscuta odorata</i> var. <i>botryoides</i>
Stems	Yellow-orange, purple-tinged or purple	Yellow-orange
Pedicels	1.5–4 mm, obconical	1–3 mm, cylindrical
Bracts	1.1–2.5 mm long	2.2–3.2 mm long
Flower length	(4–) 5–7 mm	3.8–5 mm
Calyx	2.8–4 mm long, divided 2/3–4/5 to the base, tube 0.5–1.5 mm long, lobes 2–2.5 mm long, ovate-oblong, longer than wide to as long as wide, the two external ones overlapping, usually not carinate, not auriculate at base.	2.8–3.4 mm long, divided ca. 1/2–2/3 to the base, tube 1–2 mm, lobes ovate-round, 1–2 mm long, wider than long, the two external ones carinate, broadly overlapping, auriculate at base.
Corolla	(4–) 4.5–7 mm long, tube 2.1–4.6 mm long, lobes 1.8–2.5 mm long.	Corolla 3.5–4.5; tube 2–3.5 mm long, lobes 1–1.8 mm long.
Infrastaminal scales	3–5 mm long, bridged at 0.7–1.6 mm, fimbriae 50–90, 0.5–1.2 mm long, thin-filiform without papillae.	2–3.7 mm long, bridged at 1–1.5 mm, fimbriae 60–110, 0.3–0.6 mm, often with 1–2 distal papillae.
Stamens	Anthers 0.8–1.2 mm long, filaments 0.6–0.8 mm long.	Anthers 0.6–0.8 mm long, filaments 0.4–0.6 mm long.
Pollen	3-zonocolpate, 19–29.5 µm long and 17–20 µm wide, subprolate to spheroidal or suboblate, tectum microreticulate to reticulate, lumina 0.9–3.3 µm in diameter.	(3–) 4–5 colpate, 19–26 µm long and 18–26 µm wide, sphaeroidal to subsphaeroidal, tectum perforatum; puncta 0.4–1 (1.2) µm in diameter.
Ovary	Ovary apex on both sides of styles risen to form 4 lobes or projections with stomata, 0.4–0.6 mm long.	Apex without lobes or projections but a few stomata may be present.
Styles and stigmas	Styles 0.3–0.9 mm long, 0.25–0.5 mm thick, cylindrical or slightly subulate, sometimes also with stomata at their base; stigmas 0.35–0.5 mm long and 0.5–0.7 mm wide	Styles 0.8–3 mm long, 0.25–0.35 mm thick, cylindrical; stigmas 0.3–0.4 mm long and 0.3–0.5 mm wide.
Capsule	Stomatiferous lobes form a collar around the large interstyler aperture	Without a collar around the large interstyler aperture.
Distribution	Brazil: Minas Gerais, Rio de Janeiro and São Paulo.	Argentina: Misiones; Brazil: Paraná, Santa Catarina.
Elevation	800–2360 m	3–150 m
Ecology	Montane rain forest	Resting in a forest or near rivers

and 17–20 µm wide, subprolate to spheroidal or suboblate, tectum microreticulate to reticulate, lumina 0.9–3.3 µm in diameter; *infrastaminal scales* 3–5 mm long, equalling to slightly longer than corolla tube, ovate to oblong, bridged at 0.7–1.6 mm, densely fimbriatae, fimbriae 50–80, 0.5–1.2 mm long, thin-filiform without papillae distally; *ovary* apex on both sides of styles raised to form two pairs of lobes or projections with stomata, 0.4–0.6 mm long; *styles* 0.3–0.9 mm long, 0.3–0.5 mm thick, much shorter than the ovary, cylindrical to slightly subulate, sometimes also with stomata at their base; *stigma* 0.35–0.6 mm long and 0.5–0.7 mm wide, white to purple in the fresh flowers, globose to wider than long, convoluted and lobed. *Capsules* circumscissile, 4–4.8 mm long and 4–5.5 wide mm, globose to ovoid, with a collar around the large interstyler aperture, opaque, entirely surrounded by the withered, persistent corolla. *Seeds* 2 per capsule, 1.5–2.8 mm long and 2–2.4 mm wide, dorsiventrally compressed, subrotund, hilum area lateral, 0.7–1 mm in diameter, scar 0.25–0.30 mm long, seed coat alveolate/papillate. *Chromosome number* not known.

Geographical distribution and ecology. The new species is apparently endemic to Serra da Mantiqueira in Southeastern Brazil (states of Minas Gerais, Rio de Janeiro and São Paulo), where it occurs at elevations between 800–2360 m. The climate is mesothermic, characterized by distinct dry and rainy seasons, with an average temperature

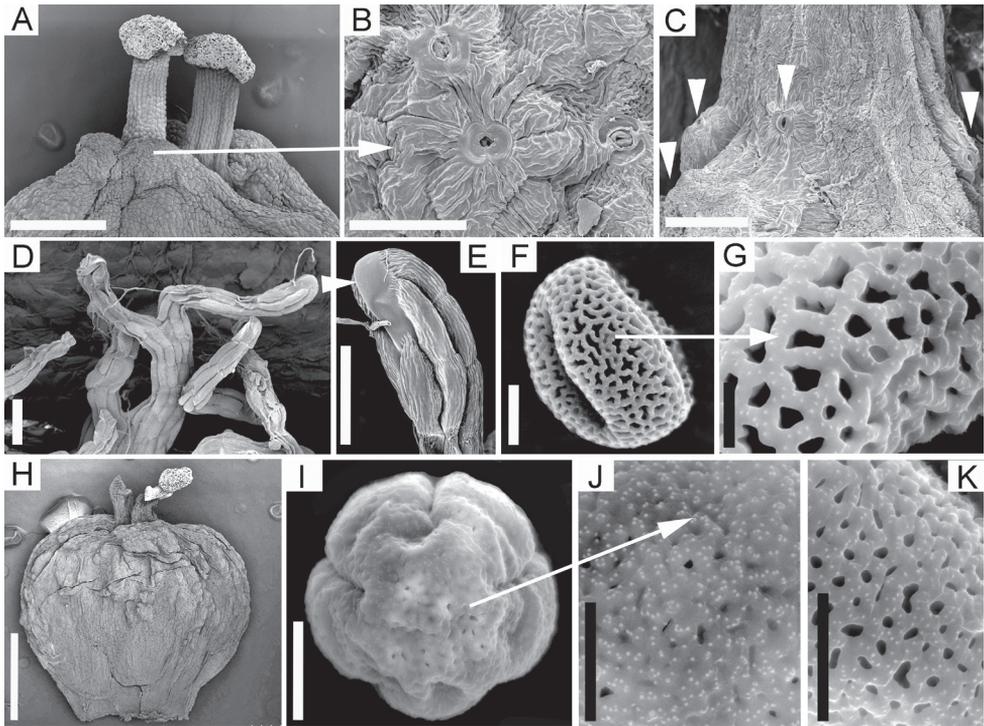


Figure 3. Micromorphology of *Cuscuta mantiqueirana* and *C. odorata* var. *botryoides* **A–G** *Cuscuta mantiqueirana* **A** distal part of ovary **B** stomata on lobes flanking styles **C** stomata (indicated by arrows) at the base of styles **D–E** infrastaminal scale fimbriae and detail **F–G** 3-colpate, reticulate pollen. All images from G.F. Árbocz et al. 2750 except C which is from Simão-Bianchini 1241 **H–K** *Cuscuta odorata* var. *botryoides* **H** gynoeceum **I–K** pollen grain: 5-colpate with perforate tectum. All images from Burkhart 1626. Scale bars: 0.5 mm (**A**); 100 μ m (**B–D**); 50 μ m (**E**); 10 μ m (**F, I**); 1 mm (**H**); 5 μ m (**G, J, K**).

that depends on the elevation, but generally exceeding 10 °C (ICMBio 2018). Serra da Mantiqueira is part of the Atlantic Forest Biome and the habitat of *C. mantiqueirana* consists of montane and upper montane cloud forests (Segadas-Vianna and Dau 1965; Ururahy et al. 1983; Veloso et al. 2012). During field work conducted in Itatiaia National Park and Monte Verde, we observed *C. mantiqueirana* at forest edges, margins of roads and clearings as well as parasitizing isolated woody plants in the forest (Fig. 4A, B). The most common host is *Fuchsia regia* (Vell.) Munz (Onagraceae; Fig. 4E), which is the most widely distributed species of this genus in Brazil, occurring throughout the distribution range of *C. mantiqueirana* (Berry 1989). *Fuchsia regia* is currently accepted to include three subspecies, *regia*, *serrae* P. Berry, and *reitzii* P. Berry (Berry 1989), and future field work will have to determine the frequency of occurrence on these subspecies. To a less extent, perhaps as secondary hosts, *C. mantiqueirana* parasitizes other herbaceous or woody plants: Asteraceae (*Baccharis* L., *Lepidaploa* (Cass.) Cass., *Mikania* Willd., including *Mikania micrantha* Kunth, and other unidentified

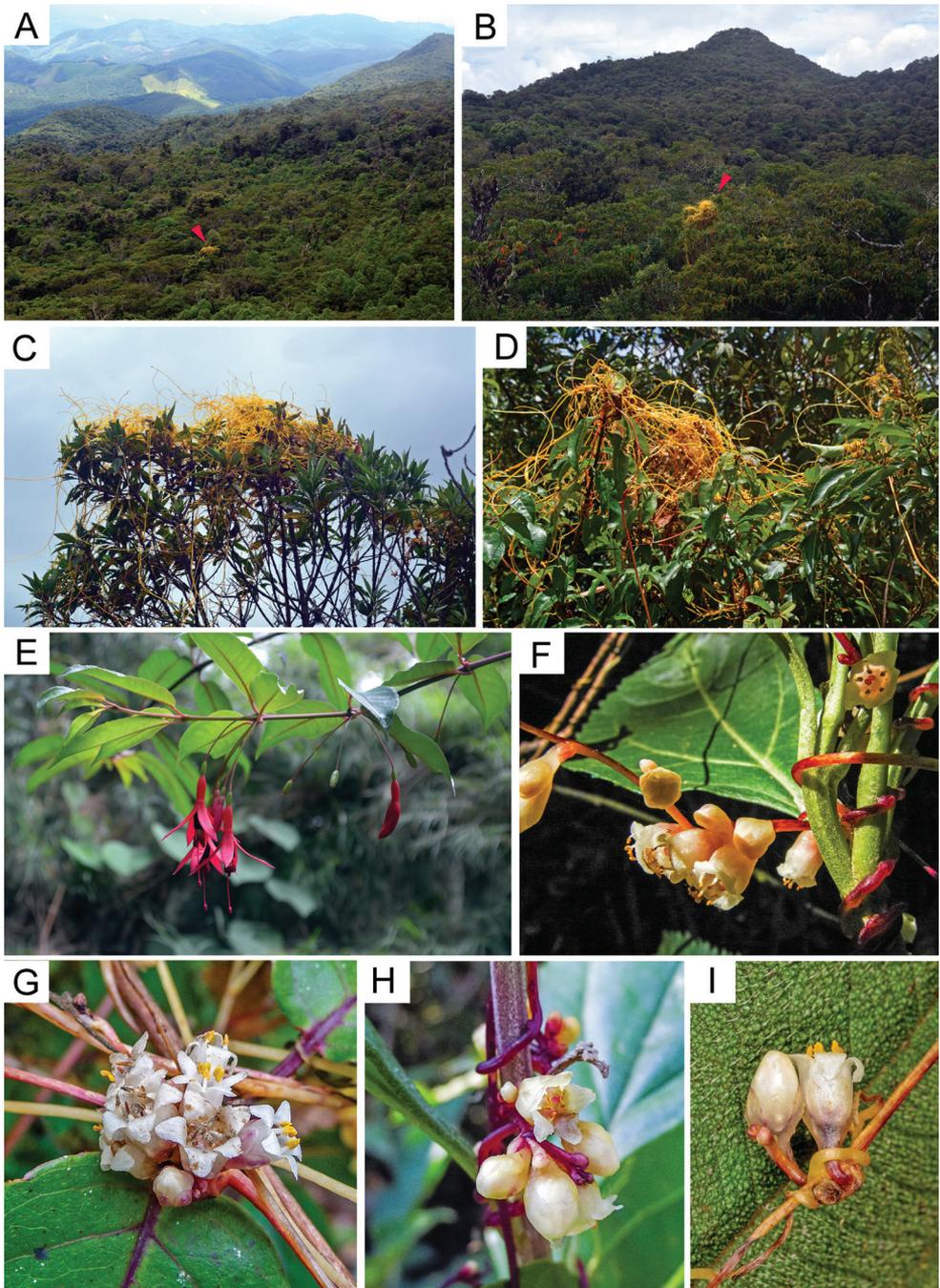


Figure 4. Habitat, habit, inflorescences and flowers of living *Cuscuta mantiqueirana* **A, B** montane cloud forest habitat (arrows indicate *C. mantiqueirana*) **C, D** habit **E** the most common host, *Fuchsia regia* **F–H** inflorescences **I** flowers (**F–I** photos: Suzana Ehlin Martins **F, H** Itatiaia **G, I** Serra do Papagaio).

Asteraceae), Euphorbiaceae (*Croton* L.), Fabaceae, Melastomataceae, Primulaceae (*Myrsine venosa* A. DC.), Polygonaceae (unidentified), Rubiaceae (cf. *Spermacoce* L.), Solanaceae (*Solanum* L.), and Styracaceae.

Phenology. Flowering in Nov-Dec and Feb-Aug, which may depend on the elevation. Very few herbarium specimens possess capsules and seeds which suggests that plants are preponderantly xenogamous and also reproducing vegetatively (Wright et al. 2012).

Etymology. The specific epithet is a feminine adjective that comes from the name of the mountain range to which the species is apparently endemic. The word “Mantiqueira” is derived from Tupi-Guarani meaning “mountains that cry” alluding perhaps to the plethora of dripping water, streams and rivers that are present during the wet season with abundant rainfall (Mendes Júnior et al. 1991).

Vernacular names. The common names used in the area are: Cipó-chumbo, fiosa-de-ovos, erva-de-passarinho (although also commonly used for other species of *Cuscuta* that occur in the region).

Provisional conservation status. The GeoCAT rapid assessment tool (Bachman et al. 2011) assigned an Endangered (EN) conservation status based on an EOO of 33011.111 Km² and a Vulnerable (VU) status based on an AOO of 504.000 Km². Further field research is necessary to investigate possible additional distributional records in the region, and assess other factors such as habitat threats to determine if this species is in need of conservation in any of the parts of the extensive Mantiqueira mountain range.

Additional specimens examined. BRAZIL. Rio de Janeiro: Itatiaia, 13 Apr 1963, *E. Pereira & C. Pereira 7559* (HB); idem, Km 7 da estrada de Registro para Planalto, 17 Feb 1969, *G.F.J. Pabst 9306* (MBM, HB); Parque Nacional de Itatiaia, 2 Nov 1965, *G.G. Eiten & L.T. Eiten 6528* (SP); idem, 2 Apr 1960, *O.M. Barth 7144* (IOC, US); idem Pico das Agulhas Negras, 2159 m, 1 May 1977, *D.M. Vital s.n.* (UEC, BR, NY); idem, Estrada Nova Km 8, 25 Mar 1942, *A.C. Brade 17266* (RB); idem, 2200 m, 1 May 1977, *J. Vasconcellos-Neto et al. s.n.* (UEC); Estrada do Ponto Zero para as prateleiras, 22°22'12"S, 44°42'31"W, 2380 m, 11 Dec 2002, *R. Marquete et al. 3437* (RB; DNA accession 1123); Resende, West side of Mt. Itatiaia, at km 9–10 km on road from “Registro” to the shelter house, “Abrigo Rebouças”, 2100 m, 7 Nov 1965, *G.G. Eiten & L.T. Eiten 6682* (K, SP, NY, MO, UBC, US); Estrada vicinal de acesso ao Pico das Agulhas Negras, 24 May 1996, *G.F. Árbocz 2750 et al.* (SP, NY, UEC, WLU). **Minas Gerais:** Alto Caparaó, Parque Nacional do Caparaó, Trilha Tronqueira ao Pico da Bandeira, 13 Mar 2010, *J.M. Silva & J. Cordeiro 7543* (MBM); Camanducaia, Monte Verde; idem, 3 Jun 1992, *R. Simão-Bianchini 317* (SPF, WLU); idem, 12 Jan 2020, *R. Simão-Bianchini 2332* (SP); idem, 16 Mar 1976, *H.F. Leitão Filho et al. 1815* (UEC, SPSF, UB, WLU); idem, Serra da Mantiqueira, 11 Dec 2001, *L.D. Meireles & R. Balinello 770* (UEC); idem, Pico do Selado, 1810 m, 17 May 2002, *L.S. Kinoshita et al. 72* (UEC); Itamonte, Parque Nacional do Itatiaia, 20 Nov 2018, *S.S. Silva et al. Itati 02, 04, 05* (SP, WLU); idem, 21 Nov 2018, *S.S. Silva et al. Itati 08* (SP, WLU; DNA accession 2436); idem, Itamonte, Serra Fina, Sítio Pierre, 2100 m, 20 Jul 2005, *L.D. Meireles et al. 1843* (SP, UEC); Idem, 4 Apr 1995, 2100 m, *I. Koch & L.S. Kinoshita 449* (UEC); **São Paulo:** Campos do Jordão, Instituto Kurihara, 8 Jun 1940, *G. Hashimoto 262* (SP); idem, Parque Estadual

Campos do Jordão, 1760 m, 8 Feb 1980, *R.A.A. Barreto 48* (SPSF); idem, Praia São José dos Alpes, 8 Jun 1992, *E. Gianotti et al. 26667* (UEC); idem, 2 Aug 1980, *A.A.B. Rubens 48* (RB) Pindamonhangaba, P.E. de Campos do Jordão, 12 Apr 1985, *M.J. Robim 277* (SPSF); idem, 12 Apr 1985, *C. Proença & M.F. Bean 496* (CEN, UB).

Discussion

Systematics of *Cuscuta mantiqueirana*-related group of taxa

Cuscuta odorata var. *boliviana* Engelm. Trans. Acad. Sci. St. Louis 1: 477. 1859.
= *C. boliviana* var. *paranensis* Hunz., Revista Argent. Agron. 14: 142.1947.

The morphological distinctiveness of *C. mantiqueirana* allows its unequivocal recognition as a new species even though we could not obtain molecular data for some morphologically similar taxa – *C. rotundiflora* and *C. odorata* var. *botryoides*. The available molecular results agree with the morphological patterns observed in section *Subulatae*, and the similarity of *C. mantiqueirana*, *C. odorata* var. *botryoides*, and *C. rotundiflora* strongly suggests a phylogenetic proximity of these taxa in clade B (Fig. 1). Nevertheless, a complete picture of the evolutionary relationships and systematics of this clade will require molecular results for all the taxa involved.

As indicated, *C. mantiqueirana* is most similar morphologically to *C. odorata* var. *botryoides* (Table 1), a taxon that was described by Engelmann (1859) from “Southern Brazil” based on a single specimen, *Lobb 49* (K, MO). Engelmann (1859) viewed this variety as “intermediate” between *C. odorata* and *C. chilensis*. Subsequently, Yuncker (1922, 1932) maintained this taxon as a variety of *C. odorata*, but compared it with *C. globiflora*. More recently, Hunziker (1947) described *C. boliviana* var. *paranensis* Hunz. from Misiones (Argentina) and Paraná (southern Brazil), which he considered to be most similar to *C. boliviana* (var. *boliviana*) and *C. cristata*. These two varietal names have been accepted by modern floristic overviews (e.g., Zuloaga et al. 2008; Flora do Brasil 2020), but the taxa themselves have remained little known until now. After examining the types and few specimens available for *C. odorata* var. *botryoides* and *C. boliviana* var. *paranensis*, we consider these names synonymous. Variety *botryoides* has priority at this rank (“the original subdivisional epithet”, a rule that had already been in place in 1947 when it was described by Hunziker (Art 55; Camp et al. 1948). Although it could not be included in the molecular study, *C. odorata* var. *botryoides* is likely not related to *C. odorata* var. *odorata* because at least some of its pollen grains are 4–5-colpate and its infrastaminal scale fimbriae have only 1–2 distal papillae. Thus, *C. odorata* is most probably polyphyletic; var. *odorata* shares affinities with the species of clade A (comprised of *C. foetida*, *C. purpurata*, *C. chilensis*, etc., Fig. 1), while var. *botryoides* is a member of clade B. The taxonomic rank and evolutionary relationships of var. *botryoides* remain to be solved by a future study.

Stomatiferous protuberances

Stomatiferous structures have been recently documented in many *Cuscuta* species (reviewed by Clayson et al. 2014); however, their presence at the apex of the ovary and base of styles in *C. mantiqueirana* is a novel feature. Stomatiferous (multicellular) protuberances (SPs) develop during anthesis on the haustorial stems of species in subgenus *Grammica*, as well as on the calyx and corolla of flowers in species from multiple clades of subgenera *Cuscuta* and *Grammica* (Clayson et al. 2014). When present on the calyx and corolla, SPs are diversely shaped (e.g., tubular, hemispherical, conical, crests) and have evolutionary and taxonomic significance (Costea and Stefanović 2010; Costea et al. 2011a, 2011b, 2013). SPs on the flowers are characteristic of species that have evolved in areas with a marked dry season (Clayson et al. 2014). The water loss through the SPs stimulates the hosts to absorb more water by increasing the negative pressure/tension in the xylem of the host, via the haustoria connection (Clayson et al. 2014). Some taxa of section *Subulatae* also have SPs on the calyx lobes; for example, the species epithets of *C. cristata* and *C. alatoloba* Yunck. refer to the presence of crests on the calyx lobes, but their authors (Engelmann 1859; Yuncker 1932) did not know that these structures bear stomata or their function. The discovery of stomatiferous structures on the ovary and style base of *C. mantiqueirana* improves the knowledge about the diversity of SPs, and strongly suggests that the morphology of floral SPs will play a significant role in the species-level systematics of section *Subulatae*.

Specimens of *C. odorata* var. *botryoides* examined

ARGENTINA. Type of *C. boliviana* var. *paranensis*; Misiones, Posadas, en la costa del río, 15 Jul 1945, *Bertoni 1502* (LIL); idem, 2 Aug 1945, *Burkhardt 1626* (CORD). BRAZIL. Type of *C. odorata* var. *botryoides*; “S. Brasil”, *Lobb 49* (K, MO); Paraná: Mun. Parana-gua, Ipanema, 27 Aug 1969, 3–5 m, *Hatschbach 22109* (K, MBM, MO); paratype of *C. boliviana* var. *paranensis*; idem, 25 Oct 1929, *Hoehne s.n.* (SP 24476).

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Appendix I

Taxa, DNA accession numbers, sources of plant material from which DNA was extracted, and GenBank accession numbers for nrITS sequences used in this study. Extraction numbers following species names are indicated on the phylogenetic tree (Fig. 1). Abbreviations of herbaria in which the vouchers are deposited follow Index Herbariorum (Thiers 2018-continuously updated). In bold are indicated accession numbers for sequences newly generated for this study.

Cuscuta aff. *chilensis* Ker Gawl.: **999**, *Hichins & Muñoz s.n.* (SGO), EF194525; **1000**, *Teiller et al 2489* (SGO), EF194524; *C. chilensis*: **567**, *Ledingham 4455* (USAS), EF194520; **715**, *Arroyo et al. 996099* (SGO), EF194521; **716**, *Morales and Cordoba s.n.* (SGO), EF194522; **967**, *Landrum 3392* (ASU), EF194523; **985**, *Muñoz and Meza 2202* (SGO), EF194519; *C. cockerellii* Yunck.: **1055**, *Straw 2267* (US), EF194518; *C. cristata* Engelm.: **939**, *Riggs 100* (F), EF194529; **1026**, *Landrum 3057* (ASU), EF194531; **1045**, *Hunziker 5047* (US), EF194530; *C. foetida* (var. *foetida*) Kunth: **496**, *Ollgaard & Balsev 8960* (F), EF194512; **922**, *Steyermark 53255* (F), EF194513; **1020**, *Sparre 16952* (TEX), EF194511; *C. foetida* Kunth var. *pycnantha* (Benth.) Yunck.: **990**, *Lira 13* (SGO), EF194527; *C. friesii* Yunck.: **1076**, *Cabrera et al. 21399* (LP), EF194536; *C. globiflora* Engelm.: **909**, *Vargas 684* (F), EF194533; **926**, *Buchtien 133* (F), EF194534; *C. grandiflora* Kunth: **540**, *Hutchinson & Wright 4305* (F), EF194535; *C. kilimanjari* Oliv.: **471**, *Knox 5020* (TRTE, IND), EF194528; *C. mantiqueirana* Costea, S.S. Silva, Sim.-Bianch., sp. nov.: **1123**, *Marquete 3437* (RB), MZ389691; 2436, *Silva et al. 08* (SP/WLU), MZ389690; 2437, *Simão-Bianchini 1241* (SP/WLU), MZ389689; *C. microstyla* Engelm.: **707**, *Muñoz et al. 3575* (SGO), EF194538; **987**, *Vargas & Farah 80* (SGO), EF194537; *C. odorata* Ruiz & Pav. var. *odorata*: **912**, *Hutchinson 1055* (F), EF194514; **1024**, *Asplund 7737* (TEX/LL), EF194515; *C. paitana* Yunck.: **940**, *Haught 63* (F), EF194516; **941**, *Weberbauer 7762* (F), EF194517; *C. parodiana* Yunck.: **512**, *Krapovickas 37354* (F), EF194532; *C. purpurata* Phil.: **1432**, *Muñoz 5132* (SGO/WLU), MZ389688.

Vitis shizishanensis, a new species of the grape genus from Hubei province, China

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Abstract

Vitis shizishanensis (Vitaceae), a new species from Hubei, China, is described and illustrated. It is morphologically similar to *V. flexuosa* and *V. bryoniifolia*, but differs in leaf lobing and pubescence. It can be easily distinguished from the two species based on its glabrous or with very sparse arachnoid tomentum on the abaxial mature leaf surface, and its unlobed to 3–7 lobed leaves. A detailed description, along with photographs for the new species, and a table for morphological comparisons with similar *Vitis* species, are also provided.

Keywords

Grape, phylogenomics, taxonomy, Vitaceae, *Vitis*

Introduction

The grapes (*Vitis vinifera* L.) represent one of the earliest domesticated and the most widely cultivated economic fruits in the world, as the source for grapes, raisins, and wine (Myles et al. 2011; Gerrath et al. 2015; Wen et al. 2018b). The grape genus *Vitis* L. contains ca. 70 species with an intercontinental disjunct distribution in North America (to northern South America), East Asia and Europe to West Asia (Galet 1988; Chen et al.

2007; Moore and Wen 2016; Wen et al. 2018a, 2018b). There are ca. 40 native species of *Vitis* in East Asia and most of them occur in China (Chen et al. 2007; Wan et al. 2008). Based on recent studies on molecular phylogeny and morphology of *Vitis*, a robust phylogenetic framework of *Vitis* has been reconstructed (Tröndle et al. 2010; Péros et al. 2011; Zecca et al. 2012; Aradhya et al. 2013; Wan et al. 2013; Liu et al. 2016; Ma et al. 2018a). However, due to rapid evolutionary radiations and extensive reticulate evolution of *Vitis* (Aradhya et al. 2013; Wan et al. 2013; Ma et al. 2018a, 2018b; Wen et al. 2018a), the species delimitation of *Vitis* is still controversial and the number of species of *Vitis* needs to be further assessed (Chen et al. 2007; Wan et al. 2008; Wen et al. 2018b; Ma et al. 2016, 2018b, 2020). Taxonomic challenges of some *Vitis* species are caused by their morphological similarity and overlapping geographic distribution (Chen et al. 2007; Moore and Wen 2016). A very complex group of *Vitis* is the *V. bryoniifolia* clade and its close allies (Ma et al. 2020). The phylogenetic relationships of the *V. bryoniifolia* clade have been reconstructed recently with robust support, which indicated that some samples previously difficult to identify need to be treated as a different species distinct from *V. bryoniifolia* based on molecular phylogenetic evidence (Ma et al. 2020) (Fig. 1). The leaf shape of this species shows a high level of phenotypic plasticity, varying from unlobed to 3–7 lobed, which caused problems for species identifications (Ma et al. 2020). After consulting relevant literature (Li et al. 1996; Wang et al. 2000; Chen et al. 2007; Wan et al. 2008) and our extensive field studies in East Asia, we herein propose to describe the new species, *Vitis shizishanensis* Z.Y.Ma, J. Wen, Q. Fu & X-Q. Liu.

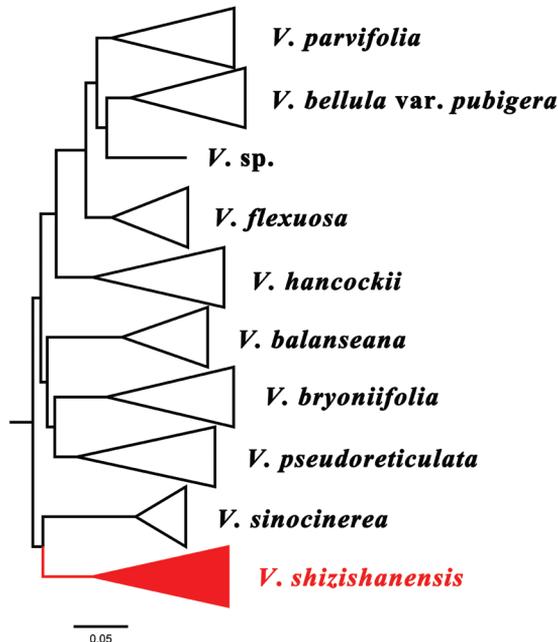


Figure 1. Simplified phylogenetic relationships of the *V. bryoniifolia* clade based on Ma et al. 2020.

Material and methods

Descriptions and measurements of morphological characters of the new species were based on field observations of living plants at the type locality and specimens in the herbarium of Huazhong Agricultural University (CCAU) and the United States National Herbarium (US). We also examined herbarium specimens of *Vitis* comparatively from the following herbaria: CCNU, CSFI, HIB, HNNU, HUNST, HZU, JIU, JXCM, NYA, PE, and WH (abbreviations following Thiers 2020), and from images of type specimens and dried herbarium specimens on the Chinese Virtual Herbarium Website (<http://www.cvh.ac.cn/>), JSTOR Global Plants (<http://plants.jstor.org>), National Specimen Information Infrastructure (<http://www.nsii.org.cn/>), and Sharing Platform of IBK (<http://www.gxib.cn/spIBK/>).

Taxonomic treatment

Vitis shizishanensis Z.Y.Ma, J.Wen, Q.Fu & X.Q.Liu, sp. nov.

urn:lsid:ipni.org:names:77221513-1

Figures 2, 3, 4, 5, 6

Type. China. Hubei: Wuhan City, Shizishan Mountain, 30°28'44"N, 114°21'48"E, 21 m, 6 May 2021, in fl., X.Q. LIU 755 (holotype: CCAU!; isotypes: CCAU!, US!).

