

Two new species of *Drymonia* (Gesneriaceae) from Northwestern South America, including the discovery of the longest flower known in the genus

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Abstract

Two new species, *Drymonia intermedia* Clavijo & J.L.Clark, **sp. nov.** and *D. longiflora* J.L.Clark & Clavijo, **sp. nov.** (Gesneriaceae, Columneinae), are described from the western Andean slopes of Ecuador and Colombia. The new species are similar to *D. fimbriata*, *D. laciniosa*, *D. macrophylla*, and *D. peponifera* because of the facultative epiphytic habit, deeply serrate to lacinate calyx margins, and fleshy bivalved capsules with tardily dehiscent endocarps. Leaves with brochidodromous venation, narrowly elongate corolla tube, and lacinate calyx margins differentiate *D. intermedia*. The longest corolla of any known *Drymonia* (> 6.5 cm long) differentiates *D. longiflora*. Digital photographs, geographic distributions, and IUCN categories are provided for the new species.

Resumen

Se describen dos especies nuevas de *Drymonia* (Gesneriaceae, Columneinae) de la vertiente occidental de los Andes ecuatorianos y colombianos: *Drymonia intermedia* Clavijo & J.L.Clark, **sp. nov.** y *D. longiflora* J.L.Clark & Clavijo, **sp. nov.** Las nuevas especies son similares a *D. fimbriata*, *D. laciniosa*, *D. macrophylla* y *D. peponifera* por ser epífitas facultativas, con márgenes de los lóbulos del cáliz fuertemente aserradas a laciniadas y los frutos en cápsulas carnosas con endocarpos de dehiscencia tardía. *D. intermedia* se diferencia por las hojas con venación broquidódroma, el tubo de la corola estrechamente elongado y los lóbulos del cáliz laciniados. *D. longiflora* se diferencia por tener la corola más larga hasta ahora reportada para el género (> 6.5 cm de largo). Se presentan láminas fotográficas, distribuciones geográficas y categorías de amenaza de la UICN para las especies nuevas.

Keywords

Andes, Chocó biogeographic region, Colombia, Columneinae, *Drymonia*, Ecuador

Introduction

The flowering plant family Gesneriaceae, with more than 3400 species and 150+ genera (Weber 2004; Weber et al. 2013), is in the order Lamiales. The family is divided into three subfamilies and seven tribes (Weber et al. 2013, 2020), which represent monophyletic lineages (Ogutcen et al. 2021). The majority of New World members are in the subfamily Gesnerioideae and are represented by 1200+ species and 77 genera (Clark et al. 2020). *Drymonia* Mart. is classified in the tribe Gesnerieae and subtribe Columneinae (Weber et al. 2013, 2020), the largest subtribe with 26+ genera and 16% (ca. 525+ spp.) of the total species' diversity in the family (Weber et al. 2013, 2020). *Drymonia* is strongly supported as monophyletic based on molecular sequence data (Clark et al. 2015). Our preliminary DNA sequence data strongly support a clade that includes the two species described here: *Drymonia intermedia* Clavijo & J.L.Clark and *Drymonia longiflora* J.L.Clark & Clavijo. Other members of this clade include *Drymonia laciniosa* Wiehler, *D. macrophylla* (Oerst.) H.E.Moore, and *D. peponifera* J.L.Clark & Clavijo. Digital images are provided to differentiate the two new species from closely related congeners. Table 1 summarizes geographic distributions and diagnostic morphological characters for differentiating *Drymonia fimbriata* C.V.Morton, *D. intermedia*, *D. laciniosa*, *D. longiflora*, *D. macrophylla*, and *D. peponifera*.

Taxonomic treatment***Drymonia intermedia* Clavijo & J.L.Clark, sp. nov.**

urn:lsid:ipni.org:names:77254937-1

Fig. 1

Diagnosis. Differs from all *Drymonia* by leaves with a submarginal collecting vein formed by the secondary venation (i.e., brochidodromous), a narrow elongate tubular corolla, and lanceolate calyx lobes with margins laciniate with unbranched filiform serrations. Similar to *D. longiflora*, but differs by smaller corolla tube and smaller calyx lobes. Similar to *D. laciniosa*, but differs by the nearly isophyllous leaves (vs. anisophyllous leaves in *D. laciniosa*).

Type. ECUADOR. Esmeraldas: cantón San Lorenzo, parroquia Alto Tambo, trail from the community Durango to Río Tululbi via trail north of highway San Lorenzo-Ibarra, forest managed by Fundación Sirua, 1°2'50"N, 78°36'54"W, 200 m, 28 May 2008, J.L. Clark, B. Bisvicutth & J. Melton III 10344 (holotype: SEL [117454]; isotypes: MO, NY, QCNE, US).

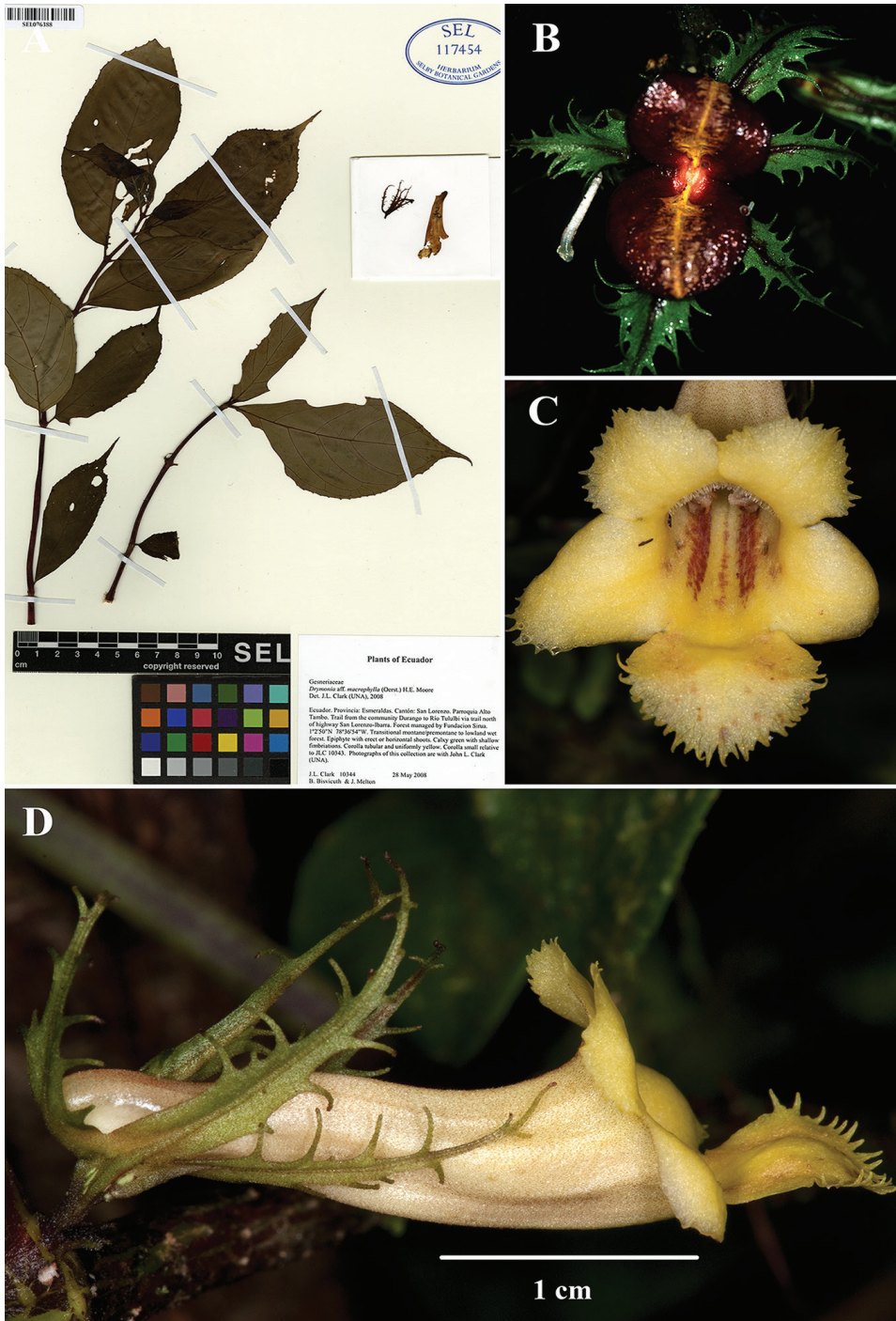


Figure 1. *Drymonia intermedia* Clavijo & J.L.Clark **A** holotype specimen **B** upper view of mature fruit **C** front view of corolla **D** lateral view of flower (**A** holotype (SEL) of J.L. Clark *et al.* 10343 **B** from J.L. Clark *et al.* 7148 **C, D** from J.L. Clark *et al.* 10344). Photos by J.L. Clark.

Description. Terrestrial, hemiepiphytic or epiphytic herb or subshrub, with scandent to horizontal shoots to 1 m long. Stems subquadrangular in cross-section, 0.3–0.5 cm in diameter, strigose to glabrate, internodes 2.5–8 cm long. **Leaves** opposite, decussate, usually evenly spaced and becoming clustered near apex, isophyllous or subequal in a pair; petioles 0.3–0.6 cm long, basal enations not evident, strigillose to strigose, terete in cross-section; blade elliptic to ovate, 6.5–12.2 × 2.6–5.5 cm, membranous to subcoriaceous, the base rounded to acute, sometimes oblique, the apex acuminate, the margin serrate, the upper surface glossy-green, glabrescent or strigose, the lower surface light green, glabrescent or strigillose, lateral secondary veins 5–6 pairs, prominent abaxially, strigose abaxially, forming a submarginal collecting vein (i.e., brochidodromous). **Inflorescence** reduced to a single axillary flower in the upper leaf axils; peduncle absent; bracts not observed. **Flowers** with calyx uniformly light green; lobes 5, fused at base, 4 nearly equal, the upper lobe slightly smaller and more narrow, membranous, lanceolate with an elongate acute apex, the margins lacinate with unbranched filiform teeth, the lobes covered with sparsely pilose trichomes, 1.4–1.6 × 0.2–0.3 cm. Corolla zygomorphic, tubular, elongate, 2.0–3.5 cm long, with slight spur (nectar chamber) at base, to 0.4 cm long; the corolla tube perpendicular relative to calyx, ampliate toward the limb, not contracted above, ca. 2.0 cm long, ca. 0.8 cm wide near middle, uniformly pale yellow outside, usually lighter yellow in the throat and limb, sometimes with brown spotting on lower portion of throat, the inside sparsely pilose or glandular, the outside uniformly sericeous; throat to 0.8 cm in diameter; limb with 5 spreading lobes, subequal, globose, rounded at apex, margins fimbriate, uniformly strigose abaxially and adaxially, lobes 3–5 × 4–5 mm, the lower lobe slightly larger. Androeceum of 4 didynamous stamens, included; filaments 1.2–2.0 cm long, adnate to the base of the corolla tube for 2–3 mm, glabrous, coiled after anthesis; the anthers at first coherent, after anthesis separating, dehiscent by basal pores, 2–4 × 1.2–1.6 mm. Gynoeceum with a single dorsal nectary gland, thick, ovate, 2–3 mm long, glabrous; the ovary superior, sericeous, green; style included, 2–3 cm long, white, puberulent; stigma stomatomorphic. **Fruit** a fleshy bivalved capsule, the valves green abaxially, dark maroon adaxially, at dehiscence reflexed 180°, revealing a central cone of fleshy, dark red funicular tissue covered by a thin, purple endocarp that remains attached and surrounds the placentae and mass of funiculi and seeds, and then dehisces at a later stage. **Seeds** numerous, initially covered by the endocarp, but immersed in the central cone of funicular tissue, each seed 0.4–0.5 × 0.2–0.3 mm, brown, subglobose, pointed at both ends, striate.

Phenology. This species has been collected with flowers in May and June. Specimens with fruits are only known from May.

Etymology. The specific epithet is in reference to the intermediate shapes and sizes of the corolla and calyx between *D. macrophylla*, *D. laciniosa*, and *D. longiflora*.

Distribution and preliminary conservation assessment. *Drymonia intermedia* is endemic to the western Andean slopes between 150 and 600 m in the Ecuadorian province of Esmeraldas. This species has not yet been found in any formally protected area in Ecuador. According to the IUCN Red List criteria (IUCN 2022)

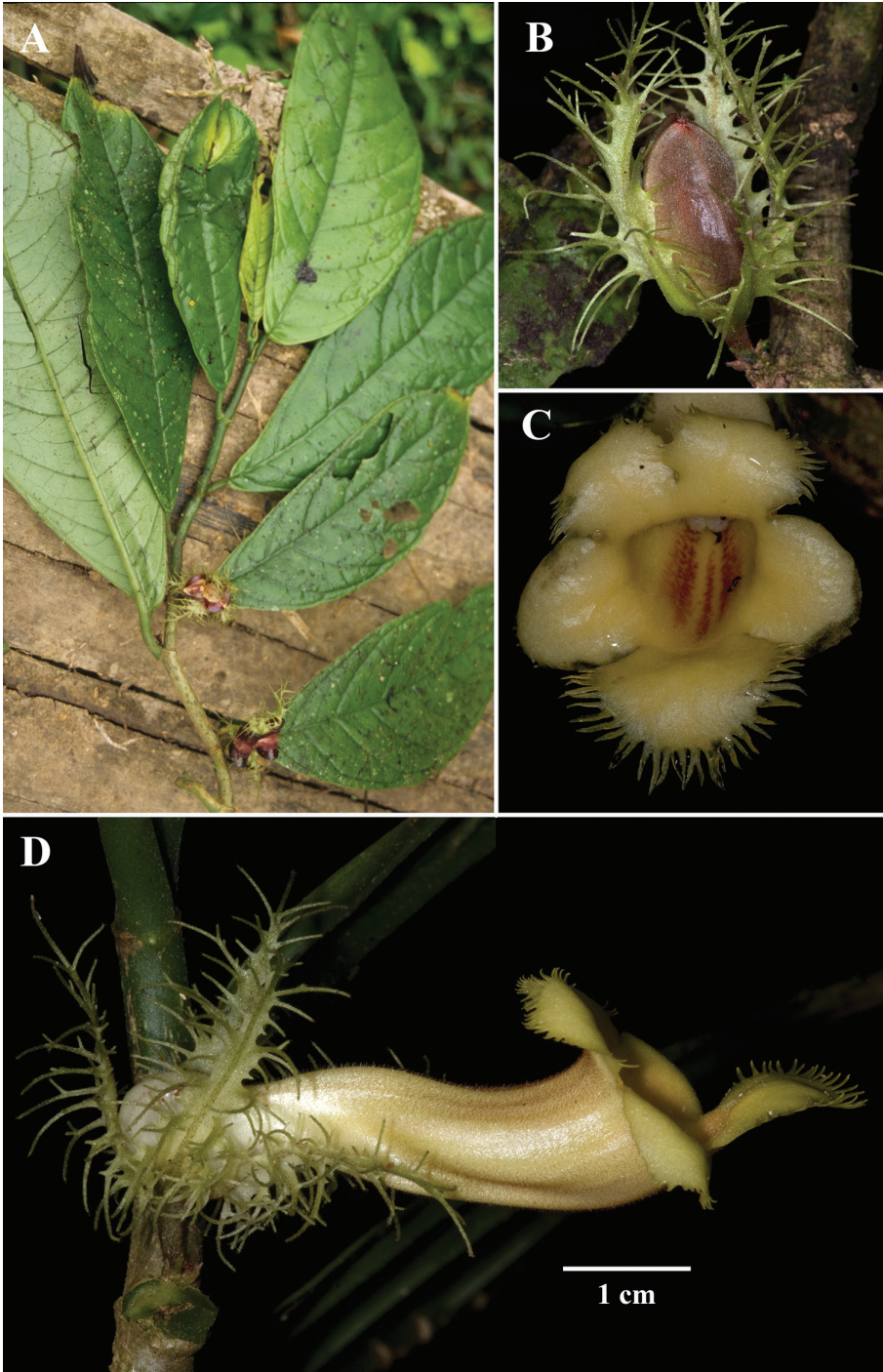


Figure 2. *Drymonia laciniosa* Wiehler **A** dorsiventral habit **B** immature cone-shaped fruit **C** front view of flower **D** lateral view of flower (**A** from J.L. Clark 1630 **B** from J.L. Clark et al. 9629 **C** from J.L. Clark et al. 10117 **D** from J.L. Clark et al. 12123). Photos by J.L. Clark.

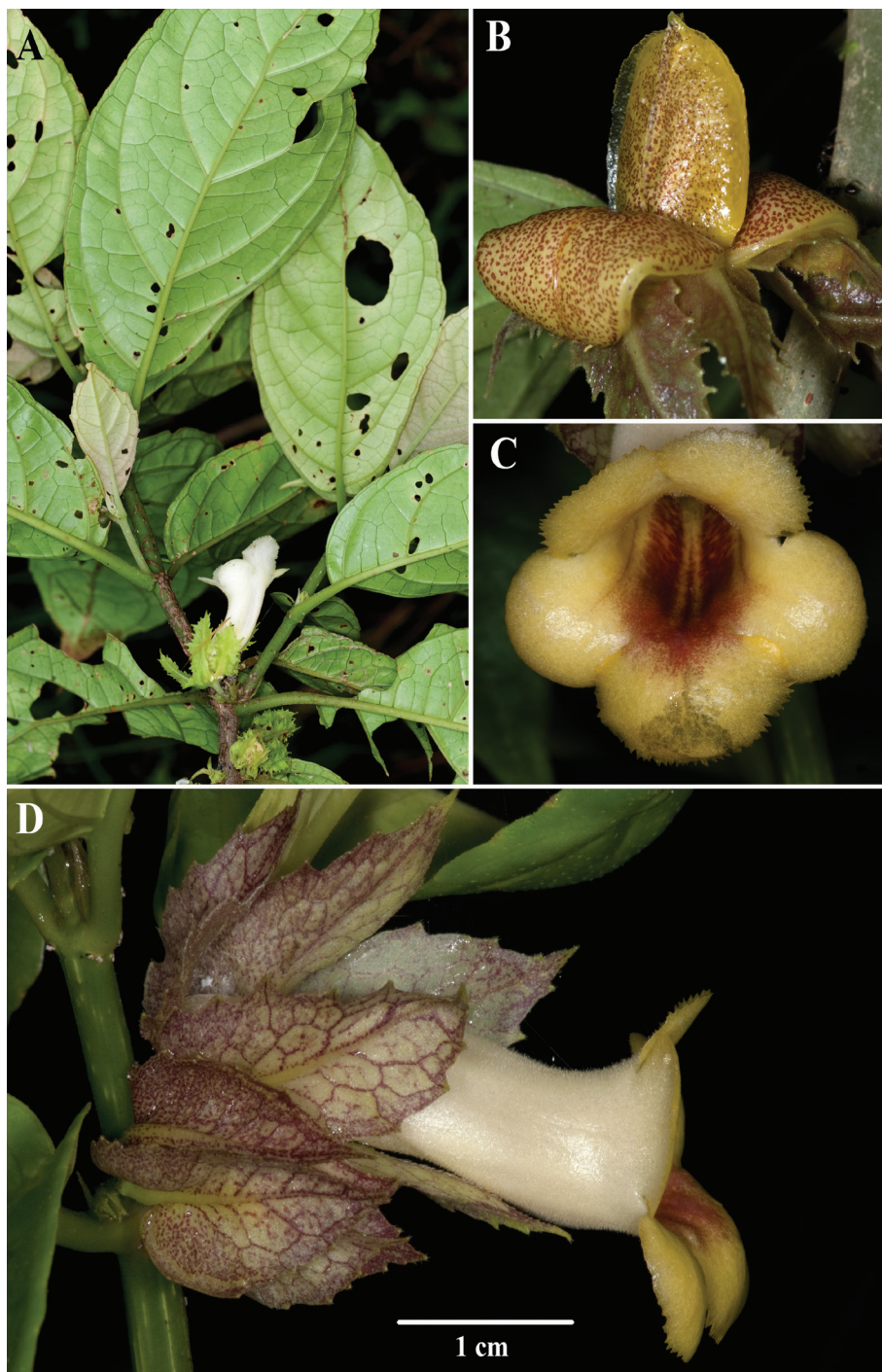


Figure 3. *Drymonia macrophylla* (Oerst.) H.E. Moore **A** erect subwoody habit **B** mature fleshy bivalved capsule **C** front view of corolla **D** lateral view of flower (**A** from *P. Pedraza et al.* 850 **B** from *J.L. Clark et al.* 12119 **C, D** from *J.L. Clark et al.* 10044). Photo **A** by P. Pedraza and photos **B–D** by J.L. Clark.

for limited geographic range (EOO <5,000 km² and AOO <500 km²) and associated subcriteria, including occurrence at fewer than five locations (B2a) and continuing decline of Andean forests (B2b), *Drymonia intermedia* should be listed in the category Endangered (EN).

Comments. The characters that differentiate *D. intermedia* are intermediate between *D. longiflora*, *D. laciniosa*, and *D. macrophylla*. Vegetatively, *D. intermedia* is distinguished by a submarginal collecting vein formed from the secondary veins that curve upwards towards the leaf margin (brochidodromous venation) (Fig. 1A). *Drymonia laciniosa* also has brochidodromous venation, but differs vegetatively by leaves that are anisophyllous (vs. leaves that are nearly isophyllous in *D. intermedia*) (Fig. 2A). Other closely related species, like *D. macrophylla*, have eucamptodromous venation that are defined by secondary veins that curve upwards, but do not form a collecting vein (Fig. 3A). The laciniolate calyx lobes of *Drymonia intermedia* are similar to *D. laciniosa*, but have unbranched filiform serrations (Fig. 1D). In contrast, the calyx lobes of *D. laciniosa* are strongly laciniolate with branched filiform serrations (Fig. 2B, D). The calyx lobes shape and margin of *D. intermedia* are similar to *D. longiflora*, but are less than 2 cm long (Fig. 1D). In contrast, the calyx lobes of *D. longiflora* are larger and usually exceed 2.5 cm (Fig. 4D). The corolla in *D. intermedia* is less than 3.5 cm long (Fig. 1D). In contrast, the corolla of *D. longiflora* is greater than 6.5 cm long (Fig. 4D). A summary of diagnostic characters is provided in Table 1.

Table 1. General geographic distribution (names in parentheses indicate provinces for Ecuador or departments for Colombia) and comparison of morphological characters to differentiate *Drymonia fimbriata*, *D. intermedia*, *D. laciniosa*, *D. longiflora*, *D. macrophylla*, and *D. peponifera*.

	<i>Drymonia fimbriata</i> C.V.Morton	<i>Drymonia intermedia</i> Clavijo & J.L.Clark	<i>Drymonia laciniosa</i> Wiehler	<i>Drymonia longiflora</i> J.L.Clark & Clavijo	<i>Drymonia macrophylla</i> (Oerst.) H.E.Moore	<i>Drymonia peponifera</i> J.L. Clark & L.Clavijo
Leaf pairs	isophyllous	isophyllous or subequal	anisophyllous	isophyllous or subequal	isophyllous or subequal	isophyllous or subequal
Petiole length	1.5–7.7 cm	0.3–0.6 cm	1.0–2.0 cm	0.7–3.0 cm	0.47–5.24 cm	0.5–3.5 cm
Leaf venation	eucamptodromous	brochidodromous	brochidodromous	eucamptodromous	eucamptodromous	eucamptodromous
Calyx lobe margins	strongly laciniolate with unbranched filiform teeth	laciniolate with unbranched filiform teeth	strongly laciniolate with branched filiform teeth	deeply serrate to slightly laciniolate	serrate to laciniolate	deeply serrate to pinnatifid
Calyx lobe folding	flat	flat	crispate	flat	flat	crispate
Corolla length	2.8–3.5 cm	2.0–3.5 cm	3.0–4.6 cm	6.5–8.0 cm	2–3.5 cm	2–4.5 cm
Geographic distribution	Costa Rica & Panama	western Andean slopes in northern Ecuador (Carchi & Esmeraldas)	western Andean slopes in northern Ecuador (Carchi & Esmeraldas)	western Andean slopes in northern Ecuador (Carchi & Esmeraldas) and Colombia (Choco, Valle de Cauca & Nariño); eastern Andean slopes in southern Ecuador (Morona-Santiago)	widespread in Central and South America	eastern Andean slopes of southern Ecuador (Morona-Santiago)

Specimens examined. Ecuador. Esmeraldas: cantón San Lorenzo, parroquia Alto Tambo, border region of Awá Indigenous Territory, entrance to the Río Bogotá community (future biological research station), near Quebrada Pambilar, 0°58'57"N, 78°35'50"W, 350–600 m, 12 Feb 2003, *J.L. Clark, G. Zapata & G. Toasa 7148* (MO, QCNE, SEL, US); Esmeraldas: cantón San Lorenzo, parroquia Alto Tambo, trail from the community Durango to Río Tululbi via trail north of highway San Lorenzo-Ibarra, forest managed by Fundación Sirua, 1°2'50"N, 78°36'54"W, 150 m, 2 Jun 2009, *J.L. Clark & The 2009 Gesneriad Research Expedition Participants 11060* (QCNE, SEL, US).

***Drymonia longiflora* J.L.Clark & Clavijo, sp. nov.**

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Fig. 4

Diagnosis. Differs from all *Drymonia* by an elongate corolla that exceeds 6.5 cm in length, the longest corolla known in the genus. The subshrub habit with elongate shoots and corolla shape are similar to *Drymonia macrophylla* and *D. peponifera*. *Drymonia macrophylla* has a corolla that rarely exceeds 3.5 cm in length and *D. peponifera* has a corolla that rarely exceeds 4.5 cm in length.

Type. ECUADOR. Esmeraldas: cantón San Lorenzo, parroquia Alto Tambo, remnant patch of primary forest on north side of road between Durango and Alto Tambo on highway San Lorenzo-Ibarra, 0°57'19"N, 78°33'30"W, 695 m, 29 May 2008, *J.L. Clark, B. Bisvicut, S. Ginzburg & J. Melton III 10442* (holotype: US; isotypes: COL, E, G, MO, NY, QCNE, SEL).

Description. Terrestrial, hemiepiphytic or epiphytic herb or subshrub, with scandent to horizontal shoots to 1 m long. Stems subquadrangular in cross-section, 0.3–0.6 cm in diameter, strigose to glabrate, internodes 1.0–8 cm long. **Leaves** opposite, decussate, usually evenly spaced and becoming clustered near apex, subequal to unequal in a pair; petioles 0.7–3.0 cm long, basal enations present, strigillose to strigose, terete in cross-section; blade elliptic to ovate, 8.0–28 × 3.5–13 cm, membranous to subcoriaceous, the base rounded to acute, sometimes oblique, the apex acute to long-acuminate, the margin serrulate, the upper surface glossy-green, glabrescent or strigose, the lower surface light green with red venation, glabrescent or strigillose, lateral secondary veins 5–7 pairs, prominent abaxially, strigose abaxially. **Inflorescence** reduced to a single flower in the upper or lower leaf axils; peduncle absent; bracts small, lanceolate, 3.0–7.0 × 1.1–1.3 mm, green, lanceolate, strigose; pedicels short, 3–7 mm long, green, strigose. **Flowers** with calyx green, dark red with green margins, or uniformly dark red, with enations at base; lobes 5, nearly free, 4 equal, the upper lobe slightly smaller and more narrow, membranous, lanceolate with an elongate acute apex, the margins deeply serrate to slightly lacinate, lobes covered with sparsely pilose trichomes, 1.5–2.7 × 0.3–0.5 cm. Corolla zygomorphic, tubular, elongate, 6.5–8.0 cm long, with slight spur (nectar chamber) at base, 0.5–0.7 cm long; the corolla tube perpendicular relative to calyx,

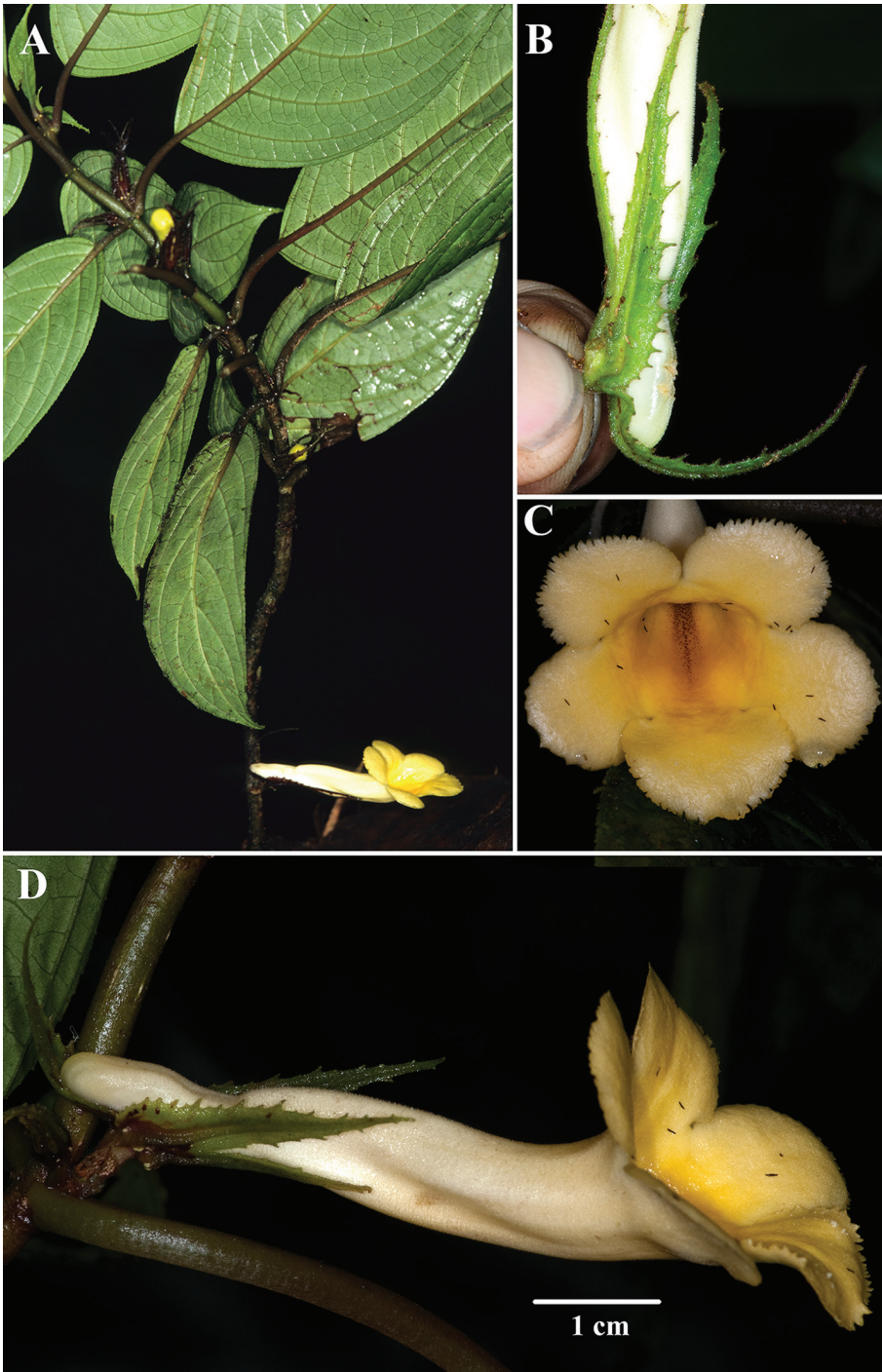


Figure 4. *Drymonia longiflora* J.L.Clark & Clavijo **A** erect subwoody habit **B** mature calyx lobes **C** front view of corolla **D** lateral view of flower (**A** from J.L. Clark et al. 7196 **B** from J.L. Clark et al. 13417 **C, D** from J.L. Clark et al. 10343). Photos by J.L. Clark.

ampliate toward the limb, not contracted above, 5.0–6.5 cm long, 0.5–1.1 cm wide at the mid portion, uniformly pale yellow and sericeous outside, usually darker yellow inside; throat to 1.4 cm in diameter, the inside yellow, sometimes with brown spotting on lower portion, sparsely pilose or glandular; limb with 5 spreading lobes, subequal, globose, rounded at apex, margins erose, glabrous abaxially and adaxially, lobes 5–10 × 4–11 mm, the lower lobe slightly larger. Androecium of 4 didynamous stamens, included; filaments 5–6 cm long, adnate to the base of the corolla tube for 2–3 mm, glabrous, coiled after anthesis; the anthers at first coherent, after anthesis separating, dehiscent by basal pores, 3–5 × 2–1.8 mm. Gynoecium with a single dorsal nectary gland, thick, ovate, 2–3 mm long, glabrous; the ovary superior, sericeous, green; style included, 5–6 cm long, white, puberulent; stigma stomatomorphic. **Fruit** and seeds not observed.

Phenology. This species has been found with flowers in two periods: February to May and August to October.

Etymology. The specific epithet is in reference to the elongate corolla tube, the longest of any known species of *Drymonia*.

Distribution and preliminary conservation assessment. *Drymonia longiflora* is locally abundant in forests along the western slopes of the northern Andes in Ecuador (Provinces Carchi and Esmeraldas) and Colombia (Departments Chocó, Valle del Cauca, and Nariño). One disjunct population is documented from southern Ecuador (Province Morona-Santiago). *Drymonia longiflora* grows in mature shaded forests, from 150 to 900 m in elevation. We provisionally assess this new species as Vulnerable (VU), according to the IUCN Red List criteria (IUCN 2022) for limited geographic range (EEO < 20,000 km² and AOO < 2,000 km²) and associated subcriteria, including occurrence at fewer than ten locations (B2a) and continuing decline of Andean forests (B2b).

Comments. *Drymonia longiflora* is distinguished by the longest known corolla recorded for *Drymonia* (> 6.5 cm). The unbranched subwoody epiphytic or terrestrial subshrub habit and the foliage are similar to *Drymonia macrophylla*, *D. fimbriata*, and *D. peponifera*. All four species are vegetatively similar, but readily differentiated by corolla tube length and calyx lobes (Fig. 5C, D). The calyx lobes of *Drymonia peponifera* appear crispate from the reflexed and undulating lobes (Fig. 5C, D). In contrast, the calyx lobes of *D. macrophylla* (Fig. 3D) and *D. longiflora* (Fig. 4D) are flat. The margins of the calyx lobes in *Drymonia longiflora* are serrate to slightly laciniate, in contrast to the strongly laciniate with unbranched filiform teeth of *D. fimbriata*. The corolla in *Drymonia longiflora* exceeds 6.5 cm in length (Fig. 4D). In contrast, the corolla rarely exceeds 3.5 cm in *D. macrophylla* (Fig. 3D) and *D. fimbriata*. The corolla is less than 4.5 cm in *D. peponifera* (Fig. 5D). *Drymonia longiflora* and *D. intermedia* share similar shapes for corolla tubes and calyx lobes, but differ in size, with *D. intermedia* much smaller. A summary of diagnostic characters is provided in Table 1.

Our preliminary DNA sequence data strongly support a close relationship in a clade that includes *D. intermedia*, *D. laciniosa*, *D. longiflora*, *D. macrophylla*, and *D. peponifera*. This clade is defined by fleshy capsular fruits with tardily dehiscent endocarps. A photographic guide and description of fruit types is summarized in Clark and Clavijo (2022). Most *Drymonia* capsules have endocarps that dehisce when

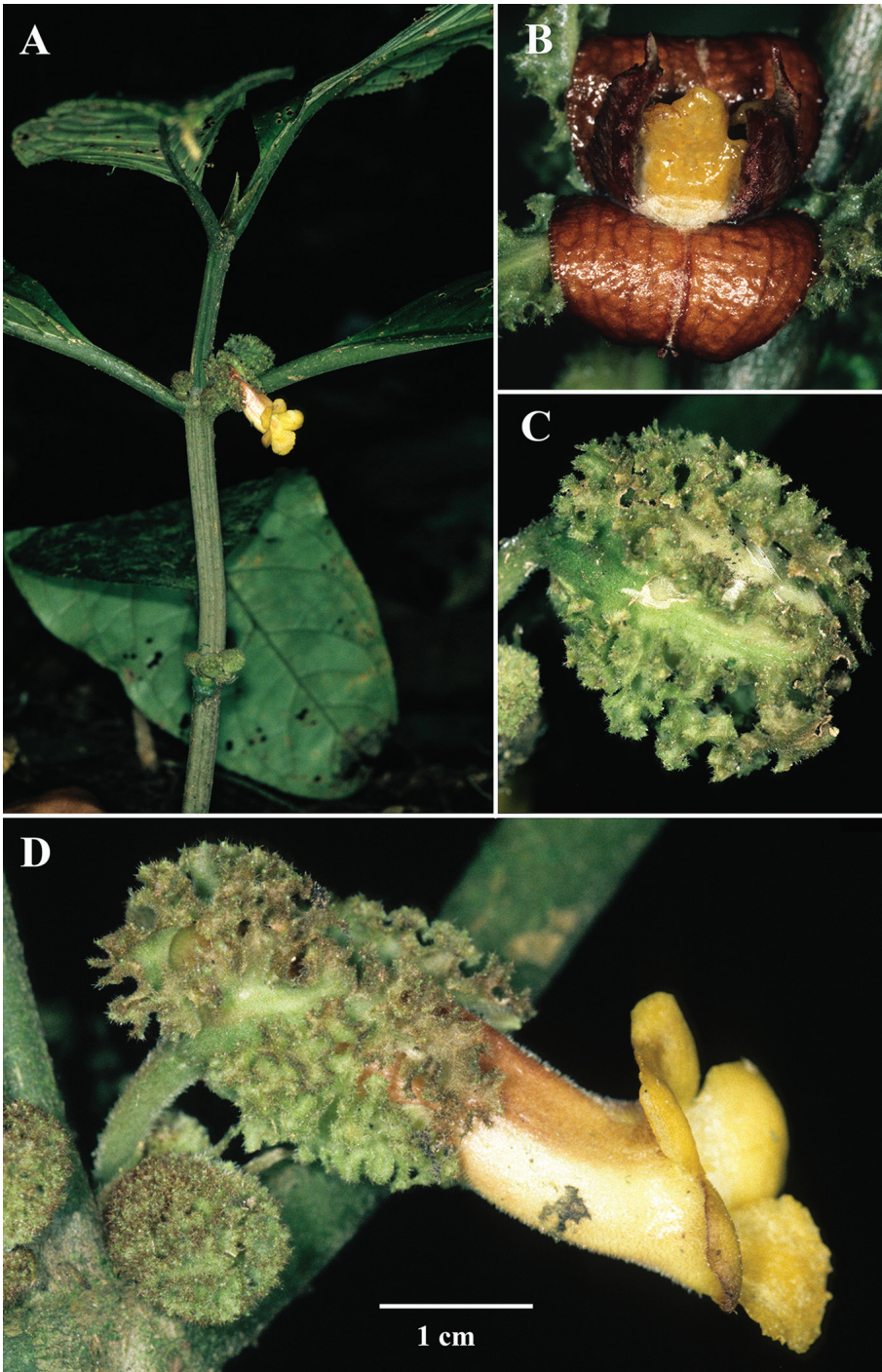


Figure 5. *Drymonia peponifera* J.L.Clark & Clavijo **A** habit **B** upper view of mature fruit **C** lateral view of immature calyx lobes **D** lateral view of axillary inflorescence with a single mature flower (**A–D** from J.L. Clark & L. Jost 6957). Photos by J.L. Clark.

mature. In contrast, the clade that includes *D. intermedia* and *D. longiflora* is defined by endocarps that remain attached and surround the placenta and mass of funiculi and seeds (Figs 1B, 5B). The endocarp eventually becomes dehiscent at a later stage when it detaches from the reflexed outer layers of the fruit wall (Fig. 5B). Although we have not observed mature fruits of *Drymonia longiflora*, we predict that the fruit type of this species shares the same characters with closely related congeners.

Specimens examined. Colombia. Chocó: 2 km south of Las Animas on road to Istmina, 150 m, 13 Aug 1976, *A. Gentry & M. Fallen 17620* (COL); **Valle del Cauca:** municipio Buenaventura, carretera vieja a Buenaventura, 900–1500 m Apr 1998, *M. Amaya-M. & J.F. Smith 594* (COL, US); municipio Buenaventura, Los Tubos, Pericos, vertiente occidental de cordillera occidental, km 43 Cali-Buenaventura, 3°51'00"N, 76°47'19"W, 521 m, 30 Aug 2011, *J. Home 228* (CUVC); Bajo Calima, concesión Pulpapel/Buenaventura, carretera a San Isidro, 3°55'N, 77°W, 100 m, 30 Sep 1987, *M. Monsalve B. 1865* (CUVC, MO); road Cali-Buenaventura, further down the road towards Buenaventura, about 4 km, 1 May 1972, *H. Wiehler, R.L. Dressler, N.H. Williams & N.F. Williams 72106* (SEL); municipio Dagua, Vereda Yatacue, sitio La Riqueza, camino desde el tunel a las torres, 3°35'40.6"N, 76°53'31.8"W, 630–780 m, 20 Jan 2019, *L. Clavijo, M. Perret, K. Arango, A. Zuluaga & W. Villar 2231* (COL, CUVC, G). **Nariño:** municipio Barbacoas, corregimiento El Diviso, vereda El Verde, western slopes of the Cordillera Occidental, remnant forest along highway between Altaquer and El Diviso, 1°21'46"N, 78°10'32"W, 795 m, 13 May 2013, *J.L. Clark, L. Clavijo, O. Marín & M. Flores 13417* (COL, CUVC, SEL, US); municipio de Ricaurte, Resguardo Indígena Nulpe Medio, camino a la quebrada La Conga, 1°6'N, 78°13'W, 750 m, 8 Jan 1996, *M.S. González & B.R. Ramírez-P. 1606* (PSO); municipio de Ricaurte, Resguardo Indígena Nulpe Medio Andalucía, quebrada La Babosa, 1°5'N, 78°14'W, 780–815 m, 12 Jan 1996, *M.S. González & B.R. Ramírez-P. 1678* (PSO). **Ecuador. Carchi:** border area between Carchi and Esmeraldas, about 30 km past Lita on road Lita-Alto Tambo, 450 m, 28 Jun 1991, *H. van der Werff, B. Gray & G. Tipas 12113* (MO, QCNE, SEL, US). **Esmeraldas:** cantón San Lorenzo, parroquia Alta Tambo, Awá Indigenous Territory, Río Bogotá community (future biological research station), 2 km S of Lita-San Lorenzo road, near Quebrada Pambilar, 0°58'57"N, 78°35'60"W, 350–600 m, 12 Feb 2003, *J.L. Clark, G. Zapata & G. Toasa 7129* (MO, QCNE, SEL, UNA); cantón San Lorenzo, parroquia Alta Tambo, border region of Awá Indigenous Territory, entrance to the Río Bogotá community (future biological research station), near Quebrada Pambilar, 0°58'57"N, 78°35'50"W, 350–600 m, 13 Feb 2003, *J.L. Clark 7197* (F, MO, QCNE, SEL, UNA, US); parroquia Alto Tambo, trail from the community Durango to Río Tululbi via trail north of San Lorenzo-Ibarra highway, forest managed by Fundación Sirua, 1°2'50"N, 78°36'54"W, 150–200 m, 28 May 2008, *J.L. Clark, B. Bisvicuth & J. Melton III 10343* (MO, NY, QCNE, SEL, US); cantón San Lorenzo, remnant patch of forest along the Ibarra-San Lorenzo highway, between Durango and Alto Tambo, 1°0'33"N, 78°35'58"W, 516 m, 3 Jun 2009, *J.L. Clark J.L. Clark & The 2009 Gesneriad Research Expedition Participants 11113* (MO, NY, QCNE, SEL, US); cantón San Lorenzo, remnant patch of forest along highway Ibarra

- San Lorenzo, between Durango and Alto Tambo, 1°0'33"N, 78°35'58"W, 516 m, *J.L. Clark & The 2009 Gesneriad Research Expedition Participants 11127* (QCNE, SEL, US); cantón Eloy Alfaro, Reserva Ecológica Cotachi-Cayapas, parroquia Luis Vargas Torres, Río Santiago, 0°49'S, 78°45'W, 250 m, 8 Dec 1993, *M. Tirado, P. Asimbaya, M.I. Corosa & V. Arroyo 776* (MO, QCNE, US); cantón San Lorenzo, San Francisco, recinto Durango, sector Colinado, terrenos propiedad Sr. Demetrio Paez, 1 km al este de la carretera Lita-San Lorenzo, 1°2'N, 78°36'W, 256 m, 17 Oct 1999, *J.C. Valenzuela & E. Freire 474* (QCNE, MO, US). **Morona-Santiago:** Región de la Cordillera del Cóndor y Cordillera de Cutucú, Cordillera de Shaime, al norte del Río Santiago, Centro Shuar Jempekat, 2°57'S, 77°50'W, 600 m, 15 Oct 2003, *G. Toasa 9343* (MO, QCNE).