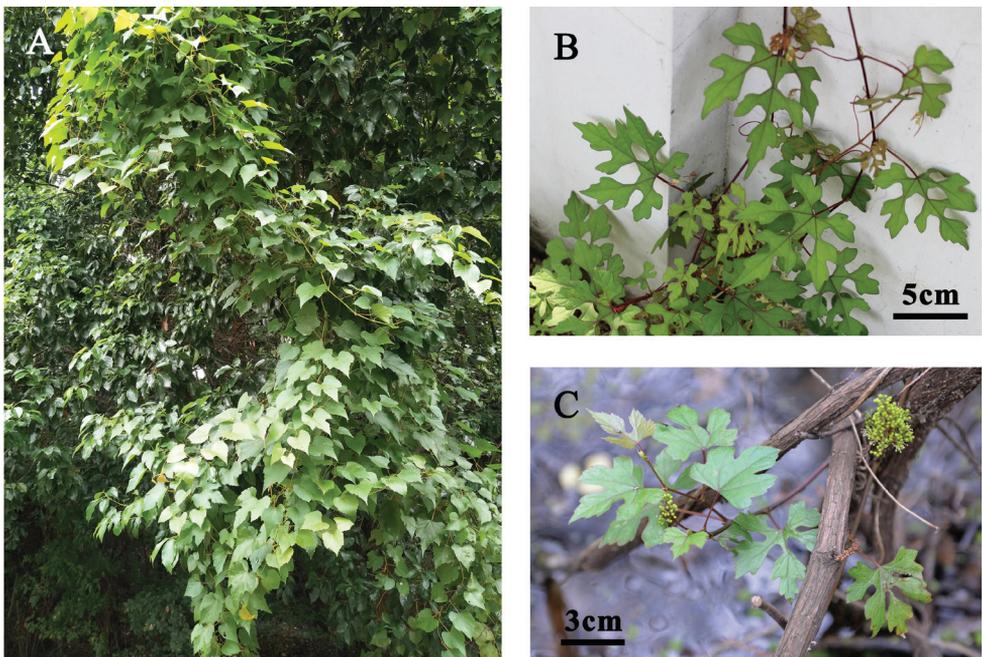


Figure 2. *Vitis shizishanensis* Z.Y.Ma, J.Wen, Q.Fu & X.Q.Liu, sp. nov. **A** habit **B** individual with 5–7 deeply lobed leaves **C** a flowering branch with 3–5 deeply lobed leaves.

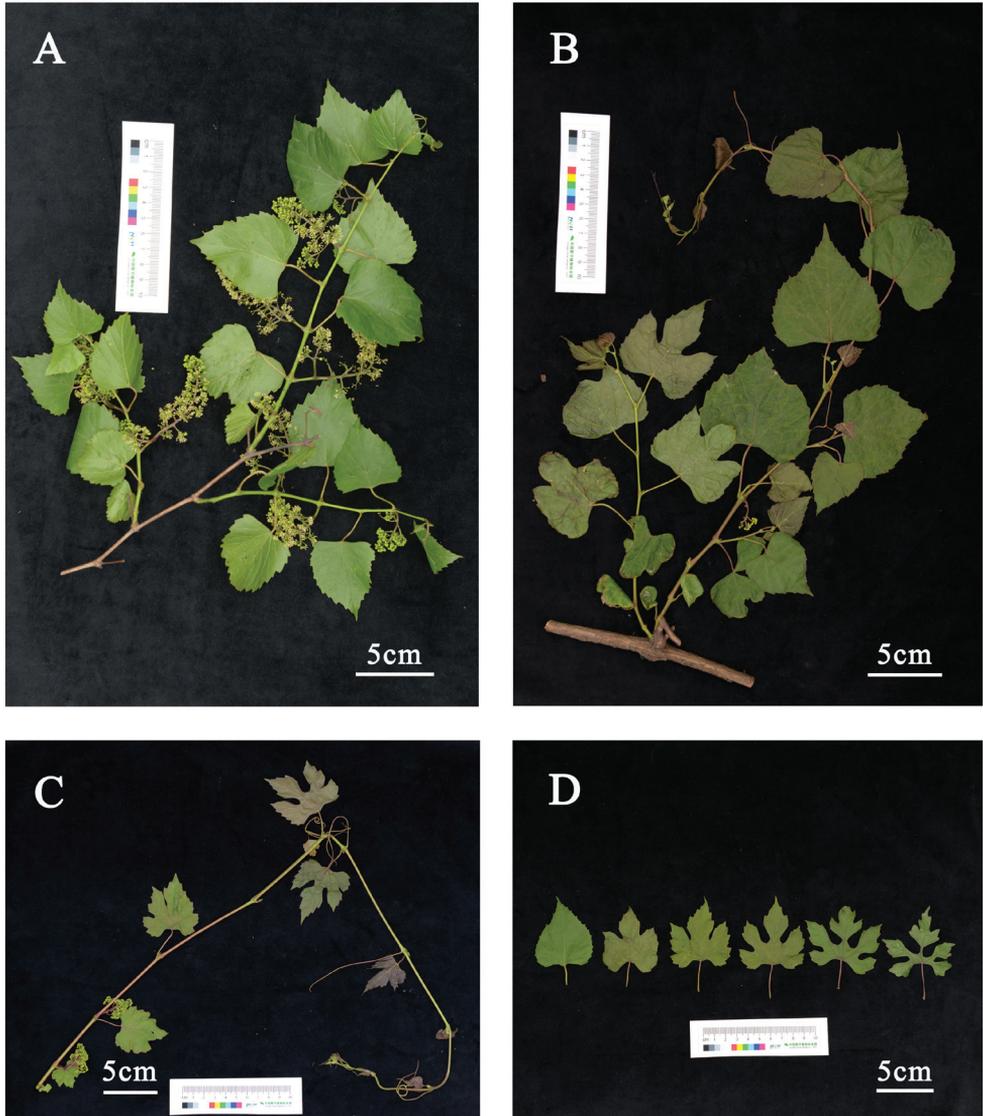


Figure 3. Branches and leaves of *Vitis shizishanensis* sp. nov. (X.Q. LIU 755) **A** branches with unlobed leaves **B** branches with unlobed to 3 lobed leaves **C** branches with 3–5 deeply lobed leaves **D** unlobed to 5–7 deeply lobed leaves.

Diagnosis. *Vitis shizishanensis* is morphologically similar to *V. bryoniifolia* Bunge, *V. flexuosa* Thunb, *V. sinocinerea* W. T. Wang, and *V. bellula* (Rehder) W. T. Wang, but differs from the *V. bryoniifolia*, *V. sinocinerea*, and *V. bellula* in its glabrous to hirtellously pubescent abaxial mature leaf surface (vs. abaxially densely arachnoid tomentose in *V. bryoniifolia*, *V. sinocinerea*, and *V. bellula*). It differs from *Vitis flexuosa* in its leaves varying from unlobed to 3–7 lobed (vs. unlobed to slightly 3-lobed leaves in *V. flexuosa*), tendrils unbranched or bifurcate from upper half (vs.

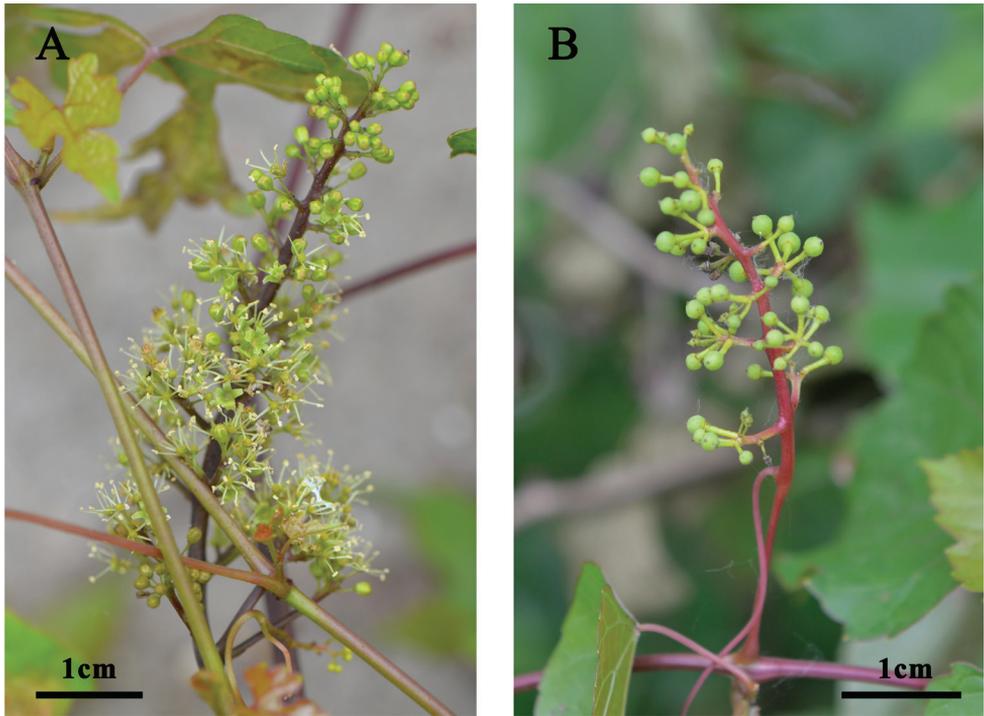


Figure 4. Inflorescences of *Vitis shizishanensis* sp. nov. **A** male flowers **B** female flowers after anthesis.



Figure 5. Seeds of *Vitis shizishanensis* sp. nov. **A** ventral view **B** dorsal view **C** lateral view **D** transverse section.

tendrils bifurcate from approximately midway in *V. flexuosa*), lack of arachnoid tomentum (vs. with sparse arachnoid tomentum to glabrescent in *V. flexuosa*), and subcordate to cordate or sometimes truncate leaf base (vs. subtruncate or slightly subcordate leaf base in *V. flexuosa*).

Description. Woody climber, sprawling to moderately high climbing, sparsely branched. Branchlets terete, glabrous, with longitudinal ridges, tendrils unbranched or bifurcate from upper half. Leaves simple; stipules ovate-elliptic or lanceolate, 1–4 mm; petiole 2–6 cm, hirtellous or glabrous; blade 3–10 × 3–9 cm, unlobed to slightly 3-lobed, or 3–7

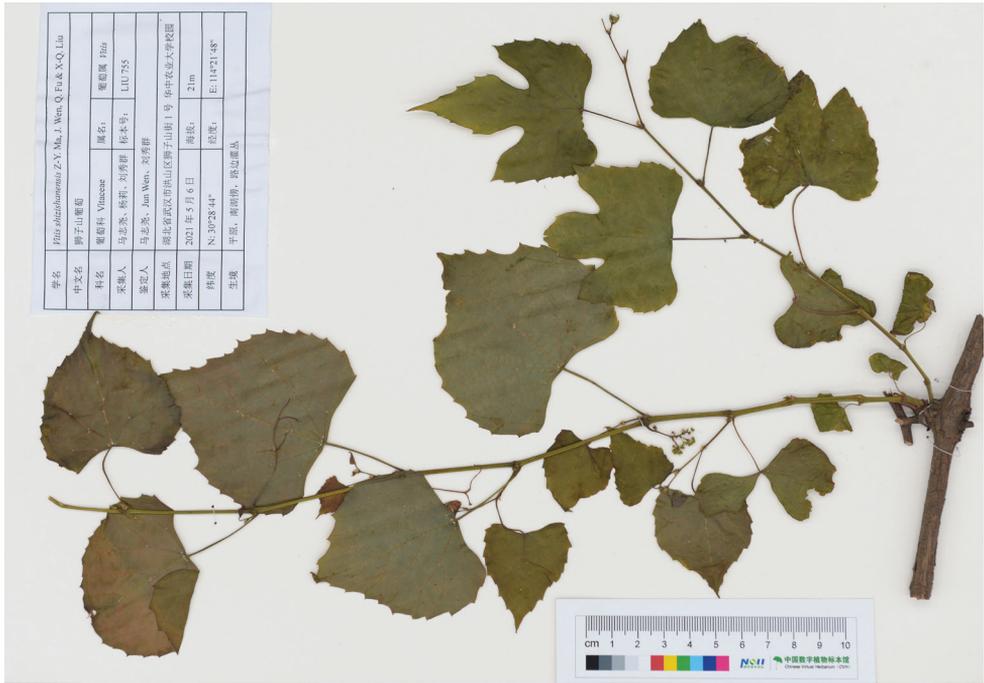


Figure 6. Holotype of *Vitis shizishanensis* sp. nov. Z.Y.Ma, J. Wen, Q. Fu & X-Q. Liu (X.Q. LIU 755).

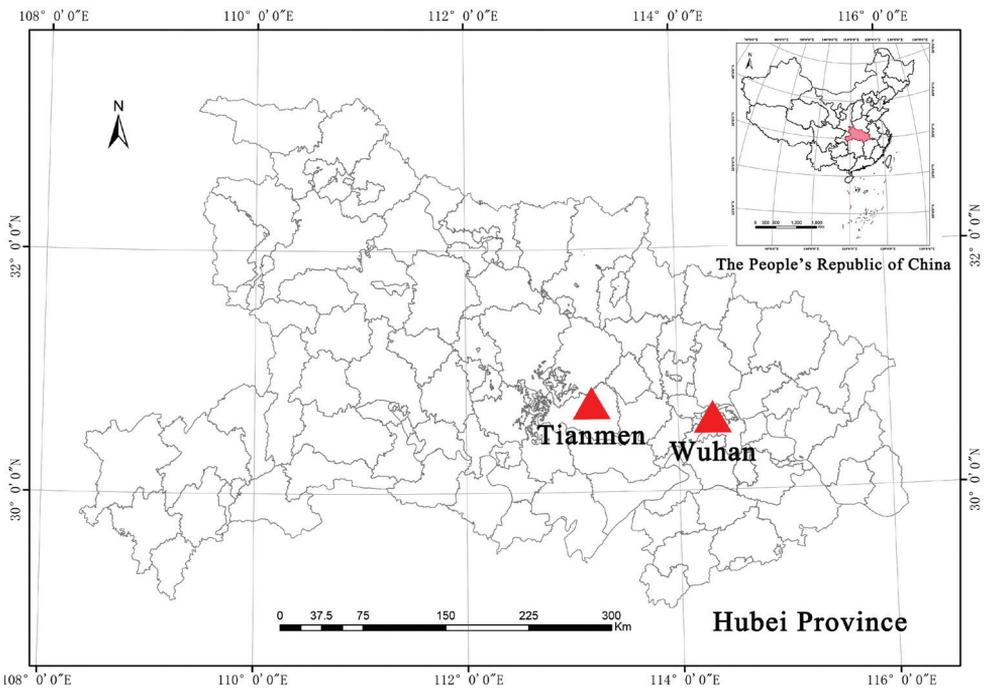


Figure 7. Distribution of *Vitis shizishanensis* sp. nov. (triangle).

Table 1. Morphological comparisons among *Vitis shizishanensis*, *V. bryoniifolia*, *V. flexuosa*, *V. sinocinerea*, and *V. bellula*.

Characters	<i>V. shizishanensis</i>	<i>V. flexuosa</i>	<i>V. bryoniifolia</i>	<i>V. sinocinerea</i>	<i>V. bellula</i>
tendrils	unbranched or bifurcate in the upper half	bifurcate to the middle	bifurcate	unbranched or bifurcate	unbranched or bifurcate
Size of leaves	ca. 3–10 × 3–9 cm	ca. 5–12 × 3.5–10 cm	ca. 2.5–8 × 2–5 cm	ca. 3–8 × 3–6 cm	ca. 3–7 × 2–4 cm
Leaf base	subtruncate or subcordate to deeply cordate	slightly subcordate or subtruncate, rarely cordate	cordate or deeply cordate	subcordate or subtruncate	subcordate, subtruncate, or subrounded
Shape of leaves	unlobed to 3–7 lobed	unlobed to slightly 3-lobed	unlobed to 3–7 lobed	3-lobed or inconspicuously divided	unlobed
Abaxial mature leaf surfaces	usually glabrous	with sparse arachnoid tomentum to glabrescent	with dense arachnoid tomentum	with dense arachnoid tomentum	with dense arachnoid tomentum
Size of fruits	5–8 mm in diam	8–10 mm in diam	5–8 mm in diam	6–10 mm in diam	6–10 mm in diam
Altitude	10–50 m	100–2300 m	100–2500 m	200–2800 m	400–1600 m
Distribution	China (Hubei)	China, India, Japan, Laos, Nepal, Philippines, Thailand, Vietnam	China (Anhui, Fujian, Guangdong, Guangxi, Hebei, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Shanxi, Sichuan, Yunnan)	China (Fujian, Hubei, Hunan, Jiangsu, Jiangxi, Taiwan, Yunnan, Zhejiang)	China (Guangdong, Guangxi, Hubei, Hunan, Sichuan)

lobed, apex acute to acuminate, base subtruncate or subcordate to cordate, abaxial surface usually glabrous, veins and vein axils hirtellous, adaxial surface glabrous, basal veins 5, with lateral veins 4–6 pairs. veinlets inconspicuous, base subcordate to cordate, occasionally truncate. Margin with 8–16 obtuse teeth on each side. Inflorescences a panicle, 3.4–9 cm, leaf-opposed, peduncle 1–6.4 cm, pedicel 1–2.5 mm, usually glabrous. Calyx shallow and saucer-shaped, glabrous. Petals 5, occasionally 6, connate distally, forming calyptra. Berries black, globose, 5–8 mm in diam. Seeds obovoid or obovoid-elliptic, 3–4 × 2–3 mm, abaxial surface with a round to elliptic chalaza, adaxial surface with 2 furrows (ventral infolds) running ½ through seed length, endosperm M-shaped in transverse section.

Additional specimens examined. China. Hubei. Tianmen City, Mawan Town, 15 Jul 2020, fr, X. Q. Liu 944 (CCAU); Wuhan, Shizhishan, 26 m, May 6, 2021, in flower, X. Q. Liu 155 (CCAU) (see photos in Suppl. material 1: Fig. S1, Suppl. material 2: Fig. S2, Suppl. material 3: Fig. S3).

Phenology. Flowering from March to May, fruiting from July to October.

Etymology. The specific epithet is derived from the type locality, Shizhishan, Wuhan, Hubei, China. The Chinese name is given as “狮子山葡萄”.

Distribution and habitat. The new species is currently known from Wuhan and Tianmen in Hubei province, China (Fig. 7). It occurs on the scrubland and the roadside of farmland at an altitude of ca. 10–50 m.

Vitis shizishanensis is morphologically similar to *V. bryoniifolia*, *V. flexuosa*, *V. sinocinerea*, and *V. bellula*. Detailed morphological comparisons among the three species are provided in Table 1. These characters were based on field observations, and herbarium and literature studies (Li et al. 1996; Chen et al. 2007; Wan et al. 2008).

Acknowledgements

We thank three master candidates (L. Yang, L. L. Gui and Y. H. Wang) in Huazhong Agricultural University for collecting some samples. This project was supported by the National Natural Science Foundation of China (Grants No. 31870193).

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Supplementary material I

Figure S1

Authors: Zhi-Yao Ma, Jun Wen, Qiang Fu, Xiu-Qun Liu

Data type: Jpg file.

Explanation note: Isotype of *Vitis shizishanensis* Z.Y.Ma, J. Wen, Q. Fu & X-Q. Liu (X.Q. LIU 155) with 5–7 lobed leaves.

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Link: <https://doi.org/10.3897/phytokeys.184.70045.suppl1>

Supplementary material 2

Figure S2

Authors: Zhi-Yao Ma, Jun Wen, Qiang Fu, Xiu-Qun Liu

Data type: Jpg file.

Explanation note: Isotype of *Vitis shizishanensis* Z.Y.Ma, J. Wen, Q. Fu & X-Q. Liu (X.Q. LIU 155) with male flowers.

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Link: <https://doi.org/10.3897/phytokeys.184.70045.suppl2>

Supplementary material 3

Figure S3

Authors: Zhi-Yao Ma, Jun Wen, Qiang Fu, Xiu-Qun Liu

Data type: Jpg file.

Explanation note: Isotype of *Vitis shizishanensis* Z.Y.Ma, J. Wen, Q. Fu & X-Q. Liu (X.Q. LIU 944) with fruits.

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Link: <https://doi.org/10.3897/phytokeys.184.70045.suppl3>

A new species of *Epidendrum* L. (Orchidaceae) of pendulous habit from Peru

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Abstract

A new species of *Epidendrum* L. from northern Peru is described, illustrated, and compared with related species. This new species belongs to the Laxicaule Group and shares morphological characteristics with *Epidendrum laxicaule* D.E. Benn & Christenson but differs in the shape and length of the dorsal sepal; the shape of the petals and the lip, ribs position of the lip, shape of the vesicle formed between the ovary and the column as well as the section of the stem.

Abstract

Se describe, ilustra y compara una nueva especie de *Epidendrum* L. del norte de Perú con otras especies relacionadas. Esta nueva especie pertenece al Grupo Laxicaule y comparte características morfológicas con *Epidendrum laxicaule* D.E. Benn & Christenson pero difiere en la forma y longitud del sépalo dorsal; la forma de los pétalos y del labio, la posición de las costillas del labio, la forma de la vesícula formada entre el ovario y la columna así como la sección del tallo.

Keywords

Cajamarca, endemic, Epidendroideae, epiphyte, neotropic, San Ignacio

Introduction

The genus *Epidendrum* Linnaeus (1763: 1347) are terrestrial, epiphytic and lithophytic plants that occur in different types of vegetation ranging from tropical forests, coastal dunes and scrubs to Andean paramos (Hágsater and Soto 2005; Chase et al. 2015). The genus ranks as one of the most diverse among Neotropical orchids, with around 2400 species (Hágsater et al. 2016), 1500 of them being recently treated and illustrated in the Icones Orchidacearum series (Hágsater and Salazar 1993; Hágsater et al. 1999; Hágsater and Sánchez 2001, 2004, 2006, 2007, 2008, 2009, 2010, 2013, 2015a, 2015b; Hágsater and Santiago 2018a, 2018b, 2019, 2020a, 2020b, 2021).

Though there have been many attempts to separate *Epidendrum* into various genera (Hágsater 1985; Hágsater and Soto 2005), it has been argued that it is best left as a single large genus reflecting its monophyly, as revealed by molecular studies (Hágsater and Soto 2005; Hágsater et al. 2019; Granados et al. 2020). Groups of species within *Epidendrum* can be cohesively aggregated based on vegetative and floral characteristics, which are also supported by molecular information. As this work is in progress, no formal sub-generic classification has been proposed to date, based on the informal groups recognized in current taxonomic practice (Hágsater 1985; Hágsater and Huayta 2018).

This is the case of the Laxicaule group, endemic to Peru, which is characterized by the pendulous, monopodial habit, with sub-apical branching, the laterally compressed to ancipitous stems, the relatively short leaves, the short racemose, few-flowered inflorescences, the large flowers and the lip with prominent ribs (Hágsater and Huayta 2018). Presently, there are only two species in the group: *Epidendrum laxicaule* D.E. Benn & Christenson and *E. megalopentadactylum* Hágsater & Huayta (Hágsater and Huayta 2018) found in central Peru. Here we are describing, illustrating and representing with photographs a new species of *Epidendrum*, *E. lufinorum* Ocupa & Hágsater, as well as photographs of the related species and two undescribed species. A table is provided with the characteristics that distinguish the three species of the Laxicaule Group for which information is available.

Material and methods

A living plant in flower of the new species was collected in March 2016 during a botanical expedition to Cerro Parcos in the department of Cajamarca, northeastern Peru. The photographs were taken in situ using a Canon Rebel T3 camera equipped with a Canon EF-S 18–55mm f/3.5–5.6 lens and were later used for preparing the line drawing and figures. Fresh flowers were preserved in 70% ethanol and 1% glycerol. The single collected plant was dried to make a herbarium specimen, which was afterwards deposited in the Herbarium Truxillense (HUT, acronym following Thiers 2021, updated continuously), Trujillo, Peru.

In order to determine the taxonomic status of the collected specimen, we examined all relevant *Epidendrum* material from USM and MOL (acronym following Thiers

2021, updated continuously), because both herbaria have a large collection of *Epidendrum* species. The original descriptions from holotypes of related species (Bennett and Christenson 1998; Hágsater and Santiago 2018a) were consulted and compared. Additionally, some online resources including the scans of relevant type specimens were accessed such as the JSTOR Global Plants web portal (<https://plants.jstor.org>). A distribution map of the proposed new species and related species, was prepared using the software QGIS 3.10 (QGIS Development Team 2020). The holotype specimen was collected under the research permit for the project “Estudios Taxonómicos Selectos de la Flora del Norte del Perú” with Resolution N° 247 –2016 –SERFOR/DGGSPFFS and the extension Resol. 430–2017.

Taxonomic treatment

Epidendrum lufinorum Ocupa & Hágsater, sp. nov.

urn:lsid:ipni.org:names:77221545-1

Figs 1, 2, 4C

Type. PERU. Cajamarca: San Ignacio, in a coffee plantation, close to the caserío Villa Rica, DDM 5°5.1607'S, 78°53.2076'W, elev. 1690 m, 03 April 2016, *Ocupa* 211 (holotype: HUT!).

Similar to *Epidendrum laxicaule* D.E.Benn & Christenson, but differs in having smaller dorsal sepal (i.e. 2.4×0.4 cm vs. 3.2×0.6 cm) which is oblong-oblancheolate (vs. narrowly oblanceolate), with an obtuse apex (vs. acute), the linear and obtuse petals (vs. narrowly linear-lanceolate and acuminate), lip transversely cordate (vs. transverse), disc with 5 parallel and central ribs (vs. 5 parallel ribs projecting distally), a gibbous vesicle (vs. globose) and the stem section terete (vs. elliptic).

Description. Epiphytic *herb*, monopodial, branching, pendulous, slender, about 72 cm long including inflorescence. **Roots** 2 mm in diameter, basal, white, fleshy. **Stems** ca. 65 cm long, terete in cross section, new shoots produced from sub-apical nodes of primary stem, enveloped by tubular, fleshy, articulated, green with vinaceous spotted sheaths, membranaceous near leaf abscission, articulate and leaf-bearing above. **Leaves** $5.5\text{--}7.2 \times 0.38\text{--}0.4$ cm, linear, sessile, distichous, fleshy, semi-terete, descending, facing downwards, sulcate below, apex obtuse; sheaths $2.5\text{--}5.0$ cm long, tubular, appressed, green with vinaceous spots as well as leaves. **Inflorescence** 7 cm long, apical, pendulous, 1–2-flowered, covered to mid portion by 3 successive, imbricating sheaths; peduncle ca. 4.4 cm long, terete, green with pale vinaceous spots; rachis 2 cm long, terete; sheaths $0.8\text{--}2.2 \times 0.2\text{--}0.3$ cm, green with vinaceous spots, ensiform, tubular, laterally compressed, ancipitous, apex acute, decreasing in size. **Floral bracts** 2.5×1.5 mm, fawn-colored with vinaceous spots, longitudinally triangular, minute, apex acuminate, base truncate. **Ovary** ca. 2 cm long, pedicellate, green with vinaceous spots, progressively thickened towards apex, slightly recurved, with 3 longitudinal furrows, one adaxially and two laterally, forming a ventral gib-

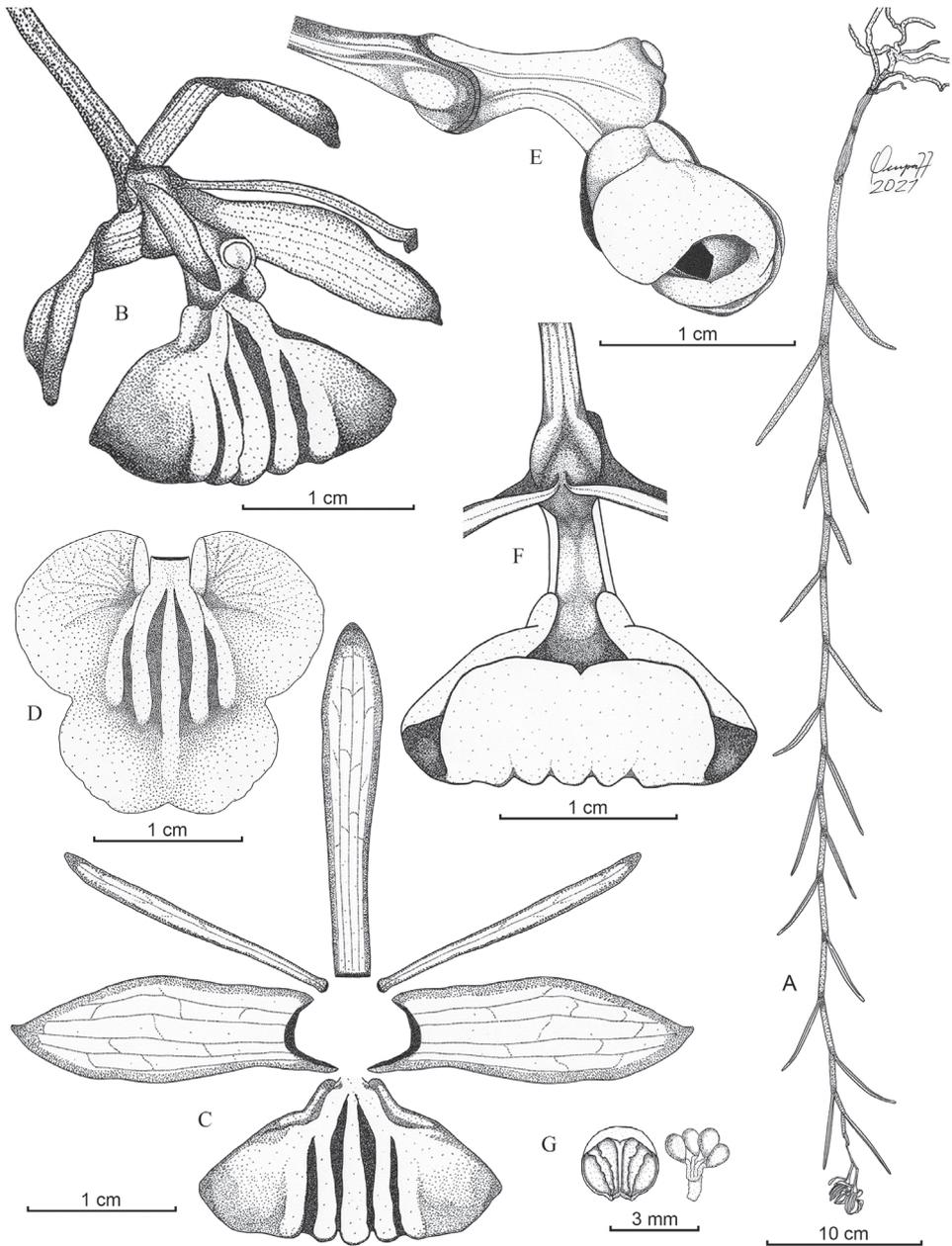


Figure 1. Drawing of *Epidendrum lufinorum* sp. nov. **A** habit **B** flower **C** dissected perianth **D** lip extended **E** column, lip and ovary, lateral view **F** lip in natural position, abaxial view **G** anther and pollinarium. Illustration by L. Ocupa from the holotype (*L. Ocupa* 211, HUT).