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Maxillaria anacatalinaportillae (Orchidaceae, Maxillariinae), a new remarkable species from Ecuador

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Abstract

Neotropical genus *Maxillaria* Ruiz & Pav. belongs to one of the most diverse and species-rich groups of orchids. Several of its representatives are popular, horticultural plants with large and showy flowers, often nicely fragranced. It is not uncommon that some distinctly colored individuals are introduced to the commercial market under names of similar, more or less related species, as informal varieties or color forms, largely causing confusion. While investigating the diversity of *Maxillaria* in Ecuador, we have encountered plants that were commercially referred to as *M. sanderiana xanthina*. In the course of conducted morphological and micromorphological analyses, we concluded that it is a new, separate species and hereby, we describe it as *M. anacatalinaportillae*.

Keywords

Ecuador, Neotropics, orchids, phylogeny, pseudopollen, SEM

Introduction

Maxillaria Ruiz & Pav. is one of the most interesting species groups in the orchid family. For many years it has been, and in some way still is quite a controversial genus. The lack of clearly defined boundaries of *Maxillaria sensu stricto* resulted in proposing several taxonomic approaches of the subtribe Maxillariinae Benth. over the past few decades. For a long time, it has been suspected that it is an assemblage of taxa, consisting of morphologically disparate groups of species (Whitten et al. 2007). Establishing the exact number of species belonging to the various genera or the subtribe itself is not easy since it depends mainly on the adopted classification system and genus concept. *Maxillaria* covers about 4/5 of species belonging to the subtribe (Senghas 2002). Depending on the applied classification, it counts from approximately 420 (Dressler 1993), through 634 (Schuiteman and Chase 2015) to 750 species (Senghas 2002).

One of the most spectacular groups of species within the genus is often referred to as ‘*Maxillaria grandiflora*-complex’ or also alliance/group (Dodson 1997), it includes species such as *M. platypetala* Ruiz & Pav., *M. molitor* Rchb. f., or *M. sanderiana* Rchb. f. ex Sander. Their common feature is the size of flowers – generally large and showy, sometimes also brightly colored. The main reason for this reference is the supposed similarity to *Maxillaria grandiflora* (Kunth) Lindl. (Christenson 2013). Dodson (1997) characterized this group as caespitose plants with unifoliate pseudobulbs and foliaceous leaf sheaths, petiolate leaf, large flowers (5–12 cm in diameter), and broad and blunt sepals and petals. Christenson (2013) pointed out, however, that the type specimen of *M. grandiflora* itself is bifoliate and several other species included by Dodson, do not fulfill the morphological criteria presented above (e.g. *M. napoensis* Dodson, *M. batemanii* Poepp. & Endl). Indeed, for many years there have been some ambiguities about the type of *M. grandiflora* (Fig. 1) and its locality, however, they have been clarified by Blanco and Stauffer in 2011. Detailed analysis of the collection time, numbers and travel route conducted by them, have led to a conclusion that the type locality of *Maxillaria grandiflora* must be somewhere in the eastern part of La Cruz municipality in the department of Nariño, Colombia at the altitude of 2,067 m. As mentioned above, some questions were raised by Christenson (2002a, b, 2013) about the number of leaves on the pseudobulb of the type specimen. It is essential to clarify that the type collection of *Maxillaria grandiflora* consists of three sheets deposited in the Bonpland herbarium in the Muséum National d’Histoire Naturelle (P) in Paris. In their paper, Blanco and Stauffer (2011) state clearly that in this case we are dealing with a mixed collection. During our visits in P herbarium, we have examined this specimen and we agree with Blanco and Stauffer that it consist of plant parts that belong to at least two different species. As the number of apical leaves per pseudobulb is an important taxonomic feature, the existence of a pseudobulb with two apical leaves as part of the type collection of *M. grandiflora* might have been a source of confusion resulting in Christenson’s belief that this taxon is not a member of the *M. platypetala* Ruiz and Pav. alliance (*sensu* Whitten et al. 2007).

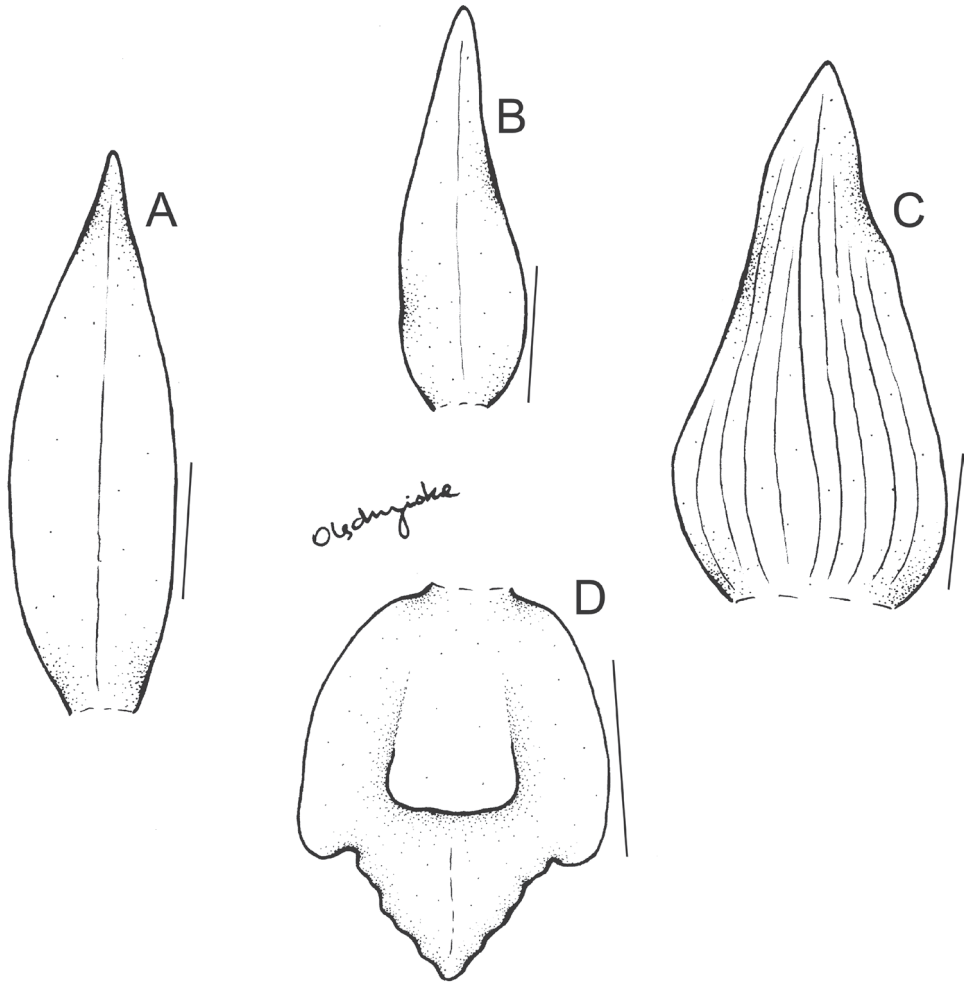


Figure 1. Drawing of the flower parts of *Maxillaria grandiflora* **A** dorsal sepal **B** petal **C** lateral sepal **D** lip. Scale bars: 10 mm. Redrawn from the type by N. Olędrzyńska.

Christenson (2013) pointed out three useful clues for the proper taxonomic identification within *Maxillaria grandiflora*-complex: 1) the orientation of tepals is crucial for understanding the species circumscription (segments reflexed, inflexed, incurved, among others), 2) flower color and lip markings, 3) the shape of the lip apex (transversely terminated, usually undulate, with lobes similar to the ruffles of a petticoat or rigidly held and V-shaped in cross-section). Most of the large-flowered Andean *Maxillaria* species are at times informally referred to as *Maxillaria grandiflora*-complex. This often causes confusion as many of them have little or nothing in common with actual *M. grandiflora* (Kunth) Lindl. The general understanding of these species remains scarce and requires intensified taxonomic investigation. Especially three

sister species can be considered as challenging, and these are *M. sanderiana* Rchb.f. ex Sander, *M. grandis* Rchb.f. (sometimes believed to be a synonym of *M. sanderiana*) and *M. wojii* Christenson (often confused with both) (Christenson 2013). However, *M. sanderiana* differs from *M. grandis* Rchb.f. by narrow and strongly undulated petals and from *M. wojii* by significantly larger flowers. *Maxillaria sanderiana* and *M. grandis* share the distribution range and occur in Ecuador and Peru. According to Christenson (2013) some may consider them to be two ends of a spectrum of variation over a broad geographic range and in this concept, the name *M. grandis* takes priority and is the correct name for the broadly defined species. The variability within *M. sanderiana* may also suggest that, in fact, it is a complex of species consisting of one or more undescribed taxa. Another theory has been mentioned by Blanco as a comment in Christenson's monograph (2013). He suggested that *M. wojii* is simply a color variation of *M. grandis* or that both are hybrids of *M. sanderiana* with some other species in the *Maxillaria grandiflora*-complex. This would expend the occurrence range of *M. grandis* as *M. wojii* is known only from Colombia.

During the past few years, we have been working intensely on the classification and especially species delimitations within *Maxillaria sensu stricto*. We have investigated herbarium materials deposited in most of the world's collections, such as AMES, B, C, COL, MA, MO, P, W, W-R to name just a few. During our expeditions to South America, our attention was drawn to the *Maxillaria grandiflora*-complex and many taxonomical ambiguities it brings. We have collected samples from both commercial and hobby growers but also purchased several plants to cultivate them in the greenhouses of the University of Gdańsk. One of them was identified as *M. sanderiana* and was referred to by Ecuagenera as "*xanthina*". Indeed, according to Christenson (2013), there are two color forms of *M. sanderiana*: *atropurpurea* (H. Williams) Christenson and *xanthina* Christenson. However, after morphological analysis of the flower, it became clear that we are facing new species and hereby we describe it as a new one. The new entity can be easily distinguished by having flat margins of petals, which are gently angled, callus extending beyond the middle of the lip and form of lip middle lobe, which is broadly cordate or triangular when spread, with fold down margins. It is known from several plants that are available commercially, however, they all originate from single (type) population.

Materials and methods

Morphological analysis

Flowering plants of the new species were collected on November 11th, 2003 in the Carchi province (northern Ecuador). The species was photographed *in situ* and taken to cultivation in the greenhouses of Ecuagenera Cia. Ltda with initial identification as *M. sanderiana* '*xanthina*'. In 2020, Ecuagenera provided plant material consisting of five plants which have been sent to Poland with corresponding CITES certificates.

Plants have been cultivated in the greenhouses of the University of Gdańsk (voucher 0148255) and used for the presented analysis. Herbarium specimen were prepared to be used as type material and deposited at UGDA. Particular parts of the flower were dissected, measured, and drawn under stereomicroscope. The line illustration of the new species was prepared from material preserved in Kew Mixture (53% ethanol: 5% formaldehyde: 5% glycerol: 37% water) and digital photos. The new entity has been compared with more than 800 herbarium specimens of other members of *Maxillaria grandiflora*-complex from the following herbaria: AMES, B, C, COL, MA, MO, NY, P, W, W-R, VALLE, QCE, and QCNE. We conducted a careful comparison of the new species with the protologues and type material of all species belonging to the complex, as well as regional floras and checklists such as Dodson and Marmol (1980), Dodson (2002), and Jørgensen and León-Yáñez (1999). The conservation status of the new species was evaluated, based on the guidelines of the International Union for Conservation of Nature (IUCN 2019).

Phylogenetic analysis

Plant material for molecular analysis has been obtained from plants provided by Ecuagenera and living orchid collection of the University of Gdańsk. Remaining sequences were obtained from NCBI database. The GenBank accession numbers of the used sequences in the study are given in the Appendix 1: Table A1.

Total genomic DNA of three species (*M. anacatalinaportillae*, *M. huebschii*, and *M. melina*) was extracted from ca. 20–25 mg of silica-dried specimens (parts of the leaves), using Sherlock AX Kit (A&A Biotechnology, Poland) and following the original protocol. Two molecular markers were used for phylogenetic reconstruction: nrITS (ITS1-5.8S-ITS2) and plastid *matK*. ITS was amplified using primers 101F and 102R (Douzery et al. 1999), while *matK* using primers 19F (Molvary et al. 2000) and 1326R (Cuénoud et al. 2002).

Polymerase chain reactions (PCR) were carried out in a total volume of 25 µl and containing 12.5 µl of StartWarm HS-PCR Mix (A&A Biotechnology, Poland), 1.0 µl of each primer (10 µM) and 1 µl of DMSO (dimethyl sulfoxide) – only for ITS. The following parameters were implemented: 94 °C – 4 min; (94 °C – 45 s; 52 °C – 45 s; 72 °C – 1 min) × 30; 72 °C – 7 min for ITS and 95 °C – 3 min; (94 °C – 45 s; 52 °C – 45 s; 72 °C – 2 min 30 s) × 33; 72 °C – 7 min for *matK* amplification. PCR products were purified using Wizard SvGel and a PCR Clean Up System (Promega, United States). The sequencing reactions were carried in an external company – Macrogen Europe B.V.

Obtained chromatograms were analyzed and edited using Finch TV (Geospiza). Two separate matrixes (ITS and *matK*) were prepared and then aligned with Mafft software (<https://mafft.cbrc.jp/alignment/server/>). Minor mistakes were additionally corrected in SeaView v.4. (Gouy et al. 2010).

Molecular substitution model was based on AIC (Akaike information criterion) and calculated with PhyML website (<http://www.atgc-montpellier.fr>). The GTR+G+I model was selected as the best one for studied matrix.

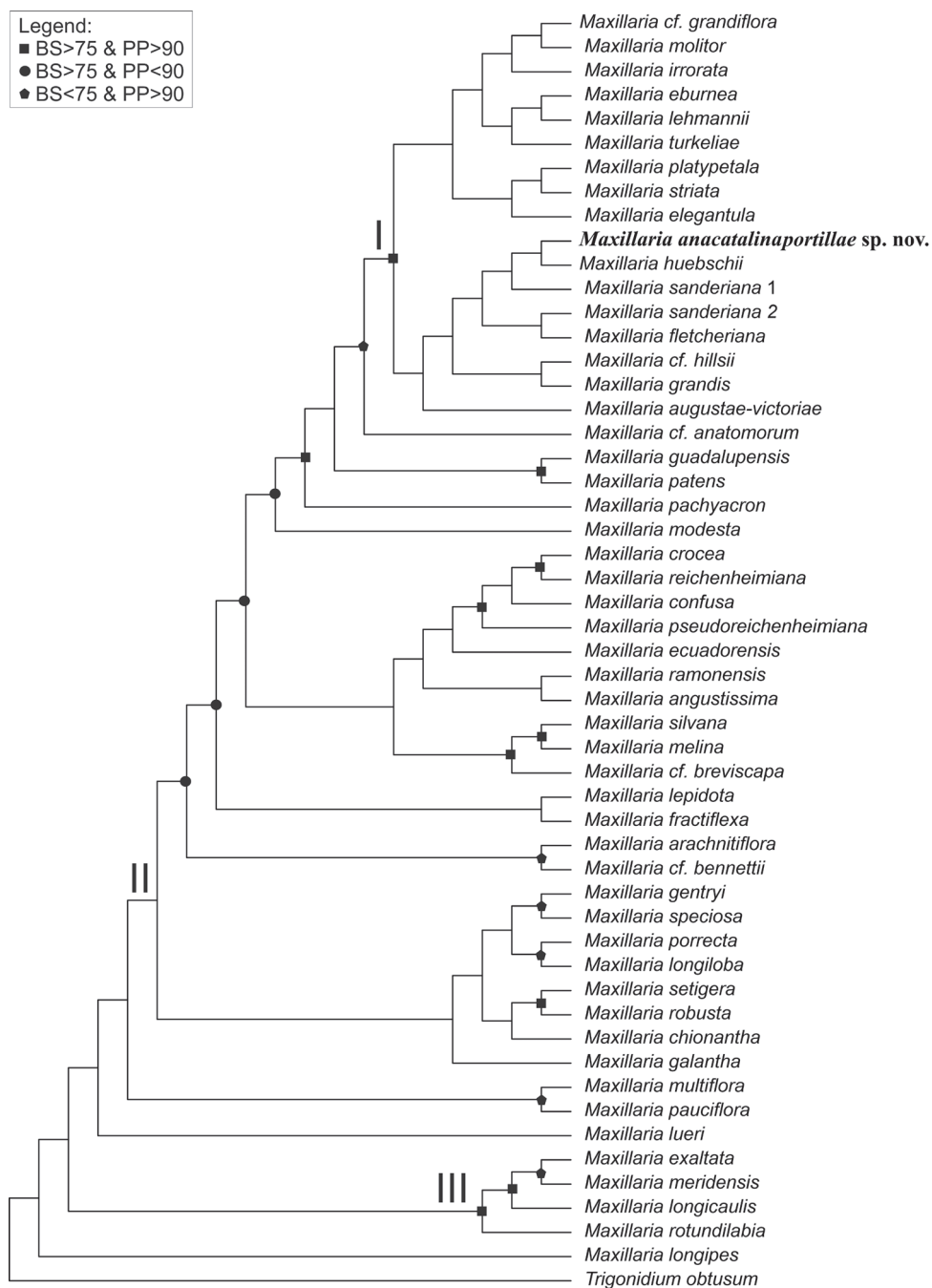


Figure 2. Phylogenetic placement of *Maxillaria anacatalinaportillae*. Maximum clade credibility tree based on combined ITS and *matK* data.

In the first step of phylogenetic tree reconstruction, two separate matrices (for ITS and *matK*) were analyzed using Bayesian Interference and maximum likelihood methods. Finally, due to the low clade support, high polytomy, and no sign of topology conflict, the combined analysis was performed (only the results of this one are shown, Fig. 2). The results of single markers analysis are available upon request.

The Bayesian analyses was performed using Markov chain Monte Carlo (MCMC) in MrBayes 3.2.7a (Huelsenbeck and Ronquist 2001) using CIPRES Science Gateway (Miller et al. 2010). The analysis was performed in two simultaneous runs of four chains for 20,000,000 generations, sampling one tree for each 100, until the average standard deviation of split ranges reached a value < 0.01. TreeAnnotator v. 1.8.1 (Drummond et al. 2012) were used to construct a maximum clade credibility tree, with a burn-in of 25%. The support of the clades was evaluated by the posterior probability (PP).

The Maximum Likelihood analysis was performed using raxmlGUI 2.0 (Edler et al. 2021) using ML + transfer bootstrap expectation + consensus option and 1000 bootstrap (BS) replications.

Micromorphological analysis

Samples for the scanning electron microscopy (SEM; voucher no. UGDA.0148255) have been preserved in 2,5% GA and 2,5% PFA in 0,05M cacodylate buffer (pH 7,0). Following dehydration in an ethanol series, they were dried by the critical point method using liquid CO₂ and coated with gold. They were observed using a Philips XL-30 scanning electron microscope.

Results

Maxillaria anacatalinaportillae Szlach. & Lipińska, sp. nov.

urn:lsid:ipni.org:names:77255006-1

Figs 3, 4

Maxillaria anacatalinaportillae Type: ECUADOR. Carchi Province, Maldonado. Alt. 1700 m. 11.04.2003. A. Portilla s.n. (Holotype: UGDA-DLSz! – spirit, drawings, photo).

Diagnosis. *M. anacatalinaportillae* appears to be similar to *M. grandis*, *M. sanderiana* and *M. wojii*. The new entity can be easily separated from *M. grandis* Rchb.f. by having flat margins of petals, which are gently angled (vs undulate and strongly recurved petals), longer lip callus extending beyond the middle of the lip (vs lip callus not reaching lip middle point) and form of lip middle lobe, which is broadly cordate or triangular when spread, with fold down margins (vs lip middle lobe oblong-elliptic, with undulate and planar margins). The lip middle lobe of *M. sanderiana* has strongly undulated and planar margins, and petals are shorter than dorsal sepal (vs equal in

length in our new species). *Maxillaria wojii* can be easily distinguished from all other species mentioned above by unique lip callus, consisting of the main part flanked by pair of subsidiary calli. Lip callus of *M. anacatalinaportillae* is very massive flanked by narrow wings on each side.

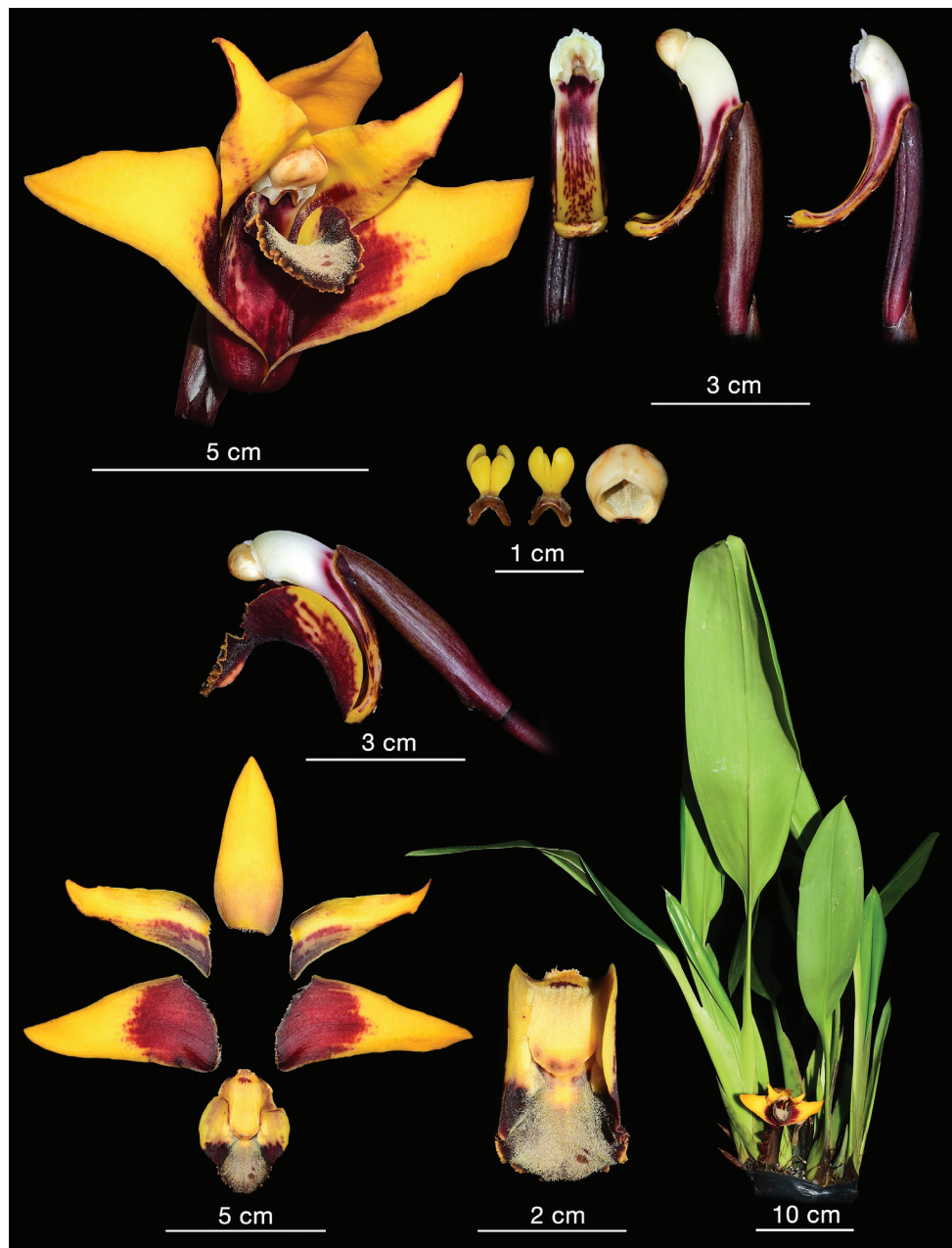


Figure 3. *Maxillaria anacatalinaportillae* **A** complete flower **B** column **C** pollinia and anther cap **D** side view of the column and lip **E** perianth parts **F** lip **G** habit. (Phot. Hugo Medina).

Description. Plants caespitose. Pseudobulbs 4–5.5 cm long, 4–4.5 cm wide, ellipsoid to almost orbicular, laterally compressed, unifoliate, supported basally by 1–2 leafy sheaths. Sheaths petiolate; petiole up to 20 cm long, conduplicate, narrow; blade up to 30 cm long and 8.5 cm wide, ligulate to oblong-elliptic, acute to shortly acuminate at apex, basally cuneate. Leaf petiolate; petiole up to 5 cm long, conduplicate; blade up to 33 cm long and 7.5 cm wide, similar in form to sheaths, ligulate to oblong-elliptic, acute to shortly acuminate. Peduncle ca 5–7 cm long, enveloped in 4–5 sheaths, erect, basal, single-flowered; sheaths elliptic-lanceolate, acute, thin, fibrous, brownish. Flowers large and showy, scentless, campanulate, not fully opened, resupinate, sepals red-wine or maroon outside, yellow inside with red-maroon basal part (Fig. 3); petals yellow with red-maroon veins and irregular dots on both sides of the middle vein, lip basal part yellow, callus yellow with dark apical part, middle lobe red-black with grayish suffusion, margins yellow, red-maroon outside, gynostemium yellowish with red-maroon on the ventral surface below stigma, anther yellow. Floral bracts ca 60 mm long, elliptic-lanceolate, greenish-brown with maroon veins. Ovary 30 mm long, glabrous. Tepals thick, fibrous. Dorsal sepal 60–62 mm long, 25–27 mm wide, elliptic-ovate, concave along midvein, apex subobtusate, canaliculated. Petals 60–62 mm long, 23–25 mm wide, oblong-lanceolate to ligulate-lanceolate, falcate at base, apex attenuate, thickened, subobtusate. Lateral sepals 75 mm long, 30–32 mm wide, obliquely oblong triangular, somewhat concave at the base, apex thickened, subobtusate. Lip hinged on the column foot, ca 45 mm long in total, 30 mm wide when spread, very stiff, gently arched, papillate in the apical half, 3-lobed in the apical third, callus very massive reaching beyond the midpoint of the lip, ligulate-ovate, flanked by narrow wing on each side; middle lobe ca 13–15 mm long, 18–20 mm wide, broadly cordate or triangular when spread, concave along midvein, margins crenulate-undulate, fold-down; lateral lobes 30 mm long, oblong-ovate in outline, canaliculated in natural position. SEM analysis revealed the presence of copious moniliform trichomes and pseudopollen grains on the lip surface, mainly middle lobe and callus (Fig. 5). Lip base and lateral lobes were rather smooth, with villiform to obpyriform papillae towards the middle part of the lip (Fig. 5B). These papillae seem to be the early stage in the development of the moniliform trichomes. Gynostemium 23 mm long, column foot 33 mm long, apically upcurved, clinandrium densely glandular.

Ecology and distribution. Grows as an epiphyte in cloud rainforest at altitude of 1700 m asl, known only from the type location.

Eponymy. Name dedicated to Ana Catalina Portilla Schröder – daughter of Alex Portilla, finder of the new entity.

Conservation status. According to the IUCN Red List criteria (IUCN 2019), the new entity should be classified as critically endangered (CR B2ab (iii)), based on the small number of known populations and restricted area of distribution. The province of Carchi in recent years suffered from problems caused by climate change, anthropogenic impact on the environment, and the lack of awareness of natural resources. The change of land use, expansion of the agricultural frontier, population growth, or the

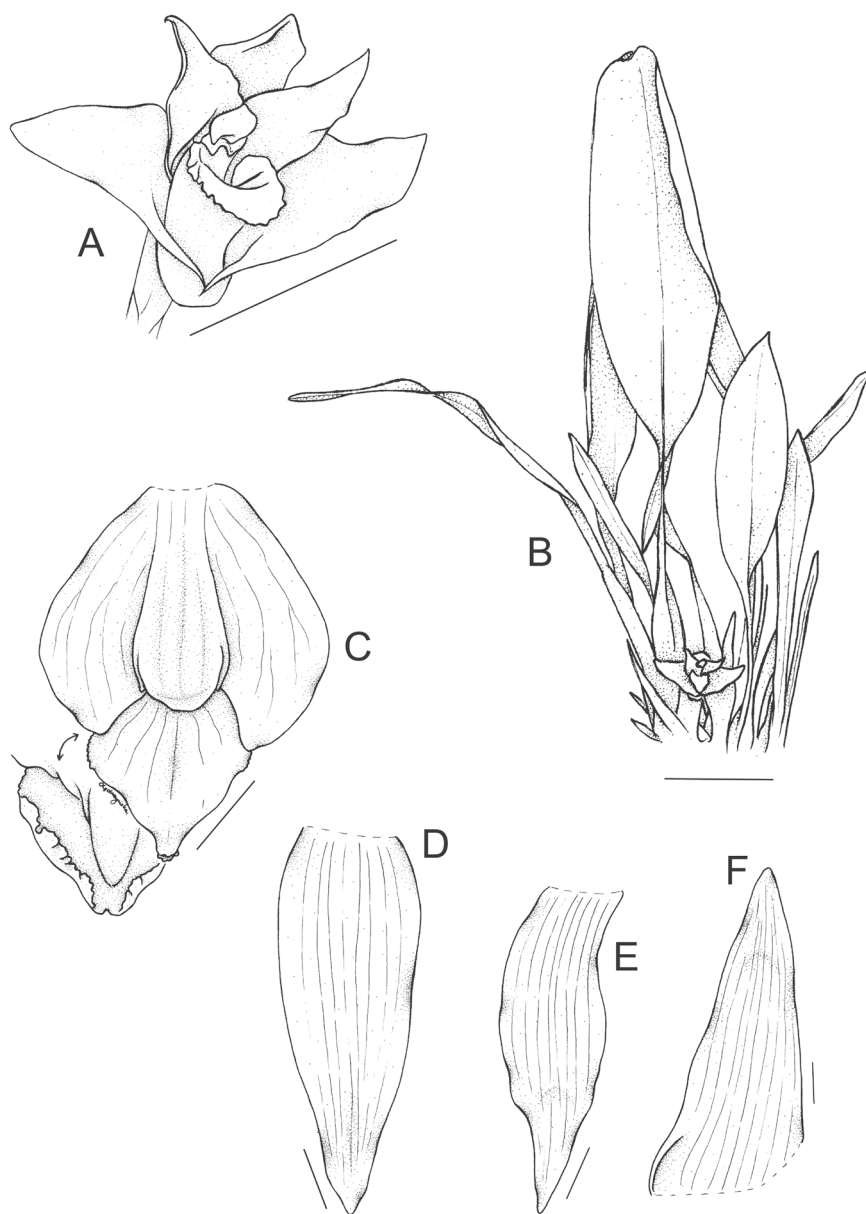


Figure 4. Drawing of the flower parts of *Maxillaria anacatalinaportillae* **A** flower **B** general habit **C** lip **D** dorsal sepal **E** petal **F** lateral sepal. Scale bars: 5 cm (**A**); 10 cm (**B**); 10 mm (**C-F**). Drawn by N. Olędzzyńska from the holotype.

opening of new roads are some dynamics that generate pressure on the ecosystems, compromising the ecological processes that take place in them. According to Global Forest Watch, from 2001 to 2020, Carchi lost 8.34 kha of tree cover, equivalent to a 3.7% decrease in tree cover since 2000.

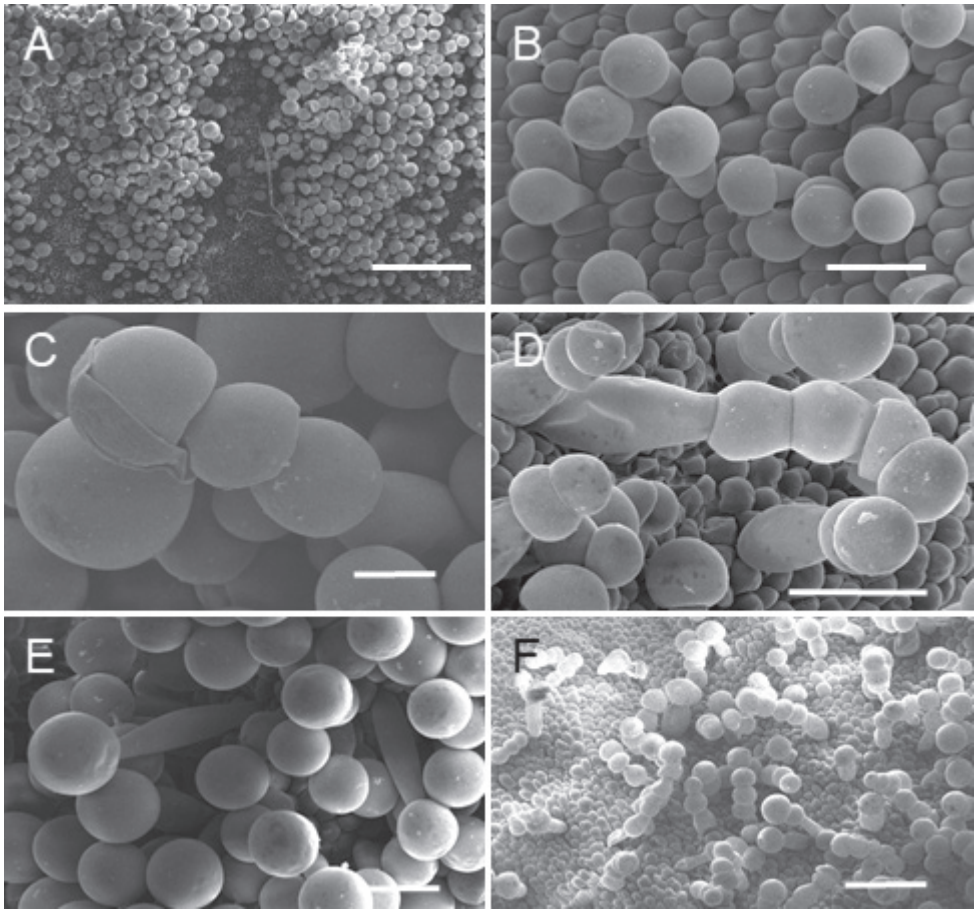


Figure 5. *Maxillaria anacatalinaportillae* **A** masses of pseudopollen accumulated on the midlobe **B** conical, villiform, and obpyriform papillae **C** grains of pseudopollen on the single celled trichome **D, E** magnification of moniliform trichomes **F** moniliform trichomes scattered on the lip surface (Phot. D. Łuszczek). Scale bars: 50µm (**A**); 100µm (**B, D, E**); 50µm (**C**); 200µm (**F**).

Notes. We know about several living collections in Ecuador that are probably representing the new entity, however, since we were not able to investigate these plants in person, we can only treat them as possible representative specimens. According to A. Hirtz, collections are located in Botanic Garden of Quito, Orquidario Las Juntas (near Gualtal at the south side of the Golondrinas Volcano, owned by Arturo and Esmeralda Mendez), Quinche (near the airport of Quito, collection of Juan Galarza), and Orquidario Casa Dracula in Quinshul (owned by Hector Yela).

Phylogeny. The results are presented on the maximum clade credibility tree obtained from Bayesian analysis. Support of particular clades (PP and additionally BS – from ML analysis) is marked with a square circle or pentagon, according to the legend given on the Fig. 2.

Obtained phylogenetic tree consists of representatives of Maxillariinae, including those recently recognized by some authors (e.g. Szlachetko et al. 2012) genera *Calawayia* (III) and *Pseudocymbidium* (represented by *M. lueri* Dodson = *Pseudocymbidium lueri* (Dodson) Szlach. and Sitko). *Maxillaria* s. str. (II) seems to be monophyletic, but there is no PP or BS support for this clade. The clade of *Maxillaria grandiflora*-complex is well supported and includes the new species *Maxillaria anacatalinaportillae*.

Discussion

Phylogeny

The main purpose of phylogenetic reconstruction in this paper was the placement of the new species, thus the phylogenetic relationship within the Maxillariinae will not be widely discussed here. Our results indicate the affinity of *Maxillaria anacatalinaportillae* to the *Maxillaria grandiflora*-complex. However, the relationships between species within the complex are still unclear. Moreover, some authors (e.g. Whitten et al. 2007) postulated to include the complex into *Maxillaria platypetala* alliance, due to the relatively low genetic differentiation among the members of these two groups, which may suggest the recent radiation of the species. In our opinion further researches are necessary to make any decision about the taxonomic position of mentioned taxa and to fully resolve the relationships between its species.

Morphology

Many representatives of *Maxillaria grandiflora*-complex (*sensu* Christenson 2013) or *M. platypetala* alliance (*sensu* Whitten et al. 2007) are superficially similar to each other but can be easily distinguished when live plants are compared side by side (Blanco and Stauffer 2011 and references their). Some of the distinguishing features, such as color patterns, may disappear or be obscured in dried herbarium specimens; especially flowers tend to become dark brown to almost black when dry, regardless of their original color (Blanco and Stauffer 2011).

Maxillaria anacatalinaportillae is the only species morphologically similar to *M. sanderiana* (Fig. 6), *M. grandis* (Fig. 7), and *M. wojii* having flowers with yellow as basic color. Flowers of all aforementioned species are primarily white with various degrees of red-wine or maroon saturation.

The forms of the flower of *Maxillaria sanderiana* and *M. anacatalinaportillae* are quite similar, but, again, the lip middle lobe of the former has strongly undulated and planar margins, and petals are shorter than dorsal sepal (vs equal in length in our new species). The micromorphology seems to be the same – in both cases, the lip surface is predominantly covered with pseudopollen, which is formed by the fragmentation of multicellular, moniliform trichomes (Davies et al. 2000). The populations of

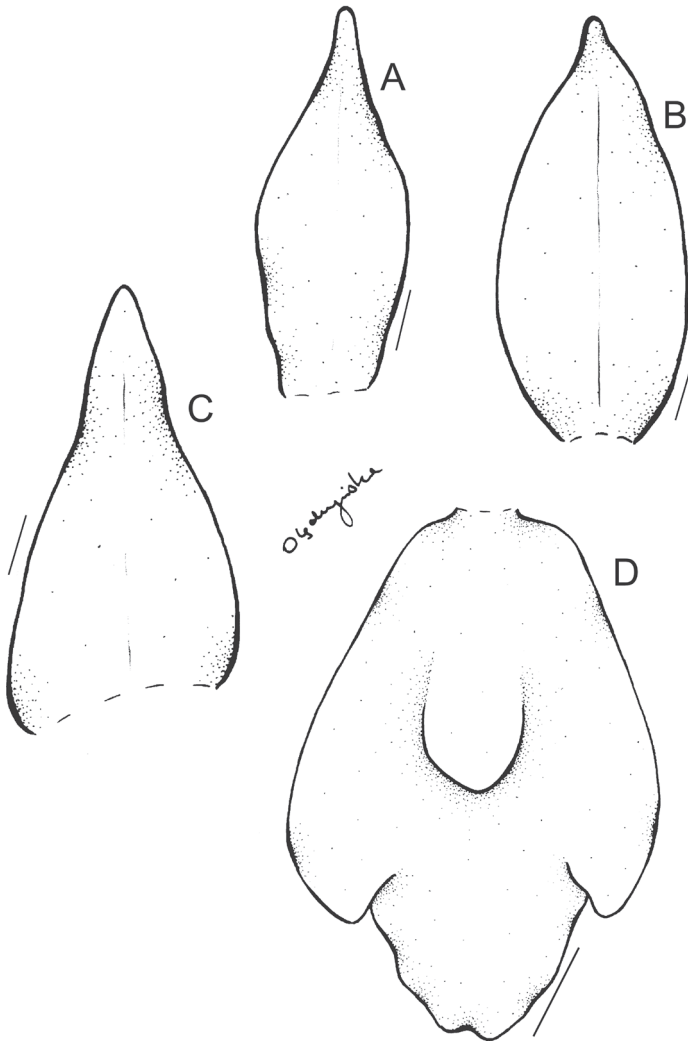


Figure 6. Drawing of the flower parts of *Maxillaria sanderiana* **A** petal **B** dorsal sepal **C** lateral sepal **D** lip. Scale bars: 10 mm. Redrawn from the type by N. Ołędryńska.

both species are isolated and are located at considerable distance from each other: *M. anacatalinaportillae* is known only from Maldonado (Carchi Province, Cantón Tulcán), whereas the closest population of *M. sanderiana* is located in the Baeza (Napo Province), ca. 400 km from Maldonado. *Maxillaria wojii* can be easily distinguished from all other species mentioned above by the unique lip callus, consisting of the main part flanked by pair of subsidiary calli. Lip callus of *M. anacatalinaportillae* is very massive, flanked by narrow wings on each side, which seems to be an intermediate state between those found in *M. wojii* and *M. sanderiana*. The morphological differences and similarities have been summarized in Table 1.

Table 1. Summary of the morphological differences between the most similar species.

	<i>M. anacatalinaportillae</i>	<i>M. grandiflora</i>	<i>M. grandis</i>	<i>M. sandieriana</i>	<i>M. wojii</i>
Distribution	Ecuador	Colombia	Ecuador, Peru	Ecuador, Peru	Colombia
Habit	Caespitose epiphytes	Caespitose to ascending epiphytes	Caespitose epiphytes	Caespitose terrestrials or epiphytes	Caespitose epiphytes
Pseudobulbs	Ellipsoid to almost orbicular, compressed	Oblong-ellipsoid, compressed	Oblong-lanceolate, compressed	Ovoid, subglobose or oblong-ellipsoid, compressed	Elliptic, compressed
Leaves	Ligulate to oblong-elliptic, acute to shortly acuminate, petiolate	Lanceolate, acuminate, petiolate	Ligulate-lanceolate to oblong-lanceolate, acute, petiolate	Oblong-elliptic to broadly elliptic, acute, petiolate	Oblong-elliptic, acute, arching, petiolate
Flowers	Large and showy, scentless, campanulate, not fully opened, resupinate	Showy, triangular in outline, resupinate	Showy, large, resupinate	Large, showy, faintly fragrant during the day, variably marked, resupinate	Showy, wide-spreading, fleshy, resupinate
Dorsal sepal	Elliptic-ovate, concave along midvein, apex subobtruse, canaliculated	Elliptic, acute, rigid, concave, keeled along the back along the midvein	Oblong elliptic-ovate, acute, lightly concave	Elliptic-lanceolate to suborbicular, acuminate to obtuse-apiculate, lightly concave	Oblong-triangular, acute, keeled, with shallowly revolute lateral margins
Lateral sepals	Obliquely oblong triangular, somewhat concave at the base, apex thickened, subobtruse	Triangular, acute-acuminate, strongly divergent, with minutely revolute lateral margins	Obliquely ovate-triangular, recurved or twisted near the middle	Obliquely ovate-triangular, recurved above the middle	Ovate-triangular, acute, with revolute lateral margins
Petals	Oblong lanceolate to ligulate-lanceolate, falcate at the base, apex attenuate, thickened, subobtruse	Elliptic-lanceolate, acute, indexed forming a chamber with the lip, with strongly recurved apices	Triangular with toothed margins, undulate and strongly recurved	Oblong-triangular to broadly ovate, abruptly acuminate	Oblong with an abruptly triangular apex, acuminate, recurved toward the apex
Lip	3-lobed, middle lobe broadly cordate or triangular when spread, concave along midvein, margins crenulate-undulate, fold-down, lateral lobes oblong-ovate in outline, canaliculated in natural position	Obscurely 3-lobed, strongly arched at the middle, lateral lobes rigidly erect, rounded, midlobe broadly ovate-triangular, obtuse, with undulate margins	Deeply 3-lobed, arching in natural position, lateral lobes obliquely elliptic, midlobe oblong-elliptic, obtuse, margins undulate and planar	3-lobed, arching, lateral lobes erect, incurved, obliquely obovate, midlobe ovate, obtuse, with undulate margins	3-lobed, arching, lateral lobes erect, transversely oblong, obtuse-rounded, midlobe ovate, obtuse, undulate-crenulate
Callus	Massive, reaching beyond the midpoint of lip, ligulate-ovate, flanked by narrow wing on each side	Ligulate, broad	Large, massive in the basal third of the lip	Large longitudinal, from the base of the lip to the middle, obtuse-rounded	Biseriate, central callus oblong-lanceolate, obtuse, flanked at the apex by a pair of low, irregular, subsidiary calli
Column	Apically upcurved	Slightly curved	Arching	Arching	Arching

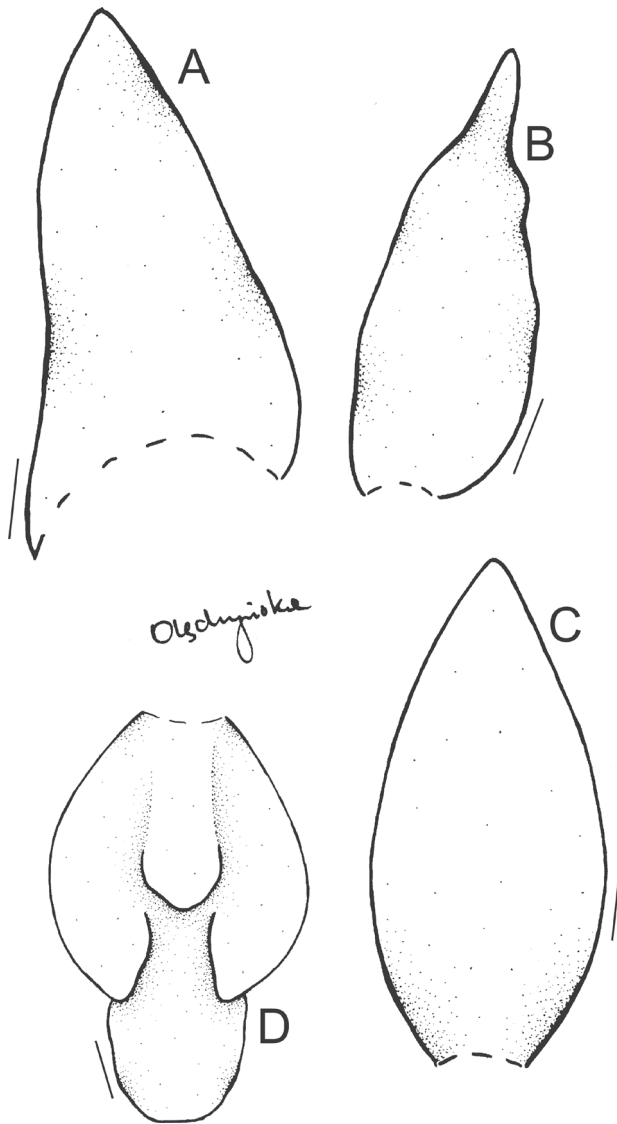


Figure 7. Drawing of the flower parts of *Maxillaria grandis* **A** lateral sepal **B** petal **C** dorsal sepal **D** lip. Scale bars: 10 mm. Redrawn from the type by N. Oleđczyńska.

Micromorphology

It is estimated that as many as 56% of the representatives of *Maxillaria sensu lato* attract pollinators with “empty promises”, which are a combination of visual, tactile, and olfactory stimuli (Davies et al. 2005 and references therein). Among the species that offer any kind of reward, there are three types: nectar, pseudopollen (*farina*), and wax-like substances (Davies et al. 2003a, b, 2005). Until now, the micromorphology of only

twelve members of *Maxillaria grandiflora*-complex has been studied. A common feature among its representatives is the presence of pseudopollen and at least 30 Ecuadorian species of *Maxillaria* produce pseudopollen (Davies et al. 2000). *M. anacatalinaportillae* is no exception and SEM analysis revealed the presence of copious moniliform trichomes and pseudopollen grains on the lip surface, mainly middle lobe and callus. Lip base and lateral lobes were rather smooth, with villiform to obpyriform papillae towards the middle part of the lip. (Davies and Turner 2004). Pseudopollen is usually produced by the fragmentation of labellar trichomes and it has the form of a whitish layer with a powder-like structure. It can be considered as a substitute reward for real pollen.

The presence of obpyriform and moniliform trichomes is typical for members of the *Maxillaria grandiflora*-complex (Davies and Turner 2004; Lipińska and Kowalkowska 2018) and is not surprising, since wherever pseudopollen-forming trichomes occur, labellar papillae tend to be obpyriform (Davies and Turner 2004).

The main pollinators of *Maxillaria* are stingless bees (Meliponini) (Roubik 2000). According to Davies et al. (2000), some of the members of the *Maxillaria grandiflora*-complex are pollinated by different insects: *Maxillaria fletcheriana* Rolfe by the bumblebee *Bombus volucelloides* Gribodo, *M. grandiflora* and *M. sanderiana* by *Eulaema cingulata* Fabricius. It is believed that bees collect pseudopollen from the flowers because of the nutrients it contains (Davies et al. 2000) and these include starch, oils, and proteins (van der Pijl and Dodson 1966). This may suggest that *M. anacatalinaportillae* is also pollinated by bees, similarly to closely related *M. grandiflora* and *M. sanderiana*.

Acknowledgements

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Appendix I

Table A1. GenBank accession numbers: taxon, accession number for ITS and matK, respectively (asterisk states for sequences obtained in this research).

Species	ITS	matK
<i>Maxillaria angustissima</i>	DQ210512	DQ210961
<i>Maxillaria arachnitiflora</i>	DQ210242	DQ210758
<i>Maxillaria augustae victoriae</i>	DQ210027	DQ210599
<i>Maxillaria anacatalinaportillae</i> (sp. nov.)	OK032114*	OK032062*
<i>Maxillaria</i> cf. <i>anatomorum</i>	DQ210483	DQ210966
<i>Maxillaria</i> cf. <i>bennettii</i>	DQ210352	DQ210849
<i>Maxillaria</i> cf. <i>Breviscapa</i>	DQ210544	DQ211019
<i>Maxillaria</i> cf. <i>Grandiflora</i>	DQ210026	DQ210598
<i>Maxillaria</i> cf. <i>Hillsii</i>	DQ210073	DQ210616
<i>Maxillaria chionantha</i>	DQ210486	DQ210792
<i>Maxillaria confusa</i>	DQ210513	DQ210840
<i>Maxillaria crocea</i>	DQ210311	DQ210634
<i>Maxillaria eburnea</i>	DQ210454	DQ210938

Species	ITS	matK
<i>Maxillaria ecuadorensis</i>	DQ210508	DQ210771
<i>Maxillaria elegantula</i>	DQ210543	DQ210921
<i>Maxillaria exaltata</i>	DQ210320	DQ210818
<i>Maxillaria fletcheriana</i>	DQ210209	DQ210739
<i>Maxillaria fractiflexa</i>	DQ210074	DQ210617
<i>Maxillaria galantha</i>	DQ210574	DQ211049
<i>Maxillaria gentryi</i>	DQ210492	DQ210845
<i>Maxillaria grandis</i>	DQ210368	DQ210862
<i>Maxillaria guadalupensis</i>	DQ210504	DQ210983
<i>Maxillaria huebschii</i>	OK032113*	OK032061*
<i>Maxillaria irrorata</i>	DQ210430	DQ210917
<i>Maxillaria lehmannii</i>	DQ210268	DQ210778
<i>Maxillaria lepidota</i>	DQ210562	DQ210857
<i>Maxillaria longicaulis</i>	DQ210510	DQ210623
<i>Maxillaria longiloba</i>	DQ210432	DQ210919
<i>Maxillaria longipes</i>	DQ210519	DQ210999
<i>Maxillaria lueri</i>	DQ210471	DQ210802
<i>Maxillaria melina</i>	OK030847*	OK032060*
<i>Maxillaria meridensis</i>	DQ210427	DQ210780
<i>Maxillaria modesta</i>	DQ210195	DQ210726
<i>Maxillaria molitor</i>	DQ210370	DQ210863
<i>Maxillaria multiflora</i>	DQ210186	DQ210716
<i>Maxillaria pachyacron</i>	DQ210489	DQ210593
<i>Maxillaria patens</i>	DQ210528	DQ210986
<i>Maxillaria pauciflora</i>	DQ210390	DQ210631
<i>Maxillaria platypetala</i>	DQ210558	DQ211033
<i>Maxillaria porrecta</i>	DQ210568	DQ210576
<i>Maxillaria pseudoreichenheimiana</i>	DQ210328	DQ210827
<i>Maxillaria ramonensis</i>	DQ210099	DQ209918
<i>Maxillaria reichenheimiana</i>	DQ210503	DQ210827
<i>Maxillaria robusta</i>	DQ210192	DQ210722
<i>Maxillaria rotundilabia</i>	DQ461792	DQ210893
<i>Maxillaria sanderiana</i>	DQ210271	DQ210781
<i>Maxillaria sanderiana</i>	DQ210453	DQ209967
<i>Maxillaria setigera</i>	DQ210230	DQ210674
<i>Maxillaria silvana</i>	DQ210516	DQ210997
<i>Maxillaria speciosa</i>	DQ210075	Q210618
<i>Maxillaria striata</i>	DQ210267	DQ210777
<i>Maxillaria turkeliae</i>	DQ210276	DQ209945
<i>Trigonidium obtusum</i>	DQ210220	DQ210641

Taxonomic identity of *Corydalis lidenii* (Papaveraceae)

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Abstract

Corydalis microflora and *C. lidenii* are recognised as separate species in “Flora of China” and the latest plant list. However, based on the examination of type specimens and field investigations, *C. lidenii* is shown to be conspecific with *C. microflora*. As a result, *C. lidenii* is synonymised with *C. microflora* in this study.

Keywords

China, *Corydalis lidenii*, *Corydalis microflora*, new synonym, Sichuan, taxonomy

Introduction

The genus *Corydalis* DC., the largest genus of Papaveraceae, contains about 465 species mainly distributed in the northern temperate zone with the highest species diversity in SW China (Wu et al. 1999; Zhang et al. 2008; Chen et al. 2018). Several new species have been described recently, and currently there are about 366 species in this genus known from China. It has been suggested that the uplift of the Qinghai Tibet Plateau and the formation of the Hengduan Mountains, led to an intensive and rapid diversification of this genus (Wang 2006; Sun et al. 2017). The high species diversity of this genus is accompanied by an extremely complex morphology that makes it notoriously difficult for taxonomy and species identification (Wu et al. 1996).

* The authors contributed equally to this study.

Many species of this genus are known only from type specimens or fewer than 10 specimens. Taking *Corydalis* sect. *Trachycarpae* (Fedde) Fedde as an example, of 56 species, 17 species are known only from type specimens, 16 species have fewer than 10 specimens, and only 23 species have more than 10 specimens in herbaria. This means that for many species we have a poor understanding of their variation.

Corydalis lidenii Z.Y.Su is one of those species and was described based on a collection from Maoxishangou, Yusaping, Detuo Town, Luding County, Sichuan Province, China (Su 2008). Su (2008) compared it to *C. pingwuensis* C.Y.Wu, a rather remotely related species. However, it does not seem possible to distinguish *C. lidenii* from the previously described *C. flexuosa* var. *microflora* C.Y.Wu in terms of morphology (Zhuang and Wu 1991); and their short rhizomes densely set with fleshy scales are unique in *Corydalis*. After its initial publication, *C. flexuosa* var. *microflora* was erected to the subspecific rank as *C. flexuosa* subsp. *microflora* (C.Y.Wu & H.Chuang) C.Y.Wu (Wu et al. 1999). Subsequently, Lidén and Su (2007) elevated it to a separate species *C. microflora* (C.Y.Wu & H.Chuang) Z.Y.Su & Lidén. In the account of the family Papaveraceae for “Flora of China” (FOC), Zhang et al. (2008) followed Lidén and Su (2007) and treated *C. lidenii* and *C. microflora* as separate species.

The main differences between *C. lidenii* and *C. microflora* stated by Zhang et al. (2008) are a thin spur longer than the inner petals in the former and a less thin spur equaling the inner petals in the latter. However, when we looked at old and new collections, we noticed that it was not possible to distinguish these two species, and we found no difference in flower and overall morphology between specimens from different localities (Table 1–2; Figs 1–5, 6a, b). Furthermore, the geographical distance between the type localities is very short (Fig. 6). This made us speculate that the two names refer to the same species.

Materials and methods

Our recently collected specimens of *Corydalis lidenii* are from the type locality: Maoxishangou, Yusaping, Detuo Town, Luding County, Sichuan Province, China. We also studied all specimens of *C. microflora* and *C. lidenii* deposited in the herbaria of PE, CDBI, KUN, SM, IBSC. Additionally, some specimens were obtained through the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>). The morphological comparison is provided in Table 1 and Fig. 6.

Results and discussion

Based on the detailed morphological comparisons of *Corydalis lidenii* and *C. microflora* in rhizome, stems, radical leaves, cauline leaves, inflorescences, flowers and capsule (Table 2), especially the proportion of spur and inner petals (Table 1) emphasized by Zhang et al. (2008), these results show that they are almost identical in overall morphology. Therefore, *C. lidenii* should be treated as a synonym of *C. microflora*.

Table 1. Morphological comparisons of *Corydalis lidenii* and *C. microflora*.

<i>C. lidenii</i>	1	2	3	4	5	6	7	8	9	10	11	12
Spur	0.40	0.60	0.7	0.75	0.85	0.90	0.90	0.95	1.03	0.95	0.95	1.10
Inner petals	0.70	0.70	0.75	0.75	0.85	0.90	0.90	0.85	0.90	0.80	0.80	0.90
<i>C. lidenii</i>	13	14	15	16	17	18	19					
Spur	1.10	1.00	1.05	1.20	1.10	1.00	0.90					
Inner petals	0.90	0.80	0.80	0.90	0.80	0.70	0.60					
<i>C. microflora</i>	1	2	3	4	5	6	7	8	9	10	11	12
Spur	0.88	0.93	0.98	0.96	1.05	0.99	1.05	1.02	1.10	1.08	1.13	1.20
Inner petals	0.85	0.88	0.88	0.84	0.90	0.84	0.87	0.84	0.90	0.85	0.88	0.93

Notes: the data of *C. lidenii* are from holotype and specimens collected from its type locality, and numbers 1–4 of *C. lidenii* indicate young flowers; the data of *C. microflora* are from holotype and isotype. Measurement unit: cm.

The following are mainly to modify some taxonomic problems and incorrect records existing in the previous publication of *C. lidenii*, and supplement some type information of *C. microflora*.

Corydalis lidenii

When *C. lidenii* was published, Su (2008) designated *Yong-jiang Li 189* (CDBI) as “holotype”. In herbarium CDBI, two sheets are found and they are not clearly labelled as the parts of a single specimens, and therefore they are not parts of a single specimen but two duplicates of a single gathering according to Art. 8.3 and footnote 1 of *Shenzhen Code* (Turland et al. 2018). Furthermore, Su did not annotate any-one as holotype as such. Therefore, no holotype was actually designated and both of them are syntypes according to Art. 9.5 and Art. 40.2 Note 1. As the specimen CDBI149418 has the anatomical drawing and anatomical records, and the flowers and fruits of this specimen are relatively more complete, it is here selected as lectotype (Fig. 1A, B).

Both specimens of *Yong-jiang Li 189* are in a poor condition; the leaves are folded and damaged and the flowers shrivelled. In May 2021, we therefore went to the type locality (Detuo Town, Luding County) to collect fresh specimens (Fig. 2) of this very delicate species, and found that very careful treatment was required to achieve the perfect condition of the type specimen of *C. microflora*.

Two paratypes were cited in the protologue (Fig. 1C, D). However, the localities for these were recorded incorrectly, and should be Ganluo County, not Dege County. The two counties are quite far apart. This error is repeated by Zhang et al. (2008). The collecting time of the paratypes was misstated to be 1997, but was in fact 1979.

Corydalis microflora

Chao-chun Hsieh’s collection records from Shimian County in 1955, show that Hsieh and Xian-xu Kong collected together. Kong is indicated as the collector of only a few specimens, while the others are marked Hsieh. Some collections record different

Table 2. Comparisons of *Corydalis lidenii* and *C. microflora* as given in FOC, and our revised data.

Characters	<i>C. lidenii</i> in FOC	<i>C. microflora</i> in FOC	<i>C. microflora</i> (Revised)
Plants	Herbs, perennial, 25–45 cm tall, glabrous.	Herbs, perennial, 16–33 cm tall, glabrous.	Herbs, perennial, (12–) 16–45 cm tall, glabrous.
Rhizome	Rhizome short, with crowded thick pale bulbous (petiolar bases).	Rhizome short, with small crowded fleshy scales or pale callosities at base.	Rhizome short, with small crowded fleshy scales at base.
Stems	Stems few, erect, very slender, simple, with 2 leaves in upper 1/2.	Stems 1 to few, erect, slender, simple, with 1 or 2 leaves in upper 1/3–1/2 (possibly also with 1 or 2 early withering small leaves at base).	Stems usually 1 or rarely 2, erect, slender, simple or with 1 tiny flower branch from upper leaf.
Radical leaves	Radical leaves early withering, few; petiole 5–9 cm, thin; blade 3–4 × 3–4 cm, thin, bi-(tri-) ternate, with long thin petiolules; leaflets obovate, 5–10 × 3–5 mm, obtuse.	Radical leaves 1 to few with thin petiole 9–12 cm; blade glaucous abaxially, biternate, ca. 3 × 3 cm; leaflets obovate, entire to shallowly 3-lobed.	Radical leaves 0 to 3 with long thin petiole 4–9 cm; blade glaucous abaxially, biternate, ca. 2.5–3 × 2.5–3 cm; leaflets obovate, entire to 3-lobed.
Cauline leaves	Cauline leaves shortly stalked or upper sessile, like radical leaves.	Cauline leaves shortly stalked to subsessile, ternate to biternate, 1.5–4 × 2–4 cm; petiolule of lateral primary leaflets 2–5 mm; petiolule of terminal leaflet 5–15 mm; ultimate leaflets broadly obovate, 8–15 mm, ± deeply divided into broad rounded lobes.	Cauline leaves 1–3, like radical leaves; upper leaves shortly stalked or sessile, the lowest leaf usually with long thin petiole.
Inflorescences	Racemes very lax, 8–12 cm, 6–8-flowered; lowermost bract leaflike, middle and upper bracts much smaller, oblanceolate, 5–10 mm, entire.	Raceme lax, 5–7-flowered, only slightly elongating in fruit; lower bract like cauline leaf, upper progressively smaller and less divided.	Raceme lax, 4–12 cm, (2–)4–7(–9)-flowered; lower bract like upper cauline leaf, upper progressively smaller and less divided to entire.
Flowers	Pedice l ca. 10 mm, 12–20 mm in fruit. Sepal minute. Corolla white to pale blue or pale purple. Outer petals elliptic, acute to shortly mucronate, without crest; upper petal 17–19 mm; spur straight or slightly downcurved, very narrow, ca. 10 mm; nectary ca. 1/2 as long as spur, thin; inner petals ca. 8 mm. Stigma broad, emarginate, basal lobes absent.	Pedice l thin, erect, 6–10 mm in flower, in fruit to 10–15 mm. Sepals 0.5–1 × 0.5–1 mm, large dentate. Corolla probably blue or pale purple. Outer petals subacute, without crest; upper petal 18–19 mm, spur straight or slightly downcurved, narrowly cylindric, 9–10 mm; nectary not recorded; inner petals 9–10 mm. Stigma subcuneate at base (without basal lobes) with 4 marginal apical simple papillae; geminate papillae 1 pair.	Pedice l thin, erect, usually 6–10 mm in flower, lower pedice l to 20 mm. Sepal minute, 0.2–0.5 × 0.2–0.5 mm, dentate. Corolla pale purple. Outer petals subacute to shortly mucronate, without crest; upper petal 17–20 mm, spur straight or slightly downcurved, narrowly cylindric, 9–10 mm; nectary ca. 1/2–3/5 as long as spur, thin; inner petals 8–10 mm. Stigma subcuneate at base with (8) 12 papillae.
Capsule and seed	Capsule 10–14 × ca. 1 mm, 8–11-seeded; style ca. 2.5 mm. Seeds in 1 row.	Capsule linear, 13–18 mm, 5–13-seeded; style ca. 2.5 mm.	Capsule linear, 10–18 × ca. 1 mm, 5–13-seeded; style ca. 2.5 mm. Seeds ca. 1 mm, in 1 row.
Flowering and fruiting period	Fl. and fr. May.	Fl. and fr. Jun.	Fl. and fr. May–Jun.
Distribution	Sichuan (Luding, Ganluo).	Sichuan (Shimian).	Sichuan (Shimian, Luding, Ganluo).

collectors, even on specimens that are obviously duplicates, as they bear the same collection number. There are few complete handwritten collection labels; most are printed labels with incomplete records (only “Shimian County 1955”). We were lucky to find two collection numbers close to the number of the type of *C. microflora* (*C.C.Hsieh* 41235), with more detailed data. One of them, No. 41231 (*Cotoneaster moupinensis* Franch), has two duplicates (PE00501194! and SZ00189464!). The collectors are



Figure 1. Photographs of the types of *Corydalis lidenii* **A** lectotype-CDBI149418, designated here! **B** isolectotype-CDBI149417 **C, D** paratypes-SM.



Figure 2. *Corydalis microflora* from the type locality of *C. lidenii*.



Figure 3. *Corydalis microflora* A holotype-PE00934552 B isotype-IBSC0133200.

Kong and Hsieh respectively, but the collection date was 19 June 1955, and a location was beside the highway of Tiezhaizi, Liziping Town, Shimian County, which was recorded in detail on the SZ specimen. Another one No. 41239 (*Valeriana* sp.), has three duplicates (PE01018029!, IBSC0498498! and HGAS029339!). The first two are marked Hsieh whereas the HGAS specimen is marked Kong. The collection date was 20 June 1955 and the locality was Haizishan, Tiezhaizi, Liziping Town, Shimian County (recorded in HGAS029339).

The collection date of the type of *C. microflora* should be 19 or 20 June 1955, and the locality should be Tiezhaizi to Haizishan, Liziping Town, Shimian County. The distance between this location and that of the type of *C. lidenii* is ca. 70 km, and the distance to the location of the paratypes is ca. 40 km (Fig. 6).

Taxonomic treatment

***Corydalis microflora* (C.Y.Wu & H.Chuang) Z.Y.Su & Lidén, Novon 17(4): 484. (Lidén and Su 2007)**

- ≡ *Corydalis flexuosa* var. *microflora* C.Y.Wu & H.Chuang, Acta Bot. Yunnan.13(2): 132 (Zhuang and Wu 1991); *C. flexuosa* subsp. *microflora* (C.Y.Wu & H.Chuang) C.Y.Wu, Fl. Reipubl. Popularis Sin. 32: 118 (Wu et al. 1999). – Type: CHINA. Sichuan: Shimian County, Liziping Town, Tiezhaizi to Haizibian, June 1955, C.C. Hsieh & X.X. Kong 41235 (holotype: PE00934552!, isotype: IBSC0133200!)
- = *Corydalis lidenii* Z.Y.Su, Acta Bot. Yunnan. 30(4): 422 (Su 2008), syn. nov. – Type: CHINA. Sichuan: Luding County, Detuo Town, Yusaping, Maoxishangou, 2,000 m, in shrubs, 7 May 1984, Y.J. Li 189 (lectotype: CDBI149418!, designated here; isoelectotype, CDBI149417!)

Description. Herbs, perennial, (12–) 16–45 cm tall, glabrous. Rhizome short, with small crowded fleshy scales at base. Stems usually 1 or rarely 2, erect, slender, simple or with 1 tiny flower branch from upper leaf. Radical leaves 0 to 3 with long thin petiole 4–9 cm; blade glaucous abaxially, biternate, ca. 2.5–3 × 2.5–3 cm; leaflets obovate, entire to 3-lobed. Cauline leaves 1–3, like radical leaves; upper leaves shortly stalked or sessile, the lowest leaf usually with long thin petiole. Raceme lax, 4–12 cm, (2–)4–7(–9)-flowered; lower bract like upper cauline leaf, upper progressively smaller and less divided to entire. Pedicel thin, erect, usually 6–10 mm in flower, lower pedicel to 20 mm. Sepals minute, 0.2–0.5 × 0.2–0.5 mm, dentate. Corolla pale purple. Outer petals subacute to shortly mucronate, without crest; upper petal 17–20 mm, spur straight or slightly downcurved, narrowly cylindric, 9–10 mm; nectary 1/2–3/5 as long as spur, thin; inner petals 8–10 mm. Stigma subcuneate at base with (8) 12 papillae. Capsule linear, 10–18 × ca. 1 mm, 5–13-seeded; style ca. 2.5 mm. Seeds ca. 1 mm, in 1 row.

Phenology. Flowering and fruiting from May–June.

Distribution and habitat. *Corydalis microflora* is a rare species with a narrow distribution in Sichuan, China (Shimian County, Luding County and Ganluo County). It grows in forest margins, open forest, or near valley stream at an eleva-

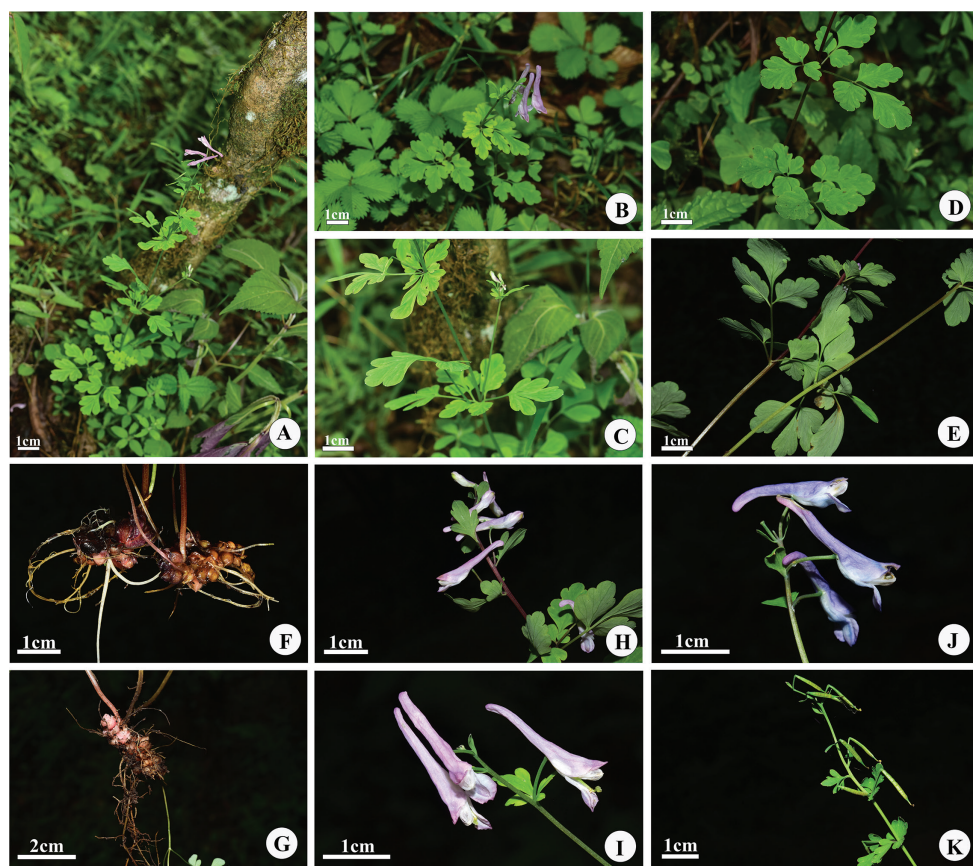


Figure 4. *Corydalis microflora* at the type locality of *C. lidenii* **A** habitat and flowering branch **B** flowering branch **C** small axillary raceme and flower **D** leaf adaxial surfaces **E** leaf abaxial surfaces **F, G** rhizome with small crowded fleshy scales at base **H–J** inflorescence and flowers **K** fruiting raceme.

tion of 2,000–2,500 m. Associated species include *Betula* sp. (Betulaceae), *Acer* sp. (Sapindaceae), *Rodgersia aesculifolia* Batalin (Saxifragaceae), *Paris* sp. (Melanthiaceae), *Veronica sutchuenensis* Franch. (Plantaginaceae), *Campylandra* sp. and *Ophiopogon* sp. (Asparagaceae), *Elatostema* sp. (Urticaceae), *Calanthe tricarinata* Lindl. (Orchidaceae), *Mimulus szechuanensis* Y.Y.Pai (Phrymaceae), *Corydalis davidii* Franch. and *Ichtyoselmis macrantha* (Oliver) Lidén (Papaveraceae), amongst others.

Additional specimens examined. – CHINA. **Sichuan:** Ganluo County, Tianba Town to Lianghe Town, elev. ca. 2,500 m, 10 May 1979, *Xichang institute of drug control* 192 (SM); Luding County, Detuo Town, Yusaping, 29.53735N, 102.26755E, elev. ca. 2,060 m, under forests and forest margins, 22 May 2021, *J.T.Chen & Z.Y.Lv Deng10838* (KUN).

Conservation status. At present, *Corydalis microflora* has been found only in three places in Sichuan (Fig. 6), and only four specimens of this species have been collected

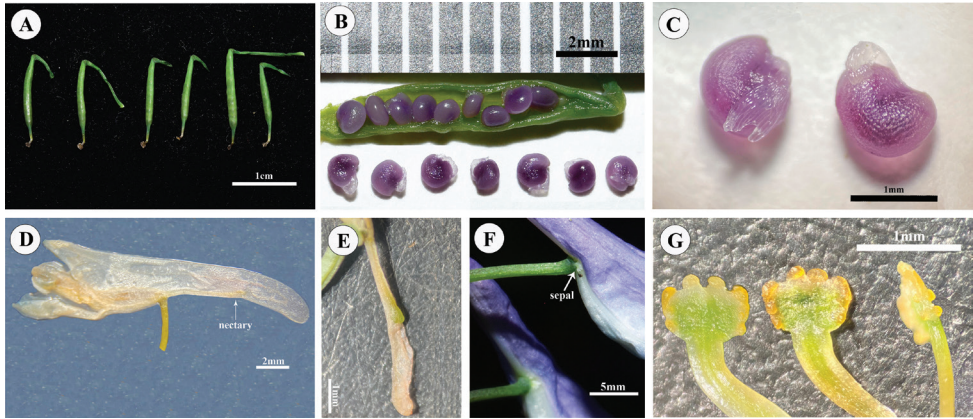


Figure 5. *Corydalis microflora* at the type locality of *C. lidenii* **A** capsule **B** longitudinal section of capsule **C** seeds with elaiosome **D** flower and nectary (arrow) **E** nectary **F** sepal **G** stigma (profile and edge).

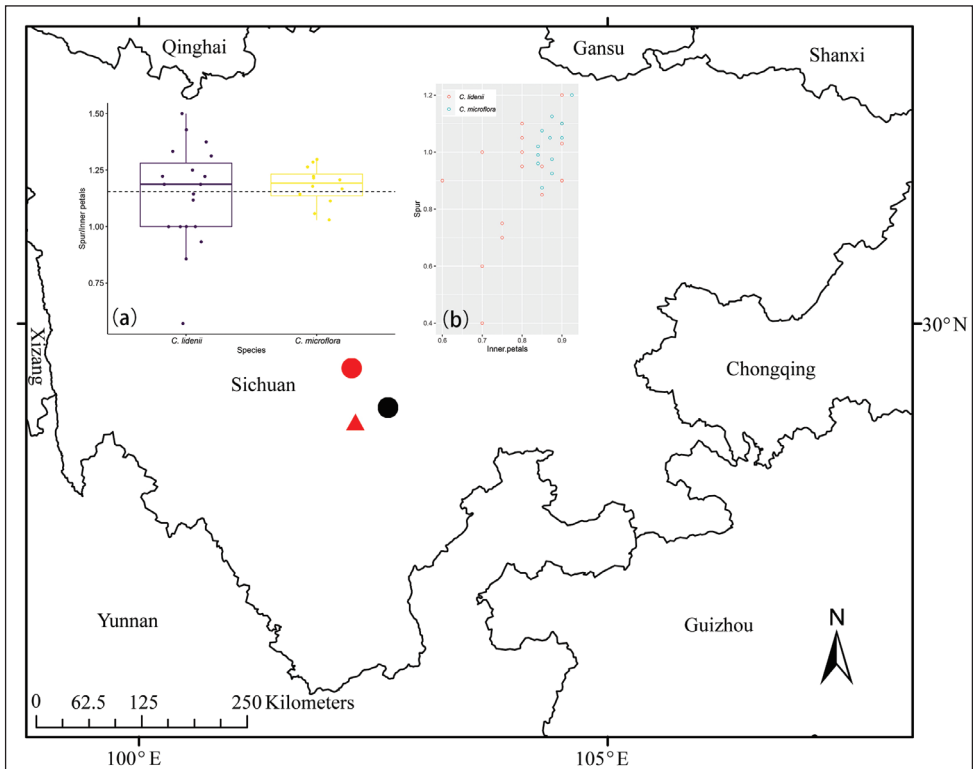


Figure 6. Distribution of *Corydalis microflora*. (Notes: the red dot represents type locality of *C. lidenii* and the black dot represents paratype locality of *C. lidenii*, the red triangle represents the type locality of *C. microflora* **a** the Box-plot of comparison of spur/inner petals of *C. lidenii* and *C. microflora* **b** the comparison of spur and inner petals of *C. lidenii* and *C. microflora*).

so far. During a field investigation in 2021, we once again visited the Yusaping, Maoxishangou in Luding. Among four ravines investigated, this species was only found in one. The population was small, ca. 30 individuals were observed, with the extent of occurrence of ca. 1 km². It was growing scattered in open deciduous broad-leaved forests near the mouth of the ravine. Further studies are needed to assess its conservation status, and we only temporarily assign it to the category ‘Data Deficient’ (DD) of the International Union for Conservation of Nature (IUCN 2019).

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Nomenclature and taxonomic identities of *Prunus zappeyana* and *P. zappeyana* var. *subsimplex* (Rosaceae)

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Abstract

The original specimens of both *Prunus zappeyana* and *P. zappeyana* var. *subsimplex* were found to belong to more than one taxon. In addition, *P. zappeyana* var. *subsimplex* was found to be invalid because, when the name was published, two separate descriptions were given to two cited collections, but not to the taxon, making the name unaccompanied with a description or diagnosis of this taxon (Art. 38.1 (a)). Therefore, a lectotype of *P. zappeyana* was designated under Art. 9.11 of ICN, by which *P. zappeyana* was placed in the synonymy of *P. veitchii*.

Keywords

Cerasus, China, taxonomy, typification

Introduction

Prunus subg. *Cerasus* (Mill.) A. Gray is a taxonomically complex group, commonly known as cherries (Liu et al. 2018). There are 39 species or varieties of China recognised in ‘*Flora of China*’ (Li and Bartholomew 2003), whose taxonomy remains partly unresolved. A recent study found that original materials containing taxonomically discordant elements may be responsible for some taxonomic confusion in this subgenus (Wu et al. 2019). In our recent work, we found another example of this kind, which we clarify below.

In 1912, Koehne (1912) described *P. zappeyana* Koehne, based on *Wilson 45* and *Wilson 70* (part). In the same work, he established a new variety named *P. zappeyana* var. *subsimplex* Koehne, based on *Wilson 45a* and *Wilson 3526*. After the publication of these two taxa, Schneider (1912) recognised *P. zappeyana*, but mentioned *P. zappeyana* var. *subsimplex* as uncertain in his famous dendrological encyclopaedia ‘*Illustriertes Handbuch der Laubholzkunde*’. Thereafter, with no explanation, Silva Tarouca and Schneider (1922) indicated *P. zappeyana* to be a synonym of *P. concinna* Koehne (which was treated as a synonym of *P. veitchii* Koehne by Wu et al. (2019)) by placing the former in parentheses following the latter. After that, these two taxa were neglected until Li and Bartholomew (2003) unexplainably synonymised them with *P. trichostoma* Koehne, which is totally different from the treatment of Silva Tarouca and Schneider (1922).

To resolve the incongruence between these two taxonomic treatments, in this study, we investigated the nomenclature and the identities of *P. zappeyana* and *P. zappeyana* var. *subsimplex* with the reference to their protologues and original materials.

Results and discussion

In the protologue of *P. zappeyana*, Koehne (1912) expressed his concern about the identities of *Wilson 45* and *Wilson 70*, saying that the leaves of the former are larger, while the leaves of the latter are smaller. Similarly, he was somewhat doubtful about the identities of *Wilson 45a* and *Wilson 3526*. He did not combine the morphological descriptions of the two collections into a single description as he did when describing other taxa, but gave two descriptions of the two collections separately.

Our examination of the original collections confirmed Koehne’s concern on the identities of the original collections, demonstrating that both the original specimens of *P. zappeyana* and *P. zappeyana* var. *subsimplex* represent at least two different taxa.

For *P. zappeyana*, we have successfully sorted out one sheet of *Wilson 45* kept at A and two sheets of *Wilson 70* kept at E and US. The specimen of *Wilson 45* (A 00032250, Fig. 1A) consists of a leafy branch with mature fruits and a small leafy branch and obviously belongs to *P. veitchii* Koehne. The leaves on this specimen are obovate-elliptic, 3.5–6.5 cm long, 1.8–3 cm broad, with netted veins that are prominent on the leaf back, with leaf margins serrate or biserrate and petioles 0.5–0.8 cm long. The peduncles are sessile. The fruits are ovoid to nearly globular and black.

In the protologue, Koehne (1912) indicated that *Wilson 70* partly belongs to *P. zappeyana* and partly to *P. pilosiuscula* var. *barbata* Koehne. We successfully traced two sheets of *Wilson 70* (E 00011305 and US 03718362) kept at E and US, both of which were determined as *P. zappeyana* by Koehne. However, we have failed to locate any specimen of *Wilson 70* that was annotated as *P. pilosiuscula* var. *barbata*. The specimen of *Wilson 70*, housed at E, was determined by T.-T. Yu in July 1948 as *P. latidentata* Koehne (which was reduced as a synonym of *P. trichostoma* in ‘*Flora Reipublicae Popularis Sinicae*’ (Yu and Li 1986)) and then was annotated by C.-L. Li in

1994 as an isotype of *P. zappeyana*, which was subsequently corrected to a syntype by herbarium curators. It is reasonable to infer that *P. zappeyana* was synonymised by Li and Bartholomew (2003), based on the specimen of *Wilson 70* kept at E. Regarding the identification of these two specimens of *Wilson 70*, we agree that they can be identified as *P. trichostoma* s.l., but we think they can also be determined as *P. stipulacea* Maxim. which is distinguished from *P. trichostoma* by blossoming slightly before the leaves (or nearly so) and ovate or auriculate stipules on vegetative branches (Li and Bartholomew 2003). Both specimens of *Wilson 70*, kept at E and US, only carry leafy branches with short branchlets and lack the important diagnostic characters.

For *P. zappeyana* var. *subsimplex*, we located one sheet each of *Wilson 45a* (A 00032252, Fig. 1B) and *Wilson 3526* (A 00032251) kept at A and E. The specimen of *Wilson 45a* carries two leafy branches with infructescences and a young leafy branch. This specimen should also be identified as *P. veitchii*. The plants on this specimen show features similar to *Wilson 45*, such as the obovate-elliptic leaves with serrate or biserrate leaf teeth, sessile peduncles and ovoid to subglobular fruits in black colour. The specimen of *Wilson 3526* contains two branches with flower buds that are mostly not open and leaves that are not expanded. This specimen could be identified as *P. clarifolia* Schneider. The leaves of this specimen are mostly simply serrate and toothed with minute apical glands. The leaf back is pubescent along veins and the petioles are glabrous. The inflorescences are umbellate with 1–2 flowers, with bracts that are toothed with capitate apical glands. The pedicels and hypanthium are glabrous.

According to Art. 9.11 of ICN (Turland et al. 2018), it is necessary to designate the lectotypes of *P. zappeyana* and *P. zappeyana* var. *subsimplex*. However, *P. zappeyana* var. *subsimplex* is invalid because, when Koehne (1912) established *P. zappeyana* var. *subsimplex*, two descriptions were separately given to the collections, but no combined description under the name directly, making the name unaccompanied by a description of this taxon (Art. 38.1(a)). Therefore, only the lectotypification of *P. zappeyana* is proposed here.

Either *Wilson 45* or *Wilson 70* can be chosen as the lectotype of *P. zappeyana* as they both correspond to the original description and match the diagnosis. We prefer to choose *Wilson 45* over *Wilson 70* as the lectotype of *P. zappeyana* for two reasons. First, as the identification of *Wilson 70* remains taxonomically ambiguous, we prefer to choose *Wilson 45* to make the identity of *P. zappeyana* more unambiguous. Second, we think Koehne apparently considered *P. zappeyana* and *P. zappeyana* var. *subsimplex* as belonging to the same species; and *Wilson 45* and *Wilson 45a* are the nomenclatural elements which provide a common taxonomy to link these two names together, though the latter was not validly published.

Therefore, we chose the specimen of *Wilson 45* as the lectotype of *P. zappeyana* and, furthermore, treated *P. zappeyana* as the synonym of *P. veitchii*, based on the lectotype. This decision deviates from the taxonomy of Li and Bartholomew (2003), but agrees with the older interpretation by Silva Tarouca and Schneider (1922). It makes no changes to the currently accepted nomenclature of Chinese cherries, but contributes to its further disambiguation.

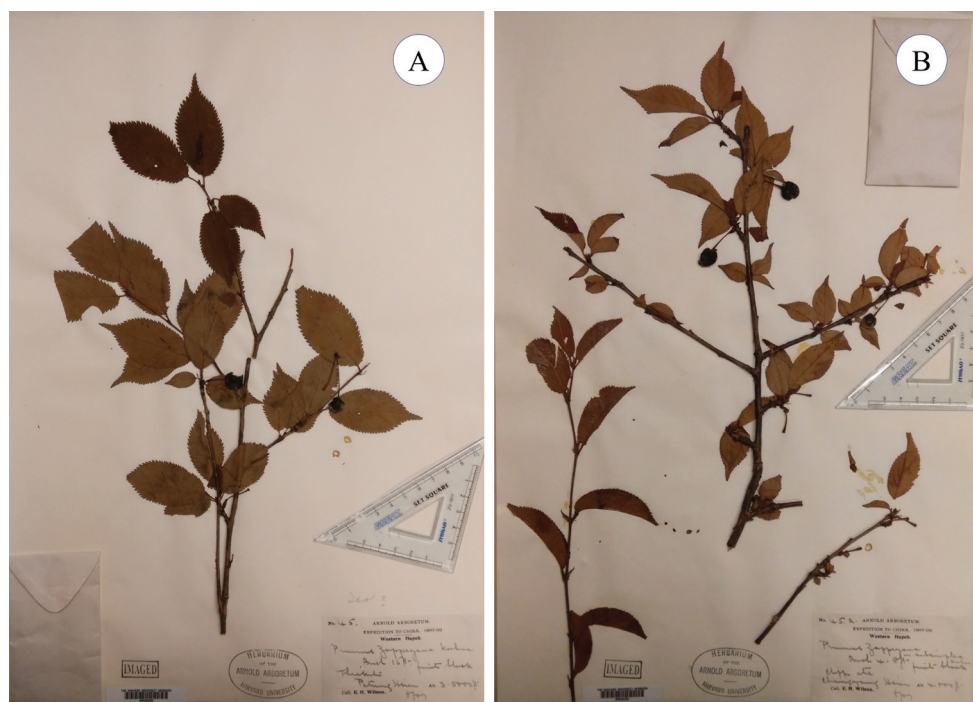


Figure 1. Original specimens of *Prunus zappeyana* and *P. zappeyana* var. *subsimplex* **A** lectotype of *P. zappeyana*, Wilson 45 (A 00032250) **B** one of the original specimens of *P. zappeyana* var. *subsimplex*, Wilson 45a (A 00032252).

Taxonomic treatment

***Prunus veitchii* Koehne, Pl. Wilson. (Sargent) 1(2): 257. 1912.**

Prunus veitchii Type: CHINA. Western Hubei, April 1900, *E.H. Wilson* 66 (lectotype designated by Wu et al. (2019: 66), US00130697, image!; isolectotypes E00417568, image!, HBG511147, image! NY00415930, image! A00032230 part, image!)

= *Prunus zappeyana* Koehne, Pl. Wilson. (Sargent) 1(2): 221. 1912, syn. nov. Type: CHINA. Hubei Province: Badong (Patung) County, alt. 1000–1600 m, June 1907, *E.H. Wilson* 45 (lectotype designated here: A00032250!) (Fig. 1A). Remaining syntype: China. Hubei, woods, alt. 1300–2000 m, June 1907, *E. H. Wilson* 70 (E 00011305, image!).

Note. For a full list of synonyms, descriptions and distribution of *Prunus veitchii*, see Wu et al. (2019).