Figure 2. *Epidendrum lufinorum* sp. nov., photographed in situ at the type locality **A** habit with stem apex including inflorescence **B** close-up of a stem segment with a new growth in early stage **C** lateral view of flower **D** abaxial view of flower with ovary and apical vesicle. Photographs by L. Ocupa (based on the holotype: *L. Ocupa* 211).

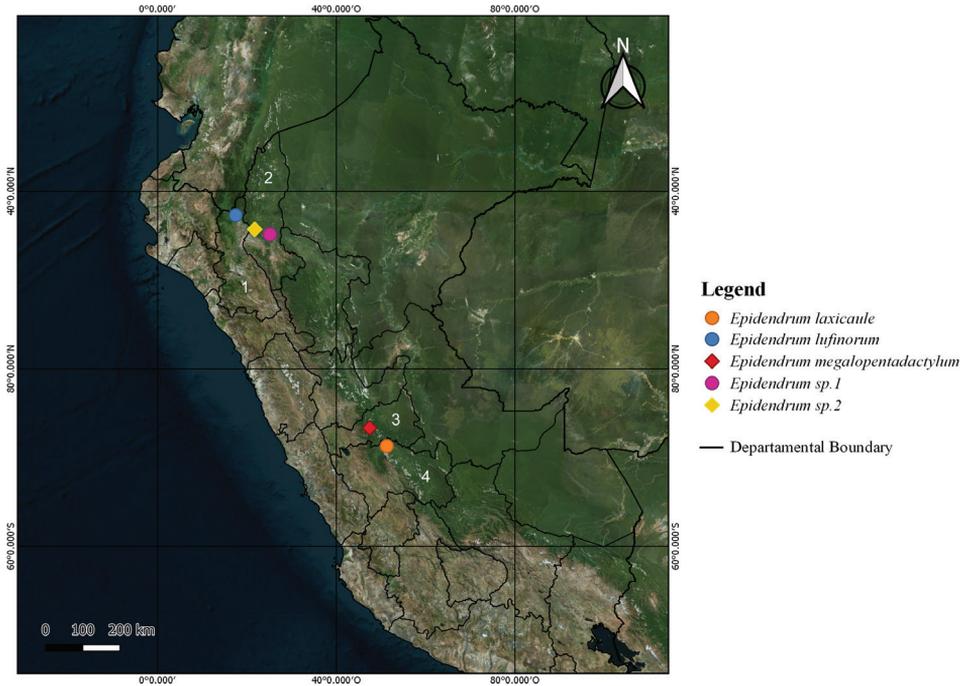


Figure 3. Species distribution map of the *Laxicaule* Group. Regions: **1** Cajamarca **2** Amazonas **3** Pasco **4** Junin Prepared by Luis Ocupa.

bose vesicle at the apex with basal portion of column. **Flowers** 1–2, lax, pendulous, resupinate, unscented; sepals and petals green, dorsally tinged reddish brown, abaxially with vinaceous spots and white margin; lip cream, becoming amber as it ages; column light green with vinaceous dorsal side. **Dorsal sepal** 2.4 × 0.4 cm, free, oblong-oblancoleate, arched forward, 5-veined, slightly concave in middle portion towards apex, 3-canalicate abaxially, margins attenuate, apex obtuse. **Lateral sepals** 2.3 × 0.75 cm, free, obliquely oblong-oblancoleate, arched forward, 5-veined, slightly convex at base towards middle portion, margin slightly revolute, involute in middle portion towards apex, apex acute, dorsally keeled. **Petals** 2.2 × 0.2 cm, free, linear, slightly incurved, slender, 1-veined, longitudinally somewhat oblique, obtuse, margins slightly recurved. **Lip** 2.2 × 2.0 cm, 3-lobed, transversely cordate, apex emarginate, fleshy, rigid, strongly revolute in natural position, margin entire; lateral lobes 11.9 × 7.2 mm, semiorbicular when expanded; mid-lobe 15 × 7.5 mm, bilobate, ecallose, disc with 5 central, parallel, prominent, fleshy and thickened ribs, fused at base, disappearing in the middle of mid-lobe, the two most lateral ribs are much less prominent. **Column** 13 × 4 mm, clavate, forming a prominent, ventral, gibbose vesicle at base with apical portion of ovary; clinandrium-hood much reduced, margin entire. **Anther** 2.5 × 2.1 mm, broadly elliptical, yellowish green. **Pollinia** 4, fulvous, in 2 nearly equal pairs, obovoid, flattened at interfaces, caudicles attaching them in pairs, granulose, viscarium semi-liquid, translucent. **Capsules** not seen.

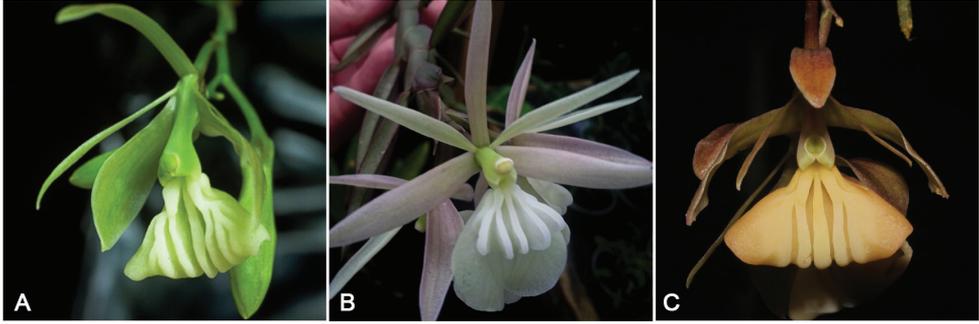


Figure 4. Comparison and flowers close-up of the *Laxicaule* Group species **A** *E. laxicaule* (Photo by I. Rolando) **B** *E. megalopentadactylum* (Photo by F. Corcuera) **C** *E. lufinorum* sp. nov. (Photo by L. Ocupa).

Eponymy. The epithet is an acronym formed by the first two letters of the names Luis (1966–), Fiorella (1993–) and Noemí (1970–), parents and sister of the first author, to whom he wishes to dedicate this species.

Distribution, habitat and comments on the conservation of the species. This species is currently known only from the type locality in the northern zone of Peru, near the base of the hill known locally as Cerro Parcos, in the village of Villa Rica, San José de Lourdes district (Fig. 3). The habitat of *Epidendrum lufinorum* is within an area with high agricultural activity such as the cultivation of *Coffea arabica* L. (Rubiaceae Juss.). No more individuals of this species were found in the surrounding areas, but a fertile individual (holotype) and some other small specimens growing as epiphytes on the trunks of *C. arabica* plants, occasionally sharing the same phorophyte with other orchid species such as *Gongora aromatica* Rchb.f., *Masdevallia glandulosa* König, *Stenia calceolaris* (Garay) Dodson & D.E.Benn. and *Telipogon astrogllossus* Rchb.f., which would support the hypothesis of a possible adaptation of this species to anthropized environments. However, its habitat continues to be fragmented as a consequence of tree felling, the expansion of agricultural crops and practices such as pruning and clearing coffee trees of epiphytic plants. The latter is a very common practice among the local population, as a way to maintain optimal conditions for the efficient production of coffee beans, however, it threatens the presence of the few individuals of *E. lufinorum* that may be growing.

Most of the coffee crops were located near secondary forest patches with some individuals of trees as *Cedrela odorata* L., *Delostoma integrifolium* D. Don, *Erythrina edulis* Triana ex Micheli and *Vochysia vismiifolia* Spruce ex Warm.

Phenology. *Epidendrum lufinorum* was observed flowering in April, at the end of the rainy season in that region.

Notes on Laxicaule Group. The combination of a monopodial pendulous habit, linear leaves, an apical inflorescence with few flowers, and a lip with a prominent ribbed disc places the new species in the informal Laxicaule Group.

There are two species in the group, both presently known from Peru: *Epidendrum laxicaule* and *E. megalopentadactylum* (Hágsater and Huayta 2018) reported in Junin and Pasco, respectively (Fig. 4A, B).

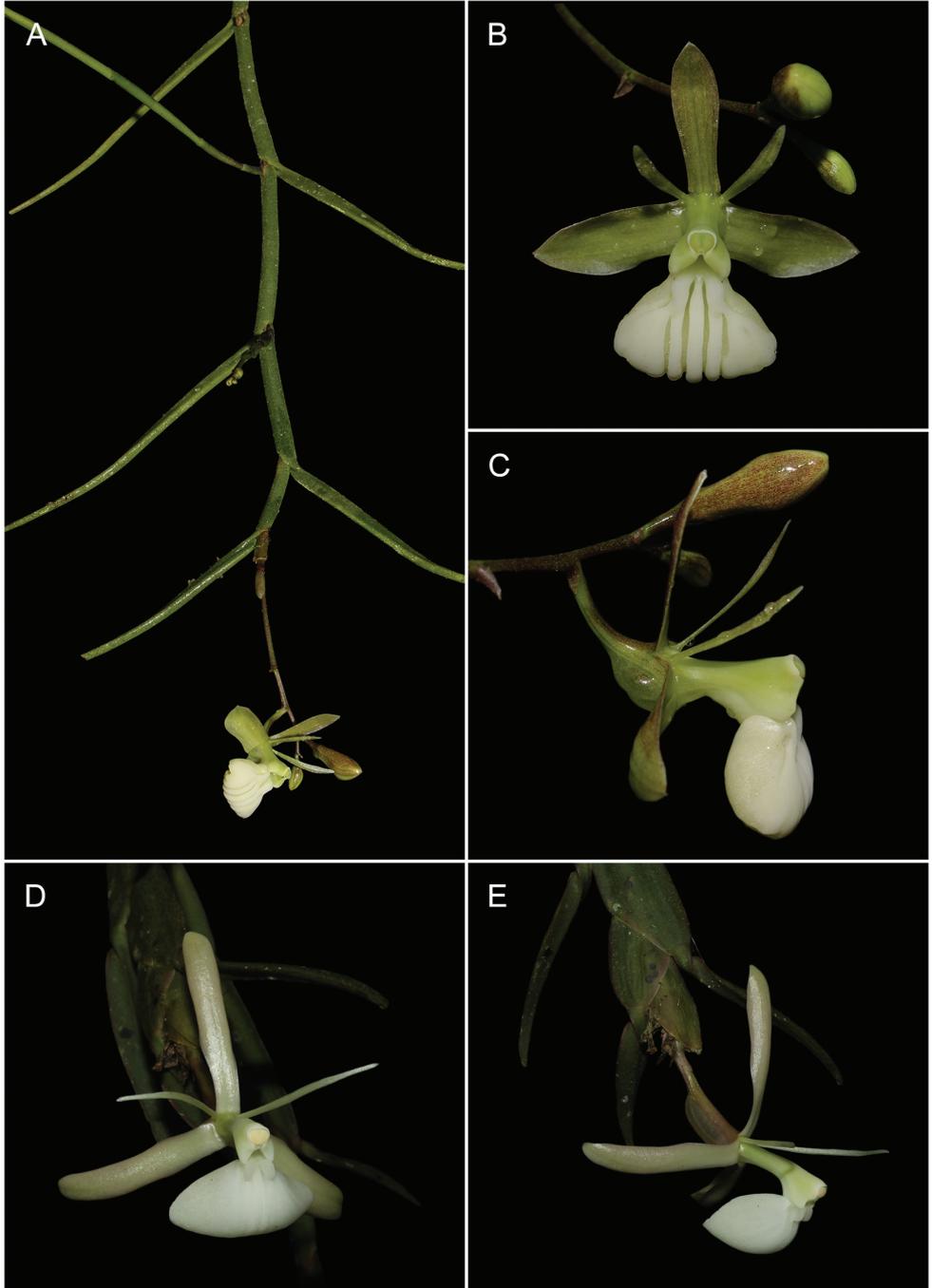


Figure 5. Other undescribed species of the Laxicaule Group found in Amazonas **A, B, C** *Epidendrum aff. laxicaule* sp. nov. and *E. lufinorum* **D, E** *Epidendrum aff. megalopentadactylum* sp. nov. Photographs by L. Pillaca (**A, B, C**) and L. Ocupa (**D, E**).

Table 1. Features distinguishing the species of Laxicaule Group.

Character	<i>E. laxicaule</i>	<i>E. megalopentadactylum</i>	<i>E. lufinorum</i>
Stem section	Elliptic	Ancipitose	Terete
Leaf size	8.5 × 0.45–0.47 cm	4.5–7.5 × 1.5–2.5 cm	5–7.2 × 0.38–0.4 cm
Inflorescence	6 cm long	Sessile, compact	7 cm long
Leaf shape	Linear	Narrowly lanceolate	Linear
Cross section leaf	Semiterete	Flat, conduplicate at base	Semiterete
Ovary length	1.3–1.5 cm	1.6 cm	2.0 cm
Ovary vesicle	Globose	Absent	Gibbose
Number of flowers	3–4	3–5	1–2
Dorsal sepal size	3.2 × 0.6 cm	3.8 × 1.0 cm	2.4 × 0.4 cm
Dorsal sepal shape	Narrowly oblanceolate	Narrowly elliptic-ovate	Oblong-oblanceolate
Dorsal sepal apex	Acute	Acute	Obtuse
Lateral sepal size	2.6 × 0.9 cm	4.0 × 1.2 cm	2.3 × 0.75 cm
Lateral sepal shape	Obliquely oblong-oblanceolate	Narrowly elliptic-ovate, slightly oblique	Obliquely oblong-oblanceolate
Petals size	2.8 × 0.2 cm	3.8 × 1.0 cm	2.2 × 0.2 cm
Petals shape	Narrowly linear-lanceolate	Narrowly elliptic	Linear
Petals apex	Acuminate	Acute	Obtuse
Lip size	2.0 × 2.6 cm	3.3 × 3.1 cm	2.2 × 2.0 cm
Lip shape and lobes	Transverse, 3-lobed	Suborbicular, entire	Transversely cordate, 3-lobed
Disc of lip	5-parallel ribs projecting distally	5-ribs	5-parallel and central ribs
Column size	1.25 cm long	1.0 cm long	1.3 cm long
Column shape	Clavate	Straight	Clavate

Among the two species belonging to the Laxicaule group, *Epidendrum lufinorum* is most similar to *E. laxicaule*, from which it differs in the shape and length of the dorsal sepal; the shape of the petals and the lip, disc ribs position of the lip; shape of the vesicle formed between the ovary and the column as well as the section of the stem. *E. lufinorum* is easily distinguished from the other species of the Laxicaule group by the characteristics indicated in the **Table 1**. It is worth mentioning that the type specimen of *E. lufinorum* we collected is a plant consisting of a flowering primary stem, with a new secondary branch clearly visible in its early stage of development (Fig. 2B).

In May 2015, a specimen of another species, *Epidendrum* aff. *laxicaule* sp. nov., was found growing as an epiphyte in a montane forest in the western part of Amazonas department in northern Peru, in the Cajaruro district of Utcubamba province, at an elevation of 1685 m. It was observed and validated with photographs (Fig. 5A, B, C) by Luis Pillaca and shows vegetative and morphological characteristics similar to those of *E. laxicaule* and *E. lufinorum*. However, it has flat and thick, narrow leaves, a much more prominent vesicle at the apex of the ovary and the narrower lip with three parallel and central ribs. Pillaca indicates that the area where the specimen was found has been destroyed due to agricultural encroachment.

A year later, in the same department, but in the province of Bagua, district of Aramango, a specimen of an additional species, *Epidendrum* aff. *megalopentadactylum* sp. nov., was found as part of the private collection of Ricardo Saens Saavedra†. This specimen observed and photographed (Fig. 5 D, E) by Luis Ocupa, presents morphological characteristics similar to *E. megalopentadactylum*, such as pendulous plants with flat leaves, conduplicate at the base, strongly laterally compressed and ancipitous stems and an entire lip, but is distinguished by the presence of a vesicle and the lip without ribs.

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Supplementary material I

A new species of *Epidendrum* (Orchidaceae) of pendulous habit from Peru

Authors: Luis Ocupa Horna, Eric Hágsater, Marco M. Jiménez

Data type: species data

Explanation note: *Epidendrum lufinorum*, found in northern Peru, it similar with *Epidendrum laxicaule* D.E.Benn & Christenson but differs in the shape and length of the dorsal sepal; the shape of the petals and the lip, ribs position of the lip, shape of the vesicle formed between the ovary and the column as well as the section of the stem.

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Link: <https://doi.org/10.3897/phytokeys.184.70844.suppl1>

Updated description of *Diospyros dussaudii* Lecomte (Ebenaceae), with lectotypification and notes on its distribution

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Abstract

Diospyros dussaudii is a poorly known species and previous descriptions lacked details about its female flowers and fruits. The species had not been recorded since type collections were made in 1913. As a result of our *Diospyros* research in Thailand, new specimens and data are now available for this species. In this study, we provide an updated morphological description, illustrations, lectotypification and a distribution map. The species was previously reported to be endemic to Laos; as such, the occurrences in Thailand greatly expand the distribution of the species. In addition, we analysed the phylogenetic relationships between *D. dussaudii* and other *Diospyros* species from Southeast Asia and other regions, using DNA sequence data from eight plastid regions. Our phylogenetic analyses indicate that *D. dussaudii* is closely related to *D. castanea*, *D. dasyphylla* and *D. insidiosa*. Their taxonomic affinities are discussed.

Keywords

Dioecy, *Diospyros*, DNA sequence data

Introduction

Although the taxonomy of the genus *Diospyros* L. in Thailand is fairly well documented, some specimens do not fit species in the known flora of Thailand (Phengkhai 1981, 2005; Duangjai et al. 2018, 2020; Eiadthong 2020). This was the case for specimens collected from Phu Langka National Park in Nakhon Phanom Province, north-eastern Thailand in 2013 and from Chumphon Province, peninsular Thailand in 2014. The leaves and buds of these specimens resemble those of *D. dasyphylla* Kurz, which also occurs in Thailand. However, their fruits are globose or depressed-globose, ca. 4.0–5.0 cm in diameter and densely covered with orange hairs, similar to those of *Sandoricum koetjape* (Burm. f.) Merr. (Meliaceae), but different from those of *D. dasyphylla*, which has glabrous fruits. When we compared these specimens with species known to occur in Indochina (Lecomte 1928, 1930), as well as specimens from the Muséum national d'Histoire naturelle (P), we observed similarities in the leaves and buds to a poorly known species from Laos, *D. dussaudii* Lecomte. However, we could not positively identify the specimens due to lack of detail on the female plants of *D. dussaudii* in the published descriptions and due to the absence of male specimens amongst our collections.

Diospyros dussaudii is one of thirty Indochinese *Diospyros* species described by the French botanist Paul Henri Lecomte (1856–1934) in 1928 (Lecomte 1928). Lecomte's description was based only on male specimens, which were collected by M. Dusaud from what is now the Lao People's Democratic Republic (PDR) on 4 October 1913. Until recently, the species was known only from one collection with three type specimens (P00721485, P00721486 and P02141495) held at P. The species is poorly known and was previously reported to be endemic to Laos (Lecomte 1928, 1930). It was not thought to occur in Thailand (Phengkhai 1981) or China (Lee et al. 1996) and is not included in a checklist of vascular plants of the Lao PDR (Newman et al. 2007a). No information was available about the female flowers and fruits, which are important in the systematics of *Diospyros* and no specific locality was mentioned in the protologue (Lecomte 1928).

In 2019, Kwanjai Khammongkol collected additional specimens of the same unknown *Diospyros* species from Tat Pho Waterfall, Phu Langka National Park, Thailand. Later, in 2020, additional populations of this species were found in peninsular Thailand during floristic surveys conducted by teams from the Protected Area Regional Office 4 (Surat Thani) and the Surat Thani National Park and Protected Area Innovation Centre. Female and male flowers were collected from Surat Thani Province and Chumphon Province, respectively. Based on examination of type specimens at P, we identified a male specimen of the unknown *Diospyros* from peninsular Thailand as *D. dussaudii*.

In late December 2020, Sukid Rueangrua and Somran Suddee found a sapling of *D. dussaudii* during a field trip with Japanese botanists on the Bolaven plateau in southern Laos, but did not collect it. However, after searching for Laotian specimens in various herbaria, another Laotian collection (Newman et al. LAO 833) of *D. dussaudii* was found in E and L (L0409075). These specimens were collected in 2005 from Khammouan and were initially identified as *Diospyros* sp.

The objectives of this study were to report the rediscovery of *D. dussaudii* in Laos and provide an updated description of the species, as well as photographs, illustrations and notes on its distribution. In addition, we typified the species name and selected a lectotype. We also determined the phylogenetic placement of the species using DNA sequence data. Finally, to facilitate the distinction of *D. dussaudii* from closely allied species, a comparison of morphological characters is presented.

Materials and methods

Morphological investigation, description and geographical distribution

Examination of *D. dussaudii* was based on specimens and preserved spirit collections obtained from north-eastern and peninsular Thailand. These voucher specimens, representing both male and female plants, were deposited in the Bangkok Herbarium (BK) and Bangkok Forest Herbarium (BKF). We also examined digital images of specimens held at BM, E, K, L and P (abbreviations follow Thiers 2020). We further compared these specimens with all published records of *Diospyros* species in Thailand and adjacent regions (Lecomte 1930; Bakhuizen van den Brink 1936–1955; Ng 1978, 2001; Phengkhai 1981, 2005; Lee et al. 1996; Singh 2005). Material collected from north-eastern and peninsular Thailand was photographed in the field. The habit, habitat, coordinates and elevation were documented in the field. Floral morphology was studied with dissecting microscopes at the Department of Forest Biology, Faculty of Forestry, Kasetsart University. An updated description of the species was developed from digital images of type specimens from P, the protologue, digital images of Lao-tian specimens held at L, specimens collected from Thailand and field observations. A distribution map, based on specimens and field observations, was created with SimpleMapp (Shorthouse 2010). The conservation status of the species was evaluated with IUCN Red List Categories and Criteria (IUCN 2019).

Phylogenetic analysis

One accession of *D. dussaudii* from north-eastern Thailand and three accessions from peninsular Thailand were compared with DNA sequences of eight plastid regions (*rbcL*, *atpB*, *matK*, *ndhF*, *trnK* intron, *trnL* intron, *trnL-trnF* spacer and *trnS-trnG* spacer). Total DNA was extracted from silica-dried leaf samples with a modified 2× cetrimonium bromide procedure (Doyle and Doyle 1987). The primers and polymerase chain reaction (PCR) protocol used for amplification are as described in Duangjai et al. (2009), except that we used 2× DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), following manufacturer's protocols. Successfully amplified products were cleaned with FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Fisher Scientific). The cleaned PCR products were sequenced with the same primers used in the initial amplifications. Sanger

sequencing was performed at the MacroGen sequencing facility (MacroGen, Inc., Seoul, South Korea).

The DNA sequences of *D. dussaudii* were manually aligned to the dataset from Duangjai et al. (in prep.). Phylogenetic analyses were carried out with Maximum Parsimony (MP) and Bayesian Inference (BI; Rannala and Yang 1996; Yang and Rannala 1997). The MP analyses were conducted with equally weighted, unordered nucleotide substitutions (Fitch 1971) in PAUP* v.4.0b10 (Swofford 2002). The most parsimonious trees were searched heuristically with 1,000 replicates of random sequence addition; the settings included tree bisection and reconnection (TBR) swapping and MulTrees = on. TBR swapping was performed on a maximum of 200 trees (nchuck = 200) per replicate. Node support was evaluated with 1,000 bootstrap replicates of 1,000 random additions. BI was performed with MrBayes v.3.2 (Ronquist et al. 2012) on the CIPRES Science Gateway platform (Miller et al. 2010). Nucleotide substitution models were selected with the Akaike Information Criterion (AIC), implemented in MrModeltest v.2.3 (Nylander 2004). We performed two independent Markov chain Monte Carlo analyses, with four simultaneous chains of 10,000,000 generations, sampling one tree per 1,000 generations. The first 25% were discarded as burn-in and the remaining trees were used to construct a majority-rule consensus tree with Bayesian Posterior Probabilities (PPs). *Euclea* L., *Lissocarpa* Benth. and *Royena* L. (Ebenaceae) species were selected as the outgroup. Genetic distances between *D. dussaudii* and closely related species were generated with the Kimura 2-parameter model (Kimura 1980), with all gaps treated as missing (complete deletion option). DNA sequences of the eight plastid regions from four individuals of *D. dussaudii* were submitted to GenBank (accession numbers: MZ457089–MZ457112).

We compared the morphology of *D. dussaudii* with that of three species determined to be closely related on the basis of the results of the phylogenetic analyses. Data for *D. castanea*, *D. dasyphylla* and *D. insidiosa* Bakh. were obtained from previous studies (Lecomte 1930; Bakhuizen van den Brink 1936–1955; Ng 1978; Phengklai 1981; Lee et al. 1996; Singh 2005) and supplemented by our own observations.

Results and discussion

After careful study of the protologue and type specimens (Fig. 1), we determined that our collections from peninsular Thailand matched the description and type specimens of *D. dussaudii*. *Diospyros dussaudii* is a poorly known species and the protologue included a description of male plants only. The rediscovery of *D. dussaudii* in Laos and the recent collections from Thailand allowed us to complete the description of the species.

Phylogenetic analysis

We investigated the phylogenetic relationships of one individual of *D. dussaudii* from north-eastern Thailand and three individuals from peninsular Thailand using DNA



Figure 1. Type specimens of *D. dussaudii* (M. Dussaud 120) deposited at P. **A, B** lectotype, P00721485, **C** isolectotype, P00721486 and **D** isolectotype, P02141495.

sequence data from eight plastid regions. In addition, we clarified the phylogenetic relationships between *D. dussaudii* and other *Diospyros* species. When sequences of four individuals of *D. dussaudii* were included in the data matrix, the concatenated alignment of the 186-terminal dataset consisted of 8,293 characters, amongst which 1,991 sites were variable and 1,150 were MP-informative. MP analysis yielded 23,000 equally parsimonious trees with 3,484 steps (consistency index = 0.66; retention index = 0.84). The results of the MP and BI analyses were generally congruent; therefore, we present only the latter (Fig. 2). Phylogenetic analyses of eight plastid regions indicate with strong support (PP 1.0) that *Diospyros* and three other genera, *Euclea*, *Lissocarpus* and *Royena*, are monophyletic. The overall phylogenetic relationships of these four genera and of the clades recovered within *Diospyros* are congruent with previous reports (Duangjai et al. 2009, 2018, 2020). We identified 11 major clades of *Diospyros*, eight of which (I, III, V, VI, VII, IX, X and XI) include Asian species. The results of our phylogenetic analyses unambiguously place the four *D. dussaudii* individuals within clade XI (Fig. 2) with 43 other species from Asia, the Americas, New Caledonia and the Pacific Islands. Although relationships within the clade are not fully resolved, the four individuals of *D. dussaudii* group together with high support (PP = 1.00) and have a well-supported sister relationship with *D. castanea* (PP = 1.00) and this clade is sister to a clade of *D. dasyphylla* and *D. insidiosa*. The genetic distance between *D. dussaudii* and *D. castanea*, *D. dasyphylla* and *D. insidiosa*, based on data from eight plastid regions, ranges from 0.0041 to 0.0080, whereas intraspecific distances amongst the four individuals of *D. dussaudii* range from 0 to 0.0009 (Table 2). Pairwise similarity for *D. dussaudii* is highest with *D. castanea* (99.58%), followed by *D. dasyphylla* (99.36%) and *D. insidiosa* (99.20%). There are 13 polymorphic characters out of 8,293 that differ within the four individuals of *D. dussaudii*. However, the four individuals have 27 characters in common that differ from the sister species *D. castanea*.

Other species in the same subclade as *D. dussaudii* are *D. buxifolia* Thouars, *D. ferox* Bakh., *D. filipendula* Pierre ex Lecomte, *D. pilosula* Wall. ex Hiern and *D. toposia* Buch.-Ham. The nine species in the subclade are all distributed in South or Southeast Asia (Lecomte 1930; Bakhuizen van den Brink 1936–1955; Ng 1978; Phengkai 1981).

Amongst the species in the subclade, the fruits of *D. dussaudii* are similar in size to those of *D. castanea*, *D. dasyphylla* and *D. insidiosa*. They are globose or depressed-globose and ca. 4.0–5.0 cm in diameter, whereas *D. buxifolia*, *D. ferox*, *D. filipendula* and *D. pilosula* have fruits that are less than 2 cm in diameter. The differences between *D. dussaudii* and *D. castanea*, *D. dasyphylla* and *D. insidiosa* are shown in Table 1. When vegetative, *D. dussaudii* may be confused with *D. dasyphylla*; however, the two species can be distinguished by leaf texture and the shape of the leaf base. *Diospyros dussaudii* has thicker leaves than *D. dasyphylla*. The leaf base of *D. dussaudii* is attenuate or cuneate, whereas that of *D. dasyphylla* is obtuse or cordate. In addition, the fruits of *D. dussaudii* are hairy, with a thin mesocarp (ca. 3.0–4.0 mm thick), whereas *D. dasyphylla* has shiny glabrous fruits with a thicker mesocarp (ca. 10.0 mm). The outer bark of *D. dussaudii* is smooth and has sparse lenticels, whereas *D. dasyphylla* has scaly bark without lenticels. When one compares leaves of *D. dussaudii* with those of *D. castanea*

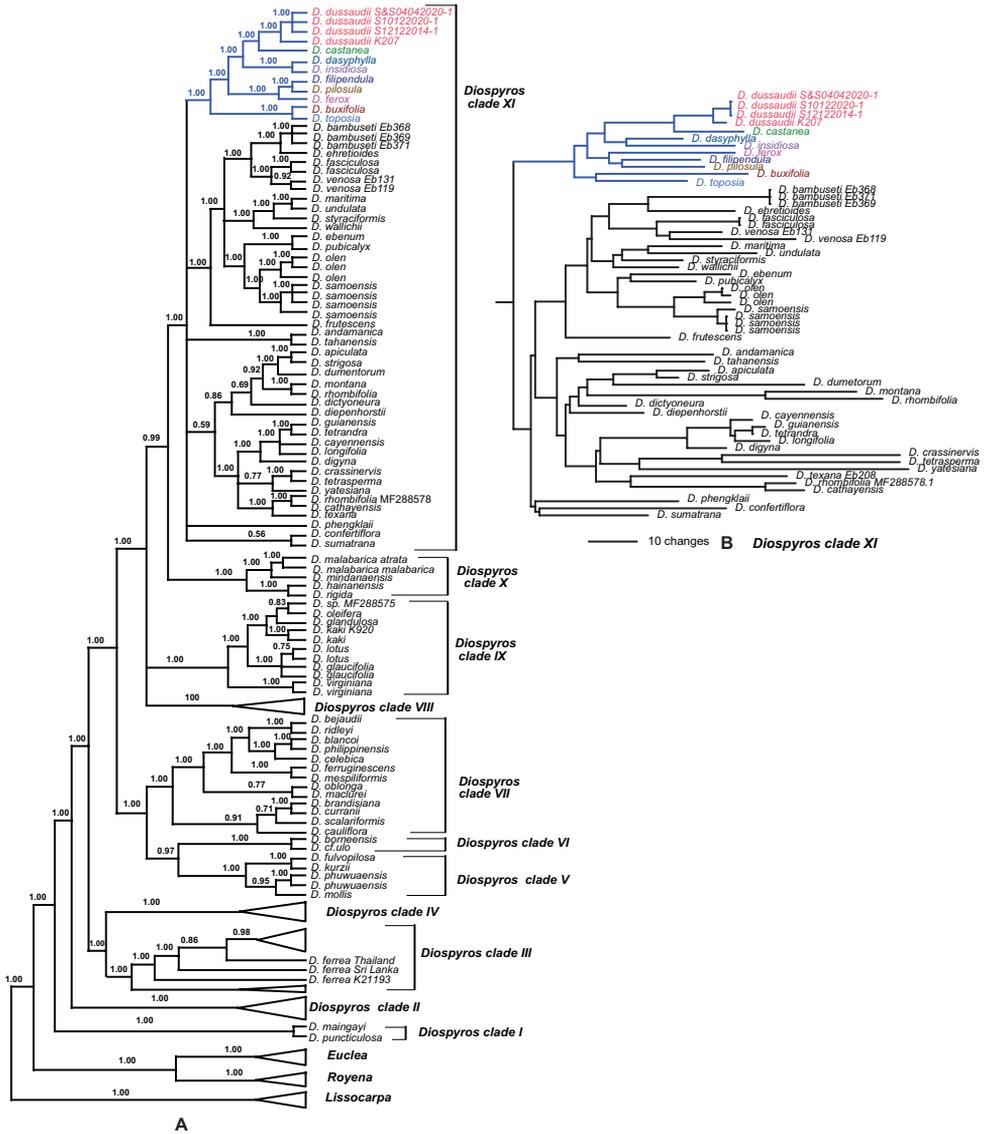


Figure 2. Phylogenetic trees of *Diospyros* and related genera, based on DNA sequence data from eight plastid regions **A** the 50% majority-rule tree from BI analysis. Posterior probabilities > 0.50 are shown above branches **B** phylogram from BI analysis, showing only the details of *Diospyros* clade XI. *Diospyros dussaudii* and closely related species are indicated in coloured text.

and *D. insidiosa*, the leaves of the latter two species are glabrous on both surfaces. The fruiting calyces of *D. dussaudii*, *D. dasyphylla* and *D. insidiosa* are similar and slightly enlarged compared to the flowering stage, with four spreading, rounded lobes, ca. 4.0 × 5.0 mm, that are divided to the base. The calyces of *D. castanea*, by contrast, are discoid and spreading, ca. 14 mm in diameter and entire or sometimes split into three lobes.

Taxonomy

***Diospyros dussaudii* Lecomte, Notul. Syst. (Paris) 4: 113. 1928; in Fl. Indo-Chine 3: 954. 1930.**

Type. Laos. reçu le 4 October, 1913, *Dussaud 120* (lectotype designated here, P barcode P00721485; isolectotypes P barcode P00721486 and P02141495). Figs 1 and 3–5.

Tree, 8–15(–20) m tall, up to 20 cm in diameter; trunk straight, without buttresses; outer bark grey, smooth and sparsely lenticellate; inner bark thick, reddish-brown or blackish-brown; sapwood white. Young branches covered with hairs, persistent or later glabrous. Buds covered by bifarious scales, ca. 3.0 cm long, outside densely pubescent. Leaves alternate; petiole 8–9 mm long, with dense short brown hairs; blade subcoriaceous, oblong or elliptic-oblong, 12.0–16.2 × 3.8–5.0 cm, base attenuate to cuneate, apex acute to acuminate; mid-rib prominent and finely pubescent above, glabrous or slightly pubescent or puberulous below; secondary veins 7–10 on each side, grooved above, raised below; glabrous adaxially, glabrous to tomentose abaxially. Male inflorescences fasciculate, axillary or on older branches below the leaves, covered with bifarious scales at base. Male flowers 4-merous; pedicel ca. 1 mm long, pubescent; calyx tubular, with 4 short lobes, rounded at apex, 6.5–8.0 mm long, pubescent outside, glabrous inside; corolla white, salverform, pubescent outside, glabrous inside, tube ca. 8.0 mm long, cylindrical, lobes 4, lanceolate, 10.0 × 3.0–3.5 mm; stamens 12–16, arranged in 2 series, attached at base of corolla tube; filaments 1–3 mm long, sparsely pubescent; anthers triangular, ca. 1.8 mm long, apex apiculate, dehiscence sublateral. Female inflorescences 1-flowered, in the axils of leaves or on older branches below the leaves. Female flowers 4-merous; sessile or subsessile, pedicel up to ca. 3.0 mm long, bracteate; calyx green, tube ca. 6.0 mm long, pubescent outside, glabrous inside, lobes rounded, ca. 5.0 × 3.0 mm, pubescent outside, glabrous inside; corolla creamy-white to pale yellow, urceolate, tube ca. 9.0 mm long, ca. 7.0 mm in diameter, lobes elliptic, ca. 10.0 × 7.0 mm, pubescent outside, glabrous inside; staminodes 8, attached at the base of corolla tube; ovary globose, pubescent, 8-locular; style 1, ca. 1 mm long, glabrous, stigmas 4. Fruits globose or depressed-globose, covered with dense orange hairs, 3.8–5.0 × 4.0–5.0 cm, apex rounded, shortly apiculate, 8-locular; seeds 3–8 per fruit, light green when immature, turning yellow and orange when ripe; fruiting calyx divided to base, lobes ovate-oblong, spreading, ca. 4.0 × 5.0 mm, pubescent outside, glabrous inside; fruit stalk ca. 5.0 mm long; mesocarp 3.0–4.0 mm thick, cream with brown dots. Seeds ellipsoid to planoconvex, two faces flat and one face convex, glabrous, ca. 4.0 × 5.0 mm, black, endosperm smooth when young, but slightly ruminated when mature.

Additional specimens examined. LAOS. Khammouan: Nam Theun, Kaeng Luang, landing area near waterfall, 17°45'46"N, 105°20'21"E, 555 m alt., 7 November 2005, fr., *Newman et al. LAO 833 (E!, L!)*. THAILAND. Nakhon Phanom Province: Ban Phaeng District, Phu Langka National Park, trail behind park headquarters, 17°59'06.60"N, 104°07'58.20"E, 197 m alt., 14 June 2013, fr., *Suddee et al. 4514*

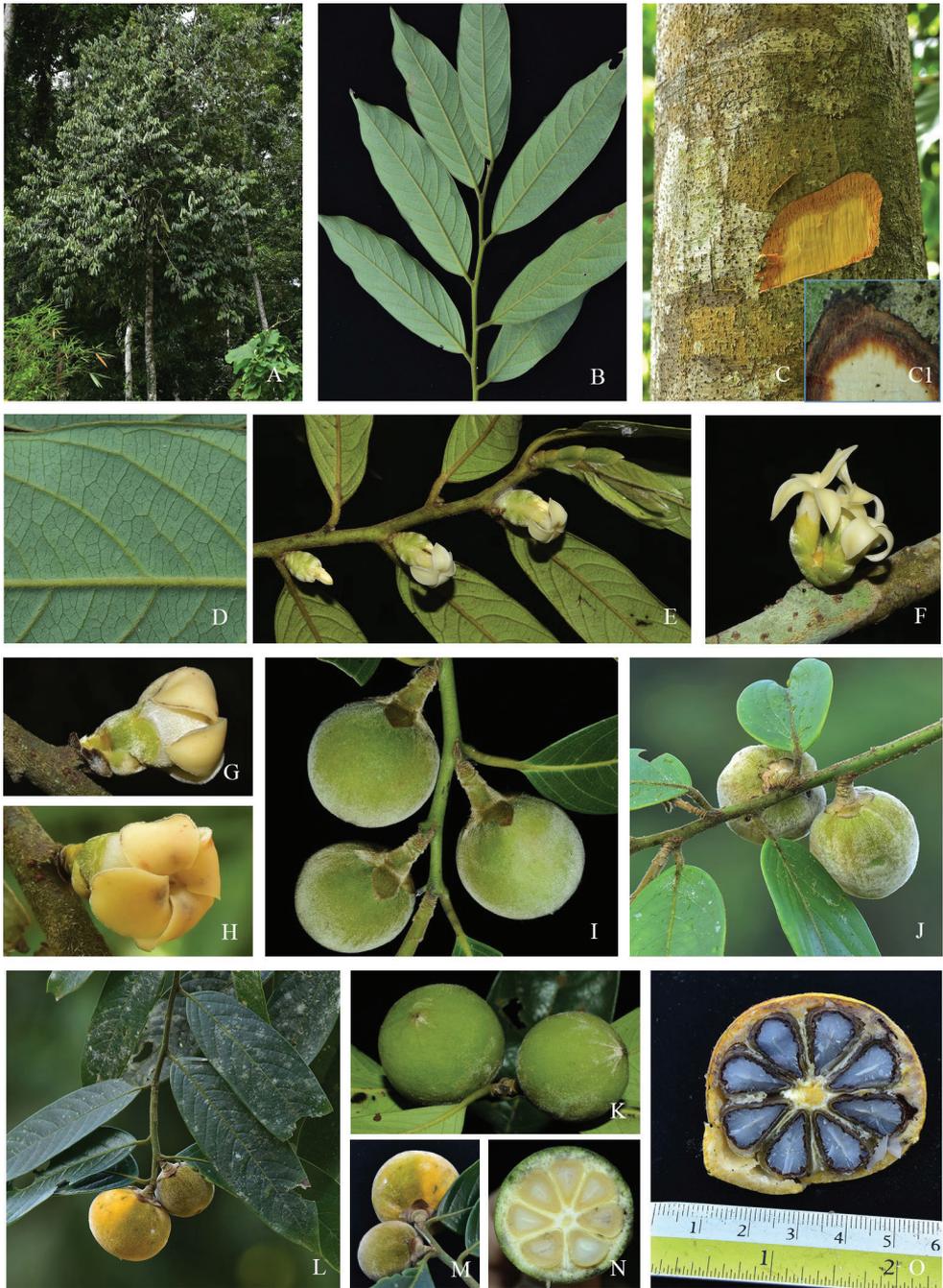


Figure 3. *Diospyros dussaudii* **A** habit **B** branch and leaves **C** trunk and bark **CI** slash of bark **D** leaf venation, abaxial view **E, F** male inflorescences **G, H** female flower **I–K** immature fruits **L, M** mature fruits **N, O** fruit in cross section showing immature seeds (**N**) and mature seeds (**O**). Photographs by Aroon Sinbumroong (**A–H, J, L–M** and **O**) and Sukid Rueangrua (**I, K** and **N**).

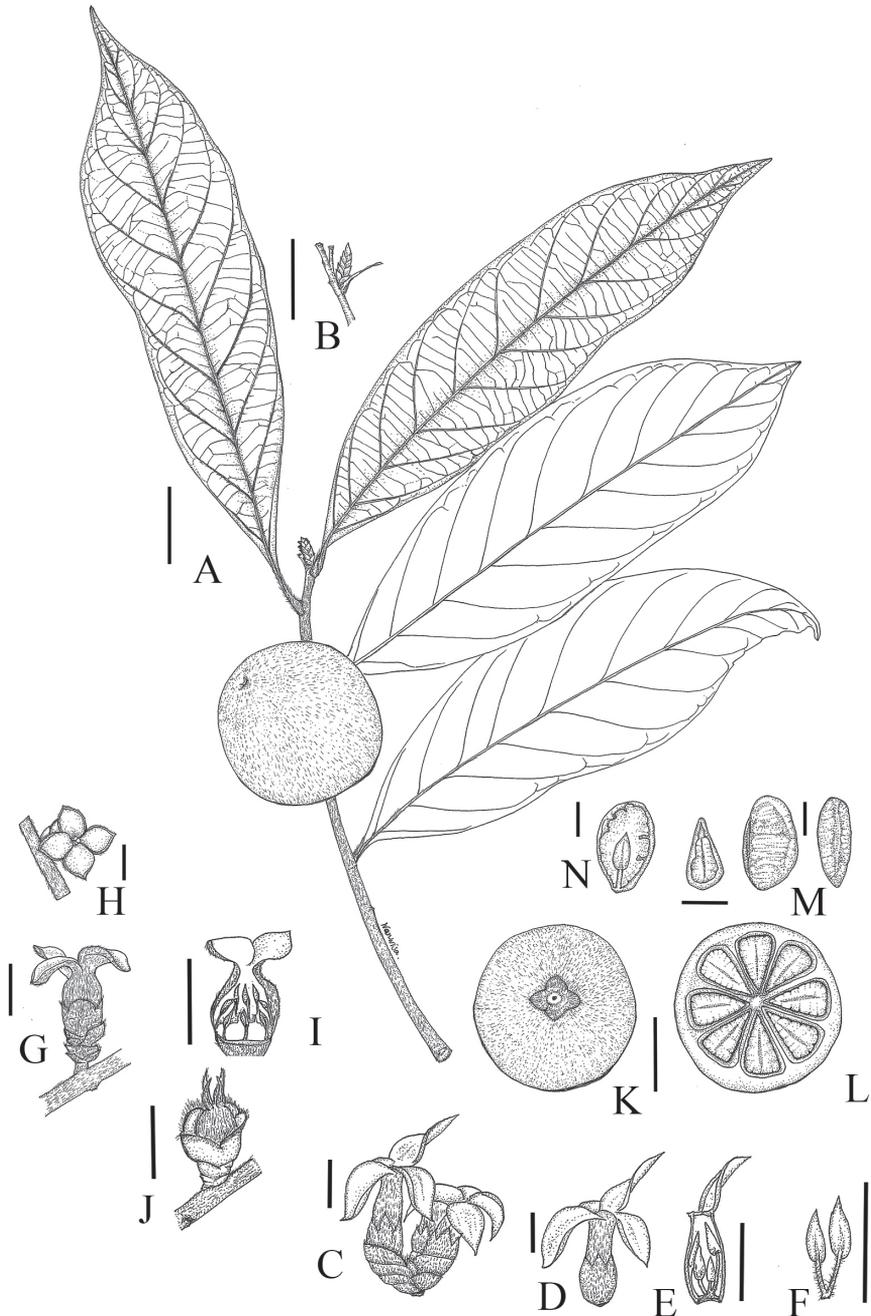


Figure 4. *Diospyros dussaudii* **A** fruiting branchlet **B** axillary bud **C** male inflorescence **D** male flower **E** opened corolla of male flower, showing stamens **F** stamens **G** female flower, side view **H** female flower, top view **I** opened corolla of female flower, showing eight staminodes attached at the base of corolla tube **J** ovary and stigma of female flower **K** fruit with four calyx **L** cross section of 8-seeded fruit **M** seeds, top view and side view and **N** the longitudinal section of seed, showing embryo. Drawn by W. Bhuchaisri from *Sinbumroong & Suekaew 04042020-1* (**A, B** and **K–N**), *Sinbumroong 12092020-1* (**C–F**) and *Sinbumroong 08052020-1* (**G–J**). Scale bars: 1 cm (**G–J, M, N**); 2 cm (**A, B, K, L**); 5 mm (**D–F**); 8 mm (**C**).

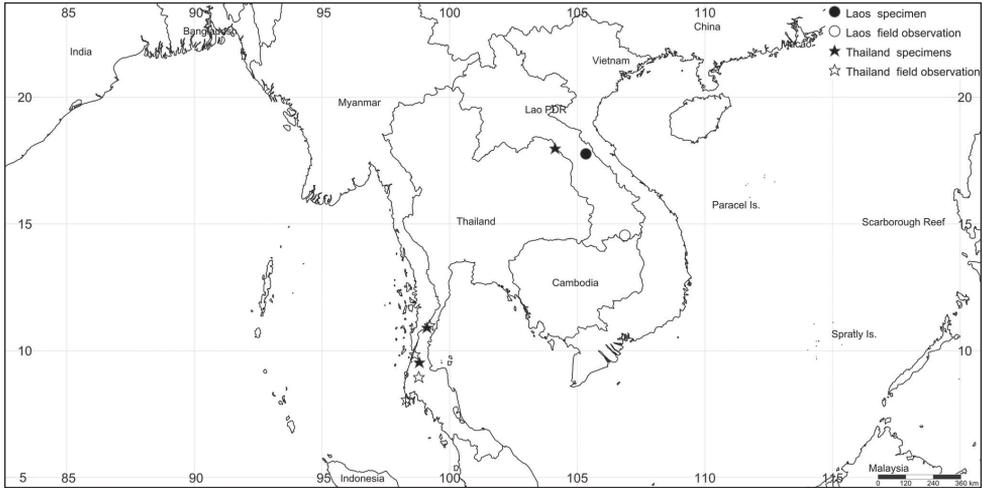


Figure 5. Geographic distribution of *D. dussaudii*. The solid star and star represent newly recorded localities in Thailand and the solid circle and circle represent localities in Laos. The map was created with SimpleMappr (<http://www.simplemappr.net>; Shorthouse 2010).

(**BKF**); Ban Phaeng District, Phai Lom, Phu Langka National Park, Tat Pho Waterfall, 26 December 2019, *Khammongkol 207* (**BKF**). Chumphon Province: Lamae District, 12 December 2014, *Sinbumroong 12122014-1* (fr.) (**BKF**); *ibid.*, 8 May 2020, *Sinbumroong 08052020-1* (female fl.) (**BKF**); Tha Sae District, 10 December 2020, *Sinbumroong 10122020-1* (fr.) (**BKF**). Surat Thani Province: Tha Chana District, 4 April 2020, *Sinbumroong & Suekaew 04042020-1* (fr.) (**BKF**); Ban Ta Khun District, Ratchaprapa Dam, 12 Sep. 2020, *Sinbumroong 12092020-1* (male fl.) (**BKF**).

Distribution. Laos, Thailand (Fig. 5).

Ecology. Scattered along streams in tropical rain forests and dry evergreen forests. The species occurs in the understorey at altitudes of 100–300 m. The canopy of the tropical rainforest in Kaeng Krung National Park, Surat Thani, where the species occurs, is dominated by *Anisoptera costata* Korth., *Artocarpus rigidus* Blume, *Dipterocarpus gracilis* Blume, *Dipterocarpus kerrii* King, *Palaquium impressionervium* Ng and *Parashorea stellata* Kurz. *Dacryodes rostrata* (Blume) H.J. Lam forms a high sub-canopy with *Hopea oblongifolia* Dyer, *Hydnocarpus castaneus* Hook. f. & Thomson, *Mesua ferrea* L. and *Xerospermum noronbianum* (Blume) Blume. Understorey species are *Barringtonia pauciflora* King, *Diospyros sumatrana* Miq., *Hydnocarpus nanus* King, *Koilodepas longifolium* Hook. f. and *Microdesmis caseariifolia* Planch. ex Hook.

Conservation status. As the Laotian population remains unknown, we have classified the species as Data Deficient (DD) based on IUCN Red List Criteria (IUCN 2019).

Phenology. Flowering May–October, fruiting May–April.

Note. When mature, specimens from peninsular Thailand exhibit hairy branches, whereas those collected from Phu Langka National Park have glabrous or glabrescent branches. The Laos specimens match those from Phu Langka National Park.

Table 1. Morphological differences between *D. dussaudii* and closely related species.

Character	<i>D. dussaudii</i>	<i>D. castanea</i>	<i>D. dasyphylla</i>	<i>D. insidiosa</i>
Bark	Outer bark grey, smooth with sparse lenticels, inner bark reddish-brown or blackish-brown, thick	Outer bark grey, cracked or scaly, inner bark brown, thick	Outer bark brownish-grey, scaly without lenticels, inner bark reddish-brown, thin	Outer bark greenish-black, smooth, inner bark blackish-brown, thick
Bifarious scales covering bud	Present	Present	Present	Absent
Leaves	Oblong or elliptic-oblong, 12.0–16.2 × 3.8–5.0 cm, subcoriaceous, glabrous adaxially, glabrous to tomentose abaxially, base attenuate or cuneate, apex acute to acuminate	Ovate, oval or elliptic, 6.0–13.0 × 2.5–8.0 cm, subcoriaceous or coriaceous, glabrous on both surfaces, base rounded, truncate or subcordate, apex acute to acuminate	Elliptic, ovate-oblong, oblong or obovate, 7.0–20.0 × 3.5–8.0 cm, chartaceous, glabrous adaxially, hispid or tomentose abaxially, base obtuse or cordate, apex acuminate to caudate-acuminate	Ovate or oblong, 5.0–17.0 × 2.0–7.0 cm, chartaceous to subcoriaceous, glabrous on both surfaces, base acute or obtuse, apex acute to acuminate
Merosity	4-merous	3-merous	4-merous	4–5-merous
Staminodes	8	Absent	Unknown	4–8
Fruit shape, covering and size	Globose or depressed-globose, covered with dense orange hairs, 3.8–5.0 × 4.0–5.0 cm	Globose or ellipsoid, glabrous or pubescent near base, 2.0–5.0 × 2.0–5.0 cm	Globose or depressed-globose, glabrous and shiny, 4.0–5.0 × 5.0–6.0 cm	Globose or depressed-globose, glabrous and shiny, 4.0–5.0 × 5.0–7.0 cm
Fruiting calyx	Lobes 4, divided to the base, ovate-oblong, spreading, ca. 4.0 × 5.0 mm	Discoid and spreading, ca. 14.0 mm in diameter, entire or sometimes split into 3 lobes	Lobes 4, divided to the base, ovate-oblong, spreading, ca. 4.0 × 5.0 mm	Lobes 4 or 5, divided to the base, ovate-oblong, spreading, ca. 4.0 × 5.0 mm
Mesocarp of mature fruits	Cream with brown dots, 3.0–4.0 mm thick	Cream with brown dots, 3.0–4.0 mm thick	Cream with brown dots, ca. 10.0 mm thick	Yellow, ca. 10.0 mm thick
Seeds	3–8 seeds per fruit, ellipsoid to planoconvex, endosperm slightly ruminated when mature	1–4 seeds per fruit, subglobose or ellipsoid, endosperm ruminated	6–8 seeds per fruit, ellipsoid to planoconvex, endosperm smooth	6–8 seeds per fruit, ellipsoid to planoconvex, endosperm smooth

Table 2. Kimura 2-parameter genetic distance amongst four individuals of *D. dussaudii* and closely related species.

	1	2	3	4	5	6
1. <i>D. dussaudii</i> S12122014-1						
2. <i>D. dussaudii</i> S&S04042020-1	0.0000					
3. <i>D. dussaudii</i> S10122020-1	0.0000	0.0000				
4. <i>D. dussaudii</i> K207	0.0009	0.0009	0.0009			
5. <i>D. castanea</i>	0.0042	0.0042	0.0042	0.0041		
6. <i>D. dasyphylla</i>	0.0064	0.0064	0.0064	0.0064	0.0063	
7. <i>D. insidiosa</i>	0.0080	0.0080	0.0080	0.0079	0.0080	0.0049

As mentioned in Newman et al. (2007b), duplicates of Laotian specimens were deposited in three other herbaria in Laos, as well as at P. However, we were unable to study the specimens in Laos due to the COVID-19 pandemic. It is unsurprising that only a few specimens were collected from Laos because Laos has a low rate of botanical collection (Middleton et al. 2019).

GenBank accession no. Sinbumroong 12122014-1: MZ457089 (*rbcL*), MZ457093 (*atpB*), MZ457101 (*matK* and *trnK* intron), MZ457097 (*ndhF*), MZ457105 (*trnL* intron and *trnL-trnF* spacer) and MZ457109 (*trnS-trnG* spacer). Sinbumroong & Suekaew

04042020-1: MZ457090 (*rbcL*), MZ457094 (*atpB*), MZ457102 (*matK* and *trnK* intron), MZ457098 (*ndhF*), MZ457106 (*trnL* intron and *trnL-trnF* spacer) and MZ457110 (*trnS-trnG* spacer). Sinbumroong 12092020-1: MZ457091 (*rbcL*), MZ457095 (*atpB*), MZ457103 (*matK* and *trnK* intron), MZ457099 (*ndhF*), MZ457107 (*trnL* intron and *trnL-trnF* spacer) and MZ457111 (*trnS-trnG* spacer). Khammongkol 207: MZ457092 (*rbcL*), MZ457096 (*atpB*), MZ457104 (*matK* and *trnK* intron), MZ457100 (*ndhF*), MZ457108 (*trnL* intron and *trnL-trnF* spacer) and MZ457112 (*trnS-trnG* spacer).

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Genetic diversity of *Salix lapponum* populations in Central Europe

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Abstract

Salix lapponum is a cold-tolerant relict species in Europe that occurs in several sites, probably reflecting previous migration routes of *S. lapponum* during the Pleistocene. However, only a few data are available on the genetic structures of populations of *S. lapponum*. In this study, we use PCR-ISSR markers to investigate genetic variation in 19 European populations of *S. lapponum* L. AMOVA analysis shows that most of the variation (55.8%) occurs within populations; variability among groups accounts for 19.7%. An AMOVA analysis based on four groups determined by STRUCTURE analysis shows similar results: variability of 54.1% within the population and variability of 18.9% between the four population groups, based on geographic regions. Within individual geographic groups, which are characterised by the studied populations, the lowest variability (as well as the highest homogeneity) was found in populations located in Belarus. The obtained results are consistent with our expectations that the European Lowland could be a significant geographic barrier for gene flow over large geographic distances for *S. lapponum*. Both the Scandinavian and Belarusian populations, as well as those coming from NE Poland, are characterised by significant genetic distinctiveness. However, some populations from NE Poland and the Sudetes show similarities with populations from other geographic regions, indicating existing genetic relationships between them. Moreover, the results suggest a fairly clear division of the population into 4 emerging geographic regions, although separated by a geographical barrier: the Polish lowland, which forms part of the larger geographic unit known as the European Lowland.

Keywords

Europe, genetic variation, phylogeography, relict plant, Salicaceae, *Salix*

Introduction

Quaternary glaciation, with numerous glaciations and deglaciations in Scandinavia and Central Europe, strongly influenced changes in the distributions of both specific plants and entire plant biomes. During Pleistocene climatic fluctuations, numerous arctic plants migrated southward, where they commonly colonised habitats primarily in European mountain ranges; in some cases, they also disappeared from these locations during warmer period called interglacials. Currently, various plant species growing in boreal areas in the Northern Hemisphere and in subalpine zones in lower latitudes in isolated mountains of the alpine system (subalpine zones) or on the lowlands – often grow on the edges of their ranges, presenting disjunct geographic distribution (Comes and Kadereit 2003; Schönswetter et al. 2003, 2005; Urbaniak et al. 2019).

Disjunctive plant populations are often attributed to isolated localities in specific habitats where the climatic and edaphic conditions have allowed for their survival as relict species until the present postglacial period (Birks 2008; Nagamitsu et al. 2014; Urbaniak et al. 2018a). The history of plant relicts relates primarily to the period of Pleistocene glaciations and climatic fluctuations. During the Ice Age, the tundra developed on a portion of the European Lowlands (Van Andel 2002; Birks and Willis 2008), where arctic and alpine plants grew on much wider territory than their current ranges. During each glaciation, species extended their ranges to lowland areas. In warmer interglacial periods, they were crowded out and retreated to specific refugia in higher mountain locations, such as alpine and subalpine zones or specific sites such as peat bogs in lowlands. Plant species that lived outside of refugia retreated northward or disappeared completely. Under these conditions, gene flow and some isolated populations could occur in between migrating populations; these were commonly also subjected to unfavourable genetic conditions such as a sharp reduction in population size due to environmental events, which reduced variation in the gene pool (known as a population bottleneck) or due to other changes in allele frequency, including genetic drift (Comes and Kadereit 1998; Hewitt 2004; Schönswetter et al. 2005; Stewart et al. 2010; Urbaniak et al. 2019). On the other hand, the effect of plant disjunction on genetic diversity and genetic structure can vary in ways that are not fully understood. However, some papers that have examined the effect of spreading disjunctive plant populations have clearly reported its negative effect (Paun et al. 2008).

Numerous phylogeographic studies have described processes including colonisations or re-colonisations from refugial zones, extinctions, or migrations across thousands of kilometres in the Northern Hemisphere (Schönswetter et al. 2004; Oliver et al. 2006). In recent years, intensive studies have sought to explain the origins of disjunct taxa (which originate from the Arctic and from alpine flora and trace their post-glacial migratory routes using various molecular tools, including AFLP techniques, microsatellite markers, or chloroplast DNA sequencing (Abbott et al. 2000; Abbott and Comes 2003; Skrede et al. 2006; Alsos et al. 2009; Allen et al. 2015). Previous research has highlighted the lack of a common model for the migration of arctic-alpine plants, even for species with similar habitat requirements (Skrede et al. 2009; Ronikier 2011).

During the Last Glacial Maximum (LGM), all natural vegetation of temperate Europe changed substantially. This also applies to the development history of various *Salix* species, for which results have been obtained from numerous paleobotanical studies and from research using modern genetic methods (Palmé et al. 2003; Reichs et al. 2007; Stamati et al. 2007; Alsos et al. 2009; Sochor et al. 2013; Berlin et al. 2014; Mirski et al. 2017; Wagner et al. 2018; Gouker et al. 2019; Hao et al. 2019; Liu et al. 2020; Wagner et al. 2020). In general, *S. lapponum* L. [Sp. Pl. 1019, 1753] belongs to sect. *Villosae* (Andersson) Rouy, subgenera *Vetrix* Dumort. (Rechinger and Akeroyd 1993; Argus 1997; Skvortsov 1999). It is a typical Euro-Siberian, boreal species, with a disjunctive arctic-alpine type of geographical distribution (Jalas and Suominen 1976; Hultén and Fries 1986; Stamati et al. 2007). *S. lapponum* can be found primarily in northern Europe and western Siberia and in isolated localities detached from the main distribution range in the mountains of central and southern Europe (the Auvergne, Pyrenees, Sudetes, Carpathians and Rhodopes). For these reasons, *S. lapponum* L. like *Andromeda polifolia* L., *Betula nana* L., *Carex bigelowii* Torr. & Schwein., *Dryas octopetala* L., *Juncus trifidus* L., *Rubus chamaemorus* L., *Salix herbacea* L. and *Saxifraga oppositifolia* L.– is considered a glacial relict (Dahl 1998; Kwiatkowski and Krahulec 2016; Dítě et al. 2018). This species grows on open or only partially shaded wet places, peat bogs, swamps, meadows at the banks of lakes and streams. It is a component of various types of vegetation such as *Caricion nigrae*, *Rhynchosporion albae*, *Molinion caeruleae* or *Adenostyllum alliariae*, *Salicion silesiacae* (Dierssen 1996; Kołos et al. 2015; Hroneš et al. 2018). It is a diploid species ($2n = 38$; Chmelař 1979). The expansion of willows has been facilitated by their ability to settle in appropriate niches, which are often small enclaves among other biotopes (Abbott et al. 2000; Birks and Willis 2008). Furthermore, due to the characteristic area of distribution of *S. lapponum* in Europe, its presence throughout diverse altitudes (lowlands, mountain areas, in subalpine zones) and its fairly narrow stenotypic habitats (wet meadows, peat bogs, and springs), *S. lapponum* constitutes a good model species for explaining the complex processes that lead to the formation of arctic-alpine disjunction. Thus, we pursue three aims in this study: 1) to determine the degree of genetic similarity between populations of *S. lapponum*; 2) to explain the origin of Central European populations situated in the contact zone with the main range and in isolated refugia; 3) to describe probable migration routes.