Acknowledgements

This study was supported by National Natural Science Foundation of China (NSFC31370246) and the Strategic Priority Research Program of Chinese Academy of Sciences (Grant No. XDB31000000). We are grateful to the curators of Herbaria A, E, US and SYS for access to the specimens and the images of specimens. We thank Dr Alexander Sennikov and one anonymous reviewer for their careful reviews.

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Allium heterophyllum (Amaryllidaceae), a new species from Henan, China

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Abstract

Allium heterophyllum D.F.Xie & X.J.He, **sp. nov.** (Amaryllidaceae), is a new species from Henan, China and is described based on morphological and molecular evidence. It is morphologically most similar to *A. dumeubuchum* in the rhomboid scape in cross-section. However, distinctive differences were detected in perianth color, leaf shape and cross-section, flowers' density as well as flowering season. Similarly, the karyotype of *A. heterophyllum* is $2n = 2x = 16$ and in *A. dumeubuchum* is $2n = 4x = 32$. Phylogenetic analysis based on nuclear ribosomal Internal Transcribed Spacers (ITS) and three cpDNA regions strongly supports that *A. heterophyllum* is a member of *Allium* section *Rhizirideum* and sister to the other species of this section (e.g. *A. senescens*, *A. spirale*, and *A. prostratum*). Currently, only one population and approximately 120 individuals were discovered; the development of scenic spots in this region may affect its growth and threaten this population. Therefore, this new species is preliminarily considered as Near Threatened (NT) according to criteria of the IUCN Red List.

Keywords

Allium, chromosome number, morphology, new species, phylogenetic analysis

Introduction

Allium L. is one of the largest genera of Amaryllidaceae (Fritsch et al. 2010; Li et al. 2010), and includes more than 950 species, that are characterized by rhizomatous or bulbous geophytes and widely used for food, medicine or as ornamental plants (e.g. garlic, leek, onion, and shallot) (Herden et al. 2016; Pellicer et al. 2017). Phylogenetic studies suggest that the genus *Allium* differentiated into three evolutionary lineages (Fritsch and Friesen 2002), and can be classified into 15 subgenera and 72 sections (Friesen et al. 2006). *Allium* species are widely distributed in the Northern Hemisphere, mostly from the dry subtropics to boreal zones, and the genus has two probable diversity centers, one stretching from the Mediterranean Basin to Central Asia and Pakistan, the other is in western North America (Fritsch and Friesen 2002). This genus is highly speciose in China with more than 150 taxa recorded and new species are frequently being discovered, such as *A. tetraploideum*, *A. xinlongense*, and *A. jingshanense* (Li et al. 2019; Xie et al. 2020a; Huang et al. 2021).

The typical section of *Allium* subgenus *Rhizirideum* (G.Don ex W.D.J.Koch) Wendelbo, section *Rhizirideum* G.Don ex W.D.J.Koch has 25 species (including the recently published new species *A. dumebuchum*) (Jang et al. 2021), and their species are characterized by membranous tunics in enclosed bulbs, which are attached to horizontal rhizomes, hemicylindrical to plain leaf, and white to purple flowers (Sinitsyna et al. 2016). Additionally, the species of section *Rhizirideum* share a similar karyotype with the basic chromosome number of $x = 8$ (Sinitsyna et al. 2016). Most previous studies suggest that this section belongs to the third evolutionary lineage of *Allium* and is mainly distributed from Europe to East Asia, especially in temperate Asia (Fritsch and Friesen 2002; Friesen et al. 2006; Li et al. 2010; Choi et al. 2012; Sinitsyna et al. 2016). In China, species of this section are mainly distributed in Northern provinces, such as Inner Mongolia, Henan, and Xinjiang.

Previous phylogenetic studies suggested that the section *Rhizirideum* is a strong monophyletic unit (Friesen et al. 2006; Li et al. 2010). Following this, the nomenclature, distribution regions, and characteristics of all species in this section were further identified and listed (Sinitsyna et al. 2016; Sinitsyna and Friesen 2018). However, given the morphological diversity and prevalent polyploidy of this section (di-, tetra-, penta-, and hexaploids were detected) (Friesen 1988, 1992; Kamelin 2004), as well as the frequent discovery of new species, further studies are required to clarify taxonomic uncertainties.

Many new *Allium* species have been described this year (Armağan 2021; Balos et al. 2021; Friesen et al. 2021; Pandey et al. 2021). During our field investigation in Songxian county in September 2021 (Henan province, China), we discovered a new *Allium* species (Fig. 1) that was similar to the members of *Allium* section *Rhizirideum* but had morphological differences. Thus, we conducted two field trips to collect fresh materials for further study. Here, we aimed to (1) investigate this new species *Allium heterophyllum* based on morphological, karyotypic data and molecular approaches, and (2) conduct a comprehensive description of this new species, and thereby confirm the taxonomic relationships with other morphologically similar species in section *Rhizirideum*.

Materials and methods

Sampling and morphological analyses

Living plants and samples of *Allium heterophyllum* were collected in Songxian county (33°41'25.61"N, 111°59'24.31"E, Altitude: 1347 m), Henan province, China. Voucher specimens were deposited at the herbarium of Sichuan University (SZ). Measurements of flowers, leaves, scapes, bulbs and rhizomes were taken from living plants and examined and measured by stereo binocular microscope (Nikon, Japan). A total of 18 diagnostic characteristics of the new species were identified and compared to six closely-related species in the *Allium* sect. *Rhizirideum*.

Karyotype analysis

Root tips were excised from the bulbs and pre-treated in saturated p-dichlorobenzene at 4 °C for 9 hours in the absence of light, then rinsed twice using distilled water and transferred to 3:1 ethanol-acetic acid for 10 hours. Subsequently, we rinsed the samples twice with distilled water and hydrolyzed in 1 mol/L HCL at 60 °C for 10 min. Finally, the samples were stained with the improved carbolfuchsin for one hour and squashed for observation. More than ten individuals were checked with three to five plates being investigated for each individual and well-spread metaphase plates were observed and further photographed using the Olympus BX43 electron microscope (Tokyo, Japan).

DNA extraction, amplification and sequencing

Total DNA was extracted from silica gel dried young leaves of the new species using the Tiangen plant genomic DNA extraction kit (Tiangen Biotech, Beijing) according to the protocols of the manufacturer. The complete nucleotide ribosomal ITS region (ITS1, 5.8S and ITS2) was amplified using the ITS primers from White et al. (1990), and three other chloroplast regions (*ndhJ-trnF*, *psbD-trnT* and *psbJ-petA*) were also collected based on corresponding primers (Taberlet et al. 1991; Shaw et al. 2007). The detailed primers and amplification information are shown in Table 1. All PCR products were visualized on 2% agarose TAE gel and sent to Sangon Biotech Institute (Shanghai, China) for sequencing. The DNA sequences generated in this study have been deposited in NCBI (Suppl. material 1: Table S1).

Sequence download, extraction and phylogenetic analysis

We downloaded an extensive dataset of ITS, cpDNA regions and chloroplast genomes from NCBI to better perform the phylogenetic analysis and confirm the systematic position of this new species. We downloaded 107 ITS sequences from 43 *Allium* species, and 69 cpDNA regions and 55 chloroplast genomes from 10 and 37 *Allium* species, respectively. To conduct the phylogenetic analysis using the three cpDNA regions, we

Table 1. Primers and amplification information were used for DNA barcoding in this study.

Fragment	Marker	Sequence 5'-3'	Tm (°C)	Reference
ITS	ITS4	TCCCTCCGCTTATTGATATGC	55.0	White et al. 1990
	ITS5	GGAAGTAAA AGTCGTAACAAGG		
ndhJ-trnF	ndhJ	ATGCCYGAAGTTGGATAGG	54.2	Shaw et al. (2007)
	tabE	GGTTCAAGTCCCTCTATCCC		
psbD-trnT	psbD	CTCCGTARCCAGTCATCCATA	54.8	Shaw et al. (2007)
	trnT ^{GGU}	CCCTTTTAACTCAGTGGTAG		
psbJ-petA	psbJ	ATAGGTACTGTARCYGGTATT	54.5	Shaw et al. (2007)
	petA	AACARTTYGARAAGGTTCAATT		

The PCR program began with 4-min initial denaturing at 94 °C followed by 35 cycles of 1-min denaturation at 94 °C, 1-min annealing at above-mentioned Tm, and 1.5-min extension at 72 °C, a final extension was run for 5 min at 72 °C.

extracted each cpDNA region from the 56 chloroplast genomes. The detailed Genbank accession information of all sequences is provided in Suppl. material 1: Table S1.

Newly sequenced ITS and cpDNA regions were assembled using the SeqMan software (Burland 2000), and then aligned with Clustal X (Jeanmougin et al. 1998) and further manually adjusted in MEGA 7.0 (Kumar et al. 2016). Two methods (Maximum likelihood and Bayesian inference) were used to perform the phylogenetic analysis. Maximum likelihood (ML) analyses were conducted in RAxML 8.2.8 (Stamatakis 2014) with GTR+G model and 1,000 bootstrap replicates. Bayesian inference (BI) was performed in MrBayes v 3.2.7 (Ronquist et al. 2012) with GTR+G being selected as the optimal model of nucleotide substitution using the Akaike information criterion (AIC; Burnham and Anderson 2002) as implemented in IQ-TREE (Trifinopoulos et al. 2016). The Markov Chains (including three heated chains and one cold chain) were run for 1 × 10⁸ generations with a sample frequency of 50 and the initial 20% of the samples discarded as burn-in to confirm the stationarity. The remaining trees were used to build a 50% majority-rule consensus tree.

Results and discussion

Taxonomy treatment

Allium heterophyllum D.F.Xie & X.J.He, sp. nov.

urn:lsid:ipni.org:names:77255008-1

Figs 1, 2, 3

Type. CHINA. Henan Province: Songxian County, Longchiman mountains, 111°59'24.31"E, 33°41'25.61"N, 1347 m alt., 04 September 2021, Anonymous, XDF20210904 (Holotype: SZ; Isotype: SZ).

Diagnosis. *Allium heterophyllum* resembles *A. dumebuchum* due to its rhomboid scape in cross-section. However, it is clearly distinguished from *A. dumebuchum* in perianth (white to light purple vs. light purple), leaves (not tortuous and not flesh vs. slight tortuous and flesh), the cross-section of leaves (two types vs. one type), flowers'

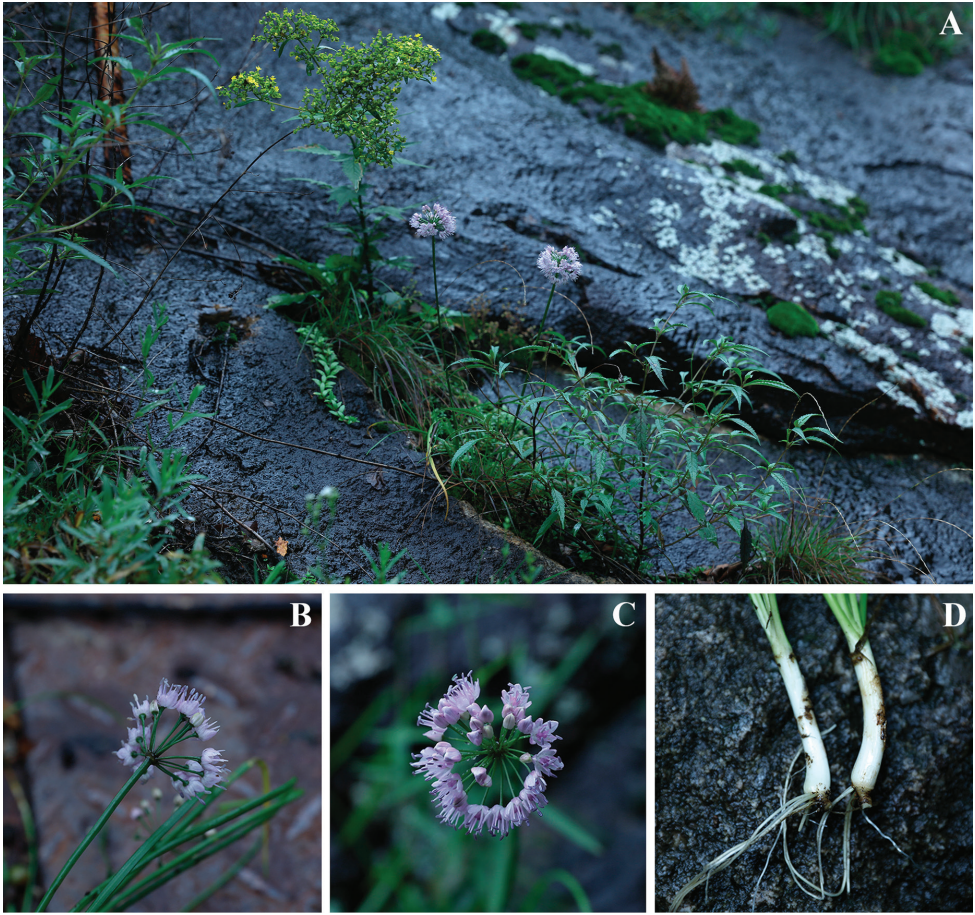


Figure 1. Living images of *Allium heterophyllum* **A** habitat, growing on the open slope of rock **B, C** inflorescence, light purple with loose flowers **D** bulb with the horizontal rhizome.

density (loose inflorescence vs. many-flowered) (Fig. 2; Table 2), flowering season (late August to September vs. late September to October), and karyotype ($2n = 16$ vs. $2n = 32$) (Fig. 4). Compared to other *Rhizirideum* species (e.g., *A. scenescens*, *A. spirale* and *A. spurium*), *A. heterophyllum* also shows distinctive morphological characters, such as rhomboid scape in cross-section, unique two types of leaves, loose flowers, white to light purple color of perianth and filaments, and flowering season.

Description. Perennial herbs, bulbs solitary, paired or clustered, ovate-cylindric or conical, 5.0–15 mm in diameter, tunics membranous, white, attached to a horizontal or oblique rhizome, 5.0–20.0 mm in diameter, surface usually blackish gray. Leaves linear, 5.0–10.0, solid in cross-section, 1.0–30.0 (–45.0) cm long and 1.5–4.0 mm wide, usually shorter than scape, sometimes equal to the length of scape, exposed sheaths in 1/7; cross-section of leaves exposed two types of morphologies, canaliculated with one bulge in the back or flat with irregularly one or two-edged

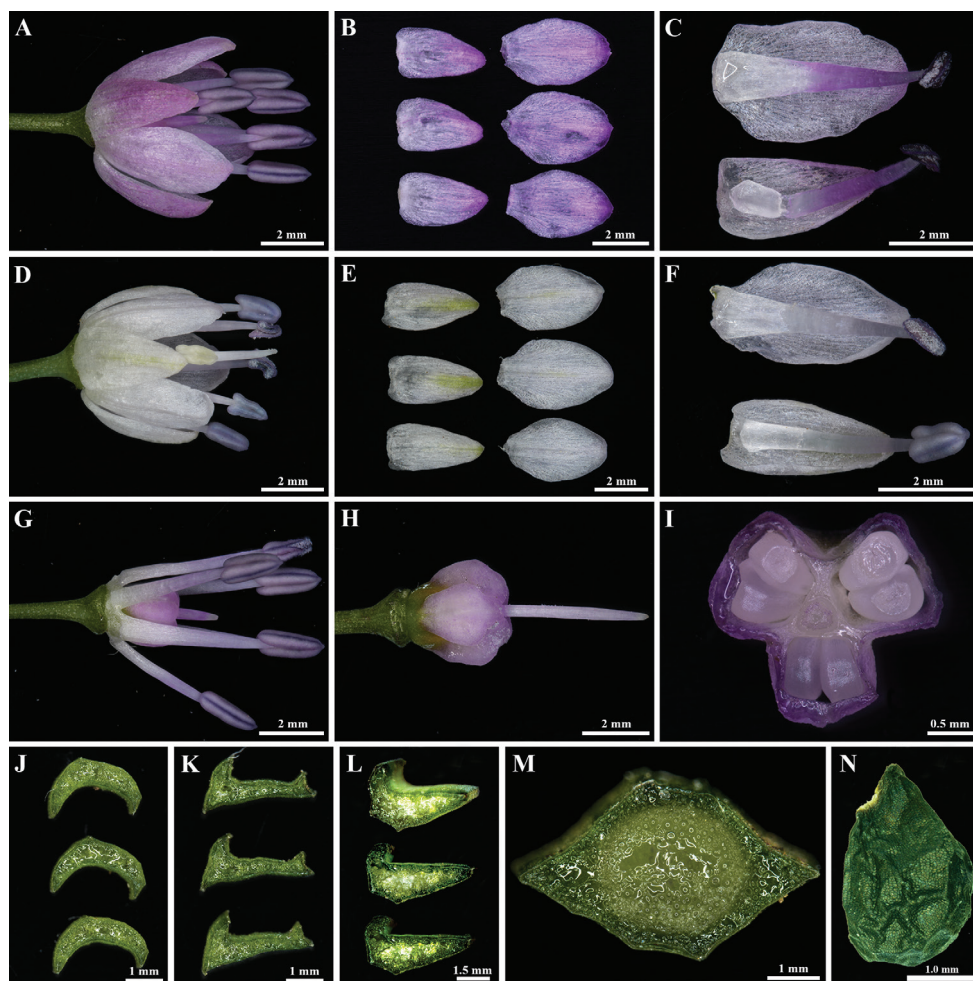


Figure 2. Morphological characters of *Allium heterophyllum* **A, D** single flower with light purple or white color **B, E** outer (left) and inner (right) tepals **C, F** inner (top) and outer (bottom) tepals and stamen **G** stamen and trait at the base **H** ovary **I** cross-section of ovary showing the carpels **J–L** the cross-section of leaf showing the blade characters **M** cross-section of rhomboid scapes **N** seeds' characters.

margin; the leaves' shape differences are most obvious in flower and fruit periods but not obvious in young leaves. Scapes rhomboid, solid in cross-section, 25.0–45.0 cm long, and 15.0–25.0 mm in diameter. Spathe 1-valved, persistent and inconspicuous; inflorescence umbellate hemispheric, loose. Pedicels equal, 10.0–15.0 mm; perianth white to light purple, inner tepals 4.0–6.0 mm, longer than outer ones, elliptical, apex obtuse; outer tepals 3.0–4.0 mm, ovate-elliptical. Filaments equal and exserted, white to light purple in the upper part, 1.5 × as long as perianth segments and connate at the base of the perianth. Outer one subulate, inner filaments narrowly triangular; anthers elliptical, purple-grey. Ovary obovoid, trigonous, white to light purple, without concave nectaries. 3 carpel and ovules 2 per locule, style exserted,



Figure 3. Photograph of the Holotype of *Allium heterophyllum*.

stigma punctiform. Capsule obovate; seeds black, rhomboidal, 1.5–2.0 mm wide and 2.5–3.0 mm long (Fig. 2; Table 2).

Etymology. The new species epithet “*heterophyllum*” is based on the unique leaves’ characters, its leaves exposed two types of morphologies, canaliculated with one bulge

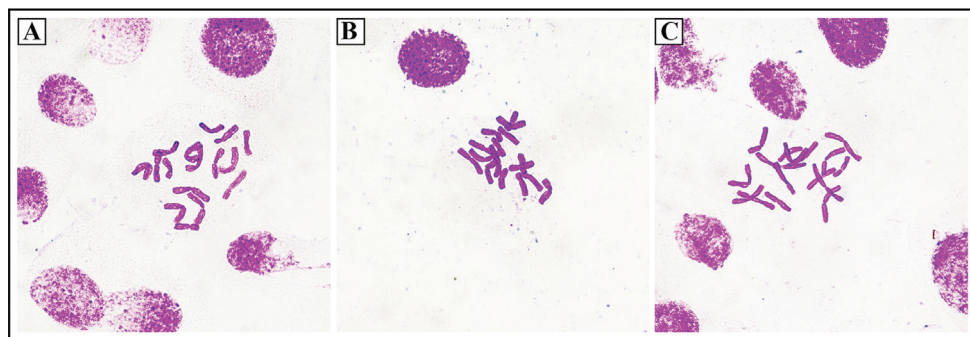


Figure 4. The chromosome complement of *Allium heterophyllum* ($2n = 2x = 16$).

in the back or flat with irregularly one or two-edged margin, and the differences in the leaves are most obvious in flowering and fruit periods. (Fig. 2).

Phenology. Through two field investigations, *A. heterophyllum* was flowering from late August to September and fruiting from late September to October.

Habitat and ecology. Currently, *A. heterophyllum* is only known from the type population in Longchiman Mountains in Songxian County, Henan, China. This species grows on the open slope of rock by the river with a small amount of soil attached, sometimes rooting in crevices, holes or steps of the rock face at an elevation from 1250 m to 1400 m.

Chinese name. Yi Ye Jiu (异叶韭).

Morphological analysis

The latest study suggested that as many as one-third of the species could face extinction within the next 50 years due to biodiversity loss resulting from climate change (Román-Palacios and Wiens 2020). Therefore, the discovery of a new species is always awe-inspiring news; in 2020, more than 300 new species were discovered in China (Du et al. 2021), which fills us with hope. In this study, we confirmed a new *Allium* species named *Allium heterophyllum* based on morphological comparisons and molecular analysis. This new species is morphologically most similar to *A. dumebuchum*, which is endemic to Ulleungdo island of Korea (Jang et al. 2021). Although these two species shared similar rhomboid scape in cross-section, their flowering times are different (late August to September vs. late September to October), and distinctive morphological characters were also detected in the perianth color, leaves' shape and cross-section, and flowers' density (Table 2). Additionally, *A. heterophyllum* is a diploid species with a somatic chromosome number of $2n = 16$, while *A. dumebuchum* is a tetraploid species with $2n = 32$ (Fig. 4). Through analyzing 18 diagnostic characteristics, we also found obvious differences between *A. heterophyllum* and other species in section *Rhizirideum* (e.g., *A. senescens*, *A. prostratum*, and *A. spirale*), such as the leaves, which in *A. dumebuchum*, *A. minus* and *A. senescens* are fleshy and glaucous and leathery and lustrous in *A. spirale* and *A. spurium*. Moreover, the flowering season is also

Table 2. The diagnostic morphological characters of *Allium heterophyllum* and related species.

Character		<i>A. heterophyllum</i>	<i>A. dumebulum</i>	<i>A. spirale</i>	<i>A. spurium</i>	<i>A. minus</i>	<i>A. senescens</i>	<i>A. nutans</i>
Bulb	growth pattern	solitary, paired or clustered	clustered	clustered	solitary or paired	clustered	solitary or paired	solitary or paired
	shape	conical to ovate-cylindric	conical to cylindric	conical to cylindric	cylindric to conical-cylindric	conical to cylindric	conical to ovate-cylindric	narrowly cylindric to subconical
	diameter (mm)	5.0–15.0	9.6–15.0	5.0–15.0	5.0–15.0	4.3–8.6	10.0–20.0	15.0–20.0
Rhizome	growth pattern	oblique to horizontal	oblique to horizontal	horizontal	horizontal	oblique	horizontal	horizontal or oblique and stout
Leaf sheath	exposed or buried	exposed	exposed	buried	buried	exposed	exposed	exposed
Leaf blade	shape	linear, solid, not fleshy, canaliculated with one bulge in the back or flat with irregularly one or two-edged margin	ascending, slightly tortuous, linear, flat and solid in cross-section, flesh, apex obtuse to rounded	linear, spirally tortuous, flat, main veins and margins minutely scabrous-denticulate, rarely smooth, fleshy, apex obtuse	narrowly linear, straight, flat to convex-flat, fleshy, margin minutely scabrous, apex acute to gradually attenuate, truncate	ascending, spirally tortuous, flat, fleshy, linear, solid, fleshy, obtuse to rounded at apex	spirally arranged, broadly linear, fleshy, sometimes slightly falcate	broadly linear, subfalcate, flat, thick, fleshy, smooth, apex obtuse
	length (cm)	15–45.0	19.5–38.0	20.0–45.0	15–30.0	11.4–24.5	23.0–45.0	30.0–55.0
	width (mm)	1.5–4.0	3.8–13.0	4.0–10.0	1.5–4.0	2.8–4.5	5.0–15.0	6.0–15.0
Umbel	shape	hemispheric, loose	subglobose, many-flowered	hemispheric to subglobose, many-flowered.	laxly hemispheric, many-flowered.	hemispheric	hemispheric to globose, many-flowered	globose, densely many-flowered
Scape	cross-section	rhomboid	rhomboid	flattened-winged	rhomboid to subterete	subterete	subterete	2-angled, narrowly 2-winged
	length (cm)	25.0–45.0	23.4–49.0	33.0–65.0	10.0–40.0	11.7–20.5	25.8–70.0	30.0–60.0
	diameter (mm)	1.5–2.5	2.5–5.6	4.0–5.1	1.5–2.5	1.5–1.6	3.0–5.5	3.5–6.0
Pedicle	length (mm)	10.0–15.0	9.8–11.2	6.0–12.4	7.6–11.1	8.7–11.1	8.0–13.0	9.0–15.5
Spathe		1-valved, persistent and inconspicuous	unknown	2-valved, persistent	2-valved, usually caducous	unknown	2-valved, persistent	2-valved, persistent
Perianth	color	white to light purple	light purple	reddish purple	strong purple or pale purple	pale purple	pale purple	pale red to pale purple
Inner tepal	shape	elliptical	elliptical to ovate-elliptical	ovate-elliptical	ovate-elliptical	elliptical	elliptical	ovate
	length (mm)	4.0–6.0	5.2–7.2	4.0–6.8	3.9–6.3	4.0–4.8	4.3–6.4	5.0–6.5
	width (mm)	2.2–2.5	3.4–4.5	2.0–4.2	2.2–3.4	1.2–1.9	1.8–2.9	2.2–3.0
Outer tepal	shape	ovate-elliptical	ovate-elliptical	ovate-elliptical	ovate-elliptical	ovate-oblong	ovate-elliptical	narrowly ovate
	length (mm)	3.0–4.0	4.8–6.1	3.1–5.0	2.9–5.2	3.7–4.6	3.1–5.2	4.5–5.5
	width (mm)	1.6–1.9	2.1–3.7	1.3–3.0	1.1–2.3	1.1–1.7	1.1–2.5	1.5–2.0
Filament	exsertion	exserted	exserted	exserted	exserted	included	exserted	exserted
	length (mm)	6.3–7.5	6.2–8.4	5.3–8.8	5.0–7.0	3.2–4.4	4.6–6.9	6.5–8.5
Base of inner filament	shape	narrowly triangular	narrowly triangular	subulate	subulate	broadened in the lower half	broadened in the lower half	broadened in the lower half, 1-toothed on each side
Anther	color	purple grey	purple	purple	yellow	reddish	black or yellowish-brown	yellow
	length (mm)	1.8–2.3	2.2–2.5	1.7–2.2	1.7–2.0	1.3–1.4	1.5–2.0	1.8–2.3
	width (mm)	0.9–1.4	0.9–1.1	0.7–1.0	0.6–0.8	0.6–0.8	0.5–0.8	0.6–0.9
Ovary	shape	obovoid	obovoid	broadly ovoid	ovoid	obovoid	obovoid	oblong-globose
Flowering season		late Aug. to Sep.	late Sep. to Oct.	Aug. to Sep.	Jul. to Aug.	May to Jul.	Jul. to Aug.	Jun. to Aug.
Chromosome number (2n)		2n = 16	2n = 32	2n = 16, 32	2n = 16, 32	2n = 16	2n = 32	2n = 16, 17, 24, 28, 32, 44, 48, 56, 64, 72

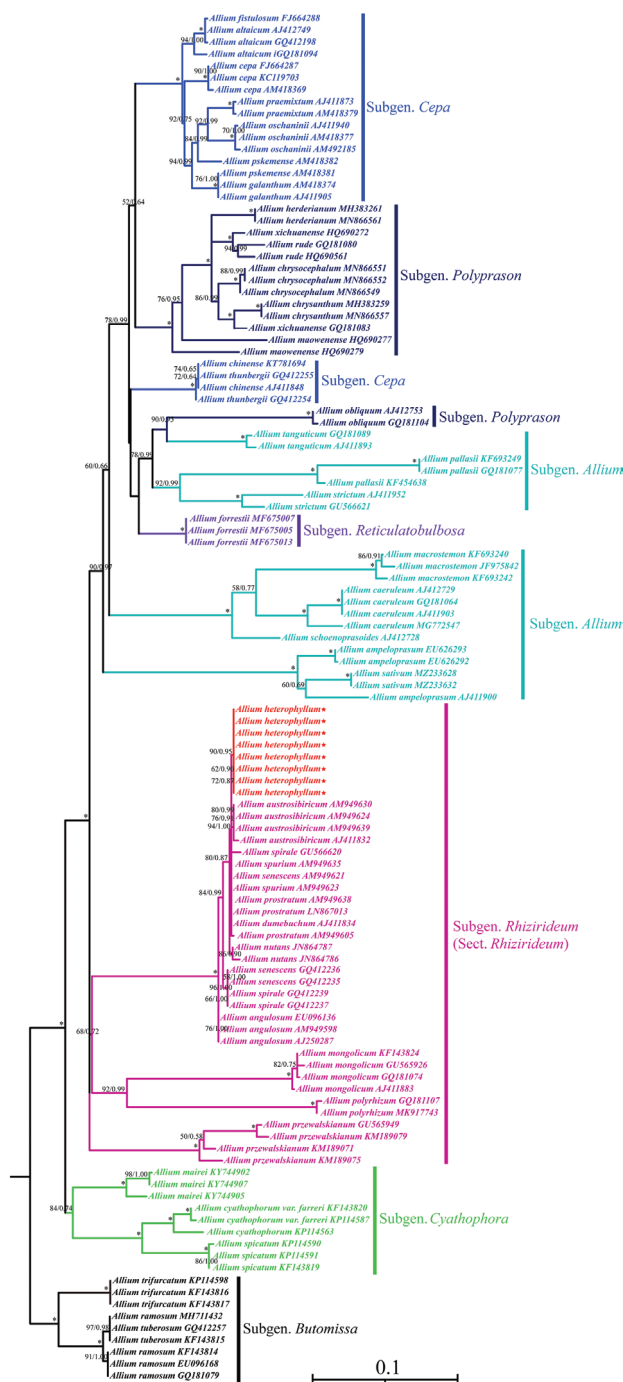


Figure 5. Phylogenetic relationships inferred from ITS. Trees constructed with maximum likelihood (ML) and Bayesian inference (BI). Support values reported above the branches are bootstrap values of ML and posterior probability of BI. * = maximum support in the two analyses. Samples of *Allium* sect. *Rhizirideum* are in rose red and the sequences of *Allium heterophyllum* are in red and marked with the star.

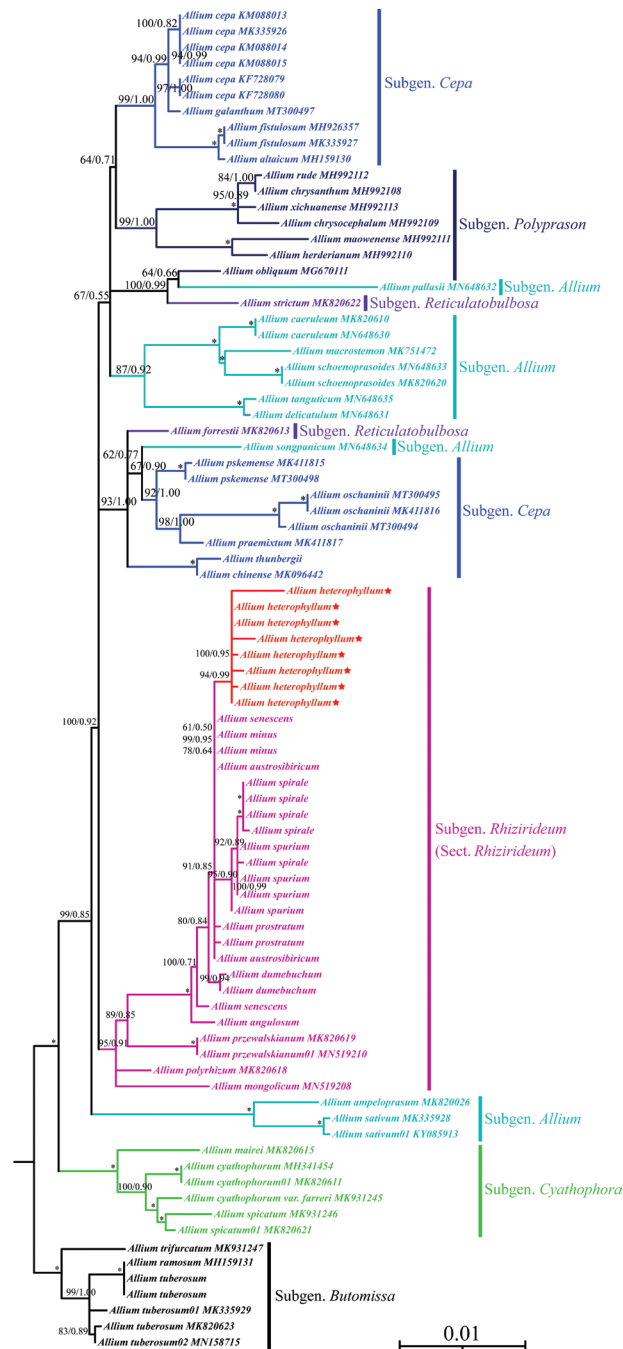


Figure 6. Phylogenetic relationships inferred from three cpDNA alignments. Trees constructed with maximum likelihood (ML) and Bayesian inference (BI). Support values reported above the branches are bootstrap values of ML and posterior probability of BI. * = maximum support in the two analyses. Branches of *Allium* sect. *Rhizirideum* are in rose red and the samples of *Allium heterophyllum* are in red and marked with the star.

different, because other species of section *Rhizirideum* usually bloom from May or July, except *A. spirale*, which blooms from August to September. Further differences are also reflected in the color of perianths, filaments and anthers (Table 2).

Phylogenetic analysis

Total ITS alignments were 703 bp in length with 446 variable sites (63.44%) and 421 parsimony-informative characters (PICs; 59.89%). Alignments of the three cpDNA regions possessed 3708 bp with 707 variable sites (19.07%) and 432 PICs (11.65%). The phylogenetic tree from ITS data was consistent with the cpDNA data set tree, in which the subgenus *Rhizirideum* is monophyletic and subgenera *Allium*, *Cepa*, and *Polyprason* are polyphyletic (Figs 5, 6). Moreover, all individuals of *A. heterophyllum* clustered into monophyly in the ITS and cpDNA trees with high support values (Figs 5, 6).

Our phylogenetic results detected a similar topology to previous studies (Friesen et al. 2006; Li et al. 2010; Choi et al. 2012; Sinitsyna et al. 2016; Xie et al. 2020b; Jang et al. 2021). ITS and cpDNA regions strongly support that *A. heterophyllum* is a member of section *Rhizirideum*, and sister to the other species of section *Rhizirideum* (e.g. *A. senescens*, *A. spirale*, and *A. prostratum*). Although *A. heterophyllum* is morphologically most similar to *A. dumeubuchum*, these two species are not closely related in the phylogenetic trees. According to previous studies, species in the section *Rhizirideum* are very widely distributed across the world and exhibit complicated relationships (Friesen et al. 2006; Choi et al. 2012; Sinitsyna et al. 2016; Jang et al. 2021), and this section is also regarded as a difficult taxon concerning classification and identification. Thus, morphological and phylogenetic analyses should be conducted at the population level in the future, thereby better investigating the species' relationships.

Conservation status

Through our field investigation, only one population with approximately 120 individuals of this species was discovered in the Longchiman Mountains. Given the development of tourism in this region, it is possible that this population may be threatened by pedestrian traffic, pollution, infrastructure development and other threatening processes associated with tourism. Therefore, this species is preliminarily considered as Near Threatened (NT) according to the IUCN Red List Categories and Criteria (IUCN 2019).

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Supplementary material I

Table S1

Authors: Deng-Feng Xie, Rui-Yu Cheng, Megan Price, Jun-Pei Chen, Jia-Qing Lei, Yi-Yang Zhang, Xing-Jin He

Data type: Sequence data accession NCBI numbers.

Explanation note: List of species accession NCBI numbers in this study.

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Link: <https://doi.org/10.3897/phytokeys.190.77449.suppl1>

Two new species of *Navicula* (Bacillariophyta) from Southeast Asia

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Abstract

We present light and scanning electron microscopical observations on two new species of *Navicula* Bory sensu stricto from Southeast Asia. *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, **sp. nov.** differs from taxa with similar outline and size by the combination of simple drop-like external raphe endings deflected to the primary valve side, presence of well-developed external longitudinal grooves and relatively low lineolae density. *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, **sp. nov.** can be discriminated from the taxa of similar valve shape and size by consistently lower lineolae density, and from the majority of them also by the structure of external proximal raphe endings with small projections in proximal parts and larger triangular insertions in distal parts. Some remarks on *Navicula* species diversity and its distribution in the Southeast Asia are given.

Keywords

Diatoms, distribution, freshwaters, morphology, *Navicula*, new species, Southeast Asia, taxonomy

Introduction

This investigation continues our description of new species from the genus *Navicula* Bory from Southeast Asia. With both freshwater and marine representatives, the genus *Navicula* Bory is the most taxon-rich genus of all diatoms (Kulikovskiy et al. 2016).

However, the number of species included in this genus has actually been decreasing over the last 20 years mainly because of the description of new genera that include species formerly in *Navicula*. The genus *Navicula* is easily distinguished from many other diatom genera by its lineolate uniseriate striae (Kulikovskiy et al. 2016). In some areas some morphologically unusual groups of species from the genus *Navicula* have been recognized, such as species with hyaline area on valve face. An example of this is *Navicula lacusbaicali* Skvortzov & Meyer from ancient Lake Baikal (Kulikovskiy et al. 2012). This group of *Navicula* formed species-flock (e.g. Kociolek et al. 2017); this is a group of closely-related taxa originated from single parent by explosive radiation (see Kociolek et al. 2019). New species from the genus *Navicula* are described often, indicating we are still gaining a better understanding of the real diversity in this genus. The taxonomy of some species in the genus is in need of clarification as well (Witkowski et al. 2010; Kulikovskiy et al. 2016).

Southeast Asia is a very interesting region for aquatic protist diversity, yet studies from the region are relatively few. More recently, we have been investigating diatoms and many other organisms of Southeast Asia (Glushchenko et al. 2021), including new genera and species, for example from Laos and Vietnam (e.g. Kulikovskiy et al. 2015, 2018, 2019; Glushchenko et al. 2016, 2017, 2018, 2019, 2020; Liu et al. 2018; Kezlya et al. 2020). A comprehensive review of diatom studies was summarized in Glushchenko et al. (2021). A few publications were dedicated to the diversity of new species from the genus *Navicula* (Chudaev et al. 2018; Kulikovskiy et al. 2020a,b, 2021) of Southeast Asia. Continuation of this work is important for understanding the diversity of the genus *Navicula* and diatoms as a whole from such an interesting region. Freshwater ecosystems from this region will need investigation in future for water quality assessment, using modern methods in ecological monitoring like DNA barcoding (Rimet et al. 2019). But a primary understanding of the biodiversity of the region is a necessary first step for future investigations and applying new methods.

The aim of this publication is to present a morphological investigation using light and scanning electron microscopy to describe two new diatom species of the genus *Navicula* from Southeast Asia.

Materials and methods

Samples from Vietnam were collected by M.S. Kulikovskiy and E.S. Gusev during expeditions organized and permitted by the Joint Russian-Vietnam Tropical Centre, Ecolan 1.2 and 3.2 projects. The sample from Laos was collected by E.L. Konstantinov during an expedition of Kaluga and Laos Joint Universities (Russia and Laos).

A list of all samples examined in this study with their geographic positions is presented in Table 1. Water mineralization and temperature measurements were performed using the Hanna Combo (HI 98129) device, Hanna Instruments, Inc., USA. Material was collected with a pipette into 15- and 50-ml polymer test tubes. Samples were fixed with 37% formaldehyde.

The samples were treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 h. Samples were subsequently boiled in concentrated hydrogen peroxide ($\approx 37\%$) to dissolve organic matter. They were washed again with deionized water four times at 12 h intervals. After decanting and filling with deionized water up to 100 ml, the suspension was spread onto coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax (refraction index = 1.73). Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective ($\times 100$, n.a. 1.4, differential interference contrast [DIC]) and Axiocam ERc 5s camera (Zeiss). Valve ultrastructure was examined by means of scanning electron microscope JEOL JSM-6380LA (JEOL Ltd., Japan) operating at 20 kV and 8 mm working distance (Faculty of Biology, M.V. Lomonosov MSU, Moscow). For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminium stubs after air-drying. The stubs were sputter coated with 50 nm of gold in an Eiko IB 3.

Fixed material and slides are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia. Isotypes are deposited in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Results

***Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov.**
Figs 1, 2

Navicula gottlandica sensu Lee, 2012 (fig. 4E) (Algal Flora of Korea vol. 3, num. 8).

Holotype. Slide 03572 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 1E.

Isotype. Slide MW-D 898s1 in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Type locality. Vietnam. Khánh Hòa Province, Suối Dầu Reservoir, benthos, 12°09.900'N, 109°03.200'E, 36 m elev., leg. E.S. Gusev, 10.08.2010.

Description. **LM** (Fig. 1A–P). Valves narrowly lanceolate with rostrate to subcapitate apices, length 29.7–49.0 μm , width 6.5–8.0 μm ($n=32$). Axial area narrow, slightly widening towards valve centre, central area transversely expanded, with irregular border due to unequal shortening of central striae, usually occupying about 1/2 of valve width. Striae radiate, becoming strongly convergent at the valve ends, 12–14/10 μm . Lineolae easy to resolve in light microscope, 24–27/10 μm . Raphe filiform to narrowly lateral, terminal fissures deflected to the secondary valve side, central pores straight or very slightly deflected to primary valve side, not close standing.

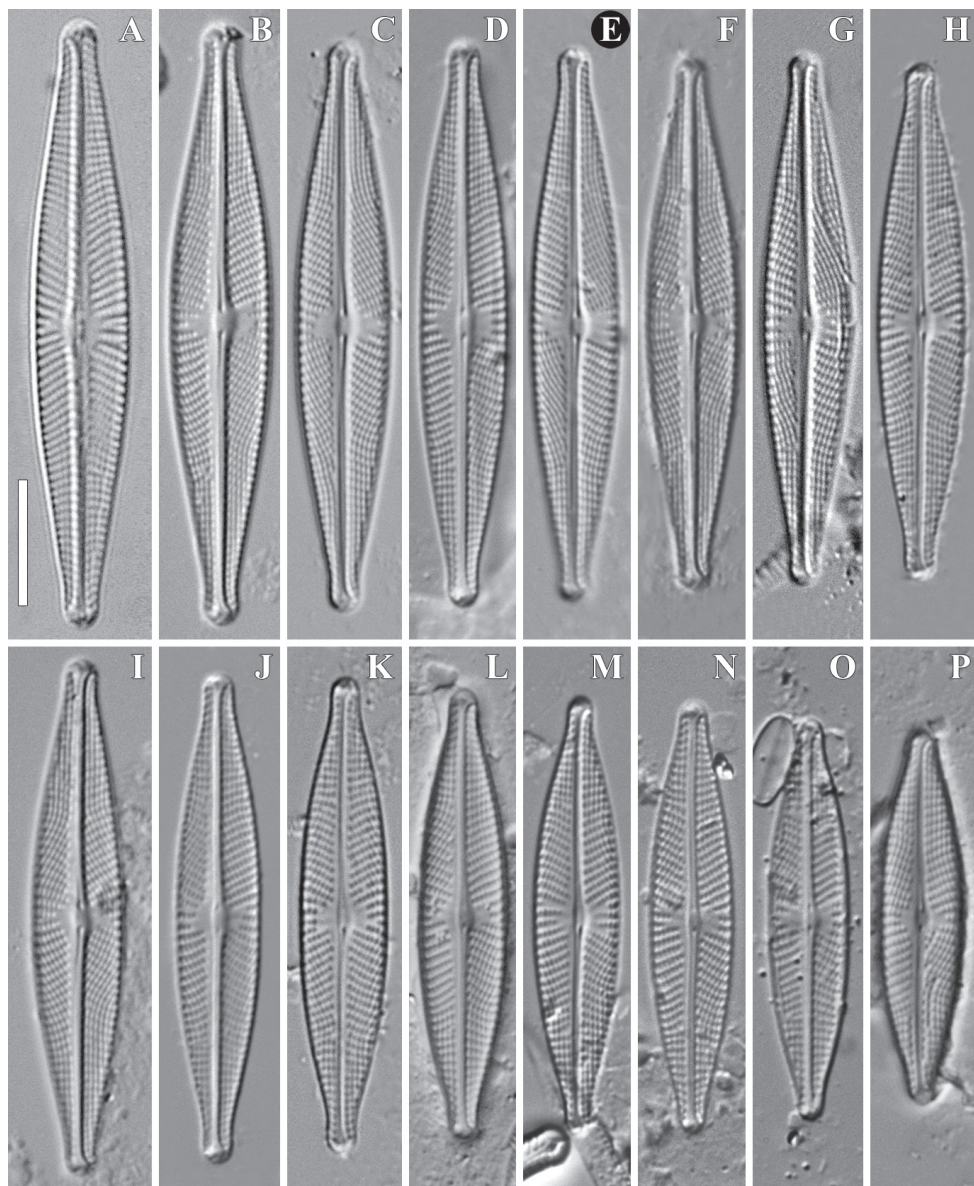


Figure 1. A–P *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Size diminution series. Vietnam, slides no 00269 (**A**), 00318 (**L, P, N**), 00323 (**I**), 00326 (**C**), 00328 (**B**), 02079 (**O**), 03572 (**E, F, H, K, M**), 03773 (**D**), 04853 (**J**). Laos, slide no 00956 (**G**). Holotype (**E**). Scale bar: 10 μ m.