Material and methods

Study regions and sampling of the populations

For this study, we used samples collected from nineteen populations of species across the geographical range in Europe (Table 1, Fig. 1).

Molecular analysis was conducted on plants from all populations. In the field, leaves of *S. lapponum* were randomly sampled in populations at distances that generally depended on the spatial extent of the populations (5–6 m). Fresh leaves were collected

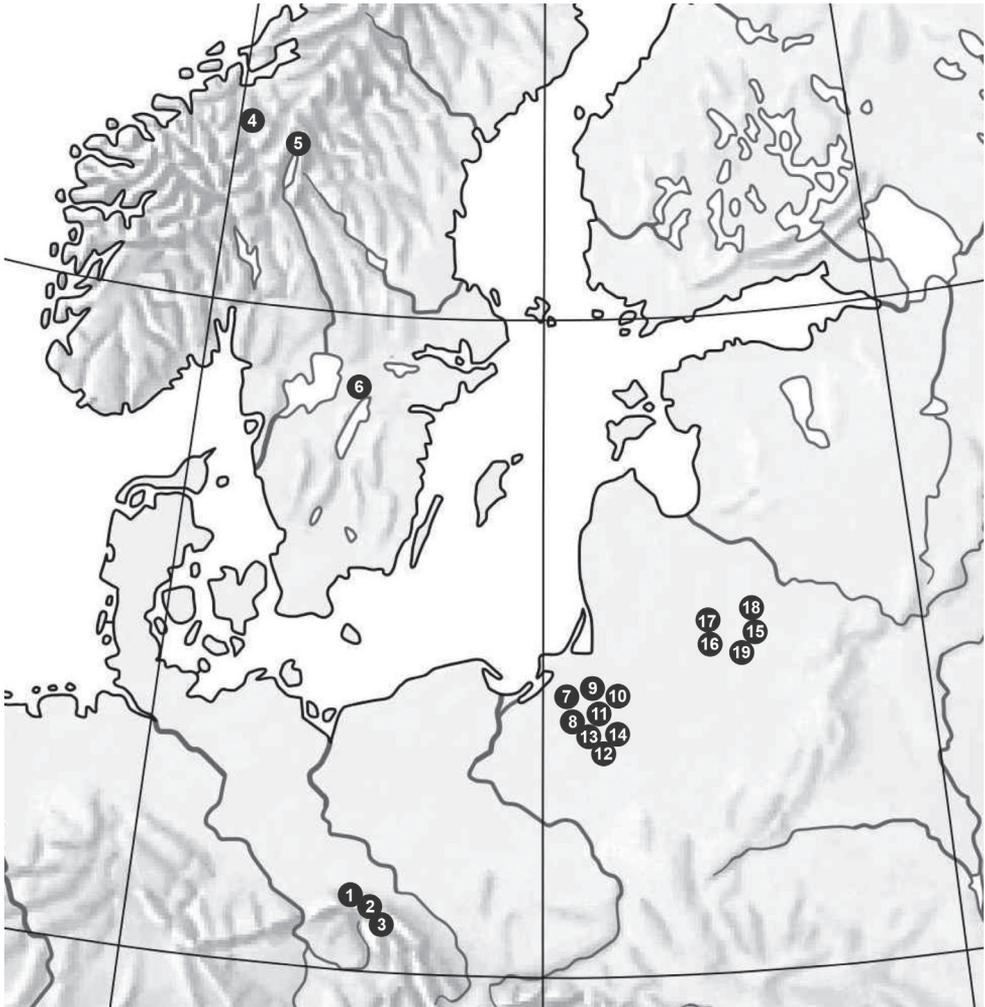


Figure 1. Location of the studied populations of *S. lapponum*. Population abbreviations are the same as in Table 1.

in plastic zip bags, dried in silica orange gel, transported to the laboratory, and stored until DNA extraction. In general, we studied eight plant samples per population and 152 specimens of *S. lapponum* in total. Whole plant material was collected in the months of June or July from 2015 to 2017. Herbarium voucher specimens that were used for DNA are stored in author collection (J. U.) deposited at WRS� Herbarium (Wrocław, Poland) and are available on request.

DNA extraction and molecular analysis

The genomic DNA was isolated after cell disrupting in a Mixer Mill MM400 (Retsch, Haan, Germany), using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) accord-

Table 1. Populations of *S. lapponum* included in the study: location in geographical regions, coordinates and Nei's gene diversity value. The populations' abbreviations are given below the table.

Region	Population (number) / abbreviation ¹	Longitude, Latitude	h = Nei's (1973)
Sudetes	(1) KWS	50°45'22"N, 15°41'29"E	0.144
	(2) POL	50°46'5"N, 15°42'35"E	0.136
	(3) KMS	50°44'51"N, 15°42'0"E	0.147
Scandinavia	(4) DOV	62°11'29"N, 9°44'59"E	0.143
	(5) FUL	61°29'58"N, 12°42'34"E	0.144
	(6) NAT	59°21'42"N, 15°8'52"E	0.121
NE Poland	(7) DWO	53°56'31"N, 23°23'16"E	0.172
	(8) WIZ	53°11'48"N, 22°23'45"E	0.110
	(9) SZT	54°7'55"N, 23°24'10"E	0.139
	(10) PRU	54°9'51"N, 22°55'24"E	0.098
	(11) BKL	53°17'9"N, 22°36'22"E	0.114
Belarus	(12) BIA	52°41'22"N, 23°44'46"E	0.147
	(13) BLA	53°16'32"N, 22°32'58"E	0.162
	(14) JMO	51°27'36"N, 23°7'14"E	0.109
	(15) GLU	55°7'38"N, 27°43'5"E	0.129
	(16) DIK	55°32'26"N, 27°50'1"E	0.163
	(17) SIE	55°47'25"N, 29°3'31"E	0.140
	(18) WIT	55°13'21"N, 29°0'11"E	0.122
	(19) BEN	55°33'37"N, 29°14'7"E	0.121

¹ KWS – Kocioł Wielkiego Stawu (Karkonosze Mts.), POL – Polana (Karkonosze Mts.), KMS – Kocioł Małego Stawu (Karkonosze Mts.), DOV – Dovre (Dovre Mts.), FUL – Fuljaflaet (Fuljaflaet Mts.), NAT – Natsverde (Natsverde Mts.), DWO – Dworczyńska (Suwałki Region), WIZ – Wizna (Suwałki Region), SZT – Sztabinki (Suwałki Region), PRU – Prudzieniszki (Suwałki Region), BKL – Kładka (Biebrza Region), BIA – Budy (Białystok Region), BLA – Ławki (Biebrza Region), JMO – Lake Moszne (Polesie Region), GLU – Głubokoye (Głębock Region), DIK – Dikoye Blota (Jelnia Region), SIE – Sierewyszczce (Rosson Region), WIT Witebszczyzna (Krasnaje Region), BEN Benczynski Zapowiednik (Połock Region).

ing to the manufacturer's protocol. The quality of the isolated DNA was determined using 1% TBE agarose electrophoresis. The Inter Simple Sequence Repeat microsatellite markers were selected to study the genetic diversity of the populations of *S. lapponum*. These markers are known to be highly polymorphic and are useful in studies on genetic diversity and species relationships; they enable easy differentiation of closely related specimens. ISSR has also been successfully employed to assess hybridisation and to detect hybrid taxa (Ziętkiewicz et al. 1994; Conte et al. 2007; Goldman 2008). These markers have some limitations in use and are less effective than, for example AFLP, but are still used in research. Prior to the study, about 80 ISSR primers were checked for their usefulness in determining population differentiation in *S. lapponum*, based on the experience of Sulima and Przyborowski (2013). A total of twelve primers: ISSR: 2, 3, 4, 5, 91, 92, 93, 94, 95, 137, 139, 142 showed a satisfactory amplification and generated an acceptable number of polymorphic bands (Suppl. material 2). The number of amplified products varied from four to nine within a size range of 100–2.000 bp, depending on the specific primer. PCR reactions were performed in 15- μ l reaction tubes that contained a Dream Taq reaction buffer containing MgCl₂, a 0.2 mM dNTP mix, 1u DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 mM ISSR primer, and 0.8 μ l of total genomic DNA. The PCR cycle consisted of an initial denaturation at 95 °C for 6 min, according to a previous study (Urbaniak et al. 2019), followed by 33 cycles at 95 °C for 30 seconds. The adequate annealing

temperature was tested using the gradient method for 30 seconds and a 72 °C elongation for 30 seconds, with a final extension of 10 min at 72 °C. For the PCR reactions, a Veriti Thermal Cycler (Life Technologies, Carlsbad, CA, USA) was used. The PCR ISSR amplification products were separated in 1% agarose gel, photographed, and compared with the DNA mass ruler (Thermo Fisher Scientific Waltham, MA, USA). All laboratory analyses were performed at the Department of Botany and Plant Ecology at Wrocław University of Environmental and Life Sciences.

Molecular data analysis

The results were analysed using CLIQS software (Totallab 2016). The markers were encoded in a binary matrix and used for computations. The AMOVA (analyses of the molecular variance) were performed using ARLEQUIN 3.5.1 with 1000 permutations to determine the distribution of genetic variation within and among the populations and to assess the importance of the main groups of populations (Excoffier and Lischer 2010). Nei's genetic identity (Nei 1978) index was calculated using POPGENE v. 1.32 (Yeh et al. 1999). Bayesian clustering was applied using STRUCTURE 2.3.4 (Pritchard et al. 2000; Evanno et al. 2005; Falush et al. 2007) based on an admixture model. The numbers of K from two to seven were tested with ten replications per K using 100,000 burn-in iteration followed by 2,000,000 MCMC iterations. Output data with multiple values of K and hundreds of iterations were analysed using STRUCTURE HARVESTER (Earl and Holdt 2012). CLUMPAK software (Kopelman et al. 2015) was used to produce graphical displays of the STRUCTURE 2.3.4 results and to compute the necessary statistics. To reconstruct the relationships among the analysed populations, we used the Neighbour Net approach as implemented in the SPLITSTREE (Huson and Bryant 2006), using 1000 bootstrap replicates.

All scientific names are given following IPNI (2021). The abbreviated author names for plant names are given as in IPNI (2021) following recommendations of ICN (Turland et al. 2018).

Results

The research markers selected by us for studying (ISSR) have some limitations in their application due to the lower number of generated markers than for example AFLP, but are still widely used in various types of phylogeographic research. Molecular variance analysis (AMOVA) indicates that most of the genetic variation in two groups of populations: Sudetes vs. NE Poland and Belarus occurs within populations (55.8%), while the variability among the groups accounts for 19.7% (Table 2).

The AMOVA analysis based on four groups (Sudetes vs. Scandinavia vs. NE Poland vs. Belarus) shows a similar result: variability of 54.1% within the population and variability of 18.9% between the four groups of the population based on geographic regions. High genetic variability was also found with the analysis of all studied populations,

Table 2. Results of AMOVA analysis studied populations *S. lapponum*.

Groups/ populations	Partitioning	d.f.	Sum of Squares	Variance components	Percentage of variation	Fst-statistic
All populations	Among populations	19	1245.5	7.3	43.8	0.43
	Within populations	134	1255.2	9.3	56.2	
Sudetes	Among populations	3	172.9	5.9	37.4	0.37
	Within populations	28	279.4	9.9	62.6	
Scandinavia	Among populations	2	151.3	8.3	48.2	0.48
	Within populations	21	188.2	8.9	51.8	
NE Poland	Among populations	7	358.7	5.4	37.2	0.37
	Within populations	54	495.1	9.2	62.8	
Belarus	Among populations	4	118.9	2.8	23.1	0.23
	Within populations	31	292.4	9.4	76.9	
Sudetes and NE Poland vs. Belarus	Among groups	1	158.4	3.4	19.7	0.44
	Among populations	7	291.8	4.2	24.5	
	Within populations	59	571.8	9.7	55.8	
Sudetes vs. Scandinavia vs. NE Poland vs. Belarus	Among groups	3	443.6	2.6	18.9	0.46
	Among populations	16	801.9	5.3	30.6	
	Within populations	134	1255.2	9.4	54.1	

Table 3. Pairwise genetic differentiation in between group of populations *S. lapponum* studied using ISSR microsatellites. Number of studied populations in each groups are given in brackets in the first column.

	All Groups	Sudetes	Scandinavia	NE Poland	Belarus
All Groups ⁽¹⁹⁾	0.46				
Sudetes ⁽³⁾		-			
Scandinavia ⁽³⁾		0.43	-		
NE Poland ⁽⁸⁾		0.40	0.44	-	
Belarus ⁽⁵⁾		0.47	0.50	0.49	-

which were treated as one group. The genetic variability value was 43.8% among populations and 56.2% within populations; the F_{st} value was 0.43 ($p < 0.001$). Within geographic groups, which are characterised by the studied populations, the lowest variability and also the highest homogeneity were found in populations located in Belarus. The genetic variation among Belarusian populations was low (23.1%). In contrast, the highest inter-population variability was found in Scandinavia (48.2%) and the variability in the Sudetes and NE Poland was similar: 37.4% and 37.2%, respectively.

High and statistically significant genetic differences between all geographical groups of the population were found; again, the greatest differentiation was noted between the Belarusian populations and the Scandinavian populations (0.5). The greatest differences were noted between the populations from Belarus and between the populations from the Sudetes and NE Poland (Table 3); these were also statistically significant. Based on the obtained results, we also calculated the genetic diversity of the studied populations of *S. lapponum* in Europe.

Both the highest and the lowest index of genetic diversity, based on Nei's (1978) index, were found in the geographic region of NE Poland, reaching the highest value in the population from Dworczyńska (DWO) (0.172) and the lowest value in the population located in Prudzeniszniki (PRU) (0.098). Correlation analysis of the relationship

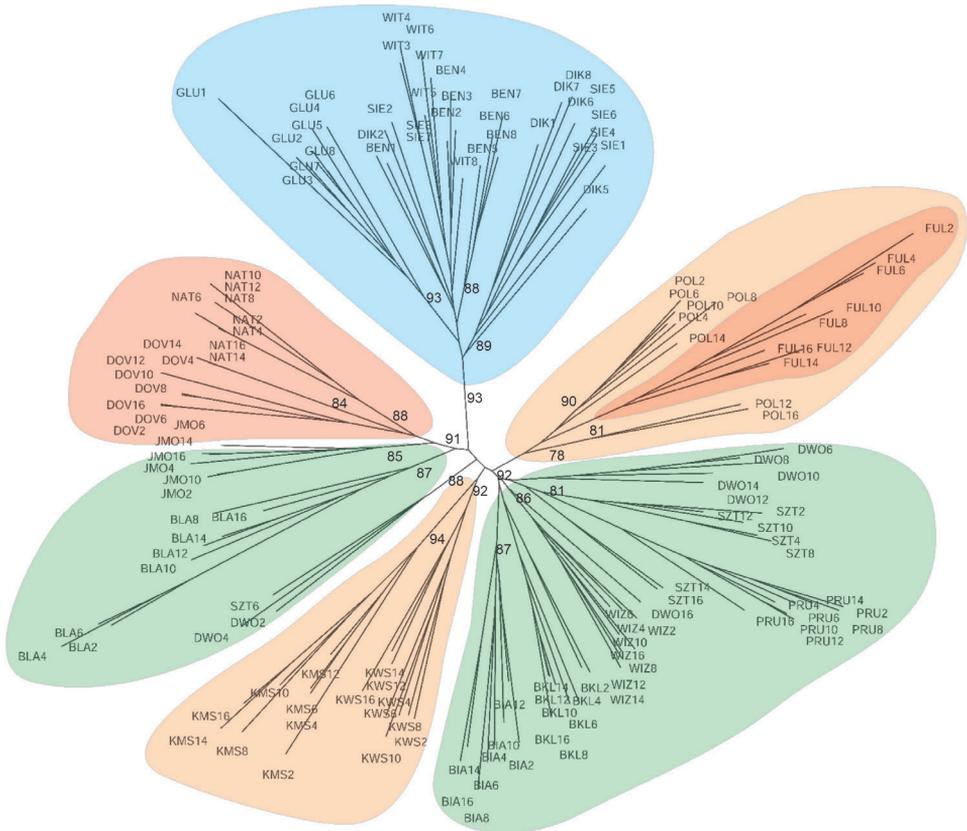


Figure 2. Neighbor-Net of *S. lapponum* individuals based on Nei (1973) coefficient. Population abbreviations are the same as in Table 1.

between geographic and genetic distances in the study area did not show any significant correlation between genetic variation and the geographic distance between the studied populations ($r = 0.121$; $p = 0.186$).

The analysis using neighbourhood joining for reconstructing the relationships among populations, revealed differences between the population samples collected from different geographical regions of *S. lapponum*'s distribution (Fig. 2). The studied populations were grouped according to their geographic distribution, creating separate groups.

Moreover, all of the selected subgroups were well-supported by bootstrap analysis. Populations from Belarus (GLU, DIK, SIE, WIT, BEN) showing a high similarity with each other (Fig. 2) were included in one of the distinguishing groups on the figure. Plants from populations collected in NE Poland (DWO, WIZ, PCS, PRU, BKL, BIA, BLA, JMO) are divided into two groups, also with quite high bootstrap support, although these groups are partially mixed with each other and some representatives of the population are placed in both groups. A separate group is composed of individuals from the populations located in the Sudetes (KWS, POL, KMS) that are grouped

in one cluster on the figure. The exception is a population from Polana (POL), which seems to be genetically similar to the Scandinavian population gathered in Fuljalfleet (FUL). Both populations form a highly distinctive group with high bootstrap support (Fig. 2). The remaining Scandinavian populations form a separate and distinct group.

Tests performed with the use of the STRUCTURE program, with which the clustering method was implemented, allowed for a more precise elucidation of the genetic variability of the *S. lapponum* population (Fig. 3). The analysis was run for $K = 2-5$; for these results, STRUCTURE HARVESTER showed that the optimal number of populations (K) was four, as reported by Evanno et al. (2005), although the highest log-likelihood value was also found for $K = 5$ (Suppl. material 3).

For $K = 2$, all of the populations studied were divided into two groups: populations from Belarus and all other geographical regions (Sudetes, Scandinavia, NE Poland). In general – not only for $K = 2$ but also for $K > 2$ the populations from Belarus appeared as most distinct in all runs. For $K = 3$, populations from the Sudetes grouped with populations from NE Poland, while Scandinavian populations formed a separate group, except for one population (FUL) that seems to be genetically closer to populations from the Sudetes. For $K = 5$, populations from Belarus and the Sudetes form nearly a consistent group; however, specimens from the Sudetes populations showed closer relations with several populations from NE Poland. Results obtained with the STRUCTURE program are almost identical to the results presented in Fig. 2.

Discussion

Climate fluctuations during the Pleistocene markedly changed distribution patterns for both plant and animal species. Consequently, full phylogenetic compatibility of species can be found only rarely, and usually only on a regional scale (Freeland 2008). When genetic research covers a much larger area, the phytogeographic variability becomes much more complex and is usually closely correlated geographically with the processes of Pleistocene glaciation (Taberlet et al. 1998; Hewitt 2004; Lindner et al. 2004; Parmesan 2006). This is the case with many species, including glacial relicts occurring in isolated sites such as mountains or in the Polish lowlands (Urbaniak et al. 2018b). The ability of *S. lapponum* to survive in such unfavourable conditions in isolated regions, such as glacial refuges, was very important for its later recolonisation and the spread of genetic lines during the interglacial period. However, the reconstruction of the glacial and postglacial history of *S. lapponum* on the basis of palaeoecological data is quite difficult, because the record of macrofossils is not known (Ralska-Jasiewiczowa et al. 2004). Fragmentation of the species occurrence area substantially reduces genetic diversity and increases inter-population genetic divergence, mostly by limiting the flow of alleles among fragmented populations, which thereby increases inbreeding and the likelihood of genetic drift in the population. Consequently, this can lead to the extinction of the species due to the reduction of allele diversity in the population, which decreases the adaptability of the species.

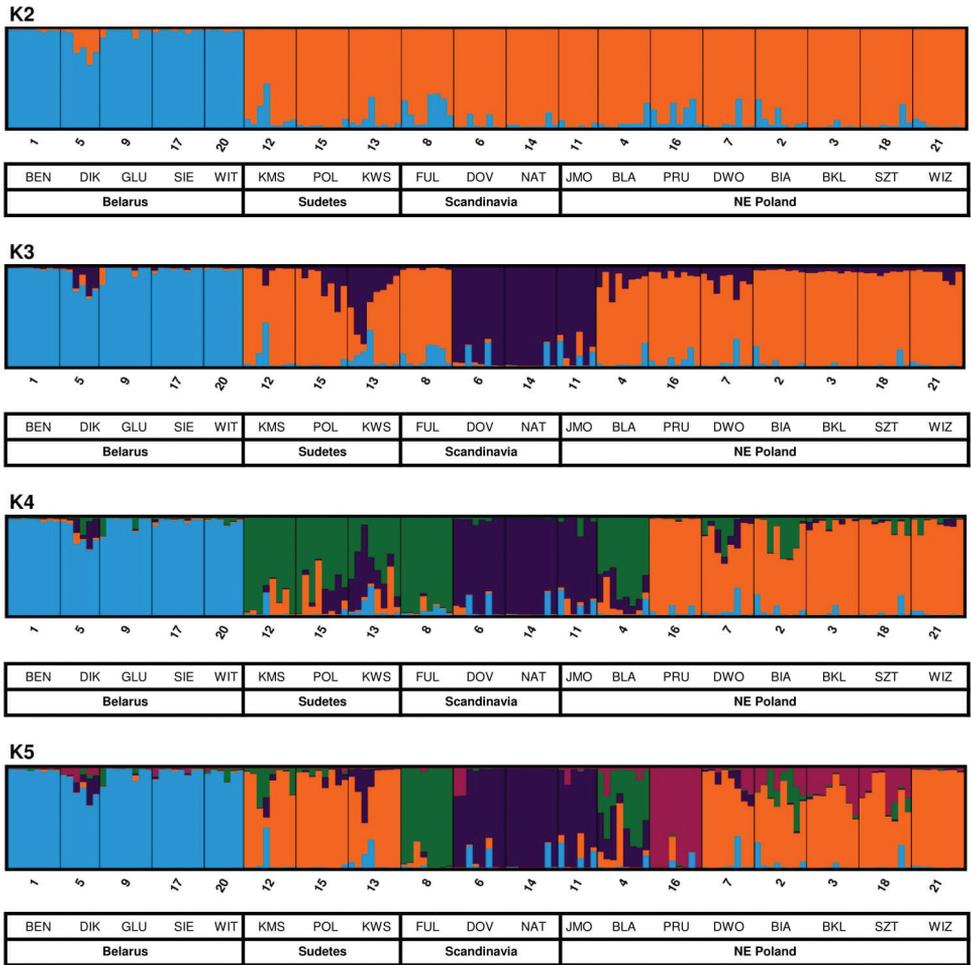


Figure 3. Results of the Bayesian admixture analysis data for populations of *S. lapponum* using STRUCTURE software. Population abbreviations are the same as in Table 1.

The results obtained from genetic analyses of the *S. lapponum* population show that genetic diversity between the studied populations in the area of the species occurrence is significant, while gene flow is clearly geographically limited. This fact may be explained by the lack of continuity in the occurrence of *S. lapponum* and by the large geographic areas that separate the studied populations, thus preventing the free flow of genes. For example, in central Poland, there are no *S. lapponum* sites – not even scattered sites. As can be seen on Fig. 2, populations from Belarus form separate clusters. It appears that this group of populations seems to be distinct, showing high genetic distinctiveness from populations from other geographic regions, while also showing low inter - population variability ($F_{st} = 23.1$, Suppl. material 1). The STRUCTURE analyses ($K = 2$) show that populations from Belarus are most separated or isolated from the other populations. Analyses at higher K indicate some similarities between the remaining populations, expressed particularly in the form of allele transmission

between them (Fig. 3). These research results also indicate a fairly high gene admission between the populations from NE Poland and other geographical regions, including Scandinavia, although some differences in genetic variation between them are also apparent. Similar results confirming the isolation of distant populations of *S. alba* are given by Puyvelde and Triest (2007) from the Alp region, and by Sulima and Przyborowski (2013) regarding *S. purpurea* from NE Poland.

The populations of *S. lapponum* in Scandinavia seem to be different from those in the rest of Europe, apart from the Fuljafleat (FUL) population. At the same time, the effects of between populations in Scandinavia and other regions are visible. In Scandinavian populations, however, there are alleles common to populations from both the Sudetes and NE Poland, demonstrating close genetic relationships and similarities between them (Fig. 2, 3). The population of Fuljafleat (FUL) is definitely most closely related to the populations of the Sudetes ($K = 3-4$) and NE Poland ($K = 3-5$). It is possible that the migrating individuals of *S. lapponum* remained in relatively small populations and, therefore, the combined effects of genetic drift and intra-population mutational processes increased differentiation between them. This is indicated by significant genetic diversity, as illustrated by the very high calculated coefficients of differentiation between the studied populations of *S. lapponum* presented in Tables 2, 3, Suppl. material 1.

There are also similarities between the analysed populations from the Sudetes and NE Poland, which may be attributed to the relatively small populations compared; this is confirmed by the results of the pairwise genetic differentiation analysis (Table 2). The calculated value between the populations from the Sudetes and NE Poland was the lowest (0.40) among all analysed population groups. This may result from a common history and species migration, especially regarding the migration of *S. lapponum* from Scandinavia to the south, which occurred during the favourable climatic periods of the Pleistocene through Poland, which was almost certainly favoured by the climatic conditions at the time. In the Sudetes, *S. lapponum* reaches its southern distribution limit, and the obtained results indicate closer relations between populations from the Sudetes and those from NE Poland. Certainly, in the past, isolated refugia existed in Central Europe. It is also possible that the separation of both population groups from the Sudetes and NE Poland took place relatively recently. Similar results have also been obtained for *S. arbutifolia* from the most NW locations in Japan. Nagamitsu et al. (2014) report a large genetic divergence between the distant geographic barrier populations between Sakhalin and Hokkaido, which entails a complicated history of migration and colonisation of this species. The most extreme populations had the lowest genetic diversity and were the most distinct from the rest. Therefore, the low genetic diversity and high genetic diversity on the extreme of ranges may suggest a significant influence of genetic drift on the genetic structure of separated populations – not only of *S. lapponum*, but also of other species.

In the Northern Hemisphere, climatic oscillations during the Quaternary period caused significant changes in plant distribution, which resulted in the repeated expansion and fragmentation of species' ranges and affected their patterns of genetic diversity. Cold-adapted plants (arctic and boreal) are believed to be more threatened during the Quaternary period than other plant groups (Comes and Kadereit 1998; Alsos et al. 2012; Eidesen et al. 2013; Paulus et al. 2013).

The currently observed decline in population sizes and geographical ranges, limited generative reproduction, and short-range spreading make boreal species more susceptible to loss of genetic diversity than, for example, plants in the temperate or Mediterranean zones. Moreover, climate scenarios (Crawford 2008) predict that the geographic range of northern species will shrink and move northwards or to higher altitudes, leading to greater isolation of their populations, or even extinction. The currently preserved disjunctive geographic range of this glacial relic is certainly related to Weichselian glaciation. Originating from the northern part of Europe and Asia, *S. lapponum* probably survived the glaciation in several isolated locations in Central Europe. Therefore, for example, populations from the Sudetes can be included in the populations found in the interglacial refugia for cold-adapted species (Lister et al. 2010; Tzedakis et al. 2013). During successive glaciations, *S. lapponum* probably colonised the areas of Belarus and NE Poland, far away from the population in the Sudetes and relatively close to the Scandinavian sites.

The clearly low and similar level of variation, especially within Belarusian populations, may result from two different processes: the founding effect and genetic drift caused by small population sizes. However, in both the Scandinavian and Sudeten populations, probably there was increased genetic variation, represented in migrants who re-settled the newly available space. In many plant species, a correlation is found between population size and genetic variation (Gaudeul et al. 2000; Despres et al. 2002), which is consistent with the hypothesis that small populations cannot maintain high genetic variation like larger populations. The high genetic diversity can be also explained by the presence of glacial refuges in the past or by the presence of a specific contact zone with different phylogenetic lines. One of these zones is Central and Eastern Europe (Taberlet et al. 1998; Hewitt 1999). Therefore, it is likely that *S. lapponum* could survive the LGM in NE Poland and Belarus on the edge of the Scandinavian glacier in the area with various phylogenetic lines. It is also possible that this part of Europe was re-occupied by the migration waves of *S. lapponum* originating in Scandinavia and the Sudetes.

These two waves of migration probably met, creating a local suture zone with a mixed haplotype character. A similar scenario has also been proposed for *Populus tremula* L. in Central Europe (Fussi et al. 2010) and for the numerous populations of *Salix herbacea* (Alsos et al. 2009). However, to reach a more thorough understanding, additional studies are needed that include not only intra- and inter-population variability studies but also cpDNA-based phylogenetic lineage studies. Results obtained with this method could further elucidate similarities or differences in the DNA haplotypes of *S. lapponum*.

Conclusions

The conducted research shows the division of the studied *Salix lapponum* populations into several genetic groups. The populations from Belarus were the most genetically different. Populations from NE Poland and the Sudetes show similarities with populations from other geographic regions, indicating existing genetic relationships between them. It is possible that there was a meeting and exchange of genes between populations in

southern Europe and Scandinavian populations and from NE Poland. However, to reach a more thorough understanding, additional studies are needed that include not only intra- and inter-population variability studies based on investigating phylogenetic lineage.

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Supplementary material I

Table S1

Authors: Jacek Urbaniak, Paweł Kwiatkowski, Paweł Pawlikowski

Data type: Docx file.

Explanation note: Pairwise Nei's genetic distances (Nei, 1978) for populations of *S. lapponum* based on ISSR analysis.

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Link: <https://doi.org/10.3897/phytokeys.184.71641.suppl1>

Supplementary material 2

Table S2

Authors: Jacek Urbaniak, Paweł Kwiatkowski, Paweł Pawlikowski

Data type: Docx file.

Explanation note: Nucleotide sequences and annealing temperatures of ISSR primers used in study.

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Link: <https://doi.org/10.3897/phytokeys.184.71641.suppl2>

Supplementary material 3

Figure S1

Authors: Jacek Urbaniak, Paweł Kwiatkowski, Paweł Pawlikowski

Data type: Jpg file.

Explanation note: Delta K values for Structure analysis.

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Link: <https://doi.org/10.3897/phytokeys.184.71641.suppl3>

Spiradiclis detianensis (Rubiaceae, Ophiorrhizeae), a new species from southwestern Guangxi, China

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Abstract

A new species of Rubiaceae, *Spiradiclis detianensis* is described from a limestone karst area of southwestern China. This new species is morphologically similar to *S. cordata* and *S. spatulata*. All of them have rosetted habit and long peduncles, but it differs from the former by the cuneate leaf bases (vs. basally cordate) and much longer corolla tubes (1.8–2.2 cm long vs. ca. 5 mm long), and from the latter mainly by its tubular-funnel shaped corolla (vs. slenderly salver shaped), 4.5–6.8 (vs. 1.5–2) mm in diam, inside throat and corolla densely puberulent (vs. glabrous except a ring of long hairs at the middle). It also resembles to *S. tubiflora*, but differs clearly by its subrosulate habit (vs. procumbent to creeping), longer leaf blades (7.0–10.5 cm vs. 0.5–2.5 cm) and longer corolla tubes (18–22 mm vs. 14–16 mm). At same time, color photos, illustrations, detailed descriptions and conservation status of the new species are provided.

Keywords

China, Guangxi, limestone, Rubiaceae, taxonomy

Introduction

Spiradiclis Blume is a morphological complex genus of Ophiorrhizeae (Rubiaceae). It is usually distinguished from its relatives by the subglobose or linear-oblong capsules with two or four twisted or straight valves when matured (Lo et al. 1983; Robbrecht 1988; Deb and Rout 1989; Lo 1999; Chen and Taylor 2011). In spite of the characteristic capsule form, the monophyly of the genus has been queried based on recent molecular evidence (Rydin et al. 2009; Razafimandimbison and Rydin 2019). Razafimandimbison and Rydin (2019) even reduced *Spiradiclis* and *Keenania* Hook.f. to the synonymy of *Ophiorrhiza* L. However, we find that the relationship between *Spiradiclis* and its relatives needs further research and thus we prefer to accept the traditional concept of *Spiradiclis* here, for the unique capsule form of the genus.

There are approximate 58 species of *Spiradiclis*, distributed in southeastern Asia and concentrated in southwestern China and northeastern India (Chen and Taylor 2011; Deng et al. 2014; Wang et al. 2015; Wen et al. 2015; Wu et al. 2015a, 2015b, 2016, 2019a, 2019b; Wang 2016a, 2016b; Pan et al. 2016, 2019; Liu et al. 2018; Zhang et al. 2018; Wen et al. 2019; Li et al. 2021). In China, 52 known species of *Spiradiclis* are recorded (Li et al. 2021).

Most representatives have a narrow habitat and prefer to grow at wet places on hill slopes or entrances of caves in limestone areas. During a field investigation to the neighboring regions between China and Vietnam in 2013, the second author came across a peculiar population of Rubiaceae. According to its calciphile habitat and rosetted habit, it is easy to associate with *Spiradiclis*. However, the flower shape of this population is very different from that of the known species of the genus. Its corollas are tubular–funnel shaped, with 1.8–2.2 cm long tubes, sharply shrunken near base, 4.5–6.8 mm in diam. at throat and 1.8–2.5 mm in diam. near the base, while the corollas of the genus are usually very short or slender. Hence, we revisited this population and collected its capsules. Their subglobose shape and dehiscence with 4 valves clearly indicate this population represents a species of the genus *Spiradiclis*. After careful comparisons with relevant literatures and examining specimens in herbaria, we found that our plant is most similar to *S. cordata* H.S. Lo & W.L. Sha and *S. spathulata* X.X. Chen & C.C. Huang, two species of subgenus *Sinospiradiclis*, but it can be distinguished from the former by the cuneate leaf bases and much longer corolla tubes, and from the latter by its tubular–funnel shaped corolla and without a villous ring inside corolla. We conclude that this population represents an undescribed species and formally treat it here.

Material and methods

Materials are deposited at the herbarium of Forest Plants in Central South University of Forestry and Technology (CSFI) and Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences (IBK) – herbarium acronyms follow Thiers (continuously updated). Morphological observations and measurements of the new species are based on living material in the field and dry specimens.

Taxonomic treatment

Spiradiclis detianensis L. Wu, Y.F. Huang & Z.J. Wen, sp. nov.

urn:lsid:ipni.org:names:77221569-1

Figs 1, 2 A–I

Type. CHINA. Guangxi: Daxin county, Shulong town, Detian village, 22°52'N, 106°43'E, elevation 650–750 m, 30 March 2018 (fl.), *Zhao-Jie Wen & Guang-Fu Mou 18033001* (holotype: CSFI [CSFI069613]; isotypes: CSFI, IBK).

Diagnosis. The new species is similar to *Spiradiclis cordata* and *S. tubiflora*, but it differs from the former mainly by the cuneate leaf base (vs. cordate) and much longer corolla tubes (1.8–2.2 cm long vs. ca. 5 mm long), and from the latter by its subrosulate habit (vs. procumbent to creeping), longer and wider leaf blades (7.0–10.5 × 2.0–3.5 cm vs. 0.5–2.5 × 0.4–1.5 cm), more secondary veins (7–10 pairs vs. 3–5 pairs) and longer corolla tubes (18–22 mm vs. 14–16 mm).

Description. Perennial herb, up to 15 cm tall; stems densely pubescent, erect or ascending, lower part rooting at nodes. Leaves subrosulate; petiole 0.8–1.7 cm long; leaf blade drying papery, obovate-lanceolate or oblong, 7.0–10.5 × 2.0–3.5 cm, obtuse or acute at apex, cuneate at base, adaxially dark green, puberulent, abaxially light green, pubescent, densely pubescent along veins at lower surface; secondary veins in 7–10 pairs; stipules persistent, pubescent, narrowly triangular, 3.2–5.7 mm long, or 2–5-lobed, lobes linear-triangular, upper part filiform. Inflorescence cymose, 2–7-flowered; peduncle 9–14 cm long, pubescent; bracts linear-triangular, 4–6 mm long, subglabrous; pedicels 0.5–5 mm long, pubescent. Flowers distylous. Calyx puberulent; hypanthium portion obconic, ca. 2 mm long; lobes triangular, 1.2–2.9 mm long, acute at apex. Corolla white to pink, tubular-funnel shaped, puberulent outside; tube 1.8–2.2 cm long, sharply enlarged at the 1/3 lower part of the corolla tube, 4.5–6.8 mm in diam. at throat, 1.8–2.5 mm in diam. near base; lobes triangular-ovate, 4.5–5.6 × 3.5–4.3 mm long. Stamens 5; anthers linear. Stigma bilobed; ovary 2-celled. Long-styled flowers: corolla tube inside with densely pubescence near base and densely puberulent above anther and on to lobes; anthers inserted near base of corolla tube, 1.9–2.3 mm long; style 1.2–1.4 cm long, puberulent; stigma inserted at between middle and throat of corolla tube, 2-lobed, lobes elliptic, 1.8–2.2 mm long. Short-styled flowers: corolla tube inside densely puberulent; anthers inserted often at or a little above middle of corolla tube, 2.6–3.3 mm long; style 1.5–3.4 mm long, glabrous; stigma near base of corolla tube, lobes ovate-triangular, 1.3–1.6 mm long. Capsules subglobose, 2.5–3.2 mm in diam., valves 4 when matured. Seeds many, angular.

Phenology. Flowering March to April, fruiting from May to July.

Etymology. The specific epithet refers to the type locality, where a famous attraction, Detian Waterfall, is situated.

Chinese name. 德天螺序草 (de-tian-luo-xu-cao in Mandarin).

Distribution and habitat. Until now, only two populations of the new species have been found. They are both known from limestone hills of southern Guangxi. Plants of

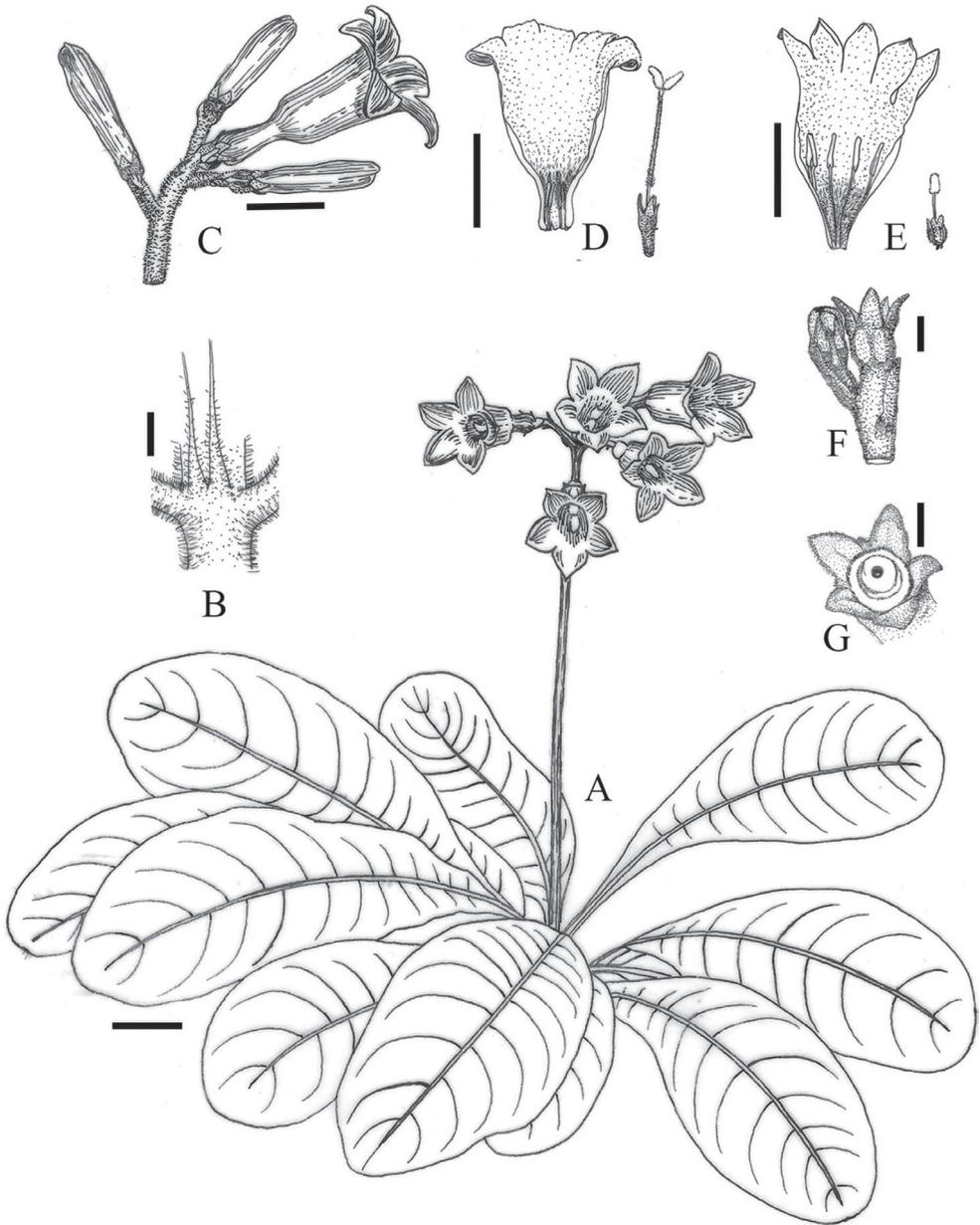


Figure 1. *Spiradiclis detianensis* **A** habit **B** stipule **C** inflorescence, side view **D** opened long-style flower **E** opened short-style flower **F** capsule, side view **G** capsule under matured, face view. Scale bar: 1 cm (**A**, **C**, **D**, **E**); 2 mm (**B**, **F**, **G**). Drawn from the holotype by X.Y. Zeng.

the new species prefer to grow at humid places at elevation range of 500–800 m, under evergreen broad-leaved forests with tree species of Fagaceae, Lauraceae, Tiliaceae, Theaceae, Myrsinaceae, Magnoliaceae and Sapindaceae.

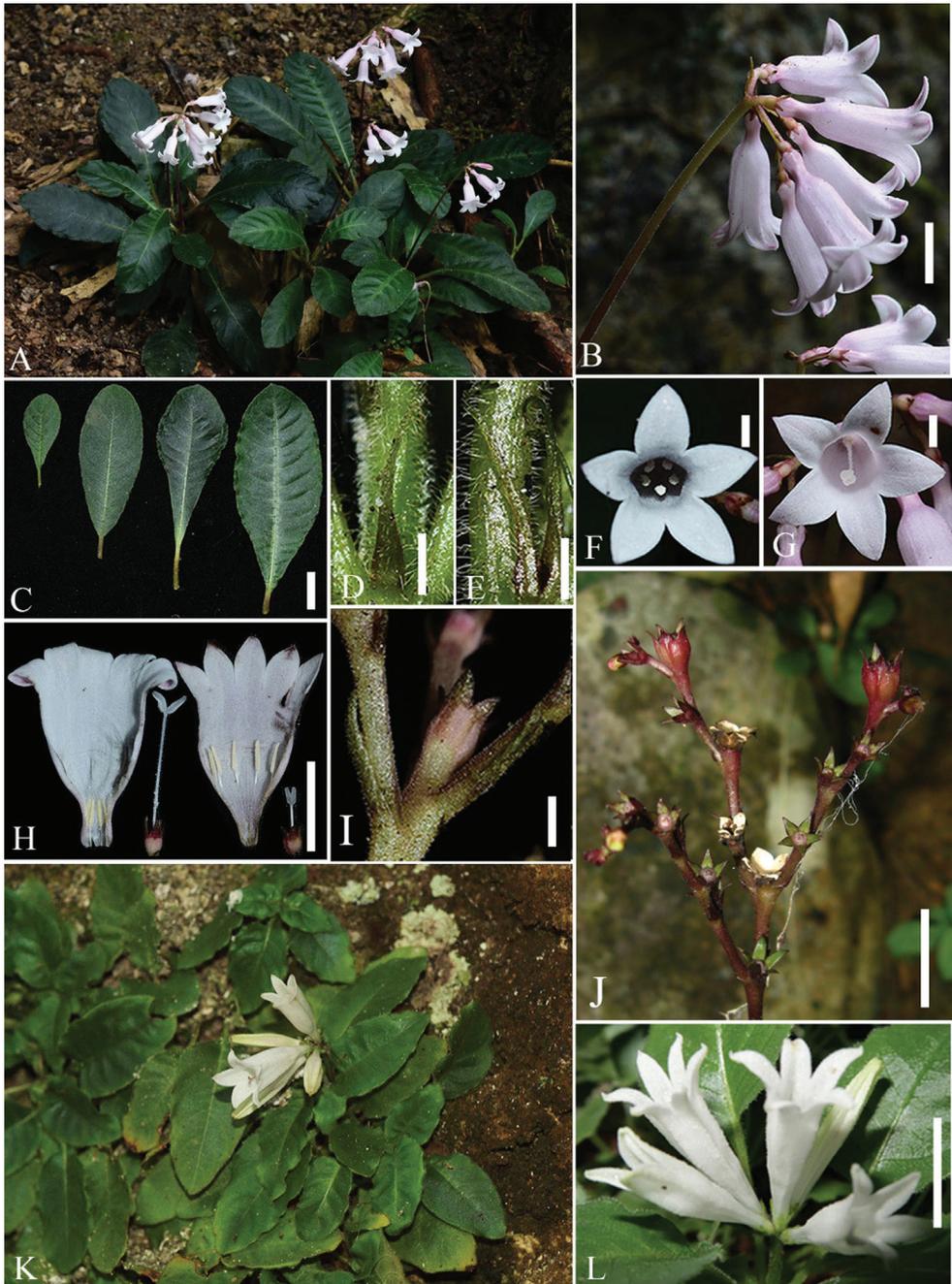


Figure 2. *Spiradiclis detianensis* **A** habit **B** inflorescence **C** leaves **D, E** stipule **F, G** short- and long-styled flower, front view **H** opened long- and short-styled flower, showing style and stamens **I** ovary, side view **J** infructescence, side view. *S. tubiflora* **K** habit **L** inflorescence. Scale bars: 1 cm (**B, C, H, J, L**); 2 mm (**D, E, F, G, I**). Photos by Z.J. Wen and L. Wu.

Provisional conservation status. During a series of field investigations at the China-Vietnam border over the past ten years (2009–2019), only three populations of *Spiradiclis detianensis* have been observed. One site with nearly 59 matured individuals is distributed in Detian Waterfall (type locality), while the other two sites with more than 250 and 114 individuals are in the Longzhou county and Ningming county, respectively. The habitats of the three sites are in good condition and have been rarely influenced by humans. Considering the above, the species can be assigned a status of ‘Least concern’ [LC] following the guidelines of IUCN (2019).

Discussion. Our unpublished molecular data indicates that *Spiradiclis detianensis* shows the closest genetic relationship with *S. cordata*. Both species prefer to grow on limestone hills from southern Guangxi, China, and have similar habit, such as short stems, subrosulate leaves, long peduncles, heterostylous flowers, funnel-shaped corollas and subglobose capsules. However, the former can be easily distinguished from the latter mainly by its leaf blade basally cuneate (vs. basally cordate) and corolla tubes 1.8–2.2 cm long (vs. ca. 5 mm long) (more detailed comparisons are listed in Table 1).

Spiradiclis detianensis is very similar to *S. spatbulata* in morphology, since both have subrosetted habit, obovate-oblancheolate leaf blades, long peduncles and subglobose capsules, but it differs mainly by its corolla tubular-funnel shaped (vs. slenderly salver shaped), 4.5–6.8 (vs. 1.5–2) mm in diam. at the middle and densely pubescence inside corolla near base and densely puberulent above anther and onto lobes (vs. glabrous except a ring of long hairs at the middle) (more detailed comparisons are listed in Table 1).

Spiradiclis detianensis is a distinct species in the genus *Spiradiclis* due to the corolla tubes 18–22 mm long and having the shape of a reversed wine bottle, with an

Table 1. Morphological comparison of *Spiradiclis detianensis*, *S. tubiflora* and *S. cordata* Lo et W. L. Sha.

	<i>Spiradiclis detianensis</i>	<i>S. cordata</i>	<i>S. spatbulata</i>	<i>S. tubiflora</i>
Habit	rosetate to subrosulate	rosetate to subrosulate	rosetate	procumbent to creeping
Leaf blade	obovate-lanceolate or oblong, 7.0–10.5 × 2.0–3.5 cm, base cuneate, apex obtuse or acute	elliptic-ovate to elliptic-oblong, 5–13 × 2–5.5 cm, base cordate, apex obtuse to rounded	spatulate or obovate-oblancheolate, 8–13 × 2–4.5 cm, base acute to cuneate, apex obtuse to rounded	ovate to elliptic, 0.5–2.5 × 0.4–1.5 cm, base rounded to obtuse, apex acute to rounded
Secondary vein	7–10 pairs	15–19 pairs	15–25 pairs	3–5 pairs
petiole	0.8–1.7 cm long	1–7 cm long	5–8 mm long	0.3–1.8 cm long
Stipule	triangular, entire or bifid	deeply 2-lobed, lobes linear	lanceolate-linear or linear	narrowly linear
Inflorescence	cymose, one per plant, 2–7-flowered	cymose to paniculate, 1–3 per plant, many flowered	cymose, 10– to many flowered	cymose, one per plant, 2–5-flowered
Peduncle	9–14 cm long	6–16 cm long	7–12 cm long	1.2–1.5 cm long
Bract	linear-triangular, 4–6 mm long	linear or subulate, 2 mm long	linear-lanceolate, 3–4 mm long	subulate, 1.8–3.0 mm long
Calyx lobe	triangular, 1.2–2.9 mm long	triangular, ca. 0.8 mm long	narrowly lanceolate, 1–1.3 mm long	triangular, 1.4–1.6 mm long
Corolla color	Purple	White	purple-reddish	white
Corolla tube	tubular-funnelform, sharply enlarged at the 1/3 lower part of the corolla tube, 18–22 mm long	tubular-funnelform, sharply enlarged at the middle or 1/3 upper part of the corolla tube, 5 mm long	slenderly salverform, tube 15–25 mm long	tubular-funnelform, slightly enlarged from the base to the throat, 14–16 mm long
Corolla inside (long-styled form)	without villous ring	with villous ring at middle	with villous ring at middle	Without villous ring

abruptly narrowed lower third, 4.5–6.8 mm in diam. at throat, 1.8–2.5 mm in diam. near base. Until now, only one other known species, *S. tubiflora* L. Wu, B.M. Wang & B. Pan (Wu et al. 2019b), has a similar corolla shape (see Fig. 2 K & L). However, *S. detianensis* differs from *S. tubiflora* principally by its rosulate habit (vs. procumbent to creeping), leaf blades longer than 7 cm and wider than 2 cm (vs. shorter than 2.5 cm and narrower than 1.5 cm), secondary veins 7–10 pairs (vs. 3–5 pairs) and longer corolla tubes (vs. 18–22 mm vs. 14–16 mm) (more detailed comparisons are listed in Table 1).

Additional specimens examined. (paratypes). CHINA. Guangxi: Longzhou county, Nonggang National Nature Reserve, 2 April 2019 (fl.), *Zheng-Quan Nong nzq0004* (CSFI); Niming county, Tingliang Town, Lixin village, 28 July 2011 (fr.), Yu-Song Huang 9422 (IBK).

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Two new species and a new combination from Zhejiang, East China

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Abstract

As the supplement of the flora of Zhejiang, East China, two new species were described with illustrations. *Cerastium huadingense* Y.F.Lu, W.Y.Xie & X.F.Jin (Caryophyllaceae) differs from *C. qingliangfengicum* in having sterile stems absent, leaves sessile, petals slightly longer than sepals, and stamens slightly shorter than sepals. *Ixeridium dimorphifolium* Y.L.Xu, Y.F.Lu & X.Cai (Asteraceae) differs from *I. beauverdianum* in having plant stoloniferous, basal leaves dimorphic, involucre 8–10 mm long, inner phyllaries 8, and florets 7–10. *Paraphlomis setulosa* C.Y.Wu & H.W.Li (Lamiaceae) was reviewed and morphological characters of the corolla and stamens of its type and the specimens collected in the field survey were critically examined. With barbate anthers and strongly divergent anther cells, *Paraphlomis setulosa* was transferred to *Sinopogonanthera*, and *S. setulosa* (C.Y.Wu & H.W.Li) H.W.Zhang & X.F.Jin was consequently combined.

Keywords

East China, nomenclatural novelty, seed plant, taxonomy, Zhejiang

Introduction

Zhejiang Province, between the area of 27°06'–31°11'N, 118°01'–123°10'E, is located on the southeastern coast of China, with the whole land area taking up about 1.1% of the country. It is a territory of diverse terrain, including the most mountains in the southwestern area with the highest peak Huangmaojian (1929 m a.s.l.) in Longquan County, central hills, northern alluvial plains and numerous eastern coastal islands. It has a typical subtropical monsoon climate with marked seasonal changes and good climatic conditions. According to the latest report, there are over 4800 species of vascular plants belonging to 1587 genera in 262 families in total, showing the abundance of plant species (Jin et al. unpublished).

Since the winter of 2014, we have prepared the new edition of *Flora of Zhejiang*. Our field surveys from Zhejiang and specimen examination and identification have resulted in the discovery of 22 new species, three subspecies and four varieties of the seed plants (Chen et al. 2017; Lu et al. 2017a, 2017b, 2019; Zhang et al. 2017; Jin et al. 2018, 2019; Xu et al. 2018; Cai et al. 2019; Xie et al. 2019a, 2019b, 2019c, 2020a, 2020b). As the supplement of the flora of Zhejiang, we herein reported the novelties below.

Results

Cerastium huadingense Y.F.Lu, W.Y.Xie & X.F.Jin, sp. nov.

urn:lsid:ipni.org:names:77221580-1

Chinese name: 华顶卷耳

Figs 1, 2

Latin diagnosis. *Species nova haec C. qingliangfengico* H.W.Zhang & X.F.Jin *affinis est, quae caulibus sterilibus frequent praesentibus, foliis petiolatis, petiolis 5–9 mm longis, petalis sepalis 2-plo ultra longioribus, 10–12 mm longis, staminibus sepalis leviter longioribus differt.*

Type. CHINA. Zhejiang (浙江): Tiantai (天台), Mount Huading (华顶山), near Huading Temple (华顶讲寺), roadside at forest margin, 29°15'06.31"N, 121°05'24.52"E, alt. 1000 m, 25 April 2020, *Xiao-Feng Jin & Yi-Fei Lu* 4583 (holotype: ZM; isotypes: HTC, PE).

Description. *Herb* perennial, 12–25 cm tall. *Roots* slender. *Stems* caespitose, unbranched, erect, green, sparsely to densely white-pubescent. *Leaves* opposite; basal and lower leaf blades spatulate or narrowly spatulate, 1.2–3.2 cm long, 0.4–0.8 cm wide, entire and sparsely ciliate at margin, apex acute, base attenuate, sparsely pubescent on both surfaces; upper leaf blades ovate, ovate-elliptic or oblong, 1.2–4 cm long, 0.5–1.5 cm wide, entire and sparsely ciliate at margin, apex acute, base slightly cordate, sparsely pubescent on both surfaces, sessile. *Cyme* terminal or axillary in upper part of stems, 6–15-flowered; bracts leaf-like but smaller, narrowly ovate or lanceolate, herbaceous, sparsely pubescent on both surfaces; pedicels slender, 3–12 mm long, white-pubescent;

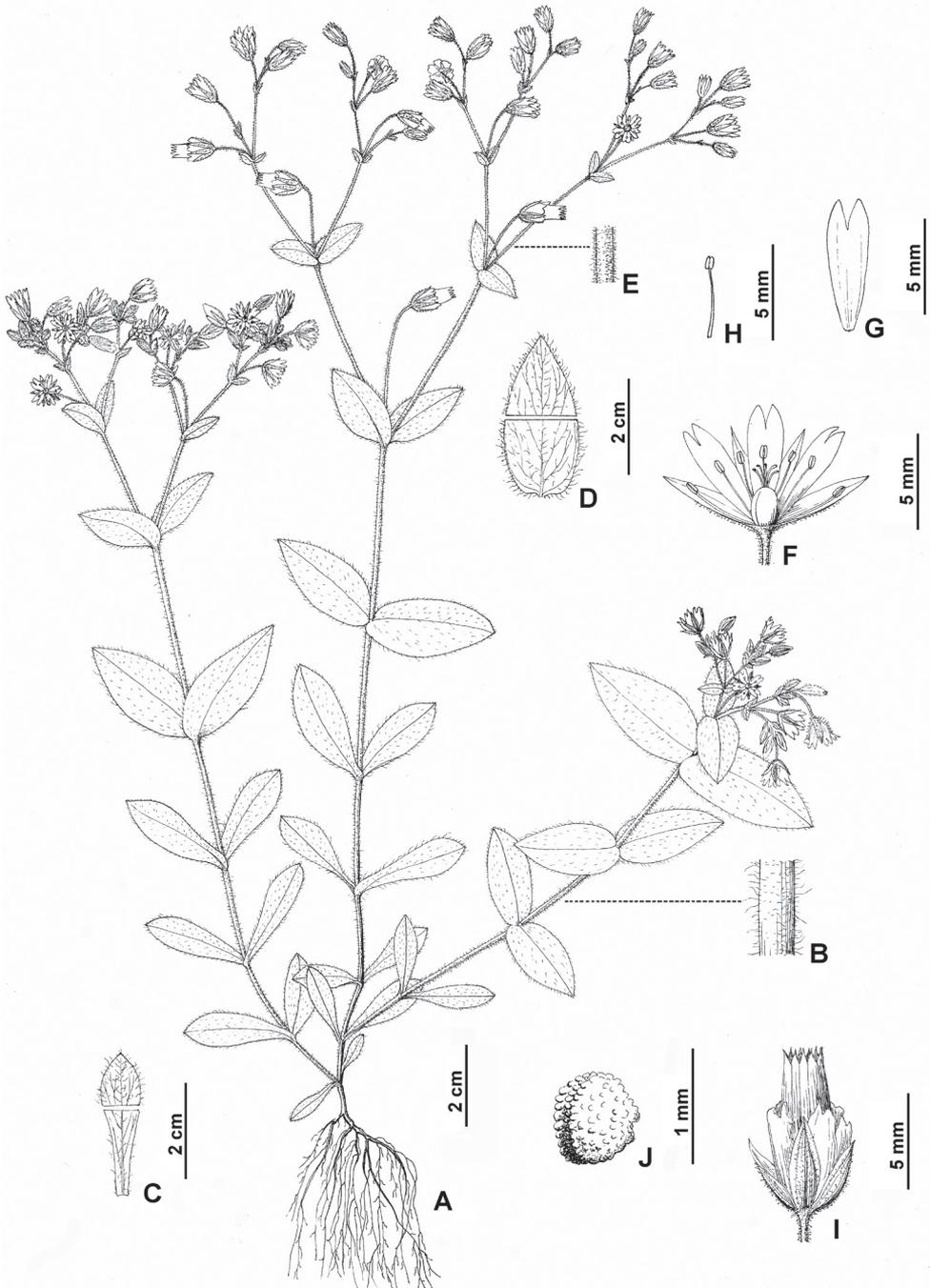


Figure 1. *Cerastium huadingense* sp. nov. **A** habit **B** indumentum on stem **C** basal leaf **D** upper leaf **E** indumentum on peduncle **F** vertical section of flower (showing sepals, petals, stamens and pistil) **G** petal **H** stamen **I** capsule with sepals and petals **J** seed (Illustrated by Hong Wang; based on Xiao-Feng Jin & Yi-Fei Lu 4583, ZM).



Figure 2. Comparison of *Cerastium huadingense* and *C. qingliangfengicum* **A–E** *Cerastium huadingense* sp. nov. **A** habitat **B** habit **C** indumentum on stem **D** flower **E** capsule **F–I** *Cerastium qingliangfengicum* **F** habitat **G** indumentum on stem **H** flower **I** capsule.

flowers white, 1.1–1.3 cm in diam. **Sepals** 5, lanceolate, green, 5–6 mm long, apex acuminate, margin membranous, abaxially pilose and glandular-pubescent. **Petals** 5, narrowly obovate, white, 6–7 mm long, slightly longer than sepals, 2-lobed for $\pm 1/4$ of length, lobes broadly ovate-oblong, apex acute, base glabrous. **Stamens** 10, filaments glabrous, slightly shorter than sepals. **Styles** 5, linear. **Capsules** cylindrical, ± 2 as long as sepals, 10-toothed. **Seeds** numerous, subtriangular-globose, slightly compressed, brown, ca. 1 mm in diam., tuberculate. Flowering and fruiting April–May.

Distribution and habitat. This new species is known from Mount Huading of Tiantai County and Mount Siming of Yuyao County, eastern Zhejiang. It grows under forest, in wet places or roadside at forest margin at the elevation of 900–1000 m.

Phenology. Flowering and fruiting from mid-April to mid-May.

Etymology. The specific epithet ‘*huadingense*’ refers to the type locality of the new species.