SEM, external view (Fig. 2A–C). Areola openings apically elongate, lying in distinct longitudinal grooves (Fig. 2C, white arrow). Four small isolated areolae with almost circular openings present at valve apices on valve mantle (Fig. 2C, white arrowheads). Raphe-sternum elevated very slightly in valve centre (Fig. 2B, black arrow). Central pores drop-like without any projections, deflected slightly to primary

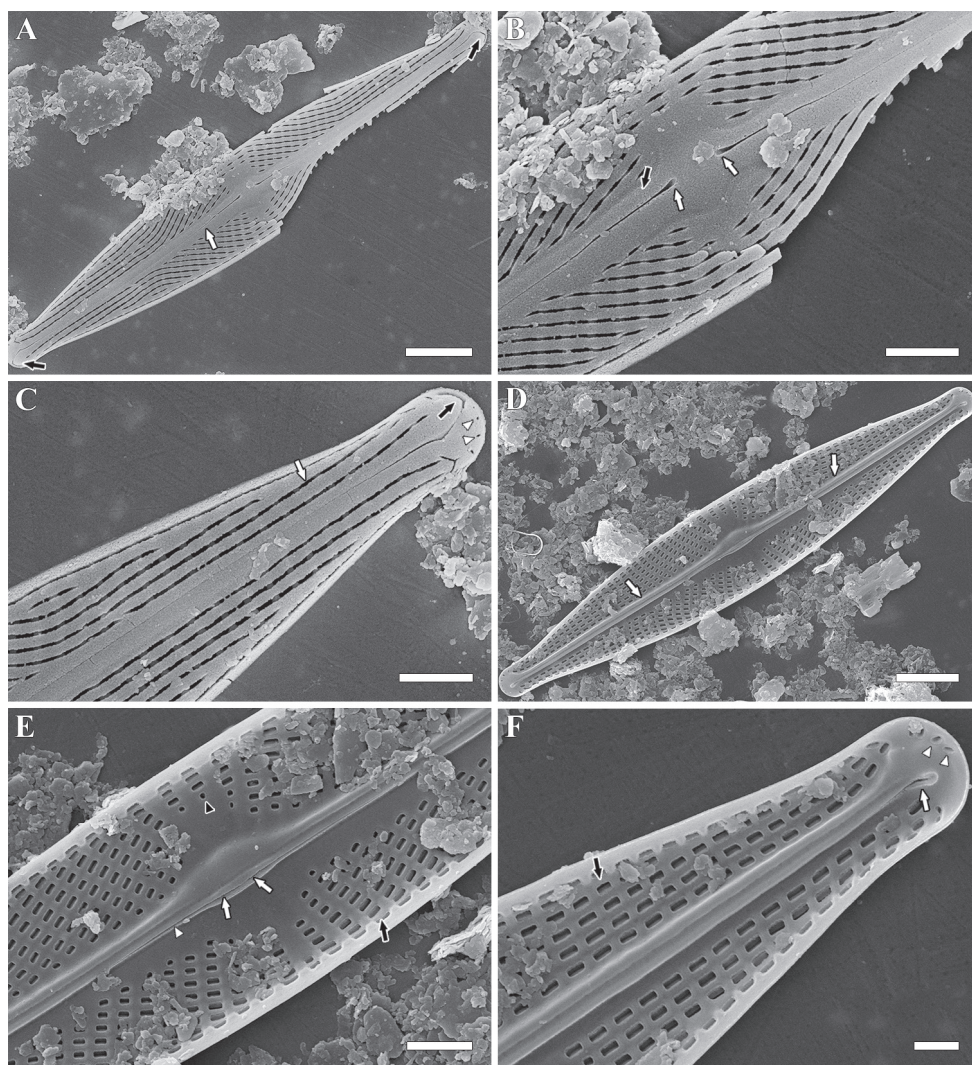


Figure 2. A–F *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Sample no 03572. A–C external views D–F internal views A, D the whole valve B, E central area C, F valve end. A black arrows show the terminal raphe fissures. White arrow shows the indistinct kink of raphe branch B white arrows show central raphe pores. Black arrow shows the very slightly elevated raphe-sternum C black arrow shows the terminal raphe fissure. White arrow shows the longitudinal grooves where areola openings are located. White arrowheads show the small isolated apical areolae D white arrows show the accessory rib E black arrow shows the rectangular areola opening. Black arrowhead shows the circular areola opening. White arrows show the central raphe endings. White arrowhead shows the raphe slit F black arrows show the stria which are slightly wider than virgae. White arrow shows the helicoglossa. White arrowheads show the small isolated apical areolae. Scale bars: 5 μm (A); 2 μm (B–E); 1 μm (F).

valve side (Fig. 2B, white arrows). Terminal fissures hooked to secondary valve side (Fig. 2A, C, black arrows). Raphe branches with indistinct kink in proximal parts (Fig. 2A, white arrow).

SEM, internal view (Fig. 2D–F). Striae slightly wider than virgae, lie at same level as latter, no transapical grooves formed (Fig. 2E, F, black arrows). Internal areola openings rectangular (Fig. 2F, black arrow) or circular (near central area) (Fig. 2E, black arrowhead), wider than external ones. Hymenes were not preserved during the material treatment. Four to five small isolated areolae present at each apex (Fig. 2F, white arrowheads). Raphe slit opens obliquely to secondary side and visible in proximal (Fig. 2E, white arrowhead) and distal (Fig. 2F, white arrow) parts only. Raphe-sternum very narrow, widened in centre, separated from accessory rib by longitudinal groove. Accessory rib well-developed (Fig. 2D, white arrows), widened unilaterally in valve centre and at valve apices. Central raphe endings straight, simple, connected by thin indistinct furrow (Fig. 2E, white arrows). Distal raphe endings well-developed helictoglossae deflected to secondary valve side (Fig. 2F, white arrow).

Etymology. Specific epithet is given due to similarity of new species to *Navicula winona* Bahls.

Distribution. Vietnam. Type locality (Suối Dầu Reservoir, slide no 03572). Suối Dầu River (slide no 00318, 00323, 03773), Suối Tiên River (slide no 00326, 00328), Cái River (slide no 04853), Mường Hoa River (slide no 02079), Da Tien Reservoir (slide 02069). Laos, Nam Lik River (slide no 00956).

Ecology. *Navicula winoniformis* sp. nov. was found in the reservoirs, and in rivers and waterfalls with different conductivity and pH values (Table 1). Below are the dominant species found in the samples along with *N. winoniformis* sp. nov.:

Sample 03572: *Neidium gracile* Hustedt, *Encyonopsis fonticola* (Hustedt) Krammer. Sample 00956: *Oricymba voronkinae* Glushchenko, Kulikovskiy & Kociolek, *Pinnularia sikkimensis* S.K. Das, C. Radhakrishnan, Kociolek & Karthick, *Pinnularia stricta* Hustedt, *Luticola muticoides* (Hustedt) D.G. Mann.

Sample 00318: *Hydrosera triquetra* Wallich, *Diadesmis confervacea* Kützing, *Luticola nipkowii* (Meister) Glushchenko & Kulikovskiy.

Sample 00323: *Gomphonema dalatica* Glushchenko, Kulikovskiy & Kociolek, *Eunotia indosinica* Glushchenko & Kulikovskiy.

Sample 03773: *Eunotia indomalaica* Glushchenko, Kulikovskiy & Kociolek, *O. voronkinae*.

Sample 00326: *Gomphonema subventricosum* Hustedt, *D. confervacea*, *Oricymba perjaponica* (Krammer & Lange-Bertalot) Kulikovskiy, Glushchenko & Kociolek, *Rhopalodia gibba* (Ehrenberg) O. Müller, *Encyonema javanicum* (Hustedt) D.G. Mann.

Sample 00328: *Placoneis paraundulata* T. Ohtsuka, *E. javanicum*, *Eunotia laeocarcus* Glushchenko, Kulikovskiy & Kociolek.

Sample 04853: *D. confervacea*, *Platessa oblongella* (Østrup) Wetzel, Lange-Bertalot & Ector, *Luticola taylorii* Levkov, Metzeltin & Pavlov, *N. gracile*, *E. javanicum*, *O. voronkinae*, *Frustulia magaliesmontana* Chohnoky.

Sample 02079: *P. oblongella*, *Reimeria sinuata* (Gregory) Kociolek & Stoermer, *E. javanicum*, *Encyonema leei* Krammer.

Sample 00269: *G. dalatica*, *Adlafia lamdongiensis* Glushchenko, Kulikovskiy & Kociolek, *Kobayasiella lamii* Glushchenko, Kulikovskiy & Kociolek.

Table 1. List of samples examined in this study. Geographic locality of samples and measured parameters indicated.

Slide	Locality	Habitat	Coordinates	Altitude, m	t, °C	pH	Conductivity, $\mu\text{S cm}^{-1}$	Coll. date
Vietnam								
00269	Lâm Đồng Province, Da Tien Reservoir	benthos	11°58.816'N, 108°26.987'E	1503	21.5	6.4	81	21.06.2012
00318	Khánh Hòa Province, Suối Dầu River	benthos	12°06.768'N, 108°59.888'E	275	24	6.7	92	02.07.2012
00323		periphyton						
00326	Khánh Hòa Province, Suối Tiên River	benthos	12°12.199'N, 109°01.694'E	68	26	6.9	101	02.07.2012
00328		periphyton						
02079	Lào Cai Province, neat the Sa Pa Town, Mường Hoa River	periphyton	22°15.415'N, 103°8.883'E	887	25.5	8.4	204	10.05.2015
03572	Khánh Hòa Province, Suối Dầu Reservoir	benthos	12°09.900'N, 109°03.200'E	36	31.4	7.3	84	10.08.2010
03773	Khánh Hòa Province, Suối Dầu River	periphyton	12°06.768'N, 108°59.891'E	275	25	6.7	119	28.05.2012
04853	Khánh Hòa Province, Cái River	plankton	12°15.983'N, 109°06.517'E	13	31.6	7.0	40	17.04.2010
Laos								
00956	Vientiane Province, Van Vieng District, Nam Lik Village, Nam Lik River	benthos	18°36.808'N, 102°24.605'E	196	23.5	6.9	98	24.11.2011
00962	Champasak Province, Bolaven Plateau, near the Pakse Town, unnamed waterfall	benthos	15°16.616'N, 106°19.935'E	1149	24.5	6.9	84	30.11.2011
01621	Champasak Province, Bolaven Plateau, near the Paksong Town, Tad Yueang Waterfall	benthos	13°57.266'N, 105°54.890'E	78	26.2	7.1	96	01.12.2011

***Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov.**
Figs 3, 4

Holotype. Slide 00962 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 3D.

Isotype. Slide MW-D 899s1 in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Type locality. Laos. Champasak Province, Bolaven Plateau, near the Pakse Town, unnamed waterfall, benthos, 15°16.616'N, 106°19.935'E, 1149 m elev., leg. E.L. Konstantinov, 30.11.2011.

Description. **LM** (Fig. 3A–N). Valves lanceolate with acutely rounded, non-protracted apices, length 33.9–56.5 µm, width 7.7–9.1 µm (n=31). Axial area narrow, widening towards valve centre, slightly wider on the secondary valve side, central area more or less round, occupying 1/3–1/2 of valve width, margins of raphe-sternum clearly visible as longitudinal lines at the central area. Striae radiate, sometimes (usually in larger valves) becoming parallel at the valve ends, 9.3–10.5/10 µm. Lineolae easy to resolve in light microscope, 17.5–19.7/10 µm. Raphe filiform to narrowly lateral, terminal fissures barely visible, deflected to the secondary valve side, central pores deflected to secondary valve side.

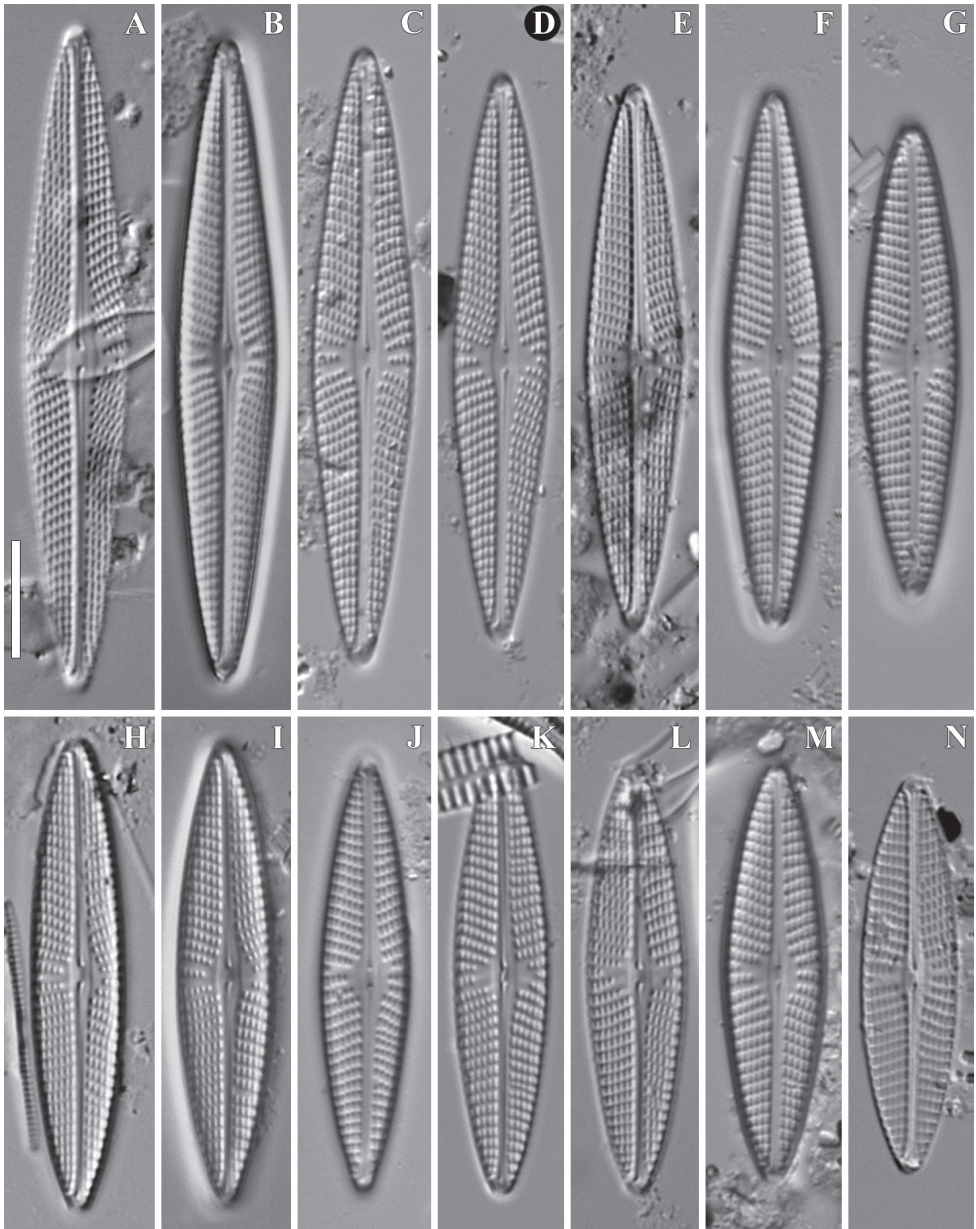


Figure 3. A–N *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Size diminution series. Laos, slides no 00962 (A, B, F, C–E, G, I, J, L, M, N), 01621 (H, K). Holotype (D). Scale bar: 10 μm.

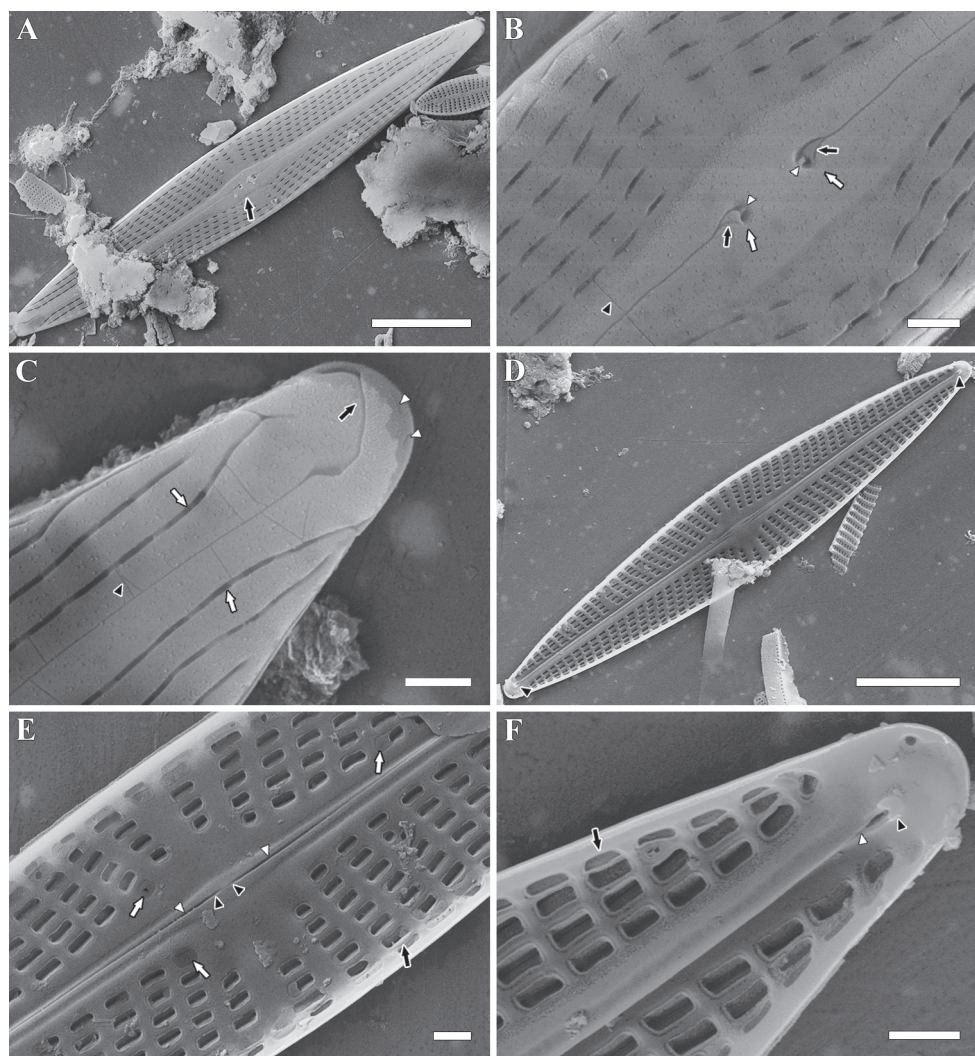


Figure 4. A–F *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Sample no 00962. **A–C** external views **D–F** internal views **A–C** external views **D–F** internal views **A, D** the whole valve **B, E** central area **C, F** valve end **A** black arrow shows the very slightly elevated raphe-sternum **B** white arrows show the drop-like central pores. Black arrows show the larger triangular insertion in distal part. White arrowheads show the small projections in proximal part. Black arrowhead shows the transapical sutures **C** black arrow shows the terminal raphe fissure. White arrows show the longitudinal grooves where areola openings are located. Black arrowhead shows the transapical sutures. White arrowheads show the small isolated apical areolae **D** black arrowheads show the helictoglossae **E** black arrow shows the stria which slightly wider than virgae. White arrows show the areola openings occluded with hymenes. Black arrowheads show the central raphe endings. White arrowheads show the raphe slits **F** black arrow show the stria which are wider than virgae. Black arrowhead shows the helictoglossa. White arrowhead shows the raphe slit. Scale bars: 10 µm (**A, D**); 1 µm (**B, C, E, F**).

SEM, external view (Fig. 4 A–C). Areolae apically elongate, areolae openings lie in shallow apical grooves more clearly expressed near valve apices (Fig. 4C, white arrows). At each apex two small isolated apical areolae present with shorter slits oriented obliquely or subparallel to valve margin (Fig. 4C, white arrowheads). Raphe-sternum elevated slightly above valve surface in centre, widened, with asymmetrical margins (Fig. 4A, black arrow). Its primary margin slightly convex, secondary margin slightly concave or straight. Central pores drop-like, deflected to secondary side. Each pore possesses small projection in proximal part (Fig. 4B, white arrowheads) and larger triangular insertion in distal part (Fig. 4B, black arrows). Terminal fissures hooked to secondary side (Fig. 4C, black arrow), proximal parts of fissures dilated. On axial area are present thin transapical sutures (Fig. 4B, C, black arrowheads).

SEM, internal view (Fig. 4D–F). Striae wider than virgae, lie at same level as latter, no transapical grooves formed (Fig. 4E, F, black arrows). Internal areola openings occluded with hymens (mostly destroyed) rectangular, wider than external ones (Fig. 4E, black arrows). Raphe slit opens obliquely to secondary side and visible in proximal and distal parts only (Fig. 4E, F, white arrowheads). Raphe-sternum very narrow, widened in centre, flanked with apical grooves from both sides. Accessory rib almost absent, especially in proximal part of valve. Central raphe endings straight, simple (Fig. 4E, black arrowheads). Distal raphe endings well-developed straight helictoglossae (Fig. 4D, F, black arrowheads).

Etymology. The specific epithet refers to the comparatively low density of areolae in the new species.

Distribution. Laos. Type locality (unnamed waterfall, slide no 00962) and Tad Yueang Waterfall (slide no 01621).

Ecology. *Navicula sparsilineolata* sp. nov. was found in a the waterfalls with low conductivity and circumneutral pH values (Table 1). Below are the dominant species found in the samples along with *N. sparsilineolata* sp. nov:

Sample 00962: *Gomphonema capitatum* Ehrenberg, *D. conservacea*, *Luticola burmensis* Metzeltin & Levkov, *L. nipkowii*, *P. sikkimensis*.

Sample 01621: *L. burmensis*, *L. nipkowii*, *P. sikkimensis*, *Platessa oblongella* (Østrup) Wetzel, Lange-Bertalot & Ector.

Discussion

The new species *Navicula winoniformis* sp. nov. is similar to a few previously described species. These include *N. cryptocephala* Kützinger, 1844, *N. krammerae* Lange-Bertalot, 1996, *N. densilineolata* (Lange-Bertalot) Lange-Bertalot, 1993, *N. wildii* Lange-Bertalot, 1993, *N. insulsa* Metzeltin & Lange-Bertalot, 1998, *N. oetzvallensis* Lange-Bertalot in Werum & Lange-Bertalot, 2004, *N. winona* Bahls, 2012 and *N. praeterita* Hustedt, 1945. A summary comparison of these taxa is presented in Table 2.

Table 2. Morphometric features of *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek sp. nov. and comparison with similar taxa.

Taxon	Outline	Valve ends	Valve length, μm	Valve width, μm	Striae in 10 μm	Areolae in 10 μm	References
<i>N. winoniformis</i> sp. nov.	narrowly lanceolate	rostrate to subcapitate	29.7–49.0	6.5–8.0	12–14	24–27	This study Lange-Bertalot 2001; Jitmer et al. 2020
<i>N. crypsocephala</i>	lanceolate to narrowly lanceolate	gradually narrowing or weakly rostrate, subcapitate to obtusely rounded	20–40	5–7	14–18	40–44	
<i>N. krammerae</i>	lanceolate	protracted and beak-like, neither distinctly nor acutely rounded	28–36	6.0–7.5	13–14	28–31	Lange-Bertalot 2001
<i>N. densilineolata</i>	narrowly lanceolate	almost acutely rounded	28–60	6.0–7.5	10–13	27–30	Lange-Bertalot 2001
<i>N. wildii</i>	(narrow) lanceolate	gradually narrowing to obtusely rounded, very rarely almost imperceptibly protracted	23–50	5.5–7.5	11.0–12.5	ca. 35	Lange-Bertalot 2001; Kulikovskiy et al. 2016
<i>N. insula</i>	rather linear-lanceolate	protracted, subcapitate to obtusely rounded	40–54	6–7	14–16	25–27	Merzlin and Lange-Bertalot 1998 Werum and Lange-Bertalot 2004
<i>N. setuallensis</i>	narrowly lanceolate to linear-lanceolate	weakly protracted	30–44	7.0–7.5	10.5–12.5	28–30	
<i>N. winona</i>	narrowly lanceolate	gradually attenuated and very narrow subcapitate	39–52	7.2–8.2	12–13	24–28	Bahls 2012
<i>N. praeterea</i>	lanceolate	rostrate-subcapitate	25–40	5.5–8.5	12–14	22–25	Lange-Bertalot 2001

Table 3. Morphometric features of *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek sp. nov. and comparison with similar taxa.

Taxon	Outline	Valve ends	Valve length, μm	Valve width, μm	Striae in 10 μm	Areolae in 10 μm	References
<i>N. sparsilineolata</i> sp. nov.	lanceolate	acutely rounded, non protracted	33.9–56.5	7.7–9.1	9.3–10.5	17.5–19.7	This study Lange-Bertalot 2001; Chudaev and Golobova 2016
<i>N. pseudolanceolata</i>	lanceolate to rhombic-lanceolate	gradually narrowed	28.7–50.0	7.0–9.8	9.5–11.2	22.0–25.7	
<i>N. oppugnata</i>	lanceolate to linear-lanceolate	usually obtusely rounded	30–60	8.5–12.0	7–12	ca. 24	Lange-Bertalot 2001
<i>N. johncaereri</i>	lanceolate	gradually narrowing to a wedge, neither distinctly acutely nor obtusely rounded	40–75	9–12	8–10	ca. 25	Lange-Bertalot 2001
<i>N. trophicatrix</i>	lanceolate to rhombic-lanceolate	gradually narrowing to a wedge, neither distinctly acutely nor obtusely rounded	25–50	7.5–10.0	11–13	21–24	Lange-Bertalot 2001
<i>N. sancti-naumii</i>	strictly lanceolate	more or less acutely rounded	28–48	7.0–8.5	10–11	28–30	Levkov et al. 2007
<i>N. weberi</i>	elliptic-lanceolate to broadly lanceolate	obtusely rounded	29–57	7.3–10.3	9–10	ca. 24	Bahls 2012

N. cryptocephala differs from *N. winoniformis* sp. nov. by having lineolae difficult to resolve in LM (40–44/10 µm) and denser striae (14–18/10 µm), no external longitudinal grooves are formed in *N. cryptocephala* and the accessory rib is not unilaterally widened internally (Lange-Bertalot 2001; Jüttner et al. 2020). *N. krammerae* has higher lineolae density (28–31/10 µm) and straight central pores (Lange-Bertalot 2001). *N. densilineolata* possesses non protracted valve ends, no external longitudinal grooves, smaller central area, striae not distinctly convergent at the valve ends, central pores that are bent to the secondary side and denser lineolae (27–30/10 µm) (Lange-Bertalot 2001). *N. wildii* has a smaller central area, coarser striae (11.0–12.5/10 µm), lineolae difficult to resolve in LM, central pores that are bent to the secondary side, and no external longitudinal grooves are formed (Lange-Bertalot 2001). *N. insulsa* has denser striae (14–16/10 µm) and a rather linear-lanceolate valve outline (Metzeltin, Lange-Bertalot 1998). *N. oetzvallensis* has coarser striae (10.5–12.5/10 µm), denser areolae (28–30/10 µm), no external longitudinal grooves and its central pores are broadly expanded and hooked to secondary side (Werum and Lange-Bertalot 2004). *N. winona* differs by gradually attenuated valve ends and proximal raphe ends deflected to the secondary valve side (Bahls 2012). Though *N. praeterita* is quite similar to *N. winoniformis* in LM appearance (Lange-Bertalot 2001), it differs clearly under SEM by the structure of external proximal raphe endings, possessing small projections and larger insertions, and by the absence of distinct external longitudinal grooves.

At the moment *Navicula winoniformis* sp. nov. is abundant and common in rivers and waterbodies of Khánh Hòa Province in Vietnam. Additionally, this species was found in Lâm Đồng Province, neighboring the previous one. We also found this species in a northern province in Vietnam – Lào Cai. Possibly this species is distributed in China too. We found this species only in the River Nam Lik in Laos. We believe that this species is widespread in freshwater systems of Vietnam, Laos and, possibly, China. Additionally, the species was found in Korea as *Navicula gottlandica* Grunow (Lee 2012).

Navicula sparsilineolata sp. nov. described herein, shows some similarity with species such as *Navicula pseudolanceolata* Lange-Bertalot, 1980, *Navicula oppugnata* Hustedt, 1945, *Navicula johncarteri* D.M. Williams, 2001 (syn. *N. concentrica* Carter in Carter & Bailey-Watts, 1981), *Navicula trophicatrix* Lange-Bertalot in Lange-Bertalot & Metzeltin, 1996, *Navicula sancti-naumii* Levkov & Metzeltin, 2007, *Navicula weberi* Bahls, 2012 (Table 3).

Navicula pseudolanceolata differs from *N. sparsilineolata* by having denser lineolae (22.0–25.7/10 µm, Lange-Bertalot 2001; Chudaev and Gololobova 2016) and simple drop-like central pores without any projections (Chudaev and Gololobova 2016, pl. 202, fig. 19). *Navicula oppugnata* is characterized by denser lineolae (c. 24/10 µm), and more obtusely rounded valve ends, raphe-sternum appears not elevated externally in light micrographs (Lange-Bertalot 2001) and these features differentiate it from our new species. *Navicula johncarteri* differs from *N. sparsilineolata* by possessing wider valves (9–12 µm) with finer lineolae (c. 25/10 µm) and central pores without larger triangular insertions (as *N. concentrica* J. Carter in Carter & Bailey-Watts; see Lange-Bertalot 2001, pl. 72, fig. 6). Though *Navicula trophicatrix* is similar to *N. sparsilineolata*

in external central pores structure, it has less curved terminal fissures (Lange-Bertalot 2001, pl. 66, fig. 1), denser striae (11–13/10 μm) and lineolae (21–24/10 μm) and rather rhombic-lanceolate than lanceolate valve outline (Lange-Bertalot 2001). In *Navicula sancti-naumii* central pores have only small proximal projections and no external longitudinal grooves are formed (Levkov et al. 2007, pl. 47, figs 3–5); areola density in this species is higher (28–30/10 μm), all features that differentiate this species from *N. sparilineolata*. *Navicula weberi* also have denser lineolae (c. 24/10 μm) and striae are distinctly convergent at valve ends (Bahls 2012), two features that distinguish this diatom from *N. sparilineolata*. *Navicula sparsilineolata* sp. nov. was found in two waterfalls in Laos. These waterfalls are 150 km from each other and situated in one Province, namely Champasak. It is possible that this species has more widespread distribution in Southeast Asia.

Description of these two new species shows that the diatom flora of Southeast Asia is a site of biodiversity discovery. The two species described herein are different from some other taxa previously described by us in that they are smaller and lack such prominent morphological features (see Kulikovskiy et al. 2020a,b). *Navicula babeiensis* Chudaev, Glushchenko, Kulikovskiy & Kociolek and *Navicula pseudokuseliana* Chudaev, Glushchenko, Kulikovskiy & Kociolek are smaller and have no special morphological peculiarities like the two species described here (Kulikovskiy et al. 2021). Two previously described species, *Navicula gogorevii* Chudaev, Glushchenko, Kulikovskiy & Kociolek and *Navicula davidovichii* Chudaev, Glushchenko, Kulikovskiy & Kociolek, are characterized by having large valves and large areolae in the striae. *Navicula davidovichii* is very interesting and characterized by having a valve shape that is more typical for the genus *Pinnularia* Ehrenberg. Even within the genus *Navicula sensu stricto* we see a diversity of ultrastructural features. And representatives with these different morphologies are present in Southeast Asia. More work is needed to sort out the morphological diversity within *Navicula*, to understand the phylogenetic relationships of these morphological groups, and to establish whether there are biogeographic patterns that correspond to the relationships.

In the book “The diatoms of Southeast Asia” we included widespread taxa from Southeast Asia (Glushchenko et al. 2021). *Navicula* species were detected from three countries including Vietnam, Laos and Cambodia. Widespread taxa are *Navicula escambia* (Patrick) Metzeltin & Lange-Bertalot, 2007, *N. simulata* Manguin 1942, *N. nielsfogedii* Taylor & Cocquyt in Taylor, Cocquyt & Mayama, 2016, *N. heimansioides* Lange-Bertalot, 1993, *N. globuliferiformis* Lange-Bertalot 1993, *N. gondwana* Lange-Bertalot, 1993, *N. tripunctata* (O.F. Müller) Bory, 1822, *N. rostellata* Kützinger, 1844, *N. subrhynchocephala* Hustedt, 1935, *N. ingapirca* U. Rumrich & Lange-Bertalot in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. recens* (Lange-Bertalot) Lange-Bertalot in Krammer & Lange-Bertalot, 1985, *N. radiosa* Kützinger, 1844, *N. avenacea* (Rabenhorst) Brébisson ex Grunow in Schneider, 1878, *N. angusta* Grunow, 1860, *N. namibica* Lange-Bertalot & U. Rumrich in Lange-Bertalot, 1993, *N. caterva* Hohn & Hellerman, 1963, *N. vandamii* Schoeman & Archibald, 1987, *N. germainii* Wallace, 1960, *N. amphiceropsis* Lange-

Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. quasidisjuncta* Lange-Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. electrolytifuga* Lange-Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000 (Glushchenko et al. 2021).

As evident from the above list of known taxa, Southeast Asia includes many species described from other areas of the Southern Hemisphere, mainly from South America (Rumrich et al. 2000). *N. nielsfogedii* is a widespread taxon on the basis of its morphospecies taxonomy (Chudaev et al. 2020). Possibly, it is cryptic species that can be evident on the basis of molecular investigation in the future. However, if we use molecular methods we can find cryptic speciation. This occurs when some small morphological features (mainly in valve dimensions and shape) are evident in different populations (Chudaev et al. 2020; Glushchenko et al. 2021). Cryptic speciation is a phenomenon that is known in diatoms (Mann 1999); however we do not know precisely how extensively it occurs in different groups of diatoms (Maltsev et al. 2021). Cryptic speciation was shown in the genus *Navicula* by Pouličková et al. (2010) on the basis investigation of *Navicula cryptocephala*. The same situation can be detected for *N. electrolytifuga* with populations from Laos, Cambodia and Vietnam (see Glushchenko et al. 2021: Pl. 86). Species such as *N. heimansioides*, *N. tripunctata*, *N. rostellata*, *N. subrhynchocephala*, *N. radiosa* are widespread across the Northern and Southern Hemispheres and found in different types of fresh waterbodies (Lange-Bertalot 2001; Kulikovskiy et al. 2016). Molecular investigation of these taxa will be important for the future research of their relationships and use in water quality analysis.

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A natural hybrid of *Sindora* (Fabaceae, Detarioideae) from Singapore

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Abstract

Sindora × *changiensis* L.M.Choo, Loo, W.F.Ang & K.Er is a new hybrid from the subfamily Detarioideae in Fabaceae. This is the first reported instance of natural hybridisation in *Sindora*. Based on population genetics analyses using ddRAD and morphological observations, this taxon represents a fertile hybrid between *Sindora coriacea* and *Sindora echinocalyx*. This new hybrid is so far only known to occur naturally from Changi at the north-eastern coast of Singapore. It has pods that are sparsely spiny. This is intermediate between the smooth, non-spiny pods of *S. coriacea*, and the densely spiny pods of *S. echinocalyx*. The calyx is smooth and unarmed, resembling *S. coriacea*. Last but not least, the ovary is entirely pubescent, different from *S. coriacea* and *S. echinocalyx*. The ovary of *S. coriacea* has a glabrous patch in the middle, while that of *S. echinocalyx* has minute spines protruding from the dense pubescence. A taxonomic description and an updated key to the *Sindora* of Singapore and Peninsular Malaysia are also provided.

Keywords

Caesalpinioideae, Changi, ddRAD, new hybrid, *Sindora coriacea*, *Sindora echinocalyx*

Introduction

Sindora Miq. is a genus of the legume family (Fabaceae: Detarioideae) and consists of 20–22 species. It has a paleotropical distribution, with one species from West Central Africa, and the rest from Asia, which are distributed from Southern China to Southeast Asia, and to as far west as the Philippines (de Wit 1949; Chen et al. 2010; Choo and Ngo 2020). The genus can be easily recognised in the field by its characteristic pods,

often with spines originating from modified glands, and dark-coloured seeds with a large yellow or reddish-brown aril of about the same size as the seed itself (de Wit 1949; Sosef 1993).

Previous work on *Sindora* in Singapore and Peninsular Malaysia reported five native species to the region, i.e. *Sindora coriacea* (Baker) Prain, *Sindora echinocalyx* Prain, *Sindora siamensis* Teijsm. ex Miq., *Sindora velutina* Baker, *Sindora wallichii* Benth. (Choo and Ngo 2020). Of these, four species, *Sindora coriacea*, *S. echinocalyx*, *S. velutina* and *S. wallichii* are present in both Singapore and Peninsular Malaysia, while *S. siamensis* is found only in northern Peninsular Malaysia, Thailand, Cambodia, Laos and Vietnam, and is only present as a cultivated tree in Singapore. In Singapore, the four native species can be found in the remaining patches of primary and mature secondary rainforest. *S. coriacea* and *S. wallichii* are relatively more abundant than *S. velutina* and *S. echinocalyx*, which are comparatively rarer in the wild in Singapore. *S. velutina* is found only in the primary rainforest at Bukit Timah Nature Reserve, while *S. echinocalyx* may be more associated with hillsides and coastal areas (Choo and Ngo 2020). Recent field observations of mature *Sindora* trees in Singapore revealed a particular 27 m-tall mature tree from Changi at the north-eastern coast of the island, which could not be satisfactorily identified to species using the taxonomic key in Choo and Ngo (2020).

From a combination of population genetics and morphological evidence, we found that this particular tree represents a natural hybrid between *Sindora coriacea* and *Sindora echinocalyx*, and is the first recorded instance of hybridisation in *Sindora*. We describe this new hybrid as *Sindora* × *changiensis* L.M.Choo, Loo, W.F.Ang & K.Er, and further explain the characteristics that distinguish this new hybrid from its parent species.

Materials and methods

Population genetics analysis

Double-digest RAD-sequencing (ddRAD) was carried out to investigate the hybrid origin of *Sindora* × *changiensis*. Of the four native species of *Sindora* in Singapore, *S. echinocalyx*, one of the likely parents as an individual of *S. echinocalyx*, was formerly collected from Changi, Singapore in 1893 as a herbarium specimen, so this species was known to exist in the area. *S. coriacea* was selected as the next most likely parent because of its smooth and spineless pods, as the hybrid had sparsely spiny pods and other characteristics which were intermediate between, or a mix between, these two species (see Results for more details). The other two native species, *S. velutina* and *S. wallichii*, were unlikely to have been the other parent because they both have spiny pods, and would not have resulted in the sparsely spiny pods of the hybrid if they had hybridised with *S. echinocalyx*.

A total of 14 individuals from Singapore were sampled and sequenced, consisting of six *S. coriacea* individuals, four *S. echinocalyx* individuals and three *S.* × *changiensis* individuals (the mature tree and two other seedlings from the tree). Leaf material from each tree was silica-dried and the resulting herbarium specimens were deposited in SING. Details of the specimens used are available in Table 1.

Table 1. Details of 14 specimens sequenced in the population genetics analysis. (BTNR = Bukit Timah Nature Reserve, CCNR = Central Catchment Nature Reserve).

Sample	Species	Collector	Voucher	Locality	Notes
Sind002	<i>Sindora coriacea</i>	Choo, L.M. & Ngo, K.M.	SING2019-840	BTNR	Wild
Sind063	<i>Sindora coriacea</i>	Ng, X.Y.	SING2021-396	CCNR	Wild
Sind064	<i>Sindora coriacea</i>	Niissalo, M.A. & Choo, L.M.	SING2021-599	CCNR	Wild
Sind065	<i>Sindora coriacea</i>	Niissalo, M.A. & Choo, L.M.	SING2021-600	CCNR	Wild
Sind066	<i>Sindora coriacea</i>	Niissalo, M.A. & Choo, L.M.	SING2021-601	CCNR	Wild
Sind067	<i>Sindora coriacea</i>	Niissalo, M.A. & Choo, L.M.	SING2021-602	CCNR	Wild
Sind068	<i>Sindora coriacea</i>	Niissalo, M.A. & Choo, L.M.	SING2021-603	CCNR	Wild
Sind006	<i>Sindora × changiensis</i>	Choo, L.M.	SING2020-649	Changi	Seedling of Sind019
Sind017	<i>Sindora × changiensis</i>	Choo, L.M.	SING2020-650	Changi	Seedling of Sind019
Sind019	<i>Sindora × changiensis</i>	Choo, L.M. et al.	SING2021-265	Changi	Wild, Mature tree
Sind010	<i>Sindora echinocalyx</i>	Choo, L.M. et al.	SING2020-1212	Changi	Cultivated
Sind011	<i>Sindora echinocalyx</i>	Choo, L.M. et al.	SING2020-1213	Changi	Cultivated
Sind020	<i>Sindora echinocalyx</i>	Choo, L.M. et al.	SING2021-266	Changi	Cultivated
Sind021	<i>Sindora echinocalyx</i>	Choo, L.M. et al.	SING2021-267	Changi	Cultivated

Genomic DNA extraction was done using the CTAB method (Doyle and Doyle 1987). ddRAD-sequencing libraries were prepared using the methods and barcodes first published by Peterson et al. (2012), and further modified by Niissalo et al. (2018, 2020). 600 ng of genomic DNA was used in the restriction digest by ApeKI and PstI, followed by a size selection using 5% SeraMag Magnetic Carboxylate-Modified Microparticles (Cytiva, USA). PCR was carried out using KAPA High-Fidelity DNA Polymerase (Roche, USA) and the concentration and library size of each sample was measured, before they were pooled together in equimolar volumes for sequencing.

The pooled libraries were sequenced by NovogeneAIT Genomics (Singapore) using Illumina NovaSeq. Sequences were demultiplexed using the process_radtags function in STACKS v1.37 (Catchen et al. 2013). STACKS was also used for de novo sequence assembly and single nucleotide polymorphism (SNP) discovery. Sequences with a minimum depth of 15 were retained, and one SNP per loci was used using the setting – write-random-snps, with SNPs present in at least 12 out of the 14 individuals selected for further analysis. Across the 14 individuals, a total of 19,856 SNPs were recovered.

To visualise the relationships between *Sindora coriacea*, *S. echinocalyx* and *S. × changiensis*, a neighbour-net plot with uncorrected p-distances was made using the Neighbour-Net function in SPLITSTREE v4.17.1 (Huson and Bryant 2006). A STRUCTURE analysis (Pritchard et al. 2000) was carried out to find out the number of genetic clusters, or K-value, in the samples sequenced. We used 10,000 randomly selected independent SNPs from the 19,856 SNPs available, with a burn-in of 10,000. This was followed by 100,000 Markov Chain Monte Carlo (MCMC) repetitions, with the values of K from 1 to 5, which was 3 more than the expected number of two populations. A total of 30 STRUCTURE iterations were run, and the optimal value of K was determined by calculating the mean $L(K)$ and the standard deviation of $L(K)$ for each value of K, and the ΔK for $K = 2$ to $K = 5$ using STRUCTURE HARVESTER (Earl and von Holdt 2012).