Conservation status. Vulnerable, VU B2ab(iii)C1 (IUCN 2019). The new species is only known from two localities, Mount Huading in Tiantai and Mount Siming in Yuyao, and occupied less than 400 km² with about 5000 mature individuals. This species is considered as Vulnerable (VU) according to IUCN Red List Categories and Criteria (IUCN 2019) based on the current data.

Specimen examined. Zhejiang (浙江): Tiantai (天台), Mount Huading (华顶山), Mochi (墨池), in wet place, alt. 900 m, 14 April 2018, *Wen-Yuan Xie* TT18041411 (PE, ZJFC, ZM), the same locality, under forest, 12 May 1978, *Chao-Fang Zhang* 3881 (ZJFC, ZM), roadside, alt. 1000 m, 25 April. 2020, *Xiao-Feng Jin & Yi-Fei Lu* 4584 (HTC, PE, ZM), 11 May 2020, *Yi-Fei Lu* 235 (HTC, PE, ZJFC, ZM); Mount Huading (华顶山), Ximaopeng (西茅蓬), roadside, alt. 900 m, 26 April 1976, *Chao-Zong Zheng* 8160 (HTC, ZM). Yuyao (余姚), Mount Siming (四明山), 28 April. 2018, *Zheng-Hai Chen & Jian-Sheng Wang* YY18042801 (HTC, ZJFC, ZM).

Notes. The genus *Cerastium* contains ca. 100 species and is almost cosmopolitan, but is mainly distributed in north temperate regions. Twenty-five species were recorded in China (Lu and Morton 2001; Zhang et al. 2008; Yao et al. 2021). A recent study revealed that the monotypic genus *Pseudocerastium* was merged in *Cerastium* (Yao et al. 2021).

Cerastium huadingense X.F.Jin, W.Y.Xie & Y.F.Lu is most similar to *C. qingliangfengicum* in pubescent stems, 5 styles and petals longer than sepals, but differs in having sterile stems absent, leaves sessile, petals slightly longer than sepals, and stamens slightly shorter than sepals. With loosely flowered cymes, pubescent pedicels, and petals 2-lobed at the apex, the new species, *C. qingliangfengicum*, *C. jiuhuashanense* and *C. wilsonii* are similar to each another and form a complex. The morphological characters distinguished from these species are shown in Table 1.

Table 1. Comparisons of *Cerastium huadingense*, *C. qingliangfengicum*, *C. jiuhuashanense* and *C. wilsonii*.

Traits	<i>Cerastium huadingense</i>	<i>C. qingliangfengicum</i>	<i>C. jiuhuashanense</i>	<i>C. wilsonii</i>
Leaf				
Petiole	sessile on sterile stems	5–9 mm long	basal leaves: 4–5 mm long; upper ones: sessile	basal leaves: 3–6 mm long; upper ones: sessile
Indumentum	sparsely pubescent on both surfaces	densely pubescent on both surfaces	sparsely pubescent on both surfaces	sparsely pubescent on both surfaces
Flower				
Sepal	5–6 mm long	6–8 mm long	5–7 mm long	ca. 6 mm long
Petal	6–7 mm long, slightly longer than sepals, 2-lobed for $\pm 1/4$ of length	10–12 mm long, ca. twice as long as sepals, 2-lobed for $\pm 1/2$ of length	10–12 mm long, ca. twice as long as sepals, 2-lobed for $\pm 1/2$ of length	ca. 12 mm long, ca. twice as long as sepals, 2-lobed for $\pm 1/2$ of length
Filament	glabrous	glabrous	villous on lower part	glabrous
Seed shape	subtriangular-globose	subtriangular-globose	subtriangular-globose	compressed globose

***Sinopogonanthera setulosa* (C.Y.Wu & H.W. Li) H.W.Zhang & X.F.Jin, comb. nov.**

urn:lsid:ipni.org:names:77221582-1

Chinese name: 小刺毛髯药草

Figs 3, 4

≡ *Sinopogonanthera zhejiangensis* H.W.Zhang & X.F.Jin, Fl. Zhejiang (New Ed.) 7: 230. 2020, nom. nud.**Basionym.** *Paraphlomis setulosa* C.Y.Wu & H.W.Li, Fl. Reipubl. Popularis Sin. 65(2): 602. 1977. Type: China. Anhui (安徽), Xiuning (休宁), 20 June 1959, *Ren-Hua Shan, Xiu Wu* 2226 (holotype: NAS 00072429!).**Note.** Based on the type specimen and the other related specimen examination, we emended the morphology of the species and described it below**Description emended.** *Herb* perennial, rhizomatous. *Stems* erect, unbranched, 30–80 cm tall, 2–3.5 mm in diam., obtusely 4-angled, shallowly canaliculate, densely retrorsely white-setulose. *Leaves* opposite; blades elliptic, oblong-ovate, elliptic-ovate or ovate, 7–19 cm long, 3–9.5 cm wide, apex acuminate, base broadly cuneate, margin serrate, adaxially green, abaxially pale green, sparsely pubescent on both sides, densely pubescent on mid-ribs, lateral veins 5–8 pairs; petioles 0.5–4.5 cm long, adaxially flat, densely white-setulose. *Verticillasters* axillary, (3–)7–14-flowered, 1.5–3 cm in diam.; pedicels 1–2 mm long or subsessile; bracteoles subulate, 1.5–3 mm long, densely setulose. *Calyx* tubular-obconical, 7–9 mm long, outside densely setulose, 5-veined, 5-lobed; teeth oblong-lanceolate or triangular, 2.5–3 mm long, apex acuminate, white-setulose. *Corolla* pale purple or pink, 10–15 mm long, outside densely appressed-pilose, inside sparsely pilose, hairy-annulate on lower part; tube 6–8 mm long, nearly straight, upper part slightly curved, amplified, with limb 2-lipped; upper lip straight, obovate-oblong, 5–5.5 mm long, apex emarginate; lower lip 3-lobed, middle lobe broadly obovate, ca. 3.5 mm long, 2.5–3 mm wide, apex emarginate, lateral lobes ovate, ca. 2.5 mm long, ca. 1.5 mm wide. *Stamens* 4, didynamous, nearly included, posterior 2 inserted in lower lip of corolla larynx, anterior 2 inserted in upper lip of corolla larynx, filaments sparsely pilose, anthers barbate, cells strongly divergent. *Ovary* glabrous; style slender, slightly exerted, apex 2-cleft. *Disc* persistent, cup-shaped. *Nutlets* obovoid, obtusely trigonous, gray-brown, ca. 2 mm long, apex truncate. Flowering and fruiting June–August.**Specimen examined.** Anhui (安徽), Xiuning (休宁), 29 June 1959, *Ren-Hua Shan, Xiu Wu* 2674 (paratype: NAS). Zhejiang (浙江): Kaihua (开化), Mount Gutian (古田山), roadside under forest, alt. 400 m, 28 June 2018, *Xiao-Feng Jin* 4230 (HTC, ZM). Lin'an (临安), Tuankou Town (湍口), Tangli Village (塘里村), in grasses under *Torreya* forest, alt. 550 m, 4 August 2014, *Xiao-Feng Jin* 3316, 3317, 3318 (ZJFC, ZM), the same locality, under forest, 5 June 2013, *Hong-Wei Zhang* 0001 (HTC, ZM), cultivated in Changhua (昌化), introduced from Tangli Village (塘里村) of Tuankou Town (湍口镇), 11 June 2021, *Yi-Fei Lu & Xiao-Feng Jin* 242 (holotype: ZM);

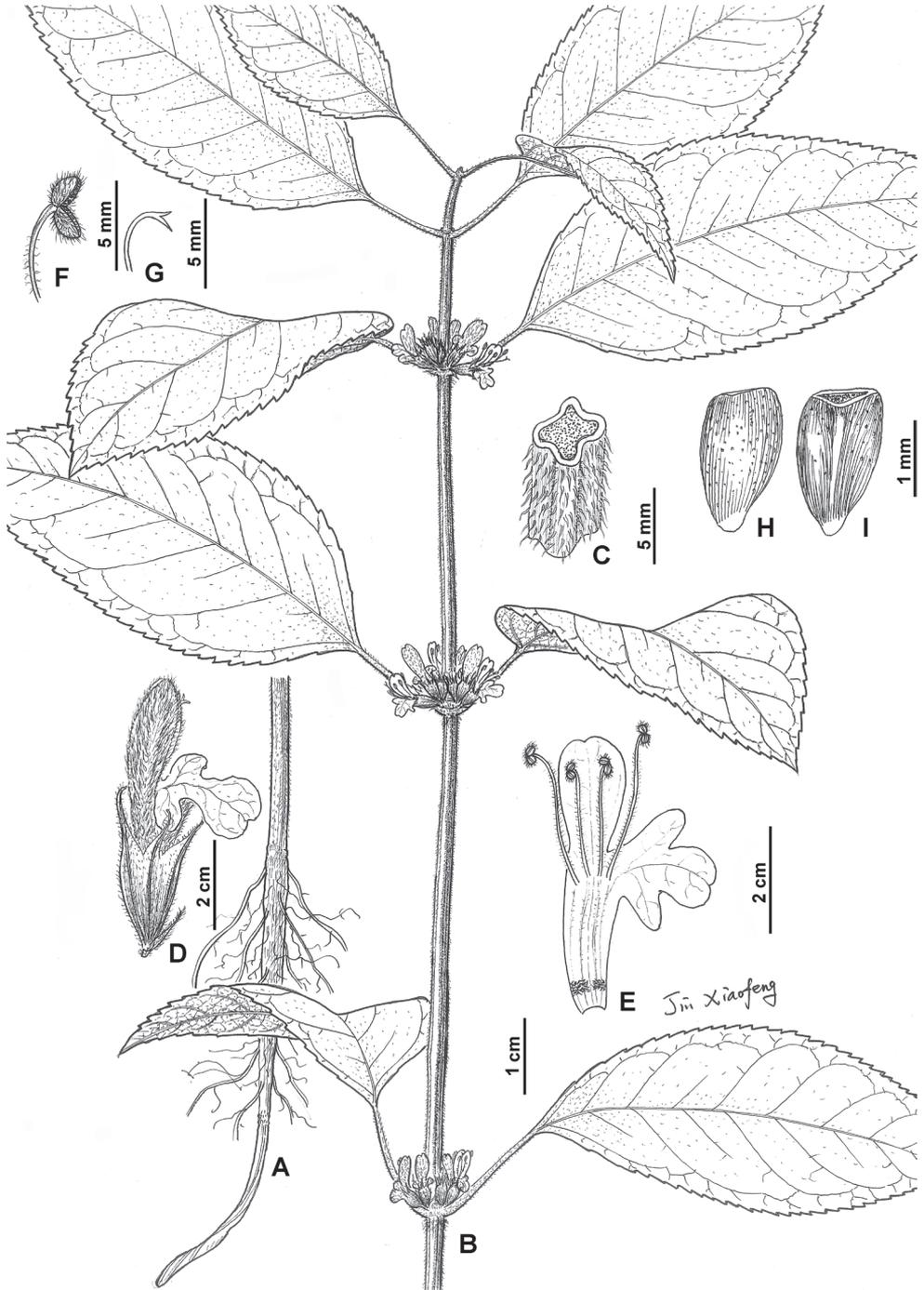


Figure 3. *Sinopogonantha setulosa* **A** lower part of habit **B** upper part of habit **C** indumentum on stem **D** flower with bracteole **E** opened corolla **F** anther **G** stigma **H** dorsal section of nutlet **I** ventral section of nutlet (Illustrated by Xiao-Feng Jin; based on Yi-Fei Lu & Xiao-Feng Jin 242, ZM).

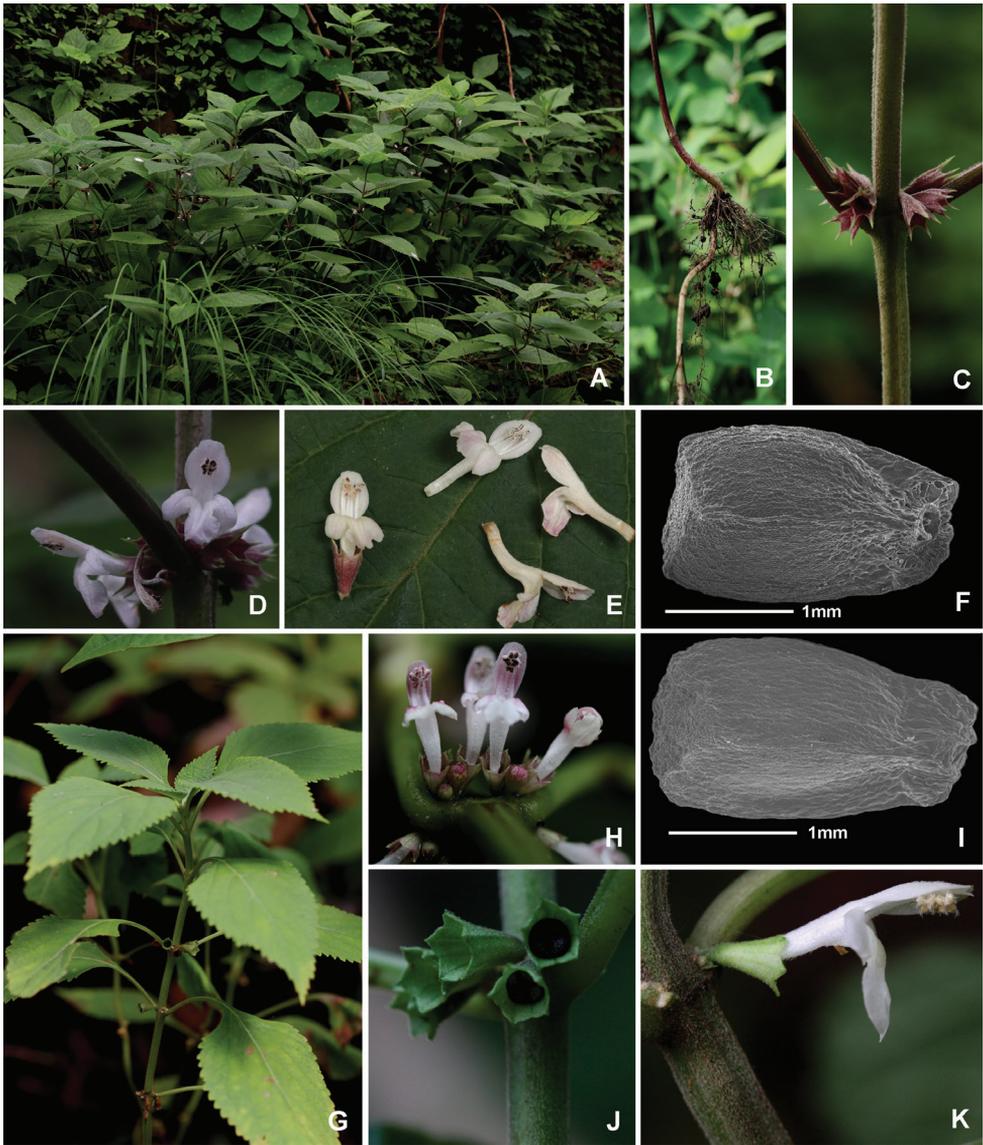


Figure 4. Comparison of *Sinopogonanthera setulosa* and *S. intermedia* **A–F** *Sinopogonanthera setulosa* **A** habitat **B** rhizome **C** calyx **D** inflorescence **E** corolla **F** nutlet **G–K** *Sinopogonanthera intermedia* **G** habit **H** inflorescence **I** nutlet **J** calyx **K** corolla.

isotypes: HTC, KUN for *Sinopogonanthera zhejiangensis*). Qujiang (衢江), Qianli-gang (千里岗), 22 July 2017, Zheng-Hai Chen s.n. (HTC).

Notes. Twenty-eight species with nine varieties within the genus *Paraphlomis* are currently recognized, which are mainly distributed in tropical and subtropical evergreen and mixed forests (Chen et al. 2021). Li (1965) revised *Paraphlomis* from China and 21 species with eight varieties were recognized. Wu & Li (1977) published *Paraphlomis setulosa* and *P. reflexa* as two new species, and they described that *Paraphlomis*

setulosa had anther cells strongly divergent. The taxonomic treatment of the genus *Paraphlomis* in *Flora of China* was just the same as Wu & Li in *Flora Reipublicae Popularis Sinicae* (Li & Hedge 1994). Guo (1993) established a new genus *Pogonanthera* close to *Paraphlomis* but mainly differs from barbate anthers and filament appendages at the apex. Unfortunately, *Pogonanthera* H.W.Li & X.H.Guo is a later homonym and Li (1993) proposed *Sinopogonanthera* H.W.Li as the replacement name, which were accepted and adopted (Guo 1995; Zheng 2005).

The genus *Sinopogonanthera* contains three species, and the key to these three species is shown as follows. *Sinopogonanthera caulopteris* H.W.Li has verticillasters up to 30-flowered, leaf blade long-elliptic, base cuneate and decurrent, petioles very short or sessile, and stems narrowly alate. *Sinopogonanthera intermedia* (C.Y.Wu & H.W.Li) H.W.Li has verticillasters 10–14-flowered, leaf blade ovate to ovate-rounded, base broadly cuneate, petioles 1–6 cm long. *Sinopogonanthera setulosa* is somewhat similar to *S. intermedia* in attenuate but not decurrent leaf blades at the base and leaf blade petiolate, but differs in having calyx lobes oblong-lanceolate, 2.5–3 mm long, corolla outside densely appressed-white-pilose, and leaves without glands. The species *Sinopogonanthera zhejiangensis* (nom. nud.) is consistent to *S. setulosa* and here reduced to synonym.

Key to the species of *Sinopogonanthera*

- 1 Stems narrowly alate; leaf base cuneate and decurrent, petioles very short or sessile; verticillasters 12–30-flowered ***S. caulopteris***
- Stems obtusely tetragonal; leaf base broadly cuneate or attenuate, but not decurrent, petioles 0.5–6 cm long; verticillasters 3–14-flowered..... **2**
- 2 Calyx lobes broadly triangular, ca. 1 mm long, outside densely pubescent; corolla outside sparsely puberulent and glabular ***S. intermedia***
- Calyx lobes oblong-lanceolate, 2.5–3 mm long, outside densely setulose; corolla outside densely pilose..... ***S. setulosa***

***Ixeridium dimorphifolium* Y.L.Xu, Y.F.Lu & X.Cai, sp. nov.**

urn:lsid:ipni.org:names:77221583-1

Chinese name: 二型叶小苦苣

Figs 5, 6

Latin diagnosis. *Species nova I. beauverdiano* (H. Lév.) Spring. *affinis est, sed planta stolonibus, foliis basilaribus dimorphis, involucris 8–10 mm longis, phyllis intimis 8, flosculis 7–10 differt.*

Type. CHINA. Zhejiang (浙江): Suichang (遂昌), Jiulongshan National Nature Reserve (九龙山国家级自然保护区), Yangmei Keng (杨梅坑), in grasses along streams, alt. 550 m, 17 April 2020, *Xiao-Feng Jin* 4568 (holotype: ZM; isotypes: HTC, ZM).

Description. *Herb* perennial, glabrous totally. *Stolons* elongate, creeping. *Stems* erect, 20–35 cm tall, slender, with sparse branches from base. *Basal leaves* crowded, present at anthesis, dimorphic; blades of early ones nearly orbicular to long-elliptic,

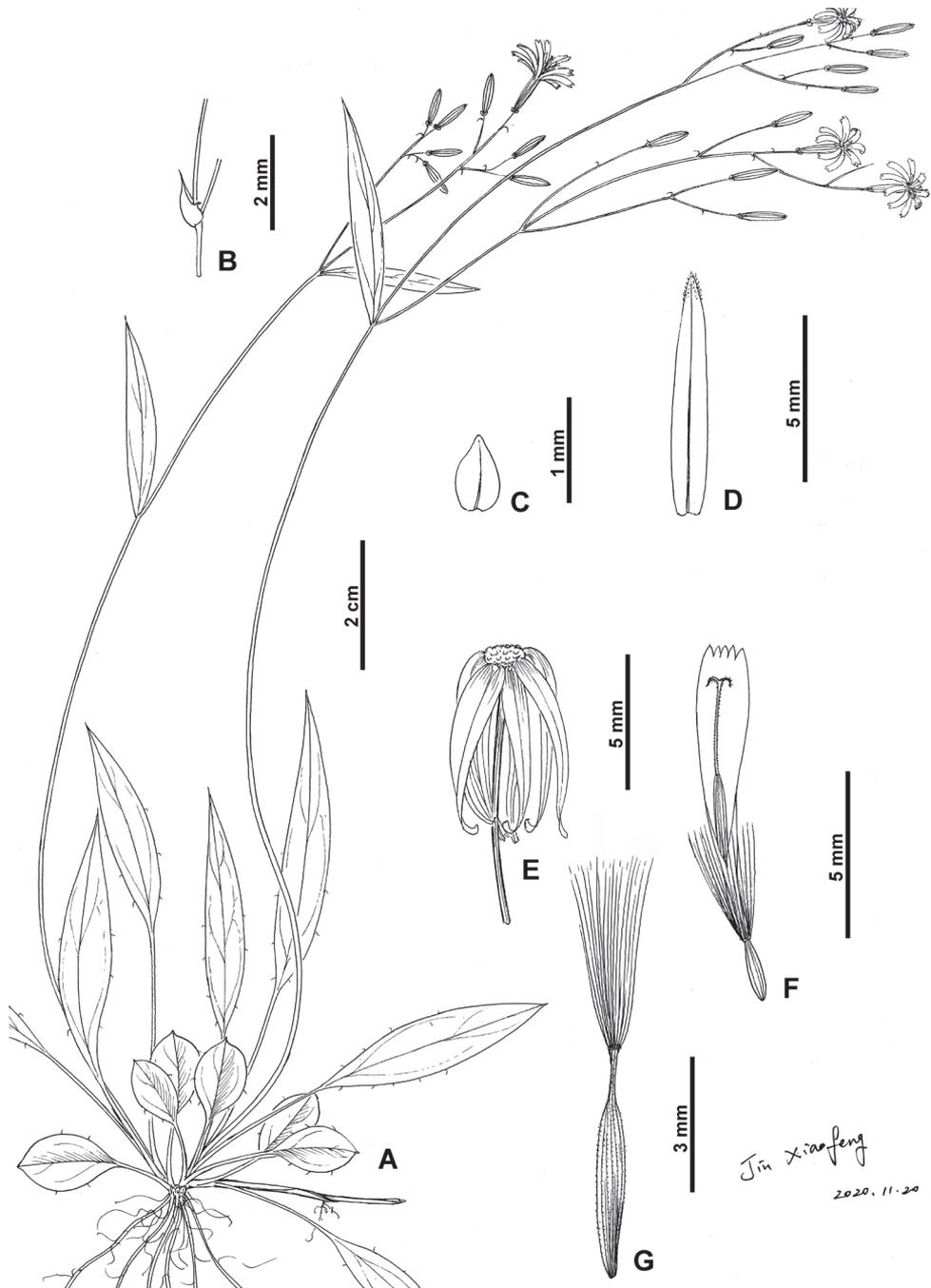


Figure 5. *Ixeridium dimorphifolium* sp. nov. **A** habit **B** bract **C** outer phyllary **D** inner phyllary **E** opened involucre (showing receptacle) **F** floret **G** achene (Illustrated by Xiao-Feng Jin; based on Xiao-Feng Jin 4568, ZM).

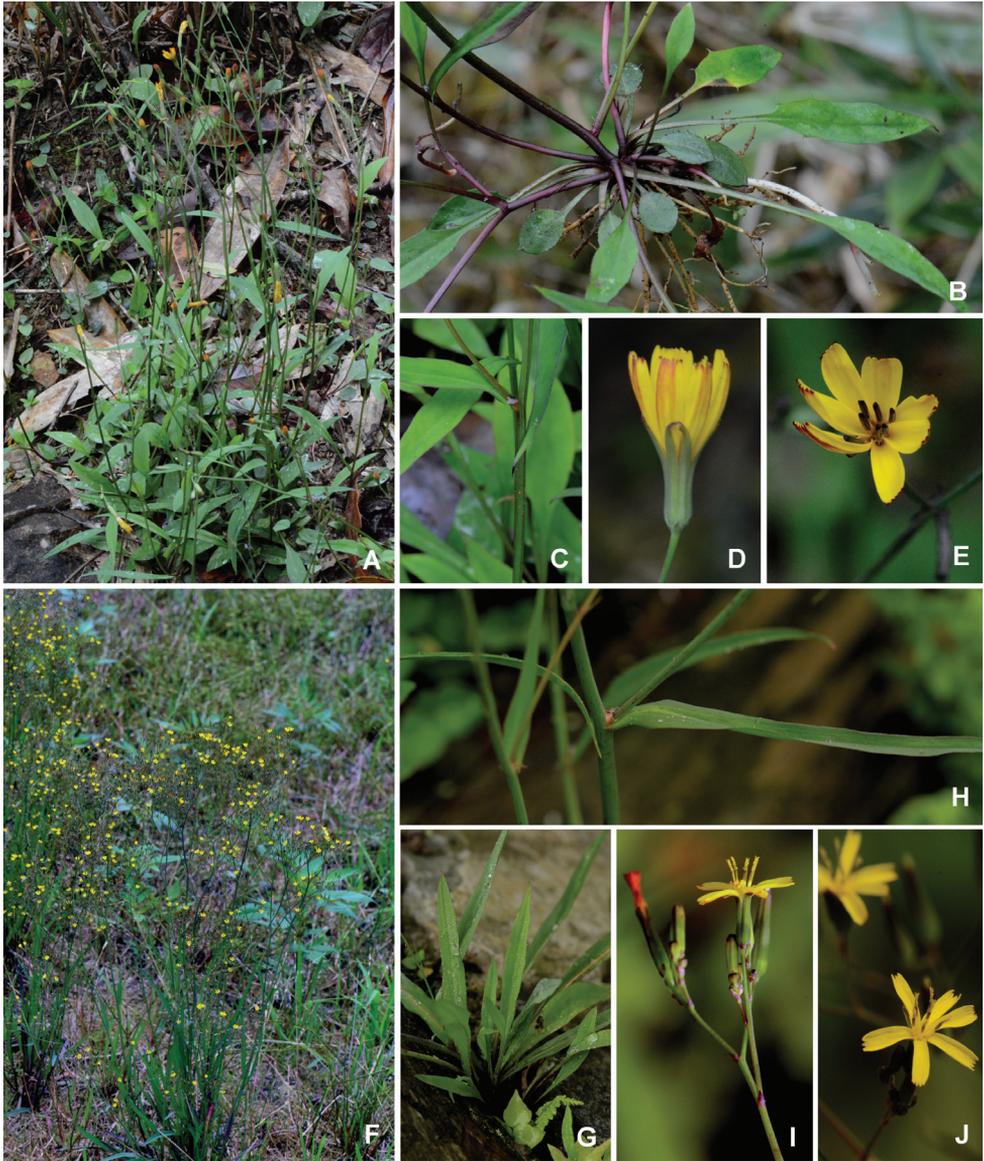


Figure 6. Comparison of *Ixeridium dimorphifolium* and *I. beauverdianum* **A–E** *Ixeridium dimorphifolium* sp. nov. **A** habit **B** basal part (showing dimorphic leaves and stolon) **C** cauline leaves **D** capitula (showing involucre) **E** capitula (showing florets) **F–J** *Ixeridium beauverdianum* **F** habit **G** basal part (showing monomorphic leaves) **H** cauline leaves **I** capitula (showing involucre) **J** capitula (showing florets).

0.9–2.5 cm long, 0.7–1.3 cm wide, apex obtuse and mucronate, base broadly cuneate to rounded, margin entire and with sparsely slender ciliate-teeth, with petioles 1–3 cm long; blades of later ones narrowly elliptic to lanceolate, 3–6 cm long, 0.6–1.1 cm

wide, apex acute, base attenuate to a 2–3 cm long petiole, margin entire or with sparsely slender ciliate-teeth below. **Cauline leaves** 1–3; blades linear-lanceolate, 2.5–7.5 cm long, 0.4–0.7 cm wide, apex acuminate, base attenuate, margin entire and with/without sparsely slender ciliate-teeth below. **Synflorescence** corymbiform, with 6–16 capitulae; capitula with 7–10 florets, base with slender, long peduncle. **Bracts** small, broadly ovate-triangular, ca. 1 mm long, margin entire or with 1-pair ciliate-teeth. **Involucre** narrowly cylindrical, 8–10 mm long. **Phyllaries** 2 series, abaxially glabrous; outer phyllaries broadly ovate, 0.5–0.7 mm long, apex obtuse; inner phyllaries 8, linear-lanceolate, 7–9 mm long, apex obtuse, short-ciliate. **Receptacle** flattened, glabrous, alveolate. **Florets** 7–10, yellow, corolla 10–11 mm long, tube 3–3.5 mm long, ligule linear, apex 5-lobed. **Achenes** brown or pale brown, narrowly fusiform or linear-fusiform, slightly compressed, 4.5–5 mm, finely spiculate, with ribs finely spiculate, apex attenuate to a 1–1.5 mm long slender beak. **Pappi** pale brown, ca. 5 mm long, scabrid. Flowering and fruiting April– July.

Distribution and habitat. *Ixeridium dimorphifolium* is currently known from only two localities in Suichang and Qingyuan counties, southwestern Zhejiang Province, East China. It grows in grassy roadside along stream or wetland at the elevation of 500–1450 m.

Phenology. Flowering and fruiting from mid-April to mid-July.

Etymology. The specific epithet ‘*dimorphifolium*’ refers to two different shapes of basal leaves.

Conservation status. Endangered, EN B2ab(iii) (IUCN 2019). The new species is only known from two localities, and occupied less than 100 km² with about 1000 mature individuals. This species is considered as Endangered (EN) according to IUCN Red List Categories and Criteria (IUCN 2019) based on the current data.

Specimen examined. Same locality as type, alt. 500 m, 5 May 2017, *Yue-Liang Xu* 234, 239, 274 (ZM), alt. 550 m, 17 April 2020, *Xiao-Feng Jin* 4569 (HTC, ZM). Qingyuan (庆元), Huangpi (黄陂), in wetland, alt. 1450 m, 12 July 2020, *Xiao-Feng Jin, Yi-Fei Lu & Dan-Qi Liu* 4600 (HTC, ZM).

Notes. *Ixeridium* (A. Gray) Tzvel. is a moderately sized genus within the tribe Cichorieae. It contains 15 species and distributes in E and SE Asia, with eight species occurring to China (Shi & Kilian, 2011). The genus *Ixeridium* is most morphologically similar to *Ixeris* (Cass.) Cass., but is distinguished in having achenes with 9–12 prominent but not wing-like ribs (vs. 10 prominent and wing-like ribs) (Shih, 1997). *Ixeridium dimorphifolium* is similar to *I. beauverdianum* in having the cauline leaf blades linear to linear-elliptic, margin nearly entire, and pappi brown or pale brown, but differs from the latter in its dimorphic basal leaves, involucre longer, 8–10 mm long, inner phyllaries 8, florets 7–10, and plant with stolons.