Morphological observations

Morphological observations of the hybrid *Sindora* × *changiensis* were made using both freshly collected and dried herbarium specimens in SING. The traits of *S. coriacea* and *S. echinocalyx* were obtained from the accounts of both species in their recent treatment in Choo and Ngo (2020), which was mainly based upon an extensive study of herbarium specimens from SING and KEP, and supplemented with measurements of recent specimens in SING collected between 2020 and 2021. Measurements of microscopic structures were made using a dissecting microscope with a calibrated eyepiece. Fresh flowers were used for the measurements where available, otherwise rehydrated flowers from dried herbarium specimens were used.

Results

Population genetics

The Neighbour-Net analysis in SPLITSTREE (Fig. 1) showed all three individuals of *S. × changiensis* to be located between *S. coriacea* and *S. echinocalyx* in the plot, which indicated that *S. × changiensis* was likely the result of hybridisation between the two parent species. The STRUCTURE analysis of the subsample of 10,000 SNPs also showed similar results at $K = 2$, where individuals of *S. coriacea* and *S. echinocalyx* were assigned to separate genetically distinct populations, and individuals of *S. × changiensis* were shown to be admixed between the two parents, as shown in Fig. 2, where the genetic composition of *S. × changiensis* is a mix of *S. coriacea* and *S. echinocalyx*. The optimal value of K for the STRUCTURE analysis, was also found to be 2, as this was the value with the highest ΔK value among the values for $K = 2$ –5 that were calculated (Suppl. material 1: Fig. S1). The value of ΔK for $K = 1$ cannot be calculated, but $K = 1$ can be excluded as the optimal value of K because there are two known species in our sampling, hence the optimal value of K has to be at least 2. As such, both the STRUCTURE analysis and the ΔK calculation suggests that the number of populations that best explains the distribution of SNPs is 2, which corresponds to the two parent species *S. coriacea* and *S. echinocalyx*, and that *S. × changiensis* is the result of a hybridisation event between the two species. These results confirm the hybrid origin of *S. × changiensis*.

Morphological analysis

Morphological observations of the *Sindora* × *changiensis* along with *S. coriacea* and *S. echinocalyx* showed that *S. × changiensis* had characters, which were both intermediate between the two parents and also a mix of characters from either parent. The pods of *S. × changiensis* are sparsely spiny, which is intermediate between the non-spiny pods of *S. coriacea*, and the densely spiny pods of *S. echinocalyx*. The calyx of *S. × changiensis*

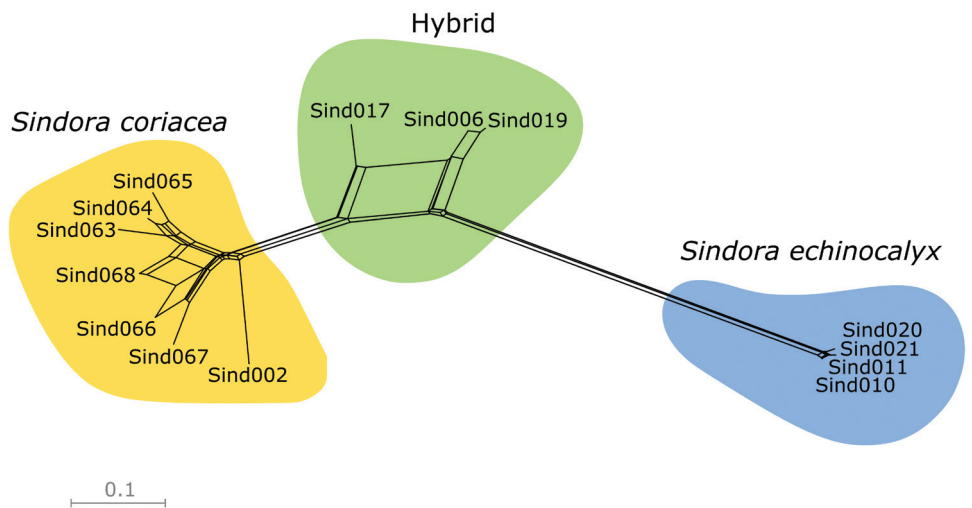


Figure 1. A neighbour-net plot with uncorrected p-distances, constructed in SPLITSTREE using the SNP dataset of all 14 individuals of *Sindora* sequenced.

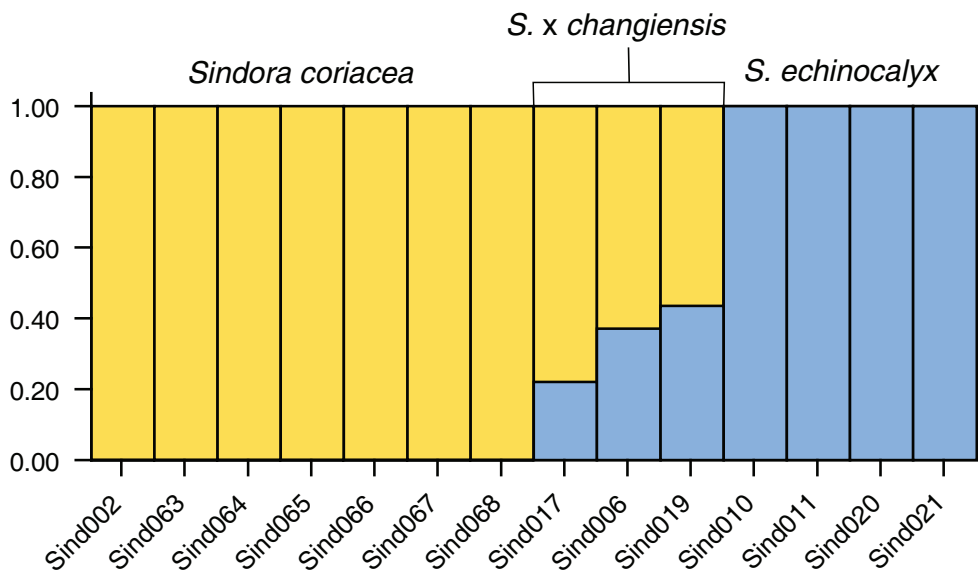


Figure 2. STRUCTURE plot generated for the optimal value of K = 2. Samples identified as *Sindora coriacea* are wholly yellow, *Sindora echinocalyx* samples are wholly blue, while the hybrid individuals of *Sindora* × *changiensis* have a mix of both yellow and blue.

is smooth and unarmed, which resembles *S. coriacea*. The ovary of *S. x changiensis* is densely villous all over and without spines on the surface, which is different from both *S. coriacea* and *S. echinocalyx*, where the ovaries are densely pubescent but with a glabrous patch in the centre, and densely villous all over but with small, blunt spines

Table 2. Differences between *Sindora* × *changiensis* and its parent species *S. coriacea* and *S. echinocalyx*.

Character	<i>Sindora coriacea</i>	<i>Sindora</i> × <i>changiensis</i>	<i>Sindora echinocalyx</i>
Stipules	Caducous, c. 4 mm long	Caducous, 1.2–1.4 cm long	Subpersistent, 0.8–1.8 cm long
Leaflet shape	Elliptic to ovate to broadly falcate, asymmetric with the midrib curved	Elliptic, slightly asymmetric with midrib slightly curved	Elliptic to obovate, slightly asymmetrical at the base, midrib generally straight
Leaflet apex	Bluntly acute to acuminate, rarely obtuse and sometimes slightly emarginate at the very tip	Acuminate to obtuse, with a very slight emarginate indent at the very tip	Apex rounded to obtuse and slightly emarginate at the very tip
Sepal size	4–5(–6.8) × 1.5–3 mm	8–9.3 × 3–5.2 mm	6–9 × 1.5–4.7 mm
Calyx surface	Unarmed	Unarmed	Armed all over with long, soft spines that are brittle when dry, spines up to 2 mm long.
Ovary surface	Densely pubescent around the edges and glabrous in the centre, surface unarmed.	Densely covered all over with long silky villous hairs, except in three strips on the surface where the hairs are less dense, spines not seen.	Densely villous, surface armed with small, blunt, spines that are visible under the hairs of the ovary
Ovary size	2.5–3.2 × 1.5–2 mm	3.5–4.5 × 2.8–3 mm	3.5–4 × 3–3.2 mm
Ovary stipe length	1.5–1.7 mm	1.8–2.2 mm	2.2–2.3 mm
Style length	9.5–10.5 mm	12–13.5 mm	9–12 mm
Pod surface	Unarmed or with few slightly raised warts	Sparsely armed with c. 20 or fewer slender spines which sometimes exude a clear resin	Densely armed with upright, regularly spaced spines, often with tipped with beads of dried resin at the ends of the spines
Aril	Aril semi-circular or trapezoid, chestnut-brown, 1.4–2 × 1.3–1.5 × 0.9–1 cm,	Aril trapezoid, yellowish brown to chestnut brown, 2.2–2.6 × 1.5–1.7 × 0.8–1 cm;	Aril narrowly trapezoid or rectangular, 1.3–1.5 × 1.3–1.8 × 0.6–0.8 cm,
Seed	1.9–2.5 × 1.4–1.8 × 0.8–0.1 cm, horizontal cracks on surface very faint and scarcely visible, chestnut brown in colour but becoming a darker shade of brown towards the centre of the seed.	2–2.5 × 1.7–1.9 × 0.8–0.9 cm, horizontal cracks on surface distinctly visible, uniformly black in colour.	1.9–2.2 × 1.1–1.9 × 0.9–0.6 cm, horizontal cracks on surface very faint and scarcely visible, uniformly black in colour.

visible under the hairs, respectively. These differences are further detailed in Table 2 and depicted in Figs 3–5. A taxonomic treatment of the new taxon, and an updated key to the *Sindora* of Singapore and Peninsular Malaysia are also provided below.

Taxonomic treatment

Sindora × *changiensis* L.M.Choo, Loo, W.F.Ang & K.Er, nothosp. nov.

Figs 3, 4, 5

= *Sindora coriacea* (Baker) Prain × *Sindora echinocalyx* Prain.

Diagnosis. Pod intermediate in character between the two parents, with a smooth surface like that of *S. coriacea* coupled with sparsely-set and fine spines which are much less dense than in *S. echinocalyx*. Flower calyx entirely smooth and without prickles, resembling *S. coriacea*. Ovary lacking the hairless patch in the centre, which is the case

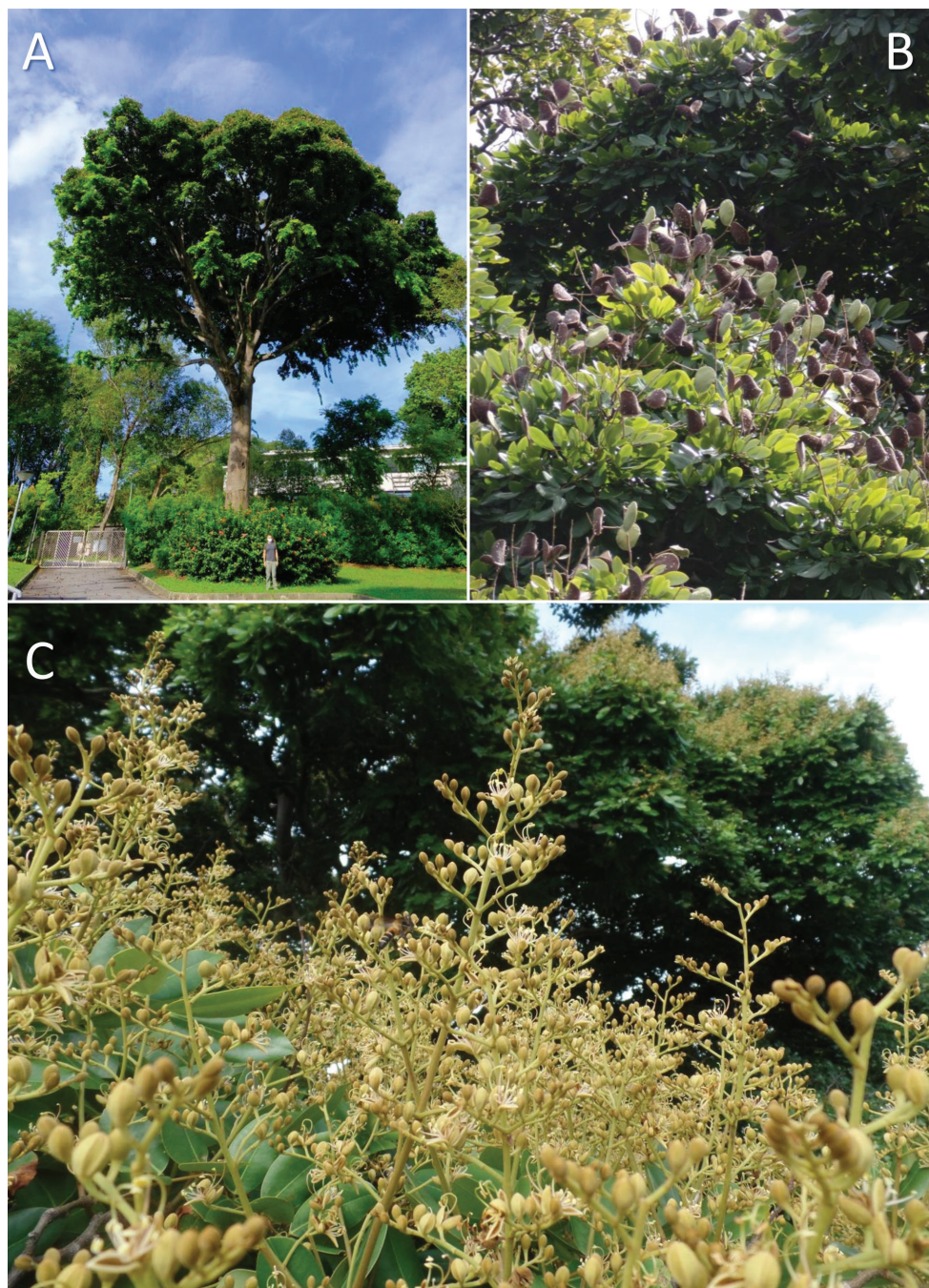


Figure 3. The hybrid tree *Sindora* \times *changiensis* **A** overview of the 27 m tall tree **B** developing (green) and ripe (brown) pods on the tree **C** inflorescences of the tree. (Photos: **A** K.B.H. Er, **B, C** L.M. Choo).

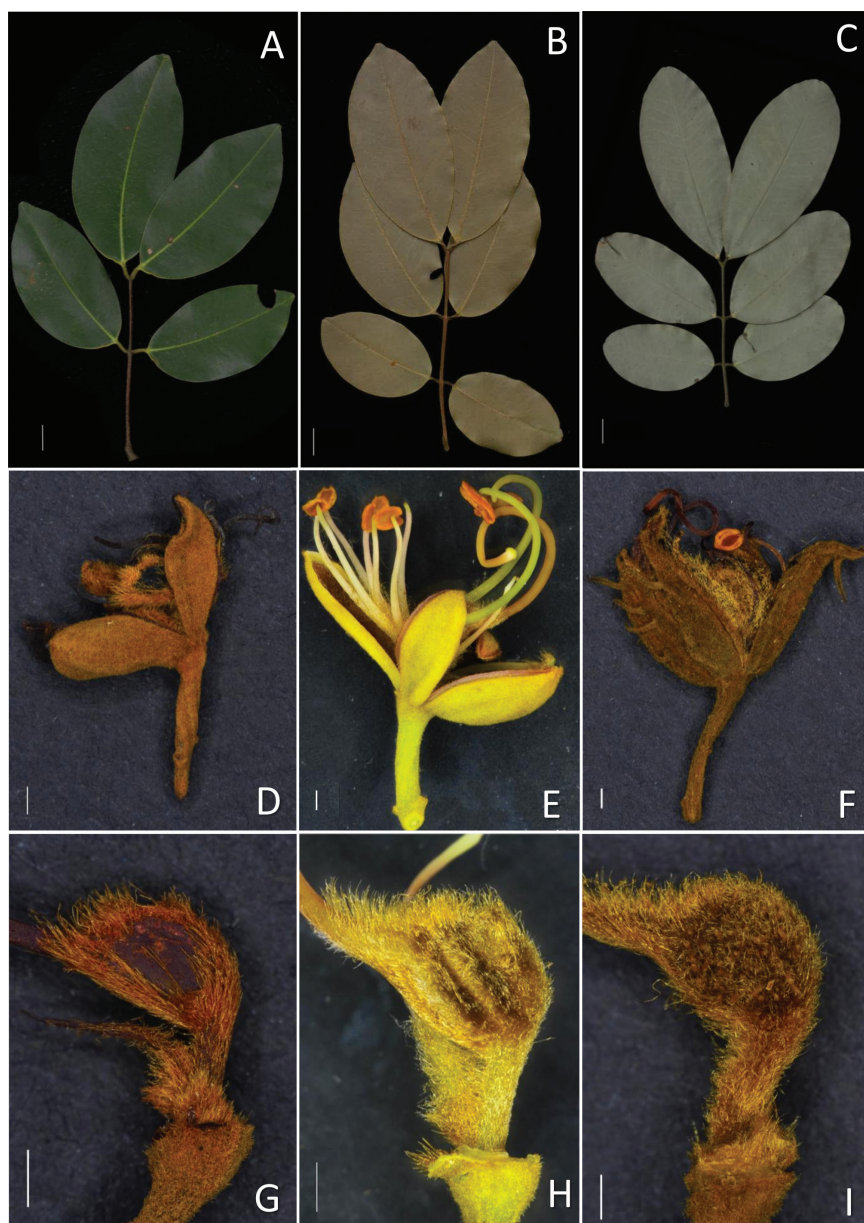


Figure 4. Comparisons of leaf and flower characters between *Sindora* × *changiensis* and its parent species. **A, B, C** leaves of **(A)** *S. coriacea*, **(B)** *S. × changiensis* and **(C)** *S. echinocalyx* respectively **D, E, F** flowers of **(D)** *S. coriacea*, **(E)** *S. × changiensis* and **(F)** *S. echinocalyx* respectively, showing the unarmed calyces of *S. coriacea*, *S. × changiensis*, and the spiny calyx of *S. echinocalyx* **G, H, I** Ovaries of **(G)** *S. coriacea*, **(H)** *S. × changiensis* and **(I)** *S. echinocalyx* respectively, showing the glabrous patch in the centre for *S. coriacea*; the densely pubescent ovary for *S. × changiensis* except for the three stripes across the width; and the densely pubescent ovary for *S. echinocalyx*, with tiny protuberances visible on the surface, which will later on develop into the spines on the fruit pods. Scale bars: 1 cm (**A, B, C**); 1 mm (**D, E, F, G, H, I**). (Photos: L.M. Choo).

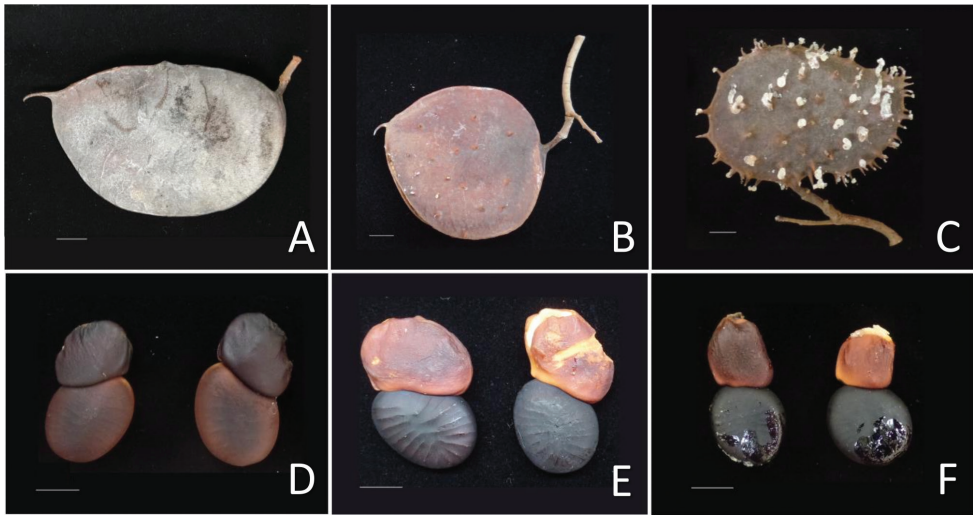


Figure 5. Comparisons of pod and seed characters between *Sindora* × *changiensis* and its parent species **A, B, C** pods of **(A)** *S. coriacea*, **(B)** *S. × changiensis* and **(C)** *S. echinocalyx* respectively, showing the unarmed pod of *S. coriacea*, the sparsely spiny pod of *Sindora* × *changiensis*, and the densely spiny pod of *S. echinocalyx* **D, E, F** seeds of **(D)** *S. coriacea*, **(E)** *S. × changiensis* and **(F)** *S. echinocalyx* respectively. Scale bars: 1 cm **(A, B, C, D, E, F)**. (Photos: L.M. Choo).

for *S. coriacea*. Instead, it is entirely pubescent with fine adpressed hairs, but without the minute protuberances or prickles that are seen in *S. echinocalyx*.

Type. SINGAPORE: Changi: 503 Cranwell Road, 1°23.335'N, 103°58.618'E, 6 May 2021, Choo et al., SING2021-265 (holotype SING, isotypes (BKF, K, KEP, L)).

Tree up to 27 m tall, dbh up to 1.5 m, bole columnar, with slightly raised rings around the girth, not buttressed, bark grey to blackish, slightly cracked or flaky. Stipules early caducous, only present in young parts, semicircular, 1.2–1.4 cm long. Leaves compound, paripinnate, 3–4 jugate, rachis puberulous, 3.5–5.6 cm long; petiole 2–3 cm long; petiolules 4–5 mm long, puberulous, grooved, greenish brown when fresh but drying dark brown to black. Leaflets opposite, coriaceous, elliptic, slightly asymmetric with midrib slightly curved, 3.5–6.8 × 2.3–3.5 cm, increasing in size up the rachis, base rounded to obtuse, apex acuminate to obtuse, with a very slight emarginate indent at the very tip; upper surface slightly glossy when fresh, but reticulations become conspicuous when dry, entirely glabrous, midrib flat to slightly sunken; lower surface glaucous, puberulous with tiny short golden hairs, midrib raised and also puberulous; thickened marginal vein either glabrous or minutely puberulous; reticulations clear and raised on both the upper and lower surfaces; one gland present on the tip of the midrib on the lower surface, another present on the thickened marginal vein close to the base of the leaflet. Inflorescence paniculate, both terminal and axillary, but mostly concentrated on the crown, growing from old stems where inflorescence branches from

the previous year have been shed, flowering rachises long and stout, measuring 11.5–25 × 5–8 cm, side branches straight but bearing scars where the flowers are attached, branches flexible but held erect in fresh specimens. Both flowering rachis and branches completely pubescent with short golden adpressed to upright hairs. Bracts not seen, caducous; bracteoles ovate, c. 2.5 × 1.3 mm, pubescent on both surfaces, caducous, only seen in inflorescences where the buds are still small and developing. Pedicels 4.5–6 mm long, pubescent, receptacle short, 1–1.5 mm long; buds obovoid to ellipsoid, suture lines of the sepals becoming evident as the bud matures, measuring 6–7.5 × 4.5–5.5 mm when mature just before anthesis. Flowers strongly zygomorphic. Sepals 4, unequal, lanceolate to elliptic, 8.0–9.3 × 3.0–5.2 mm, outer surface pubescent with small golden hairs, unarmed, inner surface densely covered with long golden brown tightly adpressed hairs. Petal 1, not exerted but nestled within the largest sepal during anthesis, rolled up and containing a drop of sweet floral-scented nectar, c. 7.5 × 2.2 mm when rolled up, top of petal with a well-defined hood fringed with long villous hairs which narrows off with the lower half of the petal with inrolled sides forming a closed tube; outer surface glabrous at the top and down the middle, densely pubescent at the sides and the lower half of the petal; inner surface glabrous; margins villous, colour pink tinged with green at the tip. Stamens 10, diadelphous, united basal portion of the stamens 2.5–3 mm long; two largest filaments 12–15 mm long, the seven in the middle of the bundle 6–7 mm long; two largest anthers elliptic, 2.5–2.7 × 1.7–1.9 mm, the others smaller and heart shaped, 1.6–2 × 1–1.4 mm, all nine mentioned here with visible pollen; final stamen on the other side of the flower is a staminode, 6–7.5 mm long but without a fertile anther. Ovary rhomboidal, densely covered all over with long silky villous hairs, except in three strips on the surface where the hairs are less dense; 3.5–4.5 × 2.8–3 mm, stipe 1.8–2.2 mm long, style glabrous except for the base where it has villous hairs like the rest of the ovary, 12–13.5 mm long, pale yellow green tinged with pink at the base, stigma capitate with small sticky papillate protuberances, c. 0.6 mm diameter. Pod a flattened, elliptic, rhomboidal or ovate two-valved dehiscent pod, surface sparsely armed with c. 20 or fewer slender spines that sometimes exude a clear resin; surface beneath the spines smooth and puberulous with short golden hairs, 7–8 × 6–6.5 cm, stipe 8–9 mm, beak 9–10 mm. Seed 1, aril trapezoid, yellowish brown to chestnut brown, 2.2–2.6 × 1.5–1.7 × 0.8–1.1 cm; seed 2–2.5 × 1.7–1.9 × 0.8–0.9 cm, surface smooth with fine horizontal cracks, black in colour.

Distribution. The hybrid is likely endemic to Singapore. It is only known to occur naturally in Changi, which is at the north-east coast of Singapore, although the offspring of the tree has been propagated and planted elsewhere in Singapore as roadside trees.

Etymology. Latin, *-ensis* = from, meaning “from Changi”.

Habitat and ecology. The species is part of the remnant vegetation of tropical lowland forest that was once present in the area, before it was cleared.

Phenology. Flowers from April to May, and fruits in August.

Conservation. Only a single tree of *S. × changiensis* is known to occur from the wild in Singapore, although the offspring of this tree have been planted elsewhere in Singapore as roadside trees.

Taxonomic notes. In *Sindora*, the leaves of seedlings, saplings and water shoots of mature trees often have a morphology different from that of the mature leaves from the crown of the tree. The leaves of *Sindora* seedlings, saplings and water shoots are usually larger in size and are pubescent on the underside and along the leaflet margins, and the shape of the leaf and the leaf apex may differ somewhat from the mature leaves (de Wit 1949; Choo and Ngo 2020). For the identification of this hybrid, pod characters are the most diagnostic, followed by flower characters, although fallen mature leaflets picked from below the tree may be useful in supplementing the fruit and flower characters.

Updated key to the *Sindora* species in Singapore and Peninsular Malaysia

- 1 Leaflets broadly elliptic, apex strongly emarginate, midrib on the lower surface with a gland located 1–3 mm away from the tip of the leaflet; calyx warty with small spines at the apex ***S. siamensis***
- Leaflets falcate, elliptic, obovate or lanceolate, apex if emarginate only very slightly notched at the tip, midrib on the lower surface with a gland at the very tip of the leaflet; calyx never warty, either unarmed or armed with spines ... **2**
- 2 Lower surface of leaflets densely pubescent or tomentose, distinctly velvety or rough to touch; leaves 4–6-jugate; rachis of young leaflets, inflorescence and stipe of pods densely tomentose with reddish brown hairs ***S. velutina***
- Lower surface of leaflets puberulous to glabrescent to glabrous, may be slightly rough like fine sandpaper but never velvety; leaves (2–)3–4-jugate; rachis of young leaflets puberulous to glabrous, inflorescence or stipe of pods pubescent to tomentose with golden brown hairs..... **3**
- 3 Lower surface of leaflets glabrous or only sparsely puberulous at the base in mature leaflets except for the midrib which is usually puberulous; pods unarmed ***S. coriacea***
- Lower surface of leaflets entirely puberulous with small, thin adpressed or strigose hairs in mature leaflets; pods armed or sparsely armed **4**
- 4 Calyx entirely smooth and without spines; pods sparsely armed, usually with c. 20 spines or much less on each surface ***S. × changiensis***
- Calyx armed, either only on the upper half or on the entire surface; pods armed with more than 20 spines on each surface **5**
- 5 Leaflets without raised reticulations above, upper surface smooth and glossy and shining; calyx armed only on the upper half or on the very tip of the bud, with small spines less than 1 mm long ***S. wallichii***
- Leaflets with raised reticulations above, upper surface not glossy; calyx armed all over the exterior of the bud with long soft spines which are brittle when dry, spines up to 2 mm ***S. echinocalyx***

Discussion

Sindora × *changiensis* is the first recorded instance of natural hybridisation in the genus *Sindora*, and is currently known to be endemic to Singapore. Both of the parent species, *S. coriacea* and *S. echinocalyx*, are native to Singapore. *S. echinocalyx* is known to occur in coastal hills and heath forests in Peninsular Malaysia (Saw 2010) and had been recorded in Changi in the past based on an herbarium specimen (*Bakar s.n.*, Year 1893, Changi, SING[SING0044593]). In addition, *S. coriacea* is a native of lowland rainforests in Singapore (Chong et al. 2009; Choo and Ngo 2020). Located at the north-eastern coast of Singapore, Changi was known to be dominated by lowland rainforest and was previously designated as a forest reserve in 1884, before it was largely cleared by the British to make way for military barracks and an airbase from 1927 to 1941 (Probert 1965; Corlett 1992). However, there are presently still a few large trees of lowland rainforest species in Changi, which are remnants of the original rainforest vegetation, such as *Shorea gibbosa*, *Dipterocarpus sublamellatus* and *Gluta malayana*. Based on early aerial photographs of the site in 1946 and 1950, it showed that the mature *Sindora* × *changiensis* tree was part of a remnant rainforest patch that was retained as part of the grounds of the existing bungalow (503 Cranwell Road) that served as the residence of senior British military officers based at Changi (National Archives of Singapore records) (Fig. 6). Carbon dating also estimated that the tree is at least approximately 226 to 364 years old, further suggesting that the tree was naturally occurring in-situ and could not have been planted (National Parks Board, unpublished data).

This hybrid individual was observed to have been pollinated by the giant honey bee (*Apis dorsata*). These bees are known to be able to transfer pollen over sizeable distances, and have also been inferred to facilitate pollen dispersal of another leguminous tree species, *Koompassia malaccensis*, between rainforest patches across a distance of more than 2.5 km within the urban landscape of Singapore (Noreen and Webb 2013; Noreen et al. 2016). Given that these bees are likely to be pollinators of other *Sindora* species, it is therefore possible that this hybrid could have come about by the transfer of pollen between *S. coriacea* and *S. echinocalyx* trees at Changi in the past.

In both the Neighbour-Net (Fig. 1) and STRUCTURE analyses (Fig. 2), the proportion of *S. coriacea* in the genetic composition of the mature hybrid tree (Sind019) is higher than that of *S. echinocalyx*. This might suggest that *S. coriacea* is the maternal parent, which contributes both plastid and nuclear genetic material to the hybrid (Greiner et al. 2015). Another possibility is that the hybrid tree or its seedlings (Sind006, Sind 017) could be backcrossed to *S. coriacea*, which could explain the larger genetic contribution of *S. coriacea* to the hybrid. However, further studies including the sequencing of the chloroplast genome and the sampling of more hybrid offspring will be necessary to ascertain this.

This hybrid is fertile, with extensive flowering and fruit set happening once a year (Fig. 3), based on field observations from August 2020 to September 2021. The hybrid characters are also stable, as an offspring of the hybrid tree which was planted in 2001 has also been observed to produce fruits with a similarly

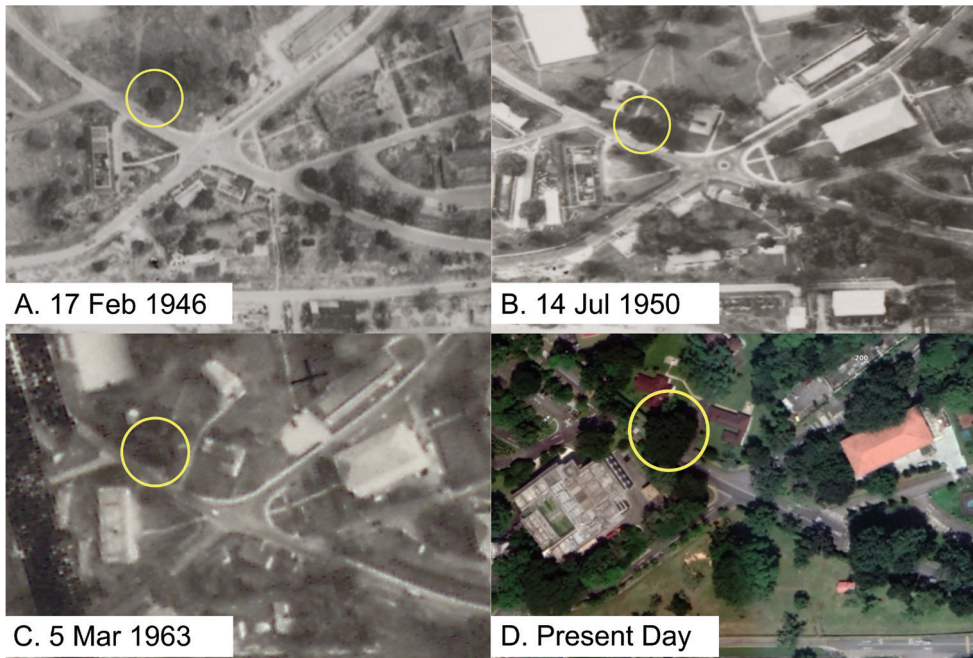


Figure 6. Aerial photo of the Changi area from various years showing *Sindora* \times *changiensis* circled in yellow **A** aerial photo on 17 Feb 1946, which is the earliest archival aerial photo of the Changi area, showing the tree as part of the rainforest remnant **B** aerial photo on 14 Jul 1950, showing the erection of the Cranwell bungalows to the left and right of the tree **C** aerial photo on 5 Mar 1963 **D** present day aerial photo showing the tree. (Images: **A–C** aerial photographs by the British Royal Air Force between 1940 to 1970s, from a collection held by the National Archives of Singapore. Crown copyright, reproduced in part **D** imagery 2021 Maxar Technologies, Map data 2021 Google).

sparse distribution of spines (Choo SING2021-677, 17 Sep 2021, outside Changi Chapel & Museum, SING). While this is unusual as hybrids often have lower fitness and are usually sterile (Ashton 1969; Hopkins 2013; reviewed in Schley et al. 2020), natural *Shorea* hybrids in the rainforest of Singapore have been documented to be fecund (Kamiya et al. 2011). The growth rates and survivability of *S. curtisii* \times *S. leprosula* seedlings were also found to be comparable to the parent species, albeit with slightly slower growth rate than *S. leprosula*, suggesting similar fitness to that of parent species in the rainforest environment (Kenzo et al. 2016, 2019). There have also been suggestions that environmental disturbance could potentially result in less favourable conditions for parental species and give rise to conditions that favour natural hybrids, with implications for conservation (Levin et al. 1996; Rieseberg and Carney 1998). This could have enabled *Sindora* \times *changiensis* to establish and persist over time, even as its habitat changed drastically from a primary rainforest to an urban environment. More research could be done to further examine the mechanisms giving rise to this fertile hybrid,

including the possibility of backcrossing with its parent species, as well as the effects of this on the evolutionary trajectory and the genetic diversity of *Sindora* in rainforest patches within anthropogenic landscapes.

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Supplementary material I

Figure S1

Authors: Le Min Choo, Adrian Hock Beng Loo, Wee Foong Ang, Kenneth Boon Hwee Er
Data type: Image.

Explanation note: ΔK value for the values of $K = 2–5$ for the 30 iterations of STRUCTURE conducted. The value of ΔK for $K = 1$ cannot be calculated. The highest value of ΔK at $K = 2$ shows that 2 is the optimal value of K for the STRUCTURE analysis.

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Link: <https://doi.org/10.3897/phytokeys.190.79185.suppl1>

A new species and new combination in basally flowering Vietnam Costaceae

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Abstract

Cheilocostus candidus **sp. nov.** (Costaceae), a basally flowering spiral ginger with cream white flowers from southern Vietnam, is described and illustrated here. A new combination, *Cheilocostus tonkinensis* (Gagnep.) Škorničk., is proposed here and the lectotype is designated. A key to *Cheilocostus* in Vietnam is included.

Keywords

Asia, *Cheilocostus candidus*, *Cheilocostus tonkinensis*, *Costus*, *Hellenia*, lectotype, spiral gingers

Introduction

The family Costaceae, with well-developed and sometimes branched true stems and leaves spirally arranged in monophyllous phyllotaxy, is easy to recognise within the Zingiberales order (see, for example, Kirchoff and Rutishauser (1990)). Based on recent phylogenetic studies, seven genera amounting to approximately 140 species are recognised in Costaceae (Specht and Stevenson 2006). The majority of this diversity is confined to African and New World tropics (Specht and Stevenson 2006, Maas-van de Kamer et al. 2016), while only a handful of species are considered native to Asia.

During this recent generic re-assessment of Costaceae, the best known and pantropically widespread Asian member of the genus, *Costus speciosus* J. König, as well as the other three Asian species (*C. lacerus* Gagnep., *C. globosus* Blume and *C. sopuensis* Maas & H. Maas), were transferred into the newly-established *Cheilocostus* (Specht and Stevenson 2006). The study, however, has not addressed the transfer of *Costus tonkinensis* Gagnep., possibly due to Maas (1979) briefly expressing a concern that 11 species described from various parts of Asia might all be mere variants of *Cheilocostus globosus* complex. While we fully agree that basally flowering Asian Costaceae are badly in need of a revision, our studies over the last decade on flowering material in SE Asia and Indochina, in particular, support the conclusion of Wu and Larsen (2000) that *Costus tonkinensis*, described from N. Vietnam, certainly represent a distinct species from *Ch. globosus* (see Fig. 1).

Specht and Stevenson (2006) overlooked the availability of two older names, *Hellenia* Retz. and *Tsiana* J.F.Gmel., both equally eligible for the clade of Asian Costaceae and proposed a new generic name, *Cheilocostus*. Govaerts (2013), while trying to fix the above mistake, unfortunately also overlooked the equally available *Tsiana* and proposed to use *Hellenia* Retz., without realising that it may very easily be confused with *Hellenia* Willd. which, although illegitimate, was used in the sister family Zingiberaceae for numerous species currently subsumed under *Alpinia* Roxb. Leong-Škorníčková and Šída (2016), therefore, put forward a proposal to conserve *Cheilocostus*, which, since 2006, quickly gained very wide usage. In line with our proposal, we, therefore, continue to uphold the genus *Cheilocostus* until the decision by the Nomenclatural Committee of Vascular Plants is reached.



Figure 1. A *Cheilocostus globosus*, Singapore **B** *Cheilocostus tonkinensis* (Gagnep.) Škorníčk., Vietnam, photographed at the type locality. Photo by Jana Leong-Škorníčková.

Only two Costaceae species are recorded to occur in Vietnam, the terminally flowering *Cheilocostus speciosus* and the basally flowering *Costus tonkinensis* (Phạm 2003). During our explorations of southern Vietnam, we have encountered an unusual radically flowering *Cheilocostus* with white flowers, which is described and illustrated below as *Cheilocostus candidus*. We also designate the lectotype *Costus tonkinensis* and effect its transfer into *Cheilocostus*. A key to the three species in Vietnam is provided.

The description of the new species is based on measurements from living flowering material. The general terminology follows Beentje (2016). The style of the description largely follows Maas-van de Kamer et al. (2016) with the following amendments. We refrain from using the term corolla tube because this term is morphologically misleading. The tubular part of the flower is primarily formed by the fused bases of the members of androecium. In fact, only the most basal part of the tube, here referred to as the floral tube, is formed by fusion/adnation of the basal part of corolla lobes (external part of the tube) and staminal whorl (internal part of the tube). After the corolla lobes diverge, the tube further extends proximally and is formed solely by the staminal whorl (i.e. labellum and stamen) and is, therefore, termed here as the staminal tube (see Fig. 2D). This usage is in line with terminology used in the sister family Zingiberaceae, particularly (e.g. Leong-Škorničková 2007, Leong-Škorničková et al. 2016). In line with the explanation provided by Newman and Kirchoff (1992), we also use the term gynopleural nectaries instead of septal nectaries.

Taxonomic treatment

***Cheilocostus candidus* Škorničk., A.Böhm. & H.Đ.Trần, sp.nov.**

urn:lsid:ipni.org:names:

Figures 2, 3

Diagnosis. Similar to *Ch. tonkinensis* by its inflorescence appearing at the base of the leafy shoots, but differs by its unbranched or very sparsely branched stems, densely puberulent leaf sheaths, densely puberulous lower side of lamina and cream-white flowers (compared to densely branched stems, glabrous leaf sheaths, glabrous lamina on both sides and yellow flowers with red markings on basal part of the labellum).

Type. Collected from the material cultivated at the Singapore Botanic Gardens, 21 September 2021, *Leong-Škorničková GRC-421* (holotype SING (inclusive flowering material in spirit), isotypes E, P, SGN). Originally collected from Vietnam, Lâm Đồng Prov., 20 June 2008, *Trần et al. 54* (living material only).

Description. Terrestrial, perennial herb in loose small clumps to ca. 2 m tall; **stems** up to 2.5 cm in diam. at base, unbranched or barely branched (in stems where the apical part was damaged) with 15–35 leaves, leafless in lower third; **sheaths** green, puberulent, becoming brown and papery with age; **ligule** 1–2 mm long, irregularly truncate, pale green, becoming pale brown with age, puberulous; **petiole** 2–5 mm long, pale green, bluntly canaliculate (kidney-shaped in cross-section), pubescent;



Figure 2. *Cheilocostus candidus* Škorničk., A.Böhm. & H.Đ.Trần sp. nov. **A** old and young inflorescence **B** flower **C** flower dissection (from left): bract, bracteole, calyx, ovary, dorsal corolla lobe, two lateral corolla lobes, floral and staminal tube with stamen attached, labellum **D** detail of floral (ft) and staminal tube (st) with stamen attached from side and dorsal view **E** detail of floral and staminal tube dissected with stamen attached to dorsal half (inset: detail of stigma). Based on plant used to prepare the type specimen Leong-Škorničková GRC-421, photo by Jana Leong-Škorničková.

lamina weakly obovate to elliptic with round to obtuse, slightly unequal base and acuminate apex, 12–28 × 4–11 cm, weakly plicate, somewhat thick, adaxially mid-green, glabrous, abaxially paler green, puberulous. **Inflorescence** radical, on a separate

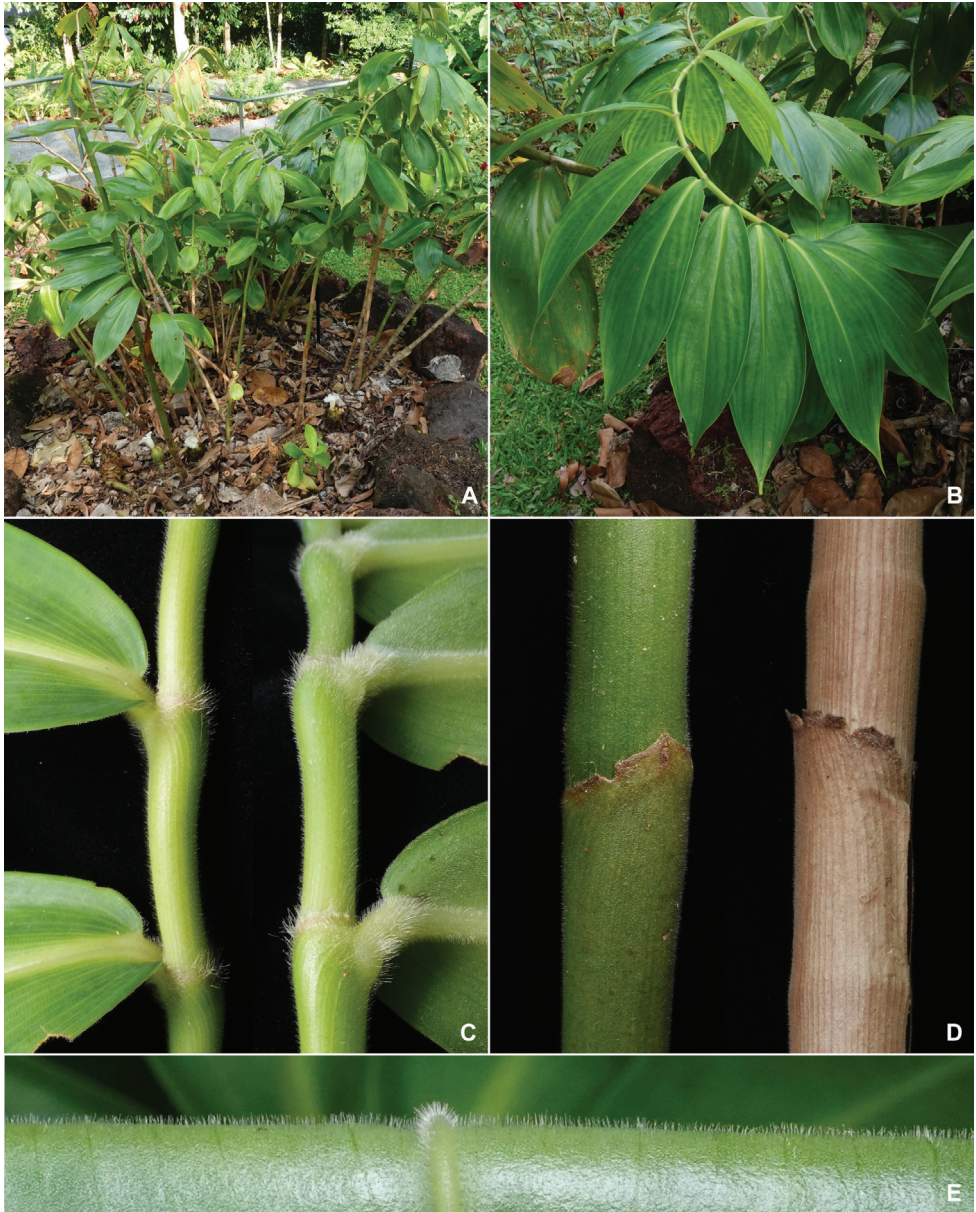


Figure 3. *Cheilocostus candidus* Škorničk., A.Böhm. & H.Đ.Trần sp. nov. **A** habit **B** leaves **C** detail of young stem with pulvini and bases of leaf blades **D** detail of young and old stems covered with leaf sheaths **E** detail of lower surface of the leaf blade. Based on plant used to prepare the type specimen *Leong-Škorničková* GRC-421, photo by Jana Leong-Škorničková.

leafless shoot emerging directly from the rhizome; **peduncle** horizontal to ascending, 3–12 cm long, sheaths \pm tubular, green, turning brown with age, densely pubescent, margin irregular; **spike** ovoid to narrowly ovoid, up to 20 cm long, 4.5–8 cm wide (narrower in very young, widening as the flowering progresses), composed of up to

ca. 120 imbricate bracts, arranged in right-handed spiral, each supporting a single bracteole and single flower (lowermost 5–10 sterile), 1–2 flowers per inflorescence open at a time; **fertile bracts** ovate to broadly ovate, to $3.2\text{--}5.5 \times 2.5\text{--}3.5$ cm, cream at base, light green distally, softly pubescent externally, glabrous internally, apex ending in very sharp callus, callus 6–7 mm long, glabrous; **bracteole** $3\text{--}4 \times 0.9\text{--}1.2$ cm long, unequally folded with single sharp keel, white to cream at base, pale green distally, externally softly pubescent, internally glabrous, apex acuminate with very sharp callus, callus ca. 5–7 mm long. **Flower** 9–11 cm long, exserted 5.5–7 cm above the supporting bract; **calyx** 3.5–4 cm long, cream to pale beige at base, light green distally, densely pubescent, apex 3-lobed, lobes 5–10 mm long, ending with very sharply mucronate callus, each callus ca. 4–5 mm long, glabrous; **floral tube** (measured from the apex of ovary to the point of divergence of corolla lobes) 2–2.5 cm long, fused solid with style in basal $\frac{1}{2}$, white to cream, externally and internally glabrous; **staminal tube** (measured from the point of divergence of corolla lobes to the point of divergence of stamen from the labellum) 8–11 mm long, white to cream, externally glabrous or with sparse hair, internally with long glandular hair; **corolla lobes** unequal, translucent cream-white, densely pubescent externally, glabrous internally, apex mucronate, mucro to 2–5 mm long, dorsal lobe obovate, symmetric, $5.1\text{--}5.8 \times 1.9\text{--}2.2$ cm, lateral lobes unequal in width, asymmetrically obovate, $4.8\text{--}5.2 \times 1.7\text{--}2$ cm; **labellum** 5.5–6 cm long, ca. 7–8 cm broad (when flattened), broadly obovate with apex often bilobed (lobes overlapping), thickened in the centre, thin towards the margins, cream white to very pale yellow in the central part, adaxially with glandular hair (more dense in central part, nearly glabrous towards the margins), abaxially mostly glabrous with some glandular hairs, margin crisped, with glandular hair; **stamen** petaloid, slightly obovate, ca. 2.6 cm long (ca. 3 cm with crest flattened), ca. 6 mm wide at base, ca. 1.3 cm wide at widest point, white to cream-white with pale yellow crest, adaxially glabrous, abaxially with sparse long glandular hair, crest 3–4 mm long, ca. 10 mm wide at base, rounded to obtuse, recurved, with glandular hair at margin; thecae 9–10 mm long, 3–4 mm across (both), dehiscent throughout their entire length. **Ovary** barrel-shaped, $13\text{--}16 \times 10$ mm, cream covered with soft dense beige hair, somewhat flattened and irregularly triangular in cross-section, trilocular with central placentation and apically embedded cream to beige coloured gynopleural nectaries, each locule with many ovules; **style** ca. 4 cm long (free part), white, glabrous; **stigma** semi-circular, 2 mm long, ca. 4 mm wide, dorso-ventrally flattened, 2-lamellate with dorsal 2-lobed appendage, cream white. **Fruits and seeds** not seen.

Etymology. The specific epithet refers to the white colour of the flower.

Distribution. Endemic to Vietnam.

Habitat and phenology. This species occurs near rocky streams in lowland broadleaved evergreen forest, at elevations about 200–300 m. Flowering in the field was observed in June, in cultivation, it extends to October; fruiting has not been so far observed.

Provisional IUCN assessment. The species is, so far, known only from the type locality, where only a few individuals were seen. A suitable habitat exists in the

proximity of the type locality and it is, therefore, most likely that the species has a wider area of distribution as it is highly unusual for Costaceae species to be stenoendemic. Nevertheless, as there is no reliable information on the population sizes or distribution of this species, we propose to treat it currently as Data Deficient (IUCN 2019).

Notes. *Cheilocostus candidus* is similar to *Ch. tonkinensis* in producing the inflorescence radically. It is, however, fairly easy to recognise it in the field even in sterile conditions by its barely branched leafy shoots, which have densely puberulent leaf sheaths and densely puberulous lower surface of lamina, compared to multi-branched stems and always glabrous leaves of *Ch. tonkinensis*.

Axillary branching of the leafy stems has been mentioned as one of the generic descriptions of *Cheilocostus* by Specht and Stevenson (2006). Since then, *Ch. borneensis* A.D. Poulsen, a species that does not have axillary branching has been described (Poulsen and Specht 2010). *Cheilocostus candidus* also does not branch or branches very sparsely (usually only after extensive damage of the main shoot by herbivory or cutting), which is yet another character helpful in the field.

No other specimens of this species were found in E, HN, K, P, SING, and VNM.

***Cheilocostus tonkinensis* (Gagnep.) Škorničk., comb. nov.**

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Figure 1B

Costus tonkinensis Gagnep., Bull. Soc. Bot. France 49: 248. 1903 [1902 publ. 1903],

Type: VIETNAM, Mount Ba Vi, *Balansa s.n.*, October 1887 (lectotype P! [P00686610], here designated).

Notes. The type locality of *Cheilocostus tonkinensis* is in northern Vietnam, Mount Ba Vi. In the protologue, Gagnepain (1902) refers to two collections made by Balansa from this locality. There are four sheets at P: a single sheet of *Balansa s.n.*, collected on 24 July 1886 [P00686610 – inflorescences & flower] and three sheets of *Balansa 4206* collected in October 1887 [P02198384 – leafy shoot & single fruit; P02198381–inflorescence only; P02198385–leafy shoot only]. Both of these collections predate the protologue and are annotated in Gagnepain’s hand as published in Bull. Bot Soc. France 49: 248. All four sheets have to be considered as syntypes and, therefore, the lectotypification is needed. Unfortunately, none of the specimens consists of both leafy shoot and an inflorescence. *Balansa s.n.* [P00686610] with inflorescence and a flower, accompanied by a pencil drawing, is selected here as the lectotype.

It is challenging to distinguish Asian radically flowering Costaceae in herbarium material as the flowers do not preserve well. Based on our observations of living flowering material and photographic records with location data, *Cheilocostus tonkinensis*, so far, occurs in S. China, northern and central Vietnam and northern Laos, but may possibly extend to Thailand.

Key to *Cheilocostus* in Vietnam

- 1 Inflorescence arising on the top of the leafy shoot, labellum white ***Ch. speciosus***
- Inflorescence arising from the base of the leafy shoot **2**
- 2 Plants well-branched, sheaths, petioles, and both surfaces of laminae glabrous, labellum yellow, with red marking in the centre ***Ch. tonkinensis***
- Plants sparsely or not branched, sheaths and abaxial surface of petioles and laminae puberulent to puberulous, labellum cream white throughout.... ***Ch. candidus***

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Nicotiana gandarela (Solanaceae), a new species of ‘tobacco’ highly endangered from the Quadrilátero Ferrífero in Brazil

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Abstract

Nicotiana gandarela Augsten & Stehmann (Solanaceae), **sp. nov.**, a small ‘tobacco’ known only from one locality at Serra do Gandarela, in the state of Minas Gerais, Brazil, is described and illustrated. It is morphologically characterized by its rosulate basal leaves, red corolla with a short tube not inflated at the apex, and the peculiar habitat, a shaded site under a rocky outcrop ledge along a forested stream. Phylogenetic analyses based on a combined dataset of nuclear (ITS) and plastid (*ndhF*, *trnLF*, and *trnSG*) DNA sequences revealed that the species belongs to the *Nicotiana* sect. *Alatae* and is sister to the clade with the remaining species in the section. A key for the identification of Brazilian species of the section is given. The unusual habitat, the small population size, and the intense pressure of mining activities in the surroundings made the species assessed as Critically Endangered (CR), needing conservation efforts to avoid its extinction.

Keywords

Endemism, IUCN, Minas Gerais, mining, molecular phylogeny

Introduction

Nicotiana L. is a Solanaceae genus known mostly because of tobacco (*N. tabacum* L.), a crop cultivated worldwide, but its diversity goes further. It is the fifth-largest genus in the family, with 82 species (Knapp 2020). *Nicotiana* occurs in South America and Australia, with one species from Africa (Namibia). The Andean region is recognized as the center of diversity of the group in the Americas, as for many other clades in Solanaceae (Goodspeed 1954; Dupin et al. 2017). Molecular phylogenies are available for the genus (Aoki and Ito 2000; Chase et al. 2003; Clarkson et al. 2004; Clarkson et al. 2017), and sectional revision is provided based on phylogenetic analyses, indicating 13 sections (Knapp et al. 2004).

In Brazil, the known native species belong to the *Nicotiana* sect. *Alatae* Goodsp. (hereafter *Alatae*). This section is morphologically characterized by herbaceous habit, rosulate and sessile leaves, usually viscid pubescent, few leaves on upper stems, and an abrupt dilatation at the throat of the corolla tube (Knapp et al. 2004). The section comprises two cytogenetic groups: one displays chromosome number $2n = 20$ and includes two species, *N. longiflora* Cav. and *N. plumbaginifolia* Viv., distributed from Argentina to the USA; the other presents chromosome number $2n = 18$ and includes *N. alata* Link & Otto, *N. bonariensis* Lehm., *N. forgetiana* Hemsl., *N. langsdorffii* Weinm., *N. mutabilis* Stehmann & Semir and *N. azambujae* L. B. Sm. & Downs, occurring in Brazil, Argentina, Uruguay, and Paraguay (Goodspeed 1954; Clarkson et al. 2004; Knapp et al. 2004). The internal phylogenetic relationships of the section are still not well resolved, with the two groups recovered as sisters (Clarkson et al. 2004) or with the $2n = 18$ group as paraphyletic (Clarkson et al. 2017). According to the plastid or nuclear markers used for the reconstruction, section diversification is proposed between 6.2 Ma and 7.7 Ma, respectively (Clarkson et al. 2017).

Nicotiana can be considered a taxonomically well-studied genus for the Brazilian flora (Vignoli-Silva and Mentz 2005; Vignoli-Silva and Stehmann 2020), in which six native, *N. alata*, *N. bonariensis*, *N. langsdorffii*, *N. forgetiana*, *N. mutabilis*, and *N. azambujae*, and two naturalized species, *N. tabacum*, and *N. glauca* Graham, are recognized. Morphometric analyses based on the corolla size and shape of *N. forgetiana* throughout its geographical distribution showed that the species seems to include two distinct taxonomic groups, one not yet formally described (Teixeira et al. 2021). Among the Brazilian species, only *N. mutabilis* is considered endangered (Vulnerable) because of its small geographic distribution and just a few known populations (Stehmann et al. 2002; CNCFlora [<http://cncflora.jbrj.gov.br/portal/pt-br/profile/Nicotiana%20mutabilis>]). *Nicotiana azambujae* needs further investigation, as it is only known from the type collection.

Within Brazil, the Southern region holds the largest number of *Nicotiana* species, which inhabit ecotonal environments between the grasslands and the *Araucaria* forest (Mixed Ombrophilous Forest) in the Atlantic Forest domain (Teixeira et al. 1986; Overbeck et al. 2007). This is an important biogeographic area for several taxa that have their evolutionary history related to this vegetation mosaic (Iganci et al. 2011;

Fregonezi et al. 2013). Species richness decreases towards the north, with *N. bonariensis* being the species with the northern-most distribution, reaching the grasslands at the high altitude regions of the Mantiqueira Range (Minas Gerais and Rio de Janeiro states) and the Espinhaço Range (Minas Gerais and Bahia states).

During floristic studies in the Quadrilátero Ferrífero, a mountainous area with large mineral reserves in Minas Gerais, with significant biological importance and high anthropic pressures (Drummond et al. 2005; Jacobi et al. 2011), samples of a small *Nicotiana* specimen with red flowers were collected. As the phenotype matched with none of the known *Nicotiana* species from Brazil, we assumed it could be a new species. In this work, we test this hypothesis using morphological and molecular data.

Methods

Taxonomy

Plant samples were collected during field expeditions in April 2018, and voucher specimens were deposited in the BHCB herbarium at the Universidade Federal de Minas Gerais, whereas flowers were preserved in Ethanol 50%. We took measures of vegetative parts from herbarium samples and of entire flowers from fixed material. Descriptive terminology was based on Radford et al. (1976). To compare the morphological characters with congeneric species and build the key, we revised the main taxonomic literature (Goodspeed 1954; Vignoli-Silva and Mentz 2005; Cocucci 2013; Vignoli-Silva and Stehmann 2020), including the protologues, as well as the materials from the following Brazilian herbaria: BHCB, HUEFS, ICN, MBM, OUPR, PAMG, RB, R, SP, SPF, and UEC. We also examined high-resolution images of the types available at Global Plants (<https://plants.jstor.org>).

Sampling for phylogenetic analyses and molecular methods

We included DNA sequences from 56 *Nicotiana* species, mostly generated in previous studies (Chase et al. 2003; Clarkson et al. 2004) and obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank). We selected species from all described sections (Knapp et al. 2004) and used two *Anthocercis* Labill. as outgroups. We generated DNA sequences for three different individuals of the new species, also for *N. mutabilis*, described in 2002 (Stehmann et al. 2002) and for which there were no DNA sequences available in the databases. For all Genbank accessions used in this work, see Suppl. material 1: Table S1. Fresh, young leaves were collected in the field and stored in silica gel. Total DNA was obtained using a modified version of the 2X-CTAB extraction method (Doyle and Doyle 1987) using 50 mg of tissue mass and washing with 70% ethanol. We used nucleotide sequences from the nuclear marker (ITS - rDNA internal transcribed spacers) and three plastid regions (*trnLF*, *trnSG*, and *ndhF*). The selection of markers was based on previous studies (Chase et al. 2003; Clarkson et al. 2004). Amplifications

were performed using the same conditions described in previous works (Chase et al. 2003; Clarkson et al. 2004). Polymerase chain reaction (PCR) products were purified by precipitation with 20% PEG (2.5 M NaCl and 20% polyethylene glycol 8000) and sequenced on demand by a specialized company using the same primers employed for amplification. We obtained bidirectional sequence reads for all the DNA regions, and consensus sequences were obtained using Geneious Prime 2020.0.3 (<https://www.geneious.com>). Sequences were aligned using CLUSTALW (Larkin et al. 2007) followed by manual adjustments in MEGA 11 (Tamura et al. 2021). Sequences were concatenated with MESQUITE (Maddison and Maddison 2019), and we obtained the best substitution model per DNA region using MRMODELTEST2 (Nylander 2004).

Phylogenetic analyses

We analyzed the data using Bayesian inference (BI) and Maximum Likelihood (ML). Initially, nuclear and plastid DNA were separately analyzed and posteriorly combined. We performed BI analyses using MRBAYES 3.2.7a (Ronquist et al. 2012) as implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal 2.0 (Miller et al. 2010), treating each DNA region as a separate partition. The “unlink” command was used to estimate model parameters separately for each partition. Each analysis consisted of two independent runs with four chains for 7,500,000 generations, sampling one tree every 1,000 generations and a temperature parameter of 0.2. Convergence between the runs was evaluated using the average standard deviation of split frequencies (< 0.01) and the Potential Scale Reduction Factor – PRSF (= 1.0) and was achieved after 715,000 generations. After discarding the first 2,500 trees (33%) as the burn-in, the remaining trees were used to assess topology and posterior probabilities (PP) in a 50% majority-rule consensus. We considered groups with PP > 0.95 as strongly supported, groups with PP ranging from 0.90–0.95 as moderately supported, and groups with PP < 0.90 as weakly supported. Maximum likelihood (ML) analysis of the concatenated dataset was performed using the online version of RAxML-HPC BlackBox (v.8.2.12) (Stamatakis 2014) through the CIPRES Science Gateway Portal (Miller et al. 2010). Bootstrap values were calculated based on 1000 replicates.

Conservation status

The conservation status was assessed based on the International Union for Conservation of Nature Criteria (IUCN 2019), considering the main threats to the *Quadrilátero Ferrífero* vegetation (Sonter et al. 2014).

Scanning electron microscopy analysis

Seeds were affixed in aluminum stubs using double-sided carbon tape, metalized with 10 nm gold-palladium (Robards 1978). The observations were performed under a JEOL JSM– 6360LV scanning electron microscope, with 5 kV (Jeol Ltd., Tokyo, Japan).

Results

Alignment characterization

The concatenated matrix consisted of 3,483 aligned characters, of which 317 (9.1%) were potentially informative. The ITS region had, proportionately, the highest number of phylogenetically-informative characters (20%). General features of the datasets and a summary of the models implemented for each partition are presented in Table 1.

Table 1. General features of the markers used in the phylogenetic analyses and models implemented for each.

Regions	Terminals	Characters	Variable uninformative characters	Informative characters (%)	Model
ITS	59	683	89	135 (20%)	GTR+I+G
<i>ndhF</i>	60	1056	49	71 (6.7%)	GTR+G
<i>trnLF</i>	60	991	41	58 (5.8%)	GTR+G
<i>trnSG</i>	60	855	38	62 (7.2%)	GTR+G
All plastid regions	60	2799	125	182 (6.5%)	–
All regions	60	3483	214	317 (9.1%)	–

Phylogenetic relationships

In the Bayesian phylogenetic tree based on ITS, *N. gandarela* appears within the section Alatae, the Alatae clade is moderately supported (PP = 0.91), and *N. gandarela* is sister of the 2n = 20 clade (*N. longiflora* and *N. plumbaginifolia*), being weakly supported (PP = 0.76) (Suppl. material 2: Fig. S1). The concatenated plastid regions recovered a Bayesian phylogenetic tree with *N. gandarela* as the sister species of section Noctiflorae, moderately supported (PP = 0.91), and Alatae appears as sister of section Repandae, weakly supported (PP = 0.84) (Suppl. material 3: Fig. S2).

Due to the combined dataset produced higher-supported trees in both ML and BI methods than using nuclear and plastid regions independently, we described the results based on this supermatrix only. As the topology was similar in ML and BI trees, we detailed the species relationships from BI results only. The three individuals of *N. gandarela* formed a well-supported clade in all obtained trees.

The *Nicotiana* species analyzed formed a strongly supported clade (PP = 1.00), and several subclades corresponded to the accepted sections for the genus (Fig. 1). The analysis confirmed the monophyly of the Alatae (PP = 0.99), which is strongly supported as the sister of the *Nicotiana* sect. *Suaveolens* Goodsp. (PP = 1.00). The two newly sequenced species, *N. gandarela* and *N. mutabilis*, were resolved as members of the Alatae. *Nicotiana gandarela* was recovered with high support (PP = 0.99) as sister to the clade with the remaining species in the Alatae, whereas *N. mutabilis* was resolved as closely related to *N. forgetiana* and *N. bonariensis* (PP = 1.00). Similar to previous analyses, low support was observed for the 2n = 18 group and for the relationship of *N. bonariensis* to *N. langsdorffii* (PP = 0.66).

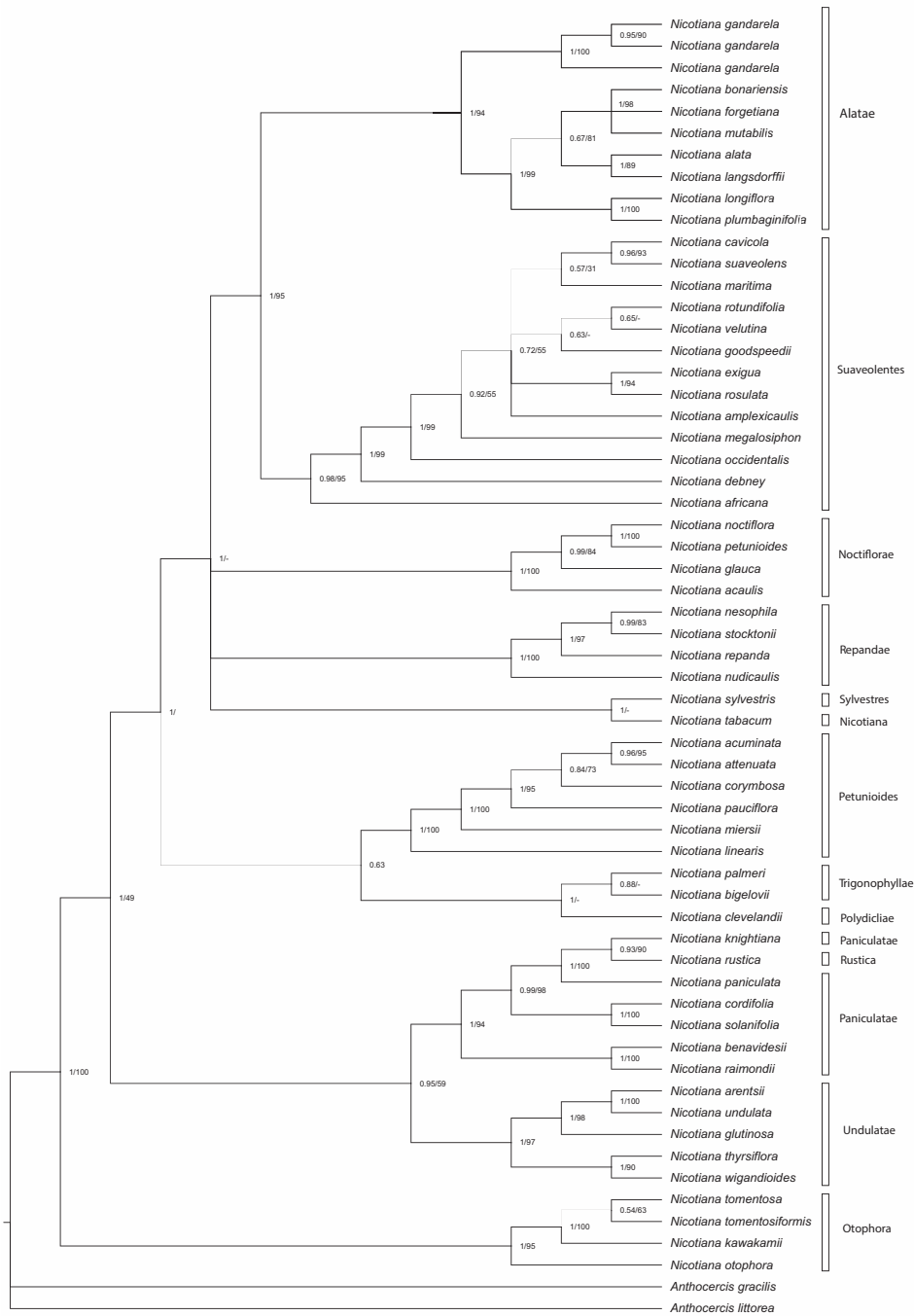


Figure 1. Bayesian 50% majority-rule consensus tree of 56 species of *Nicotiana* L. of the combined nuclear (ITS) and plastid (*ndhF*, *trnLF*, and *trnSG*) datasets. Numbers next to the nodes represent the posterior probabilities (PP) and bootstrap values. Section names (according to Knapp et al. 2004) are indicated on the right. Width of lines defined by PP values.

Taxonomic treatment

Nicotiana gandarela Augsten & Stehmann, sp. nov.

urn:lsid:ipni.org:names:

Fig. 2

Type. BRAZIL. Minas Gerais. Santa Bárbara, André do Mato Dentro, 19°59'43"S, 43°38'39"W, 17 Apr 2018 (fl, fr), *M. Augsten & J.R. Stehmann* 1078 (holotype: BHCB acc.#190958 [BHCB190958!]; isotypes: ICN, RB, to be distributed).

Diagnosis. *Nicotiana gandarela* differs from all other species of the *Nicotiana* sect. *Alatae* by its short corolla tube (< 15 mm), vivid red corolla limb, and unusual shaded, cave-mouth habitat.

Description. Annual herb to 0.5 m high, not branched, pubescent with multicellular, glandular trichomes. Leaves simple, rosulate, crowded, 6–21 cm long, (1.7–) 3.5–5.0 cm wide, persistent, spatulate, slightly discoloured, the blade hirsute throughout with simple, predominantly glandular hairs, long-attenuate at the base, margin sinuous, ciliate, midribs, and secondary veins visible at both surfaces, rounded or obtuse, sometimes acute, at the apex. Inflorescence scapose, paniculate, apical or lateral, composed of monochasial cymes; bracts lanceolate, 4.3 mm long, 1.0 mm wide, viscid-hirsute; pedicels 5.8–7.7 mm long. Calyx hirsute-glandular, deeply lobed, the tube 2.5 mm long, lobes 3–5 mm long, lanceolate, unequal, the apex acute. Corolla aestivation conduplicate, with folded basal petals covering the other three; tube 1.2–1.5 cm long, 3.0–3.4 mm diam., infundibuliform, not inflated at the apex, magenta, trichomes rare, opening ca. 3.4 mm; limb zygomorphic, red, cleft into widely-depressed ovate to very widely ovate lobes, patent or slightly reflexed. Stamens 5, didynamous, adnate ca. 5 mm from the base of the corolla tube, four longer, ca. 12.5 mm long, one shorter, ca. 11.4 mm long, all filaments glabrous; anthers 0.9–1.2 mm long, white, ellipsoid, pollen whitish; nectariferous disk present. Ovary slightly conical, glabrous, style ca. 9.4 mm long, stigma bilobed, green. Capsule 2-valved, included in the calyx, 6.0–7.5 mm long, 4.4–5.5 mm in diameter, ovoid, valves with incised apex. Seeds about 0.7–0.8 mm long, 0.6–6.5 mm wide, subglobose to obovoid, testa foveolate, anticlinal walls sinuous. Chromosome number unknown.

Etymology. The specific epithet “gandarela” refers to Serra do Gandarela, the mountain range complex where this species is endemic.

Distribution and habitat. The only known population of *Nicotiana gandarela* is at the Serra da Gandarela Mountain range, located in the northeast portion of the Quadrilátero Ferrífero in the Minas Gerais Brazilian State (Fig. 3A). The species occurs in a shaded site placed under a rocky outcrop ledge that extends for about 350 m along a stream and surrounded by a forest matrix, making this area a permanent humid environment. We recognized three subpopulations, each up to 150 individuals, including many seedlings (Fig. 2B). This environment resembles an open cave, and the individuals grow in bare powdery red soil originating from rock decomposition, usually with no other species co-occurring. The habitat is unique, and no similar microhabitat exists in this geologically diverse and complex area (Instituto Prístino: Atlas Digital

Geoambiental: Quadrilátero Ferrífero: Geodiversidade [<https://institutopristino.org.br/atlas/quadrilatero-ferrifero/>] Fig. 2A–B.

Phenology. *Nicotiana gandarela* has been collected with flowers and fruits in December and April.

Conservation status. Although the single site for *N. gandarela* would normally suggest the species was Data Deficient (DD), the precarious nature of the region

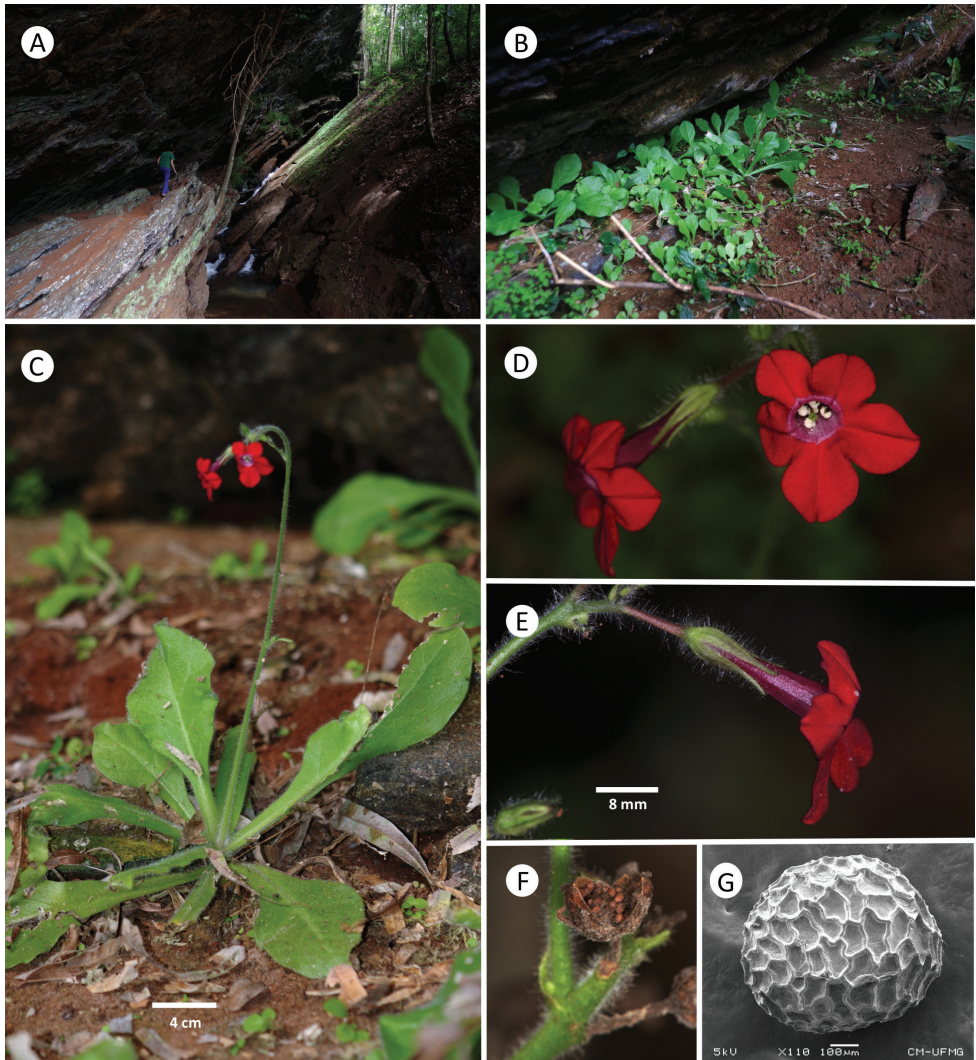


Figure 2. *Nicotiana gandarela* Augsten & Stehmann **A** peculiar habitat of the species, the shaded sites in the base of the shaded ledge **B** seedlings growing in the site **C** habit highlighting the rosulate leaves and the scapose inflorescence **D**, **E** flowers in lateral and frontal view **F** 2-valvate capsule with many seeds. **G** seed with sinuous anticlinal walls (Scanning Electron Microscopy) **D–G** were obtained from plants of the type population (Augsten and Stehmann 1078, BHCB). Photos by JR Stehmann.

in which it grows leads us to assess it more formally. The species was preliminarily assessed as Critically Endangered – CR (B1, B2a, biii, D), mainly due to its geographic range, as the extent of occurrence (EOO) and the area of occupancy (AOO) are smaller than 100 km² and 10 km², respectively. The only populations of *N. gandarela* are located on private property and not inserted in any integral conservation unit. In addition, the Quadrilátero Ferrífero suffers from constant habitat loss (Salles et al. 2018) as well as strong pressures from the mining sector (Sonter et al. 2014) with concessions required to explore iron and gold (Instituto Prístino: Atlas Digital Geoambiental 2021 [<https://institutopristico.org.br/atlas/quadrilatero-ferrifero/>]) (Fig. 3B).

Additional specimen examined. BRAZIL. Minas Gerais: Santa Bárbara. André do Mato Dentro, trilha para a cachoeira, 31 Dec 2017 (fl., fr.), *D.M.G. Oliveira, P.L. Viana & N.O. Mota* 359 (BHCB).

Identification key to the Brazilian species of *Nicotiana* sect. *Alatae*

- 1 Calyx length equal to the corolla tube.... ***N. azambujae* L.B. Sm. & Downs**
- Calyx length shorter than the corolla tube **2**
- 2 Corolla yellow-greenish or light green, actinomorphic, limb almost entire (shortly lobed), pollen blue..... ***N. langsdorffii* Weinm.**
- Corolla white, pink, magenta, purple or red, zygomorphic, limb clearly lobed; pollen green, white or yellow **3**
- 3 Corolla tube > 30 mm long, limb usually white, filaments adnate to the upper portion of the corolla..... **4**
- Corolla tube usually < 30 mm long, limb rarely white, filaments adnate to the lower portion of the corolla tube..... **5**
- 4 Leaves decurrent, limb strongly zygomorphic, stamens inserted in two levels (4 higher and 1 lower) at the corolla tube, filaments all geniculate ***N. alata* Link & Otto**
- Leaves auriculate, not decurrent, limb weakly zygomorphic, stamens inserted in three levels (2 higher, 2 middle, and 1 lower) at the corolla tube, filaments not geniculate ***N. longiflora* Cav.**
- 5 Corolla changing from white to pink or magenta during the anthesis ***N. mutabilis* Stehmann & Semir**
- Corolla keeping the same color during the anthesis..... **6**
- 6 Leaves evenly distributed and decurrent along the stem; calyx usually > 9 mm; corolla tube usually > 20 mm long, magenta or purple-red ***N. forgetiana* Hemsl.**
- Leaves usually rosulate, not decurrent on the stem; calyx usually < 9 mm long; corolla tube < 20 mm long, limb red or white..... **7**
- 7 Corolla tubular, distally ventricose; limb white ***N. bonariensis* Lehm.**
- Corolla tube gradually enlarged to the apex, not ventricose, limb red..... ***N. gandarela* Augsten & Stehmann**

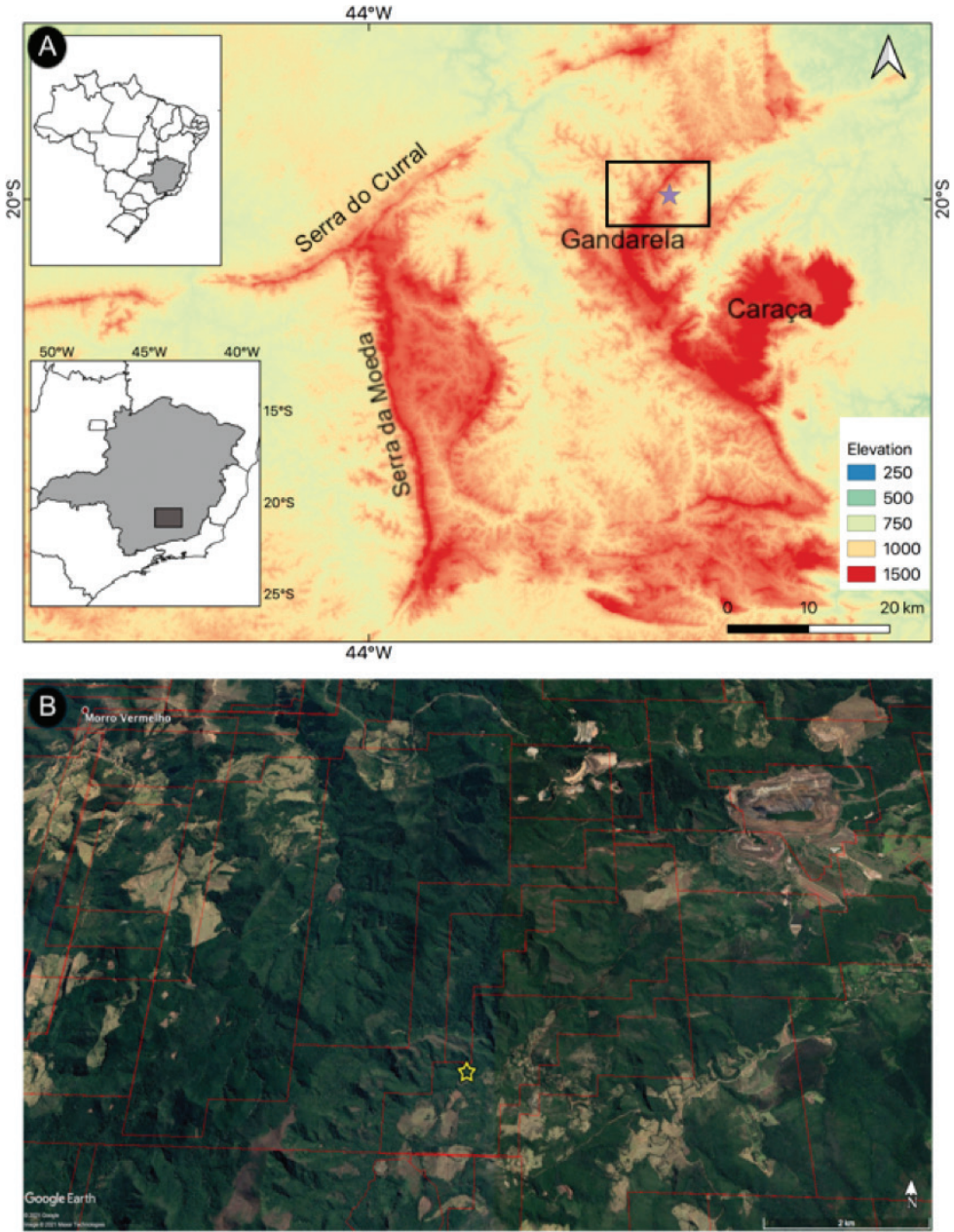


Figure 3. A main mountains of the Quadrilátero Ferrífero in Minas Gerais. Star indicates the *Nicotiana gandarela* Augsten & Stehmann occurrence site **B** Google Earth image showing the regional landscape associated with the *N. gandarela* site, the forest matrix impacted by a small village, and open mining. The red lines indicate mining concessions (Instituto Prístino: Atlas Digital Geoambiental [<https://instituto-pristino.org.br/atlas/>]).

Discussion

Overall, the recovered phylogenetic relationships were similar to those previously obtained (Chase et al. 2003; Clarkson et al. 2004; Knapp et al. 2004; Clarkson et al. 2017). Phylogenetic trees generated showed that the samples collected in the Serra da Gandarela in Minas Gerais are genetically distinct from all known species of *Nicotiana* used in our analyses. *Nicotiana gandarela* was recovered as the sister of all other species belonging to the Alatae (both of the groups with distinct cytotypes, $2n = 18$ and $2n = 20$; Clarkson et al. 2004; Knapp et al. 2004). The internal nodes of the group $2n = 18$ showed low support, and the lack of chromosome accounts to the new species does not permit any inferences about the phylogenetic relationships within the section.

The new species is an annual plant, with rosulate leaves, long-attenuate to the base, sessile, viscid-pubescent, and a zygomorphic corolla, all morphological traits associated with the species of the Alatae (Knapp et al. 2004). The decurrent leaf bases on the stem and the abrupt dilatation at the corolla throat, commonly found in the other species of the section (Goodspeed 1954), are lacking in the new species. The habit resembles that of *N. bonariensis* because of its rosulate leaves and scapose inflorescence. However, *N. gandarela* differs from *N. bonariensis* in its ombrophilous and extremely narrowly distributed habitat, its diurnal anthesis with red corollas with tube < 15 mm long, rather than being heliophilous, widespread, with nocturnal anthesis, and white corollas with a tube usually > 15 mm long.

Corolla color is variable in the Alatae, including white, yellow, pink, magenta, and purple-red. Such variation reflects an evolutionary history of radiation to distinct pollinator agents (Ippolito et al. 2004; Kaczorowski et al. 2005; Knapp 2010). Red flowers are usually associated with bird pollination (Faegri and van der Pijl 1979), and hummingbirds were already reported pollinating *N. forgetiana*, a pink to purple-red flowered species distributed in southern Brazil (Ippolito et al. 2004). Empirical data on the effective pollinator of *N. gandarela* are still necessary.

The Quadrilátero Ferrífero in Minas Gerais is located in the southern Espinhaço Range, a mountainous chain where the Cerrado (tropical Savannah) and Brazilian Atlantic Forest, two hotspots of biodiversity, connect (Mittermeier et al. 2004). The vegetation is a mosaic of topologies and vegetation, including grasslands, savannah, and forests (Spósito and Stehmann 2006), whose distributions are influenced by an altitudinal gradient, ranging from about 700 m to 2,080 m (Borges et al. 2011). In the region, besides the new species, a further two species of the Alatae are found, *N. bonariensis* and *N. langsdorffii*. The first usually inhabits the grasslands at higher altitudes known as Campos Rupestres (Silveira et al. 2016), while the second is generally associated with disturbed areas. Both grow mainly in open and sunny sites, as with most species of the Alatae, and the peculiar, shaded habitat of *N. gandarela* seems to be unique in the section. The microhabitat of the new species roughly resembles that of *Petunia exserta* Stehmann, an endemic species from Rio Grande do Sul, in

Southern Brazil, but where the geology and vegetational matrix are totally different (Lorenz-Lemke et al. 2006; Stehmann et al. 2009). In both cases, few or no species share the ground where these two species grow, meaning that they are fragile, with low, competitive capacity, but are presumably adapted to survive in these empty niches. Penumbral plant communities have also been described for the Campos Rupestres in Minas Gerais, where several species were reported as growing in small caves (Alves and Kolbek 1993), but until now, no Solanaceae species have been registered in these areas.

The new species deserves special conservation attention because it inhabits a small and rare habitat, if not unique, in the Quadrilátero Ferrífero, a region that is suffering intensive habitat loss (Salles et al. 2018) and has been the scenario of recurrent environmental disasters (Carmo et al. 2017; Rotta et al. 2019). Floristic inventories looking for new populations should be carried out in the surrounding forested areas, including the Serra do Gandarela National Park, following the drainage lines in the valleys. Also, we suggest the engagement of local people in trying to find other populations because of the impossibility of looking for similar areas since they are hidden by forest in satellite images. In the end, although we emphasize that the species should be primarily preserved in its natural habitat, ex-situ conservation measures might also be necessary (CNCFlora 2016) in order to prevent its extinction.

Acknowledgements

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Supplementary material I

Table S1

Authors: Mariana Augsten, Pablo Burkowski Meyer, Loreta B. Freitas, João A. N. Batista, João Renato Stehmann

Data type: GenBank accessions

Explanation note: Genbank accessions. ITS sequences from Chase et al. 2003, Plastidial sequences (*trnL-F*, *trnS-G* and *ndhF*) from Clarkson et al. 2004. Sequences from this work marked with an * (*Nicotiana gandarela* and *Nicotiana mutabilis*).

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Link: <https://doi.org/10.3897/phytokeys.190.76111.suppl1>

Supplementary material 2

Figure S1

Authors: Mariana Augsten, Pablo Burkowski Meyer, Loreta B. Freitas, João A. N. Batista, João Renato Stehmann

Data type: Phylogenetic

Explanation note: Bayesian 50% majority-rule consensus tree of 56 species of *Nicotiana* L. of the nuclear (ITS) dataset. Numbers next to the nodes represent the posterior probabilities (PP).