Morphology of pollens and achenes of the species in Asteraceae has taxonomic significance and sometimes was used to identify similar species (Biyiklioğlu et al. 2018; Joujeh et al. 2020; Nurgül et al. 2021). The micromorphology of achenes and pollen grains of *Ixeridium dimorphifolium* and *I. beauverdianum* was shown in Figure 7 and

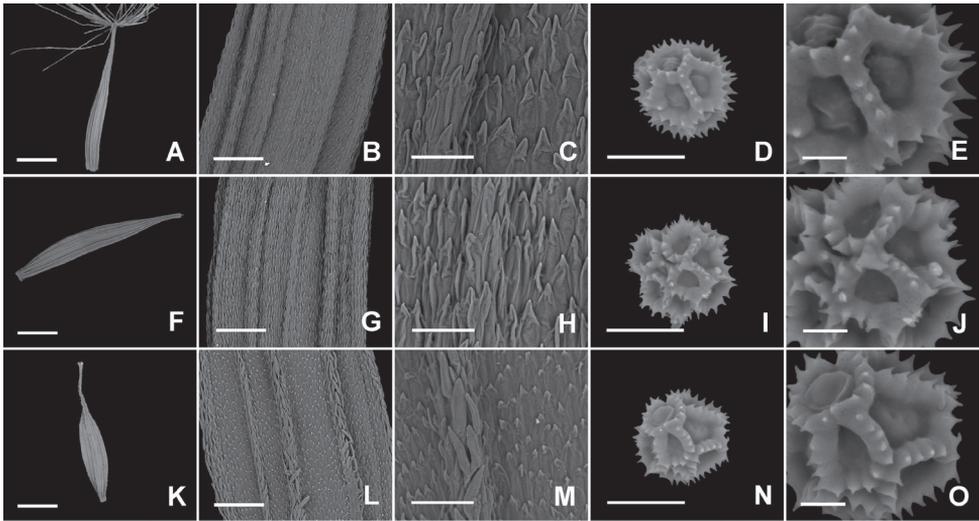


Figure 7. Micromorphology of achenes and pollen grains of *Ixeridium dimorphifolium* and *I. beauverdianum* **A–E** *Ixeridium dimorphifolium* sp. nov. (from Suichang) **F–J** *Ixeridium dimorphifolium* sp. nov. (from Qingyuan) **A** and **F** achene shape (scale bar: 1mm) **B** and **G** middle part (scale bar: 200µm) **C** and **H** spicules on surfaces and ribs (scale bar: 50µm) **D** and **I** pollen grains (scale bar: 20µm) **E** and **J** surface sculpture (scale bar: 5µm) **K–O** *Ixeridium beauverdianum* **K** achene shape (scale bar: 1mm) **L** middle part (scale bar: 200µm) **M** spicules on surfaces and ribs (scale bar: 50µm) **N** pollen grains (scale bar: 20µm) **O** surface sculpture (scale bar: 5µm).

Table 2. Comparisons of the achenes and pollen grains among *Ixeridium dimorphifolium* (two samples) and *I. beauverdianum*.

Traits/species	<i>Ixeridium dimorphifolium</i> (Suichang)	<i>I. dimorphifolium</i> (Qingyuan)	<i>I. beauverdianum</i>
Achene			
shape	narrowly fusiform, slightly compressed	linear-fusiform, slightly compressed	fusiform, slightly compressed
length	4.98±0.42 mm	5.54±0.60 mm	4.72±0.34 mm
surface	finely spiculate, with ribs finely spiculate	finely spiculate, with ribs finely spiculate	finely spiculate, with ribs finely spiculate
rib	with spicule similar to surface	with spicule similar to surface	with spicule longer than those on surface
apex	gradually narrow to a beak	gradually narrow to a beak	abruptly narrow to a beak
Pollen			
shape	tricolporate, spherical	tricolporate, spherical	tricolporate, spherical
size	27.55±3.60 µm	26.70±2.84 µm	24.62±1.95 µm
surface	echinophate	echinophate	echinophate

Table 2. The new species, *Ixeridium dimorphifolium*, has the achenes narrowly fusiform or linear fusiform, with surfaces and ribs both finely similar spiculate, apex gradually narrow to a beak. While those of *I. beauverdianum* are fusiform, with spicules on surfaces shorter than those on ribs, apex abruptly narrow to a beak. The pollen grains of *Ixeridium dimorphifolium* and *I. beauverdianum* are similar to each other.

Acknowledgements

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Plagiothecium schofieldii, a new species from the Aleutian Islands (Alaska, USA)

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Abstract

Plagiothecium schofieldii sp. nov. is described from the Aleutian Islands, Alaska, U.S.A. Some morphological features of this species correspond to *P. lamprostachys* (Southern Hemisphere species); however, *Plagiothecium schofieldii* is genetically and morphologically different from this and other common Northern Hemisphere species e.g., *P. denticulatum*, *P. platyphyllum*, or *P. ruthei*. The most important distinguishing morphological features differentiating this species are: the arrangement of the leaves on the stem; dimensions, concavity and symmetry of the leaves; dimensions of cells and their areolation; orientation of capsules. Additionally, due to the strong concavity of the leaves, they are very often badly damaged under the microscope. We present the results of DNA research of the analyzed samples, and a detailed description of the morphological features. The new species is illustrated, and its ecological preferences and currently known geographical distribution are presented. Additionally, the authors propose to add this species to *Plagiothecium* section, which is confirmed by morphological features and genetic analysis.

Keywords

Bryophyta, Plagiotheciaceae, taxonomy, W. B. Schofield

Introduction

Over the last several years, our perception has changed not only of *Plagiothecium* Schimp., but also of the whole family of Plagiotheciaceae M.Fleisch. (e.g., Pedersen and Hedenäs 2001, 2002; Wynns et al. 2018). The use of molecular methods has not only helped to understand many taxa previously considered problematic, but has also allowed for the description of a number of new taxa (e.g., Zuo et al. 2011; Wynns et al. 2018; Ignatova et al. 2019; Wolski and Nowicka-Krawczyk 2020). Nevertheless, for decades the taxonomic status of many species of this genus has been unclear and ambiguous, and those taxa currently require detailed morphological, genetic and taxonomic studies.

Although the Northern Hemisphere seems to be relatively well researched, there are still many areas (e.g., central Asia, Middle East) which remain as gaps on the world distribution map of *Plagiothecium* (Wolski et al. 2021). The results of taxonomic revisions conducted in recent years indicate the underestimation of the species richness of individual parts of the world. As a consequence of this research, many countries and regions have increased their number of known taxa of the described genus (e.g., Ellis et al. 2019a; Ellis et al. 2019b; Ellis et al. 2020, 2021; Müller and Wynns 2020; Wolski and Nowicka-Krawczyk 2020; Wolski 2020).

The Aleutian Islands, Alaska, U.S.A., are one of the many under-explored regions of the Northern Hemisphere. As a result of the taxonomic revision of *Plagiothecium* specimens from this area it was possible to describe a new species from this genus; the results are presented below.

Materials and methods

Taxonomic analyses

Material from the Missouri Botanical Garden (MO), The New York Botanical Garden (NY) and the University of British Columbia (UBC) was analyzed during the revision of *Plagiothecium* from the Aleutian Islands. For selected specimens intended for DNA analysis, appropriate consent was obtained from NY (NY02589541) and MO (MO5135779, MO5140205, MO5148015).

DNA isolation, amplification and sequencing

The molecular research was based on nuclear and chloroplast DNA markers: ITS (from the 3' end of the hypervariable nuclear spacer ITS1, through the 5.8S gDNA, to the 5' end of the ITS2 spacer); and *rpl16* cpDNA gene encoding ribosomal protein L16. Markers were selected based on Wynns et al. (2018), Ignatova et al. (2019) and Wolski and Nowicka-Krawczyk (2020) from *Plagiothecium*-focused studies.

Leafy stems of mosses were cut from dried material. Approximately 20 mg of dry tissue from each specimen in duplicates was placed in a 1.5 ml Eppendorf Safe-Lock tube and frozen (-20 °C) for homogenization. Tissue homogenization was performed using a hand-held stainless steel homogenizer (Schlüter Biologie, Eutin, Germany). Total DNA was extracted using the GeneMATRIX Plant & Fungi DNA Purification Kit (Eurx, Gdansk, Poland) following the manufacturer's protocol. DNA extracts were quantified with a BioDrop DUO Spectrophotometer (BioDrop Ltd, Cambridge, U.K.). From the duplicates, the sample with the higher quality DNA ($1.7\text{--}1.9 \text{ OD}_{260} / \text{OD}_{280}$) was selected for further analysis.

For each sample, all markers were amplified by PCR in a few replicates to obtain high quality amplicons for sequencing. PCR was performed using primers and reaction conditions as described in Wolski and Nowicka-Krawczyk (2020), with a 50 µl reaction volume with 25 µl of Color Taq PCR Master Mix (2×) (Eurx, Gdansk, Poland).

PCR products were visualized on an agarose gel (1.5%, 90V, 40 minutes) stained with GelRED fluorescent dye (Biotum, Fremont, CA, U.S.A.) and two replicates of each marker per sample were chosen for sequencing. Amplicons from the PCR reaction were cleaned using Syngen Gel/PCR Mini Kit (Syngen Biotech, Wrocław, Poland) according to the manufacturer's protocol. Samples were sequenced with Sanger sequencing using primers from amplification by SEQme s.r.o. company (Dobris, Czech Republic). The obtained sequences were assembled in Geneious 11.1.5 (Biomatters Aps, Aarhus, Denmark) (<http://www.geneious.com>). The sequences were submitted to the NCBI GenBank database (www.ncbi.nlm.nih.gov) under the accession numbers MW936654–MW936657 for ITS and MW935831–MW935834 for *rpl16*.

Phylogenetic analyses

Phylogenetic analyses of studied specimens and other species in the *Plagiothecium* group were performed based on a concatenated ITS-*rpl16* sequence matrix. Voucher information for the specimens included in this study, with corresponding GenBank accession numbers, is presented in Table 1. Sequences were aligned using the MAFFT v. 7 web server (Kato et al. 2017) (<http://mafft.cbrc.jp/alignment/server/>) where the auto strategy was applied, the scoring matrix of 200PAM with Gap opening penalty of 1.53, UniREF50 for Maft-homologs and Plot and alignment with threshold of 39 score were set. The obtained alignments were checked for poorly and ambiguously aligned regions and small corrections were made by eye. The evolutionary models were calculated using PartitionFinder 2 software (Lanfear et al. 2016) chosen according to the Akaike Information Criterion (Table 2).

Phylogenetic calculations were performed using maximum likelihood analysis (ML) in the IQ-TREE web server (Trifinopoulos et al. 2016) (<http://iqtree.cibiv.univie.ac.at/>) with the ultrafast bootstrap (UFBoot) pseudo likelihood algorithm (Hoang et al. 2018) and 10000 replicates; and Bayesian inference (BI) in MrBayes 3.2.2 (Ronquist et al. 2012) where two parallel Markov chain Monte Carlo (MCMC) runs for

Table 1. Voucher information and accession numbers for the specimens included in the phylogenetic analyses.

Taxon	Collection	Locality	ITS	rpl16
<i>Plagiothecium bergrenianum</i>	S-B44769	Russia: Pacific Siberia, Yakutiya	KY550267	KY513972
<i>Plagiothecium brasiliense</i>	E barcode E00387968	Brazil	KY550266	KY513971
<i>Plagiothecium conostegium</i>	NY: S.P. Churchill et al. 19839	Bolivia	KY550271	KY513976
	NY barcode 00845279	Guatemala	KY550318	KY514024
	S-B53327	Mexico	KY550272	KY513977
<i>Plagiothecium curvifolium</i>	DUKE barcode 0209096	Canada: BC	KY550273	KY513978
	CP: G.P. Rothero s.n.	Germany: Hochschwarzwald	KF882228	KF882328
<i>Plagiothecium denticulatum</i>	CP: J.T. Wynns 2081	Denmark: Sorø kommune, Sjælland	KF882229	KF882329
<i>Plagiothecium denticulatum</i> var. <i>bullulæ</i>	UC barcode 1947417	USA: CA	KY550277	KY513982
	UC barcode 1798690	USA: NV	KY550278	KY513983
<i>Plagiothecium denticulatum</i> var. <i>obtusifolium</i>	CP: J.T. Wynns 2842	Germany: Schauinsland, Hochschwarzwald	KF882230	KF882330
	UC barcode 1724036	USA: WA	KY550279	KY513984
<i>Plagiothecium denticulatum</i> fo. <i>pungens</i>	DUKE barcode 0150010	USA: AK	KY550280	KY513985
<i>Plagiothecium laetum</i>	CP: J.T. Wynns 2907	Germany: Schauinsland, Hochschwarzwald	KF882234	KF882334
	C barcode CP0010626	USA: NC	KY550292	KY513997
	C barcode CP0010627	USA: NC	KY550293	KY513998
	OK2066	Germany	MK934644	MK941642
<i>Plagiothecium lamprostachys</i>	OK2035	Russia: Krasnodar, Shakhe	MK934647	MK941645
	S-B54613	Australia: VIC	KY550284	KY513989
	DUKE barcode 0156846	Australia: VIC	KY550285	KY513990
	CP: I.L. Goldberg s.n.	Denmark: Holmegårds Mose, Sjælland	KF882235	KF882235
<i>Plagiothecium lucidum</i>	NY barcode 01233548	Chile	KY550298	KY514003
	BONN: J.-P. Frahm 12–6	New Zealand	KY550299	KY514004
<i>Plagiothecium membranosulum</i>	BONN: J.-P. Frahm 7756	Democratic Republic of the Congo	KY550310	KY514015
	S barcode B78514	South Africa	KY550303	KY514008
	DUKE barcode 0016754	South Africa	KY550304	KY514009
<i>Plagiothecium mollicaulis</i>	NY barcode 1596265	Brazil	KY550300	KY514005
<i>Plagiothecium ovalifolium</i>	DUKE barcode 0188886	Chile	KY550314	KY514019
<i>Plagiothecium pacificum</i>	UC barcode 1921143	USA: CA	KY550295	KY514000
<i>Plagiothecium platyphyllum</i>	CP: J. Lewinsky et al. s.n.	Finland: Haluna, Nilsiae, Savonia borealis	KF882241	KF882341
<i>Plagiothecium rossicum</i>	OIK-2019 isolate OK2054	Russia: Kunashir	MK934622	MK941625
	OIK-2019 isolate OK2032	Russia: Smolensk	MK934629	MK941630
<i>Plagiothecium ruthei</i>	CP: J.T. Wynns 1997	Denmark: Lyngby Aamose, Sjælland	KF882242	KF882342
<i>Plagiothecium svalbardense</i>	C-M-9109	Greenland: W5	KY550296	KY514001
<i>Plagiothecium angusticellum</i>	Wolski 22	Poland	MN077507	MN311142
<i>Plagiothecium longisetum</i>	Wolski 19	Poland	MN077506	MN311141
<i>Isopterygiopsis pulchella</i>	UC barcode 1947397	USA: CA	KY550336	KY514042
P1 MO5135779	MO5135779	USA: Alaska, Simeonof Island	MW936657	MW935834
P2 MO5140205	MO5140205	USA: Alaska, Simeonof Island	MW936656	MW935833
P3 MO5148015	MO5148015	USA: Alaska, Simeonof Island	MW936655	MW935832
P4 NY02589541	NY02589541	USA: Alaska, Adak Island	MW936654	MW935831

Table 2. Summary of partitions for ITS-rpl16 matrix (1574 bp) evolutionary model selection and phylogenetic interference using PartitionFinder2.

	ITS1	5.8S gDNA	ITS2	rpl16 intron	rpl16 codon
ML	JC	JC	HKY +I	TIM+I+G	JC
BI	JC	JC	HKY	F81	JC

four million generations each, with trees sampled every 1000 generations. The average standard deviation of split frequencies in both cases remained below 0.01 for the last 1000 generations and posterior probabilities were estimated from the 50% majority-rule consensus tree after elimination of the first 25% of samples as burn-in. Raw data sequences, the alignment and tree files were submitted to the figshare online database (<https://doi.org/10.6084/m9.figshare.14443697.v1>).

Haplotype network analysis was performed using Median Joining Network in PopART v. 1.7 with gap coding as a single event irrespective of length and haplotypes' geographic distribution (Leigh and Bryant 2015). The analysis included species of *Plagiothecium* sect. *Plagiothecium* with representatives of *P. longisetum* and *P. angusticellum* (sect. *Orthophyllum*).

Results and discussion

Phylogenetic analyses based on the concatenated ITS-*rpl16* matrix placed studied specimens within the branch of a *Plagiothecium* sect. *Plagiothecium* clade, or sister to it; however, the branch support was very low (BS = 49). The next branch down is to representatives of sect. *Orthophyllum* Jedd. and even more distant to sect. *Lep-tophyllum* Jedd. clade (Fig. 1). After branching off from the *Orthophyllum* clade, the internal division of sect. *Plagiothecium* was well supported by Bayesian inference (PP \geq 0.98). Maximal support from both maximum likelihood and Bayesian Inference was recorded in the clade of *Plagiothecium schofieldii*, where the representatives create a monospecific clade (PP = 1).

The haplotype network (Fig. 2) also showed internal diversity in sect. *Plagiothecium*. At the center, the analysis grouped haplotypes from the Northern Hemisphere (*P. denticulatum* and *P. ruthei*). Three branches extending from the center apply to haplotypes from Central America and the Southern Hemisphere. The position of *P. schofieldii* haplogroup is fairly isolated and consists of two haplotypes: the first refers to three specimens from Simeonof Island, while the second to a representative from Adak Island (Table 1); however, as it grows in the Aleutian Islands, Alaska, the branch vector points out the same direction as haplotypes from the Northern Hemisphere.

The individual taxonomic features of *Plagiothecium* are related to a specific level of detail in our analyses, and for example: superficial layer of the stem (epidermis layer) of large, thin-walled cells; shortly pointed leaves; serration (if present) only at apex; absence of pseudoparaphyllia; leaves clearly decurrent at the base – distinguish this genus from other genera belonging to the Plagiotheciaceae. Within *Plagiothecium*, the shape of decurrent alar regions, and the shape of their cells distinguishes the species of individual sections, while the shape and dimensions of leaf cells are the most important features distinguishing species from each other (Iwatsuki 1970; Lewinsky 1974; Noguchi 1994; Smith 2001). Therefore, based on the fact that the analyzed specimens have decurrent alar cells that are rounded, inflated, and form distinct auricles, as well as the shape and size of the

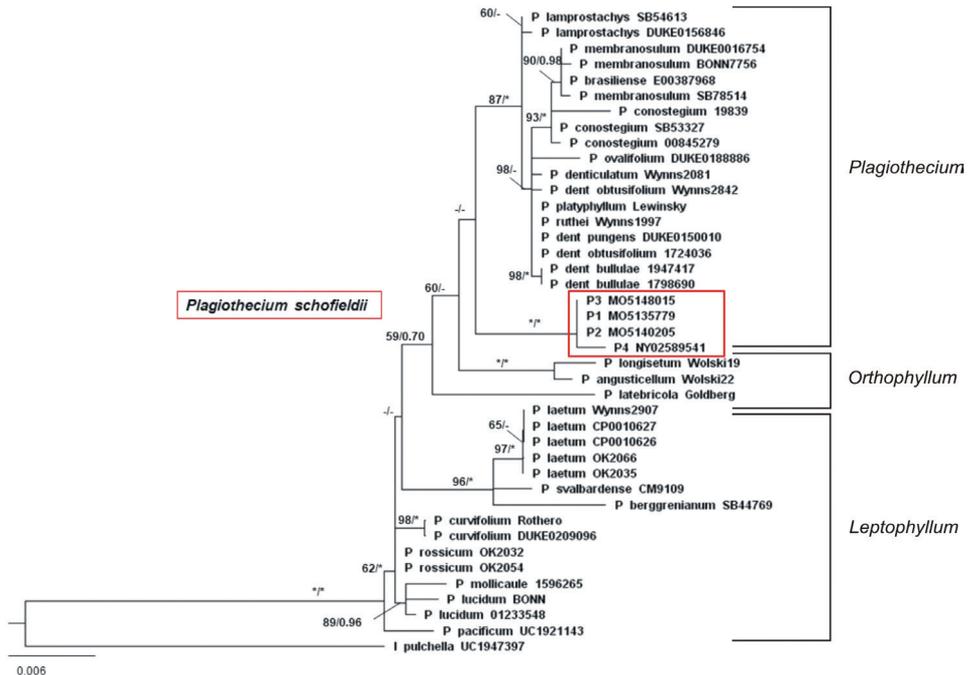


Figure 1. Phylogenetic tree of *Plagiothecium* taxa with *Isopterygiopsis pulchella* as the outgroup based on concatenated nuclear (ITS1-5.8S-ITS2) and chloroplast (*rpl16*) DNA markers (total 1574 bp). The tree presents the position of *Plagiothecium* morphotypes from Alaska among the *Plagiothecium* group which is divided into individual sections. Numbers on branches indicate bootstrap values from ML followed by posterior probabilities from BI analysis. Asterisk (*) indicates 100 (ML) and 1.00 (BI), while minus (-) indicates values below 50 (ML) and 0.7 (BI). The topology of the tree was based on ML analysis.

leaf cells of *Plagiothecium schofieldii*, we believe that this species belongs to *Plagiothecium* sect. *Plagiothecium*. This is also confirmed by molecular and haplotype network analyses.

Species that are widespread in the Northern Hemisphere: *Plagiothecium denticulatum* (Hedw.) Schimp., *P. platyphyllum* Mönk., and *P. ruthei* Limpr., significantly differ in morphology from *P. schofieldii*, which, compared to the above-mentioned species, has erect stems, while the others are usually prostrate, or sometimes prostrate to ascending (Smith 2001; Li and Ireland 2008; Wynns 2015).

Leaves of *P. schofieldii* are julateous and imbricate – very closely arranged on the stem, while in other species the leaves are strongly complanate, flaccid, and spreading on the stem. In the Northern Hemisphere only in *P. denticulatum* shoots are rarely julateous (Lewinsky 1974; Smith 2001; Li and Ireland 2008; Wynns 2015). The appearance of the mats and the arrangement of the leaves on the stem are more similar to those features in *P. cavifolium* (Brid.) Z. Iwats. (which belongs to *Plagiothecium* sect. *Orthophyllum*).

Stem leaves of *Plagiothecium schofieldii* are very strongly concave, to such an extent that under the microscope they are clearly damaged and cracked from being flattened by the coverslip. The leaves of the closely related species are rather flat. Only in the case of *P. denticulatum* are the leaves more or less concave, but never to such an extreme

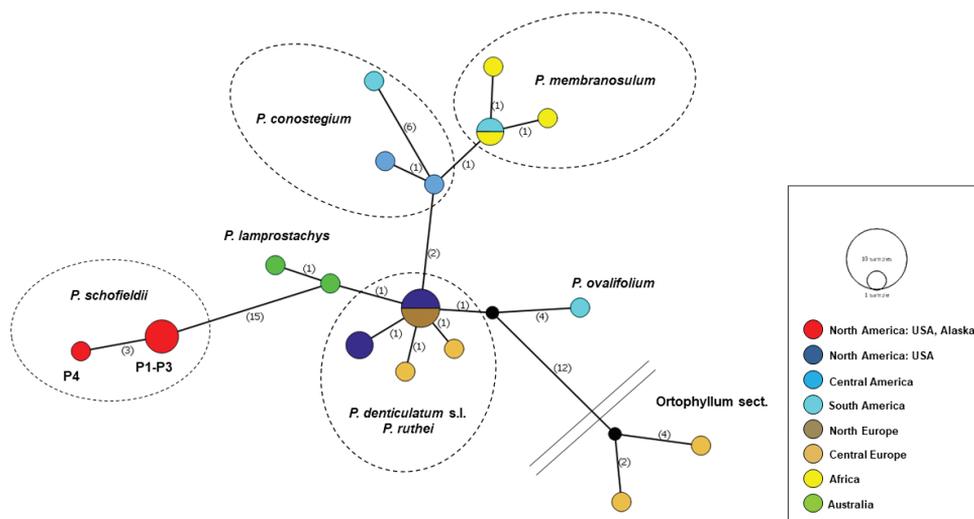


Figure 2. Median-joining haplotype network of sections *Plagiothecium* and *Orthophyllum* of *Plagiothecium* constructed in PopART. Haplotypes are represented by circles with colors indicating geographic distribution. The number on the branches indicates the mutational steps.

(Lewinsky 1974; Smith 2001; Li and Ireland 2008). *Plagiothecium schofieldii* is characterized by symmetrical leaves, and from other members of sect. *Plagiothecium* only *P. platyphyllum* has more or less symmetrical leaves, but this is the only feature common to both species. Symmetrical leaves are typical, e.g., for species from sect. *Orthophyllum* (e.g., *P. nemorale*, *P. cavifolium*) (Lewinsky 1974; Smith 2001; Li and Ireland 2008; Wynns 2015; Wolski 2020). Also, leaves of *P. schofieldii* are clearly longer and wider than those of all the species mentioned above (Smith 2001; Li and Ireland 2008).

Plagiothecium schofieldii is clearly distinguished from *P. denticulatum*, *P. platyphyllum* and *P. ruthei* by the length and width of its laminal cells. The cells located in the central part of the leaf are long and very wide (88–190 × 13–29 μm), which makes the cell areolation very loose. None of the above-mentioned species has such long and broad cells, and thus their cell areolation is tighter (Lewinsky 1974; Smith 2001; Li and Ireland 2008).

Another feature that clearly distinguishes this newly described species from the previous species in sect. *Plagiothecium* is the orientation of the capsules. In the studied specimens of *P. schofieldii*, the capsules are orientated most often more or less vertically, i.e., erect, rarely inclined. *Plagiothecium denticulatum*, *P. platyphyllum*, and *P. ruthei* have inclined capsules (Lewinsky 1974; Smith 2001; Li and Ireland 2008; Wynns 2015).

On the other hand, in terms of morphology, *P. schofieldii* looks more like *P. lamprostachys* (Hampe) A. Jaeger – a Southern Hemisphere species (Ireland 1992; Wynns 2015) – than the common Northern Hemisphere species mentioned above. Both the morphological features and molecular analyses indicate the distinctiveness of the species (Figs. 1–4).

Additionally, Wynns (2015) pointed out that *P. lamprostachys* forms a clade within *P. denticulatum sensu lato*, which is also confirmed by our research (Fig. 1). Phylogenetic analyses of concatenated nuclear and chloroplast markers placed *P. schofieldii*



Figure 3. Stems with the sporophytes of *Plagiothecium schofieldii*. Part of the turf of holotype (*W.B. Schofield 106119*, MO5135779). Scale bar: 1 cm.

within sect. *Plagiothecium* next to *P. denticulatum*; however, the branch support was very low. After branching off from the *Leptophyllum* clade (BI = 0.70), Bayesian inference highly supported the phylogenetic relations within sect. *Plagiothecium* indicating the separateness of a *P. schofieldii* clade (as well as a sect. *Orthophyllum* clade). This separation was also visible in the haplotype network, where the analysis extended the Alaskan clade far from the center of the network grouping species of *Plagiothecium* from the Northern Hemisphere.

All the above morphological data, supported by molecular studies, warrant the recognition of the Aleutian samples as a new species.

Taxonomy

Plagiothecium schofieldii G.J.Wolski & W.R.Buck, sp. nov.

Type. U.S.A. Alaska, Shumagin Islands, Simeonof Island, mainly near saddle between Hill 1436 and 1265, wet cliff chimney, 54°55'N, 159°15'W, 19 July 1996, *W.B. Schofield 106119*, Holotype MO5135779.

Description. Plants small, light green to yellow, with a delicate metallic luster, forming very dense, often homogeneous mats. Stems erect, 1.5–3.0 cm long (Fig. 3), in cross-section rounded, with a diameter of 300–380 μm , the central strand developed,



Figure 4. The most important taxonomic features of *Plagiothecium schofieldii*. Dimensions of cells from the apex **A** the middle **B** and basal part of the leaf **C** leaves **D–E** leaf apex **F** auricles **G**. Scale bar: 100 μm (**A–C**, **F–G**); 500 μm (**D–E**). Photos from the holotype (*W.B. Schofield 106119*, MO5135779).

epidermal cells 10–25 \times 6–12 μm , the parenchyma thin-walled, 20–40 \times 15–32 μm ; leaves julaceous, imbricate, very closely arranged on the stem, when dry not shrunken, very concave, therefore very often damaged under the microscope, symmetrical, ovate to elliptic, those from the middle of the stem 1.4–3.0 mm long, and the width measured at the widest point 0.9–1.9 mm; the apex obtuse and apiculate, often denticulate; costae two, thick and strong, extending usually to $\frac{1}{2}$ of the leaf length, reaching 0.5–2.0 mm; laminal cells linear, rather symmetrical, in quite regular transverse rows, the length and width very variable but dependent on location: 66–178 \times 14–33 μm at apex, 88–190 \times 13–29 μm at midleaf, and 45–221 \times 20–39 μm toward insertion, due to the very wide cells, cell areolation clearly loose; decurrencies of 4–5 rows of rounded, rounded-rectangular, inflated cells, 40–90 \times 22–48 μm , forming distinct, quite long auricles, 300–750 μm long (Fig. 4). Autoicous. Sporophytes abundant; setae dark brown at base, yellowish-orange at apex, twisted when dry, 1.8–2.3 cm long; the capsules more or less erect, 700–950 \times 280–350 μm ; operculum short and rostellate; peristome double, well developed, 450–500 μm long; exostome teeth trabeculate at the ventral side.

Etymology. The present species is named in honor of Wilfred “Wilf” Borden Schofield (1927–2008), who spent decades studying northern regions of North America, including the Aleutian Islands, and who on July 19, 1996, collected the specimen (No. 106119), chosen here as the holotype of *Plagiothecium schofieldii*. According to Stephen Talbot (pers. comm.), Schofield recognized this plant as distinct in the field.

Distribution and ecology. *Plagiothecium schofieldii* so far has only been recorded from Adak Island, Attu Island and Simeonof Island in Alaska. In this area it has been recorded on wetlands and hills, wet cliff chimney, open, moist crevice of a cliff, shaded face of hole on slope, shaded humid outcrop, along creek and adjacent slope, near saddle between hills and near base of mountain.

Additional specimens examined. U.S.A. Alaska: Adak Island, Finger Bay, along creek and adjacent slope, open, moist, crevice of cliff, 15–30 Jun 1975, *D. K. Smith 3864* (NY02589541); Attu Island, near Jaemin Pass, slopes of Ribson Ridge, shaded face of hole on slope, 52°53'N, 173°10' W, 10 Aug 2000, *W. B. Schofield & S. S. Talbot 115646*, UBC ACC# B185126; Shumagin Islands, Simeonof Islands, near base of larger mountain, N. side, 54°55'N, 159°15' W, shaded humid outcrop, 17 Jul 1995, *W. B. Schofield, S. S. Talbot & G. Argus 104056*, ACC# B159650 (MO5140205); wetlands and Hill 624, 54°55'N, 159°15'W, seepy cliff chimney, 7 Jul 1996, *W. B. Schofield 105769*, ACC# B161483 (MO5148015).

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