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Link: <https://doi.org/10.3897/phytokeys.190.76111.suppl2>

Supplementary material 3

Figure S2

Authors: Mariana Augsten, Pablo Burkowski Meyer, Loreta B. Freitas, João A. N. Batista, João Renato Stehmann

Data type: Phylogenetic

Explanation note: Bayesian 50% majority-rule consensus tree of 56 species of *Nicotiana* L. of the plastid (*trnL-F*, *trnS-G* and *ndhF*) datasets. Numbers next to the nodes represent the posterior probabilities (PP).

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Link: <https://doi.org/10.3897/phytokeys.190.76111.suppl3>

Leaf epidermal micromorphology of *Zingiber* (Zingiberaceae) from China and its systematic significance

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Abstract

Leaf epidermal characteristics are important for phylogenetic and taxonomic studies of many plants, but there is currently insufficient such data for this application in *Zingiber* species. Therefore, the leaf epidermal micromorphology of 22 species in three sections of *Zingiber* was investigated by light microscopy and scanning electron microscopy. Differences between various taxonomic groups of Zingiberaceae were also compared to assess their phylogenetic and taxonomic significance. As in other genera of Zingiberaceae, the epidermal cells in both the adaxial and abaxial epidermis of *Zingiber* species were found to be hexagonal or polygonal, with non-sinuous anticlinal walls that are arranged parallel to leaf veins. Tetracytic stomata are mostly randomly distributed in the intercostal regions of both surfaces and are more common on the abaxial surface. The stomatal density of the species in sect. *Pleuranthesis* is significantly lower than that in sects. *Zingiber* and *Cryptanthium*. There are two types of trichome in *Zingiber*: so-called “delicate” trichomes are present in most species, while “stout” trichomes with a swollen base are only found in *Z. corallinum* and *Z. montanum*. Oil cells occur in both epidermal layers of some species in sects. *Zingiber* and *Cryptanthium*, but only in the abaxial epidermis of *Z. ellipticum* in sect. *Pleuranthesis*. Crystals are found in the abaxial epidermis only in all species, but are present in both epidermal layers of *Z. corallinum* and *Z. montanum*. Although the epidermal morphology is similar in most *Zingiber* species, stomatal density, type of trichome and distribution of oil cells and crystals offer valuable information for the systematic and taxonomic studies in this genus.

* These authors contributed equally to this work.

Keywords

crystal, oil cell, stomata, trichome, Zingiberales

Introduction

The type genus *Zingiber* of Zingiberaceae was established by Miller in 1754 and contains about 150 species, widely distributed from tropical to subtropical Asia (Theilade 1999; Theerakulpisut et al. 2012). The center of *Zingiber* diversity is located in Southeast Asia, where China has 42 species (Wu and Larsen 2000). Given the recent discovery of several new species, this figure is probably an underestimate (Bai et al. 2015a, 2016, 2018; Li et al. 2020a; Wang et al. 2020). *Zingiber* has been confirmed to be monophyletic by molecular analyses (Kress et al. 2002; Li et al. 2020b) and is easily distinguished from other genera of Zingiberaceae by specific features of the flower structure; for example, the lateral staminodes are fused to the labellum; there is an elongated, horn-shaped anther crest wrapped around the upper part of style; and there is a pulvinus at the base of the petiole (Wu and Larsen 2000; Bai et al. 2015b). The traditional infrageneric classification of *Zingiber* recognizes four sections based on the habit and position of the inflorescence (Schumann 1904), namely sects. *Zingiber* (basal with long peduncle), *Cryptanthium* (radical with short procumbent peduncle), *Pleuranthesis* (spikes breaking through the leaf sheaths laterally) and *Dymczewiczia* (terminal inflorescence). However, some species (such as *Z. junceum* and *Z. barbatum*) have been found to have both basal and terminal inflorescences, making it difficult to place them unequivocally in a particular section (Kishor and Leong-Škorničková 2013). A recent study proposed that sect. *Dymczewiczia* should be merged with sect. *Zingiber* because palynological evidence suggests that the pollen is very similar in both sections, being spherical with cerebriform sculpturing, which differs from the ellipsoidal pollen grains with spira-striate sculpturing found in sect. *Cryptanthium* (Theilade et al. 1993). Phylogenetic evidence also shows that sect. *Dymczewiczia* is nested within sect. *Zingiber* in the phylogenetic tree (Theerakulpisut et al. 2012). In addition, the taxonomic identification of this genus tends to rely on floral characters, mainly flower color and labellum shape (Wu and Larsen 2000), while leaf features have received little attention. However, the flower characters are ineffective in identifying related species due to variation within the same species (Bai et al. 2015b). For example, *Z. monglaense* and *Z. flavomaculosum* have the same leaf and flower characteristics, and are considered to be merged (Tong 1998). The same was observed for *Z. nudicarpum*, *Z. peninsulare* and *Z. newmanii* (Bai et al. 2019). Moreover, the flower characters can be difficult to define precisely in *Zingiber* species, because the florescence time is short and the characters are often not well preserved in herbarium specimens (Bai et al. 2015b). Thus, more experimental studies, for example, involving leaf characteristics and leaf epidermal micromorphology, are needed on the infrageneric systematic classification of *Zingiber*. Leaves have some advantages over flowers, as they can be obtained at all stages of plant growth and leaf features are easier to preserve.

Leaf epidermal micromorphology, which describes the shape of epidermal cells, the outline of anticlinal walls, stomatal type, surface ornamentation and trichome type, has become a tool for the study of phylogeny and taxonomy in many plant species (Baranova 1972; Wilkinson 1979; Stace 1984), especially in those families where identification is complicated, such as Salicaceae (Chen et al. 2008; Ghahremaninejad et al. 2012; Wang et al. 2012), Rosaceae (Tahir and Rajput 2009; Zamani et al. 2017) and Lamiaceae (Moon et al. 2009, 2010; Eiji and Salmaki 2016; Mannethody and Purayidathkandy 2018; Gul et al. 2019). For example, papilla patterns were useful in the discrimination of several Poaceae species (Zhang et al. 2014). The type of trichome, and the form of the trichome base and anticlinal cell walls have all been valuable for the identification of Fagaceae species (Zhou and Xia 2012; Deng et al. 2013, 2014). The trichome type and stomatal length were instrumental in assessing the phylogeny of the Ranunculaceae (Hoot 1991; Shi and Li 2003). Based on evidence that the range of variation in leaf epidermis overlaps completely between the two families, den Hartog and Baas (1978) transferred Hippocrateaceae into Celastraceae. In Zingiberaceae, leaf anatomical characters have also proved useful for taxonomic studies. The subfamily Costoideae (= Costaceae) is anatomically very distinct from the remainder of the family, and thus supports the separation of Costoideae from the Zingiberaceae (Tomlinson 1956). The type of silica inclusion serves to distinguish the tribes Globbeae, Hedychieae and Alpinieae (Tomlinson 1956).

The leaf epidermal features of some genera of Zingiberaceae, such as *Amomum*, *Alpinia*, *Boesenbergia*, *Kaempferia*, *Curcuma*, *Hedychium*, *Elettaria* and *Globba*, have been described to some extent (Tomlinson 1956; Patel 1975; Hussin et al. 2000, 2001; Xiao et al. 2004; Talip et al. 2005; Jayasree 2007; Chen and Xia 2010; Martins et al. 2010; Tang et al. 2010; Kajornjit et al. 2018; Salasiah and Meekiong 2018). Nevertheless, only a few representative species in these genera have been studied. So far, studies of leaf epidermis of *Zingiber* have only covered seven species (Olatunji 1980; Nyawuame and Gill 1990; Jayasree 2007), including two widely cultivated species (*Z. officinale* and *Z. montanum*), one widely distributed species (*Z. zerumbet*), and four taxa from South India, but these accounts were not detailed. All seven species studied are from two sections, sects. *Zingiber* and *Cryptanthium* (only one species, *Z. wightianum*), which is insufficient to represent the entire genus. In this study, we used multiple samples from the three sections of *Zingiber* (sects. *Zingiber*, *Cryptanthium* and *Pleuranthesis*) in China to investigate leaf epidermal micromorphology by light microscopy (LM) and scanning electron microscopy (SEM), and then compared the leaf epidermal characters at different classification levels in Zingiberaceae. Thus, the aim of the present study was to describe the leaf epidermal features of *Zingiber* and to assess their phylogenetic and taxonomic significance.

Materials and methods

More than 300 samples from 22 *Zingiber* species (Table 1) in China were used in the study. Leaf material from mature plants was collected by the authors in the field and voucher specimens were deposited in the herbarium of South China Normal University (SN). Fresh leaves were fixed in 90% ethyl alcohol solution, 5% formaldehyde and 5%

Table 1. Comparable leaf epidermal characters of 22 *Zingiber* species. Numbers indicate mean ± standard deviation. Stomatal index = number of stomatal apparatuses/ (number of stomatal apparatuses + number of epidermal cells); Stomatal density = number of stomatal apparatuses/ mm² leaf area.

Taxa	Voucher	Adaxial epidermis				Abaxial epidermis			
		Epidermal cell size (L ×W) (μm)	Stomatal size (L ×W) (μm)	Stomatal index	Stomatal density (mm ⁻²)	Epidermal cell size (L ×W) (μm)	Stomatal size (L ×W) (μm)	Stomatal index	Stomatal density (mm ⁻²)
Sect. <i>Pleuranthesis</i>									
<i>Z. ellipticum</i>	xmh-14-23	72.46±13.15 × 37.04±5.62	37.42±3.30 × 27.57±3.28	0.16±0.26	0.28±0.44	61.38±8.43 × 46.78±6.80	36.73±2.25 × 27.56±1.92	3.67±0.95	19.30±4.94
Sect. <i>Zingiber</i>									
		64.85(53.41–76.52) × 37.01(30.54–46.59)	43.02(37.99–47.45) × 26.18(21.42–30.37)	1.53(0.22–2.87)	8.08(1.06–19.05)	49.64(40.98–56.47) × 34.98(28.65–43.45)	40.64(36.14–46.90) × 23.69(19.86–26.45)	6.70(5.12–9.17)	60.92(45.93–79.20)
<i>Z. corallinum</i>	wyq-14-46	75.27±10.41 × 46.59±6.79	47.45±2.20 × 26.57±2.21	1.54±0.69	3.93±2.38	56.47±9.05 × 43.45±6.40	46.90±3.77 × 24.26±1.69	5.67±0.79	45.93±6.89
<i>Z. neotruncatum</i>	xmh-15-16	76.52±12.36 × 30.54±4.04	44.37±3.49 × 30.37±2.43	0.22±0.16	1.06±0.78	55.46±10.12 × 28.65±4.15	38.42±1.99 × 25.29±1.86	5.12±0.69	48.60±6.99
<i>Z. nudicarpum</i>	wyq-14-22	57.44±14.90 × 34.15±2.68	37.99±1.46 × 25.20±1.30	0.41±0.28	2.33±2.06	46.25±4.82 × 32.56±2.15	36.14±2.86 × 22.59±4.54	6.82±0.59	67.74±10.38
<i>Z. montanum</i>	wyq-15-65	61.63±3.08 × 42.83±3.64	43.97±3.37 × 27.32±1.36	2.87±0.90	14.02±3.73	49.04±2.38 × 38.62±1.25	45.55±3.37 × 26.45±1.16	9.17±1.69	63.11±6.36
<i>Z. zerumbet</i>	wyq-14-44	53.41±1.74 × 30.96±1.83	41.32±2.60 × 21.42±0.38	2.60±0.51	19.05±2.31	40.98±2.56 × 31.61±2.73	36.20±0.52 × 19.86±0.21	6.70±0.52	79.20±3.98
Sect. <i>Cryptanthium</i>									
		73.49(61.10–86.03) × 36.71(28.81–45.61)	42.97(37.08–54.83) × 27.20(23.08–32.07)	1.12(0.45–2.19)	5.48(1.99–14.52)	55.12(43.36–72.89) × 39.35(31.06–49.64)	41.81(34.78–49.11) × 25.00(21.41–29.15)	6.80(4.74–9.34)	49.55(23.59–88.46)
<i>Z. atrorubens</i>	hn-zzj-14-01	72.44±15.14 × 39.86±7.24	54.83±3.47 × 28.82±2.33	2.19±0.68	7.98±2.41	55.35±5.73 × 44.39±6.19	44.50±4.22 × 26.21±2.37	6.32±1.66	28.49±11.54
<i>Z. bisectum</i>	xmh-14-15	62.18±8.02 × 37.53±2.19	44.35±2.14 × 26.51±5.54	1.39±0.35	7.50±1.82	43.36±3.23 × 33.39±1.45	42.16±3.90 × 21.58±3.92	9.34±0.58	88.46±16.44
<i>Z. cochleariforme</i>	wyq-14-56	84.36±8.46 × 36.22±2.28	42.45±2.29 × 27.69±3.50	0.55±0.34	1.99±1.73	60.29±5.44 × 41.08±1.97	42.55±3.44 × 25.16±3.42	6.89±2.33	45.27±9.26
<i>Z. densissimum</i>	wyq-14-96	84.81±6.75 × 45.15±5.65	44.30±1.98 × 32.07±3.36	0.71±0.25	2.68±1.11	55.74±10.41 × 40.61±5.42	44.52±1.54 × 29.15±2.18	7.33±1.11	50.55±7.22
<i>Z. flavomaculosum</i>	wyq-14-72	71.32±9.11 × 35.66±3.76	40.10±4.11 × 25.14±3.20	1.77±0.58	10.29±4.34	52.02±8.43 × 35.21±3.02	37.67±3.14 × 22.79±2.40	7.61±1.07	66.60±14.10
<i>Z. guangxiense</i>	wyq-15-10	85.90±7.73 × 38.59±5.70	39.92±2.68 × 27.03±2.21	0.74±0.16	3.33±0.67	60.91±4.95 × 41.88±4.29	40.84±1.73 × 24.30±2.96	7.46±0.96	48.02±8.28
<i>Z. leptorrhizum</i>	wyq-15-80	75.27±20.48 × 38.19±11.66	47.38±2.16 × 29.21±3.04	1.31±0.29	4.01±0.87	72.89±9.25 × 49.64±13.12	49.11±1.80 × 27.23±1.66	6.34±1.28	23.59±5.00
<i>Z. lingyunense</i>	xmh-14-14	61.71±11.56 × 30.16±5.37	39.46±2.42 × 24.82±2.88	0.45±0.11	3.10±0.78	50.21±7.97 × 37.51±4.43	38.79±1.56 × 23.29±1.38	4.74±0.96	35.93±5.56
<i>Z. longiglande</i>	wyq-15-02	67.45±6.28 × 38.92±1.11	41.35±0.61 × 30.47±4.23	0.61±0.03	2.50±0.39	55.86±5.17 × 39.22±5.42	43.12±4.13 × 27.90±0.65	7.08±0.08	45.45±3.72
<i>Z. longiligulatum</i>	wyq-14-124	71.47±6.28 × 37.80±5.31	45.26±5.28 × 28.84±3.07	1.10±0.39	4.73±2.70	54.93±4.42 × 41.40±3.86	44.54±5.53 × 24.48±3.01	7.28±0.77	53.99±7.36
<i>Z. orbiculatum</i>	wyq-14-62	61.10±7.42 × 28.81±6.94	37.08±1.09 × 23.08±2.05	0.94±0.68	14.52±3.36	47.97±6.74 × 31.06±4.77	34.78±0.80 × 21.41±1.89	6.25±0.63	45.97±19.12
<i>Z. recurvatum</i>	wyq-14-80	72.28±7.50 × 33.14±2.82	38.54±2.65 × 27.13±3.65	0.87±1.12	4.76±6.57	53.68±6.15 × 36.29±5.00	38.16±1.61 × 23.50±2.21	7.22±1.26	56.01±5.61
<i>Z. roseum</i>	wyq-14-122	86.03±9.60 × 45.61±6.54	42.86±2.98 × 30.08±2.16	0.83±0.24	2.67±0.97	63.33±17.15 × 40.86±5.19	42.68±2.79 × 28.56±1.85	7.42±0.70	53.46±5.91
<i>Z. teres</i>	wyq-14-119	75.28±20.29 × 31.27±2.97	38.07±1.26 × 25.27±5.41	0.85±0.59	5.43±4.35	46.99±3.42 × 35.27±1.50	37.43±3.27 × 24.03±1.79	6.47±0.23	68.04±6.42
<i>Z. tuanjiuum</i>	wyq-14-54	80.88±9.37 × 41.32±5.30	49.75±3.23 × 25.63±2.16	1.03±0.17	4.90±0.76	57.07±7.61 × 45.28±6.95	47.05±2.78 × 27.87±1.93	5.86±0.73	37.56±6.19
<i>Z. xishuangbannaense</i>	wyq-14-87	63.40±7.31 × 29.04±2.04	41.86±2.85 × 23.45±0.83	1.52±0.43	7.22±6.22	51.29±3.44 × 36.54±1.21	41.05±2.52 × 22.50±3.66	5.35±0.28	45.45±11.68

acetic acid at a ratio of 18:1:1, and subsequently epidermal tissue was obtained from the leaves by gently scraping it off with a stainless steel blade. Pieces of leaf epidermis were stained in a solution of 1% safranin in 50% ethanol, and then dehydrated in an ethanol series before being mounted in glycerine gel for light microscopy. To ensure consistency of epidermal structure, at least five slides were examined for each sample. Twenty epidermal cells and stomata from each sample were measured and a mean was calculated based on the range of variation. The stomatal index and stomatal density were obtained for an area of 0.5 mm × 0.6 mm using the following formulae: stomatal index = number of stomatal apparatuses / (number of stomatal apparatuses + number of epidermal cells); stomatal density = number of stomatal apparatuses per mm² leaf area. All statistical analyses were performed using SPSS11.5 and Microsoft Excel 2010. A confidence level of $p \leq 0.05$ was considered to be significant. Material for scanning electron microscopy was macerated in 4% glutaric dialdehyde solution for about 24 h and dehydrated in a graded alcohol series, and then mounted on stubs. After gold sputtering, the specimens were examined and photographed under a JEOL JSM-6360LV scanning electron microscope. The terminologies of the stomatal complex types used in this study are those of Fryns-Claessens and Van Cotthem (1973) and Dilcher (1974).

Results

A comparison of leaf epidermal characteristics in 22 *Zingiber* species is shown in Table 1.

Epidermal cells

When examined by LM, the epidermal cells of *Zingiber* species were found to be mostly hexagonal or polygonal, with the long axis usually perpendicular to the veins, and arranged in rows parallel to the veins; the anticlinal walls were straight to slightly curved (Fig. 1A–F). Adaxial epidermal cells were always more regularly arranged and slightly larger than abaxial epidermal cells (Table 1). Adaxial epidermal cells ranged in size from $53.41 \pm 1.74 \times 30.96 \pm 1.83$ μm (in *Z. zerumbet*) to $86.03 \pm 9.60 \times 45.61 \pm 6.54$ μm (in *Z. roseum*), and were usually elongated with the length 1.4–3.2 times longer than the width. The abaxial epidermal cells ranged from $40.98 \pm 2.56 \times 31.61 \pm 2.73$ μm (in *Z. zerumbet*) to $72.89 \pm 9.25 \times 49.64 \pm 13.12$ μm (in *Z. leptorrhizum*) in size, and also were usually elongated with the length 1.2–2.0 times longer than the width. The epidermal cells above the veins were smaller and more or less longitudinally elongated (Figs 1E, F, 2C). When examined by SEM, the cells were convex on the adaxial side of the epidermis (Fig. 2A) and concave on the abaxial side (Fig. 2B) with smooth cuticular membranes. The anticlinal cell walls were invisible or obscure.

Stomatal apparatus

The stomatal apparatus, which occurs in both the adaxial and abaxial leaf epidermis in all *Zingiber* species studied, was of the tetracytic type with four subsidiary cells

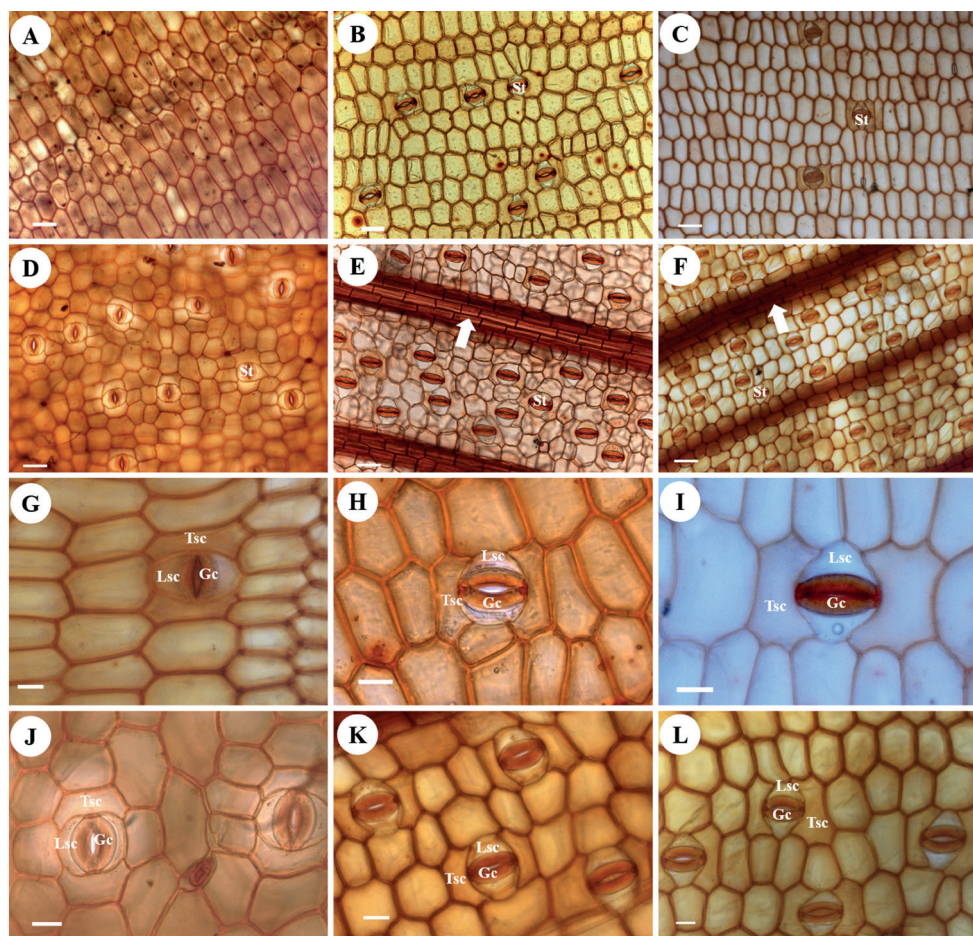


Figure 1. Leaf epidermal characters of *Zingiber* shown by light microscopy **A–C** adaxial epidermis of *Z. ellipticum* (**A**), *Z. montanum* (**B**) and *Z. flavomaculosum* (**C**) showing epidermal cells and stomatal apparatus **D–F** abaxial epidermis of *Z. ellipticum* (**D**), *Z. montanum* (**E**) and *Z. teres* (**F**) showing epidermal cells, costal epidermal cells and stomatal apparatus (arrows indicate to the costal epidermal cells) **G–I** detail of tetracytic stomatal apparatus on the adaxial epidermis of *Z. ellipticum* (**G**), *Z. montanum* (**H**) and *Z. tuanjuum* (**I**) **J–L** detail of tetracytic stomatal apparatus in the abaxial epidermis of *Z. ellipticum* (**J**), *Z. montanum* (**K**) and *Z. longiligulatum* (**L**). St: stoma; Gc: guard cell; Lsc: lateral subsidiary cell; Tsc: terminal subsidiary cell. Scale bars: 50 µm (**A–F**); 20 µm (**G–I**).

around the stoma, one on each side and one at each pole (Fig. 1G–L). The guard cells were reniform with smooth cuticular membranes (Fig. 2D). The lateral subsidiary cells were subtriangular with the long axis parallel to the stoma, while the terminal subsidiary cells were adjacent to the stoma poles. The stomatal orientation (the long axis of all stomata) was approximately parallel to the veins. The stomatal size in both leaf epidermal layers was similar in all species (Table 1), but ranged from $41.32 \pm 2.60 \times 21.42 \pm 0.38$ µm (in *Z. zerumbet*) to $54.83 \pm 3.47 \times 28.82 \pm 2.33$ µm (in *Z. atrorubens*)

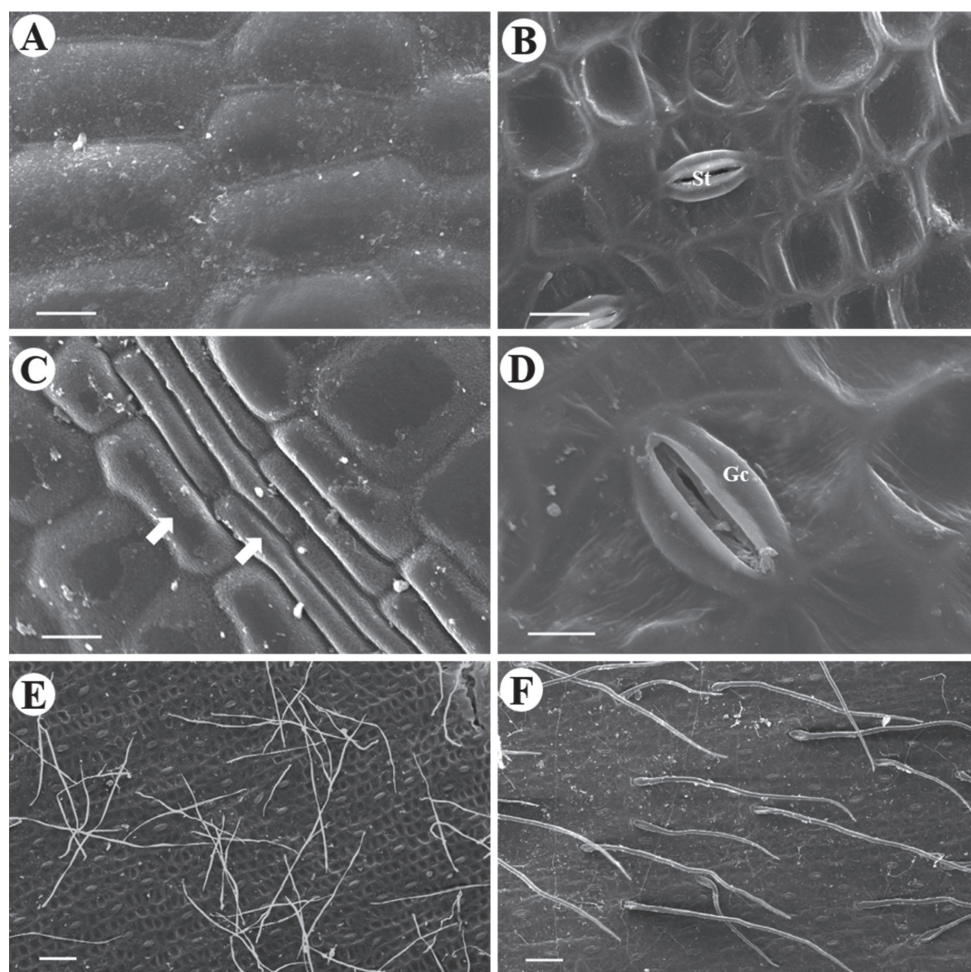


Figure 2. Leaf epidermal characters of *Zingiber* shown by scanning electron microscopy **A** adaxial epidermis of *Z. flavomaculosum* showing convex epidermal cells with smooth cuticular membranes **B** abaxial epidermis of *Z. xishuangbannaense* showing concave epidermal cells with smooth cuticular membranes **C** detail of epidermis over the vein in *Z. montanum* (arrows indicate costal epidermal cells) **D** stomatal apparatus in *Z. flavomaculosum* showing guard cells with smooth cuticular membranes **E** delicate trichomes in *Z. xishuangbannaense* **F** stout trichomes with swollen trichome base in *Z. corallinum*. St: stoma; Gc: guard cell. Scale bars: 10 μm (**D**); 20 μm (**A–C**); 100 μm (**E, F**).

in the adaxial epidermis and from $36.20 \pm 0.52 \times 19.86 \pm 0.21 \mu\text{m}$ (in *Z. zerumbet*) to $49.11 \pm 1.80 \times 27.23 \pm 1.66 \mu\text{m}$ (in *Z. leptorrhizum*) in the abaxial epidermis. Stomata occurred much more frequently in the abaxial epidermis than in the adaxial epidermis in all species studied (Table 1), and most of them were randomly distributed in the intercostal regions (Fig. 1E, F). The stomatal index of the adaxial epidermis and the abaxial epidermis ranged from $0.16\% \pm 0.26\%$ (in *Z. ellipticum*) to $2.87\% \pm 0.90\%$ (in *Z. montanum*) and from $3.67\% \pm 0.95\%$ (in *Z. ellipticum*) to $9.34\% \pm 0.58\%$ (in

Z. bisectum), respectively. The stomatal density of the adaxial epidermis and the abaxial epidermis ranged from 0.28 ± 0.44 (in *Z. ellipticum*) to 19.05 ± 2.31 (in *Z. zerumbet*) and from 19.30 ± 4.94 (in *Z. ellipticum*) to 88.46 ± 16.44 (in *Z. bisectum*), respectively.

Trichomes

Trichomes were found on the abaxial surface in all species studied (Figs 2E, F, 3A–C), and occasionally also on the adaxial surface in *Z. ellipticum*, *Z. xishuangbannaense* and *Z. bisectum*. Two types of trichome were recorded: Type 1, a delicate, simple unicellular trichome, straight or curly, and easily detached (Figs 2E, 3A, B, D, E), which was found in all species studied except for *Z. corallinum* and *Z. montanum*; Type 2, a stout, simple unicellular trichome, straight with pointed apex and swollen trichome base (Figs 2F, 3C, F), which was found in only two species, *Z. corallinum* and *Z. montanum*.

Oil cells

Oil cells occurred frequently on the abaxial surface in all samples of the *Zingiber* species studied, and also occurred infrequently in the adaxial epidermis of *Z. densissimum*, *Z. longiligulatum*, *Z. roseum* and *Z. xishuangbannaense*. The oil cells were subrotund and of small size, and usually contained yellow or translucent oil droplets (Fig. 3G–I), which were easily distinguishable from epidermal parenchyma cells.

Crystals

Crystals were found in the epidermis of all *Zingiber* species studied. There were many crystals in the epidermis of *Z. corallinum*, *Z. montanum*, *Z. longiglande*, *Z. tuanjuum*, *Z. cochleariforme*, *Z. guangxiense* and *Z. teres*, but few in *Z. atrorubens*, *Z. recurvatum* and *Z. leptorrhizum*; crystals were rare in the remaining species. The crystals were usually rhombic and square (Fig. 3J–L), and were mainly distributed above or near the veins in the abaxial epidermis (Fig. 3L), as well as in the intercostal regions (Fig. 3J, K), while only being present in the adaxial epidermis of *Z. corallinum* and *Z. montanum*.

Discussion

Our results show that the epidermal cells of *Zingiber* species are very similar in shape, i.e. hexagonal or polygonal, with non-sinuous anticlinal walls; the cells are arranged parallel to leaf veins. The results are consistent with those of previous studies on seven species of *Zingiber* (Olatunji 1980; Nyawuame and Gill 1990; Jayasree 2007) and other genus species in Zingiberaceae (Table 2).

Similarly to a number of other Zingiberaceae genera, the stomata of *Zingiber* are amphistomatic, tetracytic and aligned in a linear-axial orientation (Table 2). The distribution of the stomatal apparatus can be useful for taxonomic studies of Zingiberaceae. For example, the stomatal apparatus of three genera, *Alpinia*, *Elettaria* and *Globba*, are distributed

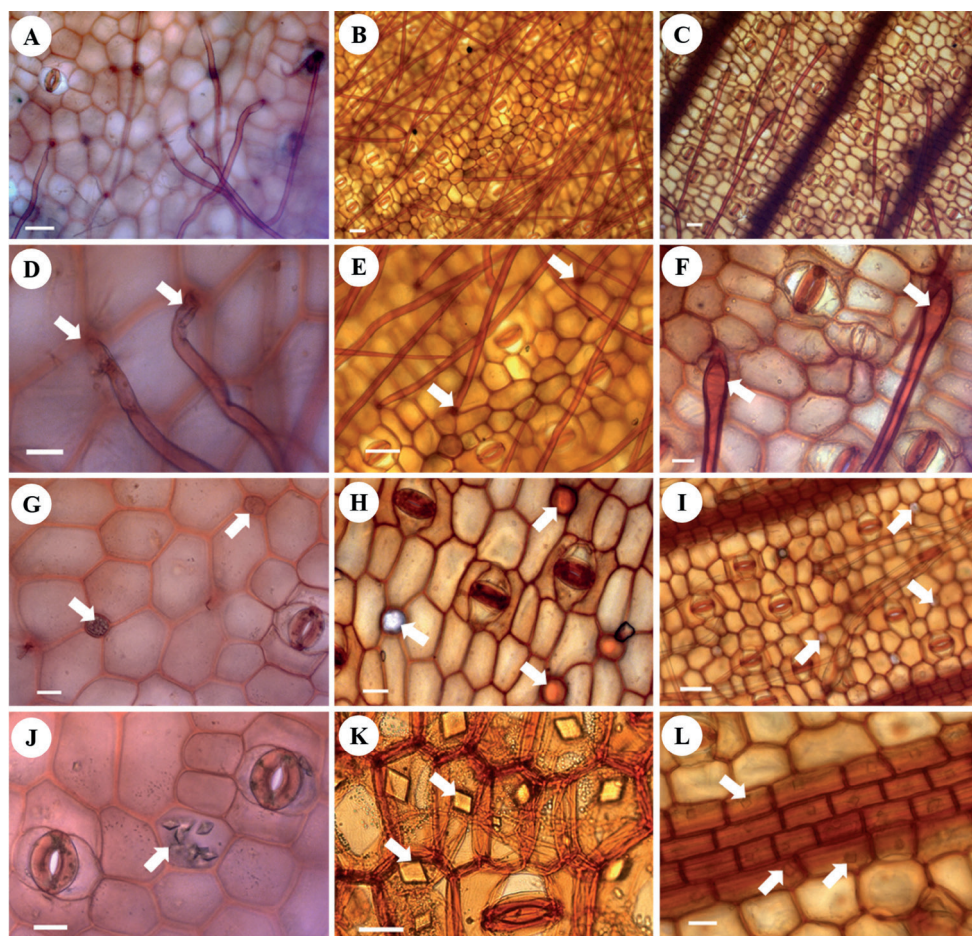


Figure 3. Characters of trichomes, oil cells and crystals in leaf epidermis of *Zingiber* shown by light microscopy **A, B** delicate trichome of *Z. ellipticum* (**A**) and *Z. densissimum* (**B**) **C** stout trichome of *Z. corallinum* **D, E** detail of delicate trichome of *Z. ellipticum* (**D**) and *Z. densissimum* (**E**) showing the trichome base (white arrows) **F** detail of stout trichome of *Z. corallinum* showing the swollen trichome base (white arrows) **G–I** oil cells (white arrows) of *Z. ellipticum* (**G**), *Z. orbiculatum* (**H**) and *Z. montanum* (**I**) **J, K** crystals distributed in the epidermal cells (white arrows) of *Z. ellipticum* (**J**) and *Z. guangxiense* (**K**) **L** crystals distributed above the veins (arrow pointing to crystal) of *Z. montanum*. Scale bars: 50 μm (**A, B, C, E, I, K**); 20 μm (**D, F, G, H, J, L**).

near the veins in rows, as well as being randomly distributed in the intercostal regions, while in most other genera of Zingiberaceae the stomata are always randomly distributed in the intercostal regions. These three genera can therefore be distinguished on this basis from other genera of Zingiberaceae. In addition, our results show that the stomatal density and stomatal index of the species in sect. *Pleuranthesis* are significantly lower than in sects. *Zingiber* and *Cryptanthium*. This suggests that stomatal density (or stomatal index) could allow species of sect. *Pleuranthesis* to be distinguished from other species of *Zingiber*.

Previous studies (Tomlinson 1956) found two types of trichome on the epidermis of Zingiberaceae, the stout trichome (“Borste”) and the delicate trichome (“Weichhaare”).

Our results also show these two types of trichomes on the epidermis of the *Zingiber* species studied. Similarly to other genera of Zingiberaceae, all trichomes on the leaf surfaces of *Zingiber* were unicellular (Table 2). However, delicate trichomes were found on the epidermis of most species of *Zingiber*, while stout trichomes (“Borste”) were only found in two species, *Z. corallinum* and *Z. montanum*. This indicates that *Z. corallinum* and *Z. montanum* are closely related and markedly different from other species of *Zingiber*. In addition, the trichomes on the epidermis of three genera (*Alpinia*, *Amomum* and *Elettaria*) from the subfamily Alpinioideae are all “Borste” (Table 2), while those of the genera in the subfamily Zingiberoideae are either “Borste” and/or “Weichhaare”. This indicates that the type of trichome can have taxonomic significance in Zingiberaceae. According to various molecular phylogenetic trees (Kress et al. 2002, Williams et al. 2004, Liang et al. 2020), the stout trichome exists in both the derived groups (such as *Kaempferia*, *Hedychium* and *Zingiber*) and basal groups (such as *Alpinia*, *Amomum* and *Elettaria*) of Zingiberaceae, while the delicate trichome is only present in the derived groups. Within the tribe Zingibereae, the genus *Curcuma* only has the stout trichome, while the other three genera, *Kaempferia*, *Hedychium* and *Zingiber*, have both types. This suggests that the latter three genera should be closely related to each other, and more distantly related to *Curcuma*, consistent with the molecular phylogenetic trees (Kress et al. 2002; Liang et al. 2020). These results also suggest that the characteristics of leaf epidermal trichomes have systematic and taxonomic significance for Zingiberaceae.

Previous studies have shown that oil cells, which often occur in the mesophyll, root and rhizome (Sherlija et al. 1998, Tang et al. 2010, Uma and Muthukumar 2014), are present in all species of Zingiberaceae (Tomlinson 1959). Oil cells are responsible for the production of volatile compounds that provide fragrance for the leaves of

Table 2. Comparable leaf epidermal characters of the nine genera in Zingiberaceae.

Genus	Epidermal cell		Stomatal apparatus		Trichome	Oil cell	References
	Shape	Anticlinal wall	Type	Distribution			
<i>Boesenbergia</i>	hexagonal or polygonal	not sinuous	tetracytic	randomly distributed in the intercostal regions	delicate trichome	present in abaxial epidermis	d, e
<i>Curcuma</i>	polygonal	not sinuous	tetracytic	distributed in the intercostal regions	stout trichome	present in abaxial epidermis	b, e, k, m, l
<i>Hedychium</i>	polygonal	not sinuous	tetracytic	distributed in the intercostal regions, sometimes above the veins	delicate trichome	present in abaxial epidermis	e, g, l
<i>Kaempferia</i>	polygonal	not sinuous	tetracytic	distributed in the intercostal regions	stout trichome and delicate trichome	present in both epidermal layers	d, e, l
<i>Globba</i>	polygonal	not sinuous	tetracytic	randomly distributed in the intercostal regions or distributed in rows near veins	delicate trichome	present in both epidermal layers	e, f, l
<i>Zingiber</i>	hexagonal or polygonal	not sinuous	tetracytic	randomly distributed in the intercostal regions	stout trichome and delicate trichome	frequently present in the abaxial epidermis; also occurs in the adaxial epidermis	a, e, l
<i>Alpinia</i>	polygonal	not sinuous	tetracytic	randomly distributed in the intercostal regions or distributed in rows near veins	stout trichome	present in abaxial epidermis	c, e, i, j, l
<i>Amomum</i>	polygonal	not sinuous	tetracytic	distributed in the intercostal regions	stout trichome	present in abaxial epidermis	e, i, k, l
<i>Elettaria</i>	hexagonal or polygonal	not sinuous	tetracytic	more frequent distributed closer to the veins	stout trichome	present in abaxial epidermis	e, i, l

Notes: a, this study; b, Chen and Xia 2010; c, Hussin et al. 2000; d, Hussin et al. 2001; e, Jayasree 2007; f, Kajornjit et al. 2018; g, Martins et al. 2010; h, Patel 1975; i, Salasiah and Meekiong 2018; j, Talip et al. 2005; k, Tang et al. 2010; l, Tomlinson 1956; m, Xiao et al. 2004.

Zingiberaceae species (Victório et al. 2011). We found that oil cells occur in the leaf abaxial epidermis of all *Zingiber* species, and also occur in the adaxial epidermis of three species from sect. *Cryptanthium*, *Z. longiligulatum*, *Z. roseum* and *Z. densissimum*, and one species from sect. *Zingiber*, *Z. xishuangbannaense*, but not at all in the leaf adaxial epidermis of the primitive sect. *Pleuranthesis*. Similarly, oil cells in most genera of Zingiberaceae are found mainly in the abaxial epidermis, but in the adaxial epidermis of only three genera of the subfamily Zingiberoideae (Table 2), *Kaempferia*, *Globba* and *Zingiber*. Thus, the distribution of oil cells is a useful characteristic in identifying species and sections of *Zingiber*, as well as genera of Zingiberaceae.

Crystals are usually rhombohedral, rod-like or acicular, sometimes occurring in clusters that resemble a coarse sand, and are commonly found in the hypodermis of the lamina in families of Zingiberales, such as Musaceae, Cannaceae and Heliconiaceae, but rarely in leaf epidermis (Tomlinson 1959, 1961, Triplett and Kirchoff 1991). Crystals were recently found in costal epidermal cells on both leaf surfaces in some genera of Zingiberaceae, such as *Globba*, *Alpinia*, *Amomum* and *Elettaria* (Tomlinson 1956; Hussin et al. 2000; Talip et al. 2005; Jayasree 2007; Kajornjit et al. 2018; Salasiah and Meekiong 2018). We also found crystals in the epidermal cells of all species of *Zingiber*. The crystals only occur in the abaxial epidermis of most species of *Zingiber*, but in both epidermal layers of *Z. corallinum* and *Z. montanum*, showing that the two species *Z. corallinum* and *Z. montanum* are closely related and markedly different from other species of *Zingiber*. Thus, crystals in leaf epidermis can also have systematic and taxonomic significance for *Zingiber*.

Z. ellipticum, the sole member of sect. *Pleuranthesis* in China, was preliminarily identified as a new species, *Plagiostachys elliptica* of the genus *Plagiostachys* by Tong and Xia (1987) based on the character of the spike inflorescence breaking through the leaf sheaths laterally. Subsequently, it was transferred to sect. *Pleuranthesis* under the genus *Zingiber* by Wu et al. (1996), because of its spherical pollen grains and two floral characters: i) the labellum has basally connate lateral staminodes; ii) the elongated anther appendage is wrapped around the style. The above analyses of leaf epidermal micromorphology of *Zingiber* spp. also show that the leaf epidermal characters of *Z. ellipticum* from sect. *Pleuranthesis* are basically consistent with other *Zingiber* species, suggesting that this species has a close interrelationship with other *Zingiber* species. However, there are obvious differences in stomatal density in leaf epidermis between *Z. ellipticum* from sect. *Pleuranthesis* and the species from the other two sections of *Zingiber*. This suggests that the species of sect. *Pleuranthesis* form a distinct taxon within the genus of *Zingiber*. This has been confirmed by molecular phylogenetics (Kress et al. 2002; Theerakulpisut et al. 2012), but further molecular, morphological, anatomical and palynological studies are needed for a systematic classification. In addition, the two species of sect. *Zingiber*, *Z. corallinum* and *Z. montanum*, both of which have stout trichomes and crystals in both epidermal layers, are markedly different from other species of *Zingiber*. Moreover, their inflorescence and leaf morphology are similar, which can make their identification confusing. These observations highlight the need for the systematic relationship between and classification of the two species to be determined after further study.

Conclusions

As in other genera of Zingiberaceae, the epidermal cells of *Zingiber* are hexagonal or polygonal, with non-sinuuous anticlinal walls, with the cells arranged parallel to leaf veins. Tetracytic stomata are distributed on both surfaces, and oil cells and crystals are common. Although the overall epidermal morphology is similar among *Zingiber* species, stomatal density, trichome type and distribution of oil cells and crystals can offer valuable systematic and taxonomic information. Two types of trichome are found in *Zingiber*: delicate trichomes are present in most species, while stout trichomes with a swollen base are present in *Z. corallinum* and *Z. montanum*, which is a novelty for *Zingiber*.

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